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The urinary metabolites of 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole

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The urine of mice, rats, dogs, and men treated with 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole has been examined for metabolic products of the drug. So far, a neutral oxadiazole derivative, which has tentatively been assigned the formula of 3-phenyl-5-hydroxyethyl-1,2,4-oxadiazole, diethylamine and small amounts of unchanged drug have been isolated.

PALAZZO and Corsi (1962) showed that 10 min after the intravenous administration of the antitussive drug 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole, none was detectable in the blood while the quantity of the drug eliminated as such from urine was not more than 2% of the dose administered. Since these results indicated the drug to be almost completely metabolised, the identification of the products of its breakdown was attempted using the urine of the mouse, rat, dog and man.

Experimental methods

The experiments were made on 400 CF1 mice, of both sexes and weighing between 18 and 30 g, and on 1200 Long-Evans or CFN rats, of both sexes and weighing from 140 to 500 g. Immediately after the treatment, the animals were placed in metabolism cages and the urine collected for 24 hr in containers in which N hydrochloric acid was provided to prevent the evaporation of diethylamine. During this time food was withheld but the animals were allowed to drink. Four dogs were also used, in two of which a permanent vesical fistula had been made. In these two animals, urine was collected directly by means of a plastic bottle placed on the abdomen at the location of the vesical fistula. The other two dogs were in normal metabolism cages. The drug was also given to 60 persons and their urine was collected for 8 or 12 hr subsequent to the dose.

THE DECOMPOSITION OF THE DRUG *IN VITRO*

The base, which is an oily liquid, was suspended in water and steam distilled for 3 to 4 hr. The alkaline vapours and the oil carried over were collected in 2N hydrochloric acid where the oil was separated and the solution remaining concentrated under reduced pressure to dryness. This residue was crystallised and analysed. The oily fractions were extracted with ether, washed with diluted hydrochloric acid, purified by distillation under reduced pressure and analysed.

DETERMINATION OF DIETHYLAMINE

Sodium hydroxide was added to the urines to give a pH of 10, the solution was then distilled for 1 to 2 hr in a boiling water-bath and the distillate collected in 2N hydrochloric acid. More than 90% of added diethylamine was recovered even in concentrations as low as 10 μg/ml.

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The quantitative determination of the drug in urine was made before and after the distillation to obviate a high result for diethylamine as some of the drug is split during distillation to give the amine. Some urine samples were treated with deactivated carbon, according to Asatoor and Dalgliesh's (1956) method, to eliminate the drug. There is some loss of diethylamine, but this experimental error has been avoided by controls made each time on urine from untreated animals. The acid distillate was then treated with a 5% solution of filtered Reinecke salt. The three times crystallised precipitate from an 80% ethanol gave m.p and mixed m.p with the reineckate, separately prepared, of 259–261°. (Aycok, Eisenbraun & Schrader, 1951, give m.p 259–61°).

To determine diethylamine quantitatively, the acid distillate was evaporated to dryness, dissolved in water and transferred to a flask which was then made up to volume with glacial acetic acid. The substance was assayed according to Morgan (1958), the readings being made at 233 mμ.

The sensitivity of the method was 5 μg/ml of the final solution in acetic acid.

QUANTITATIVE DETERMINATION OF THE NEUTRAL 3-PHENYL-OXADIAZOLE DERIVATIVE

The urine was diluted when necessary with water to about 30 ml, placed in a 100 ml separatory funnel, acidified with hydrochloric acid to a pH of 2–3 and treated with ether (30 ml) the resulting emulsion being broken with ethanol. The ether layer was retained, the extraction repeated twice with ether and the extracts transferred to a 100 ml separatory funnel and washed with a 5% Na_2CO_3 solution (10 ml). The alkaline layer was separated, the ether was washed with water (10 ml) and then slowly evaporated over a water-bath to dryness. The residue was taken up with 95% ethanol (20 ml) and poured into a 100 ml flask which was made up to volume with the ethanol used for washing. The ultra-violet spectra of the urine extracts were compared first zeroing the spectrophotometer with the control set at 238 mμ. In the presence of a 3-phenyl-1,2,4-oxadiazole compound a curve is obtained with two peaks, at 276 and 284 mμ, and with λ_{max} 238 mμ. The quantitative determinations were made assuming that the neutral 3-phenyl-oxadiazole compound was 5-methyl-3-phenyl-1,2,4-oxadiazole, E(1%, 1 cm) 780. The sensitivity of the method is around 10 μg/ml of ethanol solution. 90% of added 5-methyl-3-phenyl-oxadiazole could be recovered.

SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC DETERMINATION OF THE DRUG

The urine of controls and of treated animals was acidified, extracted according to the procedure above, and then adjusted to pH 10 with 2N sodium hydroxide. This solution was shaken with ether (25 ml) in a separatory funnel. Emulsions were broken with ethanol. The ether layer was retained and the extraction repeated with ether (25 ml). The ether extract was evaporated, the residue taken up with 15 ml of 95% ethanol, transferred to a flask and the volume made up with ethanol used for washing. The ultra-violet spectra of the extracts were then run, first

zeroing the instrument with the control at 238 mμ. In the presence of the drug a curve was obtained similar to that of the neutral oxadiazole compound. Quantitative determination was made allowing for the $E(1\%, 1\text{ cm})$ of the drug citrate being 260. A complete separation from the neutral oxadiazole compound was obtained. About 90% of the product added to the urine was detected. The sensitivity was about 10 μg/ml of ethanol solution.

For the chromatographic determination of the drug, urine (1–4 ml) was made alkaline with a saturated solution of potassium carbonate one half the urine volume. The solution was extracted twice with an equal volume and twice with one half the volume of light petroleum (b.p 60–80°). The light petroleum extracts were combined, evaporated under vacuum below 40°, the residue taken up with 0·5 N citric acid (1 ml) and chromatographed using Whatman No. 1 paper, previously washed for 24 hr with the solvent (t-butanol: acetic acid: water, 60:15:25). The front travel was about 40 cm in 15 hr at 18°. The zones were localized with ultra-violet light, cut out together with the corresponding areas of the blank, and then eluted with water (8 ml). The eluate was checked with the spectrophotometer at 239 mμ, zeroing the apparatus with the blank. About 90% of added drug is detectable. The sensitivity approximates to 5 μg/ml of eluate. The chromatographic and the spectrophotometric methods furnished superimposible results, and were used interchangeably.

CHROMATOGRAPHIC DETERMINATION OF *m*- AND *p*-HYDROXY DERIVATIVES

Urine (1–4 ml) was made alkaline with 1% $NaHCO_3$ solution (0·5 ml) and then extracted with ether (5·5 ml and 3 ml). To the extracts was added 0·1% citric acid solution (1 ml) to make the product soluble in water, and the ether was removed by an air stream at 30°. The aqueous solution remaining was slowly evaporated to dryness under vacuum and the residue taken up in water (0·2–0·3 ml). The final pH should be between 4 and 5. The solution was then chromatographed on Whatman No. 1 paper previously washed for 24 hr with t-butanol: 99% formic acid: water (70:15:15). This solvent was used for development; its travel is about 40 cm in 15 hr at 18°. The zones having an R_f corresponding to that of the derivative being examined (0·82) were localised under ultra-violet light, cut out and with the corresponding blank areas were then eluted in water. The eluate was then measured at 243 mμ with the *m*-hydroxy derivative $E(1\%, 1\text{ cm})$ 168 at 243 mμ, and at 260 mμ with the *p*- hydroxy derivative $E(1\%, 1\text{ cm})$ 315 at 260 mμ. 90% of the derivative added to biological liquids can be detected with a sensitivity of about 5 μg/ml of eluate.

Assays for glycuronic, hippuric and benzoic acid were also made using established procedures.

Results

THE BREAKDOWN OF DRUG *IN VITRO*

The oily fraction extracted with ether and purified, had b.p 85° at 0·2 mm, an ultra-violet absorption curve corresponding to that of 3-

phenyl-1,2,4-oxadiazoles with λ_{max} at 232 mμ. Found: C, 69·8; H, 4·8; N, 16·3. Calc. for $C_{10}H_8N_2O$: C, 70·0; H, 4·7; N, 16·3. The quantity of product recovered accounts for all the drug treated. A direct comparison with 3-phenyl-5-vinyl-1,2,4-oxadiazole has furnished the proof of the identity of the two substances.

