Tetrahedron Letters No. 37, pp 3349 - 3350, 1976. Pergamon Press. Printed in Great Britain.

^{13}C NMR ANALYSIS OF THE ANTITUMOR ANTIBIOTICS DAUNORUBICIN AND ADRIAMYCIN

A. Arnone, G. Fronza, R. Mondelli *
Istituto di Chimica del Politecnico, + 20133 Milano, Italy
A. Vigevani
Laboratori di Ricerche, Farmitalia, Milano, Italy

(Received in UK 20 July 1976; accepted for publication 30 July 1976)

Daunorubicin (daunomycin) **1** [1] and adriamycin (doxorubicin) **2** [2], antibiotics with potent antitumor activity, have been completely analyzed by ^{13}C NMR spectroscopy. The importance of assigning all the carbon frequencies in molecules of biological interest comes from their use as a probe for biosynthetic studies, conformational analyses or studies of biological interactions. The FT ^{13}C NMR spectra (Varian XL-100-15) have been measured in DMSO (Table 1) and D_2O. We succeeded in the attribution of all the ^{13}C signals for **1** and **2** or for their aglicones, daunomycinone **3** and adriamycinone **4** , by "gated" undecoupled spectra, ^1H noise decoupling, ^1H single frequency off-centre resonance decoupling (SFOCD), ^1H low power noise decoupling and by single frequency selective heteronuclear decoupling (SFSD)[3]. They were also compared with a series of hydroxy-and methoxyanthraquinones as models[4].

The assignment of the hydrogen bearing carbons was uniquely defined by SFSD except for C-2' and C-8 because the shift differences between the bonded protons were too small. Therefore they were identified by comparison with daunosamine methylglycoside **5** . Although their signals overlap in DMSO, C-4' and C-5' could be assigned in D_2O at 67.1 and 67.9 ppm resp. for both antibiotics. The assignment of the quaternary carbons presented major difficulties, particularly for those signals which are close to each other, or show symmetrical interactions. Therefore these were analyzed by SFSD technique, but with a particular calibration of power, as the disappearance of long-range couplings only could be observed. The protons irradiated for the identification of C-4, C-6 and C-11 are resp. OMe-4, H-7 and CH_2-10. As C-6 and C-11 interact with the chelated hydroxyl protons (J=4.0 Hz), and in the proton spectra of **3** and **4** the OH signals are well separated, we could identify C-5a vs C-11a and C-5 vs C-12 through the following procedure. After addition of a trace of TFA[5] to the DMSO solutions to sharpen the OH signals, we irradiated each hydroxyl proton, and observed which one of the two carbons, C-6 or C-11, was decoupled. Thus assigned the hydroxyl protons, irradiation of each of them allowed the identification of C-5a vs C-11a, which appear in the undecoupled spectra of **3** and **4** as sharp doublets of 5 Hz. As C-5 and C-12 are appreciably coupled, even through four bonds, to the hydroxyl protons, the irradiation of these latter provides a definite assignment of them. In fact the three-bond coupling between H-1 and C-12 is as small ($\leq$2 Hz) as a four-bond coup-

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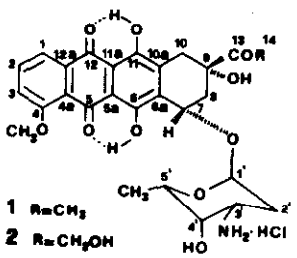
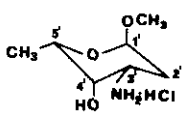
Table 1. - ^{13}C chemical shift assignments[a]

C	1	2	3	4	C	1	2	3	4	5
1	118.0	118.6	118.4	118.3	7	69.2	69.2	60.4	60.1	
2	135.3	135.8	135.6	135.5	8	35.2	35.9	35.5	36.0	
3	118.0	118.6	119.0	119.1	9	74.3	74.5	76.1	76.0	
4	159.8	160.4	160.2	160.2	10	31.1	31.7	31.8	32.4	
6	155.4	155.7	155.4	155.3	13	212.0	213.8	211.6	213.4	
11	153.8	154.2	154.4	154.3	14	23.8	63.8	24.3	63.9	
5	(184.9)	(185.5)	185.2	185.1	OMe	55.9	56.3	56.2	56.1	
12	(185.0)	(185.6)	185.4	185.3	1'	98.6	99.0			96.5
4a	118.7	119.3	119.2	119.1	2'	27.8	28.0			27.6
12a	133.4	133.9	133.8	133.7	3'	46.1	46.4			46.3
5a	(109.6)	(110.1)	110.1	110.0	4'	65.5	65.9			65.4
11a	(109.5)	(110.0)	109.8	109.8	5'	65.5	65.9			65.3
6a	(134.4)	(134.8)	136.7	136.3	Me-5'	16.3	16.6			16.6
10a	(133.7)	(133.8)	133.0	132.6						

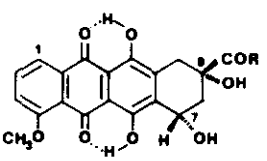
a) In ppm (δ), obtained from 0.2 M DMSO solutions containing TMS as internal reference. Similar values in parentheses may be interchanged; their tentative assignments are given on the basis of the values of **3** and **4**.



1 R=CH_3
2 R=CH_2OH



5



3 R=CH_3
4 R=CH_2OH

ling, so that the SFSD of H-1 gave ambigous results. Of the three quaternary carbons in the region 133-137 ppm, only one, C-12a, is readily located from its meta coupling (7 Hz); since the two-bond interactions in these systems are negligible[3], this signal appears in all the compounds as a neat doublet, which collapses to a singlet upon irradiation of H-2. By contrast the remaining resonances (C-6a and C-10a) are very broad, with coupling to CH_2-10, H-7 and possibly to CH_2-8 and the chelated hydroxyl protons. But both the interactions with these latter and the three-bond couplings $^3J_{C_{6a},H_8}$ are so small ($\leq$1 Hz) that SFSD experiments were unsuccessful. Since the removal of the perturbation due to the sugar moiety in **3** and **4** affected only C-6a, we could assign both C-6a and C-12a. Finally the identification of C-4a is effected through the shift value and the triplet structure of its signal, due to meta couplings with H-1 and H-3 (^3J=7 Hz).

References and Notes

(1) F. Arcamone, G. Cassinelli, G. Franceschi, R. Mondelli, P. Orezzi and S. Penco, Gazz. Chim. Ital., 100, 949 (1970).

(2) F. Arcamone, G. Franceschi, S. Penco, A. Selva, Tetrahedron Letters, 1007 (1969).

(3) The proton shifts used for SFSD of **1** and **2** have been obtained through 300 MHz analyses.

(4) A. Arnone, G. Fronza, R. Mondelli and J. Pyrek, to be published.

(5) The chemical shifts remain unchanged within 0.02 ppm for δ_H and within 0.2ppm for δ_C.