Communications to the Editor

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PULOSARIOSIDE, A NEW BITTER TRIMERIC-IRIDOID DIGLUCOSIDE, FROM AN INDONESIAN JAMU, THE BARK OF *ALYXIA REINWARDTII* BL. (APOCYNACEAE)

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A new bitter trimeric-iridoid diglucoside named pulosarioside (1) was isolated from an Indonesian folk-medicine (Jamu, "pulosari"), the air-dried bark of *Alyxia reinwardtii* BL. (Apocynaceae), and the absolute configuration of 1 has been determined on the basis of chemical and physicochemical evidence.

KEYWORDS —— pulosarioside; trimeric-iridoid diglucoside; Indonesian Jamu; *Alyxia reinwardtii*; Apocynaceae; (+)-pinoresinol glucoside; loganin

The air-dried bark of *Alyxia reinwardtii* BL. (Apocynaceae) (called "pulosari" in Indonesia) is one of the popular Indonesian folk medicines (Jamu). It has been used for various intestinal diseases and diarrhea. It is also known as a stomachic and an antispasmodic. During the course of our investigations in search of new biologically active constituents in naturally occurring drug materials in Indonesia,[1] we have chemically analyzed "pulosari." Along with the identification of known lup-20(30)-en-3β-yl acetate, (+)-pinoresinol,[2] and (+)-pinoresinol β-D-glucopyranoside,[2] we have isolated a new bitter trimeric-iridoid diglucoside named pulosarioside (1) and have elucidated the structure on the basis of the following evidence.[3]

The MeOH extract of the air-dried bark (obtained at Sukabumi, Java) was partitioned into a mixture of AcOEt and water. Chromatographic separation of the AcOEt-soluble portion using silica gel and reversed phase silica gel (Bondapak C_{18}) provided lup-20(30)-en-3β-yl acetate (0.31% from the bark), (+)-pinoresinol (0.10%), and (+)-pinoresinol β-D-glucopyranoside (0.07%). The water-soluble portion was subjected to silica gel (eluting with $CHCl_3$-MeOH-H_2O=7:3:1, lower layer) and reversed phase silica gel (Bondapak C_{18}, MeOH-H_2O=1:1) column chromatography to furnish pulosarioside (1, 0.14%), mp 132 °C (EtOH), $[\alpha]_D^{24}$ -94° (MeOH), $C_{43}H_{60}O_{23}\cdot 2H_2O$,[4] UV (MeOH): 230 nm (ε 17600), IR (KBr): 3380 (br), 2913, 1695, 1625 cm^{-1}, SIMS : m/z 967 $(M+Na)^+$, a mixture of its monoacetal (0.02%), and a diacetal (0.05%).[5]

Alkaline hydrolysis (3% NaOMe-MeOH, 25 °C, 20 min) of pulosarioside (1) and subsequent acetylation (Ac_2O-pyridine=1:2, 25 °C, 40 min) of the product yielded 2a, a white powder, $[\alpha]_D^{20}$ +129° ($CHCl_3$), $C_{14}H_{20}O_6$, IR ($CHCl_3$): 2960, 1736, 1236 cm^{-1}; 3a, a white powder, $[\alpha]_D^{20}$ -10° ($CHCl_3$), $C_{14}H_{20}O_6$, IR ($CHCl_3$): 2936, 1734. 1224 cm^{-1}, 5a, a white powder, $[\alpha]_D^{20}$ -133° ($CHCl_3$), $C_{25}H_{32}O_{14}$, IR ($CHCl_3$): 2938, 1751, 1704,

