Microbiological Hydroxylation. Part 23.[1] Bicyclic Substrates for Steroid-hydroxylating Fungi

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Bicyclic oxygen-containing substrates have been hydroxylated by *Calonectria decora* and by *Rhizopus nigricans*. Poor yields of mono- and di-oxygenated compounds were obtained. Initial attack appears to occur at carbon atoms remote from the original oxygen function.

In the preceding paper[1] we described the preparation of a set of bicyclic ketones and alcohols containing 15—16 carbon atoms. The microbiological hydroxylations of some of these compounds have now been examined to compare their behaviour with that of the C_{12-15} monocyclic ketones.[2] Two fungi, *Calonectria decora* and *Rhizopus nigricans*, have been used for this purpose and the experimental conditions were those used for the monocyclic ketones[2] and steroidal substrates.[3] The results are summarised in the Table. Since in some incubations oxidation of hydroxy-groups occurred, the broth extract was oxidised [CrO_3–Me_2CO] in all cases

[1] Part 22, A. S. Bailey, Sir Ewart R. H. Jones, and M. L. Gilpin, preceding paper.

[2] M. J. Ashton, A. S. Bailey, and Sir Ewart R. H. Jones, *J.C.S. Perkin I*, 1974, 1665.

[3] 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; for further details see M. L. Gilpin, D.Phil. Thesis, Oxford, 1975.

before preparative layer chromatography (p.l.c.); this procedure had the additional advantage that the polyketones crystallise more readily than the corresponding

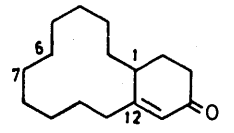
Substrate	Fungus *	Substrate recovered (%)	Main product(s) (yield) † investigated	Polar materials (%)† (unidentified)
(1)	Rn	15	(2) (4%), (3) (15%)	42
	Cd	6	(3) (10%)	81
(4)	Cd	16	(5) (5%), (6) (6%), (7) (4%)	78
(8)	Cd	32	(9) (11%), (10) (5%)	57
(8)	Rn	25	(9) (8%), (10) (3%)	42
(11)	Cd	87		
(12)	Cd	43	(13) + (14) (12%)	31
(15)	Cd	34	(16) (5%), (17) (6%)	43
(18)	Cd	15	(16) (3%), (17) (8%)	52

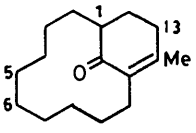
* Rn = *Rhizopus nigricans*; Cd = *Calonectria decora*.
† Calculated on the basis of starting materials consumed.

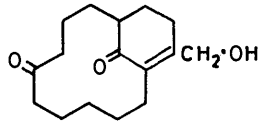
oxo-alcohols. Some of the products showed optical activity whereas none had been detected in the products obtained [2] by hydroxylation of the monocyclic ketones.

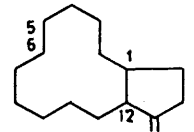
The structures of the metabolites were established by oxidative procedures similar to those used on the metabolites of the monocyclic ketones. Details are given for some of the compounds. Compound (2) ($[\alpha]_D^{20}$ −71°) showed the spectral characteristics both of a saturated ketone and of an αβ-unsaturated ketone. The n.m.r. spectrum was consistent with a structure containing 14 protons that were either allylic or α or β to a carbonyl group, suggesting that the oxygen atom had been introduced at a position remote from the cyclohexenone system. Before carrying out degradative studies the parent compound (1) was ozonised and afforded the known [4] oxo-acid (19) in 77% yield. Ozonolysis of compound (2) (Scheme 1) afforded the dioxo-acid (20); the crude material was subjected to Baeyer–Villiger oxidation [1,5] and gave a mixture of dilactones (i.r.) which, on hydrolysis, gave an acidic fraction and a small quantity of a neutral fraction, reflecting the preferred secondary alkyl migration observed in such oxidations.[6] Oxidation of the acidic fraction gave a mixture of dibasic acids; g.l.c. of the esters showed the presence of dimethyl glutarate and adipate (by enrichment with authentic samples) in the ratio 1 : 14 together with the branched-chain fragments of higher molecular weights. These were not investigated and their presence did not interfere with the analysis of the straight-chain diesters.

