Identification of 2-Chlorothioxanthen-9-one in Gastric Aspirate in a Case of Chlorprothixene Poisoning

JON ELLING PETTERSEN *§x, **GERD TEIEN** ‡, and **GUNNAR ARNFINN ULSAKER** ‡

Received November 10, 1980, from the *Institute of Clinical Biochemistry, University of Oslo, Rikshospitalet, Oslo, Norway, and the ‡National Centre for Medicinal Products Control, Oslo, Norway. Accepted for publication December 2, 1980. §Present address: Department of Clinical Chemistry, Institute of Medical Biology, University of Tromsø, N-9001 Tromsø, Norway.

Abstract □ In gastric aspirate from a case of severe chlorprothixene poisoning, large amounts (~30% of the chlorprothixene) of a previously unrecognized compound were found and identified tentatively as 2-chlorothioxanthen-9-one by combined GLC–low-resolution mass spectrometry and high-resolution mass spectrometry. The identity of the unknown compound was verified after synthesis of 2-chlorothioxanthen-9-one by two procedures. Only negligible amounts of 2-chlorothioxanthen-9-one were formed when chlorprothixene, dissolved in acids, bases, chloroform–isopropanol, methanol, or gastric fluid, was stored in the dark. However, large amounts of the drug were converted to 2-chlorothioxanthen-9-one upon exposure to UV light. Moreover, considerable quantities of unidentified degradation products were formed when chlorprothixene was exposed to lamp light as well as to UV light. Therefore, samples from cases of acute drug poisoning should be protected from light until analysis.

Keyphrases □ Chlorprothixene—degradation product identification, GLC–mass spectrometry, gastric aspirate □ GLC–mass spectrometry—analysis, chlorprothixene and degradation product, gastric aspirate □ Thioxanthene derivatives—chlorprothixene and degradation product, GLC–mass spectrometric analysis, gastric aspirate □ Tranquilizers—chlorprothixene, GLC–mass spectrometric analysis, gastric aspirate, degradation product

In our hospital, gastric lavage is performed routinely in all patients with suspected intoxication, and it is useful to analyze gastric aspirate (and unknown tablets) by combined GLC–mass spectrometry for the primary detection of the intoxicants in cases of acute drug poisoning (1). An advantage of using gastric aspirate samples is that nonmetabolized drugs may be demonstrated. This task is much simpler than identifying drug metabolites, which may be found in the urine, for example.

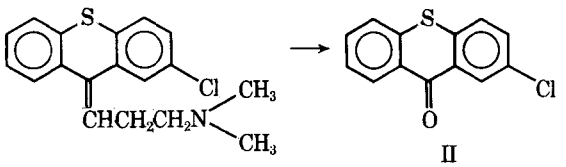
Seven thioxanthene derivatives have been described in humans after chlorprothixene ingestion. They all are probably true metabolites of the drug and not postmortem degradation products (2, 3). In the present work, an additional thioxanthene derivative, 2-chorothioxanthen-9-one, was identified in the gastric aspirate from a case of severe chlorprothixene poisoning.

EXPERIMENTAL

Chlorprothixene[1] [3-(2-chloro-9*H*-thioxanthen-9-ylidene)-*N*,*N*-dimethyl-1-propanamine, I] was purchased. 2-Chlorothioxanthen-9-one was synthesized by a convenient new method—*viz.*, oxidation of chlorprothixene by potassium permanganate in benzene using 1,4,7,10,13,16-hexaoxacyclooctadecane (18-crown-6) as a catalyst. The structure also was verified by a patented two-step synthesis (4). In the first step, 2-carboxy-4′-chlorophenylsulfide was made from 4-chlorothiophenol and 2-iodobenzoic acid. In the second step, the ring system was formed by dehydration in hot concentrated sulfuric acid.

Gastric aspirate was obtained from a case of severe chlorprothixene poisoning. The pH was adjusted to 9.5 prior to extraction of the neutral and basic compounds with chloroform–isopropanol (95:5 v/v). The extract was received by this laboratory for analysis after ~3 months. It was not possible to obtain reliable data on the storage conditions.

[1] Truxal, H. Lundbeck & Co. A/S, Copenhagen, Denmark.



Scheme I—Structural formulas of chlorprothixene (I) and its degradation product, 2-chlorothioxanthen-9-one (II).

