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TWO GRINDELANE DITERPENOIDS FROM *GRINDELIA CAMPORUM*

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Key Word Index—*Grindelia camporum*; Asteraceae; Astereae; Solidagininae; labdanes; grindelic acid derivatives; 17-carboxygrindelic acid; 7-acetyl-8,17-bisnor-8-oxagrindelic acid; chrysolic acid; strictanonic acid.

Abstract—Two new grindelane diterpenoids, termed camporic acid (a labdane-derived diterpenoid with a pyranoid B-ring) and 17-carboxygrindelic acid, were isolated from *Grindelia camporum* as methyl esters and characterized mainly by ^{1}H and ^{13}C NMR and mass spectral analyses and comparisons with related grindelanes. Chrysolic acid methyl ester and strictanonic acid methyl ester were also obtained from this species for the first time.

INTRODUCTION

During the course of a large scale isolation of grindelic acid (**1a**) from the acidic fraction of the dichloromethane extract of *Grindelia camporum* Greene, we isolated a fraction which by GC analysis contained considerable amounts of strictanonic acid (**4a**), previously isolated from *G. stricta* DC [1] and *Chrysothamnus paniculatus* (Gray) Hall [2]. We decided to isolate strictanonic acid (**4a**) as its methyl ester derivative (**4b**) from this fraction for additional biological testings. While doing so, we observed a few peaks not found or nearly absent in the gas chromatograms of the methylated acidic fractions of earlier samples of *G. camporum* [3]. We went on to show that they were due to chrysolic acid methyl ester (**5b**, a constituent of *C. paniculatus* [4]) and two new grindelanes, characterized as methyl 7-acetyl-8,17-bisnor-8-oxagrindelate (**3b**, which we name camporic acid methyl ester) and dimethyl 17-carboxygrindelate. (**2b**).

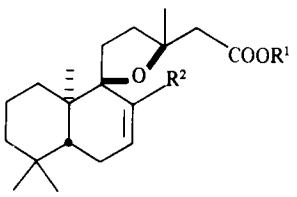
RESULTS AND DISCUSSION

Compound **2b**, $[\alpha]_D^{25}$ − 70.3° ($CHCl_3$; *c* 2.5), although homogeneous by TLC, was shown by ^{1}H NMR and GC analysis to contain a very small amount of an impurity. Its IR (neat) spectrum showed absorptions for C=O (1730 cm^{-1}) and C=C (1680, 860 cm^{-1}) groups. Its EIMS, which was very informative, displayed a small molecular ion peak at *m/z* 378 (1.1%, $C_{22}H_{34}O_5$ by high resolution MS), a minor but structurally diagnostic peak at *m/z* 305 (5.0% $[M-CH_2COOMe]^+$), highly characteristic right- and left-hand *retro*-Diels–Alder (RDA) rearrangement peaks at *m/z* 254 (90.3%, $C_{13}H_{18}O_5$) and *m/z* 124 (6.7%, C_9H_{16}), respectively, and peaks originating from RDA fragments and their daughter fragments: *m/z* 223 (25.8% $[m/z\ 254-OMe]^+$), 222 (100% $[m/z\ 254-MeOH]^+$), 190 (20.1% $[m/z\ 222-MeOH]^+$), 148 (19.8% $[m/z\ 222-MeCOOMe]^+$), 123 (25.5% $[m/z\ 124-H]^+$), 109 (40.3% $[m/z\ 124-Me]^+$) and 95 (31.6% $[m/z\ 123-C_2H_4]^+$). These diagnostic peaks led us to postulate structure **2a**, which is strongly supported by the ^{1}H (Table 1) and ^{13}C (Table 2) NMR results in comparison with those of methyl grindelate (**1b**).

