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TRITERPENOID GLYCOSIDES FROM *ADINA RUBELLA*

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Key Word Index—*Adina rubella*; Rubiaceae; saponins; glycosides; quinovic acid; rubelloside A and B.

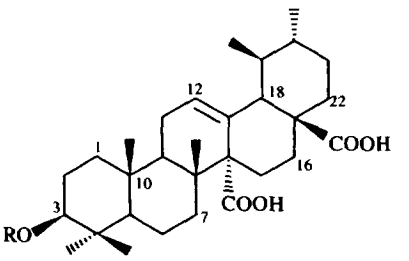
Abstract—Two new triterpenoid glycosides, quinovic acid-3β-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranoside and quinovic acid-3β-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (named rubelloside A and B, respectively), were isolated from roots of *Adina rubella*. Their structures were elucidated by spectral and chemical means. Rubelloside B exhibited immunological enhancement.

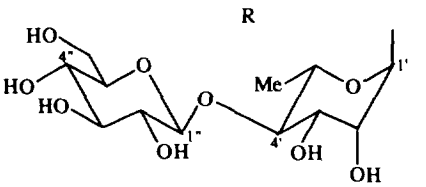
INTRODUCTION

Adina rubella Hance, a Chinese folk medicinal plant, has been used as an antibacterial agent and a cough medicine [1]. An ethanol extract was also reported to produce some restraint effect on some kinds of tumour [2], but the constituent of this has not been studied. Two new triterpenoid glycosides, quinovic acid-3β-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranoside (**1**) and quinovic acid-3β-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside (**2**), (rubelloside A and B, respectively), were obtained from the ethanol extract of roots of *A. rubella* and **2** exhibited immunological enhancement. This paper describes isolation and structure elucidation of **1** and **2**.

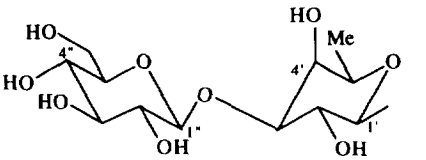
RESULTS AND DISCUSSION

Glycoside **1** was obtained as needles. The molecular formula was determined as $C_{42}H_{66}O_{14}$ by its ^{13}C NMR DEPT spectrum and FAB-mass spectral data (*m/z* 817 $[M + Na]^+$ and 795 $[M + 1]^+$). The IR spectrum of **1** indicated the presence of hydroxyl (ν_{max} 3400, 1060 cm^{-1}) and carboxyl (ν_{max} 1690 cm^{-1}) groups. The ^1H NMR spectrum showed resonances characteristic of glycosides of pentacyclic triterpene (Table 1). The ^{13}C NMR spectrum showed 42 signals (Table 2). A comparison of the ^{13}C NMR spectra of **1** and **3** [3] revealed that the aglycone of **1** was quinovic acid (**3**) and the glycosidation site was C-3 (Δ^δ = 10.3 ppm). Acid hydrolysis gave D-glucose and L-rhamnose, identified by paper chromatography and comparison with authentic samples. A careful analysis of the proton (Table 1) and carbon (Table 2) signals of the sugar moiety revealed an α-L-rhamnopyranose attached to the aglycone and a β-D-glucopyranose attached to the C-4′ of the rhamnose. The chemical shift values of the carbon signals of the rhamnose were consistent with those reported of the same linkage pattern rhamnose (δ104.2, 71.6, 72.7, 84.5, 68.1, 18.4) [4]. On irradiation of H-1″ (δ5.25), NOE was observed at H-4′ or one proton of H-6″ (δ4.38) (4.72%), but





1 R



2

3 H

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Table 1. ^{1}H NMR spectral data for **1**, **2**, and **4**

