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SESQUITERPENOID GLYCOSIDES AND AN ACETOGENIN GLUCOSIDE FROM *LESSINGIA GLANDULIFERA*

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Key Word Index—*Lessingia glandulifera*; Asteraceae; Astereae; Solidagininae; sesquiterpenoid glycosides; acetogenin glucoside; triterpenoids; steroid glucoside; flavonoid.

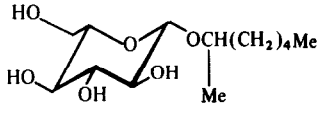
Abstract—An extract of *Lessingia glandulifera* yielded, besides five known compounds, a new acetogenin glucoside (heptane 2-*O*-β-D-glucopyranoside) and nine new sesquiterpene glycosides: 11-*O*-α-D-arabinopyranosyl-4-hydroxyisobulnesol (=lessingioside) and the 11-*O*-α-D-arabinopyranosyl and 11-*O*-α-D-(3′,4′-*O*-diacetyl)-arabinopyranosyl derivatives of the isomeric alcohols, isobulnesol, α- and β-eudesmols, and elemol.

INTRODUCTION

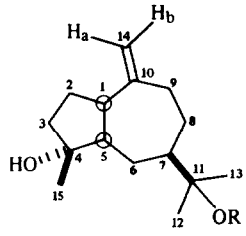
The highly unified and distinctive genus *Lessingia* (Asteraceae, Astereae, Solidagininae) contains seven species distributed in California, Mexico and Central America [1]. *Lessingia glandulifera* Gray is an annual herbaceous plant with discoid heads and markedly xerophytic leaves covered with an odorous and bitter glandular secretion. This species is characteristic of the dry, open plains and lower hills of California. As part of our ongoing phytochemical investigation of resinous, arid-adapted Astereae, we decided to examine this species.

RESULTS AND DISCUSSION

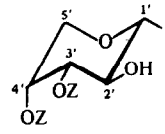
We wish to report finding a new linear acetogenin glucoside (**1**), nine new sesquiterpenoid glycosides (**2a–6a**, **3b–6b**) and five known compounds [two terpenoids (**7**, **8**), a steroid (**9**), a steroid glucoside (**10**) and a flavonoid (**11**)] in the methylenechloride extract of the aerial parts of *L.*



