Bull. Pharm. Sci., Assiut University, Vol. 25, Part 1, June 2002, pp. 85-94.

SAPONINS, NAPHTHOHYDROQUINONE AND ANTHRAQUINONE GLYCOSIDES FROM RUBIA CORDIFOLIA L.

Zedan Z. Ibraheim

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

يعتبر نبات الروبيا كورديفوليا ال. أحد نباتات العائله الفويه ذات الشهره الواسعه في علاج الكثير من الامراض وقد تم اجراء هذا البحث علي جزء البيوتانول لخلاصه الميثانول-كلوروفورم (١:١) للجذور وتم فصل مركبين من الصابونينيات هما ٣-أ-الفا-ال-هيدراجنين ارابينوبيرانوزايد و٣-أ-الفا-ال-ارابينوبيرانوزايد هيدراجنين ٢٨-أ-بيتا-دى-جلوكوبيرانوزايل (١-٦) جلوكوبيرانوزايد أستر ومركبان من جليكوزيدات النفثوهيدروكينولات هما ٢-كاربوميثوكسي-٣-بيرينيل-٤،١-نافثوهيدروكينون-٤-أ-بيتا-جليكوزيد ، ٢-كاربوميثوكسي-٣-بيرينيل-٤،١-نافثوهيدروكينون-٤،١-داى-أ-بيتا-جليكوزيد وثلاثة مركبات من الانثراكينولات الجليكوزيدية وهم ١-هيدروكسي-٢-هيدروكسي ميثيل-٩،١٠-انثراكينون-٢-بيتا-جليكوبيرانوزيل (١-٦)-بيتا-جليكوبيرانوزيد ، ٢-ميثيل-١،٣،٦-تراهيدروكسي-٩،١٠-انثراكينون-٣-أ-ألفا-ال-رامنوزيل (١-٢) (٤'-أ-اسيتيل)-بيتا-جليكوزيد ، ٢-كاربوميثوكسي-١،٣-داى هيدروكسي-٩،١٠-انثراكينون-٣-ألفا-رامنوزيل (١-٢) بيتا-جليكوزيد بالإضافة إلى الأدينوزين. وقد تم التعرف على المركبات المفصولة بإستخدام الطرق الفيزيائية والكيميائية والطيفية المختلفة.

From the butanol fraction of the chloroform-methanol extract (1:1) of the dried roots of Rubia cordifolia L, several compounds were isolated and identified viz, the saponins hederagenin-3-O-α-L-arabinopyranoside and 3-O-α-L-arabinopyranosyl-hederagenin-28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside ester, the naphthohydroquinone glycosides: 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone 4-O-β-glucoside and 2-carbomethoxy-3-prenyl-1,4-naphtho-hydroquinone 1,4-di-O-β-glucoside, the anthraquinone glycosides: 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone-11-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside, 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone-3-O-α-rhamnopyranosyl (1→2)-(4'-O-acetyl)-β-glucopyranoside and 2-carbomethoxy, 1,3-dihydroxy-9,10-anthraquinone-3-O-α-rhamno-pyranosyl-(1→2)-β-glucopyranoside and adenosine. The identification of the isolated compounds was done using different physical, chemical and spectral methods.

INTRODUCTION

Rubia cordifolia L. (Family Rubiaceae) is well known for its versatile medicinal uses.It is recommended for the treatment of hematorrhea, hematemesis, nose bleeding, traumatic bleeding, dysmenorrhea, and arthritis.[1] Many anthraquinones, naphthoquinones, naphthohydroquinones, naphthohydroquinones dimers,[2-12] triterpenes,[13-17] iridoids[18] and quinoidal derivatives[19] were isolated from the root of *Rubia cordifolia* L. in addition to cyclic hexapeptides[20-25] and polysaccharides.[26] The cytotoxic, anticancer, antibacterial, antifungal and some pharmacological activities of these compounds have been well documented.[27-31]

EXPERIMENTAL

General experimental procedure

Melting points (uncorrected) were determined by electrothermal model 550. U V. spectra were run in methanol using a Perkin-Elimer 3B UV/VIS instrument. IR spectra were measured in KBr using IR-470 Schmadzu spectrometer, Japan. ^{1}H-and ^{13}C-NMR were carried out at 400 and 100 MHz respectively on Bruker AM-400 (Germany), CIMS and FAB-MS were performed on a Joel, JMS 600 H, mass spectrometer, Japan. TLC, using silica gel G_{60} F_{254} and RP-18 pre-coated aluminum sheets (E-Merck), PC using whatman No. 1 paper. For CC, Diaion HP-20 AG (75-150 μ, Mitsubishi Chemical Industries Co. Ltd. Japan), silica gel

Received in 7/4/2002 & Accepted in 29/5/2002

(E. Merck, Germany, type 230-400 mesh) and irregular reversed phase (R 18-37, 20 μm ODS, pre-packed column) were also used. MPLC; CIG column system (22 mm. i. d. x 30 cm, Kusano Scientific Co. Tokyo, Japan) was used for final purification. The following solvent systems were used:

I) $CHCl_3$-MeOH (8:2)
II) $CHCl_3$-MeOH – H_2O (75:23:2)
III) $CHCl_3$-MeOH–H_2O (70:27:3)
IV) MeOH-H_2O (70:30)
V) MeOH-H_2O (60:40) VI) B-A-W (4:1:5)
VII) Acetonitrile-H_2O (85:15)

Plant material

The dried roots of *Rubia cordifolia* L. used in this study were purchased in India. They were kindly identified by Dr. Sang Rae Lee (Institute of Oriental Botanical Resources of Korea).

