Indonesian Medicinal Plants. XII.[1] Four Isomeric Lignan-Glucosides from the Bark of *Aegle marmelos* (Rutaceae)

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From the bark of *Aegle marmelos* CORREA (Rutaceae), an Indonesian medicinal plant, two new lignan-glucosides, (−)-lyoniresinol 2α-*O*-β-D-glucopyranoside (3) and (−)-4-epi-lyoniresinol 3α-*O*-β-D-glucopyranoside (4), have been isolated together with two known lignan-glucosides, (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (1) and (−)-lyoniresinol 3α-*O*-β-D-glucopyranoside (2).

Keywords Indonesian medicinal plant; *Aegle marmelos*; Rutaceae; lignan-glucoside; lyoniresinol glucoside; 4-epi-lyoniresinol glucoside

The bark of *Aegle marmelos* CORREA (Rutaceae), which is called "maja" in Flores Island, Indonesia, has been prescribed as a remedy for diabetes.[2] As a part of our chemical characterization studies of Indonesian medicinal plants,[1,3] we have been investigating the chemical constituents of the bark of *Aegle marmelos* collected near the seaside in Flores Island and have isolated four isomeric lignan-glucosides.

The aqueous phase, which was obtained by partition between ethyl acetate and water of the methanol extract of the bark, was further partitioned into a mixture of *n*-butanol and water. The *n*-butanol-soluble portion, which gave a residue amounting to 1.7% of the bark upon evaporation, was then subjected to silica gel column chromatography and high-performance liquid chromatography (HPLC) with a reversed-phase adsorbent to afford four lignan-glucosides, compounds **1** (0.0085% from the bark), **2** (0.0023%), **3** (0.0010%) and **4** (0.0010%). Among them, compounds **1** and **2** were identified as (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside[4] and (−)-lyoniresinol 3α-*O*-β-D-glucopyranoside,[5] respectively.

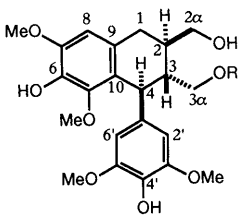
The FAB-MS of **3** and **4** gave a molecular ion peak at *m/z* 582, corresponding to $C_{28}H_{38}O_{13}$, together with two ion peaks at *m/z* 583 for $(M+H)^+$ and at *m/z* 605 for $(M+Na)^+$. The IR and UV spectra of **3** and **4** showed similar absorption patterns to those of **1** and **2**. The ^{1}H- and ^{13}C-NMR spectra of **3** and **4** exhibited signals characteristic of an aryl-tetralin type lignan-glucoside (Table I).

Enzymatic hydrolysis of **3** with cellulase liberated D-glucose ($[\alpha]_D$ +46.5° in H_2O) and an aglycone (**5**, $[\alpha]_D$ −47.9° in MeOH). The physical data for **5** were identical with those for (−)-lyoniresinol.[5,6]

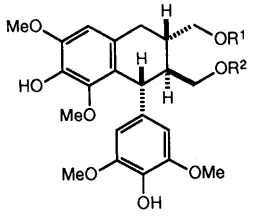
The location of the glucosidic linkage at the 2α position in **3** was confirmed by the glycosylation shifts[7] observed in the ^{13}C-NMR spectrum of **3** (as compared with **5**) for the signals assignable to C-2α (+8.2 ppm) and C-2 (−2.6 ppm) (Table I). Furthermore, the heteronuclear multiple bond correlation (HMBC) experiment on **3** revealed the presence of a cross-peak between the anomeric proton (δ 4.24, 1″-H) and the hydroxymethylene carbon (δ_c 74.9) at the 2α position. The coupling constant observed for the anomeric proton (1″-H, J=8.1 Hz) in the ^{1}H-NMR spectrum of **3** indicated the β-glycoside linkage for the D-glucose moiety. Consequently, compound **3** was concluded to be (−)-lyoniresinol 2α-*O*-β-D-glucopyranoside.

