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FLAVONOIDAL GLYCOSIDE AND ALKALOID BESIDE OTHER CONSTITUENTS FROM *BIGNONIA UNGUIS-CATI* L.

A. A. Attia

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

تم فصل مركبين لأول مرة من العائلة البجنونية (من نبات مخلب القط) وهما قلوانى هيدروكسى-٣-استاكدزين وفلافونيد جلوكوزيد بكتولنارجينين-٧-جلوكوزيد بالإضافة إلى ثلاثة مركبات فينولية وهم ليتولين ، ٣-ميثوكسى كورسيتين وكورسيتين وكذلك تم فصل مركبين آخرين هما حمض الأورسليك وبيتاسيتوستيرول.
هذا وقد تم التعرف على هذه المركبات بواسطة الطرق المختلفة للتحليل الكيماوى والطيفى.

Phytochemical investigation of Bignonia Unguis-Cati L. led to the isolation and identification of the alkaloid hydroxy-3-stachydrine and the flavonoid glycoside pectolinargenin-7-O-B-D-glucoside. Also quercetin, quercetin-3-methyl ether, luteolin, ursolic acid and B-sitosterol were isolated. The identification of the isolated compounds was estabilshed through spectral analysis as well as by direct comparison with reference materials.

INTRODUCTION

The Bignoniaceae is a family of trees and shrubs, the majority of which are Lianes. It comprises about 120 genera and 650 species.[1-3] The most common classes of phytoconstituents in this family are quinones, iridoids and flavonoids while alkaloids are rare.[4-5] In the American tropics extracts of Bignonia Unguis-Cati are used to treat contact dermatitis caused by manchineel (Hippomane mancinella). The plant is also used as a snake bite antidote.[6] Many Bignoniaceae plants were used in Folk medicine as antipyretic, hypnotic, diuretic[7], antimaleria[8] and antidiabetic.[9,10] Also to treat diarrhea, dysentery, ulcer, wounds, fungal infections of the skin[11], diseases of the throat and against stomatitis.[9,10] From the literature, nothing was reported concerning the plant under investigation except the isolation of Lapachol which was found to exhibit potent antitumor activity.[12] For the above reasons the author investigated the plant to isolate and identify its active constituents.

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EXPERIMENTAL

Plant material

The aerial parts of Bignonia Unguis-Cati L. were collected in March, 1992, from the garden of Assiut University Club. The identification of the plant was confirmed by Prof. Dr. Faid, Professor of plant Taxonomy, Dept. of Botany, Faculty of Science, Assiut University.

General methods

All mps were uncorrected, UV specta were measured in MeOH and different ionizing and complexing agents using Unicam 1750 spectrophotometer, IR, were taken in KBr with Perkin-Elmer model 457 spectrophotometer, ^{1}H-NMR spectra were run in DMSO-d_6 and CD_3OD using varian JMNG-X 500 spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR) and also JNM-l 400 MHz. Mass spectra were carried out on Hitachi M-80 and on MAT 311A, 70 ev. Spectrometer. TLC were carried out on silica plates (kieselgel 60 F254, E. Merck). For column chromatography silica gel (E. Merck) were used.

Extraction and isolation

3 kg of dried aerial parts (stems and leaves) of Bignonia Unguis-Cati L. were powdered and extracwd with cold alcohol (70%). The conc. alcoholic extract (60 g) was diluted with distilled water and extracted successively and exhaustively with n-hexane, chloroform and finally with ethyl acetate.

The n-hexane fraction was fractionated on silica gel column using n-hexane-ethyl acetate gradient. Elution with hexane-ethyl acetate (98:2 v/v) afforded compound **1** and with hexane-ethyl acetate (95:5 v/v) afforded compound **2**.

The chloroform fraction was fractionated on silica gel column using chloroform-methanol gradient to afford compounds **3,4** and **5**.

The ethyl acetate fraction was chromatographed on silica gel column and elution was performed with chloroform and increasing polarity with methanol. The fractions eluted with chloroform-methanol (7:3 v/v) afforded the major compound **6** which was purified by repeated column chromatography using silica gel and chloroform-methanol as eluent. The aqueous extract left after fractionation was concentrated under reduced pressure and chromatographed on a column of silica gel. Elution was performed with mixture of chloroform-methanol-water (65:35:5 v/v). This resulted in the isolation of compound **7** which was recrystallized from methanol.

