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COUMARINS FROM *MUSINEON DIVARICATUM**†

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Key Word Index—*Musineon divaricatum*; Umbelliferae; coumarins; isolation; HPLC; identification.

Abstract—A chemical investigation of *Musineon divaricatum* has resulted in the isolation of 19 coumarins, five of which are novel compounds; 18 are khellactone derivatives. The coumarins were isolated from the crude extracts by a combination of adsorption chromatography, gel permeation and HPLC; the more successful HPLC separations utilized a nitrile bonded phase column. The structures were determined by ^{1}H NMR and mass spectral studies and by comparisons with literature data. The relative configuration for the entire series was secured from ^{1}H NMR data, while the absolute configuration could be assigned with any certainty only in the cases of two of the compounds.

INTRODUCTION

The Crow nation utilized roots of the umbelliferous plants *Musineon hookeri* [1] and *M. divaricatum* [2] as foodstuffs. There is now indirect evidence suggesting that *M. divaricatum* may have been an important food source to semi-nomadic peoples in Montana for thousands of years. Archaeological excavations of tipi rings in a small valley in the Limestone Hills southwest of Townsend by Davis *et al.* [3] indicate periodic seasonal occupation of the site by archaeologically distinct cultures for nearly 3000 years. Not only does this site offer fresh water and plentiful game, but the eastern slopes of the valley also support a dense, stable population of *M. divaricatum*.

Noting a dearth of literature on the chemistry of *M. divaricatum* and sensing its possible archaeological and cultural significance, we undertook an examination of this plant. Described herein is the coumarin profile of *M. divaricatum* Rafin.

RESULTS

Isolation

Florisil chromatography of the organic soluble extracts of *M. divaricatum* yielded several large fractions, all eluted with a hexane–ethyl acetate gradient which, as revealed by NMR analysis, contained significant quantities of aromatic compounds. These mixtures proved to be quite complex; application of standard adsorption and gel permeation chromatographic methods led initially to only one pure compound, isosamidin (7) [4]. Three other compounds, **10**, **18** and **19**, were ultimately purified without HPLC.

Both flash and HPLC reversed-phase chromatography yielded a few additional compounds, but the poor solubility of the coumarins in the mobile phase precluded effective scale-up of what appeared to be very credible analytical separations. The solution to this separation problem lay in the use of a nitrile bonded phase HPLC column with hexane–isopropanol solvent combinations. With this system, additional coumarins could be separated from the complex mixtures. Numerous small fractions from the many experimental chromatographies were found to be related mixtures of coumarins. These fractions were combined and chromatographed on silica gel with a shallow hexane–diethyl ether gradient. Application of the aforementioned HPLC techniques afforded still more coumarins. In all, 19 coumarins were obtained from the extracts; five of these compounds had novel structures. Figures 1 and 2 provide a graphical depiction of the isolation of the coumarins described in this report and the quantities of **1**–**19** which were obtained.

Structure elucidation

The ^{1}H NMR, ^{13}C NMR and mass spectral data for the coumarins are compiled in Tables 1–4, respectively. A typical mass spectral fragmentation pattern of the khellactone coumarins is illustrated in Fig. 3.

The first compound to be identified was anomalin (**1**) [5], mp 171–173° (lit. [5] 173–174°), the most abundant and readily crystallized of the series of coumarins we encountered. UV absorption maxima at 340, 274 and 256 nm suggested a coumarin chromophore, while a fourth maximum at 219 nm indicated an additional conjugated carbonyl system. Inspection of the low-field signals in the ^{1}H NMR spectrum led to a coumarin of the khellactone (**18**) diester type; signals for three AB systems were present below δ 5.0. Two represented the four protons on the coumarin nucleus (δ 7.56 and 6.19, J = 9.5 Hz, and δ 7.33 and 6.78, J = 8.6 Hz), and the other a pair of vicinal sp^3 methines (δ 6.67 and 5.42, J = 4.7 Hz).

The ^{13}C NMR spectrum of **1** corroborated these assignments, exhibiting signals for eight aromatic carbons

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†Dedicated to the memory of Joy Yellowtail Toineeta, chronicler of Crow enthobotany.

‡B. L. Johnson Undergraduate Scholar, 1982–83; American Institute of Chemists Award, 1983.

