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LONGICORNINS A TO D, FOUR *CIS*-1(10),*CIS*-4-GERMACRADIENOLIDES FROM *MELAMPODIUM LONGICORNE*

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Key Word Index—*Melampodium longicorne*; Asteraceae; Heliantheae; sesquiterpene lactones; *cis*-1(10),*cis*-4-germacradienolides; longicornins A,B,C and D.

Abstract—The isolation and structure determination of four new *cis*-1(10),*cis*-4-germacradienolides, longicornins A–D, from *Melampodium longicorne* is described. The structure determinations involved NMR and mass spectral methods and chemical transformations. The molecular structure of longicornin A was determined by single crystal X-ray diffraction. The crystallographic data suggest revision at the chiral center C-9 of melcanthins A–G and also at C-2 in melcanthins B and C.

INTRODUCTION

In a continued biochemical systematic study of the subtribe Melampodiinae, we have analysed the aerial parts of *Melampodium longicorne* of section *Melampodium* [1] for their terpenoid constituents. The most common structural types of sesquiterpene lactones in a number of *Melampodium* species are melampolides and leucanthoiides [2]. However, the less common group of *cis,cis*-germacradienolides [3] has more recently been found in *M. leucanthum* [4], *M. cinereum* [5] and *M. rosei* [6–8]. NMR and mass spectral studies of the four new sesquiterpene lactones, which we named longicornins A–D, suggested that these compounds also belong to the *cis,cis*-cyclodecadiene subgroup.

Since our initial configurational assignments of the *cis,cis*-germacradienolide skeleton were mainly based on low-temperature ^{1}H NMR studies of the conformationally flexible melcanthin B [4], the chiralities at C-9 of melcanthins A–G and at C-2 in melcanthins B and C were tentative. The isolation of the first crystalline *cis,cis*-germacranolide, longicornin A, which was suitable for single crystal X-ray diffraction, provided an opportunity to assign unambiguously not only the chiral centers in longicornin A and its congeners but also, by ^{1}H NMR spectral correlations of the melcanthins with this benchmark molecule, the chiralities in the previously described melcanthins A–G [4, 5].

RESULTS AND DISCUSSION

The physical data for longicornins A–D are given in Table 1 while the ^{1}H NMR data for these compounds are presented in Table 2.

Longicornin A (**1**), $C_{24}H_{32}O_{10}$, mp 145–147° showed absorptions typical for an α-methylene-γ-lactone (^{1}H NMR doublets at δ6.41 and 5.63; IR band at 1760 cm^{-1}). IR absorptions at 3615 and 3595 cm^{-1} indicated the presence of hydroxyl groups. The 200 MHz ^{1}H NMR spectrum suggested a sesquiterpene lactone and exhibited signals of great similarity with melcanthin B [4]. The structure of longicornin A was initially deduced from ^{1}H NMR and mass spectral correlations with melcanthin B, and single crystal X-ray diffraction solved remaining structural ambiguities. Longicornin A showed a three-proton singlet at δ3.70 typical of carbomethoxy methyls found in melampolides and *cis,cis*-germacranolides [4, 9]. The most downfield absorption at δ7.21 corresponded to H-1 and its chemical shift suggested a *cis*-1(10)-double bond with H-1 being *cis* to the carbomethoxy group [4]. The presence of an allylic alcohol was shown by the appearance of a two-proton doublet at δ4.33 (H-15, 15′) which upon oxidation with pyridinium chlorochromate/dichloromethane gave an aldehyde (**18**). The chemical shift of the aldehyde proton at δ9.56 and the downfield shift of H-5 from δ5.74 in **1** to 6.8 in **18** indicated a 4,5-*cis*-double bond [4, 10] suggesting a *cis,cis*-germacradienolide skeleton for longicornin A. The presence of a hydroxyl group at C-2 was indicated by the appearance of a broad multiplet at δ4.77 (H-2) which was coupled to H-1. The attachment of ester side chains to C-8 and C-9 was in agreement with the chemical shifts for H-8 (δ5.86) and H-9 (δ5.63). Two one-proton multiplets at δ2.60 (H-2′) and 2.40 (H-2″) together with four pairs of doublets at δ1.15, 1.11, 1.07 and 1.02 supported the presence of two isobutyrate moieties in longicornin A. Mass spectral peaks at *m/z* 392 [M − A] and 304 [M − 2A] and a base peak at *m/z* 71 [A^1] corroborated the NMR results. The ^{13}C NMR spectral data for **1** are given in Table 3. This completed the structural arrangement of the carbon skeleton of longicornin A as shown in **1** except for the stereochemical assignments at the five chiral centers (C-2, C-6, C-7, C-8 and C-9) of the medium ring.