Acid hydrolysis of compound 6

10 mg of the isolated compound **6** was refluxed with 6% HCl (10 ml) at 100°C for 45 min. The aglycone was isolated by addition of distilled water and extracted with chloroform, dried over anhydrous calcium sulphate and crystallized from methanol. The remaining aqueous layer was evaporated under reduced pressure and dissolved in the least possible volume of isopropyl alcohol and the sugar was identified by direct TLC comparison with authentic sugar using ethyl acetate-pyridine-water (2:1:2 v/v) or n-BuOH/AcOH/H_2O (3:1:1 v/v) as developing solvent and spraying with sugar visualising reagent.

Compound 1

Needle-shaped crystals of m.p. 135-137°C, not depressed by authentic sample of B-sitosterol. Co-chromatography with authentic sample of B-sitosterol confirmed also its identity.

Compound 2

Fine needles of m.p 276-279°C, (MeOH). EIMS m/z (rel. int%) 456 (7), 410 (3), 300 (4), 248 (100), 207 (24), 188 (15), 174 (8) and 133 (30). ^{1}HNMR (DMSO-d_6): δ 5.1 (1 H, s, H-12), 4.28 (1H, d, J= 8.2 Hz, H-3) 0.66 (3H, s, CH_3), 0.74 (3H, s, CH_3), 0.80 (3H, d, J= 6.5 Hz, CH_3), 0.86 (3H, s, CH_3), 0.88 (3H, s, CH_3), 0.90 (3H, d, J= 6.2 Hz, CH_3) and 1.03 (3H, s, CH_3).

Compound 3 [FIG. 1]

Yellow amorphous powder, m.p. 330-332°C. EIMS m/z (rel. int%): 286 (100), 273 (22), 258 (18), 229 (8) and 203 (6). The UV and NMR data are listed in Tables 1 and 2 respectively.

Compound 4 [Fig. 1]

Yellow amorphous powder, m.p. 212-215°C. EIMS m/z (rel.int %): 316 (100), 298 (15), 273 (25), 203 (7), 153 (16), 135 (14) and 108 (9). The UV and NMR data are listed in Tables 1 and 2 respectively.

Compound 5 [Fig. 1]

Yellow amorphous powder, m.p. 315-316°C. EIMS m/z (rel. int %) : 302 (100), 286 (80), 273 (15), 152 (5), 153 (10), 138 (14), 137 (31), and 122 (17). The UV and NMR data are listed in Tables 1 and 2 respectively.

Compound 6 [Fig. 2]

Yellow amorphous powder, m.p. 162-164°C. ^{1}H-NMR (DMSO-d_6): δ 7.80 (2 H, d, J= 8.7 Hz, H-2′ and H-6′), 7.11 (2H, d, J= 8.7 Hz, H3′ and H-5′), 6.55 (1H, s, H-8), 6.45 (1H, s, H-3), 4.93 (1H, d, J= 7.2 Hz, anomeric proton of the sugar glucose), 3.88 (3H, s, O$\underline{CH_3}$-4′), 3.77 (3H, s, O$\underline{CH_3}$-6) and 12.66 (1H, s, OH at C-5). UV data are listed in Table 1 and ^{13}C-NMR in Table 3.

Table 1: UV spectral data of the isolated flavonoids **3, 4, 5, 6** and the aglycone of compound **6**.

Compound	MeOH	NaOMe	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3
3	254 268 290 350	268 330 402	273 301 327 425	265 275 293 388	270 325 385	260 301 369 432
4	258 270 293 358	271 326 407	277 304 442	270 305 404	272 325 385	260 296 376
5	256 270 371	273 254 410 dec.	272 304 332 440	267 300 360 401	272 328 398	262 328 390
6	272 330	290 358	270 300 355	270 300 355	272 330	271 331
Aglycone of comp. **6**	275 333	274 300 380	260 sh. 300 361	262 sh. 300 356	275 296 390	275 334

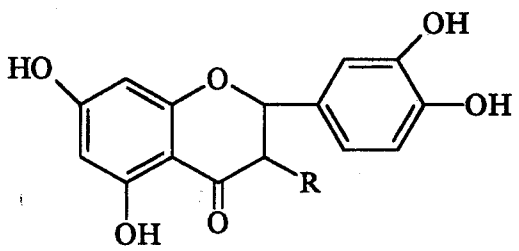
Table 2: ^{1}H-NMR data of the compound **3,4** and **5** (DMSO-d_6).

