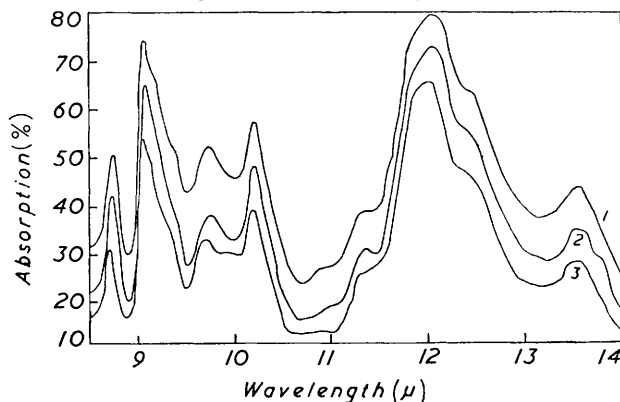


content must have been, and indeed demonstrably was,¹³ very low. Trippett's synthesis,¹³ similar to that described below, was thus the first preparation of a mixture of squalene stereoisomers almost uncontaminated with prototropic isomers, although as is shown below the all-*trans* content is unlikely to have greatly exceeded 25%. On the other hand, the synthesis by Isler, Rüegg, Chopard-dit-Jean, Wagner, and Bernhard,¹⁴ which involved major modifications of the Karrer synthesis⁴ and was submitted for publication very shortly after our own preliminary note,¹⁵ did give isolable, all-*trans*-squalene.

As a starting-point for the synthesis of squalene the crystalline calcium chloride complex of geraniol has obvious advantages. Attempts to transform the pure geraniol obtained on regeneration into reactive derivatives without loss of configuration were not successful, toluene-*p*-sulphonyl chloride in pyridine giving either geranyl chloride or the pyridinium salt rather than the desired toluene-*p*-sulphonyl ester. The pyridinium toluenesulphonate proved to be very unstable and unreactive toward sodiomalonic ester. The use of a "geranyl halide" therefore seemed necessary. As previous work showed,⁹ "geranyl chloride," obtained by conventional methods, is a mixture of the desired chloride (III) with "linalyl chloride" (IV), which on isomerisation or by reaction with nucleophilic

Infrared spectra: 1, Squalene (natural, three times regenerated). 2, Squalene (synthetic, twice regenerated). 3, Stereoisomers of squalene unaffected by thiourea under usual conditions.



Spectra were determined on neat liquids in a cell of about 0.05 mm. path-length; the "percentage transmitted" values for nos. 3 and 2 have been increased by 10% and 5%, respectively.

reagents would be converted in comparable proportions into derivatives of geraniol and nerol. Furthermore, Sørensen *et al.* have shown¹⁶ that on reductive coupling "geranyl chloride" gives a mixture containing cyclic hydrocarbons, which in all probability are formed from cyclic contaminants in the geranyl chloride. Now, in any synthetic preparation of squalene, it was necessary to minimise the formation of isomeric, including stereoisomeric, hydrocarbons, which would interfere with the formation of the squalene-thiourea clathrate; and the 6-*cis*-isomer was a particularly dangerous contaminant because of the possibility that it might form a (less stable) clathrate itself, having, for much of its length, the correct molecular cross-section. In any synthesis from geranyl chloride, a further crystalline intermediate was therefore considered essential. Geranylmalonic acid, not previously described, and geranylacetone semicarbazone were obvious possibilities.

Geranyl chloride was chosen in preference to the bromide because of the probability that the latter, but not the former, would equilibrate with its anionotropic isomers at room

¹³ Trippett, *Chem. and Ind.*, 1956, 60.

¹⁴ Isler, Rüegg, Chopard-dit-Jean, Wagner, and Bernhard, *Helv. Chim. Acta*, 1956, **39**, 879.

¹⁵ Dicker and Whiting, *Chem. and Ind.*, 1956, 351.

