Fungal Products. Part II.[1] Structure and Stereochemistry of the Acid $C_{18}H_{16}O_5$, a Degradation Product of Wortmannin

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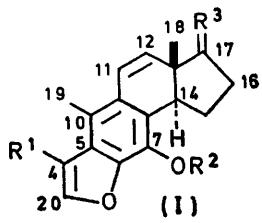
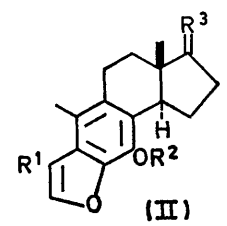
Degradation of the fungal metabolite, wortmannin, by dilute mineral acid yields, *inter alia*, an acid $C_{18}H_{16}O_5$ to which structure (Ia) is assigned from the spectroscopic properties of its derivatives. The assigned structure (Ia) is supported by dehydrogenation of the methyl ether methyl ester (Id) to methyl 2,3-dihydro-10-methoxy-6-methyl-3-oxo-1*H*-cyclopenta[7,8]naphtho[2,3-*b*]furan-7-carboxylate (VII), a previously described degradation product of the fungal metabolite, viridin (VI; X = O).

WORTMANNIN was first isolated from culture filtrates of *Penicillium wortmannii* Klocker by Brian *et al.*[2] but not characterised by them. In our hands wortmannin gave inconsistent combustion analyses but the molecular formula, $C_{23}H_{24}O_8$, was established by high resolution mass spectrometry. On treatment with boiling 2N-hydrochloric or -sulphuric acid, the metabolite yielded acetic acid (1 mol. equiv.), methoxyacetaldehyde (0·5 mol. equiv.), an acid $C_{18}H_{16}O_5$ (0·5 mol. equiv.) and an acid $C_{21}H_{22}O_7$ (0·5 mol. equiv.). This paper presents evidence [3] that the acid $C_{18}H_{16}O_5$ has the structure (Ia). Anticipating subsequent papers showing that wortmannin has the structure (IV) and that it has a steroidal biogenesis, we use the numbering system shown in formula (I) for the acid (Ia) and its derivatives.



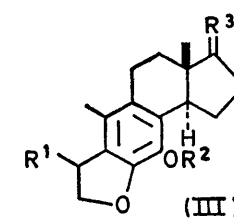
(I)

	R^1	R^2	R^3
a;	CO_2H	H	O
b;	CO_2Me	H	O
c;	CO_2Et	H	O
d;	CO_2Me	Me	O
e;	CO_2Me (16,16-$^2H_2$)	Me	O



(II)

	R^1	R^2	R^3
f;	CO_2H	Me	O
g;	CO_2Me	Ac	O
h;	CO_2Me	H	H,OH
i;	CO_2Me	Me	H,OH
j;	CO_2Me	Me	H,OMe

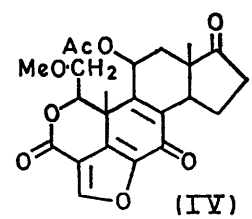


(III)

	R^1	R^2	R^3
k;	CO_2Me	Ac	H,OAc
l;	CH_2·OH	H	H,OH
m;	CH_2·OH	Me	H,OH
n;	CH_2·OAc	Ac	H,OAc

From the reaction of wortmannin and 2N-mineral acid, the acid $C_{18}H_{16}O_5$ was obtained as the free acid (Ia), the methyl ester (Ib), or the ethyl ester (Ic) depending upon whether the reaction was performed in aqueous, methanolic, or ethanolic acid. For convenience the methyl ester (Ib) was used for the structure determination. Functional derivatives of the methyl ester [compounds (I)], of the dihydro-methyl ester [compounds (II)], and of the tetrahydro-methyl ester [compounds (III)] were prepared as outlined in Scheme 1. The spectroscopic data are shown in Tables 1—5.

The i.r. spectra (Table 1) indicated the presence of benzenoid and furanoid rings in derivatives of types (I) and (II) and of a benzenoid, but no furanoid ring, in the tetrahydro-derivatives (III). These data further indicated the presence of one hydroxy- and two carbonyl groups in the methyl ester (Ib) itself. The hydroxy-group exhibited phenolic properties and the acetates (Ig, k, and n), (IIg), and (IIIg) showed carbonyl absorption typical of phenyl acetates. The presence of a benzenoid ring was also evident from the n.m.r. spectra (Tables 2—4), which contained typical aryl methyl absorption (10-Me) at τ 7·1—7·5 for all derivatives and typical aryl methyl ether singlets (7-OMe) around τ 6·0



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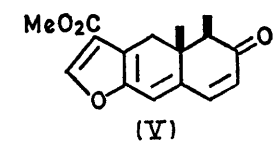

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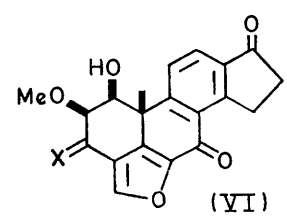

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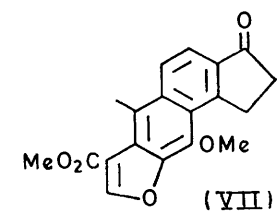


