Diterpenoids from *Acacia leucophloea*: Revision of the Structures of Leucophleol and Leucophleoxol

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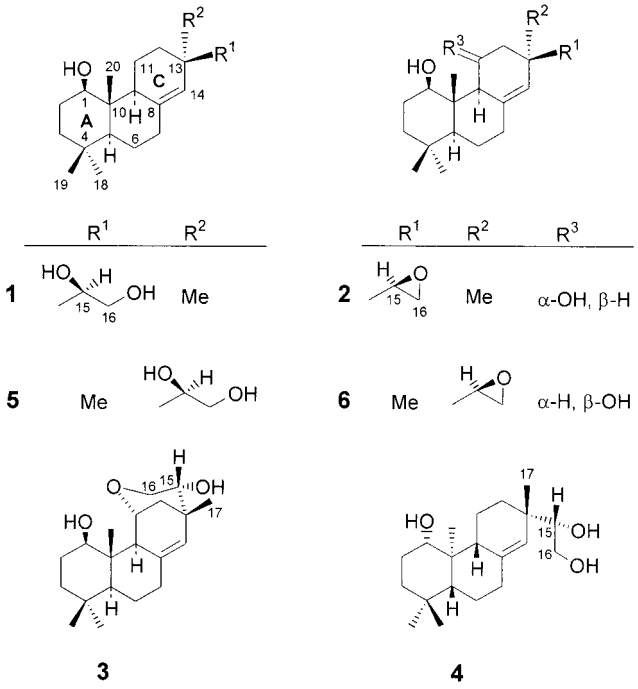
The pimarane-type structures previously suggested for leucophleol (**1**) and leucophleoxol (**2**), two diterpenoids isolated from *Acacia leucophloea*, must be amended to the isopimarane-type derivatives **5** and **6**, respectively. These corrections were supported on NMR spectroscopic studies and, in the case of **6**, by an X-ray diffraction analysis. Moreover, the unpublished complete and unambiguous ^{1}H and ^{13}C NMR assignments of **5** and **6** together with those of leucoxol (**3**), another diterpenoid from the same plant, are also reported.

Twenty years ago, one of us together with other authors reported the isolation of three diterpenoids [leucophleol, leucophleoxol, and leucoxol (**1**−**3**, respectively)] from the root bark of *Acacia leucophloea* (Roxb.) Willd. (Mimosaceae).[1,2] The pimarane-type structures of leucophleol (**1**) and leucophleoxol (**2**) were suggested[1] on the basis of some ^{1}H and ^{13}C NMR spectroscopic data, obtained at 100 and 25.2 MHz, respectively, and assigning these data only by comparison with those reported in the literature for other pimarane and isopimarane diterpenoids. On the contrary, the isopimarane-type structure of leucoxol (**3**), including its absolute stereochemistry, was firmly established from an X-ray diffraction analysis.[2] In view of those results and for biogenetic reasons, it had been pointed out[2] that the C-13 stereochemistry of **1** and **2** should be changed to that of an isopimarane hydrocarbon skeleton, such as **3**.

Recently, Subrahmanyam and co-workers[3] isolated from the marine mangrove *Bruguiera gymnorhiza*, a diterpenoid whose structure **4** [*ent*-8(14)-pimarene-1β,15*R*,16-triol][4] was rigorously established by an X-ray analysis. These authors indicated[3,4] that "it may be mentioned that 8(14)-pimarane-1,15,16-triol [leucophleol (**1**)][1] isolated from *Acacia leucophloea* differs from **4** in the physical and spectral data".[5] If the previously reported[1] structure **1** for leucophleol is correct,[6] this substance should be the enantiomer of **4**, which does not agree with the physical (mp and, particularly, $[\alpha]_D$ values) and spectroscopic data of these compounds.[1,3,5,6] In view of these facts, it was obvious that structure **1** for leucophleol,[1] and probably that of leucophleoxol (**2**),[1,2] needed to be revised.



Results and Discussion

All our attempts to grow single crystals of leucophleol or its derivatives[1] suitable for X-ray crystallography have been unsuccessful. Thus, we decided to use NMR spectroscopy, and particularly NOE experiments, to reexamine structure **1** suggested for this diterpenoid.

