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Fatal poisoning with detajmium: identification of detajmium and its metabolites and artifacts by gas chromatography–mass spectrometry and quantification by high-performance liquid chromatography

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Abstract

After ingestion of an unknown dose of detajmium, a 14-year-old female collapsed with asystolia. Resuscitation efforts were not successful. A medicolegal autopsy was carried out, and blood, liver and gastric content were extracted and analyzed by gas chromatography–mass spectrometry (GC–MS). After derivatization with acetic anhydride, detajmium and twelve of its derivatives and metabolites were identified. The main metabolic pathways include hydroxylation and subsequent O-methylation of the indol ring, and oxidation as well as reduction of the C-21 hydroxyl function. Cleavage of the N-alkyl side-chain is a further, possibly non-enzymatic degradation pathway. Artifact formation induced by acetylation included dehydratation of the hydroxyl function of C-21 and the N-alkyl side-chain. The detajmium concentration in blood of the deceased was determined by high-performance liquid chromatography with fluorimetric detection (12 μg/ml).

1. Introduction

Ajmaline and some of its derivatives such as prajmaline and detajmium are antiarrhythmic drugs of class IA/IC according to the classification of Vaughan–Williams (Fig. 1) [1–9]. Detajmium has been used in the former German Democratic Republic [10–14]. No data are available on detajmium pharmacokinetics and metabolism. Commercially available detajmium is a mixture of four stereoisomers which differ in their configuration at C-21 and at the carbon atom of the hydroxyl function of the N-diethylamino side-chain.

The main metabolic pathways of the detajmium analogue ajmaline are hydroxylation of the indol ring with subsequent O-methylation, N-oxidation, and oxidation of the 17- and 21-hydroxyl function as well as reduction of C-21 [6]. The main degradation pathways in prajmaline metabolism are hydroxylation of the indol ring with consecutive O-methylation, and

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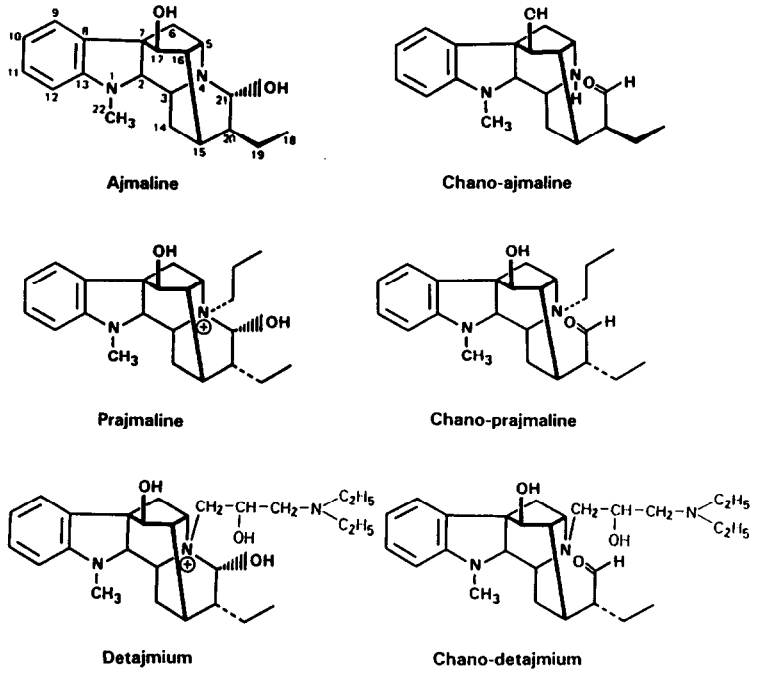


Fig. 1. Ajmaline, prajmaline, and detajmium and their corresponding chano-derivatives.

oxidation of C-21 with subsequent formation of a carbonic acid [15]. It should be noted that ajmaline and prajmaline metabolism cosegregate with debrisoquine metabolism [6,15]. Both drugs are subjected to metabolic degradation by cytochrome P-450IID6 [6,15].

