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The Structure of Americanin A

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Abstract—The structure of the neolignan americanin A was confirmed to be 2, through the application of the Selective INEPT NMR technique. Complete and unambiguous proton and ¹³C-NMR assignments are provided.

Keywords-Benzodioxane lignan · americanin A · 2D-NMR

Americanin A is a neolignan obtained from the roots of *Phytolacca americana* L. (Phytolaccaceae) whose structure was proposed to be 1, based on spectroscopic evidence. 1) In 1981, this structure was revised to 2. because americanin A provided the starting material for the partial synthesis of a derivative of the flavonolignan silandrin²⁾, and very recently³⁾, during the course of these studies, supporting chemical evidence for this structure revision has been presented. The interest of one of our groups (Woo et al.) in the constituents of P. americana led to this collaboration to determine spectroscopically whether 1 or 2 is the correct structure of americanin A. The strategy used to establish the structure was basically the same as that described for the structure elucidation of coumarinolignans4,5), since both moieties contain the 1,4-benzodioxane nucleus. For solubility reasons all studies were conducted on the triacetate derivative of americanin A.

The homonuclear COSY spectrum of americanin A triacetate (Fig. 1) revealed that the doublet of doublets at 7.14 ppm (H-6) is ortho coupled to the doublet at 6.99 ppm (H-5) with a coupling constant of 8.4 Hz, and is also meta coupled to the doublet at 7.21 ppm (H-2) with

R 2 H 3 COCH₃

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a coupling constant of 1.9 Hz. The signals for H-2 and H-6 were also weakly coupled to H-7, indicating that these protons were the 1,4-benzodioxane aromatic ring protons. The three-proton multiplet centered at 7.28 ppm, showing coupling with H-7', must therefore be H-2', H-5' and H-6' which are on the aromatic substituent attached to C-7'. The complete ¹H-NMR assignments of americanin A triacetate are shown in Table I.

In order to determine the correct regiostructure for this compound a series of selective INEPT experiments⁶⁾ was carried out. The premise of these experiments is that in isomer 2 irradiation of H-7' would result in the enhancement of the C-4 signal, and irradiation of H-8' would result in the enhancement of the C-3 signal. On the other hand in isomer 1, irradiation of H-7' would result in the enhancement of the C-3 signal and irradiation of H-8' would result in the enhancement of the C-4 signal. There-

Table I. ¹H-NMR spectral assignments for americanin A triacetate (3) (at 360 MHz in CDCl₂)

			·			
Proton M	ssignment, Iultiplicity		Coupled Protons, (coupling constant, Hz)			
2	7. 21,	d	H-6(1.9), H-5(<1), H-7(<1)			
5	6. 99,	d	H-6(8.4), H-2(<1)			
6	7. 14,	dd	H-5(8.4), $H-2(1.9)$, $H-7(<1)$			
7	7.38,	d	H-8(15.8), H-2,6(<1)			
8	6.60,	dd	H-7(15.8), H-9(7.7)			
9	9.66,	d	H-8(7.7)			
5' 6'	7. 28,	m	H-7'(<1)			
7 '	5.03,	d	H-8'(7.9), H-2',6'(<1)			
8′	4. 24,	td	H-7'(7.9), H-9'X(4.2), H-9'A(3.5)			
9' A	4. 41,	dd	H-9'X(12.4), H-8'(3.5)			
9′X	4.01,	dd	H-9'A(12.4), H-8'(4.2)			
OCOCH3	2. 31,	s				
OCOCH ₃	2. 31,	s				
CH₂OCOCH2	2.07,	3				

fore, unambiguous assignment of C-3 and C-4 is the most critical issue. Fig. 2 shows the polarization transfers induced through the irradiation of several aromatic and aliphatic protons on the compound. Irradiation of H-5 using 8 Hz for calculation of the delay resulted in the enhancement of four signals. The most intense signal at 142.7 ppm was assigned to C-3, which is three-bond coupled to H-5, and the next most intense signal at 127.9 ppm was assigned to C-1, which is also three-bond coupled to H-5. Two less intense signals at 145.7 and 122.6 ppm were assigned to C-4 and C-6, respectively. They were enhanced due to two bond coupling to H-5 with smaller coupling constants.

