Microbiological Hydroxylation. Part XII.[1] Comparative Behaviour of D-Homogonane (Perhydrochrysene) Ketones and Steroids

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rac-D-Homogonanes, with one and two oxygen substituents in positions comparable to those of certain C_{19} steroids, have been synthesised. When these C_{18} compounds, lacking the steroid angular methyl groups, are incubated with cultures of *Calonectria decora* and *Rhizopus nigricans*, the patterns of hydroxylation observed are closely analogous to those with the structurally related steroids.

MICROBIOLOGICAL hydroxylation of a large range of mono- and di-oxygenated C_{19} (and some C_{18}) steroids has been investigated [2-5] in order to ascertain the effect of varying the positions of the oxygen functions around the nucleus on the hydroxylation pattern. It is also of interest to study the extent to which the pattern depends upon the fairly precise shape and contours of the steroid nucleus. As a first step in this direction some *rac*-D-homogonanes (perhydrochrysenes, *e.g.* see the Figure), in which ring D is six- rather than five-membered and the angular methyl groups characteristic of the steroids are omitted, were synthesised and used as substrates.

When 5α-androstan-3-one is introduced into cultures of *Calonectria decora* [5] the steroid is dihydroxylated (52% yield) as indicated in the Figure; the corresponding αβ-unsaturated ketone (androst-4-en-3-one) is hydroxylated similarly but more rapidly. With the same organism the 3,12-dioxo-compound is mono-hydroxylated in the 15α-position in 37% yield.[4] With *Rhizopus nigricans*, 11α,16β-dihydroxylation (33%) is observed with the 3-ketone and the dioxygenated 11α-hydroxy-5α-androstan-3-one (Figure) undergoes smooth 16β-hydroxylation (53%). In all these instances the substituent is equatorial.

The results obtained under comparable conditions with the synthetic substrates are set out in Table 1; the most significant examples are illustrated in the Figure. (The syntheses of the substrates and the determination of the structures of the transformation products are described below.) With *C. decora* the positions, stereochemistry, and facility of the di- and mono-substitution with the first two substrates (**8**) and (**10**), which have considerable structural similarities to the normal steroid substrates (Figure), are exactly as might be expected. The dominant directing influence of the terminal ring carbonyl group is clearly seen and the distance between the two carbon atoms which are substituted (12–15) is 4·1 Å compared with 3·8 Å in the steroids. The $\Delta^{5(10)}$-ketone (5) and the enol ether are

[1] Formerly entitled Microbiological Hydroxylation of Steroids. Part XI, A. M. Bell, V. E. M. Chambers, Sir Ewart R. H. Jones, G. D. Meakins, W. E. Müller, and J. Pragnell, *J.C.S. Perkin I*, 1974, 312; for further details of the work described in this paper see M. J. Ashton, D.Phil. Thesis, Oxford, 1972.

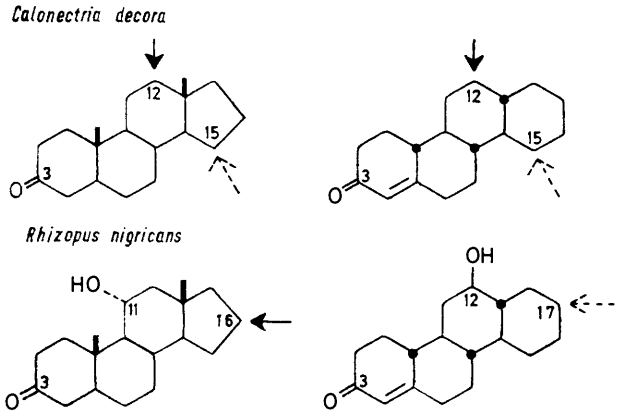
[2] Sir Ewart R. H. Jones, *Pure Appl. Chem.*, 1973, **33**, 39.

[3] A. M. Bell, J. W. Browne, W. A. Denny, Sir Ewart R. H. Jones, A. Kasal, and G. D. Meakins, *J.C.S. Perkin I*, 1972, 2930.

[4] A. M. Bell, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and W. E. Müller, *J.C.S. Perkin I*, 1972, 2579.

[5] A. M. Bell, P. C. Cherry, I. M. Clark, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and P. D. Woodgate, *J.C.S. Perkin I*, 1972, 2081.

probably hydroxylated after conversion into the αβ-unsaturated ketone (10) in the medium of pH 5·5. The 15α-substitution of the 4,12-diketone (15) is precisely in



Comparative hydroxylation behaviour of androstane and D-homogonane derivatives

line with our experience with the similarly substituted androstan-4-one. The further substitution (7α) in ring B is akin to that observed (6α) with androstan-3-one in the presence of dimethyl sulphoxide;[5] its axial orientation may be the result of inhibition of any equatorial approach to the 7-position by the 15α-hydroxy-group. Only a single substrate was used with *R. nigricans*, the 17α-substitution (equatorial—*cf.* 16β in steroids—Figure) being entirely in accord with expectations, the C(12)–C(17) distance (4·1 Å) being similar to the C(11)–C(16) distance (5·0 Å) in the steroids. The close agreement observed in the results described indicated that the changes in shape arising from ring D enlargement and the absence of the two angular methyl groups had little effect on the course of the hydroxylation process and so no more detailed exploration with closely related substrates seemed justifiable. More substantial skeletal variations in substrates have been investigated and a report of one of these is in the following paper.

TABLE 1

Microbiological hydroxylation of synthetic substrates

Substrate	Conditions	Substrate recovered (%)	Main product(s) (%) *	
Calonectria decora				
(8)	E4	30	(18)	27
(10)	E5	6	(18)	78
			(23)	17
(5)	E3	20	(18)	9
(12)	E5	29	3α-OH, 12-CO	30
(15)	E4	30	(21)	19
(4)	E3	11 †	(18)	5
Rhizopus nigricans				
(10)	E4	54	(25)	50
			(27)	4

* Yields based on unrecovered starting material. † Recovered as ketone (10).

In all these hydroxylation experiments with racemic substrates no formation of optically active products has been observed and in no case was the recovered starting material optically active. Resolution of *rac*-steroids by certain micro-organisms can be effected[6] and resolution of *rac*-19-norsteroids and 13β-alkylgonanes has been reported[7] but in general hydroxylating micro-organisms seem not to be highly substrate specific. As minor structural variations, of the kind examined in this paper, seem to be readily tolerated then differentiation between enantiomers whose general shapes would be similar apart from the angular methyl groups on one face would seem to be unlikely.

The ketone (1),[8] stable to sodium methoxide in boiling methanol, was obtained in good yield by the condensation of 6-methoxytetralone with 1-acetylcyclohexene under carefully controlled conditions; its *trans-transoid*-stereochemistry follows from the most favoured conformation of the transition state.[9] Palladium-catalysed hydrogenation of (1) yielded a mixture of hydrogenation and hydrogenolysis products (*cf.* the hydrogenation[10] of $\Delta^{1,9}$-octalin-2-one). The crude material was oxidised and poor yields of (3) and (6) were obtained. However, the required *trans*-dihydro-compound (3) was obtained in excellent yield by lithium–ammonia reduction.[11] Use of ethanol as a proton source yielded the alcohol (2) which was oxidised to the ketone (3); with ammonium chloride as proton source the ketone (3) was obtained directly. The *cis*-dihydroketone (6) results from selective[12] hydrogenation of the acetal (7) followed by hydrolysis.

