[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES, INDIANAPOLIS 6, INDIANA]

The Metabolism of Benzodioxane Derivatives. II. Ethoxybutamoxane-C^{14}

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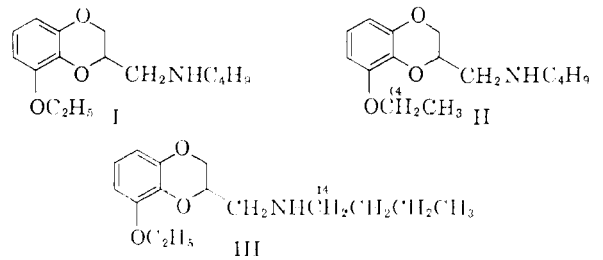
Through the use of radiotracer techniques an interesting species variation in the metabolism of ethoxybutamoxane has been demonstrated. In the dog the main route of metabolic degradation is by oxidation of the butylaminomethyl sidechain to a carboxyl group to yield 2-carboxy-8-ethoxy-1,4-benzodioxane. In the rat, however, the main route is the oxidative cleavage of the ethyl–ether linkage. In addition, experimental evidence is presented which indicates that in man the main pathway is hydroxylation of the aromatic ring.

Ethoxybutamoxane (2-butylaminomethyl-8-ethoxy-1,4-benzodioxane, I) has been shown recently to be a powerful central nervous system depressant.[1,2] Because of the potential usefulness of this compound a study has been made of its metabolic fate in laboratory animals and in man. In order to facilitate isolation and identification of metabolites in animals, radiocarbon labeling was employed. All of the work reported here was carried out on racemic material.[3]

Results and Discussion

In a previous study[4] of the metabolism of butamoxane (2-butylaminomethyl-1,4-benzodioxane), a simple analog of ethoxybutamoxane, it was found that the major route of metabolic change in both the rat and the dog was through ring hydroxylation at the 6- or 7-position. Oxidative degradation of the butylaminomethyl sidechain also occurred but it represented a minor pathway. However, in the case of ethoxybutamoxane, an additional pathway is also possible, *i.e.*, metabolic change could occur through the oxidative cleavage of the 8-ethoxy group. Oxidative cleavage of alkylaryl ethers is a well-known pathway of drug metabolism.[5]

The preparation of two differently labeled forms of I was necessary to allow an adequate study of the metabolism of this compound. One of these was labeled in the α-carbon of the O-ethyl group (II) and the other in the α-carbon of the N-butyl group (III). The preparation of these materials



was straightforward. Details of the syntheses are described in the experimental section.

Initial animal experiments were carried out in rats. The labeled forms of ethoxybutamoxane were administered to the animals and the rate and extent of elimination of radioactivity in respiratory carbon dioxide and in urine was determined. The results are summarized in Table I. In the case of the ethyl-labeled material (II), 55% of the radioactivity was recovered as respiratory radiocarbon dioxide. Thus, the major route of catabolic change which ethoxybutamoxane undergoes in the rat is cleavage of the ether group followed by oxidation of the two carbon fragment to carbon dioxide. In contrast, only 5.7% of the radioactivity appeared as carbon dioxide when the butyl-labeled material was administered. Oxidative degradation of the amino function does then occur but is not an important pathway. In this respect, the metabolism of ethoxybutamoxane is similar to that of the simple analog, butamoxane.[4]

TABLE I

ELIMINATION OF RADIOACTIVE METABOLITES OF ETHOXYBUTAMOXANE-C^{14}

Time (accum.), hr.	% of R.A. dose recovered in respired CO_2			
	Rat[a]		Dog[b]	
	II[c]	III[d]	II[c]	III[d]
0–0.5	14.4	..	..	..
0–1	29.2	2.8	2.2	6.9
0–2	41.5	3.8	5.4	26.1
0–4	47.1	4.4	8.9	37.6
0–8	50.8	4.9	10.7	42.1
0–24	54.9	5.7	..	..
Urine collec., hr.				
0–24	29	57	65	22

[a] Dose level, 10 mg./kg. [b] Dose level, 2.5 mg./kg. [c] Ethyl labeled ethoxybutamoxane. [d] Butyl labeled ethoxybutamoxane.

