1278. *Deuterium and Tritium Exchange Reactions of Phenols and the Synthesis of Labelled* 3,4-*Dihydroxyphenylalanines*

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Preparative procedures are outlined for the labelling of phenols with deuterium and tritium by exchange in alkaline solution. The use of phenols, labelled with tritium at the *ortho* and *para* positions, in biosynthetic investigations is discussed. The methods developed are illustrated by the synthesis of (±)-3,4-dihydroxyphenylalanine labelled specifically at each of the nuclear and side-chain positions.

In their early investigations Ingold, Raisin, and Wilson [1] showed that phenols, when heated in alkaline deuterium oxide, exchanged aryl hydrogens for deuterium. For example, in phenol itself three aryl hydrogens were exchanged under these conditions. These and later [2] studies established that exchange occurred predominantly at positions *ortho* and *para* to phenolic hydroxyl groups. Under more forcing conditions, for example with potassamide in liquid ammonia, *meta* exchange has also been observed.[3] The possibility [4] that some *meta* exchange might take place even in aqueous systems must be kept in mind (see below). In recent years much interest has been shown in the biosynthesis of complex phenolic compounds, especially the phenolic alkaloids.[5] For this work it was necessary to synthesise radio-labelled phenols to test as biological precursors of the natural substances. It seemed that the exchange of phenolic precursors in alkaline tritiated water would provide a convenient and inexpensive labelling procedure. With this in mind, the present investigation was undertaken to establish general tritiation methods which could be applied on a preparative scale. Recent experience in these laboratories has shown [6,7] that monohydric phenols and catechols do not exchange *ortho* and *para* tritium in biological systems, and that phenols labelled in this way may indeed be used successfully in biosynthetic studies. Resorcinol [8] and phloroglucinol [9] derivatives are, however, known to undergo nuclear exchange extremely rapidly. This would seriously limit the use of the correspondingly labelled materials in biosynthetic work. The deuteration and tritiation of a variety of simple phenols will first be described, special attention being paid to the specificity of the exchange reaction. The general methods will then be illustrated by the synthesis of (±)-3,4-dihydroxyphenylalanine labelled specifically in each of the different nuclear and side-chain positions.

Exchange Reactions of Phenols.—The exchange of *p*-cresol in deuterium oxide was followed by n.m.r. spectroscopy. At 100°, in the presence of 0·5—1·0 mole of deuteroxide (conveniently generated by addition of potassium *t*-butoxide), replacement of the *ortho* hydrogens by deuterium was observed, the original aryl quartet (4 protons) of the phenol collapsing eventually into a broad singlet (2 protons), in the expected manner. A similar

[1] C. K. Ingold, C. G. Raisin, and C. L. Wilson, *J.*, 1936, 1637.
[2] A. P. Best and C. L. Wilson, *J.*, 1938, 28; A. Murray and D. L. Williams, "Organic Syntheses with Isotopes," Part II, Interscience Publishers, New York, 1958, p. 1652.
[3] A. I. Shatenshtein and A. V. Vedeneev, *Zhur. Obshchei Khim.*, 1958, **28**, 2644; G. E. Hall, E. M. Libby, and E. L. James, *J. Org. Chem.*, 1963, **28**, 311.
[4] P. A. Small and J. H. Wolfenden, *J.*, 1936, 1811.
[5] A. R. Battersby, Tilden Lecture, *Proc. Chem. Soc.*, 1963, 189; D. H. R. Barton, Hugo Muller Lecture, *Proc. Chem. Soc.*, 1963, 293; *Pure Appl. Chem.*, 1964, **9**, 35.
[6] D. H. R. Barton, (Mrs.) A. J. Kirby, and G. W. Kirby, *Chem. Comm.*, 1965, 52.
[7] D. H. R. Barton, G. W. Kirby, W. Steglich, G. M. Thomas, A. R. Battersby, T. A. Dobson, and H. Ramuz, *J.*, 1965, 2423; L. J. Haynes, K. L. Stuart, D. H. R. Barton, D. S. Bhakuni, and G. W. Kirby, *Chem. Comm.*, 1965, 141; D. H. R. Barton, R. H. Hesse, and G. W. Kirby, *J.*, 1965, 6379; D. H. R. Barton, G. W. Kirby, and H. P. Tiwari, unpublished work.
[8] F. Munzberg, *Z. phys. Chem.*, 1936, *B*, **33**, 23; E. S. Hand and R. M. Horowitz, *J. Amer. Chem. Soc.*, 1964, **86**, 2084.
[9] H. Erlenmeyer, A. Epprecht, and H. Lobeck, *Helv. Chim. Acta*, 1936, **19**, 543; R. J. Highet and T. J. Battersham, *J. Org. Chem.*, 1964, **29**, 475.

result was observed with tyrosine (I).[10] In this example an additional mole of butoxide was added to ionise the carboxyl group of the amino-acid, and the reaction was performed in a nitrogen-filled, sealed tube. To achieve exchange under milder conditions several organic amines were tested as catalysts. Triethylamine proved effective and convenient. The effect of varying amounts of triethylamine and of varying reaction times on the exchange of *p*-cresol in deuterium oxide is illustrated in Table 1. Even with this two-phase system