Birch reduction of (3) using lithium and t-butyl alcohol–ether[13] yielded the enol ether (4) which was obtained directly from (1) using a lithium : substrate ratio of 40 : 1. The n.m.r. spectrum of the ether was consistent with this structure {$\tau = 5{\cdot}3$ [t, C(2)H[14]] and 6·3 [m, $W_{\frac{1}{2}}$ 18 Hz, C(12)H]}; the band-width shows C(12)H is axial[15] and hence the hydroxy-group is equatorial. The hydrolysis of the enol ether was examined under various conditions (see Experimental section). Perchloric acid–methanol gave the largest proportion of αβ-unsaturated material, but the most satisfactory method of obtaining (10) was by hydrolysing

[6] E. Vischer, J. Schmidlin, and A. Wellstein, *Experientia*, 1956, **12**, 50; W. S. Johnson, W. A. Vredenburgh, and J. E. Pike, *J. Amer. Chem. Soc.*, 1960, **82**, 3409.

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[8] W. S. Rapson and R. Robinson, *J. Chem. Soc.*, 1935, 1285.

[9] J. A. Marshall, H. Fanbl, and T. M. Warne, *Chem. Comm.*, 1967, 753; H. C. Odom and A. R. Pinder, *ibid.*, 1969, 26; R. E. Ireland, 'Organic Synthesis,' Prentice-Hall, Englewood Cliffs, N.J., 1969, pp. 101—103.

[10] R. L. Augustine, *J. Org. Chem.*, 1958, **23**, 1853; 1969, **34**, 1075; R. L. Augustine and D. L. Brown, *ibid.*, 1960, **25**, 802.

[11] W. F. Johns, *J. Org. Chem.*, 1963, **28**, 1856.

[12] J. E. Cole, W. S. Johnson, P. A. Robins, and J. Walker, *J. Chem. Soc.*, 1962, 244.

[13] H. L. Dryden, G. M. Webber, R. R. Burtner, and J. A. Cella, *J. Org. Chem.*, 1961, **26**, 3237.

[14] F. Bohlmann, C. Arndt, and J. Starnick, *Tetrahedron Letters*, 1963, 1605.

[15] J. E. Bridgeman, P. C. Cherry, A. S. Clegg, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, Y. Morisawa, E. E. Richards, and P. D. Woodgate, *J. Chem. Soc.* (*C*), 1970, 250.

(4) to (5) and then isomerising (5) to (10) using sodium ethoxide, this route avoiding a chromatographic separation of (10). The stereochemistry of (10) follows from its method of formation *via* (5).[16] Reduction of (10) yielded the diol (11) which, on oxidation, gave the dione (12).

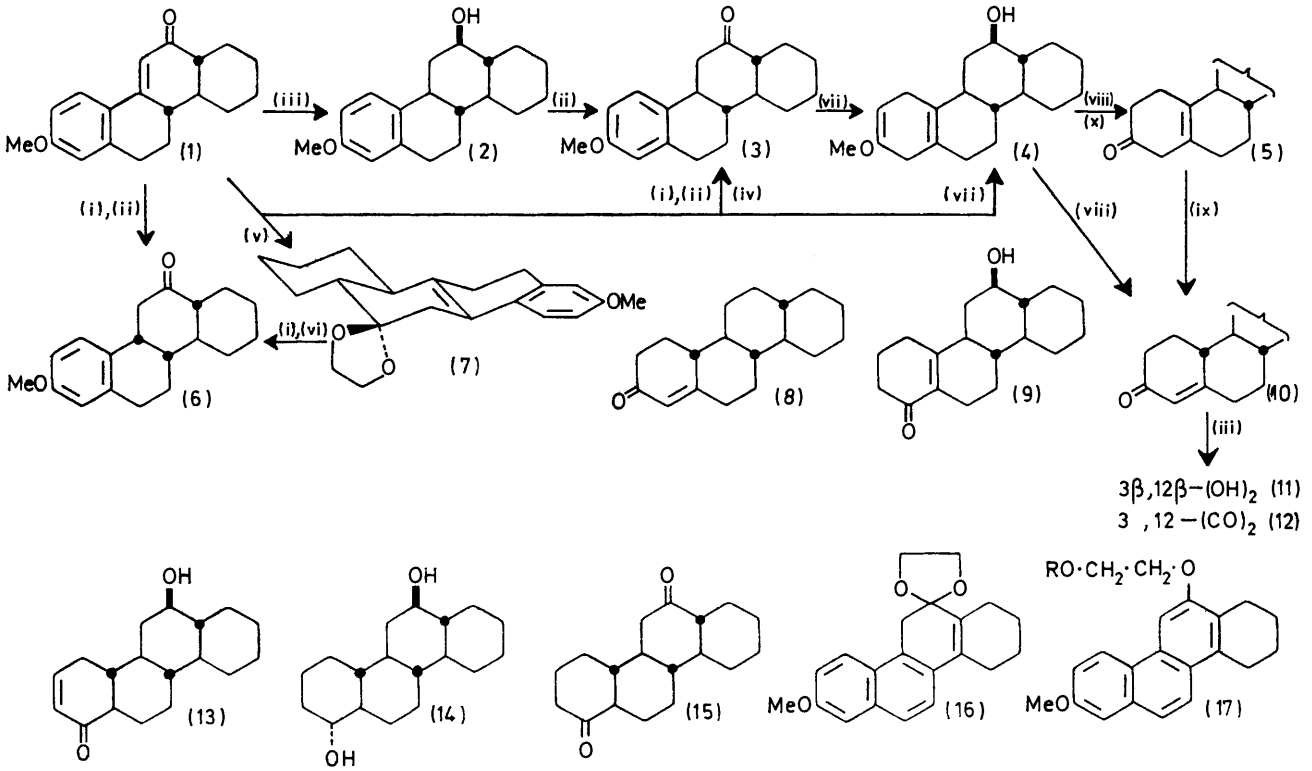
Huang-Minlon reduction of (3) afforded a mixture [12,17] of the corresponding deoxy derivative and the derived phenol; re-methylation gave the pure ether which was converted by the usual route into the unsaturated ketone (8).

Following the same route, but using 5-methoxytetralone, compound [(2), with OMe at 4-position] was prepared.

absence of the isomeric ketone (13). Reduction of (9) yielded the diol (14) which was oxidised to the diketone (15).

From the reaction between ethylene glycol and (1) to give the acetal (7) a minor product was isolated to which we assign structure (16). The compound did not contain OH or CO groups (i.r.) and in the mass spectrum the molecular ion (*m/e* 322) was the base peak. Treatment of (16) with formic acid did not hydrolyse the acetal group to form the phenol but gave the formyl ester (17; R = CHO) which was hydrolysed to the corresponding alcohol (17; R = H).

Incubation experiments were carried out using two micro-organisms, *Calonectria decora* and *Rhizopus*



Reagents: (i) H_2–Pd/C; (ii) H_2CrO_4–Me_2CO; (iii) Li–NH_3–EtOH; (iv) Li–NH_3–NH_4Cl; (v) $HOCH_2CH_2OH$–TsOH; (vi) HCl–MeOH; (vii) Li–NH_3–Bu^{t}OH; (viii) $HClO_4$–MeOH; (ix) NaOEt; (x) oxalic acid; (xi) NaOH aq; (xii) DDQ.

The compounds described in this paper are all racemates; an arbitrary choice has been made of one enantiomer for illustration.

5-Methoxytetralins are unaffected [16a,18] by metal–ammonia reduction under conditions which reduce 6-methoxytetralins. However, by using a large excess of lithium and maintaining a bronze-coloured phase [19] in the liquid ammonia we succeeded in reducing the aromatic ring of this ether. The crude material was treated with hydrochloric acid and the unsaturated ketone (9) isolated by chromatography (yield 26%). The n.m.r. spectrum of the compound contained a signal at τ 6·65 ($W_{\frac{1}{2}}$ 22 Hz) indicating that C(12)H is axial and there were no signals in the vinyl-proton region showing

[16] (*a*) A. L. Wilds and N. A. Nelson, *J. Amer. Chem. Soc.*, 1953, **75**, 5730; (*b*) S. Anachenko, V. M. Rzhenznikov, N. N. Leonov, and I. V. Torgov, *Bull. Acad. Sci. U.S.S.R., Div. Chem. Soc.*, 1961, **10**, 1789; (*c*) A. J. Birch and H. Smith, *J. Chem. Soc.*, 1956, 4909.

nigricans; the results are summarised in the Table and the evidence for the structures follows.

