Microbiological Hydroxylation. Part XIII.[1] Cyclododecanone and Cyclopentadecanone as Substrates for Steroid-hydroxylating Fungi

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Cyclododecanone and cyclopentadecanone are both hydroxylated in cultures of four steroid-hydroxylating fungi, but remarkably, neither is affected by *Aspergillus ochraceus*. Substitution is less selective and formation of only one or two major products is much less the rule than with steroid substrates; di- and poly-hydroxylation apparently proceed very rapidly. Initial attack occurs at the most remote carbon atoms, at C(8) with the C_{15} ketone and at C(6) [and C(7)] with the C_{12} ketone.

In view of the ready microbiological hydroxylation of non-steroidal polycyclic ketones (perhydrochrysenes)[2] we examined the hydroxylation of macrocyclic ketones using some of the micro-organisms that have been effective with steroids. Fonken *et al.*,[3] have described the hydroxylation of C_{12}-, C_{13}-, and C_{14}-macrocyclic alcohols using *Sporotrichum sulfurescens*, which hydroxylates steroid substrates in the 11α-position. With cyclododecanol they obtained a mixture of diols, hydroxyketones, and diketones. Oxidation to the diketones indicated that 6- and 7-hydroxylation occurred to the extent of at least 11% in each case.

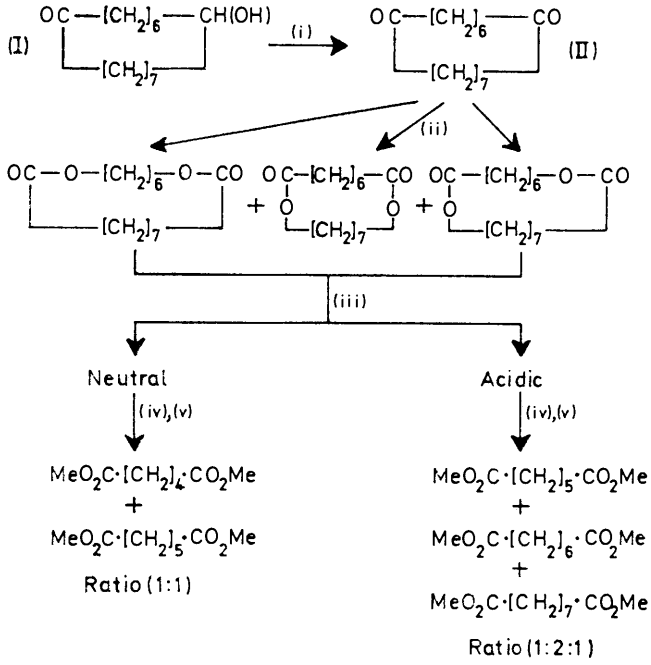
We have studied the incubation of two ketones, cyclopentadecanone and cyclododecanone, with five fungi; the results are summarised in the Tables. Great difficulty was experienced in obtaining consistent melting points with the products obtained from cyclopentadecanone; the presence of minor impurities in the hydroxyketones might be expected to depress their melting points considerably (cyclopentadecanone has been used in the Rast method of molecular weight determination). As already observed with the perhydrochrysene ketones, the products were all devoid of optical activity.

[1] Part XII, M. J. Ashton, A. S. Bailey, and Sir Ewart R. H. Jones, preceding paper.

[2] For further details see M. J. Ashton, D.Phil. Thesis, Oxford, 1972.

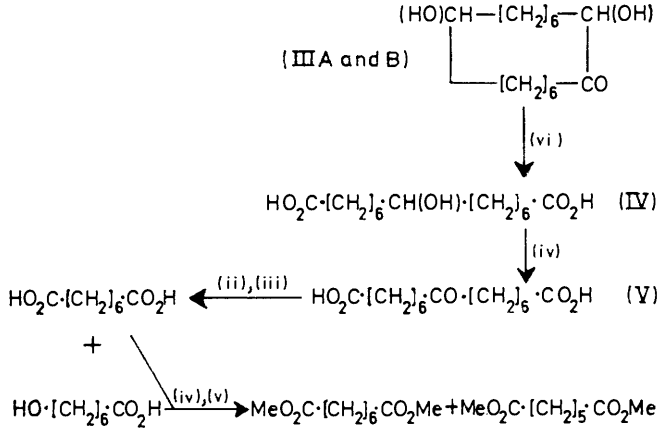
[3] G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, 1967, **89**, 672; *cf.* G. S. Fonken and R. A. Johnson, 'Chemical Oxidations with Micro-organisms,' Dekker, New York, 1972.