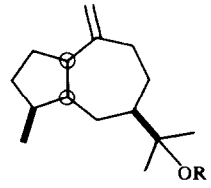
1



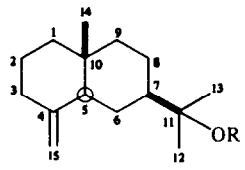
	R
2a	X
2b	H



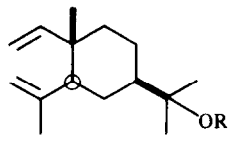
	Z
X	H
Y	Ac



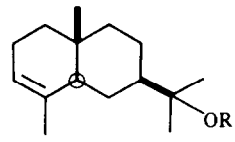
	R
3a	X
3b	Y
3c	H



	R
4a	X
4b	Y
4c	H



	R
5a	X
5b	Y
5c	H

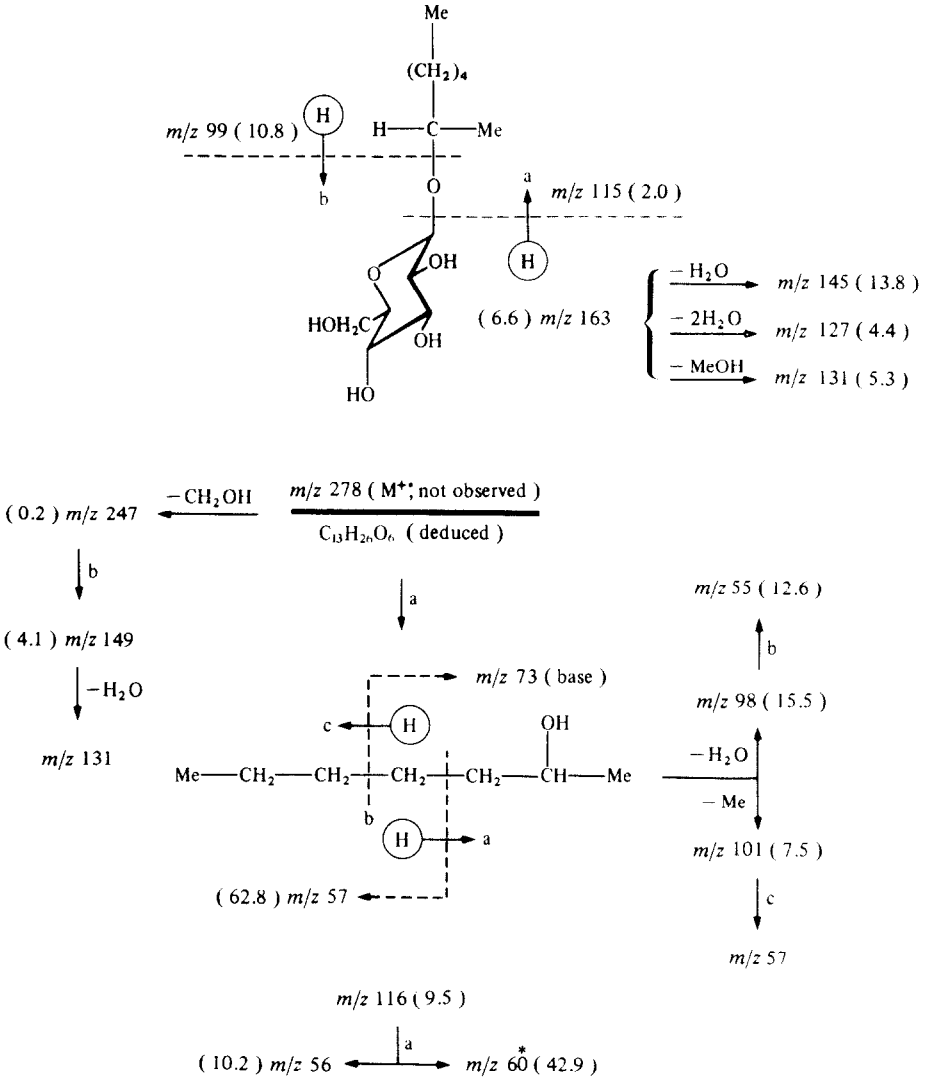


	R
6a	X
6b	Y
6c	H

glandulifera. The known compounds, which were oleanolic acid 3β-*O*-palmitate (**7**) [2] erythrodiol (**8**) [3], stigmasterol (**9**) [4], stigmasteryl β-D-glucoside (**10**) [5] and alnustin (**11**) [6], were identified by comparisons with literature data; for these compounds we report only some spectral parameters of **7** not previously available.

2-Heptanyl β-D-glucoside (**1**) was initially characterized largely from its ^{1}H NMR spectrum, which in the upfield region showed a doublet (J = 6.0 Hz) for the 1-methyl group at δ1.16, a triplet (J = 6.8 Hz) for the 7-methyl at δ0.88, a multiplet for the 3-methylene at δ1.62, and complex absorption for the 4-, 5- and 6-methylenes centered at δ1.27. The downfield region was typical of a β-D-glucoside with an added absorption for the methinyl proton of the aglycone [δ4.35 *d*, (J = 7.6 Hz), 3.78 *m*(3H), 3.48 *m* (2H), 3.28 *m* (2H)]. The EIMS of **1** did not exhibit an M^+ peak (*m/z* 278) but it did display peaks at *m/z* 116 (aglycone) and *m/z* 163 (sugar), derived from M^+ by fission at the glucosidic linkage, the former by abstracting a hydrogen from the latter. These sugar and aglycone ions then give rise to the principal fragments as shown in Scheme 1. We did not determine the configuration of the aglycone centre; 2-heptanol has long been known as a natural product [7].

