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Iridoid glycosides and phenolic glycosides from *Holmskioldia sanguinea*

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Abstract

Four new iridoids, 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol, 6-*O*-α-L-(3″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol, 6-*O*-α-L-(4″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol and 6-*O*-α-L-(4″-*O*-*cis*-feruloyl)rhamnopyranosylcatalpol, along with three known monoacyl rhamnopyranosylcatalpol derivatives have been isolated from the aerial parts of *Holmskioldia sanguinea*. Catalpol was identified by GC and GC–MS analysis. Three known phenolic glycosides, isolated in small amounts, were identified as methyl salicylate glucoside, osmanthuside H, and icariside F_2. The structures were established by spectroscopic methods. The taxonomic significance of our results is discussed.

Keywords: *Holmskioldia sanguinea*; Labiatae; Verbenaceae; Iridoids; Monoesters of rhamnopyranosylcatalpol; Phenolic glycosides; Centrifugal partition chromatography; CPC

1. Introduction

Holmskioldia sanguinea RETZ., an Asian species, is the only representative of the genus *Holmskioldia* since the African species have been transferred to the genus *Karomia* by Fernandez (1985). Traditionally, *Holmskioldia* has been included in the family Verbenaceae and tribe Clerodendreae within the subfamily Viticoideae (Briquet, 1897; Moldenke, 1971). The same position was suggested by our previous cladistic analysis (Rimpler, Winterhalter & Falk, 1992) on the basis of 56 morphological and 10 chemical characters. Cantino (1992) and Cantino, Harley and Wagstaff (1992) redefined the Labiatae, which now include a large part of the former Verbenaceae, and restricted the Verbenaceae almost entirely to the former subfamily Verbenoideae. This revised classification is used here. The genus *Clerodendrum* now belongs to the new subfamily Teucrioideae (Labiatae), whereas *Holmskioldia* was transferred to the subfamily Scutellarioideae (Labiatae). This position of *Holmskioldia* is also suggested by molecular data of cpDNA (Steane, Scotland, Mabberley, Wagstaff, Reeves et al., 1997; Wagstaff, Hickerson, Spangler, Reeves & Olmstead, 1998). Since catalpol was the only iridoid known to occur in *H. sanguinea* (Neumann, 1976) we re-investigated this species. The iridoid patterns of *Clerodendrum* s. l. (Teucrioideae) (Jacke & Rimpler, 1983; Stenzel, Rimpler & Hunkler, 1986; Stenzel, Heni, Rimpler & Vogellehner, 1988; Çalis, Hosny, Yürüker, Wright & Sticher, 1994a; Çalis, Hosny & Yürüker, 1994b) and *Scutellaria* (Scutellarioideae) (Kooiman, 1972; Cole, Paton, Harley & Fellows, 1991; Weinges, Künstler, Schilling & Jaggy, 1975; Kikuchi, Miyaichi & Tomimori, 1991; Çalis, Ersöz, Saracoglu & Sticher, 1993) differ considerably. A detailed investigation of *Holmskioldia*, therefore, may provide additional arguments for the placement of this genus. This paper describes the isolation of four new and three known monoacyl rhamnopyranosylcatalpol derivatives and of three known phenolic glycosides from the aerial parts of *Holmskioldia sanguinea* as well as the identification of catalpol by GC–MS.

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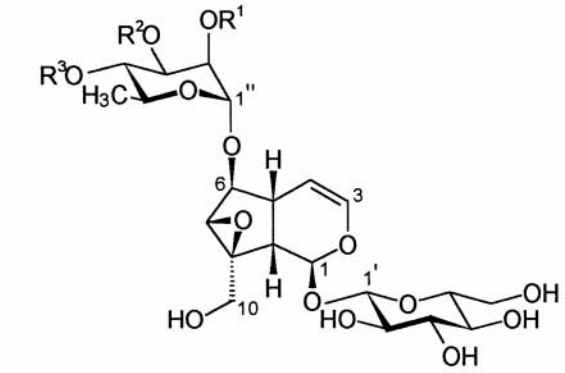

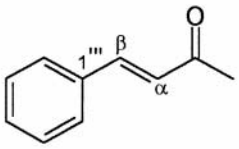

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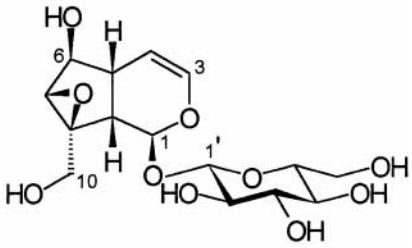

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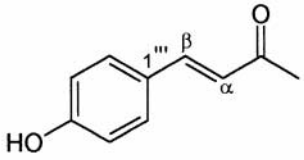

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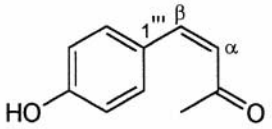

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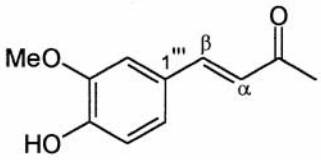

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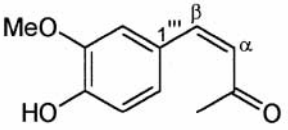
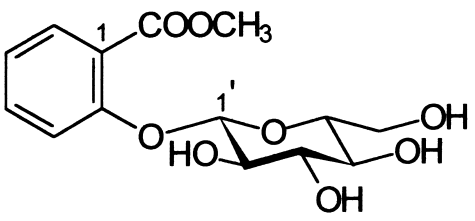


cis-feruloyl

	R^1	R^2	R^3
1	H	H	*trans*-cinnamoyl
2	*trans*-cinnamoyl	H	H
3	H	*trans*-cinnamoyl	H
4	H	H	*trans*-feruloyl
5	H	H	*cis*-feruloyl
6	H	H	*trans-p*-coumaroyl
7	H	H	*cis-p*-coumaroyl
12	H	H	H

