Bull. Pharm. Sci., Assiut University, Vol. 20, Part 1, June 1997, pp. 37-46.

PHYTOCHEMICAL INVESTIGATION OF *WEDELIA PROSTRATA* HOOK et ARN. (HEMSL.); II- THE ETHYL ACETATE SOLUBLE FRACTION OF THE METHANOL EXTRACT

S. F. Farag, N. A. El-Emary, M. A. Makboul, H. A. Hasanean and M. Niwa*

Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt
* *Faculty of Pharmacy, Meijo University, Nagoya, Japan*

باستقصاء المراجع المختلفة المتواترة عن نبات الوديليا بروستراتا هوك وارن (همسل) التابع للعائلة المركبة المنزرع فى مصر لم يعثر على أى دراسة كيميائية أو بيولوجية لهذا النبات مما حث الباحثين على دراسة هذا النبات لمعرفة مكوناته الكيميائية وقد إشتملت هذه الدراسة على الأتى: تم فصل خمسة مركبات تفصل لأول مرة من جنس الوديليا وقد تم التعرف عليها بإستخدام التحليل الطيفى بإستخدام الأشعة دون الحمراء والفوق بنفسجية والرنين النووى المغناطيسى البروتونى والكربونى بأنواعهما المختلفة وكذلك مطياف الكتلة وقد ثبت أن هذه المواد هى:

١- سلفيورين. ٢- كوريوبسين.
٣- ستيلوبسين. ٤- حامض الكونيك-٣و٥-داى كافييول.
٥- حامض الكونيك-٣و٤-داى كافييول.

كذلك تم أختبار خلاصة الميثانول للنبات كمثبط لإنزيم البروتييز وقد أعطى نسبة تثبيط ٤,١٠٪ عند تركيز حوالى ٠,١ مجم/مليلتر.

Phytochemical investigation of the ethyl acetate soluble fraction of the MeOH extract of the powdered aerial parts of Wedelia prostrata resulted in the isolation and identification of sulfurein; coreopsin; stillopsin; 3,5-dicaffeoyl quinic acid and 3,4-dicaffeoyl quinic acid. The identification of the isolated compounds was established through the different methods of spectral analysis.

INTRODUCTION

The genus *Wedelia* (*Compositae*) comprises about 50 species which are distributed in all temperate regions. The plant *Wedelia prostrata* is an ornamental plant with long creeping stems giving descending roots and ascending flowering branches at the nodes[1-4].

Few species of *Wedelia* are toxic,[5-7] on the other hand many species have valuable biological activities.[8-13] However, nothing could be traced in the literature about the biological activity of the plant of this study.

The genus *Wedelia* is characterized by the presence of tetracyclic diterpenoids[5,7,9,11] (Kaurenoic acid derivatives), sesquiterpene lactones (eudesmanolides),[11,14] flavonoids and isoflavonoids.[13]

In a previous paper[14] five sesquiterpene lactones from the chloroform soluble fraction of the title plant were reported. The present work describes the isolation and identification of five compounds from the ethyl acetate soluble fraction as first report from the genus *Wedelia*.

EXPERIMENTAL

Plant Material: The harvested aerial parts of *Wedelia prostrata* Hook. et Arn. (Hemsl.) were collected in March 1994 during the flowering stage from the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Assiut University. The identification of the plant was confirmed by Prof. Dr. Abdel-Aziz Fayed, Prof. of Plant Taxonomy, Faculty of Science, Assiut University. The plant parts were left for drying in the shade at room temperature and powdered. A voucher specimen has been deposited in the

Received in 23/9/1996 & Accepted in 1/2/1997

Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University.

General methods

Optical rotation was measured on a JASCO DIP-181 digital polarimeter at 25°. UV spectra were taken in CH_3OH with a JASCO UVIDEC 610 spectrometer and IR spectra with a JASCO FT/IR-5000 spectrometer. MS and HR-MS were obtained under FAB conditions with a JEOL DX-110 spectrometer. The ^1H-^{13}C, two dimensional (2D) NMR (HH-, CH-COSY, HMQC and HMBC) and difference NOE spectra were measured with JEOL α-400 and α-600 spectrometer. For thin layer chromatography, precoated $Kieselgel_{60}$ K_{254} (0.25 mm, E. Merck) using $CHCl_3$-MeOH-H_2O (6:4:0.5) and precoated plates of RP-18 WF_{254} (0.25 mm thick, E. Merck) using CH_3CN-1% aqueous TFA (1:2) and CH_3CN-H_2O (2:3) were used. For column chromatography: silica gel (Katayama chemical, Osaka, K_{230}) and Sephadex LH-20 (Pharmacia Biotech. AB, Upsala Sweden) were used. PTLC was carried out on precoated $kieselgel_{60}$ K_{254} (0.5 mm thick, E. Merck) using EtOAc-EtOH-H_2O (4:1:0.5) and EtOAc-MeOH (9:1).

Extraction and fractionation

The powdered air dried aerial parts (3.8 kg) were extracted by maceration in MeOH at room temperature till exhaustion. The solvent was evaporated under vacuum to give 458.7 g. The MeOH extract residue was diluted with water and successively extracted with hexane, $CHCl_3$, EtOAc and BuOH. Each fraction was evaporated under vacuum till syrupy consistency and the fractions weighed 91.8, 60.4, 41 and 45 g respectively.

