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SAPONINS FROM *COLLINSONIA CANADENSIS*

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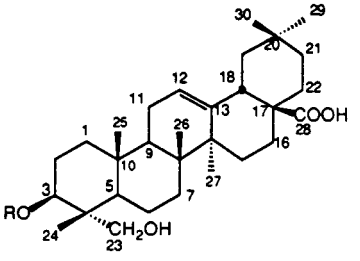
ABSTRACT.—Akeboside St_b [**1**] and two new saponins named collinsonin and collinsonidin were isolated from the roots of *Collinsonia canadensis*. On the basis of chemical and spectral studies, the structure of collinsonin [**2**] has been established as 3-*O*-α-L-arabinopyranosylcollinsogenin. 16-α-Hydroxyhederagenin, obtained by the hydrolysis of **2**, is a new sapogenin named collinsogenin [**5**]. Collinsonidin [**6**] has been identified as 3-*O*-β-D-glucopyranosyl-(1″↦3′)-α-L-arabinopyranosylhederagenin.

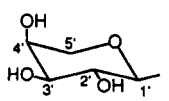
Collinsonia canadensis L. (Labiatae) (common names: horse balm, rich weed, stone root, etc.) is native to North America and grows wild from Massachusetts and Vermont to Florida and Arkansas. A tincture of the roots is reported to be used as a tonic, diuretic, and household remedy for headaches and indigestion (1,2). No chemical work has been carried out earlier on the roots of *C. canadensis*. An EtOH extract of the powdered roots showed in vitro antifungal activity. Bioassay-directed fractionation led to the isolation of three pure compounds which were responsible for the in vitro antifungal activity of the crude EtOH extract. These compounds were found to be saponins, of which two are new. We report here the structure determination of these saponins based mainly on the ^{1}H- and ^{13}C-nmr and ms data.

RESULTS AND DISCUSSION

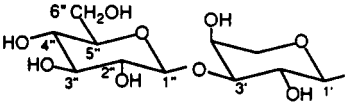
On the basis of the ms, ^{1}H- and ^{13}C-nmr spectral data, and elemental analysis ($C_{35}H_{56}O_8$), the first saponin was assigned the structure hederagenin-3-*O*-α-L-arabinopyranoside [**1**]. A literature search showed that saponin **1** has been isolated from several plant species, e.g., *Akebia quinata* Decne. (Lardizabalaceae), *Fatsia japonica* Decne et



hederagenin, R=H

1 R= 

4 R=α-L-arabinopyranosyl, 22-OH
7 R=β-D-glucopyranosyl-(1″↦4′)-α-L-arabinopyranosyl
8 R=β-D-glucopyranosyl-(1″↦2′)-α-L-arabinopyranosyl

6 R= 

Planch (Araliaceae), and *Kalopanax septemlobus* (Thunb.) Koidz. (Araliaceae). The different names reported for this saponin are: akeboside St_b (3,7,8), β_2-fatsin (4), PA (seed saponin A) (5,6), and leontoside A (9).

The ^{13}C-nmr assignments of **1** are given in Table 1. Values for C-7 (δ 33.0), C-29 (δ 33.3), and C-11, C-30 (δ 23.6), C-16 (δ 23.8) given in the literature (8) cannot be interchanged as stated, since these carbons are methylene and methyl groups. Our assignments have been confirmed by DEPT experiments. C-23 was assigned to δ 67.0 (as against δ 64.8) (8) as this was close to the assignment in hederagenin (δ 67.9), 23-hydroxyursolic acid (δ 68.1) (10), and collinsogenin (δ 68.1) (vide supra). The ^{1}H-^{1}H

TABLE 1. ^{13}C-nmr Spectral Assignments of Akeboside St_b [**1**], Collinsonin [**2**], Aglycone of Dubioside A (**3** aglycone), Kalopanax-Saponin La [**4**], and Collinsogenin [**5**] (in pyridine-d_5).