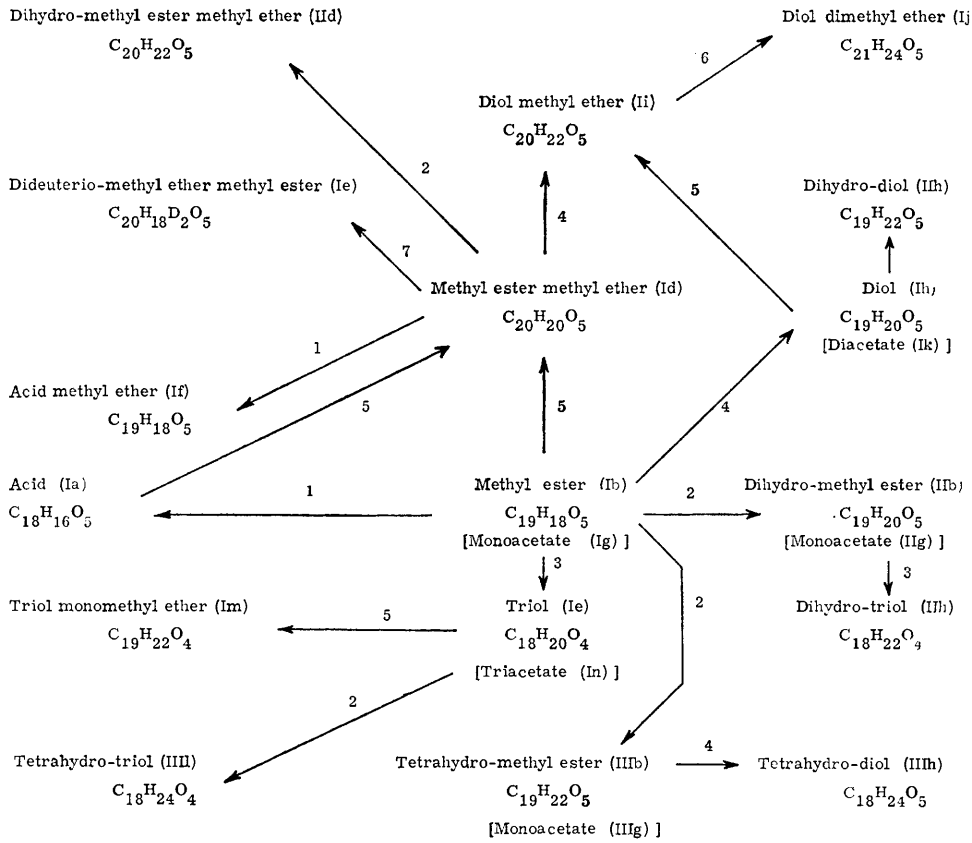
(VII)

for the derivatives (Id—f), (Ii, j, m), and (IId). The absence of aryl proton signals in the n.m.r. spectra of all

[1] Part I, D. M. Harrison and J. MacMillan, *J. Chem. Soc.* (*C*), 1971, 631.

[2] P. W. Brian, P. J. Curtis, H. G. Hemming, and G. F. L. Norris, *Trans. Brit. Mycol. Soc.*, 1957, **40**, 366.

[3] Preliminary communication, J. MacMillan, A. E. Vanstone, and S. K. Yeboah, *Chem. Comm.*, 1968, 613.



SCHEME 1 Reactions of the acid (Ia)

Reagents: 1, ^-OH; 2, Pd–$BaSO_4$; 3, $LiAlH_4$; 4, $NaBH_4$; 5, CH_2N_2; 6, MeI–Ag_2O; 7, NaOD

TABLE 1

I.r. absorption data (cm^{-1}) for the acid (Ia) and derivatives (Nujol mulls)

Compd.	ν(OH)	Furanoid ν(C–H)	Furanoid ν(C=C)	Benzenoid ν(C=C)	ν(C=O)
(Ia)	3480	*	1550	1620, 1590	1730, 1710
	3300—2600				
(Ib)	3450—3200	3150	1550	1620, 1580	1725
(Ib) †	3600, 3300br	3150	1550	1625, 1595	1740br
(Ic)	3600	3150	1520	1620, 1590	1730br
	3400—3200				
(Id)		3150	1550	1605	1740, 1720
(If)	3400—2500	*	1500	1620, 1580	1730, 1690
(Ig)		3160s	1550s	1620, 1580	1768, 1741, 1728
(Ih)	3490, 3200	3125	1540	1620, 1590	1710
(Ii)	3500	3140	1560		1710
(Ij)		3140	1550	1570	1720
(Ik)		3150s	1555s	1620w, 1580w	1770, 1742, 1725
(Il)	3500—3200	3050	1580w	1630w, 1600w	
(Im)	3300	*	1570	1610, 1590	
(In)				1620w, 1595w	1770, 1740 1715
(IIb)	3500—3200	3150	1540	1625, 1590	1720br
(IId)		3150	1550	1610, 1580	1735
(IIh)	3300br	3160	1545	1620, 1590	1735
(IIl)	3500, 3320	*		1620, 1595	
(IIg)		3150	1545		1770, 1725
(IIIb) †	3560, 3300br			1620w	1725
(IIIh) †	3570, 3400br			1625	1735, 1710
(IIIl) †	3560, 3300br			1620w, 1600w	
(IIIg) †				1610, 1600	1760, 1740

* Concealed by ν(OH). † For $CHCl_3$ solution.

derivatives showed that the benzene ring was fully substituted. The n.m.r. spectra of the derivatives (I) indicated a *cis*-disubstituted double bond. The vinylic AB-system (11-H and 12-H) had a J_{AB} value typical of a cyclohexene, showed no other coupling, and was not present after hydrogenation to the derivatives (II) and (III). Conjugation of this double bond to the aromatic system was revealed by the differences between the u.v. spectra (Table 5) of the derivatives (I) and those of their dihydro-counterparts (II). The u.v. data also showed that the furan ring was conjugated to the benzene ring; the dihydro-triol (III), in which both the methoxycarbonyl and olefinic functions were reduced, possessed the absorption of a highly substituted benzofuran. Also the tetrahydro-derivatives in which the furan ring was reduced (see later) showed the absorption expected of a fully substituted catechol.

The low-field one-proton singlet below τ 2·0 in the n.m.r. spectra of compounds (Ia—k) and (IIb, d, g, and h) could be assigned to the furanoid 20-proton, deshielded by the adjacent methoxycarbonyl group. Warburgin (V) [4] contains a similar proton absorbing at τ 1·96. In agreement with this assignment, reduction of the methyl ester grouping with lithium aluminium hydride [*e.g.* (Ih) ⟶ (Il)] caused an upfield shift (*e.g.* 0·5 p.p.m.)