Isolation

About 5 g of the ethyl acetate soluble fraction were chromatographed on 150 g flash silica column using $CHCl_3$-MeOH gradients as eluent at flow rate 4 ml/min. Four groups were obtained: E-1 (239 mg) $CHCl_3$-MeOH (9:1), E-2 (400 mg) $CHCl_3$-MeOH (8:2), E-3 (1.8 g) $CHCl_3$-MeOH (8:2) and E-4 (2.5 g) $CHCl_3$-MeOH (7:3-6:4). The major constituents were present in groups E-3 and E-4. About 600 mg of group E-3 were fractionated on sephadex LH-20 column chromatography using MeOH, to yield three fractions. Each fraction was separately purified by sephadex LH-20 column using MeOH followed by PTLC using EtOAc-MeOH (9:1) to yield pure compounds **1** (10 mg), **2** (45 mg) and **3** (50 mg). Group E-4 (1.2 g) was chromatographed on sephadex LH-20 column using MeOH to obtain 550 mg mixture of compounds **4** and **5**. Isolation of compounds **4** and **5** was achieved by rechromatography on sephadex LH-20 using MeOH-H_2O (7:3) followed by PTLC using EtOAc-EtOH-H_2O (4:1:0.5) to obtain pure compounds **4** (48 mg) and **5** (30 mg). The detection of compounds **1-5** was carried out by using the color reactions in UV before and after ammonia solution.

Compound 1

Orange powder, TLC R_f 0.33, CH_3CN-H_2O (2:3). $[\alpha]_D^{25}$-21° (MeOH; C= 0.85). UV λ_{max} (MeOH) nm (ε): 205 (31104), 255 (sh) (12960), 335 (sh) (13176), 400 (22896). IR (KBr): 3400, 1613, 890 cm^{-1}. FAB-MS m/z 431 $[M+H]^+$ $C_{21}H_{18}O_{10}$. ^{1}HNMR (CD_3OD, 600 MHz): δ 3.50 (3H, m, H-2, 3,5-Gluc), 3.63 (1H, m, H-4-Gluc), 3.72 (1H, dd, J= 6.96, 12.82 Hz, H-6 Gluc), 3.93 (1H, d, J= 11.72 Hz, H-6 Gluc), 5.11 (1H, d, J= 7.33 Hz, H-1 Gluc), 6.75 (1H, s, H-10), 6.84 (1H, d, J= 8.43 Hz, H-'5), 6.96 (1H, dd, J= 1.83, 8.79 Hz, H-5), 7.09 (1H, d, J= 1.83 Hz, H-7), 7.27 (1H, dd, J= 1.83, 8.43 Hz, H-'6), 7.52 (1H, d, J= 1.47 Hz, H-'2), 7.69 (1H, d, J= 8.79 Hz, H-4). ^{13}C-NMR (CD_3OD, 150 MHz): δ 62.5 (t, C-6 Gluc), 71.2 (d, C-4 Gluc), 74.7 (d, C-2 Gluc), 77.8 (d, C-5 Gluc), 78.4 (d, C-3 Gluc), 100.7 (d, C-7), 101.7 (d, C-1 Gluc), 114.8 (d, C-5), 115.6 (d, C-10), 116.7 (d, C-'5), 117.2 (s, C-9), 119.1 (d, C-'2), 125.3 (s, C-'1), 126.4 (d, C-4), 126.7 (d, C-'6), 146.9 (s, C-'3), 147.5 (s, C-2), 149.8 (s, C-'4), 166.6 (s, C-8), 169.1 (s, C-6), 184.6 (s, C-3).

Compound 2

Orange powder, $[\alpha]_D^{25}$-68° (MeOH; C= 1.2). UV λ_{max} (MeOH) nm (ϵ): 211 (27602), 242 (sh) (10242), 293 (sh) (10416), 369 (sh) (30380), 387 (27776). IR (KBr): 3380, 1640, 880 cm^{-1}. FAB-MS m/z 435 $[M+H]^+$ $C_{21}H_{22}O_{10}$. ^{1}H-NMR (CD_3OD; 600 MHz): see Table 1. ^{13}C-NMR (CD_3OD; 150 MHz): see Table 1.

Compound 3

Orange powder, $[\alpha]_D^{25}$-84° (MeOH; C= 1.79). UV λ_{max} (MeOH) nm (ϵ): 211 (40275), 247 (sh) (18338), 267 (25313), 257 (15188), 317 (sh) (38588), 343 (37688). IR (KBr): 3400, 1640, 899 cm^{-1}. FAB-MS m/z 451 $[M+H]^+$ $C_{21}H_{22}O_{11}$. ^{1}H-NMR (DMSO-d_6; 600 MHz): see Table 1. ^{13}C-NMR (DMSO-d_6; 100 MHz): see Table 1.

Compound 4

White powder, $[\alpha]_D^{25}$-81.7° (MeOH; C= 2.41). UV λ_{max} (MeOH) nm (ϵ): 218 (42183), 233 (sh) (10643), 243 (sh) (10965), 300 (sh) (15319), 328 (20801). IR (KBr): 3300, 1740, 1686, 1603, 812 cm^{-1}. FAB-MS (glycerol) m/z: 515 $[M-H]^-$ $C_{25}H_{24}O_{12}$. ^{1}H-NMR (CD_3OD; 600 MHz): see Table 2. ^{13}C-NMR (CD_3OD; 150 MHz): see Table 2.

