Structure Determination of Shinjulactones M and N, New Bitter Principles from *Ailanthus altissima* SWINGLE

Yoshio NIIMI, Takahiko TSUYUKI,* Takeyoshi TAKAHASHI,* and Kazuhiro MATSUSHITA†
Department of Chemistry, Faculty of Science, The University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113
†Application Center, Scientific Instrument Project, JEOL Co. Ltd.,
Nakagami, Akishima, Tokyo 196
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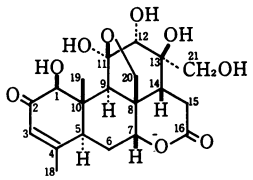
Synopsis. Two new quassinoids, shinjulactones M and N were isolated from root bark of *Ailanthus altissima* SWINGLE. The structures of these compounds were determined to be 11β,20-epoxy-1β,11α,12α,13β,21-pentahydroxypicras-3-ene-2,16-dione and 11β,20-epoxy-1β,2α,11α,12α,15β-pentahydroxypicrasa-3,13(21)-dien-16-one, respectively, by spectral and chemical means.

As a continuation of studies on the bitter principles of Simaroubaceous plants, we have been investigating bitter principles of *Ailanthus altissima* SWINGLE (Japanese name: Shinju or Niwaurushi).[1] We further examined bitter principles in aqueous extracts of root bark of *A. altissima,* and obtained two new bitter principles, shinjulactone M (**1**; 0.0005%) and shinjulactone N (**2**; 0.0002%) together with known quassinoids, ailanthone (**3**),[2] $\Delta^{13(18)}$-dehydroglaucarubolone (**4**),[3] shinjulactone A (**5**)[2], shinjulactone D[4] and shinjuglycoside D.[5] This paper describes structure determination of shinjulactones M and N (**1** and **2**) and chemical derivation of **1** from ailanthone (**3**).

Shinjulactone M (**1**), mp 260—264 °C (decomp), showed the presence of hydroxyl, δ-lactone, and α,β-unsaturated ketone by IR and UV spectra and gave a molecular peak at *m/z* 410 in the EI-MS spectrum, suggesting the molecular formula, $C_{20}H_{26}O_9$. In the ^{13}C NMR of **1**, signals were observed at $\delta_{(C)}$ 66.5 and $\delta_{(C)}$ 75.8, which were assignable to a hydroxymethyl carbon atom and a carbon atom bearing a tertiary hydroxyl group, respectively. The ^1H NMR spectrum of **1** showed the presence of a tertiary methyl and a vinyl methyl groups and the spectral feature was similar to that of ailanthone (**3**)[2] except for the absence of an *exo*-methylene group. Since the molecular formula of **1** possesses extra two hydrogen and two oxygen atoms in comparison with that of ailanthone (**3**), the structure of **1** would be deduced to be a vicinal hydroxylation product of the *exo*-methylene in ailanthone (**3**) from the spectral evidence; the structure with the primary hydroxyl group at C-21 and the tertiary hydroxyl group at C-13 would be proposed for shinjulactone M (**1**). The COSY, NOESY spectra and ^{13}C-^1H J-correlated two-dimensional spectrum for **1** confirmed the location of the tertiary hydroxyl group. The configuration of the C-13 carbon atom was determined by difference NOE measurement. Irradiation of a signal due to one proton of C-21 methylene resonating at δ 4.42, resulted in increase in area of signals at δ 3.41 and 3.49 due to C-15 methylene and at δ 4.19 due to the other proton of C-21 methylene, respectively. This observation indicates that the hydroxymethyl group at C-13 locates in α-orientation and therefore the tertiary hydroxyl group at C-13 in β-orientation. Thus the structure of shinjulactone M was determined to be 11β,20-epoxy-1β,11α,12α,13β,21-pentahydroxypicras-3-ene-2,16-dione (**1**).



