

PYRROLOPHENANTHRIDONE ALKALOIDS FROM  
CRINUM AUGUSTUM ROX. BULBS.

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ABSTRACT

Three pyrrolophenanthridone alkaloids viz, hippadine, pratorimine and pratorinine were isolated from the chloroformic fraction of the ethanolic extract of *C. augustum* Rox. bulbs. In addition (-)4-hydroxy-7-methoxy flavan was also isolated.

The identity of these compounds was confirmed through determining their physical and spectral data.

INTRODUCTION

*Crinum* species which represent an important sector in family Amaryllidaceae have been reported to elaborate a variety of alkaloids. These alkaloids have received a great deal of attention in recent years and were the subject of chemical, cytological and pharmacological investigations<sup>1,2</sup>.

Earlier investigations of *Crinum augustum* Rox.<sup>3-8</sup> resulted in the isolation of numerous alkaloids including lycorine, buphanisine, augustine, augustamine<sup>3,5,6</sup>, 6- - and 6-B-hydroxy buphanisine, 6- - and 6-B-hydroxy crinine, crinamine<sup>4</sup>, augustisine, flexinine, crinine, perlolyrine, hippadine<sup>7</sup> and pratorimine<sup>8</sup>.

The present paper includes the isolation of three nonbasic alkaloids and a flavan compound from the chloroformic fraction of *Crinum augustum* bulb.

## EXPERIMENTAL

General experimental procedures :

Melting points are uncorrected. IR spectra were taken as KBr pellets with a Perkin Elmer (Model 298) instrument, UV spectra are for solutions in methanol measured in a Perkin Elmer 550 S. NMR spectra were determined in  $\text{CDCl}_3$  or  $d_6$ -DMSO with TMS as an internal standard, with a Bruker WH 90 spectrometer, Chemical shifts are given in a (ppm) scale and coupling constants in Hz. Mass spectra were recorded in Kratos, A.E.I., MS-50 spectrometer operating at 70 ev.

TLC on silica gel plates 60 F<sub>254</sub> (E.Merck), solvent system ( $\text{CHCl}_3$  : MeOH, 95:5), spots were visualized by spraying with Dragendorff's reagent (I) and 5 %  $\text{H}_2\text{S}$  in methanol (II).

Plant Material :

The bulbs of Crinum augustum Rox. were collected from the cultivated plants at Assiut University Campus in July 1987 during flowering. The identity of the plant was confirmed by the late Prof. Dr. Vivi Tackholm (Cairo University), A voucher sample is kept in the Faculty of Pharmacy, Assiut University.

Extraction :

Air-dried powdered bulbs (2Kg) were extracted with ethanol (95 %). The solvent was evaporated under reduced pressure. The residue (500 g) was successively extracted with pet. ether (150 g),  $\text{CHCl}_3$  (50 g) and n-butanol (120 g).

The chloroformic fraction was screened on silica gel G using  $\text{CHCl}_3$ -MeOH (95:5). Spray reagents I and II were separately used. Spray I revealed 3 spots with  $R_f$  values : A ( $R_f = 0.94$  grey colour). C ( $R_f = 0.80$ , blue colour) and D ( $R_f = 0.70$ , orange colour changed to blue).

Spray II revealed only one spot B ( $R_f = 0.85$ , violet colour).

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The chloroformic fraction was chromatographed on silica gel column (120 x 2.5 cm) using gradient methanol-CHCl<sub>3</sub> up to 5 % for elution. Fractions of 100 ml each were collected and monitored by TLC using CHCl<sub>3</sub>-MeOH (95:5) as solvent system.

Fractions eluted with 2 % methanol afforded 1.3 g of A which was purified by recrystallisation from acetone to radiating needles of m.p. 213-4°C.

Fractions eluted with 3 % methanol were evaporated and the residue was crystallised from methanol to afford needle shaped crystals (350 mg of B), m.p. 142-5°C.

