THE SYNTHESIS OF COMPOUNDS ANALOGOUS TO TRANS-9-OXO-2-DECENOIC ACID (QUEEN BEE SUBSTANCE)

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ABSTRACT

Various reported synthetic schemes were tried in an attempt to find a general method for the preparation of compounds analogous to trans-9-oxo-2-decenoic acid, which is commonly known as queen bee substance. The method of choice was found to be that of Barbier and Hugel (23). A series of 1-alkyl-1-cycloalkanols, 1-alkyl-1-cycloalkenes, crude oxoaldehydes, crude α,β-unsaturated oxoacids and the purified S-benzylisothiourium salts of these acids were prepared. All of these salts, except two, gave acceptable analyses for the S-benzylisothiourium salt of the α,β-unsaturated oxoacid. In these two exceptions the S-benzylisothiourium derivative analyzed well for the saturated oxoacid which would be formed as a result of oxidation of the oxoaldehyde used as starting material.

Syntheses were also carried out in the Butler, Callow and Johnston reaction sequence (19,32) but this method was eventually abandoned because it was impossible to purify some of the intermediates by distillation due to polymerization.

Preliminary experimentation was also carried out using the Oughton synthesis (27). Experimentation using this series of reactions was stopped when the Barbier and Hugel synthesis began to produce the desired acid products.

Currently, purification of the crude acid products

obtained from the Barbier and Hugel synthesis is being attempted by gas chromatography. If separation by this method is accomplished the pure acid will be submitted to physiological testing on the immature aquatic stages of the yellow fever mosquito (*Aedes aegypti*, L.).

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INTRODUCTION

As the social organization of the honeybee, Apis mellifera, L. has developed, the honeybees have acquired the habit by which adult workers feed other adult bees as well as feeding the larvae. In this manner a material called queen substance is passed around the bee community from worker to worker. The sharing of this substance amongst the worker bees is probably the most important single factor in the social organization of the honeybee colony. The urge of the worker bee to obtain queen substance is so powerful that queenless workers will sometimes even desert young brood in order to join another group of bees which possess a queen (1).

Queen substance was originally believed to have two effects on the social organization of the honeybee. First, it was considered to be responsible for the attraction that queen bees have for worker bees as well as the attraction that worker bees from the same colony have for each other. This keeps the colony together. Secondly, this material was believed to prevent the ovary development of worker bees, as well as queen rearing by workers as long as a satisfactory queen was present in the hive. In spite of these controlling properties of queen substance the colony is allowed to replace its queen by a younger one (supersedure) or to

reproduce itself by swarming when conditions are favourable (2).

Queen substance is described as being a pheromone. This word is derived from the Greek "pherein" meaning to carry and "hormon" meaning to excite or stimulate. Pheromones are substances which are secreted to the outside of an animal and which cause a specific reaction or response in a receiving individual of the same species. The method of acceptance varies and may be either oral or olfactory (3).

Pheromones have two chief activities. They may serve as social substances of correlation between individuals of colony building insects or they may act as sexual attractants in the pairing of some male and female insects (4).

The term queen substance was first used in 1954 by Butler, an English entomologist. He observed that on removal of a queen bee from the hive several phenomena take place. There is a general restlessness of the bees present in the hive and some of the workers attach themselves to other colonies. Secondly, cells which contain young larvae destined to become worker bees are changed into emergency queen cells by the worker bees. Butler postulated from his observations that a communication takes place between bees by means of "X", a substance which is produced by queens and distributed by workers from one to another by feeding. Butler referred to "X" as queen substance (5).

A. Source of Queen Substance in the Colony

Experimental results have demonstrated that queen substance is obtained from all parts of the queen's body. The amount of this material necessary to inhibit a colony of a given size from constructing emergency queen cells is obtained only if a colony has access to a certain minimum area of the body surface of the queen (6). Other workers have reported that queen substance is particularly abundant on the queen's head, although bees do obtain it from all parts of her body (7).

Pain and her colleagues feel that queen substance is produced by the queen bee from precursors fed to the queen by the worker bees. The recent finding that royal jelly, which the workers feed to the queens, contains trans-9-hydroxy-2-decenoic acid (which is very closely related chemically to queen substance, trans-9-oxo-2-decenoic acid), suggests that the queen cannot produce queen substance without the help of workers who bring one or more required precursors in royal jelly to the queen (8).

B. Distribution of Queen Substance

Queen substance has been proved to be present, almost exclusively, in the mandibular glands of queen bees (8).

The queen and the nurse worker bees, who help the queen with her grooming, distribute the queen substance over her entire body. Some of the workers obtain queen substance by licking their queen. Part of the material thus obtained is then transferred to other members of the colony through feeding. This process is a continuous one. The rate of distribution is sufficient for the majority of bees to receive shares frequently, thus preventing the construction of emergency queen cells in the colony (6).

C. Biological Activities of Queen Substance on Worker Bees

As previously stated, the term queen substance was first used by Butler in 1954. In the same year de Groot and Voogd found that queen substance suppressed ovary development (7). In 1955 Pain described an attractant substance present on the cuticle of the queen bee which might be identical to queen substance. She observed that worker bees sought out and licked a piece of elder pith containing the substance (8).

The biological activities of crude alcohol, ether, and acetone extracts of queen bees were investigated by several workers (5,7,9). These activities were originally believed to be due to queen substance alone and are as follows:

(1) attraction for worker bees

(2) inhibition of queen rearing by workers

(3) inhibition of the ovary development of workers. However, it is now known that all the above biological properties are not due to queen substance alone. For this reason these activities will be discussed under two headings.

(1) Attraction for Worker Bees

Worker bees are attracted to dried ethanolic or ethereal extracts of queen bees. Paper or gas chromatography of the crude extracts has led to several different substances. Of these the one first isolated, purified and identified was trans-9-oxo-2-decenoic acid, often referred to as queen substance. This chemical does not attract worker bees by itself. Pain has shown there is no attraction observed when synthetic queen substance in varying concentration is presented to workers (8). Simpson has demonstrated that synthetic trans-9-oxo-2-decenoic acid presented on filter paper does not stabilize a honeybee swarm. In addition worker bees show no response toward it even when they are allowed to touch the filter paper (11). From these investigations it is obvious that the attraction the queen bee has for the worker bees is not due solely to the presence of queen substance on the queen bee.

Pain and her French colleagues believe the attraction

exhibited is due to a mixture of several components of the acid fraction of the ethereal extract of whole queen bees. They observed that this attraction was lost when the acid portion of the crude extract was subjected to chromatography. However, it reappeared when the several fractions were re-mixed after chromatography (12).

The French workers also carried out a comparative study of the mandibular glands of queen and worker bees using gas chromatography analysis techniques. They have arbitrarily divided the substances obtained from the queens into fractions which they call Pheromone I and Pheromone II.

Pheromone I	(a) trans-9-oxo-2-decenoic acid
Pheromone II	(a) three volatile substances not identified
	(b) phenylpropionic acid
	(c) phenylacetic acid

Neither of these two pheromones by itself attracts worker bees but a mixture of the two is attractive. This has led the French workers to the conclusion that the two pheromone fractions act synergistically to attract worker bees (8).

Another explanation of this attraction has been set forth by British entomologists. In 1961, Gary made the following assessments: a) attraction of normal virgin queen bees towards worker bees; b) attraction of mated laying

queens toward worker bees; c) the attraction of mated laying queens without mandibular glands towards worker bees. He concluded that a queen's attraction for workers comes from a scent produced mainly in the mandibular glands (13). The British workers do not believe that queen substance has any attraction for worker bees at all (14). In other words, they feel the attraction which the queen bee has for worker bees depends entirely on "queen scent", a substance whose chemical identification is still undetermined but which is produced in the mandibular gland of the queen bee.

(2) Inhibition of Queen Rearing and Ovary Development of Workers

Generally, the ovaries of worker bees remain undeveloped. However, when the queen bee is absent, some of the workers' ovaries develop quickly and to such an extent that the workers may lay unfertilized eggs. It appears, therefore, that in a normal queenright colony an agency which inhibits queen rearing and ovary development of workers must be obtained and circulated.

In 1961 Butler and his colleagues (15) discovered the presence of volatile substances other than queen substance in the acid fraction of crude alcohol or ether extracts of whole queens or queens' heads. One of these substances was called mated queen scent or queens' inhibitory scent. They believe this scent partially inhibits

queen rearing from emergency queen cells and also that it acts synergistically with trans-9-oxo-2-decenoic acid (queen substance) to inhibit ovary development. Under experimental conditions ovary development has been partially inhibited in queenless worker bees by synthetic queen substance. However, the extent of inhibition produced was less than that caused by an alcoholic extract of queens' heads. This suggests that there must be an active substance in the ethanol extract other than queen bee substance. It is this fact that has led the British researchers to postulate the presence of mated queen scent as the other substance which prevents queen rearing from emergency queen cells. The chemical identity of mated queen scent is still unknown.

Mated queen scent or inhibitory scent is widely distributed over the queen's body, probably by grooming. It is most likely not produced in the queen's mandibular gland since the scent of queens who have had this gland removed for several days inhibits queen rearing as much as scent from normal queens (15).

It is also possible that other physiological factors, such as the sounds produced by a queen or the workers close by her, affect the inhibition of ovary growth (15).

It has been shown experimentally that queen substance itself will inhibit emergency queen cell construction which in turn inhibits queen rearing (15).

D. Mechanism of Action of Queen Substance

Butler and Carlisle first thought that queen substance acted in one of two manners. First, it could affect the tissues of the worker bees directly, which in turn influenced the behaviour pattern of the insect. The alternative was that trans-9-oxo-2-decenoic acid set complex physiological mechanisms in motion via the central nervous system. This mechanism was believed to be triggered by taste or some other sensory stimulation (16).

It was found that worker bees pass along queen substance with the contents of their honey stomachs, thus diluting its concentration considerably. However, the amount produced by a single queen is still sufficient for tens of thousands of worker bees. From this the idea that the substance is received orally and then acts via the central nervous system has developed. It is believed that the brain then influences the behaviour of the worker directly, and indirectly influences ovarian development by endocrine methods, perhaps by neurosecretion (3).

More recently another theory regarding the mechanism of action of queen substance has been published. It deals with the physiology of the ovaries of the female worker bee. The corpora allata of the ovaries increases in volume during the first few days after removal of the queen from the hive.

Therefore the presence of the queen inhibits the growth of the worker bees' corpora allata. This suggests that the inhibition of ovary development by the pheromones present on the queen is brought about by an action upon the corpora allata which is thereby prevented from secreting the insect "gonadotrophin" hormone (17).

E. Miscellaneous Biological Properties of Queen Substance

(1) Effect on Swarming and Supersedure

Queen bee substance, or rather the lack of it, is responsible for the appearance of a new queen in both swarming and supersedure. In supersedure, the queen is producing too little queen substance and is consequently replaced by a younger queen. In swarming, although the queen's production of queen substance remains normal, the workers receive too little of it due to their vast numbers.

(2) Distinction Between Mated and Virgin Queen Bees

Worker bees are quickly able to distinguish between virgin and mated queen bees, even when the latter are deeply anaesthesized. This distinction is probably due to different types of queen substance caused by diet differences between virgin and mated laying queens (6).

In 1962, Pain et al. measured the relative amounts of queen substance present in the mandibular glands of queen

bees of various ages. They observed that older, mated queens produce far more queen substance than do virgin queens. Thus the distinction between the two queen types may be due only to the amount of queen substance produced rather than to the type (10).

(3) Chemical Mating Attractants in the Honeybee

Drones were found to be attracted to the ethereal extract of the mandibular glands of queen bees. After silica gel chromatography of the crude extract two active fractions were found. The drones were attracted to the fatty acid fraction and the phospholipid fraction. The fatty acid fraction was identified by gas chromatography as trans-9-oxo-2-decenoic acid. Synthetic queen substance was found to be attractive to drones in a concentration of 0.1 milligram per assay tube. However, the reconstituted mandibular gland complex was considerably more attractive to drones than either the fatty acid fraction or the phospholipid fraction (18). This suggests that there may be multiple mating attractants which have a synergistic action.

F. Isolation of Queen Substance

(1) Separation by Paper Chromatography

To date, two specific methods for the isolation of trans-9-oxo-2-decenoic acid from queen bees by paper

chromatography have been reported (8,16,19,20). In both cases, a crude alcoholic extract of whole queen bees was prepared and the acidic portion was separated by paper chromatography and isolated. Pure queen substance, m.p. 52.5-53°C., was obtained in each case by recrystallization after the chromatographic separation. The complete details of the methods and chromatographic systems used are given in reference (21).

(2) Separation by Gas Chromatography (8)

In 1962, Pain carried out a comparative analysis of the mandibular gland contents of queen and worker bees utilizing gas chromatography. The analysis of workers' mandibular glands at 200°C. showed the presence of an acid designated as trans-10-hydroxy-2-decenoic acid. This acid is also present in royal jelly. Sebacic acid was also found to be present in the workers' mandibular glands.

The analysis of queens' mandibular glands at 200°C. produced an entirely different result. It showed the presence of trans-9-oxo-2-decenoic acid, now commonly called queen substance. In addition sebacic and azelaic acids and methyl p-hydroxybenzoate were found present.

Gas chromatographic analysis of the queens' mandibular glands was repeated at a temperature of 150°C. The gas chromatogram showed the presence of at least five volatile substances. Two of these were identified as phenyl-

acetic and phenylpropionic acids. The other three substances were not identified (8).

FIGURE I

GAS CHROMATOGRAM ANALYSIS OF WORKERS' AND QUEENS' MANDIBULAR GLANDS (8)

Workers	Queens
A. At 200°C. (i) $HO-(CH_2)-(CH_2)_6-CH=CH-COOH$ Trans-10-hydroxy-2-decenoic acid (ii) $COOH-(CH_2)_8-COOH$ Sebacic acid	A. At 200°C. (i) $CH_3-\overset{O}{\overset{\|}{C}}-(CH_2)_5-CH=CH-COOH$ Trans-9-oxo-2-decenoic acid (ii) $COOH-(CH_2)_8-COOH$ Sebacic acid (iii) $COOH-(CH_2)_7-COOH$ Azelaic acid (iv) $HO-C_6H_4-COOCH_3$ Methyl p-hydroxybenzoate
	B. At 150°C. (i) Volatile substance A - not identified (ii) Volatile substance B - not identified (iii) Volatile substance C - not identified (iv) $C_6H_5CH_2CH_2COOH$ Phenylpropionic acid (v) $C_6H_5CH_2COOH$ Phenylacetic acid

G. Structural Elucidation of Queen Substance

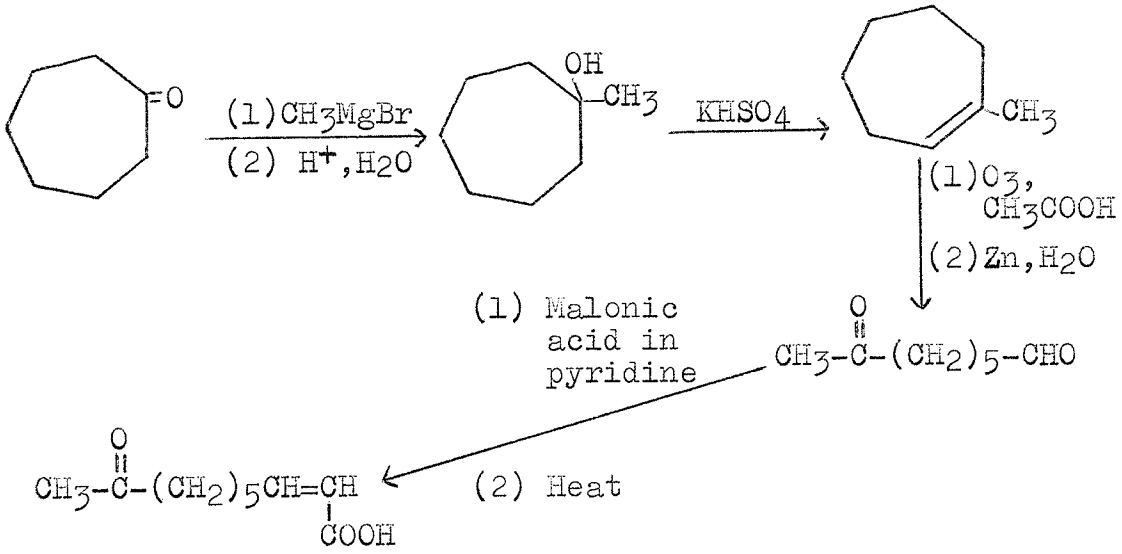
The structure of queen substance, which was isolated from the ethanolic extracts of queen bees, was found to be trans-9-oxo-2-decenoic acid. The structure was determined by the use of chemical evidence (20), infra-red (16) and ultra-violet absorption data (16,20). Synthetic trans-9-oxo-2-decenoic acid was found to be identical with the natural queen substance and hence the structure was proved conclusively (22).

H. Syntheses of Queen Bee Substance

Eight independent syntheses of this substance have so far been reported. Four of these are very similar in that they involve the preparation of 7-oxooctanal which is always converted to queen substance by the Doebner modification of the Knoevenagel condensation which utilizes malonic acid in pyridine or a pyridine-piperidine mixture. In each case the aldehyde is obtained in a different manner.