[4] C. J. W. Brooks and G. H. Daffern, *Chem. Comm.*, 1966, 393.

of this singlet to higher field (*e.g.* τ 2·23); the similar proton in viridiol (VI; X = H,β-OH) [5] absorbs at τ 2·23. In the tetrahydro-derivatives (III) the low-field singlet of the 20-proton is replaced by an AB-system of the 20-protons further coupled to the 4-proton. Thus the furan ring is reduced in the tetrahydro-derivatives (III).

signal moved upfield by about 0·10 p.p.m. on hydrogenation of the olefinic double bond in compounds (Ib, h, and e) and it moved downfield by 0·25 p.p.m. on reduction of the carbonyl group in compounds (Ib) and (IIb). The new one-proton triplet in the n.m.r. spectra of the borohydride reduction products (Ih—n), (IIh, l) was assigned

TABLE 2

Measured chemical shifts (τ) and multiplicity (*J* in Hz) of protons in the 100 MHz n.m.r. spectra [a] of compounds (I)

Compound	20-H [b]	11-H [c]	12-H [c]	14-H [d]	17-H [e]	3-OMe [b]	7-OMe [b]	13-Me [b]	10-Me [b]	Other signals
(Ia) [f]	1·65	3·25	3·75	6·90				9·26	7·34	
(Ib)	1·83	3·20	3·56	6·74		6·12		9·18	7·30	4·8 (OH)
(Ib) [g]	1·61	3·15	3·44	6·60		6·27		9·08	7·18	5·66 and 8·61 (OEt); 4·56 (OH)
(Ic)	1·89	3·25	3·61	6·80				9·17	7·33	
(Id)	1·82	3·20	3·57	6·80		6·10	5·98	9·20	7·30	
(Ie)	1·80	3·20	3·60	6·80		6·10	5·90	9·18	7·30	
(If)	1·68	3·25	3·60	6·80			6·02	9·20	7·30	
(Ig)	1·90	3·23	3·60	6·80		6·18		9·20	7·27	7·63 (OAc)
(Ih) [g]	1·63	3·15	3·35	6·80	5·54	6·28		8·82	7·14	
(Ii)	1·94	3·35	3·75	7·15	5·95	6·20	6·15	9·30	7·36	
(Ij)	1·90	3·26	3·65	7·12	6·34	6·18	6·12	9·28	7·34	6·62 (17-OMe)
(Ik)	1·96	3·36	3·72	7·03	5·02	6·20		9·26	7·32	7·70 and 7·97 (2 × OAc)
(Il) [g]	2·23	3·18	3·40	6·72	5·56			8·82	7·33	4·95 (CH_2·O)
(Im)	2·55	3·37	3·77	7·40	5·94		6·14	9·30	7·30	
(Im) [g]	2·20	3·26	3·45	6·90	5·62		6·10	8·90	7·34	4·96 (CH_2·O)
(In)	2·25	3·32	3·66	6·98	4·94			9·23	7·47	7·62 (1 × OAc); 7·91 (2 × OAc)

[a] For $CDCl_3$ solutions except where stated otherwise. [b] Singlet. [c] Doublet (*J* 10—10·5). [d] Triplet (*J* 9·5) except for (Ib) (quartet, *J* 6·0 and 12·5). [e] Triplet (*J* 8·0—9·0). [f] For [$^2H_6$]acetone solution. [g] For [$^2H_5$]pyridine solution.

Reduction of the methoxycarbonyl and olefinic functions caused upfield shifts of 0·14—0·20 and 0·12—0·4 p.p.m., respectively, in the n.m.r. signal of the aryl

TABLE 3

Measured chemical shifts (τ) and multiplicity (*J* in Hz) of protons in the 100 MHz n.m.r. [a] spectra of compounds (II)

Compound	20-H [c]	3-OMe [c]	13-Me [c]	10-Me [c]	Other signals
(IIb)	1·88	6·12	9·19	7·41	
(IIb) [b]	1·60	6·27	9·16	7·30	
(IId)	1·83	6·10	9·20	7·37	6·00 (7-OMe)
(IIg)	1·85	6·10	9·19	7·30	7·59 (7-OAc)
(IIh) [b]	1·62	6·28	8·91	7·26	5·90 (17-H) [d]
(III) [b]	2·23		8·92	7·47	5·90 (17-H) [d]

[a] For $CDCl_3$ solutions except where otherwise indicated. [b] In C_5D_5N. [c] Singlet. [d] Triplet (*J* 9·5).

methyl group, indicating the proximity of these two groups. The proximity of the tertiary methyl group in

to the 17-oxymethine proton, its multiplicity revealing the presence of two hydrogens adjacent to the carbonyl group in the methyl ester (Ib). These 16-hydrogen atoms were readily exchanged in the methyl ether (Id) to give the dideuterio-compound (Ie). Compounds (Ia—g) showed carbonyl absorption in the range 1725—1740 cm^{-1} (Table 1) consistent with the location of this carbonyl group in a five-membered ring. The latter feature was supported by the mass spectrum of the methyl ester (Ib), which showed intense ions at $M^+ - 56$ and $M^+ - 57$, characteristic of ring D fragmentation in 17-oxosteroids.[6] Furthermore, the mass spectra of both the methyl ether methyl ester (Id) and its 16,16-dideuterio-derivative (Ie) contained intense ions at *m/e* 284 ($C_{17}H_{16}O_4$, $M^+ - 56$ and $M^+ - 58$, respectively) and at *m/e* 283 ($C_{17}H_{15}O_4$, $M^+ - 57$ and $M^+ - 59$, respectively). The mass spectra of the derivatives (I)—(III) are discussed in more detail later.

