A PHYTOCHEMICAL INVESTIGATION OF ARTEMISIA BIENNIS WILLD.

A Thesis

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Master of Science

By

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The author wishes to express his appreciation to Dr. J. R. Murray and to Dr. J. W. Steele, who personally directed this work.

A B S T R A C T

A PHYTOCHEMICAL INVESTIGATION OF ARTEMISIA BIENNIS WILLD.

Screening of Artemisia biennis for general plant constituents indicated the presence of glycosides, steroidal glycosides and sterols. No indication of the presence of alkaloids, saponins and santonin was found.

Aqueous extracts of the whole plant and roots of A. biennis were found to be non-toxic to rats and had no hypoglycaemic activity in rabbits.

A white crystalline hydrocarbon-like compound (0.01% w/w) m.p. 79.5-80.0°C. was isolated from the petroleum ether extract of the whole plant. No further investigation of this compound was carried out.

The chloroform extract of the powdered root of A. biennis yielded 98 mg. (0.003% w/w) of 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene. This compound was not found to be present in the stems and leaves and inflorescence of A. biennis. Ultraviolet, infrared and nuclear magnetic resonance spectra for this compound have been recorded and were found to be identical with those recorded by Bohlmann, Kleine and Bornowski in 1962 for a compound isolated from the roots of A. arborescens.

An unsuccessful attempt was made to synthesize 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene by two methods. However a new intermediate compound, 2-acetyl-4-methoxythio-

phene (m.p. 33-35°C.) was synthesized and a 2,4-dinitrophenylhydrazone derivative prepared (m.p. 212-213°C.). Ultraviolet, infrared and nuclear magnetic resonance spectra for this new intermediate compound have been recorded.

C O N T E N T S

A PHYTOCHEMICAL INVESTIGATION OF *ARTEMISIA BIENNIS WILLD.*

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I N T R O D U C T I O N

Various members of the genus Artemisia, scattered throughout the world, have been used medicinally for a great number of years. According to John Uri Lloyd (1), several species of wormseed were mentioned by Dioscorides as being mixed with honey and employed as remedy for ascarides.

Trillianus in the sixth century, Saladinus in 1450, and Ruellius and Dodonaeus in the sixteenth century refer to the genus Artemisia as a vermifuge for children.

Chopra et al. (2) mention that the Greeks and Romans used the Artemisia to expel intestinal worms and also used it as a stomachic. The old Arabian and Persian physicians used it for the same purpose. The flowering tops of a species of Artemisia have been and are being used to this day in India as an anthelmintic, as a remedy for dropsy and as a cardiac and respiratory stimulant.

The Indians of the Missouri River region (3) used a decoction of the tops of Artemisia dracunculoides Pursh. for bathing, as a treatment for rheumatism. A decoction of Artemisia frigida Willd. was used internally by Indian women for irregular menstruation.

In northern Canada and Alaska, the mainland Eskimos (4) used infusions of Artemisia tilesii Ledeb. internally, in the treatment of hemorrhages, severe colds, and as an analgesic for rheumatism and ill-defined aches and pains. Poultices of dried leaves applied to the skin are also used as a

treatment of impetigo and sores which resist healing, or have become infected.

In a communication received from Mr. D. Prystash of Portage la Prairie, Manitoba, it was stated that an aqueous extract of Artemisia biennis Willd. was used for its hypoglycaemic activity. (5) The flowers and leaves were boiled with moderate heat for approximately thirty minutes resulting in a dark green solution. The first dose of ten to fifteen drops of this extract was taken at bedtime. The dose, taken at bedtime, was increased daily by five drops until a maximum dose of one teaspoonful was reached. In a week or two the urine test should indicate a considerable decrease in the excretion of glucose. After all traces of glucose excretion have disappeared, the daily bedtime dose should be diminished by five drops each day.

During the course of treatment dietary measures must be employed. The diet should consist of Red River cereal, whole wheat bread, soft boiled eggs, small quantities of lean meat or white poultry meat, all fruits with the exception of pears. At the same time no sugar, honey, alcoholic or coloured soft drinks should be taken. The person should also refrain from smoking.

The food should be eaten sparingly so that the individual would still feel a bit hungry. This will give the extract a chance to combat the irregularity of glucose assimilation. The diabetes symptoms should disappear in one to three months.

Due to the death of Mr. D. Prystash and the destruction of his records, detailed information on the above aspect was not available.

The investigational work was undertaken to determine the hypoglycaemic activity of infusions of A. biennis on the blood sugar of rabbits and to isolate any organic compounds that this species might contain.

Botanical Description

The genus Artemisia belongs to the natural order of Compositae. Scoggan (6) reports that there are thirteen species found in Manitoba.

Artemisia biennis (Plate I), also known as biennial wormwood, is found in the temperate zone of North America from British Columbia to eastern Quebec, south to California, Missouri and Pennsylvania. It is native to western North America but the original range is now impossible to determine.

A. biennis is a glabrous annual or biennial plant with a tap root and is found on shores, roadsides and waste ground in the southern three quarters of the province of Manitoba.

Chemical Investigation

Many species of Artemisia have been investigated with the isolation of santonin and other organic compounds. In 1830 Kahler (7) extracted santonin from Artemisia cina Berg.

PLATE I

Artemisia biennis Willd.

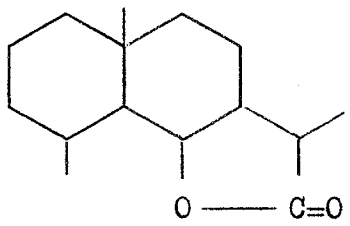
Santonin can be obtained from the unexpanded flowerheads of Artemisia maritima, Artemisia kurramensis as well as other species of Artemisia. The unexpanded flowerheads are treated with calcium oxide and then boiled with water. The solution is filtered and the cool filtrate, after acidification with hydrochloric acid, is extracted with chloroform. The filtrate is then made alkaline with four percent sodium hydroxide and re-extracted with chloroform. The combined chloroform extracts are filtered through animal charcoal and evaporated to dryness. The residue is dissolved in a minimum amount of alcohol and then diluted with boiling water. The solution is evaporated to half its volume and the santonin allowed to crystallize. (8,9)

Simonsen (10), and Krishna and Varma (11) noted that the presence of santonin depends not only on the time of harvest but also on the climatic and topographical conditions of the habitat of the species. It is due to this reason that there are contradictory claims as to whether or not a species contains santonin. Viehoever and Capen (12) indicated that positive tests were obtained on Artemisia mexicana, Artemisia neo-mexicana and Artemisia wrightii while Greenish and Pearson (13) could not isolate any santonin from other samples of Artemisia mexicana and Artemisia redolens obtained from Mexico. A summary of the species, in which santonin has or has not been found, is recorded in Appendix I.

The first chemical examination of the volatile oil

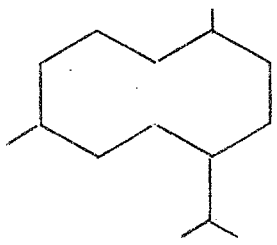
from Artemisia absinthium was done in 1845 by Leblanc (14) who isolated a compound, $C_{10}H_{16}O$, which was later identified as thujone. Other terpenes were later isolated from the volatile oils of the various species of Artemisias.

During the last two decades and especially during the last ten years, there appeared to be an increased interest in the investigation of this genus. Since 1950, a number of sesquiterpene lactones with various type structures have been isolated from members of the genus Artemisia. Balchanin (15), erivanine (16), tauremisin (17), and vulgarin (18), are examples of the santanolide type of structure I. (19)



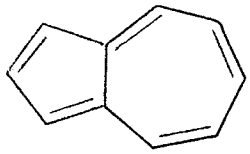
I

The germacrane type II (20) includes costunolide, hydroxycostunolide, balchanolide, hydroxybalchanolide and isobalchanolide. (21,22)



II

The azulenes III are represented by estafiatin (23) and arborescine. (24)



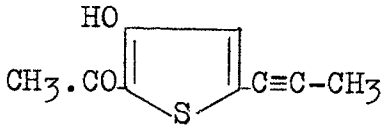
III

Of particular interest are the acetylenic derivatives found in the Artemisia species. These include the isolation of cis-dehydromatricaria ester IV by Stavholt and Sorenson in 1950. (25)

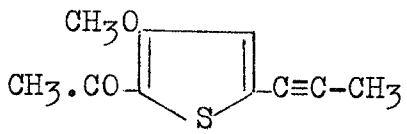
$CH_3-CH=CH-C\equiv C-C\equiv C-C\equiv C-COOCH_3$

IV

In 1962 Bohlmann, Kleine and Bornowski (26) isolated from the roots of Artemisia arborescens, two thiophene compounds, 2-acetyl-3-hydroxy-5-(1-propynyl)-thiophene V and 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene VI.



V



VI

In 1911 Rabak (27) examined the volatile oil of A. biennis obtained by steam distillation of the partially dry fresh plant oollected in South Dakota. The plant yielded 0.03 percent of oil which contained 5.6 percent of esters and 17.28

percent of free and combined alcohols. In a survey of fifty-six species of Artemisia, Viehoever and Capen (12) reported the absence of santonin in A. biennis.

A summary of the known organic compounds which have been isolated from the various species of Artemisia may be found with references in tabular form in Appendix II.

Pharmacological Investigations

Essences of Artemisia absinthium produce convulsions in experimental animals. (28-32) L. J. Boyd (33,34) studied the action of Artemisia extracts on blood pressure and respiration and on various isolated organs such as the stomach, intestines, bladder, uterus and heart.

McAlister Jr. (35) noted that aqueous infusions of Artemisia tridentata were of value in the treatment of gum infections such as pyorrhea alveolaris and obtained a United States patent for this use.

G. Madaus and Fr. E. Koch (36) found that feeding fresh herb of A. absinthium cured scabies in rats.

D. Schmahl (37) found that prolonged use of the pulverized herb of A. absinthium did not cause any liver damage or tumor formation although it diminished the mean and maximum life expectancy of rats.

Santonin together with calomel and a saline purgative is effective against Ascaris lumbricoides. It was also used against pinworms that had ascended into the intestine beyond

the reach of enemas. It is useless against taenia and not very effective against other worms. It does cause severe toxic reactions and therefore is no longer recommended in humans. Santonin decreases phlorhizin glycosuria in rabbits by raising the renal threshhold, for the blood sugar level is unaffected. (8,38)

Santonin given orally as the sodium salt produced hypoglycaemia, hypotonicity and muscular weakness when given to animals. These effects were ascribed to the degenerative changes in the liver. (39)

Tashio Kimura (40) pointed out that santonin resembles picrotoxin in its hypoglycaemic effect.

Miscellaneous

Janot and Mouton (41) found that a 1:100 infusion of the seeds of Artemisia maritima containing 1.75% santonin was better as a vermifuge than a 0.0175% solution of santonin. The parasites, Lumbricus terrestris, Carassius auratus and Ascaris megalocephala were expelled alive. Callegari and Rossi (42) also found that the oil of Libyan Artemisias was toxic to Ascaridae in hogs, and to ground worms. These were rapidly paralyzed and killed in a short time.

Gurevich (43) found that phytocides obtained in extracts of leaves and juices killed young mollusk embryos. Sublethal doses resulted in retarded development of the mollusks when they were compared with controls.

A carbohydrate isolated from paniculate wormwood possessed antibiotic activity in vitro against Staphylococcus aureus, some Proteus species, Bacillus subtilis, Escherichia coli and some fungi. It was bactericidal in dilutions of 1:500 to 1:5000 and bacteriostatic in dilutions of 1:5000 to 1:10,000. (44)

1-Phenylhex-2,4-diyne-1-one was isolated from the seed oil of Artemisia capillaris by Imai and Tanaka (45) and was found to prevent the growth of pathogenic fungi in dilutions of 1:400,000 to 1:800,000.

E X P E R I M E N T A L

The melting points are uncorrected and were determined on a Thomas Hoover capillary melting point apparatus.

The ultraviolet absorption spectra were determined in alcohol on a Beckman DU spectrophotometer or on a Hilger and Watts Ultrascan recording spectrophotometer.

Infrared absorption spectra were recorded on a Beckman IR-8 infrared spectrophotometer using the stated solvent and path length.

The molecular weight determination and the carbon, hydrogen and sulphur analyses were determined by Dr. F. Pascher and E. Pascher of Bonn, West Germany.

Aluminum Oxide for chromatographic absorption analysis (British Drug Houses) was used in column chromatography. The petroleum ether had a boiling range of 40-60°C.

The collection of the eluting solvents was done on a Towers automatic fraction collector Model A.

The author is indebted to the following persons: the late Mr. D. Prystash of Portage la Prairie for his suggestions on the uses of the species; Mr. W. Kremers, Mr. G. Beaudin and Mr. A. Siemens for their technical assistance; Miss J. Walker of the Department of Botany for the identification of A. biennis; Dr. R. Coutts formerly of the University of Saskatchewan and Dr. T. Schaefer of the Department of Chemistry for the nuclear magnetic resonance spectra; Dr. N. N. De for the toxicity tests and his help in the blood-sugar determinations; and Dr. W. E. L. Alexander, Professor R. C. S. Audette

and Professor G. Blunden, formerly of the School of Pharmacy, for their interest and helpful suggestions.

I. COLLECTION OF A. BIENNIS

A. biennis was collected on both the east and west banks of the Assiniboine River near the Trans-Canada Highway #1, ten miles east of Portage la Prairie, Manitoba. The original sample was collected in late August and early September 1960. All plant parts were air-dried and then pulverized using a small Massey-Ferguson hammer mill. The second collection took place in September 1963 in the same locality. The plants were air-dried and then separated into the following plant components: roots, stems, and leaves and inflorescence. The roots and stems were powdered separately using a Fitzpatrick Model D hammer mill so that the powdered material could pass through a #8 sieve. The leaves and inflorescence were passed through a #8 sieve.

II. SCREENING PROCEDURES

A. biennis was screened for alkaloids, glycosides, lactones, sterols, saponins and santonin, and also for toxicity and hypoglycaemic activity. The results are denoted in the following manner:

(+) - A positive colour reaction, precipitate or foam.

