

A New Glycoside, Brachynoside, Isolated from *Clerodendron brachyanthum* SCHAUER

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Eudesmin, syringaresinol dimethyl ether, kusaginins and brachynoside were isolated from the ethanol extract of the leaves of *Clerodendron brachyanthum*. The new glycoside, brachynoside, was assigned as 2-(3,4-dimethoxyphenyl)ethyl 3-O- α -L-rhamnopyranosyl-4-O-(3,4-dihydroxycinnamoyl)- β -D-glucopyranoside from studies on partial methylation and methanolysis products and from analysis of spectroscopic evidence.

Keywords *Clerodendron brachyanthum*; phenylpropanoid glycoside; brachynoside; Verbenaceae

In the previous reports,¹⁾ we described the isolation of three known compounds, clerodin, stigmasta-5,22,25-trien-3 β -ol, and 3-*epi*-glutinol, and five new diterpenes, clerodinins A, B, C, and D, and clerodiol from the hexane extract of the leaves of *Clerodendron brachyanthum*. The residue was subsequently extracted with ethanol. This extract was repeatedly chromatographed on silica gel and Sephadex LH-20 to give two lignans [eudesmin (**1a**)²⁾ and syringaresinol dimethyl ether (**1b**)³⁾] and two glycosides [kusaginins (**2a**)⁴⁾ and brachynoside (**2b**)]. In this paper we wish to report the isolation and structural elucidation of brachynoside (**2b**).

Brachynoside (**2b**) is optically active, $[\alpha]_D^{20} -98.5^\circ$, and from its elementary analysis was suggested to have the formula C₃₁H₄₀O₁₅. The infrared (IR) spectrum of **2b** shows absorption bands indicative of the existence of a hydroxyl group at 3400 cm⁻¹ and a conjugated ester group at 1700 and 1270 cm⁻¹ and the ultraviolet (UV) spectrum of **2b** in methanol shows absorption maxima characteristic of an ester of 3-(3,4-dihydroxyphenyl)-2-propenoic acid⁵⁾ at $\lambda_{\max}^{\text{MeOH}}$ 220, 234, 291, and 333 nm. From the IR spectrum together with the proton nuclear magnetic resonance

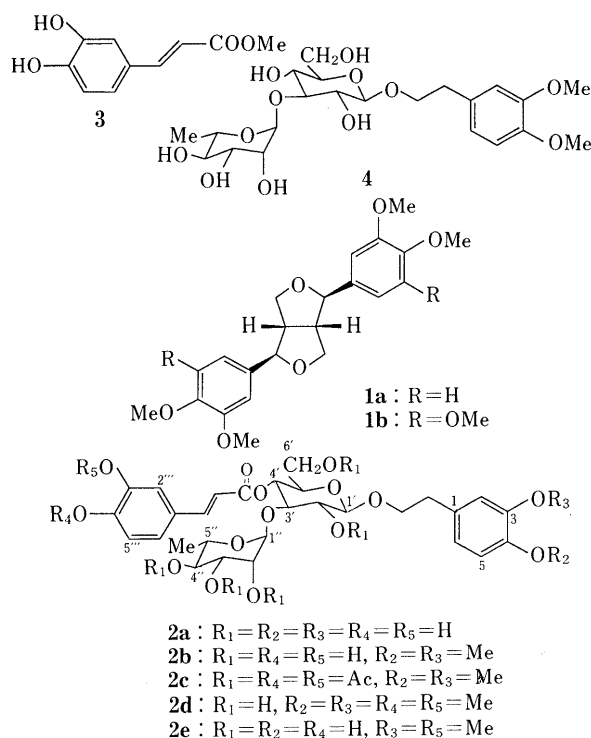
(¹H-NMR) spectrum of **2b**, which has complex signals corresponding to protons on oxygenated carbon atoms at δ 3.0—4.0, it is considered that **2b** has a sugar moiety.

