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ACETYLATED AND OTHER FLAVONOID GLYCOSIDES FROM *ARNICA CHAMISSONIS**

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Key Word Index—*Arnica chamissonis* subsp. *foliosa*; Compositae; flavonoid glycosides; 3-*O*-(6″-*O*-acetyl)-β-glucosides of patuletin, kaempferol and 6-methoxykaempferol.

Abstract—Ten flavonoid glycosides were isolated from flowers of *Arnica chamissonis* subsp. *foliosa* var. *incana*. The structures were established on the basis of acid hydrolysis and spectral data (UV, ^{1}H NMR, partially ^{13}C NMR, MS) as the 7-β-glucosides of pectolinarigenin, hispidulin, jaceosidin, and eupafolin; isorhamnetin 3-β-glucoside; the 3-β-glucuronides of patuletin and quercetin; and the 3-(6″-acetyl)-β-glucosides of kaempferol, 6-methoxykaempferol, and patuletin. The latter two compounds are isolated for the first time.

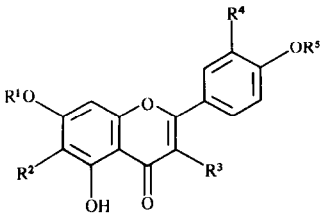
INTRODUCTION

As part of a chemotaxonomic study of the genus *Arnica* we previously reported the isolation of flavonoid aglycones and four flavonoid glycosides [1, 2] from flowers of *Arnica chamissonis* subsp. *foliosa* var. *incana* (subgenus *Chamissonis*, sect. *Euchamissonis*). In continuation of this study in the present communication the isolation and structure elucidation of 10 flavonoid glycosides including three acetylated flavonol glucosides are described, two of which are new compounds.

RESULTS AND DISCUSSION

From the ethyl acetate soluble portion of an aqueous methanolic extract of the flowers of *A. chamissonis* subsp. *foliosa* var. *incana* the 7-β-glucosides of pectolinarigenin (**4**), hispidulin (**5**), jaceosidin (**6**), eupafolin (**7**), and isorhamnetin 3-β-glucoside (**8**) were isolated. The methods used were RLCC, column chromatography and preparative TLC. The structures were determined by UV and (except for **4**) ^{1}H NMR data [2–5]. Glucose was detected as the sugar component in compounds **4**–**8** after acid hydrolysis. The MS and UV data as well as comparative TLC of the aglycones were in accordance with those of the corresponding compounds.

Additionally three acetylated flavonol glycosides, which were very unstable in methanolic solution, were isolated by the methods described above. The structure of compound **1** was established as patuletin 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside by the following spectral data. The UV spectra recorded in methanol showed two absorption maxima (256 and 265 sh nm), a characteristic of 3′,4′-oxygenated flavonols. The addition of sodium methoxide produced a bathochromic shift of 55 nm of band I with an increase of intensity, which is diagnostic



1 R¹ = H, R² = OMe, R³ = Oglc-Ac, R⁴ = OH, R⁵ = H
2 R¹ = R² = H, R³ = Oglc-Ac, R⁴ = R⁵ = H
3 R¹ = H, R² = OMe, R³ = Oglc-Ac, R⁴ = R⁵ = H
4 R¹ = Glc, R² = OMe, R³ = R⁴ = H, R⁵ = Me
5 R¹ = Glc, R² = OMe, R³ = R⁴ = R⁵ = H
6 R¹ = Glc, R² = OMe, R³ = H, R⁴ = OMe, R⁵ = H
7 R¹ = Glc, R² = OMe, R³ = H, R⁴ = OH, R⁵ = H

for the presence of a free 4′-hydroxyl group. A free 7-hydroxyl group was indicated by the appearance of band III (335 nm). The NaOMe spectrum showed degeneration only after acid hydrolysis, suggesting glycosidation at C-3. The *ortho*-diphenol system in the B ring was confirmed by $AlCl_3/AlCl_3$–HCl and NaOAc–H_3BO_3 spectra (see Table 1) [6, 7]. The MS spectrum of the aglycone showed fragments characteristic for patuletin [8]. According to TLC (after acid hydrolysis) the sugar component was found to be glucose and the aglycone patuletin. Patuletin was confirmed by the ^{1}H NMR data (see Table 2). Additionally a singlet at δ 1.833 (3 H) typical for methyl groups of acetyl groups was observed. The acetyl group must be attached to the sugar moiety because the doublet for H-1″ was slightly shifted upfield (δ 0.11) and the double doublets for H-6_A″ and H-6_B″ downfield (δ 0.45) compared to patuletin 3-β-glucoside [2]. The downfield shift suggested that the C-6″ hydroxyl was acetylated and the coupling constant of the proton at C-1″ of 7.6 Hz indicated a β-linked glycoside.