As a result of an exhaustive study of the 2D ^{1}H,^{1}H-NOESY and NOE difference spectra of leucophleol, we definitely conclude that structure **1**[1] must be amended to that of the isopimarane derivative **5** [8(14)-isopimarene-1β,15*R*,16-triol].[6] Table 1 shows some significant NOE data of leucophleol (**5**) that firmly support this conclusion. In particular, irradiation at δ 0.80 (Me-20 protons) under NOE difference experimental conditions caused NOE enhancement in both Me-17 and Me-19 proton signals (at δ 0.98 and 0.83, respectively), thus establishing that all these C-Me groups are placed on the same side of the plane defined by the A, B, and C rings of the molecule. Moreover, the *cis* spatial relationship for the Me-17 and Me-20 groups was also supported by the observed NOE cross-peak between these C-Me protons in the NOESY spectrum of **5** (see Table 1).[7]

Unlike leucophleol (**5**), we have obtained an X-ray-quality single crystal of leucophleoxol. Figure 1 shows the result of the X-ray analysis, establishing that this diterpenoid also possesses an isopimarane-type structure and that the previously reported[1] α-configuration of its secondary hydroxyl group at the C-11 position must be changed to an 11β-configuration. Thus, structure **2** attributed previously[1]

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Table 1. Significant NOE Data for Compounds **5** and **6**[a]

compd	observed proton(s)	NOE cross-peak with protons[b]
5	Me-17 (δ 0.98)	H-12β (0.4), H-14 (0.3), H_B−16 (0.8), Me-20 (0.6)
	Me-19 (δ 0.83)	H-2β (3.1), H-3β (2.3), Me-18 (0.8), Me-20 (1.1)
	Me-20 (δ 0.80)	Me-17 (0.2), Me-19 (1.1)
6	H-11α (δ 4.00)	H-1α (0.6), H-9α (9.0), H-12α (3.2), H-12β (0.8), H-15 (4.2), H_A-16 (2.0), H_B-16 (0.4)
	Me-17 (δ 1.04)	H-12β (2.5), H-14 (3.7), H_A-16 (2.3), Me-20 (1.5)
	Me-19 (δ 0.81)	H-2β (1.9), Me-18 (1.8), Me-20 (3.3)
	Me-20 (δ 0.97)	H-2β (1.5), Me-17 (1.0), Me-19 (2.4)

[a] All these data were obtained from the NOESY spectra and 1D NOE difference experiments. [b] Values in parentheses are positive NOE enhancements (in %), which were measured by the 1D NOE difference method.

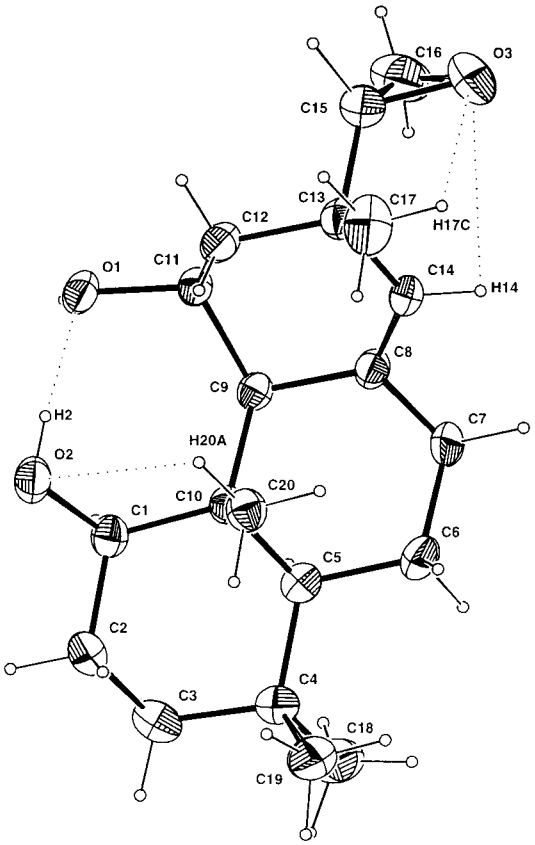

Figure 1. X-ray molecular structure of leucophleoxol (**6**), with the numbering scheme. Displacement ellipsoids are drawn at the 30% of probability level.

to this substance must be amended to **6** [15*R*,16-epoxy-8(14)-isopimarene-1β,11β-diol].[6]

In the crystalline state, bond lengths and angles of **6** are in good agreement with those found in analogous compounds.[8] Rings A and B present a chair conformation, while ring C is in the envelope form, flapping at C(11) down the overall plane of the molecule. The position of the 15,16-epoxide is determined by the torsion angle C(12)−C(13)−C(15)−C(16) of −96.5(5)°. There are four intramolecular hydrogen interactions: C(14)···O(3), C(17)···O(3), C(20)···O(2), and O(2)···O(1), plus another five intermolecular ones, that contribute to the packing in the crystal, which is governed mainly by the C(16)···O(3) ($-x+1$, $y-1/2$, $-z+1$) and C(18)···O(3) ($-x$, $y-1/2$, $-z+1$) contacts, plus other van der Waals ones, so as to give a distorted close hexagonal pattern.[9]