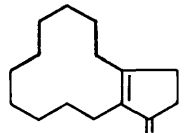
Combined g.l.c.–mass spectrometry confirmed the nature of the products by comparison of the fragmentation patterns with those of authentic samples. The large difference between the observed ratio of glutarate to adipate with that expected (*ca.* 1 : 1) probably reflects the water-solubility difference between valerolactone and adipic acid during the extractions with ether. Since we had observed the formation of valerolactone in this degradation, in subsequent degradations the aqueous hydrolysis solution was concentrated to small bulk and saturated with salt prior to extraction with ether. [For example, in the degradation of compound (3) dimethyl adipate and dimethyl pimelate were obtained in approximately 1 : 1 ratio.] The ester

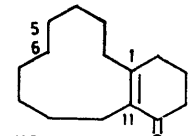

(1)
(2) O= at C(7)
(3) O= at C(6)

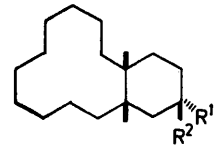

(4)
(5) HO— at C(6)
(6) HO— at C(5)

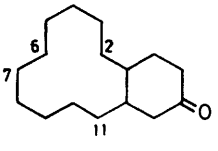

(7)


(8)
(9) O= at C(6)
(10) O= at C(5)


(11)


(12)
(13) O= at C(5)
(14) O= at C(6)


(15) R^1=OH, R^2=H
(18) R^1R^2 = O


(16) O= at C(2), C(7)
(17) O= at C(6), C(11)

from the oxidised neutral fraction consisted (g.l.c.–mass spectrometry) entirely of dimethyl succinate.

The i.r., u.v., n.m.r., and mass spectra of compound (3) ($[\alpha]_D^{20}$ +1.2°) were very similar to those of compound (2), suggesting that they were closely related. The dione was degraded in the same manner as compound (2); oxidation and esterification of the acidic fraction yielded dimethyl adipate and pimelate (1 : 1) identified by g.l.c.–mass spectrometry.

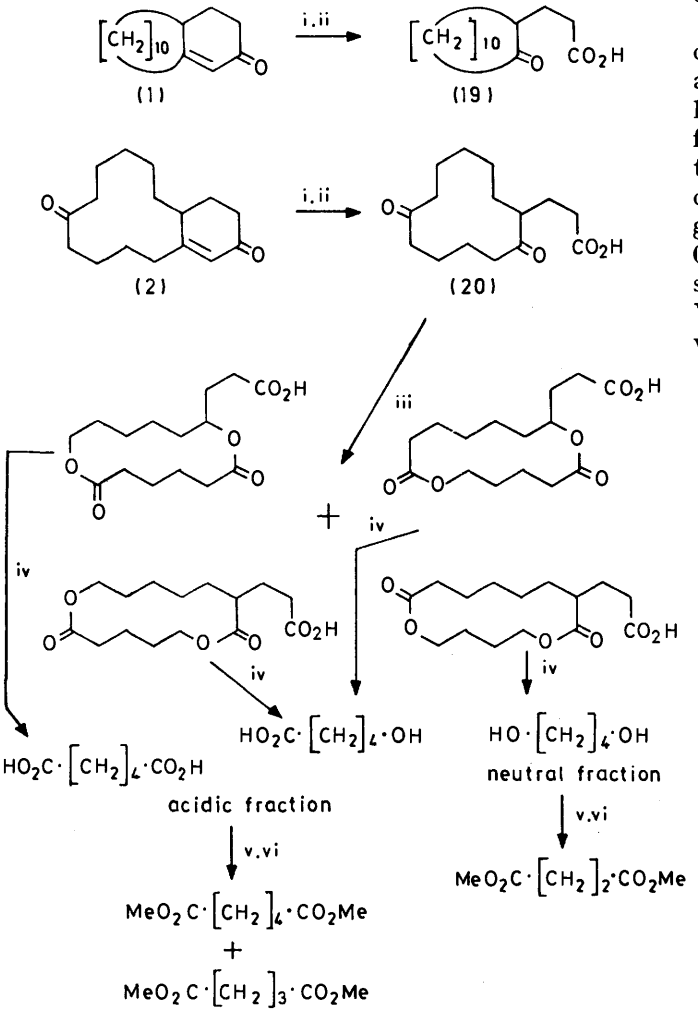
The ketol (5) ($[\alpha]_D^{20}$ +49°) was oxidised to the corresponding dione (21), the n.m.r. spectrum of which indicated that the carbonyl groups were far apart. In order to establish a suitable degradative procedure for

[4] J. R. Hargreaves, P. W. Hickmott, and B. J. Hopkins, *J. Chem. Soc.* (*C*), 1969, 592.

[5] W. D. Emmons and G. B. Lucas, *J. Amer. Chem. Soc.*, 1955, 77, 2287.

[6] J. B. Lee and B. C. Uff, *Quart. Rev.*, 1967, 21, 429.

this class of compound it was established that compound (4) could be ozonised to a triketone (22), and this trione was then oxidized with lead tetra-acetate to the dicarboxylic acid (23) in 58% yield.