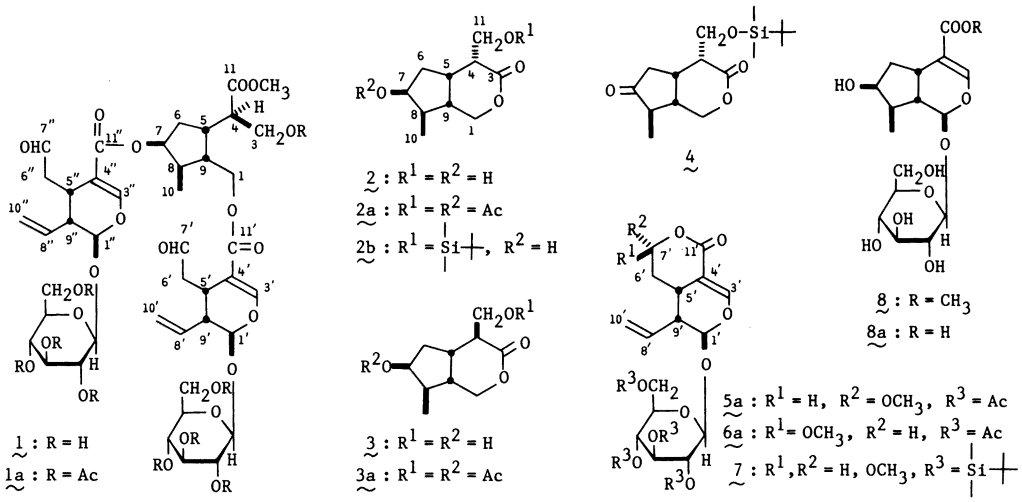
1215 cm^{-1}, and vogeloside tetraacetate (6a),[6] approximately in 1:1:2:2 ratio.[7] The unknown C-7' configuration of 6a has been determined as S on the basis of the ^1H NMR analysis[8] including the NOE experiments.[9] Furthermore, 5a has been shown to be the C-7' epimer of 6a by ^{13}C (Table I) and ^1H NMR comparisons and NOE experiments.[11]

The ^1H NMR spectrum of 2a showed, besides two acetoxyl signals (δ 2.06, 2.08), signals at δ 4.21 (d, J=12.0 Hz), 4.32 (dd, J=3.5, 12.0 Hz)(1-H_2), 3.04 (ddd, J=6.0, 7.0, 13.5 Hz, 4β-H), 2.98 (dddd, J=8.0, 10.5, 11.0, 13.5 Hz, 5β-H), 1.36 (ddd, J=3.5, 10.5, 14.0 Hz), 1.98 (dd, J=8.0, 14.0 Hz)(6-H_2), 5.20 (dd, J=3.5, 3.5 Hz, 7α-H), 2.07 (ddq, J=3.5, 7.0, 11.0 Hz, 8α-H), 2.21 (ddd, J=3.5, 11.0, 11.0 Hz, 9β-H), 1.02 (d, J=7.0 Hz, 8-CH_3), 4.06 (dd, J=7.0, 11.5 Hz), 4.48 (dd, J=6.0, 11.5 Hz)(11-H_2). The NOE's were observed between the following pairs of protons[10]: 4β-H & 9β-H (4%), 5β-H & 9β-H (11%), 9β-H & 4β-H (3%), 9β-H & 5β-H (8%), 9β-H & 8β-CH_3 (4%), and 8β-CH_3 & 9β-H (10%). Based on these physical data and detailed ^{13}C NMR examinations, the relative configuration of 2a has been elucidated as shown. In addition, by the detailed ^1H and ^{13}C NMR examinations of 3a including the NOE experiments of ^1H NMR,[12] 3a has been shown to be the C-4 epimer of 2a.

To determine the absolute configurations of 2a and 3a, loganin (8)[13] was converted to 2a and 3a. Thus, loganin (8) was first hydrolyzed to give 8a[13] by aq. 4% KOH treatment (reflux, 3 h). Enzymatic hydrolysis of 8a (β-glucosidase, H_2O, 41 °C, 64 h, with stirring) followed by $NaBH_4$ reduction in aq. 95% EtOH (25 °C, 2 h) furnished two products : 2 (11% from 8), a white powder, $[\alpha]_D^{25}$ +117° (MeOH), $C_{10}H_{16}O_4$, IR (KBr): 3464 (br), 2894, 1719 cm^{-1} and 3 (10% from 8), a white powder, $[\alpha]_D^{25}$ -35° (MeOH), $C_{10}H_{16}O_4$, IR (KBr): 3545 (br), 2879, 1721 cm^{-1}. Respective acetylation of 2 and 3 provided 2a and 3a. Thus the absolute configurations of two iridoid-lactones, which were obtained above by alkaline hydrolysis of pulosarioside (1), have been clarified.