(-) - Denotes an absence of an indicating colour, precipitate or foam.

A. Screening for General Plant Constituents

The method of Abisch and Reichstein (46) was used for general screening of plant constituents. A one gram sample of each of the powdered whole plant, powdered leaves and inflorescence and powdered root was subjected to the above procedure. Silicotungstic acid, modified Dragendorff's, Mayer's, Hager's, Sonnenschein's and Wagner's reagents were used in the test for alkaloids. According to Abisch and Reichstein the four extracts obtained through this procedure should contain the following compounds:

(i) Extract "a" - slightly polar, relatively difficult water soluble basic and neutral compounds.

(ii) Extract "b" - strongly polar, easily water soluble alkaloids and neutral glycosides but no free sugar or polysaccharides.

(iii) Extract "c" - to supplement the test for slightly polar alkaloids.

(iv) Extract "d" - to test for saponins with the help of the foam test.

The weight of residues from extracts "a" and "b" for each of the samples used is recorded in Table I.

TABLE I

WEIGHT OF RESIDUES OBTAINED PER GRAM OF SAMPLE

Sample	Extract "a"	Extract "b"
Whole plant	1.6 mg.	1.3 mg.
Leaves and Inflorescence	1.2 mg.	2.2 mg.
Root	1.3 mg.	1.8 mg.

The results of the screening procedure for the four extracts obtained from each of the different plant samples used are recorded in Table II.

TABLE II

SCREENING FOR SPECIFIC ORGANIC COMPOUNDS IN A. BIENNIS

Reagent Used	Plant Constituents	Expected Result	Whole Plant	Leaves and Inflorescence	Root
EXTRACT "a"					
Alkaloidal reagents (six used)	Alkaloids	Precipitate	-	-	-
Xanthydrol reaction	Glycosides	Red colour	-	-	+
Kiliani mixture	Glycosides	Slightly reddish-brown precipitate	+	-	-
Kedde reaction	Steroidal glycosides	Reddish-violet colour	+	+	+
Antimony trichloride reaction	Steroidal glycosides	Pink colour	+	+	+
Liebermann-Burchard reaction	Sterols	Red precipitate	+	+	+
EXTRACT "b"					
Alkaloidal reagents (six used)	Alkaloids	Precipitate	-	-	-
Xanthydrol reaction	Glycosides	Red colour	-	-	+
Kiliani mixture	Glycosides	Slightly reddish-brown precipitate	+	-	-
Kedde reaction	Steroidal glycosides	Reddish-violet colour	+	+	+
Antimony trichloride reaction	Steroidal glycosides	Pink colour	+	+	+
Liebermann-Burchard reaction	Sterols	Red precipitate	+	+	+
EXTRACT "c"					
Alkaloidal reagents (six used)	Alkaloids	Precipitate	-	-	-
EXTRACT "d"					
Foam test	Saponins	Foam	-	-	-

B. Screening for Saponins

Using the Fischer method (47), small pieces of split root, split stem, and inflorescence and leaves were placed on a blood agar plate and allowed to stand for forty-eight hours. No haemolysis of the blood occurred. Quillaja bark was used as a standard and a clear haemolyzed zone, about one inch in diameter surrounded the quillaja sample after twelve hours.

C. Screening for Santonin

Using a method of G. Wichmann (48), 1 g. of each of the following were used: leaves and inflorescence, root and whole plant. Each sample was shaken for 15 minutes with 10 ml. of benzene, then the mixture was filtered. One ml. of the filtrate was diluted with an equal quantity of benzene and 1 ml. of a saturated solution of sodium methoxide was added. The mixture was shaken for 10 seconds and then allowed to stand for 15 minutes. The colour of the lower layer was recorded in Table III. Santonica seeds were used as a standard in the above screening test. The colour produced was compared to the colour of Methyl Orange containing 2 drops of dilute hydrochloric acid.

TABLE III

COLOUR REACTION IN SCREENING TEST FOR SANTONIN

Sample	Colour of Benzene Extract	Colour Produced with Sodium Methoxide	Result
Leaves and Inflorescence	Greenish-yellow	Green	-
Root	Colourless	Colourless	-
Whole Plant	Very pale yellow	Light green	-
Santonica Seeds	Pale yellow	Dark red	+

D. Test for Toxicity

As Mr. D. Prystash reported the use of an aqueous infusion of A. biennis for its hypoglycaemic effect, a similar aqueous infusion was prepared for the test on the toxicity of the species. Five grams of powdered whole plant and powdered root were each added to 95 ml. of boiling distilled water. The mixtures were stirred and allowed to stand overnight in a refrigerator. The infusions were then filtered and the marc washed with distilled water. The filtrates were allowed to come to room temperature and made up to 100 ml. The volume of the infusion injected into the rats was calculated on the basis of the weight in milligrams of plant

material per kilogram of body weight. Twenty rats were employed in this test. The sample of whole plant infusion was injected intraperitoneally into two groups of five rats, one group receiving a dose of 50 mg./Kg. of body weight and the second group receiving 100 mg./Kg. of body weight. A similar test was done using the infusion obtained from the root of A. biennis. The test animals were observed for seventy-two hours. No deaths were recorded.

A second experiment, using a higher concentration of the same samples as reported above, was done on another twenty rats. Twenty-five percent infusions were prepared as reported above and concentrations of 500 mg./Kg. of body weight and 1000 mg./Kg. of body weight were injected intraperitoneally. No deaths were observed after seventy-two hours.

E. Test for Hypoglycaemic Activity

The test was performed on four rabbits using a ten percent aqueous infusion of whole plant (Sample A) and the roots (Sample B). Using the procedure of Marks (49), the fasting blood sugar level was first determined on the four rabbits. The same rabbits were then injected subcutaneously with the infusions previously prepared. Rabbit #1 was injected with 50 mg./Kg. of body weight of the infusion of the whole plant. Rabbit #2 was injected with 100 mg./Kg. of body weight of the same infusion. The same procedure was used for the second two rabbits using the aqueous infusion of the root of A. biennis

with exactly the same dosage as recorded above. Blood (0.1 ml.) from each rabbit was taken at hourly intervals for five hours, pooled and the blood sugar levels determined on the pooled blood samples. The results are recorded in Table IV.

TABLE IV

DETERMINATION OF BLOOD SUGAR LEVELS

Rabbit No.	Sample No.	Dose mg./Kg.	Initial Blood Sugar mg. per 100 ml.	5-Hour Pooled Blood Sugar mg. per 100 ml.
1	A	50	182	202
2	A	100	200	209
3	B	50	184	195
4	B	100	206	213

III. ISOLATION OF PLANT CONSTITUENTS OF A. BIENNIS

A. The Whole Plant

The powdered plant (4.532 Kg.) was extracted with petroleum ether using a percolator. The solvent was evaporated on a water bath leaving a dark brownish-green residue (93 g.). Forty grams of this residue were dissolved in a minimum of petroleum ether and placed on a 370 x 40 mm. column of alumina (300 g.) prepared with petroleum ether. The column was eluted with petroleum ether and other solvents. Fifty ml. fractions were collected with a Towers fraction collector and examined for colour and/or precipitates, checked for optical activity using a 2 dm. tube in a Bellingham and Stanley Polarimeter, and then combined according to the colour of the eluate and the solvent used. No optical rotation was observed. The fractions obtained by column chromatography are recorded in Table V. A crystalline residue was observed in residue number nine.

TABLE V

CHROMATOGRAPHY FRACTIONS COLLECTED FROM THE RESIDUE OF WHOLE PLANT OF A. BIENNIS

Residue Number	Eluant	Fractions Collected	Volume in Litres
1	Petroleum ether	1-4	0.20
2	Petroleum ether	5-6	0.10
3	Petroleum ether	7-13	0.35
4	Petroleum ether	14-27	0.70
5	Petroleum ether	28-67	2.00
6	Petroleum ether	68-109	2.10
7	0.5% Ethanol in petroleum ether	110-137	1.40
8	0.5% Ethanol in petroleum ether	138-143	0.30
9	0.5% Ethanol in petroleum ether	144-153	0.50
10	0.5% Ethanol in petroleum ether	154-213	3.00
11	0.5% Ethanol in petroleum ether	214-319	5.30
12	1% Ethanol in petroleum ether	320-383	3.20
13	5% Ethanol in petroleum ether	384-449	3.30
14	10% Ethanol in petroleum ether	450-484	1.75
15	25% Ethanol in petroleum ether	485-517	1.65
16	50% Ethanol in petroleum ether	518-553	1.8
17	Ethanol 95%	554-661	5.4

1. Examination of Residue Number Nine

The residue yielded white crystals (227 mg.) which melted at 79.5-80°C. (after six recrystallizations from petroleum ether). Analysis of the compound for Carbon and Hydrogen (average of three determinations) gave the following results:

C - 86.18% N - 14.55%

Ratio 1:2

∴ Empirical formula $(CH_2)_x$

The crystals dissolved in carbon tetrachloride and in benzene formed clear colourless gels. Further investigation on this compound and on the remaining fractions obtained from the chromatographic column was not carried out.

B. Leaves and Inflorescence

The dried powdered leaves and inflorescence (6.00 Kg.) of A. biennis were extracted to exhaustion with 176 litres of chloroform. The solvent was removed under reduced pressure leaving a dark solid residue (473 g.). The residue was then subjected to the process of Sanchez-Viesca and Romo (23) which they used to isolate the sesquiterpene lactone, estafiatin. The residue was dissolved in methanol, precipitated with an aqueous lead acetate solution, filtered and the filtrate extracted with chloroform. The extract was evaporated to dryness and steam distilled. The residue (20.5 g.) was dissolved in

benzene and an equal quantity of petroleum ether was added. The solution was allowed to stand overnight, then it was filtered and chromatographed on a 370 x 40 mm. column of alumina (300 g.). The column was eluted with 1:1 benzene-petroleum ether mixture and other solvents. Fifty ml. fractions were collected from the chromatographic column using a Towers fraction collector. The fractions were examined for colour and/or precipitates, and combined according to the colour of the eluate and the solvent used. The solvent was then removed under reduced pressure. The results are recorded in Table VI. As no crystalline residues were observed, no further work was done on these fractions.

TABLE VI

CHROMATOGRAPHY FRACTIONS COLLECTED FROM THE RESIDUE OF LEAVES AND INFLORESCENCE OF *A. BIENNIS*

Residue Number	Eluant	Fractions Collected	Volume in Litres
FF-1	1:1 benzene-petroleum ether	1-400	20.00
FF-2	2:1 benzene-petroleum ether	401-450	2.50
FF-3	3:1 benzene-petroleum ether	451-550	5.00
FF-4	benzene	551-640	4.50
FF-5	0.5% methanol in benzene	641-720	4.00
FF-6	5% methanol in benzene	721-829	5.45
FF-7	25% methanol in benzene	830-880	2.55
FF-8	50% methanol in benzene	881-1009	6.45
FF-9	methanol	1010-1228	10.95

C. Stems

The dried powdered stems (6.475 Kg.) of A. biennis were exhaustively extracted with 81 litres of chloroform using a percolator. The solvent was removed under reduced pressure to yield a residue (87 g.). This residue was also subjected to the process of Sanchez-Viesca and Romo (23) which gave a residue (11 g.) after steam distillation. This residue only partially dissolved in benzene leaving 7.5 g. of undissolved material. An equal amount of petroleum ether was added and the solution was allowed to stand overnight. The solution was filtered and chromatographed on a 370 x 40 mm. column of alumina (300 g.). The column was then eluted with various solvents and 50 ml. fractions were collected using a Towers fraction collector. The fractions were examined for colour and/or precipitates and then combined according to the colour of the eluate and the solvent used. The results are recorded in Table VII. As no crystalline residues were observed, no further work was done on these fractions.

TABLE VII

CHROMATOGRAPHY FRACTIONS COLLECTED FROM THE RESIDUE OF THE STEMS OF A. BIENNIS

Residue Number	Eluant	Fractions Collected	Volume in Litres
FS-1	1:1 benzene-petroleum ether	1-310	15.50
FS-2	2:1 benzene-petroleum ether	311-390	4.00
FS-3	3:1 benzene-petroleum ether	391-420	1.50
FS-4	benzene	421-530	5.50
FS-5	0.5% methanol in benzene	531-680	7.50
FS-6	5% methanol in benzene	681-760	4.00
FS-7	25% methanol in benzene	761-800	2.00
FS-8	50% methanol in benzene	801-890	4.50
FS-9	methanol	891-1036	7.30

D. Roots

The dried powdered roots (3.066 Kg.) were extracted with 44 litres of chloroform using a percolator. The solvent was removed under reduced pressure to yield a residue (31 g.). This residue was subjected to the process of Sanchez-Viesca and Romo (23). The residue after steam distillation (9 g.) was dissolved in benzene and an equal quantity of petroleum ether added. The solution was allowed to stand overnight, filtered and then chromatographed on a 370 x 40 mm. column of alumina (300 g.). The fifty ml. fractions were collected on a Towers fraction collector. They were examined for colour and/or precipitates and then combined according to the colour of the eluate and the solvent used. The results are recorded in Table VIII. Residue number FR-2 contained crystalline material. This compound was examined but no further work was done on the rest of the fractions.

TABLE VIII

CHROMATOGRAPHY FRACTIONS COLLECTED FROM THE RESIDUE OF THE ROOTS OF A. BIENNIS

Residue Number	Eluant	Fractions Collected	Volume in Litres
FR-1	1:1 benzene-petroleum ether	1-96	4.80
FR-2	1:1 benzene-petroleum ether	97-136	2.00
FR-3	1:1 benzene-petroleum ether	137-151	0.75
FR-4	2:1 benzene-petroleum ether	152-177	1.30
FR-5	3:1 benzene-petroleum ether	178-210	1.65
FR-6	benzene	211-326	5.80
FR-7	0.5% methanol in benzene	327-436	5.50
FR-8	5% methanol in benzene	437-515	3.95
FR-9	10% methanol in benzene	516-549	1.70
FR-10	25% methanol in benzene	550-599	2.50
FR-11	50% methanol in benzene	600-764	8.25
FR-12	methanol	765-901	6.85

1. Examination of Residue FR-2

After removal of the solvent under reduced pressure, the residue FR-2 contained needle-like crystals. The entire residue was dissolved in boiling petroleum ether, filtered, concentrated and cooled in a refrigerator to yield a yellow precipitate which was filtered off. The precipitate was recrystallized 3 times from petroleum ether to yield 98 mg. of fine yellow needle-like crystals (RB-1), m.p. 71.6-72.5°C. On sublimation under reduced pressure at 50°C. a white sublimate was obtained, m.p. 92.4-92.8°C., $\lambda_{max.}$ 229, 300 and 317 mμ, (ε = 7150, 13800 and 15700) in diethyl ether. The spectrum is recorded in Figure 1. On analysis of the compound, the following results were obtained:

Found: C,61.61; H,4.83; S,16.08%

Molecular Weight (Rast), 199.

