

NON-ALKALOIDAL COMPOUNDS FROM BULBS OF *CRINUM MOOREI*

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من أبصال نبات الكرينم موراي تم فصل مواد كرومونية على النحو التالي: ٥-هيدروكسي-٧-ميثوكسي-٢-ميثيل كرومون (ايوجينين) و ٧,٥-داي هيدروكسي-٢-ميثيل كرومون و ٧,٥-داي هيدروكسي-٦-ميثوكسي-٨,٢-داي ميثيل كرومون بالإضافة إلى ٤-هيدروكسي-٧-ميثوكسي فلافان ومادة البيتاسيتوستيرول وذلك لأول مرة من النبات. وقد تم التعرف على التركيب البنائي لهذه المركبات باستخدام الوسائل الطيفية المتقدمة ومقارنة النتائج بما هو منشور من قبل.

Three chromones viz. eugenin; 5,7-dihydroxy-2-methyl chromone and 5,7-dihydroxy-6-methoxy-2,8-dimethyl chromone in addition to 4'-hydroxy-7-methoxy flavan and β -sitosterol have been isolated for the first time from bulbs of *Crinum moorei*. The structures of the isolated compounds have been determined by different spectroscopic methods and comparison with published data.

INTRODUCTION

Genus *Crinum* (Amaryllidaceae) is represented by many species and has attracted a considerable attention due to the structural variation of its alkaloids and their significant therapeutic properties¹⁻⁵. It is reported that the bulbs of *Crinum* species are used as a purgative while the outer scales are poisonous⁶. The alkaloids: Lycorine, crinine, crinamidine, crinidine and dihydrocrinidine have been already isolated from the bulbs of *Crinum moorei* Hook. f. var. *Schmidtii*⁶.

However, nothing could be traced about the non-alkaloidal compounds of this plant. For this reason, the present study was planned to include the isolation and assignment of these compounds.

EXPERIMENTAL

¹H- and ¹³C-NMR spectra were recorded in DMSO using JEOL JNM-GX 400 spectrometer (400 MHz for ¹H- and 100 MHz for ¹³C-NMR) using TMS as internal standard. MS spectra were recorded by JEOL JMS-SX 102 spectrometer. UV spectra were measured on UV

spectrometer Perkin-Elmer 550 S. Thin layer chromatography (TLC) was carried out on silica gel plates (kieselgel 60 F₂₅₄, E. Merck) and for column chromatography, silica gel G (E. Merck, 230-400 mesh). Spray reagent was 10% H₂SO₄.

Plant material

The bulbs of *C. moorei* were collected from the Experimental Station, Faculty of Pharmacy, Assiut University in June, 1993. The plant was identified by Prof. A. Fayed, Dept. of Botany, Faculty of Science, Assiut University.

Extraction and isolation

The air-dried powdered bulbs (6 kg) were extracted with 95% ethanol. The solvent free residue (800 g) was partitioned between 5% HCl and CHCl₃. The concentrated chloroformic washings (80 g) were chromatographed on a column of silica gel and eluted with n-hexane-ethyl acetate in the order of increasing polarities. From 15% ethyl acetate fraction, five compounds (1-5) have been isolated as follows:

Compound (1): β -sitosterol (200 mg), needle crystals, m.p. 137-138°C.

Compound (2): Eugenin (120 mg), yellow prisms, m.p. 104-107°C. UV λ_{\max} (nm), MeOH: 220, 240, 246, 280, 304 (sh); MeOH+NaOAc: 220, 240, 246, 280. $^1\text{H-NMR}$ δ : 6.20 (1H, d, $J=2.2$ Hz, H-8), 6.15 (1H, d, $J=2.2$ Hz, H-6), 6.00 (1H, s, H-3), 3.65 (3H, s, OCH₃) and 2.2 (3H, s, CH₃). $^{13}\text{C-NMR}$ δ : 181.2 (C-4), 166.1 (C-7), 164.9 (C-5), 161.6 (C-8a), 157.8 (C-2), 108.1 (C-3), 104.7 (C-4a), 97.2 (C-6), 91.9 (C-8), 55.6 (OCH₃) and 19.8 (CH₃).

Compound (3): 5,7-dihydroxy-2-methyl chromone (100 mg), needle crystals, m.p. 266-269°C. UV λ_{\max} (nm), MeOH: 226, 248, 256, 296, 325(sh); MeOH+NaOAc: 230, 258, 270, 335. $^1\text{H-NMR}$ δ : 6.2 (1H, d, $J=2.1$ Hz, H-8), 6.18 (1H, d, $J=2.1$ Hz, H-6), 6.00 (1H, s, H-3) and 2.2 (3H, s, CH₃). $^{13}\text{C-NMR}$ δ : 171.9 (C-4), 157.7 (C-7), 154.1 (C-5), 151.9 (C-8a), 148.0 (C-2), 98.1 (C-3), 93.9 (C-4a), 89.2 (C-6), 84.1 (C-8) and 10.1 (CH₃).

