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NOTE

Complete ^{1}H and ^{13}C NMR Assignment of a Kaurane Diterpene from *Piliostigma thonningii*

M.-T. Martin,[1] M. Païs,[1]* A. O. Ogundaini,[2] J. C. Ibewuilke[2] and F. O. Ogungbamila[2]

[1] Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette Cedex, France

[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

The diterpene *ent*-16α-hydroxykauran-18-oic acid was isolated from the leaves of *Piliostigma thonningii*. Structural elucidation and complete ^{1}H and ^{13}C NMR chemical shift assignments of this compound were achieved by 2D experiments. © **1997 John Wiley & Sons, Ltd.**

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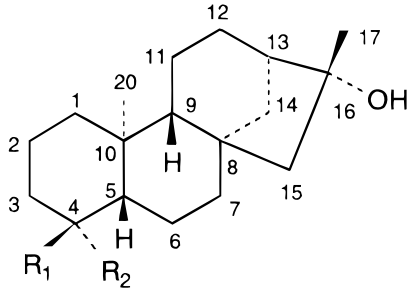
INTRODUCTION

Our previous studies on the leaves of *Piliostigma thonningii* (Schum.) Milne–Redhead (Caesalpiniaceae) had resulted in the isolation of several flavonoids.[1] In continuation of the investigation of the chemical content of leaf extracts, we have further isolated a new kaurane diterpenoid *ent*-16α-hydroxykauran-18-oic acid (**1**). To our knowledge, there has been no publication describing systematic investigations by 2D NMR of kaurane diterpenes. Such studies allow us to establish unambiguously the structure of **1**. Complete ^{1}H and ^{13}C NMR chemical shift assignments of **1** were achieved by 2D experiments and the results are reported here.

RESULTS AND DISCUSSION

Compound **1**, $[\alpha]_D$-36°, showed an $[MH]^+$ peak at *m/z* 321.2424 in the high-resolution chemical ionization mass spectrum ($\Delta - 0.6$ mu) corresponding to the molecular formula $C_{20}H_{32}O_3$, together with an intense peak at *m/z* 303.2317, $[MH - H_2O]^+$ ($\Delta - 0.7$ mu).



1 R_1 = COOH, R_2 = Me-19

2 R_1 = Me-18, R_2 = COOH

The ^{1}H NMR spectrum exhibited three tertiary methyls at δ 1.20, 1.54 and 1.69 and a series of resolved and unresolved multiplets extending from δ 1.00–2.28. The ^{13}C NMR spectrum aided by the HMQC experiment (Table 1) revealed the presence of nine methylenes, three methines, three methyls and five quaternary carbons. From chemical shift considerations, one of the quaternary carbon was linked to an oxygen (δ 78.0) and another belongs to a carbonyl group (δ 181.5). Analysis of the ^{1}H–^{1}H COSY and HMQC spectra verified three spin coupled networks, together with an isolated methylene, which could correspond to the protons of the methylenes or methines at C-1—C-2—C-3, C-5—C-6—C-7, C-9—C-11—C-12—C-13—C-14, and of the isolated 15-CH_2 respectively, belonging to an *ent*-16-hydroxykaurane skeleton. Furthermore, the ^{13}C NMR chemical shift values were close to those of the known *ent*-16α-hydroxykauran-19-oic acid (**2**)[2] except for the methyl attached to C-4 which resonated at δ_C 17.2 instead of δ_C 28.8, thus indicating an α-axial position (19-Me).[3] The HMBC correlations (Table 1), especially the key correlations observed from 17-Me, 19-Me and 20-Me to their neighbouring carbons and from H-5 to C-4, C-6, C-10 and C-19, confirmed all ^{1}H and ^{13}C assignments.

The NOESY spectrum (Table 1) exhibited the cross peaks H-5–H-9 and H-9–H-15β in accordance with the usual stereochemistry of the *ent*-kaurane skeleton. The 20-Me–19-Me correlation supported the α-axial orientation of 19-Me. The 16α-hydroxy stereochemistry had been established previously for **2** by chemical correlations involving an oxidation of a kaur-16,17-ene, which should take place most probably at the loss crowded α face of the molecule.[4,5] The NOESY correlation observed for **1** between H-15β and 17-Me (Fig. 1) proved unambiguously the 16α-hydroxy stereochemistry. Thus compound **1** is *ent*-16α-hydroxykauran-18-oic acid.

