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## A new flavonoid: tinctosid from *Anthemis tinctoria* L.

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*Received March 29, 2005, accepted April 13, 2005*

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*Pharmazie 60: 956–957 (2005)*

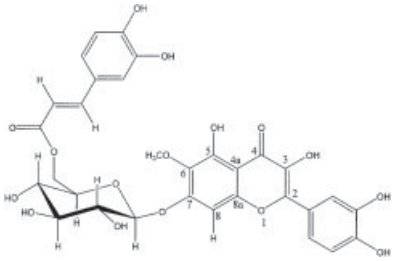
Patuletin 7-*O*-β-D-(6″-caffeoylglucoside) was isolated from the methanolic extract of the flowers of *Anthemis tinctoria* L. (Asteraceae). The isolated compound was identified by spectroscopic means and by comparison with authentic samples after enzymatic hydrolysis. A new flavonoid glycoside, tinctosid, from *Anthemis tinctoria* L. was isolated from a plant source for the first time.

*Anthemis tinctoria* L. (family Asteraceae, tribe Anthemideae) is a perrenial herb, cultivated in the Mediterranean countries.

Secondary metabolites, so far identified in this plant species, are volatile oils (Vaverková et al. 1988), triterpenes (Mašterová et al. 1995), polyacetylenes (Christensen et al. 1992), flavonoids, and other phenolics, e.g. caffeic acid (Greger et al. 1969; Kalošina et al. 1975; Wolenweber et al. 1991; Mašterová et al. 1993; Vaverková et al. 1999; Williams et al. 2001). In traditional folk medicine, the plant is used for the treatment of hepatic insufficiency and jaundice (Čižmář 1946; Liszt et al. 1972). Reports concerning medicinal or popular use of *Anthemis tinctoria* have been scarce, but several isolated flavonoids together with caffeic acid indicate the possibility of a therapeutic use, given the antioxidative and prooxidative behavior of a number of flavonoids. In general, the higher the substitution with hydroxy groups, the stronger the antioxidative and prooxidative activities. For instance, Bedir et al. (2002) confirmed that quercetin and tamarixetin, isolated from the leaves of *Ginkgo biloba*, possess antioxidative activity three times higher than ascorbic acid, employed as a standard.

We have recently reported the isolation of several flavonoids from the floral capitula of the plant species *Anthemis tinctoria* L. A new study of the plant material, and a more careful chromatography of the flavonoid-containing fractions has led to the isolation of a flavonoid glycoside esterified with caffeic acid, which is a new natural compound (derivative of patulitrin). Fractionation of the methanolic extract of the flower heads of *Anthemis tinctoria* L. with organic solvents, followed by repeated CC on Sephadex LH-20, afforded patuletin, patulitrin and its caffeoyl ester (**1**).

Compound **1** was obtained as yellow crystals. This new flavonol glycoside was analysed for $C_{31}H_{29}O_{16}$, responded to the Shinoda test, gave a ferric reaction, and a positive Molish test. The UV spectrum showed absorption maxima at 259 nm, 342 nm and 381 nm (Mabry et al. 1970), the IR spectrum displayed a wide OH band at 3400 $cm^{-1}$, an alkene band at 1598 $cm^{-1}$, and a carbonyl band at 1653 $cm^{-1}$. Hydrolysis with β-glucosidase yielded glucose, caffeic acid and an aglycone, which was identified as patuletin based on spectral and chromatographic comparison with an authentic sample.



## Experimental

### *1. Equipment*

$M_p$ is uncorr. UV spectrum was measured on a SPECORD UV VIS instrument. IR spectra were recorded on an IMPACT 400 D spectrometer (Nicolet) in anhydrous KBr discs. EI-MS analyses were done on a ZAB-EQ mass spectrometer (Micromass, Manchester, UK) using fast atom bombardment (FAB) ionization at 8 kV with Xe as a bombarding gas and glycerol as a matrix. Accurate masses of both $(M + H)^+$ ions and (aglycone + H)$^+$ ions were obtained at the resolving power of 5000. The product ions of the $(M + H)^+$ ions were recorded using linked scan maintaining B/E = const. $^1$H and $^{13}$C NMR spectra were recorded for $CD_3OD$ solutions at ambient temperature on a Varian Mercury-Vx BB 300 spectrometer operating at 300 MHz for $^1$H, 75 MHz for $^{13}$C. Chemical shifts were recorded as δ values in parts per million (ppm) and were indirectly referenced to tetramethylsilane (TMS) via the solvent signal (3.30 for $^1$H, 49,0 for $^{13}$C in $CD_3OD$). All assignments were made on the basic of pulse field gradient COSY, HSQC and HMBC experiments. Standard software, supplied by Varian, was used in recording all 2D spectra. For CC, Sephadex LH-20 (Pharmacia) was used. TLC was done on UV 254 or UV 366 plates, and silica gel 60 $F_{254}$ glass plates, using the following solvent systems: benzen-EtOH-acetone (7 : 2 : 1), and EtOAc-i-PrOH-n-BuOH-AcOH-$H_2O$ (25 : 15 : 8.75 : 8.75 : 7.5) for sugars. The chromatograms were sprayed with NEU reagents (flavonoids), and *p*-anisidine (sugars).