Compound 5

White powder, $[\alpha]_D^{25}$+26.8° (MeOH; C= 1.38). UV λ_{max} (MeOH) nm (ϵ): 218 (15480), 234 (sh) (10159), 245 (sh) (10643), 300 (sh) (14513), 328 (18383). IR (KBr): 3304,1740, 1698, 1603, 814 cm^{-1}. FAB-MS m/z: 515 $[M-H]^-$ ($C_{25}H_{24}O_{12}$). ^{1}H-, ^{13}C-NMR (CD_3OD; 600/150 MHz): see Table 2.

Screening of antiviral activity

The MeOH extract of *Wedelia prostrata* was subjected to the screening for the inhibitory effect on human immunodeficiency virus type I (HIV-I) protease using analytical HPLC method,[15] it showed moderate inhibition of the enzyme activity by 10.4% at a concentration of 0.1 mg/ml.

RESULTS AND DISCUSSIONS

Compound 1

The molecular formula of **1** was deduced as $C_{21}H_{18}O_{10}$ from its FAB-MS which showed M^+ at m/z 431 $[M+H]^+$ and from its DEPT ^{13}C-NMR which displayed signals corresponding to twenty one carbons represented by one CH_2, 12 CH and 8 quaternary carbons.

The ^{1}H-NMR (see exp.) of **1** showed a signal at δ 5.11 (1H, d, J= 7.33 Hz) assigned for a ß-anomeric proton. The sugar was identified as ß-D-glucopyranoside from comparison of its signals with those reported for ß-D-glucopyranoside[16]. Also the ^{1}H-NMR of **1** displayed a singlet at δ 6.75 (1H, s) for H-10 of aurones which is consistent with the ^{13}C-NMR signal at δ 115.5 (d)[17]. Moreover the ^{1}H-NMR showed other signals (see exp.) corresponding to a disubstituted 3',4' B-ring and a monosubstituted A-ring.

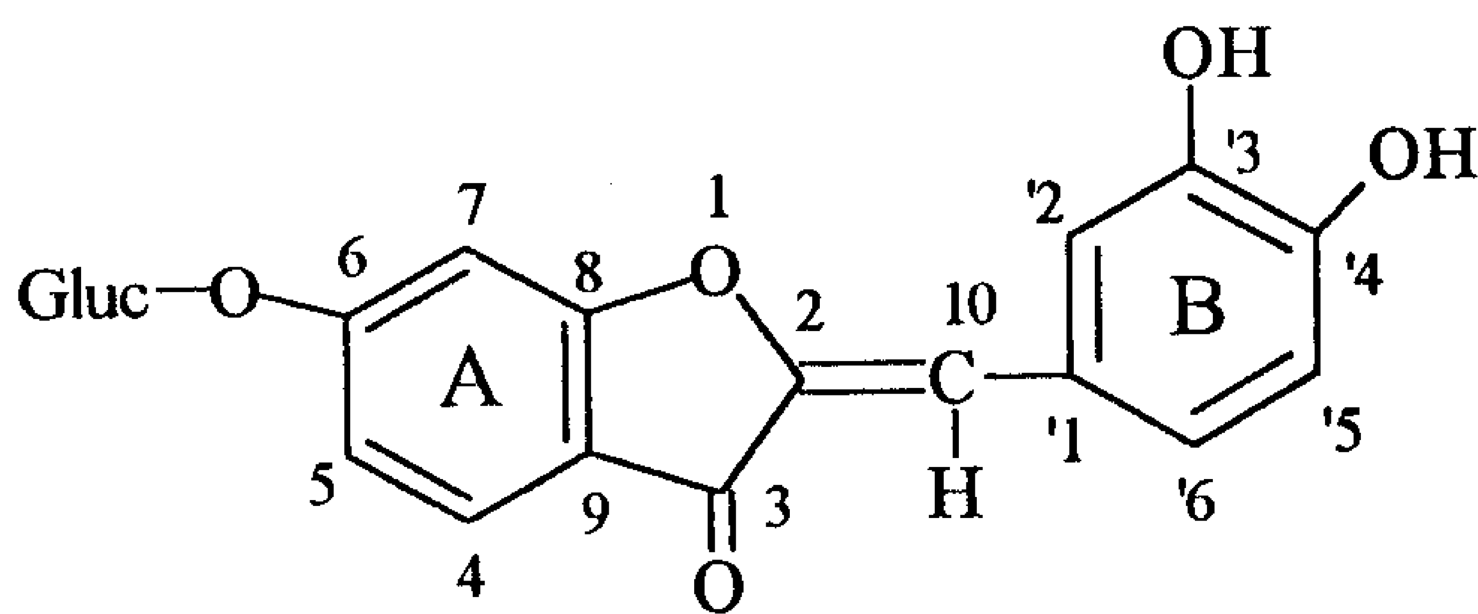
The ^{13}C-NMR signals of the aglycone part of **1** is in accordance with those reported for sulfuretin[18] and to confirm this conclusion the HH-COSY, HMQC and HMBC (Table 3) of **1** were carried out and were compatible with this conclusion.

To confirm the position of attachment of the ß-D-glucopyranoside structure at C-6 the difference NOE spectra were carried out and showed the following results: Irradiation of the signal at δ 5.11 (H-1 glucose) caused enhancement of the signal at δ 6.96 assigned for H-5 about 7.7% and also the signal at H-7 at δ 7.09 of about 7.4%. Consequently the attachment of the sugar was assigned to C-6 and **1** was identified as sulfuretin-6-O-ß-D-glucopyranoside (sulfurein).

