Complete assignment of the ^{13}C NMR spectra of a series of 5,8-disubstituted 4,4-dimethylanthracene-1,9,10(4*H*)-triones

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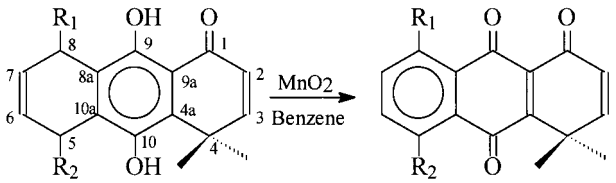
ABSTRACT: The regiosomeric quinones 5-acetyloxymethyl-4,4,8-trimethyl- (**5**) and 8-acetyloxymethyl-4,4,5-trimethylanthracene-1,9,10(4*H*)-trione (**6**) were synthesized and their regiochemistry was assigned on the basis of the unambiguous structure elucidation of 9,10-dihydroxy-5-acetyloxymethyl-4,4,8-trimethyl-5,8-dihydroanthracen-1(4*H*)-one (**2**), the precursor of **5**. The ^{1}H and ^{13}C NMR spectra of these compounds were assigned completely using two-dimensional techniques. These interpretations were used for the total assignment of the NMR spectra of the closely related 5-hydroxymethyl- and 5-formyl-4,4,8-trimethylanthracene-1,9,10(4*H*)-triones (**7** and **8**, respectively).

KEYWORDS: NMR; ^{1}H NMR, ^{13}C NMR; HMBC; HMQC; ^{5}J(CH); anthracene-1,9,10-triones

INTRODUCTION

Some natural and synthetic quinones showing strong activity against the protozoon *Trypanosoma cruzi*, the causative agent of Chagas' disease, may exert their action primarily by upsetting the parasite's anti-oxidative defense mechanisms through interaction with the enzyme trypanothione reductase[1] or by disrupting mitrochondrial electron transport.[2,3] The aromatic ring-unsubstituted 4,4-dimethylanthracene-1,9,10(4*H*)-trione **1**[4] exhibits potent lytic activity *in vitro* against *T. cruzi* and several *Leishmania* species.[5] As computational docking studies using a model of trypanothione reductase had suggested that introduction of oxymethyl substituents at C-5 or C-8 of this structure might lead to greater affinity for the enzyme's active site, precursors of such derivatives were prepared by Diels–Alder reactions of 8,8-dimethylnaphthalene-1,4,5(8*H*)-trione with appropriate dienes.[6]



2: R_1 = Me, R_2 = CH_2OAc
3: R_1 = CH_2OAc, R_2 = Me
4: R_1 = Me, R_2 = CH_2OH

1: R_1 = H, R_2 = H
5: R_1 = Me, R_2 = CH_2OAc
6: R_1 = CH_2OAc, R_2 = Me
7: R_1 = Me, R_2 = CH_2OH
8: R_1 = Me, R_2 = CHO

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RESULTS AND DISCUSSION

The quinones 5-acetyloxymethyl-4,4,8-trimethylanthracene-1,9,10(4*H*)-trione (**5**) and 8-acetyloxymethyl-4,4,5-trimethylanthracene-1,9,10(4*H*)-trione (**6**) were prepared by oxidation of 9,10-dihydroxy-5-acetyloxymethyl-4,4,8-trimethyl-5,8-dihydroanthracen-1(4*H*)-one (**2**) and 9,10-dihydroxy-8-acetyloxymethyl-4,4,5-trimethyl-5,8-dihydroanthracene-1(4*H*)-one (**3**), respectively, using active manganese dioxide.[7] In a similar manner, by oxidation of 9,10-dihydroxy-5-hydroxymethyl-4,4,8-trimethyl-5,8-dihydroanthracene-1(4*H*)-one (**4**), we recently obtained 5-hydroxymethyl- and 5-formyl-4,4,8-trimethylanthracene-1,9,10(4*H*)-trione (**7** and **8**, respectively).[6]

The ^{1}H NMR spectra of quinones **5** and **6** are almost identical and it is only possible to differentiate both regioisomers through their ^{13}C NMR spectra. The assignment of these spectra is not straightforward,

however, because the central region of these linear chemical structures consists only of quaternary and carbonyl carbon atoms whose resonances must be correlated via long-range *J*(CH). In this study we discriminated the position of the substituent on the aromatic ring by the concerted use of ^{1}H-detected one-bond (C–H) heteronuclear multiple quantum coherence (HMQC)[8] and long range C–H heteronuclear multiple bond connectivity (HMBC),[9,10] applied to **2**, which affords **5** upon oxidation with active manganese dioxide. As the substitution pattern of this compound had been demonstrated unambiguously, the structures of **5** and **6** followed. Concerted use of HMQC and HMBC also allowed the complete assignment of the ^{13}C resonances of the latter compounds. The same procedures might be expected to make the assignment of the tricyclic skeleton carbon resonances with other substituents such as formyl or hydroxymethyl possible, but in quinones bearing these groups (**7** and **8**) we were unable to observe all the necessary long-range correlations to assign their spectra completely. It was therefore necessary to compare their spectra with the data for quinones **5** and **6** in order to discriminate between the two quinone carbonyl carbon atoms.

