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GRINDELANE DITERPENOIDS FROM *GRINDELIA SQUARROSA* AND *G. CAMPORUM*

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Key Word Index—*Grindelia squarrosa*, *G camporum*, Asteraceae, Astereae, Soladiginae, diterpene acids, labdanes, methyl 13-isogrindelate, methyl 17-hydroxy grindelate, methyl 17-grindeloxy grindelate, grindelic acid derivatives, flavonoids

Abstract—An acid fraction of the dichloromethane extract of *Grindelia squarrosa* and a neutral fraction of the ethyl acetate extract of *Grindelia camporum* yielded a number of previously known grindelane diterpenoids and flavonoids Along with the known isolates, two new grindelane diterpenoids, 13-isogrindelic acid and 17-grindeloxy grindelic acid, from *G squarrosa* were isolated and identified spectroscopically

INTRODUCTION

The occurrence of grindelic acid and its 17-hydroxy derivative, flavonoids, phenolic acids and monoterpenoids in *Grindelia squarrosa* (Pursh) Dunal, a member of the tribe Astereae, subtribe Soladiginae, has been previously reported [1–3] During the course of our phytochemical investigation of this species, the acid fraction of the dichloromethane extract of the aerial parts of the plant yielded, in addition to grindelic acid and its 17-hydroxy derivative, 11 previously known and two new grindelane diterpenoids, 13-isogrindelic acid (**14**) and 17-grindeloxy grindelic acid (**15**), all isolated as methyl esters We wish to report here the structure elucidation of these two new diterpenoids

In continuation of our chemical investigations of *G camporum* [4], we now report eight naturally occurring grindelane methyl esters and four flavonoids from its neutral fraction

The presence of grindelane acids in *Grindelia* is typical of the labdane-type diterpenoids also found in *Chrysothamnus* [5, 6], *Erigeron* [7] and *Haplopappus* [8], all members of the tribe Astereae in the family Asteraceae Some grindelanes were shown to have feeding deterrent activity towards aphids [9]

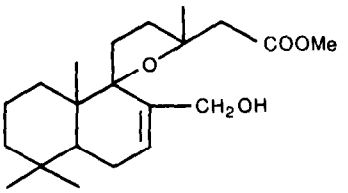
RESULTS AND DISCUSSION

The acid fraction of the dichloromethane extract of the aerial parts of *G squarrosa* was subjected to methylation and the resulting methyl ester mixture, when subjected to normal and reversed phase preparative and semipreparative HPLC, gave 15 grindelane diterpenoids (**1–15**). These included methyl grindelate (**1**) and its six derivatives [6-oxo-(**2**), 17-hydroxy-(**3**), 6-hydroxy-(**4**), 7α,8α-epoxy-(**5**), 6,8(17)-diene-(**6**), and 7-hydroxy-8(17)-dehydro-(**7**)], five 17-substituted homologs [methoxy-(**8**), acetoxy-(**9**), propionyloxy-(**10**), isobutyryloxy-(**11**), and isovaleroyloxy-(**12**)], methyl strictanonoate (**13**), methyl 13-isogrindelate (**14**), and methyl 17-grindeloxy grindelate (**15**) We have previously reported the isolation of naturally occurring acids of **1**, **2** and **13** from the acid fraction of the ethyl acetate extract of *Chrysothamnus paniculatus*, another member of the tribe Astereae of the Asteraceae, and **4–12** from the methylated acid fraction of the ethyl acetate extract of *G camporum* [4, 6 and references cited therein]

All the known diterpenoids (**1–13**) were identified by TLC, spectral and GC retention time comparison with authentic samples Structure **14** was proposed as follows The IR and mass spectra of methyl 13-isogrindelate (**14**), $C_{21}H_{34}O_3$, were very similar to those of **1** except for an intense $[M-Me]^+$ peak However, **14**, unlike **1**, gave different mp, optical rotation, R_f value, and GC retention time, strongly indicating that **14** is an isomer of **1** The ^{1}H NMR spectrum of **14** was similar to that of **1** except for the C-14 methylene protons The two C-14 protons which appear as a doublet at δ 2.67 in **1** collapsed to a singlet at δ 2 58 in **14**, indicating that the two C-14 protons have become equivalent due to free rotation, which is possible for a molecule with reverse configuration (9*R* and/or 13*R*) in contrast to the sterically hindered 9*S*,13*S*-configuration for **1**, established by O'Connell from X-ray crystallography [10] However, for biogenetic reasons the 9*S*-configuration would be preferred We therefore propose that methyl 13-isogrindelate has the 9*S*,13*R*-configuration as shown in **14**

