Biosynthesis of Aspyrone, a Metabolite of *Aspergillus melleus*. Incorporation Studies with ^{14}C- and ^{3}H-Labelled Acetates and Malonate

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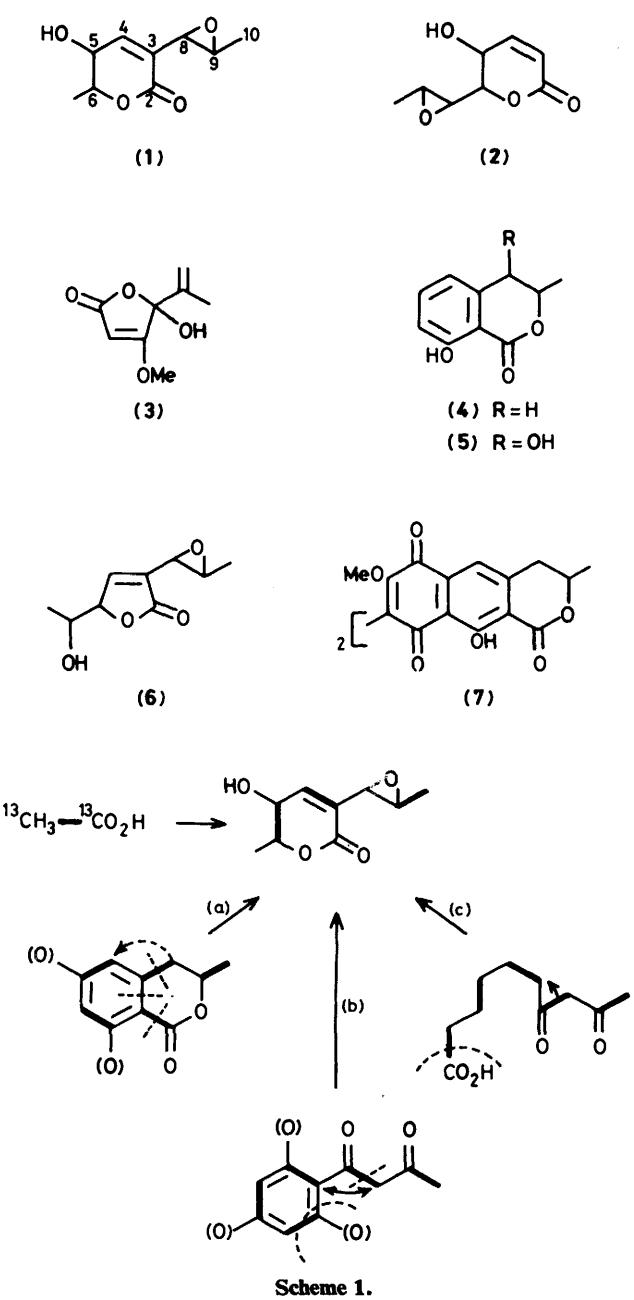
Incorporation studies with ^{14}C-labelled acetates and malonate confirm the polyketide origin of aspyrone (1), and identify the chain starter unit. Five carbons are derived from the methyl group of acetate, and the remaining four from the carboxy group. The pattern of incorporation of tritium from [2-^{3}H]acetate is inconsistent with a biosynthesis from aromatic precursors of the mellein type. Possibly advanced precursors containing a 2-methylchromone nucleus were not incorporated. The evidence suggests that aromatic precursors are not involved in aspyrone biosynthesis, and that the carbon skeleton is produced, like that of the co-metabolite asperlactone (6), by decarboxylation and rearrangement of a linear pentaketide intermediate.

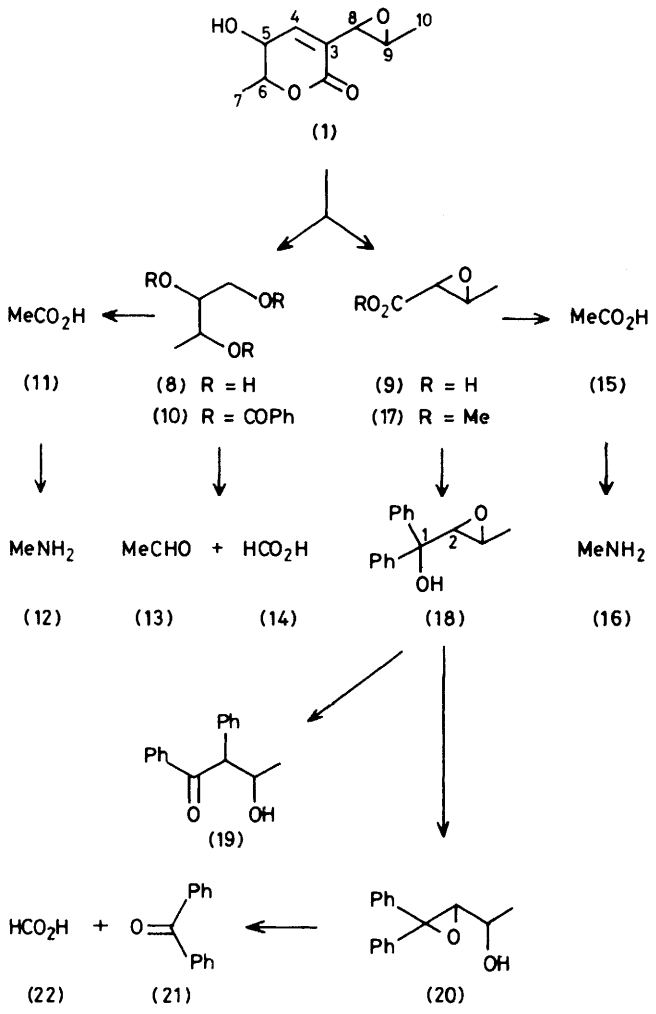
Aspyrone (1), a weak broad spectrum antibiotic, was first isolated by Mills and Turner [1] in 1967 from fermentations of *Aspergillus melleus*. More recently it has been isolated from other *Aspergillus* species [2] and its structure and stereochemistry have been investigated by further chemical and spectroscopic studies.[2b] With its unusual branched carbon skeleton aspyrone is not obviously biosynthetically related to other simpler pyrone metabolites such as asperline (2).[3] *Aspergillus melleus* produces a diverse range of metabolites along with aspyrone, including penicillic acid (3),[1,2c] mellein (4),[4] hydroxymellein (5),[5] asperlactone (6),[6] and various naphthoquinone pigments such as xanthomegnin (7).[7] Of these only (6) is obviously related to (1) since it has the same carbon skeleton and oxygenation pattern. In this and the following papers we describe experiments which explore the possibility that aspyrone (1) and asperlactone (6) are biosynthetically related to each other and also to their aromatic co-metabolites such as mellein (4) or hydroxymellein (5).