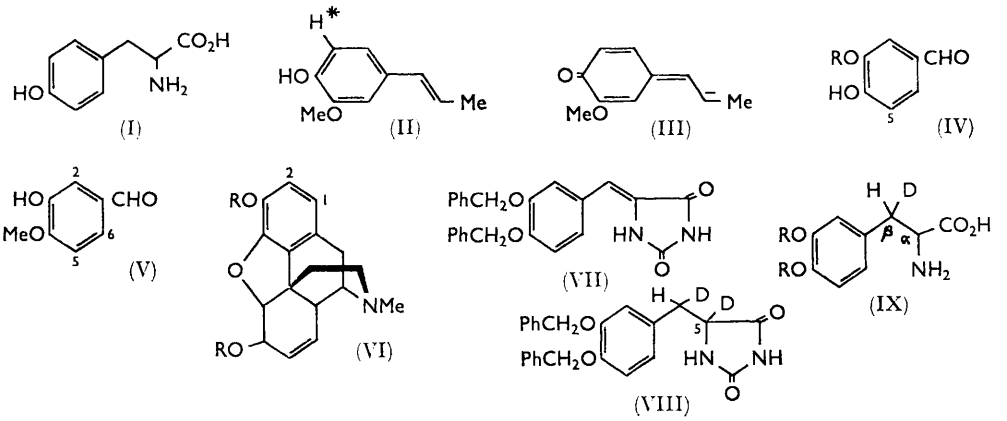
TABLE 1

Deuteration of *p*-cresol at 101—103°

Et_3N (Mole) per mole phenol	0·5	0·5	0·5	0·5	1·0	2·0	5·0
Time (hr.)	0·5	1·0	2·0	4·5	0·5	0·5	0·5
Exchange (%) at ortho positions ...	35	61	78	89	61	58	0

rapid deuteration was effected in the presence of 1 mole of amine. With excess base, exchange was very slow. This agrees with the earlier studies of Ingold, Raisin, and Wilson who showed [1] that rapid exchange occurred only when appreciable concentrations of both phenoxide and undissociated phenol (Ph OD) were present.

In the catechol monoether, isoeugenol (II), positions *ortho* and *para* to the methoxyl group are activated towards electrophilic substitution and might, in principle, be involved in the deuterium exchange reaction. However, with either deuteroxide or triethylamine as catalyst, only one aryl hydrogen [asterisk in (II)] was replaced (n.m.r. control). Also the olefinic hydrogen vinylogously *para* to the phenolic hydroxyl group [cf. (III)], was unaffected. Vanillin (IV; R = Me) likewise gave only the 5-deutero-derivative. For preparative work (see below) it was necessary to determine the stability of nuclear deuterium under the normal conditions for benzylation and debenzylation of phenolic hydroxyl groups. The monobenzylprotocatechuic aldehyde (IV; R = Ph CH_2) was converted into the monodeutero-derivative in the usual way. Benzylation, with benzyl chloride



and potassium carbonate, gave the corresponding benzyl ether without loss of deuterium. Hydrogenation over palladium–carbon, in ethanol containing hydrochloric acid, then yielded 5-deutero-3,4-dihydroxytoluene.

Although n.m.r. spectroscopy provides a convenient qualitative method for following deuteration, it is of limited value in quantitative determinations. Even with well-resolved spectra an error of 5% in the integration of proton signals is unexceptional. For this reason, tritiation was used to examine critically the specificity of *ortho* and *para* exchange. In isovanillin (V) the positions (2 and 6) *ortho* and *para* to the phenolic hydroxyl group are strongly deactivated by the aldehyde group towards electrophilic substitution. The

[10] Cf. D. Rittenberg, A. S. Keston, R. Schoenheimer, and G. L. Foster, *J. Biol. Chem.*, 1938, **125**, 1.

remaining position (5) is less deactivated and is also *ortho* to the electron-releasing methoxyl. If "*meta* exchange" of phenols in aqueous systems is at all important then it should be observed in isovanillin. Preliminary deuteration studies, under the usual conditions, showed that exchange generally was slow, but occurred more rapidly at position 2 than at position 6. The Cannizzaro reaction did not interfere to any appreciable extent. The aryl hydrogen at position 5 appeared not to exchange, but accurate integration of the n.m.r. spectrum was not possible. A sample of tritiated isovanillin, prepared in tritiated water with hydroxide as catalyst, was next examined. Benzylation and oxidation afforded 3-benzyloxy-4-methoxybenzoic acid having the same molar activity as the isovanillin. As expected, no exchange of the aldehydic proton had occurred. Demethylation of the labelled isovanillin, under mild conditions [11] with aluminium chloride and pyridine, gave protocatechuic aldehyde (IV; R = H) without significant loss of activity. Bromination gave the known 5-bromo-derivative which again had the same molar activity. Clearly, only the 2 and 6 positions were involved in the original exchange reaction.

