Biochemical Pharmacology, 1961, Vol. 7, pp. 31–34. Pergamon Press Ltd., Printed in Great Britain.

THE OXIDATION OF N-*p*-BENZOTRIDEUTERIDESULFONYL N′-*n*-BUTYLUREA (TOLBUTAMIDE-D_3) BY MAN

R. U. LEMIEUX*, K. F. SPOREK*, I. O'REILLY† AND E. NELSON†

* Department of Chemistry, University of Ottawa, Ottawa, Ontario and † School of Pharmacy, University of California Medical Center, San Francisco 22, California

(*Received* 27 *October* 1960)

Abstract—The rate of the *in vivo* oxidation of the antidiabetic drug tolbutamide (N-*p*-toluenesulfonyl-N′-*n*-butylurea) to N-*p*-carboxybenzenesulfonyl-N′-*n*-butylurea, as measured by the rate of the excretion of the oxidation product, was essentially unaffected by complete deuteration of the site of oxidation.

THE mechanism of the disposition of this compound involves oxidation of the methyl group of the toluene residue to a carboxyl group. Therefore it was reasonable to expect that, if the breaking of a C—H bond of the methyl group is involved in the rate-controlling step of the biological oxidation, replacement of the hydrogens of the methyl group of deuterium would decrease substantially the rate of the oxidation. In fact, the difference in reactivity could be in the order of from 7 to 9.[1] Also, it could be expected that the deuteration would not affect appreciably the antidiabetic activity of the compound.

When tolbutamide is administered to either normal or diabetic human subjects, the product of the oxidation, N-*p*-carboxybenzenesulfonyl-N′-*n*-butylurea (carboxytolbutamide), may be recovered from the urine in substantial amounts.[2] The oxidation proceeds at a moderate rate *in vivo*. The biological half-life of tolbutamide has been reported to be in the range of 4·7 hr[3] to 7 hr.[4]

The experimental conditions used for the preparation of the tolbutamide-D_3 are described in the experimental section. The nuclear magnetic resonance spectrum of the deuterated tolbutamide indicated that the extent of deuteration was 95 $\pm$ 1 per cent. Also, it was established that the procedures used in preparing the deuterated tolbutamide for ingestion caused no exchange of deuterium for hydrogen.

The following procedure was used for the tests *in vivo*. Five healthy adult human subjects ingested before breakfasting, 0·5-g doses of powdered sodiotolbutamide contained in hard gelatine capsules; food was not taken for 1·5 hr after drug ingestion. Urine specimens were, in most cases, collected 2, 4, 6, 8, 10, 12, 14, 24 and 36 hr after drug ingestion and assayed for carboxytolbutamide.[5] The experiment was repeated about two months later with the same test subjects taking 0·5-g doses of sodiotolbutamide-D_3 and with the collection of an additional specimen of urine after 48 hr.

For four of the five test subjects participating, the data could be treated as resulting from a first-order process. The one test subject (subject E, see Table 1) whose excretion data could not be treated in this manner gave anomalous results in both test series. In view of the established fact that carboxytolbutamide disappears from the body

approximately nine[6] to twelve[7] times faster than does tolbutamide, the rate of excretion of carboxytolbutamide can be taken as a measure of the rate of the *in vivo* oxidation of the tolbutamide. Thus, the slope, k, of the plot,

$$k\,(t_2 - t_1) = 2{\cdot}303 \log \frac{W\infty - W_1}{W\infty - W_2}$$

wherein W_1, W_2 and $W\infty$ are the amounts of carboxytolbutamide excreted after times t_1, t_2 and $t\infty$ (36 hr), respectively, can be used as a measure of the relative rates of oxidation. Since a period of 2 hr after ingestion of the dose was required for absorption of the drug and equilibrium to be established, the value for t_1 was set at 2 hr. The

