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 tetrafuranoid 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° 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 $FeCl_3$ reaction.

The proton nuclear magnetic resonance (^{1}H-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 ^{1}H–^{1}H-correlation spectroscopy (^{1}H–^{1}H-COSY) of 1 showed clear correlations among the glucosyl protons. The carbon-13 nuclear magnetic resonance (^{13}C-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 ^{13}C-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

TABLE I. ^{1}H- and ^{13}C-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 ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectra.

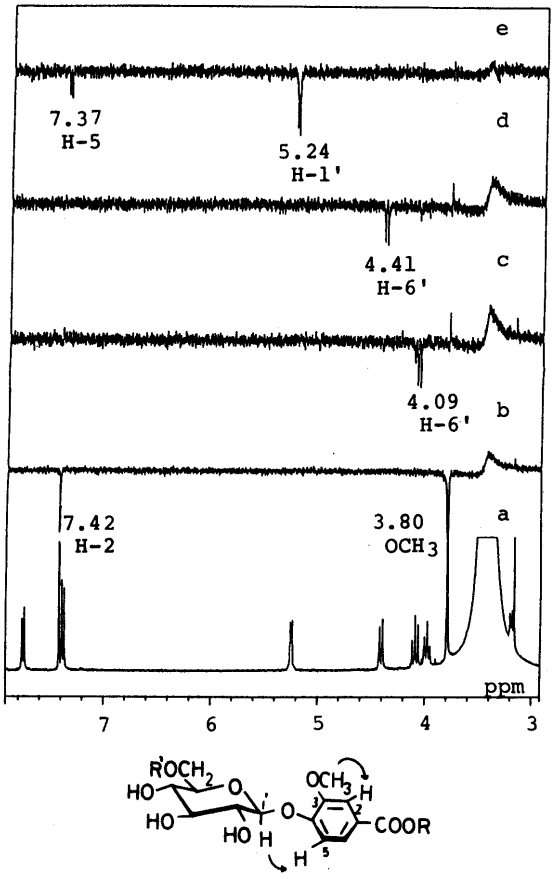


Fig. 1. ^{1}H-NMR (Normal and NOE) Spectra of Berchemolide (**1**)

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

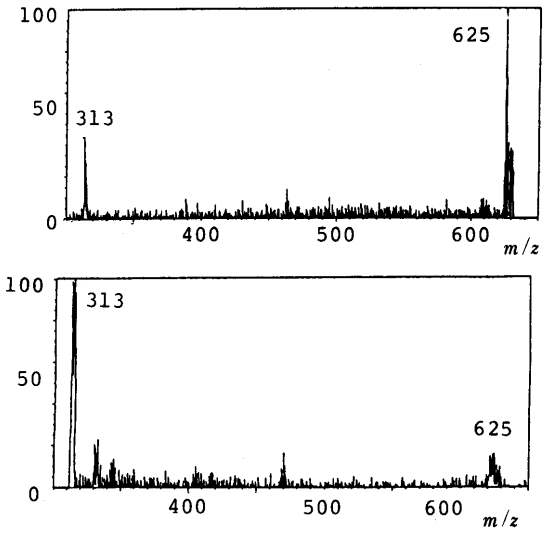


Fig. 2. FAB B/E and B^2/E Linked Scan Spectra of Berchemolide (1)

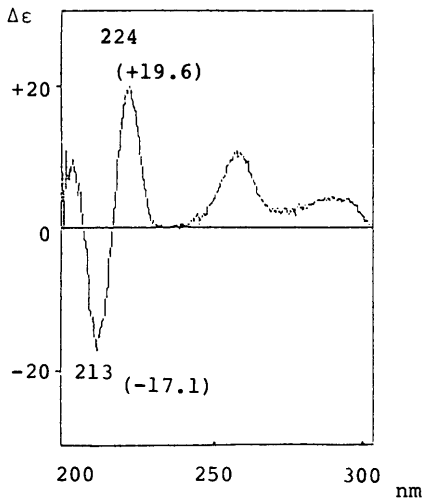


Fig. 3. CD Spectrum of Berchemolide (1)

