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HAVARDIC ACIDS A–F AND HAVARDIOL, LABDANE DITERPENOIDS FROM *GRINDELIA HAVARDII*

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Key Word Index—*Grindelia havardii*; Asteraceae; Astereae; Solidagininae; diterpenoid acids; labdanes; havardic acids A–F; havardiol.

Abstract—Seven new labdane diterpenoids, designated as havardic acids A–F (as methyl esters) and havardiol, have been obtained from the dichloromethane extract of the aerial parts of *Grindelia havardii*, and their structures have been deduced on the basis of NMR, MS and IR data. Havardic acid E has lost carbons-14 and -15, whereas havardic acid F and havardiol have lost carbon-17.

INTRODUCTION

As part of our continuing phytochemical investigation of the New World genus *Grindelia*, we have now examined the acid constituents of the resin of *G. havardii* Steyerm. from New Mexico. This paper describes the isolation and characterization of the labdane diterpenoids from the acid fraction of the dichloromethane extract of the aerial parts of *G. havardii*.

RESULTS AND DISCUSSION

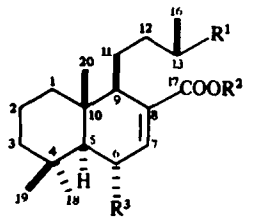
The dichloromethane extract of the aerial parts of *G. havardii* gave an acid fraction which was subsequently methylated. TLC and GC analyses of the resulting methyl ester mixture indicated total dissimilarity with the methyl ester mixture from the acid fraction of *G. camporum* [1]. Silica gel column chromatography of the methyl ester mixture, eluting with *n*-hexane–ethyl acetate, followed by preparative TLC, yielded seven new labdanoids: methyl havardates A–F (**1b**–**6b**) and havardiol (**7**). These new labdanoids were characterized spectroscopically as described below. Methyl havardates D (**4b**) and E (**5b**) were not separated from one another, but were characterized by spectral analyses of the mixture.

Methyl havardate A (**1b**)

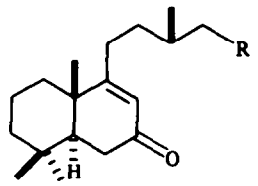
Methyl havardate A had molecular weight 320 by low-resolution mass spectrometry and molecular formula $C_{22}H_{36}O_4$ by high-resolution mass spectrometry. Its IR spectrum suggested the presence of an alkene (1648 cm^{-1}), two carbonyls (1742 and 1722 cm^{-1}) and a geminal dimethyl (1388 and 1365 cm^{-1}) grouping, but lacked hydroxyl absorptions. The electron impact (EI) mass spectrum of **1b** (Scheme 1) exhibited the characteristic feature expected from the fragmentation pattern of 7-ene labdanoids and their derivatives, which are known to undergo retro-Diels–Alder (RDA) decomposition before and/or after initial loss of neutral molecules. Losses of water, methanol and acetic acid from $[M]^+$ give a pair of peaks, one (minor) involving the ring A fragment and the other (major) corresponding to the remaining fragment. The most striking quantitative difference in the EI mass spectrum of **1b** was that the former fragment (*m/z* 124, 40.7%) and its further decomposition product (*m/z* 109, base peak) dominated the entire spectrum, overshadowing the latter fragment (*m/z* 240, 1.9%). As expected by analogy with other 17-substituted labdanoids, the molecular ion (*m/z* 364, 0.2%) was only of very low abundance. The appearance of pronounced peaks at *m/z* 332 and 300, formed by successive losses of two molecules of methanol from $[M]^+$, clearly suggested the presence of two carbomethoxyl groupings in **1b**. That the second carbomethoxyl grouping involves C-17, which is usually substituted by a methyl group in many labdanoids, was recognized by the loss of pentanoic acid methyl ester side-chain from *m/z* 364 $[M]^+$ and *m/z* 332 $[M - MeOH]^+$ ions, giving rise to peaks at *m/z* 235 and 203, respectively. The elemental compositions of major fragments above *m/z* 100 were substantiated by high-resolution mass spectrometry.

The ^1HNMR (Table 1) and ^{13}CNMR (Table 2) spectra fully support structure **1b** for this substance. Comparisons with the spectra of known labdanes [2] and of other substances in the same plant species (e.g. **2b**, which differs only in the long side-chain, and **4b**, which has the same side chain) were very helpful in making the final spectral assignments.

