PERGAMON

Phytochemistry 52 (1999) 1671–1676

PHYTOCHEMISTRY

Coumarins and dihydrocinnamic acid derivatives from *Micromelum falcatum*☆

Christine Kamperdick[a], Nguyen Minh Phuong[b], Tran Van Sung[b], Jürgen Schmidt[a], Günter Adam[a,*]

[a]*Institute of Plant Biochemistry, Weinberg 3, D-06120 Halle/Saale, Germany*
[b]*Institute of Chemistry, National Centre for Natural Science and Technology of Vietnam, Nghia Do, Tu Liem, Hanoi, Viet Nam*

Received 5 March 1999; accepted 13 April 1999

Abstract

Besides the known coumarins microminutinin and 6-methoxymicrominutinin, the new dihydrocinnamic acid derivatives 3,4-dihydro-1,2-*seco*microminutinin, 3,4-dihydro-1,2-*seco*microminutinin methylester and 3,4-dihydro-1,2-*seco*microminutinin-9-*O*-glucoside as well as the new 8-prenylated coumarin microfalcatin isovalerate were isolated from the leaves of *Micromelum falcatum* (Rutaceae). The structures were elucidated by mass and NMR spectroscopy. The relative configuration of microfalcatin isovalerate was established as 1′,2′-*threo* by analysis of the NOE effects of its acetonide. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Micromelum falcatum*; Rutaceae; Coumarins; 3,4-Dihydro-1,2-*seco*microminutinin derivatives; Microfalcatin isovalerate

1. Introduction

Micromelum falcatum (Lour.) Tan. is a shrub growing frequently in Vietnam. In folk medicine, its leaves and roots are applied externally to infected wounds, rheumatism, muscular atrophy and insect bites. Internally, the plant serves as an emmenagogue and as remedy against fever and rheumatism (Do, 1991; Perry, 1980). The genus *Micromelum* is known to contain dimeric indole alkaloids, carbazole alkaloids, polyoxygenated flavonoids and 6- and 8-prenylated coumarins. From *Micromelum falcatum*, the dimeric indole alkaloid yuehchukene, the carbazole alkaloid 5,6-pyranoglycozoline, the 6-prenylated coumarin micromelin and the 8-prenylated coumarins phebalosin and murpanidin have been isolated from the roots (Kong et al., 1988). The leaves have not been investigated until now. In continuation of our search for new biologically active compounds from Vietnamese medicinal plants (Thuy, Porzel, Ripperger, Sung, & Adam, 1999) we now report the isolation and structural elucidation of the known coumarins microminutinin (**1**) and 6-methoxymicrominutinin (**2**), the new dihydrocinnamic acid derivatives 3,4-dihydro-1,2-*seco*microminutinin methylester (**3**), 3,4-dihydro-1,2-*seco*microminutinin (**4**) and 3,4-dihydro-1,2-*seco*microminutinin-9-*O*-glucoside (**5**) as well as the new 8-prenylated coumarin microfalcatin isovalerate (**6**) from the leaves of this plant.

2. Results and discussion

The leaves of *Micromelum falcatum* were extracted with solvents of increasing polarity. Six compounds were isolated using normal phase column chromatography on silica gel. Compound **1** ($C_{14}H_{10}O_4$ $[M]^+$ m/z 242) was identified as microminutinin by comparison of its carbon shifts with reference data. This coumarin

☆ Dedicated to Professor Axel Zeeck on the occasion of his 60th birthday.

* Corresponding author. Tel.: +49-345-5582-216; fax: +49-345-5582-102.
E-mail address: gadam@ipb.uni-halle.de (G. Adam).

