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STUDIES ON THE CONSTITUENTS OF INDONESIAN *BORRERIA LATIFOLIA*[1]

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Abstract - Seven known iridoid glycosides (**1~7**), together with one known diterpenoid (**8**) were isolated from the aerial parts of *Borreria latifolia* K. Schum. (Rubiaceae) collected in Indonesia, and their structures were identified by spectroscopic methods.

Borreria latifolia is distributed widely throughout tropical Asia and is used as food or feed.[2] Among the members of this genus, *B. articularis*[3] is used to treat headache, gallstones and in a poultice upon the abdomen of very small children with stomachache in China. *B. osimoides*[3] heal wounds in Indonesia. As a continuation of the studies on the traditional medicine (Jamu) and the medicinal resources in Indonesia, we investigated the chemical constituents of *B. latifolia* collected there.

The methanol extract of the aerial parts of *B. latifolia* was passed over active charcoal packed in a column. The resulting fractions were chromatographed repeatedly on silica gel, Sephadex LH-20, MCI-gel and ODS column as described in the experimental section to isolate seven known iridoid glycosides (**1~7**),

together with acyclic diterpenoid, phytol (**8**). Here we report the isolation and identification of these compounds.

Compound (**1**) showed a brilliant reddish violet spot on the TLC upon heating with 10% sulfuric acid, before this spot subsequently changed to black, suggesting that **1** was an iridoid compound.[4] In the ^{1}H-NMR spectrum, we observed the following signals: the doublet proton signal at δ5.08 (1H, *J*=8.9 Hz) due to the presence of characteristic H-1 of the iridoid compound, two olefinic proton signals at δ 7.65 (1H, d, *J*=1.2 Hz) and δ 6.01 (1H, d, *J*=1.9 Hz), one hydroxymethylene proton signal at δ4.95 and 4.80 (each, 1H, br d, *J*=15.6 Hz), hydroxymethine proton signal at δ 4.83 (1H, m), two methine proton signals at δ 3.02 (1H, ddd, *J*=7.8, 6.2, 1.2 Hz) and δ 2.63 (1H, dd, *J*=8.9, 7.8 Hz), and one acetyl proton signal at δ 2.10 (3H, s). In addition, in the ^{13}C-NMR spectrum, we observed these signals: hydroxymethine carbon signal at δ 63.7, α,β-unsaturated carboxylic carbon at δ 172.5, double bond carbon signals at δ 155.2 and 108.4, the olefinic carbon signals at δ 131.8 and 145.7, and acetyl carbon signals at δ 172.4 and 20.8. Further, characteristic glucopyranosyl carbon signals and the coupling constant (*J*=7.8 Hz) of the anomeric proton signal at δ 4.74 (d) suggested glucose to be β-linked to the hydroxyl group at the C-1 in iridoid. Therefore, compound (**1**) was elucidated as asperulosidic acid according to values reported in the literature.[5]

Compounds (**2**) and (**3**) were identified by the comparison of the ^{1}H- and ^{13}C-NMR spectra with those of **1** to be deacetylasperulosidic acid[6] and scandoside,[6] respectively. Compound (**4**) was identified by the comparison of the ^{1}H- and ^{13}C-NMR spectra with those of **2** to be methyl deacetylasperulosidate.[7] Compound (**5**) was identified by the comparison of the ^{1}H- and ^{13}C-NMR spectra with those of **4** to be daphylloside.[8] Compound (**6**) was identified by the comparison of the ^{1}H- and ^{13}C-NMR spectra with those of **3** to be 6-*O*-acetylscandoside.

In the ^{1}H-NMR spectrum of compound (**7**), the characteristic proton signal at δ 5.15 (1H,d, *J*=6.1 Hz) due to the presence of hydroxymethine at H-1 in iridoid, and one olefinic proton signal at δ 7.45 (1H, d, *J*=1.4 Hz) were observed, but not another olefinic proton signal at C-7. Furthermore, proton signals at δ 2.27 (1H, ddd, *J*=13.4, 6.8, 1.6 Hz), δ 1.54 (1H, ddd, *J*=13.4, 9.6, 4.4 Hz) and δ 4.29 (1H, deformed t) were

detected. These findings indicated the existence of a hydroxyl group at C-7 as the loganin skeleton, which was supported by the ^{13}C-NMR spectral data. Based on the coupling constant ($W_{1/2}$=3.1) of the carbinyl methine at H-7, the hydroxyl group at C-7 was a β-configuration. In the comparison of ^{13}C-NMR of **7** with that of loganin, the existence of a carbon signal at δ 62.3 (t), because of hydroxymethylene at C-10 in **7**, indicated that compound (**7**) was 10-hydroxyloganin.[9]

