STUDIES ON THE FAWORSKY REACTION

AND ON MOULD METABOLITES.

THESIS

presented to the University of Glasgow

for the Degree of Ph.D.

by

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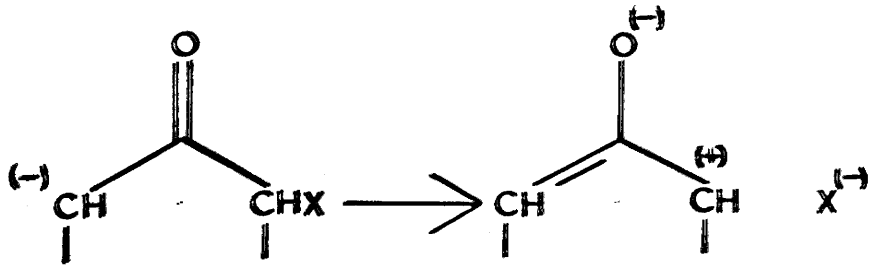
Introduction.

The Faworsky rearrangement [1, 2] involves the skeletal rearrangement of α-halogenated ketones in the presence of certain nucleophilic bases to give a variety of carboxylic derivatives.

Since the description of this rearrangement by Faworsky in 1894, successive investigations have largely clarified its scope, mechanism and more recently its stereochemistry.

The nature of the intermediate involved in such rearrangements seems now to be well established.

Following a study of the Faworsky rearrangement of 2-chlorocyclohexanone, which was labelled with C^{14} in the C_1 and C_2 positions, Loftfield [3], in order to account for the products with the isotope distributions (i) and (ii) postulated a mechanism involving a cyclopropanone intermediate. He postulated that the initial step was abstraction of a proton from the α carbon atom of the haloketone; the resulting enolate ion (iii) then underwent either concerted or consecutive intramolecular ejection of the halide ion to form a cyclopropanone intermediate (iv), cleavage of which gave the products with the expected labelling.



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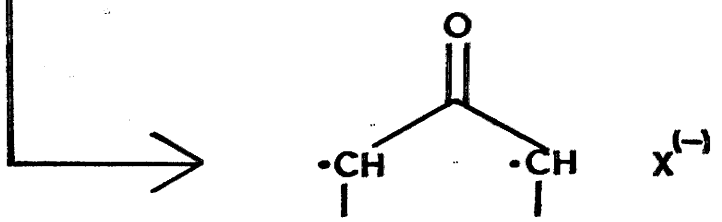
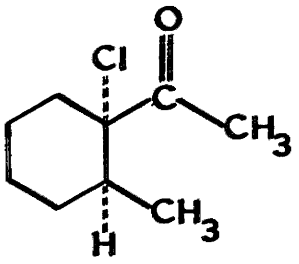


(VII)

(1)



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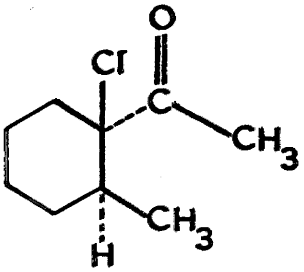

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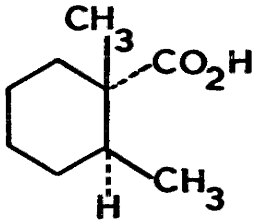

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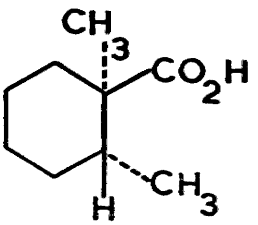
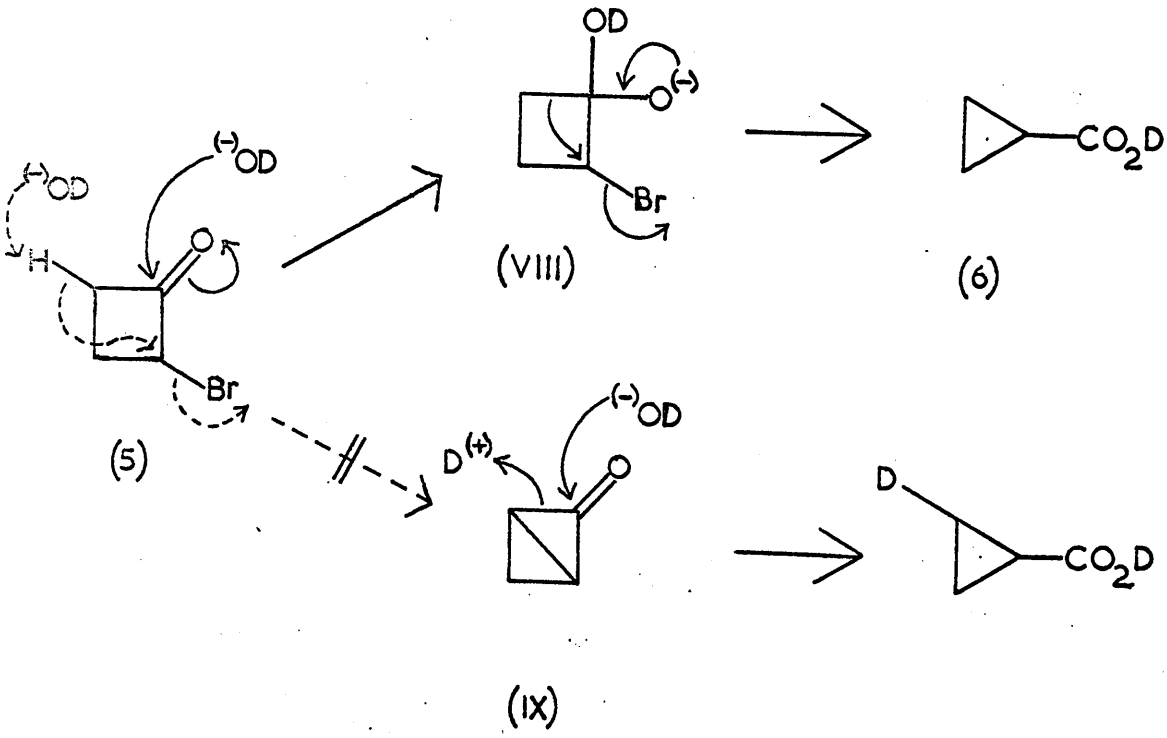


Although they accepted the concept of a cyclopropanone intermediate, Aston and Newkirk [4], and Burr and Dewar, [5] questioned the mode of formation of such an intermediate as proposed by Loftfield. They suggested that the enolate anion (v) gives rise either to a zwitterion (vi) or to a no-bond canonical form of a cyclopropanone (vii) and that such intermediates could then readily collapse to form the expected cylopropanone.

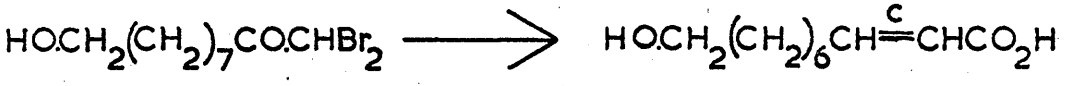
The concerted mechanism for the cyclopropanone formation as proposed by Loftfield necessitates stereochemical inversion about the carbon atom bearing the halogen, whereas the modified theory of Burr and Dewar would require racemisation about the same centre unless there was a degree of shielding by the departing halogen ion.

Experiments on cis and trans 1-acetyl-1-chloro-2-methylcyclohexanone (1 and 2 respectively) by Stork and Borowitz [6] suggested that the Faworski rearrangement was in fact stereospecific. The products, cis and trans 1, 2 -dimethylcyclohexane carboxylic acids (3 and 4 respectively), indicated that the resulting new carbon-carbon bond had the opposite configuration from that of the departing halogen, thus supporting the mechanism proposed by Loftfield. House and Gilmore [7] however, found that the rearrangement of cis-1-acetyl-


OD
(-)
Br
(VIII)
CO2D
(6)
(-)OD
H
Br
(5)
D(+)
(-)OD
D
CO2D
(IX)

1-chloro-2-methylcyclohexanone (1) was stereospecific in non polar solvents but non-stereospecific in polar media. It thus appeard most unwise to assume that a Faworsky rearrangement in an unstudied system would be stereospecific irrespective of what reaction conditions were employed.

Recent data from the rearrangement of monobromocyclobutanone (5) by Conia and Salaun [8] seem to favour the unsymmentric semi-benzylic mechanism proposed by Tchoubar and Sackur. [9] They found that the rearrangement of the bromocyclobutanone (5) in presence of heavy water gave cyclopropane carboxylic acid (6) with no deuterium on the ring carbon atoms. The cyclopropanone intermediate (ix) would necessitate the presence of a deuterium in the C_3 ring and the absence of such a product can only be explained by an unsymmetric semi-benzylic intermediate (viii). However this mechanism has not found general application apart from this isolated case.



(7) (8)

(9) (10) (11)

Discussion

A comparatively neglected aspect of the Faworsky rearrangement has been its application to geminal dihalogeno-ketones. The unexpected stereospecificity in the conversion of the α,α-dibromoketone (7) to the thermodynamically less stable cis - α,β-unsaturated carboxylic derivative (8) [10] prompted a more detailed investigation with simpler compounds of the mechanism of this little studied example of the Faworsky rearrangement.

A series of 1, 1-dibromo-ketones were prepared in high yield by treatment of the corresponding terminal acetylenes (9) with N,N-dibromodimethylhydantoin ("bromodan" in 50% aqueous acetic acid buffered with sodium acetate. The reaction was carried out at room temperature, the products being readily purified by distillation under reduced pressure. It had been reported [11] that the 1, 1-dibromo-ketones (10, R=H and CH_3) undergo rearrangement at room temperature to 1, 3-dibromo-ketones, but the higher homologues prepared above showed no such tendency to rearrange. Reaction of the dibromo-ketones with methanolic sodium methoxide (20 minutes at 0^o) and methanolic triethylamine ($2\frac{1}{2}$ hours at room temperature)

(a) $CH_3CH_2^cCH^b{=}CH^aCO_2CH_3$

Proton	Chemical Shift(τ)	Multiplicity	Jc/s
H_a	4·36	2 triplets	J_{a-b}=11·5
			J_{a-c} =1·5
H_b	3·88	2 triplets	J_{b-a} =11·5
			J_{b-c} =7·5
H_c	7·39	unresolved multiplet.	

(b) $CH_3(CH_2)_3CH_2^cCH^b{=}CH^aCO_2CH_3$

H_a	4·33	2 triplets	J_{a-b} =11·5
			J_{a-c} =1·5
H_b	3·85	2 triplets	J_{b-a} =11·5
			J_{b-c} =7·5
H_c	7·38	unresolved multiplet.	

Figure 1

$RCH_2COCHBr_2$ (10)

$RCH=\overset{c}{C}HCO_2CH_3$ (11)

R	Yield % (10)	Yield % (11) (a)	Yield % (11) (b)
CH_3CH_2-	82	65	73
$n\text{-}C_5H_{11}-$	86	61	75
$CH_3O_2C(CH_2)_7-$	87	75	73

(a) NaOMe (b) $Et_3N/MeOH$

Table 1

followed by chromatography on silica gave the pure cis- α, β -unsaturated esters (11). No trans isomer was detected at any stage by i.r., n.m.r. spectroscopy or g.l.c. The 100 megacycle n.m.r. spectra of the products were compatible with the expected cis unsaturation, the vinyl protons and the adjacent methylene protons exhibiting a perfect ABX_2 splitting pattern (figure 1). It is known that the trans-olefinic coupling constant is greater than the cis-olefinic constant, J_{trans} being about 18 c/s, J_{cis} being about 10 c/s [12]. The coupling constants observed in the spectra of the α.β -unsaturated esters was 11·5 c/s confirming the presence of a cis double bond. The i.r. spectra showed absorption at 1645 cm^{-1} (C=C stretch), and 822 cm^{-1} (=C-H out of plane deformation) [13] providing further confirmation of the cis structure of the esters. The results are summarised opposite (table 1).

For comparison, the 1, 3-dibromo compounds of pentan-2-one (12) and octan-2-one (13) were subjected to the above Faworsky conditions. The dibromo-ketones were prepared by the acid catalysed addition of bromine to the corresponding ketones. [14] This gave rise to a mixture of mono-, di-, and tribromo-ketones, which were efficiently separated using a "spinning band" column

(a) H_a, H_b $CBrCOCH_cBrCH_2CH_3$

Proton	Chemical Shift(τ)	Multiplicity	J c/s
H_a	5·969	doublet	J_{a-b} =13
H_b	5·721	doublet	J_{b-a} =13
H_c	5·43	triplet	J_{c-H} = 7

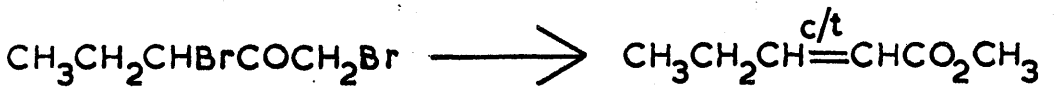
(b) H_a, H_b $CBrCOCH_cBr(CH_2)_4CH_3$

H_a	5·979	doublet	J_{a-b} =13
H_b	5·731	doublet	J_{b-a} =13
H_c	5·39	triplet	J_{c-H} = 7

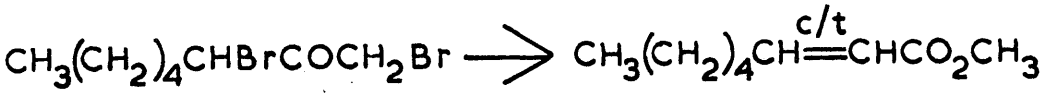
Figure 2

during distillation under reduced pressure, the yields of the purified dibromo-ketones being around 50%. The 100 megacycle n.m.r. spectra of the products confirmed that they were 1, 3-dibromo substituted ketones. (figure 2). The C_1 protons were found to be non equivalent giving rise to an AB splitting pattern, the calculated values for the protons being 5·97 τ and 5·72 τ , the geminal coupling constant (J_{A-B} = 13 c/s) being compatible with the structure. [12.] The C_3 protons gave rise to a triplet centred at 5·4 τ , having a coupling constant of 7 c/s. By comparison, the 1, 1-dibromo compounds exhibited a sharp singlet at 4·12 , attributable to the C_1 proton. Traces of tribromo impurities could be detected by the presence of signals at approximately 3·2 τ ($CHBr_2$ peak in the 1, 1, 3 -isomer) or 4·8 τ (CH_2Br peak in the 1, 3, 3 isomer). I.r. absorption frequencies of the carbonyl function also proved very characteristic, the 1, 1-dibromo compounds absorbing at 1720 cm^{-1}, the 1, 3-dibromo compounds at 1722 cm^{-1} and the 1, 1, 3- and 1, 3, 3 - tribromo compounds at 1728 cm^{-1}.

1, 3-dibromo-pentan-2-one and 1, 3-dibromo-octan-2-one on being subjected to the above rearrangement conditions did not exhibit complete stereospecificity examination of the purified products by g.l.c. (10% A.P.L. at 100°)



(12) (14)



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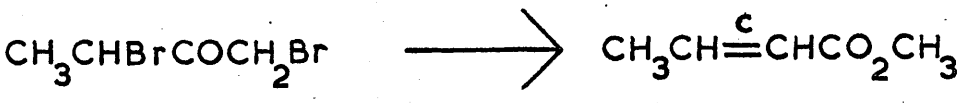


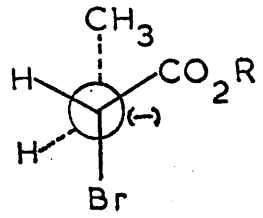
(16) (17)

indicating that both cis and trans unsaturated esters were present (14, 15). The ratio cis/trans was calculated from the area under the curves in the g.l.c. trace and in all cases was found to be 9/1.

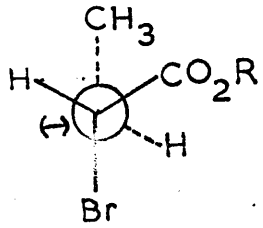
It had been previously reported [15] that the Faworsky rearrangement gave the desired products using aqueous bases such as potassium bicarbonate. Experiments on 1, 3-dibromo-pentan-2-one using sodium bicarbonate in aqueous methanolic solution gave 80% of the cis- and 20% of the trans-pent-2-enoate. The presence of methanol was necessary to give a homogeneous reaction mixture but in the absence of methanol the rearrangement did not take place.

Thus the examination of the 1, 1- and 1,3-dibromo compounds of pentan-2-one and octan-2-one under Faworsky rearrangement conditions indicated that complete stereospecificity is only achieved with the 1, 1-dibromo isomers.

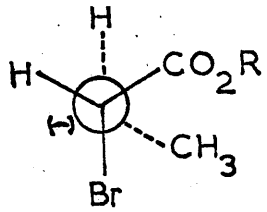
However in 1963, Rappe [15] found that the rearrangement of 1, 3-dibromo-butan-2-one (16) gave cis-crotonic acid (17) with no detectable trace of the trans isomer. In a series of experiments on polybromo-ketones, Rappe reported that in several cases the rearrangement exhibited complete stereospecificity. He offered the following argument concerning the rearrangement of 1, 3-dibromo-



XI



XII



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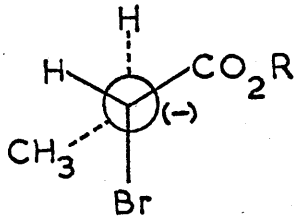
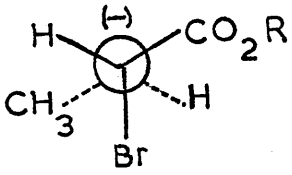
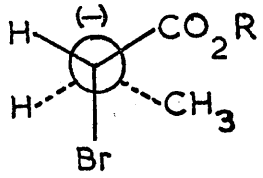
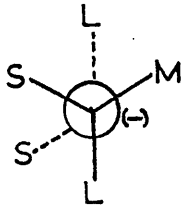


XIV







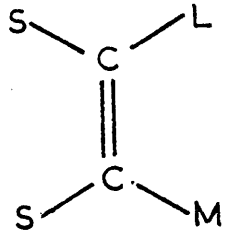


Figure 4

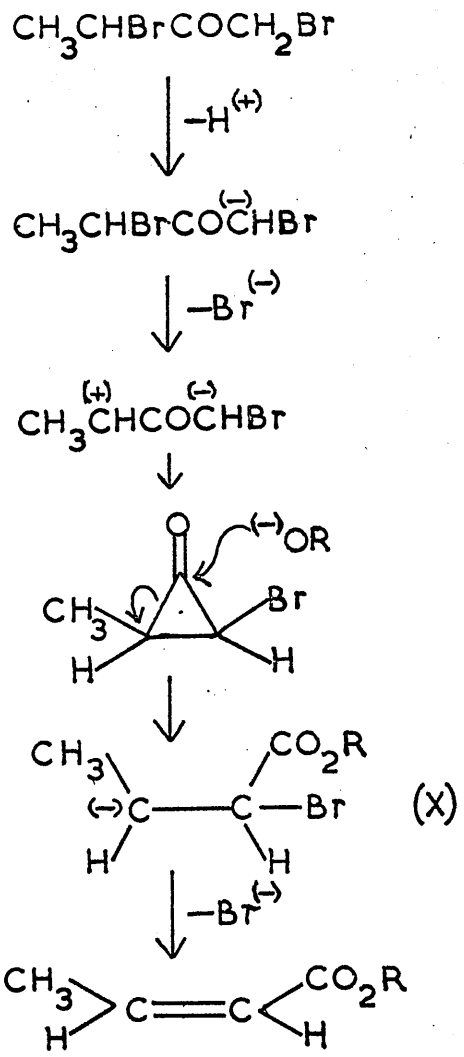

CH3CHBrCOCH2Br
−H(+)
CH3CHBrCO(−)CHBr
−Br(−)
CH3(+)CHCO(−)CHBr
(−)OR
CH3
Br
H
H
CH3
CO2R
(−)C
C
Br
H
H
(X)
−Br(−)
CH3
H
C
C
CO2R
H

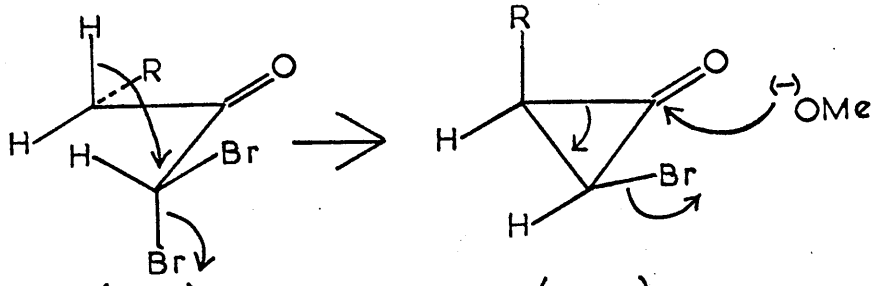
Figure 3

butan-2-one (figure 3).[22]

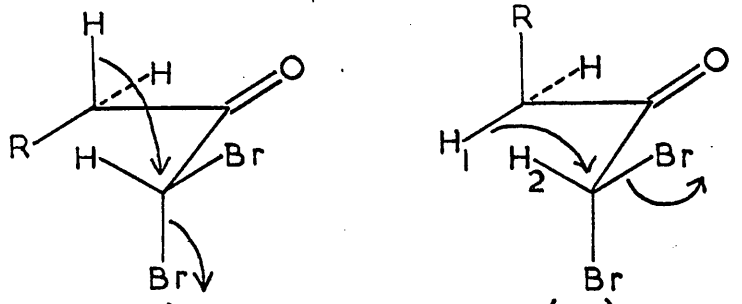
Accepting the intermediacy of a cyclopropanone, it was assumed that the formation and cleavage of this did not involve a concerted mechanism. It was proposed that the carbanion (x) is an intermediate and that this can attain six conformations (xi) to (xvi), through rapid inversion along with free rotation about the carbon-carbon bond. It was suggested that of these six configurations (xi) and (xii) are the most sterically favoured (figure 4).

In these two forms, the methyl group, the largest group at the β-carbon was placed anti to the bromine, the largest group at the α-carbon. Rappe suggested that (a) in (xi) steric repulsions are minimised and that (b) this form would give rise to the cis- α, β-unsaturated ester.

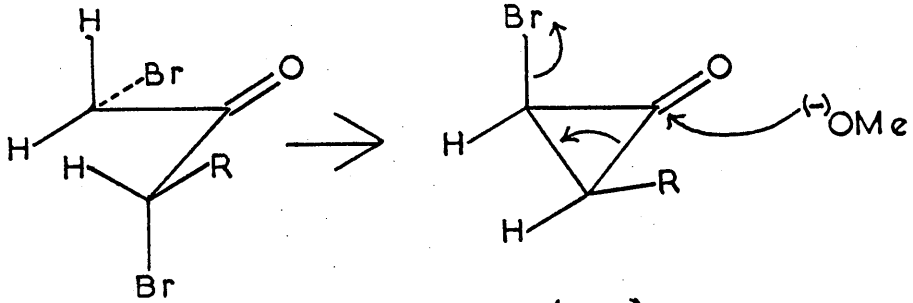
The former suggestion (a) is based on the relative spacial sizes of a proton and a lone pair of electrons. It seems unlikely that this effect would be significant in comparison with the electronic interactions of the lone pair. the carboxylate function and the bromine which would be expected to favour conformation (xii). Further, the conformation (xi) does not possess the correct geometry to allow simple expulsion of the bromine to form the



(XVII) (XVIII)



(XIX) (XX)



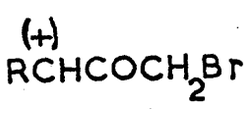
(XXI) (XXII)

double bond. In this reacting intermediate the groups are not favourably placed to allow internal nucleophilic displacement of the bromine as an anion. In fact the reacting conformations would be expected to be (xv) or (xvi), with the steric interactions of the methyl, carboxylate and bromine functions being minimised in (xv). Conformation (xv), on displacement of the bromine would give the trans- α,β -unsaturated ester!

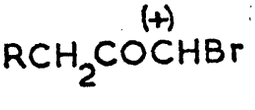
The observed stereospecificity of the rearrangement of 1, 1-dibromo-pentan-2-one (10,R=Et) and 1,1-dibromo-octan-2-one (10, R=n-C_5H_{11}) can be rationalised by assuming, in contrast to Rappe, that both the formation and cleavage of the intermediate 3-alkyl-2-bromo-cyclopropanones must involve concerted processes. This in turn implies that the exclusive production of a cis- α,β -unsaturated ester demands the exclusive intermediacy of the cis-cyclopropanone (xviii). Examination of models strongly suggests that minimal unfavourable steric interaction is attained by a trans-anti-parallel 1, 3-elimination involving the transition state (xvii). In these positions, the bulky alkyl group is as far removed as possible from the bromines, the only possible steric interactions occuring between two hydrogens nearly in the plane of the carbonyl

group but this is negligible compared with the corresponding interactions of a hydrogen and an alkyl or a bromine group in other conformations (eg. (xix) giving trans unsaturated esters). Transition state (xvii) is precisely the one which would give rise to the cis-cyclopropanone intermediate (xviii). In conformation (xx), steric interactions are minimal, but a trans coplanar 1, 3-elimination of hydrogen bromide as shown, is hindered by the proximity of H_1 and H_2. Thus in the case of 1, 1-dibromo-ketones, it appears that it is the nature of the starting dibromo-ketone which determines the geometry of the product.

This argument can be applied successfully to the predominant formation of cis-α,β-unsaturated esters from 1, 3-dibromo-ketones, the transition state (xxi) giving rise to the cis-cyclopropanone (xxii). Since the same arguments apply, the concerted reaction should lead exclusively to the cis-ester, but the fact that some proportion of the trans isomer is formed, may suggest that an alternative competing mechanism can occur in the rearrangement of the 1, 3-dibromo-ketones which is not so favourable in the case of the 1, 1-dibromo-ketones. If the initial step in the rearrangement were the expulsion of a bromine anion, the carbonium ions (xxiii) and (xxiv)



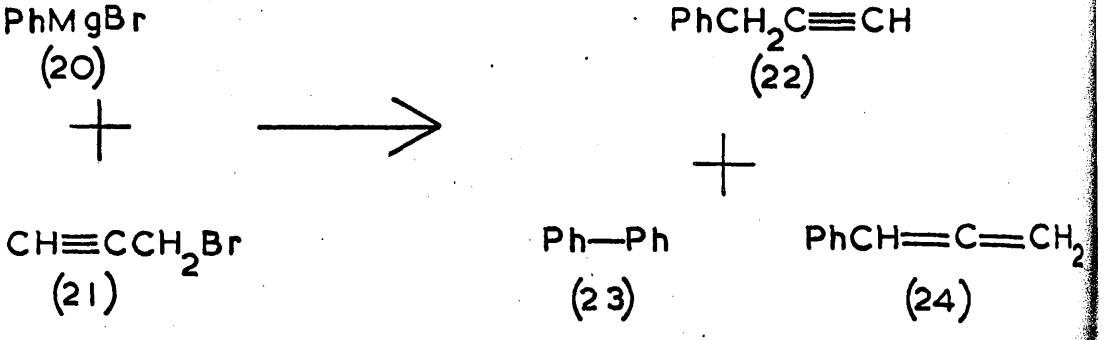
(XXIII)



(XXIV)

would be formed from the 1, 3- and 1, 1-dibromo-ketones respectively. Such carbonium ions could form both cis- and trans- cyclopropanones thus giving rise to both cis- and trans- α, β -unsaturated esters. The more thermodynamically stable trans-cyclopropanone would be expected to predominate. Carbonium ion (xxiv) has a bromine atom bonded to the charged carbon atom and a comparison of the rates of solvolysis of benzyl bromide and benzal bromide suggests [23] that in such a situation the expected increase in stability of the carbonium ion by the sharing of the positive charge with p-orbital π-overlap is cancelled out by the inductive effect of the bromine producing greater electro-negativity in the reacting carbon atom making it less able to support a positive charge. Carbonium ion (xxiii) however. has increased stability due to the electron repelling inductive effect of the neighbouring α-alkyl group. Thus the possibility of ionisation is greater in the case of the 1, 3-dibromo-ketones, the carbonium ion formed being more stable than that from the 1, 1-dibromo-ketones. This is supported by the presence of 10% trans- α, β -unsaturated ester in the products from the rearrangement of the 1, 3-dibromo-ketones.

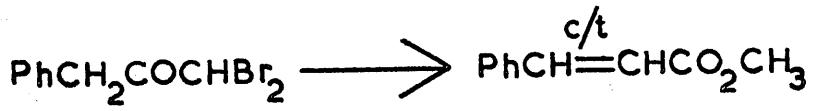
Thus, according to the above interpretation, the


PhMgBr
(20)
CH≡CCH2Br
(21)
PhCH2C≡CH
(22)
Ph—Ph
(23)
PhCH=C=CH2
(24)

previously rearrangement to give cis- α, β -unsaturated esters proceeds via the concerted formation and cleavage of a cis-cyclopropanone, while the formation of the trans isomer necessitates initial ionisation to yield a carbonium ion, the stability of this ion determining the proportion of trans- α, β -unsaturated ester in the product.

The effect of an aryl rather than an alkyl substituent was also studied. 1,1-dibromo-3-phenylacetone (18) was prepared by the reaction of "bromodan" on benzyl acetylene (22), purification of the product proving impossible since decomposition occurred on heating. Benzyl acetylene (22) could only be obtained in approximately 25% yield from the sequence shown opposite, but this still appeared to be the best preparative method. [24-26].