Ajmaline and prajmaline are highly polar substances. Both drugs may isomerize to their corresponding chano-derivatives under alkaline conditions or thermal challenge (Fig. 1). For gas chromatographic–mass spectrometric analysis of ajmaline and its analogues, derivatization prior to analysis is recommendable. A simple approach is derivatization with acetic anhydride, thereby forming acetyl derivatives. However, it has to be noted that this procedure may lead to the formation of artifacts by dehydratation [6]. The mass spectrum of a dehydration and acetylation artifact of detajmium has been published [16].

Poisoning with antiarrhythmics of class IA/IC is associated with high lethality [2]. Since the pharmacokinetics and metabolism of detajmium have not been studied, and since there is a lack of clinical experience with detajmium overdose, we report a case of fatal poisoning with detajmium and post-mortem analysis of detajmium, its derivatives and metabolites.

2. Experimental

2.1. Case report

A 14-year-old female told her classmates in school that she felt very sick after ingestion of an unknown dose of detajmium, which had been prescribed to her mother. Soon afterwards, the patient collapsed with cardiac arrest. Despite maximum prehospital resuscitation efforts over a 1-h period by an emergency physician of the Berlin Emergency Medical System, circulation could not be restored.

A medicolegal autopsy was carried out. Findings included brain edema, a dilated myocardium and pulmonary congestion. Gastric content, liver, and heart blood were stored for toxicological analysis. Since the bladder was empty, no urine was available for analysis.

2.2. Materials and chemicals

A pure sample of detajmium bitartrate was generously provided by Priv.-Doz. Dr. Terhaag, Arzneimittelwek Dresden (Dresden, Germany). All reagents, of analytical-reagent grade or better, were obtained from commercial sources and used without further purification.

Liver and gastric content were extracted according to standard procedures [17]. A toxicological screening for drugs and chemicals using gas chromatography–mass spectrometry was performed, which revealed that no other drugs than detajmium had been ingested [18]. Head-space gas chromatographic analysis of the heart blood was negative for ethanol and related volatiles.

2.3. Extraction procedure for gas chromatography–mass spectrometry (GC–MS)

For analysis of detajmium and its metabolites, the crude liver extract was adjusted to pH 8 and then extracted twice with methylene chloride–isopropanol (3:1, v/v). The organic layer was dried with sodium sulfate and the solvent was removed by a dry stream of nitrogen. The extracts were used directly and after derivatization with acetic anhydride (Merck, Darmstadt, Germany) for GC–MS analysis.

2.4. Gas chromatography–mass spectrometry

Mass spectra were run on a Model TSQ 700 gas chromatograph–mass spectrometer (Finnigan MAT, Bremen, Germany). For GC a fused-quartz silica capillary column [SE-54, 25 m × 0.32 mm I.D., 0.3 μm film thickness (Macherey-Nagel, Düren, Germany)] was used with an injection port temperature of 280°C, splitless injection and a column temperature program of 75–300°C at 15°C/min. The carrier gas was helium at a flow-rate of 1.7 ml/min. The column was coupled directly to the mass spectrometer. The ion-source pressure was $4 \cdot 10^{-5}$ Pa in the electron-impact (EI) mode and $3 \cdot 10^{-3}$ Pa in the chemical-ionization (CI) mode, using methane. The ion-source temperature was 150°C. The multiplier voltage was 1.2 kV, the dynode voltage was −5 kV.

All samples were run in the EI (70 eV) and in the CI (30 eV) mode. Structure elucidation of detajmium derivatives was based on reference mass spectra, determination of the molecular ion by CI, fragmentation pattern and formation of the corresponding derivatives after acetylation of the extracts.

2.5. In vitro experiments on the chemical stability of detajmium

The chemical stability of detajmium during the extraction procedure for the liver described above was studied under in vitro conditions with an aqueous detajmium solution (10 μg/ml). In addition, 10 ml of an aqueous solution of detajmium (10 μg/ml) were refluxed with 10 ml of 37% hydrochloric acid for 30 min and then extracted at pH 8 as described for the liver extract.