The structures of the metabolites were established by procedures, similar to those utilised by Fonken *et al.*,[3] illustrated in Schemes 1 and 2. Compound (I), a



SCHEME 1

Reagents: (i) H_2CrO_4–Et_2O; (ii) CF_3CO_3H; (iii) KOH–EtOH; (iv) H_2CrO_4–Me_2CO; (v) CH_2N_2; (vi) H_2O_2.



SCHEME 2

Reagents as in Scheme 1

monohydroxyketone, obtained from the C_{15} ketone, on oxidation with chromic acid–ether [4] yielded the diketone (II) (with this class of compounds this reagent gave better yields than CrO_3–acetone). The n.m.r. spectrum of (II) showed the presence next to the two CO groups of four α- and four β-methylene protons; hence it must be a 1,5-, 1,6-, 1,7-, or 1,8-dione. The diketone was unaffected by base, so it must therefore be a 1,8-dione since C_{12} 1,5-, 1,6-, and 1,7- and C_{15} 1,5- and 1,6-cyclodiones all undergo aldol condensations.[5] Its structure was further established (Scheme 1) by Baeyer–Villiger degradation to a mixture of glycols, hydroxy-acids, and dibasic acids.[3] Oxidation and esterification of the neutral fraction yielded a 1 : 1 mixture of dimethyl adipate and pimelate, and the acid fraction afforded the esters of pimelic, suberic, and azelaic acids in *ca.* 1 : 2 : 1 ratio.

The isomeric compounds (IIIA), m.p. 105—107°, and (IIIB), m.p. 81—83°, are dihydroxy-ketones and their n.m.r. spectra suggested that one OH group was close to the CO group and one OH group was far removed. Oxidation of (IIIA) and (IIIB) gave the same trione (non-crystalline) in poor yield; it was yellow and the change in its u.v. spectrum after addition of base [6] suggested a 1,2-dione system. The two stereoisomers (III) were unaffected by periodate; this may be due to the conformation of the macrocycle with the carbonyl group ' inside ' the ring,[7] making it difficult to form the five-membered ring intermediate [8] in the periodate oxidation (*cf.* the explanation of the behaviour of macrocyclic ketones in the Robinson annulation reaction [9]). Oxidation of (IIIA) and its isomer proceeded through the dibasic acids (IV) and (V) (Scheme 2) and eventually yielded equivalent amounts of the pimelic and suberic esters.

The structures of the three diols [(VI)—(VIII)] in Table 1 were proved in a similar manner. Oxidation of (VI) gave the known [5] dione; oxidation of (VIII) yielded (II), and (VII) afforded the unknown cyclopentadecane-1,7-dione the structure of which was proved by the Baeyer–Villiger procedure (Scheme 1) used with the 1,8-dione.

The structures of the various hydroxylation products obtained from cyclododecanone and listed in Table 2 were readily established by oxidation to the known diketones.

The highest yield (26%) of monohydroxylated product (I) from the C_{15} ketone with *C. decora* was obtained after 3 days; longer periods of incubation gave more of the dihydroxy-compounds (III) and more polar products. Only monohydroxylated products, highest combined yield 38%, could be isolated from the C_{12} ketone and, as was found by Fonken *et al.*[3] with the corresponding alcohol and *Sporotrichum sulfurescens*, attack occurred at the 5-, 6-, and 7-positions, and mainly at the last two. Initial hydroxylation occurs at positions remote from the directing keto-group; the dihydroxy-compounds (III) may be formed by hydroxylation of the enolic form of the ketone.[10] Hydroxylations with

[4] H. C. Brown, C. P. Garg, and K. T. Liu, *J. Org. Chem.*, 1971, **36**, 387.

[5] G. Ohloff, J. Becker, and K. H. Schulte-Elte, *Helv. Chim. Acta*, 1967, **50**, 705; M. E. Herr and G. S. Fonken, *J. Org. Chem.*, 1967, **32**, 4065.

[6] T. Mori, T. Nakahara, and H. Nozaki, *Canad. J. Chem.*, 1969, **47**, 3267.

[7] V. Prelog and W. Klyne, *Experientia*, 1960, **16**, 521; J. Dale, *Angew. Chem. Internat. Edn.*, 1966, **5**, 1000; T. Ledaal, *Tetrahedron Letters*, 1968, 651.

