

low as 7 g/L, the correlation with the cyanmethemoglobin method is highly significant. The same is true for methemoglobin, when compared with results by the Drabkin/Austin method.

Considering the simplicity of operation of the instrument, the obviation of reagent preparations and calculations, and the range and accuracy of the data generated, the IL 282 Co-oximeter appears to be the instrument of choice for total hemoglobin and percent methemoglobin determinations over a very wide range of concentrations.

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Isolation of 6-Demethylgriseofulvin from Urine

To the Editor:

6-Demethylgriseofulvin (6-DMG) is the principal metabolite of griseofulvin in experimental animals (1, 2) and man (1). Furthermore, in man, the amount of 6-DMG excreted in the urine is proportional to the area under the griseofulvin plasma concentration-time curve. Thus, measurement of this metabolite in urine after the administration of a griseofulvin dosage form can be used to estimate the

bioavailability of the antibiotic from that dosage form (3). During the course of such work in our laboratory, it was necessary to obtain an amount of 6-DMG for use as a standard. Our attempts to isolate this compound according to a published method (2) met with only limited success. In particular, emulsification was severe and the yield disappointing. Moreover, the procedure requires duplicate extractions with each of three solvents.

In a commonly used assay for 6-DMG (3) the extracting solvent consists of equal parts 1,2-dichloroethane and cyclohexane. This solvent provides for a high recovery of urinary 6-DMG for subsequent spectrophotometric analysis. We decided to use this solvent system in our attempts to isolate the metabolite from urine. Griseofulvin, 1.5 g, ("Grisactin," 250-mg capsules; Ayerst Laboratories, New York, NY) was ingested in divided doses during 24-h by a healthy man, who then collected urine for 24 h after the last dose, which was assayed by the above procedure (3).

Extraction of 6-demethylgriseofulvin: Urine was adjusted to pH 3.9 with HCl and extracted once with an equal volume of 1,2-dichloroethane/cyclohexane (1/1 by vol). In preliminary experiments, marked emulsification occurred, which hindered phase separation. This problem was overcome by saturating the urine with sodium chloride (about 0.3 g/mL). The extract was dehydrated with anhydrous magnesium sulfate and filtered. The filtrate was evaporated under reduced pressure at 45 °C to yield a brown crystalline residue, which was taken up in warm acetone (about 20 mg/mL). The addition of 2.5 volumes of distilled water to the acetone solution produced a white precipitate, which was filtered, dried, and recrystallized from ethanol. The precipitate (183 mg) was dissolved in a small volume of warm ethanol in a 10-mL Erlenmeyer flask and evaporated over steam until the first trace of haziness appeared (about 3 mL of ethanol remaining). Crystallization was completed overnight in the refrigerator. After three recrystallizations, 62 mg of white crystals were obtained; this represented 22% of the amount of metabolite initially assayed in the urine. Because 6-DMG is excreted over at least 72 h (4), prolonging the urine collection would result in a greater yield of the product.

The identity of the isolated produce was confirmed by established methods.

Elemental analysis (Galbraith Laboratories, Knoxville, TN) gave the following results:

Found (calculated) for $C_{16}H_{15}O_6Cl$:

C, 56.45% (56.68%); H, 4.52% (4.43%);
O, 28.57% (28.34%); Cl, 10.43% (10.48%)

Others (1, 2, 5) have reported melting points for 6-DMG ranging from 273 to 287 °C, with decomposition. Our product exhibited a melting range of 276 to 277 °C, with decomposition. Previously reported nuclear magnetic resonance spectrum (2), mass spectrum (5), and ultraviolet (6) spectrum compared favorably to the spectra of our isolated material.

We conclude that the procedure we describe offers a simple and convenient means for isolating 6-demethylgriseofulvin from urine.

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