

MICROCHEMICAL IDENTIFICATION AND DIFFERENTIATION OF AMARYLLIDACEAE ALKALOIDS

Part II: Trispheridine, 6- α ethoxybuphanisine, 6- α ethoxy-
crinine, 3-O-acetylpowelline, 3-O-acetylcrinine,
Cherylline, flexinine, bulbisine and augustisine.

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ABSTRACT

Microchemical identification of nine minor Amaryllidaceae alkaloids, trispheridine, 6- α -ethoxybuphanisine, 6- α -ethoxycrinine, 3-O-acetylpowelline, 3-O-acetylcrinine, cherylline, bulbisine, flexinine, and augustisine by microcrystal techniques and colour reactions is described.

INTRODUCTION

In Pharmacognosy Department, University of Assiut there is a great interest in the study of the alkaloids of the Amaryllidaceae plants cultivated in Egypt¹⁻¹⁴.

The Amaryllidaceae alkaloids are classified into 12 types. Each type comprises many closely related alkaloids which have similar or very near ranges of R_f values and melting points specially the isomers.

These alkaloids have minor variation in the degree of hydrogenation, oxygenation or aromatic and cyclohexene substitution in the given ring system.

Identification of such alkaloids by classical methods through the formation of some derivatives e.g. picrate, hydrochloride, O-acetyl... etc., mixed melting point, determination of optical rotation and carrying out the spectral analysis (UV, IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and MS) is somewhat difficult and time consuming, especially for those alkaloids which are obtained in microquantities.

Material :

Pure alkaloids trispheridine, 6- α -ethoxybuphanisine, 6- α -ethoxycrinine, 3-O-acetylpowelline, 3-O-acetylcrinine, bulbisine and cherylline isolated from Crinum bulbispermum Milne, and the alkaloids flexinine and augustisine isolated from Crinum augustum Rox. are used in this work. The purity of these alkaloids was verified by using UV and TLC.

EXPERIMENTAL AND RESULTS

A- Microcrystallisation^{15,16} :

A drop of the reagent was added to a drop of the test solution on a flat clean slide without application of a cover glass. Each aqueous test solution contained about 0.1 % of the alkaloid in 2 % HCl. The reagents used are; picric acid, ammonium reineckate, Wagner's reagent, Marme's reagent, mercuric chloride, gold chloride and potassium ferrocyanide.

The results are cited in Table 1 and the photograph of the crystals are shown in Fig. 1.

*Microchemical Identification and Differentiation
of Amaryllidaceae Alkaloids*

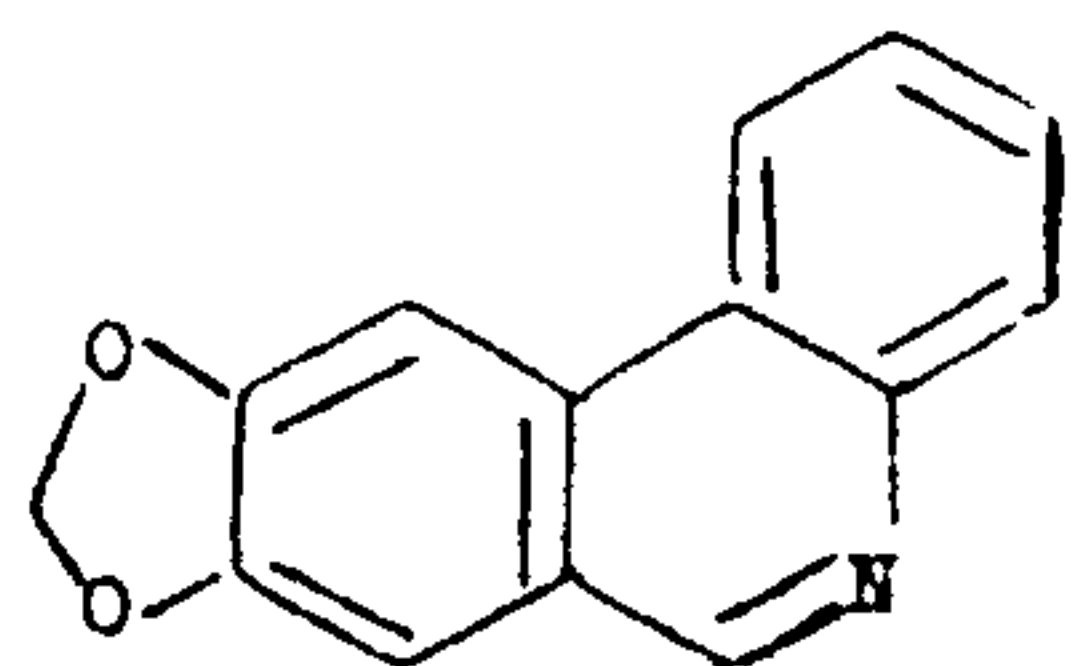
B- Colour reactions :