2. Results

Partition of the ethanol–water extract of aerial parts of *H. sanguinea* between water and methylene chloride provided a lipophilic and a hydrophilic fraction. The hydrophilic fraction was separated by a combination of column chromatography (CC) on silica gel, centrifugal partition chromatography (CPC) (Potterat, Saadou & Hostettmann, 1991) and medium pressure liquid chromatography (MPLC) on RP-18. We isolated four new monoacyl rhamnopyranosylcatalpol derivatives (**1**, **2**, **3**, **5**) along with three known iridoid glycosides (**4**, **6**, **7**). The known iridoid glycosides were identified as 6-*O*-α-L-(4″-*O*-*trans*-feruloyl)rhamnopyranosylcatalpol



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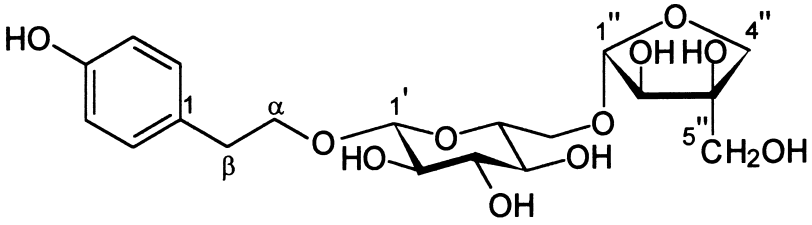

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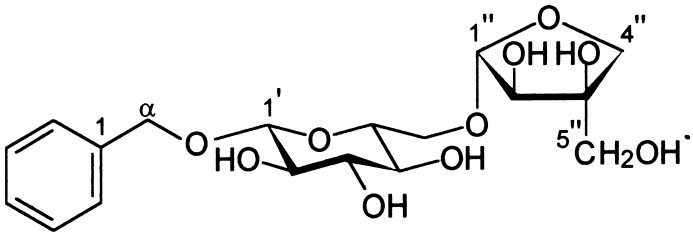

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(**4**) (Otsuka, Kubo, Sasaki, Yamasaki, Takeda et al., 1991), 6-*O*-α-L-(4″-*O*-*trans*-*p*-coumaroyl)rhamnopyranosylcatalpol (**6**) (Otsuka, Sasaki & Yamasaki, 1990a) and 6-*O*-α-L-(4″-*O*-*cis*-*p*-coumaroyl)rhamnopyranosylcatalpol (**7**) (Otsuka et al., 1990a), by comparison of their NMR spectra with published data. Another known iridoid glycoside was identified as catalpol (**8**) by GC and GC–MS, thus confirming the earlier results (Neumann, 1976). In addition we isolated three known phenolic glycosides (**9**–**11**). By comparison with reported data the structures of **9** and **10** were determined as methyl salicylate glucoside (**9**) (Ushiyama & Furuya, 1989) and osmanthuside H (**10**) (Sugiyama & Kikuchi, 1993; Ida, Satoh, Ohtsuka, Nagasao & Shoji, 1994). Compound **11** was identified as icariside F_2 by comparison of the ^{1}H NMR spectral data, recorded in pyridine-d_5 as well as in methanol-d_4, with the corresponding published data (Miyase, Ueno, Takizawa, Kobayashi & Oguchi, 1988; Ono, Ito, Ishikawa, Kitajima, Tanaka et al., 1996; Fuchino, Satoh & Tanaka, 1996) and with the data of **10** (Sugiyama & Kikuchi, 1993; Ida et al., 1994).

The ESI mass spectrum of **1** showed three quasimolecular ions at *m*/*z* 639 ($[M + H]^+$), *m*/*z* 661 ($[M + Na]^+$) and *m*/*z* 677 ($[M + K]^+$), suggesting a molecular formula of $C_{30}H_{38}O_{15}$. The ^{1}H NMR spectral data of **1** (see Table 1) indicated a catalpol unit combined with a rhamnopyranosyl unit and a cinnamoyl moiety. The two doublets of the cinnamoyl moiety at δ 6.58 (1 H, H-α) and δ 7.73 (1 H, H-β) with a coupling constant of 16 Hz indicated a *trans*-configuration of the double bond. The positive FAB mass spectrum showed signals at *m*/*z* 459 ($[M - \text{hexose}]^+$) and *m*/*z* 277 (cinnamoyldeoxyhexose) suggesting the attachment of the acyl unit to the rhamnopyranosyl moiety. The position of the *trans*-cinnamoyl moiety was determined by comparison of the ^{1}H and ^{13}C NMR spectra with those of the unsubstituted 6-*O*-α-L-rhamnopyranosylcatalpol (**12**) (Miyase, Akahori, Kohsaka & Ueno, 1991) (see Table 1 and Table 2):

Table 1
^{1}H NMR spectral data[a] (400 MHz) of compounds **1**–**3** and **12** (in CD_3OD)

