Triterpenoids from *Adina rubella*

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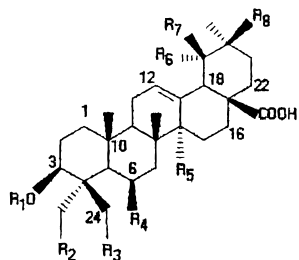
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Six new triterpenoids, $3\beta,23,24$-trihydroxyolean-12-en-28-oic acid (**1**), $3\beta,6\beta,24$-trihydroxyolean-12-en-28-oic acid (**2**), $3\beta,6\beta,19\alpha,24$-tetrahydroxyurs-12-en-28-oic acid (**3**), cincholic acid 3β-*O*-β-D-fucopyranoside (**4**), pyrocincholic acid 3β,-*O*-β-D-fucopyranoside (**5**), and pyrocincholic acid 3β-*O*-α-L-rhamnopyranoside (**6**) were isolated from the roots of *Adina rubella*. Their structures were elucidated by spectral analysis. The ^{13}C NMR signals of **5** and **6** were assigned by 2D-NMR experiments.

Adina rubella Hance (Rubiaceae), a Chinese folk medicinal plant, has been used as an antibacterial agent and a cough medicine[1] but its constituents have not yet been studied. In our investigation, six new triterpenoids $3\beta,23,24$-trihydroxyolean-12-en-28-oic acid (**1**), $3\beta,6\beta,$-24-trihydroxyolean-12-en-28-oic acid (**2**), $3\beta,6\beta,19\alpha,24$-tetrahydroxyurs-12-en-28-oic acid (**3**), cincholic acid 3β-*O*-β-D-fucopyranoside (**4**), pyrocincholic acid 3β-*O*-β-D-fucopyranoside (**5**), and pyrocincholic acid 3β-*O*-α-L-rhamnopyranoside (**6**), were isolated from the ethanol extract of the roots. This paper describes their isolation and structure elucidation.

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
1	H	OH	OH	H	Me	H	H	Me
2	H	H	OH	Me	Me	H	H	Me
3	H	H	OH	OH	Me	OH	Me	H
4	β -D-fucosyl	H	H	H	COOH	H	H	Me
7	H	OH	H	H	Me	H	H	Me
8	H	OH	H	H	Me	OH	Me	H
9	H	H	OH	H	Me	OH	Me	H
10	H	H	H	H	COOH	H	H	Me

Compound **1** was obtained as colorless needles. Its molecular formula was deduced as $C_{30}H_{48}O_5$ by ^{13}C NMR DEPT spectra (Table 2) and EIMS (m/z 488 [M]$^+$). Its ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra suggest it to be an oleanane-type triterpene with two hydroxymethyl groups. This was consistent with the information provided by its EIMS. Two characteristic peaks at m/z 240 and 248 denoted the retro-Diels−Alder cleavage fragments commonly observed for olean-12-ene or urs-12-ene derivatives possessing three hydroxyl groups in rings A/B and a carboxyl group in rings D/E. Two ions at m/z 222 and 203 indicated further loss of water and COOH from m/z 240 and 248, respectively. The ^{13}C NMR spectrum of **1** and **7**[2] were very similar except for

the substitution mode of the A ring (Table 1). On going from **7** to **1**, the signals due to C-4 and C-24 are displaced downfield by 4.2 and 50.3 ppm, respectively, and that due to C-23 was shifted upfield by 4.8 ppm, while other carbon signals remained at almost unchanged positions. These data revealed that the additional hydroxyl group of **1** must be located at C-24. In the NOESY spectrum H-23 showed cross peaks with H-3, and H-24 had cross peaks with H-25. From these results, the structure of **1** was established as $3\beta,23,24$-trihydroxyolean-12-en-28-oic acid.

