

PHYTOCHEMICAL STUDY OF *BARLERIA CRISTATA* L. AND
BARLERIA PRIONITIS L. CULTIVATED IN EGYPT

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ABSTRACT

From The hexane extract of *Barleria cristata* L. and *Barleria prionitis* L. α -amyrin, β -Sitosterol, and stigmasterol-3-O- β -D-glucoside were isolated. While acetyl barlerin (6,8-O-diacetyl shanzhiside methyl ester), shanzhiside methyl ester, apigenin and apigenin-7-O-glucoside were isolated from ethyl acetate extract of *B. cristata* L. In addition 6,8-O-diacetyl shanzhiside methyl ester, shanzhiside methyl ester, 6-O-acetyl shanzhiside methyl ester and 8-O-acetyl shanzhiside methyl ester (barlerin), were isolated from ethyl acetate extract of *B. prionitis* L. The identification of these compounds was based on physical, chemical and spectral studies including IR, UV, ^1H and ^{13}C -NMR spectra.

INTRODUCTION

Barleria cristata L. and *Barleria prionitis* L. are perennial shrubs, belonging to Family Acanthaceae. The plants are used in skin diseases, bronchitis, blood diseases and asthma ¹.

Some *Barleria* species have been investigated and proved to contain iridoids ²⁻⁶, flavonoids ⁷⁻¹¹, sterols and triterpenes ¹². This publication deals with the phytochemical study of *B. cristata* L. and *B. prionitis* L. cultivated in Egypt.

EXPERIMENTAL

General Experimental Procedure:

Melting points were determined using kofler hot stage microscope and are uncorrected, ^1H -NMR spectra were recorded in (CD₃OD) and pyridine with Bruker 400 MHz, ^{13}C -NMR were measured in (CD₃OD) using 100 MHz Bruker. Unicam SP 1025 infrared spectrophotometer was used for recording infra-red spectra and UV was measured on Unicam SP 1750 spectrophotometer.

Plant Material:

The plant material consists of the herbs of both plants, collected from Experimental Station of Medicinal Plants, Assiut University. During the flowering stage. The plants were kindly identified by Prof. Dr. N.E.El-Keltawi, Professor of Horticulture, Faculty of Agriculture, Assiut University.

Extraction:

One kg. of the powdered herbs of two *Barleria* species were separately extracted with 70% ethanol. The semisolid residue (50 g) obtained after concentration was digested with warm distilled water, successively extracted with hexane (20 g) and ethyl acetate (7 g).

Investigation of the Hexane extract of *B. Cristata* L.:

A part of hexane extract was investigated on silica gel G plates using chloroform-methanol (9:1 v/v) as developer and 15% methanolic H_2SO_4 as spray reagent.

Five spots were observed in both extracts of two plants having R_f value 0.97, 0.61, 0.55, 0.29 and 0.05.

Column Chromatography of Hexane Extract:

Nine gm. of hexane extract was transferred onto a silica gel (E-Merck, 300 g, 120x2.5 cm) column, eluted with hexane and then with mixtures of hexane-ethyl acetate in an increasing polarity. Fractions (200 ml each) were collected, concentrated and screened by TLC. Similar fractions were pooled together, concentrated and crystallized, compound H_1 was obtained from fractions (11-21) eluted with 5% ethyl acetate, H_2 from

fractions (22-32) eluted with 7% ethyl acetate followed by H_3 which was obtained from fractions (61-81) eluted with 20% ethyl acetate. The other two compounds having R_f values 0.97 and 0.61 were obtained in a small quantities.

Compound H_1 : (90 mg, $R_f=0.55$)

White needles, mp. 184-86°C (MeOH). It gave violet colour with Liebermann-Burchard's test ¹³. IR: 3310, 2910, 1480, 1360 and 1080 cm^{-1} .

This compound was identified as α -amyrin by co-chromatography and mixed m.p. with authentic sample.

Compound H_2 : (4 g, $R_f=0.29$)

White needles, mp. 135-37°C (MeOH) IR; showed: 3310, 2930, 1540, 1390, 1300, 1090 and 1030 cm^{-1} . This substance was identified as β -sitosterol by co-chromatography and mixed m.p. with authentic sample of β -sitosterol.