Recent studies on the structure [12] and the biosynthesis [6,7] of phenolic alkaloids led us to make a special examination of nuclear exchange in this class of compound. A phenolic alkaloid contains within its structure both the phenolic hydroxyl group and the amino-function required for the exchange reaction. Indeed specific *ortho* and *para* labelling of phenolic alkaloids with deuterium or tritium can be effected very simply. For example, morphine (VI; R = H), when heated alone in dimethylformamide containing deuterium oxide, was converted in good yield into the 2-deutero-derivative. The position of deuterium followed from experience with simple phenols, but was supported in an independent way. Acetylation gave the correspondingly deuterated diacetylmorphine (VI; R = Ac). The aryl protons in undeuterated material gave a pair of doublets at 3·25 and 3·47 τ (J = 8 c./sec.) in the n.m.r. spectrum. The doublet at 3·47 τ was assigned to the proton at position 1, since fine coupling with the benzylic methylene had produced broadening and shortening of its component lines in comparison with those of the other doublet. In agreement with this, the doublet at 3·25 τ disappeared on deuteration. It was possible that traces of dimethylamine in the dimethylformamide had catalysed exchange. However, a similar result was obtained with dimethylsulphoxide as solvent; also, morphine hydrochloride was not deuterated in dimethylformamide containing deuterium oxide. This procedure was used successfully for the deuteration and tritiation of sinoacutine,[6] a phenolic dienone readily decomposed by aqueous alkali or triethylamine.

Synthesis of Labelled 3,4-*Dihydroxyphenylalanine.*—*OO*-Dibenzylprotocatechuic aldehyde was selected as starting material for the synthesis of 3,4-dihydroxyphenylalanine (dopa), since we had shown (see above) that catalytic debenzylation did not remove aryl deuterium or tritium. For tracer experiments which will be described elsewhere, it was necessary to prepare dopa labelled with tritium specifically in each of the three different aryl positions.

The preparation of *OO*-dibenzyl-[5-^{2}H] protocatechuic aldehyde has already been described (see above) and the correspondingly tritiated derivative was obtained in an analogous fashion. During this preparation the opportunity was taken to confirm the specificity of labelling in the intermediate 3-benzyloxy-4-hydroxy-[5-^{3}H] benzaldehyde (IV; R = $PhCH_2$). Bromination in the presence of sodium acetate gave the corresponding 5-bromo-derivative containing $<0{\cdot}1\%$ of the original activity.

Specific labelling of *OO*-dibenzylprotocatechuic aldehyde in the two remaining aryl positions proved more difficult. Isovanillin (V), tritiated in the presence of alkali, was brominated [13,14] to give the highly crystalline 2-bromo-isovanillin, which contained tritium only at position 6 (see above). The less readily purified 6-bromo-[2-^{3}H] isovanillin was

[11] R. G. Lange, *J. Org. Chem.*, 1962, **27**, 2037.
[12] L. J. Haynes, K. L. Stuart, D. H. R. Barton, and G. W. Kirby, *Proc. Chem. Soc.*, 1964, 261.
[13] T. A. Henry and T. M. Sharp, *J.*, 1930, 2279.
[14] S. E. Hazlet and R. J. Brotherton, *J. Org. Chem.*, 1962, **27**, 3253.

also isolated from this reaction. Further bromination, in the presence of sodium acetate, of either isomer gave inactive 2,6-dibromoisovanillin in good yield. This confirms the position of labelling and removes any remaining doubt [14] concerning the structure of the dibromo-derivative. 2-Bromo-[6-^{3}H]isovanillin was demethylated (see above), and the resulting catechol converted into its dibenzyl ether. Benzylation was best effected with benzyl chloride in dimethylformamide in the presence of sodium hydride. Treatment with lithium aluminium hydride removed the bromine and reduced the aldehyde function. Oxidation of the product with manganese dioxide gave the required *OO*-dibenzyl-[6-^{3}H]-protocatechuic aldehyde. A similar series of reactions converted 6-bromo-[2-^{3}H]isovanillin into the corresponding protocatechuic aldehyde derivative. During these transformations negligible loss of tritium was observed (see Table 2). 6-Bromo-[2-^{2}H]isovanillin was most conveniently prepared by direct tritiation of the pure, inactive material. A similar procedure with 2-bromoisovanillin was frustrated by the very slow exchange at position 6 in this derivative; direct bromination of labelled isovanillin was therefore preferred.

The conversion of the labelled *OO*-dibenzylprotocatechuic aldehydes into racemic dopa

TABLE 2

Preparation of tritiated *OO*-dibenzylprotocatechuic aldehyde

(Activities in μc/mmole)

Compound	Bromoisovanillin	Bromoprotocatechuic aldehyde	*OO*-Dibenzylbromoprotocatechuic aldehyde	*OO*-Dibenzylprotocatechuic aldehyde
2-Bromo[6-^{3}H]	24	24	25	25
6-Bromo[2-^{3}H]	62	59	60	58

TABLE 3

Preparation of tritiated (±)-3,4-dihydroxyphenylalanine (dopa)

Compound	Activities (μc/mmole) at positions: 2	5	6	α	β
OO-Dibenzylprotocatechuic aldehyde	58	54	25	—	—
Dihydrohydantoin (VIII)	59	56	25	—	24 *
OO-Dibenzyl-dopa (IX; R = $PhCH_2$)	58	53	24	17	15
Dopa (IX; R = H)	50	55	21	17	16

* Some of the α-tritium was already lost during isolation of this derivative. The value (24) represents both β (15) and α (9) tritium labelling.

