Carbon-13 Nuclear Magnetic Resonance Spectra of Methaqualone Metabolites

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Carbon-13 chemical shifts are reported for methaqualone, seven hydroxylated methaqualone metabolites, and four acetate derivatives. The signals are assigned on the basis of chemical shift theory, SFORD multiplicities, signal intensities, and comparisons with model compounds.

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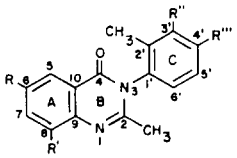
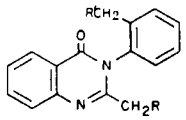
Methaqualone [2-methyl-3-*o*-tolyl-4(3*H*)quinazolinone **1**] is a widely known and frequently abused hypnotic (1). Metabolism studies have shown that methaqualone undergoes conversion to a series of hydroxylated metabolites which are excreted largely as glucuronide conjugates (2). Continued interest in methaqualone metabolism has prompted a study of the ^{13}C nmr spectra of **1** and related model compounds (3). This study has been extended to include seven hydroxylated metabolites of methaqualone. The compounds examined in the present investigation are summarized in Chart I. They include

Chart I



1, R = R′ = R″ = R‴ = H
2, R = OH; R′ = R″ = R‴ = H
3, R = OAc; R′ = R″ = R‴ = H
4, R′ = OH; R = R″ = R‴ = H
5, R″ = OH; R = R′ = R‴ = H
6, R″ = OAc; R = R′ = R‴ = H
7, R‴ = OH; R = R′ = R″ = H
8, R‴ = OAc; R = R′ = R″ = H
9, R = R″ = OH; R′ = R‴ = H
10, R = R″ = OAc; R′ = R‴ = H



11, R = OH; R′ = H
12, R = H; R′ = OH

methaqualone (**1**), the monohydroxy metabolites **2** (6-hydroxy), **4** (8-hydroxy), **5** (3′-hydroxy), **7** (4′-hydroxy), **11** (2-hydroxymethyl) and **12** (2′-hydroxymethyl), the dihydroxy metabolite **9** (3′,6-dihydroxy), and the acetate derivatives **3**, **6**, **8** and **10**. Signal assignments were made on the basis of ^{13}C nmr chemical shift theory, multiplicities as obtained by single-frequency off-resonance decoupling (SFORD) experiments, signal intensities, and comparisons to structurally related compounds. The chemical shift assignments are summarized in Table I.

The 2-methyl carbon resonance appeared consistently at 23-24 ppm (q) (4) and shifted to *ca.* 61.5 ppm (t) in the 2-hydroxymethyl derivative **11**. The 2′-methyl carbon resonance appeared normally at 16-17 ppm (q), shifted to *ca.* 60 ppm (t) in the 2′-hydroxymethyl derivative **12**, and shifted to 10-11 ppm (q) in the presence of a 3′-substituent. This upfield shift was due to steric compression (5). The acetate methyl signals were observed at 20-21 ppm (q).

The C-4 carbonyl resonance at 160-161 ppm (s) was distinguished by its consistency throughout the series. The chemical shift value was in excellent agreement with that reported for methaqualone (3). The acetate carbonyl signals came *ca.* 9 ppm further downfield.

Four other easily distinguished resonances were those assigned to C-2, C-9, C-10 and C-X (6), all of which appeared as singlets in the SFORD spectra. Since C-2 was bonded to two nitrogen atoms, the C-2 resonance was distinguished from the C-9 and C-10 signals by virtue of its downfield position (3). In addition, the C-2 resonance remained unchanged in the spectra of compounds **1**, **5-8** and **12**, and shifted downfield *ca.* 1 ppm in the 2-hydroxymethyl derivative **11**. In the case of those compounds having 3′-(**5,6**) or 4′-substituents (**7,8**), the C-2 resonance was distinguished from the C-X resonance by the upfield shift (7) of the latter resonance on going from hydroxyl to acetoxyl substituents. Hydroxylation at C-6 (compound **2**) caused a slight upfield shift in the C-2 resonance which was almost completely reversed by acetylation (compound **3**). A smaller upfield shift was observed upon 8-hydroxylation (compound **4**). The chemical shift of C-X in compounds **2-4** agreed reasonably well with the value calculated using aryl substituent constants (7). The assignments for C-2 and C-X in the 3′,6-disubstituted compounds **9** and **10** were readily transferable from the corresponding monosubstituted compounds.

