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TERPENOIDS FROM *ACAMPTOPAPPUS SPHAEROCEPHALUS* AND *A. SHOCKLEYI*

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Key Word Index—*Acamptopappus sphaerocephalus*; *A. shockleyi*; Asteraceae; Astereae; Solidagininae; labdane diterpenoids; sesquiterpenoids.

Abstract—Eight new labdane diterpenoids were isolated from the sodium carbonate-soluble acid fraction of *Acamptopappus sphaerocephalus* and their structures were deduced from the spectral properties of their methyl esters and other derivatives. They are 7,*E*-13- or 8,*E*-13-labdadienes having oxygen-containing substituents at C-2, C-7, C-15 and/or C-19. These same diterpenoids were also present in *A. shockleyi*. Eight previously reported sesquiterpenoids, namely, bisabolene-1,4-endoperoxide, caryophyllene oxide, β-eudesmol, *trans*-nerolidol, 1-oxobisabolene, shiromool, spathulenol and viridiflorol, were isolated from the non-polar fraction of *A. sphaerocephalus* and identified by their ^{1}H NMR spectra.

INTRODUCTION

Acamptopappus (Asteraceae, Astereae, Solidagininae) is comprised of two shruby species that grow in desert areas of the southwestern United States. This genus was originally treated as a section of *Haplopappus* by Gray, but was later elevated to generic rank by the same author. Taxonomy and relationships of *Acamptopappus* are presently being reviewed by Lane [1]. *Acamptopappus sphaerocephalus* (Harv. and Gray) is a low, rounded shrub with numerous erect, brittle branchlets, grayish bark and small, alternate leaves. This species flowers from March to June in rocky washes and on arid hillsides and plains from southern Utah to central Arizona and southern California. *Acamptopappus shockleyi* Gray has radiate heads solitary at the tips of the branches, in contrast to those of *A. sphaerocephalus* which are discoid and at the tips of cymosely arranged branchlets. *Acamptopappus shockleyi* grows in southern Nevada and Inyo County in California.

As part of our search for bioactive compounds from arid-adapted homochromous Astereae, we decided to investigate the genus *Acamptopappus*. This is the first report on the chemical constituents of the two species which constitute this genus.

RESULTS AND DISCUSSION

The methylenedichloride extract of the aerial part of *A. sphaerocephalus* gave a methanol soluble fraction which was concentrated, diluted with water and extracted with petrol. The aqueous methanol soluble material was separated into acidic, phenolic and neutral fractions by extraction with sodium carbonate and sodium hydroxide solutions. The acidic fraction was esterified with methyl iodide [2] and the resulting methyl ester mixture when submitted to chromatography gave seven new diterpenoid methyl esters (**4**–**8**, **11** and **12**), and a non-acidic diterpenoid (**3**). The identity of the major constituent, named acamptoic acid (Me ester, **4**), was established by spectral analyses of **4** and its reaction products **2**, **9**, and **10**. The identities of the remaining constituents, acamptodiol (**3**), desacetylacamptoic acid (Me ester, **5**), 19-isobutyryldesacetylacamptoic acid (Me ester, **6**), desacetoxyacamptoic acid (Me ester, **7**), 2-desoxyacamptoic acid (Me ester, **8**), 7α-hydroxy-8-en-acamptoic acid (Me ester, **11**) and 7-oxo-8-en-acamptoic acid (Me ester, **12**), were then established by NMR (Tables 1 and 2) and mass spectral comparisons (Fig. 1) with **4**, with one another, and with known compounds. The diketo derivative (**13**), an oxidation product of the 8,*E*-13-labdadienes **11** and **12**, helped to establish their identities.

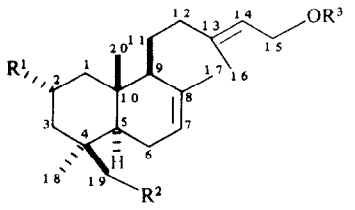
From the petrol-soluble fraction, eight previously reported sesquiterpenoids [β-eudesmol, caryophyllene oxide, *trans*-nerolidol, shiromool, 1-oxobisabolene, bisabolene-1,4-endoperoxide and a mixture of spathulenol (65%) and viridiflorol (35%)] were isolated by silica gel CC/PLC and identified by comparison of their ^{1}H NMR parameters with those of authentic samples. For these compounds we report no spectral data.

The major constituent (**4**) was established to be the methyl ester of 2α-hydroxy-15-succinyloxy-19-acetoxy-labda-7,*E*-13-diene as follows. The molecular formula ($C_{27}H_{42}O_7$) was deduced from its HRMS based on the characteristic peak at *m/z* 346 [M –HOOC$(CH_2)_2$COOMe (132 mass units)]. Its IR (neat) spectrum showed bands for OH (3500, 1025 cm^{-1}), ester (1735, 1155 cm^{-1}), acetate (1735, 1240 cm^{-1}), –CH_2O– (1410 cm^{-1}), –$C(Me)_2$– (1390, 1370 cm^{-1}) and >C=CH– (3020, 1670, 840 cm^{-1}) groups. The ^{1}H NMR spectrum (Table 1) defined the hydroxyl group to be