A study of the radioactive metabolites present in the urine was also made. Paper chromatography of the ether extract of the neutral urine from rats receiving ethyl-labeled material (II) showed that there were two extractable radioactive metabolites. One (R_f = 0.45) representing roughly 10% of the total dose appeared to be simply unchanged ethoxybutamoxane. The second metabolite (R_f = 0.20) accounted for about 5% of the total dose and gave a brilliant rose-colored spot when sprayed with diazotized sulfanilamide, indicating that it was a phenol. Extraction of the acidified urine did not yield significant amounts of radioactivity.

Extracts of the urine from rats receiving butyl-labeled ethoxybutamoxane (III) were found to contain three labeled metabolites with R_f values

(1) (a) J. Mills, R. C. Rathbun and I. H. Slater, Abstracts, Am. Chem. Soc., 132nd Meeting, September, 1957, p. 6-O. (b) J. Mills, M. M. Boren, W. E. Buting, W. N. Cannon, Q. F. Soper and M. J. Martell, *ibid.*, p. 7-O.

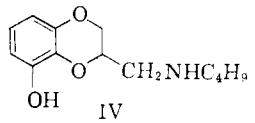
(2) (a) F. G. Henderson, B. L. Martz and I. H. Slater, *J. Pharmacol. Exptl. Therap.*, **122**, 30A (1958). (b) R. C. Rathbun, J. K. Henderson, R. W. Kattau and C. E. Keller, *ibid.*, **122**, 64A (1958). (c) I. H. Slater and G. T. Jones, *ibid.*, **122**, 69A (1958). (d) T. Verhave, J. E. Owen, Jr., D. Fadely and J. R. Clark, *ibid.*, **122**, 78A (1958).

(3) For a preliminary report on the metabolism of (−)-2-(butylaminomethyl)-8-ethoxy-1,4-benzodioxane see R. E. McMahon, J. Welles and H. Lee, ref. 1a, p. 8-O.

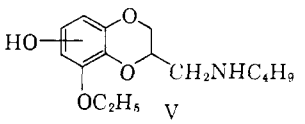
(4) R. E. McMahon, THIS JOURNAL, **81**, 5199 (1959).

(5) *Cf.*, J. Axelrod, *Biochem. J.*, **63**, 634 (1956), for a discussion of mechanism and for leading references.

of 0.45, 0.35 and 0.20. The first of these was, of course, ethoxybutamoxane. The second metabolite (R_f = 0.35) proved to be 2-butylaminomethyl-8-hydroxy-1,4-benzodioxane (IV), the product of



IV



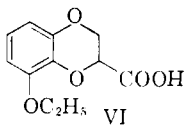
V

ether cleavage. Since IV still contains the butyl group but not the ethyl, it appears as a labeled metabolite only when the butyl-labeled material is administered. It is interesting to note that only about 10% of the dose appears as IV despite the fact that some 55% of the drug is metabolized by ether cleavage. It appears that most of the phenol (IV) which is formed undergoes further metabolic change. Actually there was a large amount of radioactive material in the urine from rats receiving the butyl-labeled drug which was not ether extractable at either acidic or basic *p*H.

The third metabolite (R_f = 0.20) was the same metabolite encountered in the urine from rats receiving ethyl-labeled ethoxy butamoxane. Thus, this phenolic metabolite contains both the ethyl and butyl groups. It is almost certainly the product of ring hydroxylation (V). The question as to the position of hydroxylation cannot be answered now.

When the same studies were carried out in the dog a surprising result was obtained. Ether cleavage was now a minor route (10.7% in 8 hr.) whereas side-chain oxidation proceeded to the extent of 42% in the first 8 hr. It is apparent that the butylaminomethyl sidechain is degraded in such a manner that the butyl grouping is oxidized to carbon dioxide.

