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FLAVONOL GLYCOSIDES FROM *DASYMASCHALON SOOTEPENSE*

ANDREA SINZ,†* RUDOLF MATUSCH,* THAWATCHAI SANTISUK,‡ SUTTIPORN CHAICHANA§ and VICHAI REUTRAKUL§

*Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, D-35032 Marburg, F.R.G.; ‡The Herbarium, Royal Forestry Department, Bankok 10900, Thailand; §Department of Chemistry, Faculty of Science, Mahidol University, Rama 6 Road, Bankok 10400, Thailand

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Key Word Index—*Dasymaschalon sootepense*; Annonaceae; flavonoids; quercetin 3,7-dimethyl ether 3′-*O*-α-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranoside; quercetin 3,7-dimethyl ether-3′-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 6)-*β*-D-glucopyranoside.

Abstract—Two novel flavonol glycosides have been isolated from a methanolic extract of *Dasymaschalon sootepense* leaves. They are the 3′-neohesperidoside and 3′(6^G-α-rhamnosylneohesperidoside) of quercetin 3,7-dimethyl ether. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Dasymaschalon sootepense Craib, Annonaceae, is a small tree growing in parts of South East Asia. Phytochemical studies on the genus *Dasymaschalon* are rare; to date, only one publication has appeared on *Dasymaschalon trichophorum* by Liu *et al.* who isolated the flavone dasytrichone [1]. In this paper we report the isolation and characterization of two novel flavonol glycosides from a leaf extract of *Dasymaschalon sootepense*. It is the first time that this species is phytochemically examined.

RESULTS AND DISCUSSION

A methanolic extract of the leaves of *Dasymaschalon sootepense* was separated by column chromatography over silica gel and preparative HPLC (see Expimental). We could obtain quercetin 3,7-dimethyl ether 3′-*O*-α-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranoside (**1**) (118 mg) and quercetin 3,7-dimethyl ether 3′-*O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 6)-*β*-D-glucopyranoside (**2**) (172 mg).

After acid hydrolysis with 2N HCl the sugars obtained were identified as glucose and rhamnose following TLC analysis using standard references. EI-MS of the aglycone revealed for both compounds **1** and **2** a molecular weight of 330, suggesting a flavone with three hydroxyl and two methoxyl groups. After UV spectral analysis of **1** and **2** with the usual shift reagents (addition of $AlCl_3$, $AlCl_3+HCl$, NaOMe, NaOAc, $NaOAc+H_3BO_3$, see Experimental) [2, 3] before and after acid hydrolysis, the aglycone was shown to be a 5,3′,4′-trihydroxy-3,7-dimethoxyflavone with the sugar moieties attached to the C-3′-hydroxyl.

Connection of the sugar moieties of **1** and **2** at C-3′ was indicated by the addition of $AlCl_3$, since in the UV spectrum of the aglycone (Band I; $\lambda_{max}^{MeOH} = 354$ nm) a bathochromic shift ($\lambda_{max} = 431$ nm) can be seen. Band I is shifted to 406 nm after addition of $AlCl_3+HCl$. In the UV spectra of the glycosides Band I ($\lambda_{max}^{MeOH} = 353$ nm) appears at 401 nm after addition of $AlCl_3$ and at 400 nm after addition of $AlCl_3+HCl$. Addition of $NaOAc+H_3BO_3$ reveals a bathochromic shift of Band I to 380 nm in the aglycone. After addition of NaOMe a bathochromic shift of Band I can be observed in both the UV spectra of the aglycone ($\lambda_{max} = 404$ nm) and the glycosides ($\lambda_{max} = 407$ nm). These results confirm that in the aglycone a 3′,4′-hydroxy substitution is present while in the glycosides there is only a free C-4′-OH group. Therefore the sugar moiety must be attached to C-3′ of the aglycone.