Compound (18) was shown to be a dihydroxy-αβ-unsaturated ketone. The u.v. spectrum of the compound was unaffected by alkali, indicating the new hydroxy-group was not at positions 1, 2, 6, or 7; the n.m.r. signal at τ 6·48 ($W_{\frac{1}{2}}$ 25 Hz) indicated two equatorial hydroxy-groups. Reduction of (18) followed by oxidation afforded the trione (19). The mass spectrum of this compound indicated clearly that the new CO group was at C(15). The molecular ion was of moderate

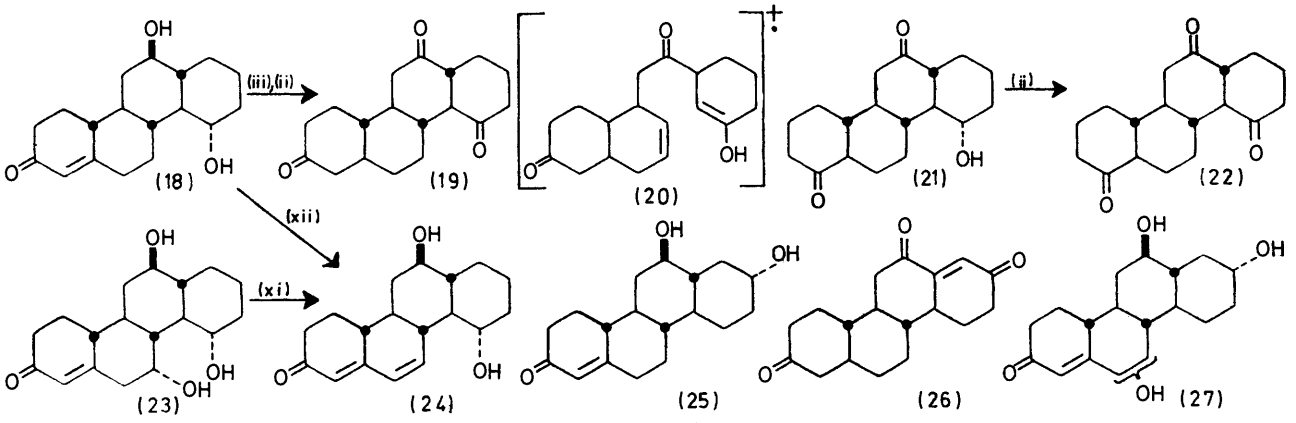
[17] M. Gates and W. G. Webb, *J. Amer. Chem. Soc.*, 1958, **80**, 1186.

[18] A. J. Birch, *J. Chem. Soc.*, 1944, 430.

[19] W. S. Johnson, B. Bannister, and R. Pappo, *J. Amer. Chem. Soc.*, 1956, **78**, 6331.

intensity, *m/e* 288 (37%), 260 (17) (*M* − CO). Three peaks arise by McLafferty rearrangement involving C(7)H and cleaving 8–14 to give an ion (20); fragmentation of this yields *m/e* 97 (100%, C_6H_9O), 124 (9, $C_7H_8O_2$), 149 (6, $C_{10}H_{13}O$), and 164 (11, $C_{11}H_{16}O$). There was also a significant peak *m/e* 205 (20%, $C_{13}H_{17}O_2$) characteristic of the cleavage of a 1,6-dioxosteroid.[20] By contrast, in the mass spectrum of the dione (12) the molecular ion was the base peak and only one peak stronger than 10% [*m/e* 109 (15%)] was observed.

The n.m.r. spectrum of (23) indicated 12β- and 15α-hydroxy-groups [τ 6·45 (2H, $W_{\frac{1}{2}}$ 24 Hz)] and a signal τ 5·65 ($W_{\frac{1}{2}}$ 9 Hz) suggested an axial hydroxy-group close to the αβ-unsaturated ketonic group. On treatment with base at room temperature the u.v. spectrum of the compound changed significantly, $\lambda_{max.}$ 241 (ε 11,500) ⟶ 282 nm (ε 29,500), the O.D. *vs.* time plots showing an isosbestic point. The dehydration product (24) was oxidation with selenium dioxide yielded a product (26) which contained a typical ene-dione chromophore [$\lambda_{max.}$ 254 (ε 10,500)]. The tentative structure (27) is assigned to the minor product obtained from *R. nigricans*. Only 9 mg of material were obtained and we assume that the compound is obtained by further hydroxylation of (25). *R. nigricans* is known[21] to hydroxylate C(6) and C(7). In the n.m.r. spectrum of the material the two C*H*OH signals we assign to C(12) and C(13) appear at τ 6·44 (m, $W_{\frac{1}{2}}$ 24 Hz) and the new C*H*OH signal appears at much lower field [τ 5·65 ($W_{\frac{1}{2}}$ 9 Hz)].



Reagents as above.

EXPERIMENTAL

Instruments used and general experimental conditions have been reported.[3] Standard isolation of neutral products involved treating the reaction mixture with water, extracting with a suitable solvent, and washing the extract with dilute acid and with alkali. After drying with magnesium sulphate the solvent was removed *in vacuo*. ' Oxidation ' implies chromic acid–acetone unless otherwise stated and acetylations were carried out using pyridine–acetic anhydride at 0° for 15 h. T.l.c. and p.l.c. were carried out using unbaked kieselgel $PF_{254/366}$ plates; petrol refers to fraction b.p. 60—80°. All compounds described are racemic.

*3-Methoxy-*D*-homogona-1,3,5(10),9(11)-tetraen-12-one* (1). —The experimental conditions are critical. Na (2·3 g) was dissolved in *anhydrous* NH_3 (500 ml), distilled from Na and containing a trace of $Fe(NO_3)_3$. After 1 h the NH_3 was evaporated in N_2, dry Et_2O (500 ml) being added simultaneously. The $NaNH_2$ suspension was boiled and stirred for 0·5 h to remove traces of NH_3. 6-Methoxytetralone (17·6 g) in Et_2O (50 ml) was added, the mixture was stirred under reflux for 12 h (N_2) and then at 0°, 1-acetylcyclohexene[22] (13·2 g) was slowly added. Next day the solid (19·7 g) was collected, washed with dilute H_2SO_4, H_2O, and with EtOH (20 ml). Crystallisation from 1,3-dimethoxypropane gave the ketone (I) (19·1 g), needles, m.p. 229—230° (lit.,[8] 230°) (Found: C, 80·5; H, 7·9. Calc. for $C_{19}H_{22}O_2$: C, 80·8; H, 7·9%); $\nu_{max.}$ (N) 1595s, 1610w, and 1645s cm^{-1}; $\lambda_{max.}$ 242 and 327 nm (ε 7500 and 17,000);

[20] R. T. Aplin and P. C. Cherry, *Chem. Comm.*, 1966, 628.
[21] J. M. Evans, E. R. H. Jones, A. Kasal, V. Kumar, G. D. Meakins, and J. Wicha, *Chem. Comm.*, 1969, 1491.
[22] J. Chanley, *J. Amer. Chem. Soc.*, 1948, **70**, 246.

τ ($CDCl_3$) 2·27 [1H, d, *J* 8·7 Hz, C(1)H], 3·23 [1H, q, *J* 8·7 and 3 Hz, C(2)H], 3·3 [1H, d, *J* 3 Hz, C(4)H], 3·45 [1H, s, C(11)H], 6·18 (3H, s, OMe), and 7·0—9·0 (15H); *m/e* 282 (M^+, 100%), 239 (17), 200 (89), and 185 (48), *m** 142 (282 ⟶ 239).

