Alkaloid Biosynthesis. Part XVI.[1,2] Colchicine: Origin of the Tropolone Ring and Studies with the C_6–C_3–C_6–C_1 System

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Colchicine (1), isolated from *Colchicum autumnale* plants which had been fed with (±)-[3-^{14}C]tyrosine, is degraded to prove that *ca.* 85% of its total activity is located in the tropolone ring at C-12. It is thereby established that the tropolone system is generated from the aromatic nucleus of tyrosine by a ring-expansion process with inclusion of the benzylic carbon atom. A biosynthetic scheme for colchicine based upon a C_6–C_3–C_6–C_1 precursor is outlined and is tested by experiments with labelled 1-(5-hydroxy-2-hydroxymethyl-4-methoxyphenyl)-3-(3-hydroxy-4,5-dimethoxyphenyl)propylamine (10). This is not incorporated into colchicine by the plants and the implications of this finding are discussed. The lactone prepared by treatment of the Windaus anhydride (7-benzamido-8,9-dihydro-2,3,4-trimethoxy-7*H*-benzocycloheptene-5,6-dicarboxylic anhydride) (5) with hydriodic acid is 7-benzamido-8,9-dihydro-2,3-dihydroxy-7*H*-benzocycloheptene-5,4-carbolactone (6).

EARLIER research [3-5] had established that ring A and carbon atoms 5, 6, and 7 of colchicine (1) are derived in nature from cinnamic acid, which is generated in turn from phenylalanine. In addition,[3,4] (±)-[3-^{14}C]tyrosine (4) was found to be used by *Colchicum autumnale* plants to form colchicine carrying 85% of its total radioactivity in the tropolone ring; suitable degradations [4] restricted the site(s) of this labelling to one or more of the atoms C-8, C-10, C-11, and C-12. The remaining 15% of the total activity was scattered at low level over other parts of the colchicine molecule (*cf.* ref. 4) and is thus of no biosynthetic significance.

Further progress depended on locating the site(s) of

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[1] Part XV, A. R. Battersby, A. R. Burnett, and P. G. Parsons, *J. Chem. Soc.* (*C*), 1969, 1193.

[2] Preliminary reports, A. R. Battersby and R. B. Herbert, *Proc. Chem. Soc.*, 1964, 260; A. R. Battersby, *Pure and Appl. Chem.*, 1967, **14**, 117.

[3] A. R. Battersby and J. J. Reynolds, *Proc. Chem. Soc.*, 1960, 346; A. R. Battersby, R. Binks, and D. A. Yeowell, *ibid.*, 1964, 86.

[4] A. R. Battersby, R. Binks, J. J. Reynolds, and D. A. Yeowell, *J. Chem. Soc.*, 1964, 4257.

[5] E. Leete and P. E. Nemeth, *J. Amer. Chem. Soc.*, 1960, **82**, 6055; E. Leete, *ibid.*, 1963, **85**, 3666.

heavy labelling in the tropolone ring and biogenetic reasoning (see later) supported C-12 as the most probable position. The following degradation based upon Windaus' work [6] was therefore carried out on the radioactive colchicine to allow C-12 to be assayed. The original methods [6,7] were considerably modified to allow work on a small scale; the changes are described in the Experimental section. Deacetylcolchiceine (2), obtained by hydrolysis of colchicine, was converted into its *N*-benzoyl derivative [6] (3); oxidation with permanganate then afforded the Windaus anhydride (5). The structure of this product is beyond question.[7,8] There was no change in molar activity during the conversion (3) ⟶ (5), which proves that no significant activity resides at positions 9, 10, or 11 of colchicine (Table 1). Only

TABLE 1

Degradation of radioactive colchicine from (±)-[3-^{14}C]tyrosine

Colchicine and degradation products		Rel. molar activities
Colchicine	(1)	1·00
Deacetylcolchiceine	(2)	0·89
Windaus anhydride	(5)	0·89
The lactone	(6)	0·91

positions 8 and 12 now remained as possible sites for the heavy labelling.

Windaus [6] heated the anhydride (5) with hydriodic acid; the product, recognised as a phenolic lactone, was assigned the formula $C_{19}H_{17}NO_5$ but its structure has since remained unknown. Repetition of this reaction afforded a lactonic product shown to have the composition $C_{19}H_{15}NO_5$ by mass spectrometry and elemental analysis; this formula is in better agreement with Windaus' analytical figures than the earlier H_{17} formulation. On this basis, structure (6) is an obvious one for the lactone and the i.r. and n.m.r. spectra (Table 2)

