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LABDANE DITERPENOIDS FROM *GRINDELIA DISCOIDEA* (ASTERACEAE)

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Key Word Index—*Grindelia discoidea*; Asteraceae; Astereae; Solidagininae; diterpenoid acids; labdanes; cordobic acid; 7-epicordobic acid; cordobic acid 18-acetate; mass spectrometry.

Abstract—The dichloromethane extract of the aerial parts of *Grindelia discoidea* afforded three new labdane diterpenoids: cordobic acid, cordobic acid 18-acetate and 7-epicordobic acid. The structures of these new isolates were deduced mainly from their spectral data, using comparisons with one another and with other labdanoids including discoidic acid, a constituent of *G. discoidea* reported earlier.

INTRODUCTION

We have previously reported the isolation and identification of two labdane diterpenoids (**4** and **5**) and three flavonoids from *Grindelia discoidea* Hook & Arn. [1]. From the same source, we now report the isolation and identification of three new bicyclic labdane diterpenoids, which we have named cordobic acid [**1**, 7α,18-dihydroxylabd-8(17)-en-oic acid or (1*R*,3*R*,4a*R*,5*R*,8a*R*,β*S*)-1,3,4,4a,5,6,7,8,8a-nonahydro-2-methylene-3-hydroxy-5-hydroxymethyl-β,5,8a-trimethyl-1-naphthalenepentanoic acid], cordobic acid 18-acetate (**2**) and 7-epicordobic acid (**3**).

