Novel Stilbene Glycosides from *Polygonum multiflorum*

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Abstract: Two new stilbene glycosides (1 and 2), together with nine known compounds (3-11), were isolated from the water extract of *Polygonum multiflorum* Thunb. The structures of the new compounds were elucidated by their chemical properties and spectroscopic analyses, including extensive 2D NMR experiments. Compound 2 showed strong DNA cleavage activity, and compounds 1, 2, and 10 (2, 3, 4, 5)-tetrahydroxy-trans-stilbene-2-O-β-D-glucopyranoside exhibited significant inhibition of lipid peroxidation.

Key words: *Polygonum multiflorum*; stilbene glycoside; DNA cleavage activity; inhibition of lipid peroxidation

A dried root tuber of *Polygonum multiflorum* (Polygonaceae) is well known as a Chinese traditional drug. He Shou Wu, which has been widely used as a tonic and antiaging ingredient in China and Japan. Stilbene glycosides (Hata et al., 1975; Zhou et al., 1994), xanthone glycosides (Zhou et al., 1994), stilbene glycoside gallates, catechins, cyanidins (Nonaka et al., 1982), anthraquinones, naphthoquinone, tricin, gallic acid and β-sitosterol (Li and Li, 1993) have been previously isolated from the root of this plant. And emodin-physicsin-β-sitosterol, emodin-8-O-β-D-glucoside (Liu et al., 1983), polygaetoxenoside, queretin-3-O-galactoside and arabinose (Yoshizaki et al., 1987) have been isolated from the stems. Inhibitions of mutagenicity and carcinogenicity (Kam, 1981; Horikawa et al., 1994) and antioxidant activities (Ip et al., 1997) of the extract of *P. multiflorum* have been reported.

In our investigation on the water extract of the roots of this plant, two new stilbene glycosides (1 and 2), together with nine known compounds were isolated. The structures of 1 and 2 were elucidated based on chemical and spectroscopic analyses, including extensive 2D NMR experiments. The known compounds were determined to be gallic acid (3), 2,6-dihydroxy-β-phenyl (4), indole-3-(L-a-amino-β-hydroxy-propionic acid) methyl-ester (5), 1,2-propanediol-1,4-hydroxy-phenyl (6) (Yamaguchi et al., 1969), emodin (7), emodin-8-O-β-D-glucoside (8) (Kato and Morita, 1987; Li and Li, 1993), (+)-lyoniresinosil-3x, O-β-D-glucopyranoside (9) (Achenbach et al., 1992; Shibuya et al., 1992), 2, 3, 4, 5-tetrahydroxy-trans-stilbene-2-O-β-D-glucopyranoside (10) (Hata et al., 1975) and 2, 3, 4, 5-tetrahydroxy-trans-stilbene-2, 3-di-O-β-D-glucopyranoside (11) (Zhou et al., 1994) as compared with the authentic samples.

1 Results and Discussion

Compound 1 was isolated as amorphous powder, \(\alpha_{D}^{20} = -22.2^\circ (c 0.18, MeOH)\). It exhibited FAB-MS ions at \(m/z 461 [M + K]^+\), indicating a molecular weight of 422, compatible with a molecular formula of \(C_{22}H_{22}O_{10}\) as determined by elemental analysis. Positive Mohile test indicated that 1 was a glycoside. The UV spectrum showed maximum absorption at 286 and 202 nm, suggesting the presence of one or more aromatic rings. Its IR spectrum showed strong bands for hydroxyl (3350 cm\(^{-1}\)) and aromatic ring (1612 and 1512 cm\(^{-1}\)).

