507. *A Stereoselective Synthesis of Squalene.*

By J. W. Cornforth, (Mrs.) R. H. Cornforth, and K. K. Mathew.

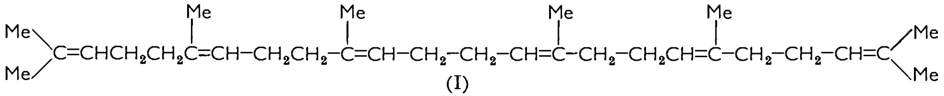
A recent[1] synthesis of olefins has been applied to the synthesis of squalene; no separation of geometrical isomers was necessary before the final stage. The three materials contributing carbon to the structure were methyl bromide, diketen, and 3,5-dichloropentan-2-one.

Interest in squalene (I), originally known only as a constituent of some fish-liver oils, was revived by the demonstration[2] that the hydrocarbon is an intermediate in biosynthesis of cholesterol (and probably of steroids and triterpenoids in general). Nicolaides and Laves[3] showed, by *X*-ray crystallographic analysis of a clathrate with thiourea, that the hydrocarbon obtained from natural sources is largely the *all-trans* geometrical isomer; and since no other isomer has yet been detected it seems appropriate to follow Dicker and Whiting[4] in reserving the name " squalene " for this isomer.

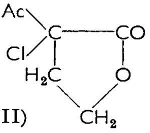
Two methods have been used successfully for the synthesis of squalene: the coupling of farnesyl bromide[5,6] and the Wittig–Schöllkopf reaction between geranylacetone and a phosphorane from 1,4-dibromobutane.[7,4,8] Each method originally gave a mixture of C_{30} hydrocarbons; refinements of procedure later permitted the separation of squalene as the clathrate in thiourea.

We now describe a third method, based on an olefin synthesis already reported;[1] the C_{30} skeleton is constructed according to the preferred scheme: $2(1 + 5 + 5) + (5 + 3) = 30$. The most important intermediate is 3,5-dichloropentan-2-one (III), which supplies 25 of the 30 carbon atoms. This ketone has previously been reported[9] to be formed from α-acetyl-α-chlorobutyrolactone (II) and hydrochloric acid in acetic acid.

The ketone with methylmagnesium bromide gave the alcohol (IV) in 94% yield. This reaction had been tried before;[10a] no pure product was obtained, but reaction conditions were different and the dichloropentanone was itself impure. Dr. L. Crombie[10b] states that the b. p. given for 3,5-dichloropentan-2-one is presumably in error (it does not agree with later values[10c] from the same laboratory). The chlorohydrin readily afforded an epoxide (V) which was reduced by sodium iodide and zinc in acetic acid–sodium acetate to



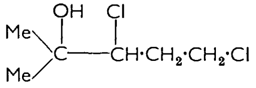
(I)



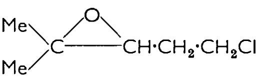
(II)

$Me{\cdot}CO{\cdot}CHCl{\cdot}CH_2{\cdot}CH_2Cl$

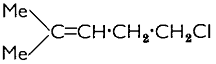
(III)



(IV)



(V)



(VI)

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[1] Cornforth, Cornforth, and Mathew, *J.*, 1959, 112.

[2] Langdon and Bloch, *J. Amer. Chem. Soc.*, 1952, **74**, 1869; *J. Biol. Chem.*, 1953, **200**, 135.

[3] Nicolaides and Laves, *J. Amer. Chem. Soc.*, 1954, **76**, 2596.

[4] Dicker and Whiting, *J.*, 1958, 1994.

[5] Karrer and Helfenstein, *Helv. Chim. Acta*, 1931, **14**, 78.

[6] Isler, Rüegg, Chopard-dit-Jean, Wagner, and Bernhard, *Helv. Chim. Acta*, 1956, **39**, 897.

[7] Trippett, *Chem. and Ind.*, 1956, 80.

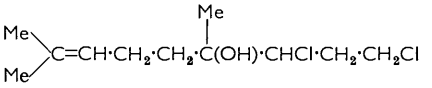
[8] Mondon, *Annalen*, 1957, **603**, 115.

[9] Yoshida and Unoki, *J. Pharm. Soc. Japan*, 1952, **72**, 1431.

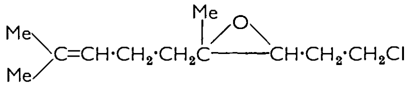
[10] (*a*) Crombie, Harper, and Stokes, *J.*, 1955, 4488; (*b*) Crombie, personal communication; (*c*) Crombie, Manzoor-i-Khuda, and Smith, *J.*, 1957, 479.

5-chloro-2-methylpent-2-ene (VI). This method of reduction was appropriate since geometrical isomerism is not possible in (VI).

A second five-carbon unit was then added. The lithium alkenyl from the chloride (VI) reacted with 3,5-dichloropentan-2-one to give the chlorohydrin (VII), from which the epoxide (VIII) was prepared. Reduction, *via* the iodohydrin, by our stereospecific process [1] using stannous chloride and phosphoryl chloride in pyridine, led to 9-chloro-2,5-dimethylnona-2,5-diene (IX). From the method of preparation this would be expected to consist largely (*ca.* 80%) of the *trans*-isomer, homogeranyl chloride.

