

Aspulvinones, a New Class of Natural Products from *Aspergillus terreus*. Re-investigation of Structures by X-Ray Crystallographic and Spectroscopic Analysis

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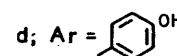
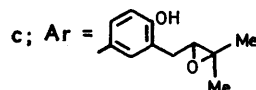
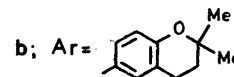
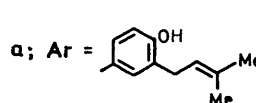
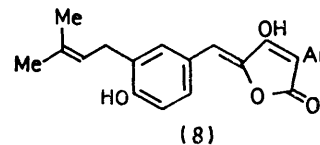
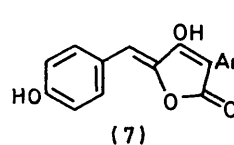
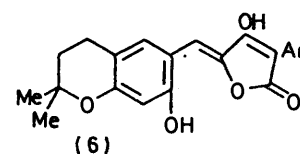
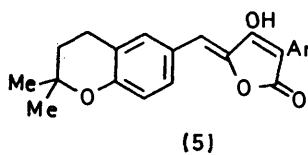
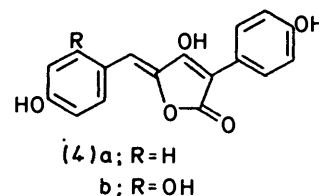
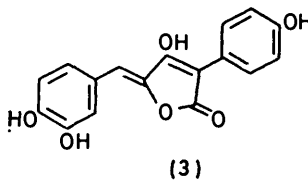
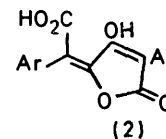
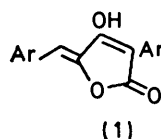
The constitutions of unsymmetrically substituted aspulvinone pigments from *Aspergillus terreus* are re-examined by a combination of X-ray crystallographic and spectroscopic analysis. The crystal structure of aspulvinone (18a) is determined by X-ray diffraction from diffractometer data by direct methods. Crystals are triclinic, space group $P\bar{1}$, with $Z = 2$ in a unit cell of dimensions: $a = 9.546 \pm 0.005$, $b = 11.019 \pm 0.005$, $c = 13.437 \pm 0.006$ Å, $\alpha = 102.94 \pm 0.05$, $\beta = 113.73 \pm 0.05$, $\gamma = 91.39 \pm 0.15^\circ$. The structure was refined by least squares to R 0.054 for 2 106 independent reflections. Comparative spectroscopic data establish constitutions (18b) and (18d) for the structurally related metabolites.

The present data, combined with synthetic studies, have led to the revision of structures assigned to all 'unsymmetrically' substituted aspulvinones found in *A. terreus*. The biosynthesis of this new class of natural products is considered.

The group of 4-ylidenetetrone acid pigments (1) known as 'pulvinones': [3-aryl-5-arylidene-4-hydroxyfuran-2(5H)-ones] are closely related to the more familiar 'pulvinic acids' (2) commonly encountered in lichens, and also found in higher fungi.¹ Although pulvinones have only very recently been reported in nature, the fundamental pulvinone carbon skeleton has been known as a product of chemical degradation of pulvinic acids since about 1895.² During 1973, Edwards and Gill³ reported the isolation of the trihydroxypulvinone (3) from stems of the common larch mushroom *Suillus grevillei* (Klotsch) Sing., and Seto and his co-workers⁴ found the dihydroxy-derivative (4a) in cultures of *Aspergillus terreus*. Two years later, the Japanese group reported the presence of six additional pulvinones [*viz.* (4b), (5a, b), (6a, b, c)] in *A. terreus*,⁵ a number of which contained isoprene residues in the aryl rings. The presence of pulvinone (4a) in *A. terreus* was subsequently confirmed by other workers,⁶ and more recently enzymatic studies of the biosynthesis of the prenylated pulvinones (5a) and (5b) have demonstrated the accumulation of the biosynthetically significant metabolites (7a or 8d) and (8a) in young cultures of *A. terreus*.^{7,8} To distinguish the group of pulvinones (4)–(8) found in *A. terreus* from other natural pulvinones, Seto has suggested the name 'aspulvinone' as the generic name for this new family of secondary metabolites.†

During contemporaneous synthetic studies amongst naturally occurring 4-ylidenebutenolides and 4-ylidene-tetrone acids,⁹ we developed an unambiguous synthetic route to the pulvinone carbon skeleton; this approach, described in the preceding paper,¹⁰ was based on aldol-type condensation between an *O*-methyl 2-aryltetrone acid and an appropriate aryl aldehyde followed by dehydration. Application of this general methodology to syntheses of permethylated derivatives of the 'symmetrical' aspulvinones (4a) and (5b) vindicated the correctness of the constitutions shown in the formulae for