(see Table 3) was accomplished by the hydantoin method, which also permitted introduction of tritium into the side chain positions. The benzylidene hydantoin (VII) was prepared in the usual way, and reduced with sodium amalgam in dioxan containing deuterium oxide. The resulting dideuterated dihydro-derivative (VIII) was hydrolysed with alkali to give the dibenzyl ether (IX; R = $PhCH_2$), which was converted by hydrogenolysis into deuterated dopa (IX; R = H). The n.m.r. spectrum showed approximately one deuterium atom at the β-carbon [as IX]; no detectable amounts of α-deuterated species were present. Loss of deuterium from the 5-position during hydrolysis of the benzylhydantoin (VIII) is not surprising, but it was necessary to show that complete loss had occurred. The undeuterated benzylhydantoin (VIII) was treated with alkaline tritiated water at room temperature to give a specimen labelled with tritium at the 5-position. Hydrolysis in hot aqueous akali, in the usual way, gave the dibenzyl ether (IX; R = $PhCH_2$) containing only 4% of the original activity. With the conditions for α-exchange clearly defined it was then possible to prepare samples of dopa labelled specifically at either the α- or β-positions.

(±)-[2,5,6-$^3H_3$] Dopa was prepared by exchange of the unlabelled amino-acid in either alkaline [15] or acidic tritiated water. The second method was preferred, since rigorous exclusion of oxygen was not so important. It was found (Table 4) that, under these conditions, the aryl hydrogens of dopa exchanged much more rapidly than the *ortho* hydrogens

[15] Cf. G. A. Swan, *Ann. New York Acad. Sci.*, 1963, **100**, 1005.

TABLE 4

Deuteration (%)* of L-tyrosine and (±)-3,4-dihydroxyphenylalanine (dopa) in 4·1N-DCl at 100°

Time (hr.)	0·25	0·5	1·0	1·5	2·0	2·5	3·5	4·5	5·5
Tyrosine	—	21	37	52	—	66	75	80	83 †
Dopa	44	64	81	82	87 †	—	—	—	—

* 100% Deuteration corresponds to the exchange of 2 and 3 aryl hydrogens in tyrosine and in dopa respectively. † Incomplete deuteration arose from introduction of atmospheric water during repeated sampling.

of tyrosine. The preparation of [2,5,6-$^3H_3$] and [6-^{3}H]dopa by reduction of suitably brominated derivatives with tritium gas has recently been described.[16]

EXPERIMENTAL

Melting points were taken on a Kofler hot-stage apparatus. All n.m.r. spectra were run on a Varian A-60 spectrometer on permanent loan to Professor D. H. R. Barton, F.R.S. from the Wellcome Trust. Deuterium oxide (99·7%) and tritiated water (200 mc. per ml.) were used in the exchange reactions.

Counting Methods.—Tritiated compounds were counted with a Tritium Scintillation Counter (Isotope Developments Ltd., Type 6012 A). Samples (typically 0·4 mg.) were dissolved in dimethylformamide (0·1 ml.) and liquid scintillator (Nuclear Enterprises Ltd., type N.E. 213) (1·2 ml.). The counting procedure was standardised using [1,2-$^3H_2$]-n-hexadecane of known activity, efficiencies of *ca.* 20% and reproducibilities of *ca.* 3% being customary. Tyrosine was converted into the ethyl ester hydrochloride by treatment with excess of cold, saturated ethanolic hydrogen chloride for *ca.* 20 hr. Solvent was removed *in vacuo* at room temperature, and the crystalline derivative obtained by addition of ether. This derivative, unlike the parent amino-acid, dissolved in dimethylformamide and was suitable for scintillation counting. Repeated treatment with cold ethanolic hydrogen chloride caused negligible loss of tritium from the *ortho* positions of tyrosine. For the same purpose, 3,4-dihydroxyphenylalanine (*ca.* 1 mg.) was converted into its hydrochloride by brief treatment with cold methanolic hydrogen chloride (*ca.* 0·2 ml.). Excess reagent was removed *in vacuo* and the residue, after drying at room temperature *in vacuo* over phosphorus pentoxide and potassium hydroxide, was dissolved in dimethylformamide (5 ml.) and aliquots (0·1 ml.) counted in the usual way. More accurate activity measurements on tyrosine and dopa were kindly made by Dr. H. Simon (Münich) using a combustion method.[17]

Exchange Reactions of Phenols.—The phenol (typically 100 mg.) in deuterium oxide (*ca.* 0·5 ml.) or tritiated water (*ca.* 0·2 ml.) containing potassium *t*-butoxide (0·5 mole equivalent) or triethylamine (1·0 mole) was heated in a sealed, nitrogen-filled tube at 100°. In general, with simple phenols, heating for 72 hr. was sufficient to ensure complete exchange, although in many experiments (see Table 1) a shorter period sufficed. With high molecular weight phenols dimethylformamide (*ca.* 0·5 ml.) was added to effect complete solution.

Deuteration of p-*Cresol.*—(a) *With triethylamine.* *p*-Cresol (100 mg.) in deuterium oxide (0·3 ml.) (two-phase system) was heated under reflux (101—103°) with varying amounts of triethylamine for varying periods of time (see Table 1). The reaction vessel was open to the air but protected from atmospheric moisture. Dioxan was added to the cooled reaction mixture to give a homogeneous solution which was examined by n.m.r. spectroscopy. The doublet, 3·38 τ (J = 9 c./sec.), corresponding to the protons *ortho* to the phenolic hydroxyl group diminished in intensity as deuteration proceeded, and the *meta*-proton doublet, 3·10 τ, was correspondingly replaced by a broad singlet. The extent of deuteration (% exchange in Table 1) was obtained from the difference in integrated areas of the *ortho*- and *meta*-proton signals.

