Mass Spectrometric Characterization of Carbamazepine-10,11-epoxide, a Carbamazepine Metabolite Isolated from Human Urine

A. FRIGERIO, R. FANELLI, P. BIANDRATE, G. PASSERINI, P. L. MORSELLI, and S. GARATTINI▲

Abstract □ Carbamazepine-10,11-epoxide was identified in human urine collected after the oral administration of 400 mg. of carbamazepine. Mass spectrometric characterization confirmed the structure of this metabolic product, which was postulated after comparison with the synthetic epoxide.

Keyphrases □ Carbamazepine-10,11-epoxide—identification as carbamazepine metabolite in human urine, mass spectrometric characterization □ Carbamazepine oral administration—identification, characterization of carbamazepine-10,11-epoxide metabolite in urine, man □ Mass spectroscopy—characterization of carbamazepine-10,11-epoxide

The metabolic fate of carbamazepine (5*H*-dibenzo-[*b*,*f*]azepine-5-carboxamide), an iminostilbene derivative with marked anticonvulsant activity (1), has not yet been determined, despite the wide use of the drug in clinical practice either as an antiepileptic agent or in the treatment of trigeminal neuralgia (2–4). Knowledge about the pharmacokinetic aspects and metabolic pathways of degradation has proved useful for the interpretation of the pharmacological action of many drugs and has provided, in some instances, a more rational basis for therapy.

The absorption, tissue distribution, and disappearance rates of carbamazepine in rats, as well as the absorption and excretion rates in humans, were the objects of previous studies (5, 6). This paper will show that after administration of carbamazepine to volunteers, carbamazepine-10,11-epoxide can be isolated from urine.

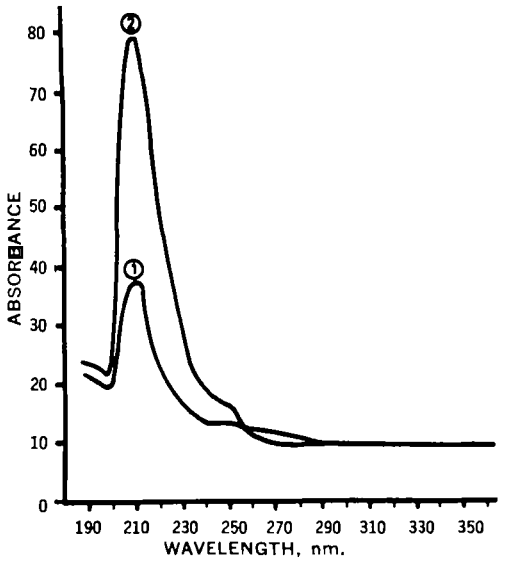


Figure 1—*UV spectrum of the TLC eluates of:* ①, *quenching zones with* R_f *values of 0.15 (System A) and 0.58 (System B); and* ②, *reference standard of carbamazepine-10,11-epoxide.*

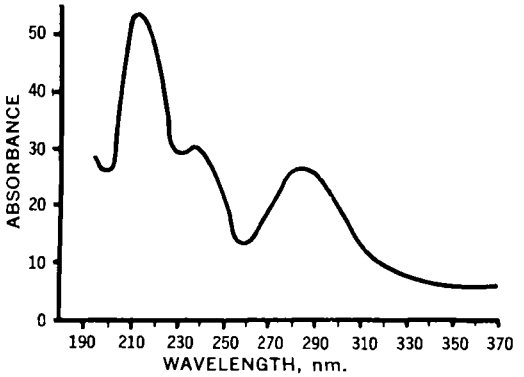


Figure 2—*UV spectrum of an ethylene chloride urine extract redissolved in 3 ml. of absolute ethanol. (For conditions, see text.)*

EXPERIMENTAL

Biological Samples—Urines of three healthy volunteers, who received 400 mg. of carbamazepine by the oral route, were collected at various times for a period of 24 hr. following drug administration and were kept frozen until the analyses were performed.

General Procedures—Thin-layer chromatograms were prepared on 20 × 20-cm. glass plates precoated with silica gel F_{254}[1] and developed at room temperature in carbon tetrachloride–methanol systems (System A, 90:10 v/v; and System B, 60:40 v/v). Carbamazepine and the possible metabolites were visualized by UV light both at 365 and 254 nm.

