Experimental

The experiments were carried out with three-weeks grown callus cultures of five plant species, namely: *Leuzea carthamoides* DC. Asteraceae (1 mg 2,4 D + 1 mg K), *Bergenia crassifolia* (L.) Fritsch Saxifragaceae (10 mg IBA + 1 mg K), *Leonurus cardiaca* L. Lamiaceae (1 mg IAA + 1 mg K), *Rhodiola rosea* L. Crassulaceae (1 mg 2,4 D) and *Datura meteloides* DC. ex Dunal Solanaceae (1 mg 2,4 D); out of the species mentioned above only *Bergenia* produces arbutin in intact plant. A 20-g aliqout of a raw callus (approx. 1 g of dried mass; size of the swelled callus particles 1–5 mm) was suspended in 300 ml of air-purged M-S medium (+ corresponding stimulators) doped with HQ (starting concentration of HQ in the medium was 0.85 mmol · l^{-1}). The biotransformation changes in the medium were monitored at 23 ± 2 °C for 48 h, the measurements of HQ concentration being performed in 30 min intervals with use of the automated amperometric FIA setup (Fig. 2). Selective FIA assay of 2 µM to 20 mM HQ in M-S medium was carried out with the use of a three-electrode flow-through amperometric cell of the wall-jet type with 0.1 M acetate buffer of pH 4.6 as the carrier stream; working electrode spectrographic graphite rod (diam. 3 mm) impregnated with epoxide resin (working potential E_w = +0.5 V vs. reference SCE); auxiliary platinum wire electrode. The overal content of arbutin in the spent calluses was determined by HPLC [2]. The biotransformation experiments were performed in duplicate; the results of the parallel runs were practically identical.

References

1 Jahodář, L.; Jílek, P.; Pátková, M.; Dvořáková, V.: Cesk. Farm. **34**, 17 (1985)
2 Holopainen, M.; Jahodář, L.; Seppanen-Laakso, T.; Laakso, I.; Kauppinen, V.: Acta Pharm. Fenn. **97**, 197 (1988)
3 Strapková, A.; Jahodář, L.; Nosál'ová, G.: Pharmazie **46**, 611 (1991)
4 Matsuda, H.; Nakata, H.; Tanaka, T.; Kubo, M.: Yakugaku Zasshi **110**, 68 (1990)
5 Matsuda, H.; Tanaka, T.; Kubo, M.: Yakugaku Zasshi **111**, 253 (1991)
6 Matsuda, H.; Nakamura, S.; Tanaka, T. Kubo, M.: Yakugaku Zasshi **112**, 673 (1992)
7 Matsuda, H.; Higashino, M.; Nakai, Y.; Iinuma, M.; Kubo, M.; Lang, F.: Biol. Pharm. Bull. **19**, 153 (1996)
8 Boonyakiat, D.; Spotts, R. A.; Richardson, D. G.; Hort Science **21**, 309 (1986)
9 Dušková, J.; Jahodář, L.; Dušek, J.: Cesk. Farm. **39**, 452 (1990)
10 Dušková, J.; Jahodář, L.; Dušek, J.: Pharmazie **49**, 624 (1994)
11 Tabata, M.; Ikeda, F.; Hiraoka, N.; Konoshima, M.: Phytochemistry **15**, 1225 (1976)
12 Yokoyama, M.; Inomata, S.; Seto, S.; Yanagi, M.: Plant Cell Physiol. **31**, 551 (1990)
13 Stoeckigt, J.; Obitz, P.; Falkenhagen, H.; Lutterbach, R.; Endress, S.: Plant Cell Tissue Organ Cult. **43**, 97 (1995)
14 Polášek, M.; Jahodář, L.; Dušková, J.; Papugová, P.; Solich, P.: Proceedings of the ICFIA 97, Orlando, Florida, Jan 12–16, 1997, p. 48

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Coumarins from *Hypericum keniense* (Guttiferae)

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Hypericum keniense Sweinf. (Guttiferae), a shrub or small tree found growing in rain forests in the tropical East Africa, is a hitherto phytochemically uninvestigated species [1]. Guttiferae plant species are widely used in folk medicine and prior investigations into some of the species of this family led to the isolation of antiviral [2], antimicrobial [3, 4], antifungal [5] and cytotoxic [6–8] bioactive compounds including coumarins. In the present study, repeated chromatographic fractionation of the *n*-hexane and ethylacetate extracts of *H. keniense* stem bark afforded 5,7-dimethoxy-8-(3′-methylbut-2′-enyl)-coumarin (15 mg, 0.0010%), 8-(3′,3′-dimethoxyoxiranyl-methyl)-5,7-dimethoxy-chromen-2-one (71 mg, 0.0046%), toddanolactone (12 mg, 0.0008%), pimpinellin (62 mg, 0.0040%), the novel coumarin **1** and betulinic acid 756 mg (0.0484%). The chemical identities of these coumarins being reported for the first time from *H. keniense*, were established by comparing their physical and spectral data with those in the literature [9–12]. Compound **1** showed the elemental composition $C_{16}H_{18}O_5$ and was identified as (*E*)-8-(3′-Hydroxy-3′-methyl-1′-butenyl)-5,7-dimethoxy-coumarin (5-methoxymurraol) from its ^{1}H NMR and ^{13}C NMR (Table 1) together with the MS, UV and IR spectroscopic data. To our knowledge, this is the first report on this position 8 substituted tertiary allylic alcohol of a 5,7-dimethoxy coumarin from a natural source. Compound **1** can be regarded as a biosynthetic intermediate of the naturally occurring 5,7-dimethoxycoumarins gleinene and gleinadiene [12].

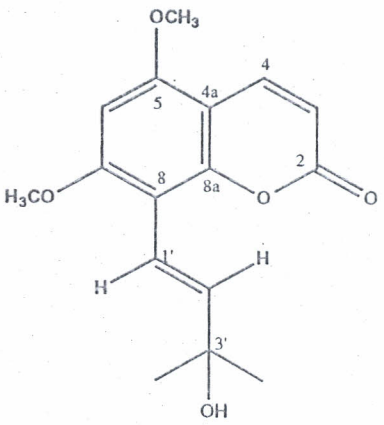

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Table: ^{1}H NMR and ^{13}C NMR data assignments and relevant HMBC correlations of (*E*)-8-(3′-hydroxy-3′-methyl-1′-butenyl)-5,7-dimethoxycoumarin (1)

Atom	^{13}C 75 MHz, $CDCl_3$	^{1}H 300 MHz, $CDCl_3$	Relevant HMBC's
2	161.1		
3	110.8	6.16, d, J = 9.6 Hz	C2, C4a
4	138.7	7.98, d, J = 9.6 Hz	C5
4a	103.6		
5	155.6		
6	90.2	6.32, s	
7	161.0		
8	106.4		
8a	153.4		
5-OCH_3	56.0	3.96, s	C5
7-OCH_3	55.9	3.94, s	C7
1′	114.2	6.86, d, J = 16.5 Hz	C8, C7, C8a, C3′
2′	141.8	6.90, d, J = 16.5 Hz	C4′, C5′
3′	77.6		
3′OH		2.02, s	
4′	30.0	1.46, s	
5′	30.0	1.46, s	