TABLE 4

Measured chemical shifts (τ) and multiplicity (*J* in Hz) of protons in the 100 MHz n.m.r. spectra [a] of compounds (III)

Compound	20-H	4-H	3-OMe	13-Me	17-Me	Other signals
(IIIb)	5·14 (*J* 4 and 9) 5·38 (*J* 9 and 9)	5·74 (*J* 4 and 9)	6·26	9·17	7·82	
(IIIg)	5·14 (*J* 4 and 9) 5·39 (*J* 9 and 9)	5·72 (*J* 4 and 9)	6·25	9·18	7·85	7·69 (7-OAc)
(IIIh)	5·12 (*J* 4 and 9) 5·39 (*J* 9 and 9)	5·74 (*J* 4 and 9)	6·25	9·25	7·82	6·17 (17-H)
(IIIl)	4·92 (*J* 1·5 and 9) 5·36 (*J* 8 and 9)	*ca.* 6·2 [b]		8·91	7·84	*ca.* 5·9 (17-H) [b] *ca.* 5·9, 6·2 (3-H_2) [b]

[a] For $CDCl_3$ solutions except for (IIIl) (in [$^2H_5$]pyridine). [b] In overlapping multiplets.

the methyl ester (Ib) to both the olefinic double bond and the second carbonyl group was likewise inferred from the n.m.r. data. Thus, in C_5D_5N solution, the tertiary methyl

The environment of the 14-hydrogen atom was established from the n.m.r. spectra of compounds (Ia—n) (see Table 2). The chemical shift was typical of a benzylic

[5] J. S. Moffat, J. D. Bu'Lock, and Tse Hing Yuen, *Chem. Comm.*, 1969, 839.

[6] H. Budzikiewic and C. Djerassi, *J. Amer. Chem. Soc.*, **1962**, **84**, 1430.

methine proton and its triplet or quartet multiplicity revealed the presence of two vicinal hydrogen atoms, the resulting AMX system being clearly identified in the 220 MHz spectrum (Figure) of the dideuterio-compound (Ie) in benzene solution. A comparison of this spectrum with the 220 MHz spectrum of the undeuteriated

TABLE 5

U.v. data for ethanolic solutions of derivatives of the acid (Ia)

Compound	$\lambda_{max.}$/nm (ε)			
(Ib)		233·5 (27,800)	262 (17,280)	286 (8050)
(IIb)	209 (41,430)	238 (17,270)	275 (3620)	299 (2540)
(IIIb)	208 (28,600)	234 * (8910)		295 (2220)
(Id)		236 (34,000)	256 (18,700)	287 (6400)
(IId)	208 (44,400)	233 (21,540)	265 (5640)	290(2560)
(Ig)		235·5 (36,800)	272·5 (13,200)	285 (8800)
(IIg)	210 (23,340)	228 (12,000)	257 (3700)	285 (900)
(IIIg)	206 (30,500)	232 * (5580)		292 (2360)
(Ih)		233 (33,000)	262·5 (19,250)	
(IIh)	210 (40,150)	238 (15,950)	255·5 (6880)	280 (3280)
(IIIh)	209·5 (25,410)	232 * (8250)		290 (1650)
(Il)		246 (25,000)	273 (15,000)	283 (10,000)
(IIl)		221 (37,500)	261 (11,000)	287 (2500)
(IIIl)	209·5 (30,400)	236 * (8080)		288 (1440)

* Shoulder.

compound (Id) showed that the 15-hydrogen atoms were further coupled to the exchangeable 16-hydrogen atoms in the methyl ether methyl ester (Id). This information provided final proof for the five-membered ring.

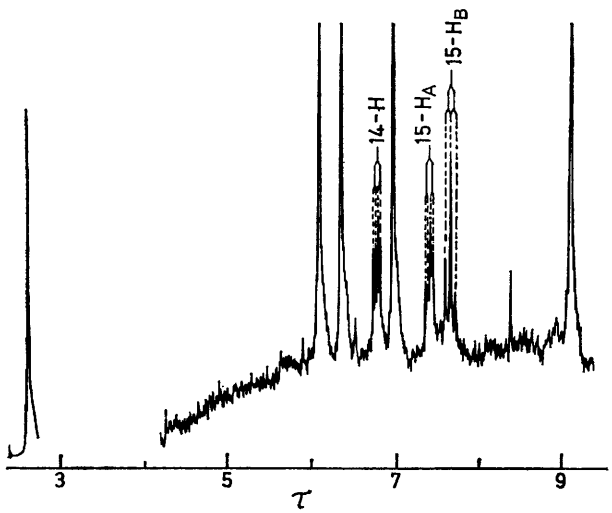
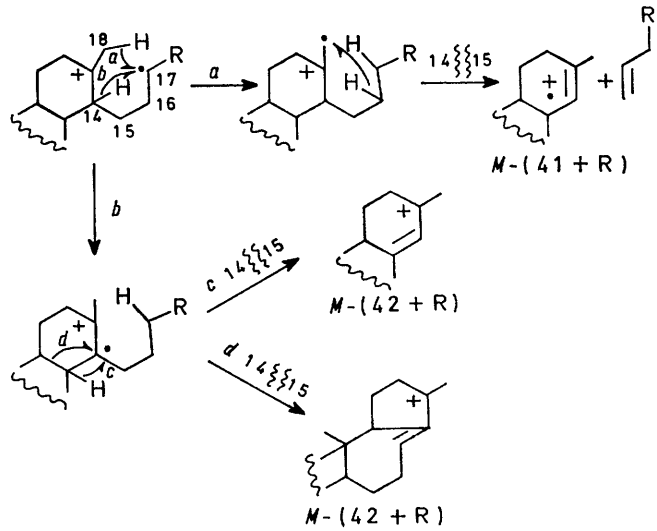


FIGURE 220 MHz n.m.r. spectrum of compound (Ie) in benzene

The absolute stereochemistry, shown in structure (I) for C/D ring junction was established by the o.r.d. behaviour of the dihydro-derivative (IId). The molecular amplitude (+134°) was typical for *trans*-14α-17-oxosteroids, such as 3β-hydroxy-5α-androstan-17-one (+138°); that for *cis*-14β-17-oxosteroids[7] is much lower (*ca.* +35°).

