New Polyketide Metabolites from *Aspergillus melleus*: Structural and Stereochemical Studies

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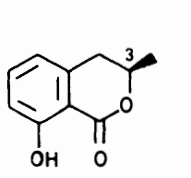
The structures of two new fungal products asperlactone (5) and isoasperlactone (6) have been deduced by comparison of their spectroscopic data with that of aspyrone (3), a known co-metabolite. All three compounds have been degraded by ozonolysis to produce crystalline derivatives of 2,3-epoxybutyric acid (9) and 1-methylglycerol (11). From measurements of optical activity it has been established that aspyrone and isopasperlactone have the (5*S*,6*R*,8*S*,9*S*) configuration, whereas that of asperlactone is (5*R*,6*S*,8*S*,9*S*). The relative stereochemistry of aspyrone has been checked by an *X*-ray crystallographic study: [$P2_12_12_1$, a = 4.427(3), b = 14.272(3), c = 14.968(3) Å, Z = 4, R = 0.033 for 1 131 unique observed reflections]. The stereochemical relationships are discussed in terms of a biosynthetic proposal by which (3) and (5) would be biosynthesised from a common intermediate *via* alternative cyclisations of a carboxy group onto an epoxide residue. It is possible that (6) is a chemical artefact derived from (3).

Aspergillus melleus is a rich source of polyketide metabolites, including (−)-(3*R*)-mellein (1) [1], (−)-(3*R*,4*R*)-hydroxymellein (2) [2,3], aspyrone (3) [4,5], and penicillic acid (4).[4] As part of a programme to investigate the biosynthesis [6] of aspyrone, we re-examined the growth of strain CMI 49108 on a sucrose-based medium. Although substantial amounts of aspyrone (*ca.* 350 mg/l) are produced during the first 10—12 days of incubation, a new compound, to which we assign the name asperlactone and structure (5), appears to be produced at the expense of aspyrone from day 10 onwards. The two metabolites, (3) and (5), are structurally related but experiments described in detail below establish that asperlactone is not a chemical artefact and can therefore be considered a natural product in its own right. A stereoisomer, isoasperlactone (6), isolated in lower yield could however be an artefact.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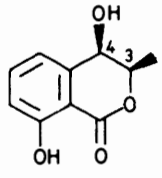
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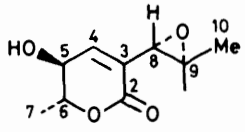
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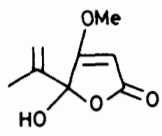
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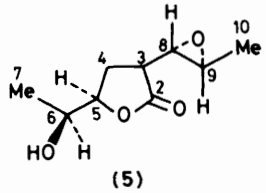
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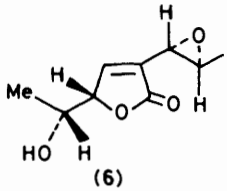
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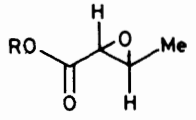
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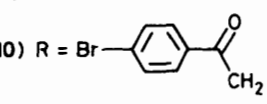
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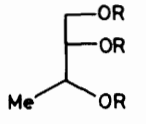

(11) R = H
(12) R = −COPh

Structural Studies.—Asperlactone, 5-(1-hydroxyethyl)-3-(2,3-epoxypropyl)butenolide, $C_9H_{12}O_4$, is a yellow oil, b.p. 140 °C/0.4 Torr, which is obtained pure by chromatographic and spectroscopic criteria following silica column chromatography using ethyl acetate–benzene as eluant. It is optically active, $[\alpha]_D$ + 61.8°, and shows a positive anomalous optical rotation curve over the range 589—365 nm. Its spectroscopic properties closely resemble those of aspyrone. Thus asperlactone gives no major u.v. absorption but has a strong carbonyl absorption in the i.r. region at 1 765 cm^{-1} together with a weaker band at 1 630 cm^{-1}, characteristic of an α,β-unsaturated γ-lactone (*cf.* aspyrone 1 709 and 1 656 cm^{-1}), as well as a strong hydroxy band. The mass spectrum reveals only a weak molecular ion at *m/z* 184 on which no satisfactory high resolution data could be obtained. Other fragment ions are at *m/z* 140 (100%, high resolution gives $C_7H_8O_3$, $M^+ - CH_3CHO$), 125, 111, and 97; this spectrum is similar to that of aspyrone.

The 90 MHz proton n.m.r. spectrum of asperlactone in deuteriochloroform reveals an olefinic proton at δ 7.28, four protons attached to carbons bearing oxygen at δ 4.82, 4.00, 3.40 and 3.15, a broad OH signal at δ 2.90, and two methyl doublets at δ 1.35 and 1.23. The olefinic proton, 4-H, is shifted downfield relative to the corresponding proton (δ 6.64) in aspyrone, consistent with the reduction in ring size.[7] Resonances due to 5-H and 6-H in aspyrone overlap at δ 4.3, whereas in asperlactone the resonance due to 5-H is shifted downfield to δ 4.82, and that to 6-H upfield to δ 4.00, again consistent with the presence of a five-membered ring. The two signals at δ 3.40 and 3.15 are assigned to 8-H and 9-H of the epoxy group; the 2 Hz coupling between them indicates a *trans* relationship, as is also the case with aspyrone. Irradiation of 9-H reduces the doublet at δ 1.35 to a singlet, enabling this signal to be assigned to 10-H whilst irradiation of 6-H