Combined GLC–low-resolution mass spectrometry was performed using a gas chromatograph[2], a molecular separator of the glass frit type, and a single-focusing low-resolution mass spectrometer[3] operated with an ionizing energy of 70 ev. The gas chromatograph was equipped with a silanized glass column (2.5 m × 2 mm i.d.) packed with 3% QF-1 on Supelcoport, 80–100 mesh. The column was maintained isothermally at 210°, and helium was used as the carrier gas with a flow rate of 30 ml/min. The instrument was connected on-line to a computer system[4].

High-resolution mass spectrometry was performed with a double-focusing instrument[5] (source temperature, 220°; ionizing energy, 70 ev; ionization current, 100 μamps). The samples were introduced by the heated direct-insertion probe. Peak-matching measurements using heptacosafluorotributylamine as a standard were performed with a resolving power of 1:15,000.

RESULTS AND DISCUSSION

GLC of the neutral and basic compounds in gastric aspirate from a case of severe chlorprothixene poisoning showed, in addition to chlorprothixene, a large peak (30% of the chlorprothixene peak area) with a relative retention volume of 0.55 compared to chlorprothixene.

The mass spectrum of the unknown compound (Fig. 1) showed prominent peaks in the high mass range and almost no fragment ions in the lower mass range, thus indicating a stable molecular structure, probably a ring structure. The peaks at *m/z* 246/248 and 218/220 indicated a chlorine atom in the compound. The direct formation of the ion at *m/z* 218 from the ion at *m/z* 246 and of the ion at *m/z* 220 from that at *m/z* 248 was shown by metastable peaks at 193.2 and 195.2, respectively.

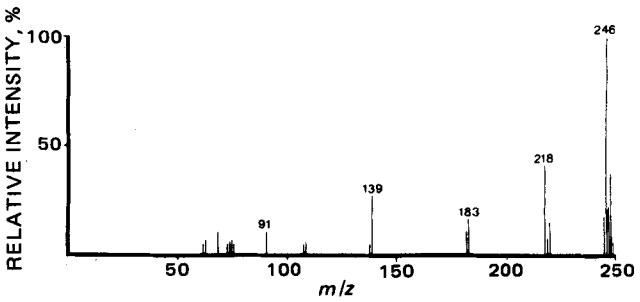


Figure 1—*Low-resolution mass spectrum of a previously unrecognized compound in gastric aspirate from a case of severe chlorprothixene poisoning. This mass spectrum was identical to that of authentic 2-chlorothioxanthen-9-one (not shown).*

[2] Varian 1440, Varian MAT, Bremen, West Germany.
[3] Varian CH 7, Varian MAT, Bremen, West Germany.
[4] Spectro System 100 MS, Varian MAT, Bremen, West Germany.
[5] MS 902, AEI, Manchester, England.

0022-3549/81/0700-0812$01.00/0

Table I—Formation of 2-Chlorothioxanthen-9-one (II) from Chlorprothixene (I) under Various Conditions

Exposure to Light	Solvent	Temperature	Days	Amount of II Formed[a], %
Darkness	2 *M* HCl	80°	9	ND
	2 *M* KOH	80°	9	ND
	1 *M* NaOH	22°	18	ND
	5 *M* NaOH	22°	18	0.2
	Gastric fluid, simulated[b]	80°	1	ND
	Gastric fluid I (pH 2.4)[c]	37°	31	0.6
	Gastric fluid II (pH 1.5)[c]	37°	38	1.5
	Gastric fluid III (pH 6.5)[c]	37°	31	0.6
	Chloroform–isopropanol (95:5 v/v)	4°	32	ND
	Chloroform–isopropanol (95:5 v/v)	4°	580	0.5
	Chloroform–isopropanol (95:5 v/v)	22°	32	ND
	Chloroform–isopropanol (95:5 v/v)	22°	580	3.5
Lamp light	Methanol	22°	34	ND[d]
	Chloroform–isopropanol (95:5 v/v)	22°	34	ND[d]
UV light (254 nm) + lamp light	Methanol	22°	4 + 16	32[d]
	Chloroform–isopropanol (95:5 v/v)	22°	4 + 16	31[d]

[a] The formation of II was measured by GLC; see *Experimental*. ND = not detectable (<0.2%). [b] Prepared according to the USP method (5). [c] Obtained by gastric aspiration in three healthy adults. [d] Considerable quantities (5–40%) of degradation products of I other than II.