The other diterpene was identified as methyl 17-grindeloxy grindelate (**15**) The molecular formula, $C_{41}H_{64}O_6$, was deduced from its elemental analysis and confirmed by its fast atom bombardment (FAB) mass spectrum which displayed a molecular ion associated peak $[MH]^+$ at *m/z* 653.5

The ^{1}H NMR spectrum of **15** revealed the presence of two one-proton vinyl broad singlets at δ 5 9 and 5 4, a two protons singlet at 4 58, a methyl ester at 3 62, a four protons broad doublet centered at 2.64 and nine C-methyl protons including a vinyl methyl at 1 78 Furthermore, the characteristic ^{13}C NMR [4] peaks for grindelane ester molecules were doubled in the ^{13}C NMR spectrum of **15**



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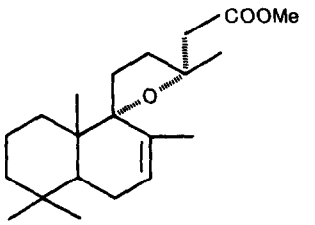

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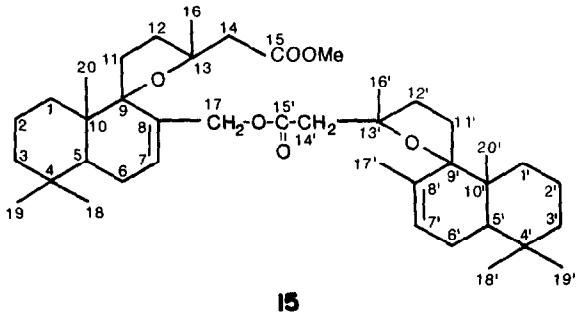


15

These data suggested that **15** was an ester derived from grindelic acid and its 17-hydroxy derivative

The fast atom bombardment (FAB) mass spectral fragmentation of **15** (Scheme 1) was especially informative and established unequivocally the M_r of **15** Allylic cleavage with charge retention by both fragments at C-17 gave a pair of peaks at m/z 333 and m/z 321 which includes an additional mass unit due to hydrogen transfer during the formation of the m/z 321 ion These ion peaks suggested that **15** consists of two grindelane units, strongly supported by their further fragmentation via retro-Diels–Alder (RDA) breakdown leading to two ions at m/z 209 and m/z 197, respectively, as shown in Scheme 1 The appearance of another peak at m/z 528 (unprotonated), stemmed from $[MH-124]^+$ via RDA breakdown involving ring B of one of the two grindelane units, further supports this view [4]

Saponification of **15** gave two grindelane units, grindelic acid and 17-hydroxy grindelic acid These and their methyl ester derivatives, prepared by diazomethane, were identical in all respects with the authentic samples Acetylation (Ac_2O–pyridine) of methyl 17-hydroxy grindelate gave **9** Direct esterification of grindelic acid with methyl 17-hydroxy grindelate in the presence of phenyl dichlorophosphate and pyridine gave a complex reaction product from which **15** was isolated by repetitive preparative TLC and shown to be identical (TLC, IR and ^{1}H NMR) with the natural sample

From the neutral fraction of the ethyl acetate extract of the aerial parts of *G. camporum*, eight grindelane diterpenoids, **1**, **2**, **4**–**7**, **9** and **10** and four previously reported flavonoids, acacetin, kumatakenin, quercetin and its 3,3′-dimethyl ether derivative [11], were isolated by silica gel column chromatography The diterpenoids were identified by direct GC retention time comparison with those isolated from *G squarrosa* We have previously reported the isolation and identification of corresponding acids from the acid fraction of the ethyl acetate extract of *G camporum* [4]