The bridgehead carbons were differentiated by the fact that C-9 resonated at lower field because it was bonded to a nitrogen atom. A similar distinction between bridgehead carbons was reported for quinazoline (8), for some 2- and 4-quinolone derivatives (9), and for methaqualone itself (3,10). Hydroxylation at C-6 (*para*) and at C-8 (*ortho*) produced the expected upfield shifts (7) in the C-9 resonance. Because of the smaller *para* effect of the acetoxyl group (7), subsequent acetylation of the 6-hydroxyl group then caused the C-9 signal to move back downfield. The C-10 resonance, on the other hand, remained essentially constant throughout the series, being

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Table I

Carbon-13 Nmr Chemical Shifts of Methaqualone Metabolites and Related Compounds (a,b)

Compound Carbon (c)	1 (d)	2 (d)	3 (e)	4 (d)	5 (d)	6 (d)	7 (d)	8 (d)	9 (d)	10 (d)	11 (d)	12 (d)
2	154.30	150.74	153.93	152.49 (f)	154.44	154.18	155.22	154.59	150.88	154.06	155.91	154.83
4	160.68	160.54	160.80	160.73	160.68	160.87	161.12	161.05	160.49	160.46	160.44	160.98
5	126.36 (b)	109.24	118.80	116.12	126.40 (b)	126.51 (b)	126.50 (b,f)	126.56 (b)	109.19	118.63	126.55 (b)	126.45 (b,f)
6	126.50 (b)	156.05	148.52	127.04 (b)	126.50 (b)	126.86 (b,f)	126.50 (b,f)	126.86 (b,f)	155.95 (b)	148.59	127.09 (b,g)	126.45 (b,f)
7	134.65	124.06	131.31	118.65	134.65	134.96	134.64	135.02	124.02	129.50	134.89	134.69
8	126.75 (b)	128.45 (f)	127.61	152.49 (f)	126.70 (b)	126.86 (b,f)	126.74 (b)	126.86 (b,f)	128.36	128.44 (f)	127.09 (b,g)	126.79 (b)
9	147.42	140.69	145.29	136.45 (b)	147.37	147.42	147.47	147.48	140.64	145.25	147.03	147.57
10	120.31	121.24	121.26	121.24	120.31	120.28	120.41	120.34	121.24 (b)	120.92	120.65	120.36
1′	136.84	137.13	136.36	136.99 (b)	137.72	137.90	128.06	134.32	138.01	137.78	135.77 (b)	135.86
2′	135.04	135.08	135.07	135.08	121.77	128.44	136.11	136.96	121.82 (b)	128.44 (f)	135.08 (b)	138.94
3′	131.04	130.99	129.43	131.04	156.44	149.95	117.38	124.33	156.39 (b)	149.95	130.89	129.33
4′	128.40	128.45 (f)	128.08	128.40	115.34	123.45	157.85	150.83	115.24	123.57	128.55	128.65 (b)
5′	127.38	127.33	127.43	127.38 (b)	127.09	127.62	114.07	120.98	126.99	127.58	127.09 (b,g)	128.55 (b)
6′	129.28	129.18	128.67	129.28	118.65	126.33 (b)	129.28	129.68	118.70	126.27	129.43	128.79 (b)
2-CH_3	23.57	23.22	23.57	23.52	23.32	23.52	23.66	23.70	23.03	23.46	61.55	23.91
2′-CH_3	16.89	16.94	17.16	16.93	10.21	10.83	17.18	17.06	10.25	10.89	17.08	59.70
CH_3CO	—	—	169.08	—	—	168.98	—	169.33	—	169.51 168.98	—	—
CH_3CO	—	—	20.81	—	—	20.70	—	21.05	—	21.00 20.70	—	—

(a) Chemical shifts are in parts per million relative to tetramethylsilane. (b) Signals in any one column may be reversed. (c) Numbering of carbons is shown in Chart I. (d) In dimethylsulfoxide-d_6 solution. (e) In deuteriochloroform solution. (f) These resonances were twice as intense as those of other similar carbons. (g) These resonances were three times as intense as those of other similar carbons.

only slightly affected by substitution at the *meta* positions.