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00243-5

Table 1
^{13}C spectral data of compounds **1–6** (75 MHz)

Pos.	**1**, $CDCl_3$	**2**[a], $CDCl_3$	**3**, $CDCl_3$	**4**, CD_3OD	**5**, CD_3OD	**6**, $CDCl_3$
2	160.5	160.8	177.1	184.3	184.1	160.1
3	112.7	113.17	35.6	39.7	39.9	113.4
4	143.9	143.8	23.6	26.9	26.7	143.8
5	129.5	109.9	131.0	131.8	131.4	128.8
6	106.9	141.5	102.2	101.8	107.1	107.8
7	162.2	151.3	158.8	159.6	159.3	160.1
8	113.4	114.7[b]	114.2	115.0	123.4	115.5
9	151.6	146.1	152.0	154.0	153.5	152.9
10	113.55	113.23[b]	120.6	124.1	129.3	113.1
1′	–	–	–	–	–	70.2
2′	113.64	114.4	112.5	113.7	113.7	75.9
3′	48.6	49.2	49.6	50.7	51.7	142.6
4′	144.2	144.1	146.3	148.1	148.2	63.4
5′	70.8	70.9	70.6	71.3	71.4	115.1
=CH_2	109.6	109.7	107.9	108.1	108.7	–
1″	–	–	–	–	107.4	172.8
2″	–	–	–	–	75.9	43.3
3″	–	–	–	–	77.9	25.6
4″	–	–	–	–	71.9	22.3
5″	–	–	–	–	78.2	22.3
6″	–	–	–	–	63.0	–
OMe	–	56.5	52.5	-	-	-

[a] Carbon shifts were assigned by comparison with those of **1** under regard of the influence of the methoxy group.
[b] Assignments interchangeable.

exhibits an unusual bicyclic prenyl residue and is a constituent of *Micromelum minutum* (Rahmani, Taufiq-Yap, Ismail, Sukari, & Waterman, 1994).

Compound **2** ($C_{15}H_{12}O_5$ $[M]^+$ *m/z* 272) is the 6-methoxyderivative of **1**, which is also known from *M. minutum*, and was identified by comparison of the ^1H NMR data with those reported (Rahmani et al., 1994). ^{13}C NMR data are to our knowledge not yet available and are added therefore in Table 1.

The NMR spectra of compounds **3**, **4** and **5** (Tables 1 and 2) show correspondence with the aromatic and the bicyclic prenyl moiety of **1**. However, instead of the characteristic complementary olefinic proton doublets of the unsaturated lactone ring, the spectra exhibit two aliphatic methylene groups suggesting the structure of 3,4-dihydrocoumarin derivatives. Compound **3** ($C_{15}H_{16}O_5$ $[M]^+$ *m/z* 276) exhibits one additional methoxy group and an ester carbonyl carbon (δ_C 177.1) which shows CH long-range correlations to these methoxy protons as well as to the methylene protons at C-3 and C-4, thus proving ring opening of the lactone to give the structure of 3,4-dihydro-1,2-*seco*microminutininmethylester.

The EIMS of compound **4** shows the peak with the highest mass at *m/z* 244 as base peak with the molecular formula $C_{14}H_{12}O_4$. Together with the prominent fragment $[M–C_2H_2O]^+$ at *m/z* 202, which is characteristic for α,β-unsaturated cyclohexanone derivatives, these data suggest a 3,4-dihydrocoumarin. However,

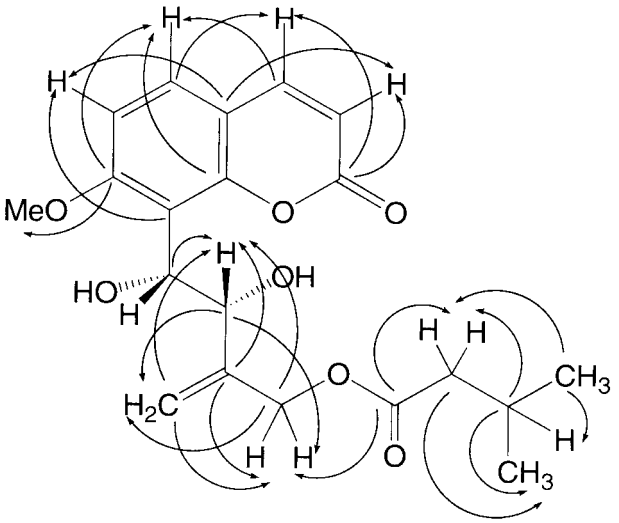


Fig. 1. CH long-range correlations from the HMBC experiment of compound **6**.

