Neocembrene-A, a Termite Trail Pheromone

By A. J. Birch, W. V. Brown, J. E. T. Corrie,* and B. P. Moore, Research School of Chemistry, Australian National University, P.O. Box 4, Canberra ACT 2600; and Division of Entomology, C.S.I.R.O., P.O. Box 109, Canberra City, ACT 2601, Australia

The trail pheromone of *Nasutitermes* is shown to be the cembrene analogue 12-isopropyl-1,5,9-trimethylcyclotetradeca-1,5,9-triene (10) by degradation, by comparison of its perhydro-derivative with perhydrocembrene, and by isomerisation with sodium methylsulphinylmethanide followed by degradation. The configurations of the double bonds are at present unknown. A key aspect of the work is the use of a quantitative micro-ozonolysis procedure.

A DITERPENE hydrocarbon, now named neocembrene-A for reasons noted later, was recognised by Moore [1] from the termite *Nasutitermes exitiosus* (Hill) and it seems to be present in *N. walkeri* (Hill) and *N. graveolus* (Hill). It has the activity of a scent-trail pheromone in extraordinarily high dilution. This activity relates to the foregoing three species but is not found, for example, with *Coptotermes lacteus* (Frogatt).

In view of possible applications of pheromones such as this in termite control, its structure has been examined. The concentration of neocembrene-A in live termites is about one part in four million,[1] so the quantities available have been only *ca.* 1 mg and deductions concerning the structure depended heavily on spectra and on biogenetic considerations.

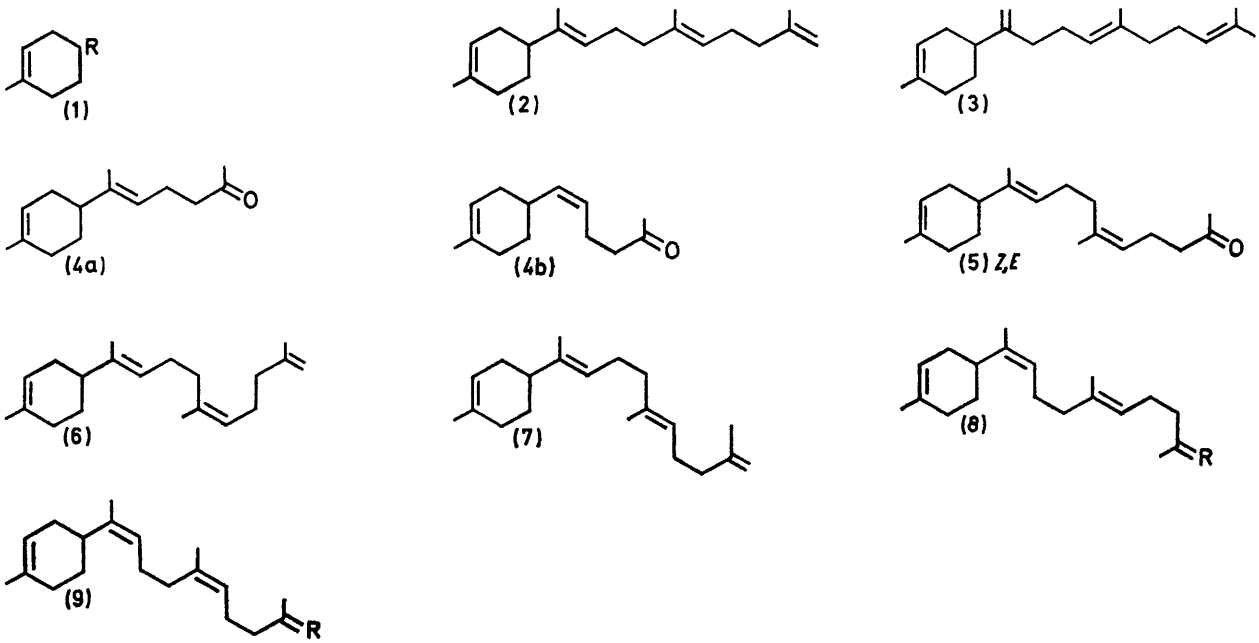
Neocembrene-A has only end absorption in the u.v. spectrum; the i.r. absorption bands at 3070, 1640, and 880 cm^{-1} are indicative of the presence of $C{=}CH_2$. The molecular ion in the mass spectrum is at *m/e* 272 ($C_{20}H_{32}$), with a fairly intense peak at *m/e* 257 (loss of Me) and a base peak at *m/e* 68, corresponding formally to the molecular ion of isoprene.[1] Catalytic hydrogenation gave an octahydro-derivative, M^+ 280, $C_{20}H_{40}$, which indicated that the pheromone has four double bonds and therefore a monocyclic structure. Ozonolysis by a specially developed micro-technique [2] produced formaldehyde and 2 equiv. of 4-oxopentanal. The production of the former confirms the presence of $=CH_2$, and that of the latter shows the presence of two $=C(Me)\cdot CH_2\cdot CH_2\cdot CH=$ units. The low-field region of the ^1H n.m.r. spectrum shows a three-proton envelope at δ 4·8—5·2 p.p.m., assigned to the olefinic protons of three trisubstituted double bonds, and a sharper two-proton envelope at δ 4·51—4·68, assigned to $=CH_2$. The high-field region, apart from a broad methylene envelope, shows four distinct methyl resonances: a quartet centred at δ 1·63 p.p.m. (J 1·4 Hz) is assigned to $MeC{=}CH_2$, and three broad singlets at δ 1·55, 1·56, and 1·58 are assigned to three MeC=CH groups.

