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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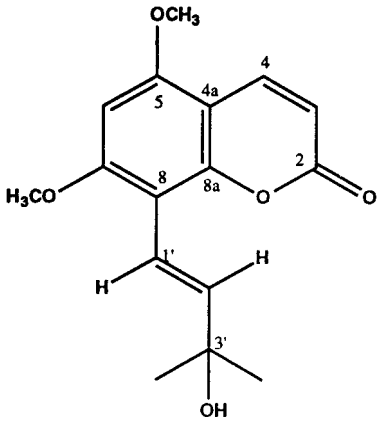
Hypericum keniense Sweinf. (Guttiferae), a shrub or small tree found growing in rain forests in the tropical East Africa, is a hitherto phytochemically uninvestigated species [1]. Guttiferae plant species are widely used in folk medicine and prior investigations into some of the species of this family 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-dimethoxycoumarin (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].



1

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	

Experimental

1. Plant material

Hypericum keniense stem bark (1.56 kg) was sampled from the slopes of mt. Kenya near Naro Moru game reserve in the central province of the Republic of Kenya. It was identified in the field by Mr. Simon Mathenge and its identity was authenticated at the University of Nairobi herbarium. A voucher specimen (coded CAA/007/96) is deposited at the University of Nairobi herbarium in Nairobi, Kenya (East Africa).

2. Extraction and purification

Repeated CC on silica gel of *n*-hexane and ethylacetate extract fractions afforded all the above mentioned compounds together with the novel coumarin **1**. Compound **1** (107 mg, 0.0068%) was recrystallized from EtOAc/*n*-hexane as yellowish green needles, mp. 213–214 °C. EIMS m/z (rel. int.): 290.1137 (M^+, 30, calculated for $C_{16}H_{18}O_5$ 290.1154), 275 ([M-Me]$^+$, 74), 247.1006 ([M-Me-CO]$^+$, 28, calc. for $C_{14}H_{15}O_4$ 247.0970), 233.0816 (29, calc. for $C_{13}H_{13}O_4$ 233.0814), 219.0673 ([M-C_4H_7O]$^+$, 100, calc. for $C_{12}H_{11}O_4$ 219.0657); IR (KBr): ν_{max} (cm^{-1}) = 3520 (br., OH), 1705 (C=O), 1600 (C=C); UV (MeOH): λ_{max} (log ε) = 210 nm (4.5), 254 (4.2), 270 (4.2), 311 (4.0); ^{1}H NMR and ^{13}C NMR see Table.

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References

1 Beentje, H.: Kenya trees, shrubs and lianas, p. 147, Kenya National Museum, Nairobi 1994
2 Jayasunya, H.; Mchesney, J. D.; Swanson, S. M.; Pezzuto, J. M.: J. Nat. Prod. **52**, 325 (1989)
3 Tada, M.; Takakuwa, T.; Nagai, M.; Yoshii, T.: Agric. Biol. Chem. **54**, 3061 (1990)
4 Chhabra, S. C.; Mahunnah, R. L. A.; Mshiu, E. N.: J. Ethnopharmacol. **28**, 255 (1990)
5 Ishiguro, K.; Yamaki, M.; Kashihara, M.; Takagi, S.; Isoi, K.: Planta Med. **56**, 274 (1990)
6 Dascoterd, L.; Stoeckli-Evans, H.; Msothi, J. D.; Hostettman, K.: Helv. Chim. Acta **70**, 1694 (1987)
7 Hegnauer, R.: Chemotaxonomie der Pflanzen, Vol. IV, 540, Birkhäuser Verlag Basel, Boston, Berlin, 1992
8 Cao, S.; Sim, K.; Pereira, J.; Goh, S.: Phytochemistry **47**, 1051 (1998)
9 Takeshi, K.; Jin-Bin, W.; Feng-Chi, H.: Phytochemistry **43**, 125 (1996)
10 Ishii, H.; Kobayashi, J.; Ishikawa, M.; Haginiwa, J.; Ishikawa, T.: Yakugaku zasshi **111**, 365 (1991)
11 Ishii, H.; Kobayashi, J.; Sakurada, E.; Ishikawa, T.: J. Chem. Soc., Perkin Trans. **13**, 1681 (1992)
12 Reich, J.; Wickramasinghe, A.; Wickramaratne Mahinda, D. M.: Liebigs Annal. **2**, 209 (1990)

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