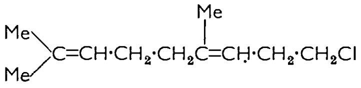

$\mathrm{Me_2C{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}\overset{Me}{C}(OH){\cdot}CHCl{\cdot}CH_2{\cdot}CH_2Cl}$

(VII)

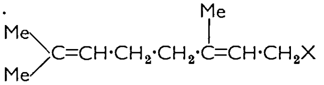

$\mathrm{Me_2C{=}CH{\cdot}CH_2{\cdot}CH_2\overset{Me}{C}\overset{O}{\text{———}}CH{\cdot}CH_2{\cdot}CH_2Cl}$

(VIII)

(IX)


$\mathrm{Me_2C{=}CH{\cdot}CH_2{\cdot}CH_2\overset{Me}{C}{=}CH{\cdot}CH_2{\cdot}CH_2Cl}$

In earlier experiments, the corresponding bromide (X), also predominantly in the *trans*-form, was prepared by a different method. The starting point was homogeranic acid (XI), prepared by dehydration of the hydroxyimino-acid (XII) to geranyl cyanide (XIII), which was hydrolysed by alkali.[11] The hydroxyimino-acid (XII) was reported [11,12] to exist in two forms, m. p. 138—139° (128—130°) and 102° (100°), the lower-melting form being obtained by repeated crystallization of the other. It was suggested [11] that these were *syn*- and *anti*-oximes; but we find the higher-melting form to be a 1 : 1 complex of the acid (XII) and its sodium salt.


$\mathrm{Me_2C{=}CH{\cdot}CH_2{\cdot}CH_2{\cdot}\overset{Me}{C}{=}CH{\cdot}CH_2X}$

(X) $\mathrm{X = CH_2Br}$
(XI) $\mathrm{X = CO_2H}$
(XII) $\mathrm{X = C(:N{\cdot}OH){\cdot}CO_2H}$
(XIII) $\mathrm{X = CN}$
(XIV) $\mathrm{X = NH_2}$
(XV) $\mathrm{X = CO{\cdot}NH_2}$
(XVI) $\mathrm{X = CO{\cdot}NH{\cdot}OH}$
(XVII) $\mathrm{X = CH_2{\cdot}OH}$

Homogeranic acid (XI) was recrystallized at low temperatures, the highest melting point attained being —13°. About 60% of the acid was crystallizable. That the crystalline acid was largely the *trans*-isomer was shown by a transformation to geranylamine (XIV) by means of reagents which would not be expected to disturb the position or geometry of the double bonds. Methyl homogeranate (from the acid and diazomethane) with ammonia gave homogeranamide (XV) (higher m. p. than recorded [11]) and this with hydroxylamine hydrochloride gave the crystalline hydroxamic acid (XVI) which was also obtained directly from methyl homogeranate in 50% yield. The barium salt of the hydroxamic acid, when heated with gypsum, gave geranylamine, identified as geranylurea.[13] The *trans*-geometry of geranylamine hydrochloride has been determined [14] by *X*-ray crystallography.

Homogeranic acid was reduced by lithium aluminium hydride to homogeraniol (XVII), from which homogeranyl bromide (X) was prepared by the consecutive action of toluene-*p*-sulphonyl bromide in pyridine and of lithium bromide in acetone.

Three methods were used to obtain the 3,6-dichloro-octane-2,7-dione (XVIII) which provided the eight central carbon atoms of squalene. (1) 3,5-Dichloropentan-2-one was converted into the ethylene ketal (XIX). The chlorine atom at position 3 was thereby inactivated, so that treatment with sodium iodide in butanone gave a preparation consisting essentially of the iodide (XX). With t-butyl sodioacetoacetate this gave a crystalline ester (XXI), which was chlorinated. The product (XXII) was hydrolysed by hydrochloric acid to the dichloro-diketone (XVIII), obtained as a mixture of a solid and a liquid

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[11] Barnard and Bateman, *J.*, 1950, 926.
[12] King and Grundon, *J.*, 1950, 3547.
[13] Forster and Cardwell, *J.*, 1913, **103**, 1342.
[14] Jeffrey, *Proc. Roy. Soc.*, 1945, *A*, **183**, 388; Cruickshank and Jeffrey, *Acta Cryst.*, 1954, **7**, 646.

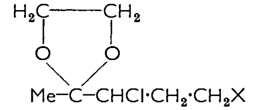
stereoisomer. This method was the best of the three. When the chlorinated ester (XXII) was heated in benzene with toluene-*p*-sulphonic acid an unstable product, largely the monoketal (XXIII), was obtained.

$Me{\cdot}CO{\cdot}CHCl{\cdot}CH_2{\cdot}CH_2{\cdot}CHCl{\cdot}COMe$

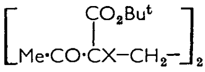
(XVIII)

$[Cl{\cdot}CO{\cdot}CHCl{\cdot}CH_2{-}]_2$

(XXVI)



(XIX) $X = Cl$
(XX) $X = I$
(XXI) $X = CH(COMe){\cdot}CO_2Bu^t$
(XXII) $X = CCl(COMe){\cdot}CO_2Bu^t$
(XXIII) $X = CHCl{\cdot}COMe$



$[Me{\cdot}CO{\cdot}C(CO_2Bu^t)X{-}CH_2{-}]_2$

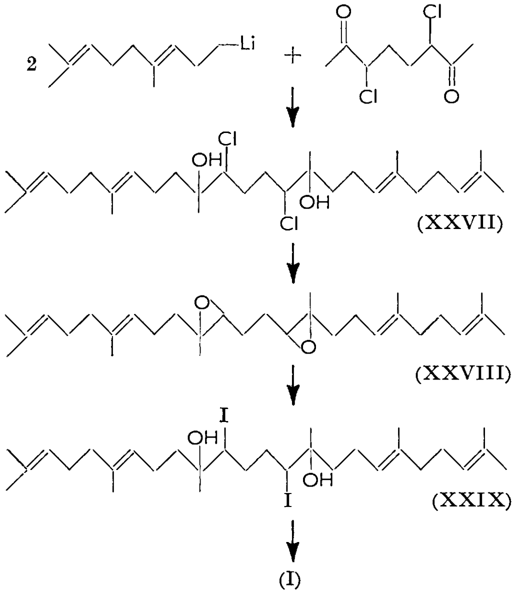
(XXIV) $X = H$
(XXV) $X = Cl$

(2) Condensation of t-butyl sodioacetoacetate with ethylene dibromide gave a small yield (15%) of t-butyl diacetyladipate (XXIV); this was chlorinated and the product (XXV) was hydrolysed.

(3) Dichloroadipoyl chloride (XXVI) gave the required dichloro-diketone (XVIII) with dimethylcadmium, but the yield was poor and the product impure.