Extraction and isolation

The dried powdered roots of *Rubia cordifolia* L. (20 kg) were extracted with chloroform-methanol (1:1) to exhaustion. The concentrated extract was diluted with distilled water and then fractionated successively and exhaustively with n-hexane, chloroform and butanol.[19] 40 g of the dried butanol extract were put over an Diaion CC. Elution was started with distilled water (1:0, fraction A), then with distilled water-methanol (4:1, fraction B), (3:1, fraction C), (1:1, fraction D), (1:3, fraction E) and (0:1, fraction F). The fractions 5 L each, were evaporated under reduced pressure at temperature below 50° using rotary evaporator. Fraction A contains mainly amino acids and sugars. Fraction B, on repeated CC over silica gel, gave compound (A) in small amount not enough for analysis and adenosine. Fractions B and C contain nearly the same major spots on TLC but differ in concentration. By using silica gel CC and chloroform-methanol gradiently, the fractions eluted with chloroform-methanol (75:25 and 70:30) are further purified by MLPC using silica gel column, (solvent systems I, II and III) and/or RP-18 (solvent systems IV and V), compounds **1**, **2** and **4** were isolated. Fraction D on repeated CC over silica gel (chloroform-methanol 80:20 and 75:25) and further purification by MLPC using pre-packed RP-18 column (solvent systems IV and V) afforded compounds **3** and **5-7**.

Acid hydrolysis

A) For saponins: Each saponin (20 mg) was autoclaved in a sealed tube with 2 ml 2N trifluroacetic acid at 120°/1 bar for 1.5 hour. The aglycone was isolated by addition of distilled water and extracted with chloroform, the chloroform was dried over anhydrous $MgSO_4$ and crystallized from anhydrous chloroform. The remaining aqueous layer was evaporated under reduced pressure and dissolved in the least amount of isopropyl alcohol and tested for its sugar contents (PC and TLC using solvent system VI and VII respectively).

B) For other glycosides: Each glycoside (10 mg) was dissolved in 5 ml methanol to which 20% H_2SO_4 solution was added and the mixture was refluxed in a boiling water bath. After complete hydrolysis, the solution was extracted with chloroform (10 ml x 3), the chloroform extract was evaporated and used for identification of the aglycone. The aqueous layer was neutralized with $BaCO_3$ and filtered. The filtrates were concentrated and examined for their sugar contents using PC and TLC using solvent system VI and VII respectively.

Alkaline hydrolysis of (2): A solution of **2** (50 mg) in 0.5 N aqueous KOH (2 ml) was heated on a boiling water bath for 0.5 h. The reaction mixture was neutralized with 0.5 N H_2SO_4 and then extracted with EtOAc-BuOH (2:1). The organic layer was washed with water and concentrated to dryness to give **1** (identified using silica gel TLC, systems I and II, and RP-18 using system IV).

Compound (1): Obtained as white powder (methanol) (180 mg), m.p 223-226°, IR ν^{KBr} 3440, 2990, 2820, 1695, 1640, 1510, 1250, 1080 and 880 cm^{-1}, negative FAB-MS showed quassi-molecular ion peak at m/z 603, other peak at m/z 471 $[(M-1)\text{-arabinose}]^-$. 400 MHz ^{1}H-NMR ($C_5D_5N$): δ 0.92 (3H, s), 0.94 (3H, s), 0.95 (3H, s), 1.01 (3H, s), 1.02 (3H, s), 1.24 (3H, s), 3.35 (1H, dd, J= 3.4 and 13.5 Hz, H-3), 3.73 (1H, d, J= 10.2 Hz, H-24a), 4.42 (1H, d, J= 10.2 Hz, H-24b), 5.22 (1H, d, J= 6.8 Hz, C_1-H arabinose), 5.48 (1H, br s, H-12). 100 MHz ^{13}C-NMR (C_5D_5N) as cited in Table 1. Acid hydrolysis afforded aglycone and arabinose.

Aglycone: Obtained in form of white needles, m.p > 300°, IR ν^{KBr} 3340, 1702, 1620 and 1100-1020 cm^{-1}, 400 MHz ^{1}H-NMR (C_5D_5N): δ 0.942 (3H, s), 0.989 (3H, s), 1.015 (3H, s), 1.064 (3H, s), 1.071 (3H, s), 1.254 (3H, s), 3.35 (1H, dd, J= 4 and 14 Hz, H-3), 3.74 (1H, d, J= 10.5 Hz, H-24a), 4.22 (1H, d, J= 10.5 Hz, H-24b) and 5.51 (1H, br. s, H-12); 100 MHz ^{13}C-NMR (C_5D_5N) as cited in Table 1.

