Tetrahedron Vol. 46, No. 6, pp. 1923-1930, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00
Pergamon Press plc

NEW ENT-PIMARANE DITERPENES

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(*Received in UK* 8 *December* 1989)

ABSTRACT: Three new ent-pimarane diterpenes were isolated from Palafoxia texana. Their structures were determined from the spectral evidence. The absolute configuration of **1** was established by means of circular dichroism analysis of a derivative.

Diterpenes with an ent-rosane skeleton[1] from Palafoxia texana D.C. (Compositae, Heleniae)[2] and their structural configurations have already been reported. There is considerable confusion about the structures of diterpenes from these species[3,4,5,6,7]. Three new ent-pimarane diterpenes have now been isolated from P. texana as acetylderivatives: 2β,3α,15β-triacetoxy-7β,(8β)-16,(14)-diepoxy-ent-pimarane (**1**); **2β,3α,14α,16-tetraacetoxy-15β-hydroxy-ent- pimarane** (**2**) and 2β,3α,14α,15β,16-pentacetoxy-ent-pimar-7-ene (**3**). Other ent-pimarane diterpenes have been reported from liverworts (Hepaticae)[8] and species of Siegesbeckia[9,10] and Palafoxia[11].

RESULTS AND DISCUSSION

Pre-acetylated alcohol-containing fractions of Palafoxia texana were repeatedly chromatographed on silica gel to yield the following compounds: **1**, $C_{24}H_{34}O_8$, mp 140-142° C, $[\alpha]_D^{20}$ +20°, no bands for alcohols in IR; signals for four methyls and three acetate groups in the ^1H NMR spectrum (consonant with MS fragments reflecting the loss of one, two and three ketenes, respectively), no vinyl proton signals. In the ^{13}C NMR spectrum, signals for seven carbons bonded with oxygen appeared between 56.1 and 88.3 ppm.

Five of the carbons were secondary, one was primary and one, quaternary. Apart from the signals for the carbons of the acetate carbonyls, no other carbonyl signal was observed. Of the eight oxygen atoms in the molecule, six must therefore belong to the acetate group and the other two to ether bridges[5]. Thus, **1** must be a pentacyclic diterpene bearing three acetate groups with two ether rings.

The ^{1}H NMR spectrum showed three signals for protons geminal to secondary acetates as: a doublet centred at δ 4.68 (J=10.0Hz), a double doublet centred on δ 4.91 (J_1=2.4,J_2=4.6Hz) and a triplet of doublets centred at δ 5.00 (J_1=2.4,J_2=10.0Hz). In a diterpene structure, the multiplicity and coupling constants of the signals centred at δ 4.68 and 5.00 are only compatible with a 2,3-diequatorial diacetoxy grouping in Ring A. These signals and that at δ 1.96 (H-1) are part of an ABX system, as has been confirmed by a COSY study.

An AB system with the A proton as a double doublet centred at δ 3.63 and the B proton as another doublet centred at δ 4.00 can be assigned to the oxymethylene of an ether bridge. A COSY experiment also showed a coupling between the oxymethylene protons and the proton geminal to the third acetoxy group centred at δ 4.91, indicating that there is a $-CH(OAc)-CH_2-O-$ grouping in the molecule. Proton singlets at δ 3.04 and 3.13 can be attributed to the protons of the ether bridge closure at C-14 and C-7 on the oxyranic ring, respectively. These data all accord with a pimarane structure for **1**. N.O.e. difference experiments (Fig. 1) confirmed this structure and established the relative configuration. The β-OAc configuration at C-15 was deduced from the fact that there was no visible n.O.e. effect between H-15 and Me-17 and this, together with the low coupling constants for H-15 (giving a pseudoaxial disposition on Dreiding models), determined the CD junction as cis with Me-17 β.

The configuration of the epimeric ent-rosene diterpenes mentioned above[1] has been determined elsewhere by x ray analysis[12]; these diterpenes, **4** and **5**, are present together with **1** in the extract. In the biogenetic pathway proposed for the formation of ent-rosane skeleton diterpenes from ent-pimaranes[13], the methyl migrates from C-10 to C-9 on the same face of the molecule, suggesting an α configuration for Me-20 and β for Me-17 for **1**, which thus should be an ent-pimarane. Data provided by Prof. Herz show that another ent-pimarane diterpene, darutigenol[14], has been isolated from a Palafoxia, P. arida[11].

