STRUCTURE-ACTIVITY RELATIONSHIPS OF CARDIOTONIC PREGNANES: SYNTHESIS, RECEPTOR BINDING AND PHARMACOLOGY

BY

YULIN YAN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

1994

ISBN 0-315-99010-4

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GLOSSARY

AcO	acetoxy
AIBN	azobisisobutyronitrile
Bz	benzoyl
CG	cardiac glycosides
CNS	central nervous system
COSY	correlated spectroscopy
CMA	chlormadinone acetate
DMF	dimethylformamide
EIMS	electron ionization mass spectrometry
EtOH	ethanol
EtSH	ethylthiol
FAB	fast atom bombardment
Fetizon's	$AgCO_3$ on Celite
GC	gas chromatography
hv	UV irradiation
HSAB	hard-soft acid and base principle
IC_{50}	inhibitory dose (50% inhibition)
IR	infrared spectrometry
LD_{50}	lethal dose (50% mortality)
LAH	lithium aluminum hydride
LTBAH	lithium tri-t-butoxylaluminohydride
LTEBH	lithium triethylborohydride
MS	mass spectrometry
MeOH	methanol
m.p.	melting point
MPA	17α-acetoxy-6α-methylpregn-4-ene-3,20-dione
NBA	N-bromoacetamide
NBS	N-bromosuccinimide
NKA	sodium potassium adenosine triphosphate
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
PDC	pyridinium dichromate
ppm	parts per million
Pyr	pyridine
RBA	radioligand binding assay
SAR	structure-activity relationship(s)
TBADC	bis(tetrabutylammonium)dichromate
TBAF	tetra-butylammonium fluoride
TBDMSCl	t-butyldimethylsilyl chloride
$TBPA^+$	tris(4-bromophenyl)-aminium hexachloroantimonate
TsOH	p-toluenesulfonic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane
v/v	volume/volume
w/v	weight/volume

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my advisor Dr. J.F. Templeton (Faculty of Pharmacy) for his valuable guidance, great patience, thoughtful encouragement and understanding during the course of my studies.

I also would like to thank my Ph.D. Advisory Committee members: Drs. N.R. Hunter and J. B. Westmore (Department of Chemistry), Dr. C.J. Briggs (Faculty of Pharmacy) and Dr. F.S. LaBella (Department of Pharmacology and Therapeutics) for their helpful advice, discussion, and help during my research and in the preparation of this thesis.

I also wish to offer my gratitude to the members of the Faculty of Pharmacy and the Department of Chemistry for the help they provided me during the period of my graduate studies.

The [^{3}H]ouabain radioligand binding assay, and pharmacological studies reported for the compounds prepared in this thesis were carried out in collaboration with Drs. F. LaBella, D. Smyth and D. Bose, in the Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba. Radioligand binding assay was completed by Mr. D. Stein, Dept. of Pharmacology and Therapeutics.

Mr. K. Marat (Department of Chemistry) recorded the COSY spectra, Mr. T. Wolowiec (Department of Chemistry) recorded the ^{1}H, ^{13}C and NOE NMR spectra, and Mr. W. Buchannon (Department of Chemistry) recorded the mass spectra.

The financial support from the Faculty of Graduate Studies (through the receipt of a Graduate Studies Fellowship, 1991-1993), the Faculty of Pharmacy (Leslie F. Buggey Graduate Scholarship, 1990-1992) and the Department of Chemistry (Teaching Assistantship) of the University of Manitoba is gratefully acknowledged.

Finally, I would like to express my deep gratitude to my wife Lisa for her understanding and encouragement throughout my graduate education and her great help in typing and preparing computer graphics for this thesis.

ABSTRACT

The research described in this thesis is aimed at the development of new cardiotonic compounds with potent inotropy but minimal cardiotoxicity and has the objective to investigate new "lead" structures for further functional group modification.

All of the compounds synthesized have been evaluated for receptor binding affinity in a [^{3}H]ouabain radioligand binding assay. Pharmacological studies of inotropic activity on the heart muscle, and diuretic effects on the kidney, were carried out on 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside (68).

17α-Acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glycosides (68 and 69) have been synthesized and show strong receptor binding affinity, together with a potassium sparing diuretic effect, a useful property for promising cardiotonic candidates as it offers a greater safety margin. As part of an ongoing effort to determine structure-activity relationships (SAR), 14ß-hydroxyprogesterone (94) has been synthesized by a newly developed approach. 14ß-Hydroxyprogesterone (94) displays an improved receptor binding affinity over progesterone (44) and represents emergence of cardiostimulant activity and a balance of positive and negative inotropy, which may be a basis of diminished cardiotoxicity.

In an effort to illustrate effects of stereochemistry and substituents with respect to establishing optimum pharmaco-

phoric patterns, the C_3 α-L-rhamnosides (11, 12 and 88) of 3ß,14ß-dihydroxy-5ß-pregn-20-one, 3ß,14ß,20ß-trihydroxy-5ß-pregnane and their analogues have been prepared from digitoxin (1). With the same objective, 3ß,14ß-dihydroxypregn-4-en-20-one (124), 14ß,20ß-dihydroxypregn-4-en-3-one (128), 14ß,15ß-epoxy-3ß-hydroxypregn-5-ene (138) and the corresponding rhamnosides, 20ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregna-5,14-diene (137) and 14ß,15ß-epoxy-20ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (139), together with related analogues, have been synthesized from the steroidal precursor, 3ß-acetoxypregna-5,16-dien-20-one (100).

Rationalization of structure-activity relationships is based on the result of receptor binding affinity and the pharmacology of the compounds synthesized.

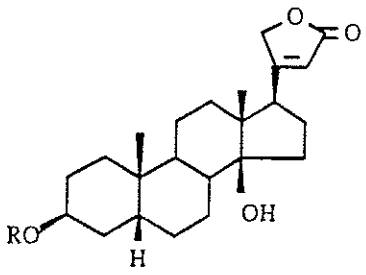
CHAPTER ONE

INTRODUCTION

1.1 Review

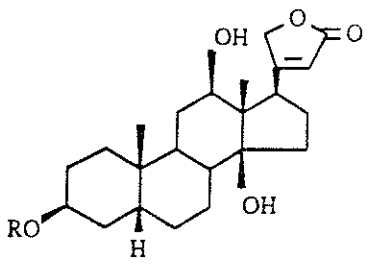
Heart disease is a major cause of death in North America. The most commonly used drugs for the treatment of congestive heart failure and atrial tachyarrhythmias are digitalis glycosides (cardiac glycosides or cardiotonic glycosides) obtained from plants, e.g. *Digitalis lanata* and *Digitalis purpurea*, such as digitoxin (1) and digoxin (2). Some naturally occurring cardiac glycosides are shown in Fig. 1. These drugs, especially digoxin (2), are the most favoured digitaloids and are extensively used clinically. Digoxin (2) is listed as one of the most commonly used prescription drugs in the United States (Flegg and Lakatta, 1984).

Unfortunately, these drugs exhibit a high incidence of toxicity (Repke and Weiland, 1988). They elicit a variety of toxic symptoms including fatigue, nausea, vomiting, anorexia, diarrhoea, dizziness, visual disturbances, other CNS effects and severe cardiotoxicity. The cardiotoxicity, or cardiac arrhythmia, is the major concern in the clinical administration of digitalis drugs. Cardiac arrhythmias may account for the high rate of death in hospitalized patients (Jick, 1974). Extensive reviews of digitalis toxicity have been given, e.g. by Smith et al., 1984. In addition to side effects, the classical cardiac glycosides only function to restore contractility to the diseased myocardium through short term therapy and probably do not reverse the progress of the disease on long term treatment (Seigl, 1986). Mainly due to



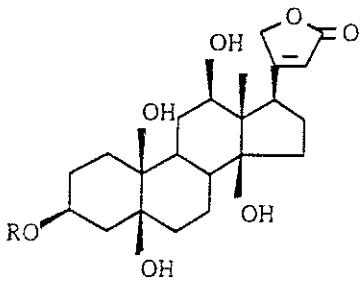
R = $(digitoxose)_3$

Digitoxin (1)



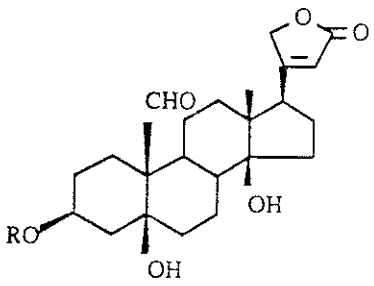
R = $(digitoxose)_3$

Digoxin (2)



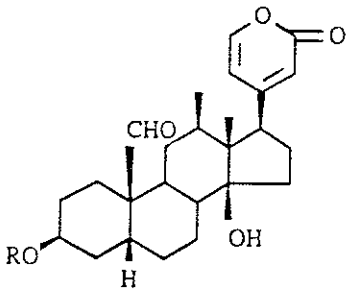
Ouabain (3) R = rhamnose

Ouabagenin (3a) R = H



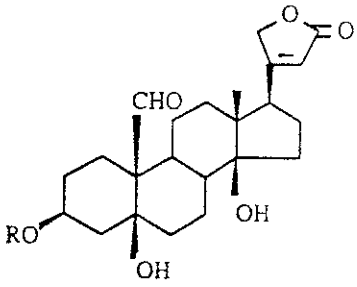
R = glucose-cymarose

K-Strophanthoside (4)



R = rhamnose-glucose

Hellebrin (5)



R = cymarose

K-β-Strophanthin (6)

Fig. 1 Some naturally occurring cardiac glycosides

the severe cardiotoxicities of the naturally occurring cardiotonic glycosides, there is a demand for new cardiotonic drugs with a more acceptable clinical profile, especially an improvement in the therapeutic index (the ratio of therapeutic to toxic dose), a reduced tendency to accumulate in the body, and a more rapid reversal of toxicity when it occurs. The conventional digitalis drugs have been in clinical use for more than two hundred years (Schoner et al., 1986).

Great effort has been made since the 1950's in attempts to develop new cardiotonic compounds (Chen, 1963). SAR studies in the period 1940-1960 were summarized by Chen, 1963, and Tamm, 1963. Thomas and his coworkers have given thorough reviews on the period 1963-1990 (Thomas et al., 1974a, b, 1990). According to Thomas et al., there is, so far, no substantive evidence to show a significant improvement in the therapeutic index despite extensive research.

Today medicinal chemists and pharmacologists face a great challenge regarding the development of new cardiotonic drugs. To ensure that SAR studies are more productive and more predictable, a clearly defined drug research strategy is required. To this end, it is necessary to summarize and extract the significant results obtained from recent metabolic, biological, pharmacological and SAR studies of cardiotonic compounds to provide a theoretical basis for drug design and synthesis. Special interest in this review is focused on

the following aspects which are of relevance to our endeavour of seeking new cardiotonic compounds for clinical use:

1) Characterization of Na^+,K^+-ATPase (NKA)
2) Mechanism of inotropy and cardiotoxicity of digitalis
3) Possible binding forces involved in drug-receptor interaction of cardiac glycosides
4) Effect of pharmacophoric patterns, conformations and stereochemistry on receptor binding affinity
5) Endogenous digitalis-like substances
6) Metabolism of cardiac glycosides
7) Methodology employed for evaluation of structure-activity relationships (SAR)

1.2 Characterization of Na^+,K^+-ATPase (NKA)

It was proposed by Repke in 1964 that NKA, a vital and universal enzyme that is present in the plasma membrane of all eukaryotic cells, is the target enzyme inhibited by cardiac glycosides (Repke, 1963a, b; Repke and Portius, 1964; Repke et al., 1965). Since then, NKA has been studied thoroughly and used as a powerful tool to investigate the drug-receptor interaction at the molecular level (Repke and Weiland, 1988). The investigation of how cardiotonic glycosides manipulate NKA, together with vital aspects of cellular homeostasis, has been consistently pursued.

Great effort has been directed towards the determination of the structure of NKA (Anner, 1985; Herbert et al., 1985), sequencing of NKA (Shull et al., 1985), location of the

digitalis binding site (Schwartz et al., 1975; Thomas et al., 1974a), definition of isozymes of NKA (Sweadner, 1979; Sweadner and Farshi, 1987) and differentiation of species sensitivity to cardiac glycosides (Ball and Lane, 1986). These studies provide profound evidence to support the assumption that NKA is a primary site of action of digitalis. In addition, an increasing amount of evidence indicates the possible existence of different isozymes responsible for initiating therapeutic and cardiotoxic effects, respectively (Sweadner, 1979; Maixent et al., 1987, 1991, 1992). Recent SAR studies show that the biological activity of cardiotonic pregnanes is not entirely dependent on inhibition of NKA (Woolfson et al., 1992); furthermore, the pregnanes which strongly bind to NKA may elicit cardiodepressive effects mediated by a two-binding-site mechanism (LaBella et al., 1989; Pamnani et al., 1991) (Fig. 2)

1.3 Mechanism of Inotropy and Cardiotoxicity of Digitalis

Since cardiotonic glycosides are targeting NKA, it is essential to find sound evidence confirming whether or not NKA can be manipulated by distinct mechanisms to initiate the desired inotropic effect. Understanding the chemical basis of the mechanism of the drug action can help in the design and synthesis of new drugs that can discriminate between substrates responsible for different biological responses, e.g. inotropic and cardiotoxic effects.

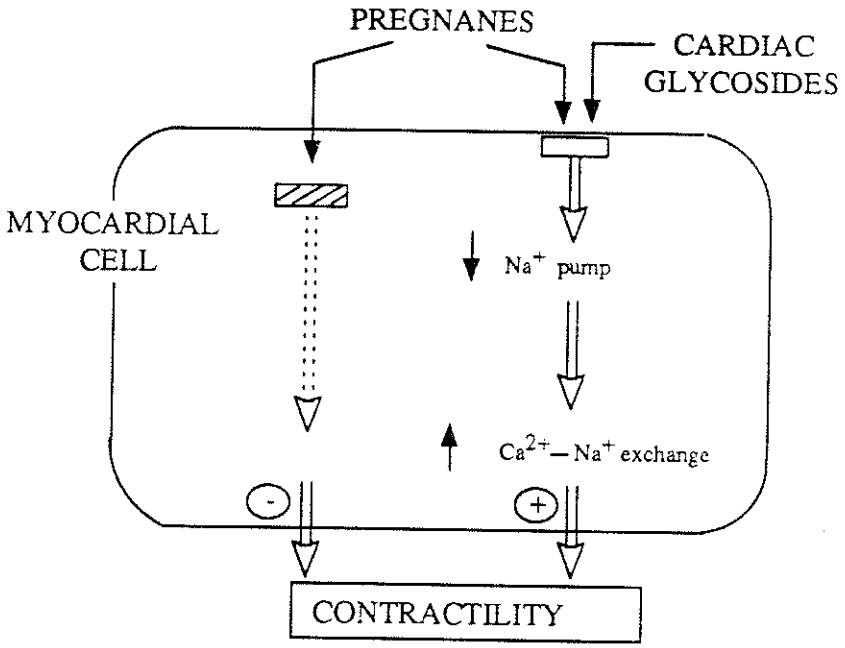


Fig. 2 Proposed two-binding-site mechanism to account for the paradoxical cardiodepression by pregnanes that bind to the digitalis receptor and inhibit the Na^+ pump (LaBella et al., 1989)

Inotropic and Cardiotoxic Effects

Much evidence obtained from pharmacological studies of digitalis compounds, e.g. digitoxin (1), seems to support the assumption that cardiotoxicity is mediated by the same mechanism as that of the therapeutic effects (Akera and Brody, 1978; Repke and Schonfelt, 1984; Repke et al., 1984; Anner, 1985). In other words, the cardiotoxicity is an extension of therapeutic efficacy when the dosage exceeds the therapeutic amount (Gillis and Quest, 1980, 1986; Wilkerson et al., 1981; Thomas et al., 1990). As illustrated in Fig. 3 (Wilkerson, 1981; Thomas et al., 1990), the interaction of digitalis compounds with NKA causes inhibition of the "sodium pump" and hence increases the concentration of Ca^{2+} ion by a sodium-calcium exchange mechanism. Eventually the calcium ion interacts with Ca-troponin C to generate positive inotropic activity. However sodium pump inhibition also causes calcium overload and consequently initiates arrhythmias, i.e. cardiotoxic effects. Clearly, if pharmacological studies continue to prove that there are no distinct mechanisms to initiate therapeutic and cardiotoxic effects, respectively, the chance of developing new cardiotonic drugs with the desired therapeutic profile is slim.

Theoretical Basis for Improving Therapeutic Index

Recently, an increasing amount of evidence indicates the possible existence of multiple specific binding sites, i.e. isozymes of NKA for cardiac glycosides (Sweadner, 1979;

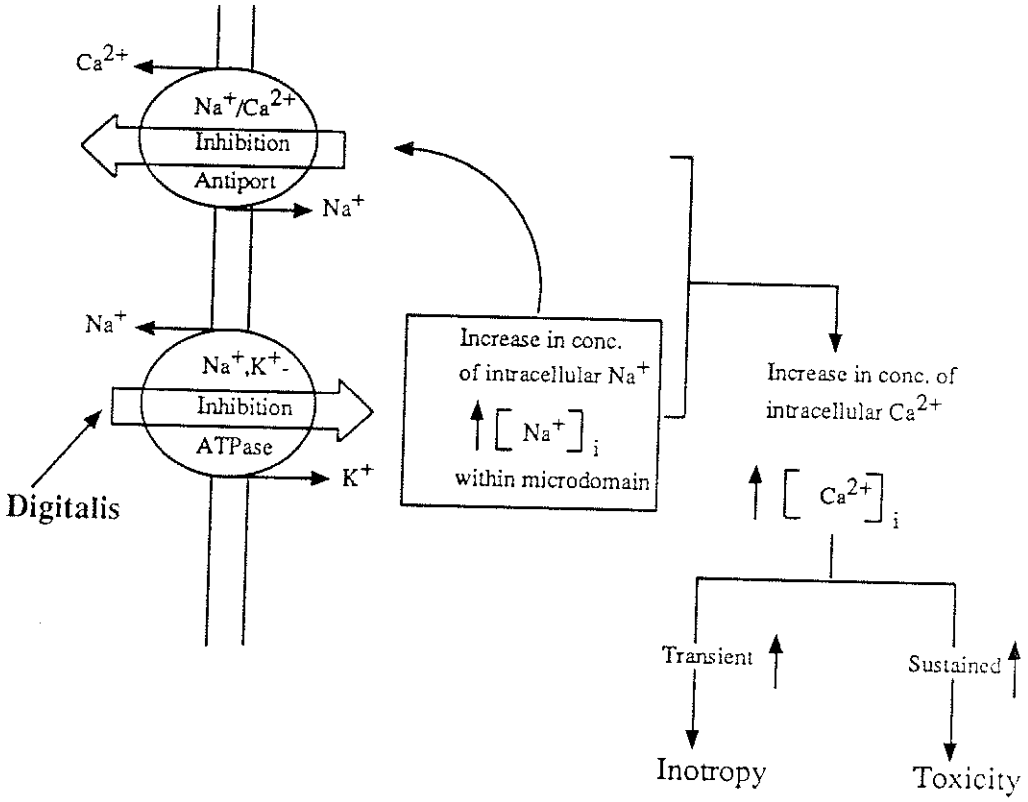


Fig. 3 A proposed mechanism of inotropy and cardiotoxicity of digitalis (Wilkerson, 1981; Thomas et al., 1990)

Erdmann et al., 1980; Sweadner and Gilkeson, 1985; Lytton, 1985; Shull et al., 1986; Fallows et al., 1987; Akera et al., 1986). It has been suggested that the high affinity digitalis binding sites on dog heart muscle NKA are associated with the inotropic effects of cardiac glycosides, while the low affinity sites are associated with toxic effects (Maixent et al., 1987, 1991). These findings were indirectly supported by the results obtained from molecular biology studies, which indicated that the isozymes of NKA have their own distinct biological role (Shull and Lingnel, 1987; Orlowski and Lingrel, 1988). In addition, it has been reported that some pregnane derivatives, with the same steroidal skeleton as digitoxin (1) but different substituents at C_{17}, display stronger inotropy and less cardiotoxicity than digitalis compounds and potassium sparing diuretic effect on the kidney (Templeton et al., 1991a, b; Smyth et al., 1992). The potassium sparing diuretic effect is considered as a useful pharmacological activity to counteract cardiotoxicity since hypokalemia is one of the predisposing factors for cardiotoxicity.

Therefore, existence of the isozymes and unique pharmacological activity, e.g. potassium sparing diuretic effect on the kidney, provides a basis for speculating that new cardiac glycosides with improved clinical profiles can be made by chemical modification of digitoxin (1) to increase selective

receptor binding affinity responsible for favourable inotropy and safety margin over digoxin (2) (Maixent et al., 1992).

Neural effects

It has been shown that the action of the heart is very sensitive to neurological control and that NKA plays a crucial role in the activity of all the neural cells. Much effort has been directed towards localizing the possible site of reaction of digitalis in the CNS and identifying the type of receptors that may be involved. These studies have provided some indirect evidence to support the assumption that an undetermined action of digitalis on the CNS is responsible for many arrhythmias associated with toxic doses of cardiac glycosides (Saxena and Bhargava, 1974, 1975; Helke et al. 1979; Gillis and Quest, 1980; Lechatt and Schmitt, 1982; Somberg, 1984, 1985; Thomas and Tripathi, 1986).

1.4 Possible Binding Forces Involved in Drug-Receptor Interaction of Cardiac Glycosides

Illustration of binding forces involved in drug-receptor interaction can provide insight into drug action and help to determine structural complementarity of drug and receptor, which is crucial for effective drug design. Structural complementarity of drug and receptor is not only recognized as a function of drug molecular shape but also as a result of mutual electronic interactions between drug and receptor. In other words, receptors recognize the electronic density of functional groups and the charge distribution in space through

drug-receptor interactions. This spacial orientation of electronic distribution around functional groups is defined as a pharmacophoric pattern (Humblet and Marshall, 1980; Gund, 1979). In general, binding forces involved in drug-receptor interaction include hydrophobic bonding, dipole moment, charge transfer (Doukas, 1975), electrostatic bonding, London dispersion force, van de Waals forces and hydrogen bonding. Both covalent and noncovalent bonding are found in drug-receptor interactions; but covalent bondings are less common in drug-receptor interactions than noncovalent ones (Kollman, 1980).

In the SAR study of cardiotonic compounds, hydrophobic bonding did not appear to play a significant role in drug-receptor interactions because the lipophilic digitoxin (1) and the hydrophilic ouabain (3) demonstrate the same pattern of kinetics in pig cardiac muscle and brain cortex (Repke, 1985). This conclusion was also supported by a study of the thermodynamics of the interaction of the steroids with cardiac enzymes (Ross and Subramanian, 1981). Short range bonding forces, i.e. London dispersion forces, are important because any variation of the shape of the steroid skeleton (3ß,14ß-dihydroxy-5ß-androstane) resulted in significant loss of interaction energy (Schonfeld et al., 1987). The electrostatic fields of drug and receptor are important, and dominate the long and intermediate range reaction (Repke, 1985). Correlation between the shape of electrostatic potential

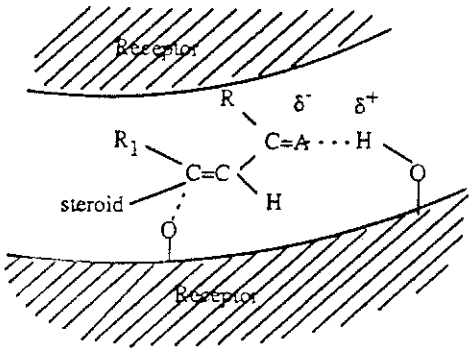
fields of cardiac glycosides and the magnitude of their Gibbs interaction energy provides useful information on the charge distribution in the drug-receptor binding site and can be used as a guide for structural modification (Repke, 1985). Based on this study, positions at C_3, C_{14} and C_{17} were proposed as three energy favourable places for conducting functional group modification (Repke, 1985).

Thomas et al. (Thomas et al., 1974a, b, 1979; Fullerton et al., 1979a, b) proposed a "two-point attachment" involving a hydrogen bond and an ion-dipole interaction (Fig. 4a). They found the fractional positive charge on C_{20} had great influence on biological activity (Fig. 5). Kupchan et al. (Kupchan et al., 1970; Kupchan, 1971) proposed a Michael reaction between a nucleophile on the enzyme and a carbon with positive charge in an enone system which initiated covalent bonding (Fig. 4b). Repke and coworkers (Repke et al., 1963a, b; Repke, 1974; Portius and Repke, 1964) assumed that the molecular dipole moment was crucial in drug-receptor interaction, and hydrogen bonding between oxygen atoms in the C_{17} side chain (a receptor site for hydrogen bonding) and K^+ binding site on NKA (an amino acid side chain of the receptor) might be involved (Fig. 4c).

1.5 Effects of Pharmacophoric Patterns, Conformations and Stereochemistry on Receptor Binding Affinity

Based on X-ray crystallography studies (LaBella et al., 1985), computer graphic comparisons (Fullerton et al., 1979a,

a. Two-point attachment (Thomas et al., 1979)



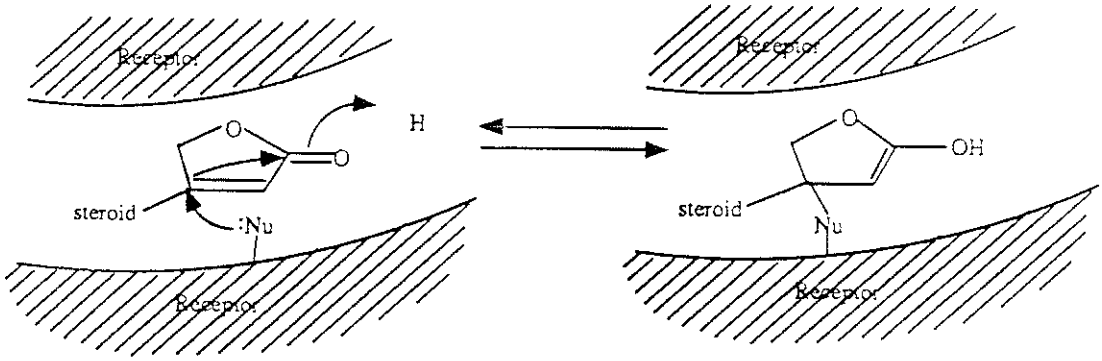
R, R_1 = rest of lactone, or

R_1 = H, R = OCH_3

(possibly H or small alkyl)

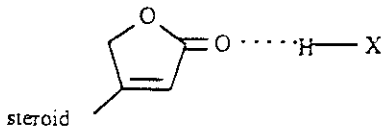
A = O, N, possibly other atoms

b. Michael attack (Kupchan et al., 1970, 1971)



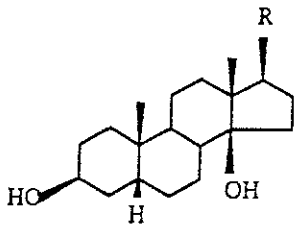
Nu = biological nucleophile such as -SH

c. Hydrogen bonding (Repke et al., 1963, 1974)



X = K^+ binding site on Na^+, K^+ -ATPase

Fig. 4 Proposed models of digitalis-receptor binding



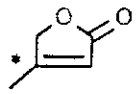
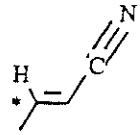
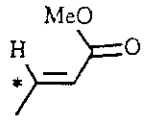
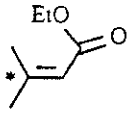
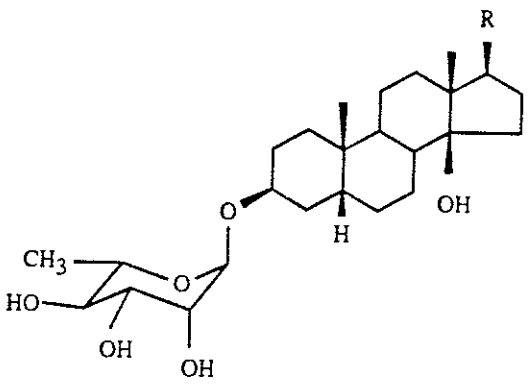
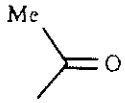
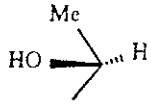
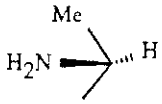
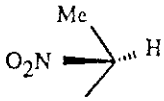
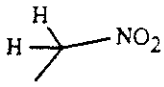
Comp. No.	R	^{13}C NMR(ppm)	Activity
7		175.3	active compounds in deceasing order of activity
8		162.6	
9		155.4	
10		155.0	

Fig. 5 Some digitoxin analogues with replacement of 17β-lactone ring by isosteric and isoelectronic groups. Charge distribution patterns allegedly due to resonance effects in these analogues correlated with their biological activities. The numbers refer to ^{13}C NMR chemical shifts for C_{20} (*) in ppm (Thomas et al., 1990).

b), potential energy calculation (Repke, 1985), functional group modification (Thomas et al., 1990; Templeton et al., 1991a, b, 1992) and characterization of endogenous digitalis-like substances (Kim et al., 1980; LaBella et al., 1985; Templeton et al., 1988; Bose et al., 1988), the structure-activity relationships of cardiotonic compounds have undergone considerable revision in the past decade. Significant conclusions are summarized as follows:

1. The C_{17}-lactone ring, although associated with strong receptor binding affinity and inotropic activity, is not essential, as previously suggested. Certain analogues with isosteric or bioisosteric groups on the C_{17} side chain instead of the lactone ring also display strong receptor binding affinity as illustrated in Fig. 5 (Thomas et al., 1974a, b, 1990) and Fig. 6 (Templeton et al., 1991a, b, 1992). Furthermore, the replaced side chain may not necessarily be coplanar and isosteric as thought previously (Thomas et al., 1990). Additionally, less sterically hindered and more freely rotating side chains with heteroatom(s) on C_{17} can enhance receptor binding affinity to a level comparable to those of digitoxin (1) and ouabain (3) (Templeton et al., 1991a, b, 1992).
2. The receptor binding affinity of progesterone derivatives is enhanced when substituents are introduced at C_{17} and C_6 which cause inversion of rings A and D to a digitalis-like conformation (Kim et al., 1980) through a long range



Comp. No.	R	IC_{50} (nM)[a]
11		150
12		75
13		72
14		45
15		12

compounds in increasing order of receptor binding affinity

[a] IC_{50} represents the concentration that inhibits binding of [^{3}H]ouabain by 50%

Fig. 6 Some digitoxin derivatives with replacement of 17β-lactone ring by non-isosteric groups and their receptor binding affinity in a [^{3}H]ouabain radioligand binding assay (Templeton et al., 1991, 1992a, b)

conformational (or conformational transmission) effect, as defined by Barton et al. (Barton et al., 1960; Blickenstaff and Sophasan, 1972). Steric rather than electronic effects of the substituents on C_6 are the determinant for receptor binding affinity (Kim et al., 1980).

3. Stereochemistry at A/B and C/D ring junctions affects receptor binding affinity. The C/D cis junction is not necessary (Weiland et al., 1991; Smyth et al., 1992); but it is usually required for high receptor binding affinity and strong inotropy.
4. The 14ß-OH group is required for strong binding affinity, but can be replaced by a 14ß-amino group (Maixent et al., 1987, 1991). Replacement of the 14ß-OH with hydrogen weakens but does not eliminate inotropy (Repke 1985; Thomas et al., 1990).
5. The sugar moiety is necessary for high binding affinity and, among the naturally occurring sugars, α-L-rhamnose (34) is superior to others (Thomas et al., 1990). The removal of the sugar moiety usually causes a decrease of activity.

Function of the Aglycone

It is known that the physiological actions of cardiotonic compounds correspond with the structure of their genins. The aglycone moiety, particularly the substituent at C_{17}, is involved in primary binding to NKA (Yoda, 1974; 1976). 3ß,14ß-Dihydroxy-5ß-androstane in the cardiac glycosides is a "lead"

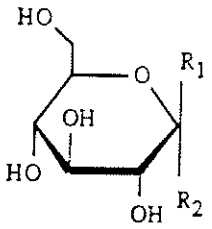
structure serving as the structural determinant for physiological action (Schonfeld et al., 1987). Most of the modified derivatives of the cardiac glycosides possess this basic structure and show a similar mechanism of inhibition (Schonfeld et al., 1987). Recently, extensive research has been focused on functional group modifications at C_3, C_{14} and C_{17}, configuration changes at the C_5 and C_{14} from A/B and C/D ring cis to trans (Thomas et al., 1990), hydroxylation of aglycones (Shigei et al., 1973), introduction of double bond at the C_4-C_5 (Templeton et al., 1988; Templeton and Yan, 1992) and/or C_5-C_6 positions (Wieland et al., 1991), amination of aglycones (Schmidt et al., 1979), replacement of 14ß-hydroxy with hydrogen (Repke, 1985; Thomas et al., 1990), the replacement of 14ß-hydroxy group with amino group (Maixent, 1987, 1991) and the replacement of C_{17} lactone ring with other functional groups (Wiesner and Tsai, 1986; Thomas et al., 1974a, b, 1979, 1990; Templeton et al., 1991a, b, 1992).

The C_{17}-lactone ring can be replaced by isosteric groups as illustrated in Fig. 5 (Thomas et al., 1990) and bioisosteric groups in Fig. 6 (Maixent et al., 1987; Templeton et al., 1991a, b, 1992). They all showed strong inhibitory activities (Maixent et al, 1987, 1991, 1992; Thomas et al., 1990; Templeton et al., 1991a, b, 1992) and also exerted potassium diuretic effects on the kidney (Templeton et al., 1991a, b, 1992; Smyth et al., 1992).

Functions of C_3-Sugar Moiety

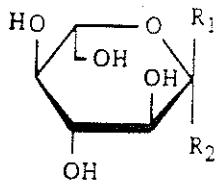
The sugar moiety is not involved in primary binding but helps to stabilize the drug-receptor complex (Yoda, 1973, 1974; Schwartz et al., 1975; Francis et al., 1985). It also facilitates distribution and transportation of the drugs to target enzymes (Repke, 1963a). A number of studies have been conducted including the definition of binding characteristics of the sugar moiety (Fullerton et al., 1990), examination of the functional groups on the sugar moiety (Erdmann and Schoner, 1974; Brown and Thomas, 1983; Fullerton et al., 1984), effect of different sugar configurations on binding (Thomas et al., 1990), conformational factors that affect the SAR (Watson et al., 1984; Chiu and Watson, 1985), enhancement of potency by introduction of mono-sides containing 6'-deoxy sugars, such as α-L-rhamnose (34) (Templeton et al., 1991a, b, 1992), effect of 6'-deoxy sugar on onset and offset time (Thomas et al., 1990), acylation of sugar hydroxyl groups (Yoda and Yoda, 1975; Haustein, 1974; Brown and Thomas, 1983), amino substitution of the sugar moiety (Caldwell and Nash, 1978), other sugars rather than pyranoses, such as furanose (Prisbe et al., 1986), and non-sugar substituents, e.g. 3ß-ester (Henderson et al., 1965), 3ß-α-amino acid (Valcavi et al., 1981), 3ß-succinate (Kossler 1963) and hydrogen (Takeda et al., 1970).

Among the sugars found in nature, which possess different configurations, (Fig. 7), α-L-rhamnose (34) is superior to



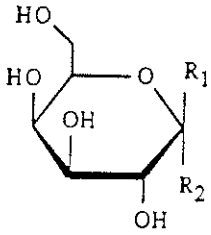
β-D-glucose (16), R_1 = OH, R_2 = H

α-D-glucose (17), R_1 = H, R_2 = OH



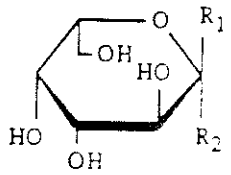
α-L-glucose (18), R_1 = OH, R_2 = H

β-L-glucose (19), R1 = H, R_2 = OH



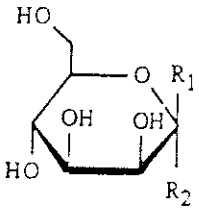
β-D-galactose (20), R_1 = OH, R_2 = H

α-D-galactose (21), R_1 = H, R_2 = OH



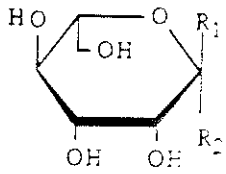
α-L-glactose (22), R_1 = OH, R_2 = H

β-L-galactose (23), R_1 = H, R_2 = OH



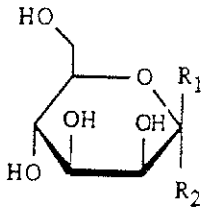
β-D-mannose (24), R_1 = OH, R_2 = H

α-D-mannose (25), R_1 = H, R_2 = OH



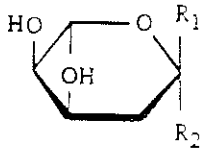
α-L-mannose (26), R_1 = OH, R_2 = H

β-L-mannose (27), R_1 = H, R_2 = H



β-D-digitoxose (28), R_1 = OH, R_2 = H

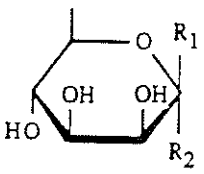
α-D-digitoxose (29), R_1 = H, R_2 = OH



α-L-digitoxose (30), R_1 = OH, R_1 = H

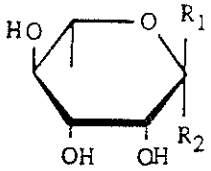
β-L-digitoxose (31), R_1 = H, R_2 = OH

Fig. 7 Some sugars found in the naturally occurring cardiac glycosides (Thomas et al., 1990)



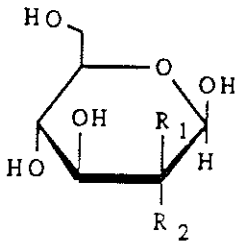
β-D-rhamnose (32), R_1 = OH, R_2 = H

α-D-rhamnose (33), R_1 = H, R_2 = OH



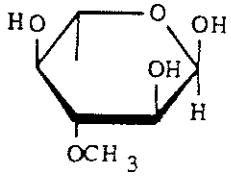
α-L-rhamnose (34), R_1 = OH, R_2 = H

β-L-rhamnose (35), R_1 = H, R_2 = OH

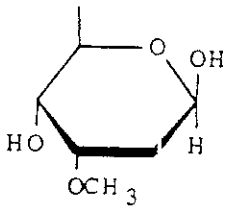


β -D-allose (36), R_1 = OH, R_2 = H

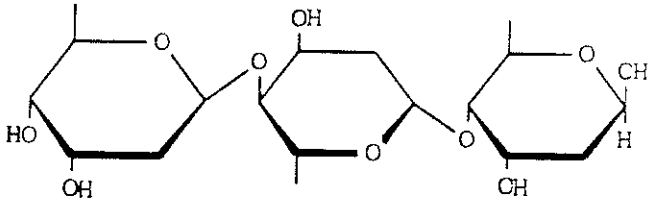
β -D-altrose (37), R_1 = H, R_2 = OH



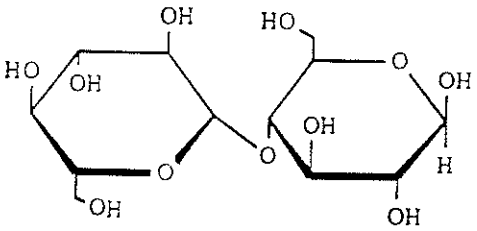
α -L-thevetose (38)



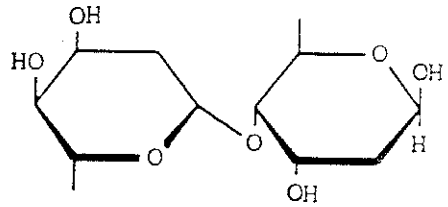
β -D-cymarose (39)



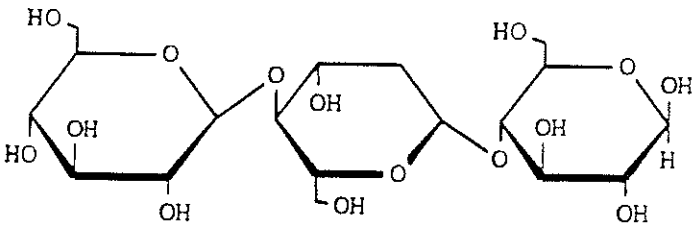
tris-digitoxose (40)



bis-glucose (41)



bis-digitoxose (42)



tris-glucose (43)

Fig. 7 (continued)

others and usually increases potency by more than ten times compared to the corresponding genins (Thomas et al., 1990) both in the cardiac glycosides (CG) and pregnane series (Templeton et al., 1991a, b, 1992). The magnitude of additive activity of the sugar moiety is also dependent on the structure of the genins, i.e. the sugar moiety plays only a secondary role in binding activity. In addition, introduction of a C_3 sugar enhances potency and alters pharmacokinetic and pharmacodynamic parameters.

1.6 Endogenous Digitalis-like Substances

Recently, research interest has been focused on endogenous digitalis-like substances (LaBella et al., 1979, 1982, 1984, 1989; Kim et al., 1980; Haber and Hanpert, 1987; Kelly and Smith, 1989). Rationalization for the existence of digitalis-like substances in humans arose mainly on three grounds:

1) Similarity of biogenesis

Both the steroidal hormones and digitalis compounds are synthesized biologically by using isopentenyl pyrophosphate as the building block via the intermediate progesterone. It is assumed that steroidal hormones can be converted to structures closer to digitalis compounds under mammalian enzymatic conditions. For example, enzymatic reduction of the steroid 4-en-3-one function may produce 5-ß metabolites with the A/B cis ring junction (Clark, 1979; LaBella, 1982; Kubli-Gafias, 1984).

2) Clinical implications (Haber and Haupert, 1987)

Some pathological conditions, such as hypertension, are found to be associated with substances in the blood that are capable of inhibiting NKA, initiating natriuresis and causing vasoconstriction (Schoner et al. 1986; Graves and Williams 1987; Haddy 1987; Haber and Haupert, 1987). A range of diverse chemical structures has been suggested (Thomas et al., 1990), including lipophilic substances likely to be steroidal compounds (LaBella, 1982; Thomas et al., 1987; Goto et al., 1988).

3) Digitalis-like activity found in mammalian heart

An endogenous digitalis-like activity was found in animal heart homogenates that demonstrated a strong inhibitory activity on NKA. The binding affinity of this substance (named Cardiodigin) was twice that of digitoxin (1) (Godfraind et al., 1982). However pure substances have not yet been isolated. The isolation procedures necessary for identification of these compounds are complicated by the possible formation of polypeptides of steroidal compounds and their instability under conditions of isolation and purification. Recently ouabain has been identified in mammals by Goto et al., 1990 and digoxin by Weinburg et al., 1992.

