

STUDY OF ALKALOIDS OF *PANCERATIUM MARITIMUM* L.
GROWING IN EGYPT

A.A. Ali, M.K. Mesbah & M.H. Mohamed
Department of Pharmacognosy, Faculty of Pharmacy, University
of Assiut, Assiut, Egypt.

ABSTRACT

The main alkaloids, viz., tazettine, pancracine, lycorine and hippadine were isolated in pure crystalline forms and their identity was confirmed through determining their physical, chemical and spectral analysis. In addition, four bases were isolated in too small quantities for identification, their picrate derivatives were prepared and investigated.

Identification of micro-quantities of the isolated compounds by microchemical techniques is described.

INTRODUCTION

Pancreatum maritimum L. (Amaryllidaceae) is a perennial herb grows wild in different localities in Egypt¹. It is also cultivated as a garden plant for its bright white flowers.

Earlier investigation on Pancreatum maritimum L.²⁻¹⁰ have resulted in the isolation of numerous alkaloids including caranine, demethylhomolycorine, dihydrolycorin, galanthamine, haemanthamine, haemanthidine, hippeastrine, homolycorine, hordenine, lycorine, N-methyl tyramine, norphlrvine, pancracine, pseudolycorine, tazettine, tyramine, and undulatine. Some of these alkaloids possess biological activity¹¹⁻¹³.

It is well known that plants having biological activity play a great role, both from the health and economic points of view. Therefore it was deemed of interest to carry out a chemical study on Pancratium mritimum L. in order to detect, separate and identify any biologically active compounds which have not been isolated before. This article deals with the investigation of alkaloidal content of Pancratium maritimum L.

EXPERIMENTAL AND RESULTS

Plant Material:

It was collected in September 1980 from plants cultivated in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Assiut University, Assiut. The whole plant was air-dried in shade, then powdered (No. 36).

Extraction and fractionation of alkaloids and/or basic nitrogenous compounds:

The air-dried powdered plant (30 kg) was extracted to exhaustion with ethanol 90% by percolation and the dried ethanol extract was partitioned in the usual manner¹⁴ to afford a crude basic fraction I (30 g.). This fraction was treated with 200 ml methanol where most of the residue went in solution leaving the alkaloid lycorine¹⁴ (7g), mp 270-272°C (methanol). The identity of this alkaloid was confirmed by direct comparison (ir, mp, mmp) with an authentic reference sample.

TLC of the mother liquor (fraction II), left after separation of lycorine on silica gel G using chloroform:methanol(9:1) as a solvent system revealed the presence of at least 14 bases (table 1).

*Study of alkaloids of *Pancreatium maritimum* L. growing in Egypt*

Fraction II (21 g) was subjected to fractionation on alumina column (600 g). Elution was started with petroleum ether (b.r. 50-70°C), then pet. ether with gradually increasing proportions of ethyl acetate, ethyl acetate, ethyl acetate with gradually increasing proportions of methanol and finally methanol. This resulted in the separation of the base A (Table 2). The effluent fractions containing more than one alkaloid were grouped according to TLC similar pattern (7 groups). Each group was fractionated on silica gel column (200 g) using chloroform: methanol in different proportions. This results in the separation of base A, B, C, D, H and M (Table 3).

The fractions of similar contents were again mixed and fractionated on silica gel G preparative layer using chloroform: methanol (9:1) as a solvent system. This resulted in the separation of base J.

Characterization of the isolated compounds:

Base A (300 mg): It occurs as colourless, odourless, bitter, needle-shaped crystals, mp 214-215°C (acetone:methanol) It gives grey colour reaction with Dragendorff's reagent and a blackish grey colour with iodine. These properties coincide with those reported for the alkaloid hippadine¹⁴. The identity was confirmed by direct comparison (IR, H-NMR, MS, mp & mmp) with an authentic reference sample and literature^{14,15}.

Base H (350 mg): mp 208-209 (acetone). It was identified as tazettine by direct comparison (IR, H-NMR, MS and mp) with literature data^{16,17}.

Base M (320 mg): mp 271-273°C (methanol). It was identified as the alkaloid pancracine^{7,18,19} by direct comparison (IR, mp and mmp) with authentic reference sample.

The bases B,C,D and J were isolated in too small quantities for identification, their picrate derivatives were prepared and investigated.

Microchemical identification of the isolated compounds:

A-Characterization by colour reactions: Few drops of each reagent were added separately to few crystals of the alkaloids hippadine, tazettine and pancracine in a porcelain slab. The results are recorded in Table 5.

B-Microcrystallization: A drop of the aqueous test solution was mixed a drop of the reagent on a plain clean slide without application of a cover glass. Each aqueous test solution contained about 0.1% of the alkaloid and 1% sulphuric acid. The reagents used are picric acid, ammonium reineckate, Mayer's, Wagner's, Kraut's, Marme's, mercuric chloride, zinc chloride, disodium hydrogen phosphate and ammonium hydroxide²⁰.