Carbon	Compound				
	1	**2**	**3** aglycone[a]	**4**[b]	**5**
		Aglycone			
C-1	38.7 t	38.9 t	38.2	38.8	38.9
C-2	26.1 t	26.2 t	24.9	26.1	27.8
C-3	81.8 d	81.9 d	82.2	81.9	73.6
C-4	43.5 s	43.5 s	55.1	42.6	42.9
C-5	47.5 d	47.3 d	48.5	47.6	48.8
C-6	18.1 t	18.2 t	20.7	18.2	18.7
C-7	33.16[c] t	33.2 t	32.8	32.8	33.4
C-8	39.7 s	40.0 s	40.3	40.0	40.0
C-9	48.1 d	47.7 d	47.1	48.2	47.4
C-10	36.9 s	37.0 s	36.3	36.9	37.3
C-11	23.8[d] t	23.9 t	23.8	23.9	23.9
C-12	122.5 d	122.5 d	122.4	122.9	122.5
C-13	144.7 s	145.1 s	144.6	144.2	145.2
C-14	42.1 s	42.1 s	42.1	42.6	42.2
C-15	28.3 t	36.2 t	36.1[c]	28.0	36.2
C-16	23.6[d] t	74.8 d	73.9	16.9	74.8
C-17	46.6 s	48.8 s	49.6	53.1	48.9
C-18	41.9 d	42.4 d	41.3	43.5	41.5
C-19	46.3 t	47.2 t	47.2	46.0	47.3
C-20	30.9 s	31.1 s	30.9	31.5	31.1
C-21	34.1 t	36.2 t	36.0[c]	43.3	36.2
C-22	32.8[c] t	32.9 t	32.1	71.5	32.9
C-23	67.0 t	67.1 t	209.3	64.5	68.1
C-24	13.6 q	13.7 q	10.9	13.6	13.2
C-25	16.1 q	16.2 q	15.8	16.1	16.1
C-26	17.4 q	17.6 q	17.5	17.4	17.6
C-27	26.1 q	27.2 q	27.2	26.7	27.2
C-28	180.1 s	180.0 s	175.8	179.4	180.0
C-29	33.21 q	33.4 q	33.3	33.4	33.4
C-30	23.7 q	24.7 q	24.8	25.2	24.8
		Sugar			
C-1′	106.7 d	106.8 d		106.6	
C-2′	73.1 d	73.1 d		73.16	
C-3′	74.7 d	74.8 d		74.7	
C-4′	69.6 d	69.7 d		69.6	
C-5′	64.4 t	64.9 t		66.9	

[a]Data for this compound are from Nagao *et al.* (15).

[b]Data for this compound are from Shao *et al.* (7).

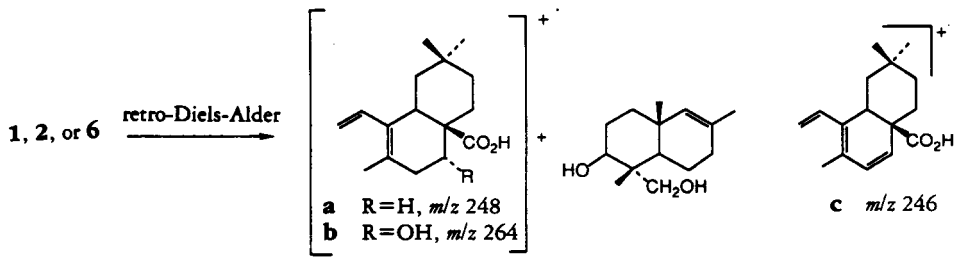
[c,d]The assignments in any vertical column may be interchanged.

COSY and the hetero COSY (HETCOR) spectra allowed correlation of the ^{13}C and ^{1}H chemical shifts as listed in Table 2. Comparison (ir, mixture mp, tlc) of compound **1** isolated by us with an authentic sample of akeboside St_b (3) showed them to be identical. The ^{13}C-nmr δ values and the physical constants of the two compounds indicated their identity.

TABLE 2. ^{1}H- and ^{13}C-nmr Chemical Shift Assignments of Compound **1** (in pyridine-d_5).