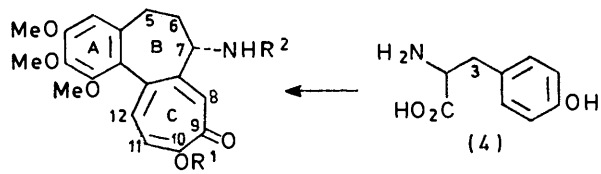
TABLE 2

Spectroscopic data on the Windaus and synthetic lactones

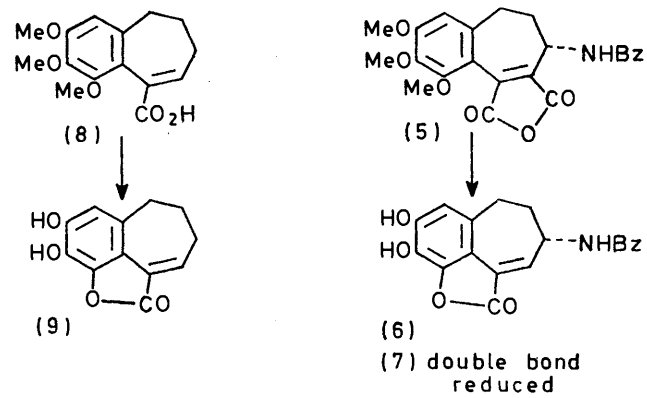
Substance	λ(MeOH)/nm	$\nu_{max.}$(KBr)/cm^{-1}	τ(CF$_3$·CO$_2$H)
Windaus lactone (6)	max. 340, 260 * min. 285	1780	3·34 (1H, s, aromatic) 2·88 (1H, d, olefinic)
Synthetic lactone (9)	max. 340, 263 min. 283	1775	3·22 (1H, s, aromatic) 2·45 (1H, t, olefinic) 3·22 (1H, s, aromatic)
Dihydro-derivative Windaus lactone (7)	max. 280 *, 240	1795	no olefinic signal.

* Shoulder.

were in full agreement with this proposal. Additional supporting evidence for the nature of the lactone was obtained by preparation of the analogous chromophoric system (9). The required acid (8) was synthesised by the published unambiguous method [9] and this product reacted with hot hydriodic acid to afford the lactone (9), $C_{12}H_{10}O_4$. The close correspondence between the spectroscopic data for this lactone and for the Windaus lactone (Table 2) leaves no doubt concerning the structure of the latter. Catalytic hydrogenation of the Windaus lactone gave the dihydro-derivative (7), which showed the expected hypsochromic shift in u.v. absorption, higher frequency carbonyl absorption in the i.r. spectrum, and loss of the olefinic n.m.r. signal (Table 2).



(1) R^1 = Me, R^2 = Ac
(2) R^1 = R^2 = H
(3) R^1 = H, R^2 = Bz



The lactone (6) is an ideal substance for further degradative work in the radioactive series in that selective loss of one carbonyl group has occurred during its formation from the anhydride (5). Accordingly, the labelled anhydride was converted as before into the lactone (6) and there was no significant change in molar activity (Table 1). Position 8 therefore is free from radioactivity. When this result is taken with earlier work [4] it follows that colchicine (1) from the experiment with (±)-[3-^{14}C]tyrosine is labelled specifically at C-12 (85% of total activity); only minor activity is present elsewhere in the molecule. These findings prove that the tropolone ring of colchicine arises by some ring-expansion process from the aromatic ring of tyrosine with inclusion of the benzylic carbon atom.

A reasonable biosynthetic scheme based on this knowledge involves the union of a C_6–C_3 precursor, derived from cinnamic acid, with a C_6–C_1 residue from tyrosine to generate the C_6–C_3–C_6–C_1 system (10). Phenol coupling would then lead to the dienone (11),

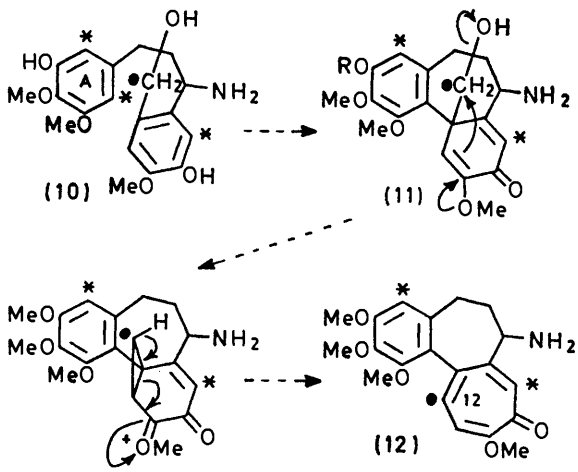
[6] A. Windaus, *Sitzungsber., Heidelberg Akad. Wiss.*, 1911, 2Abh., 1.

[7] J. W. Cook, T. Y. Johnston, and J. D. Loudon, *J. Chem. Soc.*, 1950, 537.

[8] E. C. Horning, M. G. Horning, J. Koo, M. S. Fish, J. A. Parker, G. N. Walker, R. M. Horowitz, and G. E. Ullyot, *J. Amer. Chem. Soc.*, 1950, **72**, 4840.