(b) *With deuteroxide.* Exchange was effected in a similar way using potassium *t*-butoxide

[16] M. Schreier, W. Pacha, and J. Rutschmann, *Helv. Chim. Acta*, 1963, **46**, 954; L. Birkofer and K. Hempel, *Chem. Ber.*, 1963, **96**, 1373.
[17] H. Simon and F. Berthold, *Die Atomwirtschaft*, 1962, **7**, 498.

(0·5 mole). A homogeneous reaction mixture was obtained at 100°, and heating was carried out under nitrogen (see above).

Exchange Reactions with Tyrosine and 3,4-*Dihydroxyphenylanine.*—Deuteration of L-tyrosine was carried out with deuteroxide (1·7 mole) as catalyst, as described for *p*-cresol. Exchange of L-tyrosine and (±)-3,4-dihydroxyphenylalanine (dopa) was more conveniently effected in acidic solution.[10] For this purpose, deuterium chloride was prepared by allowing the appropriate amount of thionyl chloride to react with deuterium oxide. Liberated sulphur dioxide was swept out with a stream of dry nitrogen. The amino-acid (104 mg.) in 4·1N-deuterium chloride (0·4 ml.) was heated at 100°, and the exchange determined at intervals in the usual way (Table 4). The two *ortho* hydrogens (position 3) in tyrosine and all three aryl hydrogens in dopa were eventually replaced by deuterium. Exchange at other positions was insignificant under these conditions. Tritiation of dopa was carried out in a similar manner.

Deuteration of Isoeugenol.—Exchange in deuterium oxide was carried out with triethylamine or deuteroxide exactly as described for *p*-cresol (see above). The aryl protons gave a broad signal (n.m.r.) at 3·28 τ, which diminished in area by one third upon deuteration. The splitting of the C-methyl doublet, 8·18 τ (J = 5 c./sec.), was unaffected by deuteration. A comparison of the integrated areas of the methoxyl (6·22 τ) and C-methyl signals on several spectra showed differences of up to 10%. This provides an indication of the uncertainty involved in the measurement of aryl-deuteration.

Deuteration of Vanillin and 3-*Benzyloxy*-4-*hydroxybenzaldehyde.*[18]—Both aldehydes were deuterated by the standard procedures (above) and the monodeuterated derivatives isolated and crystallised in the usual way. [5-^{2}H]Vanillin, m. p. 79—81° (from water), showed (n.m.r.) two aryl protons having the expected *meta* relationship. In deuterium oxide, containing triethylamine, these protons gave a pair of doublets (J = 2 c./sec.) situated 2·07 and 2·20 p.p.m. upfield from the aldehyde singlet. Similarly, the spectrum of 3-benzyloxy-4-hydroxy-[5-^{2}H]-benzaldehyde, m. p. 112—114°, lacked the doublet (J = 8 c./sec.) at 2·95 τ characteristic of the proton at C-5 in the starting material.

3,4-*Dihydroxy*-[5-^{2}H]*toluene.*—3-Benzyloxy-4-hydroxy-[5-^{2}H]benzaldehyde (254 mg.) in acetone (10 ml.) containing benzyl chloride (0·5 ml.) and anhydrous potassium carbonate (151 mg.) was heated under reflux for 20 hr. under nitrogen with stirring. The reaction mixture was filtered and evaporated, and excess benzyl chloride was removed from the product by steam distillation. An ether extract of the nonvolatile residue was washed with sodium hydroxide, then water, dried (Na_2SO_4), and evaporated to give the required benzyl ether (236 mg.), m.p. 88—90°. This material (195 mg.), suspended in ethanol (30 ml.) containing 6N-hydrochloric acid (0·1 ml.), was hydrogenated over 10% palladium–carbon (21 mg.) for 1 hr. Filtration and evaporation gave 3,4-dihydroxy-[5-^{2}H]toluene (60 mg.), which crystallised from benzene as prisms, m. p. 62—64°. The n.m.r. spectrum was run in deuterium oxide containing *t*-butyl alcohol (8·77 τ) as an internal standard. Bands at 3·22 and 3·41 τ were observed, arising from protons at C-2 and C-6 respectively; as expected, the low field band was a sharp doublet (J = 2 c./sec.) while the other was broadened by the neighbouring deuterium atom.

Deuteration and Tritiation of Isovanillin.—The n.m.r. spectrum of isovanillin (in dimethylsulphoxide) showed the following aryl signals: 2·59 (H_6; double doublet, J = 8 and 2 c./sec.), 2·69 (H_2; doublet, J = 2 c./sec.), and 2·88 (H_5; doublet, J = 8 c./sec.). Deuteration proceeded only slowly, both exchangeable protons (H_2 and H_6) being at positions *ortho* to the aldehyde group. Exchange occurred more rapidly at position 2 than at position 6 (n.m.r. control), but it was not possible to effect exchange exclusively at the former position. For preparative work, isovanillin was heated under nitrogen at 100° in deuterium oxide containing potassium *t*-butoxide (0·5 mole) for 3 days. The product, isolated after acidification in the usual way, was chromatographed on alumina (grade V) to remove dark impurities. Elution with benzene–chloroform (2 : 1) gave *partially deuterated* [2,6-$^2H_2$]isovanillin (60%), m. p. 113—115°. Tritiation was carried out, under the same conditions, using tritiated water (1·8 mc. per mg. atom hydrogen) to give [2,6-$^3H_2$]isovanillin (2·1 mc per mmole). Bromination experiments (see below) on several batches of labelled material showed the ratio of activities at positions 2 and 6 to be *ca.* 2 : 1.