The question of the fate of the portion of the molecule containing the benzodioxane nucleus was of particular interest. If oxidative degradation were complete, it seemed reasonable to expect that the ultimate metabolite would be 2-carboxy-8-ethoxybenzodioxane (VI). This acid, if formed, would be detectable as a radioactive metabolite



VI

in urine when the ethyl-labeled material was administered. That VI was indeed a major metabolite of ethoxybutamoxane in dogs was demonstrated by a study of the radioactive metabolites in urine. The ether extract of acidified urine from dogs receiving the ethyl-labeled material (II) was found to contain most of the radioactivity. When this activity was paper chromatographed only one radioactive spot appeared and this corresponded in R_f value to that of an authentic sample of VI prepared by permanganate oxidation of 2-hydroxymethyl-8-ethoxy-1,4-benzodioxane. This finding was confirmed when a sample of VI in pure crystalline form was isolated from dog urine. These materials were identical in melting point, X-ray, defraction pattern, R_f value and spectral properties.

Oxidative cleavage of alkyl amines yields the dealkylated amine and an aldehyde as initial products.[6] It is not possible in the present case to say whether the initial products are butyraldehyde and 2-aminomethyl-8-ethoxybenzodioxane or *n*-butylamine and 8-ethoxybenzodioxane-2-aldehyde, since further oxidation of either pair of products would lead to the observed final products.

The presence of hydroxyethoxybutamoxane (V) in urine from dogs receiving either ethyl-labeled or butyl-labeled ethoxybutamoxane was also demonstrated by paper chromatography. A small amount of IV may have been present but it was not possible to demonstrate this conclusively.

Since the study of the human urinary metabolites of ethoxybutamoxane was done with non-labeled materials the results are of a qualitative nature only. In order to find whether 2-carboxy-8-ethoxybenzodioxane (VI) was among the human metabolites, 1 mg. of the radioacid (VI) (from dog urine) was added as a tracer. The acid was now re-isolated by chromatographic methods. Since the combined weight of all of the radioactive fractions was less than 1 mg., it seems certain VI is not an important metabolite in man.

A paper chromatographic examination of the ether extract made at *p*H 8 showed a very prominent phenolic spot corresponding in color and R_f value with hydroxyethoxybutamoxane (V). This spot is not found in control urine. There were indications that the product of ether cleavage (IV) may have been present but, if so, the quantity was much less than V. Thus the metabolism of ethoxybutamoxane in man proceeds by hydroxylation of the aromatic ring. This may be the main pathway.

Experimental[7]

Materials.—The starting materials for the preparation of labeled compounds and model compounds were supplied to us by Dr. Jack Mills and his associates.[1b] The compounds supplied were: 2-hydroxymethyl-8-ethoxy-1,4-benzodioxane (m.p. 61–63°), 2-aminomethyl-8-ethoxy-1,4-benzodioxane hydrochloride (m.p. 158–159°), 2-butylaminomethyl-8-hydroxy-1,4-benzodioxane hydrochloride (m.p. 274–276°) and N-acetyl-2-butylaminomethyl-8-hydroxy-1,4-benzodioxane hydrochloride (m.p. 93–95°).

2-Butylaminomethyl-8-eth-1-C^{14}-oxy-1,4-benzodioxane Hydrochloride (II).—N-Acetyl-2-butylaminomethyl-8-hydroxy-1,4-benzodioxane (280 mg., 1 mmole), 1.5 g. potassium carbonate and 25 ml. of dry acetone were mixed with 70 mg. (0.45 mmole) of ethyl-1-C^{14} iodide containing 770 μc of radiocarbon. The reaction mixture was refluxed with stirring for 6 hr. under a Dry Ice condenser and then evaporated to dryness. To the residue was added 100 ml. of ether and 50 ml. of water. The ether layer was separated, washed with 1*N* NaOH, water, 1*N* HCl and water. The resulting "hot" amide, recovered by evaporation of the ether, was then refluxed in 90% aqueous alcohol which was saturated with hydrogen chloride. Paper chromatography showed that after 72 hr., hydrolysis was 85% complete.[8] The crude product obtained by evaporation to dryness was dissolved in 1*N* HCl and extracted with ether to remove unchanged amide. Neutralization followed by ether extraction and saturation with hydrogen chloride yielded 98 mg. (73%) of 2-butylaminomethyl-8-eth-1-C^{14}-oxy-1,4-benzodioxane hydrochloride (II), m.p. 196–198°. Paper chro-

(6) B. N. LaDu, L. Gaudette, N. Trousof and B. B. Brodie, *J. Biol. Chem.*, **214**, 748 (1955).