In an attempt to obtain evidence for the existence of endogenous digitalis-like substances, LaBella et al. (LaBella et al., 1979, 1982, 1985, 1989; Kim et al., 1980) tested a

large number of natural or semi-synthetic steroids from the pregnane, androstane and estrane families. They found that certain progesterone derivatives competitively bind to NKA (Fig. 8) and demonstrate digitalis-like action. Among these derivatives, chlormadinone acetate (45) showed a more potent receptor binding affinity than ouabagenin (3a) (Kim et al., 1980), but primarily depressed myocardial contractility, i.e. elicited negative inotropy. As a continuous effort, LaBella et al. proposed a two-binding-site mechanism to explain these differences (LaBella et al., 1989) illustrated in Fig. 2. Some progesterone derivatives with a C/D trans ring junction display, in addition to receptor binding affinity, sodium depleting and potassium sparing effects on the kidney function (Smyth et al., 1992), and are cardiostimulant (Weiland et al., 1991).

Templeton et al. synthesized 14ß-hydroxyprogesterone (94) with a C/D cis ring junction (Templeton et al., 1987a; 1988; Templeton and Yan, 1992). This compound exerted a positive inotropic effect followed by transient negative inotropic effects (Templeton et al., 1988; Bose et al., 1988). The balance of positive and negative inotropy may account for the improvement of the therapeutic index of some cardiotonic compounds (LaBella et al., 1982, 1989). It was the first time that a modified steroidal hormone has been shown to display strong cardiostimulant properties.

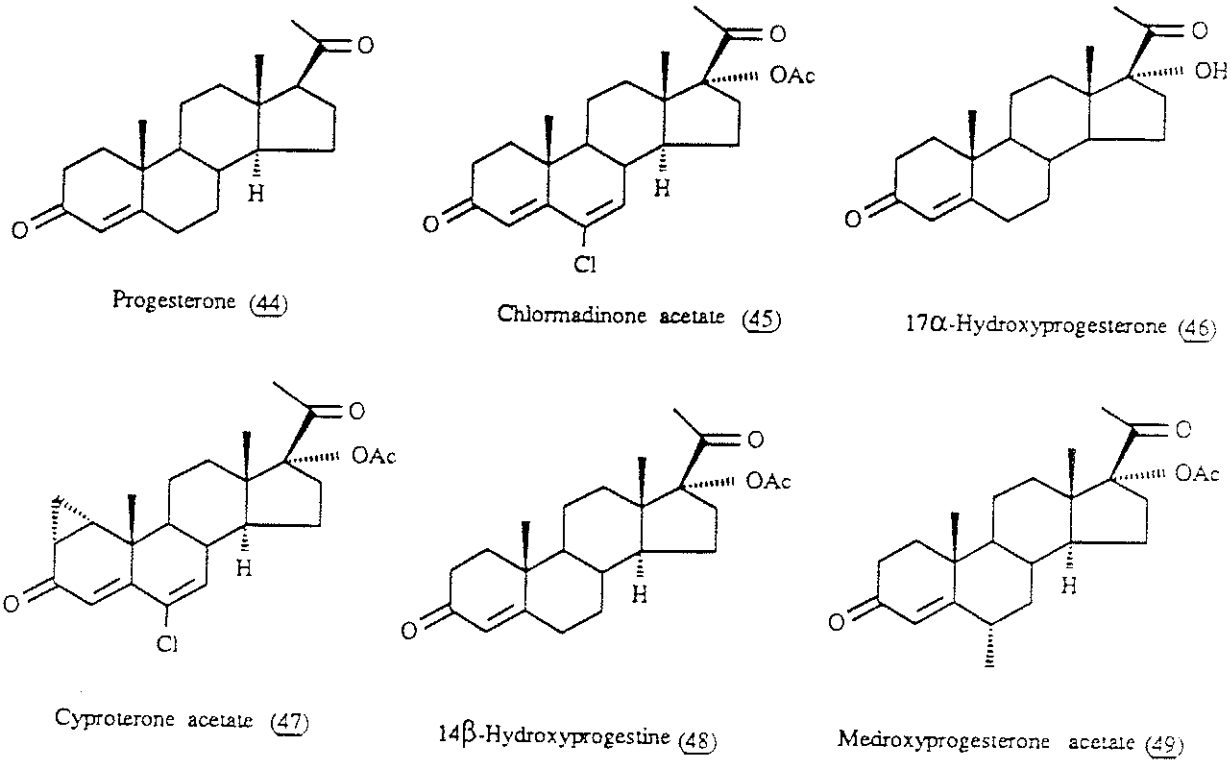


Fig. 8 Certain steroidal hormones that bind to the digitalis receptor

1.7 Metabolism of Cardiac Glycosides

Studies on the metabolism of cardiac glycosides have made great contributions to the rational use of digitalis glycosides, which is complicated by its small therapeutic index and differing individual response. The metabolism of the digitalis compounds has been well documented (Bodem and Dengler, 1976; Greef, 1981; Erdmann et al., 1986; Thomas et al., 1990). The patterns of metabolism of digitoxin (1) and digoxin (2) are similar. The metabolism of digitoxin (1) and digoxin (2) is illustrated in Fig. 9. The specific features of this metabolism are summarized as follows:

1) Digitoxin (1) and digoxin (2) have a half-life of 200 and 132 hr, respectively, in man. The half-life denotes the efficiency of elimination processes including biotransformation and excretion.
2) The metabolism of digitoxin (1) and digoxin (2) occurs mainly in kidney and liver.
3) The metabolic reactions under mammalian enzyme systems include hydrolysis, glucuronidation, hydroxylation and epimerization.
4) Lipophilicity is a determinant for the absorption and reabsorption of digitalis.
5) Cardiac glycosides are transported from plasma to bile where enterohepatic recycling occurs. The enterohepatic recycling of some deconjugated materials accounts for the

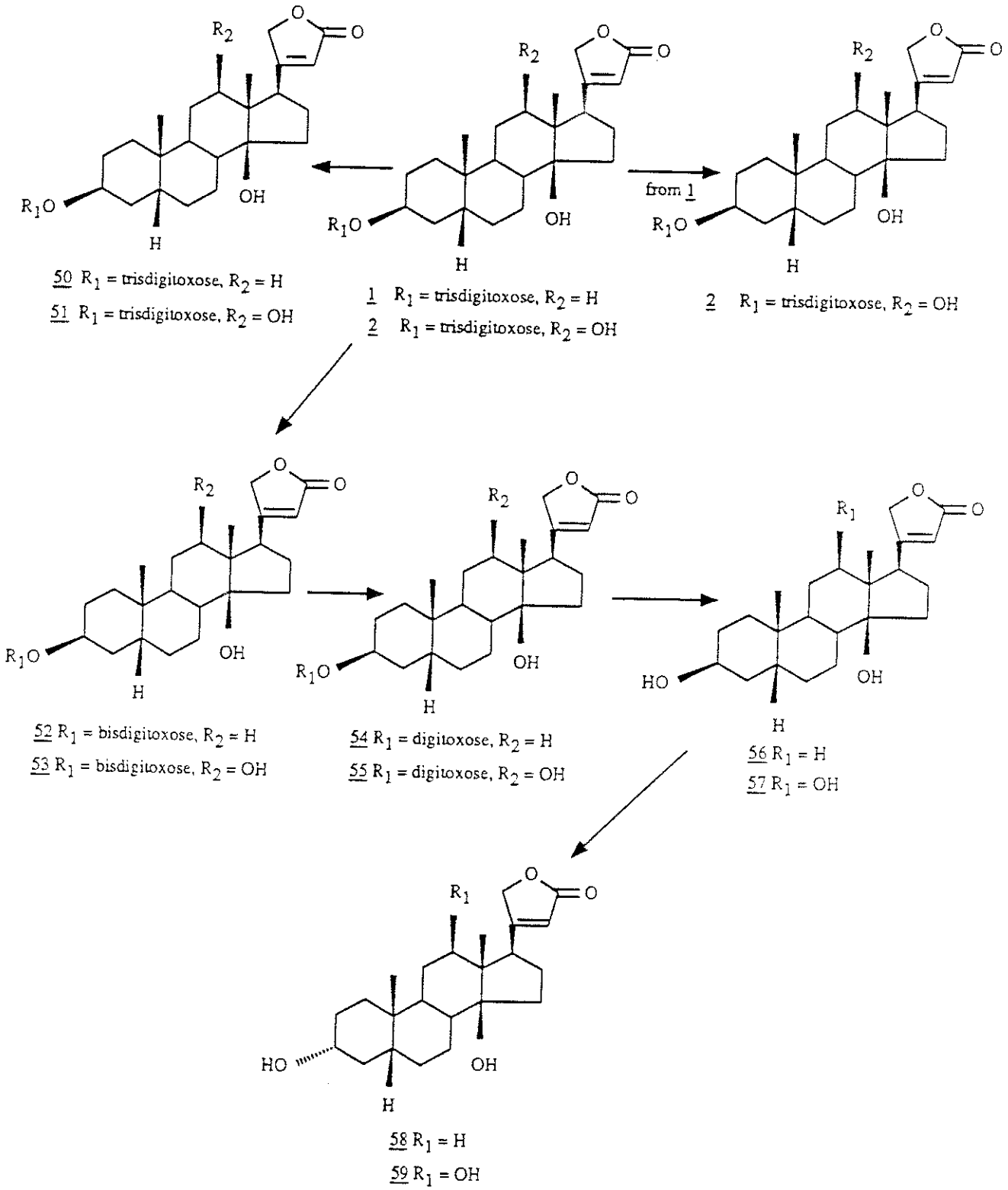


Fig. 9 Metabolites of digitoxin and digoxin (Thomas et al., 1990)

long life of cardiac glycosides, e.g. slow elimination of digitoxin (1) (Storstein, 1974).

6) As for digitoxin (1), the rate limiting step is the formation of digitoxigenin monodigitoxoside (54) derived from the didigitoxoside (52) after loss of the terminal digitoxose (Fig. 9).

The LD_{50} of the cardiac glycosides is not proportional to either the rate of biliary excretion or the concentration of CG in the myocardium (Russell and Klaassen 1972, 1973). Nevertheless the enterohepatic recycling due to reabsorption of deglucuronidated metabolites can explain the delay in eliminating cardiac glycosides from the body. Since the biliary excretion rate is proportional to lipophilicity (Russell and Klaassen, 1972, 1973), lipophobic digoxin (2) and ouabain (3), which have more hydroxyl groups, have relatively shorter life times than digitoxin (1), and hence are excreted from the body faster than digitoxin (1) (Wright, 1960).

1.8 Methodology Employed for Evaluation of Structure-Activity Relationships (SAR)

Representative methods used in the SAR studies are the computation of conformational flexibility (Hochne and Pfeiffer, 1983; Repke and Schonfeld, 1984; Repke, 1984; Ovchinnikov et al., 1985), dipole moment vector analysis (Repke, 1984; Repke and Schonfeld, 1984), calculation of molecular electrostatic potential field (Repke, 1984, 1986; Repke and Schonfeld, 1984), determination of effector and

receptor kinetics (Repke, 1984; Repke and Schonfeld, 1984), determination of equilibrium and transition state thermodynamic interactions (Beer et al., 1988), correlation of structure and potency through a thermodynamic procedure (Schonfeld et al., 1987; Beer et al., 1988), computer graphic modelling (Osman et al., 1979; Fullerton et al., 1979a, b; Stuper et al., 1979; Ahmed et al., 1983; Cohen, 1983, 1985; Watson et al., 1984; Chiu and Watson, 1985), X-ray crystallography (Chiu and Watson, 1985; LaBella et al., 1985) and proton NMR (Fesik et al., 1986, 1987, 1989; Marat et al., 1993). Other methods used include the Hansch relationship (Simon et al., 1977, 1978; Ganellin, 1977; Benlow, 1979; Martin, 1978; Hansch, 1971), Free-Wilson analysis (Free and Wilson, 1964; Wieland et al., 1991). These methods are used in attempts to correlate the structures and conformations of cardiotonic compounds to their biological activities for use in a predictable way.

1.9 Drug Research Strategies

Based on the literature survey, drug research strategies with the aim of the improvement of the therapeutic index can be outlined as follows:

1) Characterization of endogenous digitalis-like substances promises to pave a way for the development of novel cardiotonic glycosides. The balance of negative and positive inotropic effects, and potassium sparing

diuretic effects on the kidney can be considered as necessary properties to cope with cardiotoxicity.

2) The therapeutic index of cardiac glycosides can be improved by chemical modification of digitoxin (1) at C_3, C_{14} and C_{17}. It is possible that cardiotoxicity can be selectively reduced via enhancement of receptor binding affinity.

1.10 Research Objectives and Achievements

Based on the drug strategies outlined above, four goals have been set as the topic of this thesis:

1. To synthesize glycosides of steroidal hormones with typical steroidal structural features, i.e. with 14α-H and a double bond at the C_4-C_5 position. An objective of this study is to search for new "lead" compounds by the synthesis of progesterone glycosides and evaluation of their receptor binding affinity and pharmacological activity.
2. To modify digitoxin (1) at the C_3, C_{14} and C_{17} positions. The goal of this study is to collect necessary information for appropriate pharmacophores and pharmacophoric patterns required for high receptor binding affinity.
3. To design and synthesize progesterone derivatives with the combined structural features of steroidal hormones and classical cardiac glycosides, i.e. coordination of 14ß-hydroxy group and a double bond at C_4 or C_5. This study aims at the development of novel cardiac glycosides

with potential clinical value. i.e. an improvement of the therapeutic index.

4. To determine the structure-activity relationships of the compounds synthesized.

In this thesis, discussion of the chemical synthesis, the development of new synthetic methods, mechanisms of the reactions, structural identification, evaluation of receptor binding affinity and pharmacological activities have been given. An attempt has also been made to address structure-activity relationships of the compounds synthesized, and to make suggestions for further SAR studies among the pregnane derivatives.

In Chapter 2, the synthesis of 17α-acetoxy-3ß-hydroxy-pregn-4-en-20-one and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glycosides (67, 68 and 69) will be described (Scheme 1). The glycosylation of 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (63) and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) is accompanied by dehydration under Koenigs-Knorr reaction conditions and hence gives low yields. Glycosylation of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) with 1-bromo-α-L-triacetylrhamnoside (75) was found to be even more difficult than the corresponding glycosylation with 1-bromo-ß-D-tetra-acetylglucoside (72) under the Koenigs-Knorr reaction conditions. A discussion regarding this difference is given. A successful synthesis of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one rhamnoside (69) has been achieved

(>30%). The mechanism of the stereoselective and chemoselective reduction of the carbonyl group at C_3 in the genins to the corresponding 3ß-hydroxyl group, the stereoselective glycosylation, and important factors in the glycosylation will also be discussed.

In Chapter 3, the synthesis of the rhamnosides (11 and 12) and the glucoside (87) of 3ß,14ß,20ß-trihydroxy-5ß-pregnane and 3ß,14ß-dihydroxy-5ß-pregn-20-one by chemical modification of the C_{17} side chain of digitoxin (1) will be presented (Scheme 8).

In Chapter 4, the synthesis of 14ß-hydroxyprogesterone (94) will be described (Scheme 12). The key step in this synthesis, as illustrated in Scheme 12, is to reduce the triene (104) chemoselectively to give the corresponding diene (106) in high yield (83%). The triene (106) has been, for the first time, reduced chemoselectively by photochemical induction in the presence of tri-n-butyltin hydride to give the diene (106) in high yield. The diene (106), after Oppenauer oxidation, reduction with LAH, bromohydrin formation by treatment with NBA and ring closure with potassium carbonate to give the epoxide (108) followed by oxidation with PDC, eventually gave 14ß-hydroxy-progesterone (94) in an overall 30 % yield (Templeton and Yan, 1992). The intermediates 3ß-hydroxypregn-5,14-dien-20-one (106) and 14ß,15ß-epoxypregn-4-ene-3,20-dione (108) serve as starting materials

for the preparation of the glycosides (125, 133 and 142) described in Chapter 5 (Schemes 18, 19 and 21).

In Chapter 5, 3ß-hydroxypregna-5,14-dien-20-one (106) and 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) have been converted to 3ß,20ß-dihydroxypregna-5,14-diene α-L-rhamnoside (137) and 14ß,15ß-epoxy-3ß,20ß-dihydroxypregn-5-ene α-L-rhamnoside (139) (Schemes 20 and 21). Synthetic approaches to the preparation of 3ß,14ß-dihydroxypregn-4-en-20-one (124), 14ß,20ß-dihydroxypregn-4-en-3-one (128) and 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) have also been developed (Schemes 18, 19 and 21).

Receptor binding and pharmacology of the compounds synthesized will be discussed in Chapter 6. In a [^{3}H]ouabain radioligand binding assay, the rhamnoside (69) is about ten times more potent than the glucoside (68) (Table I). In addition to receptor binding affinity, 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside (68) displays potassium sparing effects on the kidney, as illustrated in Figs. 14 and 15 (Smyth et al., 1992), a significant clinical factor in the development of new cardiotonic glycosides. The receptor binding affinity of all synthesized compounds described in Chapter 3 have been compiled in Table II. The receptor binding affinities and the pharmacological properties of 14ß-hydroxy-progesterone (94) and related compounds are illustrated in Table III, and Figs. 16 and 17. The results of RBA of the synthesized compounds in Chapter 5 have been compiled in Table

IV. Structure-activity relationships of the cardiotonic compounds based on the results of the receptor binding and pharmacology have be discussed.

In conclusion, as described in Chapter 7, the significance of the results has been summarized and possible further SAR studies suggested.

CHAPTER 2

SYNTHESIS OF THE GLYCOSIDES OF 17α-ACETOXY-3ß-HYDROXY-6α-METHYLPREGN-4-EN-20-ONE AND STRUCTURALLY RELATED COMPOUNDS

2.1 Introduction

It has been generally accepted that the cardiotoxic and therapeutic effects of digitalis glycosides are mediated by the same mechanism. In other words, the therapeutic and cardiotoxic effects are inextricably linked. The cardiotoxicity is simply the consequence of the inotropic stimulation (Wilkerson, 1981; Thomas et al., 1990). However, as described in Chapter 1, LaBella and Templeton have proposed that a separation of the cardiotoxic and therapeutic effects is possible. How to reduce the cardiotoxicity becomes the core of the research in the development of new cardiotonic compounds.

Recently, research interest has been focused on the search for endogenous digitalis-like substances (Chow et al., 1979; Kim et al., 1980; LaBella et al., 1982, 1985; Kelly and Smith, 1989). Certain progesterone derivatives have been found capable of binding to NKA which recognizes these progesterone derivatives as being digoxin-like (LaBella et al., 1979, 1985; Kim et al., 1980; Templeton et al., 1988; Bose et al., 1988). These findings are important for SAR studies since the geometries of steroidal hormones are quite different from those of the classic cardiac glycosides (Fig. 10). Progesterone derivatives possess a C/D trans ring junction and a double bond at the C_4-C_5 position displaying a rather "flat" molecular geometry. Quite differently, classical cardiac

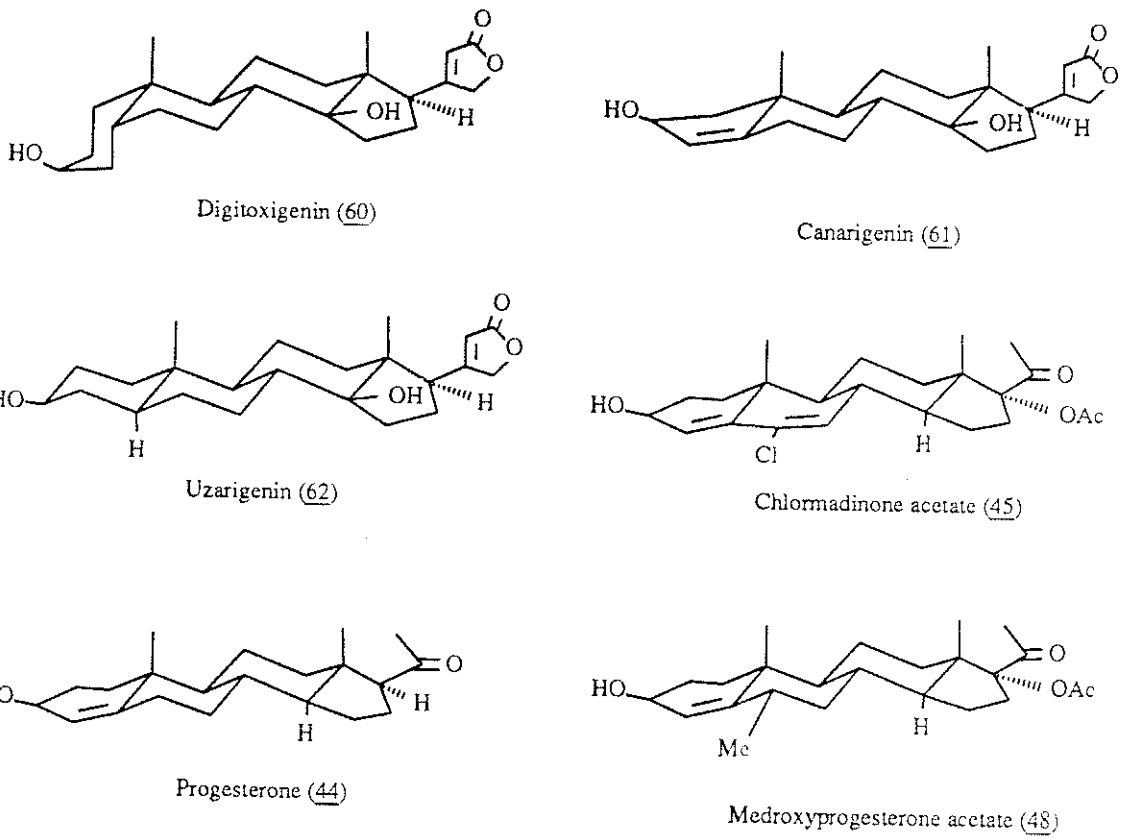


Fig. 10 Structures of digitoxigenin and progesterone derivatives that bind to the digitalis receptor

glycosides have A/B and C/D cis ring junctions and display a doubly "bent" molecular geometry as illustrated in Fig. 10. These progesterone derivatives nevertheless bind to NKA but display negative inotropy instead of positive inotropy (LaBella et al., 1979; Kim et al., 1980; LaBella et al., 1984, 1985). On the other hand, 14ß-hydroxyprogesterone (94) with a C/D trans ring junction displays cardiostimulant activity (Templeton et al., 1988; Bose et al., 1988). The variable magnitude of efficacy indicates that the pregnanes act at another receptor, different from NKA, which initiates cardiodepression (LaBella et al., 1979, 1984). Later, LaBella et al. 1985 proposed a two-binding-site mechanism in an attempt to illustrate the relationships between receptor binding specificity and biological activity (Fig. 2). This model implies that glycosylation of progesterone derivatives may prevent rapid influx across the cell membrane to the intra-cellular receptor binding site (Welshons et al., 1984; Jordan et al., 1985; Horwitz et al., 1985) and eventually lead to enhanced binding selectivity towards the extracellular receptor binding site and, as a consequence, elicit desired pharmacological effects. This assumption was supported by the results reported by Weiland et al. 1991 who synthesized a series of glycosides of steroidal hormones, i.e. the rhamnosides of pregnanes with a C_5-C_6 double bond. These compounds showed strong receptor binding affinities and the emergence of cardiostimulant effect, i.e. positive inotropy.

The negative inotropy, i.e. the antagonistic property demonstrated by progesterone derivatives, has been suggested to counteract digitalis induced arrhythmias. The balance of positive and negative inotropy accounts for the improvement of the therapeutic index of certain pregnanes as suggested by LaBella et al., 1985. The significant pattern of pharmacological activities, i.e. a balance of positive and negative inotropy and potassium sparing diuretic effects on the kidney, indicates that the progesterone derivatives possess a built-in ceiling to counteract cardiotoxicity (LaBella et al., 1989). Therefore progesterone derivatives deserve to be candidates as new "lead" compounds for further functional group modification (LaBella et al., 1985).

One of our research objectives is to search for new "lead" compounds among progesterone analogues by means of chemical synthesis (LaBella et al., 1985, 1989; Templeton et al., 1988; Weiland et al., 1991). 17α-Acetoxypregn-4-ene-3,20-dione (48) and 17α-acetoxy-6α-methylpregn-4-ene-3,20-dione (49), typical hormone derivatives, bind to NKA, and the latter (49) is more potent than the former (48) in a radioligand binding assay. Both of the compounds display exclusively negative inotropic effects. It is of interest to synthesize the glycosides of these compounds and to compare their receptor binding affinity and pharmacological activity to the corresponding genins that we have done.

This study provides insight into the specificity of the drug-receptor interaction which initiates different pharmacological activities useful for modifying cardiotoxicity in drug design. As a result, rational "lead" structures could be outlined for further functional group modification in order to develop new cardiac drugs with an improved therapeutic index.

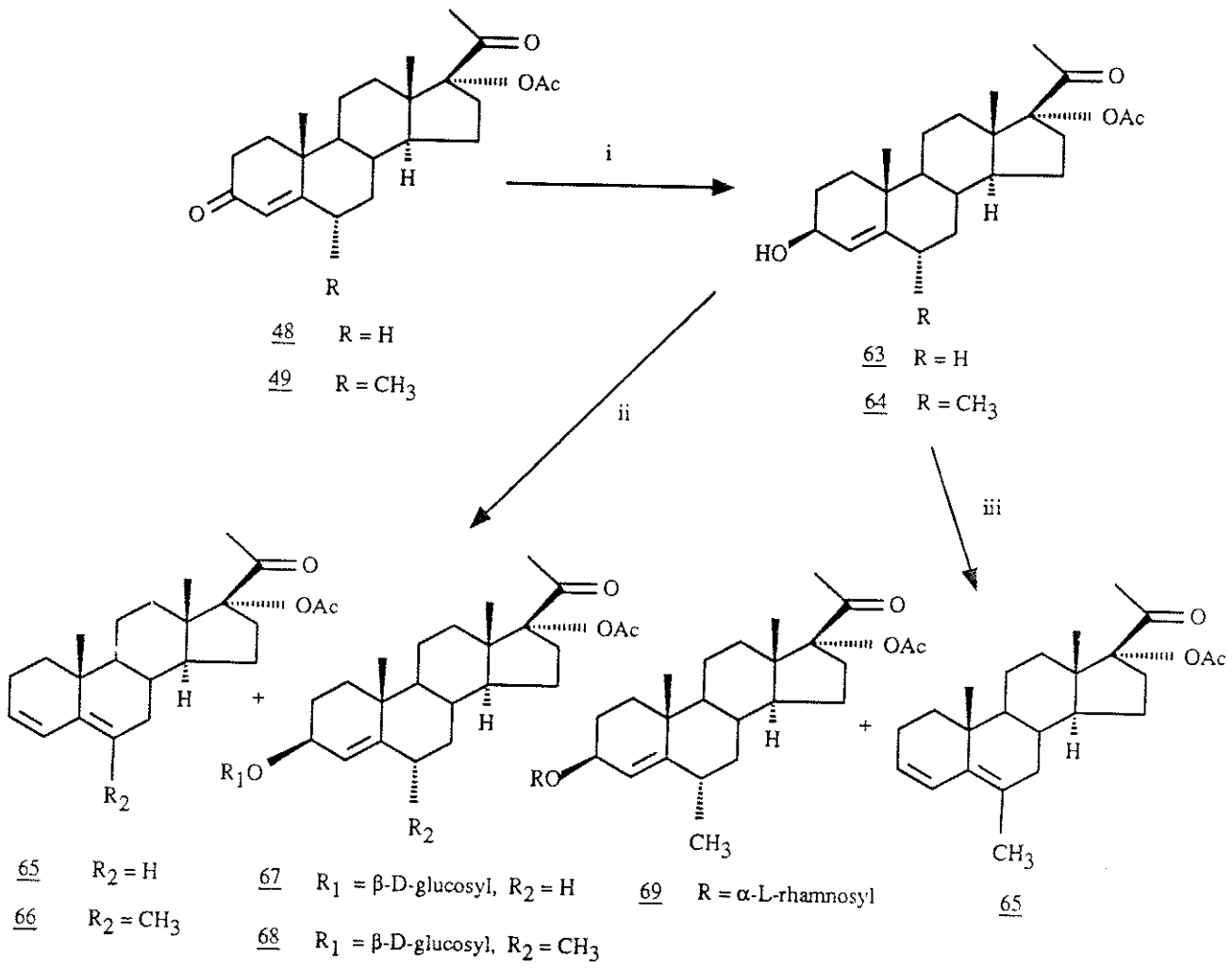
2.2 Synthesis

Introduction

The key synthetic steps in the preparation of the glycosides (67, 68 and 69) of 17α-acetoxy-3ß-hydroxypregn-4-en-20-one and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one with glucose and rhamnose include:

1) Chemoselective and stereoselective reduction of the C_3 carbonyl group to form the corresponding 3ß-OH without affecting other carbonyl groups in the molecule, i.e. the C_{20} carbonyl group and the 17α ester group.
2) Stereoselective glycosylation of the allylic alcohols, i.e. 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (63) and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) with 1-bromo-ß-D-tetraacetylglucose (72) or 1-bromo-α-L-triacetylrhamnoside (75) (Schemes 1 and 2).

Compared to LAH and LTEBH, which are strong reducing agents, LTBAH is considered a mild reducing reagent which can only reduce a ketone to a corresponding hydroxy group without reducing the conjugated double bond or the ester group (March, 1985) at the 17α position. The reduction of 17α-acetoxypregn-



48 R = H

49 R = CH_3

63 R = H

64 R = CH_3

65 R_2 = H

66 R_2 = CH_3

67 R_1 = β-D-glucosyl, R_2 = H

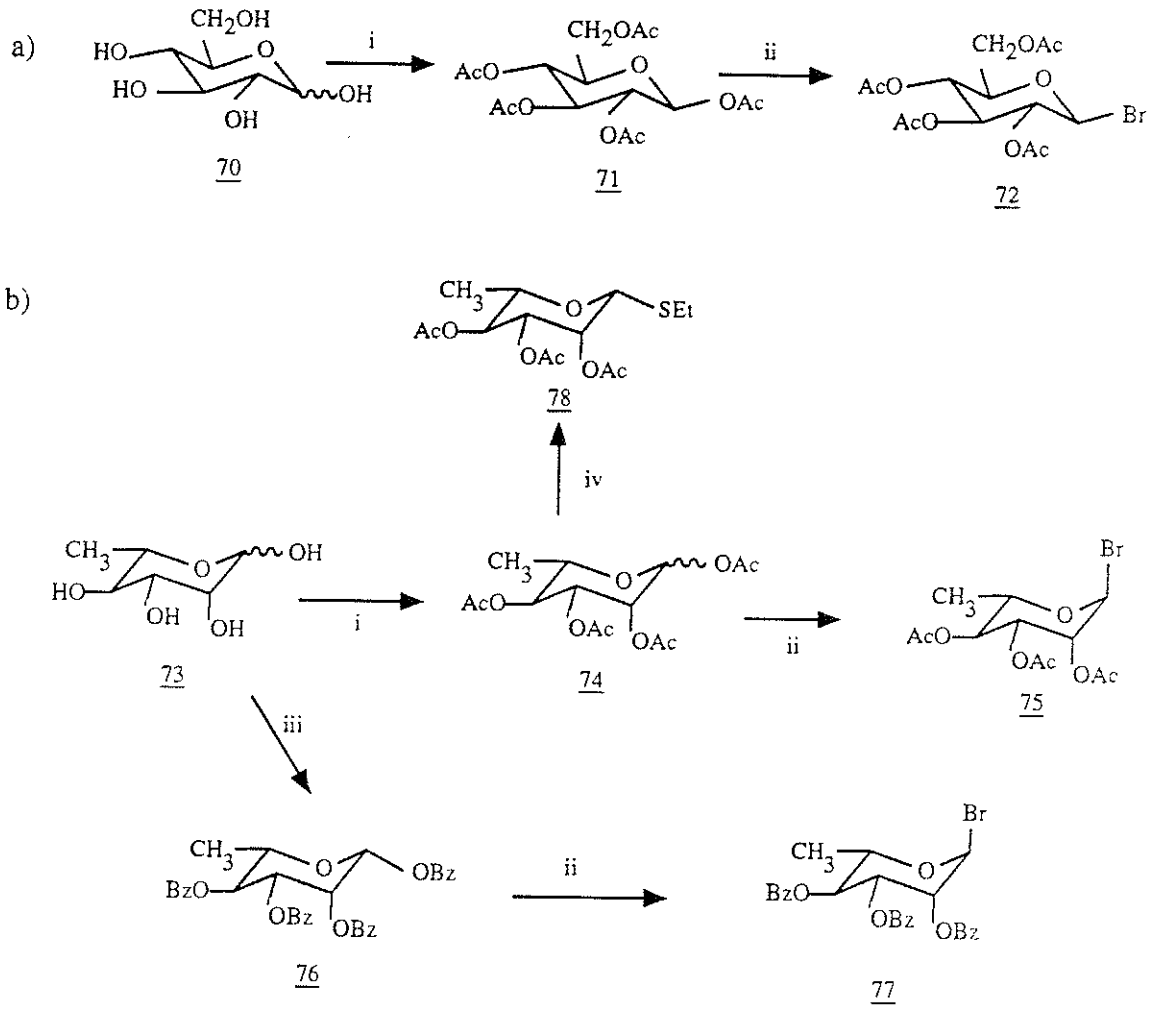
68 R_1 = β-D-glucosyl, R_2 = CH_3

69 R = α-L-rhamnosyl

65

Reagents: i) LTBA, THF; ii) 1-bromo-β-D-tetraacetylglucoside (72), Fetizon's, HgO, $HgBr_2$, molecular sieves, CH_2Cl_2; iii) 1-bromo-α-L-tribenzoylrhamnoside (75), Fetizon's HgO, $HgBr_2$, molecular sieves, diethyl ether

Scheme 1 Synthesis of 17α-acetoxy-6α-methyl-3β-(β-D-glucosyloxy)-pregn-4-en-20-one (68), 17α-acetoxy-6α-methyl-3-β-(α-L-rhamnosyloxy)-pregn-4-en-20-one (69) and related compounds

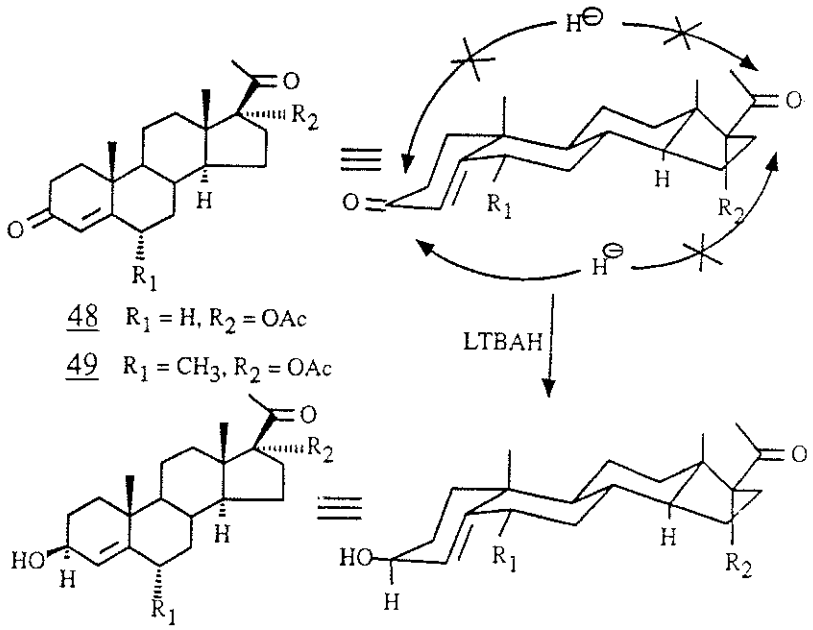


Scheme 2 Synthesis of 1-bromo-β-D-tetraacetylglucoside (72), 1-bromo-α-L-triacetylrhamnoside (75), 1-bromo-α-L-tribenzoylrhamnoside (77) and 1-ethylthio-β-L-triacetylrhamnoside (78)

4-ene-3,20-dione (48) and 17α-acetoxy-6α-methylpregn-4-ene-3,20-dione (49) is highly chemoselective and stereoselective. Only the carbonyl group at C_3 in these compounds is reduced to the corresponding 3ß-hydroxy group, while the carbonyl group at C_{20} remains intact. The high chemoselectivity and stereoselectivity are likely due to the existence of the angular methyl groups at C_{10}, C_{13} and the 17α acetoxy groups, which permits the attack of the hydride on the C_3 carbonyl group from the less steric hindered α face to give the 3ß hydroxyl groups, and block the approaching hydride attack on the C_{20} carbonyl group from either the α or ß face, as illustrated in Scheme 3.

The oldest and, so far, the most extensively used method for glycosylation is the Koenigs-Knorr reaction, which was described in 1901 (Koenigs and Knorr, 1901). Since then many new concepts have been developed with respect to this type of reaction (Juszynski and Flowers, 1972; Wulff and Roble, 1974; Igarashi et al., 1977), which are briefly summarized as follows:

1) A racemic mixture will be obtained due to the formation of an oxonium ion intermediate if there is no neighbouring group participation (Igarashi et al., 1977).
2) High stereoselectivity of glycosylation, i.e. retention of configuration at the anomeric carbon, can be achieved by the participation of neighbouring groups, such as acetyl or benzoyl groups (Juszynski and Flowers, 1972).



48 $R_1 = H, R_2 = OAc$

49 $R_1 = CH_3, R_2 = OAc$

63 $R_1 = H, R_2 = OAc$

64 $R_1 = CH_3, R_2 = OAc$

Scheme 3 A proposed mechanism for chemoselective and stereoselective reduction of the carbonyl group at C_3 in 17α-acetoxypregn-4-ene-3,20-dione (48) and 17α-acetoxy-6α-methylpregn-4-ene-3,20-dione (49)

3) The ease of formation of a cyclic oxonium ion is affected by properties of neighbouring groups and halogen atoms at the anomeric carbon (Arcamone et al., 1975) according to the hard-soft acid and base principle (HSAB) proposed by Pearson (Pearson, 1963).

4) Solvents play an important role in the reactivity and stereoselectivity of the glycosylation (Kronzer and Schuerch, 1974; Wulff and Roble, 1974).

5) The Koenigs-Knorr reaction produces an acidic proton, which affects the formation and stability of the glycosidic bond. Considering this, many organic bases have been used as acid scavengers (Hanessian and Banoub, 1977).

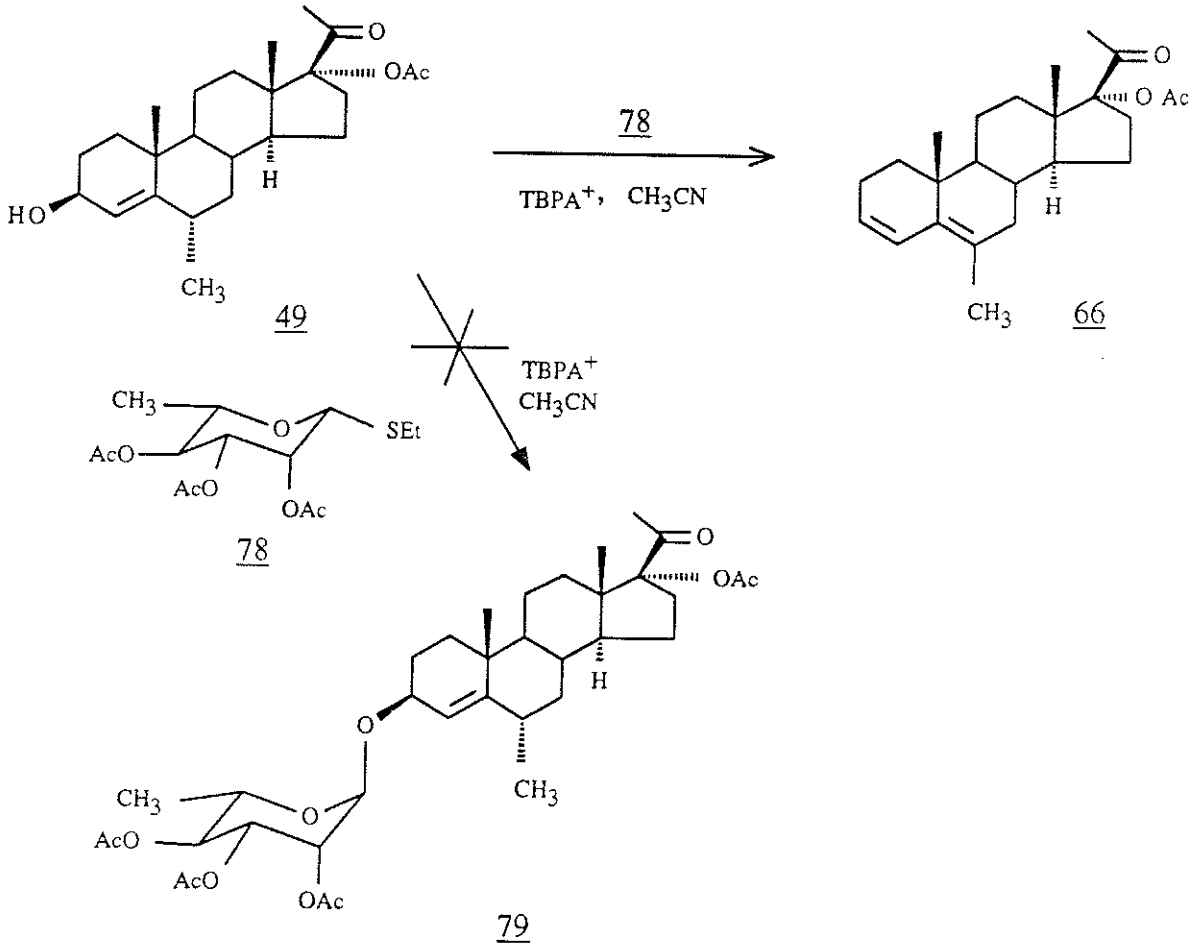
6) The thioglycosides have been used as glycosyl donors in the presence of heavy metals, through electron transfer, to form glycosides (Sinay, 1991). Other types of glycosyl donor have also been reported (Woodward et al., 1981).

