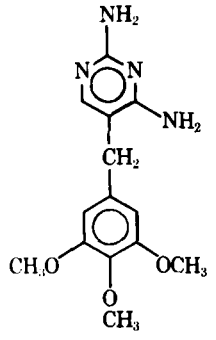
Identification of Trimethoprim 3-Oxide as a New Urinary Metabolite of Trimethoprim in Man

Keyphrases ☐ Trimethoprim 3-oxide—isolation, identification as urinary metabolite of trimethoprim, man ☐ Urinary metabolite of trimethoprim—isolation, identification of trimethoprim 3-oxide, man ☐ TLC—isolation, identification of trimethoprim 3-oxide as new urinary metabolite, man

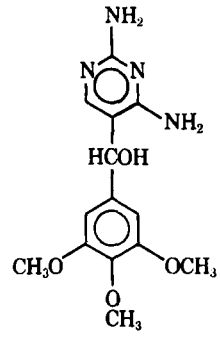
Sir:

Trimethoprim, 2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine (I), is a broad spectrum antibacterial (1) and is used in combination[1] with sulfonamides (2). The metabolism of trimethoprim in the rat, dog, and man was investigated by Schwartz *et al.* (3) using the ^{14}C-labeled drug. The ^{14}C-metabolites were separated on silica gel plates, located by fluorescence quench spectrofluorometry or by autoradiography, scraped from the plate, and eluted, and the radioactivity was measured. The isolation, identification, and quantitation of the same four metabolites were described in all three species. In our recent work on the development of a fluorescence thin-layer assay for trimethoprim and metabolites (4), we reinvestigated the thin-layer system of Schwartz *et al.* (3) and found that one of the TLC spots assigned to Metabolite II is a mixture of two compounds. A new metabolite, which is cochromatographed with II, has been isolated and identified as trimethoprim 3-oxide (III)[2].

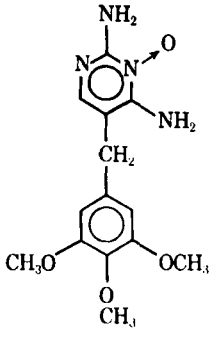
For preparative work, cumulative 24-hr. urines from four adult male volunteers who had received 160 mg. of trimethoprim[3] were combined and adjusted to pH 1.5 with 1 *N* H_2SO_4. The acidified urine was washed with chloroform, and sodium hydrogen phosphate (170 g./l.) was added. The solution was adjusted to pH 8 with 1 *N* NaOH and extracted with isopropanol–dichloromethane (1:9). The extract was evaporated under vacuum to dryness. The residue was triturated with methanol, and the methanol-soluble portion was chromatographed successively on two columns (SilicAR CC-7) by elution with methanol–chloroform and ethanol–benzene mixtures, respectively. Final purification was achieved by preparative TLC on silica gel[4]. Upon further TLC in additional solvent systems, the isolated material appeared as a single spot with the same R_f as synthetic III. A few hours after development and exposure to light, a characteristic yellow-green fluorescence under long wavelength (360 nm.) UV light developed for both the isolated and synthetic III.



I



II



III

Isolated III crystallized from water to afford colorless needles, m.p. 217–219°[2]. The IR, UV, and mass spectra of isolated and synthetic III were identical. The accurate mass of the molecular ion of isolated III was determined by peak matching at 10,000 resolution, 10% valley definition, and found to be 306.1326 (calc. for $C_{14}H_{18}N_4O_4$: 306.1328).

The amount of III excreted in human urine during 24 hr. was measured by assaying samples of urine individually from seven healthy male volunteers who had received 160 mg. of I[3]. An average of 2.1% of the administered dose was present as III. Urine levels were determined by a direct measurement of the fluorescence which developed on silica gel plates[4] for the 3-oxide spot by scanning with a spectrodensitometer[5]. Other pertinent details of the method were reported recently (4) for trimethoprim and four other metabolites and also apply directly to the assay of the 3-oxide (III).

Some revision in the reported metabolic pattern of trimethoprim in man is required. Schwartz *et al.* (3) found that II accounted for about 2.5% of the dose[6]. The present study indicates that the reported levels of Metabolite II included a substantial amount of the 3-oxide. When separated from the 3-oxide, II is barely detectable by our TLC method and is present in human urine in lower concentrations than previously determined. A more detailed report on the metabolic distribution of trimethoprim in man was presented recently (7).

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(2) S. R. M. Bushby, *Postgrad. Med. J., Suppl.*, **45**, 10(1969).

[1] Trimethoprim–sulfamethoxazole (1:5 combination); Septra, Burroughs Wellcome Co. and Bactrim, Hoffmann–La Roche.

[2] We thank Dr. L. A. Nielsen of Burroughs Wellcome Co. for a reference sample of trimethoprim 3-oxide. The sample was prepared according to the procedure of Rey-Bellet and Reiner (5) and had m.p. 216–218° [lit. (5) m.p. 225–227°] with identical spectral properties (UV, NMR, and mass spectra) to those reported (5).

[3] Two tablets of Septra (see *Footnote 1*).

[4] Plates were developed in ethanol–benzene (3:7) twice to 15 cm. followed by one development in chloroform–*n*-propanol–25% ammonium hydroxide (80:20:1). In this system, III has R_f 0.26 and is well separated from the other metabolites.