Enzymatic hydrolysis of compound **4** with cellulase liberated D-glucose ($[\alpha]_D$ +47.2° in H_2O) and an aglycone (**6**, $[\alpha]_D$ −140.8° in MeOH).

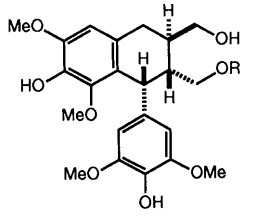
The nuclear Overhauser enhancement and exchange spectroscopy (NOESY) experiments on (−)-lyoniresinol (**5**), the aglycone of compound **3**, showed the presence of NOEs between 4-H (δ 4.30) and 2-H (δ 1.62) and between 3-H (δ 1.96) and 2′-H (δ 6.37). On the other hand, NOESY experiments on the aglycone (**6**) of **4** showed the presence



1: R= D-glcose
(+)-lyoniresinol: R= H

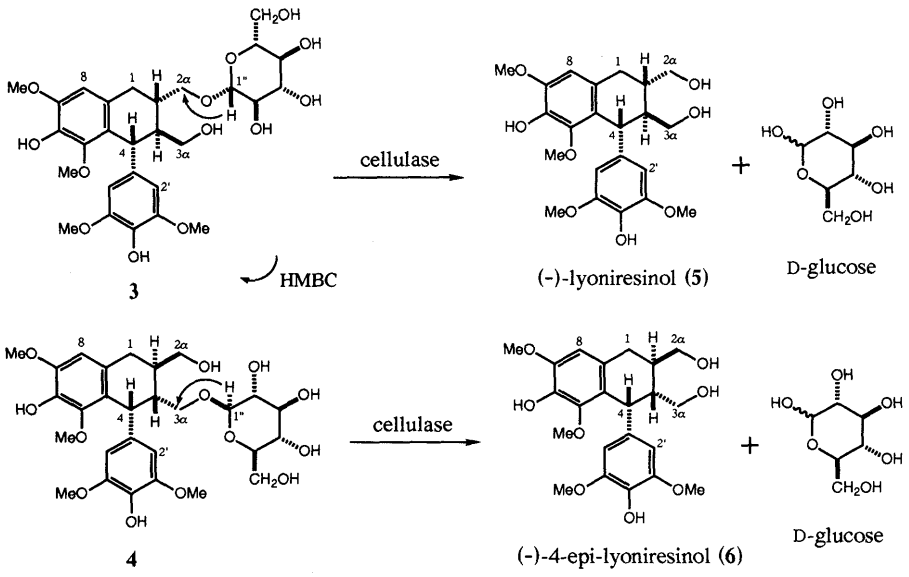


2: R^1= H, R^2= D-glucose
3: R^1= D-glucose, R^2= H
(-)-lyoniresinol (5): R^1=R^2= H



4: R= D-glucose
(-)-4-epi-lyoniresinol (6): R= H

Fig. 1



3 4

Fig. 2

TABLE I. ^{13}C-NMR Data for Four Isomeric Lignan-Glucosides (**1**, **2**, **3**, **4**) and the Aglycones (**5**, **6**) (in CD_3OD, δ_c in ppm)

