Bioactive Compounds from the Buds of *Platanus orientalis* and Isolation of a New Kaempferol Glycoside

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Abstract

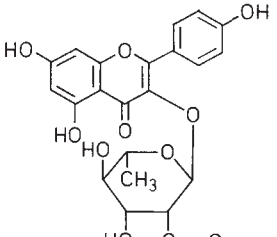
A new compound kaempferol 3-*O*-α-L-(2″-*E*-*p*-coumaroyl)-rhamnopyranoside, as well as the known flavonoids, kaempferol 3-*O*-β-D-(6″-*E*-*p*-coumaroyl)-glucopyranoside, kaempferol 3-*O*-α-L-(2″,3″-di-*E*-*p*-coumaroyl)-rhamnopyranoside, and caffeic acid were obtained from the methanolic extract of *Platanus orientalis* L. buds. All the compounds were isolated by column chromatography and identified using ^{1}H-NMR, 2D-^{1}H-NMR (COSY), ^{1}H-^{13}C-NMR, and CIDMS techniques. Cytotoxic and antimicrobial studies were carried out *in vitro* against human cell lines and against Gram-positive and Gram-negative organisms.

Key words

Plantanus orientalis L., Plantanaceae, kaempferol glycosides, antimicrobial activity, cytotoxicity.

Introduction

Plantanus orientalis L. (Plantanaceae) is a woody perennial tree, on the various parts of which some chemical investigations have been carried out (1). A chemical study on the buds of two *Platanus* species reported the isolation and characterization of some non-polar flavonoids (2, 3), while the examination of polar extracts showed the presence of a flavonoid glycoside (1) as well as acetylated and non-acetylated kaempferol monoglycosides (4). The buds of *P. orientalis* are used in folk medicine as antiseptic and antimicrobial remedies of the urinary system. In the search for bioactive compounds from natural sources, a preliminary screening on the methanolic extract of *P. orientalis* was carried out for cytotoxic and antimicrobial activity. The results prompted a closer examination of the plant in order to identify the compounds responsible for these activities and especially for the antimicrobial activity. The present paper describes the isolation and the identification of a new kaempferol glycoside **5** as well as of known compounds. The observations and the comparison of the activities of two isolated compounds and the methanolic extract are discussed.



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Materials and Methods

Apparatus

UV spectra: Hitachi (100-60) spectrometer; ^{1}H-NMR spectra Bruker HX (200 and 300 MHz); MS: NERMAG R 10-10C (90 eV).

Plant material

P. orientalis buds were collected in April 1991 on the island of Evia (Greece). They were botanically identified by Dr. D. Perdetzoglou. A voucher specimen has been deposited at the herbarium of the Division of Pharmacognosy, University of Athens (No. 203 ATPH).

Extraction and isolation

The dried buds (10 kg) were extracted at room temperature with CH_2Cl_2-MeOH mixtures of increasing polarity. The 100% MeOH fraction was subsequently concentrated under reduced pressure to a residue (80 g). An aliquot of 40 g was chromatographed over 500 g silica gel using CH_2Cl_2 and CH_2Cl_2-MeOH mixtures of increasing polarity followed by pure MeOH. A total of 45 fractions was obtained. Fractions 29–35 (× 35 ml) were examined by TLC on silica gel with CH_2Cl_2-MeOH (90 : 10), combined, and allowed to stand for 24 h at room temperature. A brown gum, insoluble in CH_2Cl_2, separated; this was subsequently crystallized from CH_2Cl_2-MeOH (99 : 1) to yield compound **1** (500 mg). Fractions 20–28 were concentrated to dryness. The residue obtained was chromatographed on a silica gel column eluted with CH_2Cl_2 and CH_2Cl_2-MeOH (99 : 1→90 : 10) to yield compounds **3** (80 mg) and **5** (3 mg). The latter compound is a new natural product. Pre-

parative TLC of the fractions 12–19 (CH_2Cl_2-MeOH 95 : 5) yielded 10 mg caffeic acid.

Kaempferol 3-*O*-β-D-(6″-*E*-*p*-coumaroyl)-glucopyranoside (tiliroside) (**1**). Yellow powder; UV (methanol); ^{13}C-NMR (4); ^{1}H-NMR (Table **1**).

