Bull. Pharm. Sci., Assiut University, Vol. 24, Part 1, June 2001, pp. 41-45.

NON ALKALOIDAL CONSTITUENTS FROM *CRINUM BULBISPERMUM* BULBS

A. A. Khalifa

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

من أبصال الكرينم بلبيسبرمم ملن تم فصل أربعة مركبات غير قلوانية منها مركبان جديدان وهما بيتا-(٤،٣-داى ميثوكسى فينيل) ألفا وبيتا-ايثان دايول وكذلك بارا-هيدروكسى بنزين خلات الايثيل. وتم كذلك فصل والتعرف على مركبين يفصلان لأول مرة من الفصيلة النرجسية وهما ٤'-هيدروكسى-٧-ميثوكسى-فلافون و٤،٤'-داى هيدروكسى-٢-ميثوكسى-شالكون. وتم التعرف على هذه المركبات بدراسة الخواص الكيميائية والفيزيائية واستخدام طرق التحليل الطيفى الحديثة.

From the bulbs of Crinum bulbispermum Milne, four non alkaloidal compounds were isolated viz, 4,4´-dihydroxy-2-methoxy-chalcone (1), and 4´-hydroxy-7-methoxy-flavone (2). In addition, two new compounds were isolated and identified as ß-(3,4-dimethoxy phenyl)-α,ß-ethanediol (3) and p-hydroxy benzene acetic acid ethyl ester (4). The structures of the isolated compounds were established by spectral evidence.

INTRODUCTION

The genus *Crinum* (Family Amaryllidaceae, subfamily Amaryllidoidae) has attracted considerable attention due to its alkaloidal content.[1] The alkaloidal content of *Crinum bulbispermum* Milne has been established.[2-5] The non-nitrogenous constituents of *Crinum bulbispermum* has not attracted much attention from phytochemists.[2,6] Phenolics appear to have desirable medicinal properties. Some have been reported to be antitumor agents and to exhibit antiviral and antimicrobial activities,[7] hypotensive effects[8] and antioxidant properties.[9]

Therefore, we are interested in identifying the phenolic constituents of the plant. Extensive column chromatography and HPLC of the defatted acidic organic layer of the ethanolic extract of the bulbs resulted in the isolation and characterisation of 4,4´-dihydroxy-2-methoxy-chalcone (1) and 4´-hydroxy-7-methoxy-flavone (2) which are reported here for the first time in the Family Amaryllidaceae.

In addition, two new compounds were isolated and identified as ß-(3,4-dimethoxy phenyl) α,ß-ethanediol (3) and p-hydroxy benzene acetic acid ethyl ester (4).

EXPERIMENTAL

General experimental procedures

NMR spectra were recorded in DMSO-d_6, unless otherwise mentioned, using JEOL JNM A-400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) with TMS as internal standard. The mass spectra were recorded by JEOL JMS-SX 102 spectrometer. IR spectra were taken in KBr using Unicam SP 1205 spectrophotometer. UV spectra were measured in MeOH and different ionizing and complexing reagents using Unicam 1750 spectrophotometer. Preparative HPLC was carried out on columns of ODS (Octadecylsilylated silica gel) (150 X 20 mm i.d., YMC) with a Tosoh refractive index (RI-8) detector. The flow rate was 6 ml/min. TLC was carried out on precoated silica gel plates (kieselgel 60 F_{254}, Merck). For CC, silica gel (E. Merck) was used.

Received in 30/9/2000 & Accepted in 13/1/2001

Plant material

The plant material was collected in April, 1997 from Experimental Station of Medicinal Plants, Pharmacognosy Dept., Assiut University. The identity of the plant was confirmed by Prof. N. EL-Hadidi, Dept. of Botany and Plant Taxonomy, Faculty of Science, Cairo University. A voucher sample is kept in the Herbarium of the Faculty of Pharmacy, Assiut University, Egypt.

