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Cytotoxic phenylethanol glycosides from *Psidium guaijava* seeds

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Abstract

Phytochemical investigations of the acetone extract of *Psidium guaijava* seeds has led to the isolation of five known flavonoid glycosides, two phenolic glycosides and two new phenylethanoid glycosides which have been identified as 1-*O*-3,4-dimethoxy-phenylethyl-4-*O*-3,4-dimethoxy cinnamoyl-6-*O*-cinnamoyl-β-D-glucopyranose and 1-*O*-3,4-dimethoxyphenylethyl-4-*O*-3,4-dimethoxy cinnamoyl-β-D-glucopyranose, on the basis of chemical, physical and spectroscopic methods of analysis.

Keywords: *Psidium guaijava*; Myrtaceae; Seeds; Phenylethanoid glycosides; NMR spectroscopy; Pharmacological activity

1. Introduction

According to the medicinal importance of *Psidium guaijava* (Watt and Breyer–Barndwijk, 1962), we continued our research on its seeds, where we previously reported the isolation of a new flavonol glycoside, quercetin-3-*O*-β-D-(2″-*O*-galloyl glucoside)-4′-*O*-vinyl-propionate together with other known flavonol and phenolic compounds from its ethanolic extract (Michael et al., 2002). Here, we report the structural elucidation of two novel phenylethanoid glycosides together with another seven known phenolic and flavonoid compounds from the acetone extract of its seeds.

The crude acetone extract and the two new isolated compounds were quantitatively tested for their pharmacological activity in vitro against Ehrlich ascites Carcinoma cells (EAC) and Leukemia P388 cells.

2. Results and discussion

The concentrated 80% aqueous acetone extract of the dried seeds was fractionated by column chromatography over Sephadex LH-20, using ethanol/acetone mixtures of increasing polarities to yield five fractions from which nine phenolic and flavonoid compounds were isolated and identified. Seven compounds were known and gave chromatographic, UV, hydrolytic and ^{1}H NMR data typical of 3,3′,4-trimethoxyellagic acid-4′-glucoside, 3,3′,4-trimethoxyellagic acid-4′-gentobioside, isorhamnetin, isorhamnetin-3-glucoside, quercetin-3-glucuronide, quercetin-3-glucoside, and luteolin-7-glucoside with the two new compounds were identified as: 1-*O*-3,4-dimethoxy-phenylethyl-4-*O*-3,4-dimethoxy cinnamoyl-6-*O*-cinnamoyl-β-D-glucopyranose ***1*** and 1-*O*-3,4-dimethoxyphenylethyl-4-*O*-3,4-dimethoxy cinnamoyl-β-D-glucopyranose ***2***.

Compound ***1*** was obtained as an amorphous off-white powder, whose UV spectrum indicated its polyphenolic nature. Complete alkaline hydrolysis gave dimethoxy cinnamic acid and cinnamic acid as the aglycone and the sugar glucose (comparative paper chromatography Co-PC). The ^{1}H NMR spectrum of ***1*** exhibited signals accounting for one 3,4-dimethoxyphenyl ethanol unit whereby it gave an ABX aromatic signals (δ 6.79 *dd*, $J = 2$ and 8 Hz, H-6; 6.8 *d*, $J = 8$ Hz, H-5 and 6.83 *d*, $J = 2$ Hz, H-2) which were more downfield than the unmethylated aglycone signals (δ 6.46 *dd*, $J = 2$ and 8 Hz, H-6; 6.63 *d*, $J = 8$ Hz, H-5 and 6.61 *d*, $J = 2$ Hz, H-2) a triplet signal at δ 2.87 ppm due to the benzylic methylene protons with the multiplet signal of the other methylene protons at δ 4.75 ppm. The two singlet signals at δ 3.8 and 3.78 ppm of two methoxyl groups

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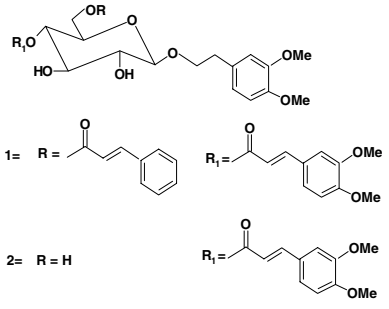
(Nonaka et al., 1982) besides a 3,4-dimethoxy cinnamic acid unit, a cinnamic acid unit (Imakura et al., 1985; Arnold et al., 2002). The anomeric proton signal of a glucose moiety at (δ 4.56, *d*, $J = 8$ Hz) with the triplet signal of H-4 of the glucose at δ 5.10 ppm and the double doublet signal of H-6 at δ 4.20 and 4.42 ppm which are more downfield than the unsubstituted H-4 and H-6 (4.0 and 3.45, resp.). Partial alkaline hydrolysis of compound ***1*** afforded the methylated cinnamic acid together with a colorless intermediate ***1a*** whose ^{1}H NMR spectrum suggested it to be 1-*O*-3,4-dimethoxy-phenylethyl-6-*O*-cinnamoyl-β-D-glucopyranose (absence of methylated cinnamoyl acid signals together with a free H-4 signal at δ 4.00 ppm). All the above data proved ***1*** to be 1-*O*-3,4-dimethoxy-phenylethyl-4-*O*-3,4-dimethoxy cinnamoyl-6-*O*-cinnamoyl-β-D-glucopyranose.

