Berchemolide, a Novel Dimeric Vanillic Acid Glucoside from *Berchemia racemosa*

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A new phenol glycoside named berchemolide was isolated together with (+)-catechin and (–)-epicatechin (as acetates), from the stems of *Berchemia racemosa* SIEB. et ZUCC. (Rhamnaceae). The structure of berchemolide, having a dimeric dilactone structure with a 22-membered ring, was determined on the basis of spectral and chemical investigations. The conformation of berchemolide was calculated by MNDO (modified neglect of diatomic overlap).

Keywords *Berchemia racemosa*; Rhamnaceae; berchemolide; cyclic dimer; vanillic acid glucoside; MNDO; CD spectrum

The stems of *Berchemia racemosa* SIEB. et ZUCC. (Rhamnaceae) have been used as a folk medicine to treat gall stones and stomach-ache in Japan. In a previous paper,¹⁾ we reported the structure elucidation of a new tetrauranoid lignan, (–)-berchemol, from the stems of *B. racemosa*. Many phenolic compounds, (–)-catechin, vanillic acid,²⁾ tachioside,³⁾ syringic acid β -glucopyranosyl ester,³⁾ etc. have been isolated from the stems of *B. racemosa*. We have now isolated a novel phenol glycoside named berchemolide (1), along with (+)-catechin (2) and (–)-epicatechin (3) (as acetates). This paper deals with the structure elucidation of berchemolide (1). The extraction and separation were carried out as described in the experimental section.

Berchemolide (1), colorless needles, mp 311–312°C, $[\alpha]_D +116^\circ$ showed hydroxyl (3500–3400 cm^{–1}) and aromatic ester (1720, 1280, 1120 cm^{–1}) absorptions in its infrared (IR) spectrum. In the ultraviolet (UV) spectrum, 1 showed absorption maxima due to aromatic rings at 218, 252 and 292 nm. The UV spectrum was similar to that of methyl 3,4-dimethoxybenzoate (4) (218, 261 and 290 nm). There was no bathochromic shift in the UV spectrum upon addition of alkali, suggesting the absence of a phenolic group in 1. Berchemolide (1) gave a negative FeCl₃ reaction.

The proton nuclear magnetic resonance (¹H-NMR) spectrum of 1 exhibited ABX-type signals at δ 7.37 (1H, d, $J=8.6$ Hz), 7.75 (1H, dd, $J=8.6, 2.0$ Hz) and 7.42 (1H,

d, $J=2.0$ Hz), one aryl methoxyl signal at δ 3.80 and glucosyl proton signals (Table I). Two-dimensional ¹H–¹H-correlation spectroscopy (¹H–¹H-COSY) of 1 showed clear correlations among the glucosyl protons. The carbon-13 nuclear magnetic resonance (¹³C-NMR) spectrum showed signals due to β -glucopyranose (δ 98.3, 72.8, 76.9, 70.6, 73.5, 65.0). On enzymatic hydrolysis the sugar moiety of 1 was confirmed to be glucose.

The ¹³C-NMR spectrum of 1 showed six aromatic carbon signals, one methoxyl carbon signal and an ester carbonyl signal, which are similar to those of vanillic acid (5). In the difference nuclear Overhauser effect (NOE) spectrum of 1, the methoxyl signal showed a negative NOE with the H-2 signal on the aromatic ring (δ 7.42) (Fig. 1). On enzymatic hydrolysis, 1 afforded vanillic acid (5), which was identified by thin layer chromatography (TLC) and high performance liquid chromatography

Fig. 1. ¹H-NMR (Normal and NOE) Spectra of Berchemolide (1)

a, normal spectrum; b–d, NOE difference spectra on irradiation at 3.80, 4.09, 4.41 and 5.24 ppm, respectively.