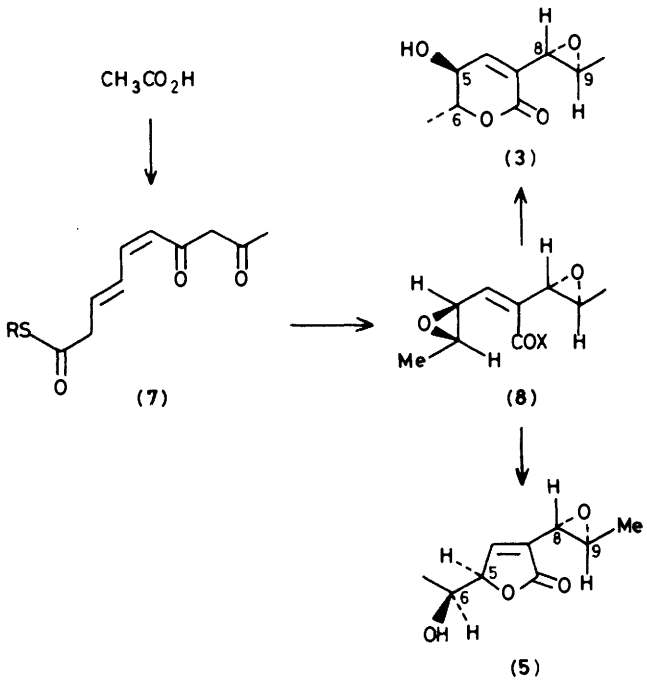
reduces the doublet at δ 1.23 to a singlet (7-H). The assignments of these two methyl groups are thus reversed relative to aspyrone, for which spin decoupling experiments show that the highfield methyl doublet is assigned to 10-H. A complete assignment of the spectra of both molecules with full coupling data is given in the Experimental section.

The proton noise decoupled ^{13}C n.m.r. spectrum of (5) in deuteriochloroform contains nine signals, δ 171 (s, C-2), 147 (d, C-4), 133 (s, C-3), 85, 68, 58, and 52 (d, C-5, C-6, C-8, and C-9), and 19, 17 (q, C-7, C-10). These assignments were rigorously checked by a series of Birdsall plots [8] and confirmed in subsequent biosynthetic experiments. As expected, the positions of the resonances for C-5 and C-6 (δ 85 and 68) are reversed in aspyrone (δ 68 and 80 respectively), owing to the change in ring size. In the ^{13}C n.m.r. spectra of both compounds, the highfield methyl signal corresponds to C-10, despite the differences in assignments of the signals for 7-H and 10-H in the proton spectra.

Several derivatives of (5) were prepared in order to confirm its composition. The acetate, $C_{11}H_{14}O_5$, is a clear yellow oil, $\nu_{max.}$ 1 760, 1 730, and 1 640 cm^{-1}, *m/z* 226. The 6-H signal is observed as a multiplet shifted downfield relative to the corresponding signal in asperlactone, so that it overlaps with that for 5-H at δ 4.95. The phenylurethane derivative,[5] $C_{16}H_{17}NO_5$, prepared by treating (5) with phenyl isocyanate at room temperature for two days, has $\nu_{max.}$ 1 760, 1 720, and 1 620 cm^{-1}, and *m/z* 303. Again in the proton n.m.r. spectrum, the signal for 6-H is shifted downfield to lie on top of that for 5-H. The benzoate, *p*-nitrobenzoate and 3,5-dinitrobenzoate derivatives were also made, with the expected spectroscopic properties. None of these derivatives crystallised. The lower yields and longer reaction times compared with those required in the preparation of the corresponding aspyrone derivatives suggest that the secondary hydroxy function of (5) is unreactive. Attempts to prepare tosyl or mesyl derivatives failed, decomposition occurring under the basic reaction conditions.

Our initial reaction to the isolation of asperlactone was to assume that it is a chemical artefact produced under the incubation conditions by hydrolysis followed by recyclisation to form a 5-membered lactone ring. This reaction has been reported on treatment of (3) with aqueous hydroxide [5] and yields a γ-lactone product, to which we assign the name isoasperlactone. Although this synthetic compound closely resembles asperlactone, the two are clearly distinguished by their ^{13}C n.m.r. spectra in which most of the resonances show small but detectable chemical-shift differences (up to 0.3 p.p.m.), which are found to vary with the relative concentrations of the two isomers. Asperlactone (5) is usually contaminated with a small amount of an isomer from which it can be separated by column chromatography. Since this isomer is indistinguishable from isoasperlactone (6) in all respects, including ^{13}C n.m.r. and optical rotation data, we conclude that some isoasperlactone is produced along with asperlactone in the incubation medium. A confirmatory ^{13}C n.m.r. experiment was carried out. A sample of (6) was prepared synthetically from (3); its ^{13}C n.m.r. spectrum contained only a single set of nine peaks. This synthetic compound was added to a biosynthetic mixture (1 : 1) of (5) and (6) and, on re-running the spectrum, the peaks assigned to isoasperlactone were increased in relative intensity. The resonances of asperlactone are at lower field of those of isoasperlactone for all carbons, except those of C-3 and C-9 which are at higher field.

