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GERMACRANOLIDES FROM *HELIANTHUS CALIFORNICUS*

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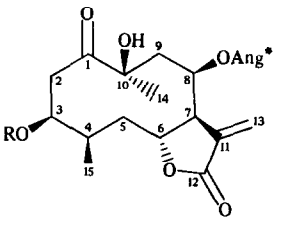
Key Word Index—*Helianthus californicus*, Asteraceae, sunflowers, sesquiterpene lactones, germacranolides, germacrolides, diterpene, trachylobane, X-ray crystallography

Abstract—Five germacranolide sesquiterpene lactones and a trachylobane diterpene were isolated from the hexaploid species *Helianthus californicus* Four of the sesquiterpene lactones are new, including a 1-oxo-3,10-diol without any carbon–carbon double bonds in the main ring whose structure was confirmed by X-ray crystallography These results require revision of the structures of several previously published compounds Two of the germacranolides have alkyl ether functions at a position β to a ketone and are believed to be artifacts of the isolation process Based on terpenoid chemistry, there are no obvious progenitors of *H californicus* among the diploid species of *Helianthus* examined to date

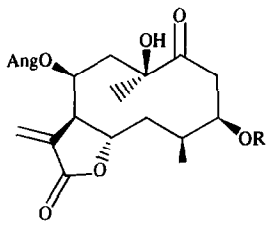
INTRODUCTION

As part of our continuing phytochemical study of the North American sunflowers, *Helianthus* (Asteraceae) [1–5], we have investigated the terpenoid chemistry of *H californicus* DC , a hexaploid perennial species of section *Divaricati*, series *Corona-solis* [6] native to central and southern California and northern Baja California [7] A substantial number of sesquiterpene lactones and diterpenes has been isolated from species of *Helianthus* [7a] Most of the sesquiterpene lactones reported are germacrolide or heliangolide-type germacranolides, while the majority of the diterpenes have kaurane, atisirane or trachylobane skeletons

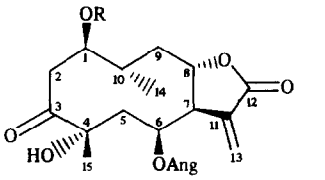
In this paper, we report the isolation and identification of a diterpene carboxylic acid, the previously characterized trachylobane carboxylic acid **19** [8] and five germacranolides from a chloroform extract of *H californicus* One of the germacranolides was the known costunolide derivative **18** [9], while the others (**1–3** and **13**) were new compounds, although **2** and **3** were probably artifacts of the isolation procedures used An X-ray analysis of compound **1** confirmed the stereochemistries of **1–3** and suggested that several previously published structures (**4–9**) needed revision



1** R = H
2 R = Me
3 R = $CHMe_2$



1a R = H
2a R = Me
3a R = $CHMe_2$



1b R = H
2b R = Me
3b R = $CHMe_2$

RESULTS AND DISCUSSION

Compound **1** gave a molecular ion at *m/z* 380 (5% relative intensity) which had a formula of $C_{20}H_{28}O_7$ (HRMS 380 1835 calculated, 380 1833 measured). Spectral data showed the presence of an α-methylene-γ-lactone (IR ν_{max} 1767 cm^{-1}, ^{13}C NMR δ169 0 *s*, ^1H NMR two narrowly-split doublets at δ5 67 and 6 27) and an angelic acid side chain [IR ν_{max} 1714 cm^{-1}, MS *m/z* 83 (base peak), ^{13}C NMR δ165 6 *s*, ^1H NMR. a spin system consisting of two methyl groups at 1 80 and 1 95 and a vinylic proton at 6 08] Of the three remaining oxygen atoms, one was in an unconjugated ketone (IR 1714 cm^{-1}, ^{13}C NMR δ213 5 *s*) and at least one was in a hydroxyl group (IR 3530 cm^{-1}, ^1H NMR: D_2O-exchangeable *br s* at 4 04) These structural features

*Ang = angelate, iVal = isovalerate, Mac = α-methylacrylate, 2-Mebut = 2-methylbutanoate

Since C-10 is depicted as a re-entry angle in these formulae, it is necessary to reverse the relative configuration at this position according to recommendations made earlier [52] The relative configuration at C-10 in **1–5, **10** and **11** is *R* (and that in the enantiomeric formulae **1a–3a** is *S*) In **1b–3b**, which are rotamers of **1a–3a**, the C-10 substituents have 'moved' to position 4 where, because this center is not drawn in re-entrant fashion, their orientations are no longer reversed Similar considerations apply to C-4, which has an *R* configuration in **1–5**, **10** and **11**, but becomes *S* in **1a–3a** (and in **1b–3b**) In **1b–3b**, the C-4 methyl group has 'moved' to a re-entrant position, C-10, so its configuration has been reversed Compounds **6–9** are depicted as in the original description It is not known if the authors had intended to reverse the stereochemistry at C-10 in these compounds or not

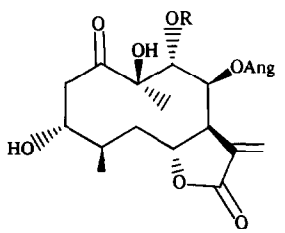
Table 1 ^{13}C NMR spectra of compounds 1–3, 10 and 13*

	1	2	3	10	13
C-1	213 5 *s*	214 8 *s*	215 2 *s*	208 2 *s*	126 0 *d*
C-2	41 9 *t*‡	37 7 *t*	40 0 *t*‡	125 5 *d*	30 5 *t*
C-3	69 1 *d*†	80 3 *d*‖	77 2 *d*‖	147 0 *d*	76 0 *d*
C-4	30 3 *d*	30 2 *d*	30 5 *d*	28 5 *d*	134 2 *s*
C-5	35 6 *t*†	35 1 *t*	35 7 *t*	40 6 *t***	130 2 *d*
C-6	75 9 *d*†	76 5 *d*‖	76 2 *d*‖	76 1 *d*	70 4 *d*
C-7	41 9 *d*	42 9 *d*	43 2 *d*	42 6 *d*	59 1 *d*
C-8	72 1 *d*†	70 0 *d*	71 4 *d*¶	73 7 *d*	79 5 *d*
C-9	40 6 *t*‡	40 2 *t*‡	40 7 *t*‡	40 4 *t***	47 7 *t*
C-10	77 6 *s*	78 1 *s*	78 6 *s*	76 7 *s*	133 6 *s*
C-11	136 8 *s*	136 3 *s*	137 1 *s*	136 1 *s*	41 2 *d*
C-12	169 0 *s*	169 3 *s*	169 7 *s*	169 5 *s*	179 2 *s*
C-13	123 5 *t*	123 4 *t*	123 6 *t*	125 1 *t*	17 7 *q*††
C-14	27 8 *q*	28 5 *q*	29 0 *q*	28 2 *q*	15 7 *q*††
C-15	20 5 *q*§	20 2 *q*§	23 8 *q*§	20 0 *q*§	16 8 *q*††
C-1′	165 6 *s*	166 3 *s*	166 6 *s*	166 2 *s*	—
C-2′	127 0 *s*	127 2 *s*	127 8 *s*	127 1 *s*	—
C-3′	138 6 *d*	139 8 *d*	139 8 *d*	140 1 *d*	—
C-4′	15 3 *q*	15 7 *q*	16 2 *q*	15 7 *q*	—
C-5′	19 8 *q*§	20 0 *q*§	22 5 *q*§	20 1 *q*§	—
OMe	—	58 1 *q*	—	—	—
$OCH(Me)_2$	—	—	70 7 *d*¶	—	—
	—	—	20 7 *q*	—	—
	—	—	20 7 *q*	—	—
Acetate	—	—	—	—	170 5 *s*
	—	—	—	—	21 3 *q*

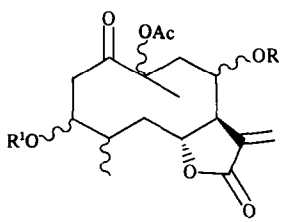
*Run at 22 6 MHz in $CDCl_3$ with TMS as an internal standard Assignments made using off-resonance decoupling experiments and by analogy with portions of model compounds **1–3** [5, 14, 15], **10** [14, 49] and **14** [33, 50, 51]

