Hydroxytryptamines. Part I. Bufotenine, 6-Hydroxybufotenine, and Serotonin.

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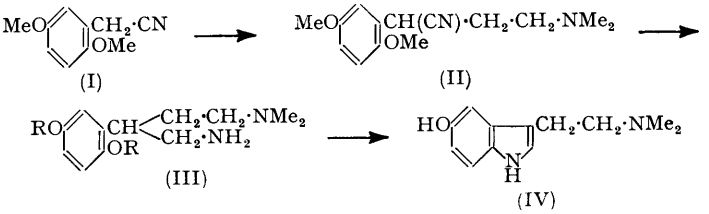
[Reprint Order No. 4804.]

Convenient syntheses of bufotenine and serotonin involving the ferricyanide oxidation of 2-(2 : 5-dihydroxyphenyl)-4-dimethylamino- and -4-amino-butylamine, respectively, are described. A similar synthesis gave 6-hydroxybufotenine, while attempts to isolate 6-hydroxyserotonin were unsuccessful.

IT has been shown (Cromartie and Harley-Mason, *J.*, 1952, 2525) that ferricyanide oxidation of 2-(2 : 5-dihydroxyphenyl)ethylamine gives 5-hydroxyindole in high yield. Application of this reaction to the synthesis of 5-hydroxytryptamine derivatives of physiological interest is now described. Wieland, Konz, and Mittasch (*Ann.*, 1934, **513**, 1) isolated bufotenine, 5-hydroxy-*NN*-dimethyltryptamine (IV), from the skin of the common toad and determined its structure; more recently it has also been obtained from the fungus *Amanita mappa* (Wieland and Motzel, *Ann.*, 1953, **581**, 10). It was first synthesised by Hoshino and Shimodaira (*Ann.*, 1935, **520**, 19). 2 : 5-Dimethoxybenzyl cyanide (I), required as starting product for a new synthesis (previously described in outline, *Chem. and Ind.*, 1952, 954), was obtained from 2 : 5-dimethoxybenzaldehyde (Gattermann, *Ann.*, 1907, **357**, 369) by catalytic hydrogenation to the alcohol, which was converted into the chloride by thionyl chloride; the chloride was then treated with potassium cyanide. Alkylation of (I) with 2-dimethylaminoethyl chloride (cf. Eisleb, *Ber.*, 1941, **74**, 1433) and sodamide gave 1-(2 : 5-dimethoxyphenyl)-3-dimethylaminopropyl cyanide (II) which was then hydrogenated to 2-(2 : 5-dimethoxyphenyl)-4-dimethylaminobutylamine (III ; R = Me) :



Boiling this with hydrobromic acid gave the hydroxy-diamine (III, R = H), which on oxidation with potassium ferricyanide gave bufotenine (IV) in good yield. The reactions involved in this oxidation have been discussed earlier (Harley-Mason, *Chem. and Ind.*, 1952, 173; Cromartie and Harley-Mason, *loc. cit.*). The ultra-violet absorption spectrum is recorded in the Figure.

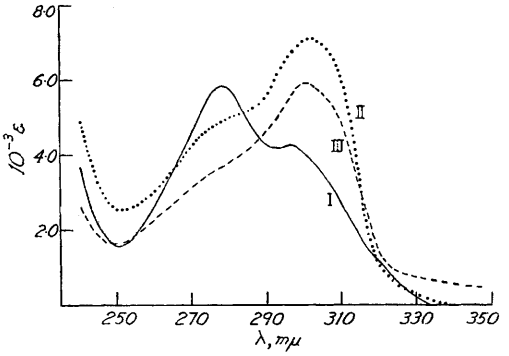
A similar reaction sequence was employed for 6-hydroxybufotenine [5 : 6-dihydroxy-*NN*-dimethyltryptamine (VI)]. In this case, the starting material, 2 : 4 : 5-trimethoxybenzyl cyanide, was obtained *via* the rhodanine condensation product of 2 : 4 : 5-trimethoxybenzaldehyde (cf. Julian and Sturgis, *J. Amer. Chem. Soc.*, 1935, **57**, 1126, 2739), since attempts to convert 2 : 4 : 5-trimethoxybenzyl alcohol into the corresponding chloride were unsuccessful. The trihydroxy-diamine (V) was obtained exactly as for (III), and a ferricyanide oxidation gave a rather low yield of (VI). The product was characterised as a picrate of rather unusual composition (2 mols. of base : 3 mols. of picric acid). Its identity was however confirmed by examination of its ultra-violet absorption spectrum (Figure), which was very similar to that of 5 : 6-dihydroxy-3-methylindole.