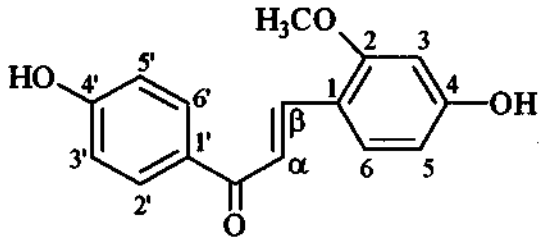
Extraction and isolation

The dried bulbs (7 kg) of *Crinum bulbispermum* Milne were extracted by maceration in EtOH for 96 h (4x15 L). The combined extracts were evaporated and the concentrated viscous extract was partitioned between $CHCl_3$ and 2% H_2SO_4. The organic layer was washed with distilled water then concentrated under reduced pressure to give viscous residue (70 g). This fraction was defatted with petrol. The defatted residue showed several $FeCl_3$-positive spots on TLC. The residue (43 g) was fractionated by flash CC (silica gel, 600x50 mm) eluting with petrol followed by EtOAc-petrol gradients. Fractions 50 ml each, were collected and monitored by TLC. Similar fractions were combined to give two main fractions (A and B). Fraction A (150 mg) contains compounds (**1**) and (**2**), while fraction B (70 mg) contains compounds (**3**) and (**4**). Fine separation of the components of each fraction was carried out by repeated Preparative HPLC on ODS columns using CH_3CN 45% for the separation of fraction B components. CH_3CN 50% is used for the fine separation of the components of fraction A. The separated compounds **1-4** have the following retention times (min), 22.2, 12.5, 12.3 and 10.2, respectively.

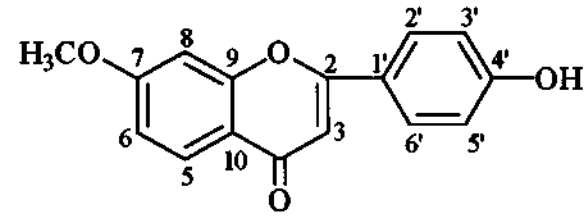
Compound **1**: Yellow needles (12 mg), m.p 210-212°. UV λ_{max} (MeOH) 235, 314, 370, +NaOMe 252, 270, 436, +$ALCl_3$ no shift. ^{1}H-NMR (400 MHz, *DMSO-d_6*): δ 3.88 (3H, s, OCH_3), 6.36 (1H, dd, *J*= 8.6 Hz, 2.5 Hz, H-5), 6.48 (1H, d, *J*= 2.5 Hz, H-3), 6.88 (2H, d, *J*= 8.6 Hz, H-3´, H-5´), 7.70 (1H, d, *J*= 15.6 Hz, H-α), 7.78 (2H, d, *J*= 8.6 Hz, H-2´, H-6´), 7.84 (1H, d, *J*= 8.6 Hz, H-6), 7.96 (1H, d, *J*= 15.6 Hz, H-ß). ^{13}C-NMR (100 MHz, *DMSO-d_6*): δ 55.87 (q, OCH_3), 98.29 (d, C-3), 106.24 (d, C-5), 115.29 (d, C-3´, C-5´), 116.11 (s, C-1), 119.19 (d, C-α), 129.44 (s, C-1´), 129.87 (d, C-6), 130.81 (d, C-2´, C-6´), 137.44 (d, C-ß), 161.90 (s, C-4), 159.73 (s, C-2), 162.76 (s, C-4´), 187.28 (s, C=O).
Compound **1** was found to be 4,4´-dihydroxy-2-methoxy chalcone.

Compound **2**: Amorphous powder (20 mg). UV λ_{max} (MeOH) 252, 328, +NaOMe 251, 364, +$AlCl_3$ 252, 328, $AlCl_3$/HCl 253, 327, +NaOAc 252, 332. ^{1}H-NMR (400 MHz, *DMSO-d_6*): δ 3.90 (3H, s, OCH_3), 6.75 (1H, s, H-3), 6.92 (2H, d, *J*= 8.5 Hz, H-3´, H-5´), 7.03 (1H, dd, *J*= 8.7 and 2.4 Hz, H-6), 7.26 (1H, d, *J*= 2.4 Hz, H-8), 7.94 (2H, d, *J*= 8.5 Hz, H-2´, H-6´), 7.90 (1H, d, *J*= 8.7 Hz, H-5). ^{13}C-NMR (100 MHz, *DMSO-d_6*): δ 56.06 (q, OCH_3), 100.90 (d, C-8), 104.61 (d, C-3), 114.40 (d, C-6), 115.93 (d, C-3´, C-5´), 117.08 (s, C-10), 121.66 (s, C-1´), 126.12 (d, C-5), 128.17 (d,C-2´, C-6´), 157.38 (s, C-9), 160.82 (s, C-4´), 162.74 (s, C-2), 163.72 (s, C-7), 176.33 (s, C=O).
Compound **2** was found to be 4´-hydroxy-7-methoxy flavone.