Lessingioside (**2a**) failed to crystallize but was judged homogeneous by TLC and NMR and had $[\alpha]_D^{25}$ +9.7° (*c* 1.3, $CHCl_3$). The structure was deduced primarily by ^{1}H (Table 1) and ^{13}C (Table 2) NMR, with the aid of heteronuclear J-resolved, homo- and heteronuclear COSY spectra. The α-arabinopyranoside grouping was evident [8]. Especially helpful in assembling the part structures for the aglycone was the upfield position (δ 0.78) of the 6β-proton, which requires a *cis*-ring juncture in order to lie over the double bond. The large couplings between the 6β-proton and the 5- and 7-protons show a *cis*-relationship between the latter two protons. The usual absolute configurations are assumed for guaiane (H-7α) and sugar (D) protons of the molecule. This compound is the α-arabinoside of an apparently unknown guaiane diol (**2b**). Evidence that the sugar is attached at



Scheme 1. Diagnostic EIMS fragments of compound **1** (relative intensities in parentheses).

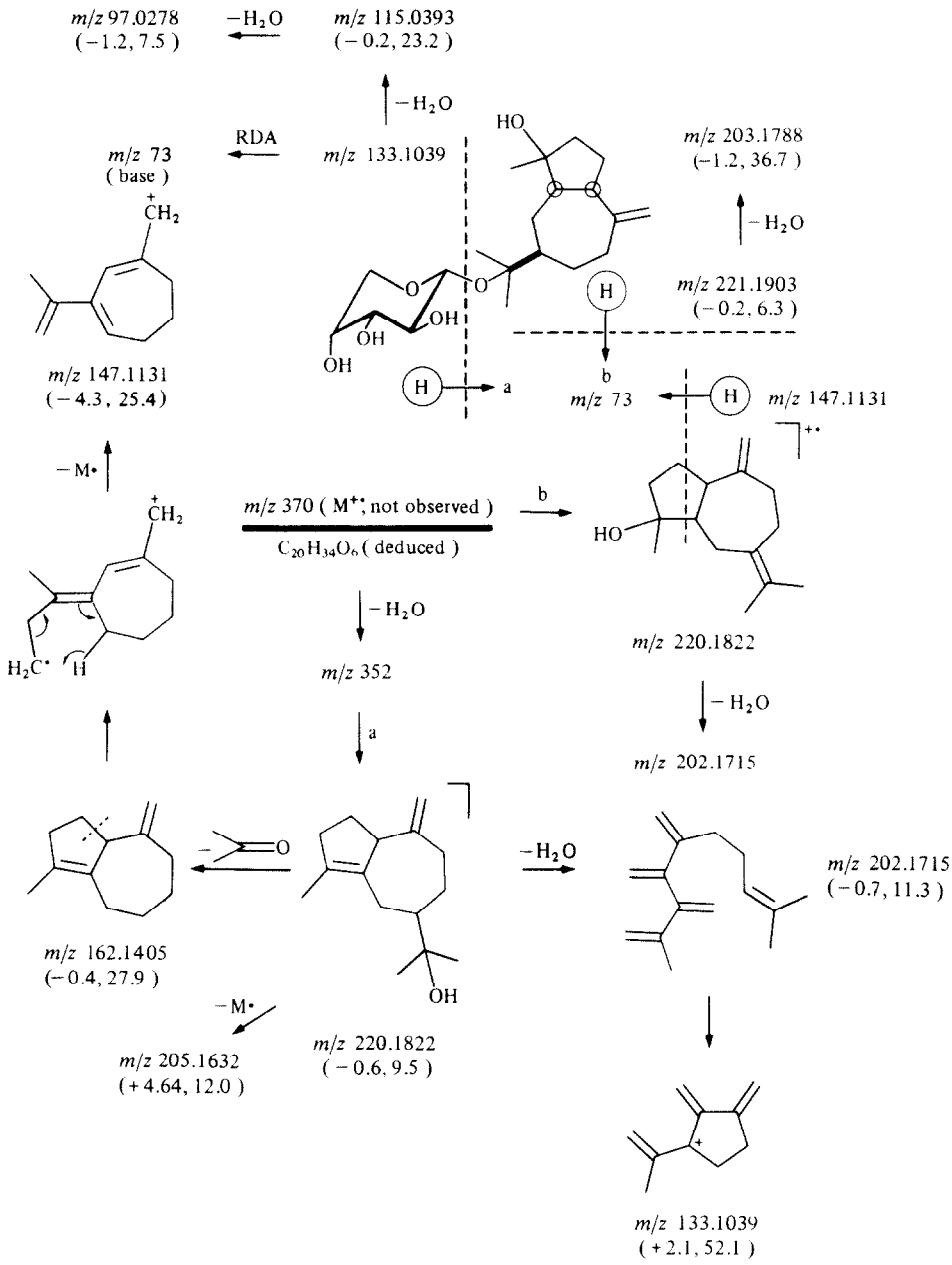
Table 1. ^{1}H NMR chemical shifts (250 MHz, δ, $CDCl_3$) and coupling constants (J, Hz) for compounds **2a**, **3a–b**, **4a–c** and **5a–c**