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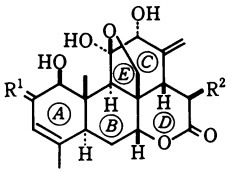
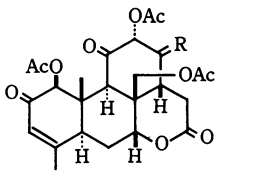


2 R¹=α-OH,β-H, R²=OH
3 R¹=O, R²=H
4 R¹=O, R²=OH
5 R¹=α-OH,β-H, R²=H



6 R=CH_2
7 R=α-CH_2OH,β-OH

Table 1. ^1H NMR Spectra of Shinjulactones M and N (**1** and **2**)[a] in C_5D_5N

	1[b]	**2**[c]
1-H	4.42 s	3.99 d (8.4)
2-H	—	4.62 m (overlapped with 7-H)
3-H	6.10 q (1.4)	5.79 br s
5-H	3.01 brd (12.6)	2.73 d (12.6)
6α-H	2.17 ddd (14.9, 2.9, 2.8)	2.08 d (14.3)
6β-H	2.07 ddd (14.9, 12.6, 2.7)	1.91 dd (14.3, 12.6)
7-H	4.61 dd (2.8, 2.7)	4.62 br s
9-H	3.39 s	3.41 s
12-H	4.32 d (1.5)	4.70 s
14-H	2.78 ddd (13.3, 6.0, 1.5)	3.01 d (11.9)
15α-H	3.41 dd (18.3, 13.3)	5.27 d (11.9)
15β-H	3.49 dd (18.3, 6.0)	—
4-Me	1.74 br s	1.61 s
10-Me	1.59 s	1.69 s
20-H	4.12 d (7.8)	3.77 d (8.4)
20-H′	4.83 d (7.8)	4.16 d (8.4)
21-H	4.19 d (11.2)	5.46 d (1.8)
21-H′	4.42 d (11.2)	5.52 d (1.8)

a) Coupling constants are expressed in Hz in parentheses. b) Measured at 400 MHz. c) Measured at 270 MHz.

Table 2. ^{13}C NMR Spectra of Shinjulactones M and N (**1** and **2**)[a] in C_5D_5N

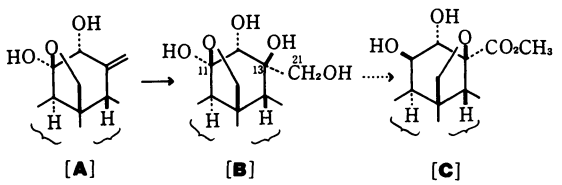
No of carbon	1[b]	2[c]	No of carbon	1	2
1	84.6 d	83.1	11	110.5 s	110.2
2	197.5 s	72.3[d]	12	80.7 d	80.7
3	126.2 d	126.7	13	75.8 s	148.6
4	162.3 s	134.4	14	46.3 d	55.5
5	42.6 d	41.5[e]	15	31.0 t	67.8
6	26.1 t	25.5	16	170.1 s	173.0
7	78.3 d	78.6	18	22.4 q	20.9
8	46.3 s[f]	45.7	19	10.6 q	10.5
9	45.3 d	47.1	20	70.9 t	72.2[d]
10	45.3 s[f]	41.7[e]	21	66.5 t	120.2

a) Chemical shifts are expressed in δ downfield from TMS as an internal standard. b) Measured at 100 MHz. c) Measured at 67.5 MHz. d), e), and f) Signals may be reversed, respectively.

The structure (**1**) proposed for shinjulactone M was firmly confirmed by a chemical conversion of ailanthone (**3**) into shinjulactone M (**1**). Ailanthone (**3**), on treatment with osmium tetraoxide, afforded a product derived from an attack of the reagent on a conjugated olefin between C-3 and C-4, because the reagent could not be accessible to the *exo*-methylene due to a steric hindrance of E-ring. Ailanthone triacetate (**6**),[6,7] however, reacted with the reagent to afford a dihydroxy derivative (**7**), $C_{26}H_{32}O_{12}$. Treatment of **7** with potassium methoxide gave shinjulactone M, identical with a natural specimen including an optical property.