Calculated for

$C_{10}H_{10}O_2S$: C,61.82; H,5.19; S,16.51%

Molecular Weight, 194.3.

The infrared spectra (c = 13.2 mg./ml. in carbon disulphide and c = 12.9 mg./ml. in chloroform) showed peaks at 3279, 3003, 2959, 2924, 2907, 2865, 2833, 2227, 1634, 1351, 1267 and 808 $cm.^{-1}$. The spectra are recorded in Figure 2. The nuclear magnetic resonance spectrum (Figure 3) in carbon tetrachloride (on a A-60 Varian spectrometer) has peaks at δ, 6.74, 3.90, 2.35 and 2.07 p.p.m.

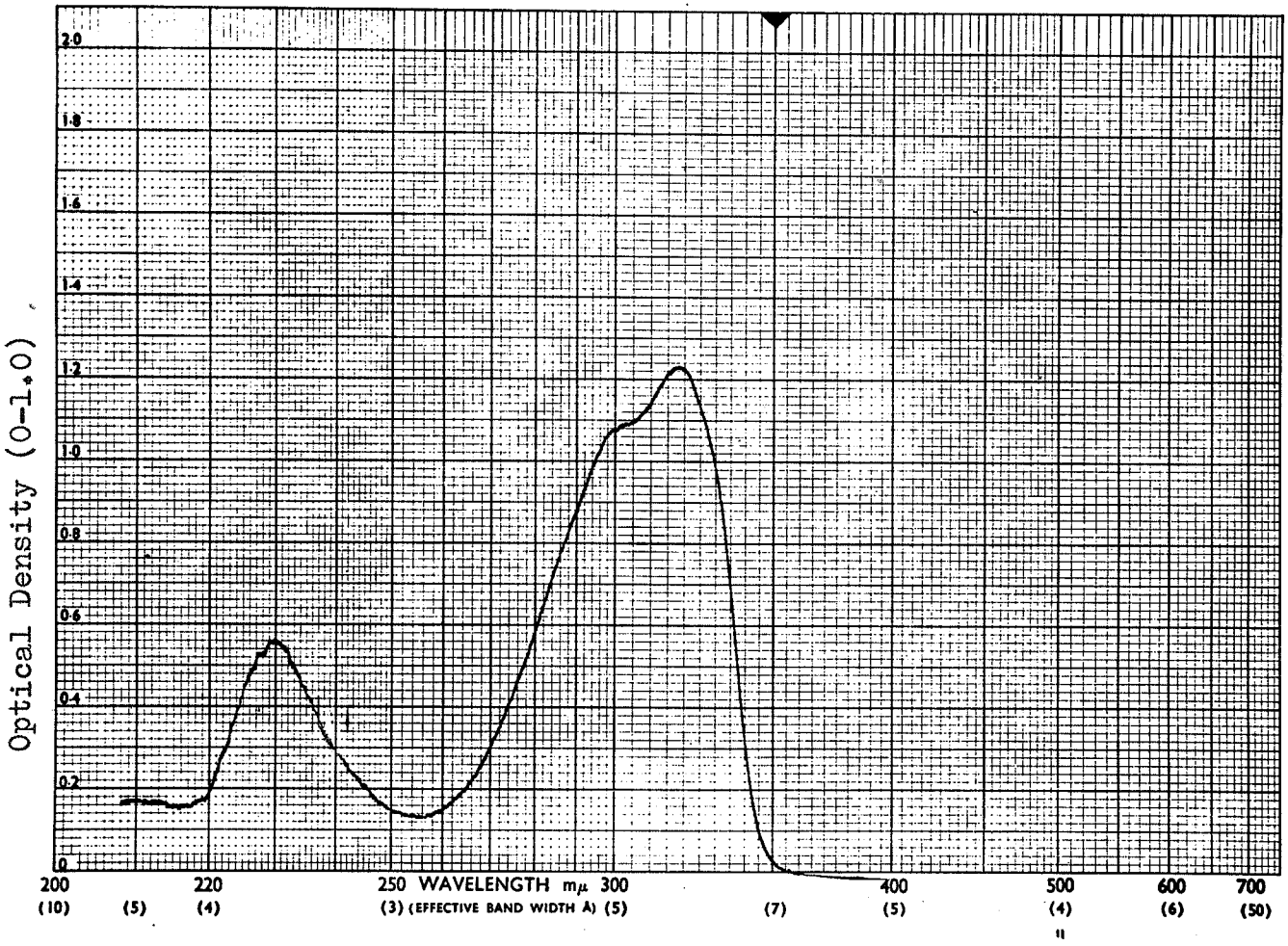


Fig. 1. Ultraviolet spectrum of 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene (RB-1)

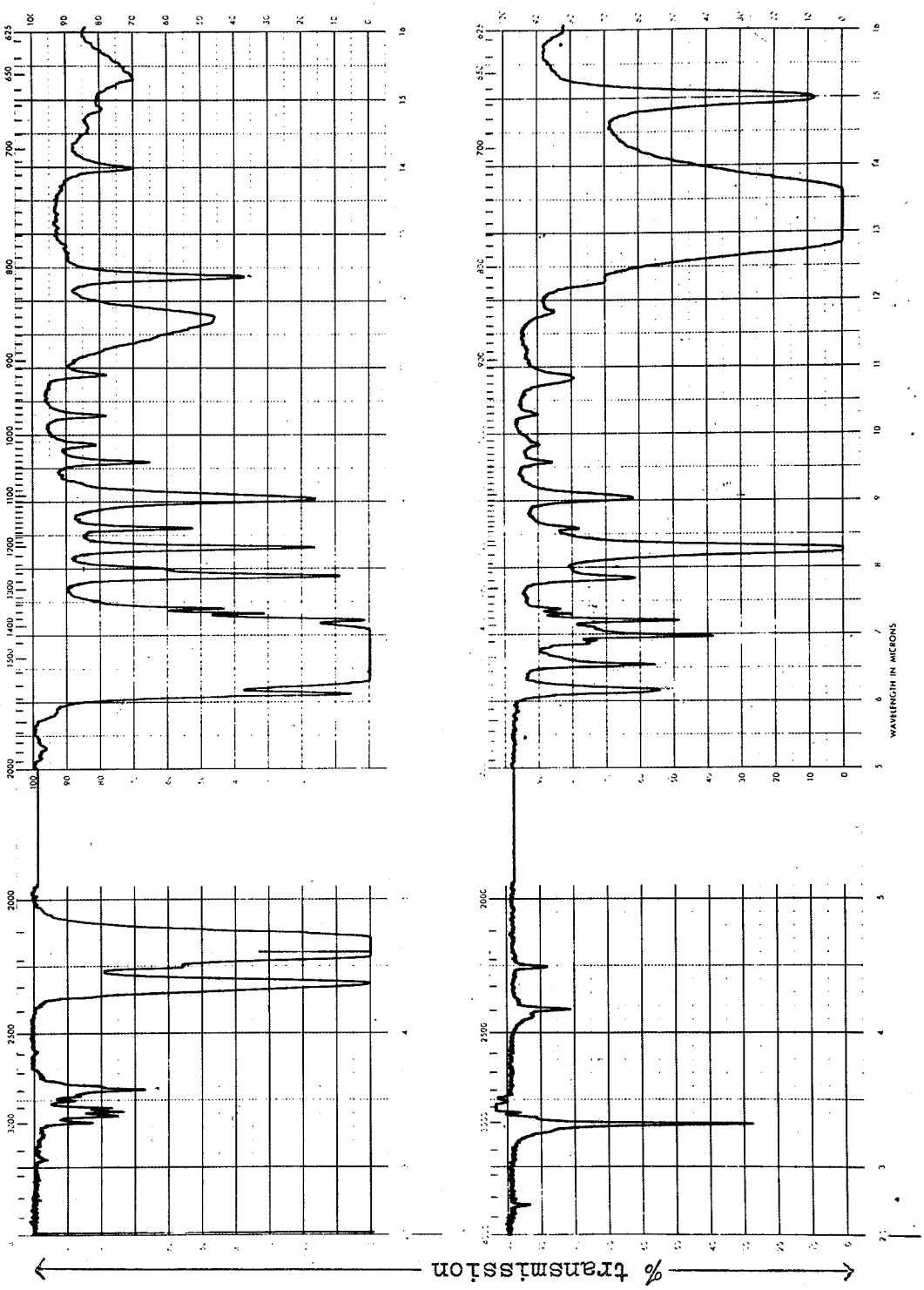


Fig. 2. Infrared spectra of 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene (RB-1): upper spectrum - in Carbon Disulphide
lower spectrum - in Chloroform

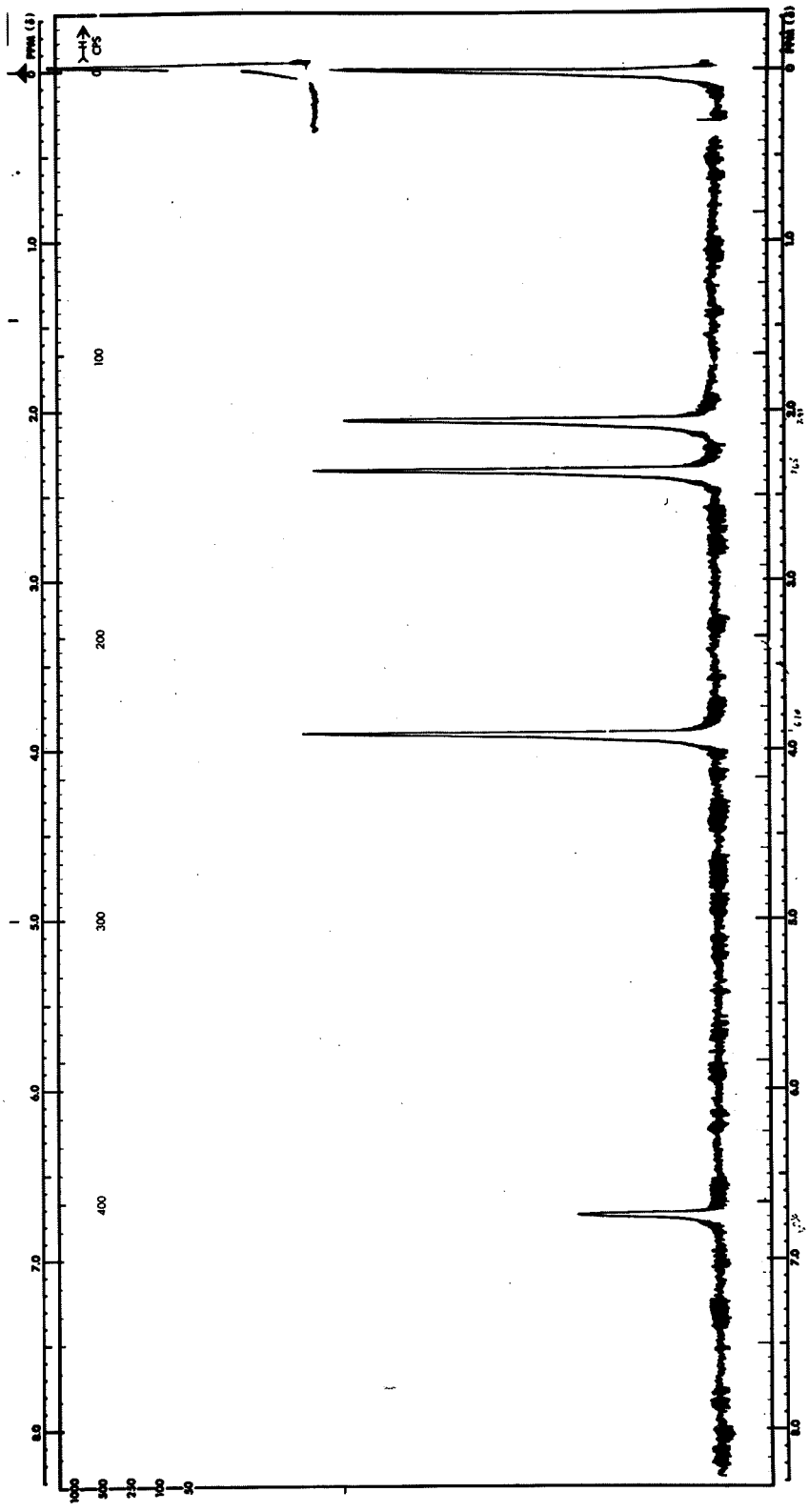


Fig. 3. Nuclear magnetic resonance spectrum of 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene (RB-1)

IV. ATTEMPTED SYNTHESIS OF COMPOUND RB-1

A. Synthesis of 2-Acetyl-4-bromothiophene

By the method of Gol'dfarb and Vol'kenshtein (50), 25 g. of 2-acetylthiophene were added dropwise to 66.3 g. of aluminum chloride, followed by dropwise addition of 12.5 ml. of bromine at 30-40°C. The solid mass was treated with ice and 66 ml. of concentrated hydrochloric acid and extracted with diethyl ether. The ether extract, dried over anhydrous sodium sulphate, was distilled yielding 47 g. of crude residue. This residue was then distilled under reduced pressure and gave 17 g. of 2-acetyl-4-bromothiophene, b.p. 133-139°C./13 mm., $n_D^{22} = 1.6068$.