The foregoing chemical and spectroscopic data provided conclusive evidence for structure (Ib) for the



SCHEME 2 Steroidal ring D fragmentation

methyl ester obtained by degradation of wortmannin. This structure (Ib) was, however, confirmed by dehydrogenation of the methyl ester methyl ether (Id) to the naphthofuran (VII), identical with the methyl ether methyl ester obtained[8] by methylation of the hydrogen peroxide oxidation product of the fungal metabolite, viridin (VI; X = O).

The mass spectral fragmentation of ring D in steroids has been studied in detail by Tokes *et al.*[9] As shown in Scheme 2 (path *a*) the ion $M^+ - (41 + R)$ involves two hydrogen transfers (18 ⟶ 17 and 16 ⟶ 18), followed by fission of the 14,15-bond. The ion $M^+ - (42 + R)$ arises by hydrogen transfer to the uncharged fragment in a random process from the 8β-, 12β-, 14α-, and 18-positions but mainly from the 14α-position (path *b*) to give either the unrearranged (path *c*) or rearranged (path *d*) ions shown in the Scheme. In the case of the wortmannin derivative (Id), formation of the $M^+ - (41 + R)$ (*i.e.* $M^+ - 56$) ion does not involve transfer of hydrogen from the 16-position, since both deuterium atoms in the derivative (Ie) remain with the unchanged fragment. Also in the formation of the $M^+ - 57$ [*i.e.* $M^+ - (42 + R)$] ions from the derivatives (I) hydrogen must be exclusively transferred from position 18. The high abundance of the $M^+ - 56$ and $M^+ - 57$ ions in the spectra of the derivatives (I) is ascribed both to the

[7] W. Klyne, *Tetrahedron*, 1961, **13**, 29.
[8] J. S. Moffatt, *J. Chem. Soc.* (*C*), 1966, 725.
[9] L. Tokes, G. Jones, and C. Djerassi, *J. Amer. Chem. Soc.*, 1968, **90**, 5465.

allylic nature of positions 13 and 14 which facilitate fission of the 13,17- and 14,15-bonds and to the aromaticity of the resulting ion. Thus these ions are of insignificant intensity in the spectra of the corresponding dihydro-derivatives (II).

Other notable fragmentations of the derivatives (I)—(III) are listed but, in the absence of definitive evidence, speculative fragmentation pathways are not presented. Of the derivatives (I), the 17-ketones are distinguished from their 17-hydroxy-analogues by the presence of $M^+ - 43$ ions, shown by mass-matching to occur by loss of C_2H_3O. Since this loss is also shown by the dideuterio-compound (Ie) and by the acid (Ia), this fragmentation probably represents a rearrangement involving the elimination of the 17-carbonyl and 18-methyl groups. The 17-hydroxy-derivatives (Ih, i, l, and m) and (IIh and l) show intense $M^+ - 44$ ions with accompanying intense ions at *m/e* 43 in the case of compounds (Ih, i, and m). Metastable ions for the transition $M^+ \longrightarrow M^+ - 44$ were observed in each case. The $M^+ - 44$ ions show metastable ions for their fragmentation to $M^+ - 59$ ions and their formation apparently involves the 17-hydroxy-group since the 17-methoxy-compounds show an intense $M^+ - 58$ ion in place of the $M^+ - 44$ ion. The dihydro-ketones show substantial $M^+ - 69$ and $M^+ - 71$ ions while the corresponding dihydro-17-hydroxy-derivatives show $M^+ - 71$ and $M^+ - 73$ ions. The four tetrahydro-derivatives (IIIb, h, l, and g) gave intense ions at *m/e* 161 ($C_{10}H_9O_2$), 85, and 83. Their spectra also contained ions corresponding to $M^+ - R'$.

EXPERIMENTAL

M.p.s were determined on a Kofler hot-stage apparatus and are corrected. N.m.r. spectra were determined with tetramethylsilane as internal standard on Varian HA100 and Varian HA220 instruments. U.v. spectra were obtained for solutions in ethanol on a Unicam SP 800 spectrometer. I.r. spectra were obtained for Nujol mulls except where stated otherwise with a Unicam SP 200 or Perkin-Elmer 257 spectrometer. Mass spectra were obtained for probe samples on an A.E.I. MS9 instrument. G.l.c. was performed on a Pye 104 chromatograph with glass columns (5 ft × 0·25 in) packed with 2% SE-33, 2% QF-1 or 2% OV-1 coatings on acid-washed and silanised Gas-Chrom A.

Acid Hydrolyses of Wortmannin.—(a) *With aqueous hydrochloric acid in methanol.* Wortmannin (2·0 g), methanol (50 ml), and 1·5N-hydrochloric acid (25 ml) were boiled for 5 h in a stream of nitrogen. No volatile carbonyl compounds (2,4-dinitrophenylhydrazine trap) and no carbon dioxide (barium hydroxide trap) were detected. After 18 h, the crystalline *methyl ester* (Ib) was collected; it recrystallised from methanol in needles (730 mg), m.p. 219—220° (Found: C, 69·9; H, 5·5%; M^+, 326·115. $C_{19}H_{18}O_5$ requires C, 69·9; H, 5·5%; *M*, 326·115); *m/e* 326(100%), 284(20), 283(24), 270(58), 269(51), 251(25), 237(56), 210(16), 182(9), 181(11), 153(12), 76(12), 69(15), 44(27), and 43(11).