Shinjulactone N (**2**), mp 245—251 °C (decomp), gave a molecular formula, $C_{20}H_{26}O_8$ by high-resolution mass spectrum and showed the presence of hydroxyl and δ-lactone groupings. Comparison of the ^1H NMR spectrum of **2** with those of shinjulactone A (**5**)[2] and $\Delta^{13(18)}$-dehydroglaucarubolone (**4**)[3] led to the proposed structure with the same A, B, C, and E ring-structures as those of **5** and with the same β-equatorial hydroxyl group at C-15 as that in **4**, the coupling constant, $J_{14,15}$, being 11.9 Hz (see Table). Therefore, 11β,20-epoxy-1β,2α,11α,12α,15β-pentahydroxypicrasa-3,13(21)-dien-16-one (**2**) was proposed for shinjulactone N. The ^{13}C NMR spectrum of shinjulactone N was compatible with the proposed structure (**2**). Shinjulactone N (**2**) is corresponding to the hydrolyzate of 13,18-dehydroexcelsin obtained from *Ailanthus excelsa*.[8]

Although more than twenty quassinoids and four quassinoid glycosides have been isolated from *A. altissima* so far, shinjulactone M (**1**) is the first example of 11β,20-epoxypicrasane which possesses a hydroxyl group at $C_{(21)}$-atom, and the picrasane [B] bearing hydroxyl groups on $C_{(13)}$- and $C_{(21)}$-atoms may be one of biogenetical intermediates yielding methyl 13β,20-epoxypicrasan-21-oate [C], such as brusatol from ailanthone-type precursor [A] (Scheme 1).



[A] [B] [C]

Scheme 1.

Experimental[9]

Extraction and Isolation of Shinjulactones M and N (1 and 2). The air-dried root bark (ca. 7 kg) was chipped and extracted with hot water twice (15 l and then 10 l). Evaporation in vacuo gave a concentrated extract (ca. 5 l), which was continuously extracted with dichloromethane (1 l×3) for 12 h. The aqueous layer was separated and concentrated to afford a solution (ca. 2 l), to which methanol (3 l) was added. After filtration, the filtrate was evaporated to give a residue (420 g), a part (317 g) of which was absorbed on silica gel (361 g) and placed on the top of silica-gel column (2 kg). Elution was performed with lower layer of chloroform–methanol–water in the following ratios successively; 50:12:3 (6.5 l), 65:35:10 (12 l), and 50:50:18 (5 l). Forty fractions (each 500 ml) were collected (column A).

Residues from fractions 19 and 20 of column A were combined (4 g) and separated by column chromatography on silica gel (80 g) eluted with 18% methanol in ethyl acetate (200 ml) and 20, 23, 26, 29, and 32% methanol in ethyl acetate (each 100 ml), and 8 fractions (each 100 ml) were collected (column B).

The residue from fraction 4 (581 mg) of column B was separated by partition chromatography on silicic acid (430 g) pretreated with water (287 g). Elution was performed with 0, 1, 3, 6, 9…24% ethanol in chloroform (each 1 l) and 33 fractions (each 250 ml) were collected (column C).

Residues from fractions 22—28 (117 mg) of column C were combined and separated by reversed phase chromatography using LiChroprep RP-8. Elution was performed with methanol–water (3:7) and 40 fractions (each 10 g) were collected. Fractions 13—17 afforded shinjulactone M (**1**; 32 mg). Combined residues (29 mg) of fractions 18—33 were further separated by gel column chromatography using Toyopearl HW-40S (2.4×100 cm) and methanol as an eluent. Seventy fractions (each 10 g) were collected. Fractions 50—52 gave shinjulactone N (**2**; 11 mg).

