Selective Estimation of Aconitine in Presence of Aconine and Benzoylaconine

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Abstract □ A selective and simple colorimetric method is presented for the estimation of aconitine in drugs in the presence of aconine and benzoylaconine. The method is based on the formation of an iron hydroxamate complex through the acetate ester group to which the biological activity is due. The color is measured at 530 nm (5–250 μg/ml). Under the experimental conditions, neither the benzoyl group of benzoylaconine nor aconine is involved in the process of hydroxylaminolysis.

Keyphrases □ Aconitine—colorimetric analysis, pharmaceutical formulations in presence of aconine and benzoylaconine □ Colorimetry—analysis, aconitine, pharmaceutical formulations in presence of aconine and benzoylaconine □ Alkaloids—aconitine, colorimetric analysis, pharmaceutical formulations in presence of aconine and benzoylaconine □ Analgesics, topical—aconitine, colorimetric analysis, pharmaceutical formulations in presence of aconine and benzoylaconine

The effectiveness of tincture of aconite in the local relief of neuralgia, sciatica, acute rheumatism, and toothache is mainly attributed to aconitine (acetylbenzoylaconine). Since aconitine gives no specific color reaction, most analytical methods depend on the determination of its relative toxicity (1–3). Other assay methods are gravimetric (4), aqueous (5, 6) and nonaqueous (7) titrimetric, and spectrophotometric (8). Paper chromatography in combination with UV spectrophotometry (9) also was reported for the assay of different aconite alkaloids.

The aim of this work was to develop a rather simple colorimetric method for the selective determination of aconitine in the presence of other aconite alkaloids such as aconine and benzoylaconine. Since the acetyl group of aconitine is essential for its biological activity (10), it was necessary to seek an assay involving the reaction of this group. Therefore, the hydroxamic acid test (11) was optimized for the quantitative assay of aconitine.

EXPERIMENTAL

Materials and Reagents—Aconite extract[1] and aconite tincture, French Codex 1949, were used as received.

Aconitine Standard Solution—Dissolve 100 mg of aconitine[2] in 100 ml of absolute ethanol.

Acetate Buffer, pH 1.5—Adjust 0.1 *M* sodium acetate to pH 1.5 with 70% perchloric acid.

Alkaline Hydroxylamine Hydrochloride Reagent (11)—Prepare Solution 1, 12.5% (w/v) methanolic sodium hydroxide, by refluxing 12.5 g of sodium hydroxide in absolute methanol for 5 min. Prepare Solution 2, 12.5% (w/v) methanolic hydroxylamine hydrochloride, by refluxing 12.5 g of hydroxylamine hydrochloride in absolute methanol for 5 min. Mix equal volumes of Solutions 1 and 2 and filter.

Ammonium Ferric Sulfate Reagent—Dissolve 20 g of ammonium ferric sulfate dodecahydrate in 100 ml of 70% perchloric acid.

Determination of Aconitine in Standards—Transfer 0.1, 0.25, 0.5, 1, 2, 4, 5, and 6 ml of aconitine standard solution to separate 25-ml flasks, and dilute to about 8 ml with absolute ethanol. Add 3 ml of alkaline hydroxylamine reagent and place the flasks in a water bath at 40–45° for 30 min. Then remove the flasks from the water bath and cool to room temperature. Add gradually 5 ml of the ammonium ferric sulfate reagent with continuous stirring. Rapidly transfer the contents of the flasks into 25-ml volumetric flasks with acetate buffer, and adjust the volume to 25 ml with the same buffer. Directly read the absorbance at 530 nm against a reagent blank.

A calibration curve of 5–250 μg of aconitine/ml *versus* color intensity obeyed Beer's law with a standard deviation of ±0.8%.

Determination of Aconitine in Products—*Extract of Aconite*—Measure accurately 1 ml of extract, dilute to 5 ml with distilled water, render alkaline with dilute ammonia solution, and extract with 20-, 10-, 10-, and 10-ml portions of ether. Dry the combined ether extract with anhydrous sodium sulfate, distill the ether to dryness, and dissolve the residue in 5 ml of absolute ethanol. Complete the assay as described for standards, beginning with: "Add 3 ml of alkaline . . . ."