Compound	H-3	H-2´	H-6´	H-5´	H-8	H-6	H-OMe
3	6.30 s	7.57 s	7.20 d (8.5)	6.82 d (8.5)	6.36 s	6.13 s	-
4	-	7.55 d (2.2)	7.45 dd (8.5, 2.2)	6.90 d (8.5)	6.40 d (2.2)	6.18 d (2.5)	3.78 s
5	-	7.66 s	7.55 d (8)	6.90 d (8)	6.44 s	6.19 s	-

Table 3: ^{13}C-NMR spectral data for compound **6**.

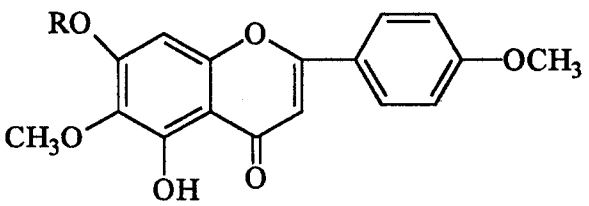
C. No.		C. No.		C. No.	
2	162	6-OCH_3	59.3	1"	99.25
3	102.8	4'-OCH_3	55.2	2"	72.20
4	181.3	1'	123.3	3"	75.89
5	151.8	2'	126.8	4"	68.89
6	131.2	3'	115.75	5"	75.70
7	157.6	4'	159.3	6"	60.22
8	89.8	5'	115.75		
9	151.4	6'	126.8		
10	104.6				

Fig. 1:



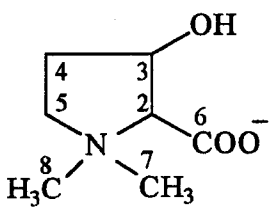
R= H = Compound **3**
R= OCH_3 = Compound **4**
R= OH = Compound **5**

Fig. 2



R= H = aglycone of compound **6** (pectolinargenine)
R= B-D-glucose = Compound **6**

Fig. 3



Compound **7**

Aglycone of compound 6

Yellow amorphous powder, m.p. 215-217°C. IR (KBr) cm^{-1}: hydroxyl at 3400 and carbonyl at 1650. ^{1}HNMR (DMOS-d_6): δ 8.05 (2 H, d, J= 8.5 Hz, H-2′ and H-6′), 7.17 (2H, d, J= 8.5 Hz, H-3′ and H-5′), 6.92 (1H, s, H-8), 6.90 (1H, s, H-3), 3.90 (3H, s, $OC\underline{H}_3$ at C-6), and 3.72 (3H, s, $OC\underline{H}_3$ at C-4′). EIMS m/z (rel. int %): 314 (100), 299 (89), 296 (16), 282 (21), 217 (67), 167 (18), 139 (20), 135 (14), and 132 (48). The UV data of the aglycone are listed in Table 1.

Compound 7 [Fig. 3]

Crystalline prisms (MeOH), m.p. 250-253°C. EIMS m/z (rel. int %): 159 (5), 111 (7), 90 (20), 69 (30), and 34 (100). ^{1}HNMR (CD_3OD): δ 2.10 (1 H, m, H-4), 2.59 (1H, m, H-4), 3.47 (1H, m, H-5), 3.74 (1H, m, H-5), 3.40 (1H, obscured, H-3), 3.31 (3H, s, $C\underline{H}_3$-7 or $C\underline{H}_3$-8), 3.38 (3H, s, $C\underline{H}_3$-8 or $C\underline{H}_3$-7), 3.96 (1H, d, J= 10.2 Hz, H-2) and 4.70 (1H, broads, OH). ^{13}C-NMR (CD_3OD): δ 168.8 (-$\underline{C}$OO), 81.3 (C-2), 71.3 (C-3), 67.1 (C-5), 53.7 (C-7 or C-8), 48.8 (C-8 or C-7), 31.2 (C-4). IR (KBr) cm^{-1}: 3450 (OH) and 1700 (C=O).

RESULTS AND DISCUSSION

Compound 1. Was identified as B-sitosterol by studying its physical properties which was found to be identical with those of the authentic B-sitosterol (m.p., m.m.p. and Co-chromatography).

Compound 2. Was identified as ursolic acid, on the basis of physical, chemical and spectroscopic evidence (NMR and MS).[13]

Compound 3 and 5 [Fig. 1]. Were identified as luteolin and quercetin respectively, by studying their physical, chemical and UV spectral data with different ionized and complexing agents[14], as well as, ^{1}H-NMR spectra Table 1 and 2 respectively.

