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FLAVONOIDS FROM *Cleome amblyocarpa*

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SUMMARY

Seven flavonoids, six of them being known compounds, named apigenin, kaempferol 3-glucoside, quercetin 3-glucoside-7-rhamnoside, isorhamnetin 3-glucoside-7-rhamnoside, kaempferol 3, 7-dirhamnoside, kaempferol 7-rhamnoside and the new derivative of kaempferol: kaempferol 7-methyl ether 3-rhamnoside were obtained from the polar fractions of *Cleome amblyocarpa.* Their structures were determined by spectral and chemical methods.

ÖZET

Cleome amblyocarpa'nın polar fraksiyonlarından yedi flavonoit bileşik elde edildi, altı tanesi daha önce elde edilmiş olan flavonoitler apigenin, kamferol 3-glikozit, kersetin 3-glikozit-7-ramnozit, izoramnetin 3-glikozit-7-ramnozit, kamferol 3,7-diramnozit, kamferol 7-ramnozit ve bir yeni kamferol türevi: kamferol 7-metil eter 3-ramnozittir. Bu bileşiklerin yapıları spektral ve kimyasal metodlarla bulunmuştur.

Key words: *Cleome amblyocarpa;* Capparaceae; flavonoids.

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INTRODUCTION

Cleome amblyocarpa and *C. brachycarpa* are used as folk medicine for the treatment of scabies, abdominal and rheumatic pains, fever and inflammation (1-5). In recent studies on the extracts of *C. amblyocarpa (=C. africana)* the subchronic effects (6), and antidiabetic activity in rats (7), as well as the anti-inflamatory, analgesic and antipyretic activities (8) were studied.

We have previously investigated *Cleome amblyocarpa* Barr. et Marb. (Synonym: *C. africana* Botsch and *C. arabia* ouct. non L.) (9) and obtained a group of dammarane triterpenoids, four of them being new compounds, amblyone, cleoamblynol A, 3α-cleoamblynol A and cleoamlynol B (10), in addition to the known compounds cleocarpanol and cabraleahydroxy lactone (5, 11) as well as the flavonoids chrysoeriol, and chrysoeriol 7-glucoside. In a previous study, we reported the isolation of kaempferol 3, 7-dirhamnoside (8).

RESULTS AND DISCUSSION

In the present study from the polar farctions of the same plant material used to isolate dammarane triterpenoids (10), we have obtained seven flavonoid derivatives. Six of them were the known compounds: apigenin, kaempferol 3-glucoside, kaempferol 3,7-dirhamnoside, kaempferol 7-rhamnoside, quercetin 3-glucoside-7-rhamnoside and isorhamnetin 3-glucoside-7-rhamnoside, the latter two compounds were previously isolated from the same plant (12) collected from Egypt under the name *C. africana*. The new compound was kaempferol 7-methyl ether 3-rhamnoside.

Kaempferol 3, 7-dirhamnoside- It has been recently isolated from the plant (8), and data in this report are collected on its acetyl derivative. It was brown under UV light, when exposed to NH_3 vapors and sprayed with NA (Naturstoffreagens A) reagent, it turned into deep yellow. UV spectra and UV shifts (13) were as follows: in MeOH λ_{max} nm: 345, 265; NaOMe: 395, 266; $AlCl_3$ and $AlCl_3$/HCl: 400, 351, 270; NaOAc: 395 (sh), 252, 264; H_3BO_3: 352, 265. The ^{1}H NMR spectrum of its acetyl derivative ($CDCl_3$) indicated the basic skeleton giving the signals at δ 7.95 (2H, d, J=8.5 Hz, H-2' and H-6'), 7.30 (2H, d, J=8.5 Hz, H-3' and H-5'), 6.67 (1H, d, J=2.5 Hz, H-8), 6.56 (1H, d, J=2.5 Hz, H-6). The protons of the acetylated sugar moieties were between δ 5.65 and 4.9. Acetyl groups were at 2.34 (3H, s, C-4' OAc), the other six acetyl groups of rhamnose moieties at δ 2.26, 2.12, 2.06, 2.02 (each 3H, s) and at δ 1.98 (6H, s), and a hydrogen-bonded hydroxyl at δ 12.11 (1H, s, 5-OH). The rhamnose moieties at C-7 and C-3 showed the methyl groups at δ 1.23 (3H, d, J=6.5 Hz) and at δ 0.85 (3H, d, J=6.5 Hz). Acid hydrolysis of the compound afforded only rhamnose as the sugar and kaempferol as the aglycone parts.