2.6. High-performance liquid chromatography (HPLC)

For quantification of detajmium by HPLC, blood was deproteinized with an aliquot of perchloric acid (6%), and the clear supernatant was injected onto the column. A HPLC system consisting of a Rheodyne 7125 50-μl loop, a P4000 pump, a Spectra Focus detector, an Fl 2000 fluorimeter and a Spectra System SN 4000 from Spectra Physics was used. The column (Lichrospher 60 RP Select B 125 × 4 mm I.D., operated at room temperature) was combined with a guard column (4 × 4 mm I.D., Merck). The mobile phase was acetonitrile (Chrom AR, Mallinckrodt, MO, USA)–0.07% orthophosphoric acid (16:84, v/v). The flow-rate was 1.5 ml/min.

The UV detector was operated at a wavelength of 245 nm with peak purity control by UV spectrum. Quantification with the fluorimetric detector was performed at an excitation wavelength of 296 nm and emission wavelength of 358 nm.

Table 1
Mass spectra of detajmium, its metabolites and artifacts

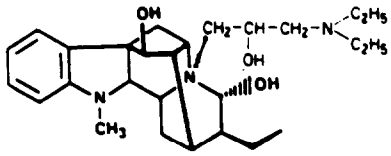
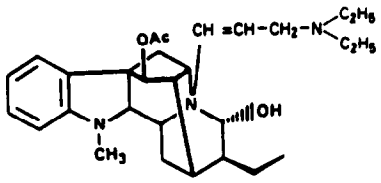
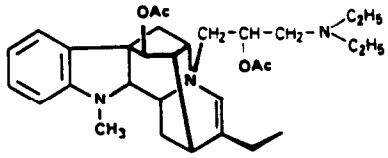
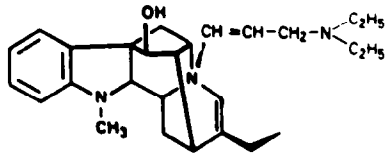
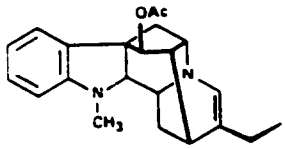
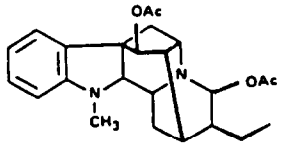
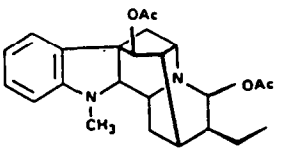
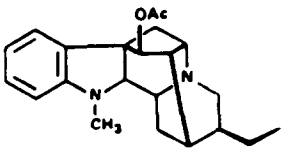
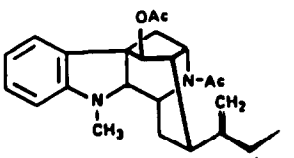
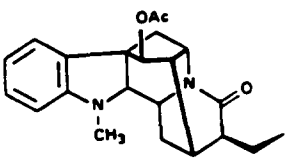
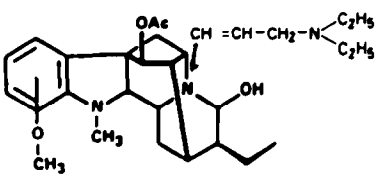
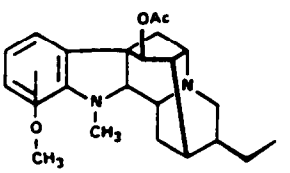
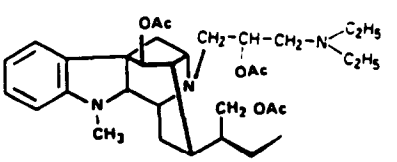
	Kovats index		*m/z* (base peak indicated in *italics*)
I Detajmium	3520		M^+ 455(1), 454(2), 437(15), 408(4), 369(38), 366(20), 365(93), 339(18), 311(18), 297(9), 224(4), 196(28), 158(21), 144(28), 112(38), *86(100)*, 58(10)
Ia acetylated $-H_2O$	3345		M^+ 479(9), 450(6), 408(21), *407(100)*, 382(7), 339(13), 335(6), 308(4), 182(8), 144(29), 112(43), 86(89)
Ib diacetylated $-H_2O$	3230		M^+ 519(6), 491(2), 448(18), 447(65), 407(24), 352(13), 281(13), 182(24), 144(50), 112(80), *86(100)*
Ic $-H_2O$	3000		M^+ 419(28), 346(16), 292(24), 182(6), 170(22), 144(6), *112(100)*, 86(22)
IIa acetylated $-H_2O$	2290		*M^+ 350(100)*, 309(10), 308(10), 292(4), 194(11), 182(48), 166(18), 144(57), 108(52)
IIb diacetylated	2620		*M^+ 410(100)*, 368(8), 367(9), 351(22), 291(21), 237(15), 194(11), 182(48), 144(36)