* *Correspondence to*: M. Païs.

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Table 1. ^{1}H and ^{13}C NMR Data of 16α-hydroxykauran-18-oic acid (pyridine-d_5)

	^{13}C[a]	^{1}H (J Hz)[b]	HMBC[c]	NOESY[b]
1	40.1	α 1.90 bd (12.8)	2, 9, 10	1β, 11, 20
		β 1.02 ddd (13.5, 12.8, 3.2)	2	3β, 5, 9
2	18.4	α 1.82 m		2β, 19, 20
		β 1.62 m	10	3α
3	37.7	α 1.87 m	5	3β, 19
		β 2.25 ddd (13.6, 12.7, 4.8)	2, 4, 18, 19	5, 9
4	47.9			
5	51.0	2.16 bd (12.0)	4, 6, 7, 10, 19, 20	6β, 7β, 9
6	24.0	α 1.70 m		6β, 19, 20
		β 1.62 dt (14.4)		
7	42.3	α 1.75 m	5	7β, 14a
		β 1.65 m	5	9
8	45.9			
9	57.6	1.24 d (6.4)	10, 11, 12, 20	12α, 15β
10	39.1			
11	18.4	α 1.70 m	12	11β
		β 1.67 m	12	
12	27.4	α 1.67 m		12β, 13, 14a
		β 1.75 m		13
13	49.6	2.30 bs		14a, 14b
14	38.2	a 2.16 d (12.0)	8, 12, 13	14b
		b 2.10 d (12.0)	8, 12, 13, 15, 16	20
15	58.9	α 2.05 d (14.3)	7, 8, 9, 13, 16	15β
		β 1.78 d (14.3)	8, 9, 14, 17	17
16	78.0			
17	25.2	1.69 s	13, 15, 16	
18	181.5			
19	17.2	1.54 s	3, 4, 5, 18, 20	20
20	18.4	1.20 s	5, 9, 10, 19	

[a] 62.5 MHz.
[b] 600.13 MHz.
[c] 400.13 MHz.

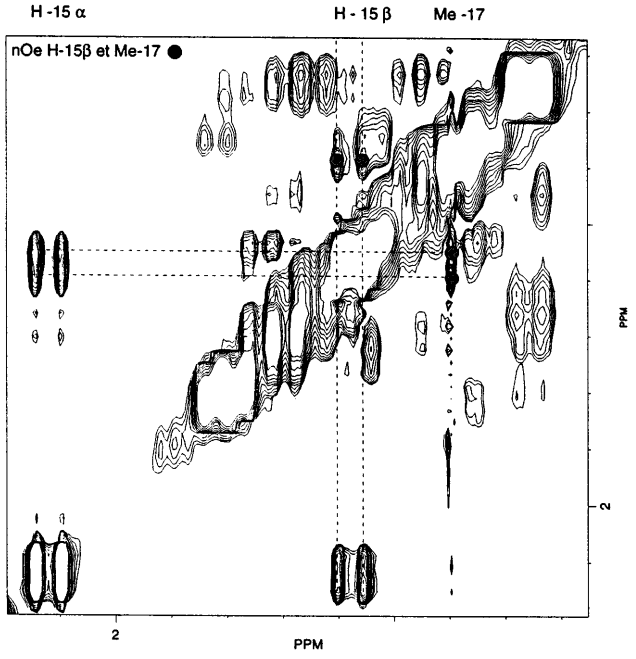

Figure 1. Partial NOESY spectrum of *ent*-16α-hydroxykauran-18-oic acid (**1**).

EXPERIMENTAL

Spectra were recorded on Bruker AC-250, AM-400 or AMX 600 spectrometers at 300 K, the last two spectrometers using a triple-resonance probe head with self-shielded gradient coils and a Bruker Z-gradient accessory delivering squared gradients. The ^{1}H and ^{13}C chemical shifts are expressed in ppm relative to TMS, but were measured against the middle solvent peak (pyridine-d_5) set at 7.58 and 135.91 ppm, respectively. The ^{1}H spectrum was recorded at 600.13 MHz and collected as a 16 K data set over a spectral width of 3.2 kHz using a 30-pulse sequence. The ^{13}C BB spectrum was recorded at 62.5 MHz and collected as a 16 K data set over a spectral width of 10.4 kHz using a 30-pulse sequence and processed using exponential multiplication with a 2 Hz line broadening.

