

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

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Received March 22, 1994; accepted May 9, 1994

From the bark of *Aegle marmelos* CORREA (Rutaceae), an Indonesian medicinal plant, two new lignan-glucosides, (–)-lyoniresinol 2 α -O- β -D-glucopyranoside (**3**) and (–)-4-epi-lyoniresinol 3 α -O- β -D-glucopyranoside (**4**), have been isolated together with two known lignan-glucosides, (+)-lyoniresinol 3 α -O- β -D-glucopyranoside (**1**) and (–)-lyoniresinol 3 α -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 3 α -O- β -D-glucopyranoside⁴⁾ and (–)-lyoniresinol 3 α -O- β -D-glucopyranoside,⁵⁾ respectively.

The FAB-MS of **3** and **4** gave a molecular ion peak at *m/z* 582, corresponding to C₂₈H₃₈O₁₃, 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 ¹H- and ¹³C-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 ([α]_D +46.5° in H₂O) and an aglycone (**5**, [α]_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 2 α position in **3** was confirmed by the glycosylation shifts⁷⁾ observed in the ¹³C-NMR spectrum of **3** (as compared with **5**) for the signals assignable to C-2 α (+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 2 α position. The coupling constant observed for the anomeric proton (1''-H, *J* = 8.1 Hz) in the ¹H-NMR spectrum of **3** indicated the β -glycoside linkage for the D-glucose moiety. Consequently, compound **3** was concluded to be (–)-lyoniresinol 2 α -O- β -D-glucopyranoside.

Enzymatic hydrolysis of compound **4** with cellulase liberated D-glucose ([α]_D +47.2° in H₂O) and an aglycone (**6**, [α]_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

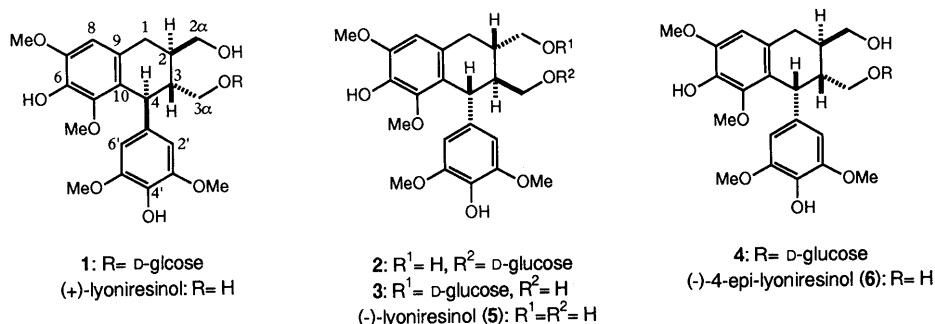


Fig. 1

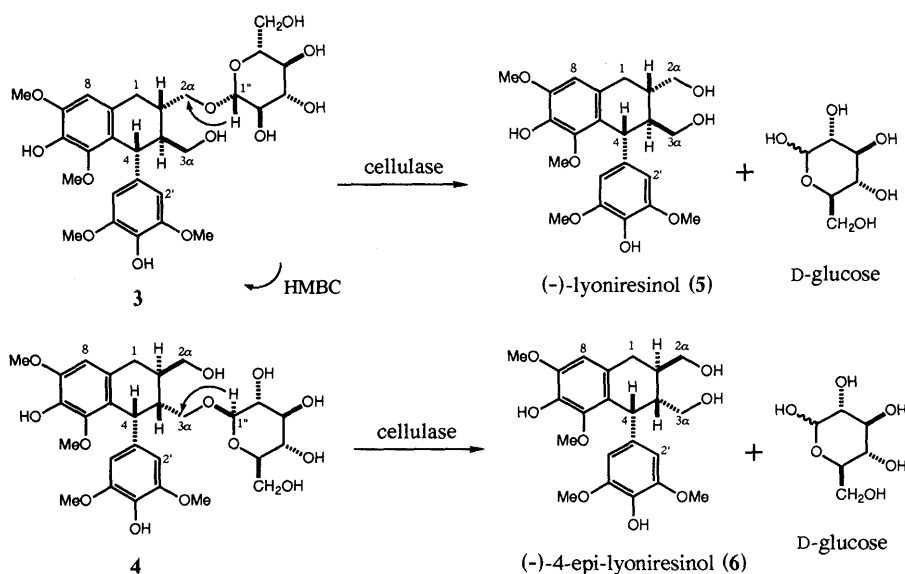


Fig. 2

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-2 α	66.4	66.2	74.9	65.2	66.7	65.6
C-3 α	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 4S for 1 and 4R for 2, 3, and 4 (Fig. 4), since the sign of the first couplet reflects the aryl substituents at C-4, namely negative for 4S and positive for 4R.⁸⁾

