Fungal Products. Part III.[1] Structure of Wortmannin and Some Hydrolysis Products

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Structure (II) is proposed for the fungal metabolite, wortmannin. The absolute stereochemistry is deduced except for that at C-1, which remains undefined. The structure of the previously described acid, $C_{21}H_{22}O_7$, obtained by acidic degradation, is determined, together with structures of the products of hydrolysis by potassium carbonate and by potassium hydrogen carbonate.

In Part II [1] it was shown that wortmannin, a $C_{23}H_{24}O_8$ metabolite of *Penicillium wortmannii*, was degraded by aqueous 2N-mineral acid to acetic acid (1 mol. equiv.), methoxyacetaldehyde (0·5 mol. equiv.), the acid (I; R = H), and an acid $C_{21}H_{22}O_7$. Evidence is now presented [2] establishing structure (II) for wortmannin. On this basis, structures are deduced for the acid $C_{21}H_{22}O_7$ (III) obtained by acidic degradation, and for the products (VII) and (VIII) of alkaline hydrolysis.

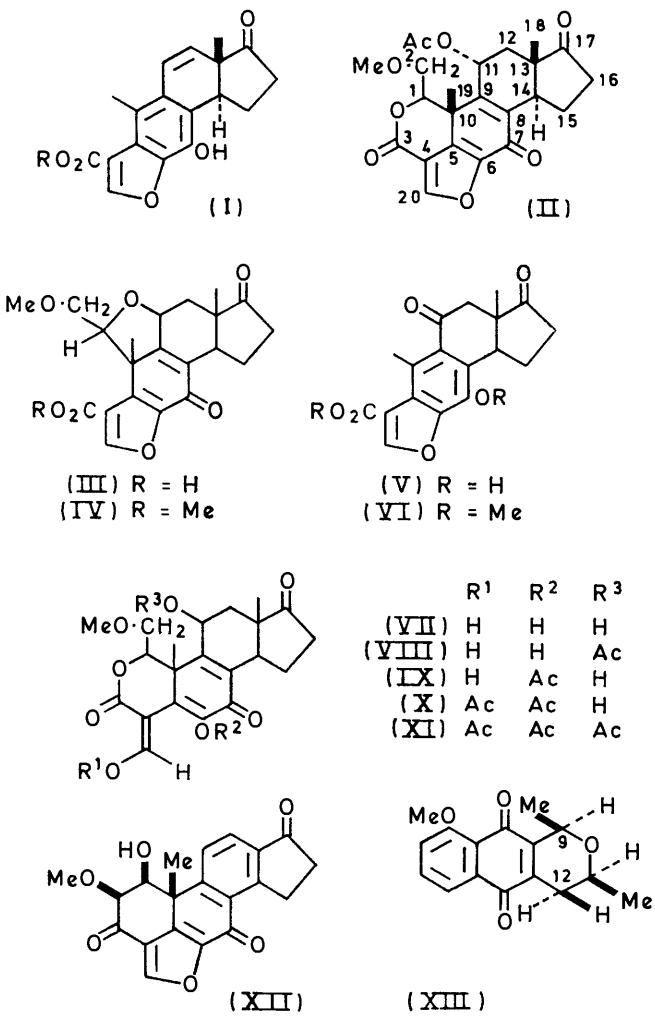
In the 220 MHz n.m.r. spectrum (Figure) of wortmannin in [^{2}H]chloroform solution, all twenty-four protons could be clearly assigned; their spin–spin interactions were established at 100 MHz by double irradiation (Tables 1 and 2). The four three-proton singlets were assigned to the 13-methyl (τ 9·06), the 10-methyl (8·29), the acetoxy- (7·91) and the methoxy- (6·87) protons. The one-proton singlet at τ 1·78 was of similar chemical shift to the furanoid proton in the acid (I; R = H) [1] and was assigned to the 20-proton in (II). An ABX-system at τ 7·02, 6·52, and 5·25 with J_{AB} 11, J_{AX} 2, and J_{BX} 6 Hz was assigned to the protons at C-2 and C-1. A double triplet at τ 3·9 was assigned to the C-11 proton, which showed an 8 Hz coupling to each of the C-12 protons at τ 7·3 and 8·4 ($J_{12A,12B}$ 12·5 Hz). In addition the 11-proton showed a homoallylic coupling of 3 Hz to the C-14 methine proton at τ 7·15. The 15-

[1] Part II, J. MacMillan, T. J. Simpson, A. E. Vanstone, and S. K. Yeboah, preceding paper.

