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# INVESTIGATION OF PETROLEUM ETHER EXTRACT OF THE BULBS OF *CRINUM AUGUSTUM* ROX.

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نبات الكرينم أوجستم من النباتات التابعة للفصيلة النرجسية والتى تشتهر باحتوائها على القلوانيات وقد تركز الاهتمام على فصل والتعرف على هذه القلوانيات. ولكن لم تجر دراسة كافية على خلاصة البترول الاثيرى ولهذا رؤى اجراء دراسة على خلاصة البترول الاثيرى لأبصال النبات. وقد أتاحت هذه الدراسة التعرف على أربعة مركبات من مجموعة الألكانات المشبعة وكذلك مركبات ٢٤-ميثيلين سيكلوأرتانول وسيكلوليوكانول. وقد أمكن التعرف على هذه المركبات باستخدام كروماتوجرافيا الغاز المرتبطة بمطياف الكتلة. وتم كذلك فصل قلواني الأنجريمين من خلاصة الكحول البيوتيلى وتم التعرف عليه بدراسة مقارنة مع ما ذكر فى المراجع بالنسبة لخواصه الطيفية.

*The bulbs of Crinum augustum Rox. Fam. Amaryllidaceae were extracted with ethanol (95%) by maceration and percolation. From the petroleum ether fraction four saturated straight chain hydrocarbons, 24-methylene cycloartanol and cycloeucalenol were isolated and identified. The identification of these compounds was based on GLC-Mass spectrometry. In addition, an alkaloid (ungeremine) was also isolated and characterised from the n-butanol extract.*

## INTRODUCTION

From the genus *Crinum* (Family Amaryllidaceae), 13 species were introduced to Egypt, being cultivated in public and private gardens.[1,2] Many *Crinum* species are used in folklore medicine or skin diseases, rheumatism, haemorroides, diuresis and as squill substitute.[1,3,4]

Reviewing the current literature, it was found that a particular attention was given to the alkaloids.[5-9] Mucilage, fatty acids,[10] as well as flavans,[9,11] chalcones[11] and ketoalcohols[12] were also reported, while the alkane and sterols composition is unknown. In this paper we report the isolation and identification of four n-alkanes ($C_{25}$-$C_{27}$ and $C_{29}$) and two $C_{30}$ and $C_{31}$ $\Delta^{24}$-monounsaturated sterols using a combination of gas chromatography-mass spectrometric technique. this paper represents the first report for the presence of 24-methylene cycloartanol and cycloeucalenol in Family Amaryllidaceae. Also the alkaloid ungeremine was isolated and characterised by spectroscopic technique.

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## EXPERIMENTAL

### Plant material

The bulbs were collected in May 1987, from the plant propagated in the Experimental Station of Medicinal plants, Faculty of Pharmacy, Assiut University, Assiut, Egypt air-dried and powdered.

### General experimental procedures

Melting points were uncorrected, IR were taken in KBr with Perkin-Elmer (Model 457), $^1$H- and $^{13}$C-NMR spectra were recorded in Bruker AM-300 Spectrometer (at 300 MHz for $^1$H- and 75 MHz for $^{13}$C-NMR) using TMS as internal standard. Mass spectra were carried out on Hitachi M-80 spectrometer (Japan). TLC were carried out on silica gel plates (Kieselgel 60 $F_{254}$, E. Merck). For column chromatography silica gel (E. Merck) were used. GC/MS was carried out using Perkin-Elmer SIGMA 3B, column: Bp1-No 20, temp. of injection 250°, Detector temp. 350°. Carrier gas Nitrogen. temp. ranging from 250-320°, increased by

6°C/min. for 20 minutes. MS: VG 7070F, 70 ev.

