A NOTE ON THE METABOLISM OF *o*-(2-ETHOXYETHOXY)-BENZAMIDE TO *o*-(CARBAMOYL)PHENOXYACETIC ACID IN MAN

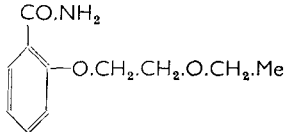
BY A. J. CUMMINGS

From the Nicholas Research Institute Limited, Slough, Bucks.

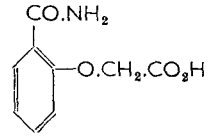
Received August 22, 1962

After the oral administration of *o*-(2-ethoxyethoxy)benzamide to man, the only metabolic product detected in the urine was *o*-carbamoylphenoxyacetic acid.

IN a study of the metabolism of derivatives of salicylamide in man, *o*-(2-ethoxyethoxy)benzamide (I) was investigated. Following the oral administration of this compound, *o*-carbamoylphenoxyacetic acid (II) was isolated from the urine.



I



II

This type of metabolic conversion, involving the rupture of an aliphatic ether linkage, does not appear to have been previously described in man.

EXPERIMENTAL

o-(2-Ethoxyethoxy)benzamide (0·5–1·0 g.) was administered orally in gelatin capsules to two male human subjects and a total urine collection was then made for 12 hr., most of the drug being eliminated within this time. The urine, acidified to approximately pH 2 with concentrated hydrochloric acid, was continuously extracted with ether for 12 hr. The white solid which accumulated in the ether reservoir was collected by filtration. The ether extract was evaporated to dryness under reduced pressure and yielded a pale brown powder. Approximately 0·7 g of solid material, from filtration and after drying, was isolated from the urine of one subject who had received 1 g. of *o*-(2-ethoxyethoxy)benzamide.

The material isolated was dissolved in 30–50 ml. of 0·1M phosphate buffer (pH 7·4) and filtered. The metabolite was precipitated from the filtrate by acidifying with 5N hydrochloric acid, and was recrystallised twice from water after decolorising with activated charcoal. The white crystalline product melted at 210–214°.

The extracted urine was hydrolysed with sulphuric acid (final concentration, 4N) by boiling under a reflux condenser for 3 hr. The hydrolysed urine was then re-extracted with ether as above.

Glucuronic acid and conjugated glycine in urine were determined by the methods of Fishman and others (1951) and of Tompsett (1961) respectively.

Chromatograms of a methanol solution of the residue of ether extracts of the urines were run on Whatman No. 1 paper using as a solvent system, butanol saturated with 1·5N aqueous ammonia solution. The chromatograms were examined in ultra-violet light after drying at room temperature.

RESULTS

The metabolite isolated from the urine was an acid, but no free phenolic group could be detected. The infra-red spectrum indicated the presence of an amido-group and a carboxyl group. Found: C. 54·9, H. 4·6 and N. 6·9 per cent. Equivalent wt. (titration) $\simeq$200. $C_9H_9ON_4$ requires C. 55·3, H. 4·6 and N. 7·2 per cent.

The compound with properties most consistent with the results and which could reasonably be expected to be derived from *o*-(2-ethoxyethoxy)-benzamide was considered to be *o*-carbamoylphenoxyacetic acid: mixed m.p. with an authentic sample ("salicylamide-*O*-acetic acid", Aldrich Chemical Co. Inc., m.p. 213–215°), was 210–214°. The infra-red spectrum of the metabolite was identical with that of the authentic compound.

A precise method for the determination of *o*-carbamoylphenoxyacetic acid in urine was not available, and the proportion of the administered *o*-(2-ethoxyethoxy)benzamide excreted in this form could not be accurately determined. However, some idea was obtained by extracting the urine (acidified to pH 2) with ether, then shaking the ether extracts with 0·1M phosphate (pH 7·4) and measuring the extinction of the buffer at 292 mμ.

The results of an excretion study in one individual indicated that about half the dose of *o*-(2-ethoxyethoxy)benzamide was excreted in 4 hr.

