(8) J. Borsy, *Arch. Int. Pharmacodyn. Ther.*, **126**, 426(1960).
(9) E. Pàlosi, C. Mészàros, and L. Szporny, *Arzneim.-Forsch.*, **19**, 1882(1969).
(10) F. Fontanini and S. Bossini, *Circ. Drugs, Proc. Int. Symp. 1967*, North-Holland Publishing, Amsterdam, The Netherlands, 1969, p. 215.
(11) G. Razzaboni, L. Setti, and R. Tamiso, *Boll. Soc. Ital. Biol. Sper.*, **44**, 1783(1968).
(12) S. Irwin, Gordon Research Conference on Medicinal Chemistry, New London, N. H., 3/7-8, 133 (1959).

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▲ To whom inquiries should be directed.

COMMUNICATIONS

Sulfamate Sweeteners: A Reappraisal

Keyphrases □ Sulfamates, alicyclic—hydrolysis rate constants determined from polar and steric parameters, equation □ Cyclamates, alicyclic—hydrolysis rate constants determined from polar and steric parameters, equation □ Sweeteners, alicyclic sulfamates—hydrolysis rate constants determined from polar and steric parameters, equation

Sir:

There is evidence from feeding experiments that *in vivo* conversion of the nonnutritive sweeteners, the cyclamates [sodium cyclohexylsulfamate (I) and calcium cyclohexylsulfamate (II)] gives cyclohexylamine (1-4), which is considered to be responsible for the induction of tumors (4). These and other experiments (5-7) resulted in a ban since 1970 on the use of cyclamates in foodstuffs, beverages, and pharmaceuticals. No work appears to have been published on the nature of the metabolic conversion of cyclamate to cyclohexylamine. However, it is possible that the ease of metabolism to primary amine may be correlative with the hydrolytic stability of the sulfamate.

We derived a Taft-Pavelich-type equation (Eq. 1) which correlates the hydrolytic rate constants (measured under identical conditions) for cleavage of the nitrogen-sulfur bond with polar (σ^*) and steric (E_s) parameters for a large number of aromatic, aliphatic, and alicyclic sulfamates (8) and is of the form:

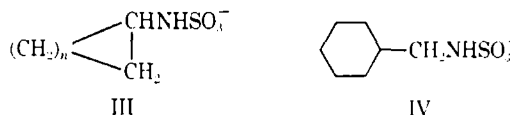
$$\log k = 2.35\Sigma\sigma^* - 1.0037\Sigma E_s + 0.6971 \quad \text{(Eq. 1)}$$

Further tests on Eq. 1 indicate that it reproduces log k values to within an average of 12%. The k values are reproduced to within 26% on average. For Compound I, the k value was reproduced with an accuracy of 5%.

By using Eq. 1, it is possible to predict the hydrolytic stability of unsynthesized sulfamates (whose σ^* and E_s values are known). Equation 1 could thus act as a guide to the choice of sulfamates that might be more resistant to *in vivo* conversion to primary amines than are I and II. Such sulfamates may lack carcinogenic or cocarcinogenic activity because of the possibility of a lower conversion to amine. A second criterion in choosing sulfamates is that they have a comparable degree of sweetness to I and II. Audrieth and Sveda (9) showed that the retention of the sulfamate function, $—NHSO_3^-$, and the presence of a reduced ring (*e.g.*, cyclohexyl) appear to be essential for sweetness in sulfamates. Later results substantiated these findings and extended them to exclude ring substitution in the reduced ring since such compounds tend to be tasteless or to leave a bitter aftertaste (10-12). These criteria for sweetness should be met by Compounds III. In fact, sodium cyclopentylsulfamate (III, n = 3) (10), sodium cycloheptylsulfamate (III, n = 5), and sodium cyclooctylsulfamate (III, n = 6) (11) are extremely sweet.

Equation 1 predicts that the cycloheptyl compound (estimated $\sigma^* = -0.10$, $E_s = -1.10$)[1] will hydrolyze almost 3 times more rapidly than I or II, the cyclopentylsulfamate ($\sigma^* = -0.20$, $E_s = -0.51$) will hydrolyze 2.5 times more slowly, and the unprepared cyclobutyl (III, n = 2) compound (estimated $\sigma^* = -0.28$, $E_s = -0.06$) will hydrolyze 11 times more slowly. Compound IV, N-(α-cyclohexylmethyl)sulfamate ($\sigma^* = -0.06$, $E_s = -0.98$) is predicted to hydrolyze 2.5 times more rapidly than I or II. Cyclopropylsulfamate (III, n = 1) was prepared (10), but its sweetness was not assessed. Polar and steric parameters are not available for this compound or for cyclooctylsulfamate.

These observations suggest that an examination of the percent metabolism to primary amine and the



[1] Polar and steric parameters were taken from K. Wiberg, "Physical Organic Chemistry," Wiley, New York, N. Y., 1966, pp. 414, 415. The σ^* values for the cycloheptylsulfamate and cyclobutylsulfamate were estimated from an examination of the values for cyclohexylsulfamate (−0.15), cyclopentylsulfamate (−0.20), and *tert*-butylsulfamate (−0.3).

carcinogenic or tumor-promoting activity of the cyclobutylsulfamate and cyclopentylsulfamate, and possibly the cyclopropylsulfamate, might be worthwhile.

(1) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.*, **14**, 971 (1965).

(2) J. S. Leahy, M. Wakefield, and T. Taylor, *Food Cosmet. Toxicol.*, **5**, 447(1967).

(3) B. L. Oser, S. Carson, E. E. Vogin, and R. C. Sonders, *Nature*, **220**, 178(1968).