Compound **2** was obtained as a white powder. The molecular formula was deduced as $C_{30}H_{48}O_5$ by ^{13}C NMR DEPT spectra (Table 2) and EIMS (m/z 488 [M]$^+$). The ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra and EIMS indicated it to be an oleanane-type triterpene with one hydroxymethyl group and two secondary alcoholic groups on rings A/B. But in its EIMS, the peak of the rings A/B possessing three alcoholic groups corresponding to the retro-Diels−Alder cleavage at m/z 240 was not observed. However, there was a peak at m/z 222, indicating the loss of one molecule of water. The ^{13}C NMR signals of rings C/D/E of **2** and **7**[2] were similar, indicating the substitution mode of the rings was identical. In the ^{1}H−^{1}H COSY spectrum, H-6 displayed cross peaks with H-5α, H-7β, and H-7α, thus indicating that the additional secondary alcoholic group on rings A/B was located at C-6. The configuration of the respective group was suggested by the shape of the proton signal of H-6 (δ 5.06, brs) in the ^{1}H NMR spectrum. In the NOESY spectrum H-6 showed a cross peak with H-23, and H-23 itself showed an NOE with H-3, while there were no cross peaks between H-6 and H-25,26. This confirmed the above suggestion. In most cases, the hydroxymethyl group of rings A/B was located at C-4 rather than C-8 and C-10. In the NOESY spectrum, H-24 showed cross peaks with H-25 and H-23, whereas H-23 revealed cross peaks with H-3 and H-6. These suggested a β-configuration of the hydroxymethyl group at C-4, which was confirmed by the *W*-type long-range coupling between the H-3α signal and one of the 4β-hydroxymethyl signals (δ 4.41, brd, $J = 10.3$ Hz) in the ^{1}H NMR spectrum. These data indicated that the structure of **2** should be $3\beta,6\beta,24$-trihydroxyolean-12-en-28-oic acid.

Table 1. ^{1}H NMR Spectral Data for **1−6**

H	1	2	3	4	5	6
3	4.40 dd (4.2, 11.5)^a	4.29 brdd (4.0, 11.4)	4.26 dd (3.6, 11.2)	3.44 dd (4.3, 14.2)	3.39 dd (4.1, 11.6)	3.19 dd (3.8, 11.6)
6		5.06 brs	5.03 brs			
12	5.47 m	5.59 m	5.67 m	6.03 m		
18	3.27 dd (3.4, 9.7)	3.34 dd (13.4, 2.8)	3.11 brs	3.17 dd (3.4, 11.2)	2.86 dd (3.1, 11.1)	2.85 dd (2.8, 11.7)
23	4.86 d (10.8)	1.73 d (2.4)	1.71 s	1.15 s^b	1.31 s	1.00 s
	4.23 d (10.8)					
24	4.63 d (11.2)	4.41 brd (10.3)	4.38 d (10.3)	1.08 s^b	0.94 s	0.78 s
	3.96 d (11.2)	4.05 dd (2.4, 10.3)	4.03 d (10.3)			
25	0.98 s	1.67 s	1.70 s	0.99 s^b	0.79 s	0.75 s
26	1.23 s^b	1.63 s	1.67 s	0.92 s^b	0.98 s	0.95 s
27	1.03 s^b	1.25 s	1.67 s			
29	0.98 s^b	0.91 s	1.46 s	0.90 s^b	0.95 s	0.91 s
30	0.91 s^b	0.99 s	1.10 d (6.0)	0.77 s^b	1.00 s	0.99 s
1′				4.57 d (7.7)	4.79 d (7.3)	5.35 brs
2′				4.29 t (7.7)	4.36 t (7.3)	4.59 brd (3.3)
3′				4.06 overlapped	4.08 overlapped	4.50 dd (3.3, 8.5)
4′				4.06 overlapped	4.08 overlapped	4.32 overlapped
5′				3.81 brd (6.2)	3.84 brd (5.9)	4.32 overlapped
6′				1.53 d (6.2)	1.53 d (5.9)	1.52 d (5.5)

^{a} Coupling constants (J in Hz) are given in parentheses; the assignments were based on ^{1}H−^{1}H COSY (**2−6**), HMQC (**2, 3, 5,** and **6**) and NOESY (**1−3**). ^{b} Assignments may be interchanged.