Compound (**8**) was obtained as colorless oil. The IR spectrum showed absorption band (1668 cm^{-1}) due to double bond, together with those due to methyl, methylen groups and hydroxyl group. In the ^{13}C-NMR, double bond carbons (δ 136.9 and 126.3) and hydroxymethylene carbon (δ 59.0) were exhibited, besides signals of methyl (5), methylene (9), methine (3). In the ^{1}H-NMR, olefinic proton signal at δ 5.74 (1H, tq, *J*=5.3, 1.3 Hz), hydroxymethylene proton signals at δ 4.45 (2H, d, *J*=6.5 Hz), isopropyl proton signals at δ 1.51 (1H, sep, *J*=6.8 Hz) and 0.90, 0.88 (each 3H, d, *J*=6.8 Hz), methyl proton signal linked double bond at δ 1.69 (3H, br s), and two methyl proton signals at δ 0.88 and 0.87 (each 3H, s) were showed. From these data, compound (**8**) was considered as the acyclic diterpenoid bearing isopropyl group and hydroxymethyl group, to identify as phytol with literature values.[10]

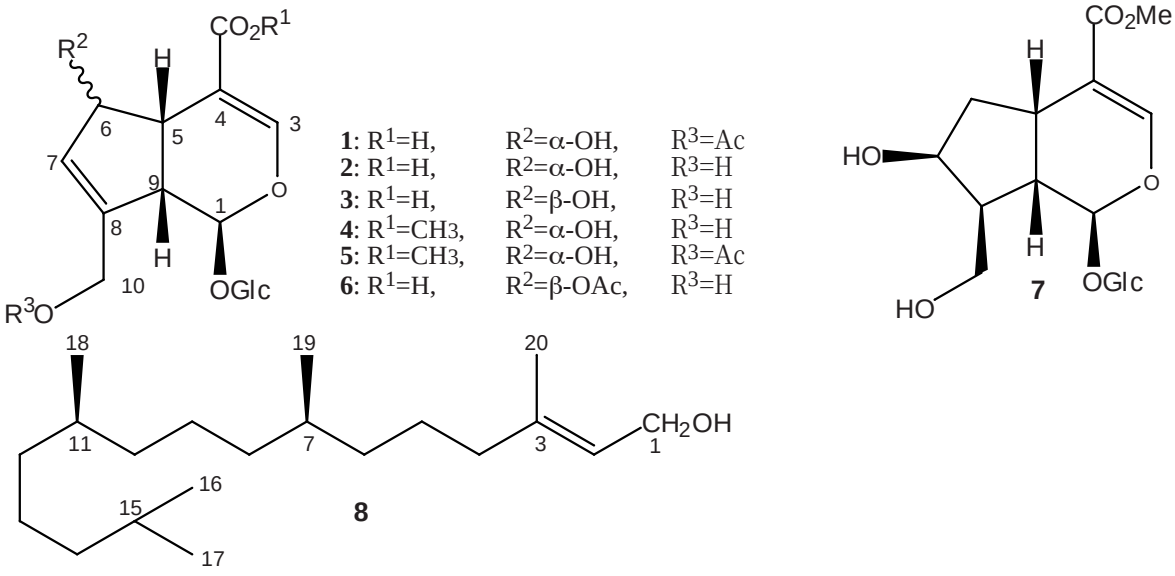

Figure 1 Chemical Structures of Compounds (**1**-**8**)