Table 1. ^{1}H NMR (250 MHz) chemical shifts (δ, $CDCl_3$) and

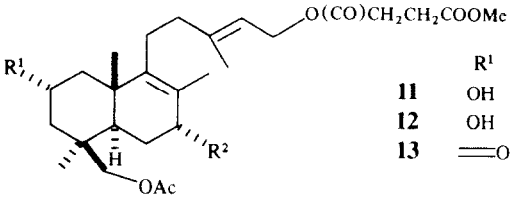
H	1	2	3	4	5	6	7
2	3.86 *tt* (11.5, 3.9)	3.86 *tt* (11.6, 3.9)	3.85 *tt* (11.4, 3.8)	3.84 *tt* (11.7, 3.3)	3.83 *m*	3.86 *tt* (11.5, 3.8)	3.85 *tt* (11.5, 3.8)
7	5.40 *br s*	5.40	5.40	5.39	5.38	5.39	5.41
14	5.42 *br t* (6.8)	5.43 *br t* (6.9)	5.42 *br t* (6.9)	5.34 *br t* (7.1)	5.33 *br t* (7.0)	5.34 *br t* (7.1)	5.34 *br t* (7.0)
15	4.15 *d* (6.8)	4.16 *d* (6.9)	4.16 *d* (6.9)	4.60 *dd* (12.3, 7.1) 4.63 *dd* (12.3, 7.1)	4.60 *dd* (12.3, 7.0) 4.62 *dd* (12.3, 7.0)	4.59 *dd* (12.4, 7.1) 4.63 *dd* (12.4, 7.1)	4.60 *dd* (12.3, 7.0) 4.63 *dd* (12.3, 7.0)
16	1.70 *s*	1.70	1.70	1.72	1.71	1.72	1.72
17	1.70 *s*	1.70	1.70	1.70	1.69	1.70	1.70
18	0.92 *s*	1.02	1.01	1.01	1.01	1.02	0.92
19	0.92 *s*	3.48 *d* (10.8) 3.74 *d* (10.8)	3.90 *d* (10.9) 4.22 *d* (10.9)	3.91 *d* (11.0) 4.21 *d* (11.0)	3.45 *d* (10.7) 3.73 *d* (10.7)	3.93 *d* (11.0) 4.20 *d* (11.0)	0.92 *s*
20	0.79 *s*	0.79	0.81	0.81	0.78	0.81	0.79
2′,3′ OMe				2.64 *s* 3.70 *s*	2.64 3.70	2.64 3.70	2.64 3.69
2″			2.07 *s*	2.07		2.57 *hept* (7.0)	
3″						1.18 *d* (7.0)	

*2-OAc at 2.08 *s*.
†1α and 3α at 2.17 *d* (13.0) and 2.22 *d* (13.0); 1β and 3β at 2.52 *d* (13.0).
‡May be reversed.



	R^1	R^2	R^3	$[\alpha]^{25}$D, ($CHCl_3$)
1	OH	H	H	+ 2.8 (*c* 1.9)
2	OH	OH	H	− 3.7[a] (*c* 0.7)
3	OH	OAc	H	−17.1 (*c* 0.7)
4	OH	OAc	X	−10.9 (*c* 0.6)
5	OH	OH	X	+ 1.0 (*c* 3.0)
6	OH	O(CO)-*i*-Bu	X	
7	OH	H	X	+ 1.5 (*c* 0.9)
8	H	OAc	X	− 3.8 (*c* 1.7)
9	OAc	OAc	X	− 4.5 (*c* 1.5)
10	=O	OAc	X	+ 8.3 (*c* 1.1)

X = $^{1'}(CO)^{2'}CH_2{}^{3'}CH_2{}^{4'}COOMe$ [a] in MeOH



	R^1	R^2	$[\alpha]^{25}$D, ($CHCl_3$)
11	OH	OH	+31.4 (*c* 1.1)
12	OH	=O	+18.2 (*c* 2.4)
13	=O	=O	+64.0 (*c* 0.2)

secondary, equatorial and at C-2 from the triplet of triplets pattern ($J=11$ and 4 Hz) for the proton bearing this hydroxy group. That the oxygen-bearing substituent at C-4 is axial was clear from an AB quartet centered at δ 4.06 ($J=11$ Hz) for the C-19 protons [3]. The *E*-configuration of the side chain double bond was evident from the shift of the C-16 methyl group. It is not clear from the NMR spectrum of **4** alone whether C-15 or C-19 contains the acetate and where the succinate resides, but the assignments were easily made by analogy with **3** and **5**, which have clearly defined positions for these groups. The relative configurations among the C-5, C-9 and C-10 positions are assumed by analogy with the known labdanes. That these new compounds are labdanes rather than *ent*-labdanes is based on the increase of 56° in molecular rotation in adding a 2-keto group in the new series ($[M]_D-17°$ for **8**→ $+39°$ for **10**) paralleling the increase of 64° which occurs in a known labdane series ($[M]_D+58°$ for (+)-manoyl oxide→ $+122°$ for 2-ketomanoyl oxide [4]).