Carbon	(ppm)	HETCOR Correlations	(ppm)	Multiplicity (Hz)	Shows Coupling with (COSY)
C-3	81.8 d	H-3	4.33	m	
C-12	122.5 d	H-12	5.49	t $J=3$	
C-18	41.9 d	H-18_β	3.30	d,d $J=14,4$	
C-23	67.0 t	H_a-23	3.73	d,d $J=12$	
		H_b-23	4.30	d,d $J=12$	H_a-23
C-1′	106.7 d	H-1′	5.05	d $J=7$	H-2′
C-2′	73.1 d	H-2′	4.45	d,d $J=9,7$	H-3′
C-3′	74.7 d	H-3′	4.08	d,d $J=9,3$	
C-4′	69.6 d	H-4′	4.27	m	H-3′
C-5′	64.4 t	H_a-5′	3.73	d,d $J=11,4$	
		H_b-5′	4.31	d,d $J=11,3$	
C-24	13.6 q	CH_3	0.93	s	
C-25	16.1 q	CH_3	0.95	s	
C-26	17.4 q	CH_3	1.05	s	
C-27	26.1 q	CH_3	1.24	s	
C-29	33.2 q	CH_3	0.95	s	
C-30	23.7 q	CH_3	1.02	s	

The new saponin, designated as collinsonin [**2**], crystallized from aqueous EtOH to afford colorless needles, mp 266–267°; $[\alpha]_D$ +26.5°. The fabms revealed *m/z* 1240 $[2M]^+$, $[M+Na]^+$ at 643, $[M+H]^+$ at 621, and $[M-H]^+$ at 619. Other important ions were observed at *m/z* 471 $[M+H-ara-H_2O]^+$, 453 $[M+H-ara-2H_2O]^+$, 435 $[M+H-ara-3H_2O]^+$, 425 $[M-ara-COOH-H_2O]^+$, 407 $[M-ara-COOH-2H_2O]^+$, 397, 341, 289, 273, 264, and 246. In the presence of KCl, *m/z* 659 $[M+K]^+$, a very strong ion, was observed (11,12). The fragment ion which is characteristic of the retro-Diels-Alder cleavage of olean-12-en-28-oic acid derivatives (13,14) was observed at *m/z* 248 (fragment **a**) in **1** and at *m/z* 264 in collinsonin [**2**] (fragment **b**) as shown below. The fragment at *m/z* 264 turned out to be less stable and the hrms was done on a fragment **c**, *m/z* 246 (calculated accurate mass 246.1619), which showed a measured mass of 246.1624 (Scheme 1). The results of the ms and the elemental analysis were consistent with the molecular formula $C_{35}H_{56}O_9$ (mol wt 620).



SCHEME 1

The ^{1}H-nmr spectrum of collinsonin showed signals for six tertiary methyl groups at δ 0.95, 0.99, 1.05, 1.08, 1.19, and 1.81, one trisubstituted olefinic proton at δ 5.66 t, J = 3 Hz, two protons of a hydroxymethyl group at δ 3.72 d, J = 12 Hz and at δ 4.30 m, one proton attached to a hydroxyl group at δ 5.26 br s, and one anomeric proton at δ 5.00 d, J = 7 Hz. The ^{13}C-nmr spectrum of collinsonin (Table 1) revealed signals of six C-C bonded quaternary carbons (δ 31.1, 37.0, 40.0, 42.1, 43.5, and 48.8), one carboxylic acid carbon (δ 180.0), a pair of olefinic carbons (δ 122.5 and 145.1), a secondary carbon attached to a hydroxyl group (δ 81.9), a methylene carbon attached to a hydroxyl group (δ 64.5), and one anomeric carbon (δ 106.8). The presence of an additional secondary hydroxyl group was indicated by a carbon signal at δ 74.8 and a proton signal at δ 5.26 (1H, br s). Selective INEPT experiments were carried out by irradiation of the proton at δ 5.26 assigned to H-16. This showed a strong three-bond polarization transfer to the signal at δ 42.1 which has been assigned to C-14 and a medium polarization transfer to the carbon signal at δ 48.8 assigned to C-17. These results confirm the position of the secondary hydroxyl group at C-16. In addition, the methyl signal in the ^{1}H-nmr spectrum also supports the location of the hydroxyl group at C-16. One of the methyl signals shifts downfield to δ 1.81 owing to 1,3-diaxial interaction to the hydroxyl group at C-16α, and this has been assigned to CH_3-27. The ^{13}C-nmr signals of the aglycone are very close to those of the aglycone (quillaic acid) in dubioside A (15,16) [**3**] and kalopanax-saponin La (3-*O*-α-L-arabinopyranosyl-22α-hydroxyhederagenin) [**4**] (7) except for the following signals. The ^{13}C-nmr signals of the aglycone of dubioside A show upfield shifts for C-14 (55.1 ppm) and C-6 (20.7 ppm) as against 43.5 ppm and 18.2 ppm in **2** and 42.6 ppm and 18.2 ppm in **4**, respectively, due to the aldehyde group at C-4 in **3** aglycone. The signal for C-15 in **2** (36.2 ppm) and **3** aglycone (36.1 ppm) appear upfield by about 4 ppm due to the presence of the hydroxyl group at C-16, when compared with the signal at 28.0 ppm in **4** which lacks the hydroxyl group. In **2** and **3** aglycone, C-16 appears at 74.8 ppm and 73.9 ppm, respectively, due to a hydroxyl group at this position. Similarly in kalopanax La [**4**], the signal for C-22 appears at 71.5 ppm due to the hydroxyl group at C-22, whereas this methylene appears at 32.9 ppm and 32.1 ppm, respectively, in **2** and **3** aglycone. In **3** aglycone, the signal for C-23 at 209.3 ppm is due to the aldehyde function (Table 1).