An alternative route to (VI), based on the observation (Harley-Mason, *J.*, 1953, 200) that 2-(2-amino-4 : 5-dihydroxyphenyl)ethylamine gives 5 : 6-dihydroxyindole on autoxidation, was explored. Alkylation of 3 : 4-dimethoxybenzyl cyanide (VII) with 2-dimethylaminoethyl chloride gave 3-dimethylamino-1-(3 : 4-dimethoxyphenyl)propyl cyanide

(VIII), which was readily nitrated to (IX). Catalytic hydrogenation followed by demethylation gave the dihydroxy-triamine (X). However, the yields of (VI) obtained on autoxidation or treatment with silver oxide of (X) were so low that this approach had to be abandoned.

(V) (VI)

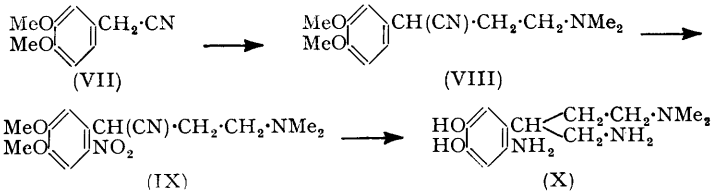
5-Hydroxytryptamine (XV) (serotonin, enteramine, thrombocytin) was isolated from ox serum by Rapport, Green, and Page (*J. Biol. Chem.*, 1948, **174**, 735; **176**, 1237), and from *Octopus vulgaris* and *Discoglossus pictus* by Erspamer and Ottolenghi (*Experientia*, 1952, **8**, 31, 152; *J. Biol. Chem.*, 1952, **200**, 311). Its distribution and pharmacological properties have been extensively studied in recent years. Four syntheses of serotonin have been reported (Hamlin and Fischer, *J. Amer. Chem. Soc.*, 1951, **73**, 5007; Speeter,



Ultra-violet absorption spectra.

I. ——— *Bufotenine (in 95% ethanol).*
II. ····· *6-Hydroxybufotenine (in 95% ethanol).*
III. — — — *5:6-Dihydroxy-3-methylindole (in buffer, pH 6·7).*

Heinzelmann, and Weisblatt, *ibid.*, p. 5514; *Chem. Eng. News*, 1953, **31**, 3861; Asero, Colo, Erspamer, and Vercellone, *Ann.*, 1952, **576**, 69) all of which involve addition of a two-carbon side-chain to a 5-alkoxy- or -benzyloxy-indole by well-known methods.

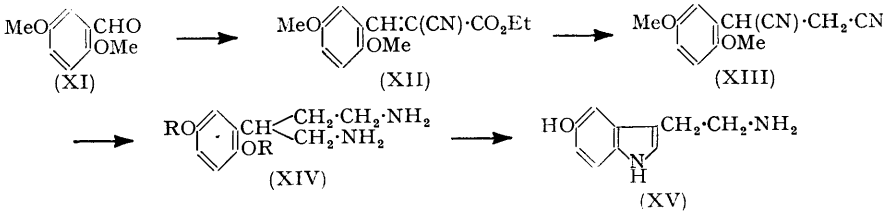


(VII) (VIII) (IX) (X)

Attempts to introduce a potential 2-aminoethyl side-chain into (I) by alkylation with *N*-2-bromoethylphthalimide or chloroacetonitrile were unsuccessful, so that an alternative route to the required diamine (XIV) was developed. 2 : 5-Dimethoxybenzaldehyde (XI) was condensed with ethyl cyanoacetate to give ethyl α-cyano-2 : 5-dimethoxycinnamate (XII) which when boiled with potassium cyanide gave 2 : 5-dimethoxyphenylsuccinonitrile (XIII) (cf. Mowry, *J. Amer. Chem. Soc.*, 1946, **68**, 2108). Catalytic hydrogenation of (XIII) in the presence of hydrochloric acid gave 1 : 4-diamino-2-(2 : 5-dimethoxyphenyl)butane (XIV; R = Me), from which (XIV; R = H) was obtained on treatment with hydrobromic acid. On ferricyanide oxidation the dihydroxy-amine gave serotonin (XV).