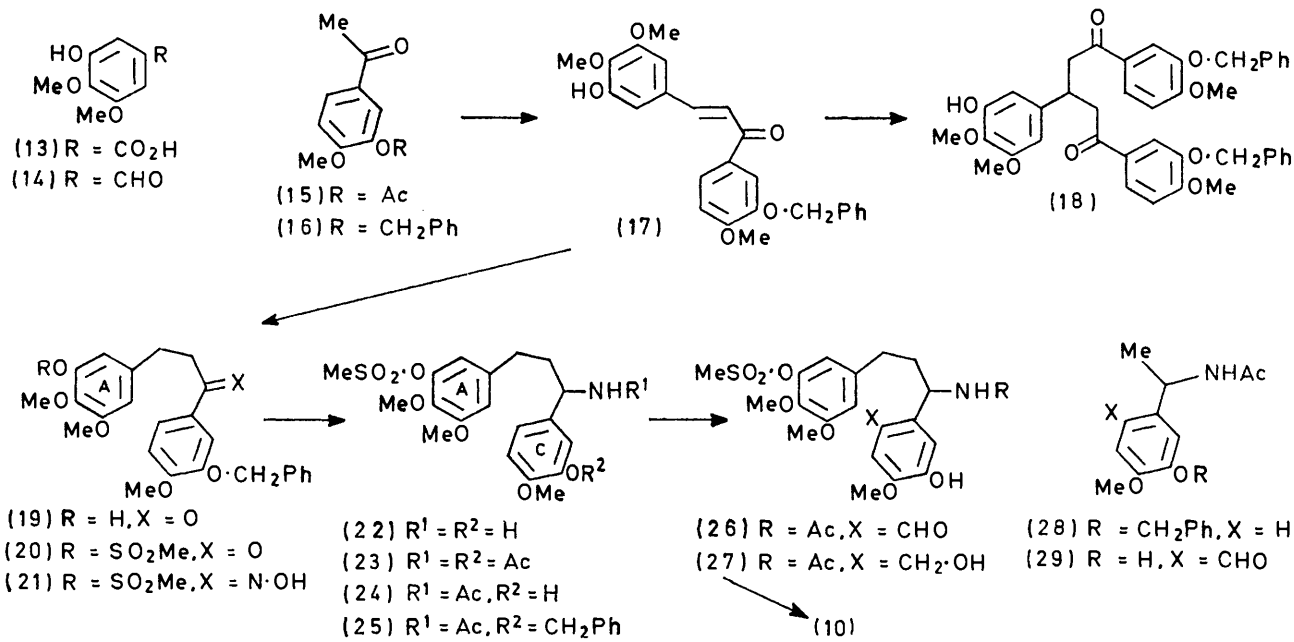
[9] J. Koo, *J. Amer. Chem. Soc.*, 1953, **75**, 720; J. Koo and J. L. Hartwell, *ibid.*, p. 1625.

which could undergo homoallylic ring expansion [10] as illustrated, possibly *via* the *O*-phosphate. Such a process would generate the colchicine skeleton (12)



specifically labelled at C-12 from [3-^{14}C]tyrosine; Leete's experiment [11] with ring-labelled tyrosine was also in agreement. Synthesis of the base (10) was therefore undertaken and our planning took into account the possible future need for ^{14}C-labelled material. Such a ^{14}C-label is best introduced late in the synthetic sequence and we decided it should be incorporated at the position dotted in structure (10). The following route was then developed, in which protection of the various labile functions and, importantly, deactivation of ring A in (10) towards electrophilic attack were achieved by selection of the correct blocking groups. Many such groups were tested before the successful combination was devised; these branches from the main synthetic sequence will be mentioned only briefly.

One starting material, 3′-benzyloxy-4′-methoxyacetophenone (16), was prepared by hydrolysis of the corresponding *O*-acetyl derivative [12] (15) followed by *O*-benzylation, and the other, 3-hydroxy-4,5-dimethoxybenzaldehyde (14), was obtained from the acid [13] (13) by Rosenmund reduction of the *O*-acetyl acid chloride. These materials underwent base-catalysed condensation under carefully controlled conditions to yield the chalcone (17). Without careful control, the yield of chalcone was variable and the by-product (18), resulting from Michael addition to the chalcone, was formed, sometimes in major amount. The structure of this substance follows from its composition ($C_{41}H_{40}O_9$) and spectroscopic properties. Mild hydrogenation of the chalcone afforded the dihydrochalcone (19), which was protected by conversion into the corresponding *O*-methylsulphonyl derivative (20). This protecting group survived all the conditions for later transformations and, moreover, served adequately to deactivate ring A. Trial experiments with the *O*-methoxycarbonyl group or the *O*-methoxymethyl group in place of the *O*-methylsulphonyl residue showed these to be unsatisfactory in one or more respects. The nitrogen atom was introduced next, by formation of the oxime (21), and catalytic hydrogenation under strongly acidic conditions then gave the amine (22) with concomitant loss of the *O*-benzyl residue. Acetylation readily yielded the *NO*-diacetyl product (23) from which the *O*-acetyl group was selectively cleaved by acid hydrolysis. *O*-Benzyl-



[10] *Cf.* O. Chapman and P. Fitton, *J. Amer. Chem. Soc.*, 1963, **85**, 41.
[11] E. Leete, *Tetrahedron Letters*, 1965, 333.
[12] R. Schwarz and K. Capek, *Monatsh.*, 1952, **83**, 883.
[13] E. Haslam, R. D. Haworth, S. D. Mills, H. J. Rogers, R. Armitage, and T. Searle, *J. Chem. Soc.*, 1961, 1836; see also A. I. Scott, F. McCapra, R. L. Buchanan, A. C. Day, and D. W. Young, *Tetrahedron*, 1965, **21**, 3605.

ation of the phenol (24) restored the phenolic protection and the system (25) was available suitably assembled for introduction of the final skeletal carbon atom in a *C*-formylation step.

