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Note

Determination of ketamine and some *in vivo* metabolites using high-pressure liquid chromatography

LARRY L. NEEDHAM and MAN M. KOCHHAR

Drug Abuse Screening Laboratory, School of Pharmacy, Auburn University, Auburn, Ala. 36830 (U.S.A.)

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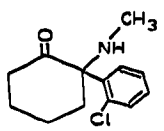
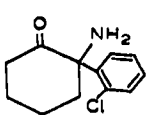
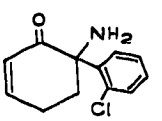
Ketamine, I [2-(*o*-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride], is a parenteral anesthetic agent with unique pharmacologic and physiologic properties[1]. It has a rapid onset of action with rapid recovery and apparent lack of convulsive properties[2,3]. In most animals it is metabolized[1,4,5] to several compounds. Two of the metabolites, 2-(*o*-chlorophenyl)-2-aminocyclohexanone and 6-(*o*-chlorophenyl)-6-aminocyclohex-2,3-en-1-one (II and III, respectively), have been identified. In recent months there have been reports[6] concerning the abuse of this drug as an hallucinogenic agent. Since very few methods of its detection are reported[7,8], it is essential that a method be developed for this purpose. This paper reports a high-pressure liquid chromatographic procedure based on the formation of the *p*-nitrobenzamide derivatives of I, II and III. Furthermore, a confirmatory method by gas–liquid chromatography (GLC) is described.



I



II



III

MATERIALS AND METHODS

Apparatus

Compounds I, II and III were supplied courtesy of Parke-Davis and Company (Detroit, Mich., U.S.A.). The liquid chromatographic analyses were performed on a Waters Ass. liquid chromatograph Model GPC/ALC 202/401 equipped with a differential ultraviolet fixed-wavelength (254 nm) detector and with the U6K universal injector. A 1-ft. × 1/4-in. stainless-steel column packed with C_{18} Microbondapak was used at room temperature in this study; the flow-rate of 1.5 ml of water–acetonitrile (1:1) resulted in a pressure of approximately 2200 p.s.i.

The *p*-nitrobenzamide derivatives were synthesized in 5-ml Regis reaction vials.

The GLC analyses were performed on a Hewlett-Packard Model 5830A gas–liquid chromatograph fitted with a flame ionization detector. A 20-in. × 1/8-in.

stainless-steel column packed with 10% UCW-982 on 80–100 mesh CWAW-DMCS was employed.

Procedure

Male Sprague-Dawley rats were given intraperitoneally 100 mg of ketamine hydrochloride per kilogram. After 6 h, the urine was collected, pooled, and 15 ml of it were made alkaline to pH 10.1 by the addition of ammonium hydroxide–ammonium chloride buffer. The mixture was extracted twice with 15 ml of a chloroform–isopropanol (3:1) solution. The organic layers were combined, dried over anhydrous magnesium sulfate, and concentrated to dryness at 65°. The extract was then dissolved in chloroform and transferred to the reaction vial. The solvent was concentrated to dryness in a hot-water bath. Then 0.2 ml of spectra-grade chloroform, 0.4 ml of 5% sodium hydroxide and 10 mg of *p*-nitrobenzoyl chloride were added. The vial was sealed and shaken at room temperature for 20 min. An aliquot of the organic layer was taken by inserting a syringe through the aqueous layer.

In order to obtain the retention volumes, the *p*-nitrobenzamides of II and III were prepared by using 1–2 mg of the metabolite, adding the reagents and proceeding as outlined above. The *p*-nitrobenzamide of I was prepared and isolated on a gram scale.

Chromatographic operation

The solvent system employed consisted of acetonitrile–distilled water (1:1) at a flow-rate of 1.5 ml/min, which resulted in a pressure of 2200 p.s.i. The column effluent was monitored with a UV detector at 254 nm. Samples of 25 μl were injected through the U6K injector.

For the GLC analyses the injector, column and detector temperatures were 290°, 270°, and 300°, respectively. Helium was used as the carrier gas at a flow-rate of 28 ml/min. The chart speed was 1 cm/min.

RESULTS AND DISCUSSION

Since ketamine is a very poor absorber (in 0.1 *N* sulfuric acid $\lambda_{max.}$ is at 269 nm with an $A^{1\%}_{1cm}$ of 23.2)[9], it is necessary, in order to detect at low concentration levels by UV, to attach a chromophore. The *p*-nitrobenzamides of I, II and III are synthesized easily and quickly and the by-products and excess of reagents do not interfere with the chromatogram. The retention volumes for the *p*-nitrobenzamides of I, II and III were found to be 10.00, 11.90 and 6.67 ml, respectively. Having established these retention volumes, 6-h urine samples from five treated rats were collected and pooled. A 15-ml aliquot of the urine was extracted and concentrated. The extract was derivatized and diluted by taking a 25-μl aliquot of the chloroform solution and adding 2 ml of tetrahydrofuran. The peaks corresponding to ketamine and its metabolites are present (Fig. 1). The large peak at 14.70 ml (10 min) has not been identified, and it is not present when a similar procedure is followed on the urine of a drug-free rat.

One of the advantages of HPLC is that the sample is not degraded. Therefore the eluate can be collected and the presence of a substance can be confirmed by another method. For this purpose, retention times on GLC were established for the *p*-nitrobenzamides of ketamine and its metabolites. The retention times for I, II and III were 5.62, 4.23 and 4.92 min, respectively.

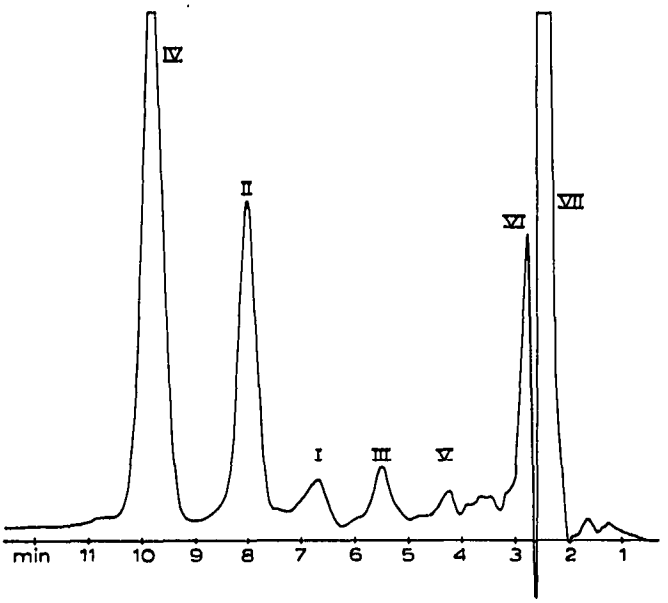


Fig. 1. The liquid chromatogram at 0.16 absorbance units at full scale of the *p*-nitrobenzamides of ketamine and its metabolites. I, II and III are compounds listed in text; IV, V = unknown peaks from sample; VI = excess *p*-nitrobenzoyl chloride; VII = peak due to a tetrahydrofuran constituent.

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