Picric acid proved to be useful for the identification of all isolated compounds and ammonium reineckate gave characteristic crystals with tazettine only. The results are given in Table 4 and the photographs of the crystals are shown in Fig. 1 and 2.

DISCUSSION

Extraction, fractionation and chromatography of Pancratium maritimum L. has now resulted in the reisolation of the alkaloids tazettine, pancracine and lycroine and the isolation of hippadine. The last alkaloid has only recently been isolated from four Amaryllidaceae plants¹⁵ and to our knowledge this represents the first reported isolation of this alkaloid from a Pancratium species. This result supports the proposal of Ali et al¹⁵ that the presence of hippadine in Amaryllidaceae

*Study of alkaloids of *Pancreatium maritimum* L. growing in Egypt*

plants may be more abundant than has been reported. This proposal was based on the assumption that hippadine might be biogenetically linked to its structurally related lycorine which is of common occurrence in the members of Amaryllidaceae.

Since the identification of microquantities of alkaloids was described to be possible by microcrystallisation and tests^{20,21}, these were applied to the compounds B,C,D and J, from each only a microquantity was obtained and was not adequate for further identification by the classical methods. These techniques were also applied to hippadine, tazettine and pancracine.

The microcrystal tests were proved to be highly efficient in identification of the investigated compounds specially in the differentiation between the closely related compounds B and C which nearly the same mp. However, the colour reactions were less specific. These techniques were proved to be simple, rapid and do not require such high purity as needed for classical methods of identification.

Table 1: TLC of fraction II.

Base	R_f	Colour with Dragendroff's reagent	Relative amount
A	0.99	grey	+++
B	0.97	orange	+
C	0.95	"	+
D	0.92	pink	+
E	0.86	Orange	+
F	0.81	"	+
G	0.77	"	+
H	0.73	"	++++
I	0.67	"	+
J	0.60	"	+
K	0.44	"	+
L	0.34	"	+
M	0.20	"	+++
N	0.12	"	+

Silica gel G, Chloroform:Methanol (9:1).

Table 2: Fractionation of the crude alkaloidal residue {fraction II}

Group No.	Fraction No.	Solvent system	Volume ml.	Separated substance	R_f value
	1-5	pet.ether	5 x 250 ml	-	-
I	6-10	pet.ether	5 x 250	{A}	0.94
II	11-12	pet.ether	2 x 250	A	0.94
				B'	0.93
				C	0.91
III	13-32	2% ethyl acetate in pet.ether	20x 250	C	0.91
				D	0.82
				E	0.63
				F	0.58
				G	0.56
IV	33-43	5% ethyl acetate in pet.ether	11x 250	F	0.58
				G	0.56
				H	0.50
				I	0.35
V	44-52	8% ethyl acetate in pet.ether	9 x 250	H	0.50
				I	0.35
				J	0.30
				K	0.26
VI	53-61	10% ethyl acetate in pet.ether	9x 250	J	0.30
				K	0.26
				L	0.17
VII	62-80	15% ethyl acetate in pet. ether	19 x250	K	0.26
				L	0.17
				M	0.13
				N	0.09

Study of alkaloids of *Pancreatium maritimum* L. growing in Egypt

Table 3: Fraction of Group II, III, IV, V and VII

Group No.	Fraction No.	Eluent	Volume ml.	Separated substance	R _f value
II	3-4	CECl ₃	2 x 50	{A}*	0.87
	5	CHCl ₃	1 x 50	A	0.87
				B	0.85
	6	CHCl ₃	1 x 50	{B}*	0.85
				7-9	CHCl ₃
III	1-5	1% CH ₃ OH in CHCl ₃	5 x 50	-	-
	6-10	2% CH ₃ OH in CHCl ₃	5 x 50	C	0.82
				D	0.74
	11-14	2% CH ₃ OH in CHCl ₃	4 x 50	{D}*	0.74
				15-19	3% CH ₃ OH in CHCl ₃
	20-25	4% CH ₃ OH in CHCl ₃	6 x 50	E	0.54
				F	0.52
{F}*				0.52	
IV	1-5	5% CH ₃ OH in CHCl ₃	5 x 50	F	0.52
				G	0.42
	6-9	6% CH ₃ OH in CHCl ₃	4 x 50	H	0.35
				{H}*	0.35
10-15	7% CH ₃ OH in CHCl ₃	6 x 50	H	0.35	
			I	0.29	
V	1-5	7% CH ₃ OH in CHCl ₃	5 x 50	H	0.35
				I	0.29
	6-10	8% CH ₃ OH in CHCl ₃	5 x 50	I	0.29
				J	0.21
	11-15	9% CH ₃ OH in CHCl ₃	5 x 50	J	0.21
				K	0.16
16-20	10% CH ₃ OH in CHCl ₃	5 x 50	J	0.21	
			K	0.16	
			L	0.11	
VII	1-5	11% CH ₃ OH in CHCl ₃	6 x 50	K	0.16
				L	0.11
	6-10	12% CH ₃ OH in CHCl ₃	5 x 50	{M}*	0.04
				11-15	13% CH ₃ OH in CHCl ₃
				N	0.01

* Separated compound.