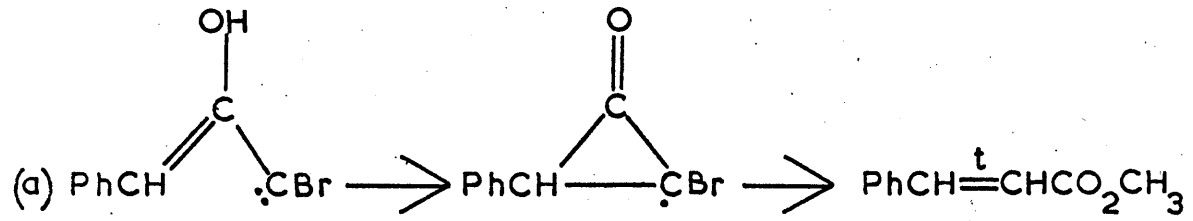
The acetylene was separated from the reaction products by preparing the silver acetylide, which was collected by filtration, the acetylene being regenerated by steam distillation from 5% hydrochloric acid solution. The acetylene was finally purified by distillation under reduced pressure. The 100 megacycle n.m.r. spectrum of the product was compatible with the expected structure, no evidence of the diphenyl (23) or phenyl allene (24) by-products being present. The acetylenic proton gave a

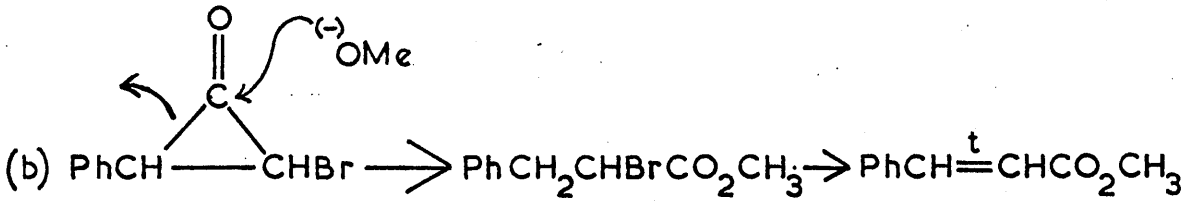


(18) (19)

sharp triplet centred at 8·01 τ with a coupling constant of 2c/s, while the benzylic protons gave rise to a doublet centred at 6·55 τ having a coupling constant of 2c/s. This long range coupling through four bonds was confirmed by spin decoupling. Infra red absorption values also indicated that the product was pure acetylene, the characteristic absorptions being 3350, 2140, 740 and 705 cm^{-1}. Although there were unattractive aspects of this method of preparation, the advantage over the other published syntheses,[27-34] was that the starting materials were readily accessible. The other synthetic routes reported in the literature appeared to be much more cumbersome and gave no information about the purity of the product.

1, 1-dibromo-3-phenylacetone (18) on rearrangement with methanolic sodium methoxide gave the expected cis-methyl cinnamate (19), as indicated by examination of the purified reaction product on g.l.c. (1% P.E.G.A. at 100°). However rearrangement with methanolic triethylamine gave only 30% cis-methyl cinnamate, 70% of the trans isomer being obtained. On repeating the reaction at -10° (ice/salt bath), the ratio of cis/trans cinnamate was found to be 1/4. This suggested that on lowering the temperature still further, the rearrangement would give trans cinnamate


(a) PhCH
OH
C
CBr
PhCH
C
CBr
PhCH=CHCO2CH3
t


(b) PhCH
CHBr
(-)OMe
PhCH2CHBrCO2CH3
PhCH=CHCO2CH3
t

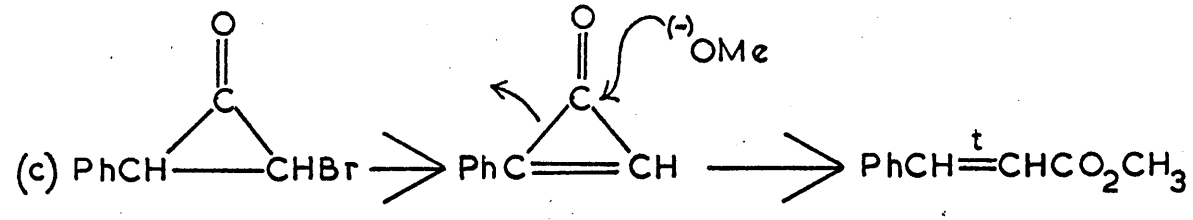

(c) PhCH
CHBr
(-)OMe
PhC=CH
PhCH=CHCO2CH3
t

Figure 5

exclusively, thus reversing the expected stereo-specificity. On repeating the reaction at -80° (acetone/CO_2 bath), the product showed only one volatile component when examined on g.l.c. which was neither cis- nor trans-methyl cinnamate. T.l.c. indicated that there were at least four compounds in the product, the major one having an R_f value similar to that of trans cinnamate. The i.r. spectrum was similar to that of cis cinnamate but the U.V. spectrum indicated end absorption only. It was thought that this product may be an intermediate in the formation of cis/trans cinnamate, but on allowing the product to stand with methanolic triethylamine at room temperature no reaction took place.

The unexpected formation of methyl trans cinnamate in the triethylamine/methanol reaction poses an interesting problem. Several mechanisms can be envisaged, as shown in figure 5, involving the intermediacy of

(a) a carbene

(b) methyl α-bromo-dihydrocinnamate

and (c) a cyclopropenone.

A reaction was carried out on 1, 1-dibromo-3-phenyl-acetone (18) with methanolic triethylamine in the presence of an equimolar quantity of cyclohexene. If a carbene was

indeed formed, it might at least be partially trapped by the cyclohexene, but the product was identical in all respects with that obtained from the normal triethylamine rearrangement. Preparation of methyl α-bromo-dihydrocinnamate and treatment of this with triethylamine in methanol at room temperature, showed that this was unlikely to be an intermediate since, after the usual reaction time, very little elimination of hydrogen bromide had occurred.

Extensive studies, detailed in the following section, were undertaken concerning the synthesis and properties of cyclopropenones. Since phenyl cyclopropenone could not be isolated, the suggestion that this may be an intermediate in the formation of methyl <u>trans</u> cinnamate cannot be ruled out.

Experimental Procedure.

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected; boiling points are uncorrected. Thin-layer chromatoplates were prepared from Kieselgel G; preparative plates from Kieselgel H (1 mm. thick). Infra red spectra were measured with a Unicam SP. 200 instrument and for high resolution spectra, (KBr discs and solution spectra), with a Unicam SP. 100 double beam infra red spectrophotometer equipped with an SP. 130 sodium chloride prism grating double-monochromater, operated under vacuum. Ultraviolet absorption spectra were measured using a Unicam SP. 800 instrument; OH^- refers to the addition of four drops of 4N sodium hydroxide solution to both the sample and reference cells. Nuclear magnetic resonance spectra were recorded with a Perkin-Elmer R.10 60 Mc/s. spectrometer and with a Varian HA.-100 100 Mc/s spectrometer, using tetramethylsilane as internal standard. Mass spectra were obtained with an A.E.I. MS 9 double focussing mass spectrometer. Molecular weight measurements were made using a Mechrolab 301 A vapour pressure osmometer. Analytical gas-liquid chromatography was performed on a Pye Argon Chromatograph and gas chromatograms/mass spectra were obtained using an LKB-9000 instrumen-

Hydrogenations were carried out at atmospheric temperature and pressure. All organic extracts were dried over anhydrous magnesium sulphate and evaporation of solvents carried out using a rotary film evaporator.

Where appropriate, all solvents and liquid reagents were purified in the prescribed manner, and all solids recrystallised. The following abbreviations are used in reporting n.m.r. spectra:- s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Experimental

1, 1-dibromo-octan-2-one (10, R=n-C_5H_{11})

Oct-1-yne (5·0g.) was added dropwise to a cooled, stirred suspension of "bromodan" (22·0g; 3 equivs.) and sodium acetate trihydrate (21·5g; 3 equivs.) in 50% aqueous acetic acid (300 ml.). The acetylene was added slowly with stirring over 15 minutes and the mixture was then stirred at 0°-3° for 6 hours and then added to water (1 litre). The whole was extracted with methylene chloride (3x200 ml.) and the combined organic extracts washed with saturated sodium meta-bisulphite solution (2x50 ml.), water (2x150 ml.) and then carefully neutralised with saturated sodium bi-carbonate solution. After washing the extract with brine, evaporation of solvent gave a yellow oil which was purified by distillation under reduced pressure to give a pale yellow oil. (11·32 g; 86%).

b.p. 68°-69°/0·09 mm. n_D^{21} 1·4958.

(literature [34]: b.p. 80°-82°/0·4 mm. n_D^{21} 1·4950).

$\nu_{max.}$ (thin film) 1720 cm^{-1}.

Tau values (CCl_4): 9·08 (3H, t, J = 7c/s), 8·66 (8H, m), 7·11 (2H, t, J = 7c/s), 4·11 (1H, s).

Octan - 1,2 - dione dioxime.

1, 1-dibromo-octan-2-one (1·46g) was dissolved in pyridine (60 ml.) and hydroxylamine hydrochloride (2·08g; 3 equivs.) added. The mixture was heated at 100° for 2 hours, poured into water and extracted with ether (4x20 ml.) After washing the combined organic extracts with 2N hydrochloric acid (2x20 ml.) and water (4x20 ml.) removal of the solvent gave a brown solid which recrystallised from benzene as fine white needles. (0·75g; 87%). m.p. 125°-126°.

$\nu_{max.}$ (KBr disc) 3300 (broad), 1100, 952, 868 cm^{-1}.

$\lambda_{max.}^{EtOH}$ 232 mμ ($\varepsilon = 1{\cdot}72 \times 10^4$)

$\lambda_{max.}^{OH^-}$ 272 mμ ($\varepsilon = 2{\cdot}41 \times 10^4$)

Tau values (pyridine) 9·2 (3H, ill defined triplet), 8·72 (6H,m), 8·15 (2H,m), 6·94 (2H, t, J = 8·5c/s), 2·35 (1H,s), -2·88 (2H, broad singlet; disappears on D_2O addition).

($C_8H_{16}N_2O_2$ requires C, 55·79; H, 9·44; N, 16·27
found C, 55·86; H, 9·32; N, 16·60%)

1, 1-dibromo-pentan-2-one (10, R = Et)

Pent-1-yne was treated as above with "bromodan" in acetic acid to give a pale yellow oil which was purified by distillation under reduced pressure (82% yield from acetylene).

b.p. 48°-50°/0·08 mm. n_D^{23} 1·5103

(literature;[35] b.p. 73°-76°/10 mm. n_D^{25} 1·5030 (98% pure)).

$\nu_{max.}$ (thin film) 1720 cm^{-1}

Tau values (CCl_4): 9·05 (3H, t, J = 7c/s), 8·25 (2H, m), 7·10 (2H, t, J = 7·5c/s), 4·14 (1H, s).

Pentan-1, 2-dione dioxime.

1, 1-dibromo-pentan-2-one was treated as above with hydroxylamine hydrochloride in pyridine to give a brown solid which recrystallised as fine white needles from benzene. m.p. 120°-121°.

$\nu_{max.}$ (KBr disc) 3300 (broad), 1258, 1092, 953, 908, 884, 856 cm^{-1}.

$\lambda_{max.}^{EtOh}$ 232 mμ ($\varepsilon = 1{\cdot}57 \times 10^4$)

$\lambda_{max.}^{OH^-}$ 271 mμ ($\varepsilon = 1{\cdot}91 \times 10^4$)

Tau values (pyridine): 8·99 (3H, t, J = 7c/s), 8·15 (2H, sextuplet, J = 7c/s), 6·98 (2H, t, J = 7c/s), 1·63 (1H, s), - 3·55 (2H, broad singlet; disappears on D_2O addition).

Octan-2-one.

Secondary octyl alcohol (120g.) was added dropwise with vigorous stirring over 1½ hours to a solution of sodium dichromate dihydrate (90g.) in water (600 ml.) and concentrated sulphuric acid (120 ml.), and the mixture was heated under reflux for 2 hours and then steam distilled

The distillate was extracted with ether (3x40 ml.) and the combined organic extracts were washed with water (3x30 ml.). Removal of solvent gave a pale yellow oil which was purified by distillation (106g: 90%).

b.p. 61°-62°/15 mm. n_D^{21} 1·4156

(literature [36]: b.p. 59°-60°/12 mm. n_D^{20} 1·4151).

1, 3-dibromo-octan-2-one (13)

48% aqueous hydrogen bromide (10 ml.) was added at 0° to octan-2-one (12·8g: 0·1M.) and bromine (32g: 0·2M.) was added dropwise with stirring, allowing the bromine colour to disappear before the addition of the next drop. Immediately after the addition, water (10 ml.) was added and the heavy organic layer separated, dried and distilled under reduced pressure. The fraction collected between 70°-90°/0·1 mm was redistilled using a "spinning band" column to give a pale yellow oil (16·58g; 58%).

b.p. 103°-105°/0·7 mm. n_D^{22} 1·5007

$\nu_{max.}$ (thin film) 1722 cm^{-1}.

Tau values ($CDCl_3$): HA-100 instrument: 9·09 (3H, t, J = 7c/s), 8·64 (6H, m), 8·0 (2H, m), 5·979 (1H, d, J = 13 c/s), 5·731 (1H, d, J = 13 c/s), 5·39 (1H, t, J = 7 c/s).

($C_8H_{14}OBr_2$ requires C, 33·57; H, 5·06; Br, 55·61

found C, 33·25; H, 4·89; Br, 55·94%).

1, 3-dibromo-pentan-2-one (12).

Pentan-2-one was treated as above with bromine in aqueous hydrogen bromide and the product purified by distillation under reduced pressure on the "spinning band" column. (53% yield).

b.p. 63°-65°/0·6 mm. n_D^{22} 1·5162

(literature [14]: b.p. 88°-89°/10 mm. n_D^{25} 1·5176)

$\nu_{max.}$ (thin film) 1722 cm^{-1}.

Tau values ($CDCl_3$): 8·95 (3H, t, J = 7 c/s), 7·93 (2H sextuplet, J = 7 c/s), 5·969 (1H, d, J = 13 c/s), 5·721 (1H, d, J = 13 c/s), 5·43 (1H, t, J = 7 c/s).

Undec-10-ynoic acid.

Undec-10-enoic acid (98g: 0·5317) was dissolved in light petroleum ether (250 ml.) and a solution of bromine (27·5 ml; 0·53 M) was dissolved in light petroleum ether (35 ml.) added slowly with stirring and cooling. Stirring was stopped after the addition and a yellowish precipitate slowly formed; this was filtered off and the filtrate concentrated to yield a further crop of solid (156 g; 85). This product was added to a concentrated solution of potassium hydroxide (250 g.) in water (150 ml.) in a 3 litre flask and heated at 150°-160°

for 8 hours. The cooled mixture was added to water (1 litre) and after acidification with concentrated sulphuric acid, the aqueous solution was extracted with ether (3x150 ml.) and the combined organic extracts thoroughly washed with water and brine. Evaporation gave an oil which solidified on standing and was purified by distillation under reduced pressure (67g; 70%).

b.p. 125°-130°/0·5 mm. m.p. 40°-41°
(Literature [10]: b.p. 177°-182°/15 mm. m.p. 42°).

11, 11-dibromo-10-keto-undecanoic acid.

A solution of undec-10-ynoic acid in 50% aqueous acetic acid was treated as above with "bromodan" for four hours at room temperature to give a brown solid which recrystallised as fine white needles from petroleum ether (52% yield). m.p. 57°-58°. (literature [10]: m.p. 58°).

The m.p. showed no depression when mixed with an authentic sample.

Methyl 11, 11-dibromo-10-keto-undecanoate (10, R = $MeO_2C(CH_2)_7$).

11, 11-dibromo-10-keto-undecanoic acid (1·99g.) in ether was esterified with diazomethane prepared from nitrosan (8·77g.)[37]. The product (1·74g) was obtained as a brown liquid.

ν_{max} (thin film) 1740, 1210, 1180 cm^{-1}.

G.l.c. on 0·5% A.P.L. at 150° indicated that the product was pure, having a retention time of 15 minutes.

Methyl cis-pent-2-enoate (14)

(a) 1, 1-dibromo-pentan-2-one (0·95g.) in anhydrous methanol (1 ml.) was added at 0° to a solution of sodium (0·3g: 3 equivs.) in anhydrous methanol (10 ml.), and the mixture was stirred at 0° for 20 minutes. After pouring into water (30 ml.), the mixture was extracted with ether (4x10 ml.) and the combined organic extracts washed with brine until neutral. Careful evaporation of solvent gave a pale yellow oil.

The product was chromatographed on silica, the following fractions being collected:

(1) Eluted with petroleum ether (40°-60°)-bromine containing by products.

(2) Eluted with benzene - a pale yellow oil (0·29g; 65%).

The product from fraction (2) was purified by distillation under reduced pressure.

b.p. 54-56°/15 mm. n_D^{25} 1·4436.

ν_{max} (CCl_4): 1726, 1641, 1194, 1177, 822 (CS_2) cm^{-1}.

λ_{max}^{EtOH}: end absorption only.

Tau values (CCl_4):- HA-100 instrument: 8·97 (3H, t,

J = 7 c/s), 7·39 (2H, triplet-J = 7 c/s - of doublets - J = 1·5 c/s), 6·38 (3H, s), 4·36 (1H, doublet - J = 11·5 c/s - of triplets - J = 1·5 c/s), 3·88 (1H, doublet - J = 11·5 c/s - of triplets - J = 7·5 c/s).

($C_6H_{10}O_2$ requires C, 63·14; H, 8·83;
found C, 63·19; H, 8·72%)

(b) 1, 1-dibromo-pentan-2-one (0·98g.) in anhydrous methanol (1 ml.) was added dropwise to a stirred solution of triethylamine (1·01g; 2·5 equivs.) in methanol (10 ml.). The mixture was stirred for 2½ hours at room temperature, poured into brine (30 ml.) and extracted thoroughly with ether (4x25 ml.). The combined organic extracts were neutralised carefully with 2N hydrochloric acid, washed with water (2x20 ml.) and evaporated to give a pale yellow oil. The product was chromatographed on silica as above, fraction (2) giving a pale yellow oil (0·31g. 73%).

G.l.c. on 10% A.P.L. at 50° indicated that the product was pure methyl cis-pent-2-enoate, (retention time being 2·8 minutes.

Methyl cis-oct-2-enoate (15).

(a) 1, 1-dibromo-octan-2-one was treated as above with sodium in methanol to give a pale yellow oil, fraction (2) from chromatography giving a pale yellow oil

(61% yield).

This product was purified by distillation under reduced pressure.

b.p. 82°-84°/15 mm. n_D^{25} 1·4366.

(literature [10]: b.p. 68°-72°/20 mm. n_D^{22} 1·4395)

$\nu_{max.}$ (CCl_4): 1726, 1645, 1200, 1172, 816 (CS_2) cm^{-1}.

$\lambda_{max.}^{EtOH}$: end absorption only.

Tau values (CCl_4): HA-100 instrument: 9·12 (3H, t, J = 7 c/s), 8·66 (6H, m), 7·38 (2H, m), 6·38 (3H, s), 4·33 (1H doublet - J = 11·5 c/s - of triplets - J = 1·5 c/s), 3·85 (1H, doublet - J = 11·5 c/s - of triplets - J = 7·5 c/s).

(b). 1, 1-dibromo-octan-2-one was treated as above with triethylamine in methanol, purification of the product by chromatography on silica as above giving a pale yellow oil from fraction (2) (75% yield).

G.l.c. on 10% A.P.L. at 100° indicated that the product was pure methyl cis-oct-2-enoate, retention time being 4·8 minutes.

Dimethyl cis-undec-2-ene-1, 11-dioate (11, R = $MeO_2C.(CH_2)_7$).

(a) Methyl 11, 11-dibromo-10-keto-undecanoate was treated as above with sodium in methanol to give a light brown oil, fraction (2) from chromatography giving a pale

yellow oil (75% yield).

The product was purified by distillation under reduced pressure.

b.p. 130°-132°/0·15 mm. n_D^{22} 1·4415

G.l.c. on 0·5% A.P.L. at 150° indicated that the product was pure, retention time being 2·5 minutes.

($C_{13}H_{22}O_4$ requires C, 64·44; H, 9·15

found C, 64·14; H, 8·92%)

(b) Methyl 11, 11-dibromo-10-keto-undecanoate was treated as above with triethylamine in methanol, chromatography of the product on silica giving fraction (2) as a pale yellow oil (73% yield).

G.l.c. on 0·5% A.P.L. at 150° indicated that the product was pure dimethyl cis-undec-2-ene-1, 11-dioate, retention time being 2·5 minutes.

Faworsky rearrangements of 1, 3-dibromo-pentan-2-one.

1, 3-dibromo-pentan-2-one was treated as above with (a) sodium in methanol (60% yield), and (b) triethylamine in methanol (75% yield).

The products were purified as above by chromatography on silica, g.l.c. indicating that both products contained 90% methyl cis-pent-2-enoate and 10% of the trans isomer, retention times being 2·8 and 4·4 minutes respectively.

Faworsky rearrangements of 1, 3-dibromo-octan-2-one.

1, 3-dibromo-octan-2-one was treated as above with (a) sodium in methanol (60% yield). and (b) triethylamine in methanol (75% yield).

The products were purified as above by chromatography on silica, g.l.c. indicating that both products contained 90% methyl cis-oct-2-enoate and 10% of the trans isomer, retention times being 4·8 and 7·4 minutes respectively.

Benzyl acetylene. (22).

To a stirred mixture of magnesium turnings (31g.) in anhydrous ether (500 ml.), bromobenzene (190g.) was added dropwise with cooling, the reaction being conducted under nitrogen. The reagent was cooled to -20° (CCl_4/CO_2 bath) and propargyl bromide (47·2g.) in ether (150 ml.) added during $1\frac{1}{2}$ hours and after the addition the mixture was kept at -5° for 18 hours and then poured carefully into 6N sulphuric acid (300 ml.) and crushed ice. After the vigorous reaction had subsided, the aqueous layer was extracted with ether (4x50 ml.) and the combined organic extracts washed with water (100 ml.), saturated sodium bicarbonate solution (100 ml.) and water (2x100 ml.) Evaporation of the solvent gave a pale yellow oil.

$\nu_{max.}$ (thin film) 3325, 2120, 1947, 1695, 1600, 770, 710 cm^{-1}.

This oil was dissolved in absolute ethanol (300 ml.) and added to silver nitrate (150g.) dissolved in water (350 ml.). The mixture was stirred for 1 hour and the precipitate filtered at the water pump, the solid being washed with large quantities of water, methanol and ether. The silver acetylide was added to 5% hydrochloric acid (600 ml.) and the mixture steam distilled until oily drops ceased to collect in the distillate. The distillate was extracted with ether (6x100 ml.), and, after washing the combined organic extracts with water until neutral, evaporation of solvent gave a yellow oil which was purified by distillation under reduced pressure. (8·41g; 20% from propargyl bromide).

b.p. 28°-20°/0·2 mm. n_D^{23} 1·5291.

(literature [33]: b.p. 68°-69°/17 mm n_D^{20} 1·509).

$\nu_{max.}$ (CCl_4): 3350, 2140, 1600, 740, 705 cm^{-1}.

Tau values: (CCl_4) :- HA-100 instrument :- 8·01 (1H, t, J = 2 c/s), 6·55 (2H, d, J = 2 c/s), 2·87 (5H,s).

1, 1-dibromo-3-phenyl acetone (18)

Benzyl acetylene (8·41 g.) was added dropwise to a cooled, stirred suspension of "bromodan" (22·5 g; 3 equivs.) and sodium acetate trihydrate (21·75 g.; 3 equivs.) in 50% aqueous acetic acid (450 ml.). The mixture was stirred for 16 hours at 0°, poured into water (650 ml.) and extracted

with methylene chloride (4x100 ml.), the combined organic extracts being washed with saturated sodium metabisulphite solution (2x100 ml.), carefully neutralised with saturated sodium bicarbonate solution and washed with water (3x50 ml.). Evaporation of solvent yielded a yellow oil which rapidly darkened at room temperature and was stored at 0° (18·1g; 87%). The product decomposed on attempted distillation.

$\nu_{max.}$ (thin film) 1738. 1240, 1045, 770, 705 cm^{-1}.

Tau values (CCl_4): 6·24 (2H, m), 4·12 (1H, s), 2·82 (5H. m).

The product formed a red crystalline compound on treatment with 2; 4-dinitrophenylhydrazine. m.p. 208° - 210° (dioxan/methanol).

Faworsky rearrangements of 1, 1-dibromo-3-phenylacetone.

1, 1-dibromo-3-phenylacetone was treated as above with (a) sodium in methanol.

The product was purified as above by chromatography on silica (57% yield), g.l.c. on 1% P.E.G.A. at 100° indicating that the product was pure methyl *cis* cinnamate, retention time being 3·2 minutes, on comparison with an authentic sample:-

(b) triethylamine in methanol at room temperature.

The product was purified as above by chromatog-

raphy on silica (55% yield), g.l.c. indicating that the product contained 30% methyl cis cinnamate and 70% methyl trans cinnamate, retention times being 3·2 minutes and 5·75 minutes respectively, on comparison with authentic samples.

(c) triethylamine in methanol at -8° (ice/salt bath).

G.l.c. analysis indicated that the product contained 20% methyl cis cinnamate and 80% methyl trans cinnamate.

(d) triethylamine in methanol at -80° (CO_2/acetone bath).

T.l.c. of the purified product indicated that it contained at least four compounds, the major one having an R_f similar to that of trans cinnamate, but g.l.c. analysis indicated that the product contained only one volatile component which was neither cis nor trans methyl cinnamate.

This compound has not yet been fully characterised.

(b). Synthesis of monosubstituted cyclopropenones.

Introduction.

In organic chemistry, the term aromatic is used rather loosely to designate a certain group of characteristic physical and chemical properties. Ever since the discovery of benzene by Farady in 1825, there has been great interest in the theoretical aspects of this and related cyclic, unsaturated aromatic systems.

The idea of aromaticity originated in relation to the observed chemical behaviour of particular compounds in a wide variety of reactions and also to some extent in connection with the physical properties in which aromatic molecules were exceptional.

A well defined concept of what is characteristic of the chemical behaviour of aromatic molecules was arrived at empirically many years ago. A number of properties appeared by that time to be common to aromatic compounds and several of these are still accepted today as useful criteria of aromatic character e.g.

(1) Thermal stability which is exemplified by the far greater stability which aromatic compounds possess to heat compared with acyclic conjugated polyolefins.

(2) Special chemical reactivity, exemplified by a reluctance to undergo addition reactions at the

multiple bonds and a readiness to undergo electrophilic substitution.

(3) Thermodynamic stability, exemplified by the great tendency of aromatic systems to form from a wide variety of reactions and the tendency to survive as such through various reactions.

Other properties, often associated with aromatic character but whose general diagnostic values are limited, are:

(a) the shift of the light absorption of aromatic compounds to longer wavelengths compared with olefinic systems.

(b) the low experimental values of heats of combustion and hydrogenation of aromatic compounds compared with the calculated values, the differences being identified as a resonance energy. It has been pointed out that this criterion has only limited value, because the value for the resonance energy is empirical, derived as it is from thermochemical data. The total stabilisation represented by the thermochemical data is certainly not only related to the dynamic behaviour of the electrons, which largely determines the properties of a molecule.[38]

(c) the additional bonding energy which results from the delocalisation of the electrons as compared to

those constrained to isolated double bonds. A high degree of electron delocalisation, i.e. a strong ring current of π-electrons does not always guarantee benzene-like reactivity and stability (compare the high degree of reactivity of the "aromatic" 18-annulene).

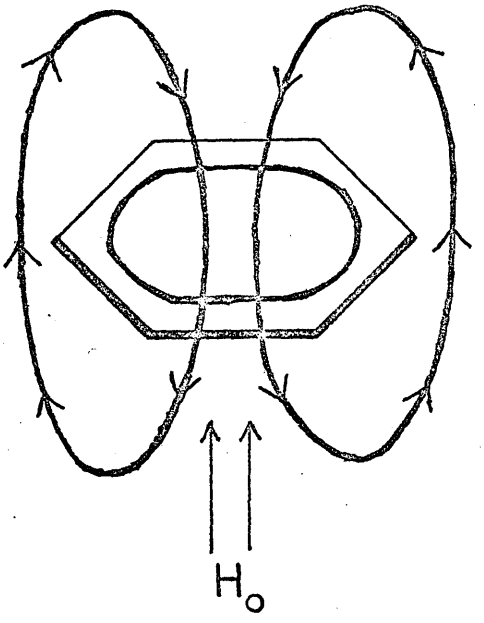
The above criteria are associated with the excited state of the molecules and are therefore subject to the peculiarities of each individual structure.

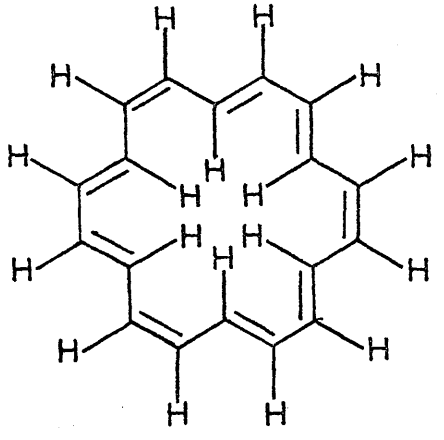
In the case of unsubstituted monocyclic carbocyclic systems a special criterion can be applied, the equivalence of all carbon-carbon bonds and the equivalence of all the carbon atoms. This was proved ingeniously by the case of tropylium ion by Volpin [56] who, after reaction of cycloheptatriene, labelled specifically at the methylene carbon atom, with phenyllithium followed by oxidation, found almost exactly $^{1}/_{7}$ of the activity in the resulting benzoic acid.

A number of properties associated with the ground state can also be found experimentally and thus are the most reliable indications available for aromaticity.

(1) The character of the carbon-carbon bond which is intermediate between single and double bonds, particularly noticeable in interatomic distances (X-ray studies).

(2) The planar or almost planar structure of the





inner H 11·8 τ

outer H 1·5 τ

normal olefinic proton 4–5 τ

ring (X-ray studies).

(3) The anisotropy of diamagnetic susceptibility e.g. under an applied magnetic field the π-electron ring current acts like a solenoid and develops a sizeable diamagnetic field, i.e. one which opposes the applied field. This is evident in the n.m.r. values for protons of aromatic systems, a proton on the outside of the ring being lower than the normal values for olefinic protons and one on the inside of a ring being higher (see opposite).

By the 1920's, the concept of aromaticity had begun to be interpreted and expressed in terms of the electronic theories of chemistry being developed by Ingold [39] and Robinson [40]. The change in emphasis in the study of aromaticity from chemical behaviour to concern with physical properties, reflects the much greater success, at least until the last few years, of semi-quantitative calculations of ground state compared with those of reactivity, transition states and reaction rates.