Irradiation of H-6 using the same delay time as for irradiation of H-5 resulted in the enhancement of five signals. The most intense

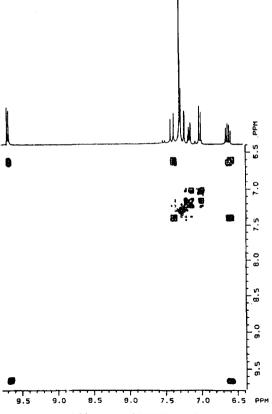


Fig. 1. Downfield region of ¹H-¹H correlation spectrum for americanin A triacetate(3) in CDCl₃.

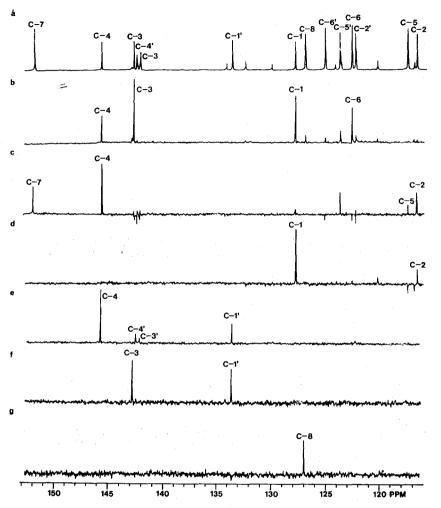


Fig. 2. Downfield region of the ¹³C-NMR spectrum of americanin A triacetate(3). a, Proton noise decoupled spectrum: b-g, SINEPT spectra obtained by irradiation of H-5(J=8 Hz), H-6(8 Hz), H-8(8 Hz), H-7'(1 Hz), H-8'(1 Hz) and H-9(10 Hz), respectively.

signal at 145.7 ppm was assigned to C-4, which is three-bond coupled to H-6, and the next most intense signal at 151.9 ppm was assigned to C-7 for the same reason. (7) The enhanced signals at 116.7 and 117.5 ppm were assigned to C-2 and-5, respectively, due to three-bond and two-bond coupling to H-6. The signal observed at 123.7 ppm which was assigned to C-2', C-5' or C-6' was regarded as a residual signal due to one of the ¹³C satellites of either H-2', H-5' or H-6'.

Irradiation of H-8 using the same delay time as for the irradiation of H-6 and H-5 resulted

in the enhancement of two signals. The intense signal at 127.9 ppm was assigned to C-1, which is three-bond coupled to H-8. The small enhancement of the carbon signal at 116.7 ppm (C-2) indicated that this carbon is also coupled to H-8, but with a smaller coupling constant.

Irradiation of H-7' with $J_{\rm CH}=1$ Hz resulted in the enhancement of four signals. The most intense signal, at 145.7 ppm assigned to C-4, was also the resonance found to be the most enhanced when H-6 was irradiated, thus strongly suggesting that 3 is the correct structure of americanin A triacetate rather than structure 4. The next most intense signal, at 133.6 ppm, was assigned to C-1' which is two-bond coupled to H-7'. The same signal (C-1') was also enhanced in coumarinolignans whenever H-7' was irradiated. Two small signals at 142.5 and 142.2 ppm, were assigned to C-4' and C-3', respectively, based on their intensity. Although C-4' is five-bond coupled to H-7', according to a model compound-toluene, the value of $^5J_{\rm CH}$ is larger than $^4J_{\rm CH}$. It therefore seemed reasonable to attribute the signal at 142.5 ppm to C-4', since the $J_{\rm CH}$ value used in this experiment was 1 Hz.

Irradiation of H-8' with $J_{\rm CH}=1$ Hz resulted not only in the enhancement of C-1' at 133.6 ppm, but also in the enhancement of the C-3 signal at 142.7 ppm, that is the same carbon (C-3) as did irradiation of H-5. The suggestion that 3 is the correct structure for americanin

A triacetate is therefore further confirmed. Due to the overlap of H-2', H-5' and H-6', it was difficult to assign these protons. However, irradiation of H-7' with $J_{\rm CH}$ =6 Hz resulted in the enhancement of the signals at 125.1 and 122.3 ppm, which were assigned to C-6' and C-2', respectively.