†Assignments confirmed by single-frequency off-resonance decoupling experiments

‡§‖¶**††Assignments interchangeable



4 R = Ac
5 R = 2–Mebut



	3–OR¹	4–Me	8–OR	10–OAc
6 R = Mac R¹ = H	β	α	α	β
7 R = Ang R¹ = H	β	β	α	α
8 R = Ang R¹ = H	α	α	β	β
9 R = Mac R¹ = Me	β	α	α	β

accounted for six of the seven degrees of unsaturation calculated from the molecular formula Since all of the ^{13}C NMR resonances for sp^2-hybridized carbons were accounted for (Table 1), the final degree of unsaturation was a ring This ring had to be carbocyclic and the remaining oxygen had to be in a second hydroxyl group since there was only one more ^{13}C NMR signal for an sp^3-hybridized carbon atom bonded to oxygen

Spin decoupling experiments were undertaken to assign the remainder of the ^1H NMR signals (Tables 2 and 3) Irradiation at δ6 27 (H-13a) located H-7 (2 66), which was coupled to signals at 4 93 and 5 55 Since there were no carbon–carbon double bonds in the macrocycle, the signal at 4 93 seemed to be that of the proton at the site of lactone ring fusion and the signal at 5 55 was probably that of the proton at the position of attachment of the ester side chain Irradiation at 5 55 collapsed a sharp two-proton doublet at 2 40 into a singlet, while irradiation at 4 93 simplified methylene protons at 1 46 and 2 18 These methylene protons seemed to be spin-coupled to a partially-obscured multiplet at *ca* 1 95, which was in turn coupled to a saturated methyl group (1 12 *d*) and a *ddd* at 4 28, a signal assignable to a proton adjacent to a hydroxyl group Irradiation at 4 28 collapsed double doublets at

Table 2 ^{1}H NMR spectra of the sesquiterpene lactones **1–8*** chemical shifts

	1	**1** (C_5D_5N)	**2**	**3**	**4** [10]	**5** [10]	**6** [11]	**7** [12]	**8** [13]
H-2a†	3 12 *dd*	3 75	2 96 (2H) *d*	3 05 *dd*	3 25	3 25	3 15	3 17	3 16
H-2b	2 85 *dd*	3 19	—	2 76 *dd*	2 96	2 95	2 89	2 91	2 90
H-3	4 28 *ddd*	4 63	3 73 *dt*	3 92 *ddd*	4 34	4 31	4 23	4 23	4 24
H-4	1 95‡	2 39 *dddq*	1 95‡	‡	‡	1 75‡	2 10‡	2 08‡	2 06
H-5a	2 18 *ddd*	2 67	2 14	2 19	‡	2 12	2 10	2 08	2 10
H-5b	1 46 *ddd*	1 53	1 46	1 45	‡	1 6‡	1 51	1 53	1 53
H-6	4 93 *ddd*	5 55	4 88	4 91	4 90	4 92	4 48	4 46	4 45
H-7	2 66 *dddd*	3 28	2 64	2 65	2 59	2 60	2 98	2 95	2 94
H-8	5 55 *td*	6 11 *ddd*	5 54 *td*	5 56	5 80 *dd*	5 94	4 94 *ddd*	5 01	5 00
H-9a	2 40 (2H) *d*	3 01 *dd*	2 40 (2H) *d*	2 41 (2H)	5 54 *d*	5 85	2 64 *dd*	2 60	2 61
H-9b	—	2 57 *dd*	—	—	—	—	2 19 *dd*	2 15	2 15
H-13a	6 27 *d*	6 27	6 26	6 26	6 31	6 29	6 33	6 33	6 32
H-13b	5 71 *d*	5 48	5 79	5 69	5 80	5 79	5 71	5 70	5 70
H-14	1 39 (3H) *s*	1 49 (3H)	1 37 (3H)	1 36 (3H)	1 28 (3H)	1 27 (3H)	1 88 (3H)	1 89 (3H)	1 90 (3H)
H-15	1 12 (3H) *d*	1 24 (3H)	1 11 (3H)	1 09 (3H)	1 14 (3H)	1 14 (3H)	1 13 (3H)	1 13 (3H)	1 14 (3H)
H-3′	6 08 *qq*	5 96	6 17	6 08	—				
H-4′	1 95 (3H) *dq*	2 01 (3H)	1 95 (3H)	1 95 (3H)	—				
H-5′	1 80 (3H) *br s*	1 80 (3H)	1 79 (3H)	1 79 (3H)	—				
10-OH	4 04 *br s*	—	4 03	4 02	—				
OMe	—	—	3 39 (3H) *s*	—	—				
$OCH(Me)_2$	—	—	—	3 63 *septet*	—				
	—	—	—	1 16 (3H) *d*	—				
	—	—	—	1 15 (3H) *d*	—				

*Run in $CDCl_3$ (except as noted) with TMS as an internal standard Compounds **1–5** were measured at 200 MHz, **7** at 270 MHz and **6** and **8** at 400 MHz Data for **4–8** taken from literature refs given Signals for the side chain esters in these compounds are omitted Multiplicities are the same as those in the previous column, except as noted

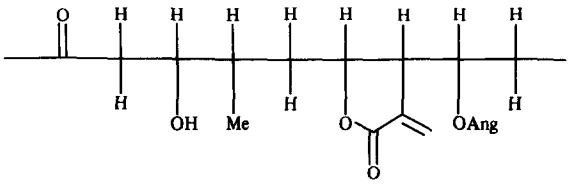
†Methylene proton assignments—**1–3** 2a = 2β, 2b = 2α, 5a = 5β, 5b = 5α, **7** 2a = 2β, 2b = 2α, 5a = 5α, 5b = 5β, 9a = 9α, 9b = 9β Other methylene protons not assigned

‡Obscured by overlapping signals

Table 3 ^{1}H NMR spectra of the sesquiterpene lactones **1–8** coupling constants

	1	**1** C_5D_5N	**2**	**3**	**4**[10]	**5**[10]	**6**[11]	**7**[12]	**8**[13]
H-2a, 2b	18	18	—	18	18	18	19	—	19
H-2a, 3	10 5	10 5	8 5	9	10 5	10	—	—	—
H-2b, 3	6	6	8 5	7	6 5	6 5	—	—	—
H-3, 4	~2	~2	~2	~2	2 5	2 5	1 5	—	—
H-4, 5a	8 5	9 5	8 5	8	—	—	9	—	~7 5
H-4, 5b	4 5	4	4	4	—	4	3	3 5	~3
H-4, 15	7 5	6 5	7	7	6 5	6 5	7	7	—
H-5a, 5b	16	15	16	14	—	16	15	15	13
H-5a, 6	4 5	4 5	4 5	4 5	6 5	6 5	4	5 5	~4
H-5b, 6	8 5	8 5	8 5	8 5	6 5	6 5	7	7 5	~10
H-6, 7	4 5	3 5	4 5	4 5	3 5	4	—	4 5	~7
H-7, 8	2	2	2	2	1 5	1 5	~2	5	4
H-7, 13a	2	2	2	2	2	2	2 5	2 5	2 5
H-7, 13b	1 5	1 5	1 5	2	2	2	2	2	2 3
H-8, 9a	8	10	8	8	10	10 5	8 5	8 5	9
H-8, 9b	8	4	8	8	—	—	~1	1 5	~1
H-9a, 9b	—	14 5	—	—	—	—	—	15	14

*See footnotes to Table 2 Where couplings are not listed, they can be assumed to have been obscured, or in the case of geminal couplings, both protons have the same chemical shift Couplings for angelate side chain in **1–3** 3′, 4′ = 7 5, 3′, 5′ = 1 5 and 4′, 5′ = 1 5



A

2 85 and 3 12 into an isolated AB pattern These results are summarized in partial structure **A** The chemical shifts of the signals at 2 85 and 3 12 suggested that they were adjacent to the ketone The remaining position in the ten-membered ring, then, had to accomodate another methyl group (^{1}H NMR 1 39 *s*) and the second hydroxyl group, giving the general structure shown in formula **1**, exclusive of stereochemistry