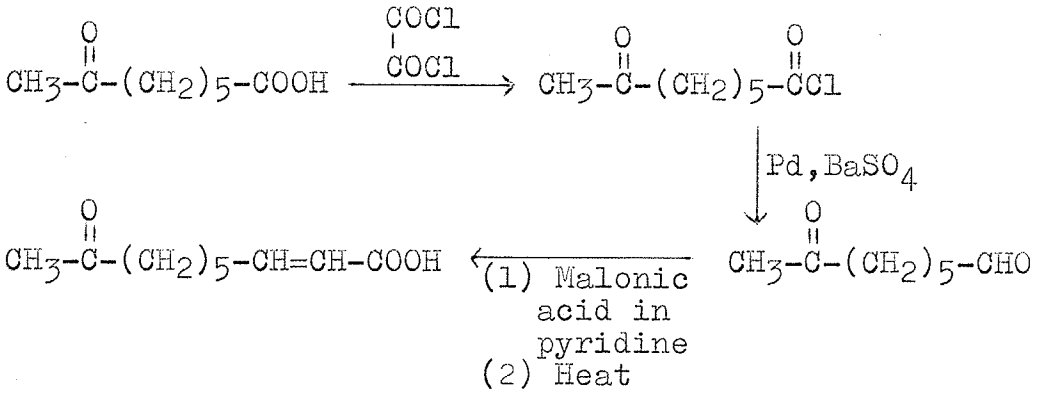
(1) Methods Involving a Knoevenagel Condensation

(a) Method of Barbier and Hugel (23)



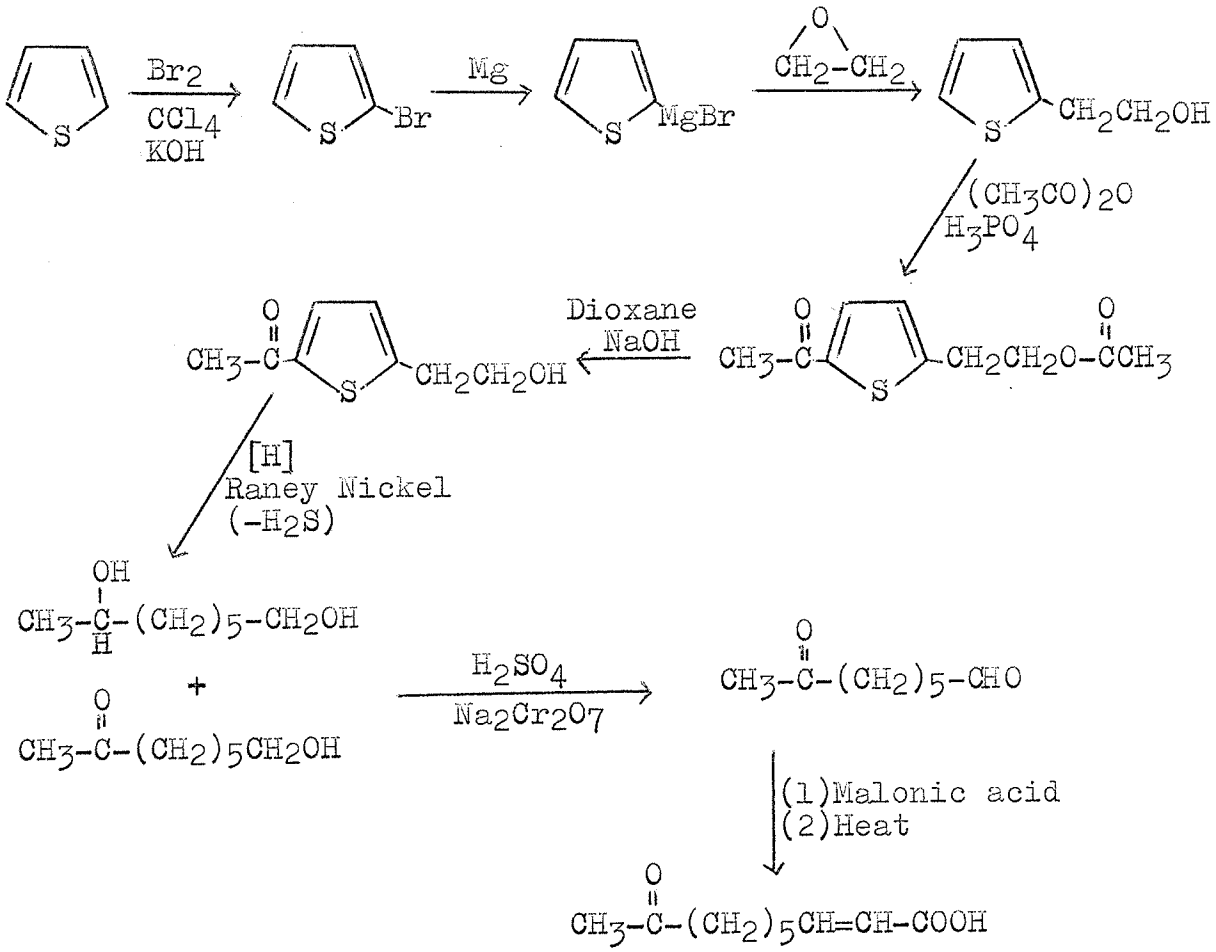
Cycloheptanone was treated with methylmagnesium bromide in a Grignard reaction to yield 1-methylcycloheptanol. This was dehydrated by refluxing with potassium hydrogen sulphate, yielding, according to the authors, pure 1-methyl-1-cycloheptene which was then ozonized to yield 7-oxooctanal. Malonic acid in anhydrous pyridine at 30°C. was added immediately to the 7-oxooctanal. The mixture was heated to 50°C. for fifteen minutes and then brought to a boil over a thirty minute period. Finally, the mixture was refluxed for one hour to yield synthetic queen substance.

(b) Method of Jaeger and Robinson (24,25)



7-Oxooctanoic acid dissolved in dry benzene under a nitrogen atmosphere was treated with oxalyl chloride. After heating and removing excess solvent and excess reagent in vacuo, the residue was fractionated to yield 7-oxooctanoyl chloride. This chemical was dissolved in dry xylene and was heated with 5% palladium-barium sulphate in a stream of hydrogen gas. The filtered solution was evaporated in vacuo and the residue fractionated to yield 7-oxooctanal. A solution of malonic acid was dissolved in a definite volume of pyridine. A similar solution of 7-oxooctanal was prepared. These two solutions were cooled to 0°C. and then mixed together. A small amount of piperidine was added and the mixture was heated to a temperature of 50°C. for forty-eight hours to yield trans-9-oxo-2-decenoic acid.

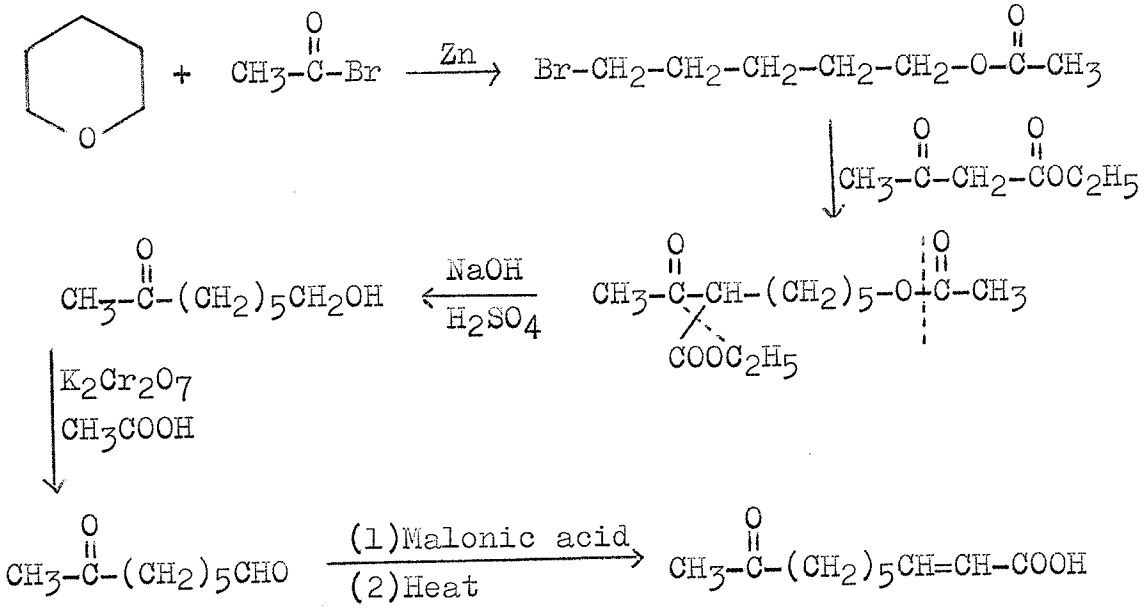
(c) Method of van der Plas and Persons (26)



A solution of bromine in carbon tetrachloride was added to a solution of thiophene in the same solvent. The mixture was kept at room temperature for twelve hours and the solvent was removed in vacuo. The residue was treated with potassium hydroxide. The solution was filtered and fractionally distilled to yield 2-bromothiophene. Dry magnesium turnings, and 2-bromothiophene were caused to react in dry ether. Ethylene oxide was added and the reaction mixture then purified to yield 2-(2-thienyl)-ethanol, which was

reacted with acetic anhydride and 85% phosphoric acid to yield 2-(5-acetyl-2-thienyl)ethyl acetate. The latter was treated with 2N aqueous sodium hydroxide in dioxane to yield 2-(5-acetyl-2-thienyl)ethanol. This substance was refluxed with freshly prepared Raney nickel in ethanol. The reaction was purified to yield a mixture of 1,7-octanediol and 7-oxo octan-1-ol. This mixture was oxidized to 7-oxooctanal by the use of sulphuric acid and potassium dichromate dissolved in water. The 7-oxooctanal was converted into queen substance as in reaction (b).

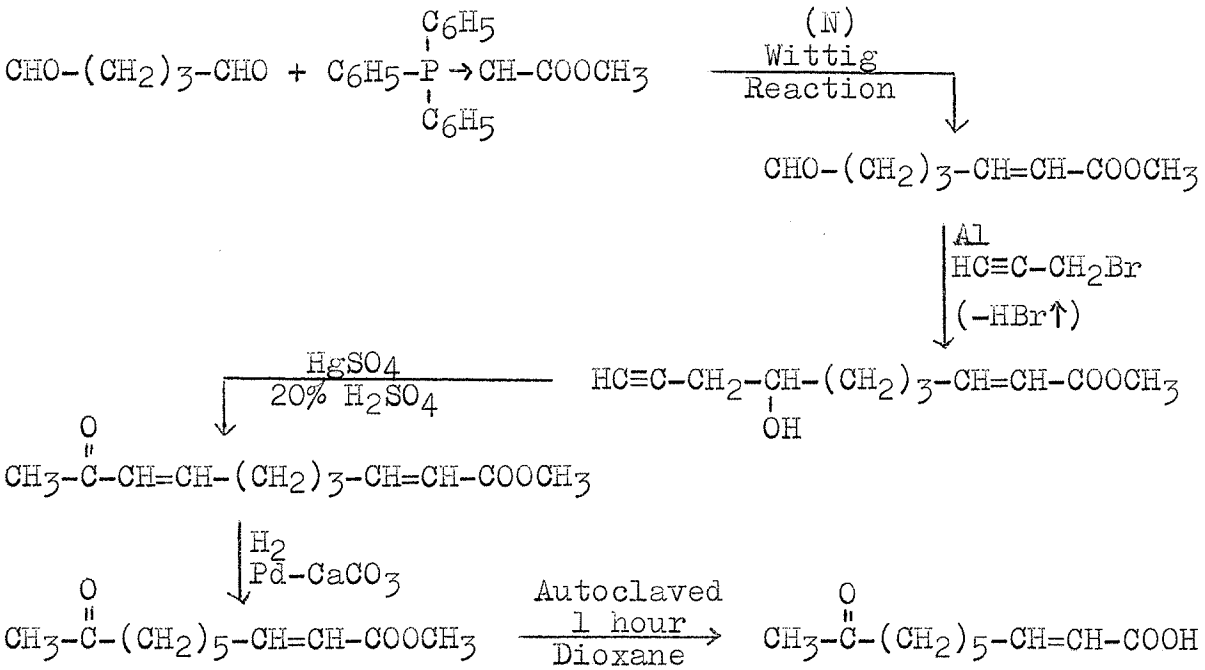
(d) Method of Oughton (27)



Acetyl bromide was added to tetrahydropyran and zinc dust and the mixture was heated to yield 5-bromoamyl acetate. Ethyl acetoacetate was added to a solution of sodium ethoxide. The 5-bromoamyl acetate was added to this solution followed by stirring and refluxing over a four hour period, yielding octan-1-ol-7-one on hydrolysis and purification of the reaction mixture. This substance was heated with powdered potassium dichromate in glacial acetic acid at 85-90°C. for one hour producing 7-oxooctanal. The 7-oxooctanal was then condensed in a pyridine-piperidine solution with malonic acid at 30°C. for twenty hours to produce queen substance.

(2) Other Methods of Synthesizing Queen Substance

(a) Method of Eiter (28,29)

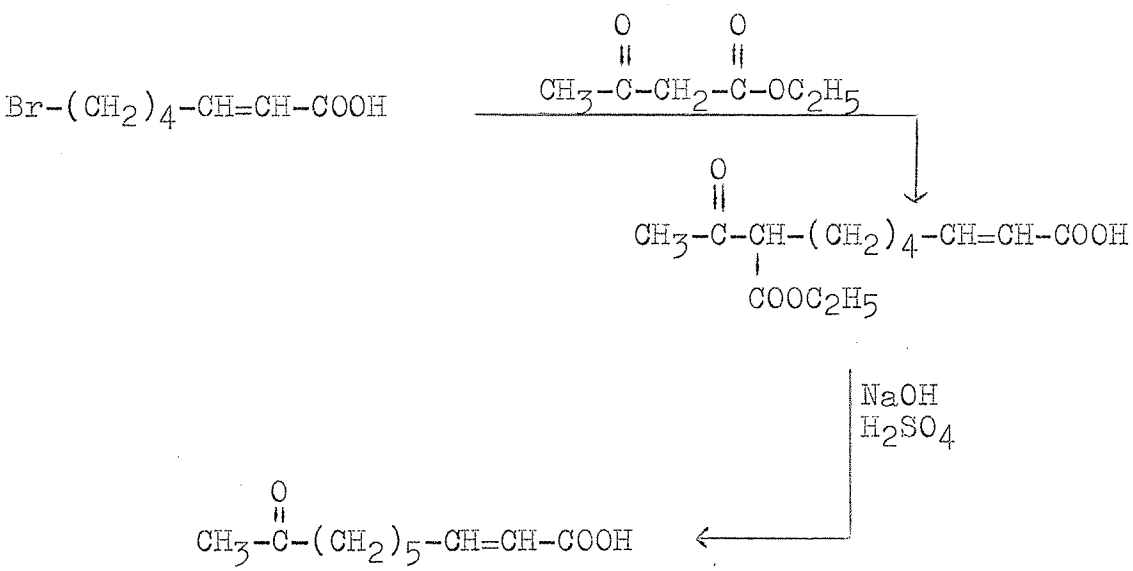


Freshly distilled glutardialdehyde was stirred in a nitrogen atmosphere with proportionate addition of dry powdered triphenylphophinecarbomethoxymethylene. The purified product was a mixture of methyl cis,trans-2-heptene-7-al-1-oate.

Aluminum flakes activated with mercuric chloride and a trace of iodine were stirred in anhydrous tetrahydrofuran in a water-free nitrogen atmosphere while propargyl bromide was carefully added dropwise. The methyl 2-heptene-7-al-1-oate was added with vigorous stirring. The mixture was decomposed and the resulting oil on distillation yielded methyl 7-hydroxydec-2-ene-9-yneoate. This was dissolved in dioxane and stirred in a nitrogen atmosphere under reflux with basic mercuric sulphate. This, while boiling, was treated with 20% sulphuric acid. The residue was chromatographed from 1:1 petroleum ether-benzene on an aluminum oxide column. Fractions 1-3 yielded methyl 9-oxo-2,7-decadienoate.

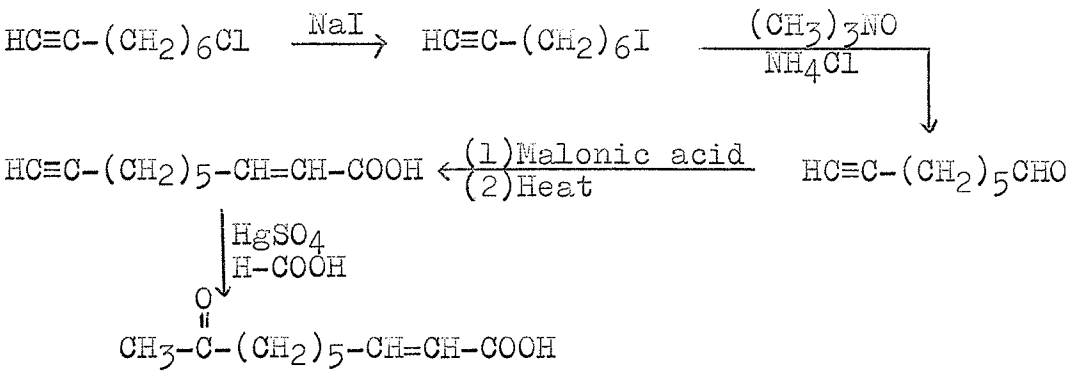
This substance was hydrogenated with 5% palladium-calcium carbonate mixture and the distilled product was methyl trans-9-oxo-2-decenoate. The methyl ester of queen substance was placed in dioxane and autoclaved with 2N aqueous sodium carbonate. The mixture was freed from dioxane in vacuo, diluted with water, and the methyl ester was removed by ether extraction. The aqueous layer was saturated with sodium chloride and extracted with ether. The product received from the ether extraction was trans-9-oxo-2-decenoic acid.

(b) Method of Kennedy, McCorkindale and Raphael (30)



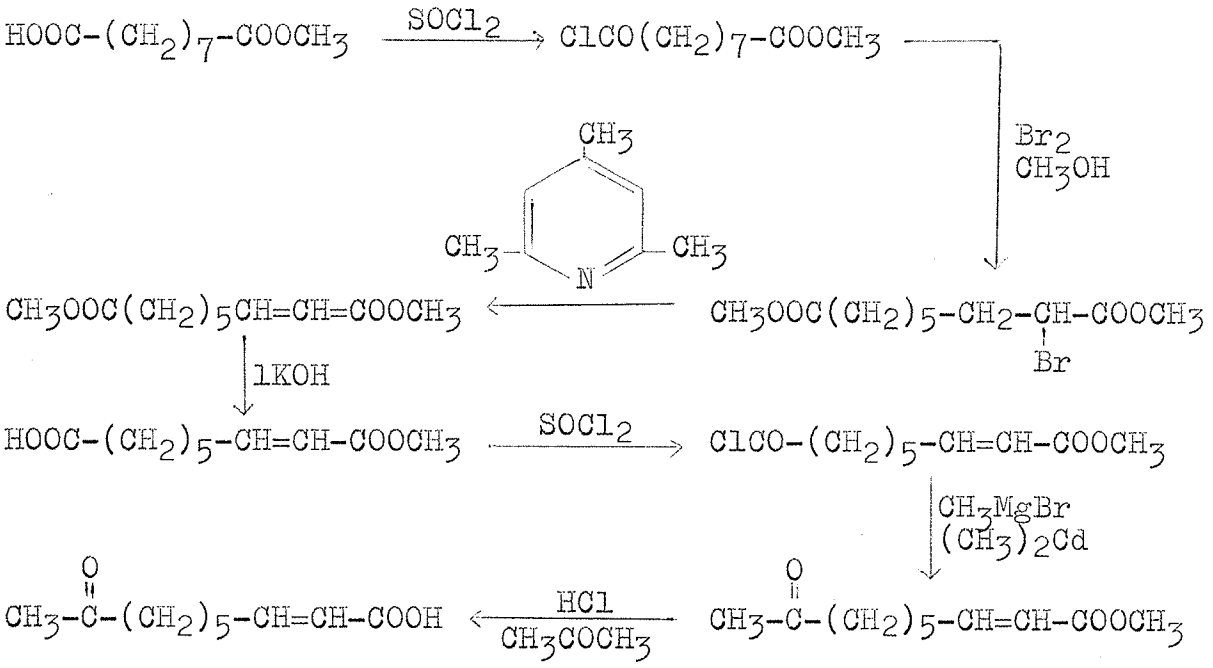
Trans-7-bromohept-2-enoic acid was dissolved in absolute ethanol and sodium (2 moles) was added to form sodium ethoxide. To this solution was added 1 mole of ethyl acetoacetate. The condensation mixture was diluted, acidified and concentrated to yield a yellow oil. This oil was treated with 5% sodium hydroxide solution and then sulphuric acid. The ether extract obtained was extracted with saturated aqueous sodium carbonate solution. This extract was acidified and extracted with ether to yield on ether removal, trans-9-oxo-2-decenoic acid.