An important part in the foundation of modern theory was played by Huckel [41], who was the first to apply the molecular orbital method to aromatic systems. In this method the π electrons of the conjugated system are regarded as common to all the carbon atoms of the system

and occur in common molecular orbitals. The number of such orbitals is equal to the number of atoms taking part in the formation of the conjugated system.

The following three types of molecular orbital are possible:

(1) The bonding orbital, in which energy of the electrons is less than their energy in the atomic orbitals and consequently their occurence in the orbitals renders the system more stable.

(2) The non-bonding orbital, in which the energy of electrons is equal to their energy in the atomic orbitals and,

(3) The anti-bonding orbital, in which the energy of electrons is greater than their energy in the atomic orbitals, so that their occurrence in these orbitals renders the system less stable. The number of bonding, non-bonding and anti-bonding orbitals and their energies depend on the number of atoms in the molecule and its symmetry. In conformity with the Pauli exclusion principle, not more than two electrons can be allocated to each molecular orbital.

Calculation by the molecular orbital method showed that benzene contains three bonding and three anti-bonding orbitals. In the ground state, the six π electrons occupy the most favourable, lowest energy levels, namely

the three bonding orbitals. Huckel deduced from this simple treatment that benzene contained a stable, "closed" system of π electrons.

The rule formulated by Huckel can be expressed as follows:-

"A monocyclic conjugated polyene having the symmetry of a regular polygon will possess a closed electron shell and thus aromatic stability if the number of π electrons is $4n + 2$".

Thus for the first time a definition having physical significance was given to the concept of an aromatic system. Also the peculiar stability of the aromatic sextet of π electrons, which had been noted but not explained by classical theory, was explained as a particular case of the $(4n + 2)$ π rule with $n = 1$.

Huckels rule gave the incentive to numerous investigations which led ultimately to a new definition of aromatic character and which added greatly to the knowledge of the properties and reactivity of aromatic compounds. Guided by the $(4n + 2)$ rule, much of the chemistry of monocyclic ring systems and their derivatives was evaluated in a qualitative fashion. These predictions were developed by theoretical chemists and it has remained for the synthetic organic chemists to test the

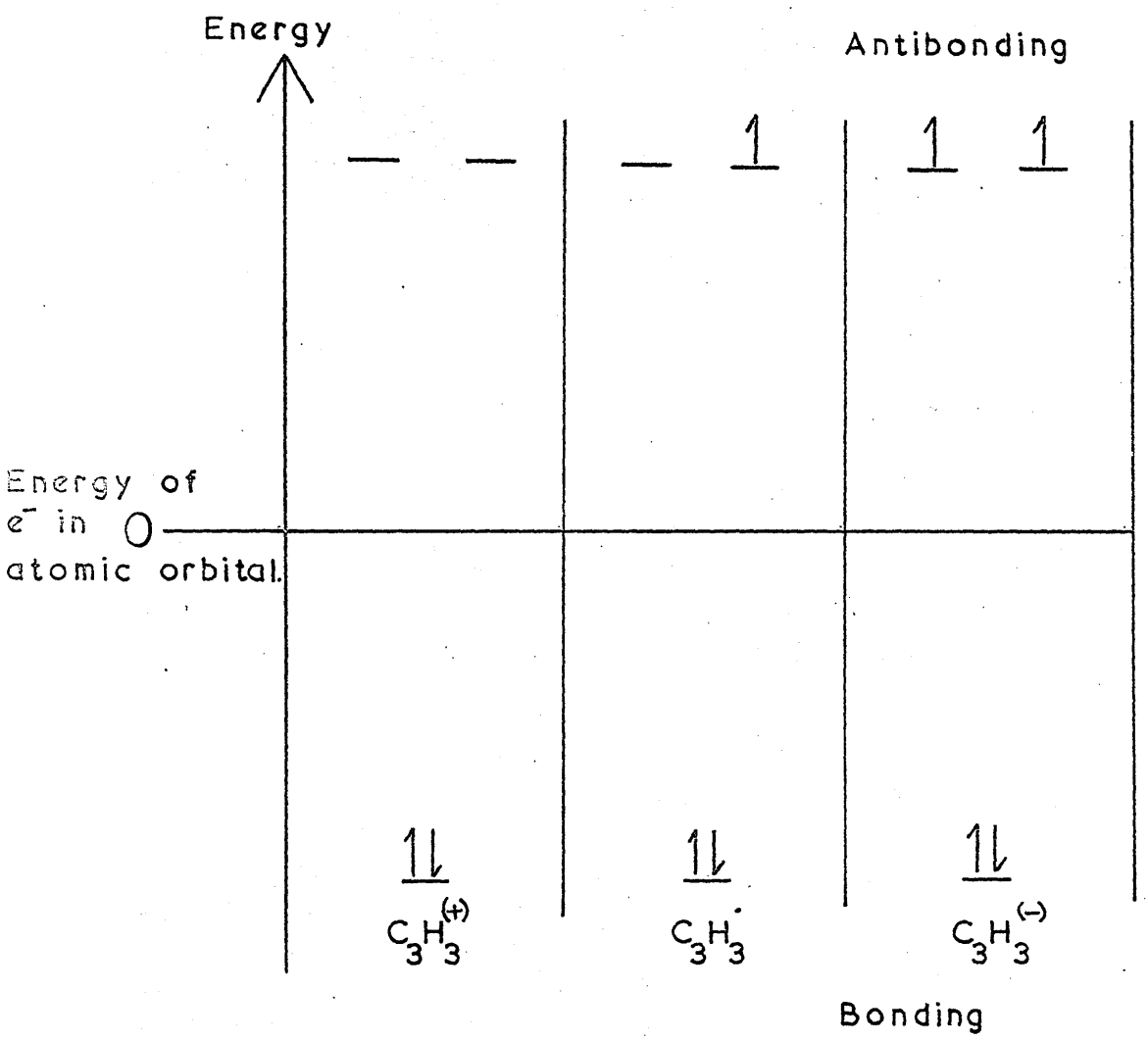

Energy
Antibonding
Energy of e⁻ in atomic orbital
0
1
1 1
1↓
1↓
1↓
$C_3H_3^{(+)}$
$C_3H_3^{\cdot}$
$C_3H_3^{(-)}$
Bonding

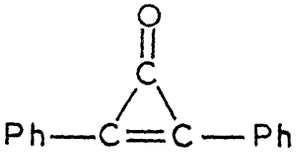
Figure 6

theories.

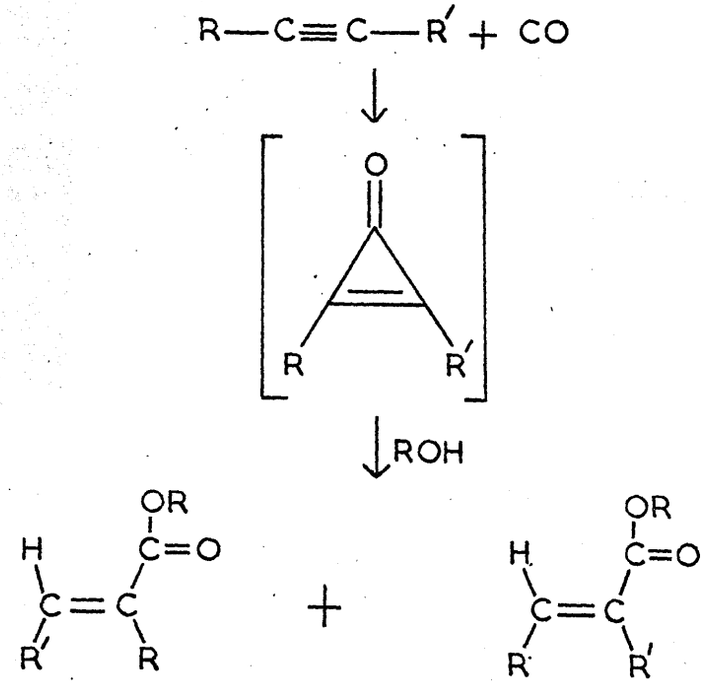
In recent years a number of remarkable compounds have been synthesised in a brilliant and fascinating chapter of organic chemistry. Roberts, Streitwieser and Regan [42] were the first to suggest the theoretically expected stability of the simplest aromatic system, namely the cyclopropenylium cation, with n = 0. Their predictions can be represented diagrammatically as shown opposite (figure 6).

In the ground state, the 2 π electrons of the cyclopropenylium system occupy the most favourable, lowest energy level, namely the bonding orbital. The radical C_3H_3 and the anion $C_3H_3^{(-)}$ contain one and two electrons respectively in anti-bonding orbitals. Thus it is only in the cation that all the π electrons are arranged in bonding molecular orbitals, and form a closed electron shell. The cyclopropenylium cation obeys the $(4n + 2)\,\pi$ rule and should be stable.

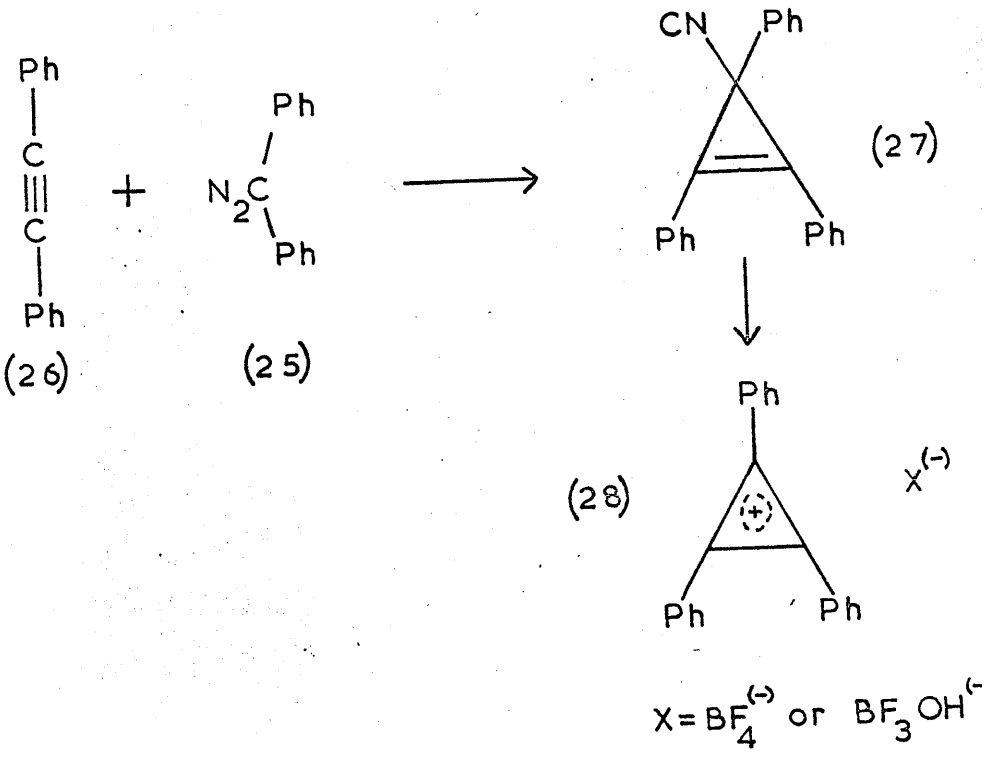
The first derivatives of this new aromatic system were obtained by Breslow [43, 44] in 1957. The starting materials for all syntheses were cyclopropenes which are generally obtained by addition of carbenes to substituted acetylenes. Thus by reacting phenyldiazonitrile (25) with diphenylacetylene (26), Breslow obtained 1, 2,

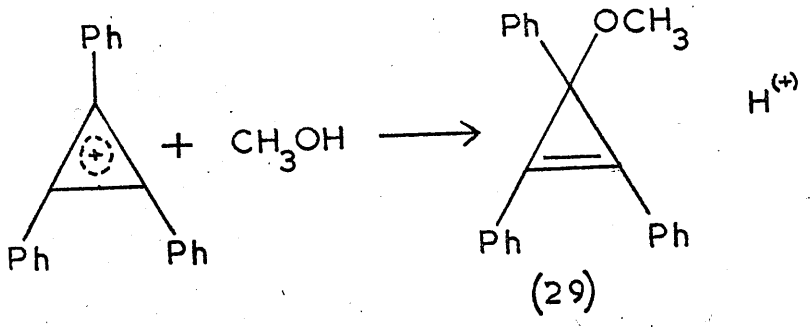


(34)



(30)


Ph
C
C
Ph
(26)
+
Ph
N2C
Ph
(25)
CN
Ph
Ph
Ph
(27)
Ph
(28)
X(-)
Ph
Ph
X = BF4(-) or BF3OH(-)


Ph
+
CH3OH
Ph
Ph
Ph
OCH3
H(+)
Ph
Ph
(29)

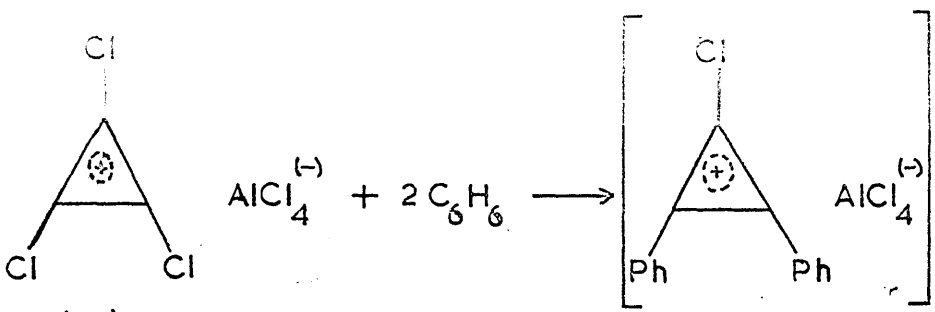
Scheme a

3, - triphenylcyclopropenyl cyanide (27) which yielded the mixed fluoroborate/hydroxyfluoroborate of the 1, 2, 3 - triphenylcyclopropenyl cation (28) on treatment with moist boron trifluoride etherate. (scheme c).

1, 2, 3 - triphenylcyclopropenyl methyl ether (29) was found to be a suitable starting material for the preparation of cyclopropenylium salts, giving 1, 2, 3 - triphenylcyclopropenylium bromide on reaction with hydrogen bromide. [44] Attempts to prepare the unsubstituted cyclopropenylium cation by hydride abstraction from cyclopropene with the triphenylmethyl cation were unsuccessful. [45]

Whereas in the case of seven membered ring compounds, tropone was synthesised before the tropylium cation [46], the first cyclopropenone, namely diphenylcyclopropenone (31) [47, 48] was only described after the first cyclopropenylium salt. However cyclopropenones had previously been postulated as intermediates, although with no conclusive evidence, particularly in the catalytic carbonylation of acetylenes, which in the presence of water or alcohols leads to acrylic acid or acrylates (30). [49]

According to classical concepts, the more highly strained cyclopropenones should be much less stable than the cyclopropanones as is the case with the corresponding four-membered ring compounds. [50] However only recently



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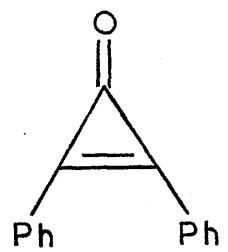
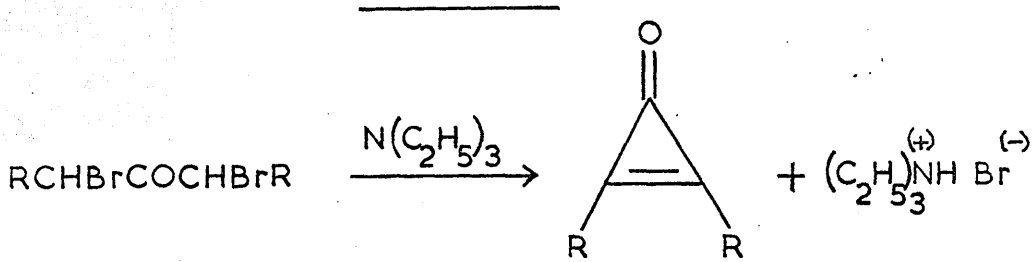


(34)

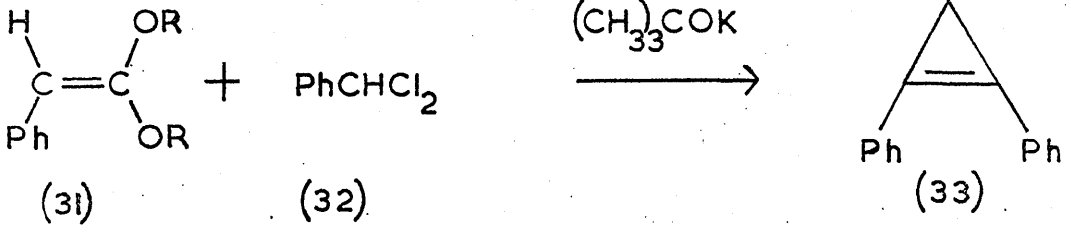
Scheme d



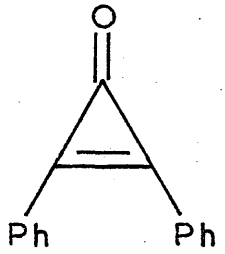
(38) (39)

$R = C_6H_5, n\text{-}C_4H_9$

Scheme e

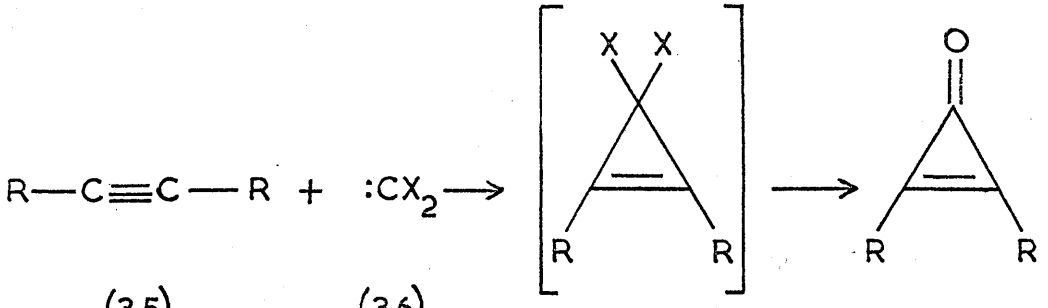


(31) (32) (33)



(34)

Scheme b



(35) (36)

R = Ph, n-C_3H_7 ; X = Cl, Br

Scheme c

has the first stable cyclopropanone, namely tetramethyl cyclopropanone been isolated.[51]

In the first synthesis of diphenylcyclopropenone (34), Breslow [47] treated phenylketene acetal (31) with phenylchlorocarbene (32) which yielded, on elimination of HCl, the cyclopropene ketal (33) which is readily hydrolysed. (scheme b).

A simpler synthesis of cyclopropenones was based on the addition of dichloro- or dibromo-carbenes (36) to disubstituted acetylenes (35) and hydrolysis of the resulting product. (scheme c).[48, 52, 53.]

Diphenylcyclopropenone (34) was also formed from benzene and the triphenylcyclopropenylium cation (37) by a Friedel Crafts reaction followed by hydrolysis. (scheme d).[54]

The best procedure on a preparative scale was developed by Breslow. [55] It has been established that the Faworsky reaction of α-halogenated ketones proceeds through an intermediate with the symmetry of a cyclopropanone.[2] Breslow, under certain experimental conditions, was able to intercept the intermediate cyclopropanone when the starting material was a dibromoketone, by dehydrobromination to the very stable cyclopropenone system. As starting materials Breslow used α , α'-dibromo-ketones (38) which on treatment with triethylamine or other bases lost two

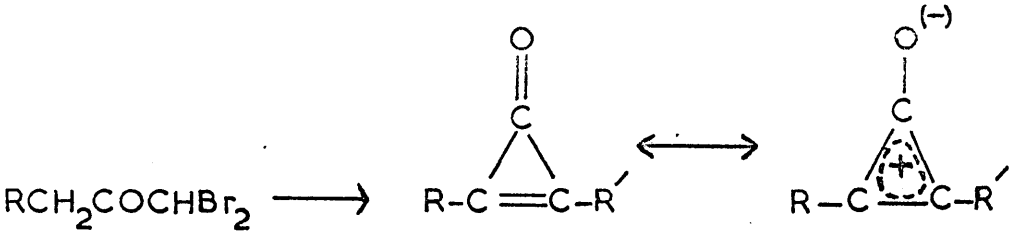
moles of hydrogen bromide to form the corresponding cyclopropenones (39) (scheme e).

At the beginning of this work no synthesis of a monosubstituted cyclopropenone system had been recorded.

Discussion.

The present studies were directed towards the preparation of monosubstituted cyclopropenones in reasonable, reproducible yields by means of a double elimination of hydrogen bromide from the readily available 1, 1-dibromo-ketones.

The 1, 1-dibromo-ketones were prepared as previously from the corresponding terminal acetylenes (part one) by the action of bromodan in buffered aqueous acetic acid. The bases which were used to effect the dehydrobromination were triethylamine, trioctylamine, Eiter's base (1, 5-diaza-bicyclo-(3, 5, 0) -non-5-ene) and collidine in acetonitrile or methylene chloride. It was found that the best preparative method involved the use of acetonitrile as solvent in high dilution with triethylamine as base for $2\frac{1}{2}$ hours at 55°-60°, the reactions being carried out under a continuous flow of nitrogen. The progress of the reaction was followed by titration and by infra-red studies, the cyclopropenones having two very characteristic absorptions. (see below) After evaporation of solvent and triethylamine, the products were purified firstly by extraction from the residual triethylamine hydrobromide into carbon tetrachloride, and then from this into 30% hydrochloric acid,



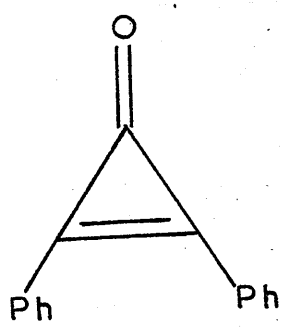
	R.	R'.	Yield%.	τH.	i.r. peaks.
(42)	CH_3CH_2-	H	10	–	1830[b], 1595[b].
(43)	$n\text{-}C_5H_{11}-$	H	21	1·53	1830[a], 1595[b].
(44)	$CH_3O_2C.(CH_2)_7-$	H	10	–	1852[a], 1650[a].
(45)	Ph–	H	5	–	1850[b], 1596[b].
(46)	CH_3CH_2-	$CH_3(CH_2)_2-$	15	–	1848[a], 1643[a].

(a) CCl_4 solution. (b) Liquid film.

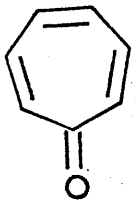
Table 2

careful neutralisation of the aqueous acidic extracts with solid sodium bicarbonate yielding the free cyclopropenones. The readiness with which cyclopropenones form salts of acids reflects the high degree of basicity of such compounds; the molecule in forming the salt attains the structure of a substituted cyclopropenylium cation (41), which, by obeying Huckel's rule for aromaticity, (n = 0),[41] has a considerably enhanced stability. After acid extraction, the products were purified by short path distillation under reduced pressure, the purified samples being stored at 0^{o} under nitrogen. Under these conditions, samples of cyclopropenones remained in a high state of purity, but the least trace of contamination or lengthy exposure to the atmosphere led to a rapid and complete decomposition. This route was employed for the synthesis of various monosubstituted cyclopropenones and also proved adaptable to unsymmetrically disubstituted cyclopropenones, as exemplified by the preparation of ethyl propyl cyclopropenone from the symmetrical acetylene oct-4-yne. The general results are shown opposite (table 2).

The monosubstituted cyclopropenones exhibit two very distinct bands in the infra red at 1830 and 1595 cm^{-1} while the disubstituted cyclopropenones prepared previously



(34)



(47)

by Breslow absorb at 1850 and 1640 cm^{-1} [57]. On the basis of solvent shifts, the 1850 cm^{-1} band has recently been assigned to the C = C stretch in the cyclopropenone system and the 1600 cm^{-1} band to the C = O stretch. [58] Since cyclopropanone probably has its carbonyl stretching frequency at 1825 cm^{-1} [59], a shift to 1600 cm^{-1} would represent considerable weakening of the C = O bond, suggesting that the cyclopropenone system exists largely in the cyclopropenylium cation form (41). Agranat [60] compared the i.r. frequencies of diphenylcyclopropenone (34) with those of tropone (47), (which also shows two bands in the carbonyl region), in which the charge distribution is expected to be similar to that prevalent in the cyclopropenone system.[61] Comparison of figures showed that only the 1640 cm^{-1} band of diphenylcyclopropenone showed the same solvent dependence as that of the stretching frequency of the carbonyl of tropone. Thus it was suggested that the 1640 $cm.^{-1}$ band was the carbonyl stretching frequency of cyclopropenones. It was also proposed that, since the 1850 cm^{-1} band did not show a dependence on solvent comparable to the higher band of tropone, this absorption is due to a "molecular frequency"[62] not of R.C = C.R [58] but of the disubstituted cyclopropenone in its dipolar form. Recent work on O^{18}

substituted cyclopropenones (cf. ref. 63) indicated that this affected the high-energy transition most strongly, thus suggesting that the assignment of the peaks should be reversed. This conflicting evidence suggests that the two modes (carbonyl stretch and olefin stretch) are so strongly coupled that the two infra-red bands cannot be uniquely assigned.

The highly reactive nature of the monosubstituted cyclopropenones prepared as above led to difficulties in characterisation. Amylcyclopropenone however appeared to have a chain length long enough for stability and short enough for ease of distillation, and gave satisfactory analyses, molecular weight (osmometer) and n.m.r. spectrum.

The ethyl and phenylcyclopropenones on the other hand, did not survive the conditions of n.m.r. spectroscopy (37° in $CDCl_3$ or 20% CF_3COOH/CCl_4) as indicated by the disappearance of the characteristic i.r. spectrum. (7-carbomethoxyhentyl)-cyclopropenone (44) gave the expected i.r. spectrum but neither the crude reaction product nor the distilled product showed an ethylenic proton in the n.m.r. spectra although satisfactory otherwise. The analyses of several different samples could be reconciled with the persistence of small varying amounts of water.

The disubstituted ethyl propyl cyclopropenone (46) proved much easier to purify and this was fully characterised. It will be seen later that the problem of characterisation was partially overcome by the preparation of crystalline derivatives (except in the case of phenylcyclopropenone).

Another feature of the monosubstituted cyclopropenones was the lack of any recognisable parent molecular ion in the mass spectra, and it was assumed that the compounds did not survive the conditions necessary for effecting entry into the mass spectrometer. Ethyl propyl cyclopropenone did however give a mass spectrum, a parent ion at $^{m}/e$ 124, with analysis from mass measurement as $C_8H_{12}O$, being observed.

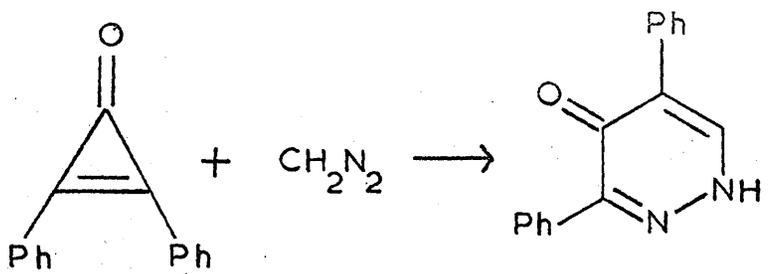
The n.m.r. spectrum ($CDCl_3$) of amycyclopropenone showed a sharp singlet assigned to the ring proton at 1·53 τ (1H), a triplet at 7·3 τ (2H, J = 7 c/s), a triplet of triplets centred at 8·25 τ (2H, $J_1 = J_2 =$ 7 c/s), a multiplet at 8·53 τ (4H) and a triplet at 9·08 τ (3H, J = 6 c/s). Of these resonances, only those due to the protons in the immediate environment of the ring showed appreciable shifts in 20% CF_3CO_2H/CCl_4, the α-methylene protons appearing as a triplet at 7·17 τ and the ring proton as a singlet at 1·32 τ. The small shifts

of the ring proton and the α-methylene protons in acid reflects the high degree of polarisation of the cyclopropenone system. This is in keeping with the high dipole moment of the various cyclopropenones.[57]

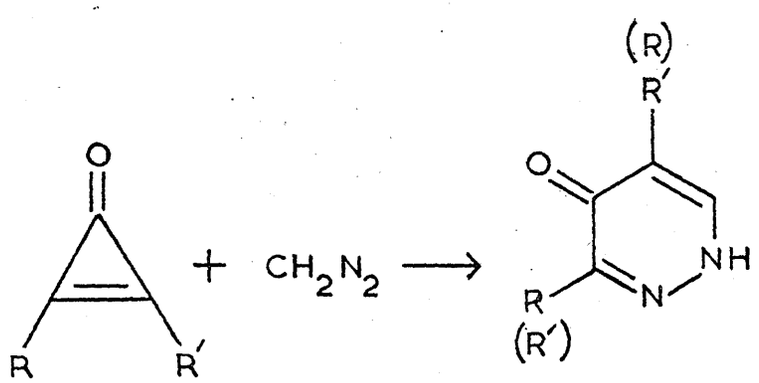
Breslow [57] found that dialkyl cyclopropenones are in fact more basic than the diaryl cyclopropenones, in spite of the fact that the dipole moments of the diaryl compounds are higher. However the dipole moment of diphenylcyclopropenone will be larger even with a smaller contribution of charge-separated resonance forms, since delocalisation into the phenyl rings increases the length of the dipole.

Thus the largely polar structures of the mono-substituted cyclopropenones are consistent with the i.r. and n.m.r. figures.