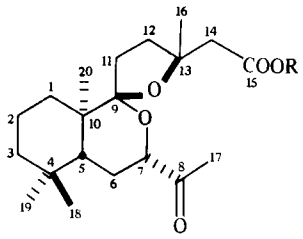
Compound **3b**, $[\alpha]_D^{25}$ − 5.7° ($CHCl_3$; *c* 3.4), homogeneous by TLC, was >90% pure by GC analysis. Its IR (neat) spectrum showed a split broad intense carbonyl band at 1720 (>C=O) and 1740 (ester) cm^{-1}. Its EIMS displayed a barely discernible M^+ peak at *m/z* 366 (0.4%), determined by HRMS to be $C_{21}H_{34}O_5$, the same molecular formula as strictanonic acid methyl ester (**4b**). The fragmentation pattern with the base peak at *m/z* 109 (*m/z* 43 in **4b**) was different to that of **4b**. The loss of 58 mass units from M^+ in **4b** (*m/z* 308, $[M-MeCOMe]^+$) was not observed in **3b**. Instead, a loss of 43 mass units from M^+ (*m/z* 323, 17%), corresponding to −COMe by HRMS, was observed. This basic difference between **3b** and **4b** together with the characteristic left-hand *retro*-Diels–Alder (RDA) fragment (*m/z* 124, 31.4%), its daughter ion at *m/z* 109 ($[m/z\ 124-Me]^+$ 100%) and a small but diagnostic peak at *m/z* 293 ($[M-73]^+$, $-CH_2COOMe$ by HRMS), suggested that **3b** was an isomer of **4b** differing only in the B ring. This led us to postulate structure **3b**. The presence of the -COMe group at C-7 and an ethereal oxygen between C-7 and C-9 permits the formation of *m/z* 194 ($C_{13}H_{22}O$), 136 ($C_{10}H_{16}$) and 155 ($C_8H_{11}O_3$) fragments (Scheme 1).

The ^{1}H (Table 1) and ^{13}C (Table 2) NMR parameters observed, strongly support the constitution indicated in **3b**, and show the acetyl group to be equatorial (H-7 has an axial–axial coupling constant of 12.0 Hz to the H-6 axial proton). The usual grindelic configurations are assumed at positions 5, 10, and 13, and the configuration shown at position 9 was supported by a strong peak between the OMe and H-7 in the NOESY spectrum of **3b**.

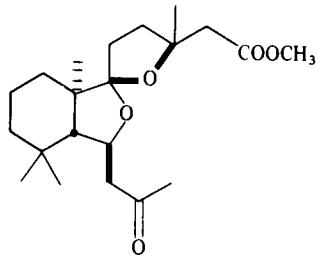
The biosynthesis of **3a** from grindelic acid (**1a**) like that of **4a** [2] requires two oxidations, and may proceed *via* intermediates **6–8** as shown. Compound **3b** has not been previously reported from a natural source, but it has been reported in an epimeric mixture at C-7 as a synthetic product without chemical or spectral properties [6].



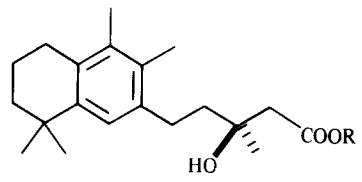
	R^1	R^2
1a	H	Me
1b	Me	Me
2a	H	COOH
2b	Me	COOMe