The ^{13}C NMR spectrum of **4** (Table 2) supported the proposed structure. The assignments were greatly facilitated by comparison with the spectrum of the methyl ester of 15-succinyloxy-*ent*-labd-*E*-13-en-8β-ol [5].

coupling constants (Hz, in parentheses) for compounds **1–13**

8	9*	10†	11	12	13
	4.92 *tt* (11.9, 3.8)		3.94 *br s*	4.01 *m*	
5.37	5.38	5.44	3.94 *br s*		
5.34 *br t* (7.1)	5.34 *br t* (6.9)	5.33 *br t* (6.9)	5.36 *br t* (7.0)	5.40 *br t* (6.9)	5.39 *br t* (7.0)
4.62 *d* (7.1)	4.62 *d* (6.9)	4.61 *d* (6.9)	4.62 *d* (7.0)	4.63 *d* (6.9)	4.63 *d* (7.0)
1.71	1.71	1.73	1.74	1.78	1.76
1.69	1.71	1.70	1.74	1.78	1.81
0.95	1.01	1.11	1.07	1.16	1.17‡
3.94 *d* (10.9)	3.98 *d* (11.1)	3.86 *d* (11.2)	3.86 *d* (11.1)	3.92 *d* (11.3)	4.00 *s*
4.30 *d* (10.9)	4.19 *d* (11.1)	4.09 *d* (11.2)	4.20 *d* (11.1)	4.25 *d* (11.3)	
0.77	0.86	0.81	0.96	1.07	1.15‡
2.64	2.64	2.64	2.64	2.64	2.65
3.69	3.69	3.69	3.70	3.70	3.70
2.05 *s*	2.05	2.05	2.07	2.09	2.08

Table 2. ^{13}C NMR (22.63 MHz) chemical shifts (δ, $CDCl_3$) for compounds **4**, **11** and **12**

C	4	11	12
1	47.7	45.2	44.9
2	63.9	64.5	63.7
3	44.5	44.8	44.5
4	38.2	37.9	38.1
5	50.1	45.7	49.6
6	23.1*	26.6	34.8
7	121.8	70.0	198.8
8	135.0	127.7	130.2
9	54.2	144.1	165.9
10	38.2	40.9	42.0
11	25.4*	28.4	28.1
12	41.5	39.1	38.0
13	142.3	142.3	141.1
14	118.4	117.9	118.8
15	61.5	61.5	61.3
16	16.5	16.5	16.5
17	20.8	19.8	11.4
18	27.1	27.2	26.8
19	66.9	67.4	67.0
20	15.3	17.4	19.4
1′	172.2	172.2	172.2
2′	28.8	28.8	28.8
3′	29.0	29.0	29.0
4′	172.7	172.8	172.8
1″	171.2	171.3	171.0
2″	21.9	20.9	20.8
OMe	51.7	51.8	51.8

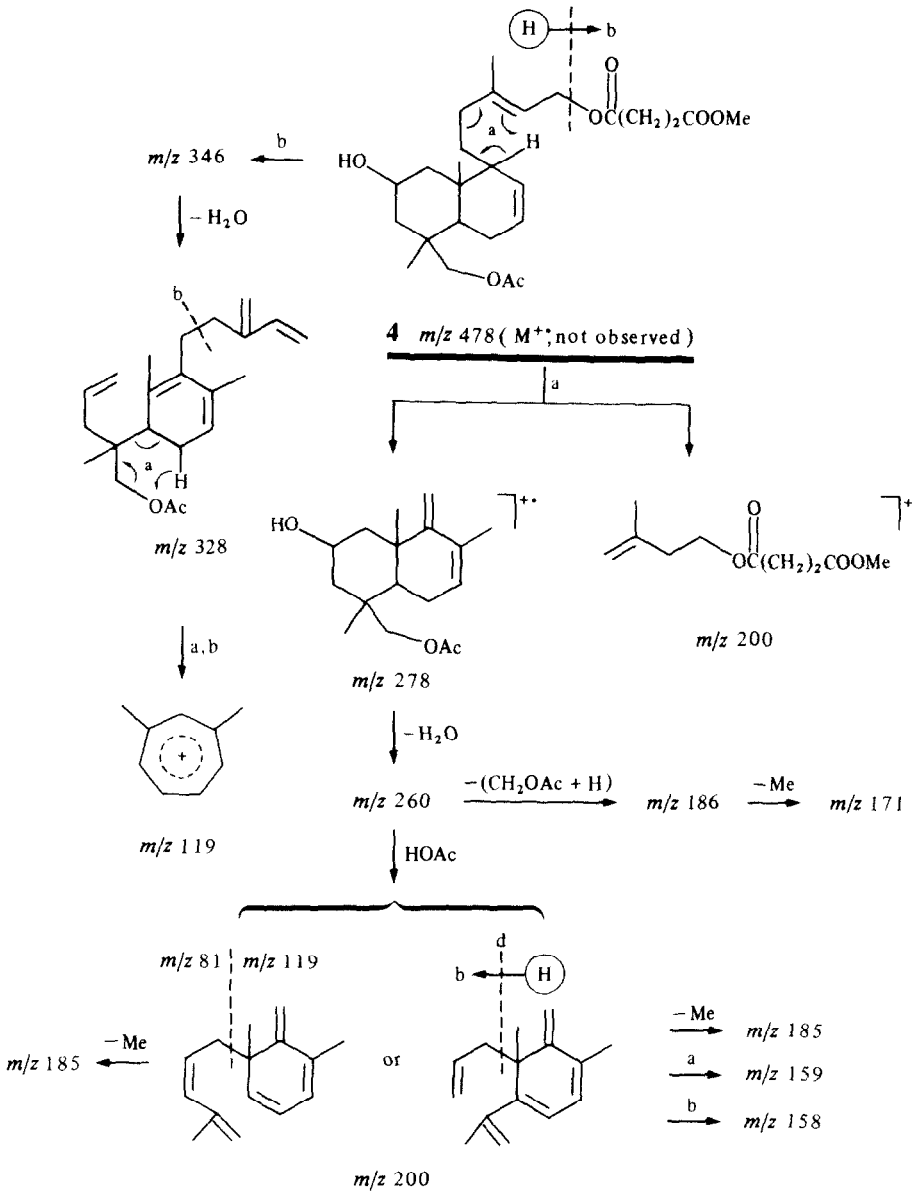
*May be reversed.