Tincture of Aconite—Measure 10 ml of tincture, concentrate to 1–2 ml, and complete the assay as under *Extract of Aconite*.

[1] Les Laboratoires Givaudan, Lavirotte & Cie, Lyon, France.
[2] E. Merck, Germany.

Table I—Assay of Aconitine by Proposed Method in Synthetic Mixtures of Aconitine and Benzoylaconine

Mixture		
Aconitine, mg	Benzoylaconine, mg	Aconitine Found, mg
3	1	2.98
2	2	2.00
1	3	0.99
2	1	1.98

RESULTS AND DISCUSSION

Aconitine and benzoylaconine cannot be separated from each other by any of the usual methods (6). However, the hydroxamic acid method proved to be selective for aconitine; benzoylaconine did not interfere under the specified conditions. Application of the hydroxamic acid method to benzoylaconine, prepared according to Majima *et al.* (12), showed that hydroxylaminolysis of benzoylaconine required a longer time and a higher temperature. Moreover, investigation of the visible spectrum of the iron hydroxamate of benzoylaconine showed λ_{max} 558 nm, which was sufficiently far from that of aconitine–iron hydroxamate (530 nm).

With synthetic mixtures of both aconitine and benzoylaconine, the hydroxamic acid method under the specified conditions gave only the expected amount of aconitine (Table I). Also, aconine, being an amino alcohol, is very soluble in water and, unlike aconitine, is unextractable with ether. Therefore, it does not interfere. Furthermore, aconine does

Table II—Assay of Aconitine in Aconite Preparations by Proposed Method

Preparation	Amount Declared, mg/ml	Amount Found, mg/ml	Amount Added, mg/ml	Amount Found, mg/ml	Recovery, %
Extract of aconite	3.00	3.60	2.00	5.580	99
Tincture of aconite	0.320	0.350	0.500	0.844	98.8
Average recovery					98.9
SD					0.1

not contain an ester group or any other group that may react with the hydroxylamine reagent.

Therefore, the method is rather specific and is preferably used in the analysis of aconitine in aconite preparations that generally contain benzoylaconine and aconine. The method showed good recovery (99.8%) and a reasonable standard deviation (±0.8%) (Table II).

REFERENCES

(1) C. C. Haskell, *Am. Drug.*, **4**, 596 (1916).

(2) R. Paris and J. Vavasseur, *Ann. Pharm. Fr.*, **9**, 718 (1951).

(3) "The United States Pharmacopeia," 10th rev., Lippincott, Philadelphia, Pa., 1926.

(4) M. Ribaut, *Bull. Sci. Pharmacol.*, **17**, 634 (1910).

(5) "The Egyptian Pharmacopoeia" (The English Text), Cairo University Press, Cairo, Egypt, 1963.

(6) G. Baker and C. B. Jordan, *J. Am. Pharm. Assoc., Sci. Ed.*, **25**, 291 (1936).

(7) L. G. Chatten, M. Pernarowski, and L. Levi, *ibid.*, **44**, 332 (1955).

(8) E. Berman and H. N. Wright, *Arch. Ind. Hyg. Occup. Med.*, **8**, 518 (1935).

(9) H. Yoshida, S. Kuwano, Y. Matsunaga, and S. Takagi, *J. Pharm. Soc. Jpn.*, **85**, 709 (1965).

(10) F. Bamford, "Poisons: Their Isolation and Identifications," 3rd ed., J. and A. Churchill Ltd., London, England, 1951, p. 41.

(11) R. F. Goddu, N. F. Le Blanc, and C. M. Wright, *Anal. Chem.*, **27**, 1251 (1955).

(12) R. Majima, H. Suginome, and S. Mario, *Ber.*, **57**, 1456 (1924).