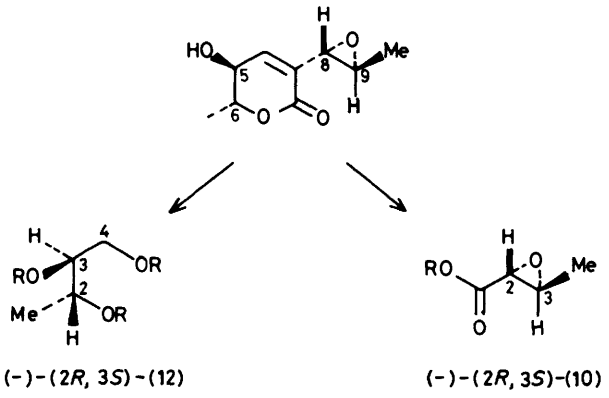
Stereochemical Studies.—The co-occurrence of aspyrone, asperlactone, and isoasperlactone raises interesting biosynthetic questions. An intriguing hypothesis is that all three



Scheme 1.

compounds originate from the same intermediate (8), itself derived from a linear polyketide precursor (7) by oxidation and rearrangement, as shown in Scheme 1. Attack of the carboxylate group in (8), specifically on one of the two epoxide residues, can lead to either the aspyrone or asperlactone nucleus depending on the site of attack. The relative stereochemistry indicated for aspyrone has been suggested from spectroscopic studies [5] and, if correct, requires that the configuration of the epoxide group under attack must be *trans*. If this biosynthetic hypothesis is correct, the relative stereochemistry predicted for asperlactone (5) would be as shown, with two centres, C-8 and C-9, having the same configuration as the corresponding centres in aspyrone (3), the remaining centres, C-5 and C-6, being opposite in configuration; the proton n.m.r. coupling data discussed above support this idea by establishing that the relative configuration of the two centres in the epoxy-propyl side-chain is the same in all three molecules. The isoasperlactone isolated from the incubation mixture can arise chemically from aspyrone and on this basis these two molecules would be expected to have the same absolute stereochemistry at all four centres. In order to provide evidence for these proposals, we have determined the absolute configuration of all three molecules by chemical correlation with known compounds. Additionally, since single crystals of aspyrone are available [from chloroform–light petroleum (b.p. 60—80 °C)] a sample was submitted for *X*-ray crystallographic analysis to check its overall relative stereochemistry.

A detailed analysis of the stereochemical properties of the fungal products was achieved by degradation into smaller chiral fragments. It was most important to separate C-5/C-6 from C-8/C-9 and in this respect the degradative sequence (Scheme 2) developed during earlier biosynthetic work on aspyrone [6] served admirably. The ozonolytic degradation was carried out without modification on rigorously purified samples of aspyrone, asperlactone, and isoasperlactone. The optical rotation of the *p*-bromophenacyl derivatives (10) of the epoxy acid samples derived from each of the three natural products were identical, both in sign and in magnitude. The



Scheme 2.

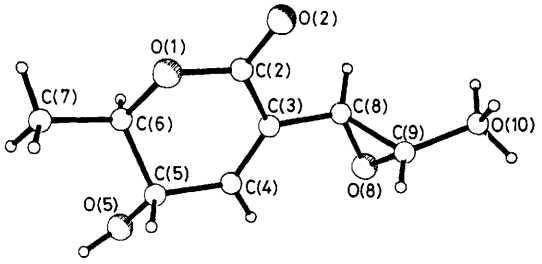


Figure 1. Perspective view of the molecule showing the atom numbering scheme. Oxygen atoms are shaded.

optical rotation curves of the 1-methylglycerol tribenzoates (12) from aspyrone and isoasperlactone were identical in all respects, but that of the asperlactone-derived tribenzoate, although equal in magnitude, was opposite in sign. Hence aspyrone and isoasperlactone have the same configuration at all four centres but asperlactone differs in configuration at both C-5 and C-6. The implications of these results are discussed in more detail in the accompanying paper on the biosynthesis of asperlactone.

Subsequently, the absolute configuration of all three molecules was determined. Optically-active (+)-*erythro*-1-methylglycerol, otherwise (+)-(2*R*,3*S*)-butane-1,2,3-triol or (+)-(2*S*,3*R*)-1-deoxyerythritol, has been prepared from D-glucose.[9] The triol from asperlactone had a small positive sign of rotation while that from aspyrone was, as expected, negative. In view of the difficulty in the purification of this compound and the small $[\alpha]_D$ values observed, the triol from aspyrone was converted into the known tris(*p*-nitrobenzoate) derivative;[9,10] the optical rotation of this ester is larger than that of the triol and is therefore less subject to error. The ester from aspyrone had $[\alpha]_D$ − 79°; both the sign and magnitude of the optical rotation are identical with those of the corresponding derivative of (−)-(2*R*,3*S*)-1-deoxyerythritol. This result establishes that aspyrone is the (5*S*,6*R*) isomer as shown in Scheme 2; the configuration at C-6 is in agreement with that suggested on the basis of the octant rule.[5] It follows from the above data that the configuration of asperlactone is (5*R*,6*S*).

The epoxy acid (9) from aspyrone had a negative sign of rotation and formed a brucine salt, as does (−)-(2*R*,3*S*)-2,3-epoxybutyric acid [11] suggesting that the configuration of the epoxypropyl side-chain is (8*S*,9*S*). The naturally derived epoxy acid samples resisted crystallisation, hence it was decided to check the overall relative stereochemistry of aspyrone through an *X*-ray crystallographic study; this, together with the results for C-5/C-6, defines completely the absolute stereochemistry of (3), and therefore that of (5) and (6). The details of the *X*-ray analysis are given in the Experimental section. Atomic co-ordinates are given in Table 1 and derived bond lengths and angles in Tables 2 and 3. A picture of the molecule is shown in Figure 1 and Figure 2 shows a packing plot. The *trans*-epoxypropyl side-chain is clearly visible; with the centres at C-5 and C-6 defined as *S* and *R* respectively, the configurations at C-8 and C-9 are deduced to be both *S*, in agreement with the preliminary chemical studies. Thus aspyrone and isoasperlactone both have the configuration (5*S*,6*R*,8*S*,9*S*), whereas that of asperlactone is (5*R*,6*S*,8*S*,9*S*).