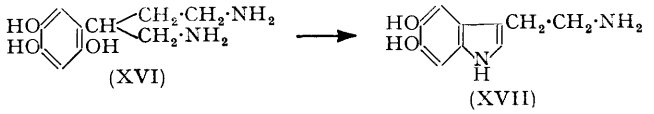
The overall yield of (XV) from (XI) was 25% and in view of its simplicity and of the few stages involved, this synthesis is recommended as the best available for serotonin. For pharmacological purposes serotonin has usually been isolated as the double salt with creatinine sulphate. We have found that the hydrogen oxalate is a more readily prepared crystalline salt and is stable and non-hygroscopic. It is noteworthy that oxidative ring-closure of (XIV; R = H) could theoretically lead either to an indole or a dihydroquinoline

derivative depending on which of the two amino-groups was involved; however, no indication of the formation of the latter was found.



(XI) (XII) (XIII) (XIV) (XV)

A similar reaction sequence from 2 : 4 : 5-trimethoxybenzaldehyde was used in an attempt to prepare 6-hydroxyserotonin (XVII). The trihydroxy-diamine (XVI) was obtained in exactly the same manner as (XIV), and the initial stage of oxidation with potassium ferricyanide appeared to proceed normally. However, attempts to isolate a pure product were unsuccessful, though the extracts gave a strongly positive Ehrlich reaction similar to that given by (VI).



(XVI) (XVII)

Experimental

2 : 5-*Dimethoxybenzyl Cyanide*.—2 : 5-Dimethoxybenzaldehyde (15 g.) was hydrogenated in ethanol (150 c.c.) over platinum oxide (0·15 g.) at 50° and 50 atm. After filtration and removal of the solvent, 2 : 5-dimethoxybenzyl alcohol (13·2 g.) was distilled at 110°/1 mm. Thionyl chloride (20 c.c.) in ether (80 c.c.) was added during 15 min. to a solution of the above alcohol (13 g.) and pyridine (1 c.c.) in ether (150 c.c.) with stirring. After a further 15 min. the solution was extracted with water (2 × 100 c.c.) and dried ($MgSO_4$). Removal of the solvent and recrystallisation from light petroleum (b. p. 80—100°) gave 2 : 5-dimethoxybenzyl chloride (crude yield 13·1 g., 92%) as needles, m. p. 70—72°. Baumann and Fraenkel (*Z. physiol. chem.*, 1895, **20**, 20) give m. p. 72—73°.

A solution of the above chloride (12·5 g.) and potassium cyanide (30 g.) in ethanol (240 c.c.) and water (60 c.c.) was refluxed for 3 hr. The dark red solution was cooled and poured on ice (300 g.), and the oil extracted with ether. The ethereal extract was dried ($MgSO_4$) and the solvent removed. Distillation of the residue at 115°/2 mm. gave 2 : 5-dimethoxybenzyl cyanide (9·8 g.), large prisms, m. p. 52—53°. Sugasawa and Shigehara (*Ber.*, 1941, **74**, 459) give m. p. 54—55°.

1-(2 : 5-*Dimethoxyphenyl*)-3-*dimethylaminopropyl Cyanide*.—A solution of 2-dimethylaminoethyl chloride hydrochloride (8·7 g.) in water (25 c.c.) was treated with 2N-sodium hydroxide (30 c.c.), then saturated with potassium carbonate and extracted with benzene (3 × 15 c.c.). The dried (K_2CO_3) extracts were added to a solution of 2 : 5-dimethoxybenzyl cyanide (10·6 g.) in benzene (25 c.c.). Finely powdered sodamide (2·4 g.) was slowly added with stirring, the temperature being kept below 40°. The mixture was then heated under reflux on the water-bath with vigorous stirring for 2 hr. After cooling, the mixture was extracted with water to remove the separated sodium chloride, the benzene layer dried (K_2CO_3), and the solvent removed. The residual oil was distilled at 150—155°/2·5 mm. giving the amino-cyanide (7·5 g., 51%) as a pale yellow oil. A portion was treated with hydrogen chloride giving 1-(2 : 5-*dimethoxyphenyl*)-3-*dimethylaminopropyl cyanide hydrochloride*, which formed white prisms from ethanol–ether, m. p. 161—162° with softening at 150° (Found : C, 59·3; H, 7·7; N, 9·6. $C_{14}H_{20}O_2N_2$,HCl requires C, 59·0; H, 7·4; N, 9·6%).