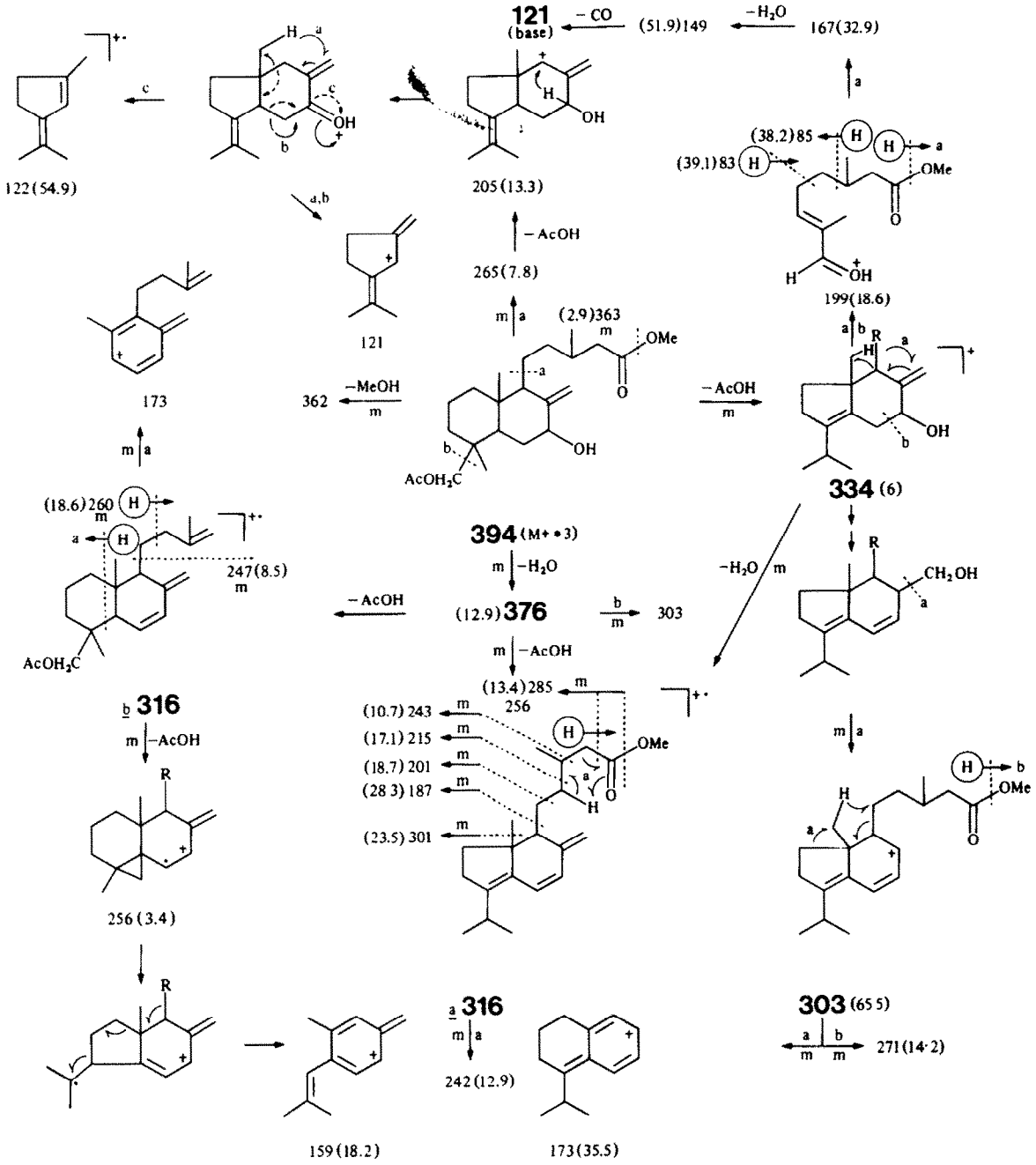
RESULTS AND DISCUSSION

The new diterpenoids (**1–3**) were isolated from the same chromatographic column that gave **4** and **5** [1]. The natural products reported here were not separated as free acids but were converted into their methyl ester derivatives (**1a**, **2a**, **3a**) which were separated by preparative TLC and characterized by spectroscopic methods. From the IR and EIMS fragmentation pattern of **2a** and by correlation of the molecular formulae of major fragments in the HRMS of **4a** with those of **2a**, the structures of **1a**, **2a** and **3a** (except for their stereochemistry) were deduced without the aid of NMR data.

The evidence for structure **2a** follows. The IR ($CHCl_3$) spectrum of **2a** showed clearly the presence of hydroxyl (3620 and 1015 cm^{-1}), methyl (1372 cm^{-1}, singlet) and exocyclic methylene (3070, 1650 and 905 cm^{-1}) groupings but, like **4a**, the lack of a gem-dimethyl group. From the shape and location of the carbon–oxygen stretching (1740–1710 and 1250–1150 cm^{-1}), we suspected that **2a** might contain more than one ester group.

The EIMS of **2a** showed an $[M]^+$ peak at *m/z* 394, 58 amu higher than that of **4a**, indicating the presence of an additional ester grouping. This conclusion was supported by the elemental composition of the $[M]^+$ peaks of **4a** ($C_{21}H_{36}O_3$) and **2a** ($C_{23}H_{38}O_5$) by HRMS. The fragmentation pattern in the EIMS of **2a** was noticeably different from that of discoidic acid methyl ester (**4a**). Neither the characteristic retro-Diels–Alder rearrangement leading to the base peak at *m/z* 122 nor the pronounced loss of β-methylpentanoic acid methyl ester ($C_5H_{10}COOMe$) side chain at C-9 from $[M]^{+\cdot}$ in **4a** was observed in **2a**. The base peak in **2a** occurred at *m/z* 121 rather than 122 (suggesting the absence of a Δ^7 double bond) and the loss of C-9 side chain was reduced to a secondary process, as demonstrated by the low abundances of peaks at *m/z* 265 (7.8%), 247 (8.5%) and 205 (13.3%). This latter process was intensified after the loss of H_2O and AcOH from $[M]^+$ as shown by the high abundance of the peak at *m/z* 187 (28%). The C-9 side chain unit was seen directly as a peak at *m/z* 129 ($C_7H_{13}O_2$) which confirmed the presence of acetate and hydroxyl groupings in the bicyclic moiety. Peaks at *m/z* 376 $[M-H_2O]^+$, 334 $[M-AcOH]^+$ and 316 $[376-AcOH]^+$ and $[334-H_2O]^+$ also confirmed the presence of these groupings. The pronounced loss of 73 amu from the fragment *m/z* 376 to give a peak at *m/z* 303 (65.5%) and the lack of gem-dimethyl group absorptions in the IR spectrum of **2a** clearly suggested that it was the primary hydroxyl group that was acetylated and must therefore be attached to C-4 replacing the C-19 methyl of gem-dimethyl group. The presence of the hydroxyl function adjacent to the exocyclic methylene group appears to be the structural prerequisite for the formation of an intense *m/z* 199 fragment (18.6%, $C_{11}H_{19}O_3$) that results from decomposition of *m/z* 334 initiated by McLafferty rearrangement followed by allylic cleavage. From the transitions shown in Scheme 1, which are substantiated by metastable peaks, the unit $C_2H_4O_2$ (60 amu) was lost from the *m/z* 376 fragment in two ways to give the *m/z* 316 peak (species *a* and *b*). Other structures are possible for some of the fragments but the molecular formulae of all fragments shown in Scheme 1 are substantiated by HRMS.