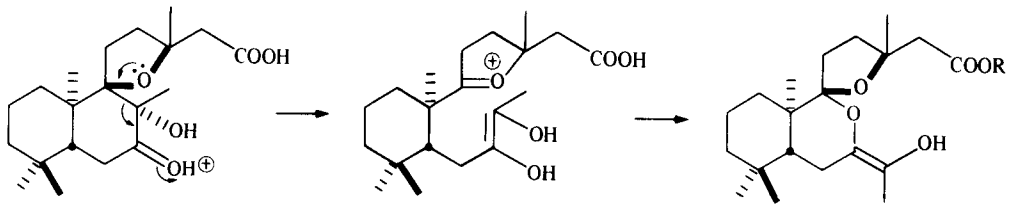
	R
3a	H
3b	Me



	R
4a	H
4b	Me



	R
5a	H
5b	Me



6 **7** **8**

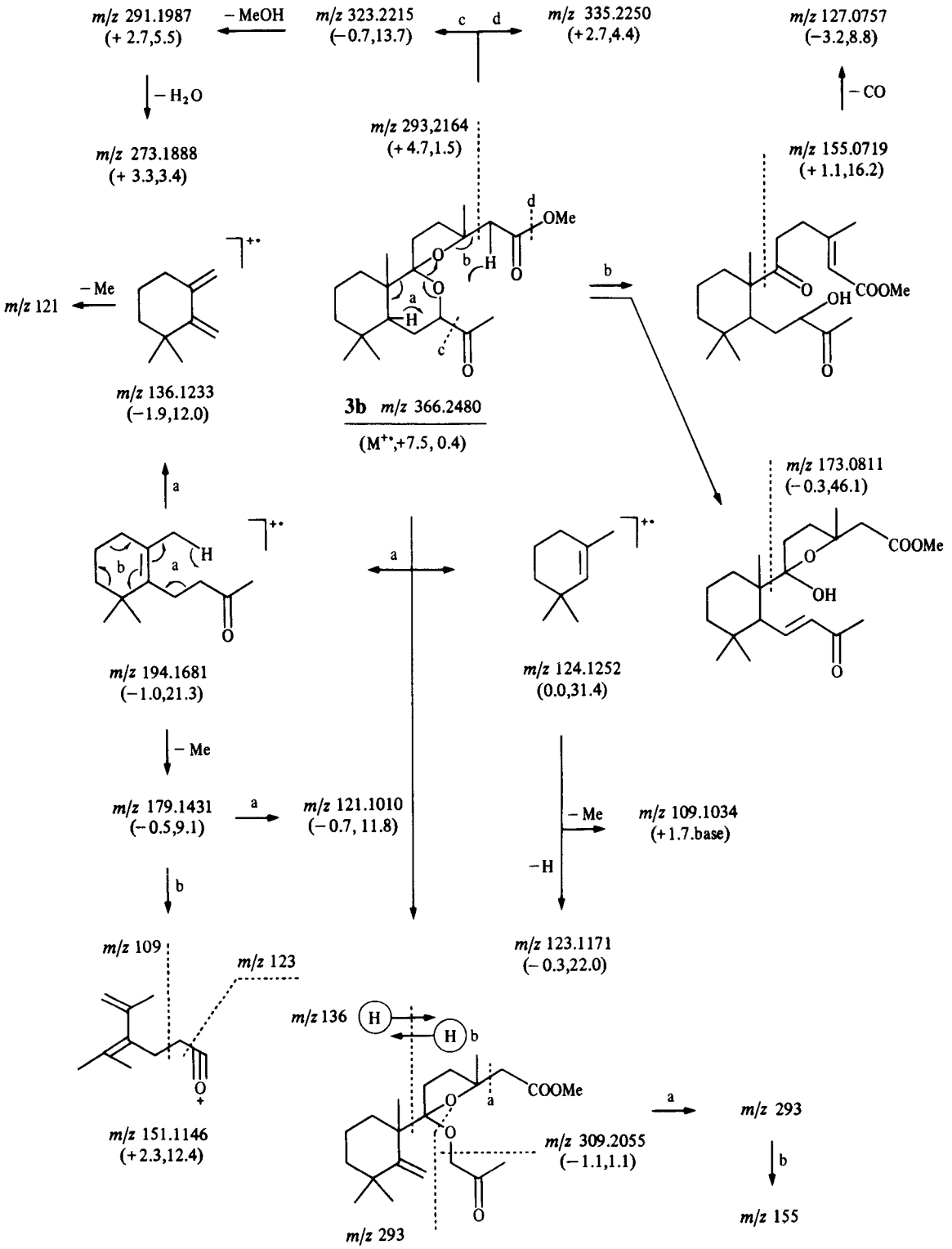
Table 1. ^{1}H NMR chemical shifts (δ, $CDCl_3$) and coupling constants (Hz, in parentheses) for compounds **1b–4b**

H	1b	2b	3b	4b [2]
7	5.51 *m*	6.45 *dd* (4.8, 2.7)	4.27 *dd* (12.0, 3.2)	2.73 *m*
14a	2.62 *d* (14.2)	2.66 *d* (14.1)	2.58 *d* (14.1)	2.68 *s*
14*b*	2.75 *br d* (14.2)	2.81 *br d* (14.1)	2.68 *d* (14.1)	2.68 *s*
16	1.33 *s*	1.33 *s*	1.30 *s*	1.22 *s*
17	1.76 *br s*	—	2.21 *s*	2.23 *s*
18	0.90 *s*	0.90	1.03	1.02
19	0.87 *s*	0.88	0.88	1.00
20	0.81 *s*	0.83	0.84	0.93
15-OMe	3.66 *s*	3.64	3.65	3.64
17-OMe	—	3.73 *s*	—	—

Unfortunately, no authentic sample was available for direct comparison.

EXPERIMENTAL

Plant material. Plant material used in this study was harvested during the spring of 1982 from the University of Arizona's Overpass Farm. The field was planted with seeds collected at the Environmental Research Laboratory, University of Arizona, Tucson, in January 1981. Herbarium specimens have been deposited at the University of Arizona. All plant material was air-dried, ground to 3 mm particle size and stored at 5° prior to extraction.



Scheme 1. Diagnostic fragment ions, established by HRMS, in the EIMS of compound **3b**. The figures in parentheses represent difference in mmu and relative intensities.