Structure **6** for leucophleoxol was also in agreement with NOE experiments. The NOE cross-peaks observed in the NOESY spectrum of **6** between the Me-20 protons (δ 0.97) and the Me-17 and Me-19 protons (at δ 1.04 and 0.81, respectively)[7] were consistent with an isopimarane-type structure. Moreover, the H-11α proton of **6** (δ 4.00) showed cross-peaks of NOE with the H-1α, H-9α, and the C-15 and C-16 epoxide protons, all of them placed on the α face of the molecule, whereas no NOE was observed between the H-11α proton and those of the Me-17 and Me-20 groups (see Table 1).

To obtain a reliable interpretation of the NOESY data of **5** and **6**,[7] we have needed to make an unambiguous and complete assignment of the ^{1}H and ^{13}C NMR spectra of these compounds. In addition to conventional 1D NMR methods, 2D shift-correlated experiments [^{1}H,^{1}H−COSY, ^{1}H,^{1}H-TOCSY, ^{1}H,^{13}C-HSQC−^1J(C,H), and ^{1}H,^{13}C-HMBC−^nJ (C,H) (n = 2 and 3)] were used for this purpose. The ^{1}H and ^{13}C NMR assignments of **5** and **6** are included in Tables 2 and 3, together with those of leucoxol (**3**), because only partial ^{1}H NMR data of **5** and **6**, a calculated ^{13}C NMR spectrum of **5**, the ^{13}C NMR spectrum of **6** (with erroneous assignments), and incomplete ^{1}H and ^{13}C NMR data of the diacetyl derivative of **3** have been reported previously.[1,2]

Experimental Section

Samples of the Diterpenoids. The samples of leucophleol (**5**), leucophleoxol (**6**), and leucoxol (**3**) used for this study were small amounts of crystals that remained from the respective original work.[1,2]

Acquisition of the NMR Spectral Data of the Diterpenoids. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA-400 spectrometer operating at 400 and 100 MHz, respectively, using $CDCl_3$ (**5** and **6**) or pyridine-d_5 (**3**) as solvent at 22 °C. Chemical shifts are given in the δ scale and were referenced to residual $CHCl_3$ or pyridine at 7.25 ppm or 8.71, 7.55, and 7.19 ppm for proton, respectively, and to the solvent at 77.00 ppm or 149.9, 135.5, and 123.5 ppm for carbon, respectively. One-dimensional ^{1}H and ^{13}C NMR spectra were acquired with standard conditions. The pulse programs of the COSY, TOCSY, NOESY, HSQC, and HMBC experiments were taken from the Varian software library. The COSY and NOESY 2D NMR spectra were acquired in the phase-sensitive mode. Data were collected in a 1024 × 256 matrix with a spectral width of 2485 Hz and a 2 s recycle delay and processed in a 1024 × 1024 matrix. The NOESY spectra were generated with a mixing time of 0.5 s. The TOCSY experiments were acquired with a mixing time of 20−80 ms and processed in the phase-sensitive mode using parameters very similar to those given above for the COSY and NOESY experiments. The data for the HSQC spectra were collected in a 1024 × 256 matrix with a spectral width of 2485 Hz in the proton domain and 10 000 Hz in the carbon domain and processed in a 1024 × 512 matrix. The null time following the BIRD pulse was 400 ms. The HMBC experiments were optimized for long-range coupling constants of 8 Hz, and the data were processed using parameters very similar to those used in the HSQC experiments.

X-ray Crystallographic Analysis of Leucophleoxol (6). Crystals of **6** suitable for X-ray diffraction analysis were obtained by recrystallization from MeOH−H_2O. A colorless prism of **6** (1.0 × 0.7 × 0.8 mm) was selected for the data collection. Crystal data: $C_{20}H_{32}O_3$; M_r = 320.46 g.mol^{-1}; monoclinic a = 10.946(1) Å, b = 6.650(1) Å, c = 13.090(2) Å, β = 103.27(1)°, V = 927.3(2) Å^3, space group $P2_1$ (no. 4), Z = 2, D_{cal} = 1.148 Mg m^{-3}. Data collection: Seifert XRD 3000S diffractometer; 3584 independent reflection intensities were collected between 4° and 67° in the $\omega/2\theta$ scan mode, with Cu Kα monochromated radiation (λ = 1.54180 Å). No decay was observed in two reference reflections measured every 150 min, and 3155 reflections were considered as observed at the 2$\sigma(I)$ level.