Treatment of longicornin A (**1**) with sodium borohydride not only provided the expected 11,13-dihydrolongicornin A (**12**), but in addition compound **16** was obtained as the major product. In this reaction the 1,10-double bond rearranged to the 9,10-position with loss of the isobutyrate group at C-9, a reaction that is common for

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Table 1. Physical data of longicornins A–D

Compound	Empirical formula	Mp	UV, ε	λ_{max}^{MeOH}, nm (ε)	CD, c	CD, λ_{max}^{MeOH}, nm ([θ])	IR, ν_{max}^{film}, cm^{-1}
1	$C_{24}H_{32}O_{10}$	145–147°	3.94×10^{-5}	213 (1.8×10^4)	1.9×10^{-4}	212 (-7.0×10^4), 235 ($+3.8 \times 10^3$), 262 (-3.8×10^3)	3615, 3595, 3010, 1760, 1725, 1710, 1200, 1130
2	$C_{24}H_{32}O_9$	135–136.5	3.77×10^{-5}	207 (2.1×10^4)	1.9×10^{-4}	205 (-6.5×10^4), 214 ($+6.0 \times 10^4$), 234 ($+1.4 \times 10^4$)	3495, 2995, 1740, 1730, 1715, 1700, 1601, 1185, 1120
3	$C_{25}H_{34}O_{10}$	Gum	4.25×10^{-5}	208 (1.8×10^4)	2.1×10^{-4}	211 (-6.1×10^4), 233 ($+5.9 \times 10^3$), 254 (-3.5×10^3)	3495, 3000, 1755, 1705, 1645, 1210, 1125
4	$C_{25}H_{34}O_9$	Gum	4.52×10^{-5}	215 (2.5×10^4)	3.1×10^{-4}	209 (-3.9×10^4), 231 ($+1.8 \times 10^4$), 265 (-7.8×10^2)	3600, 3000, 1755, 1705, 1635, 1205, 1130

Table 2. ^{1}H NMR spectral data* for longicornins A–D and derivatives (200 MHz, TMS as internal standard)

Assignment	**1**	**2**	**3**	**4**	**12**§	**13**	**14**	**15**	**16**§	**16**†	**18**	**19**
H-1/H-1′	7.21 *d* (7.0)	7.16 *dd* (8.0)	7.25 *d* (6.5)	7.19 *dd* (9.0)	7.07 *d* (7.0)	7.1 *d* (9.0)	7.01 *dd* (9.5; 8.0)	7.05 *dd* (8.5)	3.02 *dd*/2.19 *dd* (12.0; 3.5)/(12.0)	2.84 *dd*/1.84 *dd* (12.5; 3.0)/(12.5)	7.29 *d* (6.0)	7.01 *d* (8.0)
H-2	4.77 *br*	2.43–2.90‡	4.80 *br m*	4.55 *br m*	4.55 *br m*	4.58 *br m*	—	—	3.78 *br m*	3.66 *br l*	4.49 *br*	4.95 *br m*
H-3	2.81 *dd* (15.0; 5.0)	2.80 *dd* (17.0; 9.0)	2.81 *dd* (15.0; 5.0)	2.90–2.30‡	2.82 *d*‡	‡	‡	‡	2.94 *dd* (15.0; 6.0)	2.61 *dd* (15.0; 6.0)	2.91 *d* (16.0)	2.6 *d* (16.0)
H-3′	2.53 *d* (15.0)	2.43–2.90‡	2.62 *d* (15.0)	—	2.28‡	‡	‡	‡	2.27 *d* (15.0)	2.01 *d* (15.0)	2.68 *dd* (16.0; 4.0)	1.9 *dd* (16.0; 4.0)
H-5	5.74 *d* (9.5)	5.65 *d* (10.0)	5.75 *d* (10.0)	5.67 *d* (10.0)	5.75 *d* (10.0)	5.74 *d* (10.0)	5.82 *d* (9.5)	5.69 *d* (8.3)	5.56 *d* (10.0)	5.34	6.8 *d* (9.0)	3.19 *d* (10.0)
H-6	5.20 *dd* (9.5; 7.0)	5.46 *dd* (10.0; 5.0)	5.22 *dd* (9.5; 7.0)	5.44 *dd* (10.0; 4.5)	5.23 *dd* (10.0, 2.0)	5.24 *dd* (10.0; 3.0)	5.67 *d* (9.5)	5.85 *br*	5.38 *dd* (10.0; 3.0)	5.34	5.39 *d* (9.0)	4.42 *dd* (10.0; 5.0)
H-7	3.16 *m*	3.12 *m*	3.16 *m*	3.13 *m*	‡	2.68 *ddd* (11.0; 3.0; 2.0)	2.67 *dd* (9.5; 2.0)	2.69 *dd* (10.0; 1.0)	2.68 *dt* (11.0; 3.0)	2.55 *dt* (11.0; 3.0)	3.24 *m*	3.39 *br s*
H-8	5.86‡	5.80 *dd* (4.5; 2.5)	5.88‡	5.81 *dd* (4.5; 2.5)	5.64	5.66 *d* (2.0)	5.38 *dd* (3.0; 2.0)	5.39 *dd* (4.0; 1.0)	6.03 *dd* (5.0; 3.0)	6.23 *dd* (5.0; 3.0)	5.96 *d* (6.5)	5.72
H-9	5.63 *d* (4.0)	6.02 *d* (4.5)	5.67 *d* (4.0)	6.03 *d* (4.5)	5.49	5.51	6.22 *d* (3.0)	6.26 *br s*	6.15 *d* (5.0)	5.98 *d* (5.0)	5.46 *d* (6.5)	5.72