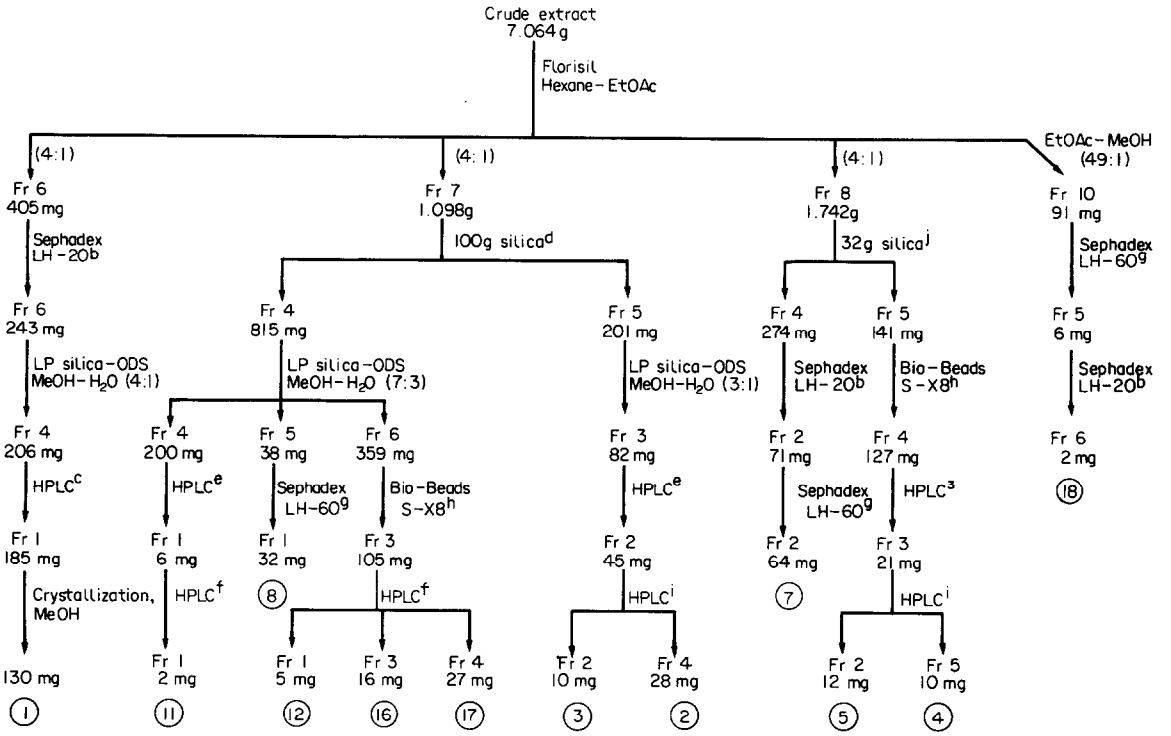


Fig. 1. Initial separation scheme for isolation of coumarins from *M. divaricatum*. [b]$CHCl_2$–MeOH (1:1); [c]Ultrasphere-ODS, MeOH–H_2O (4:1); [d]Hexane–Me_2CO (17:3); [e]Ultrasphere-ODS, MeOH–H_2O (7:3); [f]Ultrasphere-Cyano, hexane–i-PrOH (7:1); [g]CH_2Cl_2–MeCN (3:1); [h]CH_2Cl_2–cyclohexane (3:2); [i]Ultrasphere-Cyano, hexane–i-PrOH (5:1); [j]hexane–Et_2O (1:4).

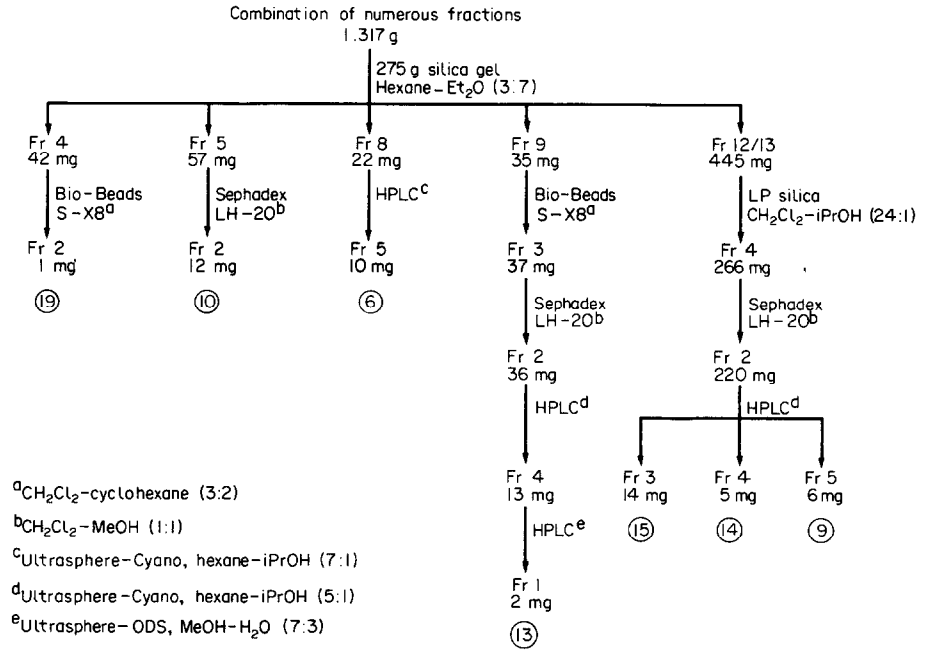


Fig. 2. Secondary separation scheme for isolation of coumarins from *M. divaricatum*.