Compound 4 [Fig. 1]. Was obtained as a yellow amorphous powder m.p. 212-215°C. The ^{1}H-NMR spectrum showed the presence of one methoxyl group (δ 3.78). In addition, NMR signals were observed for five aromatic protons in accord with quercetion type flavonol (δ 7.55, 7.45 and 6.90 for the B-ring, 6.40 and 6.18 for the A-ring protons). The presence of a hydroxyl group at C-5 was evident since the compound appears as a purple spot on the paper chromatogram when viewed in UV light and also the $AlCl_3$/HCl shift in UV relative to methanol (Table 1). The presence of a second hydroxyl group at C-7 was indicated by a bathochromic shift (+14 nm) of band II in NaOAc relative to band II in methanol.[14] The bathochromic shift of band I (+18) in the presence of NaOAc/H_3BO_3, indicates the presence of free orthodihydroxy groups in ring B. The EIMS showed molecular ion peak at m/z (316). So compound **4** was identified as quercetin 3-methyl ether.

Compound 6 [Fig. 2]. Was obtained as yellowish amorphous powder, m.p. 162-164°C. The IR (KBr) spectrum showed the presence of a hydroxyl group at 3400 cm^{-1} and carbonyl at 1650 $cm.^{-1}$ The UV spectral data (Table 1), indicated that compound **6** is a flavone with no orthodihydoxy function in ring B (there is no bathochromic shift in band II with NaOAc/H_3BO_3), absence of a free hydroxyl group at C-7 is indicated by the shift in NaOAc of band II relative to band II in methanol and the presence of a hydroxyl group at C-5 was evident since the compound appears as a purple spot on paper chromatogram when viewed in UV light and $AlCl_3$/HCl shift (Table 1). The substituted hydroxyl at C-4′ was indicated by the decreased intensity of band I with $NaOCH_3$ in UV spectra. ^{1}H-NMR spectrum of compound **6** showed the presence of two methoxyl groups (δ 3.88 and 3.77) and one anomeric proton for the sugar glucose moiety at δ 4.93 (d, J= 7.2 Hz). In addition, signals for 6 aromatic protons, 2′,6′-proton signals at δ 7.80, 3′,5′-proton signals at δ 7.11 (protons of ring B), signal at δ 6.55 (C-8 proton of ring A), showing that 5,6,7 position of ring B are substituted and signal at δ 6.45 (C-3 proton of ring C). The ^{13}C-NMR showed that the sugar is a B-D-glucose and linked to the aglycone with the hydroxyl group at C-7 (C-1′ at δ 99.25),[15,16] two methoxyl groups at C-4′

(δ 55.2) and C-6 (δ 59.3). Other signals are listed in Table 3. Acid hydrolysis of the glycoside gave an aglycone and the sugar glucose (TLC and PC). The UV, NMR data and m.p. of the aglycone were in agreement with those reported for pectolinargenin (5,7-dihydroxy-6,4´-dimethoxy flavone).[17,18,19] The UV data of the aglycone are listed in Table 1. Thus the glycoside **6** was identified as pectolinargenin-7-O-B-D-glucoside. It is the first report of this compound in the family Bignoniaceae.

Compound 7 [Fig. 3]. Was obtained as crystalline prisms. It is very hygroscopic and the anhydrous crystals melted at 250-253°C. It is soluble in polar solvent and insoluble in chloroform, ether and benzene. Compound **7** gave orange red colour with modified dragendorff's reagent. The IR spectrum showed the presence of hydroxyl group at 3450 cm^{-1} and carbonyl group (C=O) at 1700 cm^{-1}. The positive results with modified dragendorff's reagent and odd number molecular weight (M^+= 159) suggesting the alkaloidal nature of compound **7**. ^{1}H-NMR spectrum showed the presence of two methyl groups at δ 3.31 and 3.38 (each 3H, s). Four protons (each 2H, m) assigned for **4** and **5** methylene protons, one proton (d, 10.2 Hz) assigned for methine proton at C-2 and one proton (m) indicated the presence of one proton (CH) at C-3. Since the proton at C-2 appeared in the NMR as doublet, the most probable location of the hydroxyl group is at C-3. The ^{13}C-NMR spectrum howed the presence of seven carbon signals at δ 168.8 assigend to (COO), 81.3, 71.3, 67.1 and 31.2 assigned to C-2, C-3, C-5 and C-4 respectively, signals at δ 53.7, 48.8 assigned to C-8 and C-7. The above physical, chemical and spectral data are consistent with the identity of the compound **7** as hydroxy-3-stachydrine alkaloid. This is the first report of this compound in the family Bignonaceae but it was reported before in other family.[20]

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