The glycosylation of allylic alcohols under the Koenigs-Knorr reaction conditions has not been extensively explored and only a few papers related to this subject have been published (Wulff et al., 1974, Templeton et al., 1988). The reaction readily causes dehydration, to give the diene as a major by-product (Smyth et al., 1992). Furthermore, we have found that 1-bromo-α-L-triacetylrhamnoside (75) is more difficult to couple with the genins than 1-bromo-ß-D-tetra-acetylglucoside (72) under Koenigs-Knorr conditions.

An attempt was made to couple the genins with a thioglycoside (78) as glycosyl donor under mild conditions (Sinay 1991). However the reaction failed to give the expected glycoside (79) and, instead, gave the diene (66) as the major product (Scheme 4). The mechanism of the formation of this diene (66) is not clear.

Results and Discussion

Reduction of 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (48) by treatment with LTBAH in THF gave 17α-acetoxy-3ß-hydroxylpregn-4-en-20-one (63) as the major compound and its 3α isomer as the minor compound. The ratio of the α to the ß isomers is about 5:95 as observed in the ^{1}H NMR spectrum. The stereoselectivity of the reduction can be rationalized by Dunitz's approach geometry (Dunitz et al., 1974), which describes how an approaching nucleophile will attack the carbonyl group at a certain angle (109°). As is seen in Scheme 3, the angular methyl group at C_{10} blocks the approach of the hydride from the ß face and hence allows the nucleophilic attack of the hydride on the carbonyl group at C_3 from the less sterically hindered α face. The stereoselectivity seems to be less important in the formation of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) on treatment with LTBAH compared to 17α-acetoxy-3ß-hydroxy-pregn-4-en-20-one (63). The ratio of the α to ß isomer, i.e. 17α-acetoxy-3α-hydroxy-6α-methylpregn-4-en-20-one and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64), is about 15:85. Lower stereo-



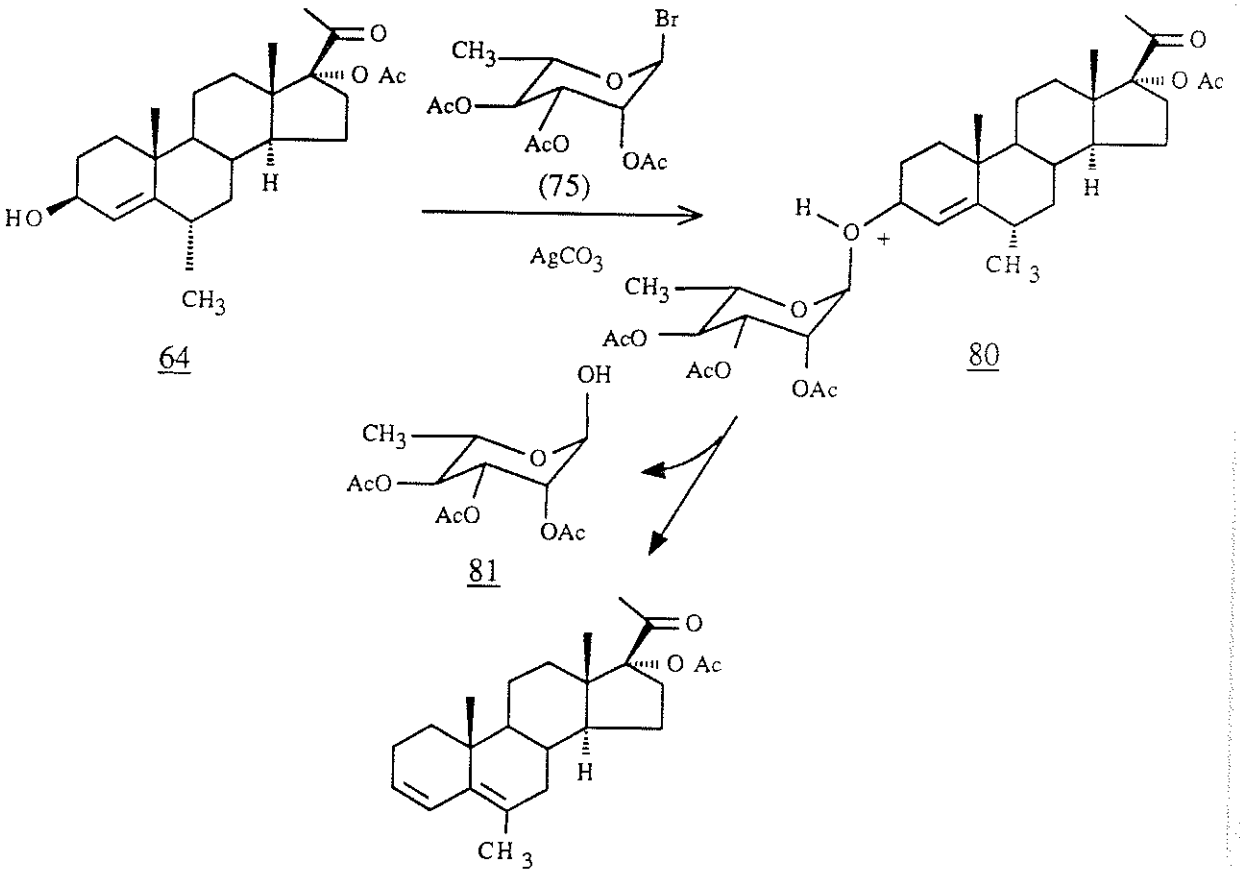
Scheme 4 Formation of 17α-acetoxy-6α-methylpregna-3,5-diene-20-one (66) in the presence of $TBPA^+$

selectivity is possibly because of the introduction of the quasi-equatorial methyl group at C_6 which, either directly or indirectly (through a conformational transmission effect), causes additional steric hindrance at the α face (Duax et al., 1978; Duax and Strong, 1979).

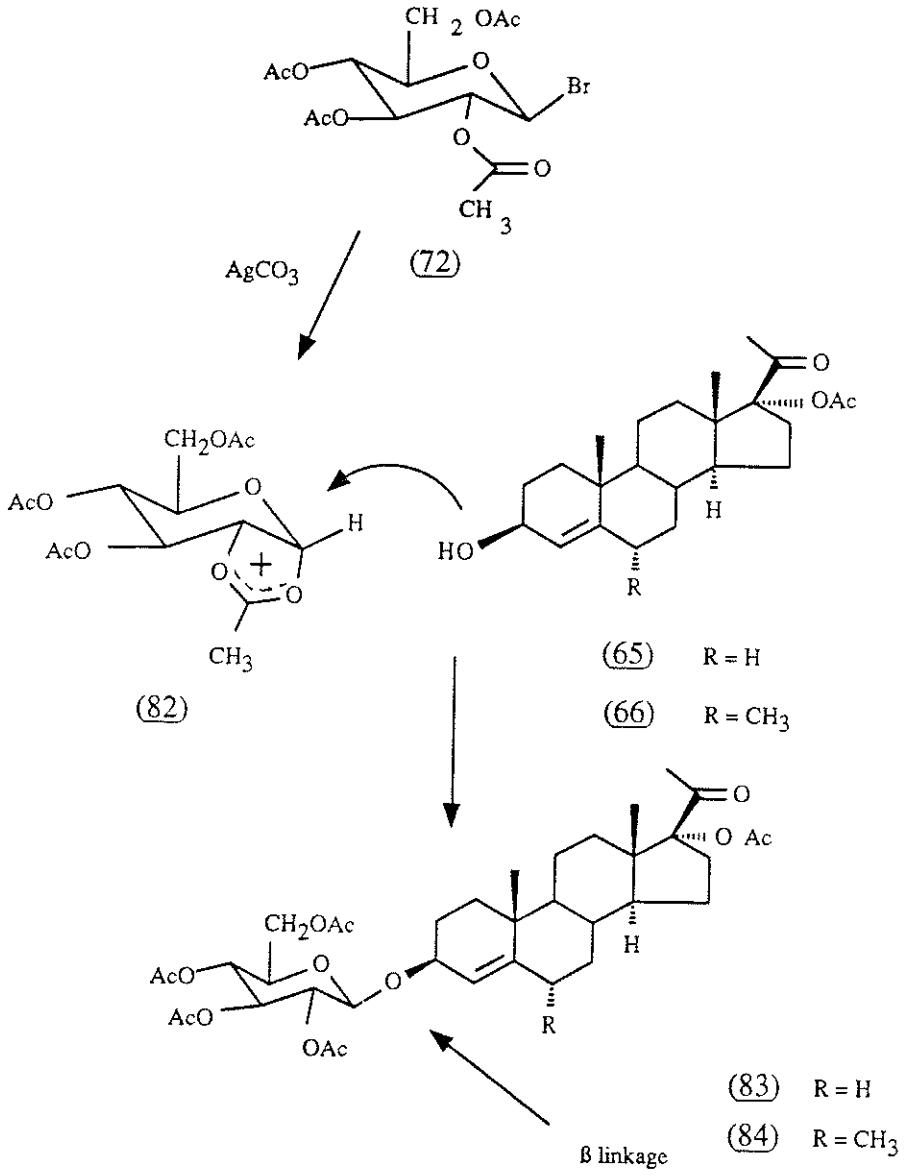
Normally saturated ketones react faster than unsaturated ketones because of conjugation. However, it was found that the carbonyl group at C_{20} in both compounds survived the reduction conditions. This also can be readily explained by Dunitz's approach geometry (Dunitz et al., 1974). As illustrated in Scheme 3, both the ß and α face in the preferred conformers are blocked by the angular methyl group at C_{13} and the quasi-axial acetyl group at C_{17}, respectively. Therefore, the attack of the nucleophile on the carbonyl group at C_{20} is inhibited. Additionally, LTBAH is a weak reducing reagent and does not affect the C_4 double bond and the ester group at the 17α positions (March, 1985). Glycosylation of 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (63) and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) with 1-bromo-α-D-tetraacetyl-glucoside (72) was achieved, but in low yield (15%), under modified Koenigs-Knorr reaction conditions (Smyth et al., 1992). The major compounds obtained were the corresponding dienes, viz. 17α-acetoxy-3ß-hydroxypregna-3,5-dien-20-one (65) and 17α-acetoxy-3ß-hydroxy-6α-methylpregna-3,5-dien-20-one (66) (Scheme 1).

The low yield from glycosylation of these allylic alcohols is associated with their liability to dehydration under acidic conditions. The acidic proton produced under Koenigs-Knorr conditions is able to cause dehydration as illustrated in Scheme 5. It was found that the protected bromorhamnoside (75) was less reactive than the bromoglucoside (72) in coupling with the allylic alcohols. Under the same conditions, none of the expected rhamnoside was isolated when 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) was coupled with 1-bromo-α-L-triacetylrhamnoside (75). It has been rationalized that the lower reactivity of 1-bromo-α-L-triacetylrhamnoside (75) compared to 1-bromo-α-D-tetraacetylglucoside (72) can be attributed to the geometry differences of the two sugars. As calculated by a computer modelling program (PCMODEL), the distance from the oxygen of the acetoxyl group on C_2 to the anomeric carbon in 1-bromo-ß-D-tetraacetylglucoside (72) is shorter than that in 1-bromo-α-L-triacetylrhanmoside (75); this probably facilitates the formation of the cyclic acetonium ion (82) as illustrated in Scheme 6. With respect to this consideration, an excess of 1-bromo-α-L-triacetylrhamnoside (75) (> 6 eq.) was employed for rhamnosylation and this eventually gave 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one rhamnoside (69), but in very low yield (5%).

It was observed that solvents (Reichardt, 1979) played an important role in rhamnosylation of the allylic alcohols. In



Scheme 5 Proposed mechanism for the formation of dehydration product (66) under Koenigs-Knorr reaction conditions

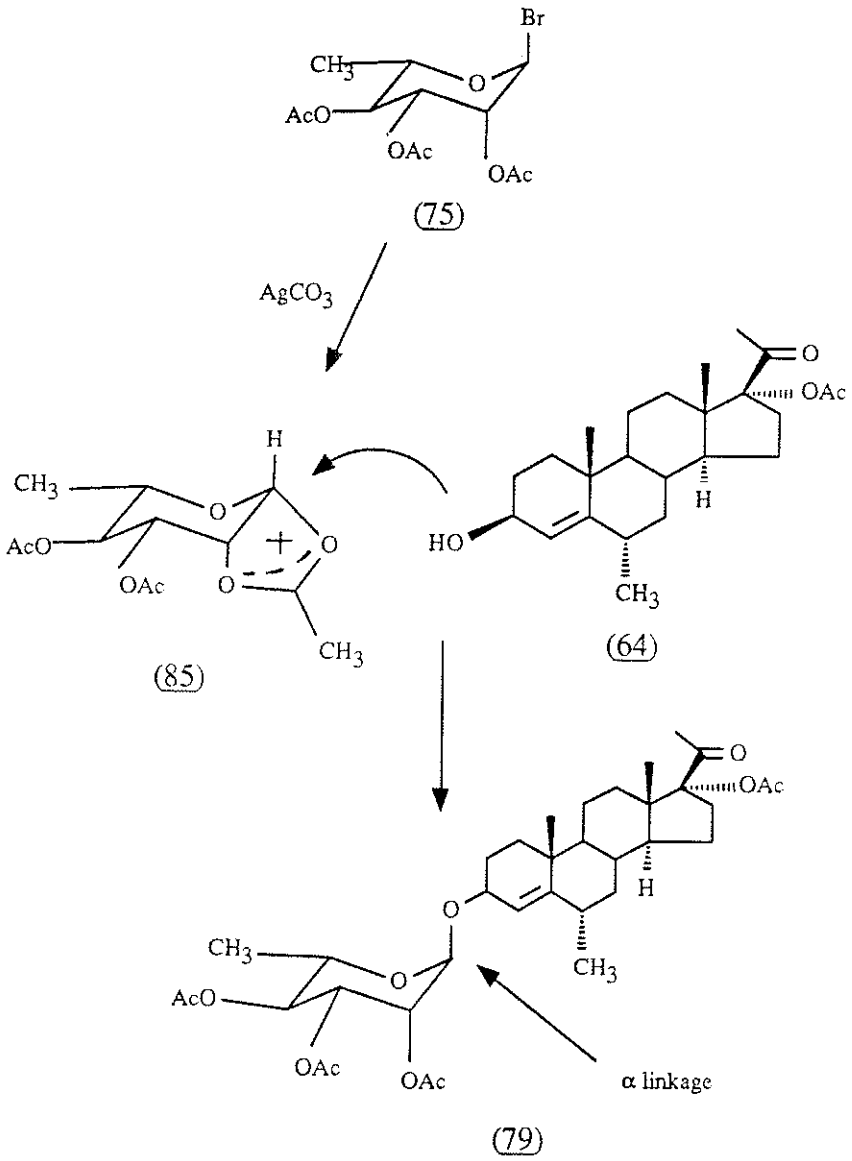


Scheme 6 Proposed mechanism for stereoselective formation of the β linkage glucosides

diethyl ether, the formation of dehydration product was significantly suppressed (Scheme 7) and hence a higher yield of glycosylation was obtained (>30%). In dichloromethane, more dehydration product (66) was formed, and this led to a poor yield on rhamnosylation (5%).

The acetyl and benzoyl groups in the protected sugars are apparently involved in the neighbouring group effect, which facilitates the formation of the ß-glycosidic linkage in the glucosides (83 and 84) (Scheme 6) and the α-linkage in the rhamnoside (79) (Scheme 7). The function of the C_2 acetyl and benzoyl substituents is to form a five membered cyclic oxonium ion. This intermediate would be attacked by the incoming nucleophile, i.e. the 3ß-hydroxy aglycone, to form a 1,2-trans-oriented glycoside. Consequently, retention of the configuration of the anomeric carbons has been achieved as illustrated in Schemes 6 and 7. Removal of the acetyl protecting groups in the sugar moieties is carried out by hydrolysis under mildly basic conditions (triethylamine-methanol-water). Under stronger basic conditions, like KOH-methanol, epimerization may occur at the C_{17} position as well as hydrolysis of the ester groups.

All structures have been unequivocally determined by ^1H, ^{13}C NMR and MS. The anomeric configuration and conformation of the sugars, as well as the structure of the glycosides, can be readily determined by analysis of their ^1H and ^{13}C NMR spectra



Scheme 7 Proposed mechanism for stereoselective formation of the α inkage rhamnoside (79)

(Ferrier and Collins, 1972; Breitmaier and Voelter, 1974; Gunther, 1987; Khadem, 1988).

In the ^1H NMR spectrum of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64), the two singlet signals of six methyl protons, at δ = 0.65 ppm and δ = 1.05 ppm, are assigned to C_{13}-CH_3 and C_{10}-CH_3 (Templeton et al., 1987d). The two singlet signals of six methyl protons, belonging to the methyl group in the 17α side chain and in C_{20}-CH_3, appear at δ = 2.03 ppm and 2.08 ppm respectively (Templeton et al., 1987d).

The anomeric proton resonance of the glycosides (67, 68 and 69) is readily identified at the low-field end of the ^1H NMR spectra, because it is the only proton in the molecule that is attached to a carbon atom bearing two oxygen atoms and hence is significantly less shielded than other protons. In addition, as it is coupled only to one proton, its splitting is simple, which permits first-order resolution and accurate measurement of the coupling constant. In the ^1H NMR spectrum of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one rhamnoside (69), the α linkage of the glycosidic bond is confirmed by the chemical shift (4.92 ppm), and the small coupling constant (1.3 Hz) indicates that the dihedral angle between the anomeric proton and vicinal proton is slightly less than 60° (Khadem, 1988).

The corresponding doublet signal of one proton at 4.48 ppm, with J = 7.5 Hz, in the ^1H NMR spectrum of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside (68) is

assigned to the axial anomeric proton. The larger coupling constant (J = 7.5 Hz) reveals two vicinal protons at C_1 and C_2 at about 180°, and hence the ß linkage of the glucoside (68) is confirmed. Compared to the glucoside, it is not surprising to observe that the anomeric proton in the rhamnoside (69) is shifted to lower field if one consider that the shielding behaviour of the C-C single bond has more effect on the axial proton than the equatorial one (Gunther, 1987). Therefore, the equatorial proton in the rhamnoside (69) is shifted to lower field (δ = 4.92 ppm) than the axial proton of glucoside (δ = 4.45 ppm). In the ^{1}H NMR spectrum of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one rhamnoside (69), the signals responsible for the six methyl groups can be readily differentiated by analysis of the electronic inductive effect, anisotropic effect of the neighbouring groups, and the spin-spin interaction with the neighbouring groups. The magnitude order of electronic inductive effect is : -O-C < -CO- < -$OCOCH_3$. Therefore, the signals at 1.29 ppm as a doublet with J = 6.4 Hz, 2.12 ppm as a singlet and 2.05 ppm as singlet are assigned to the $C_{5'}$-CH_3, C_{20}-CH_3 and 17α-$OCOCH_3$ respectively (Templeton et al., 1987d). A triplet signal of one proton at 3.42 ppm is assigned to the proton at $C_{4'}$ with $J_{4',5'} = J_{4',3'}$ = 9.5 Hz. The proton signals at $C_{3'}$ and $C_{5'}$ are overlapped at 3.72 ppm as a multiplet. A quartet signal of one proton at 3.86 ppm is due to the proton at $C_{2'}$ with $J_{2',1'}$ = 1.3 Hz and $J_{2',3'}$ = 3.3 Hz. Signals of the proton at C_3 and the olefinic

proton at C_4 are at 4.14 ppm as a broad triplet and 5.29 ppm as a singlet, respectively.

In the ^1H NMR spectrum of 17α-acetoxy-6-methylpregna-3,5-dien-20-one (66), the two olefinic proton signals on C_3 and C_4 appear at 5.65 ppm as a broad multiplet and 6.33 ppm as a broad doublet, respectively. The calculated δ values for the protons at C_3 and C_4, 5.71 ppm and 6.25 ppm, respectively are based on an empirical equation (Pascual et al., 1969) and are in agreement with the literature (Smyth et al., 1992). The signal of the methyl group at C_6 is shifted to lower field (1.69 ppm) compared to the aglycone (64) (1.00 ppm) due to the deshielding effect of the C_5-C_6 double bond. The three olefinic protons at C_3, C_4 and C_6 in the ^1H NMR spectrum of 17α-acetoxypregna-3,5-dien-20-one (65) appear at 5.61 ppm, 5.95 ppm and 5.40 ppm, in agreement with the calculated signals, i.e. 5.71 ppm, 6.25 ppm and 5.51 ppm (Pascual et al., 1969).

In the ^1H NMR spectrum of 1-ethylthio-α-L-triacetyl-rhamnoside (78), all proton signals are shifted to lower field compared to sugars 74 and 76. The proton singlet at 4.75 ppm, J = 1 Hz, is assigned to the 1ß-H. The C_2-H signal appears at δ = 5.50 ppm, with $J_{2,1}$ = 1 Hz and $J_{2,3}$ = 3 Hz, the lowest field. The signals of C_3-H is at δ = 5.02 ppm as double doublet with $J_{3,2}$ = 3 Hz and $J_{3,4}$ = 10 Hz. The proton signal at δ = 5.10 ppm, a triplet with $J_{4,3} = J_{4,5}$ = 10 Hz, is assigned to the C_4-H. The chemical shift and spin-spin coupling which lead to interpret the chemical structure of 1-ethylthio-ß-L-

triacetylrhamnoside (78) was further confirmed by a COSY spectrum.

2.3 Summary

1) 17α-Acetoxy-3ß-hydroxypregn-4-en-20-one glucoside (67), 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside (68), and rhamnoside (69) have been synthesized for biological evaluation in terms of receptor binding affinity and pharmacological activity.
2) Rhamnosylation of allylic alcohols has not been extensively explored. Under the modified Koenigs-Knorr reaction conditions previously described (Smyth et al., 1992), rhamno-sylation of 17α-acetoxy-6α-methylpregn-4-en-20-one rhamno-side, only the diene was isolated because of dehydration. Efforts have been made towards successful synthesis of 17α-acetoxy-6α-methylpregn-4-en-20-one rhamnoside (69) in 30% yield. Solvent effect plays an important role in the rhamnosylation reaction of allylic alcohols.
4) An effort has made to prepare the rhamnoside (69) by using 1-ethylthiol-α-L-triacetylrhamnoside as glycosyl donor without success.

2.4 Experimental

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H, ^{13}C and COSY NMR spectra were recorded in $CDCl_3$ on a Bruker AM300 instrument operating at 300 MHz for hydrogen and 75 MHz for carbon. All ^{13}C chemical

shifts data are reported in Tables V-IX Appendix. TMS was used as internal standard. EI Mass spectra were determined on a VG-7070E-HF instrument at 70 eV. Reactions were monitored by TLC on silica gel plates (Merck type 60H) and visualized with a UV lamp where appropriate and/or by dipping in 8% conc. sulfuric acid in ethanol followed by heating. Flash chromatography was carried out on silica gel (Terochem, silica gel 20-45 microns for column chromatography). The interatomic distances were measured by aid of a computer modelling program (PCMODEL, Version 2.0).

17α-Acetoxy-3ß-hydroxypregn-4-en-20-one (63)

To a stirred solution of 17α-acetoxy-3ß-hydroxypregn-4-en-3,20-dione (48) (10 g) in anhydrous diethyl ether (500 mL) was added lithium tri-t-butoxyaluminohydride (LTBAH) (20 g, 3 eq.). The reaction mixture was stirred at room temperature under argon for 15 hr, and then was cooled in an ice-bath followed by addition of water (20 mL). The solution was stirred for an additional 30 min and evaporated to reduce the volume of the solvent to about 250 mL. Then the solution was diluted with dichloromethane (200 mL) and washed with an ice cooled saturated aqueous sodium hydrogen carbonate (3 X 100 mL) and water (3 X 50 mL), dried over anhydrous sodium sulfate and filtered. The filtrate was evaporated to dryness to give crystals of the product (63), which were recrystallized from dichloromethane and diethyl ether, (6.5 g, 64%), m.p. 198-201 °C, lit. m.p. 198-200.5 °C (Marshall et al., 1964).

^{1}H NMR ($CDCl_3$) δ: 0.65 (s, 1H, C_{13}-CH_3), 1.06 (s, 1H, C_{10}-CH_3), 2.03 (s, 3H, C_{17}-OCOCH3), 2.09 (s, 3H, C_{20}-CH_3), 2.93 (m, 1H, 16ß-H), 4.15 (m, 1H, C_3-H), 5.30 (d, H, J = 1.5 Hz, C_4-H).

17α-Acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64)

To a stirred solution of 17α-acetoxy-6α-methylpregn-4-ene-3,20-dione (49) (5 g, 12.94 mmol) in anhydrous diethyl ether (250 mL) was added LTBAH (9.8 g, 3 equivalent). The solution was stirred at room temperature under argon overnight followed by addition of water. The solution was stirred for an additional 25 min and extracted with dichloromethane (200 mL). The extract was washed with 5% HCl (2 X 25 mL), saturated aqueous sodium hydrogen carbonate (2 X 30 mL) and water (2 X 30 mL). The organic layer was dried over sodium sulfate, filtered and evaporated to dryness to give the crystalline product (64) (3.4 g, 68%) which was recrystallized from dichloromethane and diethyl ether, m.p. 183-185 °C (lit. 182-185 °C, Marshall et al., 1964).

^{1}H NMR ($CDCl_3$) δ: 0.65 (s, 3H, C_{13}-CH_3), 1.00 (d, 3H, J = 6.4 Hz, C_6-CH_3), 1.05 (s, 3H, C_{10}-CH_3), 2.03 (s, 3H, C_{17}-$OCOCH_3$), 2.08 (s, 3H, C_{20}-CH_3), 2.20 (m, 1H, C_{15}-H), 2.92 (m, 1H, 16ß-H), 4.20 (m, 1H, C_3-H), 5.29 (d, 1H, J = 1.5 Hz, C_4-H).

EIMS m/z (%RA): 370 (M^+-H_2O, 15), 267 (66), 119 (100).

17α-Acetoxy-3ß-(ß-D-glucosyloxy)-pregn-4-en-20-one (67)

To a stirred solution of 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (63) (200 mg) in dry dichloromethane (15 mL) were added Fetizon's reagent (3.2 g), pulverized molecular sieves

(3 Å, 1g), followed by addition of red mercuric oxide (700 mg) and mercuric bromide (200 mg). 1-Bromo-α-D-tetraacetylglucose (72) (1.5 g) in dry dichloromethane (10 mL) was divided into two equivalent portions, and added through a funnel over 30 min at the beginning of the reaction and after 4 hr, respectively. The reaction process was monitored by TLC (ethyl acetate-hexane, 1:4, v/v). After completion of the reaction, the solution was filtered through a pad of Celite. The filtrate was washed with ice cooled saturated aqueous sodium hydrogen carbonate (2 X 20 mL) and ice-water (2 X 20 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue obtained was dissolved in a solution of methanol (20 mL), triethylamine (15 mL) and water (2 mL), and stirred under argon for 3 days. The solution was evaporated under reduced pressure at 30-35 °C to dryness to afford a syrupy residue (1.4 g). The residue was chromatographed over silica (15 g). A less polar component from elution with 2% methanol in dichloromethane was identified as 17α-acetoxypregna-3,5-dien-20-one (65) (120 mg) which was recrystallized from methanol and water (Rf = 0.46, acetone-dichloromethane, 1:3, v/v), m.p. 176-180 °C.

^{1}H NMR ($CDCl_3$) δ: 0.67 (s, 3H, C_{13}-CH_3), 0.96 (s, 3H, C_{10}-CH_3), 2.05 (s, 3H, C_{17}-$OCOCH_3$), 2.11 (s, 3H, C_{20}-CH_3), 2.25 (m, 1H, C_{15}-H), 2.95 (m, 1H, 17α-H), 5.40 (d, 1H, C_6-H), 5.61 (m, 1H, C_3-H, 5.95 (d, 1H, J = 7.5 Hz, C_4-H).

EIMS m/z (%RA): 356 (M^+, 1), 341 (M^+-15, 1), 253 (4), 149 (100).

The fractions on elution with 4% methanol in dichloromethane were collected to give a crystalline product, (43 mg) in 15 % yield. This was further recrystallized from methanol-water to give the glycoside (67) (32 mg), m.p. 203-205 °C. ^{1}H NMR ($CDCl_3$:CD_3OD, 1:1) δ: 0.66 (s, 3H, C_{13}-CH_3), 1.07 (s, 3H, C_{10}-CH_3), 2.05 (s, 3H, C_{17}-$OCOCH_3$), 2.13 (s, 3H, C_{20}-CH_3), 2.25 (broad t, 1H, C_{15}-H), 2.90 (t, 1H, 16ß-H), 3.20-3.50 (m, 4H, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H and $C_{5'}$-H), 3.80 (dq, 2H, $C_{6'}$-2H), 4.30 (m, 1H, $C_{3'}$-H), 4.45 (d, 1H, J = 7.5 Hz, $C_{1'}$-H), 5.45 (s, 1H, C_4-H). Elemental Analysis ($C_{29}H_{44}O_9$ 1.5 H_2O): Calcd. C, 61.79 %, H 8.14 %; Found C 61.75 %, H 8.09 %.

RBA (IC_{50}): 10 μM

17α-Acetoxy-3ß-(ß-D-glucosyloxy)-6α-methylpregn-4-en-20-one (68)

To a stirred solution of 17α-acetoxy-3ß-hydroxy-6α-methyl-pregn-4-en-20-one (64) (1 g) in dichloromethane (100 mL) were added molecular sieves (4 Å, 3 g) and Fetizon's reagent (13.5 g), followed by addition of red mercuric oxide (500 mg) and mercuric bromide (500 mg). 1-Bromo-α-D-tetraacetylglucose (72) (6.25 g) in dichloromethane (25 mL) was divided into two portions, one of 15 mL and the other of 10 mL; and each was added through a funnel over 30 min, at the beginning of the reaction and after 3 hr, respectively. The reaction solution was stirred under argon in the dark for 15 hr.

The solution was filtered through a pad of Celite and the filtrate was evaporated to dryness to give a gummy residue. The residue was dissolved in a solution of methanol (75 mL), triethylamine (50 mL) and water (5 mL). The solution was stirred under argon for 3 days and then evaporated to give a residue (1.4 g). The residue was purified through flash column chromatography over silica gel (20 g). The fractions from 10% ether in hexane gave 17α-acetoxy-3ß-hydroxy-6α-methylpregna-3,5-dien-20-one (66) (427 mg), m.p. 124-125 °C from dichloromethane-methanol (lit. m.p. 132-133° C, Schroff, 1968).

^{1}H NMR ($CDCl_3$) δ: 0.66 (s, 3H, C_{13}-CH_3), 0.92 (3H, s, C_{10}-CH_3), 1.69 (s, 3H, C_6-CH_3), 2.05 (s, C_{17}-$OCOCH_3$), 2.11 (s, 3H, C_{20}-$COCH_3$), 2.95 (dd, 1H, J = 11.4 Hz, 13.5 Hz, C_{16}-H), 5.65(m, 1H, C_3-H), 6.33 (d, 1H, J = 10.0 Hz, C_4-H).

EIMS m/z (%RA): 370 (M^+, 20.8), 267 (100).

The fractions from elution with 10% methanol in dichloromethane were combined and evaporated to dryness to give a crystalline product (68) (225 mg) in 16 % yield. This was recrystallized from methanol-water to give the glycoside (185 mg), m.p. 228-229 °C.

^{1}H NMR ($CDCl_3$-CD_3OD, 1:1), δ: 0.65 (s, 1H, C_{13}-CH_3), 1.03 (d, 3H, J= 6.4 Hz, C_6-CH_3), 1.07 (s, 3H, C_{10}-CH_3), 2.05 (s, 3H, C_{17}-$OCOCH_3$), 2.12 (s, 3H, C_{20}-CH_3), 2.24 (broad m, 1H, C_{15}-H), 2.90 (t, 1H, 16ß-H), 3.20-3.40 (m, 4H, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H and $C_{5'}$-H),

3.80 (dq, 2H, $C_{6'}$-2H), 4.28 (t, 1H, C_3-H), 4.45 (d, 1H, J = 7.5 Hz, $C_{1'}$-H), 5.42 (s, 1H, C_4-H).

Elemental Analysis ($C_{30}H_{46}O_9$): Calcd. C 67.39%, H 8.67%; Found C 67.22%, H 8.79%.

RBA (IC_{50}): 3.0 μM

17α-Acetoxy-6α-methyl-3ß-(α-L-rhamnosyloxy)-pregn-4-en-20-one (69)

Method A:

To a stirred solution of finely ground molecular sieves (4 Å, 5g), Fetizon's reagent (4g), red mercuric oxide (700 mg), mercuric bromide (200 mg) and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) (300 mg) in anhydrous diethyl ether (25 mL), was added freshly prepared 1-bromo-α-L-tribenzoylrhamnose (77) (3.54 g, 10 eq.) in 20 mL of dichloromethane in two equal portions at the interval of 0 and 1 hr respectively. The reaction solution was stirred under argon in the dark for 3 hr at 0 °C and then at room temperature for 10 hr. The reaction process was monitored by TLC (hexane-acetone, 75:25, v/v).

The solution was filtered through a pad of Celite and the filtrate was washed by water with crushed ice (2 X 15 mL), saturated aqueous sodium hydrogen carbonate with crushed ice (3 X 15 mL) and water with crushed ice (2 X 15 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give a syrupy residue. The residue was dissolved in a solution of triethylamine (8

mL), methanol (40 mL) and water (8 mL), and the solution was stirred under reflux and a flow of argon for 20 hr. The evaporation of the solution to dryness gave a residue which was subjected to column chromatography (silica gel 10 g). The earlier fraction from dichloromethane and 5% methanol in diethyl ether yielded 17α-acetoxy-6α-methylpregana-4,6-dien-20-one (66) (40 mg). The latter fractions on elution with 4% methanol in dichloromethane afforded 17α-acetoxy-6α-methyl-3ß-(α-L-rhamnosyloxy)-pregn-4-en-20-one as crystals (69) (130 mg, 35%). Further recrystallization from methanol and water gave 89 mg of the product (69), m.p. 202-205 °C.

^{1}H NMR (CCl_3D-CD_3OD, 1:1) δ: 0.67 (s, 3H, C_{13}-CH_3), 1.02 (d, 3H, J = 6.4 Hz, 6α-CH_3), 1.08 (s, 3H, C_{10}-CH_3), 1.29 (d, 3H, $C_{5'}$-CH_3, J = 6.4 Hz), 2.05 (s, 3H, 17α-$OCOCH_3$), 2.12 (s, 3H, C_{20}-CH_3), 2.89 (t, 1H, 16ß-H), 3.40 (t, 1H, $J_{4',3'} = J_{4',5'}$ = 9.5 Hz, $C_{4'}$-H), 3.67-3.76 (m, 2H, $C_{3'}$-H and $C_{5'}$-H), 3.86 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $J_{2',3'}$ = 3.3 Hz, $C_{2'}$-H), 4.14 (m, 1H, $W_{1/2}$ = 19 Hz, 3α-H), 4.92 (d, 1H, J = 1.3 Hz, $C_{1'}$-H), 5.29 (s, 1H, C_6-H).

Elemental analysis ($C_{30}H_{46}O_6$): Calcd. C 67.39%, H 8.67%; Found C 67.22%, H 8.79%.

RBA (IC_{50}): 0.36 μM

Method B: To a stirred solution of finely pulverized molecular sieves (4 Å, 2 g), Fetizon's reagent (4 g), red mercuric oxide (700 mg), mercuric bromide (200 mg) and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) (300 mg) in dry dichloromethane (20 mL) was added freshly prepared 1-bromo-α-

L-triacetylrhamnose (75) (4.7 g) in 20 mL of dichloromethane in two equal portions at the interval of 0 and 1 hr respectively. The reaction solution was stirred under argon in the dark for 20 hr. The reaction process was monitored by TLC (hexane-acetone, 75:25, v/v).

The solution was filtered through a pad of Celite and the filtrate was washed by water with crushed ice, saturated aqueous sodium hydrogen carbonate with crushed ice (3 X 15 mL) and water with crushed ice (2 X 15 mL). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give a syrupy residue. The residue was dissolved in a solution of triethylamine (18 mL), methanol (20 mL) and water (1.5 mL), and the solution was stirred under argon at room temperature for 3 days. The evaporation of the solution under reduced pressure to dryness gave a residue which was subjected to column chromatography (silica gel 10 g). The earlier fraction from dichloromethane with 2% methanol gave the by-product, 17α-acetoxy-6α-methylpregana-3,5-dien-20-one (66), as a major product (85 mg). The latter fraction from the solution of dichloromethane with 4% methanol afforded 17α-acetoxy-3ß-hydroxy-6α-methyl-3ß-(α-L-rhamnosyloxy)-pregn-4-en-20-one (69) (35 mg). Recrystallization from methanol and water gave the product (69), m.p. 198-201°C.

Pentaacetyl-ß-D-glucose (71)

D-Glucose (70) (20 g) was treated with acetic anhydride and pyridine by the method described for the preparation of

tetraacetyl-α-L-rhamnose (74) to give pentaacetyl-ß-D-glucose (71) (37.2 g, 85%) which was recrystallized from dichloromethane and diethyl ether.

^1H NMR ($CDCl_3$) δ: 2.01, 2.03, 2.08, 2.11 (4 singlet, 12H, 4 $COCH_3$), 3.84 (m, 1H, C_5-H), 4.10-4.30 (dq, 2H, C_6-2H), 5.10-5.30 (m, 3H, C_2-H, C_3-H and C_4-H), 5.71 (d, 1H, J = 8.3 Hz, 1α-H).

1-Bromo-α-D-tetraacetylglucoside (72) (Jeremias et al., 1948)

Pentaacetyl-ß-D-glucose (71) (2 g) was treated with hydrogen bromide in acetic acid by the method described for the preparation of 1-bromo-α-L-triacetylrhamnoside (75) to give a residue (72). This compound was used to couple with the aglycone without delay.

^1H NMR ($CDCl_3$) δ: 2.03, 2.05, 2.09, 2.10 (4 singlet, 12H, 4 CH_3CO), 4.10-4.35 (dq, 2H, C_6-2H), 4.30 (m, 1H, C_5-H), 4.85 (dd, 1H, $J_{2,1}$ = 4 Hz, $J_{2,3}$ = 10 Hz, C_2-H), 5.15 (t, 1H, $J_{3,2} = J_{3,4}$ = 10 Hz, C_3-H), 5.55 (t, 1H, $J_{4,3} = J_{4,5}$ = 10 Hz, C_4-H), 6.70 (d, 1H, J = 4 Hz, 1ß-H)

Tetraacetyl-α-L-rhamnose (74) (Fischer et al., 1920; Bebault et al., 1974)

To a stirred solution of L-rhamnose (73) (16 g) in acetic acid (20 mL) and pyridine (50 mL) was added acetic anhydride (140 mL) at about 0 °C. The solution was stirred at room temperature for 15 hr. Then dichloromethane (100 mL) was added to the solution, followed by washing with water (3 X 100 mL). The organic layer was further washed with saturated aqueous

sodium hydrogen carbonate (3 X 100 mL) and water (3 X 100 mL). The combined organic portion was dried over sodium sulfate and filtered. The filtrate was evaporated to dryness under reduced pressure to dryness to give a syrupy product (74) (25.6 g, 98%).

^{1}H NMR ($CDCl_3$) δ: 1.21 (d, 3H, J = 6.4 Hz, C_5-CH_3), 1.98, 2.04, 2.14, 2.15 (4 singlet, 12H, 4 CH_3CO), 3.93 (m, 1H, C_5-H), 5.10 (t, 1H, $J_{4,3} = J_{4,5}$ = 10 Hz, C_4-H), 5.23 (dd, 1H, $J_{2,1}$ = 2 Hz, $J_{2,3}$ = 3 Hz, C_2-H), 5.29 (dd, 1H, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 10 Hz, C_3-H), 6.00 (d, 1H, J = 2 Hz, C_1-H).

1-Bromo-α-L-triacetylrhamnoside (75) (Bebault et al., 1974)

To a stirred solution of dichloromethane (2 mL), acetic acid (3 mL) and HBr in acetic acid (30-32%) (5 mL) was added tetraacetyl-α-L-rhamnose (74) (2 g). The solution was stirred at room temperature for 2.5 hr and the process was monitored by TLC (ethyl acetate-hexane, 1:1.5, v/v). The solution was diluted with dichloromethane (50 mL) and washed by shaking vigorously with ice-water (3 X 25 mL), saturated aqueous sodium hydrogen carbonate with crushed ice (3 X 20 mL), and ice water (3 X 10 mL). The organic layer was dried over sodium sulfate, filtered and evaporated to dryness to give a gum. Then the residue was dissolved in dichloromethane (15 mL) followed by addition of anhydrous diethyl ether (15 mL) and evaporated to dryness to give a syrupy residue (2.3 g). This compound was allowed to react with the allylic alcohols without delay.

^{1}H NMR ($CDCl_3$) δ: 1.28 (d, 1H, J = 6.4 Hz, C_5-CH_3), 2.00, 2.08, 2.16 (3 singlet, 9H, 3 CH_3CO), 4.10 (m, 1H, C_5-H), 5.15 (t, 1H, $J_{4,3} = J_{4,5}$ = 10 Hz, C_4-H), 5.44 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $J_{2',3'}$ = 3.3 Hz, C_2-H), 5.65 (dd, 1H, $J_{3,2}$ = 3.5 Hz, $J_{3,4}$ = 10 Hz, C_3-H), 6.26 (d, 1H, J = 0.8 Hz, C_1-H).

Tetrabenzoyl-ß-L-rhamnose (76) (Ness et al., 1951)

To a stirred solution of L-rhamnose (73) (20 g) in dichloromethane (250 mL) and pyridine (250 mL) was added benzoyl chloride (90 mL) dropwise over a 3 hr period at 0-5 °C. Then the solution was stirred at room temperature for 24 hr. Ice-water was added to the solution, and the solution was stirred for additional one hr followed by extraction with dichloromethane. The extract was washed with dilute sulfuric acid (3 M), sodium hydrogen carbonate solution and water. Workup gave an amorphous tetrabenzoate (76) (24 g).

^{1}H NMR ($CDCl_3$) δ: 1.47 (d, 1H, J = 6.4 Hz, C_5-CH_3), 4.45 (m, 1H, C_5-H), 5.75 (t, 1H, $J_{4,5} = J_{4,3}$ = 9.8 Hz, C_4-H), 5.92-6.00 (m, 2H, C_2-H and C_3-H), 6.60 (d, 1H, J = 2.6 Hz, 1α-H), 7.95-8.40 (m, 20H, aromatic protons)

1-Bromo-α-L-tribenzoylrhamnoside (77)

To a solution of 29 g of ß-L-rhamnose tetrabenzoate (76) in dichloromethane (20 mL) and glacial acetic acid (30 mL) was added 100 mL of a solution of hydrogen bromide in glacial acetic acid (30-32% HBr). The solution was stirred for 20 hr at room temperature and then dichloromethane was added. The resulting solution was washed with ice-water, aqueous sodium

hydrogen carbonate, and ice-water. The organic phase was dried over sodium sulfate, filtered and evaporated under reduced pressure at 40 °C to dryness. The residue was recrystallized from hexane and acetone to give 24 g of rhamnoside (77), m.p. 169-171 °C, lit. 163-164 °C (Ness et al., 1951).