The filtrate was extracted with chloroform which, in turn, was extracted with aqueous sodium carbonate. The recovered neutral oil (130 mg) yielded the methyl ester (Ib) in needles (60 mg). The recovered acidic material yielded a yellow foam (930 mg), crystallised from ether to yield the acid ($C_{21}H_{22}O_7$) as pale yellow needles (827 mg), m.p. 236—238° (Found: M^+, 386·134. Calc. for $C_{21}H_{22}O_7$: *M*, 386·136).

(b) *With aqueous hydrochloric acid in ethanol.* Wortmannin (650 mg), ethanol (30 ml), and 1·5N-hydrochloric acid (30 ml) were boiled for 5 h under nitrogen; work-up as in (a) gave the acid $C_{21}H_{22}O_7$ and the *ethyl ester* (Ic), needles (210 mg), m.p. 231—235° (from methanol) (Found: M^+, 340·130. $C_{20}H_{20}O_5$ requires *M*, 340·131).

(c) *With aqueous sulphuric acid: identification of acetic acid.* Wortmannin (425 mg) and 1·5N-sulphuric acid were heated on a water bath for 5 h under nitrogen. The *acid* (Ia) was collected; m.p. 253—256° (from methanol) (Found: M^+, 312·099. $C_{18}H_{16}O_5$ requires *M*, 312·099).

The filtrate was steam-distilled. Titration of the steam distillate with standard potassium hydroxide indicated 0·91 mol. equiv. of a monobasic acid. The alkaline distillate was evaporated to dryness and the residue in aqueous ethanol was boiled for 1 h with *p*-bromophenacyl bromide to give *p*-bromophenacyl acetate, plates (from ethanol), m.p. and mixed m.p. 82—83°.

The residue from steam-distillation was extracted with chloroform. The acidic portion of the extract gave a yellow gum, crystallising from ether in rosettes, m.p. 232—234° identical (mixed m.p. and i.r. spectrum) with the acid $C_{21}H_{22}O_7$ obtained in (a).

(d) *Identification of methoxyacetaldehyde.* As in experiment (a), the *methyl ester* (Ib) was filtered off. The filtrate was steam-distilled and the distillate treated with 2,4-dinitrophenylhydrazine sulphate in ethanol. The precipitated methoxyacetaldehyde 2,4-dinitrophenylhydrazone crystallised from ethanol in orange plates, m.p. 123·5—124·5°, identified by mixed m.p. and by i.r. and n.m.r. spectroscopy.

Derivatives of the Acid (Ia).—(i) *The methyl ether methyl ester* (Id). Prepared from the methyl ester (Ib) and diazomethane, it crystallised from ethanol in *needles*, m.p. 156—158° (Found: M^+, 340·131. $C_{20}H_{22}O_5$ requires *M*, 340·131); $\nu_{max.}$ ($CHCl_3$) 3150, 1740, 1620, 1580, and 1550 cm^{-1}; *m/e* 340(100%), 326(47), 325(44), 297(34), 284(67), 283(66), 282(34), 281(31), 270(47), 269(35), 268(30), 267(25), 237(66), 181(31), 165(32), 153(36), 152(36), 139(21), and 44(60). The methyl ether methyl ester was also prepared (*a*) from the acid (Ia) and diazomethane, and (*b*) by boiling the methyl ester (Ib) with dimethyl sulphate and 2N-sodium hydroxide for 2 h.

(ii) *The 16,16-dideuterio-methyl ether methyl ester* (Ie). To the foregoing methyl ether methyl ester (116 mg) in acetonitrile (7 ml) was added potassium hydroxide (5·5 mg) in deuterium oxide (3·5 ml) and the mixture was stirred under nitrogen at 45—50° for 4 days. Extraction of the purple solution with ether, and recovery, afforded the 16,16-*dideuterio-derivative* (Ie) as pink needles, m.p. 144—150° (from methanol) (Found: M^+, 342·142. $C_{20}H_{18}D_2O_5$ requires *M*, 342·143); *m/e* 342(100%), 341(23), 327(27), 284(62, $C_{17}H_{16}O_4$ and/or $C_{17}H_{12}D_2O_4$), 283(63, $C_{17}H_{15}O_4$ and/or $C_{18}H_{15}D_2O_3$), 268(23), and 237(42).

(iii) *The methyl ether* (If). The methyl ether methyl ester (Id) (90 mg), methanol (10 ml) and 2N-potassium hydroxide (16 ml) were heated under nitrogen at 60° for 4·5 h. After extraction with methylene dichloride, the solution was acidified and the acidic fraction was recovered in methylene dichloride as a foam (90 mg). Preparative

t.l.c. on pre-washed silica gel with 3% acetic acid in methylene dichloride gave the *methyl ether* (If), needles (42 mg), m.p. 189—192° (from ether) (Found: M^+, 326·115. $C_{19}H_{18}O_5$ requires M, 326·115).

(iv) *The acetate* (Ig). Prepared by heating the methyl ester (Ib) (50 mg) in acetic anhydride (1 ml) and pyridine (3 drops) for 15 min on a water-bath, the *acetate* (Ig) crystallised from methanol in needles (39 mg), m.p. 236—237·5° (Found: M^+, 368·125. $C_{21}H_{20}O_6$ requires M, 368·126); $\nu_{max.}$ ($CHCl_3$) 3150, 1768, 1736, 1625, 1580, and 1552 cm^{-1}; *m/e* 368, 326, 311, 283, 270, 269, and 237.