Compound (4): 5,7-dihydroxy-6-methoxy-2,8-dimethyl chromone (70 mg), needle crystals, m.p. 203-206°C. UV λ_{\max} (nm), MeOH: 228, 290, 326 (sh); MeOH+NaOAc: 235, 304, 326. $^1\text{H-NMR}$ δ : 6.00 (1H, s, H-3), 3.57 (3H, s, OCH₃), 2.5 (3H, s, CH₃-2) and 1.65 (3H, s, CH₃-8). $^{13}\text{C-NMR}$ δ : 201.0 (C-4), 170.8 (C-7), 170.0 (C-5), 163.2 (C-8a), 162.2 (C-6), 160.1 (C-2), 104.1 (C-8), 101.9 (C-4a), 90.1 (C-3), 55.2 (OCH₃), 31.9 (CH₃-2) and 7.2 (CH₃-8).

Compound (5): 4'-hydroxy-7-methoxy flavan (40 mg), needle crystals, m.p. 140-143°C. UV λ_{\max} (nm), MeOH: 222, 280, 282. $^1\text{H-NMR}$ δ : 7.22 (2H, d, $J=8.5$ Hz, H-2',6'), 6.78 (2H, d, $J=8.5$ Hz, H-3',5'), 6.92 (1H, d, $J=8.5$ Hz, H-5), 6.4 (1H, dd, $J=8.5,2.2$ Hz, H-6), 6.35 (1H, d, $J=2.2$ Hz, H-8), 4.90 (1H, m, H-2), 2.65 (2H, m, 2H-4), 2.00 (2H, m, 2H-3) and 3.65 (3H, s, OCH₃). $^{13}\text{C-NMR}$ δ : 158.0 (C-7), 155.0 (C-4'), 154.7 (C-8a), 133.1 (C-1'), 129.3 (C-5), 127.1 (C-2',6'), 114.7 (C-3',5'), 113.1 (C-4a), 107.0 (C-6), 100.9 (C-8), 77.1 (C-2), 55.1 (OCH₃), 29.2 (C-4) and 24.1 (C-3). EI-MS m/z : 256 (C₁₆H₁₆O₃), 255, 241, 239, 225 and 150.

RESULTS AND DISCUSSION

From the chloroformic washings after addition of 5% HCl to the ethanolic extract of *C. moorei* bulbs, five compounds (1-5) have been isolated.