Several other combinations of protective groups were tested. Thus, the *N*-phthaloyl derivative of base (22) could not be *C*-formylated even under forcing conditions nor could the benzoyl analogues of (23) and (24). It is evident that these groups seriously reduce the reactivity of ring C by steric or electronic effects and the spectra of the products showed that rapid *O*-formylation occurred when the phenolic hydroxy-group in ring C was left unprotected. Though these experiments did not yield the desired product, they served to demonstrate that the protected ring A is unaffected under forcing conditions in attempted *C*-formylations.

C-Formylation of the system (25) was studied with tin(IV) chloride and bischloromethyl ether.[14] The crude product showed a new carbonyl band at 1680 cm^{-1} and ester carbonyl absorption in the 1750 cm^{-1} region, but after chromatography on alumina a homogeneous fraction was obtained showing only the former absorption. These results suggested that *C*-formylation of ring C had been accomplished and that *O*-debenzylation by the tin chloride had occurred with subsequent *O*-formylation. Cleavage of the *O*-formyl group on the alumina would then leave only the aldehydic absorption in the spectrum of the final product. Structure (26) is the most probable one for this substance and all the spectroscopic evidence was in agreement. However, substitution at a different position in ring C could not be excluded on chemical grounds, and overlapping signals in the aromatic region of the n.m.r. spectrum prevented assignment of structure with the necessary certainty by this method. For this reason, and because the aldehyde (26) and the subsequent product were amorphous materials characterised spectroscopically, the amide (28) was prepared like the amide (25) from 3′-hydroxy-4′-methoxyacetophenone. This allowed the formylation step to be studied in a simpler but strictly analogous series yielding crystalline solids. The amide (28) was subjected to the formylation conditions previously used and the product was the aldehyde (29), as shown by its n.m.r. spectrum. Two singlets appeared at τ 2·5 and 2·95 (aromatic protons). The two alternative products would have shown splitting of the signals from aromatic protons with the demonstrated differing chemical shifts.

These experiments allowed structure (26) to be assigned to the aldehyde in the main synthetic sequence; only reduction of the aldehyde residue and removal of the protecting groups were now necessary. The former step was achieved by catalytic hydrogenation of the aldehyde (26) over platinum in the presence of iron(II) chloride.[15] This method afforded the alcohol (27) and so overcame the difficulties we had experienced in attempting to reduce *p*-hydroxybenzaldehydes with borohydride. Removal of the *O*-methylsulphonyl and *N*-acetyl groups from compound (27) was effected with base to yield the unstable diphenol (10). This was labelled by mild base-catalysed exchange with tritiated water.[16] Two separate batches of labelled material (0·03 and 0·6 mCi) were prepared. Experience of tritiations with many different phenols [16,17] makes it certain that only the positions *ortho* and *para* to the phenolic groups are involved in the labelling step, as indicated by stars for structure (10). Two of these labels would survive biological conversion of compound (10) into the colchicine skeleton (12) *via* (11) as illustrated. The two batches of labelled diphenol (10) were fed independently to seed capsules of *Colchicum autumnale* plants, and after 2 weeks the colchicine was extracted. The product from both experiments was totally radioinactive. Other precursors fed at the same time were incorporated efficiently and so the sum of evidence pointed against the biosynthetic scheme based upon the C_6–C_3–C_6–C_1 precursor (10). However, the knowledge and ideas arising from the foregoing research allowed immediate advantage to be taken of clues derived from structural work on androcymbine (see Part XVII [18]).

EXPERIMENTAL

Cultivation of *Colchicum autumnale* plants, administration of labelled materials, and extraction of colchicine were described in Part VI.[4] The methods used to obtain proof of purity of the radioactive materials fed and isolated were outlined in Parts III [19] and IV.[20] Assays of radioactivity were carried out as reported in Part VIII.[21] For general directions, see ref. 22.

The Windaus Anhydride (5).—A solution of *N*-benzoyldeacetylcolchiceine (3) (0·5 g) in aqueous 3% potassium hydroxide (5 ml) was treated at 5—10° for 45 min with aqueous 4% potassium permanganate (32·5 ml). The temperature was then allowed to rise to 20° and the mixture was stirred for 12 h before being acidified with sulphuric acid. The manganese dioxide was dissolved by passing sulphur dioxide into the mixture, which was then exhaustively extracted with methylene chloride. The extract (0·35 g) was fractionated on silica gel G plates (0·04 in thick) with acetic acid–benzene (1 : 4 v/v). Elution of the yellow band and crystallisation of the product from benzene afforded the Windaus anhydride (84 mg), m.p. 206—208° (Found: C, 64·9; H, 4·9. Calc. for $C_{23}H_{21}NO_7$: C, 65·2;

[14] H. Fross, A. Reiche, and G. Matthey, *Chem. Ber.*, 1963, **96**, 308.
[15] R. Adams and W. H. Carothers, *J. Amer. Chem. Soc.*, 1924, **46**, 1675.
[16] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6914.
[17] *E.g.* D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *J. Chem. Soc.*, 1965, 6379; A. R. Battersby, R. B. Herbert, E. McDonald, R. Ramage, and J. H. Clements, *Chem. Comm.*, 1966, 603; A. R. Battersby, E. Brochmann-Hanssen, and J. A. Martin, *J. Chem. Soc.* (*C*), 1967, 1785.
[18] A. R. Battersby, R. B. Herbert, L. Pijewska, F. Santavy, and P. Sedmera, following paper.
[19] A. R. Battersby, R. Binks, S. W. Breuer, H. M. Fales, W. C. Wildman, and R. J. Highet, *J. Chem. Soc.*, 1964, 1595.
[20] A. R. Battersby, R. Binks, R. J. Francis, D. J. McCaldin, and H. Ramuz, *J. Chem. Soc.*, 1964, 3600.
[21] A. R. Battersby, D. M. Foulkes, and R. Binks, *J. Chem. Soc.*, 1965, 3323.
[22] A. R. Battersby, J. C. Byrne, H. Gregory, and S. P. Popli, *J. Chem. Soc.*, 1967, 813.