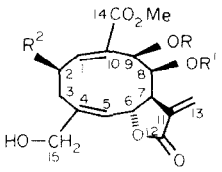
H-13	6.41 *d* (3.0)	6.35 *d* (2.9)	6.42 *d* (3.5)	6.35 *d* (3.0)	—	—	—	—	—	—	6.5 *d* (3.0)	6.45 *d*
H-13′	5.86 *d* (3.0)	5.77 *d* (2.9)	5.9 *d* (3.5)	5.79 *d* (3.0)	—	—	—	—	—	—	6.3 *d* (3.0)	5.95 *d*
H-15	4.33 *d* (3.5)	4.13 *br*	4.36 *br*	4.14 *br*	4.22	4.24	4.02	—	4.11	4.1	9.56	4.06 *d*; 3.82 *d* (13.0)
CO_2Me	3.70	3.72	3.70	3.71	3.74	3.72	3.76	3.78	3.76	3.78	3.71	3.70
H-2′	2.60 *h* (7.0)	2.59 *h* (7.0)	2.41 *h* (7.0)	2.40 *h* (7.0)	2.48 *h* (7.0)	2.44 *h* (7.0)	2.61 (7.0)	‡	2.6 *h* (7.0)	2.38 *h* (7.0)	2.45 *h* (7.0)	2.62 *h* (7.0)
H-2″	2.40 *h* (7.0)	2.4 *h* (7.0)	2.45 *h* (7.0)	2.43 *m* (7.0)	2.33 *h* (7.0)	2.45 *q* (7.0)	2.46 *h* (7.0)	‡	—	—	2.57 *h* (7.0)	2.39 *h* (7.0)
H-3″	—	—	1.55 *m* (7.0)	1.57 *m* (7.0)	—	1.55 *m* (7.0)	—	1.57 *m*	—	—	—	—
C-2′-Me	1.11 *d*; 1.15 *d* (7.0)	1.16 *d*; 1.19 *d* (7.0)	1.08 *d*; 1.13 *d* (7.0)	1.07 *d*; 1.16 *d* (7.0)	1.16 *d*; 1.15 *d* (7.0)	1.16 *d*; 1.14 *d* (7.0)	1.21 *d*; 1.17 *d* (7.0)	1.18 *d*; 1.15 *d* (7.0)	1.18 *d*; 1.19 *d* (7.0)	1.08 *d* (7.0)	1.11 *d*; 1.13 *d* (7.0)	1.18 *d*; 1.15 *d* (7.0)
C-2″-Me	1.02 d; 1.07 *d* (7.0)	1.03 *d*; 1.07 *d* (7.0)	1.05 *d* (7.5)	1.03 *d* (7.0)	1.12 *d*; 1.11 *d* (7.0)	1.09 *d* (7.0)	1.14 *d*; 1.12 *d* (7.0)	1.12 *d* (7.0)	—	— (7.0)	1.06 *d*; 1.07 *d* (7.0)	1.07*d*; 1.02 *d* (7.0)
C-3″-Me	—	—	0.84 *t* (7.0)	0.86 *t* (7.0)	—	0.85 *t* (7.0)	—	0.90 *t* (7.0)	—	—	—	—
H-11	—	—	—	—	2.82 *dq*	‡	2.88 *dq* (9.5; 7.0)	2.87 *dq* (10.0; 7.0)	2.82 *dq* (11.0; 7.0)	2.37 *dq* (11.0; 7.0)	—	—
C-11-Me	—	—	—	—	1.09 *d* (7.0)	1.13 *d* (7.0)	0.97 *d* (7.0)	1.08 *d* (7.0)	1.21 *d* (7.0)	1.17 *d* (7.0)	—	—

*Spectra were run at 298 K in $CDCl_3$ at 200 MHz, and TMS was used as internal standard. Values are recorded in ppm relative to TMS. Singlets are unmarked and multiplets are designated as follows: *d*, doublet; *t*, triplet; *q*, quarter; *p*, pentet; *h*, heptet; *m*, multiplet whose center is given; *br*, broad. Figures in parentheses are coupling constants or line separations in hertz.

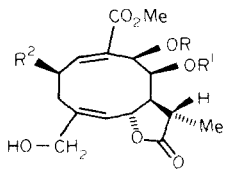
‡Obscured by other signals.

†Run in benzene-d_6 at 348 K.

§Run at 328 K.



		R	R'	R²
Longicornin A	**1**	**A'**	**A'**	OH
Longicornin B	**2**	**A'**	**A'**	H
Longicornin C	**3**	**B'**	**A'**	OH
Longicornin D	**4**	**B'**	**A'**	H
Melcanthin A	**5**	Ac	**C'**	H
Melcanthin B	**6**	Ac	**C'**	OH
Melcanthin C	**7**	Ac	**A'**	OH
Melcanthin D	**8**	Ac	Mac	H
Melcanthin E	**9**	Ac	**A'**	H
Melcanthin F	**10**	Ac	**B'**	H
Melcanthin G	**11**	**B'**	Ac	H



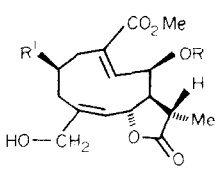
	R	R'	R²
12	**A'**	**A'**	OH
13	**B'**	**A'**	OH
14	**A'**	**A'**	H
15	**B'**	**A'**	H

these types of compounds [6, 11]. The structure was confirmed by ^{1}H NMR spectroscopy, which showed a downfield shift for the H-9 signal from δ5.63 in **1** to δ6.15 in **16**. The stereochemistry of the C-11 methyl group must be α-oriented since the coupling constant ($J_{7,11}$ = 11 Hz) suggested an antiperiplanar orientation of H-11 and H-7.

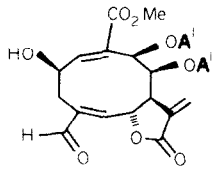
Reaction of longicornin A (**1**) with *m*-chloroperbenzoic acid gave the 4,5-epoxide (**19**) which exhibited a diagnostic doublet at δ3.19 due to H-5. The coupling constant ($J_{5,6}$ = 10 Hz) suggested an antiperiplanar orientation of H-5 and H-6 as in structure **1**. Since backside attack of the reagent to the 4,5-double bond is most unlikely for steric reasons, the stereochemical structure of the resulting epoxide must be as shown in **19** and its conformation must be [^{1}D^{14}, $_{15}$E$_5$] [12].

