Chemistry of Tanning Materials and Proteins Department, National Research Centre, Dokki, Cairo, Egypt

Acylated flavonol glycoside from *Psidium gauijava* L. seeds

H. N. Michael, J. Y. Salib and M. S. Ishak

Ten phenolic and flavonoid compounds including one new acylated flavonol glycoside were isolated from *Psidium gauijava* seeds. The structures of the new compound quercetin-3-*O*-β-D-(2″-*O*-galloyl glucoside)-4′-*O*-vinylpropionate and of the known compounds were elucidated by different chemical and physical methods, ^{1}H- and ^{13}C NMR spectral analysis.

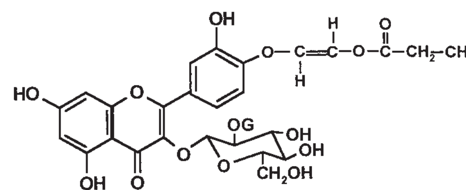
1. Introduction

Polyphenolic compounds and various flavonoid classes are widely distributed in the family Myrtaceae [1]. The leaves and flowers of *P. guaijava* in Hawaii show a considerable degree of antibacterial activity [2] as they yield antibacterial substances, namely: avicularine, guajaverin and quercetin [3, 4]. Ellagitannins [5] and novel gauvins [6] were also isolated. Glycosides of ellagic acid were found from the stem as well as the leaves [7], while the fruit, which are much eaten in Southern Africa, is rich in vitamin-C [2]. From the leaves of an Egyptian species we isolated a new flavonol glycoside; quercetin-4′-glucuronide besides five known compounds [8]. Investigation of the seeds of *Psidium guaijava* (about which only limited information had been reported) led to the isolation of nine known phenolic and flavonoid components namely; ellagic, gallic and caffeic acid, methyl gallate, 8,3′-dimethoxy gossypetin, quercetin-3-*O*-glucoside with the aglycones kaempferol, quercetin and myricetin along with the new natural acylated flavonol glycoside. This paper deals with the elucidation of the structure of the new compound. The pharmacological evaluation of the extract showed a significant inhibitory activity against leukemia P388 and Ehrlich ascites carcinoma cells (EAC).

2. Investigations, results and discussion

The *P. guaijava* seeds were first extracted with chloroform, followed by diethyl ether. The concentrated diethyl ether extract was chromatographed on a Sephadex LH-20 column eluted by ethanol followed by ethanol/water mixtures to give five fractions including the compounds **1–10** which were further purified as required.

The new natural compound, quercetin-3-*O*-β-D-(2″-*O*-galloyl glucoside)-4′-*O*-vinylpropionate (**8**), which was eluted in the fourth fraction of the column (50% ethanol), had UV absorption bands at λ_{max} 258 and 358 nm in MeOH. After testing with different diagnostic reagents a 3,4′-disubstituted flavonol was suggested [10]. Complete acid hydrolysis of **8** gave the aglycone quercetin, gallic acid and the sugar glucose (Co-PC). On controlled acid hydrolysis it yielded an intermediate (**8a**), chromatographic and spectral analyses of which gave the quercetin-4′-*O*-vinylpropionate structure suggesting that both the glucosyl and galloyl moieties are present in position-3 of **8**. The ^{1}H NMR spectrum showed a doublet signal at δ 5.4 ppm of the glucose anomeric proton indicating its attachment to the 3-hydroxyl of quercetin [9] while the downfield appearance of the glucose H-2 as a doublet signal at δ 5.3 ppm together with the singlet signal at δ 6.9 ppm (H-3,5 of galloyl) proved its substitution by a galloyl moiety [10]. On the other hand the substitution of the 4′-position by the vinyl proprionate group was assigned from the upfield shift in the signal of H-5′ besides the appearance of the following signals at δ 7.7 (d, J = 16 Hz, H-1‴), 6.8 (d, J = 16 Hz, H-2‴), 4.15 (q, J = 7 Hz, the CH_2 group), and 1.28 (t, J = 7 Hz, the methyl group) while the H-1‴ was found downfield of the H-2‴ indicating its linkage to the 4′-OH [11]. The ^{13}C NMR data finally confirmed the presence of a 3,4′-disubstituted quercetin; in which the 3-position was occupied by 2″-galloylglucose (the anomeric carbon of β-glucopyranoside at δ 100.49 and a downfield shift of C-2″ at δ 77.1 ppm), and the 4′-position by a vinyl propionate group whose signals appeared at δ 170 (C-3‴), 125 (C-1‴), 110.47 (C-2‴), 39.7 (C-4‴) and 15.42 (C-5‴) ppm showing a downfield shift in C-1‴ and an upfield one in C-2‴ [12].



G = galloyl

Quercetin-3-*O*-β-D-(2″-*O*-galloyl glucoside)-4′-*O*-vinylpropionate

Examination of the pharmacological activity of the diethylether extract of the seeds of *P. gauijava* showed that the survival time of mice inoculated intraperitoneally with 2.5×10^{6} cell/mouse of EAC cells, and fed on the extract increased to 23 days (T/C = 230%) in comparison with those fed on the standard control pellet, where the survival time was 10 days only (T/C = 100%) i.e. the extract possesses highly inhibitory effects *in vitro* on EAC. On the other hand, it gave a moderate cytotoxic activity against P388 ($ED_{50} = 16.7\ \mu g\ m^{-1}$) when the results are expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}). Compounds having an $ED_{50} < 20$ μg/ml were considered active.