Exact mass measurements in a high-resolution mass spectrometer showed that the empirical formula of the ion at *m/z* 246 was $C_{13}H_7ClOS$ (theoretical mass, 245.9906; observed mass, 245.9906). Similarly, the ions at *m/z* 218 and 183 were shown to be $C_{12}H_7ClS$ (theoretical mass, 217.9957; observed mass, 217.9959) and $C_{12}H_7S$ (theoretical mass, 183.0268; observed mass, 183.0264), respectively.

From these data, the unknown compound was identified tentatively as 2-chlorothioxanthen-9-one (II). This compound was synthesized by two methods. The synthetic compounds cochromatographed with the unknown in GLC, and their mass spectra were identical. Thus, the identity of the unknown compound was established as II.

Table I shows the formation of II from chlorprothixene (I) under various storage conditions. Only negligible amounts were formed when the drug was stored in the dark or in lamp light. However, when I was treated with UV light for 4 days followed by storage in lamp light for 16 days, ~30% of I was converted to II. Moreover, considerable quantities of unidentified degradation products of I were formed when I was exposed to lamp light and UV light. These findings are in accordance with unpublished data (6). Therefore, samples from cases of acute drug poisoning should be stored in the dark.

REFERENCES

(1) E. Saltvedt, E. Wiik-Larsen, B. Skuterud, J. E. Pettersen, P. K. M. Lunde, and E. Enger, *Tidsskr. Nor. Laegeforen.*, **97**, 227 (1977).

(2) H. Christensen, *Acta Pharmacol. Toxicol.*, **34**, 16 (1974).

(3) J. Raaflaub, *Arzneim.-Forsch.*, **17**, 1393 (1967).

(4) U.S. pat. 2,951,082 (1960).

(5) "The United States Pharmacopeia," 20th rev., United States Pharmacopeial Convention, Rockville, Md., 1980, p. 1105.

(6) B. C. Rudy and B. Z. Senkowski, in "Analytical Profiles of Drug Substances", vol. 2, K. Florey, Ed., Academic, New York, N.Y., 1973, p. 77.

GLC Analysis of Phenylalkyl Primary Amines Using Nitrogen Detector

J. I. JAVAID ˣ **and J. M. DAVIS**

Received August 21, 1980, from the *Illinois State Psychiatric Institute, 1601 West Taylor Street, Chicago, IL 60612.* Accepted for publication December 2, 1980.

Abstract □ A comprehensive method for the analysis of several phenylalkyl primary amines of biological interest was developed. The amines were derivatized with perfluoro acid anhydrides or carbon disulfide in ethyl acetate, and the respective acyl derivatives or isothiocyanate derivatives were analyzed by GLC using nitrogen-specific detection. The described procedure was used to measure the amphetamine concentrations in rat serum, brain, and liver after intraperitoneal injection (5 mg/kg).

Keyphrases □ Phenylalkyl primary amines—GLC analysis using nitrogen detection, rat serum, brain, and liver □ Amphetamines—phenylalkyl primary amines, GLC analysis using nitrogen detection, rat serum, brain, and liver □ GLC—nitrogen detection, analysis, phenylalkyl primary amines in rat serum, brain, and liver

The use of nitrogen–phosphorus detection in GLC has many applications in biomedicine for the trace analyses of nitrogen-containing compounds. The nitrogen–phosphorus detector is generally 10–100 times more sensitive than the flame-ionization detector and has very little response to organic compounds that do not contain phosphorus or nitrogen atoms. Thus, therapeutic levels of many drugs containing a secondary or tertiary nitrogen, *e.g.*, anticonvulsants (1, 2), analgesics (3), tricyclic antidepressants (4–6), antipsychotics (7, 8), and drugs of abuse (9–11), can be measured. However, this approach has not yet been exploited for the analysis of primary amines. The use of GLC with nitrogen detection was suggested for the identification of amphetamine in forensic toxicology (9).

In this report, a comprehensive method for the analysis of several phenylalkyl primary amines is described. The amines were derivatized with acid anhydrides or carbon disulfide, and the *N*-acyl and isothiocyanate derivatives were analyzed by GLC with nitrogen detection.

EXPERIMENTAL

Materials—All of the phenylalkyl primary amines were obtained

0022-3549/81/0700-0813$01.00/0