TABLE I. ¹H- and ¹³C-NMR Chemical Shifts (δ ppm) and Coupling Constants (J /Hz in Parentheses) of Berchemolide (1) in DMSO-*d*₆

No.	Carbon	Proton
Vanillic acid		
1	122.50 s	
2	112.19 d	7.417 d (2.0)
3	148.43 s	
4	149.85 s	
5	114.42 d	7.374 d (8.6)
6	122.91 d	7.752 dd (8.6, 2.0)
7	165.06 s	
Glucose		
1'	98.33 d	5.242 d (7.1)
2'	72.80 d	3.39 dd (7.1, 9.0)
3'	76.92 d	3.39 t (9.0)
4'	70.59 d	3.176 t (9.0)
5'	73.46 d	3.974 ddd (9.6, 9.0, 2.0)
6'	64.99 t	4.087 dd (11.2, 9.6)
		4.409 dd (11.2, 2.0)
OCH ₃	55.50 q	3.796 s

Signal assignments were made based on the ¹H–¹H and ¹H–¹³C COSY spectra.

Fig. 2. FAB B/E and B²/E Linked Scan Spectra of Berchemolide (1)

(HPLC). The anomeric proton signal at δ 5.24 showed a negative NOE with the H-5 signal on the aromatic ring at δ 7.37. Based on the above data, berchemolide (1) was considered to be a derivative of vanillic acid glucoside (6).

From a comparison of the ¹³C-NMR spectrum of 1 with those of vanillic acid glucoside (6)⁴) and vanilloyl glucose (7),⁵) both glucoside and ester linkage were considered to be present in 1. In the ¹³C-NMR spectrum of 1, an acylation shift was observed at the signals of C-6 (+4.7 ppm) and C-5 (−2.5 ppm) of glucose, suggesting that the C-6 hydroxyl group of glucopyranose was esterified with the carbonyl group of vanillic acid (5).

The ¹³C-NMR spectrum of 1 showed fourteen signals (vanillic acid (C₈) + glucose (C₆)). In the electron impact mass spectrum (EI-MS), 1 showed a fragment peak at m/z 312 (C₁₄H₁₆O₈), and in the chemical ionization MS (CI-MS), 1 showed a peak at m/z 313. The molecular formula of 1 was estimated to be C₁₄H₁₆O₈, but the positive fast atom bombardment mass spectrum (FAB-MS) of 1 exhibited the [M+H]⁺ ion peak at m/z 625 and the [M+Na]⁺ ion peak at m/z 647. The negative FAB-MS of 1 exhibited the [M−H][−] ion peak at m/z 623. The B/E and B²/E linked scan of 1 was run to confirm that the peak at m/z 313 in the FAB-MS was not the peak due to the monomer of the dehydrated derivative of vanillic acid glucoside. The results obtained from the scanning suggest that the daughter ion (m/z 313) was produced from the [M+H]⁺ ion (m/z 625) of 1. Based on the positive FAB-high resolution MS (FAB-HRMS) (m/z 625.1747), the molecular formula of 1 was determined to be C₂₈H₃₂O₁₆, indicating the presence of a C₂ axis of symmetry in 1.

The circular dichroism (CD) spectrum of 1 exhibited a distinct positive split Cotton effect owing to exciton coupling with a maximum at 224 nm ($\Delta\epsilon$ +19.6) and a minimum at 213 nm ($\Delta\epsilon$ −17.1) (Fig. 3), indicating the existence of two chromophores in the molecule.⁶) We also performed modified neglect of diatomic overlap (MNDO) semiempirical molecular orbital calculation to obtain the equilibrium structure for 1 with the MOPAC Ver. 5 program (Fig. 4).^{7,8}) Optimization was carried out for all geometrical parameters, including hydrogen atoms. The

Fig. 3. CD Spectrum of Berchemolide (1)

Fig. 4. Structure of Berchemolide (1)

Chart 1

calculated result predicts 1 to exist in the potential energy minimum.

Alkaline hydrolysis of 1 with sodium hydroxide in dimethyl sulfoxide yielded vanillic acid glucoside (6). Vanillic acid glucoside (6), which was synthesized by the Koenigs–Knorr reaction of methyl vanillate (8) with α -acetobromoglucose,⁹) was identical with 6 derived from 1 with respect to *R_f* value on TLC and *t_R* on HPLC.