The ¹H-NMR spectrum of **2b** in dimethyl sulfoxide-*d*₆ (DMSO-*d*₆) shows the signals attributed to the two phenolic protons at δ 9.61 and 8.80 (each 1H, s, disappeared on D₂O addition), protons attached to two 1,3,4-trisubstituted benzene rings at δ 7.28 and 6.67 (each 1H, s, 2'''-H, 2-H), 7.09 and 6.81 (each 1H, d, *J*=8.3 Hz, 6'''-H, 5'''-H), and 6.79 and 6.63 (each 1H, d, *J*=8.1 Hz, 6-H, 5-H), two *trans* olefinic protons at δ 7.53 and 6.40 (each 1H, d, *J*=15.8 Hz), two anomeric protons of sugars at δ 5.02 (1H, br s, 1''-H) and 4.36 (1H, d, *J*=7.8 Hz, 1'-H), a proton attached to a carbon atom bearing an ester group at δ 4.71 (1H, t, *J*=9.7 Hz, 4'-H), two phenolic methyl ether groups at δ 3.80 and 3.71 (each 3H, s), methylene protons of a benzyl group at δ 2.73 (2H, t, *J*=6.7 Hz), and methyl protons at δ 0.96 (3H, d, *J*=6.0 Hz). The ¹H-NMR spectrum of brachynoside (**2b**) is similar to that of kusaginins (**2a**) except for two methoxyl groups in place of two phenolic hydroxyl groups. Acetylation of **2b** with acetic anhydride and pyridine gave a heptaacetate **2c** as an amorphous powder and its ¹H-NMR spectrum shows signals at δ 1.84, 1.90, 1.99, 2.00, 2.05 (each 3H, s, aliphatic acetoxy), 2.22, and 2.27 (each 3H, s, aromatic acetoxy). In its IR spectrum, the hydroxyl group absorption band is replaced by a strong ester absorption band at 1745 cm⁻¹. Partial methylation of brachynoside (**2b**) was achieved as follows. The reaction mixture of **2b**, anhydrous potassium carbonate, and a few drops of (CH₃)₂SO₄ was stirred at room temperature and yielded **2d** as an amorphous solid [$\nu_{\max}^{\text{KBr}}$ 3450, 1720, and 1605 cm⁻¹; ¹H-NMR (CD₃COCD₃) δ : 3.77, 3.79, 3.86, and 3.88 (each 3H, s)] which was identical with the product formed from the tetramethyl ether of kusaginins under similar reaction conditions. The product is also identical with the partial methylation product of cistanoside D (**2e**).⁶⁾

When brachynoside was subjected to methanolysis with sodium methoxide in dry methanol at room temperature, it gave methyl caffeate (**3**)⁵⁾ and **4** [an amorphous powder; $\nu_{\max}^{\text{KBr}}$ 3430, 1602, and 1516 cm⁻¹; ¹H-NMR (CD₃OD) δ : 1.23 (3H, d, *J*=6.0 Hz), 2.86 (2H, t, *J*=7.0 Hz), 3.77 and 3.80 (each 3H, s), 4.30 (1H, d, *J*=7.8 Hz), 5.14 (1H, d, *J*=1.5 Hz), 6.78 (1H, dd, *J*=8.2, 1.9 Hz), 6.85 (1H, d, *J*=8.2 Hz), and 6.89 (1H, d, *J*=1.9 Hz)]. Therefore, the structure of brachynoside was determined as **2b**.

Experimental

Melting points were determined on a Yanagimoto micro melting point



apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter at room temperature. IR spectra were recorded on a Perkin-Elmer 781 spectrometer. $^1\text{H-NMR}$ spectra were run on a Bruker AM 300 at 300 MHz. Chemical shifts are given in δ -values and coupling constants (J) are given in hertz (Hz). UV spectra were taken on a Hitachi U-3200 instrument.

Extraction and Isolation The air-dried leaves of *Clerodendrum brachyanthum* SCHAUER (0.68 kg), collected in Taipei, were extracted three times with hexane (8 l) and yielded three known compounds, clerodin, stigmasta-5,22,25-trien-3 β -ol, and 3-*epi*-glutinol together with five new diterpenes, clerodinins A, B, C, and D, and clerodiol.¹⁾ The residue was successively extracted with ethanol. The extract was concentrated to a syrup under reduced pressure and the syrup was subjected to chromatography on silica gel. The eluate with chloroform gave eudesmin (**1a**) (35 mg) and syringaresinol dimethyl ether (**1b**) (9 mg), and the eluate with 20% MeOH in CHCl_3 was repeatedly chromatographed on Sephadex LH-20 and silica gel to yield kusaginin (**2a**) (26 mg) and brachynoside (**2b**) (38 mg).

Eudesmin (1a)²⁾ Colorless needles, mp 105–107°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3060, 1610, 1580, 1500, 1260, 1220, 1138, 1025, 820. MS m/z (%): 386 (M^+ , 70), 205 (8), 189 (11), 177 (65), 165 (100), 151 (52), 138 (20). $^1\text{H-NMR}$ (CDCl_3) δ : 3.09 (2H, m), 3.85 and 3.88 (each 6H, s), 3.88 (2H, obscured by signals of -OMe), 4.24 (2H, dd, $J=9.0, 6.6$ Hz), 4.74 (2H, d, $J=4.3$ Hz), 6.81 (2H, d, $J=9.0$ Hz), 6.86 (2H, dd, $J=9.0, 2.0$ Hz), 6.89 (2H, d, $J=2.0$ Hz).

Syringaresinol Dimethyl Ether (1b)³⁾ Colorless needles, mp 120–122°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3050, 1630, 1590, 1510, 1260, 1230, 1120, 1020, 820, 755. MS m/z (%): 446 (M^+ , 62). $^1\text{H-NMR}$ (CDCl_3) δ : 3.08 (2H, m), 3.80, 3.80, and 3.85 (each 6H, s), 3.87 (2H, obscured by signals of -OMe), 4.23 (2H, dd, $J=9.0, 6.7$ Hz), 4.70 (2H, d, $J=4.3$ Hz), 6.54 (4H, s).