Compound (2): Obtained as colorless needles (430 mg), m.p 232-234° (dec.) IR ν^{KBr} 3440, 2992, 2828, 1725, 1640, 1512, 1255, 1080 and 880 cm^{-1}, negative FAB-MS showed quassi-molecular ion peak at m/z 927 $[(M-1)]^-$, other peaks at m/z 795 [(M-1)-arabinose]$^-$, 777 [(M-1)-arabinose-H_2O]$^-$, 633 [(M-1)-arabinose-glucose]$^-$, 603 [(M-1)-2 glucose]$^-$, 471 [(M-1)-arabinose-2 glucose]$^-$ and 427 [(M-1)-arabinose-2 glucose-COO]$^-$. 400 MHz ^{1}H-NMR (C_5D_5N): δ 0.87 (3H, s), 0.88 (3H, s), 0.94 (3H, s), 0.995 (3H, s), 1.13 (3H, s), 1.18 (3H, s), 5.13 (1H, d, J= 7.2 Hz, C_1-H of the terminal glucose unit) 5.38 (1H, d, J= 6.6 Hz, C_1-H arabinose), 5.42 (1H, br s, H-12) and 6.28 (1H, d, J= 7.1 Hz, C_1-H of the inner glucose unit); 100 MHz ^{13}C-NMR (C_5D_5N) as cited in Table 1. Acid hydrolysis gave the same aglycone as **1** and two sugars identified as arabinose and glucose, while alkaline hydrolysis afforded **1**.

Compound (3): Obtained as pale yellowish fine needle crystals (40 mg), m.p 170-172°, ^{13}C-NMR (CD_3OD) as cited in Table 2. Other data (UV, IR, ^{1}H-NMR and MS) are identical to those reported for 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone 4-O-glucoside.[4]

Compound (4): Very fine needles (57 mg), m.p 234-237° (methanol). ^{13}C-NMR (CD_3OD) as cited in Table 2. Other data (UV, IR, ^{1}H-NMR and MS) are identical to those reported for 2-carbomethoxy-3-prenyl-1,4-naphthohydro-quinone 1,4-di-O-β-glucoside.[3]

Compound (5): Yellow needles (methanol) (320 mg), m.p 170-171°, UV λ_{max}^{MeOH} 202, 222, 253, 277, 330; IR ν^{KBr} 3440, 2920, 2882, 1672, 1635, 1590, 1433, 1362, 1290, 1260, 1166, 1075, 1050 and 1015 cm^{-1}; CIMS m/z (% rel. int.):579 [M+1] (8), 255 (80), 238 (55), 209 (23) 181 (44), 152 (65) and 139 (70); 400 MHz ^{1}H-NMR (DMSO-d_6): δ 2.99 (1 H, m, H-2"), 3.04 (H-3' and H-4"), 3.06-3.25 (4H, m, H-2', H-4', H-3" and H-5"), 3.35 (1H, t, H-5'), 4.30 (1H, d, J= 7.8 Hz, H-1"), 4.36 (1H, d, J= 7.8 Hz, H-1'), 4.73 and 4.92 (1H each, d, J= 14.8 Hz, CH_2-11), 7.72 (1H, d, J= 8.5 Hz, H-4), 7.95 (2H, m, H-6 and H-7), 8.17 (1H, d, J= 8.5 Hz, H-3), 8.20 (2H, m, H-5 and H-8) and 12.75 (1H, s, OH at C-1). Other sugar proton appears between δ 4.00 and 5.30 ppm. ^{13}C-NMR (DMSO-d_6) see Table 3.

Compound (6): Obtained as yellowish powder (32 mg), m.p 222-224° (from methanol), CIMS m/z (% rel. int.) 621 $[M+1]^+$ (33), 577 (12), 553 (53), 461 (84), 271 (100) and 241 (45). UV (methanol) λ_{max} nm: 276 and 305; IR ν^{KBr} cm^{-1}: 3440 (OH), 1722, 1672, 1630 (C=O), 1590 and 1573 (aromatic C=C); ^{1}H-NMR (DMSO-d_6): δ 1.11 (3H, d, 6.1 Hz, rhamnose-CH_3), 2.05 (3H, s, Ac-Me), 2.17 (3H, s, CH_3-11), 3.53-4.05 (other sugar protons), 5.31 (1H, d, J= 2.1 Hz, rhamnose H-1"),5.51 (1H, d, J= 7.4 Hz, glucose H-1'), 7.26 (1H, dd, J= 8.1 & 2.0 Hz, H-7), 7.44 (1H, s, H-4), 7.52 (1H, d, J= 2.0 Hz, H-5), 8.13 (1H, d, J= 8.1 Hz, H-8) and 12.87 (1H, s, OH at C-1) ^{13}C-NMR as cited in Table 3.

Compound (7): Obtained as yellowish powder (23 mg), m.p 187-188°, UV (methanol) λ_{max} nm: 252, 286 and 385; IR ν^{KBr} cm^{-1}: 3880 (OH), 1725, 1662, 1626 (C=O), 1592 and 1571 (aromatic C=C), ^{1}H-NMR (DMSO-d_6): δ 1.10 (3H, d, J= 6.2 Hz, CH_3-rhamnose), 3.98 (3H,s, OCH_3), 5.03 (1H, d, J= 2.0 Hz, rhamnose H-1), 5.24 (1H, d, J= 7.4 Hz, glucose-H-1), 7.02 (1H, s, H-4), 7.98 (2H, m, H-6 and H-7), 8.12 (2H, m, H-5 and H-8) and 13.08 (1H, s, OH at C-1). Other sugar proton appears between δ 4.00 and 5.20 ppm. ^{13}C-NMR as cited in Table 3.