The relative orientation of the ring substituents in **1** was difficult to determine because the conformational flexibility of molecular models made applications of the Karplus relationship unreliable and because there were no appropriate analogues whose stereochemistry had been unequivocally established For example, the ^{1}H NMR shifts and coupling constants for protons at H-2, H-3 and H-4 in the related compounds **4–8** [10–13] are very much like those for the analogous protons in **1** (Tables 2 and 3) This might suggest that all of these compounds share the same stereochemistry at C-3 and C-4 However, the configurations of these three centers in compounds **4–8** have been assigned in all possible ways

The elucidation of the structure of a derivative of **1**, compound **10**, helped overcome these problems The dehydrated derivative **10** was the major product of an attempt to acetylate **1** with acetic anhydride in pyridine Spectral data showed the presence of a C-2–C-3 double

Table 4 ^{1}H NMR spectra of compounds **10** and **11***

	10	**11** [14]
H-2	6 51 *d* (12)	6 52 *d* (11)
H-3	5 91 *dd* (11, 12)	5 96 *d* (11)†
H-4	3 10 *dddq* (5, 11, 12, 6)	3 09 *m*
H-5α	1 43 *ddd* (5, 12 5, 13)	‡
H-5β	1 83 *ddd* (5 5, 12, 13)	‡
H-6	4 54 *br dd* (5 5, 12 5)	4 50 *dd* (5, 12)
H-7	2 68 *br ddd* (1 5, 2, 2 5)	‡
H-8	5 36 *ddd* (2 5, 5, 9 5)	5 32 *dt* (2, 7)
H-9α	2 35 *dd* (5, 16)	2 26 (2H) *d* (7)
H-9β	2 26 *dd* (9 5, 16)	—
H-13a	6 38 *d* (2)	6 27 *d* (1)
H-13b	5 78 *d* (1 5)	5 77 *d* (1)
H-14	1 46 (3H) *s*	1 44 (3H) *s*
H-15	1 13 (3H) *d* (6)	1 12 (3H) *d* (7)
OH	4 08 *br s*	3 70 *br s*
H-3′	6 05 *qq* (7 5)	
H-4′	1 95 (3H) *dq* (7 5, 1 5)	
H-5′	1 77 (3H) *br s* (1 5, 1 5)	

*Run in $CDCl_3$ with TMS as an internal standard at 200 MHz (**10**) and 100 MHz (**11**) Data for **11** is from ref [14] Signals for the ester side chain in this compound are omitted Numbers in parentheses are coupling constants in Hz

†Probably a misprint—should read 'triplet'

‡Signal not reported

1685 cm^{-1}, ^{13}C NMR upfield shift of carbonyl to 208 2, ^{1}H NMR new signals at 5 91 and 6 51) With the exception of signals for the side chain, the NMR spectra of **10** were very similar to those of neurolenin A (**11**) (Tables 1

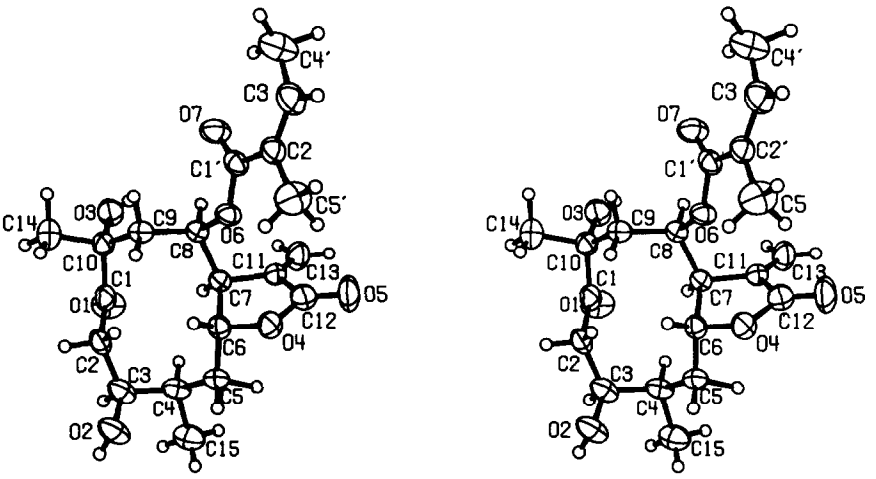
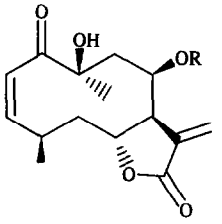
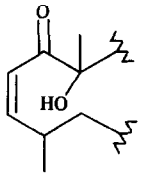


Fig 1 Stereoscopic view of the molecule showing the atom labeling scheme The thermal ellipsoids are 50% equiprobability envelopes, with hydrogens as spheres of arbitrary diameter Hydrogens are numbered the same as the atom to which each is attached



10 R = Ang
11 R = iVal



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lography [14] The stereochemistry of the main skeleton of **10** therefore seemed to be the same as that of **11**, which appeared to fix the configurations at C-4, C-6, C-7, C-8 and C-10 in the parent compound **1**

To confirm these findings and to determine the configuration at C-3, an X-ray analysis of **1** was obtained As seen in Fig 1, the orientation of substituents at C-4, C-6, C-7, C-8 and C-10 is the same as in **11** and the 3-hydroxyl group is β-disposed The bond distances and angles in the ten-membered ring system (Tables 5 and 6) are not unusual and show good agreement with corresponding fragments in similar compounds [15–17] The geometry of the C-8 side chain also compares well with that of other angelate residues in natural products [18–20] Some degree of shortening of the angelate methyl bonds due to high thermal motion is seen, although not to the same extent as noted in our previous studies [19, 21] The α-methylene-γ-lactone ring of **1** is decidedly non-planar, as evidenced by the sum of the endocyclic torsion angle

Table 5 Intramolecular bond distances (Å)

O1-C1	1 208 (4)	C4-C15	1 536 (6)
O2-C3	1 444 (4)	C5-C6	1 523 (5)
O3-C10	1 434 (5)	C6-C7	1 548 (4)
O4-C6	1 474 (4)	C7-C8	1 520 (4)
O4-C12	1 352 (4)	C7-C11	1 515 (5)
O5-C12	1 202 (4)	C8-C9	1 533 (5)
O6-C8	1 462 (4)	C9-C-10	1 521 (5)
O6-C1'	1 344 (4)	C10-C14	1 534 (6)
O7-C1'	1 222 (4)	C11-C12	1 479 (5)
C1-C2	1 510 (5)	C11-C13	1 306 (5)
C1-C10	1 546 (5)	C1'-C2'	1 468 (5)
C2-C3	1 517 (5)	C2'-C3'	1 335 (5)
C3-C4	1 535 (5)	C2'-C5'	1 501 (6)
C4-C5	1 529 (5)	C3'-C4'	1 496 (6)

Table 6 Intramolecular bond angles (°)

C6-O4-C12	110 6 (3)	C7-C8-C9	118 0 (3)
C8-O6-C1'	118 4 (3)	C8-C9-C10	117 4 (3)
O1-C1-C2	121 5 (4)	O3-C10-C1	107 6 (3)
O1-C1-C10	120 4 (4)	O3-C10-C9	111 6 (3)
C2-C1-C10	118 0 (4)	O3-C10-C14	105 8 (4)
C1-C2-C3	115 7 (4)	C1-C10-C9	114 5 (3)
O2-C3-C2	106 1 (3)	C1-C10-C14	107 5 (3)
O2-C3-C4	110 3 (4)	C9-C10-C14	109 3 (4)
C2-C3-C4	115 8 (3)	C7-C11-C12	107 0 (3)
C3-C4-C5	116 9 (3)	C7-C11-C13	130 5 (4)
C3-C4-C15	109 6 (4)	C12-C11-C13	122 5 (4)
C5-C4-C15	107 9 (4)	O4-C12-O5	122 0 (4)
C4-C5-C6	120 6 (3)	O4-C12-C11	109 3 (3)
O4-C6-C5	106 2 (3)	O5-C12-C11	128 7 (4)
O4-C6-C7	104 6 (3)	O6-C1'-O7	122 2 (3)
C5-C6-C7	114 9 (3)	O6-C1'-C2'	110 7 (3)
C6-C7-C8	115 5 (3)	O7-C1'-C2'	127 1 (4)
C6-C7-C11	101 1 (3)	C1'-C2'-C3'	121 9 (4)
C8-C7-C11	110 5 (3)	C1'-C2'-C5'	117 4 (4)
O6-C8-C7	105 5 (3)	C3'-C2'-C5'	120 6 (4)
O6-C8-C9	106 5 (3)	C2'-C3'-C4'	129 4 (5)

