Isolation and Identification of a Metabolite of Methyl 1-(Butylcarbamoyl)-2-benzimidazolecarbamate in Rat Urine

Methyl 5-hydroxy-2-benzimidazolecarbamate was found as a major metabolite of methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate in the urine of rats fed a diet containing 2500 p.p.m. of the parent compound for 6 months. Approximately 1200 p.p.m. of the metabolite were found in the urine after enzyme hydrolysis to liberate glucuronide and/or sulfate conjugates.

Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (Figure 1), Du Pont Fungicide 1991, has recently been introduced as a fungicide and mite ovicide candidate (Delp and Klopping, 1968). In connection with mammalian toxicity studies with this compound, preliminary work on its metabolism in rats has been performed.

EXPERIMENTAL

Rat Feeding Method. A group of Charles River-CD male rats were fed a diet of ground Purina Laboratory Chow to which 1% corn oil and 2500 p.p.m. of Fungicide 1991 had been added. After the rats had been on this diet for approximately 6 months, a composite urine sample was taken for analysis. A sample of urine from rats receiving only the chow plus 1% corn oil was taken for comparison.

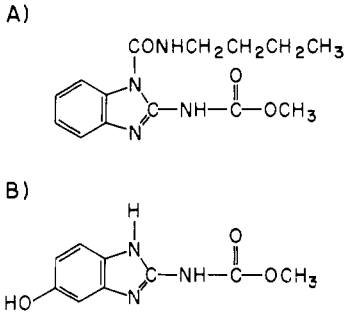

Figure 1. Structural formulas

A. Fungicide 1991
B. Methyl 5-hydroxy-2-benzimidazolecarbamate

Enzymatic Hydrolysis of Rat Urine. Fifty-milliliter aliquots of treated and control urine were adjusted to pH 5.0 by addition of 6*N* HCl, and 2 ml. of β-glucuronidase–aryl sulfatase enzyme solution (Boehringer and Mannheim Corp., New York, N.Y.) were added to each sample. Both samples were incubated for 16 hours at 35° C. to liberate glucuronides and sulfates in the urine.

Extraction Procedure. The hydrolyzed urine samples were diluted to 200 ml. with water and 20 ml. of 6.5*N* NaOH were added to each sample. Samples were extracted three times with 200-ml. portions of ethyl acetate, and the combined ethyl acetate extracts were evaporated to dryness. (Under these conditions, ethyl acetate neutralizes the sodium hydroxide, so that the extraction is accomplished from an aqueous phase with pH between 7 and 9.) Residues (from the ethyl acetate evaporation) were dissolved in 25 ml. of 1*N* NaOH, washed once with 50 ml. of hexane, and extracted three times with 25-ml. portions of ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness and the residues treated with 4 ml. of methanol for thin-layer chromatographic (TLC) analysis. At this point, the extract from control animals was colored but contained no insoluble material, whereas the extract from animals which received methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate contained considerable solid material which did not dissolve completely in the small volume of methanol used. This material would dissolve in additional amounts of methanol.

Identification of Metabolite. The urine extracts were examined on 250-micron silica gel GF TLC plates which

contained an incorporated phosphor. Plates were developed in an ethyl acetate–chloroform–acetic acid mixture (100:100:4 by volume) for 15 cm. When examined under ultraviolet light, only one material was detected in the extract from the treated animal which was not present in the control extract. Its R_f was 0.6, which corresponds to that of synthetically prepared methyl 5-hydroxy-2-benzimidazolecarbamate. TLC plates were also sprayed with $KMnO_4$ solution. No additional materials were detected in the treated urine extract by this more general technique, indicating that no other major metabolites or large amounts of parent compound were present in the urine from treated animals.

The infrared spectra of the isolated metabolite and synthetic methyl 5-hydroxy-2-benzimidazolecarbamate are identical (Figure 2). Both materials give the same mass spectrum with a molecular ion at 207 *m/e*. The solid metabolite in the treated sample described above was recrystallized once from acetonitrile and subjected to elemental analysis for final confirmation (Table I).

Synthesis of Metabolite. The method of synthesis is outlined in Figure 3.

3,4-DINITROPHENOL. This compound was prepared by the nitration of 60 grams of *m*-nitrophenol (Eastman) according to the procedure of Holleman and Wilhemy (1902), with the following exception. After the alcohol-soluble fraction had been recrystallized from benzene, it showed an unsatisfactory melting point; so it was subjected to steam distillation; 700 ml. of distillate was collected. The nonvolatile fraction was collected and recrystallized from benzene (Darco) to give 5 grams of the desired material (m.p. 135–6°; lit. 134°).

METHOXYCARBONYL-*S*-METHYLISOTHIOUREA. The compound was prepared from 3.5 grams of *S*-methylisothiouronium sulfate and 2.5 grams of methyl chloroformate according to procedures described by Loux (1961) (Example 2). The compound, in 50 cc. of water, was not isolated.

METHYL 5-HYDROXY-2-BENZIMIDAZOLECARBAMATE. 3,4-Dinitrophenol (5 grams) was dissolved in a mixture of 100 cc. of ethanol and 5 cc. of acetic acid. Palladium on charcoal (10%) (1 gram), was added and the mixture reduced in a Parr shaker with hydrogen until absorption ceased. The catalyst was removed by filtration and the filtrate was quickly added to the above described solution of methoxycarbonyl-*S*-methylisothiourea. The resulting mixture was boiled under reflux for 1/2 hour and cooled, and the precipitate collected. The solids were dissolved in an excess of 1*N* sodium hydroxide. The solution was treated with Darco, filtered, and then acidified with acetic acid to yield a good grade of crude subject compound. One gram could be purified by recrystallization from 1000 ml. of acetonitrile. The compound shows an indefinite melting point near 300°. Analytical data are shown in Table I.