The homonuclear ^{1}H–^{1}H shift correlated 2D NMR spectrum was obtained using the COSY program from the Bruker software library and was recorded at 400.13 MHz. The spectral widths were $F_2 = 680$ and $F_1 = 340$ Hz. The spectrum was collected as 1024 × 128 blocks of

data and was processed by sinusoidal multiplication in each dimension followed by symmetrization of the final data matrix, after zero filling in the F_1 dimension. The homonuclear dipolar correlated 2D NMR NOESY spectrum, in the phase-sensitive mode using TPPI, was obtained using the NOESYPH program from the Bruker software library and was recorded at 600.13 MHz. The spectral widths were $F_2 = 3268$ and $F_1 = 1634$ Hz. The spectrum was collected as 4096×512 blocks of data. A squared sine-bell window multiplication shifted by $\pi/3$ was used in the F_2 dimension and a sine-bell window multiplication shifted by $\pi/2$ in the F_1 dimension. Other parameters were as follows: number of increments in t_1, 512; scans, 96; phase cycling, 16 steps; relaxation delay, 1 s; and mixing time 0.6 s.

Two-dimensional heteronuclear shift correlation spectra were recorded in the inverse mode at 400.13 MHz. The one-bond 2D XH correlation experiment via heteronuclear zero- and double-quantum coherence (HMQC) used the sequence D_1–90(^{1}H)–D_2–90(^{13}C)–D_0–t_g*2–t_m–180(^{1}H)–t_g*2–t_m–D_0–90(^{13}C)–t_g–D_2–t_2 and GARP decoupling during acquisition;[6,7] relaxation delay $D_1 = 1$ s, evolution delay $D_2 = 3.70$ ms and gradient on time $t_g = 400$, after a gradient recovery time $t_m = 300$ μs. The F_2 spectral width was 680 Hz over 2 K real data using 64 transients per t_1 increment and the F_1 spectral width was 5.8 kHz over 256 t_1 increments (zero-filled to 1 K). A squared sine-bell window multiplication shifted by $\pi/3$ was applied in each dimension.

The long-range 2D heteronuclear shift correlations, via heteronuclear zero- and double-quantum coherences with a low-pass *J*-filter to suppress one-bond correlations (HMBC), were obtained using the following sequence: D_1–90(^{1}H)–D_2–90(^{13}C)–D_4–90(^{13}C)–D_0–t_g*2–t_m–180(^{1}H)–t_g*2–t_m–D_0–90(^{13}C)–t_g–t_m–t_2.[6,8] Delays D_1 and D_2 and times t_g and t_m were the same as in the previous HMQC experiment while the evolution delay D_4 for CH long-range coupling was adjusted to 70 ms. Typically, 2048 data points were acquired using a 680 Hz spectral width for the proton dimension and 256 time increments for a 9.05 kHz spectral width for carbons. The data were then zero-filled to give a final $2K \times 1K$ data matrix. A squared sine-bell window multiplication shifted by $\pi/3$ was applied in each dimension.

High-resolution chemical ionization mass spectrometry (HR-CI-MS) of **1** was performed on a Kratos MS 80 mass spectrometer.

Plant material

See Ref. 1.

Isolation of *ent*-16α-hydroxykauran-18-oic acid (1)

Extraction and preliminary purification have been reported previously.[1] Successive column chromatography of fraction IVb (1.2 g) on Sephadex LH-20 with toluene–ethanol (7:3) and accelerated gradient chromatography using an increasing gradient of chloroform in toluene (0–100%) followed by gradient of ethyl acetate in chloroform (0–100%) yielded **1** (10 mg).

***ent*-16α-Hydroxykauran-18-oic acid (1).** Amorphous solid, $[\alpha]_D$-36° [*c*. 0.4, $CHCl_3$–MeOH (4:1)]. IR (KBr), ν_{max} (cm^{-1}), 3383 (OH), 1692 (C=O); HR-CI-MS, *m/z* 321.2424 ($C_{20}H_{32}O_3$, Δ-0.6 mu), 303.2317 ($C_{20}H_{30}O_2$, Δ-0.7 mu). For ^{1}H and ^{13}C NMR data, see Table 1.

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