Table 1. ^{1}H NMR data for the coumarins of *M. divaricatum* (250 MHz, $CDCl_3$, TMS as internal standard)

Compound	H-3	H-4	H-5	H-6	H-5′/H-6′	H-3′	H-4′	R^1	R^2
1	6.19	7.56	7.33	6.78	1.46/1.43	5.42	6.67	6.09 (1H, *br q*, J = 7.3)	6.00 (1H, *br q*, J = 7.3)
	(9.6)*	(9.5)	(8.6)	(8.6)		(4.7)	(4.7)	1.95 (3H, *dq*, J = 7.3, 2.5)	1.95 (3H, *dq*, J = 7.3, 2.5)
								1.83 (3H, *br q*, J = 2.5)	1.80 (3H, *br q*, J = 2.5)
2	6.19	7.57	7.33	6.78	1.46/1.41	4.06 (*br d*)	6.46	6.08 (1H, *br q*, J = 7.2)	2.84 (1H, *br s*)
	(9.7)	(9.7)	(8.8)	(8.8)		(4.7)	(4.7)	1.99 (3H, *dq*, J = 7.2, 2.5)	
								1.86 (3H, *br q*, J = 2.5)	
3	6.23	7.62	7.32	6.77	1.49/1.41	5.45	5.20 (*br d*)	3.17 (1H, *br s*)	6.14 (1H, *br q*, J = 7.3)
	(9.5)	(9.5)	(8.6)	(8.6)		(4.7)	(4.7)		1.97 (3H, *dq*, J = 7.3, 2.5)
									1.92 (3H, *br q*, J = 2.5)
4	6.19	7.57	7.32	6.78	1.44/1.42	4.02 (*dd*)	6.42	5.70 (1H, *br s*)	2.96 (1H, *d*, J = 2.6)
	(9.5)	(9.5)	(8.6)	(8.6)		(4.7, 2.6)	(4.7)	2.22, 1.89 (each 3H, *br s*)	
5	6.21	7.61	7.30	6.75	1.46/1.37	5.17	5.39 (*br d*)	3.37 (1H, *br s*)	5.76 (1H, *br s*)
	(9.3)	(9.3)	(8.6)	(8.6)		(4.6)	(4.6)		2.17, 1.88 (each 3H, *br s*)
6	6.20	7.57	7.34	6.78	1.43/1.41	5.33	6.60	6.02 (1H, *br q*, J = 7.5)	2.07 (3H, *s*)
	(9.5)	(9.5)	(8.7)	(8.7)		(5.0)	(5.0)	1.98 (3H, *dq*, J = 7.5, 2.5)	
								1.84 (3H, *br q*, J = 2.5)	
7	6.18	7.56	7.32	6.76	1.43/1.39	5.27	6.55	5.61 (1H, *br s*)	
	(9.7)	(9.7)	(8.6)	(8.6)		(4.8)	(4.8)	2.20, 1.87 (each 3H, *br s*)	2.06 (3H, *s*)
8	6.21	7.57	7.33	6.77	1.43/1.40	5.28	6.52	2.40–2.08 (3H, *m*)	2.08 (3H, *s*)
	(9.3)	(9.3)	(8.7)	(8.7)		(4.9)	(4.9)	0.97 (2 overlapping 3H doublets)	
9	6.21	7.58	7.33	6.77	1.45/1.40	4.02 (*dd*)	6.40	2.44–2.09 (3H, *m*)	2.74 (1H, *d*, J = 2.4)
	(9.5)	(9.5)	(8.6)	(8.6)		(4.9, 2.4)	(4.9)	0.97 (2 overlapping 3H doublets)	
10	6.20	7.56	7.32	6.77	1.45/1.42	5.37	6.60	2.40–2.05 (3H, *m*)	6.10 (1H, *br q*, J = 8.7)
	(9.6)	(9.6)	(8.7)	(8.7)		(4.3)	(4.3)	0.93 (2 overlapping 3H doublets)	1.95 (3H, *dq*, J = 8.7, 2.6)
									1.84 (3H, *br q*, J = 2.6)
11	6.22	7.62	7.33	6.77	1.46/1.39	5.11	5.41 (*dd*)	3.01 (1H, *d*, J = 4.0)	2.66 (1H, *hept.* J = 6.9)
	(9.6)	(9.6)	(8.6)	(8.6)		(4.7)	(4.7, 4.0)		1.23, 1.22 (each 3H, *d*, J = 6.9)
12	6.20	7.57	7.33	6.78	1.45/1.42	5.40	6.57	2.55 (1H, *hept.*, J = 7.1)	6.10 (1H, *br q*, J = 8.7)
	(9.5)	(9.5)	(8.6)	(8.6)		(4.9)	(4.9)	1.18, 1.15 (each 3H, *d*, J = 7.1)	1.95 (3H, *dq*, J = 8.7, 2.6)
									1.84 (3H, *br q*, J = 2.6)
13	6.21	7.57	7.34	6.78	1.42/1.39	5.30	6.51	2.59 (1H, *hetp.* J = 7.8)	2.06 (3H, *s*)
	(9.4)	(9.4)	(8.7)	(8.7)		(5.0)	(5.0)	1.21, 1.17 (each 3H, *d*, J = 7.8)	

Table 1. (*Continued*)