The absolute configuration of **1** was established by means of a CD analysis of a derivative, **6**. Compound **1** was hydrolysed with KOH/MeOH (5%) for 13 hr at room temp giving compound **7** (see Table 2). This product was then treated with benzoyl chloride in pyridine with DMAP as catalyst for 24 hr and after

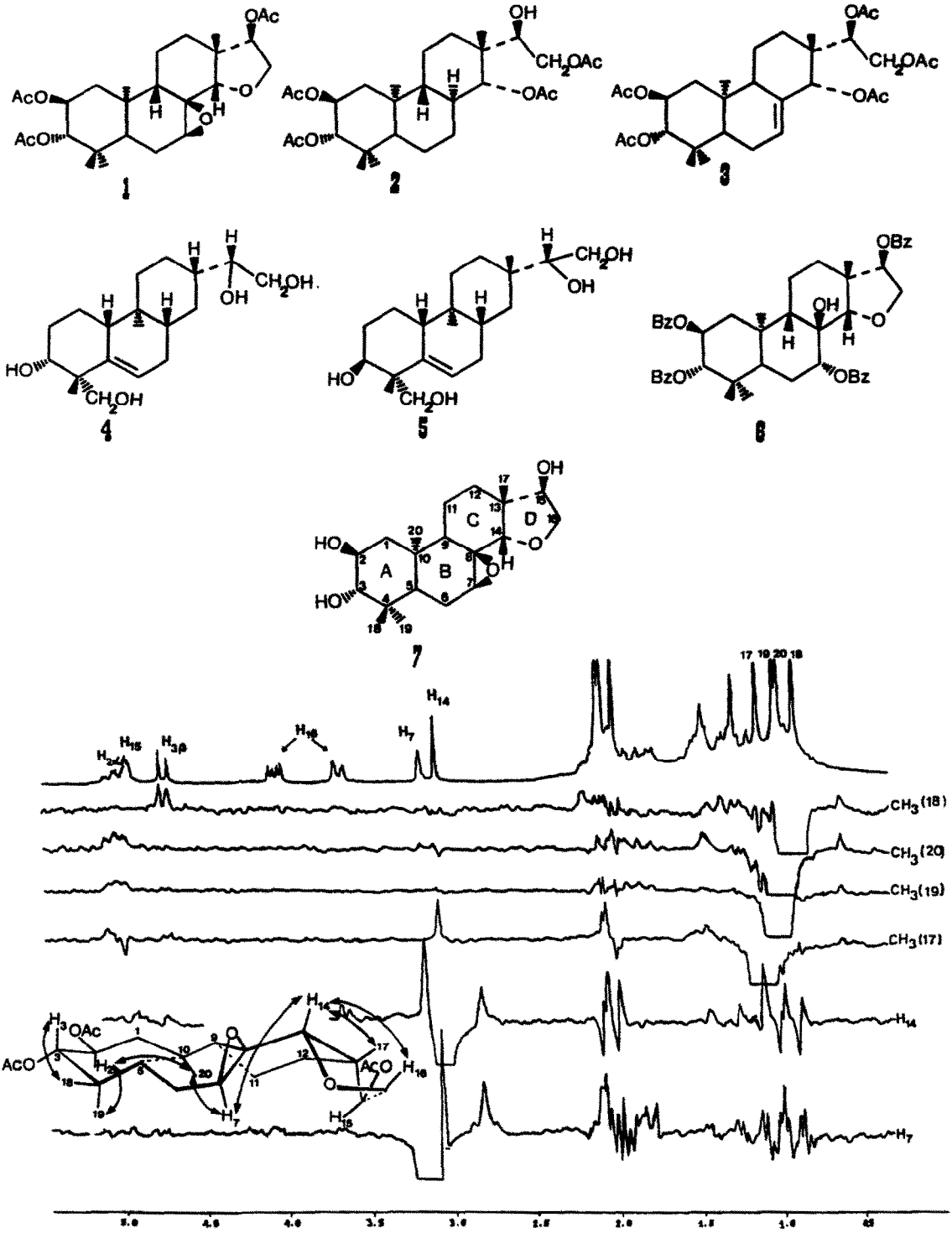

FIG 1

work-up, product **6** was obtained. The structure of **6** was established from its spectral data (see Tables). The antiperiplanar attack leading to the 7α-benzoate-8β-hydroxyderivative is particularly noteworthy. The CD spectrum of **6** exhibited a positive first Cotton effect at 234 nm ($\Delta\epsilon$ =+28.9) and a negative second Cotton effect at 219 nm ($\Delta\epsilon$=-2.6) and according to the dibenzoate chirality rule[14], an extension of the exciton chirality method[15], the sign and amplitude of split Cotton effects depend on the absolute disposition of the benzoate chromophores. Therefore, the split Cotton effects observed for compound **6** must be due mainly to the chiral exciton coupling between the 2β- and 3α-benzoate chromophores. The contribution of the 7α- and 15β-benzoate groups is negligible given the distance between them and the nearest dibenzoate moiety.

The positive sign of the 234 nm Cotton effect thus indicated the absolute configuration shown for **6** and **1** was therefore established as: (2S,3S,5R,7S,8R,9S,10R,13S,14S,15S,2,3,15-triacetoxy-7(8),14(16)-diepoxy-ent-pimarane). The most important signals in ^{13}C NMR spectrum (Table 1) were for the secondary oxygen-bearing C-2, C-3, C-7, C-14 and C-15, the primary C-16 and the quaternary C-8. The ^{13}C NMR spectrum was assigned from DEPT experiments and correlation with data published, notably by Prof. Wenkert[17] who synthesized and studied a tetrahydrofuran derivative with an isopimarane skeleton[18].