GLC determinations were performed on a chromatograph[2] equipped with a flame-ionization detector. The chromatographic

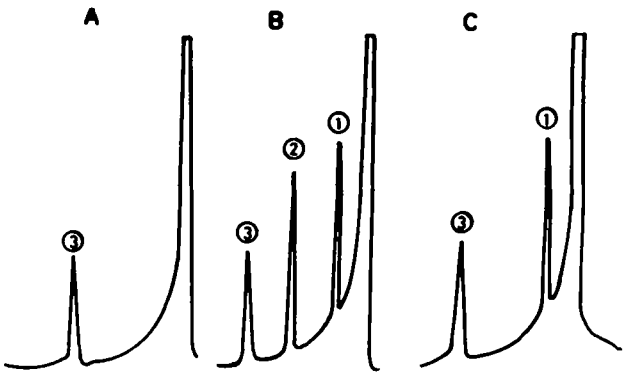


Figure 3—*GLC analysis of urine ethylene chloride extract. Key: A, urine blank extracts; B, urine extracts after carbamazepine administration; C, standard carbamazepine-10,11-epoxide;* ①, *carbamazepine-10,11-epoxide;* ②, *carbamazepine; and* ③, *N-demethyldiazepam (internal marker).*

[1] Merck.
[2] Carlo Erba Fractovap G1.

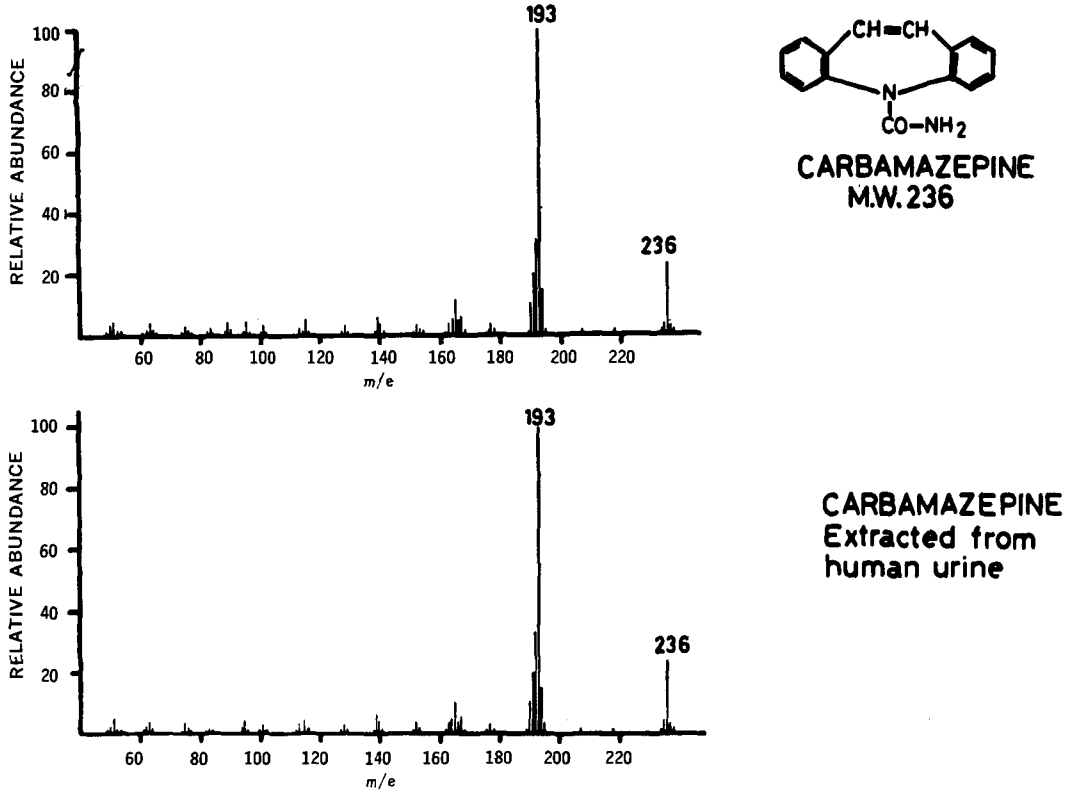


Figure 4—*Mass spectra of a compound extracted from urine (lower panel) and authentic carbamazepine (upper panel) (direct inlet system: probe temperature 70°).*

column was glass tubing, 2 m. long and 4 mm. i.d., packed with 100–120-mesh Chromosorb Q and coated with 3% OV-17[3]. The operating conditions were: injection port temperature, 285°; oven temperature, 260°; nitrogen (carrier gas) flow rate, 50 ml./min.; hydrogen flow rate, 20 ml./min.; and air flow rate, 300 ml./min.