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

proton signals were located at τ 6·8 and 7·8 and those of the 16-protons at τ 7·4 and 7·9.

From the foregoing n.m.r. data and the i.r. spectrum seven of the eight oxygen atoms in wortmannin were

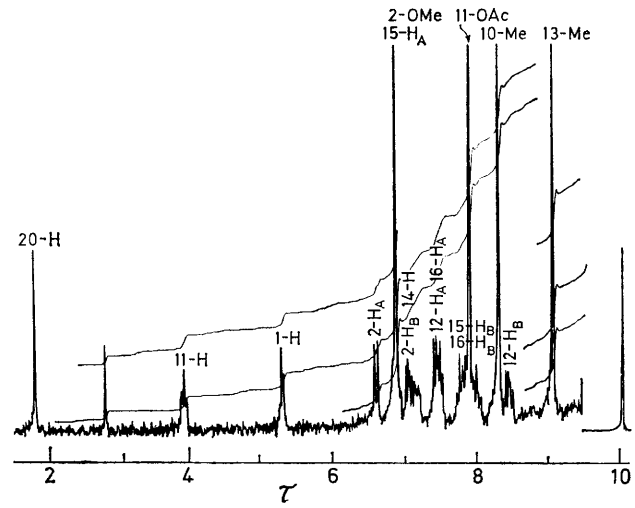


	R^1	R^2	R^3
(VII)	H	H	H
(VIII)	H	H	Ac
(IX)	H	Ac	H
(X)	Ac	Ac	H
(XI)	Ac	Ac	Ac

identified in the following functions: (*a*) five-membered ring ketone (1750 cm^{-1}); (*b*) an acetoxy-group (1732 cm^{-1}; formation of acetic acid on acidic hydrolysis; and three-proton singlet at τ 7·91); (*c*) an unsaturated carbonyl group (1684 and 1656 cm^{-1}) in a cyclohexadienone chromophore; (*d*) a saturated carbonyl group (1732 cm^{-1}) of the six-membered-ring lactone; and (*e*) a methoxy-group (n.m.r.) associated with the production of methoxyacetaldehyde on acidic degradation. The presence of a furanoid ring was indicated by absorption at 3110 (ν_{C-H}) and 1540 ($\nu_{C=O}$) cm^{-1}, accounting for the eighth oxygen function.

High resolution mass spectroscopy of wortmannin showed a weak molecular ion at *m/e* 428 with losses of CH_3 (*m/e* 413), $CH_3{\cdot}CO$ (*m/e* 385), $CH_3{\cdot}CO_2H$ (*m/e* 368), and $CH_3{\cdot}O{\cdot}CH_2{\cdot}CHO$ (*m/e* 354). The base peak at *m/e* 266 corresponded to the overall loss from the molecular ion of $CH_3{\cdot}CO_2H$, $CH_3{\cdot}O{\cdot}CH_2{\cdot}CHO$, and CO. Ions at *m/e* 45 ($CH_3{\cdot}O{\cdot}CH_2$) and 43 ($CH_3{\cdot}CO$) were also present. Unlike the acid (I) but like its 11,12-dihydro-derivative,[1] the mass spectrum of wortmannin showed only weak ions at $M^+ - 56$ and $M^+ - 57$ from the fragmentation of the cyclopentanone ring.

The foregoing spectroscopic data, together with the degradation by acid to methoxyacetaldehyde, acetic acid,