The presence of an aliphatic acetyl group was deduced from the signals at δ 20.451 (Me) and 172.665 (C=O) in

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Table 1. UV data of compounds 1–3

	1	2	3
MeOH	256, 265 *sh*	265, 300 *sh*	269, 295 *sh*
	352	348	337
NaOMe	270, 335	273, 325	272, 325
	407	397	397
$AlCl_3$	275, 305 *sh*	272, 305	275, 303 *sh*
	342 *sh*, 438	353, 397	365, 385*sh*
$AlCl_3$–HCl	267, 300 *sh*	274, 304	278, 295 *sh*
	375, 400 *sh*	345, 395	357, 385 *sh*
NaOAc	270, 325 *sh*	272, 304	272, 305
	383	377	384
NaOAc–H_3BO_3	262, 378	265, 300 *sh*	269, 345
		350	

the ^{13}C NMR spectrum (see Table 3). The acetylation at the C-6″ hydroxyl of the sugar was evidenced by the downfield shift of δ 1.7 in the C-6 signal and an upfield shift of δ 2.9 in the C-5 signal [2, 9]. The other signals for the sugar and the aglycone were identical with those from patuletin 3-β-glucoside [2].

Compound **2** was identified as kaempferol 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside. The UV spectrum (Table 1) exhibited a bathochromic shift of 49 nm of band I with an increase of intensity after addition of sodium methoxide, which demonstrated a free 4′-hydroxyl group. The appearance of band III at 325 nm indicated the presence of a free 7-hydroxyl group, which was further supported by the fact that band I in the sodium acetate spectrum appeared at a shorter wavelength than in the sodium methoxide spectrum [10]. On the other hand, the similarity between the spectra in presence of $AlCl_3$ and $AlCl_3$ + HCl, together with the absence of a significant shift after addition of NaOAc + H_3BO_3, indicated the absence of an *ortho* dihydroxy system on the B ring. After acid hydrolysis the sodium methoxide spectrum was unstable, which showed that the sugar was attached at the 3-position. The aglycone was identified by MS data and comparative TLC as kaempferol and the sugar component as glucose (by TLC). Kaempferol was confirmed by ^1H NMR (see Table 2). The 4′-oxygenated B ring appeared as an AA′BB′ system with signals at 6.854 (2H, *d*, J = 9 Hz) and 8.020 (2H, *d*, J = 9 Hz), which were assigned to the protons H-3′, H-5′ and H-2′, H-6′, respectively. The signals of H-6 and H-8 (6.204 ppm, d, 6.409 ppm, d) were in the same region as those for kaempferol 3-β-glucoside [2]. A singlet at 1.833 ppm suggested the presence of an acetyl group, which was attached to glucose at the C-6″ hydroxyl because of the downfield shifts of H-6_A″ and H-6_B″ compared to kaempferol 3-β-glucoside [2]. The presence of an aliphatic acetyl group was evident from the signals at 20.597 ppm (Me) and 172.646 ppm (C=O) in the ^{13}C NMR spectrum. The site of the acetyl group was at the C-6″ hydroxyl of the sugar because all signals for glucose were in the same region as those for **1** (Table 3). A downfield shift in the C-6 signal as well as an upfield shift in the C-5 signal according to [9] were observed. Furthermore glucose in the pyranose form and the aglycone (except for C-7) were confirmed (Table 3). It is surprising that the signal for C-7 could not be detected but, its absence can possibly be explained by H/D exchange effects during the measurement which took a long time because of the small amount of the compound. The exchange of H-6 and H-8 protons with deuterium atoms was observed in the ^1H NMR spectrum taken after the