Acetylation of **2a** with acetic anhydride–pyridine gave **2b** which did not display a recognizable $[M]^+$ peak but did exhibit a strong peak at *m/z* 394, corresponding to **2a**, and a very similar fragmentation pattern but with a base peak at *m/z* 187.



Scheme 1. Fragmentation pathways of some characteristic ions (*m/z* ratios) established by exact mass measurements, in the mass spectrum of cordobic acid 18-acetate methyl ester (**2a**).

Further support was provided by the virtually identical mass spectra of cordobic acid methyl ester (**1a**) and its 7-epimer (**3a**), which essentially followed the fragmentation pattern outlined for cordobic acid 18-acetate methyl ester (**2a**). Their spectra showed a base peak at *m/z* 123, an $[M]^+$ peak at *m/z* 352, as required by the substitution of a hydroxyl for an OAc group at C-18 in **1a** and **3a**, and intense peaks at *m/z* 334, 321, 316 and 303, corresponding to losses of H_2O, OMe, $2 \times H_2O$ and $H_2O - OMe$ from $[M]^+$, respectively.

The constitution of **1–3** deduced from IR and mass spectral data was confirmed by ^1H and ^{13}C NMR spectroscopy (Table 1), which also aided in establishing their stereochemistry. The ^1H NMR parameters for H-7, H-17a and H-17b for 7-ols (**1a**, **2a** and **7a** [2]) and their acetates (**2b** and **7b** [2]) were very close to one another and quite

Table 1. ^{13}C and ^{1}H NMR data of compounds **1a**, **2a**, **2b**, **3a**, **7a**, **7b** and **8** ($CDCl_3$, TMS as int. standard)

	^{13}C		^{1}H					
Atom	**2a**	**8**	**1a**	**2a**	**2b**	**3a**	**7a**†	**7b**†
1	38.6	38.6						
2	18.9	18.6	1.0–2.0					
3	35.4	35.9						
4	36.8	36.9						
5	48.2	49.6	1.0–2.0					
6	30.8	24.4						
7	73.7	38.1	4.36 *t* (2.9)*‡	4.36 *t* (2.8)	5.38 *s* (*br*)	3.92 *m*	4.36 *m*	5.40 *m*
8	149.2	147.9						
9	51.3	57.3	1.0–2.0					
10	39.7	39.6						
11	20.7	21.1	1.0–2.0					
12	36.3	36.0						
13	31.1	30.9				1.92 *octet* (6.5)‖		
14	41.4	41.4	2.11 *dd* (14.7, 8.0) 2.32 *dd* (14.7, 6.0)	2.11 *dd* (14.7, 8.0) 2.32 *dd* (14.7, 6.1)	2.10 *dd* (14.6, 8.2) 2.32 *dd* (14.6, 5.8)	2.11 *dd* (14.7, 8.1) 2.32 *dd* (14.8, 6.0)		
15	173.6	178.5						
16	19.9	20.0	0.94 *d* (6.6)	0.94 *d* (6.6)	0.94 *d* (6.7)	0.94 *d* (6.6)	0.92 *d* (6)	0.92 *d* (6)
17	110.0	106.8	4.62 *t* (1.5) 5.03 *t* (1.2)	4.63 *s* (*br*) 5.04 *s* (*br*)	4.73 *s* (*br*) 5.16 *s* (*br*)	4.66 *s* (*br*) 5.16 *s* (*br*)	4.62 *s* (*br*) 5.02 *s* (*br*)	4.72 *m* 5.14 *m*
18	66.8	?§	3.40 *d* (10.9) 3.75 *d* (10.9)	3.84 *d* (10.9) 4.21 *d* (10.9)	3.82 *d* (11.0) 4.20 *d* (11.0)	3.39 *d* (10.9) 3.73 *d* (10.9)	0.77 *s*	0.78 *s*
19	27.2	17.5?§	0.99 *s*	0.97 *s*	0.92 *s*	1.00 *s*	0.80 *s*	0.86 *s*
20	14.1	14.9	0.63 *s*	0.66 *s*	0.68 *s*	0.64 *s*	0.64 *s*	0.65 *s*
MeO	51.3		3.66 *s*	3.66 *s*	3.66 *s*	3.67 *s*	3.64 *s*	3.64 *s*
Me(CO)	20.9	?§		2.04 *s*	2.02 *s* 2.04 *s*		2.02 *s*	2.02 *s*
Me(CO)	171.3	171.1						
HO			1.62 *s* (*br*) 2.09 *s*	1.44 *s*		1.78 *s* (*br*) 2.07 *s*		