2-(2 : 5-*Dihydroxyphenyl*)-4-*dimethylaminobutylamine* (III; R = H).—A solution of the amino-cyanide (7 g.) in ethanol (50 c.c.) saturated with ammonia was hydrogenated over Raney nickel at 100° and 100 atm. for 5 hr. The catalyst was filtered off, the solvent removed, and the residual oil distilled at 145—150°/2·5 mm., giving the diamine (III; R = Me) (5·8 g.) as a pale yellow oil. The *dipicrate* formed yellow prisms, m. p. 198—199° (decomp.), from methanol (Found : C, 43·9; H, 4·2; N, 16·0. $C_{14}H_{24}O_2N_2$,$2C_6H_3O_7N_3$ requires C, 43·9; H, 4·2; N, 15·8%).

The dimethoxy-diamine (1·3 g.) was refluxed with hydrobromic acid (*d* 1·49; 8 c.c.) for 45 min., and the resulting solution diluted with water and evaporated to dryness on the water-bath under reduced pressure of hydrogen. The residual dark brown solid was dissolved in water and boiled with charcoal, and the solution again evaporated under hydrogen. The last traces of water and hydrobromic acid were removed by storage in a vacuum desiccator over phosphoric oxide and sodium hydroxide, leaving the dihydroxy-diamine dihydrobromide as a light brown hygroscopic glass which did not crystallise.

Bufotenine (IV).—To a solution of the dihydroxy-diamine dihydrobromide (1·9 g.) in water (60 c.c.), a solution of potassium ferricyanide (3·2 g.) and sodium hydrogen carbonate (1·65 g.) in water (60 c.c.) was added with stirring during 5 min. The mixture darkened at first, but became paler after 20 min. A little sodium dithionite was added and, after filtration from a small amount of flocculent precipitate, the light yellowish-brown solution was extracted continuously with peroxide-free ether for 2 days. Removal of the ether and drying of the residue in a vacuum over phosphoric oxide gave bufotenine (0·45 g.; 45%) as a hard brown glass, purified by sublimation at 160°/10^{-4} mm. The picrate formed red needles, m. p. 177° (decomp.), from methanol (Found: C, 43·6; H, 3·6; N, 17·2. Calc. for $C_{12}H_{16}ON_2,2C_6H_3O_7N_3$: C, 43·5; H, 3·3; N, 16·8%). Hoshino and Shimodaira (*loc. cit.*) give m. p. 178°.

2 : 4 : 5-*Trimethoxybenzyl Alcohol*.—2 : 4 : 5-Trimethoxybenzaldehyde (5 g.), dissolved in ethanol (100 c.c.), was hydrogenated over platinum oxide (0·2 g.), the reaction being interrupted when one mol. of hydrogen had been absorbed. After filtration from the catalyst, evaporation of the solvent left an oil from which 2 : 4 : 5-*trimethoxylbenzyl alcohol* was obtained as small prisms, m. p. 70—71°, after recrystallisation from benzene–light petroleum (Found: C, 60·5; H, 7·4. $C_{10}H_{14}O_4$ requires C, 60·6; H, 7·1%).

Reaction with Thionyl Chloride.—The above alcohol (1·5 g.), dissolved in dry ether (25 c.c.) and pyridine (0·1 c.c.), was treated with thionyl chloride (2·25 g.) in ether (10 c.c.). After 20 min. at room temperature the mixture was extracted with water (2 × 10 c.c.), the ethereal solution was dried (Na_2SO_4), and the solvent was removed. From the residue a halogen-free substance (1·2 g.), m. p. 101°, was isolated as prisms by recrystallisation from light petroleum (b. p. 80—100°) [Found: C, 65·1; H, 6·4; OMe, 45·3%; *M* (Rast), 287].

2 : 4 : 5-*Trimethoxybenzylidenerhodanine*.—Rhodanine (15 g.) and 2 : 4 : 5-trimethoxybenzaldehyde (15 g.) were dissolved in acetic acid (100 c.c.), finely powdered anhydrous sodium acetate (20 g.) was added, and the mixture refluxed for 4 hr. The mixture, from which most of the product had crystallised during the reaction, was poured into water, and the product filtered off and washed with water, alcohol, and ether. 2 : 4 : 5-*Trimethoxybenzylidenerhodanine* (12 g.) formed red needles, very sparingly soluble in most organic solvents. A small quantity recrystallised from glycol monoethyl ether had m. p. 250—252° (decomp.) (Found: C, 50·1; H, 4·1. $C_{13}H_{13}O_4NS_2$ requires C, 50·2; H, 4·1%).