Table 7 Torsion angles (°)

C12-O4-C6-C7	−21 4
O4-C6-C7-C11	26 5
C6-C7-C11-C12	−23 3
C7-C11-C12-O4	11 8
C11-C12-O4-C6	6 4
O5-C12-C11-C13	11 9
C1-C2-C3-C4	56 3
C2-C3-C4-C5	63 6
C3-C4-C5-C6	−64 9
C4-C5-C6-C7	−54 3
C5-C6-C7-C8	151 1
C6-C7-C8-C9	−57 3
C7-C8-C9-C10	−65 5
C8-C9-C10-C1	68 3
C9-C10-C1-C2	53 6
C10-C1-C2-C3	−157 6
C7-C8-O6-C1′	145 9
C8-O6-C1′-C2′	−176 6
O6-C1′-C2′-C3′	174 7
C1′-C2′-C3′-C4′	0 1
C5′-C2′-C3′-C4′	178 5

moduli (89°, see Table 7) This is the usual case for a *trans*-fused ring [19, 22], quite unlike the nearly planar *trans*-fused ring found in niveusin C-2′,3′-epoxide [21] The bonding geometry around the ring is in excellent agreement with literature values [20, 21, 23, 24]

The packing of the molecules in the unit cell is shown in Fig 2 Both of the hydroxyl hydrogens participate in hydrogen bonding to an oxygen of a neighboring molecule, with the interatomic separations being O-2 O-1 = 3 023 A and O-3 O-7 = 2 897 A Both of these would be classified as relatively weak hydrogen bonds [25, 26]

The absolute configuration of **1** was not assignable from the results of the X-ray investigation alone, since there was no heavy, anomalously scattering atom in the molecule The CD data were of little help in this connection either, since it was not possible to definitely determine the sign of the diagnostic $n \rightarrow \pi^*$ transition of the α-methylene-γ-lactone system (240–265 nm) [27] due to interference from the chromophore of the angelate side chain The CD curve showed a broad shallow minimum from 235–260 nm Therefore, both Fig 1 (represented in **1**) and its mirror image (**1a**) are equally plausible When rotated to a more conventional representation (**1b**), the mirror image is seen to be 12,8-lactonized with its substituents at differently numbered positions than in **1** Biosynthetic considerations do not permit one to decide between **1** and **1b** because *H. californicus* produces both 12,6 and 12,8-lactonized sesquiterpene lactones (see below) and because there are no carbon–carbon double bonds in the macrocycle of **1** to indicate its mode of biogenesis In this paper, the data for **1**–**3** are discussed as if these were 12,6-lactonized compounds to be consistent with the representation of the analogues **4**–**9** and **11** in the literature Compound **4** is considered to be 12,6-lactonized based on its conversion to a furanone-type sesquiterpene lactone closely related to zexbrevin [10] However, the absolute configuration has not yet been determined for any of these compounds

With the relative configuration of **1** established, the close correspondence of the H-2, H-3 and H-4 ^{1}H NMR signals in **4**–**8** to those of **1** (Tables 2 and 3) suggest that the substituents at C-3 and C-4 in **4**–**8** are all probably β-oriented The signals of H-5–H-8, however, show consistent differences between **6**–**8**, on the one hand, and **1**, **4** and **5** on the other (Tables 2 and 3), probably attributable to a change in the orientation of the C-8 side chain Since the side chain is β-oriented in **1**, it is likely to have a β-orientation in **4** and **5** and an α-orientation in **6** and **7**, as originally proposed, but it is probably also β-oriented in **8**† A reversal of the configuration at C-10 in **6**–**8** might

†Striking similarities in the ^{1}H NMR spectra reported for **7** [12] and **8** [13] indicate that these compounds are probably identical

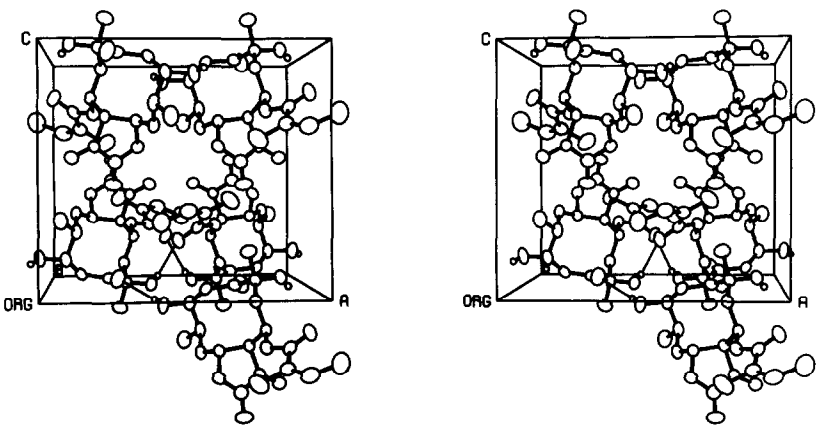


Fig 2 Stereoscopic view of the molecular packing in the unit cell, as viewed along the *b* axis Hydrogen bonds are included as thin solid lines

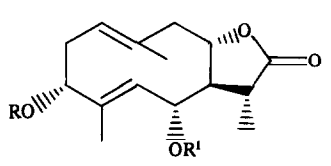
also account for the ^{1}H NMR differences at H-5–H-8, but this seems less likely, as such a change should lead to a shift in the conformation of the C-1 ketone function to maintain its hydrogen bonding with the 10-hydroxyl group which, according to models, should also affect the conformation at C-2 and C-3 As stated above, the ^{1}H NMR signals for protons at C-2 and C-3 are very similar in all of these compounds

Two close analogues of **1**, compounds **2** and **3** (or **2a** and **3a**) were also isolated during this study Structures were assigned to them based on comparisons of their spectral data with those of **1** (Tables 1–3) The presence of an unprecedented isopropyl group in **3** suggested that this compound might be an artifact of our isolation and purification procedures which employed both isopropanol and isopropyl ether If so, both **1** and **2** might also be considered artifacts derived from the reaction of precursors like **12** with methanol or water Reactions of this type have been reported for sesquiterpene lactones *in vitro* [28] Portions of the *H californicus* extract were exposed to methanol during thin-layer chromatography and water was used in the precipitation of phenolics and chlorophyll with lead acetate during the basic extraction procedure [29]

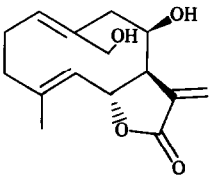
In order to resolve this question, a second collection of *H californicus* leaves, gathered from the same plants as the initial collection, was extracted and the resulting syrup worked up without exposure to methanol, isopropanol or isopropyl ether (see Experimental section) Compound **1** was isolated from this second extract, but compounds **2** and **3** were not detected As a second test, a small amount of leaves from the second collection was ground in chloroform for 60 sec and the resulting extract analyzed directly, without being subjected to lead acetate treatment Thin-layer chromatography again showed the presence of compound **1** (as well as **13**, **18** and **19**), but not **2** or **3** Therefore, it appears that **1** and, by implication, related sesquiterpene lactones with β-hydroxyketone moieties (such as **4**–**8**) are probably naturally-occurring compounds, but that **2** and **3** were artifacts of the purification procedures Methyl ethers have been reported to occur in a few sesquiterpene lactones [30, 31], including **9** [11], which is closely related to **3** (The C-4 methyl group of **9** is probably β-oriented, as discussed above) In these methyl ethers, the methoxyl group is always β to a carbonyl function