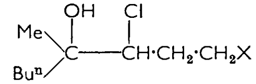
The preparation of squalene from homogeranyl-lithium (from IX or X) and the dichloro-octanedione was carried through, without isolation of intermediates, *via* the chlorohydrin (XXVII), the epoxide (XXVIII), and the iodohydrin (XXIX), which was reduced by the stereospecific procedure. When the hydrocarbon fraction was treated with thiourea, 18—20% entered a clathrate. From the complex, squalene was obtained; its infrared spectrum was essentially identical with that of purified natural material and with the two published spectra [4,6] of synthetic squalene.

If the expected stereoselectivity (*ca.* 80% for each double bond) was obtained in this synthesis, the product could have contained *ca.* 40% of *all-trans*-isomer. With a stereoselectivity of 70% *trans*, 24% of *all-trans*-isomer would be expected. Since even pure squalene does not form a thiourea clathrate quantitatively (70—80% is the usual recovery), and a lower recovery would be expected from less pure material, a stereoselectivity higher than 70% was presumably reached.

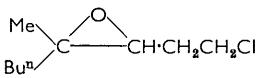


Some orienting experiments on the condensation of n-butyl-lithium with 3,5-dichloropentan-2-one and with 5-acetoxy-3-chloropentan-2-one are recorded below. The chlorohydrins (XXX) and (XXXI) and the epoxide (XXXII) were prepared.

(XXX) X = Cl
(XXXI) X = OAc



$Me(Bu^n)C(OH)\text{—}CHCl\cdot CH_2\cdot CH_2X$



$Me(Bu^n)\overset{O}{C\text{—}CH}\cdot CH_2CH_2Cl$

(XXXII)

Experimental

3,5-*Dichloropentanone* (III).—This was prepared [9] from α-acetyl-α-chlorobutyrolactone and redistilled through a 12″ Widmer column; it was a colourless liquid, b. p. 76°/15 mm., $n_D^{21\cdot5}$ 1·4670 (Found: C, 39·0; H, 5·2; Cl, 46·0. Calc. for $C_5H_8OCl_2$: C, 38·8; H, 5·2; Cl, 45·8%), and did not darken on storage.

3,5-*Dichloro*-2-*methylpentan*-2-*ol* (IV).—Methylmagnesium bromide, from magnesium (24·3 g.) and methyl bromide (62 ml.) in ether (500 ml.), was cooled to −70° and stirred during addition (10 min.) of 3,5-dichloropentan-2-one (104 g.) in ether (100 ml.). After a further 10 min. acetic acid (65 ml.) in ether (100 ml.) was added. The mixture was brought to room temperature and stirred with water (300 ml.). The aqueous layer was washed once with ether which was combined with the ether layer, washed with aqueous sodium hydrogen carbonate, dried ($MgSO_4$) and distilled. 3,5-*Dichloro*-2-*methylpentan*-2-*ol* (109 g.; b. p. 92—96°/15 mm.) had b. p. 94—95°/15 mm. on redistillation (Found: C, 42·3; H, 7·3; Cl, 41·3. $C_6H_{12}OCl_2$ requires C, 42·1; H, 7·1; Cl, 41·5%).

5-*Chloro*-2,3-*epoxy*-2-*methylpentane* (V).—The above chlorohydrin (109 g.) in methanol (100 ml.) was treated gradually at 0° with well-cooled methanolic 2·15N-potassium hydroxide (310 ml.). After ½ hr. without further cooling, 1 equiv. of alkali had been consumed; water (1 l.) was then added. Three extractions with ether, which was dried ($CaCl_2$) before evaporation, and distillation gave the epoxide (81 g.), b. p. 52—56°/15 mm. On redistillation 5-*chloro*-2,3-*epoxy*-2-*methylpentane* had b. p. 53—54°/15 mm., n_D^{25} 1·4358 (Found: C, 53·6; H, 8·4; Cl, 25·7. $C_6H_{11}OCl$ requires C, 53·5; H, 8·2; Cl, 26·4%).

5-*Chloro*-2-*methylpent*-2-*ene* (VI).—(*a*) The above epoxide (81 g.) was added during 25 min. to a stirred, ice-cooled mixture of sodium iodide (90 g.), sodium acetate (15 g.), zinc powder (150 g.), and acetic acid (400 ml.). After 1½ hr. the zinc was removed and washed with acetic acid and ether. Water (1 l.) was added to the filtrate which was extracted thrice with ether. The extract was neutralized at −20° by concentrated potassium hydroxide solution, washed with water, dried ($CaCl_2$), and distilled through a 12″ Widmer column, finally under reduced pressure. The *iso*pentenyl chloride (50·5 g.) had b. p. 80—82°/130 mm., 134°/750 mm., n_D^{20} 1·4458 (Found: C, 60·8; H, 9·5; Cl, 29·6. Calc. for $C_6H_{11}Cl$: C, 60·7; H, 9·3; Cl, 29·9%). (*b*) The epoxide (2 g.) was added at −20° to propionic acid (10 ml.) containing sodium iodide (4 g.), sodium acetate (0·4 g.), and acetic acid (4 ml.). After ½ hr. at −20° the mixture was poured into excess of aqueous sodium hydrogen carbonate overlaid by ether. The ether extract was dried ($MgSO_4$) and evaporated. The iodohydrin (3·5 g.) was added to a cooled solution of anhydrous stannous chloride (4 g.) in pyridine (20 ml.). After addition of phosphoryl chloride (1·1 ml.) in pyridine (3 ml.) the mixture soon solidified; after 1 hr. water (50 ml.) was added and the mixture distilled so long as oil came over. The distillate (20 ml.) was neutralized by 3N-hydrochloric acid. The oil was separated, dried, and distilled to give the isohexenyl chloride (1·3 g.), b. p. 132—135°.