EXPERIMENTAL

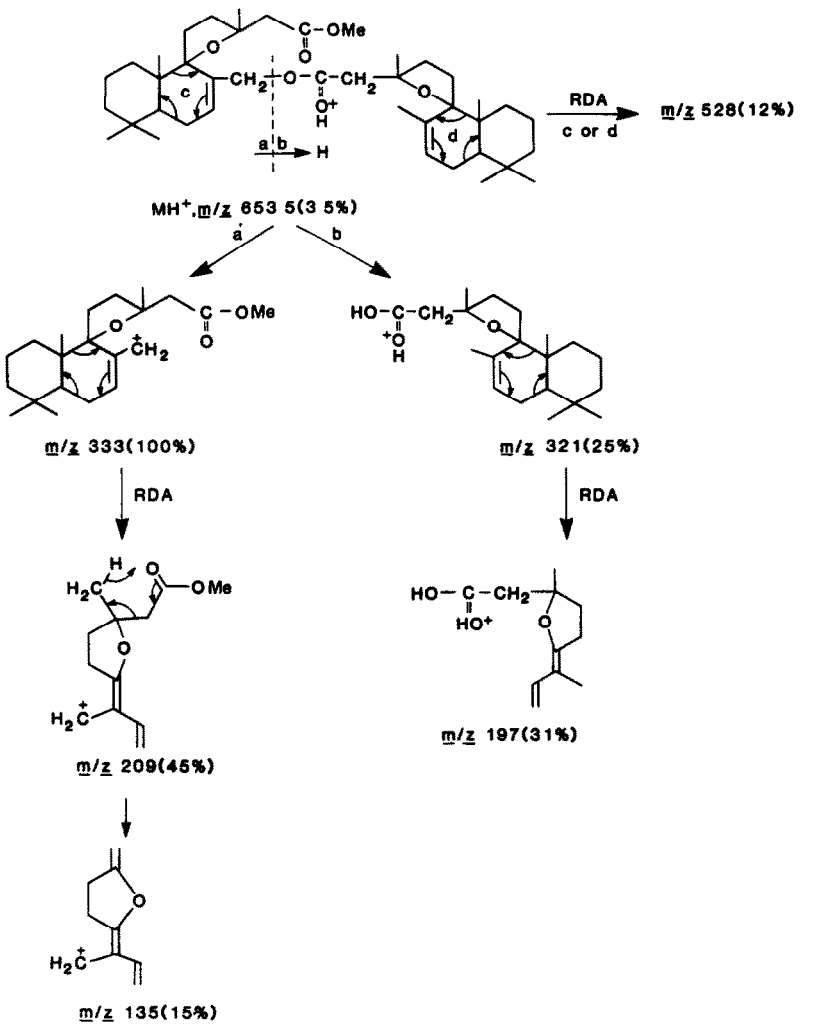
NMR measurements were recorded on JEOL FX90 Q spectrometer with TMS as an int standard The FAB mass spectra were recorded on a Varian MAT 311A mass spectrometer fitted with an 11NF-FAB Ion-Tech Saddle Field gun using Xe as an ionizing gas at a pressure of 10^{-5} torr with an emission current of 1 0 mA and a potential of 8 0 kV in the positive mode Samples were deposited as a thin film on a stainless steel probe tip by dissolving 1–5 μg of sample in 5–10 μl of tetraethylene glycol dimethyl ether (Aldrich Gold Label) and introduced at ambient temp For other instrumentation see ref [4]

Known diterpenoids were identified by direct GC retention time and spectral comparison with authentic samples Flavonoids were identified by co-TLC and spectral comparison with authentic samples

Isolation of compounds **14** *and* **15** *from* G squarrosa The plant material used in this work was collected in Reno, NV, in 1981, extracted exhaustively with CH_2Cl_2 and the extract, after removal of solvent, was stored at $-10°$ before work-up

The procedure used for the isolation of the acid fraction from the dichloromethane extract, methylation (MeI–Me_2CO–K_2CO_3) of the isolated acid fraction and separation of the resulting complex methyl ester mixture into four fractions (I–IV) by HPLC on a Waters PrepPAK-500/silica cartridge column was essentially similar to that described for the EtOAc extract of *G camporum* in ref [4] The GC analysis pattern of the methyl ester mixture obtained from *G squarrosa* was close to that of the methyl ester mixture obtained from *G camporum* [4]

Methyl isogrindelate (**14**) was isolated from fraction I, which contained **1**, **6** and **14**, by silica gel 60 column chromatography (hexane–EtOAc, 98 2) followed by prep TLC (silica gel 60 PF-



Scheme 1 Major fragment ions in the FAB mass spectrum of **15**

254) using the same solvent system From fraction IV, which contained **7**, **9–13** and **15** (major component), methyl 17-grindeloxy grindelate (**15**) was separated by HPLC on a Waters PrepPAK-500 reverse-phase cartridge column followed by a semi-preparative reverse-phase column using MeOH The purity of **15** was assessed only by TLC since it could not be detected by GC under our standard experimental conditions The diterpenoids of fractions II (**1**) and III (**3–5** and **8–12**) were isolated by repetitive prep and semi-prep HPLC on a Waters PrepPAK-500/Silica cartridge column and PrepPAK/Silica packed in a stainless steel column (1 in × 12 in), respectively, using various solvent systems