(c) Method of Shishido et al. (31)



8-Chlorooct-1-yne and sodium iodide were refluxed in acetone. The filtered solution on evaporation yielded 8-iodooct-1-yne. This material was treated with trimethyl-nitroso in chloroform in an ice-bath. The mixture was decomposed and the organic layer washed and dried producing oct-7-yne-1-al. This liquid was added dropwise to malonic acid in pyridine. The reaction yielded trans-dec-2-ene-9-yneoic acid. Mercuric sulphate in 80% formic acid was stirred with the trans-dec-2-ene-9-yneoic acid. The waxy product received on evaporation of the ethereal extract of the above reaction mixture was recrystallized to yield synthetic queen substance.

(d) Method of Callow and Johnston (22,32)



While methyl hydrogen azelate and thionyl chloride were being refluxed bromine was added. The resulting mixture was cooled and poured into methanol. Addition of water and ether extraction yielded dimethyl bromoazelate. This product was refluxed in 2,4,6-collidine to give dimethyl trans-2-nonenedioate. The monomethyl ester was obtained by partial hydrolysis with potassium hydroxide solution. This monomethyl ester treated with thionyl chloride yielded methyl trans-2-nonenedioate acid chloride. Reaction of this product with methylmagnesium bromide and dimethyl cadmium produced methyl trans-9-oxo-2-decenoate. The methyl ester of queen substance was treated with concentrated hydrochloric

acid in acetone at room temperature yielding trans-9-oxo-2-decenoic acid.

I. Biological Activity of Synthetic Queen Substance (synthetic trans-9-oxo-2-decenoic acid)

(1) Attraction for Worker Bees

Pain has clearly demonstrated that neither cis nor trans-9-oxo-2-decenoic acid has any attraction for female worker bees (10).

(2) Inhibition of Ovary Development of Worker Bees

Butler (16) has found that synthetic queen substance and its methyl ester both inhibit the development of the ovaries of queenless worker bees, and that these substances inhibit ovarian growth to a lesser extent than the crude extract of the queen's head.

Pain has reported, however, that there is no ovary growth inhibition in workers in the presence of queen substance alone (33).

(3) Effect on Emergency Queen Cell Production or Queen Rearing

Butler (16) has shown that trans-9-oxo-2-decenoic acid and its methyl ester inhibit the construction of emergency queen cells. However, since the amount of synthetic queen

substance required is much higher than is ever present in the queen, these compounds inhibit to a lesser degree than the contents of the queen's mandibular gland.

Callow and Johnston (22) have reported that synthetic queen substance inhibits the construction of queen cells in a concentration of 0.13 microgram per bee.

Pain (10) has screened several compounds for the inhibition test described by Butler (16). She has found the test to be very specific, since only natural and synthetic Queen substance and its cis isomer were found to be active. Other similar fatty acids that were tested and found to be inactive were trans-8-oxo-2-nonenoic acid, trans-8-oxo-2-decenoic acid, trans-9-hydroxy-2-decenoic acid, 6-oxoheptanoic acid, 7-oxooctanoic acid, 8-oxononanoic acid, 9-oxodecanoic acid, trans-9-oxo-9-phenyl-2-decenoic acid and 9-oxo-2-decynoic acid.

Thus synthetic queen substance has been found to have no attraction for worker bees. However, it does inhibit the development of worker bees' ovaries and the production of emergency queen cells to some extent.

J. Metabolism of trans-9-oxo-2-decenoic acid by Worker Bees (34)

Trans-9-oxo-2-decenoic acid(2-C^{14}) was prepared in radioactive form and fed to worker bees. Three major fatty

acid metabolites were found in the gut and abdomen. These included 9-oxodecanoic acid, 9-hydroxydecanoic acid and trans-9-hydroxy-2-decenoic acid.

K. Biological Activity of Queen Bee Substance on Other Genera and Species

(1) Natural Queen Substance

(a) Effect on Crustaceans (in the prawn Leander serratus)

A single injection of queen substance produced a strong but incomplete inhibition of ovary development in the prawn. Similarly, in the worker honeybee, an extract of the prawn sinus gland appeared to inhibit ovary development. Thus a reciprocity of action has been observed between queen substance and the ovary inhibiting hormone of prawns from the sinus gland (16,35).

(2) Synthetic Queen Substance

(a) Effect on Mammals

Synthetic queen substance has been tested for physiological effect in various mammals. It has been found that this compound has no activity on the guinea-pig ileum, the rat uterus, the rat colon, cat blood pressure, cat respiration,

blood flow through the cat hind limb, nervous transmission through the superior cervical ganglion in cats or the permeability of blood vessels in the skin of guinea pigs and rabbits (16).

Furthermore, the substance has been shown to have no detectable effect on ovary activity or reproduction in female rats or mice, or on ovulation in the rabbit (36).

(b) Effect on the Ant (Myrmica rubra L.)

Synthetic trans-9-oxo-2-decenoic acid was tested for its influence on the brood growth in ants. In the experiments carried out it was found that queen substance did not prevent oviposition by worker ants. However, queen substance was found to stimulate brood growth in the ant in some cases (37).

(c) Effect on the House-fly (Musca domentica L.)

In 1962 Dr. J. K. Nayar (38) of the University of Manitoba Entomology Department showed that the continuous presence of synthetic queen substance in the fly insect body will cause inhibition of the ovary development in the house-fly. Under the experimental conditions newly-emerged flies were injected in the thorax with 5 microliters per fly of a dilute ethanolic solution of synthetic queen substance containing 2 milligrams per milliliter. It was demonstrated that higher concentrations were toxic and lower concentra-

tions did not show any appreciable effect on the inhibition of ovary development in the house-fly.

(d) Effect on the Aquatic Stages of the Yellow Fever Mosquito (Aedes aegypti L.)

Recently (39) Dr. Quraishi and Dr. Thorsteinson of the University of Manitoba have conducted a series of tests on mosquito larvae and pupae with synthetic queen substance and some similar fatty acids. Their results have shown that queen substance does not affect Aedes aegypti in its immature stages, except during the larval-pupal molt, when the mosquito larvae are raised in a 0.011 molar aqueous solution of trans-9-oxo-2-decenoic acid. If this is done during the larval-pupal molt a delayed toxic effect takes place during metamorphosis of the larvae causing them to sicken and die in the pupal stage.

Using the same test trans-2-decenoic acid, 2-oxodecanoic acid and 2-oxooctanoic acid are lethal to both aquatic stages of the mosquito (larval and pupal). Preliminary evidence indicates that both the unsaturated carbon linkage and the oxo group influence the modification of toxicity that is observed.

It is presumed that the toxic effects of queen substance in the mosquito are closely associated with metamorphosis, which is a change peculiar to insects. Hence, since queen bee substance has no demonstrated physiological effects

on mammals, there is an excellent possibility of utilizing queen substance or one of its more active analogs as a toxic agent toward insects such as the mosquito without causing any harm to mammals.

L. Aims of This Thesis

Queen substance and some of the other fatty acids tested have been found to exhibit interesting metabolic effects on mosquitoes. They cause deformity of pupae formation and introduce tanning of the larvae, which normally takes place in the pupal stage, although the larvae do not contract to form a normal pupa.

In the tests performed with the saturated fatty acids it was found that the toxicity increased with chain length from hexanoic to nonanoic acids. From nonanoic to dodecanoic it decreased to the extent that dodecanoic acid was nontoxic to the larvae. Thus the nine carbon acid was the most active of the series. In the light of this observation, the purpose of this thesis was to synthesize some α,β unsaturated keto fatty acids which were analogous to queen bee substance and which would be suitable for biological testing by Dr. Quraishi and Dr. Thorsteinson.

Of these acids only three had been synthesized when this work was begun. These included trans-9-oxo-2-decenoic acid, trans-8-oxo-2-nonenoic acid (23) and trans-8-oxo-2-

decenoic acid (23). Recently it has been reported that three more have been synthesized. These include 7-oxo-2-octenoic acid, 7-oxo-2-decenoic acid and 10-oxo-2-undecenoic acid (40,41). These compounds were prepared using the Barbier and Hugel synthesis (23).

EXPERIMENTAL PROCEDURES

All melting points were determined using a Thomas Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were determined on a Beckman IR-8 instrument using carbon disulphide solutions or potassium bromide pellets.

All carbon, hydrogen, and nitrogen microanalyses were determined by Organic Microanalyses (Dr. C. Daessle), Montreal, P.Q., Canada.

Refractive indices were determined with a Zeiss refractometer at the stated temperature.

Thin layer chromatography was carried out with silica gel G and silica gel GF (fluorescent) manufactured by Warner-Chilcott Laboratories and E. Merck respectively.

I. BARBIER AND HUGEL SYNTHESIS (23)

A. General Preparation of the 1-Alkyl-1-cycloalkanols

(1) A solution of alkyl halide in 250 ml. of anhydrous ether was added dropwise, with frequent shaking, to a mixture of magnesium filings in 100 ml. dry ether. The rate of addition was such that the reaction refluxed gently. After addition of the alkyl halide the reaction mixture was refluxed for fifteen minutes and cooled.

The cycloalkanone, dissolved in 100 ml. of anhydrous

ether, was added dropwise with frequent shaking to the prepared Grignard reagent at a rate sufficient to maintain a smooth reflux. The reaction mixture was refluxed for thirty minutes following the addition. The cooled mixture was poured slowly into a stirred mixture of 750 g. crushed ice and 25 ml. concentrated hydrochloric acid. The ethereal layer was separated and the aqueous layer extracted with ether. The combined ether solutions were washed with 10% hydrochloric acid, water, 10% aqueous sodium carbonate solution, and finally with water until neutral. The ether solution was dried (anhydrous sodium sulphate) and the solvent was removed. Distillation of the crude product yielded the 1-alkyl-1-cycloalkanol.

Compounds (A-1)-(A-7) which appear in the following table (Table I) were prepared by this procedure.

TABLE I

REACTANTS, YIELDS, AND PHYSICAL PROPERTIES OF THE 1-ALKYL-1-CYCLOALKANOLS

Compound	Name of Product	Magnesium mol. (g.)	Alkyl Halide mol. (g.)	Cycloalkanone mol. (g.)	Yield % (g.)	Boiling Point °C./mm.	Refractive Index
(A-1)	1-Methyl-1-cycloheptanol	1.0 (24.4)	Methyl iodide 1.0 (142.0)	Cycloheptanone 0.89 (100.0)	85.6 (97.8)	76-80/15	n_D^{23} 1.4697
(A-2)	1-Methyl-1-cyclohexanol	1.0 (24.4)	Methyl iodide 1.0 (142.0)	Cyclohexanone 0.58 (57.2)	92.4 (61.5)	56-65/15-20	$n_D^{23.5}$ 1.4609
(A-3)	1-Ethyl-1-cyclohexanol	1.0 (24.4)	Ethyl iodide 1.0 (156.0)	Cyclohexanone 0.58 (57.2)	76.5 (57.2)	61-63/10	n_D^{23} 1.4639
(A-4)	1-Methyl-1-cyclopentanol	1.0 (24.4)	Methyl iodide 1.0 (142.0)	Cyclopentanone 0.58 (49.0)	86.9 (50.7)	44-50/18 m.p. 26.5-27.5	
(A-5)	1-Ethyl-1-cyclopentanol	1.0 (24.4)	Ethyl iodide 1.0 (156.0)	Cyclopentanone 0.58 (49.0)	36.8[a] (24.5)	59-60/11	n_D^{23} 1.4542
(A-6)	2-Chloro-1-ethyl-1-cyclohexanol	1.0 (24.4)	Ethyl iodide 1.0 (156.0)	2-Chlorocyclohexanone 0.57 (75.8)	0	Synthesis unsuccessful	
(A-7)	1-Propyl-1-cyclopentanol	1.0 (24.4)	Propyl iodide 1.0 (170.0)	Cyclopentanone 0.58 (49.0)	44.6[b] (33.3)	68-71/15	$n_D^{25.5}$ 1.4518

(a) The breakage of a funnel during the reaction work up was responsible for the low yield.

(b) Due to the low yield received from this reaction, subsequent propyl, butyl, and pentyl Grignard reagents were prepared in a larger excess than generally used in an attempt to increase the yields. It is well known that an increase in alkyl halide chain length makes the preparation of a Grignard reagent more difficult (42).

(2) The above procedure was modified slightly for compounds (A-8) to (A-12) in Table II. A mechanical stirrer was used to stir the reaction contents continuously and the times required for the reactions were reduced substantially. The remaining alcohols (A-8)-(A-20) were not distilled but were dehydrated in the crude form. This procedure was first used for 1-methyl-1-cyclooctanol which began to spontaneously dehydrate during attempted distillation. The dehydration of the crude alcohol worked so successfully and saved so much time that this procedure was subsequently used for the remaining alcohols.

(3) A further time-saving modification was used in the synthesis of crude alcohols (A-12)-(A-20). The reaction flask was immersed in a cold water bath (15-20°C.) throughout the reaction. This decreased the time necessary for the reactions by an hour on the average (from four hours to three hours). This increase in rate was due to the fact that the alkyl halides and the cycloalkanones could be added more quickly since the water bath decreased the amount of refluxing.

TABLE II

REACTANTS, YIELDS, AND PROCEDURE USED FOR THE PREPARATION OF THE REMAINING 1-ALKYL-1-CYCLOALKANOLS

Compound	Name of Product	Magnesium mol. (g.)	Alkyl Halide mol. (g.)	Cycloalkanone mol. (g.)	Yield % (g.)
(A-8)	1-Methyl-1-cyclo-octanol [a]	0.98 (24.0)	Methyl iodide 1.13 (160.0)	Cyclooctanone 0.78 (100.0)	86.6 (97.6)
(A-9)	1-Ethyl-1-cyclo-octanol [a]	0.98 (24.0)	Ethyl iodide 1.12 (175.0)	Cyclooctanone 0.69 (87.4)	89.6 (97.0)
(A-10)	1-Ethyl-1-cyclo-heptanol [a]	0.98 (24.0)	Ethyl iodide 1.02 (160.0)	Cycloheptanone 0.89 (100.0)	89.6 (113.6)
(A-11)	1-Propyl-1-cyclo-heptanol [a]	2.00 (48.6)	Propyl iodide 2.00 (340.0)	Cyclpheptanone 0.89 (100.0)	82.8 (115.3)
(A-12)	1-Ethyl-1-cyclo-hexanol [a]	1.00 (24.3)	Ethyl bromide 1.31 (143.0)	Cyclohexanone 1.02 (100.0)	82.8 (106.2)
(A-13)	1-Propyl-1-cyclo-hexanol [a]	2.00 (48.6)	Propyl iodide 2.00 (340.0)	Cyclohexanone 1.08 (106.0)	75.6 (116.2)
(A-14)	1-Butyl-1-cyclo-hexanol [a]	2.00 (48.6)	Butyl iodide 2.00 (370.0)	Cyclohexanone 1.10 (108.1)	79.9 (137.6)
(A-15)	1-Pentyl-1-cyclo-hexanol [a]	2.00 (48.6)	Pentyl iodide 2.00 (400.0)	Cyclohexanone 1.23 (120.4)	72.8 (152.2)
(A-16)	1-Methyl-1-cyclo-pentanol [a]	1.50 (36.5)	Methyl iodide 1.50 (213.0)	Cyclopentanone 1.43 (120.0)	64.4 (92.0)
(A-17)	1-Ethyl-1-cyclo-pentanol [a]	1.50 (36.5)	Ethyl iodide 1.65 (257.0)	Cyclopentanone 1.43 (120.0)	70.5 (114.9)
(A-18)	1-Propyl-1-cyclo-pentanol [a]	2.00 (48.6)	Propyl iodide 2.00 (340.0)	Cyclopentanone 1.43 (120.0)	57.4 (105.0)
(A-19)	1-Butyl-1-cyclo-pentanol [a]	2.00 (48.6)	Butyl iodide 2.00 (370.0)	Cyclopentanone 1.43 (120.0)	75.0 (152.3)
(A-20)	1-Pentyl-1-cyclopentanol [a][b]	2.00 (48.6)	Pentyl iodide 2.00 (400.0)	Cyclopentanone 1.43 (120.0)	82.1 (183.0)

(a) Since larger molar quantities were being used a greater volume of anhydrous ether was used in these reactions. The alkyl halide was dissolved in 250 ml. ether and the cycloalkanone in 200 ml. ether. The magnesium was placed in 250 ml. ether in the reaction flask.

(b) The crude 1-pentyl-1-cyclopentanol was later found to contain n-decane which had been formed by the coupling of the pentylmagnesium-iodide Grignard reagent (43).

B. General Preparation of the 1-Alkyl-1-cycloalkenes

(1) Dehydration with Potassium Bisulphate or Pyrosulphate

The 1-alkyl-1-cycloalkanol was refluxed with freshly pulverized potassium bisulphate or potassium pyrosulphate for thirty minutes according to the method of Barbier and Hugel (23). Following this the reaction mixture was distilled at which time an azeotropic mixture of water and alkene was usually obtained. The distillate was extracted with ether and the resulting ethereal solution was dried (anhydrous sodium sulphate). Removal of solvent from the filtered solution and distillation of the residual liquid yielded the 1-alkyl-1-cycloalkene. Compounds (B-1) and (B-2) in Table III were prepared by this method.

Due to the low yields of product obtained in these first two reactions the procedure was altered. Following distillation of the product from the reaction vessel a gummy mass of potassium pyrosulphate was left behind. This was extracted thoroughly with ether and the combined extracts were bulked with the ether soluble distillate of the reaction mixture. By distillation of the dried extract, as described above, alkenes (B-3)-(B-6) were obtained.