Few drops of each reagent (Table 2) were added separately to few crystals of the alkaloids, trispheridine, flexinine, 3-O-acetylpowelline, 3-O-acetylcrinine, bulbisine, cherylline and augustisine and to the residue of 6- α -ethoxybuphanisine and 6- α -ethoxycrinine in clean porcelain slap.

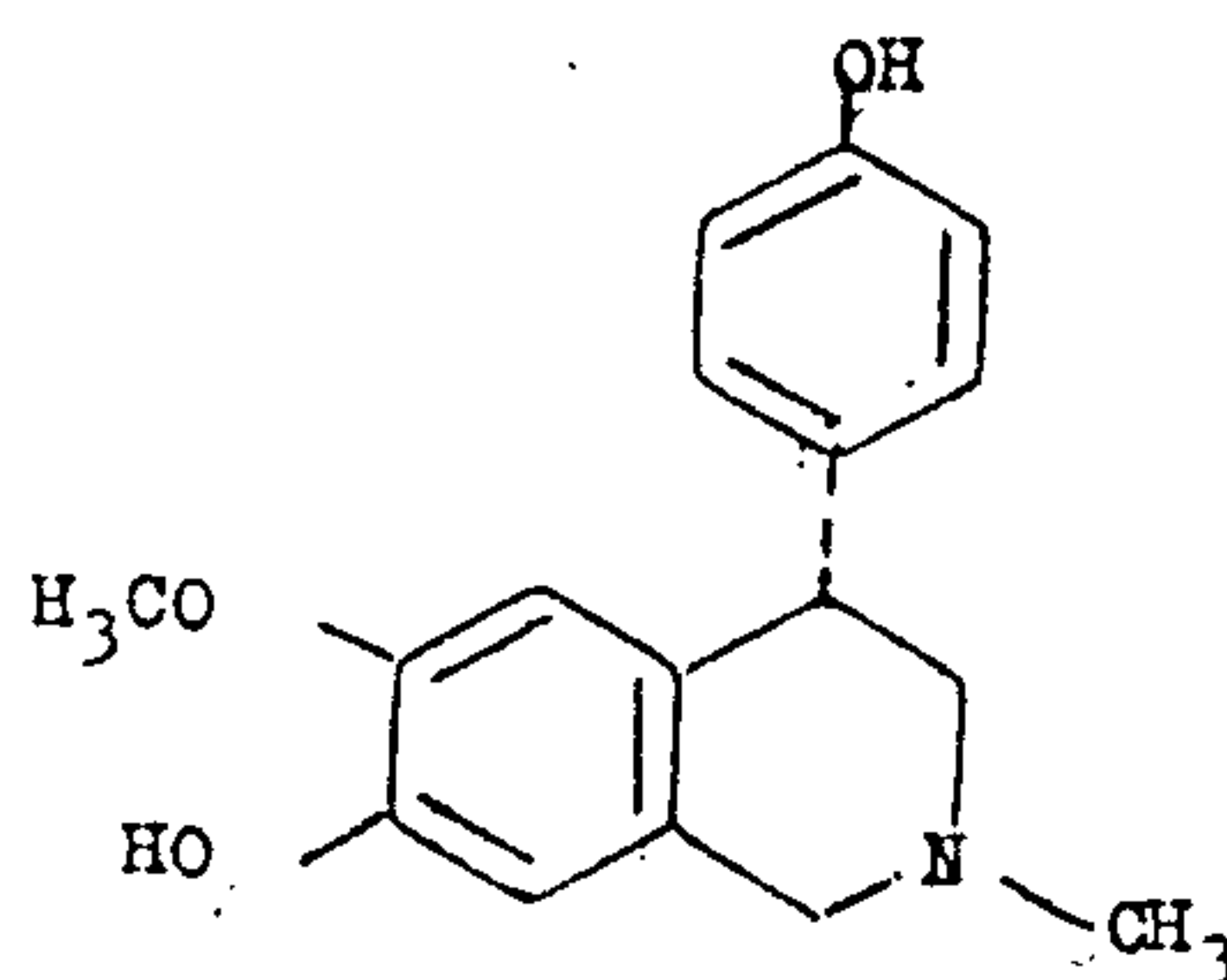
DISCUSSION

All the investigated alkaloids belong to the (-) crinane skeleton (5,10-b-ethanophenanthridine nucleus) except