B. Attempted Synthesis of 2-Acetyl-4-methoxythiophene (51)

Sodium (6 g.) was dissolved in 75 ml. of absolute methanol in a 500 ml. three-necked flask, fitted with a mercury-seal stirrer and a reflux condenser with a calcium chloride tube. To this was added 83 mg. of potassium iodide, 15 g. of 2-acetyl-4-bromothiophene and 3.7 g. of cupric oxide. The mixture was refluxed with continuous stirring for 100 hours. On cooling, 250 ml. of cold water were added and the aqueous mixture was extracted with diethyl ether. The ether extract

was filtered, dried over anhydrous sodium sulphate and then distilled, leaving a tarry residue from which no product was obtained.

C. Synthesis of Dimethyl Acetylenedicarboxylate (52)

To 1600 g. of methanol, 800 g. of concentrated sulphuric acid were added slowly with continuous cooling. To the cool solution, 400 g. of the monopotassium salt of acetylenedicarboxylic acid were added and left for four days at room temperature, with occasional swirling. Due to the vesicant nature of the product, rubber gloves and a respirator were used in the following procedure. The solution was filtered and the solid washed with 200 ml. of cold water. The filtrates were combined and extracted with diethyl ether (5 x 750 ml.). The ether extracts were washed successively with 800 ml. of water, 600 ml. of saturated sodium bicarbonate solution, 800 ml. of water and then dried over anhydrous calcium chloride. After distillation of the ether on a water bath, the residue was distilled under reduced pressure to yield 245 g. of product, b.p. 94-99°C./12 mm., n_D^{25}= 1.4448-1.4459.

D. Synthesis of Methyl Thioglycollate (53)

Thioglycollic acid (150 g.) was mixed with 200 ml. of

methanol, 300 ml. of chloroform and 20 ml. of concentrated sulphuric acid in a litre flask. The mixture was refluxed for sixteen hours using a Soxhlet apparatus containing 90 g. of anhydrous magnesium sulphate. The solution was washed with water, dried over anhydrous sodium sulphate, and then distilled using a small column. The resulting residue was then distilled under reduced pressure, yielding 110 g. of liquid, b.p. 55°C./20 mm.

E. Synthesis of Dimethyl 3-Hydroxy-2,5-thiophenedicarboxylate (54)

Seventy-four grams (0.7 mole) of methyl thioglycollate in 300 ml. of methanol were added slowly with stirring and cooling in ice, to 100 g. (0.7 mole) of dimethyl acetylenedicarboxylate. To this cold mixture, one litre of 1N methanolic potassium hydroxide solution was added slowly with constant stirring over a period of one-half hour and the resulting mixture was further stirred for another half hour. The yellow reaction mixture was diluted with two litres of water and then acidified with dilute hydrochloric acid to yield a white precipitate. The precipitate was filtered, washed with water and dried. Yield: 115 g., m.p. 109-110°C.

F. Synthesis of 4-Hydroxy-2-thiophenecarboxylic Acid (55)

Dimethyl 3-hydroxy-2,5-thiophenedicarboxylate (77 g.) was refluxed for one hour with 500 ml. of 4N sodium hydroxide solution. On cooling, the solution was acidified with hydrochloric acid to yield 66 g. of white solid, m.p. 194°C., with evolution of carbon dioxide and remelting at 202°C. This solid was then refluxed with 500 ml. of 2N sodium hydroxide solution for one hour and then acidified with hydrochloric acid, to yield on cooling 30 g. of pale brown solid, m.p. 202°C.

G. Synthesis of 4-Methoxy-2-thiophenecarboxylic Acid (51)

4-Hydroxy-2-thiophenecarboxylic acid (10 g.) was stirred with 80 ml. of 10% w/v sodium hydroxide solution and 10 ml. of dimethyl sulphate at room temperature for half an hour. The mixture was then refluxed for two hours. After cooling, the alkaline solution was extracted once with ether. On acidification with hydrochloric acid, 10 g. of crude product precipitated out which, after recrystallization from aqueous alcohol, yielded 9.5 g. of the acid, m.p. 165-167°C.

H. Synthesis of 2-Acetyl-4-methoxythiophene

Using the method of Cason (56), a Grignard reagent was

prepared using 16.5 g. (0.68 mole) of magnesium and 82.0 g. (0.86 mole) of methyl bromide in 450 ml. of anhydrous diethyl ether. To this Grignard reagent 70.0 g. (0.38 mole) of anhydrous cadmium chloride were added and the mixture was stirred for one hour at room temperature. The diethyl ether was distilled over on a water bath and 300 ml. of anhydrous benzene were added. The mixture was refluxed and 30 g. (0.17 mole) of 4-methoxy-2-thienoyl chloride in 75 ml. of benzene were added over a twenty minute period. (The 4-methoxy-2-thienoyl chloride was previously prepared from 27 g. of 4-methoxy-2-thiophenecarboxylic acid and an excess of thionyl chloride.) After the addition of the 4-methoxy-2-thienoyl chloride was complete, the resulting mixture was refluxed for one hour, cooled and then poured onto cracked ice in hydrochloric acid. The benzene layer was separated, the aqueous portion was washed once with benzene and the benzene extracts combined. The benzene solution was washed with water until it was neutral to litmus and then dried over anhydrous sodium sulphate. The benzene was distilled over leaving 26 g. of residue which was chromatographed using an alumina column with petroleum ether as solvent. The residue obtained from the chromatographic eluate was recrystallized from ether to yield yellow needle-like crystals, m.p. 33-35°C.

On analysis of the compound, the following results were obtained:

Found: C,54.06; H,5.12; S,19.92%;

Molecular Weight, 163 (by Kofler method in camphor).

Calculated for

$C_7H_8O_2S$: C,53.83; H,5.16; S,20.52%;

Molecular Weight, 156.2.

$\lambda_{max.}$ 259 and 324 mμ. (ε = 15450 and 7400) in ethanol (Figure 4). The infrared spectra (Figure 5) (c = 24 mg./ml. in carbon disulphide and c = 45.4 mg./ml. in chloroform) showed peaks at 3003, 2950, 2933, 2817, 2169, 2155, 1664, 1351 and 1269 $cm.^{-1}$. The nuclear magnetic resonance spectrum (Figure 6) indicated peaks at δ = 7.14, 7.11, 6.48, 6.45, 3.72 and 2.35 p.p.m.

I. <u>Preparation of the 2,4-Dinitrophenylhydrazone of 2-Acetyl-4-methoxythiophene</u>

Using the method of Vogel (57), 0.2 g. of 2-acetyl-4-methoxythiophene dissolved in ethanol was added to a clear solution obtained by warming 0.25 g. of 2,4-dinitrophenylhydrazine, 0.5 ml. of concentrated hydrochloric acid and 5 ml. of ethanol. The mixture was refluxed for a few minutes and then cooled. Dark red needle-like crystals were obtained, m.p. 212-213°C., after recrystallization from ethanol.

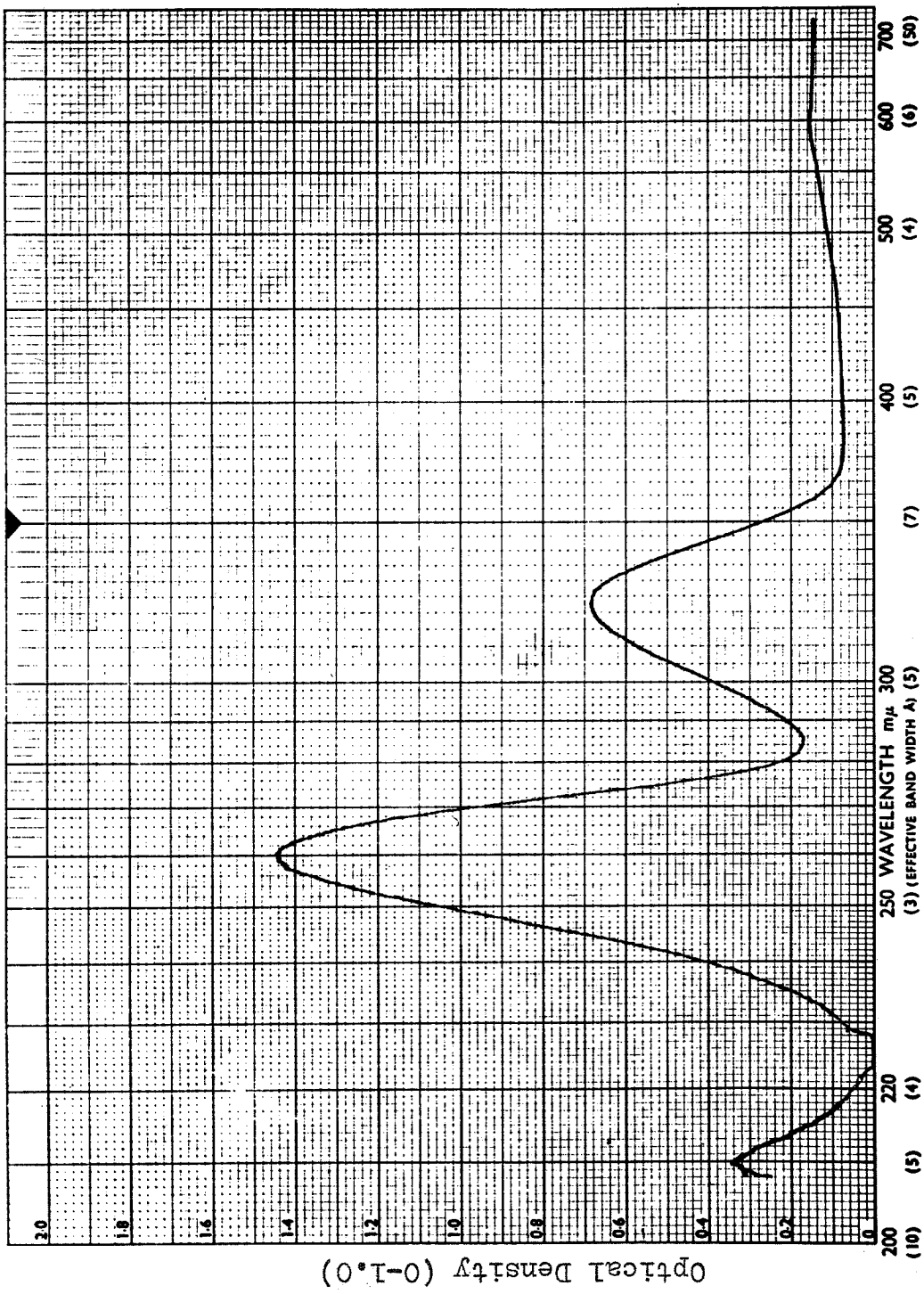


Fig. 4. Ultraviolet spectrum of 2-acetyl-4-methoxythiophene (RB-2)

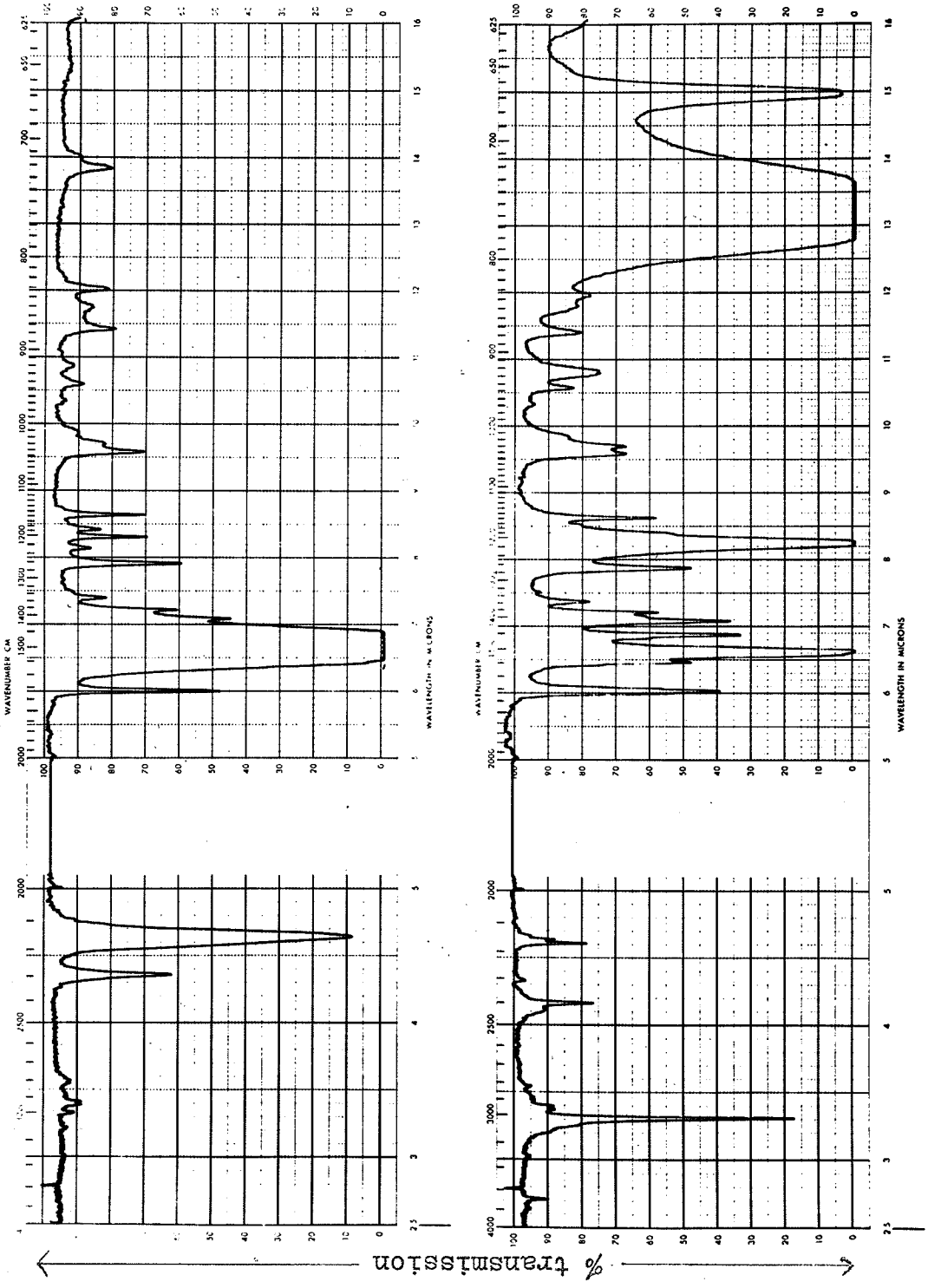


Fig. 5. Infrared spectra of 2-acetyl-4-methoxythiophene (RB-2):
upper spectrum - in Carbon Disulphide
lower spectrum - in Chloroform