Compound	H-3	H-4	H-5	H-6	H-5′/H-6′	H-3′	H-4′	R^1	R^2
14	6.21 (9.5)	7.58 (9.5)	7.34 (8.7)	6.78 (8.7)	1.47/1.39	4.05 (*dd*) (4.9, 2.7)	6.37 (4.9)	2.45 (1H, *m*) 1.75 (2H, *m*) 1.18 (3H, *d*, $J = 7.3$) 0.95 (3H, *t*, $J = 7.6$)	2.78 (1H, *d*, $J = 2.7$)
15	6.22 (9.5)	7.61 (9.5)	7.32 (8.6)	6.77 (8.6)	1.46/1.38	5.18 (4.6)	5.40 (*dd*) (4.6, 4.4)	2.16 (1H, *d*, $J = 4.4$)	6.91 (1H, *qq*, $J = 7, 1.5$) 1.86 (3H, *d*, $J = 1.5$) 1.78 (3H, *d*, $J = 7$)
16	6.19 (9.5)	7.56 (9.5)	7.33 (8.7)	6.78 (8.7)	1.44/1.41	5.37 (4.8)	6.64 (4.8)	5.63 (1H, *br s*) 2.13, 1.86 (each 3H, *br s*)	5.98 (1H, *br q*, $J = 7.2$) 1.95 (3H, *dq*, $J = 7.2, 2.5$) 1.82 (3H, *br q*, $J = 2.5$)
17	6.18 (9.4)	7.56 (9.4)	7.32 (8.6)	6.77 (8.6)	1.46/1.42	5.37 (4.8)	6.62 (4.8)	6.08 (1H, *br q*, $J = 7.2$ 1.94 (3H, *dq*, $J = 7.2, 2.5$) 1.83 (3H, *br q*, $J = 2.5$)	5.58 (1H, *br s*) 2.15, 1.84 (each 3H, *br s*)
18	6.24 (9.5)	7.64 (9.5)	7.31 (8.6)	6.78 (8.6)	1.45/1.38	3.85 (*br d*) (3.3)	5.20 (*br d*) (3.3)	4.00 (1H, *br s*)	3.30 (1H, *br s*)
19	6.22 (9.5)	7.61 (9.5)	7.24 (8.5)	6.78 (8.5)	1.38/1.37	5.18 (*t*) (2.5)	3.24, 2.99 (each *dd*) (18.5, 2.5)	OR^1 replaced by H	6.07 (1H, *br q*, $J = 7.4$) 1.89 (3H, *dq*, $J = 7.4, 2.5$) 1.83 (3H, *br q*, $J = 2.5$)

*Coupling constants (J in Hz) are given in parentheses.

Table 2. ^{13}C NMR data for the khellactone ring carbons in the more abundant coumarins from *M. divaricatum* (62.83 MHz, $CDCl_3$, TMS as internal standard)

C	1	2	4	5	6	7	12
2	159.69	159.93	160.06	160.52	160.02	159.80	159.75
3	114.37	114.54	114.53	114.62	114.44	114.39	114.36
4	143.11	143.32	143.30	143.87	143.13	143.09	143.18
4a	107.73	107.23	107.36	110.79	107.50	107.67	107.54
5	129.16	129.28	129.21	128.76	129.25	129.13	129.11
6	113.35	112.98	113.08	112.65	113.39	113.38	113.30
7*	156.88	157.04	157.14	156.20	156.77	156.80	156.86
8	112.56	112.27	112.36	112.49	112.60	112.61	112.55
8a*	154.29	154.27	154.39	154.55	154.40	154.24	154.50
2′	78.20	78.56	78.76	77.78	78.70	77.21	78.18
3′	60.28	63.35	63.05	60.25	60.22	59.65	60.79
4′	70.26	71.56	71.73	71.52	70.61	70.78	69.99
5′/6′	25.34, 22.51	25.61, 20.81	25.50, 21.17	25.54, 22.50	25.30, 22.14	25.37, 22.19	24.97, 22.68

*May be interchanged.

Table 3. ^{13}C NMR data for the ester carbons in coumarins from *M. divaricatum* (62.83 MHz, $CDCl_3$, TMS as internal standard)

Compound	Carbonyls	Other
1	166.57, 166.37	139.63 *d*, 138.28 *d*, 127.56 *s*, 127.21 *s*, 20.22 (2C) *q*, 15.65 *q*, 15.45 *q*
2	169.11	138.83 *d*, 127.46 *s*, 20.31 *q*, 15.63 *q*
4	167.59	159.58 *s*, 115.07 *d*, 27.51 *q*, 20.51 *q*
5	165.62	159.06 *s*, 115.29 *d*, 27.43 *q*, 20.39 *q*
6	170.98, 167.10	137.72 *d*, 127.80 (2), 20.62 *q*, 20.29 *q*, 15.47 *q*
7	169.94, 165.29	159.80 *s*, 115.20 *d*, 27.31 *q*, 20.57 *q*, 20.30 *q*
12	175.65, 166.47	139.65 *d*, 127.11 *s*, 34.05 *d*, 29.63 *d*, 20.34 *q*, 18.70 (2C) *q*, 15.65 *q*

(four doublets and four singlets), along with three ester carbonyls, four olefinic carbons, six methyl groups, and two sp^3 methines and one quaternary carbon bearing heteroatoms. The quaternary carbon, two methyl singlets at δ1.46 and 1.43 and the considerable shielding of one of the aromatic protons (δ6.78) completed the ^{13}C NMR characterization of the khellactone skeleton (part structure **1a**).

Two ester groups accounted for the remaining signals; in the case of anomalin, two identical esters were present, but one set of signals was shifted to a slightly higher field than those of the second ester. Each olefinic proton was a quartet of quartets near δ6.0 and each exhibited vicinal coupling to one methyl group and allylic coupling to another. These characteristic signals, corroborated by associated ^{13}C NMR spectral data, indicated the presence of angelate esters, thus establishing the identity of **1** with anomalin [5].