The basic fraction from distillation has been identified as diethylamine. The quantities obtained tally with the theoretical ones.

IN VIVO BREAKDOWN

In experiments on rats treated with 1000 mg/kg of drug orally the following fractions were isolated from urines:

(i) unchanged drug in amounts from 1 to 5% of the administered dose;

(ii) diethylamine, in amount corresponding to the metabolism of about 10% of the administered drug;

(iii) a neutral residue, with ultra-violet spectrum corresponding to a 3-phenyl-1,2,4-oxadiazole, in amounts corresponding to the metabolism of 1 to 5% of the administered drug;

(iv) hippuric acid, in amounts corresponding to the theoretical metabolism of 5 to 15% of the administered drug;

(v) a glycuronide in quantities corresponding to about 150 mg for every kg of animal weight.

The neutral 3-phenyl-oxadiazole compound is detectable in urines in larger amount after acid hydrolysis, which suggests that it may be eliminated, acetylated or conjugated with glycuronic acid. On the basis of the results obtained from the *in vitro* decomposition of the drug, the neutral oxadiazole derivative might be 5-hydroxyethyl-3-phenyl-oxadiazole, from the addition of H_2O to 3-phenyl-5-vinyl-oxadiazole. In the fractions containing the glycuronide there are large amounts of benzoic acid and this metabolite may be identified as a benzoylglycuronide. No *m* or *p* hydroxy derivatives were found in urines. The urines of a second group of rats treated orally with 500 mg/kg of the drug citrate yielded the same products as those in the preceding experiment in quantities proportional to the administered dose. A third group treated orally with 50 mg/kg furnished similar results again except that diethylamine was absent or present only in slight traces, while the glycuronide was present in quantities corresponding to about 50 mg/kg of animal weight.

In mice given an oral dose of 500 mg/kg of the drug, hippuric acid, a glycuronide, and traces of diethylamine were found in the urine. But neither drug nor the neutral 3-phenyl-oxadiazole residue was found. In the dog given 100 or 200 mg/kg of drug orally, diethylamine appeared in the urine in amounts of about 5 to 12% of theoretical. Elimination was in the first 6 hr after administration. Hippuric acid, conjugated glycuronic acid, and a neutral 3-phenyl-oxadiazole derivative were also observed.

In man treated with single doses of 100 to 300 mg of the drug citrate, 8 and 12 hr urines contained hippuric acid and conjugated glycuronic

acid. Hippuric acid was nearly all eliminated within 4 hr while glycuronic acid was excreted in greater amounts in the subsequent 4 hr. The drug was present in quantities up to 1–2% of the doses. Diethylamine was absent. The amounts of secondary amines found after treatment did not differ from control values.

Discussion

The 5-(2-diethylaminoethyl)-3-phenyl-1,2,4-oxadiazole molecule can be broken down *in vitro* with the formation of diethylamine and 3-phenyl-5-vinyl-1,2,4-oxadiazole. In the urine of treated animals the fractions so far isolated are diethylamine, a neutral 3-phenyl-1,2,4-oxadiazole derivative and small quantitites of unchanged drug.

The presence of diethylamine on one hand and the other fractions on the other suggest that the metabolism of the drug *in vivo* initially resembles that observed *in vitro*, i.e. the rupture of the lateral alkylamino-ethyl chain. In its turn, the 3-phenyl-oxadiazole nucleus seems to be mainly transformed to benzoic acid, which is excreted in the form of hippuric acid or glycuronide.

The amounts of metabolites, allowing for the experimental loss, account for much of the drug administered, so far as the phenyl-oxadiazole moiety is concerned. Diethylamine was found in about 10% of theoretical quantity for high doses and was either found only in traces or not at all, for lower doses.

It is possible that diethylamine can be eliminated in other ways. Few examples exist of studies on the formation of diethylamine during the metabolism of drugs but we believe this is due more to a lack of specific investigations, rather than to a peculiarity of the metabolism of the drug.

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