X-Ray data of longicornin A

Single crystal X-ray diffraction of longicornin A gave the relative configuration and solid-state conformation of the molecule. The coordinates for non-hydrogen atoms and torsion angles in longicornin A are summarized in Tables 4 and 5, and Fig. 1 shows the β-face of the molecule. Based on the findings that higher plants produce sesquiterpene lactones with 7α-H [3], the shown stereostructures (**1** and Fig. 1) most likely represent the absolute configuration of the molecule. As *cis*-1(10),*cis*-4-germacradienolides appear to be biogenetically derived from melampolides [*cis*-1(10),*trans*-4-germacradienolides], it is of interest to observe the structural changes in going from a typical melampolide, melampodin A, [13] to longicornin A. Both structures contain a cyclodeca-1,5-diene skeleton with the lactone ring fused C-12 to C-6, and bear oxygen functions at C-8 and C-9. In contrast to melampodin A, which exhibits a typical [$_1$D$_{14}$, ^{15}D$_5$] conformation, longicornin A adopts a [^{1}D^{14}, $_{15}$D$_5$] arrangement with the oxygen functions at C-2, C-8 and C-9 being



16 R = **A'**, R' = OH
17 R = **A'**, R' = H



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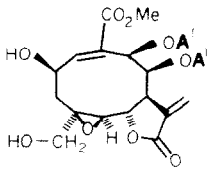

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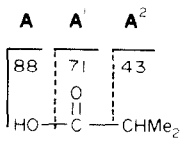

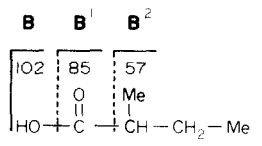

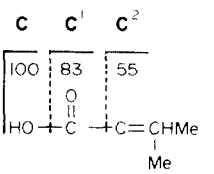

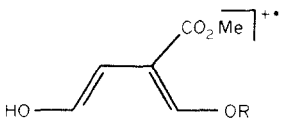

D

1 R = **A'**
3 R = **B'**

Table 3. ^{13}C NMR spectral data* of longicornins A (1) and B (2)

Carbon	1	2
1	152.87 *d*	147.31 *d*
2	69.24 *d*	25.87 *t*†
3	39.21 *t*	25.74 *t*†
4	124.93	128.03
5	124.64 *d*	123.37 *d*
6	73.75 *d*	73.80 *d*
7	48.77 *d*	48.09 *d*
8	70.74 *d*†	71.52 *d*‡
9	70.40 *d*†	69.92 *d*‡
10	141.91	143.31
11	133.18	134.0
12	176.88	176.01
13	125.85 *t*	127.07 *t*
14	174.46	174.7
15	65.84 *t*	65.36 *t*
16	168.78‡	168.98§
16′	165.1‡	165.63§
17	33.83 *d*	33.63 *d*
17′	33.83 *d*	33.63 *d*
C_{17}(2Me)	19.13–18.35 *q*	18.93–18.06 *q*
OMe	51.77 *q*	51.68 *q*

*Run at 50.00 MHz in $CDCl_3$ with $CDCl_3$ as internal standard. Values are in ppm. Unmarked signals are singlets.

†,‡,§Assignments interchangeable.

β-oriented. In melampolides, the C-9 oxygen configuration is consistently α [3].

The intra-annular torsion angles for longicornin A are given in Table 5. Of particular interest is the flatness of the α-methylene-γ-lactone ring of longicornin A, characterized by the small values of the torsional angles. The maximum magnitude of these is 3.9°, and the sum of the five magnitudes is only 12.5°. In contrast, the α-methylene-γ-lactone ring of melampodin A is distinctly puckered, with torsion angle magnitudes summing to 117.5°, and the maximum value 35.0° for the angle about the ring fusion bond C6–C7. Torsion angles and best planes calculations involving the two *cis*-double bonds of longicornin A suggest that the cyclodecadiene ring is less strained than that of melampodin A. Intra-annular torsion angles involving the double bonds differ from ideal values by only 1.8° at the 4-double bond and 2.0° at the 1(10)-double bond. Corresponding deviations for melampodin A are 24.6° and 4.4°, respectively. In addition to torsional distortions in melampodin A, distortions also occur in which ring carbon atoms adjacent to the double bonds are pulled out of the plane of the other four atoms associated with the bond. Angular deviations are 4.6° for C-2, 1.5° for C-9, 6.7° for C-3, and 14.0° for C-6. In longicornin A, such distortions are present, but consistently small, having respective values of 4.5°, 3.1°, 3.2° and 1.7°. The dihedral angle formed by the two double bond planes is 46.2° in melampodin A and 26.7° in longicornin A.