The mass spectral fragmentation of **4** complemented the above findings. Under GCMS conditions (see Experimental) **4** gave two peaks (A and B), eluted at R_t values 12.68 and 13.04 min, respectively. A and B fragmented differently as shown in Schemes 1 and 2. In both, the molecular ion peak (*m/z* 478) was not observed, the ion at *m/z* 43 ($MeC{\equiv}O^+$) gave the base peak, and elimination from $[M]^+$ of methyl succinate (*m/z* 346) followed by one mole of water (*m/z* 328) gave key peaks. In the mass spectrum of A, the most characteristic pair of peaks adding up to the M_r (*m/z* 278 and 200) were not derived from the *retro*-Diels–Alder (RDA) fragmentation, usually diagnostic for labd-7-enes, but instead, from the fragmentation depicted. The further decomposition products of the ion *m/z* 278 as well as other important ions are rationalized as shown in Scheme 1. **B**, which is probably the allylic ester rearrangement product of **4**, undergoes allylic cleavage of the C-9–C-11 bond leading to an ion of mass *m/z* 265, the precursor for major fragment ions as shown in Scheme 2.

Acetylation of **4** gave the diacetate **9** (IR: free of OH absorption; ^1H NMR: additional MeCOO–signal, Table 1; and MS peak at *m/z* 278 in **4**, shifted to higher mass number by 42). Oxidation of **4** with manganese dioxide gave the corresponding 2-keto derivative **10** [IR ($CHCl_3$): no OH absorption, new C=O band at 1660 cm^{-1}: ^1H NMR: no C-2 proton signal; and MS: *m/z* 278 peak shifted to lower mass number by 2]. Hydrolysis of **4** with Na_2CO_3–MeOH gave the triol **1** [IR ($CHCl_3$): no C=O band; ^1H NMR: no MeCOO– or $-CH_2CH_2COOMe$ signals; and MS *m/z* 278 peak shifted to lower mass number by 42].

Compounds **3** and **5-8** were readily characterized by comparing their ^1H NMR (Table 1) and mass spectra with those of **4**. Unlike **4**, compound **3** gave a single GCMS peak, at 15.32 min. It did not display an $[M]^+$ peak at *m/z* 364 but its fragmentation pattern below *m/z* 278 $[M-86]^+$, except for relative intensities, was very similar to that of GC-MS/peak A of **4** (Scheme 1). The EIMS of the remaining compounds similarly showed no $[M]^+$ peak but, like **4**, gave characteristic peaks due to an ion of the type shown in Fig. 1. Their subsequent principal bond fissions follow the paths outlined in Scheme 1 and hence will not be discussed further.

Hydrolysis of **7** with Na_2CO_3–MeOH gave the diol **1** [IR (neat): no C=O band; ^1H NMR (no