It remained only to clarify the origin of the two γ-lactone isomers in view of the facile chemical conversion of aspyrone into isoasperlactone. Spectroscopic and chromatographic studies indicate clearly that aspyrone alone is produced during the first 10 days of growth, but that thereafter asperlactone and isoasperlactone are produced. In control experiments aspyrone decomposed slowly over 3—5 days when in contact with sterilised culture medium in the absence of the fungus, but no isoasperlactone was produced. The *in vivo* production of this compound may therefore be enzymically controlled although the possibility cannot be ruled out that it is a chemical artefact produced by decomposition of aspyrone during incubation or isolation. In a related experiment ^{14}C-labelled

Table 1. Atom co-ordinates (× 10^4) and isotropic temperature factors (Å^2 × 10^3)

	x/a	*y/b*	*z/c*	*U*
O(1)	10 592(3)	3 417(1)	7 858(1)	62(1) *
C(2)	10 026(5)	3 992(1)	7 171(1)	57(1) *
O(2)	11 244(5)	4 751(1)	7 153(1)	80(1) *
C(3)	8 097(5)	3 650(1)	6 444(1)	57(1) *
C(4)	7 427(5)	2 745(1)	6 396(2)	64(1) *
H(4)	6 311	2 517	5 891	80
C(5)	8 362(6)	2 074(1)	7 106(1)	58(1) *
H(5)	10 264	1 787	6 964	70
O(5)	6 093(4)	1 376(1)	7 184(1)	76(1) *
H(51)	6 831(64)	863(14)	7 389(14)	89(7)
C(6)	8 620(6)	2 604(1)	7 978(1)	58(1) *
H(6)	6 584	2 770	8 131	74
C(7)	9 930(7)	2 043(1)	8 738(2)	76(1) *
H(7a)	8 611	1 525	8 861	91
H(7b)	10 064	2 436	9 256	91
H(7c)	11 902	1 812	8 588	91
C(8)	7 153(6)	4 351(1)	5 758(2)	69(1) *
H(8)	6 294(63)	4 901(16)	5 989(17)	84
O(8)	5 588(6)	3 994(1)	5 006(2)	120(1) *
C(9)	8 482(8)	4 414(2)	4 881(2)	76(1) *
H(9)	10 032(66)	3 999(18)	4 781(17)	92
C(10)	8 584(9)	5 286(2)	4 345(2)	93(1) *
H(10a)	10 582	5 508	4 475	111
H(10b)	7 127	5 736	4 549	111
H(10c)	8 370	5 201	3 712	111

* Equivalent isotropic *U* calculated from anisotropic *U*.

Table 2. Bond lengths (Å)

C(2)–O(1)	1.341(3)	C(2)–O(2)	1.209(3)
C(2)–C(3)	1.467(4)	C(3)–C(4)	1.327(4)
C(3)–C(8)	1.494(4)	C(4)–C(5)	1.489(4)
C(5)–O(5)	1.419(4)	C(5)–C(6)	1.513(4)
H(51)–O(5)	0.858(23)	C(6)–O(1)	1.463(3)
C(6)–C(7)	1.507(4)	C(8)–H(8)	0.939(25)
C(8)–O(8)	1.415(5)	C(8)–C(9)	1.441(5)
C(9)–O(8)	1.426(5)	C(9)–H(9)	0.919(29)
C(10)–C(9)	1.482(4)		