2 : 4 : 5-*Trimethoxybenzyl Cyanide*.—The foregoing rhodanine derivative (12 g.) was warmed at 100° with 15% sodium hydroxide (80 c.c.) for 20—30 min. until it had all dissolved, giving a deep yellowish-brown solution, and the solution was then cooled. 10% Hydrochloric acid (120 c.c.) was added and the voluminous precipitate of the thiopyruvic acid was collected and dried. Sodium (2·7 g.) was dissolved in dry ethanol (80 c.c.) and added to hydroxylamine hydrochloride (8 g.) dissolved in water (8 c.c.). The sodium chloride was filtered off and the filtrate poured on to the thiopyruvic acid. The mixture was heated on the water-bath for 20 min. with stirring; a homogeneous solution was obtained. The alcohol was removed under vacuum the residue taken up in 5% sodium hydroxide (40 c.c.), and the solution filtered and cooled in ice. Addition of 10% hydrochloric acid precipitated 2 : 4 : 5-trimethoxyphenylpyruvic acid oxime, which was collected, washed, and dried. The oxime was treated with acetic anhydride (50 c.c.), and the mixture warmed continuously on the water-bath until all had dissolved. The acetic anhydride was then removed under vacuum and the residue was shaken with ether and water. The ethereal layer was separated, washed with sodium carbonate solution, and dried ($MgSO_4$). The solvent was removed and the residue distilled at 80—90°/10^{-4} mm. giving 2 : 4 : 5-trimethoxybenzyl cyanide (2·8 g.) as blunt needles, m. p. 84° [after recrystallisation from light petroleum (b. p. 80—100°)] (Found: N, 6·75. Calc. for $C_{11}H_{13}O_3N$: N, 6·8%). Robertson and Rusby (*J.*, 1935, 1371) give m. p. 85°.

3-*Dimethylamino*-1-(2 : 4 : 5-*trimethoxyphenyl*)*propyl Cyanide*.—2-Dimethylaminoethyl chloride hydrochloride (2 g.) dissolved in water (5 c.c.) was treated with 2N-sodium hydroxide (6·6 c.c.), and extracted with xylene (3 × 10 c.c.) after saturation with potassium carbonate. 2 : 4 : 5-Trimethoxybenzyl cyanide (2·5 g.) was dissolved in the dried (K_2CO_3) xylene extracts, and the solution cooled to 0° before the addition of powdered sodamide (0·54 g.). The mixture

was heated slowly to 100°, kept at this temperature for 30 min., and then boiled under reflux for 5 hr. The cooled solution was shaken with dilute hydrochloric acid (40 c.c.), and the aqueous layer separated and basified with sodium hydroxide before it was extracted with ether (3 × 25 c.c.). The dried ($MgSO_4$) ethereal extract was evaporated and the residual oil distilled from a small retort (external temperature 140°) at 0·1 mm., yielding a viscous yellow oil (1·5 g.). Treatment of a portion with hydrogen chloride gave *3-dimethylamino-1-(2 : 4 : 5-trimethoxyphenyl)propyl cyanide hydrochloride* as small white prisms, m. p. 172—174°, from ethanol–ether (Found : C, 57·4; H, 7·6; N, 8·9. $C_{15}H_{22}O_3N_2,HCl$ requires C, 57·2; H, 7·4; N, 8·9%).

4-Dimethylamino-2-(2 : 4 : 5-trimethoxyphenyl)butylamine.—The foregoing amino-cyanide (1·5 g.), dissolved in dry ether (200 c.c.), was added slowly during 1 hr. to a stirred solution of lithium aluminium hydride (1 g.) in dry ether (50 c.c.). The mixture was refluxed for 2½ hr., cooled, and then decomposed with a saturated solution of sodium potassium tartrate (25 c.c.). The dried ($MgSO_4$) ether layer was evaporated, leaving the diamine as a yellow oil. Treatment of a portion with picric acid gave *4-dimethylamino-2-(2 : 4 : 5-trimethoxyphenyl)butylamine dipicrate* which formed yellow prisms, m. p. 187—190°, from methanol (Found : C, 43·6; H, 4·1; N, 14·6. $C_{15}H_{26}O_3N_2,2C_6H_3O_7N_3$ requires C, 43·8; H, 4·3; N, 15·1%). The diamine was demethylated as described above for the dimethoxy-compound (III; R = Me), giving the trihydroxy-diamine (V) dihydrobromide as a hygroscopic light brown glass.