The intense ' isoprene ' ion in the mass spectrum could be the result of a retro-Diels–Alder reaction such as that first invoked in arriving at the structure of geijerene,[3] suggesting a possible component (1) in the structure. From this postulate, structure (2), with an isoprenoid skeleton, was considered for neocembrene-A. No steric assignments [4] to the MeC=CH units could be made on the basis of the ^1H n.m.r. spectrum, which is complex with the Me groups resonating at slightly higher field than normally expected. However, structure (2) represents a relatively simple synthetic problem, and the synthesis of all four stereoisomers was examined. Other biogenetically more appealing structures such as (3) and related structures are ruled out by lack of formation of acetone and formation of two molecules of 4-oxopentanal during ozonolysis. Because of the small quantity available, optical activity was not examined.

4-Acetyl-1-methylcyclohexene [5] was treated with the ylide from 4,4-ethylenedioxypentyltriphenylphosphonium iodide [6] to give, after deacetalisation, a 5 : 1 ratio of the *E*- and *Z*-isomers (4a and b). Careful distillation of the mixture separated (5*E*)-6-(4-methylcyclohex-3-enyl)hept-5-en-2-one (4a), which showed three distinct methyl resonances: δ 2·12 (COMe), 1·65 (MeC=CH in the cyclohexene, identical with that in the methylcyclohexene precursor), and 1·60, which in the light of literature comparisons [4,6] represents a methyl group *cis* to the aliphatic chain. A fraction was also obtained from the distillation containing a *ca.* 1 : 1 mixture of isomers (4a and b), but the latter was not obtained pure. The pure *E*-isomer (4a) was again treated with the same ylide. The product, after deacetalisation, contained the 5*Z*- and 5*E*-isomers of the ketone (5) in the ratio 2 : 1. The total material was treated with methylenetriphenylphosphorane, to convert C=O into $C{=}CH_2$, and the product was chromatographed on silver nitrate–alumina, which removed some C_{15} compound derived from structure (4) present as impurity in the ketone (5). The tetraenes (6) and (7) could not be separated in this way, but the mixture was readily resolved by preparative g.l.c. Since the stereochemistry of the starting material (4a) was defined, it was possible by ^1H n.m.r. spectra to distinguish between compounds (6) and (7). Both showed a broad singlet at δ 1·72 ($MeC{=}CH_2$); in the case of structure (6) (*Z,E*-configuration) there were two coincident methyl resonances at δ 1·67 and a single methyl signal at δ 1·61, whereas the *E,E*-isomer (7) showed one resonance at δ 1·66 and two coincident resonances at δ 1·61.

[1] B. P. Moore, *Nature*, 1966, **211**, 746.
[2] B. P. Moore and W. V. Brown, *J. Chromatog.*, 1971, **60**, 157.
[3] A. J. Birch, J. Grimshaw, A. R. Penfold, N. Sheppard, and R. N. Speake, *J. Chem. Soc.*, 1961, 2286.
[4] R. B. Bates, D. M. Gale, and B. J. Gruner, *J. Org. Chem.*, 1963, **28**, 1086; J. W. K. Burrell, R. F. Garwood, L. M. Jackman, E. Oskay, and B. C. L. Weedon, *J. Chem. Soc.* (*C*), 1966, 2144.
[5] A. Manjarrez, T. Rios, and A. Guzman, *Tetrahedron*, 1964, **20**, 333.
[6] G. W. K. Cavill and P. J. Williams, *Austral. J. Chem.*, 1969, **22**, 1737.

The pure *Z*-isomer (4b) was separated by preparative g.l.c., but the Wittig condensation failed to yield the expected hydrocarbon. However, similar treatment of the 1:1 mixture of the isomers of (4), followed by deacetalisation, gave material showing four peaks on g.l.c., two coincident with the *Z,E*- and *E,E*-isomers of (5); the other two presumably correspond to structures (8; R = O) and (9; R = O). The mixture was treated



with methylenetriphenylphosphorane and the product separated on g.l.c. into three peaks, the first corresponding to compound (6), the second to a mixture of (7) and (8; R = CH_2), and the third to (9; R = CH_2). The configuration of the last was again defined by its ^1H n.m.r. spectrum.

