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TRITERPENOID GLYCOSIDES FROM *ADINA RUBELLA*

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Key Word Index—*Adina rubella*; Rubiaceae; quinovic acid glycosides; triterpenoids.

Abstract—From the roots of *Adina rubella*, four new quinovic acid glycosides, quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-fucopyranoside, quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-fucopyranosyl-(28→1)-*β*-D-glucopyranosyl ester, quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl-(28→1)-*β*-D-glucopyranosyl ester and quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-(28→1)-*β*-D-glucopyranosyl ester, were isolated. Their structures were elucidated on the basis of hydrolytic and spectral methods. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

Adina rubella Hance, a Chinese folk medicinal plant, has been shown to contain an extensive series of triterpenoid saponins [1–3]. Our further investigation of the roots led to the isolation of four new triterpenoid glycosides, quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-fucopyranoside (**1**), quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→4)-*β*-D-fucopyranosyl-(28→1)-*β*-D-glucopyranosyl ester (**2**), quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→4)-α-L-rhamnopyranosyl-(28→1)-*β*-D-glycopyranosyl ester (**3**), quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-(28→1)-*β*-D-glycopyranosyl ester (**4**). This paper describes the isolation and structure elucidation of compounds **1–4**.

RESULTS AND DISCUSSION

The compounds **1–4** were obtained as powders. Their structures were determined by FAB-mass spectrometry, ^{1}H and ^{13}C NMR spectroscopy and sugar analysis. On acid hydrolysis, **1** and **2** afforded D-glucose and D-fucose, respectively, **3** afforded D-glucose and α-rhamnose; **4** yielded D-glucose only identified by PC comparison with authentic samples.

The FAB-mass spectrum of **1** showed peaks at *m/z* 817 $[M+Na]^+$ and 795 $[M+1]^+$, and fragment ions at *m/z* 617 $[(M+1)-178]^+$, resulting from the cleavage of a glucose unit with the glycosidic oxygen, and a fragment at *m/z* 487 $[(M+1)-162-146]^+$, resulting from the loss of a glucose and a fucose unit without the glycosidic oxygens.

The FAB-mass spectrum of **2** showed molecular peaks at *m/z* 979 $[M+Na]^+$ and 975 $[M+1]^+$ shifting 162 mass units relative to **1** and fragments at *m/z* 795 $[(M+1)-162]^+$ and 779 $[(M+1)-178]^+$, suggesting the presence of an extra glucose moiety with respect to **1**. The ion peaks at *m/z* 633 $[(M+1)-2\times162]^+$ and 617 $[(M+1)-162-178]^+$ showed clearly the further loss of a glucose unit while a fucose remained attached to the aglycone. The fragment at *m/z* 487 $[(M+1)-2\times162-146]^+$ corresponded to the subsequent loss of a fucose unit.

Compound **4**, in its FAB-mass spectrum gave molecular ions at *m/z* 995 $[M+Na]^+$ and 973 $[M+1]^+$ and a fragment ion at *m/z* 811 $[(M+1)-162]^+$, showing the loss of a glucose unit without the glycosidic oxygen; a fragment at *m/z* 649 $[(M+1)-2\times162]^+$, showing the subsequent loss of a glucose unit that was confirmed by the fragments at *m/z* 605 $[(M+1-44)-2\times162]^+$ and 589 $[(M+1-44)-162-178]^+$, suggesting the facile loss of a carboxyl group and the loss of a glucose unit with or without the glycosidic oxygen. Starting from the peak *m/z* 649, the loss of the third glucose unit led to the peak at *m/z* 487 $[(M+1)-3\times162]^+$.

The FAB-mass spectrum of **3** showed the same fragments pattern observed in **2** and indicated that **3** was isomeric to **2**. The molecular formulae $C_{42}H_{66}O_{14}$ for **1**, $C_{48}H_{76}O_{19}$ for **2** and **3**, $C_{48}H_{76}O_{20}$ for **4**, and the aglycone formula $C_{30}H_{46}O_5$ were deduced by the FAB mass spectrum (Table 1) and ^{13}C NMR DEPT spectrum.

