Phytochemistry, Vol. 20, No. 9, pp. 2249–2253, 1981.
Printed in Great Britain.

0031-9422/81/092249-05 $02.00/0
Pergamon Press Ltd.

GRINDELANE DITERPENOID ACIDS FROM *GRINDELIA HUMILIS*: FEEDING DETERRENCY OF DITERPENE ACIDS TOWARDS APHIDS

ALLAN F. ROSE*, KENNETH C. JONES, W. F. HADDON and DAVID L. DREYER

U.S. Department of Agriculture, Science and Education Administration, Western Regional Research Center, Berkeley, CA 94710, U.S.A.

(*Received* 28 *January* 1981)

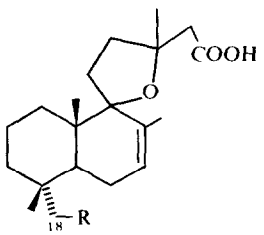
Key Word Index—*Grindelia humilis*; Compositae; diterpene acids; 6α-hydroxygrindelic acid; 6β-hydroxygrindelic acid; structure determination; *Schizaphis graminum*; Aphididae; Homoptera; insect feeding deterrents.

Abstract—Two new labdane diterpene acids have been isolated from *Grindelia humilis*. The spectroscopic properties of the acids and their derivatives, as well as conversion to methyl 6-oxogrindelate, showed that they were the epimeric pair 6α-hydroxy- and 6β-hydroxygrindelic acid. A series of 11 diterpene acids were surveyed for their feeding deterrency towards the aphid *Schizaphis graminum*. The substances tested showed a wide range of activity.

INTRODUCTION

A previous paper from this laboratory reported [1] the isolation and structure determination of two new grindalane diterpene acids from *Chrysothamnus nauseosus* (Pall.) Britt (Compositae). These diterpene acids showed antifeeding properties towards the Colorado potato beetle and were formulated as 18-hydroxygrindelic acid (**1**) and 18-succinyloxygrindelic acid (**2**) based on spectroscopic considerations and chemical interconversions.

To confirm the structures and define the stereochemistry it was desirable to relate these diterpene acids chemically to a known substance. One possible way would be their conversion to grindelic acid (**3**) by the removal of the functional group at C-18. Accordingly, the locally available *Grindelia humilis* (Hook. and Arn.) was examined as a possible source of a reference sample of grindelic acid. Grindelic acid was not found but instead two new labd-7-ene diterpene acids, **4** and **8**, were isolated and their structures are reported in this paper as well as the feeding deterrent properties of 11 diterpene acids towards the aphid *Schizaphis graminum* (Rondani).



1 R = OH
2 R = $OCOCH_2CH_2COOH$
3 R = H

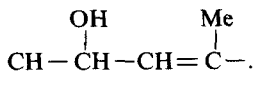
* Present address: Chevron Chemical Co., Ortho Agrochemical Division, 940 Hensley St., Richmond, CA 94803, U.S.A.

RESULTS AND DISCUSSION

Structure elucidation of the new labd-7-ene diterpene acids

The bicarbonate-soluble portion of the crude plant extracts was chromatographed on silica gel. Two of the fractions which were obtained crystallized. The high-resolution MS and carbon count in the ^{13}C NMR (Table 1) indicated that both substances were C_{20} diterpenes of the formula $C_{20}H_{32}O_4$.

The less polar acid, **4**, showed hydroxy and carbonyl bands in the IR. The ^1H NMR spectrum showed a one-proton vinyl multiplet at δ 5.83, a one-proton doublet of multiplets centred at δ 4.38, a two-proton AB quartet, δ 2.52, 2.59 (J = 15 Hz), and five C-methyl resonances, one of which must be due to a vinyl methyl (δ 1.85). Irradiation of the vinyl methyl signal at δ 1.85 caused the multiplet assigned to a alcohol methine proton (δ 4.38) to collapse into a clean AB quartet, J = 3, J = 9 Hz, while the vinyl multiplet collapsed into a clean doublet, J = 3 Hz. These decoupling results indicated that both the vinyl proton and the alcohol methine proton were subject to long-range coupling with the vinyl methyl. Since the decoupled alcohol methine proton signal was a doublet of doublets there had to be in addition to the vinyl proton, one other proton adjacent to the methine hydrogen. These conclusions were supported by decoupling of the vinyl signal at δ 5.83. These results showed that the acid contained the following part structure:



Methylation of **4** with diazomethane gave a monomethyl ester (**5**).

