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A FURANOCOUMARIN GLUCOSIDE FROM STEMBARK OF *SKIMMIA JAPONICA**

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Key Word Index—*Skimmia japonica* ssp. *japonica*; Rutaceae; coumarin glucosides; structural determination.

Abstract—From the stembark of *Skimmia japonica* the new furanocoumarin glucoside, 2,3,-dihydro-9-hydroxy-2-[1-(6-feruloyl)-β-D-glucosyloxy-1-methylethyl]-7H-furo[3,2g] [1]-benzopyran-7-one has been isolated along with three known coumarin glucosides. They have been found in female and male plants of the species.

INTRODUCTION

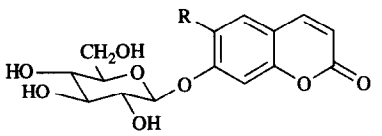
Skimmia japonica (Rutaceae) originates from East Asia and is cultivated for ornamental purposes in Europe [1]. Previous investigations [2] of the dioecious *S. japonica* ssp. *japonica* showed differences in the methylenechloride extracts from the stembark of female and male plants. In this report the isolation and characterization of a new furanocoumarin glucoside together with three known glucosides from the stembark of the female cultivar *S. japonica* ssp. *japonica* 'Oblata' is discussed.

RESULTS AND DISCUSSION

The concentrated water-soluble portion of the ethanolic extract from the stembark was chromatographed on silica gel (CC). Two well-known coumarin glucosides, skimmin (**1**) earlier isolated from *S. japonica* [3] and scopolin (**2**) [4], were the main constituents obtained from the column.

Separation of a third fraction by prep. TLC (system i) afforded two furanocoumarin glucosides. One of them, compound **3**, mp 163–166°, is the 6′-ester of the glucoside isorutarin [5] and sinapic acid which has been isolated from the seeds of *Apium graveolens* (Umbelliferae) [6]. This is the first report of the occurrence of such a kind of ester in Rutaceae.

The second glucoside of this fraction, compound **4**, was obtained by crystallization from water–acetone as needles, mp 150–153°. Comparison of the spectral data with those of **3** confirmed that **4** is of a similar structure. The mass spectrum showed the $[M]^+$ peak at *m/z* 600 in the latter and *m/z* 630 in the former and a further peak *m/z* 262 in both corresponding to rutaretin (**5**) as a fragment ion. A singlet at δ3.89 in the ^{1}H NMR spectrum of **4** and three signals at δ6.83 (*d*, $J = 8.17$ Hz), 7.05 (*dd*, $J = 1.96$ and 8.17 Hz) and 7.27 (*d*. $J = 1.96$ Hz) are typical for *trans*-ferulic acid, whereas *trans*-sinapic acid is the substituent in furanocourmarin glucoside **3**. The ^{1}H NMR and ^{13}C NMR data (Table 1) of **4** were also compared with those taken from an authentic sample of ferulic acid. Acid hydrolysis of **4** followed by alkaline hydrolysis gave **5**, ferulic acid and D-glucose. Rutaretin (**5**) and ferulic acid were identified by co-TLC with authentic samples. glucose by co-HPTLC. The structure of **4** was confirmed as 6′-*O*-*trans*-ferulolylisorutarin. By TLC (systems i and ii) the presence of all isolated compounds **1**–**4** in the male cultivar *S. japonica* ssp. *japonica* 'Rubella' was also established.



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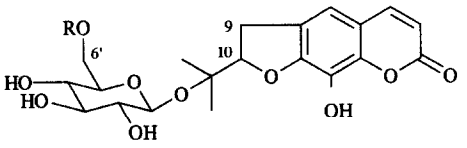

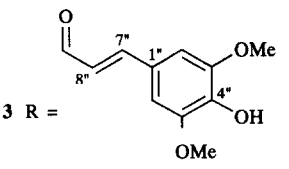

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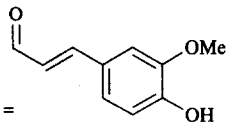

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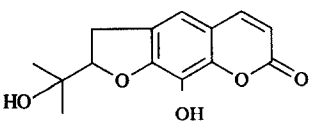

5

*Part 152 in the series 'Natural Products Chemistry'. For Part 151 see Reisch, J., Voerste, A. A., Top, M., Dziemba, P. (1992) *Monatsh. Chem.* **123**, 473.

