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A note on the identification of *N*-hydroxyphenmetrazine as a metabolic product of phendimetrazine and phenmetrazine

A. H. BECKETT AND M. A. SALAMI

Department of Pharmacy, Chelsea College (University of London), Manresa Road, London, S.W.3., U.K.

Phenmetrazine and the *N*-oxide of phendimetrazine have been demonstrated as metabolites in urine in man after oral phendimetrazine (Beckett & Watson, to be published). Recently, in studies of the *in vitro* metabolism of phenmetrazine and phendimetrazine by liver microsomes of various animal species, we observed a new product in high yields which could be separated from both amines by g.l.c. and t.l.c.; the new metabolic product gave a black spot on t.l.c. when sprayed with ammoniacal silver nitrate. The structure and characteristics of this new metabolic product are now reported.

METHODS

Thin-layer chromatography (t.l.c.) was on glass plates (20 × 20 cm) spread to a thickness of 0·5 mm with a mixture of Silica Gel G (Merck) and water (1:2) and heated for 1 h at 110°. The solvent systems used were: A, benzene–methanol–diethylamine (70:15:15); B, chloroform–acetone–diethylamine (80:5:15). Spray reagents were Dragendorff reagent, ammoniacal silver nitrate solution.

Gas-liquid chromatography (g.l.c.) was carried out using a Perkin-Elmer Model F11 Gas Chromatograph (F.I.D.); Hitachi Perkin-Elmer Model 159 recorder. Columns and conditions: 1 metre $\frac{1}{4}''$ o.d. glass tubing, Gas Chrom Q (100-120 mesh) A/W, DMCS treated, coated with 10% Apiezon L; oven temp. 140°; H_2, 25 lb/in²; air, 20lb/in²; N_2, 70ml/min—Column A. 2 metre $\frac{1}{4}''$ o.d. glass tubing, Gas Chrom W (80–100 mesh) A/W, DMCS treated, coated with 7·5% Carbowax 20 M; oven temp. 155°; H_2, 20 lb/in² ; Air, 20 lb/in², N_2, 80 ml/min—Column B.

Polarography was carried out using a Cambridge Pen-Recording Polarograph Model C. The supporting electrolyte was Na_2SO_3/NaOH solution and polarograms were recorded after deoxygenation with oxygen-free N_2 for 15 min; potentials were recorded with reference to the saturated calomel electrode. The dropping mercury electrode had the following characteristics: h(height of mercury column) = 50 cm; $m^{2/3}\, t^{1/6} = 1{\cdot}70$ mg/s.

All mass spectra were obtained using a Perkin-Elmer model 270 gas chromatograph mass spectrometer system at an electron energy of 70 eV.

Nmr spectra in $CDCl_3$ were recorded using a Perkin-Elmer R-10 nmr spectrometer plus a Northern Scientific 544 CAT with tetramethylsilane as the internal standard.

Infrared spectroscopy was on Nujol mulls using a Unicam SP 200 infrared spectrometer.

Isolation of the metabolic product

After phenmetrazine or phendimetrazine had been incubated separately with 10 000 *g* liver preparations, the incubation mixture was adjusted to pH 10–11 with ammonia solution (10% v/v) and the mixture extracted with freshly distilled diethyl ether. The ethereal extract was concentrated and analysed by g.l.c. and t.l.c.

When the extract was examined by t.l.c., phenmetrazine, phendimetrazine and *N*-hydroxyphenmetrazine were run as reference compounds. Spots were visualized by the spray reagents.

After preparative t.l.c., the band corresponding to the spot later identified as *N*-hydroxyphenmetrazine was scraped off, extracted with diethyl ether and the compound crystallized as the hydrochloride.

RESULTS AND DISCUSSION

The metabolite obtained from t.l.c. and synthetic *N*-hydroxyphenmetrazine gave identical results in all the tests described. The properties of the synthetic compound which allow its separation from mixtures of the metabolite with the precursor drugs and the evidence for its structure are as follows:

(i) The elemental analysis.

(ii) The compound showed different t.l.c. and g.l.c. characteristics from those of the amine i.e. t.l.c. System A, R_F0·76; System B, R_F0·90 (phenmetrazine-R_F0·63 and 0·87 respectively). G.l.c., Column A, T_R (retention time) 9·0 min; Column B, T_R 43 min (phenmetrazine; T_R 5·0 min and 7·3 min respectively).

(iii) The OH absorption at 3,600 cm^{-1} in the infrared and the nmr signal at 6·7τ are consistent with the proposed structure; the absence of the N–H band of the amine indicate, that the compound was not phenmetrazine.

(iv) Upon treatment with $TiCl_3$ and $LiAlH_4$, the compound was reduced *quantitatively* to phenmetrazine.

(v) A cathodic polarographic reduction wave, $E_{\frac{1}{2}}$ of–1·38 V was obtained for the compound, whereas phenmetrazine did not undergo any reduction.