Earlier biosynthetic studies [8] with ^{13}C-labelled acetates have established that aspyrone is an extensively modified polyketide. Thus, in incorporation experiments with [1,2-$^{13}C_2$]acetate, all the carbons were enriched and the pattern of incorporation determined by ^{13}C n.m.r. was as indicated in Scheme 1. In addition to the intact units marked by heavy lines, there was evidence for long-range coupling between the two carbons C-2 and C-8 which was interpreted in terms of a rearranged C_2 unit.[8b,c] Since the remaining carbon, C-7, had lost its partner it showed in the spectrum as a singlet. On the basis of these results a number of hypothetical biosynthetic schemes have been proposed.[8] Three fundamentally different possibilities which served as working hypotheses in the present investigation are indicated in Scheme 1; other schemes based on the combination of two separately formed polyketide chains are also possible. Firstly, pathway (a) shows in outline how the aspyrone skeleton could be derived from that of one of its co-metabolites mellein or a related compound. In this connection it is interesting to note that another co-metabolite, penicillic acid (3), is known to be produced by cleavage of an aromatic system.[9] Pathway (b) also invokes an aromatic system as intermediate, this time an acylphloroglucinol, a standard type of pentaketide folding pattern.[10] Finally, pathway (c) indicates a most unusual type of process by which aspyrone would be produced by rearrangement and cleavage of an uncyclised non-aromatic pentaketide.

We now describe experiments aimed at testing the validity of these proposals. Radioisotopes were used as tracers in the study and it was therefore necessary to devise an efficient series of degradations to locate sites of labelling. These are shown in Scheme 2.



Scheme 1.



Scheme 2.

The key step in the scheme is cleavage of the carbon-carbon double bond by ozonolysis, followed by work-up with either sodium borohydride to give 1-methylglycerol (8), or with aqueous sodium hydroxide to give 2,3-epoxybutyric acid (9), which were converted for radioactivity determination into the crystalline tribenzoate ester (10) and the *p*-bromobenzoate ester respectively. Between them these two major fragments contain eight of the nine carbons of aspyrone. Each was degraded further to locate the precise sites of labelling. Thus the triol (8) was subjected either to Kuhn-Roth oxidation to give acetic acid (11) followed by Schmidt degradation to give methylamine (12), or to periodate oxidation from which acetaldehyde (13) and formic acid (14) were isolated.

Controlled degradation of the epoxy acid (9) proved more troublesome. First the *p*-bromophenacyl derivative was subjected to Kuhn-Roth oxidation to give acetic acid (15) which was converted by Schmidt degradation into methylamine (16). To determine the distribution of activity further the methyl ester (17) was treated under carefully controlled conditions in tetrahydrofuran with excess of phenylmagnesium bromide to give the tertiary alcohol (18). This was treated with potassium t-butoxide in t-butyl alcohol, and also with sodium ethoxide in ethanol in the hope of causing fragmentation to give benzophenone but a migration corresponding to a pinacol rearrangement took place instead leading to the formation of the isomeric ketone (19); the same ketone was formed directly by carrying out the Grignard reaction in diethyl ether under more vigorous conditions. The ease of this migration of a phenyl group from C-1 to C-2 of (18) means that any degradation scheme in which benzoic acid is produced by oxidation would not provide an unambiguous method of determining the amount of radioactivity located at C-3 of the pyrone. In order to rule out the possibility that migration had occurred it was essential to isolate benzophenone instead. It was reasoned that this could be achieved by periodate oxidation. Since direct oxidation of (18) proved fruitless, prior treatment with potassium hydroxide in aqueous methanol was explored. Instead of undergoing hydrolysis to a triol as desired, (18) rearranged to the isomeric epoxide (20); there is precedent for transposition of α-hydroxy epoxide groups in this way.[11] Fortunately this product proved to be the key to success, for unlike (18), (20) underwent oxidative cleavage on treatment with periodate to give a quantitative yield of benzophenone (21). Formic acid (22) deriving from C-8 of (1) could also be isolated as its *p*-bromophenacyl ester from this reaction. Hence the degradations in Scheme 2 allow the degree of labelling at each of the nine carbons of aspyrone to be determined uniquely either by direct measurement or by difference.

The first biosynthetic experiments involved [1-^{14}C]acetate and [2-^{14}C]acetate. Each precursor was incorporated well and from experiments 1 and 2 in Tables 1, 2 and 3, it can be seen that the results confirm the labelling pattern deduced from ^{13}C labelling studies. Thus there are four carbons in (1) uniformly labelled by [1-^{14}C]acetate: C-2, C-4, C-6, and C-9, and five uniformly labelled by [2-^{14}C]acetate C-3, C-5, C-7, C-8, and C-10.