Methyl isogrindelate (**14**) Colorless crystals, mp 41–42° IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1730 (CO_2R) $[\alpha]_D^{25}$ −17 98° ($CHCl_3$, *c* 2 05) Found C, 73 04, H, 10 13 $C_{21}H_{34}O_3$ requires C, 75 4, H, 10 17 MS *m/z* (rel int) 334 $[M]^+$ (0 54), 319 $[M-Me]^+$ (0 71), 210 $[M-124]^+$ (100), 178 $[210-MeOH]^+$ (3 70), 150 $[210-AcOH]^+$ (3 77), 136 $[210-AcOMe]^+$ (11 33) ^1H NMR (90 MHz, $CDCl_3$) δ5 32 (1H, *br s*, H-7), 3 59 (3H, *s*, C-15 Me), 2 58 (2H, *s*, H-14), 1 65 (3H, *s*, H-17), 1 32 (3H, *s*, H-16), 0 85 (3H, *s*, H-18), 0 84 (3H, *s*, H-19), 0 78 (3H, *s*, H-20)

Methyl 17-grindeloxy grindelate (**15**) Colorless resin, mp 44–46° IR ν_{max}^{KBr} cm^{-1} 1740 (CO_2R) UV λ_{max}^{MeOH} 210 85 nm $[\alpha]_D^{25}$ −123 15° ($CHCl_3$, *c* 1 5) Found C, 75 36, H, 9 40 $C_{41}H_{64}O_6$ requires C, 75 41, H, 9 87 FAB MS *m/z* (rel int) 653 5 $[MH]^+$ (3 50), 528, 333, 321, 209, 197, 135 (see Scheme 1) ^1H NMR (90 MHz, $CDCl_3$) δ5 9 (1H, *br s*, H-7), 5 48 (1H, *br s*, H-7'), 4 58 (2H, *s*, H-17), 3 62 (3H, *s*, H-15 Me), 2 64 (4H, *br d*, H-14, H-14'), 1 78 (3H, *s*, H-17'), 1 35 (6H, *s*, H-16, H-16'), 0 85 (18H, *br s*, H-18, H-18', H-19, H-19', H-20, H-20') ^{13}C NMR ($CDCl_3$) 38 862 (C-1)*, 38 592 (C-1')*, 18 871 (C-2, C-2'), 42 275 (C-3, C-3', C-5'); 33 282 (C-4, C-4'), 42 709 (C-5); 32 741 (C-6, C-18); 33 012 (C-6', C-18'), 132 908 (C-7), 126 569 (C-7'), 135 020 (C-8), 134 912 (C-8'), 90 760 (C-9)†, 89 839 (C-9')†, 40 975 (C-10, C-10'), 24 559 (C-11, C-11'), 27 755 (C-12)‡; 28 405 (C-12')‡, 81 659 (C-13, C-13'), 47 854 (C-14), 48 612 (C-14'), 171 371 (C-15), 170 992 (C-15'), 27 73 (C-16), 27 539 (C-16'), 66 436 (C-17), 22 284 (C-17'), 22 013§ (C-19), 21 092§ (C-19'), 16 866 (C-20, C-20'), 51 050 (OMe) The shift values with the same superscript symbol may be interchanged

Saponification of compound **15** To a soln (100 ml) of 10% KOH in MeOH was added **15** (120 mg) and the mixture refluxed under argon for 2 hr The reaction mixture was cooled, acidified and extracted with Et_2O The Et_2O extract, after removal of solvent, when subjected to prep TLC (silica gel 60 PF-254, C_6H_6–Et_2O–AcOH, 40 10 1), gave grindelic acid and 17-hy-

droxy grindelic acid, identical with the authentic samples Methylation (CH_2N_2) of hydrolysed products gave the corresponding methyl esters, identified by spectral comparison with authentic samples spectra Acetylation (Ac_2O–pyridine) of methylated 17-hydroxy grindelic acid gave **9**

Preparation of compound **15** To a soln of grindelic acid (68 mg) in 1,2-dimethoxyethane (15 ml) at 0°, were added sequentially distilled pyridine (0 24 ml), phenyl dichlorophosphate [$C_6H_5OPOCl_2$] (0 5 ml) and methyl 17-hydroxy grindelate (88 0 mg) [12] The mixture was stirred at room temp under argon for 18 hr The soln was poured into ice-cold 1 N HCl (40 ml) and extracted with $CHCl_3$ (4 × 30 ml) The combined extracts were dried ($MgSO_4$) and concd Repetitive prep TLC (silica gel 60 PF-254) of the reaction mixture with toluene–EtOAc (9 1) gave, among other products, **15** (8 mg, 5% yield) whose IR and ^{1}H NMR spectra were identical to those of naturally occurring **15**

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