cherylline and trispheridine. Therefore the identification and differentiation of such closely related alkaloids (Fig. 2) separated in minor quantities is carried out by using the microcrystal technique.

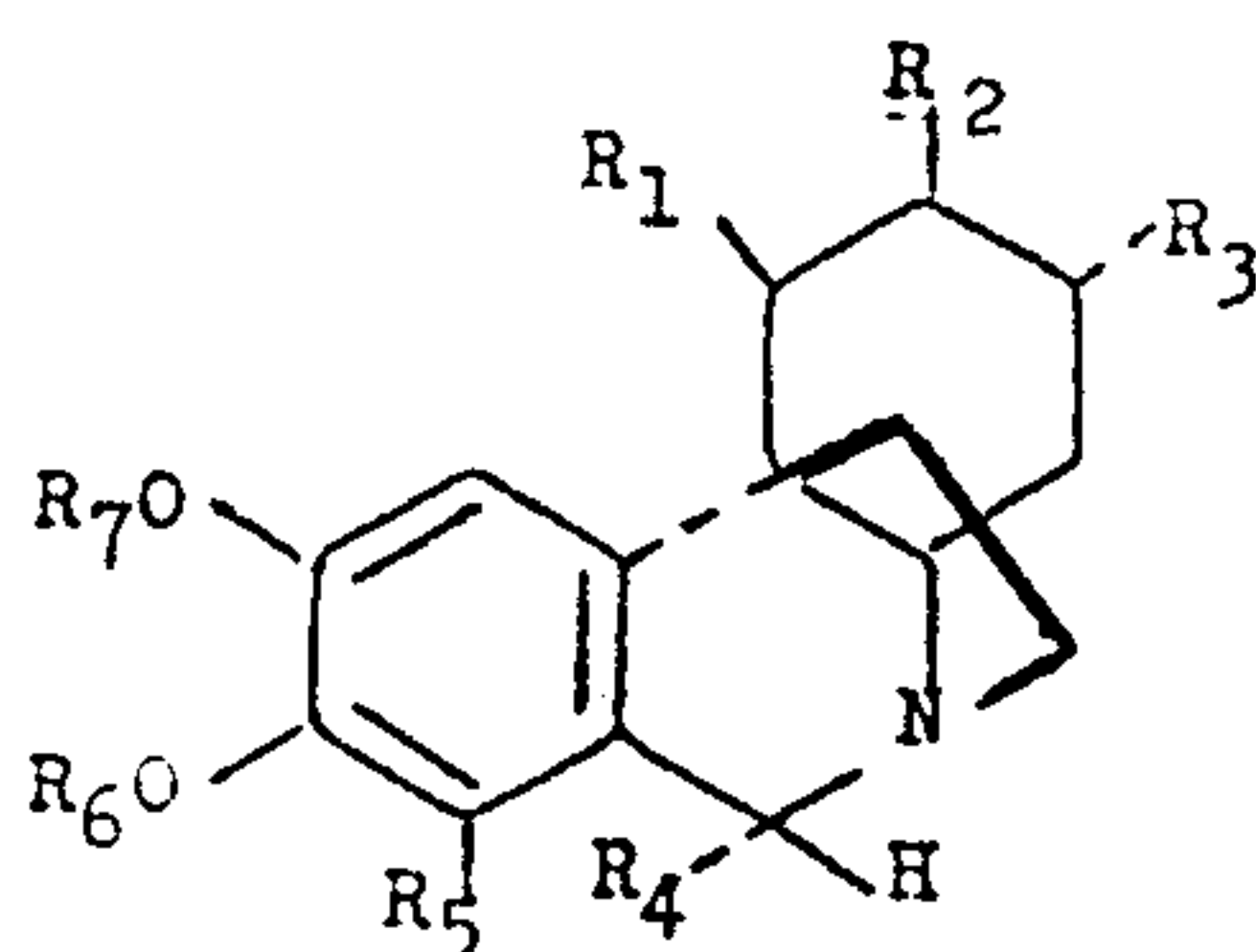
However the colour reactions were less specific than microcrystal tests and thus may be suitable for preliminary testing or adding an additional character to the identification of the investigated alkaloids.