Laserpitin (**2**) [6] and the known **3** [6], each differing from anomalin in that one of the angelate esters was replaced by a free hydroxyl group, were identified next. The structure elucidation was straightforward; molecular formulae were gleaned from mass spectral and ^{1}H NMR data, while characteristic shifts of the alcohol-bearing methines to higher field than their ester-bearing counterparts delineated the location of the hydroxyl group. In **3** the benzylic methine appeared at δ5.21 (cf. δ6.67 in **1** and 6.46 in **2**); on the other hand, the methine vicinal to the benzylic position in laserpitin (**2**) resonated at δ4.06 (cf. δ5.42 in **1** and δ5.45 in **3**).

The identification of the known khellactone monosenecioates, **4** [7] and **5** [7], followed. Mass spectrometry revealed that **2**–**5** were constitutional isomers and ^{1}H NMR analyses established, as detailed above for **2** and **3**, the location of the hydroxyl and ester groups. The major difference between the two pairs of compounds, and the key to the structures, lay in the ^{1}H NMR signals attributed to the esters. In **4** and **5**, the olefinic protons in the esters were very broad singlets, rather than the broad quartets in **1**–**3**; in addition, both vinyl methyls encountered only allylic coupling.

The identification of the mixed esters was not quite as straightforward, but could be accomplished by reliance on the known mass spectral fragmentation patterns in this class of coumarins [8, 9] (see Fig. 3 and Table 4) and consideration of NMR spectral data. For example, coumarin **6** was clearly a khellactone diester with one angelate and one acetate, but only a very weak molecular ion was observed in the mass spectrum. The largest observed ion, at *m/z* 326, corresponded to the loss of acetic acid from the expected molecular ion. Since the dihydropyranocoumarins are known to undergo a preferential first loss of the ester moiety attached to C-3′ [8, 9], the acetate was placed at C-3′ and **6**, then, had to be pteryxin [4, 10]. Isosamidin (**7**) [4], with acetate and senecioate esters suggested by the ^{1}H NMR spectrum,

Table 4. Major ions in the mass spectra of coumarins from *M. divaricatum* (see Fig. 1)*

Compound	$[M]^+$	$[M-R^2OH]^+$	$[M-R^2OH, Me]^+$	$[M-R^2OH, Me, (R^1-H)]^+$	Other
1	426.1667 ($C_{24}H_{26}O_7$ requires 426.1678)	327	311	229	
2	344.1240 ($C_{19}H_{20}O_6$ requires 344.1260)	245/244	229	229†	326 $[M-H_2O]^+$ 311 $[M-H_2O, Me]^+$
3	344.1252 ($C_{19}H_{20}O_6$ requires 344.1260)	245/244	229	229†	311 $[M-H_2O, Me]^+$ 261 $[M-C_5H_7O]^+$
4	344.1256 ($C_{19}H_{20}O_6$ requires 344.1260)	244	229	229†	326 $[M-H_2O]^+$ 311 $[M-H_2O, Me]^+$ 261 $[M-C_5H_7O]^+$
5	344.1259 ($C_{19}H_{20}O_6$ requires 344.1260)	244	229	229†	311 $[M-H_2O, Me]^+$ 261 $[M-C_5H_7O]^+$
6	386.1373 ($C_{21}H_{22}O_7$ requires 386.1365)	326	311	229	287 $[M-C_5H_7O_2]^+$
7	386.1366 ($C_{21}H_{22}O_7$ requires 386.1365)	326	311	229	287 $[M-C_5H_7O_2]^+$
8	388.1522 ($C_{21}H_{24}O_7$ requires 388.1522)	328.1303 ($C_{19}H_{20}O_5$ requires 328.1311)	311	229	287 $[M-C_5H_9O_2]^+$
9	346.1411 ($C_{19}H_{22}O_6$ requires 346.1416)	328	312	229	245 $[M-C_5H_9O_2]^+$
10	428.1842 ($C_{24}H_{28}O_7$ requires 428.1835)	328.1339 ($C_{19}H_{20}O_5$ requires 328.1311)	313	229	
11	332.1283 ($C_{18}H_{20}O_6$ requires 332.1260)	244	229	229	314 $[M-H_2O]^+$ 299 $[M-H_2O, Me]^+$ 261 $[M-C_4H_7O]^+$
12	414.1676 ($C_{23}H_{26}O_7$ requires 414.1678)	314	299	229	
13	374.1403 ($C_{20}H_{22}O_7$ requires 374.1366)	314.1121 ($C_{18}H_{18}O_5$ requires 314.1154)	299	229	
14	346.1400 ($C_{19}H_{22}O_6$ requires 346.1416)	328	313	229	
15	344.1259 ($C_{19}H_{20}O_6$ requires 344.1260)	244	229	229†	326 $[M-H_2O]^+$ 311 $[M-H_2O, Me]^+$
16	426.1683 ($C_{24}H_{26}O_7$ requires 426.1678)	327/326	311	229	
17	426.1687 ($C_{24}H_{26}O_7$ requires 426.1678)	327/326	311	229	
18	276.1023 ($C_{15}H_{16}O_5$ requires 276.0998)	N.O.‡	244	229	262 $[M-CH_2]^+$, 175
19	328.1324 ($C_{19}H_{20}O_5$ requires 328.1311)	228	213	N.O.‡	

*Positive ion electron impact spectra.
†$R^1 = H$.
‡Not observed.