The ^{13}C NMR spectrum of ***1*** finally confirmed its structure as its spectrum exhibited signals similar to those of phenylethanoid glycosides spectra (Suzuki et al., 1993; Franzyk et al., 1998) with a β-glucopyranosyl moiety carrying additional groups at C-4 (δ 74.2 ppm) and C-6 (δ 63.5 ppm). Comparing the above spectral data with those of the tetramethylated campnoside I (Imakura et al., 1985) confirmed the attachment of the cinnamoyl moiety at C-6 of the glucose with the dimethoxy cinnamic moiety at C-4.

The UV, ^{1}H NMR and ^{13}C NMR and spectral data of ***2*** together with the chemical methods were very similar to those of ***1*** except that the signals of the cinnamoyl group were absent and position-6 of the glucose moiety was unsubstituted as the H-6 signal appeared at δ 3.4 ppm and C-6 at δ 62.3 ppm, thus proving the structure of ***2*** to be 1-*O*-3,4-dimethoxyphenylethyl-4-*O*-3,4-dimethoxy cinnamoyl-β-D-glucopyranose.



The biological assay of the acetone extract and the two new isolated compounds showed that the extract has moderate inhibition activity against both the EAC and Leukemia P388 (180% and $ED_{50} = 14.6$), while the two new isolated compounds (***1*** and ***2***) showed high inhibition activity against EAC (220% and 240%) and a low one against Leukemia P388 ($ED_{50} = 17.3$ and 16.1); whereby the results expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}), compounds having an ED_{50} μg/ml < 20 were considered active. As well as a high inhibitory effect in vitro Ehrlich ascites carcinoma cells which showed that the survival time of mice fed on the extract and the two compounds increased to (18, 22 and 24 days, respectively) in comparison with those fed on the standard control pellet which survived for 10 days only ($T/C = 100\%$).

3. Experimental

^{1}H and ^{13}C NMR (TMS as int. standard): 200 and 50 MHz, respectively, were recorded on Varian Gemint-200 NMR spectrometer. Samples were run in Me_2CO-d_6 or DMSO-d_6. UV spectra were obtained on a Shimadzu spectrometer. Column Chromatography: Sephadex LH-20. PC: Whatman No. 1 and 3 MM using solvent systems (1) *n*-butanol–acetic acid–water (6:1:2); (2) aqueous acetic acid; (3) 30% AcOH (acetic acid–water 30:70); (4) H_2O.

3.1. Plant material

Seeds of *P. guaijava* were collected from Helwan, Cairo, Egypt in September 1999. They were identified at the National Research Centre Herbarium (CAIRC), where voucher specimens are deposited.

3.2. Pharmacological methods

3.2.1. Antitumor test in vitro against Ehrlich ascites carcinoma

Female Swiss albino mice weighing 18–20 g obtained from the breeding unit of the National Research Centre, Cairo, Egypt, were used in this study. EAC cells were supplied through Dr. C. Benkhuiisen, Netherlands Cancer Institute, Holland. The tumor line was maintained in female mice by weekly intraperitoneal transplantation of 2.5×10^6 cells. At the start of experiment the EAC cells were tested for viability and contamination using the trypan blue dye exclusion technique. The mice were inoculated with ascitic fluid diluted with normal saline so that each 0.2 ml contained 2.5×10^6 cells. The cells were counted microscopically, using a hemocytometer. The data were expressed as $T/C =$ (mean treated survival time/mean control survival time) $\times$ 100.

3.2.2. Cytotoxic activity against P388 leukemia cells

The cell line employed in the present investigation (P388 leukemia cells) was obtained from the American Type Culture Collection (Rockville, MD). This cell line was cultured in Fisher's medium containing 10% horse inactivated serum at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity).

The cultured cells at a long phase of their growth cycle were treated in triplicate with various concentrations (0.5–100 μg/ml) of the extracted compounds dissolved in DMSO by adding 100 μl DMSO to each tested compound followed by gentle shaking. The culture cells were incubated for 18 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by counting the P388 cells in a hemocytometer.

