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Report on a Lethal Antazoline Intoxication

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Summary. A lethal antazoline intoxication is reported and a method for isolation and determination of this compound and two hydrolysis products in autopsy material is described. Analytical findings in liver tissue, kidney tissue and blood are given. Definite identification of the compounds found was obtained by mass spectrometry.

Zusammenfassung. Ein Fall von tödlicher Vergiftung mit Antazolin und eine Methode zur Isolierung und Bestimmung dieser Verbindung und zweier Hydrolyseprodukte in Leichenmaterial wurden beschrieben. Es wurden die analytischen Ergebnisse aus dem Blut, Leber- und Nierengewebe referiert. Die definitive Identifikation der Komponenten wurde mit Hilfe der Massenspektrometrie durchgeführt.

Key words: Antazoline, determination — Intoxication, antazoline.

Antazoline also called imidamine or phenazoline is an antihistamine drug. The recommended oral dose in human medicine is 100 mg up to 4 times a day for adults. Antazoline is chemically: 2-(N-Benzylanilinomethyl)-2-imidazoline and is distributed as the hydrochloride for oral use. As far as we know there is only 1 case of lethal antazoline intoxication reported. In that case no quantitative analysis was reported [1]. Clarke [2] reports the estimated acute lethal dose in man to be 25 to 250 mg/kg and that LD_{50} (oral) in mice is 250 to 1000 mg/kg. Sunshine [3] gives LD_{50} (subcutaneous) in rat to 500 mg/kg for antazoline phosphate. To our knowledge there are no data on the therapeutic levels in tissues or body fluids of antazoline in man published nor any reports on the metabolism of antazoline. This report gives the concentration of antazoline in liver and kidney tissue and in blood in a case of lethal intoxication with this drug. Hydrolysis products of antazoline found in extracts of liver and kidney tissue have been isolated and identified.

Methods

In literature there are some methods for analyzing antazoline reported. Cochin *et al.* [4] have proposed an extraction method followed by identification and isolation by thin-layer chromatography used for phenothiazine tranquillizers and antihistaminics (including antazoline) in body fluids and tissues. The extraction is carried out at pH 9.0 by shaking with 4 volumes of ethylenedichloride containing 10% isoamylalcohol. Four different solvent systems for the thin-layer chromatography are presented. The spots were detected by spraying with a idoplatinate reagent. Goenechea [5] has published a thin-layer chromatographic method for

four important imidazolines, including antazoline, where four different reagents for detecting the spots were used. Gas chromatographic determinations of antazoline have been done on different columns [6—9] and seem to meet no special difficulties, though MacDonald *et al.* [9] have reported decomposition in some cases. Jensen *et al.* [10] have published a method for fluorimetric determination of antazoline.

The methods used by us are as follows:

Extraction

Blood was extracted with 3 × 100 ml ethylether at pH 9.5. The pH was adjusted with dilute ammonia. The collected ether phases were washed with 10 ml aq. dest The ether was evaporated to about 100 ml and then extracted with 3 × 25 ml 0.1 N H_2SO_4. The acid phase was alkalized to about pH 9.5 with dilute ammonia and then extracted with 2 × 150 ml chloroform. Evaporation of the chloroform was carried out to a final volume of about 2—3 ml. The volume was then adjusted to 5 ml by adding chloroform. The blood remaining from the extraction was neutralized with 1 N HCl and then hydrolyzed with 0.1 vol. konc. HCl by boiling at elevated pressure for 30 min. The extraction of this solution was performed as for unhydrolyzed blood, except that pH was about 8.5 (adjusted with sodium hydrogen carbonate). The resulting chloroform extract was brougth to 5 ml.

Liver tissue was extracted with 70% aqueous ethanol according to Bonnichsen [11]. An aliquot of the ethanol extract was made slightly acidic and was then evaporated to nearly dryness on a boiling water bath. The residue was dissolved in 30 ml aq. dest. This solution was extracted and hydrolyzed exactly as described for the blood. Kidney tissue was handled the same way as liver tissue.