The ^{13}C-nmr signals for the sugar part of the molecule are very similar to those of arabinose, as in the spectrum of the saponin **1**. The carbon-proton connectivities (Table 3) were based on the HETCOR spectrum. On the basis of the above data, structure **2** was assigned to collinsonin. The only difference between the aglycone in **2** and quillaic acid is that the functional group at C-23 is a primary alcohol in **2**, whereas it is an aldehydic group in quillaic acid. The aglycone of the new saponin, designated collinsogenin, is a new triterpene. Alkaline hydrolysis of **2** afforded collinsogenin [**5**]. The ^{13}C-spectral assignments of **5** are given in Table 1.

The second new saponin, named collinsonidin, was obtained as colorless crystals, mp 250–252°; [α]D +55.6°. Fabms revealed an $[M + Na]^+$ ion at *m/z* 789, indicating a mol wt of 766. Other useful ions were observed at *m/z* 767 $[M + H]^+$, 605 $[M + H - glc\ (C_6H_{11}O_5)]^+$, 472 $[M + H - glc - ara\ (C_{11}H_{19}O_9)]^+$, 471 $[M - glc - ara]^+$, 455 $[M + H - glc - ara - OH]^+$. Ions were also observed at *m/z* 437, 409, 391, 368, 355, 341, 327, 295 (glc-ara), 248, 203, 201, and 189. The retro-Diels-Alder fragment **a**, discussed earlier, appeared at *m/z* 248. The hrms for the ion *m/z* 248 indicated a measured mass of 248.1776 (calcd accurate mass 248.1775). In the sims negative mode, ions were observed at *m/z* 765 $[M - H]^-$, 603 $[M - H - glc\ (C_6H_{10}O_5)]^-$, 585 $[M - H - glc - H_2O]^-$, 471 $[M - H - glc - ara\ (C_{11}H_{18}O_9)]^-$, 453 $[M - H - ara - glc - H_2O]^-$, 423 $[M - H - glc - ara - H_2O - CH_2O]^-$, and 247 [retro-Diels-Alder product]$^-$. The ms and elemental analyses suggested the molecular formula

TABLE 3. ^{1}H- and ^{13}C-nmr Chemical Shift Assignments of Collinsonin [2] (in pyridine-d_5).