H	**1**[b]	**2**	**3**	**12**[c]
1	5.08 *d*	5.08 *d*	5.10 *d*	5.07 *d*
	$J_{1,9} = 10$	$J_{1,9} = 10$	$J_{1,9} = 10$	$J_{1,9} = 10$
3	6.38 *dd*	6.37 *dd*	6.38 *dd*	6.35 *dd*
	$J_{3,4} = 6$	$J_{3,4} = 6$	$J_{3,4} = 6$	$J_{3,4} = 6$
	$J_{3,5} = 2$	$J_{3,5} = 2$	$J_{3,5} = 2$	$J_{3,5} = 2$
4	5.05 *dd*	5.07 *dd*	5.13 *dd*	5.05 *dd*
	$J_{4,3} = 6$	$J_{4,3} = 6$	$J_{4,3} = 6$	$J_{4,3} = 6$
	$J_{4,5} = 5$	$J_{4,5} = 5$	$J_{4,5} = 5$	$J_{4,5} = 5$
5	2.42 *m*	2.45 *m*	2.45 *m*	2.38 *m*
6	4.04 *dd*	4.03 *dd*	4.05 *dd*	3.99 *dd*
	$J_{6,5} = 8$	$J_{6,5} = 8$	$J_{6,5} = 8$	$J_{6,5} = 8$
	$J_{6,7} = 2$	$J_{6,7} = 2$	$J_{6,7} = 2$	$J_{6,7} = 2$
7	3.65 *d*	3.65 *br s*	3.66 *br s*	3.62 *d*
	$J_{7,6} = 2$			$J_{7,6} = 2$
8	–	–	–	–
9	2.56 *dd*	2.56 *dd*	2.57 *dd*	2.54 *dd*
	$J_{9,1} = 10$	$J_{9,1} = 10$	$J_{9,1} = 10$	$J_{9,1} = 10$
	$J_{9,5} = 8$	$J_{9,5} = 8$	$J_{9,5} = 8$	$J_{9,5} = 8$
10A	3.81 *d*	3.81 *d*	3.83 *d*	3.81 *d*
	$J_{10A,10B} = 13$	$J_{10A,10B} = 13$	$J_{10A,10B} = 13$	$J_{10A,10B} = 13$
10B	4.13 *d*	4.15 *d*	4.15 *d*	4.13 *d*
	$J_{10B,10A} = 13$	$J_{10B,10A} = 13$	$J_{10B,10A} = 13$	$J_{10B,10A} = 13$
1′	4.77 *d*	4.77 *d*	4.78 *d*	4.77 *d*
	$J_{1',2'} = 8$	$J_{1',2'} = 8$	$J_{1',2'} = 8$	$J_{1',2'} = 8$
2′	3.25 *dd*	3.22–3.29[d]	3.22–3.29[d]	3.25 *dd*
	$J_{2',1'} = 8$			$J_{2',1'} = 8$
	$J_{2',3'} = 9$			$J_{2',3'} = 9$
3′	3.40 *dd*	3.38 *t*	3.39 *t*	3.38 (3.40) *t*[e]
	$J_{3',2'} = 9$	$J = 9$	$J = 9$	$J = 9$
	$J_{3',4'} = 8$			
4′	3.24 *dd*	3.22–3.29[d]	3.22–3.29[d]	3.24 *dd*
	$J_{4',3'} = 8$			$J_{4',3'} = 8$
	$J_{4',5'} = 10$			$J_{4',5'} = 10$
5′	3,3 *m*[f]	3,3 *m*[f]	3,3 *m*[f]	3,3 *m*[f]
6′A	3.64 *dd*	3.63 *dd*	3.64 *dd*	3.61 *dd*
	$J_{6'A,6'B} = 12$	$J_{6'A,6'B} = 12$	$J_{6'A,6'B} = 12$	$J_{6'A,6'B} = 12$
	$J_{6'A,5'} = 6$	$J_{6'A,5'} = 6$	$J_{6'A,5'} = 6$	$J_{6'A,5'} = 6$
6′B	3.91 *dd*	3.89 *dd*	3.91 *dd*	3.91 *dd*
	$J_{6'B,6'A} = 12$	$J_{6'B,6'A} = 12$	$J_{6'B,6'A} = 12$	$J_{6'B,6'A} = 12$
	$J_{6'B,5'} = 2$	$J_{6'B,5'} = 2$	$J_{6'B,5'} = 2$	$J_{6'B,5'} = 2$
1″	4.99 *d*	5.04 *d*	4.98 *d*	4.92 *d*
	$J_{1'',2''} = 2$	$J_{1'',2''} = 2$	$J_{1'',2''} = 2$	$J_{1'',2''} = 2$
2″	3.88–3.95[d]	5.16 *dd*	4.09 *dd*	3.84 *dd*
		$J_{2'',1''} = 2$	$J_{2'',1''} = 2$	$J_{2'',1''} = 2$
		$J_{2'',3''} = 4$	$J_{2'',3''} = 4$	$J_{2'',3''} = 3$
3″	3.88–3.95[d]	3.92 *dd*	5.12 *dd*	3.67 *dd*
		$J_{3'',2''} = 4$	$J_{3'',2''} = 4$	$J_{3'',2''} = 3$
		$J_{3'',4''} = 9$	$J_{3'',4''} = 9$	$J_{3'',4''} = 9$
4″	5.09 *t*	3.48 *t*	3.69 *t*	3.38 (3.39) *t*[e]
	$J = 10$	$J = 9$	$J = 9$	$J = 9$
5″	3.88–3.95[d]	3.73–3.86[d]	3.73–3.86[d]	3.63–3.69 *m*[e,g] (3.65)
				$J_{5'',4''} = 9$
				$J_{5'',6''} = 6$
6″ (3H)	1.17 *d*	1.30 *d*	1.32 *d*	1.25 *d*
	$J_{6'',5''} = 6$	$J_{6'',5''} = 6$	$J_{6'',5''} = 6$	$J_{6'',5''} = 6$
2‴/6‴	7.62 *m*	7.63 *m*	7.63 *m*	
3‴/4‴/5‴	7.41 *m*	7.41 *m*	7.41 *m*	
α	6.58 *d*	6.60 *d*	6.61 *d*	
	$J_{\alpha,\beta} = 16$	$J_{\alpha,\beta} = 16$	$J_{\alpha,\beta} = 16$	
β	7.73 *d*	7.75 *d*	7.78 *d*	
	$J_{\beta,\alpha} = 16$	$J_{\beta,\alpha} = 16$	$J_{\beta,\alpha} = 16$	

[a] Chemical shift values (δ) in ppm. Coupling constants (J) in Hz. Assignments were confirmed by ^{1}H–^{1}H COSY.
[b] Assignments of the ^{1}H signals of the rhamnopyranoysyl moiety were confirmed by ^{13}C–^{1}H COSY.
[c] Data are identical to published data (Miyase et al., 1991).
[d] Overlapped by other signals; therefore, the coupling constants could not be accurately determined.
[e] Chemical shifts in paranthesis and coupling constants were determined by recording at 50°.
[f] Overlapped by the CD_2HOD signal.
[g] Coupling constants were determined by double resonance technique.