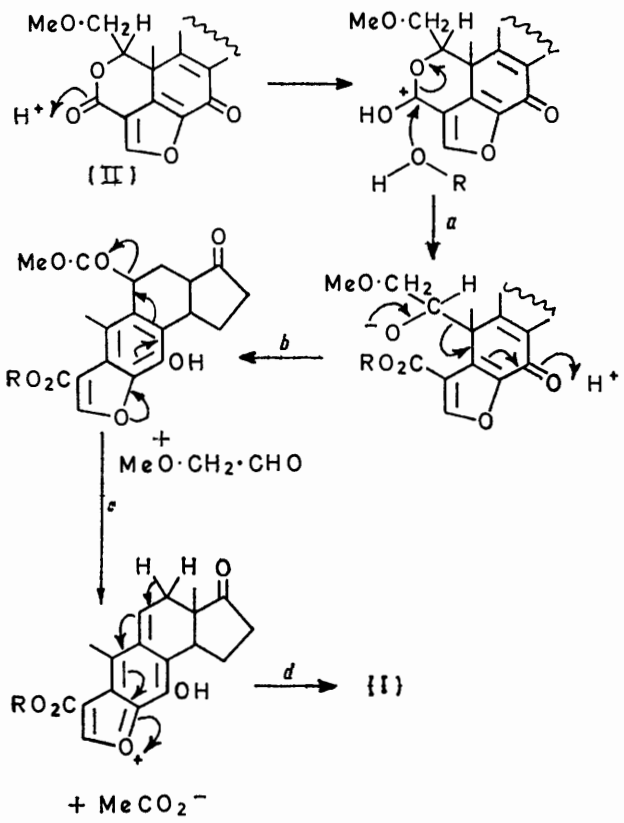


220 MHz N.m.r. spectrum of wortmannin (II) in [^{2}H]chloroform

and the acid (I), is satisfactorily accommodated in structure (II) for wortmannin. The conversion of wortmannin (II) into methoxyacetaldehyde and the acid (I; R = H) may be represented by the sequence in Scheme 1 where hydrolysis of the lactone by *O*-acyl fission (step *a*) leads to the observed [1] formation of the free acid (I; R = H), the methyl ester (I; R = Me), or the ethyl ester (I; R = Et) depending upon whether aqueous, methanolic, or ethanolic acid is used. In step *b* (Scheme 1) a vinologous retro-aldol reaction leads to the formation of methoxyacetaldehyde and aromatisation of ring B. Finally, loss of acetic acid is depicted as the expulsion of acetate ion by the lone pair on oxygen (step *c*) followed by loss of a 12-proton (step *d*). Before discussing the absolute stereochemistry shown for wortmannin in structure (II) it is necessary to consider the structures of the acid $C_{21}H_{22}O_7$ (III), obtained by acidic degradation, and of the products (VII) and (VIII) of alkaline hydrolyses of wortmannin.

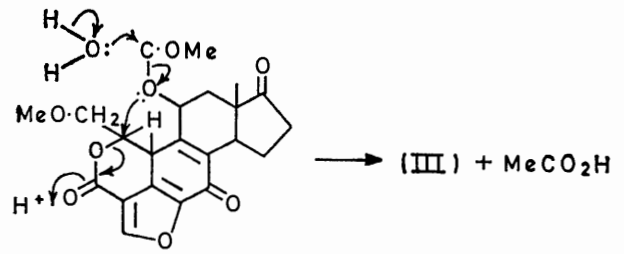
Formation of the acid $C_{21}H_{22}O_7$ from wortmannin involves the loss of the 11-acetyl group as shown by the n.m.r. spectrum [(III) Table 1]. However this loss was not a simple hydrolytic cleavage of the 11-acetoxy-group; the product was a carboxylic acid, forming a methyl ester which showed no hydroxy-absorption in the i.r. spectrum. The absence of alcoholic or enolic hydroxy-groups, together with the stability of the acid $C_{21}H_{22}O_7$ to further treatment with acid, suggested that the potential methoxyacetaldehyde grouping was part of an inert

ether linkage at positions 1 and 11 as in structure (III). This structure is in complete agreement with the spectroscopic data for the acid (III) and its methyl ester and, in particular, with the n.m.r. data (Tables 1 and 2). It is suggested that the formation of the acid (III) from



SCHEME 1

wortmannin (II) occurs by *O*-alkyl fission with participation of the 11-oxygen function as represented in Scheme 2. This mechanism accounts for the observation that,



SCHEME 2

unlike the acid (I) which is formed in the same reaction by *O*-acyl fission, the free acid is always obtained even in the presence of an alcohol as co-solvent. With refluxing N-sodium hydroxide, the acid (III) afforded in 2·5% yield an acidic product, $C_{18}H_{10}O_6$. The latter compound is formulated as the keto-acid (V) on the basis of the i.r., u.v., and n.m.r. (Tables 1 and 2 and Experimental section) spectra of the acid and its methyl ester methyl ether (VI). These spectra were similar to those of the acid (I) and its methyl ester methyl ether [1] except for the absence of 11-proton signals in the n.m.r. and the presence of a conjugated carbonyl absorption at 1668 cm^{-1} in the i.r. spectrum. This carbonyl group was shown to be conjugated to the benzenoid ring by the intensity of the $\nu_{C=C}$ absorption at 1600 cm^{-1} and by the change in the u.v. absorption spectrum of the methyl ester methyl ether on addition of sodium borohydride. This carbonyl group was thus placed at position 11, leading to structure (V). No satisfactory mechanism for the formation of the keto-acid (V) can be offered; opening of the ether bridge by attack of hydroxide or of the carboxylic anion at C-1 and subsequent retro-aldol reaction could lead to the 11-alcohol corresponding to (V) which might be oxidised by the methoxyacetaldehyde in a Cannizzaro reaction.