The ^{1}H-NMR spectrum of **1** showed aromatic signals at δ 7.84 (1H, *d*, $J = 2.0$ Hz), 7.73 (1H, *dd*, $J = 8.5$ Hz, 2.0 Hz), 7.02 (*d*, 1H, $J = 8.5$ Hz), 6.81 (*d*, 1H, $J = 2.2$ Hz) and 6.39 (*d*, $J = 2.2$ Hz). These signals could be assigned to H-2′, H-6′, H-5′, H-8 and H-6, respectively. Besides two singlets at δ 3.89 and 3.85 indicated the presence of two methoxy groups. The

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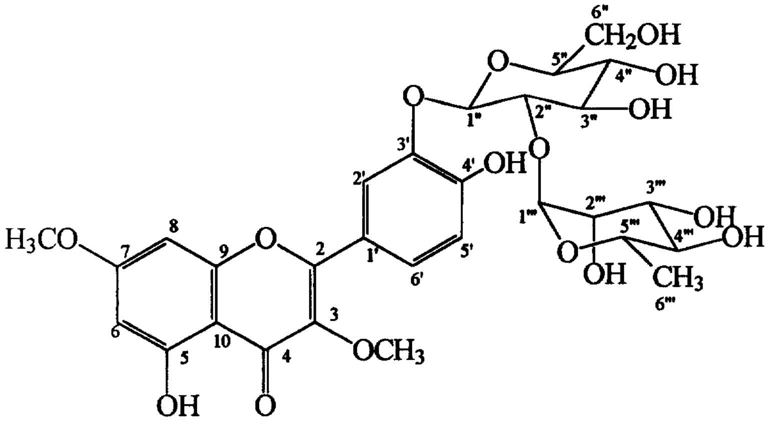
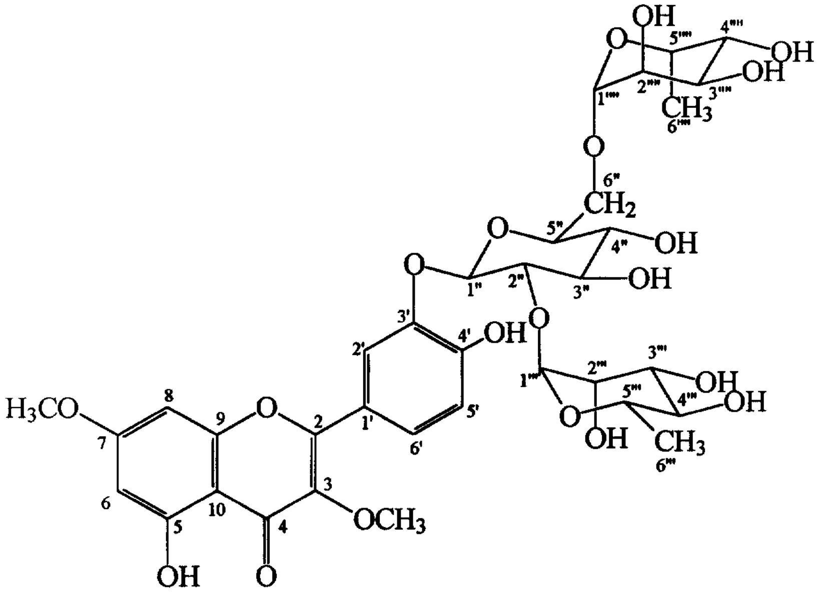
† Author to whom correspondence should be addressed.



1



2

signal at δ 5.24 (1H, *d*, $J = 1.4$ Hz) clearly belongs to a rhamnosyl anomeric proton suggesting an α-linkage, while the signal at δ 5.11 (1H, *d*, $J = 7.6$ Hz) was assigned to a glucosyl anomeric proton with a β-linkage [4].

In the ^{13}C-NMR spectrum, signals of a β-glucopyranosyl and an α-rhamnopyranosyl moiety could be detected. The downfield shift of the C-2″ and the upfield shift of C-1″ suggested the position of attachment of the rhamnosyl moiety at C-2″ of glucose [5]. This linkage was proven by the HMBC spectrum which showed a correlation between C-2″ (glucose) and C-1‴ (rhamnose).

The connection of the sugar moieties with the C-3′-hydroxyl, that had already been predicted by UV spectral data, was confirmed by NOE-difference-MR spectra as a Nuclear Overhauser Effect can be seen between C-H-1″ (glucose) and C-H-2′ of ring B of the flavonoid.