Another sesquiterpene lactone isolated in this study, compound **13**, was a saturated α-methyl-γ-lactone [IR ν_{max} 1773 cm^{-1}, ^{13}C NMR δ179 2 *s*, ^{1}H NMR δ1 46 (3H) *d*] with an acetate function [IR ν_{max} 1738 cm^{-1}, ^{13}C NMR δ170 5 *s*, ^{1}H NMR δ2 10 (3H) *s*], two carbon–carbon double bonds (^{13}C NMR δ126 0 *d*, 130 2 *d*, 133 6 *s*, 134 2 *s*) and a hydroxyl group (IR ν_{max} 3450 cm^{-1}) Irradiation at H-13 in the ^{1}H NMR spectrum (1 46) (Table 8) located H-11 (2 81), which was also shown to be spin-coupled to a signal at *ca* 2 1 (H-7) Irradiation at 2 1 simplified two signals for protons adjacent to oxygen atoms (4 08 and 4 36) The signal at 4 36 was next to a hydroxyl group since it shifted downfield to 5 55 in the acetylation product **14** The proton at 4 08, then, must be the one at the site of lactone fusion Further decoupling experiments established partial structures **B** and **C** In joining these fragments, placing the double bonds in their usual locations from a biosynthetic perspective (C-1–C-10 and C-4–C-5) resulted in general structure **13** with lactone fusion to C-8

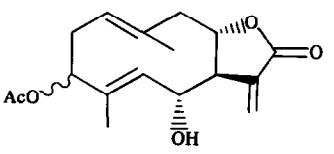
The stereochemistry at C-3, C-6, C-8 and C-11 in **13** was deduced from ^{1}H NMR data (Table 8) and models, assuming a typical germacrolide conformation with both methyl groups projecting above the β-face of the molecule [32] The large $J_{6,7}$ (9 5 Hz) and $J_{7,8}$ (8 Hz) values were indicative of a C-6α-hydroxyl group and a *trans*-fused lactone ring, respectively, assuming that H-7 was α-oriented as in all known sesquiterpene lactones of authenticated absolute stereochemistry The small $J_{2,3}$ coupling constants (3 and 4 Hz) correlated well with H-2–H-3 couplings in other 3α-oxygenated germacrolides [33, 34], but were quite different from those reported for 3β-oxygenated germacrolides (**4**–**6** and **8**–**11**) [e g 35–37] The configuration at C-11 in 11,13-dihydro-sesquiterpene lactones is often determined from the magnitude of the diamagnetic (upfield) shifts of the H-13 signal in benzene or pyridine [38] The pyridine shift of H-13 in **13** was downfield by 0 30 ppm from its location in chloroform,



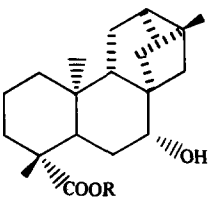
13 R = Ac, R¹ = H
14 R = Ac, R¹ = Ac
15 R = H, R¹ = H



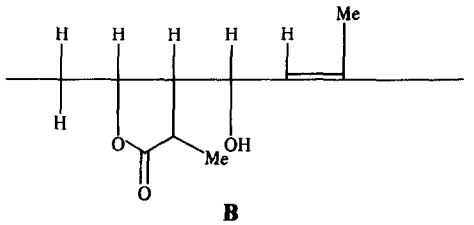
18



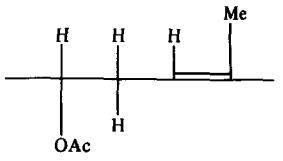
16 R = 3β–OAc
17 R = 3α–OAc



19 R = H
20 R = Me



B



C

Table 8 ^{1}H NMR spectra of compound **13***

	$CDCl_3$	C_5D_5N
H-1	5 10 *br dd* (4, 11)	5 25
H-2α	2 34 *ddd* (3, 4, 13)	2 33
H-2β	2 50 *ddd* (4, 11, 13)	2 52
H-3	5 17 *dd* (3, 4)	5 30
H-5	5 02 *br d* (1 5, 10)	5 39
H-6	4 36 *dd* (9 5, 10)	4 54
H-7	2 1† (8, 9 5, 10 5)	2 33
H-8	4 08 *ddd* (2, 8, 9 5)	4 21
H-9α	2 44 *dd* (9 5, 13)	2 54
H-9β	2 79 *br d* (2, 13)	2 81
H-11	2 81 *dq* (10 5, 7 5)	3 15
H-13	1 46 (3H) *d* (7 5)	1 76 (3H)
H-14	1 56 (3H) *br s*	1 50 (3H)
H-15	1 65 (3J) *br s* (1 5)	1 54 (3H)
acetate methyl	2 10 (3H) *s*	2 00 (3H)

*Run at 200 MHz with TMS as an internal standard Numbers in parentheses are coupling constants in Hz Multiplicities in C_5D_5N were similar to those in $CDCl_3$

†Partially obscured due to overlapping signals

Table 9 Data collection and processing parameters

Space group	$P2_12_12$, orthorhombic
Cell constants	$a = 12\,736\,(7)$ Å
	$b = 13\,587\,(8)$
	$c = 11\,302\,(4)$
	$V = 1956$ Å^3
Molecular formula	$C_{20}H_{28}O_7$
Molecular weight	380 42 g/mol^{-1}
Molecules per cell	$Z = 4$
Density	$\rho = 1\,29$ g/cm^{-3}
Absorption coefficient	$\mu = 0\,6$ cm^{-1}
Radiation (MoKα)	$\lambda = 0\,71073$ Å
Collection range	$4° \leqslant 2\theta \leqslant 60°$
Scan width	$\Delta\theta = (1\,00 + 0\,35 \tan\theta)°$
Maximum scan time	240 s
Scan speed range	0 4 to 5 0° min^{-1}
Total data collected	3191
Independent data, $I > 3\sigma(I)$	1294
Total variables	344
$R = \Sigma\|\|F_o\|-\|F_c\|\|/\Sigma\|F_o\|$	0 027
$R_w = [\Sigma w(\|F_o\|-\|F_c\|)^2/\Sigma_w\|F_o\|^2]^{1/2}$	0 021
Weights	$w = \sigma(F_o)^{-2}$
Goodness-of-fit	1 22

which, for *trans*-fused lactones, is closer to the range for α-oriented C-11 methyl groups (smaller upfield shifts) than that for β-oriented C-11 methyl groups (larger upfield shifts) [38] More significantly, the downfield shift of the C-11 methyl group in pyridine can be explained by its close proximity to the 6α-hydroxyl group [39], a fact which clearly points to its α-orientation

The structure of **13** was confirmed by its conversion to the diacetate **14**, identified by comparison of its spectral data with those presented in the literature [33] Compound **14** was previously prepared from the diol **15** isolated from *H pumilus* An 11,13-dehydro-analogue of **13**, compound **16**, has been reported [40], but comparison of its $J_{2,3}$ values (2 and 3 Hz, presumably from the pyrazoline derivative) with those of other 3-oxygenated germacrolides (see above) suggests that it has the same configuration as **13–15**, and so should have its structure revised to **17** However, in contrast to the published report, Bohlmann [personal communication] mentioned that the ^{1}H NMR spectrum of **16** shows broad signals and that the couplings of H-3 "seem to be large" He further noted that the acetylation product of **16** did not give chamissonin diacetate, the expected product from **17**

The two other compounds isolated in this investigation were the sesquiterpene lactone **18** and the diterpene **19**, both known from other species of *Helianthus* Compound **18**, a simple costunolide derivative, was reported from *H grossesserratus* [9] and *H niveus* subsp *niveus* [Whittemore, A T, Gershenzon, J and Mabry, T J, unpublished results], while **19**, a hydroxytrachylobane acid first isolated from *H ciliaris* [8], has also been isolated from eight other species of *Helianthus* [3, 5, 9, 41–43 and Lee, E, Gershenzon, J and Mabry, T J, unpublished results]