H	2a	3a	3b	4a	4b	4c	5a	5b	5c
1	2.95	2.74	2.75				5.80	5.81	5.80
2	~1.75						4.88	4.89	4.89
3	~1.70			2.30	2.31	2.31	4.57, 4.81	4.59, 4.82	4.59, 4.82
5	2.01								1.96
6α	2.03								
6β	0.78	0.77	0.76						
7	1.50								
8α	1.80								
8β	1.08								
9α	2.54	2.41	2.41						
9β	1.85								
12, 13	1.09, 1.25	1.15, 1.22	1.15, 1.22	1.22, 1.25	1.23, 1.25	1.21, 1.21			1.20, 1.20
14a	4.76	4.71	4.71	0.69	0.69	0.70	0.97	0.98	0.98
14b	4.84	4.77	4.78						
15	1.10	0.93	0.93	4.43, 4.71	4.43, 4.71	4.44, 4.71	1.71	1.70	1.71
1′	4.38	4.44	4.44	4.43	4.45				
2′	3.63	3.67	3.78	3.67	3.78				
3′	3.60	3.67	4.96	3.67	4.97				
4′	3.91	3.92	5.24	3.92	5.24				
5′ax	3.49	3.50	3.61	3.50	3.62				
5′eq	3.93	3.90	3.95	3.70	3.96				
Ac			2.08, 2.12		2.07, 2.13				
1,2	~9, ~9	~9, ~9	~9, ~9				17.8, 10.6	17.6, 10.5	17.8, 10.5
1, 5	9.0	8.5	8.5						
3α, 3β				14.0	14.0	13.9			
4, 15		6.9	6.8						
5, 6α									2.4
5, 6β	12.2	10.0	10.0						11.6
6α, 6β	12.2	12.2	12.2						11.6
6β, 7	8.6	10.0	10.0						
7, 8β	~9								
8α, 9α	~3	3.6	3.6						
8α, 9β	~9	7.4	7.4						
9α, 9β	12.0	13.2	13.4						
1′,2′	6.2	5.1	7.6	5.1	7.5				
2′,3′	9.3		10.2		10.2				
3′,4′			3.6		3.6				
4′,5′ax	2.0	2.0	1.3	2.0	1.3				
4′,5′eq			2.2		2.2				
5′ax, 5′eq	11.3	11.7	13.4	11.7	13.4				

C-11 rather than at C-4 as in some guaianolides [8] is the downfield shift of C-11.