Thin-layer Chromatography

An aliquot of the 5 ml chloroform solution resulting from the extraction procedure is spotted on a thin-layer plate (0.2 mm layer fluorescent silica gel) together with appropriate standards. The plate is developed in methanol, 25% ammonia (100:1.5). The spots are visualized under an UV lamp and then sprayed with Dragendorffs reagent. The reagent was produced according to Clarke [2].

Ultraviolet Spectrometry

Spots resulting from thin-layer chromatography of aliquots of the chloroform extracts are visualized under an UV lamp and then eluated with methanol (the recovery of 100 μg antazoline standard was more than 90%). The methanol is carefully evaporated under a stream of filtered air. The residue is dissolved in ammoniacal aqueous ethanol (0.025 N ammonia in 75% w/v ethanol). The ultraviolet spectrum is recorded with an Unicam SP 8000 ultraviolet recording spectrophotometer. The solution is then acidified with 6 N sulphuric acid (0.1 volumes of the ammoniacal aqueous ethanol) and then the spectrum is recorded again.

Mass Spectrometry

A LKB 9000 gas chromatograph-mass spectrometer with a silanized glass column 2.5 m × 2 mm (I.D.) filled with 1% SE-30 on Gas Chrom P was used. Flash heater and ion source temperatures were respectively 210 and 270°C. The electron energy was 70 eV and the trap current was 120 μA.

Case Report

The deceased, a 53-year-old man suffering from asthma, was found dead on the floor in his apartment. In the kitchen an almost empty bottle of potato-spirit was found. There were no signs of physical violence on the body nor any disorder in the apartment. The time of death could not be established, but the man was alive 2 days before the body was found.

Autopsy was performed on the fourth day after the body was found and the autopsy material (blood, liver tissue and kidney tissue) arrived at our laboratory the following day.

Autopsy Findings

The phatological-anatomical findings were non-specific with pulmonary edema and the histologic picture of an asthmatic bronchitis. There were no signs of an acute attack of asthma (status asthmaticus). Otherwise the autopsy revealed stasis of the inner organs, slight myocardial fibrosis and slight fatty degeneration of the liver.

Results and Discussion

According to Miescher *et al.* [12] 2-imidazolines are very easily hydrolyzed in water and alkaline aqueous solutions, while their salts are quite stable in water and in acid solutions. When antazoline is boiled with water it is hydrolyzed to (Phenyl-benzyl-amino-acetyl)-ethylenediamine [12]. Alkaline hydrolysis of antazoline performed by Boon *et al.* [6] yielded a mixture of two basic substances not having imidazoline rings but with ultraviolet absorption spectra similar to antazoline.

We refluxed antazoline in excess of 1 N KOH for 30 min. This yielded (Phenyl-benzyl-amino-acetyl)-ethylenediamine and minor amounts of a substance identified by GC-MS as benzylaniline (see Fig. 4a and b). We also found that about 50% of the antazoline is hydrolyzed to (Phenyl-benzyl-amino-acetyl)-

Table 1

Substance	Rf value[a]	λ max. in ammoniacal aqueous ethanol[b]	$E^{1\%}_{1\,cm}$ at λ max.
Antazoline	0.33	248 nm	543
(Phenyl-benzyl-amino-acetyl)-ethylenediamine	0.54	248 nm	496
Benzylaniline	0.81	246 nm	606

[a] Conditions: see methods.
[b] For the absorption maxima used for calculations.

Table 2

Substance	mg/100 g blood		mg/100 g liver tissue		mg/100 g kidney tissue	
	UV[a]	TLC[b]	UV	TLC	UV	TLC
Antazoline	0.5	0.4	11.8	14	6.5	6
(Phenyl-benzyl-amino-acetyl)-ethylenediamine	—	—	4.6	4	3.6	4
Benzylaniline	—	—	—	traces	—	traces

[a] UV = results according to ultraviolet spectrometry.
[b] TLC = results according to estimations of spots on thin-layer plates.