The following solvent systems were used:
System I : Chloroform
System II: Chloroform-Methanol (98:2)

**Extraction and isolation of pet. ether constituents**

About 10 kg of the powdered air-dried bulbs was extracted with EtOH (95%), first by maceration and then by percolation. The concentrated extract was defatted with pet. ether (b.r. 40-60°C) and the solvent was evaporated under reduced pressure. The yield was 7.5%. About 300 g of the dried pet. ether extract was saponified by refluxing with 2N ethanolic KOH for 3 hours. The unsaponifiable fraction was chromatographically examined using silica gel G. plates (E. Merck 0.2-0.5 mm) and systems I & II. The unsaponifible matter was subjected to silica gel column chromatography. Elution was carried out using benzene and benzene / methanol gradients. Fractions eluted with benzene afforded a single component (TLC, silica gel G, systems I&II) [Compound **I**]. Fractions eluted with benzene - methanol (97:3) afforded [Compound **II**], while those eluted with benzene - methanol (95:5) afforded [Compound **III**]. The defatted concentrated ethanol extract was fractionated by EtOAc and was refregerated and reserved for further investigation. The EtOAc insoluble fraction was extracted with n-butanol, concentrated and chromatographed on silica gel column. Fractions eluted with chloroform - methanol (85:15) aforded compound **A** which was purified by repeated chromatography on pre-packed columns (LiChroprep. $SiO_2$) using chloroform - methanol (8:2) as an eluent where compound **A** could be isolated as yellow crystals.

**Compound I**: It was crystallised from acetone as white flakes, m.p 53-55°. IR ($\nu_{max}$, $cm^{-1}$) 2920, 2850, 1460 and 720. GC/MS analysis indicated that compound **I** consists of four components. The MS of each component shows successive fragmentation of $CH_2$ units and the following respective parent ion peaks ($M^+$) at 352, 366, 380 and 408. The percentage of each component was determined by triangulation method and is shown in Table 2.

**Compound II**: Crystallised from MeOH as radiating needles, m.p 124-126° showed single component (GC). $[\alpha]_D^{20}$ = + 45.6°. IR ($\nu_{max}$, $cm^{-1}$) 3400,3050, 1635, 1450, 1370, 1070 and 883. $^1H$ NMR ($CDCl_3$, $\delta$) 0.28 and 0.52 (each 1 H, d, J= 4.5 Hz, $H_2$-19), 0.80 (3H, s, 29-$CH_3$), 0.84 (3H, d, J= 6.2 Hz 21-$CH_3$), 0.88 (3H, s, 31-$CH_3$), 0.95 (3H,s, 18-$CH_3$), 1.02 (3H, s, 30-$CH_3$), 1.15 (6H, d, J= 6.2 Hz, 26-$CH_3$ and 27-$CH_3$). HRMS, m/z 440.4003, 425.3755, 422.3918, 407.3673, 379.353, 315, 300, 297 and 173. The acetate was prepared by refluxing in acetic anhydride-pyridine. Crystallisation of the product from acetone afforded fine needles, m.p 116°. MS, m/z 482 ($M^+$), 467, 422, 407, 379, 357, 353 and 297. The 3-keto derivative was prepared by $CrO_3$ oxidation in glacial acetic acid at room temperature. Crystallisation from acetone afforded fine needles, m.p 116-117°.

$[\alpha]_D^{20}$ = + 14.4°.

**Compound III**: crystallised from MeOH as long radiating needles, m.p 142.3-143.8° showed single component (GC). IR ($\nu_{max}$, $cm^{-1}$) 3400, 3050, 1630, 1460, 1380, 1080 and 880. $^1H$ NMR ($CDCl_3$, $\delta$) 0.16 and 0.48 (each 1 H, d, J= 4 Hz, $H_2$-19), 0.82 (3H, s, 29-$CH_3$), 0.96 (3H, d, J= 6.2 Hz 21-$CH_3$), 1.0 (3H, s, 31-$CH_3$), 1.20 (3H, s, 18-$CH_3$), 1.35 (6H, d, J= 6.2 Hz, 26-$CH_3$ and 27-$CH_3$), 4.67 and 4.72 (each 1H, d, J= 2.3 Hz, $H_2$-28), 2.32 (1H, disappeared after $D_2O$, OH) and 3.23 (1H, d, J= 5 Hz, H-3$\alpha$). HRMS, m/z 426.3834, 411.3634, 408.3802, 393.3520, 365,353, 343,300 and 286. The acetate was prepared as mentioned before to give fine needles, m.p 108-110°. MS, m/z 468 ($M^+$), 453, 408, 393, 353, 343 and 300.