the chemical shift of the carbonyl carbon (δ 184.3) is too high for such a lactone and proposes the free acid, the molecular ion of which could be obtained from the negative ESI MS spectrum ($[M–H]^-$ *m/z* 261). Thus, **4** represents the acid corresponding to **3**, which, under EIMS conditions, easily loses one water molecule to give the lactone. Analysis of the NOE effects, observed in the NOE difference spectra, as well as the CH long-range correlations from the HMBC of compounds **3** and **4** confirmed the substitution pattern and afforded the carbon assignments (Table 1).

Compound **5** is a glycoside, which shows no molecular ion in the EIMS, but exhibits the same peaks as **4** and an additional small peak (2%) for the aglycone at *m/z* 262. The molecular ion was obtained from the negative ESI MS as $[M–H]^-$ peak at *m/z* 423 corresponding to the molecular formula $C_{20}H_{24}O_{10}$. The identity of the aglycone moiety with **4** was confirmed by analysis of the CH long-range correlations. C-9 (δ 153.5) shows one additional correlation to the anomeric proton (δ 4.68) of the sugar moiety, which proves the structure of a 9-*O*-glycoside. The carbon shifts of the sugar moiety together with the HH coupling constants characterize a β-glucopyranoside as sugar moiety.

Compounds **3**, **4** and **5**, which are new compounds, represent 3,4-dihydro-1,2-*seco*microminutinin derivatives, suggesting a biosynthesis identical to a large extent to that of microminutinin (**1**). The co-occurrence of coumarins and the related dihydrocinnamic acid derivatives is also known from *Micromelum minutum*, the roots of which contain micromelin and 1,2-*seco*dihydromicromelin (Rahmani et al., 1994).

While compound **6** does not show a molecular ion peak in the EIMS, the ESI MS gave a $[M+Na]^+$ peak at *m/z* 399. Compound **6** forms a methylboronate (**6b**)

Table 2
^{1}H spectral data of compounds **1**–**6a**

Pos.	**1**[a], $CDCl_3$	**2**[a], $CDCl_3$	**3**[b], $CDCl_3$	**4**[b], CD_3OD	**5**[b], CD_3OD	**6**[b], $CDCl_3$	**6a**[b], CD_3OD
3	6.24 d (9.6)	6.26 d (9.6)	2.74 m, 2.67 m	2.48 m	2.46 m, 2.38 m	6.26 d (9.5)	6.28 d (9.8)
4	7.64 d (9.6)	7.61 d (9.6)	2.88 m, 2.74 m	2.77 m, 2.73 m	3.15 m, 2.82 m	7.62 d (9.5)	7.89 d (9.8)
5	7.32 d (8.5)	6.82 s	6.86 d (8.2)	6.84 d (8.2)	7.04 d (8.2)	7.41 d (8.6)	7.64 d (8.9)
6	6.79 d (8.2)	–	6.38 d (8.2)	6.20 d (7.9)	6.50 d (8.2)	6.88 d (8.6)	7.10 d (8.6)
1′	–	–	–	–	–	5.32 t (7.9)	5.54[e] d (8.9)
2′	6.56 d (6.1)	6.61 d (5.8)	6.39 d (5.8)	6.29 d (5.8)	6.28 d (5.8)	4.64 d (8.2)	5.19[e] d[f]
3′	4.67 dm (5.8/1.7)	4.70 dm (5.9, 1.5)	4.49 d (5.8)	4.40 d (5.5)	5.08 d (5.5)	–	–
4′	–	–	–	–	–	4.57 m	4.52 s
5′	4.51 dt (12.5, 1.7), 4.42 dq(12.4, 1.7)	4.52 dt (12.7, 1.6), 4.45 dm (12.6, 1.9)	4.42 br s	4.35 dm (12.2, 1.4), 4.28 dm (12.2, 2.1, 1.5)	4.34 d (12.2), 4.24 dm (12.1, 1.5)	5.03 br s, 5.02 br s	5.29 s, 5.18 s
$=CH_2$ (E)[c]	5.74 m (1.7)	5.73 m (1.7)	5.56 m (1.5)	5.48 d (1.5)	5.62 s	–	–
$=CH_2$ (Z)[c]	5.20 m (1.8)	5.20 m (1.8)	5.11 m (1.5)	5.05 s	5.04 s	–	–
1″	–	–	–	–	4.68 d (7.6)	–	–
2″	–	–	–	–	3.52 dd (9.5, 7.6)	2.19 d (7.0)	2.00 m
3″	–	–	–	–	3.47 dd (9.2, 8.6)	2.08 m	1.89 m
4″	–	–	–	–	3.37 dd (9.5, 8.9)	0.93 d (6.4)	0.849 d (6.7)
5″	–	–	–	–	3.26[d]	0.93 d (6.4)	0.846 d (6.7)
6″	–	–	–	–	3.90 dd (11.9, 2.1), 3.69 dd (11.7, 6.3)	–	–
OMe	–	3.92 s	3.71 s	–	–	3.96 s	3.95 s
OH	–	–	7.97 s	–	–	3.74 d (9.1)	–
OH	–	–	–	–	–	3.23 br s	–