Table 1 *(continued)*

	Kovats index		*m/z* (base peak indicated in *italics*)
IIc diacetylated (stereoisomer of IIb)	2660		*M^+ 410(100)*, 368(16), 367(12), 353(22), 291(6), 237(6), 194(6), 182(44), 144(38)
IIIa acetylated	2420		*M^+ 352(100)*; 351(4), 323(4), 309(8), 293(27), 253(9), 236(19), 182(10), 144(56), 136(10), 115(10)
IIIb diacetylated	2870		*M^+ 394(100)*, 351(23), 334(23), 291(10), 242(18), 194(44), 144(23)
IVa acetylated	2445		*M^+ 366(100)*, 323(24), 293(26), 273(78), 194(58)
Va acetylated $-H_2O$	3110		M^+ 511(8), 481(3), 451(19), 379(28), 352(50), 292(5), 182(17), 144(10), *112(100)*, 86(44)
VIa acetylated	2520		M^+ 382(20), 353(24), *352(100)*, 323(4), 309(21), 293(55), 237(6), 182(39), 144(24)
VIIa triacetylated	3370		M^+ 523(4), 494(3), 451(61), 407(28), 393(8), 352(6), 351(6), 293(4), 282(4), 194(6), 182(16), 144(32), 112(92), *86(100)*

3. Results

Under the GC–MS conditions described here, unchanged detajmium could be detected in gastric content and liver extract. Acetylation of a detajmium reference sample and the liver extract led to the formation of the derivates Ia, Ib, and Ic (Table 1, Fig. 2). Acetylation was associated with dehydratation of hydroxyl functions of C-21 and the N-alkyl side-chain. Analysis of extracts without acetylation gave evidence that no acetyl derivatives were formed in vivo.

The in vitro extraction experiments using the extraction procedure of Klug [17] led to the formation of traces of a reaction product (IIb) with cleavage of the N-alkyl side-chain. Substantial amounts of IIb were formed after hydrochloric acid hydrolysis of detajmium.

The mass spectra of detajmium, its metabolites, artifacts, and derivates are summarized in Table 1. The molecular ions were identified by chemical ionization with methane, which yielded the typical $[M+1]^+$, $[M+29]^+$, and $[M+41]^+$ ions. Unchanged detajmium was identified in the gastric content. Detajmium (I) and 12 derivatives and artifacts (II to VII) were identified in the liver extract (Fig. 2, Table 1).

Under the HPLC conditions described here, the retention time of detajmium was 3.9 min with a small shoulder with a retention time of 4.3 min (Fig. 3). The UV spectrum of the detajmium peak and the shoulder were identical (Fig. 4). The blood concentration of detajmium determined by HPLC was 12 μg/ml. The recovery of the HPLC procedure was 70%, the coefficient of variation was 7% (at a detajmium concentration of 200 ng/ml), the limit of quantification 50 ng/ml using fluorimetric detection (signal-to-noise ratio 13:1).

4. Discussion

Overdose with antiarrhythmics and betablockers has occasionally been observed in the former German Democratic Republic [19]. In the case reported here, a fatal overdose of