Table 2. ^{1}H NMR Spectral Data for Compounds **3**, **5**, and **6**[a]

proton(s)	**3**[b]	**5**[c]	**6**[c]	$J_{H,H}$ (Hz)	**3**[b]	**5**[c]	**6**[c]
H-1α	3.84 (ddd)[d]	3.46 (dd)	3.57 (dd)	1α,2α	5.1	6.9	7.0
H-2α	1.85 (m)[e]	1.57 (m)[e]	1.63 (m)[e]	1α,2*β*	10.4	8.8	9.0
H-2*β*	1.87 (m)[e]	1.57 (m)[e]	1.63 (m)[e]	2α,2*β*	*e*	*e*	*e*
H-3α	1.32 (ddd)	1.31 (m)[e]	1.30 (m)[e]	2α,3α	4.0	*e*	*e*
H-3*β*	1.36 (m)[e]	1.38 (m)[e]	1.39 (m)[e]	2α,3*β*	*e*	*e*	*e*
H-5α	1.09 (dd)	0.99 (dd)	1.09 (dd)	2*β*,3α	13.2	*e*	*e*
H-6α	1.58 (dddd)	1.56 (m)[e]	1.68 (m)[e]	2*β*,3*β*	*e*	*e*	*e*
H-6*β*	1.40 (dddd)	1.38 (m)[e]	1.47 (dddd)	3α,3*β*	13.4	*e*	*e*
H-7α	2.23 (dddd)	2.05 (dddd)	1.97 (dddd)	5α,6α	2.0	2.6	2.7
H-7*β*	2.35 (ddd)	2.28 (ddd)	2.27 (ddd)	5α,6*β*	12.4	14.0	12.5
H-9α	2.16 (br s)[f]	1.94 (m)[e]	2.03 (br d)	6α,6*β*	12.9	*e*	13.0
H-11α		1.94 (m)[e]	4.00 (ddd)	6α,7α	5.2	5.6	4.1
H-11*β*	6.09 (dd)	1.73 (dddd)		6α,7*β*	2.4	2.0	2.0
H-12α	1.79 (ddd)	1.45 (ddd)	1.63 (dd)	6*β*,7α	13.3	13.8	13.1
H-12*β*	1.72 (dd)	1.31 (m)[e]	1.73 (dd)	6*β*,7*β*	4.3	4.6	4.6
H-14	5.73 (t)	5.26 (d)	5.02 (br s)[f]	7α,7*β*	13.5	14.5	12.8
H-15	3.77 (ddd)[g]	3.42 (dd)	2.79 (dd)	9α,11α		*e*	5.3
H_A-16	3.71 (t)[h]	3.51 (dd)	2.58 (dd)	9α,11*β*	<0.5	13.5	
H_B-16	4.04 (dd)[i]	3.73 (dd)	2.63 (dd)	11α,11*β*		13.7	
Me-17	1.36 (3H, s)	0.98 (3H, s)	1.04 (3H, s)	11α,12α		3.1	4.4
Me-18	0.83 (3H, s)	0.86 (3H, s)	0.85 (3H, s)	11α,12*β*		*e*	12.5
Me-19	0.82 (3H, s)	0.83 (3H, s)	0.81 (3H, s)	11*β*,12α	3.9	13.0	
Me-20	1.11 (3H, s)	0.80 (3H, s)	0.97 (3H, s)	11*β*,12*β*	1.9	3.8	
OH-1*β*	6.12 (d)[j]	*k*	*k*	12α,12*β*	12.6	13.4	13.2
OH-15α	6.06 (d)[j]	*k*		14,7α	1.6	1.6	1.3
				14,9α	1.6	0	<0.5
				15,16A	10.2	9.0	2.7
				15,16B	4.8	2.6	3.8
				16A,16B	10.2	10.9	4.9
				9α,12α	1.4	0	0
				1α,1*β*OH	4.7[j]	*k*	*k*
				15,15OH	4.7[j]	*k*	

[a] All these assignments were in agreement with COSY, TOCSY, HSQC, and HMBC spectra and NOE experiments. [b] In pyridine-d_5 solution. [c] In $CDCl_3$ solution. [d] This signal collapsed into a dd (J = 10.4, 5.1 Hz) after addition of D_2O. [e] This is an overlapped signal; approximate δ value was measured from the HSQC spectrum. [f] This signal showed a $W_{1/2}$ = 4 Hz. [g] This signal collapsed into a dd (J = 10.2, 4.8 Hz) after addition of D_2O. [h] This is the H-16 axial hydrogen. [i] This is the H-16 equatorial hydrogen. [j] This signal disappeared after addition of D_2O. [k] This signal was not observed.