The EIMS of **2a** (Scheme 2) did not exhibit an M^+ peak but fragmentation peaks at m/z 352 $[M-H_2O]^+$ and m/z 334 $[M-2H_2O]^+$ were visible. Except for m/z 294 $[M-58]^+$ no significant peaks were observed above the peaks at m/z 221 ($C_{15}H_{25}O$), 220 ($C_{15}H_{24}O$), 203 ($C_{15}H_{23}$) and 202 ($C_{15}H_{22}$) from the aglycone. This, and a peak at m/z 133, supported the molecular formula $C_{20}H_{34}O_6$ (m/z 370) arranged as $C_{15}H_{25}O$ (aglycone, m/z 221)-O-$C_5H_9O_4$ (sugar, m/z 133). The base peak at m/z 73 is derived either from m/z 133 *via* the *retro*-Diels–Alder (RDA) breakdown or from m/z 220 as shown in Scheme 2, and other diagnostic peaks at m/z 115 ($C_5H_7O_3$) and m/z 97 ($C_5H_5O_2$) originate from m/z 133 ($C_5H_9O_4$) by successive loss of water. These mass spectral peaks and the IR bands for OH (3400 cm^{-1}), >C =CH_2 (3090, 1640, 880 cm^{-1}) and –$C(Me)_2$ –(1380, 1370 cm^{-1}) support structure **2a**.

The isomeric compounds, occurring in mixture 'A' [**3a** (50%), **4a** (40%), **5a** (5%) and **6a** (5%)], were not separated from one another due to their very similar R_f values on chromatography (single spot on TLC with various solvent systems), but were characterized by spectral analysis of the mixture. The IR (CCl_4) spectrum of the mixture indicated the presence of >C=CH_2 (3080, 1640, 880 cm^{-1}) and OH (3620 cm^{-1}) groups. The ^{1}H and ^{13}C NMR spectra yielded the parameters in Tables 1 and 2. Compound **3a**, identical to **2a** except for lacking the C-4 hydroxy group, exhibits the same diagnostic high-field absorption for the 6β-proton but has a doublet rather than a singlet for the 15-methyl protons; strong NOE's between the 12- and 13-methyl protons and both the 15-methyl and 14-methylene protons require the rela-



Scheme 2. Diagnostic fragment ions, estabished by HRMS, in the EIMS of lessingioside (**2a**). The figures in parentheses represent difference in mmu and relative intensities.

tive configurations shown at C-4 and C-7. Compounds **4a**–**6a** were characterized as β-eudesmyl, elemyl, and α-eudesmyl α-D-arabinopyranosides, respectively, by NMR spectral comparisons with β-eudesmol (**4c**), elemol (**5c**), and α-eudesmol (**6c**). Compound **6a**, not included in Table 1, had vinyl proton absorption at δ5.32 which matched that of **6c** in location and appearance.

The EIMS of the mixture of **3a**–**6a**, which was very similar to that of **2a**, provided further support, exhibiting no M^+ peak (*m/z* 354), a peak at *m/z* 296 $[M-58]^+$, a base peak at *m/z* 73 and pairs of abundant peaks adding up to the M_r: *m/z* 133 (sugar)–*m/z* 221 (aglycone-O) and *m/z* 149 (sugar-O)–*m/z* 205 (aglycone).

The isomeric compounds, occurring in mixture 'B', also were not separated from one another due to their very similar R_f values on TLC, but their structures and percentages [**3b** (45%), **4b** (40%), **5b** (10%) and **6b** (5%)] were determined by spectral analysis of the mixture B. The IR (CCl_4) spectrum of the mixture clearly indicated the presence of $>C=CH_2$ (3080, 1640, 880 cm^{-1}), OH (3620 cm^{-1}), $-\overset{O}{\overset{\|}{C}}-O-$ (1745, 1235 cm^{-1}) and Me

Table 2. ^{13}C NMR chemical shifts (62.9 MHz, δ, $CDCl_3$) for compounds **2a**, **3a**, **4a** and **4c**