¹⁶ N. A. Sørensen, Gillebo, Holtermann, and J. S. Sørensen, *Acta Chem. Scand.*, 1951, **5**, 757.

temperature.¹⁷ In the halogenation much hydrocarbon (presumably myrcene and ocimene, although cyclic products may also be present) is formed concurrently; to separate the chloride, distillation has been customary. Indeed Barnard and Bateman⁹ say that, on heating, "a more homogeneous product" results, implying that the equilibration of the three chlorides gives a mixture containing less of the tertiary isomer than does the product of the kinetically controlled reaction. This, however, must mean the formation of a large proportion—probably nearly 50%—of the *cis*-isomer (V). We therefore chose to treat the crude, undistilled chloride-hydrocarbon mixture with sodio-malonic or -acetoacetic ester solutions, and distil the stable products (after hydrolysis to geranylacetone in the latter case).

Geranylmalonic ester has been prepared before, but gave a syrupy acid on hydrolysis.^{18,19} Our specimen was hydrolysed to an acid which solidified on cooling; about a third could be obtained crystalline, and when pure it melted at 50.5–51.5°. The low yield from constant-boiling ester reveals the presence of isomeric impurities in the latter, although no doubt they amounted to much less than two-thirds of the whole product; the non-crystalline acid in the mother-liquors had an infrared spectrum not greatly different from that of the crystalline product, which at least excluded the presence of $\text{H}_2\text{C}=\text{C}<$ groupings. The crystalline acid absorbed two mols. of hydrogen and gave in good yield a crystalline perhydro-acid; it is assumed to be the *trans*-acid (VI). Decarboxylation gave an 82% yield of a monocarboxylic acid, and a 9% yield of a neutral isomer which was clearly the δ -lactone (VIII). The acid had m. p. -11° to -10° , raised inefficiently to a constant -1.5° on recrystallisation. Evidently part of the acidic product was formed *via* the lactone, and presumably consisted of up to five isomers. This complication lowered the overall yield of the pure geranylacetic acid (VII) and, having obtained poor preliminary results in the reaction with methyl-lithium, we turned our attention to the known geranylacetone semicarbazone; however, the acid may prove useful in other synthetic work.

Despite the precautions taken, geranylacetone, prepared as above and by methods of Dauben and Bradlow¹² for hydrolysis of the keto-ester, gave a semicarbazone with m. p. *ca.* 70° , which was raised to the published value of $96-97^\circ$ only after about five crystallisations from aqueous ethanol, the best solvent found. The yield, on each recrystallisation, then reached a constant value, and the melting point also remained constant. Treatment with 2:4-dinitrophenylhydrazine sulphate gave a derivative which crystallised readily and had m. p. $54-55^\circ$ (cf. the liquid 2:4-dinitrophenylhydrazone obtained by Carroll,²⁰ and by ourselves from the crude ketone*). We therefore assumed that the semicarbazone was in fact homogeneous. The yield was only 48–62% from the crude ketone, whereas regeneration gave a ketone which was reconverted into a semicarbazone, m. p. $96-97^\circ$, after only one crystallisation, in high yield. Again, it is clear that the crude ketone from geranyl chloride, despite the *a priori* reasonable methods used, was far from pure. Since the completion of the present work Stadler *et al.*²¹ have reached a similar conclusion regarding "geranylacetone" prepared *via* the bromide, and have described a method of purification by rigorous fractional distillation.

Preliminary attempts to convert the regenerated *trans*-ketone into farnesic acid *via* the ethoxyacetylenic alcohol were discouraging in that the product apparently underwent partial cyclisation on acid-catalysed rearrangement; and neither the crude ethyl farnesate,

* Naves (*Helv. Chim. Acta*, 1949, **32**, 1801) obtained a 2:4-dinitrophenylhydrazone, m. p. $72-73^\circ$, from a "geranylacetone" obtained by a reverse aldol fission of natural farnesal. This suggests the presence of a *cis*-linkage in the latter.