RESULTS AND DISCUSSION

The butanol fraction obtained from the chloroform-methanol extract (1:1) of *Rubia cordifolia* L. upon repeated chromatographoic fractionation and fine separation resulted in the isolation of two saponins, two napthohydro-quinone glucosides, three anthraquinone glycosides in addition to adenosine (identified by TLC co-chromatography and spectral data).

Table 1: 100 MHz ^{13}C-NMR data of compounds **1**, **2** and aglycone (C_5D_5N).

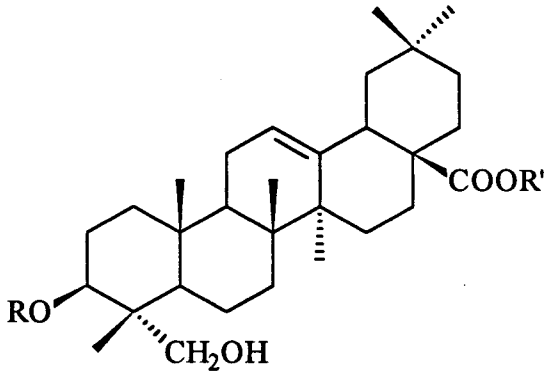
C No.	1	2	agly.	C No.	1	2
1	38.76	38.83	38.79	Arabinose		
2	26.12	26.09	27.65	1'	106.58	106.57
3	81.92	81.95	73.48	2'	73.07	73.13
4	43.46	43.50	42.86	3'	74.66	74.71
5	47.61	47.64	48.15	4'	69.54	69.49
6	18.15	18.20	18.59	5'	66.86	66.89
7	32.88	32.81	32.99			
8	39.76	39.98	39.77	Glucose (inner)		
9	48.15	48.20	48.67	1"		95.68
10	36.94	36.90	37.23	2"		73.91
11	23.76	23.87	23.75	3"		78.40
12	122.53	122.94	122.58	4"		70.99
13	144.81	144.16	144.81	5"		77.98
14	42.14	42.17	42.00	6"		69.49
15	28.32	28.31	28.33			
16	23.67	23.37	23.70	Glucose		
17	46.63	47.07	46.65	(terminal)		
18	41.69	41.68	42.19	1'''		105.29
19	46.41	46.20	46.45	2'''		75.18
20	30.92	30.75	30.93	3'''		78.74
21	33.22	33.97	33.20	4'''		71.61
22	32.87	32.55	32.98	5'''		78.43
23	64.51	64.55	68.02	6'''		62.67
24	13.56	13.59	13.07			
25	16.07	16.25	15.95			
26	17.45	17.62	17.48			
27	26.12	26.05	26.14			
28	180.19	176.52	180.13			
29	33.22	32.82	33.21			
30	23.76	23.68	23.75			

Table 2: 100 MHz ^{13}C-NMR data of compounds **3** and **4** (CD_3OD).

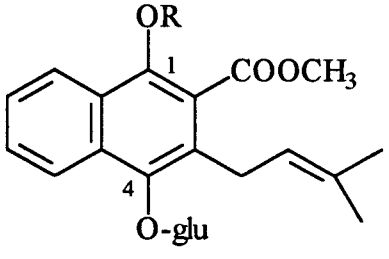
C No.	3	4
1	157.39	149.85
2	109.63	111.22
3	126.94	128.69
4	143.76	148.85
5	124.63a	125.27
6	130.11	129.54
7	126.56	125.88
8	124.23	125.54
9	131.79	131.90
10	132.39	133.86
1'	28.53	28.77
2'	125.90	125.27
3'	132.30	129.29
4'	18.57	16.65
5'	25.89	19.20
$COO\underline{CH_3}$	52.95	53.76
$\underline{CO}OCH_3$	173.23	171.93
1"(1"')	106.25	107.06 (107.26)
2"(2"')	75.98	76.41 (76.68)
3"(3"')	78.11	78.68 (79.66)
4"(4"')	71.83	72.42 (72.74)
5"(5"')	78.15	78.76 (78.88)
6"(6"')	62.84	63.42 (64.12)

Table 3: 100 MHz ^{13}C-NMR data of compounds 5, 6 and 7 (DMSO-d_6).

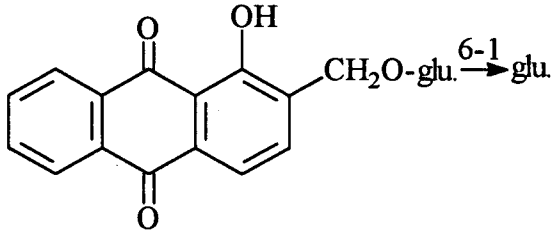
C. No.	5	6	7	C. No.	5	6	7
1	158.74	163.65	167.81		Glucose	Glucose	Glucose
2	131.88	120.62	112.73	1'	102.58	97.31	97.62
3	135.15	159.73	166.68	2'	73.48	73.21	73.31
4	118.63	104.99	107.39	3'	76.59	74.06	75.12
5	126.87	112.60	125.88	4'	70.08	71.82	70.29
6	135.20	161.22	135.01	5'	76.01	75.12	76.04
7	134.63	121.42	132.54	6'	68.32	60.42	60.34
8	126.60	129.73	125.91				
9	188.55	186.31	185.61		Glucose	Rhamnose	Rhamnose
10	181.75	181.61	181.16	1"	103.24	101.22	101.21
9a	115.08	110.66	106.67	2"	73.55	70.03	70.32
4a	132.74	135.23	136.14	3"	76.82	70.41	70.22
10a	134.02	131.90	135.23	4"	70.01	71.73	71.83
8a	133.18	124.24	131.93	5"	76.74	69.06	68.64
(11) side	64.13	8.55	172.11	6"	61.08	17.88	17.85
chain				$\underline{CH_3CO}$	-----	20.77	-----
				$CH_3\underline{CO}$	-----	170.10	-----
				$O\underline{CH_3}$	-----	-----	52.05