Finally, it should be pointed out that the stereochemical and conformational orientations found in longicornin A (Fig. 1) represent the arrangement required for the facile transformation of *cis,cis*-germacradienolides to the

Table 4. Coordinates for non-hydrogen atoms in longicornin A

Atom	*X*	*Y*	*Z*
O1	−0.0080 (1)	0.3722 (1)	0.8130 (1)
O2	0.1411 (2)	0.0394 (2)	0.7054 (2)
O3	0.4524 (1)	0.2375 (2)	0.6606 (1)
O4	0.5964 (2)	0.2116 (2)	0.5720 (2)
O5	0.3825 (1)	0.4695 (1)	0.5946 (1)
O6	0.4270 (2)	0.5500 (2)	0.4692 (1)
O7	0.1325 (2)	0.5025 (2)	0.5154 (1)
O8	−0.0319 (2)	0.4510 (2)	0.5614 (2)
O9	0.2582 (2)	0.6410 (2)	0.6100 (2)
O10	0.2053 (2)	0.6442 (1)	0.7517 (1)
C1	0.1407 (2)	0.4585 (2)	0.7487 (2)
C2	0.0894 (2)	0.3608 (2)	0.7615 (2)
C3	0.1690 (2)	0.2867 (2)	0.8057 (2)
C4	0.1923 (2)	0.1998 (2)	0.7475 (2)
C5	0.2657 (2)	0.1965 (2)	0.6821 (2)
C6	0.3410 (2)	0.2755 (2)	0.6549 (2)
C7	0.3256 (2)	0.3109 (2)	0.5561 (2)
C8	0.2972 (2)	0.4179 (2)	0.5480 (2)
C9	0.1832 (2)	0.4446 (2)	0.5853 (2)
C10	0.1803 (2)	0.4973 (2)	0.6742 (2)
C11	0.4347 (2)	0.2903 (2)	0.5139 (2)
C12	0.5054 (2)	0.2419 (2)	0.5817 (2)
C13	0.4720 (3)	0.3124 (3)	0.4334 (2)
C14	0.2197 (2)	0.6014 (2)	0.6731 (2)
C15	0.1187 (2)	0.1146 (2)	0.7652 (2)
C16	0.4443 (2)	0.5321 (2)	0.5461 (2)
C17	0.5355 (3)	0.5710 (2)	0.6037 (3)
C18	0.6198 (3)	0.4940 (4)	0.6177 (4)
C19	0.5853 (4)	0.6601 (3)	0.5626 (5)
C20	0.2389 (3)	0.7461 (2)	0.7562 (3)
C21	0.0239 (2)	0.4978 (2)	0.5102 (2)
C22	−0.0167 (4)	0.5615 (4)	0.4335 (3)
C23	0.0366 (5)	0.5419 (6)	0.3493 (3)
C24	−0.1310 (4)	0.5777 (6)	0.4386 (5)

Table 5. Selected torsion angles (°) of longicornin A

Atoms				Angle
C10	C1	C2	C3	102.1
C1	C2	C3	C4	−119.0
C2	C3	C4	C5	83.0
C3	C4	C5	C6	1.8
C4	C5	C6	C7	−118.7
C5	C6	C7	C8	120.6
C6	C7	C8	C9	−67.4
C7	C8	C9	C10	105.6
C8	C9	C10	C1	−112.8
C9	C10	C1	C2	−2.0
O3	C6	C7	C11	−0.8
C6	C7	C11	C12	2.8
C7	C11	C12	O3	−3.9
C11	C12	O3	C6	3.4
C12	O3	C6	C7	−1.6
O4	C12	C11	C13	−5.8

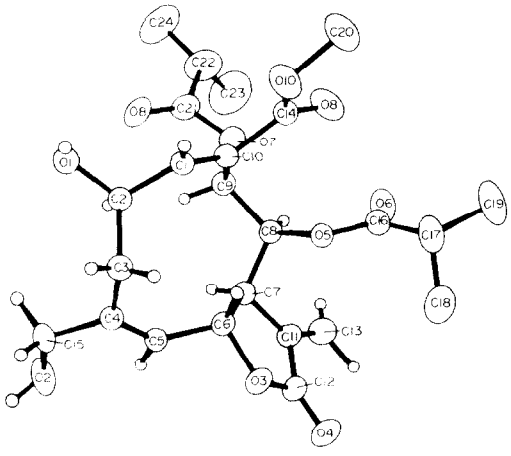

Fig. 1. ORTEP representation of the longicornin A molecule. Hydrogen atoms of methyl groups have been omitted for clarity.

leucantholides [6], which are typically represented by melampodin B, a compound with known X-ray structure [14].

Longicornin B (**2**), $C_{24}H_{32}O_9$, mp 135–136.5°, showed a ^{1}H NMR spectrum and a mass spectral fragmentation pattern similar to those of **1**. The major difference in the medium ring skeleton of the two compounds was suggested by the upfield shift of H-2 from δ4.77 in **1** to δ2.5 in **2** indicating the absence of a hydroxyl group at C-2 in **2**. The appearance of a doublet of doublets at δ7.16 (H-1) was also indicative of the presence of two protons at C-2. The other parameters in the ^{1}H NMR spectrum of **2** were very similar to **1** and are summarized in Table 2. The mass spectral fragmentation and the ^{1}H NMR spectral data suggested that the two ester side chains had to be attached to C-8 and C-9 and represent isobutyrates as in **1**.