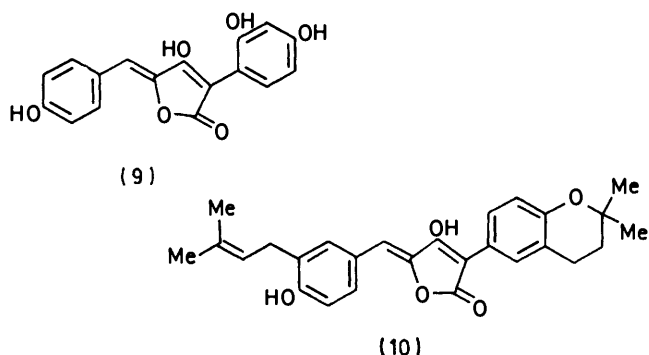
these metabolites. Furthermore the synthetic studies permitted us to establish conclusively structure (3) for the 'unsymmetrically' substituted pulvinone found in



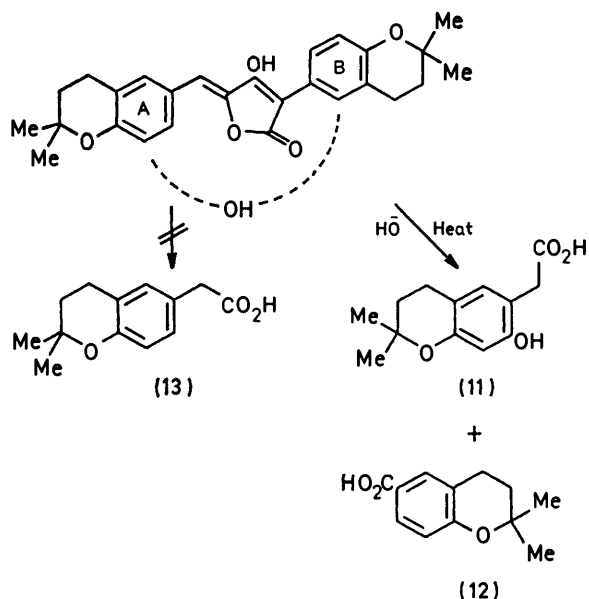
† The name 'aspergillide' has also been suggested.⁶ To avoid any confusion, the name 'aspulvinone' is used throughout this paper.

S. grevillei.* We were unable, however, to confirm the structures (4b) and (5a) assigned by Seto *et al.* to two unsymmetrical aspulvinones isolated from *A. terreus*; accordingly these structures were revised to (9) and (10) respectively.

Seto and his co-workers obtained information on the substitution patterns in the aryl rings of unsymmetrically substituted aspulvinones from an analysis of the constitutions of the products of their degradation in hot alkali.⁵ Thus, treatment of the bischromanated aspulvinone [(6b) or isomer] with sodium hydroxide in



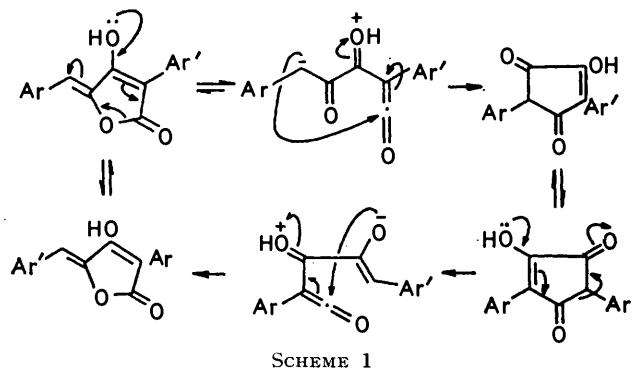
refluxing ethanol for two days produced a mixture of phenylacetic and benzoic acids (11) and (12) respectively, which led to the assignment of structure (6b) for the metabolite, containing the 'extra' hydroxy-group in aryl ring-A. The assignment of structure amongst this class of compound based on degradations in hot alkali



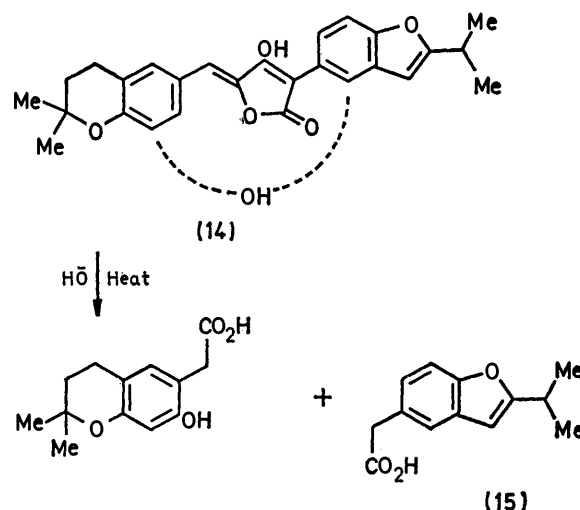
however, is fraught with difficulties, and tantamount to tempting providence, in view of the known incidence of the equilibria depicted in Scheme 1 for the chemical

* Throughout this paper the terms 'symmetrical' and 'unsymmetrical' refer to the relative substitution patterns in the two aryl rings of the aspulvinones; thus (4a) is symmetrically substituted, whereas (3) is unsymmetrically substituted.

system.¹¹ On this basis, alkali degradation of the aspulvinone (6b) would be expected to result in the formation of phenylacetic acid (13) in addition to (11).