TABLE 1. RATES OF EXCRETION OF CARBOXYTOLBUTAMIDE

Time (hr)	A*		B*		C*		D*		E*	
	W (mg)	k (hr^{-1})	W (mg)	k (hr^{-1})	W (mg)	k (hr^{-1})	W (mg)	k (hr^{-1})	W (mg)	k (hr^{-1})
A. Tolbutamide										
2	36		29		57		51		8	
4	112	0·116	108	0·116	147	0·139	172	0·199	41	
6	177	0·120	179	0·095	205	0·116	211	0·087	80	
8	220	0·099	239	0·120	246	0·104	239	0·070	116	
10	257	0·104	280	0·100	288	0·135	287	0·150	144	
12	291	0·120	314	0·095	308	0·075	331	0·203	171	
14	316	0·116	346	0·127	330	0·104	347	0·100	201	
24	383	0·111	424	0·119	400	0·134	375	0·050	274	
36	416		458		425		419		355	
Average k (hr^{-1})		0·112		0·110		0·115		0·123		
B. Tolbutamide-D_3										
2	35		98		65		32		26	
4	108	0·091	206	0·150	145	0·123	116	0·127	84	
6	172	0·104	273	0·123	205	0·116	186	0·139	126	
8	220	0·091	331	0·135	248	0·104	237	0·131	174	
10	256	0·083	365	0·123	284	0·108	277	0·135	201	
12	287	0·087	396	0·116	314	0·115	306	0·127	232	
14	313	0·087	421	0·120	339	0·120	325	0·108	260	
24	393	0·089	471	0·076	404	0·122	376	0·102	336	
36	431		498		420		402		370	
48	449		514		431		405		407	
Average k (hr^{-1})		0·090		0·120		0·115		0·124		

* Test subjects.

increasing amount of carboxytolbutamide, W, found in the urine at the times indicated for the four normal patients (A, B, C and D), together with the calculated velocity constants, are presented in Table 1. The half-lives for the *in vivo* oxidations of both the tolbutamide and the tolbutamide-D_3 for the four test subjects were calculated from the data in Table 1. The mean value of these half-lives are 6·0 and 6·2 hr for the oxidation of tolbutamide and the tolbutamide-D_3, respectively. Thus, these values indicate an apparent isotope effect of 1·03. Although apparently normal biolgocal variations are shown in both cases, it is obvious from these results that no significant difference exists in the *in vivo* oxidation rates of the two forms of tolbutamide. The mean half-life obtained in studies to be reported[7] from one of these laboratories (University of California Medical Center) using the same test subjects as in the work reported now but giving tolbutamide as a free acid was 5·2 hr. Using this value for the

mean half-life for oxidation of tolbutamide gives an apparent isotope effect of 1·19. Assuming that tolbutamide-D_3 undergoes the oxidation at the same rate as does tolbutamide and that the total amount of hydrogen in the methyl group which is attached to the benzene ring is present in this form, then the maximum apparent isotope effect may be some 15 per cent greater.

Any attempt to attach theoretical significance to the present results would necessarily be highly speculative, even if the rates determined were known definitely to be directly related to the rate-controlling stage of the biological oxidation. Presumably, this stage would be concerned with the oxidation of the methyl group in either a one- or two-electron process. Nevertheless, the results are not without interest since they do limit the possible mechanisms and can serve as a guide in future investigations related to the oxidation of tolbutamide *in vivo*. It is of interest to contrast the present results to those of Belleau *et al.*[8] They found that deuteration of the carbon *alpha* to the amine in both tyramine and tryptamine caused a two- to three-fold increase in the intensity of adrenergic responses in cats. In *in vitro* experiments, the deuteration decreased the rate of oxidation by a monoamineoxidase from rat liver by a factor of 2·3.