Proof of Labelling Pattern in [2,6-$^3H_2$]*Isovanillin.*—The labelled isovanillin (270 mg., 29 μc per mmole) in dimethylformamide (2 ml.) containing benzyl chloride (1 ml.) was treated, under

[18] D. H. R. Barton, G. W. Kirby, J. B. Taylor, and G. M. Thomas, *J.*, 1963, 4545.

nitrogen, with sodium hydride (53% dispersion in mineral oil, 100 mg.) at room temperature for 30 min. The reaction mixture was then heated at 100° for 30 min. and steam distilled to remove excess benzyl chloride. The neutral product was isolated in the usual way, and crystallised from methanol to give *O*-benzyl-[2,6-$^3H_2$]isovanillin (29 μc per mmole) as needles (275 mg.), m. p. 61—63° (lit.,[19] 62—63°). Oxidation of this material with potassium permanganate in acetone [20] gave the correspondingly labelled *O*-benzylisovanillic acid (28 μc per mmole), m. p. 177—178° (lit.,[20] 177—178°).

Demethylation [11] of the labelled isovanillin gave [2,6-$^3H_2$] protocatechuic aldehyde (29 μc per mmole). Bromination [21] gave 5-bromo-[2,6-$^3H_2$] protocatechuic aldehyde (29 μc per mmole) which crystallised from aqueous ethanol as needles, m. p. 228—230° (lit.,[21] 230°). The n.m.r. spectrum of 5-bromoprotocatechuic aldehyde (in dimethylsulphoxide) showed aryl proton signals at 2·42 and 2·72 τ. Both were well resolved doublets (J = 2 c./sec.) confirming the *meta* orientation of the corresponding protons.

Deuteration of Morphine.—Morphine (200 mg.) in dimethylformamide (2 ml.) or dimethylsulphoxide (2 ml.) containing deuterium oxide (0·5 ml.) was heated at 100° in a nitrogen-filled, sealed tube. The exchange was slower (*ca.* 70% in 66 hr.) than that observed (above) with *p*-cresol and triethylamine. [2-^{2}H]Morphine (160 mg.) was obtained in pure form by dilution of the cooled reaction mixture with water. Acetylation, in the usual way, gave diacetyl-[2-^{2}H]morphine, m. p. 171—173° (lit.,[22] 173°). The n.m.r. spectrum has already been discussed (above). An experiment in dimethylformamide, under these conditions but using morphine hydrochloride instead of morphine, gave undeuterated alkaloid.

3-*Benzyloxy*-5-*bromo*-4-*hydroxybenzaldehyde*.—3-Benzyloxy-4-hydroxybenzaldehyde [18] (109 mg.) in acetic acid (2 ml.) containing anhydrous sodium acetate (80 mg.) was treated with bromine (100 mg.) in acetic acid (0·2 ml.) at room temperature. The 5-*bromo-derivative* separated as plates (103 mg.) and was recrystallised from ethanol to give material, m. p. 163—165° (Found: C, 54·7; H, 3·7. $C_{14}H_{11}BrO_3$ requires C, 54·7; H, 3·6%).

OO-*Dibenzyl*-[5-^{3}H]*protocatechuic Aldehyde*.—3-Benzyloxy-4-hydroxybenzaldehyde [18] was tritiated under the conditions described above for deuteration. Bromination (as above) gave the corresponding 5-bromo-derivative containing <0·1% of the ^{3}H activity. Benzylation, with benzyl chloride in refluxing acetone containing excess potassium carbonate, proceeded without loss of tritium to give the required labelled dibenzylprotocatechuic aldehyde.[23]

Bromination of Isovanillin.—Bromination [13] in acetic acid gave a mixture of 2- and 6-bromoisovanillin. The 2-bromo-derivative was readily purified. 6-Bromoisovanillin, even when obtained as well-formed crystals, was shown by thin-layer chromatography to contain traces of the 2-isomer. On silica gel G (Merck) plates developed in chloroform the 2- and 6-isomers (revealed by iodine vapour) had R_F values of 0·7 and 0·6 respectively. Repeated recrystallisation of 6-bromoisovanillin monohydrate from aqueous ethanol eventually gave pure material.

Conversion of 2-*Bromoisovanillin into* OO-*Dibenzylprotocatechuic Aldehyde*.—2-Bromoisovanillin (100 mg.) was demethylated [11] by heating under reflux in methylene chloride (7·5 ml.) and pyridine (1·5 ml.) containing anhydrous aluminium chloride (65 mg.) for 24 hr. The cooled reaction mixture was decomposed with 6N-hydrochloric acid in the usual way. Extraction of aqueous phase with ether gave 2-*bromoprotocatechuic aldehyde* (86%), which crystallised from water as needles, m. p. 183—185° (after drying *in vacuo* at room temperature) (Found: C, 38·6; H, 2·1. $C_7H_5BrO_3$ requires C, 38·7; H, 2·3%). The n.m.r. spectrum (in dimethylsulphoxide) showed the expected AB quartet at 2·64 and 3·03 τ (J = 8 c./sec.).