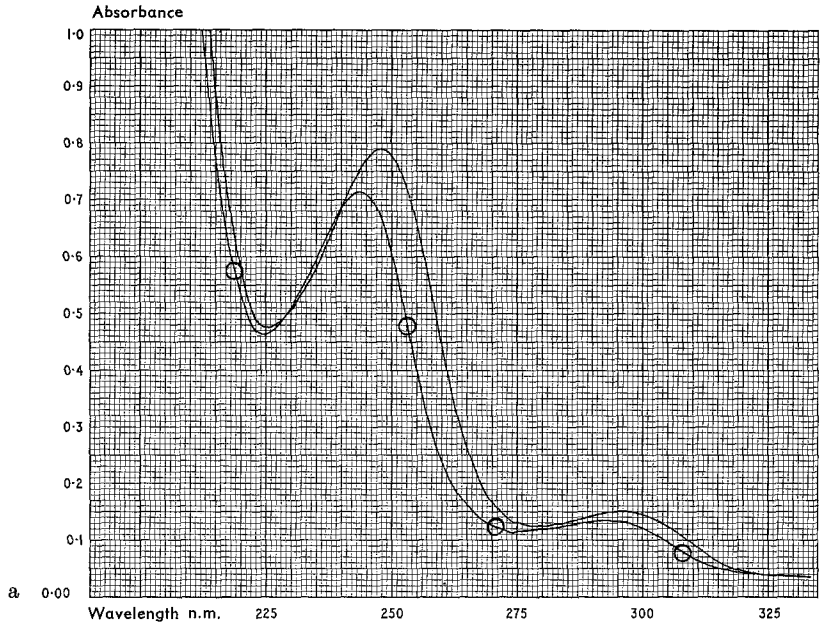
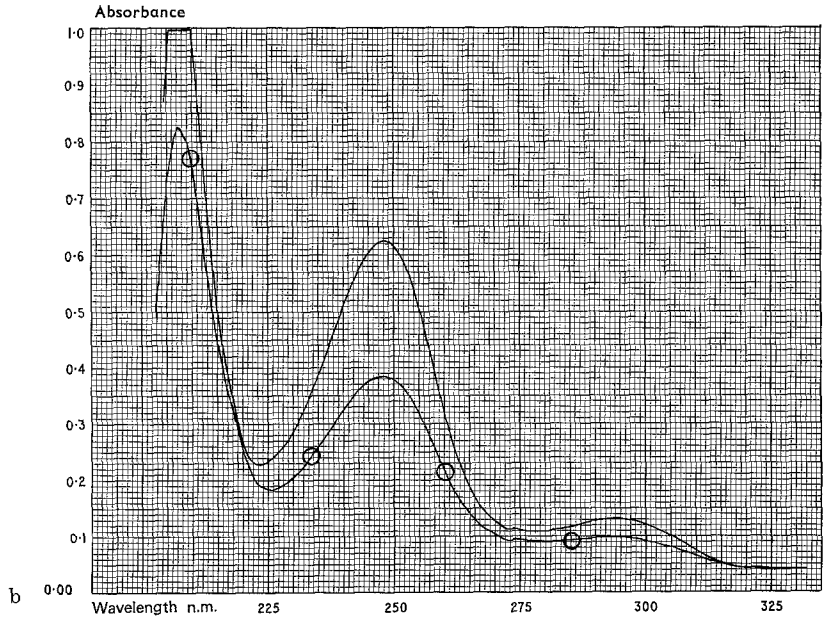