(7) All melting points are corrected.

(8) This amide which was unusually resistant to hydrolysis was found to form a nicely crystalline "hydrochloride," m.p. 95–98° when treated with dry hydrogen chloride in ether. *Anal.* Calcd. for C$_{17}$H$_{26}$O$_4$NCl: Cl, 10.31. Found: Cl, 9.94.

matography showed only one radioactive spot which corresponded with an authentic sample of I.

2-Butyl-1-C^{14}-aminomethyl-8-ethoxy-1,4-benzodioxane Hydrochloride (III).—Sodium butyrate-1-C^{14} (0.5 mmole, 860 μc), 5 ml. of dry benzene, 0.05 ml. of thionyl chloride and one drop of pyridine were stirred at room temperature for 2 hr. One and one-half grams of 2-aminomethyl-8-ethoxy-1,4-benzodioxane (free base) in 5 ml. of benzene was added and the mixture refluxed for 5 hr. After cooling, ether and 1N NaOH were added and the whole transferred to a separatory funnel. The organic layer was separated and washed with additional 1N NaOH, 1N HCl and water. By evaporation to dryness the radioactive amide was recovered as an oil which crystallized upon standing, m.p. 99–101°.

Anal. Calcd. for $C_{15}H_{21}O_4N$: N, 5.02. Found: N, 4.84.

Reduction of the intermediate amide was effected by refluxing 16 hr. with 100 mg. of lithium aluminum hydride in a mixture of 25 ml. of ether and 25 ml. of benzene. After cooling, 1N NaOH (10 ml.) was added and the organic layer recovered by centrifugation. The product was extracted into 1N HCl and after neutralization re-extracted into ether. The crude hydrochloride obtained by saturation of the ether with dry hydrogen chloride was purified by recrystallization from ethyl ether–methanol. The product, 2-butyl-1-C^{14}-aminomethyl-8-ethoxy-1,4-benzodioxane hydrochloride (III), weighed 134 mg. (89%) and melted at 196–198°. This material had the same X-ray pattern as II and an authentic sample of I and was one spot material when paper chromatographed.

2-Carboxy-8-ethoxy-1,4-benzodioxane (VI).—This acid was prepared by the procedure described by Koo[9] for the preparation of the unsubstituted analog. Oxidation of 9.5 g. of 2-hydroxymethyl-8-ethoxy-1,4-benzodioxane yielded 5.9 g. (58%) of 2-carboxy-8-ethoxy-1,4-benzodioxane (VI). The acid after purification by sublimation melted at 126–127°.

Anal. Calcd. for $C_{11}H_{12}O_5$: C, 58.93; H, 5.40. Found: C, 58.72; H, 5.43.

Animal Experiments.—Methods for collecting and counting urine and respiratory carbon dioxide samples from rats[10] and from dogs[4] (chihuahua) have been described. The dose level in rats was 10 mg./kg. and 2.5 mg./kg. in dogs. The dose was administered intraperitoneally in aqueous solution. Each set of rat data in Table I represents the mean of four animals (the standard deviation was less than 5% of observed mean values in most cases). The dog data is based on a single animal.

