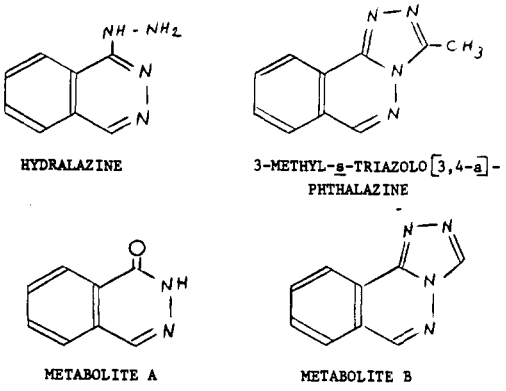
Communications to the Editor

Identification of Two New Metabolites of Hydralazine from Human Urine

Sir:

Hydralazine hydrochloride (Apresoline),† a potent peripherally acting vasodilator, has been used for the treatment of essential hypertension for many years. It has been recognized that the compound undergoes extensive metabolism in man,[1] but the nature of the metabolic products has not been investigated in detail. We wish to report the isolation and identification of two new metabolites of hydralazine from human urine (Chart I).

Chart I. Structures of Hydralazine and Three of Its Metabolites



Hydralazine-^{14}C (100 mg, 80 μCi, labeled in the 1 position) was administered orally in a gelatin capsule to each of four healthy male subjects following an overnight fast. The time course of plasma levels of hydralazine in these subjects will be reported elsewhere.[2] Urine was collected for 72 hr postdose with a recovery of the administered radioactivity in the range of 54–84%.

A 50-ml aliquot of urine was adjusted to pH 9 and extracted with 300 ml of benzene–methanol (93:7). The extract was evaporated to dryness in a stream of nitrogen and the residue dissolved in 2 ml of methanol. The solution was subjected to preparative tlc by streaking on methanol-washed silica gel GF plates (250 μ, Analtech, Inc.). After development in cyclohexane–acetone (1:1), visualization under short-wave ultraviolet light, and radioscanning, two new metabolites of hydralazine were detected.

Each compound was eluted with acetone and rechromatographed in benzene–ethanol–acetic acid (220:10:1). The purity of the two metabolites was ascertained by analytical tlc (spotting on silica gel GF plates) in the above solvent systems as well as in toluene–ethyl acetate (9:10).

An additional spot was found to be indistinguishable from authentic 3-methyl-*s*-triazolo[3,4-*a*]phthalazine by comparison of their tlc behavior. This metabolite has been seen by other investigators in the urine of humans and several animal species.[1,3-5]

The structures of the two new metabolites were deduced from their mass spectra.‡

Metabolite A. The mass spectrum afforded a molecular ion at *m/e* 146, which upon high-resolution analysis furnished a molecular formula of $C_8H_6N_2O$ (obsd, 146.0473; calcd, 146.0480). The molecular ion therefore established the absence of the hydrazine side chain, while fragment ions at $M^+ - CO$ and $M^+ - CHO$ demonstrated the presence of a cyclic carbonyl group. Thus, the mass spectral data suggested the 1(2*H*)-phthalazinone structure. The latter was confirmed by the nmr spectrum which showed an aromatic pattern identical with that reported for 1(2*H*)-phthalazinone.[6]

Metabolite B. High-resolution mass spectral analysis afforded a $C_9H_6N_4$ formula for the molecular ion at *m/e* 170 (obsd, 170.0595; calcd, 170.0592). The only other peak in the spectrum of significant intensity occurred at *m/e* 115. The latter furnished an elemental composition of C_8H_5N (obsd, 115.0414; calcd, 115.0421) corresponding to elimination of HCN and N_2 from the molecular ion. These data indicated that metabolite B was *s*-triazolo[3,4-*a*]phthalazine. The assignment was confirmed by comparing the metabolite with authentic *s*-triazolo[3,4-*a*]phthalazine. Both samples exhibited identical mass spectra and tlc behavior in three solvent systems.

Hydralazine is unstable at pH 9. In order to ascertain that the two new metabolites were not artifacts, extraction was also carried out at pH 6.5. Furthermore, an aliquot of urine was acidified and allowed to react with *p*-methoxybenzaldehyde at 70° to form a derivative of hydralazine[2] before extraction at pH 9. In another control experiment, ethanol was substituted for methanol in the extraction and all subsequent operations. The new metabolites were unequivocally detectable under all of these conditions. Conversely, when human urine from untreated subjects was spiked with hydralazine, extracted, and chromatographed using methanol that had been mixed with 1% of 37% aqueous formaldehyde, *s*-triazolo[3,4-*a*]phthalazine was not detectable. These observations show that 1(2*H*)-phthalazinone and *s*-triazolo[3,4-*a*]phthalazine are true *in vivo* metabolites of hydralazine.