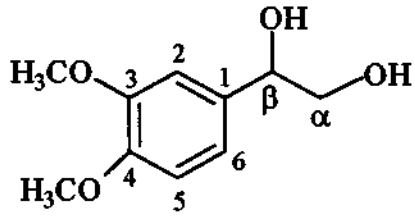
Compound **3**: Amorphous powder (6 mg). ^{1}H-NMR (400 MHz, *CD_3OD*): δ 6.94 (1H, d, *J*= 2 Hz, H-2), 6.80 (1H, dd, *J*= 8.3 Hz, 2 Hz, H-6), 6.75 (1H, d, *J*= 8.3 Hz, H-5), 4.76 (1H, m, H-ß), 4.31 (1H, m, H-αax), 3.82 (1H, m, H-αeq), 3.84 (6H, s, 2x OCH_3). ^{13}C-NMR (100 MHz, *CD_3OD*): δ 55.36 (q, OCH_3), 56.44 (q, OCH_3), 72.61 (t, C-α), 87.50 (d, C-ß), 111.02 (d, C-2), 116.09 (d, C-5), 120.07 (d, C-6), 133.82 (s, C-1), 147.32 (s, C-5), 149.13 (s, C-4); EIMS (m/z, rel.int.%), 198 (M^+, 4.4), 182 (M^+-16, 10.1), 181 (M^+-OH, 11.4), 167(M^+-CH_2OH, 11.5), 151 (M^+-47, 59.2), 107 (26.1), 121 (base peak); IR (KBr, ν cm^{-1}), 3410 (OH), 1594, 1507 (aromatic system).
Compound **3** was found to be ß-(3,4-dimethoxy phenyl) α,ß-ethanediol.



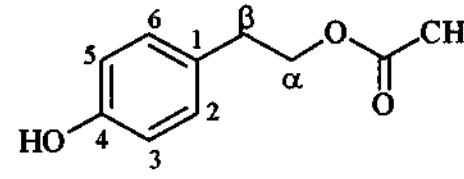
Compound 1



Compound 2



Compound 3



Compound 4

Compound **4**: Amorphous powder (10 mg). ^{1}H-NMR (400 MHz, *DMSO-d_6*): δ 7.60 (1H, br.s, OH), 7.20 (2H, d, *J*= 6.1 Hz, H-3, H-5), 7.14 (2H, d, *J*= 6.1 Hz, H-2, H-6), 4.31 (2H, t, *J*= 7.1 Hz, H_2-α), 2.86 (2H, t, *J*= 7.1 Hz, H_2-ß), 1.96 (3H, s, CO<u>CH_3</u>). ^{13}C-NMR (100 MHz, *DMSO-d_6*): δ 170.69 (s, C=O), 116.35 (d, C-3, C-5), 130.46 (d, C-2,C-6), 65.51 (t, C-α), 34.52 (t, C-ß), 20.76 (q, CO-<u>CH_3</u>), 157.65 (s, C-4), 128.69 (s, C-1). EIMS (m/z, rel.int.%): 180 (M^+, 7.1), 138 (M^+-42, 6.5), 137 (M^+-$COCH_3$, 1.3), 121 (M^+-$COOCH_3$, 37.2), 120 (M^+-$HO.CO.CH_3$, base peak), 107 (M^+-CH_2-O-$CO.CH_3$, 98), 43 ($CO.CH_3$, 82.6). IR (KBr, ν cm^{-1}), 3450 (OH), 1728 (C=O), 1600, 1512, 1432 (aromatic system).

Compound **4** was found to be p-hydroxy benzene acetic acid ethyl ester.

RESULTS AND DISCUSSION

Compound **1** was obtained as yellow needles, the chalcone character of this compound was shown from its UV spectrum (methanol) λ_{max} 235, 314 and 370 nm.[10] It gave a positive response to the $FeCl_3$ test for phenols. The absence of C-2′ and C-6′ hydroxy groups was confirmed, as there was no shift on addition of $AlCl_3$ and also from the upfield shift of the carbonyl group in the ^{13}C-NMR (δ 187.28). The ^{1}H-NMR spectrum showed the presence of methoxyl group, two olefinic protons of trans-α,ß unsaturated ketone (δ 7.70, d, *J*= 15.6 Hz, H-α) and (δ 7.96, d, *J*= 15.6 Hz, H-ß) and seven aromatic protons forming an ABX and AA′BB′ pattern of substitution on the two aromatic rings.