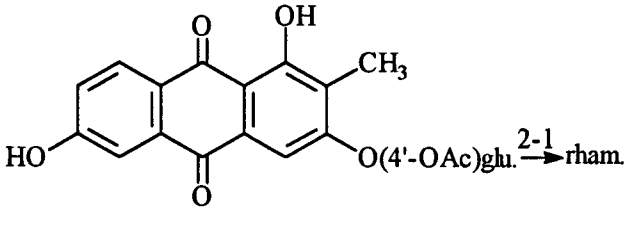
	R	R'
Compound **1**	arabinose	H
Compound **2**	arabinose	glucose $\xrightarrow{6\text{-}1}$ glucose
Aglycone	H	H



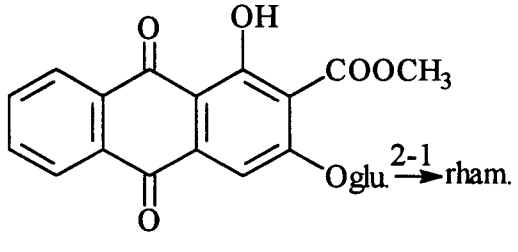
Compound **3** R = H
Compound **4** R = glu.



Compound **5**



Compound **6**



Compound **7**

List of Compounds Isolated from *Rubia cordifolia*

Acid hydrolysis of compounds **1** and **2** afforded the same aglycone identified as hederagenin by compairing its spectral data (IR, MS, ^{1}H-NMR and ^{13}C-NMR) with the reported data.[32]

Compound 1: Showed the molecular formula $C_{35}H_{56}O_8$ deduced from the negative FAB-MS and DEPT ^{13}C-NMR. The negative FAB-MS showed a quasi-molecular ion peak at m/z 603 $[(M-H^+)]^-$ and further peak at m/z 471 $[(M-H^+)-132]^-$ for the loss of pentose sugar. ^{1}H-NMR showed six singlet methyl signals, one olefinic proton at δ 5.48 assigned for H-12, and one anomeric proton at δ 5.22 (1H, d, J= 6.8 Hz) for α-sugar, and the ^{13}C-NMR showed anomeric sugar carbon at δ 106.58 for D-sugar. Acid hydrolysis of **1** gave only one sugar identified as D-arabinose (PC and TLC, using solvent systems VI and VII respectively) and aglycone identified as hederagenin (by comparing its physical and spectral data with published data).[32] ^{13}C-NMR aided by DEPT experiment showed 30 carbon signals for the aglycone and five carbon signals for pentose sugar indicating that **1** is a monodesmosidic saponin. The location of the sugar to the aglycone was deduced to be at C-3 due to the downfield shift of C-3 (+ 7.46 ppm) and the upfield shift of C-2 (-1.53 ppm) compared with the aglycone.[33,34] As so, compound **1** is identified as hederagenin-3-O-α-L-arabinopyranoside (δ-hederin). This compound was previously isolated from some plants including *Hedera rhombea,* and *Kalopanax pictus* (F. Araliaceae) and *Akebia quinata* (F. Larizabalaceae),[35-38] but this is the first report of the isolation of this compound from the genus *Rubia.*

Compound 2: The molecular formula of **2** was deduced to be $C_{47}H_{76}O_{18}$ from negative FAB-MS and DEPT ^{13}C-NMR. The negative FAB-MS showed a quasi-molecular ion peak at m/z 927 $[(M-H^+)]^-$, other peaks at m/z 795 [(M-1)-arabinose]$^-$, 777 [(M-1)-arabinose-H_2O]$^-$, 633 [(M-1)-arabinose-glucose]$^-$, 603 [(M-1)-2 glucose]$^-$, 471 [(M-1)-arabinose-2 glucose]$^-$ and 427 [(M-1)-arabinose-2 glucose-COO]$^-$ indicating the presence of one molecule of arabinose and two molecules of glucose. ^{1}H-NMR showed the presence of six singlet methyls and three anomeric protons, two for β-sugars (at δ 5.13, 1 H, d, J= 7.2 Hz and δ 6.28, 1 H, d, J= 7.1 Hz) and one for α-sugar (at δ 5.38, 1H, d, J= 6.6 Hz). The ^{13}C-NMR spectral data showed that compound 2 is a triterpene containing three monosaccharides due to the presence of three anomeric carbon signals at δ 106.57, 95.68 and 105.29, and these signals with the other sugars signals (Table 1) indicating the presence of the three sugar moieties in pyranose forms. Acid hydrolysis of **2** afforded aglycone identical to that obtained from compound 1 and two sugars identified as arabinose and glucose (PC and TLC, using solvent systems VI and VII respectively), while alkaline hydrolysis afforded **1**. Comparing the ^{13}C-NMR of **2** with **1** (Table 1) showed that the C-28 carbonyl signal of **2** was upfield shifted (-3.67 ppm), while C-3 in both are nearly similar, indicating that **2** is a bidesmosidic sapoinin. The ^{13}C-NMR (Table 1) and FAB-MS indicated that the linkage of one of the glucose moieties to the aglycone was at C-28 and the two glucose moieties were linked together througth (1→6) linkage, and this was deduced from the downfield shift of C-6 and upfield shift of C-5 of the inner glucose moiety comparing with the terminal glucose (Table 1). From all of the above mentioned data compound **2** was identified as 3-O-α-L-arabinopyranosyl-hederagenin-28-O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside ester (akebia saponin D). This compound was previously isolated from some plants including *Hedera rhombea* (F. Araliaceae), *Akebia quinata* (F. Larizabalaceae) and *Dipsacus asper* (F. Dipsaceae),[35,37,38] but this is the first report of the isolation of this compound from the genus *Rubia.*