Acetylation of **1**

Compound **1** was dissolved in Ac_2O-Py and left for 48 h at room temperature to yield heptaacetyltiliroside (**2**). Yellow powder; ^{1}H-NMR (300 MHz, Me_2CO-d_6) δ: 6.77 (1H, d, J = 2 Hz, H-6), 7.21 (1H, d, J = 2 Hz, H-8), 7.96 (2H, d, J = 8 Hz, H-2′,6′), 7.11 (2H, d, J = 8 Hz, H-3′,5′), 5.53 (1H, d, J = 8 Hz, H-1″), 5.17 (1H, dd, J = 9 and 8 Hz, H-2″), 5.29 (1H, t, J = 9 Hz, H-3″), 5.07 (1H, t, J = 9 Hz, H-4″), 3.67 (1H, m, H-5″), 4.08 (2H, m, H-6″A,6″B), 7.50 (2H, d, J = 8 Hz, H-2‴,6‴), 7.16 (2H, d, J = 8 Hz, H-3‴,5‴), 7.51 (1H, d, J = 16 Hz, H-7‴), 6.24 (1H, d, J = 16 Hz, H-8‴), 2.46–2.02 (7 × OAc).

Kaempferol 3-*O*-α-L-(2″,3″-di-*E*-*p*-coumaroyl)-rhamnopyranoside (platanoside) (**3**). Yellow powder; UV (methanol); ^{13}C-NMR (4); ^{1}H-NMR (Table **1**). The ^{13}C-NMR assignments have been revised on the basis of a ^{1}H-^{13}C-NMR spectrum. Thus it was found that the carbon signal at δ = 72 ppm corresponds to C-5″ and that at δ = 71 ppm to C-2″, in contrast to those mentioned in the literature (4).

Acetylation of **3**

Compound **3** was dissolved in Ac_2O-Py and left for 48 h at room temperature to yield hexaacetylplatanoside (**4**). Yellow powder; ^{1}H-NMR (300 MHz, Me_2-CO-d_6) δ: 6.84 (1H, d, J = 2 Hz, H-6), 7.29 (1H, d, J = 2 Hz, H-8), 7.98 (2H, d, J = 8 Hz, H-2′,6′), 7.33 (2H, d, J = 8 Hz, H-3′,5′), 5.71 (1H, d, J = 2 Hz, H-1″), 5.87 (1H, dd, J = 4 and 2 Hz, H-2″), 5.45 (1H, dd, J = 10 and 4 Hz, H-3″), 5.10 (1H, t, J = 10, H-4″), 3.50 (1H, m, H-5″), 0.94 (3H, d, J = 6 Hz, CH_3-6″), 2.45 (3H, s, OAc-5), 2.31–2.36 (4 × OAc), 1.97 (3H, s, OAc-4″), 7.56 and 7.47 (d, J = 8 Hz, H-2,6 coum.), 7.14 and 7.09 (d, J = 8 Hz, H-3,5 coum.), 6.49 and 6.30 (d, J = 16 Hz, H-8 coum.), 7.68 and 7.60 (d, J = 16 Hz, H-7 coum.).

Kaempferol 3-*O*-α-L-(2″-*E*-*p*-coumaroyl)-rhamnopyranoside (**5**). Yellow powder; ^{1}H-NMR (Table **1**). The ^{1}H-NMR spectrum of **5** exhibited two doublets with large coupling constants (J = 16 Hz) at δ = 6.38, and 7.68 (5). The doublet at δ = 5.4 with the 1.6 Hz coupling constant was assigned to the anomeric proton of α-rhamnose and confirmed the linkage of kaempferol at C-3 (6).

Table 1 ^{1}H-NMR spectral data of compounds **1**, **3**, and **5**[a].

H	**1**	**3**	**5**
6	6.05 d (2)	6.10 br. s	6.1 br. s
8	6.19 d (2)	6.26 br. s	6.3 br. s
2′6′	7.91 d (8.6)	7.81 d (8.8)	7.8 d (8.5)
3′5′	6.76 d (8.6)	6.93 d (8.8)	6.9 d (8.5)
1″	5.19 d (7.6)	5.55 d (1.6)	5.4 d (1.6)
2″	3.45 m	5.77 dd (3.4, 1.6)	5.66 dd (3.4, 1.6)
3″	3.45 m	5.25 dd (9.6, 3.4)	3.9 dd (9.6, 3.4)
4″	3.45 m	3.59 t (9.6)	3.4 m
5″	3.50 m	3.50 m	3.4 m
6″	4.29 dd (11.9, 1.9) 4.15 dd (11.9, 6.8)	0.99 d (6)	1 d (5.8)
2,6 coum.	7.21 d (8.5)	7.42 and 7.33 d (8.6)	7.2 d (8.3)
3,5 coum.	6.71 d (8.5)	6.76 and 6.71 d (8.6)	6.88 d (8.3)
7 coum.	7.34 d (15.9)	7.58 and 7.56 d (15.9)	7.68 d (16)
8 coum.	6.02 d (15.9)	6.33 and 6.24 d (15.9)	6.38 d (16)

