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Poikilothermia induced by a 2-furancarboxylic acid derivative

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The furans comprise an important class of heterocyclic compounds exerting a wide range of useful biological and pharmacological properties. They are found both in natural and many commercial products, pharmaceuticals, dyes and fragrances [1–2]. In this communication we briefly describe the synthesis and some intriguing biological properties of a newly synthesised 2-furancarboxylic acid derivative: 5-methyl-*N*-(2-hydroxyethyl)-2-furamide monohydrate (MHF). The compound is formed by addition of an equimolar amount of water to the previously synthesised 5-methyl-*N*-(2-hydroxyethyl)-2-furamide. Using mice of CBA/H strain, the MHF has been assayed for its toxicity *in vivo* both in terms of survival and the effect on the lymphohematopoietic cells. To examine the hypothermic activity of the compound, body temperature was monitored at various concentrations and various ambient temperatures. The compound was shown to be highly nontoxic since the animals survived the dose as high as 3000 mg $\times$ kg^{-1} (16 mmol $\times$ kg^{-1}), and remained healthy. As shown in the Table, MHF did not affect the growth and differentiation of hematopoietic stem cells in radiation chimeras, and also it did not exert any suppressive effect on the spleen lymphocytes in their response to polyclonal mitogens, phytohemagglutinin and lipopolysaccharide.

Most intriguing, MHF at this high concentration (3000 mg $\times$ kg^{-1}; 16 mmol $\times$ kg^{-1}) consistently, induces poikilothermia, a hibernation-like physiological status within one hour. As shown in the Fig., the colon temperature of the MHF-treated mice mirrored that of the ambient, being negligibly higher (for 0.8 °C). Lower concentrations of MHF (down to 200 mg $\times$ kg^{-1}; 1 mmol $\times$ kg^{-1}) induced only hypothermia. The reflex reaction, however, in the "hybernating" animals remained unaffected. They respond vigorously when the footpad was pinched with the forceps. After the first four hours, the body temperature began to rise gradually ($\sim$ 0.1 °C every two minutes). Extremely low toxicity and high concentration of the compound required to induce poikilothermia may be due to the methyl group at position five of the furane ring. The similar compound lacking only that group is several time more toxic exerting also hypothermia.

To propose possible mechanisms of this remarkable property of MHF, additional biochemical experiments to explain interactions at the receptor level are clearly needed.

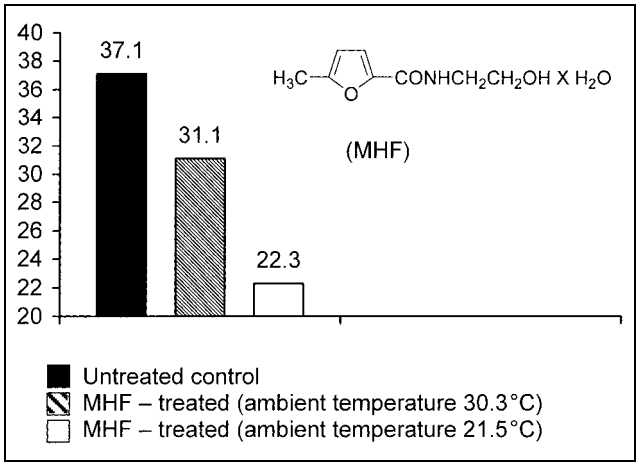

Fig.: Colon temperature in CBA/H mice 1 h after intraperitoneal injection of 3000 mg $\times$ kg^{-1} of 5-methyl-*N*-(2-hydroxyethyl)-2-furamide monohydrate (MHF). For each bar n = 2 animals. The values between the individual animals of the group did not differ. The poikilothermic effect of MHF has been confirmed by repeated experiments

Alternatively, the poikilothermic effect of MHF may not be mediated by receptors since very high concentrations of the compound are needed. As even five metabolites, along with the original compound were detected in the urine by TLC, very complex interactions should be considered to explain the observed effect. Physiologically, this poikilothermic effect of MHF could be explained as if the hypothalamic thermostat has been somehow temporarily switched off. This was facilitated by concomitant muscle relaxation so that this tissue, as the main thermogenic generator (shivering) in the case of hypothermia simply did not respond. It may well be that metabolic thermogenesis was absent as well. The lack of thermogenetic responses, which are stressful by itself, may also explain why a significant lowering of the body temperature was not harmful for the animals. There are many reports on hypothermia induced by various compounds [3–5], but according to our knowledge, MHF seems to be unique in its ability to induce poikilothermia. However, the compound appears to be virtually nontoxic even at this high concentrations both in long-term animal survival and at the cellular level.

There could be many potential applications for MHF in biomedicine and as a research tool for studying thermoregulatory mechanisms.

Experimental

1. Synthesis

5-Methyl-*N*-(2-hydroxyethyl)-2-furamide has been synthesised by condensation of ethylester-5-methyl-2-furancarboxylic acid with monoethanolamine: b. p. 175–180 °C at 533.3 Pa, yield 75–90%; ^{1}H NMR ($CDCl_3$): δ 7.00 (1 H, d, $J_{3,4}$ = 3.2, H, furane ring), 6.82–6.21 (1 H, m, NH), 6.08

Table: The effect of 5-methyl-N-(2-hydroxyethyl)-2-furamide monohydrate (MHF)

Parameter monitored	Compound assayed		
	Media control (Hanks)	Cy*	MHF
Spleen cellularity ($\times 10^6$ cells)	138 $\pm$ 12	19 $\pm$ 4	130 $\pm$ 22
CFU-s** (No. per spleen)	18 $\pm$ 1	0	15 $\pm$ 2
Mitogens*** PHA (cpm)	128 639 $\pm$ 32 689	22 388 $\pm$ 4922	149 426 $\pm$ 26 629
LPS (cpm)	26 223 $\pm$ 8078	354 $\pm$ 97	21 523 $\pm$ 6502

* In all experiments the effect of MHF was compared to cyclophosfamide (Cy), well known immunosuppressor.
** The number of colony forming units (CFU-s), as a measure of stem cell potential of the bone marrow, was assayed by the method of Till and McCulloch [6].
*** The mitotic response of spleens T- and B-lymphocytes to polyclonal mitogens (PHA, LPS) is expressed as counts per minute (cpm) when measured by a scintillation counter. The cpm values correlate with rate of tritiated thymidine incorporation into DNA of dividing cells.

(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.

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

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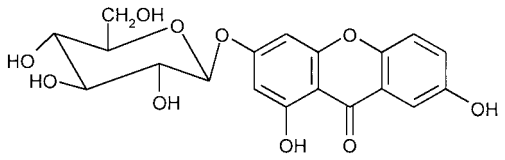
Flavonoids and xanthones possess a broad spectrum of pharmacological activities and are widespread in many *Hypericum* species [1, 2]. *Hypericum annulatum* Moris subsp. *annulatum* also known as *H. degenii* Bornm. is a herbaceous plant, growing on the Balkan peninsula and Sardinia [3, 4]. Some flavonols, 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].