Thus, berchemolide (1) was concluded to be a dimer of vanillic acid glucoside, having a cyclic dilactone structure with a 22-membered ring as shown in Chart 1.

Glucoside and glucosyl esters of vanillic acid have been found in some plants,¹⁰) but it is of interest that a dimer such as berchemolide has been isolated as a natural product.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-181 automatic polarimeter in a 1 dm tube. CD spectra were recorded on a JASCO J-500C spectrometer. IR spectra were recorded with a Hitachi IR 260-10 spectrometer. UV spectra were recorded with

a Shimadzu UV-250 spectrometer. MS and FAB-MS were measured on JEOL JMS-D-300 and JMS-SX 102 spectrometers. ^1H - and ^{13}C -NMR spectra were recorded with a JEOL JNM GX-400 spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts are recorded in δ (ppm), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Column chromatography was performed on Kieselgel 60 (Merck, 70–230 mesh) and Sephadex LH-20 (Pharmacia Fine Chemicals). Gas-liquid chromatography (GLC) was performed with a Shimadzu GC-4CM. HPLC was performed with a Shimadzu LC-3A. TLC was carried out with precoated Kieselgel 60 F₂₅₄ plates (Merck) and detection was carried out by UV irradiation and by spraying 10% H_2SO_4 followed by heating.

Isolation The dried stems (3 kg) of *B. racemosa* were extracted with hexane, acetone and MeOH (each 7 l $\times$ 3) under reflux, successively. The MeOH solution was concentrated *in vacuo* to afford the MeOH extract (118 g). The MeOH extract was washed with EtOAc (300 ml $\times$ 3) under reflux and the residue was dissolved in H_2O . The aqueous solution was passed through an Amberlite XAD-2 column. The MeOH eluate was concentrated *in vacuo*. The residue was chromatographed on silica gel with EtOAc–MeOH (1:0–0:1) and CHCl_3 –MeOH (5:1–1:1) to afford **1** (1 mg), and a mixture of **2** and **3** (92 mg). The mixture (92 mg) was acetylated with Ac_2O (3 ml) in pyridine (3 ml) at room temperature overnight. After usual work-up, the crude product was chromatographed on silica gel with benzene–acetone (9:1) to afford (+)-catechin pentaacetate (**9**) (21 mg), mp 124–126°C, $[\alpha]_D^{32} + 29.3^\circ$ ($c=0.5$, acetone) and (–)-epicatechin pentaacetate (**10**) (13 mg), mp 148–151°C, $[\alpha]_D^{32} - 13.7^\circ$ ($c=0.5$, acetone), which were found to be identical with authentic samples (TLC, mixed melting point, $[\alpha]_D$, IR and ^1H -NMR spectra).

The dried stems (2 kg) of *B. racemosa* were extracted with water (5 l $\times$ 3). The water extract was passed through an Amberlite XAD-2 column, with H_2O and then MeOH. The MeOH eluate was concentrated *in vacuo* and chromatographed on Sephadex LH-20 with MeOH to afford **1** (5.8 mg).