The remaining oxygen atom was identified as a cyclic ether by ^{13}C NMR. The ^{13}C NMR spectrum of **4** showed signals for one carbonyl group, two vinyl carbons and three carbons in the range of δ 60–90. The cyclic ether was implied by two off-resonance singlets (δ 81.2 and 91.3)

Table 1. ^{13}C NMR data of **4**–**6**, **8** and **9** (25 MHz, $CDCl_3$, TMS as int. standard)

C atom	4	5	8	9	6*
1	39.3	38.3	39.0	38.2	38.4
2	18.9	18.9	18.9	18.9	18.1
3	42.5	42.8	43.1	43.2	43.0
4	33.6	33.4	33.6	33.6	32.5
5	44.7	44.9	51.2	51.0	56.4
6	80.7	81.6	68.0	68.0	200.2
7	128.4	126.1	131.4	130.7	129.5
8	139.2	139.8	136.2	136.5	154.9
9	91.3	89.7	91.2	90.1	89.8
10	43.9	43.3	43.5	43.2	45.4
11	27.0†	27.9†	27.4†	27.8†	28.6†
12	33.3†	33.4†	33.6†	33.6†	33.6†
13	81.2	81.7	81.5	81.5	82.6
14	47.1	47.6	47.5	47.6	47.7
15	171.9	171.8	173.4	171.5	171.3
16	26.8	27.5	27.1	27.5	27.6†
17	22.5	22.8	22.7	22.8	21.7
18	34.4	34.9	35.6	35.5	32.8
19	19.0‡	18.6‡	18.6‡	18.6‡	19.8‡
20	21.1‡	20.7‡	20.7‡	20.7‡	20.9‡
Me ester		51.5		51.5	

* Proton noise decoupled spectrum not available.
† Interchangeable.
‡ Interchangeable.

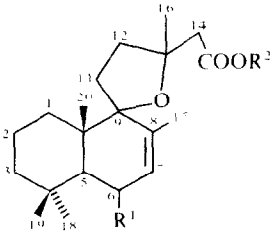
assignable to tertiary carbons bearing oxygen. The exceptionally low field resonance of one of these signals (δ 91.3) required that this carbon be adjacent to a quarternary centre. These data suggested that **4** contains a C-9, C-13 oxygen bridge. Jones oxidation of the methyl ester gave a product (**6**) which showed two carbonyl bands, 1738, 1665 cm^{-1}, in the IR and lacked the hydroxy band. The IR and NMR spectra suggested that this substance was an α,β-unsaturated ketone. This was supported by the UV spectrum. This ketone is a known substance [1, 2], methyl 6-oxogrindelate (**6**), and the IR spectrum was identical with that previously published [2]. The corresponding 6-oxogrindelic acid (**7**) occurs naturally in *G. robusta* [2]. These data showed that the non-polar acid was 6-hydroxygrindelic acid.

The ^1H NMR spectrum of the more polar acid (**8**) showed signals for one vinyl hydrogen, a multiplet at δ 4.05 for a secondary alcohol methine proton, two exchangeable protons, a two-proton AB quartet centred at δ 2.58 and five C-methyl resonances. One of the C-methyl signals appeared to be due to a vinyl methyl (δ 1.77). Irradiation of the vinyl methyl at δ 1.77 in the D_2O exchanged spectrum caused a collapse of the vinyl proton signal to a clean doublet, J = 3 Hz, while the signal at δ 4.05 collapsed to a clean doublet of doublets (J = 8 and 3 Hz). On the other hand, irradiation of the δ 4.05 signal caused the vinyl proton signal at δ 5.55 to take the form of a broadened singlet indicating the presence of allylic coupling. Moreover, the vinyl methyl resonance at δ 1.77 was sharpened. Finally, irradiation of the δ 5.55 vinyl resonance caused the δ 1.77 vinyl methyl resonance to sharpen and the δ 4.05 methine resonance to collapse to a doublet of two broadened singlets. Therefore, the methine proton was not only adjacent to the vinyl proton but was adjacent to one more aliphatic hydrogen. These data again suggested the presence of the same allyl alcohol part structure as encountered in **4**. The ^{13}C NMR spectrum confirmed the presence of an acid group, one trisubstituted double bond, a hydroxy group and, in addition, indicated the presence of a cyclic ether. The lack of additional signals in the δ 3.5–4.0 region of the ^1H NMR and the lack of multiplicity in the undecoupled ^{13}C spectrum indicated that the ether oxygen terminated at fully substituted carbons. The close similarity of the ^{13}C NMR spectra for the two acids, **4** and **8** (Table 1) with that from the 18-substituted grindelic acids from *Chrysothamnus* [1] suggested that they all possessed the same carbon skeleton as well as the C-9, C-13 oxygen bridge.