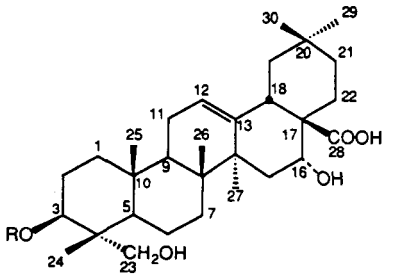
Carbon	(ppm)	HETCOR Correlations	(ppm)	Multiplicity (Hz)	Shows Coupling with (COSY)
C-1	38.9	H-1	1.32		
C-3	81.8 d	H-3	4.33	m	
C-9	47.7	H-9	1.75		
C-12	122.5 d	H-12	5.66	t J = 3	
C-16	74.8 d	H-16	5.26	br s	
C-18	41.4 d	H-18β	3.64	d,t J = 14, 1.5, 1.5	
		H_a-19	2.84	t J = 13	
C-23	64.5 t	H_a-23	3.72	d,d J = 12	
		H_b-23	4.33	d,d J = 12	
C-16		(OH)	6.38	d J = 3	H-16
C-1′	106.8 d	H-1′	5.00	d J = 7	H-2′
C-2′	73.2 d	H-2′	4.46	d,d J = 9, 7	H-3′
C-3′	74.8 d	H-3′	4.08	d,d J = 9, 3	
C-4′	69.7 d	H-4′	4.26	m	H-3′
C-5′	67.1 t	H_a-5′	3.74	d,d J = 11, 2	
C-5′	67.1 t	H_b-5′	4.30	d,d J = 11, 3	
C-24	13.7 q	CH_3	0.95	s	
C-25	16.2 q	CH_3	0.99	s	
C-26	17.6 q	CH_3	1.08	s	
C-27	27.2 q	CH_3	1.81	s	
C-29	33.4 q	CH_3	1.05	s	
C-30	24.7 q	CH_3	1.19	s	

$C_{41}H_{66}O_{13}$ for collinsonidin. The ^{1}H (Table 4) and the ^{13}C (Table 5) nmr spectra showed signals for six quaternary methyls: ^{1}H-nmr signals at δ 0.94 (6H, 2 × Me), 1.01, 1.03, 1.04, and 1.22 (each 3H, s) and ^{13}C-nmr signals at 33.3, 26.2, 23.7, 17.5, 16.1, and 13.5 ppm. The spectra also exhibited signals due to the following groups at positions similar to those of hederagenin: a carboxylic acid group (δ 180.2), a trisubstituted double bond (δ 122.6 and 144.8, a proton signal at δ 5.48 t), a primary alcoholic group (δ 64.9, proton signals at δ 3.77 and 4.25 each (1H, d, J = 11 Hz) and a secondary alcoholic hydroxyl group (δ 81.5, proton signal at δ 4.20, m). The one-proton signal due to H-18β, characteristic of olean-12-en-28-oic acid type triterpenes (7,10) was observed at δ 3.30 (1H, d, d, J = 14.0, 4.0 Hz). Comparison of the ^{13}C-nmr spectrum of collinsonidin with that of the saponin **1** and collinsonin [**2**] demonstrated the same glycosylation shift around C-3 in both these saponins (Tables 1 and 5). The anomeric carbons at δ 106.0 and 104.0 in collinsonidin indicated the presence of two sugar units. Observation of a fragment ion at m/z 605 $[M + H - glc]^+$ in the positive fabms of collinsonidin indicated the presence of a terminal glucose unit. Acid hydrolysis of collinsonidin gave hederagenin. On the basis of the above data, structure **6**, 3-*O*-β-D-glucopyranosyl-(1″↦3′)-α-L-arabinopyranosylhederagenin, has been proposed for collinsonidin. When the ^{13}C-nmr signals of **6**, **7**, and **8** are compared (in pyridine-d_5), an upfield shift of about 8 to 10 ppm is observed for the carbons (C-3′, C-4′, and C-2′, respectively, to which the C-1″ of glucose is attached. The other ^{13}C signals due to the sugar moiety of **6** were in positions similar to those of α-fatsin [**7**], 3-*O*- β-D-glucopyranosyl-(1″↦4′)-α-L-arabinopyranosylhederagenin (4), and Pf (seed saponin C) [**8**], 3-*O*-β-D-glucopyranosyl-(1″↦2″)-α-L-arabinopyranosylhederagenin (5).

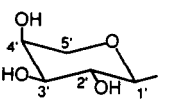
In order to assign unambiguously the carbon signals at δ 81.5 and 82.2, selective INEPT experiments were carried out. Irradiation of the anomeric proton at δ 5.18 and

TABLE 4. ^{1}H-nmr Chemical Shift Assignments of Collinsonidin [**6**].