From the n.O.e. difference[19] study and analysis of the coupling constants[20] it was deduced that Ring A is in the chair conformation[1] while B is a deformed chair due to the presence of the oxyranic ring where C-8 and C-9, C-11, C-13 and C-14 form part of a plane with Ring C which is an envelope the with C-12 out of plane. The trans AB junction was anti and the cis CD junction, syn to the cis BC junction. In Ring D, C-13 is outside the plane.

The slightly more polar compound **2** proved to be another diterpene and its structure was established as follows: in MS, the molecular ion was not visible although there was a fragment at m/z 446 for the successive loss of one, two and three acetates and another at m/z 420 corresponding to the successive loss of H_2O, one, two and three acetates. Both fragments may be explained if the virtual molecular ion of **2** is [M^+] m/z 524, so that the signal at m/z 446 would correspond to the loss of H_2O and acetates, and that at m/z 420 to the loss of a methyl acetate and two methyls.

The ^1H NMR spectrum shows signals for four angular methyls between δ 0.82 and 1.22, four acetate methyls between δ 1.97 and 2.08 and a double doublet centred at δ 3.84 which can be assigned to the proton geminal to the secondary alcohol group. Two signals attributable to the geminal protons of secondary acetates appear as a doublet centred at δ 4.75 and a triplet of doublets centred at δ 5.05 has the same chemical shift, the same

multiplicity and the same coupling constants as H-2α and H-3β in **1**, thus confirming the existence of a 2β,3α-diacetoxy in the molecule. Signals for two protons as double doublets centred at δ 4.17 and δ 4.28 could be charged to a $-CH_2OAc$ grouping. Double resonance experiments demonstrated that these protons are coupled with the proton geminal to a secondary alcohol group centred at δ 3.84 and thus confirm the presence of the $-CHOH-CH_2OAc$ group characteristic of the side chain of an ent-pimarane with hydroxy groups at C-15 and C-16. Finally, the geminal proton of the third secondary acetoxy group appeared as a doublet centred at δ 4.96 (J=2.6Hz) and the multiplicity and coupling constants of this signal are compatible, in a pimarane skeleton with the standard trans BC junction, only with an α-acetoxy group on C-14.