*Reference [3].
†Reference [2].
‡*J* (Hz) in parentheses.
§In the Table in ref. [3], there is some ambiguity regarding these values.
‖The C-13 proton in **1a**, **2a**, and **2b** absorbs here as well, but is largely obscured by other absorptions.

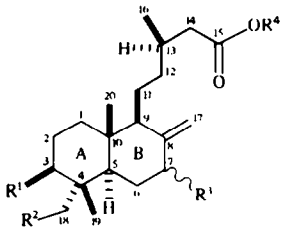
different from the values for any other known labdane. This indicated close similarity except possibly for enantiomerism. The fact that they have the same absolute configuration was shown by the similar change in molecular rotation (in $CHCl_3$) upon acetylation of the 7-OH for each pair: $[-5°(\mathbf{7b})]-[-57°(\mathbf{7a})] = +52°$, and $[-20°(\mathbf{2b})]-[-54°(\mathbf{2a})] = +34°$. Thus the new compounds **2a** and **2b** are in the labdane series and, by analogy with the known labdanes, have fixed configurations at C-5, C-9, C-10 and C-13. The C-7 OH or OAc was clearly axial from the small values of $J_{6\alpha,7}$ and $J_{6\beta,7}$, and because the ^{1}H NMR parameters match those of **7a** and **7b**. The remaining question, the configuration at C-4, was answered by noting the close match of the 18-methylene and 19-methyl ^{1}H NMR absorptions of **1a** and **3a** with those of **4a**, known to have the C-4 configuration shown [1]; all of these compounds showed the same broadening of the C-18 methylene absorptions, especially of the upfield doublet, due to *W*-coupling with the C-19 methyl protons, indicating that the upfield doublet was due to the underlined proton in Fig. 1, the major rotamer expected about the C-4 to C-18 bond. Since **3a** differed from **1a** only in configuration at C-17, the ^{1}H NMR absorption of **3a** showed the expected differences (7-methinyl and 17-vinyl) and similarities (all of the other values in Table 1). The ^{13}C shifts reported for **8** [3] given in Table 1 were helpful in assigning the ^{13}C spectrum of **2a**. The changes in absorptions for C-5 through C-9 are consistent with the addition of a 7-OH grouping in **2a**.

A reasonable biosynthetic scheme for **1**–**5**, all of which occur in the same plant species, consists of oxidation of cativic acid (**6**) [4] to **5** and **4**, oxidation of the latter to **3** and **1**, and acetylation of **1** to **2**.

EXPERIMENTAL

See ref. [5] for description of instrumental procedures used. The plant material used and the extraction, fractionation and chromatography procedures employed were the same as described in our previous work [1].