Longicornin C (**3**), $C_{25}H_{34}O_{10}$, was a gum which on the basis of ^{1}H NMR spectral patterns had to be structurally closely related to **1** and contain the same substitution pattern at the medium ring portion of the molecule. This suggested that the difference between compounds **1** and **3** had to be in the ester side chains attached to C-8 and C-9. Besides ^{1}H NMR and mass spectral signals typical of an isobutyrate moiety, the appearance of multiplets centered at δ1.55 (H-3″) and 2.45 (H-2″), a doublet at 1.05 (Me-2″), and a triplet at 0.84 (Me-3″) suggested the presence of a 2-methylbutanoate group. Mass spectral peaks at *m/z* 392 $[M-\mathbf{B}]^+$ and 85 $[\mathbf{B}^1]^+$ were in agreement with a five-carbon ester group. The site of attachment of the two different ester functions was established by two independent methods. The mass spectrum of longicornin C exhibited a strong peak at *m/z* 228 which was assigned to radical ion **D** [4]. This required that the 2-methylbutanoate had to be attached to C-9 and the isobutyrate moiety to C-8. This assignment was verified by the following chemical transformation. Treatment of longicornin C with sodium borohydride provided, besides the 11,13-dihydro product **13**, the reduction–rearrangement product **16** which also had been obtained from longicornin A (**1**) under similar conditions. Therefore, this transformation not only demonstrated that in **3** the 2-methylbutanoate group is attached to C-9 and the isobutyrate moiety to C-8 but also established unambiguously that the stereochemical centers at C-2, C-6, C-7 and C-8 of longicornin C (**3**) are the same as in **1**. The facile reductive rearrangement of longicornin C necessitates a 9α-H [6] and the nearly identical ^{1}H NMR parameters for H-9 of **1** and **3** strongly suggest that longicornin C is represented by structure **3**.

Longicornin D (**4**), $C_{25}H_{34}O_9$, was obtained as a gum which was not completely free of a structural analog with an angelate moiety (mass spectrometry and ^{1}H NMR). The ^{1}H NMR and mass spectral parameters for the medium ring portion of **4** showed close similarities with the data obtained for compound **2**. In addition, the presence of an isobutyrate moiety and a 2-methylbutanoate group was indicated by the spectral data. The appearance of a mass spectral peak at *m/z* 212 (**D**) and the lack of a peak at *m/z* 198 suggested that the 2-methylbutanoate group be attached to C-9 as shown in structure **4**. However, the above low resolution mass spectral assignments could not be verified by the high resolution mass spectral data. Therefore, longicornin D was subjected to a reductive rearrangement reaction as described for the other longicornins. Treatment of longicornin D (**4**) with sodium borohydride in methanol provided 11βH,13-dihydrolongicornin D (**15**) as a major product, and in very low yield the rearranged product **17**. The high resolution mass spectrum of the latter compound exhibited a parent peak at *m/z* 380 ($C_{20}H_{28}O_7$) which indicated that the isobutyrate group was still present in **17** and the 2-methylbutanoate moiety at C-9 of the longicornin D molecule was extruded in the reductive rearrangement reaction. This demonstrated that in longicornin D the 2-methylbutanoate group is attached to C-9 and the isobutyrate moiety to C-8. Consequently, longicornin D is represented by structure **4**.

Comments on the structures of melcanthins A–G

The previous assignments of the melcanthins [4] were based on low-temperature ^{1}H NMR studies and resulted in a tentative major conformation [$_1$D$_{14}$, $_{15}$D$_5$]-melcanthin B, which in return was the basis for the configurational assignments at C-2 and C-9. The basic skeletal arrangement of the conformationally flexible melcanthin B and its analogs could be clearly established from spectral and chemical data, but the conformation as well as the configurations at C-9 (melcanthins A–G) and C-2 (melcanthins B and C) could not be unambiguously determined.

Chemical studies of *cis,cis*-germacradienolides with nucleophilic reagents resulted in the formation of leucantholides of the melampodin B type [6]. Stereochemical and mechanistic considerations of these addition–rearrangement reactions suggested a conformation [^{1}D^{14}, $_{15}$D$_5$] for the *cis,cis*-germacradienolide precursors as well as a β-configuration of the leaving group at C-9 in the medium ring [6].

Earlier attempts to obtain X-ray data on melcanthin B failed. Therefore, the X-ray structure of longicornin A (**1**) not only allowed complete structural assignments of the four newly described longicornins but by ^{1}H NMR correlations of melcanthins A–G with the spectral parameters of [^{1}D^{14}, $_{15}$D$_5$]-longicornin A also permitted a correct structural description of the melcanthins. Since the ^{1}H NMR spectral data for the medium ring portion of the longicornins were nearly identical with the analogs of

the melcanthin series, it is strongly suggestive that the major conformation of the melcanthins is [$^1D^{14}$, $_{15}D_5$], as shown in Fig. 1, and consequently the chiral centers C-9 of melcanthins A–G and also at C-2 in melcanthins B and C should be reversed. The corrected structures for the melcanthins A–G are shown (**5**–**11**).

EXPERIMENTAL

Melampodium longicorne A. Gray was collected on 23 September 1976 in Pima County, Arizona, 3 miles west of Highway 83 or 2 miles north of Greaterville (R. Hartman and V. Funk No. 4385; voucher at O.S., U.S.A.). Dried leaves (130 g) were extracted and worked up as previously described [15], providing 1.12 g crude syrup which was chromatographed over 60 g silica gel using Et_2O–petrol followed by mixtures of Et_2O–Me_2CO with increasing polarity; 45 fractions of 50 ml were taken and all fractions were monitored by TLC.

Fractions 16–20 (134 mg) contained a mixture of **2** and **4**. Rechromatography by prep. TLC using Et_2O–petrol (3:1) yielded 48 mg **2** and 22 mg **4** which was slightly contaminated with an unidentified analog containing an angelate moiety. Fractions 21–24 gave **2** (44 mg) which was recrystallized from EtOAc. Fractions 25–28 (53 mg), after rechromatography by prep. TLC using Et_2O–petrol (17:3), afforded 24 mg pure **3**. Fractions 29–35 gave 189 mg pure **1** which was recrystallized from EtOAc to be used for X-ray analysis.