When undried ammonia was used a brown solid (13·5 g) was obtained. Chromatography on silica gave 6-methoxytetralone (8·95 g) and self-condensation products of 1-acetylcyclohexene.[23] From the ethereal mother-liquors some of the dihydro-compound (3) (2·7 g) (see below) was isolated.

Hydrogenation of (1).—The compound (1) (1·0 g) in AcOEt (200 ml) was hydrogenated at 20° (Pd–C, 10%; 400 mg; 3 atm). Oxidation gave product (0·75 g; m.p. 135—151°) and then by fractional crystallisation afforded trans-transoid-trans-3-*methoxy*-D-*homogona*-1,3,5(10)-*trien*-12-*one* (3) (100 mg), needles (from MeOH), m.p. 153—155° (Found: C, 80·4; H, 8·6. $C_{19}H_{24}O_2$ requires C, 80·2; H, 8·5%); $\nu_{max.}$ ($CHCl_3$) 1609s and 1705s cm^{-1}; $\lambda_{max.}$ 228, 280, and 287 nm (ε 5300, 1400, and 1250); τ ($CDCl_3$) 2·81 [1H, d, *J* 8·7 Hz, C(1)H], 3·28 [1H, q, *J* 8·7 and 3·0 Hz, C(2)H], 3·36 [1H, d, *J* 3 Hz, C(4)H], 6·23 (3H, s, OMe), and 7·1—9·0 (18H); *m/e* 284 (M^+, 100%), 199 (24), 173 (38), 171 (19), and 160 (20). The more soluble cis-transoid-trans-*ketone* (6) (80 mg) formed needles (from MeOH), m.p. 146—147° (Found: C, 80·3; H, 8·7. $C_{19}H_{24}O_2$ requires C, 80·2; H, 8·5%); $\nu_{max.}$ ($CHCl_3$) 1610 and 1705 cm^{-1}; $\lambda_{max.}$ 228, 277, and 286 nm (ε 5850, 1800, and 1650); τ ($CDCl_3$) 2·87 [1H, d, *J* 8·8 Hz, C(1)H], 3·31 [1H, q, *J* 8·8 and 3 Hz, C(2)H], 3·37 [1H, d, *J* 3 Hz, C(4)H], 6·23 (3H, s, OMe), and 7·0—8·9 (18H); *m/e* 284 (M^+, 100%), 240 (15), 199 (27), and 173 (39). The separation was followed by g.l.c., R_t (3) 17 min, R_t (6) 20 min, gas flow 40 ml min^{-1}, *T* 215°, 2% polyethylene glycol adipate column, Pye 104 instrument.

3-*Methoxy*-D-*homogona*-1,3,5(10)-*trien*-12β-*ol* (2).—A slurry of the ketone (1) (4·5 g) in Et_2O (25 ml) was poured into NH_3 (500 ml; distilled from sodium) containing Et_2O (115 ml). Li (360 mg), and 3 h later, EtOH (10 ml) was added slowly. This gave *compound* (2) (3·95 g) as needles (from MeOH), m.p. 132—134° (Found: C, 79·9; H, 9·3. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·15%); $\nu_{max.}$ (CCl_4) 1610 and 3625 cm^{-1}; $\lambda_{max.}$ 222, 279, and 288 nm (ε 5250, 1500, and 1500); τ ($CDCl_3$) 2·29 [1H, d, *J* 9 Hz, C(1)H], 3·20 [1H, q, *J* 9 and 3 Hz, C(2)H], 3·30 [1H, d, *J* 3 Hz, C(4)H], 6·20 (3H, s, OMe), 6·85 (1H, m, $W_{\frac{1}{2}}$ 21 Hz, C*H*OH), 7·3 [m, C(6)H_2 and C(9)H], and 7·8—9·0 (16H); *m/e* 286 (M^+, 30%), 268 (100), and 225 (8). Oxidation of this alcohol (4 g) gave ketone (3) (3·8 g), needles (from MeOH), m.p. and mixed m.p. 153—154°. When the above Li reduction of (1) (6 g) was repeated replacing the EtOH by NH_4Cl (10 g) the ketone (3) (5·7 g) was obtained directly.

Reaction of (1) *with Ethylene Glycol*.—A solution of the ketone (1 g) in benzene (180 ml) containing ethylene glycol (1·5 ml) and $TsOH,H_2O$ (100 mg) was boiled under reflux (Dean–Stark) for 14 h. Chromatography (silica; Et_2O–light petroleum 1 : 9) yielded the *acetal* (7), small needles, m.p. 104—105° (845 mg) (petrol) (Found: C, 77·3; H, 8·0. $C_{21}H_{26}O_3$ requires C, 77·3; H, 8·0%); $\nu_{max.}$ (CCl_4) 1462 cm^{-1}; $\lambda_{max.}$ 274 nm (ε 16,000); τ ($CDCl_3$) 2·95 [1H, d, *J* 8·3 Hz, C(1)H], 3·3 [1H, q, *J* 8·3 and 2·8 Hz, C(2)H], 3·35 [1H, d, *J* 2·8 Hz, C(4)H], 6·03 (4H, m, OCH_2CH_2O), 6·21 (3H, s, OMe), 7·3 [m, C(6)H_2 and C(11)H_2], and 7·6—8·8 (12H); *m/e* 326 (M^+, 100%), 265 (75), and 254 (12). Elution with Et_2O–petrol (1 : 4) gave starting material (30 mg) and Et_2O–petrol (1 : 1) gave 12,12-*ethylenedioxy*-3-*methoxy*-D-*homogona*-1,3,5(10),6,8(9),13(14)-*hexaene* (16) (89 mg), needles (from MeOH), m.p. 170—171° (Found: C, 78·3; H, 6·8. $C_{22}H_{22}O_3$ requires C, 78·2; H, 6·9%); $\nu_{max.}$ (N) 1602 and 1625 cm^{-1}; $\lambda_{max.}$ 221, 223, 257, 273, 285, 313, 339, and 353 nm (ε 30,000, 28,000, 43,000, 31,500, 22,500, 9150, 500, and 550); τ ($CDCl_3$) 1·5 [1H, d, *J* 9·9 Hz, C(6)H], 2·12 [1H, d, *J* 9·9 Hz, C(1)H], 2·24 [1H, d, *J* 3·0 Hz, C(4)H], 2·45 [1H, d, *J* 9·9 Hz, C(7)H], 2·78 [1H, q, *J* 9·9 and 3·0 Hz, C(2)H], 5·80 (4H, m, OCH_2CH_2O), 6·06 (3H, s, OMe), 6·85 [2H, s, C(11)H_2], 7·18 [4H, m, C(15)H_2 and C(17a)H_2], and 8·1 [4H, m, C(16)H_2 and C(17)H_2]; *m/e* 322 (M^+, 100%) and 278 (47%), *m** 240 (322 ⟶ 278).

The acetal (7) (660 mg) was hydrogenated (Pd–C, 10%; 1 atm, ethanol, room temp.). P.l.c. (EtOAc) gave an oil (R_F 0·6) (560 mg.) This was dissolved in light petroleum and the solution cooled to −80° and the solvent removed from the solid by pipette. This was repeated three times giving 12,12-*ethylenedioxy*-3-*methoxy*-D-*homogona*-1,3,5(10)-*triene* as an oil (539 mg) (Found: C, 77·5; H, 7·2. $C_{21}H_{24}O_3$ requires C, 77·8; H, 7·5%); $\nu_{max.}$ (CCl_4) 1450 and 1460 cm^{-1}; τ (CCl_4) 2·96 [1H, d, *J* 9·0 Hz, C(1)H], 3·47 [1H, q, *J* 9·0 and 3·0 Hz, C(2)H], 3·54 [1H, d, *J* 3 Hz, C(4)H], 6·12 (4H, m, CH_2CH_2O), 6·29 (3H, s, OMe), and 7·0—9·0 (18H); *m/e* 328 (M^+, 100%) and 267 (50). This acetal (500 mg) was hydrolysed (MeOH–HCl) to yield the ketone (6) (479 mg), needles from MeOH, m.p. and mixed m.p. 146—147°, identical with material described earlier (i.r., g.l.c.).