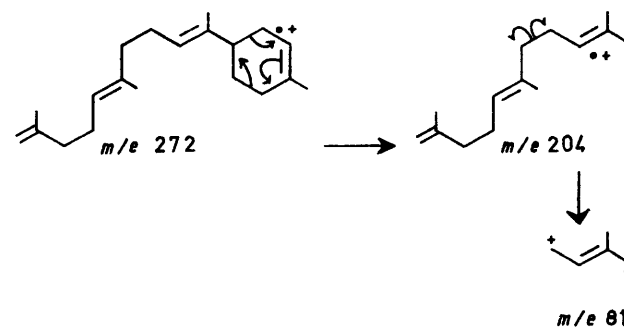
A comparison of the ^1H n.m.r. spectra of the three tetraenes obtained pure with that of neocembrene-A showed that the latter has a basically different type of structure. In particular for the synthetic compounds the ring olefinic proton signal is clearly downfield of those of the other two protons on the trisubstituted double bonds. The aliphatic methylene signals largely fall into a broad envelope, whereas those in neocembrene-A form a complex multiplet. The methyl resonances of the synthetic compounds all fall in the expected positions;[4] those of neocembrene-A are more complex.

The initial interpretation of the significance of the peak at *m/e* 68 was incorrect, since in the mass spectra of the synthetic compounds this ion is of low intensity; all the tetraenes have a base peak at *m/e* 81, the suggested route to which is shown.

Bioassay of the synthetic substances also excluded possible identity with neocembrene-A. The most active was compound (9) but in all cases activity was hundreds of times less than that of the natural material.

In considering possible modes of biogenesis of neocembrene-A we assumed that the precursor is geranyl-geranyl pyrophosphate and that double bond migration does not occur. These assumptions led immediately to a possible structure (10) (no significance is attached to the particular stereochemistry represented; all possible stereoisomers can be constructed with models without undue strain). This structure (10), which is the theoretical precursor of cembrene (11), isolable from haploid pines,[6] would account for the complexity of the ^1H n.m.r. spectrum, which could be due to transannular interactions.

Confirmation that neocembrene-A has the skeleton of (10) was obtained by comparison of perhydroneocembrene-A with the perhydro-derivative of cembrene

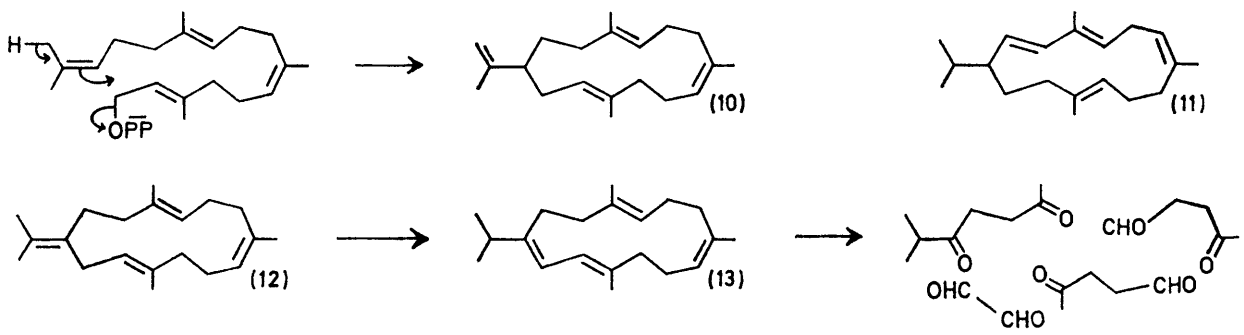


(11),[7] provided by Dr. W. G. Dauben (Berkeley). Although these saturated hydrocarbons can exist in four diastereoisomeric (±)-forms, the hydrogenation may well lead to much the same mixture in both cases, and the mass spectrum is probably insensitive to isomerism of this type. The compounds from the two sources could not be distinguished by g.l.c. or by mass spectrometry. In contrast, hydrogenation of a mixture

[7] W. G. Dauben, W. E. Thiessen, and P. R. Resnick, *J. Org. Chem.*, 1965, **30**, 1693.

of the synthetic tetraenes already obtained gave a product which differed from octahydroneocembrene-A in both g.l.c. behaviour and mass spectrum.