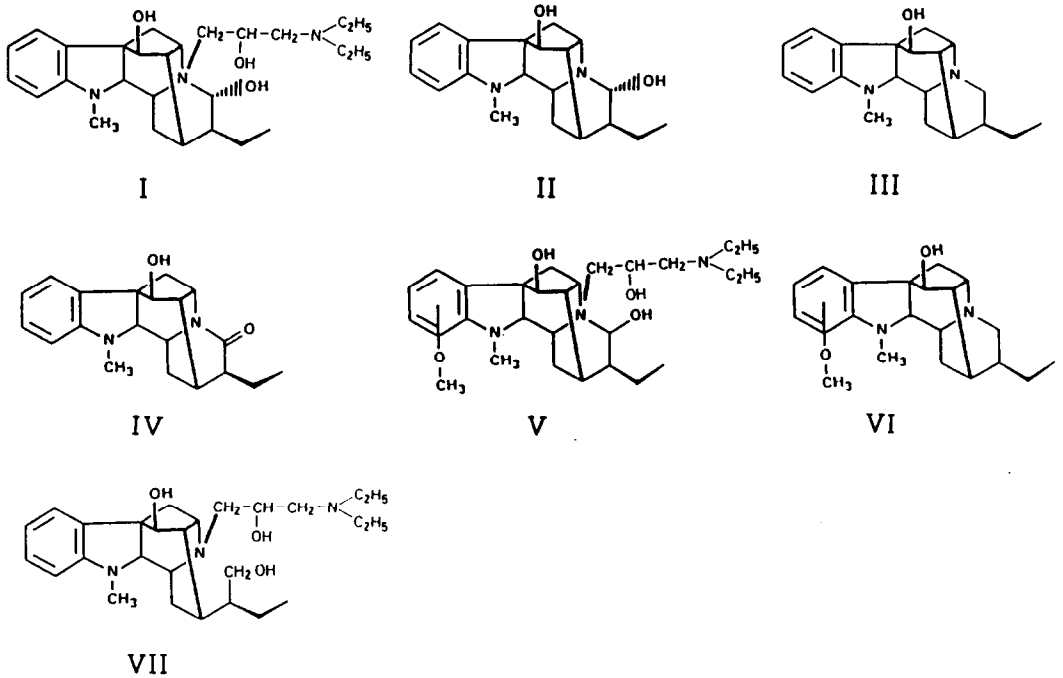


Fig. 2. Detajmium (I), its metabolites (V, VII) and artifacts or metabolites (II–IV, VI).

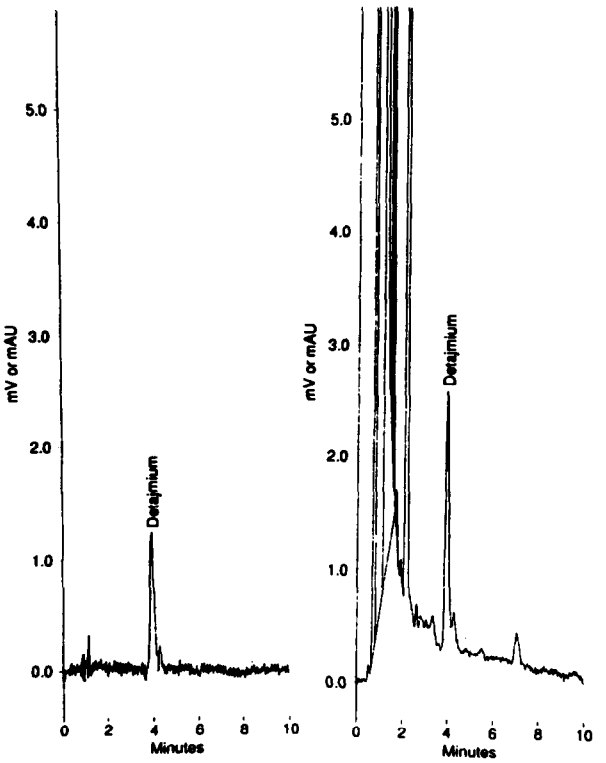


Fig. 3. HPLC (with UV-detection) of a standard detajmium solution (70 ng) and of the blood extract (12 μg/ml).

detajmium was the cause of death. The macroscopic findings at autopsy suggested failure of the left half of the heart. Detajmium could be identified in the gastric content, liver, and blood. The detajmium blood concentration was 12 μg/ml. In six unpublished cases of fatal detajmium overdose, detajmium blood concentrations determined by a fluorimetric assay ranged from 1.5 to 35 μg/ml (U. Demme, Friedrich Schiller Universität Jena, Germany, personal communication). The applied fluorimetric assay consisted of a rapid extraction of detajmium at pH 11 with ethylacetate and a subsequent re-extraction into an aqueous phase of pH 2 (0.05 *M* H_2SO_4). The fluorimetric measurement of this aqueous re-extract was done at an excitation wavelength of 295 nm and an emission wavelength of 350 nm. This simple fluorimetric assay probably also included detajmium metabolite concentrations due to a lack of specificity (Table 2).