Berchemolide (1) Colorless needles (from dimethylsulfoxide (DMSO)–MeOH (1:1)), mp 311–312°C. Optical rotatory dispersion (ORD) ($c=0.1$, pyridine), $[\alpha]_D^{32}$ nm: +116° (589), +124° (577), +141° (546), +294° (435), +605° (365). FeCl_3 reaction: negative. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 218 (4.29), 252 (4.05), 292 (3.67); $\lambda_{\text{max}}^{\text{NaOMe}}$ nm: no shift. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3500–3400, 1720, 1600, 1510, 1420, 1300, 1280, 1120, 1080. EI-MS m/z (%): 312 (3), 168 (90), 153 (44), 151 (88), 40 (100). HR-MS m/z : Calcd for $\text{C}_{14}\text{H}_{16}\text{O}_8$: 312.0845, $\text{C}_8\text{H}_8\text{O}_4$: 168.0423, $\text{C}_7\text{H}_5\text{O}_4$: 153.0186, $\text{C}_8\text{H}_7\text{O}_3$: 151.0395. Found: 312.0847, 168.0425, 153.0183, 151.0403. CI-MS m/z (%): 313 (5), 169 (100). Positive FAB-MS m/z : 625 $[\text{M}+\text{H}]^+$, 647 $[\text{M}+\text{Na}]^+$. [Negative FAB-MS m/z : 623 $[\text{M}-\text{H}]^-$. Positive FAB-HRMS: Calcd for $\text{C}_{28}\text{H}_{33}\text{O}_{16}$: 625.1769. $\text{C}_{28}\text{H}_{32}\text{O}_{16}\text{Na}$: 647.1588. Found: 625.1747, 647.1624. ^1H - and ^{13}C -NMR (Table I).

Enzymatic Hydrolysis of 1 Compound **1** (1 mg) in 0.2 M Na_2HPO_4 –0.1 M citric acid buffer (pH 4.1) (2 ml) was hydrolyzed with molsin (4 mg) at 37°C for 6 d, monitoring the product with TLC. The reaction mixture was passed through an Amberlite MB-3 column and the eluate was chromatographed on Sephadex LH-20 (H_2O –MeOH (1:0–0:1)) to afford **5** and the sugar moiety. The aglycone (**5**) was methylated with ethereal CH_2N_2 to give **4**. Compound **5** and **4** were identified as vanillic acid and methyl 3,4-dimethoxybenzoate by TLC [**5** and **4**: R_f : 0.29, 0.61

(CHCl_3 –MeOH (19:1))] and HPLC [**4**: $t_R=2.8$, column; Wakosil 5C₁₈-200 ODS (4.6 $\times$ 250 mm), flow rate; 0.8 ml/min, mobile phase; acetonitrile–water (1:2)].

The sugar moiety in water was reduced with NaBH_4 (2 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite MB-3 column and evaporated to dryness. Boric acid was removed by distillation with MeOH and the residue was acetylated with Ac_2O (1 ml) in pyridine (1 ml) at room temperature overnight. The solvent was evaporated off *in vacuo*. Glucitol acetate was identified by GLC [$t_R=11.6$ min, column; 2% OV-17 (Support, Gas Chrom Q) (3 mm $\times$ 2 m); column temperature, 200°C; carrier gas, N_2].

Alkaline Treatment of Berchemolide (1) A solution of **1** (2 mg) in DMSO (2 ml) was heated with powdered NaOH (10 mg) at 95°C for 2 h. The solution was deionized with Amberlite IR-120 (H^+) resin. The eluate was concentrated *in vacuo* to give vanillic acid glucoside (**6**). TLC: R_f : 0.30 (CHCl_3 –MeOH (8:5)). HPLC: t_R (min) A: 3.3 B: 2.2; column, Nishio Neopack C₁₈ ODS (4.6 $\times$ 150 mm), flow rate; 0.5 ml/min; mobile phase A: MeOH, B: H_2O : EtOH (2:1).

Synthesis of Vanillic Acid Glucoside (6) A solution of **8** (100 mg) in dry pyridine (2 ml) was stirred with α -acetobromoglucose (200 mg) and dry silver oxide (200 mg) at room temperature in the dark for 3 h. The insoluble silver salts were filtered off, and the filtrate was concentrated *in vacuo*. The residue was diluted with H_2O and extracted with CHCl_3 . The CHCl_3 solution was dried over Na_2SO_4 and concentrated. A solution of the residue in methanol was decolorized with charcoal and evaporated to dryness, and the residue was crystallized from EtOH to give 4-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl) vanillic acid methyl ester (**11**), mp 142–143°C. A solution of **11** (20 mg) in methanol (2 ml) was treated with 0.1 M sodium methoxide (2 ml). Removal of methanol at 40°C *in vacuo* and addition of water yielded **6**. Compound **6** gave **5** upon acid hydrolysis.

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