Mild alkaline hydrolysis of wortmannin opens the furan ring. With potassium carbonate the triol (VII) is obtained. The three hydroxy-groups were characterised by the formation of a triacetate (XI) showing enol acetate absorption at 1802 and 1774 cm^{-1} and three acetate methyl singlets in the n.m.r. spectrum (Tables 1 and 2). The enolic hydroxy-protons appeared at τ 2·74 and −3·50, the lower field signal being coupled to the C-20 proton at τ 1·34 with J 13·0 Hz. This coupling was removed by the addition of D_2O. Of the two possible stereochemical arrangements of the 4(20)-double bond the one shown is preferred from the i.r. (3270 cm^{-1}) and n.m.r. (τ −3·5) evidence for a strongly hydrogen-bonded 20-hydroxy-group, and by the shift of +0·55 p.p.m. of the 20-proton signal on acetylation of the 6-hydroxy-group [see (IX)]. The two enolic hydroxy-groups were readily acetylated by sodium acetate and acetic anhydride to yield the diacetate (X), which showed carbonyl absorption in the i.r. spectrum at 1804 and 1774 cm^{-1}, typical of enol acetates, and hydroxy-absorption at 3458 cm^{-1}. The n.m.r. data for the diacetate are listed in Tables 1 and 2. The diacetate was readily de-acetylated on silica gel layers to the monoacetate (IX), characterised by its i.r. and n.m.r. (Tables 1 and 2) spectra; the acetoxy-group was located at position 6 by the presence of an enol acetate carbonyl absorption (1772 cm^{-1}), by the presence of the enolic hydroxy-absorption at τ −3·5 similar to that of the 20-hydroxy-group in the triol (VII), and by the absence of the enolic hydroxy-signal at τ 2·74, assigned to the 6-hydroxy-group in the triol (VII). Coupling between the 20-proton and the 20-enolic hydroxy-proton was not observed in the monoacetate (IX) although the signal of the 20-proton at τ 1·89 was sharpened on addition of D_2O.

The triol (VII) was converted by boiling methanolic N-hydrochloric acid into the methyl ester of (I) and the acid (III), the known products of similar treatment of wortmannin. In this case, the methyl ester of (I) was

the minor product, lending support to the proposed step *d* (Scheme 1) in the formation of the acid (I) from wortmannin.

Hydrolysis of wortmannin (II) with N-potassium hydrogen carbonate gave the diol (VIII) as an intractable foam, characterised by t.l.c., n.m.r. spectrum (Tables 1 and 2), and acetylation to give the triacetate (XI) of the triol (VII). The presence of two enolic hydroxy-groups was shown by signals at τ 2·82 and −3·76, the acid (I) does not disturb the C/D ring junction (see Scheme 1), the same *trans*-13β-configuration is assigned to wortmannin (II). The replacement of an 11α-acetoxy-group by an 11α-hydroxy-group in *trans*-13β-steroids has been reported [5] to cause upfield shifts of the order of 0·03 p.p.m. in the n.m.r. signals of the 18-protons whereas for the corresponding change from 11β-OAc to 11β-OH downfield chemical shift differences of about 0·13 p.p.m. have been observed. The negligible

TABLE 1

Measured chemical shifts (τ) of protons in wortmannin (II) and derivatives [a]