1,3-*Dichloro*-4,8-*dimethylnon*-7-*en*-4-*ol* (VII).—Lithium (2·5 g.; thin strips freshly cut) and ether (200 ml.) under nitrogen were stirred during the slow addition of 5-chloro-3-methylpent-2-ene (21 g.) in ether (100 ml.); when reaction began, a cooling bath (−20°) was applied. Stirring was continued at −15° until nearly all the lithium had reacted (2 hr.). To the solution at −70°, 3,5-dichloropentan-2-one (22 g.) in ether (100 ml.) was added (5 min.). Ten minutes later the Gilman test was negative; acetic acid (16 ml.) in ether was added and the mixture was treated as in the preparation of 3,5-dichloro-2-methylpentan-2-ol (above), the chlorohydrin (22 g.) distilling at 78—85°/0·005 mm. A redistilled specimen of the 1,3-*dichloro*-4,8-*dimethylnon*-7-*en*-4-*ol* had b. p. 70—72°/0·001 mm. (Found: C, 55·0; H, 8·2; Cl, 30·3. $C_{11}H_{20}OCl_2$ requires C, 55·2; H, 8·4: Cl, 29·7%). When magnesium was used instead of lithium, little of the desired product was obtained; there was extensive reduction of the ketone by the Grignard reagent.

9-*Chloro*-6,7-*epoxy*-2,6-*dimethylnon*-2-*ene* (VIII).—The above chlorohydrin (22 g.) was treated with methanolic 1·1N-potassium hydroxide as in the preparation of 5-chloro-2,3-epoxy-2-methylpentane (above). The *product*, a colourless pleasant-smelling liquid (17·4 g.), had, after redistillation, b. p. 124—125°/15 mm. n_D^{22} 1·4668 (Found: C, 65·3; H, 9·6; Cl, 17·6. $C_{11}H_{19}OCl$ requires C, 65·2; H, 9·5; Cl, 17·5%).

9-*Chloro*-2,6-*dimethylnona*-2,6-*diene* (*Homogeranyl Chloride*) (IX).—The above epoxide (12 g.) was added to a cold (−30°) solution of sodium iodide (28 g.) and sodium acetate (3 g.) in a mixture of acetic acid (30 ml.) and propionic acid (80 ml.). After 2 hr. at −20° and 1 hr. at −10° the mixture was poured into excess of aqueous sodium hydrogen carbonate covered by ether. The ether, after being decolorized by sodium hydrogen sulphite, was dried ($MgSO_4$) and evaporated at low pressure. To the iodohydrin (18·6 g.) was added anhydrous stannous chloride (20 g.) in dry pyridine (80 ml.). Phosphoryl chloride (5 ml.) in pyridine (20 ml.) was added slowly to the ice-cooled mixture, which became clear and then solidified. After 1 hr. ether (300 ml.) was added and after trituration the solid was collected and washed with ether. The ice-cooled ethereal filtrate was acidified (Methyl Orange) with dilute hydrochloric acid, washed with water, treated with water and small quantities of iodine until excess of iodine indicated that all phosphorus had been destroyed, washed with alkaline (Na_2CO_3) sodium thiosulphate and with water, dried ($CaCl_2$), and evaporated. Distillation gave the product (9·6 g., 86% calc. on epoxide), b. p. 105—110°/17 mm. Redistilled through a short column, the 9-*chloro*-2,6-*dimethylnona*-2,6-*diene* had b. p. 106—107°/17 mm., n_D^{22} 1·4740 (Found: C, 70·7; H, 10·3; Cl, 18·9. $C_{11}H_{19}Cl$ requires C, 70·8; H, 10·2; Cl, 19·0%).

Homogeranic Acid (XI).—The method of Barnard and Bateman was found more convenient than that of King and Grundon. " Meranol " geraniol from A. Boake Roberts & Co. needed no purification before use; it was converted into geranyl chloride, geranylmalonic ester, and α-hydroxyiminogeranylacetic acid essentially as described. The " higher-melting form " of the hydroxyimino-acid, m. p. 132—133° (from acetone), was found by titration and by qualitative test for sodium to be an approximately equimolecular complex of the acid and its sodium salt; it was, however, suitable for the preparation [11] of geranyl cyanide. This nitrile was hydrolysed [11] to homogeranic acid, which was crystallized twice from light petroleum (b. p. 40—60°) at −70° and then had m. p. −13° to −14°. This acid was correlated with 1-amino-3,7-dimethyl-2-*trans*-octa-2,6-diene (geranylamine) as follows: The acid (1 g.) was esterified by ethereal diazomethane, and the ester was left for 4 days with saturated methanolic ammonia (10 ml.). Light petroleum (b. p. 40—60°) and water were added and the crystalline product (homogeranamide) (XV) (0·43 g.) was collected; it had m. p. 64—65° before and after crystallization from light petroleum (b. p. 40—60°). This product (318 mg.) and hydroxylamine hydrochloride (140 mg.) in ethanol (2 ml.) were kept in a sealed tube at 37° for 6 days. The product was separated from unchanged amide by extraction from ethereal solution by aqueous sodium hydroxide; acidification then gave N-(4,8-*dimethylnona*-3,7-*dienoyl*)*hydroxylamine* ([*homogeranylhydroxamic acid*] (XVI), which formed long needles, m. p. 75°, from light petroleum (b. p. 60—80°) (Found: C, 67·4; H, 9·8; N, 6·95. $C_{11}H_{19}O_2N$ requires C, 67·0; H, 9·65; N, 7·1%). The substance gave an intense red colour with ferric chloride. The hydroxamic acid (500 mg.) in a little ethanol was added to aqueous barium hydroxide (1 equiv.). The mixture was evaporated at low pressure; the dried residue was heated carefully with powdered gypsum (1 g.) at 15 mm. with a free flame until visible reaction ceased. The basic product (243 mg.), separated from the distillate by means of acid, alkali, and ether, was dissolved in N-hydrochloric acid (1·6 ml.) and treated with a slight excess of potassium cyanate. After 36 hr. the solid product was collected and recrystallized from aqueous acetone. The long white needles (197 mg.) had m. p. 82° (Found: N, 14·1. Calc. for $C_{11}H_{20}ON_2$: N, 14·3%); a mixture with geranylurea, m. p. 83°, prepared from authentic geranylamine, had m. p. 82—83°.