In view of the difficulty experienced in trying to characterise the monosubstituted cyclopropenones, it seemed desirable to be able to obtain a stable crystalline derivative. However, attempts at the preparation of sulphates, oxalates, perchlorates and antimony hexachlorides all proved unsuccessful. None of the products were crystalline. They had lost the characteristic i.r. absorptions of the cyclopropenone system but exhibited none which were indicative of the formation of a salt.[58]



(34) (48)

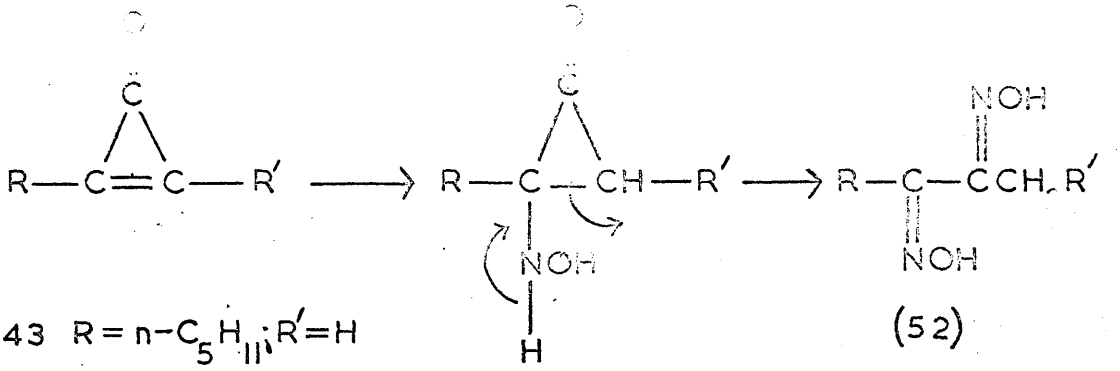


46. $R = C_2H_5$; $R' = n\text{-}C_3H_7$ (49)

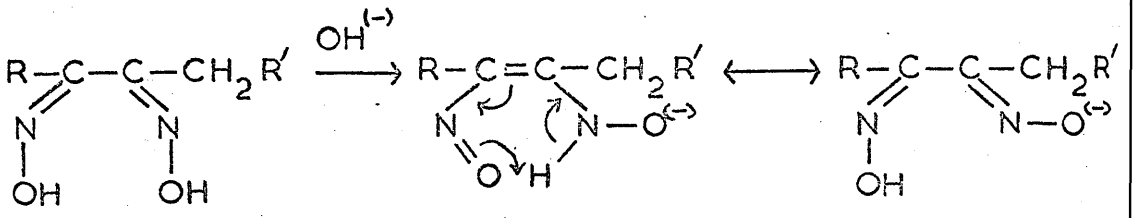
43. $R = n\text{-}C_5H_{11}$; $R' = H$

Neutralisation of the reaction mixtures did not allow recovery of any cyclopropenone, suggesting that these compounds were unstable under the conditions employed.

The reported formation [64] of the crystalline 3, 5-diphenylpyridaz-4-one (48) from the reaction of diazomethane on diphenylcyclopropenone (34) was applied in the present work. Ethyl propyl cyclopropenone (46), on treatment with diazomethane for 24 hours at 0^{o}, gave a highly crystalline white solid which exhibited the u.v. maximum reported for pyridaz-4-ones, [65] namely, 276 mμ ($\varepsilon = 1{\cdot}22 \times 10^{4}$). The product also underwent a reversible bathochromic shift in alkali, the maxima being 258 and sh. 278 mμ ($\varepsilon = 1{\cdot}04 \times 10^{4}$ and $7{\cdot}2 \times 10^{3}$ respectively). The mass spectrum of the product showed a parent ion at $^{m}/e$ 166, which together with the appropriate analyses, suggested that it was the desired substituted pyridaz-4-one (49). The mass spectral breakdown suggested that both possible isomers were present in the product in approximately equal amounts. Amylcyclopropenone (43), on similar treatment with diazomethane, gave a viscous oil which exhibited the required u.v. characteristics ($\lambda_{max.}^{EtOH}$ 269 mμ ,$\lambda_{max.}^{OH^{-}}$ 252 and sh. 278 mμ), but the product did not crystallise. Formation of the N-acetate by heating with acetic anhydride and



43 R = n-C_5H_{11}; R′ = H (52)

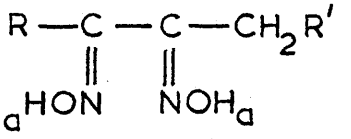


	R	R′	EtOH λ_{max} mμ.	EtOH λ_{max} mμ.
(51)	CH_3CH_2	H	229	264
(52)	n-C_5H_{11}	H	229	261
(53)	$CH_3O_2C(CH_2)_7$	H	229	261
(54)	CH_3CH_2	$CH_3(CH_2)_2$	229	261
(55)	$CH_3(CH_2)_2$	CH_3CH_2	229	261

Table 3

pyridine also gave a viscous oil which defied all attempts at crystallisation. Since no comparable results were obtained from the more unstable cyclopropenones, this approach was discontinued.

Treatment of amylcyclopropenone (43) with hydroxylamine in ethanol gave a high yield of a neutral compound. This compound was shown to be identical with an authentic sample of octan-2,3-dione dioxime (52), a tentative mechanism for this ring fission being shown opposite. From the product obtained, it is obvious that hydroxylamine must attack the carbon atom of the cyclopropenone ring bearing the alkyl group, no trace of the octan-1,2-dione dioxime which would be formed from attack on the unsubstituted carbon atom, being found. All the monosubstituted cyclopropenones, except phenylcyclopropenone (45), gave the comparable 2, 3-dione dioxime on treatment with hydroxylamine. The u.v. spectra of the products (table 3) were characteristic, all absorbing at the same wavelength and all exhibiting a most noticeable, reversible bathochromic shift in alkali. The basic structur of the glyoximes is akin to that of a simple diene, the maximum of the chromophore being of a comparable value. In base the maximum becomes comparable to that expected for an N-nitroso chromophore with a double bond in conjugation[66]



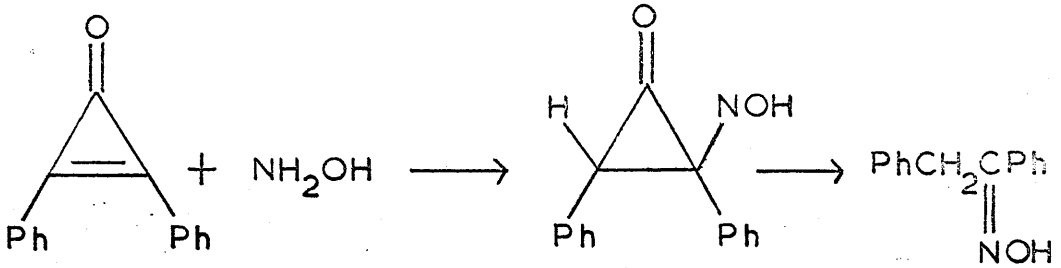
	R	R′	τH_a	τα-CH_2	τβ-CH_2
(51)	CH_3CH_2	H	−3·3	6·91	—
(52)	n-C_5H_{11}	H	−3·3	6·9	8·15
(53)	$CH_3O_2C.(CH_2)_7$	H	−3·34	6·88	8·2

Table 4

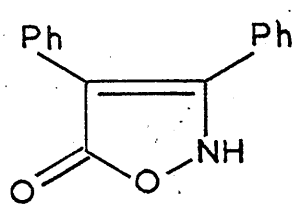
(233 mμ + 30 mμ for the double bond), thus suggesting that the resonating structures shown form in base.

The n.m.r. spectra (pyridine) of the products showed extensive deshielding by the oxime groups, the β-methylene groups absorbing at 8·2 τ and the α-methylene groups absorbing at 6·9 τ, compared with the observed 8·7 τ for chain methylene groups. The hydroxyl protons absorbed at the unusually low value of -3·3 τ, the singlet peak disappearing on D_2O addition. The general n.m.r. figures are shown in table 4. Another noticeable feature of these compounds was the non-appearance in the i.r. (KBr disc) of any C=N absorption and also of a series of sharp peaks in the 900-1100 cm^{-1} region. The mass spectra of the products showed no standard cracking pattern, the only feature common to all being a fairly abundant ion at (P-17) mass units which was assumed to be the loss of hydroxyl.

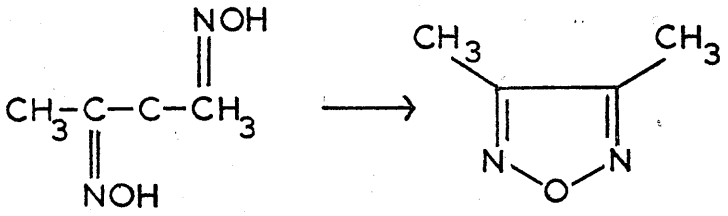
Ethyl propyl cyclopropenone (46), as expected gave a mixture of two isomeric dioximes on treatment with hydroxylamine, namely octan-4, 5- and octan-3, 4-dione dioxime. The n.m.r. spectrum of the product indicated that the ratio of the products was approximately 1/2 the symmetrical 4, 5-dione dioxime being the major component.


O
Ph
Ph
+ NH2OH
H
NOH
Ph
Ph
PhCH2CPh
NOH

(34) (56)


Ph
Ph
NH
O
O

(57)

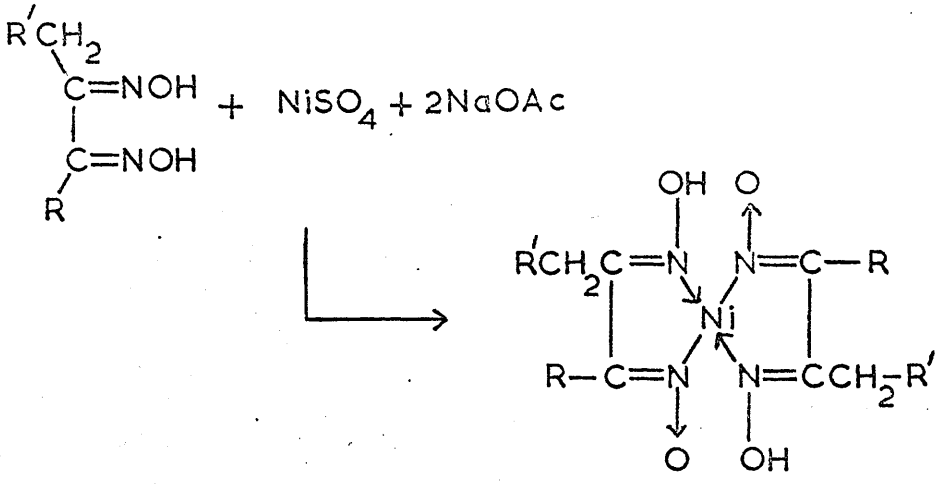

NOH
CH3–C–C–CH3
NOH
CH3
CH3
N
O
N

(58) (59)

Treatment of diphenylcyclopropenone (34) with hydroxylamine in ethanol gave no trace of the corresponding glyoxime , but, as had been reported previously[57], gave two unusual products, deoxybenzoin oxime (30%) (56) and 3, 4-diphenylisoxazolone (60%) (57).

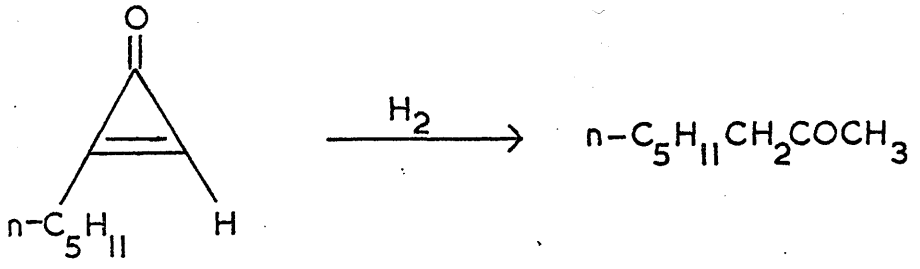
To finally prove the structure of the above glyoximes, the preparation of the dehydrated substituted furazans was attempted.[67] This type of compound can be obtained from butan-2, 3-dione dioxime (58) by heating to 150° with a molar equivalent of succinic anhydride, the colourless product, dimethylfurazan (59) being distilled directly from the reaction mixture. Under these conditions octan-2, 3-dione dioxime (52) did not give any substituted furazan. It was felt that this was a rather vigorous method for effecting the dehydration and that a synthesis of furazans under milder conditions was desirable. However, heating the dimethylglyoxime with either acetic anhydride or N, N-dicyclohexylcarbodiimide gave no dimethylfurazan.

Tschugaeff's reaction[68] was known to be extremely sensitive to and characteristic of glyoximes, intense colourations being observed in the presence of very small quantities of reagent. This method depends on the fact that a coloured nickel complex is formed from



R	R′	Colour of complex
CH_3	H	red
CH_3CH_2	H	orange
$n-C_5H_{11}$	H	orange/yellow
$CH_3O_2C(CH_2)_7$	H	orange
CH_3CH_2	$CH_3(CH_2)_2$	yellow

Table 5



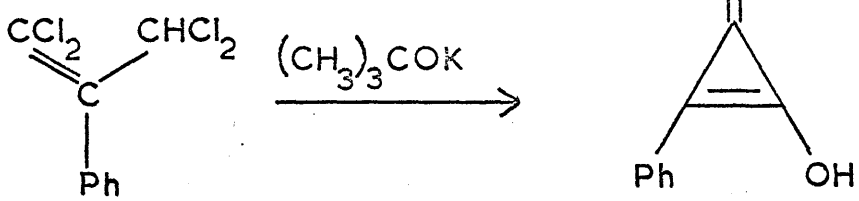
(43) (60)

nickel sulphate and any glyoxime in ethanolic solution. This proved successful with the products, the colours observed being shown opposite (table 5).

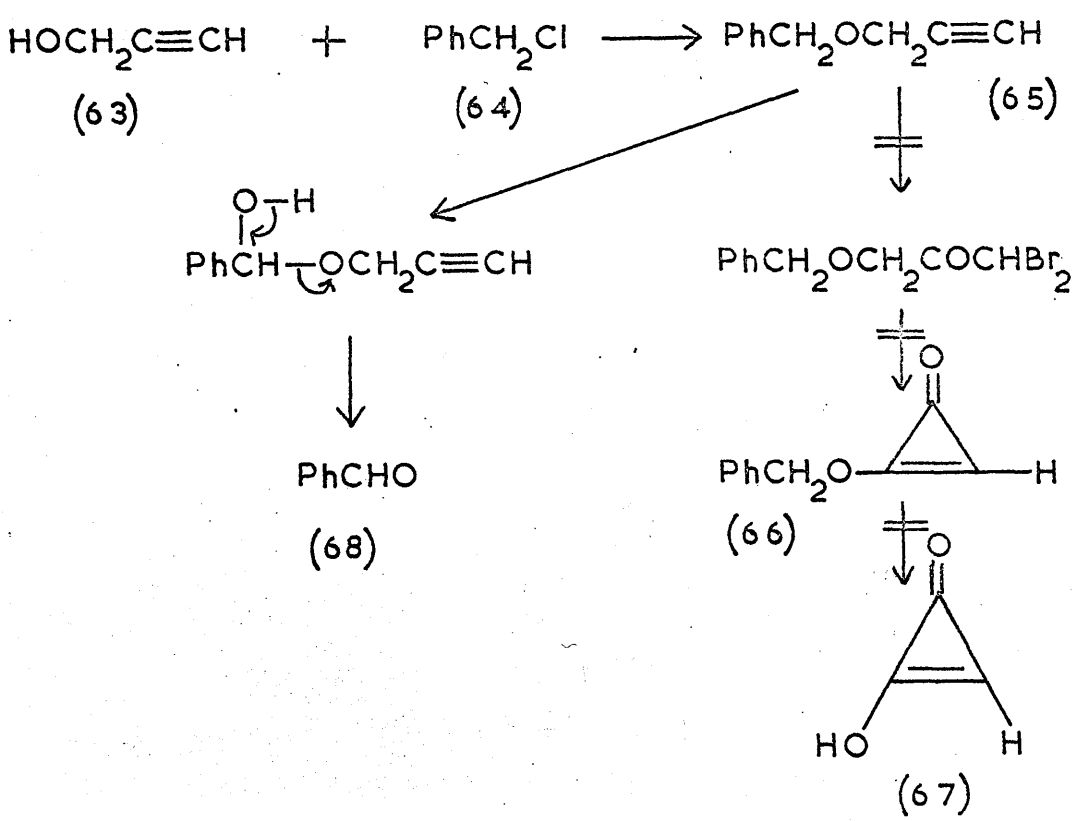
Thus additional confirmation of the glyoxime structure of the products from the reaction of hydroxylamine on the above cyclopropenones was obtained.

Further proof of the structure of the mono-substituted cyclopropenones was obtained by catalytic hydrogenation of amycyclopropenone (43) over pre-reduced platinum oxide, using dioxan as solvent. The product was expected to be mainly octan-2-one (60) and g.l.c. investigations on a variety of columns (cyano-P, 10% A.P.L. and 5% Q.F.I. at 100°) showed that the product had a retention time identical to that of an authentic sample of the straight chain ketone, octan-2-one. The i.r. spectrum of the purified product (preparative t.l.c.) showed carbonyl absorption at 1718 cm^{-1}, identical to that of octan-2-one. The 2, 4-dinitrophenylhydrazone of the product gave an identical u.v. spectrum ($\lambda_{max.}^{EtOH}$ 361mμ $\lambda_{max.}^{OH^-}$ 432 mμ), an identical i.r. spectrum and had a melting point which was undepressed on mixing with an authentic sample.

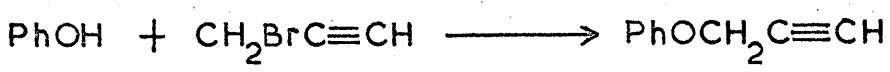
The reported synthesis of the very stable phenyl-


CCl2
CHCl2
C
Ph
(CH3)3COK
O
Ph
OH

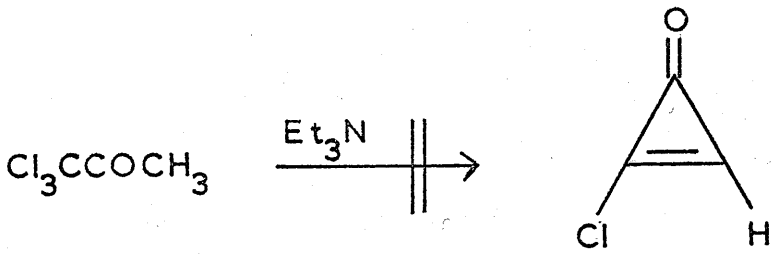
(61) (62)


HOCH2C≡CH
PhCH2Cl
PhCH2OCH2C≡CH
(63)
(64)
(65)
O—H
PhCH—OCH2C≡CH
PhCHO
(68)
PhCH2OCH2COCHBr2
O
PhCH2O
H
(66)
O
HO
H
(67)

hydroxycyclopropenone (62)[69] from 2-phenyltetrachloropropene (61) with potassium t-butylate prompted an investigation into the possibility of synthesising hydroxycyclopropenone (67). It was assumed that the preparation of benzyl propargyl ether (65) and treatment of this compound with "bromodan" as before would give a dibromoketone which would, on treatment with triethylamine give benzyloxycyclopropenone (66). It is well known that benzyl ethers are very readily decomposed and it was hoped that mild cleavage of this ether would give hydroxycyclopropenone (67). Benzyl propargyl ether was prepared in high yield from propargyl alcohol (63) and benzyl chloride (64) by heating with potassium hydroxide in acetone. The initial conditions for hydrobromination of the acetylene employed 50% aqueous acetic acid as solvent, the acid being a catalyst for the reaction. Under these conditions, the major product formed appeared to be benzaldehyde (68), a tentative mechanism for this reaction being shown. In spite of reducing the percentage of acetic acid in the reaction mixture and shortening the reaction time, even up to the point of only permitting 30-40% conversion of the acetylene, the major product was always benzaldehyde. Thus it appeared that the reaction conditions favoured benzylic oxidation rather



(69) (70) (71)

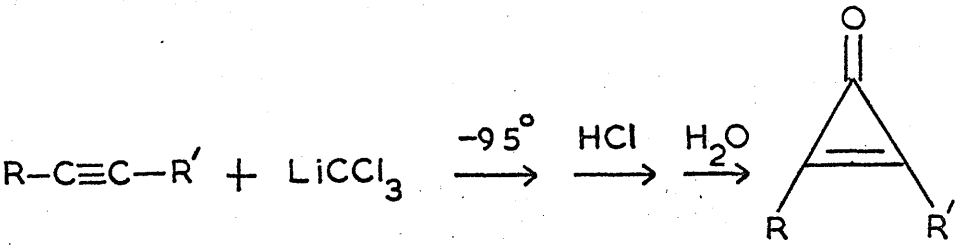


(72) (73)

than the desired addition reaction.

It was hoped that phenyl propargyl ether (71) would survive the reaction conditions for conversion of the acetylenic grouping. Phenyl propargyl ether was prepared in high yield by heating propargyl bromide (70) and phenol (69) with potassium carbonate in acetone. However, on treatment of the product with bromodan in buffered acetic acid as before, no reaction occurred, the acetylene being recovered unchanged. Increasing the reaction time failed to induce any reaction.

A different approach to the synthesis of hydroxycyclopropenone was then attempted. 1,1,1-trichloroacetone (72) on treatment with triethylamine, might be expected to give chlorocyclopropenone (73), which on solvolysis under mild conditions should give the desired product. In these studies, aliquots were removed from the reaction mixture in order to monitor the formation of the cyclopropenone system by i.r. However, on reaction at room temperature with triethylamine in acetonitrile, even for 24 hours, no evidence of cyclopropenone was found. Under these conditions the starting material was essentially unchanged, while on heating at 55° it was eventually consumed (disappearance of carbonyl



(35)

R= n-C_3H_7; R′=H

R= CH_3; R′= H

absorption in i.r. spectra), without formation of detectable quantities of cyclopropenone.

Thus a route of some generality to the synthesis of monosubstituted cyclopropenones has been developed, and a novel and unexpected cleavage reaction with hydroxylamine has allowed characterisation of these unstable compounds as the corresponding glyoximes. Routes to hydroxycyclopropenone did not give encouraging results.

During the completion of this work, Breslow [63] reported an independent synthesis of monosubstituted cyclopropenones; he treated various acetylenes (35) with lithium trichloromethide at -95° giving 15-20% yields of the corresponding cyclopropenones.

Experimental.

4, 4-dibromo-octan-5-one.

The compound was prepared from oct-4-yne and bromodan in a buffered sodium acetate, acetic acid solution as in previous experiments. (80% yield).

b.p. 78°-80°/0·9 m.m. n_D^{20} 1·4960

$\nu_{max.}$ (thin film) 1720 cm^{-1}

Tau values (CCl_4): 9·03 (3H, t, J = 7 c/s), 9·00 (3H, t, J = 7 c/s), 8·32 (6H, m), 7·5 (2H, t, J = 7 c/s), 6·91 (2H, t, J = 7 c/s).

Octan-4, 5-dione dioxime.

4, 4-dibromo-octan-5-one was treated as previously with hydroxylamine hydrochloride in pyridine to give a brownish solid which recrystallised from benzene as white needles. m.p. 169°-170°

(literature [70]; m.p. 186°-187° (175°))

$\nu_{max.}$ (KBr disc) 3200 (broad), 1146, 1043, 1020, 926, 892, 870 cm^{-1}.

$\lambda_{max.}^{EtOH}$ 228·5 mμ ($\varepsilon = 1{\cdot}79 \times 10^4$).

$\lambda_{max}^{OH^-}$ 261 mμ ($\varepsilon = 1{\cdot}40 \times 10^4$).

Tau values (pyridine): 8·99 (6H, t, J = 7 c/s), 8·13 (4H, m), 6·90 (4H, t, J = 7 c/s), - 3·23 (2H, broad

singlet; disappears on D_2O addition).

($C_8H_{16}N_2O_2$ requires C, 55·79; H, 9·36; N, 16·27

found C, 56·03; H, 9·19; N, 15·80%)

Amylcyclopropenone (43)

1, 1-dibromo-octan-2-one (cf. part one) (2·49 g.) dissolved in acetonitrile (40 ml.) was added dropwise over 15 minutes to a solution of triethylamine (8·75 ml., 7·5 equivs.) in acetonitrile (125 ml.) at 55°-60°. The reaction mixture was kept under nitrogen, a slow stream being used as a means of stirring. After stirring at 55°-60° for 2½ hours, solvent and excess triethylamine were evaporated and the residual mass extracted with carbon tetrachloride (80 ml.). The solid was filtered off and the filtrate extracted with 30% hydrochloric acid (4x50 ml.). The combined acid extracts were washed with carbon tetrachloride (30 ml.), and then carefully neutralised with solid sodium bicarbonate, the aqueous layer being covered with ether during the neutralisation. The aqueous layer was thoroughly extracted with ether and the combined ethereal extracts washed with water (3x25 ml.) and dried. Removal of solvent gave a brownish oil (0·23g.; 21%) which was purified by short path distillation.

b.p. 40°-45°/0·01 mm.

$\nu_{max.}$ (CCl_4) 1830; (liquid film) 1830, 1595 cm^{-1}.

Tau values ($CDCl_3$): 9·08 (3H, t, J = 6 c/s), 8·53 (4H, m), 8·25 (2H, triplet of triplets, $J_1 = J_2$ = 7 c/s), 7·3 (2H t. J = 7 c/s), 1·53 (1H, s).

Tau values (20% CF_3CO_2H/CCl_4): 9·08 (3H, t, J = 6 c/s), 8·53 (4H, m), 8·21 (2H, triplet of triplets, $J_1 = J_2$ = 7 c/s) 7·17 (2H, t, J = 7 c/s), 1·32 (1H, s).

Molecular weight was determined by osmometry using CCl_4 and $CHCl_3$ as solvents. These gave 248·8 and 125·1 respectively (required 124).

($C_8H_{12}O$ requires C, 77·43; H, 9·69

found C, 77·56; H, 9·89%)

Ethyl propyl cyclopropenone. (46)

4, 4-dibromo-octan-5-one was treated with triethylamine in acetonitrile as above (15% yield).

b.p. 45–50°/0·04 mm.

$\nu_{max.}$ (CCl_4): 1848, 1643 cm^{-1}.

Tau values ($CDCl_3$): 8·95 (3H, t, J = 7 c/s), 8·7 (3H, triplet, J = 7 c/s), 8·24 (2H, triplet of quartets, $J_1 = J_2$ = 7 c/s), 7·38 (4H, triplet upon quartet, $J_1 = J_2$ = 7 c/s).

Tau values (20% CF_3CO_2H/CCl_4): 8·95 (3H, t, J = 7 c/s), 8·7 (3H, t, J = 7 c/s), 8·22 (2H, triplet of quartets, $J_1 = J_2$ = 7 c/s), 7·28 (4H, m).

Molecular weight was determined by osmometry using

CCl_4 as solvent and was found to be 125·2 (required 124).

Parent molecular ion at $^m/_e$ 124 (mass measurement gave analysis as $C_8H_{12}O$).

($C_8H_{12}O$ requires C, 77·43; H, 9·68

found C, 71·19; H, 9·94%)

Despite repeated purifications, the carbon content was consistently low on analysis.

Ethyl cyclopropenone (42)

1, 1-dibromo-pentan-2-one (cf. part one) was treated with triethylamine in acetonitrile as above (10% yield).

b.p. 45°- 50°/0·3 mm.

$\nu_{max.}$ (thin film) 1830, 1595 cm^{-1}.

Satisfactory analysis and n.m.r. figures were unable to be obtained, but the formation of the cyclopropenone system was evident from the i.r. spectrum. The compound was characterised as the glyoxime (see later).

(7-carbomethoxyheptyl) - cyclopropenone (44)

Methyl 11, 11-dibromo-10-keto-undecanoate (cf. part one) was treated with triethylamine in acetonitrile as above (10% yield).

b.p. 140°-160°/0·05 mm.

ν_{max} (CCl_4): 1852, 1740, 1650 cm^{-1}.

($C_{12}H_{18}O_3$ requires C, 68·58; H, 8·57

found C, 65·42; H, 8·44)

Satisfactory analysis and n.m.r. figures were unable to be obtained, but the formation of the cyclopropenone system was evident from the i.r. spectrum. The compound was characterised as the glyoxime (see later).

Treatment of 1, 1-dibromo-3-phenylacetone with triethylamine.

1, 1-dibromo-3-phenylacetone (cf. part one) was treated with triethylamine in acetonitrile as above (5% yield).

$\nu_{max.}$ (thin film) 1850, 1596 cm^{-1}.

This product was extremely unstable and all attempts at purification failed. It was not able to be characterised as the glyoxime.

Attempted preparation of solid salt derivaties of cyclopropenones.

Amylcyclopropenone was dissolved in methylene chloride and 50% sulphuric acid was added dropwise with cooling. No solid precipitated and on neutralisation the starting material was not regenerated.

The above procedure was repeated with oxalic acid, perchloric acid and antimony pentachloride/hydrochloric acid solutions but in no case was any precipitate obtained.

Reaction of diazomethane with ethyl propyl cyclopropenone.

A dry ethereal solution of diazomethane was added at

0° to a solution of ethyl propyl cyclopropenone (0·05 g.) in ether (10 ml.). A large excess of diazomethane was used. The flask was loosely stoppered, and, after leaving for 24 hours at 0°, evaporation gave a yellow oil. The petroleum ether insoluble fraction was recrystallised from benzene to give fine white needles (0·035 g; 71%) m.p. 135°-136°.

$\nu_{max.}$ (nujol) 3295, 1605, 1546, 1500, 1480, 1122 cm^{-1}

$\lambda^{EtoH}_{max.}$ 276 mμ ($\varepsilon = 1{\cdot}21 \times 10^4$)

$\lambda^{OH^-}_{max.}$ 258 and sh. 278 mμ ($\varepsilon = 1{\cdot}04 \times 10^4$ and $7{\cdot}24 \times 10^3$ respectively).

Parent molecular ion at $^m/_e$ 166.

($C_9H_{14}N_2O$ requires C, 65·03; H, 8·49; N, 16·85

found C, 64·86; H, 8·35; N, 16·60%)

The mass spectrum indicated a mixture of both possible isomers 3-ethyl-5-propyl- and 3-propyl-5-ethylpyridaz-4-one

Treatment of amylcyclopropenone with diazomethane.

Amylcyclopropenone was treated with diazomethane as above. The petroleum ether insoluble fraction gave a viscous oil which could not be crystallised (45%).

$\nu_{max.}$ 3295, 1600, 1535, 1495 cm^{-1}.

$\lambda^{EtOH}_{max.}$ 271 mμ

$\lambda^{OH^-}_{max.}$ 253, sh. 278 mμ

Treatment of the crude reaction product with acetic

anhydride in pyridine gave an oil which could not be crystallised.