Cpd.	1-H	2-H	11-H	12-H	14-H	20-H	18-H	19-H	2-OMe	3-OMe	7-OMe	20-OAc	6-OAc	11-OAc	20-OH	6-OH
(II) [b]	5·25	6·52, 7·02	3·90	7·30, 8·0	7·15	1·78	9·06	8·29	6·87	*c*	*c*	*c*	*c*	7·91	*c*	*c*
(III)	5·47	6·92, 7·04	4·88	7·75, 8·62	7·35	1·58	8·98	8·24	7·07	*c*	*c*	*c*	*c*	*c*	*c*	*c*
(IV)	5·40	6·95, 7·11	4·95	7·61, 8·58	7·42	1·87	8·99	8·27	7·07	6·17	*c*	*c*	*c*	*c*	*c*	*c*
(VI)	*c*	*c*	*c*	*d*	6·80	1·82	9·13	7·16	*c*	6·14	6·00	*c*	*c*	*c*	*c*	*c*
(VII)	4·74	6·46, 6·82	5·18	7·84, 8·08	*d*	1·34	9·16	8·44	6·77	*c*	*c*	*c*	*c*	*c*	−3·50	2·74
(VIII)	5·55	6·70, 6·90	4·04	7·66, 8·15	*d*	1·30	9·17	8·48	6·77	*c*	*c*	*c*	*c*	7·96	−3·76	2·86
(IX)	4·64	6·42, 6·63	5·24	7·84, 8·24	*d*	1·89	9·18	8·43	6·80	*c*	*c*	*c*	7·70	*c*	−3·60	*c*
(X)	4·50	6·45, 6·70	5·22	*d*, 8·49	*d*	1·37	9·11	8·35	6·83	*c*	*c*	7·81	7·76	*c*	*c*	*c*
(XI)	5·39	6·58, 6·78	4·03	*d*, 8·30	*d*	1·36	9·12	8·49	6·84	*c*	*c*	7·82	7·79	7·96	*c*	*c*

[a] In $CDCl_3$ except for compound (III) in $[(CD_3)_2CO]$. [b] 15-Protons at τ 6·80 and 7·80; 16-protons at τ 7·40 and 7·90. [c] Absent. [d] Not located in overlapping multiplets.

TABLE 2

Measured coupling constants (Hz) of protons in wortmannin (II) and derivatives [a]

Compd.	$J_{1,2}$	$J_{1,2'}$	$J_{2,2'}$	$J_{11,12}$	$J_{11,12'}$	$J_{12,12'}$	$J_{11,14}$	$J_{14,15}$	$J_{14,15}$
(II)	2·0	7·0	11·0	8·0	8·0	12·5	3·0	6·0	12·2
(III)	4·6	4·6	10·6	7·0	10·2	11·5	3·2	*b*	*b*
(IV)	4·0	4·0	10·0	7·0	10·2	11·5	3·0	*b*	*b*
(VII)	4·0	7·6	10·0	6·0	6·0	13·6	*b*	*b*	*b*
(VIII)	2·0	7·0	11·6	8·0	4·0	15·0	*b*	*b*	*b*
(IX)	4·0	8·8	10·0	7·0	7·0	13·8	*b*	*b*	*b*
(X)	3·0	9·0	10·0	9·0	9·0	12·0	2·5	*b*	*b*
(XI)	3·2	5·4	10·8	6·2	8·0	*b*	2·0	*b*	*b*

[a] In $CDCl_3$ solution except for compound (III) [in $(CD_3)_2CO$]. [b] Not determined in overlapping multiplets.

latter being coupled to the 20-proton at τ 1·30 (*J* 13 Hz). Treatment of the diol (VIII) with methanolic 2N-hydrochloric acid yielded a complex mixture from which the following compounds were identified by t.l.c.: wortmannin (II), the methyl ester of the acid (I), the acid (III), and the methyl ester (IV).

The ease of opening of the furan ring by mild alkaline treatment is due to the activation by the 3- and 7-carbonyl groups which assists nucleophilic attack at positions 6 and 20. In the closely related fungal metabolite, viridin (XII), the furan ring is also opened by alkali, as shown by the formation of formic acid.[3,4] The weak reducing properties of wortmannin towards Tollens reagent can be ascribed to the formation of the triol (VII) or diol (VIII), both of which rapidly reduce this reagent.

The *trans*-13β-configuration of the C/D ring junction in the acid (I) has been established [1] by o.r.d. studies. On the reasonable assumption that the formation of the

shifts (see Table 1) of the 18-protons in going from the 11-acetate (VIII) to the triol (VII), and from the triacetate (XI) to the diacetate (X), indicate a 11α-acetoxy-grouping in these derivatives and in wortmannin itself. A pseudoaxial (β) configuration for the 11-hydrogen in a half-chair cyclohexene is also consistent with the magnitude of the observed coupling to the 12-protons and to the 14α-proton (see Table 2). The latter value of 3·0 Hz is similar to that observed [6] for the coupling between the pseudoaxial 9- and 12-protons in eleutherin (XIII).