(2) Dehydration with Iodine

Since dehydration with potassium bisulphate or pyro-

sulphate produced low and variable yields of the 1-alkyl-1-cycloalkenes the remaining 1-alkyl-1-cycloalkanols were dehydrated in the presence of a trace of iodine according to the procedure described by Diaper (44). The crude alcohol was used in each case and the alkene was redistilled using a 36 cm. by 2 cm. Dufton fractionating column which was insulated with a heating tape.

The syntheses were carried out in the apparatus illustrated in Figure II which was designed to allow the removal of water formed during the reaction. The 1-alkyl-1-cycloalkanol was refluxed with a trace of iodine for forty minutes to three hours, depending upon the rate of dehydration. When no more water was collected in the side arm the alkene was allowed to distill and was collected. This distillate was dissolved in ether and dried (anhydrous sodium sulphate). The ether was removed and the product fractionally distilled until pure. All of the crude products except compounds (B-15)-(B-18) were distilled in the presence of sodium metal to remove moisture. These alkenes had been dried (anhydrous sodium sulphate) for at least one week and showed no traces of moisture. Their physical characteristics agreed well with those reported after the second distillation.

Compounds (B-7)-(B-19) were prepared by this procedure.

FIGURE II

DIAGRAM OF APPARATUS DESIGNED TO ALLOW WATER REMOVAL WHILE DEHYDRATION REACTIONS WERE PROCEEDING

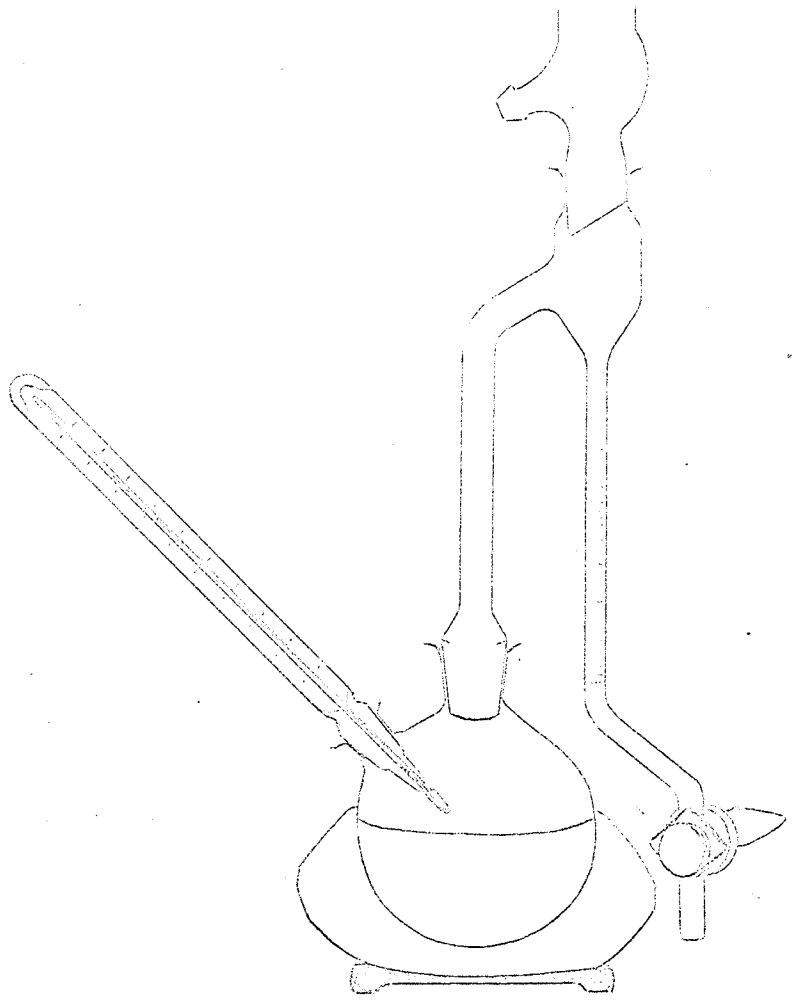


TABLE III (CONT'D)

Compound	Name of Product	1-Alkyl-1-cycloalkanol mol. (g.)	Catalyst mol. (g.)	Yield % (g.)	Reflux Time Hours	Reported b.p., °C./mm.	Observed b.p., °C.	Reported Refractive Index	Observed Refractive Index
(B-16)	1-Ethyl-1-cyclopentene[b]	1-Ethyl-1-cyclopentanol 1.01 (114.9)	Iodine 0.00197 (0.5)	55.8 (54.0)	1.5	106.5-107 (47)	103-6.5	n_D^{20} 1.4429 (47)	$n_D^{30.5}$ 1.4388
(B-17)	1-Propyl-1-cyclopentene[b]	1-Propyl-1-cycloptenanol 0.819 (105.0)	Iodine 0.00197 (0.5)	48.9 (44.1)	2.0	131.1 (55)	123-8	n_D^{20} 1.4448 (55)	$n_D^{25.5}$ 1.4427
(B-18)	1-Butyl-1-cyclopentene[b]	1-Butyl-1-cyclopentanol 1.07 (152.3)	Iodine 0.00197 (0.5)	38.5 (51.2)	2.0	157.5-8 (47)	152-4	n_D^{20} 1.4488 (47)	n_D^{29} 1.4449
(B-19)	1-Pentyl-1-cyclopentene[b][e]	1-Pentyl-1-cyclopentanol 1.17 (183.0)	Iodine 0.00197 (0.5)	0	2.0	179 (56)		n_D^{20} 1.4513 (56)	

(a) Potassium pyrosulphate (Fisher Scientific Co.), which is a mixture of potassium bisulphate and potassium pyrosulphate, was used as the catalyst. The molar concentration was calculated by using the molecular weight of potassium sulphate for the reagent.

(b) The alcohols in these reactions were not distilled prior to distillation.

(c) Since the physical characteristics of this compound have not been reported in the literature only the constant boiling fraction of the distillate was used. This compound was previously synthesized by Khromov *et al*. (57)

(d) On the distillation prior to the final redistillation the physical characteristics of 1-pentyl-1-cyclopentene were b.p. 197-9°C., n_D^{24} 1.4591. The boiling point of the compound during the final distillation was 186-7°C., [$n_D^{29.5}$ 1.4581]. The refractive index remained constant (if the differences in temperature of the two determinations are considered) and hence the compound was used for ozonolysis although the boiling point recorded on the final distillation was much lower than the reported.

(e) Upon fractional distillation of the reaction product a mixture of n-decane (b.p. 174°C., n_D^{20} 1.4120) (58) formed as a by-product by the coupling of the Grignard reagent (43), and 1-pentyl-1-cyclopentene was obtained.

TABLE III (CONT'D)

Compound	Name of Product	1-Alkyl-1-cycloalkanol mol. (g.)	Catalyst mol. (g.)	Yield % (g.)	Reflux Time Hours	Reported b.p., °C./mm.	Observed b.p., °C.	Reported Refractive Index	Observed Refractive Index
(B-8)	1-Ethyl-1-cyclooctene[b]	1-Ethyl-1-cyclooctanol 0.621 (97.0)	Iodine 0.00197 (0.5)	55.7 (47.8)	3.0	186.9 (51)	182-4	n_D^{25} 1.4720[51]	n_D^{24} 1.4713
(B-9)	1-Ethyl-1-cycloheptene[b]	1-Ethyl-1-cycloheptanol 0.834 (118.6)	Iodine 0.00197 (0.5)	71.3 (73.9)	0.7	159-61/750[52]	162-4	n_D^{20} 1.4646[52]	n_D^{25} 1.4626
(B-10)	1-Propyl-1-cycloheptene[b][c]	1-Propyl-1-cycloheptanol 0.738 (115.3)	Iodine 0.00197 (0.5)	26.5 (27.0)	3.0		174.5		n_D^{25} 1.4629
(B-11)	1-Ethyl-1-cyclohexene[b]	1-Ethyl-1-cyclohexanol 0.828 (106.2)	Iodine 0.00197 (0.5)	58.1 (53.0)	2.0	136.3 (49)	132-4	n_D^{20} 1.4558[49]	n_D^{25} 1.4551
(B-12)	1-Propyl-1-cyclohexene[b]	1-Propyl-1-cyclohexanol 0.817 (116.2)	Iodine 0.00197 (0.5)	60.8 (67.1)	2.0	156 (53)	146-8	n_D^{20} 1.4577[53]	n_D^{25} 1.4566
(B-13)	1-Butyl-1-cyclohexene[b]	1-Butyl-1-cyclohexanol 0.881 (137.6)	Iodine 0.00197 (0.5)	37.8 (46.0)	2.0	178-9 (54)	173-4	n_D^{20} 1.4568[54]	n_D^{26} 1.4565
(B-14)	1-Pentyl-1-cyclohexene[b][d]	1-Pentyl-1-cyclohexanol 0.894 (152.2)	Iodine 0.00197 (0.5)	47.8 (65.1)	2.0	203.4-5 (53)	186-7	n_D^{20} 1.4605[53]	$n_D^{29.5}$ 1.4581
(B-15)	1-Methyl-1-cyclopentene[b]	1-Methyl-1-cyclopentanol 0.919 (92.0)	Iodine 0.00197 (0.5)	35.0 (26.4)	2.0	74.5-6.5 (46)	72-3	n_D^{20} 1.4300[46]	$n_D^{23.5}$ 1.4299

TABLE III

REACTANTS, YIELDS, AND PHYSICAL PROPERTIES (FOUND AND REPORTED) OF THE 1-ALKYL-1-CYCLOALKENES

Compound	Name of Product	1-Alkyl-1-cycloalkanol mol. (g.)	Catalyst mol. (g.)	Yield % (g.)	Reflux Time Hours	Reported b.p., °C./mm.	Observed b.p., °C.	Reported Refractive Index	Observed Refractive Index
(B-1)	1-Methyl-1-cycloheptene	1-Methyl-1-cycloheptanol 0.176 (22.6)	Potassium Bisulphate 0.0734 (10.0)	54.6 (10.6)	0.5	133.5/720 (45)	134-6	n_D^{20} 1.4575 (45)	n_D^{24} 1.4566
(B-2)	1-Methyl-1-cyclopentene	1-Methyl-1-cyclopentanol 0.506 (50.7)	Potassium Bisulphate 0.220 (30.0)	20.4 (8.5)	0.5	74.5-6.5 (46)	71-2	n_D^{20} 1.4300 (46)	$N_D^{23.5}$ 1.4290
(B-3)	1-Methyl-1-(a) cycloheptene	1-Methyl-1-cycloheptanol 0.702 (90.0)	Potassium Pyrosulphate 0.441 (60.0)	71.3 (55.2)	0.5	133.5/720 (45)	135-7	n_D^{20} 1.4575 (45)	n_D^{23} 1.4578
(B-4)	1-Ethyl-1-(a) cyclopentene	1-Ethyl-1-cyclopentanol 0.215 (24.5)	Potassium Pyrosulphate 0.110 (15.0)	37.3 (7.7)	0.5	106.5-7 (47)	106-8	n_d^{20} 1.4429 (47)	$n_D^{22.4}$ 1.4421
(B-5)	1-Methyl-1-(a) cyclohexene	1-Methyl-1-cyclohexanol 0.534 (61.0)	Potassium Pyrosulphate 0.294 (40.0)	37.8 (19.4)	0.5	110.3-110.4 (48)	103-7	n_D^{20} 1.4510 (48)	n_D^{24} 1.4491
(B-6)	1-Ethyl-1-(a) cyclohexene	1-Ethyl-1-cyclohexanol 0.446 (57.2)	Potassium Pyrosulphate 0.257 (35.0)	41.9 (20.6)	0.5	136.3 (49)	123-4	n_D^{25} 1.4558 (49)	n_D^{24} 1.4569
(B-7)	1-Methyl-1-(b) cyclooctene	1-Methyl-1-cyclooctanol 0.686 (97.6)	Iodine 0.00394 (1.0)	62.4 (53.2)	1.0	158-60 (50)	160	$n_D^{13.5}$ 1.4720 (50)	n_D^{24} 1.4688

(3) Additional Dehydration and Closely Related Experiments

(a) Preparation of 1-Methyl-1-cyclopentene

Magnesium filings (1.0 mole), methyl iodide (1.0 mole) and cyclopentanone (0.58 mole) were reacted as described in procedure A (1). On attempted distillation of the alcohol, dehydration occurred yielding two alkene fractions. Both gave a positive unsaturation test with tetranitromethane reagent. The first fraction (7.20 g., b.p. 61-2°C., $n_D^{24.5}$ 1.4097) was not identified. The second fraction (11.91 g., b.p. 74°C., $n_D^{24.5}$ 1.4299) was identified as 1-methyl-1-cyclopentene. Since two products and a low yield were obtained and the alcohol dehydrated during distillation, a new method of synthesis was adopted. This is the reason that alkenes (B-7)-(B-19) in Table III were prepared by iodine dehydration.

(b) Separation of n-Decane and 1-Pentyl-1-cyclopentene

Iodine catalyzed dehydration of 1-pentyl-1-cyclopentanol gave rise to a mixture of n-decane (b.p. 170°C., n_D^{20} 1.4120)(58) and 1-pentyl-1-cyclopentene (b.p. 179°C., n_D^{20} 1.4513)(56). This mixture could not be separated by distillation due to the similarities in boiling points of the two constituents. Therefore the distillate (121.0 g.) was

dissolved in chloroform (150 ml.) and bromine (60 ml.) in chloroform (150 ml.) was added dropwise to the stirred hydrocarbon solution until excess bromine was present. The reaction was stirred at room temperature for ninety minutes and the chloroform removed by distillation. During the distillation of n-decane from the reaction mixture the dibromo derivative of 1-pentyl-1-cyclopentene decomposed to a black gummy tar. Consequently this derivative was not distilled with zinc to regenerate the alkene. The experiment was not repeated.

C. Ozonolysis of the 1-Alkyl-1-cycloalkenes

The ozonolysis experiments were carried out using a Gallenkamp GE-150 ozonizer. This instrument is designed to provide a continuous supply of ozonized oxygen (up to 170 ml./minute) containing 6-7% ozone. The rate of oxygen flow into the ozonizer was regulated at less than one litre/hour.

(1) Preliminary Ozonolysis Experiments

(a) Quantitative Determination of Ozone Formation

After the ozonizer had been allowed to operate for fifteen minutes the oxygen-ozone mixture produced was bubbled through a solution of 5% aqueous potassium iodide (100 ml.) for fifteen minutes. The solution was acidified by the addition of 5% sulphuric acid (35 ml.) and the liberated iodine

titrated with decinormal sodium thiosulphate solution.

After numerous trials the ozonizer was found to deliver from 0.0286 to 0.209 moles of ozone per hour. The average value of all trials was found to be 0.097 moles of ozone per hour.

(b) Ozonolysis of 1-Methyl-1-cycloheptene According to the Method of Barbier and Hugel (23)

(i) 1-Methyl-1-cycloheptene (3.00 g., 0.0272 mole) dissolved in glacial acetic acid was ozonized for two hours forty-five minutes. The ozonolysis system consisted of the reaction vessel, two solvent traps (25 ml.) and a trap containing 5% aqueous potassium iodide (150 ml.) in series. The oxygen gas was passed through concentrated sulphuric acid and a mechanical trap containing glass wool before introduction into the ozonizer. From the ozonizer the ozone-oxygen mixture was passed through an empty trap and then into the reaction vessel. The completion of the reaction was indicated by the potassium iodide trap solution which developed a definite brown colour due to the liberation of iodine. The reaction vessel and first acetic acid trap were cooled in an ice bath (0-5°C.) during the reaction.

On completion of the reaction the acetic acid trap solutions were bulked with the reaction mixture. Ether (50 ml.) was added and the mixture was cooled to 0°C. in an ice bath with stirring. Powdered zinc and water (0.05 ml.) were

added alternately with stirring until one drop of mixture did not cause colour formation on starch-iodide test paper. Water (80 ml.) was added and the ethereal layer was separated. The aqueous layer was extracted with ether and the combined ethereal extracts washed thoroughly with water to remove the acetic acid. The extracts were further washed with 10% aqueous sodium bicarbonate solution and finally with water until neutral. After drying the ethereal solution over anhydrous sodium sulphate, removal of the ether produced 1.47 grams of crude 7-oxooctanal which gave a positive aldehyde test with Schiff's reagent.

The above procedure was repeated and on the second trial three hours twenty minutes were required for the ozonolysis. Crude 7-oxooctanal (1.71 g.) was obtained.

(ii) 1-Methyl-1-cycloheptene (10.0g., 0.091 mole) was dissolved in glacial acetic acid (25 ml.) and ozonized as described in (i) above. Three such trials were carried out. They required ten hours forty-five minutes, twelve hours fifteen minutes, and eleven hours five minutes respectively. The solvent traps and reaction mixtures of all three trials were bulked. Ether (150 ml.) was added and the reaction mixture was hydrolyzed as described previously. On ether removal 17.6 grams of residue were obtained.

The residue was fractionally distilled under reduced pressure. Two distillate fractions were obtained plus a large quantity of residue, which would not distill. This residue

had been formed during the distillation and was believed to be a polymer.

Fraction I of the distillate weighed 2.51 grams (b.p. 70-4°C./13 mm.). It showed a negative Schiff's test, denoting the absence of an aldehyde. It had a sweet, slightly sickening odour identical to that of cycloheptanone. The reported boiling point of cycloheptanone (179-81°C./760 mm.) (59) agrees fairly well with the observed. Cycloheptanone dissolved in carbon disulphide showed peaks at 3.41 μ (methylene) and 5.90 μ (ketone carbonyl) (64). The I.R. spectrum of the unknown was identical in all respects to that of cycloheptanone.

Fraction II weighing 3.60 grams (b.p. 118-30°C./13 mm.) gave a strongly positive Schiff's test. The reported boiling point of 7-oxooctanal is 62-4°C./0.2 mm. (24). The reported infrared spectrum had peaks at 3.65 μ (aldehydic C-H) and 5.79 μ (aldehydic and ketonic carbonyl) (24). The infrared spectrum of this substance in carbon disulphide showed absorption bands at 3.70 μ and 5.86 μ. The slight differences in wavelength were believed to be due to the different instruments used.

While distillation was proceeding a rapid and prolonged rise in temperature took place, until no more distillate was obtained. Upon cooling a gelatinous semisolid was present in the distillation flask. This undistilled product was believed to be some sort of polymer.

(c) Ozonolysis of 1-Methyl-1-cyclohexene in Acetic Acid

1-Methyl-1-cyclohexene (10.0 g., 0.10 mole) was dissolved in glacial acetic acid (25 ml.) and was ozonized to completion (twenty-four hours). The reaction was hydrolyzed as explained in (b) above. The sodium bicarbonate washings of the ethereal solution were acidified with concentrated hydrochloric acid. The resulting acidic mixture was extracted with ether, and the ethereal extracts were washed with water until neutral. The ethereal solution was dried over anhydrous sodium sulphate and the solvent was removed to yield 3.10 grams of acidic substance, probably 6-oxoheptanoic acid.