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Synthesis and Antituberculosis Activity of Thiocarboxamide Derivatives of Schiff Bases

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Abstract □ Synthesis of some *N*-(4-thiocarboxamidobenzylidene)arylamines and *N*-(substituted benzylidene)-*p*-thiocarboxamidoanilines from various arylaldehydes and arylamines is described. Fourteen representative compounds were tested *in vitro* on the ground $H_{37}RV$.

Keyphrases □ Thiocarboxamides, various—synthesized, screened for antituberculosis activity □ Antituberculosis activity—screened in various thiocarboxamide derivatives □ Structure–activity relationships—various thiocarboxamide derivatives screened for antituberculosis activity

In a search for antituberculosis agents (1–3), some thiocarboxamide derivatives of Schiff bases of series I and II were prepared from various arylaldehydes and arylamines and studied for biological activity.

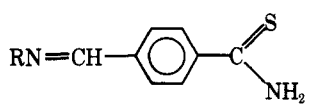
EXPERIMENTAL[1]

An attempt was made to prepare Schiff bases of series I through the thiolysis of corresponding nitriles (III) in anhydrous medium, but the reaction took an unusual path, yielding disulfide IV (4) (Scheme I). Consequently, the following route was established to synthesize the compounds: preparation of the key intermediate, *p*-thiocarboxamidobenzaldehyde, from *p*-cyanobenzylidene acetate, followed by condensation of the key intermediate with amines.

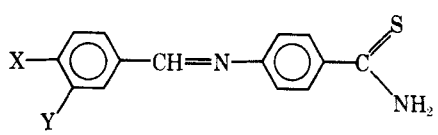
Schiff bases of series II were obtained by heating *p*-thiocarboxamidoaniline with aromatic aldehydes.

Fourteen representative compounds were tested *in vitro* on the ground $H_{37}RV$. None showed significant antituberculosis activity against either 0.01 or 0.05 mg of the bacillus at 40 μg/ml.

***p*-Cyanobenzylidene Acetate (V)**—Compound V was obtained by the method of Lieberman and Connor (5). The crude product was crystallized from alcohol and obtained in 73% yield, mp 100°.



Ia: R = C_6H_5
Ib: R = *p*-IC_6H_4
Ic: R = *p*-ClC_6H_4
Id: R = $C_6H_5CH_2$
Ie: R = *p*-$CH_3C_6H_4$
If: R = *p*-$CH_3OC_6H_4$
Ig: R = *p*-$C_2H_5OC_6H_4$
Ih: R = *p*-$CONH_2C_6H_4$



IIa: X = Y = H
IIb: X = CH_3, Y = H
IIc: X = Cl, Y = H
IId: X = CH_3O, Y = H
IIe: X = $(CH_3)_2N$, Y = H
IIf: X = H, Y = NO_2
IIg: X = S=CNH_2, Y = H

***p*-Thiocarboxamidobenzylidene Acetate (VI)**—Compound V (23.3 g, 0.1 mole) was dissolved in anhydrous pyridine (120 ml), and 20 ml of triethylamine was added. Dry hydrogen sulfide was passed through the solution for 4 hr. The reaction mixture was left at room temperature for 5 hr and was then poured into cold water and extracted with ether. The ether solution was treated with dilute hydrochloric acid, washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue, which was either solid or oily, was suitable for the next step. Recrystallization from methyl ethyl ketone gave yellow crystals in 70% yield, mp 220°.

Anal.—Calc. for $C_{12}H_{13}NO_4S$: C, 53.94; H, 4.87; N, 5.24; S, 11.99. Found: C, 53.55; H, 4.90; N, 5.40; S, 12.34.

***p*-Thiocarboxamidobenzaldehyde (VII)**—Hydrolysis of VI was

[1] Melting points were measured on a Kofler hot-bench apparatus. A Beckman IR-20A spectrophotometer was used for IR spectra, which were run in potassium bromide. Microanalyses were performed by Service Central de Microanalyse, 94-Thias, France.