The terpenoid chemistry of *H californicus* follows the general patterns of sesquiterpene lactone and diterpene constituents isolated from other species of *Helianthus* Both germacranolide sesquiterpene lactones and trachylobane diterpenes are common in the genus The species considered to be most closely related to *H californicus* on morphological grounds [7, 44] is *H nuttallii*, another member of section *Divaricati*, series *Corona-solis* [6] This taxon, a native of the Rocky Mountains and southern Canada with a diploid chromosome number, has been suggested as a likely progenitor of the hexaploid *H californicus* [7] The chemical evidence for these relationships is equivocal *Helianthus nuttallii* has been shown to contain a distinctive 12,8-*cis*-lactonized eudesmanolide and a furanoheliangolide [Lee, E, Gershenzon, J and Mabry, T J, unpublished results] However, no eudesmanolides or furanoheliangolides were isolated in this study, although compounds **1–3** might be considered heliangolide derivatives Interestingly, *H californicus* has chemical similarities with two *Helianthus* taxa of the western United States which are classed in other sections

of the genus *Helianthus niveus* subsp *niveus* (section *Helianthus*), found along the coast of Baja California, also produces **18** [Whittemore, A, Gershenzon, J and Mabry, T J, unpublished results], while *H pumilus* (section *Ciliares*), native to Colorado and Wyoming, contains **15**, an 11,13-dehydro-12,8-lactonized germacrolide very similar to **13** [33] Investigations of the terpenoid chemistry of additional species of *Helianthus* are in progress

EXPERIMENTAL

Plant material Leaves of *Helianthus californicus* were collected at the U S Dept of Agriculture research facility, Bushland, Texas on 14 October, 1978 (J G #35) and on 11 August, 1979 (J G #44, vouchers on deposit at the Herbarium of the University of Texas) from plants growing from rootstock originally collected on the west side of Lake Berryessa, Napa Co, California, on Knoxville Rd (01 00 marker), north of state highway 128, 27 August, 1977 (C E Rogers and T E Thompson #772)

First extraction Plant material collected in 1978 (700 g) was air-dried, ground and extracted with $CHCl_3$ at room temp for 24 hr Standard workup [29] gave 5 6 g of crude syrup which was applied to a silica gel column (140 g) packed in $CHCl_3$ The column was eluted with a $CHCl_3$–*iso*-PrOH gradient Fractions that eluted with 5% *iso*-PrOH were combined and separated by repeated prep TLC (silica gel, 2 mm) in several solvent systems [$CHCl_3$–*iso*-PrOH (15 1), $CHCl_3$–MeOH (20 1), toluene–EtOAc (1 1)] to give 87 mg of **2** and 34 mg of **3** These compounds were recrystallized from mixtures of EtOAc and *iso*-Pr_2O

A ppt from fractions that eluted with 10% *iso*-PrOH was washed with $CHCl_3$ and MeOH leaving 104 mg of **19** as powdery crystals Methylation of 50 mg of **19** with CH_2N_2 gave 16 mg of **20** as long white needles Compounds **19** and **20** were identified by comparison of their mps and spectral data with those in the literature [18] and with those obtained from an authentic specimen isolated from *H niveus* subsp *canescens* and its methylated derivative [3] Crystals formed in the filtrate from the 10% *iso*-PrOH fractions on standing These were recrystallized from hot CH_2Cl_2 to give 244 mg of **1** TLC comparisons showed that compounds **13** and **18**, which were isolated in the second extraction, were also present in this first extract, although they were not purified

Second extraction Plant material collected in 1979 (750 g) was extracted and worked up as before The crude syrup (10 g) was applied to a silica gel column (200 g) packed in toluene, which was eluted with a toluene–EtOAc gradient Fractions that eluted with 40% EtOAc were combined and separated by repeated prep TLC to give 340 mg **13** as a pale yellow oil Fractions that eluted with 50% EtOAc crystallized when triturated with CH_2Cl_2 Recrystallization from hot CH_2Cl_2 gave *ca* 2 g of **1** Separation of fractions that eluted with 65% EtOAc by repeated prep TLC gave 25 mg **18** as a pale gum that was unstable on standing at room temp TLC showed that **19** was also present in this extract, but **2** and **3** were not detectable These TLC plates, like many others run during the course of this work, were visualized with an acidified vanillin spray [45]

8β-Angeloyloxyternifolin (**1**) Mp 168–170° (CH_2Cl_2) CD (MeOH) $[\theta]_{284}$ +1900 (ketone), broad shallow minimum 260–225 nm (e g $[\theta]_{237}$ −4200), $[\theta]_{211}$ −16000 IR ν_{max}^{Nujol} cm^{-1} 3530 (OH), 1767 (lactone >C=O), 1714 (ester side chain >C=O and ketone >C=O), 1666, 1267, 1242, 1221, 1170, 1124, 1083, 1047, 1026, 920, 818 MS (probe) 70 eV, *m/z* (rel int) 380 (5) $[M]^+$, 362 (3) $[M-H_2O]^+$, 281 (10) $[M-C_5H_7O_2]^+$ side chain cleavage at ether oxygen, 263 (9) $[281-H_2O]^+$, 245 (3) $[281-H_2O-H_2O]^+$, 237 (8) $[281-CO_2]^+$, 235 (12) $[263-CO]^+$, 165 (16), 137 (14), 83 (100) $[C_5H_7O]^+$ side chain acylium ion, 55 (86) $[83-CO]^+$, 43 (72)

Dehydration of **1** In an attempt to acetylate **1**, 150 mg was left in 3 ml Ac_2O and 1 5 ml pyridine for 12 hr at room temp and the reaction mixture worked up in the usual fashion [1] Separation by prep TLC (CH_2Cl_2–*iso*-PrOH, 12 1) gave 90 mg of **10** as the principal product, colorless oil, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3500 (OH), 1760 (lactone >C=O), 1715 (side chain >C=O), 1685 (α,β-unsaturated ketone), 1650, 1635, 1280, 1240, 1140, 1135, 1120, 1050, 1040, 1000, 950, 885, 850, 815 MS *m/z* (rel int) 362 (59) $[M]^+$, 344 (25) $[M-H_2O]^+$, 318 (8) $[M-CO_2]^+$, 279 (14) $[M-C_5H_7O]^+$ α-cleavage of side chain, 262 (65) $[M-C_5H_8O_2]^+$ McLafferty rearrangement and side-chain cleavage, 261 (36), 245 (35), 219 (51), 165 (78), 123 (63), 83 (86) $[C_5H_7O]^+$ side chain acylium ion, 55 (100) $[83-CO]^+$

X-ray analysis of **1** All measurements were made using an Enraf-Nonius CAD-4 automatic diffractometer equipped with a MoKα target tube and a dense graphite crystal monochromator Final cell constants, as well as other information pertinent to data collection and refinement, are given in Table 9 The Laue symmetry was determined to be mmm, and from the systematic absences noted the space group was shown unambiguously to be $P2_12_12$, which is quite uncommon Intensities were measured using the θ-2θ scan technique, with the scan rate depending on the net count obtained in rapid pre-scans of each reflection In reducing the data, Lorentz and polarization factors were applied, but no absorption correction was made

The structure was solved by use of MULTAN [46], which revealed the positions of all of the non-hydrogen atoms The usual sequence of isotropic and anisotropic refinement was followed, after which all hydrogens were located in difference Fourier syntheses The thermal parameters of the H-4′ hydrogens had to be fixed in the final cycles of refinement due to slight disorder and/or high thermal motion After all shift/esd ratios were less than 0 3, the full-matrix least squares converged to the agreement factors listed in Table 9 The atomic scattering factors for C and O were computed from numerical Hartree-Fock wave functions [47], for H those of Stewart, Davidson and Simpson were used [48] Bond lengths, angles and torsion angles are given in Tables 5–7, based on the final positional parameters (Table 10)