The presence of (13) was not reported amongst the products of alkali degradation of (6b), which is perhaps even the more surprising in view of the somewhat conflicting observation, reported concurrently, that alkali degradation of the aspulvinone (14) [obtained by acid



treatment of (6c)?) did lead to a mixture of phenylacetic acids (11) and (15). Together with our synthetic studies amongst pulvinones, these latter conflicting observations, led us naturally to question the structures assigned to the unsymmetrically substituted aspulvinones (6a, b, c) from *A. terreus*. In this paper we report the results of a re-examination of the structures of these metabolites using a combination of X-ray analysis [of aspulvinone (6a)?] and comparative ¹H n.m.r. and mass spectral data.¹²

The aspulvinone assigned structure (6a) by Seto *et al.* crystallised from methanol as a hydrate, C₂₇H₂₈O₆·1.5H₂O, m.p. 257–258 °C, suitable for crystallographic analysis. The structure was solved from diffractometer data (2 106 reflections) by direct methods, and refined by least-squares and difference Fourier methods to R 0.054. A general view of the resulting molecular structure is illustrated in Figure 1.

This shows that Seto's formulation for the metabolite is incorrect, the difference lying in the distribution of the substituents in the two aryl rings of the pulvinone carbon skeleton.

For crystallographic purposes only, the numbering scheme in Figure 2 was adopted. Bond lengths and bond angles are displayed in Figures 3 and 4. Figure 1 reveals the overall planar nature of the molecule that would be expected from such a highly conjugated system. There are, however, several significant deviations from overall planarity caused by van der Waals repulsions. The central butenolide ring and its immediately attached atoms [atoms (1)—(7) and (20)—(22)] are approximately planar, with no atom further than 0.04 Å

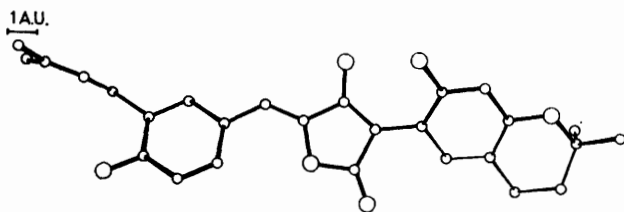


FIGURE 1 General view of the aspulvinone molecule

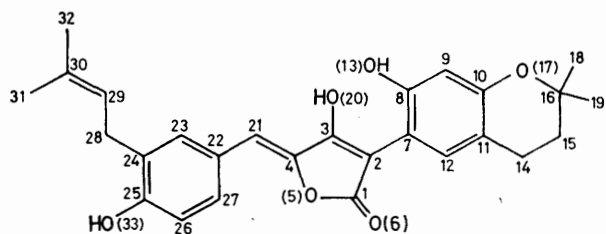


FIGURE 2 Crystallographic numbering system

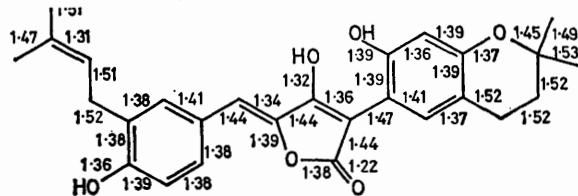


FIGURE 3 Bond lengths (Å); all standard deviations less than 0.01 Å

from the mean plane through these ten atoms. However, both the phenyl rings are twisted out of conjugation with the plane of the butenolide ring. The degree of twist is best described by the torsion angles about the C(2)–C(7) bond (24°) and C(21)–C(22) bond (20°) in opposite senses such that the dihedral angle between the mean planes through the phenyl rings is 48° . This strain is emphasised by the unusually large bond angles such as O(20)–C(3)–C(2) (132°), C(3)–C(2)–C(7) (131°), and C(4)–C(21)–C(22) (129°). The C(2)–C(7) torsion angle is also affected by a short intramolecular hydrogen bond OH(20)–O(13) of length 2.56 Å. The hydrogen atom was not located along the inter oxygen line but such that the O–H–O angle was 149° . The twist about the C(21)–C(22) bond has enlarged the O(5)–C(27) separation to 3.01 Å.

Both the phenyl rings are planar with the benzylidene ring [ring A; C(22)—C(27)] forming a better plane ($\chi^2 = 2.5$) than the strained chroman ring, C(7)—C(12)

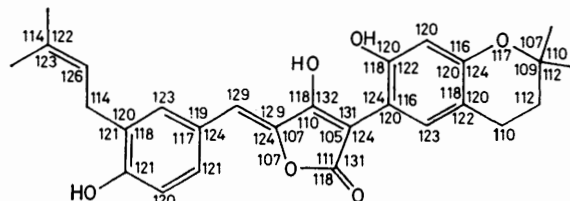


FIGURE 4 Bond angles ($^\circ$). All standard deviations less than 0.5°. Additional angles C(15)–C(16)–C(18) 115° ; O(17)–C(16)–C(19) 103°

($\chi^2 = 27.8$). The heterocyclic ring of the chroman adopts the expected half-chair conformation. The prenyl side-chain, C(28)—C(32), forms a very flat plane ($\chi^2 0.3$). This plane is twisted to form a dihedral angle of 79° to the benzylidene ring by torsion angles about the C(24)–C(28) bond (73°) and about the C(28)–C(29) bond (15°).