The neutral fraction obtained from the hydrolysis of the ozonide in the usual manner consisted of 2.25 grams of crude 6-oxoheptanal.

This experiment indicated that oxidation was occurring when the hydrolysis was carried out due to the fact that all of the hydrogen peroxide formed was not being destroyed by the metallic zinc. In several of the preliminary ozonolysis experiments the acetic acid in the reaction vessel had solidified from the cooling supplied from the ice bath. The subsequent ozonolysis reactions were carried out in chloroform using a different method of hydrolysis in an attempt to overcome these difficulties.

(d) Ozonolysis of 1-Methyl-1-cyclohexene in Chloroform

1-Methyl-1-cyclohexene (3.0 g., 0.03 mole) was dissolved in chloroform (35 ml.). Ozonolysis was carried out for eight hours. When reaction was complete the chloroform was removed under reduced pressure with extreme care in the fume hood. The ozonide was dissolved in glacial acetic acid and decomposed by the alternate addition of small amounts of zinc and water as described previously in (b). Working up the reaction mixture as described in (c) yielded 1.10 grams of crude 6-oxoheptanal and 1.20 grams of acidic product, which was probably 6-oxoheptanoic acid.

Since on hydrolysis of the ozonide utilizing zinc and water, more than half of the ozonide was being converted to the corresponding acid the remaining ozonides were hydrolyzed using a variation of the Whitmore and Church procedure (60).

(e) Ozonolysis of 1-Methyl-1-cyclohexene Utilizing the Hydrolysis Method of Whitmore and Church (60)

1-Methyl-1-cyclohexene (25.0 g., 0.260 mole) was dissolved in chloroform (50 ml.) and was ozonized to completion (22.5 hours). The bulked reaction mixture and trap solutions were added dropwise to a stirred boiling mixture of 400 ml. of water and 17.0 grams of zinc (0.260 mole) with traces of hydroquinone and silver nitrate. Nitrogen gas was passed

through the reaction flask continuously. The chloroform distilled over as the ozonide solution was added. When all of the chloroform solution of the ozonide had been added the reaction flask was allowed to cool. The contents were extracted with chloroform. The chloroform extract was dried (anhydrous sodium sulphate) and the solvent was removed to yield 25.1 grams of crude 6-oxoheptanal, which gave a positive Schiff's test immediately.

The crude aldehyde was fractionally distilled in a nitrogen atmosphere under reduced pressure. Three fractions were obtained.

Fraction	Weight(g.)	b.p.°C./pressure(mm.)	Refractive Index
I	1.65	52-60/0.05	n_D^{23} 1.4516
II	2.40	60-64/0.05	n_D^{23} 1.4443
III	1.50	64-190/0.05	n_D^{25} 1.4750

As well as these fractions (I-III) a residue was left in the distillation flask which refused to distill (15.0 g.). It is believed that the rapid rise in temperature and refractive index in fraction III is due to polymerization.

Due to the results obtained during the attempted distillation of 7-oxooctanal and 6-oxoheptanal it was decided to condense the crude aldehydes with malonic acid rather than to distill them since this led to an undesired polymerization-like side reaction.

(2) General Procedure for the Preparation of the Oxoaldehydes by Ozonolysis of the 1-Alkyl-1-cycloalkenes

(a) The 1-alkyl-1-cycloalkene (25.0 g.) was dissolved in chloroform (100 ml.). An ozone-oxygen gas mixture was bubbled through the system until ozonolysis was complete. This system was the same one used in (C)(1)(b) except that a trap containing light liquid petroleum (150 ml.) was inserted between the second solvent trap and the potassium iodide trap and the solvent traps each contained 150 ml. of chloroform. The completion of ozonolysis was detected by the development of a dark brown colour in the potassium iodide trap solution due to the liberation of iodine. During ozonolysis the reaction vessel contained a white cloud which disappeared when the reaction was almost complete. The reaction vessel and the first chloroform trap were cooled in an ice-bath (0-5°C.) throughout the ozonolysis. On completion of ozonolysis the reaction mixture was bulked with the chloroform in the solvent traps and the combined solution was stored in the refrigerator.

This chloroform solution was added dropwise to a stirred boiling mixture of water (250 ml.), excess metallic zinc and traces of hydroquinone and silver nitrate. Nitrogen gas was passed through the reaction flask continually. The rate of addition was adjusted so that the chloroform distilled over at the same rate as the ozonide solution was added. Upon

completion of ozonide addition and hydrolysis the reaction mixture was cooled with stirring and the contents of the reaction vessel were extracted with chloroform. An emulsion formed which was separated on a centrifuge. The aqueous portion obtained on centrifugation was further extracted with chloroform. These extracts were bulked with the chloroform fraction that had been received on centrifugation of the emulsion. The combined chloroform solution was dried over anhydrous sodium sulphate. The chloroform was removed from the filtered solution with a Rinco rotary evaporator. The crude aldehyde obtained gave a positive Schiff's test in each case. The crude aldehyde was condensed with malonic acid in pyridine.

Compounds (C-1) and (C-2) in the following table were prepared using this general procedure.

(b) A further modification was introduced since an emulsion formed on chloroform extraction of the hydrolysis reaction mixture. Ether was added to the cooled hydrolysis mixture, with mechanical stirring for fifteen minutes. The layers were separated in a separatory funnel. The aqueous layer was extracted with ether (8-10 times), the extracts added to the ethereal layer and the combined solutions were dried (anhydrous sodium sulphate). The crude aldehyde was obtained by removal of solvent in the cold, using a Rinco evaporator.

The aqueous layer that had been subjected to ether

extraction was further extracted with chloroform since it still gave a positive Schiff's test. Drying of this solution and evaporation yielded 5.35 grams of polymerized 8-oxononanal. The product was a slightly yellow semisolid whereas the aldehyde was a free-flowing liquid. The polymer still gave a positive Schiff's test. This procedure was only used for compound (C-3) in the following table.

(c) Procedure (a) above was repeated for compound (C-4). On chloroform extraction of the aqueous layer 14.75 grams of 7-oxooctanal that had begun to polymerize were obtained.

(d) The remaining aldehydes, compounds (C-5) to (C-16) were prepared as outlined in procedure C(2)(a) (page 48) using 250 ml. of water in the hydrolysis mixture instead of 300 ml. The aqueous fraction was not extracted with chloroform after ethereal extraction since this had led to polymerization in (C-3) and (C-4). Instead, a saturated solution of sodium bisulphite was added to the filtered aqueous solution obtained from compounds (C-8) to (C-16) in the following table. A solid precipitate was received in each instance, which still had not melted at 300°C. These derivatives were believed to be the bisulphite addition salts of the aldehydes. The derivative received from 6-oxodecanal (C-11) was purified by recrystallization from water (only very sparingly soluble). The analysis data showed conclusively that the solid derivative formed was not the aldehyde bisulphite compound, and

the analysis indicated the product may be inorganic in nature.

During the ether extraction of the reaction mixtures which yielded aldehydes (C-7), (C-10), (C-14) and (C-15) emulsions were formed. These were separated on a centrifuge. The aqueous fraction recovered after centrifugation was further extracted with ether. The ethereal extracts were bulked with the original ether solution in each case and were dried. The crude aldehyde was obtained by solvent removal in the usual manner. All crude aldehydes received were sweet-smelling free-flowing liquids with a colour ranging from light yellow to yellow.

Compound	Name of Product	1-Alkyl-1-cycloalkene mol. (g.)	Zinc mol.	Ozonolysis Time (hours)	Yield of Crude Product % (g.)	Method of Synthesis (Experiment No.)
(C-1)	7-Oxooctanal	1-Methyl-1-cycloheptene 0.227 (25.0)	0.25	23.7	52.1 (16.8)	C (2)(a)
(C-2)	6-Oxooctanal	1-Ethyl-1-[(a)] cyclohexene 0.180 (19.9)	0.21	17.3	30.3 (7.8)	C (2)(b)
(C-3)	8-Oxononanal	1-Methyl-1-cyclooctene 0.201 (25.0)	0.21	20.5	71.6 (22.5)	C (2)(b)
(C-4)	7-Oxooctanal	1-Methyl-1-cycloheptene 0.227 (25.0)	0.24	19.5	47.1 (15.2)	C (2)(c)
(C-5)	8-Oxodecanal	1-Ethyl-1-cyclooctene 0.181 (25.0)	0.21	17.3	97.3 (30.8)	C (2)(d)
(C-6)	7-Oxononanal	1-Ethyl-1-cycloheptene 0.201 (25.0)	0.24	19.5	63.8 (20.1)	C (2)(d)
(C-7)	7-Oxodecanal	1-Propyl-1-cycloheptene 0.181 (25.0)	0.24	14.0	63.2 (19.5)	C (2)(d)
(C-8)	6-Oxoheptanal	1-Methyl-1-cyclohexene 0.260 (25.0)	0.31	25.7	33.2 (11.1)	C (2)(d)
(C-9)	6-Oxooctanal	1-Ethyl-1-cyclohexene 0.227 (25.0)	0.28	22.5	49.4 (16.0)	C (2)(d)
(C-10)	6-Oxononanal	1-Propyl-1-cyclohexene 0.201 (25.0)	0.24	13.5	63.5 (20.0)	C (2)(d)
(C-11)	6-Oxodecanal	1-Butyl-1-cyclohexene 0.181 (25.0)	0.24	21.1	76.5 (23.6)	C (2)(d)
(C-12)	6-Oxoundecanal	1-Pentyl-1-cyclohexene 0.164 (25.0)	0.20	20.3	68.9 (20.9)	C (2)(d)
(C-13)	5-Oxohexanal	1-Methyl-1-cyclopentene 0.309 (25.0)	0.35	37.0	39.8 (14.1)	C (2)(d)
(C-14)	5-Oxoheptanal	1-Ethyl-1-cyclopentene 0.260 (25.0)	0.31	27.3	38.1 (12.7)	C (2)(d)
(C-15)	5-Oxooctanal	1-Propyl-1-cyclopentene 0.227 (25.0)	0.28	17.6	64.3 (20.8)	C (2)(d)
(C-16)	5-Oxononanal	1-Butyl-1-cyclopentene 0.201 (25.0)	0.24	23.6	65.5 (20.6)	C (2)(d)

(a) Only 19.85 grams of 1-ethyl-1-cyclohexene were ozonized instead of the usual 25.0 grams.

TABLE IV

REACTANT, CRUDE YIELDS, OZONOLYSIS TIMES, AND METHOD OF SYNTHESIS OF THE OXOALDEHYDES

Compound	Name of Product	1-Alkyl-1-cycloalkene mol. (g.)	Zinc mol.	Ozonolysis Time (hours)	Yield of Crude Product % (g.)	Method of Synthesis (Experiment No.)
(C-1)	7-Oxooctanal	1-Methyl-1-cycloheptene 0.227 (25.0)	0.25	23.7	52.1 (16.8)	C (2)(a)
(C-2)	6-Oxooctanal	1-Ethyl-1-[a] cyclohexene 0.180 (19.9)	0.21	17.3	30.3 (7.8)	C (2)(b)
(C-3)	8-Oxononanal	1-Methyl-1-cyclooctene 0.201 (25.0)	0.21	20.5	71.6 (22.5)	C (2)(b)
(C-4)	7-Oxooctanal	1-Methyl-1-cycloheptene 0.227 (25.0)	0.24	19.5	47.1 (15.2)	C (2)(c)
(C-5)	8-Oxodecanal	1-Ethyl-1-cyclooctene 0.181 (25.0)	0.21	17.3	97.3 (30.8)	C (2)(d)
(C-6)	7-Oxononanal	1-Ethyl-1-cycloheptene 0.201 (25.0)	0.24	19.5	63.8 (20.1)	C (2)(d)
(C-7)	7-Oxodecanal	1-Propyl-1-cycloheptene 0.181 (25.0)	0.24	14.0	63.2 (19.5)	C (2)(d)
(C-8)	6-Oxoheptanal	1-Methyl-1-cyclohexene 0.260 (25.0)	0.31	25.7	33.2 (11.1)	C (2)(d)
(C-9)	6-Oxooctanal	1-Ethyl-1-cyclohexene 0.227 (25.0)	0.28	22.5	49.4 (16.0)	C (2)(d)
(C-10)	6-Oxononanal	1-Propyl-1-cyclohexene 0.201 (25.0)	0.24	13.5	63.5 (20.0)	C (2)(d)
(C-11)	6-Oxodecanal	1-Butyl-1-cyclohexene 0.181 (25.0)	0.24	21.1	76.5 (23.6)	C (2)(d)
(C-12)	6-Oxoundecanal	1-Pentyl-1-cyclohexene 0.164 (25.0)	0.20	20.3	68.9 (20.9)	C (2)(d)
(C-13)	5-Oxohexanal	1-Methyl-1-cyclopentene 0.309 (25.0)	0.35	37.0	39.8 (14.1)	C (2)(d)
(C-14)	5-Oxoheptanal	1-Ethyl-1-cyclopentene 0.260 (25.0)	0.31	27.3	38.1 (12.7)	C (2)(d)
(C-15)	5-Oxooctanal	1-Propyl-1-cyclopentene 0.227 (25.0)	0.28	17.6	64.3 (20.8)	C (2)(d)
(C-16)	5-Oxononanal	1-Butyl-1-	0.24	23.6	65.5	C (2)(d)

D. Preparation of the α,β-Unsaturated Oxoacids by Condensation of the Crude Aldehyde with Malonic Acid

The pyridine used in all condensation reactions was dried over potassium hydroxide pellets for at least one week and was filtered just prior to use.

The malonic acid was dried in the oven at 90°C. for one hour and was stored in a vacuum dessicator (14 mm.) until use.

(1) Comparison of Various Reported Methods for Condensing an Aldehyde with Malonic Acid in Pyridine or a Pyridine-Piperidine Mixture (Doebner Modification of the Knoevenagel Condensation)

(a) Method of Allen and Van Allan (61)

Heptaldehyde (10.0 g., 0.0875 mole) was dissolved in pyridine (9.11 g., 0.115 mole). To this solution was added malonic acid (9.11 g., 0.0175 mole) and the mixture was refluxed for three hours on an oil bath. The reaction mixture was cooled, acidified by the addition of 10% hydrochloric acid and extracted with ether. The ethereal solution was washed with 10% hydrochloric acid and water and was then extracted with 10% sodium carbonate solution to obtain the acidic ether soluble material. The combined sodium carbonate extracts were washed with ether and acidified with 10% hydrochloric acid. The resulting mixture was extracted with

ether. The combined ethereal extracts were washed with water and dried (anhydrous sodium sulphate). On ether removal 10.6 g. (77.7% of theoretical) crude trans-2-nonenoic acid were obtained. The crude product was distilled to yield trans-2-nonenoic acid b.p. 149-53°C./13 mm., n_D^{21} 1.4581. The reported physical characteristics for this compound are b.p. 135-8°/5 mm., n_D^{25} 1.4561 (62).

(b) Method of Rajagopalan and Raman (63)

Heptaldehyde (10.0 g., 0.0875 mole) was dissolved in pyridine (4.68 g., 0.0592 mole). Malonic acid (9.11 g., 0.0875 mole) was added to the resulting solution. The reaction mixture was heated on a boiling water bath for two hours, then treated as in (a) (page 53). Crude trans-2-nonenoic acid (9.7 g., 70.8%) was obtained.

(c) Method of Jaeger and Robinson (24)

Heptaldehyde (10.0 g., 0.0875 mole) was dissolved in a solvent mixture consisting of pyridine (10 ml.) and piperidine (1.5 ml.). The solution was cooled in ice for thirty minutes. Malonic acid (9.11 g., 0.0875 mole) was dissolved in pyridine (10 ml.) and was also cooled to 0°C. in ice. The aldehyde solution was added to that of the acid and the reaction was heated for forty-eight hours at 50°C. Treatment of the reaction mixture as described in (a) (page 53) yielded 11.30 g. (82.7%) crude trans-2-nonenoic acid.

(d) Method of Barbier and Hugel (23)

Heptaldehyde (10.0 g., 0.0875 mole) was dissolved in 30 ml. pyridine. Excess malonic acid (11.00 g., 0.106 mole) was added to the aldehyde solution at 30°C. in a reaction vessel fitted with an internal thermometer. The reaction contents were heated at 50°C. for fifteen minutes, then the temperature was slowly increased to the reflux point over thirty minutes. The reaction mixture was refluxed for one hour. Treatment of the reaction mixture in the usual manner (a) (page 53) yielded 7.7 g. (56.4%) of crude trans-2-nonenoic acid.

(e) Method of Oughton (27)

Heptaldehyde (10.0 g., 0.0875 mole) was dissolved in pyridine (10 ml.) and piperidine (1 ml.). Excess malonic acid (13.51 g., 0.130 mole) was added. The reaction mixture was placed in a constant temperature water bath at 30°C. for twenty hours, and was then refluxed for four hours. When the reaction mixture was treated as described in (a) (page 53), 10.5 g. (76.9%) of crude trans-2-nonenoic acid were obtained.

The crude acid obtained from reactions (b) to (e) was bulked and distilled under reduced pressure. From 16.2 grams of the crude acid 11.6 grams of trans-2-nonenoic acid b.p. 151-6°C./13 mm., n_D^{23} 1.4584 were obtained. The crude yields

were used as an indication of the success of each reaction since a large portion of the acid was lost on fractional distillation of small quantities of the crude acid. The reaction worked successfully in all instances.

(2) Preliminary Condensation Experiments Leading to the Formation of α,β-Unsaturated Oxoacids

(a) Preparation of Trans-9-oxo-2-decenoic Acid According to the Procedure of Barbier and Hugel (23)

Crude 7-oxooctanal (1.47 g., 0.0103 mole) was dissolved in pyridine (20 ml.). Malonic acid (2.00 g., 0.0192 mole) was added slowly to this solution with shaking. The reaction was carried out as described in (d) (page 55) and 138 mgm. of crude trans-9-oxo-2-decenoic acid were obtained.

(b) Preparation of Trans-9-oxo-2-decenoic Acid

Distilled 7-oxooctanal, b.p. 118-30°C./13 mm. (3.60 g., 0.0253 mole), was condensed with malonic acid (3.00 g., 0.0288 mole) according to Jaeger and Robinson (24). From this reaction 1.80 grams of crude trans-9-oxo-2-decenoic acid were obtained.