Octan-2, 3-dione dioxime (52)

A mixture of hydroxylamine hydrochloride (1·70g; 10 equivs.) in water (9 ml.), 20% aqueous sodium acetate trihydrate solution (6 ml.) and amylcyclopropenone (0·3 g.) in absolute ethanol (25 ml.) was refluxed for 30 minutes. The ethanol was removed, and the residue poured into water (50 ml.) and the whole extracted with ether (3x30 ml.). The combined organic extracts were washed with saturated sodium bicarbonate solution (2x20 ml.), 2N hydrochloric acid (2x20 ml.) and water (2x20 ml.). The dried solution was evaporated to give a brown solid, which recrystallised from benzene as fine white needles (0·22g; 52%). m.p. 169°-171°.

(literature [71]: m.p. 173°)

$\nu_{max.}$ (KBr disc) 3200 (broad), 1012, 990, 960, 916. 892 cm^{-1}.

$\lambda^{EtOH}_{max.}$ 229 mμ ($\varepsilon = 1{\cdot}56 \times 10^4$)

$\lambda^{OH^-}_{max.}$ 261 mμ ($\varepsilon = 9{\cdot}9 \times 10^3$)

Tau values (pyridine): 9·2 (3H, t, ill defined), 8·7 (4H, m), 8·15 (2H, m), 7·56 (3H, s), 6·9 (2H, t, J = 8 c/s), -3·3 (2H, broad singlet; disappears on D_2O addition)

Parent molecular ion at $^m/_e$ 172.

($C_8H_{16}N_2O_2$ requires C, 55·79; H, 9·36; N, 16·27

found C, 55·77; H, 8·97; N, 14·78%)

Despite repeated attemps the nitrogen analysis was always low.

Pentan-2, 3-dione dioxime (51).

Crude ethyl cyclopropenone was treated as above with hydroxylamine in ethanol to give a solid which recrystallised from benzene as white needles (17% yield). m.p. 169°-171°

(literature [72]: m.p. 172°-173°).

$\nu_{max.}$ (KBr disc) 3200 (broad), 1142, 1008, 966, 899 cm^{-1}.

$\lambda_{max.}^{EtOH}$ 229 mμ ($\varepsilon = 1{\cdot}51 \times 10^4$)

$\lambda_{max.}^{OH^-}$ 264 mμ ($\varepsilon = 1{\cdot}58 \times 10^4$)

Tau values (pyridine): 8·68 (3H, t, J = 7 c/s), 7·57 (3H, s), 6·91 (2H, q, J = 7 c/s), -3·3 (2H, broad singlet; disappears on D_2O addition).

Parent molecular ion at $^m/_e$ 130.

($C_5H_{10}N_2O_2$ requires C, 46·14; H, 7·74; N, 21·52

found C, 46·14; H, 8·12; N, 21·50%)

Methyl 9, 10-diketo-undecanoate dioxime (53)

Crude (7-carbomethoxyheptyl)-cyclopropenone was treated as above with hydroxylamine in ethanol, to give an off-white solid which recrystallised as plates from aqueous ethanol (45% yield). m.p. 116°-117°.

$\nu_{max.}$ (KBr disc) 3200 (broad), 1734, 1170, 1076, 1014, 978, 912 cm^{-1}.

$\lambda^{EtOH}_{max.}$ 229 mμ ($\varepsilon = 1{\cdot}62 \times 10^4$)

$\lambda^{OH^-}_{max.}$ 261 mμ ($\varepsilon = 1{\cdot}55 \times 10^4$)

Tau values (pyridine); 8·68 (8H, m), 8·2 (2H, broad multiplet), 7·72 (2H, t, J = 7 c/s), 7·53 (3H, s), 6·88 (2H, t, J = 8 c/s), 6·38 (3H, s), -3·34 (2H, broad singlet; disappears on D_2O addition).

Parent molecular ion at $^m/_e$ 258

($C_{12}H_{22}N_2O_4$ requires C, 55·80; H, 8·58; N; 10·84

found C, 55·93; H, 8·83; N; 10·94%)

Treatment of ethyl propyl cyclopropenone with hydroxylamine hydrochloride.

Ethyl propyl cyclopropenone was treated as above with hydroxylamine in ethanol, to give a white solid which recrystallised from aqueous ethanol as fine white needles (90% yield). m.p. 152°-153°.

The n.m.r. indicated a mixture of the two possible isomers octan-3, 4- and octan-4, 5- dione dioxime, and the following physical data refers to the unseparated mixture.

$\nu_{max.}$ (KBr disc): 3200 (broad), 1030, 1021, 969, 930, 908, 894 cm^{-1}.

$\lambda^{EtOH}_{max.}$ 229 mμ ; $\lambda^{OH^-}_{max}$ 261 mμ .

Parent molecular ion at $^{m}/e$ 172.

($C_8H_{16}N_2O_2$ requires C, 55·79; H, 9·36; N, 16·27.
found C, 55·85; H, 9·44; N, 16·06%).

3-isonitroso-octan-2-one.

Octan-2-one (cf. part one) (9·5g.) was added to concentrated hydrochloric acid (1·2 ml.) and cooled in an ice bath. Amyl nitrite (8·7g; 1 equiv.) was added slowly with vigorous stirring, and the mixture was then stirred for an additional 30 minutes at 0°. The mixture was then added to ether (50 ml.), extracted with 2N potassium hydroxide (2x20 ml.), the basic extracts washed thoroughly with ether, and, after being heated on a steam bath for 5 minutes, were acidified with 6N hydrochloric acid. The aqueous layer was extracted with ether (3x20 ml.) and the combined organic extracts were washed with brine until neutral. Drying and evaporation of the solvent gave a pale green oil which was purified by distillation to give a pale green oil (3·27g; 28%).

b.p. 138°-142°/15 mm.

(literature: b.p. 133°/11 mm)

ν_{max} (thin film) 3450 (broad), 1680, 1075, 1008 cm^{-1}.

Octan-2, 3-dione dioxime (52).

3-isonitroso-octan-2-one (0·5g.) in ethanol (4 ml.) was added to a solution of hydroxylamine hydrochloride

(1·1g; 5 equivs.) and sodium acetate trihydrate (2g; 5 equivs.) in water (8 ml.). The mixture was refluxed for 30 minutes and then worked up as before to give an off-white solid which recrystallised from benzene as fine white needles (0·52g; 95%). m.p. 169°-171°.

(literature[71]: m.p. 173°).

The product was identical in all respects with the product from the reaction of hydroxylamine on amyl-cyclopropenone.

1, 3-dibromo-1, 3-diphenylacetone (38, R = C_6H_5)

To a solution of dibenzyl ketone (70g.) in glacial acetic acid (250 ml.), a solution of bromine (110g; 2 equivs.) in glacial acetic acid (500 ml.) was added with stirring over 15 minutes. The mixture was stirred for an additional 5 minutes and then poured into water (1 litre). Solid sodium metabisulphite was added slowly until the bromine colouration was discharged and the mixture allowed to stand for 1 hour. The aqueous layer was extracted with ether (4x100 ml.) and the combined extracts washed with water (4x50 ml.). Drying and evaporation gave a light yellow oil which solidified on standing, and which re-crystallised from petroleum ether as white needles, (120g; 95%). m.p. 79°-83°.

(literature[57]: m.p. 79°-83°).

Diphenylcyclopropenone (34)

1, 3-dibromo-1, 3-diphenylacetone (120 g.) was dissolved in methylene chloride (500 ml.) and added to a solution of triethylamine (70g; 2 equivs.) in methylene chloride (250 ml.) over a period of 1 hour. The mixture was stirred for an additonal 30 minutes, and then extracted with 3 N hydrochloric acid (2x150 ml.). The organic phase was then cooled 0° and an ice-cold solution of concentrated sulphuric acid (50 ml.) in water (25 ml.) was added slowly with stirring. The precipitate was filtered and washed with a large volume of methylene chloride; the solid was then added to methylene chloride (250 ml.) and water (500 ml.), and sodium carbonate (5g.) added slowly with stirring. The mixture was then thoroughly extracted with methylene chloride and the combined organic extracts washed with water. Drying and evaporation of the solvent gave a dark brown gum, which was extracted with boiling cyclohexane, the organic layer being decanted from the oily impurity. On cooling, a light brown solid crystallised which was recrystallised from cyclohexane as fine white needles. (34g; 50%). m.p. $119{\cdot}5^{\circ}$-120°

(literature [57]: 119°-120°).

$\nu_{max.}$(nujol) 1845, 1625 cm^{-1}.

Treatment of diphenylcyclopropenone with hydroxylamine hydrochloride.

Diphenylcyclopropenone (2·06g.) dissolved in ethanol (125 ml.) was treated with hydroxylamine in ethanol as before. The product was split into acidic (10% sodium bicarbonate solution) and neutral fractions. Evaporation of the neutral fraction gave a white crystalline solid which recrystallised from aqueous ethanol as fine white needles. (0·62g; 30%) m.p. 94·5°-96°

(literature [57]: m.p. 94°-96°)

This product was deoxybenzoin oxime (56).

The basic solution was acidified with dilute hydrochloric acid and the aqueous layer extracted with ether. Washing, drying and evaporation of solvent gave a white solid which recrystallised from aqueous ethanol as white plates (1·39; 60%). m.p. 158°-159°

(literature [57]: 158°-159°).

This product was diphenylisoxazolone (57).

Dimethylfurazan (59)

Dimethylglyoxime (58g; 0·5M) and succinic anhydride (50g; 0·5M) were ground together and heated with stirring to approximately 150°. At this temperature a pale yellow oil distilled and was collected. When the temperature of the reaction mixture reached 200°, it was allowed to cool

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and after addition of water, the mixture was then steam distilled. The distillate was added to ether (100 ml.) and the ether extracts washed with water (2x50 ml.), dried and evaporated to give a pale yellow oil. This was purified by distillation at atmospheric pressure to give a colourless oil (25g; 52%).

b.p. 154^{o}-158^{o} n_D^{25} 1·4234.

(literature [67]: b.p. 156^{o}-159^{o} n_D^{25} 1·4243).

$\nu_{max.}$ (thin film): 1600, 1220, 1055, 902, 718 cm^{-1}.

$\lambda_{max.}^{EtOH^-}$ 217·5 mμ ($\varepsilon = 1{\cdot}9 \times 10^3$)

$\lambda_{max.}^{OH^-}$ 217·5 mμ and 278 mμ ($\varepsilon = 2{\cdot}3 \times 10^3$, and $1{\cdot}3 \times 10^2$).

Heating dimethylglyoxime with either acetic anhydride or N, N-dicyclohexylcarbodiimide failed to give any dimethylfurazan.

Treatment of octan-2, 3-dione dioxime with succinic anhydride.

Octan-2, 3-dione dioxime, on being heated with a molar equivalent of succinic anhydride as above failed to give any distillable product.

Octan-2-one (60)

Amylcyclopropenone (0·15g.) in dioxan (10 ml.) was added slowly to platinum oxide (39·8 mg.) in purified dioxan (5 ml.) which had been pre-reduced with hydrogen. Uptake of hydrogen ceased after 1 3/4 hours when 23 ml. had

been absorbed (ie. 0·7 mole equivalents). The solution was filtered through glass paper and the dioxan was carefully removed using a fractionating column at atmospheric pressure. The residue was poured into water (50 ml.) and extracted with ether (4x10 ml.). Drying and evaporation of the solvent gave a pale yellow oil (0·04g; 25%).

The oil was purified by preparative t.l.c., (silica gel H; 10% ethyl acetate/petroleum ether) and the product examined by g.l.c. on cyano-P, 10% A.P.L. and 1% Q.F.I. at 100°. The major product had an identical retention time to that of an authentic sample of octan-2-one on all three columns.

$\nu_{max.}$ (thin film): 1718 cm^{-1}.

The 2, 4-dinitrophenyl hydrazone of the product was recrystallised from aqueous methanol m.p. 57°-58°.

(literature [73]: m.p. 58°).

$\lambda_{max.}^{EtOH}$ 260·5 mμ ($\varepsilon = 1{\cdot}36 \times 10^4$).

$\lambda_{max.}^{OH^-}$ 428 mμ ($\varepsilon = 1{\cdot}01 \times 10^4$).

An authentic sample of 2, 4-d.n.p. of octan-2-one gave $\lambda_{max.}^{EtOH}$ 260·5 mμ and $\lambda_{max.}^{OH^-}$ 428 mμ.

Benzyl propargyl ether (65)

A mixture of propargyl alcohol (14g.), benzyl chloride (31·6 g; 1 equiv.), acetone (40 ml.) and potassium hydroxide

(14g; 1 equiv.) was heated for 8 hours at 50°-70°. After evaporating the solvent, the solid mass was thoroughly extracted with ether. The combined ethereal extracts were washed with water (6x100 ml.), dried and evaporated to give a light yellow oil which was purified by distillation. (16·8g.; 45%).

b.p. 98°-100°/15 mm. n_D^{22} 1·4316.

$\nu_{max.}$ (thin film): 3320, 2130, 1456, 1360, 1095, 760, 710 cm^{-1}.

($C_{10}H_{10}O$ requires C, 82·34; H, 7·05 %).

Treatment of benzyl propargyl ether with bromodan.

Benzyl propargyl ether was treated as before with bromodan in buffered acetic acid. The product was obtained as a deep red oil, which was shown by t.l.c. to contain at least 5 compounds, the major one having R_f value identical to that of benzaldehyde.

$\nu_{max.}$ (thin film) 1697 cm^{-1}

In spite of varying the reaction conditions eg. lowering the acetic acid percentage and reducing the time of reaction, benzaldehyde was always the major product.

Phenyl propargyl ether. (71)

A mixture of phenol (23·5 g.), propargyl bromide (30g; 1 equiv.), acetone (40 ml.) and anhydrous potassium carbonate (35 g; 1 equiv.) was refluxed for 8 hours. After

evaporation of the acetone, the solid mass was poured into water (100 ml.) and extracted thoroughly with ether. The combined organic extracts were washed with 4N sodium hydroxide solution (2x50 ml.) and then with water (6x50 ml.), dried and evaporated. This gave a light yellow oil which was purified by distillation under reduced pressure (29·4g.; 88%).

b.p. 88°-90°/15 mm. n_D^{23} 1·5321

(literature[74]: b.p. 92°/25 mm. n_D^{20} 1·532).

$\nu_{max.}$ (thin film): 3350, 2120, 1604, 1495, 1230, 1045, 763, 695 cm^{-1}.

Tau values ($CDCl_3$): 7·51 (1H, t, J = 2 c/s), 5·3 (2H, d, J = 2 c/s), 2·93 (5H, m).

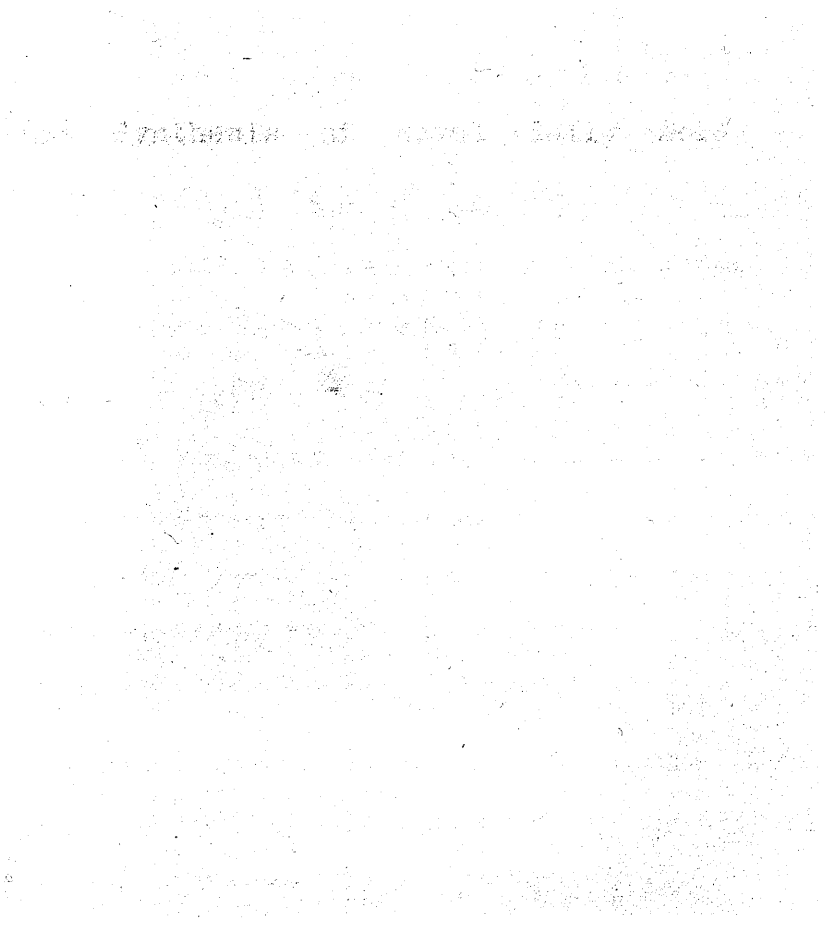
Treatment of phenyl propargyl ether with bromodan.

Phenyl propargyl ether was treated as before with bromodan in buffered acetic acid. The product was a light yellow oil which was shown by t.l.c. and i.r. to be unchanged starting material. Extending the reaction time still gave no addition reaction, the acetylene being recovered essentially unchanged.

Treatment of 1, 1, 1-trichloroacetone with triethylamine.

1, 1, 1-trichloroacetone was treated with triethylamine in acetonitrile as previously. The reaction mixture was stirred for 24 hours at room temperature, the progress of

the reaction being followed by i.r. studies of aliquots removed from the reaction. No reaction had occurred after 24 hours and subsequent heating eventually destroyed the starting material without formation of any cyclopropenone.



(c) Synthesis of Royal Jelly Acid.

Introduction.

The chemistry of "Royal Jelly" has been the subject of intensive investigations in recent years especially since the components have a profound effect on the organisation of a hive of honeybees.

A normal colony of honeybees (Apis melifera) is composed of three main types of adult members, the Queen bee, the worker bee and the drone.

The Queen and the workers are female bees, the drone being a fertile male. The Queen honeybee is responsible for the laying of the eggs from which all members of the colony arise. The Queen is capable of laying both fertilised and unfertilised eggs, the latter always producing drones whereas the former invariably produces female honeybees.

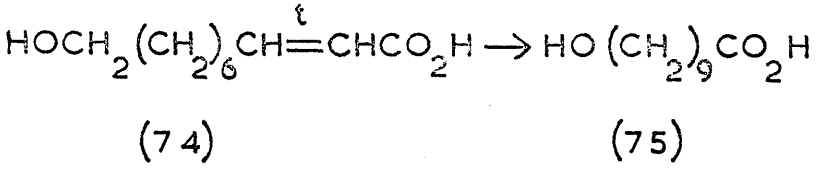
The brood food of the larvae is a highly nutritious protein rich food which is produced by the worker nurse bees,[75] and has been called "Royal Jelly". After three days the larvae which are destined to become worker bees are no longer supplied with this food but the supply of "Royal Jelly" to the potential Queens is maintained and continues throughout the span of their lives.

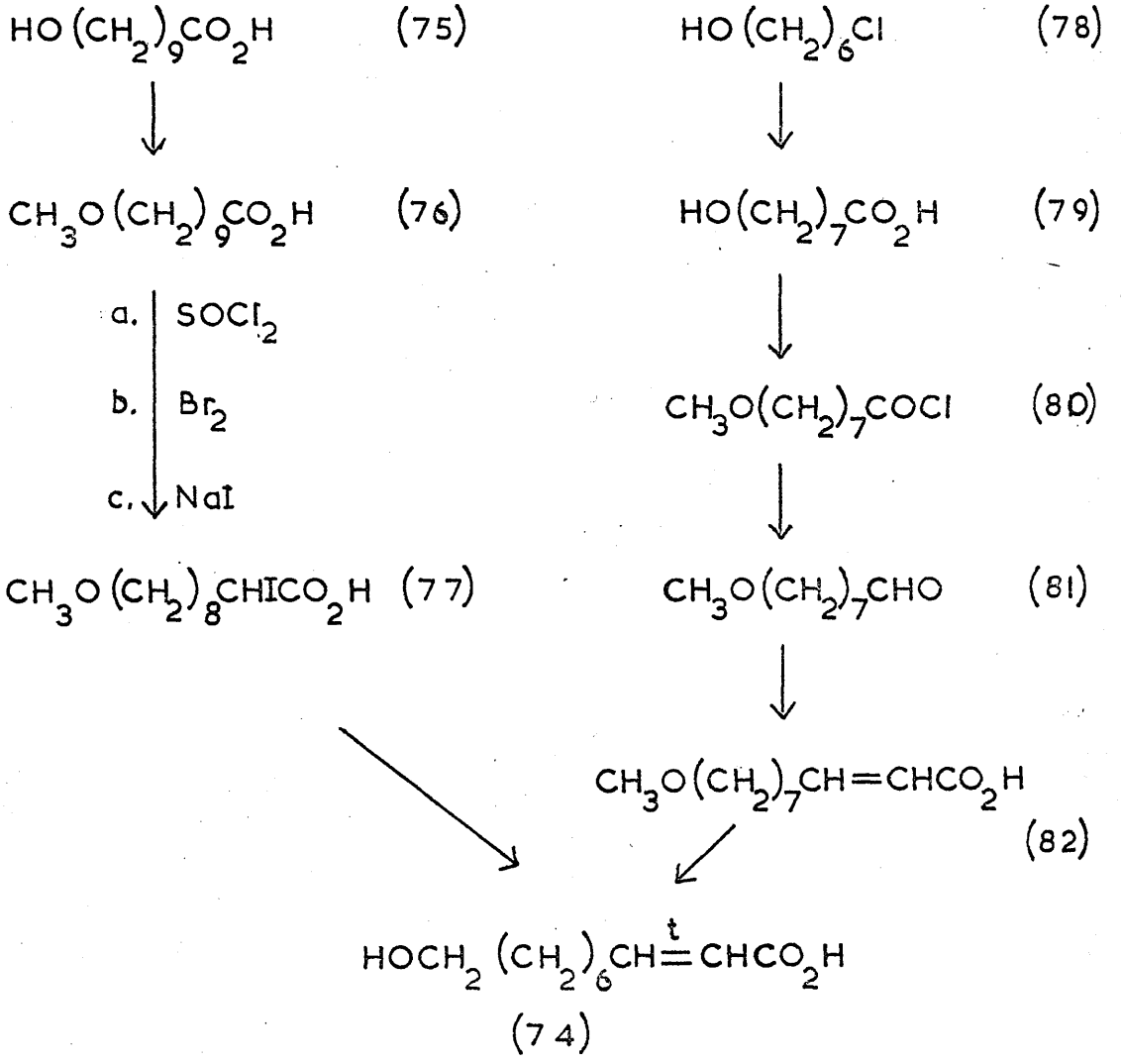
It was postulated [75] that some active constituent in

this material was responsible for the differentiation of the female honeybees. This proposed physiological activity led to a thorough investigation of the compounds of "Royal Jelly" in an attempt to find the active constituent. The lipid soluble fraction from the ethereal extract of dried "Royal Jelly" had a distinct effect on the sexual behaviour of the fruit fly, thus suggesting that the active constituent of "Royal Jelly" might be in the ether soluble fraction.[76, 77]

A carboxylic acid was isolated from the lipids in high yield [75] and the chemical structure of this acid was determined by Butenandt and Rembold. They reported a melting point of 54°-56° and a molecular formula of $C_{10}H_{18}O_3$, the ultraviolet and infra-red absorption spectra suggesting that the acid contained a hydroxyl group (3600 cm^{-1}) and a conjugated double bond (1650 cm^{-1}).

The stereochemistry about the double bond was elucidated by a study of the n.m.r. spectrum of the methyl ester [79], the π values and the coupling constants, which were found for the olefinic protons, being those expected of a trans double bond. The uptake of one mole of hydrogen on catalytic hydrogenation and the subsequent formation of 10-hydroxydecanoic acid (75) a known compound, showed conclusively that the active constituent of "Royal


HOCH2(CH2)6CH=CHCO2H → HO(CH2)9CO2H
(74)
(75)


HO(CH2)9CO2H (75)
CH3O(CH2)9CO2H (76)
a. SOCl2
b. Br2
c. NaI
CH3O(CH2)8CHICO2H (77)
HO(CH2)6Cl (78)
HO(CH2)7CO2H (79)
CH3O(CH2)7COCl (80)
CH3O(CH2)7CHO (81)
CH3O(CH2)7CH=CHCO2H (82)
HOCH2(CH2)6CH=CHCO2H (74)

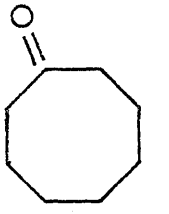
Jelly" was trans-10-hydroxydec-2-enoic acid (74).[78]

The interest in Royal Jelly acid has been quickened recently by the findings that Royal Jelly inhibited the development of transplantable mouse leukaemia and the formation of tumours in mice and that Royal Jelly acid was 100 times more active than "Royal Jelly" itself.[80]

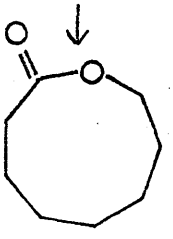
The first reported syntheses of Royal Jelly acid appeared in 1960 and are outlined below.[81, 82, 83.]

In one synthesis 10-hydroxydecanoic acid (75) was acetylated to give 10-acetoxydecanoic acid (76) the acid chloride of which was brominated, hydrolysed with water and treated with sodium iodide in ethanol to give the iodo-acid (77). Aqueous ethanolic sodium hydroxide converted this compound to trans-10-hydroxydec-2-enoic acid (74), which was shown to be identical with natural Royal Jelly acid.

The same authors also described an alternative synthesis, in which 1-chloro-6-hydroxyhexane (78) was converted by a malonic ester synthesis to 8-hydroxy- octanoic acid (79). The acetate of the corresponding acid chloride (80) was reduced over palladized barium sulphate to give 8-acetoxyoctanal (81). A Doebner condensation of this aldehyde with malonic acid yielded 10-acetoxydec-2-enoic acid (82) which on hydrolysis gave trans-10-hydroxydec-2-



(83)



(84)

$HO(CH_2)_7COCH_2CN$ (85)

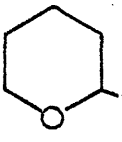
$HO(CH_2)_7COCH_2CO_2CH_3$ (86)

$HO(CH_2)_7CHOHCH_2CO_2H$ (87)

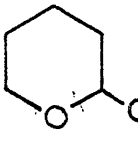
$HO(CH_2)_7CH{=}^{t}CHCO_2H$

(74)

$HO(CH_2)_7Cl$ (88)



$O(CH_2)_7Cl$ (89)



$O(CH_2)_7C{\equiv}CH$ (90)

$HO(CH_2)_7C{\equiv}CCO_2H$ (91)

$HO(CH_2)_7CH{=}^{c}CHCO_2H$

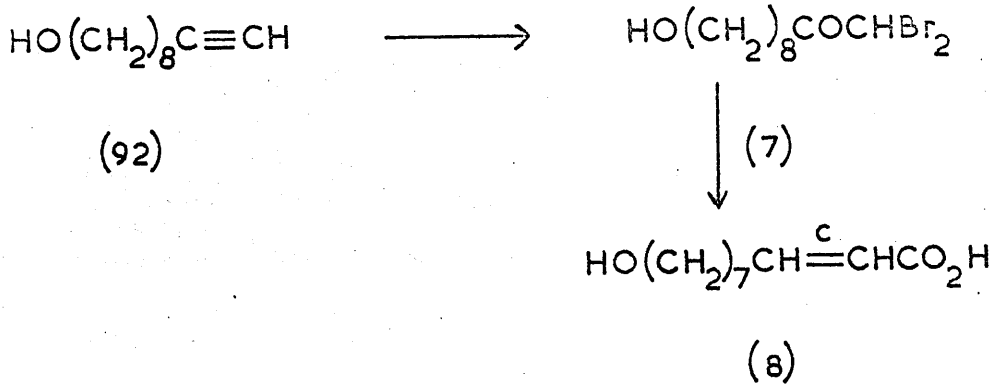
(8)

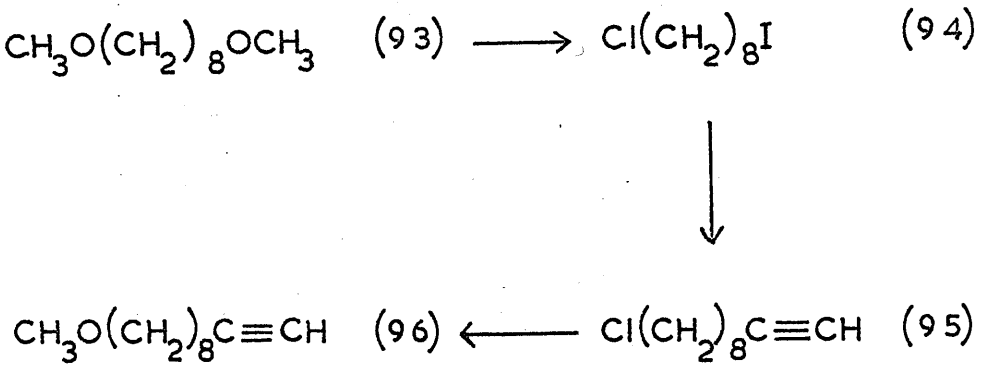
enoic acid (74).

Similar approaches to Royal Jelly acid have been reported by Fijii, Koga, Osawa and Ohieman. [84].

Huren [85] treated cyclo-octanone (83) with trifluoroperacetic acid to obtain the lactone (84), which was opened and condensed with the sodium salt of acetonitrile to give 10-hydroxy-3-keto-decanonitrile (85) which on methanolysis yielded the ester (86). Catalytic reduction followed by hydrolysis gave 3, 10-dihydroxydecanoic acid (87) which was readily dehydrated to trans-10-hydroxydec-2-enoic acid (74).

A synthesis of cis-10-hydroxydec-2-enoic acid (8) was also reported. [82, 86] 7-chloroheptanol (88) was converted into the corresponding tetrahydropyranyl ether (89) and then, after halogen exchange to give the corresponding iodo compound, condensation with sodium acetylide gave tetrahydropyranyloxy-non-1-yne (90), the magnesium derivative of which was carboxylated with carbon dioxide to yield 10-hydroxydec-2-ynoic acid (91). Hydrogenation over Lindlar catalyst gave cis-10-hydroxydec-2-enoic acid (8).