Table 3. ^{13}C NMR Spectral Data for Compounds **3**, **5**, and **6**[a]

carbon	**3**[b]	**5**[c]	**6**[c]
C-1	78.4 (d)	79.2 (d)	76.1 (d)
C-2	30.9 (t)	30.0 (t)	28.1 (t)
C-3	40.0 (t)	39.7 (t)	39.8 (t)
C-4	33.6 (s)	33.3 (s)	33.2 (s)
C-5	54.5 (d)	54.0 (d)	55.2 (d)
C-6	23.2 (t)	22.3 (t)	24.3 (t)
C-7	36.4 (t)	36.2 (t)	36.6 (t)
C-8	140.9 (s)	140.0 (s)	139.9 (s)
C-9	57.4 (d)	51.7 (d)	55.8 (d)[d]
C-10	44.8 (s)	43.9 (s)	47.4 (s)
C-11	68.8 (d)	21.9 (d)	69.4 (d)
C-12	38.5 (t)	29.2 (t)	38.2 (t)
C-13	34.9 (s)	37.8 (s)	36.8 (s)
C-14	128.3 (d)	128.1 (d)	123.7 (d)
C-15	74.8 (d)	79.6 (d)	59.3 (d)[d]
C-16	65.4 (t)	62.7 (t)	44.9 (t)
C-17	25.8 (q)	22.7 (q)	24.9 (q)
C-18	33.5 (q)	33.2 (q)	33.5 (q)
C-19	21.9 (q)	21.7 (q)	21.4 (q)
C-20	11.3 (q)	8.5 (q)	12.4 (q)

[a] All these assignments were in agreement with HSQC and HMBC spectra. [b] In pyridine-d_5 solution. [c] In $CDCl_3$ solution. [d] These assignments are reversed with respect to those reported previously.[1]

The structure was solved by direct methods (SHELXS97)[10] and difference Fourier techniques; no absorption correction was applied (μ = 0.589 mm^{-1}). The structure was refined using full matrix least-squares on F^2. All non-H atoms were refined with anisotropic thermal parameters. Since **6** crystallizes in a polar space group, polar axis restraints were applied.[11] The H atoms were assigned geometrically and treated using appropriate riding models. The refinement converged to R = 0.070. All calculations were done with the program SHELX97.[10] All the geometric calculations were performed with the program PARST,[12] and scattering factors and anomalous dispersions were taken from the *International Tables for X-ray Crystallography*.[13,14]

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(4) There is an error in the formula of **4** depicted in ref 3, p 84 (where it is described with the number **6**); the C-15 asymmetric center shows a wrong 15*R* (*ent*-15*S*) absolute configuration, whereas it is correctly depicted as 15*S* (*ent*-15*R*) on p 86 (Figure 1, X-ray structure diagram).[3] Moreover, the semisystematic name for this compound (**4** in this work and **6** in ref 3) written in the text of ref 3 is in agreement with a 15*S* (*ent*-15*R*) absolute stereochemistry, although the prefix *ent* has been placed incorrectly after the descriptor 15*R* (personal communication from Prof. C. Subrahmanyam).
(5) Leucoplheol had mp 176−178 °C (Me_2CO−*n*-hexane), $[\alpha]^{19}_D$ +6.5° (*c* 0.25, EtOH).[1] Compound **4**: mp 220 °C (C_6H_6−$CHCl_3$), $[\alpha]^{30}_D$ −35° (*c* 0.2, MeOH).[3] For the ^{1}H and ^{13}C NMR spectral data differences between these compounds, see refs 1 and 3. For this work, we have measured the optical rotation of leucopheol in MeOH solution giving $[\alpha]^{28}_D$ +2.7° (*c* 0.183).
(6) It is of interest to indicate that the *normal*-pimarane absolute stereochemistry of leucopheol and leucopheoxol and their 15*R* absolute configuration were established by application of the Horeau's method (Horeau, A.; Nouaille, A. *Tetrahedron Lett.* **1971**, *12*, 1939−1942) to suitable derivatives possessing only one free hydroxyl group at the C-1 equatorial or C-15 position.[1] These results are also valid for structures **5** and **6**, possessing a *normal*-isopimarane absolute configuration and a 15*R* stereochemistry.

(7) The unambiguous assignment of the C-Me groups of **5** and **6** was firmly established from their HSQC and HMBC spectra (see Table 2).
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(14) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained (CCDC 159182), free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

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