Position	1	2	4
3	2.98 *dd* (11.7, 4.4)	3.15 *dd* (11.7, 4.3)	3.00 *dd* (11.1, 4.7)
12	6.00 *m*	6.00 *m*	5.67 *m*
18	2.79 *d* (11.3)	2.81 *d* (11.4)	1.21 *d* (10.1)
23	1.10 *s**	1.12 *s*	0.88 *s**
24	0.78 *s**	0.94 *s**	0.87 *s**
25	0.72 *s**	0.90 *s**	0.82 *overlap**
26	0.90 *s**	1.09 *s*	0.82 *overlap**
29	1.22 *d* (5.8)†	1.22 *d* (6.0)	0.82 *overlap**
30	0.80 *d* (6.0)†	0.80 *d* (6.3)	0.38 *s**
1′	5.10 *brs*	4.55 *d* (7.6)	4.37 *d* (7.8)
2′	4.49 *brs*	4.28 *t* (7.6)	5.01 *overlap*
3′	4.51 *dd* (9.1, 2.6)	4.08 *overlap*	4.85 *dd* (10.4, 2.4)
4′	4.38 *overlap*	4.05 *overlap*	3.81 *d* (2.4)
5′	4.20 *overlap*	3.78 *m*	3.58 *overlap*
6′	1.22 *d* (5.8)	1.62 *d* (6.3)	1.21 *d* (5.9)
1″	5.25 *d* (7.6)	5.17 *d* (7.8)	4.46 *d* (8.0)
2″	4.12 *t* (7.6)	4.05 *overlap*	4.98 *overlap*
3″	4.20 *overlap*	4.21 *overlap*	5.20 *t* (9.5)
4″	4.28 *t* (8.8)	4.09 *overlap*	5.01 *overlap*
5″	3.80 *m*	3.89 *m*	3.58 *overlap*
6″	4.38 *overlap*	4.51 *overlap*	4.19 *dd* (11.9, 1.9)
	4.25 *overlap*	4.38 *m*	4.05 *m*
–OAc			2.13 (*s*), 2.10 (*s*), 2.05 (*s*), 2.00 (*s*), 1.99 (*s*), 1.98 (*s*)

Coupling constants (*J* in Hz) are given in parentheses; the assignments were based on ^{1}H-COSY (**1**, **2**, **4**) and ^{13}C-^{1}H COSY (**2**).

*†Assignments may be interchanged within each column.

the latter was less likely. These further confirmed the linkage pattern of the sugar moiety. From these considerations, the structure of **1** was determined as quinovic acid-3β-*O*-β-D-glucopyranosyl-(1 → 4)-α-L-rhamnopyranoside.

Glycoside **2** was obtained as a white powder. Its molecular formula was also determined as $C_{42}H_{66}O_{14}$ by the ^{13}C NMR DEPT spectrum and FAB-mass spectral data (*m/z* 817 $[M + Na]^+$ and 795 $[M + 1]^+$). Its IR spectrum showed absorption bands due to hydroxyl ν_{max} 3400, 1070 cm^{-1}) and carboxyl (ν_{max} 1690 cm^{-1}) groups. A comparison of the ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra of **2** and **1** showed identical aglycones and glycosidation sites. Acid hydrolysis gave D-glucose and D-fucose. After the assignment of the proton and carbon signals of the sugar moiety by ^{1}H and ^{13}C–^{1}H COSY, we concluded that a β-D-fucopyranose was attached to the aglycone and a β-D-glucopyranose was attached to C-3′ of the fucose by analysing the coupling constants of the proton signals (Table 1) and the chemical shift values of the carbon signals (Table 2). The cross peaks between H-1″, H-3″, H-5″, between H-2″, H-4″, between H-1′, H-3′, H-5′, between H-1′, H-3 and between H-1″, H-3′ were observed in the NOESY. After assignment of the proton signals of the sugar moiety of the peracetylate of **2** (**4**) by ^{1}H-COSY, we analysed the coupling constants of its proton signals (Table 1) and the constituents of the sugars were further confirmed. On irradiation of the signal of H-1″ of **4**, NOE was observed at H-3′, confirming the linkage pattern of the two sugars. Considering the above results, **2** was established to be quinovic acid-3β-*O*-β-D-glucopyranosyl-(1 → 3)-β-D-fucopyranoside.

EXPERIMENTAL

General. mps: uncorr. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-400 and AM-300 spectrometers, respectively, with TMS as internal standard, pyridine-d_5 as solvent.

Extraction and isolation. The roots of *A. rubella* were collected in Jiang-su, China. A voucher specimen is deposited at our institute. The air-dried roots (5.0 kg) were extracted with EtOH to yield 228 g extract, which was subjected to partition with petrol, Et_2O, $CHCl_3$, EtOAc and *n*-BuOH successively from a MeOH-H_2O soln. The Et_2O fr. (107 g) was chromatographed on a silica gel column using MeOH-$CHCl_3$ as eluent. The frs eluted with $CHCl_3$-MeOH (1 : 1) were further chromatographed on a silica gel column eluted with EtOAc and gave **1** (84 mg) and **2** (62 mg) consecutively.