The high degree of uniformity in the distribution of the isotope, which is established within narrow limits by these radiotracer studies, points to a pathway which involves a single polyketide chain rather than one which involves a combination of two separately formed chains. This point was confirmed by administering [2-^{14}C]malonate to the organism. The results of experiment 3 in Tables 1, 2, and 3 show that aspyrone was labelled evenly over four sites, C-3, C-5, C-7, and C-8, but the level of incorporation at the fifth site, C-10, was markedly lower. In addition to confirming that only one chain is involved, this experiment identifies C-9 + C-10 as the chain starter unit. Recently it was reported [8d] that [2-^{13}C]malonate failed to produce differential incorporation into the starter unit. This is presumably due to overloading of the metabolism of the organism by the much larger dose of precursor which is normally used in a ^{13}C labelling experiment.

At this stage all three pathways of Scheme 1 are consistent with all the available evidence. Further experiments using tritium-labelled precursors were then carried out to provide a more searching test. [2-^{3}H]Acetate was administered to the organism in two separate experiments, one with, and the other without, an internal standard in the form of [2-^{14}C]acetate. A substantial retention of tritium was observed (see experiment 4 in Tables 1, 2, and 3, and Table 4). The ^{3}H-labelled compounds were degraded in the same way as the carbon labelled compounds (it was checked that no significant exchange of tritium with the medium had occurred in those steps where this could be a problem by carrying out the reactions in D_2O). With the exception of C-3, which does not carry a hydrogen, all the carbons derived from C-2 of acetate retain some tritium. The striking feature of these results is the marked variation in the amount of tritium retained at the four sites, C-7, C-5, C-8, and C-10. It is not possible at this stage to give an explanation or to draw any conclusion from the variation in tritium level; apart from essential biosynthetic reactions which necessarily remove a hydrogen (*e.g.* carbon-carbon bond formation, and oxidation reactions), loss of hydrogen isotope by exchange will also occur in the many intermediates

Table 1. Incorporation of singly-labelled precursors into aspyrone (1) and ozonolysis results

			Relative molar activities (%)		
				Epoxy acid [a] (9)	Triol [b] (8)
Expt	Precursor	Incorporation (%)	Aspyrone (1)	C-(3 + 8 + 9 + 10) [c]	C-(4 + 5 + 6 + 7) [c]
1	[1-^{14}C]Acetate	0.7	100	24.6	50.2
2	[2-^{14}C]Acetate	10	100	59.0	40.5
3	[2-^{14}C]Malonate	13	100	52.0	48.0
4	[2-^{3}H]Acetate	0.3	100	52.0	42.0

[a] Counted as *p*-bromophenacyl ester. [b] Counted as the tribenzoate ester. [c] Carbons of aspyrone (1) in degradation product.

Table 2. Degradations of labelled samples of acid (7) (following Table 1)

	Molar activities (%) relative to aspyrone				
	Epoxy acid [a] (9)	Acetate [a] (15)	Methylamine [b] (16)	Benzophenone [c] (21)	Formate [a] (22)
Expt	C-(3 + 8 + 9 + 10) [d]	C-(9 + 10) [d]	C-10 [d]	C-3 [d]	C-8 [d]
1	24.6	24.6	<0.5	—	—
2	59.0	21.0	19.4	19.3	19.3
3	52.0	7.1	7.5	—	—
4	52.0	39.0	—	—	—

[a] Counted as the *p*-bromophenacyl ester. [b] Counted as the *p*-bromobenzamide. [c] Counted as the oxime. [d] Carbons of aspyrone (1) in degradation product.

Table 3. Degradations of labelled samples of triol (6) (following Table 1)

	Molar activities (%) relative to aspyrone			
	Triol [a] (8)	Acetate [b] (11)	Methylamine [c] (12)	Formate [a] (14)
Expt	C-(4 + 5 + 6 + 7) [d]	C-(6 + 7) [d]	C-7 [d]	C-5 [d]
1	50.2	25.2	0.5	—
2	40.5	21.5	19.4	19.28
3	48.0	22.2	24.3	—
4	42.0	31.0	—	10.5

[a] Counted as the tribenzoate. [b] Counted as the *p*-bromophenacyl ester. [c] Counted as the *p*-bromobenzamide. [d] Carbons of aspyrone (1) in degradation product.

Table 4. Incorporation of [2-^{14}C,2-^{3}H]acetate into aspyrone (1)

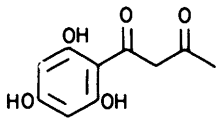
	^{3}H : ^{14}C Ratios (percent relative to administered acetate)				
Precursor	Aspyrone (1)	Epoxy acid [a] (9)	Acetate [a] (15)	Triol [b] (8)	Acetate [a] (11)
8.7 (100)	2.9 (33)	2.4 (27)	5.8 (67)	3.2 (37)	4.4 (50)

[a] Counted as the *p*-bromophenacyl ester. [b] Counted as the tribenzoate.

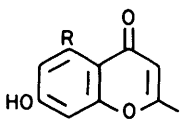
where C–H groups derived from the methyl group of acetate are acidic, and are therefore susceptible to exchange with the medium (*e.g.* in a C–H group flanked by two carbonyl groups). It is worth noting, however, that the distribution proved to be reproducible within experimental error over two separate incorporation experiments.