Assignment of formula (10) rests on the ozonolysis results also, and was uncertain to the extent that, apart from 4-oxopentanal and formaldehyde, a C_9 diketo-aldehyde must be formed, which is not readily available for comparison. On the basis of the rules for acidity of unsaturated hydrocarbons,[8] a very strong base might be expected to remove protons from structure (10) in only two types of position to give mesomeric anions: from a methyl group, in which case re-protonation should regenerate the original structure, and from CH_2=C-(Me)CH$^-$ since the resulting mesomeric allylic anion would then contain a CH_2 group in the structure.[8] Migration of the isolated double bonds in the nucleus should therefore be slow in comparison with isomerisation to structure (12). Furthermore, since structure (12) contains the highly acidic system C=CH·CH_2·C=C, proton abstraction from this should be easy,[8] with eventual production of the conjugated isomer (13). The action of sodium methylsulphinylmethanide in dimethyl sulphoxide solution,[9] which is a more convenient and powerful reagent than the original potassamide in liquid ammonia,[8] caused partial conversion of neocembrene-A into compound (13), which was separated by preparative g.l.c. The micro-ozonolysis procedure gave two equivalents of 4-oxopentanal and one each of glyoxal and 6-methylheptane-2,5-dione. The last compound was obtained for comparison by ozonolysis of *p*-mentha-1,3-diene. These fragments constitute all the carbon atoms of neocembrene-A, and the structure, apart from the configurations of the trisubstituted double bonds, is thus firmly established.



Since this work was completed two isolations of a hydrocarbon from plant oils with the structure (undefined stereochemistry) of neocembrene-A have come to our notice. One of these,[10] of a substance named neocembrene, from two conifers, differs slightly in the published n.m.r. spectrum from neocembrene-A, which may indicate either some difference in double-bond stereochemistry or small proportions of impurity. It is for this reason we have added the suffix A to the Russian name; this can be dropped if identity is established. We have not been able to obtain a specimen for direct comparison. The other isolate[11] is identical with neocembrene-A; a specimen provided by Dr. Dev had the same g.l.c. retention time, n.m.r. spectrum, and biological activity. This material can more conveniently be used for determination of stereochemistry. It is also available in large quantities for assessment of utility in termite control.

[8] A. J. Birch, *J. Chem. Soc.*, 1947, 1642.
[9] E. J. Corey and M. Chaykovsky, *J. Amer. Chem. Soc.*, 1965, **87**, 1345.
[10] E. N. Shmidt, N. K. Kashtanova, and V. A. Pentegova, *Khim. prirod. Soedinenii*, 1970, **6**, 694.

EXPERIMENTAL

I.r. spectra were examined for solutions in carbon tetrachloride with a Perkin-Elmer 257 instrument, u.v. spectra for solutions in ethanol with a Unicam SP 800 machine, and ^{1}H n.m.r. spectra for solutions in [^{2}H]chloroform with a Varian HA100 spectrometer (tetramethylsilane as internal standard). Mass spectra were obtained with an A.E.I. MS902 instrument. Unless noted otherwise g.l.c. was carried out, in the synthetic work, with a PE881 chromatograph (6 ft × 0·25 in glass columns packed with 5% Carbowax 20M or 5% BDS on 80—100 mesh Chromosorb W; nitrogen carrier at 30 ml min^{-1}) or with an Aerograph 202-1C instrument (5 ft × 0·25 in stainless steel column packed with 20% Carbowax 20M, 20% SE30, 20% Ucon 50 LB 550X or 10% Apiezon L on 60—80 mesh Chromosorb W; helium carrier at 60 ml min^{-1}). In the isolation work, the separation of isoneocembrene-A, and the separations of ozonolysis products,[2] g.l.c. was conducted in a Varian 2100–40 machine (2 m × 4 mm glass columns and a 1 : 10 splitter for preparative work). The columns were (A) 5% Carbowax-20M on GasChrom Z, and (B) 5% OV-1 on GasChrom Q.

Isolation of Neocembrene-A.—The identification of the *Nasutitermes* scent-trail pheromone [1] as the diterpene component of the termite lipid fraction led to considerable simplification of the isolation procedure and obviated the necessity, in large scale runs, for frequent screening of fractions in the biological test.[12]

The termites (mixed castes) were separated from mounds of *N. exitiosus* (Hill) according to a standard technique [13] and were stored in ethanol (95%) until about 12 kg (live weight) had accumulated. The preserved termites were

[11] S. Dev, Indian Department of Scientific and Industrial Research, Central Research Laboratories, Poona, personal communication.
[12] A. J. Birch, K. B. Chamberlain, B. P. Moore, and V. H. Powell, *Austral. J. Chem.*, 1970, **23**, 2337.
[13] F. J. Gay, T. Greaves, F. G. Holdaway, and A. H. Wetherly, *Bull. Commonwealth Sci. Ind. Res. Organisation*, 1955, **277**, 1.

then homogenised in the medium, with addition of further ethanol, to form a thin slurry, which was centrifuged, in batches, at 7000 rev. min^{-1} for 15 min, in a high-capacity machine. The supernatant aqueous-ethanolic layer showed little activity in the biological test [12] and was therefore discarded. The solid residues were broken up mechanically and steeped in light petroleum (b.p. $<40°$) for 24 h. The extract was recovered by filtration and the solids were re-extracted with fresh solvent in the same way. The combined extracts were freed from the bulk of the solvent on a water-bath, to yield reddish-yellow lipid material (*ca.* 750 g).