Table 2. ^{13}C NMR Spectral Data for **1−10**

C	1	2	3	4	5	6	7[2]	8[3]	9[3]	10[4]	DEPT
1	38.6	40.1	41.3	39.3	38.5	38.2	38.5	38.9	38.8	39.2	CH$_2$
2	28.0	27.3	28.1	25.2	26.8	26.0	27.4	27.7	28.5	25.3	CH$_2$
3	74.1	72.6	73.9	88.2	88.9	88.8	73.3	73.7	80.3	77.8	CH
4	46.8	43.4	44.0	39.7^a	39.6	39.3	42.6	42.9	43.2	39.1^a	C
5	48.3	48.7	49.9	55.7	55.8	55.4	48.5	48.8	56.5	55.7	CH
6	19.0	66.9 (CH)	68.0 (CH)	18.4	18.7	18.8	18.3	18.9	19.3	18.7	CH$_2$
7	33.0	40.4	41.6	37.2	39.6	39.5	32.7	33.4	34.0	37.4	CH$_2$
8	39.6	38.5	39.9	39.3^a	37.9	37.9	39.5	40.4	40.4	39.8^a	C
9	48.1	48.0	48.5	47.3	56.5	56.3	47.9	47.9	47.9	47.5	CH
10	36.8	36.3	37.1	36.9	37.2	37.2	37.0	37.3	37.2	37.3	C
11	23.5	23.2	24.4	23.3	18.1	18.1	23.5	24.1	24.3	23.4	CH$_2$
12	122.3	122.3	128.5	125.8	32.2 (CH$_2$)	32.1 (CH$_2$)	122.2	128.1	127.9	125.9	CH
13	144.6	143.5	139.5	138.0	130.7	130.7	144.6	140.0	140.0	138.1	C
14	41.8	42.0	42.5	56.4	136.9	136.8	42.0	42.2	42.1	56.5	C
15	28.1	27.6	29.4	26.6	21.2	21.2	28.1	29.4	29.1	28.1	CH$_2$
16	23.9	23.1	26.7	24.8	24.1	24.5	23.5	26.5	26.5	24.9	CH$_2$
17	46.3	46.0	48.5	47.6	45.2	45.2	46.2	48.3	48.4	47.7	C
18	41.9	41.4	54.9	44.1	39.8	39.8	41.8	54.7	54.7	44.2	CH
19	46.5	45.8	73.0 (C)	43.9	41.7	41.7	46.4	72.7 (C)	72.8 (C)	44.0	CH$_2$
20	30.8	30.3	42.8 (CH)	30.8	30.8	30.8	30.7	42.4 (CH)	42.4 (CH)	30.9	C
21	34.0	33.5	27.1	33.9	34.6	34.6	34.0	27.0	27.0	34.0	CH$_2$
22	33.2	32.6	38.6	32.7	31.7	31.7	33.0	38.5	38.6	32.8	CH$_2$
23	63.1	14.1 (Me)	14.6 (Me)	27.9 (Me)	28.2 (Me)	28.2 (Me)	67.9	68.2	23.7 (Me)	28.5 (Me)	CH$_2$
24	63.1	66.4	68.0	16.9^b (Me)	16.6 (Me)	16.5 (Me)	12.8 (Me)	13.1 (Me)	64.6	16.5^b (Me)	CH$_2$
25	15.7	16.8	17.6	16.4^b	16.6	16.5	15.7	17.3	17.2	16.4^b	Me
26	17.1	17.9	18.5	18.6	20.8	20.8	17.2	16.8	16.8	18.7	Me
27	25.9	25.6	24.8	178.4 (C)			25.9	24.9	24.7	178.5 (C)	Me
28	180.0	179.5	180.9	180.1	180.2	180.2	179.9	180.7	180.8	180.2	C
29	33.0	32.6	27.3	33.0	32.5	32.5	33.0	27.2	27.2	33.1	Me
30	23.6	23.1	16.8	23.6	25.1	25.1	23.6	16.0	16.1	23.7	Me
1′				107.0	107.3	104.5					CH
2′				72.6	72.5	72.5					CH
3′				75.3	75.5	73.0					CH
4′				72.5	72.8	74.1					CH
5′				71.0	71.2	69.9					CH
6′				17.3	17.5	18.5					Me

^{a,b} Assignments may be interchanged.

