(1 H, d, $J_{4,3} = 3.2$, H-4, furane ring), 3.84–3.53 (4 H, m, H′-1, H′-2, chain), 3.21–2.96, m, OH), 2.32 (1 H, s, CH_3) ppm., M.S. 169 (M^+), 151, 138, 109 (base).
$C_8H_{11}NO_3$ (169.16)
5-Methyl-*N*-(2-hydroxyethyl)-2-furamide monohydrate (MHF) has been obtained from 5-methyl-*N*-(2-hydroxyethyl)-2-furamide with an equimolar amount of water: m.p. 60–63 °C, yield 92%. The results of elemental analyses for both compounds were in an acceptable error range.
$C_8H_{13}NO_4$ (187.2)

2. Pharmacological analysis

2.1. Effect on mouse lymphohematopoietic cells

In all experiments mice of CBA/H strain were used. Cyclophosphamide (Cy), a well known immunosuppressor served as control. MHF and Cy were given to the mice in equimolar concentrations (1 mmol × kg^{-1}, i.p.). For spleen celularity MHF and Cy were given to the mice (n = 6 in each group) on day minus four prior to counting the cells. The cells were counted and the results were expressed as number of cells per spleen. The spleen colony forming capacity of the bone marrow cells (CFU-s) was determined according to the method ofTill and McCulloch [6]. Mice (n = 6 in each group) were subjected to 8.5 Gy of whole body x-irradiation. Several hours latter, 10^5 syngeneic bone marrow cells were grafted intravenously into irradiated hosts. Two days after bone marrow transplantation, the compounds were given to the animals. On day 7 after irradiation splenic colonies were counted. To show the mitotic response of spleen cells to phytohaemagglutinin (PHA) and lipopolisaccharide (LPS), spleen cell suspensions (25×10^6) were prepared in Parker's medium, supplemented with 5% human serum one day after administration of the compounds. The suspension was cultured in a flat-bottomed Microtiter plates. Each well contained suspension of 5×10^6 cells in 200 µl of medium. Some of them were withouth mitogen to the others PHA (4 µg per well) or LPS (5 µg per well) were added. Each combination consisted of 5 samples. The cell cultures were incubated in a humidified (5% CO_2) atmosphere at 37 °C for 48 h. Tritiated tymidine 3.7×10^4 Bq in 25 µl) was added and the cell cultures were incubated for additional 24 h.The amount of radioactivity incorporated was counted (cpm) using a liquid scintilation counter. The results are shown in the Table.

2.2. Poikilothermic effect

MHF (3.000 mg × kg^{-1}; 16 mmol × kg^{-1}) was given to the mice of the CBA/H strain at various ambient temperatures. The colon body temperature was monitored using an electronic thermomeer (Fig.).

3. Statistical analysis

The results were compared by student T test for statistical significance.

References

1 D'Auria, M.: Heterocycles **50**, 1115 (1999)
2 Dunlop, A. P.; Peters, F. N.: The Furans, Am. Chem. Soc. Monograph, No 19, Washington, D.C.
3 Farfel, G. M.; Saiden, L. S.: J. Pharmacol. Exp. Ther. **272**, 868 (1995)
4 Sanchez, C.; Lembol, H. L.: Pharmacol. Toxicol. **74**, 35 (1994)
5 Unal, C. B.; Demiral, Y.; Ulus, I. H.: Eur. J. Pharmacol. **363**, 121 (1998)
6 Till, J. E.; McCulloch, E. A.: Radiat. Res. **14**, 213 (1961)

Received July 29, 1999
Accepted November 4, 1999

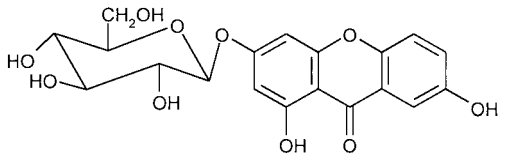
Dr. Antonia Vlahov
Department of Organic Chemistry
Marulićev trg 20
P.O. box 177
10000 Zagreb
Croatia
avlahov@pierre.fkit.hr

Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Sofia, Sofia, Bulgaria

Xanthohypericoside, a new xanthone-*O*-glucoside from *Hypericum annulatum*

G. M. KITANOV and P. T. NEDIALKOV

Flavonoids and xanthones possess a broad spectrum of pharmacological activities and are widespread in many *Hypericum* species [1, 2]. *Hypericum annulatum* Moris subsp. *annulatum* also known as *H. degenii* Bornm. is a herbaceous plant, growing on the Balkan peninsula and Sardinia [3, 4]. Some flavonols, catechins and large amounts of gentisein have been found in the aerial parts of this species [5, 6]. Gentisein was found to exhibit a tuberculostatic activity [2]. The reinvestigation of this taxa has now led to the isolation of a new xanthone glycoside (**1**) together with eleven known xanthone and flavonoid aglycones and glycosides.



1

The TSP-MS spectrum of **1** showed a $[M + H]^+$ peak at *m/z* 407 consistent with the molecular formula $C_{19}H_{18}O_{10}$ and an ion peak at *m/z* 245 $[M_{agl} + H]^+$ with the formula $C_{13}H_8O_5$. The IR absorption spectra established the presence of hydroxyl groups (3380 cm^{-1}), conjugated carbonyl (1651 cm^{-1}) and aromatic double bounds (1614 and 1580 cm^{-1}). The UV spectrum showed typical absorption maxima for xanthones at 236, 260, 302 and 376 nm. The bathochromic shift of band IV with $AlCl_3$/HCl (59 nm) reveals the presence of a free hydroxyl group at C-1 (and/or C-8) position. No change on addition of NaOAc was observed which indicates substituted hydroxyl at C-3 (and/or C-6) position of compound **1** [7]. Both total acid hydrolysis and treatment with β-glucosidase gave D-glucose and an aglycone in a ratio of 1 : 1, identified as 1,3,7-trihydroxyxanthone (gentisein) [6]. It shows a bathochromic shift (band III) with NaOAc (35 nm) which suggests the attachment of the sugar moiety at C-3 of gentisein [7]. The ^{1}H NMR spectrum of **1** showed the presence of a chelated hydroxyl (δ 12.53), a broad singlet due to the hydroxyl proton at C-7 and signals of five aromatic protons due to the gentisein moiety. The signal at δ 5.08 (*d*, $J = 7.3$ Hz) and a multiplet at δ 3.16–3.73 were assigned to the sugar residue. Thus, the structure of **1** was established as 1,7-dihydroxyxanthone-3-O-β-D-glucopyranoside, named xanthohypericoside.
The EtOAc and *n*-BuOH extracts gave also the known compounds norathyriol, isomangiferin, isoquercitrin and I-3,II-8-biapigenin together with gentisein, kaempferol, quercetin, myricetin, hyperoside, quercetrin and rutin, earlier reported for this plant. The chromatographic and spectroscopic data of biapigenin are similar to those given in the literature [8, 9].
Although xanthones are frequently reported for *Hypericum*, to date only one xanthone-O-glycoside (1,5-dihydroxyxanthone-6-O-glucoside) has been isolated from a native species of this genus [10].

Experimental

1. General procedures

M.ps. uncorr. The spectra were recorded on Brucker ARX 300 (^{1}H NMR, 300 MHz, DMSO-d_6, TMS), FTIR-8101M Shimadzu (IR, nujol) and Specord UV-VIS (UV, MeOH and shift reagents) instruments. Thermospray MS (TSP-MS) were registered on a LC/MS HP 5989 A mass spectrometer (Hewlett Packard) in positive ion mode. Hydrolysis of the glycosides was carried out with 2% HCl (100°, 30 min) and β-glucosidase. Polyamide (Woelm), silica gel (Merck) and Sephadex LH-20 (Farmacia) were used for CC and TLC was carried out on silica gel 60 F_{254} plates (Merck).

2. Plant material

The aerial parts of *Hypericum annulatum* were collected during the flowering season from wild habitat at the Central Rodope Mountains in July 1997. A voucher specimen # 144296 was deposited at Herbarium of the Botany Institute of Sofia (SOM).