The mixed lipids were saponified, in portions of 150 g by refluxing for 1·5 h with potassium hydroxide (90 g) in methanol (1·2 l). Water (350 ml) was added to each of the cooled hydrolysates, and each was extracted successively with portions (1 × 350, 2 × 150, and 1 × 50 ml) of light petroleum. The mixtures separated into three layers in equilibrium and only the uppermost was collected at each stage. The combined extracts from each hydrolysate were washed by shaking successively with portions (1 × 100 and 2 × 50 ml) of aqueous 4% potassium hydroxide, and then by decantation, repeatedly with water, until significant amounts of soap were no longer removed. Undue agitation of the mixtures at the latter stage of the washing led to formation of intractable emulsions.

The combined petroleum layers from all hydrolysates were reduced to small bulk on a water-bath, then chilled to 4° for several hours and freed from flocculent soaps by centrifugation at the same low temperature. The solution was further clarified by chromatography, in the same grade of light petroleum, on a dry-packed column (25 mm i.d.) of alumina (100 g; activity *ca.* III on the Brockmann [14] scale); all material which emerged ahead of the mobile, yellow zone of β-carotene was retained as a single fraction. Removal of the solvent on a water-bath left mixed, colourless hydrocarbons (*ca.* 1·5 g). n-Eicosane (200 mg) was added to this material, to serve as a volatile carrier, and the mixture was subjected to short-path distillation, firstly at 25 mmHg and a bath-temperature of 110°, when monoterpenes [15] distilled over, and then at 0·05 mmHg, when all material distilling over at a bath-temperature of 180° was collected. The volatile hydrocarbon fraction thus obtained (*ca.* 250 mg) was dissolved in light petroleum and chromatographed on silica gel (2 g in a column of 9 mm i.d.) activated by previous heating at 120° for 5 h. Elution was monitored by means of a Pye–Unicam liquid chromatograph. Saturated hydrocarbons soon emerged, without a change of solvent, and unsaturated materials were then eluted with 10% ether–light petroleum. G.l.c. of the latter fraction [column (A), 175°] showed a strong, well-separated peak (t_R *ca.* 12·5 min) in the C_{20} region, corresponding to neocembrene-A, together with a cluster of peaks in the C_{15} region (t_R *ca.* 3 min) and minor ones in the intermediate zone. Material corresponding to the neocembrene-A peak was collected from repeated preparative injections to give *ca.* 2—3 mg of material. As thus prepared, neocembrene-A formed a colourless oil, with a faint wax-like odour; g.l.c. analyses [columns (A) and (B)] indicated a purity of greater than 99%. This material was active in the trail-laying test over the approximate concentration range (in ether) of 10^{-5} to 10^{-8} g ml^{-1}.

Quantitative Micro-ozonolysis of Neocembrene-A.—Approximately equivalent solutions of neocembrene-A and of cembrene in ethyl acetate (containing *ca.* 1 μg in 2 μl) were separately treated with an aliquot portion of a similar solution of n-tetradecane, to serve as an internal standard. The solutions of olefin and standard were analysed by g.l.c. [column (A), 110° for 5 min, then temperature-programmed at 6° min^{-1}] to determine the relative amounts of test materials and internal standard. Micro-ozonolyses were performed on 100 μl samples of these mixtures, according to the method of Moore and Brown,[2] and the relative responses of (undegraded) standard and resulting 4-oxopentanal were determined on the gas chromatograph. The response recoveries, after ozonolysis, could then be calculated. Cembrene, which is known to produce 1 mol. equiv. of 4-oxopentanal, gave a value of 15·2%, whereas neocembrene-A gave 33·5%, a recovery to be expected from 2 mol. equiv. of the keto-aldehyde. Since a carbonyl carbon system is known to give little response in the flame-ionisation detector, 4-oxopentanal would be expected to respond essentially as a C_3 unit, thus leading to a figure of 15% recovery per mole when derived from a C_{20} hydrocarbon. Formaldehyde was also detected.[2]

Ozonolysis of Isoneocembrene-A.—This was carried out similarly, but the use of the C_8 diketone as an internal standard for response recoveries made unnecessary the addition of another standard. Glyoxal, the C_8 diketone (alternatively produced from *p*-mentha-1,3-diene), and 4-oxopentanal were compared with authentic materials and identified in the molar ratios 1 : 1 : 2. The details of identification and comparison methods are given in ref. 2.