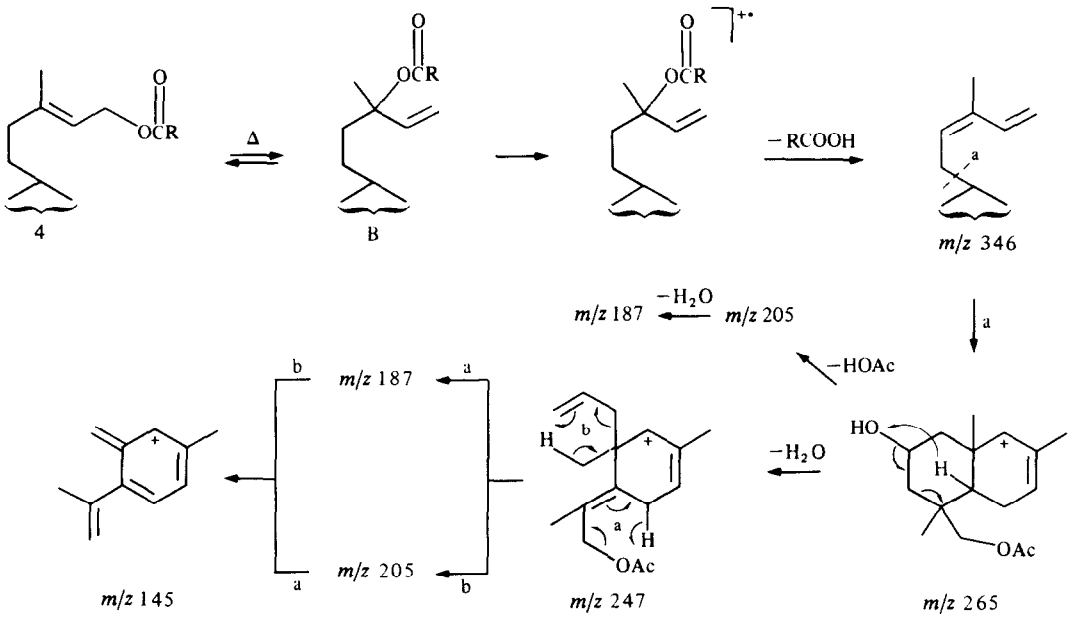


Scheme 1. Diagnostic fragment ions of peak A (R_t = 12.68 min) in the GCMS of **4**.

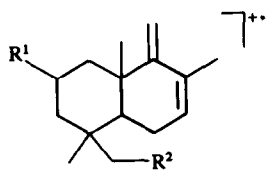
$-CH_2CH_2COOMe$ signals); and MS *m/z* 220 peak in **7** did not shift and appeared as base peak in **1** (Fig.1)].

Compounds **11** and **12** lacked C-7 vinyl proton absorption in their ^1H NMR spectra (Table 1) but still had absorption for the methyl groups attached to double bonds. That a tetrasubstituted double bond, presumably between C-8 and C-9, was present, was clear in their ^{13}C NMR spectra (Table 2). Compound **12** contained a ketone grouping (δ 198.8), conjugated with the tetrasubstituted alkene from the down field location (δ 165.9) for the β-carbon as well as from its IR spectrum ($CHCl_3$) which showed a strong band at 1660 cm^{-1} in addition to bands for the presence of many of the structural elements of **4**. Compound **11**, whose IR spectrum ($CHCl_3$) was very similar to that of **12** but lacked a conjugated C=O band, is one of the corresponding alcohols since it gives the same diketone **13** (free of OH absorption, conjugated C =O band at 1662 cm^{-1} in the IR spectrum) on oxidation. The α-(pseudoaxial)-configuration is depicted for it from the narrowness of the absorption for H-7 in the ^1H NMR spectrum.

The main fragmentation pattern in the EIMS of **12** was very different from those **1**–**10**. In the EIMS of **12**, no $[M]^+$ peak (*m/z* 492) was seen but a peak at *m/z* 477 $[M-Me]^+$ was visible. Aside from ubiquitous ions at *m/z* 360 $[M-HOOC(CH_2)_2COOMe]^+$, 345 $[360-Me]^+$, 342 $[360-H_2O]^+$ and 115 $[O{\equiv}C(CH_2)_2COOMe$, base], unexpected peaks at *m/z* 294 $[M-198]^+$ and *m/z* 201 dominated the spectrum. The former may be seen as arising from $[M]^+$ by a thermally induced Cope rearrangement, followed by a fragmentation or by cleavage of a doubly allylically activated C-11–C-12 bond with transfer of a hydrogen atom from the side chain, and the latter ion from *m/z* 294 by further loss of methyl, acetic



Scheme 2. Diagnostic fragment ions of peak B (R_t = 13.04 min) in the GCMS of **4**.



	R¹	R²	m/z
1,7	OH	H	220
2,5	OH	OH	236
3,4	OH	OAc	278
6	OH	—O(CO)-*i*-Bu	306
7	OH	H	220
8	H	OAc	262
9	OAc	OAc	320
10	O	OAc	276

Fig. 1.