3. Experimental

3.1. Instruments and materials

PC: Whatman No. 1 and 3 MM using the solvent systems: (1) BAW (n-butanol: AcOH: H_2O, 6 : 1 : 2); (2) 6 & 30% AcOH (AcOH: H_2O) (3) H_2O, CC: Sephadex LH-20, complete and controlled acid hydrolysis: 2 N HCl for 60 min and 0.1 N HCl at 100 °C respec., UV: Shimadzu spectrophotometer model UV-240, NMR: Varian GEMINT-200 spectrometer. ^{1}H and ^{13}C chemical shifts operating at 200 and 50 MHz, repectively. Samples were run in DMSO-d_6. TMS as internal standard.

The plant material was collected from Helwan. It was identified at the National Research Centre Herbarium (CAIRC), where voucher specimens are deposited.

3.2. Extraction, isolation and purification

The air-dried and powdered seeds were first extracted with chloroform followed by diethyl ether. The concentrated ether extract was applied to a Sephadex LH-20 column and eluted first with ethanol and then ethanol/water mixtures giving five fractions. The fourth fraction was further applied on a Sephadex LH-20 column and eluted with an ethyl acetate : ethanol (3 : 1) mixture to give the new compound quercetin-3-*O*-β-D-(2″-*O*-galloyl glucoside)-4′-*O*-vinylpropionate (**8**) and quercetin-3-*O*-D-glucoside (**9**).

3.3. Characteristics of compound 8; Quercetin-3-O-β-D-(2″-O-galloyl glucoside)-4′-O-vinylpropionate

R_f values × 100: 54 (BAW); 28 (6% AcOH); 19 (H_2O); 75 (30% AcOH). UV (λ_{max} nm) MeOH: 258, 268 sh., 292, 358; + NaOMe: 271, 327 sh., 408; + NaOAc: 267, 368; + NaOAc/H_3BO_3: 260, 291 sh., 372; + $AlCl_3$: 274, 432; + $AlCl_3$/HCl: 272, 302 sh., 357 sh., 402. ^{1}H NMR: aglycone moiety δ (ppm) 7.71 (d, J = 16 Hz, H-1‴); 7.70 (dd, J = 2.5 Hz and J = 9 Hz, H-6′); 7.59 (d, J = 2.5 Hz, H-2′); 6.92 (s, H3,5-galloyl); 6.86 (d, J = 9 Hz, H-5′); 6.81 (d, J = 16 Hz, H-2‴); 6.41 (d, J = 2.5 Hz, H-8); 6.21 (d, J = 2.5 Hz, H-6); 4.15 (q, J = 7 Hz, H-4‴); 1.28 (t, J = 7 Hz, H-5‴); sugar moiety δ 5.4 (d, J = 7.5 Hz, H-1″); 5.3 (d, J = 9 Hz, H-2″); 3.0–3.9 (m, the other sugar protons). ^{13}C NMR: aglycone moiety δ 158.2 (C-2); 133.5 (C-3); 177.5 (C-4); 162 (C-5); 97.8 (C-6); 163.14 (C-7); 95.27 (C-8); 158.3 (C-9); 103.5 (C-10); 123.9 (C-1′); 117.29 (C-2′); 146.7 (C-3′); 150.4 (C-4′); 117.65 (C-5′); 122.68 (C-6′); 125 (C-1‴); 110.47 (C-2‴); 170 (C-3‴); 39.79 (C-4‴); 15.42 (C-5‴); sugar moiety δ 100.49 (C-1″); 77.15 (C-2″); 75 (C-3″); 69.9 (C-4″); 77.5 (C-5″); 61 (C-6″); galloyl moiety δ 166 (CO); 147.2 (C-3, 5); 137 (C-4); 117.89 (C-1); 108.3 (C-2, 6).

3.4. Pharmacological methods

3.4.1. Antitumor test in vitro against Ehrlich ascites carcinoma

Female swiss albino mice weighing 18–20 obtained from the breeding unit of the National Research Centre, Cairo, Egypt, were used in this study. They were kept in plastic cages where they were fed on special food pellets, consisting of proteins, essential amino acids, minerals and vitamins.

EAC cells, were supplied through Dr. C. Benckhuiisen, 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, EAC cells were tested for viability and contamination using the trypan blue dye exclusion technique. The mice were inoculated with the tumor cells at a concentration of 2.5×10^6 cells/mouse.

The ascitic fluid was 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) × 100.

3.4.2. Cytotoxic activity against P 388 leukemia cells

The cell line employed in the present investigation (P 388 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 P 388 cells in a hemocytometer.

Acknowledgements: The authors are grateful to the Medicinal Chemistry Department, National Research Centre, Cairo, Egypt, for measuring the pharmacological activity.

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Received December 12, 2001
Accepted May 13, 2002

Dr. H. N. Michael
National Research Centre
Chemistry of Tanning Materials
and Proteins Department
Sh. El-Tahir
Dokki-Cairo
Egypt