[a] Measured at 300 MHz in CD_3OD, chemical shifts (ppm), rel. to TMS = 0, in brackets J values in Hz.

The downfield shift of H-2″ (δ = 5.66, dd, J = 3.4 and 1.6 Hz) indicated that *p*-coumaric acid was attached to C-2″. Further evidence for this structure was given by the HH-Cosy experiments. The CID mass spectrum of **5** exhibited a molecular ion at m/z = 579 [M + H]$^+$. This agreed to a molecular formula $C_{30}H_{26}O_{12}$ and hence characterized **5** as a flavonol coumaroyl glycoside.

Biological activities

Cytotoxic activity

The MeOH extract and compounds **1** and **3** were tested for cytotoxic activity. The following cell lines have been used: MOLT3 and MOLT4 cells (9); a human T cell line originating from a patient with acute lymphoblastic leukemia (ALL)-KM3 cells (10); a pre-B cell line originating from a patient with "common" ALL-SDK cells (11); a B-cell line originating from a patient with ALL-RAJI cells (12); a pre-B cell line originating from a patient with Burkitt lymphoma-HL60 cells (13); a cell line from a patient with promyelocytic leukemia-JURKAT cells; human T cell line cells from a patient with leukemic lymphoblast and DAUDI cells (14); and a human B cell line from a patient with Burkitt lymphoma. The cell lines were grown as exponentially proliferation cultures in suspension. All cells were cultured in RPMI 1640 medium (Gibco Europe Ltd., Scotland), supplemented with fetal calf serum (Myoclone Gibco) 2 mM L-glutamine (Gibco), and 50 μg/ml gentamycin and incubated at 37 °C in a humidified atmosphere and 5 % CO_2. Viability was assessed by trypan blue dye exclusion and was always greater than 98 %.

Treatment of cells with compounds **1**, **3** and with the MeOH extract: Compounds **1**, **3** and the MeOH extract were dissolved in absolute DMSO at the appropriate concentrations and stored at −40 °C. To determine cell proliferation, the compounds were added at the same time to each cell line culture of 2 × 10^6 cells/ml. An equivalent amount of DMSO was added to control cultures. The cells were cultured for 3 days in culture medium in a moist atmosphere of 5 % CO_2 in air. 16 h before termination of the cultures, 1 μCi [^3H]-thymidine (Amersham, U.K.) was added to each culture. The cells were harvested in an automatic cell harvester and the amount of radioactivity incorporated into macromolecules was measured in a liquid scintillation counter (Packard IL) and expressed as counts per minute (CPM). All tests on the samples were evaluated at three concentrations (15, 7.5, and 3.75 μg/ml).

Antimicrobial activity

In vitro antimicrobial studies were carried out by disc diffusion method (7, 8), against four Gram-positive (*Staphylococcus aureus, Streptococcus* group B, *Streptococcus* group F, *Staphylococcus epidermides*) and five Gram-negative (*Neisseria gonorrhoeae, Haemophilus influenzae, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) organisms. Paper discs with a diameter of 6 mm containing 300, 100, 50, and 10 μg of samples to be assayed, were deposited on blood agar, except for *Neisseria gonorrhoeae* and *Haemophilus influenzae* which were deposited on chocolate blood agar.

The plates with *Staphylococcus aureus, Staphylococcus epidermides, E. coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were incubated for 24 h at 37 °C. The plates with *Streptococcus* group B, *Streptococcus* group F, *Neisseria gonorrhoeae, Haemophilus influenzae* were deposited into the incubator (10 % CO_2) for 24 h at 37 °C. The isolated compounds and the methanol extract were dissolved in MeOH. Nitrofurantoin, norfloxacin, and trimethoprime were used as standard antibiotics for comparison. For each experiment a control disc with the solvent was used. The experiments were repeated four times and the results were expressed as average values.