UV spectra were determined with a double-beam grating spectrophotometer[4] operated at a scanning speed of 60 mm./min. and a sensitivity of 10 mv.

A mass spectrometer[5] equipped with a gas chromatograph was used at the following conditions: energy of the ionization beam, 70 ev.; ion-source temperature, 290°; accelerating voltage, 3.5 kv.; and trap current, 60 μamp. The introduction of the sample was carried out either by a direct inlet system (probe temperature 70°) or by a GLC procedure on a glass spiral column, 1 m. long and 4 mm. i.d., packed with 3% OV-17 on 100–120-mesh Gas Chrom Q, at the following conditions: injector temperature, 250°; oven temperature, 220°; helium (carrier gas) flow, 30 ml./min.; and detector total ion monitor.

Synthesis of Carbamazepine-10,11-epoxide—Carbamazepine, 236 mg., and *m*-chloroperbenzoic acid, 200 mg., were dissolved in 10 ml. of ethylene chloride. After 24 hr., the excess peracid was destroyed by addition of a sodium sulfite solution. The organic layer was separated, washed with a 5% sodium bicarbonate solution, dried over sodium sulfate, and evaporated. The residue was suspended in 10 ml. of ether and filtered. The filtrate contained the carbamazepine-10,11-epoxide. The yield of epoxide, crystallized from ether, was 35%. The melting point was 190–195°.

The compound yielded R_f 0.15 in System A and R_f 0.58 in System B. An ethanolic solution showed a UV spectrum with maximum absorbance at 212 nm. (Fig. 1). GLC analysis revealed a peak with a retention time of 1.56 min. Similar results were obtained with a reference standard of carbamazepine-10,11-epoxide[6].

Treatment of Biological Samples—Urines (10–20 ml.) were extracted with ethylene chloride as previously described (5). The organic phase was washed twice with 1 *N* NaOH and once with 1 *N* HCl and then concentrated to dryness in a water bath at 65° under a gentle stream of nitrogen. This material was then used for UV, TLC, GLC, and mass spectrometry.

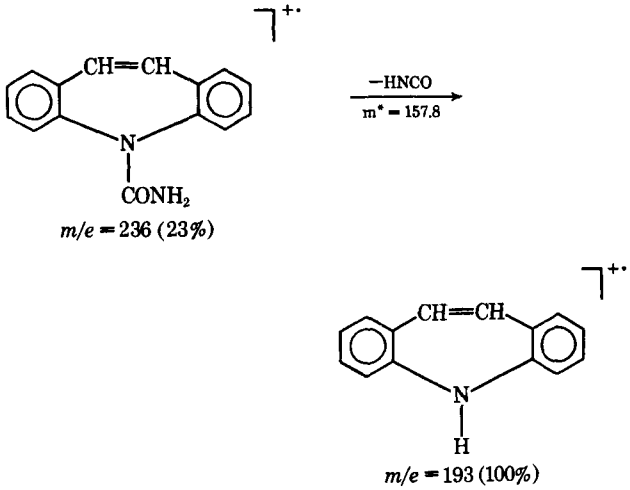
[3] Applied Science Lab.
[4] Perkin-Elmer model 124.
[5] LKB model 9000.
[6] Donated by Ciba-Geigy, Basel, Switzerland.

RESULTS AND DISCUSSION

The UV spectrum of the ethylene chloride residue redissolved in 3 ml. of absolute ethanol revealed the presence of two peaks, at 286 nm. (corresponding to carbamazepine) and at 212 nm. (Fig. 2).

The GLC analysis yielded a peak with a retention time of 4.07 min. (corresponding to carbamazepine) and a second intense peak, with a retention time of 1.56 min., not present in the extract of urines obtained from the same subjects before the treatment with carbamazepine (Fig. 3).

The ethylene chloride extract of 20 ml. of urine was concentrated to dryness and redissolved in 100 μl. of freshly distilled acetone for chromatography on silica gel F_{254} plates in Systems A and B.