4,8-*Dimethylnona*-3,7-*dien*-1-*ol* (*Homogeraniol*) (XVII).—Homogeranic acid (25 g.; m. p. −15°) in dry ether (300 ml.) was added dropwise at room temperature to a stirred suspension of lithium aluminium hydride (7·5 g.) in ether (400 ml.). After 21 hr. water (15 ml.) was added in small portions and after a further 24 hr. the solid was removed and washed with warm ether. The ethereal solution on distillation gave 4,8-*dimethylnona*-3,7-*dien*-1-*ol* (21·5 g.), b. p. 124—126°/17 mm., n_D^{22} 1·4722 (Found: C, 78·5; H, 12·2. $C_{11}H_{20}O$ requires C, 78·6; H, 11·9%); it was a colourless oil, much less odorous than geraniol. The 3,5-*dinitrobenzoate* crystallized from acetone in light yellow prisms, m. p. 30—31° (Found: N, 7·7. $C_{18}H_{22}O_6N_2$ requires N, 7·7%).

9-*Bromo*-2,6-*dimethylnona*-2,6-*diene* (*Homogeranyl Bromide*) (X).—Homogeraniol (27·5 g.) in dry pyridine (110 ml.) was stirred and cooled in ice during the addition (20 min.) of toluene-*p*-sulphonyl bromide (53 g.). After a further 20 min. in ice and 20 min. without cooling, the ice-bath was replaced and water was added until the solid precipitate dissolved. After 5 min. the mixture was poured into ice-cold 2N-sulphuric acid (850 ml.) and extracted thrice with ether which was then washed with water and evaporated at low pressure. The residue (53 g.) was stirred and boiled under reflux for 2 hr. with lithium bromide (40 g.) in acetone (240 ml.). Distillation in steam and extraction of the distillate with ether gave 9-*bromo*-2,6-*dimethylnona*-2,6-*diene* (36 g.), b. p. 64°/0·4 mm., n_D^{21} 1·4952 (Found: C, 57·4; H, 8·6; Br, 34·0. $C_{11}H_{19}Br$ requires C, 57·1; H, 8·3; Br, 34·6%).

t-Butyl Acetoacetate.—T-butyl alcohol (74 g.) and triethylamine (1 ml.) were heated under reflux (bath 90°) with stirring during addition of diketen (84 g.) at such a rate that the internal temperature remained below 100° (1·5 hr.). After 30 min. more, the product was cooled, united with the reaction mixture from a duplicate run, diluted with ether, washed with water, sodium hydrogen carbonate solution, and again water, dried ($MgSO_4$), and distilled, to give the ester (295 g., 93%), b. p. 78—80°/16 mm. (Found: C, 60·5; H, 8·9. Calc. for $C_8H_{14}O_3$: C, 60·8; H, 8·9%). The yield, which was still 90% on twice the scale, is somewhat superior to that obtained by Treibs and Hintermeier.[15]

2-(1,3-*Dichloropropyl*)-2-*methyl*-1,3-*dioxolan* (XIX).—3,5-Dichloropentan-2-one (15·5 g.), ethylene glycol (6·5 ml.; redistilled), toluene-*p*-sulphonic acid (55 mg.), and dry toluene (20 ml.) were boiled under a Dean–Stark water-separator for 4 hr., and then for 2 hr. after addition of ethylene glycol (2 ml.). The cooled mixture was diluted with ether, washed as above, dried ($MgSO_4$), and distilled, to give a fraction (12—16 g.), b. p. 110—120°/15 mm. Redistillation afforded the *dioxolan*, b. p. 116—118°/15 mm. (Found: C, 42·3; H, 5·8; Cl, 35·1. $C_7H_{12}O_2Cl_2$ requires C, 42·2; H, 6·0; Cl, 35·7%). The lower fractions from several runs were treated again with ethylene glycol, the final yield of ketal being 90%.

t-Butyl α-*Acetyl*-δ-*chloro*-δ-(2-*methyl*-1,3-*dioxolan*-2-*yl*)*valerate* (XXI).—The chloro-ketal (XIX) (25 g.) was boiled with sodium iodide (45 g.) in butanone (400 ml.) for 24 hr. The filtered solution was concentrated under slightly reduced pressure, diluted with ether, washed with 0·1N-sodium thiosulphate, water, and aqueous sodium chloride, dried ($MgSO_4$), and distilled. The product (32·2 g.), b. p. 70—76°/0·005 mm., was not the pure (XX) (Found: C, 30·9; H, 4·4; I, 38·1. Calc. for $C_7H_{12}O_2ClI$: C, 28·9; H, 4·1; I, 43·7%) but was suitable for the next step. After redistillation (b. p. 70—71°/0·001 mm.) the second of the two equal fractions collected contained 40·6% of iodine.

Sodium (2·3 g.; finely sliced) was dissolved in boiling t-butyl alcohol (80 ml.), and t-butyl acetoacetate (21 g.) followed by crude 2-(1-chloro-3-iodopropyl)-2-methyl-1,3-dioxolan (32 g.) were added. The mixture was stirred at the b. p. for 24 hr., cooled, neutralized with acetic acid, diluted with ether, washed with water, aqueous sodium hydrogen carbonate, and water again, dried ($MgSO_4$), and distilled. The product (25 g.; b. p. 116—118°/0·01 mm.) was nearly pure (Found: C, 55·8; H, 7·5; Cl, 11·2%) but was later found to contain a little phenolic material which was extractable by 2N-sodium hydroxide.