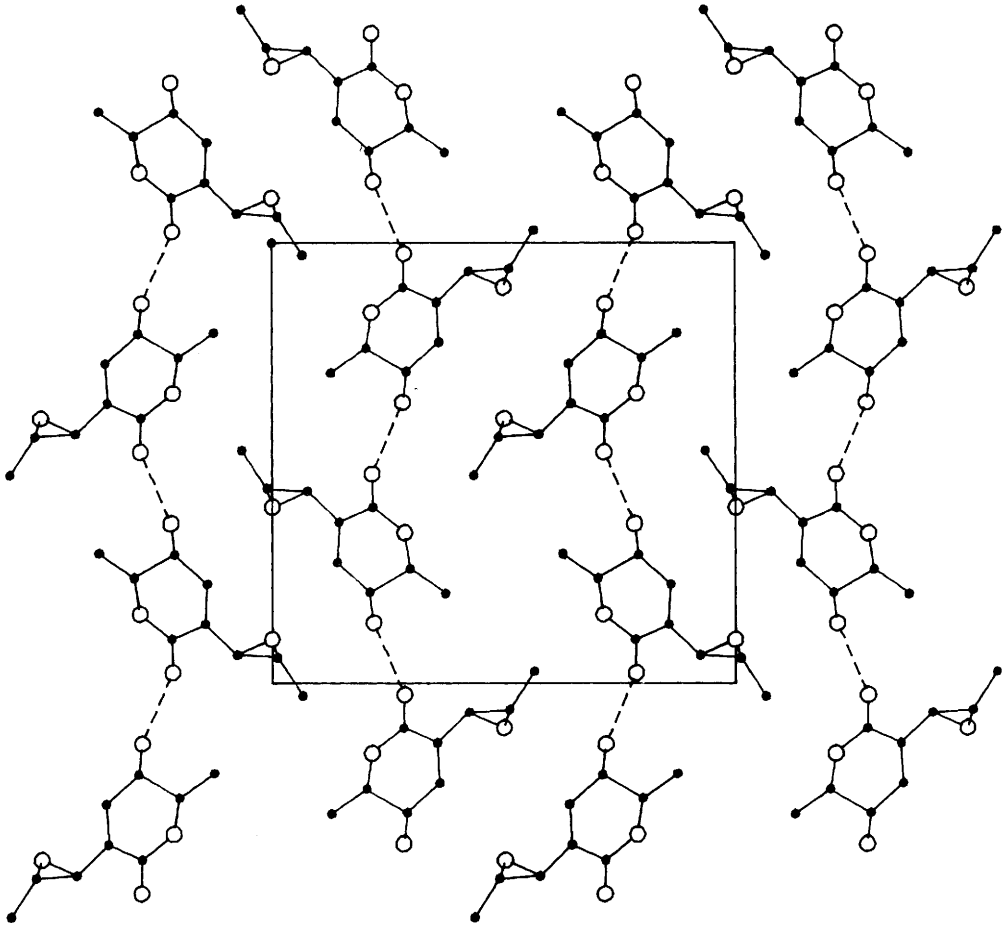


Figure 2. Packing plot projected down the x axis. H atoms omitted for clarity. H bonds are indicated by dotted lines [O(5) · · · O(2) 2.79 Å; O(2) at $2 - x$, $-0.5 + y$, $1.5 - z$].

Table 3. Bond angles (°)

C(2)–O(1)–C(6)	117.9(3)	O(1)–C(2)–O(2)	118.8(3)
O(1)–C(2)–C(3)	118.3(3)	O(2)–C(2)–C(3)	122.8(3)
C(2)–C(3)–C(4)	119.7(3)	C(2)–C(3)–C(8)	116.7(3)
C(4)–C(3)–C(8)	123.5(3)	C(3)–C(4)–H(4)	119.2(2)
C(3)–C(4)–C(5)	121.7(3)	H(4)–C(4)–C(5)	119.2(2)
C(4)–C(5)–H(5)	111.0(2)	C(4)–C(5)–O(5)	108.2(3)
H(5)–C(5)–O(5)	109.9(2)	C(4)–C(5)–C(6)	108.3(3)
H(5)–C(5)–C(6)	109.8(2)	O(5)–C(5)–C(6)	109.5(3)
C(5)–O(5)–H(51)	111.0(19)	O(1)–C(6)–C(5)	109.7(3)
O(1)–C(6)–H(6)	113.2(2)	C(5)–C(6)–H(6)	105.0(2)
O(1)–C(6)–C(7)	106.5(3)	C(5)–C(6)–C(7)	114.5(3)
H(6)–C(6)–C(7)	108.2(2)	C(6)–C(7)–H(7a)	108.7(2)
C(6)–C(7)–H(7b)	108.9(2)	C(6)–C(7)–H(7c)	110.9(2)
C(3)–C(8)–H(8)	114.8(16)	C(3)–C(8)–O(8)	116.2(3)
H(8)–C(8)–O(8)	113.3(17)	C(3)–C(8)–C(9)	123.6(3)
H(8)–C(8)–C(9)	116.7(16)	O(8)–C(8)–C(9)	59.9(3)
C(8)–O(8)–C(9)	60.9(3)	C(8)–C(9)–O(8)	59.2(3)
C(8)–C(9)–H(9)	114.4(17)	O(8)–C(9)–H(9)	115.0(18)
C(8)–C(9)–C(10)	123.9(3)	O(8)–C(9)–C(10)	116.8(4)
H(9)–C(9)–C(10)	115.5(18)	C(9)–C(10)–H(10a)	101.2(3)
C(9)–C(10)–H(10b)	111.6(3)	C(9)–C(10)–H(10c)	115.2(2)

aspyrone derived from [1-^{14}C]acetate was supplied to the organism, but no conversion into asperlactone or isoasperlactone was detected. Shortage of material precluded separation of the two isomers and the specific activities of the individual isomers were not therefore determined.

Experimental

Solutions were dried over sodium sulphate (anhydrous). M.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded with a Perkin-Elmer 257 spectrophotometer for solutions in chloroform. U.v. spectra were recorded on a Unicam SP8000 B for solutions in 95% ethanol. Mass spectra were recorded on an AEI MS9 or MS30 mass spectrometer. ^{1}H N.m.r. spectra were recorded on a Perkin-Elmer EM390 spectrometer for solutions in deuteriochloroform with $SiMe_4$ as internal standard. ^{13}C N.m.r. spectra were recorded on a Varian XL 100 A spectrometer for solutions in deuteriochloroform with $SiMe_4$ as internal standard. Column chromatography was carried out on silica gel (Merck, 500 mesh) and preparative t.l.c. on glass plates coated with Merck Kieselgel GF_{254}. Radioactive samples were counted as solutions in aqueous or organic scintillator solution (7 ml) on a Packard Tri-Carb 3385 scintillation counter and standardised internally with radio-labelled n-hexadecane. Optical activities were measured for solutions in chloroform unless otherwise stated on a Perkin-Elmer 241 polarimeter using a 0.1 dm path length.