Scheme I—Fragmentation pathway of carbamazepine

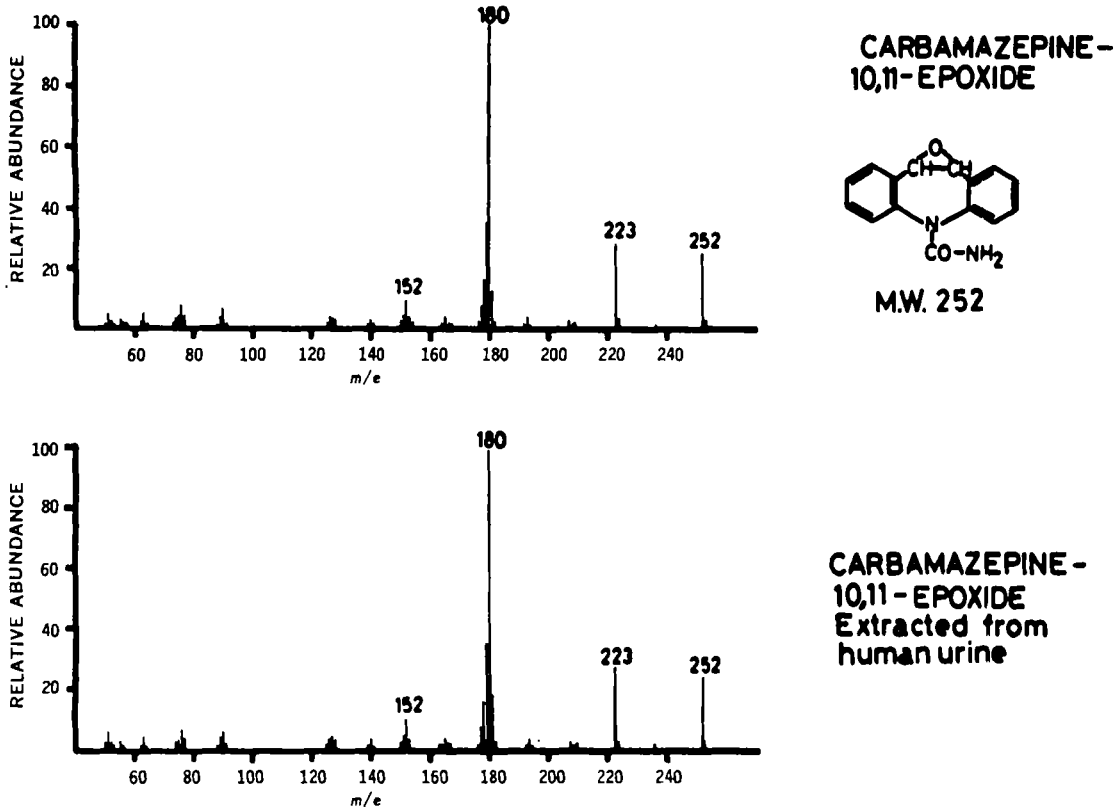
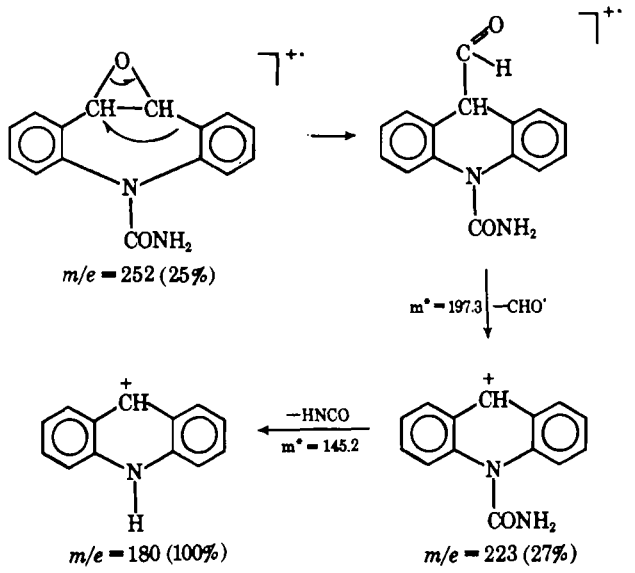