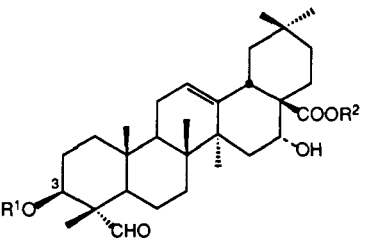
Proton	C_5D_5N (ppm)	Multiplicity (Hz)	DMSO + CD_3OD (ppm)	Multiplicity (Hz)
H_a-2, H_b-2			1.46, 1.67	m
H-3	4.20	m	3.49	m
H_b-11	2.00	d,d J = 10, 3		
H_a-11	1.82	d,d J = 10, 3		
H-12	5.48	t, J = 3	5.18	t, J = 3
H-18β	3.30	d,d J = 14, 4	2.74	d,d J = 13, 4
H_a-19, H_b-19			1.06, 1.64	d,d
H-23	3.77, 4.25	d,d $J_{a,b}$ = 11		
	1.60	t, J = 12, 12		
	1.56	d,t J = 12, 3, 3		
H-1′	5.18	d, J = 6	4.34	d J = 8
H-2′	4.59	d,d J = 7, 6	2.98	t J = 8, 8 (coupled to OH at 5.38 d)
H-3′	4.26	m	3.14	m
H-4′	4.24	m	3.08	m
H_a-5′	3.71	d,d J = 11, 1	3.47, 3.66 ($5'_a$, $5'_b$)	m
H_b-5′	4.29	d,d J = 11, ~2		
H-1″	5.20	d, J = 7	4.46	d J = 5
H-2″	4.10	d,d J = 9, 7		
H-3″	4.18	t J = 9, 9		
H-4″	4.26	m		
H-5″	3.83	m		
H_a-6″	4.39	d,d J = 12, 4		
H_b-6″	4.48	d,d J = 12, 3		
CH_3	1.22	s	1.11	s
CH_3	1.04	s	0.89	s
CH_3	1.03	s	0.88 × 2	s
CH_3	1.01	s	0.72	s
2 × CH_3	0.94	s		
OH(sugars)			4.61 d, 4.92 d, 4.98 d, 4.90	
23-OH			4.37 d,d	



2 R=



5 R=H



3 R^1=β-D-galactopyranosyl-(1″↦2′)-β-D-glucopyranosyl
R^2=α-L-rhamnopyranosyl-(1″↦2′)-α-L-arabinopyranosyl

TABLE 5. ^{13}C-nmr Spectra of Collinsonidin [**6**], α-Fatsin [**7**] and Pf (seed saponin C) [**8**].

Carbon	Aglycone				Carbon	Sugar			
	6		**7**[a]	**8**[b]		**6**		**7**[a]	**8**[b]
	DMSO-d_6	Pyridine-d_5	Pyridine-d_5	Pyridine-d_5		DMSO-d_6	Pyridine-d_5	Pyridine-d_5	Pyridine-d_5
C-1	37.8 t	38.7 t	38.8	38.8	C-1′	103.6 d	106.0 d	106.7	103.7
C-2	25.0 t	26.0 t	26.0	26.0					
C-3	78.7 d	81.5 d	82.2	82.2	C-2′	71.1 d	73.7 d	73.6	81.0
C-4	42.2 s	43.5 s	43.4	43.4					
C-5	46.0 d	48.1 d	47.7	47.7	C-3′	79.8 d	82.2 d	74.6	73.4
C-6	17.1 t	18.2 t	18.2	18.2					
C-7	32.0 t	32.9[c] t	32.9[c]	32.9[c]	C-4′	66.2 d	68.3 d	79.7	68.1
C-8	38.7 s	39.7 s	39.8	39.8					
C-9	47.0 d	47.9 d	48.2	48.2	C-5′	62.7 t	65.1 t	66.2	64.7
C-10	35.9 s	36.9 s	37.0	37.0					
C-11	22.5 t	23.7[d] t	23.7[d]	23.7[d]	C-1″	102.3 d	104.0 d	106.3	105.6
C-12	121.4 d	122.6 d	122.6	122.6					
C-13	143.7 s	144.8 s	144.8	144.8	C-2″	74.4 d	76.3 d	75.7	76.0
C-14	41.3 s	42.1 s	42.2	42.2					
C-15	27.1 t	28.3 t	28.3	28.3	C-3″	76.7[c] d	78.3[c] s	78.6[c]	78.1
C-16	22.8 t	23.9[d] t	23.8[d]	23.8[d]					
C-17	45.3 s	46.6 s	46.5	46.5	C-4″	69.7 d	71.3 d	71.4	71.4
C-18	40.7 d	42.0 d	42.0	42.0					
C-19	45.6 t	46.4 t	46.7	46.7	C-5″	76.2[c] d	78.2[c] d	78.3[c]	78.1
C-20	30.3 s	31.0 s	30.9	30.9					
C-21	33.2 t	34.2 t	34.3	34.3	C-6″	60.8 t	62.5 t	62.6	62.5
C-22	31.8 t	33.3[c] t	33.3	33.3					
C-23	63.0 t	64.9 t	64.7	64.7					
C-24	12.6 q	13.5 q	13.5	13.5					
C-25	15.4 q	16.0 q	16.1	16.1					
C-26	16.8 q	17.4 q	17.5	17.5					
C-27	25.5 q	26.1 q	26.2	26.2					
C-28	178.4 s	180.2 s	180.1	180.1					
C-29	32.7 q	33.3 q	33.3[c]	33.3[c]					
C-30	23.3 q	23.8[d] q	23.7[d]	23.7					