Table 2 Cytotoxic activity of the MeOH extract and compounds **1** and **3**[a].

Compounds	Cell lines KM3	HL60	DAUDI	JURKAT	SDK	RAJI	MOLT3	MOLT4
MeOH extr.	–	14.7	–	–	N.T.[b]	–	15.3	–
1	–	N.T.	–	–	N.T.	–	–	N.T.
3	8.9	5.8	9.8	8.7	8.2	10.4	10	10.9

[a] Results are expressed as IC_{50} values (μg/ml).
[b] N.T. = not tested in higher doses.

Results and Discussion

Cytotoxic activity

The results obtained on cytotoxic activity are summarized in Table **2**. We have investigated compounds **1** and **3** and the MeOH extract, for their *in vitro* action on different "frozen" established cell lines, at different stages of development of the human immune system. As we have found after the *in vitro* treatment the total extract seems to be active only in two cell lines: HL60 (a promyelocytic cell line) and MOLT3 (a T-ALL with phenotypic characteristics of cortical thymocytes). However, it is unclear why MOLT4 did not respond under the same conditions. On the other hand the absence of any response to the treatment with compound **1** together with the significant modulation of all cell lines proliferation in the presence of **3** suggests that the latter is one of the active components of the MeOH extract. It is interesting that the MeOH extract seems to have a major influence on the HL60 cell line, suggesting that probably the main target is the immature cell of the myeloid lineage. We have experiments with Balb/c mice in progress in order to determine the influence of these compounds *in vivo*.

Antimicrobial activity

The results obtained on antimicrobial activity are summarized in Tables **3** and **4**. In contrast to **1** and the MeOH extract, compound **3** was inactive against *Neisseria gonorrhoeae, Haemophilus influenzae, Staphylococcus epidermides, Streptococcus* group F; however, it was more active than the other two towards the other organisms. Comparing the activities of compounds **1** and **3** to the antibiotics trimethoprime and nitrofurantoin, compound **1** proved to be active against *Streptococcus* group B, *Neisseria gonorrhoeae, Haemophilus influenzae*, contrary to the standard antibiotic trimethoprime, and more active than nitrofurantoin against *Pseudomonas aeruginosa*. Also, compound **3** was active against *Streptococcus* group B, *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* in contrast to antibiotics Nitrofurantoin and trimethoprime (Table **4**).

Table 3 Results of the antimicrobial activity.

Tested organisms[a]	μg/paper disc	Zones of inhibition in mm Compounds E[b]	**1**	**3**
Staphylococcus aureus	10	–	–	10
	50	10	–	12
	100	10	8	15
	300	12	10	16
Streptococcus group B	10	8	15	15
	50	8	19	17
	100	10	10	20
	300	13	12	22
Streptococcus group F	10	8	12	–
	50	8	14	–
	100	10	18	7
	300	11	20	8
Neisseria gonorrhoeae	10	8	10	–
	50	11	12	–
	100	15	13	–
	300	17	15	–
Haemophilus influenzae	10	–	8	–
	50	–	9	–
	100	10	11	–
	300	12	15	–
Escherichia coli	10	–	–	17
	50	8	8	18
	100	10	11	20
	300	12	13	22
Klebsiella pneumoniae	10	–	7	10
	50	–	8	15
	100	8	8	20
	300	12	10	23
Pseudomonas aeruginosa	10	7	–	–
	50	9	8	10
	100	14	11	14
	300	14	12	16
Staphylococcus epiderm.	10	8	–	–
	50	8	8	–
	100	10	8	–
	300	12	10	–

[a] Tested organisms were clinically isolated at the Dept. of Bacteriology of the Hellenic Pasteur Institute.
[b] MeOH extract.

Table 4 Results of the standard antibiotics[a].

Tested organisms	Zones of inhibition in mm F (300 μg/paper disc)	N (5 μg/paper disc)	T (5 μg/paper disc)
Staphylococcus aureus	21	26	15
Streptococcus group B	18	12	6
Streptococcus group F	30	20	30
Neisseria gonorrhoeae	28	40	–
Haemophilus influenzae	26	12	–
Escherichia coli	18	34	14
Klebsiella pneumoniae	18	25	28
Pseudomonas aeruginosa	–	15	–
Staphylococcus epiderm.	23	25	13

[a] F: Nitrofurantoin, N: norfloxacin, T: trimethoprim. Commercial products from Diagnostics Pasteur (92430) Marnes-la-Coquette France).

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