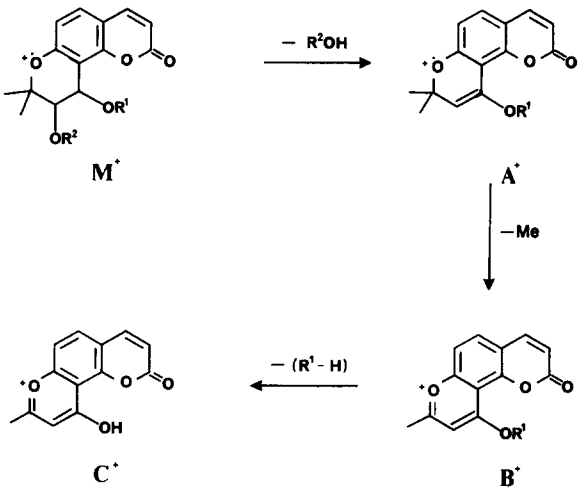
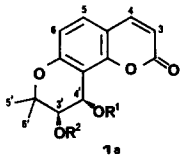
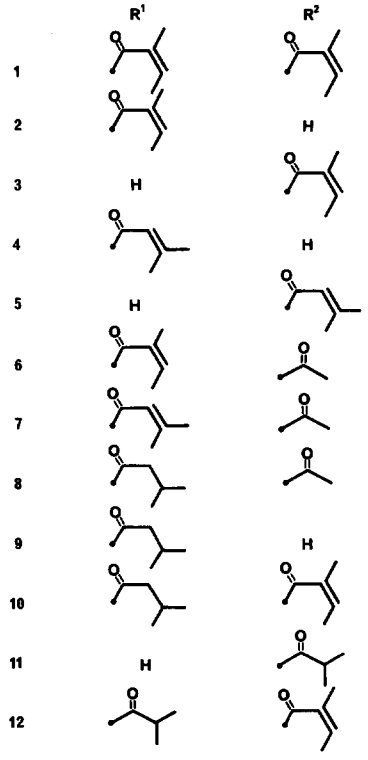
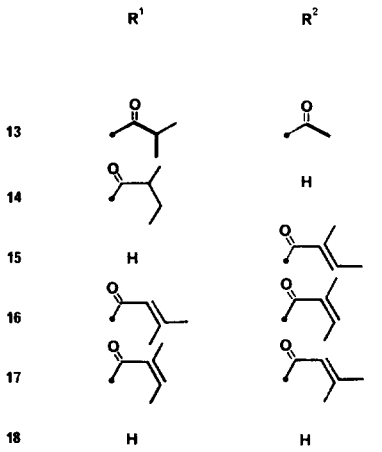
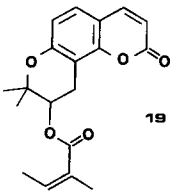


Fig. 3. Key mass spectral fragmentations of khellactone coumarins.





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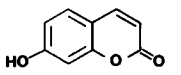
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*Both **10** and **12** are reported in ref. [6], but no data are provided to support the proposed structures.

exhibited a mass spectrum identical to that of pteryxin. A third diester proved to be suksdorfin (**8**) [4], again with an acetate at C-3′, but with an isovalerate at C-4′; instead of ^{1}H NMR signals for a conjugated ester, high-field signals for an isopropyl group coupled to a methylene adjacent to a carbonyl were observed.

Once the signals for the isovalerate ester had been assigned, several new coumarins could be identified. The structure of the monoisovalerate **9** was easily deduced from the ^{1}H NMR spectral data; two overlapping methyl doublets at δ0.97 and a three-proton multiplet in the δ2.09–2.24 range coincided with signals observed for **8**, while the upfield shift of the C-3′ methine and its coupling to a hydroxyl proton at δ2.74 secured the position of the ester at C-4′.

^{1}H NMR analyses revealed that praeruptorin E (**10**) [11, 12]* contained angelate and isovalerate esters and the mass spectrum indicated that the angelate had to be at C-3′, since the first fragment ion observed was $[M-100]^+$ (loss of angelic acid). No initial loss of isovaleric acid, $[M-102]^+$, was observed.

Three minor components, two of them novel compounds incorporating an isobutyrate ester, were then identified. Coumarin **11** had a free hydroxyl at C-4′, since the benzylic methine was shifted to higher field; the ester at C-3′ exhibited ^{1}H NMR signals for an isopropyl group (methyl doublets at δ1.23 and 1.22, along with a one-proton heptet at δ2.66). The position of the methine absorption required placement adjacent to a carbonyl and major fragments in the mass spectrum at *m/z* 261 and 244 (losses of 71 and 88 amu from the molecular ion) cor-

roborated the assignment of the isobutyrate, a relatively uncommon ester in the coumarins [13].

The ^{1}H NMR spectrum of coumarin **12*** exhibited the now familiar signals for angelate and isobutyrate esters. The mass spectrum of **12** revealed a molecular ion of modest intensity and a first fragment ion at m/z 314 $[M-100]^+$, indicating that the angelate resided on C-3′. The third member of this subgroup was comprised of acetate and isobutyrate esters. Again, mass spectral fragmentation was the key to placement of the esters; a first loss of acetic acid from the molecular ion put the acetate at C-3′ and the isobutyrate at C-4′ in seravshanin, (**13**) [14].