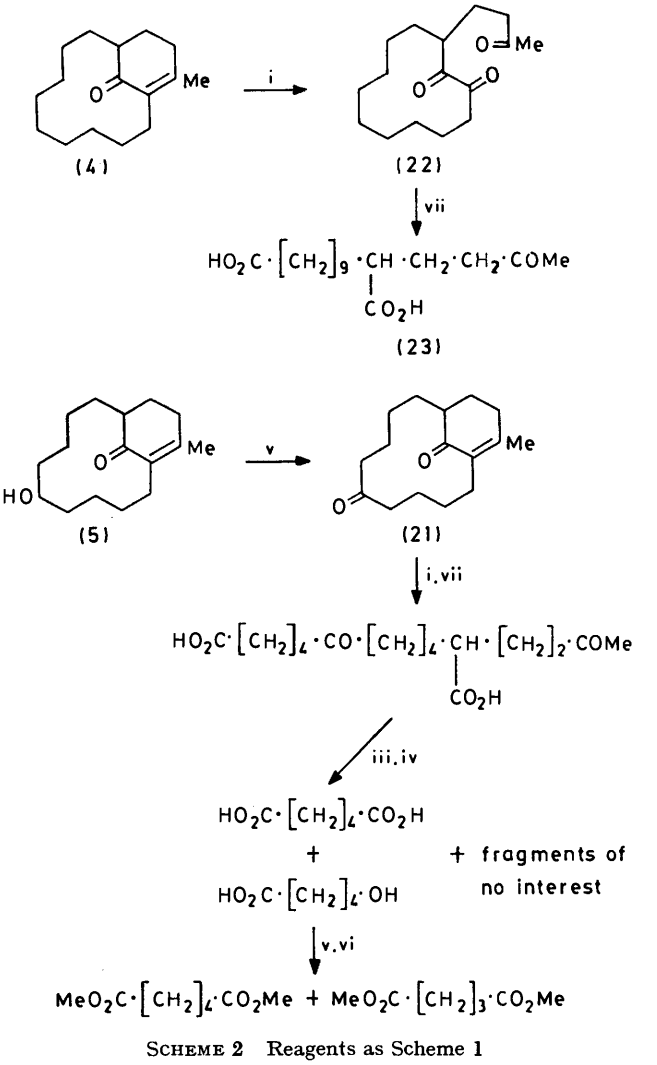


SCHEME 1 Reagents: i, O_3; ii, H_2O_2–H_2O; iii, $CF_3{\cdot}CO_3H$; iv, NaOH–MeOH; v, CrO_3–Me_2CO; vi, CH_2N_2–Et_2O; vii, $Pb(OAc)_4$

Application of this procedure (Scheme 2) to compound (21) afforded dimethyl glutarate and adipate (ratio 1 : 3.8). The accompanying metabolite (6) was an oil, but oxidation afforded a crystalline dione which on degradation afforded dimethyl adipate and pimelate (ratio 1 : 1.0). The n.m.r. spectrum of the other metabolite (7) showed that hydroxylation of the methyl group had occurred and oxidation yielded the corresponding carboxylic acid, degradation of which led to a mixture of dimethyl adipate and pimelate. It seems likely, therefore, that compound (7) is formed by further hydroxylation of either (5) or (21).

The structures of the saturated diketones (9) and (10) were established by a double Baeyer–Villiger degradation (see Scheme 3). For example (9) ultimately yielded a mixture containing dimethyl glutarate, adipate, and pimelate (ratio 0.2 : 1.0 : 0.8), and compound (10) afforded dimethyl adipate, pimelate, and suberate (ratio 0.2 : 1.0 : 0.8); the ratios of the esters suggested 80% secondary alkyl migration in the second Baeyer–Villiger oxidation.