Fractions eluted with 4% methanol yielded 70 mg of C which was crystallised from methanol to afford needle shaped crystals, m.p. 225-6°C.

Fractions eluted with 5 % methanol afforded 50 mg of D which was crystallised from methanol to light brown radiating needles, m.p. 264-5°C.

Compound B (-) 4-hydroxy-7-methoxy flavan :

Crystallised from methanol (350 mg). mp 142-5°C.

$[\alpha]_D^{20} = -14.7$  (C = 0.8 in ethanol).

UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log E): 225.5 (4.28), 281 (3.72) and 287 (3.64).

IR  $\text{cm}^{-1}$ : 3380 (OH), 1620, 1590 (arom. system), no C=O; <sup>1</sup>H NMR (90 MHz, CDCl<sub>3</sub>):

7.30 (2H, m H-2, H-6), 6.97 (1H, d,  $J_{5,6} = 8.5$  Hz, H-5), 6.80 (2H, m H-3, H-5)  
6.45 (1H, dd,  $J_{5,6} = 8.5$  Hz,  $J_{6,8} = 2.5$  Hz, H-8), 6.50 (1H, d, H-8), 5.18  
(1H, br. s., exchangeable, OH), 4.95 (1H, dd, H-2), 3.75 (3H, s, OCH<sub>3</sub>),  
2.8 (2H, m, H<sub>2</sub>-4) and 2.1 (2H, m, H<sub>2</sub>-3). MS, m/z (rel. int.):

256 ( $C_{16}H_{16}O_3$ , base peak), 255 ( $M^+-H$ )<sup>+</sup> (10), 241 ( $M-CH_3$ )<sup>+</sup> (5), 239 ( $M^+-OH$ )<sup>+</sup> (9), 225 ( $M^+-OCH_3$ )<sup>+</sup> (7), 150 ( $M^+-C_7H_6O$ )<sup>+</sup> (22), 137 ( $M^+-C_8H_7O$ )<sup>+</sup> (67), 120 ( $M^+-C_8H_8O_2$ )<sup>+</sup> (35), 107 ( $M^+-C_9H_9O_2$ )<sup>+</sup> (11) and 91 ( $C_7H_7$ )<sup>+</sup> (11),

Compound D (Pratorinine) :

Crystallised from methanol (50 mg), mp. 264-5°C.

IR  $cm^{-1}$ : 3380 (OH), 1675 (CON), 1620, 1580. 1355, 1280 (arom. system).

UV  $\lambda_{max}^{MeOH}$  nm (log E): 226 (4.54), 235 (4.55), 248 (4.62),  
256 (4.61), 285 (4.35), 294 (4.52),  
336 (4.09), 346 (4.17), 361 (4.06).

<sup>1</sup>H NMR ( $d_6$ -DMSO, 90 MHz):

8.26 (1H, dd,  $J_{1,2} = 7.6$  Hz,  $J_{1,5} = 1.0$  Hz, H-1), 7.46 (1H, dd,  $J_{2,1} = 7.6$  Hz,  $J_{2,3} = 7.6$  Hz, H-2), 7.80 (1H, dd, H-3), 7.00 (1H, d,  $J_{4,5} = 3.6$  Hz, H-4), 8.03 (1H, d, H-5), 7.73 (1H, s, H-11), 7.90 (1H, s, H-8), 9.93 (1H, s br., OH), 4.03 (3H, s,  $OCH_3$ ). MS, m/z (rel. int.):

265.0763 ( $M^+$ ,  $C_{16}H_{11}NO_3$ , base peak), 250 ( $M^+-CH_3$ ) (15).

222 ( $C_{14}H_8NO_2$ , 45), 194 ( $C_{13}H_8NO$ , 10).

## RESULTS AND DISCUSSION

The concentrated chloroformic fraction of the ethanolic extract of the bulbs of Crinum augustum Rox. was chromatographed on silica gel column to yield four pure components designated A-D.

