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

Mahmoud A. Ramadan

Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

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 cycloartenol and cycloeucalenol were isolated and identified. The identification of these compounds was based on GLC-Mass spectrometry. In addition, an alkaloid (angeremine) 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. Many Crinum species are used in folklore medicine or skin diseases, rheumatism, haemorrhoides, diuresis and as squill substitute.

Reviewing the current literature, it was found that a particular attention was given to the alkaloids. Mucliates, fatty acids, flavans, chalcones and ketaolcohols were also reported, while the alkane and sterol composition is unknown. In this paper we report the isolation and identification of four n-alkanes (C_{25},C_{27} and C_{30}) and two C_{30} and C_{31} Δ^{27} monounsaturated steros using a combination of gas chromatography-mass spectrometric technique. This paper represents the first report for the presence of 24-methylene cycloartenol and cycloeucalenol in Family Amaryllidaceae. Also the alkaloid ugeremine was isolated and characterised by spectroscopic technique.

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), ¹H- and ¹³C-NMR spectra were recorded in Bruker AM-300 Spectrometer (at 300 MHz for ¹H and 75 MHz for ¹³C-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

Received in 12/6/1998 & Accepted in 28/6/1998
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 unsaponifiable 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 refrigerated 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) afforded compound A which was purified by repeated chromatography on pre-packed columns (LiChroprep. SiO2) 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 (νmax, 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 CH₃ 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). [α]D²⁰ = + 45.6°. IR (νmax, cm⁻¹) 3400,3050, 1635, 1450, 1370, 1070 and 883. 'H NMR (CDCl₃, δ) 0.28 and 0.52 (each 1 H, d, J = 4.5 Hz, H₂-19), 0.80 (3H, s, 29-CH₃), 0.84 (3H, d, J = 6.2 Hz 21-CH₃), 0.88 (3H, s, 31-CH₃), 0.95 (3H,s, 18-CH₃), 1.02 (3H, s, 30-CH₃), 1.15 (6H, d, J = 2.6 Hz, 26-CH₃ and 27-CH₃). 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 CrO₃ oxidation in glacial acetic acid at room temperature. Crystallisation from acetone afforded fine needles, m.p 116-117°. [α]D²⁰ = + 14.4°.

Compound III: crystallised from MeOH as long radiating needles, m.p 142.3-143.8° showed single component (GC). IR (νmax, cm⁻¹) 3400, 3050, 1630, 1460, 1380, 1080 and 880. 'H NMR (CDCl₃, δ) 0.16 and 0.48 (each 1 H, d, J = 4 Hz, H₂-19), 0.82 (3H, s, 29-CH₃), 0.96 (3H, d, J = 6.2 Hz 21-CH₃), 1.0 (3H, s, 31-CH₃), 1.20 (3H, s, 18-CH₃), 1.35 (6H, d, J = 6.2 Hz, 26-CH₃ and 27-CH₃), 4.67 and 4.72 (each 1H, d, J = 2.3 Hz, H₂-28), 2.32 (1H, disappeared after D₂O, OH) and 3.23 (1H, d, J = 5 Hz, H₂-3α). 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 (νmax, cm⁻¹), 3400-2600 (OH or salt), 1620 and 1510 (C = N),
1045 and 930 (OCH₂O). 1H-NMR (DMSO-d₆, δ) 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, OCH₃), 5.18 (2H, t, J = 7 Hz, H₂-5), 3.66 (2H, dt, J = 7.2 Hz, H₂-4). 13C-NMR (DMSO-d₆, δ) 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, OCH₃), 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⁺, C₁₀H₁₄NO₃, 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 cycloauralen [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 saponifiable 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 CH₃ (14) units characteristic for relevant hydrocarbon (Table 2).

Compound II

Showed only one spot on TLC (Table 1) and gave red colour with Salkowski’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. The IR showed pronounced bands at 1635 and 883 cm⁻¹ demonstrating the presence of a terminal methylene group (C=CH₂). 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). 1H-NMR showed typical singals for cytoстерol, with six signals at δ 0.80 (3H, s, 29-CH₃), 0.84 (3H, d, J = 6.2 Hz 21-CH₃), 0.88 (3H, s, 31-CH₃), 0.95 (3H, s, 18-CH₃), 1.02 (3H, s, 30-CH₃), 1.15 (6H, d, J = 6.2 Hz, 26-CH₃ and 27-CH₃) corresponding to seven methyl groups. A pair of doublet centered at δ 0.28 and 0.52 (each 1H, J = 5 Hz) indicating the presence of two diasterometric protons in the cyclopropane ring as well as a signal at δ 4.6 which is typical of the 1H chemical shift of the proton of C-28. The α-orientation of H-3 was assigned on the basis of 1H-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 molecular formula C₃₅H₅₄O (calculated 440.4018) and other significant peaks at m/z 425.3755 corresponding to the composition C₃₄H₅₃O (M⁺-CH₃). A peak at m/z 407.3673 was in accordance with the composition C₃₄H₄₇ 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 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. TLC of acetate on AgNO₃-impregnated alumina using hexane-ethyl acetate (20:1) showed one component with Rf 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⁺-CH₃), 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>
<thead>
<tr>
<th>Name</th>
<th>Structure</th>
<th>Molecular Formula</th>
<th>Relative Molecular Weight</th>
<th>Relative Amount</th>
<th>Strain</th>
<th>No.</th>
<th>Reaction</th>
<th>GC Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonacosane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heptacosane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hexacosane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pentacosane</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The Components of Fractions Identified by GC/MS of C. aquatilis pulp.

<table>
<thead>
<tr>
<th>Spot</th>
<th>Rf</th>
<th>System I</th>
<th>System II</th>
<th>Colour Reaction</th>
<th>Solution of</th>
<th>Crystals with 50% H2SO</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Ethanol</th>
<th>Test for Starch</th>
<th>Water</th>
<th>pH</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.95</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.33</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
identity of 4,4-dimethyl sterol as 24-methylene cycloartanol, mp. 124-126°C (lit. 125-128°C), $[\alpha]_D^{20} = +45.6^\circ$ lit. $+45^\circ$. 

GC-MS confirmed the presence 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$_{28}$H$_{46}$O. On the basis of the above physical, chemical and spectral studies, the 4-atom methyl sterol was identified as cycloegenic.$^{15,17,20-24}$

Compound [A]:

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

REFERENCES