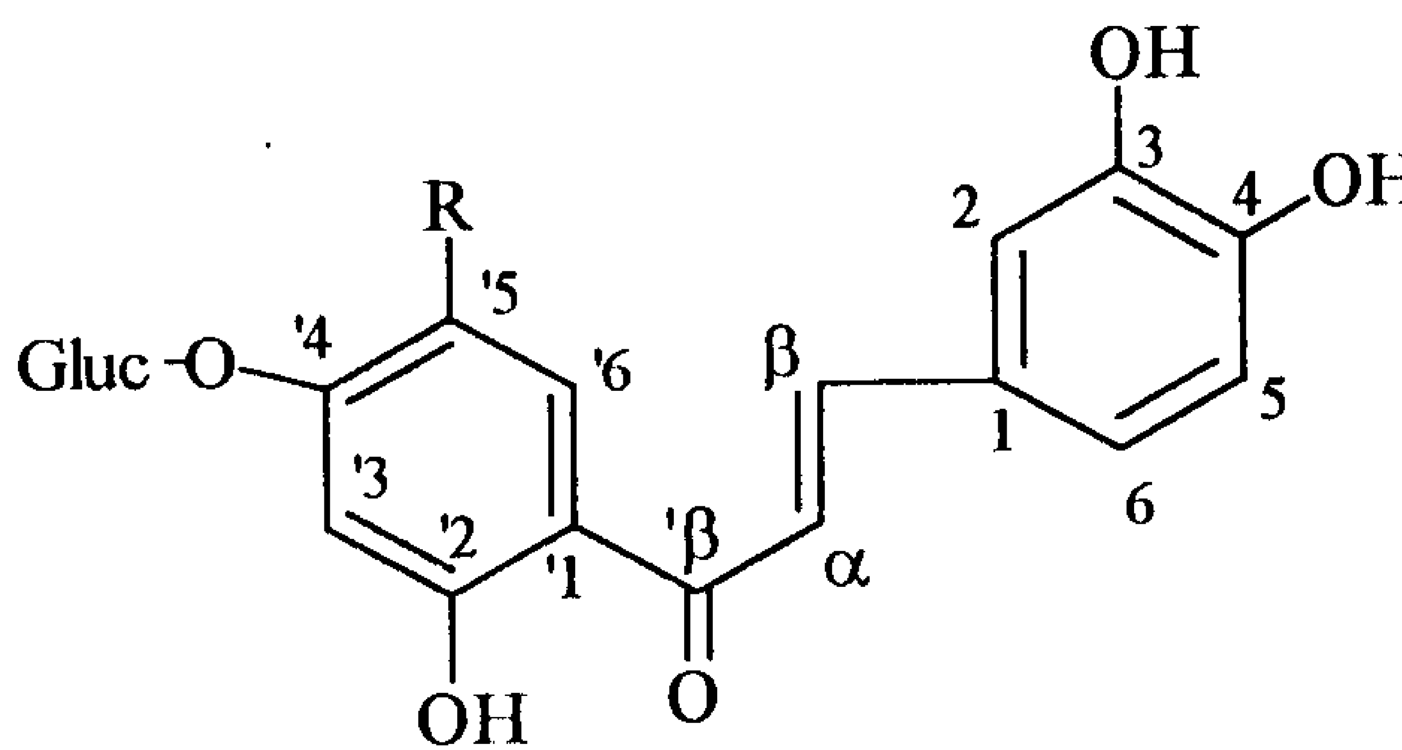
Compounds **2** and **3** were identified as chalcone monoglucosides where each of them displayed signals in the ^{1}H-NMR for two trans olefenic protons characteristic for H-α and H-ß of chalcones[18] and one anomeric proton for ß-D glucose (see Table 1).

A: Compound 2

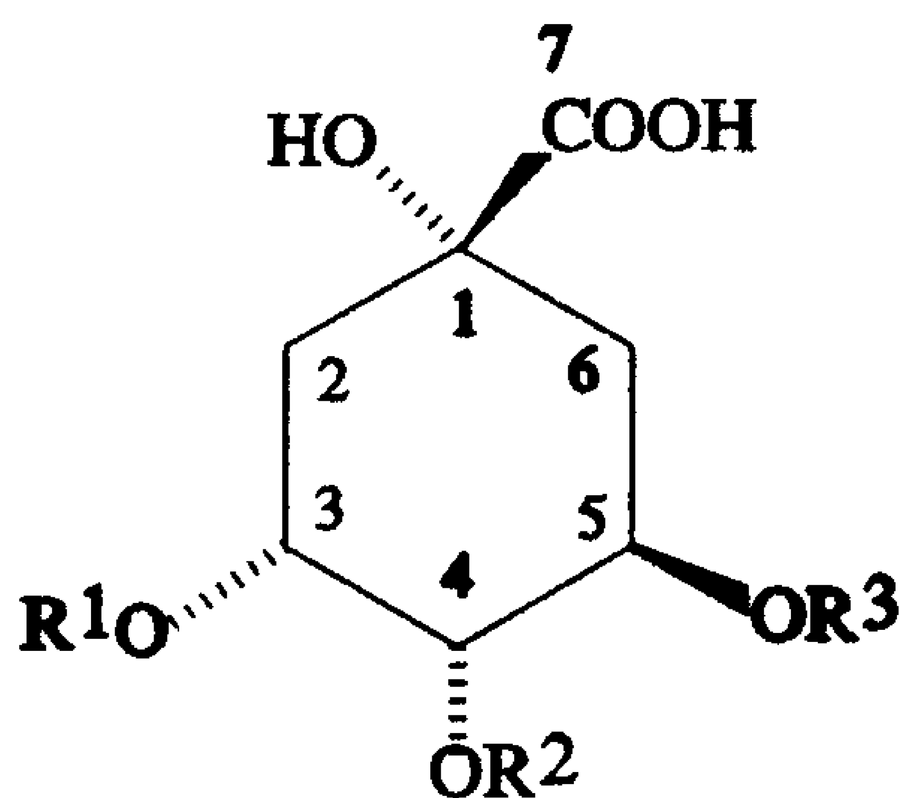
The molecular formula of **2** was deduced from its FAB-MS as $C_{21}H_{22}O_{10}$ which showed M^+ at m/z 435 $[M+H]^+$ and was confirmed by its ^{13}C- and DEPT ^{13}C-NMR which showed signals