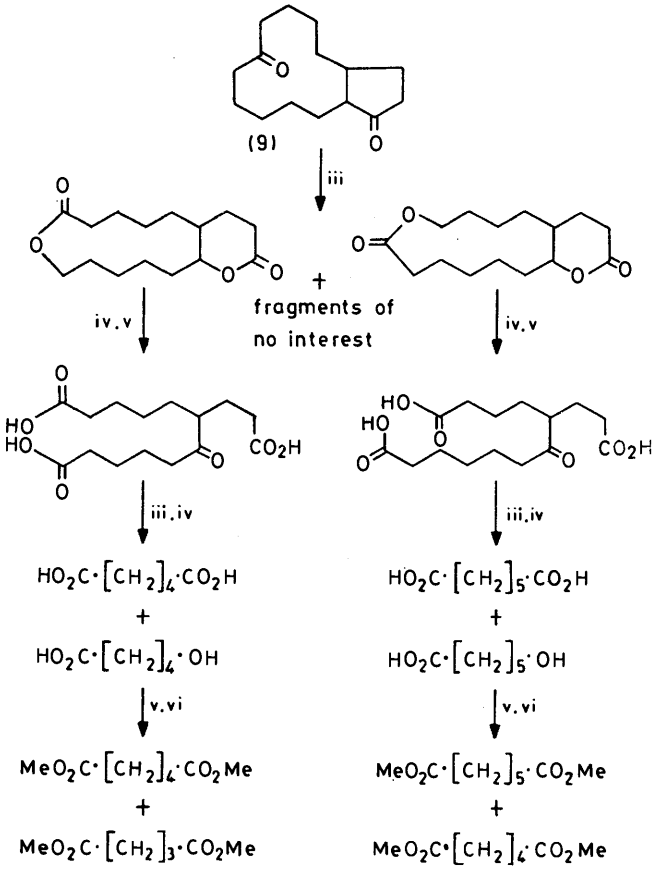
Compounds (13) and (14) were obtained as a non-crystalline mixture consisting (p.l.c.) of *ca.* 75% of (13) and *ca.* 25% of (14). The mixture of ketones was hydrogenated to saturate the C=C bond, and then followed a double Baeyer–Villiger degradation akin to that shown in Scheme 3 for the cyclopentanone-derived compounds. The resulting dimethyl esters (succinate, glutarate, adipate, and pimelate) were in the ratio 0.06 : 0.46 : 1 : 0.59. On the assumption that 80% secondary alkyl migration occurs in the second Baeyer–Villiger oxidation, a mixture of 70% (13) and 30% (14) would give the observed ratio of esters.



SCHEME 2 Reagents as Scheme 1

The triones (16) and (17) had neither α- nor β-diketone groupings; hence introduction of both oxygen functions had occurred in the larger ring. Standard degradation of (16) afforded a 1 : 1 mixture of

dimethyl glutarate and adipate but no dimethyl succinate. The spectral data of the trione (17) were very similar to those of compound (16), apart from an n.m.r. signal at τ 6.84 (1 H) suggesting the close proximity of two of the carbonyl groups. Degradation of the trione (17) gave dimethyl succinate, glutarate, and adipate (0.7 : 1.6 : 1.0). Since hydroxylation of monocyclic ketones [1] and also of compounds (1), (4), and (8) occurs at carbon atoms remote from the C=O group it might be expected that the initial attack on compound (15) would be at carbon atom 6 or 7, and would be



SCHEME 3 Reagents as Scheme 1

followed, since degradation of the triones affords adipic acid, by hydroxylation 5 carbon atoms away from the first hydroxy-group. This suggests the two structures (16) and (17) for the triones; the low-field triplet observed in the n.m.r. spectrum of compound (17) is assigned to C(12)H.

The yields of simple hydroxylation products from all these bicyclic substrates were disappointing; substantial quantities of more polar (polyhydroxylated?) materials were formed, much more than had been observed in the hydroxylation of perhydrochrysenes and steroids and particularly with the monocyclic ketones.[2] Attempts to improve yields by variation of conditions were not considered to be justifiable. It is possible that the substrates containing an oxygen-substituted six-membered ring are converted by alternative dehydrogenation processes into phenolic materials which would be very prone to further hydroxylation. The presence of an angular methyl group as in the steroids might afford a useful degree of protection in such cases.

In all these incubations initial monohydroxylation has occurred at carbon atoms remote from the supposed binding site. The flexibility of the macrocycles renders it impossible precisely to estimate the HO · · · CO distances but they are probably in the range 7—9 Å, distances which are comparable to those involved in the hydroxylation of steroids and of perhydrochrysenes.[7] With the macrocyclic ketones and *rac*-D-homogonane derivatives, *Calonectria decora* and *Rhizopus nigricans* did not give the slightest indication of stereoselectivity leading to optical activity. Encountering quite sizeable rotations in some of the products in the present study is intriguing, but also exasperating in view of the relative inaccessibility of the products.

EXPERIMENTAL

General directions and instruments used have been described.[2,3] The mycelium was collected by filtration through a salt bed then soaked (24 h) twice in acetone. The acetone was removed by evaporation and the residue partitioned between chloroform and water, the organic layer yielding the ' mycelial extract.' The aqueous filtrate from the mycelium was saturated with salt and either extracted by shaking with ethyl acetate (extraction A) or continuously extracted with ether (extraction B). The usual abbreviated form will be used to describe incubations.[3]

Standard Degradation Procedures.—A stirred solution of the ketone (5—100 mg) in AcOH (5 ml) and EtOAc (5 ml) was cooled (−10 °C) and ozonised oxygen (5—8%; flow rate 18 l h^{-1}) passed through the solution (8 min). Water (1 ml) was added followed by hydrogen peroxide (0.15 ml; 30%) if oxidative work-up was needed. After 18 h at room temperature ether (120 ml) was added, and the extract was washed with a little water and evaporated. Baeyer–Villiger oxidations with trifluoroperacetic acid, hydrolysis of the lactone mixtures, separations, oxidations, and esterifications (CH_2N_2) were carried out as described for the monocyclic ketones.[2]

