Anal. Calcd. for $C_{26}H_{30}N_2O_2$: C, 77.58; H, 7.51; N, 6.96. Found: C, 77.77; H, 7.59; N, 6.76.

The carbanilate melted at 171.5–173° after recrystallization from methyl ethyl ketone.

Anal. Calcd. for $C_{26}H_{31}N_3O_2$: C, 74.79; H, 7.48; N, 10.06. Found: C, 74.67; H, 7.37; N, 9.83.

1,1-Bis-(*p*-dimethylaminophenyl)-butanol-2 Dihydrochloride.—The procedure described above when carried out with an equivalent amount of 1,1-bis-(*p*-dimethylaminophenyl)-butanone-2 gave a quantitative yield of the dihydrochloride. After recrystallization from absolute ethanol–ethyl acetate (3:1) the material melted at 215° dec.

Anal. Calcd. for $C_{20}H_{28}N_2O.2HCl$: C, 62.33; H, 7.85; N, 7.27. Found: C, 62.21; H, 8.10; N, 7.29.

KALAMAZOO, MICH.

[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

The Metabolism of 2-(Butylaminomethyl)-1,4-benzodioxane-C^{14}

BY ROBERT E. MCMAHON

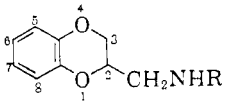
RECEIVED MAY 8, 1959

The metabolism of 2-(butylaminomethyl)-1,4-benzodioxane-C^{14} has been studied in dogs and in rats. Both metabolize the drug by the same pathways. The major route is *via* hydroxylation to 6(or 7)-hydroxy-2-(butylaminomethyl)-1,4-benzodioxane. Oxidative degradation of the butylamino side chain represents a minor route.

Introduction

The adrenolytic activity of the 2-(dialkylaminomethyl)-1,4-benzodioxanes was first observed in 1933 by Fourneau and Bovet.[1] Interest was recently renewed when Mills and co-workers[2] found that the 2-(monoalkylaminomethyl)-1,4-benzodioxanes produced pronounced behavioral changes in experimental animals.

No studies on the metabolic fate of drugs which contain the benzodioxane nucleus have as yet been published.[3] The present study concerns the *in vivo* metabolism of I, 2-(butylaminomethyl)-1,4-benzodioxane, which contains the basic structural features of the active compounds reported by Mills and co-workers.[2]



I, R = *n*-butyl
II, R = *n*-butyl-1-C^{14}

Results and Discussion

Radiocarbon labeling was employed in order to simplify the experimental work. 2-(Butyl-1-C^{14}-aminomethyl)-1,4-benzodioxane (II) was prepared in good yield through the reaction of butyryl-1-C^{14} chloride with 2-aminomethyl-1,4-benzodioxane followed by reduction of the resulting amide with lithium aluminum hydride.

The initial animal studies were performed in the dog (chihuahua). After administration of the radioactive drug by the intraperitoneal route the rate and extent of elimination of radioactivity in respiratory carbon dioxide and in urine was followed (Table I).

About 10% of the radioactivity was recovered as radiocarbon dioxide during the 10 hour collection period. This was an interesting finding since radiocarbon dioxide represents the final product of oxidative degradation of the butylaminomethyl side chain and this route of metabolism has rarely been observed to occur.[4]

TABLE I

ELIMINATION OF RADIOACTIVE METABOLITES OF II

Dose: rat, 10 mg./kg.; dog, 2.5 mg./kg.

Time (accumulated), hr.	% of R.A. dose recovered in— Respired CO_2 Rat	Dog	Urine Rat	Dog
0–1	2.0	3.3	..	..
0–2	2.8	7.0	38.8	..
0–4	3.2	8.7	48.0	..
0–10	3.8	10.5	65.9	42.2
0–24	4.0	..	77.8	58.1
0–48	..	..	..	66.6