The structure of the most polar product, **3**, was established as 2β,3α,14α,15β,15-pentacetoxy-entpimar-7-ene from the following data: in MS, the molecular ion appeared at m/z 564 ($C_{30}H_{44}O_{10}$ by HRMS). No hydroxy bands were observed in IR. The ^1H NMR spectrum showed signals for four angular methyls, five acetate groups, a vinyl proton as a narrow triplet centred at δ 5.32, and six acetate group geminal protons, two for a primary acetate and the others for secondary acetates. The $^1H-^1H$ couplings were determined from COSY experiments. If the spectrum was compared with that of **2**, the same sequences for H-2 and H-3 were observed, signifying the same substitution process on Ring A. The H-15 appeared as a double doublet centred at δ 5.07 coupled with the H-16 but the most striking difference between **2** and **3** was the low chemical shift of the proton geminal to the remaining secondary acetate group, which appeared as a singlet at δ 5.62 making it allylic to the double bond. In an ent-pimarane skeleton, the only possible relative position is the H-14 with a C-7-C-8 double bond. The ^{13}C spectral data confirmed this structure.

TABLE 1: ^{13}C NMR for **1** and **3**

C	**1**	**3**	C	**1**	**3**	C	**1**	**3**
1	42.7	42.4	7	57.0	134.9	14	88.3	74.2
2	69.4	69.6	8	56.1	136.6	15	83.4	74.7
3	83.4	80.4	9	52.1	47.4	16	71.3	63.6
4	35.3	37.4	10	39.0	39.0	17	27.8	28.5
5	45.2	46.4	11	18.3	18.0	18	17.6	17.7
6	22.1	27.4	12	34.5	31.3	19	21.0	21.0
			13	43.2	39.1	20	15.8	14.9
		1					**3**	
$CH_3-CO-O-$	(170, 170, 170.7)			(170.1, 170.4, 170.5, 170.5, 170.9)				
$CH_3-CO-O-$	(20.9, 21.0, 21.2)			(20.9, 21.0, 21.7, 21.7, 23.1)				

EXPERIMENTAL

Mp's are uncorr. ^{1}H NMR were run at 200 MHz, ^{13}C NMR at 50.3 MHz in $CDCl_3$ with TMS as internal standard; IR were taken in $CHCl_3$ and dry chromatography with silica gel.

Isolation of the Products The methanol extract (60 g) of the aerial part of Palafoxia texana D.C., collected in Mexico (voucher specimen No. 7819 on file with the Dept. of Botany of the Instituto Tecnológico de Monterrey), was chromatographed on silica gel and eluted with C_6H_{14}-EtOAc mixtures. 250 ml fractions were collected. The mother liquor of Fractions 336-348 (400 mg), which had no signals for acetate groups in ^{1}H NMR, was dissolved in pyridine and an excess of acetic anhydride was added. After extraction with ether and evaporation of the solvent, the mixture was subjected to centrifugal chromatography (Chromatotron) using 4 mm silica gel disc and C_6H_{14}-EtOAc (63:35) as solvent. 5 ml fractions were collected to yield the products:

2β,3α,15β-Triacetoxy-7β,8β,14α,(16)-diepoxy-ent-pimarane (**1**) (10.5 mg): mp 140-142° (from EtOAc); $[\alpha]_D^{20}$ = +20° ($CHCl_3$; c 0.05); [M$^+$] m/z 478.2435 (calc. for $C_{24}H_{34}O_8$, 478.2304); IR ϑ_{max} cm^{-1}: 3010, 1730, 1500, 1470, 1430, 1380, 1200, 1040, 920; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); MS m/z (rel. int. %) 478 [M$^+$], 460 (2), 418 (8), 403 (4), 400 (2), 385 (1), 376 (6), 358 (5), 343 (8), 340 (3), 325 (2), 301 (2), 255 (18), 237 (9), 225 (53), 223 (10), 209 (5), 194 (38), 183 (9), 179 (5), 169 (10), 165 (29), 159 (16), 147 (47), 134 (97), 120 (37), 105 (60), 95 (66), 81 (78), 69 (56), 55 (98).

2β,3α,14α,16-Tetracetoxy-15β-hydroxy-ent-pimarane (**2**) (7 mg): $[\alpha]_D^{20}$ = +24.2° ($CHCl_3$; c 0.078); IR ϑ_{max} cm^{-1}: 2920, 1730, 1370, 1050; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); MS m/z (rel. int. %) 446 [M$^+$-HOAc-H_2O] (1), 420 (5), 402 (6), 384 (4), 374 (2), 360 (3), 326 (2), 267 (4), 239 (5), 225 (4), 159 (7), 145 (8), 133 (7), 119 (11), 105 (11), 95 (9), 91 (8), 81 (10), 69 (15), 55 (15), 43 (100).

