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Note

Identification of carbamate derivatives formed during chloroform extraction of tricyclic antidepressants from urine

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During the isolation and identification procedure of octriptyline metabolites from monkey urine, two metabolites were subsequently identified as the methoxy and ethoxy carbamate derivatives. As unknown metabolites they were chromatographed in a series of thin-layer chromatographic (TLC) systems and then analyzed by gas chromatography–mass spectrometry (GC–MS) using the GC conditions given in Table I. The mass spectrum of the methoxy carbamate had a base peak at m/z 102, characteristic peaks at m/z 244, 231, 216, 117 and 44, and a small molecular ion at m/z 333. The ethoxy carbamate spectrum had a base peak at m/z 116, characteristic peaks at m/z 244, 231, 216, 102 and 44, and a molecular ion at m/z 347.

TABLE I

RETENTION TIME OF METHOXY AND ETHOXY CARBAMATE DERIVATIVES OF OCTRIPTYLINE

Sample	*GC conditions*	*Retention time (min)*
Methoxy carbamate	1.83 m × 2 mm I.D. 1.5% OV-17 column programmed from 180 to 285°C at 7°C/min	11.6
Ethoxy carbamate	1.83 m × 2 mm I.D. 1.5% OV-17 column programmed from 190 to 250°C at 5°C/min	13.7

The methoxy and ethoxy carbamate derivatives of octriptyline were synthesized and their mass spectra, GC retention times, characteristic proton nuclear magnetic resonance (^{1}H NMR) peaks, and carbonyl infrared (IR) absorption bands were all identical to those of the isolated derivative.

Sheehan and Haythorn[1] reported that nortriptyline and desipramine were chemically altered under various urinary extraction conditions. An ion at m/z 116 was found to be the base peak and second largest in the mass spectra of nortriptyline and

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desipramide derivatives, respectively. This appeared very similar to the ethoxy carbamate which we had isolated. Our compound originated from a secondary N-alkyimethylamine. The only similarity in our procedures and that of Sheehan and Haythorn was the chloroform used to extract urine. We postulated that phosgene in chloroform was responsible for forming the carbamate derivatives. To test this, both octriptyline and nortriptyline were extracted from aqueous solutions using the procedure of Sheehan and Haythorn. Each was extracted with chloroform containing ethanol preservative and with chloroform not containing ethanol preservative. Subsequent TLC (ethyl acetate–methanol–ammonium hydroxide, 17:2:1) showed ultraviolet light sensitive spots having the same R_F values as authentic nortriptyline and octriptyline. However, the extracts using chloroform without ethanol preservative had additional ultraviolet light sensitive spots near the solvent front. The silica gel from one of these zones was extracted with methanol and a GC–MS profile obtained. The methoxy carbamate derivatives of both octriptyline and nortriptyline were identified.

Our studies and those of Sheehan and Haythorn show that derivatives of tricyclic antidepressants can be formed under various extraction procedures. Our studies further suggest that the derivatives are carbamates formed during chloroform extraction from phosgene which can be present in chloroform if the ethanol preservative is missing. When possible, chloroform extraction of tricyclic antidepressants should be avoided.

MATERIALS AND METHODS

The derivatives were isolated as part of an identification procedure for metabolites in monkey urine[2]. The monkeys had been orally dosed with an aqueous solution of [^{14}C]octriptyline (specific activity 19.4 μCi/mg). The ethoxy carbamate was isolated from a chloroform extraction of urine at pH 5. The urine had been treated previously with glucuronidase/sulfatase (Calbiochem, Los Angeles, CA, U.S.A.) at pH 5 and 37°C for 24 h. The chloroform extract was evaporated to dryness and the extract residue was placed on a 5-g silica gel column in chloroform. The ethoxy carbamate was eluted from the column with chloroform.

The methoxy carbamate was isolated from other monkey urine which also was treated previously with glucuronidase/sulfatase at pH 5. This mixture was placed on an Amberlite XAD-2 column, and then eluted with methanol. The methanol extract was evaporated to dryness and chromatographed on a silica gel column. The methoxy carbamate was eluted with methanol–chloroform (5:95).

Thin-layer chromatography was on silica gel (250 μm, HF; Woelm, Eschwege, G.F.R.) plates. The plates were then scanned for the presence of radioactivity zones (Actigraph III, Searle Analytic), which were scraped and the silica gel eluted with methanol.

GC (Searle Analytic) analysis was performed on a 1.83 m × 2 mm I.D. 1.5% OV-17 column which was temperature programmed.