^{1}H NMR ($CDCl_3$) δ: 1.43 (d, 3H, J = 6.4 Hz, C_5-CH_3), 4.45 (m, 1H, C_5-H), 5.78 (t, 1H, $J_{4,3} = J_{4,5}$ = 10 Hz, C_4-H), 5.85 (dd, 1H, $J_{2,1}$ = 1.5 Hz, $J_{2,3}$ = 3 Hz, C_2-H), 6.20 (dd, 1H, $J_{3,2}$ = 3.2 Hz, $J_{3,4}$ = 10 Hz, C_3-H), 6.55 (d, 1H, J = 1.2 Hz, 1ß-H), 7.25-8.10 (m, 15H, aromatic protons).

1-Ethylthio-α-L-triacetylrhamnoside (78)

To a stirred solution of tetraacetyl-α-L-rhamnose (74) (2.6 g) in dichloromethane (4 mL) was added trifluoroborane etherate (BF_3 in diethyl ether, 1 M). The solution was stirred at room temperature for 20 hr and then washed with saturated aqueous sodium hydrogen carbonate followed by water. The organic phase was dried over magnesium sulfate, filtered and evaporated to dryness under reduced pressure to give a gummy residue. Column chromatography over silica on elusion with 20% acetone in hexane gave crystalline 1-ethylthio-α-L-triacetyl-rhamnose (78) (1.8 g) which was recrystallized from dichloro-methane and diethyl ether, m.p. 166-168 °C.

^{1}H NMR ($CDCl_3$) δ: 1.30 (t, 3H, CH_3), 1.29 (d, 3H, C_5-CH_3), 1.98, 2.05, 2.18 (3 singlet, 9H, 3 $COCH_3$), 2.72 (q, 2H, CH_2), 3.55 (m, 1H, C_5-H), 4.75 (d, 1H, J = 1 Hz, 1ß-H), 5.00-5.10 (m, 2H,

C_3-H and C_4-H), 5.50 (dd, 1H, $J_{2,1}$ = 1 Hz, $J_{2,3}$ = 3 Hz, C_2-H).
EIMS m/z (%RA): 273 (M^+-61, 28.5), 153 (66), 111 (100).

CHAPTER 3

SYNTHESIS OF THE GLYCOSIDES OF PREGNANES AND STRUCTURALLY RELATED COMPOUNDS WHICH BIND TO Na^+,K^+-ATPASE

3.1 Introduction

The research program described in this thesis, with the aim to improve the therapeutic index of cardiotonic compounds, has been carried out along the following two lines:

1) Search for new "lead" compounds among steroidal hormones based on pharmacological studies for further functional group modification.
2) Chemical modification of conventional cardiac glycosides, e.g. digitoxin (1).

In Chapter 2, the synthesis of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glycosides (68 and 69) and its analogues have been described as part of our ongoing effort to search for new cardiotonic compounds. In this chapter, research is focused on functional group modification of digitoxin (1). The theoretical basis for this study is drawn from results of recent SAR studies:

1) Recently, different NKA isozymes have been proposed to be responsible for the initiation of therapeutic and cardiotoxic effects (Sweadner, 1979; Maixent et al., 1987, 1991). This finding suggests that inhibition of the isozymes may initiate specific therapeutic effects, i.e. the inotropic effect observed in modified digitoxin derivatives (Maixent et al., 1992). In this sense, functional group modification is not only expected to show an increase in receptor binding affinity but also to demonstrate useful pharma-

cological activity with respect to an improvement in the safety margin (Maixent et al., 1992).

2) Some pregnanes have been found to bind to NKA and have an effect on heart muscle and the kidney, i.e. they elicited a balance of positive and negative inotropy on the heart, and potassium sparing diuretic effects on the kidney (Templeton et al., 1988, 1991a, b; Smyth et al., 1992). Among these pharmacological activities the latter can account for an improvement in the safety margin of certain pregnanes (LaBella et al., 1979, 1984, 1985; Templeton et al., 1988; Bose et al., 1988).

3) Recent SAR studies indicate that the relative potency of cardiotonic compounds are not only related to receptor binding affinity but also include other effects initiated by these compounds, e.g. the regulation of muscular tone in the heart (Woolfson et al., 1992).

Interest in functional group modification of cardiac glycosides is focused on three areas of the molecule that are directly or indirectly involved in interaction with active site recognition of NKA: i) the C_3 sugar moiety, ii) the steroid rings in which the stereochemistry of the A/B and C/D ring junctions are important for high potency, and iii) the 17ß substituent.

Among the different types of C_3 glycosylation, α-L-rhamnosylation has been shown to be capable of reinforcing receptor binding affinity more significantly than other types

of glycosylation, e.g. ß-D-glucosylation (Thomas et al., 1990; Templeton et al., 1991a). Nevertheless, the sugar moiety at C_3 may not be essential for receptor binding affinity (Repke, 1985; Templeton et al., 1991a, b) and may not be involved in primary binding to NKA (Yoda et al., 1973). However, its existence facilitates drug distribution and transportation (Repke, 1963a), stabilization of the primary drug-receptor binding complex (Schwartz et al., 1975), optimization of binding conformations which mimic that of digitoxin (1) (Watson et al., 1984; Chiu and Watson, 1985), enhancement of inotropic potency (Repke and Weiland, 1988) and binding selectivity towards an extracellular receptor site (e.g. NKA) (LaBella et al., 1989; Weiland et al., 1991; Smyth et al., 1992). In addition, glycosides are less liable to inactivation in the liver than the C_3 alcohol of the genins (Repke et al., 1988). The steroid ring junctions and stereochemistry of C_4, C_5 and C_{14} in rings A/B and C/D, are known to be a determinant of receptor binding affinity (Kim et al., 1980; Templeton et al., 1992). Pregn-4-ene derivatives show an unique pharmacological activity, i.e., a balance of positive and negative inotropy which counteracts cardiotoxicity, a property which had not been observed in the natural cardiac glycosides (Templeton et al. 1988; LaBella et al., 1989).

The activity of the 5α-pregnane derivatives is more controversial. The 5α-pregnane derivatives have shown weaker receptor binding affinity (Templeton et al., 1993), similar

(Thomas et al., 1990) and stronger inotropic activity (Watson et al., 1984) compared to the 5ß series. Some steroidal hormones, all of which possess the 14α stereochemistry, display exclusively cardiodepression, while some of these compounds elicit positive inotropy when they are in the form of glycosides (Weiland et al., 1991; Smyth et al., 1992). Replacement of the 14ß-OH with hydrogen reduced the potency of digitoxigenin (60) to 1/10 (Thomas et al., 1990) but did not destroy activity. It was found that 14-epi-digitoxigenin, in which there is a conversion of the stereochemistry of the C/D ring junction from cis to trans compared to digitoxigenin (60), caused complete loss of activity (Zurcher et al., 1969).

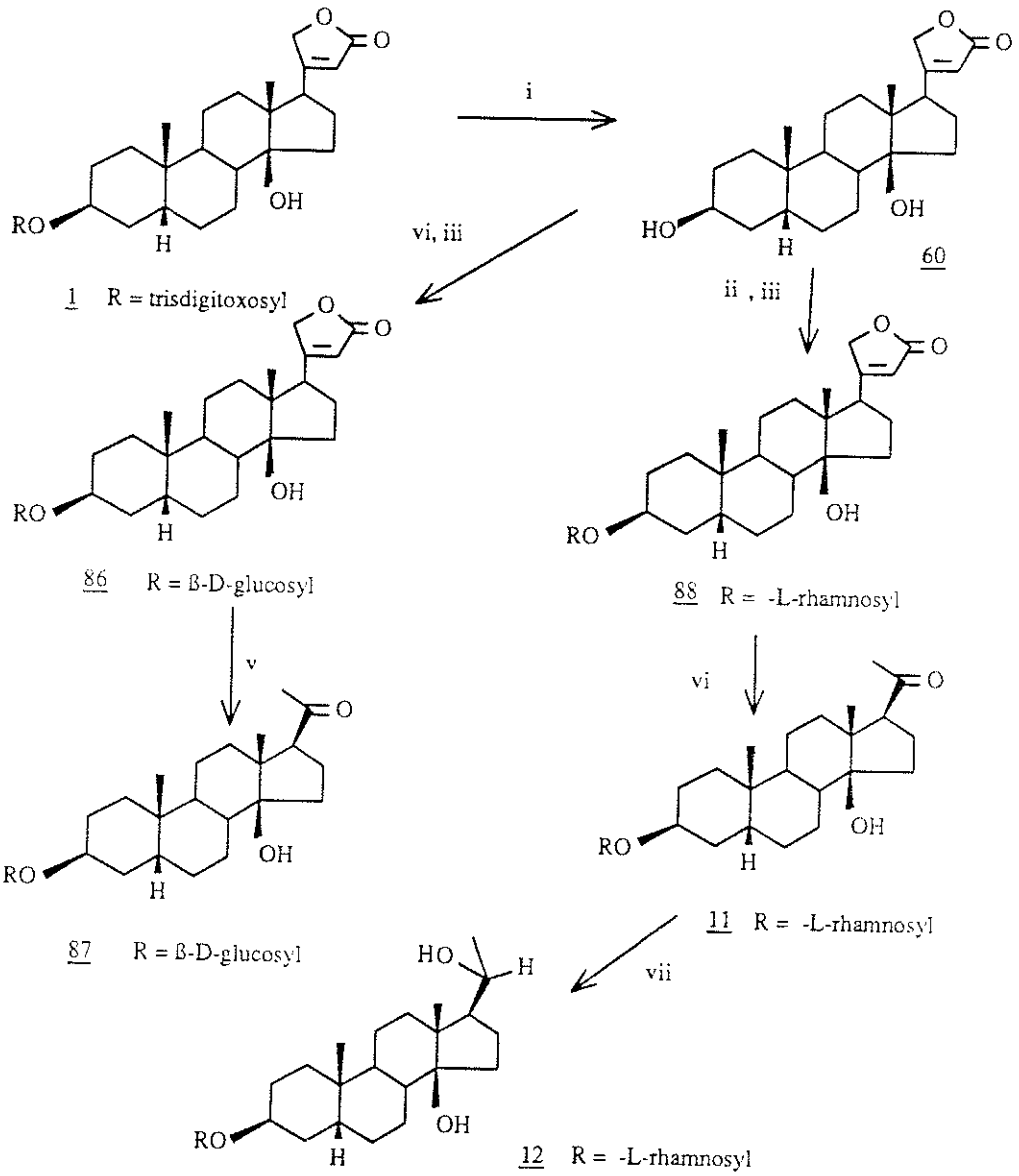
At the 17ß position, an α,ß-unsaturated lactone ring or unsaturated isostere (Wiesner and Tsai, 1986; Thomas et al., 1974b, 1990) was considered necessary for potent receptor binding (Thomas et al., 1974b, 1990). Recently, Templeton et al. (1991a, b, 1992) have shown that the lactone ring can be replaced by a saturated alkyl side chain to form saturated pregnane derivatives and 21-norpregnane derivatives which retain strong receptor binding affinity. Some of these pregnane derivatives display similar diuretic effects on the kidney to progesterone derivatives, e.g. 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside (68) in Chapter 2 (Symth et al., 1992).

The research described in this chapter, which is focused on functional group modification of digitoxin (1) at C_3, C_{14}

and C_{17}, has been carried out so that a potential pharmacophoric pattern can be outlined based on corresponding receptor binding affinity of the compounds studied. To this end, the glycosides of 3ß,14ß,20ß-trihydroxy-5ß-pregnane and related compounds, e.g. compounds 87, 11 and 12, have been synthesized (Scheme 8). Receptor binding affinity was determined in a [^{3}H]ouabain radioligand binding assay. The results will be discussed in Chapter 6.

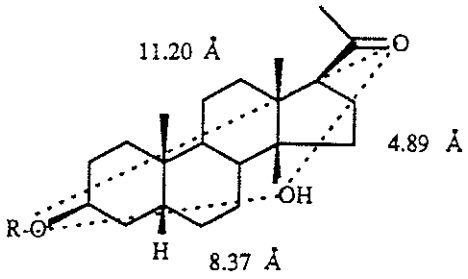
For further illustration of the differences in the spacial orientation among compounds with similar receptor affinities but different substituents and stereochemistry, interatomic distances between oxygen atoms of the substituents at C_3, C_{14} and C_{17} have been measured with the aid of a computer modelling program (PCMODEL). Coordination of the receptor binding affinity with the interatomic distances reveals a similar spacial orientation of the substituents accommodated in the compounds with similar receptor affinities (Fig. 11). The outcome of these studies calls for further investigation of the spacial orientation of these pharmacophores. This study promises to provide more specific information of the pharmacophoric pattern, which is essential for the development of new cardiotonic compounds with useful clinic value.

To determine how the configuration at the A/B and C/D ring junctions and substituents at C_{14} affect receptor binding affinity, canarigenone (91), 14,15-didehydrocanarigenone (92), 20ß-acetoxy-3ß-hydroxypregn-4-ene (14α) (99) and its



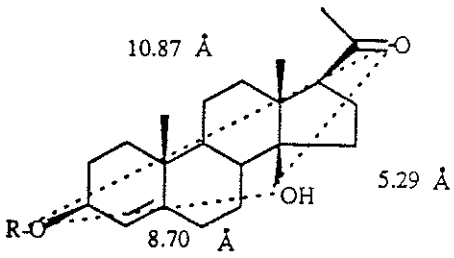
Reagents:
- i) methanol/0.05 M sulfuric acid
- ii) 1-bromo- α-L-triacetylrhamnoside (75)/ Fetizon's reagent/ CH_2Cl_2
- iii) triethylamine/methanol/water
- iv) 1-bromo-ß-D-tetraacetylglucoside (72)/ Fetizon's reagent /CH_2Cl_2
- v) O_3/$P(OCH_3)_3$/Zn, HOAc
- vi) O_3/Zn/HOAc
- vii) LTBAH/THF

Scheme 8 Synthesis of 3β-(β-D-glucosyloxy)-14β-hydroxy-5β-pregn-20-one (87) and 14β,20β-dihydroxy-3β-(α-L-rhamnosyloxy)-5β-pregnane (12)



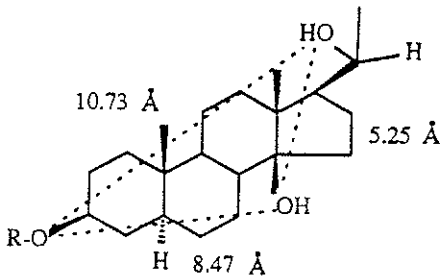
11 R = α-L-rhamnosyl

IC_{50} = 0.2 μM



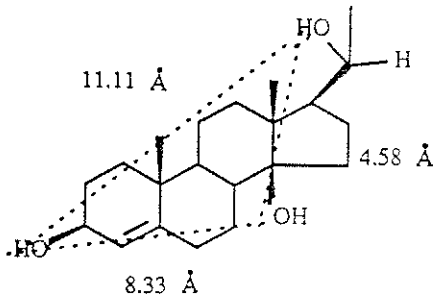
87 R = β-D-glucosyl

IC_{50} = 1.2 μM

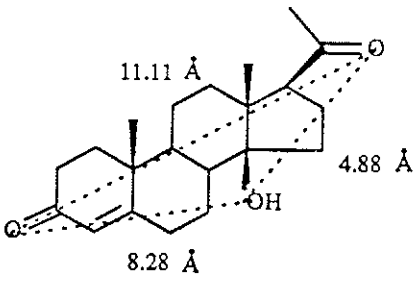


129 R = α-L-rhamnosyl

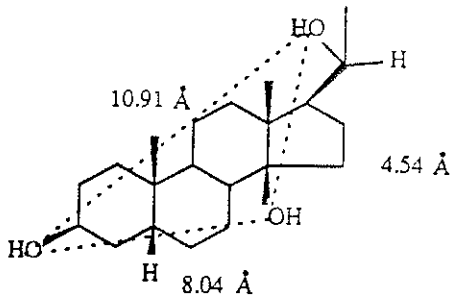
IC_{50} = 1.0 μM



93 IC_{50} = 4.0 μM



94 IC_{50} = 3.9 μM



95 IC_{50} = 6.4 μM

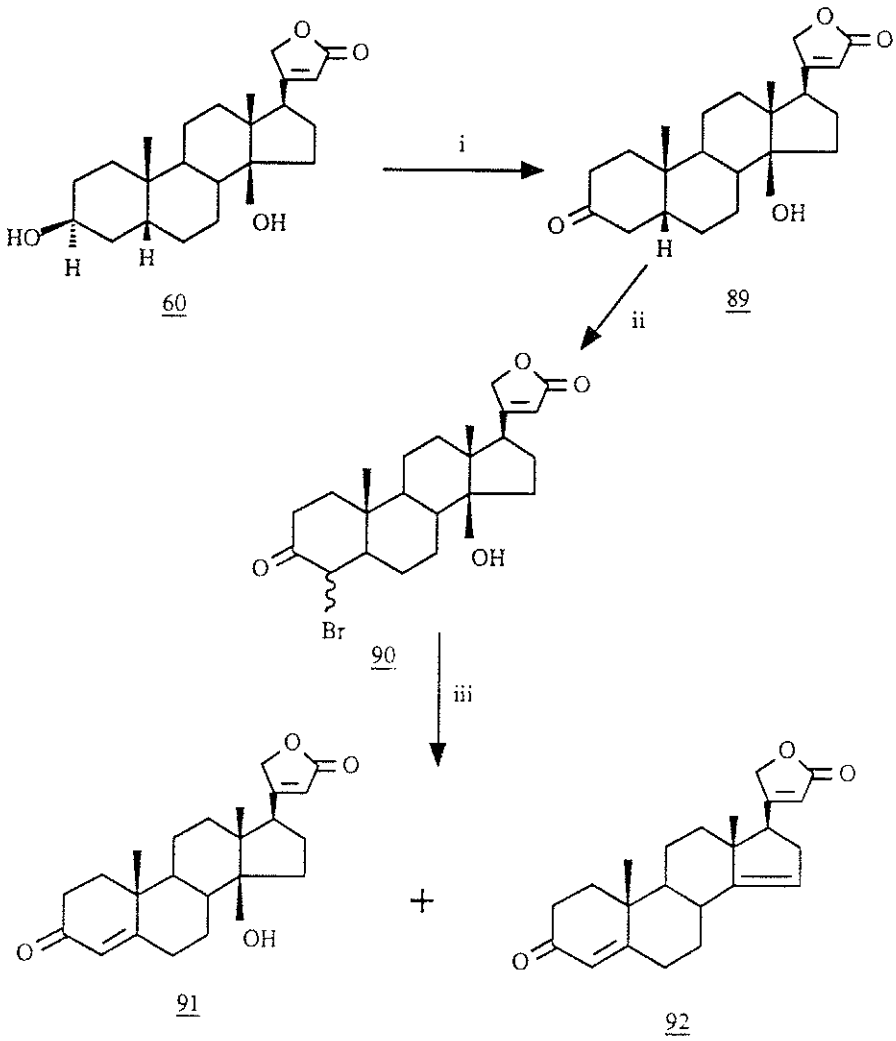
Fig. 11 Interatomic distances among the oxygen atoms at C3, C4 and the C17 side chain in some pregnanes with A/B, C/D ring cis junctions and the corresponding receptor binding affinity in a [^{3}H]ouabain radioligand assay

derivatives have also been synthesized (Schemes 9 and 10).

3.2 Functional Group and Stereochemical Modification of Pregnanes at the C_3, C_5, C_{14} and C_{17} Positions

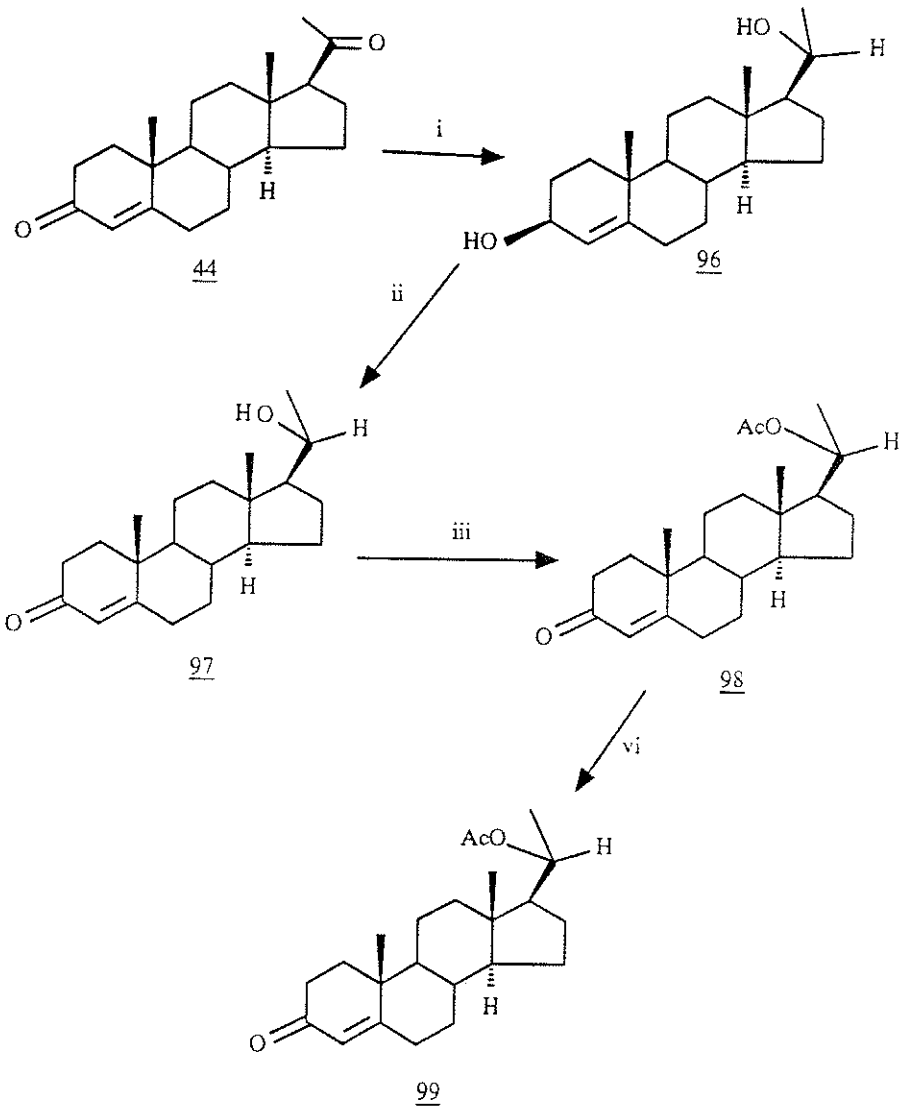
Synthesis of 3ß,14ß,20ß-Trihydroxy-5ß-pregnane C_3-Glycosides (12) and Related Compounds (11 and 87)

Functional group modification of digitoxin (1) has been focused on the C_3 and C_{17} positions, the energetically favourable positions for substitution based on Gibbs free energy calculations (Repke, 1985). It was thought, until recently, that the unsaturated lactone ring and isosteres at C_{17} are necessary for high receptor binding affinity and strong inotropy (Thomas et al., 1974b, 1990). Pregnanes with the substituents at C_{17}, which are saturated but not isosteric with respect to the lactone ring, also demonstrate strong receptor binding affinity and inotropy (Templeton et al., 1991a, b, 1992). The stereochemistry of the steroid rings has been considered as a determinant for potent receptor binding affinity (Kim et al., 1980). For example, changes of configuration at C_5 and C_{14} (e.g. 5ß/5α and 14ß/14α series) or introduction of a double bond at ring A, B or D (i.e. the 4-ene and 5-ene series) cause a major variation in the magnitude of receptor binding affinity and different pharmacological activities (e.g. negative inotropy, a balance of positive and negative inotropy, and potassium sparing diuretic effects) (LaBella et al., 1979, 1989; Templeton et al., 1988; Weiland et al., 1991; Smyth et al., 1992). It seems clear that the



Reagents: i) Jones' reagent, ii) Br_2/DMF, iii) LiCl/dichloromethane

Scheme 9 Synthesis of canarigenone [14β-hydroxy-3-oxo-carda-4,20(22)-dienolide] (91) and 14,15-didehydrocanarigenone [3-oxocarda-4,14,20(22)-trienolide] (92)



Reagents: i) LAH/THF, ii) MnO_2/CH_2Cl_2, iii) acetic anhydride/Py, vi) LTBAH/THF

Scheme 10 Synthesis of 20β-acetoxypregn-4-en-3-one (99) and related compounds

effect of substituents at C_3, C_{14} and C_{17} on receptor binding affinity can be attributed to their different electronic configurations (Repke et al., 1985; Thomas et al., 1990; Templeton et al., 1992), steric hindrances (Templeton et al., 1993), and possibly through the resulting conformational transmission effects of the substituents (Barton et al., 1960) as illustrated in Figs. 12 and 13 (Fullerton et al., 1979a; LaBella et al., 1985; Chiu and Watson, 1985). Conformational effects, the nature of the substituents, and changes in stereochemistry at the A/B and C/D ring junctions alter the spacial orientation of pharmacophores (i.e. the substituents at C_3, C_{14} and C_{17}), and hence cause differences in receptor binding affinity. This is not surprising if one considers that receptors recognize the spacial orientation of pharmacophores by interaction with the electrons located on the pharmacophoric substituents. It is of interest to further illustrate these factors so that specific pharmacophoric patterns required for improved potency compared to digitoxin (1) can be outlined. To this end, the glycosides of 3ß,14ß,20ß-trihydroxy-5ß-pregnane (12) and related compounds (11, 87, 91, 92, 97 and 99) have been synthesized (Schemes 8, 9 and 10).

Results and Discussion

Digitoxin (1) has been used as the starting material for synthesis of 3ß,14ß,20ß-trihydroxy-5ß-pregnane C_3-glycoside (12) and related compounds (11 and 87). Digitoxigenin (60) was obtained by acidic hydrolysis of digitoxin (1) (Brown et al.,

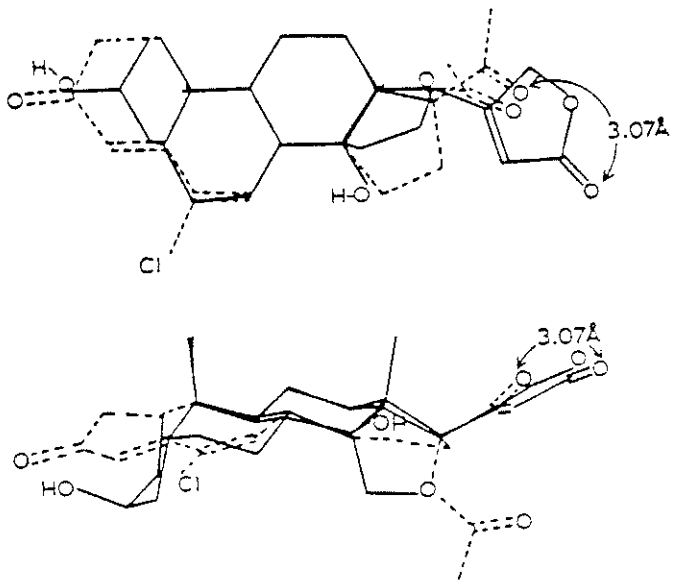


Fig. 12 Superimposition of atoms in B and C rings of digitoxigenin and chlormadinone acetate. Solid and dashed lines represent digitoxingenin and chlormadinone acetate respectively (LaBella et al., 1985).

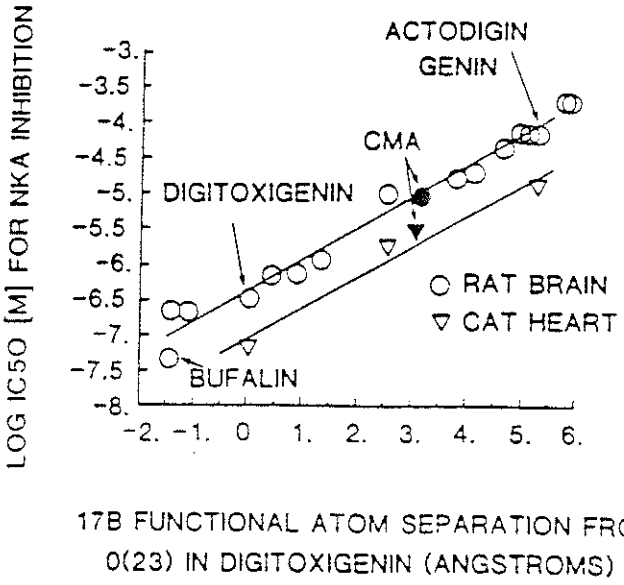
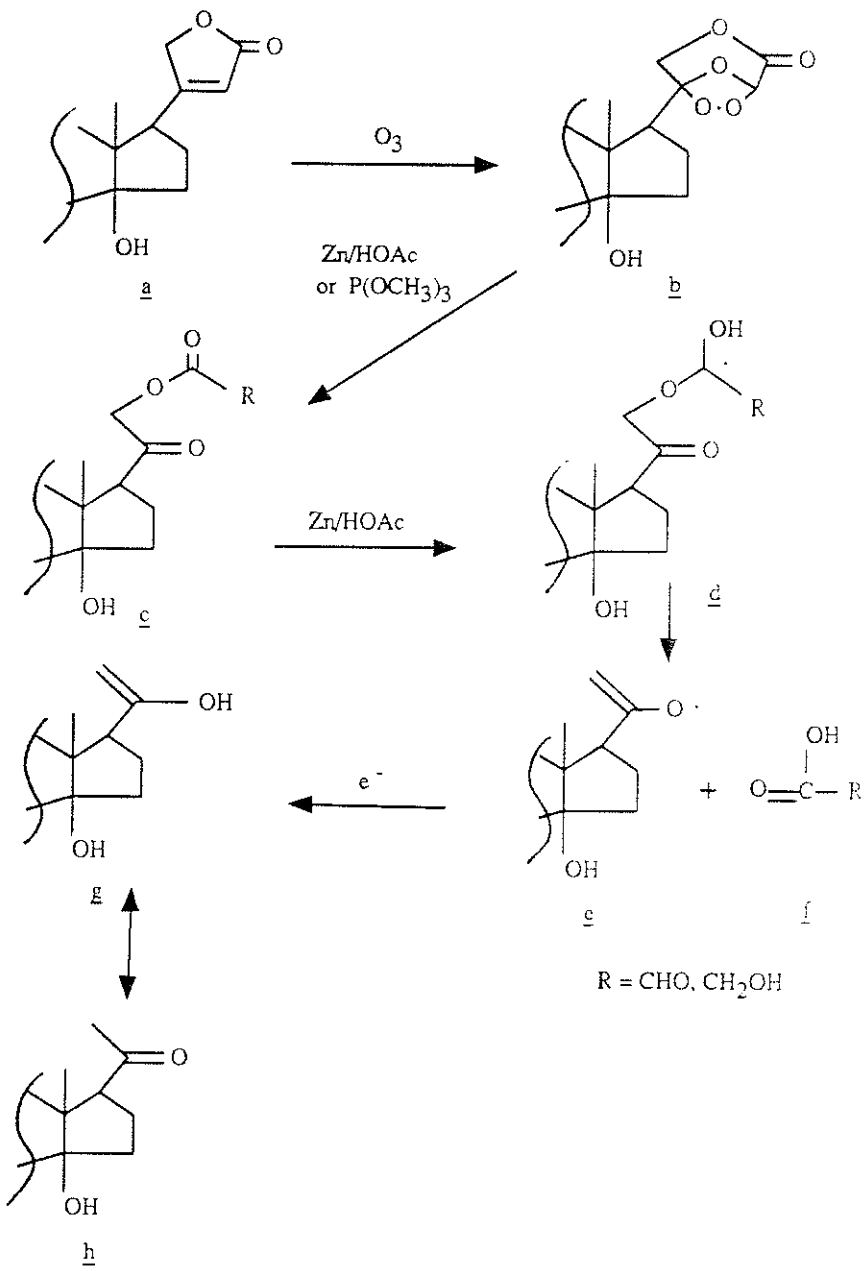


Fig. 13 Relationship of cardiac steroidal genins and carbonyl oxygen positions to the potency (IC_{50}, log molar concentration) to inhibit Na^+,K^+-ATPase (LaBella et al., 1985).

1981). The concentration of acid (0.005 M) used is important since a higher concentration of acid may cause not only cleavage of the glycosidic bond but also dehydration at the C_{14} position. Digitoxigenin (60) was coupled with 1-bromo-α-D-tetraacetylglucoside (72) and 1-bromo-α-L-triacetylrhamnoside (75) in the presence of Fetizon's reagent (Brown et al., 1981) followed by basic hydrolysis to give digitoxigenin ß-D-glucoside (86) and α-L-rhamnoside (88). The reaction was complete within one hour, but a longer reaction time may cause dehydration of the C_{14}-OH. To simplify the purification process, the gummy residue of glucoside tetraacetate and rhamnoside triacetate, without further purification, was dissolved in a solution of triethylamine, methanol and water (12:12:1, v/v) to give digitoxigenin ß-D-glucosides (86) and α-L-rhamnoside (88). Under strongly basic conditions epimerization at C_{17} and reaction between the 14ß-OH and the 17ß-lactone may also occur (Thomas et al., 1990). Ozonolysis of digitoxigenin glycosides (86 and 88) was carried out in dichloromethane by passing ozone/O_2 at -60 to -65 °C for up to one hour. Longer reaction times were employed to ensure complete formation of the ozonide (Scheme 11, b) since conjugated double bonds are usually more stable than unconjugated double bonds. Conversion of the digitoxigenin glycosides to the C_{20}-ketone derivatives (86 and 88) via ozonolysis followed by removal of the hydroxyl group at C_{21} through tosylation, iodination and reduction is lengthy and



Scheme 11 Proposed mechanism of the reductive cleavage of a C-O bond in the α-acetoxylketone

overall yields were low. A more efficient synthetic approach for the preparation of the C_{20}-ketone derivatives has been developed (Templeton et al., 1991a, b). In this approach, the formation of the C_{20}-ketone was achieved by reduction, with excess zinc and acetic acid for 18 hr, of C_{21}-$OCOCH_2OH$, an intermediate derived from short treatment of the ozonide with zinc and acetic acid. If the C_{21}-OH is formed during the reduction of the ozonide, it can not be removed by treatment with zinc and acetic acid (Cohnen et al., 1982; Templeton et al., 1991a). Obviously the -$OCOCH_2OH$ group is a better leaving group than -OH under treatment with zinc and acetic acid and facilitates the cleavage of the C-O bond. Conversion of the digitoxigenin glycosides (86 and 88) to the corresponding C_{20}-ketones (87 and 11) was also achieved by ozonolysis followed by treatment with trimethylphosphite and additional treatment with excess zinc and acetic acid. A possible mechanism for this reaction is suggested in Scheme 11. Conversion of the C_{20}-ketone (11) to the corresponding 20ß-hydroxy derivative (12) as major product (20ß:20α, 9:1) can be achieved by treatment with LTBAH in THF (Scheme 8).

Synthesis of Canarigenone (91) and Related Compounds

Canarigenin (91) (C_4-C_5 double bond) and uzarigenin (92) (5α) are structurally similar, with only stereochemical difference at the A/B ring junction (Fig. 10). However, these compounds show quite different degrees of cardioactivity. In general, the uzarigenin glycosides (5α) show only weak

cardioactivity (Fieser et al., 1959). Unlike cardenolides of the uzarigenin type, the closely related canarigenin family are biologically more active (Studer et al., 1963). Canarigenin glycosides have a relatively flat geometry (like 5α) due to introduction of a double bond at the C_4-C_5 position, but different from digitoxigenin (60) which has the 5ß configuration (Fig. 10). It is known that the geometry defined by stereochemistry of genins is a determinant for the attachment of the substituents at C_3, C_{14} and C_{17} with respect to interaction energy with NKA (Repke and Weiland, 1988) and receptor binding affinity (LaBella et al., 1985; Templeton et al., 1987c).

For a further illustration of stereochemical effects of the A/B ring junction, digitoxigenone (89), canarigenone (91) and 14,15-didehydro-14-deoxycanarigenone (92) were synthesized (Scheme 9) for further receptor binding evaluation. To find how significant the spacial orientation of the substituents at C_3, C_4 and C_{14} is in affecting receptor binding affinity, interatomic distances among the oxygen atoms at C_3, C_4 and C_{17} of some pregnanes have been measured and coordinated with the corresponding receptor binding affinity measured by a [^{3}H]ouabain radioligand binding assay, as illustrated in Fig. 11.

Results and Discussion

Digitoxin (1) was used as the starting material for preparation of canarigenone (91) and 14,15-didehydrocanari-

genone (92). Digitoxigenin (60) was obtained by acidic hydrolysis as described by Brown et al. (1981). Oxidation of digitoxigenin (60) with Jones' reagent gave digitoxigenone (89) in high yield. Formation of an α,ß-unsaturated ketone has been reported to occur by treatment of a saturated ketone with DDQ (Shimizu et al., 1966), benzeneseleninic anhydride (Barton et al., 1978) or t-butylhypochlorite (Beereboom et al., 1953). Application of these reagents to the preparation of canarigenone was unsuccessful. Treatment of digitoxigenone with bromine in DMF, followed by treatment with lithium chloride (Kamano et al., 1975), gave canarigenone (91) as the major product and 14,15-didehydro-14-deoxycanarigenone (92) as a minor product (Scheme 9).

For further illustration of the relationships between the spacial orientation of the substituents and receptor binding affinity, interatomic distances of the oxygen atoms at C_3, C_{14} and C_{17} of some pregnanes were calculated by means of a computer modelling program (PCMODEL). The data of the interatomic distances obtained have been coordinated with the corresponding values of receptor binding affinity. It is of interest to note that the compounds displaying comparable magnitude of receptor binding affinity have similar patterns for their interatomic distances (Fig. 11). Therefore, the spacial orientation of the substituents at C_3, C_{14} and C_{17} in the compounds with similar receptor binding affinity can be anticipated to be similar. These results parallel the results

of previous SAR studies (LaBella et al., 1985, Fullerton et al., 1979a). It has been reported that the spacial location of the carbonyl oxygen in the C_{17}-side chain relative to the ring skeleton is a determinant of receptor binding affinity (Figs. 12 and 13) based on crystallographic analysis (LaBella et al., 1985) and computer graphic estimates (Fullerton et al., 1979a). To formulate quantitative SARs, many other factors need to be addressed, e.g. the boundary of the cleft of NKA which accommodates digitalis for drug-receptor interaction. Repke claimed that the depth of the cleft is about 1.9 nm (Repke, 1985). The width or boundary of the cleft has not been reported.

Synthesis of 20ß-acetoxy-3ß-hydroxypregn-4-ene (99) and Structurally Related Compounds

To illustrate the importance of the C/D ring junction, 20ß-acetoxy-3ß-hydroxypregn-4-ene (14α) (99) and related compounds have been synthesized, as illustrated in Scheme 10.

Progesterone (44) was treated with LTBAH in THF to give the diol (96). Selective oxidation of the diol with manganese dioxide (Mancera et al., 1953) gave 20ß-hydroxypregn-4-en-3-one (97). 20ß-Acetoxy-3ß-hydroxypregn-4-ene (99) was obtained by treatment of 20ß-hydroxypregn-4-en-3-one (97) with acetic anhydride and pyridine, followed by treatment with LTBAH in THF.

3.3 Summary

1) Chemical modification of digitalis glycosides to pregnane derivatives promises to develop new cardiotonic drugs with enhanced receptor binding affinity and inotropic potency that have improved therapeutic efficacy and safety.
2) To illustrate how the nature and stereochemistry of steroidal ring system and the substituents at C_3 and C_{17} affect receptor binding affinity, 3ß,14ß-dihydroxy-5ß-pregn-20-one glucoside (87), rhamnoside (11), 3ß,14ß,20ß-trihydroxy-5ß-pregnane rhamnoside (12), canarigenone (91), 14,15-didehydrocanarigenone (92), 20ß-hydroxypregn-4-en-3-one (14α) (97) and related compounds have been synthesized.
3) The results of receptor binding and interatomic distance measurement of some pregnane derivatives indicate that compounds with similar receptor binding affinities have similar pharmacophoric patterns, i.e. a similar spacial orientation for the substituents at C_3, C_{14} and C_{17}.

3.4 Experimental

General methods and materials are described previously in Chapter 2.

14ß-Hydroxy-3ß-(α-L-rhamnosyloxy)-5ß-pregn-20-one (11)

Digitoxigenin α-L-rhamnoside (88) (50 mg) was dissolved in dichloromethane (50 mL) and acetone (5 mL) and the solution was cooled in a dry ice-acetone solution to about -50 °C for 10 min. Then an ozone/dioxygen mixture produced from an ozonizer was passed into the stirred solution through a

dispersion tube for 45 min while the temperature remained at about -50 °C. Completion of the reaction was determined by the disappearance of the starting material on the TLC when visualized under UV light.

After 45 min, nitrogen was bubbled into the solution for 10 min at -50 °C to remove the remaining ozone and terminate the reaction. Trimethylphosphite (1 mL) was added to the solution. The solution was stirred for 15 hr at room temperature and then evaporated under reduced pressure to remove the solvent and give a residue. The residue was dissolved in acetic acid (50 mL) followed by addition of activated zinc dust (2.5 g) and the solution was shaken on a shaker at room temperature overnight. The solution was filtered to remove zinc dust and the cake of zinc dust was washed with 10% methanol in dichloromethane (20 mL). The filtrate and washing was combined and diluted with the same volume of hexane (70 mL) and evaporated azeotropically to dryness to afford a crystalline product (11) (30 mg, 65%) which was recrystallized from methanol and chloroform, m.p. 246-250 °C.