Compound H_3 : (90 mg, $R_f=0.05$)

White powder, mp. 263-65°C (MeOH). IR (ν cm^{-1}) 3420 (OH), 2940, 1470, 1380 (CH_2 & CH_3), 1280, 1105, 1075, 1030 (CH bending). ¹H-NMR (400 MHz, pyridine) δ 5.35(1H, br. s.) for H-6, 5.07 (2H, dd) for H-22, 23, 5.07 (1H, d, J=7.6) for H-1', 4.58 (1H, dd, J=10.9, 2.5) for H-6'a, 4.4 (1H, dd, J=11.4, 5.5) for H-6'b, 4.3 (2H, m) for H-3' and H-4', 4.0(1H,t, J=8,15.8) for H-2', 3.9(2H, m) for H-3, 5, 1.0-2.8 (CH_2 and CH protons), 1.01 (3H,d, J=6.5) for CH_3 -21, 0.86 (3H, s) for CH_3 -19, 0.91 (3H, t) for CH_3 -29, 0.64(3H, s) for CH_3 -18, 0.95 (3H, d) for CH_3 -26, 0.86 (3H, d) for CH_3 -27.

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The above mentioned physical, chemical and spectral data of compound H-3, are similar to those reported for stigmaterol 3-O- β -D-glucoside¹⁴. Compounds H₁, H₂ and H₃ were also separated from hexane extract of *B. prionitis* L. and identified as α -amyrin, β -sitosterol and stigmaterol glucoside.

Investigation of Ethyl Acetate Extract of *B. cristata* L.:

TLC investigation of ethyl acetate extract using silica gel G, solvent system chloroform-methanol-water (8:2:1 v/v) and spray reagent vanillin H₂SO₄ revealed the presence of two spots having R_f values: 0.7 and 0.38 representing Bc₁ & Bc₂. Two spots having R_f values 0.08 and 0.18 were revealed by spray reagent AlCl₃ in methanol representing compounds F₁ and F₂. Column chromatographic fractionation on silica gel column was performed, eluting with ethyl acetate and then with mixture of ethyl acetate-methanol in an increasing polarity. Fractions were collected, concentrated, and screened by TLC, similar fractions were pooled together.

Preparative TLC using silica gel G and chloroform-methanol-water (8:2:1 v/v) succeeded for isolation of two iridoids Bc₁ and Bc₂, as well as preparative paper chromatography using whatman No. 3MM and 45% acetic acid was used for isolation of two flavonoids (F₁ and F₂).

Four iridoids were isolated from *B. prionitis* L. Bc₁, Bc₂, Bc₃ and Bc₄. The two iridoids Bc₁ and Bc₂ are identical with those isolated from *B. cristata* L.

Acid Hydrolysis:

The isolated flavonoidal glycoside (5 mg) was dissolved in 5.0 ml MeOH to which 20% H₂SO₄ solution was added and the mixture was refluxed on a boiling water bath for 8 hr. A sample of the hydrolysate was withdrawn every 30 minutes and subjected to TLC study. After complete hydrolysis, the mixture was cooled and the aglycone was separated by successive extraction with chloroform. Chromatographic studies of aglycone and sugar were carried out using chloroform-methanol (85:15 v/v) as system for aglycone, acetone-pyridine-water (3:1:1 v/v) and thymol H₂SO₄ as spray reagent for sugars.

Compound F₁: (35 mg)

Yellow powder, m.p. 343-345°C (MeOH) soluble in most organic solvents, insoluble in water. From UV-spectrophotometric data with shift reagents Table 1 and by co-chromatography with authentic sample, it was concluded that this compound F₁ is Apigenin.

Compound F₂: (60 mg)

Yellow powder, m.p. 178-180°C (MeOH), soluble in water and methanol. The UV-spectral data Table I proved that this substance is a flavonoid glycoside. Partial and complete acid hydrolysis indicated that it is a monoside giving apigenin aglycone (AF₂) and the sugar moiety was identified as glucose. From the above data compound F₂ was found to be apigenin-7-O-glucoside.

Compound Bc₁: (90 mg)

Yellowish brown residue R_f 0.7, in (CHCl₃-MeOH-H₂O 8:2:1) UV $\lambda_{\text{MeOH}}^{\text{max}}$ 232 nm. for iridoids with carboxy-

methyl group at C-4. IR (ν cm^{-1}) 3450 (OH), 1710, 1690 (C=O), 1660 (C=C) for iridoids.

$^1\text{H-NMR}$ (400 MHz) and $^{13}\text{C-NMR}$ are listed in Tables II and III.

From physicochemical and spectral data of compound Bc_1 , it was proved to be identical to 6,8-O-di-acetyl shanzhiside methyl ester, (Acetyl barlerin) ⁵.

Compound Bc_2 : (80 mg)

Yellowish powder, m.p. 116-118°C. (MeOH) R_f 0.38
UV $\lambda_{\text{max}}^{\text{MeOH}}$ 233 nm. for iridoids with carboxymethyl group at C-4.

IR (ν cm^{-1}) 3455 (OH), 1690 (C=O), 1650 (C=C) for iridoids. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ are listed in Table II and III.