H, 5·0%); τ 2·0—3·0 (6H), 3·33 (1H, s), 5·95 (3H, s), 6·05 (3H, s), and 6·13 (3H, s); i.r. spectrum identical with that of an authentic sample provided by Prof. J. D. Loudon (Glasgow).

Radioactive counting was carried out on a solution prepared as follows. The weighed sample of the anhydride (5) was kept in methanol (0·2 ml) overnight. The solution became colourless and scintillator was then added as usual.

7-Benzamido-8,9-dihydro-2,3-dihydroxy-7H-benzocycloheptene-5,4-carbolactone (6).—The foregoing anhydride (0·2 g) in constant-boiling hydriodic acid (5·0 ml) and acetic acid (2·5 ml) was heated for 2 h under reflux in a bath at 120°. Water (150 ml) was then added and distillation was carried out under normal pressure until crystallisation occurred. After 16 h at 0° the crystals were collected and recrystallised from aqueous acetic acid (93 mg); the *lactone* had m.p. >350° (Found: C, 67·7; H, 4·6. $C_{19}H_{15}NO_5$ requires C, 67·6; H, 4·5%); *m/e* 337, 216, and 105 (base peak).

The sample for counting was dried for 5 h at 100° and 0·1 mmHg and treated in methanolic solution (0·5 ml) in the counting vial with an excess of ethereal diazomethane (1·0 ml). Evaporation after 20 h left a residue which was treated again with ethereal diazomethane (0·5 ml) for 3 h. The residue left by evaporation was readily soluble in methanol (0·5 ml) and the scintillator solution in toluene.[21]

7-Benzamido-6,7,8,9-tetrahydro-2,3-dihydroxy-5H-benzocycloheptene-5,4-carbolactone (7).—A solution of the foregoing lactone (6) (18·6 mg) in methanol (5 ml) was shaken with hydrogen and platinum [from platinum dioxide (20 mg)] at 765 mmHg and 20° (uptake 0·98 mol. equiv. complete in 1 h). The product gave the *tetrahydro-lactone* (7), m.p. >350° (from aqueous acetic acid) (Found: C, 63·7, 64·0; H, 5·4, 5·1. $C_{19}H_{17}NO_5,H_2O$ requires C, 63·9; H, 5·3%).

8,9-Dihydro-2,3-dihydroxy-7H-benzocycloheptene-5,4-carbolactone (9).—Constant b.p. hydriodic acid (1·25 ml) was added to a solution of 8,9-dihydro-2,3,4-trimethoxy-7*H*-benzocycloheptene-5-carboxylic acid [9] (8) (50 mg) in acetic acid (0·63 ml) and the mixture was heated under reflux for 3 h in a bath at 120°. After addition of water (30 ml), distillation at normal pressure was carried out until the product separated. Recrystallisation from water afforded the *lactone* (9) (24 mg), m.p. 175—185° (decomp.) (Found: C, 66·1; H, 4·4. $C_{12}H_{10}O_4$ requires C, 66·0; H, 4·6%).

3′-Benzyloxy-4′-methoxyacetophenone (16).—3′-Acetoxy-4′-methoxyacetophenone [12] (15 g) in ethanol (40 ml) was treated under nitrogen with 2N-sodium hydroxide (4 equiv.) for 1 h at 20°. The ethanol was evaporated off, the residue was treated with an excess of 2N-hydrochloric acid, and the product was extracted into ether. It was recrystallised from aqueous ethanol to give the phenol (12 g), which in dry acetone (50 ml) was heated under reflux for 24 h with anhydrous potassium carbonate (15 g) and benzyl chloride (12 ml). The solids were then filtered off, the filtrate with washings was evaporated, and the residue was triturated with light petroleum. Recrystallisation of the resultant solid from methanol gave *3′-benzyloxy-4′-methoxyacetophenone* (11 g), m.p. 79—79·5° (Found: C, 74·9; H, 6·2. $C_{16}H_{16}O_3$ requires C, 75·0; H, 6·3%); $\nu_{max.}$ 1675 cm^{-1}; τ 7·52 (3H, s), 6·12 (3H, s), 4·85 (2H, s), 3·15 (1H, d, *J* 9 Hz), and 2·3—2·8 (7H, m).