Isolation of Metabolites.—Aspergillus melleus (CMI 49108) was grown at 27 °C in static cultures, each flask containing 500 ml of an aqueous solution with potassium dihydrogen phosphate (1 g), magnesium sulphate heptahydrate (0.5 g), potassium chloride (0.5 g), urea (0.7 g), glucose (75 g), and yeast extract powder (5 g, Oxoid) per litre of glass-distilled water. After 21 days, the cultures were filtered through Celite

then extracted with ethyl acetate to give a brown gum (*ca.* 0.5 g/l) which was further purified by either column chromatography or preparative t.l.c. using ethyl acetate–benzene (1 : 1) or diethyl ether–light petroleum (b.p. 60—80 °C) (1 : 1) as eluants. Four bands were scraped off and eluted with ethyl acetate to give the following in order of decreasing R_F value: (a) *mellein*, (−)-(3R)-3,4-*dihydro*-8-*hydroxy*-3-*methylisocoumarin* (1), white needles from light petroleum (b.p. 40—60 °C), m.p. 56—58 °C (lit.,[1] 58 °C); $[\alpha]_D^{22}$ −80.0° (*c* 0.020), R_F 0.8 (ethyl acetate–benzene, 1 : 1); $\lambda_{max.}$ (EtOH) 246 and 312 nm; $\nu_{max.}$ 3 150br m, 1 680s, 1 620m, 1 585m, 1 470m, and 1 220s cm^{-1}; δ 7.37 (1 H, t, *J* 7 Hz, 6-H), 6.84 (1 H, d, *J* 8 Hz, 7-H), 6.66 (1 H, d, *J* 7 Hz, 5-H), 4.70 (1 H, m, *J* 7 Hz, 3-H), 2.89 (2 H, d, *J* 7 Hz, 4-H), and 1.49 (3 H, d, *J* 7 Hz, Me); *m/z* 178 (M^+).

(b) 4-*Hydroxymellein*, (−)-(3R,4R)-3,4-*dihydro*-4,8-*dihydroxy*-3-*methylisocoumarin* (2), white needles from benzene, m.p. 133.5—135 °C (lit.,[3] 133—135 °C), $[\alpha]_D$ −27.5° (*c* 0.04), R_F 0.7 (ethyl acetate–benzene, 1 : 1); $\lambda_{max.}$ 217, 245, and 313 nm; $\nu_{max.}$ 3 300br s, 1 680s, 1 620m, 1 585s, 1 460s, and 1 220 cm^{-1}; δ 11.5 (1 H, s, OH), 7.48 (1 H, t, *J* 8 Hz, 6-H), 7.05 (1 H, d, *J* 7 Hz, 5-H), 6.95 (1 H, d, *J* 9 Hz, 7-H), 4.47 (2 H, m, 3-H and 4-H), 3.02 (1 H, s, OH), and 1.38 (3 H, d, *J* 5 Hz); *m/z* 194 (M^+), 150, 122, 105, 101, 85, 83, and 59.

(c) *Aspyrone*, 3-(1,2-*epoxypropyl*)-5,6-*dihydro*-5-*hydroxy*-6-*methylpyran*-2-*one* (3), white needles from benzene, m.p. 110—112 °C (lit.,[4] 109—112 °C), $[\alpha]_D$ −10.5° (*c* 0.03); R_F 0.45 (ethyl acetate–benzene, 1 : 1); $\lambda_{max.}$ 205 nm; $\nu_{max.}$ 3 410s, 1 709s, and 1 656 cm^{-1}; δ 6.64 (1 H, dd, *J* 1, 2.5 Hz, 4-H), 4.30 (1 H, m, *J* 8.5, 7 Hz, 6-H), 4.14 (1 H, m, *J* 2.5, 8.5 Hz, 5-H), 3.72 (1 H, br, OH), 3.45 (1 H, d, *J* 1, 2 Hz, 8-H), 2.80 (1 H, dq, *J* 5, 2 Hz, 9-H), 1.43 (3 H, d, *J* 7 Hz, 7-H), and 1.36 (3 H, *J* 5 Hz, 10-H); *m/z* 184 (M^+), 167 (M−OH), 140, 125, 111, 97, 83, 68, and 40.

(d) *Asperlactone*, 3-(1,2-*epoxypropyl*)-5-(1-*hydroxyethyl*)-*butenolide* (5), b.p. 140 °C/0.4 Torr, $[\alpha]_D$ +61.84° (*c* 0.11); R_F 0.35 (ethyl acetate–benzene, 1 : 1); $\lambda_{max.}$ 205 nm; $\nu_{max.}$ 3 600sh, 3 480br, 2 990, 2 920, 1 765s, 1 630, 1 380, 1 350, 1 130, and 1 070 cm^{-1}; δ 7.28 (1 H, d, *J* 2 Hz, 4-H), 4.82 (1 H, m, *J* 2, 2.5 Hz, 5-H), 4.00 (1 H, m, *J* 6, 2.5 Hz, 6-H), 3.40 (1 H, d, *J* 1.5 Hz, 8-H), 3.15 (1 H, dq, *J* 5, 1.5 Hz, 9-H), 2.90 (1 H, br, OH), 1.35 (3 H, d, *J* 5 Hz, 10-H), and 1.23 (3 H, d, *J* 6 Hz, 7 H); *m/z* 185, 184 (weak), 140 M^+ − CH_3CHO), 125, 111, 97 (100%), 95, 69, 68, 55, 45, 41, 39, and 27.