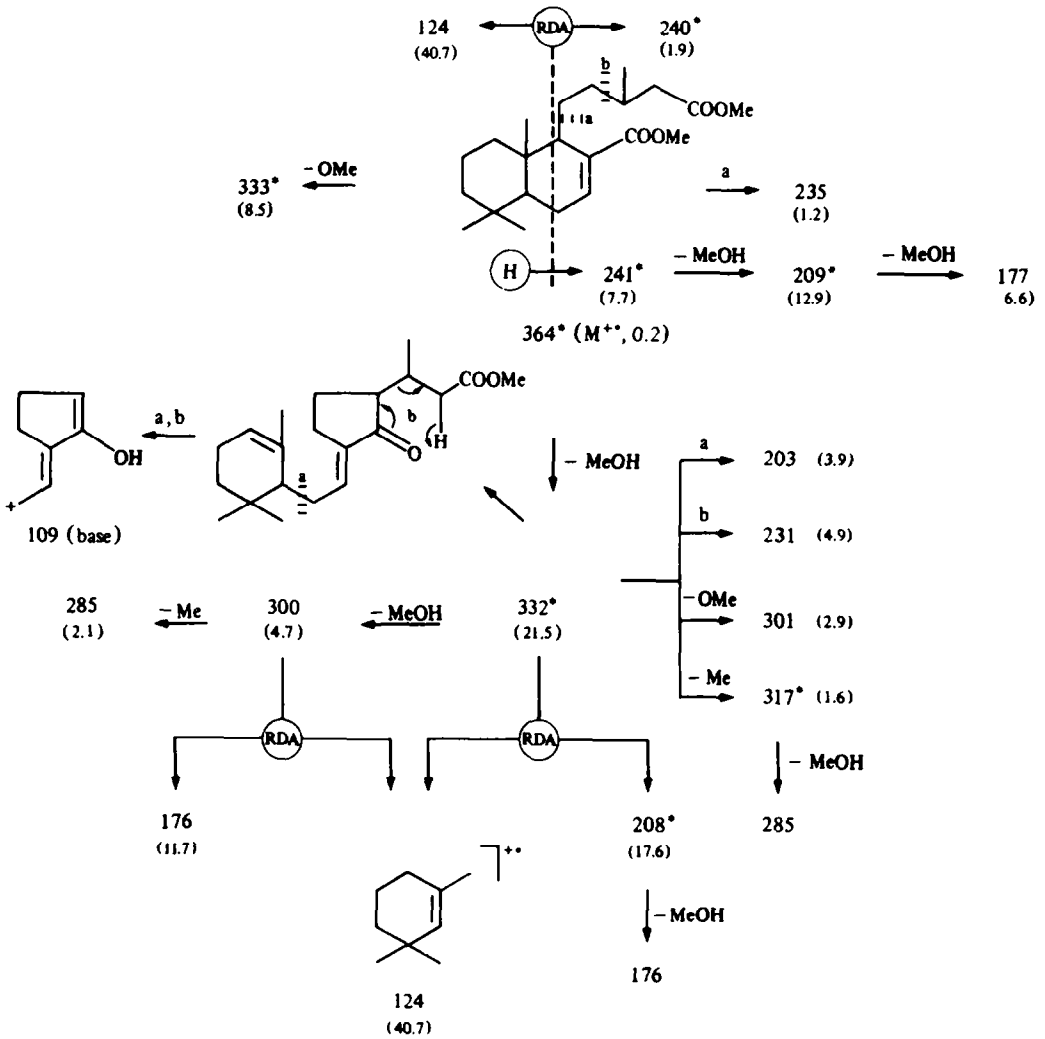
That these diterpenoids have the labdane stereochemistry shown (fixing the configurations at C-5, C-9, C-10 and C-13) is expected by analogy with all other diterpenoids so far found in the genus *Grindelia* [1–11] and supported by the large positive change in $[\alpha]_D$ caused by the addition of an equatorial hydroxyl group at C-6 (**2b** → **3b** compared to methyl grindelate → methyl 6-hydroxygrindelate [6]).