3-Methoxy-8β-angeloyloxyternifolin (**2**) Colorless oil IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3845 (OH), 1756 (lactone >C=O), 1720 (side chain >C=O), 1703 (ketone >C=O), 1670, 1618, 1228, 1154, 1090, 1042, 1000, 852, 817 MS *m/z* (rel int) 394 (2) $[M]^+$ $C_{21}H_{30}O_4$ (HRMS 394 1991 calc, 394 1989 meas), 295 (2) $[M-C_5H_7O_2]^+$, 277 (3) $[295-H_2O]^+$, 263 (10) $[295-MeOH]^+$, 235 (33) $[263-CO]^+$, 165 (30), 123 (35), 83 (100) $[C_5H_7O]^+$, 55 (95) $[83-CO]^+$

3-Isopropoxy-8β-angeloyloxyternifolin (**3**) Colorless oil IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3485 (OH), 1756 (lactone >C=O), 1719 (side chain >C=O), 1701 (ketone >C=O in 7-membered ring or larger), 1671, 1617, 1227, 1136, 1110, 1102, 1035, 1000, 858, 818 MS *m/z* (rel int) 422 (1) $[M]^+$ $C_{23}H_{34}O_7$ (HRMS 422 2304 calc, 422 2301 meas), 380 (1) $[M-CH_2CO]^+$, 362 (1) $[M-iso\text{-}PrOH]^+$, 281 (11) $[380-C_5H_7O_2]^+$ side chain cleavage at ether oxygen, 263 (14) $[281-H_2O]^+$, 245 (7) $[281-H_2O-H_2O]^+$, 235 (32) $[263-CO]^+$, 165 (30), 83 (100) $[C_5H_7O]^+$, 55 (70) $[83-CO]^+$

3α-Acetoxy-11,13-dihydrochamissonin (**13**) Pale yellow oil IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3450 (OH), 1773 (saturated lactone >C=O), 1738 (acetate >C=O), 1262, 905, 880 MS *m/z* (rel int) 308 (1) $[M]^+$ $C_{17}H_{24}O_5$ (HRMS 308 1624 calc, 308 1626 meas), 282 (1 5), 266 (5) $[M-CH_2CO]^+$, 248 (9) $[M-HOAc]^+$, 230 (6), $[248-H_2O]^+$, 202 (7) $[248-H_2O-CO]^+$, 173 (15), 139 (27), 121 (43), 107 (58), 95 (73), 43 (100)

Acetylation of **13** Compound **13** (70 mg) was acetylated with

Table 10 Atomic coordinates and thermal parameters (× 1000)

Atom	X/A	Y/B	Z/C	U11	U22	U33	U12	U13	U23
O1	0 2316 (2)	0 2746 (2)	0 9882 (3)	55 (2)	46 (2)	90 (2)	−8 (2)	16 (2)	14 (2)
O2	0 5501 (2)	0 3343 (3)	0 8998 (3)	30 (2)	68 (2)	79 (3)	2 (2)	−8 (2)	16 (2)
O3	0 0901 (2)	0 4095 (3)	1 0300 (3)	34 (2)	85 (2)	52 (2)	−4 (2)	5 (2)	11 (2)
O4	0 3465 (2)	0 5227 (2)	0 6079 (2)	40 (2)	49 (2)	42 (2)	−6 (1)	5 (1)	14 (2)
O5	0 2510 (2)	0 5467 (2)	0 4445 (2)	78 (2)	85 (2)	35 (2)	−3 (2)	−3 (2)	19 (2)
O6	0 1762 (2)	0 6134 (2)	0 7540 (2)	38 (1)	32 (1)	46 (2)	7 (1)	6 (1)	9 (1)
O7	0 0196 (2)	0 6631 (2)	0 8234 (2)	41 (2)	54 (2)	66 (2)	10 (2)	13 (2)	12 (2)
C1	0 2673 (3)	0 3569 (3)	0 9928 (3)	44 (3)	54 (3)	32 (2)	−3 (2)	3 (2)	11 (2)
C2	0 3834 (3)	0 3760 (3)	0 9776 (4)	36 (2)	54 (3)	39 (3)	−1 (2)	−9 (2)	7 (3)
C3	0 4426 (3)	0 3008 (3)	0 9042 (4)	32 (2)	47 (3)	60 (3)	−1 (2)	−3 (2)	15 (3)
C4	0 4005 (3)	0 2835 (3)	0 7787 (4)	29 (2)	39 (2)	61 (3)	2 (2)	2 (2)	2 (2)
C5	0 4048 (3)	0 3707 (3)	0 6932 (4)	30 (2)	50 (3)	47 (3)	7 (2)	4 (2)	0 (3)
C6	0 3399 (3)	0 4629 (3)	0 7164 (3)	31 (2)	42 (2)	36 (3)	−4 (2)	−2 (2)	4 (2)
C7	0 2210 (3)	0 4446 (3)	0 7342 (3)	27 (2)	32 (2)	36 (2)	−2 (2)	1 (2)	5 (2)
C8	0 1648 (3)	0 5183 (3)	0 8131 (3)	27 (2)	31 (2)	37 (2)	−3 (2)	0 (2)	1 (2)
C9	0 2054 (3)	0 5322 (3)	0 9397 (4)	34 (2)	36 (3)	44 (3)	2 (2)	1 (2)	−2(2)
C10	0 1959 (3)	0 4452 (3)	1 0237 (3)	35 (2)	55 (3)	34 (3)	0 (2)	2 (2)	5 (2)
C11	0 1814 (3)	0 4523 (3)	0 6082 (3)	31 (2)	42 (2)	35 (2)	2 (2)	2 (2)	−1 (2)
C12	0 2587 (3)	0 5119 (3)	0 5419 (4)	50 (3)	45 (3)	46 (3)	7 (2)	0 (3)	−4 (2)
C13	0 0976 (4)	0 4153 (4)	0 5585 (4)	46 (3)	88 (4)	40 (3)	−8 (3)	−3 (3)	0 (3)
C14	0 2240 (5)	0 4784 (5)	1 1494 (4)	69 (4)	86 (4)	41 (3)	9 (4)	−7 (3)	−2 (3)
C15	0 4597 (4)	0 1973 (4)	0 7214 (5)	53 (3)	56 (3)	82 (5)	12 (3)	−2 (3)	−6 (3)
C1′	0 0972 (3)	0 6786 (3)	0 7627 (4)	41 (2)	36 (2)	42 (3)	3 (2)	−12 (2)	−4 (2)
C2′	0 1196 (3)	0 7670 (3)	0 6923 (4)	54 (3)	36 (2)	40 (3)	6 (2)	−4 (2)	1 (2)
C3′	0 0494 (4)	0 8391 (3)	0 6794 (4)	81 (4)	47 (3)	56 (3)	11 (3)	−6 (3)	9 (3)
C4′	−0 0592 (4)	0 8473 (4)	0 7288 (5)	80 (4)	82 (4)	104 (5)	38 (3)	−5 (4)	1 (4)
C5′	0 2244 (5)	0 7725 (4)	0 6319 (6)	84 (4)	39 (3)	82 (4)	2 (3)	25 (4)	17 (3)
H(O2)	0 584 (4)	0 290 (3)	0 921 (5)	112 (25)					
H(O3)	0 062 (3)	0 428 (4)	0 994 (4)	100 (0)					
H2A	0 400 (2)	0 452 (2)	0 947 (3)	46 (10)					
H2B	0 411 (2)	0 377 (2)	1 056 (3)	44 (11)					
H3	0 438 (2)	0 231 (2)	0 945 (3)	58 (12)					
H4	0 323 (2)	0 260 (2)	0 786 (2)	19 (8)					
H5A	0 387 (2)	0 344 (2)	0 595 (3)	58 (11)					
H5B	0 480 (2)	0 391 (2)	0 681 (3)	48 (11)					
H6	0 370 (2)	0 505 (2)	0 777 (2)	14 (8)					
H7	0 208 (2)	0 374 (2)	0 767 (2)	18 (8)					
H8	0 085 (2)	0 503 (2)	0 818 (2)	18 (8)					
H9A	0 166 (2)	0 589 (2)	0 971 (3)	50 (12)					
H9B	0 280 (2)	0 559 (2)	0 938 (3)	58 (12)					
H13A	0 048 (2)	0 367 (2)	0 607 (3)	52 (11)					
H13B	0 081 (2)	0 427 (2)	0 485 (3)	42 (12)					
H14A	0 173 (3)	0 542 (3)	1 170 (4)	108 (18)					
H14B	0 291 (2)	0 507 (3)	1 152 (3)	60 (15)					
H14C	0 225 (3)	0 421 (3)	1 205 (4)	110 (20)					
H15A	0 417 (3)	0 173 (3)	0 653 (3)	80 (0)					
H15B	0 527 (3)	0 213 (3)	0 694 (4)	101 (20)					
H15C	0 464 (3)	0 138 (3)	0 779 (3)	70 (0)					
H3′	0 076 (3)	0 894 (3)	0 631 (3)	59 (13)					
H4′A	−0 090 (0)	0 785 (0)	0 770 (0)	120 (0)					
H4′B	−0 105 (0)	0 858 (0)	0 649 (0)	120 (0)					
H4′C	−0 064 (0)	0 912 (0)	0 783 (0)	120 (0)					
H5′A	0 236 (3)	0 835 (3)	0 597 (3)	67 (14)					
H5′B	0 234 (3)	0 723 (3)	0 566 (3)	81 (16)					
H5′C	0 287 (3)	0 753 (3)	0 678 (4)	75 (17)					