C	2a	3a	4a	4c
1	46.7	47.4	41.0	41.1
2	26.2	25.0	22.2	22.3
3	40.7	32.0	36.8	36.9
4	81.0	38.9	151.1	151.1
5	52.5	49.9	49.8	49.7
6	27.2	28.3	24.7	25.0
7	50.6	49.8	48.1	49.4
8	33.1	32.2	23.4	23.5
9	39.0	34.8	41.8	41.8
10	151.6	154.0	35.9	35.9
11	81.0	81.7	80.7	73.0
12	20.3	22.6	23.7	27.1
13	26.3	24.7	24.0	27.1
14	109.3	109.9	16.3	16.3
15	23.6	16.1	105.4	105.3
1′	97.5	96.9	97.1	—
2′	71.1	71.2	71.2	—
3′	73.3	72.8	72.9	—
4′	68.3	67.7	67.8	—
5′	65.8	64.8	64.9	—

(1370 cm^{-1}) groups. ^1H and ^1H–^1H COSY NMR spectra yielded the parameters in Table 1; the downfield locations of the 3′- and 4′-protons showed the acetates to be attached to the 3′- and 4′-carbons. The FAB mass spectrum of the mixture did not exhibit an MH^+ peak but the diagnostic protonated and unprotonated fragments originating from the aglycone and sugar moieties, as illustrated in Scheme 3, provided essential information which fully supported the above findings. The sugar peak at *m/z* 133 in mixture A was shifted to a mass number 84 higher in mixture B (*m/z* 217), indicating the presence of diacetylated sugar moieties in the latter. The aglycones in both mass spectra gave peaks at *m/z* 221.

The biosynthesis of the sesquiterpene portion of **2a–6a** and **3b–6b** from hedycaryol [9] are easily rationalized; only **2a** requires an oxidation step.

Compound **7**, mp 123–124°, colourless needles from acetone-diethyl ether, was identified as oleanolic acid 3β-*O*-palmitate, previously reported from *Madhuca longifolia* [2] and *M. butyraceae* [10] with very little spectral information. Its IR (KBr) [3000–2500, 1695 (COOH), 1730 (–C(=O)–O–), 1415 ($\underline{CH_2}CO$), 1385–1370 doublet ($-CMe_2-$), series of evenly spaced bands between 1360–1180, and 720 [$(CH_2)_n$, $n = >4$]cm^{-1}, EIMS, ^1H and ^{13}C NMR spectra (see Experimental) were in accord with the structure.