Figure 5 illustrates the molecular packing and shows the arrangement of the molecules in the unit cell projected down the *c*-axis. In addition to the intramolecular hydrogen bond already mentioned, two strong intermolecular hydrogen bonds are found. One of these is formed to a symmetry related aspulvinone molecule OH(33)—O(6') of length 2.69 Å, and the other is formed to a molecule of water OH(13)—O(34) length 2.67 Å indicating that the water molecule is tightly bound to the aspulvinone. Both of these hydrogen atoms were located along the line of oxygen centres. In addition to these short hydrogen bonds the water molecule forms two rather longer, weaker hydrogen bonds, one to an aspulvinone molecule OH(34)—O(33') 2.88 Å, and the other to the second water molecule OH(34)—O(35) 3.19 Å. Apart from this very weak bond the second water

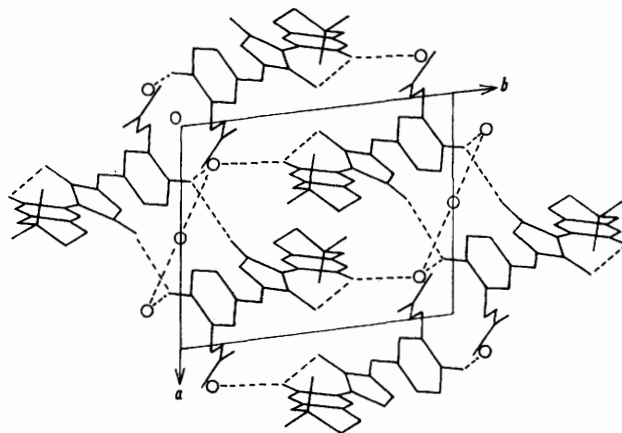
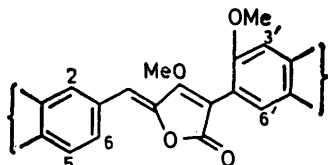


FIGURE 5 Arrangement of molecules in unit cell projected along *c* axis

molecule, located on a crystallographic centre of symmetry, forms no close intermolecular contacts, appears disordered from large temperature factors, and appar-

ently merely occupies a 'hole' in the tightly bound intermolecular framework of hydrogen bonds.

The aspulvinones assigned constitutions (6a), (6b), and (6c) by Seto and his co-workers,⁵ show closely comparable electronic absorption spectra [λ_{\max} 242–245 (log ϵ 4.23–4.31) and 375–377 (log ϵ 4.47–4.74) nm], and their i.r. spectra are very similar in the carbonyl region. The corresponding permethylated derivatives



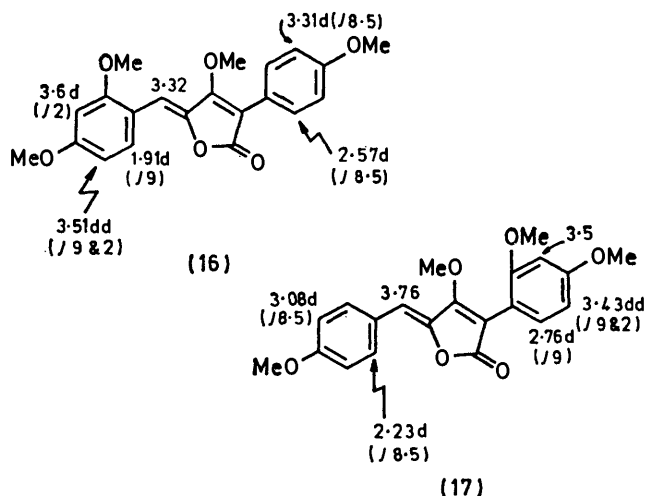
also exhibit closely similar electronic and i.r. absorption properties. ¹H N.m.r. data for the aryl and benzylic hydrogens in the permethylated derivatives of the three aspulvinones are summarised in Table 1. The data

TABLE 1

¹H N.m.r. data for aryl and benzylic hydrogens in permethylated aspulvinones (18a), (18b), and (18d)

	Benzylic :CH	H-2	H-5	H-6	H-3'	H-6'
(6a) (18a)	3.76	2.43	3.08	2.17	3.56	2.93
(6b) (18b)	3.84	2.45	3.23	2.53	3.63	3.01
(6c) (18d)	3.84	2.28	3.27	2.59	3.65	3.03