HO(CH2)8C≡CH
HO(CH2)8COCHBr2
(92)
(7)
HO(CH2)7CH=CHCO2H
(8)

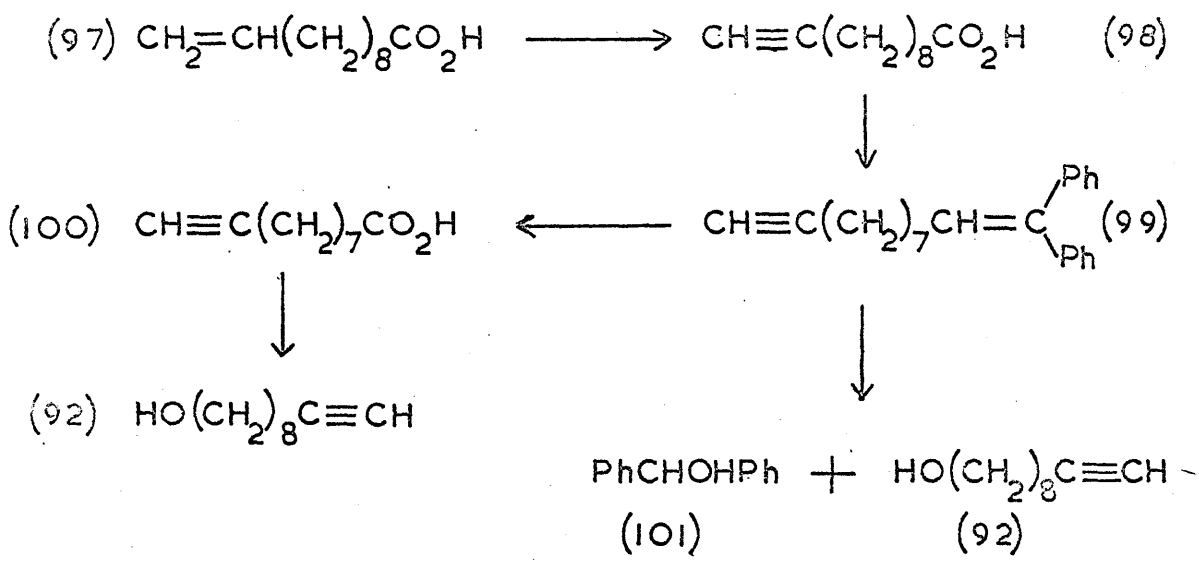

CH3O(CH2)8OCH3
(93)
Cl(CH2)8I
(94)
CH3O(CH2)8C≡CH
(96)
Cl(CH2)8C≡CH
(95)

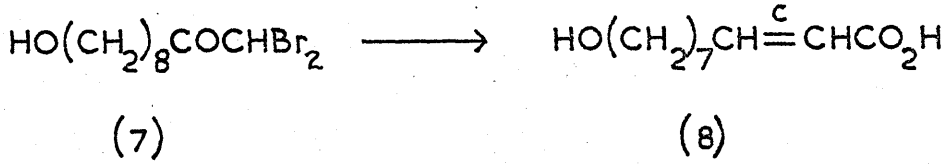
Discussion.

The present work was concerned with the synthesis of 10-hydroxydec-1-yne (92) which, by treatment with hypobromous acid to give the dibromoketone (7), can be converted to cis-10-hydroxydec-2-enoic acid (8) by a Faworsky reaction.[10] This serves to exhibit the application of the above rearrangement to the synthesis of natural products.

The routes available to this intermediate were somewhat cumbersome and gave poor overall yields.[10] Thus, one route involved the conversion of 1, 8-dimethoxyoctane (93) to the dihydroxy compound by means of the dibromide and the diacetoxy compound. The diol was converted to the dichloro compound which on treatment with sodium iodide in acetone gave 1-chloro-8-iodo-octane (94). Condensation with sodium acetylide in liquid ammonia gave 10-chloro-dec-1-yne (95) which yielded the corresponding acetoxy compound (96) on treatment with potassium acetate in acetic acid (8% overall yield).[10].

An alternative route involved the conversion of undec-10-enoic acid (97) to undec-10-ynoic acid (98). The methyl ester, when subjected to the Barbier-Wieland


(97) CH2=CH(CH2)8CO2H
CH≡C(CH2)8CO2H (98)
CH≡C(CH2)7CH=C(Ph)(Ph) (99)
(100) CH≡C(CH2)7CO2H
(92) HO(CH2)8C≡CH
PhCHOHPh + HO(CH2)8C≡CH
(101) (92)


HO(CH2)8COCHBr2
HO(CH2)7CH=C=CHCO2H
(7)
(8)

degradation process, yielded 1, 1-diphenylundec-1-ene-10-yne (99), which was oxidised to dec-9-ynoic acid (100). Lithium aluminium hydride reduction gave 10-hydroxydec-1-yne (92) (9% overall yield). [10].

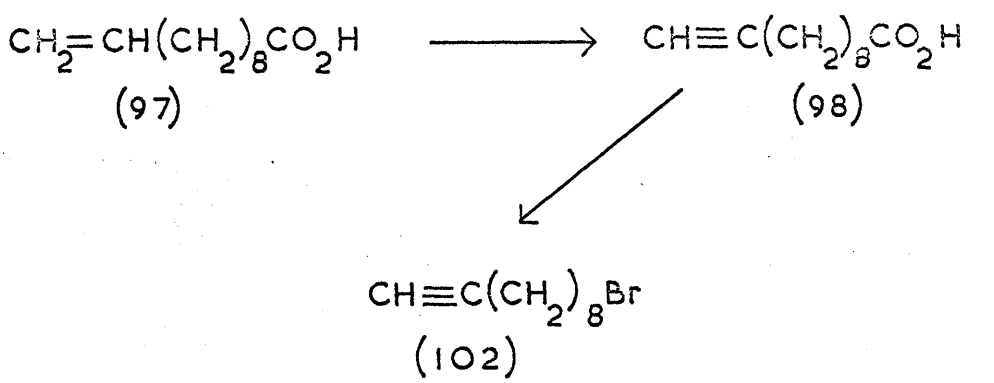
The Barbier Wieland product (99), on careful ozonolysis and reduction with sodium borohydride, gave a mixture of diphenylcarbinol (101) and 10-hydroxydec-1-yne (92) (19% overall yield). [10].

Formation of the dibromo-ketone (7) from the acetylenic alcohol, followed by a Faworsky reaction, gave cis-10-hydroxydec-2-enoic acid (8).[10]

The findings[80] concerning the inhibitory action of Royal Jelly acid towards mice tumours revived the interest in a synthetic route which might be of more practical value.

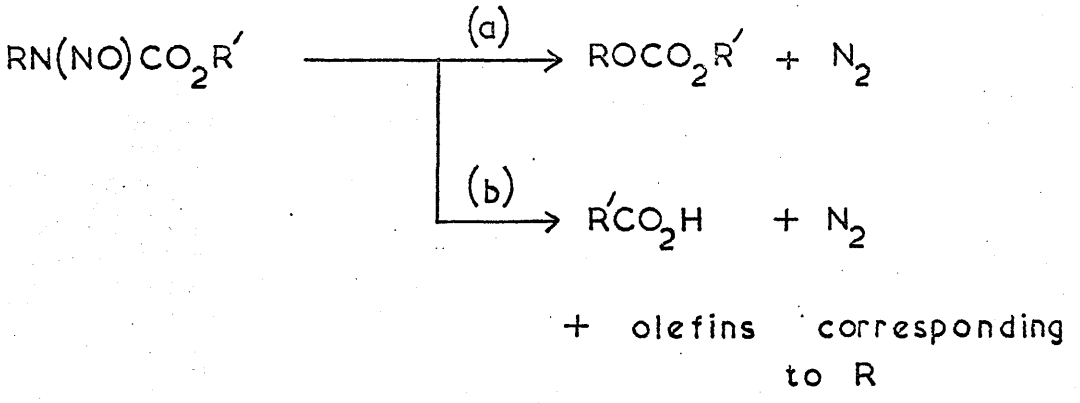
In the present work, four approaches to the key intermediate 10-hydroxydec-1-yne were investigated all designed to shorten the sequence and improve the overall yield.

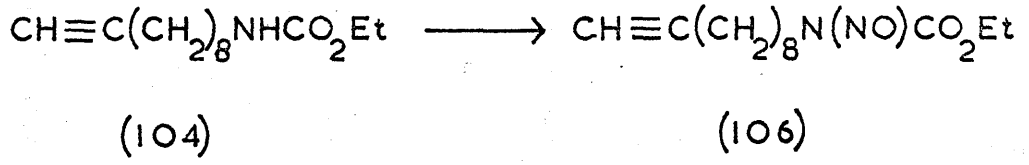
1. The readily available undec-10-enoic acid (97) was brominated and dehydrobrominated to give undec-10-ynoic acid (98). Attempts to convert this acid directly to 10-bromo-dec-1-yne (102) by a Hunsdiecker reaction.[87, 88]


CH2=CH(CH2)8CO2H
(97)
CH≡C(CH2)8CO2H
(98)
CH≡C(CH2)8Br
(102)
CH≡C(CH2)8CONHNH2
(103)
CH≡C(CH2)8NHCO2Et
(104)
HO(CH2)8C≡CH
(92)
CH≡C(CH2)8NH2
(105)

were all unsuccessful. The modified version described by Cristol and Firth [89] using red mercuric oxide and bromine with the exclusion of light gave, not a neutral compound as expected, but a saturated acid which gave a positive Bielstein test suggesting that reaction had occurred preferentially at the triple bond.

2. The methyl ester of undec-10-ynoic acid was treated with hydrazine hydrate, [90, 91] the acid hydrazide (103) being formed as a highly crystalline white solid in high yield. The hydrazide was converted to the urethan, 10-carbethoxyaminodec-1-yne (104) by careful treatment with nitrous acid at 0°, [92, 93] the azide formed in situ being converted to the urethan by reaction with anhydrous ethanol. The purified urethan (column chromatography) proved difficult to convert to the free amine. 10-aminodec-1-yne (105) by treatment with methanolic barium hydroxide. [93] Only once was the hydrochloride of the amine obtained crystalline, but complete characterisation was not possible since the crystals rapidly darkened on exposure to air. It was found that the amine could be stored in a solution of 6N hydrochloric acid but the free amine, on exposure to air, rapidly became encrusted with a whitish solid, presumably the amine carbonate. The yields in the conversion of the urethan


RN(NO)CO2R′
(a)
ROCO2R′ + N2
(b)
R′CO2H + N2
+ olefins corresponding to R

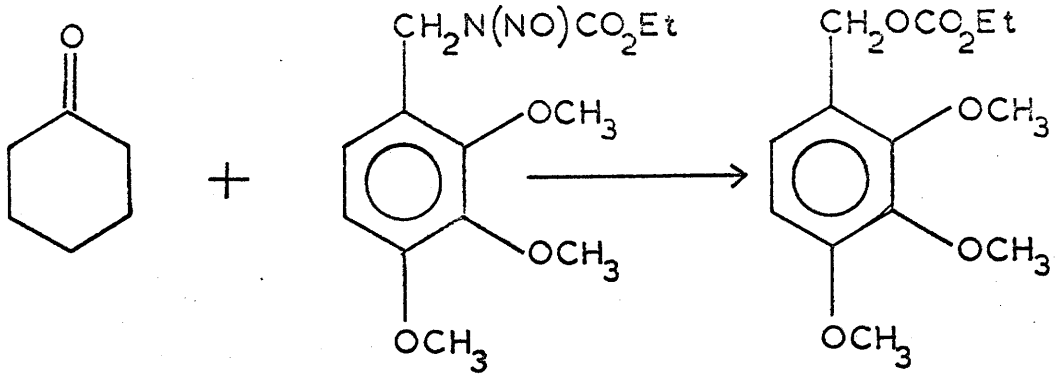

CH≡C(CH2)8NHCO2Et
CH≡C(CH2)8N(NO)CO2Et

(104) (106)

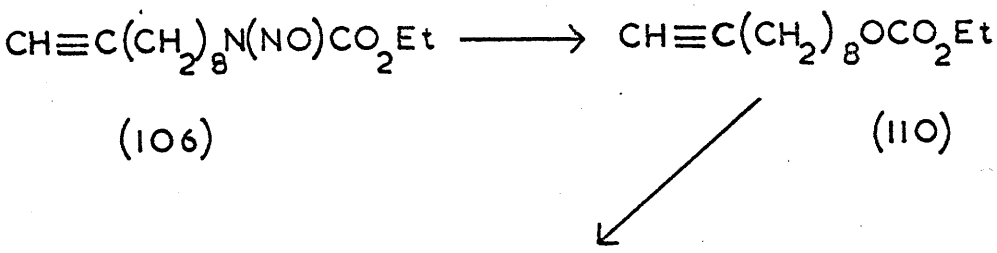
to the amine were always low (25-30%) and could not be increased by lengthening the reflux time.

Treatment of the amine, with sodium nitrite in hydrochloric acid at 0^{o} gave a complex mixture of products. The petroleum ether soluble fraction was purified by column chromatography on silica gel and finally by preparative t.l.c. The final product appeared to exhibit the required spectral characteristics of the acetylenic alcohol, 10-hydroxydec-1-yne (92) but the yields were too low to tempt further study. Variations in the experimental conditions for the conversion of the amine to the desired alcohol did not improve the yield. This type of direct conversion of aliphatic amines into alcohols is a reaction which seldom gives good yields.

3. The next approach employed the facile thermal elimination of nitrogen from N-alkyl-N-nitrosamides.[94] (See opposite). 10-carbethoxyaminodec-1-yne (104), when treated with sodium nitrite in acetic anhydride/acetic acid solution at 0^{o}, gave a high yield of 10-(N-nitroso)-carbethoxyaminodec-1-yne (106). The product decomposed on attempted distillation but i.r. spectral absorptions (1745 cm^{-1} and 1520 cm^{-1}) indicated that the N-nitroso compound had been formed. T.l.c. investigations showed



(107) (108) (109)

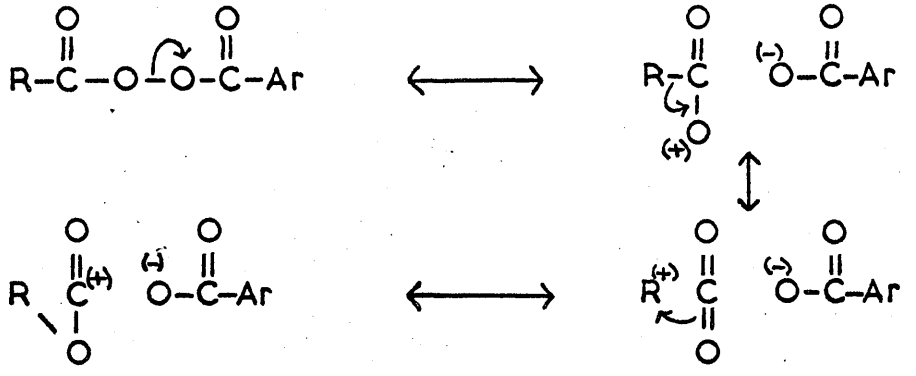


(106) (110)

(92)

that the reaction gave a pure product. The i.r. values compared favourably with reported values for this type of compound [94] viz. 1754 and 1527 cm^{-1}. Refluxing the N-nitroso compound in non-polar solvents (petroleum ether, benzene, pentane) did not cause the desired elimination of nitrogen. With benzene, the product was seen to contain some unsaturated material, probably the alkene as indicated by pathway (b) opposite. Gutsche, [95] on attempting a ring expansion of cyclohexanone (107) with N-carbethoxyamino-N-nitroso-2, 3, 4-trimethoxybenzylamine (108), found that he obtained a 75% yield of 2, 3, 4-trimethoxybenzyl ethyl carbonate (109), thus suggesting that cyclohexanone might be a possible solvent for the nitrogen elimination. Heating 10-(N-nitroso)-carbethoxyaminodec-1-yne (106) in cyclohexanone for 4 hours at 115° gave an almost quantitative yield of nitrogen, collected by downward displacement of water. The i.r. spectra of the product indicated that the N-nitroso group had been eliminated and that a carbonate ester had been formed (1785 and 1735 cm^{-1}). The unpurified ethyl decynyl carbonate (110) was hydrolysed with potassium hydroxide in methanol, giving 10-hydroxydec-1-yne (92). (25% overall yield.).

4. Finally, the best and most direct method for



$$CH\equiv C(CH_2)_8COCl \longrightarrow CH\equiv C(CH_2)_8CO.OO.COC_6H_4Cl$$

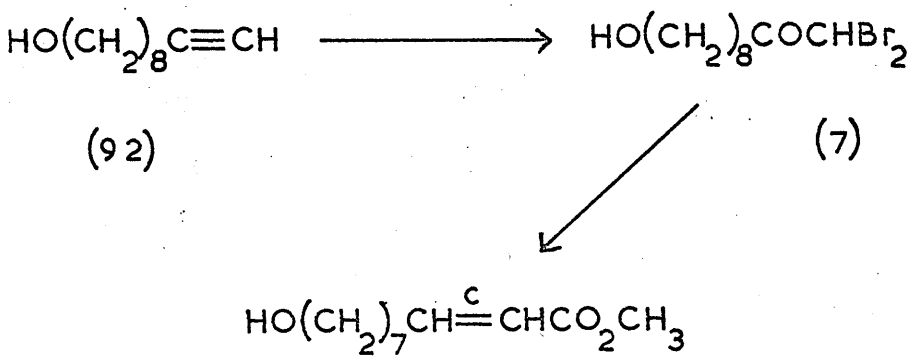
(111) (112)

$$CH\equiv C(CH_2)_8OCO.OCO.C_6H_4Cl$$

(113)

the preparation of the intermediate alcohol involved a carboxy-inversion reaction of an acyl-aroyl peroxide [96], which gives the corresponding acyl-aroyl carbonate. It was known[96] from consideration of experimental data that the peroxide linkage breaks to give the potentially more stable carboxylate anion which in the case of acyl-aroyl peroxides is the aroyl carboxylate anion. (See opposite).

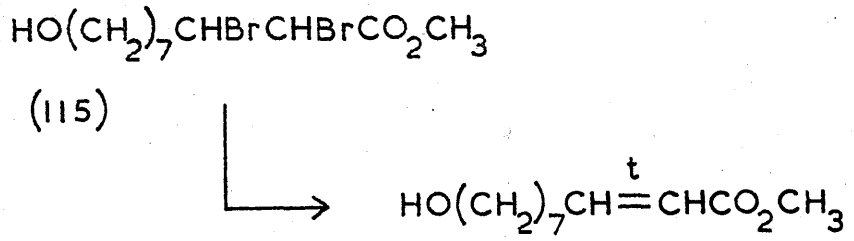
Undec-10-ynyl chloride (111) was prepared in high yield by the reaction of thionyl chloride on the corresponding acid. On treatment with metachloroperbenzoic acid and pyridine in hexane at -20° (CCl_4/CO_2 bath), the acid chloride was converted to the mixed peroxide (112) (1810 and 1775 cm^{-1}). It was known[96] that the rates of the rearrangement of mixed peroxides varied according to the nature of the alkyl group present, the peroxides with primary alkyl groups being the slowest. Since the mixed peroxide prepared above involved the migration of the primary undecynyl group, the carboxy-inversion reaction was achieved by "forcing conditions",[96] namely, refluxing in hexane/cyclohexane solution for 13 hours. The product the corresponding mixed carbonate (113), showed carbonyl absorption in the i.r. at 1810 and 1720 cm^{-1} (broad). The persistence of the peak at


HO(CH2)8C≡CH
HO(CH2)8COCHBr2
(92)
(7)
HO(CH2)7CH=CHCO2CH3
(114)

1810 cm^{-1} suggested that the rearrangement had not gone to completion but qualitative peroxide tests with starch iodide paper and potassium iodide/starch aqueous solutions proved negative. Neither the peroxide nor the carbonate were purified since both products were extensively decomposed on attempted distillation. The hydrolysis of the carbonate, metachlorophenyl dec-9-ynyl carbonate with potassium hydroxide in methanol gave the desired 10-hydroxydec-1-yne (92), (42% overall yield).

An additional advantage of this route is the speed with which the reaction sequence can be carried through, the complete synthesis being achieved in less than 3 days.

The synthesis of Royal Jelly acid was then completed as follows. Treatment of the acetylenic alcohol with bromodan in buffered acetic acid gave 1, 1-dibromo-10-hydroxydecan-2-one (7) as a viscous red oil which decomposed on attempted distillation. The product was characterised as the bis 2, 4-dinitrophenylhydrazone. Treatment of the dibromo-ketone with sodium methoxide in methanol gave the desired methyl cis-10-hydroxydec-2-enoate (114), which was purified by column chromatography on silica gel followed by short path distillation. The n.m.r. and i.r. spectra (813 cm^{-1}) confirmed the presence


HO(CH2)7CHBrCHBrCO2CH3
(115)
t
HO(CH2)7CH=CHCO2CH3
(116)

of a cis double bond, no absorptions indicative of a trans double bond being present.

The n.m.r. spectrum exhibited a clearly defined ABX_2 splitting pattern for the olefinic and adjacent methylene protons the value of the coupling constant, J_{cis}=12 c/s being in accord with the presence of a cis double bond. [12]

The reported[10] conversion of methyl cis-oct-2-enoate to the trans isomer by column chromatography on grade I alumina could not be repeated in spite of many variations in the type of alumina used. Methyl cis-10-hydroxydec-2-enoate (114), on being subjected to the above treatment, also failed to give any of the trans ester, the starting material being unchanged even after repeated elutions from alumina.

However, the cis ester was eventually converted to the corresponding trans ester as follows.

Addition of bromine in carbon tetrachloride to methyl cis-10-hydroxydec-2-enoate gave methyl 2, 3-dibromo-10-hydroxydecanoate (115), the i.r. spectrum of the product indicating that bromine had added across the double bond as desired. The carbonyl absorption frequency had risen from 1725 cm^{-1} to 1743 cm^{-1}, a value expected for a saturated ester. Several attempts

were made to dehalogenate this ester with zinc, but in spite of a variety of methods of activating the zinc being employed. [97, 98, 99] no unsaturation was evident in the i.r. spectra of the products.

The desired methyl trans-10-hydroxydec-2-enoate (116) was finally prepared by refluxing the saturated dibromo ester (115) with sodium iodide in absolute ethanol [100], the vicinal di-iodide formed being unstable, readily eliminating iodine to form a trans double bond. The product had a retention time on g.l.c. (10% P.E.G.A. at 150°), identical to that of methyl cis-10-hydroxydec-2-enoate but the i.r. spectrum confirmed the presence of trans unsaturation (1654 and 978 cm^{-1}).

Experimental.

Attempted Hunsdiecker reaction on undec-10-ynoic acid.

To a slurry of red mercuric oxide (1·94 g; 0· 009 M.) in a refluxing solution of undec-10-ynoic acid (2·05g; 0·0113 M.) in anhydrous carbon tetrachloride (50 ml.), bromine (1·80g; 0·0113 M.) in carbon tetrachloride (10 ml.) was added dropwise with stirring. The reaction mixture was refluxed in the dark for 1 hour with continuous stirring and, after cooling, was filtered through glass paper and the filtrate thoroughly washed with sodium metabisulphite solution, 4N sodium hydroxide solution (4x50 ml.) and water (4x20 ml.). Evaporation gave only very small quantities of an off white solid. The basic washings were acidified with 6N hydrochloric acid solution, extracted thoroughly with ether and the combined organic extracts washed with brine until neutral. Evaporation gave a light brown oil (2·67 g.) which gave a positive Beilstein test.

$\nu_{max.}$ (thin film): 3100 (broad), 1710 cm^{-1} - no peak at 2120 cm^{-1}.

Methyl undec-10-ynoate.

Undec-10-ynoic acid (4·95 g.) was dissolved in anhydrous methanol (42 ml.) containing concentrated

sulphuric acid (0·25 ml.) and the solution was allowed to stand in a stoppered flask for 16 hours at room temperature. Most of the methanol was removed and the residual solution poured into water (25 ml.) and extracted with ether (4x15 ml.). The combined organic extracts were washed thoroughly with water and evaporated to give a pale yellow oil. The product was purified by distillation under reduced pressure to give a colourless sweet smelling oil (5·1 g; 95%).

b.p. 96°-97°/0·5 mm. n_D^{25} 1·4465.

(literature[10]: b.p. 99°/0·5 mm. n_D^{24} 1·4460).

$\nu_{max.}$ (thin film): 3350, 2230, 1740 cm^{-1}.

Undec-10-ynoic acid hydrazide (103).

Methyl undec-10-ynoate (5·1 g; 0·027 M.) was added dropwise to boiling 100% hydrazine hydrate (2·1g; 0·04 M.). The mixture was refluxed for 3½ hours and then poured into ice-cold water (100 ml.), the resulting precipitate being filtered and washed with water and 10% ethanol/water. The product recrystallised from water as white needles. (3·4 g; 68%). m.p. 82°-83°.

(literature [90]: m.p. 84°-85°).

$\nu_{max.}$ (nujol): 3355, 3220, 2220, 1633, 1542 cm^{-1}.

($C_{11}H_{20}N_2O$ requires C, 67·31; H, 10·27; N, 14·27

found C, 67·25; H, 10·16; N, 14·01%)

10-carbethoxyaminodec-1-yne (104)

Undec-10-ynoic acid hydrazide (3·4 g; 0·017 M.) was dissolved in dilute hydrochloric acid, ethanol being added to aid solution. After cooling the solution to 0°-5° in an ice bath, a concentrated aqueous solution of sodium nitrite (1·24g; 0·017 M.) was added slowly with vigorous stirring, the temperature of the reaction mixture being kept below 10°. Before the addition, the aqueous layer was covered with ether, the azide formed *in situ* passing into the ether layer. Immediately after the addition, the layers were separated and the ethereal layer washed with saturated sodium bicarbonate solution (2x25 ml.), water (2x25 ml.) and brine (2x25 ml.). To the dried ethereal solution, anhydrous ethanol (250 ml.) was added, the ether evaporated and the remaining ethanolic solution refluxed for 3½ hours. Evaporation of the ethanol and column chromatography on silica gel gave the urethan as a pale yellow oil (1·85g: 50%).

$\nu_{max.}$ (thin film) 3400 (broad), 2130, 1720, 1535, 1250 cm^{-1}.

($C_{13}H_{23}NO_2$ requires C, 69·29; H, 10·29; N, 6·22

found C, 68·95; H, 10·14; N, 6·18%)

Reaction of 10-carbethoxyaminodec-1-yne with barium hydroxide.

10-carbethoxyaminodec-1-yne (1·48 g.) was refluxed with a saturated methanolic solution of barium hydroxide (25 ml.) for 24 hours. The barium carbonate precipitate was filtered off and water (150 ml.) added to the filtrate which was then extracted with ether (4x25 ml.) and the combined organic extracts washed with water (4x25 ml.). An ethereal solution of gaseous hydrochloric acid was added to the dried ethereal solution of the product until it was acidic, the mixture cooled in an ice bath and the precipitate filtered and dried. (0·40g; 40%). m.p. 149°-150°.

Attempts at purification of the amine hydrochloride for analysis were unsuccessful since rapid decolourisation of the crystals occurred on exposure to air or to heat.

$\nu_{max.}$ (nujol) 3480, 3300, 2100, 1615 cm^{-1}.

On evaporation of the ether a light red oil was obtained which appeared from i.r. spectra to consist mainly of unchanged 10-carbethoxyaminodec-1-yne. This oil was again refluxed as above and the isolation procedure repeated. Since this failed to yield the hydrochloride as a solid. the ethereal solutions containing the amine were extracted with 6N hydrochloric acid (4x20 ml.), the acid extracts washed with ether (4x20 ml.), carefully neutralised with saturated sodium bicarbonate

solution and re-extracted with ether (4x20 ml.). The combined organic extracts were thoroughly washed with brine and evaporated. Since, on exposure to air, the oily amine rapidly became encrusted with a whitish solid, the amine was stored in a solution of 6N hydrochloric acid.

Attempted preparation of 10-hydroxydec-1-yne.

A solution of the amine in dilute hydrochloric acid was cooled to 0° and a 10% aqueous sodium nitrite solution (50 ml.) added slowly with stirring, the temperature of the mixture being kept below 10°. The mixture was then extracted with ether (3x20 ml.) and the combined organic extracts thoroughly washed with water and evaporated. Extraction with light petroleum ether gave a yellow mobile liquid (0·98 g.) which appeared to contain olefinic material from i.r. spectral absorptions (sharp peak at 1645 cm^{-1} and peaks at 980 and 922 cm^{-1}). Separation by column chromatography on silica gel and preparative t.l.c. gave a yellow oil (0·29g.) which appeared to be impure 10-hydroxydec-1-yne.

$\nu_{max.}$ (thin film): 3450 (broad), 3300, 2130, 1060 cm^{-1}.

This was not investigated further.

Cis-methyl-10-carbethoxyaminodec-2-enoate.

10-carbethoxyaminodec-1-yne (1·11g.) was added over 5 minutes to a cooled, stirred suspension of bromodan (2·15g; 3 equivs.) and sodium acetate trihydrate (2·04g; 3 equivs.) in 50% aqueous acetic acid (60 ml.) and the reaction mixture stirred at room temperature for 2½ hours. The reaction mixture was added to water (300 ml.) and the whole extracted with ether, until the ether extracts were colourless. The combined organic extracts were washed with saturated sodium metabisulphite solution (30 ml. saturated solution in 100 ml. water) until the bromine colour disappeared, water (2x150 ml.), carefully neutralised with saturated sodium bicarbonate solution, and finally washed with water (2x150 ml.). Evaporation of the solvent gave the crude dibromo-ketone as a brownish oil (1·89g; 92%).

$\nu_{max.}$ (thin film) 3350, 1720, 1705, 1540, 1260 cm^{-1}.

No purification of the product was attempted.