6-Hydroxybufotenine (VI).—The above dihydrobromide (0·89 g.), dissolved in water (40 c.c.), was treated with a solution of potassium ferricyanide (1·32 g.) and sodium hydrogen carbonate (0·84 g.) in water (40 c.c.). The deep red solution was kept for 1·5 hr. under hydrogen, the colour changing to dark grey. A little sodium dithionite was added to prevent further oxidation, and a small amount of dark precipitate was filtered off. The solution was then continuously extracted with peroxide-free ether for 2 days. The extract, which showed a marked bluish-violet fluorescence, was evaporated and the residue dried in a vacuum over phosphoric oxide. Sublimation at 135°/10^{-4} mm. gave an almost colourless waxy solid of indefinite m. p. Its ultra-violet spectrum is given in the Figure. With Ehrlich's reagent it gave a bluish-green colour which slowly changed to a very intense blue. Treatment with methanolic picric acid gave *6-hydroxybufotenine picrate*, red needles, m. p. 139—140° (decomp.) (Found : C, 44·6; H, 4·1; N, 15·7. $2C_{12}H_{16}O_2N_2,3C_6H_3O_7N_3$ requires C, 45·3; H, 3·9; N, 16·0%).

1-(4 : 5-Dimethoxy-2-nitrophenyl)-3-dimethylaminopropyl Cyanide (IX).—3 : 4-Dimethoxybenzyl cyanide was alkylated with 2-dimethylaminoethyl chloride as described for the 2 : 5-dimethoxy-compound, except that xylene was used as solvent. 1-(3 : 4-Dimethoxyphenyl)-3-dimethylaminopropyl cyanide distilled at 115—120°/0·1 mm. and was characterised as the *picrate* which formed yellow prisms, m. p. 156—158°, from aqueous ethanol (Found : N, 14·8. $C_{14}H_{20}O_2N_2,C_6H_3O_7N_3$ requires N, 14·7%). The amino-cyanide (7 g.) was dissolved in dilute nitric acid (15 c.c.), and the solution added slowly with stirring and cooling to concentrated nitric acid (30 c.c.). After storage overnight at 0° the mixture was poured into water and basified with sodium hydroxide. The nitro-compound was extracted with ether and the extract dried (Na_2SO_4). After removal of the solvent the residue was taken up in ethanol (30 c.c.), and hydrogen chloride passed in. The precipitated *1-(4 : 5-dimethoxy-2-nitrophenyl)-3-dimethylaminopropyl cyanide hydrochloride* was collected and recrystallised from ethanol giving very pale yellow needles, m. p. 189—190° (decomp. with softening at 183°) (Found : C, 51·1; H, 6·3; N, 12·5. $C_{14}H_{20}O_4N_3Cl$ requires C, 51·0; H, 6·1; N, 12·7%).

2-(4 : 5-Dihydroxy-2-aminophenyl)-4-dimethylaminobutylamine (X).—The above hydrochloride (2·5 g.) dissolved in ethanol (225 c.c.) and 2N-hydrochloric acid (25 c.c.) was hydrogenated over platinum oxide (0·5 g.) at 4—5 atm. Hydrogen uptake was complete after 2·5 hr.; the catalyst was then filtered off and the solvent removed under reduced pressure of hydrogen at 40°. The pale blue residue was dried in a vacuum desiccator and then triturated with a small quantity of propanol and ether, which removed most of the colour. Recrystallisation from propanol–ether yielded *2-(4 : 5-dimethoxy-2-aminophenyl)-4-dimethylaminobutylamine trihydrochloride* (1·9 g.) as tiny needles, m. p. 237—239° (decomp.) (Found : N, 11·6. $C_{14}H_{25}O_2N_3,3HCl$ requires N, 11·2%). The trihydrochloride was demethylated with hydrobromic acid as for (III; R = Me) above. The dihydroxy-trihydrobromide formed a pale greenish solid. Autoxidation or oxidation of this compound with silver oxide yielded only traces of 6-hydroxybufotenine.

