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Constitution, Stereochemistry and Conformational Behaviour of the Photoreaction Product of 7-Deacetoxy-7-oxokhivorin†

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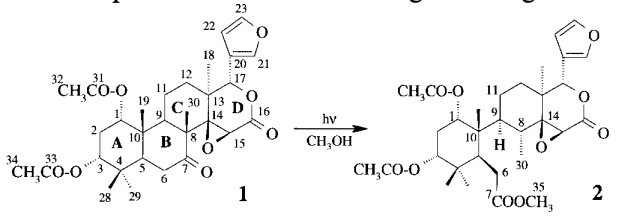
ABSTRACT: By means of 2D NMR experiments (COSY, HMQC, HMBC, ROESY), the constitution of the photoreaction product 3-deoxo-1α,3 α-diacetoxy-1,2,3,3,8,30-hexahydro-18β*H*-andirobin (**2**), obtained by photolysis of 7-deacetoxy-7-oxokhivorin (**1**) in methanol, was shown to be a ring B seco-limonoid with the 14,15-epoxide intact. The configuration of C-8 is *R*. Ring C adopts a twist-boat conformation. No conformational preferences for the rotation of ring A in **2** with respect to rings C/D and for the side-chain C-6/C-7 could be detected. Nearly all ^{1}H and all ^{13}C resonances were assigned unambiguously. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: limonoids; NMR; ^{1}H NMR; ^{13}C NMR; gs-COSY; gs-HMQC; gs-HMBC; ROESY; ^{1}H-detected 1D gradient-selected long-range ^{13}C, ^{1}H correlation; conformational analysis; boat conformation

INTRODUCTION

In an earlier report[1] we showed that the photolysis of 7-deacetoxy-7-oxokhivorin (**1**) (Scheme 1) in methanol gave the ring B seco-limonoid methyl ester 3-deoxo-1α,3 α-diacetoxy-1,2,3,3,8,30-hexahydro-18β*H*-andirobin (**2**), the structure of which was similar to that of andirobin.[2] The reaction was therefore different from a similar photoreaction product of limonin which gave a ring B seco-aldehyde along with the C-8 epimer of limonin.[3] Doubts were raised about the constitution of the ester **2** because it failed to give the 14,15-deoxy derivative on reaction with chromium(II) chloride[4] as is usual with gedunin,[4] khivorin,[4] andirobin[2] and related compounds. It also failed to react with the zinc–copper couple and zinc in acetic acid, unlike similar compounds.[5] The reaction of **2** with boron trifluoride and hydriodic acid failed to give the expected rearrangement products[6] through opening of the 14,15-epoxide ring. The presence of the 14,15-epoxide ring in the photoreaction product was therefore not certain and rested only on microanalytical results and low-resolution NMR spectra of **2** and its derivatives. The stereochemistry at C-8 and the conformation of **2** also could not be verified, and therefore even the constitution remained tentative.[1] In this paper, we report NMR experiments with both the starting material **1** and the photo-product **2** which enabled us to determine the complete structure of **2** unequivocally. In addition and for comparison, we assigned all ^{1}H and ^{13}C signals of **1**.



Scheme 1. Structures of **1** and **2**

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† This paper is dedicated to the 65th birthday of Professor Donald E. U. Ekong, BSc (London), Dr rer. nat. (Heidelberg), FAS (Nigeria), FAAS, FTWAS, former Secretary General, Association of African Universities, who about 25 years ago as Head of the University of Ibadan, F103 Natural Products Organic Chemistry Research Group, first posed the problem solved in this work.

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EXPERIMENTAL

Spectroscopy

The IR spectrum was measured in KBr on a Bruker IFS-25 instrument.

NMR spectra were recorded in pyridine-d_5 at room temperature using a Bruker Avance DRX-500 or

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DRX-600 spectrometer. Chemical shifts are given on the δ-scale and were referenced to the solvent (C-β, $\delta = 123.4$ and H-β, $\delta = 7.17$). In the 1D measurements (^{1}H, ^{13}C, DEPT), 64K data points were used for the FID.