^{1}H NMR (CCl_3D/CD_3OD, 1:1) δ: 0.95 (s, 3H, C_{10}-CH_3), 0.97 (s, 3H, C_{13}-CH_3), 1.27 (d, 3H, J = 6.4 Hz, $C_{5'}$-H), 2.27 (s, 3H, C_{20}-CH_3), 2.95 (dd, 1H, J = 4.2 Hz, 9.3 Hz, 17α-H), 3.40 (t, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 9.5 Hz, $C_{4'}$-H), 3.64-3.76 (m, 2H, $C_{3'}$-H and $C_{4'}$-H), 3.82 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $J_{2',3'}$ = 3.3 Hz, $C_{2'}$-H), 3.95 (s, 1H, 3α-H), 4.80 (d, 1H, J = 1.5 Hz, $C_{1'}$-H).

Elemental analysis ($C_{28}H_{44}O_7$): Calcd. C 65.30%, H 8.93 %; Found C 65.09%, H 9.17%.

14ß,20ß-Dihydroxy-3ß-(α-L-rhamnosyloxy)-5ß-pregnane (12) (Templeton et al., 1991a)

14ß-Hydroxy-3ß-(α-L-rhamnosyloxy)-5ß-pregn-20-one (11) (22 mg) was dissolved in anhydrous THF (15 mL) and treated with LTBAH (100 mg). The solution was stirred at room temperature overnight. Then water (10 mL) was added to the solution and the mixture was extracted with dichloromethane to give the C_{20}-alcohol (12) (7 mg, 32%), which was crystallized from methanol and water, m.p. 238-244 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 0.96 (s, 3H, C_{10}-CH_3), 1.17 (s, 3H, C_{13}-CH_3), 1.24 (d, 3H, J = 6.6 Hz, C_{20}-CH_3), 3.91 (m, 1H, C_3-H), 3.79 (m, 1H, C_{20}-H), 4.81 (d, 1H, J = 1.2 Hz, $C_{1'}$-H), 3.82 (dd, 1H, $J_{2',1'}$ = 1.6 Hz, $J_{2',3'}$ = 3.3 Hz, $C_{2'}$-H), 3.64-3.75 (m, 1H, $C_{3'}$-H), 3.39 (t, 1H, $J_{4',3'}$ = $J_{4',5'}$ = 9.5 Hz, $C_{4'}$-H), 3.64-3.76 (m, 1H, $C_{5'}$-H), 1.27 (d, 3H, J = 6.2 Hz, $C_{5'}$-CH3).

Digitoxigenin (60) (Brown et al., 1981)

Digitoxin (1) (10 g) was dissolved in a solution of methanol (1000 mL) and conc. sulfuric acid (2.7 mL). The solution was stirred at room temperature for 4 hr and neutralized by addition of saturated aqueous sodium hydrogen carbonate. The solution was evaporated to remove methanol and filtered to collect crude crystalline digitoxigenin. The cake of digitoxigenin was washed with water (20 mL X 2).

Recrystallization from ethanol and water gave pure digitoxigenin (60) (5.2 g), m.p. 242-246 °C.

Digitoxigenin glucoside (86)

To a stirred solution of digitoxigenin (60) (2 g) in dichloromethane (250 mL) were added 1-bromo-α-D-tetra-acetylglucoside (72) (13 g), Fetizon's reagent (15 g) and molecular sieves (4 Å, 5 g). The solution was stirred at room temperature in the dark for 50 min and filtered through a pad of Celite, which was then washed with dichloromethane. The combined filtrates were washed with saturated aqueous sodium hydrogen carbonate and water. The organic phase was dried over sodium sulfate, filtered and evaporated to dryness to give a residue. The residue was dissolved in a solution of triethylamine (16 mL), methanol (20 mL) and water (2 mL) and stirred at room temperature under argon for 3 days. The reaction solution was evaporated to dryness to give a residue which was subjected to flash chromatography over silica. The fractions, on elution with 15% methanol in dichloromethane, gave digitoxigenin glucoside (86) (1.94 g), which was then crystallized from dichloromethane and acetone, m.p. 241-243 °C (lit. 242-246 °C, Kihara et al., 1984).

3ß,14ß-Dihydroxy-3ß-(ß-D-glucosyloxy)-5ß-pregnan-20-one (87) (Templeton et al., 1991a)

To a stirred solution of digitoxigenin ß-D-glucoside (86) (50 mg) in acetone (5 mL) and dichloromethane (50 mL) was passed ozone/dioxygen through a dispersion tube at -50 °C.

After 45 min, TLC showed the disappearance of the starting material (dichloromethane-methanol, 9:1, v/v). Dry nitrogen was bubbled into the solution for 15 min, to remove remaining ozone, followed by addition of trimethylphosphite (1 mL) and the solution was stirred for 30 min while the temperature remained at about -50 °C. Then the solution was stirred at room temperature for 15 hr, when TLC indicated the conversion of the more polar component to a less polar component (dichloromethane-methanol, 85:15, v/v). The solution was evaporated under reduced pressure at about 45 °C, to give a residue which was dissolved in glacial acetic acid (50 mL) followed by addition of zinc dust (2.5 g). The solution was vigorously stirred at room temperature for 25 hr. At an interval of 12 hr, additional zinc dust (2.5 g) was added. The solution was filtered through a pad of Celite and the zinc dust was washed with ethyl acetate (15 mL), dichloromethane (15 mL) and methanol (5 mL). The combined filtrate and washings were washed with saturated aqueous sodium chloride (25 mL X 3), saturated aqueous sodium hydrogen carbonate (25 mL X 3) and saturated aqueous sodium chloride solution (20 mL X 3). The organic layer was dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated to dryness, to give a residue. Column chromatography of the residue (silica gel 2 g; solvent system: i, cyclohexane, ii, 2-12% methanol in dichloromethane) gave the C_{20}-ketone (87) (33 mg, 77%) from methanol and dichloromethane, which was

recrystallized from a solution of acetone and water (9:1, v/v), m.p. 232-235 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 0.96 (s, 6H, C_{13}-CH_3 and C_{10}-CH_3), 2.26 (s, 3H, C_{20}-CH_3), 2.96 (dd, 1H, J = 4.2 Hz, 9.3 Hz, 17α-H), 3.20-3.30 (m, 2H, $C_{2'}$-H and $C_{5'}$-H), 3.36-3.44 (m, 2H, $C_{3'}$-H and $C_{4'}$-H), 3.71 (dq, 2H, J_{AB} = 12 Hz, J_{AX} = 2.6 Hz, J_{BX} = 5.2 Hz, $C_{6'}$-2H), 4.07 (s, 1H, C_3-H), 4.33 (d, 1H, J = 7.7 Hz, C_1-H).

Digitoxigenin α-L-Rhamnoside (88)

To a stirred solution of digitoxigenin (60) (1.43 g), Fetizon's reagent (19 g), and molecular sieves (4 Å, 5 g) in dry dichloromethane (220 mL) was added 1-bromo-α-L-tri-acetylrhamnoside (75) (9 g) in dichloromethane (30 mL) in one portion and stirred under argon in the dark for 50 min. The reaction progress was monitored by TLC (acetone : hexane, 25 : 75, v/v). The solution was filtered through a pad of Celite to remove the solid residue and the filtrate was washed with ice-cooled saturated aqueous sodium hydrogen carbonate (50 mL X 2) and water (50 mL X 2). The organic layer was dried over sodium sulphate and filtered. The filtrate was evaporated to dryness, to give a syrupy residue (11 g). The residue was dissolved in a solution of methanol (120 mL), triethylamine (120 mL) and water (10 mL) and stirred under argon at room temperature for 3 days. The solution was evaporated to dryness to afford a syrupy residue, which was subjected to flash chromatography (50 g silica gel).

The fractions, on elution with 8-15% methanol in dichloromethane, afforded digitoxigenin 3ß-α-L-rhamnoside (88) (1.58 g), which was recrystallized from ethanol and water, m.p. 219-220 °C (lit. 215-217 °C, Brown et al., 1981).

^{1}H NMR ($CDCl_3$-CD_3OD, 1:1) δ: 0.88 (s, 3H, C_{13}-CH_3), 0.94 (s, 3H, C_{10}-CH_3), 1.27 (d, 3H, J= 6.0 Hz, $C_{5'}$-CH_3), 2.80 (m, 1H, 17α-H), 3.40-3.85 (m, 4H, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H and $C_{5'}$-H), 3.95 (s, 1H, C_{14}-OH), 4.00 (s, 1H, C_3-H), 4.30 (d, 1H, J = 1.5 Hz, $C_{1'}$-H), 4.95 (q, 2H, C_{21}-2H), 5.85 (s, 1H, C_{22}-H).

Digitoxigenone [14ß-hydroxy-3-oxocard-20(22)-enolide] (89)

To a solution of digitoxigenin (60) (1 g) in DMF (50 mL) was added Jones' reagent dropwise with stirring until the solution became slight brown. The solution was stirred at room temperature for 2 hr and the mixture was poured into ice-water, washed with saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane. The combined organic extracts were washed with water, dried over sodium sulphate, filtered and evaporated to dryness under reduced pressure to give a residue. Flash chromatography of the residue over silica, on elution with 25% acetone in hexane, gave the C_3-ketone (89) (780 mg) from dichloromethane and diethyl ether, m.p. 194-200 °C (lit. 194-197 °C, Fritsch et al. 1969).

^{1}H NMR ($CDCl_3$) δ: 0.91 (s, 3H, C_{13}-CH_3), 1.03 (s, 3H, C_{10}-CH_3), 2.60 (t, 1H, C_{17}-H), 2.80 (m, 1H, C_{16}-H), 4.90 (dq, 2H, C_{21}-2H, J_{gem} = 18 Hz, $J_{21\text{-}20}$ = 1.7 Hz), 5.90 (m, 1H, C_{22}-H).

EIMS m/z (%RA): 372 (M^+, 52) 354 (M^+-H_2O, 9), 108 (100).

Canarigenone [14ß-hydroxy-3-oxocard-4,20(22)-enolide] (91) and 14,15-Didehydro-14-deoxycanarigenone [3-oxocard-4,14,20(22)-enolide] (92)

To a stirred solution of digitoxigenone (89) (1 g) in DMF (70 mL), was added bromine (560 mg) in DMF (30 mL). The solution was stirred at room temperature for 6 hr, then diluted with dichloromethane, and then washed with water. The organic phase was dried over sodium sulphate, filtered and evaporated to dryness. The residue obtained was dissolved in DMF (50 mL) and treated with lithium chloride under reflux for 3 hr. The solution was diluted with dichloromethane and washed with water. The organic phase was dried over sodium sulphate, filtered and evaporated to dryness to give a residue which was subjected to column chromatography over silica. The fractions on elution with 20% ethyl acetate in dichloromethane, gave 14,15-didehydro-14-deoxycanarigenone (92) (200 mg), which was recrystallized from dichloromethane and diethyl ether, m.p. 278-285 °C (lit. 288-292 °C, Kamano et al., 1975).

^{1}H NMR ($CDCl_3$) δ: 0.87 (s, 3H, C_{13}-CH_3), 1.22 (s, 3H, C_{10}-CH_3), 4.74-4.78 (dq, 2H, J_{gem} = 17 Hz, $J_{21,22}$ = 1.8 Hz, C_{21}-2H), 5.77 (d, 1H, J = 1 Hz, C_4-H), 5.91 (d, 1H, J = 1.5 Hz, C_{22}-H).

RBA (IC_{50}): 0.3 μM

The later fractions gave canarigenone (91) (400 mg), which was recrystallized from dichloromethane and diethyl ether, m.p. 248-254 °C (lit. 257-263 °C, Kamano et al., 1975).

^{1}H NMR ($CDCl_3$) δ: 0.94 (s, 3H, C_{13}-CH_3), 1.19 (s, 3H, C_{10}-CH_3), 4.75-5.05 (dq, 2H, J_{gem} = 18 Hz, J_{21-22} = 2Hz, C_{21}-2H), 5.75 (s, 1H, C_4-H), 5.90 (m, 1H, C_{22}-H).

EIMS m/z (%RA): 370 (M^+, 76), 352 (M^+-18, 79), 355 (M^+-15, 14), 260 (20), 245 (27), 217 (54), 161 (100).

RBA (IC_{50}): 0.18 μM.

3ß,20ß-Dihydroxypregn-4-ene (96)

Progesterone (44) (200 mg) was dissolved in diethyl ether (50 mL) and treated with LTBAH (486 mg, 3 eq.) and stirred for 20 hr. The reaction was quenched by addition of water and the solution was stirred for an additional hr. The solution was diluted with diethyl ether and the aqueous phase was further extracted with diethyl ether. The combined organic extracts were dried over sodium sulfate, filtered and evaporated to dryness to give the crystalline alcohol (96) (195 mg), which was recrystallized from methanol and diethyl ether, m.p. 157-159 °C.

RBA (IC_{50}): 71 μM

20ß-Hydroxypregn-4-en-3-one (97)

3ß,20ß-Dihydroxypregn-4-ene (96) (1 g) was dissolved in dichloromethane (200 mL) and treated with manganese dioxide (410 mg, 1.5 eq.) at room temperature for 20 hr. Workup by filtration, evaporation and chromatography over silica on elution with 25% acetone in hexane gave the unsaturated ketone (97) (400 mg), which was recrystallized from methanol and diethyl ether, m.p. 135-142 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 0.74 (s, 3H, C_{13}-CH_3), 0.81 (s, 3H, C_{10}-CH_3), 1.13 (d, 3H, C_{20}-CH_3), 3.65 (m, 1H, C_{20}-H), 5.75 (s, 1H, C_4-H).

EIMS m/z (%RA): 316 (M^+, 14), 298 (M^+-18, 25), 124 (100).

RBA (IC_{50}): 110 μM

3ß-Hydroxy-20ß-acetoxypregn-4-ene (99)

20-Hydroxypregn-4-en-3-one (97) (130 mg) was dissolved in acetic anhydride (30 mL) and pyridine (10 mL). The solution was stirred overnight and then diluted with water and stirred for an additional hr. The solution was poured into water and extracted with dichloromethane. The organic phase was further washed with dilute HCl solution (pH 3), water, saturated aqueous sodium hydrogen carbonate and water. The organic phase was dried over sodium sulphate, filtered and evaporated to dryness to give a residue. The residue was dissolved in anhydrous diethyl ether and treated with LTBAH (320 mg) by stirring at room temperature for 20 hr. Workup gave the ester (99) (72 mg) which was recrystallized from dichloromethane and diethyl ether, m.p. 198-201 °C.

^{1}H NMR ($CDCl_3$) δ: 0.67 (s, 3H, C_{13}-CH_3), 1.06 (s, 3H, C_{10}-CH_3), 1.15 (d, 3H, C_{20}-CH_3), 2.03 (s, 3H, $COCH_3$), 4.12 (m, 1H, C_3-H), 4.85 (m, 1H, C_{20}-H), 5.25 (d, 1H, C_4-H).

EIMS m/z (%RA): 360 (M^+, 3), 342 (M^+-H_2O, 44), 300 (13), 282 (36), 267(45), 91 (100).

Elemental Analysis ($C_{23}H_{36}O_3$): Calcd. C 76.62%, H 10.07%; Found C 76.44%, H 10.18%.

RBA (IC_{50}): No activity; IC_{50} > 200 μM

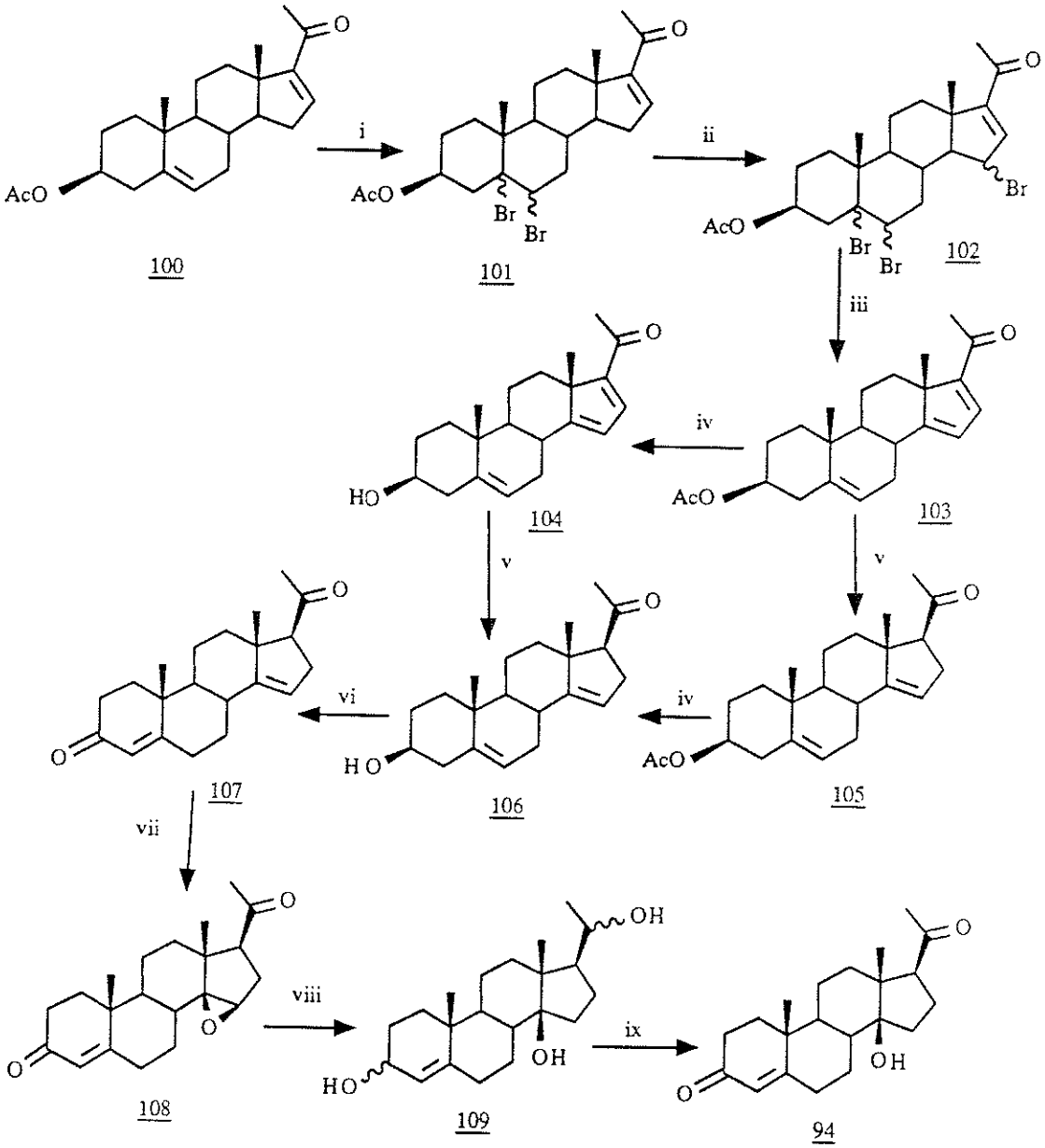
CHAPTER 4

SYNTHESIS OF 14ß-HYDROXYPREGN-4-ENE-3,20-DIONE AND STRUCTURALLY RELATED COMPOUNDS

4.1 Introduction

In an attempt to find endogenous digitalis-like substances (Kim et al., 1981; Jarreau et al., 1984; LaBella et al., 1987; Habert and Haupert, 1987; Weiland et al., 1987; Hamlyn et al., 1991), LaBella et al. found that certain steroidal hormones such as progesterone (44), chlormadinone acetate (45) and their derivatives bind competitively to the digitalis receptor (NKA) in a [^{3}H]ouabain radioligand binding assay (LaBella et al., 1985, 1989) and elicit negative inotropic activity. Based on these findings, LaBella et al. 1989 proposed a hypothetical two-binding-site mechanism (Fig. 2) to explain the paradoxical cardiodepression by some pregnanes that bind to the digitalis receptor and inhibit the sodium pump. As part of our SAR studies described in Chapter 2, glycosides of 3ß-hydroxy-17α-acetoxypregn-4-en-20-one and 3ß-hydroxy-6α-methyl-17α-acetoxypregn-4-en-20-one (67, 68 and 69) were synthesized for comparison of their receptor binding affinity with the corresponding genins (Smyth et al., 1992). However these compounds display only weak, if any, inotropic activity compared to digitoxin. If cardiodepression can be lowered while retaining positive inotropy the compounds should be inotropic at lower concentration. The question can now be raised: How can these steroidal hormones be converted to cardiotonic compounds with clinical value, i.e. strong inotropy and little or no cardiotoxicity? Templeton et al. reported the synthesis of 14ß-hydroxypregn-4-ene-3,20-dione

(14ß-hydroxyprogesterone) (94) and its glucoside (Templeton et al., 1987a, b, 1988) with the structural combination of a 14ß-OH and a C_4 double bond, the structural features found in classic cardiac glycosides and in steroid hormones, e.g. progesterone (44). The synthesis of 14ß-hydroxyprogesterone (94) from 14α-hydroxyprogesterone (116) obtained from microbial oxidation of progesterone (44) (Singh et al., 1965; Templeton et al., 1987a) has been reported. Microbial oxidation of progesterone (44) was a time consuming and tedious procedure which proved to be difficult to reproduce (Templeton et al., 1987a). Here we report a chemical synthesis of 14ß-hydroxyprogesterone (94) from 3ß-acetoxypregna-5,16-dien-20-one (100) (Templeton and Yan, 1992). This synthesis of 14ß-hydroxypregn-4-ene-3,20-dione (94) was completed in six steps with an overall yield of 30 % from 3ß-acetoxypregna-5,16-dien-20-one (100) (Scheme 12). 3ß-Hydroxypregna-5,14-dien-20-one (106) and pregna-4,14-diene-3,20-dione (107) are important synthetic intermediates required for the synthesis of a series of other progesterone analogues, such as 3ß,14ß-dihydroxypregn-4-en-20-one (124) and 14ß,15ß-epoxy-20ß-hydroxypregn-5-ene rhamnoside (139) as described in Chapter 5. These compounds are required as part of our systematic SAR studies of progesterone analogues to develop new cardiac glycosides with an improved therapeutic index.



Reagents: i) Br_2, ii) NBS/hυ, iii) NaI, iv) KOH/MeOH, v) n-Bu_3SnH/xylene/hυ/A
vi) $(i\text{-}PrO)_3Al$/cyclohexanone, vii) NBA/HOAc/K_2CO_3, viii) LAH/THF
ix) PDC/acetone/DMF

Scheme 12 Synthesis of 14β-hydroxypregn-4-ene-3,20-dione (94) from 3β-acetoxypregna-5,16-dien-20-one (100) (Templeton and Yan, 1992)

4.2 Results and Discussion

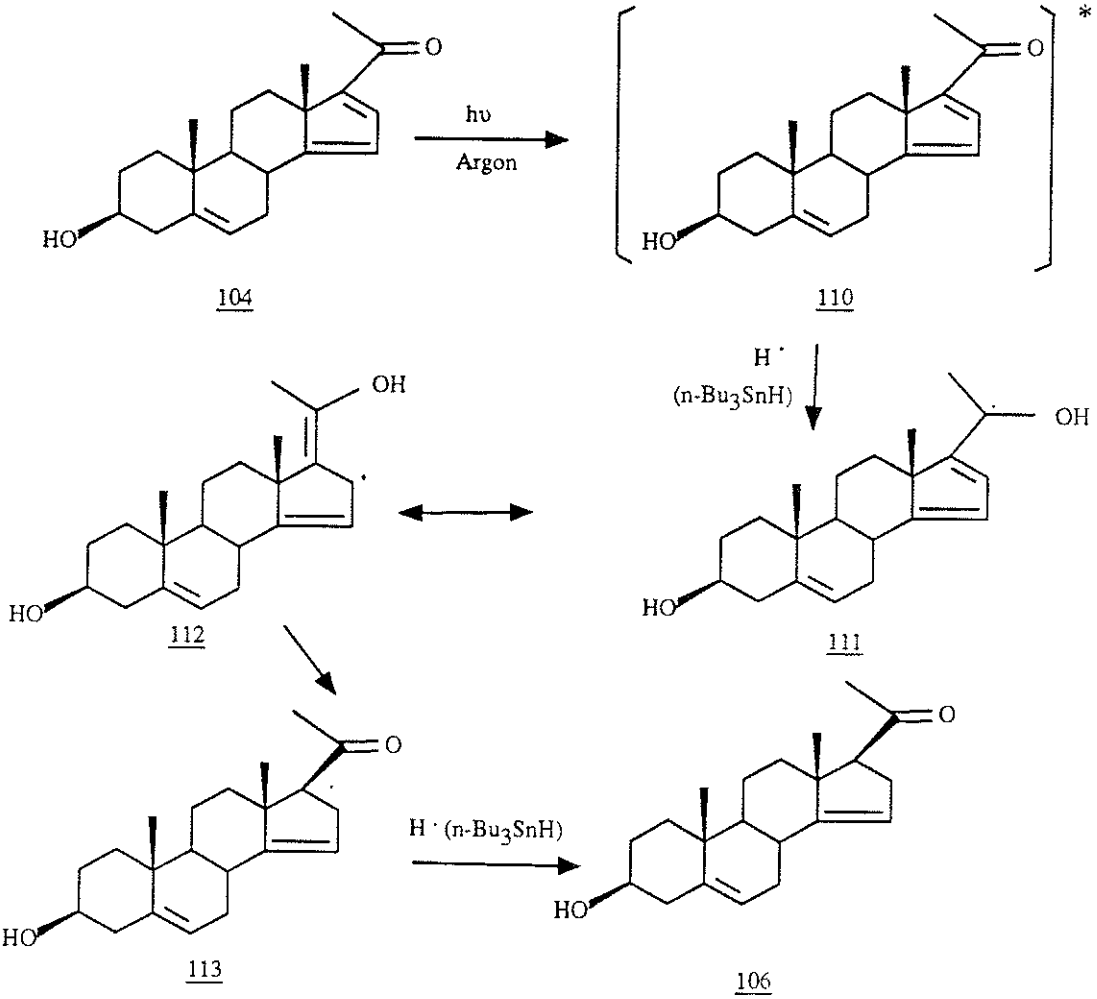
As seen in Scheme 12, conversion of 3ß-acetoxypregna-5,16-dien-20-one (100) to 3ß-acetoxypregna-5,14,16-trien-20-one (103) was carried out by the modified method reported by Solo and Singh in 68 % yield (Solo and Singh, 1965). Treatment of 3ß-acetoxypregna-5,16-dien-20-one (100) with bromine was carried out to protect the isolated double bond at C_4-C_5 while the conjugated double bond at C_{16}-C_{17} remained intact. Without isolation, the corresponding dibromo compound (101) was further treated with NBS under UV light, giving the tribromo compound (102). Debromination was achieved, without further purification, by treatment with sodium iodide to give 3ß-acetoxypregna-5,14,16-trien-20-one (103). The basic hydrolysis of 3ß-acetoxypregna-5,14,16-trien-20-one (103) with potassium hydroxide in methanol gave 3ß-hydroxypregna-5,14,16-trien-20-one (104) in quantitative yield. Chemoselective reduction of the C_{16}-C_{17} double bond in the triene is of particular value with respect to the synthesis of a series of analogues of cardiotonic glycosides. Reduction of the C_{16}-C_{17} double bond in 3ß-acetoxypregna-5,14,16-trien-20-one (103) has been reported using lithium in ammonia (Back et al., 1968) and sodium in propanol (Hensser et al., 1955) as reducing agents. However, these methods gave very poor yields. Trialkyltin hydride (Yoshii and Yamaseki, 1968; Nambara et al., 1970; Pommerenk et al., 1972; Yoshii et al., 1975), silicon hydride (Yoshii et al., 1972, 1975, 1977) and dialkyldisiloxanes (Yoshii et al.,

1977) have also been used. Yoshii and Yamasaki (1968) treated the triene with triphenyltin hydride in toluene in the presence of AIBN to give the diene. This method proved to be inconvenient as addition of triphenyltin hydride several times during the reaction to a sealed tube was required. Furthermore, this method consumed a large amount of triphenyltin hydride, required a long reaction time, and failed to give reproducible results (Yang et al., 1966; Yoshii and Yamasaki, 1968; Yoshii et al., 1972; Brimage et al., 1971). The difficulty in the formation of the diene from the triene on thermal treatment with triphenyltin hydride may be attributed to the instability of the reagent under the reaction conditions. A higher energy and more thermally stable tin hydride reagent are necessary to initiate the reaction.

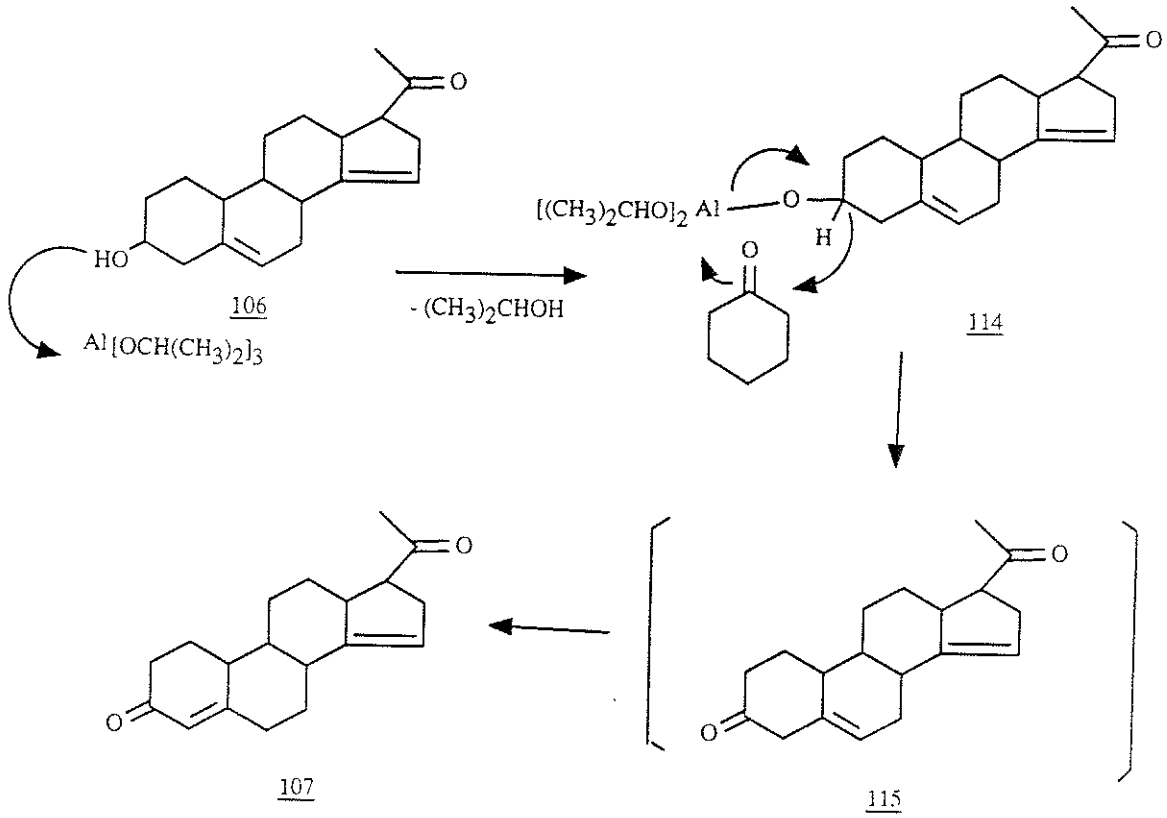
Tri-n-butyltin hydride is found to be more thermally stable than other alkyl and aryltin hydrides. However, the reaction proceeded sluggishly on thermal treatment with this reagent even in the presence of the radical initiator AIBN (Yoshii et al., 1972). Palladium-catalyzed conjugate reductions of α,ß-unsaturated carbonyl compounds with tri-n-butyltin hydride have been reported (Four and Guibe, 1982; Keinan and Gleize, 1982). However, these methods failed to work in our case.

When the triene alcohol (104), under irradiation in an argon atmosphere, was treated with tri-n-butyltin hydride in xylene under reflux, it was converted to the diene alcohol

(106) in high yield (> 83 %) (Scheme 12). The diene (106) was the predominant product as determined by GC-MS spectrometry; no by-products were isolated, e.g. dimeric compounds. Under irradiation, photochemically induced reduction can proceed favourably via an excited state (triplet state) of the triene (110) (Scheme 13), a more energetic state than the ground state (Kropp, 1966, 1967). Consequently the excited triene (110) in the triplet state can abstract a hydrogen atom from tri-n-butyltin hydride (Brimage et al., 1971) to give the radical (113). This radical (113) effectively competes with the formation of the dimer or the alcohol addition products in the presence of tri-n-butyltin hydride to give the diene (106) due to the reactivity of the weak tin-hydrogen bond (Hammond and Leermakers, 1963) as illustrated in Scheme 13. Similar treatment of 3ß-acetoxypregna-5,14,16-trien-20-one (103) gave the corresponding diene (105). The reaction time of two hours was sufficiently short to allow reduction of the triene to go to completion despite the instability of the hydride to heat (Yoshii et al., 1972). Finally, treatment of the 3ß-hydroxy-pregna-5,14-dien-20-one (106) with aluminium isopropoxide under Oppenauer oxidation conditions gave pregna-4,14-diene-3,20-dione (107) in 80 % yield as illustrated in Schemes 12 and 14. In the previously described method (Templeton et al., 1989), dehydration of 14α-hydroxyprogesterone (116) in benzene in the presence of p-toluenesulfonic acid at 60 °C afforded pregna-4,14-diene-3,20-dione (107) in low yield probably due

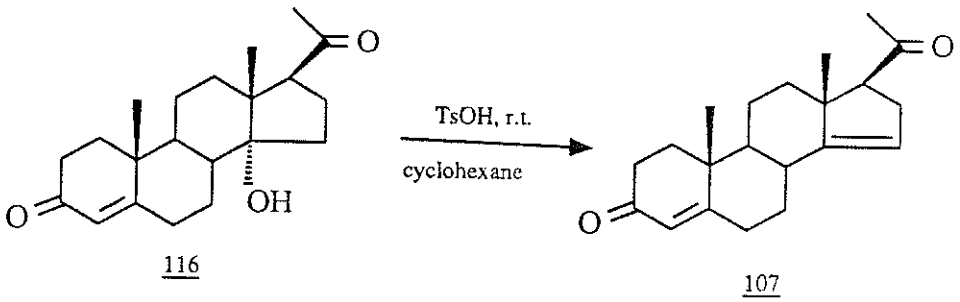


Scheme 13 Proposed mechanism of the chemoselective reduction of 3β-hydroxypregna-5,14,16-trien-20-one (104) to 3β-hydroxy-pregna-5,14-dien-20-one (106) by photochemical induction in the presence of tri-n-butyltin hydride.



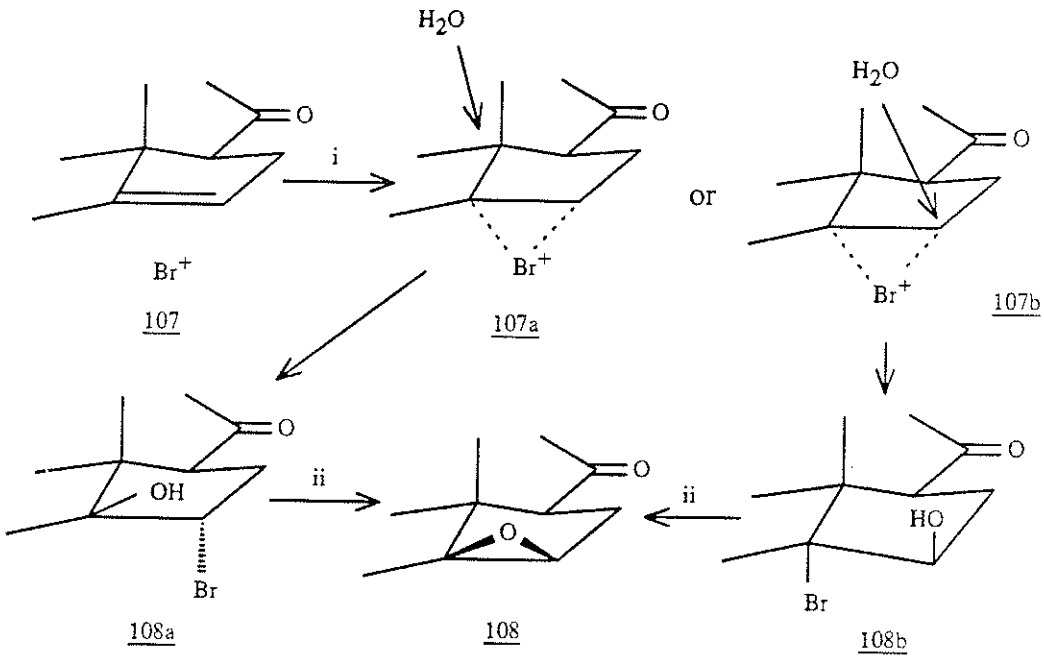
Scheme 14 Proposed mechanism of the conversion of 3β-hydroxypregna-5,14-dien-20-one (106) to pregna-4,14-diene-3,20-dione (107) under Oppenauer reaction conditions

to the migration of the double bond under acidic conditions. This procedure has been improved by using cyclohexane instead of benzene (Scheme 15). The dehydration of 14α-hydroxypregn-4-en-20-one (116) proceeded readily at room temperature to give pregna-4,14-diene-3,20-dione (107) in a higher yield (80%) probably because of effect of the different solvent polarity. Due to steric hindrance on the ß face of the steroid, treatment of the pregna-4,14-diene-3,20-dione (107) with m-chloroperbenzoic acid gave mainly the α-epoxide (Wiesner, 1974). Introduction of the 14ß-hydroxy group was achieved by the treatment with NBA and acetic acid [milder conditions than previously described (Templeton et al., 1989)] followed by base treatment to give the 14ß,15ß-epoxide (108) (Wiesner, 1974; Wiesner and Tsai, 1986; Templeton et al., 1987b). The mechanism with respect to stereoselectivity of the epoxidation is proposed (Scheme 16). Lithium triethylborohydride was reported to produce better chemoselectivity and stereo-selectivity than other reducing reagents (Corey and Varma, 1971; Brown and Krishnamurthy, 1973). However, a disadvantage of this reagent is its high sensitivity to moisture and air. Considering that there is no stereochemical concern about C_3 and C_{20} since the hydroxyl groups with either α or ß configuration will eventually be converted to the carbonyl groups with sp^2 configuration, the more commonly used lithium aluminium hydride was employed. This reagent gave a diastereomeric mixture of 3ξ,14ß,20ξ-trihydroxypregn-4-ene



116 107

Scheme 15 Synthesis of pregna-3,14-diene-3,20-dione (107) from 14α-hydroxyprogesterone (116)

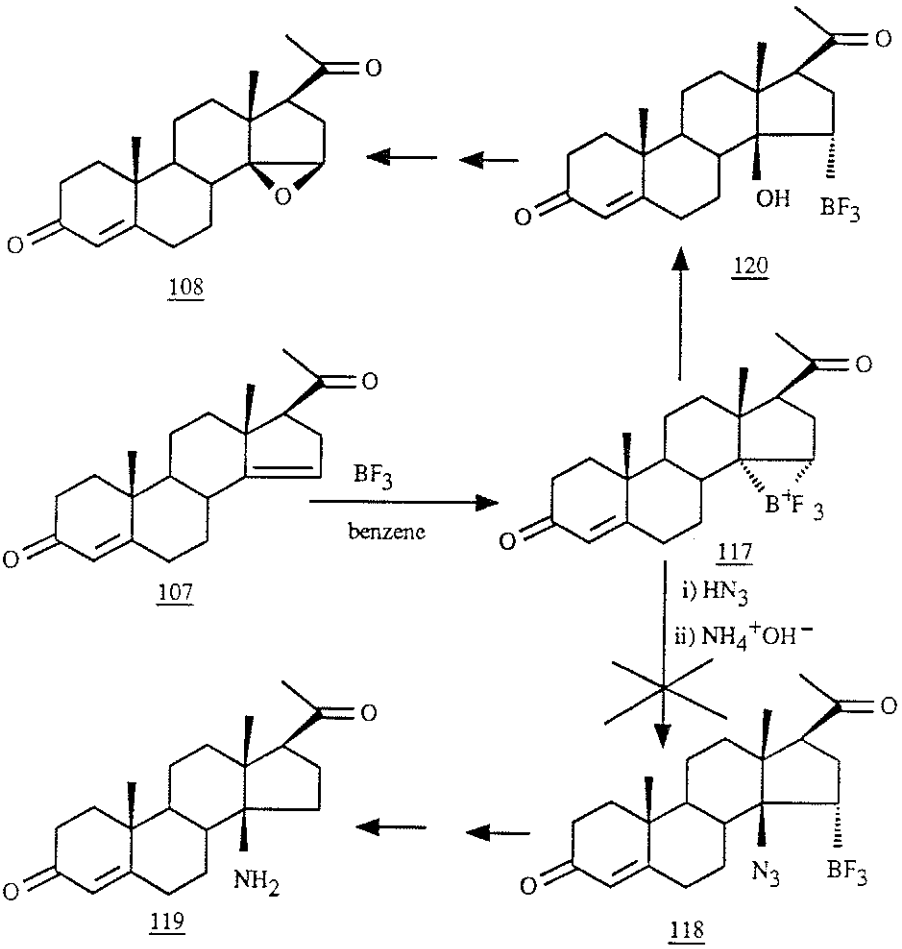


Reagents: i) NBS/acetone/water, ii) K_2CO_3

Scheme 16 Proposed mechanism of chemoselective and stereo-selective formation of the 14β,15β-eposide (108)

(109) with the 3α-OH to the 3ß-OH ratio of about 15:85. This triol mixture was treated with PDC in DMF to give 14ß-hydroxypregn-4-ene-3,20-dione (94). An excess of PDC (> 10 equivalents) was used to ensure complete oxidation of the 3α-hydroxy isomer, which is quasi-axial conformation and is therefore less reactive to the chromate (Parish and Scott, 1983).

In an attempt to synthesize 14ß-aminopregn-4-ene-3,20-dione (119) (Scheme 17), pregn-4-ene-3,20-dione (107) was treated with hydrazoic acid in benzene in the presence of boron trifluoride (Adeoti et al., 1989; Astier et al., 1978), followed by reduction with lithium aluminium hydride and oxidation with PDC. However, the product isolated was 14ß,15ß-epoxypregn-4-ene-3,20-dione (108) rather than the expected 14ß-aminopregn-4-ene-3,20-dione (119) (Scheme 17). The structure of compound 108 obtained from the reaction was confirmed by the analysis of ^{1}H, ^{13}C NMR, EIMS and FAB mass spectra. This result implies that a higher concentration of HN_3 is required for the reaction to proceed (Adeoti et al., 1989). However, the reaction was not pursued further due to concern about the safety of preparation of HN_3 in the laboratory since commercial HN_3 was not available due to new regulations for chemical shipment. Trimethylsilyl azide (Kabalka et al., 1988) also proved to be unsuccessful in converting pregn-4,14-ene-3,20-dione (107) to 14ß-amino-progesterone (119). Receptor binding and pharmacology



Scheme 17 Attempt to prepare 14β-aminoprogesterone (119)

measurement on 14ß-hydroxyprogesterone and related compounds have been carried out. The results will be discussed in Chapter 6.