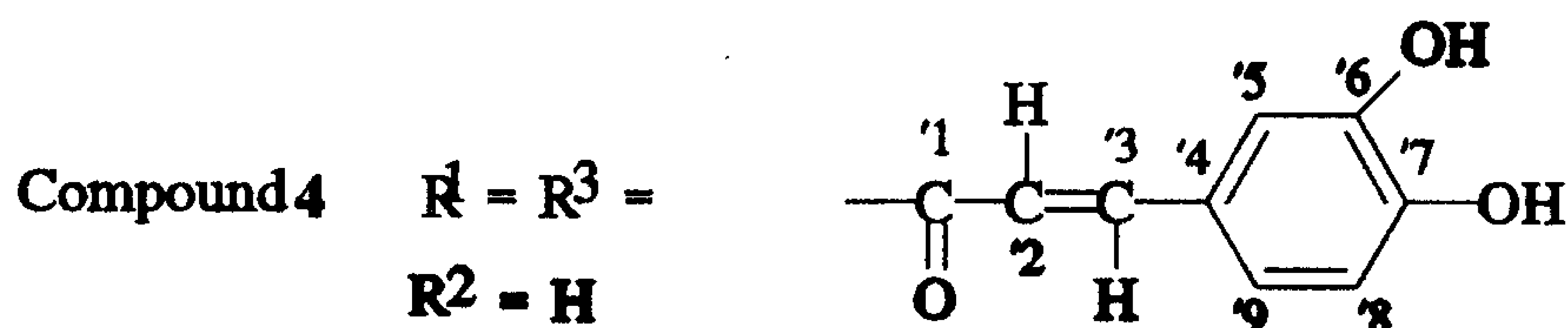


Compound **1**



Compound **2** R = H
Compound **3** R = OH





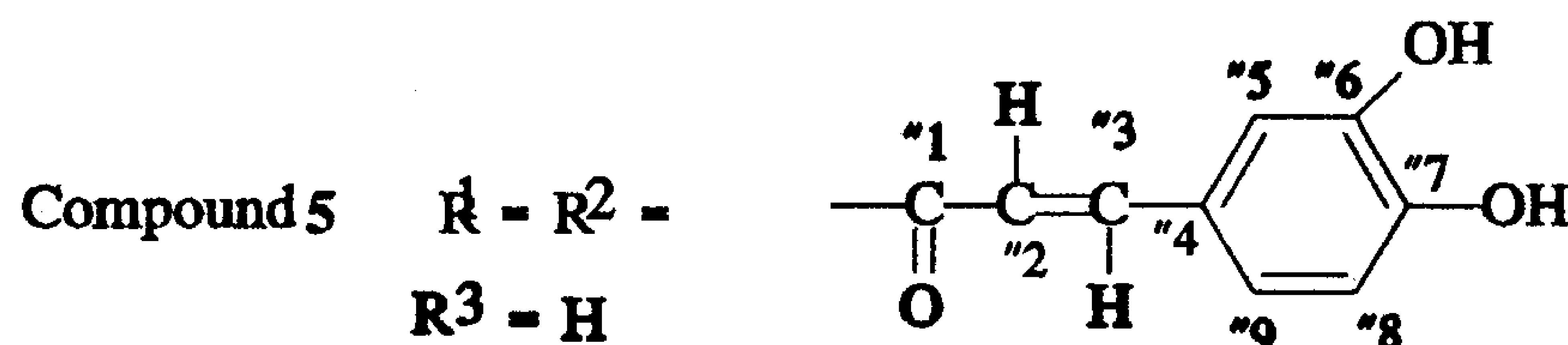


Table 1: ^{13}C-, ^{1}H-NMR spectral data of compounds **2** and **3**.