	R^1	R^2	R^3
1a	CH_2COOH	H	H
1b	CH_2COOMe	Me	H
2a	$(CH_2)_2OH$	H	H
2b	$(CH_2)_2OH$	Me	H
3a	$(CH_2)_2OH$	H	OH
3b	$(CH_2)_2OH$	Me	OH
4a	CH_2COOH	H	=O
4b	CH_2COOMe	Me	=O
5a	=O	H	H
5b	=O	Me	H



	R
6a	COOH
6b	COOMe
7	CH_2OH



Scheme 1. Major fragment ions (m/z ratios, relative intensities in parentheses), established by high-resolution exact mass measurements, in the EI mass spectrum of **1b**. *Decreased by 28 and 12 mu in **2b** and **3b**, respectively.

Table 1. ^{1}H NMR chemical shifts (δ, TMS–$CDCl_3$) and coupling constants (Hz, in parentheses) for compounds **1b–6b** and **7**

Proton	**1b**	**2b**	**3b**	**4b**	**5b**	**6b**	**7**
1 β	1.83 *br d* (13.0)	1.85 *br d* (12.9)	1.77 *br d* (12.6)	1.85 *br d* (13.0)	1.85 *br d* (13.0)	1.87 *br d* (12.4)	1.88 *br d* (12.1)
5	1.19 *dd* (11.7, 2.9)	1.20 *dd* (11.7, 4.9)	1.15 *d* (10.2)	2.10 *s*	1.19 *dd* (11.2, 5.4)	1.73 *dd* (13.5, 4.3)	1.73 *dd* (13.4, 4.2)
6	2.02 *m* 2.16 *m*	2.02 *m* 2.16 *m*	4.36 *ddd* (10.2, 3.3, 2.3)	—	2.02 *m* 2.16 *m*	α2.47 *dd* (17.5, 4.3) β2.34 *dd* (17.5, 13,5)	2.47 *dd* (17.6, 4.2) 2.34 *dd* (17.6, 13.4)
7	6.63 *m*	6.63 *m*	6.43 *t* (2.3)	6.25 *d* (3.2)	6.71 *m*	—	—
8	—	—	—	—	—	5.71 *br s*	5.73 *br s*
9	2.06 *m*	2.06 *m*	2.06 *m*	2.44 *m*			
11					~ 1.40 *m* ~ 1.85 *m*	2.21 *dddd* (17.0, 10.3, 5.4, 1.3)	2.21 *dddd* (17.0, 10.3, 5.4, 1.3)
12					2.43 *m* 2.88 *ddd* (17.3, 11.2, 4.8)		
13	1.87 *m*			1.90 *m*		2.01 *m*	
14	2.08 *dd* (14.7, 8.2) 2.30 *dd* (14.7, 5.9)			2.08 *dd* (14.8, 7.9) 2.29 *dd* (14.8, 6.2)		2.19 *dd* (15.1, 7.4) 2.32 *dd* (15.1, 6.1)	
15	—	~ 3.70 *m*	~ 3.70 *m*	—	—	—	3.70 *m*
16	0.92 *d* (6.9)	0.89 *d* (7.5)	0.88 *d* (6.3)	0.94 *d* (6.6)	2.12 *s*	0.98 *d* (6.6)	0.94 *d* (6.5)
18	0.90 *s*	0.90 *s*	1.14 *s*	1.18 *s*	0.90 *s*	1.12 *s*	1.12 *s*
19	0.86 *s*	0.86 *s*	1.07 *s*	1.10 *s*	0.87 *s*	0.94 *s*	0.94 *s*
20	0.81 *s*	0.82 *s*	0.85 *s*	0.90 *s*	0.82 *s*	0.90 *s*	0.90 *s*
15-OMe	3.66 *s*	—	—	3.67 *s*	—	3.68 *s*	
17-OMe	3.71 *s*	3.70 *s*	3.72 *s*	3.80 *s*	3.70 *s*	—	—

Table 2. ^{13}C NMR chemical shifts (δ, TMS–$CDCl_3$) for compounds **1b–6b** and **7**