The ^1H NMR spectrum (in d_5-pyridine-D_2O) of pulosarioside (1) showed signals due to one *sec*.CH_3 group (δ 1.06, d, J=7 Hz), one methoxycarbonyl group (δ 3.75), two aldehydic protons (δ 9.85, 9.87) and two olefinic protons (δ 7.71, 7.76, both br s, 3'-H, 3"-H). In addition to accumulated spectral properties of pulosarioside (1), the product ratio (*vide supra*) of two iridoid-lactones (2a, 3a) and two secoiridoid-glucosides (5a, 6a) obtained by alkaline treatment of pulosarioside (1), have shown that 1 contains one mole of iridoid and two moles of secoiridoid-glucosides connecting through two ester-linkages in its molecule.

Silylation of 1 (*t*-butyldimethylsilyl chloride-imidazole in DMF, 35 °C, 72 h) and subsequent alkaline treatment of the product (2% NaOMe-MeOH, 25 °C, 1 h) provided two products: 2b, a white powder, $[\alpha]_D^{22}$ +6.0° ($CHCl_3$), $C_{16}H_{30}O_4Si$, IR ($CHCl_3$) : 3680, 3425, 2929, 2856, 1754, 1604 cm^{-1} and 7 (a mixture of C-7' epimers), IR ($CHCl_3$): 2914, 1721 cm^{-1}, ^1H NMR: δ 3.67, 3.70 (totally 3H, both s, 7'-OCH_3), 7.49 (br s, 3'-H). Oxidation of 2b with pyridinium chlorochromate (PCC) in CH_2Cl_2 gave 4, a white powder, $[\alpha]_D^{22}$ +16° ($CHCl_3$), $C_{16}H_{28}O_4Si$, UV (MeOH): 238 nm (ε 1000), IR ($CHCl_3$): 2927, 2857, 1741, 1080 cm^{-1}. The ^1H NMR spectrum of 4 showed, besides signals due to one *t*-butyldimethylsilyl group (δ 0.07, 6H, s; δ 0.89, 9H, s), signals at δ 4.31 (d, J=12.0 Hz), 4.49 (dd, J=3.5, 12.0 Hz)(1-H_2), 3.04 (ddd, J=5.0, 7.0, 10.0 Hz, 4-H), 3.24 (dddd, J=3.0, 7.0, 10.0, 10.0 Hz, 5-H), 2.15 (ddd, J=2.0, 10.0, 18.0 Hz), 2.35 (dd, J=10.0, 18.0 Hz)(6-H_2), 2.47 (ddd, J=2.0, 6.5,



1 : R = H
1a : R = Ac

2 : $R^1 = R^2$ = H
2a : $R^1 = R^2$ = Ac
2b : R^1 = Si, R^2 = H

3 : $R^1 = R^2$ = H
3a : $R^1 = R^2$ = Ac

4

8 : R = CH_3
8a : R = H

5a : R^1 = H, R^2 = OCH_3, R^3 = Ac
6a : R^1 = OCH_3, R^2 = H, R^3 = Ac
7 : R^1, R^2 = H, OCH_3, R^3 = Si

Table I. ^{13}C NMR Data

	1 a)	1a b)	2a b)	3a b)		1 a)	1a b)	5a b)	6a b)
1	64.3	64.5	67.4	69.2	1', 1"	96.6	96.9	95.4	96.2
3	62.9	64.5	172.4	172.4	3', 3"	152.7	151.0	151.5	151.5
4	46.1	46.0	41.6	43.2	4', 4"	108.9, 109.1	110.2	105.3	104.7
5	41.0	41.2	35.2	36.9	5', 5"	26.9, 27.3	26.3	21.9	24.2
6	36.0	36.1	35.4	39.0	6', 6"	44.1, 44.4	43.7	29.1	30.4
7	76.3	76.7	77.9	78.9	7', 7"	200.6	200.4, 200.5	101.4	103.5
8	37.5	37.9	40.5	41.0	8', 8"	133.9	133.2	131.2	130.8
9	44.3	44.2	43.3	43.5	9', 9"	49.3	49.4	41.7	42.1
10	14.5	14.6	12.6	13.3	10', 10"	119.6	120.6	121.1	121.4
11	174.8	173.7	62.3	62.3	11', 11"	166.3, 166.5	166.3, 166.6	164.3	164.3

a), b) Measured at 125 MHz a) in d_5-pyridine or b) in $CDCl_3$.