Compound A :

The physical, chemical properties and spectroscopic data (UV, IR <sup>1</sup>H NMR and MS) of compound A indicated its identity as hippadine which had been previously isolated from Crinum augustum<sup>7</sup>, Crinum pratense<sup>9</sup>, Crinum americanum<sup>10</sup> and Hippestrum vittatum<sup>11</sup>.

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Compound B :

This compound, mp 142-5°C  $C_{16}H_{16}O_3$ , exhibited UV maxima which indicated an unconjugated aromatic system<sup>12,13</sup>. The IR spectrum revealed the presence of an aromatic system and hydroxyl group, but no carbonyl bands were observed.

The  $^1H$  NMR spectrum of B is typical for flavans<sup>12,13</sup>. It showed resonances at 3.75 (3H) corresponding to OMe. The aromatic protons of ring B (H-2, H-3, H-5 and H-6) exhibit an  $A_2B_2$  system at 6.80 and 7.30 (2H each). The aromatic protons of ring A, H-5 and H-6 protons appears as two ortho-coupled doublets ( $J=8.5$  Hz) at 6.88 and 6.45 respectively. The H-6 further meta couples ( $J=2.5$  Hz) with H-8 proton at 6.5. The aliphatic H-2 appears at 4.95 as a double doublet and the remaining aliphatic protons H-3 and H-4 appear as methylene multiplets at 2.1 and 2.8 respectively.

In the MS, a peak at  $m/z$  256 confirms the parent formula  $C_{16}H_{16}O_3$ . Other significant peaks at  $m/z$  241 ( $M^+-CH_3$ ), 239 ( $M^+-OH$ ), 225 ( $M^+-OCH_3$ ), 150 ( $M^+-C_7H_6O$ ) and the base peak at  $m/z$  137 ( $M^+-C_8H_7O$ ) which may be originated through retro-Diels-Alder pathway. The MS of compound B was in accord with the recorded spectra of flavans<sup>14,15</sup>.

From the abovementioned physical and spectroscopic data compound B was identified as (-)-4-hydroxy-7-methoxy flavan, identical with that previously reported from Stypandra grandis<sup>16</sup> and Crinum americanum<sup>17</sup>. Its spectral study has been fully explored with the aim of its complete structural investigation and to assist in the identification of related compounds in the Family Amaryllidaceae in future on comparative basis.

This is the first report of compound B in Crinum augustum Rox.