Carbon	1b	2b	3b	4b	5b	6b	7
1	39.6	39.6	39.5	38.9	39.3	35.4	35.3
2	18.5	18.5	18.3	18.0	18.4	18.6	18.5
3	42.1	42.1	43.5	42.9	42.0	41.5	41.3
4	32.7	32.8	33.1	32.2	32.7	33.4	33.4
5	49.5	49.5	56.7	52.4	49.4	51.0	51.0
6	23.9	23.9	68.7	199.8	23.9	35.4	35.3
7	136.7	136.7	139.4	131.6	137.9	200.2	*
8	135.4	135.6	135.7	150.2	134.7	123.8	123.6
9	51.2	51.2	50.9	63.9	50.4†	175.2	176.0
10	36.9	36.9	39.6	42.6	37.0	40.4	40.3
11	25.5	25.7	25.7	25.8	21.9	28.0	28.0
12	38.0	38.3	38.5	38.2	45.4	34.7	35.1
13	31.2	30.6	30.5	30.9	208.8	30.4	29.6
14	41.4	39.7	39.6	41.2	29.7	41.3	39.8
15	173.6	61.1	60.9	173.3	—	173.1	60.9
16	19.7	19.7	19.6	19.7	—	19.7	19.5
17	169.6	169.8	169.5	168.4	169.2	—	—
18	33.1	33.1	36.4	33.2	33.0	32.6	32.6
19	21.9	21.9	22.4	21.5	21.9	21.4	21.4
20	14.3	14.3	15.4	15.0	14.0	18.5	18.4
15-OMe	51.1‡	—	—	51.2	—	51.3	—
17-OMe	51.0‡	51.3	51.4	52.4†	52.0†	—	—

*Not visible above noise.

†,‡Values with the same symbol may be interchanged.

Methyl havardate B (**2b**)

A similar approach was employed successfully to assign structure **2b** for methyl havardate B, molecular weight 336 by low-resolution mass spectrometry. The IR spectrum of **2b** showed bands for the presence of a hydroxyl group (3580 cm^{-1}) in addition to C=C (1648 cm^{-1}), $-C(Me)_2-$ (1390 and 1368 cm^{-1}) and C=O (1720 cm^{-1}) groupings, and the EI mass spectrum essentially followed the fragmentation pattern outlined for **1b**. The $[M]^+$ (*m/z* 336, 0.7%), *m/z* 304 ($[M-MeOH]^+$, 19.9%), *m/z* 203 (*m/z* 304 – 101 amu), *m/z* 124 (43.0%) and *m/z* 109 (base) peaks indicated that the only difference between **2b** and **1b** was that the COOMe grouping in **1b** was replaced by a CH_2OH grouping in **2b**. Fragments which do not contain this side chain were found in the spectra of both **1b** and **2b** with the same *m/z* value, while those containing the CH_2OH grouping were lowered by 28 mu in the latter, as shown in Scheme 1. The NMR spectra (Tables 1 and 2) were in complete accord with structure **2b**.

Methyl havardate C (**3b**)

Methyl havardate C, molecular weight 352 by low-resolution mass spectrometry, gave an overall IR spectrum [3440 (OH), 1725 (C=O), 1650 (C=C) and 1385–1365 ($-C(Me)_2-$) cm^{-1}] and fragmentation pattern resembling that of **2b**. From the $[M]^+$ (*m/z* 352, 5.8%), which indicated an additional oxygen atom, and two high mass range peaks at *m/z* 334, $[M-H_2O]^+$ and *m/z* 302 (*m/z* 320 $[M-MeOH]^+ - H_2O]^+$, the presence of a hydroxyl group as an additional substituent in **3b** was apparent. So was a $CH_2CH_2CH(Me)CH_2CH_2OH$ (101 amu) grouping, inferred from *m/z* 233 (*m/z* 334 – 101 amu, 59.6%) and *m/z* 201 (*m/z* 302 – 101 amu, 25%) peaks. Evidence that the new hydroxyl group was at C-6 came from the most characteristic peaks at *m/z* 196 (73.7%, shifted from *m/z* 180 in **2b** and *m/z* 208 in **1b**), 124 (16.0%) and 109 (77.3%), arising from RDA breakdown of the *m/z* 320 fragment (Scheme 1). In the ^1H NMR spectrum (Table 1), decoupling confirmed that the proton attached to the hydroxyl-bearing carbon was coupled to the C-7 vinyl proton, confirming the location of the hydroxyl at C-6, and that it had a large coupling (J = 10.2 Hz) with the axial proton at C-5, showing that the hydroxyl at C-6 was equatorial.

