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

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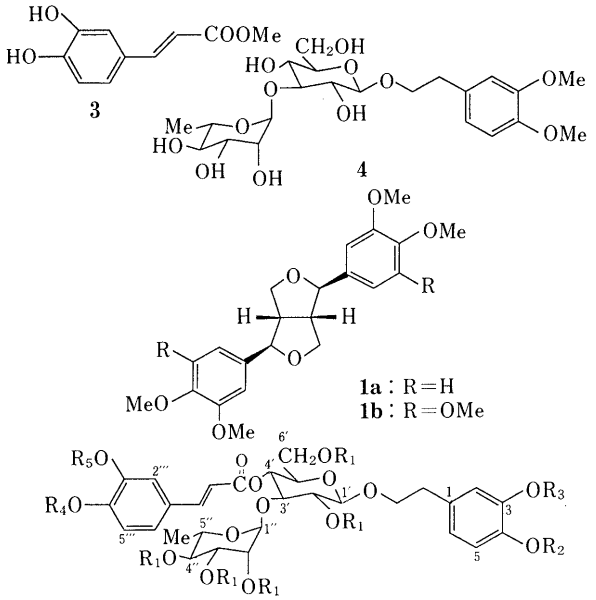
Eudesmin, syringaresinol dimethyl ether, kusaginin and brachynoside were isolated from the ethanol extract of the leaves of *Clerodendron brachyanthum*. The new glycoside, branchynoside, was assigned as 2-(3,4-dimethoxyphenyl)ethyl 3-*O*-α-L-rhamnopyranosyl-4-*O*-(3,4-dihydroxycinnamoyl)-β-D-glucopyranoside 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-3β-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 were 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 [kusaginin (**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°, and from its elementary analysis was suggested to have the formula $C_{31}H_{40}O_{15}$. 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 acid[5] at λ_{max}^{MeOH} 220, 234, 291, and 333 nm. From the IR spectrum together with the proton nuclear magnetic resonance (^{1}H-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 ^{1}H-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 D_2O 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 ^{1}H-NMR spectrum of brachynoside (**2b**) is similar to that of kusaginin (**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 ^{1}H-NMR spectrum shows signals at δ 1.84, 1.90, 1.99, 2.00, 2.05 (each 3H, s, aliphatic acetoxyl), 2.22, and 2.27 (each 3H, s, aromatic acetoxyl). 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 $(CH_3)_2SO_4$ was stirred at room temperature and yielded **2d** as an amorphous solid [ν_{max}^{KBr} 3450, 1720, and 1605 cm^{-1}; ^{1}H-NMR (CD_3COCD_3) 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 kusaginin 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; ν_{max}^{KBr} 3430, 1602, and 1516 cm^{-1}; ^{1}H-NMR (CD_3OD) 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}H-NMR spectra were run on a Brucker 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 *Clerodendrun 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-3β-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 ν_{max}^{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}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 ν_{max}^{KBr} cm^{-1}: 3050, 1630, 1590, 1510, 1260, 1230, 1120, 1020, 820, 755. MS m/z (%): 446 (M^+, 62). ^{1}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 ν_{max}^{KBr} cm^{-1}: 3400, 3050, 1700, 1630, 1595, 1515, 1270,1245, 1155, 1130, 1030, 810, 760. ^{1}H-NMR (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, br s), 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 ν_{max}^{KBr} cm^{-1}: 1750, 1610, 1500, 1250, 1210, 1110, 1035, 895, 930. ^{1}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, br s), 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 λ_{max}^{MeOH} nm (log ε): 220 (4.15), 234 (4.05), 291 (4.21), 333 (4.12). IR ν_{max}^{KBr} cm^{-1}: 3400, 1700, 1630, 1595, 1515, 1270, 1245, 1155, 1130. *Anal.* Calcd for $C_{31}H_{40}O_{15}$: 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 ν_{max}^{KBr} cm^{-1}: 1745, 1605, 1500, 1245, 1220, 1100, 1035, 890. ^{1}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, ArC$\underline{H}_2$–), 3.58 (2H, m, 5′-H, 5″-H), 3.83 (2H, m, –OC$\underline{H}_2$CH_2Ar), 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 ν_{max}^{KBr} cm^{-1}: 3440, 3240, 1675, 1630, 1265, 1170, 950, 840. ^{1}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 ν_{max}^{KBr} cm^{-1}: 3430, 1602, 1516, 1140, 1060, 1020).

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References

1) *a*) Y. L. Lin, Y. H. Kuo and Y. L. Chen, *Chem. Pharm Bull.*, **37**, 2191 (1989); *b*) *Idem*, *Heterocycles*, **29**, 1489 (1989).
2) I. Wahlberg, K. Karlsson and C. R. Enzell, *Acta Chem. Scand.*, **26**, 1383 (1972).
3) *a*) H. Fujimoto and T. Higuchi, *Mokuzai Gakkaishi*, **23**, 405 (1977); *b*) E. E. Dickey, *J. Org. Chem.*, **23**, 179 (1958).
4) *a*) A. Sakurai and T. Kato, *Bull. Chem. Soc. Jpn.*, **56**, 1573 (1983); *b*) H. Kobayashi, H. Karasawa, T. Miyase and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 3009 (1984).
5) H. J. Klosterman and R. Z. Muggli, *J. Am. Chem. Soc.*, **81**, 2188 (1959).
6) H. Kobayashi, H. Karasawa, T. Miyase, and S. Fukushima, *Chem. Pharm. Bull.*, **32**, 3880 (1984).