Isolate **3**, a white powder, molecular formula $C_{30}H_{48}O_6$, was also deduced by ^{13}C NMR DEPT spectra (Table 2) and EIMS (m/z 504 [M]$^+$). The structure of an urs-12-ene derivative possessing three alcoholic groups on rings A/B and one alcoholic group and one carboxyl group on rings D/E was revealed by its ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra and EIMS. As with **2**, an ion at m/z 222, but not at m/z 240, was observed in the EIMS. The carbon signals of rings A/B of **2** and **3** were very similar, indicating the structures of rings A/B were identical, as confirmed by ^{1}H−^{1}H COSY and NOESY

spectra. In the ^{1}H NMR spectrum, the H-18 signal was a broad single peak, suggesting that one hydroxyl and one methyl groups were positioned at C-19. This substitution mode was confirmed by the fact that the carbon signals of rings D/E of **3** and **8**[3] were similar. From these results, the structure of **3** was established as $3\beta,6\beta,19\alpha,24$-tetrahydroxyurs-12-en-28-oic acid.

When the ^{13}C NMR signals of the rings A/B of **2** and **3** were compared to those of **7**,[2] **8**,[3] and **9**,[3] it seemed that the configurations of the hydroxymethyl group at C-4 of **2** and **3** should be α rather than β (see Table 2),

which was contradictory to the spectral evidence. In order to confirm the structures of these two compounds, we measured the NOE difference spectra of **2** after carrying out the assignment of ^{1}H NMR spectrum by the ^{1}H−^{13}C COSY spectrum and an HMBC experiment (see Table 3). On irradiation of H-24 (δ 4.41, brd), NOE enhancement was observed at H-23 (5.63%) and H-25 (5.0%), and NOE enhancement was also observed at H-23 (3.5%) on irradiation of H-3. Thus, the discrepancy probably resulted from the introduction of a hydroxyl group at C-6.

Compound **4** was obtained as a white powder. Its molecular formula, $C_{36}H_{56}O_9$, was deduced by ^{13}C NMR DEPT spectra (Table 2) and FABMS (655 [M + Na], 633 [M + 1]). The structure of pentacyclic triterpenoid monoglycoside was revealed by ^{1}H (Table 1) and ^{13}C (Table 2) NMR spectra. In the ^{13}C NMR spectrum, carbon signals of the aglycon moiety of **4** were very similar to those of **10**[4] except that the C-3 signal of **4** differed from that of **10** by a deshielding of 10.4 ppm, which was a result from glycosidation. The signals of the sugar moiety of **4** were in agreement with the signals of the sugar moiety of 3β,16β,23,28-tetrahydroxyolean-9(11),12-diene 3β-O-β-D-fucopyranoside (δ 106.3 C-1′, 73.0 C-2′, 75.5 C-3′, 72.8 C-4′, 71.3 C-5′, 17.4 C-6′),[5] indicating that the sugar was β-D-fucopyranose. This was also consistent with the coupling constants of the proton signals in its ^{1}H NMR (Table 1). Thus, **4** should be cincholic acid 3β-O-β-D-fucopyranoside.