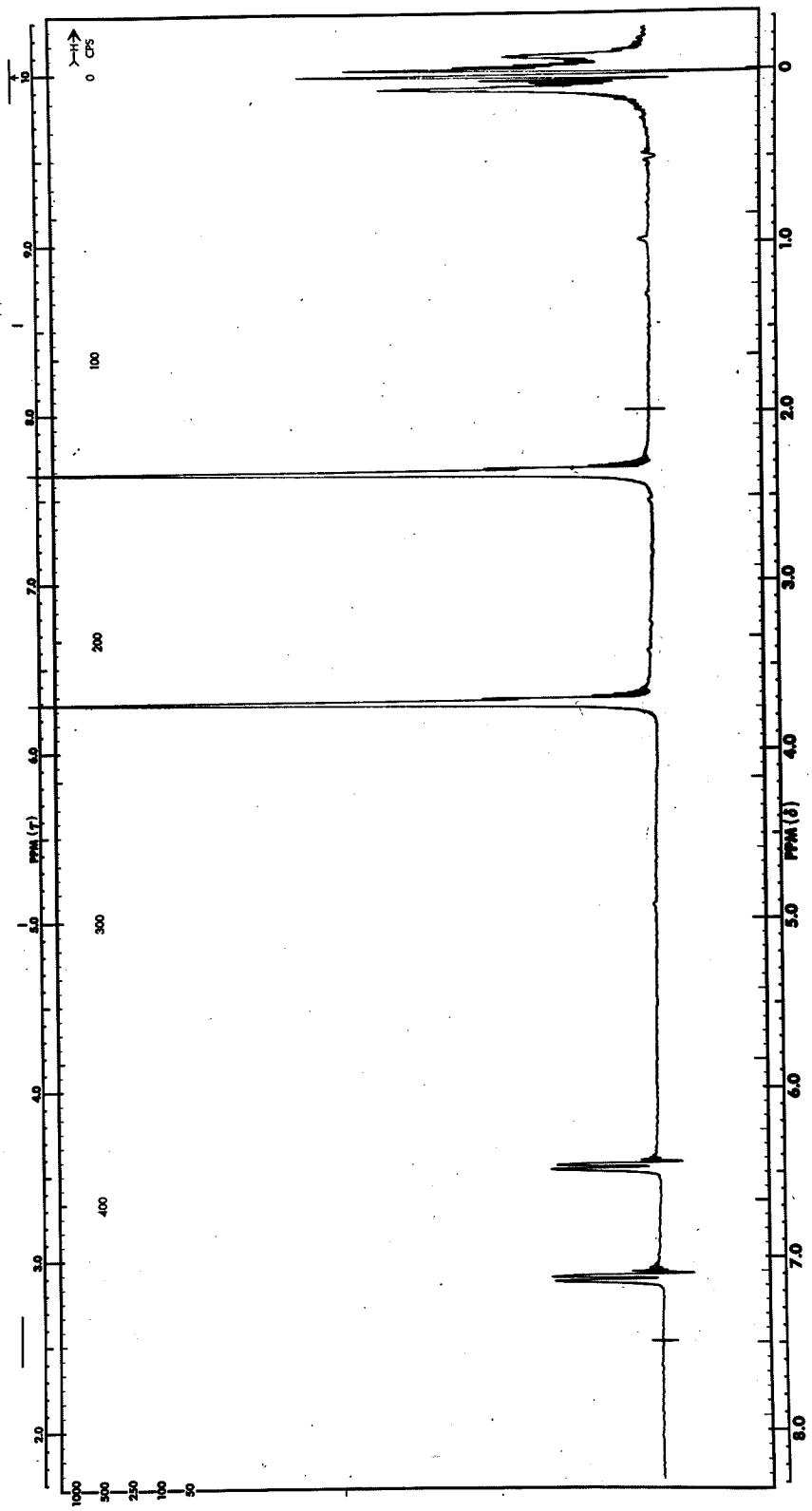


Fig. 6. Nuclear magnetic resonance spectrum of 2-acetyl-4-methoxythiophene (RB-2)

J. Attempted Synthesis of 2-(1,1-Dichloroethyl)-4-methoxy-thiophene

By the method of Keskin, Miller and Nord (58), 83 g. (0.40 mole) of phosphorous pentachloride were covered with 100 ml. of anhydrous benzene in a three-necked round bottom flask equipped with a stirrer and reflux condenser. To the prepared flask, 46.9 g. (0.30 mole) of 2-acetyl-4-methoxy-thiophene were added and the mixture refluxed with stirring on a water bath. The solution was cooled and then poured carefully into a mixture of 250 g. of ice and 75 ml. of diethyl ether. The ethereal layer was separated, washed with water until the aqueous layer was clear, and then dried over anhydrous sodium sulphate. On distillation of the diethyl ether a tarry residue was obtained from which no products were obtained.

D I S C U S S I O N

I. COLLECTION OF A. BIENNIS

A. biennis is found throughout the temperate zone of North America. It is an annual or biennial plant ranging in size from 1 to 4 feet (61). The colour of the leaves varies from light to dark green but this is usually due to the nitrogen content of the soil in which it grows. A. biennis was picked along the banks of the Assiniboine River, which is indicative of the usual areas in which it grows, i.e. moist places, slough margins, roadsides and cultivated fields.

II. SCREENING PROCEDURES

The method of Abisch and Reichstein (46) was found to be satisfactory in indicating the presence of specific organic constituents of A. biennis (Table II, page 14). However, the quantity of powdered plant material had to be increased to 10 g. in order to obtain sufficient residue for use in the tests, the result of which indicated that no alkaloids or saponins were present. The absence of saponins was also confirmed by Fischer's method (47) using a blood agar plate. The various plant parts of A. biennis did not produce a haemolyzed zone while quillaja bark, used as a control produced a clear haemolyzed zone of about one inch in diameter.

A positive Xanthydrol test and a positive Kiliani

reaction indicated the presence of glycosidal materials in the root and whole plant respectively, while steroidal glycosides (positive Kedde and antimony trichloride reactions) and sterols (positive Liebermann-Burchard test) appeared to be present in all plant parts of A. biennis.

No santonin was detected in any of the plant parts of A. biennis when examined by the method of Wichmann (48). A control sample of santonica seeds gave a positive test when examined at the same time.

Extracts of the powdered whole plant and the powdered root were found to be non-toxic to rats even when the volume of infusion injected was equivalent to 1000 mg. of plant material per kilogram of body weight. At this concentration only one rat had a ruffled appearance although no deaths were recorded.

Using the procedure of Marks (49) it was found that there was no significant change in the blood-sugar levels of the rabbits tested, when compared with their fasting blood-sugar levels. It was noted that the quantity of oxalic acid used to prevent coagulation of the blood samples was critical. Too much oxalic acid prevented the complete precipitation of the blood proteins, while too little did not prevent the blood samples from coagulating before being used in the procedure. The solution of potassium ferricyanide used must also be freshly prepared so that its oxidizing properties would not be diminished due to reduction to potassium ferrocyanide.

III. ISOLATION OF PLANT CONSTITUENTS OF A. BIENNIS

When the dark greenish-brown petroleum ether fraction was chromatographed on an alumina column, a number of residues were obtained. Residue number nine, eluted from the column with 0.5% ethanol in petroleum ether, yielded white crystals m.p. 79.5-80.0°C. The analysis of this compound indicated a Hydrogen-Carbon ratio of 2:1 $(CH_2)_x$. As the percentage content of carbon and hydrogen totalled 100.7%, no other element was present. This indicated that the compound was one of the higher hydrocarbons or possibly a mixture of related hydrocarbons but no further investigation was carried out.

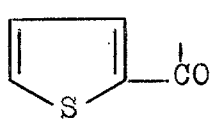
The leaves and inflorescence, stems and roots of A. biennis were separately subjected to the extraction procedure and chromatographic method of Sanchez-Viesca and Romo (23). No crystalline residues were obtained from the extracts of the leaves and inflorescence or stems, but needle-like crystals were obtained from the extract of the root (residue number FR-2, Table VIII, page 27).

The entire residue (FR-2) was recrystallized from petroleum ether to yield 98 mg. of fine yellow needle-shaped crystals (RB-1) m.p. 71.6-72.5°C. These crystals were sublimed under reduced pressure at 50°C. to yield a white sublimate m.p. 92.4-92.8°C., molecular weight 199 (Rast). As a small quantity of brown residue was left after sublimation it is

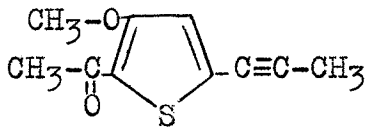
believed that the rise in melting point was due to the removal of contaminating materials.

The analysis of this compound indicated a Carbon-Hydrogen-Oxygen-Sulphur ratio of 10:10:2:1 ($C_{10}H_{10}O_2S$). The ultraviolet spectrum in diethyl ether (Figure 1, page 29) showed maxima at 229, 300 and 317 mμ (ϵ = 7150, 13800 and 15700). The infrared spectra indicated peaks at 3279 (>C=) overtone); 2959, 2924 and 2907 (C-H stretching); 2833 ($-OCH_3$); 2227 (R-C≡C-R'); 1634 (C=C-$\overset{O}{\overset{\|}{C}}$-); 1351 ($CH_3$-$\overset{O}{\overset{\|}{C}}$-); 1267 (=C-O-$CH_3$); and 808 $cm.^{-1}$ (C-H out of plane deformation).

The nuclear magnetic resonance spectrum indicated peaks at 6.74 p.p.m. (δ) (aromatic C-H, 1 proton), 3.90 p.p.m. ($-OCH_3$, 3 protons), 2.34 p.p.m. ($-COCH_3$, 3 protons) and 2.07 p.p.m. (-C≡C-CH_3, 3 protons).

Thus the above spectra in conjunction with the elemental analysis and the molecular weight determination pointed to the fact that the compound isolated from the root of A. biennis was a trisubstituted thiophene. Bohlmann, Kleine and Bornowski (26) also isolated a trisubstituted thiophene from the root of A. arborescens. They reported ultraviolet spectrum maxima at 300 and 317 mu, infrared spectrum peaks at 1650 (-$\overset{O}{\overset{\|}{C}}$-) and 1550 $cm.^{-1}$ (), and nuclear magnetic resonance spectrum peaks at 6.80 p.p.m. (δ) (aromatic C-H, 1 proton), 3.90 p.p.m. (-O-CH_3, 3 protons), 2.47 p.p.m. ($-COCH_3$) 3 protons), and 2.10 p.p.m. (-C≡C-CH_3, 3 protons)

Comparison of the two groups of results for a trisubstituted thiophene indicated that the compound RB-1 isolated from the roots of A. biennis was 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene.



VI

IV. ATTEMPTED SYNTHESIS OF 2-ACETYL-3-METHOXY-5-(1-PROPYNYL)-THIOPHENE

Synthesis of the isolated compound (RB-1) was attempted in order to completely confirm the structure. The intended scheme of synthesis is shown in Figure 7.

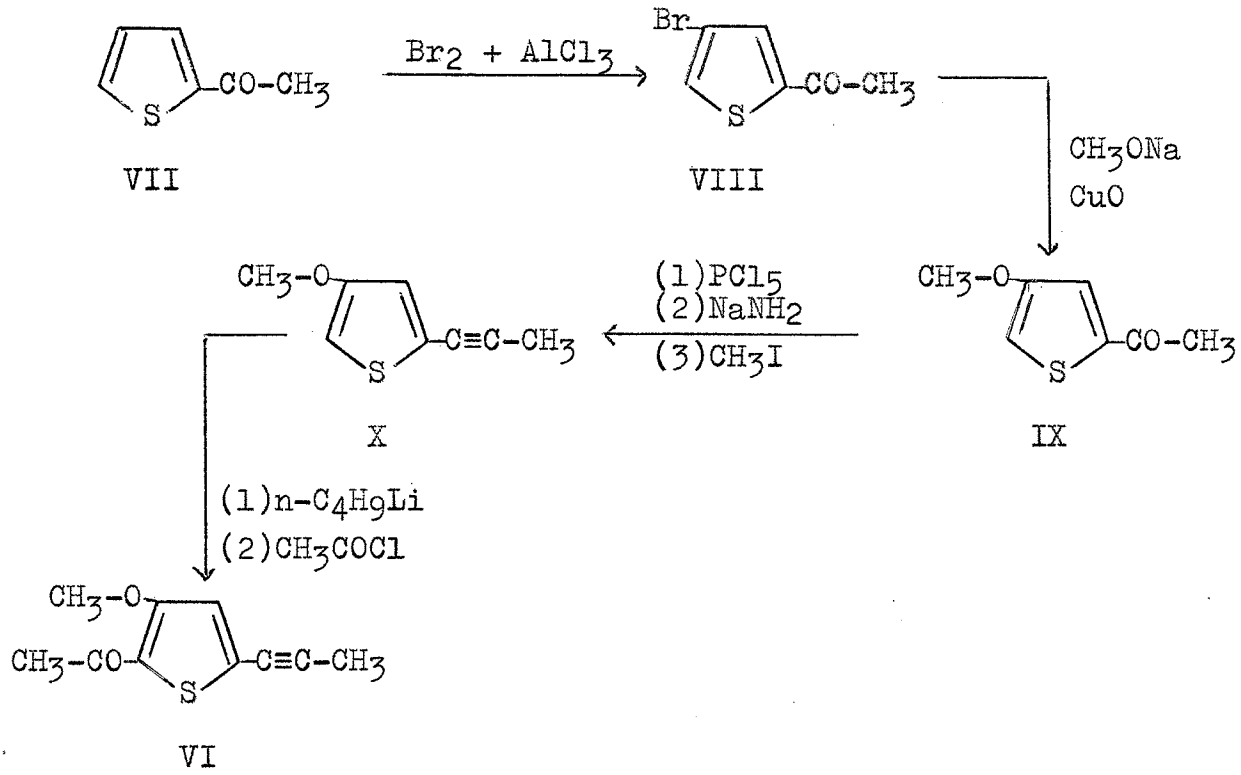


Fig. 7. First scheme for synthesis of 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene

The bromination of the 2-acetylthiophene VII at C_4 was accomplished by the use of the swamping catalyst effect (bromination in the presence of excess $AlCl_3$ in absence of solvent) as shown by Pearson and Pope (59) and applied to thiophene compounds by Gol'dfarb and Vol'kenshtein (50). The resulting compound VIII was then subjected to the Williamson reaction as reported by Gronowitz (51). However, this reaction resulted in a tarry product from which no compounds were isolated.