[a] 300 MHz.
[b] 500 MHz.
[e] Assignment exchangeable.
[f] Overlapped.
[c] The differentiation between the (*E*) and (*Z*) protons was gained from the NOESY experiments (**3**, **5**) or by comparison with **3**.
[d] Overlapped with CD_3OD.

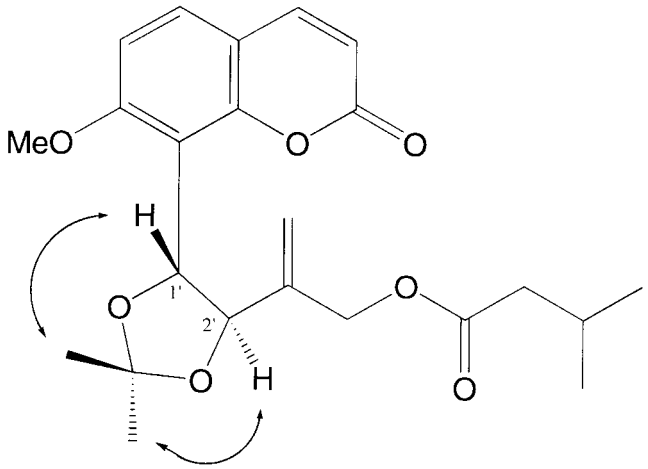


Fig. 2. NOE effects obtained from the NOE difference spectra of compound **6a**.

indicating the vicinal position of two hydroxy functions. The derivative **6b**, investigated by GCMS, displays a M^+ peak at *m/z* 400 confirming the molecular mass of 376 for **6**. The NMR spectra exhibit an intact coumarin skeleton. Analysis of the CH long-range correlations from the HMBC experiment (Fig. 1) afforded three subunits: an 8-substituted 7-methoxycoumarin (the substitution pattern was confirmed by the NOESY experiment), an isovaleryl moiety and a 3-methylenebutyl residue with 3 oxygen substituents (deduced from the high shifts of the three carbons) in positions 1, 2 and 4. Two of them are hydroxyl groups, which appear in the ^{1}H NMR-spectrum at δ 3.74 and 3.24. The ester linkage between the isovaleric acid and the oxygen substituent in position 4′ is deduced from the CH long-range correlation between the carboxyl carbon (C-1″) at δ 172.8 and the protons at δ 4.57 (H_2-4′). The connection of the aliphatic and the coumarin part is only possible between C-8 of the coumarin moiety and C-1′ of the aliphatic part. No CH long-range correlations were observed between these parts. However, the EIMS with the base peak at *m/z* 205, $C_{11}H_9O_4$, corresponding to a 7-methoxy-8-hydroxymethylene coumarin fragment, confirms this connection resulting in the structure of 7-methoxy-8-[1,2,4-trihydroxy-3-methylene-4-*O*-isovaleryl)-butyl]-coumarin for **6**. The relative configuration of the two hydroxyl groups in positions 1′ and 2′ could not be determined by NMR spectroscopy because of the flexibility of the side chain. Therefore the acetonide **6a** was prepared. Its proton spectrum contained the two additional methyl groups as 3H singlets at δ 1.50 and 1.65. H-1′ and H-2′ both appeared as doublets at δ 5.54 and 5.19. The NOE difference spectra showed for the doublet at δ 5.54 a significant NOE effect to the methyl group at δ 1.50 and no effect to the proton at δ 5.19. Analogously for the doublet at δ 5.19 a significant effect to the methyl group at δ 1.65 and no effect to the proton at δ 5.54 was observed. Thus, the NOE effects indicate an opposite arrangement of both protons H-1′ and H-2′ (Fig. 2). This proves for **6a** and **6** the *threo*-configuration of the diol subunit.