[8] H. O. House, 'Modern Synthetic Reactions,' 2nd edn., W. A. Benjamin, Menlo Park, California, 1972, p. 357.

[9] V. Prelog, *J. Chem. Soc.*, 1950, 420.

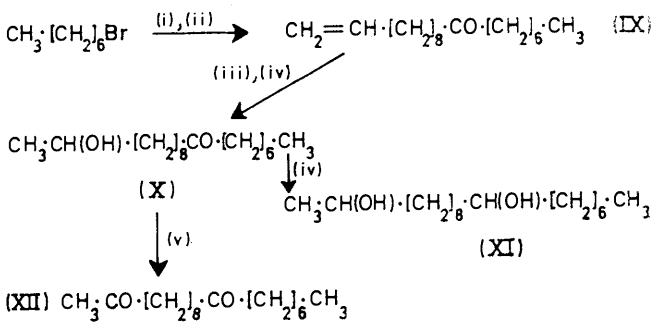
[10] S. Baba, H. J. Brodie, M. Hayano, D. H. Peterson, and O. K. Sebek, *Steroids*, 1973, **1**, 151.

Rhizopus nigricans gave products in which the CO group had been reduced; similar results have been obtained in the hydroxylation of steroidal ketones with this organism.[11]

With *Daedalea rufescens* hydroxylation also occurs at positions remote from the CO group and reduction of this group also occurs; similar results have been observed using steroids.[12] *Ophiobolus herpotrichus* hydroxylates 3-oxosteroids in ring D;[13] it attacked cyclododecanone and cyclopentadecanone in places remote from the CO groups and gave 6-hydroxycyclododecanone in high yield (34%) with recovery of cyclododecanone (20%) and cyclododecanol (10%). In striking contrast was the failure to effect hydroxylation with *Aspergillus ochraceus* although this organism readily hydroxylates steroids.[14]

It seemed desirable to investigate the behaviour of some aliphatic ketonic compounds under comparable conditions. For this purpose di-n-heptyl ketone was synthesised and an appropriate diketone, octadecane-2,11-dione was prepared as shown in Scheme 3. The incubation results were disappointing; no simple hydroxylated materials were formed, only starting materials or very polar products being evident on t.l.c.

These aliphatic ketones are probably oxidised to carboxylic acids which then get into the normal fatty acid β-oxidation pathway and are rapidly degraded.



SCHEME 3

Reagents: (i) Mg–Et_2O; $CdCl_2$–PhH; (ii) CH_2=CH·$[CH_2]_8$·COCl; (iii) $Hg(OAc)_2$; (iv) $NaBH_4$; (v) H_2CrO_4–Me_2CO.

TABLE 1

Cyclopentadecanone incubated with fungal cultures

	C. decora			*R. nigricans*	*D. rufescens*	*O. herpotrichus*
Time (days)	3	4	6	3	3	3
Yields (%) *						
8-Hydroxy-(I)	26	20	8·4		5·1	14
2,9-Dihydroxy-{(IIIA)	4·9	10	5·0			
2,9-Dihydroxy-{(IIIB)	6·0	9·2	2·9			
Cyclopentadecanol				4·5	1·1	15
1,6-Diol (VI)				6·1	1·8	
1,7-Diol (VII)				5·8	1·5	
1,8-Diol (VIII)				8·3	6·8	
Polar products	23	28	28		5·4	22
Starting material (%)	35	36	11	32·5	6·3	60

* Calculated on unrecovered ketone (+ alcohol).

TABLE 2

Cyclododecanone incubated with fungal cultures

	C. decora			*R. nigricans*	*D. rufescens*	*O. herpotrichus*
Time (days)	3	5	2 †			
Yields (%) *						
5-Hydroxycyclododecanone	2·4	1·2	3	8·2		5·5
6-Hydroxycyclododecanone	18	8·5	14·5	18	16	50
7-Hydroxycyclododecanone	17·5	7·5	12	8·2	5	
Cyclododecane-1,5-diol					1·6	
Cyclododecane-1,6-diol					4·2	
Cyclododecane-1,7-diol					2·3	
Cyclododecanol				6·7	9·8	12·3
Polar products	5	30	30	16	6·4	
Starting material (%)	50	20	20	40	14	20

* Calculated on unrecovered ketone (+ alcohol). † Fermenter run (10 g).

EXPERIMENTAL

General reaction conditions, the nutrient media, and the hydroxylation techniques have been reported.[2,15] Runs using the large-scale fermenter [16] are marked (F). Petrol refers to light petroleum, b.p. 40—60°.