4.3 Summary

1) Synthesis of 14ß-hydroxyprogesterone (94) was achieved in six steps in 30% overall yield from 3ß-acetoxypregna-5,16-dien-20-one (100). Chemoselective reduction of the C_{16}-C_{17} double bond in 3ß-hydroxypregna-5,14,16-trien-20-one (104) was made by photochemically induced reduction with tri-n-butyltin hydride in xylene in 83% yield, higher than other methods reported. The highly efficient synthetic approach developed for the preparation of 14ß-hydroxyprogesterone (94), especially photoreduction of the triene to the diene, provides a solid synthetic basis for the preparation of other 14ß-hydroxyprogesterone derivatives from steroidal precursors.

2) Intermediates such as 3ß-hydroxypregna-5,14-dien-20-one (106) and pregna-4,14-diene-3,20-dione (107) can be used for the synthesis of the 4-ene and 5-ene analogues described in Chapter 5, such as 3ß,14ß-dihydroxypregn-4-en-20-one (124), 14ß,20ß-dihydroxypregn-4-en-3-one (128), 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) and 14ß,15ß-epoxy-3ß,20ß-dihydroxypregn-5-ene 3-rhamnoside (139).

3) Attempts to synthesize 14ß-aminoprogesterone (119) from pregna-4,14-diene-3,20-dione (107) were unsuccessful.

However, this preliminary work indicates that a higher concentration of hydrazoic acid may be required.

4.4 Experimental

General methods and materials are described previously in Chapter 2. GC-MS and FAB mass spectra were obtained on a VG-7070E-HF instrument at 70 eV. A commercial 275 Watt sun-lamp was used for irradiation of reactions in a Pyrex glass flask. 3ß-Acetoxypregna-5,16-dien-20-one (100) was obtained from the Tienjin No.1 Pharmaceutical Co., Tienjin, P.R. China.

14ß-Hydroxyprogesterone (14ß-Hydroxypregn-4-ene-3,20-dione) (94)

To a stirred solution of 14ß,15ß-epoxypregn-4-ene-3,20-dione (108) (166 mg) in dry THF (30 mL) was added LAH (300 mg). The solution was stirred under reflux and argon for 5 hr and then mixed with water followed by extraction with dichloromethane. The combined extracts were washed with water, dried over sodium sulfate, filtered and evaporated to dryness to give a residue. The residue was dissolved in DMF (20 mL) and treated with PDC (800 mg) for 18 hr. Flash chromatography over silica on elution with dichloromethane gave 14ß-hydroxyprogesterone (94) (145 mg, 87%), after recrystallization from dichloromethane and acetone, m.p. 181-184 °C (lit. 180-181 °C, Templeton et al., 1988).

^{1}H NMR ($CDCl_3$) δ: 1.02 (s, 3H, C_{13}-CH_3), 1.18 (s, 3H, C_{10}-CH_3), 2.25 (s, 3H, C_{20}-CH_3), 2.94 (dd, 1H, J = 4.1 Hz, 8.2 Hz, 17α-

H), 4.51 (s, 1H, 14ß-OH), 5.74 (d, 1H, J = 1 Hz, C_4-H). EIMS m/z (%RA): 330 (M^+, 9), 312 (M^+-H_2O, 81), 302 (100).

3ß-Acetoxypregna-5,14,16-trien-20-one (103) (Solo et al., 1965)

To a stirred solution of 3ß-acetoxypregna-5,16-dien-20-one (100) (5.02 g) in anhydrous diethyl ether (170 mL) was added anhydrous potassium acetate (10 g) in glacial acetic acid (100 mL). The solution was cooled in an ice-bath (0-3 °C) while bromine (2.25 g) in acetic acid (50 mL) was added dropwise over a time period of 3 hr. The solution was stirred at 0-15 °C for an additional 4 hr and at room temperature for 15 hr. The solution was extracted with dichloromethane (200 mL X 2) and the combined extracts were washed with water (300 mL X 2), saturated aqueous sodium hydrogen carbonate (300 mL X 3) and water (300 mL X 3). The organic phase was dried over anhydrous sodium sulfate, filtered and evaporated to dryness to give a yellowish gummy residue (7.5 g).

The residue was dissolved in carbon tetrachloride (75 mL) and NBS (4.84 g, 0.028 mole) was added to the solution. The solution was brought to reflux with stirring under irradiation (UV) and argon for 40 min and under reflux for an additional hr. The solution was cooled to room temperature and filtered. The filtrate was evaporated to dryness to give a foamy pale yellowish residue. The residue was dissolved in acetone (75 mL) followed by addition of sodium iodide (10 g). The solution was heated to reflux under argon for 5 hr and extracted with

dichloromethane (200 mL X 2). The combined extracts was washed with saturated aqueous sodium thiosulphate (250 mL X 4) and water (250 mL X 3). The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to give a brown solid (5.6 g), which was subjected to flash chromatography over acid-washed alumina (80 g) (i, hexane, ii, benzene-ethyl acetate, 98:2, v/v), to give pale yellowish crystals (103) when recrystallized from acetone. m.p. 157-159 °C, (lit. 159-160 °C, Solo et al., 1965).

^{1}H NMR ($CDCl_3$) δ: 0.89 (dt, 1H, J = 5.5, 12.6, 12.6 Hz, C_5-H), 1.15 (s, 1H, C_{10}-CH_3), 1.20 (s, 1H, C_{13}-CH_3), 2.10 (s, 3H, $OCOCH_3$), 2.33 (s, 3H, C_{20}-CH_3), 4.60 (m, 1H, C_3-H), 5.48 (m, 1H, C_6-H), 6.03 (t, 1H, J = 2, 1 Hz, C_{15}-H), 7.22 (dd, J = 2.3, 1 Hz, C_{16}-H).

EIMS m/z (%RA): 294 (M^+-HOAc, 4), 171 (6), 84 (100).

3ß-Hydroxypregna-5,14,16-trien-20-one (104)

To a stirred solution of methanol (800 mL) and 700 mL of 0.5 M potassium hydroxide was added 3ß-acetoxypregna-5,14,16-trien-20-one (103) (13.6 g). The solution was stirred at room temperature for 20 hr, diluted with water (800 mL), and then extracted with dichloromethane (500 mL X 2). The organic phase was washed with water (500 mL X 2), dried over sodium sulfate, filtered, and evaporated to dryness to give pale crystals (104) which were recrystallized from dichloromethane and methanol, m.p. 199-202 °C (lit. 185-187 °C, Plattner et al., 1948).

^{1}H NMR ($CDCl_3$) δ: 0.82 (dt, 1H, J = 5.5, 12.6, 12.6 Hz, C_9-H), 1.15 (s, 1H, C_{10}-CH_3), 1.20 (s, 1H, C_{13}-CH_3), 2.33 (s, 1H, C_{20}-CH_3), 3.54 (m, 1H, C_3-H), 5.45 (m, 1H, C_6-H), 6.02 (t, 1H, J = 2 Hz, C_{15}-H), 7.23 (d, 1H, J = 2 Hz, C_{16}-H).

EIMS m/z (%RA): 312 (M^+, 29), 279 (M^+-H_2O, 16), 269 (8), 122 (100).

3ß-Acetoxypregna-5,14-dien-20-one (105)

To a stirred solution of 3ß-acetoxypregna-5,14,16-trien-20-one (103) (100 mg) in xylene (20 mL) was added tri-n-butyltin hydride (0.4 mL); the solution was stirred under reflux and irradiated in an argon atmosphere for 1 hr and then heated at reflux for an additional hr. Methanol (10 mL) was added and stirring was continued for 1 hr to terminate the reaction. The reaction product was flash chromatographed over silica and eluted with hexane to remove hexa-n-butylditin followed by 10 % methanol in dichloromethane which yielded 3ß-acetoxypregna-5,14-dien-20-one (105) (50 mg, 50%), m.p. 61 °C (dichloromethane-methanol) (lit. m.p. 158-161 °C, Nambara et al., 1970).

^{1}H NMR ($CDCl_3$) : 0.88 (s, 3H, C_{13}-CH_3), 1.04 (s, 3H, C_{10}-CH_3), 2.03 (s, 3H, C_{20}-CH_3), 2.16 (s, 3H, C_3-OAc), 2.92 (dd, J = 7.9, 9.9 Hz, 1H, 17α-H), 4.60 (m, 1H, C_3-H), 5.18 (d, 1H, J = 2 Hz, C_{15}-H), 5.43 (t, 1H, J = 2 Hz, C_6-H).

3ß-Hydroxypregna-5,14-dien-20-one (106)

Method A: To a stirred solution of 3ß-hydroxypregna-5,14,16-trien-20-one (104) (1.6 g) in xylene (60 mL) was added tri-n-

butyltin hydride (8.6 g). The solution was gradually brought to reflux and irradiated under argon for 1 hr, and reflux continued under argon for 2 hr. Then methanol (20 mL) was added to the solution and stirred for an additional 1.5 hr at room temperature. The solution was mixed with water and evaporated azeotropically to remove xylene under reduced pressure. The aqueous solution was extracted with dichloromethane. The combined extracts were dried over magnesium sulfate, filtered and evaporated under reduced pressure to give a colourless residue. The fractions, on elution with hexane, gave the ditin compound. The fractions on elution with 10% methanol in dichloromethane gave 3ß-hydroxypregna-5,14-dien-20-one (106) which was recrystallized from dichloromethane and diethyl ether (1.35 g), m.p. 212-215 °C (lit. m.p. 215-218 °C, Kocovsky et al., 1981).

IR (cm $^{-1}$, CH_2Cl_2): 3700 (OH), 1700 (C=O), 1080, 1320.

^{1}H NMR ($CDCl_3$) δ: 0.87 (s, 3H, C_{13}-CH_3), 1.03 (s, 3H, C_{10}-CH_3), 2.17 (s, 3H, C_{20}-CH_3), 2.92 (dd, 1H, J = 7.9, 9.9 Hz, 17α-H), 3.52 (m, 1H, 3α-H), 5.17 (m, 1H, C_{15}-H), 5.40 (m, 1H, C_6-H).

EIMS m/z (%RA): 314 (M^+, 96), 296 (M^+-OH, 37), 84 (100).

Method B: 3ß-Acetoxypregna-5,14-dien-20-one (105) (50 mg) in 0.025 M methanolic potassium hydroxide (12 mL) was allowed to stand at room temperature for 18 hr; flash chromatography over silica with 10 % dichloromethane in hexane, gave fractions of 3ß-hydroxypregna-5,14-dien-20-one (106) (38 mg, 86%) as determined by ^{1}H NMR.

Pregna-4,14-diene-3,20-dione (107)

Method A: To dry toluene (145 mL) containing 3ß-hydroxy-pregna-5,14-dien-20-one (106) (1 g) and freshly distilled cyclohexanone (50 mL) was added a solution of aluminum isopropoxide (3 g) in dry toluene (50 mL) over 1.5 hr duration. Sodium potassium tartrate solution was added and the mixture was steam distilled. The aqueous layer was extracted with chloroform; flash chromatography over silica, with 25 % ethyl acetate in hexane, gave pregna-4,14-diene-3,20-dione (107) (800 mg, 80 %) which was recrystallized from diethyl ether and methanol, m.p. 142-145 °C (lit. 143-145 °C, Templeton et al., 1987).

^{1}H NMR ($CDCl_3$) δ: 0.91 (s, 3H, C_{13}-CH_3), 1.21 (s, 3H, C_{10}-CH_3), 2.16 (s, 3H, C_{20}-CH_3), 2.92 (t, 1H, J = 8, 10 Hz, 17α-H), 5.21 (dd, 1H, J = 4, 8 Hz, C_{15}-H), 5.76 (d, 1H, J = 1 Hz, C_4-H).

EIMS m/z (%RA): 312 (M^+, 28), 269 (M^+-CH_3CO0), 149 (36), 81 (55), 69 (100).

Method B: To a stirred solution of 14α-hydroxyprogesterone (116) (1 g) in cyclohexane (600 mL) was added p-toluene-sulfonic acid (500 mg). The solution was stirred at room temperature for 6 hr and then washed with saturated aqueous sodium hydrogen carbonate (100 mL X 2) and water (100 mL X 2). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated to give compound 107 as crystals (650 mg), which were recrystallized from methanol and water, m.p. 142-145 °C (lit. m.p. 143-145 °C, Templeton et al., 1987).

Flash chromatography (silica gel) of the mother liquor gave an additional 203 mg of the product. The total yield was 90 %.

14ß,15ß-Epoxypregn-4-ene-3,20-dione (108)

To a stirred solution of pregna-4,14-diene-3,20-dione (107) (150 mg) in glacial acetic acid (10 mL), acetone (30 mL) and water (20 mL), was added N-bromoacetamide (NBA, 200 mg). The solution was stirred under argon at room temperature for 40 min. Then the solution was cooled in an ice bath and adjusted to pH 9 by addition of saturated aqueous potassium carbonate. The solution was stirred for 15 hr at room temperature and extracted with diethyl ether (50 mL X 2). The combined extracts were washed with saturated aqueous sodium hydrogen carbonate (30 mL X 3) and water (20 mL X 2), dried over anhydrous sodium sulfate and evaporated to dryness to give a syrupy residue (159 mg). Crystallization from methanol and water gave 14ß,15ß-epoxypregna-3,20-dione (108) (127 mg, 80%), m.p. 186-188 °C (lit. 187-188 °C, Templeton et al., 1987).

^{1}H NMR ($CDCl_3$) δ: 1.08 (s, 3H, C_{13}-CH_3), 1.22 (s, 3H, C_{10}-CH_3), 2.19 (s, 3H, C_{20}-CH_3), 3.51 (s, 1H, 15α-H), 5.74 (s, 1H, C_4-H)

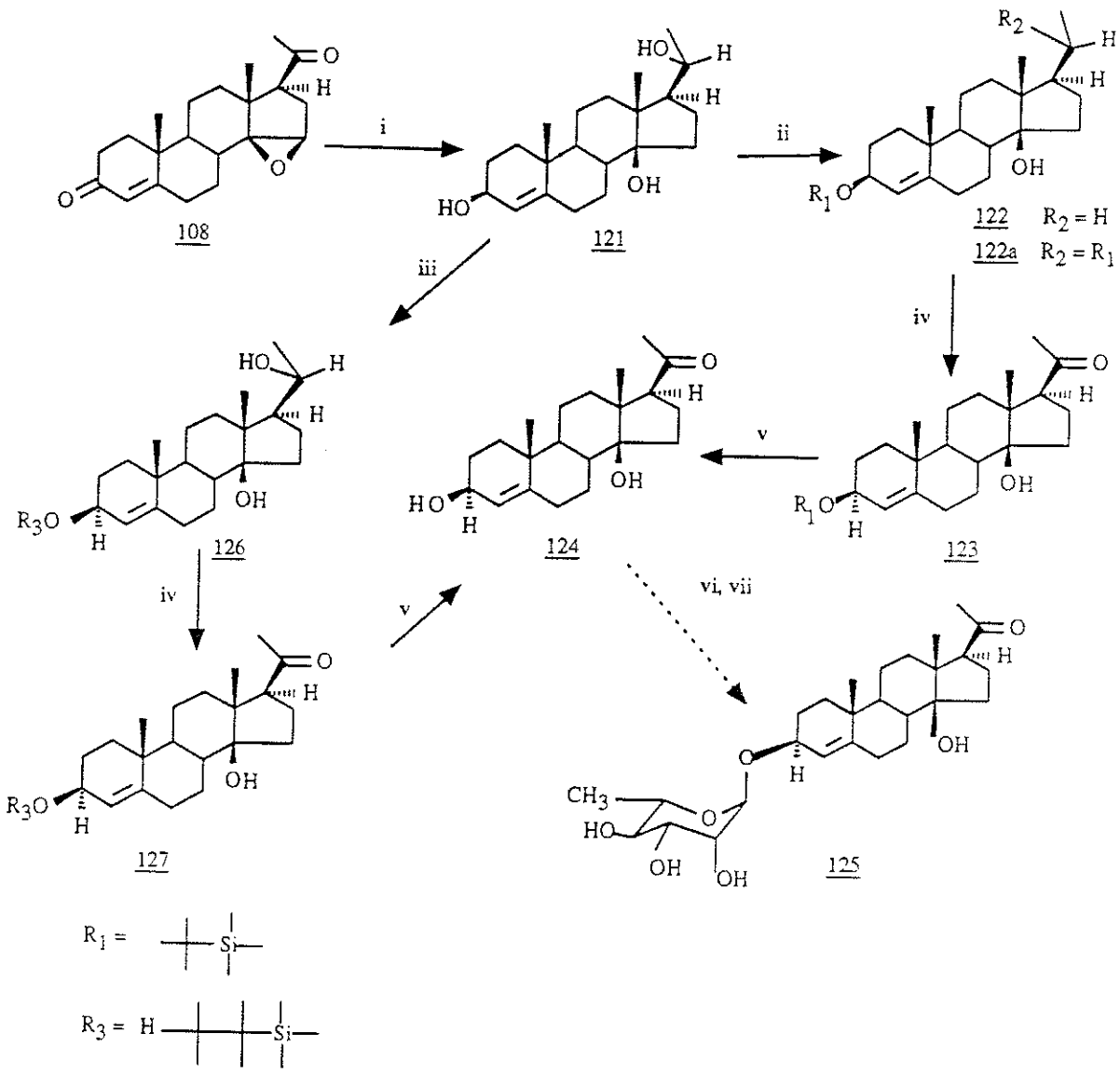
EIMS m/z (%RA): 328 (M^+, 16), 285 (M^+-CH_3CO), 267 (18), 246 (100), 83 (75).

CHAPTER 5

SYNTHESIS OF 14ß,15ß-EPOXY-20ß-HYDROXY-3ß-(α-L-RHAMNOSYLOXY)-PREGN-5-ENE AND STRUCTURALLY RELATED PREGN-4-ENE AND PREGN-5-ENE DERIVATIVES

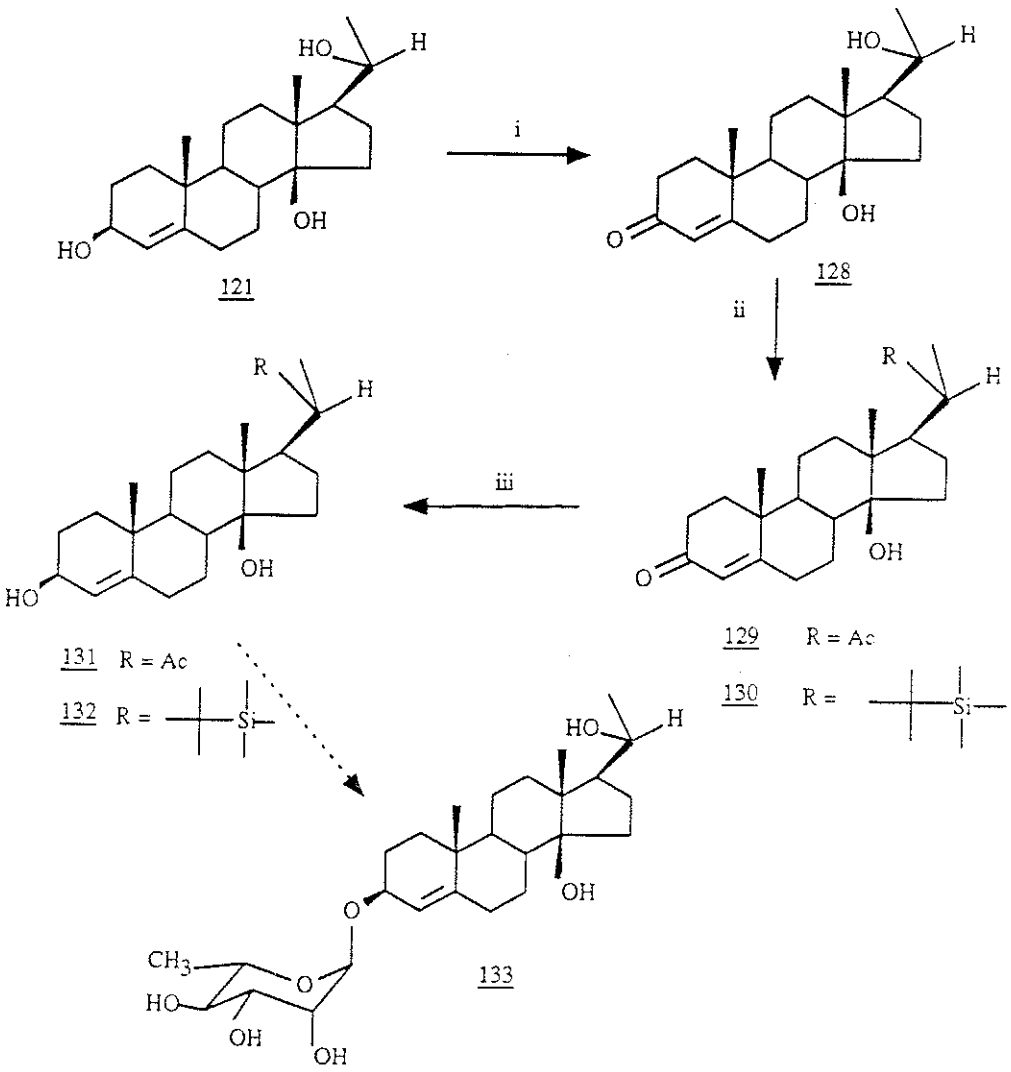
5.1 Introduction

As pointed out in Chapter 3, the nature and spacial coordination of the substituents at C_3, C_{14} and C_{17} are determinants of receptor binding affinity and, cooperatively, stereochemical differences of the steroidal skeleton, i.e. a 5α, 5ß, C_4 or C_5 double bond, inevitably vary the spacial coordination or pattern of the substituents. Therefore, the spacial pattern of C_3, C_{14} and C_{17} substituents required for optimum receptor binding affinity may be different among pregnane derivatives with different stereochemistry at the steroid ring junctions, i.e. 5α, 5ß ring junctions and C_4 or C_5 double bond(s). Illustration of differences in receptor binding affinity between pregnanes with the same substituents at C_3, C_{14} and C_{17}, but different stereochemistry at C_4 or C_5, may help to delineate the specific pharmacophoric pattern necessary for progesterone derivatives to display potent receptor binding affinity. One of our objectives has been to synthesize C_4 and C_5 unsaturated derivatives to this end. For these SAR studies, we required the genins with different stereochemistry at ring A/B and C/D junctions with similar functional groups at C_3, C_{14} and C_{17}, e.g. 3ß,14ß-dihydroxy-pregn-4-en-20-one (124), 14ß,20ß-dihydroxypregn-4-en-3-one (128), 14ß,15ß-epoxy-20ß-hydroxypregn-5-ene (138) and related rhamnosides 137 and 139 (Schemes 18, 19, 20 and 21). Efforts have been made to synthesize rhamnosides 125, 133 and 142 (Schemes 18, 19 and 20).



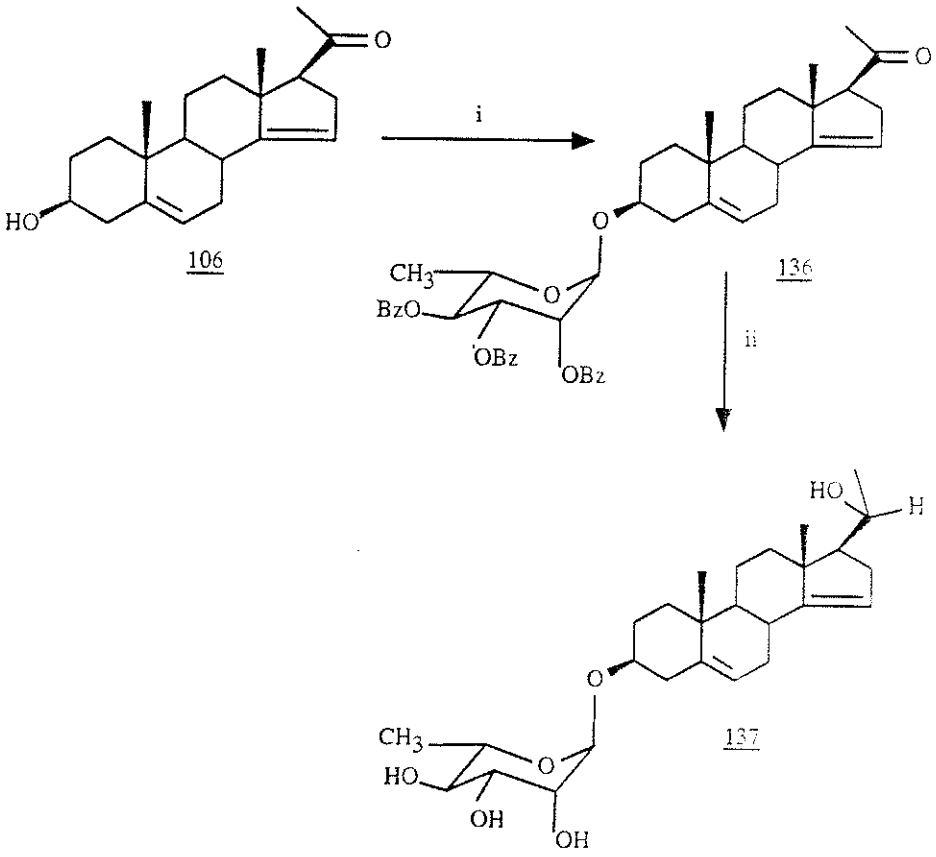
Reagents: i) Lithium diethylborohydride/THF; ii) t-butyldimethylsilyl chloride/imidazole/THF iii) dimethylthexylsilyl chloride/imidazole/DMF; iv) pyridinium dichromate/DMF v) tetra-n-butylammonium fluoride/THF; vi) 1-bromo-α-L-triacetylrhamnoside/ Fetizon's/HgO/$HgBr_2$/2,6-di-t-butyl-4-methylpyridine; vii) triethylamine/MeOH/ water

Scheme 18 Synthesis of 14β-hydroxy-3β-(α-L-rhamnosyloxy)-pregn-4-en-20-one (125)



Reagents : i) bis(tetrabutyl)ammonium dichromate/CH_2Cl_2/DMF; ii) t-butyldimethylsilyl chloride imidazole/DMF; iii) LTBAH/THF

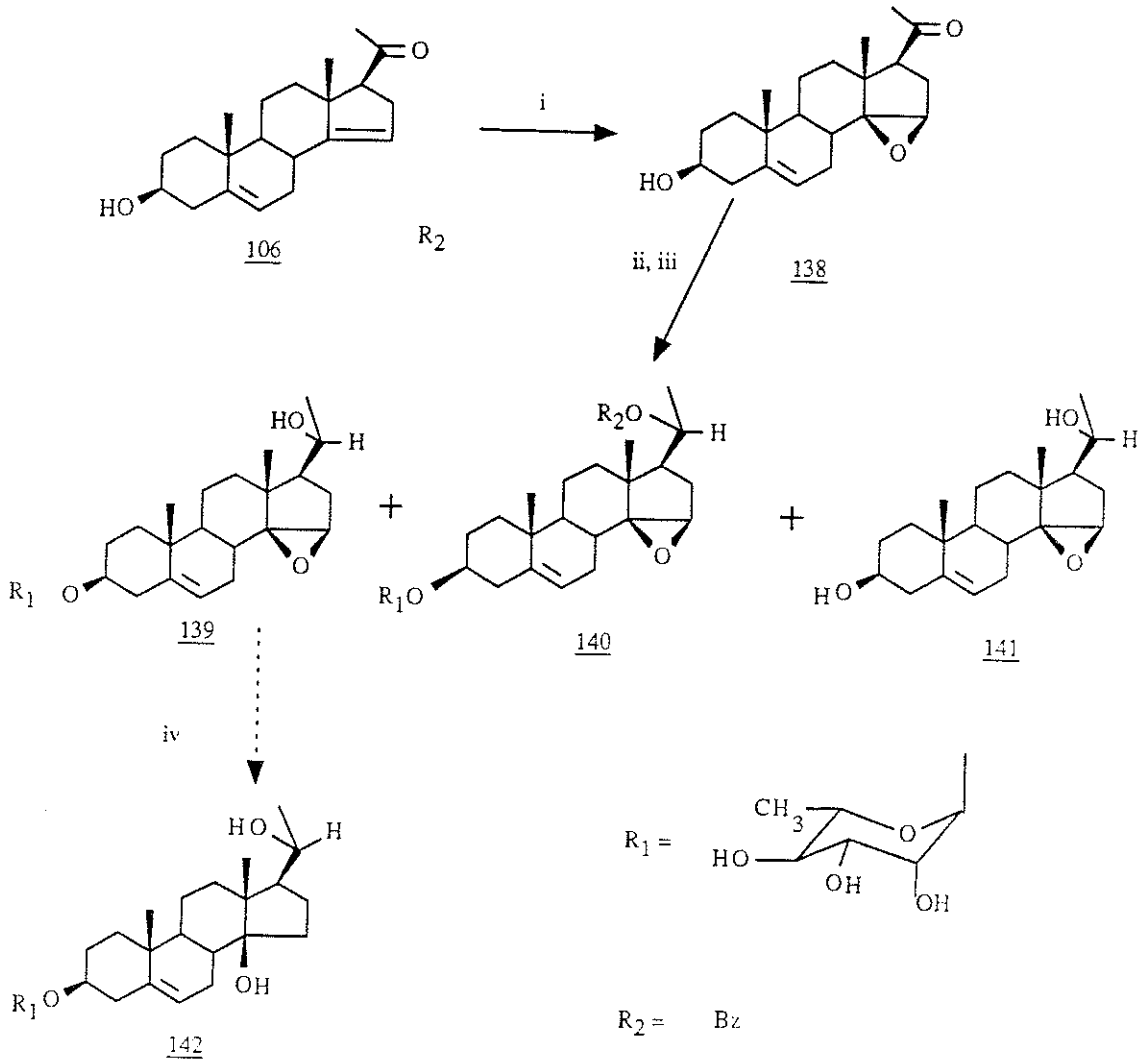
Scheme 19 Synthetic approach to 20β-acetyl-3β,14β-dihydroxy-pregn-4-ene (131) and 20β-t-butyldimethylsilyloxy-3β,14β-dihydroxypregn-4-ene (132)



Reagents: i) 1- bromo-α-L-tribenzoylrhamnoside/Fetizon's/CH_2Cl_2

ii) lithium triethylborohydride/THF

Scheme 20 Synthesis of 20β-hydroxy-3β-(α-L-rhamnosyloxy)-pregna-5,14-diene (137) from 3β-hydroxypregna-5,14-dien-20-one (106)



Reagents:
i) NBA/acetic acid/acetone
ii) 1-bromo-α-L-tribenzoylrhamnoside/Fetizon's/CH_2CH_2
iii) 10% ammonia in MeOH
iv) lithium triethylborohydride/THF

Scheme 21 Synthesis of 14β, 20β-dihydroxy-3β-(α-L-rhamnosyloxy)-pregn-5-ene (142) from 3β-hydroxypregna-5,14-dien-20-one (106)

The synthetic approaches (Schemes 12, 18, 20 and 21), designed for the synthesis of these rhamnosides proved to be difficult. The difficulty can be attributed to the following factors:

1) The preparation of the required compounds, in the case of preparation of 3ß,14ß-dihydroxypregn-4-en-20-one rhamnoside (125), starting from 3ß-acetoxypregna-5,14-dien-20-one (100) requires 14 steps (Schemes 12 and 18). In almost every step, separation and purification, via chromatography and recrystallization are required, to ensure the purity of the compounds for measurements in the RBA tests.
2) The linear synthetic approach carried out suppresses the overall yield (Schemes 12, 18, 19, 20 and 21).
3) Glycosylation of allylic alcohols has not been extensively investigated. Under Koenigs-Knorr conditions, the major product is the dehydrated diene (Smyth et al., 1992). The rhamnosylation of 14ß-hydroxyprogesterone (94) has not been reported previously. In addition, the rhamnosylation is nearly the last step and this creates additional difficulty in carrying out model experiments. The use of heavy metal salts in diethyl ether for rhamnosylation did not prove useful, as the optimum conditions determined for 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (69) did not give the same result with 3ß,14ß-dihydroxypregn-4-en-20-one (124).

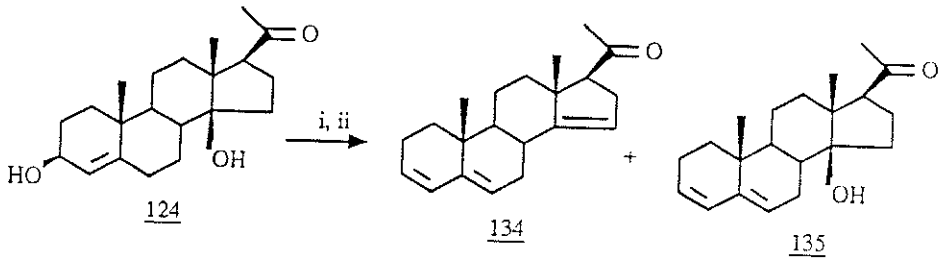
The 3ß-OH precursors (124, 131 and 132) required for the rhamnosylation were prepared by two routes, as illustrated in Schemes 18 and 19. In Scheme 18, the triol (121) was treated with one equivalent of t-butyldimethylsilyl chloride to give 3ß-t-butyldimethylsilyl-14ß,20ß-dihydroxypregn-4-ene (122) chemoselectively (Corey and Venkateswaralu, 1972; Templeton et al., 1988). Oxidation of this compound (122) by treatment with PDC (Corey and Venkateswaralu, 1972), followed by removal of the silyl group by reaction with tetra-n-butylammonium fluoride (Santaniello and Ferraboschi, 1980), gave 3ß-hydroxy-pregn-4-en-20-one (124), which was used to couple with the activated rhamnose. Alternatively, dimethylthexylsilyl chloride (Wetter and Oertle, 1985) was used instead of t-butyldimethylsilyl chloride as the silylating reagent, to give 3ß,14ß-dihydroxypregn-4-en-20-one (124) (Scheme 18). The advantage of this reagent is that it is less costly and easier to handle (Wetter and Oertle, 1985). However, a longer reaction time was required to remove the dimethylthexylsilyl group by treatment with tetra-n-butylammonium fluoride than the t-butyldimethylsilyl group (20 hr vs 12 hr).

In Scheme 19, the triol (121) was chemoselectively oxidized at C_3 by treatment with bis(tetrabutyl)ammonium dichromate (Landini and Rolla, 1979; Santaniello and Ferraboschi, 1980) in dichloromethane and DMF to give 14ß,20ß-dihydroxypregn-4-en-3-one (128). Silylation of this compound was carried out by treatment with t-butyldimethylsilyl

chloride (Corey and Venkateswaralu, 1972) followed by reduction with LTBAH to give 3ß,14ß-dihydroxy-20ß-t-butyldimethylsilyloxypregn-4-ene (131). The diol (128) was also acylated by treatment with acetic anhydride and pyridine, and then reduced by reaction with LTBAH to give 3ß,14ß-dihydroxy-20ß-acetoxy-pregn-4-ene (132). These compound are suitable for rhamnosylation with the activated sugars.

The conditions developed for the rhamnosylation of 17α-acetoxy-6α-methyl-3ß-hydroxypregn-4-en-20-one (64) (Smyth et al., 1992), as described in Chapter 2, failed completely for the rhamnosylation of 3ß,14ß-dihydroxypregn-4-en-20-one (124). Thus, as a model the 14α compound was unsatisfactory for the 14ß-OH analogue. Under the same conditions, rhamnosylation of 3ß,14ß-dihydroxypregn-4-en-20-one (124) gave the dehydrated compounds (134 and 135) and none of the desired product was isolated (Scheme 22). Attempts have been made to synthesize 3ß,14ß-dihydroxypregn-4-en-20-one rhamnoside (125) by treatment of compound 121 with Fetizon's reagent, HgO, $HgBr_2$ and a non-nucleophilic organic base (2,6-di-t-butyl-4-methyl-pyridine) in dichloromethane (Scheme 18). However, chemical characterization of this compound has not be fully established due to lack of the starting material, the preparation of which requires twelve synthetic steps.

The rhamnosides of 3ß,20ß-dihydroxypregna-5,14-diene and 3ß-hydroxy-14ß,15ß-epoxypregn-5-ene, e.g. compounds 137 and 139, were synthesized under Koenigs-Knorr reaction conditions



124 134 135

Reagents : i) 1-bromo-α-L-triacetylrhamnoside/Fetizon's/HgO/$HgBr_2$/diethyl ether
ii) Et_3N/MeOH/H_2O/reflux 20 hr

Scheme 22 Formation of pregna-3,5,14-trien-20-one (134) and 14β-hydroxy-pregna-3,5-dien-20-one (135) under Koenigs-Knorr reaction conditions in diethyl ether

using 3ß-hydroxypregna-5,14-dien-20-one (106) as starting material (Schemes 20 and 21).

5.2 Results and Discussion

To prepare 14ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregn-4-en-20-one (125), 14ß,20ß-dihydroxy-3ß-(α-L-rhamnosyloxy)-pregn-4-ene (133) and 14ß,20ß-dihydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (142), appropriate genins were first required for coupling with the activated rhamnose, i.e. 1-bromo-α-L-triacetyl-rhamnoside (75) or 1-bromo-α-L-benzoylrhamnoside (77). Two synthetic approaches, as described in Schemes 18 and 19, have been developed for this purpose, starting from 3ß-hydroxy-pregna-5,14-dien-20-one (106) and 3ß,14ß,20ß-trihydroxypregn-4-ene (121), the intermediates in the synthesis of 14ß-hydroxyprogesterone (94) (Scheme 12 in Chapter 4).

In Scheme 18, the triol (121) was treated with t-butyl-dimethylsilyl chloride in the presence of imidazole (Corey and Venkateswaralu, 1972) to give 3ß-t-butyldimethylsilyloxy-14ß,20ß-dihydroxypregn-4-ene (122) as the major compound, and the disilyl ether (122a) as the minor compound. Dimethyl-thexylsilyl chloride was used to replace t-butyldimethylsilyl chloride for preparation of 3ß,14ß-dihydroxylpregn-4-en-20-one (124) in comparable yield. The procedure was similar to the t-butyldimethylsilyl chloride procedure except that a longer reaction time was required for removal of the silyl protecting group by treatment with tetra-n-butylammonium fluoride (20 hr vs 12 hr). The chemoselectivity on silylation is due to

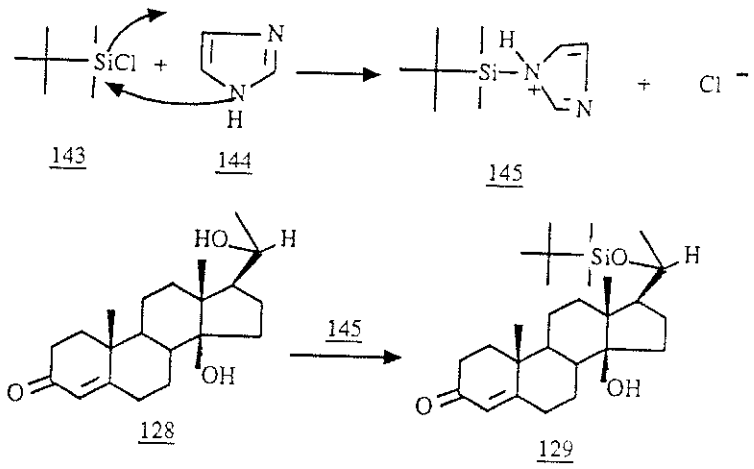
different steric environment around the two hydroxyl groups, the 3ß-OH being less hindered than the 20ß-OH.

14ß,20ß-Dihydroxy-3ß-t-butyldimethylsilyloxypregn-4-ene (122) was then oxidized by treatment with PDC to give 3ß-t-butyldimethylsilyloxy-14ß-hydroxypregn-4-en-20-one (123). The removal of the t-butyldimethylsilyl group was achieved by treatment with tetra-n-butylammonium fluoride to give 3ß-hydroxypregn-4-en-20-one (124), which was used for rhamnosylation.

The selective oxidation of allylic alcohols using manganese dioxide (Mancera et al., 1953) and DDQ to form α,ß-unsaturated ketones (Walker and Hiebert, 1967; Burn et al., 1960) have been reported. However, these reagents failed to convert the triol (121) to 14ß,20ß-dihydroxypregn-4-en-3-one (128) with high chemoselectivity in this case. The utility of 3,5-dimethylpyrazole complexed with chromium trioxide, for oxidation of alcohols to carbonyl compounds and for benzylic oxidation has been reported (Corey et al., 1973; McDonald et al., 1975; Salmond et al., 1975; Chorvat et al., 1979). This method gave better chemoselectivity compared to other reagents, e.g. MnO_2.

As shown in Scheme 19, the triol (121) was oxidized to the enone (128) by treatment with bis(tetrabutyl)ammonium dichromate (Lanadini and Rolla, 1979; Santaniello and Ferraboschi, 1980) in dichloromethane and DMF in 80% yield. In the ^1H NMR spectrum of compound 128, the two singlet signals

of three methyl protons each, at δ = 1.20 ppm and δ = 1.25 ppm, are assigned to C_{10}-CH_3 and C_{10}-CH_3 respectively, based on NOE enhancement between the C_{17}-H and C_{13}-CH_3. The two proton singlet, at δ = 2.51 ppm, is assigned to the 14ß-OH and the 20ß-OH from treatment of the sample with D_2O. In a resolution enhanced ^1H NMR spectrum of compound 128, the spin-spin coupling constant between 17α-H and 20α-H was determined to be 4.3 Hz which is identical to that of analogous R epimers in compounds studied previously (Marat et al., 1993). The product, 20ß-hydroxypregn-4-en-3-one (128), was then acetylated by treatment with acetic anhydride and pyridine to give 20ß-acetoxy-pregn-4-en-3-one (129). By treatment with LTBAH in THF, 20ß-acetoxylpregn-4-en-3-one (129) was converted to 3ß-hydroxy-20ß-acetoxypregn-4-ene (131), a precursor for rhamnosylation. 20ß-Hydroxypregn-4-en-3-one (128) was also treated with t-butyldimethylsilyl chloride in the presence of imidazole (Corey et al., 1972) to give the silyl ether (130) (Scheme 19). The use of imidazole as catalyst and DMF as solvent results in conversion of alcohols to t-butyl-dimethyl-silyl ethers in high yield at ambient conditions. This process may proceed via N-t-butyldimethylsilylimidazole (145) (Corey et al., 1972), a conjugate acid of imidazole, which can be expected to be a very reactive silylating reagent, as illustrated in Scheme 23. The silyl ether (130) was then converted to the alcohol (132) by reduction with LTBAH in THF.