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Table 1. ^{13}C NMR spectral data for compounds 1–4

C	1	2	3	4
2	160.2	160.4	161.4	161.0
3	113.1	113.2	112.7	112.4
4	144.1	144.1	145.8	145.4
4a	113.2	112.2	114.6	114.3
5	129.3	109.8	115.4	115.0
6	113.6	146.0	126.5[a]	126.5
7	160.1	149.9[a]	152.2	151.7
8	103.2	103.0	129.9	129.5
8a	155.0	148.9[a]	144.3	143.8
9	—	—	31.2	30.9
10	—	—	91.5	91.2
Me	—	—	22.3	22.1
			24.1	23.9
C (Me)$_2$	—	—	78.9	78.7
OMe	—	56.0	56.9	56.4
1′	100.0	99.6	98.9	98.6
2′	73.1	73.0	75.0	74.8
3′	77.1	77.1	75.2	75.0
4′	69.6	69.6	71.8	71.6
5′	76.5	76.7	78.3	78.1
6′	60.6	60.6	64.4	64.2
1″	—	—	126.8[a]	127.5
2″	—	—	107.3	111.4
3″	—	—	149.4	150.2
4″	—	—	140.0	148.8
5″	—	—	149.4	116.0
6″	—	—	107.3	124.0
7″	—	—	146.6	145.8
8″	—	—	116.3	115.8
9″	—	—	167.9	167.4

1, 2 in DMSO-d_6; 3, 4 in acetone-d_6; TMS as internal standard.

[a]These values may be interchanged in the same column.

EXPERIMENTAL

Mps: uncorr. IR: KBr disc. ^1H NMR spectra were run at 200 MHz, ^{13}C NMR at 50 MHz using TMS as int. standard. MS were recorded at 70 eV. TLC: silica gel using the solvent systems: (i) EtOAc–MeOH–H_2O (20:3:2), (ii) CH_2Cl_2–MeOH (9:1), (iii) CH_2Cl_2–MeOH (19:1), (iv) EtOAc.

Extraction and isolation. Air-dried stembark of *Skimmia japonica* ssp. *japonica* 'Oblata' Thunb. (110 g) was extracted with EtOH at room temp. for 3 weeks, evapd to dryness (11 g) and partitioned between CH_2Cl_2 and H_2O. The concd H_2O-sol. extract (7.3 g) was chromatographed on silica gel. Elution with CH_2Cl_2–MeOH (9:1) afforded 298 mg of skimmin (**1**), 281 mg of scopolin (**2**) and a fr. which contained 2 compounds. This fr. was sepd by prep. TLC (silica gel 60 F_{254}, system i); 29 mg of **3** and 10 mg of **4** were obtained.

Skimmin (**1**). Mp 224–226°, from H_2O–Me_2CO, hydrolysed with 0.5 M HCl for 1.5 hr under reflux, gave umbelliferone (mp 226–227°, lit. [7] 229–231° and D-glucose.

Scopolin (**2**). Mp 217–219° from EtOAc, was hydrolysed as compound **1**. Scopoletin (mp 202–203°, lit. [8] 205°) and D-glucose were obtained.