6-(4-*Methylcyclohex*-3-*enyl*)*hept*-5-*en*-2-*one* (4).—4-Acetyl-1-methylcyclohexene, prepared according to ref. 5, gave a single peak on each of the columns noted in connection with synthetic work. The ^1H n.m.r. spectrum showed δ 5·37 (1H, envelope, =CH), 2·3—2·7 (1H, m, CO·CH), 2·17 (3H, s, Ac), and 1·65br (3H, s, $C{=}C{-}CH_3$) superimposed on 1·4—2·3(6H, m, aliphatic H) p.p.m. 4,4-Ethylenedioxypentyltriphenylphosphonium iodide, made by the method of ref. 6, had m.p. 216—219°.

A solution of sodium methylsulphinylmethanide,[9] prepared from 50% sodium hydride dispersion (9·73 g) and dry dimethyl sulphoxide (290 ml), was cooled to 40° and the phosphonium salt (100 g) was added slowly under nitrogen. The solution was then stirred for a further 20 min at 45°, treated with 4-acetyl-1-methylcyclohexene (26·5 g), stirred for 3 h at 45°, kept overnight at room temperature, and poured into ice–water (1·5 l). Triphenylphosphine oxide was filtered off and the filtrate was extracted with pentane. The extracts were washed with water and brine, dried, and evaporated to leave a pale yellow oil (38 g) which was added to a solution of toluene-*p*-sulphonic acid (1 g) in acetone (500 ml) and left at 25° for 2 h. After neutralisation with solid potassium carbonate and filtration, the solvent was removed under reduced pressure and the residue was distilled to give a colourless liquid (23·8 g), b.p. 60—120° at 0·4 mmHg. G.l.c. analysis (5% Carbowax 20M; 183°) showed four peaks, t_R 0·6, 4·2, 4·5, and 5·1 min, which correspond to unchanged starting material, an unidentified compound, and the *E*- and *Z*-isomers of (4), respectively. Fractional distillation on a Nester–Faust spinning-band apparatus gave initially the starting ketone (4 g), then a series of fractions which contained varying proportions of the unidentified compound and the *E*-ketonic product (total 7·5 g), followed by pure (5E)-6-(4-*methylcyclohex*-3-*enyl*)*hept*-5-*en*-2-*one* as a colourless liquid (4·5 g), b.p. 80° at 0·25 mmHg (Found: C, 81·9; H, 10·8%; M^+, 206.

[14] H. Brockmann and H. Schodder, *Ber.*, 1941, **74**, 73.
[15] B. P. Moore, *J. Inst. Physiol.*, 1964, **10**, 371.

$C_{14}H_{22}O$ requires C, 81·5; H, 10·8%; *M*, 206); $\nu_{max.}$ 1720 cm^{-1}; δ 5·40 (1H, envelope, 3′-H), 5·05br (1H, t, $J_{4,5}$ 6·9 Hz, 5-H), 2·12 (3H, s, Ac), 1·65br (3H, s, 4′-CH_3), and 1·60br (3H, s, 6-CH_3) superimposed on 1·4—2·8 (11H, m, aliphatic H) p.p.m.; *m/e* 41(16%), 43(69), 55(10), 79(14), 81(13), 93(15), 94(11), 95(100), 96(12), 119(10), 121(19), 132(12), and 138(10).

The *semicarbazone* formed colourless needles (from aqueous ethanol), m.p. 126—127° (Found: C, 68·5; H, 9·8; N, 15·9%; M^+, 263. $C_{15}H_{25}N_3O$ requires C, 68·4; H, 9·6; N, 16·0%; *M*, 263); $\nu_{max.}$ ($CHCl_3$) 3530, 3410, 3385, 1675, and 1560 cm^{-1}.

A portion of the residual 1 : 1 mixture of the *E*- and the *Z*-isomers (4) was separated into its components by preparative g.l.c. (7 ft × 0·25 in 20% Carbowax 20M; nitrogen flow rate 100 ml min^{-1}; temperature programmed from 140° to 200° at 4° min^{-1}). The ketones had t_R 33·0 and 35·4 min, respectively. The collected *Z*-ketone was purified by short-path distillation at 0·2 mmHg (bath temperature 90°). (5Z)-6-(4-*Methylcyclohex*-3-*enyl*)*hept*-5-*en*-2-*one* was a colourless liquid (Found: C, 81·3; H, 10·6%; M^+, 206); $\nu_{max.}$ 1720 cm^{-1}; δ 5·38 (1H, envelope, 3′-H), 5·11br (1H, t, $J_{4,5}$ 6·6 Hz, 5-H), 2·12 (3H, s, Ac), and 1·61 and 1·63 (each 3H, s,br, allylic CH_3) superimposed on 1·4—2·7 (11H, m, aliphatic H) p.p.m.