Compound **6** and also the parent alcohol 7-methoxy-8-(1,2,4-trihydroxy-3-methylenebutyl)-coumarin, named microfalcatin, are hitherto unknown (*threo*- and *erythro*-isomers). Some other mono- and di-isovalerates of the similar compounds murrangatin (*threo*) and minumicrolin (*erythro*) are known from *Murraya paniculata* (Ito, Furukawa, Ishii, Ishikawa, & Haginiwa, 1990).

3. Experimental

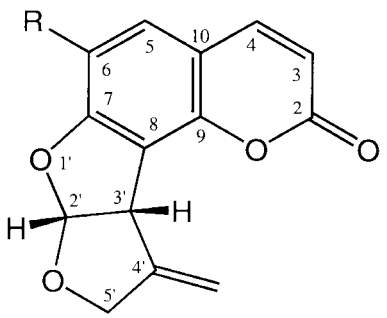
EIMS: AMD 402, 70 eV. ESI MS: Finnigan TSQ 700. GC–MS: MD 800 (Fisons Instruments), 70 eV EI, source temp. 200°C; columns DB-5MS (J&W, 15 m × 0.32 mm, 0.25 μm film thickness), inj. temp. 260°C, interface temp. 300°C, carrier gas He, flow rate 1 ml/min, splitless injection, column temp. program: 170°C for 1 min, then raised to 270°C at a rate of 25°C min^{-1} and then to 290°C at a rate of 2°C min^{-1}, then hold at this temp. for 15 min. NMR: Varian Gemini 300, Unity 500. CC: silica gel 60, 40–63 μm (Merck), Lichroprep RP-18, 25–40 μm (Merck). Prep. TLC: precoated plates, silica gel 60, F_{254}, thickness 0.5 mm (Merck).

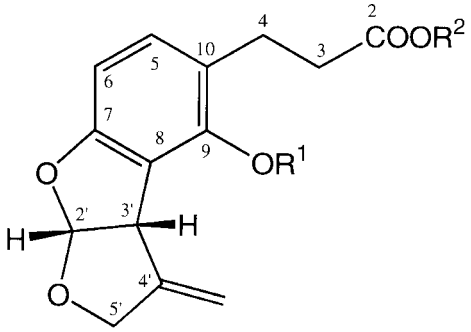
3.1. Plant material

Leaves and branches of *Micromelum falcatum* (Lour.) Tan. were collected in August 1997 in Tam Dao, Vinh phuc, North Vietnam and identified by Ngo Van Trai, Institute of Materia Medica, Hanoi. A voucher specimen is deposited at the Institute of Ecology, National Centre for Natural Science and Technology, Hanoi, Vietnam.