EXPERIMENTAL

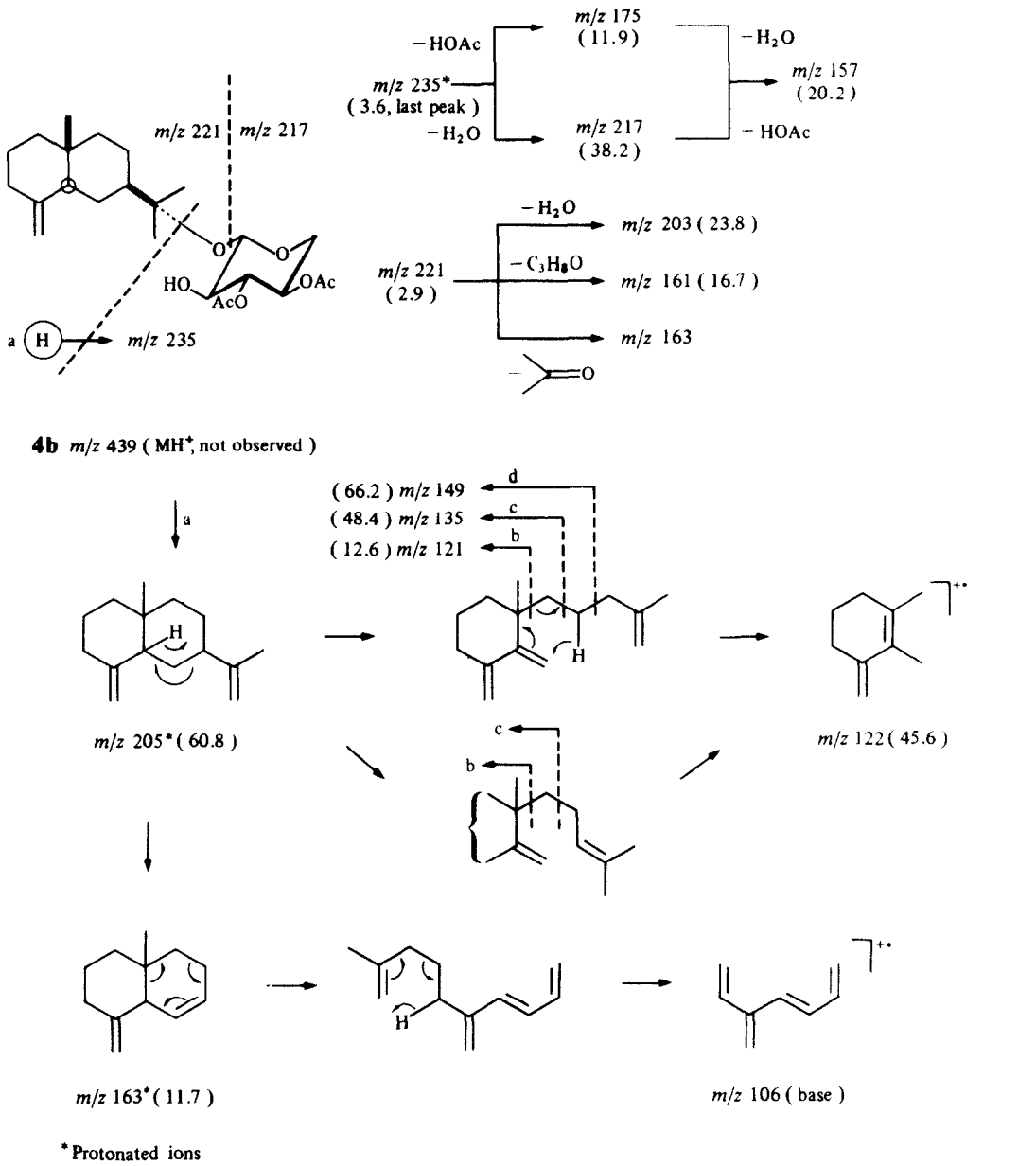
Plant material. Plant material was collected on 12 September 1985 in San Diego County, California, along Highway 522, 3 miles east of the junction with 52, southeast of Warner Springs. A voucher specimen (SPM 3063) is on deposit in the Herbarium of the University of Arizona. All plant material was air dried, ground to 3 mm particle size and stored at 5° prior to extraction.

Extraction, fractionation and isolation. The ground material of *L. glandulifera* (aerial parts, 876 g) was extracted with CH_2Cl_2 by percolation at room temp. for 24 hr. The solvent-free CH_2Cl_2 extract (61 g) was educed with Et_2O (600 ml) by stirring (2 hr), left in the refrigerator overnight and the supernatant Et_2O-soluble portion was decanted. This step was repeated with more Et_2O (500 ml) and the combined Et_2O-soluble portion was extracted with 5% aq. Na_2CO_3. This gave a non-acidic fraction (26.5 g) and an interphase (10.6 g). From the interphase, compounds **1**, **2a**, **3a–6a** (mixture A) and **10** were isolated and the remaining compounds [**3b–6b** (mixture B), **7**, **8**, **9**, and **11**] were isolated from the non-acidic fraction. The isolation of these compounds was performed qualitatively.

