

Semialactone, Isofouquierone Peroxide and Fouquierone, Three New Dammarane Triterpenes from *Rhus javanica*

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Three new dammarane triterpenes and semialactic acid were isolated from the stem bark of *Rhus javanica*. The structures of these triterpenes, named semialactone, isofouquierone peroxide and fouquierone, were elucidated by 2D-NMR analysis (HMQC, ^1H - ^1H COSY and HMBC), and the ^{13}C -NMR data of semialactic acid is revised.

Key words *Rhus javanica*; dammarane triterpene; semialactic acid; semialactone; fouquierone; isofouquierone peroxide

Rhus javanica L. (Anacardiaceae) is a tall, broad leaved tree that is distributed in Korea, Japan and China. Its barks and leaves are used as traditional remedies of dysentery and diarrhea in Korea.¹⁾ Recently, tannic acid production by cell cultures,²⁾ prophylactic activity related to the Herpes simplex virus type 1³⁾ and the antineoplastic effect of *R. javanica*⁴⁾ have been studied. However, the chemical components of this plant have not been fully investigated.

In the course of investigations into the constituents of *R. javanica*, three new dammarane triterpenes were isolated from the stem bark of this plant. Based upon various 2D-NMR techniques (HMQC, ^1H - ^1H COSY, HMBC and NOESY), the structures of three new triterpenes were elucidated as semialactone (**2**), isofouquierone peroxide (**3**) and fouquierone (**4**); in addition, semialactic acid (**1**) was also isolated for the first time. Herein, we report the structural assignments of three new triterpenes as well as the revised ^{13}C -NMR data for semialactic acid.

Results and Discussion

Semialactic acid (**1**) was isolated as a white powder, $[\alpha]_{\text{D}} +71^\circ$ ($c=0.40$, CHCl_3). The ^1H - and ^{13}C -NMR spectra of semialactic acid from *Rhus semialata* have been previously assigned,⁵⁾ however, revisions based upon 2D-NMR were found to be necessary to some of the ^{13}C -NMR assignments. In the ^1H - ^1H COSY, H-24 (δ 6.04) was correlated with H-23 (δ 2.16) and H-27 (δ 1.89); and H-21 (δ 4.88, 4.92) was correlated with H-17 (δ 2.64) and H-22 (δ 2.02, 2.16). In the HMBC spectrum, H-27 (δ 1.89) was correlated with C-24 (δ 145.65), C-25 (δ 126.47) and C-26 (δ 172.68); and H-21 (δ 4.88, 4.92) was correlated with C-17 (δ 43.66), C-20 (δ 151.31) and C-22 (δ 37.81) (Fig. 1). These results indicated that two quaternary carbons (δ 126.47, δ 151.31), a methine carbon (δ 43.66) and a methylene carbon (δ 37.81) were located at C-25, C-20, C-17 and C-22, respectively (Table 1).

Compound **2** was isolated as a white powder, $[\alpha]_{\text{D}} +73^\circ$ ($c=0.15$, CHCl_3). Its high resolution FAB-MS spectrum showed the $[\text{M}(\text{C}_{30}\text{H}_{45}\text{O}_4)+\text{H}]^+$ ion peak at m/z 469.3317 (requires: 469.3318). Its IR spectrum contained absorption bands for a hydroxyl group (3515 cm^{-1}) and a carbonyl group (1705 cm^{-1}). The ^1H - and ^{13}C -NMR spectra were very similar to those of compound **1**, except for the side chain relationships. That is, the protons of a methylene (δ 2.34, 2.53) and two methines (δ 4.75, 6.60) with a carbonyl group indicated the presence of an α,β -unsaturated- δ -lactone.⁶⁾ In the