3.2. Extraction and isolation

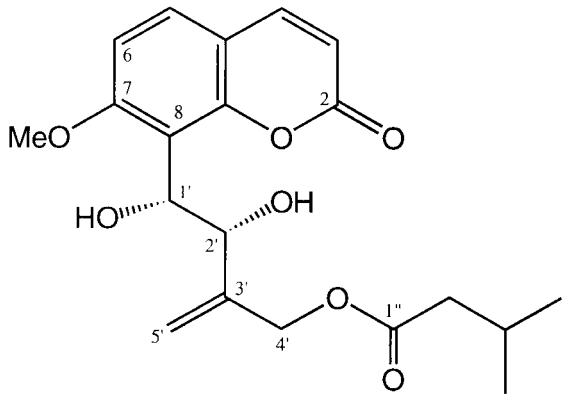
The plant material was dried (1.54 kg), ground and extracted 3× for 12 h with MeOH–H_2O (95:5). The organic solvent was evaporated under red. pres. and the aq. residue extracted with *n*-hexane, EtOAc and *n*-BuOH, successively (each 3×) giving 8.9 g *n*-hexane extract, 9.7 g EtOAc extract and 8.7 g *n*-BuOH extract. The EtOAc extract was separated on silica gel (300 g, 63–200 μm) with solvents of increasing polarity: 10–90% acetone in *n*-hexane followed by 10–100% MeOH in $CHCl_3$ (65 frs). Frs 15–22 (1.35 g, eluted with *n*-hexane–acetone 7:3) were crystallized from MeOH yielding 309 mg of **1**. A part of the



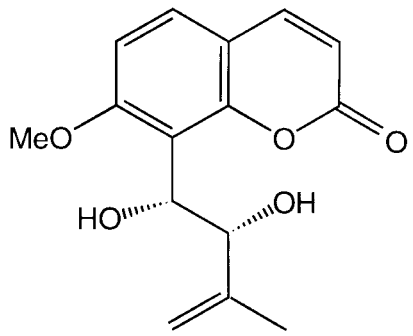


1 R = H
2 R = OMe

3 R^1 = H R^2 = Me
4 R^1 = H R^2 = H
5 R^1 = glc R^2 = H



6



Murrangatin

mother liquid was concentrated under red. pres. to dryness (173 mg) and fractionated on silica gel with *n*-hexane–EtOAc (7:3) to yield 35 mg of **3**. Frs 25–30 (356 mg, eluted with *n*-hexane–acetone 6:4) gave after crystallization from acetone 110 mg of **2**. Frs 40–42 (252 mg, eluted with *n*-hexane–acetone 1:1) were chromatographed on silica gel using *n*-hexane–acetone (4:6) and afforded 54 mg of **6**. Frs 61–62 (373 mg, eluted with $CHCl_3$–MeOH 7:3) were fractionated on silica gel with $CHCl_3$–MeOH (75:25) to give 135 mg residue. Subsequent reversed-phase chromatography on RP-18 with MeOH–H_2O (1:1) afforded 41 mg of **4**. Frs 63–65 (1.1 g, eluted with 100% MeOH) were fractionated on silica gel using $CHCl_3$–MeOH–H_2O (65:35:4) to give 165 mg residue, the subsequent reversed reversed-phase chromatography of which on RP-18 (MeOH–H_2O 3:7) yielded 64 mg of **5**.

3.2.1. Microminutinin (*1*)

Crystals from MeOH, m.p. 114–115°C. $[\alpha]_D^{22}$ +59° ($CHCl_3$, *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 322 (4.15), 260 (3.64). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2868, 1733, 1617, 1577, 1487, 1455, 1406, 1338, 1265, 1115, 1061, 1021, 949, 923, 835. Positive ESI-MS *m/z* (rel. int.): 243 $[M+H]^+$ (100).

3.2.2. -Methoxymicrominutinin (*2*)

Crystals from acetone, m.p. 225–230°C. $[\alpha]_D^{21}$ +336° ($CHCl_3$, *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 341 (3.93), 254 (3.56). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2937, 2869, 1723, 1617, 1581, 1497, 1464, 1410, 1310, 1230, 1194, 1154, 1111, 1028,

940, 915, 851, 820. Positive ESI MS *m/z* (rel. int.): 295 $[M+Na]^+$ (95), 273 $[M+H]^+$ (100).