Analysis of ^{13}C and ^{1}H NMR data (Tables 2 and 3,

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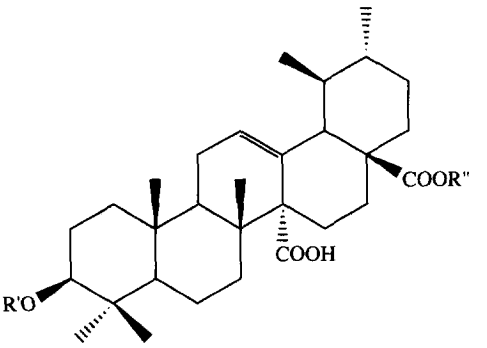
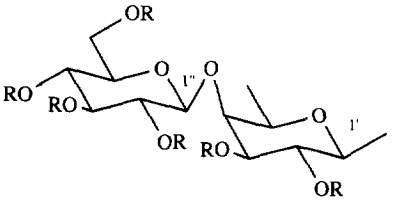
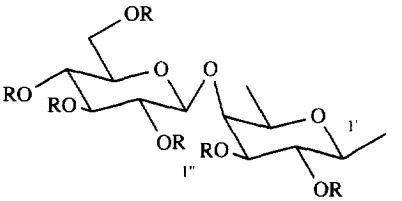
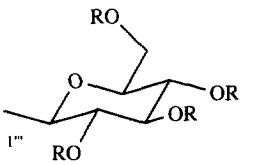


Compound	R	R'	R"
1	H		H
1a	Ac		
2	H		
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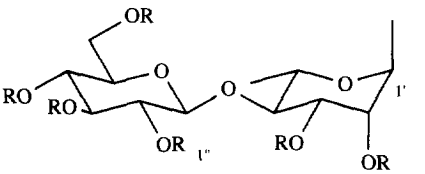
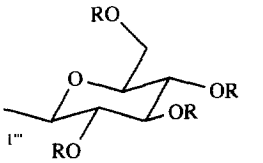
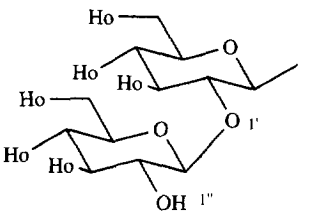
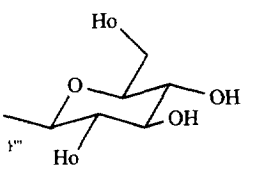
3	H		
3a	Ac		
4			

Table 1. FAB-mass spectral data for compounds **1–4**

m/z	**1**	*m/z*	**2** and **3**	*m/z*	**4**
817	$[M+Na]^+$	979	$[M+Na]^+$	995	$[M+Na]^+$
795	$[M+1]^+$	957	$[M+1]^+$	973	$[M+1]^+$
617	$[(M+1)-178]^+$	817	$[(M+Na)-162]^+$	951	$[(M+Na)-44]^+$
487	$[(M+1)-162-146]^+$	795	$[(M+1)-162]^+$	811	$[(M+1)-162]^+$
		779	$[(M+1)-178]^+$	789	$[(M+Na-44)-162]^+$
		633	$[(M+1)-2\times162]^+$	773	$[(M+Na-44)-178]^+$
		617	$[(M+1)-162-178]^+$	649	$[(M+1)-2\times162]^+$
		487	$[(M+1)-2\times162-146]^+$	605	$[(M+1-44)-2\times162]^+$
				589	$[(M+1-44)-162-178]^+$
				487	$[(M+1)-3\times162]^+$

Table 2. ^{13}C NMR spectral data for the aglycones of compounds **1–4**

C	1	2	3	4	*	DEPT
1	39.1	38.9	39.1	39.2	39.4	CH_2
2	26.9	26.6	26.5	26.9	26.5	CH_2
3	88.7	88.4	88.4	89.0	78.1	CH
4	39.5	40.0	39.3	39.6	39.4	C
5	56.1	55.7	55.7	56.3	55.9	CH
6	18.8	18.4	18.9	18.8	19.0	CH_2
7	37.7	37.3	37.2	37.7	37.7	CH_2
8	40.2	40.0	40.4	40.3	40.2	C
9	47.4	47.1	47.4	47.3	47.4	CH
10	37.2	36.8	37.2	36.7	37.5	C
11	23.5	23.2	23.7	23.6	23.5	CH_2
12	129.2	129.4	129.7	129.5	129.1	CH
13	134.3	133.1	133.6	133.7	134.2	C
14	57.0	56.6	57.0	57.1	56.9	C
15	26.5	26.0	26.2	26.4	28.3	CH_2
16	25.7	25.4	25.8	25.8	25.6	CH_2
17	48.9	48.8	49.2	49.2	48.9	C
18	55.1	54.5	54.9	54.9	55.1	CH
19	39.3	39.3	39.3	39.3	39.5	CH
20	37.9	37.3	37.7	37.8	37.8	CH
21	30.8	30.1	30.4	30.5	30.7	CH_2
22	37.2	36.3	36.7	37.1	37.2	CH_2
23	28.2	27.8	28.3	28.2	28.7	Me
24	17.2	16.9	17.0	17.1	16.8	Me
25	16.7	16.5	16.8	16.8	16.8	Me
26	19.1	19.1	19.4	19.4	18.4	Me
27	178.2	177.9	178.4	178.5	178.2	C
28	180.3	176.3	176.8	176.7	180.2	C
29	18.4	18.0	18.6	18.4	19.1	Me
30	21.4	21.1	21.5	21.4	21.7	Me