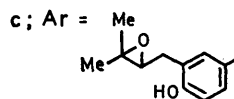
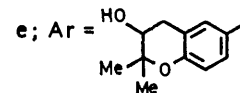
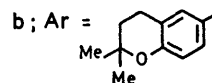
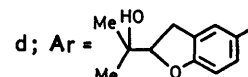
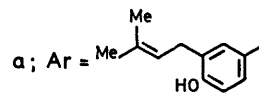
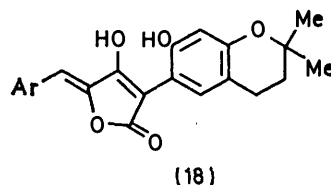
show striking similarities to each other, and also to those of the permethylated derivative of the non-prenylated metabolite (9) which co-occurs in *A. terreus*; it is recalled that constitution (9) followed for this aspulvinone from comparison of physical and spectral data for the permethylated derivative with those of the totally synthetic isomer (16).¹⁰ A significant and distinguishing feature between the ¹H n.m.r. spectra of the methoxypulvinones (17) and (16), is that whereas the former displays a prominent absorption for the benzylic proton at τ 3.76,



the corresponding proton in (16) is markedly deshielded by the proximate 2-OMe group, and absorbs at τ 3.32. The ¹H n.m.r. data for these pulvinones are summarised

in diagrams (16) and (17) respectively; assignments in (16) followed from specific decoupling experiments.

The close similarity between the ¹H n.m.r. data of permethylated derivatives of the aspulvinones containing an 'extra' phenolic-OH group, summarised in Table 1 and formula (17), supports a similar orientation of the aryl substituents about the aspulvinone carbon skeleton, with the additional OH-group in aryl ring B in all three metabolites. The remaining structural uncertainty amongst this group of metabolites concerns the nature of the phenyl residue in ring A of the aspulvinone which contains an extra oxygen atom to aspulvinone [(6a) now (18a)]. Seto *et al.*⁵ have formulated this metabolite as an epoxide [(6c)?, now (18c)]; to our knowledge prenyl-epoxides of this type have not previously been isolated from nature. Since treatment of the metabolite with diazomethane, produces only a dimethylated derivative,* it seems more likely that this aspulvinone has the dihydrobenzofuran structure (18d); the alternative dihydrobenzopyran structure (18e) is

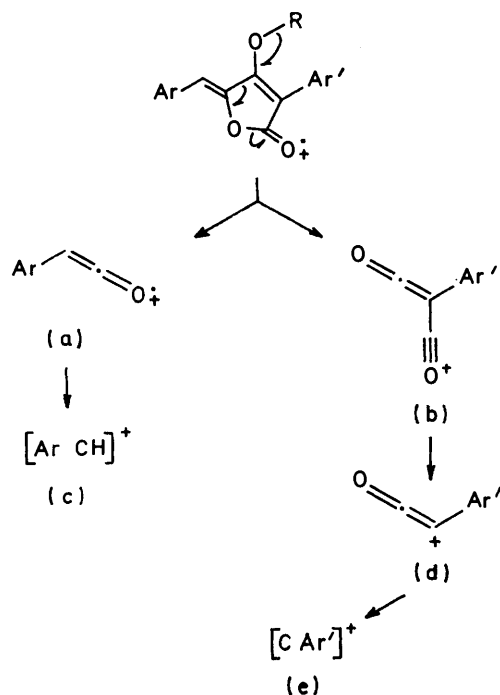


less likely, since acid-treatment of the metabolite leads to the benzofuran derivative,⁵ rather than to a chromen. The revised structures (18a), (18b), and (18d) therefore follow for the prenylated aspulvinones containing the 'extra' phenolic hydroxy group, found in *A. terreus*.

The mass spectral fragmentation patterns of the aspulvinones all follow that summarised in Scheme 2. Since positional isomers of the unsymmetrically substituted aspulvinones (18a), (18b), and (18d) were in thermal equilibrium (*via* the corresponding cyclopentatriene; see Scheme 1) in the mass spectrometer, it was not possible however to distinguish one isomer from another by simple inspection of fragmentation patterns. During investigations of the structure of pulvinone (3)

* A structurally similar compound has been isolated by Golding *et al.* from *A. terreus*. These authors have also demonstrated that the metabolite produces a dimethylated derivative, which was shown to be laevorotatory, and to contain hydroxy-absorption in the i.r. region, ν_{\max} 3 450 (Nujol); 3 570 (CCl₄) (personal communication from Dr. Golding).

found in *Suillus grevillei*, Edwards and Gill³ were able to demonstrate that equilibration between the positional isomers (19) and (22) of the corresponding permethylated derivatives [via *O*-methylcyclopentenediones (20) and (21) respectively], was negligible in the mass spectrometer, and direct comparison of their fragmentation patterns could be used to distinguish between them. Accordingly we prepared the permethylated derivatives of the aspulvinones (4a), (8b), (3), (5d), and (18a, b, d), and their relevant mass spectral fragmentation data are collected in Table 2. The data relate to spectra recorded at an inlet temperature of 140 °C, the lowest temperature at which the molecular ions were visible, but do not vary in detail to those recorded at temperatures between 140 and 215 °C. Although fragments corresponding to the presence of isomeric *O*-methyl-