	1	2	3	4	5	6
C-1	33.8	33.9	34.1	34.1	33.6	34.2
C-2	40.7	41.3	38.3	35.3	40.9	35.3
C-3	46.7	46.6	50.1	42.1	48.7	44.9
C-4	42.8	43.3	42.8	41.9	42.3	41.8
C-5	147.7	147.6	147.7	146.4	147.7	146.4
C-6	139.0	139.5	139.6	138.6	139.3	138.6
C-7	148.7	148.7	148.7	149.1	148.6	149.1
C-8	108.0	107.8	107.7	107.6	107.7	107.5
C-9	130.3	130.2	130.1	128.1	130.2	128.0
C-10	126.5	126.3	126.6	127.5	126.2	127.5
C-1′	134.6	134.6	134.5	134.9	134.5	134.9
C-2′,6′	107.1	107.1	107.0	109.5	106.8	109.0
C-3′,5′	149.1	149.0	149.0	148.5	149.0	148.5
C-4′	139.4	138.9	140.0	135.1	138.9	135.0
C-2α	66.4	66.2	74.9	65.2	66.7	65.6
C-3α	71.6	72.0	63.3	71.6	64.1	63.5
5-OMe	60.3	60.1	60.0	60.0	60.1	59.9
7-OMe	56.7	56.6	56.6	56.5	56.6	56.5
3′,5′-OMe	57.0	56.9	56.9	57.0	56.7	56.8
C-1″	104.9	104.3	104.7	104.7		
C-2″	75.3	75.1	75.2	75.5		
C-3″	78.3	78.2	78.2	78.3		
C-4″	71.8	71.6	71.7	72.0		
C-5″	78.0	78.0	78.0	78.2		
C-6″	62.9	62.7	62.8	63.1		

of NOEs between 4-H (δ 4.57) and 3-H (δ 1.93) and between 2-H (δ 1.99) and 2′-H (δ 6.39). It was concluded that the aglycone (**6**) is a new lignan, the 4-epi-derivative of lyoniresinol.

The absolute configuration of compound **4** was determined by examination of the circular dichroism (CD) spectra of compounds **1**, **2**, **3**, and **4**, which showed the first couplets corresponding to the B-absorption band. The signs of their CD spectra indicated that the absolute configurations of the aryl substituent at C-4 are 4*S* for **1** and 4*R* for **2**, **3**, and **4** (Fig. 4), since the sign of the first couplet reflects the aryl substituents at C-4, namely negative for 4*S* and positive for 4*R*.[8]

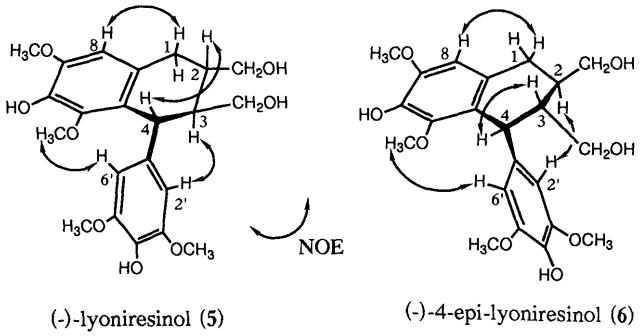


Fig. 3

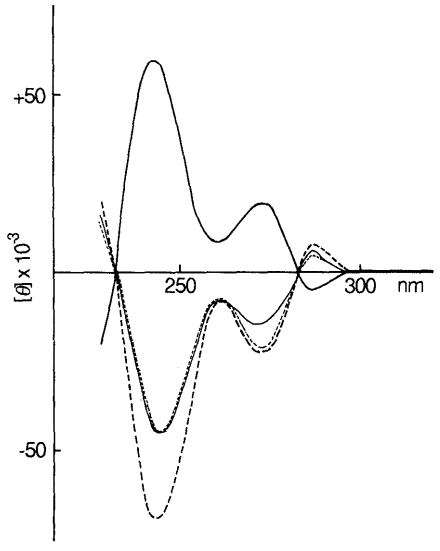


Fig. 4. CD Curves of **1**, **2**, **3** and **4**
——, **1**; – – –, **2**; ——, **3**; ------, **4**.

The location of the D-glucose moiety at the 3α position was confirmed by the glycosylation shifts[7] observed in the ^{13}C-NMR spectrum of **4** (as compared with **6**) for the signals assignable to C-3α (+8.1 ppm) and C-3 (−2.8 ppm) (Table I). Furthermore, the HMBC spectrum of **4** exhibited

a cross-peak between the anomeric proton (δ 4.31, 1″-H) and the hydroxymethylene carbon (δ_c 71.6) at the 3α position. The coupling constant of the anomeric proton (1″-H, J=7.4 Hz) in the ^{1}H-NMR spectrum of **4** indicated the β-glycoside linkage for the D-glucose moiety.