The pulse programs of the following 2D experiments were taken from the Bruker software library and the parameters were as follows:

Gradient-selected HMQC[7] spectra: relaxation delay $D_1 = 2.0$ s; evolution delay $D_2 = 3.45$ ms; 90° pulse, 11.5 μs for ^{1}H, 10.0 μs for ^{13}C hard pulses and 65.0 μs for ^{13}C GARP decoupling; 1K points in t_2; spectral width 8 ppm in F_2 and 130 ppm in F_1; 256 experiments in t_1; linear prediction to 512 and zero-filling up to 1K.

Gradient-selected HMBC[8] spectra: relaxation delay $D_1 = 1.5$ s; evolution delay $D_2 = 3.45$ ms; delay for evolution of long-range coupling $D_6 = 70$ ms ($J = 7$ Hz); 1K points in t_2; spectral width 8 ppm in F_2 and 180 ppm in F_1; 256 experiments in t_1, linear prediction to 512 and zero-filling up to 1K.

ROESY[9] spectra: relaxation delay $D_1 = 2.0$ s; 90° pulse for ^{1}H; spin lock 300 ms; 2K points in t_2; spectral width 8 ppm in both dimensions; 512 experiments in t_1, linear prediction to 2K.

TOCSY[10] spectra: relaxation delay $D_1 = 1.4$ s; 90° pulse for ^{1}H; 90° pulse for MLEV; TRIM pulse 2.5 ms; mixing time 80 ms; 1K points in t_2; spectral width 8 ppm in both dimensions; 256 experiments in t_1, linear prediction to 512 points, zero filling up to 1K.

Gradient-selected ^{1}H,^{1}H COSY[10] spectra: relaxation delay $D_1 = 1.0$ s; 90° pulse for ^{1}H; 2K points in t_2; spectral width 8 ppm in both dimensions; 256 experiments in t_1, linear prediction to 512 points, zero filling up to 2K.

500/125 MHz HMQC-TOCSY[11] spectra: relaxation delay $D_1 = 1.5$ s, evolution delay $D_2 = 3.45$ ms, 90° pulse for ^{1}H and ^{13}C; 90° pulse for ^{13}C GARP decoupling; 90° pulse for MLEV; TRIM pulse 80 μs; 1K points in t_2; spectral width 8 ppm in F_2 and 130 ppm in F_1; 512 experiments in t_1, linear prediction to 1K.

The selective 1D long-range ^{13}C–^{1}H correlation measurement was performed applying a Bruker standard program optimized for a 7.5 Hz ^{13}C, ^{1}H coupling constant.

The mass spectrum (EI, 70 eV) was measured on a VG Autospec instrument.

Isolation of 7-deacetoxy-7-oxokhivorin (1)

7-Deacetoxy-7-oxokhivorin was isolated from an extract of *Khaya senegalensis* as reported earlier.[12]

Photolysis of 7-deacetoxy-7-oxokhivorin (1)[1,13]

7-Deacetoxy-7-oxokhivorin (**1**, 0.5 g) was dissolved in 500 ml of dry methanol in a Pyrex flask clamped 2–3 cm over a medium-pressure Hg lamp and irradiated for 10 h at reflux. On concentrating the solution, the product gave crystals of 3-deoxo-1α,3 α-diacetoxy-1,2,3, 3,8,30-hexahydro-18βH-andirobin (**2**), recrystallized from methanol to produce needles (0.2 g), m.p. 240 °C (lit.:[1] 227–230 °C). IR: ν(KBr) 3434 (MeOH from recrystallization), 3100, 1503, 874 (furan), 1735 (C=O), 1260 (C–O–C) cm^{-1}. UV (CH_3CN): 209 cm^{-1}. CD (CH_3CN): 233 nm ($\Delta\varepsilon = -45.6$). EI–MS, m/z (relative intensity, %): 574 (M^+, 5), 463 (7), 451 (17), 391 (7), 331 (27), 313 (13), 299 (52), 253 (18), 211 (82), 195 (42), 133 (100), 121 (49), 95 (49).

RESULTS AND DISCUSSION

Table 1 gives the ^{1}H and ^{13}C chemical shifts and HMBC and ROESY correlations of **1** and **2**.