Extraction and fractionation. The procedures used for the extraction of ground aerial parts of *G. camporum* with CH_2Cl_2, separation of the acidic fraction (Na_2CO_3-soluble) from the MeOH-soluble portion of the CH_2Cl_2 extract and silica gel CC of the acidic fraction were similar to those described for the EtOAc extract of *G. camporum* [3]. The column was eluted with CH_2Cl_2 containing increasing concentrations of MeOH and finally with 100% MeOH. The fraction leached with 1% MeOH in CH_2Cl_2 and 100% MeOH contained strictanonic acid (**4a**), as judged by TLC and GC analyses.

Isolation of **2b–5b**. The fraction (57 g) containing strictanonic acid (**4a**) was stirred with Et_2O (1400 ml, 8 hr), left in the

Table 2. ^{13}C NMR chemical shifts (δ, $CDCl_3$) of compounds **1b–4b**

C	1b	2b	3b	4b [2]
1	32.7	31.7	32.7	36.2
2	18.7	18.5	18.3	19.4
3	41.7	41.7	41.3	42.0
4	33.1	33.1	32.7	32.7
5	42.6	41.6	44.0	57.2
6	24.1	24.2	23.8*	73.5
7	126.5	137.5	76.6	48.1
8	134.8	135.4	210.4	208.1
9	90.5	88.4	112.8	117.6
10	40.6	40.6	39.8	46.5
11	28.4	27.9	31.6*	30.8
12	38.1	37.6	35.4*	31.2
13	81.5	82.0	82.9	81.4
14	47.9	47.1	47.1	52.5
15	171.8	172.0	171.1	171.6
16	27.3	27.1	25.9	26.0
17	22.3	169.8	25.9	30.4
18	32.9	32.6	32.9	34.3
19	21.2	21.9	21.7	22.2
20	16.7	16.7	17.0	18.1
15-OMe	51.3	51.2	51.3	51.3
17-OMe	—	51.7	—	—

*May be interchanged.

refrigerator overnight, filtered, the solvent removed and the Et_2O-soluble residue submitted to silica gel CC (1200 g, packed in *n*-hexane; eluant *n*-hexane–Me_2CO, 3:1). Fractions containing strictanonic acid (**4a**) were combined, the solvent removed, the residue (31 g) methylated with MeI [4] and the methylated product (30.2 g) submitted to silica gel CC (1 kg packed in *n*-hexane). The column was eluted with *n*-hexane containing increasing concns of Et_2O and fractions (4–78) of 125 ml size were collected after initial collection of three 1 l size fractions (1–3). *n*-Hexane–Et_2O (9:1) eluted compounds **2b**, **3b**, and **5b** and fractions (4–16) containing these compounds were combined (3.27 g). A portion of this fraction (1.0 g) when submitted to prep TLC on silica gel (*n*-hexane–Et_2O, 2:1) gave two fractions, A (410 mg) and B (550 mg). Chrysolic acid methyl ester (**5b**, 98 mg) was isolated from fraction B by a further prep TLC (*n*-hexane–Et_2O, 8:7) and purified by a second and third prep. TLC [*n*-hexane–Et_2O (5:2), 3 developments; (2:1), 5 developments]. Its identity was established by direct comparison with an authentic sample.

Fraction A (400 mg) when submitted to prep. TLC (*n*-hexane–Et_2O, 4:1) gave three fractions, A1 (55 mg), A2 (184 mg) and A3 (130 mg). Compound **2b** (87 mg) was isolated from fr. A2 by prep TLC (*n*-hexane–Et_2O, 2:1, 4 developments). Compound **3b** (97 mg) was isolated from fr. A3 by prep TLC (*n*-hexane–Et_2O, 3:1, 2 developments).

n-Hexane–Et_2O (17:3) eluted strictanonic acid methyl ester (**4b**) together with 6-oxogrindelic acid methyl ester in fractions 36–50 (5.5 g), the latter as the major component. A portion of this fraction (3.1 g), when submitted to prep TLC on silica gel (*n*-hexane–Et_2O, 3:2, 2 developments) in three batches, gave > 98% GC pure strictanonic acid methyl ester (**4b**, 0.79 g), identical in all respects with an authentic sample.

Dimethyl 17-carboxygrindelate (**2b**) *and methyl 7-acetyl-8,17-bisnor-8-oxagrindelate* (**3b**). The spectral properties of these compounds which are described in the text, were in accord with the shown structures. The NMR spectra were obtained on Bruker AM- and WM-250 spectrometers.

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