Incubations with Calonectria decora.—(a) *Cyclopentadecanone.* The ketone (2 g) in EtOH (250 ml), 50 flasks, medium B,[15] 3 days, extraction (2) [2] yielded broth extract (1·1 g) and mycelial extract (0·96 g). These were combined and chromatographed on silica (60 g). Elution with

[11] V. E. M. Chambers, W. A. Denny, J. M. Evans, Sir Ewart R. H. Jones, A. Kasal, G. D. Meakins, and J. Pragnell, *J.C.S. Perkin I*, 1973, 1500.

[12] V. E. M. Chambers, W. A. Denny, Sir Ewart R. H. Jones, G. D. Meakins, and A. Pendlebury, in preparation.

[13] Sir Ewart R. H. Jones, G. D. Meakins, *et al.*, unpublished work.

[14] 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.

[15] J. W. Blunt, I. M. Clark, J. M. Evans, Sir Ewart R. H. Jones, G. D. Meakins, and J. T. Pinhey, *J. Chem. Soc.* (*C*), 1971, 1136.

[16] 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.

Me_2CO–hexane (1 : 20, 1 l) gave s.m. (700 mg); Me_2CO–hexane (1 : 10, 1 l) gave an oil (390 mg) which after p.l.c. (Me_2CO–hexane, 1 : 4) was recrystallised from hexane at −10° and yielded 8-*hydroxycyclopentadecanone* (I) (340 mg) as needles, m.p. 73—74° (Found: C, 74·6; H, 11·4. $C_{15}H_{28}O_2$ requires C, 75·0; H, 11·7%); $\nu_{max.}$ (CCl_4) 1711s and 3620 cm^{-1}; τ (CCl_4) 6·41 (1H, m, C*H*OH), 7·65 (4H, t, *J* 6·5 Hz, CH_2·CO·CH_2), and 8·37—8·8 (23H, m); *m/e* 240 (M^+, 5%), 222 (22), 113 (41), 98 (50), and 66 (100).

Elution with Me_2CO–hexane (1 : 5—1 : 3) gave an oil which was further separated by p.l.c. (Et_2O). The less polar band gave more (I) (20 mg) and the more polar band (300 mg) was further resolved on plates (CH_2Cl_2–Me_2CO–EtOH, 16 : 2 : 1). The fast running band after crystallisation from Me_2CO–hexane gave 2,9-*dihydroxycyclopentadecanone* (IIIA) (78 mg); the slow running band yielded the isomeric *dihydroxy-ketone* (IIIB) (90 mg) after recrystallisation from Et_2O–petrol. Compound (IIIA) formed needles, m.p. 105—107° (Found: C, 70·5; H, 11·0. $C_{15}H_{28}O_3$ requires C, 70·3; H, 11·0%); $\nu_{max.}$ ($CHCl_3$) 1709s and 3460 cm^{-1}; τ ($CHCl_3$) 5·65 (1H, m, C*H*OH), 6·38 (1H, m, C*H*OH), 7·5 (2H, m, CH_2·CO), and 7·9—9·0 (24H, m); *m/e* 256 (M^+, 12%), 238 (10), 206 (40), 135 (36), 98 (90), and 81 (100). The isomer (IIIB) formed needles, m.p. 81—83° (Found: C, 70·1; H, 11·0%); $\nu_{max.}$ ($CHCl_3$) 1709s and 3460 cm^{-1}; τ ($CHCl_3$) 5·85 (1H, m, C*H*OH), 6·29 (1H, m, C*H*OH), 7·6 (2H, m, CH_2·CO), and 8·15—8·85 (24H, m); *m/e* 256 (M^+, 13%), 238 (11), 149 (30), 111 (48), 98 (100), and 81 (90).

Results of incubating cyclopentadecanone (2 g) with *C. decora* for varying times are summarised in Table 3.

TABLE 3

Incubation of cyclopentadecanone with *C. decora*

Incubation time (days)	3	4	6	3 *
Broth extract (g)	1·1	1·2	2·0	8·1
Mycelial extract (g)	0·96	0·8	0·7	1·0
(I) (mg)	360	270	160	600
(IIIA) (mg)	78	140	100	85
(IIIB) (mg)	90	135	60	81
Polar products (mg)	300	360	500	4800
Recovered s.m. (mg)	700	717	22	2000

* Cyclopentadecanone (10 g) in EtOH (500 ml), 50 flasks, medium B (F).