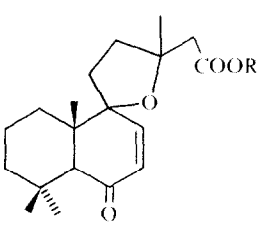
Treatment of **8** with diazomethane gave the corresponding methyl ester (**9**). The methyl ester showed a carbonyl band in the IR at 1740 cm^{-1} and a hydroxy band at 3400 cm^{-1}. Proton spin–spin decoupling results on **9** paralleled those on the parent acid (**8**). Jones oxidation of the methyl ester (**9**) gave the same α,β-unsaturated ketone (**6**) [2] as was obtained from the methyl ester of the less polar acid (**5**). Since this ketone (**6**) had the same optical rotation whether obtained by oxidation of **5** or **9** then **4** and **8** must have differed only in the configuration of the 6-hydroxy group and the stereochemistry at all the other centres had to be the same.

The rotational data (see Experimental) indicated that **6** was stereochemically homogeneous with other naturally occurring 6-oxolabd-7-enes; 6-oxogrindelic acid [2, 3], dihydrohedychenone [4] and 6-oxocativic acid [5].

The MS of the acids, **4** and **8**, and their methyl esters supported the presence of the functional groups assigned in each case. In addition, a major fragmentation pattern



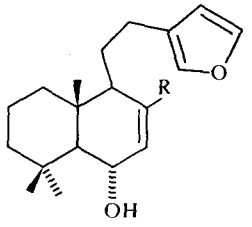
4 $R^1 = \alpha$ OH; R^2 = H
5 $R^1 = \alpha$ OH; R^2 = Me
8 $R^1 = \beta$ OH; R^2 = H
9 $R^1 = \beta$ OH; R^2 = Me



6 R = Me
7 R = H

was attributed to a reverse Diels–Alder reaction associated with the double bond in the B-ring [6] (*m/z* 124 and 212 for **4** and **8**). This indicated that the double bond was correctly located at C-7.

The multiplicity of the H-6 resonance in both **4** and **8** was very similar and the coupling constants extracted from the decoupled spectra were $J_{H-5,H-6} = 9$ Hz in **4** and 8 Hz in **8**. These values did not permit assignment of stereochemistry to H-6 based on the Karplus expression since such large coupling constants would allow either a small or large dihedral angle between H-5 and H-6. On the other hand, there was a striking difference between the chemical shifts of H-6 in **4** (δ 4.38) and **8** (δ 4.08). The stereochemistry of H-6 in **10** and its derivatives had been assigned axial on the basis of a large coupling constant ($J_{H-5,H-6} = 11$ Hz) [7]. The chemical shift of H-6 was δ 4.29 in the related derivative, **11** [7]. This compared well with the chemical shift of H-6 in **4** (δ 4.38) relative to that of **8** (δ 4.08). However, the lack of NMR data on the β-isomer of **11** made the assignment based on chemical shift values uncertain since cases were known [8] where there were very small differences in the chemical shifts of the methine protons or carbons in epimeric 2-cyclohexen-1-ols. Since there was a large difference in the optical rotation of the epimers **4** and **8** assignment of configuration at the 6-position could be made using Mills' rule [9, 10]. Especially analogous was the case of the epimeric cholest-5-en-7-ols [10, 11]. Thus the β-isomer should be much more laevorotatory than the equatorial alcohol. On this basis **4** is a 6α-hydroxygrindelic acid and **8** is 6β-hydroxygrindelic acid.