The major portion of the radioactivity (66%) was, however, found in the urine collections. After the urine had been hydrolyzed with acid, most of the radioactivity could be extracted into ether at *p*H 8. Paper chromatography of extracted material showed it to contain only one radioactive component, and the fact that it could be readily visualized by spraying with a phenol reagent (diazotized sulfanilamide)[5] suggested strongly that the metabolite was a phenol, *i.e.*, the product of ring hydroxylation, a commonly observed pathway of metabolism of aromatic compounds. Four isomeric hydroxy derivatives of I are possible. Of these, two, the 5- and the 8- have been prepared by Mills and co-workers[2b] who have kindly supplied us with samples of each. The 6- and 7-isomers are unknown. The 8-OH compound had an R_f value of 0.35, while the value for the 5-OH was 0.22. Both gave a rich orange color with the phenol reagent. The metabolite, however, had an R_f value of 0.28 and gave a rose colored spot when sprayed. Thus the major metabolite of I in the dog appeared to be one of the two unknown phenols (III).

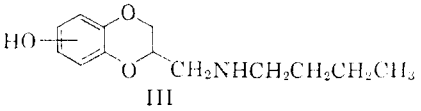
(1) E. Fourneau and D. Bovet, *Arch. internat. pharmacodyn. therap.*, **46**, 178 (1933).

(2) (a) J. Mills, R. C. Rathbun and I. H. Slater, Abstracts, A.C.S., 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, ref. 2a, p. 7-O.

(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. 2a, p. 8-O.

(4) The *in vitro* dealkylation of butylaminoantipyrine has been reported by B. N. La Du, L. Gaudette, N. Trousof and B. B. Brodie, *J. Biol. Chem.*, **214**, 748 (1955).

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



III

Further evidence for the structure of the metabolite came from isolation studies. A small amount of the metabolite was isolated as the N-acetyl derivative from the urine of dogs receiving I. This material, an oil, had an infrared spectrum which was consistent with that expected for the N-acetyl derivative of III. Conclusive evidence for the proposed structure came from ultraviolet absorption data. In Table II comparative ultraviolet data is presented for the N-acetyl derivative of the metabolite, 2 - (butylaminomethyl) - 5 - hydroxy-1,4 - benzodioxane, 2 - (butylaminomethyl) - 8 - hydroxy-1,4-benzodioxane, 5-hydroxy-1,4-benzodioxane and 6-hydroxy-1,4-benzodioxane. It is seen that the spectrum of the metabolite is practically identical to that of 6-hydroxy-1,4-benzodioxane,[6] whereas it differs markedly from that of the others in which the phenolic hydroxyl is *ortho* to the benzodioxane ring. Thus the metabolite is either the 6- or 7-hydroxy isomer (III) or possibly a mixture of the two. No effort was made to distinguish between these possibilities.

TABLE II

ULTRAVIOLET SPECTRA IN ETHANOL

Compound	Neutral λ (mμ)	Neutral ε	0.02 *N* NaOH λ (mμ)	0.02 *N* NaOH ε
Metabolite	~220	Shoulder	240	Shoulder
	290	3455	306	3880
6-Hydroxy-1,4-benzodioxane	~220	Shoulder	240	Shoulder
	291	3700	306	4000
2-(Butylaminomethyl)-5-hydroxy-1,4-benzodioxane	~230	Shoulder	244	8200
	268	658	286	2070
2 (Butylaminomethyl)-8-hydroxy-1,4-benzodioxane	~224	Shoulder	244	7100
	267	422	286	1820
5-Hydroxy-1,4-benzodioxane	~225	Shoulder	244	8900
	268	630	286	2240

The metabolism of II was also studied in male albino rats, and it was found that the general pattern of metabolism closely resembled that found in the dog. Side chain oxidation was found to occur, but only to the extent of about 4%. As in the dog most of the radioactivity appeared in urine. The major urinary metabolite was shown by paper chromatography and by ultraviolet studies to be III. This metabolite appeared to be present mainly as a glucuronide, since incubation with β-glucuronidase greatly increased the amount of III that was extractable. As was true for the dog, there was present in the urine smaller amounts of more polar metabolites which were not ether extractable and were not investigated.