DEPT spectrum, an oxy-methine carbon (δ 80.78) was found instead of the methylene (δ 37.81) of **1**, which also implied an ester linkage with a carboxyl (δ 165.99). In the HMBC spectrum, H-21 (δ 5.23, 5.28) was correlated with C-17 (δ 40.03) and C-22 (δ 80.78); and C-20 (δ 149.22) was correlated with H-13 (δ 2.18), H-16 (δ 1.70, 2.18), H-17 (δ 2.96) and H-22 (δ 4.75) (Fig. 1). The configuration at C-22 was deduced as *S*, based on the H-22 being positioned as an axial by its coupling constants (H-22: dd, $J=12.5, 3.5\text{ Hz}$) in the ^1H -NMR spectrum, and observation of the nuclear Overhauser effect spectroscopy (NOESY) between the following proton signals; δ 4.75 (H-22) and δ 2.96 (H-17); δ 2.53 (H-23b) and δ 1.70 (H-16a). Therefore, the structure of **2** was

Table 1. ^{13}C -NMR Chemical Shifts of Triterpenes from *Rhus javanica* (75 MHz, CDCl_3).

Carbon no.	1	2	3	4
1	29.56	30.06	39.89	39.88
2	35.52	35.55	34.12	34.11
3	98.41	98.13	218.19	218.20
4	35.46	35.47	47.44	47.44
5	49.89	49.33	55.35	55.34
6	19.81	19.83	19.65	19.64
7	33.01	33.08	34.53	34.52
8	39.58	39.67	40.29	40.27
9	45.38	45.36	49.98	49.95
10	40.50	40.44	36.84	36.82
11	23.09	23.11	22.00	22.00
12	25.29	25.29	27.50	27.49
13	45.00	44.99	42.58	42.46
14	49.44	49.91	50.28	50.31
15	32.97	33.11	31.11	31.13
16	28.09	29.49	24.88	24.78
17	43.66	40.03	50.14	50.03
18	15.37	15.41	15.20	15.19
19	68.00	67.99	16.03	16.03
20	151.31	149.22	75.08	75.25
21	109.59	113.41	25.78	25.28
22	37.81	80.78	43.38	36.16
23	28.65	29.01	127.10	25.07
24	145.65	139.13	137.48	89.69
25	126.47	128.37	82.06	143.72
26	172.68	165.99	24.14 ^{a)}	114.01
27	20.46	16.99	24.48 ^{a)}	17.62
28	26.75	26.76	26.72	26.70
29	18.50	18.45	21.02	21.02
30	16.32	16.54	16.33	16.40

* Assignments may be interchangeable.

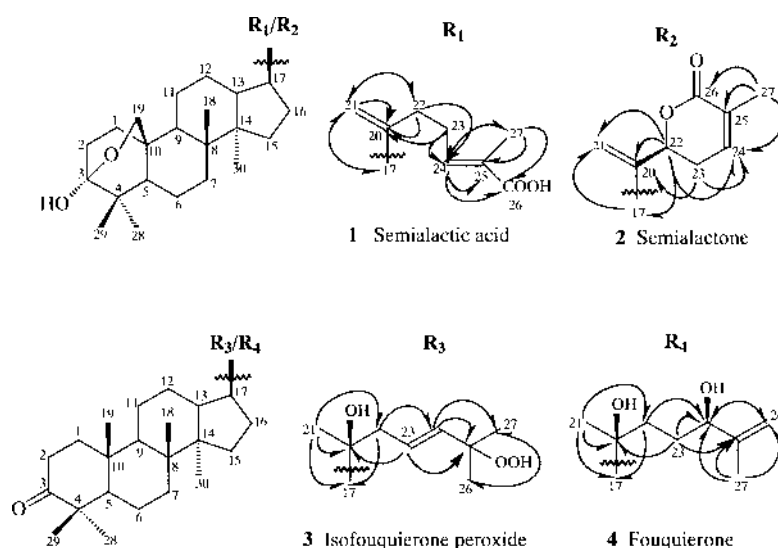


Fig. 1. HMBC Correlations of Triterpenes Isolated from *Rhus javanica*

proposed to be a 22*S*-lactone form of **1**, thus named an semialactone.