The ketol (I) (160 mg) in Et_2O (10 ml) was oxidised with chromic acid.[4] *Cyclopentadecane-1,8-dione* (II) formed needles (149 mg), m.p. 75—76°, from petrol at −30° (Found: C, 75·9; H, 11·2. $C_{15}H_{26}O_2$ requires C, 75·6; H, 11·0%); $\nu_{max.}$ (CCl_4) 1712 cm^{-1}; τ (CCl_4) 7·71 (8H, t, *J* 6 Hz, CH_2·CO), 8·47 (8H, m, CH_2·CH_2CO), and 8·80br (10H); *m/e* 238 (M^+, 15%), 181 (21), 126 (21), 112 (69), 111 (68), 98 (100), and 84 (95).

Degradation of the diketone (II). The diketone (II) (95 mg) in CH_2Cl_2 (10 ml) was added slowly to cooled (0°) peroxytrifluoroacetic acid [prepared from the anhydride (1 ml) and hydrogen peroxide (90%; 0·2 ml)] in CH_2Cl_2 (2 ml) containing disodium hydrogen orthophosphate (230 mg). After 30 min at 20° and reflux for 1 h aqueous KI (2 ml; saturated) was added and, after 5 min $NaHSO_3$ in H_2O. Work-up with CH_2Cl_2 gave an oil (104 mg) with a pronounced 'musk' odour; $\nu_{max.}$ (CCl_4) 1205 and 1745s cm^{-1}. This was hydrolysed [MeOH (20 ml), NaOH (2M; 4 ml), 2 h], and after saturation with NaCl continuous Et_2O extraction (2 h) gave an oil (a) (42 mg), $\nu_{max.}$ ($CHCl_3$) 3450s and 3600w cm^{-1}. The aqueous layer was acidified and extracted with Et_2O (8 h) yielding an oil (b) (73 mg), $\nu_{max.}$ ($CHCl_3$) 1728s and 3450 cm^{-1}. The neutral fraction (a) was oxidised (CrO_3–Me_2CO) and continuous extraction (Et_2O, 6 h) gave the acids (46 mg) which with CH_2N_2 gave an oil (38 mg), $\nu_{max.}$ (CCl_4) 1740 cm^{-1}. These esters were shown (g.l.c., enrichment with authentic samples) to be dimethyl pimelate and adipate in 1 : 1 ratio. The acid fraction (b) was oxidised and methylated to a mixture of esters (63 mg), $\nu_{max.}$ 1738 cm^{-1}. This was similarly shown to consist of dimethyl pimelate, suberate, and azelate in the ratio 1 : 2 : 1. G.l.c. analyses were carried out on a Pye-104 instrument (flame-ionisation detector) using a 10% silicone oil column. Authentic specimens of dibasic acid methyl esters showed the following retention times (min) (temp. 130°, flow rate 40 ml min^{-1}): malonic 2, succinic 2·4, glutaric 3·4, adipic 6, pimelic 9·5, suberic 16, azelaic 24, and sebacic 32. As a blank test pimelic acid was oxidised by CrO_3–Me_2CO and the product esterified; g.l.c. showed only one component (R_t 9·5 min) and no shorter chain esters were detected.

Oxidation of the diol (IIIA). The diol (IIIA) (100 mg) was oxidised (CrO_3–Me_2CO) and yielded a neutral yellow oil (20 mg); t.l.c. afforded 9 mg of apparently pure material which did not crystallise, $\nu_{max.}$ (CCl_4) 1712s cm^{-1}; $\lambda_{max.}$ 276 (ε 76) nm, (EtOH–NaOH) 295 (ε 200); τ (CCl_4) 7·68 (8H, m, CH_2·CO), 8·40 (8H, m, CH_2CH_2CO), and 8·75 (8H, m); *m/e* 252 (M^+, 24%), 234 (14), 140 (56), 112 (58), and 97 (100). Compound (IIIB) (100 mg) gave identical material (14 mg) (i.r., and t.l.c. in two solvent systems).