[a]Data for this compound are from Tanemura and Takamura (4) and Li *et al.* (8).

[b]Data for this compound are from Higuchi and Kawasaki (5) and Li *et al.* (8).

[c,d]The assignments in any vertical column may be interchanged.

5.20 (in pyridine-d_5) did not distinguish between the carbons appearing at 81.5 and 82.2 ppm. Therefore, the ^1H and ^{13}C carbon spectra were determined in DMSO-d_6 (Tables 4 and 5). Irradiation at δ 4.45 (anomeric proton of glucose) relaxed the carbon signal at δ 79.8, and irradiation of the proton at δ 4.33 (anomeric proton of arabinose) relaxed the signal at δ 78.7. The upfield carbon signal at 78.7 ppm was therefore assigned to C-3 and the downfield carbon appearing at 79.9 was assigned to C-3′. It is of interest to note that none of the saponins and sapogenins listed in the reviews (17–21) have been isolated from plants belonging to the Labiatae family.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fischer-Kopfler hot stage apparatus fitted with a microscope and polarizer. Ir spectra were recorded with a Perkin-Elmer model 1421 spectrophotometer and specific rotations were measured on a Perkin-Elmer model 141 polarimeter. ^1H- and ^{13}C-nmr spectra were recorded on Varian XL 300 (300 MHz for ^1H, and 75.5 MHz for ^{13}C) and Varian XL 400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometers. Chemical shift data are given in ppm downfield from TMS. Fabms was obtained on a VG-ZAB SE double focusing mass spectrometer, operating at an acceleration voltage of 8 kV. The sample dissolved in DMSO was deposited on a copper probe tip and then mixed with a thin layer of *m*-nitrobenzyl alcohol or glycerol/thioglycerol. The sample was ionized by bombardment with Xenon atoms produced by a saddle field ion source from Ion Tech. Corporation, Teddington, UK, operating with tube current of 2 mA at an energy of 8 keV. The temperature of the source was maintained at room temperature. Separations were carried out by vlc (22) on Si gel, Merck 60H.

PLANT MATERIAL.—Roots of *C. canadensis* were identified, collected and supplied by Wilcox Drug Co., Boone, North Carolina. An authenticated voucher specimen of this plant is deposited in the offices of the Wilcox Drug Co., Boone, North Carolina.

ISOLATION OF THE SAPONINS.—The powdered roots of *C. canadensis* (240 g) were extracted in a Soxhlet apparatus with hexane (2 liters) to afford a hexane extract (3.1 g). The roots were then extracted with 95% EtOH (2 liters × 2) and the extract evaporated in vacuo to give a residue (57.8 g).

Part of the 95% EtOH extract (12.5 g) was dissolved in EtOH (50 ml) and stirred with Si gel and the EtOH removed in vacuo. The dried material was loaded on a vlc column and eluted with a gradient of $CHCl_3$ and increasing percentages of MeOH. Fractions (each 100 ml) were collected: fractions 1–3, $CHCl_3$; 4–9, $CHCl_3$-MeOH (92:8); 10, $CHCl_3$-MeOH (90:10); 11, $CHCl_3$-MeOH (90:10); 12–14 $CHCl_3$-MeOH (88:12); 15, $CHCl_3$-MeOH (85:15); 16, $CHCl_3$-MeOH (82:18); 17–20, $CHCl_3$-MeOH (80:20).