Compound Bc_3 : (25 mg)

Yellowish-brown residue insoluble in chloroform and hexane, soluble in methanol, acetone, R_f 0.67 in ($\text{CHCl}_3\text{-MeOH-H}_2\text{O}$ 8:2:1). The UV absorption at 235 nm, the IR showed bands at: 3400 (OH), 1630 (C=C), 1690 (C=O) and 1730 cm^{-1} . The ^1H and $^{13}\text{C-NMR}$ data are listed in Table IV.

Compound Bc_4 : (50 mg)

Yellowish-brown residue, R_f 0.56. The UV absorption at 233 nm. The IR showed, 3400, 1730, 1690 and 1625 cm^{-1} .

RESULTS AND DISCUSSION

The ethanolic extract of *Barleria cristata* L. and *Barleria prionitis* L. were separately fractionated into

hexane and ethyl acetate soluble fractions. The hexane extract of both plants was chromatographed over silica gel column, three compounds were isolated ($\text{H}_1\text{-H}_3$) from each plant which are similar in both plants. On the basis of co-chromatography, mixed m.p., physical properties, chemical tests and comparison of IR and $^1\text{H-NMR}$ spectra compounds H_1 , H_2 and H_3 were found to be α -amyrin, β -sitosterol and stigmasterol-3-O-glucoside in both plants under investigation.

Fractionation of ethyl acetate extract of both plants separately over silica gel column followed by preparative TLC and PC, afforded four compounds F_1 , F_2 , Bc_1 and Bc_2 from *Barleria cristata* L. and *B. prionitis* L. while other two iridoids Bc_3 and Bc_4 were isolated from *B. prionitis* L. only.

The compounds F_1 and F_2 were proven to be flavonoids. Their chromatographic characters, spectral data, partial and complete acid hydrolysis proved that compound F_1 and F_2 are apigenin and apigenin-7-O-glucoside respectively.

Compounds ($\text{Bc}_1\text{-Bc}_4$) were proven to be iridoids in nature ¹⁵, the UV showed absorption maxima at 232 nm characteristic for a conjugated enol-ether system and typical for iridoid glycosides with C-4 methoxy carbonyl group ⁶. IR showed bands at 1730 and 1639 cm^{-1} . $^1\text{H-NMR}$ spectra of all compounds exhibits only minor differences. A signal at δ 1.55 assignable to a tertiary methyl group attached to oxygenated carbon (C-8) ⁵. This was confirmed from $^{13}\text{C-NMR}$ which exhibited signal at δ 90.44 ppm. The methylene protons at δ 2.15 and 2.35 assignable for H-7, which was confirmed by