Ethyl α-Cyano-2 : 5-dimethoxycinnamate.—2 : 5-Dimethoxybenzaldehyde (5·6 g.) and ethyl cyanoacetate (3·7 g.) were dissolved in warm ethanol (10 c.c.), piperidine (0·1 c.c.) was added, and the solution was kept at 60° for an hour. On cooling of the solution to 0°, *ethyl α-cyano-2 : 5-dimethoxycinnamate* (7·9 g., 95%) crystallised as beautiful orange needles, which were

collected and washed with a little alcohol. The bulk of the product was used directly for the next stage; for analysis, a small portion was recrystallised from aqueous ethanol, and had m. p. 81° (Found: C, 64·4; H, 5·8; N, 5·7. $C_{14}H_{15}O_4N$ requires C, 64·4; H, 5·8; N, 5·4%).

2 : 5-*Dimethoxyphenylsuccinonitrile* (XIII).—The foregoing ester (7·9 g.) and A.R. potassium cyanide were dissolved in 90% ethanol (120 c.c.), and the mixture refluxed for 2 hr. The resulting solution was cooled and filtered, the solvent was removed under reduced pressure of nitrogen, and the residue was twice recrystallised from aqueous ethanol, giving 2 : 5-*dimethoxyphenylsuccinonitrile* (4·6 g., 70%) as short white needles, m. p. 85° (Found: C, 66·5; H, 5·4; N, 13·1. $C_{12}H_{12}O_2N_2$ requires C, 66·7; H, 5·5; N, 12·9%).

1 : 4-*Diamino*-2-(2 : 5-*dimethoxyphenyl*)*butane Dihydrochloride*.—The succinonitrile (4 g.), dissolved in ethanol (195 c.c.) and concentrated hydrochloric acid (5 c.c.), was hydrogenated at 4—5 atm. over platinum oxide (0·5 g.) Hydrogen uptake ceased after about 5 hr., and the solution was then filtered and evaporated under vacuum. The residual solid was triturated with a little propanol and ether, and then recrystallised from ethanol–ether. 1 : 4-*Diamino*-2-(2 : 5-*dimethoxyphenyl*)*butane dihydrochloride* (XIV; R = Me) (4·6 g., 85%) formed small prisms, m. p. 219—220° (decomp.) (Found: C, 48·7; H, 7·4; N, 9·3. $C_{12}H_{20}O_2N_2$,2HCl requires C, 48·5; H, 7·4; N, 9·4%).

Similar reduction of phenylsuccinonitrile (Mowry, *loc. cit.*) gave 1 : 4-*diamino*-2-*phenylbutane dihydrochloride*, m. p. 295—297° (decomp.) (Found: C, 51·1; H, 7·7; N, 12·0. $C_{10}H_{16}N_2$,2HCl requires C, 50·6; H, 7·6; N, 11·8%).

Demethylation.—The dimethoxy-diamine dihydrochloride (4 g.) was demethylated as for (III, R = Me); after the evaporation the residue was triturated with warm propanol and ether, and the 1 : 4-*diamino*-2-(2 : 5-*dihydroxyphenyl*)*butane dihydrobromide* (4·3 g., 90%) then crystallised. (On the first occasion, crystallisation was extremely slow; subsequently when seed-crystals were available, no difficulty was encountered.) Recrystallised from ethanol–ether, it formed prisms, m. p. 254—257° (decomp.) (Found: C, 33·6; H, 5·1; N, 7·5. $C_{10}H_{16}O_2N_2$,2HBr requires C, 33·5; H, 5·0; N, 7·8%).