Table 4: Picrate derivatives of the bases B,C,D and J

<i>Pictate derivatives</i>	<i>mp^oC</i>
B	217-219
C	118-120
D	206-209
J	157-159

Table 5: Colour reactions

<i>Alkaloid</i>	<i>Formaldehyde+ sulphuric acid</i>	<i>Ammonium vanadate + sulphuric acid</i>	<i>Ammonium molybdate sulphuric acid⁽¹²⁾</i>
Hippadine	Light brown	Violet	Orange brown
Tazettine	- ve	Greenish-black	Green
Panracine	- ve	Violet	Violet

Table 4: Forms of crystals

<i>Base</i>	<i>Picric acid</i>	<i>Ammonium reineckate</i>
Hippadine	Elongated plates, clustered (Fig. 1,a)	-ve
B	Trapezoids (Fig. 1,b)	-ve
C	Bird or insect-like crystals (Fig.1,c)	-ve
D	Chips, thick, tend to rectangular (Fig. 1,d)	-ve
Tazettine	Characteristic rosettes of needles and rods (Fig. 2,a)	Dendrites nearly circular in outline (Fig. 2,b)
J	Triangular plates (Fig. 2,c)	-ve
Panracine	Irregular blades, mottled (Fig. 2,d)	-ve

*Study of alkaloids of *Pancreatium maritimum* gL. growing in Egypt*

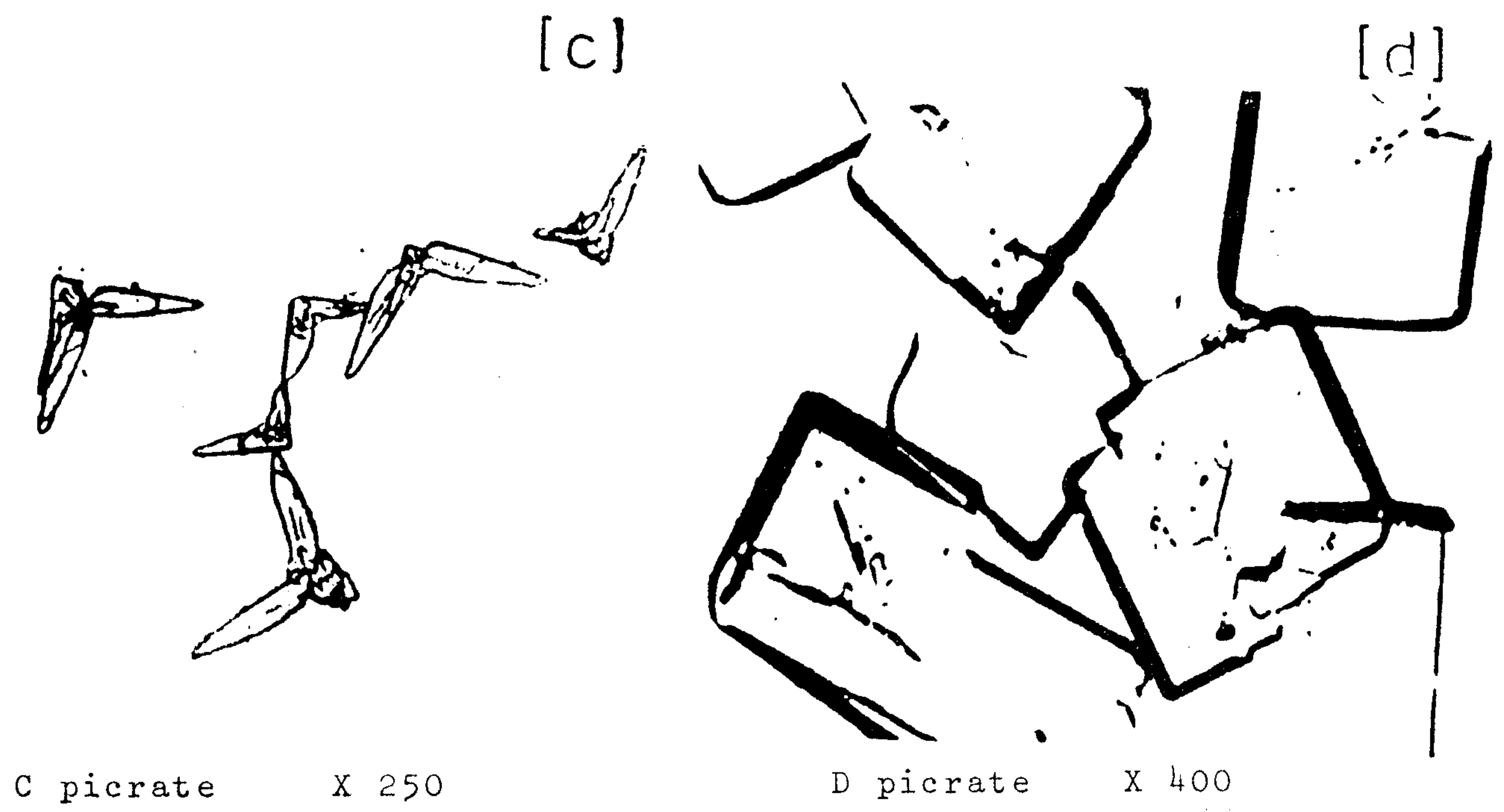
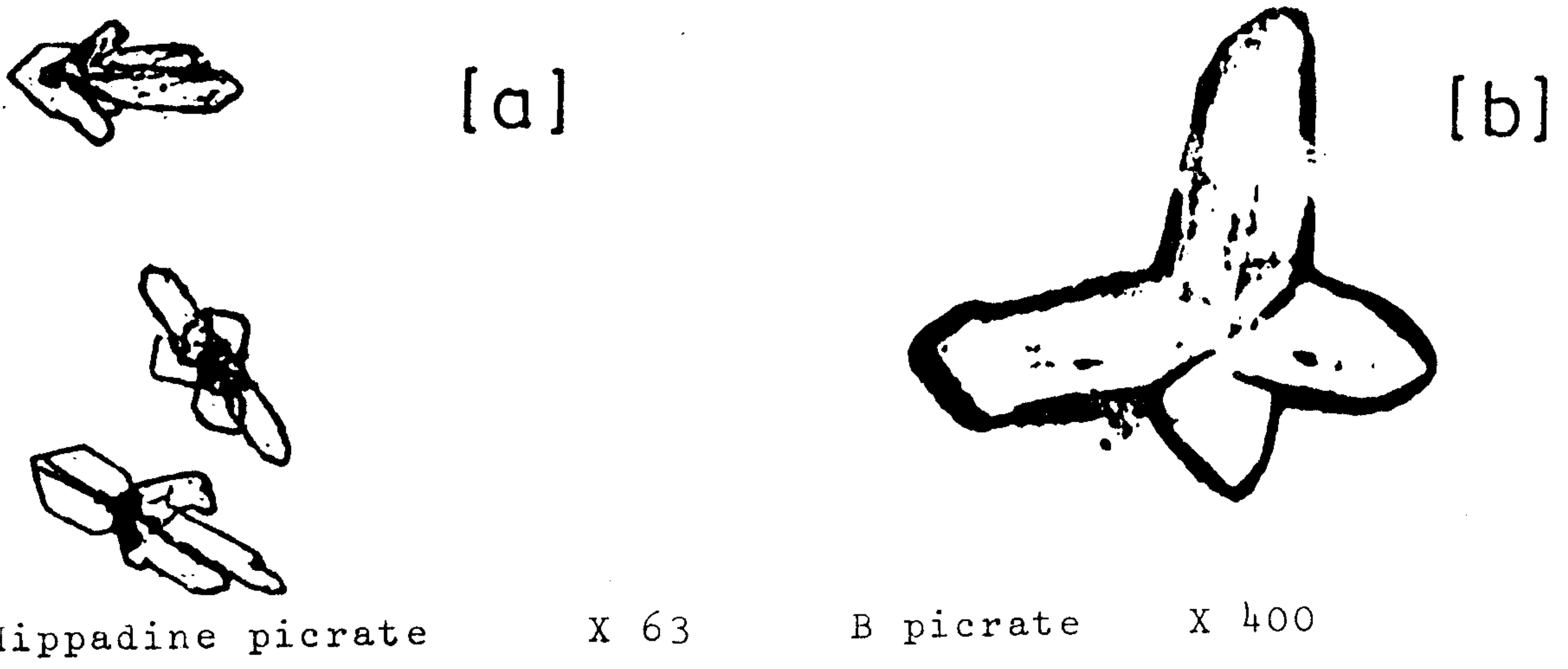
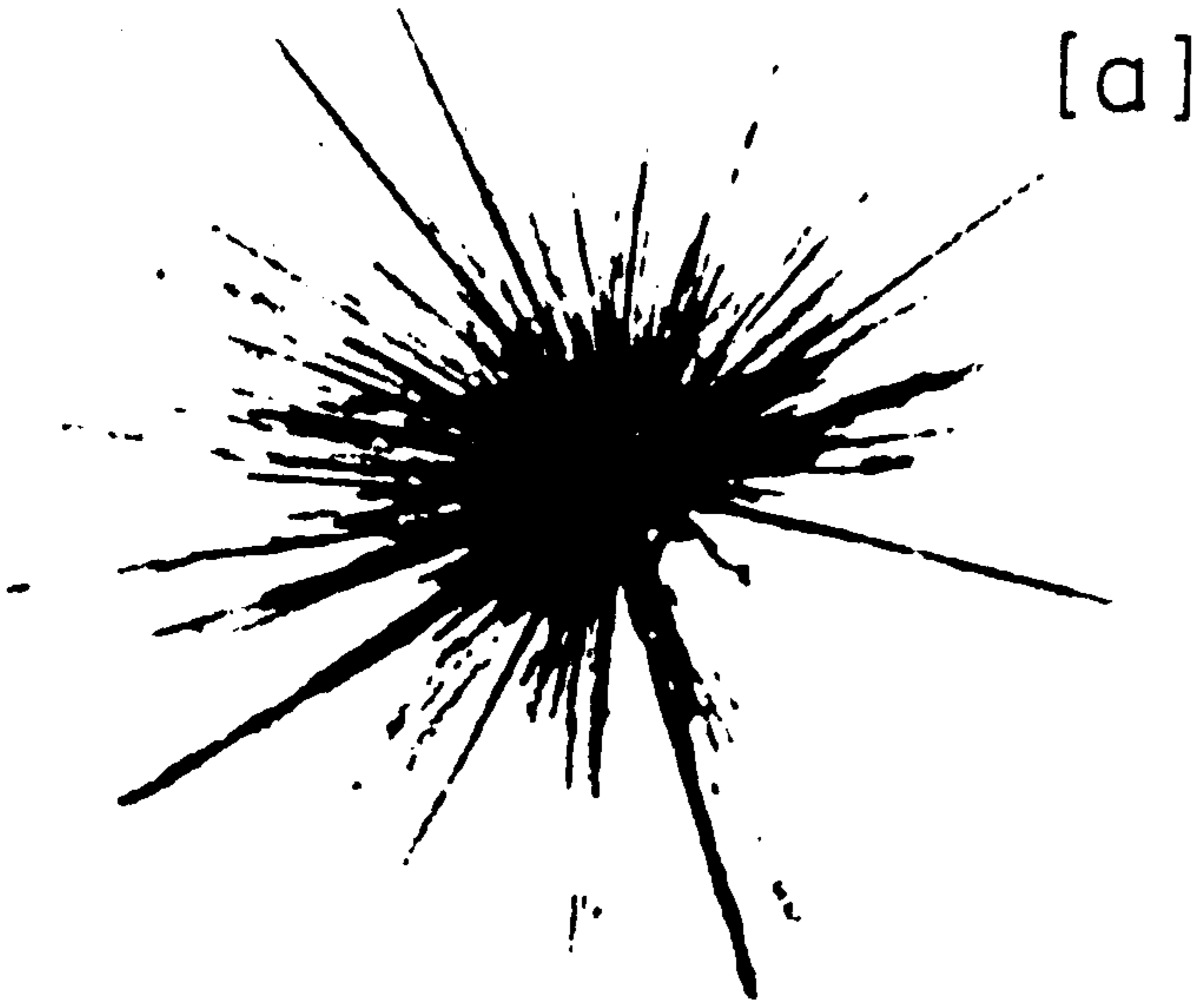
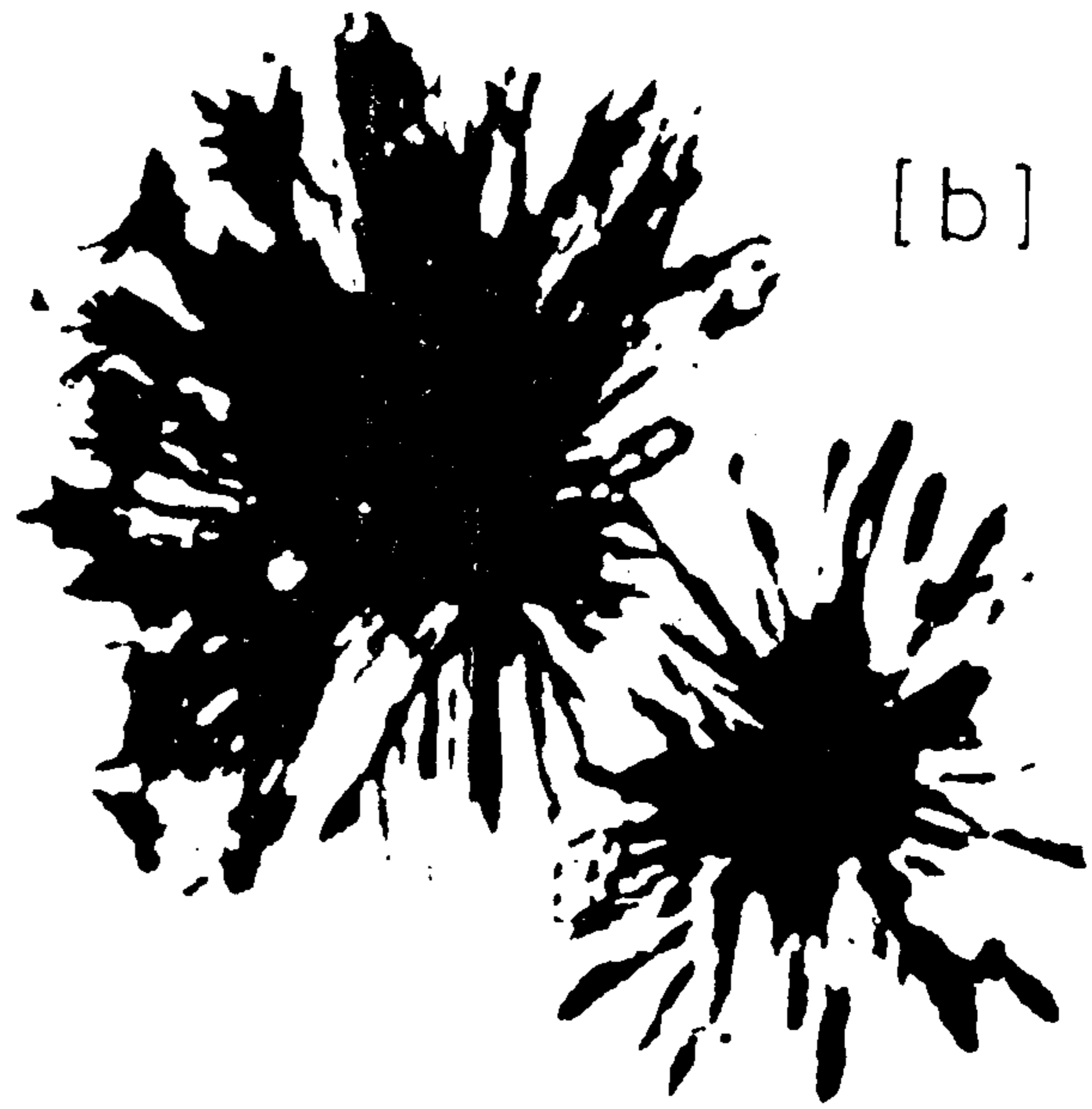