Scheme 23 Proposed mechanism for imidazole as a catalyst in the silylation reaction

t-Butyldimethylsilyl ethers are generally not affected by metal hydride reagents, e.g. LAH (Corey et al., 1972).

The chemoselective oxidation of the hydroxyl groups in the triol (121) with chromate reagents is due to the formation of the energetically favoured allylic carbocation (House, 1972). The 3ß-allylic alcohol is in the quasi-equatorial configuration and more liable to chromate oxidation (Parish and Scott, 1983) than the 20ß-OH group. It has been reported that an allylic alcohol with a quasi-axial configuration, e. g., the 3α-hydroxypregn-4-ene system, has a slower rate of oxidation by chromate (House, 1972; Parish and Scott, 1983). However both ß and α epimers can be oxidized to the corresponding ketone if the chromate reagent is in excess (Parish and Scott, 1983).

For allylic alcohols, three factors may affect the yield of the glycosylation reaction:

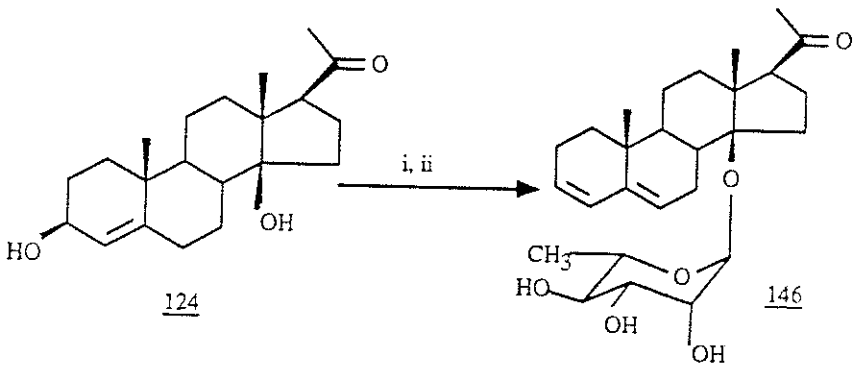
1) Solvent (Wulff and Rohle, 1974)
2) Catalyst (Lemieux et al., 1975; Paulsen, 1982)
3) Acid scavenger (Lemieux and Morgan, 1963; Hanessian et al., 1975a, b, 1976, 1977)

In Chapter 2, a glycosylation method involving the use of diethyl ether as solvent for synthesis of the allylic alcohol rhamnoside, i.e. 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one rhamnoside (69), has been described. In diethyl ether, the rate of formation of the dehydrated diene, i.e. 17α-acetoxy-6α-methylpregna-3,5-dien-20-one (66), was signifi-

cantly suppressed as monitored by TLC and hence increased the yield from 5% (in dichloromethane) to 30% (in diethyl ether).

However, under the same conditions, the glycosylation of 3ß,14ß-dihydroxypregn-4-en-20-one (124) with 1-bromo-α-L-tribenzoylrhamnoside (77) gave dehydrated triene and diene, i.e. pregna-3,5,14-triene (134) and 14ß-hydroxypregna-3,5-dien-20-one (135), and none of the desired glycoside was isolated (Scheme 22). It was found that under a high concentration of the sugar, e.g. 1-bromo-α-L-triacetylrhamnoside (75) (>6 eq.), and lack of the organic base (<1 eq.), 14ß-hydroxypregna-3,5-dien-20-one rhamnoside (146) was formed (Scheme 24). The structure of compound 146 has been confirmed by ^{1}H, ^{13}C and COSY45 NMR spectra.

1-Bromo-α-L-triacetylrhamnoside (75) and 1-bromo-α-L-tribenzoylrhamnoside (77) have similar reactivities regarding rhamnosylation (Paulsen, 1982). Compared to bromorhamnoside triacetate (75) (Farkas et al., 1965), bromorhamnoside tribenzoate (77) is more readily crystallized and more stable (Ness et al., 1951). Under the method developed, 1-bromo-α-L-tribenzoylrhamnoside (77) was also used to couple with 3ß,14ß-dihydroxypregn-4-en-20-one (124) to afford 3ß,14ß-dihydroxypregn-4-en-20-one rhamnoside tribenzoate (147). When this compound was treated with NH_3 and methanol, it was found that one of the benzoyl groups, which was probably at $C_{2'}$, was difficult to remove. In addition, the resulting compound (148)



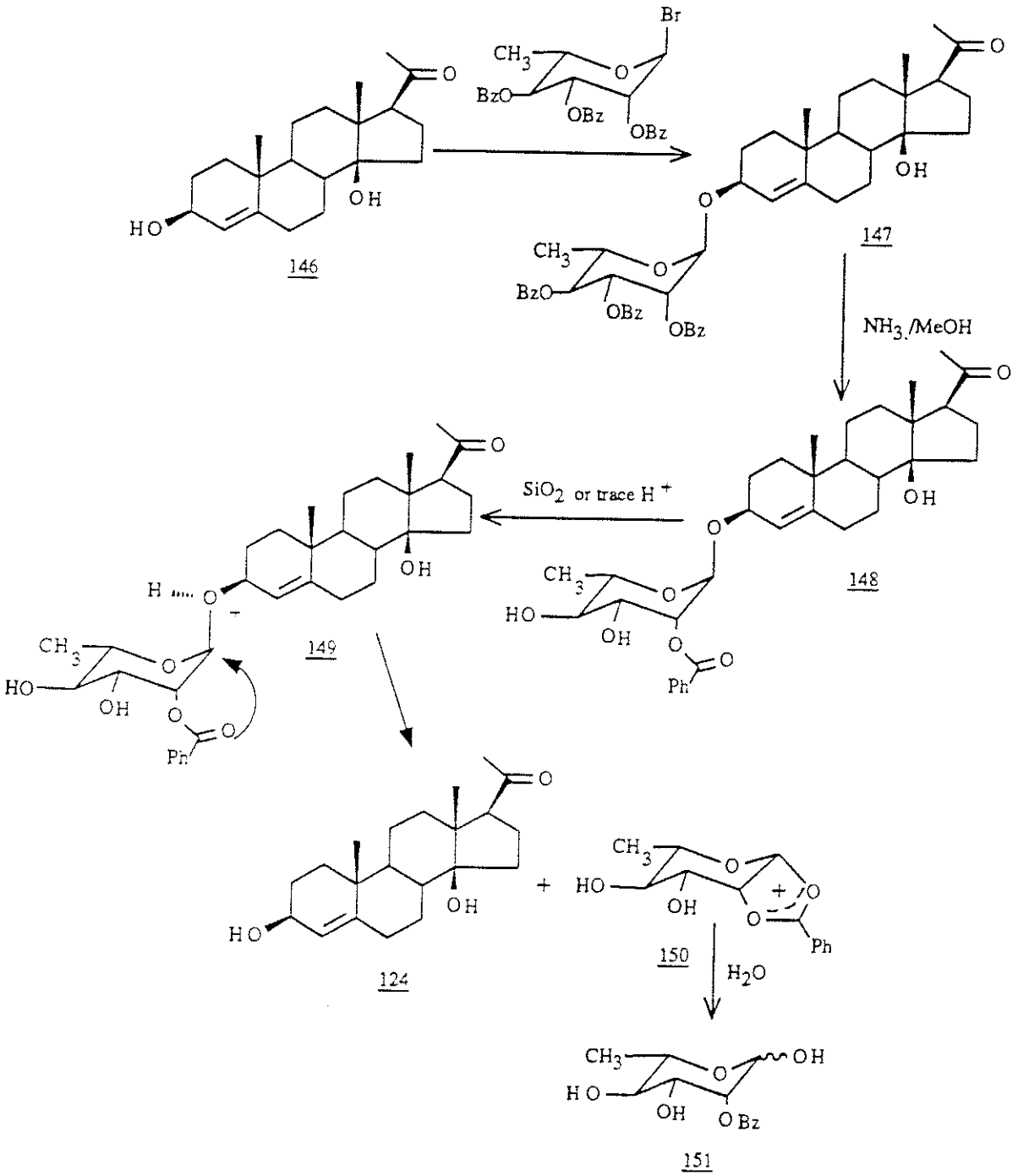
124 146

Reagents: i) 1-bromo-α-L-triacetylrhamnoside (75) (> 6 equivalent)/Fetizon's/HgO/$HgBr_2$/CH_2Cl_2/2,6-di-t-butyl-4-methylpyridine
ii) Et_3N/MeOH/H_2O/reflux 20 hr

Scheme 24 Formation of 14β(α-L-rhamnosyloxy)-pregna-3,5-dien-20-one (146) under Koenigs-Knorr reaction conditions with excess 1-bromo-α-L-triacetylrhamnoside (75)

seems to be very unstable on chromatography over silica, or in deuteriochloroform, and decomposed to give 3ß,14ß-dihydroxy-pregn-4-en-20-one (124) and the sugar (151). Based on this observation, a tentative mechanism was proposed (Scheme 25). An attempt at conversion of 3ß,14ß-dihydroxypregn-4-en-20-one rhamnoside tribenzoate (147) by treatment with sodium boro-hydride to 3ß,14ß,20ß-trihydroxypregn-4-ene rhamnoside tribenzoate, followed by hydrolysis in methanolic potassium hydroxide solution to give 3ß,14ß,20ß-trihydroxypregn-4-ene rhamnoside (133) has not been completed, due to a lack of the starting material which requires twelve synthetic steps.

Synthesis of the glycosides of pregn-5-ene analogues was carried out starting from 3ß-hydroxypregna-5,14-dien-20-one (106) (Schemes 20 and 21). Treatment of 3ß-hydroxylpregna-5,14-dien-20-one (106) with 1-bromo-α-L-tribenzoylrhamnoside (77) in the presence of Fetizon's reagent gave 3ß-hydroxy-pregna-5,14-dien-20-one rhamnoside benzoate (136). Reduction of this compound with lithium triethylborohydride gave 3ß,20ß-dihydroxy-3ß-(α-L-rhamnosyloxy)-pregna-5,14-diene (137) (Scheme 20). Formation of 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) was achieved by treatment of 3ß-hydroxy-pregna-5,14-dien-20-one (106), with one equivalent of NBA in the presence of acetic acid at 0 °C followed by treatment with saturated aqueous potassium carbonate (Scheme 21). At room temperature, and with excess of NBA, more by-products were formed. 14ß,15ß-Epoxy-3ß-hydroxypregn-5-en-20-one (138) was



Scheme 25 Proposed mechanism for cleavage of the rhamnoside linkage after addition of $CDCl_3$ or chromatography over silica

treated with 1-bromo-α-L-tribenzoylrhamnoside (77) in the presence of Fetizon's reagent followed by treatment with lithium triethylborohydride gave 14ß,15ß-epoxy-20ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (139), 14ß,15ß-epoxy-20ß-benzoyloxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (140) and 14ß,15ß-epoxy-3ß,20ß-dihydroxy-pregn-5-ene (141). However, the structure of compound 142 obtained by treatment of 14ß,15ß-epoxy-20ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (139) with lithium triethylborohydride has not be confirmed due to restricted amount of material.

The receptor binding affinities of the synthesized compounds were determined by a [^{3}H]ouabain radioligand binding assay. The results will be discussed in Chapter 6.

5.3 Summary

1) 14ß,15ß-Epoxy-3ß-hydroxypregn-5-en-20-one (138) has been synthesized by treatment of 3ß-hydroxypregna-5,14-dien-20-one (106) with one equivalent of NBA in a solution of acetone, acetic acid and water at low temperature (0-5 °C). This compound can be used as a precursor for preparation of a series of pregn-5-ene derivatives, e.g. 14ß,15ß-epoxy-3ß,20ß-dihydroxypregn-5-ene rhamnoside (139) and 3ß,14ß,20ß-trihydroxypregn-5-ene rhamnoside (142).
2) 3ß,14ß-Dihydroxypregn-4-en-20-one (124), a precursor designed for preparation of 3ß,14ß-dihydroxypregn-4-en-20-one 3ß-rhamnoside (125), has been synthesized based on a method described previously (Templeton et al., 1988).

Dimethylthexylsilyl chloride, which is more convenient to handle and less costly, has been used to replace the silylating reagent t-butyldimethylsilyl chloride for silylation of the triol (121).

3) 14ß,20ß-Dihydroxypregn-4-ene-3-one (128), an alternative precursor for preparation of 3ß,14ß,20ß-trihydroxypregn-4-ene (133) has been synthesized by treatment of 3ß,14ß,20ß-trihydroxypregn-4-ene (109) with one equivalent of TBADC in dichloromethane and DMF in 80% yield.

4) The rhamnosides 137 and 139 have been synthesized by rhamnosylation of 3ß-hydroxypregna-5,14-diene-20-one (106) and 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) under Koenigs-Knorr reaction conditions followed by stereoselective reduction with $LiEt_3BH$.

5.4 Experimental

General methods and materials are described previously in Chapter 2. NOE and COSY45 NMR spectra were recorded in $CDCl_3$ on a Bruker AM 300 and AM 500 instrument, respectively.

3ξ,14ß,20ξ-Trihydroxypregn-4-ene (109)

To a stirred solution of 14ß,15ß-epoxypregn-4-ene-3,20-dione (108) (810 mg) in anhydrous THF (116 mL) was added lithium triethylborohydride in THF (16 mL, 1.0 M in THF). The solution was refluxed with stirring under argon for 3 hr and after cooling in an ice-bath, then 5% sodium hydroxide solution (16 mL) and hydrogen peroxide (30%, 16 mL) were added. The solution was stirred overnight at room temperature

and diluted with water followed by extraction with dichloromethane. The combined extracts were dried over anhydrous sodium sulphate, filtered and evaporated to dryness to give the triol (109) (728 mg) which was recrystallized from diethyl ether and methanol, m.p. 185-191 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 1.06 (s, 3H, C_{13}-CH_3), 1.22 (s, 3H, C_{10}-CH_3), 1.26 (d, 3H, J = 6 Hz, C_{20}-CH_3), 3.85 (m, 1H, C_{20}-H), 4.15 (m, 1H, C_3-H), 5.30 (d, 1H, J = 0.6 Hz, C_4-H).

3ß-t-Butyldimethylsilyl-14ß,20ß-dihydroxypregn-4-ene (122)

To a stirred solution of 3ß,14ß,20ß-trihydroxypregn-4-ene (121) in anhydrous DMF (12 mL) were added imidazole (194 mg) and t-butyldimethylsilyl chloride (206 mg, 1.2 eq.). The solution was stirred under argon at room temperature for one hour and diluted with cold saturated aqueous sodium hydrogen sulphate (10 mL) followed by extraction with diethyl ether. The extract was washed with dilute HCl solution (0.3 M) followed by saturated aqueous sodium hydrogen carbonate, and then water. The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated under reduced pressure to dryness to give a gummy residue. Flash chromatography of the residue over silica on elution with dichloromethane gave the disilyl ether (122a) (133 mg), which was crystallized from diethyl ether and methanol, m.p. 105-107 °C.

^{1}H NMR ($CDCl_3$) : 0.06 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3), 0.11 (s, 6H, Si-CH_3), 0.90 [s, 9H, $C(CH_3)_3$], 0.92 [s, 9H, $C(CH_3)_3$], 1.03 (s, 3H, C_{10}-CH_3), 1.18 (s, 3H, C_{13}-CH_3), 1.31 (d,

3H, J = 6.5 Hz, C_{20}-CH_3), 3.85 (m, 1H, C_{20}-H), 3.90 (s, 1H, C_{14}-OH), 4.18 (m, 1H, C_3-H), 5.20 (d, 1H, J = 1.2 Hz, C_4-H).

The fractions on elution with methanol gave the monosilyl ether (122) (497 mg) which did not crystallize.

^{1}H NMR ($CDCl_3$) δ: 0.06 (s, 3H, Si-CH_3), 0.07 (s, 3H, Si-CH_3), 0.89 [m, 9H, $C(CH_3)_3$], 1.03 (s, 3H, C_{10}-CH_3), 1.19 (s, 3H, C_{13}-CH_3), 1.26 (d, 3H, J = 6.6 Hz, C_{20}-CH_3), 3.85 (m, 1H, C_{20}-H), 4.15 (m, 1H, CH_3), 5.20 (d, 1H, J = 1 Hz, C_4-H).

14ß-Hydroxy-3ß-t-butyldimethylsilylpregn-4-en-20-one (123)

3ß-t-Butyldimethylsilyl-14ß,20ß-dihydroxypregn-4-ene (122) (497 mg) was dissolved in dichloromethane (30 mL) and DMF (5 mL), followed by treatment with PDC (3 g). The solution was stirred under argon for one hour and then washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to give the silyl ketone (123) (344 mg), which did not crystallize.

^{1}H NMR ($CDCl_3$) δ: 0.07 (s, 3H, Si-CH_3), 0.08 (s, 1H, Si-CH_3), 0.90 [s, 9H, $C(CH_3)_3$], 0.99 (s, 3H, C_{13}-CH_3), 1.04 (s, 3H, C_{10}-CH_3), 2.23 (s, 3H, C_{20}-CH_3), 2.90 (m, 1H, C_{17}-H), 4.20 (m, 1H, C_3-H), 4.34 (s, 1H, C_{14}-OH), 5.21 (d, 1H, J = 1 Hz, C_4-H).

EIMS m/z (%RA): 446 (M^+, 12), 389 [M^+-$C(CH_3)_3$, 20].

3ß,14ß-Dihydroxypregn-4-en-20-one (124)

Method A: 3ß-t-Butyldimethylsilyl-14ß-hydroxypregn-4-en-20-one (123) (344 mg) was dissolved in anhydrous THF, followed by treatment with tetra-n-butylammonium fluoride (TBAF) (3.2 mL, 1 M in THF). The solution was stirred for 12 hr at room

temperature, then diluted with water (15 mL) followed by exhaustive extraction with dichloromethane. The combined extracts were dried over sodium sulfate, filtered and evaporated to dryness to give a residue which was subjected to flash chromatography over silica. The fractions on elution with diethyl ether, gave 3ß,14ß-dihydroxypregn-4-en-20-one (124) (108 mg) which was recrystallized from dichloromethane and acetone, m.p. 211-214 °C, (lit. 200-202 °C, Templeton et al., 1988).

^{1}H NMR ($CDCl_3$) δ: 1.00 (s, 3H, C_{10}-CH_3), 1.03 (s, 3H, C_{13}-CH_3), 2.24 (s, 3H, C_{20}-CH_3), 2.90 (m, 1H, C_{17}-H), 4.15 (m, 1H, C_3-H), 4.38 (s, 1H, C_{14}-OH), 5.29 (d, 1H, J = 1.4 Hz, C_4-H).

EIMS m/z (%RA): 332 (M^+, 1), 314 (M^+-H_2O, 37), 296 (35), 228 (43), 91 (98), 105 (100).

RBA (IC_{50}): 4.0 μM

Method B: To a stirred solution of 3ß,14ß,20ß trihydroxypregn-4-ene (121) (100 mg) in anhydrous DMF (4 mL) was added imidazole (51 mg) and dimethylthexylsilyl chloride (64 mg). The solution was stirred under argon for 2 hr, then diluted with saturated aqueous sodium hydrogen carbonate (10 mL) followed by extraction with dichloromethane. The organic phase was washed with water, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to give a gummy residue which was subjected to flash chromatography over silica. The fractions, on elution with methanol, gave the monosilyl ether (108 mg) which did not crystallize. The monosilyl ether was

dissolved in dichloromethane (8 mL) and DMF (2 mL), followed by treatment with PDC (800 mg). The solution was stirred under argon at room temperature for 1 hr and then passed through a column packed with silica. The fractions, on elution with dichloromethane and methanol, gave a residue. The residue was dissolved in anhydrous THF (8 mL) followed by treatment with TBAF (4 mL). The solution was stirred at room temperature for 20 hr and was then diluted with water (8 mL), followed by extraction with dichloromethane. The combined extracts were washed with saturated aqueous sodium hydrogen carbonate (20 mL) and water (20 mL X 2). The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness, to give a residue which was subjected to flash chromatography over silica. The fractions, on elution with diethyl ether, gave the crystalline product (124) (25 mg) from dichloromethane and methanol, m.p. 207-213 °C. The structure of 124 was confirmed by ^{1}H NMR.

14ß,20ß-Dihydroxypregn-4-en-3-one (128)

3ß,14ß,20ß-Trihydroxypregn-4-ene (121) (371 mg) was dissolved in dichloromethane (25 mL) and DMF (3 mL), followed by addition of bis(tetrabutyl)ammonium dichromate (1.80 g). The solution was brought to reflux under argon for 12 hr. Evaporation of the solution under reduced pressure to dryness gave a residue, which was subjected to flash chromatography over silica. Fractions on elution with 50% ethyl acetate in

hexane gave the 3-ketone (128) (341 mg). The product was recrystallized from acetone and hexane, m.p. 198-203 °C.

^{1}H NMR ($CDCl_3$) δ: 1.19 (s, 3H, C_{10}-CH_3), 1.25 (s, 3H, C_{13}-CH_3), 1.29 (d, 3H, J = 6.4 Hz, C_{20}-CH_3), 2.71 (s, 2H, C_{14}-OH and C_{20}-OH), 3.90 (m, 1H, C_{20}-H), 5.73 (s, 1H, C_4-H).

EIMS m/z (%RA): 332 (M^+, 16), 314 (M^+-H_2O, 35), 296 (49), 149 (100).

Elemental Analysis ($C_{21}H_{32}O_3$): Calcd. C 75.86%, H 9.70%; Found C 76.07%, H 9.74%.

RBA (IC_{50}): 47 μM

20ß-Acetoxy-14ß-hydroxypregn-4-en-3-one (129)

Compound 128 (50 mg) was treated with acetic anhydride (5 mL) and pyridine (5 mL) at room temperature for 6 hr. The solution was diluted with ice-water and stirred for an additional hr and then extracted with dichloromethane. The extracts were washed with water, saturated aqueous sodium hydrogen carbonate and water. The organic phase was dried over sodium sulfate, filtered and evaporated to dryness to give a syrupy product (35 mg) which did not crystallize.

^{1}H NMR ($CDCl_3$) δ: 1.02 (s, 3H, C_{13}-CH_3), 1.18 (s, 3H, C_{10}-CH_3), 1.20 (d, 3H, J = 6.4 Hz, C_{20}-CH_3), 2.02 (s, 3H, CH_3CO), 4.95 (m, 1H, C_{20}-H), 5.73 (s, 1H, C_4-H).

20ß-t-Butyldimethylsilyloxy-14ß-hydroxypregn-4-en-3-one (130)

14ß,20ß-Dihydroxypregn-4-en-3-one (128) (150 mg) was treated with t-butyldimethylsilyl chloride (244 mg) and imidazole (236 mg) in DMF (15 mL). The solution was stirred

under argon for 3 hr. Evaporation and workup gave a residue which was subjected to flash chromatography over silica. Fractions on elution with 30% ethyl acetate in hexane gave the silyl ether (130) (135 mg) which did not crystallize.

^{1}H NMR ($CDCl_3$) δ: 0.13 [s, 6H, $Si(CH_3)_2$], 0.93 [s, 9H, $C(CH_3)_3$], 1.18 (s, 3H, C_{13}-CH_3), 1.21 (s, 3H, C_{10}-CH_3), 1.34 (d, 3H, J = 6.5 Hz, C_{20}-CH_3), 3.88 (m, 1H, C_{20}-H), 4.15 (s, 1H, C_{14}-OH), 5.72 (s, 1H, C_4-H).

20ß-Acetoxy-3ß,14ß-dihydroxypregn-4-ene (131)

Compound 129 (35 mg) was dissolved in anhydrous THF (20 mL) and treated with LTBAH (100 mg) at room temperature for 20 hr. Similar workup procedure as that described in the preparation of 63 gave a residue. Flash chromatography over silica on elution with 10% methanol in dichloromethane gave compound 131 (15 mg) which did not crystallize.

^{1}H NMR ($CDCl_3$) δ: 0.96 (s, 3H, C_{13}-CH_3), 1.17 (s, 3H, C_{10}-CH_3), 1.97 (s, 3H, CH_3CO), 4.05 (m, 1H, C_3-H), 5.75 (s, 1H, C_4-H).

20ß-t-Butyldimethylsilyloxy-3ß,14ß-dihydroxypregn-4-ene (132)

14ß-Hydroxy-20ß-t-butyldimethylsilyloxypregn-4-en-3-one (130) (320 mg) was treated with LTBAH (600 mg) in THF and the solution was stirred at room temperature for 26 hr. The solution was diluted with water and extracted with dichloromethane. The organic phase was dried over sodium sulfate, filtered, and evaporated to dryness to give a residue, which was subjected to flash chromatography over silica. Fractions

on elution with 50% diethyl ether in dichloromethane, gave the 3-alcohol (132) (305 mg) which did not crystallize.

^{1}H NMR ($CDCl_3$) δ: 1.12 (s, 3H, Si-CH_3), 1.30 (s, 3H, Si-CH_3), 0.92 [s, 9H, $C(CH_3)_3$], 1.20 (s, 3H, C_{13}-CH_3), 1.33 (s, 3H, C_{10}-CH_3), 1.41 (d, 3H, J = 6.4 Hz, C_{20}-CH_3), 3.85 (m, 1H, C_3-H), 5.40 (q, 1H, C_{20}-H), 5.75 (s, 1H, C_4-H).

Pregna-3,5,14-trien-20-one and 14ß-Hydroxypregna-3,5-dien-20-one (134 and 135)

To a stirred solution of 3ß,14ß-dihydroxypregn-4-en-20-one (124) (141 mg), Fetizon's reagent (1.88 g), HgO (329 mg), $HgBr_2$ (94 mg) and molecular sieves (3 Å, 2 g) in anhydrous diethyl ether (25 mL) was added 1-bromo-α-L-tribenzoyl-rhamnoside (77) (1.34 g). The solution was stirred under argon in the dark at 0-5 °C for 5 hr and then filtered through a pad of Celite. The filtrate was evaporated to dryness to give a residue which was dissolved in a solution of methanol (14 mL), triethylamine (4 mL) and water (4 mL). The solution was refluxed under argon for 20 hr and then worked up by evaporating to dryness to give a residue which was subjected to flash chromatography over silica. The fractions, on elution with 15% ethyl acetate in hexane, gave pregna-3,5,14-trien-20-one (134) (16 mg) after recrystallization from dichloromethane and methanol, m.p. 148-151 °C.

^{1}H NMR ($CDCl_3$) δ: 0.91 (s, 3H, C_{10}-CH_3), 1.12 (s, 3H, C_{13}-CH_3), 2.17 (s, 3H, C_{20}-CH_3), 5.20 (m, 1H, C_{15}-H), 5.45 (m, 1H, C_6-H), 5.62 (m, 1H, C_3-H), 5.93 (d, 1H, J = 10 Hz, C_4-H).

EIMS m/z (%RA): 296 (M^+, 93), 281 (M^+-CH_3, 38), 253 (M^+-CH_3CO), 32), 91 (100).

The later fractions on elution with 10 % methanol in dichloromethane gave 14ß-hydroxypregna-3,5-dien-20-one (135) (26 mg) after recrystallization from dichloromethane and methanol, m.p. 176-180 °C.

^{1}H NMR ($CDCl_3$) δ: 0.93 (s, 3H, C_{13}-CH_3), 1.03 (s, 3H, C_{10}-CH_3), 2.25 (s, 3H, C_{20}-CH_3), 4.46 (s, H, C_{14}-OH), 5.45 (m, 1H, C_6-H), 5.60 (m, 1H, C_3-H), 5.95 (d, 1H, J = 9.8 Hz, C_4-H).

EIMS m/z (%RA): 314 (M^+, 18), 296 (M^+-H_2O, 53), 281 (296-CH_3, 21), 253 (296-CH_3CO, 36), 145 (100).

3ß-[α-L-(Tribenzoyl)-rhamnosyloxyl-pregna-5,14-dien-20-one (136)

To a stirred solution of 3ß-hydroxypregna-5,14-dien-20-one (106) (200 mg) and pulverized molecular sieves (3 Å, 2 g) in dry dichloromethane (30 mL) were added Fetizon's reagent (2.4 g) and 1-bromo-α-L-tribenzoylrhamnoside (77) (1.4 g). The solution was stirred under argon in the dark at room temperature for 5 hr, and then passed through a pad of Celite. The filtrate was evaporated to dryness under reduced pressure at 40 °C to give a residue. Flash chromatography over silica on elution with 50% ethyl ether in dichloromethane gave the glycoside tribenzoate (136) (210 mg) on recrystallization from hexane and acetone, m.p. 174-176 °C.

^{1}H NMR ($CDCl_3$) δ: 0.90 (s, 3H, C_{13}-CH_3), 1.08 (s, 3H, C_{10}-CH_3), 1.30 (d, 3H, J = 6.4 Hz, $C_{5'}$-CH_3), 2.17 (s, 3H, C_{20}-CH_3), 3.60

(m, 1H, $C_{5'}$-H), 4.28 (m, 1H, $C_{3'}$-H), 5.16 (d, 1H, J = 1 Hz, $C_{1'}$-H), 5.17 (s, 1H, C_{15}-H), 5.45 (m, 1H, C_6-H), 5.60 (dd, 1H, $J_{2',1'}$ = 1 Hz, $J_{2',3'}$ = 3 Hz, $C_{2'}$-H), 5.65 (t, 1H, $J_{4',5'}$ = 10 Hz, $J_{4',3'}$ = 10 Hz, $C_{4'}$-H), 5.84 (dd, 1H, $J_{3',4'}$ = 10 Hz, $J_{3',2'}$ = 3 Hz, $C_{3'}$-H), 7.20-8.20 (m, 15H, aromatic protons).

Elemental Analysis ($C_{48}H_{52}O_9$): Calcd. C 74.59%, H 6.78%; Found C 74.29%, H 6.91.

14ß,20ß-Dihydroxy-3ß-(α-L-rhamnosyloxy)-pregna-5,14-diene (137)

3ß-[α-L-(Tribenzoyl)-rhamnosyloxy]-pregna-5,14-dien-20-one (136) (171 mg) was dissolved in anhydrous THF (30 mL) and treated with lithium triethylborohydride (4 mL, 1 M in THF). The solution was brought to reflux for 2 hr and then treated with 5% sodium hydroxide solution (4 mL) and hydrogen peroxide (30%, 4 mL). The solution was stirred for an additional 15 hr at room temperature and extracted with dichloromethane. The extract was washed with water, and the organic phase was dried over sodium sulfate, filtered and evaporated to dryness to give a residue which was subjected to flash chromatography over silica. The fractions from elution with i) hexane, ii) 20% ethyl acetate in hexane, and iii) 10% methanol in dichloromethane, after evaporation, gave the product (137) (75 mg) on recrystallization from methanol and water, m.p. 232-234 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 1.00 (s, 6H, C_{13}-CH_3 and C_{10}-CH_3), 1.13 (d, 3H, J = 6.4 Hz, C_{20}-CH_3), 1.23 (d, 3H, J =6.5 Hz, $C_{5'}$-

CH_3), 3.35 (t, 1H, $J_{4',3'} = J_{4',5'}$ = 10 Hz, $C_{4'}$-H), 3.45 (m, 1H, C_{20}-H), 3.65 (m, 2H, $C_{3'}$-H and $C_{5'}$-H), 3.75 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $J_{2',3'}$ = 3 Hz, $C_{2'}$-H), 3.85 (m, 1H, C_3-H), 4.83 (d, 1H, J = 1.3 Hz, $C_{1'}$-H), 5.10 (s, 1H, C_{15}-H), 5.35 (m, 1H, C_6-H).

Elemental Analysis ($C_{27}H_{42}O_6$): Calcd. C 70.10%, H 9.15%; Found C 70.09%, H 9.25%.

RBA (IC_{50}): No activity; IC_{50} > 200 μM

14ß,15ß-Epoxy-3ß-hydroxypregn-5-en-20-one (138)

To a stirred solution of 3ß-hydroxypregna-5,14-dien-20-one (106) (420 mg) in acetone (100 mL), acetic acid (33 mL) and water (62 mL) was added NBS (203 mg). The solution was stirred at 0-5 °C under argon for 5 hr and then basified with cool saturated aqueous potassium carbonate to pH 9-10 and stirred at room temperature overnight. The reaction mixture was extracted with diethyl ether (100 mL X 3) and the combined extracts were washed with water. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to give a residue which was flash chromatographed over silica by elution with i) hexane, ii) 20% diethyl ether in hexane, and iii) 10% methanol in dichloromethane, to give starting material (106) (103 mg) and the epoxide (138) (200 mg) which was recrystallized from dichloromethane and methanol, m.p. 196-201 °C.

^{1}H NMR ($CDCl_3$) δ: 1.04 (s, 3H, C_{13}-CH_3), 1.06 (s, 3H, C_{10}-CH_3), 2.20 (s, 3H, C_{20}-CH_3), 3.50 (m, 1H, C_3-H), 3.55 (s, 1H, C_{15}-H), 5.35 (m, 1H, C_6-H).

EIMS m/z (%RA): 330 (M^+, 17), 314 (M^+-O, 36), 287 (M^+-CH_3CO, 22), 248 (95), 91 (100).

Elemental Analysis ($C_{21}H_{30}O_3$): Calcd. C 76.33%, H 9.15%; Found C 76.05%, 9.11%.

RBA (IC_{50}): No activity; IC_{50} > 200 μM

14ß,15ß-Epoxy-20ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (139), 14ß,15ß-Epoxy-20ß-benzoyloxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (140) and 14ß,15ß-Epoxy-3ß,20ß-dihydroxypregn-5-ene (141)

To a stirred solution of 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) (107 mg) in dichloromethane (15 mL) were added 1-bromo-α-L-tribenzoylrhamnoside (77) (742 mg), Fetizon's reagent (1.28 g) and pulverized molecular sieves (3 Å, 1.2 g). The solution was stirred under argon, in the dark, for 3.5 hr and then passed through a pad of Celite. The filtrate was evaporated to dryness under reduced pressure at about 40 °C. The residue was dissolved in dichloromethane and the solution was washed with saturated aqueous sodium hydrogen carbonate and water. The organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to give a residue. The residue was dissolved in anhydrous THF and treated with lithium triethyl borohydride (7 mL, 1 M in THF). The solution was brought to reflux for 2 hr then aqueous sodium hydroxide (5%, 7 mL) and hydrogen peroxide (30%, 7 mL) were added to the solution while it was cooled in an ice-bath. Stirring was continued for an additional 15 hr. The solution

was diluted with water, followed by extraction with dichloromethane. The combined extracts were washed with water and the organic phase was dried over anhydrous sodium sulfate, filtered, and evaporated to dryness to give a residue. The residue was dissolved in 10% NH_3 in methanol (w/v) and the solution was kept in refrigerator at 4 °C overnight and then evaporated to dryness to give a residue which was subjected to flash chromatography over silica. The earlier fractions on elution with 2% diethyl ether in dichloromethane and 10% methanol in dichloromethane gave 14ß,15ß-epoxy-3ß,20ß-dihydroxypregn-5-ene (141) (68 mg) which was recrystallized from dichloromethane and methanol, m.p. 216-220 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 1.07 (s, 3H, C_{13}-CH_3), 1.20 (d, 3H, J = 6.5 Hz, C_{20}-CH_3), 1.28 (s, 3H, C_{10}-CH_3), 3.45 (m, 1H, C_{20}-H), 3.48 (s, 1H, 15α-H), 3.83 (m, 1H, C_3-H), 5.35 (m, 1H, C_6-H).

RBA (IC_{50}): No activity; IC_{50} > 200 μM.

The fractions on elution with 10% methanol in dichloromethane gave 14ß,15ß-epoxy-20ß-benzoyloxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (140) (71 mg) on recrystallization from dichloromethane and methanol, m.p. 216-218 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 1.04 (s, 3H, C_{13}-CH_3), 1.10 (s, 3H, C_{10}-CH_3), 1.28 (d, 3H, J = 6.3 Hz, C_{20}-CH_3), 1.30 (d, 3H, J = 6.3 Hz, $C_{5'}$-CH_3), 3.40 (t, 1H, $J_{4',3'} = J_{4',5'}$ = 10 Hz, $C_{4'}$-H), 3.45 (m, 1H, C_3-H), 3.50 (s, 1H, 15α-H), 3.68 (m, 2H, $C_{3'}$-H and $C_{5'}$-H), 3.88 (dd, 1H, $J_{2',1'}$ = 1.5 Hz, $J_{2',3'}$ = 3.3 Hz, $C_{2'}$-H), 4.86

(d, 1H, J = 1.5 Hz, $C_{1'}$-H), 5.25 (m, 1H, C_{20}-H), 5.35 (m, 1H, C_6-H), 7.35-8.00 (m, 5H, aromatic protons).

The later fractions on elution with 10% methanol in dichloromethane gave 14ß,15ß-epoxy-20ß-hydroxy-3ß-(α-L-rhanmosyloxy)-pregn-5-ene (139) (82 mg), which was re-crystallized from dichloromethane and methanol, m.p. 225-228 °C.

^{1}H NMR ($CDCl_3/CD_3OD$, 1:1) δ: 1.07 (s, 3H, C_{13}-CH_3), 1.20 (d, 3H, J = 6.5 Hz, C_{20}-CH_3), 1.28 (d, 3H, J = 6 Hz, $C_{5'}$-CH_3), 1.28 (s, 3H, C_{10}-CH_3), 3.38 (t, 1H, $J_{4',3'} = J_{4',5'}$ = 10 Hz, $C_{4'}$-H), 3.45 (m, 1H, C_{20}-H), 3.45 (s, 1H, 15α-H), 3.70 (m, 2H, $C_{3'}$-H and $C_{5'}$-H), 3.82 (m, 2H, C_3-H and $C_{2'}$-H), 4.87 (d, 1H, J = 1.2 Hz, $C_{1'}$-H), 5.35 (m, 1H, C_6-H).

Elemental Analysis ($C_{27}H_{42}O_7$): Calcd. C 67.78%, H 8.85%; found C 68.01%, H 8.91%.

RBA (IC_{50}): 55 μM

14ß-(α-L-Rhamnosyloxy)-pregna-3,5-dien-20-one (146)

To a stirred solution of 3ß,14ß-dihydroxypregn-4-en-20-one (124) (51 mg) and molecular sieves (3 Å, 1.5 g) in dichloromethane were added 2,6-di-t-butyl-4-methylpyridine (15.3 mg), Fetizon's reagent (673 mg), mercury oxide (123 mg), mercury bromide (31 mg) and 1-bromo-α-L-triacetylrhamnoside (75) (660 mg). The solution was stirred for 15 hr and filtered through a pad of Celite. The filtrate was evaporated to dryness under reduced pressure at about 40 °C to give a residue. The residue was dissolved in a solution of methanol

(11 mL), triethylamine (8 mL) and water (1 mL) and stirred under argon for 3 days. A similar workup procedure to that described in the preparation of 17α-acetoxy-6α-methylpregn-4-en-20-one rhamnoside (12) in Chapter 2 (Method A) gave the diene (5 mg) and 14ß-(α-L-rhamnosyloxy)-pregna-3,5-dien-20-one (146) (24 mg), which did not crystallize.

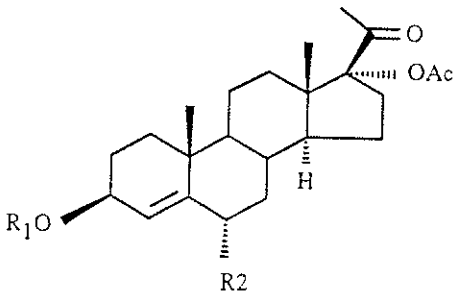
^{1}H NMR ($CDCl_3$) δ: 0.94 (s, 3H, C_{10}-CH_3), 1.22 (d, 3H, J = 6.5 Hz, $C_{5'}$-CH_3), 1.31 (s, 3H, C_{10}-CH_3), 2.16 (s, 3H, C_{20}-CH_3), 3.40 (t, 1H, $J_{4',3'} = J_{4',5'}$ = 10 Hz, $C_{4'}$-H), 3.70-3.80 (m, 3H, $C_{2'}$-H, $C_{3'}$-H and $C_{5'}$-H), 4.92 (d, 1H, J = 1.5 Hz, $C_{1'}$-H), 5.35 (m, 1H, C_6-H), 5.55 (m, 1H, C_3-H), 5.92 (d, 1H, J = 10 Hz, C_4-H).

CHAPTER 6

RECEPTOR BINDING, STRUCTURE-ACTIVITY RELATIONSHIPS AND PHARMACOLOGY

In the literature, cardioactivity is measured by different methods, the reliability and accuracy of which have been a source of disagreement (Thomas et al., 1990). A [^{3}H]ouabain radioligand binding assay has been considered an appropriate method, among others (Thomas et al., 1974, 1990), for screening potential cardiotonic compounds at the earlier stage of the SAR study. Considering that most cardiac glycosides have demonstrated strong receptor binding in a RBA which is a very sensitive measurement of drug-receptor fit, this method is applied in our case for screening potential cardiotonic compounds among progesterone and pregnane derivatives. However, it is necessary to point out that the receptor binding affinity measured by RBA even though can be interpreted in term of drug-receptor fit but do not necessarily reflect intrinsic inotropic activity. As suggested by Watson and coworkers (Watson et al., 1992), cardiotonic activity may not be only initiated by inhibition of NKA.