To a solution of sodium (0·068g; 3 equivs.) in anhydrous methanol (5 ml.), the crude dibromo-ketone (1·89g.) in anhydrous methanol (1 ml) was added slowly with vigorous stirring. After stirring at room temperature for 20 minutes, the reaction mixture was poured into water (100 ml.) and extracted with ether (4x20 ml.). The

combined organic extracts were washed with brine until neutral and evaporated to give a dark brown oil, which was purified by column chromatography on silica gel to give a pale yellow oil (0·2g; 16%). b.p. (short path) 125°-130°/0·1 mm. n_D^{22} 1·4638.

$\nu_{max.}$ (thin film) 3400, 1720, 1705, 1645, 1540, 1280, 840 cm^{-1}.

Tau values ($CDCl_3$): 8·79 (3H, t, J = 7 c/s). 8·66 (10H, m), 7·69 (1H, s), 5·91 (3H, s), 5·90 (2H, q, J = 7 c/s), 4·33 (1H, doublet - J = 11·5 c/s - of triplets - J = 1·5 c/s), 3·83 (1H, doublet - J = 11·5 c/s - of triplets - J = 7·5 c/s).

($C_{14}H_{25}NO_4$ requires C, 61·97; H, 9·29; N, 5·16

found C, 61·78; H, 9·18; N, 5·39%)

10-hydroxydec-1-yne (92)

(a) To an ice cold solution of 10-carbethoxyaminodec-1-yne (11g.) in acetic anhydride (250 ml.) and acetic acid (50 ml.), granular sodium nitrite (71g; 21 equivs.) was added over 4½ hours with continuous stirring. The mixture was maintained at 0° for 18 hours, and allowed to warm to 10° over 30 minutes. The solution was then poured into ice water (1 litre) and extracted thoroughly with ether, the combined organic extracts being washed with water (2x50 ml.), saturated sodium bicarbonate solution (2x50 ml.),

and water (2x50 ml.). Evaporation of solvent gave a yellow oil (12·3 g; 95%). A sample of the product largely decomposed on attempted distillation. b.p. 95°-100°/0·3 mm.

$\nu_{max.}$ (thin film) 3380, 2130, 1745, 1520, 1135 cm^{-1}.

The crude 10-(N-nitroso)-carbethoxyaminodec-1-yne (1·14 g.) in cyclohexanone (125 ml.) was heated at 115°-120° with stirring for 4 hours, the nitrogen evolved (95 ml; 95%) being collected by downward displacement of water. Excess cyclohexanone was removed by distillation and the residue poured into ether (250 ml.), the ethereal solution being washed with saturated sodium bisulphite solution (5x50 ml.) and water (2x50 ml.).

Evaporation of solvent gave a yellow oil.

$\nu_{max.}$ (thin film) 3380, 2130, 1785, 1735, 1280 cm^{-1}.

The crude mixed carbonate (1·14g.) was refluxed with 1N methanolic potassium hydroxide solution (50 ml.) for 18 hours. Most of the methanol was removed and the residue poured into water (200 ml.) and extracted thoroughly with ether. The combined organic extracts were washed with brine till neutral and evaporated to give a pale yellow oil. (0·55g; 65% from 10-carbethoxyaminodec-1-yne). The

product was purified by distillation.

b.p. 79°-80°/0·15 mm. n_D^{23} 1·4570

(literature[10]: b.p. 74°-80°/0·2 mm. n_D^{25} 1·4575).

$\nu_{max.}$ (CCl_4): 3628, 3306, 2108, 1048 cm^{-1}.

Tau values (CCl_4): 8·63 (12H, m), 8·25 (1H, doublet - J = 4 c/s - of triplets - J = 2 c/s), 7·85 (2H, m),
6·75 (1H, s, disappears on D_2O addition), 6·47 (2H, t, J = 7 c/s).

($C_{10}H_{18}O$ requires C, 77·87; H, 11·76

found C, 77·57; H, 11·61%)

(b) Undec-10-ynoic acid (8·5g.) was added to an excess of thionyl chloride and the mixture heated on a steam bath for 30 minutes. The excess thionyl chloride was removed at the water pump to give a brown oil which was purified by distillation under reduced pressure to give a straw coloured liquid (6·0 g; 70%)

b.p. 133°-135°/15 mm.

$\nu_{max.}$ (thin film): 3380, 2160, 1805 cm^{-1}.

The acid chloride (6·0g; 0·03 M.) was added to metachloroperbenzoic acid (6·01g; 0·03 M.) in hexane (150 ml.) and the stirred mixture was treated dropwise

with a solution of pyridine (2·31g; 0·03 M.) in hexane (15 ml.). The temperature of the reaction mixture was maintained at -20° to -24° (CO_2/CCl_4 bath) during the addition and during the stirring of the mixture for 5 hours thereafter. The hexane solution was then filtered to remove the pyridine hydrochloride and the filtrate washed with water (2x50 ml.), 10% sodium bicarbonate solution (50 ml.), water (2x50 ml.), 10% hydrochloric acid solution (50 ml.) and water (2x50 ml.). Evaporation gave a colourless oil (9·80g; 93%).

$\nu_{max.}$ (thin film) 3380, 2150. 1810, 1775, 1580, 1235, 1080 (broad) cm^{-1}.

The mixed peroxide (9·80g; 0·029 M.) was refluxed in hexane (60 ml.) and cyclohexane (40 ml.) for 13 hours, and evaporation of solvent gave a colourless oil. Qualitative peroxide tests (starch iodide paper and aqueous potassium iodide/starch solution) proved negative.

$\nu_{max.}$ (thin film) 3380, 2150, 1810, 1720 (broad), 1580, 1260, 1060 (broad) cm^{-1}.

The mixed carbonate (9·80g; 0·029 M.) was added to 1N methanolic potassium hydroxide solution (175 ml.) and refluxed for 19 hours. Most of the methanol was

evaporated and the residue poured into water (500 ml.) and extracted thoroughly with ether. The combined organic extracts were washed with brine till neutral and evaporated to yield the acetylenic alcohol as a pale yellow oil (1·93g; 42% from acid chloride).

10-hydroxydec-1-yne was purified by distillation under reduced pressure.

b.p. 77°-80°/0·15 mm. n_D^{24} 1·4573.
(literature[10]: b.p. 74°-80°/0·2 mm. n_D^{25} 1·4575).

The i.r. and n.m.r. spectra (CCl_4 solutions) were identical in all respects with those of a sample prepared from 10-carbethoxyaminodec-1-yne.

(Acidification of the aqueous layer gave a quantitative recovery of metachlorobenzoic acid).

1,1-dibromo-10-hydroxydecan-2-one (7)

10-hydroxy-dec-1-yne (1·28g.) was added dropwise to a cooled, stirred suspension of "bromodan" (3·75g; 3 equivs.) and sodium acetate trihydrate (3·39g; 3 equivs.) in 50% aqueous acetic acid (100 ml.) and left stirring overnight at room temperature. The reaction mixture was poured into water (100 ml.) and extracted thoroughly with ether, the combined ethereal extracts being washed with saturated sodium metabisulphite solution until the bromine colour disappeared, and then being carefully neutralised

with saturated sodium bicarbonate solution. After washing with brine, the solvent was evaporated to give a pale yellow oil (2·11g; 77%).

$\nu_{max.}$ (thin film) 3520 (broad), 1718 cm^{-1}.

The product formed a red bis-dinitrophenylhydrazone m.p. 175°-176° (chloroform/methanol). The melting point was undepressed on being mixed with an authentic sample. (literature[10]: m.p. 174°-176°).

Methyl cis-10-hydroxydec-2-enoate.

1, 1-dibromo-10-hydroxydecan-2-one (0·9g.) in methanol (10 ml.) was added at 0° to a solution of sodium (0·18g: 3 equivs.) in methanol (20 ml.), and the reaction mixture stirred at room temperature for 30 minutes. The mixture was then poured into water (250 ml.) and extracted with ether (4x25 ml.), the combined ethereal extracts being washed with brine until neutral. Evaporation of solvent gave a pale brown oil which was purified by column chromatography on silica gel to give a pale yellow oil (0·25g; 48%).

b.p. 115°-120°/0·7 mm. n_D^{22} 1·4346.

$\nu_{max.}$ (CCl_4): 3625, 1725, 1644, 1193, 1172, 1040 (broad), 813 (CS_2) cm^{-1}.

Tau values (CCl_4): 8·62 (10H, m), 7·34 (2H, d, J = 7c/s) 6·3 (2H, t, J = 7 c/s), 6·25 (3H, s), 4·19 (1H, doublet -

J = 12 c/s - of triplets - J = 2 c/s), 3·71 (1H, doublet -J = 12 c/s - of triplets - J = 7 c/s).

($C_{11}H_{20}O_3$ requires C, 65·97; H, 10·07

found C, 65·79; H, 9·80%).

Methyl trans -10-hydroxydec-2-enoate (116).

Methyl cis-10-hydroxydec-2-enoate (0·05 g.) was dissolved in carbon tetrachloride (5 ml.) and a solution of bromine in carbon tetrachloride was added dropwise with stirring until the bromine colour persisted. The mixture was poured into water (10 ml.) and extracted thoroughly with carbon tetrachloride, the combined organic extracts being washed with saturated sodium metabisulphite solution (10 ml.) and brine (10 ml.) Evaporation of the solvent gave a pale yellow oil (0·09 g.; 85%).

$\nu_{max.}$ (thin film): 3550, 1743 cm^{-1}. - no peak at 1644 cm^{-1}.

The dibromo-decanoate (0·04 g.) in absolute ethanol (5 ml.) was added to crushed sodium iodide (0·5 g.) in absolute ethanol (10 ml.) and the suspension refluxed for 13 hours. Most of the ethanol was removed and the residue poured into water (20 ml.) and extracted thoroughly with ether, the combined organic extracts being washed with

saturated sodium metabisulphite solution until the iodine colour disappeared, with water (2x10 ml.) and with brine. Evaporation of solvent gave a brown oil which was purified by chromatography on silica gel to give a pale yellow oil (0·025g; 75%).

b.p. 115°-120°/0·6 mm.

ν max. (CCl_4): 3628, 1724, 1654, 1193, 1172, 1040 978 cm^{-1}.

This was identical with that of an authentic sample.

G.l.c. on 10% P.E.G.A. at 150° indicated that the compound was pure, having the same retention time as the cis isomer, namely 36 minutes.

References.

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Part II

Studies on Mould Metabolites.

(a) Examination of the Sterols of various orders of the Phycomycetes.

Introduction

For millenia the fungi have contributed to the ease and disease of mankind. The action of yeast in promoting the fermentation of diverse carbohydrate sources was known to the Philistines ca. 1100 B.C., while the unleavened bread of the Israelites was produced by the deliberate omission of yeast from the baking mixture. It is certain that the controlled use of fungi has played a major part in food production from the earliest periods of civilisation.

Baking and brewing still represent the most significant applications of micro-organisms but, in recent years, great advances have been made in the field of chemotherapeutics by the direct use of fungal metabolites.

The Fungi (Phylum Mycophyta) form one of the larger groups within the plant kingdom. Although the more familiar members of this group have fairly large fruiting bodies, most species are inconspicuous, appearing only as small dark dots or colourless cobweb-like coverings; many in fact are only discernible under the microscope.

All fungi lack the pigment chlorophyll,

which gives the characteristic green colour to true plants, and thus do not possess the ability to carry out photosynthesis. For this reason fungi, in order to grow must live as parasites on living organisms or as saprophytes on their dead tissues. This enables the fungi to secure an adequate supply of essential carbohydrates which they further transform as required.

The absorption of nutrients takes place via the mycelium which consists of minute tubes called hyphae, usually much thinner than a cobweb. The hypha consists of an elongated cyclindrical wall of polysaccharide material containing a mass of cytoplasm and many nucleii, which may or may not be separated by cross walls, the septa.

Classification of fungi is based on morphological criteria and need not be detailed here. However the four main groups are:

Phycomycetes (tube fungi)

Ascomycetes (sac fungi)

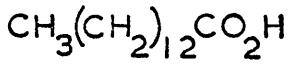
Basidiomycetes (club fungi)

Fungi Imperfecti.

The Saprolegniales, one of the orders of the Phycomycetes, is of particular interest as it contains

species that are parasitic on and toxic to fish. As a group they are unusual in that their cell walls consist of cellulose rather than the usual chitin. It was therefore of interest to determine whether this difference in cell wall constitution was reflected in the type of metabolite produced.

The following work was initiated in this department by Dr. B. Parker and Dr. R. Wheeler. [1, 2].



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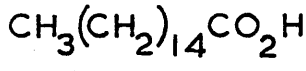

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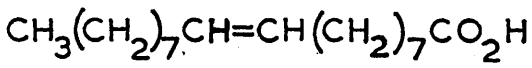

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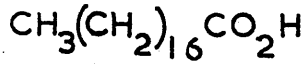


(4)

Discussion.

The species of the order Saprolegniales initially investigated were those native to Scotland, the fungi being grown on a medium containing 2.5% glucose and 0·5% peptone (a trypsin hydrolysate of beef, with added vitamins and salts). Petroleum ether, chloroform and ethanol extracts of the dried mycelia were examined, the most interesting results being obtained from the petroleum ether fraction.[1].

This extract was chromatographed on neutral, grade III, alumina using a step wise elution gradient. The initial fractions from the column were shown to contain esters of myristic (1), palmitic (2), oleic (3), and stearic acids (4) (g.l.c.), the quantities of each varying with the individual species. The polar fractions eluted from the columns appeared to contain amides, Saprolegnia ferax for example, containing a crystalline, high molecular weight amide which was tentatively assumed from mass spectral data to be a long chain acetamide probably containing more than one acetamide group. The yields of purified material (less than 0·09% of the mycelial extract) did not permit a more complete examination.[1].

The middle fractions consisted mainly of sterol esters and sterols, and, after hydrolysis with aqueous methanolic base, the sterols were isolated via their digitonin complex. The sterols isolated in this way were examined by g.l.c. [1].

Isolation of the sterols by preparative t.l.c. was later found to give results comparable with the digitonin method. Since the digitonin separation was tedious, and involved the handling of expensive toxic material, the preparative t.l.c. procedure was employed [2] subsequently and in the present work.

In this modified treatment, the petroleum ether extract of the mycelium was separated into three main fractions by chromatography on deactivated alumina: viz.

(A) Non polar fats.

(B) Sterol esters and sterols.

(C) Polar material.

Fraction (B) was hydrolysed and the neutral fraction examined by t.l.c. for the presence of sterols, the final separation of the sterols being achieved by preparative t.l.c.

A detailed gas chromatographic analysis of these compounds and their trimethyl silyl ethers and their

Phycomycetes previously studied

Fungus	Sterol content.
(a) Leptomitales	
Apodachlya brachynema	-
Apodachlya completa	+
Apodachlyella minima	+
(b) Saprolegniales.	
Saprolegnia ferax	+
Leptolegnia 74	+
Pythiopsis cymosa	+
Achlya caroliniana	+
Isoachlya monolifera	+
Saprolegnia megasperma	+
(c) Peronosporales.	
Phytophthora infestans	-
Pythium debaryanum	-
Pythium ultimum	-

Table 1.

Fungus.	Sterol content.
(d) Mucorales	
Absidia glauca (+), (-).	+ (trace)
Cunninghamella echinulata	-
Mortierella rammaniana	+ (trace)
Phycomyces blakesleeanus (+), (-).	+
Rhizopus stolonifer (+), (-).	+
Thamnidium elegans	+ (trace)
Syncephalastrium racemosum	-
Zygorhynchus moelleri	+ (trace)

Table 1.

trifluoroacetates suggested that three of the four major constituents were cholesterol (5), desmosterol (6), and fucosterol (7) [1]. The fourth sterol, the major component of the sterol fraction of Saprolegnia ferax, Saprolegnia megasperma and Leptolegnia 74 was inseparable (g.l.c.) from an authentic sample of 24-methylene cholesterol (8) synthesised from 24-keto cholesterol (9) by a Wittig reaction. [2].

The survey was extended to other orders of the Phycomycetes as shown in table (1).

However no definite conclusions were reached about the nature of the sterols in those species of the order Mucorales from which isolable quantities were obtained.

The first phase of the present work involved a more rigorous examination of the sterols of two species of the order Saprolegniales and also the first determination of the type of sterol contained in six species of the order Mucorales (g.l.c. results - table 2).

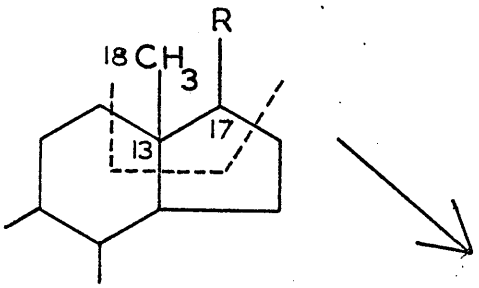
G.l.c. and mass spectral studies of the mixtures of sterols suggested that the species Saprolegnia ferax and Aplanopsis terrestris of the order Saprolegniales contained cholesterol (5), desmosterol (6), 24-methylene cholesterol (8), and fucosterol (7) in varying quantities. Previous attempts to separate

G.l.c. retention times.

Fungus	g.l.c. retention times relative to cholestane on 1%F-60 at 225°
(a) Saprolegniales.	
Saprolegnia ferax	2·22, 2·44, 2·92, 3·92
Aplanopsis terrestris	2·22, 2·95, 3·96
(b) Mucorales.	
Mucor hiemalis (+)	2·92, 3·42, 3·84
Mucor hiemalis (+) - shake culture	2·92, 3·42, 3·95
Mucor plumbeus	—
Mucor globosum	—
Mucor dispersus	2·91, 3·42, 3·81
Mucor fragilis	—
(c) Sterol	
cholesterol	2·2
desmosterol	2·45
24-methylene cholesterol	2·93
ergosterol	2·90
β-sitosterol	3·85
fucosterol	3·98

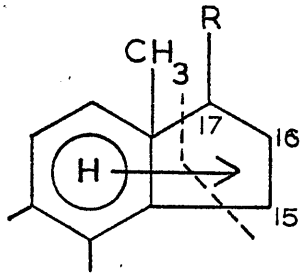
Table 2.

(a)



P—(side chain + 42 mass units)

(b)



Parent ions

Figure 1

the mixtures of sterols into the individual, pure components had been unsuccessful. [1] Preparative t.l.c. on silica gel impregnated with a pyrene dye was found to be best, as the dye was not soluble in the eluting solvents and was very sensitive to sterols when irradiated under U.V. light. Elution of the plates several times allowed a clean separation of cholesterol from the other sterols, but the latter compounds could not be separated by this method.

The sterol fractions were studied on the combined gas chromatograph-mass spectrometer, L.K.B. - 9000, which allowed the mass spectra of the individual components to be obtained. For reference purposes, detailed examinations of the mass spectra of standard samples of the sterols which were thought to be present were carried out.

The cracking pattern of all the sterols investigated showed many features typical of compounds of this type. Abundant ions corresponding to the loss of the complete side chain plus 42 mass units occurred in all the spectra. This had previously been regarded as a characteristic elimination of the side chain together with C_{13}, C_{17} and C_{18}, a suggestion which was criticised by Biemann and Djerassi[3] (Figure 1). They pointed out that such

a process required the rupture of three bonds without creating a particularly stable fragment. Biemann [4] proposed that the loss of C_{15}, C_{16} and C_{17}, which involves the breaking of only two bonds with the transfer of one hydrogen atom, perhaps from C_{14}, might account for this fragmentation (Figure 1).

Another abundant ion occurred at (P-33) mass units in all the spectra (methyl radical plus water). Djerassi [5], by deuterium labelling in the cholestane series, has shown that 3β - hydroxy steroids, similar to the compounds under study, exhibited a random loss of water after elimination of the C_{19} methyl group. He found that 40% of the hydrogen lost came from the 1β and 5α position with the remainder being abstracted from some position other than in ring A. He envisaged this being possible by rupture of the 1,2 bond or preferentially the 4,5 bond yielding ionised double bonds, thus allowing hydrogen abstraction from some other ring.

Another general feature of the mass spectral breakdown was the loss of 111 mass units from the sterols with a 5,6 double bond, other than compounds related to ergosterol (10) with a 5,7 diene. This loss corresponds to the entire loss of ring A with

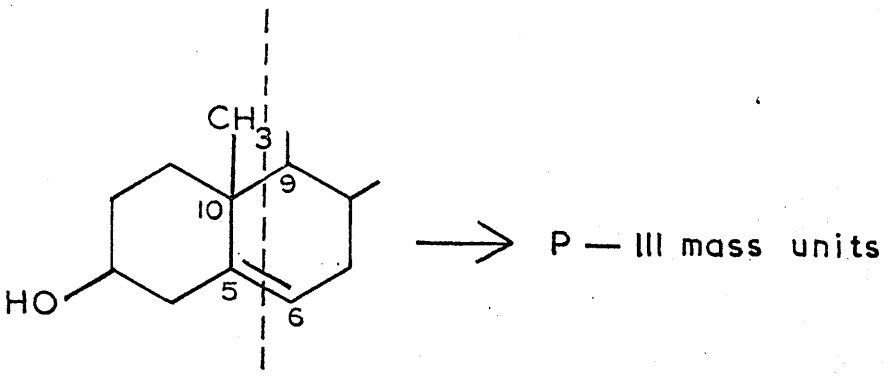


Figure 2

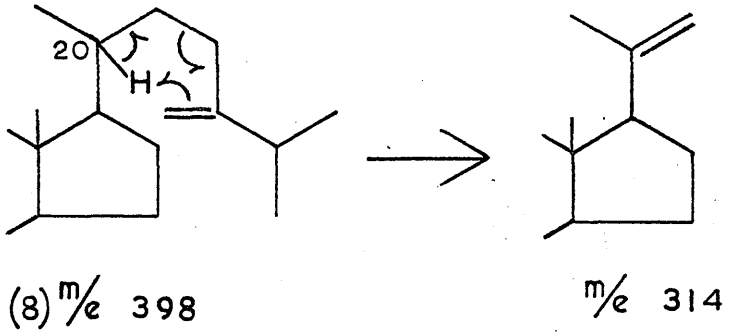


Figure 3

cleavage of the 9, 10 bond and the 5, 6 double bond [6] (Figure 2).

Fucosterol (7) and 24-methylene cholesterol (8) had their base peaks at $^{m}/_{e}$ 314. This could be explained by a McLafferty rearrangement in the C_{17} side chain (Figure 3). It had been suggested [7] that in a rearrangement of this type in a sterol side chain containing a 24-methylene or 24-ethylidene group, the proton which was transferred came from the C_{20} position. This rearrangement would leave an isopropenyl group on ring D but the presence of an abundant ion at $^{m}/_{e}$ 271 corresponding to the loss of 43 mass units ($C_3 H_7$) from the rearranged ion necessitates migration of the double bond into the nucleus prior to the cleavage of the 17, 20 bond. This migration would then allow cleavage of a bond β to a double bond, a most favoured process, with the resultant loss of the newly formed isopropyl group.

Desmosterol (6) gave rise to a base peak at $^{m}/_{e}$ 271, corresponding to a loss of $C_5 H_{10}$ plus 43 mass units from the parent molecular ion. This breakdown is analogous to the rearrangement observed in the spectra of fucosterol and 24-methylene cholesterol, namely a McLafferty rearrangement involving the side chain.

However, in desmosterol, the base peak is the ion produced by the initial McLafferty rearrangement (loss of $C_5 H_{10}$) followed by the loss of 43 mass units, the peak at $^{m}/e$ 314 being negligible (3·8%). The breakdown from this stage followed the usual course.

Amongst the fungi of the order Saprolegniales, Aplanopsis terrestris was found to give a good yield of sterols. G.l.c. and mass spectral analyses of the crude sterol mixtures from the mycelial extract had suggested that the sterols present were cholesterol (5), 24-methylene cholesterol (8), and fucosterol (7), the relative percentage distribution being 18%, 12% and 70% respectively. The mass spectra from G.C.M.S. studies were completely compatible with the above assignments (table 3) the spectra from the individual peaks being identical with the reference spectra.

Saprolegnia ferax had been thought to contain cholesterol (5), desmosterol (6), 24-methylene cholesterol (8), and fucosterol (7) from g.l.c. and mass spectral analyses of the crude mixtures, the percentage distribution being 2%, 22%, 50% and 26% respectively. G.C.M.S. analysis (table 4) confirmed the above assignments.

Species of the order Mucorales, in contrast to species of the order Saprolegniales, produced relatively

G.C.M.S. Mass spectra of sterols from Aplanopsis terrestris.

Cholesterol m/e	% relative intensity.	Fucosterol m/e	% relative intensity.
386	100	412	8
371	49·6	397	4·6
368	54	394	3
353	49	314	100
301	54·5	299	31·8
275	88	296	21
273	35·2	281	41·5
255	41·2	271	19
247	24·2	255	14
231	37·6	231	22
213	67·2	229	35·6
		213	31
		211	25

Table 3.

24--methylene cholesterol. m/e	% relative intensity.
398	14
383	17·5
380	7
365	10
314	100
299	35·5
296	17
281	34·8
271	58
255	18
231	21
229	33·5
213	37·7

Table 3.

G.C.M.S. Mass spectra of sterols from Saprolegnia ferax.

Desmosterol. m/e	% relative intensity.	Fucosterol m/e	% relative intensity.
384	22	412	8
369	32	397	6·5
366	5	394	5
351	15·3	379	6
314	3·8	314	100
299	30	299	25·8
298	20·2	296	15·2
281	16·1	281	30
273	14·2	273	5
271	100	271	18
255	10·8	255	11·2
253	16·7	253	5·8
231	13	231	14
213	25·2	299	29·8
207	29	213	20·2
		211	12·7

Table 4.

24-methylene cholesterol m/e	% relative intensity.
398	11
383	17
380	5·2
365	9·8
314	100
299	38·8
296	17
281	42·6
271	51
255	13
231	17·5
229	32
213	43·4

Table 4

small quantities of sterols, if any. From the U.V. spectra of the sterols of different species of Mucor (table 5), it was evident that Mucor hiemalis (+) and Mucor dispersus produced sterols containing the 5, 7 diene chromophore of ergosterol (10). The mass spectral and g.l.c. analyses of the sterol mixtures suggested the presence of ergosterol (10), and a dihydroergosterol together with traces of 24-methylene cholesterol (8, $^{m}/e$ 398), fucosterol (7, $^{m}/e$ 412), β-sitosterol (11, $^{m}/e$ 414) and campesterol (12, $^{m}/e$ 400). The mass spectrum of an authentic sample of ergosterol showed the base peak at $^{m}/e$ 363, corresponding to a loss of 33 mass units (methyl radical plus water) from the parent molecular ion. The rest of the breakdown followed the general pattern for sterols, abundant ions corresponding to loss of the side chain and loss of side chain plus 42 mass units being observed.

Examination by G.C.M.S. of the sterol fraction of Mucor hiemalis (+) grown in shake flasks, indicated, as discussed below, the presence of ergosterol (10) (table 6) and 22-dihydroergosterol (13) together with traces of fucosterol (7) and/or 24-methylene cholesterol (8) and β-sitosterol (11). It must be stressed that only ergosterol was present in sufficient quantity to

U.V. Spectra.

Fungus	$\lambda_{max.}^{EtOH}$ mμ
M. hiemalis (+)	261, 272, 282, sh. 292.
M. hiemalis (+) - shake culture.	262, 271·5, 282, 292·5.
M. plumbeus.	end absorption
M. globosum.	end absorption
M. dispersus.	260, 271, 281, 292.
M. fragilis.	end absorption
cf. Ergosterol[8]	261, 271, 281·5, 292·5.

Table 5.

G.C.M.S. Mass Spectrum of sterol from Mucor hiemalis (+).

Ergosterol m/e	% relative intensity.
396	69·5
381	4
378	7
363	100
337	37
281	9·8
271	22·5
253	45·5
237	17
211	34
207	21·5

Table 6.

give a pure mass spectrum, the other peaks giving mass spectra with at least two parent molecular ions. The mass spectrum obtained from the second peak on the g.l.c. chart appeared to contain a mixture of ergosterol ($^{m}/e$ 396) and a dihydroergosterol ($^{m}/e$ 398). From a consideration of the ions which did not involve complete loss of the side chain, it was evident that the hydrogenated bond in dihydroergosterol was the 22,23 bond, since the peaks corresponding to the loss of an angular methyl radical and water showed an increase of two mass units compared with the appropriate ions in the spectrum of ergosterol. Peaks corresponding to ions formed by the complete loss of the side chain did not show an increase of two mass units as compared to ergosterol.

At first sight, the retention times of ergosterol and 22-dihydroergosterol did not appear to agree with the relative values observed for fucosterol (7) and β-sitosterol (11). Fucosterol has a considerably longer retention time than β-sitosterol, suggesting that the double bond in the 24, 28 position hinders the passage of the compound down the column, but ergosterol has a retention time which is shorter than that for the corresponding 22-dihydro compound. However,

a better comparison appears to be the retention values for stigmasterol (14), relative to that of β-sitosterol [9]. It appears from this data that double bonds in the side chain in the 22, 23 position result in shorter retention times than those of the corresponding saturated compounds. Thus, whereas unsaturation in the 24, 28 position prolongs the retention time, unsaturation in the 22, 23 position shortens the retention time in comparison to that observed for the corresponding saturated compounds.

The G.C.M.S. investigation of Mucor hiemalis (+) also indicated the presence of small amounts of fucosterol (7), and/or 24-methylene cholesterol (8), peaks at $^{m}/e$ 314, 271, 255, 231 and 211 being characteristic of the breakdown of both of these sterols. There was also a parent molecular ion of low abundance at $^{m}/e$ 414 which could possibly be due to β-sitosterol (11).

Mucor hiemalis (+) grown in surface culture was not examined by G.C.M.S. since the crude sterol mixture could not be purified (partly decomposing on sublimation) but it was apparent from g.l.c. and mass spectral analyses of the crude product that 22-dihydroergosterol (13) and ergosterol (10) were present, the major component being

22-dihydroergosterol. Trace amounts of sterols (parent molecular ions at $^{m}/e$ 400 and $^{m}/e$ 414) were also present and with the g.l.c. evidence were suggested to be campesterol (12) and β-sitosterol (11) respectively.

Sterol fractions, obtained from extracts of Mucor dispersus grown on surface cultures, exhibited the 5, 7 diene chromophore and g.l.c. and mass spectral analyses confirmed the presence of ergosterol (10) as the major component together with small traces of a dihydroergosterol and β-sitosterol (11). Once again purification for study by G.C.M.S. was unsuccessful the characteristic g.l.c. trace being no longer obtained after attempted sublimation.