Compound (16) (160 mg) was dissolved in HCO_2H (20 ml; 90%). After 3 h the solution was poured into water. P.l.c. of the product [Et_2O–hexane (1 : 1), 2 elutions] gave 12-(2-*formyloxyethoxy*)-8-*methoxy*-1,2,3,4-*tetrahydrochrysene* (17; R = CHO) (130 mg), blades, m.p. 178—179°, from Et_2O–light petroleum (Found: C, 75·1; H, 6·5. $C_{22}H_{24}O_4$ requires C, 75·4; H, 6·3%); $\nu_{max.}$ (CCl_4) 1665w and 1735 cm^{-1}; $\lambda_{max.}$ 257, 273, 285, 313, 339, and 353 nm (ε 31,400, 12,200, 10,200, 4400, 790, and 550);[24] τ (CS_2) 2·03 (1H, s, O·CO·H), 1·8—3·40 (5H, m, Ar), 3·42 [1H, s, C(11)H], 5·65 (2H, t, *J* 6 Hz, Ar·O·CH_2), 5·84 (2H, t, *J* 6 Hz, ·CH_2·O·CH), 6·29 (3H, s, OMe), 7·39 [4H, m, C(1)H_2 and C(4)H_2], and 8·27 [4H, m, C(2)H_2 and C(3)H_2]; *m/e* 350 (M^+, 100%), 278 (93), and 276 (50). Hydrolysis of the formyl ester (100 mg) with cold aqueous alcoholic alkali gave 12-(2-*hydroxyethoxy*)-8-*methoxy*-1,2,3,4-*tetrahydrochrysene* (17; R = H), needles (84 mg), m.p. 179—181°, from aqueous MeOH (Found: C, 78·3; H, 6·7. $C_{21}H_{22}O_3$ requires C, 78·2; H, 6·9%); $\nu_{max.}$ ($CHCl_3$) 1615w, 3450m, and 3625w cm^{-1}; $\lambda_{max.}$ 257, 273, 285, 313, 339, and 353 nm (ε 27,300, 18,700, 12,400, 5000, 840, and 620); τ ($CDCl_3$) 1·8—3·4 (5H, m, Ar), 3·44 [1H, s, C(11)H], 5·66 (2H, t, *J* 6 Hz, ArO·CH_2), 5·88 [2H, t, *J* 6 Hz, CH_2OH], 7·40 [4H, m, C(1)H_2 and C(4)H_2], and 8·27 [4H, m, C(2)H_2 and C(3)H_2]; *m/e* 322 (M^+, 100%), 278 (18), 277 (17), and 250 (14).

3-*Methoxy*-D-*homogona*-2,5(10)-*dien*-12β-*ol* (4).—(*a*) A slurry of the ketone (3) (2 g) in Et_2O–Bu^tOH (1 : 1; 80 ml) was added to anhydrous NH_3 (400 ml) containing Et_2O–Bu^tOH (320 ml). After stirring for 20 min Li (1 g) was added in small portions. After refluxing for 5 h the NH_3 was allowed to evaporate. Next day the residue was stirred for 1 h with EtOH (100 ml) and poured into water. Isolation ($CHCl_3$, neutral conditions) gave an oil which crystallised on trituration with petroleum. The solid was recrystallised from petroleum (b.p. 60—80°) yielding the

[23] E. R. H. Jones and H. P. Koch, *J. Chem. Soc.*, 1942, 393; E. R. Clark, *ibid.*, 1959, 2345.

[24] G. N. Walker, *J. Amer. Chem. Soc.*, 1958, **80**, 645.

enol ether (4) (1·65 g), needles, m.p. 130—131°, from aqueous MeOH (Found: C, 78·9; H, 9·6. $C_{19}H_{28}O_2$ requires C, 79·1; H, 9·8%); $\nu_{max.}$ (CCl_4) 1668w, 1698m, and 3600m cm^{-1}; τ ($CDCl_3$) 5·27 [1H, t, *J* 3 Hz, C(2)H], 6·24 (3H, s, OMe), 6·85 [1H, m, $W_{\frac{1}{2}}$ 18 Hz, C(12)H], and 7·0—9·0 (23H, m); *m/e* 288 (M^+, 47%), 270 (33), 242 (33), 122 (66), and 41 (100).

(*b*) The unsaturated ketone (1) (4 g) was reduced using NH_3 (800 ml), Et_2O–Bu^tOH (320 ml), and Li (4 g); compound (4), m.p. and mixed m.p. 130—131° (3·3 g), was obtained.

12β-*Hydroxy*-D-*homogon*-5(10)-*en*-3-*one* (5).—The enol ether (4) (2 g) was dissolved in EtOH (250 ml) and a solution of oxalic acid (3 g) in water (20 ml) was added. After 3 h the solution was diluted with water; isolation ($CHCl_3$) gave an oil which crystallised on trituration with hexane. Recrystallisation from Me_2CO–hexane yielded the *unsaturated ketone* (5) as needles (1·69 g), m.p. 144—145°. The analytical sample was recrystallised from aqueous MeOH (m.p. 145—146°) (Found: C, 78·8; H, 9·5. $C_{18}H_{26}O_2$ requires C, 78·8; H, 9·6%); $\nu_{max.}$ ($CHCl_3$) 1712s and 3450m cm^{-1}; τ ($CDCl_3$) 6·95 [1H, m, $W_{\frac{1}{2}}$ 18 Hz, C(12)H] and 7·26—9·0 (25H, m); *m/e* 274 (M^+, 10%), 256 (100), and 214 (15).

12β-*Hydroxy*-D-*homogon*-4-*en*-3-*one* (10).—The enol ether (4) (1 g) was dissolved in MeOH (250 ml) containing conc. HCl (2 ml) and H_2O (3 ml) and the solution was heated under reflux (N_2 atmosphere) for 3 h. The solvent was removed *in vacuo* and isolation (Et_2O) gave an oil which was chromatographed on silica (50 g). Elution with PhH–Et_2O mixtures (20 : 1—10 : 1) gave (5) (375 mg), m.p. and mixed m.p. 144—145°. Benzene–Et_2O (5 : 1) eluted the αβ-*unsaturated ketone* (10) (380 mg), m.p. 150—152°. The analytical sample (aqueous MeOH) had m.p. 152—153° (Found: C, 78·6; H, 9·5. $C_{18}H_{26}O_2$ requires C, 78·8; H, 9·6%); $\nu_{max.}$ ($CHCl_3$) 1618, 1666s, 3445, and 3600w cm^{-1}; $\lambda_{max.}$ 243 nm (ε 15,000); τ ($CDCl_3$) 4·14 [1H, s, C(4)H], 6·54 [1H, m, $W_{\frac{1}{2}}$ 24 Hz, C(12)H], and 7·4—9·0m (24H); *m/e* 274 (M^+, 21%), 256 (43), 228 (18), 147 (40), and 110 (100).

The reaction was carried out under different conditions and results are summarised in Table 2; yields are of material isolated.

TABLE 2

Treatment of the enol ether (4) in acid

Wt. of ether (g)	Acid	Solvent [volume (ml)]	Time of reflux (h)	Yield (%) (5)	(10)
1	HCl	MeOH (250)	3	40	38
1	HCl	MeOH (250)	5	11	32
0·08	H_2SO_4 (6 ml; 5M)	MeOH (25)	2	19	60
0·1	Oxalic (400 mg)	Dioxan (20) + water (3)	27	16	70
0·1	Perchloric (0·3 ml; 30%)	MeOH (10)	2	5	84

The βγ-unsaturated ketone (5) (2·09 g) was dissolved in EtOH (200 ml) and N_2 passed through the solution for 30 min. A solution of Na (1·68 g) in EtOH (50 ml) was added. Next day HOAc (2 ml) was added and isolation ($CHCl_3$) gave an oil which crystallised on trituration with hexane yielding the ketone (10), m.p. 151—152° (1·3 g). The material (0·5 g) from the mother liquors was separated (p.l.c.) into (5) (35 mg) and (10) (200 mg) and unidentified non-polar materials.