C	$^{a}\delta$ ^{13}C of compd. **2** [ppm]	$^{b}\delta$ ^{13}C of compd. **3** [ppm]	H	$^{a}\delta$ ^{1}C of compd. **2** [ppm]	M	J [Hz]	$^{b}\delta$ ^{1}C of compd. **3** [ppm]	M	J [Hz]
1	128.3(s)	126.2(s)	-	-	-	-	-	-	-
2	115.9(d)	115.9(d)	2	7.18	d	1.47	7.29	d	1.83
3	146.8(s)	145.2(s)	-	-	-	-	-	-	-
4	150.1(s)	149.1(s)	-	-	-	-	-	-	-
5	116.6(d)	115.8(d)	-	6.81	d	8.06	6.80	d	8.06
6	123.9(d)	122.6(d)	6	7.09	dd	8.06, 1.47	7.21	dd	8.06, 1.83
α	118.1(d)	117.5(d)	α	7.52	d	15.4	7.60	d	15.38
ß	146.8(d)	145.7(d)	ß	7.72	d	15.4	7.66	d	15.38
‘ß	193.8(s)	191.8(s)	-	-	-	-	-	-	-
‘1	116.6(s)	113.7(s)	-	-	-	-	-	-	-
‘2	165.0(s)	158.2(s)	‘2-OH	-	-	-	13.06	brs	-
‘3	105.1(d)	103.5(d)	‘3	6.59	d	2.2	6.65	s	-
‘4	166.7(s)	152.6(s)	-	-	-	-	-	-	-
‘5	109.3(d)	139.0(s)	‘5	6.66	dd	8.8, 2.2	-	-	-
‘6	132.9(d)	115.3(d)	‘6	7.98	d	8.8	7.65	s	-
1-Gluc	101.3(d)	100.7(d)	1-Gluc	5.03	d	7.33	4.91	d	7.33
2-Gluc	74.7(d)	73.2(d)	2-Gluc	3.50	m	-	3.32	m	-
3-Gluc	77.8(d)	75.7(d)	3-Gluc	3.50	m	-	3.32	m	-
4-Gluc	71.1(d)	69.7(d)	4-Gluc	3.43	d	9.53	3.16	m	-
5-Gluc	78.2(d)	77.3(d)	5-Gluc	3.50	m	-	3.41	m	-
6-Gluc	62.4(t)	60.6(t)	6-Gluc	3.78	dd	5.86, 12.45	3.71	d	10.63
				3.90	dd	9.53, 12.45	3.46	m	-
				-	-	-	-	-	-

a) measured in CD_3OD at 150/600 MHz.
b) measured in DMSO-d_6 at 100/600 MHz.

Table 2: ^{13}C- and ^{1}H-NMR spectral data of compounds **4** and **5** (CD_3OD; 150/600 MHz).

C	δ ^{13}C of compd **4** [ppm]	δ ^{13}C of compd **5** [ppm]	H	δ ^{1}H of compd **4** [ppm], J [Hz]	δ ^{1}H of compd **5** [ppm], J [Hz]
1	76.1(s)	75.6(s)	-		
2	37.5(t)	38.1(t)	2	2.1 (1H,m) 2.27 (1H, m)	2.07 (1H, m) 2.19 (1H, m)
3	74.2(d)	70.0(d)	3	5.38 (1H, ddd, J= 3.41, 3.41, 3.41)	5.62 (1H, ddd, J= 3.42, 8.05, 7.08)
4	72.9(d)	74.6(d)	4	3.91 (1H, dd, J= 3.17, 9.76)	5.18 (1H, dd, J= 2.93, 6.35)
5	72.5(d)	67.7(d)	5	5.5 (1H, ddd, J= 5.37, 9.76, 9.75)	4.13 (1H, m)
6	40.5(t)	38.1(t)	6	2.1 (1H, m)	2.07 (2H, m)
7	180.9(s)	181.8(s)	-	-	-
'1	168.9(s)	168.4(s)	-	-	-
'2	115.6(d)	115.3(d)	'2	6.29 (1H, d, J= 16.12)	6.24 (1H, d, J= 16.12)
'3	146.8(d)	147.1(d)	'3	7.57 (1H, d, J= 16.12)	7.50 (1H, d, J= 16.12)
'4	127.9(s)	127.8(s)	-	-	-
'5	115.2(d)	115.1(d)	'5	7.07 (1H, d, J= 1.47)	7.01 (1H, d, J= 2.2)
'6	146.8(s)	146.7(s)	-	-	-
'7	149.5(s)	149.5(s)	-	-	-
'8	116.5(d)	116.5(d)	'8	6.77 (1H, d, J= 8.06)	6.76 (1H, d, J= 8.79)
'9	122.9(d)	123.2(d)	'9	6.94 (1H, dd, J= 8.06, 1.46)	6.92 (1H, dd, J= 2.2, 8.79)
"1	169.3(s)	168.4(s)	-	-	-
"2	116.0(d)	115.0(d)	"2	6.39 (1H, d, J= 16.12)	6.29 (1H, d, J= 16.12)
"3	146.9(d)	147.3(d)	"3	7.60 (1H, d, J= 16.12)	7.57 (1H, d, J= 16.12)
"4	128.1(s)	127.8(s)	-	-	-
"5	115.2(d)	115.1(d)	"5	7.05 (1H, d, J= 1.47)	7.05 (1H, d, J= 2.20)
"6	146.7(s)	146.8(s)	-	-	-
"7	149.3(s)	149.6(s)	-	-	-
"8	116.5(d)	116.5(d)	"8	6.77 (1H, d, J= 8.06)	6.73 (1H, d, J= 8.06)
"9	122.9(d)	123.1(d)	"9	6.96 (1H, d, J= 8.06, 1.46)	6.87 (1H, d, J= 2.2, 8.06)

Table 3: Significant correlations of aglycone of **1** from HMBC (CD_3OD; 400/100 MHz).