(vi) pKa of the compound was 7·2* whereas pKa of phenmetrazine was 8·45 (Vree, Muskins & van Rossum, 1969).

(vii) In the mass spectrum the highest peak observed had *m/e* value of 176 suggesting an M^+–17 ion from the hydroxylamine, whilst the spectrum of phenmetrazine showed a molecular ion at *m/e* 177.

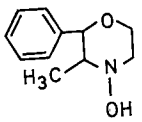
(viii) Extract of the t.l.c. spot sprayed with Dragendoff reagent gave unchanged compound (t.l.c. and g.l.c. evidence.)

(ix) The black spot obtained by spraying with $AgNO_3$ (ammoniacal) constituted a complex mixture (t.l.c. and g.l.c. evidence) in which there was no unchanged *N*-hydroxy compound (contrast the reaction of the corresponding *N*-hydroxyamphetamine and with ammoniacal $AgNO_3$ which give oximes exclusively upon the same treatment (Beckett & Al-Sarraj, 1972).

Stability. *N*-Hydroxyphenmetrazine was stable at pH 1, 7 and 11 in aqueous solution (contrast *N*-hydroxyamphetamine which is relatively stable only at pH7), allowing extraction from biological fluids without decomposition, and it did not decompose upon extraction with ether, chloroform or ethyl acetate (contrast *N*-hydroxyamphetamine which decomposes upon extraction with ethylacetate).

* Determined potentiometrically.

Synthesis of N-*Hydroxyphenmetrazine*



Phenmetrazine (5·0 g, 0·028 mol) in dry acetone (20 ml) was treated with *m*-chloroperbenzoic acid (9·632 g, 0·056 mol) in an ice-bath for 24 h. The excess unreacted acid was decomposed by addition of water gently to the mixture. 5 ml 2 N HCl was added to bring the pH of the solution to 1 and the aqueous layer was washed thoroughly with ether (3 × 10 ml). The pH of the aqueous layer was then carefully adjusted to 9 with 10% ammonia solution and the free base of the *N*-hydroxyphenmetrazine was extracted with ether (5 × 10 ml). The combined ether extracts were dried (K_2CO_3), and removal of ether under reduced pressure gave a yellow solid (3·5 g.), m.p. 89–91°. Recrystallization from methanol gave *N*-hydroxyphenmetrazine as yellow prisms (2·2 g., 44% yield), m.p. 92–93°. Found: C, 67·9; H, 7·6; N, 7·8. $C_{11}H_{15}NO_2$ requires C, 68·4; H, 7·7; N, 7·3%. Infrared: ν_{max} (Nujol) 3600 cm^{-1} (OH); nmr: OH, 6·7; 3–CH_3, 1·3τ. The hydrochloride salt melted at 104–106°.

Reactions of N-*hydroxyphenmetrazine*

(a) *$TiCl_3$ reduction to phenmetrazine.* A mixture of *N*-hydroxyphenmetrazine in water (50 μg/ml, 2 ml), 2 N HCl (1 ml) and 30% w/v $TiCl_3$ (0·2 ml) was added to a glass stoppered centrifuge tube and stored in the dark at room temperature (20°) for various times (5 min–2 h). At the end of each reaction, benzophenone (20 μg/ml, 1 ml) was added as the internal standard, the mixture was adjusted to pH 10–11 (10% ammonia solution) and extracted with freshly distilled ether (3 × 3 ml). The ether extract was concentrated and injected on Column A for quantitative analysis.

(b) *$LiAlH_4$ reduction to phenmetrazine.* A mixture of *N*-hydroxyphenmetrazine in ether (50 μg/ml, 2 ml) and $LiAlH_4$ (0·002 g) was added to a glass-stoppered centrifuge tube and stored at various time intervals (5 min–2 h). At the end of each reaction, distilled water (2 ml) was slowly added followed by benzophenone (20 μg/ml, 1 ml); the mixture was made alkaline and extracted for quantitative analysis as above.

(c) *Stability and extractibility at different pH values.* 100 μg of *N*-hydroxyphenmetrazine in distilled water (2 ml) was introduced into three separate glass-stoppered centrifuge tubes, and the pH of the solutions was adjusted to 1,7 and 11 respectively. Each solution was then extracted with diethyl ether (3 × 3 ml) and benzophenone in ether (20 μg/ml) was added as internal standard to each ethereal extract before concentration and analysis. Then the aqueous layers at pH 1 and 7 were brought to pH 11 (10% v/v ammonia solution), extracted, the internal standard added before concentration and analysis by quantitative g.l.c.

(d) *Interaction with sprays.* When the t.l.c. of *N*-hydroxyphenmetrazine had been carried out, Dragendorff reagent and $AgNO_3$ (ammoniacal) solution were used to spray the plates. The sprayed spots were scraped off, and extracted with diethyl ether (3 × 3 ml); the ethereal extract was concentrated and a t.l.c. and g.l.c. analysis carried out.

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