Methyl havardates D (**4b**) *and E* (**5b**)

Although methyl havardates D and E were not separated owing to their very similar R_f values on chromatography, their structures were evident from the NMR and mass spectra of the 70% D–30% E mixture obtained. The IR (CCl_4) spectrum of the mixture indicated the presence of an alkene (3010 and 1642 cm^{-1}), three carbonyls including an α,β-unsaturated C=O (1730, 1715 and 1685 cm^{-1}) and a geminal dimethyl (1385 and 1360 cm^{-1}) but lacked hydroxyl absorptions. The NMR spectra of the mixture (Tables 1 and 2) confirmed the keto group in **4b** to be at C-6 by its effects on the ^1H and ^{13}C shifts and ^1H–^1H coupling constant for the nearby atoms, and that otherwise, the compound was like **1b**. Similarly, it led to the recognition that **5b** was also identical to **1b** except for a side chain shortened by two atoms and possessing a keto group at C-13 (δ208.8; H-16 now giving a singlet at δ2.12). The EI mass spectrum of the mixture, which was very informative, fully supported these findings. The molecular ion peaks and diagnostic fragments,

corresponding to **5b**, were sorted out and rationalized as shown in Scheme 2.

Methyl havardate F (**6b**)

The IR (CCl_4) spectrum of **6b** showed bands for the presence of many of the structural elements of **1b** [1740 (C=O), 3010, 1615 (C=C) and 1375–1365 ($-C(Me)_2-$) cm^{-1}]; the most striking difference was the appearance of a strong band at 1670 cm^{-1} in **6b**, indicating the presence of a conjugated carbonyl group. The EI mass spectrum of **6b** displayed a strong molecular ion peak at *m/z* 320 (24.6%), determined by high-resolution mass spectrometry to be $C_{20}H_{32}O_3$; since the compound is a methyl ester, one of the 20 labdane carbons had been lost, presumably C-17 via decarboxylation. The major fragments observed and their genesis are depicted in Scheme 3. The operation of RDA fragmentation from $[M]^+$ was not observed, which ruled out the C=O group being at C-6 with C=C at C-7–C-8, but it did operate from the *m/z* 219 (base peak) ion ($[M]^+ - 101$ amu), resulting in the appearance of a second major peak at *m/z* 95 (44.8%). These characteristic peaks, which were of great help in the structure elucidation, demonstrated that ring A was unsubstituted and that the trisubstituted double bond was not conjugated to the methyl ester carbonyl of the side chain. This left two possibilities for the location of the double bond with the C=O group at C-7, one at C-5–C-6 and the other at C-8–C-9. The ease of decomposition of the molecular ion with the formation of an ion at *m/z* 219 (base peak) and its further decomposition products could be explained only if the double bond was placed at C-8–C-9, as shown in Scheme 2. The elemental compositions of major fragments above *m/z* 100 were substantiated by high-resolution mass spectrometry. The AMX coupling pattern for the protons at C-5 and C-6 (Table 1) provided strong evidence for the location of the α,β-unsaturated ketone system, and the rest of the NMR parameters (Tables 1 and 2) were in accord with structure **6b**.

Havardiol (**7**)