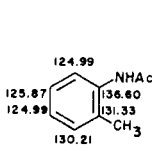
At this point it was necessary to differentiate the resonances of the remaining ring A carbons from those of the ring C carbons. Comparison of the spectra of compounds **1-4** revealed that six of the remaining unassigned resonances were unaffected by substitution in ring A. These six signals, which included two singlets and four doublets (SFORD), were therefore attributed to the ring C carbons of these four compounds. Furthermore, comparison of the spectra of compounds **1**, **5-8**, **11** and **12** indicated that four of the remaining resonances, all doublets (SFORD), were unaffected by substitution in rings B or C. Consequently, these signals were attributed to the ring A carbons of these seven compounds. In this manner, the ring A and ring C carbons were completely distinguished from each other.

Once the ring A carbon resonances had been identified, their assignment was straightforward. The signal at 134-135 ppm in the spectra of compounds **1**, **5-8**, **11** and **12** was attributed to C-7 by analogy to the assignments in quinazoline (3,8). The resonances assigned to C-5, C-6 and C-8 in these seven compounds were interchangeable with each other. However, upon 6-hydroxylation (compound **2**), the C-6 signal (C-X) became a downfield singlet (SFORD) at *ca.* 156 ppm while the C-8 resonance (*meta*) moved downfield slightly and the C-5 and C-7 resonances (*ortho*) shifted upfield *ca.* 15 and 11 ppm, respectively. These shifts were of the magnitude expected for introduction of a hydroxyl substituent (7). Similar but smaller shifts were observed for the 6-acetoxy derivative **3** (7). Hydroxylation at C-8 (compound **4**) also shifted the C-5-C-8 resonances and led to the assignments shown in Table I. In this case, the signal at *ca.* 116 ppm was attributed to C-5 (*para*) because this carbon was upfield from C-7 (*ortho*) initially and because *para* effects from hydroxylation were smaller than *ortho* effects (7). The chemical shifts for the ring A carbons in the 3′,6-disubstituted compounds **9** and **10** were obtained by comparison with compounds **2** and **3**, respectively.

The off-resonance experiments conveniently divided the ring C carbons into two groups. In the spectra of compounds **1-4**, the two singlets (SFORD) attributable to carbons C-1′ and C-2′ appeared in the 135-137 ppm region. Of these, the downfield signal was assigned to C-1′ on the basis of the nitrogen substituent (3) and comparison to model compound **13** (11). From this assignment and the aryl substituent constants (7) it was then possible to calculate chemical shift values for C-1′ and C-2′ in compounds **5-10** and **12**. The signals observed in the spectra of these latter compounds were in excellent agreement with the calculated values, a fact lending considerable credence to the assignments. Only in the spectrum of the 2-hydroxymethyl derivative **11** were the C-1′ and C-2′ resonances interchangeable. Those derivatives having a 3′- or 4′-substituent had an additional ring C singlet (SFORD) in their spectra. The assignment of this carbon (C-X) was described earlier.

The remaining ring C carbon resonances all appeared as doublets in the SFORD spectra. Comparison of the spectrum of methaqualone (**1**) to those of the 3′-substituted derivatives revealed that the signal at *ca.* 127 ppm was affected only slightly by the 3′-substitution. Since the *meta* substituent effects were the smallest (7), it followed that this was the C-5′ resonance. A similar comparison with the 4′-substituted derivatives led to the identification of the C-6′ resonance at *ca.* 129 ppm. Using the above chemical shift value for the C-5′ of methaqualone and the *ortho* substituent constant (7), the chemical shifts for the C-5′ of the 4′-substituted derivatives were calculated. Due to their close agreement with the calculated values, the observed resonances at *ca.* 114 and 121 ppm in the spectra of compounds **7** (4′-hydroxy) and **8** (4′-acetoxy) were subsequently attributed to C-5′. The remaining signal in the spectra of these compounds was thus identified as the C-3′ resonance by process of elimination. The above procedure was then repeated starting with the above chemical shift value for the C-6′ of methaqualone. Comparison with the calculated chemical shift value thus identified the C-6′ resonance in the 3′-hydroxy compounds **5** and **9** and the 3′-acetoxy derivatives **6** and **10** at *ca.* 118.5 and *ca.* 126 ppm, respectively. Again by process of elimination, the remaining signal in the spectra of the 3′-substituted derivatives was assigned to C-4′. At this point all of the ring C carbon resonances in the spectra of compounds **5-10** had been assigned.