A reference range for therapeutic concentrations of detajmium is lacking. Recommended therapeutic doses of detajmium bitartrate (3 × 25 mg/day) are similar to those of prajmaline bitartrate (3–6 × 20 mg/day). Therapeutic doses of prajmaline are associated with serum concentrations in the range 20–200 ng/ml [15].

In the liver extract, a total of 12 derivatives and artifacts could be detected besides unchanged detajmium (I). Analysis of extracts without acetylation gave no evidence for in vitro formation of acetyl derivatives. Since small amounts of IIb were formed during the in vitro extraction experiment, it remains open whether degradation of the N-alkyl side-chain is due to an in vivo reaction or whether this is exclusively a non-enzymatic degradation.

Compound Ia is formed by acetylation of the OH function at C-17 and dehydratation of the N-alkyl side-chain. Compound Ib is an acetylation product of detajmium with loss of water from the OH function of C-21. Compound Ic is an artifact formed from detajmium with elimina-

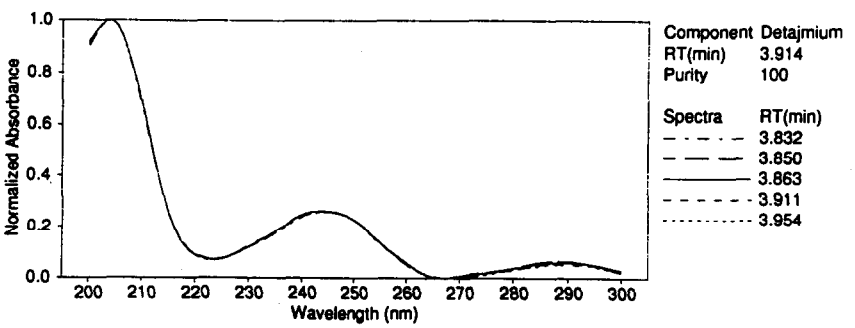


Fig. 4. UV spectrum of a reference detajmium solution (70 ng).

Table 2
Post-mortem detajmium blood concentrations determined by fluorimetry in six cases of detajmium overdose

Case	Age (years)	Sex	Detajmium in blood (μg/ml)	Ethanol or other drug
1	23	Female	35	1.0‰ ethanol
2	18	Female	12	–
3	42	Female	25	–
4	36	Female	1.5	2.1‰ ethanol
5	71	Male	2.0	Nifedipine
6	16	Female	28	–

tion of water from the N-alkyl side-chain and the OH function of C-21 under acetylating conditions. Cleavage of the N-alkyl side-chain and subsequent loss of water from C-21 produces IIa. Two isomers of diacetylajmaline (IIb and IIc) could be detected. Compound IIIa is generated by reduction of C-21 with ring opening to a primary alcohol and subsequent loss of water with reclosure of the 6-membered ring. Reduction of C-21, diacetylation, and dehydratation lead to the formation of IIIb, cleavage of the N-alkyl side-chain and oxidation at C-21 to IVa. Compound Va is formed after O-methylation of the indol ring and dehydratation of N-alkyl side-chain. Compound VIa is formed by O-methylation of the indol ring and reduction of C-21 combined with cleavage of the N-alkyl side-chain. The exact position of the O-methyl group in the indol ring of V and VI could not be derived from the mass spectrum. The triacetyldehydroajmaline derivative VIIa is generated after reduction of C-21.

The main metabolic pathways of detajmium are hydroxylation of the indol ring system with subsequent O-methylation, reduction of C-21 as well as oxidation of C-21, and cleavage of the N-alkyl side-chain. However, the latter reaction might be due to non-enzymatic degradation.

The HPLC procedure described here allowed specific quantitation of detajmium. Separation of the four stereoisomers of detajmium could not be achieved under the HPLC conditions chosen here. A small shoulder, probably due to a detajmium isomer, appeared at a retention time of 4.3 min.

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