Thus the main pathway of metabolism of the simple benzodioxane derivative I was found to be hydroxylation of the aromatic nucleus. Studies are now underway to determine the effect of ring substitution upon the course of the metabolic reactions.

(6) The ultraviolet spectrum of this system appears to be quite specific as the closely related phenol, 3,4-dimethoxyphenol, shows a maximum at 287 mμ (ε 3430) which shifts to 299 mμ (ε 3590) in alkali.

Experimental[7]

2-(Butyl-1-C^{14}-aminomethyl)-1,4-benzodioxane-Hydrochloride (II).—Finely ground sodium butyrate-1-C^{14} (1.5 mmoles, 1.0 mcurie), 10 ml. of dry benzene, 0.12 ml. of thionyl chloride and one drop of pyridine were placed in a 50-ml. flask fitted with reflux condenser and drying tube. The mixture was stirred magnetically at room temperature for 2 hr., and 1.5 g. of 2-aminomethyl-1,4-benzodioxane[8] in 10 ml. of dry benzene was then added. After 1 hr. of refluxing the reaction mixture was cooled and 15 ml. of 1 *N* NaOH added. Both layers were transferred to a separatory funnel with the aid of ether washes. The organic layer was washed with additional base and with 1 *N* hydrochloric acid. Evaporation of the solvent yielded 350 mg. of 2-(butyryl-1-C^{14}-aminomethyl)-1,4-benzodioxane as a thick oil which crystallized upon standing, m.p. 59–60°.

Anal. Calcd. for $C_{13}H_{17}O_3N$: C, 66.36; H, 7.29; N, 5.95. Found: C, 66.81; H, 7.24; N, 5.70.

The crude amide was reduced in a mixture of 50 ml. of ether and 25 ml. of benzene with 200 mg. of lithium aluminum hydride by refluxing the solution for 2 hr. One normal sodium hydroxide (10 ml.) was added dropwise with stirring, and the organic layer was removed by centrifugation. The product was then extracted into 1 *N* hydrochloric acid from the organic layer. The acid solution was neutralized and the product was extracted again into ether. Saturation of the ether solution with dry hydrogen chloride yielded the product as a crystalline solid which was recrystallized from ethanol–ether. The yield of 2-butyl-1-C^{14}-aminomethyl-1,4-benzodioxane hydrochloride,[9] m.p. 222–223.5°, was 295 mg.

Anal. Calcd. for $C_{13}H_{20}O_2NCl$: N, 5.43; Cl, 13.76. Found: N, 5.19; Cl, 13.64.

5-Hydroxy-1,4-benzodioxane.—This preparation was similar to that reported earlier by Magatti.[10] A solution of 126 g. of pyrogallol and 280 g. of potassium carbonate in 1 l. of acetone was prepared, and to it was added 80 ml. of 1,2-dibromoethane. The reaction mixture was stirred with refluxing for 20 hr. and the acetone then removed under vacuum. The residue was taken up in water, and the solution was saturated with carbon dioxide. The product was extracted into ether and the ether extract washed four times with water. The product weighed 36 g. and had a boiling point of 120–121° (1 mm.) (reported[10] 267°).

Anal. Calcd. for $C_8H_8O_3$: C, 63.15; H, 5.30. Found: C, 63.42; H, 5.21.

The 3,5-dinitrobenzoate prepared by the method of Brewster[11] melted at 112–114°.

Anal. Calcd. for $C_{15}H_{10}O_8N_2$: N, 8.09. Found: N, 8.02.

6-Hydroxy-1,4-benzodioxane.—This phenol was prepared by the diazotization of 27.6 g. of 6-amino-1,4-benzodioxane[12] using the procedure described by Thompson and Symon.[13] The yield of 6-hydroxy-1,4-benzodioxane, b.p. 140–144° (3 mm.) (reported[13] 115–125° (1 mm.)) was 12.5 grams.

Anal. Calcd. for $C_8H_2O_3$: C, 63.15; H, 5.30. Found: C, 63.24; H, 5.86.

The 3,5-dinitrobenzoate ester melted at 181–183°.

Anal. Calcd. for $C_{15}H_{10}O_8N_2$: N, 8.09. Found: N, 8.03.