The *semicarbazone* separated from aqueous ethanol as colourless plates, m.p. 132—134° (Found: C, 68·9; H, 9·8; N, 16·2%; M^+, 263); $\nu_{max.}$ ($CHCl_3$) 3530, 3410, 3385, 1685, and 1560 cm^{-1}.

2,6-*Dimethyl*-10-(4-*methylcyclohex*-3-*enyl*)*undeca*-1,5,9-*triene*.—To a solution of sodium methylsulphinylmethanide [from 50% sodium hydride dispersion (1·05 g) and dimethyl sulphoxide (30 ml)] was added a solution of 4,4-ethylenedioxypentyltriphenylphosphonium iodide (10·7 g) in dimethyl sulphoxide (25 ml). After 10 min, the *E*-ketoolefin (4a) (4·2 g) was added and the solution was kept at 40° for 3 h, then at 25° overnight. It was worked up as before and the residue was shown by its i.r. spectrum to contain a large amount of unchanged starting material. The total material was added to an identical solution of the ylide and the solution was stirred at 50° for 36 h; after work-up the residue was deacetalised as before to leave a yellow oil (2·4 g). G.l.c. (5% Carbowax 20M; 223°) showed three peaks, t_R 0·9, 4·7, and 5·9 min, which were respectively due to the starting ketone, and the (5*Z*,9*E*)- and (5*E*,9*E*)-isomers of (5). The total mixture was added to a solution of methylenetriphenylphosphorane, prepared by the addition of methyltriphenylphosphonium iodide (12·8 g) in dimethyl sulphoxide (20 ml) to sodium methylsulphinylmethanide [from 50% sodium hydride dispersion (1·45 g) and dimethyl sulphoxide (40 ml)]. The solution was kept at 50° for 15 h and worked up as before. The residue (2·1 g) was chromatographed in pentane over 20% silver nitrate-impregnated alumina (65 g). Pentane–ether (99 : 1) eluted the C_{15} triene derived from the compound (4) present (0·5 g), which was discarded; the remainder of the material was stripped from the column with pentane–ether (1 : 1) to afford the mixed tetraenes (6) and (7) as a colourless liquid (1·3 g). Small-scale preparative g.l.c. (20% Carbowax 20M; 195°) gave (5Z,9E)-2,6-*dimethyl*-10-(4-*methylcyclohex*-3-*enyl*)*undeca*-1,5,9-*triene* (6) as a colourless oil, t_R 15·2 min, followed by (5E,9E)-2,6-*dimethyl*-10-(4-*methylcyclohex*-3-*enyl*)*undeca*-1,5,9-*triene* (7) as a colourless oil, t_R 17·5 min. Tetraene (6) had $\nu_{max.}$ 3080, 1650, and 890 cm^{-1}; δ 5·40 (1H, envelope, 3′-H), 5·13br (2H, t, 5-H and 9-H), 4·68br (2H, s, $=CH_2$), 2·6—2·8 (1H, envelope, 1′-H), 1·5—2·3 (14H, envelope, aliphatic H), 1·72 (3H, s, 2-CH_3), 1·67 (6H, s, 6-CH_3 and 4′-CH_3), and 1·61 (3H, s, 10-CH_3) p.p.m.; *m/e* 41(32%), 53(18), 55(61), 68(12), 69(15), 77(11), 79(22), 81(100), 91(12), 93(69), 94(21), 95(19), 107(28), 119(12), 121(26), and 149(31).

Tetraene (7) had $\nu_{max.}$ 3080, 1650, and 890 cm^{-1}; δ 5·42 (1H, envelope, 3′-H), 5·12 (2H, envelope, 5-H and 9-H), 4·67br (2H, s, $=CH_2$), 2·5—2·8 (1H, envelope, 1′-H), 1·4—2·3 (14H, envelope, aliphatic H), 1·72 (3H, s, 2-CH_3), 1·66 (3H, s, 4′-CH_3), and 1·61 (6H, s, 6-CH_3 and 10-CH_3); *m/e* 41(32%), 53(18), 55(61), 67(21), 68(12), 69(15), 77(11), 79(22), 81(100), 91(12), 93(69), 94(21), 95(19), 107(28), 119(12), 121(26), and 149(31).