10 R = COOH
11 R = CH_2OH

Biological activity

The larval growth inhibition of a series of diterpene acids towards the sunflower moth (*Homeosoma electellum* H.) as well as other Lepidoptera species had been reported [12, 13]. Using a previously described [14] bioassay towards the aphid, *Schizaphis graminum*, a series of diterpene acids were measured for their feeding deterrent activity.

The bioassay consisted of a synthetic diet to which substances to be tested were added. Each substance was tested at a series of concentrations so that a dose–response curve could be constructed. From this curve a concentration could be obtained at which half of the aphids would not feed, ED_{50}. As the results in Table 2 show, a wide range of responses were obtained. Some of the compounds were extremely active at levels far below that often found in plants. Aphids, however, are often very selective about the tissues on which they feed. As a result, the effect of such substances in nature depends upon the actual concentration in those tissues on which the insect actually feeds. This may be very low in spite of high concentrations of the acids in special storage tissues which the aphid can easily avoid.

Table 2. Feeding deterrency of some diterpene acids

Compound	ED_{50} (% of diet)	Compound	ED_{50} (% of diet)
4	0.02	**14**	0.1
8	0.02	**15**	0.03
1	0.002	**16**	0.006
2	0.003	**17**	0.01
12	Inactive at 1	**18**	0.004
13	Inactive at 1		

EXPERIMENTAL

Isolation. Grindelia humilis was collected along the shore-line of San Francisco Bay; along Hoffman Blvd., about 0.5 mile N of Albany junction with Buchanan St. Extraction of 285 g of dried and ground inflorescences with $CHCl_3$ in a Soxhlet extractor followed by partitioning with aq. $NaHCO_3$ yielded 10 g of an acid fraction. The acid fraction was chromatographed on Si gel with C_6H_6 containing increasing amounts of Et_2O. Two of the 27 fractions which were collected cryst. and were used for the structural studies.

6α-Hydroxygrindelic acid (**4**) was the least polar crystalline component, mp 106–108° (hexane–EtOAc); IR ν_{max}^{Nujol} cm^{-1}: 3310, 1705; UV λ_{max}^{EtOH}: end adsorption; ^1H NMR (90 MHz, $CDCl_3$): δ 5.83 (*m*, H-7) 4.38 (*d* of multiplets, H-6), 2.52 (1 H, *d*, $J = 15$ Hz), 2.59 (1 H, *d*, $J = 15$ Hz, H-14) 1.85 (3 H, *t*, $J = 2$ Hz, H-17), 1.38 (3 H, *s*), 1.08 (3 H, *s*), 0.98 (3 H, *s*), 0.84 (3 H, *s*); MS (probe) *m/z* (rel. int.): 336 $[M]^+$ (4), 318 (19), 212 (32), 210 (35), 189 (98), 183 (28), 123 (24), 109 (32), 69 (52), 55 (44), 43 (100); high resolution MS *m/z* 336.2300 (calc. for $C_{20}H_{32}O_4$, 336.2300);

$[\alpha] =$	589	578	546	436	365 nm
	+19.3°	+20.1°	+22.7°	+37.8°	+59.8°

($c = 0.586$, $CHCl_3$).

6β-Hydroxygrindelic acid (**8**) was the more polar crystalline component; mp 147–150° (hexane–EtOAc); IR ν_{max}^{Nujol} cm^{-1}: 3340, 1706; UV λ_{max}^{EtOH} end adsorption; ^1H NMR (90 MHz, $CDCl_3$): δ 5.9 (2 H, *s* (*br*) exchangeable with D_2O), 5.64 (1 H, *m*, H-7), 4.08 (2 H, *m*, H-6), 2.53 (1 H, *d*, $J = 14$ Hz), 2.63 (1 H, *d*, $J = 14$ Hz, H-14), 1.78 (3 H, *t*, $J = 2$ Hz, H-17), 1.37 (3 H, *s*), 1.12 (3 H, *s*), 1.01 (3 H, *s*), 0.84 (3 H, *s*); MS (probe) *m/z* (rel. int.): 336 $[M]^+$ (1), 318 (18), 212 (15), 187 (100); high resolution MS *m/z* 336.2302 (calc. for $C_{20}H_{32}O_4$, 336.2300);

$[\alpha] =$	589	578	546	436	365 nm
	−42.9	−45.0	−51.7	−93.4	−151.5°

($c = 0.573$, $CHCl_3$).