Consequently, the structure of compound **4** was determined as (−)-4-epi-lyoniresinol 3α-*O*-β-D-glucopyranoside.

Experimental

Instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[1]

Isolation of Four Lignan-Glucosides The air-dried bark (2.0 kg) of *Aegle marmelos* CORREA (Rutaceae), which was collected at the Larantuka area in Flores Island, Indonesia, in August 1988, was extracted with MeOH at room temperature and the solvent was evaporated under reduced pressure to give the MeOH extract (200 g). The MeOH extract was partitioned into EtOAc–H_2O (1 : 1). Furthermore the water phase was shaken with *n*-BuOH and the *n*-BuOH phase was taken and concentrated under reduced pressure to give the *n*-BuOH extract (32 g). The *n*-BuOH extract (22 g) was subjected to column chromatography (SiO_2 2 kg, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1, lower phase), and reversed-phase HPLC (LiChrosorb RP-18, 0.25 m × 10 mm, MeOH : H_2O = 1 : 2) to afford (+)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**1**, 175 mg, 0.0085% from the bark),[4] (−)-lyoniresinol 3α-*O*-β-D-glucopyranoside (**2**, 45 mg, 0.0023%),[5] (−)-lyoniresinol 2α-*O*-β-D-glucopyranoside (**3**, 20 mg, 0.0010%), and (−)-4-epi-lyoniresinol 3α-*O*-β-D-glucopyranoside (**4**, 20 mg, 0.0010%). (+)-Lyoniresinol 3α-*O*-β-D-glucopyranoside (**1**): CD ($c=5.25\times10^{-5}$, MeOH) $[\theta]^{18}$ (nm): +59900 (243), +18500 (273), −4700 (286). ^{13}C-NMR: as given in Table I. (−)-Lyoniresinol 3α-*O*-β-D-glucopyranoside (**2**): CD ($c=1.62\times10^{-5}$, MeOH) $[\theta]^{18}$ (nm): −69100 (243), −22700 (272), +7200 (286). ^{13}C-NMR: as given in Table I.

(−)-Lyoniresinol 2α-*O*-β-D-Glucopyranoside (**3**): A white amorphous solid, $[\alpha]_D$ −51.7° (c=0.41, in MeOH at 27 °C). IR (KBr) cm^{-1}: 3400, 1620, 1515. UV (MeOH) nm (log ε): 224 (sh), 276 (4.18), 282 (4.18). CD ($c=1.72\times10^{-5}$, MeOH) $[\theta]^{18}$ (nm): −48800 (244), −15000 (272), +5200 (286). ^{1}H-NMR (CD_3OD) δ: 1.83 (1H, m, 2-H), 1.87 (1H, m, 3-H), 2.57 (1H, dd, J=10.9, 15.0 Hz, 1-H_a), 2.88 (1H, dd, J=4.0, 15.0 Hz, 1-H_b), 3.17 (1H, t, J=8.1 Hz, 2″-H), 3.24—3.36 (3H, m, 3″-H, 4″-H, 5″-H), 3.32 (3H, s, 5-OCH_3), 3.49—3.63 (3H, m, 3α-H_2, 2α-H_a), 3.94 (1H, dd, J=5.4, 9.9 Hz, 2α-H_b), 3.65 (1H, dd, J=5.3, 11.9 Hz, 6″-H_a), 3.74 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.85 (3H, s, 7-OCH_3), 3.86 (1H, dd, J=1.8, 11.9 Hz, 6″-H_b), 4.24 (1H, d, J=8.1 Hz, 1″-H), 4.30 (1H, br d, J=6.0 Hz, 4-H), 6.38 (2H, s, 2′-H, 6′-H), 6.57 (1H, s, 8-H). ^{13}C-NMR: as given in Table I. FAB-MS m/z: 583 $(M+H)^+$, 582 (M^+). High-resolution FAB-MS m/z: Calcd for $C_{28}H_{38}O_{13}$: 582.2312. Found: 582.2327 (M^+).