SCHEME 2

aspulvinones/cyclopentenediones were present in most spectra (percentage abundances are represented in

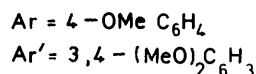
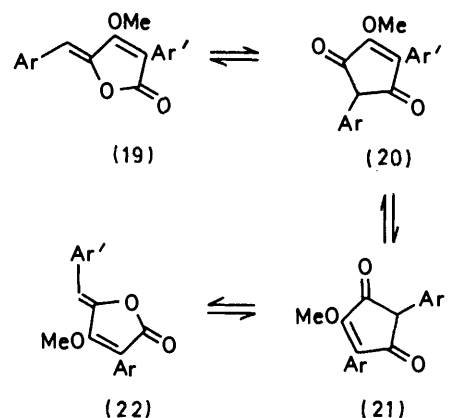
TABLE 2

Intensity data of major fragments in mass spectra of permethylated aspulvinones

Fragment	(4a)	(8b)	(3)	(5d)	(18a)	(18b)	(18d)
M^+	100	61.4	84.4	94.7	20	100	100
(a)	31.4	3.1	7.0	2.0	13	10	4
(b)	14.7	1.3	4.8	5.4	13	5.0	4
(c)	25.4	1.1	1.5	1.2	15	3.2	3
(d)	43.8	2.0	10.7	36	20	10.3	5
(e)	52.3	4.1	13.0	21.3	29	17	4
		(1.5)	(1.4)	(1)	(10)	(4)	(9)

parentheses in the Table) the data show that, within limits, the fragmentation patterns can be used to provide

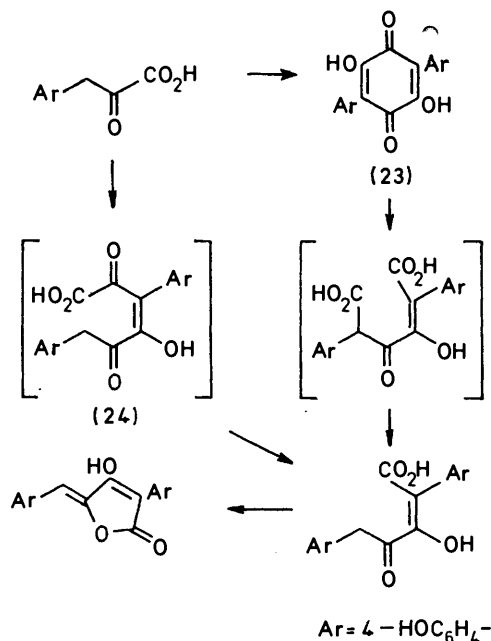
a clue to the orientations of the aryl ring substituents in aspulvinones (8b), (3), and (5d). The corresponding



SCHEME 3

data for the aspulvinones (18a, b, d) containing an extra OMe substituent in aryl-ring B, however, were more complex and, by themselves, were clearly less useful in establishing unambiguously the orientations of the substituents in the metabolites.

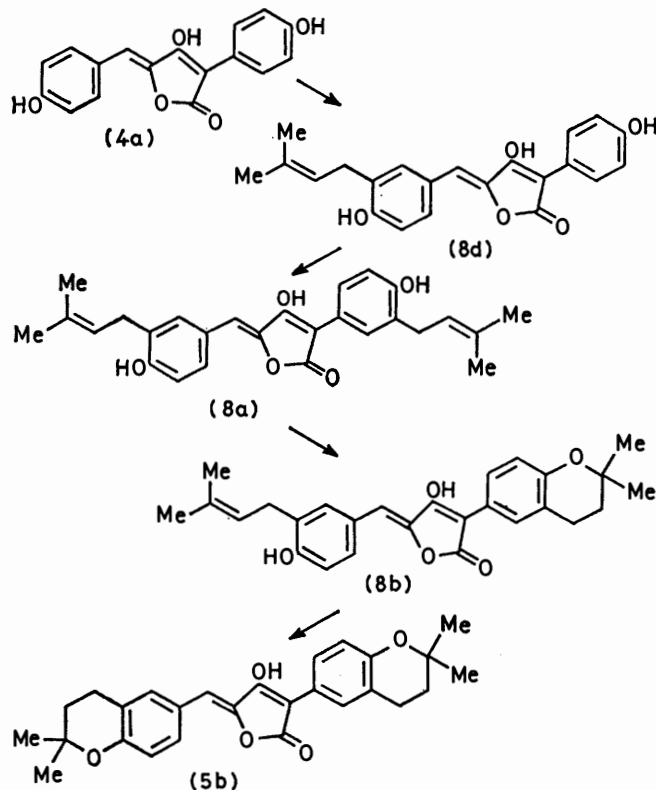
The present X-ray and spectroscopic data, combined with our synthetic studies, have thus led to the revision of structures of all the unsymmetrically substituted aspulvinones found in *A. terreus*. Although no studies of the early stages of the biosynthesis of aspulvinones have been reported, it seems probable that this follows a



