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NMR CHEMICAL SHIFT CORRELATIONS IN 16, 17-DIHYDROXY-KAURANOIDS: IMPLICATION FOR STEREOCHEMICAL ASSIGNMENTS*

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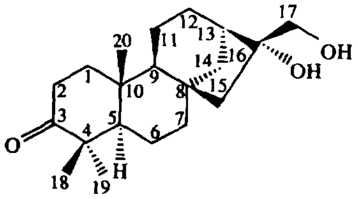
Key Word Index—*Callicarpa macrophylla*; Verbanaceae; 16,17- dihydroxykauranoids; NMR analysis; stereochemical assignments.

Abstract—The unambiguous ^1H and ^{13}C NMR assignments for 16α,17-dihydroxy-3-oxo-phyllocladane (calliterpenone) were achieved by the concerted application of homo- and heteronuclear correlation techniques. Comparison of these with the data for related *ent*-kauranoids established the characteristic NMR shielding patterns applicable for differentiation between the phyllocladane skeleton and the *ent*-kauranoid skeleton as well as for the assignment of the stereochemistry of the hydroxyl groups at C-16 and C-17. These studies also led to a revision of the stereochemistry of the hydroxy groups at C-16 and C-17 of a diterpenoid isolated from *Euphorbia sieboldiana*.

INTRODUCTION

Recently, we reported the characterization of a diterpenoid from *Callicarpa macrophylla*, the structure of which was deduced as 16α,17-isopropylidenocalliterpenone on the basis of its spectroscopic data and by a comparison of its spectral data with those of isopropylidene derivative derived from calliterpenone (1), a major diterpenoid constituent of the aerial parts of the plant [1]. It is worth mentioning that diterpenoid constituents so far characterized from this plant are oxy-substituted at C-3, C-16 and C-17 [2, 3]. The fact that the ^{13}C NMR spectral data for **1** could not be definitively assigned [1] by analogy with the reported values for abbeokutone (2), having an identical substitution pattern but an ent-kaurane skeleton [4], prompted us to carry out homo- and heteronuclear correlation studies on **1**. These studies led not only to the assignment of the ^1H and ^{13}C NMR frequencies of **1** but also led to a revision of the stereochemistry of a tetracyclic diterpenol isolated from *Euphorbia sieboldiana* [5] in view of the characteristic shielding pattern noticed for 16α,17β- and 16β,17α-dihydroxylated kauranoids.



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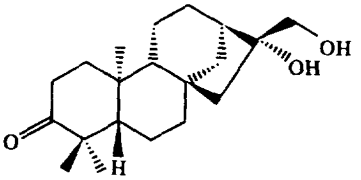

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RESULTS AND DISCUSSION

The ^1H NMR spectrum of **1** contained the signals for three tertiary methyl groups (δ0.84, 0.94, 1.07) and a AB pair of doublets ($J = 11.4$ Hz) at δ4.01 and 4.05 corresponding to a hydroxymethylene group (H_2-17). The proton-noise decoupled spectrum in conjunction with DEPT editing established the presence of 5 × C, 3 × CH, 9 × CH_2 and 3 × Me. The ^1H and ^{13}C NMR assignments were deduced by means of the combined use of ^1H –^1H COSY, ^1H–^1H NOESY and HETCOR spectroscopy in combination with standard 1D NMR techniques [6, 7]. The general strategy adopted for spectral analysis of **1** required the assignment of the isolated ^1H NMR resonances, followed by the tracing of the cross-peak connectivities in the ^1H–^1H COSY spectrum and then their correlation in the ^{13}C-dimension in the one-bond HETCOR spectrum to give the ^{13}C NMR assignments for the protonated carbon resonances. The HETCOR spectrum also led to the identification of the geminal proton partners in several cases, even though some were severely overlapped in the δ1.1–2.1 range (Table 1). The orienta-

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Table 1. ^{13}C and ^{1}H NMR spectral data for compounds **1** and **2** [4]