For the purposes of paper chromatography studies, each urine sample was extracted with ether at neutral pH and again at pH 2. The neutral extracts were chromatographed using the buffered system described earlier.[4,11] The acid extracts were chromatographed on Whatman #1 paper using a 1:1 1-butanol–1.5 N ammonium hydroxide system. Carboxylic acids were visualized by spraying with brom phenol blue. The radioactive spots on chromatograms were located in an automatic scanning device. Phenol spots were found by spraying with diazotized sulfanilamide.[12]

Isolation of 2-Carboxy-8-ethoxy-1,4-benzodioxane (VI) **from Dog Urine.**—A total of 400 mg. of I containing tracer amounts of II was administered to dogs at a dose level of 2.5 mg./kg. (i.p.) and 24-hr. urine collections made. The urine (800 ml.) was then made pH 2 and extracted continuously with diethyl ether to yield after evaporation a black tar (150 mg.) When warmed with benzene (25 ml.) part of the tar dissolved. The benzene solution after cooling was placed on a prepared silica gel (Davidson) column and the column developed with benzene–ethylacetate mixtures. The fractions containing peak radioactivity were combined and sublimed at 90° (0.1 mm.) to yield 15 mg. of white crystals m.p. 124–127°. These were identical in X-ray diffraction pattern, infrared and ultraviolet spectra and R_f value to authentic 2-carboxy-8-ethoxy-1,4-benzodioxane.

Studies on Human Urine.—Morning urine collections from 12 patients receiving 25 mg. of ethoxybutamoxane per day were made. The urine samples were then extracted with ether first at pH 2 and then at pH 8.

Whether or not VI was present in the acid extract could not be settled by paper chromatography so that it was necessary to resort to column chromatography. To the acid extract was added, as a tracer, 1 mg. of radioactive acid VI obtained from dog urine (see above). The acid extract was then chromatographed in the same manner as described above. The peak radioactive fractions were combined and found to weigh slightly less than 1 mg., showing that no VI was present in human urine.

The extracts made at pH 8 were paper chromatographed and sprayed with phenol reagent.

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(9) J. Koo, S. Avakian and G. J. Martin, THIS JOURNAL, **77**, 5373 (1955).

(10) R. E. McMahon, *ibid.*, **80**, 411 (1958).

(11) A. Brossi, O. Hafliger and O. Schnider, *Arzneim. Forsch.*, **5**, 62 (1955).

(12) R. J. Block, R. LeStrange and G. Zweig, "Paper Chromatography," Academic Press, Inc., New York, N. Y., 1952, p. 64.

[CONTRIBUTION OF THE CHEMISTRY RESEARCH DEPARTMENT, U. S. NAVAL ORDNANCE LABORATORY, WHITE OAK, SILVER SPRING, MD.]

Transesterification in Sulfuric Acid[1a]

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2,4,6-Trichlorophenol or its acetate and some exemplary halogen substituted alcohols and their acetates produce good yields of esters by transesterification with negatively substituted methyl esters or the free acids in 100% or fuming sulfuric acid solution. Evidence is presented that the reaction proceeds by cleavage of the starting ester in the sulfuric acid solution followed by establishment of new equilibria which favor the formation of the product ester.

Introduction

Previous studies have shown that simple esters of carboxylic acids in general undergo cleavage in sulfuric acid and the free acids can be recovered by pouring the reaction solutions into water.[2-4] However we have observed that esters such as 2,4,6-trichlorophenyl and 2,2,2-tribromoethyl 4′-nitrobenzoates were only partly cleaved in 100% sulfuric acid and, in an extreme case, 2,4,6-trichlorophenyl 3′,5′-dinitrobenzoate was almost com-

(1) Presented in part before the Organic Division, 131st National A.C.S. Meeting, Miami, Fla., April, 1957.

(1a) Stanford Research Institute, Menlo Park, California.

(2) For a good review of organic bases in sulfuric acid see J. R. Gillespie and J. A. Leisten, *Quart. Revs.*, **8**, 40 (1954).

(3) M. S. Newman, R. A. Craig and A. B. Garrett, THIS JOURNAL, **71**, 869 (1944).

(4) (a) M. S. Newman, H. G. Kuivila and A. B. Garrett, *ibid.*, **67**, 704 (1945); (b) L. P. Kuhn and A. H. Corwin, *ibid.*, **70**, 3370 (1948); (c) A. Bradley and M. E. Hill, *ibid.*, **77**, 1575 (1955).