Obtained as a white powder, **5** had a molecular formula of $C_{35}H_{56}O_7$, as deduced by ^{13}C NMR DEPT spectra (Table 2) and FABMS (611 [M + Na], 589 [M + 1]). Its ^{1}H (Table 1) and ^{13}C NMR spectra showed that the compound was a nortriterpene glycoside containing one sugar moiety. No olefinic proton signal was observed in its ^{1}H NMR spectrum. The ^{13}C NMR spectrum revealed 29 carbon signals of the aglycon, including two quaternary olefinic carbons (δ 136.9, 130.7). A comparison of the ^{13}C NRM spectra of **5** and that of pyrocincholic acid 3β-O-β-6-deoxy-D-glucopyranosyl-(28→1)-β-D-glucopyranoside (**11**)[4]revealed their carbon

compound	R$_1$	R$_2$
5		H
6		H
11		
12	H	H
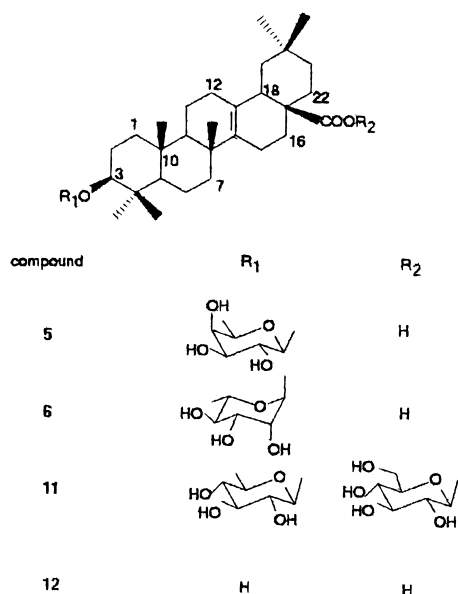
signals of the aglycon were almost identical except for the chemical shift of the carboxylic carbon of **5** which was 180.2 ppm compared to 176.5 ppm in **11**. This is

Table 3. Long-Range Connectivities Observed in the HMBC Experiment of **2**

C	H at C	C	H at C	C	H at C
3	23, 24	10	25	17	18
4	23, 24	11	12	18	12, 27
5	23, 24	12	18	19	18, 29, 30
7	26	13	18, 27	20	29, 30
8	26	14	18, 26, 27	23	3, 24
9	12, 25, 26	15	27	24	3, 23

resulted from the deglycosidation of the carboxylic group. This indicated that the aglycon of **5** was pyrocincholic acid (**12**) with a sugar moiety attached at C-3. The ^{1}H (Table 1) and ^{13}C (Table 2) NMR signals of the sugar moiety of **5** were very similar to those of **4** (Tables 1 and 2), indicating the sugar of **5** to be β-D-fucopyranose. All these considerations led us to assign the structure of pyrocincholic acid 3β-O-β-D-fucopyranoside to **5**.

Compound **6** was obtained as colorless needles. Its molecular formula, $C_{35}H_{56}O_7$, was deduced by ^{13}C NMR DEPT spectra (Table 2) and FABMS (611 [M + Na], 589 [M + 1]). The carbon signals of the aglycon of **6** were almost identical with those of **5**, and thus it was identified as pyrocincholic acid, with the site of the sugar attachment being at C-3. The carbon signals of the sugar moiety (Table 2) were in agreement with those of methyl rhamnopyranoside (102.1, 71.2, 71.5, 73.3, 69.5, 17.9).[6] The sugar of **6** therefore was α-L-rhamnopyranose, which was also indicated by the coupling constants of the protons of the sugar moiety (Table 1). The structure of **6** was thus shown to be pyrocincholic acid 3β-O-α-L-rhamnopyranoside. Compounds **4**, **5**, and **6** were subjected to acid hydrolysis, and the identities of the sugars were confirmed by PC comparison with authentic samples.

In order to assign the ^{13}C NMR signals of the aglycon of **5** and **6**, that is, pyrocincholic acid (**12**), we measured the ^{1}H−^{1}H COSY, TOCSY, HMQC, and HMBC spectra of these two compounds. Their structures were further confirmed.