Besides, in the ^{13}C-NMR spectrum two methoxy groups at 59.7 and 56.0 ppm were striking [see Table 1]. The presence of the methoxy groups at C-3 and C-7 could be confirmed by the downfield shift of the methoxy group at C-3 [2] and HMBC-NMR spectral data showing correlations between C-3 and C-7 and the protons of the methoxy groups.

The ^{1}H-NMR spectrum of **2** showed almost the same signals as **1**: the aromatic signals at δ 7.84 (1H, *d*, $J = 2.1$ Hz), 7.74 (1H, *dd*, $J = 8.5$ Hz, 2.1 Hz), 7.06 (*d*, 1H, $J = 8.5$ Hz), 6.78 (*d*, 1H, $J = 2.2$ Hz) and 6.40 (*d*, $J = 2.2$ Hz) could be assigned to H-2′, H-6′, H-5′, H-8 and H-6, respectively. As in **1**, two singlets at

Table 1. ^{13}C-NMR spectral data of **1** and **2**, 125 MHz, DMSO-d_6

	1	**2**
Aglycone		
2	155.6	155.4
3	137.9	137.9
4	178.0	177.9
5	160.9	160.8
6	97.7	97.6
7	165.1	165.0
8	92.4	92.2
9	156.3	156.2
10	105.2	105.2
1′	120.5	120.5
2′	116.7	117.0
3′	145.0	144.6
4′	150.7	150.7
5′	116.2	116.3
6′	123.7	123.7
C-3-OCH_3	59.7	59.6
C-7-OCH_3	56.0	56.0
Glucose		
1″	99.4	99.4
2″	77.0	77.0
3″	77.0	77.0
4″	69.9	69.7
5″	77.2	75.2
6″	60.7	65.7
Rhamnose		
1‴	100.5	100.5
2‴	70.5	70.5
3‴	70.6	70.4
4‴	72.1	71.9
5‴	68.5	68.5
6‴	17.9	17.8
1⁗		100.5
2⁗		70.6
3⁗		70.2
4⁗		72.0
5⁗		68.2
6⁗		17.6

δ 3.91 and 3.86 indicated the presence of two methoxyl groups. The signals at δ 5.23 (1H, *d*, *J* = 1.4 Hz) for the α-rhamnosyl anomeric proton and the signal at δ 5.14 (1H, *d*, *J* = 7.6 Hz) for the β-glucosyl anomeric proton were similar to those of **1**, but in addition to these signals another at δ 4.56 (1H, *d*, *J* = 1.4 Hz) could be assigned to a further α-rhamnosyl anomeric proton.

In the ^{13}C-NMR spectrum, the two methoxy groups at δ 59.3 and 56.0 ppm suggested the same substitution pattern as in **1**, but in addition signals of a β-glucopyranosyl and two α-rhamnopyranosyl moieties could be detected [see Table 1]. Downfield shift of the C-6 and upfield shift of the C-5 in the glucosyl moiety of **2** in comparison with **1** suggested the presence of an α-L-rhamnopyranosyl-(1 → 2)-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucospyranoside moiety in **2** [5]. The assignments of the sugar signals of **1** and **2** were made by HMQC-NMR spectra. Connection of the sugar moieties with the aglycone at C-3′-OH could be established by HMBC-NMR spectral data in addition to UV spectral analysis.

EXPERIMENTAL

General. Mps: uncorr.; NMR spectra: Jeol Eclipse + 500 spectrometer, using TMS as internal standard; EI-mass spectra: 70 eV; CC: silica gel 60 (15–40 μm, Merck); prep. HPLC: Waters prep LC 4000 system with 990 Diode Array Detector.

Plant material. Leaves were collected in November 1992 at Doi Tung (Thailand). The plant material was identified by Dr P. Kessler, Rijksherbarium Leiden (L.), where a voucher specimen has been deposited.