Yet another new compound proved to be the C-4′ monoester of 2-methylbutyric acid. As before, ^{1}H NMR arguments placed the hydroxyl at C-3′ and decoupling experiments and mass spectral data established the constitution of the new ester group. Two methyl groups were observed, one a doublet at δ1.18 and the other a triplet at δ0.95. The methyl doublet was coupled to a methine at δ2.45 (α to a carbonyl) which was, in turn, coupled to a methylene at δ1.75; the methylene was coupled to the methyl triplet. Thus, the structure **14** was assigned to this dihydro derivative of laserpitin (**2**).

The final novel coumarin to be identified was the monoester **15**; ^{1}H NMR chemical shift data required the hydroxyl to be at C-4′. At first glance, the signals for the ester protons appeared to represent an angelate ester, but this compound was not identical to **3**. The olefinic proton was considerably farther downfield in **15** (δ6.91) and the resonances for the vinyl methyl groups were sharper (no homoallylic coupling), while the one vicinally coupled to the olefin was shifted to higher field (δ1.78 in **15** vs δ1.97 in **3**). This new compound, then, had to be a tiglate ester; examination of the reported differences between angelate and tiglate ^{1}H NMR data confirmed this assessment [15].

Rounding off this montage of khellactone esters from *M. divaricatum* were the two known mixed esters (angelate and senecioates) calypteryxin (**16**) [16] and **17** [17]. The ^{1}H NMR and mass spectra unequivocally defined the constitution of this pair of isomers, and comparison of these data with the literature [16, 17] permitted differentiation of the two.

The parent diol khellactone (**18**) [18] was found in the most polar coumarin fraction and was identified in the same manner as the other coumarins; both dihydropyran methines were shifted to higher field than was observed in the diesters. In addition, signals for two hydroxyl protons were observed between δ3.0 and 4.0.

The lone member of the group not derived from khellactone was **19**. The angelate ester at C-3′ was evident from the NMR and mass spectral data. The interesting difference between **19** and the other isolates was the AMX-type pattern for the dihydropyran ring protons. H-3′ appeared as a triplet at δ5.18, with coincidentally equivalent coupling to both protons on C-4′. The C-4′ protons were observed as doublets of doublets at δ3.24 and 2.99, with a large (18.5 Hz) geminal coupling. It was apparent, then, that this compound was a lomatin ester of angelic acid, probably identical to selinidin [19].

Stereochemistry

The ester substituents in anomalin (**1**), as well as the other khellactone derivatives, were assigned the *cis* configuration on the basis of the 4.7 Hz coupling constant between H-3′ and H-4′ and the small (0.03 δ) difference in the geminal dimethyl resonances [4]. These values prevail throughout this group of coumarins; thus, all 18 of the khellactone esters were assigned *cis* configuration. The optical rotation of **1**, $[\alpha]_D$ −46.5°, compared favourably with the literature value ($[\alpha]_D$ −37°) [20] for anomalin, assigned the 3′ *R*, 4′ *R* configuration. Similarly, the observed rotation for **3**, $[\alpha]_D$ −56.8°, was also of the proper sign for the 3′ *R*, 4′ *R* configuration (lit. [7]: $[\alpha]_D$ −91.8°). While it seems likely that all the isolated khellactone derivatives have the same absolute configuration, limited quantities of most of the isolates precluded an accurate evaluation of the optical rotations.

The configuration of **19** was not determined because the paucity of material negated efforts to measure the optical rotation with any confidence. Since all the known lomatin derivatives have been shown to have the 3′ *R* configuration, it is likely that **19** is the known selinidin.

DISCUSSION

The most surprising aspect of this work is the number of new coumarins isolated from a single plant. In view of the very large number of coumarins already known, this must be considered a significant find. Since the new compounds are, for the most part, minor metabolites comprised of esters from acids uncommon in the coumarins, we suspect that such compounds may not be unique to *M. divaricatum*. Instead, it seems likely that development of new isolation methods has made such minor metabolites more accessible.

The advent of HPLC and its dramatic resolution capabilities have revolutionized separation science, but natural products chemists have only recently begun to exploit this powerful technique. An important consideration for isolation chemists is that reverse-phase HPLC, the method of choice for qualitative and quantitative analyses, is sometimes not amenable to scale-up for preparative separation because the compounds under investigation are not very soluble in the aqueous eluant. In this case, the nitrile bonded phase column provided an attractive alternative to normal and reversed-phase columns. Irreversible adsorption and solubility problems were avoided, and the bonded phase provided excellent separations based on partition effects.

The only previous study [21] of *M. divaricatum* resulted in the isolation of umbelliferone (**20**), as the sole coumarin; curiously, we found no trace of this simple precursor to the khellactone esters in our extracts.

Finally, it is noteworthy that several of the known khellactone esters we encountered have been reported to exhibit anti-spasmodic and vasodilatory activity [22–24]. Since *M. divaricatum* has been an important food source to the Crow [2] and may have served tne same purpose for earlier Native American cultures, it would be interesting to ascertain the vasodilatory effects engendered by routine consumption of such pharmacologically active compounds as part of a standard diet.

EXPERIMENTAL

NMR spectra were obtained with a Bruker WM-250 Fourier transform spectrometer. Chemical shifts are expressed as δ units, relative to TMS ($\delta = 0$), using $CDCl_3$ as solvent and internal standard. IR spectra were obtained on a Beckman IR-20; UV spectra with a Varian G34 spectrophotometer. Mass spectra were recorded with VG MM16F and 7070 EHF spectrometers

operating at 70 eV in the electron impact mode. Optical rotations were determined with a Carl Zeiss circle polarimeter.