Signal and structural assignments

^{1}H and ^{13}C signal assignments were achieved by extensive application of various 1D and 2D NMR methods, such as gs-COSY, gs-HMQC, gs-HMBC and ROESY at 500.1 and 125.7 MHz (**1**) and 600 and 150.9 MHz, respectively (**2**). The general data evaluation procedure has been described in earlier papers[14,15] so we restrict the discussion here to some peculiarities.

With only one exception (see below), all signals of **1** and **2** could be assigned unequivocally, even those of the two acetyl groups in both instances. For example, one of the two acetyl methyl signals of **2** shows a ROESY cross peak with H-9, proving that it belongs to the substituent at C-1 (see Fig. 1). Correlations were possible within each of the acetyl groups, but there were no HMBC cross peaks linking the C(=O)CH_3 atoms to the C-1/H-1 and C-3/H-3 fragments. No ROESY signals were detectable for the two methyl proton signals for contact between each other. Apparently, the two acetoxy groups strongly prefer conformations in which they are extended outwards, escaping the severe

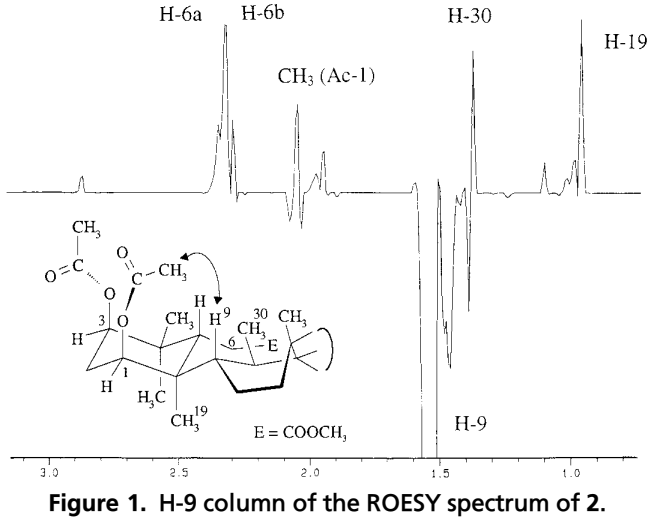


Figure 1. H-9 column of the ROESY spectrum of **2**.

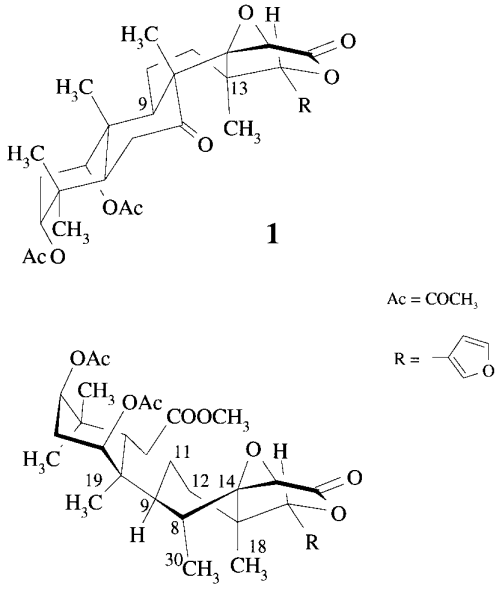
Table 1. 1H and ^{13}C chemical shifts and HMBC and ROESY correlations of 1 and 2[a]