### *2. Plant material*

*Anthemis tinctoria* L. (Asteraceae) flowers, were collected from plants cultivated in the agricultural cooperative of Plavnica in Eastern Slovakia. A voucher specimen has been deposited at the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University.

### *3. Extraction and isolation*

Air-dried flowers (600 g) were ground, and the powder was completely extracted with MeOH. The MeOH extract (24 g) was dissolved in $H_2O$, and the resultant mixture was washed successively with $CHCl_3$, $Et_2O$, EtOAc, and n-BuOH. After purification through a Sephadex LH-20 column (elution with MeOH), patuletin, identical to a standard, was obtained from the ether-soluble (4 l) fraction. Similarly, the EtOAc soluble part (12 g) was purified through a column of Sephadex LH-20, using MeOH-$H_2O$ (8 : 2) as the mobile phase. Patulitrin was identified on comparison with an authentic sample by TLC and m.p., as well as by acidic hydrolysis. In addition, a new pure compound (**1**) was obtained, and crystallized from MeOH (30 mg).

Patuletin 7-*O*-β-D-(6″-caffeoylglucoside) (**1**): Yellow crystalls (methanol), m.p. 178–182 °C. UV($\chi^{MeOH}$nm): 259, 342, 381. IR ($\upsilon^{KBr}_{max}$ $cm^{-1}$: 3400, 1686, 1653, 1598, 1557, 1517, 1481, 1278, 1198, 1074, 994, 955, 811.

$(M + H)^+$ ions were recorded at m/z 657; the accurate mass measured was 657.1442, which corresponds to the elemental composition of $C_{31}H_{29}O_{16}$ (657.1445 calc.) $(M + H)^+$ ions eliminated neutral molecules of caffeic acid to give fragment ions at m/z 495, followed by the loss of hexose to produce the $(aglycone + H)^+$ ions at m/z 333. The value of 333.0619, corresponding to the elemental composition of $C_{16}H_{13}O_8$ (333.0610 calc.), was the accurate mass measured.

The comparison of routine $^1H$ and $^{13}C$ NMR spectra with the standards of patuletin and caffeic acid revealed that the compound is composed of patuletine, caffeic acid and D-glucose. According to the coupling constant of the olefinic protons in the caffeic acid residue (15.8 Hz), the double bond is in the *E* configuration.

In the $^1H$ NMR spectrum, the chemical shifts of H6 and H6′ of the glucosyl residue (4.31 and 4.62 ppm) indicated that the primary hydroxyl has been acylated. This was unequivocally corroborated by a gHMQC experiment, where these two protons displayed a clear correlation to the CO group of the caffeic acid residue at 169.0 ppm. Thus, the primary OH group in D-glucose is esterified with caffeic acid.

As regards the attachment of D-glucose to patuletin, the H1 (hemiacetal) proton in D-glucose at 5.12 ppm showed a cross peak to a patuletin carbon at 157.3 ppm, which was further identified from the correlations of patuletin H8. This hydrogen (at 6.72 ppm) displayed cross peaks to five carbons, at 177.4, 157.3, 152.9, 133.3 and 106.6 ppm. The carbon at 177.4 is the C4 carbonyl, the signal at 133.3 must be C6 since it showed another correlation to the protons of the $CH_3O$ group at 3.88 ppm, and the resonance at 106.6 is C4a based on the chemical shift. Hence, the signals at 157.3 and 152.9 belong to C7 and C8a, respectively, and D-glucose is attached to the C7 hydroxy group of patuletin via the hemiacetal hydroxy function. Given the coupling constant of glucose H1 (7.3 Hz), the glycosidic bond is in the β configuration.