Compound **3** was isolated as a white powder, $[\alpha]_D^{25} +35^\circ$ ($c=0.29$, CHCl_3). Its high resolution FAB-MS spectrum showed the $[\text{M}(\text{C}_{30}\text{H}_{50}\text{O}_4)+\text{Na}]^+$ ion peak at m/z 497.3603 (requires: 469.3607). Its IR spectrum contained hydroxyl (3421 cm^{-1}) and carbonyl (1704 cm^{-1}) groups. The ^1H -, ^{13}C -NMR spectra and the specific rotation were typical of the isofouquierone from *Commiphora dalzielii*,⁷⁾ however, comparison of the ^{13}C -NMR signals of **3** with the data of isofouquierone revealed that C-25 (δ 82.06) was shifted downfield, and C-26 (δ 24.14) and C-27 (δ 24.48) were shifted upfield. (isofouquierone: C-25, δ 70.62; C-26, δ 29.83; C-27, δ 29.88). These results suggested that the quaternary carbinol of C-25 was peroxidized, which was in close agreement with the result of chemical shifts of 25-hydroperoxy- $4\alpha,14\alpha$ -dimethyl-cholesta-8,23-dien-3 β -ol from *Xanthosoma robustum*.⁸⁾ Thus, the structure of **3** was proposed to be an isofouquierone with a hydroperoxyl substitution, named an isofouquierone peroxide.

Compound **4** was isolated as a white powder, $[\alpha]_D^{25} +58^\circ$ ($c=0.16$, CHCl_3). The spectral characteristics of EI-MS, IR and NMR indicated that **4** was also a 3-oxodammarane. In the ^1H -NMR spectrum, singlets of methyl protons occurred at δ 0.88, 0.94, 0.98, 1.04, 1.08, 1.13 and 1.75 (each 3H, s), which typically represent the dammaran skeleton,⁷⁾ and a singlet of methylene protons at δ 5.01 (2H, s) suggested the presence of an exo-methylene moiety in the side chain. In the HMBC spectrum, the quaternary carbinol carbon at δ 75.25 (C-20) was correlated with H-22 (δ 1.48, 1.55) and H-23 (δ 1.63); and H-26 (δ 5.01) was correlated with C-25 (δ 143.72), C-24 (δ 89.69) and C-27 (δ 17.62) (Fig. 1). These results were in close agreement with the literature data on the side chain of fouquierol from *Fouquieria splendens*.⁹⁾ Thus, the structure of **4** was proposed to be dammar-25-ene-20,24-diol-3-one, named a fouquierone.

Experimental

Melting points were measured using an Electrothermal 9100 and are uncorrected. IR was recorded with a Magna 550. FAB-MS, HR-FAB-MS and

EI-MS spectra were recorded using a JEOL JMS-HX 110A, a JEOL JMS-HX 110A and a JEOL JMS-HX 110A-Hewlett-Packard 5889A spectrometer, respectively. The ^1H - (300 MHz), ^{13}C -NMR and DEPT (75 MHz) spectra were recorded on a Bruker DRX-300 NMR instrument and the chemical shifts are quoted with TMS as an internal standard. HMQC, ^1H - ^1H COSY, HMBC and NOESY data were recorded on a Bruker DMX-600 spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck No. 9385 or 7729). HPLC separation was carried out on a Macherey-Nagel column (ET 250/17/20 Nucleosil) with an ELS Detector (Alltech 500).

Plant Material Plant material was collected in Taejon, Korea during July 1998, and dried at room temperature. The voucher specimen is deposited in our laboratory as NDC-208.

Extraction and Isolation The ground stem bark (2 kg) was extracted three times with MeOH (20 l) to afford a crude extract (550 g), which was partitioned between *n*-hexane (10 l) and H_2O (10 l) to yield an *n*-hexane soluble fraction (116 g). The *n*-hexane extract (100 g) was chromatographed on a silica gel column and eluted with CHCl_3 -MeOH of increasing polarity to yield 14 fractions. Fractions 3 (1.3 g) and 4 (5.0 g) were further chromatographed on a silica gel column using CHCl_3 -MeOH (99:1—9:1, step gradient) to yield compounds **2** (194 mg) and **1** (1.5 g), respectively. Fraction 10 (3.2 g) was chromatographed on a silica gel column using *n*-hexane-EtoAc (4:1) to yield a mixture of compounds **3** and **4** (80 mg), and each of them was purified using a HPLC column: (silica gel; solvent, *n*-hexane: acetone, 9:1; compound **3**, 23 mg; **4**, 19 mg).