Incubation of Bicyclo[10.4.0]*hexadec-12-en-14-one* (1) *with* Rhizopus nigricans.—The ketone (1) (4 g) in EtOH (100 ml) (200 flasks; 4 days) gave a broth extract (2.10 g) (extraction A) and mycelial extract (2.88 g). Chromatography (silica) of the mycelial extract [EtOAc–petrol (1 : 4)] gave s.m. (594 mg). Oxidation (CrO_3–Me_2CO) of the broth extract followed by p.l.c. (Et_2O; 2 runs) of the product gave 2 bands. The less polar band (148 mg) gave *bicyclo*[10.4.0]-*hexadec-12-ene-7,14-dione* (2), prisms (76 mg) (EtOAc–hexane), m.p. 128.5—130°, $[\alpha]_D^{20}$ −71° ($CHCl_3$) (Found: C, 77.5; H, 9.7. $C_{16}H_{24}O_2$ requires C, 77.4; H, 9.7%); $\nu_{max.}$ 1 715, 1 680, and 1 628 cm^{-1}; $\lambda_{max.}$ 239 nm (ε 15 500); τ ($CDCl_3$) 4.19 (1 H, s, HC=C), 6.92—7.50 (2 H, complex), 7.60—8.30 (12 H, complex), and 8.38—8.55 (9 H); *m/e* 248 (M^+, 50%), 163 (10%), 136 (35%), 110 (77%), 79 (59%), and 41 (100%). The more polar band (505 mg) gave the *6,14-dione* (3), needles (244 mg) (EtOAc–hexane), m.p. 133—

[7] M. J. Ashton, A. S. Bailey, and Sir Ewart R. H. Jones, *J.C.S. Perkin I*, 1974, 1658.

135°, $[\alpha]_D^{20}$ +1.2° ($CHCl_3$) (Found: C, 77.3; H, 9.6%); $\nu_{max.}$ 1 718, 1 680, and 1 625 cm^{-1}; $\lambda_{max.}$ 239 nm (ε 14 200); τ ($CDCl_3$) 4.14 (1 H, s, HC=C), 7.38—8.00 (9 H, complex), 8.04—8.40 (6 H, complex), and 8.55br (8 H); *m/e* 248 (M^+, 57%), 163 (11%), 135 (31%), 110 (100%), 79 (49%), and 41 (74%). Incubation of the ketone (1) (1 g) in EtOH (100 ml) (50 flasks; 3 days) with *Calonectria decora* gave (extraction A) a broth extract (0.94 g) and a mycelial extract (0.95 g). The mycelial extract gave s.m. (60 mg). Chromatography (SiO_2; Et_2O) of the broth extract gave a yellow oil (178 mg). This oil was oxidised ($Na_2Cr_2O_7$–AcOH) and the product separated (p.l.c.; Et_2O; 2 runs) affording compound (3) (90 mg) as the only material identified.

3-(2-*Oxocyclododecyl*)*propanoic Acid* (19).—The ketone (1) (100 mg) was ozonised. The ethereal solution of the crude product was extracted with 2M-NaOH, and the aqueous extract acidified and extracted (Et_2O). The extract was evaporated to 1 ml and petrol added, yielding (19), needles (83 mg), m.p. 104—104.5° (lit.,[4] 103°), $\nu_{max.}$ (Nujol) 3 300—2 500 and 1 710 cm^{-1}.

Degradation of Compounds (2) *and* (3).—The dione (2) (50 mg) was ozonised and the product (50 mg) oxidised (Baeyer–Villiger) giving an oil (40 mg); $\nu_{max.}$ 3 300—2 500, 1 741, 1 716, 1 177, and 1 155 cm^{-1}. The oil was hydrolysed and separated giving a neutral fraction (8 mg) and an acidic fraction (32 mg). The acidic fraction was oxidised and the product esterified (CH_2N_2–Et_2O). This product contained dimethyl glutarate and adipate (g.l.c.–mass spectrometry). Oxidation and esterification of the neutral fraction gave an oil containing dimethyl succinate (8 mg). The dione (3) (100 mg) afforded a mixture of the dimethyl esters of adipic and pimelic acids (g.l.c.–mass spectrometry). The neutral fraction (2 mg) in this degradation was discarded.

