Plasma concentrations of compound I were measured by a modification of the radioisotope derivative technique described by Hammer & Brodie (1967). The biological half-life was 18·1 ± 1·0 h as estimated by exponential regression analysis of the mean plasma levels observed from 4 to 31 h. That of amantadine was 9 to 15 h, based on urinary excretion data (Bleidner, Harmon & others, 1965).

Urinary concentrations of unchanged compound I and two metabolites (structure II, isomers a, b) were assayed by g.l.c. using a flame ionization detector, after chloroform extraction of the urine at pH 9 before and after acid hydrolysis. Compound I, unlike amantadine, was metabolized in man by mono-hydroxylation of the adamantane nucleus followed by conjugation (Chatfield & Green, unpublished observations).

After a single oral dose (100 mg) of compound I to five subjects the mean 48 h urinary excretion of unchanged I and its metabolites accounted for 33·3% (range 16·0–53·9%) of the dose. Less than 1% of the unchanged compound was excreted, most of the urinary material being present in two isomers of the hydroxylated product II, a and b, corresponding to different but as yet unknown positions of hydroxylation on the adamantane nucleus. The major urinary metabolite, considered as compound IIa, accounted for 30·5% (range 15·2–48·7% of the dose, only small amounts (1·9%, range 0·3–4·3%) of the isomer IIb being recovered. About half the total amounts of metabolites IIa and IIb were excreted as acid-labile conjugates.

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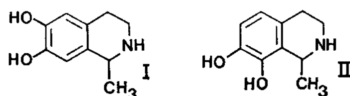
Isosalsolinol formation: a secondary reaction in the Pictet-Spengler condensation

1,2,3,4-Tetrahydroisoquinolines are formed non-enzymatically in the Pictet-Spengler condensation (Whaley & Govindachari, 1958) of a carbonyl compound with a phenethylamine. The condensation is almost certainly the first step in the biosynthesis of this series of alkaloids in plants, and a similar reaction has recently been described in man (Sandler, Bonham Carter & others, 1973). Typically, dopamine and acetaldehyde condense to form 1,2,3,4-tetrahydro-6,7-dihydroxy-1-methylisoquinoline (salsolinol, I) with cyclization occurring *para* to a hydroxyl group which is then at the 6 position of the resulting alkaloid.

Compounds belonging to this series have recently aroused considerable biomedical interest (Lancet, 1973) although three representatives only have so far come under close scrutiny in this context. Salsolinol itself (Heikkila, Cohen & Dembiec, 1971) and its non-methylated analogue, norsalsolinol (Cohen, Mytilineou & Barrett, 1972), are taken up into catecholamine binding sites and may act as false neurochemical transmitters (Greenberg & Cohen, 1973). There has been speculation that tetrahydropapaveroline, the pharmacologically active (Holtz, Stock & Westermann, 1964)



condensation product of dopamine with the aldehyde formed from the oxidative deamination of another molecule of dopamine, is important in alcoholism (Davis & Walsh, 1970) and in the L-dopa treatment of parkinsonism (Sourkes, 1971).

When this condensation occurs at physiological pH, the reaction is driven by the hydroxyl group *para* to the point of condensation. It is, however, theoretically possible for a hydroxyl group *ortho* to the point of condensation to bring about this reaction, and yield a group of compounds with potential pharmacological activity. A variant of this reaction is in fact known; 3-hydroxy-4,5-dimethoxyphenethylamine condenses with carbonyl compounds, resulting in 8-hydroxy-6,7-dimethoxyisoquinolines (Kapadia, Subba Rao & others, 1970). We now wish to describe the condensation of dopamine with acetaldehyde to yield not only salsolinol but 1,2,3,4-tetrahydro-7,8-dihydroxy-1-methylisoquinoline (isosalsolinol, II).

Dopamine was condensed with a slight excess of acetaldehyde at pH 1, 4·5 and 8·5 at room temperature (20°). The first two reaction mixtures were examined after 3 days and the reaction at pH 8·5 after 1 day. The isoquinolines were detected by g.l.c. as their pentafluoropropionyl derivatives (Karoum, Cattabeni & others, 1972; Sandler & others, 1973). At pH 1, salsolinol was detected; the reaction was incomplete but no other product was present. At pH 8·5, a small amount of a second product (<5%) was present, and at pH 4·5 this minor component represented about 10% of the product.