Table 2. ^1H NMR spectral data of compounds 1–3

H	1	2	3
6		6.204 *d*	
		(1.9)	
8	6.506 *s*	6.409 *d*	6.431 *s*
		(1, 9)	
2′	7.604 *d*	8.020 *d*	8.005 *d*
	(2)	(9)	(9)
3′		6.854 *d*	6.847 *d*
		(9)	(9)
5′	6.826 *d*	6.854 *d*	6.847 *d*
	(8, 5)	(9)	(9)
6′	7.574 *dd*	8.020 *d*	8.005 *d*
	(2; 8, 5)	(9)	(9)
1″	5.144 *d*	5.144 *d*	5.074 *d*
	(7, 6)	(7, 3)	(7, 3)
2″-5″	3.300–	3.353–	3.328–
	3.497 *m*	3.472 *m*	3.501 *m*
6_A″	4.166 *dd*	4.155 *dd*	4.141 *dd*
	[2; (−) 11, 8]	[1, 7; (−) 11, 8]	2; (−) 11, 8]
6_B″	4.031 *dd*	4.055 *dd*	4.030 *dd*
	[5, 4; (−) 11, 8]	[5, 7; (−) 11, 8]	[5, 2; (−) 11, 8]
OMe at C-6	3.875 *s*		3.856 *s*
Me (Ac)	1.833 *s*	1.833 *s*	1.847 *s*

At 360, 135 MHz in CD_3OD, chemical shifts (ppm) relative to TMS as internal standard, in parenthesis, coupling constants in Hz.

Table 3. ^{13}C NMR spectral data of compounds **1** and **2**

C	1*	2
2	159.559[a]	159.311[a]
3	135.149	135.227
4	179.668	179.014
5	153.775[a]	162.757[a]
6	132.724	100.989
7	158.806[a]	— †
8	95.044	95.540
9	153.578[a]	158.695[a]
10	106.067	104.806
1′	123.399[b]	122.700
2′	117.479[c]	132.235
3′	145.894	116.017
4′	149.794	161.690[a]
5′	115.860[c]	116.017
6′	123.152[b]	132.235
[Glc 1″]	104.423	104.601
2″	75.666[d]	75.528[b]
3″	77.956	77.841
4″	71.337	71.237
5″	75.533[d]	75.465[b]
6″	64.299	64.305
OMe at C-6	60.974	
Me COO^-	20.451	20.597
Me COO^-	172.665	172.646

[a–d] Assignment interchangeable.
† Not detected.
75 MHz (*100 MHz), CD_3OD, TMS as internal standard

^{13}C NMR measurement by the decrease in the intensities of the corresponding peaks [11].

The structure of compound **3** was established as 6-methoxykaempferol 3-*O*-(6″-*O*-acetyl)-β-D-glucoside. After acid hydrolysis are aglycone was identified by MS data and TLC analysis as 6-methoxykaempferol [12], and the sugar component by TLC as glucose. Free hydroxyl groups at C-5, 7 and 4′ and a substituted hydroxyl group at C-3 were concluded from the UV spectra with diagnostic reagents before and after hydrolysis. 6-Methoxykaempferol was confirmed by the ^1H NMR data which also indicated the presence of an acetyl group at the C-6″ hydroxyl of the glucose. Compared to 6-methoxykaempferol 3-β-glucoside [2] the doublet for H-1″ was shifted upfield (δ 0.18) and the double doublets for H-6_A″ and H-6_B″ downfield (δ 0.44 resp. 0.5). These signals were in the same region as those for **1** and **2** (Table 2). Therefore acetylation must have taken place at the C-6″ hydroxyl. From the coupling constant of H-1″ (7.3 Hz) a β-glycosidic linkage was deduced (Table 2). Insufficient material was available for a ^{13}C NMR spectrum.

In addition to these compounds two flavonoid glucuronides were isolated from the aqueous residue. Their structures were established by UV and ^1H NMR data and acid hydrolysis as patuletin 3-β-glucuronide (**9**) and quercetin 3-β-glucuronide (**10**) [13].

Compounds **1–4** and **6** are new for the genus *Arnica* while compounds **1** and **3** are to the best of our knowledge new natural products. Patuletin 7-(6″-acetyl)-β-glucoside has been reported [14] but not compound **1**. Compound **2** has been found previously only in *Senecio aureus* [15] but no spectral data were given.