(v) *The diol* (Ih). To the methyl ester (Ib) (367 mg) in ethanol (30 ml), sodium borohydride (150 mg) was added in portions. After stirring at 20° for 15 min water (30 ml) and 2N-sulphuric acid (5 ml) were added. The usual work-up gave the *diol* (Ih), plates (350 mg), m.p. 228—229° (from ethyl acetate) (Found: M^+, 328·132. $C_{19}H_{20}O_5$ requires M, 328·131); $\nu_{max.}$ ($CHCl_3$) 3600, 3350br, 1720, 1630, and 1593 cm^{-1}; *m/e* 328(79%), 313(14), 311(19), 310(36), 298(19), 297(20), 295(34), 284(100), 281(20), 271(56), 270(58), 269(77), 257(46), 256(64), 255(21), 237(56), 210(20), 192(22), 182(22), 181(27), 165(22), 153(33), 152(35), 139(21), 128(22), 115(23), 95(19), and 43(21).

(vi) *The diol monomethyl ether* (Ii). To the methyl ether methyl ester (Id) (100 mg), in ethanol (15 ml), was added with stirring a suspension of sodium borohydride (100 mg) in ethanol (15 ml). After 10 min at 20°, the usual work-up gave the *diol monomethyl ether* (Ii) as needles (96 mg), m.p. 170—173° (from ether) (Found: M^+, 342·149. $C_{20}H_{22}O_5$ requires M, 342·147). When the reaction was performed at 20° for 20 min a mixture of the diol monomethyl ether and the triol monomethyl ether (Im) (see later) was obtained.

(vii) *The diol dimethyl ether* (Ij). Dry silver oxide (400 mg) was added in portions during 1 h to a boiling solution of the diol monomethyl ether (Ii) (35 mg) in methyl iodide. After heating under reflux for 17 h, the silver oxide was filtered off and washed with methylene chloride. Recovery from the filtrate and washings gave the *diol dimethyl ether* (Ij) (28 mg), m.p. 102—104° (from ether) (Found: M^+, 356·164. $C_{21}H_{24}O_5$ requires M, 356·162); *m/e* 356(100%), 325(27), 324(29), 309(30), 298(50), 285(34), 283(64), 270(52), 268(22), 237(58), 181(20), 165(24), 153(24), 152(22), 44(28), 43(20), and 41(30).

(viii) *The diol diacetate* (Ik). Prepared by heating the diol (Ih) in acetic anhydride and pyridine at 100° for 1 h, *the diacetate* (Ik) was obtained as rods, m.p. 180—183° (from methanol) (Found: M^+, 412·153. $C_{23}H_{24}O_7$ requires M, 412·152); *m/e* 412, 381, 370, 352, 328, 327, 310(100%), and 295.

(ix) *The triol* (Il). Lithium aluminium hydride (500 mg), suspended in tetrahydrofuran (100 ml) was added in portions to a stirred solution of the methyl ester (Ib) (500 mg) in tetrahydrofuran (50 ml). After 1 h at 20° then 1 h under reflux, ethyl acetate, water, and 2N-sulphuric acid were added. The usual work-up gave a yellow gum (380 mg) which crystallised from ethyl acetate to give the *triol* (Il), needles, m.p. 211—213° (Found: M^+, 300·136. $C_{18}H_{20}O_4$ requires M, 300·136); *m/e* 300(92%), 282(39), 267(26), 256(100), 243(40), 242(34), 241(68), 228(49), and 223(67).

(x) *The triol monomethyl ether* (Im). The methyl ester (Id) (20 mg) in ether (6 ml) was added slowly to a stirred suspension of lithium aluminium hydride (14 mg) in ether. After 1 h at 20°, ethyl acetate and ethanol were added with vigorous stirring. The mixture was then poured over 2N-sulphuric acid and ice. Recovery in ether gave the *triol monomethyl ether* (Im) (17 mg), m.p. 208—210° (from ether) (Found: M^+, 314·151. $C_{19}H_{22}O_4$ requires M, 314·152).

(xi) *The triol triacetate* (In). Prepared from the triol (Il), acetic anhydride, and pyridine at 100° for 30 min, it was obtained as an intractable yellow oil (Found: M^+, 426·167. $C_{24}H_{20}O_7$ requires M, 426·167); $\lambda_{max.}$ 242, 259, 270, and 281 nm (ε 45,900, 16,120, 9350, and 6800); *m/e* 426(35%), 384(60), 343(10), 324(100), 309(35), 282(27), 281(13), 265(20), 263(11), 249(40), 237(15), 225(12), and 223(12).

(xii) *The dihydro-methyl ester* (IIb). The methyl ester (Ib) (220 mg), 10% palladium–barium carbonate (75 mg), and ethyl acetate (50 ml) were shaken at 20° for 10 min with hydrogen. The usual work-up gave needles (220 mg), shown by g.l.c. (2% OV-1; 239°) to be a mixture of the dihydro- (IIb) (80%) and tetrahydro- (IIIb) (20%) compounds. Recrystallisation from methanol gave the *dihydro-compound* (IIb) as needles m.p. 196—198° [containing no tetrahydro-compound (IIIb) by g.l.c.] (Found: M^+, 328·133. $C_{19}H_{20}O_5$ requires M, 328·131); $\nu_{max.}$ ($CHCl_3$) 3580, 3300br, 3150w, 1725, 1620, 1595, and 1545 cm^{-1}; *m/e* 328(100%), 326(2·5), 313(21), 297(10), 285(55), 272(40), 271(48), 259(38), 257(22), and 43(28).

(xiii) *The dihydro-methyl ether* (IId). The methyl ether (Id) (35 mg), in ethyl acetate (5 ml) was shaken at 18° for 2 h with hydrogen and 5% palladium–barium carbonate (30 mg). The usual work-up gave the *dihydro-compound* (IId), needles (30 mg), m.p. 146—148° (from methanol) (Found: M^+, 342·147. $C_{20}H_{22}O_5$ requires M, 342·147); $\nu_{max.}$ ($CHCl_3$) 3150, 1735, 1610, 1580, and 1550 cm^{-1}; *m/e* 342(100%), 327(17), 311(15), 299(21), 286(37), 285(24), 273(24), and 271(10).