Kaempferol 7-rhamnoside- The color of the compound under UV light was yellow and it did not change by exposure to NH_3 vapors or NA spray reagent, indicating a free 3-OH group. UV spectra also indicated the free 3-OH as well as free 4'-OH (13). UV in MeOH λ_{max} nm: 365, 253; NaOMe: 421, 350, 266; $AlCl_3$ and $AlCl_3$/HCl: 420, 350, 268; NaOAc: 374, 253; H_3BO_3: 365, 257. ^{1}H NMR of its acetyl derivative ($CDCl_3$) δ 7.88 (2H, d, J=8.5 Hz, H-2' and H-6'), 7.30 (2H, d, J=8.5 Hz, H-3' and H-5'), 6.8 (1H, d, J=2.5 Hz, H-8), 6.67 (1H, d, J=2.5 Hz, H-6), 12.12 (1H, s, 5-OH), 1.22 (3H, d, J=6.5 Hz, CH_3 rhamnose). Four acetyl groups were at δ 2.35, 2.20, 2.07 and 2.05 (each 3H, s). Acid hydrolysis afforded rhamnose and kaempferol.

Kaempferol 7-Methyl ether 3-rhamnoside- The color of the compound under UV light was purple, on exposure to NH_3 vapors and NA spray reagent, the color turned to yellow indicating a free 4'-OH group. UV spectra (13) in MeOH λ_{max} nm: 346, 265; NaOMe: 400, 266; $AlCl_3$ and $AlCl_3$/HCl: 400, 350, 300, 272; NaOAc: 405 (sh), 350, 264; H_3BO_3: 347, 266. The ^{1}H NMR of the acetyl derivative of the compound ($CDCl_3$) indicated the structure clearly as follows: δ 12.37 (1H, br s, C-5 OH hydrogen bond), 7.95 (2H, d, J=8.5 Hz, H-2' and H-6'), 7.30 (2H, d, J=8.5 Hz, H-3' and H-5'), 6.66 (1H, d, J=1.5 Hz, H-8), 6.55 (1H, d, J=1.5 Hz, H-6), 3.95 (3H, s, OMe), 2.34 (3H, s, OAc), 2.12, 2.05, 2.02 (each 3H, s, OAc), 1.23 (3H, d, J=6.5 Hz, Rh Me). Acid hydrolysis yielded 7-OMe kaempferol and only rhamnose as the sugar moieties thus indicating the proposed structure.

EXPERIMENTAL

General Experimental Procedures- The spectra were recorded with the following instruments: UV, Varian Techtron 635; ^{1}H NMR, Bruker AC 200L. Kieselgel $60F_{254}$ (E. Merck) plates were used for preparative separation, Sephadex LH-20 (Fluka) for further separation.

Plant Material- The aerial parts of *Cleme amblyocarpa* were collected from the Quassim province of Saudi Arabia in May 1993. The plant material was identified by the Botany Dept. College of Science, King Saud University. A voucher specimen is deposited in the Herbarium of the Collece of Agriculture and Veterinary Medicine of the same University.

Extraction and Fractionation- Powdered air-dried aerial parts of the plant (800 g) were exhaustively extracted with 95% EtOH at room temparature. Upon evaporation under reduced pressure, a dark green residue (75 g) was obtained. The residue dissolved in EtOH, the waxes were filtered off. The solvent was evaporated and the residue was dissolved in EtOH-H_2O (1:4), than extracted with petrol (31 g), $CHCl_3$ (11.5 g), EtOAc (3.5 g), the remaining aqueous layer was discarded. The EtOAc soluble fraction was fractionated on Sephadex LH-20 column in MeOH, followed by purification on preparative TLC plates. Seven flavonoidal compounds, six of them are being known, namely: apigenin, kaempferol 3-glucoside, quercetin 3-glucoside 7-rhamnoside, isorhamnetin 3-glucoside 7-rhamnoside, kaempferol 3, 7-dirhamnoside, kaempferol and kaempferol 7-rhamnoside, and one new derivative of kaempferol: Kaempferol 7-methylether 3-rhamnoside were obtained.

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FURTHER SESQUITERPENE LACTONES AND FLAVONES FROM *Centaurea bruguierana*

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SUMMARY

A further investigation of the aerial parts of *Centaurea bruguierana* (DC.) Hand.-Mazz. afforded four sesquiterpene lactones namely, cnicin, 11,13-dehydromelitensin, 8α-(5-hydroxy)-angeloyloxy-11,13-dehydromelitensin, 8α-(3',4'-dihydroxyethyl)-acryloxy-11,13-dehydromelitensin and five flavonoids; eupatilin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone, jaceosidin, pectolinarigenin and cirsilineol. The structures were determined by spectral methods especially by high field NMR spectroscopy.