Compounds **5** and **8–10** have already been found in *A. montana* [2, 13], **7** in *Arnica* species of subgenus *Austromontana* [16]. The flavonoid pattern in this plant proved to be similar to that of the previously reported for *A. montana* [2, 13]. However one remarkable difference is the occurrence of acetylated flavonol glycosides in *A. chamissonis* subsp. *foliosa* var. *incana*, which could not be detected in flowers of *A. montana*. In both species sugar attachments are all at the 3 position in the flavonols and the 7 position in the flavones. Comparative examinations of the flavonoid pattern within the section *Euchamissonis* are in progress but preliminary analysis show no notable differences.

EXPERIMENTAL

Plant material see [17].

General techniques see [2].

Isolation of flavonoids. 13 g of the EtOAc extract (total 24 g, preparation see [17] were chromatographed on Sephadex LH-20 (Pharmacia) with MeOH yielding 16 fractions. Fr. 5 was rechromatographed on XAD-2-Adsorberharz (Serva) with H_2O (pH 2), H_2O, and H_2O–MeOH (1:1). Subsequent separation on silica gel TLC plates with EtOAc–HOAC–HCO_2H–H_2O (100:11:11:27) afforded **3** (5 mg) and **4** (10 mg). Fr. 6 was separated by RLCC (ascending, solvent system *n*-PrOH–EtOAc–H_2O (2:7:4), flow rate 1, 8, rotation 5, angle 5°). CC on Polyclar AT (GAF, Köln) with CH_2Cl_2–MeOH–EtOAc–Me_2CO (20:10:5:1) (with increasing MeOH portion) and XAD-2-Adsorberharz (solvent mentioned above), prep. TLC (see above), and recryst. gave **1** (60 mg), **2** (22 mg), **5** (3 mg), **6** (17 mg), **7** (47 mg) and **8** (3 mg). A part of the aqueous residue was chromatographed on XAD-2-Adsorberharz (see above). From the MeOH–H_2O fraction **9** (14 mg) and **10** (53 mg) were isolated by RLCC (descending, solvent system $CHCl_3$–*n*-BuOH–MeOH–H_2O (10:1:10:6), and subsequently prep. TLC (solvent system, see above). Finally all isolated compounds were purified on columns of Sephadex LH-20 with MeOH to remove Polyclar and/or silica gel residues. Acid hydrolysis was carried out as previously described [2, 13].

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REFERENCES

1. Merfort, I., Marcinek, C. and Eggert, A. (1986) *Phytochemistry* **25**, 2901.
2. Merfort, I. and Wendisch, D. (1987) *Planta Med.* **53**, 434.
3. Krishnaswamy, N. R., Seshadri, T. R. and Tahir, P. J. (1968) *Indian J. Chem.* **6**, 676.
4. Wagner, H., Hörhammer, L., Höer, R., Murakami, T. and Farkas, L. (1969) *Tetrahedron Letters*, 3411.
5. Williams, C. (1979) *Phytochemistry* **18**, 803.
6. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, Heidelberg.

7. Wollenweber. E. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds). Chapman & Hall, London.
8. Merfort, I. (1985) *Planta Med.* 136.
9. Markham, K. R. and Chari, V. M. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds). Chapman & Hall, London.
10. Bacon, J. D. and Mabry, T. J. (1976) *Rev. Latinoam. Quim.* **7**, 83.
11. Cheminat, A. and Brouillard, R. (1986) *Tetrahedron Letters* **27**, 4457.
12. Lebreton, P., Wollenweber, E., Southwick, L. and Mabry, T. J. (1971) *C. R. Acad. Sc. Paris, Ser. C* **272**, 1529.
13. Merfort, I. and Wendisch, D. (1988) *Planta Med.* (in press).
14. Harborne, J. and Williams, C. A. (1982) in *The Flavonoids: Advances in Research* (Harborne, J. B. and Mabry, T. J., eds), Chapman & Hall, London.
15. Piegay, I., Reynaud, J. and Raynaud, J. (1984) *Pharmazie* **41**, 524.
16. Wolf, S. J. and Denford, K. E. (1984) *Biochem. Syst. Ecol.* **12**, 183.
17. Merfort, I. (1984) *Planta Med.* 107.