Diol (IIIA) (128 mg) in dioxan (10 ml) was oxidised by alkaline H_2O_2. After 1 h, acidification, saturation (NaCl), and extraction (Et_2O, 2 h) gave a solid (124 mg); $\nu_{max.}$ ($CHCl_3$) 1720s and 3450w cm^{-1}. Oxidation (CrO_3–Me_2CO) gave a keto-acid (117 mg), $\nu_{max.}$ ($CHCl_3$) 1709 and 1720s cm^{-1}. This was subjected to Baeyer–Villiger oxidation and the lactones saponified. The resulting oil (83 mg) was oxidised and the mixture of acids methylated to yield an oil (54 mg), $\nu_{max.}$ (CCl_4) 1738s cm^{-1}, shown (g.l.c.) to be a 1 : 1 mixture of dimethyl pimelate and suberate. The isomer (IIIB) (128 mg) likewise yielded the same two esters in 1 : 1 ratio.

(b) *Cyclododecanone*. The ketone (4 g) in EtOH (500 ml), 100 flasks, 3 d, medium B, extraction 2, gave broth (2·55 g) and mycelial extracts (2·2 g); these were combined and chromatographed on silica (200 g). Me_2CO–hexane (1 : 20) gave s.m. (2·0 g). Me_2CO–hexane (1 : 10—1 : 3) gave an oil (830 mg) which was subjected to p.l.c. (Et_2O, 3 runs). The fast-running band gave *5-hydroxycyclododecanone* (48 mg), needles, m.p. 84—85°, from Et_2O–petrol (Found: C, 72·6; H, 11·2. $C_{12}H_{22}O_2$ requires C, 72·7; H, 11·2%); $\nu_{max.}$ (CCl_4) 1709, 3450, and 3616 cm^{-1}; τ (CCl_4) 6·65 (1H, m, C*H*OH), 7·60 (4H, m, CH_2CO), and 8·2—8·85 (17H, m); *m/e* 198 (M^+, 3%), 180 (60), 98 (60), and 81 (100). Oxidation of the ketol gave the known 1,5-dione, m.p. 64—65° (lit.,[3] 64—65°), with identical i.r. spectrum. The slow-running band was divided into two equal parts: the upper part was rechromatographed (Et_2O, 3 runs) and the product recrystallised from Et_2O–petrol as needles (360 mg) of *7-hydroxycyclododecanone*, m.p. 91·5—92·5° (Found: C, 72·5; H, 11·1. $C_{12}H_{22}O_2$ requires C, 72·7; H, 11·2%); $\nu_{max.}$ (CCl_4) 1707 and 3620 cm^{-1}; τ (CCl_4) 6·45 (1H, m, C*H*OH), 7·40 (4H, t, *J* 6 Hz, CH_2CO), and 7·7—8·8 (17H, m); *m/e* 198 (M^+, 3%), 180 (60), 109 (34), 81 (55), and 55 (100). Oxidation gave the 1,7-dione,[3] m.p. and mixed m.p. 134—135°; $\nu_{max.}$ (CCl_4) 1708 cm^{-1}, *m/e* 196 (M^+, 13%) and 98 (100). The lower part of the slow-running band gave after p.l.c. (Et_2O, 3 runs) *6-hydroxydodecanone*, needles

(375 mg), m.p. 65·5—66·5° (Me_2CO–petrol) (Found: C, 72·6; H, 11·0. $C_{12}H_{22}O_2$ requires C, 72·7; H, 11·2%); $\nu_{max.}$ (CCl_4) 1707 and 3620 cm^{-1}; τ 6·47 (1H, m, C*H*OH), 7·39 (4H, t, *J* 6 Hz, CH_2CO), and 7·8—9·0 (17H, m). Oxidation of this ketol yielded the 1,6-dione,[3] m.p. and mixed m.p. 94—95°: $\nu_{max.}$ 1708 cm^{-1}; *m/e* 196 (M^+, 13%) and 98 (100).

Elution of the main column with Me_2CO–hexane (1 : 1) gave highly polar non-crystalline material (270 mg). The fermenter run (10 g) (medium A) yielded 5- (250 mg), 6- (1·2 g), and 7-hydroxycyclododecanone (1·0 g).