The infrared spectrum of the crude acid in carbon disulphide showed peaks at 3.0-4.0 μ (bonded hydroxyl of acid) (64), 5.92 μ (α,β-unsaturated acid carbonyl) (64), and 6.10 μ

(conjugated ethylene group) (64). The spectrum was identical to that of authentic trans-9-oxo-2-decenoic acid obtained courtesy of Glaxo Research Limited, Greenford, Middlesex, England. Attempted recrystallization of the crude acid from ether-petroleum ether resulted in oil formation.

The crude acid product and the authentic sample of trans-9-oxo-2-decenoic acid were submitted to thin layer chromatography. Silica Gel G plates of 200 μ thickness were used. They were prepared according to Stahl (65). The plates were allowed to air dry overnight before use and were not activated. Development of the plates was carried out in a pre-saturated tank with a filter-paper liner utilizing a solvent system of benzene (90), methanol (16) and glacial acetic acid (8) by volume. The solvent front was allowed to migrate 10 cm. from the origin in all cases. Five drops of a solution containing 2 mgm./ml. were applied at the origin from a micropipette (made by pulling out a melting point capillary).

After development the chromatoplate was removed from the tank and was heated at 95°C. for forty-five minutes to remove all traces of solvent. It was then sprayed with 0.01% bromocresol green in alcohol (ethanol/butanol, 9:1) which had been previously basified by the addition of 1 drop sodium hydroxide solution (20% w/v). The acids were detected as yellow spots against a blue background.

The crude trans-9-oxo-2-decenoic acid isolated from the reaction mixture was identical to the authentic sample of

the same compound received from Glaxo on the same chromatoplate. There was some variation in the Rf of both compounds from one plate to another, however. The average Rf of trans-9-oxo-2-decenoic acid was found to be 0.53 in this solvent system.

(c) Preparation of Trans-8-oxo-2-nonenoic Acid

Distilled 7-oxoheptanal (b.p. 60-4°C./0.05 mm.),(2.40 g., 0.0187 mole) was condensed with malonic acid (2.20 g., 0.0211 mole) in the presence of pyridine (14 ml.) and piperidine (0.25 ml.) according to the procedure of Allen and Van Allan (61). Crude trans-8-oxo-2-nonenoic acid (500 mgm.) was obtained. The crude acid was recrystallized once from ether-petroleum ether to yield slightly yellow, wax-like needles, m.p. 44-8°C. (Reported m.p. 47-51°C. (23)). The infrared spectrum showed peaks at 3.0-4.0μ, 5.93 μ and 6.09 μ and was hence demonstrated to be the correct compound due to the m.p. and the similariries between its infrared spectrum and that of authentic trans-9-oxo-2-decenoic acid (pages 56-7).

Thin-layer chromatography was also carried out on the trans-8-oxo-2-nonenoic acid using 200 μ plates as described under (b) (pages 56-7). The average Rf of trans-8-oxo-2-nonenoic acid was found to be 0.51, and this compound ran very similarly to trans-9-oxo-2-decenoic acid in all cases.

(3) General Method for the Preparation of α,β-Unsaturated Oxoacids by Condensation of the Crude Oxoaldehyde with Malonic Acid

As previously mentioned the oxoaldehydes were condensed with malonic acid in the crude form since on distillation most of the aldehyde was lost due to a polymerization-like reaction.

The oxoaldehyde was dissolved in pyridine at room temperature, malonic acid was added slowly with frequent shaking to this solution and the reaction mixture was refluxed for two hours on an oil bath (temp. 125-40°C.). The reaction mixture was cooled, acidified with 10% hydrochloric acid and extracted with ether. The combined ether extract was washed with water, 10% hydrochloric acid and then again with water. Extraction of the ethereal solution with 10% aqueous sodium carbonate solution removed the acidic ether-soluble material. This extract was washed with ether and was then acidified with constant stirring using 10% hydrochloric acid. The resulting mixture was extracted with ether, the combined ether extracts were washed with water until neutral, and dried (anhydrous sodium sulphate). The crude acid was obtained on solvent removal, which was accomplished using a Rinco rotary evaporator.

The crude acids were subjected to thin-layer chromatography on 250 μ fluorescent silica gel GF_{254} plates. The

procedure used was identical with that explained in (b) (pages 56-7). Authentic trans-9-oxo-2-decenoic acid was used as the standard. Five drops of a solution containing 10 mgm./ml. were applied to the plate. All R_f values reported are the averages of several trials. On the same plate all of the acids ran very close together, indicating that the solvent system used was not a good one for the separation of these acids. After drying of the plates the spots were detected under ultraviolet light. The R_f values of the acids are given in Table V (page 62).

The S-benzylisothiourium salt of each of the crude acids was prepared according to Vogel (66). The crude acid (500 mgm.) was suspended in water (10 ml.). Phenolphthalein indicator (2 drops) was added and the acid was neutralized by the careful addition of normal sodium hydroxide solution. Hydrochloric acid (0.1 normal) was added until the phenolphthalein was almost decolourized. S-Benzylisothiourium hydrochloride (2.0 g.) dissolved in water (10 ml.) was filtered into the aqueous solution of the sodium salt of the acid. The crude derivative formed immediately and was purified by repeated recrystallization from water (three to six recrystallizations). The derivatives were analyzed and all but two gave satisfactory results for the expected structures.

The two exceptions were trans-9-oxo-2-dodecenoic acid and trans-8-oxo-2-tridecenoic acid. The S-benzylisothiourium salt of the supposed trans-9-oxo-2-dodecenoic acid (calculated for $C_{20}H_{30}N_2O_3S$: 63.46% C; 7.99% H; found: 61.69% C;

(7.88% H) gave analyses which agree very well for the S-benzylisothiourium salt of 7-oxodecanoic acid (calculated for $C_{18}H_{28}N_2O_3S$: 61.33% C; 8.01% H). This compound would appear in the crude acid product due to the oxidation of 7-oxodecanal, which was the starting material for the condensation reaction with malonic acid.

The analysis data of the S-benzylisothiourium derivative of crude trans-8-oxo-2-tridecenoic acid (calculated for $C_{19}H_{30}N_2O_3S$: 64.25% C; 8.22% H; found: 62.22% C; 8.61% H) was found to agree very well with the analysis of the S-benzylisothiourium salt of 6-oxoundecanoic acid (calculated for $C_{19}H_{30}N_2O_3S$: 62.26% C; 8.25% H). From these analysis results it appears that a greater amount of saturated acid was present in the crude acid product than the desired α,β-unsaturated acid. On recrystallization of these two crude products the S-benzylisothiourium salt of the desired acid was eliminated leaving only the salt of the saturated acid.

The S-benzylisothiourium salts were examined by infrared spectroscopy using a potassium bromide pellet which contained 1-2 mgm. of derivative in 400 mgm. of potassium bromide. All spectra showed the same peaks at 3.0-4.0 μ (anion of acid and primary amine as well as the possibility of a quaternary salt) (64) and 5.88-5.91 μ (carboxylate ion of α,β-unsaturated or saturated acid) (64). The spectra were quite complex and a complete interpretation was not attempted.

The spectrum of trans-7-oxo-2-octenoic acid did not

TABLE V (CONT'D)

Compound	Name of Product	Aldehyde mol. (g.)	Malonic Acid mol. (g.)	Volume of Pyridine (ml.)
(D-9)	Trans-8-oxo-2-dodecenoic acid	6-Oxodecanal 0.138 (23.6)	0.154 (16.0)	40
(D-10)[b]	Trans-8-oxo-2-tridecenoic acid	6-Oxoundecanal 0.113 (20.9)	0.125 (13.0)	35
(D-11)[c]	Trans-7-oxo-2-octenoic acid	5-Oxohexanal 0.123 (14.1)	0.135 (14.0)	30
(D-12)	Trans-7-oxo-2-nonenoic acid	5-Oxoheptanal 0.099 (12.7)	0.111 (11.5)	30
(D-13)	Trans-7-oxo-2-decenoic acid	5-Oxooctanal 0.145 (20.7)	0.159 (16.5)	40
(D-14)	Trans-7-oxo-2-undecenoic acid	5-Oxononanal 0.132 (20.6)	0.144 (15.0)	40

(a) The S-benzylisothiourium salt of (D-5) (trans-9-oxo-2-dodecenoic acid) analyzed well for the S-benzylisothiouri salt of 7-oxodecanoic acid, which would be formed due to oxidation of 7-oxodecanal.

(b) The derivative of (D-10) (trans-8-oxo-2-tridecenoic acid) analyzed well for the S-benzylisothiourium salt of 6-oxoundecanoic acid which would be formed due to the oxida tion of 6-oxoundecanal.

(c) After two recrystallizations there was not enough sample left for further purification. The 500 mgm. of trans-7-oxo-2-octenoic acid remaining was retained for attempted gas chromatographic purification (of the methyl ester) at a later date.

Average R_f of Acid	S-Benzyliso-thiourium Salt m.p.°C.	S-Benzylisothiourium Salt Analyses					
		Calculated			Found		
		%C	%H	%N	%C	%H	%N
0.65	141-141.5	62.61	7.74	7.69	62.16	7.73	-
0.67	136.5-7	63.46	7.99	7.40	63.82	7.84	7.29
0.63	136.5-7.5	61.69	7.48	7.99	61.83	7.38	-
0.68	135-5.5	62.61	7.74	7.69	62.29	7.52	7.63
0.68	135-5.5	63.46	7.99	7.40	61.69	7.88	-
0.60	148.5	60.69	7.19	8.33	60.84	7.37	8.12
0.63	140-140.5	61.69	7.48	7.99	62.22	7.31	8.18
0.70	137.5-8	62.61	7.74	7.69	62.78	7.28	7.75

TABLE V

REACTANTS, CRUDE YIELDS, AND R_f VALUES (THIN-LAYER CHROMATOGRAPHY) OF THE α,β-UNSATURATED OXOACIDS AND THE MELTING POINTS AND MICROANALYSES (CALCULATED AND FOUND) OF THEIR S-BENZYLISOTHIOURIUM SALTS

Compound	Name of Product	Aldehyde mol. (g.)	Malonic Acid mol. (g.)	Volume of Pyridine (ml.)
(D-1)	Trans-10-oxo-2-undecenoic acid	8-Oxononanal 0.144 (22.5)	0.154 (16.0)	35
(D-2)	Trans-10-oxo-2-dodecenoic acid	8-Oxodecanal 0.176 (30.0)	0.178 (18.5)	40
(D-3)	Trans-9-oxo-2-decenoic acid	7-Oxooctanal 0.107 (15.2)	0.115 (12.0)	30
(D-4)	Trans-9-oxo-2-undecenoic acid	7-Oxononanal 0.128 (20.1)	0.135 (14.0)	40
(D-5)[a]	Trans-9-oxo-2-dodecenoic acid	7-Oxodecanal 0.114 (19.5)	0.125 (13.0)	40
(D-6)	Trans-8-oxo-2-nonenoic acid	6-Oxoheptanal 0.0862 (11.1)	0.0961 (10.0)	25
(D-7)	Trans-8-oxo-2-decenoic acid	6-Oxooctanal 0.112 (16.0)	0.120 (12.5)	30
(D-8)	Trans-8-oxo-2-undecenoic acid	6-Oxononanal 0.128 (20.0)	0.139 (14.5)	30

Average R_f of Acid	S-Benzylisothiourium Salt m.p. °C.	S-Benzylisothiourium Salt Analyses					
		Calculated			Found		
		%C	%H	%N	%C	%H	%N
0.68	134.5-135	63.46	7.99	7.40	63.32	7.78	7.17
0.69	136	64.25	8.22	7.14	62.22	8.61	-
0.69	138-8.5	-	-	-	-	-	-
0.64	147.5	60.69	7.19	8.33	60.27	6.99	8.28
0.65	141.5	61.69	7.48	7.99	61.69	7.40	7.86
0.69	140	62.61	7.74	7.69	62.71	7.57	7.56

have distinct bands since it had only been recrystallized twice. However, it was still obvious that the same principal areas of absorption were present.

II. SYNTHESIS BY BUTLER, CALLOW AND JOHNSTON

A. Preparation of Dimethyl α-Bromoadipate (67)

Adipic acid (292.3 g., 2.00 mole), chloroform (500 ml.), and excess thionyl chloride (300 ml.) were refluxed together until all of the adipic acid had dissolved (four hours). Bromine (540 g., 2.13 mole) was added carefully to the stirred solution from a dropping funnel and the reaction was refluxed for four hours. Methanol (300 ml.) was added slowly, with external cooling in a water bath, at such a rate that the reaction refluxed briskly. After addition of the methanol was complete the reaction was refluxed an additional fifteen minutes. The reaction mixture was then cooled and washed with water. The organic solution was dried over anhydrous sodium sulphate, the solvent removed and the crude product was fractionally distilled three times using a 30 cm. insulated Vigreux column. A reflux distillation head adjusted to a ratio of 9:1 (reflux: distillation) was used for the final distillation. Pure dimethyl α-bromoadipate (143.1 g., 28.2%), b.p. 153-6°C./13 mm., n_D^{25} 1.4670 was obtained.

The infrared spectrum, determined as a liquid film,

showed bands at 5.78 μ (saturated aliphatic ester carbonyl) (68), 8.71 μ (ester carbonyl) (68) and 7.98 μ and 8.38 μ (unassigned).

The reported physical characteristics of this compound are b.p. 141-4°C./9 mm., n_D^{20} 1.4656 (67).

B. Attempted Preparation of Dimethyl Trans-2-hexenedioate (19,32)

(1) Dimethyl α-bromoadipate (66.3 g., 47.5 ml., 0.262 mole) was refluxed with sym.-collidine (95.0 ml.), which had been freshly distilled and dried over potassium hydroxide pellets, for forty-five minutes. A solid precipitated out during the course of the reaction.

The reaction mixture was cooled and the supernatant liquid poured into water. The solid was dissolved in water and this aqueous solution was added to the above mixture. The oil which separated was removed and the aqueous solution extracted with ether. The ether extracts were added to the separated oil which resulted in an ethereal solution. This solution was washed with 10% hydrochloric acid, water, 10% sodium carbonate and finally with water until neutral, then dried over anhydrous sodium sulphate. Solvent removal from the filtered solution yielded 15.8 grams of crude product. This oil was fractionally distilled under reduced pressure and 1.70 grams (3.8%) of the desired product (b.p. 135-7°C./

22 mm., n_D^{25} 1.4589) were obtained. The infrared spectrum as a liquid film showed bands at 5.81 μ (α,β-unsaturated ester carbonyl) (64), 6.05 μ (-CH=CH- conjugated with acid carbonyl) (64), and 7.95 μ and 8.35 μ (unassigned).

A second fraction (b.p. 137-240°C./22 mm.) was obtained in which the boiling point of the distillate increased rapidly. The remainder of the product polymerized in the distillation flask. Similar polymerization has been reported for dimethyl α,α'-dimethylmuconate (69). The infrared spectrum, as a liquid film, showed bands at 5.80 μ (α.β-unsaturated ester carbonyl) and 6.06 μ (-CH=CH conjugated with -C=O) (64).

(2) Dimethyl α-bromoadipate (43.4 g., 0.171 mole, 31 ml.) and dried redistilled sym.-collidine were gently refluxed for one hour. The reaction mixture turned yellow initially, and then gradually to a dark brown colour. The reaction was cooled and the supernatant liquid was decanted. The solid material remaining in the reaction flask was washed thoroughly with ether. This residue (33.7 g.) was recrystallized from alcohol-ether to form an off-white crystalline solid, m.p. 74-6°C., which was sym.-collidine hydrobromide.

The supernatant liquid was dissolved in the ethereal washings and the resulting solution was washed with 10% hydrochloric acid and then with water until neutral. On drying of the solution and solvent removal, 16.2 grams of crude product were obtained. The crude product was fractionally

distilled under reduced pressure in a nitrogen atmosphere in an attempt to prevent the polymerization which had occurred in the previous experiment. Three fractions were obtained. Fraction I (2.90 g., 9.8%), b.p. 76-80°C./0.03 mm., n_D^{25} 1.4522, was believed to be the desired product. The infrared spectrum determined as a liquid layer had bands at 5.82 μ (α,β-unsaturated ester carbonyl) (64) and 6.08 μ (-CH=CH- conjugated with C=O) (64).

Fraction II (0.60 g., b.p. 80-188°C./0.03 mm., n_D^{25} 1.4579) and fraction III (2.70 g., b.p. 180-250°C.,/0.03 mm., n_D^{25} 1.4673) were polymerization products of the desired compound (69).

As well as the three fractions of distillate received there was a residue left in the flask which refused to distill. It existed as a dark brown, fairly mobile liquid.

C. Preparation of Dimethyl α-Bromosebacate (67)

Sebacic acid (404.5 g., 2.00 moles), chloroform (500 ml.) and excess thionyl chloride (300 ml.) were stirred together at room temperature overnight. The following morning the reaction mixture was refluxed for two and one-half hours (until all of the solid acid had dissolved) and bromine (540 g., 2.13 moles) was added over a twenty minute period. The reaction was refluxed until all of the bromine vapour had disappeared (3 hours), methanol (300 ml.) was added over a

twenty minute period with external cooling of the reaction flask in a water bath (15-18°C.), and the reaction was refluxed for fifteen minutes. The cooled reaction mixture was washed with water and dried. On solvent removal and fractional distillation as described in A (page 63), 176.9 g. (28.6%) of dimethyl α-bromosebacate, b.p. 119-123°C./0.04-0.05 mm., n_D^{25} 1.4658, were obtained. The reported refractive index for this compound is n_D^{20} 1.4638 (67). The infrared spectrum determined as a liquid film, showed absorption at 5.78 u (saturated aliphatic ester carbonyl) and 8.73 u (ester carbonyl) (64,68).

D. Attempted Preparation of Dimethyl Trans-2-decenedioate (19,32)

(1) Since polymerization had been encountered in B and C (pages 64-66), anthraquinone (0.5 g.) was added to the reaction mixture as a free radical inhibitor. Dimethyl α-bromosebacate (37.3 g., 0.121 mole) was refluxed with sym.-collidine (60 ml.) for one hour. Reaction treatment as described in B (page 64), yielded 27.8 g. sym.-collidine hydrobromide and 15.4 g. of crude product. The crude ester was fractionally distilled under reduced pressure in a nitrogen atmosphere in the presence of anthraquinone. The first fraction of distillate weighed 3.55 g. (12.9%, b.p. 106-10°C./0.10 mm., n_D^{23} 1.4570). A second fraction weighing 0.50 g.

(b.p. 162-240°C./0.10 mm.) was also obtained. The first fraction's infrared spectrum (liquid film) had bands at 5.82 μ (α,β-unsaturated ester carbonyl) and 6.05 μ (-CH=CH- in conjugation with $-\overset{O}{\overset{\|}{C}}-OCH_3$) (64,68). The infrared data showed conclusively that the dehydrohalogenation had taken place.