Whatever the significance of the relative retentions of tritium, there is one important piece of information to emerge from these experiments: tritium is retained to a significant extent at C-7 of aspyrone (1). This would not be expected for pathway (a) in Scheme 1 since all three hydrogens originally present in the methyl group of the acetate precursor are necessarily removed from C-7 in forming the aryl ring of the early intermediates. An alternative rearrangement of the mellein skeleton in which the C_3 side-chain migrates in the opposite direction would satisfactorily account for the observed tritium retentions but it would lead to the wrong pattern of incorporation of intact C_2 units from acetate. Therefore, all variants of this proposal are eliminated, as is an earlier proposal [8a] based on an alternative chain-folding pattern. That the levels of tritium retention were sufficiently high to be significant is apparent from the experiment in which an internal ^{14}C standard was used (Table 4). As can be seen, the methyl group at C-10 retains 69% of the tritium originally present, and that at C-7, 50%. The value for the incorporation of tritium at C-7 is too high for it to be attributed to random intra- or inter-molecular transfer of the isotope from one labelled site to another because the tritium would be expected to be heavily diluted by protium from the medium in the course of such a process.

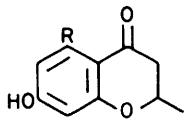
It is not possible to discriminate between the two remaining schemes on the basis of these results, even though two of the three hydrogens of acetate may be retained at C-7 on pathway (c) and only one on pathway (b). This is because isotope effects



(23)



(24) R = H
(25) R = OH



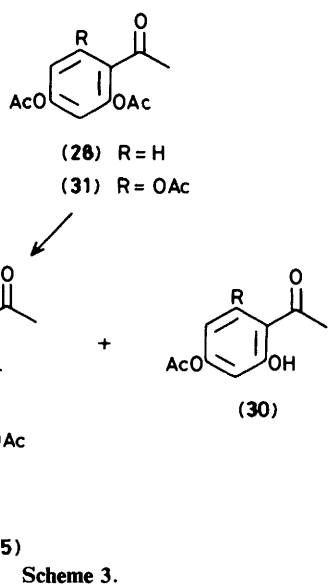
(26) R = H
(27) R = OH

of unknown size will favour removal of protium over tritium from the CH_2T group of the administered acetate. Therefore, even if pathway (b) of Scheme 2 is the correct one, as much as 50% of the tritium could be retained relative to ^{14}C at C-7 of (1).

In an attempt to test the validity of pathway (b) feeding experiments were carried out with possible advanced precursors. In selecting candidates for synthesis, it was necessary to bear in mind the results of experiments with [1,2-$^{13}C_2$]-acetate which eliminate symmetrical compounds such as (23) from consideration, though the analogous chromone (25) is allowed as long as no symmetrical intermediate is involved in an earlier step of the biosynthesis; this would be possible if formation of the heterocyclic ring were to take place prior to release of the aromatic system from the polyketide synthase.

Four candidates (24), (25), (26), and (27) were synthesised to test this hypothesis. The general method is indicated for (24) in Scheme 3. Treatment of the diacetoxy derivative (28) with sodamide resulted in cyclisation and intermolecular acetyl group transfer to give the chromone (29); (30), the deacetylation product of (28), was also formed. Base-catalysed deacetylation of (29) gave one of the desired chromones, (24), from which the chromanone (26) was obtained by hydrogenation. The phloroglucinol analogues, (25) and (27), were similarly prepared from (31) *via* (32). For the radiochemical syntheses, [2-^{14}C]acetic anhydride was used in the preparation of (28) and (31). Thus the four potential precursors (24), (25), (26) and (27) were obtained specifically labelled with ^{14}C in the C-methyl group.

All four labelled compounds were administered to growing cultures of *A. melleus*. In each experiment little radioactivity (< 0.01%) was recovered in aspyrone (1). These negative incorporation results do not, of course, eliminate pathway (b) from contention, although they do favour the alternative, pathway (c), in which no aromatic intermediates are involved. In subsequent work on the related metabolite asperlactone (6), positive evidence has been obtained to show that the carbon skeleton arises in the same way as that of aspyrone and also to eliminate any pathway in which aromatic intermediates are involved.[6] Given the close structural similarity between aspyrone and asperlactone, it is highly likely that they share a common biosynthesis and therefore that aspyrone also is derived by a pathway of type (c). The implications of this relationship are discussed further in following papers concerning the structure of asperlactone and its biosynthesis.



(28) R = H
(31) R = OAc

(29) R = H
(32) R = OAc

(30)

(24) or (25)

Scheme 3.

Experimental

M.p.s were determined with a Reichert-Kofler hot-stage apparatus. Preparative t.l.c. was carried out on glass plates coated with Merck Kieselgel GF_{254}. Radioactive samples were counted in 7 ml of organic scintillator or aqueous scintillator on a Packard Tri-Carb 3385 instrument and standardised with radiolabelled n-hexadecane as internal standard. Unless otherwise stated, u.v. spectra were recorded with a Unicam SP 800 spectrometer for solutions in 95% ethanol, i.r. spectra for solutions in chloroform with a Unicam SP 200 or SP 1 000 spectrometer, and n.m.r. spectra for solutions in deuteriochloroform with a Perkin-Elmer R12B or Varian HA-100 spectrometer (tetramethylsilane standard). Mass spectra were determined with an A.E.I. MS9, MS12, or MS902 spectrometer by direct insertion at appropriate temperatures.

Isolation of Aspyrone (1) *from* Aspergillus melleus.—The mould *Aspergillus melleus* (Commonwealth Mycological Institute No. 49 108) was grown as still cultures at 25 °C in glass vessels each containing approximately 400 ml of an aqueous solution of potassium dihydrogen phosphate (0.1%), magnesium sulphate heptahydrate (0.05%), potassium chloride (0.05%), urea (0.07%), and sucrose (7.5%). The cultures were inoculated by mycelium transfer and incubated for 21 days. The fermentation liquors were then filtered off and extracted in portions (1l) with ethyl acetate (3 × 500 ml). Evaporation of the solvent under reduced pressure after drying over anhydrous sodium sulphate, gave a brown gum. The gum was chromatographed on a column of silica gel (200—300 mesh) using ethyl acetate–benzene (2 : 1) as eluant.