Complete assignment of the ^{13}C NMR spectra of compounds such as distamycin A has required the correlation of carbonyl resonances with aromatic ring protons via ^{4}J(CH). This long range correlation is generally weak and requires high sensitivity.[11,12] Inverse-detected heteronuclear shift correlation efficiency can be augmented significantly by the incorporation of pulsed field gradients.[13] This technique results in high resolution, allowing the detection of the crucial low intensity correlations via ^{4-5}J(CH) which have now made the present assignments possible.

The key feature for the assignment of the ^{13}C NMR spectrum of **2** was the presence of the chelated hydroxyl group at C-9. The corresponding proton, resonating at 13.18 ppm, does not show any C–H correlation. Nevertheless, the hydroxyl proton resonating at 6.81 ppm correlates with a quaternary carbon nucleus (C-10a) which also shows a ^{3}J(CH) correlation with the acetyloxymethyl substituent methylene protons. Other correlations shown in Table 1 reinforce this assignment.

The ^{1}H NMR spectra of the common tricyclic skeleton of the four quinones consist of two AB systems corresponding to the enone moiety and the aromatic ring protons. In addition, only singlets due to the methyls and alternatively formyl, hydroxymethyl and acetyloxymethyl groups are observed. In all cases the two-spin system of the enone moiety may be easily assigned from the chemical shifts and the vicinal coupling constants by comparison with closely related compounds.[4] This spin system corresponds to H-2 and H-3, and serves as the entry point for spectral interpretation.

The ^{1}H NMR spectrum of **5** shows the enone AB system at 6.35 and 6.80 ppm with a coupling constant of 10.2 Hz, with H-3 at lower field due to the deshielding effect of the conjugated carbonyl group. The AB system corresponding to the aromatic ring protons appears at 7.49 and 7.63 ppm with an *ortho* coupling constant of 8.1 Hz. The proton resonating at 7.63 ppm is located at C-6, as it lies close to the acetyloxymethyl substituent. Additionally, four singlets may be observed at 1.61,

Table 1. ^{1}H and ^{13}C NMR data (δ in ppm, *J* in Hz) for 9,10-dihydroxy-5-acetyloxymethyl-4,4,8-trimethyl-5,8-dihydroanthracene-1(4*H*)-one (**2**)

	$\delta(^{1}H)$	$\delta(^{13}C)$	^{2}J(CH) ^{3}J(CH) ^{4}J(CH) ^{5}J(CH)
1	—	191.39	
2	6.25 (d, J = 10.0 Hz)	123.91	C-4; C-9a
3	6.85 (d, J = 10.0 Hz)	161.37	Me-4; Me′-4; C-4; C-4a; C-1
4	—	38.34	
Me-4	1.62	25.19	Me′-4; C-4; C-3; C-4a;
Me-4′	1.68	24.91	Me-4; C-4; C-3; C-4a;
4a	—	132.88	
5	3.82 (m)	35.18	CH_2 ; C-8a; C-10a; C-6; C-7;
CH_2	4.31–3.98	71.05	C-10a; C-6; C-5; C=O (Ac)
C=O (Ac)		171.8	
CH_3 (Ac)	2.18	20.89	C=O (Ac)
6	5.95 (dd, J_1 = 9.7, J_2 = 4.9 Hz)	123.07	Me (Ac); C-8; C-10a; C-5; C-7
7	6.19 (dd, J_1 = 9.7, J_2 = 5.1 Hz)	134.75	CH_2 ; C-8a; C-8a; C-5
8	3.75 (m)	29.64	Me-Ar; C-9; C-7; C-10a; C-8a; C-6
Me-Ar	1.34	22.36	C-8; C-7; C-8a
8a	—	127.87	
9	—	153.99	
9a	—	113.67	
10	—	143.87	
10a	—	130.3	
OH-9	13.18		
OH-10	6.81		

Table 2. ^{1}H and ^{13}C NMR data (δ in ppm, J in Hz) for 5-acetyloxymethyl-4,4,8-trimethylanthracene-1,9,10(4*H*)-trione (**5**)