Extraction and isolation. Dried leaves (100g) were crushed and percolated at 30° with MeOH. After evpn of MeOH the residue (13,8 g) was sepd in a CH_2Cl_2 soluble and a CH_2Cl_2 insol fr. The CH_2Cl_2 insol fr (11.4 g) was subjected to silica gel CC with solvents of increasing polarity beginning with CH_2Cl_2, progressively adding MeOH and finally water affording 13 frs. Fr.9 eluted with a mixt of CH_2Cl_2 MeOH (3:7) was sepd by prep. HPLC on 250 * 25 mm, LiChrospher 60 RPselectB, 15 μm (Merck), 40 ml min^{-1}, MeOH H_2O (60:40) affording compound **1** (118 mg) and **2** (172 mg).

Hydrolysis. Each compound **1** and **2** (1 mg) was refluxed in 2N HCl (10 ml) for 4 hr. The identification of the sugars was performed by TLC on silica gel. Solvent system: toluene–$CHCl_3$–Me_2CO (8:5:7) with authentic samples as references.

Quercetin 3, *7-dimethyl ether* 3′-O-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**1**). Colourless crystals (118 mg), mp: 239°, $[\alpha]_D^{20}$ −44.2° (MeOH, c 0.45), UV λ_{max}^{MeOH} nm: 254 (log ε 4.08), 269 (log ε 4.08), 297 (log ε 3.89), 353 (log ε 4.11); + $AlCl_3$: 269, 277, 302, 363, 401; + $AlCl_3$/HCl: 269, 278, 302, 358, 400; + NaOMe: 263, 302 *sh*, 407; + NaOAc: 254, 269, 354; + NaOAc/H_3BO_3: 254, 269, 352; UV λ_{max}^{MeOH} nm (aglycone): 256, 268 *sh*, 297, 354; + $AlCl_3$: 275, 302 *sh*, 332, 431; + $AlCl_3$/HCl: 267, 300, 362, 406 *sh*; + NaOMe: 267, 302 *sh*, 404; + NaOAc: 258, 296, 404; + NaOAc/H_3BO_3: 262, 380. FD-MS *m/z*: 639 $[M+H]^+$. EI-MS (70 eV): *m/z* (rel. int.) 331 $[M, aglycone+H]^+$ (13), 330 $[M, aglycone]^+$ (100), 329 $[M, aglycone-H]^+$ (62), 287 (20), 191 (15), 173 (19), 167 (11), 147 (32), 144 (22), 137 (12), 129 (38), 113 (19), 102 (12). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.65 (1H, *brs*, 5-OH), 7.84 (1H, *d*, *J* = 2.0 Hz, 2′-H), 7.73 (1H, *dd*, *J* = 8.5 Hz, 2.0 Hz, 6′-H), 7.02 (1H, *d*, *J* = 8.5 Hz, 5′-H), 6.81 (1H, *d*, *J* = 2.2 Hz, 8-H), 6.39 (1H, *d*, *J* = 2.2 Hz, 6-H), 5.24 (1H , *d*, *J* = 1.4 Hz, 1‴-H), 5.11 (1H, *d*, *J* = 7.6 Hz, 1″-H), 3.91 (1H, *dq*, *J* = 9.3 Hz, 6.0 Hz, 5‴-H), 3.89 (3H, *s*, 7-OCH_3), 3.85 (3H, *s*, 3-OCH_3), 3.78 (1H, *m*, 2‴-H), 3.74 (1H, *dd*, *J* = 9.8 Hz, 1.5 Hz, 6″-H), 3.63 (1H, *dd*, *J* = 8.8 Hz, 8.2 Hz, 2″-

H), 3.57–3.49 (3H, *m*, 5″-H, 6″-H, 3‴-H), 3.40 (1H, *m*, 3″-H), 3.41–3.30 (7H, *m*, unresolved sugar-OH), 3.27 (1H, *dd*, *J* = 9.3 Hz, 9.3 Hz, 4″-H), 3.23 (1H, *dd*, *J* = 9.5 Hz, 9.3 Hz, 4‴-H), 1.11 (3H, *d*, *J* = 6.0 Hz, 6‴-H). ^{13}C NMR (125 MHz, DMSO-d_6): Table 1. Anal. calc. for $C_{29}H_{34}O_{16}$: C, 54.54; H, 5.33 (found: C, 54.85; H, 5.41).