Fig. 1a—c. Absorption spectra in ammoniacal aqueous ethanol. The marked curves are the spectra after acidification with 6 N sulphuric acid as described under methods. a Absorption spectrum of antazoline. b Absorption spectrum of (Phenyl-benzyl-amino-acetyl)-ethylene-diamine. c Absorption spectrum of benzylaniline



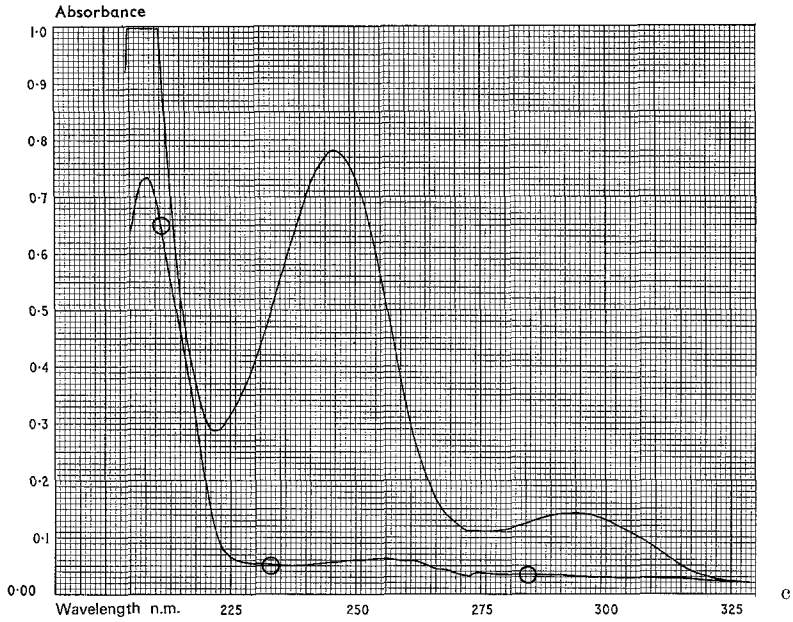


Fig. 1c

ethylenediamine when left in excess of destilled water at 37° C for 4 hrs. Traces of benzylaniline was also found. Thus when analyzing antazoline in blood or tissue it must be avoided to keep aqueous, neutral or alkaline solutions stored or to heat such solutions.

Acid hydrolysis for release of eventual conjugated metabolites as described under methods did not destroy antazoline (more than 90% recovered and none of the mentioned hydrolysis products appeared).

The recovery of antazoline added to ethanol extracts of liver is by our described method about 80%. Trace amounts of (Phenylbenzyl-amino-acetyl)-ethylenediamine also appeared according to thin-layer chromatography. Rf values and spectrophotometrical data are shown in Table 1. The ultraviolet spectra are shown in Fig. 1a—c.

Toxicological Findings

The results of the analysis performed on tissues and blood are listed in Table 2.

Thin-layer chromatographic and spectrophotometric investigations of the extracts after acid hydrolysis (see methods) revealed no conjugated metabolites.

The identity of the compounds found was established by mass spectrometry. The mass spectra are shown in Figs. 2—4. As seen all spectra of the substances isolated from kidney tissue agree well with their respective reference spectra. The identity of (Phenyl-benzyl-amino-acetyl)-ethylenediamine isolated from kidney tissue was also confirmed by high resolution mass spectrometry (calculated mass 283.1685, found mass 283.1681).

The amount of (Phenyl-benzyl-amino-acetyl)-ethylenediamine found can perhaps to some extent be due to post mortem hydrolysis and hydrolysis during