The H-4″ signal of **1** was shifted downfield by 1.71 ppm; the C-4″ signal was also shifted downfield by 1.66 ppm, whereas the signals of C-3″ and C-5″ were shifted upfield by 2.09 and 1.93 ppm, respectively. These features were only compatible with the attachment of the acyl group to the 4″-oxygen. This assignment was confirmed by comparison with the data of the known 4″-acyl rhamnopyranosylcatalpol derivatives **4** (Otsuka et al., 1991), **6** (Otsuka et al., 1990a) and **7** (Otsuka et al., 1990a). Therefore, the structure of **1** was determined as 6-*O*-α-L-(4″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol.

The ESI mass spectrum of the mixture of compounds **2** and **3** showed only two quasimolecular ions at m/z 661 ($[M + Na]^+$) and m/z 677 ($[M + K]^+$) suggesting a molecular formula of $C_{30}H_{38}O_{15}$ for both compounds. The ^{1}H NMR spectrum of the mixture showed two sets of signals with a ratio of *ca* 1:2, corresponding to compounds **2** and **3**, respectively. Hydrolysis of the mixture of **2** and **3** afforded 6-*O*-α-L-rhamnopyranosylcatalpol (**12**) (Miyase et al., 1991) and cinnamic acid. The ^{1}H NMR spectra of **2** and **3** were very similar (see Table 1), only the signals of the rhamnopyranosyl moieties differed substantially. Compared to **12** a downfield shift (1.32 ppm) of the H-2″ signal was observed for **2** and a downfield shift (1.45 ppm) of the H-3″ signal was observed for **3**. These data proved the attachment of the cinnamoyl moiety to O-2″ in **2** and to O-3″ in **3**. Thus, the structures were established as 6-*O*-α-L-(2″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**2**) and 6-*O*-α-L-(3″-*O*-*trans*-cinnamoyl)rhamnopyranosylcatalpol (**3**).

Compounds **4** and **5** were also obtained as a mixture. The FAB mass spectra showed quasimolecular ions at m/z 685 ($[M + H]^+$) in the positive mode and at m/z 683 ($[M–H]^-$) in the negative mode, indicating the presence of two isomers with the molecular formula of $C_{31}H_{40}O_{17}$. The ratio of **4** to **5** was determined as 5:2 by the intensities of the corresponding proton signals in the ^{1}H NMR spectrum. The main compound (**4**) was identified as 6-*O*-α-L-(4″-*O*-*trans*-feruloyl)rhamnopyranosylcatalpol by comparison of the ^{1}H NMR data with those of the literature (Otsuka et al., 1991). The ^{1}H NMR spectrum of **5**, too, exhibited the typical

Table 2
^{13}C NMR spectral data[a] (100 MHz) of compounds **1**, **6**, **7** and **12** (in CD_3OD)

C	**1**[b]	**6**[b]	**7**	**12**
1	95.22	95.21	95.21	95.23
3	142.32	142.32	142.33	142.18
4	103.46	103.47	103.45	103.64
5	37.35	37.34	37.36	37.37
6	84.21	84.17	83.92	83.69
7	59.49	59.48	59.36	59.38
8	66.59	66.58	66.60	nd
9	43.36	43.35	43.32	43.35
10	61.48	61.48	61.49	61.50
1′	99.76	99.75	99.76	99.77
2′	74.85	74.85	74.87	74.88
3′	77.73	77.72[c]	77.74[c]	77.76[d]
4′	71.80	71.80	71.82	71.83
5′	78.63	78.63[c]	78.65[c]	78.65[d]
6′	62.96	62.95	62.97	62.97
1″	100.49	100.47[c]	100.32[c]	100.38
2″	72.49	72.50	72.47	72.28*
3″	70.29	70.33	70.29	72.38*
4″	75.59	75.28	75.01	73.93
5″	68.26	68.34	68.21	70.19
6″	17.86	17.87	17.87	17.94
1‴	135.78	126.90[c]	127.24[c]	
2‴/6‴	129.28	131.24	133.82[c]	
3‴/5‴	130.03	117.05[c]	116.09[c]	
4‴	131.56	161.96[c]	161.07[c]	
α	118.87	114.86[c]	116.20[c]	
β	146.66	147.03[c]	145.74[c]	
C=O	168.30	168.99	167.84	

[a] Chemical shift values (δ) in ppm.
[b] Assignments were confirmed by ^{13}C–^{1}H COSY.
[c] The data of the rhamnopyranosyl moiety are almost identical with published data obtained from a mixture of the *trans* (**6**)- and *cis* (**7**) isomers (Otsuka et al., 1990), but the chemical shifts reported previously for the C-1″ signals of the *trans*- and *cis* isomers must be interchanged. The chemical shifts of the C-3′ and C-5′ signals in the spectra reported for **6** as well as for **7** must also be interchanged. Small differences were also observed for some olefinic and aromatic protons.
[d] Data are identical to published data (Miyase et al., 1991) except the chemical shift values reported previously for C-3′ and C-5′ must be interchanged.
nd Signal was not detectable.
*Values are interchangeable.