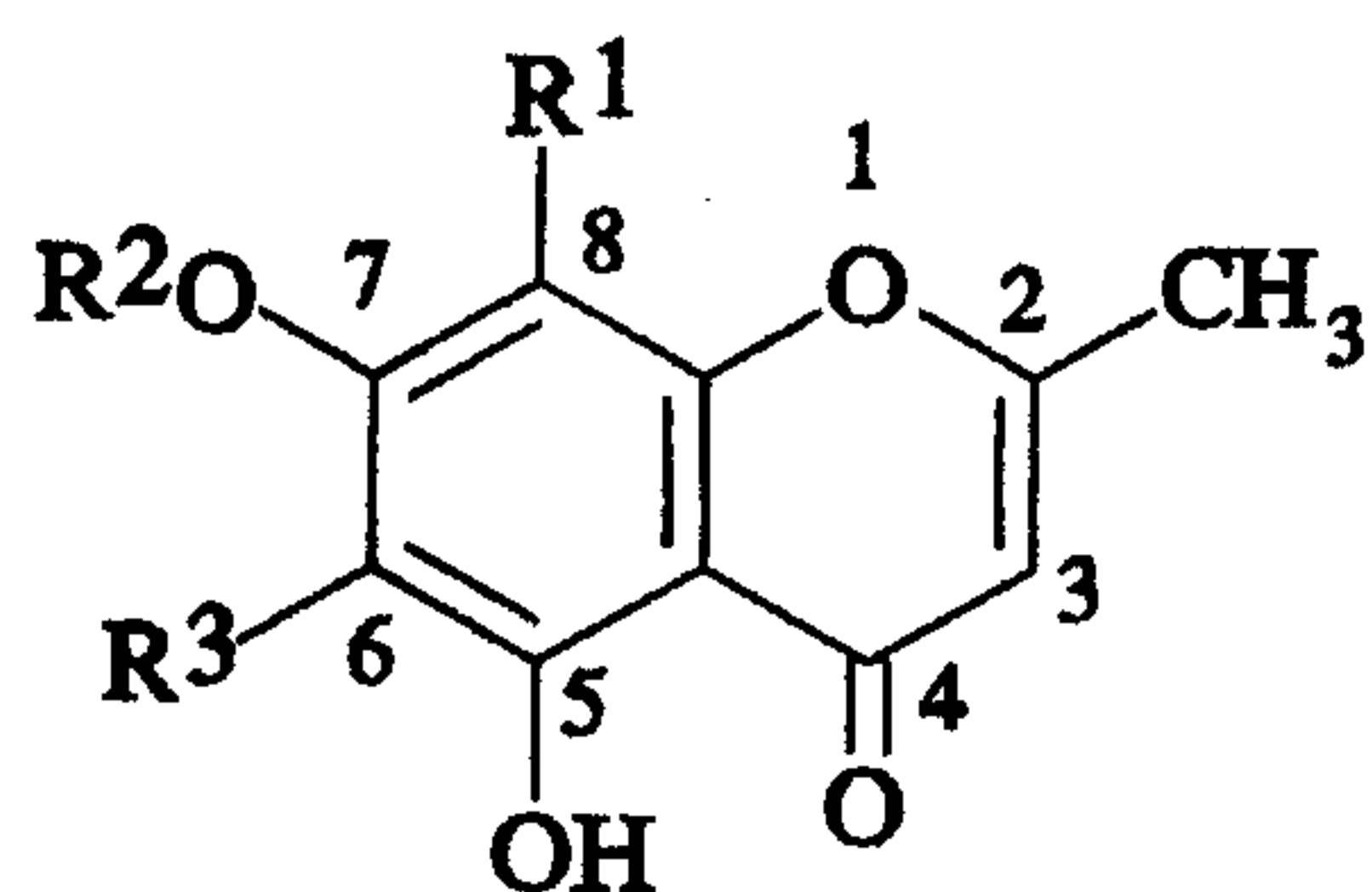
Compound (1) was identified as β -sitosterol by direct comparison of its m.p. and IR spectrum with reported data^{7,8}.

The UV spectra of compounds (2-4) were found to be characteristic for chromones⁹. Upon addition of NaOAc, the bathochromic shifts were only observed in compounds (3) and (4) indicating the absence of any substitution at C-7 OH groups of these compounds and vice versa in case of compound (2).

The inspection of $^{13}\text{C-NMR}$ spectrum of compound (2) revealed the presence of 11 carbon signals. The most downfield signal (δ 181.2) was obvious for the carbonyl group, while the most upfield one (δ 19.8) was attributed to C-2 methyl group. The methoxyl group at C-7 was indicated from the signal at δ 55.6. This assignment was substantiated by $^1\text{H-NMR}$ analysis which showed two doublets at δ 6.20 and 6.15 ($J=2.2$ Hz) for the meta coupling of H-8 and H-6 respectively. The singlet at δ 6.00 was clear for H-3 while the singlets at δ 3.56 and 2.2 were assigned for OCH₃ and CH₃ respectively. The structure assignment was confirmed by EI-MS analysis which revealed M^+ at m/z 206 and other significant peaks at m/z : 178 ($M^+-\text{CO}$), 177 (178-H), 163 (178-CH₃) and 148 (163-CH₃). Therefore, the structure of compound (2) was identified as 5-hydroxy-7-methoxy-2-methyl chromone (eugenin).

$^{13}\text{C-NMR}$ spectrum of compound (3) was identical to that of (2) except absence of the methoxyl signal. This was also confirmed from the absence of the singlet at δ 3.65 in its $^1\text{H-NMR}$ spectrum. EI-MS analysis of (3) revealed M^+ at m/z 192 (206-14) and other significant peaks at m/z : 152 (192-C₃H₄), 124 (152-CO), 164 (192-CO) and 43 (C₂H₃O). Consequently, the structure of compound (3) was assigned as 5,7-dihydroxy-2-methyl chromone.