Trispheridine
mp 139-140°C
m.W 223.0631



Cherylline
mp 212-213 °C
m.w 285.1316



Alkaloid	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	R ₇	m.p	mol. wt.
6- O -ethoxybuphanisine			OCH ₃	OCH ₂ CH ₃	H	-CH ₂ -		---	329.1622
6- O -ethoxycrinine			OH	OCH ₂ CH ₃	H	-CH ₂ -		---	315.1727
3-O-acetylpowelline			OH	H	OCH ₃	-CH ₂ -		59-60	343.1420
3-O-acetylcrinine			OH	H	H	-CH ₂ -		140-1	313.1316
Bulbisine	OH	OH	H	H	OCH ₃	-CH ₂ -		182-3	319.1422
Flexinine	1,2epoxy		OH	H	H	-CH ₂ -		233-5	287.1167
Augustisine			OCH ₃	H	OCH ₃	OH	OCH ₃	173-5	287.1527

Figure 2: Structure of the investigated alkaloids.

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Table 1 : Forms of Crystals

Alkaloid	Forms of Crystals with						
	Picric acid	Amn. reineckate	Wagner's reagent	K. ferrocyanide	HCl ₂	Marme' reagent	HAuCl ₄
Tripheredine	Insect clusters, mosquito clusters	Needles scattered at the edge of the drop	-ve	-ve	-ve	-ve	-ve
6- α -ethoxy-buphanisine	Large rods	-ve	Diamond shaped tablets	-ve	-ve	-ve	-ve
6- α -ethoxy-crinine	Straight sided narrow blades	-ve	-ve	highly angular blades	-ve	-ve	-ve
3-O-acetyl powelline	Clusters of separate plates	Spidder prisms	-ve	-ve	Characteristic rosettes of needles	-ve	-ve
3-O-acetylerinine	Complex branching aggregates of dendrites	-ve	-ve	-ve	needles in fans	-ve	-ve
Cherrylline	Serrate irregular blades	-ve	-ve	-ve	long, large rods	Rectangular and square blades	-ve
Bulbisine	Trapezoids	Stem branching needles	-ve	-ve	-ve	-ve	-ve
Ploxinine	Paddle-shaped crystals	-ve	-ve	-ve	-ve	-ve	Tripartite crystals and staples
Augustisine	Large needles	Rectangular plates	-ve	-ve	-ve	-ve	-ve

Table 2 : Colour Reactions

Alkaloid	Mandalin's Reagent	Froed's Reagent	Marguies Reagent	Erdmann's Reagent	Conc. HNO ₃
Trispheridine	red	yellow	yellow	yellow	yellow
6- α -ethoxybuph nistine	violet \rightarrow red \rightarrow yellow	green \rightarrow yellow	yellow	violet \rightarrow yellow	yellow
6- α -ethoxycrinine	violet \rightarrow yellow	-----	-----	-----	yellow
3-O-acetylpowelline	violet \rightarrow green	bluish violet	violet	violet	yellow
3-O-acetylcrinine	red \rightarrow brown	yellow \rightarrow green	-----	yellow	yellow
Cherylline	red \rightarrow dirty green \rightarrow yellow	reddish violet \rightarrow yellow \rightarrow yellowish green	violet	pink	yellow
Bulbisine	violet \rightarrow brown \rightarrow bluish green	emerald green \rightarrow brownish yellow	brick red	reddish-brown	yellow
Flexinine	orange	violet \rightarrow bluish green	pale yellow	yellow	yellow \rightarrow orange
Augustisine	violet	violet \rightarrow brown \rightarrow bluish green	pale yellow	yellow	yellow