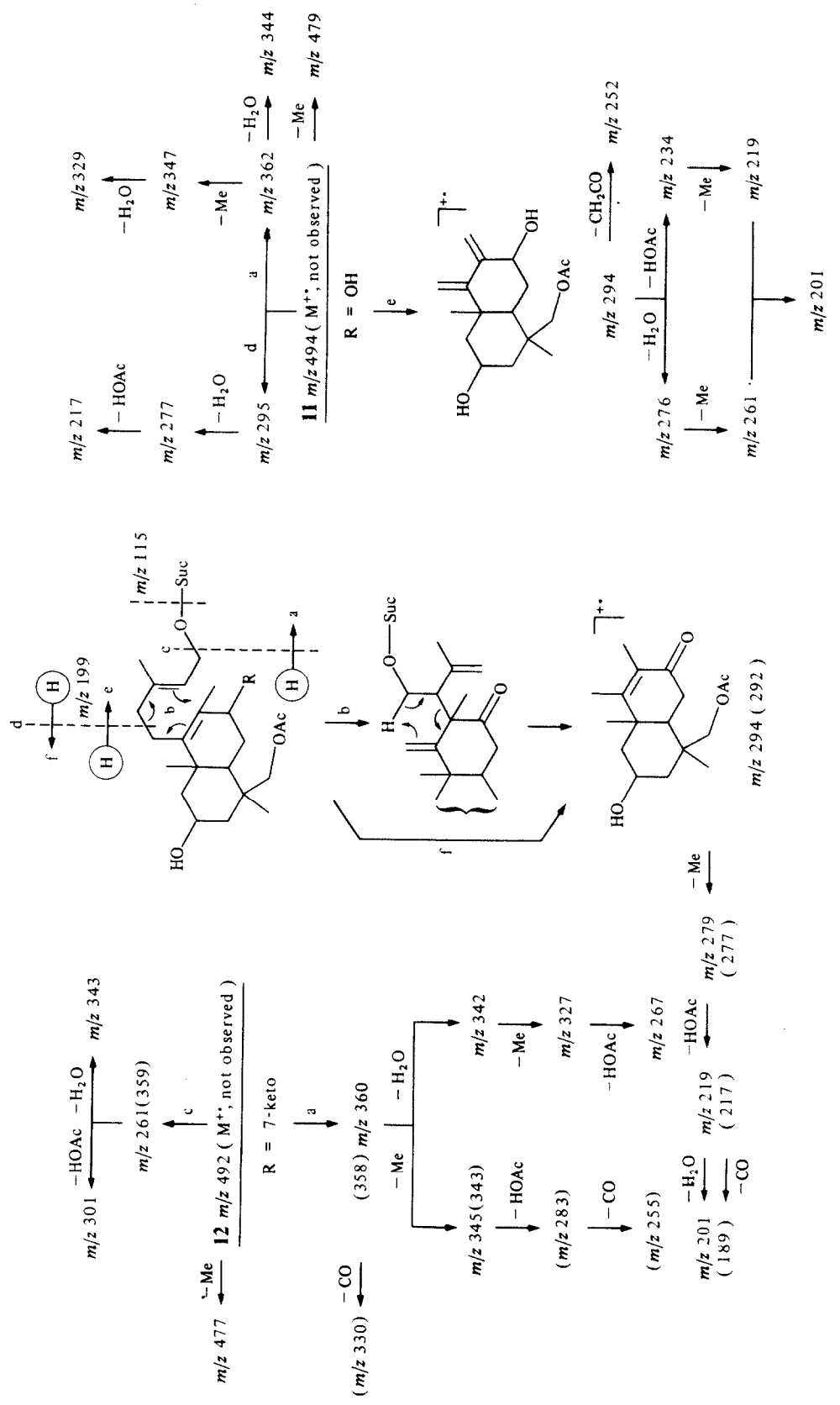
acid and water as depicted in Scheme 3. Compound **11**, like **12**, displayed no $[M]^+$ peak (*m/z* 494) but the peaks at *m/z* 477, 360, 345 and 342 in **12** were shifted to higher mass numbers by 2 in **11**, as required by the substitution of a carbonyl for a hydroxyl group at C-7 in **11**. Surprisingly, the Cope rearrangement ion (*m/z* 294) and its further decomposition product (*m/z* 201) did not shift but remained dominant in **11**, suggesting that **11**, unlike **12**, did not undergo [3, 3] sigmatropic shifts. Instead, the main decomposition route involved fragmentation to the ion at *m/z* 294 (base) followed by loss of water, methyl and acetic acid leading to the ion at *m/z* 201 as shown in Scheme 3. However, the diketone **13** ($[M]^+$ at *m/z* 490 not observed), derived from **11**, exhibited the typical fragmentation of **12** with appropriate shifts to lower mass numbers as shown in Scheme 3. This is clearly seen from the Cope rearrangement ion which occured at *m/z* 292 rather than *m/z* 290.

Following the procedure outlined for *A. sphaerocephalus*, the sodium carbonate soluble acid fraction was isolated from the methylenedichloride extract of *A. shockleyi* and methylated. The methyl ester mixtures of *A. sphaerocephalus* and *A. shockleyi* were nearly identical on TLC (petrol–Et_2O–MeOH, 20:25:1). The diterpenoid constituents of the methyl ester mixture of *A. shockleyi* were not isolated but their presence were assumed after gas chromatography (GC) analysis of the methyl ester mixtures of both plants. The chromatograms were identical except for the relative proportions of the individual constituents. The validity of our assumption was enhanced by spiking each mixture with **4** and injecting a 1:1 mixture of the two methylated acid fractions, whose retention times were superimposable with the gas chromatograms obtained from each plant sample. The major constituent in both plant extracts was **4**.

EXPERIMENTAL

A Hewlett–Packard 5% phenyl methyl silicone (stationary phase) fused silica capillary column (25 m × 0.2 mm i.d.) as programmed 100–300° at 20°/min with an initial and final hold of 1 and 10 min, respectively, was used. The glass capillary column was BP-1 (9.9 m × 0.22 mm) with He as the carrier gas regulated at a flow rate of 30 ml/min. The injector port temp was maintained at 200° and the detector at 340°. The column oven was temp. programmed from 100 to 300° at 8°/min after an initial 1 min hold at the starting temp. See ref. [6] for other analytical procedures used.