D-*Homogonane*-3β,12β-*diol* (11).—The ketone (10) (2 g) was reduced (Li–NH_3–EtOH) yielding a solid (1·9 g). Recrystallisation from tetrahydrofuran–hexane gave needles (1·8 g), m.p. 212—214°. The *diol* formed large needles, m.p. 214—215°, from aqueous MeOH (Found: C, 77·6; H, 10·6. $C_{18}H_{30}O_2$ requires C, 77·7; H, 10·9%); $\nu_{max.}$ (N) 3460s cm^{-1}; τ ($CDCl_3$) 6·4 [2H, m, $W_{\frac{1}{2}}$ 20 Hz, C(3)H and C(12)H] and 7·0—9·0 (28H, m); *m/e* 278 (M^+, 1%), 260 (41), and 242 (100). The diol was oxidised and the crude material purified by passing an Me_2CO–hexane (3 : 7) solution through alumina (yield 78%). Recrystallisation (Me_2CO–hexane) yielded D-*homogonane*-3,12-*dione* (12), needles, m.p. 157—158° (Found: C, 78·4; H, 9·3. $C_{18}H_{26}O_2$ requires C, 78·8; H, 9·6%); $\nu_{max.}$ ($CHCl_3$) 1711 cm^{-1}; *m/e* 274 (M^+, 100%), 256 (9), and 124 (7).

3-*Methoxy*-D-*homogona*-1,3,5(10)-*triene*.—The ketone (3) (250 mg) was reduced (Huang-Minlon, total reaction time 7 h, solvent diethylene glycol). The resulting oil was dissolved in hexane and next day the phenol (44 mg) was collected. 3-*Hydroxy*-D-*homogona*-1,3,5(10)-*triene* formed large needles, m.p. 98—99°, from MeOH (Found: C, 84·0; H, 9·3. $C_{18}H_{24}O$ requires C, 84·3; H, 9·4%); $\nu_{max.}$ (CCl_4) 1612 and 3620s cm^{-1}; τ (CCl_4) 2·8 [1H, d, *J* 8·9 Hz, C(1)H], 3·38 [1H, q, *J* 8·9 and 2·8 Hz, C(2)H], 3·41 [1H, d, *J* 2·8 Hz, C(4)H], 5·35 (1H, s, ArO*H*), and 7·0—9·0 (20H, m); $\lambda_{max.}$ 223 and 287 nm (ε 3170 and 785), (EtOH–NaOH) 247 and 300 (ε 4100 and 1300); *m/e* 256 (M^+, 100%), 228 (12), and 185 (22). The residue from hexane was chromatographed (p.l.c.; PhH–EtOAc, 3 : 1). The band (R_F 0·49) yielded the *title ether* (81 mg), needles, m.p. 82—83°, from aqueous MeOH (Found: C, 84·3; H, 9·5. $C_{19}H_{26}O$ requires C, 84·4; H, 9·7%); $\nu_{max.}$ (CCl_4) 1608 and 3010w cm^{-1}; $\lambda_{max.}$ 223, 279, and 288 nm (ε 7500, 1990, and 1950); τ (CCl_4) 2·90 [1H, d, *J* 8·6 Hz, C(1)H], 3·45 [1H, q, *J* 8·6 and 2·7 Hz, C(2)H], 3·51 [1H, d, *J* 2·7 Hz, C(4)H], 6·28 (3H, s, OMe), and 7·0—9·0 (20H, m), *m/e* 270 (M^+, 100%), 199 (36), and 173 (43). The above reaction was repeated and the crude material methylated, yielding the ether directly.

D-*Homogon*-4-*en*-3-*one* (8).—The foregoing ether (2·4 g) was reduced (Li–NH_3–Bu^tOH) and the product (2·35 g) dissolved in petrol and cooled to −30°. After 72 h the solid was collected and recrystallised (petrol) at low temp. 3-*Methoxy*-D-*homogona*-2,5(10)-*diene* formed cubes, m.p. 56—59° (Found: C, 83·8; H, 10·4. $C_{19}H_{28}O$ requires C, 83·8; H, 10·4%); $\nu_{max.}$ (CCl_4) 1665w and 1695 cm^{-1}; τ ($CDCl_3$) 5·27 [1H, t, *J* 3 Hz, C(2)H], 6·42 (3H, s, OMe), and 7·0—9·0 (22H, m); *m/e* 272 (M^+, 90%), 244 (27), and 122 (100). The ether was hydrolysed with oxalic acid (room temp., 3 h) yielding D-*homogon*-5(10)-*en*-3-*one*, needles, m.p. 45—47°, from petroleum (−30°) (Found: C, 83·8; H, 10·1. $C_{18}H_{26}O$ requires C, 83·7; H, 10·1%); $\nu_{max.}$ (CCl_4) 1725 cm^{-1}; τ (CCl_4) 6·9—7·6 [4H, m, C(2)H_2 and C(4)H_2], and 7·7—9·0 (22H, m); *m/e* 258 (M^+, 100%), 200 (65), 188 (80), 162 (92), and 148 (85). This ketone (500 mg) was heated (1 h) in MeOH (10 ml) containing conc. HCl (1 ml). P.l.c. of the product (Me_2O–hexane, 1 : 5) gave recovered βγ-unsaturated ketone (140 mg), R_F 0·59. and the desired product (308 mg), R_F 0·48. The αβ-*unsaturated ketone* (8) formed needles (287 mg), m.p. 46—48° (petrol, −30°) (Found: C, 83·3; H, 10·15. $C_{18}H_{26}O$ requires C, 83·7; H, 10·1%); $\nu_{max.}$ (CCl_4) 1618w and 1678 cm^{-1}; $\lambda_{max.}$ 241 nm (ε 15,400), τ (CCl_4) 4·25 [1H. s, C(4)H] and 6·0—9·0 (25H, m); *m/e* 258 (M^+, 40%), 148 (91), 123 (43), and 110 (100).

D-*Homogonan*-3-*one*.—The ketone (8) (200 mg) was

reduced (Li–NH_3–EtOH) and the crude product oxidised. P.l.c. (Et_2O–petrol, 1 : 2) and recrystallisation from petrol gave the *saturated ketone* as cubes (115 mg), m.p. 86—87° (Found: C, 82·8; H, 10·7. $C_{18}H_{28}O$ requires C, 83·0; H, 10·8%); $\nu_{max.}$ (CCl_4) 1706s cm^{-1}; *m/e* 262 (M^+, 100%), 244 (10), 147 (81), and 110 (91).

4-*Methoxy*-D-*homogona*-1,3,5(10),9(11)-*tetraen*-12-*one*.— 5-Methoxytetralone (22 g) was condensed with 1-acetylcyclohexene yielding the *unsaturated ketone*, rhomboids (19·2 g), m.p. 168—170°, from 1,2-dimethoxyethane (Found: C, 80·4; H, 7·9. $C_{19}H_{22}O_2$ requires C, 80·8; H, 7·9%); $\nu_{max.}$ (N) 1579s, 1610w, and 1654s cm^{-1}; $\lambda_{max.}$ 242 and 297 nm (ε 9000 and 18,800); τ ($CDCl_3$) 2·62 [1H, q, *J* 8·0 and 1·9 Hz, C(1)H], 2·81 [1H, t, *J* 8·0 Hz, C(2)H], 3·14 [1H, q, *J* 8·0 and 1·9 Hz, C(3)H], 3·4 [1H, s, C(11)H], 6·17 (3H, s, OMe), 6·7—6·9 [2H, m, $C(6)H_2$], and 7·0—9·0 (14H, m); *m/e* 282 (M^+, 100%), 239 (14), and 200 (70), *m** 203 (282 ⟶ 239) and 142 (282 ⟶ 200). 5-Methoxytetralone (1·9 g) was recovered. The ketone was unaffected by NaOMe in MeOH.