Collection and extraction of M. divaricatum. Whole flowering plants were collected in May 1981 at the Montana State University Agricultural Experimental Station at Red Bluff, Montana. The plants were divided into above- and below-ground parts and treated separately. The plant materials were ground in a Waring blender with MeOH. After the solvent was removed by filtration, the plant residues were steeped in fresh MeOH for 24 hr. Again the MeOH extracts were removed by filtration; the marc was then steeped in CH_2Cl_2 for 24 hr (2 ×). The MeOH extracts were reduced, *in vacuo*, to an aq. suspension which was equilibrated with the CH_2Cl_2 extracts. The CH_2Cl_2 phase of the above-ground parts was evapd to give a thick green gum, 7.06 g (dry wt after extraction, 52.6 g), while that from the roots gave 1.39 g brown gum (from 19.7 g dried plant residue). ^{1}H NMR analysis of the extracts showed virtually identical constituent profiles in the two extracts.

Fractionation of crude extract. The extract from the above-ground parts was chromatographed on a Florisil column (210 g) with a hexane–EtOAc–MeOH gradient. Fourteen fractions were collected; fractions 6, 7 and 8, all eluted with hexane–EtOAc (4:1), accounted for nearly one-half of the total extract. Fraction 10, eluted With EtOAc–MeOH (49:1) yielded khellactone (**18**). The isolation of the various individual coumarins from these fractions is outlined in Figs. 1 and 2. ^{1}H NMR data are presented in Table 1, ^{13}C NMR data in Tables 2 and 3, and mass spectral data in Table 4. The mass spectral fragmentation pattern of the khellactone esters is depicted in Fig. 3.

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REFERENCES

1. Blankinship, J. W. (1905) *Montana Agric. College Exp. Station Bull.* **56**, 56.
2. Joy Yellowtail Toineeta (1970) M. Ed. Thesis, Montana State University p. 14.
3. Davis, L. B., Aaberg, S., Wilson, M. and Ottersberg, R. (1982) *Stone Circles in the Montana Rockies.* Report to the Montana Army National Guard by Montana State University.
4. Lemmich, J., Lemmich, E. and Nielsen, B. E. (1966) *Acta Chem. Scand.* **20**, 2497.
5. Hata, K., Kozawa, M. and Yasumasa, I. (1967) *Yakugaku Zasshi* **87**, 18; (1968) *Chem. Abstr.* **68**, 10231.
6. Bohlmann, F., Bhaskar Rao, V. S. and Grenz, M. (1968) *Tetrahedron Letters* 3947.
7. Gonzalez, A. G., Barroso, J. T., Lopez Dorta, M., Luis, J. R. and Rodriguiz-Luis, F. (1979) *Phytochemistry* **18**, 1021.
8. Shaath, N. A., Soine, T. O. and Shipchandler, M. T. (1976) *J. Pharm. Sci.* **65**, 1028.
9. Zakharov, P. I., Terent'ev, P. B., Nikonov, G. K. and Bankovskii, A. I. (1971) *Khim. Prir. Soedin.* **7**, 704; (1972) *Chem. Abstr.* **76**, 125962.
10. Willette, R. E. and Soine, T. O. (1962) *J. Pharm. Sci.* **51**, 149.
11. Okuyama, T. and Shibata, S. (1981) *Planta Med.* **42**, 89.
12. Ye, J., Zhang, H. and Yuan, C. (1982) *Yoxue Xuebao* **17**, 431; (1982) *Chem. Abstr.* **97**, 178696.
13. Murray, D. H., Mendez, J. and Brown, S. A. (1982) *The Natural Coumarins. Occurrence, Chemistry and Biochemistry*, p. 40. John Wiley, Chichester.
14. Dukhovlinova, L. I., Sklyar, Y. E. and Pimenov, M. G. (1980) *Khim. Prir. Soedin.* 832.
15. Fraser, R. R. (1960) *Can. J. Chem.* **38**, 549.
16. Nielsen, B. E. and Soine, T. O. (1967) *J. Pharm. Sci.* **56**, 184.
17. Nielsen, B. E., Larsen, P. K. and Lemmich, J. (1971) *Acta Chem. Scand.* **25**, 529.
18. Lemmich, J., Pedersen, P. A. and Nielsen, B. E. (1969) *Tetrahedron Letters* 3365.
19. Seshadri, T. R., Sood, M. S., Handa, K. L. and Vishwapaul (1967) *Tetrahedron* **23**, 1883.
20. Murray, D. H., Mendez, J. and Brown, S. A. (1967) *Tetrahedron* **23**, 365.
21. Crowden, R. K., Harborne, J. B. and Heywood, V. M. (1969) *Phytochemistry* **8**, 1963.
22. Smith, E., Hosansky, N., Bywater, W. G. and van Tamelen, E. E. (1947) *J. Am. Chem. Soc.* **79**, 3534.
23. Call, T. G. and Fischer, E. B. (1958) *Northwest Sci.* **32**, 96.
24. Call, T. G. and Green, J. (1956) *Proc. Montana Acad. Sci.* **16**, 49.