Compound 3: The UV, IR, MS, ^{1}H-NMR and ^{13}C-NMR (Table 2) were identical with those reported for 2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone 4-O-β-glucoside which was isolated previously from *Rubia oncotricha*[4] but this is the first report for isolation of this compound from *Rubia cordifolia* L.

Compound 4: This compound was identifiesd as 2-carbomethoxy-3-prenyl-1,4-naphthohydro-quinone 1,4-di-O-β-glucoside by comparing its physical, chemical and spectral data (UV, IR, ^{1}H-NMR, ^{13}C-NMR and MS), with the published data.[3] compound **4** was isolated from *Rubia cordifolia* var. *pratensis*[3] but this is the

first report for isolation of this compound from *Rubia cordifolia* L. The aglycone of compounds **3** and **4** (the same aglycone) was not isolated from the plant sources or by acid hydrolysis from **3** and **4**, because the removal of the sugar moiety from C-4 will lead to cyclization.[4]

Compound 5: Obtained as yellow needle crystals, m.p170-171°. IR spectrum showed bands at 1672 and 1635 cm^{-1} indicate the presence of free and chelated carbonyl groups respectively.[39] The CIMS showed $[M+1]^+$ at m/z 579 calculated for the molecular formula $C_{27}H_{30}O_{14}$ and a peaks at m/z 255 and 238 probably due to a cleavage of the glycosidic bond. ^{1}H-NMR showed the presence of two independent spin systems in the aromatic region, the first was a four-spin system associated with H-5 to H-8, the second was AX system (J= 8.5 Hz) indicating two *ortho*-protons, the remaining two positions on the anthraquinone residue being occupied by a hydroxyl group at C-1 and an oxymethylene group at C-2. The latter protons constitute an AB system at δ 4.73 and 4.92 (1H each, d, J= 14.8 Hz). Thus, the aglycone of compound **5** is digiferruginol and a comparison with its ^{1}H-NMR data[7,10] showed that the glycosidation site is the oxymethylene group. Also the ^{1}H-NMR showed the presence of two anomeric protons at δ 4.30 (1H, d, J= 7.8 Hz) and 4.36 (1H, d, J= 7.8 Hz) for two β-linked sugars. Acid hydrolysis of **5** gave only one sugar identified as glucose (PC and TLC, using solvent systems VI and VII respectively), since the ^{1}H-NMR showed the presence of two anomeric protons and the mass spectra showed a prominent peak at 255 $[(M+1)-2 \times 162]^+$ for the loss of two glucose moieties, this confirm the presence of two glucose moieties in **5**. ^{13}C-NMR showed the presence of 14 carbon signals for anthraquinone moiety, signal for oxymethylene and twelve carbon signals assigned for two glucose moieties (Table 3). The linkage of the two glucose moieties was deduced to be (1→6) from the ^{13}C-NMR, since C-6 of the inner glucose was downfield shifted (+ 7.24 ppm). The aglycone was identified as 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone (digiferruginol) previously isolated from the same plant.[10] So compound **5** was identified as 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone-11-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside. This compound was isolated once before from *Rubia schumanniana,*[40] and this is the first report for isolation of this compound from *Rubia cordifolia* L. and the second report for its isolation from natural source.