Compound **3**. Mp 163–166° from H_2O–Me_2CO. $[\alpha]_D^{20}$ +59.0° (MeOH; *c* 0.3). UV (MeOH) λ_{max} nm (log ε) 330 (4.37), 264 (3.74), 244sh (4.08), 210 (4.56). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1705 (C=O), 1615, 1585, 1510, 1420, 820. ^1H NMR (acetone-d_6): δ 1.36 and 1.37 (3H each, *s*, *gem* dimethyl); 3.13–3.55 (6H, *m*, H_2-9 and 4 glucose protons); 3.87 (6H, *s*, 2 × OMe); 4.10–4.30 (2H, *m*, H_2-6′); 4.71 (1H, *d*, *J* = 7.64 Hz, anomeric proton); 4.86 (1H, *dd*, *J* = 8.43 and 8.49 Hz, H-10); 6.10 (1H, *d*, *J* = 9.44 Hz, H-3); 6.39 (1H, *d*, *J* = 15.89 Hz, H-8″); 6.91 (1H, *s*, H-5); 6.96 (2H, *s*, H-2″ and H-6″); 7.67 (1H, *d*, *J* = 15.89 Hz, H-7″); 7.74 (1H, *s*, *J* = 9.44 Hz, H-4). EIMS *m/z* (rel. int.): 630 $[M]^+$ (1), 368 $[M-C_{14}H_{14}O_5]^+$ (14), 262 $[C_{14}H_{14}O_5]^+$ (16), 224 $[C_{11}H_{12}O_5]^+$ (29), 207 $[224-OH]^+$ (100).

Compound **3** (15 mg) was hydrolysed with 1.5 M HCl for 1.5 hr under reflux. After extraction of the hydrolysate with EtOAc and sepn by prep. TLC (system iii), rutaretin (**5**) (4 mg) was obtained.

Rutaretin (**5**). Mp 191–193° from CH_2Cl_2 (lit. [9] 192–193°). EIMS *m/z* (rel. int.): 262 $[M]^+$ (46), 229 $(M-H_2O-Me]^+$ (15), 203 $[M-C_3H_7O]^+$ (100).

Compound **4**. Mp 150–153° from H_2O–Me_2CO. $[\alpha]_D^{20}$ +37.9° (MeOH; *c* 0.33). UV (MeOH) λ_{max} nm (log ε) 328 (4.26), 264 (3.62), 232sh (4.00), 210 (4.43). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1695 (C=O), 1620, 1580, 1500, 1420, 820. ^1H NMR (acetone-d_6): δ 1.34 and 1.38 (3H each *s*, gem dimethyl); 3.13–3.56 (6H, *m*, H_2-9 and 4 glucose protons); 3.89 (3H, *s*, OMe); 4.15–4.28 (2H, *m*, H_2-6′); 4.70 (1H, *d*, *J* = 7.68 Hz, anomeric proton); 4.87 (1H, *dd*, *J* = 8.40 Hz, H-10); 6.10 (1H, *d*, *J* = 9.51 Hz, H-3); 6.35 (1H, *d*, *J* = 15.92 Hz, H-8″); 6.83 (1H, *d*, *J* = 8.17, H-5″); 6.91 (1H, *s*, H-5); 7.05 (1H, *dd*, *J* = 1.96 and 8.17 Hz, H-6″); 7.27 (1H, *d*, *J* = 1.96 Hz, H-2″); 7.56 (1H, *d*, *J* = 15.92 Hz, H-7″); 7.74 (1H, *d*, *J* = 9.51 Hz, H-4). EIMS *m/z* (rel. int.): 600 $[M]^+$ (1), 262 $[C_{14}H_{14}O_5]^+$ (16), 229 $[262-H_2O-Me]^+$ (28), 204 $[262-C_3H_7O+1]^+$ (100); 203 $[262-C_3H_7O]^+$ (98).

After acid hydrolysis of compound **4** (2 mg), rutaretin (**5**) was identified by co-TLC (systems iii and iv) from the EtOAc extract. The aq. residue was hydrolysed again for 1.5 hr with 2 M NaOH. After neutralization and extraction with EtOAc *trans*-ferulic acid was identified by co-TLC (systems iii and iv) and D-glucose by co-HPTLC (silica gel 50000, MeCN–H_2O, 17:3, developed twice, detection alkaline soln of $KMnO_4$) from the H_2O-sol. portion.

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