The location of methoxyl group at C-2 was confirmed from the upfield shift of C-ß (δ 137.44). This is because the chemical shift of C-ß exert dependence on the substituent at C-2 position and get shifted 3-5 ppm upfield by an oxy substituents such as methoxyl group at C-2 position.[11] The above mentioned data are in agreement with the data reported for echinatin previously isolated from the cultured cells of *Glycyrrhiza echinata*[12] and from *Bauhinia manca*[13] but reported here for the first time in the family Amaryllidaceae. Consequently, it has been established that isoliquritigenin, a normal chalcone, is an efficient precursor of echinatin[12] whose A-ring is derived from the B-ring of isoliquritigenin by conversion of carbonyl group. The isolation of isoliquritigenin from *Crinum bulbispermum*[6] agrees with this establishment.

Compound **2** exhibited UV absorption maxima at 252 and 328 nm indicating that it is a flavone.

The ^{1}H-NMR spectrum showed an ABX and AA´BB´ pattern of substitution on two aromatic rings and a singlet at δ 6.75 for the olefinic proton H-3. The spectrum did not exhibit a hydrogen-bonded phenolic hydroxyl which indicated that H-5 was free or substituted by OCH_3. The spectrum also showed one signal for one aromatic methoxyl at δ 3.90 which appeared as quartet in the ^{13}C-NMR at δ 56.06. The methoxyl and hydroxyl groups are positioned at C-7 and C-4´, respectively. This could be confirmed from the non-bathochromic shift with NaOAc and bathochromic shift with NaOMe. Furthermore, the ^{13}C-NMR spectrum showed resonances for 16 carbons. The DEPT experiments revealed the presence of one quartet (OCH_3-7), six doublets (C-3, C-5,C-6, C-8, C-2´/C-6´ and C-3´/C-5´) and seven singlets, among which were four oxygenated carbons which appeared downfield (δ 157.38 to 163.72) and corresponded to C-9, C-4´, C-2 and C-7 together with the resonances at 176.33, 121.66 and 117.08 for C-4, C-1´ and C-10, respectively. The above mentioned data are in agreement with the data reported for 4´-hydroxy-7-methoxy flavone isolated from *Medicago sativa*[14] and reported here for the first time in the family Amaryllidaceae.

Compound **3** was obtained as amorphous powder. Its IR spectrum exhibited absorption bands for OH group and an aromatic system. The mass spectrum revealed a molecular ion peak at m/z 198 corresponding to the molecular formula $C_{10}H_{14}O_4$ (deduced from MS, ^{13}C-NMR, ^{1}H-NMR and DEPT experiments).

The ^{1}H-NMR spectrum displayed the aromatic signals of this compound in the region δ 6.75-6.94 ppm as an ABX system. The chemical shift of the multiplet at δ 4.76 assignable to the proton at the ß-carbon indicated that this carbon is oxygenated, which further confirmed from the chemical shift of its ^{13}C-NMR signal at δ 87.5. The two multiplets centered at δ 4.31 and 3.84 were assigned for the α-CH_2 of the aromatic side chain.

The ^{1}H-NMR study indicated that the aromatic region integrated for three protons forming an ABX system along with two phenoxymethyl groups and the substitution pattern was evident by the presence of two ortho-coupled protons (H-5 and H-6) at δ 6.75 and 6.80 (J= 8.3 Hz), respectively. The later proton was further meta coupled (J= 2 Hz) with H-2 at δ 6.94.

Its ^{13}C-NMR spectrum showed the resonances for 10 carbons. These signals, based on DEPT experiments, were classified to three singlets (C-4, C-3, and C-1), four doublets (C-6, C-5, C-2, C-ß), one tirplet (C-α) and two quartets (2XOCH_3). Among the singlets were 2 oxygenated ones appeared at δ 149.13 and 147.32 for C-4 and C-3, respectively.

From the above mentioned data compound **3** was identified as ß-(3,4 dimethoxyphenyl)-α,ß-ethanediol.

To best of our knowledge, compound **3** has not encountered before in nature nor has it been prepared synthetically.