**Compound [A]**: Obtained as yellow crystals (MeOH), m.p. 260-261°(dec.). IR ($\nu_{max}$, $cm^{-1}$), 3400-2600 (OH or salt), 1620 and 1510 (C=N),

1045 and 930 ($OCH_2O$). $^1$H-NMR (DMSO-$d_6$, $\delta$) 9.45 (1H, s, H-7), 8.2 (1H, s, H-11), 7.8 (1H, s, H-8), 7.71 (1H, d, J= 2 Hz, H-1), 7.36 (1H, dt, J= 2 Hz, H-3), 6.40 (2H, s, $OCH_2O$), 5.18 (2H, t, J= 7 Hz, $H_2$-5), 3.66 (2H, dt, J= 7,2 Hz, $H_2$-4). $^{13}$C-NMR (DMSO-$d_6$, $\delta$) 160.79 (s, C-2), 155.62 (s, C-10), 150.19 (s, C-9), 141.47 (d, C-7), 138.99 (s, C-3a), 131.43 (s, C-11a), 130.89 (s, C-11c), 124.96 (s, C-11b), 122.65 (s, C-7a), 116.99 (d, C-3), 107.46 (d, C-8), 104.13 (t, $OCH_2O$), 103.63 (d, C-1), 101.56 (d, C-11), 55.96 (t, C-5) and 27.46 (t, C-4). HRMS, m/z: 265.0722 [$M^+$, $C_{16}H_{11}NO_3$, 59 %], base peak at m/z 264.

## RESULTS AND DISCUSSION

Silica gel column chromatography of the unsaponifiable fraction obtained from the petroleum ether fraction of the alcoholic extract of *Crinum augustum* bulbs furnished three crystalline compounds. These compounds are saturated hydrocarbon [compound **I**], 24-methylene cycloartanol [compound **II**] and cycloeucalenol [compound **III**].

### Compound I

It gave only one spot on TLC using different solvent systems (Table 1) and eluted with petroleum ether (up to 8% of the non saponifible lipid). It did not decolourise bromine water suggesting saturation and gave negative Liebermann-Burchard test. IR spectrum showed no absorption bands corresponding to hydroxyl or carbonyl group or double bond and GC-MS indicated that this compound consists of four aliphatic alkanes. The MS of each component showed successive fragmentation of $CH_2$ (14) units characteristic for relevant hydrocarbon (Table 2).