signals of a rhamnopyranosylcatalpol ester. The acyl moiety was also linked to the 4″-oxygen since the H-4″ signal showed a characteristic downfield shift of 1.69 ppm compared to **12**. Two doublets at δ 6.90 and δ 5.83 with a coupling constant of 13 Hz, assignable to two olefinic protons of a *cis* double bond, three aromatic protons (AMX system with coupling constants of 2 Hz and 8 Hz) and a methoxyl group were observed for the acyl part, suggesting a *cis*-feruloyl or a *cis*-isoferuloyl moiety. Apart from the olefinic proton signals the ^{1}H NMR spectra of **4** and **5** differed only in the chemical shifts of the aromatic protons. The H-2‴ signals showed the highest difference: The spectrum of **5** showed a signal at δ 7.76 ppm whereas the H-2‴ signal of **4** was observed at δ 7.20 ppm. Smaller differences were observed for the aromatic protons H-5‴ and H-6‴: The H-5‴ signal of **5** showed an upfield shift of 0.06 ppm and the H-6‴ signal of **5** a downfield shift of 0.03 ppm compared to **4**. These differences are in agreement with the differences expected for *trans*- and *cis* feruloyl moieties. Indeed, the ^{1}H NMR data of the acyl moiety in **5** are identical with those of the feruloyl moiety in 2′-*O*-acetyl-4′-*O*-*cis*-feruloylswertiamarin (Kikuzaki, Kawasaki, Kitamura & Nakatani, 1996). Therefore, the structure of **5** was determined as 6-*O*-α-L-(4″-*O*-*cis*-feruloyl)rhamnopyranosylcatalpol.

The ratio of compounds **2** and **3** was determined as 1:1.7 in the fraction containing **1**, **2** and **3**. On an attempt to separate these compounds by semipreparative HPLC this ratio was shifted to about 1:1 by transacylation during the evaporation of the solvent (*in vacuo*, temp. $<40^{\circ}$). Since it seemed possible that some transacylation had already occurred during the extraction or in earlier steps of the separation procedure we repeated the extraction on an analytical scale under modified conditions that minimize the possibility of interconversions. We refluxed the plant material only once with ethanol for 10 min. After dilution with water and extraction with acid-free chloroform the aqueous layer was directly lyophilized providing the raw iridoid fraction (RIF). The RIF was examined by HPLC–UV analysis as well as by co-chromatography with authentic samples. Although the peak of **2** was partly overlapped by other peaks, the ratio of **2** to **3** could be determined as approximately 1:2; this ratio is in the same range as in the fraction containing **1**, **2** and **3**. Since a transacylation seems rather unlikely under the mild extraction conditions used, we assume that both compounds are genuine plant constituents.

However, we noticed that compound **5** could not be detected in the RIF. Additionally, the ratio of compounds **6** and **7** was observed as 6.0:1 in the RIF in contrast to the ratio of 3.3:1 in the fraction containing **6** and **7**. These findings suggested *trans* to *cis* isomerizations of **4** to **5**, and of **6** to **7** during the extraction and/ or isolation procedure.

3. Discussion

Our re-investigation of *Holmskioldia sanguinea* demonstrated that rhamnopyranosylcatalpol esters are the main iridoid constituents present in this species. Similar compounds have previously been isolated from several *Premna* species (Viticoideae–Labiatae) (Otsuka et al., 1991, 1990a; Otsuka, Kubo, Yamasaki & Padolina, 1989a; Otsuka, Sasaki, Yamasaki, Takeda & Seki, 1989b; Otsuka, Sasaki, Yamasaki, Takeda & Seki, 1990b) as well as from species of the

families Buddlejaceae (Miyase et al., 1991) and Scrophulariaceae (Çalis, Gross, Winkler & Sticher, 1988; Bhandari, Mishra, Roy & Garg, 1992; Çalis, Zor, Basaran, Wright & Sticher, 1993; Fernandez, Diaz, Ollivier, Faure & Balansard, 1995; Seifert, Lien, Schmidt, Johne, Popov et al., 1989; Warashina, Miyase & Ueno, 1991). Thus, the significance of acyl rhamnopyranosylcatalpol derivatives as taxonomic markers is limited since they obviously evolved several times independently in different families. However, at the level of genera and tribes the substitution pattern of these iridoids like the 7,8-oxido group and the 10-hydroxyl group as well as acylation of the iridoids with unsubstituted or substituted cinnamic acids might be useful characters. Within the Labiatae this substitution pattern was found in species of the genera *Scutellaria* (Scutellarioideae) (Kooiman, 1972; Cole et al., 1991; Weinges et al., 1975; Kikuchi et al., 1991; Çalis et al., 1993), *Premna* (Viticoideae) (Sudo, Ide, Otsuka, Hirata, Takushi et al., 1997), *Leucosceptrum* and *Pogostemon* (Pogostemonoideae) (Schmidt, 1997) as well as in species of the tribe Prostanthereae (Chloanthoideae) (Kooiman, 1972; Schmidt, 1997; Dellar, Conn, Cole & Waterman, 1996).

The iridoid pattern of *Holmskioldia* is similar to *Scutellaria* and clearly different from *Clerodendrum* s. l. In *Holmskioldia* and *Scutellaria* 11-nor iridoids with 7,8-oxido, 6-hydroxy and 10-hydroxy substitution acylated with unsubstituted or substituted cinnamoyl moieties are accumulated, whereas in the genus *Clerodendrum* s. l. no 11-nor iridoids with a 7,8-oxido group have been found. Species of *Clerodendrum* s. str. accumulate 11-nor iridoids or iridoid-11-acids characterized by 7,8-dehydro, 8β-hydroxy/ 8α-methyl, 8α-methyl, and 7β-hydroxy substitution or they do not accumulate iridoids (Jacke & Rimpler, 1983; Stenzel et al., 1988; Çalis et al., 1994a,b). In the new genus *Cyclonema*, which has been segregated from Clerodendrum (Cantino, 1992; Cantino et al., 1992; Steane et al., 1997), 11-oxo iridoids with 8,10-dehydro, 8β-hydroxy/ 8α-methyl, and 8α-methyl substitution were found (Jacke & Rimpler, 1983; Stenzel et al., 1986). The results of our phytochemical investigation, therefore, are better compatible with the placement of *Holmskioldia* near *Scutellaria* within the subfamily Scutellarioideae than with a position near the genus *Clerodendrum*. They confirm the revised classification by Cantino (1992) and Cantino et al., (1992) as well as the results of the cpDNA investigations (Steane et al., 1997; Wagstaff et al., 1998).