Compound 6: Obtained as yellow fine needles m.p 222-224°, IR spectrum showed bands at 1722, 1672 and 1635 cm^{-1} for acetyl group, free and chelated carbonyl groups. The molecular formula of **6** was deduced to be $C_{29}H_{32}O_{15}$ from the MS and DEPT ^{13}C-NMR. CIMS showed a peak at m/z 621 $[M+1]^+$ and base peak at m/z 271 $[(M+1)$-glucose-rhamnose$]^+$ for the aglycone part. ^{1}H-NMR showed a pattern characteristic for 1,3,6-trihydroxy-2-methyl-9,10-anthraquinone derivatives,[3] the signal at δ 12.87 (1H,s) was assigned for the chelated hydroxyl group at C-1, the signal at δ 2.17 (3H, s) was assigned for CH_3 at C-2 and the signal at δ 5.51 (1H, d, J= 7.4 Hz) was assigned for β-glucose anomeric proton while the signal at δ 5.31 (1H, d, J= 2.1 Hz, amoneric proton) together with the doublet methyl signal at δ 1.11 (3H, d, J= 6.1 Hz, CH_3-6) were assigned for α-rhamnose. Acid hydrolysis of **6** gave an aglycone identified as 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone (m.p, m.m.p and co-chromatography) previously isolated from the same plant[10] and two sugars identified as rhamnose and glucose (TLC and PC). ^{13}C-NMR (Table 3) showed the presence of 2-methyl-anthrquinone nucleus in addition to fourteen carbon signals i.e, eight signals for acetylated hexose and six signals for methyl pentose sugars, the two signals at δ 20.77 and 170.10 were assigned for the acetate moiety, the other signals were assigned for the glucose and rhamnose moieties (Table 3), the sugars must be attached to the C-3 hydroxyl group,[41] since one of the carbonyl groups resonated more downfield than the other and C-1 hydroxyl group was chelated. The two sugars are linked by (1→2) linkage and the acetyl group was attached to C-4 of the glucose moiety, since C-4 was downfield shifted and C-3 and C-5 were upfield shifted comparing with the non-acetylated sugar according to the known chemical shift rules.[2,40-41] Many glycosides having the aglycone 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone were isolated from the

genus *Rubia*.[2-4, 40] So, compound **6** was confirmed to be 2-methyl-1,3,6-trihydroxy-9,10-anthraquinone 3-O-α-rhamnopyranosyl (1→2)-(4'-O-acetyl)-β-glucopyranoside. Although many antharquinones with acetylated sugars were isolated from the genus *Rubia*, but according to the available literature, this is the first report for the isolation of this compound from natural source.

Compound 7: Obtained as yellowish powder, m.p 187-188°, IR spectrum showed bands at 1725, 1662 and 1626 cm^{-1} for acetyl group and free and chelated carbonyl groups. ^{1}H-NMR showed the presence of trisubstituted ring C and non-substituted ring A anthraquinone nucleus. The signal at δ 13.08 (1 H, s) was assigned for chelated hydroxyl group at C-1, while the signal at δ 7.02 (1H, s) was assigned for H-4 and the signal at δ 3.98 (3H, s) for the methoxy group in the tri-substituted ring. The non-substituted ring showed signal at δ 7.98 (2H, m) assigned for H-6 and H-7 and signal at δ 8.12 (2H, m) assigned for H-5 and H-8.^{13}C-NMR (Table 3) showed signals for cabomethoxy group, tri-substituted 9,10-anthraquinone nucleus and two sugar moieties; the signals at δ 97.62, 73.31, 75.12, 70.29, 76.04 and 60.34 were assigned for β-glucose (C_1 H, d, J= 7.4 Hz) and the signals at δ at 101.21, 70.32, 70.22, 71.83, 68.64 and 17.85 were assigned for α-rhamnose (C_1 H, d, J= 2.0 Hz). Acid hydrolysis afforded an aglycone and two sugars identified as glucose and rhamnose (TLC and PC). The attachment of the sugar to the aglycone was deduced to be at C-3 hydroxyl group since in the ^{13}C-NMR spectrum one of the carbonyl groups resonated more downfield than the other and the hydroxyl group at C-1 appeared at δ 13.08 in ^{1}H-NMR spectrum. The two sugars were linked together through (1→2) linkage and this is deduced from the downfield shift of C-2 and the upfield shift of C-3 of glucose according to the known chemical shift rule.[2,42-44] From all of the above mentioned data compound **7** was identified as 2-carbomethoxy, 1,3-dihydroxy 9,10-anthraquinone-3-O-α-rhamnopyranosyl (1→2)-β-glucopyranoside. Although 1,3-dihydroxy-2-carboxyanthraquinone (rubiadin) was isolated from *Rubia cordifolia*,[3,9] but according to the available literature, this is the first report for the isolation of this compound from natural source.

Acknowledgment

The author would like to thank Prof. Dr. H. Itokawa, Tokyo University of Pharmacy and Life Science for providing the plant material and measuring NMR and MS spectra.

REFERENCES

1- W. Tang and G. Eisenbrand, "Chinese Drugs of Plant Origin", Chemistry, Pharmacology, and Use in Traditional and Modern Medicine, Springer-Verlag, Berlin, Heidelberg, (1992), pp. 885-895.
2- H. Itokawa, K. Mihara and K. Takeya, Chem. Pharm. Bull., 31, 2352 (1983).
3- H. Itokawa, Y.-F. Qiao and K.Takeya, Phytochemistry, 28, 3465 (1989).
4- H. Itokawa, Y.-F. Qiao and K. Takeya, Phytochemistry, 30, 637 (1991).
5- Y.-F Qiao, K. Takeya and H. Itokawa, Chem. Pharm. Bull., 38, 2896 (1990).
6- R. Wijnsma, R. Verpoorte, Th. Mulder-Krieger and A. B. Svendsen, Phytochemistry, 23, 2307 (1984).
7- R. Y.-F Wijnsma, and R. Verpoorte, "Progress in the Chemistry of Organic Natural Products" Vol. 49, ed. By W. Herz, H. Griesebach, G. W. Kirby and Ch. Tamm, Springer-Verlag, Wien-New York, (1986), pp. 79-149 and references cited therein.
8- N. Warma, P. Painuly, S. C. Sharma and J. S. Tandon, Ind. J. Chem., 24B, 791 (1985).
9- Ch. Dosseh, A. M. Tessier and P. Delaveau, Planta Medica, 43, 141 (1981).
10- H. Itokawa, Z. Z. Ibraheim, Y.-F. Qiao and K. Takeya, Chem. Pharm. Bull., 41, 1869 (1993).
11- P. P. Gupita, R. C. Srimal, N. Verma and J. S. Tandon, Pharm. Biol., 37, 46 (1999).
12- L. C. Chang, D. Chavez, J. J. Gills, H. H. S. Fang, J. M. Pezzuto and A. D. Kinghorn, Tetrahedron Lett., 41 (37), 7152 (2000).
13- S. K Talapatra, A. S. Sarkar and B. Talapatra, Phytochemistry, 20, 1923 (1981).