### Compound II

Showed only one spot on TLC (Table 1) and gave red colour with Salkowiski's test, violet colour with vanillin/HCl and blue colour with trichloroacetic acid reagent indicating its steroidal nature. A yellowish-red colouration with Liebermann-Burchard reagent is identical to that of 24-methylene cycloartanol.[13] The IR showed pronounced bands at 1635 and 883 $cm^{-1}$ demonestrating the presence of a terminal methylene group ($C=CH_2$). A definite shoulder at 3050 $cm^{-1}$ indicating a steroid containing a cyclopropane ring and a terminal methylene group, with other bands at 3400 (OH) and 1070 (C-O). $^1$H-NMR showed typical singals for cytosterol,[14] with six signals at $\delta$ 0.80 (3H, s, 29-$CH_3$), 0.84 (3H, d, J= 6.2 Hz 21-$CH_3$), 0.88 (3H, s, 31-$CH_3$), 0.95 (3H, s, 18-$CH_3$), 1.02 (3H, s, 30-$CH_3$), 1.15 (6H, d, J= 6.2 Hz, 26-$CH_3$ and 27-$CH_3$) corresponding to seven methyl groups. A pair of doublet centered at $\delta$ 0.28 and 0.52 (each 1H, J= 5 Hz) indicating the presence of two diasterometric protons in the cyclopropane ring[15] as well as a signal at $\delta$ 4.6 which is typical of the $^1$H chemical shift of the proton of C-28.[16] The $\alpha$-orientation of H-3 was assigned on the basis of $^1$H-NMR signal appearing at 3.4 (t, J= 5.1 Hz). The HRMS afforded the molecular ion at m/z 440.4003 corresponding to the molcular formula $C_{31}H_{52}O$ (calculated 440.4018) and other significant peaks at m/z 425.3755 corresponding to the composition $C_{30}H_{49}O$ ($M^+$-$CH_3$). A peak at m/z 407.3673 was in accordance with the composition $C_{30}H_{47}$ formed by the loss of one methyl group and one molecule of HOH from the molecular ion. Other peaks at 379, 353, 315, 313, 300 (M-ring A), 297 (M- side chain-HOH) and 175 (loss of side chain from m/z 300). The fragment found at m/z 300 is a good evidence for the presence of 9,19 cyclopropane ring[15,17] and makes it necessary to conclude that the two methyl groups are found in ring A. The placing of the side chain methylene group at C-24/28 is supported by the appearance of a fragment m/z 83 corresponding to the allylic radical resulting from a favourable cleavage at the C-22/23 bond. This fragmentation pattern is consistent with that of 24-methylene cycloartanol.[15,18-21] TLC of acetate on $AgNO_3$-impregnated alumina using hexane-ethyl acetate (20:1) showed one component with $R_f$ 0.35 of 24-methylene cycloartanol acetate. Mass spectrometry confirmed this result; Molecular ion at m/z 482 with main fragments at m/z 467 ($M^+$-$CH_3$), 422 ($M^+$-60), 407 ($M^+$-60-15), 379, 357 ($M^+$-side chain), 353 ($M^+$-ring A) and 297 ($M^+$-side chain-Acetate). The above physical, chemical and spectral studies are consistent with the

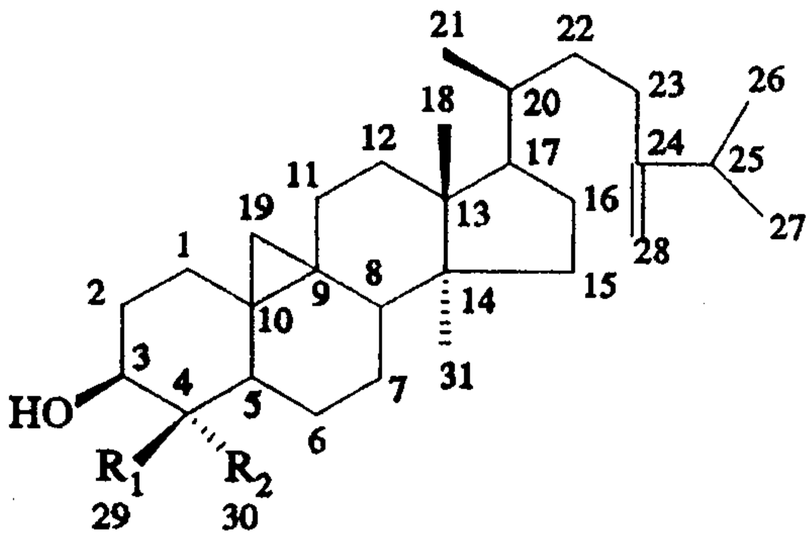
**Table 1:** TLC, Physical and Chemical Study of the Separated Fractions of the unsaponifiable matter of *C. augustum* Rox.

| Spot | $R_f$ | | Colour reaction with 50% $H_2SO_4$ | Solvent of crystallisation | Shape of crystals | Test for sterols | mp |
|---|---|---|---|---|---|---|---|
| | System I | System II | | | | | |
| 1 | 0.95 | -- | Brow | Acetone | Flakes | Negative | 53-56° |
| 2 | 0.53 | 0.82 | Red-violet | Methanol | Radiating needles | Positive | 124-126° |
| 3 | 0.42 | 0.76 | Red-violet | Methanol | Long needles | positive | 142.3-143.8° |