$^1H$ NMR (300 MHz, $CD_3OD$): δ 3.45 (1 H, t, J = 9.3 Hz, H4 glc), 3.50–3.66 (2 H, m, H2 and H3 glc), 3.78–3.92 (1 H, m, H5 glc), 3.88 (3 H, s overlapped, $CH_3O$), 4.31 (1 H, dd, $J_1 = 12.00$ Hz, $J_2 = 7.3$ Hz, H6 glc), 4.62 (1 H, dd, $J_1 = 12.0$ Hz, $J_2 = 2.4$ Hz, H6′ glc), 5.12 (1 H, d, J = 7.3 Hz, H1 glc), 6.13 (1 H, d, J = 15.8 Hz, caffeic CH=), 6.51 (1 H, s overlapped, caffeic H-Ar), 6.52 (1 H, d overlapped, caffeic H-Ar), 6.71 (1 H, d, J = 1.1 Hz, caffeic H-Ar), 6.72 (1 H, s, patuletin H8), 6.82 (1 H, d, J = 8.7 Hz, patuletin H-Ar), 7.38 (1 H, d, J = 15.8 Hz, caffeic CH=), 7.58 (1 H, dd, $J_1 = 8.7$ Hz, $J_2 = 2.2$ Hz, patuletin H-Ar), 7.70 (1 H, d, J = 2.2 Hz, patuletin H-Ar).

$^{13}C$ NMR (75 MHz, $CD_3OD$): δ 177.4, 169.0, 157.3, 153.1, 152.9, 149.3, 148.9, 148.8, 147.5, 146.5, 146.0, 137.3, 133.3, 127.3, 123.9, 122.2, 121.9, 116.4, 116.2, 116.1, 115.5, 114.4, 106.6, 101.5, 95.1, 77.8, 75.5, 74.6, 72.0, 64.6, 61.6.

#### *4. Enzymatic hydrolysis*

A sample of **1** (5 mg) was hydrolysed with β-glucosidase in the MeOH-$H_2O$ (1 : 1) mixture at room temperature over 15 h. The reaction mixture was evaporated in vacuo, and, after the addition of $H_2O$, extracted with EtOAc. Caffeic acid and patuletin were clearly visible as a couple of spots on the chromatogram ($R_F$ value of patuletin 0.7, and caffeic acid 0.5 in the benzen-aceton-EtOH solvent system (7 : 2 : 1), twice developed) of the EtOAc phase. The presence of glucose was detected in the water phase, upon spraying the silica gel plate with a solution of p-anisidine, followed by heating for 5 min.

Acknowledgement: This work was supported by the Scientific Grant Agency of the Ministry of Education of the Slovak Republic (project No. 1/1185/04). NMR work was supported by the Ministry of Education of the Czech Republic (project No. MSM0021620822).

### References

Bedir E, Tatli II et al. (2002) Biologically active secondary metabolites from Ginkgo biloba. J Agric Food Chem 50: 3150–3155.

Christensen LP (1992) Acetylenes and related compounds in Anthemideae. Phytochemistry 31: 7–49.

Čižmář J (1946) "Lidové lékařství v Československu" (Tradicional medicine in Czechoslovakia) Melantrich Brno 2: 95.

Greger H (1969) Flavonoide und Systematik der Anthemideae (Asteraceae). Naturwissenschaften 56: 467–468.

Kalošina NA, Glyzin VI et al. (1975) Flavonoidy iz *Anthemis tinctoria*. Chim Prir Soedin 11: 654.

List PM, Hörhammer L (1972) Hagers Handbuch der pharmazeutischen Praxis. Springer Verlag Heidelberg III: 113.

Mabry TJ, Markham KR, Thomas MB (1970) The Systematic Identification of Flavonoids. Springer Verlag Berlin.

Mašterová I, Grančaiová Z et al. (1993) Phenolic substances in flowers of *Anthemis tinctoria*. Fitoterapia 64: 277.

Mašterová I, Vaverková Š et al. (1995) Komparatívne štúdium vybraných obsahových látok v kvetných úboroch *Anthemis tinctoria* L. Čes a Slov farm 44: 22–24.

Vaverková Š, Hollá M et al. (1988) Variabilita kvalitatívnych vlastností *Anthemis tinctoria* L. v priebehu ontogenézy. Čes a Slov farm 47: 40–43.

Vaverková Š, Mašterová I et al. (1999) The qualitative pharmacological properties of *Anthemis tinctoria* of Slovak provenience. Biologia (Bratislava): 443–445.

Williams CA, Greenham J et al. (2001) The role of lipophilic and polar flavonoids in the classification of temperate members of the Anthemideae. Biochem Syst Ecol 29: 929–945.

Wollenweber E, Mayer K (1991) Exudate flavonoids of *Anthemis nobilis* and *Anthemis tinctoria*. Fitoterapia 62: 365–366.