3.2.3. 3,4-Dihydro-1,2-secomicrominutinin methylester (**3**)

Amorphous. $[\alpha]_D^{26}$ −3.6° ($CHCl_3$, *c* 0.5). UV λ_{max}^{EtOH} nm (log ε): 282 (3.29), 215 (4.08). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 3309, 2955, 2866, 1711, 1628, 1607, 1480, 1441, 1371, 1234, 1203, 1163, 1084, 1036, 955. EIMS *m/z* (rel. int.): 276 $[M]^+$ (84), 258 $[M–H_2O]^+$ (13), 247 $[M–CHO]^+$ (36), 244 $[M–MeOH]^+$ (80), 215 (21), 203 (100), 202 $[244\text{-}C_2H_2O]^+$ (91), 187 (18), 175 (30), 174 $[202\text{-}CO]^+$ (56), 173 (62), 161 (34). HRMS 276.1003 $[M]^+$ ($C_{15}H_{16}O_5$ requires 276.0980).

3.2.4. 3,4-Dihydro-1,2-secomicrominutinin (**4**)

Amorphous. $[\alpha]_D^{25}$ +6.0° (MeOH, *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 281 (3.30), 212 (4.07). IR ν_{max}^{KBr} cm^{-1}: 3420 (broad), 2932, 2864, 1609, 1554, 1476, 1460–1400, 1057, 1100–1040, 955, 839, 801. EIMS *m/z* (rel. int.): 244 $[M–H_2O]^+$ (100), 215 (7), 202 $[M–H_2O–C_2H_2O]^+$ (57), 187 (24), 174 $[202\text{-}CO]^+$ (49), 173 (42), 159 (16), 145 (12), 131 (11), 115 (20), 91 (39), 77 (21). Negative ESI MS *m/z* (rel. int.): 261 $[M–H]^-$ (100), 243 $[M–H–H_2O]^-$ (49), 217 $[M–H–CO_2]^-$ (100), 189 (94), 174 (9). HRMS 244.0748 $[M–H_2O]^+$ ($C_{14}H_{10}O_4$ requires 244.0736).

3.2.5. 3,4-Dihydro-1,2-secomicrominutinin-9-O-glucoside (**5**)

Amorphous. $[\alpha]_D^{26}$ +11.5° (MeOH, *c* 1.0). UV λ_{max}^{MeOH} nm (log ε): 279 (3.55), 203 (4.41). IR ν_{max}^{KBr} (cm^{-1}): 3403 (broad), 2927, 2869, 1600, 1559, 1473–1419, 1261, 1212, 1072, 953, 815. EIMS *m/z* (rel. int.): 262 [aglycone]$^+$ (2), 244 [aglycone–H_2O]$^+$ (100), 215 (11), 202 $[244\text{-}C_2H_2O]^+$ (39), 187 (16), 174 $[202\text{-}CO]^+$ (57), 173 (71), 159 (76), 145 (9), 131 (8), 115 (15), 102 (19), 91 (18), 60 (20). Negative ESI MS *m/z* (rel. int) 423 $[M–H]^-$ (69), 261 [aglycone-H]$^-$ (100).

3.2.6. Microfalcatin-4′-isovalerate (threo-7-methoxy-8-[1,2,4-trihydroxy-3-methylene-4-O-isovaleryl-butyl]-coumarin, **6**)

Crystals from *n*-hexane–acetone, m.p. 100–105°C. $[\alpha]_D^{24}$ 0° ($CHCl_3$, *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 322 (4.14), 211 (4.22). IR $\nu_{max}^{CHCl_3}$ (cm^{-1}): 2963, 2873, 1733, 1608, 1567, 1497, 1464, 1405, 1292, 1250, 1120, 1094, 834. EIMS *m/z* (rel. int.): 275 $[M–O–CO–CH_2–CH(CH_3)_2]^+$ (0.2), 257 $[275\text{-}H_2O]^+$ (3), 205 [8-hydroxymethylene-7-methoxycoumarin]$^+$ (100), 191 (7), 175 $[205\text{-}CHOH]^+$ (19), 85 [isovaleryl]$^+$ (10), 57 [isobutyl]$^+$ (19), 43 (14), 42 (26). Positive ESI MS *m/z* (rel. int.): 399 $[M+Na]^+$ (64), 257 (100), 229 (16), 189 (32), 131 (31), 115 (34). HRMS 257.0818 $[M–O–CO–CH_2–CH(CH_3)_2–H_2O]^+$ ($C_{15}H_{13}O_4$ requires 257.0814), 205.0486 [7-methoxy-8-hydroxymethylene coumarin fragment]$^+$ ($C_{11}H_9O_4$ requires 205.0501).