4-*Methoxy*-D-*homogona*-1,3,5(10)-*trien*-12-*one*.—Reduction of the unsaturated ketone (14 g) (Li–NH_3–EtOH) yielded 4-*methoxy*-D-*homogona*-1,3,5(10)-*trien*-12β-*ol*, needles, m.p. 187—188° (13·1 g), from aqueous MeOH (Found: C, 79·9; H, 9·0. $C_{19}H_{26}O_2$ requires C, 79·7; H, 9·2%); $\nu_{max.}$ (N) 1588 and 3400 cm^{-1}; $\lambda_{max.}$ 221, 272, and 281 nm (ε 8400, 1600, and 1700); τ ($CDCl_3$) 2·88 [1H, t, *J* 8 Hz, C(2)H], 3·10 [1H, q, *J* 8 and 1·8 Hz, C(1)H], 3·27 [1H, q, *J* 8 and 1·8 Hz, C(3)H], 6·21 (3H, s, OMe), 6·58 (1H, m, $W_{\frac{1}{2}}$ 23 Hz, C*H*OH), and 7·0—9·0 (19H, m); *m/e* 286 (M^+, 100%) and 268 (10), *m** 251 (286 ⟶ 268). Oxidation of this alcohol (10 g) gave the *ketone* (9·7 g), m.p. 169—171°, from MeOH (Found: C, 80·3; H, 8·5. $C_{19}H_{24}O_2$ requires C, 80·2; H, 8·5%); $\nu_{max.}$ (N) 1592 and 1711s cm^{-1}; $\lambda_{max.}$ 221, 273, and 280 nm (ε 7950, 1530, and 1580); τ ($CDCl_3$) 2·88 [1H, t, *J* 8 Hz, C(2)H], 3·21 [1H, q, *J* 8·0 and 1·7 Hz, C(3)H], 3·32 [1H, q, *J* 8·0 and 1·7 Hz, C(4)H], 6·20 (3H, s, OMe), and 6·8—9·0 (18H); *m/e* 284 (M^+, 100%).

D-*Homogonane*-4,12-*dione* (15).—The foregoing ketone (7·6 g) was reduced under ' forcing conditions ' [NH_3 (1 l), EtOH (600 ml), Li (50 g)] maintaining a ' bronze phase ' by adding NH_3 and EtOH as required. Trituration of the product with Et_2O–light petroleum (1 : 1) removed the alcohol formed by reduction of the C=O and C=C bonds. The gum (6·4 g) was boiled with HCl–MeOH (3 h) and the product was chromatographed on silica (250 g). Elution with Me_2CO–petrol (1 : 50, 1 l) gave hydrogenolysis products (2·62 g); Me_2CO–petrol (1 : 16, 1 l) yielded more alcohol (1·0 g); Me_2CO–petroleum (1 : 10 and 1 : 5, 2 l) gave 12β-*hydroxy*-D-*homogon*-5(10)-*en*-4-*one* (9) (2·0 g). Recrystallisation from $CHCl_3$–hexane gave blades, m.p. 169—170° (Found: C, 78·5; H, 9·4. $C_{18}H_{16}O_2$ requires C, 78·8; H, 9·6%); $\nu_{max.}$ (CS_2) 1672s and 3625 cm^{-1}; $\lambda_{max.}$ 247 nm (ε 12,000); τ ($CDCl_3$) 6·65 [1H, m, $W_{\frac{1}{2}}$ 22 Hz, C*H*OH] and 7·6—9·0 (26H, m); *m/e* 274 (M^+, 30%), 256 (15), 111 (45), and 84 (100). This ketone (1·79 g) was reduced (Li–NH_3–EtOH) to the diol (14), needles from aqueous MeOH, m.p. 203—206° (1·41 g). Sublimation gave D-*homogonane*-4α,12β-*diol* (14), long needles, m.p. 206—207° (Found: C, 77·4; H, 10·7. $C_{18}H_{30}O_2$ requires C, 77·7; H, 10·9%); $\nu_{max.}$ (N) 3450 cm^{-1}; *m/e* 278 (M^+, 7%), 260 (100), and 242 (40).

Oxidation of the diol yielded the *diketone* (15), small needles (Me_2CO–Et_2O, 0°), m.p. 200—201°. Sublimation gave a sample, m.p. 202—203° (Found: C, 79·1; H, 9·5. $C_{18}H_{26}O_2$ requires C, 78·8; H, 9·6%); $\nu_{max.}$ (N) 1702 cm^{-1}; *m/e* 274 (M^+, 100%), 256 (38), 219 (67), 124 (63), and 109 (61).

Details of the nutrient media (A and B) and of the technique used in these microbiological hydroxylations have been published. The mycelia were collected, extracted with Me_2CO, and the culture fluid (broth) was saturated with salt and extracted either by shaking with EtOAc (extraction 1) or by continuous extraction with Et_2O (extraction 2).[3,4]

Incubations with Calonectria decora.—Ketone (10) (440 mg), 3 d, medium B, extraction 1, yield broth extract 646 mg, mycelial extract 126 mg. P.l.c. (EtOAc) of mycelial extract gave s.m. (35 mg). The broth yielded s.m. (R_F 0·63) (9 mg) and a band (R_F 0·43) (322 mg). Recrystallisation from EtOAc afforded 12β,15α-*dihydroxy*-D-*homogon*-4-*en*-3-*one* (18), needles, m.p. 219—221° (312 mg), m.p. 224—226° (aqueous MeOH) (Found: C, 74·3; H, 8·7. $C_{18}H_{26}O_3$ requires C, 74·4; H, 9·0%); $\nu_{max.}$ ($CHCl_3$) 1618w, 1662s, 3450m, and 3620w cm^{-1}; $\lambda_{max.}$ 242 nm (ε 15,000); τ ($CDCl_3$) 4·10 [1H, s, C(4)H], 6·48 [2H, m, $W_{\frac{1}{2}}$ 25 Hz, C(12)H and C(15)H], and 7·0—8·8 (23H, m); *m/e* 290 (M^+, 2%), 272 (20), and 254 (100), *m** 254 (290 ⟶ 272) and 238 (272 ⟶ 254). A band (R_F 0·34) (84 mg) yielded 7α,12β,15α-*trihydroxy*-D-*homogon*-4-*en*-3-*one* (23), needles from $CHCl_3$–hexane, m.p. 187—189° (Found: C, 70·6; H, 8·4. $C_{18}H_{26}O_4$ requires C, 70·6; H, 8·6%); $\nu_{max.}$ ($CHCl_3$) 1616w, 1660s, 3450s, and 3620w cm^{-1}; $\lambda_{max.}$ 241 nm (ε 11,500); τ ($CDCl_3$) 4·05 [1H, s, C(4)H], 5·65 [1H, m, $W_{\frac{1}{2}}$ 9 Hz, C(7)H], 6·45 [2H, m, $W_{\frac{1}{2}}$ 24 Hz, C(12)H and C(15)H], and 7·0—9·0 (22H, m); *m/e* 306 (M^+, 45%), 288 (21), 154 (32), 123 (45), and 110 (100).

The unsaturated ketone (18) (127 mg) was reduced (Li–NH_3–EtOH) and the crude product oxidised yielding D-*homogonane*-3,12,15-*trione* (19), blades, m.p. 141—142° (67 mg) (Et_2O–light petrol) (Found: C, 75·0; H, 8·3. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%); $\nu_{max.}$ ($CHCl_3$) 1708 cm^{-1}. Samples (10—20 mg) of the trione were treated with selenium dioxide and with DDQ. The neutral fraction recovered had no u.v. adsorption above 220 nm.