Derivatives of Asperlactone (5).—(a) *Acetyl derivative*. Asperlactone (172 mg) was stirred with freshly distilled acetic anhydride (1 ml) and pyridine (2 drops) for 2 h, then ethyl acetate (1 ml) was added and the mixture purified by preparative t.l.c. using ethyl acetate–benzene (1 : 1) as eluant to give the acetyl derivative (102 mg, 48%) as a pale oil, R_F 0.4; $\nu_{max.}$ 1 760, 1 730, and 1 640 cm^{-1}; δ 7.08 (1 H, d, *J* 1 Hz, 4-H), 4.90 (2 H, m, 5-H and 6-H), 3.34 (1 H, d, *J* 1.5 Hz, 8-H), 3.15 (1 H, dq, *J* 1.5, 5 Hz, 9-H), 2.08 (3 H, s, $OCOCH_3$), 1.42 (3 H, d, *J* 5.5 Hz, Me), and 1.28 (3 H, d, *J* 6 Hz, Me); *m/z* 226 (weak), 198, 182, 167, 158, 140, 125, 97, 55, and 43.

(b) *Phenylurethane derivative*.[5] Asperlactone (90 mg) and phenyl isocyanate (0.05 ml) were stirred in dry chloroform for 2 days. The crude reaction mixture was purified by preparative t.l.c. using ethyl acetate–benzene (1 : 1) and the band with R_F 0.7 eluted with chloroform to give an oil (61 mg, 41%); $\nu_{max.}$ 3 420, 2 960, 2 780, 1 765, 1 735, and 1 600 cm^{-1}; δ 7.5—6.6 (5 H, m, ArH), 7.20 (1 H, s, 4-H), 5.04 (2 H, m, 5-H and 6-H), 3.40 (1 H, d, *J* 1 Hz, 8-H), 3.16 (1 H, dq, *J* 1, 5 Hz, 9-H), 1.46 (3 H, d, *J* 5 Hz, Me), and 1.27 (3 H, d, *J* 5 Hz, Me); *m/z* 303 (Found: M^+, 303.1107. $C_{16}H_{17}NO_5$ requires M, 303.1102).

(c) p-*Nitrobenzoate derivative*. Asperlactone (78 mg), *p*-nitrobenzoyl chloride (150 mg), and pyridine (2 drops) were stirred in dry pyridine for 2 h. The mixture was then poured into water (2 ml) and extracted into diethyl ether (3 × 3 ml). The combined ether layers were washed with 5% hydrochloric acid (2 × 1 ml), saturated sodium hydrogen carbonate (2 × 1 ml), and water (1 ml), and then dried and evaporated to give an oil (100 mg) which was purified using ethyl acetate–benzene (1 : 1) as eluant to yield an oil (50 mg, 39%), R_F 0.4; $\nu_{max.}$ 1 760, 1 717, and 1 610 cm^{-1}; δ 8.30—8.00 (4 H, d, *J* 4 Hz, ArH), 7.20 (1 H, d, *J* 2 Hz, 4-H), 5.30 (1 H, m, 6-H), 5.20 (1 H, m, 5-H), 3.45 (1 H, s, 8-H), 3.20 (1 H, q, *J* 5 Hz, 9-H), 1.46 (3 H, d, *J* 5 Hz, Me), and 1.40 (3 H, d, *J* 5 Hz, Me).

Base Hydrolysis of Aspyrone (3): *Preparation of Synthetic Isoasperlactone* (6).[5]—Aspyrone (25 mg) was stirred with NaOH (1M; 1 ml) at room temperature for 30 min. The solution was then acidified with dilute hydrochloric acid and extracted into diethyl ether (4 × 2 ml). The combined organic layers were dried and evaporated to give isoasperlactone (20 mg) as a pale oil, $[\alpha]_D$ −51.6° (*c* 0.124).

Degradation of Asperlactone (5).—Asperlactone (100 mg) was dissolved in AnalaR ethyl acetate (75 ml) and cooled to −78 °C. A stream of ozonised oxygen was passed through for 5 min until a pale blue colour appeared. Excess of ozone was removed by passing dry nitrogen through the solution until the effluent gases gave a negative starch–iodide test. The solution was brought to room temperature then evaporated under reduced pressure to give an oil (140 mg).

(+)-(2R,3S)-1-*Methylglycerol* (9).—To the ozonide (140 mg) was added sodium borohydride (210 mg). The mixture was cooled in a liquid nitrogen bath and treated with sodium hydroxide (1M; 4 ml) dropwise. The mixture was warmed to room temperature, stirred for 20 h, then passed down a column of Amberlite Monobed Resin (M.B.I., 50 g) and eluted slowly with water (500 ml). The solvent was removed under reduced pressure and the residue azeotroped with ethanol to give a viscous oil (48 mg, 83%), b.p. 94 °C/0.05 Torr, $[\alpha]_D$ +5.6° (*c* 0.033 in MeOH); $\nu_{max.}$ (film) 3 600s and 2 900 cm^{-1}; δ (D_2O) 3.65 (4 H, m) and 2.22 (3 H, d, *J* 5.5 Hz).