6.5 Hz, 8-H), 2.38 (ddd, J=3.0, 3.5, 6.5 Hz, 9-H), 1.14 (d, J=6.5 Hz, 8β-CH_3), 3.64 (dd, J=10.0, 10.0 Hz), 4.09 (dd, J=5.0, 10.0 Hz)(11-H_2). The NOE's were observed between the following pairs of protons: 4β-H & 5β-H (11%), 5β-H & 4β-H (4%), 5β-H & 9β-H (9%), 9β-H & 5β-H (17%), 9β-H & 8β-CH_3 (3%), and 8β-CH_3 & 9β-H (12%). Based on these findings together with ^{13}C NMR analysis, the stereostructures of 2b and 4 with 4(R) configurations have been elucidated.[14] In addition, it has been shown that 1-OH and 7-OH groups in the iridoid moiety are respectively esterified with the carboxyl residues of two secoiridoid-glucoside moieties in pulosarioside (1).

Furthermore, enzymatic hydrolysis of 1 with lipase (Triacylglycerol lipase from Porcine pancreas, Type II Crude, Sigma) in a phosphate buffer (pH 5.5)(38 °C, 72 h) followed by treatment with ionic resin (Dowex 50w x 8, H^+ form) provided 2. On alkaline treatment (2% NaOMe-MeOH, 20 °C, 10 h), 2 was epimerized to 3, while 3 was not affected by a similar alkaline treatment. Consequently, the C-4 configuration in 1 has been confirmed as R which is preserved in 2. Finally, the above-mentioned findings and the ^{13}C NMR examinations in detail of 1, 1a,[15] 2a, 3a, 5a, and 6a, have substantiated the absolute configuration of pulosarioside (1) as shown.

It is interestingly pointed out here that pulosarioside (1) is very bitter comparable to swertiamarin which is the bitter principle of *Swertia japonica* Makino[16]

and this property may be important for the use of "pulosari" as a stomachic.

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4) The molecular composition of the compound given with the chemical formula was determined by elemental analysis or high resolution mass spectrometry.

5) The aq. 50% acetone extract of the air-dried bark ("pulosari") contained pulosarioside (1) but lacked these monoacetal and diacetal derivatives as shown by TLC and HPLC, so that these acetals were considered secondarily formed during the MeOH extraction. Monoacetal mixture, IR (KBr) : 3494 (br), 2920, 1696, 1625, 1275, 1062 cm^{-1}, ^1H NMR (d_5-pyridine-D_2O, δ) : 1.09 (d, J=6.5 Hz, 8-CH_3), 3.35, 3.58 (both s, OCH_3x2), 3.74 (s, $COOCH_3$), 9.86 (s, CHO), ^{13}C NMR (d_5-pyridine, δ_C) : 14.6 (10-C), 51.4, 52.0, 53.0 (OCH_3x3), 103.1 ($^1/_2$C), 103.3 ($^1/_2$C), 200.6 (7', 7"-C), SIMS : m/z 1013 $(M+Na)^+$. Diacetal, mp 104-105 °C (EtOH), $[\alpha]_D^{25}$ -71° (MeOH), $C_{47}H_{72}O_{25}\cdot 2H_2O$, IR (KBr) : 3380 (br), 2917, 1701, 1634 cm^{-1}, ^1H NMR (d_5-pyridine-D_2O, δ) : 1.12 (d, J=4.0 Hz, 8-CH_3), 3.31 (3H), 3.33 (9H) (both s, OCH_3x4), 3.70 (s, $COOCH_3$), ^{13}C NMR (d_5-pyridine, δ_C) : 51.4, 52.0 (2C), 52.9 (2C) (OCH_3x4), 103.0, 103.2 (7', 7"-C), SIMS : m/z 1059 $(M+Na)^+$.