3. Extraction and isolation

Dried and powdered aerial parts (1.7 kg) were defatted with *n*-hexane and extracted with hot MeOH. The crude MeOH residue was dissolved in hot H_2O, filtered and treated with $CHCl_3$. The aq. phase was partitioned successively with EtOAc and *n*-BuOH, respectively. The EtOAc fraction (52 g) was chromatographed on a polyamide column using a 0–60% EtOH linear gradient and gave a mixture of **1** and other components (H_2O fraction), flavonoid glycosides (10–30% EtOH) and a mixture of xanthone and flavonoid aglycones (40–60% EtOH). The *n*-BuOH extract was separated by the same procedure and afforded crude isomangiferin and rutin. The H_2O fraction was rechromatographed on polyamide using a gradient system $CHCl_3$–MeOH (0–20%). Subsequent purification by prep. TLC and Sephadex LH-20 gel filtration gave pure **1** (20 mg). All other compounds were separated by usual techniques followed by purification on Sephadex LH-20 and were identified by spectral methods and co-chromatography with authentic samples [11].

Xanthohypericoside **1**. Yellow needles, m.p. 233–234 °C. TSP–MS *m/z* (rel. int.): 407 $[M+H]^+$ $(C_{19}H_{18}O_{10}+H)^+$ (22), 245 $[C_{13}H_8O_5+H]^+$ (100). IR ν_{max}^{nujol} cm^{-1}: 3380, 1651, 1614, 1580,1464. UV λ_{max}^{MeOH} nm: 205, 236, 260, 302, 376; + $AlCl_3$ 234, 253sh, 278, 324, 435; + $AlCl_3$/HCl 233, 253sh, 276, 324, 435; +NaOAc 260, 302, 376; + NaOAc/H_3BO_3 260, 302, 376. ^{1}H NMR (DMSO-d_6, δ): 12.53 (1H, *s*, 1-OH), 10.12 (1H, br. *s*, 7-OH), 7.53 (1H, *d*, $J=9$ Hz, H-5), 7.44 (1H, *d*, $J=3$ Hz, H-8), 7.33 (1H, *q*, $J_{6,8}=3$ Hz, $J_{5,6}=9$ Hz, H-6), 6.67 (1H, *d*, $J=2.17$ Hz, H-4), 6.44 (1H, *d*, $J=2.17$ Hz, H-2), 5.08 (1H, *d*, $J=7.3$ Hz, H-1′), 3.16–3.73 (*m*, sugar protons).

Acknowledgements: The authors are indebted to Dr. Ulrich Girreser (Kiel, Germany) and to Prof. Ivo Ivanov, (Sofia, Bulgaria) for measurement and providing the MNR and MS spectra. This work was supported by a grant from the Medical Science Council (M.S.C.) at Medical University of Sofia.

References

1 Kitanov, G. M.; Blinova, K. F.: Khim. Prir. Soedin. (2), 185 (1987)
2 Bennet, G. J.; Lee, H.-H.: Phytochemistry **28**, 967 (1989)
3 Jordanov, D.; Kožucharov, S.; in: Jordanov, D. (Ed.): Flora of R. P. Bulgaricae Vol. **4**, p. 224, BAS, Sofia 1970
4 Robson, N. K. B.: Bull. Nat. Hist. Mus. London (Botany) **23** (2), 67 (1993)
5 Akhtardzhiev, Ch.; Nakov, N.; Atanasov, G.: Farmatsiya (Sofia) **22** (6), 35 (1972)
6 Kitanov, G.; Akhtardzhiev, Ch.: Pharmazie **34**, 447 (1979)
7 Hostettmann, K.; Wagner, H.: Phytochemistry **16**, 821 (1977)
8 Kitanov, G. M.: Farmatsiya (Sofia) **35** (5), 13 (1985)
9 Berghöfer, R.; Hölzl, J.: Planta Med. **53**, 216 (1987)
10 Wu, Q. L.; Wang, S. P.; Liao, Y. H.; Wang, L. W.; Feng, Y. X.; Yang, J. S.; Xiao, P. G.: Chin. Chem. Lett. **7**, 1011 (1996)
11 Markham, K. R.: Techniques of Flavonoid Identification Academic Press, London, New York 1982

Received May 26, 1999
Accepted October 5, 1999

Assoc. Prof. Dr. Gerassim M. Kitanov
Department of Pharmacognosy
Medical University of Sofia
Dunav str. 2
1000 Sofia
Bulgaria
gkitanov@mbox.pharmfac.acad.bg