The failure of the above approach to obtain the inter-

mediate compound IX resulted in another approach to the attempted synthesis, as outlined in Figure 8.

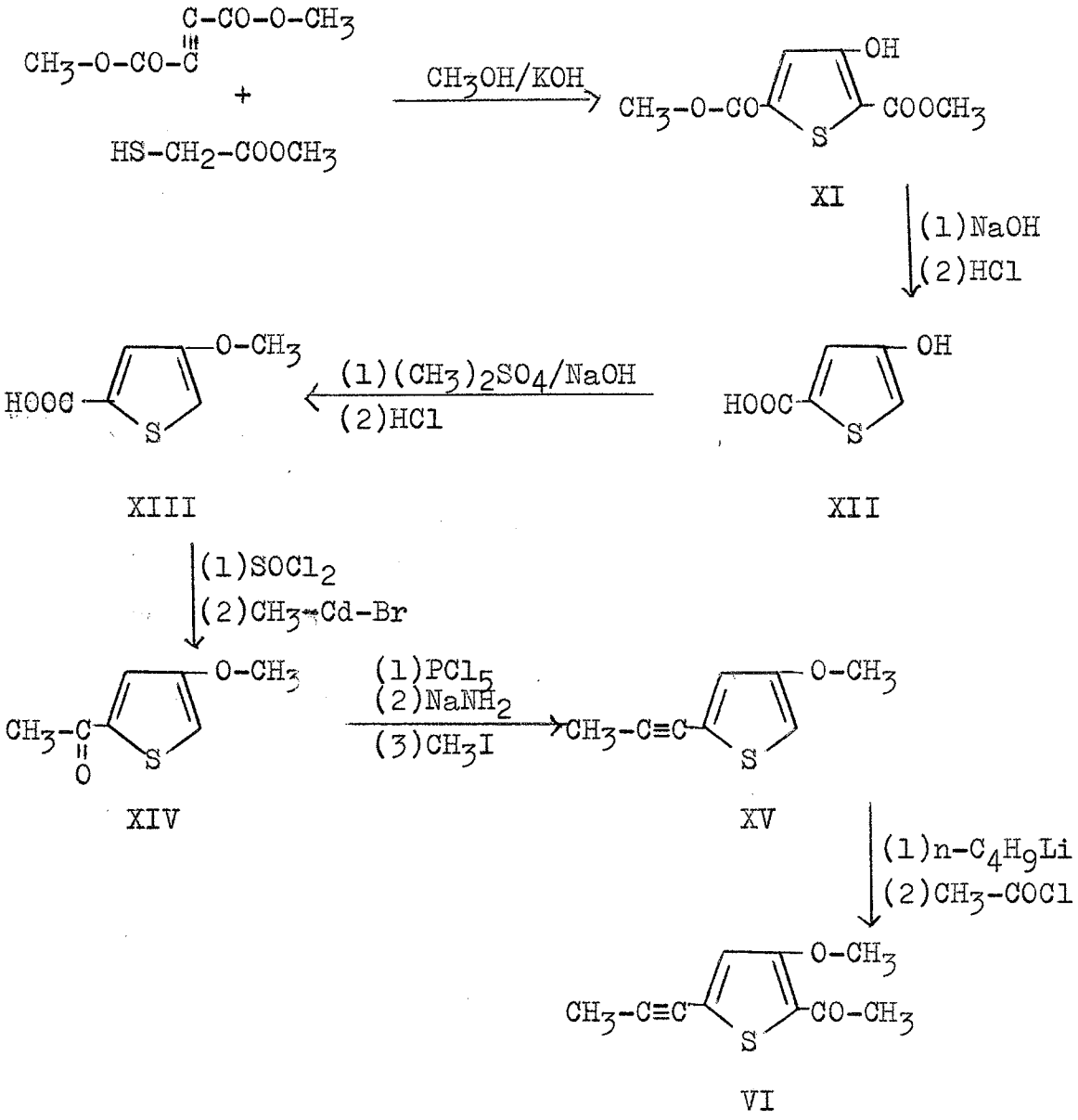


Fig. 8. Second scheme for synthesis of 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene

The starting materials - monopotassium salt of acetylenedicarboxylic acid (52) and the thioglycollic acid (53) - were both esterified with methanol. The two products were then condensed (54) to form dimethyl 3-hydroxy-2,5-thiophenedicarboxylate XI. Saponification followed by acidification resulted in elimination of the methoxycarbonyl group adjacent to the hydroxyl group (55). The resulting compound XII was then methylated with dimethyl sulphate in an alkaline medium (51) to form 4-methoxy-2-thiophenecarboxylic acid XIII. The acid chloride of compound XIII was treated with a Grignard reagent prepared with cadmium chloride (56). The resulting crude product was chromatographed on an alumina column prepared with petroleum ether. The residue from the chromatographic eluate was recrystallized from ether, to give XIV as yellow needle crystals, m.p. 33-35°C. (97%).

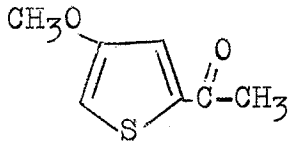
The ultraviolet spectrum (Figure 4, page 38) had maxima at 259 and 324 mμ (ϵ = 15,450 and 7400). The infrared spectra (Figure 5, page 39) showed peaks at 3003 (=C-H stretching), 2950 and 2933 (C-H stretching of CH_3), 2817 ($-OCH_3$), 2169 and 2155 (not assigned; these bonds are possibly due to impurities), 1664 ($-C=C-\overset{O}{\overset{\|}{C}}-$), 1351 ($CH_3-\overset{O}{\overset{\|}{C}}-$) and 1269 $cm.^{-1}$ ($=C-OCH_3$). The nuclear magnetic resonance spectrum (Figure 6, page 40) had chemical shifts at 7.13 p.p.m. (δ), (1 proton doublet J = 1.8 c.p.s.), 6.46 p.p.m. (1 proton doublet J = 1.8 c.p.s.), 3.72 p.p.m. (3 protons), and 2.35 p.p.m. (3 protons).

The doublets at δ = 7.13 and 6.46 p.p.m. indicate

spin-spin coupling between 2 protons with different chemical shifts. The coupling constant indicates a 3,5 proton spin-spin coupling of a thiophene ring (60). The position of the other peaks confirmed the presence of methoxy and acetyl groups.

Further confirmation of the carbonyl group was provided by the preparation of a 2,4-dinitrophenylhydrazone m.p. 212-213°C.

The spectra, elemental analysis and molecular weight determination (156.2) prove that a new compound, 2-acetyl-4-methoxythiophene (XIV) was synthesized.



XIV

An attempt was made to prepare 4-methoxy-2-(1-propynyl)-thiophene (XV) by the method of Keskin, Miller and Nord (58) but the resulting residue was a tar from which no product could be isolated.

C O N C L U S I O N

Screening tests on A. biennis indicated the presence of glycosides, steroidal glycosides and sterols and the absence of alkaloids, saponins and santonin. Infusions of the plant were found to be non-toxic to rats and had no hypoglycaemic action when tested on rabbits.

The extraction of the whole plant with petroleum ether and chromatography of the resulting residue on an alumina column, yielded 227 mg. of a white crystalline compound m.p. 79.5-80.0°C. (recrystallized from petroleum ether. Analysis of this compound indicated that it was a hydrocarbon. It was not examined further.

The leaves and inflorescence, stems and roots were each extracted with chloroform and the residues were subjected to the process of Sanchez-Viesca and Romo (23). The residue from the root yielded 98 mg. (0.003% w/w) of fine yellow needle-like crystals m.p. 71.6-72.5°C. (recrystallized from petroleum ether). This compound sublimed under reduced pressure at 50°C. to yield a white sublimate m.p. 92.4-92.8°C.

Based on the ultraviolet, infrared and nuclear magnetic resonance spectra and in conjunction with the elemental analysis and molecular weight determination, it was concluded that this compound was 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene and was identical with the compound isolated from the roots of A. arborescens by Bohlmann, Kleine and Bornowski (26).

An attempt was made to synthesize 2-acetyl-3-methoxy-5-(1-propynyl)-thiophene. Using the swamping catalyst effect,

4-bromo-2-acetylthiophene was prepared. However the methylation of this compound was unsuccessful by the Williamson reaction as reported by Gronowitz (51). A new approach of synthesis was attempted. Using the ring closure method of Fiesselmann and Shipprak (53) an intermediate compound, dimethyl 3-hydroxy-2,5-thiophenedicarboxylate was prepared. A number of other intermediate compounds were also prepared ending in a new synthetic intermediate compound, 2-acetyl-4-methoxythiophene, m.p. 33-35°C. (recrystallized from ether); 2,4-dinitrophenylhydrazone m.p. 212-213°C.

Further attempts to synthesize the next intermediate compound, 2-(1-propynyl)-4-methoxythiophene were unsuccessful.

A P P E N D I X I

A. Species of the Genus Artemisia Reported to Contain Santonin

A. arctica (62)

A. brevifolia Wall. (48, 63 to 68)

A. caerulescens L. (48,69,70)

A. camphorata Vill. (48)

A. cina (Berg) Willkomm. (48, 71 to 77)

A. finita Kitagawa (48, 78 to 79)

A. fragrans Willd. (48,80)

A. gallica Willd. (48,81,82)

A. glaucina (83)

A. hybrida Sag. (48,84)

A. incana (85)

A. kurramensis Qasilbash (48,64,70,86 to 102)

A. maritima L. (48,77,81,82,87,103 to 115)

A. maritima var. diffusa (116,117)

A. maritima var. monogyna (48,84,86,88,118,119)

A. mexicana Willd. (12,48)

A. mogoltavica (83)

A. neo-mexicana Wooton (12,48)

A. pauciflora Weber (48,80)

A. ramosa C. Sm. (48,120)

A. salina Willd. (48,84)

A. wrightii Gray (12,48)

A. terrae albae (121)

A. transiliensis (48)

A. new pentaloidal-cross of maritima var. monogyna and lercheana var. duhunia (122)

B. Species of the Genus Artemisia Reported to Contain No Santonin

A. absinthium L. (12)

A. albula Wooton (12)

A. annua L. (12)

A. arborescens L. (12)

A. aromatica A. Nels. (12)

A. atomifera Piper (12)

A. biennis Willd. (12)

A. bigelovii A. Gray (12)

A. caerulescens var. augustifolia (70)

A. californica Less. (12)

A. cana Pursch. (12)

A. canadensis Michx. (12)

A. caudata Michx. (12)

A. carruthii A. Wood. (12)

A. dracunculina S. Wats. (12)

A. dracunculoides Pursch. (12)

A. filifolia Torr. (12)

A. forwoodii Watson (12)

A. frauserioides Greene (12)

A. frigida Willd. (12)

A. gallica Willd. (12)

A. gnaphalodes Nutt. (12)

A. heterophyllea Nutt. (12)

A. judaica (123)

A. mexicana Bakeri (12)

A. mexicana Willd. (12,23)

A. microcephale Wooton (12)

A. neo-mexicana Wooton (12)

A. pontica L. (12)

A. redolens Gray (12,13)

A. rhizomatus pubularis (12)

A. tridentata Nutt. (12)

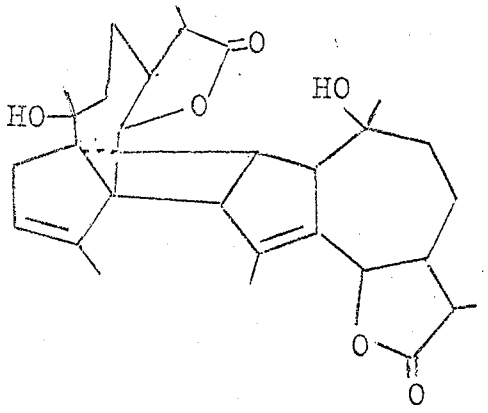
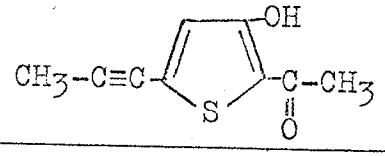
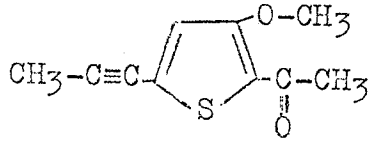
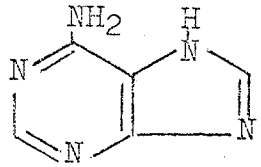
A. vulgaris L. (12)