Quercetin 3,7-*dimethyl ether* 3′-O-α-L-rhamnopyranosyl-(1 → 2)-O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranoside (**2**). Yellow powder (172 mg), mp: 170°, $[\alpha]_D^{20}$ −58.1° (MeOH, c 0.5), UV λ_{max}^{MeOH} nm: 254 (log ε 4.08), 269 (log ε 4.08), 297 (log ε 3.89), 353 (log ε 4.11); +$AlCl_3$: 269, 277, 302, 363, 401; +$AlCl_3$/HCl: 269, 278, 302, 358, 400; +NaOMe: 263, 302 *sh*, 407; +NaOAc: 254, 269, 354; +NaOAc/H_3BO_3: 254, 269, 352; UV λ_{max}^{MeOH} nm (aglycone): 256, 268 *sh*, 297, 354; +$AlCl_3$: 275, 302 *sh*, 332, 431; +$AlCl_3$/HCl: 267, 300, 362, 406 *sh*; +NaOMe: 267, 302 *sh*, 404; +NaOAc: 258, 296, 404; +NaOAc/H_3BO_3: 262, 380. FD-MS *m/z*: 785 $[M+H]^+$, 639 $[M+H-\text{rhamnosyl}]^+$. EI-MS (70 eV): *m/z* (rel. int.) 331 $[M, \text{aglycone}+H]^+$ (19), 330 $[M, \text{aglycone}]^+$ (100), 329 $[M, \text{aglycone}-H]^+$ (78), 287 (36), 203 (13), 167 (17), 147 (20), 144 (10), 137 (19), 128 (61), 113 (36), 102 (18). ^{1}H-NMR (500 MHz, DMSO-d_6): δ 12.65 (1H, *br s*, 5-OH), 7.84 (1H, *d*, *J* = 2.1 Hz, 2′-H), 7.74 (1H, *dd*, *J* = 8.5 Hz, 2.1 Hz, 6′-H), 7.06 (1H, *d*, *J* = 8.5 Hz, 5′-H), 6.78 (1H, *d*, *J* = 2.2 Hz, 8-H), 6.40 (1H, *d*, *J* = 2.2 Hz, 6-H), 5.35–4.29 (7H, unresolved sugar OH), 5.23 (1H, *d*, *J* = 1.4 Hz, 1‴-H), 5.14 (1H, *d*, *J* = 7.8 Hz, 1″-H), 4.56 (1H, *d*, *J* = 1.4 Hz, 1⁗-H), 3.91 (3H, *s*, 7-OCH_3), 3.89 (1H, *dq*, *J* = 9.3 Hz, 6.1 Hz, 5‴-H), 3.86 (3H, *s*, 3-OCH_3), 3.85 (1H, *dd*, *J* = 9.6 Hz, *J* = 1.5 Hz, 6″-H), 3.80 (1H, *m*, C-2‴-H), 3.64 (1H, *dd*, *J* = 8.0 Hz, 7.8 Hz, 2″-H), 3.57–3.49 (5H, *m*, 3″-H, 5″-H, 6″-H, 3‴-H, 3⁗-H), 3.41 (1H, *dq*, *J* = 9.3 Hz, 6.1 Hz, 5⁗-H), 3.35 (1H, *m*, 2⁗-H), 3,30 (1H, *m*, 4″-H), 3.24 (1H, *dd*, J = 9.6 Hz, 9.3 Hz, 4‴-H), 3.14 (1H, *dd*, *J* = 9.5 Hz, 9.3 Hz, 4⁗-H), 1.12 (3H, *d*, *J* = 6.1 Hz, 6‴-H), 1.06 (3H, *d*, *J* = 6.1 Hz, 6⁗-H). ^{13}C NMR (125 MHz, DMSO-d_6): Table 1. Anal. calc. for $C_{35}H_{44}O_{20}$: C, 53.57; H, 5.61 (found: C, 53.02; H, 5.58).

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