The fractions corresponding to the pyrone were collected and on removal of eluant gave a pale brown solid which recrystallised from benzene to give aspyrone as white needles, m.p. 110—112 °C (lit.,[1] 109—112 °C); $[\alpha]_D^{22}$ − 10.5 °C (*c* 0.03 in $CHCl_3$); $\lambda_{max.}$ 205 nm; $\nu_{max.}$ 3 410, 1 709, and 1 656 cm^{-1}; δ 6.6 (1 H, dd, *J* 1, 2.5 Hz), 4.3 (1 H, m, *J* 7, 8.5 Hz), 4.1 (1 H, m, *J* 2.5, 8.5 Hz), 3.7 (1 H, br), 3.5 (1 H, dd, *J* 1, 2 Hz), 2.8 (1 H, dq, *J* 2, 5 Hz), 1.43 (3 H, d, *J* 7 Hz), and 1.36 (3 H, d, *J* 5 Hz); *m/z* 184 (M^+), 167, 140, 125, 111, and 97.

Incorporation Experiments.—The radiolabelled precursor was administered in aqueous solution to 10 glass vessels each containing 400 ml of nutrient medium which had been inoculated 10 days previously. After 21 days the fermentation liquors were filtered off. The work-up procedure was as described above except that the metabolite was recrystallised to constant activity.

Ozonolysis of Aspyrone (1).—(a) *Preparation of the ozonide.* A stream of ozonised oxygen (6%) was passed into a solution

of aspyrone (100 mg) in ethyl acetate (75 ml) at −78 °C for *ca.* 7 min, when the solution turned pale blue indicating an excess of ozone. The excess ozone was removed by passing dry nitrogen through the solution. Removal of the solvent under reduced pressure gave the ozonide as a colourless oil which crystallised as white needles (130 mg). T.l.c. on silica with benzene–ethyl acetate (1 : 1) as eluant showed no trace of aspyrone.

(b) 2,3-*Epoxybutyric acid* (9). The foregoing ozonide was cooled in a liquid nitrogen bath and a solution of sodium hydroxide (1M; 4 ml) and water (2 ml) added dropwise. The temperature of the reaction was allowed to rise to −2 °C and the solution was left to stand at this temperature for 12 h. The solution was then adjusted to pH 4 with 2M-hydrochloric acid and extracted with diethyl ether (8 × 10 ml). The ethereal solution was dried (Na_2SO_4), and evaporated to give a white crystalline solid, 2,3-expoxybutyric acid (53 mg), m.p. 84—85 °C (lit.,[12] 84.5—85 °C) (Found: C, 47.15; H, 5.85. Calc for $C_4H_6O_3$: C, 47.05; H, 5.90%); $\nu_{max.}$ 1 724 cm^{-1}; δ 7.9 (1 H, br), 3.3 (2 H, m), and 1.4 (3 H, d, *J* 5 Hz).

p-*Bromophenacyl* 2,3-*epoxybutyrate*. A solution of 2,3-epoxybutyric acid (9) (50 mg) and *p*-bromophenacyl bromide (160 mg) in AnalaR ethyl acetate (5 ml) and triethylamine (50 mg) was stirred at room temperature during 18 h and then filtered to remove the hydrobromide salt of the triethylamine. Removal of the solvent left a gum which was purified by preparative layer chromatography in benzene–ethyl acetate (10 : 1) to give p-*bromophenacyl* 2,3-*epoxybutyrate* which recrystallised from cyclohexane–ethanol as white plates (140 mg), m.p. 85—86 °C (Found: C, 48.1; H, 3.45. $C_{12}H_{11}BrO_4$ requires C, 48.17; H, 3.68%); $\nu_{max.}$ 1 750, 1 700, and 1 590 cm^{-1}; *m/z* 298/300 (*M*+); δ 7.7 (4 H, m), 5.3 (2 H, s), 3.3 (2 H, s), and 1.4 (3 H, d, *J* 5 Hz).

(c) *Preparation of* (10), *the tribenzoate of* 1-*methylglycerol*. To the foregoing ozonide was added sodium borohydride (210 mg). The mixture was cooled in a liquid nitrogen bath and sodium hydroxide (1M; 4 ml) added dropwise. The reaction was then allowed to warm to room temperature. After being stirred for 24 h the reaction solution was passed down a column of ion-exchange resin (50 g Amberlite Monobed Resin M.B.I.) and eluted with water (500 ml). The water was removed by evaporation under reduced pressure and the product heated at 110 °C for 3 min to remove final traces of water, leaving 1-methylglycerol (8) as a viscous sweet-smelling oil (45 mg); $\nu_{max.}$ 3 600 and 2 900 cm^{-1}; δ (D_2O) 3.65 (4 H, m) and 1.2 (3 H, d, *J* 5 Hz).

A mixture of 1-methylglycerol (45 mg), benzoyl chloride (220 mg), and anhydrous pyridine (30 mg) was warmed gently during 30 min and then evaporated. The remaining solid was purified by preparative layer chromatography on silica using benzene as eluant. Removal of the solvent left the *tribenzoate* as an oil which recrystallised from cyclohexane as white needles, m.p. 80—81 °C (Found: C, 71.9; H, 5.5. $C_{25}H_{22}O_6$ requires C, 71.76; H, 5.26%); $\nu_{max.}$ 3 000, 1 720, and 1 110 cm^{-1}; δ 8.1 (5 H, m), 7.5 (10 H, m), 5.7 (2 H, m), 4.7 (2 H, m), and 1.6 (3 H, d, *J* 6 Hz).