(4) F. J. C. Roe, L. S. Levy, and R. L. Carter, *Food Cosmet. Toxicol.*, **8**, 135(1970).

(5) E. Bajusz, *Nature*, **223**, 406(1969).

(6) G. T. Bryan and E. Erturk, *Science*, **167**, 996(1970).

(7) G. T. Bryan and E. Erturk, *Chem. Eng. News*, **47**, 20(1969).

(8) W. J. Spillane, C. B. Goggin, N. Regan, and F. L. Scott, *Int. J. Sulfur Chem. A*, in press.

(9) L. F. Audrieth and M. Sveda, *J. Org. Chem.*, **9**, 89(1944).

(10) B. Unterhalt and L. Böschemeyer, *Z. Lebensm.-Unters.-Forsch.*, **145**, 93(1971).

(11) F. F. Blicke, H. E. Millson, Jr., and N. J. Doorenbos, *J. Amer. Chem. Soc.*, **76**, 2498(1954).

(12) K. M. Beck, in "Encyclopedia of Chemical Technology," vol. 19, 2nd ed., Wiley, New York, N. Y., 1969, p. 593.

WILLIAM J. SPILLANE
Chemistry Department
University College
Galway, Ireland

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Determination of Trimethoprim and Its N-Oxide Metabolites in Urine of Man, Dog, and Rat by Differential Pulse Polarography

Keyphrases □ Trimethoprim and *N*-oxide metabolites—analysis in urine of man, dog, and rat, differential pulse polarography □ Polarography, differential pulse—analysis, trimethoprim in urine of man, dog, and rat

Sir:

An analytical method was recently reported for the determination of trimethoprim [2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine] in blood and urine of man and dog by differential pulse polarography (1). It was qualitatively noted that a portion of the dose was excreted as the unconjugated N_1-oxide, N_3-oxide, and hydroxy-methyl metabolites of trimethoprim, all of which are resolved by TLC. However, due to low recoveries, the measurement of these metabolites was not previously reported. The purposes of this work were to quantitate the *N*-oxide metabolites and to determine if a species difference existed regarding the formation of the *N*-oxides. Since recovery of the hydroxy-methyl metabolite remained low with this modified assay; its measurement is not reported.

A modification of the above-mentioned assay is reported, which includes a "salting-out" procedure to facilitate the extraction of the *N*-oxides into chloroform. In the modified procedure, 1 ml. urine and 4 ml. distilled water are added to a 50-ml. centrifuge tube to which 6 g. anhydrous potassium carbonate is then added. The method from this point onward is identical to the previously reported one (1), with the exception that polarography is performed in 1 *M* pH 3.0 phosphate buffer rather than in 0.1 *N* H_2SO_4. Five and ten micrograms of trimethoprim and the *N*-oxides added to urine as internal standards should be processed with the unknowns. The choice of pH 3.0 phosphate buffer for the urinary assay is dictated by the need for maximal separation in potential of the two *N*-oxide peaks for qualitative identification. This supporting electrolyte was completely free of background interferences in the region of the polarographic reduction of the *N*-oxides.

Polarography is a useful means of identifying and quantitating the *N*-oxides (Fig. 1). The *N*-oxides of trimethoprim possess two distinct polarographic peaks at −1.095 and −0.950 v. *versus* a saturated calomel electrode (SCE) in 1 *M* pH 3 phosphate buffer due to the reduction of the N_1- and N_3-oxide functional groups, respectively. These peaks are easily distinguished from the reduction peak of the azomethine bond in the pyrimidine ring of all three compounds, which occurs at −1.190 v. *versus* SCE in the pH 3.0 phosphate buffer. Although each *N*-oxide possesses a distinct functional group, the *N*-oxide compounds cannot be determined without prior separation because large quantities of trimethoprim in the urine result in a large analytical peak that masks the *N*-oxide peaks. Thus, TLC separation, in which the compounds are identified by comparison with the R_f values of the authentic compounds

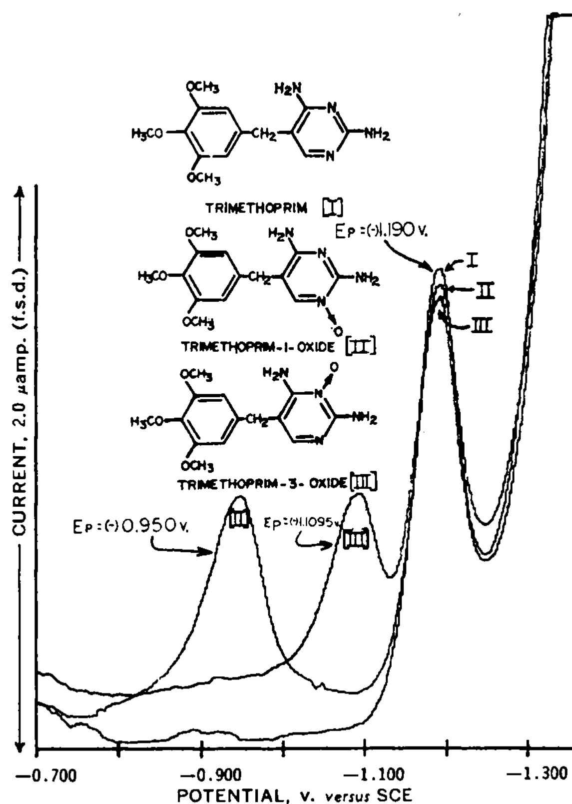

Figure 1—*Polarograms of trimethoprim and its* N*-oxide metabolites in 1* M *phosphate buffer (pH 3).*