In the present study, 4–14% of the dose was excreted as unchanged hydralazine, shown by a specific spectrophotometric assay based on derivatization with *p*-methoxybenzaldehyde.[2] These findings are at variance with the 54% value reported by Lesser, *et al.*,[7] but agree with an excretion of less than 10% reported by several authors.[1,8-10] The amounts excreted as 1(2*H*)-phthalazinone, *s*-triazolo[3,4-*a*]phthalazine, and 3-methyl-*s*-triazolo[3,4-*a*]phthalazine amounted to 12–18, 6–9, and 6–9%, respectively. Small amounts of other, unidentified products also were noted.

Additional radioactive material, amounting to 8–22% of the dose, became extractable after acidic hydrolysis of the urine. This fraction probably represented glucuronides or other conjugates.

The mechanism of the biotransformation of hydralazine to *s*-triazolo[3,4-*a*]phthalazine has not been clarified to date. A possible mechanism might involve the one-carbon pool of the organism with subsequent oxidation and cyclization. An alternative pathway might arise from the metabolite 3-methyl-*s*-triazolo[3,4-*a*]phthalazine. This compound is formed by the acetylation of hydralazine, a pathway subject to genetic control,[11-14] and subsequent cyclization. Oxidation of the 3-methyl side chain to a carboxy group and decarboxylation could lead to *s*-triazolo[3,4-*a*]phthalazine. Additional work is required to elucidate the exact mechanism.

† Apresoline is a trademark of CIBA-GEIGY Corp.

‡ Mass spectra were obtained with an AEI MS-902 mass spectrometer by means of a direct insertion probe. High-resolution mass spectral data were acquired with the AEI DS-30 data acquisition system. Nmr spectra were obtained with a Varian Model XL-100 spectrometer by time-averaging successive scans of a sample contained in a microcell and using tetramethylsilane as an internal standard.

Acknowledgment. The authors are indebted to Dr. John E. Francis, CIBA-GEIGY Corp., for the synthesis of *s*-triazolo[3,4-*a*]phthalazine and 3-methyl-*s*-triazolo[3,4-*a*]phthalazine.

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Book Reviews

Methods of Neurochemistry. Vol. 5. Edited by Rainer Fried with eight contributors. Marcel Dekker, New York, N. Y. 1973. $19.50.

If it weren't for the excellent review of the state of the art and future challenges of denervation and reinnervation studies to interested neurochemists or neurobiologists generally, volume 5 of "Methods of Neurochemistry" would be difficult to justify acquiring. E. Gutman's succinct presentation, marred only by the occasional lapse of editorial proofing and/or typographical accidents which appear elsewhere in the book as well, offers insights into areas unrelated to neuromuscular transmission specifically but relative generally to problems of molecular biology, ontogenesis and tropism, and relationships between intracellular transport and intercellular communication.

The only continuity evident in this volume is the short chapter by Sampson and coworkers on microtubules and microtubular proteins. Better editorial planning would have put this chapter (as well as the chapter on Culture of Nerve Tissue by J. F. Schneider, which appeared in volume 4) after the one by Guttman.

Although the chapter by Seiden on Behavioral Methods in Pharmacology is an excellent review on operant techniques and experimental design for studying drug-behavior interactions, it falls short of the mark for describing in detail how the disciplines of neurochemistry and behavioral pharmacology could shed more light on the biochemical-cellular mechanism of action by which drugs modify mood and behavior. For this reason, it does not depart significantly from the chapter on Behavior Techniques by Simon and Freedman in volume 4. The same objection can be put forward regarding the chapter by Abdel-Latif on Ion Transport in Synaptosomes in that the chapter by Whittaker and Barker on Synaptosomes in volume 2 of "Methods of Neurochemistry" offers essentially the same methodological approaches.

Lastly and also leastly is the superficial overview of Prenatal Diagnosis of Genetic Disorders Leading to Mental Retardation. In the authors' (Melancon and Nadler) own words, "Hopefully, every laboratory and hospital will not attempt to duplicate facilities already available in trying to monitor 'high risk' pregnancies for rare biochemical disorders, but will send the appropriate material to nearby centers that have gained expertise with the particular disorder." These authors should have chosen one or two disorders and gone into greater details of methodologies and associated problems rather than attempted to cover the entire area in 42 pp. The reader does not feel comfortable with the bits and pieces and would be hard-pressed to even attempt to set up an experimental project for lack of specific information for doing so, which should be within a treatise on methods.

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