Cordobic acid 18-acetate methyl ester (**2a**). Cordobic acid 18-acetate (**2**), which had a lower R_f than discoidic acid (**4**), was isolated from fraction 55 of the EM silica gel CC of the acid fraction that followed discoidic acid (**4**) and was obtained from



	R^1	R^2	R^3	R^4	
1	H	OH	α-OH	H	
1a	H	OH	α-OH	Me	
2	H	OAc	α-OH	H	
2a	H	OAc	α-OH	Me	
2b	H	OAc	α-OAc	Me	
3	H	OH	β-OH	H	
3a	H	OH	β-OH	Me	
4	H	OH	H	H	Δ^7 not $\Delta^{8(17)}$
4a	H	OH	H	Me	
5	OH	H	H	H	
6	H	H	H	H	
7a	H	H	α-OH	Me	
7b	H	H	α-OAc	Me	
8	H	OAc	H	H	

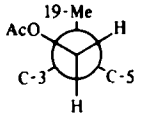


Fig. 1.

the previously reported isolation procedure [1]. Fraction 55 (300 mg), when dissolved in Et_2O and treated with petrol, gave an oily residue which was filtered off. The Et_2O–petrol soluble fraction (100 mg), after evaporation of the solvent to dryness under vacuum, was esterified with MeI (3 ml) in dry Me_2CO (8 ml) and anhydrous K_2CO_3 (188 mg) at 56–60° for 5 hr [6]. The esterified mixture, after work-up, when chromatographed by repetitive prep. TLC [silica gel 60 PF-254, toluene–EtOAc–AcOH (50:25:1)] followed by decolourization afforded **2a** (60 mg, oil), homogeneous by TLC, $[\alpha]_D^{25}$ −13.9° ($CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: see text; ^1H and ^{13}C NMR: Table 1 and MS *m/z* (rel. int.): Scheme 1; in accord with structure **2a**.

The ^1H NMR data (Table 1) of the acetate of **2a** [**2b**, prepared by treatment with Ac_2O–C_5H_5N at room temp. overnight, $[\alpha]_D$ −4.5° ($CHCl_3$)] was in accord with structure **2b**.

Cordobic acid methyl ester (**1a**) *and 7-epicordobic acid methyl ester* (**3a**). Compounds **1** and **3** were present in fraction 60 (6.2 g), which was complex. Esterification of this fraction with MeI in Me_2CO–K_2CO_3 [6] followed by silica gel 60 (250 g) CC of the resulting methyl ester mixture (*n*-hexane with increasing concns of EtOAc) gave fractions (8–10) from which **1a** (81 mg, oil) and **3a** (35 mg, oil) were isolated and purified by repetitive prep. TLC (silica gel 60 PF-254; CH_2Cl_2–EtOAc–AcOH, 40:10:1).

Cordobic acid methyl ester (**1a**). $[\alpha]^{25}$ −12.7 ($CHCl_3$). IR $\nu_{max}^{CHCl_3}$: 3460, 3090, 1730, 1645, 1370, 900 cm^{-1}; ^1H NMR: (Table 1); MS *m/z* (rel. int.): 352 $[M]^+$ (1.8), 334 (15), 321 (4.3), 316 (13.3), 304 (26.7), 303 (47.6), 301 (5), 289 (6.7), 271 (13.3), 223 (10.8), 187 (12.3), 173 (27.2), 167 (17), 159 (18.2), 151 (58.5), 149 (11.8), 147 (22.5), 133 (22.2), 123 (100), 122 (20.4), 121 (44.2), 119 (31.3), 109 (60), 107 (45.3), 105 (40.5), 95 (70.6), 93 (57.4), 91 (42.7), 83 (23.5), 81 (83.2), 79 (49), 69 (52.2), 67 (55.9), 59 (39.4), 55 (85.7); spectra were in accord with structure **1a**.

7-Epicordobic acid methyl ester (**3a**). $[\alpha]^{25}$ +14.9° ($CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: superimposable with **1a**; ^1H NMR (Table 1) and MS *m/z* (rel. int.): similar to **1a**, spectra were in accord with structure **3a**.

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