Berchemolide, a Novel Dimeric Vanillyl Acid Glucoside from Berchemia racemosa

Nobuko SAKURAI, Masato KOBAYASHI, Atsushi SHIGIHARA and Takao INOUE*

Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41, Shinagawa-ku, Tokyo 142, Japan. Received August 6, 1991

A new phenol glucoside 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 MINDO (modified neglect of diatomic overlap).

Keywords Berchemia racemosa; Rhamnaceae; berchemolide; cyclic dimer; vanillyl 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 tetrafluoroan 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 glucoside 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, [x]D 111.2° 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 methoxy 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 methoxy 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)](image_url)

Table I. ¹H- and ¹³C-NMR Chemical Shifts (δ ppm) and Coupling Constants (J/Hz in Parentheses) of Berchemolide (1) in DMSO-d₆

<table>
<thead>
<tr>
<th>No.</th>
<th>Carbon</th>
<th>Proton</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vanillic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>122.50 s</td>
<td>7.417 d (2.0)</td>
</tr>
<tr>
<td>2</td>
<td>112.19 d</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>148.43 s</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>149.85 s</td>
<td>7.374 d (8.6)</td>
</tr>
<tr>
<td>5</td>
<td>114.42 d</td>
<td>7.752 dd (8.6, 2.0)</td>
</tr>
<tr>
<td>6</td>
<td>122.91 d</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>165.06 s</td>
<td></td>
</tr>
</tbody>
</table>

| 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 | 3.391 (9.0) |
| 4° | 70.59 d | 3.176 (9.0) |
| 5° | 73.46 d | 3.974 dd (9.6, 9.0, 2.0) |
| 6° | 64.99 t | 4.087 dd (11.2, 9.6) |
| OCH₃ | 55.50 q | 3.796 s |

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

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(HPLC). The anemeric 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) and vanilloyl glucose (7), 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$_8$)). 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. 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). 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, 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 di lactone structure with a 22-membered ring as shown in Chart 1.

Glucoside and glucosyl esters of vanillic acid have been found in some plants, 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 a 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$_2$SO$_4$ followed by heating.

**Isolation**

The dried stems (3 kg) of B. racemosa were extracted with hexane, acetone and MeOH (each 7 × 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$_2$O. 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 (1:0–0:6) and CHCl$_3$–MeOH (1:1–1:1) to afford 1 (1 g), and a mixture of 2 and 3 (92 mg). The mixture (92 mg) was acetylated with Ac$_2$O (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, [α]$_D^{25}$ +29.3° (c=0.5, acetone) and (−)-epicatechin pentaacetate (10) (15 mg), mp 146–151°C, [α]$_D^{25}$ −13.7° (c=0.5, acetone), which were found to be identical with authentic samples (TLC, mixed melting point, [α]$_D$ IR and $^{13}$C-NMR spectra).

The dried stems (2 kg) of B. racemosa were extracted with water (510 l). The water extract was passed through an Amberlite XAD-2 column, with H$_2$O 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), [α]$_D^{25}$ nm: +116° (589), +124° (577), +141° (546), +294° (435), +605° (365). FeCl$_3$ reaction: negative. UV $^{1}$H-NMR nm (log$\varepsilon$): 218 (4.29), 252 (4.05), 292 (3.67). $^{13}$C-NMR nm: no shift. IR $\nu_{\text{max}}$ cm$^{-1}$: 3500–3400, 1720, 1600, 1510, 1420, 1300, 1200, 1120, 1080. EI-MS m/z (%): 312 (3), 168 (90), 153 (44), 151 (88), 40 (100). HR-MS m/z: Calculated for C$_{26}$H$_{20}$O$_{10}$: 512.0845. C$_{26}$H$_{18}$O$_{10}$: 168.0423, C$_{26}$H$_{18}$O$_{9}$: 151.0183, C$_{26}$H$_{18}$O$_{9}$: 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: C$_{26}$H$_{20}$O$_{16}$, 625.1769. C$_{26}$H$_{18}$O$_{16}$Na: 647.1588. Found: 625.1747, 647.1624. $^{1}$H- and $^{13}$C-NMR (Table 1).

**Enzymatic Hydrolysis of Compound 1**

Compound 1 (1 mg) in 0.2 ml Na$_2$PO$_4$, 0.1 M citric acid buffer (pH 4.1) (2 ml) was hydrolyzed with molisin (4 mg) at 37°C for 6d, 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$_2$O–MeOH (1:0–0:1)) to afford 5 and the sugar moieties. The aglycone (5) was methylated with ethereal CH$_3$$_2$N$_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 (CHC$_3$–MeOH (19:1))] and HPLC [4: t$_R$=2.8, column: Wakosil SC$_{18}$-200 ODS (4.6×250 mm), flow rate: 0.8 ml/min, mobile phase: acetonitrile–water (1:2)].

The sugar moieties 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$_2$O (1 ml) in pyridine (1 ml) at room temperature overnight. The solvent was evaporated off in vacuo. Glucosyl 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: R$_f$: 0.30 (CHC$_3$–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$_2$O–EtOH (2:1).

**Synthesis of Vanillary Acid Glucoside (6)**

A solution of 8 (100 mg) in dry pyridine (2 ml) was stirred with 2-acetobromoglucoside (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$_2$O and extracted with CHCl$_3$. The CHCl$_3$ solution was dried over Na$_2$SO$_4$ 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-β-D-glucopyranosyl vanillyl acid methyl ester (II), 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.

**Acknowledgements**

We thank the staff of the Analytical Division of this University for measurement spectra.

**References and Notes**


7) MOPAC Ver. 5 program; J. J. P. Stewart, QCPE 4455 (1989).

8) This result was added on 1991, 11. 5.