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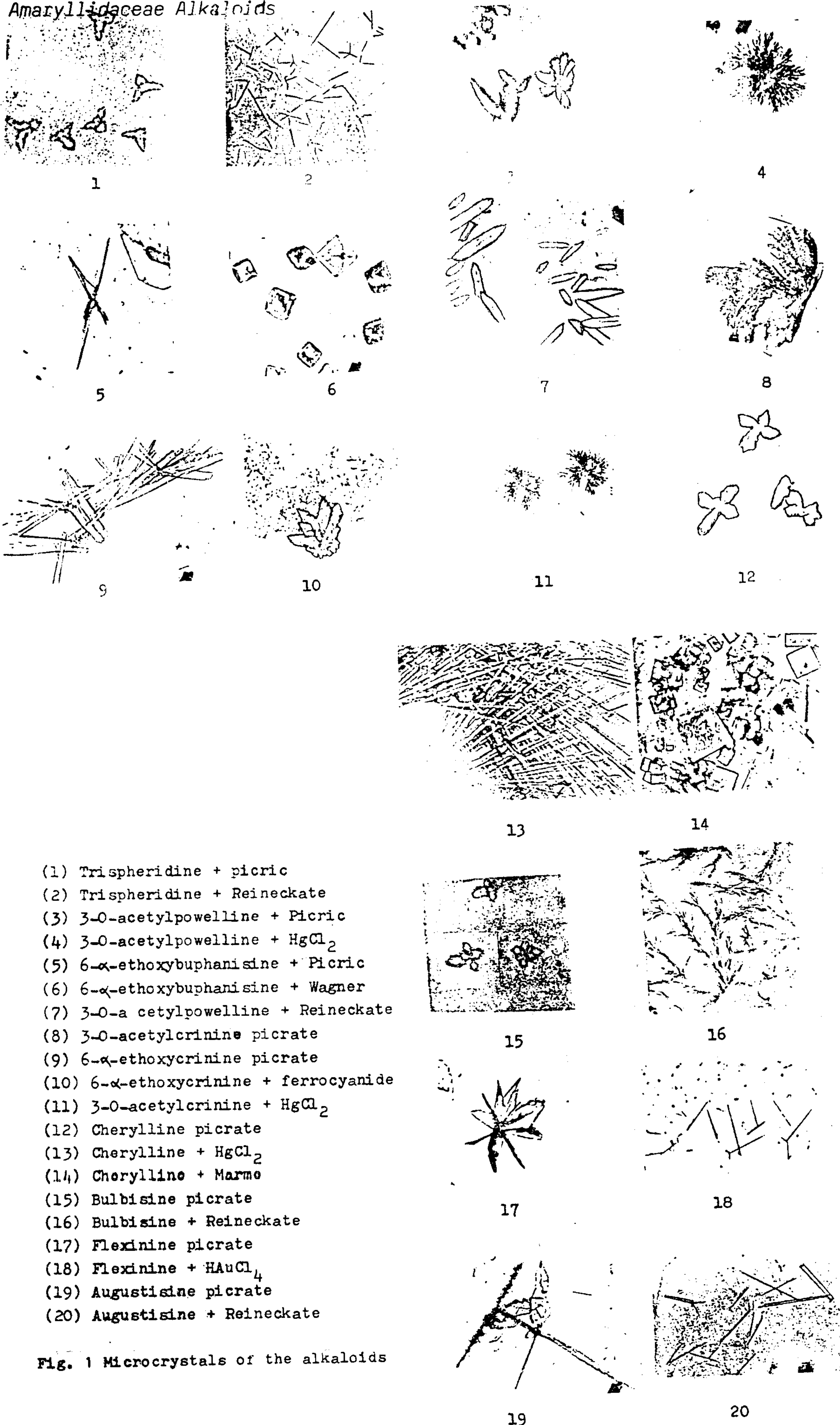


Fig. 1 Microcrystals of the alkaloids

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الدراسة البلورية والتفاعلات اللونية
لبعض قلويدات الفصيلة النرجسية

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تم تكوين بالورات ذات صفات مميزة للقلوانيات الاتية :

- ١ - تريسرديين
- ٢ - ٦ - الفا - ايثوكسي بوفانيزين .
- ٣ - ٦ - الفا - ايثوكسي كرينين .
- ٤ - ٣-أ - خلاات البوليين
- ٥ - ٣-أ - خلاات الكرينين
- ٦ - شيريلين
- ٧ - بلبيزين
- ٨ - فلكسينين .
- ٩ - أوحستيزين .

وذلك باستخدام المحاليل مرساب القلوانيات وتم فحصها مجهريا . وكذلك عمل
تفاعلات الالوان لها وذلك بقصد التعرف عليها وخصوصا اذا تم الحصول عليها بكميات
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