3-Hydroxy-4,5-dimethoxybenzaldehyde (14).—Dimethyl sulphate (40 ml) and aqueous sodium hydroxide (60 ml, containing 25 g) were added during 20 min simultaneously and dropwise to a vigorously stirred suspension of gallic acid (100 g) in water (300 ml). This process was repeated five further times, with 30 min between each addition. The mixture was stirred for 15 h after the final addition, then acidified and extracted with ether (4 × 250 ml). The extract in anhydrous methanol (500 ml) was mixed with sulphuric acid (10 ml); the solution was heated under reflux for 24 h and then concentrated to *ca.* 150 ml before being poured on ice and sodium carbonate (35 g). The alkaline mixture was extracted with ether and the combined ethereal solution was shaken at 0° with 4N-sodium hydroxide until the aqueous extracts were almost colourless. Each alkaline extract was immediately acidified and extracted with ether. These extracts afforded methyl 3-hydroxy-4,5-dimethoxybenzoate (*ca.* 30 g), m.p. 79—83° (*cf.* ref. 13).

The methyl ester (29 g) was heated under reflux with 4N-sodium hydroxide (100 ml) for 1·5 h. The solution was acidified, and 3-hydroxy-4,5-dimethoxybenzoic acid separated (25 g); m.p. 188—190°. This acid (24 g) was heated under reflux for 6 h with acetic anhydride (100 ml); the solution was evaporated and the residue mixed with water (100 ml). After 2 h, 3-acetoxy-4,5-dimethoxybenzoic acid (26 g) was extracted by ether. The acetoxy-acid (25 g) was converted into the chloride by heating for 1 h with thionyl chloride (70 ml) and a few drops of pyridine; evaporation left the chloride, which was immediately dissolved in dry xylene (100 ml). After dry nitrogen had been passed through the solution to remove hydrogen chloride, 10% palladised barium sulphate (5 g) was added and the stirred mixture was heated under reflux as dry hydrogen was passed through. Evolution of hydrogen chloride ceased after 6 h, and the filtered solution was then evaporated. A solution of the residue in ethanol (40 ml) and aqueous 2N-sodium hydroxide (3 equiv.) was left under nitrogen for 2 h; the ethanol was then evaporated off and the solution was acidified. Extraction with ether afforded a gum which was distilled at 140° and 0·5 mmHg to yield 3-hydroxy-4,5-dimethoxybenzaldehyde [23] (15 g), m.p. 70—72°; $\nu_{max.}$ 1690 and 3510 cm^{-1}; τ 6·08 and 6·0 (each 3H, s), 2·91 and 2·84 (each 2H, d, *J* 3 Hz), and 0·30 (1H, s).

3′-Benzyloxy-3-hydroxy-4,4′,5-trimethoxydihydrochalcone (19).—3-Hydroxy-4,5-dimethoxybenzaldehyde (4·25 g) and 3′-benzyloxy-4′-methoxyacetophenone (6·4 g) in anhydrous methanol (25 ml) were added to a solution of sodium (4·0 g) in anhydrous methanol (25 ml). After 48 h at 20°, a deep orange-red solution had formed, but occasionally the sodium salt of the chalcone crystallised out. In either event, the mixture was poured into an excess of 2N-hydrochloric acid and the product extracted into 3 : 1 ether–chloroform. This afforded the chalcone (17) as a resin (10·1 g) which was homogeneous on t.l.c.; $\nu_{max.}$ 3505, 1652, and 1588 cm^{-1}.

A solution of the chalcone (10 g) in methanol (100 ml) was shaken at 760 mmHg and 20° with hydrogen and platinum [from platinum dioxide (150 mg)]; uptake 1·0 mol. equiv. in 3 h. Filtration and evaporation gave the *dihydrochalcone* (19) (8·7 g), m.p. 76—77° (from methanol) (Found: C, 71·3; H, 6·4. $C_{25}H_{26}O_6$ requires C, 71·1; H, 6·2%); $\nu_{max.}$ 3510, 1672, and 1600 cm^{-1}; τ 6·7—7·2 (4H, m), 6·20, 6·16, and 6·11 (each 3H, s), 4·85 (2H, s), 3·65 (1H, d, *J* 3 Hz), 3·51 (1H, d, *J* 3 Hz), 3·10 (1H, d, *J* 9 Hz), and 2·3—2·8 (7H, m).

1,5-Bis-(3-benzyloxy-4-methoxyphenyl)-3-(3-hydroxy-4,5-dimethoxyphenyl)pentane-1,5-dione (18).—When the fore-

[23] A. I. Scott, F. McCapra, R. L. Buchanan, A. C. Day, and D. W. Young, *Tetrahedron*, 1965, **21**, 3605.

going reaction was carried out in undried methanol or if aqueous potassium hydroxide was used as the base, the yield of chalcone (17) was low (*ca.* 15%) and varying amounts of an ether-insoluble by-product were formed. This crystallised from methanol to yield the *pentane*-1,5-*dione* (18), m.p. 144—146° (Found: C, 72·6; H, 6·1; OMe, 19·3. $C_{41}H_{40}O_9$ requires C, 72·8; H, 6·0; OMe, 18·4%); $\nu_{max.}$ 3510, 1670, and 1600 cm^{-1}; τ 6·6—7·0 (4H), 6·25 and 6·20 (each 3H, s), 6·10 (6H, s), 4·84 (4H, s), 3·65 (1H, d, *J* 3 Hz), 3·48 (1H, d, *J* 3 Hz), 3·11 (2H, d, *J* 9 Hz), and 2·2—2·9 (14H, m).