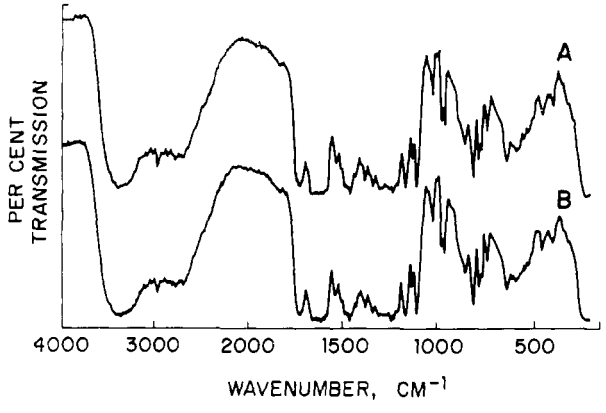

Figure 2. Infrared spectra

A. Isolated metabolite
B. Methyl 5-hydroxy-2-benzimidazolecarbamate

Table I. Results of Elemental Analyses

	Carbon	Hydrogen	Nitrogen
Calculated for $C_9H_9N_3O_3$	52.17	4.38	20.28
Synthetic compound	52.33	4.37	20.12
Isolated metabolite	52.54	4.46	20.76

NMR was used to prove unequivocally that the synthetic material contained a 1,3,4-trisubstituted benzene ring. In the aromatic region, the following values were obtained: τ = 2.78 doublet (J = 8 cps.); τ = 3.16 doublet (J = 2 cps.); τ = 3.45 quartet (J = 2.8 cps.). The solvent used was d_6 DMSO with TMS.

Estimation of Concentration of Metabolite in Rat Urine. Ten-milliliter aliquots of control and treated rat urine were analyzed both with and without the enzyme hydrolysis step to liberate glucuronides and sulfates. In all cases, urine samples were extracted with ethyl acetate as described. The ethyl acetate extracts were concentrated to 100 ml., and the metabolite was extracted into 0.1*N* HCl by partitioning the ethyl acetate twice with 10 ml. of 0.1*N* HCl. The aqueous extracts were made alkaline by adding 2.0 ml. of 6.5*N* NaOH. The metabolite was again extracted into ethyl acetate, which was evaporated to dryness. Residues were redissolved and analyzed by liquid chromatography using equipment and techniques similar to those described by Kirkland (1968).

Recoveries of methyl 5-hydroxy-2-benzimidazolecarbamate added to control urine at the 50- and 500-p.p.m. level were 68 and 75%, respectively. In fresh urine from the treated animals, 40 p.p.m. of metabolite were found. In urine hydrolyzed to liberate glucuronides and sulfates, 1200 p.p.m. of metabolite were found. Both results were calculated assuming an average recovery of 70%. No metabolite was detected in control urine.

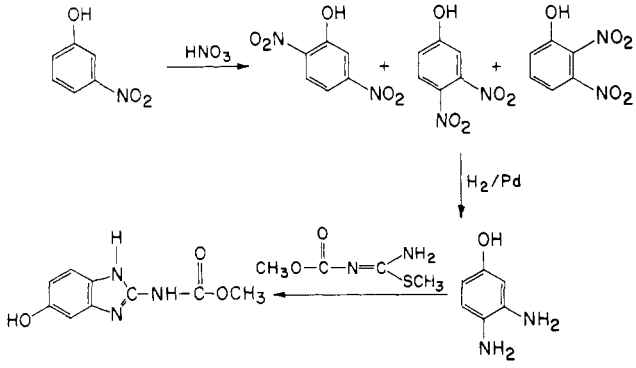

Figure 3. Method of synthesis

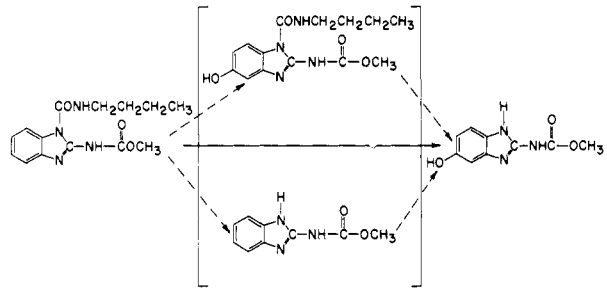

Figure 4. Possible routes of metabolism

DISCUSSION

This study shows that methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate undergoes a metabolic transformation in the rat to produce methyl 5-hydroxy-2-benzimidazolecarbamate, which is excreted in the urine as a conjugate. Since this over-all conversion involves removal of the 1-butylcarbamoyl group as well as hydroxylation, two possible pathways are suggested (Figure 4). However, neither of the possible intermediates was isolated in this study, and conclusions cannot be drawn at this time regarding the order in which these reactions occur. Hydroxylation of the phenyl ring in benzimidazoles has been reported by Tocco *et al.* (1966), who reported that thiabendazole [2-(4′-thiazolyl)benzimidazole] is metabolized in animal systems to the corresponding 5-hydroxy compound, which is eliminated in the urine as glucuronide and sulfate conjugates.

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Correction

BIOLOGICAL ACTIVITY AS AN EFFECT OF STRUCTURAL CHANGES IN ARYL *N*-METHYLCARBAMATES

In this article by R. P. Miskus *et al.* [J. AGR. FOOD. CHEM. **16,** 605 (1968)], in Table III, column 3, compound VIII should have Spruce Budworm Mortality, LD_{50}, μg./G., designated as 15 rather than 75.