Compound **4** was obtained as amorphous powder and gave a positive response to $FeCl_3$ test for phenols. The Mass spectrum exhibited a molecular ion peak at m/z 180 corresponding to the molecular formula $C_{10}H_{12}O_3$ with five double bond equivalents (deduced from ^{1}H-NMR, ^{13}C-NMR and DEPT experiments). The IR spectrum exhibited absorption bands for hydroxyl and carbonyl groups at 3450 and 1728 cm^{-1}, respectively.

The ^{1}H-NMR spectrum in C_5H_5N revealed signals at δ 2.86 and 4.31 (2H, each, triplet, J= 7.1 Hz, phenyl-CH_2-CH_2-O). In the aromatic region, the ^{1}H-NMR spectrum revealed an AA´BB´ pattern of substitution at δ 7.14 (2H, d, J= 6.1 Hz, H-2/H-6) and δ 7.20 (2H, d, J= 6.1 Hz, H-3/H-5) and were confirmed from the the ^{13}C-NMR by the two doublets at 130.46 (C-2/C-6) and δ 116.35 (C-3/C-5). The ^{1}H-NMR spectrum also showed one signal for methyl group at δ 1.96 which appear as quartet in the ^{13}C-NMR (DEPT) at δ 20.76 for $\underline{CH_3}$-CO. The ^{13}C-NMR also revealed three singlet carbons at δ 170.69, 157.65 and 128.69 for CH_3-$\underline{CO}$, C-4 and C-1, respectively.

The location of the acetyl group in the side

chain was deduced from the positive response with $FeCl_3$ and from the Mass spectrum which exhibited ions at m/z 120 (base peak, M^+-HOOC-CH_3) and m/z 107 (M^+-H_2C-OOC-CH_3).

The above mentioned data are consistent with para substituted phenyl derivative. Consequently compound **4** was assigned as p-hydroxy benzene acetic acid ethyl ester.

To best of our knowledge, compound **4** has not encountered before in nature nor has it been prepared synthetically.

Acknowledgements

The author is grateful to Dr. M.S. Kamel, Associate professor of pharmacognosy, Assiut University for carrying out NMR spectral analysis.

REFERENCES

1- K. Ghosal, S. Saini and S. Razdan, Phytochemistry, 24, 2141 (1985).
2- M. A. Ramadan, Phytochemical investigation of the minor alkaloids and phenolic compounds of *Crinum Bulbispermum* and *Crinum Augustum* (from the family Amaryllidaceae) cultivated in Egypt, A Ph.D Thesis, Faculty of Pharmacy, Assiut Univ., Egypt (1986).
3- A. M. El-Moghazi and A. A. Ali, Planta Medica, 30, 369 (1976).
4- A. M. Abd El-Baky, Pharmacognostical study of *Crinum bulbispermum* Milne growing in Egypt, A Master Thesis, Faculty of Pharmacy, Assiut Univ., Egypt (1976).
5- A. A. Ali, M. A. Ramadan and A. W. Frahm, Planta Medica, 50, 424 (1984).
6- M. A. Ramadan, M. S. Kamel, K. Ohtani, R. Kasai, and K. Yamasaki, Assiut University Second Pharmaceutical Sciences Conference, Assiut, Egypt, 8-9 March, 2000 p. 51.
7- R. Robbins, Medical and nutritional aspects of citrus bioflavonoids. In S. Nagy and J. Attaway, eds., Citrus Nutrition and Quality, pp. 43–59. American Chemistry Society, Washington, DC. (1980).
8- Y. Matsubara, H. Kumamoto and Y. Iizuka, Agricultural and Biological Chemistry, 49: 909 (1985).
9- J. Robak and R. J. Gryglewski, Biochemistry and Pharmacology, 37, 837 (1988).
10- S. I. Ayabe, M. Kobayashi, M. Hikichi, K. Matsumoto and T. Furuya, Phytochemistry, 19, 2179 (1980).
11- P. K. Agrawal, Carbon-13 NMR of Flavonoids, Elsevier Science Publishers, Oxford, 365 (1989).
12- T. Saitoh, H. Noguchi and S. Shibata, Chemical and Pharmaceutical Bulletin, 26, 144(1978).
13- H. Achenbach, M. Stöcker and M. A. Constenla, Phytochemistry, 27, 1835 (1988).
14- C. A. Maxwell, U. A. Hartwig, C. M. Joseph and D. A. Phillips, Plant Physiology, 91, 842 (1989).