A small portion of the interphase, when submitted to silica gel prep TLC (CH_2Cl_2–MeOH, 6:1) gave three fractions (A–C). From fraction A, compounds **3a–6a** were isolated as a TLC-homogeneous mixture A (oil, the minor component of the interphase) by a further prep TLC (CH_2Cl_2–MeOH, 8:1). From fraction C, compound **2a** (the major component of the interphase) was isolated as colourless foamy substance, homogeneous by TLC, by prep TLC (CH_2Cl_2–MeOH, 6:1). From another portion of the interphase, after removal of **2a–6a**, a very small amount of compound **1** was isolated by repetitive silica gel prep TLC (various conc. of MeOH in CH_2Cl_2, multiple developments). A third portion of the interphase was submitted to a small silica gel CC and the fraction eluted with CH_2Cl_2–MeOH (19:1) gave compound **10** which crystallized out as colourless flakes (mp 309–310°) when treated with CH_2Cl_2.

The non-acidic fraction (26.5 g) was submitted to silica gel CC (1 kg packed in *n*-hexane) and the column was eluted with *n*-hexane containing various concentrations of Et_2O. *n*-Hexane–Et_2O (3:2 and 2:3) eluted compounds **9** and **11** which were separated by silica gel prep. TLC [*n*-hexane-Et_2O (1:1]. Compound **9** was obtained as colourless long needles from Et_2O (mp 175–176), while compound **11** crystallized as yellow thick rods from Et_2O (mp 184–185). *n*-Hexane–Et_2O (3:2) eluted compound **7**. The fr. containing **7** was treated with petrol and the petrol-soluble portion when submitted to silica gel prep. TLC [*n*-hexane–Et_2O (2:1)] gave **7** as colourless long needles, homogeneous by TLC. *n*-Hexane–Et_2O (2:3) eluted compounds **8** and **3b–6b** (mixture B). Compound **8** was isolated by silica gel prep. TLC [*n*-hexane–Et_2O (1:2)]; the fraction containing **8** when treated with petrol–Et_2O (1:1) gave **8** as a colourless solid. Mixture B (**3b–6b**), homogeneous by TLC, was isolated as an oil by repetitive silica gel prep TLC [*n*-hexane–Et_2O (2:3), multiple developments]. Et_2O (100%) eluted more **2a**.

The physical and spectral properties of **2a–6a** and **3b–6b** are described in the text. Spectral parameters of **7** are given below. ^1H NMR ($CDCl_3$, δ): Me's (0.75*s*, 0.85*s*, 0.86*s*, 0.88*t*, 0.90*s*, 0.93*s*, 0.94*s*, 1.13*s*); many CH_2's, 1.25*br s*; 2′-CH_2, 2.29*t* (7.5 Hz); H-18, 2.82 *dd* (13.6, 4.1); H-3, 4.49*t* (7.8); H-12, 5.27 *br t* (2.7). ^{13}C NMR ($CDCl_3$, δ): 1–30 (in order), 38.0, 23.4, 80.5, 37.7, 55.3, 18.2, 32.5, 39.2, 47.5, 37.0, 22.7, 122.5, 143.6, 41.5, 27.6, 23.5, 46.5, 40.9, 45.8, 30.7, 33.8, 32.4, 28.0, 17.2, 15.3, 16.7, 25.9, 184.3, 33.0, 23.6; 1′, 173.7; 2′, 34.8; 3′, 25.2; 4′-6′ and 13′, 29.2, 29.3, 29.4, 29.5; 7′-12′, 29.7; 14′, 32.0; 15′, 22.8; 16′, 14.1. EIMS (*m/z*): 694 (M^+; not observed), 649 (M – COOH, last peak), pair of peaks adding up to the mol. wt. (445 and 249), 438 [M – Me $(CH_2)_{14}COOH]^+$, peaks originating from 438 [423 (–Me), 249 and 189, 248 (base) and 190 (via RDA)], peaks originating from 248 [233 (–Me), 204 ($-CO_2$), 203 ($-CO_2H$)] and 190 [175 (–Me)].



Scheme 3. Major fragment ions (relative intensities in parentheses) related to compound **4b** in the FABMS of a mixture of compounds **3b**–**6b**.

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