A sample of the ester (20·3 g.) became largely crystalline after some weeks and the solid (12·5 g.; m. p. 64—66°) was recovered by washing with light petroleum (b. p. 40—60°). Recrystallization from the same solvent gave the *ketal ester* (XXI) in prisms, m. p. 65—66° (Found: C, 56·3; H, 7·9; Cl, 10·9. $C_{15}H_{25}O_5Cl$ requires C, 56·2; H, 7·8; Cl, 11·1%). This was evidently a keto-form as the ferric reaction was weak and slow to develop.

t-Butyl α-*Acetyl*-αδ-*dichloro*-δ-(2-*methyl*-1,3-*dioxolan*-2-*yl*)*valerate* (XXII).—The crystalline ester (XXI) (6·4 g.) in acetic acid (20 ml.) containing acetic anhydride (1 ml.) and powdered anhydrous potassium acetate (4 g.) was treated with a solution (21·2 ml.) of chlorine (1·63 g.) in carbon tetrachloride and shaken intermittently in a stoppered flask. After several hours the mixture was poured into excess of aqueous sodium carbonate, and the product, recovered by extraction with ether, was purified by flash-distillation in a high vacuum. The colourless *t-butyl ester* (XXII) (6·65 g.) had b. p. 120—124°/0·03 mm. (Found: C, 50·9; H, 6·9; Cl, 20·4. $C_{15}H_{24}O_5Cl_2$ requires C, 50·7; H, 6·8; Cl, 20·0%). This ester could be used without distillation.

3,6-*Dichloro*-6-(2-*methyl*-1,3-*dioxolan*-2-*yl*)*hexan*-2-*one* (XXIII).—This compound was never

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[15] Treibs and Hintermeier, *Chem. Ber.*, 1954, **87**, 1163.

obtained pure. A solution of the dichloro-ester (XXII) (3·0 g.) in dry benzene (30 ml.) containing toluene-*p*-sulphonic acid (75 mg.) was boiled for 80 min., cooled, diluted with ether, washed with sodium hydrogen carbonate solution and water, dried ($MgSO_4$), and flash-distilled to give 1·65 g. of material, b. p. 88—96°/0·01—0·1 mm., and a black residue. Redistillation, again attended by some decomposition, gave 1·2 g. of ketone, b. p. 74—82°/0·001—0·02 mm. (Found: C, 48·2; H, 6·7; Cl, 26·5. Calc. for $C_{12}H_{20}O_4Cl_2$: C, 47·1; H, 6·3; Cl, 27·8%). This compound after several weeks changed to a black oil containing crystals, which were isolated by washing them with ether and ethanol and recrystallized from ethanol (charcoal). The colourless crystals, m. p. 151°, were shown by analysis to be 1,4-*dichloro*-1,4-*di*-(2-*methyl*-1,3-*dioxolan*-2-*yl*)*butane* (Found: C, 48·4; H, 6·7; Cl, 23·4. $C_{12}H_{20}O_4Cl_2$ requires C, 48·2; H, 6·7; Cl, 23·7%).

t-Butyl 2,5-*Diacetyladipate* (XXIV).—Sodium (11·6 g.; thin slices) dissolved during 7 hr. in boiling t-butyl alcohol (400 ml.). To the cooled mixture t-butyl acetoacetate (80 g.) was added fairly rapidly. After addition next of ethylene dibromide (50 g.) the mixture was stirred and boiled overnight, cooled, neutralized with acetic acid (2—3 ml.), filtered, and concentrated at 250 mm. (bath 70°). The residue in ether was washed with aqueous sodium hydrogen carbonate and brine, dried ($MgSO_4$), and distilled. A fraction (39·5 g.) containing t-butyl 1-acetylcyclopropane-1-carboxylate and t-butyl acetoacetate was collected below 70°/0·5 mm.; the residue (23 g.) was dissolved in ether, washed four times with 2N-sodium hydroxide and once with saturated aqueous sodium chloride, dried ($MgSO_4$), and recovered. It (23 g.) crystallized from 2 parts of acetone at −70°. The product (13·4 g.; m. p. 63°) was collected next day, washed with cold methanol, and dried. Recrystallization from light petroleum gave *t-butyl* 2,5-*diacetyladipate*, m. p. 72° (Found: C, 62·9; H, 8·8. $C_{18}H_{30}O_6$ requires C, 63·2; H, 8·8%). M. p.s ranging from 50° to 90° were observed in different experiments and were attributed to tautomerism.

Benzyl 2,5-*Diacetyladipate*.—Ethyl 2,5-diacetyladipate (19·25 g.; prepared according to Perkin [16]) was heated for 6 hr. at 180—185°, ethanol being allowed to distil. After concentration at low pressure the residue was distilled in steam to remove traces of benzyl alcohol; then ether was added and the solution was washed with sodium hydrogen carbonate solution and water, dried ($MgSO_4$), and evaporated, finally at 100°/15 mm. The residue consisted essentially of *benzyl* 2,5-*diacetyladipate* (Found: C, 70·6; H, 6·8. $C_{24}H_{26}O_6$ requires C, 70·2; H, 6·3%). A "di-imide," 2,7-*diamino*-3,6-*di*(*benzyloxycarbonyl*)*octa*-2,6-*diene*, prepared by keeping the ester in ethanolic ammonia for 2 days, crystallized from dioxan in prisms, m. p. 189—192° (Found: C, 70·9; H, 7·0; N, 6·9. $C_{24}H_{28}O_4N_2$ requires C, 70·6; H, 6·9; N, 6·9%); from this, the keto-ester was regenerated by dissolution in a little concentrated hydrochloric acid, dilution with water, and warming to 50°.

t-Butyl 2,5-*Diacetyl*-2,5-*dichloroadipate* (XXV).—Acetic acid (96 ml.) containing sodium acetate trihydrate (20 g.) was saturated with chlorine (6·1 g.). T-butyl diacetyladipate (13·9 g.) was added and the mixture was shaken intermittently in a stoppered flask for 4 hr. The mixture, still containing a little chlorine, was diluted with water and extracted with ether, which was washed thrice with water (250 ml.), then with aqueous sodium hydrogen carbonate until neutral, and finally with water. The dried ($MgSO_4$) extract was evaporated and the residue (16·95 g.) taken up in light petroleum (b. p. 40—60°). After some hours the crystals (7·65 g.; m. p. 112—115°) were collected and the filtrate was distilled, to give 8·4 g. of product, b. p. 118—122°/0·03 mm. The crystalline *t-butyl* 2,5-*diacetyl*-2,5-*dichloroadipate* recrystallized from light petroleum (b. p. 40—60°) or ether and then had m. p. 117—119° (Found: C, 52·8; H, 7·0; Cl, 16·9. $C_{18}H_{28}O_6Cl_2$ requires C, 52·6; H, 6·8; Cl, 17·3%). The liquid ester was less pure (Found: C, 53·4; H, 7·1; Cl, 15·9%).