	1		2	
Atom	^{13}C	^{1}H	^{13}C	^{1}H
1	38.0	1.66, 1.18	39.2	1.34, 1.95
2	33.9	2.47, 2.39	33.9	2.44 × 2
3	216.2		218.2	—
4	46.4		47.1	—
5	55.1	1.21	54.2	1.38
6	21.3	1.10, 1.04	21.6	1.42, 1.49
7	40.9	1.70, 1.42	40.8	1.47, 1.63
8	43.9	—	44.3	—
9	55.8	0.85	55.3	1.08
10	37.0		38.5	—
11	19.8	1.26, 1.26	18.8	1.57, 1.57
12	26.5	1.92, 1.36	26.0	1.50, 1.60
13	44.3	2.31	45.2	2.04
14	48.5	2.47, 1.00	36.8	1.61, 1.87
15	44.9	2.15, 1.57	52.6	1.39, 1.52
16	84.1	—	81.7	—
17	65.5	4.01, 4.05	66.1	3.67, 3.77
18	26.7	1.07	27.2	1.06
19	21.3	0.94	20.9	1.01
20	14.3	0.84	17.7	1.06

tions of H-5, H-9, H-13, H_2-17 and H_3-20 were ascertained from the NOE connectivities observed in the ^{1}H–^{1}H NOESY spectrum and led to the confirmation of the structure of calliterpenone as 16α, 17-dihydroxy-3-oxo-phyllocladane (**1**).

Both **1** and **2** possess identical substitution at C-3, C-16, and C-17, i.e. C-3 oxo group and vicinal dihydroxyl groups at C-16α and C-17. However, they differ in the orientations of the H and Me at the ring junctions. A comparison of the ^{13}C NMR chemical shifts revealed that this skeletal difference was reflected in the chemical shifts for C-14, C-15, C-16, C-17 and C-20 and the chemical shift difference $\Delta\delta_1 - \delta_2 = -11.7, +7.7, -2.4, +0.6$ and $+3.45$ ppm for the above carbon resonances, respectively. The chemical shift of H_2-17 was also valuable in the differentiation of these skeletal types as these absorb at 0.28–0.34 ppm lower field in **1** as compared with **2**, in which they were found to absorb at δ 3.67 and 3.77 [4] as a pair of AB doublets (Table 1).

A survey of the literature for 16, 17-dihydroxy-*ent*-kauranoids [4, 5, 8–12] revealed that the chemical shifts for tertiary hydroxyl group-bearing C-16 and hydroxymethylene (C-17) positions are sensitive towards the relative orientations of these substituents at C-16. In general, C-16 and C-17 absorb between δ 79.7–79.8 (C) and 69.5–70.4 (CH_2) and between δ 81.6–81.8 (C) and 66.2–66.5 (CH_2) in *ent*-kauranoids with 16β,17α- and 16α,17β-diol groups respectively. This characteristic ^{13}C NMR shielding behaviour led us to revise the stereochemistry of the diterpenol isolated from *Euphorbia sieboldiana* [5]. Thus, the C-16 and C-17 signals for this compound were found to appear at δ 79.7 and 69.7 reflecting the β-orientation of the tertiary hydroxyl group at C-16. This meant that the diterpenol is ent-kaurane-3-oxo-16β,17-diol and not ent-kaurane-3-oxo-16α,17-diol [5], a compound which had been already reported in the literature as abbeokutone (**2**) [4]. Moreover, the appearance of H_2-17 at δ 3.39 and 3.42 [5] as an AB pair of doublets is in complete accordance with the revised stereochemical assignment [8, 10, 13].

EXPERIMENTAL

General. NMR: GE (QE-300) or Varian (VXR-300) NMR spectrometer, $CDCl_3$ soln, under the experimental conditions as reported elsewhere [14].

Plant material and isolation. Leaves of *Callicarpa macrophylla* were collected from Pantnagar, India, and were authenticated by the Botanical survey of India, Calcutta. Calliterpenone (**1**) was available from our earlier studies [1].

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