EXPERIMENTAL

p-*Benzotrideuteride sulfonamide*

The directions for the preparation of benzotrideuteride are an adaption of the method reported by Renaud and Leitch.[9] A mixture of acetic anhydride (freshly distilled over magnesium metal) (135 g) and 99·7 per cent deuterium oxide (30 g) was heated at a 100 °C for 1 hr. Benzotrichloride (169 g) in dry ether (200 ml) was added dropwise over a period of about 5 hr to a stirred mixture of the acetic acid-D (183 g), Merck 94 per cent pure zinc dust (183 g), and dry ether (200 ml) kept in an ice–water bath. The reaction temperature was maintained between 3 °C and 5 °C throughout the addition. After the addition was complete, the mixture was stirred at room temperature for 2 hr. Water (200 ml) was added and the ethanol layer was washed twice with water, then with a 1 per cent solution of sodium carbonate, and again with water. After drying over anhydrous sodium sulfate, the ether was removed using an efficient column for fractional distillation. The yield of benzotrideuteride deuterated with the methyl group to the extent of 95 $\pm$ 2 per cent was 60 per cent.

Benzotrideuteride (57·5 g) was heated to boiling and chlorosulfonic acid (80 ml) was added at a rate sufficient to maintain refluxing. After addition was completed the mixture was stirred for 15 min, cooled to 0 °C, and poured into 500 ml of ice–water mixture. The crystalline product was gathered and pressed as free as possible of the oily phase. After drying, the product was recrystallized from petroleum ether, after decolorization of the solution with silica gel. The total yield (61·5 g) of *p*-benzotrideuteridesulfonyl chloride, melting at 63–66 °C, was added, in lots of from 2 to 3 g, to a nearly boiling mixture of concentrated ammonium hydroxide (250 ml) and water (250 ml). After the addition was complete, the mixture was cooled to about 5 °C. The near quantitative yield of crystalline product, *p*-benzotrideuteridesulfonamide, was collected, washed with water and dried *in vacuo* at room temperature.

Tolbutamide-D_3

The above-described precursor, after conversion to the sodium salt by neutralization with sodium methoxide, was condensed with an equimolar amount of

n-butylisocyanate in acetone at from 15 to 20 °C. The tolbutamide-D_3 crystallized on neutralization with acetic acid. After washing with water, the product was dried before recrystallization from ethyl acetate–petroleum ether. *Anal.* Calcd. for $C_{12}H_{15}D_3N_2O_3$ S, 11·7; N, 10·25; equiv. wt. 273·4. Found: S, 11·63; N, 10·21; equiv. wt. 274. Analysis of the compound by nuclear magnetic resonance spectroscopy indicated that 16 ± 2 per cent of the atoms substituted on the carbon of the methyl group were hydrogen. Therefore, the extent of deuteration was 95 ± 2 per cent.

Acknowledgement—The authors wish to thank the Upjohn Company for financial assistance and analytical results on extent of deuteration.

REFERENCES

1. K. B. WIBERG, *Chem. Rev.* **55**, 713 (1955).
2. L. H. LOUIS, S. S. FAJANS, J. W. CONN, W. A. STRUCK, J. B. WRIGHT and J. L. JOHNSON, *J. Amer. Chem. Soc.* **78**, 5701 (1956).
3. R. E. KNAUFF, S. S. FAJANS, E. RAMIREZ and J. W. CONN, *Metabolism* **8**, 606 (1959).
4. K. M. WEST and P. C. JOHNSON, *Metabolism* **8**, 596 (1959).
5. E. NELSON, I. O'REILLY and T. CHULSKI, *Clin. Chim. Acta* **5**, 774 (1960).
6. R. E. KNAUF, S. S. FAJANS, E. RAMIREZ and J. W. CONN, *Ann. N.Y. Acad. Sci.* **74**, 603 (1959).
7. E. NELSON and I. O'REILLY. To be published.
8. B. BELLEAU, J. BURBA, M. PINDELL and J. REIFFENSTEIN, *Science*. In press.
9. R. RENAUD and L. C. LEITCH, *Canad. J. Chem.* **34**, 98 (1956).