3′-Benzyloxy-3-methylsulphonyloxy-4,4′,5-trimethoxydihydrochalcone Oxime (21).—A solution of the dihydrochalcone (19) (9·0 g) in dry pyridine (50 ml) was treated at 0° for 16 h, with methanesulphonyl chloride (9·0 ml); the mixture was then poured into an excess of 2N-hydrochloric acid. Extraction with chloroform gave the methylsulphonyl derivative (9·7 g), which was dissolved in ethanol (200 ml) and mixed with a solution (adjusted to pH 5 with sodium acetate) of hydroxylamine hydrochloride (10 g) in water (50 ml).

After being heated under reflux for 8 h, the solution was diluted with water (200 ml) and extracted with chloroform to yield the *dihydrochalcone oxime* (21) (5·7 g), m.p. 125—126° (from methanol) (Found: C, 60·4; H, 5·7; N, 2·8; S, 6·3. $C_{26}H_{29}NO_8S$ requires C, 60·6; H, 5·7; N, 2·7; S, 6·2%); τ 6·9—7·3 (4H, m), 6·86 (3H, s), 6·20, 6·15, and 6·10 (each 3H, s), 4·85 (2H, s), 3·29 (1H, d, *J* 3 Hz), 3·17 (1H, d, *J* 3 Hz), and 2·3—3·1 (8H, m).

1-(*3-Hydroxy-4-methoxyphenyl*)-*3-(4,5-dimethoxy-3-methylsulphonyloxyphenyl)propylamine* (22).—The foregoing oxime (1·03 g) in acetic acid (16 ml) and concentrated hydrochloric acid (AnalaR; 0·6 ml) was added to a pre-reduced suspension of 10% palladised charcoal (640 mg) in acetic acid (6 ml). The mixture was shaken under hydrogen at atmospheric pressure for 18 h (uptake 93% of theory). After removal of the catalyst, the solution was evaporated to dryness and the residue in water (10 ml) was extracted with ethyl acetate. The aqueous solution was then made basic with sodium hydrogen carbonate and extracted with chloroform–propan-2-ol (4 : 1 v/v). The latter extracts afforded the propylamine (22), which crystallised from methanol as the *monohydrate*, m.p. 158° (Found: C, 52·5; H, 6·4; N, 3·1; S, 7·1. $C_{19}H_{25}NO_7S,H_2O$ requires C, 53·1; H, 6·3; N, 3·3; S, 7·7%); τ 6·82 (3H, s) and 6·13 (9H, s).

This product was heated at 120° for 3 min with an excess of benzoic anhydride, then cooled, and, as a solution in chloroform, washed with sodium hydrogen carbonate. The residue left after evaporation of the chloroform crystallised from methanol to give the *NO*-dibenzoyl derivative, m.p. 158—160°; $\nu_{max.}$ 3500, 1688 (NH·CO), and 1745 cm^{-1} (O·COAr).

1-(*5-Hydroxy-2-hydroxymethyl-4-methoxyphenyl*)-*3-(3-hydroxy-4,5-dimethoxyphenyl)propylamine* (10).—A solution of the foregoing amine (3·5 g) and anhydrous sodium acetate in acetic anhydride (25 ml) was heated under reflux for 1 h, cooled, and diluted with water (75 ml); the product was extracted into 3 : 1 ether–chloroform. The organic solution was washed with N-hydrochloric acid and aqueous sodium hydrogen carbonate, then dried, and evaporated to yield the *NO*-diacetyl derivative as a resin (3·5 g), homogeneous by t.l.c.; $\nu_{max.}$ 3420, 1760, and 1660 cm^{-1}.

Ethanolic 2N-hydrochloric acid was prepared by diluting concentrated acid with the appropriate volume of ethanol and the foregoing diacetyl derivative (3·5 g) was heated under reflux with this solution (100 ml) for 2 h. After concentration to *ca.* 50 ml, the solution was mixed with water (50 ml), adjusted to pH 11—12 by addition of 4N-potassium hydroxide, and extracted with 3 : 1 ether–chloroform. The aqueous layer was acidified and re-extracted with 3 : 1 ether–chloroform to yield the *N*-acetylphenol (24) as a gum (2·5 g); $\nu_{max.}$ 3550, 3420, and 1660 cm^{-1}.

Sodium (123 mg) was dissolved in a solution of the foregoing *N*-acetylphenol (2·29 g) in anhydrous methanol (25 ml), and benzyl chloride (0·68 g) was then added. The mixture was heated under reflux for 4 h, then diluted with water, and extracted with 3 : 1 ether–chloroform. The acetamide (2·54 g) so obtained failed to crystallise but was homogeneous by t.l.c.; $\nu_{max.}$ 3420 and 1660 cm^{-1}.

The foregoing acetamide (116 mg) in methylene dichloride (4 ml) was treated at −78° with tin(IV) chloride (0·1 ml) and bischloromethyl ether (0·5 ml). The mixture was allowed to warm to 20° and, after 30 min at 20°, was shaken with ice (10 g) and ether (20 ml). The ethereal solution was washed with water, saturated sodium hydrogen carbonate solution, and water again, dried, and evaporated to a gum (81·7 mg). Fractionation on alumina in 1 : 1 benzene–chloroform gave three components, the last to be eluted (38 mg) being the aldehyde (26); $\nu_{max.}$ 3520, 3400, 1685, and 1660 cm^{-1}; τ 7·96 (3H, s), 6·66 (3H, s), 6·20, 6·11, and 6·08 (each 3H, s), 2·6—3·4 (4H, overlapping), and 0·0 (1H, s).