4. Experimental

4.1. Plant material

Holmskioldia sanguinea was cultivated in the Botanical Garden, Freiburg. The aerial parts of the plant were collected in the flowering period. A voucher specimen (1276) has been deposited at the herbarium of the Institut für Pharmazeutische Biologie, Freiburg.

4.2. General

NMR: 400 MHz (^{1}H) or 100 MHz (^{13}C), chemical shifts as δ values (ppm) relative to the CD_2HOD signal at 3.30 ppm (^{1}H) and 49.0 ppm (^{13}C), CD_3OD as solvent. FAB–MS: positive and negative ion mode; glycerol as matrix, rel. int. after background subtraction of glycerol, ionization with Xe (5–6 keV, 3 kV). ESI–MS: positive ion mode, flow rate of 2 μl min^{-1}, temp. of capillary 200° (spray 4.5 kV). TLC: Silica gel 60 F_{254}, CH_2Cl_2–MeOH–H_2O (70:30:3) and RP-18 MeCN–MeOH–H_2O (25.0:7.7:67.3); spray reagent vanillin (3%) and H_2SO_4 (1%) in EtOH followed by heating at 110° for 5–10 min. CC: Silica gel 60, 63–200 μm (Merck). HPLC: Column I: LiChrospher 100 C-18 (10 μm; 8×250 mm), flow 2 ml min^{-1}, pressure 430–480 psi; column II: LiChrosorb C-18 (10 μm; 16×250 mm), flow 6 ml min^{-1}, pressure 2500 psi; photodiode array detector, 193–400 nm, Waters 996; solvent system A: MeCN–MeOH–H_2O (27.5:8.5:64.0); solvent system B: MeCN–MeOH–H_2O (25.0:7.7:67.3); solvent system C: MeCN–MeOH–H_2O (20.8:9.0:70.2); solvent system D: MeCN–MeOH–H_2O (22.5:6.9:70.6); solvent system E MeCN–MeOH–H_2O (21.3:6.6:72.1). The solvents were optimized with OPTISOLVE (Geiger & Rimpler, 1990). MPLC: LiChroprep C-18 (15–25 μm; 26×500 mm); flow 4.5 ml min^{-1}, pressure 70–180 psi; solvent systems A, B and C; frs. were monitored by TLC and UV detection at 254 nm. CPC: ITO multi-layer coil separator–extractor (capacity 380 ml; i.d. 2.6 mm; 700 rpm); $CHCl_3$–MeOH–*iso*-PrOH–H_2O (5:6:1:4) in the descending mode (4 ml min^{-1}) (Potterat et al., 1991).

4.3. Isolation

The lyophilized powdered aerial parts (130 g) were extracted by refluxing for 30 min successively with 96, 80, 70 and 50% EtOH. The combined extracts were conc. *in vacuo* and partitioned between H_2O and CH_2Cl_2 into a hydrophilic and a lipophilic fr. The hydrophilic fr. (31 g) after concentration *in vacuo* and lyophilization was sepd by CC on silica gel with CH_2Cl_2–MeOH–H_2O mixts. of increasing polarity [(90:10:1) → (50:50:5)] providing 7 frs. (I–VII). Fr.I (330 mg) was obtained by elution with the mixts.

90:10:1, 80:20:2 and 70:30:3. Elution with the mixt. 60:40:4 yielded frs. II (380 mg), III (1120 mg), IV (2070 mg), V (3000 mg), and VI (1930 mg). Fr. VII (1300 mg) was eluted with the mixt. 50:50:5. Frs. I–VII were conc. *in vacuo* and lyophilized. TLC of frs. III and IV indicated the presence of iridoids. Separation of 500 mg of fr. III by CPC afforded 4 frs. (III.1–III.4) which were lyophilized after removal of the $CHCl_3$ *in vacuo* and dilution of the residue with H_2O. MPLC of fr. III.2 (230 mg) on RP-18 with solvent system A gave 6 frs. (III.2.1–III.2.6). The organic solvents of all frs. were removed by extraction with $CHCl_3$ ×3 and the aq. layers were directly lyophilized. Fr. III.2.6 afforded compound **1** (110 mg); frs. III.2.3 (4 mg) and III.2.4 (6 mg) were combined since they both contained **2** and **3** in different concentrations. Purification of the combined frs. (8 mg) by HPLC (column I) on RP-18 with THF–MeCN–MeOH–H_2O (0.9:23.7:7.7:67.1) yielded after evaporation of the solvent (*in vacuo*, temp. $<40^\circ$) a 1:1 mixture of **2** and **3**. Therefore, the structure elucidation of **2** and **3** was carried out on the small amount of fr. III.2.4 (2 mg). In order to avoid transacylation during work-up all subfractions of fr. IV obtained by MPLC and HPLC were diluted with H_2O and directly lyophilized; from subfractions obtained by CPC the $CHCl_3$ was removed in the rotary evaporator (temp. $<30^\circ$) and the residue was diluted with H_2O and lyophilized. Separation of fr. IV (1700 mg) by MPLC with solvent system B provided five frs. (IV.1–IV.5). Fr. IV.3 (265 mg) was separated by CPC affording a mixt. of **4** and **5** (60 mg) and another fr. (IV.3.7; 105 mg) which was purified by MPLC. Elution with solvent system C gave compounds **6** (30 mg) and **7** (10 mg). Fr. IV.1 (580 mg) contained compound **8** which was identified by GC and GC–MS analysis. Fr. IV.2 (340 mg) was also separated by CPC providing the frs. IV.2.1–IV.2.13. Fr. IV.2.4 (5 mg) was purified by HPLC (column II) on RP-18 with MeCN–MeOH–H_2O (12.5:15.4:72.1) affording compound **9** (1 mg). Separation of fr. IV.2.12 (95 mg) by MPLC with MeCN–MeOH–H_2O (3.7:18.5:77.8) provided compound **10** (6 mg). MPLC of fr IV.2.8 (60 mg) with MeCN–MeOH–H_2O (4.4:21.5:74.1) gave compound **11** (4 mg).