Chromatography of the ether extracts of the original urines, before and after hydrolysis, failed to detect any salicylic acid, salicylamide or gentisic acid. There was no significant increase in the amount of glucuronic acid or conjugated glycine excreted in the urine during the period of maximum excretion of *o*-carbamoylphenoxyacetic acid. No other metabolites or unchanged drug have so far been detected in the urine.

DISCUSSION

The metabolic conversion of *o*-(2-ethoxyethoxy)benzamide to *o*-carbamoylphenoxyacetic acid is of interest since it indicates the presence in man of a mechanism capable of breaking an aliphatic ether linkage. While many examples of the *in vivo* dealkylation of methyl and ethyl aromatic ethers are known (Brodie and Axelrod, 1949; Smith and Williams, 1949; Bray, Craddock and Thorpe, 1955) little has been reported on the metabolic fate of aliphatic ethers. Buckle and Saunders (1949) have obtained some indirect evidence that fluorinated aliphatic ethers of the type $F{\cdot}[CH_2]_m{\cdot}O{\cdot}[CH_2]_n{\cdot}CO_2H$ undergo rupture of the ether linkage *in vivo*. Patterson (1949) has stated that the toxicity results obtained with ω-fluoroalkyl ethers provide circumstantial evidence of the rupture of these ethers in the animal body, and suggested that it is not unreasonable to extend these conclusions to their unfluorinated analogues.

Axelrod (1956) studied the enzymic systems, found in the liver of some mammalian species, which cleave alkyl-aromatic ethers to a phenol and

an aldehyde. Attempts to cleave aliphatic ethers such as 3-methoxypropylamine, methoxyacetic acid and dimethoxyethane with the same systems were unsuccessful.

Duncan and Scales (1961) have shown that 2-(2-methoxyethyl)pyridine is metabolised almost exclusively to pyrid-2-ylacetic acid in five animal species. This metabolic conversion is remarkably similar to the conversion of *o*-(2-ethoxyethoxy)benzamide to *o*-carbamoylphenoxyacetic acid. There is little evidence at present to indicate the metabolic pathway by which this latter conversion is accomplished. It is possible that the first step is a dealkylation, by a mechanism similar to that which cleaves alkyl-aromatic ethers, to yield a primary alcohol and an aldehyde. (It is noteworthy that (ω-1) hydroxylation would give a hemi-acetal, which would be expected to yield readily an alcohol and an aldehyde.) The primary alcohol so formed would then be expected to be rapidly oxidised to the corresponding acid, since it is known that 2-phenylethanol is largely converted to phenaceturic acid (Smith, Smithies and Williams, 1954).

The conversion of *o*-(2-ethoxyethoxy)benzamide to *o*-carbamoylphenoxyacetic acid is also of interest in providing evidence of the *in vivo* stability of the amido-group of salicylamide derivatives in man.

Acknowledgements. I wish to thank Dr. R. W. Temple for the synthesis of the *o*-(ethoxyethoxy)benzamide, Mr. D. Manning for the infra-red spectroscopy and Mr. P. A. P. Peachey for the elemental analyses.

REFERENCES

Axelrod, J. (1956). *Biochem. J.*, **63**, 634–639.
Bray, H. G., Craddock, V. M. and Thorpe, W. V. (1955). *Biochem. J.*, **60**, 225–232.
Brodie, B. B. and Axelrod J. (1949). *J. Pharmacol.*, **97**, 58–67.
Buckle, F. J. and Saunders, R. C. (1949). *J. chem. Soc.*, 2774–2778.
Duncan, W. A. M. and Scales, B. (1961). *Biochem. J.*, **80**, 1 P.
Fishman, W. H., Smith, M., Thompson, D. B., Bonner, C. D., Kasdon, S. C. and Homburger, F. (1951) *J. clin. Invest.*, **30**, 685–696.
Paterson, F. L. M. (1959). *Toxic Aliphatic Fluorine Compounds*, p. 138, Elsevier Monograph.
Smith, J. N., Smithies, R. H. and Williams, R.T. (1954). *Biochem. J.* **56**, 320–324.
Smith, J. N. and Williams, R. T. (1949). *Biochem. J.*, **44**, 239–242.
Tompsett, S. L. (1961). *J. Pharm. Pharmacol.*, **13**, 115–120.