In order to assist in the assignment of C-8, the aldehyde proton (H-9) was irradiated with $J_{\rm CH}{=}10$ Hz. The only carbon signal observed at 127.0 ppm was thus assigned to C-8 which is two-bond coupled to H-9. It is established⁸⁾ that large, positive two-bond coupling constants can be observed for α,β -unsaturated aldehydes. CSCM 1D experiments⁽⁹⁾ were used to initially assign C-7' and C-8' in 3. Thus irradiation of the proton at 5.03 ppm*, which from its chemical shift, multiplicity and the COSY spectrum must be H-7' led to a specific enhancement of the

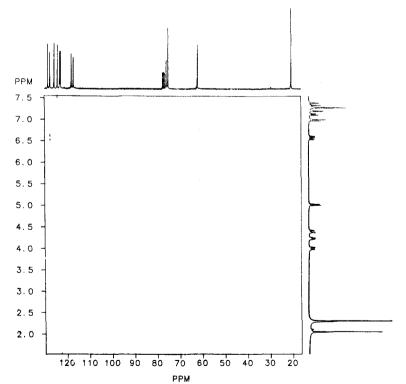


Fig. 3. ¹H-¹⁸C Correlation spectrum for americanin A triacetate (3) in CDCl₃.

^{*} Irradiations were conducted 75 Hz upfield and downfield of the center of the proton resonances with identical results in terms of enhancement.

	Americanin A	Americanin A triacetate			Americanin A	Americanin A triacetate		
Carbon	(DMSO-d ₆) ²	(DMSO-de initial assignmen	revised	Carbon	$(DMSO-d_{\theta})^a$	(DMSO-d ₆) ^a initial assignments		(CDCl ₃) ^b revised assignments
. 1	127.6°	128.1 (s) 127.9(s)	4'	145. 9e	142. 1 ^g	(s)	142.5(s)
2	116.8	116.9 ^f (d) 116.7(d)	5′	115. 5 ^d	123. 9 ^h	(d)	123.7(d)
3	143. 5	142.7 ^g (s) 142.7(s)	6'	118.9	126.0	(d)	125.1(d)
4	146.6	145.8 (s) 145.7(s)	7′	76. 1	75. 4	(d)	76.0(d)
5	117.3	117.5f (1) 117.5(d)	8'	78. 1	74.3	(d)	75.8(d)
6	122.6	123.0h (1) 122.6(d)	9'	60. 1	62.0	(t)	62.0(t)
7	126.8	127.2	1) 151.9(d)	COCH₃		168.0	(q)	167.6(q)
8	153. 0	152.6 (d) 127.0(d)	COCH₃		168.0	(q)	167.6(q)
9	194. 0	193.9 (d) 193.2(d)	COCH₃		169.8	(p)	169.9(q)
1'	127. 2°	134.2 (s) 133.6(s)	COCH3		20. 2	(q)	20.4(q)
2'	115.0 ^d	123.0 (d) 122.3(d)	COCH₃		20. 2	(q)	20.4(q)
3′	145. 3°	142.5 ^g (s	s) 142.2(s)	COCH ₃		20. 2	(p)	20.4(q)

Table II. 13C-NMR spectroscopic data for americanin A and its triacetate

signal at 76.0 ppm. On the other hand irradiation at 4.24 ppm* (H-8') only enhanced the signal at 75.8 ppm which could therefore be assigned to C-8'. Finally, direct confirmation of the assignment of all the protonated carbon atoms was achieved through a 2D-heteronuclear chemical shift correlation experiment (Fig. 3).

Comparison of our data with the literature ¹³C-NMR data of americanin A and its triacetate (3) is shown in Table II. The revised^{2,3)} structure of americanin A is therefore confirmed as being that shown in 2.

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a) Data are from reference 1.

b) Recorded at 90.8 MHz using TMS as an internal standard.

c-h) Assignments may be reversed.