14- M. Arisawa, H. Ueno, M. Nimura, T., Hayashi and N. Morita, J. Nat. Prod., 49, 1114 (1986).
15- H. Itokawa Y.-F. Qiao K. Takeya and Y. Iitaka, Chem. Pharm. Bull., 37, 1670 (1989).
16- H. Itokawa, Y.-F. Qiao and K. Takeya, Chem. Pharm. Bull., 38, 1435 (1990).
17- H. Inouye, Y. Takeda, H. Nishimura, A. Kanomi, T Okuda, and C. Puff, Phytochemistry, 27, 2591 (1988).
18- A. Bianco, M. Guiso, C. Iavarone, P. Passacantilli and C. Trogolo, Gazz.Chim. Ital., 108, 13 (1978).
19- H. A. Hassanean, Z. Z. Ibraheim, K. Takeya and H. Itokawa, Pharmazie, 54, 12, 1999.
20- H. Morita, T. Yamamiya, K. Takeya and H. Itokawa, Chem. Pharm. Bull., 40, 1352 (1992).
21- H. Itokawa, T. Yamamiya, H. Morita and K. Takeya, J. Chem. Soc., Perkin Trans., 1, 455 (1992).
22- H. Itokawa, H. Morita, K. Takeya, N. Tomioka, A. Itai and Y. Iitaka, Tetrahedron, 47, 7007, (1991).
23- H. Itokawa, K. Takeya, N. Mori, T. Sonobe, S. Mihashi and T. Hamanaka, Chem. Pharm. Bull., 34, 3762 (1986).
24- H. Itokawa, K. Takeya, N. Mori, T. Hamanaka and K. Mihara, Chem. Pharm. Bull., 32, 284 (1984).
25- K. Takeya, T. Yamamiya, H. Morita and H. Itokawa, Phytochemistry, 33, 613 (1993).
26- H. X. Wang and B. J. Wang, Chin. Tradit. Herb. Drugd, 29, 219 (1998).
27- M. K. Adwankar and M. P. Chitnis, Biomed. Pharmachother., 36, 104 (1982).
28- Y. B. Tripathi, M. Sharma and M. Manickam, Indian J. Biochem. Biophys., 34, 302 (1997).
29- L. K. Ho, M. J. Don, H. C. Chen, S. F. Yeh and J. M. Chen, J. Nat. Prod., 59, 330 (1996).
30- H. A. Galani, K. H. Janbaz, M. Zaman, A. Lateef, A. Suria and H. R. Ahmed, J. Pak. Med. Association, 44, 82 (1994).
31- Y.-F. Qiao, S.X. Wang, L. J. Wu, X. Li and T. R. Zhu, Yao Hsuch Hsuch Pao, 25, 834 (1990).
32- V. U. Ahmad and Att-ur-Rahman, "Handbook of Natural Products Data, II". Pentacyclic triterpenoids. In: A.U. Rahman, editor. Amsterdam, Elsevier, (1994), pp. 223-4.
33- R. Casai, M. Okihara, J. Asakawa, K. Mizutani and O. Tanaka, Tetrahedron, 35, 1427 (1979).
34- K. Mizutani, R. Kasai and O. Tanaka, Carbhydr. Res., 87, 19 (1980).
35- D. H. Kim, K. W. Yu, E. A. Bae, H. J. Park and J. W. Choi, Biol. Pharm. Bull., 21, 360 (1998).
36- H. KIizu, S. Hirabayashi, M. Suzuki and T. Tomimori, Chem. Pharm. Bull., 33 (8), 3473 (1985).
37- R. Higuchi and T. Kawasaki, Chem. Pharm. Bull., 20, 2143 (1972)
38- I. Kouno, A. Tsuboi, M. Nanri and N. Kawano, Phytochemistry, 29, 338 (1990).
39- E. Okuyama, K. Sato and K. Yashihara, Phytochemistry, 29, 3973 (1990)
40- Y.-L. Liu, B.-Z. Chen, Y.-L Bai, H. Duddeck and M. Hiegemann, Phytochemistry, 30 (3), 947 (1991).
41- Y. Berger and A. Castonguay, Org. Magn. Reson., 11, 375 (1978).
42- K. Yoshimoto, Y.Itatani and Y. Tsuda, Chem. Pharm. Bull., 28, 2065 (1980).
43- S. B. Kalidhar, Phytochemictery, 28, 2455 (1989).
44- S. B. Kalidhar, Phytochemictery, 28, 3459 (1989).