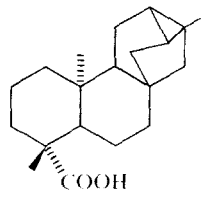
The Me esters were prepared by treatment of the acids **4** and **8**, with CH_2N_2 in Et_2O.

Methyl 6α-hydroxygrindelate (**5**) was an oil; IR ν_{max}^{film} cm^{-1}: 3400, 1740; ^1H NMR (90 MHz, $CDCl_3$): δ 5.49 (*m*, H-7), 4.05 (1 H, *m*, H-6), 3.63 (3 H, *s*, OMe), 2.53 (1 H, *d*, $J = 15$ Hz, H-14), 2.66 (1 H, *d*, $J = 15$ Hz, H-14), 1.76 (3 H, *t*, $J = 2$ Hz, H-17), 1.30 (3 H, *s*), 1.12 (3 H, *s*), 0.99 (3 H, *s*), 0.81 (3 H, *s*); MS (probe) *m/z* (rel. int.): 350 $[M]^+$ (9), 332 (25), 226 (70), 224 (59), 197 (50), 187 (63), 135 (53), 82 (49), 69 (60), 55 (62), 43 (100); high resolution MS *m/z* 350.2462 (calc. for $C_{21}H_{34}O_4$, 350.2457);

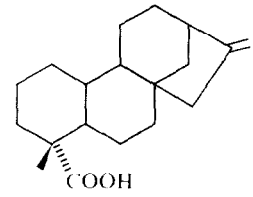
$[\alpha] =$	589	578	546	436	365 nm
	−24.0	−25.2	−29.2	−55.3	−94.1°

($c = 1.33$, $CHCl_3$).

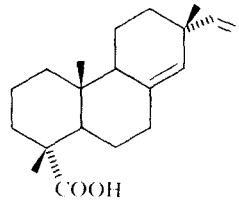
Methyl 6β-hydroxygrindelate (**9**): mp 149–150° (hexane–EtOAc); IR ν_{max}^{Nujol} cm^{-1}: 1725; ^1H NMR (90 MHz,



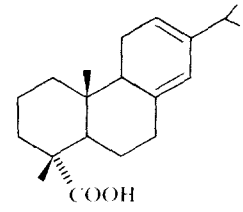
12 Trachyloban-19-oic acid



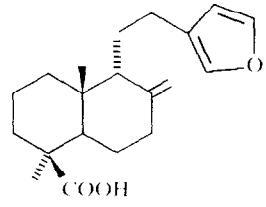
13 Kaur-16-en-19-oic acid



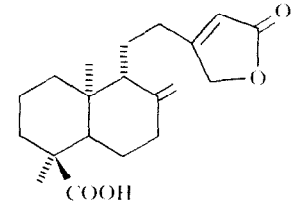
14 Sandaracopimaric acid



15 Levopimaric acid



16 Lambertianic acid
17 Daniellic acid (antipode of **16**)



18 Eperu-8(17)-en-15,19-dioic acid butenolide

$CDCl_3$): δ 5.68 (*m*, H-7), 4.37 (1 H, *d* of multiplets $J = 9$ Hz, H-6), 3.61 (3 H, *s*, OMe), 2.50 (1 H, *d*, $J = 15$ Hz, H-14, 2.63 (1 H, *d*, $J = 15$ Hz, H-14). 1.80 (3 H, *t*, $J = 2$ Hz, H-17), 1.30 (3 H, *s*), 1.06 (3 H, *s*), 0.98 (3 H, *s*), 0.81 (3 H, *s*); MS (probe) *m/z* (rel. int.): 350 $[M]^+$ (5), 332 (19), 226 (36), 224 (42), 187 (55), 135 (35), 111 (35), 95 (43), 82 (32), 69 (52), 55 (50), 43 (100); high resolution MS *m/z* 350.2456 (calc. for $C_{21}H_{34}O_4$, 350.2457);

[α] =	589	578	546	436	365 nm
	−66.5	−69.6	−79.7	−142.7	−235.1°

($c = 0.487$, $CHCl_3$).