Compound	Atom	^{1}H	J(Hz)	ROESY	^{13}C	HMBC correlations (^{13}C partners)
1	1	4.79 t	3.0	2α; 2β; 11α; 19	72.4	3; 5; 10; 31
	2α	2.03 dt	partial overlap	1; 2β; 3	25.9	1; 3; 4; 10
	2β	2.28 dt	16.7; 3.0	1; 2α; 3; 19; 28		
	3	4.76 t	3.0	2α; 2β; 28; 29	75.7	1; 5; 33
	4				37.3	4; 7; 10; 19; 28
	5	2.35 m	2nd-order spin system	9; 29	45.8	
	6α	2.38 m	2nd-order spin system	6β; 29	35.7	4; 8; 10
	6β	2.76 t	15.1	5; 6α; 19; 28; 30		4; 5; 7; 10
	7				209.3	
	8				53.3	
	9	2.54 d	11.0	5; 11α; 18	44.4	5; 7; 8; 10; 11; 12; 19; 30
	10				40.6	
	11α	1.50 br m	2nd-order spin system	1; 9; 12α	16.2	8; 9; 12; 13
	11β	1.57 m	2nd-order spin system	19; 30		
	12α	1.18	partial overlap	11β; 12β	32.2	18
	12β	1.78 dd	13.7; 7.5	12α; 17		9; 11; 13; 18
	13				37.5	
	14				65.4	
	15	3.79 s		30	53.4	14; 16
	16				167.1	
	17	5.45 s		12β; 21; 22	78.1	12; 13; 14; 18; 20; 21; 22
	18	1.17 s		9; Ac-1	21.0	12; 13; 14; 17
	19	1.17 s		1; 2β; 6β; 11α; 11β; 28	17.1	1; 5; 9; 10
	20				120.3	
	21	7.40 t	1.7		143.1	20; 22; 23
	22	6.34 dd	1.7; 0.8	23	109.7	20; 21; 23
	23	7.41 dd	1.7; 0.8	22	141.0	20; 21
	28	0.99 s		2β; 3; 6β; 19; 29	20.9	3; 4; 5; 29
	29	0.90 s		3; 5; 6α; 28	27.0	3; 4; 5; 28
	30	1.15 s		6β; 15	16.9	7; 8; 9; 14
	Ac-1	1.96 s		18	169.4	
					21.1	
	Ac-3	2.02 s			170.1	
					21.1	
2	1	4.76 t	3.0	8, 9, 19; 30; Ac-1; Ac-3	73.3	
	2α	2.14 dt	16.4; 3.0	2β	26.3	
	2β	2.02 dt	16.4; 3.3	19; 28		
	3	4.75 t	3.0	28; 29; Ac-1; Ac-3	75.4	
	4				38.3	
	5	2.88 dd	7.9; 2.7	9; 6α; 6β; 11β; 28; 29; Ac-1	38.4	
	6α	2.36 dd	14.8; 7.9	9; 19; 28; 29	30.0	4; 5; 7
	6β	2.31 dd	14.8; 2.7			5
	7				174.6	
	8	1.47 quint	7.2	19; 30	36.7	9; 10; 13; 14; 30
	9	1.54 ddd	12.2; 6.4; 3.5	6α; 6β; 12α; 19; 30; Ac-1	53.2	
	10				43.1	
	11α	2.05 m	9; 11β; 12β			
	11β	1.96 dddd	14.0; 12.0; 12.0; 3.0	9; 11α; 12α;	21.8	
	12α	0.99 ddd	14.0; 13.8; 4.0	9; 12β; 18		
	12β	1.87 dt	14.5; 3.8	9; 12α	32.8	
	13				37.3	
	14				70.5	
	15	3.43 s		8; 18; 30	53.8	14; 16
	16				167.7	
	17	5.44 s			77.6	13; 14; 18; 20; 21; 22
	18	1.10 s			20.0	12; 13; 14; 17
	19	0.96 s			21.4	1; 5; 9; 10
	20				120.1	
	21	7.40 dd	1.7; 0.8		141.3	22; 23
	22	6.39 dd	1.8; 0.8		110.4	20; 21; 22; 23
	23	7.39 t	1.7		143.0	21; 22
	28	1.05 s		19; 29	23.1	3; 4; 5; 29
	29	0.86 s			28.0	3; 4; 5
	30	1.38 d	7.3	18; 19	25.1	8; 9; 14
	Ac-1	2.04 s			21.2	
					170.2	CO of Ac-1
	Ac-3	2.11 s			22.0	
					170.1	CO of Ac-3
	OMe	3.76 s		29	51.9	

[a] In $CDCl_3$; for referencing and recording conditions, see Experimental section. Abbreviations: s = singlet, bs = broad singlet, d = doublet; t = triplet; quint = quintet, m = multiplet.