Ethyl 2,5-*Diacetyl*-2,5-*dichloroadipate*.—This was prepared by addition of sulphuryl chloride (10 g.) to ice-cooled ethyl diacetyladipate (10 g.); the mixture was heated for 15 min. at 100°, cooled, diluted with ether, washed as usual, dried ($MgSO_4$), and distilled. The product, b. p. 132°/0·01 mm., partially crystallized; recrystallization from light petroleum (b. p. 40—60°) gave one isomer of the *dichloro-ester* as prisms, m. p. 66—68° (Found: C, 47·4; H, 5·8; Cl, 20·4. $C_{14}H_{20}O_6Cl_2$ requires C, 47·3; H, 5·6; Cl, 20·0%). This ester proved highly resistant to hydrolysis by acid.

3,6-*Dichloro-octane*-2,7-*dione* (XVIII).—(*a*) Crystalline t-butyl diacetyldichloroadipate (11 g.), toluene-*p*-sulphonic acid (290 mg.), and dry benzene (100 ml.) were boiled under reflux until evolution of gas was slight (1·5 hr.), cooled, washed with aqueous sodium hydrogen carbonate

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[16] Perkin, *J.*, 1890, **57**, 215.

and water, dried ($MgSO_4$), and distilled. The product (5·45 g.), b. p. 64—74°/0·03—0·06 mm., was dissolved in ether and cooled to −70°. After 1 hr. the crystals (1·5 g.; m. p. 70—72°) were collected; recrystallization from light petroleum (b. p. 60—80°) gave the crystalline *dichloro-diketone*, m. p. 77° (Found: C, 45·7; H, 5·6; Cl, 34·1. $C_8H_{12}O_2Cl_2$ requires C, 45·5; H, 5·7; Cl, 33·7%). The liquid *isomer* was recovered from the ethereal mother-liquor (Found: C, 45·9; H, 5·9; Cl, 33·4%).

(*b*) Liquid t-butyl diacetyldichloroadipate (7·5 g.) was treated similarly to give 2·3 g. of diketone, b. p. 50—65°/0·005—0·2 mm., from which solid (0·6 g.) and liquid isomers were obtained as before.

(*c*) Crystalline t-butyl diacetyldichloroadipate (4 g.) was boiled with ethanol (30 ml.) and hydrochloric acid (30 ml.; *d* 1·18) for 1 hr. The cooled, nearly colourless solution was diluted with water, and the product, recovered by means of ether, was distilled to give 1·95 g., b. p. 65—75°/0·01 mm., from which the crystalline (0·67 g.) and the liquid isomer were obtained (Found, for liquid isomer: C, 45·9; H, 5·9; Cl, 33·8%).

(*d*) The liquid dichloro-ester (7·5 g.) was decarboxylated similarly to give, from a dark reaction mixture, somewhat impure diketone (2·0 g.) and thence the crystalline isomer (0·6 g.).

(*e*) The ester (XXII) (7·2 g.; crude product from chlorination) was hydrolysed as above with ethanol (35 ml.) and hydrochloric acid (35 ml.). The product (3·15 g.), b. p. 60—70°/0·005 mm., gave the solid isomer (0·9 g.) and the liquid isomer as before.

(*f*) Crystalline 2,5-dichloroadipic acid (17·2 g.) was warmed for ½ hr. with phosphorus pentachloride (35 g.) in chloroform (30 ml.). The solvent and phosphoryl chloride were removed at low pressure and a final temperature of 110°. The acid chloride (20·2 g.) was a colourless liquid.

Methylmagnesium bromide (from 5·2 g. of magnesium) in ether (100 ml.) under nitrogen was cooled in ice and stirred with cadmium chloride (22 g.) until the Gilman test was negative (20 min.). Most of the ether was distilled; dry benzene (50 ml.) was added and the mixture frozen (−20°). The acid chloride in benzene (50 ml.) was added and the mixture gently stirred as it came to room temperature. After 18 hr. ice and dilute hydrochloric acid were added. The residue from evaporation of the benzene layer was stirred for 2 hr. with water to hydrolyse residual acid chlorides, taken up in ether, washed as in (*a*), dried ($MgSO_4$), evaporated, and flash-distilled to give a somewhat impure product (4·5 g.), b. p. 68—72°/0·01 mm., from which a crystalline solid (0·35 g.), m. p. 130—132°, separated and was removed. The liquid portion in ether (20 ml.) was cooled to −70°, to give the crystalline isomer (0·6 g.; m. p. 68—70°) of 2,6-dichloro-octane-2,7-dione. The identity of this and the liquid isomer with material prepared by the other methods (*a*—*e*) was confirmed by preparation of the bis-aminothiazole (below).