Incubations with R. nigricans.—Cyclopentadecanone (4 g) gave, in order of elution, s.m. (1·3 g), cyclopentadecanol (120 mg), *cyclopentadecane*-1,7-*diol* (160 mg), needles, from Me_2CO–hexane, m.p. 99—101° (Found: C, 74·0; H, 12·5. $C_{15}H_{30}O_2$ requires C, 74·3; H, 12·5%); $\nu_{max.}$ (CCl_4) 3620 cm^{-1}; τ ($CDCl_3$) 6·25 (2H, m, C*H*OH) and 8·3—8·8 (28H, m); *m/e* 242 (M^+, 1%), 224 (16), 206 (14), 98 (75), and 82 (100); *cyclopentadecane*-1,8-*diol* (230 mg), needles, m.p. 107—108° (Found: C, 74·0; H, 12·3%); $\nu_{max.}$ 3610 cm^{-1}; τ 6·28 (2H, m, C*H*OH) and 8·3—8·8 (28H); *m/e* 242 (M^+, 2%), 224 (14), 110 (38), and 82 (100); and *cyclopentadecane*-1,6-*diol*, needles (170 mg), m.p. 121—122°, from Et_2O–petrol (Found: C, 74·0; H, 12·4%); $\nu_{max.}$ (CCl_4) 3610 cm^{-1}; τ 6·25 (2H, m, C*H*OH) and 8·3—8·8 (28H, m); *m/e* 242 (M^+, 1%), 135 (37), 96 (80), and 82 (100). Oxidation of the 1,8-diol gave the dione (II), m.p. and mixed m.p. 75—77°; the 1,6-diol gave cyclododecane-1,6-dione, m.p. 77—78° (lit.,[5] 77—78°), identical i.r. spectrum.* The 1,7-diol gave *cyclopentadecane*-1,7-*dione*, needles, m.p. 88—89° (aqueous MeOH) (Found: C, 75·9; H, 11·2. $C_{15}H_{26}O_2$ requires C, 75·6; H, 11·0%); $\nu_{max.}$ 1710 cm^{-1}; τ (CCl_4) 7·7 (8H, m, CH_2·CO), 8·45 (8H, m, CH_2CH_2·CO), and 8·6—8·9 (10H, m). The ketone (100 mg) was subjected to Baeyer–Villiger degradation as described above. The esters obtained *via* the neutral extract were dimethyl glutarate and dimethyl suberate. The dimethyl esters obtained *via* the acidic fraction were those of adipic, pimelic, azelaic, and sebacic acids.

Cyclododecanone (4 g) with *R. nigricans* (medium B, 3 days, extraction 2) gave s.m. (1·6 g) and an oil (1·45 g) by column chromatography (Me_2CO–hexane mixtures). The oil was separated by p.l.c. (Me_2CO–$CHCl_3$–EtOH, 16 : 4 : 1) giving cyclododecanol (160 mg), m.p. and mixed m.p. 79—80°, 5-hydroxycyclododecanone (200 mg), m.p. and mixed m.p. 84—85°, 7-hydroxycyclododecanone (200 mg), m.p. and mixed m.p. 91·5—92·5°, and 6-hydroxycyclododecanone (430 mg), m.p. and mixed m.p. 65—66°.

Incubations with Daedalia rufescens.—Cyclopentadecanone (4 g), medium B, 3 d, extraction 2, gave s.m. (230 mg), cyclopentadecanol (40 mg), 8-hydroxycyclopentadecanone (200 mg), cyclopentadecane-1,7-diol (60 mg), cyclopentadecane-1,8-diol (269 mg), cyclopentadecane-1,6-diol (74 mg) and polar material (300 mg). Cyclododecanone (4 g) gave s.m. (540 mg), cyclododecanol (340 mg), 7-hydroxycyclododecanone (174 mg), 6-hydroxycyclododecanone (580 mg), *cyclododecane*-1,6-*diol*, needles (Me_2CO–hexane) (54 mg), m.p. 120—121° (Found: C, 71·8; H, 12·0. $C_{12}H_{24}O_2$ requires C, 72·0; H, 12·1%); $\nu_{max.}$ ($CHCl_3$) 3450s cm^{-1}; τ ($CDCl_3$) 6·41 (2H, m, C*H*OH) and 7·3—9·0 (22H, m), *cyclododecane*-1,5-*diol* (145 mg), fine needles, m.p. 113—115° (Found: C, 72·3; H, 12·3%); $\nu_{max.}$ 3450 cm^{-1}; τ 6·40 (2H, m, C*H*OH) and 7·4—8·9 (22H, m), and *cyclododecane*-1,7-*diol* (78 mg), needles, m.p. 136—139° (Found: C, 71·7; H, 12·0%); $\nu_{max.}$ 3450 cm^{-1}; τ 6·36 (2H, m, C*H*OH) and 7·4—9·0 (22H, m). The three diols were identified by oxidation to the known diones (see earlier description of oxidation of monohydroxycyclododecanones).