¹⁷ See, *inter al.*, Winstein and Young, *J. Amer. Chem. Soc.*, 1936, **58**, 104.

¹⁸ Dupont and Labaune, *Wiss. und Industrie Ber.*, Rour-Bertrand Fils, 1911, (III), **3**, 3; *Chem. Zentralblatt*, 1911 (II), **15**, 138.

¹⁹ Forster and Cardwell, *J.*, 1913, 1338.

²⁰ Carroll, *J.*, 1940, 704.

²¹ Stadler, Nechvatal, Frey, and Eschenmoser, *Helv. Chim. Acta*, 1957, **40**, 1373.

nor the derived farnesol, formed a thiourea clathrate. Attention was turned to the possible use of the Wittig reagent from 1 : 4-dibromobutane to give squalene directly. Triphenylphosphine and the dihalide gave a good yield of the 4-bromobutyltriphenylphosphonium bromide in boiling butan-2-one, and this reacted with another molecule of triphenylphosphine in boiling cyclohexanone, giving a salt of which the dihydrate, m. p. 146—149°, was converted with some difficulty into an anhydrous product, m. p. 300—308°. Addition of ethereal butyl-lithium solution to an excess of the salt gave only a transient yellow coloration; but when the salt was added gradually to the butyl-lithium solution (in tetrahydrofuran) it dissolved almost completely, and a deep red solution of the Wittig reagent resulted. Addition of the ketone decolorised the solution with the formation of a precipitate, which was decomposed under nitrogen at 60°. Extraction with light petroleum and chromatographic purification gave a hydrocarbon (15% yield) of which the infrared spectrum was identical with squalene.

Trippett's brief description¹³ of the reaction between geranylacetone and 1 : 4-dibromobutane appeared when the Wittig reaction was under investigation in these laboratories, and his use of tetrahydrofuran as solvent prompted us to employ it.

The hydrocarbon obtained gave a crystalline precipitate when treated with thiourea in methanol-benzene, from which the regenerated all-*trans* hydrocarbon was obtained in 6—13% yield. Treatment of the mother-liquors with further quantities of thiourea failed to produce more clathrate; on the other hand the once regenerated hydrocarbon gave the same yield (75—80%) in a further purification cycle as did once regenerated natural squalene in duplicate small-scale experiments. Infrared spectra of the regenerated synthetic and the natural substance are illustrated, together with that of the hydrocarbon which refused to form a clathrate. Such spectra are evidently unsuitable for distinguishing between stereoisomers of the polyisoprene type, although they would reveal the presence of even a small proportion of any prototropic isomer with a $>C=CH_2$ grouping.

Dr. N. Nicolaidis and Dr. F. Laves have examined the clathrate of synthetic squalene, and report that the length of the hydrocarbon chain is the same, within experimental error of $\pm 0.1 \text{ \AA}$, as that of natural squalene. He was, however, able to prepare, from a sample of the hydrocarbon which we had tried to free from the all-*trans*-form by treatment with thiourea, a certain amount of thiourea clathrate which showed an inclusion chain-length measurably shorter than that of the *cis*-hydrocarbon. This would correspond to the presence of one or more *cis*-linkages,⁶ with some distortion of the molecule from its most stable conformation which is apparently unsuited to clathrate formation.

Although we believe that, in the combination of infrared scrutiny and the formation and X-ray examination of clathrates, definitive physical methods for the comparison of stereoisomeric unconjugated polyenes are at last available, it remained necessary, for complete satisfaction, to prove the identity of the natural and synthetic hydrocarbons toward an enzymic system. Dr. K. Bloch, Dr. T. T. Tchen, and Dr. R. K. Maudgal have now obtained conclusive evidence on this point, which will be described in detail elsewhere.