(+)-(2R,3S)-1-*Methylglycerol Tribenzoate* (12).—1-Methylglycerol (48 mg) was treated with benzoyl chloride (220 mg) and anhydrous pyridine (30 mg). The reaction mixture was warmed over 30 min, then diluted with ice–water (5 ml) and extracted into diethyl ether (3 × 3 ml). The combined organic extracts were washed with aqueous sodium hydrogen carbonate (3 ml), dried, and evaporated to give a precipitate (160 mg) which was purified by preparative t.l.c. using benzene as eluant to give 1-methylglycerol tribenzoate (white needles from cyclohexane), m.p. 80—81 °C (lit.,[6] 80—81 °C), $[\alpha]_D$ 3.60° (*c* 0.04); $\nu_{max.}$ 3 000s, 1 720s, and 1 100 cm^{-1}; δ 8.1 (5 H, m, ArH), 7.5 (10 H, m, ArH), 5.7 (2 H, m), 4.7 (2 H, m), and 1.6 (3 H, d, *J* 6 Hz); *m/z* 418 (M^+).

(−)-(2R,3S)-2,3-*Epoxybutyric Acid* (9).—The ozonide prepared as described above from asperlactone (100 mg) was cooled in a liquid nitrogen bath and a solution of sodium hydroxide (1M; 4 ml) and water (2 ml) added dropwise. The temperature was allowed to rise to −2 °C and the solution left at this temperature for 12 h, then taken to pH 4 with dilute hydrochloric acid, and extracted into diethyl ether (8 × 10 ml). The dried organic layers were evaporated to give the acid (9) [6] as a gummy solid (53 mg), $[\alpha]_D$ −78° (*c* 0.06 in benzene); $\nu_{max.}$ 1 724 cm^{-1}; δ 7.9 (1 H, br, OH), 3.3 (2 H, m), and 1.4 (3 H, d, *J* 5 Hz).

(+)-(2R,3S)-p-*Bromophenacyl* 2,3-*Epoxybutyrate* (10).—(−)-2,3-Epoxybutyric acid (9) (50 mg) was dissolved in AnalaR ethyl acetate (5 ml). Triethylamine (50 mg) and *p*-bromophenacyl bromide (160 mg) were added and the mixture stirred at room temperature for 18 h; it was then filtered to remove the hydrobromide salt. After removal of the solvent, the residue was purified by preparative t.l.c. using ethyl acetate–benzene (10 : 1) as eluant to give a white solid which was recrystallised from cyclohexane containing a trace of ethyl alcohol to give the ester as white plates (140 mg), m.p. 85 °C (lit.,[6] 85—86 °C), $[\alpha]_D$ +0.88° (*c* 0.011); $\nu_{max.}$ 1 750s, 1 700s, and 1 590 cm^{-1}; δ 7.7 (4 H, m, ArH), 5.32 (2 H, s, $COCH_2O$), 3.3 (2 H, s, CHCH), and 1.4 (3 H, d, *J* 5 Hz, Me).

Brucine Salt of (−)-trans-2,3-*Epoxybutyric Acid.*—(−)-(2*R*,3*S*)-Epoxybutyric acid (9) (21 mg) and brucine (80 mg) were dissolved in hot methanol (*ca.* 0.5 ml). The solution was filtered and the residual semisolid dissolved in hot methanol (*ca.* 0.5 ml). The solution was filtered and the residual semisolid dissolved in hot methanol–acetone (1 : 9). On cooling, crystals, m.p. 165—170 °C separated and were recrystallised from methanol–acetone to give the brucine salt, m.p. 171—173 °C (lit.,[11] 173 °C); $[\alpha]_D$ −26.0° (*c* 2.1 in H_2O).

Tris(p-*Nitrobenzoate*) *Derivative of* (−)-(2R,3S)-1-*Methylglycerol.*—Aspyrone (100 mg) was converted into (−)-(2*S*,3*R*)-1-methylglycerol (30 mg) by the above procedure. This was treated with pyridine (2 ml) and *p*-nitrobenzoyl chloride (160 mg). After being stirred at room temperature for 24 h, the solution was treated with ice-cold dilute sulphuric acid (3 ml) and filtered. The solid was washed with dilute sulphuric acid, water, aqueous sodium hydrogen carbonate, and water and then air-dried to give the product (47 mg), m.p. 160—162 °C from acetone (lit.,[10] 161—162 °C), $[\alpha]_D$ −79.0° (*c* 0.02, $CHCl_3$).

X-*Ray Crystallographic Analysis.*—*Crystal data.* $C_9H_{10}O_4$, M = 182.18, Orthorhombic, Space group $P2_12_12_1$, a = 4.427(3), b = 14.272(3), c = 14.968(3) Å, U = 945.7 $Å^3$, Z = 4, D_x = 1.279 g cm^{-3}, F_{000} = 384, Mo-K_α radiation, λ = 0.710 69 Å, μ = 1 cm^{-1}. Calculations performed with program system SHELXTL.

Data collection. 2178 Reflexions from the layers 0—5*kl* were collected to 2θ = 50° on a Stoe two-circle diffractometer. After application of Lp corrections, averaging equivalents gave 1 322 unique reflexions, 1 131 of which with F 4σ(F) were used for structure solution and refinement. The structure was solved by direct methods and refined to R 0.033, R' 0.037. All non-hydrogen atoms were refined anisotropically, OH and epoxide ring H freely and isotropically, other H with the constraints C–H 0.96 Å, H–C–H 109.8°, U(H) = 1.2 U(C). The weighting scheme was $w^{-1} = \sigma^2(F) + 0.0005F^2$. Observed and calculated structure factors and anisotropic thermal parameters are deposited as a Supplementary publication [Sup No. 23827 (10 pp.)].*

* For details of the Supplementary publications scheme, see Instructions for Authors (1984), *J. Chem. Soc., Perkin Trans. 1*, 1984, Issue 1.

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