Incubation of Compound (4) *with* Calonectria decora.—The ketone (2 g) in EtOH (100 ml) (50 flasks; 4 days; extraction A) gave a broth extract (1.54 g) and a mycelial extract (1.06 g). P.l.c. (EtOAc–petrol) of the mycelial extract gave s.m. (327 mg). The broth extract was separated by p.l.c. [6 plates; Et_2O–petrol (3 : 1); 5 runs]. The fastest running band (81 mg) was recrystallised (EtOAc–hexane) to give 6-*hydroxy*-12-*methylbicyclo*[9.3.1]*pentadec*-11-*en*-15-*one* (5) (37 mg), needles, m.p. 149.5—150°, $[\alpha]_D^{20}$ +49° (EtOH) (Found: C, 76.6; H, 10.3. $C_{16}H_{26}O_2$ requires C, 76.8; H, 10.4%); $\nu_{max.}$ 3 620, 1 670, and 1 632 cm^{-1}; $\lambda_{max.}$ 246 nm (ε 10 000); τ ($CDCl_3$) 6.42br (1 H, m, C*H*·OH), 7.22—8.00 (5 H, complex), 8.13 (3 H, s, Me), and 8.21—9.00 (17 H); *m/e* 250 (M^+, 100%), 235 (15%), 232 (56%), 217 (22%), and 67 (56%). The middle band gave the ketol (6) (106 mg) as an oil not obtained crystalline. The slow running band gave 12-*hydroxymethylbicyclo*[9.3.1]-*pentadec*-11-*ene*-5,15-*dione* (7) (68 mg). Recrystallisation (EtOAc–hexane) gave prisms (35 mg), m.p. 122.5—123° (Found: C, 72.7; H, 9.1. $C_{16}H_{24}O_3$ requires C, 72.7; H, 9.1%); $\nu_{max.}$ ($CHCl_3$) 3 550—3 440, 1 710, and 1 668 cm^{-1}; τ ($CDCl_3$) 5.65 and 5.83 (2 H, AB system, *J* 14 Hz, CH_2·OH), 6.98—7.51 (6 H, complex, CH_2·CO and OH), 7.72—8.18 (8 H, complex), and 8.38—9.25 (8 H, complex); *m/e* 264 (M^+, 15%), 246 [82%; *m** 229 (264 ⟶ 246)], 228 [25%; *m** 211.5 (246 ⟶ 228)], 121 (64%), 91 (63%), and 41 (100%).

2-(3-*Oxobutyl*)*dodecanedioic Acid* (23).—The ketone (4) (2.0 g) was ozonised to give the diketone (22), bright yellow oil (1.4 g); $\nu_{max.}$ 1 729, 1 715, and 1 167 cm^{-1}. This diketone (266 mg) in HOAc (5 ml; 90%) was stirred with $Pb(OAc)_4$ (0.6 g) at 55—60 °C for 1.5 h. Dilute H_2SO_4 (10 ml) was added and the mixture extracted (Et_2O). Evaporation gave solid (186 mg). The *oxo-acid* (23) formed needles (174 mg) (Et_2O–hexane), m.p. 82.5—84° (Found: C, 64.3; H, 9.5. $C_{16}H_{28}O_5$ requires C, 64.0; H, 9.3%); $\nu_{max.}$ ($CHCl_3$) 3 300—2 500 and 1 713 cm^{-1}; τ ($CDCl_3$) −1.2 (2 H, s, CO_2H), 7.45—7.76 (5 H, complex, C*H*·CO), 7.89 (3 H, s, Me), 8.08—8.55 (6 H, complex), and 8.74br (12 H); *m/e* 282 (2%, *M* − 18), 225 (22%), 168 (20%), 98 (97%), 43 (100%), and 41 (29%).

Degradation of Compounds (5)—(7).—Oxidation of the ketol (5) (37 mg) afforded the diketone (21). 12-*Methylbicyclo*[9.3.1]*pentadec*-11-*ene*-6,15-*dione* formed needles (35 mg) (EtOAc–hexane), m.p. 125—130° (Found: C, 77.1; H, 9.7. $C_{16}H_{24}O_2$ requires C, 77.4; H, 9.7%); $\nu_{max.}$ 1 712, 1 675, and 1 630 cm^{-1}; τ ($CDCl_3$) 6.95—8.03 (13 H, complex), 8.13 (3 H, s, Me), and 8.27—9.25 (8 H); *m/e* 248 (M^+, 100%), 230 (41%), 163 (73%), 147 (58%), and 79 (58%). This dione (24 mg) was ozonised and the product treated with $Pb(OAc)_4$ giving an oil (28 mg). Baeyer–Villiger oxidation and hydrolysis gave an acidic fraction which was esterified. G.l.c. showed the presence of dimethyl glutarate and adipate. The oily ketol (6) was oxidised giving 12-*methylbicyclo*[9.3.1]*pentadec*-11-*ene*-5,15-*dione*, prisms (EtOAc–hexane), m.p. 97—99° (Found: C, 77.5; H, 9.9. $C_{16}H_{24}O_2$ requires C, 77.4; H, 9.7%); $\nu_{max.}$ 1 710, 1 670, and 1 632 cm^{-1}; τ ($CDCl_3$) 6.93—8.10 (13 H, complex), 8.18 (3 H, s, Me), and 8.35—8.90 (8 H); *m/e* 248 (M^+, 60%), 230 (21%), 187 (36%), 121 (54%), and 43 (100%). This dione (38 mg) was degraded as described for its isomer (21), giving dimethyl esters (16 mg) of adipic and pimelic acids. The hydroxy-diketone (7) (20 mg) gave the corresponding acid (18 mg), plates, m.p. 180—196° (decomp.) (Me_2CO–hexane), decomposing on attempted recrystallisation; $\nu_{max.}$ ($CHCl_3$) 3 500—2 600, 1 712, 1 684, and 1 265 cm^{-1}; τ ($CDCl_3$) 0.70br (1 H, CO_2H), 7.12—7.47 (5 H, complex), and 7.60—8.65 (16 H); *m/e* 278 (M^+, 51%), 260 (88%), 232 (48%), and 91 (49%). The acid (5 mg) was degraded as above affording ultimately dimethyl adipate and pimelate (2 : 1).