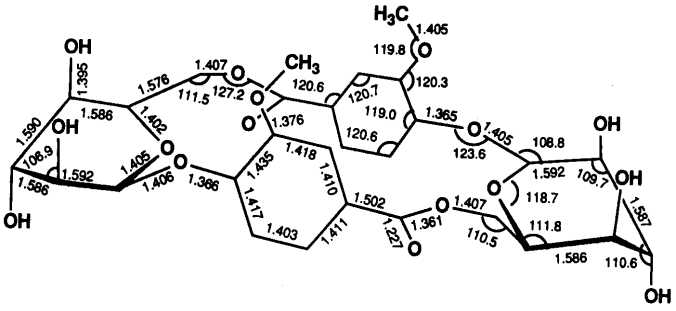
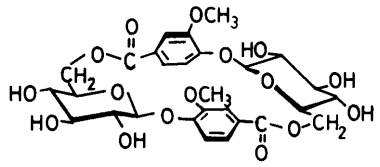


Fig. 4. Structure of Berchemolide (1)



berchemolide (1)

Chart 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 ^{13}C-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 ^{13}C-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 ^{13}C-NMR spectrum of **1** showed fourteen signals (vanillic acid (C_8)+glucose (C_6)). In the electron impact mass spectrum (EI-MS), **1** showed a fragment peak at *m/z* 312 ($C_{14}H_{16}O_8$), 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 $C_{14}H_{16}O_8$, 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 B^2/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 $C_{28}H_{32}O_{16}$, indicating the presence of a C_2 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 ($\Delta\varepsilon$+19.6) and a minimum at 213 nm ($\Delta\varepsilon$−17.1) (Fig. 3), indicating the existence of two chromophores in the molecule.[6] We also performed modified neglect of diatomic overlap (MNDO) semiemperical 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 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 *Rf* 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 was 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. ^{1}H- 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 F_{254} 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 × 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 × 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° (c=0.5, acetone) and (−)-epicatechin pentaacetate (**10**) (13 mg), mp 148—151 °C, $[\alpha]_D^{32}$ −13.7° (c=0.5, acetone), which were found to be identical with authentic samples (TLC, mixed melting point, $[\alpha]_D$, IR and ^{1}H-NMR spectra).

The dried stems (2 kg) of *B. racemosa* were extracted with water (5 l × 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), $[\alpha]^{32}$ nm: +116° (589), +124° (577), +141° (546), +294° (435), +605° (365). $FeCl_3$ reaction: negative. UV λ_{max}^{MeOH} nm (log ε): 218 (4.29), 252 (4.05), 292 (3.67); λ_{max}^{NaOMe} nm: no shift. IR ν_{max}^{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 $C_{14}H_{16}O_8$: 312.0845, $C_8H_8O_4$: 168.0423, $C_7H_5O_4$: 153.0186, $C_8H_7O_3$: 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 $[M+H]^+$, 647 $[M+Na]^+$. [Negative FAB-MS m/z: 623 $[M-H]^-$. Positive FAB-HRMS: Calcd for $C_{28}H_{33}O_{16}$: 625.1769. $C_{28}H_{32}O_{16}Na$: 647.1588. Found: 625.1747, 647.1624. ^{1}H- 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**: Rf; 0.29, 0.61 ($CHCl_3$–MeOH (19:1))] and HPLC [**4**: t_R=2.8, column; Wakosil 5C_{18}-200 ODS (4.6 × 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 × 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: Rf; 0.30 ($CHCl_3$–MeOH (8:5)). HPLC: t_R (min) A: 3.3 B: 2.2; column, Nishio Neopack C_{18} ODS (4.6 × 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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References and Notes

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