Compound 1 (Semialactic Acid): White powder, $[\alpha]_D^{25} +71^\circ$ ($c=0.40$, CHCl_3). IR (KBr) cm^{-1} : 3480, 2850, 1680, 1630, 1450, 1275, 1060, 895. Pos. FAB-MS m/z : 471 $[\text{M}+\text{H}]^+$. HR-FAB-MS m/z : 471.3474 ($[\text{M}(\text{C}_{30}\text{H}_{47}\text{O}_4)+\text{H}]^+$, requires: 471.3481). ^1H -NMR (CDCl_3) δ : 0.85 (3H, s, H₃-18), 0.86 (3H, s, H₃-30), 0.98 (3H, s, H₃-29), 1.02 (3H, s, H₃-28), 1.09 (1H, m, H-11a), 1.13 (1H, m, H-11b), 1.12 (4H, m, H-5, H₂-7, H-12a), 1.46 (4H, m, H-6a, H-9, H₂-15), 1.73 (2H, m, H-6b, H-12b), 1.79 (2H, m, H₂-16), 1.89 (3H, s, H₃-27), 2.02 (2H, m, H-13, H-22a), 2.16 (3H, m, H-22b, H₂-23), 2.64 (1H, m, H-17), 3.72 (1H, d, $J=7.9$ Hz, H-19a), 4.33 (1H, d, $J=6.4$ Hz, H-19b), 4.88 (1H, s, H-21a), 4.92 (1H, s, H-21b), 6.04 (1H, t, $J=6.3$ Hz, H-24). For ^{13}C -NMR (75 MHz, CDCl_3) details refer to Table 1.

Compound 2 (Semialactone): White powder, $[\alpha]_D^{25} +73^\circ$ ($c=0.15$, CHCl_3). Pos. FAB-MS m/z : 469 $[\text{M}+\text{H}]^+$. HR-FAB-MS m/z : 469.3318 ($[\text{M}(\text{C}_{30}\text{H}_{45}\text{O}_4)+\text{H}]^+$, requires: 469.3318). IR (KBr) cm^{-1} : 3515, 1705, 1450, 1372, 1244, 1129, 1094, 904. ^1H -NMR (CDCl_3) δ : 0.88 (3H, s, H₃-18), 0.90 (3H, s, H₃-30), 0.99 (3H, s, H₃-29), 1.03 (3H, s, H₃-28), 1.05 (1H, m, H-2a), 1.13 (1H, m, H-5), 1.25 (1H, m, H-12a), 1.28 (2H, m, H₂-15), 1.49 (4H, m, H-6a, H-9, H₂-15), 1.70 (6H, m, H-1a, H-6b, H₂-11, H-12b, H-16a), 1.93 (3H, s, H₃-27), 1.97 (1H, m, H-1b), 2.18 (3H, m, H-2b, H-16b, H-13), 2.34 (1H, m, H-23a), 2.53 (1H, m, H-23b), 2.96 (1H, dd, $J=19.0, 9.0$ Hz, H-17), 3.73 (1H, dd, $J=9.0, 1.5$ Hz, H-19a), 4.23 (1H, dd, $J=8.5, 2.5$ Hz, H-19b), 4.75 (1H, dd, $J=12.5, 3.5$ Hz, H-22), 5.23 (1H, s, H-21a), 5.28 (1H, s, H-21b), 6.60 (1H, d, $J=6.0$ Hz, H-24). ^{13}C -NMR (75 MHz, CDCl_3)

see Table 1.