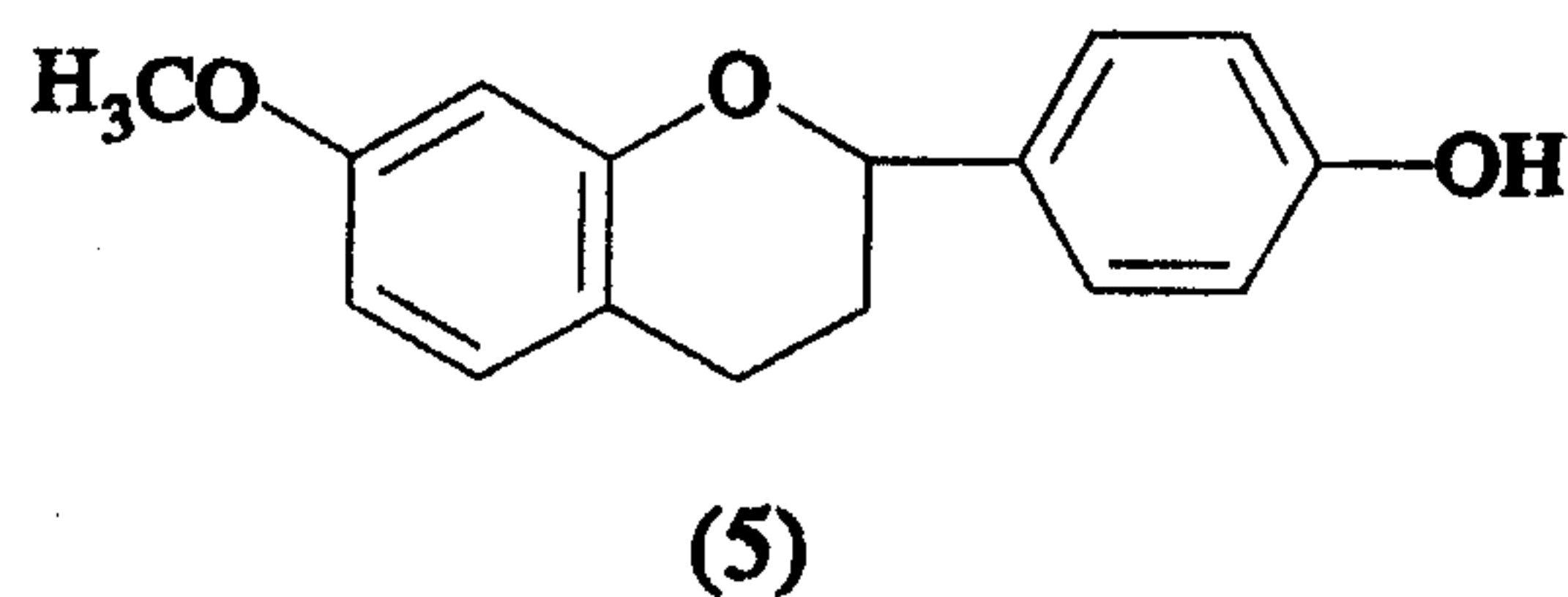
^{13}C -NMR spectrum of compound (4) was almost similar to that of (3). However, it showed two additional signals at δ 55.2 and 7.2 for OCH_3 at C-6 and CH_3 at C-8 respectively. This suggestion was supported by the absence of H-8 and H-6 doublets in ^1H -NMR spectrum. The EI-MS spectrum of (4) exhibited M^+ at m/z 236 consistent with the molecular formula $\text{C}_{12}\text{H}_{12}\text{O}_5$ and other significant peaks at m/z 196 ($\text{M}^+ - \text{C}_3\text{H}_4$), 181 ($196 - \text{CH}_3$) and 166 ($181 - \text{CH}_3$). From the above mentioned data, the structure of (4) was interpreted as 5,7-dihydroxy-6-methoxy-2,8-dimethyl chromone.



	R ¹	R ²	R ³
(2)	H	CH ₃	H
(3)	H	H	H
(4)	CH ₃	H	OCH ₃

UV spectrum of compound (5) was characteristic for flavanes¹⁰. Inspection of its ^1H -NMR revealed an A_2B_2 coupling system for 3',5' and 2',6' protons from the two doublets at δ 6.78 and 7.2 ($J = 8.5$ Hz) respectively. The doublet at δ 6.35 ($J = 2.2$ Hz) was characteristic for H-8 while that at δ 6.9 ($J = 8.5$ Hz) was diagnostic for H-5. The proton at C-6 was clear from the signal (dd) at δ 6.4 ($J = 8.5, 2.2$ Hz), while the three multiplet signals at δ 4.9, 2.0 and 2.65 were assigned for the protons at C-2, C-3 and C-4 respectively. The methoxyl group at C-7 was determined from the singlet at δ 3.65. Consequently, the structure of compound (5) was determined as 4'-hydroxy-7-methoxy flavan. The structural assignment has been confirmed by comparison of its ^{13}C -NMR and EI-MS spectra with those reported in literature^{11,12}.

To our knowledge this study represents the first reported isolation of compounds (1-5) from *C. moorei*.



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