Incubation of Bicyclo[10.3.0]*pentadecan*-13-*one* (8) *with* Calonectria decora.—The ketone (8) (2 g) in EtOH (100 ml) (50 flasks; 4 days; extraction A) gave a mycelial extract (1.47 g) and a broth extract (1.0 g). P.l.c. [EtOAc–petrol (1 : 4)] gave s.m. (640 mg). The broth extract was oxidised and p.l.c. [Et_2O–petrol (1 : 1); 2 runs] gave 2 bands. The less polar band gave the 6,13-*dione* (9) (150 mg), m.p. 75—91° (from hexane), $[\alpha]_D^{20}$ (EtOH) −4.5° (Found: C, 76.6; H, 10.1. $C_{15}H_{24}O_2$ requires C, 76.3; H, 10.2%); $\nu_{max.}$ 1 746 and 1 717 cm^{-1}; τ (CCl_4) 7.22—7.48 (2 H, m), 7.75—8.33 (10 H, complex), and 8.42—8.66 (12 H); *m/e* 236 (M^+, 28%), 218 (34%), 137 (25%), 109 (27%), and 83 (100%). The more polar band afforded the 5,13-*dione* (10) (65 mg). Recrystallisation (hexane) gave needles, m.p. 80—88° (Found: C, 76.3; H, 10.2%); $\nu_{max.}$ 1 746 and 1 717 cm^{-1}; τ (CCl_4) 4.40—7.44 (4 H, complex), 7.87—7.09 (4 H, complex), and 8.20—8.76 (16 H); *m/e* 236 (M^+, 48%), 218 (30%), 151 (31%), 150 (31%), 109 (39%), and 83 (100%). The ketone (8) (2 g) was incubated with *Rhizopus nigricans* yielding s.m. (503 mg). Jones oxidation of the broth extract followed by p.l.c. gave the diketones (9) (126 mg) and (10) (49 mg).

Degradation of Bicyclo[10.3.0]*pentadecane*-6,13-*dione* (9).—Baeyer–Villiger degradation of the diketone (9) (30 mg) gave a mixture of lactones (36 mg). This mixture was hydrolysed and the alkaline liquid made acidic, evaporated

to small bulk, and extracted (Et_2O). The extract was oxidised (CrO_3–Me_2CO) to give an oil (18 mg). This mixture was subjected to a second Baeyer–Villiger oxidation followed by hydrolysis, oxidation, and esterification. G.l.c. and g.l.c.–mass spectrometry showed the presence of dimethyl glutarate, adipate, and pimelate (0.2 : 1.0 : 0.8). Bicyclo[10.3.0]pentadecane-5,13-dione (10) (21 mg) was similarly degraded to give the dimethyl esters (12 mg) (g.l.c.–mass spectrometry) of adipic, pimelic, and suberic acids (0.2 : 1.0 : 0.8).