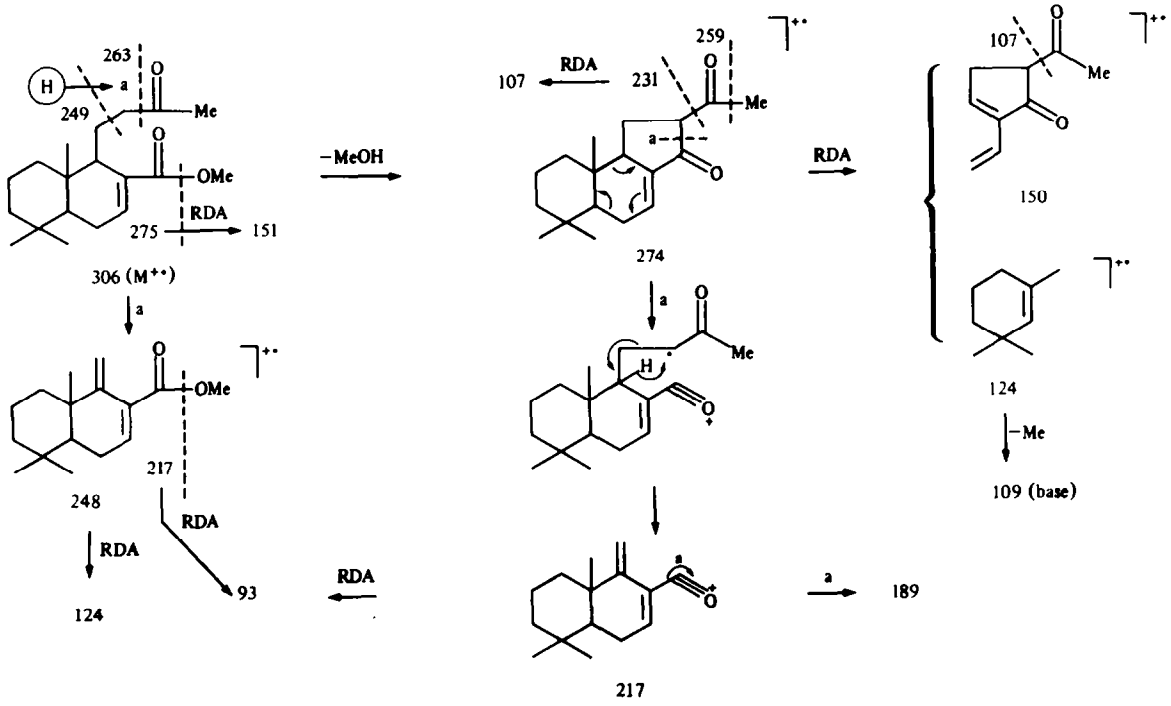
This compound contained a small amount of a persistent impurity giving an extra carbonyl band in the IR spectrum, but the combined spectral methods (especially NMR, Tables 1 and 2) clearly showed that the major component was **6b** with the reduced side-chain of **2b** and **3b**, i.e. **7**. The IR (CCl_4) spectrum showed hydroxyl (3480 cm^{-1}), alkene (1615 cm^{-1}), α,β-unsaturated ketone (1670 cm^{-1}) and $-C(Me)_2-$ (1378–1368 cm^{-1}) groupings. The EI mass spectrum showed the expected molecular ion peak at *m/z* 292 and the fragmentation pattern with the base peak at *m/z* 219 was very similar to that of **6b**.