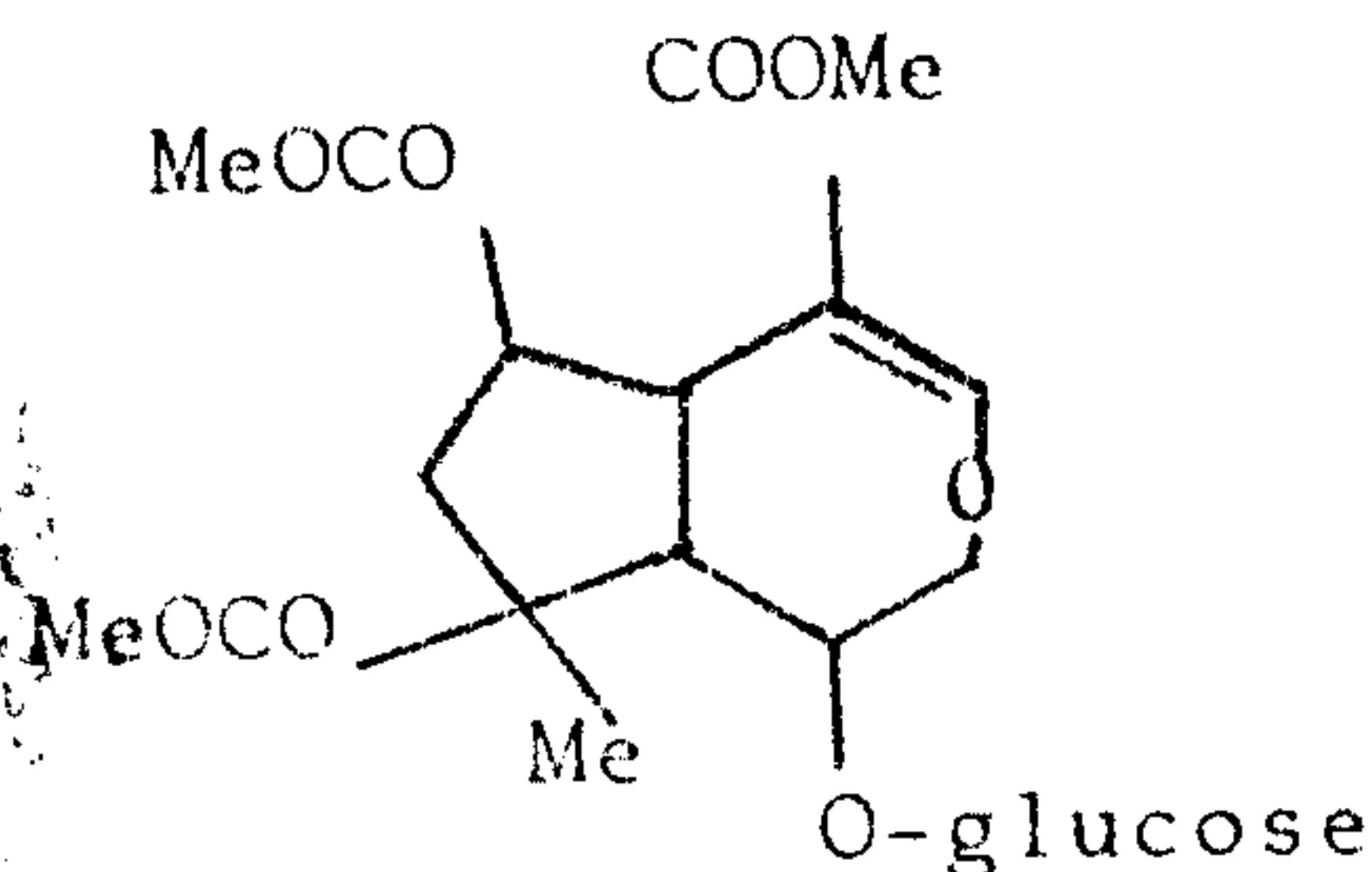
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^{13}C -NMR located at δ 45.8 (c.f. C-7 substituted iridoids) ⁶ in all compounds, also two signals at δ 3.11 and δ 2.95 ppm assignable for H-5, and H-9 respectively. ^{13}C -NMR at δ 40.7 indicated the absence of substitution at C-5, the signal at δ 51.11 attributable to C-9 unsubstituted. ^1H -NMR indicated the presence of two acetyl groups at δ 2.0 and δ 2.05 ppm., which was confirmed from ^{13}C -NMR located at δ 173. δ 173.6. According to the previous data, these two acetate groups must be located at C-6 and C-8 respectively in compound Bc₁.

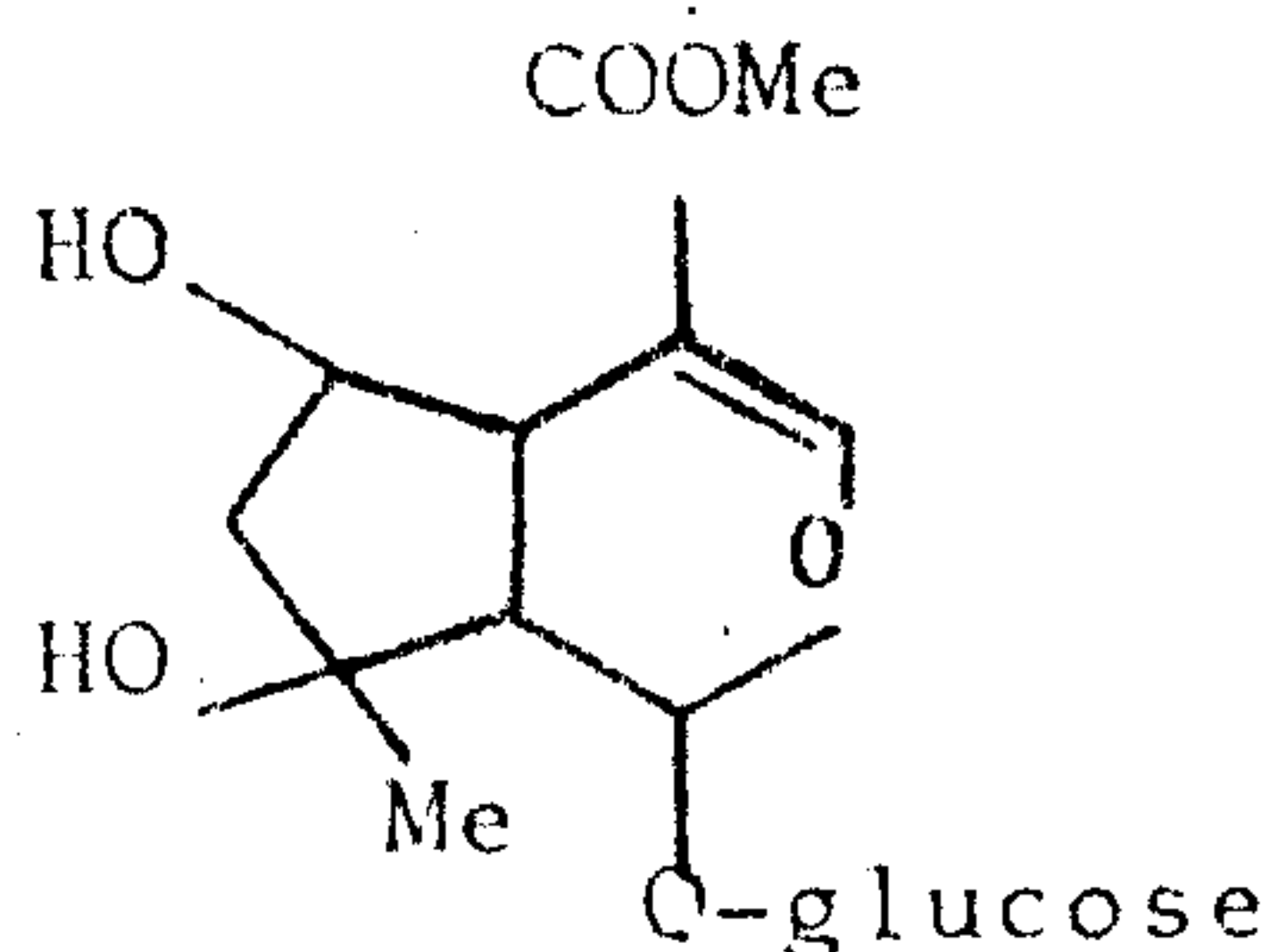
The ^1H -NMR spectrum of Bc₂ showed, the absence of any signal corresponding to acetyl residue, so that C-6 and C-8 are substituted with hydroxy groups. This was confirmed from the chemical shift of H-5 and H-9 which were present at δ 3.01 and δ 2.60 ppm respectively (c.f. 6,8-O-diacetyl iridoids, δ 3.1 and δ 2.95 respectively) ⁶. The compound Bc₂ is 6,8-di-hydroxy iridoid.