2β,3α,14α,15β,16-Pentacetoxy-ent-pimar-7-ene (**3**) (12 mg): $[\alpha]_D^{20}$ = -12.7° ($CHCl_3$; c 0.011); [M$^+$] m/z 564.3151 (calc. for $C_{30}H_{44}O_{10}$, 564.3368); IR ϑ_{max} cm^{-1}: 2940, 1730, 1370, 1250, 1050; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); MS m/z (rel. int. %) 564 [M$^+$] (1), 522 (1), 462 (1), 444 (6), 402 (1), 384 (2), 359 (2), 299 (4), 257 (2), 239 (17), 119 (5), 105 (6), 95 (7), 83 (5), 71 (5), 55 (10), 43 (100).

2β,3α,15β-Trihydroxy-7β,8β,14α,(16)-diepoxy-ent-pimarane (**7**) The structure of this compound was established from its spectra. (For ^{1}H NMR, see Table 2.) **7** was treated with BzCl directly to yield compound **6**.

2β,3α,7α,15β-Tetrabenzoate-14α,(16)-epoxy-8β-hydroxy-ent-pimarane (**6**) Purified by HPLC with a column of μ-Porasil, n-hexane-EtOAc (30%), λ=254nm. IR ϑ_{max} cm^{-1}: 3380, 2910, 2840, 1715, 1270, 1110, 1020; ^{1}H NMR (see Table 2); ^{13}C NMR (see Table 1); MS FAB m/z: [M$^+$-COC_6H_5-H_2O], 663, 609, 582, 551, 517, 491, 451, 425, 405, 393, 341, 333, 315, 297, 257, 241, 207, 167, 149, 131, 122, 105. UV (CH_3CN): λ_{max} 229 nm. CD (CH^3CN) λ_{ext} = 234 nm ($\Delta\epsilon$ = +28.9); 219 nm ($\Delta\epsilon$ = -2.6).

ACKNOWLEDGEMENTS

The authors are indebted to the AIETI and the CAICYT (Grant FAR88-0501/89-91) for financial support.

TABLE 2: ^{1}H NMR for **1**, **2**, **3**, **6** and **7**

H	**1**	**2**	**3**	**6**	**7**
1α	1.96m*			2.49dd (4.0,12.0)	
1β	1.96m*			1.82m*	
2α	5.0td (2.4,10.0)	5.05td (3.9,10.3)	5.06td (4.3,10.0)	5.57m	
3β	4.68d (10.0)	4.75d (10.3)	4.79d (10.0)	5.24d (10.4)	2.96d (8.0)
5β	1.45m*			1.26m*	
6α	1.76m*			1.60dd (9.0, 6.0)	
6β	1.85m*			2.33d* (6.0,12.0)	
7	3.13br s		5.32br s	6.01br s	3.15br s
14β	3.04s	4.95d (2.6)	5.62br s	4.07s	3.60s
15α	4.91dd (2.4,4.6)	3.84dd (4.2,8.4)	5.07dd (2.5,9.0)	5.17d (4.0)	3.86d (4.0)
16α	3.63dd (2.4,10.0)	4.17dd (4.2,12.5)	4.00dd (9.0,11.7)	3.82dd (1.4,11.0)	3.63dd (8.0)
16β	4.00dd (4.6,10.0)	4.28dd (8.5,12.5)	4.33dd (2.5,11.7)	4.53dd (5.0,11.0)	3.95dd (4.0,8.0)
17β	0.85s	0.82s	0.85s	1.22s	1.00s
18	0.95s	0.92s	0.92s	1.02s	0.88s
19	0.97s	0.98s	0.96s	1.03s	0.88s
20α	1.08s	1.12s	0.98s	1.26s	1.18s
OAc	1.96s	1.97s	1.98s		
	2.03s	2.04s	2.01s		
	2.05s	2.05s	2.05s		
		2.08s	2.06s		
OBz				7.42m	
				7.97m	

* Partially overlapping another signal

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