ÖZET

Centaurea bruguierana (DC.) Hand.-Mazz. bitkisinin toprak üstü kısımlarından dört seskiterpen lakton; 11,13-dehydromelitensin, cnicin, 8α-(5-hidroksi)-angeloyiloksi-11,13-dehidromelitensin, 8α-(3',4'-dihidroksietil)-akriloksi-11,13-dehidromelitensin ve beş flavonoid bileşik; eupatilin, 5-hidroksi-6,7,3',4'-tetrametoksiflavon, jaceosidin, pectolinarigenin ve cirsilineol elde edilmiştir. Bileşiklerin yapıları spektroskopik yöntemler ile açıklanmıştır.

Key words: *Centaurea bruguierana;* Compositae; sesquiterpene lactones; flavones.

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INTRODUCTION

In course of our research of the genus *Centaurea,* we have examined *Centaurea bruguierana* (DC.) Hand.-Mazz. *(Syn. C. phllocephala* Boiss.) a Saudi Arabian plant, which was distributed in Najid province of Saudi Arabia.

The plant has been investigated previously by one of us, F.M. Harraz, and the presence of a germacranolide; cnicin, an eudesmanolide; 11,13-dehydromelitensin 8α-acetate and three flavonoids; circilineol, cirsimaritin and hispudilin 7-O-rutinoside were reported (1). In the present paper, we report the isolation and characterization of four sesquiterpene lactones **(1-4)** and five flavones**(5-9)** from the aerial parts of *C. bruguierana.* The sesquiterpene lactone, cnicin **(1)** and the flavon, cirsilineol **(9)** were previously isolated from many *Centaurea* species (1-3) while the others 8α-(3',4'-dihydroxyethyl)-acryloxy-11,13-dehydromelitensin **(2)** (4,5), 8α-(5-hydroxy)-angeloyl-11,13-dehydromelitensin **(3)** (6), 11,13-dehydromelitensin **(4)** (7) and flavones, eupatilin **(5)** (8), 5-hydroxy-6,7,3',4'-tetramethoxyflavone **(6)** (9), jaceosidin **(7)** (10), pectolinarigenin **(8)** (11) were reported for the first time from *Centaurea bruguierana.*

RESULTS AND DISCUSSION

Compound 1 was cnicin, a common sesquiterpene lactone of *Centaurea* species, it was identified by comparison of its spectral data with those reported in the literature (2,3).

Compound 2 was obtained as glassy gum. The IR spectrum showed the characteristic doublets of α,β-unsaturated-γ-lactone carbonyl at 1770 cm^{-1}, an ester carbonyl at 1725 and 1270 cm^{-1}, hydroxyl(s) at 3450 cm^{-1} and unsaturations at δ 1630, 980, 860 cm^{-1}. Extensive ^1H NMR spin decoupling experiments (Table 1), ^1H NMR (Table 2) and ^{13}C NMR spectra were evident for the structure of compound **2.** Starting irradiation of the typical doublets of exocyclic methylene group at δ 6.16 (d, J=3 Hz, H-13) and 5.56 (d, J=3.5 Hz, H-13') led to the unambiguous sequences H_7-H_9 and H_7-H_5. The signals at δ 5.77 (dd, J= 10.5 and 17 Hz, H-1), δ 5.07 (dd, J=1 and 10.5 Hz, H-2a) and δ 5.03 (dd, J=1 and 17 Hz, H-2b) were easily assigned as a vinyl group and the broadened singlets at δ 5.42 and 4.96 indicated the presence of a terminal methylene group in the molecule. Further spin decoupling experiments permitted identification of the ester function, irradiation of the signal at δ 4.64 (dd, J=4 and 7 Hz, H-3')

Table 1: Spin-decoupling experiments on Compound 2 ($CDCl_3$)

Irradiated H	**Observed H**	**Multiplicities changes**
6.16 (H-13)	2.97 (H-7)	dddd ----- ddd (J= 3.5, 11, 11 Hz)
2.97 (H-7)	4.24 (H-6)	t--------d (J= 11 Hz)
	5.29 (H-8)	ddd-----dd (J= 4,10 Hz)
4.24 (H-6)	2.58 (H-5)	d-------s
5.29 (H-8)	2.05 (H-9b)	dd-----d (J= 14 Hz)
	1.69 (H-9a)	dd-----d (J= 14 Hz)
5.77 (H-1)	5.07 (H-2a)	dd-----br s
	5.03 (H-2b)	dd-----br s
4.64 (H-3')	3.85 (H-4'a)	dd-----d (J= 11 Hz)
	3.60 (H-4'b)	dd-----d (J= 11 Hz)