1,2-*Bis*-(2-*amino*-4-*methyl*-5-*thiazolyl*)*ethane*.—3,6-Dichloro-octane-2,7-dione (300 mg.; crystalline or liquid isomer), thiourea (300 mg.), and ethanol (2·5 ml.) were boiled for 1 hr. The solid hydrochloride was collected, washed with ethanol, dissolved in water, and treated with aqueous sodium carbonate to precipitate the *bis-aminothiazole* which was recrystallized from ethanol and melted at 218—220° (Found: C, 47·0; H, 5·3; N, 21·8. $C_{10}H_{14}N_4S_2$ requires C, 47·2; H, 5·5; N, 22·0%). This derivative was obtained from the solid and liquid isomers prepared according to procedures (*a*), (*e*), and (*f*).

all-trans-2,6,10,15,19,23-*Hexamethyltetracosa*-2,6,10,14,18,22-*hexaene* (*Squalene*) (I).—Freshly cut lithium (526 mg.) was stirred in ether (25 ml.) under nitrogen and treated with part of a solution of homogeranyl chloride (IX) (7·4 g.) in ether (35 ml.). When reaction began, a temperature of −15° was maintained during the slow addition of the rest of the chloride. After 2 hr. the solution was cooled to −70° and 3,6-dichloro-octane-2,7-dione (2·2 g.; mixed isomers) in ether (20 ml.) was added. After ½ hr. acetic acid (2·5 ml.) in ether, and then water, were added. The ethereal layer was washed with water, aqueous sodium hydrogen carbonate, and again water, dried ($MgSO_4$), and evaporated. The crude product was treated with methanolic N-potassium hydroxide (25 ml.). Titration after ½ hr. indicated consumption of 20 ml. of N-alkali. Water was added and the product was isolated by means of ether. Distillation (bath at 160—165°; 0·001 mm.) removed volatile by-products; squalene diepoxide could be distilled (b. p. 160—175°/0·001 mm.) at this stage but distillation had no advantage. The residue (1·5—2 g.) was added to a solution of sodium iodide (5 g.) in a mixture of acetic acid (10 ml.), sodium acetate (1 g.), and propionic acid (10 ml.) at −20°. After 2 hr. the mixture was brought to room temperature and poured into excess of sodium hydrogen carbonate. The crude iodohydrin, usually weighing 0·7 g. more than the crude epoxide, was recovered by means of ether and mixed with anhydrous stannous chloride (5 g.) in pyridine (20 ml.). Phosphoryl chloride

(1·5 ml.) in pyridine (5 ml.) was added dropwise to the cooled mixture, which was left for 1 hr. at room temperature and diluted with ether (50 ml.). The solids were removed, pyridine was washed out of the filtrate by means of dilute hydrochloric acid, and the ether was washed with aqueous sodium carbonate and evaporated. The residue was boiled under reflux for 2 hr. with ethanolic 2N-potassium hydroxide (25 ml.) under nitrogen (this removed traces of iodine compounds). After dilution with water the product was recovered, dissolved in light petroleum (b. p. 60—80°; 15 ml.), and put on a column of alumina (35—40 g.). Elution by light petroleum (150 ml.) gave a pale yellow oil (600—700 mg.) which was distilled over metallic sodium to give a colourless product (500—600 mg.), b. p. 145—150°/0·001 mm. This material had an infrared spectrum very similar to that of natural squalene. With hydrogen chloride in acetone[17] it readily gave a solid hexahydrochloride, separable (like the hydrochlorides of squalene) into two products, m. p. 107—108° and 144—145°, by crystallization from acetone and ethyl acetate.

The distilled hydrocarbons (500—600 mg.) were dissolved in benzene (6 ml.), poured into a saturated solution (50 ml.) of thiourea in methanol, and cooled to 0°. The needle-shaped crystals were collected after 1 hr. and washed with a little light petroleum. Treatment with water then liberated squalene, which was extracted with light petroleum (b. p. 40—60°). The water-washed, dried ($MgSO_4$) extract was evaporated, leaving squalene (100—110 mg.) as a colourless mobile liquid, n_D^{22} 1·4950 (Found: C, 87·4; H, 12·4. Calc. for $C_{30}H_{50}$: C, 87·7; H, 12·3%). The infrared spectrum was identical with that of natural squalene purified *via* the clathrate with thiourea.

1,3-*Dichloro*-4-*methyloctan*-4-*ol*.—This was prepared from n-butyl-lithium and 3,5-dichloropentan-2-one (9 g.), essentially as in the preparation of 3,5-dichloro-2-methylpentan-2-ol (above). The *dichloro-alcohol* (8·45 g.) had b. p. 60°/0·05 mm. (Found: C, 50·3; H, 8·5; Cl, 33·3. $C_9H_{18}OCl_2$ requires C, 50·7; H, 8·5; Cl, 33·3%).

1-*Chloro*-3,4-*epoxy*-4-*methyloctane*.—The above chlorohydrin (8·45 g.) reacted rapidly with methanolic N-potassium hydroxide (47 ml.). After 15 min. water and ether were added and the product, isolated in the normal manner, was distilled. The *epoxide* (6·55 g.), a colourless oil, had b. p. 104—106°/18 mm. (Found: C, 61·3; H, 10·0; Cl, 20·7. $C_9H_{17}OCl$ requires C, 61·2; H, 9·6; Cl, 20·1%).

1-*Acetoxy*-3-*chloro*-4-*methyloctan*-4-*ol*.—Ethereal n-butyl-lithium, prepared in the usual way from lithium (1·2 g.) and n-butyl bromide (9·2 ml.), was transferred to a dropping funnel filled with nitrogen and cooled by solid carbon dioxide held in place by a jacket of cotton wool. The solution was added during 10 min. to 5-acetoxy-3-chloropentan-2-one (15·3 g.) in ether (75 ml.) at −70° with stirring. After 10 min. acetic acid (5 ml.) in ether (15 ml.) was added and the product was isolated in the normal manner. Distillation gave 9·4 g., b. p. 70—80°/0·001 mm., which was redistilled to give the *acetoxychloro-octanol* (7·2 g.), b. p. 68—70°/0·001 mm. (Found: C, 55·6; H, 9·0; Cl, 15·0. $C_{11}H_{21}O_3Cl$ requires C, 55·8; H, 8·9; Cl, 15·0%).

We offer grateful thanks for generous gifts of chemicals from Roche Products Ltd. We also thank the Government of Kerala, the Government of India, and the British Council for enabling K.K.M. to participate in this work.

NATIONAL INSTITUTE FOR MEDICAL RESEARCH,
LONDON, N.W.7.

[*Received, March* 16*th*, 1959.]

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[17] Heilbron, Kamm, and Owens, *J.*, 1926, 1630.