*4.3.1. 6-O-α-L-(4″-O-trans-cinnamoyl)rhamnopyranosylcatalpol (**1**)*

White amorphous powder. HPLC (solvent system A) R_t 17.9 min. $[\alpha]_D^{24}$ −150° (MeOH; *c* 0.20). ESIMS, *m/z* (rel. int.): 677 $[M + K]^+$ (59.3), 661 $[M + Na]^+$ (100.0), 639 $[M + H]^+$ (7.4). FABMS, negative mode, *m/z* (rel. int.): 637 $[M–H]^-$ (18.4), 147 (100.0). FABMS, positive mode, *m/z* (rel. int.): 639 $[M + H]^+$ (1.3), 459 $[M - \text{glucose}]^+$ (1.6), 277 (58.7), 131 (100.0). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2.

*4.3.2. Mixture of 6-O-α-L-(2″-O-trans-cinnamoyl)-rhamnopyranosylcatalpol (**2**) and 6-O-α-L-(3″-O-trans-cinnamoyl)rhamnopyranosylcatalpol (**3**)*

Pale yellow powder. HPLC (solvent system A) R_t 10.5 min (**2**) and 12.1 min (**3**). ESIMS, *m/z* (rel. int.): 677 $[M + K]^+$ (78.3), 661 $[M + Na]^+$ (100.0). ^{1}H NMR: see Table 1.

Hydrolysis of **2** and **3** in CD_3OD/CD_2HOD and traces of H_2O in the NMR tube during 30 h at room temperature afforded compound **12** and cinnamic acid:

4.3.3. Cinnamic acid

^{1}H NMR: δ 6.52 (1 H, *d*, *J* = 16 Hz, H-α), 7.40 (3 H, H-3‴, H-4‴, H-5‴), 7.60 (2 H, H-2‴, H-6‴), 7.69 (1 H, *d*, *J* = 16 Hz, H-β).

*4.3.4. 6-O-α-L-Rhamnopyranosylcatalpol (**12**)*

ESIMS, *m/z* (rel. int.): 547 $[M + K]^+$ (28.7), 531 $[M + Na]^+$ (78.6). ^{1}H NMR: see Table 1. ^{13}C NMR: see Table 2.

*4.3.5. Mixture of 6-O-α-L-(4″-O-trans-feruloyl)rhamnopyranosylcatalpol (**4**) and 6-O-α-L-(4″-O-cis-feruloyl)-rhamnopyranosylcatalpol (**5**)*

Pale yellow powder. HPLC (solvent system D) R_t 13.2 min (**4**) and 17.7 min (**5**). ESIMS, *m/z* (rel. int.): 723 $[M + K]^+$ (66.5), 707 $[M + Na]^+$ (100.0). FABMS, negative mode, *m/z* (rel. int.): 683 $[M–H]^-$ (90.6), 193 (73.6). FABMS, positive mode, *m/z* (rel. int.): 685 $[M + H]^+$ (3.2), 323 (55.6), 177 (65.7). ^{1}H NMR (**4**, **5**): δ 1.15 (3 H, *d*, *J* = 6 Hz, **5**: H-6″), 1.17 (3 H, *d*, *J* = 6 Hz, **4**: H-6″), 2.41 (2 H, *m*, **4**, **5**: H-5), 2.55 (1 H, *dd*, *J* = 10, 8 Hz, **5**: H-9), 2.57 (1 H, *dd*, *J* = 10, 8 Hz, **4**: H-9), 3.24 (2 H, *dd*, *J* = 10, 8 Hz, **4**, **5**: H-4′), 3.25 (2 H, *dd*, *J* = 9, 8 Hz, **4**, **5**: H-2′), 3.30 (**4**, **5**: H-5′, overlapped by the CD_2HOD signal), 3.39 (2 H, *t*, *J* = 9 Hz, **4**, **5**: H-3′), 3.62 (2 H, *dd*, *J* = 12, 6 Hz, **4**, **5**: H-6′A), 3.64 (1 H, *br s*, **5**: H-7), 3.65 (1H, *br s*, **4**: H-7), 3.81 (1 H, *d*, *J* = 13 Hz, **5**: H-10A), 3.82 (1 H, *d*, *J* = 13 Hz, **4**: H-10A), 3.85-3.93 (8 H, *m*, **4**, **5**: H-6′B, H-2″, H-3″, H-5″), 3.86 (3 H, *s*, **5**: $-OCH_3$), 3.89 (3 H, *s*, **4**: $-OCH_3$), 4.02 (1 H, *dd*, *J* = 8, 2 Hz, **5**: H-6), 4.04 (1 H, *dd*, *J* = 8, 2 Hz, **4**: H-6), 4.14 (1 H, *d*, *J* = 13 Hz, **5**: H-10B), 4.15 (1 H, *d*, *J* = 13 Hz, **4**: H-10B), 4.75 (1 H, *d*, *J* = 8 Hz, **5**: H-1′), 4.76 (1 H, *d*, *J* = 8 Hz, **4**: H-1′), 4.98 (1 H, *d*, *J* = 2 Hz, **5**: H-1″), 5.00 (1 H, *d*, *J* = 2 Hz, **4**: H-1″), 5.03–5.10 (4 H, *m*, **4**, **5**: H-4, H-4″), 5.07 (1 H, *d*, *J* = 10 Hz, **5**: H-1), 5.09 (1 H, *d*, *J* = 10 Hz, **4**: H-1), 5.83 (1 H, *d*, *J* = 13 Hz, **5**: H-α), 6.38 (2 H, *dd*, *J* = 6, 2 Hz, **4**, **5**: H-3), 6.40 (1 H, *d*, *J* = 16 Hz, **4**: H-α), 6.75 (1 H, *d*, *J* = 8 Hz, **5**: 5‴), 6.81 (1 H, *d*, *J* = 8 Hz, **4**: 5‴), 6.90 (1 H, *d*, *J* = 13 Hz, **5**: H-β), 7.10 (1 H, *dd*, *J* = 8, 2 Hz, **4**: 6‴), 7.13 (1 H, *dd*, *J* = 8, 2 Hz, **5**: 6‴), 7.20 (1 H, *d*, *J* = 2 Hz, **4**: 2‴), 7.65 (1 H, *d*, *J* = 16 Hz, **4**: H-β), 7.76 (1 H, *d*, *J* = 2 Hz, **5**: 2‴). Assignments were confirmed by ^{1}H–^{1}H COSY.