In an attempt to increase the percentage yield of the sterols, the effect of varying the growing conditions was studied. It was found that the fungus grown in shake flasks gave an extract containing an equivalent amount of sterols to that obtained from a surface culture, the ratio of sterols obtained being identical except in the case of Mucor hiemalis (+) which gave a smaller quantity of sterols. Differences

in the relative percentages of sterols obtained from Mucor hiemalis (+) were also noticed, the extract from shake flasks giving ergosterol (10) as the major component whereas the extract from surface culture gave 22-dihydroergosterol (13) as the major component. Variation in the desaturation of ergosterol derivatives produced by yeasts as growing conditions are changed is well known.[10].

It is evident from the above studies that there is some taxonomic value in a knowledge of the constituent sterols of the genera within different orders of the Phycomycetes. Thus fungi of the orders Saprolegniales and Leptimotales, lacking chitin in the cell wall, give rise to what are generally regarded as plant sterols cholesterol (5), desmosterol (6), 24-methylene cholesterol (8), and fucosterol (7), while, on the other hand, fungi of the order Mucorales produce typically fungal sterols ergosterol (10) and its more or less saturated derivatives. 22-dihydroergosterol (13) does not appear to have been reported before as a constituent of other fungi but it remains to be seen whether it is a characteristic metabolite of species of Mucor.

Experimental

Examination of fungi for sterols.

The petroleum ether (60° - 80°) extract of the dried mycelium of the fungus was chromatographed on alumina (Woelm, neutral, grade III - 40 times weight of extract). The eluant was collected in three fractions:-

(A) petroleum ether/benzene - 1/1 - 200 ml./g. of extract. The column was made up in this system and the sample introduced in as small a volume as possible.

(B) benzene/ether - 95/5 - 100 ml./g. of extract.
benzene/ether - 1/1 - 100 ml./g. of extract.

(C) ether - 100 ml./g of extract.

The second fraction was divided as above into two solvent systems in order to avoid the effects which might result from the heat generated by a sudden large change in solvent composition.

The three fractions were examined by t.l.c. on silica gel G in chloroform, using cholesterol as a marker for 3 β-hydroxy steroids, and a dye mixture for reference purposes (azo-benzene (50 mg.), sudan red (50 mg.), p-amino-azo-benzene (50mg.), p-hydroxy-azo benzene (50 mg.). "quinolone yellow" (50 mg.) in benzene (25 ml.) and ethanol (50 ml.)). If fraction (B) was

fairly large and showed signs that it contained steroid material, it was hydrolysed with aqueous methanolic potassium hydroxide solution (10% H_2O, 10% KOH in methanol, 40 x v/w) overnight. The methanol was removed and the solution extracted with ether to give a neutral fraction. The aqueous alkaline solution was acidified and re-extracted with ether to give an acidic fraction. The neutral fraction was separated by preparative t.l.c., the position of the sterol zone being defined by its relationship to the reference dye system. The material obtained from this zone was then examined by g.l.c. on 1% F.-60 and 1% S.E.-30 columns at 225°. If fraction (B) was low in weight. the hydrolysis steps were neglected and preparative t.l.c. was carried out immediately using 0·8 mm. layers of silica gel H. Prior to examination by G.C.M.S. on 1% S.E. - 30 at 225° the sterol mixtures were sublimed under reduced pressure (170° - 200°/0·1 mm.)

The experimental data for the fungi examined are shown in table (7).

Fungus	Petroleum ether extract of mycelium (g.)	Column (g.)			Hydrolysis (g.)		Fraction from prep. plate (mg.)	% sterol in mycelium extract
		A	B	C	Acid	Neutral		
Aplanopsis terrestris	2·56	1·38	0·40	0·055	0·295	0·055	25	0·97
Saprolegnia Ferax	3·9	1·73	0·23	0·09	0·107	0·090	43	1·1
Mucor hiemalis (+)	2·63	1·91	0·19	0·078	0·011	0·080	18	0·68
Mucor dispersus	1·22	1·153	0·017	0·002	-	-	5·6	0·46
Mucor plumbeus	0·50	0·254	0·116	0·012	0·08	0·026	1	-
Mucor globosum	1·24	1·05	0·16	0·028	0·107	0·060	1	-
Mucor fragilis	6·5	2·03	0·490	0·065	0·240	0·059	5·4	-
Mucor hiemalis (+) - shake flasks	0·90	0·810	0·080	0·005	0·060	0·011	4	0·44

Experimental Data

Table 7.

Biogenesis of Lanosterol

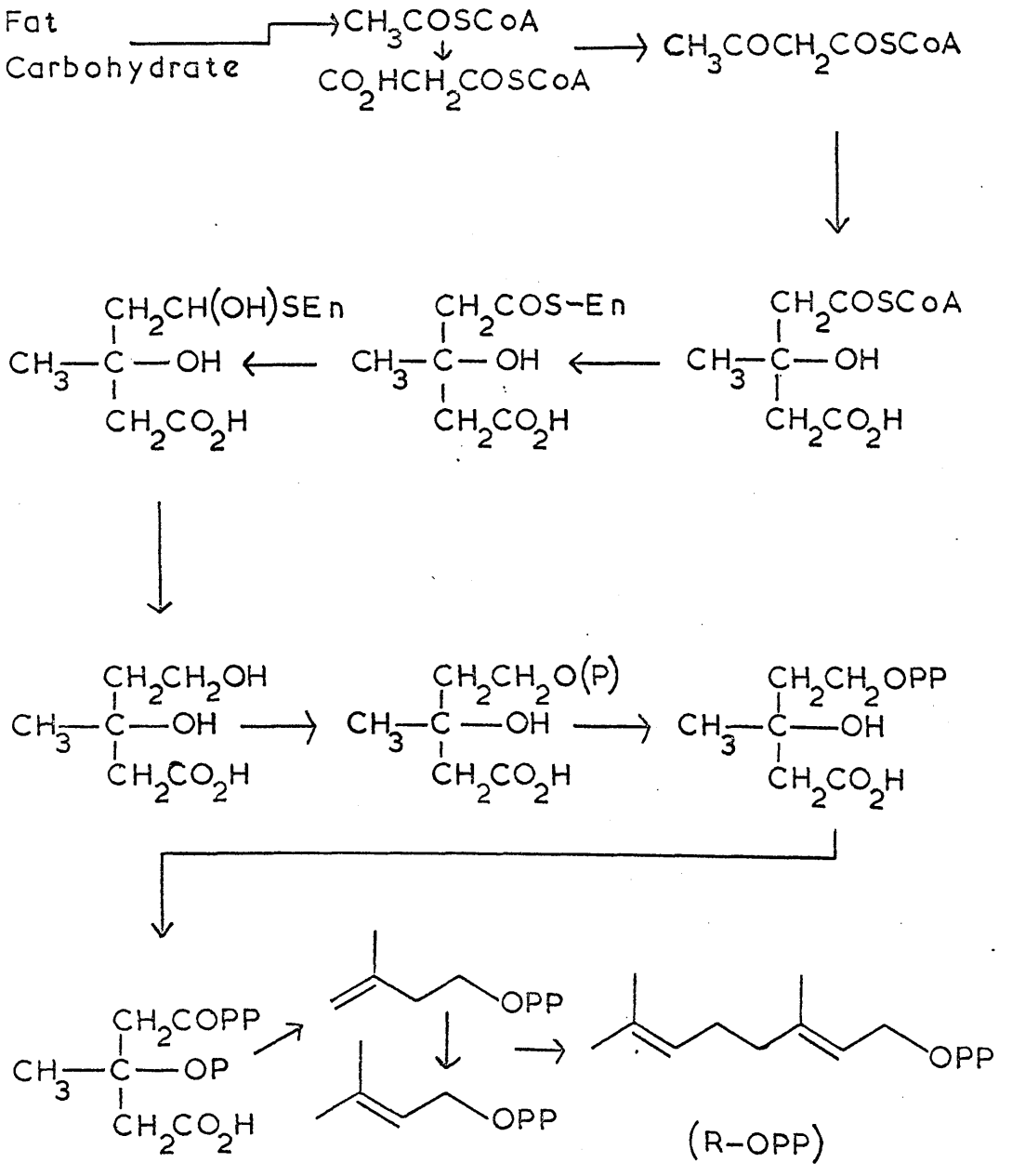
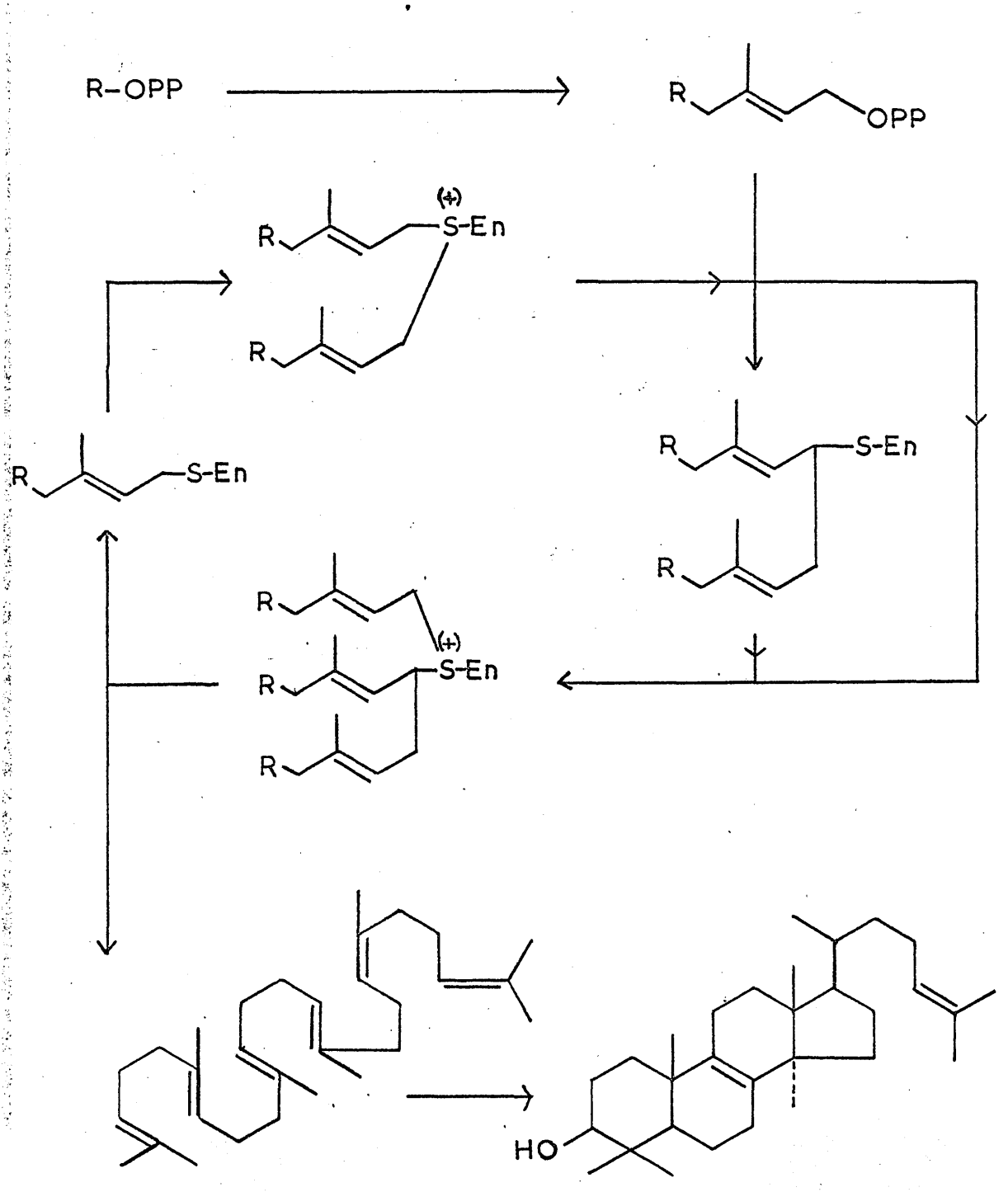


Figure 4



(15)

Biosynthesis of Phytosterols.

The brilliant investigations of Bloch, Cornforth, Popjak and others [11, 12] have elucidated the pathway of biogenesis of sterols in yeast and higher plants and animals and only the basic elements of the processes need be considered here.

The sequence of reactions commences with the derivation of acetyl coenzyme A from dietary fat or carbohydrate. As is shown in figure (4), one molecule of this substance is carboxylated to yield malonyl coenzyme A which is then coupled with a second molecule of acetyl coenzyme A to yield acetoacetyl coenzyme A. Condensation with a third molecule of acetyl coenzyme A provides β-hydroxy-β-methylglutaryl coenzyme A which is then reduced to yield mevalonic acid. The conversion of mevalonic acid to isopentenyl pyrophosphate requires three distinct phosphorylation reactions followed by a concerted 1,2 elimination of carbon dioxide and the elements of phosphoric acid.

The elaboration of the C_{30} isopentenyl polymer squalene from isopentenyl pyrophosphate proceeds via dimethylallyl, geranyl and farnesyl pyrophosphates, the detailed mechanisms of the reactions being not yet fully

understood. However some of the stereochemical aspects of the sequence have been elucidated by the Popjak, Cornforth group. [13] No totally authenticated mechanism for the union of the two farnesyl units is presently available however, the scheme, based on the well known Stevens rearrangement due to Popjak and Cornforth [14] which is in accord with all experimental data, is shown in figure (4). The cyclisation of the squalene precursor to lanosterol (15), involves a series of concerted hydrogen and methyl migrations, all of which have been carefully studied with the aid of radio tracers.[13] This then undergoes a series of oxidation and decarboxylation reactions which lead to the removal of the methyl groups on carbon atoms C_4 and C_{14}. Isomerisation yields desmosterol (6) the immediate precursor of cholesterol (5) in the animal organism. [15]

It has been shown that the additional carbon atom of ergosterol (10) is derived from methionine [16]. Further work with ergosterol shows that when Me-D_3-methionine was fed to a methionine deficient strain of Neurospora crassa, only two of the three deuterium atoms were incorporated into ergosterol. [17] It has also recently been shown that when ergosterol is biosynthesised from 2-^{14}C-4S-^{3}H-mevalonate a tritium atom is retained at C_{24}.[18]

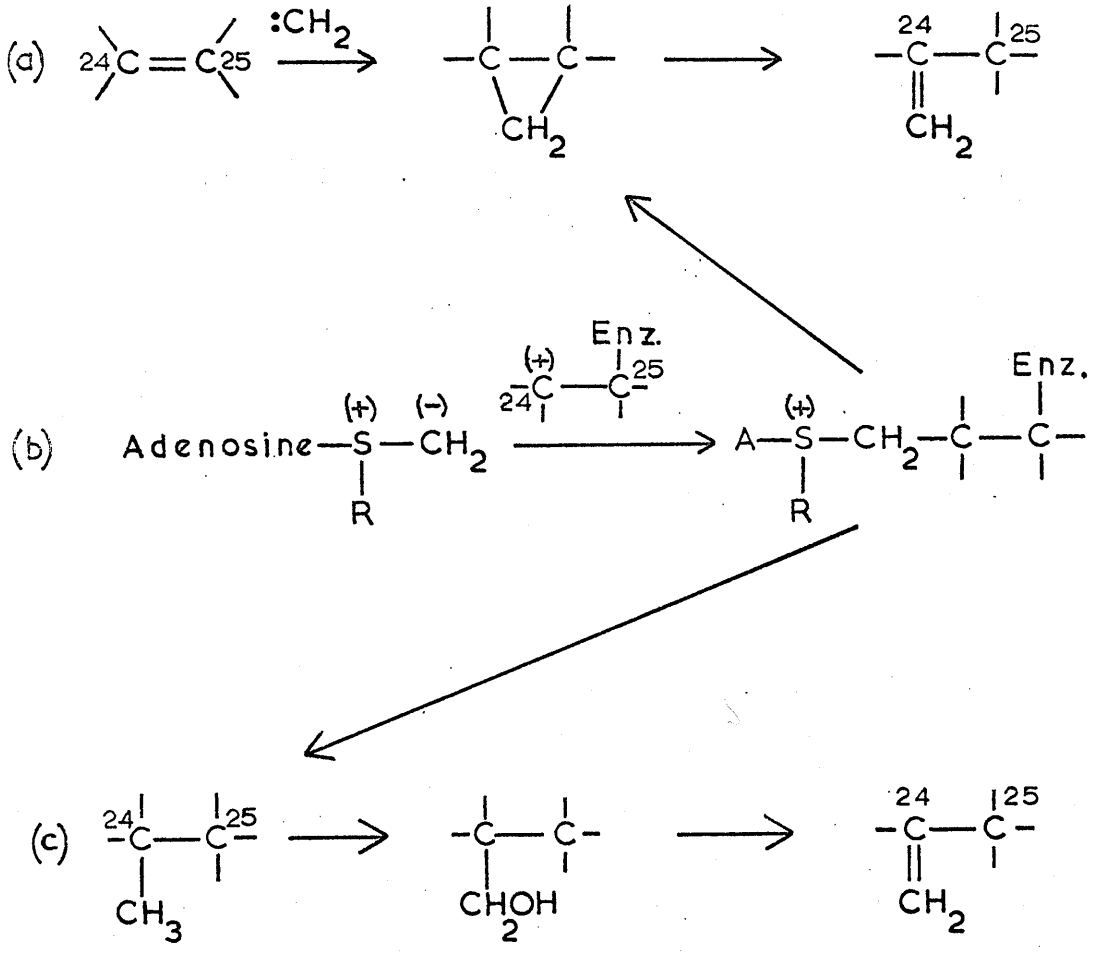


Figure 5

These findings suggest that the "extra" carbon atom of the C_{28} sterols may be introduced via a 24, 25 cyclopropanoid intermediate which may have been formed by the addition of a carbene like species to the 24, 25 double bond. (figure 5). [12].

Another attractive mechanism for the incorporation of the C_1 unit has been proposed by Lederer [12] and involves the addition of an ylide, generated by the oxidation of S-adenosylmethionine to the 24, 25 double bond which has been suitably polarised with an enzyme (figure 5). Either of these above mechanisms can lead directly to 24-methylene cholesterol (8). A further mechanism for the biosynthesis of this sterol, proposed by Lederer [19], involves the oxidation of the "extra" C_1 unit attached to C_{24}, the resulting alcohol being dehydrated to yield 24-methylene cholesterol (figure 5).

After some initial speculations,[20, 21] the origin of the "extra" two carbon atoms of the C_{29} sterols has been studied by several groups of authors. By analogy with the role of methionine as methyl donor in ergosterol biosynthesis, ethionine could have been assumed to be an ethyl donor for the biosynthesis of C_{29} sterols. This "rather improbable pathway" was ruled out after

incorporation experiments with ethyl-labelled L-ethionine.[22, 23] Bader et al.[24] fed DL-(Me-^{14}C)-methionine to rhizomes of Menyanthes trifoliata and isolated radioactive spinasterol (16). Degradation showed that all the isotope was in the ethyl side chain and that both carbon atoms were equally labelled, thus proving conclusively that C_{28} and C_{29} came from methionine. It was proposed that the C_{29} sterols were derived directly from 24-methylene cholesterol (8) by addition of an "extra" carbon atom to the 24, 28 double bond, via the ylide or cyclopropanoid pathways described previously.[12]

Since several of the fungi studied have been shown to produce both fucosterol (7) and 24-methylene cholesterol (8) this permitted a study of the possible biogentic interelationship of 24-methylene cholesterol and fucosterol

Discussion.

^{14}C-24-methylene cholesterol (8) was synthesised via a Wittig reaction using ^{14}C-methyl iodide on 24-ketocholesterol (9), pure sterol being obtained in about 50% yield [2].

From the studies outlined previously, it was decided that Aplanopsis terrestris would prove the most useful for feeding experiments since it was the best producer of fucosterol (7) relative to 24-methylene cholesterol (8).

As was expected, 24-methylene cholesterol proved to be insoluble in water and various methods of feeding the labelled sterol were investigated. Control experiments were carried out on shake cultures of the fungus with increasing percentages of methanol added and it was found that concentrations in excess of 0·1% completely inhibited growth. However this was not a sufficient quantity to appreciably change the solubility of the sterol in the culture medium.

On addition of silicone emulsion to the culture medium increasing percentages caused corresponding delays in the initiation of growth in the flasks, 2½% emulsion causing a delay of four days compared with visible

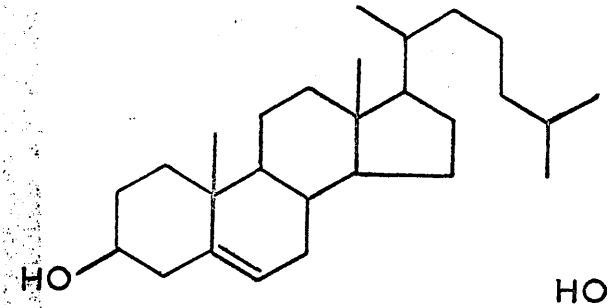
growth after 24 hours in a normal culture. This delay in growth greatly increased the possibility of contamination of the culture thus making this approach less desirable.

Finally, addition of the labelled sterol adsorbed onto celite proved to be the most successful method, the fungus showing appreciable growth after 30 hours.

Having fed the labelled sterol in this manner and isolated the sterols by the method used previously, treatment with osmium tetroxide/sodium periodate, followed by flushing with methanol allowed the formaldehyde and acetaldehyde liberated to be trapped as their 2, 4-dinitrophenylhydrazones. It was found that the best method for separation of the d.n.p.'s was paper chromatography using downward elution with heptane saturated with methanol. This showed that the mixture contained a small quantity of acetaldehyde d.n.p. which showed appreciable activity over background. Three possible conclusions can be drawn from this preliminary experiment.

1. The labelled sterol was not taken up by the fungus.
2. The incorporation was so low (eg. less than 0·1%) and the quantity of fucosterol isolated so small that the activity could not be detected.

3. 24-methylene cholesterol is not an intermediate in the biosynthesis of fucosterol but a branch in the pathway. Alternative routes might involve 24-methylene lanosterol recently shown by Akhtar et al. to be an intermediate in the biosynthesis of ergosterol (cf. Chem. Comm., 565, (1966).



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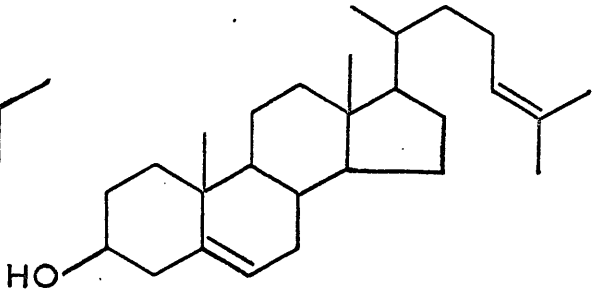

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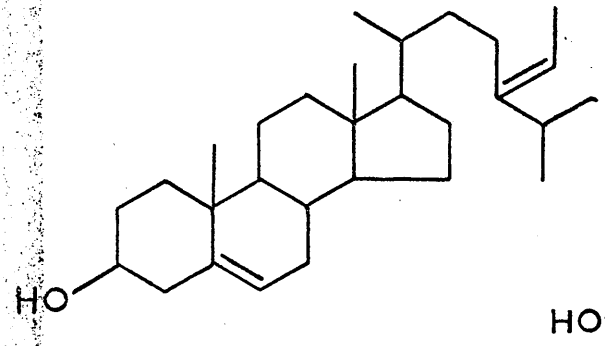

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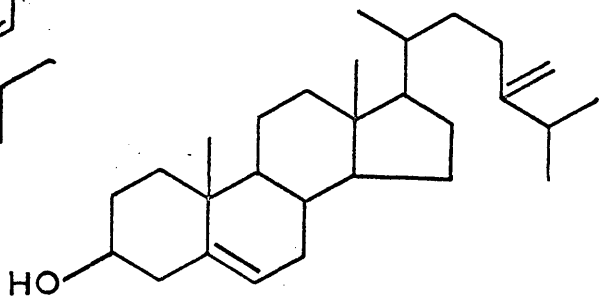

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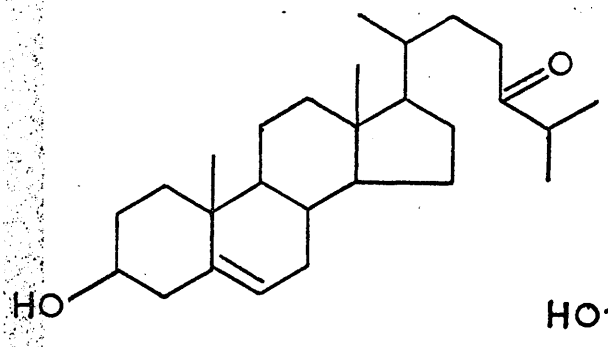

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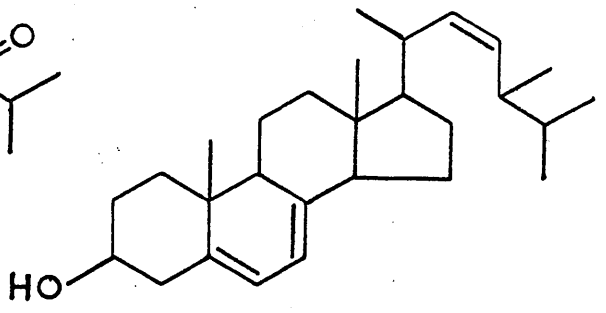

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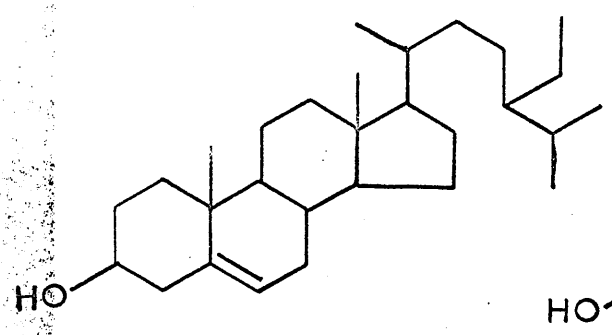

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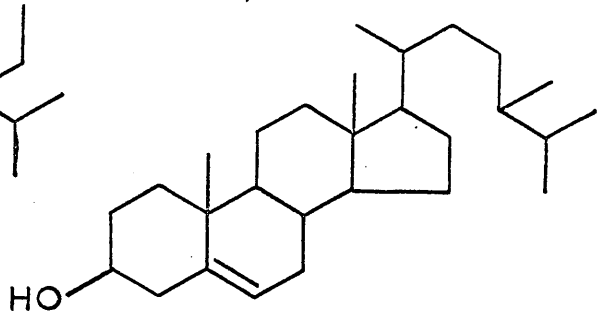

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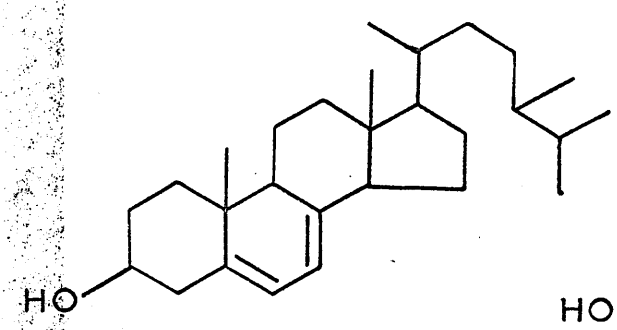

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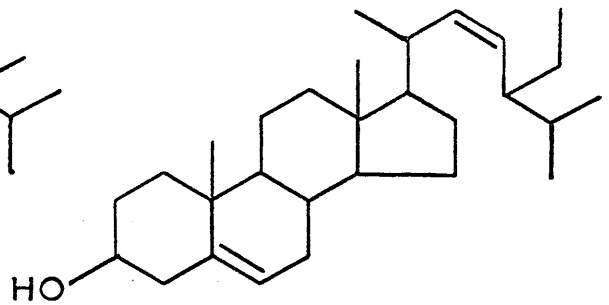

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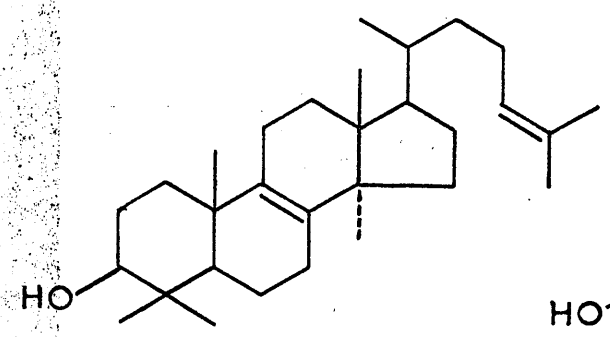

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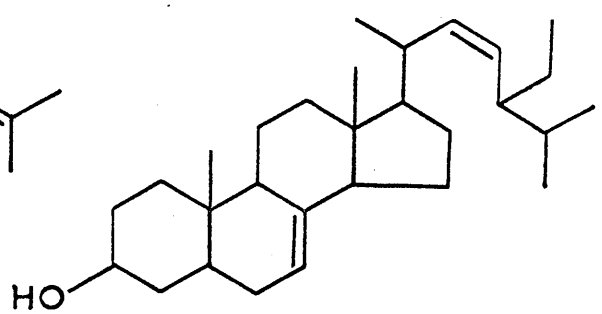

(16)

Experimental.

Treatment of sterol mixture with osmium tetroxide.

The isolated sterol mixture was added to a stirred suspension of osmium tetroxide (1 mole %), water (1 ml.) and dioxan (3 ml.) and stirred for 5 minutes at room temperature. Sodium periodate (210 mole %) was added over 30 minutes and the slurry stirred for a further 2 hours. The solid was filtered off and methanol added to the filtrate which was then distilled, the distillate being collected in methanolic 2, 4-d.n.p. solution. The d.n.p. solution was evaporated almost to dryness and added to water, the mixture then being thoroughly extracted with chloroform. Final isolation of the mixture of d.n.p.'s was achieved by preparative t.l.c. on silica gel H. Separation and identification of the d.n.p.'s was achieved by downward elution on paper using heptane saturated with methanol as the solvent. This permitted identification of radioactive formaldehyde and small quantities of inactive acetaldehyde.

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