On the basis of an 11α-acetoxy-configuration, the formation of the cyclic ether (III) from wortmannin (II) (see Scheme 2) requires a 10β-methyl stereochemistry. The 10β-methyl configuration is supported by the observed differences in chemical shifts of the 1- and 2-protons on changing from an 11α-acetoxy- to an 11α-hydroxy-group (see Table 1). For the pairs (VIII) ⟶ (VII), (XI) ⟶ (X), and (II) ⟶ (IV), the observed

[3] J. F. Grove, P. McCloskey, and J. S. Moffatt, *J. Chem. Soc.* (*C*), 1966, 743.
[4] J. F. Grove, *J. Chem. Soc.* (*C*), 1969, 549.
[5] N. S. Bhacca and D. H. Williams, 'Applications of N.M.R. Spectroscopy,' Holden-Day, San Francisco, 1964, p. 19.
[6] D. W. Cameron, D. G. L. Kingston, N. Sheppard, and Lord Todd, *J. Chem. Soc.*, 1964, 98.

shifts are downfield and in the range 0·79—0·89 p.p.m. for the 1-proton and 0·12—0·14 for one of the 2-protons. Also the small downfield shifts of the 10-methyl signals in the change from the 11α-acetoxy- to 11α-hydroxy-substituent are consistent with a 10β-methyl group.

The foregoing arguments lead to the absolute stereochemistry shown for wortmannin (II). The stereochemistry at C-1 cannot be defined on the present evidence. A nuclear Overhauser increase in the integrated intensities of the 1- and 11-proton signals was not observed on irradiation of the 19-protons. This result is not surprising with adjacent 2- and 12-protons. Biosynthetic studies designed to confirm the proposed stereochemistry (II) and to elucidate the stereochemistry at C-1 are in progress.

EXPERIMENTAL

For general experimental details see Part II.[1]

Wortmannin.—The metabolite, provided by I.C.I. Ltd., Pharmaceuticals Division, was crystallised from methanol or benzene to give *needles*, m.p. 238—239°, $[\alpha]_D^{26}$ +89° (*c* 1·1 in $CHCl_3$) [Found (after crystallisation from methanol and drying *in vacuo* at 100° for 15 h): C, 63·5, 64·1, 63·9; H, 5·6, 5·9, 5·9%. Found (after crystallisation from benzene and drying *in vacuo* at 100° for 6 h): C, 64·8, 65·0, 65·4, 64·3; H, 5·7, 5·7, 5·6, 5·9%; M^+, 428·145. Found (after sublimation): C, 63·8, 63·6; H, 5·8, 5·8%. $C_{23}H_{24}O_8$ requires C, 64·5; H, 5·6%; *M*, 428·147], $\lambda_{max.}$ 257 and 292 nm (ε 11,770 and 7700); $\nu_{max.}$ 3112, 1751, 1732, 1684, 1656, and 1547 cm^{-1}; $\nu_{max.}$ ($CHCl_3$) 3150, 1750, 1683, 1653, and 1553 cm^{-1}; *m/e* 428 (0·1%, $C_{23}H_{24}O_8$), 413 (8·0, $C_{22}H_{22}O_8$), 385 (19·0, $C_{21}H_{21}O_7$), 368 (46·0, $C_{21}H_{20}O_6$), 354 (68, $C_{20}H_{18}O_6$), 323 (94, $C_{19}H_{15}O_5$), 294 (32, $C_{18}H_{14}O_4$), 268 (55), 266 (100, $C_{17}H_{14}O_3$), 239 (41), 238 (62), 223 (40), 45 (37), 44 (45), and 43 (55).

The *mono-2,4-dinitrophenylhydrazone* crystallised from acetic acid in orange needles, m.p. 266—268° [Found (after drying *in vacuo* at 100° for 12 h): C, 56·3; 56·5, 56·7; H, 4·6, 4·7, 5·1; N, 9·0, 8·9. $C_{29}H_{28}N_4O_{11}$ requires C, 57·2; H, 4·6; N, 9·2%]; $\lambda_{max.}$ 362 nm; $\nu_{max.}$ 3100, 1745br, 1675, 1650, 1620, 1590, 1550, and 1523 cm^{-1}.