**Table 2:** The Components of Fraction 1 identified by GC/MS of *C. augustum* bulbs.

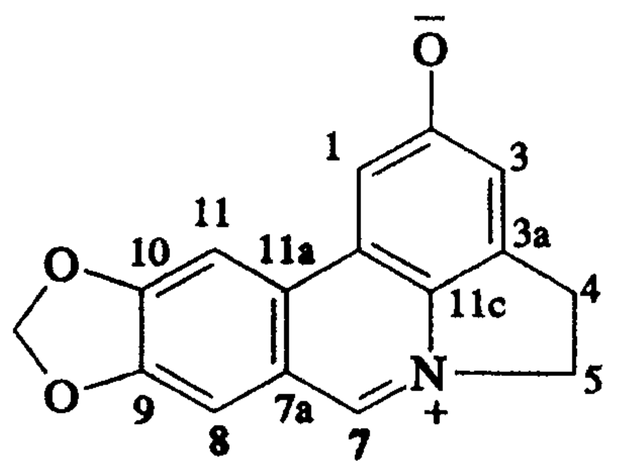
| GC Peak No. | Retension time | molecular weight | Relative amount% | Emperical formula | Structure | Name |
|---|---|---|---|---|---|---|
| 1 | 35.00 | 352.0 | 18.339 | $C_{25}H_{52}$ | $H_3C\text{-}(CH_2)_{23}\text{-}CH_3$ | Pentacosane |
| 2 | 37.69 | 366.0 | 6.169 | $C_{26}H_{54}$ | $H_3C\text{-}(CH_2)_{24}\text{-}CH_3$ | Hexacosane |
| 3 | 40.85 | 380.0 | 47.454 | $C_{27}H_{56}$ | $H_3C\text{-}(CH_2)_{25}\text{-}CH_3$ | Heptacosane |
| 4 | 49.29 | 408.0 | 28.024 | $C_{29}H_{60}$ | $H_3C\text{-}(CH_2)_{27}\text{-}CH_3$ | Nonacosane |

identity of 4,4-dimethyl sterol as 24-methylene cycloartanol,[13,15,17-19,21-22] mp. 124-126°C (lit. 125-128°C), $[\alpha]_D^{20} = +45.6°$ lit. + 45°.[17]




**Compound II** (24-methylene cycloartanol)
$R_1 = R_2 = CH_3$

**Compound III** (cycloleucalenol)
$R_1 = H$, $R_2 = CH_3$




Compound [A]

**Compound III**:

It also showed a single spot on TLC (Table 1) and gave a yellowish brown colour with Liebermann-Burchard reagent and had identical IR spectrum to 24-methylene cycloartanol. Its $^1$H-NMR spectrum showed five signals corresponding to six methyl groups at δ), 0.82 (3H, s, 29-$CH_3$), 0.96 (3H, d, J= 6.2 Hz 21-$CH_3$), 1.0 (3H, s, 31-$CH_3$), 1.20 (3H, s, 18-$CH_3$), 1.35 (6H, d, J= 6.2 Hz, 26-$CH_3$ and 27-$CH_3$), two one proton doublets centered at δ 0.I6 and δ 0.48 (J= 4.0 Hz) were indicative of a cyclopropyl grouping bearing two geminal protons only and two one proton doublets at δ 4.67 and δ 4.72 (J= 2.3 Hz) indicated the presence of a terminal methylene group. In addition, it showed one hydroxyl proton at δ 2.32 (disappeared after $D_2O$) and one carbinol proton of the secondary alcohol group appearing as a broad doublet at δ 3.23 (J= 5 Hz) suggesting the α-orientation of H-3.

GC-MS confirmed the presence of of a single component with $M^+$ 426. The mass spectrum was found to be similar to the spectrum of 24-methylene cycloartanol, except that the ions for $M^+$, $M^+$-15, $M^+$-18, $M^+$-33, were all 14 mass units less. High resolution mass spectrum showed $M^+$ at m/z 426.3834 corresponding to the molecular formula $C_{30}H_{50}O$. On the basis of the above physical, chemical and spectral studies, the 4-α-methyl sterol was identified as cycloeucalenol.[15,17,20-24]

**Compound [A]**:

Obtained as yellow crystals melted at 260° (dec.), showed mass, $^1$H-, and $^{13}$C-NMR spectra in full agreement with those reported for ungeremine,[25,26] and this is the first report of this alkaloid in the title plant.

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