* Literature data of quinovic acid from [4].

respectively) suggested the identity of the aglycone moiety as quinovic acid. A comparison of the ^{13}C NMR spectrum of **1–4** with the literature data of quinovic acid [4] revealed that the glycosidation site of **1** was C-3 (Δ^{δ} = 10.6 ppm), and the sites of glycosidation of **2–4** were C-3 and C-28.

The ^1H NMR spectrum of compound **1** displayed a proton anomeric signal at δ5.26 (*d*, *J* = 7.8 Hz), and its attached carbon signal was located at δ107.1 in the ^{13}C NMR spectrum. Another proton anomeric signal of a fucose was overlapped. Thus the peracetylation of **1** was carried out leading to **1a**. The two proton anomeric signals were revealed at δ4.40 (*d*, *J* = 7.9 Hz) and 4.50 (*d*, *J* = 7.9 Hz). The *J* values of the anomeric protons indicated that the sugar moieties had a β-configuration. The position of attachment of the sugar chain to the aglycone was revealed by the NOESY correlations of **1a** between H-1′ (δ4.40) and H-3 (δ3.00), H-1″ (δ4.50) and H-4′ (δ3.82), as 3-*O*-β-D-glucopyranosyl(1→4)-β-D-fucopyranoside. Thus, saponin **1** was concluded to be quinovic acid 3-*O*-β-D-glucopyranosyl(1→4)-β-D-fucopyranoside.

Basic hydrolysis of **2** yielded **1** identified by TLC. The ^1H and ^{13}C NMR spectrum displayed three anomeric signals at δ4.63 (overlap), 5.25 (*d*, *J* = 7.8 Hz), 6.44 (*d*, *J* = 8.0 Hz) and δ106.8, 106.8, 95.5, respectively. The anomeric signals of one glucose appearing at δ6.44 (*J* = 8.0 Hz) in its ^1H NMR spectrum and δ95.5 in the ^{13}C NMR spectrum indicated that the glucose moiety gave a β-configuration and was attached to the 28-carboxyl group of the aglycone. Since the anomeric proton signal of fucose was overlapped, **2a** was obtained by peracetylating **2**. The NOESY cross peaks of **2a** between H-1′ (δ4.52) and H-3 (δ2.95), H-1″ (δ5.02) and H-4′ (δ4.13), revealed that the sugar moiety attachments were 3-*O*-β-D-glucopyranosyl(1→4)-β-D-fucopyranosyl. Therefore, saponin **2** was identified as quinovic acid 3-*O*-β-D-glucopyranosyl(1→4)-β-D-fucopyranosyl-(28→1)-β-D-glucopyranosyl ester.

Basic hydrolysis of **3** yielded quinovic acid 3-*O*-β-D-glucopyranosyl(1→4)-α-L-rhamnopyranoside (**5**) [2] identified by TLC. Three anomeric signals of saponin **3** were shown at δ5.11 (*s*), suggesting the sugar had an α-configuration, 5.23 (*d*, *J* = 7.7 Hz), 6.40 (*d*, *J* = 8.0 Hz) in its ^1H NMR and δ104.0, 107.0, 95.5 in the ^{13}C NMR spectrum, respectively. The upfield