2 ml Ac_2O in 1 ml pyridine for 12 hr at room temp and the reaction worked up in the usual manner [1] The reaction mixture was separated by prep TLC (CH_2Cl_2–*iso*-PrOH, 15 1) to give 22 mg **14** as a pale gum Spectral data for **14** were very similar to those given in the literature [33]

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REFERENCES

1 Ohno, N and Mabry, T J (1979) *Phytochemistry* **18**, 1003
2 Ohno, N, Mabry, T J, Zabel, V and Watson, W H (1979) *Phytochemistry* **18**, 1687
3 Ohno, N and Mabry, T J (1980) *Phytochemistry* **19**, 609
4 Ohno, N, Gershenzon, J, Neuman, P and Mabry, T J (1981) *Phytochemistry* **20**, 2393
5 Watanabe, K, Ohno, N, Yoshioka, H, Gershenzon, J and Mabry, T J (1982) *Phytochemistry* **21**, 709
6 Schilling, E E and Heiser, C B (1981) *Taxon* **30**, 393
7 Heiser, C B, Jr (1969) *Mem Torr Bot Club* **22**, 1
7a Gershenzon, J and Mabry, T J (1984) *Phytochemistry* **23**, 1959 and refs therein
8 Bjeldanes, L F and Geissman, T A (1972) *Phytochemistry* **11**, 327
9 Herz, W and Kumar, N (1981) *Phytochemistry* **20**, 99
10 Lee, I-Y, Olivier, E J, Urbatsch, L E and Fischer, N H (1982) *Phytochemistry* **21**, 2313
11 Bohlmann, F, Zdero, C, Robinson, H and King, R M (1982) *Phytochemistry* **21**, 1087
12 Bohlmann, F, Gupta, R K, Jakupovic, J, Robinson, H and King, R M (1981) *Phytochemistry* **20**, 1609
13 Bohlmann, R, Wallmeyer, M, King, R M and Robinson, H (1982) *Phytochemistry* **21**, 1439
14 Manchand, P S and Blount, J F (1978) *J Org Chem* **43**, 4352
15 Baruah, R N, Sharma, R P, Thyagarajan, G, Herz, W, Govindan, S V and Blount, J F (1980) *J Org Chem* **45**, 4838
16 Watkins, S F, Korp, J D, Bernal, I, Perry, D L, Bhacca, N S and Fischer, N H (1978) *J Chem Soc Perkin Trans* 2, 599
17 Hamilton, J A, McPhail, A T and Sim, G A (1962) *J Chem Soc* 708
18 Herz, W, Kumar, N and Blount, J F (1980) *J Org Chem* **45**, 489
19 Korp, J D, Bernal, I, Fischer, N H, Leonard, C, Lee, I-Y and LeVan, N (1982) *J Heterocycl Chem* **19**, 181
20 Herz, W, de Groote, R, Murari, R, Kumar, N and Blount J F (1979) *J Org Chem* **44**, 2784
21 Gershenzon, J, Liu, Y-L, Mabry, T J, Korp, J D and Bernal, I (1984) *Phytochemistry* **23**, 1281
22 Asher, J D M and Sim, G A (1965) *J Chem Soc* 1584
23 Schmalle, H W, Klaska, K H and Jachow, O (1977) *Acta Crystallogr* **B33**, 2213
24 Quick, A and Rogers, D (1976) *J Chem Soc Perkin Trans* 2, 465
25 Whuler, A, Brouty, C and Spinat, P (1980) *Acta Crystallogr* **B36**, 1267
26 Wallwork, S C (1962) *Acta Crystallogr* **15**, 758
27 Stocklin, W, Waddell, T G and Geissman, T A (1970) *Tetrahedron* **26**, 2397
28 Herz, W and Sharma, R P (1975) *J Org Chem* **40**, 3118
29 Mabry, T J, Miller, H E, Kagan, H B and Renold, W (1966) *Tetrahedron* **22**, 1139
30 Kondo, Y, Hamada, R and Yoshizaki, F (1976) *Heterocycles* **5**, 373
31 Saleh, A A, Cordell, G A and Farnsworth, N R (1980) *J Chem Soc Perkin Trans* 1, 1090
32 Fischer, N H, Olivier, E J and Fischer, H D (1979) *Prog Chem Org Nat Prod* **38**, 47
33 Herz, W and Kumar, N (1981) *Phytochemistry* **20**, 1339
34 Yoshioka, H, Renold, W, Fischer, N H, Higo, A and Mabry, T J (1970) *Phytochemistry* **9**, 823
35 Bohlmann, F and Dutta, L N (1979) *Phytochemistry* **18**, 847
36 Bohlmann, F, Dutta, L N, Robinson, H and King, R M (1979) *Phytochemistry* **18**, 1401
37 Bohlmann, F, Jakupovic, J, King, R M and Robinson, H (1981) *Phytochemistry* **20**, 1613
38 Narayanan, C R and Venkatasubramanian, N K (1968) *J Org Chem* **33**, 3156
39 Demarco, P V, Farkas, E, Doddrell, D, Mylari, B L and Wenkert, E (1968) *J Am Chem Soc* **90**, 5480
40 Bohlmann, F, Zdero, C, King, R M and Robinson, H (1980) *Phytochemistry* **19**, 115
41 Ortega, A, Ayala, A, Guerrero, C and Romo de Vivar, A (1972) *Rev Soc Quim Mex* **16**, 191
42 Herz, W, Govindan, S V and Watanabe, K (1982) *Phytochemistry* **21**, 946
43 Herz, W, Kulanthaivel, P and Watanabe, K (1983) *Phytochemistry* **22**, 2021
44 Heiser, C B, Jr (1965) *Ann Mo Bot Gard* **52**, 364
45 Picman, A K, Ranier, R L, Towers, G H N and Lam, J (1980) *J Chromatogr* **189**, 187
46 Germain, G, Main, P and Woolfson, M M (1971) *Acta Crystallogr* **A27**, 368
47 Cromer, D T and Mann, J B (1968) *Acta Crystallogr* **A24**, 321
48 Stewart, R F, Davidson, E R and Simpson, W T (1965) *J Chem. Phys* **42**, 3175
49 Herz, W and Kumar, N (1980) *Phytochemistry* **19**, 593
50 Herz, W, Kalyanaraman, P S, Ramakrishnan, G and Blount, J F (1977) *J Org Chem* **42**, 2264
51 Tori, K, Horibe, I, Tamura, Y, Kuriyama, K, Tada, H and Takeda, K (1976) *Tetrahedron Letters* 387
52 Rogers, D, Moss, G P and Neidle, S (1972) *J Chem Soc Chem Commun* 142