Figure 5—*Mass spectrum of a compound extracted from urine (lower panel) and authentic carbamazepine-10,11-epoxide (upper panel) (direct inlet system: probe temperature 70°).*

Two quenching zones not found in the urine blank were detected in each system: at R_f 0.15 and 0.22 in System A and at R_f 0.58 and 0.68 in System B. The R_f 0.22 and 0.68 spots corresponded to carbamazepine. The quenching zones were eluted with 2 ml. of acetone. The eluate was brought to dryness and analyzed spectrophotometrically and gas chromatographically. The zones corresponding to R_f values of 0.22 (System A) and 0.68 (System B) yielded UV spectra and GLC retention times identical to those of a reference standard of carbamazepine. The zones corresponding to R_f values of 0.15 (System A) and 0.58 (System B) yielded UV spectra with maximum absorbance at 212 nm. (Fig. 1) and a GLC retention time of 1.56 min. A known sample of carbamazepine-10,11-epoxide had the same mobility on TLC (R_f 0.15 for System A and 0.58 for System B) and yielded identical results with UV and GLC analyses (Figs. 1 and 3).



Scheme II—Fragmentation pathway of carbamazepine-10-11-epoxide

The mass spectrum of standard carbamazepine (Fig. 4) showed the molecular ion at *m/e* 236; it was the second most intense peak in the spectrum (relative intensity 23%). The base peak (*m/e* 193) was produced by the loss of HNCO from the molecular ion, as evidenced by a metastable ion m* at *m/e* 157.8. This fragmentation pathway is shown in Scheme I.

Mass spectrometric analysis of the acetone eluates obtained from urine submitted to TLC revealed a mass spectrum identical to that of carbamazepine for the zone with R_f 0.22 (System A) and R_f 0.68 (System B) (Fig. 4).

The mass spectrum of the eluate from the zones with R_f 0.15 (System A) and R_f 0.58 (System B) exhibited the molecular ion at *m/e* 252 (relative intensity 25%). It showed a loss of 29 mass units to give an intense peak at *m/e* 223 (relative intensity 27%). This transition was confirmed by a metastable ion m* at *m/e* 197.3. The loss of 43 mass units from the fragment ion at *m/e* 223 gave rise to the base peak at *m/e* 180. The presence of a metastable peak m* at *m/e* 145.2 confirmed this latter fragmentation.

The presence of the molecular ion at *m/e* 252 with an increase of 16 mass units with respect to the carbamazepine mass spectrum suggests the gain of an oxygen atom during biotransformation. The fragmentation pathway suggests that the oxygen atom may be located at carbons 10 and 11 to form the epoxide derivative of carbamazepine. Scheme II shows the probable fragmentation pattern of this molecule. The hypothesized structure was confirmed by the fact that the mass spectrum of authentic carbamazepine-10,11-epoxide was identical to that of the metabolite (Fig. 5).

The mass spectrum of the GLC peak with retention time 4.07 min. was identical to that of the carbamazepine standard. The mass spectrum of the peak with retention time 1.56 min. showed an intense ion at *m/e* 207 and a fragmentation pattern difficult to interpret. This spectrum was identical to that obtained when the mass spectrometric analysis was carried out on the peak resulting from the injection of the authentic carbamazepine-10,11-epoxide in the GLC column. Carbamazepine-10,11-epoxide probably undergoes a thermal degradation during the GLC analysis. Further studies are in progress to elucidate the structure of the thermolysis product(s).

The identification of a stable epoxide as a major metabolite of carbamazepine is of interest as a possible precursor of hydroxylated metabolites which are now being identified and may be present in the urines of subjects treated with carbamazepine.

Although the pharmacological properties of carbamazepine-10,11-epoxide are still unknown, it is tempting to speculate that this metabolite may be involved in the cases of agranulocytosis ascribed to the administration of carbamazepine (2).

REFERENCES

(1) W. Theobald and H. A. Kunz, *Arzneim.-Forsch.*, **13,** 122 (1963).

(2) S. Livingston, "Drug Therapy for Epilepsy," Charles C Thomas, Springfield, Ill., 1966, p. 125.

(3) W. Amols, *N. Y. J. Med.*, **1,** 2429(1970).

(4) D. J. Dalessio and K. H. Abbott, *Headache*, **5,** 103(1966).