steric crowding in the molecular skeleton. This explains the absence of HMBC cross peaks because the molecular fragments (O=)C—O—C-1—H-1 and (O=)C—O—C-3—H-3 are expected to be far from coplanar and the three-bond couplings therefore have to be small. A safe stereochemical assignment of the two H-6a and H-6b signals was not possible, however, although they displayed distinctly different vicinal couplings to H-5 [^3J(H-5,H-6a) = 7.9 Hz and ^3J(H-5,H-6b) = 2.7 Hz]. This has to be ascribed to the mobility of the $CH_2CO_2CH_3$ side-chain as revealed by the ROESY peaks (see below). There are at least two conceivable conformations which agree with the observed coupling constants but the resulting assignments of the two H-6 signals are opposite.

The existence of the 14,15-epoxide ring is proved by the magnitude of ^1J(C-15,H-15) = 190 Hz, which was detected by a ^{13}C-coupled HMQC (see Fig. 2). This value is characteristic of a three-membered oxirane ring.[16] Figure 2 contains two more cross peak pairs for comparison: C-9/H-9 and OCH_3).



Scheme 2. Stereoprojections of **1** and **2**.

Ring conformations and configurations at C-8 and C-9

The most crucial points in the structure of **2** are the conformations of the rings A and C and the configurations at C-8 and C-9.

In the starting material **1**, ring C is forced into a twist-boat conformation. This is similar in **2**; however, ring C adopts another twist-boat conformation. Whereas the two bowsprit carbon atoms in **1** are C-9 and C-13 (directed towards the α-side), the corresponding atoms in **2** are C-11 and C-14 directed towards the β-side (Scheme 2). This is evident from the signal of the boat-axial H-11 (H-11β), which appears as a double quartet showing coupling constants of *ca.* 14, 12, 12 and 3 Hz. These correspond to one geminal and three vicinal couplings, and their magnitudes show that H-11β is in a boat-axial position and has two antiperiplanar hydrogen neighbours. This is possible only in the boat conformation mentioned. Similar couplings would be observed in a ring C chair conformation, but this would imply an axial position of ring A at C-9, which is impossible for steric reasons (cf. conformational behaviour of *tert*-butyl substituents). Our conclusion is supported by a significant ROESY peak proving the spatial proximity of H-9 and H-12 in boat-axial positions (H-12α) (cf. Table 1).

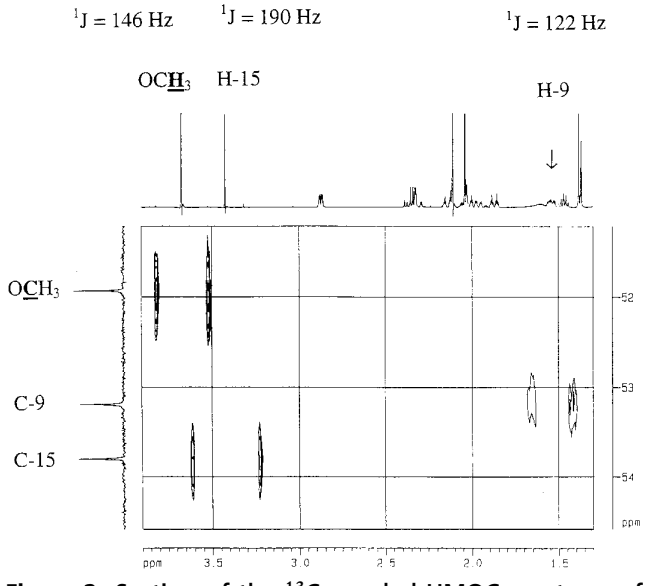


Figure 2. Section of the ^{13}C-coupled HMQC spectrum of **2**. One-bond ^{13}C,^{1}H coupling constants are $\pm$4 Hz.

The C-8 configuration is *R*, i.e. the methyl group 30 is in a boat-axial position as evidenced by the ROESY peaks H-18/H-30 and H-19/H-30. Hence a configurational inversion at C-8 has taken place during the ring B opening. Apparently, C-30 flips down to the α-side of the molecule after the irradiation-induced homolytic cleavage of the C-7—C-8 bond, and a hydrogen atom moves from C-6 to C-8 (Norrish type I reaction). The resulting C-6/C-7 ketene is attacked by a methanol molecule to give the methyl ester **2**.