Serotonin (XV).—The foregoing dihydrobromide (1·78 g.), dissolved in water (80 c.c.), was treated with a solution of potassium ferricyanide (3·2 g.) and sodium hydrogen carbonate (1·69 g.) in water (80 c.c.). The resulting greenish-brown solution was extracted with peroxide-free ether (300 c.c.) in a continuous extractor for 2 days; the aqueous solution had then become yellow and a small amount of amorphous precipitate had settled out, while the ether was almost colourless and a small amount of yellowish-brown oily material (*A*) had separated from it. The ethereal solution was decanted and evaporated under hydrogen, leaving crude serotonin as a brown gum. Sublimation of a portion at 150°/10^{-4} mm. gave serotonin as a very pale brown glass, which could not be crystallised. The bulk of the material was dissolved in a little warm ethanol and treated with an ethanolic solution of oxalic acid. A small amount of brownish amorphous solid was filtered off, and to the warm filtrate dry ether was added until the product began to separate. After being kept at 0°, the *serotonin* [5-*hydroxytryptamine*] *hydrogen oxalate* (0·55 g.), which had separated as pale buff micro-crystals, was collected. More (0·08 g.) of less pure material was obtained by treating the oily material (*A*) with ethanolic oxalic acid, the total yield being 0·63 g. (48%). A further recrystallisation from ethanol–ether (charcoal) gave a colourless product, m. p. 195—197° (decomp.) (Found: C, 54·3; H, 5·3; N, 10·6. $C_{10}H_{12}ON_2$,$C_2H_2O_4$ requires C, 54·1; H, 5·3; N, 10·5%). By treatment of serotonin with the calculated amount of ethanolic oxalic acid, *serotonin oxalate*, large plates, m. p. 194—196° (decomp.) from ethanol, was obtained (Found: C, 59·7; H, 5·85; N, 12·75. $2C_{10}H_{12}ON_2$,$C_2H_2O_4$ requires C, 59·7; H, 5·9; N, 12·7%). With Ehrlich's reagent a pinkish-violet colour was given initially, slowly becoming an intense deep blue. The ultra-violet absorption spectrum of the free base was found to agree with that reported by Hamlin and Fischer (*loc. cit.*). The picrate formed orange-red needles, m. p. 196—197° (decomp., with sintering at 130°) from aqueous methanol; Asero *et al.* (*loc. cit.*) give m. p. 191—193° (Found: C, 45·7; H, 4·3; N, 16·7 Calc. for $C_{10}H_{12}ON_2$,$C_6H_3O_7N_3$,H_2O: C, 45·4; H, 4·0; N, 16·6%).

Ethyl α-Cyano-2 : 4 : 5-*trimethoxycinnamate*.—2 : 4 : 5-Trimethoxybenzaldehyde (6·6 g.) and ethyl cyanoacetate (3·8 g.) were dissolved in ethanol (40 c.c.), and the warm solution treated with two drops of piperidine. The solution became bright yellow, heat was evolved, and the product rapidly crystallised. *Ethyl α-cyano*-2 : 4 : 5-*trimethoxycinnamate* (9 g.) formed feathery, bright yellow needles, m. p. 161°, from ethanol (Found: C, 62·3; H, 6·1; N, 4·8. $C_{15}H_{17}O_5N$ requires C, 61·8; H, 5·8; N, 4·8%).

1 : 4-*Diamino*-2-(2 : 4 : 5-*trihydroxyphenyl*)*butane* (XVI).—The foregoing ester (9·0 g.) was treated with potassium cyanide (4·0 g.) in ethanol (400 c.c.; 90%) as in the case of the di-

methoxy-compound (XII). 2 : 4 : 5-*Trimethoxyphenylsuccinonitrile* formed needles, m. p. 107°, from aqueous ethanol (Found : N, 11·5. $C_{13}H_{14}O_3N_2$ requires N, 11·4%). The nitrile was reduced in the same manner as the corresponding dimethoxy-compound, yielding 1 : 4-*diamino*-2-(2 : 4 : 5-*trimethoxyphenyl*)*butane dihydrochloride*, prisms, m. p. 248—250° (decomp.) (Found : C, 45·2; H, 7·2. $C_{13}H_{22}O_3N_2,2HCl,H_2O$ requires C, 45·2; H, 7·1%).

Demethylation was carried out as in the previous examples, giving 1 : 4-*diamino*-2-(2 : 4 : 5-*trihydroxyphenyl*)*butane dihydrobromide*, prisms, m. p. 229—231° (decomp.) (Found : C, 31·4; H, 5·2; N, 7·6. $C_{10}H_{16}N_2O_3,2HBr$ requires C, 31·8; H, 4·8; N, 7·5%). Oxidation with potassium ferricyanide or silver oxide gave a deep red solution : however, on continuous extraction with ether, only traces of material were obtained, though a strongly positive Ehrlich reaction indicated that a small amount of the expected product, 6-hydroxyserotonin (XVII) was probably formed.

The authors thank Glaxo Laboratories Ltd. for gifts of 2 : 5-dimethoxybenzaldehyde and 2 : 5-dimethoxybenzyl cyanide, and Dr. R. I. T. Cromartie for the spectrum of 5 : 6-dihydroxy-3-methylindole. One of us (A. H. J.) thanks the Department of Scientific and Industrial Research for a maintenance grant.

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