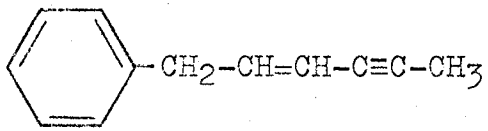
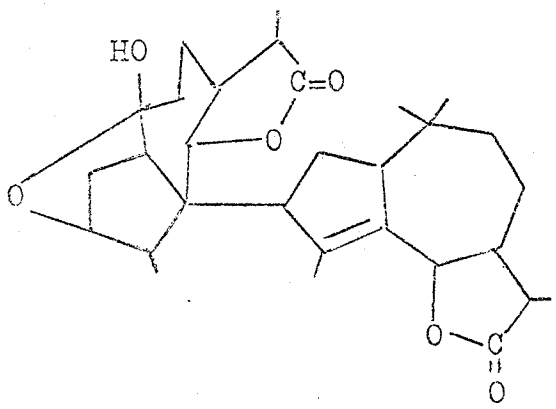
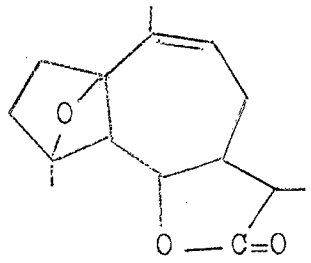
A. wrightii Rausch. (12)

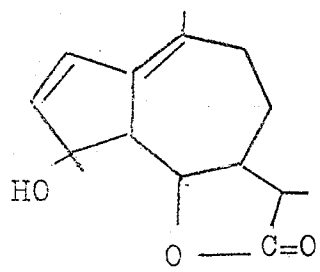
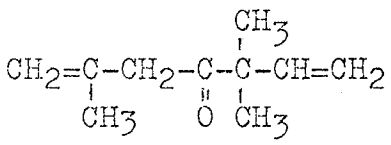
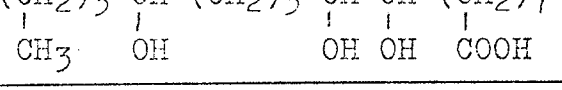
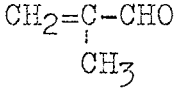
A. wrightii Gray (12)

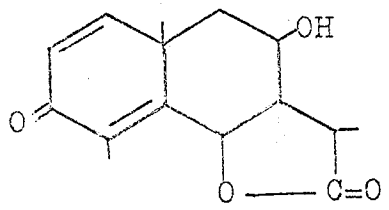
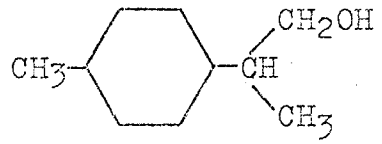
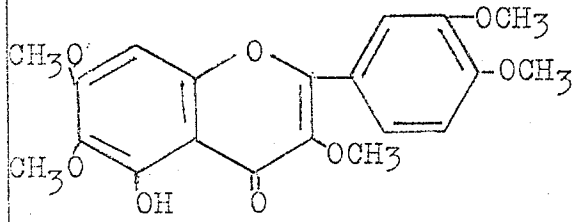
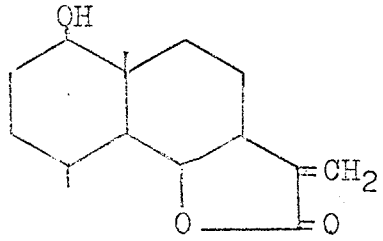
A P P E N D I X I I

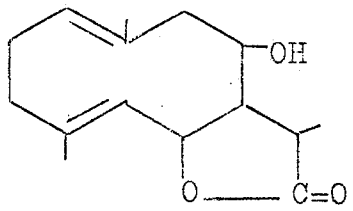
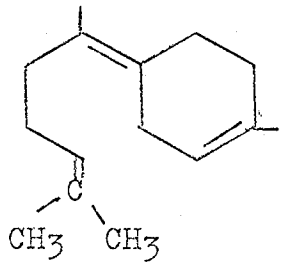
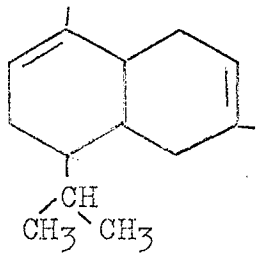
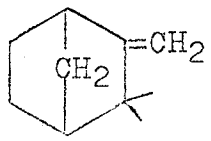
ORGANIC COMPOUNDS FOUND IN THE GENUS ARTEMISIA

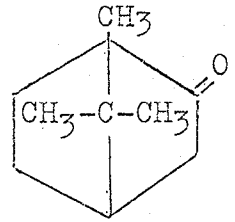
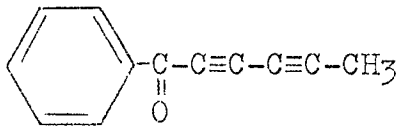
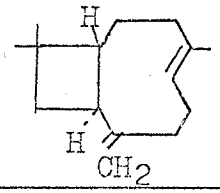
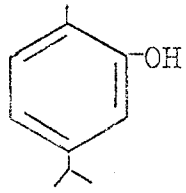
Name	b.p. °C.	m.p. °C.	Molecular Formula	Structural Formula	Species
Absinthin		179-80d	$C_{30}H_{40}O_6$		A. absinthium (124,125,126)
2-Acetyl-3-hydroxy-5-(1-propynyl)-thiophene		100.5	$C_9H_8O_2S$		A. arborescens (26)
2-Acetyl-3-methoxy-5-(1-propynyl)-thiophene		90-91	$C_{10}H_{10}O_2S$		A. arborescens (26)
Adenine		260-265	$C_5H_5N_5$		A. artemisiafolia (127)

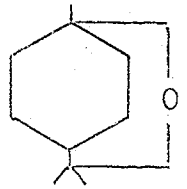
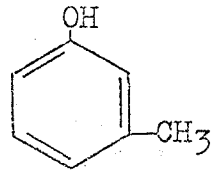
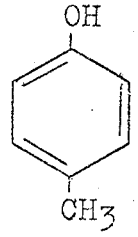
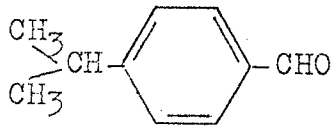
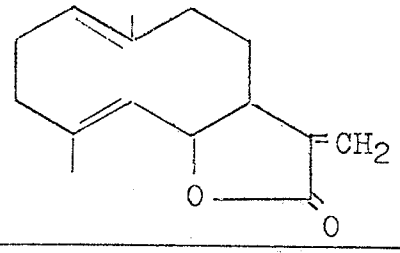
Agropyren	140-3		$C_{12}H_{12}$		A. capillaris (128)
Anabsinthin		260	$C_{30}H_{40}O_6$		A. absinthium (124,129)
Anisic Acid				refer to p-Methoxybenzoic acid	
Arachidyl Alcohol				refer to Eicosanol-1	
Arborescine		145	$C_{15}H_{20}O_3$		A. arborescens (24,130)

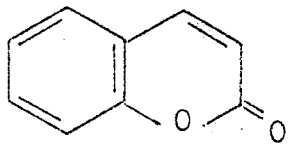
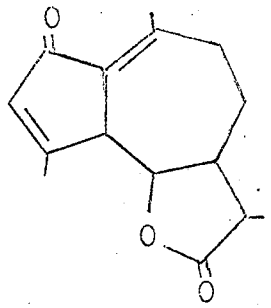
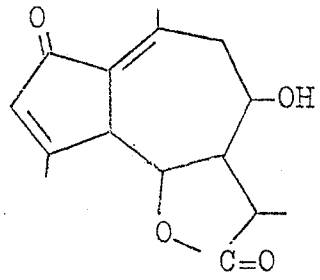
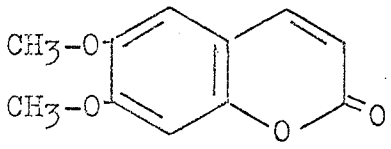
Artabsin		135	$C_{15}H_{20}O_3$		A. absinthium (131)
Artamarin		95-6		Not reported	A. absinthium (132)
Artamaridin		72		Not reported	A. absinthium (132)
Artamaridinin				Not reported	A. absinthium (132)
Artamarinin		82		Not reported	A. absinthium (132)
Artemisia-ketone	182-3		$C_{10}H_{16}O$		A. annua (133,134,135)
Artemisic Acid		108	$C_{18}H_{36}O_5$		A. monogyna (136)
Artemisal	73.5		C_4H_6O		A. tridentata (137,138,139)

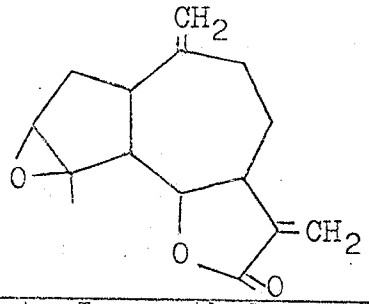
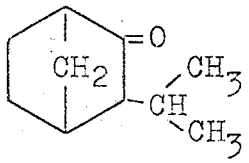
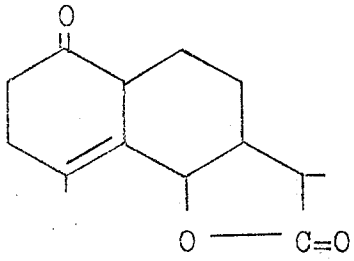
Artemisin		203	$C_{15}H_{18}O_4$		A. maritima (140-156)
Artemisol	ca 115/ 17mm.		$C_{10}H_{18}O$		A. tridentata (137)
Artemitin		161.5	$C_{20}H_{20}O_8$		A. absinthium (157,158,159) A. arborescens (160)
Artilesin A				Refer to Matricarin	
Austricine		149-150.5	$C_{15}H_{18}O_4 \cdot H_2O$	Not reported	A. austriaca (161) A. leucodes (162)
Ayapanin				Refer to 7-Methoxycoumarin	
Balchanin		142	$C_{15}H_{22}O_3$		A. balchanorum (22)

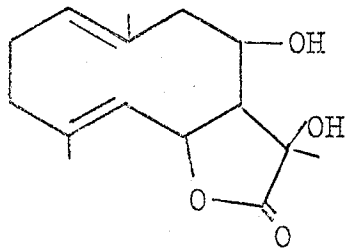
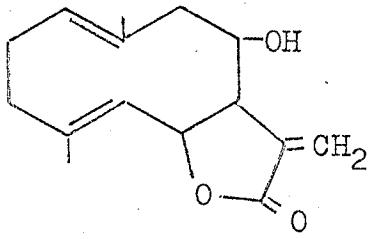
Balchanolide		154	$C_{15}H_{21}O_3$		A. balchanorum (22)
Bisabolen	262-3		$C_{15}H_{24}$		A. absinthium (163)
Brevifolin				Refer to Xanthoxylin	
n-Butyraldehyde	75-6		C_4H_8O	$CH_3-CH_2-CH_2-CHO$	A. scoparia (164)
Cadinene	274-5		$C_{15}H_{24}$		A. annua (165) A. absinthium (166)
Camphene	51-52		$C_{10}H_{16}$		A. annua (165)

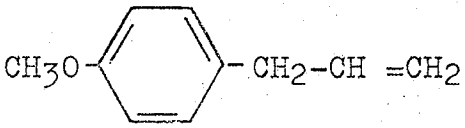
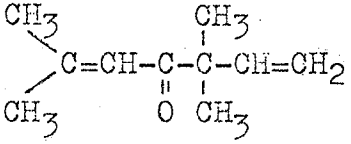
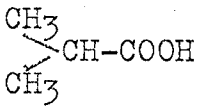
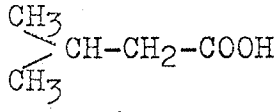
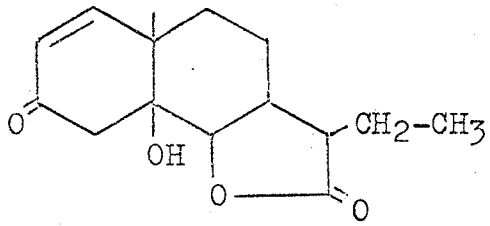
Camphor		179-80	$C_{10}H_{16}O$		A. cina (167) A. afra (168) A. annua (169) A. arborescens (170) A. maritima var. erivanica(171)
Capillin		81	$C_{12}H_8O$		A. capillaris (172)
n-Caproic acid		31.5	$C_{10}H_{20}O_2$	$CH_3-(CH_2)_8-COOH$	A. vulgaris (173)
Caryophyllene	258-9 /752 mm.		$C_{15}H_{24}$		A. annua (165) A. absinthium (174)
Caryophyllene monoxide		61	$C_{15}H_{24}O$	Not reported	A. absinthium (163)
Carvacrol	237-8		$C_{10}H_{14}O$		A. cina (167)
Centaur X3		trans 18° cis -12°	$C_{17}H_{18}$	$CH_3-(C\equiv C)_3-(CH=CH)_2-(CH_2)_4-CH=CH_2$	A. vulgaris (25,176)

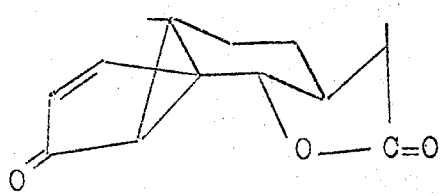
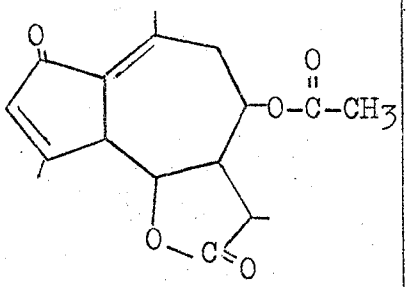
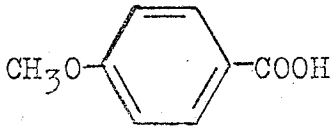
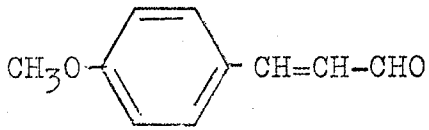
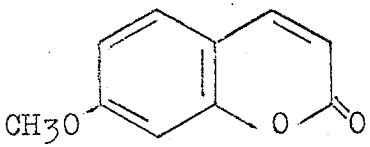
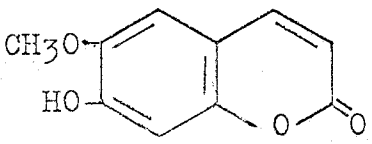
Ceryl Cerotate		81.5-82.5	$C_{52}H_{104}O_2$	$C_{25}H_{51}\overset{O}{\overset{\|}{C}}-O-C_{26}H_{53}$	A. afra (168)
Cineol	176-7		$C_{10}H_{18}O$		A. cina (167) A. vulgaris (177-179) A. herba alba (178) A. annua (165)
m-Cresol	202-3		C_7H_8O		A. transiliensis (180)
p-Cresol		35-36.5	C_7H_8O		A. santolinofolia (181)
Cuminaldehyde	236-7		$C_{10}H_{12}O$		A. annua (165)
Costunolide		106-7	$C_{15}H_{10}O_2$		A. balchanorum (182,183)