This aldehyde (0·1 g) in ethanol (5 ml) and aqueous 0·1% sodium hydroxide (1·4 ml) was treated with 0·2M-iron(II) chloride (0·05 ml) and then shaken with platinum oxide (17 mg) and hydrogen at 20° and *ca.* 760 mmHg. Uptake (1·06 mol. equiv.) was complete in 4 h. Evaporation of the filtered solution left the crude product (27) as a gum; $\nu_{max.}$ 3510, 3400, and 1660 cm^{-1}. This material (94 mg) was heated under reflux in nitrogen with methanolic 10% potassium hydroxide (5 ml) for 6 h. After addition of water (15 ml), the solution was adjusted to pH 8·7 with acetic acid and extracted four times with ethyl acetate. The combined extracts were shaken twice with 2N-acetic acid and the aqueous solution was made basic with sodium hydrogen carbonate before being extracted five times with ethyl acetate. Evaporation of these final ethyl acetate extracts left the *aminophenol* (10) as a gum (16 mg), homogeneous by t.l.c.; $\nu_{max.}$ 3550 and 3400 cm^{-1} (no bands at 1660 and 1370 cm^{-1} corresponding to acetamide or methylsulphonyloxy-groups).

The [aryl-^{3}*H]Diphenolic Base* (10).—The foregoing diphenol (15·7 mg) was dissolved in aqueous 0·1% sodium hydroxide (3·46 ml) and the solution was evaporated; traces of water were removed by azeotropic distillation with ethanol and benzene. A solution of the residue in tritiated water (0·1 ml; 100 mCi) was kept for 12 h then evaporated to dryness. The residue was dissolved in ethanol and the solution evaporated. This final stage was repeated 14 times to afford the sodium salt of the [^{3}H]diphenolic base (10; 0·03 mCi) free from labile tritium. Purity and absence of chemical change were established by chromatographic comparison with starting material on Whatman No. 1 paper in pyridine–ethyl acetate–water.

A repeat preparation run for a longer period yielded more highly labelled material (0·6 mCi).

N-(3-Acetoxy-4-methoxy-α-methylbenzyl)acetamide.— 3′-Acetoxy-4′-methoxyacetophenone [12] (10 g) was treated with hydroxylamine as for the preparation of the oxime

(21) to yield 3′-hydroxy-4′-methoxyacetophenone oxime (7·8 g), m.p. 140° (from methanol); $\nu_{max.}$ 3520, 3300, and 1695 cm^{-1}; τ 7·75 (3H, s), 6·10 (3H, s), 3·11 (1H, d, *J* 9 Hz), 2·80 (1H, dd, *J* 9 and 3 Hz), and 2·70 (1H, d, *J* 3 Hz). This was reduced catalytically by the method used for the preparation of the amine (22) and the basic product (4·9 g) was acetylated in the normal way [*cf.* preparation of (25)]. Crystallisation of the neutral product from acetone–light petroleum (b.p. 60—80°) yielded the *amide* (4·75 g), m.p. 158—159° (Found: C, 62·2; H, 6·9. $C_{13}H_{17}NO_4$ requires C, 62·1; H, 6·8%); $\nu_{max.}$ 3420, 1760, and 1610 cm^{-1}; τ 8·58 (3H, d, *J* 7 Hz), 8·10 (3H, s), 7·72 (3H, s), 6·22 (3H, s), 4·90 (1H, q, *J* 7 Hz), and 2·6—3·3 (3H, m).

N-(*3-Benzyloxy-4-methoxy-α-methylbenzyl*)*acetamide* (28).—The foregoing diacetyl derivative was selectively *O*-deacetylated with ethanolic hydrogen chloride as in the preparation of compound (25) to yield the corresponding phenol (2·94 g). This was *O*-benzylated as for compound (25) and the neutral product was recrystallised from acetone–light petroleum (b.p. 40—60°) to give the *amide* (28) (2·95 g), m.p. 171° (Found: C, 72·4; H, 7·1. $C_{18}N_{21}NO_3$ requires C, 72·2; H, 7·1%); $\nu_{max.}$ 3420 and 1658 cm^{-1}; τ 8·58 (3H, d, *J* 7 Hz), 8·08 (3H, s), 6·12 (3H, s), 4·90 (1H, q, *J* 7 Hz), 4·85 (2H, s), 3·12 (3H, s), and 2·4—2·8 (5H, m).

N-(*2-Formyl-5-hydroxy-4-methoxy-α-methylbenzyl*)*acetamide* (29).—Formylation of the foregoing *O*-benzyl derivative (0·1 g) was carried out under the exact conditions used for the preparation of compound (26). Combination of the appropriate chromatographic fractions and crystallisation of the product from chloroform afforded the *aldehyde* (29), m.p. 147—150° (43 mg) (Found: C, 61·0; H, 6·2. $C_{12}H_{15}NO_4$ requires C, 60·7; H, 6·4%); $\nu_{max.}$ 3410, 3300, 1668, and 1658 cm^{-1}; τ 8·0 (3H, s), 7·18 (3H, d, *J* 5 Hz), 6·03 (3H, s), 5·1 (1H, q, *J* 5 Hz), 2·91 (1H, s), 2·51 (1H, s), and −0·03 (1H, s).

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