Longicornin A (**1**). EIMS (probe) *m/z* (rel. int.): 480 $[M]^+$ (not present), 392 $[M-A]^+$ (0.2), 321 $[M-A-A^1]^+$ (0.1), 304 $[M-2A]^+$ (1.5), 286 $[M-2A-H_2O]^+$ (1.6), 272 $[M-2A-MeOH]^+$ (3.0), 254 $[M-2A-MeOH-H_2O]^+$ (2.4), 214 $[D]^+$, 71 $[A^1]^+$ (100), 43 $[A^2]^+$ (53.4). (Found: (MS) 480.2022. $C_{24}H_{32}O_{10}$ requires: 480.1992.)

Longicornin B (**2**). EIMS (probe) *m/z* (rel. int.): 464 $[M]^+$ (not present), 376 $[M-A]^+$ (0.6), 305 $[M-A-A^1]^+$ (1.0), 288 $[M-2A]^+$ (4.9), 270 $[M-2A-H_2O]^+$ (4.4), 238 $[M-2A-H_2O-MeOH]^+$ (4.8), 71 $[A^1]$ (100), 43 $[A^2]$ (62). (Found: (MS) 464.2025. $C_{24}H_{32}O_{10}$: requires 464.2043.)

Longicornin C (**3**). EIMS (probe) *m/z* (rel. int.): 494 $[M]^+$ (not present), 392 $[M-B]^+$ (0.4), 321 $[M-B-A^1]^+$ (0.3), 304 $[M-B-A]^+$ (4.5), 286 $[M-B-A-H_2O]^+$ (4.9), 228 $[D]^+$ (33.8), 85 $[B^1]^+$ (83.2), 71 $[A^1]^+$ (100), 57 $[B^2]^+$ (88.8), 43 $[A^2]^+$ (64.8); CIMS (isobutane) *m/z*: 495 $[M+1]$.

Longicornin D (**4**). EIMS (probe) *m/z* (rel. int.): 478 $[M]^+$ (not present), 390 $[M-A]^+$ (0.4), 376 $[M-B]^+$ (0.9), 305 $[M-B-A^1]^+$ (2.0), 288 $[M-A-B]^+$ (7.5), 85 $[B^1]^+$ (71.5), 71 $[A^1]^+$ (100), 57 $[B^2]^+$ (88.8), 43 $[A^2]^+$ (68.5). (Found: (MS) 478.2204. $C_{25}H_{34}O_9$: requires 478.2203.)

Oxidation of longicornin A (**1**) *with pyridinium chlorochromate* (*PCC*). To a suspension of 91 mg PCC in 2 ml CH_2Cl_2, 50 mg **1** was added. The mixture was stirred at room temp. for 1 hr and monitored by TLC. After 1 hr the reaction product was worked up to give 9.1 mg aldehyde (**18**): EIMS (probe) *m/z* (rel. int.): 478 $[M]^+$ (not present), 390 $[M-A]^+$ (0.6), 319 $[M-A-A^1]^+$ (0.3), 302 $[M-2A]^+$ (2.7), 284 $[M-2A-H_2O]^+$ (1.6), 270 $[M-2A-MeOH]^+$ (6.6), 252 $[M-2A-MeOH-H_2O]^+$ (1.4), 214 $[D]^+$ (12), 71 $[A^1]^+$ (100), 43 $[A^1]^+$ (42). CIMS (isobutane) *m/z*: 479 $[M+1]$.

$NaBH_4$ *reduction of longicornin A*. To a soln of 50 mg **1** in 10 ml of dry MeOH at 0° was added 9.6 mg $NaBH_4$. The mixture was stirred for 45 min, neutralized with 5% aq. HCl and evapd to dryness. H_2O was added and the soln extracted with $CHCl_3$, washed with H_2O and dried ($CaCl_2$). The oily residue was separated by prep. TLC (EtOAc–petrol, 3:1). The band with R_f = 0.33 gave 17.7 mg **12** and the band at R_f = 0.2 provided 22 mg **16**. 11βH,13-Dihydrolongicornin A (**12**), $C_{24}H_{34}O_{10}$, IR ν_{max}^{film} cm^{-1}: 3565 and 3500 (OH), 1780 (γ-lactone), 1760, 1740, 1730 (esters); EIMS (probe) *m/z* (rel. int.): 482 $[M]^+$ (not present), 306 $[M-2A]^+$ (2.2), 288 $[M-2A-H_2O]^+$ (2.1), 274 $[M-2A-MeOH]^+$ (5.1), 256 $[M-2A-MeOH-H_2O]^+$ (4.4), 71 $[A^1]^+$ (98.9), 43 $[A^2]^+$ (100). CIMS (isobutane) *m/z*: 483 $[M+1]$. Rearrangement product (**16**), $C_{20}H_{28}O_8$, IR ν_{max}^{film} cm^{-1}: 3590 and 3485 (OH), 1770 (γ-lactone), 1735 and 1710 (esters); EIMS (probe) *m/z* (rel. int.): 396 $[M]^+$ (not present), 308 $[M-A]^+$ (1.8), 290 $[M-A-H_2O]^+$ (2.1), 272 $[M-A-2H_2O]^+$ (1.8), 240 $[M-A-2H_2O-MeOH]^+$ (2.0), 71 $[A^1]^+$ (88.0), 43 $[A^2]^+$ (100). (Found: (MS) 396.1789. $C_{20}H_{28}O_8$: requires: 396.1784.)