collapsed the two double doublets at δ 3.82 (J=4 and 11 Hz, H-4'a) and 3.60 (J= 7 and 11 Hz, H-3'b) to doublets and sharpened the vinylic singlets at δ 6.39 (H-5'a) and 6.08 (H-5'b) indicating that the nature of the ester function as (3',4'-dihydroxyethyl)-acryloic acid. The lower chemical shift of H-8 (ddd, 5.29) indicated that the ester function is at C-8. ^{13}C NMR (APT) spectrum ($CDCl_3$) of compound **2** showed 20 carbon singlets, 1xCH_3, 7xCH_2, 6xCH and 6xC. Multiplicities were assigned by DEPT spectra. 145.53 (C-1), 113.20 (C-2), 115.15 (C-3), 143.58 (C-4), 50.46 (C-5), 78.63 (C-6), 52.31 (C-7), 69.69 (C-8), 44.97 (C-9), 41.88 (C-10), 136.60 (C-2'), 169.51 (C-12), 120.163 (C-13), 18.60 (C-14), 67.35 (C-15), 165.14 (C-1'), 139.10 (C-11), 71.17 (C-3'), 65.76 (C-4'), 127.63 (C-5'). In the view of spectral data **2** was identified as 8α-(3',4'-dihydroxyethyl)-acryloxy-11,13-dehydromelitensin. All spectral data of **2** were in good agreement with those reported by Bruno and Herz (4).

Compound 3 has the same structural skeleton with that of compound **2,** it only differs on the nature of ester function. The ^{1}H NMR spectrum indicated the presence of an angeloyloxy group as ester function at C-8 showing the signals of a methine quartet at δ 6.45, a methyl doublet at δ 2.07 and a methylene singlet adjacent to a hydroxyl group at δ 4.27. Spin decoupling experiments clearly showed the relations between these protons. Irradiation of H-3' turned the doublet at δ 2.08 (H-4') to a br singlet conversly, the former turned to a br singlet. On the basis of all spectral data compound **3** was identified as 8α-(5-hydroxy)-angeloyl-11,13-dehydromelitensin.

Table 2: ^{1}H NMR spectra of Compounds **2-8**, 200 MHz, $CDCl_3$

H	**2**	**3**	**4***
1	5.78 dd	5.77 dd	5.79 dd
2a	5.07 dd	5.08 dd	5.03 dd
2b	5.03 dd	5.04 dd	4.99 dd
3a	5.42 br s	5.44 br s	5.39 br s
3b	4.98 br s	4.97 br s	4.90 br s
5	2.59 d	2.59 d	2.49 d
6	4.23 t	4.25 t	4.16 t
7	2.97 ttt	2.98 ttt	2.65 ttt
8	5.29 ddd	5.36 ddd	3.60 ?
9a	2.03 dd	2.12 dd	1.83 dd
9b	1.69 dd	1.67 dd	1.62 dd
13	6.16 d	6.18 d	6.16 d
13'	5.56 d	5.69 d	6.03 d
14	1.19 s	1.18 s	1.19 s
15a	4.11 d	4.11 d	4.04 d
15b	3.99 d	4.00 d	3.95 d
3'	4.64 dd	6.47 q	–
4'a	3.85 dd	2.08 d	–
4'b	3.60 dd	–	–
5'a	6.39 br s	4.29 br s	–
5'b	6.08 br s	–	–

H	**5**	**6**	**7**	**8**
2'	7.34 d	7.35 d	7.33 d	7.82 d br
6'	7.53 dd	7.54 dd	7.47 dd	
3'				6.94 d br
5'	6.98 d	6.98 d	7.04 d	
3	5.59 s	5.98 s	6.55 s	6.54 s
8	6.63 s	6.62 s	6.60 s	6.59 s
12-OH	13.07 s	12.76 s	13.10 s	12.77 s
OCH_3	3.96 s	3.94 s	4.00 s	3.92 s
	3.97 s	3.97 s	4.03 s	3.97 s
	4.05 s	3.98 s	–	–
	–	4.01 s	–	–

J (Hz) = **2**:2a, 1= 10; 2b, 1= 17; 2a, 2b= 1; 5, 6=11; 6, 7= 11; 7, 8= 10; 9a, 8= 11; 9b, 8= 4; 9a, 9b= 14; 3', 4'a= 4; 3', 4'b= 7; 4'a, 4'b= 11; **3:** 3', 4'= 7; **5-8:** 2', 6'= 2.5; 3', 5'= 2.5; 5', 6'= 8.