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7) TLC examination of the reaction mixture showed that only iridoid-lactone 2 was liberated at the initial stage of alkaline treatment and 3 was formed gradually at the time proceeded.

8) The ^1H NMR spectra were measured at 500 MHz in $CDCl_3$ unless otherwise specified.

9) 6a : ^1H NMR (δ) : 5.37 (d, J=1.5 Hz, 1'α-H), 7.59 (d, J=2.5 Hz, 3'-H), 3.22 (dddd, J=1.5, 2.5, 5.5, 13.5 Hz, 5'β-H), 1.67 (ddd, J=3.0, 13.5, 13.5 Hz, 6'α-H), 1.82 (ddd, J=1.5, 5.0, 13.5 Hz, 6'β-H), 5.29 (dd, J=3.0, 5.0 Hz, 7'α-H), 5.46 (ddd, J=9.5, 9.5, 17.0 Hz, 8'-H), 2.61 (ddd, J=1.5, 5.5, 9.5 Hz, 9'β-H), 5.11 (dd, J=7.5, 17.0 Hz), 5.28 (dd, J=7.5, 9.5 Hz)(10'-H_2). NOE experiments : 5'β-H & 6'β-H (6%), 5'β-H & 9'β-H (10%), 6'β-H & 5'β-H (9%), 6'α-H & 7'α-H (7%), 7'α-H & 6'α-H (4%), 7'β-OCH_3 & 6'β-H (3%).[10]

10) The magnitude of NOE (%) given in the parenthesis was obtained when the underlined proton was irradiated.

11) 5a : ^1H NMR (δ) : 5.34 (d, J=1.5 Hz, 1'α-H), 7.56 (d, J=2.5 Hz, 3'-H), 2.82 (dddd, J=1.0, 2.5, 5.5, 9.5 Hz, 5'β-H), 1.54 (ddd, J=9.5, 10.0, 13.5 Hz, 6'α-H), 1.94 (ddd, J=1.0, 2.5, 13.5 Hz, 6'β-H), 5.22 (dd, J=2.5, 10.0 Hz, 7'β-H), 5.43 (ddd, J=9.5, 10.0, 17.0 Hz, 8'-H), 2.67 (ddd, J=1.5, 5.5, 9.5 Hz, 9'β-H), 5.28 (dd, J=7.5, 10.0 Hz), 5.29 (dd, J=7.5, 17.0 Hz)(10'-H_2). NOE experiments : 5'β-H & 6'β-H (4%), 6'β-H & 5'β-H (8%), 6'β-H & 7'β-H (5%), 7'β-H & 6'β-H (11%).[10]

12) 3a : ^1H NMR (δ) : 3.99 (dd, J=11.5, 11.5 Hz), 4.41 (dd, J=6.0, 11.5 Hz)(1-H_2), 2.65 (ddd, J=5.0, 6.0, 11.0 Hz, 4α-H), 2.48 (dddd, J=7.5, 10.0, 11.0, 11.0 Hz, 5β-H), 1.70 (ddd, J=4.0, 10.0, 14.0 Hz), 2.28 (ddd, J=1.0, 7.5, 14.0 Hz)(6-H_2), 5.26 (ddd, J=1.0, 4.0, 4.0 Hz, 7α-H), 1.87 (ddq, J=4.0, 7.0, 9.5 Hz, 8α-H), 2.35 (dddd, J=6.0, 9.5, 11.0, 11.5 Hz, 9β-H), 1.02 (d, J=7.0 Hz, 8β-CH_3), 4.24 (dd, J=5.0, 11.5 Hz), 4.43 (dd, J=6.0, 11.5 Hz)(11-H_2). NOE experiments : 5β-H & 11-H (δ 4.24)(5%), 9β-H & 11-H (δ 4.43)(6%), 8β-CH_3 & 9β-H (5%).[10]

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14) When the C-4 carbinyl group was protected with a *t*-butyldimethylsilyl group, the C-4 configuration seemed hardly epimerized on alkaline treatment.

15) 1a, a white powder, $[\alpha]_D^{20}$ -85° ($CHCl_3$), $C_{61}H_{78}O_{32}$, was prepared from 1 by acetylation [Ac_2O-pyridine (1:1), 20 °C, 4 h].

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