Incubation of Bicyclo[9.4.0]*pentadec*-1(11)-*en*-12-*one* (12) *with* Calonectria decora.—The ketone (12) (2 g) in EtOH (100 ml) (50 flasks; 4 days; extraction A) gave a broth extract (0.49 g) and mycelial extract (1.94 g). Oxidation and chromatography (Et_2O) gave s.m. (860 mg) and a mixture of diones (13) and (14) (131 mg) as an oil (Found: C, 76.8; H, 9.5. Calc. for $C_{15}H_{22}O_2$: C, 76.9; H, 9.4%); $\nu_{max.}$ 1 715, 1 673, and 1 620 cm^{-1}; τ (CCl_4) 7.54—7.86 (12 H, complex), 7.93—8.37 (6 H, complex), and 8.46—8.75 (4 H); *m/e* 234 (M^+, 100%), 191 (19%), 163 (42%), and 126 (45%). Chromatography of a small quantity (18 mg) of the mixture [1 plate; C_6H_6–CH_2Cl_2–Me_2CO–MeOH (80 : 80 : 8 : 2); 2 runs] gave 2 bands; the less polar gave an oil (13 mg) and the more polar an oil (4 mg). The mixture of diones (49 mg) was hydrogenated [EtOH (30 ml) + 2M-NaOH (2 drops); PtO_2 (10 mg); 1 atm; 24 h] giving an oil which was oxidised to a mixture of saturated diketones; $\nu_{max.}$ 1 718 cm^{-1}. A double Baeyer–Villiger degradation was carried out as described for compound (9), giving the dimethyl esters (31 mg) ($\nu_{max.}$ 1 748, 1 205, and 1 175 cm^{-1}) of succinic, glutaric, adipic, and pimelic acids (0.06 : 0.46 : 1.00 : 0.59).

Incubation of (1RS,12SR,14SR)-*Bicyclo*[10.4.0]*hexadecan*-14-*ol* (15) *with* Calonectria decora.—Compound (15) (2 g) in EtOH (100 ml) (50 flasks; 4 days; extraction B) gave a broth extract (1.06 g) and mycelial extract (1.76 g). The mycelial extract gave s.m. (690 mg). The broth extract was separated [p.l.c.; 3 large plates; Et_2O–petrol (2 : 1); 2 runs] into 6 bands. The two least polar bands (28 and 18 mg) were discarded. The next band gave the 2,7,14-*trione* (16) (68 mg), needles (Et_2O–Me_2CO), m.p. 93—97° (Found: C, 72.2; H, 9.1. $C_{16}H_{24}O_3$ requires C, 72.7; H, 9.1%); $\nu_{max.}$ 1 720 cm^{-1}; τ ($CDCl_3$) 7.50—7.75 (12 H, complex, CH_2·CO), 8.06—8.40 (10 H, complex, CH_2·CH_2·CO), and 8.70 (2 H); *m/e* 264 (M^+, 18%), 246 (10%), 123 (45%), 110 (40%), 55 (100%), and 41 (58%). The fourth band yielded the 6,11,14-*trione* (17) (84 mg), needles (Et_2O–Me_2CO), m.p. 135—140° (Found: C, 72.6; H, 9.3%); $\nu_{max.}$ 1 720 cm^{-1}; τ ($CDCl_3$) 6.84 (1 H, t, *J* 6 Hz, CO·C*H*·CH_2·CO), 7.02—7.97 (10 H, complex, CH_2·CO), 8.03—8.32 (8 H, complex, CH_2·CH_2·CO), and 8.62br (5 H); *m/e* 264 (M^+, 22%), 246 (6%), 123 (27%), 110 (33%), 55 (100%), and 41 (68%). The fifth band gave a trione (132 mg), needles (Et_2O–Me_2CO), m.p. 90—92° (Found: C, 72.8; H, 9.0%); $\nu_{max.}$ ($CHCl_3$) 1 710 cm^{-1}; τ ($CDCl_3$) 7.10—7.78 (12 H, complex), 7.90—8.34 (8 H, complex), 8.73 (2 H, t, *J* 6 Hz), and 8.96 (2 H, t, *J* 6 Hz); *m/e* 264 (M^+, 38%), 246 (30%), 136 (75%), 123 (39%), 110 (100%), and 55 (95%), which was not investigated further. The most polar band afforded a mixture (167 mg) of ketonic material, needles (Me_2CO–Et_2O), m.p. 95—130° (Found: C, 72.6; H, 9.0%); $\nu_{max.}$ 1 710 cm^{-1}; τ ($CDCl_3$) 7.15—7.90 (12 H, complex) and 7.95—8.90 (12 H, complex); *m/e* 264 (32%), 246 (21%), 136 (35%), 123 (66%), and 110 (100%), which was not investigated further.

Bicyclo[10.4.0]hexadecane-2,7,14-trione (16) (6 mg) was subjected to Baeyer–Villiger oxidation followed by the standard work up. G.l.c. showed the presence of dimethyl glutarate and adipate (1 : 1). Similar degradation of bicyclo[10.4.0]hexadecane-6,11,15-trione (17) afforded dimethyl succinate, glutarate, and adipate (0.7 : 1.6 : 1.0).

The ketone (18) (2.0 g) was incubated with *Calonectria decora* [EtOH (100 ml); 50 flasks; 4 days]. The mycelial extract yielded s.m. (295 mg); the broth extract was oxidised and then separated [p.l.c.; Et_2O–petrol (2 : 1); 2 runs] giving the triones (16) (54 mg) and (17) (177 mg).

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