Plant material. Aerial plant material of *A. sphaerocephalus* (SPM 4259) was collected on 23 April 1987 in Clark County, Nevada, 13.9 miles north of Searchlight, and *A. shockleyi* (BNT 869) was collected on 18 May 1986 in Clark County, Nevada, 9.2 miles east of Toiyabe National Forest boundary on Kyle Canyon Road, towards Hwy US 95. Voucher specimens are on deposit in the Herbarium at the University of Arizona, Tucson. All plant material was air-dried, ground to 3 mm particle size and stored at 5° prior to extraction.



Scheme 3. Diagnostic EIMS fragments of **11** and **12**. Figures in parentheses represent analogous ions in the EIMS of **13**.

Extraction and separation of the acidic fraction. Percolation of ground *A. sphaerocephalus* (2779 g) with CH_2Cl_2 at room temp (43 hr) gave an extract (145 g) after freeing from solvent. The dry extract (112 g) was stirred with MeOH (1800 ml) for 6 hr, left in the refrigerator overnight, filtered, concd to 400 ml, diluted with H_2O (100 ml) and extracted with petrol (500 ml × 5). The aq. MeOH phase, freed from MeOH, was taken up in Et_2O and washed with 5% aq. Na_2CO_3 soln until the washings were light yellow. The combined washings were acidified with 25% aq. HCl to pH 5 and the liberated acids were taken up in Et_2O, dried and removal of the Et_2O under vacuum gave the acid mixture (27.2 g).

Further extraction of the ethereal soln (after separation of the acidic fraction) with 5% aq. NaOH soln followed by acidification gave a phenolic fraction (2.4 g). The extracted Et_2O soln after washing with H_2O, drying and evapn gave the neutral fraction (19.0 g).

Isolation of **3–8**, **11** *and* **12**. The acid mixture (27.2 g) was esterified with MeI [2] and the resulting Me ester mixture (25.2 g) was chromatographed on a silica gel column (700 g packed in petrol). Initial elution of the column with petrol–Et_2O (3:2) followed by gradually increasing concentrations of Et_2O to 100% and final elution with CH_2Cl_2–MeOH (1:1) gave 37 fractions. From these fractions **3–8**, **11** and **12** were isolated qualitatively by PLC as shown in Table 3.

Isolation of sesquiterpenoids. The petrol-soluble fr (32.0 g), freed from solvent, was submitted to silica gel CC (1500 g, packed in petrol). Elution with petrol, petrol–Et_2O (various concn), Et_2O and CH_2Cl_2–MeOH (1:1) gave 34 frs from which sesquiterpenoids were isolated qualitatively by PLC as summarized in Table 4.

Hydrolysis of **4**. To a soln of **4** (0.8 g) in MeOH (140 ml) was added a soln of Na_2CO_3 (1.0 g) in H_2O (3 ml) and the mixture was stirred (24 hr) at room temp. The reaction mixture was filtered, solvent freed, taken up in Et_2O, washed with H_2O and dried. Evapn of the solvent gave a residue (0.53 g) which was stirred with Et_2O (1 hr), cooled and filtered. A colourless residue (homogeneous by TLC) was collected (334 mg), an analytical sample was obtained by crystallization from Et_2O: mp 149–150°, $[\alpha]_D$ (see text). IR (KBr): strong OH absorption (3330, 1025 cm^{-1}), free of C=O absorption >C=CH (1670 cm^{-1}), ^1H NMR (Table 1) and EIMS *m/z* (rel. int.): 322 $[M]^+$ (not observed), 273 $[M-H_2O-CH_2OH]^+$ (1.5), 255 $[273-H_2O]^+$ (2.7), 237 $[255-H_2O]^+$ (11.1), 236 [M−86 (MLR)]$^+$ (74.0), 221 $[236-Me]^+$ (13.2), 218 $[236-H_2O]^+$ (14.2), 205 $[236-CH_2OH]^+$ (11.4), 200 $[236-(2\times H_2O)]^+$ (14.6), 187 $[218-CH_2OH]^+$ (24.2), 185 $[200-Me]^+$ (33.3), 171 (27.7), 159 (21.3), 145 (29.0), 133 (35.5), 119 (56.2), 107 (67.7), 93 (39.6), 81 (100), 67 (15.1), 55 (36.5). The spectra were in accord with the triol structure 2α,15,19-trihydroxylabda-7,*E*-13-diene (**2**).

Table 3. PLC procedures for separation of compounds **3–8**, **11** and **12**

Compound	CC Fr.	No. of PLC	Solvent/developments*
8	2	2	A/1. B/4
7	5	2	F/2, F/2
6	5	3	H/multiple
4	7–18	2	F/1, F/1
12	29–32	3	C/1, D/1, G/multiple
3	29–32	2	C/1, G/multiple
11	33–34	2	E/1, C/1
5	33–34	2	E/1, C/2

*A Petrol–Et_2O (2:1), B: petrol–Et_2O (3:1), C: $CHCl_3$–MeOH (23:2), D: $CHCl_3$–MeOH (50:3), E: $CHCl_3$–MeOH (47:3), F: petrol–Et_2O–MeOH (20:25:1), G: petrol–Et_2O–MeOH (20:25:2), H: petrol–Et_2O–MeOH (25:20:1).