Table 3. ^1H NMR spectral data of compounds **1–4**

H	1	2	3	4
H-3	3.33 *dd* (4.2, 11.6)	3.25 *dd* (4.2, 11.5)	2.97 *dd* (4.2, 11.5)	3.10 *dd* (4.2, 11.5)
H-12	6.10 *s*	6.05 *s*	5.98 *s*	5.95 *s*
H-18	2.90 *d* (11.1)	2.77 *d* (11.3)	2.69 *d* (13.8)	2.67 *d* (11.0)
Me-23	1.19 *s*	1.22 *s*	0.69 *s*	1.09 *s*
Me-24	1.00 *s*	1.04 *s*	0.90 *s*	1.04 *s*
Me-25	0.97 *s*	1.00 *s*	0.71 *s*	0.86 *s*
Me-26	1.25 *s*	1.32 *s*	1.25 *s*	1.20 *s*
Me-29	1.30 *d* (5.8)	1.25 *d* (6.0)	1.15 *d* (6.0)	1.18 *d* (5.5)
Me-30	0.90 *d* (6.1)	0.85 *d* (6.5)	0.70 *br*, *s*	0.75 *d* (5.7)
H-1′	4.60 overlap	4.63 overlap	5.11 *s*	4.74 *d* (7.4)
H-1″	5.26 *d* (7.8)	5.25 *d* (7.8)	5.23 *d* (7.7)	5.30 *d* (7.5)
H-1‴		6.44 *d* (8.0)	6.40 *d* (8.0)	6.36 *d* (7.9)
Me-6′	1.70 *d* (6.0)	1.70 *d* (5.9)	1.70 *d* (5.9)	

shift of one glucose anomeric signal (C-1‴, δ95.5 in ^{13}C NMR) indicated the esterifying unit of C-28 aglycone. Compared with methyl-α-L-rhamnopyranoside [5], the C-4′ signal of the rhamnose unit was shifted to lowfield. Thus, another glucose unit was linked to the 4′-hydroxy of the rhamnose. All these attachments were confirmed by the NOESY correlations of **3** and **3a** between H-1′ and H-3, H-1″ and H-4′. The structure of **3** was quinovic acid 3-*O*-β-D-glucopyranosyl(1 → 4)-α-L-rhamnopyranosyl-(28 → 1)-β-D-glucopyranosyl ester.

In the ^1H NMR spectrum of saponin **4**, three proton anomeric signals of glucose were displayed at δ4.74 (*d*, *J* = 7.4 Hz), 5.30 (*d*, *J* = 7.5 Hz), 6.36 (*d*, *J* = 7.9 Hz). Comparison of the ^{13}C NMR signals of the three glucose units, showed an upfield shift of C-1‴ (δ95.8), corresponding to the ester-linked anomeric carbon; and a downfield shift of C-2′ (at δ83.5), suggesting the glycosidation site of its glucose unit. The above conclusion was confirmed by the NOESY cross peaks between H-1′ (δ4.74) and H-3 (δ3.10), H-1″ (δ5.30) and H-2′ (δ4.15). Therefore, saponin **4** was elucidated to be quinovic acid 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-(28→1)-β-D-glycopyranosyl ester.

Table 4. ^{13}C NMR spectral data of the sugar moieties for compounds **1–4**

C	1	2	3	4	DEPT
3-*O*-fuc			3-*O*-rha	3-*O*-glc	
1′	107.1	106.8	104.0	105.1	CH
2′	73.5	73.2	72.9	83.5	CH
3′	75.8	75.4	72.1	79.4	CH
4′	83.2	83.2	85.5	71.8	CH
5′	70.5	70.1	68.3	78.3	CH
6′	17.8	17.5	18.4	63.0[a]	CH_3/CH_2
3-*O*-glc					
1″	107.1	106.8	107.0	106.1	CH
2″	76.0	74.0	74.4	77.2	CH
3″	78.8	79.1	79.5	79.1	CH
4″	71.7	71.3	71.7	71.8	CH
5″	78.8	78.4	78.8	78.0	CH
6″	63.0	62.6	62.9[a]	62.9[a]	CH_2
28-*O*-glu					
1‴		95.5	95.9	95.8	CH
2‴		76.0	76.7	74.3	CH
3‴		78.7	79.1	78.5	CH
4‴		71.0	71.4	71.4	CH
5‴		78.4	78.7	78.0	CH
6‴		62.1	62.6[a]	62.6[a]	CH_2

[a] Assignments may be interchanged in each column.