In a [^{3}H]ouabain radioligand binding assay (Table I and Fig. 14), the glucosides (67 and 68) of 17α-acetoxy-3ß-hydroxypregn-4-en-20-one, 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one and the rhamnoside (69) of 17α-acetyl-3ß-hydroxy-6α-methylpregn-4-en-20-one gave IC_{50} values of 10 μM, 3 μM and 0.4 μM, respectively, and our results have been published (Smyth et al., 1992). Considering the major change in stereochemistry between 14ß-OH and 14α-H compounds, this



Compond No.	Structure Variation		RBA IC_{50}[a] (uM)
	R_1	R_2	
63	H	H	52.0
64	H	CH_3	7.2
67	β-D-glucosyl	H	10.0
68	β-D-glucosyl	CH_3	3.0
69	α-L-rhamnosyl	CH_3	0.4

[a] IC_{50} represents the concentration that inhibits binding of [^{3}H]ouabain by 50%

Table I The receptor binding affinity of 17α-acetoxy-3β-hydroxy-6α-methylpregn-4-en-20-one glycosides (67, 68 and 69) and structurally related compounds in a [^{3}H]ouabain radioligand binding assay

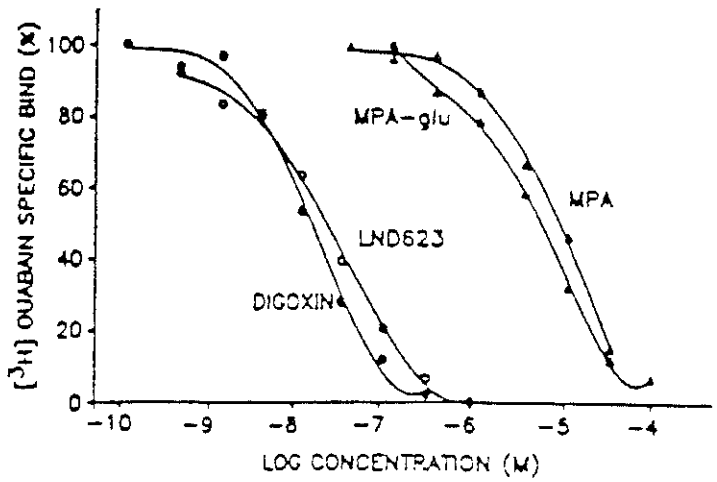


Fig. 14 Inhibition by 17α-acetoxy-3β-hydroxy-6α-methylpregn-4-en-20-one glucoside (MPA-glu, 68) and other steroids of specific binding of [^{3}H]ouabain to cardiac membranes (Smyth et al., 1992)

result could not be predicted. The glycosylation of the genins enhances the receptor binding affinity in which the rhamnoside (69) is more potent (by about 10 times) than the corresponding glucoside (68). Greater binding affinity among the glycosides is consistent with the results of previous studies on pregnane derivatives (Templeton et al., 1991a, b, 1992). The genins, 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (63) and 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64), have IC_{50} values of 52 μM and 7.2 μM, respectively. The enhancement of receptor binding affinity of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one (64) compared to 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (63) may be attributed to the conformational transmission effect introduced by the 6α-methyl group, which leads to the adoption of the A ring to a conformation more closely approximating the digitoxigenin structure (Duax et al., 1978, 1979; Kim et al., 1980). Compared to the corresponding genins, the IC_{50} values of the glycosides (67, 68 and 69) are significantly increased but are still much lower than digoxin (2) (Fig. 14).

The pharmacology of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside (68, MDP-glu) comprises a unique diuretic effect on the kidney, i.e. sodium depleting in the presence of potassium sparing effects (Smyth et al, 1992), compared with the natural cardiac glycosides which cause sever loss of sodium and potassium ions. As illustrated in Fig. 15, 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside

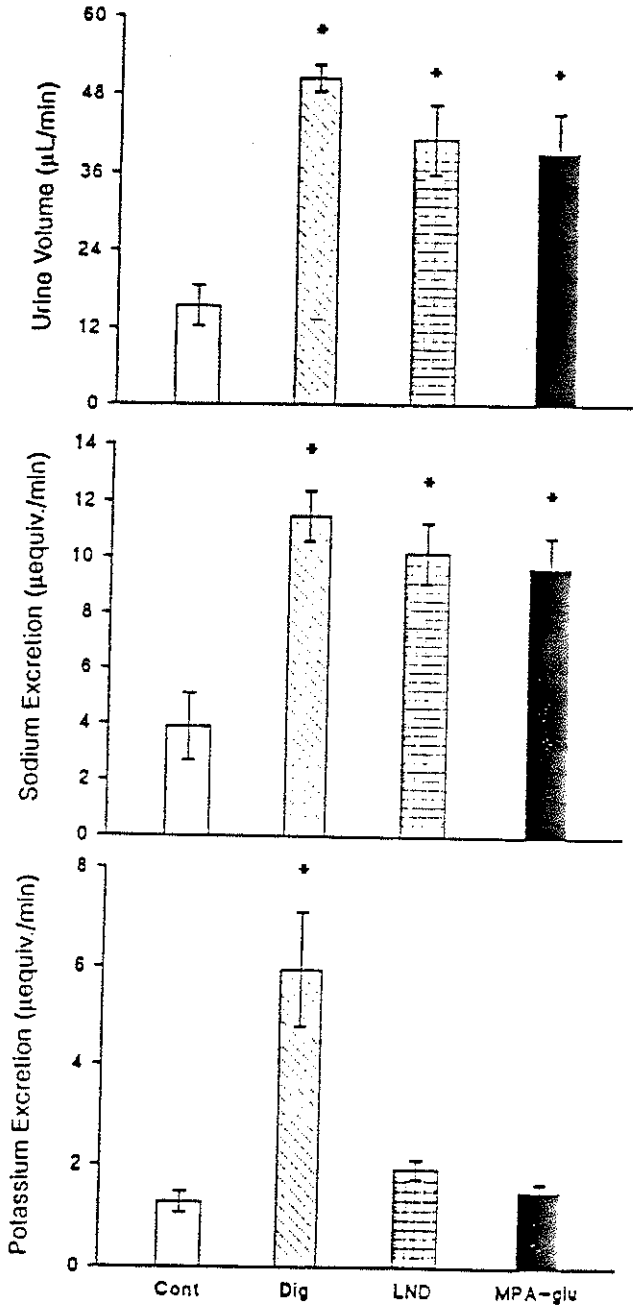


Fig. 15 Effect of saline, digoxin (Dig, 0.5 mg/kg), LND 623 (LND 0.7 mg/kg, and 17α-acetoxy-3β-hydroxy-6α-methylpregn-4-en-20-one glucoside (MPA-glu, 68) on urine volume, sodium excretion and potassium excretion (Smyth et al., 1992)

(68) demonstrates similar effects on urine volume and sodium excretion as digoxin (2), a classic cardiac glycoside. However, unlike digitoxin, it shows little effect on potassium excretion. These results have significant clinical implications as it is known that excessive loss of potassium ions is associated with cardiotoxicity or arrhythmias induced by the cardiac glycosides. Because of hypokalemia symptom, potassium salts have been used for the treatment of arrhythmias induced by cardiac glycosides. Therefore, potassium sparing diuresis provides a theoretic basis for minimizing cardiotoxicity in the SAR studies of cardiotonic compounds. Pharmacological studies of 17α-acetoxy-3ß-hydroxy-6α-methyl-pregn-4-en-20-one rhamnoside (69) has not yet been carried out.

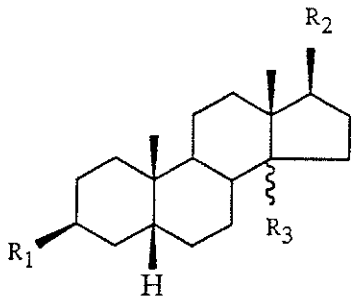
Three conclusions can be drawn based on the pharmacology of 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one glucoside (68, MDP-glu):

1) The results provide further evidence supporting the previous conclusion that a endogenous digitalis-like substances may exist with a steroidal hormone origin.
2) Potassium diuretic effects on the kidney demonstrated by compound 68 and 14ß-amino-20ß-hydroxy-3ß-(α-L-rhamnosyl-oxy)-5ß-pregnane (LND623) (Fig. 15) provide an additional reference for evaluation of cardiotonic compounds with potential improvement in the therapeutic index.
3) A similar diuretic pattern shared by compound 68 (C_4-C_5 unsaturated steroidal hormone) and certain pregnane, such

as LND623 and compound 12 (5ß and 17ß-saturated substituents) but not digitoxin (1) (5ß, 17ß-lactone), clearly indicates that stereochemistry at C5 and substitution occurred at the 17ß position are determinants for potassium sparing diuretic effect while retain receptor binding affinities.

These conclusions are contributed to construction of a working hypothesis for further chemical modification of pregnane and progesterone derivatives as described in Chapter 3, 4 and 5.

The results of the radioligand binding assay of the compounds described in Chapter 3 have been compiled in Table II. Replacement of the tridigitoxosyl with the rhamnosyl group at C_3 in digitoxin (1) enhances receptor binding affinity in a [^{3}H]ouabain radioligand binding assay. Digitoxigenin rhamnoside (88) also displays stronger receptor binding affinity than digitoxigenin glucoside (86) and digitoxigenin monodigitoxoside (Templeton et al., 1991a). Replacement of the α,ß-unsaturated lactone ring at C_{17} with planar isosteres (Thomas et al., 1974) is not necessary for strong receptor binding affinity. Compounds containing a saturated alkyl group with a heteroatom(s) at C_{17}, such as 3ß,14ß-dihydroxy-5ß-pregn-20-one and 3ß,14ß,20ß-trihydroxy-5ß-pregnane rhamnosides (11 and 12), display strong receptor binding affinity. The 20ß-OH (12) shows stronger binding affinity than the 20α-OH or the



Comp. No.	Structure Variation				RBA IC_{50} [a] (μM)
	R_1	R_2	R_3	Others	
97	C=O	$CH_3CHOH(\beta)$	14α-H	Δ^4	110,000
96	OH	$CH_3CHOH(\beta)$	14α-H	Δ^4	71,000
99	OH	$CH_3CHOAc(\beta)$	14α-H	Δ^4	N/A
87	β-D-glucosyloxy	$CH_3C=O$	14β-OH	5β	500
92	C=O	γ-lactone	—	Δ^4, Δ^{14}	300
91	C=O	γ-lactone	14β-OH	Δ^4	180
11	α-L-rhanmosyloxy	$CH_3C=O$	14β-OH	5β	150
12	α-L-rhanmosyloxy	$CH_3CHOH(\beta)$	14β-OH	5β	75
60	OH	γ-lactone	14β-OH	5β	20
86	β-D-glucosyloxy	γ-lactone	14β-OH	5β	7
88	α-L-rhanmosyloxy	γ-lactone	14β-OH	5β	3

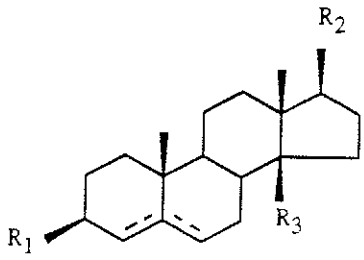
[a] Represents the concentration that inhibits binding of [^{3}H]ouabain by 50%.

Table II The receptor binding affinity of 3β,14β,20β-trihydroxy-5β-pregnane rhamnoside (12) and related compounds in a [^{3}H]ouabain radioligand binding assay

20-ketone (11) (Templeton et al., 1991a, 1992). Without the 14ß-OH group, as in the cases of 14,15-didehydro-14-deoxy-canarigenone (92) and 20ß-hydroxypregn-4-en-3-one (14α-H) (97), receptor binding affinities are weaker in comparison with canarigenone (91) (14ß-OH).

The receptor binding affinity differences between the 20ß-OH (12) and 20α-OH, digitoxigenone (89) and canarigenone (91), the 20ß-OH (12) and LND623, and other lactone ring replacements (Templeton et al., 1991a, b, 1992; Smyth et al., 1992) indicate that the nature, e.g. dipole-dipole moment, electronic configuration, steric hindrance and spatial orientation, of pharmacophores at C_3, C_{14} and C_{17} is important for high potency in receptor binding affinity.

14ß-Hydroxyprogesterone (94), as described in Chapter 4, displays a strong receptor binding affinity in a [^{3}H]ouabain radioligand binding assay (Table III). Comparisons of the magnitudes of receptor binding affinity among 14ß-hydroxyprogesterone (94), progesterone (44) and other cardiotonic compounds are illustrated in Fig. 16. As seen in Fig. 16, 14ß-hydroxyprogesterone (94) shows stronger receptor binding affinity than progesterone (44) but still weaker than ouabain (3) or chlormadinone acetate (45). Nevertheless, this compound elicits a cardiostimulant activity, as illustrated in Fig. 17. Interestingly, this compound, in addition to strong receptor binding affinity and cardiostimulant activity, displays a



Comp. No.	Structure Variation				RBA IC_{50} [a] (μM)
	R_1	R_2	R_3	Others	
106	OH	$CH_3C=O$	—	Δ^5,Δ^{14}	N/A
107	C=O	$CH_3C=O$	—	Δ^4,Δ^{14}	134.0
121	OH	$CH_3CHOH(\beta)$	14β-OH	Δ^4	4.0
94	C=O	$CH_3C=O$	14β-OH	Δ^4	3.9

[a] Represents the concentration that inhibits binding of [^{3}H]ouabain by 50%.

Table III The receptor binding affinity of 14β-hydroxypregn-4-ene-3,20-dinoe (94) and related compounds in a [^{3}H]ouabain radioligand binding assay

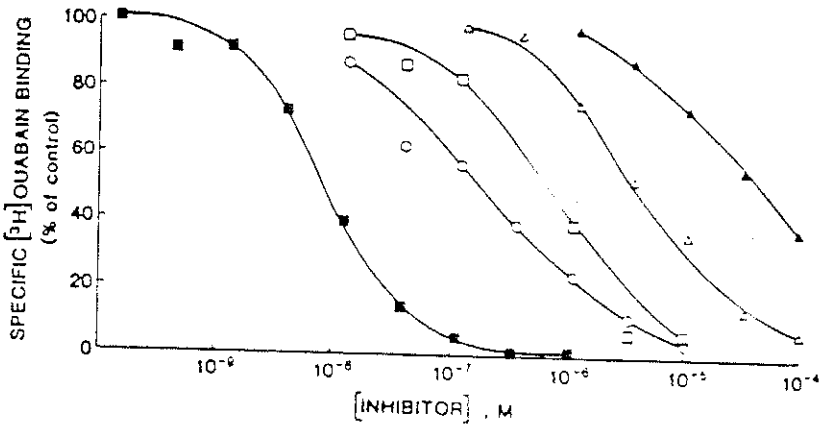


Fig. 16 Inhibition of specific binding of [^{3}H]ouabain to cardiac trabecular tissue by various steroids: (■) ouabain, (o) chlormadinone acetate, (□) ouabagenin, (△) 14ß-hydroxyprogesterone, (▲) progesterone (Templeton et al., 1987).

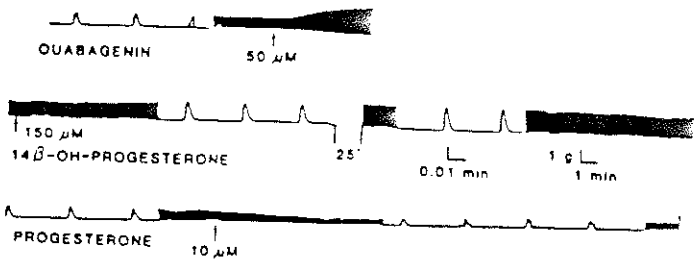
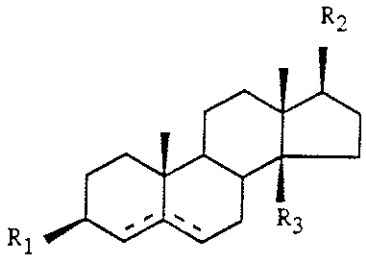


Fig. 17 Change in active tension of electrically driven right ventricular trabecula by steroids dissoved in 20 µL of dimethylsulfoxide and added to the bath at the arrow (↑). Final bath concentrations are indicated (Templeton et al., 1987).

potassium sparing diuresis on the kidney and a balance of positive and negative inotropy which may account for the improvement of therapeutic index of certain pregnane derivatives (LaBella et al., 1985). This unique pattern of pharmacological activities makes 14ß-hydroxyprogesterone (94) a prominent candidate for further functional group modification in the effort to develop new cardiotonic compounds with improved clinical profile.

The pregn-4-ene and 5-ene derivatives described in Chapter 5 have been compiled in Table IV. The receptor binding affinities of pregn-5-ene derivatives, such as 3ß,20ß-dihydroxy-14ß,15ß-epoxypregn-5-ene (141), 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) and 14ß,15ß-epoxy-20ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (139) are all show weak binding affinity.



Comp. No.	Structure Variation				RBA IC_{50} [a] (μM)
	R_1	R_2	R_3	Others	
138	OH	$CH_3C=O$	—	Δ^5, 14β,15β-epoxy	N/A
141	OH	$CH_3CHOH(\beta)$	—	Δ^5, 14β,15β-epoxy	N/A
139	α-L-rhamnosyloxy	$CH_3CHOH(\beta)$	—	Δ^5, 14β,15β-epoxy	55
128	C=O	$CH_3CHOH(\beta)$	14β-OH	Δ^4	47
124	OH	$CH_3C=O$	14β-OH	Δ^4	4
137	α-L-rhamnosyloxy	$CH_3CHOH(\beta)$	—	Δ^5, Δ^{14}	N/A

[a] Represents the concentration that inhibits binding of [^{3}H]ouabain by 50%.

Table IV The receptor binding affinity of 3β,20β-dihydroxy-3β-(α-L-rhamnosyl-oxy)-pregna-5,14-diene (137), 14β,15β-epoxy-20β-hydroxy-3β-(α-L-rhamnosyloxy)-pregn-5-ene (139) and related compounds in a [^{3}H]-ouabain radioligand binding assay

CHAPTER 7

CONCLUSIONS

The research reported in this thesis was carried out with the following four objectives:

1) Synthesis, receptor binding and pharmacology of the glycosides of 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (67) and its 6α-Me derivatives (68) and (69),
2) Synthesis of 14ß-hydroxyprogesterone (94), and its C_4 and C_5 unsaturated derivatives,
3) Synthesis, receptor binding and pharmacology of 5ß-pregnanes derived from digitoxin (1),
4) Rationalization of the structure-activity relationships of the compounds synthesized.

The results obtained are summarized as follows:

1) The progesterone derivatives, 17α-acetoxy-3ß-hydroxypregn-4-en-20-one (67), 17α-acetoxy-3ß-hydroxy-6α-methylpregn-4-en-20-one ß-D-glucoside (68) and α-L-rhamnoside (69) have been synthesized. These allylic glycosides were obtained by treatment of the 3ß-alcohols (63 and 64) with 1-bromo-α-L-triacetylrhamnoside (75) under modified Koenigs-Knorr reaction conditions. These allylic glycoside pose a problem as dehydration to the diene occurs as a major byproduct and there are few successful synthesis reported and those gave very low yield. These glycosides have a structural geometry different from the conventional cardiac glycosides, can bind to NKA, the target enzyme receptor in heart muscle recognized by cardiac glycosides. These compounds display, in addition to receptor binding affinity, remarkable

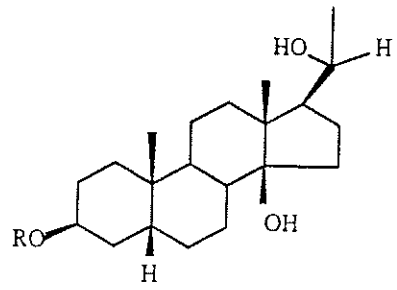
potassium-sparing diuretic effects on the kidney, which can act as a built-in protection against cardiotoxicity induced by cardiac glycosides.

2) A new chemical synthesis of 14ß-hydroxyprogesterone (94) has been carried out. Intermediates in this synthesis were designed as starting materials for the synthesis of the C_4 and C_5 unsaturated glycosides. This synthetic scheme can also be adapted to the preparation of 5α- and 5ß-pregnane glycosides. Thus this synthesis can be used to prepare genin and glycoside derivatives of all saturated and unsaturated derivatives at C_5. Therefore the receptor binding and SAR of the four compounds can be compared. The C_5 unsaturated derivatives have been converted to pregna-5,14-diene rhamnosides and to the 20ß-hydroxy-14ß,15ß-epoxypregn-5-ene rhamnosides. However, synthesis of 14ß-hydroxy-3ß-(α-L-rhamnosyloxy)-pregn-4-ene-20-one (125) has not been successful because of the ease of dehydration of the allylic alcohol to the diene under the reaction conditions employed. The linear synthetic approach employed (14 steps in all) has made the preparation of the starting material, e.g. 14ß-hydroxypregn-4-en-20-one (124), ready for the rhamnosylation, more difficult and consequently limited the attempts to explore the optimum reaction conditions. Attempts at a variety of methods (Smyth et al., 1992; Sinay, 1991; Wulff and Roble, 1974) proved unsuccessful.

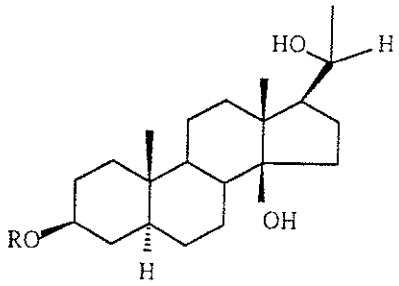
3) Synthesis of 5ß-pregnane glycosides were carried out from digitoxin (1) using an improved method developed in our group of conversion of the 17ß-lactone to the pregnane side chain. Synthesis of 3ß,14ß-dihydroxy-5ß-pregn-20-one glucoside (87), 3ß,14ß-dihydroxy-5ß-pregn-20-one rhamnoside (11) and 3ß,14ß,20ß-trihydroxy-5ß-pregnane (12) has been achieved. These compounds (11 and 87) display strong receptor binding affinity and compound 11 shows potassium sparing diuretic effects, as demonstrated among other progesterone derivatives.

4) Rationalization of SAR by evaluation of stereochemical effects on receptor binding affinity was also carried out. The IC_{50} results and interatomic distance measurement indicate that the stereochemistry of the steroid rings, spacial orientation of the substituents at C_3, C_{14} and C_{17}, and the nature of the substituents at C_{14} and C_{17}, are all determinants of high receptor binding affinity. The outcome of this research calls for further investigation into the pharmacophoric pattern required for favourable inotropy and improved therapeutic indexes of new cardiotonic compounds over conventional cardiac glycosides, such as digoxin (2).

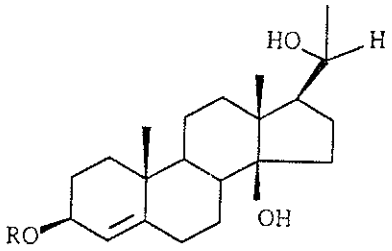
Our objective to compare to the structure-activity relationships of compounds (12, 129, 133 and 142) with the same substituents but different steroid ring stereochemistry at C_5 (Fig. 18) is uncompleted as we were not able to prepare the C_4 unsaturated glycoside 133. Correlation of spacial



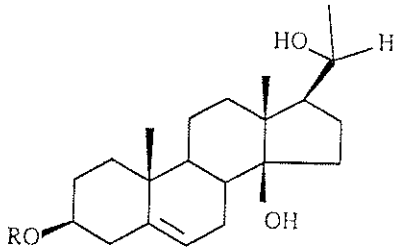
12



129



133



142

R =

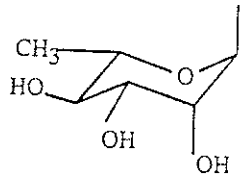


Fig. 18 Pregnane and progesterone derivatives with the same substituents at C_3, C_{14} and C_{17} but different stereochemistry at steroid rings

orientation parameters of the substituents, which could be measured by means of x-ray crystallography and computer-aided modelling analysis, with the corresponding receptor binding affinity may provide a clearer picture of the drug-receptor interaction and hence lead to the formulation of quantitative structure-activity relationships for new cardiotonic drug design.

ADDENDUM TO CHAPTER 5

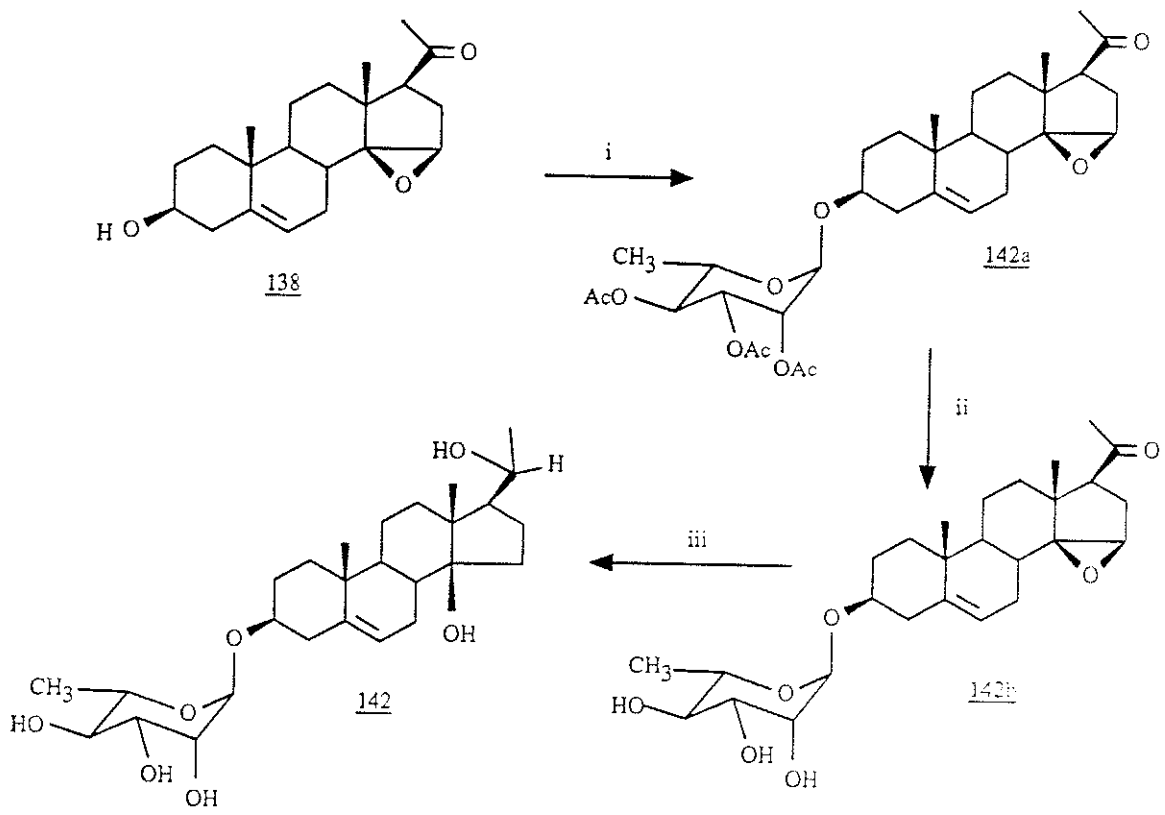
14ß,15ß-Epoxy-3ß-hydroxypregn-5-en-20-one (138) has been converted to the 20-ketone (142b) by coupling with 1-bromo-α-L-triacetylrhamnoside (75) in the presence of Fetizon's reagent followed by basic hydrolysis and flash chromatography over silica. The 20-ketone (142b) was further treated with lithium triethylborohydride in THF to give 14ß,20ß-dihydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (142) (Scheme 27).

Experimental

General methods and materials are described previously in Chapter 2.

14ß,15ß-Dihydroxy-3ß-(α-L-rhamnosyloxy)-pregn-5-ene (142)

To a stirred solution of 14ß,15ß-epoxy-3ß-hydroxypregn-5-en-20-one (138) (150 mg) in dry dichloromethane (30 mL) in the presence of Fetizon's reagent (2.45 g) and molecular sieves (3 Å, 2.40 g) was added 1-bromo-α-L-triacetylrhamnoside (75) (1.42 g) in dichloromethane (10 mL). The solution was stirred under argon in the dark for 2 hr when an additional portion of 1-bromo-α-L-triacetylrhamnoside (75) (1.07 g) in dichloromethane (2 mL) was added to the solution. The reaction mixture was stirred for an additional hr. The solution was filtered through a pad of Celite and the filtrate was evaporated to dryness to give the rhamnoside triacetate (142a). The rhamnoside triacetate (142a) was dissolved in a solution of triethylamine, methanol and water and stirred under argon at room temperature for 3 days. The solution was



Reagents: i) 1-bromo-α-L-triacetylrhamnoside (75), Fetizon's reagent, CH_2Cl_2

ii) triethylamine, methanol, water, r.t., 3 days

iii) LTEBH, THF, reflux, 3 hr

Scheme 26 Synthesis of 14β,20β-dihydroxy-3β-(α-L-rhamnosyloxy)-pregn-5-ene (142)

evaporated to dryness to give a residue which was chromatographed over silica. The fractions on elution with 5% methanol in dichloromethane gave the rhamnoside 142b (67 mg). The rhamnoside 142b (67 mg) was dissolved in THF (20 mL) and treated with lithium triethylborohydride (15 mL, 1 M in THF), and refluxed for 2 hr. A similar workup procedure to that described in the preparation of 3ß,14ß,20ß-trihydroxypregn-4-ene (121) gave the rhamnoside (142) (15 mg) which was recrystallized from methanol and water, m.p. 218-220 °C.

^{1}H NMR ($CDCl_3$) δ: 1.03 (s, 3H, C_{13}-CH_3), 1.20 (s, 3H, C_{10}-CH_3), 1.25 (d, 3H, J = 6.5 Hz, C_{20}-CH_3), 1.27 (d, 3H, J = 6.4 Hz, $C_{5'}$-CH_3), 3.35 (t, 1H, $J_{4',3'} = J_{4',5'}$ = 9.5 Hz, $C_{4'}$-H), 3.50 (m, 1H, C_3-H), 3.70 (m, 2H, $C_{3'}$-H and $C_{5'}$-H), 3.80 (m, 2H, $C_{2'}$-H and $C_{20'}$-H), 4.86 (d, 1H, J = 1.3 Hz, $C_{1'}$-H), 5.40 (m, 1H, C_6-H).

Elemental Analysis ($C_{27}H_{44}O_7$ H_2O: Calcd. C 65.03%, H 9.30%; Found C 65.06%, H 9.29%.

APPENDIX

Table V ^{13}C Chemical shifts of 17α-acetoxypregnane derivatives [a]

Carbon no.	Compounds						
	63	64	65	66	67[b,f]	68[b,g]	69[b,h]
1	33.0	35.7	33.8	34.1	36.1	36.4	36.4
2	29.5	29.2	23.0	23.1	27.7	27.4	27.3
3	67.9	63.3	128.8	124.5[c]	76.6[c]	76.8	72.0[c]
4	123.7	121.2	125.1	125.3 [c]	121.3	118.9	118.7
5	147.0	150.5	141.3	133.8	148.2	151.6	151.6
6	32.0	32.3	122.5	126.8	32.7	33.0	33.0
7	35.9	41.8	31.9	38.9	33.6	42.4	42.4
8	36.0	35.7	31.7	31.8	36.7	36.3	36.4
9	53.8	54.0	47.8	48.1	54.5	54.7	54.6
10	37.3	37.6	35.2	35.4	38.0	38.2	38.2
11	20.7	20.8	20.6	20.8	21.3	21.2	21.3
12	31.2	31.3[c]	31.1[c]	31.3[d]	31.8[d]	31.7	31.8
13	46.9	46.9	46.9	47.0	47.6	47.6	47.6
14	51.3	51.2	52.1	52.2	52.0	51.8	51.9
15	23.9	23.8	24.0	24.0	24.4	24.3	24.3
16	30.3	31.2[c]	30.5 [c]	30.7[d]	30.9[d]	30.8	30.9
17	96.9	96.9	97.0	97.2	97.8	97.6	97.7
18	14.4	14.4	14.4	14.5	14.9	14.7	14.7
19	18.9	18.9 [d]	18.8	18.7 [e]	19.2	19.0	19.1
20	204.1	204.1	204.1	204.3	206.2	206.1	206.1
21	26.4	26.4	26.4	26.5	26.8	26.7	26.7
$\underline{C}OCH_3$	170.7	170.7	170.7	170.9	172.1	172.0	172.1
$CO\underline{C}H_3$	21.2	21.2	21.3	21.4	21.5	21.3	21.3
C_6-$\underline{C}H_3$		19.8 [d]		18.7[e]		19.9	20.0

[a] In $CDCl_3$: CD_3OD (1:1). [b] In $CDCl_3$. [c, d, e] Values are interchangeable or overlapping. [f] 102.7 (1'), 74.3 (2'), 76.8 (3')[c], 71.0 (4'), 77.3 (5')[c], 62.5 (6'). [g] 102.8 (1'), 74.3 (2'), 77.2 (3'), 70.9 (4'), 77.3 (5'), 62.3 (6'), [h] 100.2 (1'), 69.2 (2'), 72.0 (3')[c], 73.5 (4'), 75.1 (5'), 17.7 (6').

Table VI ^{13}C Chemical shifts of β-D-glucose and α-L-rhamnos derivatives [a]

Carbon no.	Compounds		
	75	77	78
1	83.7	83.9	82.0
2	70.3	71.0	70.5
3	71.1	71.5	75.0
4	72.4	73.4	72.0
5	67.9	68.9	70.8
6		17.2	17.7
$\underline{C}H_2CH_3$			25.7
$CH_2\underline{C}H_3$			15.0
$\underline{C}O$	169.8	165.7	169.8
	169.7	165.3	170.2
	169.5	165.1	170.2
$\underline{C}H_3OCO$	20.6		20.6
	20.7		20.7
	20.8		20.8
1'		133.7	
		133.6	
		133.3	
2', 6'		128.7	
		128.5	
		128.3	
3', 5'		129.9	
		129.7	
		129.7	
4'		128.9	
		128.8	
		128.7	

[a] In $CDCl_3$.

Table VII ^{13}C Chemical shifts of 5β-pregnane and pregn-4-ene derivatives [a]

Carbon no.	Compounds					
	11 [e]	12 [f]	87 [g]	89 [b]	91 [b]	99
1	30.2	20.2 [c]	30.4 [c]	37.1	35.8	36.1
2	27.2	27.0 [d]	27.1 [d]	20.9	27.5	25.9
3	72.6	72.7	74.3	212.4	174.3	73.8
4	31.0	31.0 [c]	30.7 [c]	33.1	117.9	12 4.0
5	37.3	37.3	36.8	41.6	169.6	147.3
6	27.0	26.7 [d]	27.0 [d]	26.9	33.0	33.6
7	22.1	21.1	22.0	26.5	32.8	39.9
8	40.6	40.9	40.6	43.6	41.9	36.4
9	35.8	36.1	35.9	36.6	49.5	55.2
10	35.7	35.8	35.7	35.2	38.7	37.8
11	21.3	21.9	21.3	21.2	21.1	21.5
12	39.7	42.0	39.7	39.8	39.5	32.7
13	50.0	50.1	50.0	49.6	85.1	42.8
14	86.2	85.9	86.2	85.2	160.4	55.5
15	34.3	32.3	34.3	36.6	33.9	24.7
16	25.4	27.3 [d]	25.4	42.0	26.8	29.3
17	63.0	57.0	63.0	50.8	73.4	56.1
18	15.5	16.6	15.5	15.8	15.7	12.8
19	24.1	24.1	23.9	22.5	17.5	19.15
20	219.4	71.8	219.4	174.3	174.2	67.9
21	33.1	23.2	33.0	73.4	73.4	20.0
22				117.7	124.9	
23				174.4	199.1	
$\underline{C}OCH_3$						171.8
$CO\underline{C}H_3$						21. 6

[a] In $CDCl_3$: CD_3OD (1:1). [b] In $CDCl_3$. [c, d] Values are interchangeable. [e] 98.8 (1'), 72.0 (2'), 72.0 (3'), 73.5 (4'), 69.1 (5'), 17.7 (6'). [f] 98.8 (1'), 72.0 (2'), 72.0 (3'), 73.5 (4'), 69.1 (5'), 17.7 (6'). [g] 101.9 (1'), 75.0 (2'), 77.3 (3'), 71.0 (4'), 76.7 (5'), 62.2 (6').

Table VIII ^{13}C Chemical shifts of 14β-hydroxypregn-4-ene-3, 20-dione and related derivatives [a]

Carbon no.	Compounds					
	94	103	104	106	107	108
1	35.9	37.3	37.5	37.1	35.6	35.7
2	33.9	27.7	31.6	31.6	33.9	33.9
3	199.4	73.7	71.7	71.7	199.3	199.1
4	123.8	38.0	42.2	42.2	124.1	124.3
5	171.0	139.4	140.5	139.9	170.4	169.5
6	33.1	121.7	120.7	121.3	32.6	32.2
7	27.9	28.9	28.9	29.7 [b]	31.4 [b]	27.9
8	40.0	32.3	32.4	30.9	34.9	33.6
9	42.9	54.0	54.1	50.1	53.4	52.7
10	38.7 [b]	37.5	37.4	36.9	38.6	38.7
11	20.7	20.7	20.7	21.7	21.9	20.7
12	38.7 [b]	35.7	35.7	41.3	41.7	38.9
13	49.0	53.5	53.5	48.2	48.1	45.4
14	84.5	173.0	173.2	151.0	150.1	73.3
15	34.0	119.1	119.0	118.2	118.1	60.1 [b]
16	24.9	141.7	141.7	31.2 [b]	29.9	27.1
17	62.1	154.8	154.8	65.2	65.1	60.4 [b]
18	15.2	18.4	18.4	18.3	18.5	15.7
19	17.6	19.4	19.5	19.1	17.5	17.7
20	217.8	192.6	192.6	209.3	209.1	211.5
21	33.4	26.7	26.7	31.4	31.4 [b]	29.9
$\underline{C}OCH_3$		170.4				
$CO\underline{C}H_3$		21.4				

[a] In $CHCl_3$. [b] Values are interchangeable or overlapping.

Table IX ^{13}C Chemical shifts of pregn-4-ene and pregn-5-ene derivatives [a]

Carbon no.	Compounds						
	122	123	124	128	129	130	134
1	35.9	36.0	35.5	35.9	35.9	35.9	33.6
2	29.6	29.6	29.5	34.0	34.0	34.0	23.1
3	68.9	68.3	67.9	199.4	199.2	199.5	128.1
4	124.3	124.5	123.4	123.8	123.9	123.6	125.1
5	145.5	145.6	147.1	170.8	170.5	171.6	140.6
6	32.3	32.3	32.3	33.2	33.1	33.2	122.6
7	28.7	28.9	28.9	27.8	27.8	27.9	31.2
8	40.9	40.3	40.3	40.7	41.6	39.7	31.0
9	50.3	49.9	49.7	49.5	49.6	49.5	48.4
10	37.4	37.4	37.5	38.8	38.7	38.8	35.6
11	20.3	20.7	20.7	20.5	20.7	20.5	21.5
12	41.3	39.1	39.1	41.1	41.2	32.5	41.4
13	47.6	49.2	49.1	47.5	46.5	48.1	48.3
14	85.3	84.7	84.7	84.7	85.3	83.5	151.4
15	32.0	33.9	33.9	32.1	31.7	41.1	117.9
16	26.2	24.9	24.9	26.2	25.1	26.0	29.6
17	56.3	62.2	62.2	56.1	54.0	56.5	65.3
18	16.3	15.3	15.3	16.3	15.1	17.0	18.4
19	18.8	18.9	19.0	17.6	17.6	26.0	18.5
20	71.9	217.7	217.7	72.0	74.2	74.0	209.3
21	25.7	33.4	33.4	23.4	21.6	23.5	31.4
$\underline{C}OCH_3$					170.4		
$CO\underline{C}H_3$					19.3		
$SiCH_3$	-4.6	-4.1				-4.2	
	-4.4	-4.4				-4.5	
$\underline{C}(CH_3)_3$	23.3	26.0					
$C(\underline{C}H_3)_3$	18.4	18..4				17.6	
						18.2	

[a] In $CHCl_3$.

Table IX (continued)

Carbon no.	Compounds						
	135 [b]	136 [b]	137 [b]	138 [b]	139 [b]	140 [b, c]	141 [b]
1	33.8	37.1	37.6	37.6	37.7	37.8	37.6
2	23.0	31.3	30.3	31.4	30.4	29.8	31.4
3	128.8	77.9	71.2	71.5	76.6	68.9	71.5
4	124.7	41.3	42.0	42.1	39.3	38.9	42.2
5	139.9	139.3	140.2	140.6	140.1	140.1	140.6
6	123.8	121.7	122.2	120.9	121.4	121.5	120.9
7	27.4	29.8	29.9	27.1	29.8	29.8	30.4
8	36.3	30.9	31.5	30.2	29.7	29.8	29.7
9	44.1	50.1	50.7	49.9	50.4	50.1	49.9
10	35.4	37.2	37.7	37.6	37.7	37. 6	37.6
11	20.8	21.7	22.0	21.1	20.7	20.9	20.7
12	38.7	38.4	38.9	39.2	38.7	38.8	39.4
13	49.2	48.2	47.6	46.0	44.7	44.3	44.7
14	85.5	151.0	155.2	74.9	76.9	75.7	76.6
15	34.2	118.2	118.1	61.0	61.2	28.1	61.2
16	24.6	29.4	34.1	27.1	26.8	2 7.0	26.8
17	62.6	65.2	60.5	61.4	53.4	51.4	53.4
18	15.22	18.3	17.7	15.8	15.4	14.8	15.4
19	18.7	19.2	19.4	19.7	19.6	19.4	19.7
20	217.8	209.3	68.9	213.9	71.8	60.8	70.8
21	33.4	31.4	123.6	30.0	22.8	19.6	22.8
1'		95.9	98.8		98.7	98.9	
2'		71.5	71.8		70.8	71.8	
3'		70.1	71.8		71.8	73.4	
4'		72.1	73.5		73.4	74.9	
5'		66.7	69.3		68.8	68.9	
6'		17.1	17.2		17.7	17.7	

[b] In $CHCl_3$: CD_3OD (1:1). [c] 129.3, 129.4, 129.5 (1''), 129.7, 129.7, 129.9 (2'', 6''), 128.2, 128.4, 128.5 (3'', 5'' 133.2, 133.0, 133.4 (4''), 165.5, 165.7, 165.8 (COPh).

Table IX (continued)

Carbon no.	Compounds	
	142 [b]	146
1	37.8	34.9
2	32.8 [c]	23.9
3	77.1	123.9
4	38.8	125.6
5	139.8	141.5
6	122.7	129.8
7	29.9	32.5
8	37.2	38.1
9	47.0	48.2
10	37.9	36.7
11	20.9	21.0
12	41.2	28.5
13	50.1	50.9
14	85.9	96.1
15	32.8 [c]	26.9
16	27.6	23.1
17	57.1	63.4
18	16.5	19.0
19	19.6	20.6
20	71.8 [d]	212.3
21	23.3	32.0
1'	98.9	96.8
2'	71.8 [d]	72.5
3'	71.9	73.8
4'	73.5	74.0
5'	69.0	70.3
6'	17.7	18.0

[b] In $CDCL_3$: CD_3OD (1:1). [c, d] Values are overlapping.

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