Compound **3** (Isofouquierone Peroxide): White powder, $[\alpha]_D^{25} + 35^\circ$ ($c = 0.29$, CHCl_3). EI-MS m/z (rel. int.): 474 $[\text{M}]^+$ (1), 359 $[\text{M}-\text{C}_6\text{H}_{11}\text{O}_2, \text{side chain}]^+$ (56), 315 $[\text{M}-\text{C}_2\text{H}_4\text{O}+\text{side chain}]^+$ (35), 205 (47), 143 (6), 125 (20), 82 (91), 55 (100). HR-FAB-MS m/z : 497.3603 ($[\text{M}(\text{C}_{30}\text{H}_{50}\text{O}_4)+\text{Na}]^+$, requires: 497.3607). IR (KBr) cm^{-1} : 3421, 1704, 1458, 1381, 1263, 1148, 737. $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (3H, s, H₃-30), 0.95 (3H, s, H₃-19), 1.00 (3H, s, H₃-18), 1.04 (3H, s, H₃-29), 1.08 (3H, s, H₃-28), 1.09 (1H, m, H-15a), 1.15 (3H, s, H₃-21), 1.26 (1H, m, H-12a), 1.27 (1H, m, H-11a), 1.29 (1H, m, H-7a), 1.34 (3H, s, H₃-27), 1.37 (3H, s, H₃-26), 1.40 (1H, m, H-5), 1.48 (4H, m, H-1a, H-6a, H-9, H-15b), 1.53 (1H, m, H-11b), 1.55 (1H, m, H-16a), 1.57 (2H, m, H-6b, H-7b), 1.73 (1H, m, H-13), 1.76 (2H, m, H-16b, H-17), 1.89 (1H, m, H-12b), 1.95 (1H, m, H-1b), 2.25 (2H, m, H₂-22), 2.43 (1H, m, H-2a), 2.54 (1H, m, H-2b), 5.62 (1H, d, $J = 15.8$ Hz, H-24), 5.76 (1H, dt, $J = 15.8, 7.0$ Hz, H-23). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) see Table 1.

Compound **4** (Fouquierone): White powder, $[\alpha]_D^{25} + 58^\circ$ ($c = 0.16$, CHCl_3). EI-MS m/z (rel. int.): 458 $[\text{M}]^+$ (1), 359 $[\text{M}-\text{C}_6\text{H}_{11}\text{O}, \text{side chain}]^+$, 315 $[\text{M}-\text{C}_2\text{H}_4\text{O}, \text{side chain}]^+$ (13), 205 (40), 143 (17), 125 (100). HR-FAB-MS m/z : 481.3657 ($[\text{M}(\text{C}_{30}\text{H}_{50}\text{O}_3)+\text{Na}]^+$, requires: 481.3658). IR (KBr) cm^{-1} : 3414, 1701, 1457, 1381, 1265, 1112, 1014, 900. $^1\text{H-NMR}$ (CDCl_3) δ : 0.88 (3H, s, H₃-30), 0.94 (3H, s, H₃-19), 0.98 (3H, s, H₃-18), 1.04 (3H, s, H₃-28), 1.08 (3H, s, H₃-29), 1.10 (1H, m, H-15a), 1.13 (3H, s, H₃-21), 1.24 (1H, m, H-12a), 1.29 (1H, m, H-11a), 1.30 (1H, m, H-7a), 1.39 (1H, m, H-5), 1.45

(1H, m, H-9), 1.48 (4H, m, H-1a, H-15b, H-16a, H-22a), 1.53 (6H, m, H₂-6, H-7b, H-11b, H-16b, H-22b), 1.63 (3H, m, H-13, H₂-23), 1.73 (1H, m, H-17), 1.75 (3H, s, H₃-27), 1.82 (1H, m, H-12b), 1.90 (1H, m, H-1b), 2.43 (1H, m, H-2a), 2.45 (1H, m, H-2b), 4.31 (1H, dd, $J = 12.4, 6.3$ Hz, H-24), 5.01 (2H, s, H₂-26). $^{13}\text{C-NMR}$ (75 MHz, CDCl_3) see Table 1.

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