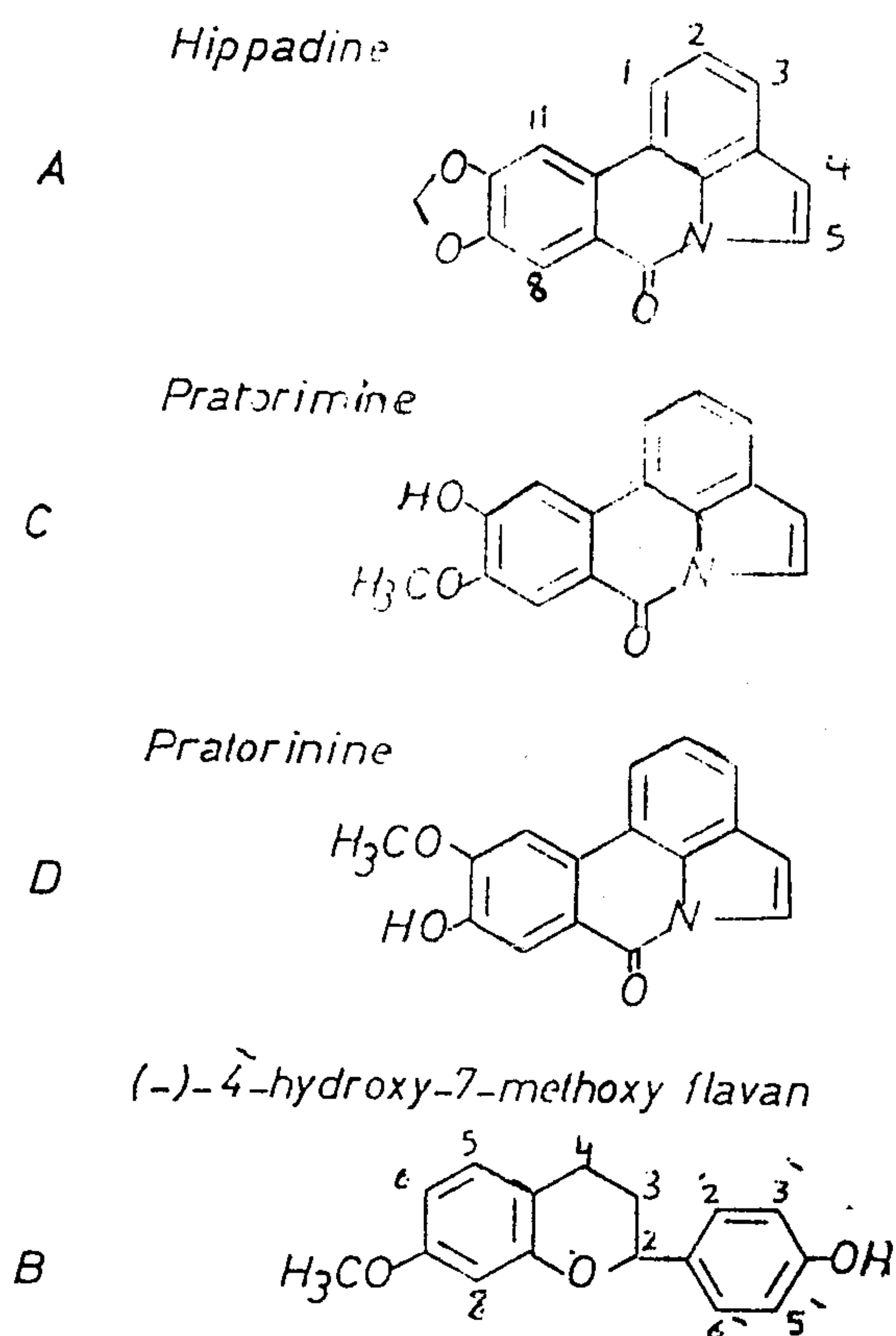
Compound C :

On the basis of physical, chemical and spectral properties (UV, IR,  $^1\text{H}$  NMR and MS), compound C was found to be pratorimine which had been isolated from Crinum americanum<sup>10</sup>, C. Asiaticum<sup>18</sup>, C. latifolium<sup>9</sup> and C. augustum<sup>8</sup> growing abroad.

Compound D :

Compound D was identified as pratorinine through its physical and chemical properties as well as its spectral data (UV, IR,  $^1\text{H}$  NMR and MS).

This compound was previously isolated from Crinum latifolium<sup>9</sup>, C. americanum<sup>10</sup> and C. bulbispermum<sup>7</sup>, but this is first report of this alkaloid in the title plant.



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قلويدات بيرولوفيننثريدون المفصولة

من أبصال الكرينم أوجستم روكسى

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أحمد عبدالرحمن على - محمود أحمد رمضان - محمد عبدالمطلب عبدالحافظ  
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تم فى هذا البحث فصل ثلاث مركبات قلوانية ( هيبادين -  
براتوريمين - براتورينين ) ومركب فلافونيدى ( ٤ - هيدروكسى  
- ٧ - ميزوكسى - فلافان ) من خلاصة الكلوروفورم لابصـال  
الكرينم أوجستم روكسى .  
وأمكن التعرف على هذه المركبات بدراسة خواصها  
الطبيعية والتحليل الطيفى لهما .

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