The triol (23) (45 mg) was dissolved in EtOH (5 ml) and NaOH (0·4 ml; 2M) added. After 30 min the product was isolated (44 mg). P.l.c. ($CHCl_3$–MeOH, 4 : 1) yielded 12β-15α-*dihydroxy*-D-*homogona*-4,6-*dien*-3-*one* (24), blades, m.p. 158—159°, from Me_2CO–hexane (Found: C, 74·9; H, 8·5. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%); $\nu_{max.}$ ($CHCl_3$) 1621s, 1655s, 3445, and 3600w cm^{-1}; $\lambda_{max.}$ 282 nm (ε 29,500); τ ($CDCl_3$) 3·61 [1H, q, *J* 11 and 5 Hz, C(7)H], 3·90 [1H, d, *J* 11 Hz, C(6)H], 4·14 [1H, s, C(4)H], 6·46 [2H, m, C(12)H and C(15)H], and 7·0—9·0 (19H, m); *m/e* 298 (M^+, 100%), 270 (71), 252 (10), and 110 (80). The enone (18) (58 mg) was dissolved in dry dioxan (6 ml; saturated with HCl gas) and DDQ (50 mg) added. After 2 h isolation ($CHCl_3$) gave the dienone (24) (44 mg), m.p. and mixed m.p. 158—159°. The enol ether (4) (800 mg) medium B, extraction gave broth extract (1·2 g) and mycelial extract (300 mg). The mycelial extract yielded ketone (10) (80 mg); the broth extract contained (18) (40 mg) and a complex mixture of polar products (200 mg).

Incubation of the βγ-unsaturated ketone (5) (400 mg), medium B, extraction 1 produced s.m. (80 mg) from the mycelium; the broth yielded (10) (20 mg), (18) (28 mg), and a polar mixture (200 mg). *rac*-D-Homogon-4-en-3-one (8) (320 mg), medium B, extraction 1 gave broth extract (500 mg) and mycelial extract (110 mg). The latter

contained s.m. (84·5 mg); and the broth afforded s.m. (10 mg) and (18) (64·5 mg).

From the saturated diketone (12) (200 mg, medium B, extraction 1) was obtained s.m. (33 mg from mycelial extract) and from broth s.m. (25 mg) and 3α-*hydroxy*-D-*homogonan*-12-*one*, needles (45 mg), m.p. 125—131°, from Et_2O–light petroleum (Found: C, 78·0; H, 10·1. $C_{18}H_{28}O_2$ requires C, 78·2; H, 10·2%); $\nu_{max.}$ (CCl_4) 1707s and 3620 cm^{-1}; τ ($CDCl_3$) 5·85 [1H, q, *J* 2·5 Hz, $W_{\frac{1}{2}}$ 9 Hz, C(3)H*eq*], 7·5 [3H, m, C(11)H_2 and C(13)H], and 7·9—9·0 (24H, m); *m/e* 276 (M^+, 75%), 258 (100), 240 (29), 133 (70), and 109 (45). Oxidation of the hydroxy-ketone (10 mg) gave the dione (12) (9 mg). The hydroxy-ketone (30 mg) was reduced (Huang-Minlon) and the product oxidised to the known D-homogonan-3-one (see earlier) (16 mg), m.p. 88—90°.

Incubation of the 4,12-dione (15) (440 mg, medium B, extraction 1) gave s.m. (110 mg from mycelium, 21 mg from broth). P.l.c. of broth (Me_2CO–Et_2O, 1 : 2) gave 15α-*hydroxy*-D-*homogonane*-4,12-*dione* (21), small needles (61 mg), from $CHCl_3$–hexane, m.p. 216—217° (Found: C, 74·1; H, 9·1. $C_{18}H_{26}O_3$ requires C, 74·4; H, 9·3%); $\nu_{max.}$ (N) 1709s and 3450s cm^{-1}; τ ($CDCl_3$) 6·40 [1H, m, $W_{\frac{1}{2}}$ 24 Hz, C(15)H] and 7·0—9·0 (25H, m); *m/e* 290 (M^+, 41%), 272 (100), 171 (10), 142 (15), 133 (26), and 110 (47). Oxidation of the compound (30 mg) produced D-*homogonane*-4,15,15-*trione* (22), cubes (19 mg), m.p. 140—141°, from Me_2CO–hexane (Found: C, 74·8; H, 8·3. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%); $\nu_{max.}$ ($CHCl_3$) 1709 cm^{-1}; *m/e* 288 (M^+, 40%), 270 (6), 205 (20), 164 (21), 149 (19), 124 (8), and 97 (100).

Incubation with Rhizopus nigricans.—The unsaturated ketone (10) (440 mg) (medium B, extraction 1) gave a mycelial extract (510 mg) and a broth extract (1·0 g). The mycelial extract (p.l.c., $CHCl_3$–MeOH, 9 : 1) gave s.m. (220 mg). P.l.c. of the broth extract gave s.m. (16 mg). The next polar band afforded a white solid (117 mg). 12β,17α-*Dihydroxy*-D-*homogon*-4-*en*-3-*one* (25) formed needles (107 mg), m.p. 230—231°, from Me_2CO–hexane (Found: C, 74·2; H, 9·0. $C_{18}H_{26}O_3$ requires C, 74·4; H, 9·0%); $\nu_{max.}$ (N) 1617w, 1665s, and 3450 cm^{-1}; $\lambda_{max.}$ 242 nm (ε 14,700); τ ($CDCl_3$) 4·1 [1H, s, C(4)H], 6·45 [2H, m, $W_{\frac{1}{2}}$ 23 Hz, C(12)H and C(17)H], and 7·5—9·0 (23H, m); *m/e* 290 (M^+, 17%), 272 (18), 254 (15), 145 (28), and 110 (100). The least polar band gave the *triol* (27) as needles, m.p. 217—218°, from Me_2CO–hexane (Found: C, 70·5; H, 8·4. $C_{18}H_{26}O_4$ requires C, 70·6; H, 8·6%); $\nu_{max.}$ ($CHCl_3$) 1616w, 1661s, 3450, and 3620w cm^{-1}; $\lambda_{max.}$ 237 nm (ε 14,000); τ ($CDCl_3$) 4·11 [1H, s, C(4)H], 5·65 [1H, m, $W_{\frac{1}{2}}$ 9 Hz, C(6)H or C(7)H], 6·44 [2H, m, $W_{\frac{1}{2}}$ 24 Hz, C(12)H and C(17)H], and 7·5—8·9 (22H, m); *m/e* 306 (M^+, 15%), 256 (24), 204 (48), 124 (40), and 110 (100).

The unsaturated ketone (25) (70 mg) was reduced (Li–NH_3–EtOH) and the crude product oxidised. D-*Homogonane*-3,12,17-*trione* formed needles, m.p. 179—181° (43 mg) (Found: C, 75·0; H, 8·2. $C_{18}H_{24}O_3$ requires C, 75·0; H, 8·4%); $\nu_{max.}$ ($CHCl_3$) 1711s cm^{-1}; τ ($CDCl_3$) 7·41 [5H, m, C(11)H_2, C(13)H, and C(17a)H_2], and 7·6—9·0 (19H, m); *m/e* 288 (M^+, 100%), 270 (13), 201 (17), 146 (19), and 110 (17). This trione (25 mg) was boiled in glacial HOAc for 14 h with freshly sublimed SeO_2. The Et_2O extract was filtered through Celite and evaporated. The yellow oil so obtained had $\lambda_{max.}$ 254 nm (ε 10,500). T.l.c., Et_2O–light petroleum (1 : 1), Me_2CO–hexane (1 : 5) (2 runs), and $CHCl_3$ (3 runs) showed one spot only.

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