Animal Experiments.—Methods for collecting and counting radioactive urine and respiratory carbon dioxide samples

(7) All melting points are corrected.

(8) This material was synthesized by Mrs. Wilma McCarthy by the procedure of G. B. Marmi-Bettolo, R. Landi-Vittory and D. Bovet, *Gazz. chim. ital.*, **83**, 144 (1953).

(9) The preparation of 2-(butylaminomethyl)-1,4-benzodioxane as the free base has been reported by D. Bovet and A. Simon, *Arch. internat. pharmacodyn. therap.*, **55**, 15 (1937).

(10) G. Magatti, *Ber.*, **12**, 1860 (1879).

(11) J. H. Brewster and C. J. Ciotti, Jr., THIS JOURNAL, **77**, 6214 (1955).

(12) P. M. Heertjes and L. J. Revallier, *Rec. trav. chim.*, **69**, 262 (1950).

(13) R. B. Thompson and T. Symon, *J. Am. Oil Chem. Soc.*, **33**, 414 (1956).

from rats have been described.[14] An aqueous solution of the radioactive drug was administered by intraperitoneal injection to rats at a dose level of 10 mg./kg. of body weight. The dog experiments were performed on a female chihuahua weighing two kilograms. The collection of urine and respiratory carbon dioxide was done through the use of a specially designed all glass metabolic cage. The dose employed for the dog study was 5.0 mg./dog (i.p.).

Urine samples were hydrolyzed by refluxing 1 hr. after adding 10% by volume of concentrated hydrochloric acid. In the case of the rat urine hydrolysis by incubation with β-glucuronidase was also employed.[14] The hydrolyzed urine samples were first extracted at *p*H 2, and then a second extract was made at *p*H 8. Ether was used as the extraction solvent. The *p*H 8 extracts from the dog urine contained 55–65% of the urinary radioactivity. The figure was 75–85% in the case of the rat. The amount of radioactive material in the acid extracts was negligible and was not investigated further.

The material which was extractable at *p*H 8 was examined by chromatographic separation on Whatman No. 1 paper, which had been buffered by treatment with 0.1 *M* phosphate buffer (*p*H 6.1). The papergrams were developed with *t*-amyl alcohol–*n*-butyl ether–water (80:7:13).[15] The radioactive spots were located in an automatic scanner equipped with a windowless gas flow counter. Phenols were located by spraying with diazotized sulfanilamide.[5]

(14) R. E. McMahon, THIS JOURNAL, **80**, 411 (1958).

(15) A. Brossi, O. Haflinger and O. Schnider, *Arzneim. Forsch.*, **5**, 62 (1955).

Isolation of the Metabolite III from Urine.—Two dogs were given a total of 320 mg. of II by i.p. injection, and the urine was collected for 36 hr. The urine was made 1 *N* with hydrochloric acid and refluxed for 1 hr. An extract of the acidic solution was made and discarded, and the urine was then adjusted to *p*H 8 and extracted with methylene chloride.

The alkaline extract was a dark oil which was acetylated by dissolving in a mixture of 3 ml. of pyridine and 0.5 ml. of acetic anhydride. The diacetate so obtained was heated to boiling with 1 *N* alcoholic NaOH to hydrolyze the ester grouping. The crude N-acetyl derivative of the metabolite was then purified by chromatography on silica gel (Davidson) using benzene–ether mixture for elution. The peak radioactive fractions were combined to give 10 mg. of radioactive metabolite which had an infrared spectrum quite consistent with the proposed structure, *i.e.*, the N-acetyl derivative of III. Other properties of this material are discussed in the text.

The same material was also isolated from the urine of rats which had received a total of 50 mg. of II by intraperitoneal injection. The physical properties (infrared, ultraviolet and paper chromatographic behavior) were identical to those of the material isolated from dogs.

Acknowledgments.—Thanks are due both Dr. Jack Mills and Dr. E. C. Kornfeld for invaluable advice, to Mr. Warren Miller for technical assistance and to Mr. Lee Howard and D. O. Woolf for interpretation of physical data.