*4.3.6. 6-O-α-L-(4″-O-trans-p-coumaroyl)rhamnopyranosylcatalpol (**6**)*

Pale yellow powder. HPLC (solvent system C) R_t 13.5 min. $[\alpha]_D^{24}$ −129° (MeOH; *c* 0.17). ESIMS, *m/z* (rel. int.): 693 $[M + K]^+$ (21.9), 677 $[M + Na]^+$ (100.0). FABMS, negative mode, *m/z* (rel. int.): 653 $[M–H]^-$ (66.0), 163 (53.0). FABMS, positive mode, *m/z* (rel. int.): 677 $[M + Na]^+$ (5.5), 655 $[M + H]^+$ (4.2), 475 $[M - glucose]^+$ (2.3), 293 (66.7), 147 (100.0). ^{1}H NMR: The data are identical with published data, recorded at 100 MHz, as far as they have been reported (Otsuka et al., 1990a). ^{13}C NMR: see Table 2.

4.3.7. 6-O-α-L-(4″-O-cis-p-coumaroyl)rhamnopyranosylcatalpol (7)

Pale yellow powder. HPLC (solvent system C) R_t 19.5 min. $[\alpha]_D^{24}$ −104° (MeOH; *c* 0.05). ESIMS, *m/z* (rel. int.): 693 $[M + K]^+$ (35.5), 677 $[M + Na]^+$ (100.0). FABMS, negative mode, *m/z* (rel. int.): 653 $[M–H]^-$ (47.0), 163 (40.0). FABMS, positive mode, *m/z* (rel. int.): 677 $[M + Na]^+$ (4.5), 655 $[M + H]^+$ (2.0), 293 (29.5), 147 (43.0). ^{1}H NMR: The data are identical with published data, recorded at 100 MHz, as far as they have been reported (Otsuka et al., 1990a). ^{13}C NMR: see Table 2.

*4.3.8. Methyl salicylate glucoside (methyl-2-O-β-D-glucopyranosyloxybenzoate) (**9**)*

FABMS and ^{1}H NMR: the data agreed with published data (Ushiyama & Furuya, 1989).

*4.3.9. Osmanthuside H (2-(4-hydroxyphenyl)ethyl-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside) (**10**)*

FABMS, ^{1}H NMR and ^{13}C NMR: the data agreed with published data (Sugiyama & Kikuchi, 1993; Ida et al., 1994).

*4.3.10. Icariside F_2 (phenylmethyl-β-D-apiofuranosyl-(1 → 6)-β-D-glucopyranoside) (**11**)*

^{1}H NMR: δ 3.25 (2 H, *m*, H-2′, H-4′), 3.33 (1 H, *t*, *J* = 9 Hz, H-3′), 3.38 (1 H, *m*, H-5′), 3.59 (2 H, *s*, H-5″), 3.63 (1 H, *dd*, *J* = 11, 6 Hz, H-6′A), 3.76 (1 H, *d*, *J* = 10 Hz, H-4″A), 3.92 (1 H, *d*, *J* = 2 Hz, H-2″), 3.98 (1 H, *d*, *J* = 10 Hz, H-4″B), 4.10 (1 H, *dd*, *J* = 11, 2 Hz, H-6′B), 4.33 (1 H, *d*, *J* = 8 Hz, H-1′), 4.65 (1 H, *d*, *J* = 12 Hz, H-αA), 4.88 (1 H, *d*, *J* = 12 Hz, H-αB), 5.05 (1 H, *d*, *J* = 2 Hz, H-1″), 7.26 (1 H, *m*, H-4), 7.33 (2 H, *m*, H-3, H-5), 7.43 (2 H, *m*, H-2, H-6). FABMS and ^{1}H NMR (400 and 500 MHz, pyridine-d_5): The data agreed with published data (Miyase et al., 1988; Ono et al., 1996).

*4.3.11. Catalpol (**8**)*

GC–MS: EI 70 eV, TMSi derivative, He as carrier gas (6 psi, split vent flow of 10 ml min^{-1}); WCOT Rtx®-200 (trifluoropropylmethyl polysiloxane, 30 m×0.32 mm), film thickness 0.25 μm; operation conditions: 220° for 10 min, then 3° min^{-1} to 260°. GC: same column and operation conditions, linear velocity 22 cm min^{-1} (set at 220°), split ratio 85:1; guard column (5 m×0.32 mm). GC–MS *m/z* (rel. int.): 416 $[M - glucose(TMSi)_5]^+$ (1.0), 81 [pyrylium–ion]$^+$ (11.8). GC: R_t 18.24 min. Catalpol was identified by comparison of the mass spectrum and R_t as well as by co-chromatography with authentic catalpol.

4.4. Analytical-scale extraction

The lyophilized powdered plant material (10 g) was extracted by refluxing for 10 min with 96% EtOH. The extract was diluted with H_2O in a ratio of 1:3 and extracted ×5 with $CHCl_3$ (purified by CC on basic Al_2O_3, Bunge, 1959). The aq. layer was directly lyophilized and analysed by HPLC with solvent system B, D and E (sample: *c* 10.0%, 70 μl). The compounds were identified by comparison of their R_ts and UV spectra with those of authentic samples as well as by co-chromatography with authentic samples.

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