Evaporation of fraction 10 gave a solid (600 mg) which was crystallized twice from EtOH and a few drops of H_2O to afford colorless needles of akeboside St_b [**1**]: mp 233–234°, $[\alpha]_D$ +53.2° ($c = 0.5$, MeOH); ir (Nujol) ν max 3380, 1680, 1460, 1375, 1300, 1270, 1235, 1210, 1170, 1135, 1080, 1050, 1000, 950, 915, 870, 780 cm^{-1}; fabms *m/z* $[M + Na]^+$ 627 (604 + 23); $[M + K]^+$ 643 (604 + 39). Other ions appeared at *m/z* 604 $[M]^+$, 455 $[M + H - ara - H_2O]^+$, 437 $(M + H - ara - 2H_2O]^+$, 409 $[M - ara - COOH - H_2O]^+$, 423, 379, 355, 285, 248, 227. Found C 68.53, H 9.37; calcd for $C_{35}H_{56}O_8 \cdot 1/2\ H_2O$, C 68.50, H 9.30%. The saponin **1** was found to be identical in its tlc (Si gel, $CHCl_3$/20% MeOH, iodine vapors, R_f 0.5), mp, and ir spectral comparison with akeboside St_b (3).

Fractions 12–14 on evaporation in vacuo gave a residue (1.5 g) which was rechromatographed on vlc on Si gel and eluted with $CHCl_3$ containing increasing percentages of MeOH. Fractions 10–15 obtained by elution with $CHCl_3$-MeOH (93:7) gave a solid (200 mg) which was crystallized thrice from EtOH containing a few drops of H_2O. The saponin collinsonin [**2**] was obtained as colorless needles (95 mg): mp 266–267°, $[\alpha]_D$ +26.5° ($c = 0.48$, MeOH); ir (Nujol) ν max 3510, 3360, 1720, 1690, 1460, 1375, 1300, 1250, 1210, 1165, 1130, 1110, 1075, 1060, 1040, 1000, 940, 910, 850, 790 cm^{-1}. Found C 65.82, H 9.09; $C_{35}H_{56}O_9 \cdot H_2O$ requires C 65.83, H 9.09%.

Evaporation of fraction 16 gave 1.2 g of a solid which was crystallized twice from EtOH to afford collinsonidin [**6**] (100 mg) as colorless needles: mp 250–252°; $[\alpha]_D$ +55.6° [$c = 0.36$, EtOH·H_2O (4.5 ml:1.5 ml)]; ir (Nujol) ν max 3660, 3500, 3420, 3300, 1690, 1620, 1460, 1375, 1300, 1265, 1240, 1210, 1200, 1170, 1130, 1090, 1060, 1030, 1000, 785 cm^{-1}. Found C 63.92, H 8.74; $C_{41}H_{66}O_{13}$ requires C 64.22, H 8.62%.

HYDROLYSIS OF COLLINSONIN [**2**] AND COLLINSONIDIN [**6**].—Collinsonin (40 mg) was heated on a steam bath with 2 M H_2SO_4 (1.25 ml), and EtOH (1.25 ml) for 3 h. When cooled, a crystalline solid separated. This was collected, washed with 70% EtOH (1 ml × 3) and crystallized from EtOH to afford

colorless needles of collinsogenin [**5**] (15 mg): mp 313–314° (dec); ir (Nujol) ν max 3580, 3442, 3280, 1700, 1470, 1455, 1380, 1330, 1300, 1265, 1250, 1240, 1210, 1180, 1170, 1150, 1100, 1080, 1060, 1040, 1018, 1008, 990, 980, 960, 940, 915, 880, 852, 830, 810, 790 cm^{-1}; ms *m/z* (%) $[M]^+$ 488 (0.5), $[M+-H_2O]^+$ 470 (1), 264 ($C_{16}H_{24}O_3$; **b**, 8.5), 246 (**b** − H_2O, **c**, 31), 219 (264 − COOH, 6), 201 (219 − H_2O, 28), 42 (100).

Similarly, hydrolysis of collinsonidin (50 mg) gave hederagenin, identical in its tlc, ir and mass spectral comparison with an authentic sample.

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