A 1 : 1 mixture of the unsaturated *E*- and *Z*-ketones (4) (2 g) was stirred for 16 h at 50° with 3 equiv. of the ylide from 4,4-ethylenedioxypentyltriphenylphosphonium iodide, and the mixture was worked up as before. The crude product was treated with a fresh solution of the ylide (3 equiv.) for a further 16 h at 50°, after which g.l.c. analysis showed complete consumption of the starting ketones. The acetal group was removed as before to leave a mixture of ketones (1·8 g). The mixture was added to a threefold excess of methylenetriphenylphosphorane solution and kept at 50° for 15 h, then worked up as before. The residue (1·7 g) was separated by small-scale preparative g.l.c. (20% Carbowax 20M; 190°). The isomer (6) was eluted first, t_R 18·3 min, followed by a broad peak, t_R *ca.* 22·7 min, which was a mixture of (7) and (8; R = CH_2); finally (5Z,9Z)-2,6-*dimethyl*-10-(4-*methylcyclohex*-3-*enyl*)*undeca*-1,5,9-*triene* (9; R = CH_2) was obtained as a colourless oil, t_R 25·5 min. Trial runs on BDS and QF-1 columns gave no better resolution of the isomers (7) and (8).

Tetraene (9; R = CH_2) had $\nu_{max.}$ 3080, 1651, and 890 cm^{-1}; δ 5·40 (1H, envelope, 3′-H), 5·16 (2H, envelope, 5-H and 9-H), 4·69br (2H, s, $=CH_2$), 1·4—2·4 (15H, envelope, aliphatic H), 1·72 (3H, s, 2-CH_3), and 1·62br (9H, s, 6-CH_3, 9-CH_3, and 4′-CH_3); *m/e* 41(59%), 43(36), 44(58), 53(18), 55(67), 67(39), 68(12), 69(26), 77(13), 79(34), 80(10), 81(100), 83(16), 84(29), 91(17), 93(76), 94(27), 95(24), 96(15), 97(21), 98(20), 105(12), 107(40), 108(11), 109(16), 110(29), 111(13), 119(21), 121(31), 122(13), 123(22), 132(10), 133(11), 149(39), and 272(10).

Neocembrene-A.—The pheromone showed *m/e* 41(43%), 45(20), 53(24), 55(33), 59(30), 67(41), 68(100), 69(27), 77(12), 79(25), 80(15), 81(64), 91(17), 92(13), 93(73), 94(29), 95(32), 105(27), 106(10), 107(53), 108(30), 109(23), 119(23), 120(17), 121(55), 122(22), 123(20), 133(23), 134(20), 135(26), 136(13), 147(20), 148(15), 149(12), 161(18), 175(11), 189(15), 257(24), and 272(38).

Octahydrocembrene.—(i) Neocembrene-A (0·25 mg) was hydrogenated for 16 h at 20° and atmospheric pressure in ethanol (1 ml) over prereduced platinum oxide (1 mg). The solution was filtered through a small bed of Celite and the solvent was removed to afford octahydrocembrene, t_R 7·6 min (5% Carbowax 20M; 140°) and 6·5 min (5% BDS; 140°); *m/e* 41(67%), 42(14), 43(74), 55(100), 56(33), 57(84), 67(14), 68(12), 69(88), 70(25), 71(47), 81(20), 82(14), 83(69), 84(14), 85(27), 95(18), 96(10), 97(73), 109(12), 110(10), 111(49), 125(25), 139(10), 236(78), 237(55), and 280(M^+, 6).

(ii) Cembrene (1 mg) was similarly hydrogenated to afford octahydrocembrene, t_R 7·6 min (5% Carbowax 20M; 140°) and 6·5 min (5% BDS; 140°); *m/e* 41(70%), 42(12), 43(77), 55(100), 56(41), 57(87), 67(11), 68(11),

69(93), 70(29), 71(43), 81(14), 82(14), 83(71), 84(14), 85(29), 95(14), 97(77), 111(57), 125(30), 139(12), 236(100), 237(66), 238(11), and 280(M^+, 9).

2,6-*Dimethyl*-10-(4-*methylcyclohexyl*)*undecane*.—A mixture of the synthetic tetraenes was similarly hydrogenated to afford the saturated hydrocarbon, t_R 6·0 min (5% Carbowax 20M; 140°) and 4·7 min (5% BDS; 140°); *m/e* 41(18%), 43(26), 55(62), 56(12), 57(40), 69(33), 71(21), 81(19), 83(14), 85(10), 96(83), 97(100), 125(19), 182(10), and 280(M^+, 9).

Isomerisation of Neocembrene-A.—Sodium methylsulphinylmethanide [from 50% sodium hydride dispersion (0·11 g) and dimethyl sulphoxide (2 ml)] was cooled to 50° and a solution of neocembrene-A (2 mg) in dimethyl sulphoxide (0·5 ml) was added. The mixture was stirred at 55° for 15 h then cooled, diluted with water, and extracted with ether. The extracts were washed with water and brine, dried, and evaporated. The residue was filtered in ether through a small column of alumina to remove coloured material. Preparative g.l.c. separation recovered about 90% of neocembrene-A, the remainder being a uniform product, isoneocembrene-A [column (A)].

We acknowledge the award of a research scholarship from C.S.I.R.O. (to J. E. T. C.).

[2/1096 *Received*, 15*th May*, 1972]