INDIANAPOLIS, INDIANA

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF WISCONSIN, AND THE C.S.I.R.O. CHEMICAL RESEARCH LABORATORIES, ORGANIC CHEMISTRY DEPARTMENT, UNIVERSITY OF ADELAIDE, SOUTH AUSTRALIA]

The Alkaloids of *Senecio jacobaea L.* IV. The Structures of Jacobine, Jaconine, Jacoline and their Constituent Acids

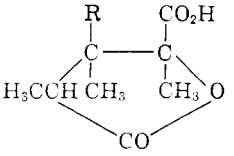
BY R. B. BRADBURY AND SATORU MASAMUNE

RECEIVED MARCH 2, 1959

Degradation of jaconecic acid with lead tetraacetate at 100° yielded carbon dioxide, acetaldehyde, β-methyllevulinic acid and β-methyl-γ-carboxy-γ-valerolactone. Under the same conditions *iso*jaconecic acid gave a ketonic acid $C_9H_{14}O_4$. The lithium aluminum hydride reduction product of dimethyl jaconecate is almost inert to periodic acid, whereas that from dimethyl *iso*jaconecate consumes more than one mole, and liberates formaldehyde. These results, together with the ferric chloride test and active hydrogen determinations establish that *iso*jaconecic acid is an α-hydroxy acid whereas jaconecic acid is not. The dimethyl ester from the acid $C_9H_{14}O_6$ obtained by nitric acid oxidation of jaconecic acid, on reduction with lithium aluminum hydride, consumes two moles of periodic acid and liberated formaldehyde. These, together with previous results, have been reinterpreted in terms of new formula, which are supported by nuclear magnetic resonance data.

A number of different structures have been proposed for jaconecic acid (Ia),[1] (Ib),[2] (II),[3] *iso*jaconecic acid (III)[1b] and the chlorodilactone (IV),[1b] (V).[3,4] These formulas were based on the assumption that, in the chlorodilactone, the presence of an infrared carbonyl band at 1781 cm.$^{-1}$ and the absence of a band between 1750 and 1735 cm.$^{-1}$[5] indicated the presence of two five-membered lactone rings. Relevant data are collected in Table I. However, it is possible for spiro-type δ-lactones to show absorption in the region 1793–1786 cm.$^{-1}$,[7] and this shift can occur where there is ring strain.[5] In addition, since it has hitherto been necessary to postulate methyl group migration[2] to explain the conversion of Ib to β-methyl-γ-carboxy-γ-valerolactone, and since the formulas proposed by Adams[3,4,8] cannot be satisfactorily explained in terms of the experimental data, the

TABLE I

INFRARED CARBONYL FREQUENCIES, CM.$^{-1}$ (CHLOROFORM)		
Chlorodilactone		1781
Jacolinecic dilactone acetate		1780, (1742)
p-Bromophenacyl derivative of β-methyl-γ-carboxy-γ-valerolactone		1779, (1751, 1702)
Monocrotalic acid[6]		
	[α]	
	acid, 5.6°, R = H	1774
	acid, −60.0°	1782
	acid, −5.0°, R = OH	1780

(1) (a) R. B. Bradbury, *Chemistry & Industry*, 1021 (1954); (b) R. B. Bradbury and J. B. Willis, *Aust. J. Chem.*, **9**, 258 (1956).

(2) R. B. Bradbury, *Tetrahedron*, **2**, 363 (1958).

(3) R. Adams, M. Gianturco and B. L. van Duuren, THIS JOURNAL, **78**, 3513 (1956).

(4) R. Adams and M. Gianturco, "Festschrift Prof. Arthur Stoll," Druck von Birkhauser Ag., Basel, Switzerland, 1957, p. 77.

(5) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed., Methuen and Co., Ltd., London, 1958, pp. 179, 185, 186.

(6) R. Adams, P. R. Shafer and B. H. Braun, THIS JOURNAL, **74**, 5612 (1952).

(7) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

(8) R. Adams and M. Gianturco, *Angew. Chem.*, **69**, 1252 (1954).