Table 1 ^{13}C-NMR Spectral Data for Compounds (**1**-**7**) in CD_3OD

	1	**2**	**3**	**4**	**5**	**6**	**7**
C-1	101.2	101.5	98.9	101.6	101.3	93.4	99.0
C-3	155.2	155.4	153.9	155.3	155.3	150.2	152.9
C-4	108.4	108.5	111.0	108.3	108.1	106.2	113.0
C-5	42.3	42.8	47.1	42.7	42.4	37.5	33.5
C-6	75.3	75.4	82.5	75.4	75.3	86.3	43.1
C-7	131.8	130.0	130.0	129.9	131.9	129.0	73.3
C-8	145.7	151.4	147.4	151.5	145.9	144.2	42.2
C-9	46.2	45.9	46.1	45.9	46.3	45.3	49.9
C-10	63.7	61.7	61.1	61.7	63.7	61.9	62.3
C-11	172.5	170.8	171.9	169.4	169.3	172.5	169.4
C-1'	100.5	100.5	100.3	100.5	100.6	100.1	100.6
C-2'	74.8	75.0	74.7	75.0	74.9	74.6	74.8
C-3'	78.2	78.5	78.3	78.5	78.5	78.4	78.3
C-4'	71.4	71.7	71.5	71.7	71.6	71.6	71.5
C-5'	77.7	77.8	77.8	77.9	77.9	77.9	78.0
C-6'	62.8	62.8	62.6	62.9	63.0	62.8	62.3
CH_3O				51.7	51.8		51.7
$CH_3\underline{C}O$	172.4				172.5	172.2	
$\underline{C}H_3CO$	20.8				20.7	20.6	

EXPERIMENTAL

General Procedures and Plant Material Optical rotations were measured using a Jasco DIP-1000 digital polarimeter. IR and UV spectra were measured on a Shimadzu FT-IR 8300 infrared spectrometer and a Hitachi U-3000 spectrometer, respectively. The NMR spectra were recorded in CD_3OD or C_5D_5N with TMS as internal standard on a Bruker DPX-400. Column chromatography was carried out on Kieselgel 60 (70-230 mesh, Merck Co.), Sephadex LH-20 (Pharmacia Co.). Medium pressure liquid chromatography (MPLC, micro pump KP-7, Kusano Scientific Co. Tokyo) was carried out on a CIG column [ODS (C-18)]. HPLC (JASCO HPLC system; pump:880-PL, detector:875 UV/VIS detector) was

carried out on a SiO_2 packed column (20 mm i. d.×250 mm, YMC Co.).

Borreria latifolia K. Schum. was collected in July, 1992 at Dien in Indonesia and the plant was identified by Y. Saiki. A voucher specimen has been deposited in the Herbarium of Kobe Gakuin University.

Isolation of Compounds (***1***~***8***) The air-dried aerial parts of *B. latifolia* (860 g) was extracted 7 times with methanol (3 L) under 60 °C for 8 h. The combined methanol extracts were passed over active charcoal packed in a column (fraction I), and were eluted with methanol (fraction II) and 30% $CHCl_3$/ MeOH (fraction III), successively.

Fraction I (66.18 g) was subjected repeatedly to column chromatography on SiO_2 ($CHCl_3$-MeOH gradient solvent system), Sephadex LH-20 (MeOH : H_2O, 2 : 1) and ODS (Rp-18, MeOH : H_2O, 1 : 2) to yield compounds (**1**) (203.5 mg), (**2**) (30.0 mg), (**3**) (70.9 mg), (**4**) (37.2 mg), (**5**) (37.8 mg), (**6**) (24.0 mg), and (**7**) (41.3 mg). Fraction III (13.55 g) was subjected to repeat column chromatography on SiO_2 ($CHCl_3$-MeOH gradient solvent system) and Sephadex LH-20 ($CHCl_3$: MeOH, 2 : 1), and finally was purified by HPLC (hexane : EtOAc, 9 : 1) to give compound (**8**) (95.7 mg).