Kuhn-Roth Oxidation of 1-*Methylglycerol Tribenzoate* (10). —A solution of the tribenzoate (50 mg) in chromic acid (5M; 10 ml) and concentrated sulphuric acid (2 ml) was heated during 1 h at 100 °C and then steam distilled. The distillate (200 ml) was boiled to remove dissolved carbon dioxide, then cooled in an ice-bath before being neutralised with sodium hydroxide (0.5M; 4.5 ml) using phenolphthalein as indicator.

Kuhn-Roth Oxidation of p-*Bromophenacyl* 2,3-*Epoxybutyrate*.—The conditions of the Kuhn-Roth oxidation for this ester were the same as for the tribenzoate above except that silver sulphate (100 mg) was added to remove hydrogen bromide formed in the oxidation.

Preparation of p-*Bromophenacyl Acetate*.—The residue obtained from the Kuhn-Roth oxidation was taken up in water (1 ml) and ethanol (9 ml). *p*-Bromophenacyl bromide (50 mg) was added, and the solution refluxed for 75 min. The ethanol was removed, water (10 ml) added and the solution extracted with ether (3 × 10 ml). The organic phase was dried over anhydrous sodium sulphate and the solvent then removed to give a gum which was purified by preparative layer chromatography using benzene–ethyl acetate (10 : 1) as eluant. The *p*-bromophenacyl acetate (18 mg) was recrystallised from light petroleum (b.p. 60—80 °C) as plates, m.p. 85—86 °C (lit.,[13] 86 °C); $\nu_{max.}$ 1 740, 1 705, and 1 600 cm^{-1}; *m/z* 256/258 (*M*+).

Schmidt Degradation of Sodium Acetate.—A mixture of sodium acetate (15 mg), chloroform (1.5 ml), and sulphuric acid (0.5 ml) was heated to 50 °C and sodium azide (70 mg) added. After 90 min at this temperature the solution was neutralised with 40% potassium hydroxide solution (3 ml) and then distilled to dryness, the distillate being collected in hydrochloric acid (5M; 15 ml). Removal of the water under reduced pressure to give methylamine hydrochloride (9 mg). A mixture of this with *p*-bromobenzoyl chloride (50 mg) and potassium hydroxide solution (2M; 10 ml) was shaken vigorously for 15 min and then the reaction mixture was extracted with ether (3 × 10 ml). The ethereal solution was dried and evaporated to give a white solid (45 mg). Purification by preparative layer chromatography on silica with ethyl acetate–benzene (1 : 3) as eluant gave, after removal of solvent, *N*-methyl-*p*-bromobenzamide (23 mg) which recrystallised from ethyl acetate–cyclohexane as white needles, m.p. 165—167 °C (lit.,[14] 167 °C); $\lambda_{max.}$ 1 665, 1 595, and 1 485 cm^{-1}.

Sodium Metaperiodate Oxidation of 1-*Methylglycerol* (8).—1-Methylglycerol (58 mg) and sodium metaperiodate (210 mg) were dissolved in a buffer solution (pH 7; 50 ml) prepared by mixing potassium dihydrogen phosphate (0.1M; 1 l) and sodium hydroxide (0.1M; 582 ml). The reaction was heated to 50 °C and a stream of nitrogen gas passed through the solution, the exhaust gases being passed into an aqueous solution of phenylsemicarbazide hydrochloride (80 mg) and sodium acetate (80 mg), where a dense white precipitate formed. This was filtered off and recrystallised from water to give the phenylsemicarbazone of acetaldehyde as white needles (21 mg), m.p. 150—151 °C (lit.,[15] 151 °C); $\nu_{max.}$ 1 690 and 1 545 cm^{-1}.

The reaction solution was acidified with sulphuric acid (5M) and then steam distilled. The distillate was neutralised with sodium hydroxide (0.1M; 4.3 ml) and the solvent removed. Following the procedure described for the corresponding acetate derivative the residue was converted into *p*-bromophenacyl formate (17 mg); this recrystallised from light petroleum (b.p. 60—80 °C) to give white plates, m.p. 99 °C (lit.,[16] 99 °C); $\nu_{max.}$ 1 738, 1 705, and 1 595 cm^{-1}; *m/z* 242/244 (*M*+).

1,2-*Diphenyl*-3-*hydroxybutan*-1-*one* (19).—A solution of diazomethane in ether was prepared by dropwise addition of an ethereal solution of Diazald * (20.5 g) to potassium hydroxide (8.5 g) in ethanol (25 ml) and water (8 ml). The reaction mixture was heated to 65 °C and the distillate collected.

The ethereal solution of diazomethane was added dropwise to a solution of 2,3-epoxybutyric acid (9) (5 g) in ether until a pale yellow colour persisted. The excess diazomethane was

* *N*-Methyl-*N*-nitrosotoluene-*p*-sulphonamide.

destroyed by the dropwise addition of glacial acetic acid and then the solvents removed to give a liquid (5.1 g). This was distilled under reduced pressure to give the methyl ester (17) (4.5 g), b.p. 58 °C/10 mmHg (lit.,[17] 85—86 °C/48 mmHg); $\nu_{max.}$ 1 750 and 1 450 cm^{-1}; δ 3.7 (1 H, s), 3.2 (2 H, m), and 1.3 (3 H, d, *J* 5 Hz).

To magnesium (51 mg) in dry ether (25 ml) was added a crystal of iodine. The solution was heated to reflux temperature under an atmosphere of nitrogen and then a solution of bromobenzene (340 mg) in diethyl ether (10 ml) added dropwise. The reaction mixture was heated until all the magnesium had dissolved.