The Acid $C_{21}H_{22}O_7$ (III).—Prepared as described in Part II,[1] the *acid* had m.p. 236—238° (Found: M^+, 386·134. $C_{21}H_{22}O_7$ requires *M*, 386·136); $\lambda_{max.}$ 258 and 298 nm (ε 9500 and 6900); $\nu_{max.}$ 3400, 2500, 3150, 1744, 1728, 1660, 1640, 1585, and 1540 cm^{-1}; $\nu_{max.}$ ($CHCl_3$) 3600, 2600, 1730, 1720sh, 1650, 1580, and 1540 cm^{-1}; *m/e* 386 (45%, $C_{21}H_{22}O_7$), 371 (100, $C_{20}H_{20}O_7$), 341 (8, $C_{19}H_{17}O_4$), 313 (23, $C_{18}H_{17}O_5$), 312 (12), 285 (11), 284 (14), 271 (13), 269 (14), 267 (16), 257 (22, $C_{15}H_{13}O_4$), 256 (11, $C_{15}H_{12}O_4$), 255 ($C_{15}H_{11}O_4$), 253 (10), 225 (5, $C_{15}H_{13}O_2$ and $C_{14}H_9O_3$, doublet), 193 (13), 45 (42), and 44 (11).

The Methyl Ester (IV).—Treatment of the acid (III) [1] in methanol with ethereal diazomethane gave a colourless foam which was homogeneous by t.l.c. and g.l.c. [Found: OMe, 17·4%; M^+, 400·152. $C_{22}H_{24}O_7$ requires OMe, 15·5% (for 2); *M*, 400·152]; $\nu_{max.}$ ($CHCl_3$) 3120, 1735, 1663, 1584, 1540, 970, and 840 cm^{-1}.

The Keto-acid (V).—The acid (III) (400 mg) in methanol (20 ml) and N-potassium hydroxide (20 ml) was boiled for 4 h in a stream of nitrogen. The mixture was acidified and extracted with chloroform. Recovery yielded a brown gum (280 mg) from which, by repeated crystallisation from methanol, was obtained the *keto-acid* (V) as plates (9 mg) subliming at 282·5° (Found: M^+, 328·093. $C_{18}H_{10}O_6$ requires *M*, 328·095); $\lambda_{max.}$ 240, 255, 275, and 335 nm (ε 21,450, 17,980, 6780, and 2680); $\lambda_{max.}$ (+0·1N-KOH) 238, 277, 298, and 384 nm (ε 17,040, 15,460, 5679, and 2996); $\lambda_{max.}$ (after addition of $NaBH_4$) 228, 275, and 295 nm (ε 16,000, 7500, and 5160); $\nu_{max.}$ 3300—2700, 1730—1715, 1663, and 1608 cm^{-1}; τ 1·34 (1H, s), 6·60 (3H, s), 6·3—7·9 (11H, m), and 9·04 (3H, s).

The Methyl Ester Methyl Ether (VI).—Obtained by treatment with diazomethane, this crystallised from methanol as *needles*, m.p. 235·5—236·5° [Found: OMe, 15·6%; M^+, 356·127. $C_{20}H_{20}O_6$ requires OMe, 17·4%; (for 2). *M*, 356·126]; $\lambda_{max.}$ (with and without added KOH) 235, 250sh, 270sh, and 317 nm (ε 26,700, 17,730, 7730, and 1395); $\lambda_{max.}$ (after addition of $NaBH_4$) 232, 249, and 268 nm (ε 13,350, 5830, and 3110); $\nu_{max.}$ 3140, 1720br, 1668, 1600, and 1540 cm^{-1}.

Potassium Carbonate Hydrolysis of Wortmannin; Preparation of the Triol (VII).—Wortmannin (200 mg), methanol (10 ml), and N-potassium carbonate (10 ml) were stirred at 18° for 5 h. Acidification with 2N-sulphuric acid then extraction with chloroform gave a yellow gum (185 mg) which crystallised from ether to give the *triol* (VII) as yellow needles, m.p. 160—161° (Found: M^+, 404·145. $C_{21}H_{24}O_8$ requires *M*, 404·147); $\lambda_{max.}$ 244, 275sh, and 398 nm (ε 18,050, 5490, and 7846); $\nu_{max.}$ 3510, 3460, 1744, 1670, 1655w, 1620, and 1580 cm^{-1}.