The ^1H -NMR spectrum of Bc₃ showed only one acetyl residue at δ 1.8 ppm which is located at C-6, this was supported by the downfield shift of δ 0.70 ppm in the signal (5.05 ppm) of H-6 compared with the corresponding signal (4.35) for the reported 6-hydroxy iridoids ⁶. So this compound is 6-acetyl-8-hydroxy iridoid. Comparing the spectral data of Bc₄ with those of Bc₁, it was found that they are identical except that, in the ^1H -NMR spectrum of compound Bc₄ the presence of one acetyl residue, which located at C-8, this was supported by the position of H-6 (4.35) which was identical to the reported 6-hydroxy iridoids ⁶. So that compound Bc₄ is 6-hydroxy-8-acetyl iridoid.

From the previous physical, chemical and spectral analysis, compound Bc₁ proved to be 6,8-O-di-acetylshanzhiside methyl ester (Acetyl barlerin), which was isolated previously from *Barleria* species, compound Bc₂ is shanzhiside methyl ester. This is the first report of isolation of this compound from *Barleria cristata* L. Compound Bc₃ is 6-O-acetyl shanzhiside methyl ester, this was previously isolated from *Barleria lupulina* Lindl., and compound Bc₄ is 8-O-acetyl shanzhiside methyl ester, which was also isolated from *Barleria lupulina* ⁶.