Compound (***1***) (*Asperulosidic acid*) A white amorphous powder, anthrone / H_2SO_4 reaction positive, $[\alpha]_D^{26}$+22.7 ° (*c*=2.0, MeOH), IRν_{max} (KBr) cm^{-1} : 3448, 1728, 1685, 1636; UVλ_{max} (MeOH) nm (log ε) : 201.5 (3.8), 239.1 (4.0); ^{1}H-NMR (δ, CD_3OD) : 5.08 (1H, d, *J*=8.9 Hz, H-1), 7.65 (1H, d, *J*=1.2 Hz, H-3), 3.02 (1H, ddd, *J*=7.8, 6.2, 1.2 Hz, H-5), 4.83 (1H, m, H-6), 6.01 (1H, d, *J*=1.9 Hz, H-7), 2.63 (1H, dd, *J*=8.9, 7.8 Hz, H-9), 4.95, 4.80 (each 1H, d, *J*=15.6 Hz, H-10), 2.10 (3H, s, $COCH_3$), 4.74 (1H, d, *J*=7.8 Hz, H-1'); ^{13}C-NMR : Table1

Compound (***2***) (*Deasperulosidic acid*) A white amorphous powder, anthrone / H_2SO_4 reaction positive, $[\alpha]_D^{27}$+4.13 ° (*c*=3.0, MeOH). IRν_{max} (KBr) cm^{-1} : 3432, 1685, 1670, 1636. UVλ_{max} (MeOH) nm (log ε) : 202.0 (3.7), 233.0 (3.8); ^{1}H-NMR (δ, CD_3OD): 5.05 (1H, d, *J*=8.9 Hz, H-1), 7.65 (1H, d, *J*=1.2 Hz, H-3), 3.00 (1H, ddd, *J*=7.7, 6.4, 1.2 Hz, H-5), 4.82 (1H, m, H-6), 6.02 (1H, d, *J*=1.9 Hz, H-7), 2.58 (1H, dd, *J*=8.9, 7.7 Hz, H-9), 4.46, 4.21 (each 1H, d, *J*=15.6 Hz, H-10), 4.72 (1H, d, *J*=7.8 Hz, H-1');

^{13}C-NMR : Table I

Compound (**3**) (*Scandoside*) A white amorphous powder, anthrone / H_2SO_4 reaction positive, $[\alpha]_D^{26}$ -32.7 ° (*c*=0.7, MeOH). IRν_{max} (KBr) cm^{-1}: 3432, 1682, 1670, 1636; UVλ_{max} (MeOH) nm (log ε): 201.6 (3.8), 233.7 (3.9); ^{1}H-NMR (δ, CD_3OD): 5.09 (1H, d, *J*=6.7 Hz, H-1), 7.54 (1H, d, *J*=1.1 Hz, H-3), 5.82 (1H, d, *J*=1.0 Hz, H-7), 2.96 (1H, dd. *J*=9.0, 7.7 Hz, H-9), 4.35, 4.10 (each, 1H, d, *J*=15.4 Hz, H-10), 4.70 (1H, d, *J*=7.9 Hz, H-1'); ^{13}C-NMR : Table 1.

*Compound (**4**) (Methyl deacetylasperulosidate)* A white amorphous powder, anthrone / H_2SO_4 reaction positive, $[\alpha]_D^{27}$+17.5 ° (*c*=0.7, MeOH); IRν_{max} (KBr) cm^{-1}: 3424, 1696, 1670, 1634; UVλ_{max} (MeOH) nm (log ε): 201.1 (3.7), 234.9 (3.9); ^{1}H-NMR (δ, CD_3OD): 5.05 (1H, d, *J*=9.0 Hz, H-1), 7.65 (1H, d, *J*=1.6 Hz, H-3), 3.02 (1H, ddd, *J*=7.3, 6.0, 1.6 Hz, H-5), 6.02 (1H, d, *J*=2.1 Hz, H-7), 2.56 (1H, dd, *J*=9.0, 7.4 Hz, H-9), 4.45, 4.20 (each,1H, d, *J*=15.6 Hz, H-10), 3.74 (3H,s, $COCH_3$), 4.71 (1H, d, *J*=4.71 Hz, H-1'); ^{13}C-NMR : Table 1.