Incubations with Ophiobolus herpotrichus.—Cyclopentadecanone (2 g) yielded s.m. (1·2 g), cyclopentadecanol (125 mg), and 8-hydroxycyclopentadecanone (100 mg). Cyclododecanone (2 g) gave combined extracts (2·4 g) which in Et_2O were filtered through alumina (30 g). The ethereal solution was concentrated (5 ml), hexane (5 ml) was added, and after 30 min the solid, 6-hydroxycyclododecanone (460 mg), was collected. Chromatography of the residues gave s.m (400 mg), cyclododecanol (200 mg), 5-hydroxycyclododecanone (82 mg), and 6-hydroxycyclododecanone (285 mg).

Di-n-heptyl Ketone.—To a solution of n-heptylmagnesium bromide [from Mg (4 g)] in Et_2O (250 ml) was added $CdCl_2$ (16·0 g) and the Et_2O replaced by PhH. Octanoyl chloride (27 g) in PhH was added at 0° and the mixture boiled for 2 h. The usual work-up gave di-n-heptyl ketone (29 g), plates, m.p. 39—41°, from EtOH (lit.,[17] 40°).

Octadec-1-*en*-11-*one* (IX).—A suspension of di-n-heptylcadmium in PhH (150 ml) was prepared from heptyl bromide (11·8 g), Mg, and $CdCl_2$ (5·7 g) in the usual way. Undecenoyl chloride (10 g) was added, the PhH boiled for 2 h, and the reaction worked up in the usual way. The crude ketone was boiled with alkali to remove traces of ester present [18] ($\nu_{max.}$ 1735m cm^{-1}). Recrystallisation at −30° from petrol gave the *unsaturated ketone* (IX) (4·8 g) m.p. 27—29° (Found: C, 80·9; H, 12·9. $C_{18}H_{34}O$ requires C, 81·1; H, 12·9); $\nu_{max.}$ (CCl_4) 912m, 1645w, and 1718s cm^{-1}; *m/e* 266 (M^+, 5%), 127 (35), and 57 (100).

2-*Hydroxyoctadecan*-11-*one* (X).—To a suspension of $Hg(OAc)_2$ (3·19 g) in water (10 ml) and tetrahydrofuran (10 ml) was added the alkenone (IX) (2·66 g) in tetrahydrofuran (10 ml); the mixture was stirred for 1 h, NaOH (3M; 10 ml) was added, followed by dropwise addition (10 min) of $NaBH_4$ (379 mg) in NaOH (3M; 10 ml). Brine (25 ml) was added and the solid from the organic layer was recrystallised (petrol) yielding the *hydroxy-ketone* (X) (2·3 g), needles, m.p. 65—66° (Found: C, 76·3; H, 12·9. $C_{18}H_{36}O_2$ requires C, 76·0; H, 12·8%); $\nu_{max.}$ (CCl_4) 1717 and 3620 cm^{-1}; τ (CCl_4) 6·25 (1H, m, C*H*OH), 7·66 (4H, t, *J* 7 Hz, CH_2·CO), 8·45—8·8 (25H, m), 8·81 (3H, d, *J* 7 Hz, CH_3·CH), and 9·14 (3H, t, *J* 7 Hz, CH_3·CH_2); *m/e* 284 (M^+, 3%), 71 (42), and 57 (100). Reduction of this ketone with $NaBH_4$ in MeOH gave 2,11-*dihydroxyoctadecane* (XI) (prisms from petrol), m.p. 65—67° (Found: C, 75·3; H, 13·3. $C_{18}H_{38}O_2$ requires C, 75·5; H, 13·4%); $\nu_{max.}$ (CCl_4) 1380 and 3620 cm^{-1}; *m/e* 250 (M^+, 3%), 94 (74), and 68 (100).

Octadecane-2,11-*dione* (XII).—Oxidation of the hydroxy-ketone (X) gave the *diketone* (XII), plates, m.p. 72—73°, from petrol (Found: C, 76·4; H, 11·9. $C_{18}H_{34}O_2$ requires C, 76·5; H, 12·1%); $\nu_{max.}$ (CCl_4) 1721 cm^{-1}; τ (CCl_4) 7·68 (6H, m, CH_2·CO), 7·94 (3H, s, CH_3·CO), 8·6—8·9 (22H, m), and 9·12 (3H, t, *J* 7 Hz, CH_3·CH_2); *m/e* 282 (M^+, 4%), 225 (12), 198 (22), 142 (39), 127 (29), and 57 (100).

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