(2) Experiment (1) was repeated using 59.8 g. (0.193 mole) dimethyl α-bromosebacate and 100 ml. sym.-collidine in the absence of anthraquinone. The reaction mixture was heated on a boiling water bath for two hours. From this reaction 37.3 g. (84.5%) crude dimethyl trans-2-decenedioate and 34.7 g. sym.-collidine hydrobromide were isolated.

Since polymerization had occurred on all previous attempted distillations of products of this type, the crude ester was submitted to the next step in the reaction sequence.

E. Attempted Preparation of Monomethyl Ester of Trans-2-decenedioic Acid (19,32)

Crude dimethyl trans-2-decendioate (20.0 g., 0.0876 mole) was dissolved in a solution of potassium hydroxide (4.9 g., 0.0873 mole) in water (35 ml.) and methanol (70 ml.) and left overnight at room temperature. The reaction was diluted with water and the unchanged ester was removed by ether extraction. Acidification of the aqueous solution with 10%

hydrochloric acid and ether extraction yielded the crude half-ester. The combined ethereal extracts were dried over anhydrous sodium sulphate and the solvent removed. The crude product (16.9 g., 90.0%) was obtained.

The equivalent weight of the crude half-ester was determined by dissolving it in 50% v/v aqueous ethanol and titrating with decinormal sodium hydroxide solution to phenol red indicator. The equivalent weight was found to be 258.1. The theoretical equivalent weight of the half-ester is 214.7 g. and of the dibasic acid 100.1.

F. Reaction of Crude Monomethyl Ester of Trans-2-decenedioic Acid with Thionyl Chloride (19,32)

The crude monomethyl ester of trans-2-decenedioic acid (16.2 g., 0.0756 mole) was stirred overnight with thionyl chloride (52.0 g., 0.437 mole) at room temperature and was refluxed on a boiling water bath for thirty minutes the following morning. The excess thionyl chloride was removed in vacuo leaving the acid chloride as a liquid residue.

G. Attempted Synthesis of Methyl Trans-10-oxo-2-undecenoate (70,71)

A Grignard reagent was prepared from magnesium filings (8.00 g., 0.329 mole) and methyl bromide (42.0 g., 0.442 mole)

in anhydrous ether (250 ml.). The reaction vessel was immersed in an ice-water bath and anhydrous cadmium chloride (32.0 g., 0.146 mole) was added slowly to the cooled Grignard. The reaction was refluxed for two hours and the ether was removed by distillation. Anhydrous benzene (150 ml.) was added and some of the benzene was distilled off to insure complete removal of the ether. The acid chloride of the monomethyl ester of trans-2-decenedioic acid (F) (page 69) was dissolved in 25 ml. anhydrous benzene. This benzene solution was added to the Grignard reagent over twenty minutes. A canary yellow colour developed in the reaction flask. The reaction mixture was refluxed for twenty minutes on a boiling water bath, cooled and hydrolyzed by pouring it into a stirred mixture of crushed ice and concentrated hydrochloric acid. The resulting mixture was extracted with ether. The combined ether extracts were washed with water, dried and the solvent was removed to yield 9.8 g. of crude product. This was fractionally distilled under reduced pressure.

The first fraction (0.75 g., b.p. 128-34°C./0.35 mm., n_D^{23} 1.4458) was mainly forerun. Fraction II (1.35 g., b.p. 128-30°/0.21 mm., n_D^{23} 1.4488) was initially believed to be the desired product. The infrared spectrum (liquid film) of the second fraction had peaks at 5.78 μ (possibly saturated ester carbonyl or α,β-unsaturated ester carbonyl), 5.87 μ (acyclic ketone), 8.37 μ and 8.5 μ (ester carbonyl), and 7.37 μ ($CH_3-\overset{O}{\overset{\|}{C}}-$) (64,68). However, the peak which should have been

present at 6.02-6.05 μ, which is characteristic of $-CH=CH-\overset{O}{\overset{\|}{C}}-OCH_3$, was absent (32,68). Thus the evidence indicates that a saturated ester was obtained instead of the desired α,β-unsaturated ester (methyl trans-10-oxo-2-undecenoate). The saturated ester was not identified since it obviously was not the correct product. Due to the difficulties which had been encountered with the Butler, Callow and Johnston synthesis, no further experiments were carried out.

III. OUGHTON SYNTHESIS

A. Preparation of 5-Bromoamyl Acetate (27)

Acetyl bromide (126.5 g., 1.029 mole) was added slowly to a stirred mixture of anhydrous tetrahydrofuran (93.9 g., 1.090 mole) and zinc dust (0.4 g.). The temperature rose to 42°C. The reaction mixture was warmed and a vigorous reaction took place which was controlled by external cooling. The reflux temperature of the reaction mixture increased slowly to 145°C. at which point the temperature became constant. After cooling to room temperature, with stirring, the residue was distilled through a 30 cm. Vigreux column. 5-Bromoamyl acetate (190.0 g., 88.4%), b.p. 131.5-132°C./37 mm., n_D^{24} 1.4622 was obtained. The reported physical characteristics of this compound are b.p. 112-3°C./17 mm., n_D^{20} 1.4610 (27).

B. Preparation of 7-Oxooctanol (27)

Sodium ethoxide was prepared by the careful addition of metallic sodium (10.35 g., 0.45 mole) to stirred absolute ethanol (250 ml.) and ethyl acetoacetate (60.0 g., 0.45 mole) was added to the hot ethoxide solution. To this 5-bromoamyl acetate (105.0 g., 0.50 mole) was added from a dropping funnel over a period of fifteen minutes. The reaction mixture was then refluxed for five hours. The alcohol was removed by distillation on a boiling water bath. Water (50 ml.) was added to dissolve the sodium bromide and the organic layer which formed was separated and added to a solution containing 40.0 grams of sodium hydroxide in a litre of water. This mixture was stirred at room temperature for six hours, then refluxed for thirty minutes. A solution of sulphuric acid (25 ml.) in water (100 ml.) was added slowly to the cooled solution. The mixture was distilled and the product was salted out of the distillate with potassium carbonate, and was extracted with ether. The combined ethereal extracts were washed with 10% sodium bicarbonate solution, then with water until neutral, and were dried. The ether was removed leaving a residue (30 g.) which was distilled under reduced pressure to yield 7-oxooctanol (20.0 g., 31%), b.p. 164°C./40 mm., n_D^{20} 1.4459. (Reported b.p. 100°C./0.5 mm., n_D^{20} 1.4450) (27)

C. Preparation of 7-Oxooctanal (27)

A solution of 7-oxooctanol (18.0 g., 0.125 mole), powdered potassium dichromate (15.1 g., 0.513 mole) and glacial acetic acid (189 ml.) was stirred for one hour on a steam bath. The mixture was poured into three litres of water and extracted with ether. The ethereal extracts were washed with 10% sodium bicarbonate solution and then with water until neutral. The extract was dried and the solvent removed to yield 4.1 grams of residue. On distillation of this residue a light yellow fragrant oil, b.p. 154-80°C./43 mm., was obtained. The bisulphite compound, which was a white, crystalline solid, was prepared. The reported boiling point of 7-oxooctanal is 62-64°C./0.2 mm. (24).

D. Preparation of 4-Bromobutyl Acetate (27)

(1) Acetyl bromide (400.0 g., 3.2 moles) was added slowly to a stirred mixture of anhydrous tetrahydrofuran (230.4 g., 3.2 moles) and zinc dust (1.28 g.) over a period of one hour. The reaction mixture was refluxed for one hour and then distilled through a 30 cm. Vigreux column. The resulting distillate was fractionally distilled.

Fraction	b.p.°C.(15.5 mm.)	n_D^{25}
I	90-5	1.4872
II	96-9	1.4778
III	100	1.4627
IV	100-102	1.4571

From the refractive indices it is obvious that the product was a mixture.

(2) Acetyl bromide (50.0 g., 0.407 mole), anhydrous tetrahydrofuran (28.8 g., 0.399 mole) and powdered zinc (0.20 g.) were reacted as above. The reaction mixture was cooled and poured into a litre of water and was extracted with ether. The combined ethereal extracts were washed with water, 10% sodium bicarbonate and then again with water until neutral. The ether solution was dried over anhydrous sodium sulphate. Evaporation gave 53.3 grams of residue which was submitted to fractional distillation under reduced pressure using a 30 cm. Vigreux column.

Fraction	b.p.°C.(17 mm.)	n_D^{25}
I	94-6	1.4872
II	96-8	1.4824
III	98-101	1.4732
IV	101-104	1.4515

Since the product was obviously a mixture the reaction was not attempted again.

E. Preparation of 4-Chlorobutyl Acetate (72)

Acetyl chloride (130 g., 1.151 mole) was added dropwise to a stirred mixture of anhydrous tetrahydrofuran (100.0 g., 1.386 mole) and zinc powder (0.4 g.). The reaction mixture was refluxed for one hour and was then subjected to fractional distillation under reduced pressure. 4-Chlorobutyl acetate (161.5 g., 77.5%, b.p. 84-87.5°C./13 mm., n_D^{25} 1.4330) (Reported b.p. 103°C./18 mm., n_D^{20} 1.4344 (72)), was obtained.

Further syntheses were not carried out using the Oughton synthesis since the Barbier and Hugel synthesis was now leading to a greater variety of the desired α,β-unsaturated oxoacids than could be prepared using Oughton's method.

IV. MISCELLANEOUS SYNTHESES

A. Reduction of Trans-9-oxo-2-decenoic Acid with Lithium Aluminum Hydride in Anhydrous Ether

Lithium aluminum hydride (1.12 g., 0.0295 mole) was mixed with anhydrous ether (100 ml.) and the stirred mixture was cooled to 0°C. using an ice-salt mixture. A solution of trans-9-oxo-2-decenoic acid (20.0 g., 0.109 mole) dissolved in 350 ml. dry ether was added dropwise at such a rate that the temperature of the reaction mixture did not exceed 5°C.

The reaction was stirred for forty-five minutes after the addition was complete.

The complex was decomposed by the addition of dilute (10%) hydrochloric acid with stirring for thirty minutes. The layers were separated and the aqueous layer was extracted with ether. The combined ether solutions were washed with water and extracted with 10% sodium bicarbonate solution. The sodium bicarbonate extracts were washed with ether, acidified, and extracted with ether. The ether extracts were washed, dried and the solvent removed to yield 19.7 grams of crude trans-9-hydroxy-2-decenoic acid. The crude product was fractionally distilled under reduced pressure and 10.6 grams (52.7%) of product b.p. 146-9°C./0.05 mm. were obtained. The distillate was originally believed to be pure trans-9-hydroxy-2-decenoic acid. However, by thin-layer chromatography, using 200 μ Silica Gel G plates as described in (b) (page 56)it was found that the product consisted of trans-9-hydroxy-2-decenoic acid plus a trace of unreduced trans-9-oxo-2-decenoic acid. Authentic trans-9-oxo-2-decenoic acid was used as the standard for thin-layer chromatography. The thin-layer chromatogram of the distilled reaction product showed two spots. The spot of higher R_f always ran identical to trans-9-oxo-2-decenoic acid on the same chromatogram. It was a very weak spot in intensity compared to the one having a lower R_f, which was due to trans-9-hydroxy-2-decenoic acid.

B. Reduction of Trans-9-oxo-2-decenoic Acid with Lithium Aluminum Hydride in Anhydrous Tetrahydrofuran

Lithium aluminum hydride (0.224 g., 0.00590 mole) was mixed with anhydrous tetrahydrofuran (25 ml.), at room temperature. To this stirred mixture was added trans-9-oxo-2-decenoic acid (1.0 g., 0.00543 mole) dissolved in tetrahydrofuran (15 ml.) and the reaction mixture was refluxed for three hours. One millilitre of the reaction mixture was removed and hydrolyzed with dilute hydrochloric acid. This was extracted with ether (2 ml.) and the resulting ether solution was used for thin-layer chromatography. On the resulting chromatogram run with trans-9-oxo-2-decenoic acid as a standard, the reaction mixture showed only one spot having an average R_f of 0.31 (0.31, 0.30 and 0.31). This spot was due to the reduced acid, trans-9-hydroxy-2-decenoic acid. On the same chromatoplate the reference compound, trans-9-oxo-2-decenoic acid had an average R_f of 0.37 (0.37, 0.36 and 0.37). The thin-layer chromatography was carried out as described in (b) (page 56).

The remaining reaction mixture was hydrolyzed with dilute hydrochloric acid (10%) as described in A above. As a result 0.65 gram (64.4%) of crude trans-9-hydroxy-2-decenoic acid was prepared, which on thin-layer chromatography showed the absence of any starting material (trans-9-oxo-2-decenoic acid).

C. Reaction of Trans-9-oxo-2-decenoic Acid with Bromine in Chloroform

Trans-9-oxo-2-decenoic acid (1.0 g., 0.00543 mole) in chloroform was mixed with an excess of bromine in the same solvent and the solution was kept at room temperature, with intermittent shaking, for twelve hours. There was an evolution of white vapours which indicated that substitution was probably taking place as well as addition. Excess bromine and chloroform were removed in vacuo in the cold, ether was added to the residue and the ethereal solution was washed with 10% aqueous sodium thiosulphate solution and water. The ether was removed from the dried extract and a residue (2.90 g.) was obtained. The infrared spectrum, in carbon disulphide, showed bands at 3.0-4.0 μ (carboxylic acid), 5.79 μ (saturated aliphatic acid carbonyl) and 15.4 μ (C-Br). No bands were observed at 6.02-6.05 μ ($\alpha.\beta$-unsaturation conjugated with carbonyl) or 7.63-7.75 μ ($CHR_1=CHR_2$) (64,68).

Since the starting material is a methyl ketone, the hydrogen bromide vapour given off is an indication that substitution is taking place at this group (73). Thus this reaction could not be used for the preparation of 9-oxo-2,3-dibromodecanoic acid, which was the desired product. Hence the synthesis was not repeated.

DISCUSSION

As a result of a literature survey it was decided that the Barbier and Hugel procedure (23) was the method of choice for synthesis of the desired α,β-unsaturated oxoacids. There were two governing factors for this decision. First, this synthesis used starting materials, the cycloalkanones and alkyl halides, which are readily available commercially. Second, it is a general method of synthesis for compounds of this type and a wide range of acids analogous to trans-9-oxo-2-decenoic acid could potentially be prepared by varying the cycloalkanone and the Grignard reagents used to prepare the 1-alkyl-1-cycloalkanol. Also, all steps in the reaction sequence seemed to be very straightforward.

During preliminary experimentation, however, it was discovered that the oxoaldehydes were polymerized during attempted distillation. Two other reported methods of synthesis of α,β-unsaturated oxoacids were then attempted. These were the Butler, Callow and Johnston synthesis (19,32) and the Oughton synthesis (27). In the final analysis the Barbier and Hugel synthesis was reverted to, but, since the intermediary aldehydes could not be distilled they were carried through the final reaction step without purification.

I. BARBIER AND HUGEL SYNTHESIS

A. 1-Alkyl-1-cycloalkanols

These tertiary cyclic alcohols were prepared in good yield from the corresponding Grignard reagent and cycloalkanone. From preliminary experiments it was found necessary to use a large excess of propyl, butyl or pentyl-magnesium iodide in order to receive a good yield of cyclic alcohol (yield calculated on basis of cycloalkanone).

The alcohols which were prepared all had a camphor-like odour. Upon distillation they frothed a great deal and it was necessary to use oversized flasks to prevent the froth from creeping up the distillation column and into the distillate. The addition of a few drops of silicone oil did not appreciably decrease this frothing. Since some alcohols, 1-methylcyclopentanol and 1-methylcyclooctanol, began to dehydrate during distillation under reduced pressure, alcohols (A-8) to (A-20) in Table II (page 35) were dehydrated in the crude form to yield the corresponding 1-alkyl-1-cycloalkenes.

In the preparation of 1-pentyl-1-cyclopentanol (A-20) (page 34), some of the pentylmagnesium iodide coupled to form n-decane (43). The crude alcohol, which contained n-decane as an impurity was dehydrated using a trace of iodine as the catalyst. The boiling points of n-decane [174°C. (58)] and

1-pentyl-1-cyclopentene [179°C.(56)] are very close which explains why this mixture could not be separated by fractional distillation (pages 40,41). However, the desired alcohol could have been prepared by removing the n-decane from the 1-pentyl-1-cyclopentanol (reduced pressure) and then dehydrating the n-decane-free alcohol.

B. 1-Alkyl-1-cycloalkenes

In the earlier dehydration experiments potassium bisulphate (later potassium pyrosulphate) was used as a catalyst. However, this method was found to have several disadvantages. When the 1-alkyl-1-cycloalkene was distilled at the completion of the reaction low yields of product were obtained. It was found that the wet gummy residue of potassium bisulphate was holding much of the product by absorption. This problem was overcome by ethereal extraction of the residue after the distillable portion had been collected. The ether extract was bulked with the distillate, the bulked solution was dried and the residue was redistilled after solvent removal yielding the 1-alkyl-1-cycloalkene product [(B-3) to (B-9) (page 38)]. Another disadvantage of this dehydration method was that an azeotropic mixture of water and the alkene product was often received. This was overcome by using an apparatus which allowed the water formed during the dehydration to be removed while the reaction was proceeding (Figure

II, page 37a). The final difficulty experienced while using potassium bisulphate as a proton donor for dehydration experiments was the preparation of 1-methyl-1-cyclopentene (page 40). Two alkene products were received.from this dehydration reaction. For the above reasons potassium bisulphate was replaced by iodine as a dehydration catalyst for 1-alkyl-1-cycloalkenes (B-9) to (B-19) (page 38). Another advantage of iodine was that only a trace of it was required. The 1-alkyl-1-cycloalkanols which were dehydrated to form the alkenes (B-7) to (B-19) were dehydrated in the crude form since two of the alcohols had dehydrated during distillation under reduced pressure.

The iodine dehydrations all gave the corresponding 1-alkyl-1-cycloalkene in good yield. Since the 1-alkyl-1-cycloalkanols used as starting materials frothed a great deal when refluxed, an oversized flask was used for the reaction (five hundred ml. flask for approximately one hundred ml. of 1-alkyl-1-cycloalkanol). The alkenes prepared all gave a positive test for unsaturation with tetranitromethane. The 1-methyl, 1-ethyl and 1-propyl-1- cycloalkenes had characteristic olefin odours whereas the 1-butyl and 1-pentyl derivatives had sweet rather sickening odours. The products were all mobile, colourless, low-density liquids (d = 0.8-0.9).