Potassium Hydrogen Carbonate Hydrolysis of Wortmannin; Preparation of the Diol (VIII).—A suspension of wortmannin (170 mg) in methanol (7 ml) was stirred at 18° for 10 min under nitrogen. N-Potassium hydrogen carbonate (1·5 ml) was added and stirring was continued at 18° for 2·5 h. Extraction of the red solution with chloroform yielded an intractable mixture (31 mg). After acidification with 2N-sulphuric acid, chloroform extraction gave the diol (VIII) as an intractable foam (137 mg); $\lambda_{max.}$ 246, 275sh, and 410 nm (ε 19,900, 5529, and 8477); $\nu_{max.}$ 3350, 3100, 1740, 1665, 1630, and 1598 cm^{-1}.

Acetylation of the diol (30 mg) with sodium acetate (10 mg), dissolved in the minimum of water (0·3 ml) and acetic anhydride (0·4 ml) gave the triacetate (XI) (15 mg), m.p. 185—187°, identical (n.m.r., i.r., and mass spectra) with the triacetate obtained from the triol (VII) as described later.

Acetylation of the Triol (VIII).—(a) *The diacetate* (X). The triol (100 mg) and sodium acetate (30 mg) were dissolved in the minimum of water. Acetic anhydride (1·2 ml) was added dropwise with shaking and cooling until a cream coloured solid separated. Crushed ice was added with vigorous shaking and the collected solid was purified by repeated crystallisation from ethyl acetate to give the *diacetate* (X), m.p. 195·5—198° (Found: M^+, 488·166. $C_{25}H_{28}O_{10}$ requires 488·168); $\lambda_{max.}$ 254, 310sh, and 388 nm (ε 16,940, 6077, and 6077); $\nu_{max.}$ 3458, 3070, 1804, 1774, 1738, 1646, 1630sh, and 1558 cm^{-1}.

(b) *The monoacetate* (IX). The triol (VIII) was acetylated as already described. The crude product showed two spots on t.l.c., both with 5% acetic acid–methylene chloride and with ethyl acetate–methylene chloride–acetic acid (15 : 5 : 1) as solvent. Preparative t.l.c. with the latter solvent system and elution with the same solvent mixture gave two fractions which appeared to be identical when re-run in both solvent systems. The R_F value of the original faster moving spot corresponded to that of the diacetate (X), which appeared to be hydrolysed to the monoacetate

(IX). Recrystallisation of each fraction from ether gave the *monoacetate* (IX) as yellow needles, m.p. 182—185° (Found: M^+, 446·154. $C_{23}H_{20}O_9$ requires M, 446·155); $\lambda_{max.}$ 254, 275sh, and 388 nm (ε 17,070, 10,280, and 8535); $\nu_{max.}$ 3460, 3090, 1772, 1738, 1670, 1652, and 1595 cm^{-1}.

(c) *The triacetate* (XI). The diacetate (X) (26 mg), in pyridine 0·2 ml) and acetic anhydride (0·4 ml), was left for 16 h at 18°, then the mixture was poured into 1·5N-hydrochloric acid (1 ml) at 0° and shaken vigorously. After the addition of crushed ice (2 g), the cream-coloured solid (19 mg) was collected and crystallised from ethyl acetate–ether to give the *triacetate* as yellow prisms, m.p. 185—187° (Found: M^+, 530·175. $C_{27}H_{30}O_{11}$ requires M, 530·179); $\lambda_{max.}$ 254, 275, and 390 nm (ε 17,120, 9326, and 7645); $\nu_{max.}$ 3070, 1803, 1778, 1736, 1660, 1638w, and 1606w cm^{-1}.

Reaction of the Triol (VII) *with Hydrochloric Acid.*—(*a*) A solution of the triol (5 mg) in methanol (1 ml) and 3N-hydrochloric acid (0·5 ml) was boiled for 3 h. The mixture was analysed by t.l.c. with ethyl acetate–methylene chloride–acetic acid (15 : 5 : 1). The plates were sprayed with 4% cerium(IV) sulphate in 20% sulphuric acid and heated at 120°. The major spot co-chromatographed with acid (III); two minor spots co-chromatographed with the methyl ester (IV) and the methyl ester of the acid (I).

(*b*) When this reaction was repeated without methanol, the acid (III) was the only product.

Reaction of the Diol (VIII) *with Hydrochloric Acid.*—A solution of the diol (10 mg) in methanol (1 ml) and 2N-hydrochloric acid (1 ml) was boiled for 4 h under nitrogen. Analysis of the mixture at intervals by t.l.c. as in the previous experiment showed the initial formation of wortmannin (II), followed by the acid (III), the methyl ester (IV), and the methyl ester of the acid (I).

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