EXPERIMENTAL

General. Mps are uncorr. The NMR, NOESY spectra of **1–4** were recorded on a Bruker AM-400 spectrometer, all with TMS as internal standard and pyridine-d_5 as solvent. The FAB-MS were obtained using glycerol as matrix on a MAT-95 double focusing mass spectrometer.

Plant materials. The roots of *A. rubella* were collected in Jiang-su, China and authenticated by vice-Professor Huang Xu-lan. A voucher specimen is deposited at Shanghai Institute of Materia Medica.

Extraction and isolation. The air-dried roots (5.0 kg) were extracted with EtOH and 228 g of extract was obtained, which was partitioned with petrol, Et_2O, $CHCl_3$, EtOAc and *n*-BuOH successively from a MeOH–H_2O soln. The *n*-BuOH fr. (92 g) was chromatographed on a silica gel column using EtOAc–MeOH as eluent. The frs, eluted with EtOAc–MeOH (9:1, fr. A) and EtOAc–MeOH (3:1, fr. B), were further chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (300:100:10) and on an ODS column with MeOH–H_2O (3:2) to obtain from fr. A compounds **1** (52 mg), **2** (273 mg) and **3** (40 mg) and from fr. B compound **4** (34 mg).

Compounds **1**, **2** and **3** were acetylated as usual with Ac_2O–pyridine to give **1a**, **2a** and **3a**, respectively.

Compound **1**. Powder, mp 230–232°; $[\alpha]_D^{27}$ + 18.44° (MeOH; *c* 0.003253). IR ν_{max}^{KBr} cm^{-1}: 3400 (*br*), 2940, 1730, 1690, 1074. FAB-MS: see Table 1. ^1H and ^{13}C NMR: see Tables 2–4.

Compound **1a**. ^1H NMR: H-1′ (δ4.40, *d*, *J* = 7.9 Hz), H-4′ (δ3.82, *d*, *J* = 2.6 Hz), H-1″ (δ4.50, *d*, *J* = 7.9 Hz), H-3 (δ3.00, *dd*, *J* = 4.2, 11.8 Hz).

Compound **2**. Powder, mp 225–230°; $[\alpha]_D^{20}$ + 32.61° (MeOH; *c* 0.006900). IR ν_{max}^{KBr} cm^{-1}: 3396, 2927, 1697, 1456, 1227, 1072. FAB-MS: see Table 1. ^1H and ^{13}C NMR: see Tables 2–4.

Compound **2a**. ^1H NMR: H-1′ (δ4.52, *d*, *J* = 7.9 Hz), H-3′ (δ5.33, *dd*, *J* = 2.5, 10.8 Hz), H-4′ (δ4.13, *d*, *J* = 2.1 Hz), H-1″ (δ5.02, *d*, *J* = 7.9 Hz), H-1‴ (δ6.32, *d*, *J* = 8.1 Hz), H-3 (δ2.95, *dd*, *J* = 4.2, 11.8 Hz).

Compound **3**. Powder, mp 232–235°; $[\alpha]_D^{24}$ + 6.280° (MeOH; *c* 0.003917). IR ν_{max}^{KBr} cm^{-1}: 3419, 2927, 1699, 1456, 1389, 1070. FAB-MS: see Table 1. ^1H and ^{13}C NMR: see Tables 2–4.

Compound **3a**. ^1H NMR: H-1′ (δ5.10, *s*), H-1″ (δ5.45, *d*, *J* = 7.9 Hz), H-1‴ (δ6.40, *d*, *J* = 8.1 Hz), H-3 (δ3.05, *dd*, *J* = 4.2, 11.5 Hz).

Compound **4**. Powder, mp 206–209°; $[\alpha]_D^{24}$ + 21.88° (MeOH; *c* 0.006400). IR ν_{max}^{KBr} cm^{-1}: 3400, 2900, 1726, 1550, 1454, 1225, 1074. FAB-MS: see Table 1. ^1H and ^{13}C NMR: see Tables 2–4.

Acid hydrolysis of **1–4**. Compounds **1–4** (5 mg of each) were submitted to acid hydrolysis in the usual manner [6]. The sugars were identified by comparison with authentic samples of L-rhamnose, D-glucose and D-fucose by PC.

Basic hydrolysis of **2** *and* **3**. Compounds **2** and **3** (5 mg, respectively) were submitted to alkaline hydrolysis in the usual manner [6]. Compound **2** yielded **1**, and compound **3** gave **5** [2] identified by TLC.

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