Benzylation was carried out in dimethylformamide with benzylchloride and sodium hydride (see above). The crude neutral product was chromatographed on alumina (grade III), elution with benzene giving OO-*dibenzyl*-2-*bromoprotocatechuic aldehyde* (75%) which crystallised from ethanol as needles, m. p. 142—144° (Found: C, 63·2; H, 4·15. $C_{21}H_{17}BrO_3$ requires C, 63·5; H, 4·25%).

The dibenzyl ether (500 mg.) in tetrahydrofuran (15 ml.) was heated under reflux for 19 hr.

[19] E. Späth, A. Orechoff, and F. Kuffner, *Ber.*, 1934, **67**, 1214.
[20] A. Lovecy, R. Robinson, and S. Sugasawa, *J.*, 1930, 817.
[21] R. Pschorr, *Annalen*, 1912, **391**, 29.
[22] K. W. Bentley, "The Chemistry of the Morphine Alkaloids," Clarendon Press, Oxford, 1954, p. 32.
[23] H. Burton and P. F. G. Praill, *J.*, 1951, 522.

with lithium aluminium hydride (250 mg.). The reaction mixture was decomposed with water, acidified with excess 6N-hydrochloric acid, and the product extracted into chloroform. The extract was dried (Na_2SO_4) and evaporated to give crude 3,4-dibenzyloxybenzyl alcohol (440 mg.). The material was shaken in benzene (20 ml.) with manganese dioxide [24] (4 g.) for 2 hr. Filtration and evaporation gave *OO*-dibenzylprotocatechuic aldehyde which was crystallised from methanol to give material (230 mg.), m. p. and mixed m. p. 89—91° (lit.,[23] 91°).

Conversion of 6-Bromoisovanillin into OO-*Dibenzylprotocatechuic Aldehyde.*—The procedure followed in detail that given above for the 2-isomer. Similar yields of intermediates were obtained. *6-Bromoprotocatechuic aldehyde* crystallised from water as needles, m. p. 225—227° (decomp.) (Found: C, 38·8; H, 2·85. $C_7H_5BrO_3$ requires C, 38·7; H, 2·3%).

OO-*Dibenzyl-6-bromoprotocatechuic aldehyde* was obtained from ethanol as needles, m. p. 105—107° (Found: C, 63·3; H, 4·2. $C_{21}H_{17}BrO_3$ requires C, 63·5; H, 4·25%).

2-*Bromo*-[6-^{3}H]*isovanillin.*—Separate samples of [2,6-^{3}H]isovanillin (18·1 and 28·6 μc per mmole) were brominated, in the usual way, to give corresponding samples of 2-bromo-[6-^{3}H]-isovanillin (5·9 and 9·5 μc per mmole). Higher activities were employed for the conversion of the labelled bromo-compound into *OO*-dibenzyl-[6-^{3}H]protocatechuic aldehyde (Table 2).

6-*Bromo*-[2-^{3}H]*isovanillin.*—6-Bromoisovanillin monohydrate [13] (102 mg.) in deuterium oxide (0·2 ml.) containing triethylamine (43 mg.) was heated at 100° for 14·5 hr. Work-up in the usual way gave anhydrous 6-bromo-[2-^{2}H]isovanillin (68 mg.), m. p. 110—112°. The n.m.r. spectrum, in dimethylsulphoxide, showed a singlet at 2·71 τ (1 proton) and a weak line at 2·66 τ. With the undeuterated material both signals were of similar integrated intensity although the high field signal (from H_5) was broader and, therefore, lower than its companion. In a similar way 6-bromo-[2-^{3}H]isovanillin (1·3 mc per mmole) was prepared using tritiated water (3·6 mc per mmole). Conversion of a diluted specimen into *OO*-dibenzyl-[2-^{3}H]protocatechuic aldehyde is recorded in Table 2.

2,6-*Dibromoisovanillin.*—2-Bromoisovanillin (97 mg.) in dimethylformamide (1 ml.) and acetic acid (1 ml.) containing anhydrous sodium acetate (60 mg.) was treated with bromine (70 mg.) for 40 min. at room temperature with stirring. Water (10 ml.) was added, and after 1 hr. the product (79 mg.) was collected. Crystallisation from aqueous ethanol gave 2,6-dibromoisovanillin as needles, m. p. 162—164° (lit.,[14] 160—161°). Bromination of 6-bromoisovanillin, in the same system but without dimethylformamide, gave the same dibromo-compound which must, therefore, be the 2,6-isomer. In this experiment the product crystallised directly from the reaction mixture. Bromination in hot acetic acid gave, in accord with earlier work,[14] a poor yield of this derivative. When 2-bromo-[6-^{3}H]isovanillin and 6-bromo-[2-^{3}H]isovanillin were brominated, in the presence of sodium acetate, the resulting specimens of 2,6-dibromoisovanillin contained <3 and $<0·4$%, respectively, of the original tritium.

5-(3,4-*Dibenzyloxybenzylidene*)*hydantoin.*—3,4-Dibenzyloxybenzaldehyde [23] (1 g.) and hydantoin (450 mg.) in acetic anhydride (9 ml.) were heated under reflux for 70 min. in the presence of anhydrous sodium acetate (450 mg.). The hot reaction mixture was poured into excess hot water and the resulting suspension heated for 10 min., and then filtered. The dark yellow product (1·3 g.), after washing with hot water, was sufficiently pure for the next step (see below). Crystallisation from ethyl acetate gave yellow needles of the *benzylidene-hydantoin*, m. p. 197—200° (Found: C, 72·4; H, 5·3. $C_{24}H_{20}N_2O_4$ requires C, 72·0; H, 5·0%).