3.2.7. Acetonide of **6** (**6a**)

20.5 mg of **6** were stirred overnight in 30 ml dry acetone with catalytic amounts of *p*-TsOH. After neutralisation with $NaHCO_3$ the solvent was removed under red. pres. The residue (20 mg) was separated by prep. TLC. After development with mixtures of *n*-hexane–acetone (2× with 8:2 followed by 7:3) two products were obtained. One of them (1.6 mg) was identified as the desired acetonide **6a** by ^1H NMR spectroscopy (Table 1) and MS. EIMS *m/z* (rel. int.): 416 $[M]^+$ (1), 401 $[M–Me]^+$ (11), 359 (3), 275 (4), 257 (59), 246 (100), 205 (28), 189 (38), 110 (43). Positive ESI MS *m/z* (rel. int.): 439 $[M+Na]^+$ (46), 359 (8), 275 (4), 257 (100), 229 (8), 189 (8). The other product (3.5 mg) was supposed to be the corresponding epoxide from its EIMS spectrum ($[M]^+$ *m/z* 358).

3.2.8. Microfalcatin isovalerate methylboronate (**6b**)

The methylboronation was carried out by treatment of the sample with pyridine containing methaneboronic acid for 30 min at 70° (Takatsuto, Ying, Morisaki & Ikekawa, 1982). R_t = 17.65 min. EIMS (*m/z*, rel. int): 400 $[M]^+$ (0.5), 358 [M–isopropyl]$^+$ (0.7), 315 [M–isovaleryl]$^+$ (0.8), 298 [M–isovaleric acid]$^+$ (4), 230 (100), 215 $[230\text{-}Me]^+$ (31), 205 $[230\text{-}B\text{-}CH_2]^+$ (45), 202 $[230\text{-}CO]^+$ (21), 187 $[215\text{-}CO]^+$ (85), 127 (13), 85 [isovaleryl]$^+$ (62), 57 [isobutyl]$^+$ (47). The base peak at *m/z* 230 ($C_{12}H_{11}O_4B$), originated by cleavage between C-1′ and C-2′, contains the coumarin part with C-1′ carrying the O–B–Me residue.

Acknowledgements

We thank the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, Bonn and the VW-Stiftung, Hannover, for financial support.

References

Ito, C., Furukawa, H., Ishii, H., Ishikawa, T. & Haginiwa, J. (1990). *Journal of the Chemical Society Perkin Transactions 1*, 2047.

Kong, Y.-C., But, P. P.-H., Ng, K.-H., Li, Q., Cheng, K.-F., & Waterman, P. G. (1988). *Biochemical Systematics and Ecology*, *16*, 485.

Do, Loi Tat (1991). In *Nhung cay thuoc va vi thuoc Viet Nam* (Glossary of Vietnamese medicinal plants) (p. 589). Hanoi, Vietnam: Nha xuat ban Khoa hoc va Ky thuat (Science and Technics Publication).

Perry, L. M. (1980). In *Medicinal plants of East and Southeast Asia* (p. 366). Cambridge: MIT Press.

Rahmani, M., Taufiq-Yap, Y. H., Ismail, H. B. M., Sukari, A., & Waterman, P. (1994). *Phytochemistry*, *37*, 561.

Takatsuto, S., Ying, B., Morisaki, M., & Ikekawa, N. (1982). *Journal of Chromatography*, *239*, 233.

Thuy, T. T., Porzel, A., Ripperger, H., Sung, T. V., & Adam, G. (1999). *Phytochemistry*, *50*, 903.