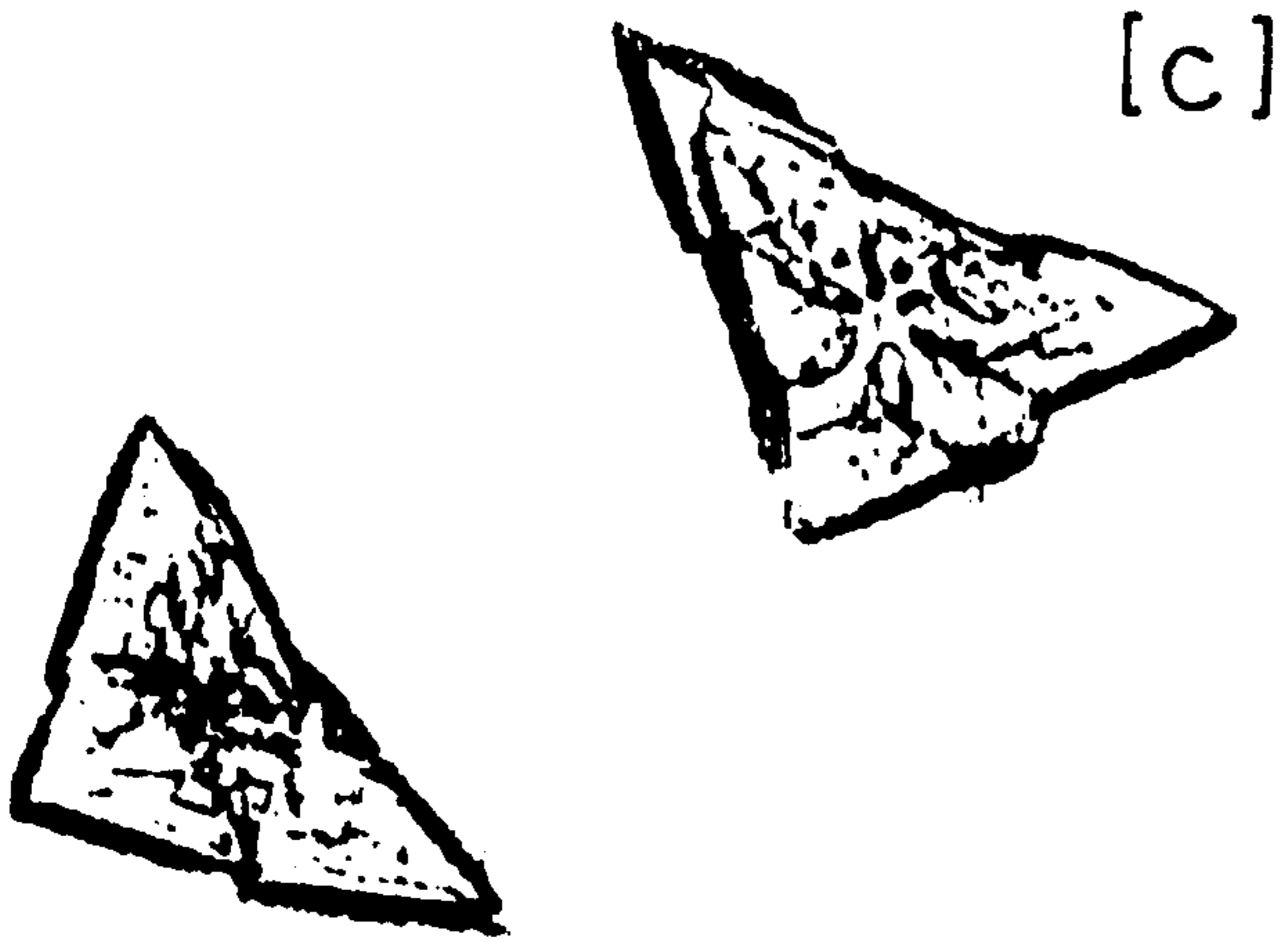
Fig. 1: Photographs of Crystals



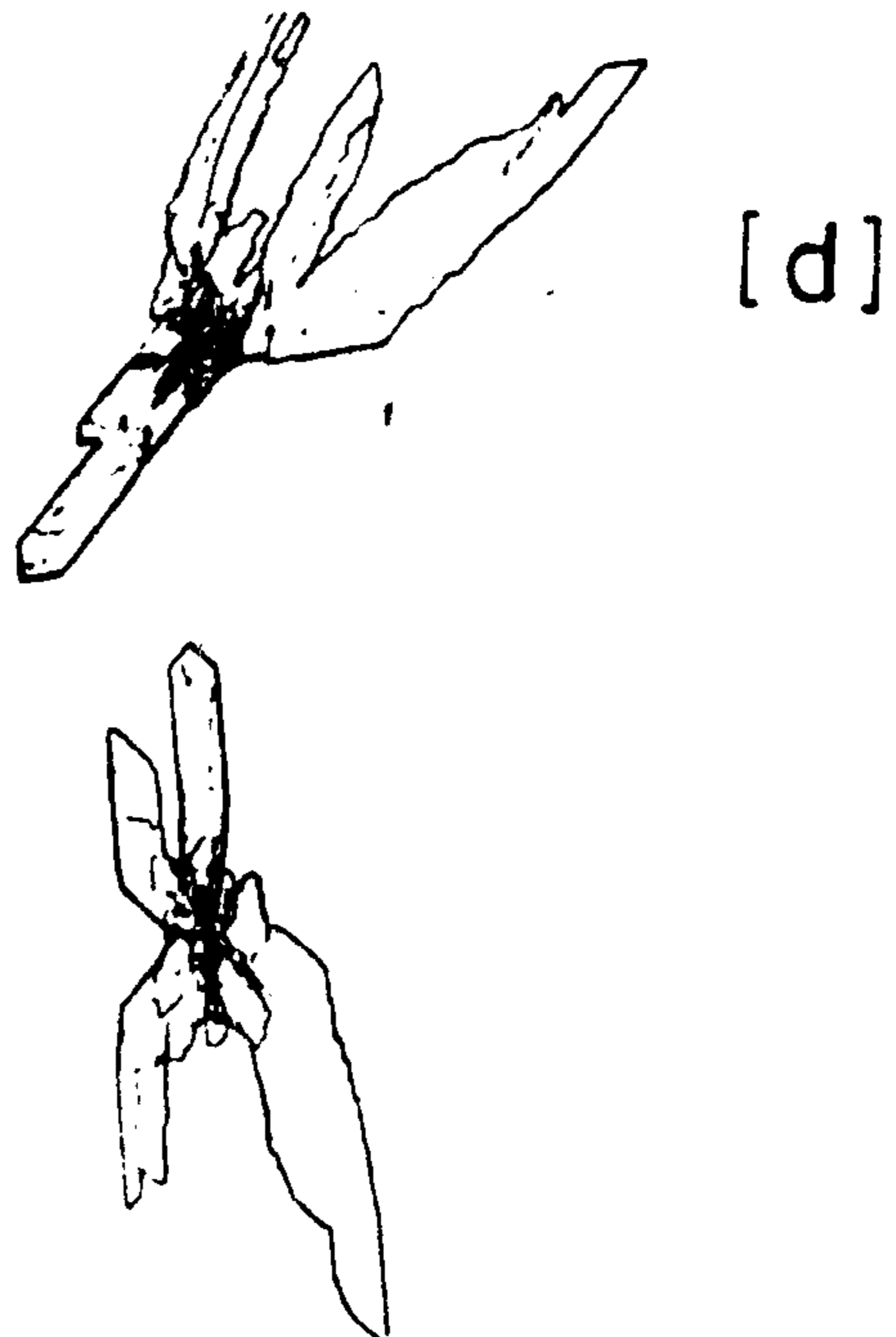
Tazettine reineckate X 160



Tazettine picrate X 160



J picrate X 250



Pancracine picrate X 63

Fig. 2: Photographs of Crystals

Study of alkaloids of *Pancreaticum maritimum* L. growing in Egypt

REFERENCES

- 1) R. Muschler; "A manual flora of Egypt", Verlag von J. Cramer, New York, 23 (1970).
- 2) N.F. Proskurnina; Zhun Obshech Khim, 25: 834-6 (1955). Through C.A. 50, 2667
- 3) N.F. Proskurnina; Doklady Akad. Nauk. U.S.S.R., 103:851-852 (1955). Through C.A. 50, 9434.
- 4) H.G. Boit and H. Ehmke; Chem. Ber., 89, 2093 (1956). Through C.A. 50, 7384.
- 5) F. Sandberg, and S. Ageurell; Tidskr, 63:657-66 (1959), Through C.A. 54, 4787.
- 6) F. Sandberg and K.H. Michel; Naturwissen-schaften, 50, 333-4 (1963). Through C.A. 59, 1437.
- 7) F. Sandberg and S. Ageurell ; Abhandl-Deut. Wiss, Berlin, Kl., CHEM. Geol. Biol. (4) 383-387 (1963), Through C.A. 61, 1708.
- 8) F. Sandberg and K.H. Michel; Lloydia, 26, 78-90 (1963) .
- 9) Z.F. Ahmed, A.M. Rizk and F.M. Hammouda; Lloydia, 27, 115 (1964).
- 10) Sandberg and K. Heinz Acta Pharm. Suecica, 5(2) 61-66 (1968). Through C.A. C.A. 69, 25043.