Methyl 6-oxogrindelate (**6**). Oxidation of **5** or **9** with Jones reagent gave **6**; mp 70–71° (hexane–EtOAc); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1665; λ_{max}^{EtOH} nm: 232; ^1H NMR (90 MHz, $CDCl_3$): δ 5.69 (1 H, *s br*, H-7), 3.68 (3 H, *s*, OMe), 2.78 (1 H, *s*, H-5), 2.72 (1 H, *d*, $J = 14$ Hz, H-14), 2.57 (1 H, *d*, $J = 14$ Hz, H-14), 1.99 (3 H, *d*, $J = 1$ Hz, H-17), 1.43 (3 H, *s*), 1.24 (3 H, *s*), 1.14 (3 H, *s*), 0.98 (3 H, *s*); ^1H NMR (90 MHz, C_6D_6); δ 5.70 (1 H, (*br*), H-7), 3.27 (3 H, *s*, OMe), 2.91 (1 H, *s*, H-5), 2.57 (1 H, *d*, $J = 14$ Hz, H-14), 2.39 (1 H, *d*, $J = 14$ Hz, H-14), 1.61 (*d*, $J = 1$ Hz, H-17), 1.33 (3 H, *s*), 1.31 (3 H, *s*), 1.30 (3 H, *s*), 0.76 (3 H, *s*); MS (probe) *m/z* (rel. int.): 348 $[M]^+$ (4), 224 (100), 164 (16), 150 (32), 114 (27), 111 (80), 109 (19), 82 (73), 69 (25), 55 (31), 43 (44); high resolution MS *m/z* 348.2309 (calc. for $C_{21}H_{32}O_4$, 348.2300). For **6** from oxidation of **9**,

[α] =	589	578	546	436	365 nm
	−84.4	−86.4	−95.5	−102.5	+363.8°

($c = 0.995$, $CHCl_3$).

For **6** from oxidation of **5**,

[α] =	589	578	546	436	365 nm
	−85.8	−87.4	−98.4	−107.5	+350.8°

($c = 0.254$, $CHCl_3$).

Acknowledgements—The authors are indebted to John Strother for identification of plant material, to M. Benson for the ^{13}C NMR data, to S. Tillin for the MS data and to Carl Elliger for samples of some of the resin acids.

REFERENCES

1. Rose, A. F. (1980) *Phytochemistry* **19**, 2689.
2. Mangoni, L. and Belardini, M. (1962) *Gazz. Chim. Ital.* **92**, 983.
3. O'Connell, A. M. (1973) *Acta Crystallogr.* **B29**, 2232.
4. Sharma, S. C., Tandone, J. S., Upredty, H., Shukla, Y. N. and Dhar, M. M. (1975) *Phytochemistry* **14**, 1059.
5. Halsall, T. G. and Moyle, M. (1960) *J. Chem. Soc.* 1324.
6. Enzell, C. R. and Ryhage, R. (1965) *Ark. Kemi* **23**, 367.
7. Bohlmann, F., Zdero, C., King, R. M. and Robinson, H. (1979) *Phytochemistry* **18**, 1533.
8. Wahlberg, I., Almqvist, S., Nishida, T. and Enzell, C. R. (1975) *Acta Chem. Scand.* **29B**, 1047.
9. Mills, J. A. (1952) *J. Chem. Soc.* 4976.
10. Klyne, W. (1952) *Helv. Chim. Acta* **35**, 1224.

11. Shoppee, C. W. and Newman, B. C. (1968) *J. Chem. Soc. C* 981.
12. Waiss, A. C., Jr., Chan, B. G., Elliger, C. A., Garrett, V. A., Carlson, E. and Beard, B. H. (1977) *Naturwissenschaften* **64**, 341.
13. Elliger, C. A., Zinkel, D. F., Chan, B. G. and Waiss, A. C., Jr. (1976) *Experientia* **32**, 1364.
14. Dreyer, D. L., Reese, J. C. and Jones, K. C. (1981) *J. Chem. Ecol.* **7**, 273.