The solution of phenylmagnesium bromide was added dropwise to a solution of the above methyl ester (100 mg) in dry diethyl ether (10 ml). A dense white precipitate was observed. This was heated at reflux for 4 h. A saturated solution of ammonium chloride was added to destroy excess of Grignard reagent. The ethereal extract was dried and the solvent removed to give a white solid (153 mg). The *ketone* (19) recrystallised from cyclohexane as white needles, m.p. 103—105 °C (Found: C, 79.9; H, 6.7%. $C_{16}H_{16}O_2$ requires C, 80.0; H, 6.7%); $\nu_{max.}$ 3 600 and 1 665 cm^{-1}; δ 7.4 (10 H, m), 4.5 (2 H, m), 2.9 (1 H, s), and 1.1 (3 H, d, *J* 5 Hz); *m/z* 196 ($M^+ - 44$).

1,1-*Diphenyl*-2,3-*epoxybutanol* (18).—To magnesium turnings (51 mg) in dry tetrahydrofuran (25 ml) was added a crystal of iodine. The solution was heated at reflux temperature and then a solution of bromobenzene (340 mg) in dry tetrahydrofuran (10 ml) added dropwise. The solution was refluxed until all of the magnesium had dissolved.

The solution of phenylmagnesium bromide was cooled and added dropwise to a solution of methyl 2,3-epoxybutyrate (17) (100 mg), prepared as described above, in tetrahydrofuran (10 ml). The resulting solution was stirred at room temperature overnight. Saturated aqueous ammonium chloride was added to destroy excess of Grignard reagent. The organic phase was dried and the solvent removed under reduced pressure to give the *alcohol* (18) as a white solid; this was recrystallised from cyclohexane to give white needles (173 mg), m.p. 163 °C (Found: C, 79.9; H, 6.8%. $C_{16}H_{16}O_2$ requires C, 80.0; H, 6.7%); $\nu_{max.}$ 3 400 and 1 465 cm^{-1}; δ 7.4 (10 H, m), 6.0 (1 H, s), 3.5 (1 H, d, *J* 2 Hz), 3.3 (1 H, m), and 1.3 (3 H, d, *J* 5 Hz); *m/z* 240 (M^+).

Base Catalysed Rearrangement of 1,1-*Diphenyl*-2,3-*epoxybutanol* (18).—(a) *With potassium t-butoxide*. The epoxy alcohol (25 mg) was dissolved in t-butyl alcohol (15 ml), and potassium t-butoxide (50 mg) added. The solution was refluxed for 4 h. Work-up by preparative layer chromatography gave benzophenone (2 mg) and 1,2-diphenyl-3-hydroxybutan-1-one (19) (18 mg), the latter displaying physical properties identical with those for the authentic material described earlier.

(b) *With sodium ethoxide*. The epoxy alcohol (18) (25 mg) was dissolved in absolute ethanol (15 ml) and sodium ethoxide (53 mg) added. The solution was refluxed for 2 h. Work-up by preparative layer chromatography on silica gel gave benzophenone (21) (1 mg), and 1,2-diphenyl-3-hydroxybutan-1-one (19) (16 mg).

(c) *With potassium hydroxide followed by periodate oxidation of the product*. A solution of the epoxy alcohol (18) (50 mg) in methanol (20 ml) and aqueous potassium hydroxide (1M; 1 ml) was stirred during 3 h. The solvent was removed and the residue partitioned between diethyl ether and water. The organic phase was dried and the solvent removed to give a clear gum (48 mg). This was purified by p.l.c. using benzene–ethyl acetate (10 : 1) as solvent. The product, 4,4-diphenyl-3,4-epoxybutan-2-ol (20), was obtained as an unstable colourless gum (41 mg); $\nu_{max.}$ 3 600, 1 600, 1 500, and 1 450 cm^{-1}; δ 7.3 (10 H, m), 3.2 (2 H, m), 1.7 (1 H, s), and 1.3 (3 H, d, *J* 5 Hz).

To this alcohol (41 mg) was added a solution of sodium metaperiodate (200 mg) in water (10 ml). The solution was stirred during 18 h, and then extracted with diethyl ether (5 × 10 ml). The ethereal extract was dried and the solvent removed under reduced pressure to give a gum (28 mg). This was converted into the oxime as described below.

The aqueous layer was steam distilled and the distillate neutralised with dilute sodium hydroxide solution. Removal of the solvent gave a solid which was used to prepare *p*-bromophenacyl formate which recrystallised from ethanol (8 mg), m.p. 99 °C (lit.,[16] 99—100 °C).

Hydroxylamine hydrochloride (80 mg) was dissolved in water (1 ml) and sodium hydroxide (2M; 1 ml) added. To this solution was added the crude benzophenone from above in ethanol (1 ml). The solution was refluxed during 1 h. Addition of water caused the oxime to precipitate. This was filtered off and recrystallised from light petroleum (b.p. 68—80 °C) to give benzophenone oxime as white needles, m.p. 143—144 °C (lit.,[18] 144 °C).

7-*Acetoxy*-3-*acetyl*-2-*methylchromen*-4-*one* (29).—A solution of 2,4-dihydroxyacetophenone (20 g) in pyridine (50 ml) and acetic anhydride (40 ml) was stirred at room temperature for 16 h. The mixture was evaporated to a small volume and the residue dissolved in diethyl ether (100 ml) and the solution washed with water (2 × 50 ml), dilute hydrochloric acid (2 × 50 ml), water (50 ml), and aqueous sodium hydrogen carbonate (2 × 50 ml); it was then dried and evaporated to give 2,4-diacetoxyacetophenone (28) as a viscous oil (25 g), m.p. 37—38 °C (lit.,[19], 38 °C), $\lambda_{max.}$ (EtOH) 211 and 247 nm; $\nu_{max.}$ (film) 1 775s, 1 695s, 1 610s, 1 375s, and 1 210s cm^{-1}; δ 7.80 (1 H, d, *J* 8 Hz), 7.00 (2 H, m), 2.49 (3 H, s), 2.30 (3 H, s), and 2.26 (3 H, s); *m/z* 236 (M^+).