- 11) M. Ieven, J. Totte, D. Van den Berghe and A.J. Vlietinck; Planta medica, 33, 284 (1978).
- 12) D. Van den Berghe, M. Ieven, F. Mortens and A.J. Vlietinck; Lloydia, 41, 463 (1978).
- 13) M. Ieven, D. Van den Berghe and A.J. Vlietinck; Planta medica, 36, 254 (1979).
- 14) A.M. El-Moghazi, A.A. Ali and M.K. Mesbah; Planta Medica, 28, 336 (1975).
- 15) A.A. Ali, M.K. Mesbah and A.W. Frahm; Planta medica, 43, 407 (1981).
- 16) H.G. Boit; Chem. Ber., Humboldt Univ. Berlin, 1704-7 (1954).
- 17) W.C. Wildman and C.J. Kaufman; J. Am. Chem. Soc., 76, 5185-16 (1954).
- 18) G.G. De Angelis and W.C. Wildman; Tetrahedron, 25, 5099 (1969).
- 19) A.A. Ali, M.K. Mesbah and W.A. Frahm; Planta medica, 50, 188 (1984).
- 20) C.C. Fulton; "Modern microcrystal tests for drugs", Wiley-Interscience, New York, P. 39 (1969).
- 21) A.M. El-Moghazi and A.A. Ali; Planta medica, 30, 369 (1976).

دراسة قلويدات نبات البانكرشيم مارثيم
الذى ينمو فى مصر

أحمد عبدالرحمن على - مصطفى كامل محمد مصباح - محمود حامد محمد
قسم العقاقير - كلية الصيدلة - جامعة أسيوط

تم فصل والتعرف على قلويد التازتين والبانكرسبين
والهبادين والليكورين والتعرف عليهم بالأشعة تحت الحمراء
والرنين النووى ومطياف الكتله - كما تم فصل أربعة مركبات
قاعديه ودراستها عن طريق الأشعة تحت الحمراء وكذلك بواسطة
محاليل مرسبات القلويدات وفحصها مجهرياً .