EXPERIMENTAL

trans-4 : 8-Dimethylnona-3 : 7-diene-1 : 1-dicarboxylic ("Geranylmaleonic") Acid.—Geraniol (25 g.; regenerated from its calcium chloride complex), dry pyridine (13.8 g.), and dried ether (100 c.c.) were stirred at -20° to -10° while thionyl chloride (22.3 g.; redistilled over quinoline, then linseed oil) was added during 1 hr. Dry ether (100 c.c.) was added, and the solution was stirred overnight at room temperature. The supernatant liquid was decanted from the pyridine hydrochloride, which was washed with ether (2×50 c.c.), and the ethereal solution was washed successively with much water, sodium hydrogen carbonate solution to neutrality, dilute sulphuric acid until no more pyridine was removed, sodium hydrogen carbonate solution, and water, and dried (Na_2SO_4). Evaporation at 10 mm. gave a liquid (25.0 g.) which showed bands at 835, 895, 925, 990 cm^{-1} attributable respectively to $>C=CH-$, unconjugated vinyl, conjugated vinyl, and unconjugated vinyl groupings. This was added dropwise to a solution of

the sodium derivative of ethyl malonate, prepared from sodium (6.7 g.) and the ester (57.6 g.) in dry ethanol (200 c.c.). The mixture was heated under reflux during 6 hr. with mechanical stirring. Water (400 c.c.) was added and the neutral fraction was isolated with ether. After removal of the solvent, distillation from a Kon flask gave (a) ethyl malonate, (b) a mixture (of terpene hydrocarbons, geraniol, and C₁₀ ethyl ethers), b. p. 90—97°/0.15 mm., and (c) the required ester (17.5 g.), b. p. 97—100°/0.15 mm., n_D^{20} 1.4610 (Barnard and Bateman⁹ give b. p. 126—129°/0.2 mm., n_D^{20} 1.4610).

This was heated with 10% potassium hydroxide solution (150 c.c.) under reflux for 15 hr., the upper layer disappearing. Isolation of the acidic fraction gave a syrup (12.7 g.), which crystallised partly at -78°. A solution in methylene dichloride (30 c.c.) was held at -78° for 18 hr., and the liquid was removed as completely as possible from the separated solid. After repetition of this process (necessarily inefficient because of the fibrous crystals obtained) from 30 c.c., then from 10 c.c., of methylene dichloride the product (5.0 g.; m. p. 41—45°) was crystallised from light petroleum containing a little ether at 0°, giving 4.0 g. of the essentially pure acid, m. p. 46—48°, finally raised to 50.5—51.5° on further crystallisation (Found: C, 64.75; H, 8.25. C₁₃H₂₀O₄ requires C, 65.0; H, 8.35%). (The overall yield of essentially pure acid from geraniol varied from 11 to 13%.)

4 : 8-Dimethylnonane-1 : 1-dicarboxylic Acid.—Crystalline geranylmalonic acid (65 mg.) was hydrogenated in glacial acetic acid (10 ml.) in the presence of palladium-calcium carbonate (35 mg.) for 30 min. at 20° [uptake 2.3 mols.]. After filtration and evaporation the product solidified. Crystallisation from pentane at -78° gave the acid, m. p. 56—57° (Found: C, 63.85; H, 10.05. C₁₃H₂₄O₄ requires C, 63.95; H, 9.85%).