SCHEME 4

closely similar 'phenylpropanoid' pathway to that worked out for the related pulvinic acids (2).¹³ Thus,

oxidative rearrangement of, and decarboxylation from the terphenyl quinone 'atromentin' (23), produced from tyrosine *via* dimerisation of *p*-hydroxyphenylpyruvate, could furnish 4,4'-dihydroxyaspulvinone (4a); alternatively either a 'half-protected' form of the quinone or the open-chain oxo-acid (24) might be implicated in the sequence (Scheme 4). Seto's and our own studies^{7,8,10} have suggested that the transfer of prenyl moieties to (4a) leading to (8a) is a step-wise process proceeding *via* (8a), and that the conversion of (8a) to the bis-chroman (5b) ('chromanation') is also step-wise, proceeding *via* the mono-chromated aspulvinone (8b).



SCHEME 5

EXPERIMENTAL

Samples of aspulvinones from *A. terreus* were obtained through the generosity of Dr. S. Seto (Tohoku University, Japan). Permethylated derivatives of the aspulvinones were prepared using ethereal solutions of diazomethane, and were purified by chromatography. Mass spectral data were recorded on a VG 70-70F spectrometer.

Crystallographic Analysis of Aspulvinone (18a).—Suitable specimens of the aspulvinone were recrystallised from methanol. Oscillation and Weissenberg photographs were taken about the *a*-axis to establish approximate unit-cell dimensions and space group. For intensity measurement a crystal of dimensions *ca.* 0.5 × 0.2 × 0.1 mm was mounted about the *a*-axis on a Hilger and Watts four-circle diffractometer. The unit-cell dimensions were refined by a least-squares fit on the positions of 18 peaks found on the diffractometer. Intensity data were collected with Mo-*K*_α radiation for 2θ ≤ 50° by use of an ω/2θ scan. Of the

4 409 independent reflections measured, 2 106 were considered observed (net count > 3σ) and these were used in the structure refinement. No absorption corrections were

TABLE 3

Fractional atomic co-ordinates with standard deviations in parentheses; values multiplied by 10⁴ (10³ for hydrogen atoms)

Atom	<i>x/a</i>	<i>y/b</i>	<i>z/c</i>
C(1)	6 266(5)	2 508(4)	-1 665(3)
C(2)	7 135(4)	3 694(4)	-0 946(3)
C(3)	8 018(5)	4 022(4)	-1 449(3)
C(4)	7 738(5)	3 070(4)	-2 456(3)
O(5)	6 621(3)	2 147(3)	-2 581(2)
O(6)	5 325(4)	1 810(3)	-1 591(3)
C(7)	7 022(4)	4 319(4)	0 106(3)
C(8)	7 375(5)	5 603(4)	0 592(3)
C(9)	7 305(5)	6 137(4)	1 584(3)
C(10)	6 783(5)	5 411(4)	2 115(3)
C(11)	6 394(5)	4 122(4)	1 663(3)
C(12)	6 505(5)	3 609(4)	0 670(3)
O(13)	7 847(4)	6 338(3)	0 044(3)
C(14)	5 775(6)	3 330(4)	2 224(4)
C(15)	5 281(6)	4 173(5)	3 053(4)
C(16)	6 482(5)	5 300(4)	3 783(3)
O(17)	6 679(4)	6 046(3)	3 075(2)
C(18)	8 036(6)	4 981(5)	4 454(4)
C(19)	5 932(6)	6 211(5)	4 553(4)
O(20)	9 030(4)	5 014(3)	-1 168(3)
C(21)	8 410(5)	3 020(4)	-3 166(3)
C(22)	8 105(5)	2 098(4)	-4 203(3)
C(23)	9 238(5)	2 018(4)	-4 626(3)
C(24)	9 033(5)	1 177(4)	-5 619(3)
C(25)	7 654(5)	0 400(4)	-6 222(3)
C(26)	6 500(5)	0 459(4)	-5 834(4)
C(27)	6 739(5)	1 298(4)	-4 832(4)
C(28)	10 315(5)	1 103(4)	-6 019(4)
C(29)	9 988(6)	1 637(4)	-7 019(4)
C(30)	10 737(7)	1 466(5)	-7 662(4)
C(31)	12 037(13)	0 724(11)	-7 476(10)
C(32)	10 338(10)	2 032(9)	-8 651(7)
O(33)	7 464(4)	-0 431(3)	-7 195(3)
O(34)	8 155(8)	8 822(4)	0 877(5)
O(35)	5 000(—)	0 000(—)	0 000(—)
H(9)	762(4)	699(4)	190(3)
H(12)	622(4)	269(3)	031(3)
H(13)	790(6)	712(5)	035(4)
H(14a)	487(5)	261(4)	158(4)
H(14b)	662(4)	-289(3)	261(3)
H(15a)	514(5)	367(4)	352(4)
H(15b)	430(6)	460(5)	269(5)
H(18a)	842(6)	444(5)	397(5)
H(18b)	800(7)	455(5)	501(5)
H(18c)	878(8)	589(7)	507(6)
H(19a)	670(6)	708(5)	498(5)
H(19b)	481(5)	620(4)	397(4)
H(19c)	627(7)	574(6)	522(5)
H(20)	890(5)	568(5)	-069(4)
H(21)	926(4)	368(3)	-293(3)
H(23)	1 019(4)	263(3)	-424(3)
H(26)	556(4)	-009(4)	-628(3)
H(27)	589(6)	131(5)	-458(4)
H(28a)	1 131(5)	150(4)	-539(4)
H(28b)	1 038(4)	023(4)	-623(3)
H(29)	908(5)	212(4)	-725(4)
H(31a)	1 121(9)	036(8)	-715(7)
H(31b)	1 244(11)	074(9)	-773(8)
H(31c)	1 271(20)	049(17)	-645(16)
H(32a)	1 118(9)	254(7)	-851(6)
H(32b)	930(7)	244(6)	-885(5)
H(32c)	1 015(13)	133(10)	-944(9)
H(33)	652(5)	-083(4)	-763(4)
H(34a)	831(12)	944(10)	134(9)
H(34b)	733(5)	855(4)	061(3)