Experimental Section

Apparatus. Melting points are uncorrected. The ^{1}H and ^{13}C NMR spectra of **1**, **2**, **3**, and **4** were recorded on Bruker AM-400 and AM-300 spectrometers, respectively; the other NMR spectra were recorded on a Bruker AMX-600 spectrometer, all with TMS as internal standard and pyridine-d_5 as solvent. L-Rhamnose was purchased from Shanghai Chemical Reagent Corp., and D-fucose was available in our laboratory.

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 the 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 petroleum ether, Et$_2$O, CHCl$_3$, EtOAc, and *n*-BuOH successively from a MeOH−H$_2$O solution. The ether fraction (107 g) was chromatographed on a silica gel column using MeOH−CHCl$_3$ as eluent. The fractions eluted with CHCl$_3$−MeOH (14:1, fraction A) and CHCl$_3$−MeOH (9:1, fraction B) were further chromatographed on a silica gel column with EtOAc to obtain from fraction A com-

pounds **1** (33 mg), **6** (77 mg), and **5** (73 mg) and from fraction B compounds **2** (16 mg), **4** (27 mg), and **3** (17 mg).

3β,23,24-trihydroxyolean-12-en-28-oic acid (**1**): mp 267−8 °C; colorless needles from MeOH−H$_2$O; [α]$^{17}_D$ +82.86° (c = 0.070, MeOH); IR ν max (KBr) cm^{-1} 3400 (br), 2940, 1690, 1460, 1385, 1240, 1030; EIMS 488 (M$^+$), 470, 452, 440, 422, 394, 339, 249, 248, 240, 222, 203, 191, 133; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

3β,6β,24-trihydroxyolean-12-en-28-oic acid (**2**): mp 283−5 °C; white powder; [α]$^{22}_D$ +32.60° (c = 0.077, MeOH); IR ν max (KBr) cm^{-1} 3460, 2940, 1700, 1460, 1357, 1300, 1270, 1230, 1200, 1050, 1030; EIMS 488 (M$^+$), 470, 452, 425, 409, 393, 249, 248, 222, 203, 189, 133; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

3β,6β,19α,24-tetrahydroxyurs-12-en-28-oic acid (**3**): mp 245−6 °C; white powder; [α]$^{17}_D$ −35.00° (c = 0.020, MeOH); IR ν max (KBr) cm^{-1} 3400, 2930, 1700, 1540, 1460, 1380, 1070, 1030; EIMS 504 (M$^+$), 486, 468, 458, 438, 386, 264, 246, 230, 222, 218, 201, 173, 146, 133; ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

Cincholic acid 3β-*O*-β-D-fucopyranoside (**4**): mp 204−5 °C; white powder; [α]$^{20}_D$ +25.34° (c = 0.174, MeOH); IR ν max (KBr) cm^{-1} 3450 (br), 2940, 1700, 1450, 1390, 1070 (br); FABMS 655 (M + Na), 633 (M + 1); ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

Pyrocincholic acid 3β-*O*-β-D-fucopyranoside (**5**): mp 190−1 °C; white powder; [α]$^{22}_D$ −31.56° (c = 0.352, MeOH); IR ν max (KBr) cm^{-1} 3400 (br), 2940, 1700, 1640, 1460, 1390, 1050 (br); FABMS 611 (M + Na), 589 (M + 1); ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

Pyrocincholic acid 3β-*O*-α-L-rhamnopyranoside (**6**): mp 280−2 °C; colorless needles from MeOH−H$_2$O; [α]$^{22}_D$ −58.32° (c = 0.445, MeOH); IR ν max (KBr) cm^{-1} 3400 (br), 2930, 1730, 1700, 1450, 1380, 1050; FABMS 611 (M + Na), 589 (M + 1); ^{1}H and ^{13}C NMR, see Tables 1 and 2, respectively.

Acid Hydrolysis of 4, 5, and 6. Isolates **4**, **5**, and **6** (10 mg, respectively) were submitted to acid hydrolysis in the usual manner, and the sugars were identified by comparison with authentic samples of L-rhamnose and D-fucose by TLC and PC.

References and Notes

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