trans-5 : 9-Dimethyldeca-4 : 8-dienoic (Geranylacetic) Acid.—Geranylmalonic acid (6.6 g.; m. p. 46—49°) was heated in nitrogen at 0.05 mm.; decomposition began at 145° and was completed by raising the bath-temperature during 1 hr. to 165°. The distillate (4.9 g.) was dissolved in ether and shaken with sodium carbonate solution. The latter was acidified and the acidic fraction was isolated with ether, giving, after removal of solvent at 0.05 mm., the crude acid (4.45 g., 83%), m. p. -11° to -10°, raised economically to -7° on one crystallisation from pentane, or in ca. 25% yield after five crystallisations and redistillation to -1.5° [b. p. 115° (bath-temp.)/0.05 mm., n_D^{25} 1.4718]. The S-benzylisothiuronium salt formed plates, m. p. 128—129°, from aqueous ethanol (Found: N, 7.40. C₁₉H₃₀O₂N₂S requires N, 7.73%). Distillation of the neutral fraction gave 5-hydroxy-5 : 9-dimethyldec-8-enoic lactone (450 mg.), b. p. 100—120° (bath-temp.)/0.01 mm. (Found: C, 73.0; H, 10.1. C₁₂H₂₀O₂ requires C, 73.45; H, 10.2%).

Similar decarboxylation of the syrup from the geranylmalonic acid mother-liquors (7.16 g.) gave a monocarboxylic fraction (3.6 g., 61%) which failed to solidify even at -78° or on treatment with light petroleum at low temperatures and seeding. Its S-benzylisothiuronium salt had m. p. 119—125° initially, raised to 124—131° after four crystallisations, but still evidently heterogeneous.

trans-6 : 10-Dimethylundeca-5 : 9-dien-2-one ("Geranylacetone").—The crude geranylchloride (211 g.) obtained from geraniol (200 g.) was added to a solution prepared from sodium (36.8 g.) and ethyl acetoacetate (273 g.) in dried ethanol (750 c.c.), and the mixture was heated under reflux with mechanical stirring for 6 hr. Water (3 l.) was added, and the neutral fraction was isolated with ether. After removal of the solvent at 10 mm., the residue (279 g.) was added to a solution of sodium hydroxide (70 g.) in water (2 l.) and ethanol (3 l.). The mixture was heated under reflux for 48 hr., water (5 l.) was added, and the neutral fraction was isolated with ether. Addition of benzene and removal of solvents at 10 mm., followed by distillation from a Kon flask, gave a series of fractions (45.5 g.), b. p. 53—61°/0.1 mm., n_D^{18} 1.4596—1.4640, which were rejected, followed by others (107.5 g.), b. p. 62—68°, n_D^{18} 1.4655—1.4686, which were added to a solution of semicarbazide hydrochloride (67 g.) and sodium acetate trihydrate (100 g.) in water (650 c.c.) and ethanol (750 c.c.). The solution was warmed at 50° for 30 min. and cooled to 0°; the precipitated semicarbazone was collected, then crystallised five times from 1 : 1 v/v water-ethanol (10 c.c. per g.). The recovery rose gradually from 84% to a constant 94%, the crystals, originally small and indefinite, became large, transparent plates, and the m. p. rose from 85—94° to 96.5—97.5° (yield 66 g.) (Forster and Cardwell¹⁹ give m. p. 97°).

The semicarbazone (66 g.) dissolved when shaken with 2N-sulphuric acid (1.5 l.) and light petroleum (b. p. 40—60°; 1.5 l.) for 24 hr. at ~22°. Evaporation of the solvent and distillation gave the trans-ketone (48.2 g., 19% from geraniol), b. p. 60—61°/0.05 mm., n_D^{23} 1.4661, n_D^{16}

1.4688 (Found: C, 80.35; H, 11.2. Calc. for $C_{18}H_{22}O$: C, 80.3; H, 11.4%). It gave a semi-carbazone which after one crystallisation had m. p. 96—97°. The 2:4-dinitrophenylhydrazone formed orange plates, m. p. 54—55°, from ethanol (Found: C, 61.2; H, 7.05. $C_{19}H_{26}O_4N_4$ requires C, 60.9; H, 7.0%).