	$\delta(^{1}H)$	$\delta(^{13}C)$	$^{2}J(CH)$ $^{3}J(CH)$ $^{4}J(CH)$ $^{5}J(CH)$
1	—	182.32	
2	6.35 (d, J = 10.2 Hz)	127.00	C-4; C-9a; C-1
3	6.80 (d, J = 10.2 Hz)	157.68	Me × 2; C-4; C-4a; C-9
4	—	38.51	
Me × 2	1.61	26.03	Me × 2; C-4; C-3; C-4a; C-2
4a	—	156.37	
5	—	135.75	
6	7.63 (d, J = 8.1 Hz)	132.04	CH_2 ; C-8; C-10a
7	7.49 (d, J = 8.1 Hz)	136.79	Me-Ar; C-8a; C-5
8		139.99	
Me-Ar	2.68	22.43	C-8; C-7; C-8a; C-9
8a	—	131.76	
9	—	187.75	
9a	—	133.95	
10	—	184.77	
10a	—	131.22	
CH_2	5.55	63.91	C-10a; C-6; C-5; C-10; C=O (Ac)
C=O (Ac)		170.29	
CH_3 (Ac)	2.16	20.78	C=O (Ac); CH_2

2.16, 2.68 and 5.55 ppm, corresponding to the two equivalent 4-methyl groups, the acetyl methyl, the 8-methyl and the 5-acetyloxymethyl methylene groups. The HMBC method allowed the observation of a $^{4}J(CH)$ coupling from the methyl group linked to the aromatic ring to the C-9 carbonyl carbon and a $^{5}J(CH)$ coupling from this nucleus to H-3, in addition to a $^{4}J(CH)$ coupling between the 5-acetyloxymethyl methylene group and C-10. The assignments of the carbon resonances obtained from the analysis of long-range correlations are presented in Table 2. It may be noted that C-7 resonates about 5 ppm downfield from C-6, the carbon nucleus neighboring the acetyloxymethyl group.

The ^{1}H NMR spectrum of **6** is almost identical with that of **5**, and differs only slightly in the chemical shifts of the signals of the enone and aromatic AB systems. In this case the HMBC method also allows the observation of a $^{3}J(CH)$ coupling from C-1 to H-3 and a $^{5}J(CH)$ coupling from the latter to C-9. The 5-methyl-C-10 $^{4}J(CH)$ coupling may also be seen but, unlike the case of **5**, no long-range coupling can be observed between the 8-acetyloxymethyl methylene protons and

Table 3. ^{1}H and ^{13}C NMR data (δ in ppm, J in Hz) for 8-acetyloxymethyl-4,4,5-trimethylanthracene-1,9,10(4*H*)-trione (**6**)

	$\delta(^{1}H)$	$\delta(^{13}C)$	$^{2}J(CH)$ $^{3}J(CH)$ $^{4}J(CH)$ $^{5}J(CH)$
1	—	182.67	
2	6.35 (d, J = 10.2 Hz)	127.27	C-4; C-9a
3	6.80 (d, J = 10.2 Hz)	157.81	Me × 2; C-4; C-4a; C-1; C-9
4	–	38.60	
Me × 2	1.61	26.24	Me × 2;C-4; C-3; C-4a
4a	–	155.62	
5	–	131.31	
Me-Ar	2.7	21.67	C-10a; C-6; C-8a; C-10
6	7.53 (d, J = 8.1 Hz)	137.51	Me-Ar; C-8; C-10a
7	7.65 (d, J = 8.2 Hz)	131.55	CH_2 ; C-8a; C-5
8		136.24	
CH_2	5.54	64.46	C-7; C-8; C=O (Ac)
C=O (Ac)		170.51	
CH_3 (Ac)	2.17	20.96	C=O (Ac)
8a	–	139.64	
9	–	188.13	
9a	–	134.43	
10	–	184.92	
10a	–	132.07	

Table 4. ^{1}H and ^{13}C NMR data (δ in ppm, J in Hz) for 5-hydroxymethyl-4,4,8-trimethylanthracene-1,9,10(4*H*)-trione (**7**)

	$\delta(^{1}H)$	$\delta(^{13}C)$	^{2}J(CH) ^{3}J(CH) ^{4}J(CH) ^{5}J(CH)
1		182.95	
2	6.36 (d, $J = 10.2$ Hz)	127.73	C-4; C-6
3	6.79 (d, $J = 10.2$ Hz)	158.16	Me × 2; C-4; C-4a; C-1
4		38.96	
Me × 2	1.63	26.56	Me × 2; C-4; C-3; C-4a
4a		156.01	
5		141.02	
CH_2	4.81 (d, $J = 7.0$ Hz)	64.82	C-8a; C-6; C-5
OH	3.33 (t, $J = 7.0$ Hz)		CH_2
6	7.66 (d, $J = 7.7$ Hz)	134.52	CH_2 ; C-10a; C-8
7	7.52 (d, $J = 7.7$ Hz)	138.47	Me-Ar; C-8a; C-5
8		140.31	
Me-Ar	2.7	22.06	C-8a; C-7; C-8
8a		132.7	
9		190.23	
9a		135.38	
10		185.26	
10a		132.7	

C-9. The full assignment of the carbon resonances obtained from the analysis of long-range correlations is presented in Table 3.