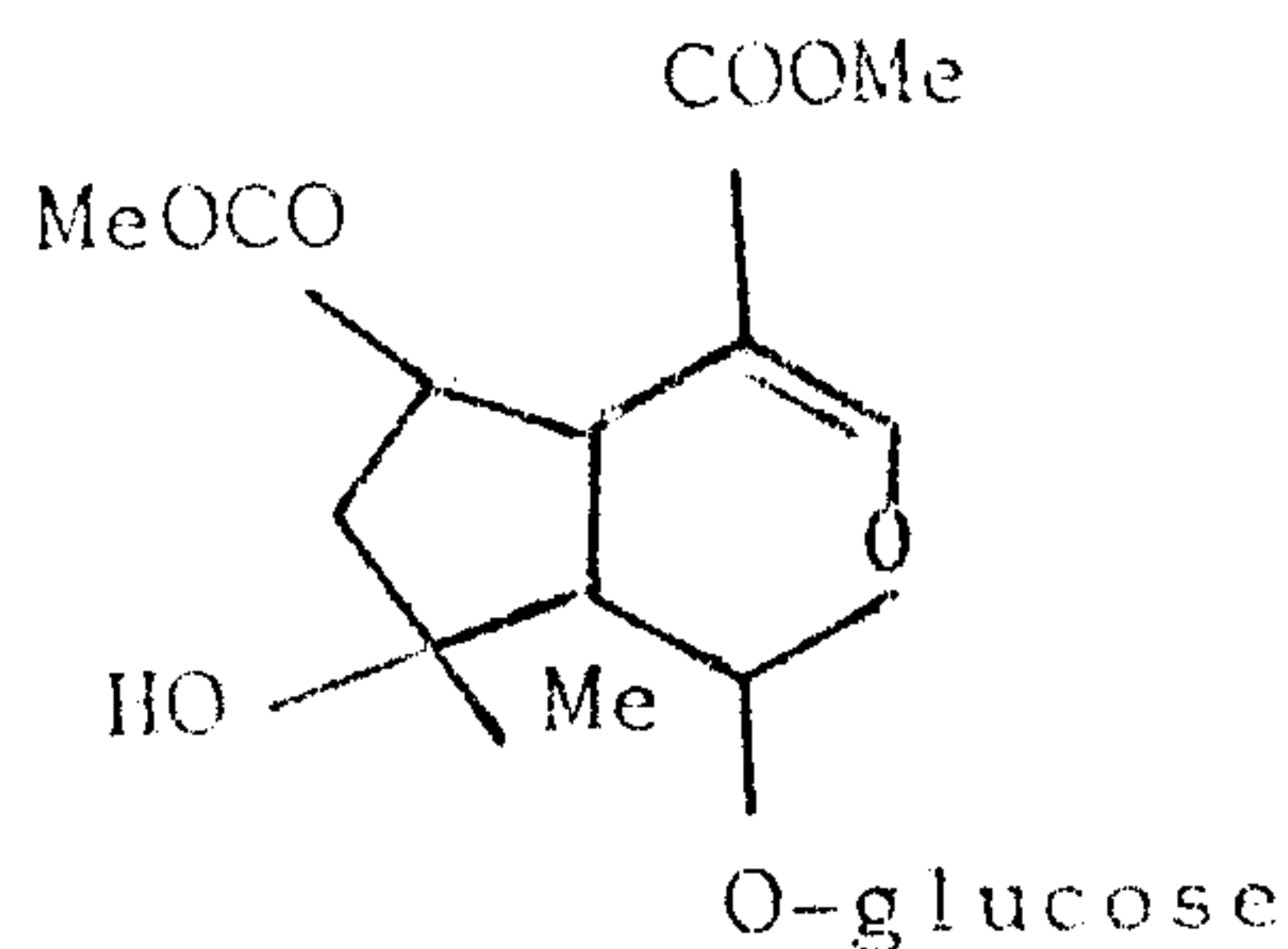
Bc₁-6,8-diacetyl shanzhiside methyl ester (acetyl barlerin).