Sodium amide (3 g) was added to a solution of the diacetoxyacetophenone (28) (10 g) in toluene (150 ml) and the mixture was heated at reflux for 6 h. The toluene was decanted and evaporated and dilute hydrochloric acid (50 ml) was added to the residue; this was extracted with ether (2 × 50 ml). The extract was dried and evaporated to give the chromone (29), which recrystallised from ethyl acetate–hexane (5.0 g), m.p. 127—128 °C (lit.,[20] 127 °C); $\lambda_{max.}$ 241, 291, and 301 nm; $\nu_{max.}$ ($CHCl_3$) 1 775s, 1 695s, 1 650s, 1 630s, and 1 150s cm^{-1}; δ 8.17 (1 H, d, *J* 9 Hz), 7.21 (1 H, s), 7.13 (1 H, dd, *J* 2 and 9 Hz), 2.61 (3 H, s), 2.49 (3 H, s), and 2.34 (3 H, s); *m/z* 260 (M^+).

7-*Hydroxy*-2-*methylchromen*-4-*one* (24).—7-Acetoxy-3-acetyl-2-methylchromen-4-one (29) (5 g) was heated at reflux in 10% aqueous sodium carbonate for 15 min. The solution was cooled, acidified with concentrated hydrochloric acid, and set aside at 0 °C for 2 h. The product (24) was collected and recrystallised from aqueous ethanol (2.6 g), m.p. 249—250 °C (lit.,[20] 249—250 °C); $\lambda_{max.}$ 215, 240, 248, and 294 nm; $\nu_{max.}$ (Nujol) 3 000br s, 1 650s, 1 615s, and 1 260s cm^{-1}; δ (CF_3CO_2H) 8.38 (1 H, d, *J* 10 Hz), 7.44 (1 H, dd, *J* 2 and 10 Hz), 7.37 (1 H, d, *J* 2 Hz), 7.17 (1 H, s), and 2.85 (3 H, s); *m/z* 176 (M^+).

7-*Hydroxy*-2-*methylchroman*-4-*one* (26).—A solution of 7-hydroxy-2-methylchromen-4-one (24) (1 g) in ethanol (50 ml) was hydrogenated in the presence of 10% palladium on charcoal (100 mg). When rapid uptake of hydrogen had ceased the palladium on charcoal was removed by filtration and the solution evaporated to give the product (26) which was recrystallised from ethanol (960 mg), m.p. 175—176 °C

(lit.,[21] 176 °C); $\lambda_{max.}$ 223, 283, and 289sh nm; $\nu_{max.}$ (Nujol) 3 400br s, 1 620s, 1 590s, and 1 510s cm^{-1}; δ (CF_3CO_2H) 7.03 (1 H, d, *J* 8 Hz), 6.59 (2 H, m), 4.40 (1 H, m), 2.81 (1 H, m), 2.00 (1 H, m), and 1.48 (3 H, d, *J* 6 Hz); *m/z* 178 (M^+).

5,7-Diacetoxy-3-acetyl-2-methylchromen-4-one (32).—A solution of 2,4,6-trihydroxyacetophenone (10 g) and sodium acetate (10 g) was heated in acetic anhydride (60 g) at reflux for 10 h. The acetic anhydride was removed by evaporation and the residue purified by chromatography on silica gel using diethyl ether as eluant. The fraction corresponding to 5,7-diacetoxy-3-acetyl-2-methylchromen-4-one was collected and the product recrystallised from ethanol (11.8 g), m.p. 130—131 °C (lit.,[22] 131 °C); $\lambda_{max.}$ 246 and 301 nm; δ 7.19 (1 H, d, *J* 2 Hz), 6.82 (1 H, d, *J* 2 Hz), and 2.52, 2.41, 2.40, and 2.31 (each 3 H, s); *m/z* 318 (M^+).

5,7-Dihydroxy-2-methylchromen-4-one (25).—A solution of 5,7-diacetoxy-3-acetyl-2-methylchromen-4-one (32) (3 g) in aqueous sodium carbonate (2M; 30 ml) was heated at reflux for 1 h. After cooling the solution was acidified with concentrated hydrochloric acid and extracted with ethyl acetate (3 × 50 ml). The dried organic extracts were evaporated to give the chromone (25) which recrystallised from ethanol (1.4 g), m.p. 278—279 °C (lit.,[22] 279 °C); $\lambda_{max.}$ 227, 255, and 294 nm; $\nu_{max.}$ (Nujol) 3 400br s, 1 640s, 1 560m, and 1 160s cm^{-1}; δ[$(CD_3)_2SO$] 6.32 (1 H, d, *J* 2 Hz), 6.20 (1 H, d, *J* 2 Hz), 6.11 (1 H, s), and 2.35 (3 H, s); *m/z* 192 (M^+).

5,7-Dihydroxy-2-methylchroman-4-one (27).—A solution of 5,7-dihydroxy-2-methylchromen-4-one (25) (1 g) in ethyl acetate (50 ml) was hydrogenated in the presence of 10% palladium on charcoal (100 mg). When rapid uptake of hydrogen had ceased, the palladium on charcoal was removed by filtration and the solution evaporated to give the chroman-one (27) which recrystallised from ethanol (900 mg), m.p. 176—177 °C (lit.,[22] 176—177 °C); $\lambda_{max.}$ 226, 249, 256, and 293 nm; $\nu_{max.}$ (Nujol) 3 400br s, 1 620s, 1 600s, 1 510s, and 1 160s cm^{-1}; δ (CF_3CO_2H) 6.90 (2 H, m), 4.65 (1 H, m), 2.90 (1 H, m), 2.22 (1 H, m), and 1.58 (3 H, d, *J* 6 Hz); *m/z* 194 (M^+).

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