(−)-4-Epi-lyoniresinol 3α-*O*-β-D-Glucopyranoside (**4**): A white amorphous solid, $[\alpha]_D$ −144.8° (c=0.42, in MeOH at 26 °C). IR (KBr) cm^{-1}: 3400, 1620, 1520. UV (MeOH) nm (log ε): 224 (sh), 276 (4.20), 282 (4.17). CD ($c=1.74\times10^{-5}$, MeOH) $[\theta]^{18}$ (nm): −44700 (244), −22800 (272), +4800 (286). ^{1}H-NMR (CD_3OD) δ: 2.00 (1H, m, 2-H), 2.13 (1H, m, 3-H), 2.71 (1H, dd, J=11.5, 16.8 Hz, 1-H_a), 2.96 (1H, dd, J=5.7, 16.8 Hz, 1-H_b), 3.21—3.39 (4H, m, 2″-H, 3″-H, 4″-H, 5″-H), 3.22 (3H, s, 5-OCH_3), 3.48 (1H, dd, J=5.9, 9.9 Hz, 3α-H_a), 3.53 (1H, dd, J=5.9, 11.1 Hz, 2α-H_a), 3.60 (1H, dd, J=3.9, 11.1 Hz, 2α-H_b), 3.65 (1H, dd, J=5.7, 12.0 Hz, 6″-H_a), 3.74 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.81—3.89 (2H, m, 3α-H_b, 6″-H_b), 3.85 (3H, s, 7-OCH_3), 4.31 (1H, d, J=7.4 Hz, 1″-H), 4.60 (1H, br d, J=4.5 Hz, 4-H), 6.42 (2H, s, 2′-H, 6′-H), 6.57 (1H, s, 8-H). ^{13}C-NMR: as given in Table I. FAB-MS m/z: 583 $(M+H)^+$, 582 (M^+). High-resolution FAB-MS m/z: Calcd for $C_{28}H_{38}O_{13}$: 582.2312. Found: 582.2301 (M^+).

Enzymatic Hydrolysis of (−)-Lyoniresinol 2α-*O*-β-D-Glucopyranoside (3) Giving (−)-Lyoniresinol (5) A solution of (−)-lyoniresinol 2α-*O*-β-D-glucopyranoside (**3**, 10 mg) in H_2O (1 ml) was treated with cellulase (Sigma, *ca.* 10 mg) at 37 °C for 24 h. The reaction mixture was extracted with EtOAc, and the EtOAc extract was evaporated under reduced pressure to give (−)-lyoniresinol[5,6] (**5**, 6 mg). The aqueous phase was passed through a silica gel column (SiO_2 3 g, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1, lower phase) to afford D-glucose ($[\alpha]_D$ +46.5°, c=0.10, 24 h after dissolution in H_2O).

Enzymatic Hydrolysis of (−)-4-Epi-lyoniresinol 3α-*O*-β-D-Glucopyranoside (4) Giving (−)-4-Epi-lyoniresinol (6) A solution of (−)-4-epi-lyoniresinol 3α-*O*-β-D-glucopyranoside (**4**, 10 mg) in H_2O (1 ml) was treated with cellulase (Sigma, *ca.* 10 mg) at 37 °C for 24 h. The reaction mixture was extracted with EtOAc, and the EtOAc extract was evaporated under reduced pressure to give (−)-4-epi-lyoniresinol (**6**, 6 mg). The aqueous phase was passed through a silica gel column (SiO_2 3 g, $CHCl_3$: MeOH : H_2O = 7 : 3 : 1, lower phase) to afford D-glucose ($[\alpha]_D$ +47.2°, c=0.09, 24 h after dissolution in H_2O).