The \(^1H\)-NMR spectra of 1 indicated the occurrence of two independent aromatic rings. One ring showed AA'BB' type signals resonating at \(\delta 7.18\) and \(8.63\) (2H each, d, \(J = 8.2\) Hz), which could be assigned to the protons on a 1, 4-disubstituted phenyl group, while the other only showed one proton resonating at \(\delta 6.37\) (1H). The anomic proton signal at \(\delta 4.83\) (1H, d, \(J = 7.8\) Hz) indicated the occurrence of a sugar moiety which was diagnosed as β and pyranose according to its anomeric coupling constant and carbon resonance described below (Agrawal, 1992). Beside that, two aliphatic protons (\(\delta 4.89\) (br. s, \(H-β\)) and 4.46 (br. s, \(H-α\))) appeared as singlet (but in \(^1H\)-H COSY spectra, they had weak correlation). \(^13C\)-NMR spectrum exhibited 20 carbons. The signals in aromatic field supported the conclusion mentioned above: the two aliphatic carbons resonating at \(\delta 53.5\) and 60.0 were directly coupled to the two aliphatic protons mentioned above, respectively. The remaining signals were attributed to a hexose (anomic carbon \(δ 106.0\)). \(^1H\) and \(^13C\)-NMR spectra data suggested that 1 was a stilbene derivative. In HMBC experiments, H-β showed correlations with \(δ 150.8\) (C-5), 125.8 (C-6), 143.8 (C-1), 139.7 (C-1') and 130.2 (C-2'), 6'), which strongly suggested that C-β was linked to C-6 and C-1'. And the expected correlations observed between H-α and δ 125.8, 143.8, 136.2 (C-2) and 139.7 confirmed the linkage of C-α with C-1. Thus 1 possesses a benzocyclobutene skeleton which may be formed via cyclization of the double bond and the aromatic ring of a stilbene precursor. Hydrolysis of 1 afforded D-glucose (determined by co-TLC and GC analysis in comparison...
with an authentic sample). The sugar linkage was resolved by means of HMBC experiment. Long-range correlation between the anomeric proton and C-2 indicated that the glucose was attached to C-2. NOESY correlation observed between the two aliphatic protons suggested the cis configuration of H-α and H-β which is in accord with their small coupling constants (Fraenkel et al., 1964). Thus the structure of 1 was elucidated as shown in Fig. 1.

![Fig. 1. Significant HMBC (from H to C) and NOE correlations of 1 and 2.](attachment:image)

Compound 2 was obtained as amorphous powder, $\delta_\ell = 58.4^\circ$ (c 0.15, MeOH) with a molecular formula of C$_{26}$H$_{32}$O$_{14}$, which was indicated by FAB-MS and elemental analysis. Positive Mohish test indicated that 2 was also a glycoside. The UV spectrum showed maximum absorption at 288 and 203 nm and the IR spectrum suggested the presence of hydroxyl (3 420 cm$^{-1}$), aromatic ring (1 624 and 1 458 cm$^{-1}$) and double bond (1 630 cm$^{-1}$). FAB-MS showed ions at m/z 568 [M]$^+$, 407 [M - 162 + 1]$^+$ and 244 [M - 162 + 2]$^+$.

The $^1$H-NMR spectra of 2 contained a set of ortho-coupled protons assignable to one para-hydroxy phenyl group in an AA'BB' type of arrangement ($\delta$ 7.16 and 6.79 (2H each, d, J = 8.5 Hz)), a set of meta-coupled protons assignable to a 1, 2, 3, 5-tetrasubstituted phenyl ($\delta$ 6.46 and 6.25 (1H each, d, J = 2.3 Hz)) and a cis double bond ($\delta$ 6.66 and 6.60 (1H each, d, J = 12.0 Hz)). The other signals could be assigned to two pyranoses that were determined as D-glucoses according to the results of the acidic hydrolysis and the subsequent GC analysis. $^1$C-NMR spectrum exhibited 26 carbons. The signals in aromatic field exhibited 14 carbons which were due to two aromatic rings and a double bond, the rest signals were due to two glucose moieties (anomeric carbons $\delta$ 107.2 and 100.7). The UV data, $^1$H- and $^1$C-NMR spectra data suggested that 2 was a cis stilbene glycoside. The interpretation of the COSY, HMOC and HMBE experiments confirmed the conclusion and finally led to the elucidation of the complete structure as shown in Fig. 1. The linkage of the sugar moieties was obtained from HMBE experiment. The glucose (H-1'' $\delta$ 4.78) was attached to C-2 ($\delta$ 138.0) of the aglycone which was apparently evidenced by a strong long-range correlation between H-1'' ($\delta$ 4.78 (1H, d, J = 7.4 Hz)) and C-2. While the other glucose was linked to C-6'' of the former glucose as suggested by the glycosidation shift of C-6'' ($\delta$ 67.8) and the HMBC correlations observed between H-1'' ($\delta$ 4.73 (1H, d, J = 7.0 Hz)) and C-6'', between H-6'' and C-1'' ($\delta$ 100.7). Furthermore, the β linkage of the sugar moieties was evidenced by the coupling constants of the anomeric protons (Agrawal, 1992).

Biological assays demonstrated that 1 and 2 possessed strong inhibition of lipid peroxidation. Compounds 1 and 2 also exhibited strong DNA cleavage activity. This inhibitory activity may be attributed to the phenolic functions in the molecules. However, 1 and 2 did not show any cytotoxicity against three human cancer cell lines KB-Hela and A549 in vitro.