made. Data reduction and subsequent crystallographic calculations were performed using the 'X-Ray '70' system

of programs.¹⁴ Atomic scattering factors were taken from ref. 15.

Crystal Data.— $C_{27}H_{28}O_6 \cdot 1.5H_2O$, $M = 475.545$, m.p. 257–258 °C. Triclinic, $a = 9.546 \pm 0.005$, $b = 11.019 \pm 0.005$, $c = 13.437 \pm 0.006$ Å, $\alpha = 102.94 \pm 0.05$, $\beta = 113.73 \pm 0.05$, $\gamma = 91.39 \pm 0.15^\circ$, $U = 1\,250.69$ Å³, $Z = 2$, $D_c = 1.26$ g cm⁻³, $F(000) = 5.6$. Space group $P\bar{1}$ from intensity statistics, and structure refinement; Mo- K_α radiation $\lambda = 0.710\,69$ Å, $\mu = 0.99$ cm⁻¹.

Structure Solution and Refinement.—Normalised structure factors were calculated using a procedure that follows the experimental $k(s)$ points at low $\sin\theta$, the $k(s)$ curve at medium $\sin\theta$ and a constant value at high $\sin\theta$. The intensity statistics suggested the presence of a centre of symmetry with $|E^2 - 1| = 0.891\,5$ compared to the theoretical values of 0.968 0 for centrosymmetric and 0.736 0 for non-centrosymmetric. The 350 largest normalised structure factors ($E > 1.67$) were then used to determine the structure by direct methods with the aid of the Multan program of Germain, Main, and Woolfson.¹⁶ The best set of phases obtained with this program had a figure of merit 1.111 3, after the all positive set had been eliminated. A subsequent E -map based on these phases revealed 31 peaks in chemically reasonable positions among the 35 largest peaks.

A structure-factor calculation following two cycles of block-diagonal least-squares refinement showed an agreement factor R of 0.24. A subsequent difference-Fourier synthesis revealed 3 large peaks corresponding to the remaining 2 atoms in the pulvinone molecule and one molecule of water [O(34)]. Four further cycles of least-squares refinement of atomic positions and isotropic temperature factors followed using all data and unit weights. The agreement factor R was lowered to 0.16 and in subsequent refinement the atomic temperature factors were allowed to vary anisotropically. Four more cycles lowered R to 0.103 and a difference-Fourier synthesis was then calculated. This revealed the positions of all 30 hydrogen atoms together with a large diffuse peak on a centre of symmetry assumed to be a second molecule of water [O(35)]. The hydrogen atoms and O(35) were then included in the refinement with isotropic temperature factors but the position of O(35) was fixed at the centre of symmetry. During the course of this refinement the temperature factor for O(35) became rather large and its population parameter was also refined. However, the population parameter did not refine significantly away from 1.0 and it was subsequently fixed at unity again. Finally

the temperature factor of O(35) was allowed to vary anisotropically to better account for its disordered nature. A total of 12 further cycles of refinement were calculated in this way lowering R to 0.060. Analysis of the agreement of F_o and F_c suggested the adoption of a weighting scheme of the form $w = 1.0/\{1 + [(F_o - 13.0)/9.75]\}^2$. This was used in the final three cycles of refinement with full-matrix calculations. The largest parameter shifts were then $< 0.5\sigma$ indicating that refinement had converged at R 0.054 after a total of 25 cycles. The accuracy of the structure was confirmed by computing a final difference map which showed no peaks or depressions > 0.2 eÅ⁻³. Final atomic co-ordinates are listed in Table 3; temperature factors and observed and calculated structure factors are listed in Supplementary Publication No. 22327 (28 pp.).*

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* For details of the Supplementary publication scheme see Notice to Authors No. 7, *J.C.S. Perkin I*, 1977, Index issue.