C	δ^{13} C [ppm]	$^2J_{CH}$ correlated with H (δ ^{1}H [ppm])	$^3J_{CH}$ correlated with H (δ ^{1}H [ppm])
2	147.5	10 (6.75)	
3	184.6		10 (6.75)
4	126.4		
5	114.8		7 (7.09)
6	169.1	7 (7.09)	
7	100.7		5 (6.96)
8	166.7	7 (7.09)	
9	117.2		5 (6.96), 7 (7.09)
10	115.5		'6(7.27), '2 (7.52)
'1	125.3		'5 (6.84)
'2	119.1		10 (6.75), '6 (7.27)
'3	146.9	'2 (7.52)	'5 (6.84)
'4	149.8		'6 (7.27), '2 (7.52)
'5	116.7		
'6	126.7		10 (6.75), '2 (7.52)

corresponding to twenty one carbons and revealing the presence of one CH_2, 13CH and 7 quaternary carbons. The nature of the glucose moiety was in accordance with a ß-D-glucopyranoside structure from the pattern of the signal of ^{1}H-NMR for the anomeric proton and the ^{13}C-NMR signals of glucose.[16,17] The proton of the sugar moiety was deduced to be at C-'4 from difference NOE spectra which were as follows: irradiation of H-1 glucose at δ 5.03 showed NOE at H-'3 (δ 6.59) about 5.5% and H-'5 (δ 6.66) about 2.9%.

The ^{13}C-NMR data of the aglycone part of compound **2** are similar to those reported in literature[19] for similar chalcone aglycones. The CH-COSY and HMBC spectra were recorded to confirm the structure. The HMBC correlations are listed in Table 4. Consequently compound **2** was identified as coreopsin.

B- Compound 3

The molecular formula of **3** was deduced from its FAB-MS which showed a peak at m/z 451 for $[M+H]^+$. Calculated for $C_{21}H_{22}O_{11}$. This formula was confirmed also by ^{13}C-NMR analysis (Table 1).

The nature of the ß-glucose unit in **3** was deduced to be ß-D-glucopyranoside structure from comparison of the ^{1}H-NMR and ^{13}C-NMR data with those reported in literature.[16,17] The glucose moiety was located at C-'4 from the results of difference NOE spectra as follows: irradiation of the proton at δ 4.91 (H-1 glucose) showed NOE at δ 6.65 (H-3) about 16.2%.

The ^{13}C-NMR data of the aglycone part of compound **3** are in agreement with that reported in literature[19] for similar chalcones. Consequently compound **3** should to be identified as stillopsin. The HH-COSY, CH-COSY and HMBC data (Table 5) were taken to confirm this conclusion.

Compounds 4 and 5

The ^{1}H-NMR spectrum of compound **4** (Table 2) (CD_3OD; 600 MHz) exhibited signals for quinic acid[20-22] appeared at: δ 5.51 (1H, ddd, J= 5.37, 9.76, 9.75 Hz), 5.38 (1H, ddd, J= 3.41, 3.41, 3.41 Hz), 3.91 (1H, dd, J= 3.17, 9.76 Hz), 2.1 (3H, m) and 2.27 (1H. m). In addition to signals belonging to 2 caffeic acid moieties:[20-22] four trans olefinic protons at δ 7.60, 7.57, 6.39 and 6.29 (J= 16.12 Hz) and six aromatic protons at δ 7.07 (1H, J= 1.47 Hz), 7.05 (1H, J= 1.47 Hz), 6.96 (1H, dd, J= 8.06, 1.46 Hz), 6.94 (1H, dd, J= 8.06, 1.46 Hz), 6.77 (2H, d, J= 8.06 Hz). ^{1}H-NMR spectrum of compound **5** (CD_3OD; 600 MHz) exhibited the same pattern of compound **4** as shown in Table 2.

Table 4: Significant correlations of compound **2** from HMBC (CD_3OD; 600/100 MHz).

C	δ^{13} C [ppm]	$^2J_{CH}$ correlated with H (δ ^{1}H [ppm])	$^3J_{CH}$ correlated with H (δ ^{1}H [ppm])
1	128.3		5 (6.81)
2	115.9		6 (7.09)
3	146.8	2 (7.18)	5 (6.81)
4	150.1		2 (7.18), 6 (7.09)
5	116.6		
6	123.9		
α	118.1		
ß	146.8		2 (7.18), 6 (7.09)
'ß	193.8	α (7.52)	'6 (7.98)
'1	116.6		'6 (6.66), '3 (6.59)
'2	165.0		'6 (7.98)
'3	105.1		
'4	166.7		'6 (7.98)
'5	109.3		'3 (6.59)
'6	132.9		

Table 5: Significant correlations of compound **3** from HMBC (DMSO-d_6; 400/100 MHz).

C	δ^{13} C [ppm]	$^2J_{CH}$ correlated with H (δ ^{1}H [ppm])	$^3J_{CH}$ correlated with H (δ ^{1}H [ppm])
1	126.2		5 (6.80), α (7.60)
2	115.9		6 (7.21)
3	145.2	2 (7.29)	5 (6.80)
4	149.1		6 (7.21), 2 (7.29)
5	115.8		
6	122.6		2 (7.29)
α	117.5		
ß	145.7		6 (7.21), 2 (7.29)
'ß	191.8	α (7.60)	ß (7.66)
'1	113.7		'3 (6.65)
'2	158.2	'3 (6.65)	'6 (7.65)
'3	103.5		
'4	152.6	'3 (6.65)	'6 (7.65)
'5	139.0	'6 (7.65)	'3 (6.65)
'6	115.3		

^{13}C-, DEPT ^{13}C-NMR spectra of both compounds (CD_3OD; 150 MHz) showed signals corresponding to twenty five carbons for each of them, revealed by DEPT experiment as follows: two CH_2, 13CH, 10 quaternary carbons including two carbonyl ester carbons and one carboxylic carbon. Their chemical shifts are illustrated in Table 2.