6: A white amorphous solid, $[\alpha]_D$ −140.8° (c=0.23, in $CHCl_3$ at 27 °C). IR ($CHCl_3$) cm^{-1}: 3430, 1615, 1455, 1495. ^{1}H-NMR (CD_3OD) δ: 1.85—2.05 (2H, m, 2-H, 3-H), 2.66 (1H, dd, J=11.0, 16.9 Hz, 1-H_a), 2.97 (1H, dd, J=5.5, 16.9 Hz, 1-H_b), 3.24 (3H, s, 5-OCH_3), 3.42 (1H, dd, J=8.0, 10.5 Hz, 3α-H_a), 3.59 (1H, dd, J=5.7, 10.4 Hz, 2α-H_a), 3.67 (1H, dd, J=3.9, 10.4 Hz, 2α-H_b), 3.73 (6H, s, 3′-OCH_3, 5′-OCH_3), 3.84 (3H, s, 7-OCH_3), 4.57 (1H, d, J=4.0 Hz, 4-H), 6.39 (2H, s, 2′-H, 6′-H), 6.57 (1H, s, 8-H). ^{13}C-NMR: as given in Table I. EI-MS m/z (%): 420 (M^+, 2.8), 57 (100). High-resolution EI-MS m/z: Calcd for $C_{22}H_{28}O_8$: 420.1784. Found: 420.1791 (M^+).

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References

1) *a*) Part XI: I. Kitagawa, P. Simanjuntak, T. Watano, H. Shibuya, S. Fujii, Y. Yamagata, M. Kobayashi, *Chem. Pharm. Bull.*, submitted; *b*) Part X: K. Ohashi, T. Tanikawa, Y. Okumura, K. Kawazoe, N. Tatara, M. Minato, H. Shibuya, I. Kitagawa, *ibid.*, **42**, 1791 (1994); *c*) Part IX: K. Ohashi, H. Kojima, T. Tanikawa, Y. Okumura, K. Kawazoe, N. Tatara, H. Shibuya, I. Kitagawa, *ibid.*, **42**, 1596 (1994).
2) I. Kitagawa, "Research Report of Investigation of Naturally Occurring Drug Materials in Indonesia-2," Osaka, 1990.
3) *a*) H. Shibuya, R.-s. Zhang, J. D. Park, N. I. Baek, Y. Takeda, M. Yoshikawa, I. Kitagawa, *Chem. Pharm. Bull.*, **40**, 2647 (1992), and preceding papers cited therein; *b*) M. Yoshikawa, E. Harada, S. Aoki, J. Yamahara, N. Murakami, H. Shibuya, I. Kitagawa, *ibid.*, **41**, 2101 (1993); *c*) I. Kitagawa, P. Simanjunrtak, K. Hori, N. Nagami, H. Shibuya, M. Kobayashi, *ibid.*, **42**, 1050 (1994); *d*) I. Kitagawa, T. Mahmud, P. Simanjuntak, K. Hori, T. Uji, H. Shibuya, *ibid.*, **42**, 1416 (1994).
4) *a*) K. Yoshimoto, Y. Itatani, Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 2065 (1980); *b*) O. Tanaka, *Yakugaku Zasshi*, **105**, 323 (1985); *c*) H. Shibuya, Y. Takeda, R.-s. Zhang, A. Tanitame, Y. Tsai, I. Kitagawa, *Chem. Pharm. Bull.*, **40**, 2639 (1992).
5) H. Achenbach, M. Lowel, R. Waibel, M. Gupta, P. Solis, *Planta Med.*, **58**, 270 (1992).
6) D. Dada, A. Corbani, P. Manitto, G. Speranza, L. Lunazzi, *J. Nat. Prod.*, **52**, 1327 (1989).
7) R. Kasai, M. Suzue, J. Asakawa, O. Tanaka, *Tetrahedron Lett.*, **1977**, 175.
8) *a*) J. Sakakibara, H. Ina, M. Yasue, *Yakugaku Zasshi*, **94**, 1377 (1974); *b*) P. B. Hulbert, W. Klyne, P. M. Scopes, *J. Chem. Res.* (*S*), 27 (1981).