Acetylation of **4**. Treatment of **4** (74 mg) with Ac_2O–pyridine in the usual manner followed by PLC (petrol–Et_2O–MeOH, 15:30:1; one development) gave 2α,19-diacetoxy-15-succinyloxylabda-7,*E*-13-diene (**9**). IR (neat): free of OH absorption, ^1H NMR (Table 1) and EIMS *m/z*: 520 $[M]^+$ (not observed), 320, 260 $[320-HOAc]^+$, 200 $[260-HOAc]^+$, 185 $[200-Me]^+$, 171, 159, 145, 133, 119, 115 (base), spectra were in accord with the structure shown.

Oxidation of **4**. A mixture of **4** (100 mg), CCl_4 (7 ml) and MnO_2 (1.0 g) was stirred (24 hr), filtered, solvent freed and the residue when submitted to PLC (petrol–Et_2O–MeOH, 15:30:1, single development) gave the corresponding 2-keto derivative, 2-oxo-19-acetoxy-15-succinyloxylabda-7,*E*-13-diene (**10**) in addition to some starting material **4**. The IR (text), ^1H NMR (Table 1) and EIMS *m/z*: 476 $[M]^+$ (not observed), 344 $[M-132]^+$, 216 $[276-HOAc]^+$, 201 $[261-Me]^+$, 115 (base), spectra were in accord with the structure shown.

Hydrolysis of **7**. Hydrolysis of **7** (100 mg) with Na_2CO_3–MeOH as described above for **4** followed by PLC (petrol–Et_2O–MeOH, 20:25:1, two developments) gave the diol, 2α,15-dihydroxy-19-desacetyllabda-7,*E*-13-diene (**1**). Its IR (neat): no C=O band, ^1H NMR (Table 1) and EIMS *m/z*: 306 $[M]^+$ (not observed), 288 $[M-H_2O]^+$, 270 $[M-2H_2O]^+$, 255 $[M-(2H_2O+Me)]^+$, 220 (base), 207 [M−C-9 side chain]$^+$, 205 $[220-Me]^+$, 202 $[220-H_2O]^+$, 189 $[207-H_2O]^+$, 187 $[202-Me]^+$, spectra were in accord with the structure shown.

Oxidation of **11** *and* **12**. Oxidation of both **11** and **12** with MnO_2 as described above for **4** followed by PLC ($CHCl_3$–MeOH, 20:1, two developments) gave the same diketo derivative, 2,7-dioxo-19-acetoxy-15-succinyloxylabda-8,*E*-13-diene

Table 4. Separation of sesquiterpenes

Compound	CC Fr.	No. of PLC	Solvent/developments*
trans-Nerolidol	3	2	A/1, A/2
Caryophyllene oxide	3	4	A/1, B/3, C/3, C/4
Spathulenol-viridiflorol	3	3	A/1, B/3, D/2
Bisabolene-1,4-endoperoxide	3	5	A/1, B/3, C/3, C/4, F/1
β-Eudesmol	7	3	A/2, A/2, A/3
1-Oxobisabolene	8	1	A/2
Shiromool	26–30	2	E/2, E/3

*A: Petrol–Et_2O (4:1), B: petrol–Et_2O (47:3), C: petrol–Et_2O (23:2), D: petrol-Et_2O (9:1), E: petrol–Et_2O–MeOH (35:15:1), F: petrol–CH_2Cl_2 (1:1).

(13). IR (text), ^{1}H NMR (Table 1) and MS (Scheme 3) were in accord with the structure shown.

Percolation of ground *A. shockleyi* (656 g) with CH_2Cl_2 at room temp (1 hr) gave an extract (6.2 g) after freeing from solvent. A portion (2.6 g) of this extract, following the procedure outlined for *A. sphaerocephalus*, gave 0.33 g of Me ester mix which on TLC (petrol–Et_2O MeOH, 20:25:1) and GC was very similar to that of the Me ester mix obtained from *A. sphaerocephalus*.

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REFERENCES

1. Lane, M. (1988) Madroño (in press).
2. Timmermann, B., Hoffmann, J., Jolad, S., Schram, K., Klenck, R. and Bates, R. (1982) *J. Org. Chem.* **47**, 4114.
3. Dentali, S., Hoffmann, J., Jolad, S. and Timmermann, B. (1987) *Phytochemistry* **26**, 3025.
4. Devon, T. and Scott, A. (1972) in *The Handbook of Naturally Occurring Compounds* **II**, pp. 203 and 204.
5. Hoffmann, J., Jolad, S., Timmermann, B., Bates, R., Camou, F. and Siahaan, T. (1987) *Phytochemistry* **26**, 2861.
6. Jolad, S., Hoffmann, J., Schram, K., Cole, J., Tempesta, M. and Bates, R. (1981) *J. Org. Chem.* **46**, 4267.