Shinjulactone M (1). Colorless prisms crystallized from methanol–ethyl acetate, mp 260—264 °C (decomp); $[\alpha]_D^{25}$ +37° (*c* 0.46, MeOH); IR (KBr) 3500, 2900, 1730, 1670, 1260, 1225, 1060, and 1030 cm^{-1}; UV (EtOH) 238 nm (ε 15600); ^1H and ^{13}C NMR (Tables 1 and 2); MS (EI) *m/z* (%) 410 (M^+; 2.2), 392 (3), 374 (13), and 107 (100); Found: *m/z* 410.1580. Calcd for $C_{20}H_{26}O_9$: M, 410.1577.

Shinjulactone N (2). Colorless amorphous solid from methanol–ethyl acetate, mp 245—251 °C; $[\alpha]_D^{25}$ +60.6° (*c* 0.32, MeOH), IR (KBr) 3420, 2870, 1720, 1625, 1610, and 1050 cm^{-1}; ^1H and ^{13}C NMR (Tables 1 and 2); MS (EI) *m/z* (%) 394 (M^+; 3.9), 376 (18), 361 (10), 332 (23), 105 (79), and 57 (100); Found: *m/z* 394.1616. Calcd for $C_{20}H_{26}O_8$: M, 394.1626.

Conversion of Ailanthone Triacetate (6) into Shinjulactone M (1). Ailanthone triacetate (**6**; 417.5 mg)[7] was treated with osmium tetraoxide (199 mg) in a mixture (8 ml) of pyridine–THF (1:1) at −72 °C for 9 h. After addition of sodium hydrogensulfite (0.36 g) in water (6 ml) and pyridine (3 ml), THF was removed by distillation in vacuo. The remaining solution was stirred at room temperature for 1 h. The usual work-up followed by separation by column chromatography on silica gel afforded shinjulactone M triacetate (**7**; 141 mg) together with the starting material (**6**; ca. 90 mg). **7**; colorless

needles from chloroform–ethyl acetate, mp 261—263 °C (decomp); $[\alpha]_D^{24}$ +10° (*c* 0.10, pyridine); IR (KBr) 3490, 3280, 1750, 1710, 1680, 1380, 1210, 1060, 1040, 900, and 600 cm^{-1}; UV (EtOH) 238 nm (ε 8800); ^1H NMR (C_5D_5N) δ 1.65 (3H, s; 10-Me), 1.79 (3H, s; 4-Me), 1.95, 2.10, and 2.15 (each 3H; OAc), 3.1—3.8 (3H, m; 14-H, 15-H, and 15-H′), 4.03 (2H, s; 21-H and 21-H′), 4.68 (1H, d, *J*=10.8 Hz; 20-H), 4.97 (1H, br s; 7-H), 5.31 (1H, d, *J*=10.8 Hz; 20-H′), 5.47 (1H, s; 1-H or 12-H), 5.66 (1H, s; 12-H or 1-H), and 6.03 (1H, br s; 3-H); ^{13}C NMR (C_5D_5N) δ 203.4, 191.6, 170.9, 170.3, 169.5, 169.2, 161.7, 126.5, 84.9, 81.0, 80.0, 78.0, 64.5, 64.1, 48.8, 44.7, 44.3, 42.1, 38.2, 28.4, 25.4, 21.9, 21.0, 20.7, 20.6, and 12.0; MS (EI) *m/z* (%) 536 (M^+; 6.2), 518 (2.4), 494 (5.6), 476 (13), 463 (4.3), 458 (5.4), 434 (9.6), 416 (11), 398 (20), 151 (19), 95 (32), and 60 (100); Found: *m/z* 536.1891. Calcd for $C_{26}H_{32}O_{12}$: M, 536.1892.

Shinjulactone M triacetate (**7**; 118 mg) was hydrolyzed with 0.3 M (1 M=1 mol dm^{-3}) potassium methoxide in methanol (10 ml) at room temperature for 1 h and, after the usual work-up, the hydrolyzate was purified by column chromatography on silica gel (5 g). Elution with 15% methanol in chloroform afforded shinjulactone M (**1**; 75.7 mg); mp 263—266 °C (decomp); $[\alpha]_D^{14}$ +20° (*c* 0.46, MeOH); the spectral data were completely identical with those of a natural specimen.

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