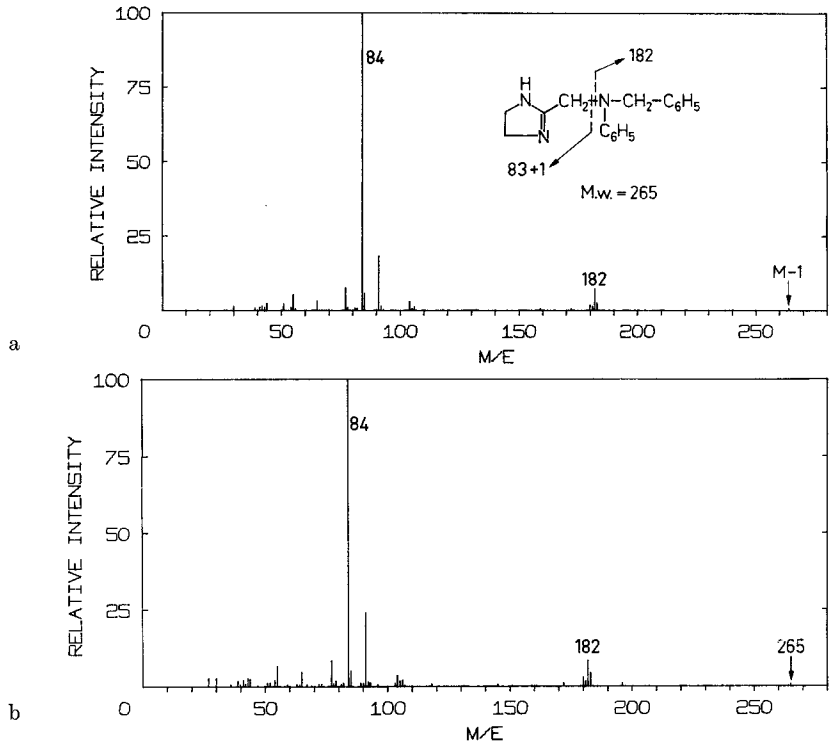


Fig. 2. a Reference mass spectrum of antazoline. b Mass spectrum of compound isolated from kidney tissue. Column temperature was 180°C in both cases

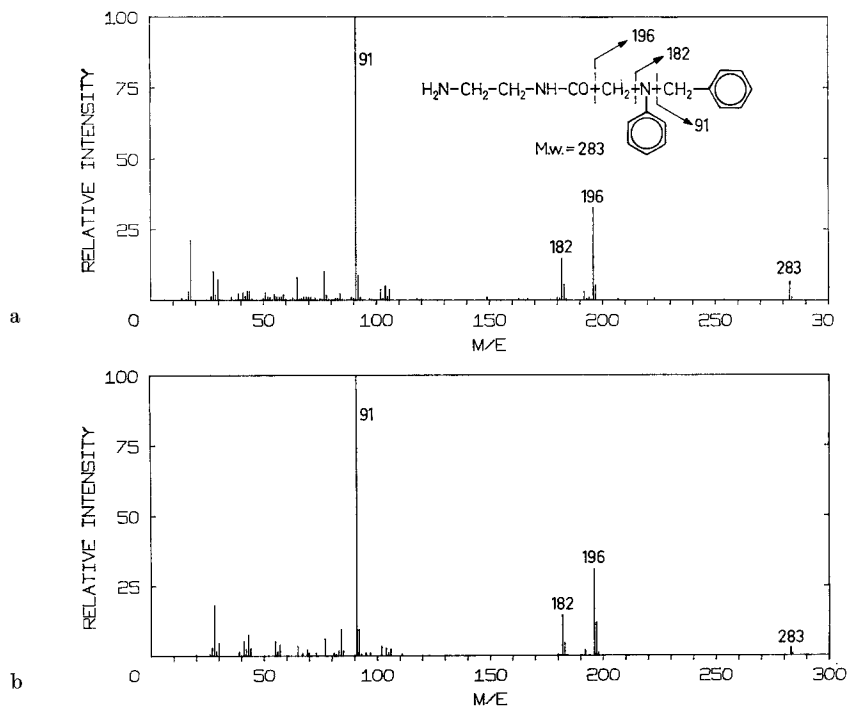


Fig. 3. a Mass spectrum of (Phenyl-benzyl-amino-acetyl)-ethylenediamine prepared by boiling antazoline with water according to Miescher *et al.* [12]. b Mass spectrum of compound isolated from kidney tissue. In both cases the direct inlet systeme was used