Ring A adopts a chair conformation with the two acetoxy groups at C-1 and C-3 in axial positions. This is evident from the small couplings (3 Hz) between H-1 and H-3 and the two H-2 atoms, proving that of H-1 and H-3 are equatorial.

Further evidence for the structural assignments arises from ^{1}H-detected 1D gradient-selected long-range ^{13}C,^{1}H correlation experiments under decoupling of C-19 [Fig. 3(a) and (b)] and of C-9 [Fig. 3(c)–(e)][17] displaying the following vicinal ^{13}C,^{1}H coupling constants: C-19/H-1, 5 Hz (*gauche*); C-19/H-5, 8.5 Hz (antiperiplanar); C-9/H-12β, 9 Hz (antiperiplanar); C-9/H-30, 4.5 Hz (averaged value for rotating H-30 atoms); C-9/H-19, 4.5 Hz (averaged value for rotating H-19 atoms).

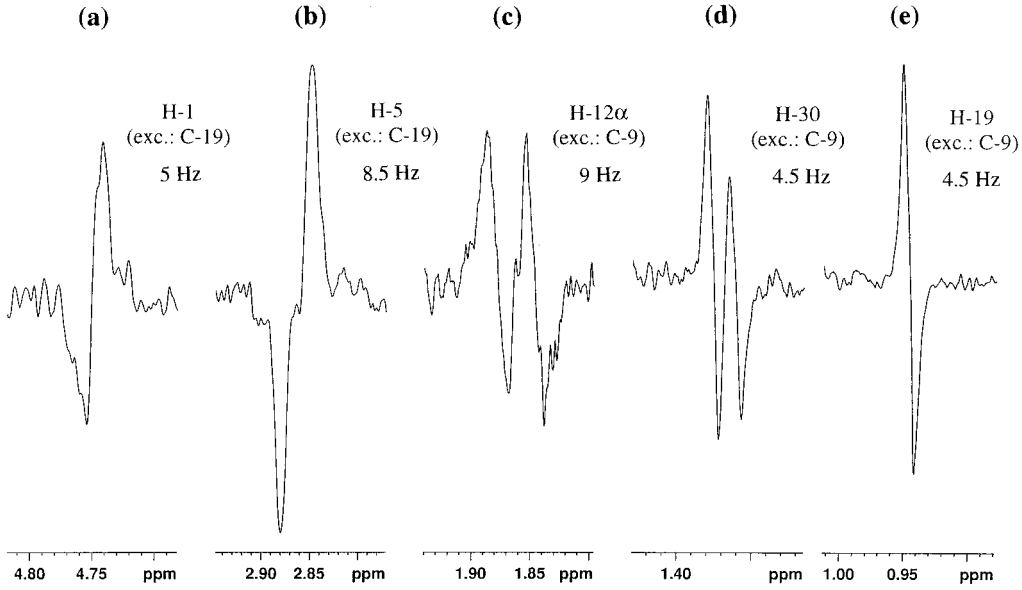


Figure 3. ^{1}H-detected 1D gradient-selected long-range ^{13}C,^{1}H correlation experiments of **2** under decoupling of C-19 and of C-9.

Conformational behaviour

Finally, the question had to be answered of whether there is a perferred conformation in **2** with respect to the rotation about the C-9—C-10 bond. The observation of ROESY cross peaks connecting the 19-methyl protons with H-6a, H-8, H-9 and H-30 (Table 1) clearly shows that there are more than one preferred conformation. This excludes a safe stereochemical assignment of the diastereotopic H-6a and H-6b signals although their vicinal ^{1}H,^{1}H coupling constants (see above) and their ROESY cross peak patterns differ.

The presumed conformational preferences of the two acetoxy groups have been mentioned above.

CONCLUSION

Application of extensive 1D- and 2D-NMR techniques resulted in the complete signal and structure assignment of the flexible photoreaction product **2**, including some evidence about its dynamic behaviour. This allowed us to rationalize the mechanism of its photo-induced rearrangement.

Acknowledgements

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