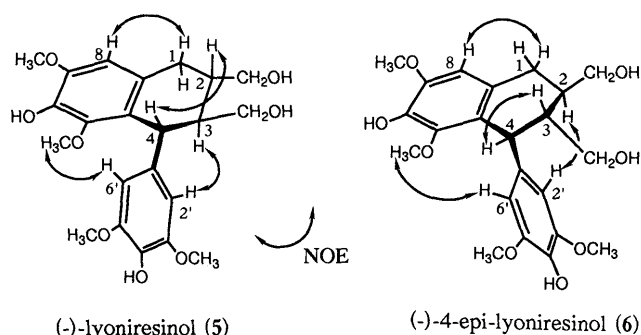
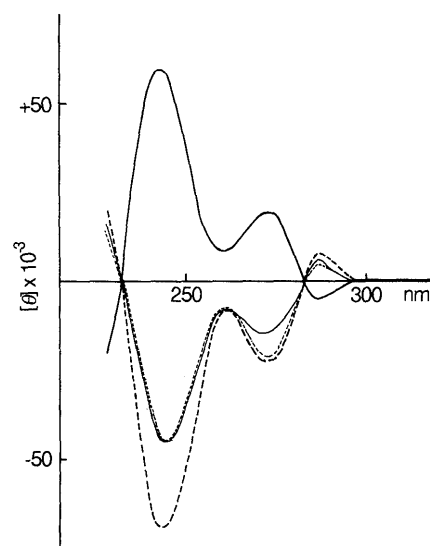


Fig. 3

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

The location of the D-glucose moiety at the 3 α position was confirmed by the glycosylation shifts⁷⁾ observed in the ^{13}C -NMR spectrum of 4 (as compared with 6) for the signals assignable to C-3 α (+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 ^1H -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 Lantuka 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–H₂O (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 (SiO₂ 2 kg, CHCl₃:MeOH:H₂O=7:3:1, lower phase), and reversed-phase HPLC (LiChrosorb RP-18, 0.25 m $\times$ 10 mm, MeOH:H₂O=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 ($c=5.25 \times 10^{-5}$, 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 ($c=1.62 \times 10^{-5}$, 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, $[\alpha]_D -51.7^\circ$ ($c=0.41$, 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 ($c=1.72 \times 10^{-5}$, MeOH) $[\theta]^{18}$ (nm): –48800 (244), –15000 (272), +5200 (286). ^1H -NMR (CD₃OD) 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-OCH₃), 3.49–3.63 (3H, m, 3 α -H₂, 2 α -H_a), 3.94 (1H, dd, $J=5.4$, 9.9 Hz, 2 α -H_b), 3.65 (1H, dd, $J=5.3$, 11.9 Hz, 6''-H_a), 3.74 (6H, s, 3'-OCH₃, 5'-OCH₃), 3.85 (3H, s, 7-OCH₃), 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 C₂₈H₃₈O₁₃: 582.2312. Found: 582.2327 (M⁺).

(–)-4-Epi-lyoniresinol 3α -O- β -D-glucopyranoside (**4**): A white amorphous solid, $[\alpha]_D -144.8^\circ$ ($c=0.42$, 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 ($c=1.74 \times 10^{-5}$, MeOH) $[\theta]^{18}$ (nm): –44700 (244), –22800 (272), +4800 (286). ^1H -NMR (CD₃OD) 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-OCH₃), 3.48 (1H, dd, $J=5.9$, 9.9 Hz, 3 α -H_a), 3.53 (1H, dd, $J=5.9$, 11.1 Hz, 2 α -H_a), 3.60 (1H, dd, $J=3.9$, 11.1 Hz, 2 α -H_b), 3.65 (1H, dd, $J=5.7$, 12.0 Hz, 6''-H_a), 3.74 (6H, s, 3'-OCH₃, 5'-OCH₃), 3.81–3.89 (2H, m, 3 α -H_b, 6''-H_b), 3.85 (3H, s, 7-OCH₃), 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 C₂₈H₃₈O₁₃: 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 H₂O (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 (SiO₂ 3 g, CHCl₃:MeOH:H₂O=7:3:1, lower phase) to afford D-glucose ($[\alpha]_D +46.5^\circ$, $c=0.10$, 24 h after dissolution in H₂O).

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 H₂O (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 (SiO₂ 3 g, CHCl₃:MeOH:H₂O=7:3:1, lower phase) to afford D-glucose ($[\alpha]_D +47.2^\circ$, $c=0.09$, 24 h after dissolution in H₂O).

6: A white amorphous solid, $[\alpha]_D -140.8^\circ$ ($c=0.23$, in CHCl₃ at 27°C). IR (CHCl₃) cm^{-1} : 3430, 1615, 1455, 1495. ^1H -NMR (CD₃OD) 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-OCH₃), 3.42 (1H, dd, $J=8.0$, 10.5 Hz, 3 α -H_a), 3.59 (1H, dd, $J=5.7$, 10.4 Hz, 2 α -H_a), 3.67 (1H, dd, $J=3.9$, 10.4 Hz, 2 α -H_b), 3.73 (6H, s, 3'-OCH₃, 5'-OCH₃), 3.84 (3H, s, 7-OCH₃), 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 C₂₂H₂₈O₈: 420.1784. Found: 420.1791 (M⁺).

Acknowledgement The authors thank the Ministry of Education, Science and Culture of Japan for financial support (Grant-in-Aid No. 63041083).

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