Kusaginin (2a) Colorless needles, mp 153–155°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 3050, 1700, 1630, 1595, 1515, 1270, 1245, 1155, 1130, 1030, 810, 760. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 0.95 (3H, d, $J=6.0$ Hz), 2.73 (2H, t, $J=6.5$ Hz), 4.34 (1H, d, $J=8.0$ Hz), 4.71 (1H, t, $J=9.6$ Hz), 5.03 (1H, brs), 6.21 and 7.53 (each 1H, d, $J=15.8$ Hz), 6.59 (1H, dd, $J=8.1, 1.9$ Hz), 6.67 (1H, d, $J=8.1$ Hz), 6.73 (1H, d, $J=1.9$ Hz), 6.81 (1H, d, $J=8.0$ Hz), 6.98 (1H, dd, $J=8.0, 2.0$ Hz), 7.09 (1H, d, $J=2.0$ Hz). Nonaacetate of kusaginin: mp 94–95°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1750, 1610, 1500, 1250, 1210, 1110, 1035, 895, 930. $^1\text{H-NMR}$ (CDCl_3) δ : 1.00 (3H, d, $J=6.1$ Hz), 1.85, 1.96, 2.00, 2.07, 2.09, 2.22, 2.24, 2.27, and 2.27 (each 3H, s), 2.84 and 3.85 (each 2H, m), 3.62 (2H, m, 5'-H, 5''-H), 4.05–4.20 (3H, m), 4.35 (1H, d, $J=8.1$ Hz), 4.80 (1H, brs), 4.91 (1H, t, $J=9.8$ Hz), 5.00–5.10 (3H, m), 5.17 (1H, t, $J=9.4$ Hz), 6.31 and 7.62 (each 1H, d, $J=15.9$ Hz), 6.90–7.40 (6H, m).

Brachynoside (2b) An amorphous powder. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 220 (4.15), 234 (4.05), 291 (4.21), 333 (4.12). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3400, 1700, 1630, 1595, 1515, 1270, 1245, 1155, 1130. Anal. Calcd for $\text{C}_{31}\text{H}_{40}\text{O}_{15}$: C, 57.05; H, 6.17. Found: C, 56.91; H, 6.21.

Acetylation of 2b with Acetic Anhydride and Pyridine Brachynoside (5 mg) was allowed to react with Ac_2O (0.5 ml) in pyridine (0.5 ml) at room temperature overnight. Usual work-up gave a heptaacetate **2c** (5 mg), an amorphous power. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1745, 1605, 1500, 1245, 1220, 1100, 1035, 890. $^1\text{H-NMR}$ (CDCl_3) δ : 0.98 (3H, d, $J=6.4$ Hz), 1.84, 1.90, 1.99, 2.00, 2.05, 2.22, 2.27, 3.76, 3.81 (each 3H, s), 2.78 (2H, m, ArCH_2 -), 3.58 (2H, m, 5'-H, 5''-H), 3.83 (2H, m, $-\text{OCH}_2\text{CH}_2\text{Ar}$), 4.00–4.20 (3H, m, 3'-H, 6'-H), 4.35 (1H, d, $J=8.9$ Hz, 1'-H), 4.82 (1H, d, $J=1.8$ Hz, 1''-H), 4.92 (1H, t, $J=9.4$ Hz), 4.98–5.12 (3H, m), 5.14 (1H, t, $J=9.4$ Hz), 6.32 and 7.63 (each 1H, d, $J=15.9$ Hz), 6.78–7.13 (6H, m, aromatic H).

Partial Methylation of Kusaginin (2a) and Brachynoside (2b) Dimethyl sulfate (2 drops) was added to a solution of **2a** or **2b** (each 15 mg) in dry acetone (5 ml) containing anhydrous potassium carbonate (100 mg). The reaction mixture was stirred at room temperature for 25 h, then filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography to give the tetramethyl ether (**2d**) 8 mg from **2a**, 7 mg from **2b** as an amorphous powder. The product was identical with the partial methylation product of cistanoside D (**2e**).⁶⁾

Methanolysis of 2b with Sodium Methoxide Brachynoside (10 mg) and sodium methoxide (40 mg) were dissolved in dry methanol (3 ml), and the solution was stirred at room temperature for 4 h under a nitrogen atmosphere. Then 20 ml of dry MeOH was added, and neutralized with Amberlite IR-120. The product was subjected to chromatography on a column of Sephadex LH-20 to give **3** (3 mg) [mp 151–153°C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3440, 3240, 1675, 1630, 1265, 1170, 950, 840. $^1\text{H-NMR}$ (CD_3OD) δ : 3.72 (3H, s), 6.21 and 7.52 (each 1H, d, $J=16.0$ Hz), 6.74 (1H, dd, $J=8.0, 2.0$ Hz), 6.91 (1H, d, $J=2.0$ Hz), 7.02 (1H, d, $J=8.0$ Hz)] and **4** (6 mg) (an amorphous powder. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3430, 1602, 1516, 1140, 1060, 1020).

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