4-Bromobutyltriphenylphosphonium Bromide.—Triphenylphosphine (78 g.), 1:4-dibromobutane (43 g.), and butan-2-one (300 c.c.) were heated under reflux for 12 hr. After cooling, the product (93 g., 98%) was collected, washed with ether, and dried. It formed plates, m. p. 207—212°, from water (Found: C, 55.3; H, 4.7. $C_{22}H_{23}PBr_2$ requires C, 55.25; H, 4.8%).

Tetramethylene-1:4-bistriphenylphosphonium Bromide.—The above salt (103 g.), triphenylphosphine (173 g.), and cyclohexanone (1600 c.c.) were heated under reflux for 48 hr. On cooling, two layers formed; the lower layer was dissolved in hot water (200 c.c.), the hydrated salt (84 g.), m. p. 146—149° with resolidification, separating on cooling. Further quantities of hydrate separated from the cyclohexanone layer, bringing the total yield to 122 g., i.e., 77% of the dihydrate. Prolonged drying of the finely ground hydrate at 100°/0.05 mm. over phosphoric oxide gave the anhydrous salt, m. p. 300—308°.

all-trans-2:6:10:15:19:23-Hexamethyltetracosane-2:6:10:14:18:22-hexaene (Squalene).—A solution of butyl-lithium in ether was prepared by the action of *n*-butyl bromide on lithium (6.45 g.) at -10°, and estimated, after filtration through glass wool into a flask filled with nitrogen, by the double-titration method.²² A portion (45 c.c., containing 0.062 mole) was added to purified tetrahydrofuran (100 c.c.), and the mixture was stirred under nitrogen while the finely powdered dibromide (22.9 g., 0.031 mole) was added during 10 min. The solution became deep red, but some of the salt remained undissolved. *trans*-6:10-Dimethylundecane-5:9-dien-2-one (12.0 g.) in anhydrous tetrahydrofuran (50 c.c.) was at once added dropwise; a white precipitate was formed, the solution becoming almost colourless. The mixture was heated under reflux for 1 hr., the solvent removed at 10 mm., and the semi-solid residue heated in nitrogen for 5 hr. at 60°. The resultant material was extracted with light petroleum (b. p. 60—80°; 5 × 100 c.c.), the extract was concentrated, and insoluble matter was removed by centrifugation. Evaporation gave an oil (11.2 g.) which was chromatographed on alumina (500 g.; grade "H") with light petroleum (b. p. 60—80°) as eluant. The hydrocarbon fraction so obtained (1.95 g., 15%) had an infrared spectrum indistinguishable from that of squalene; yields varied from 13 to 22%.

The hydrocarbon mixture in benzene (24 c.c.) was added in one portion to a saturated solution of thiourea in methanol (160 c.c.) with swirling. After 1 hr. at 0° the adduct (1.50 g.) formed fine needles; a further crop (0.97 g.) separated overnight. The first crop was decomposed by shaking it with water (50 c.c.) and light petroleum (b. p. 40—60°; 50 c.c.), and the extract was washed with water, dried ($MgSO_4$), and evaporated, giving the hydrocarbon (122 mg., 6.3%) (in other experiments yields, based on the hydrocarbon mixture, were 10.5—13%) (Found: C, 87.8; H, 12.2. Calc. for $C_{30}H_{50}$: C, 87.7; H, 12.3%).

Mixture of the Δ^{10} -cis- and the $\Delta^{10:14}$ -Di-cis-isomer.—Recovery of the hydrocarbon by dilution of the methanol-benzene-thiourea solution with water and extraction with light petroleum gave an oil (1.7 g.) which was again treated with benzene (20 c.c.) and thiourea-methanol (135 c.c.), giving a slight precipitate which was removed. Re-isolation and re-chromatography gave a colourless liquid (1.40 g.) with the infrared spectrum illustrated (Found: C, 87.6; H, 12.1%).

Infrared spectra were determined with a Perkin-Elmer Model 21 spectrophotometer with sodium chloride optics.

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²² Gilman and Haubein, *J. Amer. Chem. Soc.*, 1944, **66**, 1515.