[5] Schoeffel.

[6] The data of Schwartz *et al.* (3) expressed in "percent urinary concentrations" were converted to a "percent of dose" figure by using their observation that 59% of the dose is present in the urine in 24 hr. as unchanged drug and metabolites (6).

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(7) C. W. Sigel, M. E. Grace, and C. A. Nichol, presented at the Trimethoprim–Sulfonamide Conference, Boston, Mass., Dec. 8, 1972.

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Participation of Micelle at Crystal–Solution Interface in Rate-Determining Step for Cholesterol Gallstone Dissolution in Unsaturated Bile Media

Keyphrases ☐ Cholesterol gallstone dissolution rate in unsaturated bile media—effect of micelle at crystal–solution interface ☐ Gallstone dissolution rate in unsaturated bile media—effect of micelle at crystal–solution interface ☐ Micellar effect—cholesterol gallstone dissolution rate in unsaturated bile media ☐ Bile salt–lecithin micelle—effect on transport of cholesterol in interface, effect on gallstone dissolution rate

Sir:

Recent investigations (1–3) in this laboratory have established that the *in vitro* dissolution of human cholesterol gallstones in bile salt–lecithin media is largely controlled by crystal–solution interfacial kinetics rather than by bulk solution diffusion. Apparent interfacial barriers for dissolution were determined (2, 3) for a variety of cholesterol stones; these were typically 15–20 times greater than bulk diffusion barriers in the *in vitro* experiments (2, 3). Similar dissolution rate experiments with compressed pellets of cholesterol monohydrate crystals have yielded comparable results—*viz.*, interfacial barriers 20 times greater than bulk diffusion in sodium taurocholate–lecithin and sodium cholate–lecithin solutions (2, 3). These results have encouraged the mechanistic exploration of the rate-determining step(s) at the cholesterol monohydrate crystal–solution interface.

This communication reports data showing that the bile salt–lecithin micelle is critically involved in the "activated complex" for the transport of cholesterol in the interfacial region. Table I gives the results of a dissolution rate study showing the relative constancy of the effective interfacial transport coefficient, P, when the bile salt–lecithin concentration is changed while the bile salt–lecithin ratio is kept constant. As can be seen, P remained essentially constant when the solubility, C_s, varied by about a factor of 10. Since it was already demonstrated (2, 3) that the process is interface-kinetics controlled, these data can only be interpreted by some mechanism in which the bile salt–lecithin–cholesterol micelle is involved in the rate-determining step.

Table I—Dissolution Rate[a] of Cholesterol Monohydrate Pellets Showing the Direct Dependence of the Rate, J/A, upon the Solubility, C_s, and the Relative Independence of the Rate upon the Interface Transport Coefficient, P

Sodium Cholate + Lecithin, % (in 0.1 M Phosphate, pH 7.4)	$(J/A)^b 10^4$, mg./cm.²/sec.	C_s[c], mg./cm.³	$D^d \times 10^{6e}$, cm.²/sec.	$P^f \times 10^5$, cm./sec.
1 + 0.5	0.057	0.30	1.46	1.90
2 + 1.0	0.16	1.05	1.49	1.52
2 + 1.0 + 0.07 M NaCl	0.14	1.00	1.49	1.40
ionic strength (correct to 5% cholate)				
5 + 2.5	0.56	3.00	1.30	1.87

[a] Experimental methods for determining J/A, C_s, and D are given in *Reference 3*. [b] $J/A = PC_s$ = dissolution rate (mg./cm.²/sec.). [c] C_s = total solubility of cholesterol monohydrate. [d] D = effective diffusivity of cholesterol in micellar solution. [e] Diffusion coefficient data are presented mainly to show that the size of the micelle is not changing greatly over the concentration range studied. [f] P = interface transport coefficient (cm./sec.).

This finding is exciting for a number of reasons. The recent studies of Surpuriya and Higuchi (4, 5) showed that the oil–water transfer of a variety of sterols in several bile salt–lecithin systems is also interfacial-barrier controlled and that the bile salt–lecithin–cholesterol micelle is involved in the rate-determining step. Thus the basic mechanisms may be very similar for the two situations, and other parallel characteristics are anticipated. For example, Surpuriya[1] showed that the presence of 0.01 M calcium chloride increases P by a factor of 5 in his oil–water system. It would indeed be interesting to see similar effects in the gallstone and/or cholesterol monohydrate dissolution.

There is a significant clinical aspect with regard to the present findings. Recently, Danzinger *et al.* (6) showed that oral administration of chenodeoxycholic acid to patients suffering from gallstone disease can lead to *in vivo* dissolution of gallstones. In four of the seven patients studied, these investigators found that gallstones either disappeared completely or diminished significantly in size during the 14–22 months of chenodeoxycholic acid treatment. In the remaining three patients, no changes in gallstone size could be determined. These clinical studies support the idea that increasing the *in vivo* dissolution rate by a factor of 10 or more should yield material patient benefits in that practical medical treatment times may be approached.

The present findings and the parallel work on the oil–water transport of sterols could be of value in seeking out agents, additives, and other biopharmaceutical factors important to the safe and efficacious treatment of gallstone sufferers.

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[1] Data to be published.