Compound (***5***) (*Daphylloside*) A white amorphous powder, anthrone / H_2SO_4 reaction positive , $[\alpha]_D^{32}$ +10.7 ° (*c*=2.0, MeOH); IRν_{max} (KBr) cm^{-1}: 3456, 1716, 1696, 1670, 1636; UVλ_{max} (MeOH) nm (log ε): 201.9 (3.7), 234.9 (4.0); ^{1}H-NMR (δ, CD_3OD): 5.06 (1H, d, *J*=9.0 Hz, H-1), 7.65 (1H, d, *J*=1.4 Hz, H-3), 3.03 (1H, ddd, *J*=7.4, 6.4, 1.4 Hz, H-5), 6.02 (1H, d, *J*=1.8 Hz, H-7), 2.64 (1H, dd, *J*=9.0, 7.4 Hz, H-9), 4.95, 4.80 (each,1H, d, *J*=15.6 Hz, H-10), 2.09 (3H, s, $COCH_3$), 3.74 (3H, s, CH_3), 4.72 (1H, d, *J*=7.8 Hz, H-1'); ^{13}C-NMR : Table 1.

Compound (***6***) (*6-O-Acetylscandoside*) A white amorphous powder, anthrone / H_2SO_4 reaction positive, $[\alpha]_D^{32}$-15.6 ° (*c*=2.0, MeOH); IRν_{max} (KBr) cm^{-1}: 3392, 1738, 1702, 1664; UVλ_{max} (MeOH) nm (log ε): 202.4 (3.8), 234.5 (3.8); ^{1}H-NMR (δ, CD_3OD): 5.56 (1H, d, *J*=6.0 Hz, H-1), 7.30 (1H, d, *J*=2.2 Hz, H-3), 3.35 (1H, dd, *J*= 6.1, 2.2 Hz, H-5), 5.95 (1H, d, *J*=1.4 Hz, H-7), 3.68 (1H, m, H-9), 4.77, 4.66 (each, 1H, d, *J*=14.3 Hz, H-10), 2.08 (3H, s, $COCH_3$), 4.68 (1H, d, *J*=7.9 Hz, H-1'); ^{13}C-NMR : Table 1

Compound (***7***) (*10-Hydroxyloganin*) A white amorphous powder, anthrone / H_2SO_4 reaction positive, $[\alpha]_D^{27}$-93.8 ° (*c*=1.4, MeOH); IRν_{max} (KBr) cm^{-1}: 3432, 1702, 1634; UVλ_{max} (MeOH) nm (log ε): 193.6 (3.5), 193.7 (3.5), 234.5 (4.0); ^{1}H-NMR (δ, CD_3OD): 5.15 (1H, d, *J*=6.1 Hz, H-1), 7.45 (1H, d, *J*=1.4 Hz,

H-3), 3.64 (1H, m, H-5), 2.27 (1H, ddd, *J*=13.4, 9.6, 4.4 Hz, H-6), 1.54 (1H, ddd, *J*=13.4, 9.6, 4.4 Hz, H-6), 4.29 (1H, t, *J*=2.1 Hz, H-7), 3.69 (3H, s, $COCH_3$), 4.56 (1H, d, *J*=7.9 Hz, H-1'); ^{13}C-NMR : Table 1. Compound (**8**) (Phytol) A colorless oil, $[\alpha]_D^{27}$+0.2 (*c*=10, $CHCl_3$), IRν_{max} (CCl_4) cm^{-1} : 3628, 2956, 2928, 2868, 1668, 1464, 1380; ^{1}H-NMR (δ, C_5D_5N) : 5.74 (1H, tq, *J*=5.3, 1.3 Hz, H-2), 4.45 (2H, d, *J*=6.5 Hz, H-1), 2.02 (1H, t, *J*=7.1 Hz, H-4), 1.69 (3H, br s, H-20), 1.51 (1H, sep, *J*=6.8 Hz, H-15), 0.90, 0.88 (each 3H, d, *J*=6.8 Hz, H-16 and H-17), 0.88, 0.87 (each 3H, s, H-18 and H-19); ^{13}C-NMR (δ, C_5H_5N) : 136.9 (C-3), 126.3 (C-2), 59.0 (C-1), 40.3 (C-4), 39.7(C-14), 37.8 (C-8), 37.8 (C-10), 37.7 (C-12), 37.1 (C-6), 33.2 (C-7), 33.1 (C-11), 28.3 (C-15), 25.6 (C-5), 25.2 (C-13), 24.9 (C-9), 22.9 (C-17), 22.8 (C-16), 20.0 (C-18), 20.0 (C-19), 16.3 (C-20).

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