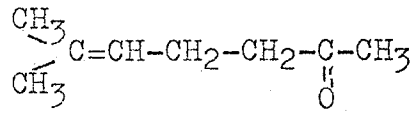
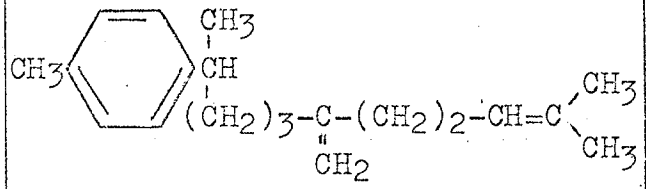
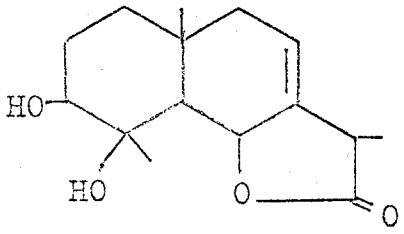
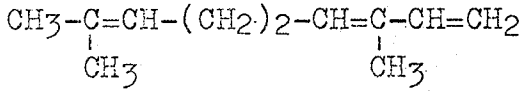
Coumarin		68-70	$C_9H_6O_2$		A. dracunculus (184)
Dehydromatricaria ester		trans 105-6 cis 112-3	$C_{11}H_8O_2$	$CH_3-(C\equiv C)_3-CH=CH-CO-O-CH_3$	A. vulgaris (25)
Desacetoxymatricarin		202-3	$C_{15}H_{18}O_3$		A. leukodes Shrenk.(185)
Desacetylmatricarin		143-6	$C_{15}H_{18}O_4 \cdot H_2O$		A. tilesii Ledeb. (186)
6,7-Dimethoxycoumarin		146-7	$C_{11}H_{10}O_4$		A. scoparia (187-191) A. dracunculus (191, 194) A. capillaris (191-3)

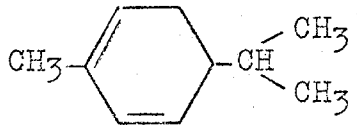
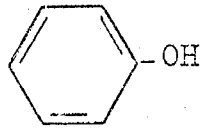
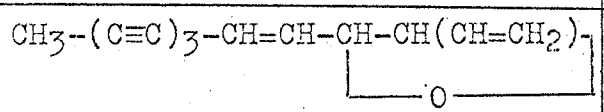
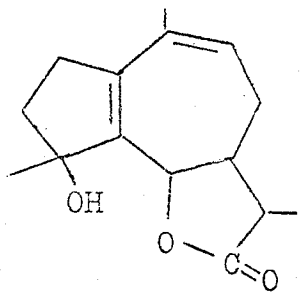
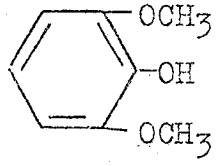
Eicosanol-1		70-1	$C_{20}H_{42}O$	$CH_3-(CH_2)_{18}-CH_2OH$	A. vulgaris (173)
Erivanine		203-5	$C_{15}H_{22}O_4$	No structure available	A. fragrans Willd. var. erivanica Bess. (16)
Esdragol				Refer to Isoanethol	
Estafiatin		104-6	$C_{15}H_{18}O_3$		A. mexicana Willd. (23)
Estragol				Refer to Isoanethol	
Esculetin-dimethylether				Refer to 6,7-Dimethoxy-coumarin	
Fenchon	193.5	5-6	$C_{10}H_{16}O$		A. frigida(181) A. santolino-folia(181) A. verlotanum (181)
Finitin		153-5	$C_{15}H_{20}O_3$		A. finita (79)

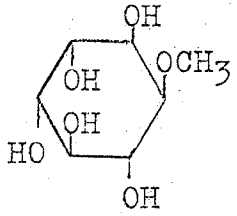
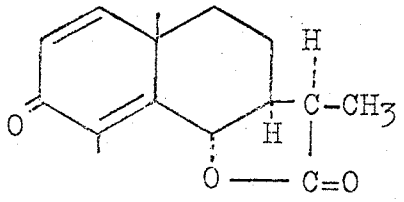
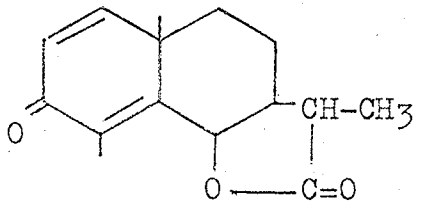
Formic Acid	100.5	8.4	CH_2O_2	H-COOH	A. transiliensis (180)
Humulen				Refer to Caryophyllene	
α-Hydroxysantonin				Refer to Artemisin	
9-Hydroxy-trans-10-cis-12-octadecadienoic Acid			$C_{18}H_{32}O_3$	$(CH_2)_4$-CH=CH-CH=CH-CH-$(CH_2)_7$ CH_3 OH COOH	A. absinthium (197)
13-Hydroxy-cis-9-trans-11-octadecadienoic Acid			$C_{18}H_{32}O_3$	$(CH_2)_4$-CH-CH=CH-CH=CH-$(CH_2)_7$ CH_3 OH COOH	A. absinthium (197)
Herniarin				Refer to 7-Methoxycoumarin	
Hydroxy-balchanolide		163	$C_{15}H_{22}O_4$		A. balchanorum (22)
Hydroxy-costunolide		acetate derivative 98	$C_{15}H_{20}O_3$		A. balchanorum (22)
l-Inosit-2-methylether				See Quebrachitol	

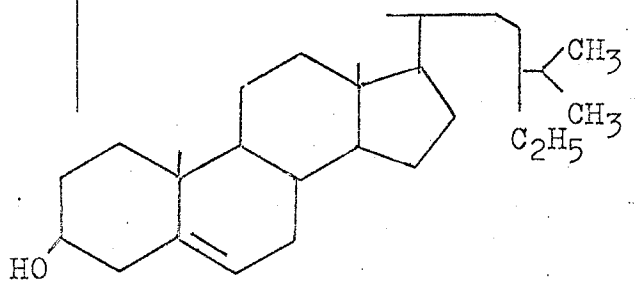
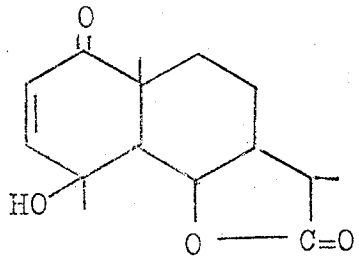
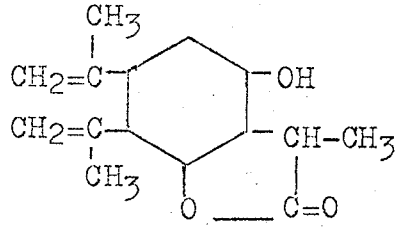
Isoanethol	215-6		$C_{10}H_{11}O$		A. dracunculus (198)
Isoartemisia-ketone	182-3		$C_{10}H_{16}O$		A. annua (165,199)
Isobalchan-olide		133		Not available	A. balchanorum (22)
Isobutyric Acid	153-5	-47	$C_4H_8O_2$		A. transilien-sis (200)
Isovaleric Acid	175-7	-37	$C_5H_{10}O_2$		A. absinthium (201)
Judaicin		177-8	$C_{15}H_{20}O_4$		A. judaica (123)
Leucomysine		196-8	$C_{15}H_{18}O_3$	Not reported	A. austriaca (161) A. leukodes (161)
Limen				See Bisabolen	

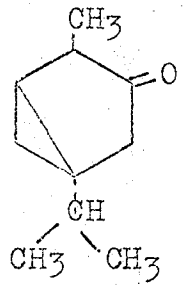
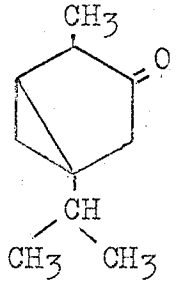
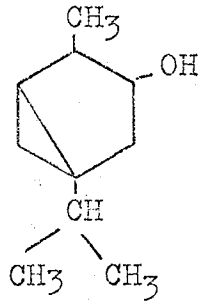
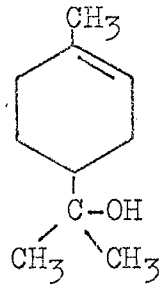
Lumisantonin		153.5-155	$C_{15}H_{18}O_3$		A. kurramensis (202)
Matricarin		190-1	$C_{17}H_{20}O_5$		A. tilesii Ledeb. (186)
p-Methoxy-benzoic Acid		184	$C_8H_8O_3$		A. dracunculus (203)
p-Methoxy-cinnamaldehyde		58	$C_{10}H_{10}O_2$		A. dracunculus (204)
7-Methoxy coumarin		117-9	$C_{10}H_8O_3$		A. dracunculus (191,194)
6-Methoxy-7-oxycoumarin		205-6	$C_{10}H_8O_4$		A. persica(206) A. santolinifolia (206) A. afra(168) A. capillaris (229)

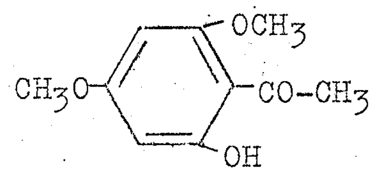
α-Methyl-acrolein				Refer to Artemisal	
Methylacryl-aldehyde				Refer to Artemisal	
β-Methyl-aesculetin				Refer to 6-Methyl-7-oxy-coumarin	
2-Methyl-hepten-2-one-6	173-4		$C_8H_{14}O$		A. scoparia (208)
Methyl-n-dec-enetryonate				Refer to Dehydromatricaria ester	
2-Methyl-6-methylen-10-p-tolylundecene-2	124/ .1 mm.		$C_{20}H_{30}$		A. absinthium (163,209)
Mibulactone		228-9	$C_{15}H_{22}O_4$		A. monogyna (211-3)
Monogynin		138	$C_{15}H_{20}O$	Not available	A. monogyna (211,212,214)
Ocimene	72-4		$C_{10}H_{16}$		A. dracunculus (215)

Pelargonic Acid	253-5	15	$C_9H_{18}O_2$	$CH_3-(CH_2)_7-COOH$	A. arborescens (216)
Pelinlactone		237-9	$C_{15}H_{22}O_4$	Not reported	A. caerulescens (48)
α-Phellandrene	175-6		$C_{10}H_{16}$		A. absinthium (166)
Phenol	182-3	41-42	C_6H_6O		A. annua (167)
Pontica Epoxide		66	$C_{13}H_{10}O$		A. pontica (217) A. sacrorum viridis (217) A. annua (217) A. ludoviciana (217)
Prochamazulenogen		129-132d	$C_{15}H_{20}O_3$		A. absinthium (218)
Pyrogallol-1,3-dimethyl-ether		55.6	$C_8H_{10}O_3$		A. herba alba var. densiflora (219)

Quebrachitol		190-2	$C_7H_{14}O_6$		A. absinthium (125,220) A. afra (168) A. vulgaris(220) A. compestus (220) A. tridentata (220) A. cina (220) A. abrotanum (220) A. procera(220) A. arborescens (221) A. camphorata (221)
α-Santonin		170	$C_{15}H_{18}O_3$		see Appendix I
β-Santonin		216-8	$C_{15}H_{18}O_3$		see Appendix I
Scoparilene		as nitro-sate 216	$C_{12}H_{24}$	Not reported	A. scoparia (222)

Scoparone			Refer to 6,7-Dimethoxycoumarin		
Scopoletin			Refer to 6-Methoxy-7-oxycoumarin		
Sesquiarte-misol	Not reported		$C_{12}H_{24}O$	Not reported	A. cina (167)
β-Sitosterol		140	$C_{29}H_{50}O$		A. vulgaris (223)
Succinic Acid		185-7	$C_4H_6O_4$	$CH_2\text{-}COOH$ $CH_2\text{-}COOH$	A. absinthium (224)
Tauremisin		176-7	$C_{15}H_{20}O_4$		A. taurica (17,162,225) A. vulgaris (18)
Temisin		228	$C_{15}H_{22}O_3$		A. maritima (226)

α-Thujone	74.5/ 9 mm.		$C_{10}H_{16}O$		A. absinthium (227)
β-Thujone	200-202		$C_{10}H_{16}O$		A. absinthium (227)
Thujyl Alcohol	99/ 6 mm.		$C_{10}H_{18}O$		A. absinthium (228) A. arborescens (228) A. transiliensis (228) A. scoparia (228)
α-Terpineol	210-218		$C_{10}H_{18}O$		A. cina (210)

Tetracosanol-1		76.5-77.5	$C_{24}H_{50}O$	$CH_3-(CH_2)_{22}-CH_2OH$	A. vulgaris (223)
Tetradecatri-yn-2,4,6-en-8-one-12		57.5	$C_{14}H_{14}O$	$(C{\equiv}C)_3-CH{=}CH-CH_2-CH_2-C(=O)-CH_2$ with CH_3 on the first C and CH_3 on the last CH_2	A. vulgaris (25)
Tricosanol-12		75-6	$C_{23}H_{48}O$	$CH_3-(CH_2)_{10}-CH(OH)-(CH_2)_{10}-CH_3$	A. vulgaris (173,207)
n-Triacontane	65.5-66.5		$C_{30}H_{62}$	$CH_3-(CH_2)_{28}-CH_3$	A. afra (168)
Undecanoic Acid		29-30	$C_{11}H_{22}O_2$	$CH_3-(CH_2)_9-COOH$	A. frigida(205)
Vachanoic Acid		173-5	$C_{15}H_{24}O_3$	Not reported	A. vachanica (196)
Vulgarin				Refer to Tauremisin	
Xanthoxylin		78-80	$C_{10}H_{12}O_4$		A. brevifolia (175,195)

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