IR spectra of compounds **4** and **5** showed characteristic absorption bands for hydroxyl, carboxylic, ester and aromatic groups: compound **4** (ν_{max} (KBr): 330-2500 (br), 1740, 1686, 1603 and 812 cm^{-1}). Compound **5** (ν_{max} (KBr): 3304-3500 (br), 1740, 1668, 1603 and 814 cm^{-1}).

FAB-MS of both compounds showed m/z at 515 $[M-H]^+$, this molecular weight was consistent with the molecular formula $C_{25}H_{24}O_{12}$, consequently the number of double bond equivalents calculated for each compound is 14.

The full assignment of all signals in the ^{13}C-NMR spectrum of compound **4** was performed by HMQC and HMBC experiments.

The signals of H-3, H-4 and H-5 of quinic acid moiety of compound **4** were assigned by HH-COSY spectral data (CD_3OD; 600 MHz) as follows: There are cross signals between the protons on carbons at δ 72.9, 72.5 and 74.2. Furthermore, the cross signals between the protons on the carbons at δ 72.5 and 40.5, in addition to the cross signal between the protons on carbons at δ 74.2 and 37.5. Consequently compound **4** should be identified as 3,5-dicaffeoyl quinic acid.

The HH-COSY spectrum of compound **5** (CD_3OD; 600 MHz) exhibited cross signals between the proton on carbons at δ 74.6, 70.0 and 67.7. Furthermore the cross signal between the protons on carbons at δ 67.7 and 38.1 in addition to the cross signal between the protons on carbons at δ 70.0 and 38.1. Also CH-COSY and HMBC spectra of compound **5** confirmed the structure to be 3,4-dicaffeoyl quinic acid, i.e. isomeric with compound **4**.

Acknowledgements

We wish to thank Mr. K. Terashima and Miss. J. Ito, Faculty of Pharmacy, Meijo University, Nagoya, Japan for the mass spectra and NMR spectral analysis on Jeol α-400 spectrometers. We are thankful to Prof. Dr. M. Hattori and Mrs. A.S. Ahmed, Traditional Sino-Japanese Medicines, Toyama Medical and Pharmaceutical University, Toyama, Japan for the screening of MeOH extract of *W. prostrata* for the inhibitory effects on HIV-type I protease.

REFERENCES

1- L. Benson, "Plant Classification", 2nd. Ed., IBH. Publishing Co., Oxford, 295-302 (1976).
2- G. H. M. Lawrence, "Taxonomy of Vascular Plants", 2nd. Ed., IBH. Publishing Co., Oxford, 726-32 (1976).
3- E. L. Core, "Plant Taxonomy", Englwood, Cliffs, N.J. Prentice Hall, Inc., 421-28 (1962).
4- H-L. Li, T-S. Liu, T. C. Huang, T. Koyama and C. E. Devol, "Flora of Taiwan", Volume IV, Epoch Publishing Co., Ltd. Taipei, Taiwan, 958-61 (1978).
5- C. D. Schteingart and A. B. Pomilio, J. Nat. Prod., 47 (6), 1046 (1984).
6- P. B. Oelrichs, P. J. Vallely, J. K. Macleod and I. A. S. Lewis, J. Nat. Prod., 43 (3), 414 (1980).
7- J. V. Eichholzer, I. A. S. Lewis and J. K. Macleod, Tetrahedron, 37 (10), 1881 (1981).
8- L. M. Perry, "Medicinal Plants of East and Southeast Asia", Massachusetts and London, England, 101 (1980).
9- L. L. Yang, K.-Y. Yen, C. Konno, Y. Oshima, Y. Kiso and H. Hikino, Planta Med., (6), 499 (1986).
10- S. Gopalakrishnan, J. Sadique and T. Chandra, Fitoterapia, LX (5), 456 (1989).
11- C. Y. Ragasa, W. G. Padolina, B. F. Bowden, S. Li, D. M. Tapiolas and J. C. Coll, J. Nat. Prod., 56 (3), 386 (1993).
12- D. H. Miles, V. Chittawong, P. A. Hedin and U. Kokpol, Phytochemistry, 32 (6), 1427 (1993).
13- D. H. Miles, V. Chittawong and A. M. Payne, J. Agric. Food Chem., 38 (7), 1591 (1990).
14- S. F. Farag, N. A. El-Emary and M. Niwa, Chem. Pharm. Bull. 44 (4), 661 (1996).

15- N. Tsunueo, *et. al*, Shoyakugaku Zasshi, 46 (2), 190 (1992).

16- E. Breitmaier, W. Volter, " Carbon-13 NMR Spectroscopy", 3 rd Ed.; VCH Publishers, 1987.

17- J. B. Harborne "The Flavonoids: Advances in Research Since 1986", Chapman and Hall, 2-6 Boundary Row, London. SE 18 HN, 464 (1994).

18- J. B. Harborne and T. J. Mabry, "The Flavonoids: Advances in Research", Chapman and Hall Ltd, London, New York, 313-416 (1982).

19- P. K. Agrawal, "Carbon-^{13}NMR of Flavonoids", Elsevier Science Publishing Co., Inc., New York, NY10010, U.S.A., 106 (1989).

20- A. Rumbero-Sanchez and P. Vazquez, Phytochemistry, 30 (1), 311 (1991).

21- I. Merfort, Phytochemistry, 31 (6), 2111 (1992).

22- M. N. Clifford, Phytochemistry, 25 (7), 1767 (1986).