In both **5** and **6**, C-9 resonates several ppm downfield from C-10, regardless of the arrangement of the aromatic substituents. A ^{5}J(CH) coupling is also seen in both compounds between C-9 and H-3. Hence the enone system seems to have a decisive influence upon the general characteristics of the ^{13}C NMR spectra and the long-range couplings, unlike the relatively unimportant effect of exchanging the aromatic ring substituents.

The ^{1}H and ^{13}C resonances of quinone **7** show similar chemical shift trends to those discussed for quinones **5** and **6** regarding H-6 and H-7, C-7 and C-6. However, the HMBC experiment showed only one satisfactory correlation for the carbonyl carbons: a strong ^{3}J(CH) cross peak corresponding to C-1 and H-3. The assignment of the quinone carbonyl signals was made by comparison with the spectra of quinones **5** and **6** that show that the C-9 carbonyl carbon nucleus resonates at lower field. The complete spectra assignment is shown in Table 4.

The ^{1}H NMR spectrum of formylquinone **8** shows the usual two AB systems, aside from the *gem*-dimethyl, the aromatic ring methyl and the formyl resonances. In this case, the aromatic AB system shows H-6 at lower field than H-7 owing to the vicinity of the aldehyde carbonyl group. In the ^{13}C NMR spectrum, the signals

Table 5. ^{1}H and ^{13}C NMR data (δ in ppm, J in Hz) for 5-formyl-4,4,8-trimethylanthracene-1,9,10(4*H*)-trione (**8**)

	$\delta(^{1}H)$	$\delta(^{13}C)$	^{2}J(CH) ^{3}J(CH) ^{4}J(CH) ^{5}J(CH)
1		182.76	
1	6.27 (d, $J = 10.2$ Hz)	127.91	C-9a
3	6.69 (d, $J = 10.2$ Hz)	157.99	Me × 2; C-4; C-9; C-1; C-4a
4		38.99	
Me × 2	1.52	26.52	Me × 2; C-4; C-4a; C-3
4a		155.47	
5		136.64	
CHO	10.26	191.49	C-10a; C-6
6	7.78 (d, $J = 8.1$ Hz)	132.37	CHO; C-10a; C-8
7	7.53 (d, $J = 8.1$ Hz)	138.28	Me-Ar; C-5; C-6; C-8a
8		134.87	
Me-Ar	2.66	22.61	C-8a; C-7; C-10a
8a		131.52	
9		188.07	
9a		135.61	
10		184.04	
10a		145.48	

corresponding to C-6 and C-7 follow the previously observed trend according to which the resonance of the aromatic ring carbon nucleus lying closer to the oxygen substituent appears at lower field. The HMBC experiment showed long-range correlations between H-3 and the C-1 and C-9 carbonyl carbons [^3J(CH) and ^5J(CH), respectively], as seen for the most closely related acetyloxymethyl compound (**5**). Finally, C-9 resonates downfield from C-10, as is also the case for **5** and **6**, thus confirming our assignment presented in full in Table 5.

EXPERIMENTAL

Proton and ^{13}C NMR spectra were acquired using a Bruker AVANCE DRX 300 spectrometer operating at 300.13 MHz (^{1}H) or 75.47 MHz (^{13}C). All measurements were carried out at a probe temperature of 300 K, using solutions of **5**, **6**, **7** or **8** (300, 125, 100 and 150 mg ml^{-1}, respectively) in $CDCl_3$ containing tetramethylsilane (TMS) as an internal standard. All two-dimensional spectra were acquired with a Bruker inverse 5 mm Z gradient probe. The HMQC spectra were recorded using standard Bruker software (inv4gstp). These spectra were collected with 512 × 512 data points, a data acquisition of 8 scans × F and 256 increments in t_1. Spectral widths of 3000 and 18 000 Hz were employed in the F_2 (^{1}H) and F_1 (^{13}C) domains, respectively. The HMBC spectra were obtained using the inv4gslplrnd pulse sequence in the Bruker software. The spectral widths were 3000 Hz (F_2) and 18 000 Hz (F_1) and the delays Δ_1 and Δ_2 were set to 3.45 and 65 ms, respectively. In addition, another experiment was performed at 3.45 and 100 ms for Δ_1 and Δ_2, respectively. Data were processed using an exponential window in F_2 with lb = 5 Hz and a Qsine window in F_1.

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