5-(3,4-*Dibenzyloxybenzyl*)*hydantoin.*—The corresponding benzylidenehydantoin (1 g.) in dioxan (10 ml.) and water (2 ml.) was treated at room temperature with successive small portions of 3% sodium amalgam (10 g.) during 2 hr., with stirring. After a further 1 hr. the solution was decanted from the mercury, diluted with water (100 ml.), filtered, and acidified with 6N-hydrochloric acid. After 1 hr. at 0° the precipitated product (800 mg.) was collected, dried, and crystallised from benzene to give the *benzylhydantoin* as colourless plates, m. p. 136—138°, $\nu_{max.}$ 3450, 1775, and 1730 cm.$^{-1}$ (in chloroform) (Found: C, 71·9; H, 5·5. $C_{24}H_{22}N_2O_4$ requires C, 71·6; H, 5·5%).

(±)-3,4-*Dibenzyloxyphenylalanine.*—The corresponding benzylhydantoin (400 mg.) in 2-methoxyethanol (6 ml.) and water (2 ml.) containing potassium hydroxide (1 g.) was heated under reflux for 15 hr. The solvent was evaporated *in vacuo* and the residue dissolved in water (50 ml.). Acidification with 6N-hydrochloric acid gave a precipitate (390 mg.) of the required

[24] J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jansen, and T. Walker, *J.*, 1952, 1094.

amino-acid hydrochloride. After treatment with charcoal (in ethanol) (±)-**3,4-***dibenzyloxyphenylalanine hydrochloride* crystallised from ethanol–ether as small prisms, m. p. 182—185° (Found: C, 67·2; H, 5·7. $C_{23}H_{24}ClNO_4$ requires C, 66·7; H, 5·8%).

Hydrolysis of the benzylhydantoin with potassium hydroxide in aqueous ethanol gave 5-(**3,4-***dibenzyloxybenzyl*)*hydantoic acid* which crystallised from acetonitrile as needles, m. p. 175—176° (Found: C, 68·2; H, 6·1. $C_{24}H_{24}N_2O_5$ requires C, 68·6; H, 5·75%).

(±)-**3,4-***Dihydroxyphenylalanine* (*dopa*).—The corresponding dibenzyl ether (212 mg.) in ethanol (20 ml.) containing 6N-hydrochloric acid (0·2 ml.) was hydrogenated over 10% palladium–charcoal at room temperature for 50 min. After removal of the catalyst and evaporation of the solvent, at room temperature, the crude product was dissolved in water (0·5 ml.). The solution was adjusted to pH 4—5 by slow addition of aqueous ammonia at 0°. The (±)-3,4-dihydroxy-phenylalanine which crystallised out on standing was collected, washed with ethanol and acetone, and dried *in vacuo* to give material (60 mg.), m. p. and mixed m. p. 272—275°. The infrared spectrum (Nujol) was identical with that of authentic (±)-dopa.

Hydrolysis of 5-(**3,4-***Dibenzyloxybenzyl*)-[5-^{3}H]*hydantoin*.—The unlabelled benzylhydantoin (207 mg.) in 2-methoxyethanol (3 ml.) and tritiated water (1 ml.) containing potassium hydroxide (504 mg.) was kept at room temperature for 24 hr. The solvent was removed *in vacuo* and the residue dissolved in water (20 ml.). Acidification, in the usual way, gave the [5-^{3}H]hydantoin (160 mg.). Crystallisation from benzene gave plates (124 mg.), m. p. 136—138°. The activity of this material was not significantly altered by repeated addition and evaporation of methanol. Hydrolysis in refluxing 2-methoxyethanol, as above, gave 3,4-dibenzyloxyphenylalanine hydrochloride containing 3·9% of the original activity. A duplicate experiment gave a 4·1% retention of activity.

Preparation of Tritium-Labelled (±)-**3,4-***Dihydroxyphenylalanine*.—Conversion of the three labelled specimens of *OO*-dibenzylprotocatechuic aldehyde into the desired amino-acid was carried out, as described above, with the results given in Table 3. (±)-3,4-Dihydroxy-[2,5,6-$^2H_3$]phenylalanine was prepared by heating dopa (106 mg.) in 4·1N-deuterium chloride (0·4 ml.) for 3 hr. at 100°. L-Tyrosine (100 mg.) was converted into the 3,5-dideutero-derivative by 6 hr. heating under the same conditions (see Table 4). (±)-3,4-Dihydroxy-[2,5,6-$^3H_3$]phenylalanine was obtained in the same way using tritiated hydrochloric acid. Reduction of the benzylidenehydantoin (VII) with sodium amalgam, in dioxan containing tritiated water (see above), and hydrolysis of the resulting benzylhydantoin (as VIII) gave β-labelled *OO*-dibenzyl-dopa. Hydrogenation then gave (±)-3,4-dihydroxy-[β-^{3}H]phenylalanine (Table 3). Hydrolysis of the non-radioactive benzylhydantoin (as VIII) in the presence of tritiated water, and hydrogenation of the product, afforded (±)-3,4-dihydroxy-[α-^{3}H]phenylalanine.

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