EXPERIMENTAL

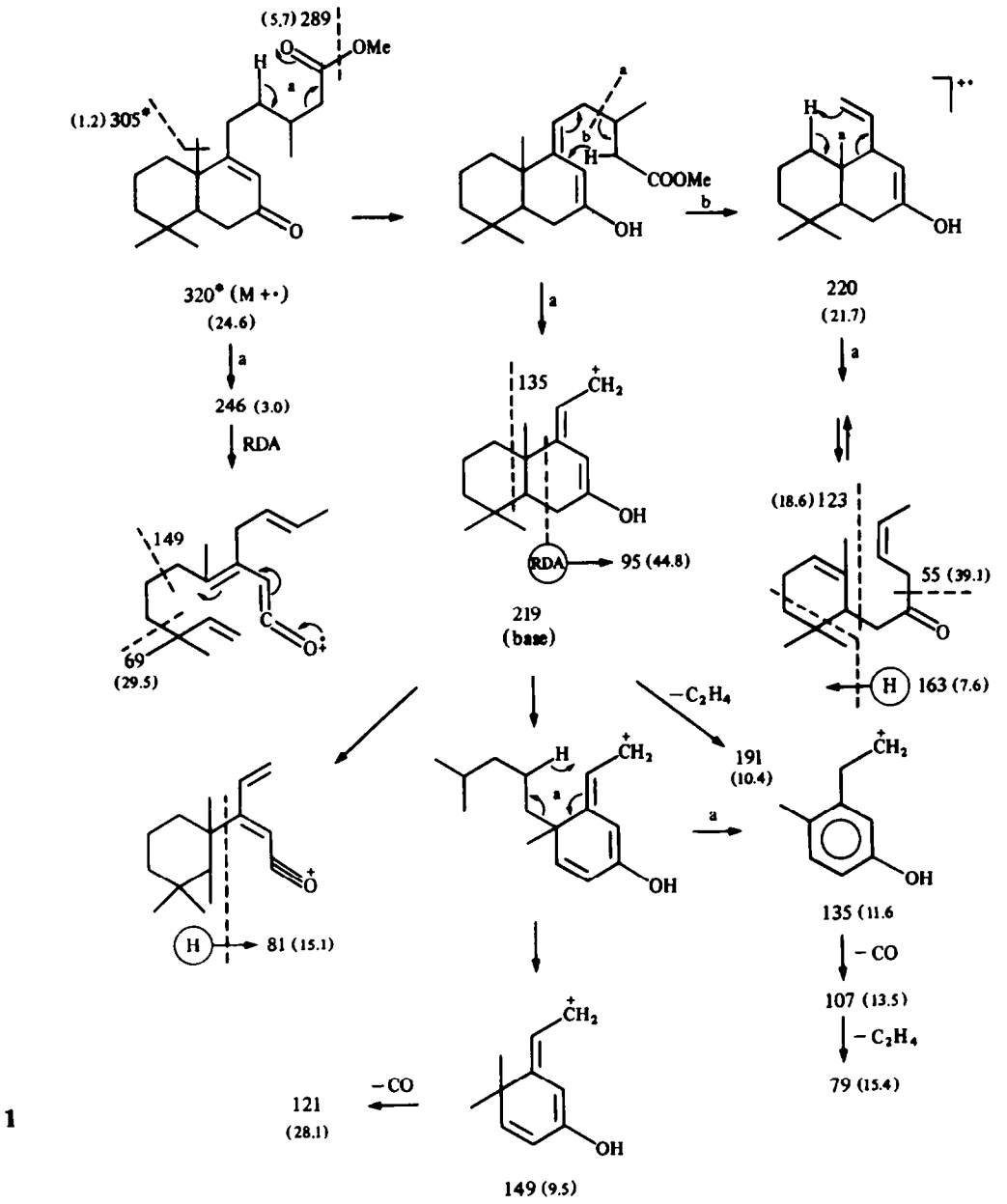
General. For instrumental procedures, see ref. [12].

Plant material. The plant material used in this study was collected in New Mexico, Eddy County, on US Highway 285 outside Carlsbad city limits in August 1984. A herbarium specimen has 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.

Extraction. The ground aerial parts of *G. havardii* (340 g) were extracted exhaustively with CH_2Cl_2 in a Soxhlet apparatus and solvent freed. The dry extract (26.9 g) was extracted with MeOH (800 ml) by stirring at room temp. (4 hr), left in the refrigerator overnight and filtered. The MeOH-soluble filtrate



Scheme 2. Major fragment ions (*m/z* ratios) related to **5b** in the EI mass spectrum of a mixture of **4b** and **5b**.



Scheme 3. Major fragment ions (*m/z* ratios, relative intensities in parentheses), established by high resolution exact mass measurements, in the EI mass spectrum of **6b**. * Decreased by 28 mu in **7**.

(24.0 g), after drying under vacuum, was separated into Et_2O-soluble and -insoluble fractions by stirring with Et_2O (600 ml) at room temp. (2 hr). The Et_2O-soluble fraction was then separated into acidic (16.2 g) and non-acidic (5.3 g) fractions using 5% aq. Na_2CO_3 followed by neutralization of the alkaline phase with 25% aq. HCl.

Methylation. The dry acid fraction (16.2 g) was methylated with MeI in dry Me_2CO–K_2CO_3 under the conditions described earlier [1].

Isolation of **1b–6b** *and* **7**. The methylated product (16.0 g) was dissolved in CH_2Cl_2, adsorbed on silica gel 60 and subjected to silica gel 60 (500 g packed in *n*-hexane) CC. The column was eluted with *n*-hexane–EtOAc (19:1) followed by increasing concns of EtOAc, and 30 fractions [1–25 (200 ml) and 26–30 (1000 ml)] were collected.