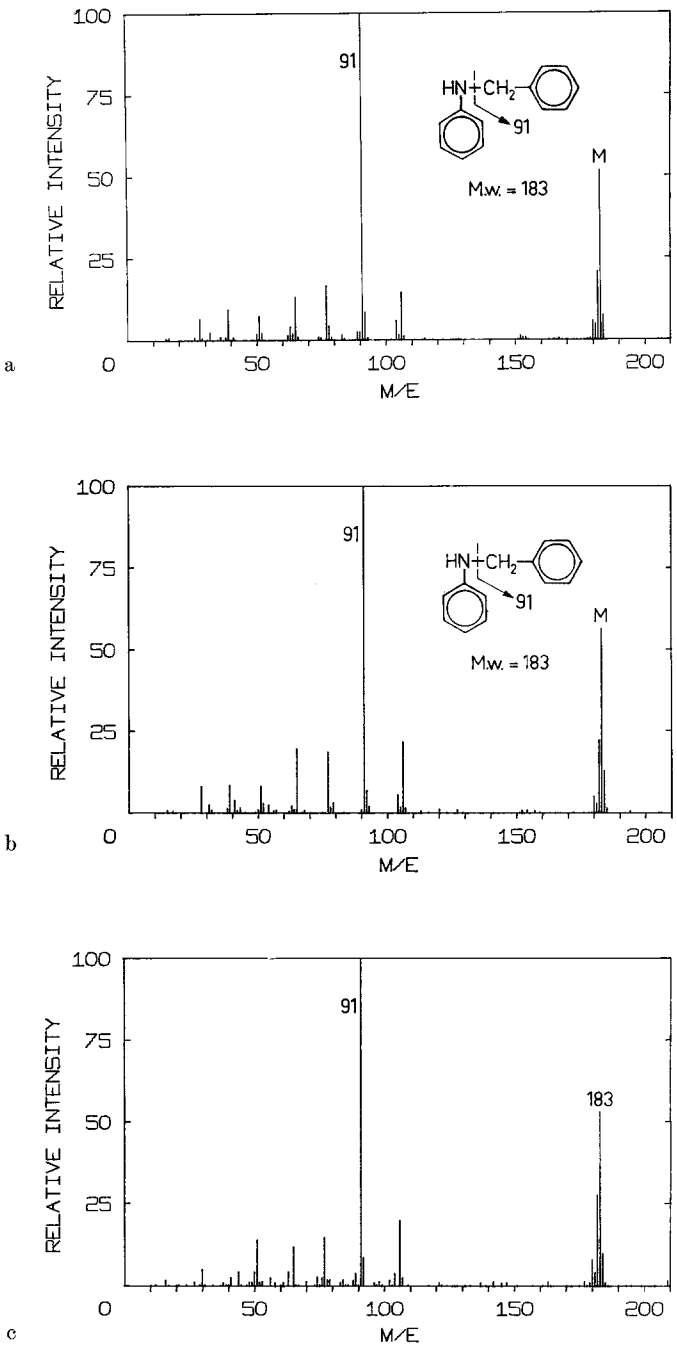


Fig. 4. a Reference mass spectrum of benzylaniline. b Mass spectrum of the same compound produced by alkaline hydrolyzis of antazoline (see text). c Mass spectrum of compound isolated from kidney tissue. Column temperature was 130°C in all cases

storage and analysis of blood and tissues. All material was stored at +4°C after received at our laboratory.

Screening procedure according to Maehly *et al* [13] (somewhat modified) performed on liver tissue revealed no other drugs than antazoline, 0.06% ethanol was, however, found in blood. Police report and autopsy did not suggest an other cause of death than an overdose of antazoline.

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