(5) P. L. Morselli, M. Gerna, and S. Garattini, *Biochem. Pharmacol.*, **20,** 2043(1971).

(6) P. L. Morselli, P. Biandrate, F. De Nadai, R. Fanelli, A. Frigerio, M. Gerna, A. Guaitani, G. Rutigliano, and G. Zanda, *Abstr. Proc. World Congr. Psychiat., 5th, 1971*, p. 435. La Prensa Medica Mexicana, 1971, 20, Mexico D.F.

ACKNOWLEDGMENTS AND ADDRESSES

Received November 30, 1971, from the *Istituto di Ricerche Farmacologiche 'Mario Negri,' Via Eritrea, 62-20157 Milan, Italy.*

Accepted for publication March 7, 1972.

Supported by Grant 1 P01-GMI 8376 01 PTR from the National Institutes of Health, Bethesda, MD 20014

The authors thank Mr. F. De Nadai for preparing the mass spectra and Dr. P. Martelli for the synthesis of carbamazepine epoxide. They also thank Ciba-Geigy, Basel, Switzerland, for the generous gift of a reference standard of carbamazepine-10,11-epoxide.

▲ To whom inquiries should be directed.

DRUG STANDARDS

Rapid Analysis of Iodochlorhydroxyquin and Related Halogenated 8-Hydroxyquinolines *via* GLC of Their Trimethylsilyl Ethers

M. P. GRUBER, R. W. KLEIN▲, M. E. FOXX, and J. CAMPISI

Abstract □ A rapid GLC analysis procedure of the trimethylsilyl ethers of 8-hydroxyquinoline and related halogenated 8-hydroxyquinolines, with significantly greater accuracy and precision than official procedures, is presented. A 5–20-fold increase in sensitivity is obtained, allowing detection, identification, and quantitation of impurities at low levels. The method is free of apparent interferences and may be extended to provide rapid, accurate analyses of diiodohydroxyquin and other halogenated 8-hydroxyquinolines.

Keyphrases □ Iodochlorhydroxyquin and related 8-hydroxyquinolines—rapid GLC analysis □ 8-Hydroxyquinolines, halogenated—rapid GLC analysis □ GLC—analysis, iodochlorhydroxyquin and related halogenated 8-hydroxyquinolines □ *N*-Trimethylsilylimidazole—silylating reagent for rapid GLC analysis of halogenated 8-hydroxyquinolines

The procedure employed in the USP XVIII (1) for the assay of iodochlorhydroxyquin marks a significant departure from that listed in an earlier edition (2), which employed an oxygen flask combustion followed by a potentiometric titration of the liberated halides. Iodochlorhydroxyquin in creams, ointments, and suppositories was measured spectrophotometrically in glacial acetic acid at 325 nm. Both procedures have given way to the IR technique of Urbanyi *et al.* (3), which involves the absorbance peak of 14.4 μ (694 cm.$^{-1}$) peculiar to iodochlorhydroxyquin among the halogenated 8-hydroxyquinolines.

Previous investigations with spectrophotometric, colorimetric, titrimetric, polarographic, and chromatographic procedures suffered from one common failing; each was unable to distinguish one or more impurities from one another or from the parent compound. The TLC method of Korzun *et al.* (4) provided the best separation of halogenated 8-hydroxyquinolines but failed to separate 5,7-dichloro-8-hydroxyquinoline,

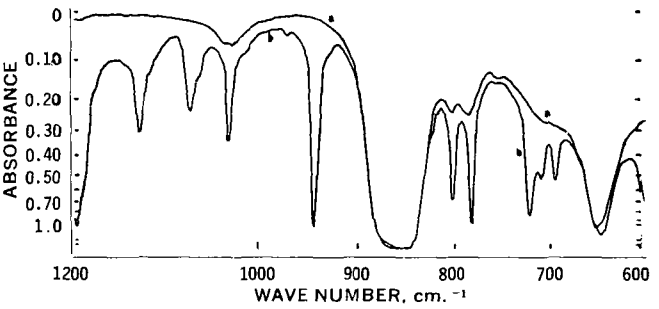


Figure 1—*IR spectrum of iodochlorohydroxyquin (5.00 mg./ml.) in carbon disulfide (b) superimposed on carbon disulfide (a) in 3-mm. sodium chloride cell.*