Dopamine hydrochloride (5 g) in 30 ml water was adjusted to pH 4·5 with a little aqueous ammonia and 1·6 ml cold acetaldehyde was added; the mixture was left at room temperature for 3 days. After evaporation to dryness, the product was dissolved in a minimum volume of hot ethanol, and ethyl acetate was added until there was a faint cloudiness. Overnight, 3·1 g of salsolinol hydrochloride separated. The mother liquors were evaporated to dryness and the procedure repeated, to yield 2·1 g of a material which contained about 30% of the "minor component". On recrystallization a mixture of brown granules containing about 50% of the "minor component" and white needles of salsolinol HCl was obtained. About 25 mg of the granules were chromatographed on t.l.c. cellulose plates (8 × 20 cm square, with a slurry depth of 1 mm). The plates were developed in butanol–acetic acid–water (4:1:1). Strips were sprayed with diazotised sulphanilamide and sodium carbonate to detect phenols, and two zones were detected. The faster moving zone was the "minor component" and this was eluted with methanol containing HCl. The eluate was evaporated to dryness and the residue, in aqueous solution, was extracted with ethyl acetate which removed some pigment and then decolourized with charcoal. It was recrystallized twice from methanol by addition of about 4 volumes of ethyl acetate. A flocculent precipitate which formed initially was discarded. The product behaved on g.l.c. in the same way as the "minor component", and contained only a trace of salsolinol. It was tentatively given the structure of isosalsolinol. To confirm this identification, salsolinol and the "minor component" were examined as their pentafluoropropionyl derivatives, by combined gas chromatography - mass spectrometry, using an LKB 9000S instrument. The salsolinol derivative had a Kovats (retention) index (Kovats, 1958) of 1710 at 175° on a 1% OV1 HP Chromosorb W, 2 mm internal diameter, 12′ column, and the derivative of the "minor component" displayed an index of 1665 under the same conditions. The mass spectra of the two derivatives (scanned at the top of the g.l.c. peaks at 70 eV) were almost

indistinguishable. The predominant fragmentation process corresponded to the loss of the 1-methyl group from the molecular ion at *m/e* 617 (20%) to give a base peak at *m/e* 602.*

Infrared spectra of the hydrochlorides of salsolinol and the "minor component" as Nujol mulls indicated that the latter had absorption maxima consistent with the presence of two adjacent hydrogens on the aromatic ring (at 870 and 895 cm^{-1}). The remaining regions of the spectra were similar.

The 100 MHz proton magnetic resonance spectra of the hydrochlorides of salsolinol and the "minor component" in D_2O were obtained, using the tertiary butanol methyl resonance at τ8·70 as an internal lock signal. The pmr spectrum of salsolinol contained two aromatic singlet signals at τ3·20 and τ3·17 with no coupling apparent between the two protons. The spectrum of the "minor component" contained a two-proton AB quartet due to two aromatic proton signals at τ3·21 and τ3·05 with a coupling constant of 8 Hz. Otherwise the spectra were very similar; the methyl doublet occurred at τ8·33 (3H, $J = 7$ Hz) and multiplets were observed at τ6·94 (2H) and τ6·45 (2H), attributable to the aliphatic ring protons. In the spectrum of salsolinol, the methine quartet at τ5·40 (1H, $J = 7$ Hz) was apparent but this signal was obscured by the residual H_2O resonance in the spectrum of the "minor component". The AB quartet observed for the aromatic protons of the "minor component" when compared with the corresponding signal for salsolinol confirmed that the structure was that of isosalsolinol which has two *ortho* hydrogen atoms on the benzene ring.

Thus, under favourable conditions, isoquinolines can be formed from suitably activated phenethylamines, e.g. dopamine, by condensation with carbonyl compounds at C-2 in competition with C-6 of the aromatic nucleus, and this finding indicates that a hydroxyl group can activate its *ortho* position sufficiently for the condensation to occur.

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* These spectra have been submitted to the Mass Spectrometry Data Centre at Aldermaston Berkshire, U.K. Copies are available upon request.