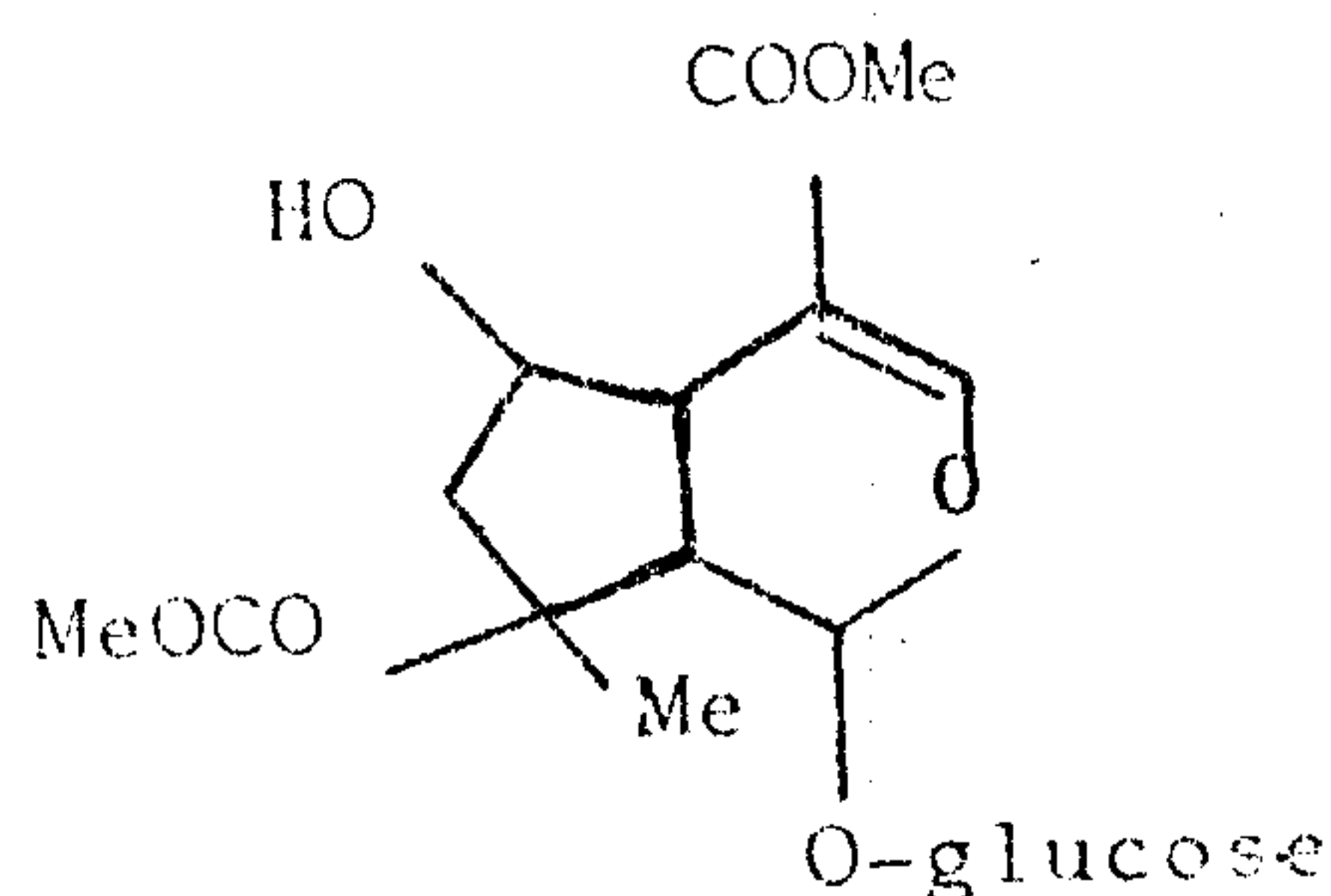
Bc₂-Shanzhiside methyl ester.

Table 1: UV-Spectra of the isolated flavonoids F₁ and F₂ with different ionizing and complexing reagents.

| Sub No. | Band | max. | | | | | |
|-----------------|------|------|----------------------------|--------------------|--|--------|---------|
| | | MeOH | +AlCl ₃ +HCl | +AlCl ₃ | NaOAc +H ₃ BO ₃ | +NaOAc | +NaOMe |
| | | max | max | max | max | max | max |
| F ₁ | I | 328 | 340 +12 | 340 +12 | 328 - | 328 - | 386 +58 |
| | II | 268 | 276 +8 | 276 +8 | 275 +7 | 268 - | 273 +5 |
| F ₂ | I | 328 | 342 +14 | 342 +14 | 334 +6 | 328 - | 386 +58 |
| | II | 268 | 278 +10 | 278 +10 | 268 - | 268 - | 268 - |
| AF ₂ | I | 329 | 342 +14 | 342 +14 | 329 - | 329 - | 390 -62 |
| | II | 268 | 277 +9 | 277 +9 | 276 +8 | 268 - | 273 +5 |



Bc₃-5-acetyl shanzhiside methyl ester.



Bc₄-8-acetyl shanzhiside methyl ester (barlerin).

Table II: ¹H-NMR of compounds Bc₁ and Bc₂.

| Proton | Bc ₁ | Bc ₂ |
|--------------------------|---|--|
| H-3 | 7.5 (s) | 7.5 (d, J _{3,5} =1.1) |
| H-1 | 5.8 (d, J _{1,9} =3.5) | 5.56 (d, J _{1,9} =2.7) |
| H-6 | 5.31 (m.) | 4.04 (m.) |
| H-5 | 3.1* (m.) | 3.01 (m.) |
| H-9 | 2.95 (dd, J _{9,1} =3.4, J _{9,5} =5.5) | 2.6 (d, J _{9,1} =2.6) |
| H-7 _a | 2.35 (d, J _{7a,7b} =15.7) | 2.1 (d, J _{7a,7b} =14) |
| H-7 _b | 2.15 (dd, J _{7b,7a} =15.6, J _{7b,6} =5.6) | 1.85 (dd, J _{7b,7a} =14, J _{7,6} =6) |
| O-CH ₃ | 3.7 (s) | 3.7 (s) |
| CH ₃ -CO, 6,8 | 2, 2.05 (s) | |
| CH ₃ -8 | 1.55 (s) | 1.31 (s) |
| H-1' | 4.7 (d, J=7.9) | 4.65 (d, J=7.8) |
| H-6' _a | 3.9 (dd, J=2.1, 15.2) | 3.9 |
| H-6' _b | 3.65 | 3.66 |
| Sugar proton | 3.4-3.1 | 3.1-3.6 |

* Overlapped with sugar protons.

