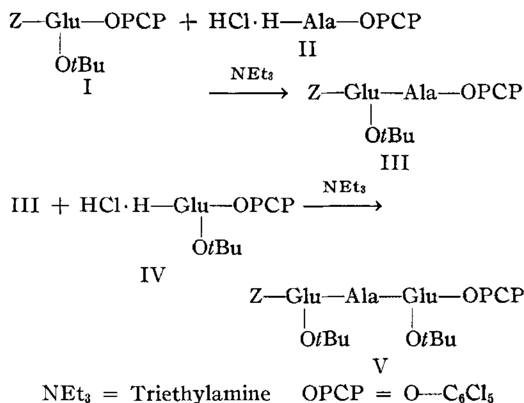

Scheme I

mental analysis identical to those previously reported (3).

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A. KAPOOR

Department of Pharmaceutical Chemistry
College of Pharmacy
St. John's University
Jamaica, NY 11432

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Assay of Chlorpromazine Glucuronides in Human Urine

Sir:

Recently we reported a routine assay for conjugated and unconjugated chlorpromazine metabolites in human urine (1). The group of conjugated chlorpromazine metabolites was assayed by passing a small volume of urine through an ion-exchange resin to remove unconjugated drug metabolites and some contaminating endogenous urinary constituents. The eluate was then made up to 50% sulfuric acid content, and the conjugated drug metabolites were estimated spectroscopically. The procedure was satisfactory for conditions of chronic drug administration of 100 to 1400 mg. chlorpromazine per day. However, studying the excretion of chlorpromazine glucuronides in patients during the initial 4 days of low dosage chlorpromazine therapy (300 mg. or less per day), the method was found lacking in specificity and therefore inaccurate. In a typical determination, *i.e.*, with adequate amounts of chlorpromazine glucuronides, a certain amount of endogenous urinary chromogens can be compensated for by the background cancellation technique. At a starting level of 300 mg. chlorpromazine per day, an adequate urinary level of chlorpromazine glucuronides was reached only after about 1 week. A more selective assay was needed to measure the initial low levels of chlorpromazine glucuronides in the presence of excessive amounts of endogenous urinary chromogens. The following modification of the original procedure was found satisfactory.

Instead of adding 5 ml. of concentrated sulfuric acid to 5 ml. of effluent from the ion-exchange column, as previously specified, it was found practical to add 5 ml. of 50% sulfuric acid solution containing 75 mcg. $FeCl_3 \cdot 6H_2O$ per ml., in a 10-ml. volumetric flask immersed in an ice water bath. After thorough mixing under cooling, the solution was removed from the ice water bath, made up to volume with 25% H_2SO_4, and the absorption spectrum recorded between 400 and 700 mμ, when the mixture had reverted to room temperature. Maximum absorption was read as previously specified, at 550 mμ against a standard calibration curve prepared from 7-methoxychlorpromazine.

Sharper maxima, cleaner purple colors, and lower background readings were obtained by this modification of color development which lowered the over-all acidity from the previously specified 50% to 25%. Maximum color development was completed at 5 min., and colors remained stable for 60 min., the longest period tested.

The modification was therefore adopted for de-

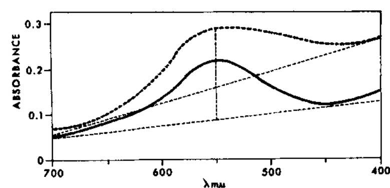

Fig. 1—Comparison of two reagents. Determination of chlorpromazine O-glucuronides in 0.5 ml. urine of a patient dosed with 4.8 mg. chlorpromazine/Kg. per day. Key: - - -, previously reported determination in 50% sulfuric acid medium; ——, proposed modification using 25% sulfuric acid medium in the presence of ferric chloride.

termination of urinary chlorpromazine glucuronides over the entire clinical range, from trace amounts as seen in initial drug therapy, to the substantial daily amounts excreted during chronic drug dosage.

Figure 1 illustrates the determination of chlorpromazine glucuronides in the urine of a patient chronically dosed with 4.8 mg. chlorpromazine per Kg. daily, according to the previously reported procedure (broken line) and the present modification (solid line). Using the background cancellation technique, the proposed modification yields an insignificantly higher reading for absorbance, but a lower background and a sharper maximum. As a rule, differences in absorbance due to the method of determination amounted to less than 5%, and did not exceed ±10% in any specimen examined.

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A. G. Bolt
I. S. Forrest

Biochemical Research Laboratory
Veterans Administration Hospital
Palo Alto, CA 94304

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Proper Identification of the *n*-Alkane from *Arctostaphylos patula*

Sir:

In a recent phytochemical investigation reported in *J. Pharm. Sci.*, an *n*-alkane was isolated from *Arctostaphylos patula* Greene (*Ericaceae*) which was tentatively identified as *n*-nonacosane (1). This identification was made on the basis of solubility characteristics and a sharp melting point of 64° for the isolate. Since this report was published, we have gained additional information on the identity of this subtance, which is the subject of this communication.

In order to obtain an exact molecular weight for the isolate, it was subjected to mass spectral analysis and was found to be a mixture of nine *n*-alkanes.[1] All members of the C_{25}–C_{33} series were present with the odd carbon atom compounds being predominant. This finding is in agreement with recent extensive studies on plant *n*-alkanes reported by others (2–4).

Furthermore, the sample was subjected to a gas chromatographic analysis using a Packard FID instrument fitted with a 4 mm. × 2 M. glass column and packed with 3% SE-30 on Shimalite W (80–100 mesh). Argon (60 ml./min.) was the carrier gas, and a flame ionization detector with a sensitivity setting of 1×10^{-7} was used. The column temperature was maintained at 230° and the injection port was 235°. Estimation of the concentration of *n*-alkanes in the sample was by planimeter (5).

Under these conditions, it was found that the sample separated into nine distinct peaks, each corresponding to the mass spectral data. These peaks were identified as *n*-pentacosane, $C_{25}H_{52}$ (<1%); *n*-hexacosane, $C_{26}H_{54}$ (<1%); *n*-heptacosane, $C_{27}H_{56}$ (3%); *n*-octacosane, $C_{28}H_{58}$ (4%); *n*-nonacosane, $C_{29}H_{60}$ (17%); *n*-triacontane, $C_{30}H_{62}$ (5%); *n*-hentriacontane, $C_{31}H_{64}$ (60%); and *n*-dotriacontane, $C_{32}H_{66}$ (6%); as well as *n*-tritriacontane, $C_{33}H_{68}$ (5%). No peaks representative of *iso*alkanes were observed. An infrared absorption spectrum (KBr) of the original sample was typical for *n*-alkanes, with no evidence of OH or carbonyl absorption.

The qualitative and relative quantitative distribution of *n*-alkanes in the mixture is consistent with data reported for other ericaceous species (3).

Therefore, on the basis of the data reported at this time, the material reported earlier as "*n*-nonacosane" is a mixture containing *n*-hentriacontane (60%) as the major constituent (1).

[1] The authors thank Dr. William Hargrove, Eli Lilly and Co., for the initial mass spectral data used in this study.

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Norman R. Farnsworth
Hildebert Wagner
Ludwig Hörhammer
Hanns-Peter Hörhammer
G. H. Constantine, Jr.*

Institut für Pharmazeutische Arzneimittellehre der Universität München
Munich, Germany
*Department of Pharmacognosy
Oregon State University
Corvallis, OR 97331

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