C. Oxoaldehydes

It was found that the Gallenkamp GE-150 ozonizer, which was used for all the ozonolysis experiments in this thesis, gave yields of ozone which varied substantially from trial to trial even on the same day. The instrument delivered from 0.0286 mole to 0.209 mole of ozone per hour. The average value of all determinations made was 0.097 mole of ozone per hour. This explains why some compounds such as 1-propyl-1-cycloheptene (C-7) and 1-propyl-1-cyclohexene (C-10) were ozonized much more rapidly than others of the same molecular weight.

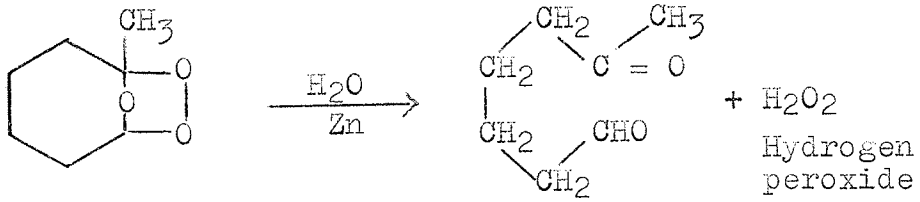
The original ozonolysis experiments were carried out using acetic acid as the solvent (23). However, on several trials the reaction vessel, which was immersed in an ice bath, solidified. This is not surprising since the melting point of acetic acid is 16.7°C. (74). For this reason, later experiments were carried out using chloroform as the ozonolysis solvent.

It was also demonstrated that the Barbier and Hugel method of hydrolysis (23) was producing as much acid as aldehyde from the ozonide (pages 45-46). This method of hydrolysis was carried out by adding small amounts of powdered zinc and water alternately to the stirred, cooled acetic acid solution of the ozonide. The purpose of the zinc was

to destroy the hydrogen peroxide which is formed during hydrolysis (75). Otherwise the peroxide present will oxidize the aldehyde to the corresponding acid. The equations in Figure III illustrate the reactions which are taking place:

FIGURE III

HYDROLYSIS OF AN OZONIDE IN THE PRESENCE OF ZINC



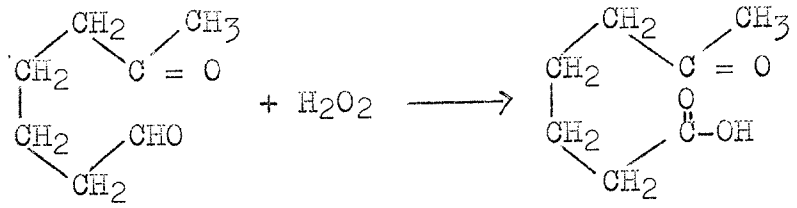
Ozonide of 1-methyl-1-cyclohexene

6-Oxoheptanal

If zinc reacts with hydrogen peroxide immediately:

$$H_2O_2 + Zn \longrightarrow ZnO + H_2O$$

If hydrogen peroxide is left unreacted the corresponding saturated acid is formed:



6-Oxoheptanal

6-Oxoheptanoic acid

Since more acid than aldehyde was being obtained using the hydrolysis procedure of Barbier and Hugel (23) it was replaced by that of Whitmore and Church (60). A chloroform

solution of the ozonide was dropped slowly into a stirred mixture of boiling water which contained excess metallic zinc, hydroquinone and silver nitrate (trace). The hydroquinone and silver nitrate are present to prevent oxidation and polymerization.

The greatest difficulty that was encountered during the course of this thesis was the polymerization-like reaction which occurred when distillation of the oxoaldehydes was attempted. A small amount of aldehyde would distill over and then the boiling point would increase very rapidly until, finally, distillation ceased. Upon cooling, the residue left in the flask became semi-solid and varied in colour from dark yellow to dark brown. It still gave a weakly positive test with Schiff's reagent. Since distillation of the aldehydes represented a very difficult problem it was decided to react the crude aldehyde with malonic acid and then to attempt purification of the final acid product. Dr. L. Schmid recently reported the synthesis of three of these α,β-unsaturated acids using the Barbier and Hugel synthesis (40,41). In a very recent letter (76) to us Dr. Schmid mentioned that the same difficulty was encountered in his laboratory on attempted distillation of the aldehydes. He also found it necessary to condense the oxoaldehyde with malonic acid and then to purify the final product as was attempted in this thesis. This was not reported in his research paper (41).

The crude oxoaldehydes (C-1) to (C-16) were pale

yellow to yellow in colour. All were mobile, very sweet smelling oils which gave vivid Schiff's tests immediately. Comparison of the crude aldehyde yields showed that the yield generally increased as the homologous series was ascended. The reason for this was believed to be that the water solubility of the oxoaldehydes was decreasing with an increase in alkyl chain length. This explanation is supported by the fact that even after many (8-10) ethereal extractions the aqueous hydrolysis mixture always gave a strong Schiff's test, indicating that there was still some aldehyde present. A saturated aqueous solution of sodium bisulphite was added to the extracted aqueous solution in several cases and a solid derivative precipitated out of solution in each case. These derivatives were thought to be the sodium bisulphite addition complexes of the oxoaldehydes but analysis of one of the recrystallized compounds showed that this was not so (page 50).

D. α,β-Unsaturated Oxoacids

These acids were prepared using the Doebner modification of the Knoevenagel condensation. The preliminary experiments (pages 53-6) involved a comparison of various reported methods for carrying out this reaction. Using heptaldehyde it was demonstrated that trans-2-nonenoic acid was obtained in good yield in all cases. All of these methods consisted of the condensation of the aldehyde with malonic acid,

followed by heating to decarboxylate the unstable dibasic acid. Each used different conditions. From these preliminary experiments the general synthesis for the α,β-unsaturated oxoacids (page 59) was derived.

Thin-layer chromatography was used to check the reaction products for the presence of acids closely related to trans-9-oxo-2-decenoic acid. The crude reaction product was spotted on silica gel thin-layer chromatography plates along with authentic trans-9-oxo-2-decenoic acid (page 59). The plate was developed in a solvent system of benzene/methanol/acetic acid (90:16:8). This solvent system was demonstrated to be excellent for the detection of fatty acids of this type, but since the R_f values were all very similar (page 62), this system could not be used for the separation of a mixture of acids analogous to trans-9-oxo-2-decenoic acid. Also, only one spot was present on the chromatoplates of the crude acid products. Since the crude oxoaldehyde was used in the condensation, it seems very likely that there should be at least two spots present; one due to the α,β-unsaturated oxoacid prepared by condensation and the other due to saturated oxoacid that would be present due to oxidation of the aldehyde used as starting material. The analysis data of the S-benzylisothiourium salts of the crude acid mixtures indicated that a mixture of these two possible products was indeed produced (page 62). Thus, since only one spot was present on the thin-layer chromatogram of all α,β-unsaturated oxoacid reaction mixtures,

this solvent system could not even differentiate between the α,β-unsaturated oxoacid and the saturated oxoacid containing two less carbon atoms.

Purification of the crude acid products by recrystallization was attempted but was unsuccessful due to oil formation. For this reason the S-benzylisothiourium salt of the crude reaction mixture was prepared in all cases (page 60). This derivative was a solid which separated as soon as the aqueous solution of S-benzylisothiourium hydrochloride was added. The derivatives were recrystallized from water to constant melting point (3-7 recrystallizations). These salts were analyzed and with the exception of two all gave acceptable results for the derivative of the expected α,β-unsaturated oxoacid (page 62). The S-benzylisothiourium salts of the two exceptions, trans-9-oxo-2-dodecenoic acid (D-5) and trans-8-oxo-2-tridecenoic acid (D-10) analyzed well for the saturated oxoacid that would be received from the oxoaldehyde by oxidation. In other words, the derivative from the reaction mixture which was expected to yield trans-8-oxo-2-tridecenoic acid analyzed well for the S-benzylisothiourium salt of 6-oxoundecanoic acid while that of trans-9-oxo-2-dodecenoic acid analyzed well for the salt of 7-oxodecanoic acid. This experimental evidence seemed to indicate that in all of the crude acid products a mixture of α,β-unsaturated oxoacid and saturated oxoacid (having two less carbon atoms in the chain) was prepared. Hence, on recrystallization of the S-benzylisothiourium salt, a derivative

was obtained which analyzed for the salt of the α,β-unsaturated oxoacid or the saturated oxoacid, depending on which product was present in larger concentration in the crude acid product. It is interesting to note that the two products whose derivatives analyzed well for the saturated oxoacids were prepared from the 1-alkyl-1-cycloalkenes with the longest alkyl sidechains (butyl and amyl), although this may simply be a coincidence.

The following table (Table VI) shows the difference between the theoretical analyses of the S-benzylisothiourium salts of the α,β-unsaturated oxoacids and the corresponding saturated oxoacids which contain two less carbon atoms. As can be seen from the values listed in the table there is a difference of approximately 2% in the theoretical carbon content between all α,β-unsaturated and saturated oxoacid derivatives. All of the oxoacids of a series have the same theoretical analysis. That is trans-7-oxo-2-decenoic acid has the same empirical formula as trans-8-oxo-2-decenoic acid and hence has the same theoretical analysis. The 2% difference in carbon content between α,β-unsaturated and saturated oxoacid derivatives appears to be large enough to give an analysis which verifies the identity of the compound, provided that the theoretical and experimental values agree well with each other. Naturally in any cases where the actual carbon analysis falls between the two theoretical values, there is a possibility that a mixture of the two derivatives has been obtained and

recrystallized to a mixture of fixed composition with a constant melting point.

TABLE VI

COMPARISON OF THEORETICAL ANALYSES OF S-BENZYLISOTHIOURIUM SALTS OF α,β-UNSATURATED OXOACIDS AND CORRESPONDING SATURATED OXOACIDS (HAVING TWO LESS CARBON ATOMS)

α,β-Unsaturated Oxoacid	α,β-Unsaturated Oxoacid Derivative			Corresponding Saturated Oxoacid Derivative		
	%C	%H	%N	%C	%H	%N
Nonenoic	60.69	7.19	8.33	58.04	7.14	9.03
Decenoic	61.69	7.48	7.99	59.23	7.46	8.64
Undecenoic	62.61	7.74	7.69	60.63	7.74	8.28
Dodecenoic	63.46	7.99	7.40	61.33	8.01	7.95
Tridecenoic	64.25	8.22	7.14	62.26	8.25	7.64

Another experimental peculiarity was noted. The S-benzylisothiourium salt of authentic trans-9-oxo-2-decenoic acid melted at 155°C., whereas the same salt isolated from the crude reaction mixture and recrystallized to constant melting point melted at 137-8°C. These two derivatives had identical infrared spectra and both analyses agreed very well with the theoretical. Also, Dr. L. Schmid (41) prepared the S-benzylisothiourium salt of trans-7-oxo-2-decenoic acid and reported a melting point of 151°C. The same compound prepared in this

laboratory melted at 141.5°C. (page 62). A possible explanation for these discrepancies could be that the melting point of our products is being depressed due to the presence of a trace of the S-benzylisothiourium salt of the saturated oxoacid, even though there is not enough of this derivative present to affect the analysis results.

Due to the possibility that these derivatives do contain some of the salt of the saturated oxoacid, further work is being continued. Gas-chromatographic separation of the crude acid products will be attempted. There is an excellent possibility that this can be accomplished since the separation of trans-9-oxo-2-decenoic and trans-9-oxo-3-decenoic acid has been reported in the literature by this method (34). Upon separation by preparative scale gas chromatography the α,β-unsaturated oxoacids will be converted to their S-benzylisothiourium salts and the melting point will be compared with that of the derivative which has already been synthesized and reported (page 62). The purified α,β-unsaturated oxoacids will then be tested for their effect on immature stages of the yellow-fever mosquito (Aedes aegypti, L.) according to the procedure of Quraishi and Thorsteinson (38).

II. BUTLER, CALLOW AND JOHNSTON SYNTHESIS (19,32)

Originally the desired α,β-unsaturated oxoacids were to be synthesized by the Barbier and Hugel method (23).

However, due to the fact that the oxoaldehydes polymerized on attempted distillation, experiments were carried out using this second method of synthesis.

A. Dimethyl α-Bromoadipate and Dimethyl α-Bromosebacate

These two starting materials were prepared by a standard synthetic route (67). Both products were obtained in fair yield in pure form. Their identity was verified by the comparison of the observed infrared spectra and physical properties to the reported values. Both α-bromoesters were colourless, fairly mobile liquids.

B. Dimethyl Trans-2-hexenedioate and Dimethyl Trans-2-decenedioate

The chief difficulty encountered in these syntheses was once again found to be polymerization. On attempted dehydrobromination of both dimethyl α-bromoadipate and dimethyl α-bromosebacate with sym.-collidine there was no doubt that the reaction had taken place since a solid residue of sym.-collidine hydrobromide was formed in the reaction flask (pages 64-8). However, on attempted distillation of the isolated crude products only a small forerun of the desired compounds was obtained. Then the boiling point of the liquid being distilled began to rise rapidly indicating that polymerization was taking

place in the distillation flask. The residue which refused to distill remained mobile. Polymerization of the same type has been reported with similar compounds such as dimethyl α,α'-dimethyl muconate (69).

Several distillation modifications were attempted to prevent this undesirable side reaction from taking place. An inert nitrogen atmosphere and high vacuum were employed. One distillation of dimethyl trans-2-decendioate (page 67) was even attempted in the presence of anthraquinone as a free-radical inhibitor. In spite of these precautions polymerization resulted and very low yields of product were obtained.

Infrared absorption spectra of the distillates recovered before polymerization began to take place always showed peaks in the range 6.02-6.08 μ which can be attributed to -CH=CH- in conjugation with a carbonyl (64,68). Since this characteristic absorption was absent in both starting materials it demonstrated conclusively that dehydrobromination had taken place.

C. Methyl Trans-10-oxo-2-undecenoate

Since the only difficulty appeared to be polymerization during distillation it was decided to dehydrobrominate dimethyl α-bromosebacate and then to submit the crude dimethyl trans-2-decenedioate to the next step in the reaction sequence. This involved a partial hydrolysis of the dimethyl ester with one

mole of potassium hydroxide in aqueous methanol (page 68). The equivalent weight of the half-ester product (monomethyl ester of trans-2-decenedioic acid) was found to be 258.1. The theoretical equivalent weight of the product was 214.7. This is a large difference, even for a crude product. However, it was decided to continue with this crude ester through the remaining steps of the reaction (pages 69-71). The final product obtained from this treatment was distilled under reduced pressure. The desired product was methyl trans-10-oxo-2-undecenoate, a new compound. The infrared spectrum which was determined both as a liquid film and as a carbon disulphide solution showed no absorption at 6.02-6.08 μ. Thus the ester prepared did not have unsaturation in the 2 position (64,68). The infrared data further showed the presence of an acyclic ketone, a saturated ester carbonyl and an acetyl grouping (page 70). The ester obtained was not identified since it was obviously not the desired product. Further experiments were not carried out using this method of synthesis, due to the difficulties that had been encountered.

III. OUGHTON SYNTHESIS (27)

This sequence of reactions was attempted because of the polymerization difficulties which were encountered using the other two synthetic routes.

A. 5-Bromoamyl Acetate

This compound was prepared following Oughton's method (27) and its physical characteristics agreed very well with the reported.

B. 7-Oxooctanol

This oxoalcohol was also prepared by following the procedure of Oughton and its identity was verified by comparison of its physical characteristics to the reported values.

C. 7-Oxooctanal

Oxidation of the 7-oxooctanol with potassium dichromate in glacial acetic acid (pages 72-73) produced a very low yield of 7-oxooctanal. This product was purified by distillation under reduced pressure and had a fairly large boiling range (154-180°C./43 mm.). The sodium bisulphite addition compound was prepared and it was a white, crystalline solid.

D. 4-Bromobutyl Acetate

Two attempts were made to synthesize this compound (pages 73-74) by the same method used for the preparation of

5-bromoamyl acetate (27). In both experiments a mixture that could not be separated by fractional distillation was obtained. This verified the work of Cloke and Pilgrim (72) who found that the material responsible for the difficulties met in separation was tetramethylene dibromide. This reaction was not repeated due to the formation of this mixture.

E. 4-Chlorobutyl Acetate

As a substitute for 4-bromobutyl acetate, 4-chlorobutyl acetate was prepared by the reaction of acetyl chloride with anhydrous tetrahydrofuran (72). The reaction was successful and fractional distillation yielded a pure product whose physical properties agreed very well with those reported for this compound.

Since the Barbier and Hugel synthesis (23) was now yielding the desired α,β-unsaturated oxoacids, further syntheses of this sequence were not carried out.

IV. MISCELLANEOUS SYNTHESES

A. Trans-9-hydroxy-2-decenoic Acid

Trans-9-oxo-2-decenoic acid was successfully reduced by lithium aluminum hydride in anhydrous ether (page 75) (77) and the product was distilled under reduced pressure. Thin-

layer chromatography on silica gel plates using benzene/methanol/acetic acid (90:16:8) showed that the distilled product still contained a small amount of unreduced trans-9-oxo-2-decenoic acid. The lithium aluminum hydride reduction was repeated using anhydrous tetrahydrofuran as the solvent with refluxing for three hours (page 77). Under these more drastic conditions all of the starting material was reduced to trans-9-hydroxy-2-decenoic acid. This was demonstrated by thin-layer chromatography of the reaction mixture.

B. Reaction of Trans-9-oxo-2-decenoic Acid with Bromine

An attempt was made to add bromine across the double bond of this α,β-unsaturated oxoacid. The infrared spectrum of the isolated product did not have an absorption band at 6.02-6.08 μ and hence did not possess α,β-unsaturation (64,68). The spectrum also showed the presence of a broad band at 15.4 μ, which can possibly be attributed to C-Br (64). The evolution of white fumes during the reaction indicated that substitution was probably occurring simultaneously (73). Also since the yield of product (2.90 g.) was greater than the theoretical yield (1.86 g.), (on the basis of 9-oxo-2,3-dibromodecanoic acid), it was shown that substitution was accompanying addition. The product was not identified. Since the reaction did not yield the desired 9-oxo-2,3-dibromodecanoic acid it was not repeated.

CONCLUSION

The 1-alkyl-1-cycloalkenes which were prepared as intermediates in the Barbier and Hugel synthesis are currently being investigated for their effect on certain insects by Dr. M.S. Quraishi*.

Many problems were encountered during the course of this study and several of these remain unresolved. The polymerization of the oxoaldehydes, dimethyl trans-2-hexenedioate and dimethyl trans-2-decenedioate possibly warrant further study.

Currently, investigation is being carried out regarding the possible purification of the crude acid products by gas chromatographic methods. During the course of this work it is hoped to find if the crude products are indeed mixtures of the unsaturated and saturated oxoacids. If separation and purification are possible using this technique the purified unsaturated acids will be submitted to insect physiological study by Dr. Quraishi.

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