2 Experimental

2.1 General experimental procedures

Optical rotations were recorded in CH$_2$OH using a Perkin-Elmer 241 automatic digital polarimeter. $^1$H- and $^1$C-NMR, $^1$H-1$^H$ COSY, HMOC, HMBE and NOESY spectra were recorded on a Bruker DRX-400 spectrometer. The carbon multiplicities were obtained by DEPT experiment. FAB-MS were obtained using a Finnigan MAT-90 instrument. UV was carried out on a Varian Cary 300 Bio instrument. IR was recorded on a Hitachi 275-50 IR spectrometer. Elemental analysis was carried out on an Elementar Vario EL instrument. Gas chromatography (GC) was run on an HP 1890 gas chromatography. Sephadex LH-20 (Pharmacia), Toyopearl HW40F (Tosoh), MCI-gel CHP20P (Mitsubishi) and Cosmosil ODS (40 – 60 μm, Nacalai Tesque Inc.) were used for column chromatography.

2.2 Plant material

The roots of Polygonum multiflorum Thunb. were collected from Sichuan Province of China in October 1997, and was identified by Dr. XU Ya-Ming. A voucher specimen (No. PC002) is deposited at Shanghai Institute of Materia Medica. The Chinese Academy of Sciences.

2.3 Extraction and isolation

The water extract of the dried roots of P. multiflorum was precipitated with EtOH (1:1) and was
filtered. The solution was evaporated in vacuum to remove EtOH and was concentrated to a suitable volume. Then it was subjected to chromatography on MCI gel CH2P20 column eluted with H2O and aqueous MeOH (10% - 80%) successively. The sugar eluted by water was discarded and the MeOH eluates were subjected to Sephadex LH-20 chromatography eluted with water to 80% aqueous MeOH gradiently to give five fractions, which were subjected to a combination of column chromatography on Sephadex LH-20 - MCI gel CH2P20 - Cosmosol ODS and Toyopearl HW-40F to give compounds 1 (8 mg), 2 (8 mg), 3 (30 mg), 4 (10 mg), 5 (80 mg), 6 (15 mg), 7 (16 mg), 8 (10 mg), 9 (17 mg), 10 (300 mg) and 11 (160 mg), respectively.

2.4 Identification

**Compound 1** White amorphous powde/\[\alpha\]_D^25 -22.2° (c 0.18, MeOH); UV \(\lambda_{max}\) nm: 286, 225 (sh); 202; IR \(\nu_{max}\) cm\(^{-1}\): 3 400 - 1 612, 1 512, 1 456, 1 356, 1 259, 1 072; \(^1H-NMR\) (D_2O, 400 MHz) \(\delta\): 7.18 (2H, d, J = 8.8 Hz, H-2', 6'), 6.83 (2H, d, J = 8.8 Hz, H-3', 5'), 6.37 (1H, s, H-4'), 4.89 (1H, s, H-3), 4.83 (1H, d, J = 7.6 Hz, H-1'), 4.36 (1H, s, H-6a'), 3.74 (1H, d, J = 7.6 Hz, H-2, 6'), 3.65 (1H, dd, J = 11.4, 2.0 Hz, H-6a'), 3.55 (1H, dd, J = 9.1, 9.2 Hz, H-3'), 3.31 (1H-m, H-6b'), 3.22 (1H, dd, J = 9.4, 9.2 Hz, H-4'), 3.12 (1H, m, H-5'), 13-C-NMR (D_2O, 100 MHz) \(\delta\): 151.5 (s, C-4'), 150.8 (s, C-5'), 150.2 (s, C-3'), 143.8 (s, C-1'), 139.7 (s, C-1'), 136.2 (s, C-2'), 130.2 (C-2', C-6'), 125.8 (s, C-6), 116.5 (C-3', C-5'), 106.0 (C-1'), 77.1 (C-3'), 77.1 (C-5'), 75.4 (C-2'), 71.4 (C-4'), 62.6 (t, C-6'), 60.0 (C-2'), 53.5 (C-6'), FAB-MS m/z: 461 [M + K]^+; Anal. C 52.30%, H 5.68%, calcd. for C_{20}H_{20}O_{15}: C 52.40%, H 5.72%.