GC–MS analyses (Kratos-AEI MS-30 mass spectrometer) were performed at 20 eV with a source temperature of 180°C and molecular separator temperature of 206°C.

The nuclear magnetic resonance spectra were determined in [$^2H_4$]methanol

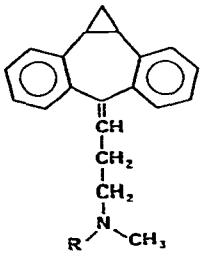

Fig. 1. Structures of octriptyline and ethoxy and methoxy carbamate derivatives. R = H: octriptyline (Ia); R = $\overset{O}{\overset{\|}{C}}$–O–$CH_2$–$CH_3$ ethoxy carbamate (IIa, *syn*; IIb, *anti*); R = $\overset{O}{\overset{\|}{C}}$–O–$CH_3$ methoxy carbamate (IIIa, *syn*; IIIb, *anti*). *Syn* conformer has cyclopropane and *exo*-propylidine amino group on same side of 7-membered ring. *Anti* conformer has them on opposite sides.

using a Varian XL-100-15 instrument, and the infrared absorption spectra were determined in chloroform solution using a Beckman IR-12.

SYNTHESIS

The synthesis of ethyl[3-(1a,10b-dihydrodibenzo[*a*,*e*]cyclopropa[*c*]cycloheptan-6(1H)-ylidene)propyl]ethylcarbamate (II, Fig. 1) is as follows. To a solution of octriptyline (free base, 3.0 g, 9.3 mmole; I) in 50 ml of chloroform and 1.4 ml of triethylamine was added ethylchloroformate (1.14 g, 10.5 mmole). After 15 min, the solution was washed successively with water, dilute hydrochloric acid and saturated sodium bicarbonate. The chloroform solution was dried over magnesium sulfate and solvents were removed. The residual oil was distilled to yield 700 mg of a 70:30 mixture of conformers IIa and IIb at 203°C (0.06 mmHg). IR($CHCl_3$) 1695, 1602 cm^{-1}. NMR (C^2HCl_3, ppm), 7.17 (8H, m), 5.60 (1H, t, J = 7.5 Hz, IIa), 5.77 (1H, t, J = 7 Hz, IIb), 4.10 (2H, q, J = 7 Hz, IIa), 4.03 (2H, q, J = 7 Hz, IIb), 3.38 (2H, t, NCH_2), 2.83 (3H, s), 2.6–2.2 (5H, cmplx band, allylic-CH_2 and 3 cyclopropyl protons, IIa and IIb), 1.4 (1H, two m, IIa and IIb), 1.18 [^{3}H, t, J = 7, $CH_3(CH_2O)$], 0.63 (1H, td, J = 5.5, 3.5 Hz). NMR ($C^2H_3O^2H$) 5.55, 4.06, and 2.80 correspond to the 5.60, 4.10, and 2.83 signals in C^2HCl_3 spectrum, otherwise identical. Mass spectrum, M^+ 347, 0.5% of base. Analysis calculated for $C_{23}H_{25}NO_2$:C, 79.50; H, 7.25; N, 4.03. Found: C, 79.79; H, 7.41; N, 4.01.

The synthesis of methyl[3-(1a,10b-dihydrodibenzo[*a*,*e*]cyclopropa[*c*]cycloheptan-6(1H)-ylidene)propyl]methylcarbamate (III) from octriptyline (3.23 g, 10 mmole) and methylchloroformate (1.16 g, 12.3 mmole) gave 950 mg of IIIa and IIIb at 205°C (0.1 mmHg). IR($CHCl_3$) 1700, 1600 cm^{-1}. NMR (C^2HCl_3) 7.17 (8H, m), 5.72 (1H, t, J = 7.5 Hz, IIIb), 5.58 (1H, t, J = 7.5 Hz, IIIa), 3.65 (3H, s), 3.38 (2H, t), 2.81 (3H, s), 2.6–2.2 (5H, cmplx band), 1.4 (1H, 2m), 0.6 (m, trace). Mass spectrum, M^+ 333, 0.9% of base. Anal. calcd, for $C_{22}H_{23}NO_2$:C, 79.25; H, 6.95; N, 4.20. Found: C, 79.16; H, 7.03; N, 4.15.

The cyclopropylcycloheptane ring has stable *syn* and *anti* conformers which are differentiated by the ^{1}H NMR spectra. The *syn* conformer (a) exhibits a vinyl

proton resonance upfield from the *anti* conformer (b). Additionally, the *anti* conformer exhibits an upfield cyclopropyl proton which is absent in the *syn* conformer. Octriptyline is the phosphate salt of the *syn* conformer.

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