3.3. *l-O-3,4-dimethoxy-phenylethyl-4-O-3,4-dimethoxy cinnamoyl-6-O-cinnamoyl-β-D-glucopyranose (**1**)*

R_f-values × 100: 84.9(1), 87.6(2), 85.9(4); UV λ_{max} nm (MeOH): 217, 232sh, 247sh, 289, 329; ^{1}H NMR (acetone-d_6) *Aglycone moiety* δ 6.85 (*d*, *J* = 2 Hz, H-2); 6.8 (*d*, *J* = 8 Hz, H-5); 6.75 (*dd*, *J* = 2 and 8 Hz, H-6); 3.87 (*m*, H-8); 2.87 (*t*, *J* = 7 Hz, H-7); 3.8 and 3.78 (*s*, 2 OMe gp.); *Glucose moiety*: δ 4.56 (*d*, *J* = 8 Hz, H-1); 5.1 (*t*, *J* = 9 Hz, H-4); 4.4 and 4.2 (*dd*, *J* = 12 and 2 Hz, H-6); 3.10–4.12 (sugar protons); *Dimethoxy cinnamoyl moiety*: δ 7.57 (*d*, *J* = 16 Hz, H-β); 7.18 (*d*, *J* = 2 Hz, H-2); 7.03 (*d*, *J* = 8 Hz, H-5); 6.95 (*dd*, *J* = 2 and 8 Hz, H-6); 6.44 (*d*, *J* = 16 Hz, H-α); 3.76 (*s*, 2 OMe); *Cinnamoyl moiety:* δ 7.7 (*d*, *J* = 16 Hz, H-β); 7.63 (*d*, H-2 and 6); 7.41 (*br t*, H-3 and 5); 7.4 (*d*, H-4); 6.58 (*d*, *J* = 16 Hz, H-α); ^{13}C NMR (acetone-d_6) *Aglycone moiety*: δ 132.9(C-1); 113.1(C-2); 148.8(C-3); 150.1(C-4); 114.0(C-5); 122.5(C-6); 36.5(C-7); 71.4(C-8); 56.8(OMe); *Glucose moiety:* δ 103.9(C-1); 74.5(C-2); 75.2(C-3); 72.0(C-4); 72.5(C-5); 63.5(C-6); *Dimethoxy cinnamoyl moiety:* δ 128(C-1); 112.3(C-2); 152.3(C-3); 152.3(C-4); 123.8(C-5); 126.2 (C-6); 147.1(C-β); 115.4(C-α); 167.7(CO); 55.7(OMe); *Cinnamoyl moiety* δ 136.09(C-1); 129.1(C-2 and 6); 129.8(C-3 and 5); 131.3(C-4); 146.6(C-β); 118.4(C-α); 168.5(CO).

3.4. *l-O-3,4-dimethoxy-phenylethyl-6-O-cinnamoyl-β-D-glucopyranose (**2**)*

R_f-values × 100: 79.6(1), 87.8(2), 85.2 (3), 81.7(4); UV λ_{max} nm (MeOH): 217, 234sh, 244sh, 298, 329; ^{1}H NMR (acetone-d_6): *Aglycone moiety*: δ 6.85 (*d*, *J* = 2 Hz, H-2); 6.81 (*d*, *J* = 8 Hz, H-5); 6.75 (*dd*, *J* = 2 and 8 Hz, H-6); 3.87 (*m*, H-8); 2.87 (*t*, *J* = 7 Hz, H-7); 3.8 and 3.78 (*s*, 2 OMe group); *Glucose moiety*: δ 4.52 (*d*, *J* = 8 Hz, H-1); 4.94 (*t*, *J* = 9 Hz, H-4); 3.00–4.12 (sugar protons); *Dimethoxy cinnamoyl moiety*: δ 7.57 (*d*, *J* = 16 Hz, H-β); 7.18 (*d*, *J* = 2 Hz, H-2); 7.03 (*d*, *J* = 8 Hz, H-5); 6.95 (*dd*, *J* = 2 and 8 Hz, H-6); 6.44 (*d*, *J* = 16 Hz, H-α); 3.76 (*s*, 2OMe); ^{13}C NMR (acetone-d_6): *Aglycone moiety*: δ 132.9(C-1); 113.1(C-2); 148.8(C-3); 150.1(C-4); 114.0(C-5); 122.5(C-6); 36.5(C-7); 71.4(C-8); 56.8(OMe); *Glucose moiety*: δ 103.5(C-1); 74.2(C-2); 77.2(C-3); 71.4(C-4); 74.5 (C-5); 62.3 (C-6); *Dimethoxy cinnamoyl moiety:* δ 128(C-1); 112.3(C-2); 152.3(C-3); 152.3(C-4); 123.8(C-5); 126.2(C-6); 147.1(C-β); 115.4(C-α); 167.7(CO); 55.7(OMe).

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