(xiv) *The monoacetate* (IIg). Prepared from the dihydro-methyl ester (IIb) (40 mg), acetic anhydride (1 ml), and pyridine (2 drops), the *acetate* (IIg) crystallised from methanol in prisms (36 mg), m.p. 201—203° (Found: M^+, 370·143. $C_{21}H_{22}O_6$ requires M, 370·141); *m/e* 370(14%), 328(100), 313(11), 285(30), 272(19), 271(21), 259(18), 257(8), and 43(17).

(xv) *The dihydro-diol* (IIh). The dihydro-compound (IIb) (50 mg), in methanol (10 ml), was stirred at 20° for 10 min with sodium borohydride (50 mg). The usual work-up gave the *dihydro-diol* (IIh) as needles (40 mg), m.p. 215—217° (from methanol) (Found: M^+, 330·149. $C_{19}H_{22}O_5$ requires M, 330·147); *m/e* 330(100%), 315(27), 297(30), 286(31), 271(83), 259(30), 257(18), 243(19), and 219(19).

(xvi) *The dihydro-triol* (IIl). To a suspension of lithium aluminium hydride (46 mg) in tetrahydrofuran (10 ml) was added the dihydro-methyl ester (IIb) (54 mg). The mixture was stirred at 20° for 45 min then under reflux for 45 min. The usual work-up gave the *dihydro-triol* (IIl), needles (40 mg) m.p. 219—221° (from ethyl acetate) (Found: M^+, 302·155. $C_{18}H_{22}O_4$ requires M, 302·152); *m/e* 302(100%), 287(26), 269(27), 258(17), 244(16), 243(53), 237(18), 229(9), 191(10), and 43(52).

(xvii) *The tetrahydro-methyl ester* (IIIb). The methyl ester (Ib) (120 mg), in ethyl acetate (25 ml), was shaken with hydrogen and 10% palladium–barium sulphate (125 mg) at 20° for 1 h. The usual work-up gave the *tetrahydro-derivative* (IIIb) as a gum (110 mg) showing a single g.l.c. peak on a 2% OV-1 column at 240° (Found: M^+, 330·147. $C_{19}H_{22}O_5$ requires M, 330·147); *m/e* 330(100%), 328(27), 326(16), 301(11), 300(18), 299(6), 298(21), 287(19), 285(10),

283(12), 273(17), 272(15), 271(50), 269(15), 261(15), 241(24), 161(70), 85(60), 83(100), 47(27), 43(40), and 41(34).

The acetate (IIIg), prepared with acetic anhydride in pyridine, was also an intractable gum (Found: M^+, 372·157. $C_{21}H_{24}O_6$ requires M, 372·155); *m/e* 372(18%), 330(100), 287(11), 271(30), 161(67), 85(10), 83(16), and 43(>100).

(xviii) *The tetrahydro-diol* (IIIh). The tetrahydro-methyl ester (IIIb) (30 mg) in methanol (3 ml) was stirred at 20° for 15 min with sodium borohydride (30 mg). The usual work-up gave the *tetrahydro-diol* (IIIh) as an intractable gum (33 mg) (Found: M^+, 332·162. $C_{19}H_{24}O_5$ requires M, 332·161); *m/e* 332(100%), 330(12), 273(40), 258(74), 257(23), 256(32), and 161(64).

(xix) *The tetrahydro-triol* (IIIl). The triol (Il) (20 mg), in ethyl acetate (12 ml), was hydrogenated as before to give a gum (18 mg) which, on trituration with chloroform, gave the *tetrahydro-triol* (IIIl) as an amorphous solid (Found: M^+, 304·167. $C_{18}H_{24}O_4$ requires M, 304·166); *m/e* 304(53%), 302(5), 273(74), 161(100), 85(37), 83(58), 47(19), and 43(14%).

Alkaline Hydrolysis of the Methyl Ester (Ib).—The methyl ester (200 mg), methanol (15 ml) and 2N-potassium hydroxide (20 ml), were heated at 90° for 3 h. The usual work-up gave an acidic solid (117 mg), identical (m.p. and i.r. spectrum) with the acid (Ia) already described and obtained from wortmannin by aqueous acidic hydrolysis.

Dehydrogenation of the Methyl Ether Methyl Ester (Id).—An intimate mixture of the methyl ether methyl ester (62 mg) and 20% palladium–charcoal (72 mg) was heated at 190° for 40 min under nitrogen. Heating was continued at 196—200° for 1 h. Extraction of the cooled mixture with ether, then recovery, gave a pink gum (44 mg). A portion (16 mg) of this product was subjected to preparative t.l.c. on silica gel G, pre-washed with benzene–ethyl acetate (3 : 1) and re-activated at 100° for 0·5 h, and developed with benzene–ethyl acetate (3 : 1). The band, fluorescing blue and corresponding in R_F value to the authentic derivative (VII) from viridin,[8] was collected and extracted with ether. Recovery gave methyl 2,3-dihydro-10-methoxy-6-methyl-3-oxo-1*H*-cyclopenta[7,8]naphtho[2,3-*b*]furan-7-carboxylate (VII), as yellow needles (1 mg), m.p. 236—238° (sublimation from 190°) (from ethanol), not depressed on admixture with the viridin derivative (m.p. 235·5—237°). The i.r. spectrum (Nujol) ($\nu_{max.}$ 3150, 2990, 2970, 2850, 1735, 1730, 1710—1690, 1625, 1604, 1510, 1465, 1440, 1405, 1390, 1370, 1330, 1305, 1272, 1250, 1152, 1136, 1090, 1075, 1024, 990, 918, and 900 cm^{-1}) and g.l.c. retention times (10·5 min on 2% SE-33 at 238° and 75 ml N_2 min^{-1}; 11·8 min on 2% QF-1 at 238° and 60 ml N_2 min^{-1}) were identical with those of the viridin derivative (VII).

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