Compound **1b** (oil) was isolated from fraction 4 by prep. TLC using *n*-hexane–EtOAc (47:3, single development). Compounds **2b** (oil) and **6b** were isolated from combined fractions 21–24 by prep. TLC using *n*-hexane–EtOAc (7:3, single development); **6b** crystallized from n-hexane. Compounds **3b** (oil) and **5b** (oil) were isolated from fraction 26 by repetitive prep. TLC using CH_2Cl_2–EtOAc (7:3, single development) and CH_2Cl_2–EtOAc–HOAc (40:10:1, multiple developments). The mixture of **4b** and **5b** was isolated from combined fractions 11–12

by repetitive prep. TLC using *n*-hexane–EtOAc (22:3, multiple developments).

Labd-7-en-15,17-dioic acid dimethyl ester (**1b**). $[\alpha]_D^{25} - 56.0°$ (*c* 4.3; $CHCl_3$); IR: see text; ^1H NMR and ^{13}C NMR: see Tables 1 and 2; MS (Scheme 1): calculated for $C_{21}H_{32}O_3$ ([M − MeOH]$^+$): 332.2352; measured: 332.2357.

Labd-7-en-15-ol-17-oic acid methyl ester (**2b**). $[\alpha]_D^{25} - 65.0°$ (*c* 4.2; $CHCl_3$); IR: see text; ^1H NMR and ^{13}C NMR: see Tables 1 and 2; MS *m/z* (rel. int.): 336 [M]$^+$ (0.7), 305 (5.8), 304 (19.9), 235 (2.5), 213 (11.6), 181 (18.2), 180 (12.2), 163 (2.4), 149 (2.6), 135 (3.7), 124 (43.0), 109 (100), 95 (10.5), 91 (11.5), 81 (17.9), 79 (10.7), 69 (15.3), 55 (17.3).

Labd-7-en-6α,15-diol-17-oic acid methyl ester (**3b**). $[\alpha]_D^{25} - 2.4°$ (*c* 3.1; $CHCl_3$); IR: see text; ^1H NMR and ^{13}C NMR: see Tables 1 and 2; MS *m/z* (rel. int.): 352 [M]$^+$ (5.83), 337 (2.6), 334 (2.3), 321 (8.4), 320 (32.0), 305 (6.2), 302 (16.6), 293 (5.8), 287 (13.6), 276 (8.4), 234 (13.2), 233 (59.6), 219 (10.1), 201 (25.0), 196 (73.7), 178 (20.1), 163 (23.3), 153 (31.1), 151 (24.6), 149 (28.2), 135 (27.8), 124 (16.0), 123 (45.0), 109 (77.3), 95 (52.3), 91 (37.6), 82 (58.7), 81 (73.6), 79 (36.6), 69 (93.4), 55 (100).

Labd-7-en-6-oxo-15,17-dioic acid methyl ester (**4b**) *and labd-7-en-12-acetyl-17-oic acid methyl ester* (**5b**). IR: see text; ^1H NMR and ^{13}C NMR: see Tables 1 and 2; MS (for **4b**) *m/z* (rel. int.): 378 [M]$^+$ (0.86), 347 (2.5), 346 (4.6), 331 (1.9), 314 (3.0), 299 (0.6), 254 (4.4), 222 (9.9), 190 (17.5), 124 (19.3), 109 (100); see Scheme 2 for **5b**.

Labd-8-en-7-oxo-15-oic acid methyl ester (**6b**). Needles, mp 82–83°; $[\alpha]_D^{25} + 31.3°$ (*c* 0.5; $CHCl_3$); IR: see text; ^1H NMR and ^{13}C NMR: see Tables 1 and 2; MS (Scheme 3): calculated for $C_{20}H_{32}O_3$: 320.2351; measured: 320.2359.

Labd-8-en-7-oxo-15-ol (**7**). $[\alpha]_D^{25} + 22.4°$ (*c* 0.3; $CHCl_3$); IR: see text; ^1H NMR and ^{13}C NMR: see Tables 1 and 2; MS *m/z* (rel. int.): 292 [M]$^+$ (22.6), 277 (3.3), 275 (3.6), 274 (1.9), 219 (100), 191 (22.7), 176 (11.9), 163 (19.6), 151 (18.1), 149 (25.5), 135 (27.3), 124 (15.1), 123 (58.2), 121 (53.3), 109 (61.1), 95 (61.6), 91 (35.0), 81 (50.9), 79 (33.9), 69 (55.7), 55 (61.8).

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