Rubelloside A (**1**). Needles (MeOH–H_2O), mp 246–247°. $[\alpha]_D^{17}$ + 16.2° (MeOH; *c* 0.222). IR ν_{max} cm^{-1}: 3400, 2930, 1690, 1550, 1450, 1390, 1310, 1220, 1060, 980. FAB-MS *m/z*: 817 $[M + Na]^+$, 795 $[M + 1]^+$, ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2.

Rubelloside B (**2**). Powder, mp 252–253°, $[\alpha]_D^{17}$ + 36.2° (MeOH; *c* 0.423). IR ν_{max} cm^{-1}: 3400, 2940, 1690, 1650,

Table 2. ^{13}C NMR spectral data for compounds **1**, **2**, and **3***

Position	1	2	3	DEPT
1	38.8	39.3	39.1	CH_2
2	25.7	26.4	26.2	CH_2
3	88.1	88.6	77.8	CH
4	38.7	39.1	39.1	C
5	55.3	55.9	55.5	CH
6	18.1	18.6	18.7	CH_2
7	37.2	37.5	37.4	CH_2
8	39.8	40.0	39.8	C
9	47.0	47.2	47.1	CH
10	36.9	37.1	37.1	C
11	23.2	23.3	23.2	CH_2
12	128.7	128.8	128.8	CH
13	133.9	134.2	133.9	C
14	56.6	56.9	56.6	C
15	26.2	26.7	27.9	CH_2
16	25.3	25.5	25.3	CH_2
17	48.5	48.7	48.5	C
18	54.8	55.0	54.7	CH
19	37.5	37.7	37.5	CH
20	39.2	39.4	39.2	CH
21	30.4	30.6	30.4	CH_2
22	36.8	37.0	36.9	CH_2
23	27.9	28.0	28.4	Me
24	16.3	16.5	16.5	Me
25	16.6	16.9	16.5	Me
26	18.7	18.8	18.7	Me
27	177.8	178.0	177.8	C
28	180.1	180.2	179.9	C
29	18.1	18.1	18.1	Me
30	21.1	21.3	21.2	Me
1′	103.4	106.8		CH
2′	71.4	73.3		CH
3′	71.7	83.2		CH
4′	84.7	75.5		CH
5′	67.9	70.2		CH
6′	18.1	17.5		Me
1″	106.3	106.8		CH
2″	76.2	76.0		CH
3″	78.3	78.4		CH
4″	72.4	71.6		CH
5″	78.1	78.4		CH
6″	62.5	62.8		CH_2

*Data from [3].

1550, 1450, 1390, 1070. FAB-MS *m/z*: 817 $[M + Na]^+$ 795 $[M + 1]^+$. ^1H NMR: see Table 1. ^{13}C NMR: see Table 2. Compound **2** was acetylated with Ac_2O-pyridine at room temp. and worked up in the usual manner to afford **4** (mp 282–284°. ^1H NMR: see Table 1).

Acid hydrolysis of **1** *and* **2**. Compounds **1** and **2** (10 mg, respectively) were submitted to acid hydrolysis in the usual way. The quinovic acid and sugars were identified with authentic samples by TLC and PC, respectively.

Immunity test. These tests were made on the T lymphocyte transformation model, the concentration was 0.01 $\mu g\,ml^{-1}$, and only **2** exhibited an immunological enhancement within and outside the body.

REFERENCES

1. (1985) in *Dictionary of Chinese Medicine* pp. 530, 546. Jiangsu, Jiangsu New Medical Institute.
2. Shanghai Anti Tumor Research Institute (1977) in *Handbook of Practical Anti Tumor Drugs* p. 10. Institute of Blood Transfusion and Hematology, Chinese Academy of Medical Sciences (1972) in *Dev. Hem. Res.* **5**, 4.
3. Ghulam, A. Miana, and Hassan, M. G. Al-Hazimi, (1987) *Phytochemistry* **26**, 225.
4. Higuchi, R., Tokimitsu, Y., Fujioka, T., Komori, T., Kawasaki, T. and Oakenful, D. G. (1987) *Phytochemistry* **26**, 229.