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Table III: ^{13}C -NMR of compound Bc₁ and Bc₂.

| Carbon atom | Bc ₁ | Bc ₂ |
|-------------------------|-----------------|-----------------|
| 1 | 96.2 | 95.5 |
| 3 | 155 | 153.3 |
| 4 | 109.4 | 112 |
| 5 | 40.7 | 41.6 |
| 6 | 79.7 | 79.6 |
| 7 | 45.8 | 46.0 |
| 8 | 90.4 | 82.0 |
| 9 | 51.1 | 52.1 |
| 10 | 21.9 | 24.1 |
| 1' | 101.1 | 100.4 |
| 2' | 75.4 | 75 |
| 3' | 78.7 | 78 |
| 4' | 72.5 | 72 |
| 5' | 79.1 | 79 |
| 6' | 63.8 | 63 |
| O-C-CH ₃ 6.8 | 173.6, 173 | |
| C-O-CH ₃ | 169.2 | 169.5 |
| O-CH ₃ | 52.6 | 52.5 |
| C-CH ₃ | 23, 22.6 | -- |

Table V: ^{13}C -NMR (CD₃OD, 100 MHz) spectrum of compound Bc₄

| Carbon | Bc ₄ |
|---------------------|-----------------|
| C-1 | 97.4 |
| C-3 | 153.6 |
| C-4 | 110.06 |
| C-5 | 42.3 |
| C-6 | 76.1 |
| C-7 | 47.7 |
| C-8 | 89.6 |
| C-9 | 51.8 |
| C-10 | 22.3 |
| O-C-CH ₃ | 173.1 |
| CO-CH ₃ | 22.2 |
| C-CH ₃ | 169.2 |
| O-CH ₃ | 53 |
| C-1' | 100.4 |
| C-2' | 74.7 |
| C-3' | 78.3 |
| C-4' | 71.7 |
| C-5' | 78.1 |
| C-6' | 63.05 |

Table IV: ^1H -NMR (CD₃OD-400 MHz) spectra of compounds Bc₃ and Bc₄.

| Proton | Bc ₃ | Bc ₄ |
|--------------------|--|--|
| H-3 | 7.3 (s) | 7.45(d, J _{3,5} =1.4) |
| H-1 | 5.55(d, J _{1,9} =2.3) | 5.9 (d, J _{1,9} =2.4) |
| H-6 | 5.05(m.) | 4.35(m.) |
| H-5 | 2.9* | 3.09(dd, J _{5,1} =1.5, J _{5,9} =9) |
| H-9 | 2.7 (dd, J _{9,1} =3.1, J _{9,5} =8.3) | 3.01(dd, J _{9,1} =2.3, J _{9,5} =8.8) |
| H-7 _a | 2.1(d, J _{7b} =16.6) | 2.2 (d, J _{7b} =14.9) |
| H-7 _b | 2.05(dd, J _{7b,6} =7.2, J _{7b,7a} =13) | 2.05(dd, J _{7b,6} =7.2, J _{7b,7a} =13). |
| O-CH ₃ | 3.4 (s) | 3.8 (s) |
| CO-CH ₃ | 1.8 (s) | 2.0 (s) |
| CH ₃ -8 | 1.3 (s) | 1.5 (s) |
| H-1' | 4.5 (d, J=7.8) | 4.65(d, J=7.9) |
| 2', 3', 4', 5' | 3 - 3.5 | 3.2 - 3.55 |
| H-6' | 3.2 (d, J=12.1) | 6' _a =3.9(dd, J=2.2, 12.2) 6' _b =3.6 (m.) |

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دراسة الكيمياء العقاقيرية لنباتى البارليريا كريستاتا ل.

والبارليريا بريونييتس ل. المنزرعين فى مصر

نصر أحمد العمرى - مقبول أحمد مقبول - محمد عبد المطلب عبد الحافظ

أمانى سيد أحمد

قسم العقاقير - كلية الصيدلة - جامعة أسيوط

تم فصل الفا أميرين وبيتا سيتوستيرون وستيجمما ستيرول-3-أ-جلوكوزيد من خلاصة الهكسان لكلا النباتين.

تم فصل شنهيزيد ميثيل استر، 6، 8-داى اسيتيل شنهيزيد ميثيل استر، ابيجينين، ابيجينين-7-أ-جلوكوزيد من خلاصة خلات الايثيل لنبات بارليريا كريستاتا ل.

وكما تم فصل شنهيزيد استر، 6-أ-اسيتيل شنهيزيد ميثيل استر، 8-أ-اسيتيل شنهيزيد ميثيل استر من خلاصة خلات الايثيل لنبات البارليريا بريونييتس ل.

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