The final problem was differentiation of the C-3′ and C-4′ resonances of the remaining compounds. Calculations based on the C-3′ and C-4′ assignments made for compounds **5-10** and the appropriate substituent constants (7) gave ambiguous results. However, the position of C-4′ relative to the nitrogen (*para*) and methyl (*meta*) substituents suggested that it should resonate at higher field than C-3′. Moreover, this reasoning was supported by the assignments for the model compound **13** (11). Consequently, the resonance at *ca.* 131 ppm in the spectra of compounds **1-4** and **11** was assigned to C-3′ and the



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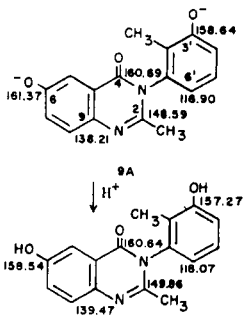

9A

9B

resonance at *ca.* 128.5 ppm to C-4′. In the spectrum of the 2′-hydroxymethyl derivative **12**, the resonance at *ca.* 129.5 ppm was attributed to C-3′ on the basis of the expected downfield shift of *ca.* 1.5 ppm (7). The C-4-C-6 resonances were interchangeable.

The spectrum obtained on the initial sample of the 3′,6-dihydroxy derivative **9** contained several signals with chemical shifts different from those expected on the basis of values obtained for compounds **2** and **5**. Moreover, the facile darkening of the nmr sample suggested that the material contained some diphenolate salt (*cf.* **9A**). Addition of acid to the sample discharged the color and caused the signals in question to shift in the direction expected (7) from protonation of the diphenolate (**9A** → **9B**). That the observed shifts were not artifacts of the altered medium was indicated by the consistency of the C-4 resonance. This experiment provided additional evidence for the assignments shown in Table I for C-2, C-6, C-9, C-3′ and C-6′ in the 3′- and 6-hydroxy compounds.

Continued advances in ^{13}C nmr technology make this technique increasingly useful in areas such as metabolism studies where small samples are not uncommon (12). The results of the present study indicate that ^{13}C nmr definitely would be useful in the unambiguous identification of methaqualone metabolites.

EXPERIMENTAL

Chemicals.

Compounds **1**, **2** (13), **4** (13), **5** (13), **7** (13), **9** (14) and **12** (15) were prepared using literature procedures. To prepare compound **11**, 2-nitrobenzo-*o*-toluidide (16) was first reduced to the amine, which was then coupled with 2-acetoxyacetic acid in the presence of EEDQ. Cyclization of the resultant intermediate in the presence of phosphorus trichloride afforded 2-acetoxymethyl-3-*o*-tolyl-4(3*H*)quinazolinone (17), which was subsequently hydrolyzed to **11**. The acetate derivatives **3**, **6**, **8**, **10** and **13** were prepared using pyridine and acetic anhydride.

The 3′,6-dihydroxy derivative **9** initially obtained had m.p. 313-314° [lit. m.p. 312-313° (15)]. However, the ^{13}C nmr spectrum suggested the presence of some diphenolate salt in the sample (*cf.* text). Consequently, the compound was redissolved in methanol, and hydrochloric acid was added dropwise to the solution until *p*H 7. Addition of benzene followed by gradual solvent evaporation then afforded **9** as off-white crystals, m.p. 318-321°. This material was used to obtain the chemical shifts reported in Table I.

Spectral Measurements.

The ^{13}C nmr spectra were determined at 25.03 MHz on a modified JEOL JNM-PS-100 FT NMR interfaced with a Nicolet 1085 Fourier-transform computer system. The samples (50 mg./0.3 ml.) were spun in 5 mm o.d. tubes. The spectra were recorded at ambient temperature by using the deuterium resonance of the solvent as the internal lock signal and tetramethylsilane as reference. All proton lines were decoupled by a broad band (*ca.* 2500 Hz) irradiation from an incoherent 99.528 MHz source. Interferograms were stored in 8K of computer memory (4K output data points in the transformed phase corrected real spectrum), and chemical shifts were measured on 5000 Hz sweep width spectra. Typical pulse widths were 17 μs (45° flip angle), and the delay time between pulses was fixed at 1.0 s. The precision of the chemical shifts is ± 0.05 ppm.

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