**Compound 2** White amorphous powde/\[\alpha\]_D^25 +58.4° (c 0.15, MeOH); UV \(\lambda_{max}\) nm: 288, 203; IR \(\nu_{max}\) cm\(^{-1}\): 3 420, 1 630, 1 624, 1 458, 1 400; \(^1H-NMR\) (D_2O, 400 MHz) \(\delta\): 7.16 (2H, d, J = 8.5 Hz, H-2', 6'), 6.79 (2H, d, J = 8.5 Hz, H-3', 5'), 6.66 (1H, d, J = 12.0 Hz, H-3), 6.60 (1H, d, J = 12.0 Hz, H-3'), 6.46 (1H, d, J = 2.3 Hz, H-4'), 6.25 (1H, d, J = 2.3 Hz, H-6'), 4.78 (1H, d, J = 7.4 Hz, H-1'), 4.73 (1H, d, J = 7.0 Hz, H-1'), 3.86 (1H, m, H-6a'), 3.84 (1H, observed, H-6a'), 3.77 (1H, dd, J = 12.3, 4.9 Hz, H-6b'), 3.66 (1H, m, H-2'), 3.65 (1H, m, H-3'), 3.59 (1H, m, H-4'), 3.56 (1H, m, H-5'), 3.52 (1H, m, H-2'), 3.48 (1H, dd, J = 9.1, 9.3 Hz, H-3'), 3.46 (1H, m, H-4'), 3.39 (1H, m, H-6b'), 3.37 (1H, m, H-6a'), 13-C-NMR (D_2O, 100 MHz) \(\delta\): 157.6 (s, C-4'), 155.5 (s, C-5'), 152.3 (s, C-3'), 138.0 (s, C-2'), 136.1 (C-1'), 133.8 (d, C-3), 133.3 (d, C-2', C-6'), 126.5 (d, C-6), 117.8 (d, C-3', C-5'), 110.3 (d, C-6), 107.2 (d, C-1'), 105.5 (d, C-4'), 100.7 (d, C-7'), 78.5 (d, C-3'), 77.3 (d, C-5'), 76.0 (d, C-5), 75.8 (d, C-3'), 74.3 (d, C-2'), 74.1 (d, C-2'), 72.1 (d, C-4'), 71.8 (d, C-4'), 67.8 (t, C-6'), 63.0 (t, C-6'), FAB-MS m/z: 607 [M + K]^+, 407 [M - 162 + 1]^+, 244 [M - 162 x 2]^+, Anal. C 55.50%, H 5.62%, calcd. for C_{20}H_{20}O_{15}: C 54.93%, H 5.67%.

2.5 Acid hydrolysis of compounds 1 and 2

A solution of 1 and 2 (2 mg each) in 7% HCl-EtOH (3:7) was refluxed for 4 h, respectively. The mixture was diluted with H2O and extracted with Et2O, respectively. The aqueous layer was neutralized with 1 mol/L NaOH and a sample of it was then subjected to TLC analysis on Kieselgel 60 F_{254} (Merck) with 9 mL CHCl_3-MeOH-H_2O (30:12:4) and 1 mL HOAc, and paper chromatography (using n-BuOH-HOAc-H_2O (4:1:5)) with standard sugars, in each case the presence of glucose was established. The aqueous layers were then passed through an Amberlite IRA-60E column, the aqueous eluate was concentrated and treated with thionyl chloride as described previously (Xiao et al., 1999). Only the D-glucose derivative was detected by GC. (GC conditions: column: Supelco SPB-1, 0.25 mm x 27 m, column temperature 230 °C; carrier gas: N_2; t_r, D-glucose derivative 17.9 min. L-glucose derivative 17.3 min.)

2.6 Biological assays

The inhibition of lipid peroxidation assay was evaluated using the previously described protocol (Okawa et al., 1979). A modified Hecht procedure was adapted to evaluate the inhibition of DNA cleavage activity (Huang et al., 1996). Cytotoxicity was carried out according to previously described MTT protocols against three human cancer cell lines KB, Hela and A549 (Likhitwitaywud et al., 1993).

References:


何首乌中新的二苯乙烯甙

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摘要：用反相层析法从何首乌（Polygonum multiflorum Thunb.）根茎的水提物中分得2个新的二苯乙烯甙（1、2）及9个已知化合物：没食子酸（3）、2,6-二羟基苯甲酸（4）、吲哚-3-(L-α-氨基-α-羟基-丙酸)甲酯（5）、1, 2-二羟基-4-(4-羟基-苯基)（6）、大黄素（7）、大黄素-8-O-β-D-葡萄糖甙（8）、(+)-lyoniresinol-3-O-β-D-葡萄糖甙（9）、2, 3, 4', 5-四羟基反式二苯乙烯-2-O-β-D-吡喃葡萄糖甙（10）和2, 3, 4', 5-四羟基反式二苯乙烯-3, 2-二-O-β-D-吡喃葡萄糖甙（11）。新化合物结构通过理化性质与波谱分析特别是2D NMR得以确定。化合物2表现出很强的DNA裂解活性，化合物1, 2与10具有很强的抗肿瘤活性。

关键词：何首乌；二苯乙烯甙；DNA裂解活性；抗肿瘤活性

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