Epoxidation of longicornin A (**1**). To 89 mg **1** in 5 ml CH_2Cl_2 was added a soln of 56 mg *m*-chloroperbenzoic acid dissolved in 1 ml CH_2Cl_2. After 48 hr, 5 ml of a 10% aq. soln of $NaHCO_3$ was added. Usual work-up provided an oily residue which was separated by prep. TLC (EtOAc–Et_2O, 1:1). The fraction with R_f = 0.33 was unreacted **1** (21 mg) and the band with R_f = 0.27 gave 12.2 mg 4,5-epoxylongicornin A (**19**), $C_{24}H_{32}O_{11}$; IR ν_{max}^{film} cm^{-1}: 3600 (OH), 1770 (γ-lactone), 1750 and 1730 (esters); EIMS (probe) *m/z* (rel. int.): 496 $[M]^+$ (not present), 337 $[M-A-A^1]^+$ (0.3), 320 $[M-2A]^+$ (1.1), 302 $[M-2A-H_2O]^+$ (1.8), 288 $[M-2A-MeOH]^+$ (3.7), 270 $[M-2A-MeOH-H_2O]^+$ (3.4), 71 $[A^1]^+$ (100), 43 $[A^2]^+$ (52). CIMS (isobutane) *m/z*: 497 $[M+1]$.

$NaBH_4$ *reduction of longicornin C*. The reduction of 16 mg **3** by the procedure described for longicornin A afforded 3 mg **13** and 1.7 mg **16**. 11β-H,13-Dihydrolongicornin C (**13**), $C_{25}H_{36}O_{10}$; EIMS probe *m/z* (rel. int.): 496 $[M]^+$ (not present), 394 $[M-B]^+$ (0.5), 323 $[M-B-A^1]^+$ (0.3), 306 $[M-B-A]^+$ (3.1), 288 $[M-B-A-H_2O]^+$ (3.0), 228 $[D]^+$ (32.8), 85 $[B^1]^+$ (100), 71 $[A^1]$ (79.7), 57 $[B^2]^+$ (85), 43 $[A^2]^+$ (47.4). CIMS (isobutane) *m/z*: 497 $[M+1]$.

$NaBH_4$ *reduction of longicornin B*. Longicornin B (86 mg) upon $NaBH_4$ reduction afforded 55 mg 11βH,13-dihydrolongicornin B (**14**), $C_{24}H_{34}O_9$; IR ν_{max}^{film} cm^{-1}: 3635 (OH), 1770 (γ-lactone), 1750 (esters); EIMS (probe) *m/z* (rel. int.): 466 $[M]^+$ (0.2), 378 $[M-A]^+$ (0.5), 307 $[M-A-A^1]^+$ (1.0), 290 $[M-2A]^+$ (2.8), 272 $[M-2A-H_2O]^+$ (1.8), 240 $[M-2A-H_2O-MeOH]^+$ (1.4), 71 $[A^1]^+$ (100), 43 $[A^2]^+$ (67). CIMS (isobutane) *m/z*: 457 $[M+1]$.

Reduction of longicornin D (**4**). Compound **4** (20 mg) was reduced with 32 mg $NaBH_4$ in dry MeOH for 20 min, followed by a work-up as described above for the reduction of **1**. The reaction mixture was separated by prep. TLC (EtOAc–cyclohexane, 3:2). The band with R_f = 0.51 gave 5.1 mg of 11βH,13-dihydrolongicornin D (**15**) and the band at R_d 0.53 provided less than 1 mg of the reduction–rearrangement product (**17**). 11βH,13-Dihydrolongicornin D (**15**), $C_{25}H_{36}O_9$, IR ν_{max}^{film} cm^{-1}: 3540 (OH), 1770 (γ-lactone), 1740 (esters); EIMS (probe) *m/z* (rel. int.): 480 $[M]^+$ (0.2), 392 $[M-A]^+$ (0.2), 378 $[M-B]^+$ (0.7), 307 $[M-B-A^1]^+$ (2.5), 290 $[M-A-B]^+$ (5.0), 85 $[B^1]^+$ (100), 71 $[A^1]^+$ (88), 57 $[B^2]^+$ (86), 43 $[A^2]^+$ (40). Rearrangement product (**17**), $C_{20}H_{28}O_7$, EIMS (probe) *m/z* (rel. int.): 380 $[M]^+$ (0.3), 292 $[M-A]^+$ (3.3), 291 $[M-A^1-H_2O]^+$ (1.4), 274 $[M-A-H_2O]^+$ (1.4). (Found: (MS) 380.1860. $C_{20}H_{28}O_7$ requires: 380.1836.)

The crystal structure of longicornin A (**1**) was determined using an Enraf–Nonius CAD4 diffractometer with CuK_α radiation (λ = 1.54184Å). Crystal data are: $C_{24}H_{32}O_{10}$, MW 480.5, orthorhombic space group $P2_12_12_1$, a = 12.278 (1), b = 13.797 (2), c = 14.867 (2) Å, z = 4, d_{calcd} = 1.267 g/cm^3, μ(MoKα) = 8.38 cm^{-1}, R = 0.050 for 2613 data having $2° \leqslant \theta \leqslant 75°$. Coordinates for non-hydrogen atoms are given in Table 4.

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