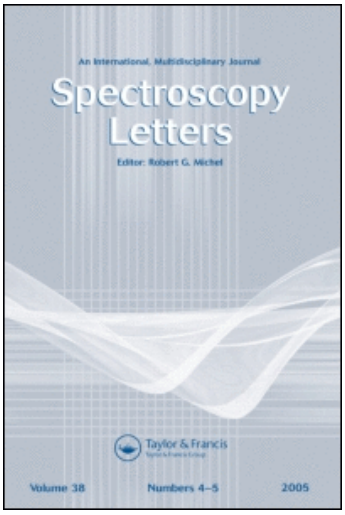


Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

^{13}C NMR Assignments of the Antimalarials, Chloroquine, 4-Methylprimaquine, 5-Methoxy-4-methylprimaquine and 5-Methoxyprimaquine

Charles D. Hufford[a]; John K. Baker[b]
[a] Department of Pharmacognosy, School of Pharmacy University of Mississippi, University, MS [b] Department of Medicinal Chemistry, School of Pharmacy University of Mississippi, University, MS

To cite this Article Hufford, Charles D. and Baker, John K.(1986) '^{13}C NMR Assignments of the Antimalarials, Chloroquine, 4-Methylprimaquine, 5-Methoxy-4-methylprimaquine and 5-Methoxyprimaquine', Spectroscopy Letters, 19: 6, 595 — 602
To link to this Article: DOI: 10.1080/00387018608069265
URL: http://dx.doi.org/10.1080/00387018608069265

SPECTROSCOPY LETTERS, 19(6), 595-602 (1986)

^{13}C NMR ASSIGNMENTS OF THE ANTIMALARIALS, CHLOROQUINE, 4-METHYLPRIMAQUINE, 5-METHOXY-4-METHYLPRIMAQUINE AND 5-METHOXYPRIMAQUINE

Charles D. Hufford* and John K. Baker+
*Department of Pharmacognosy and
+Department of Medicinal Chemistry
School of Pharmacy
University of Mississippi
University, MS 38677

Key Words: Chloroquinine, 4-Methylprimaquine, 5-Methoxy-4-Methylprimaquine, 5-Methoxyprimaquine, Antimalarial Drugs, ^{13}C NMR Assignments

Abstract

The ^{13}C nmr assignments for the antimalarial drugs chloroquine, 4-methyl primaquine, 5-methoxy-4-methylprimaquine and 5-methoxyprimaquine were established. These assignments were based on comparison with those of primaquine, proton-coupled data, and selective long-range proton decoupling.

INTRODUCTION

Studies on the metabolism of the antimalarial drug, primaquine (1), has yielded valuable information regarding the fate of primaquine in mammalian systems.[1] The metabolites have

 0038-7010/86/1906-0595$3.50/0

been characterized primarily by utilizing ^{13}C-nmr spectral data.[2,3] The ^{13}C nmr assignments for primaquine originally reported[4] were later corrected,[2] while the ^{13}C nmr assignments for chloroquine (2) base[4] and salts[5] have also been reported. However, even though 5-methoxyprimaquine (3)[6], 4-methylprimaquine (4)[7], and 5-methoxy-4-methylprimaquine (5)[8] have all been prepared as potential new antimalarials, their complete ^{13}C-nmr assignments have not been reported.

As a part of our ongoing study of the metabolism of potential new antimalarial drugs, it was deemed important to report here the complete ^{13}C-nmr assignments for compounds **2-5** since this information is invaluable for the structure elucidation of key metabolites.

RESULTS AND DISCUSSION

Even though the ^{13}C-nmr assignments for chloroquine (2) have been reported previously[4,5], we deemed it necessary to report our results since numerous erroneous assignments were made by these same authors with primaquine.[4] The other published report[5] was done on the hydrochloride salts in D_2O where significant shifts occur in the aromatic carbon signals.

The ^{13}C-nmr assignments for **2** ($CDCl_3$) are listed in Table 1. They differ from those reported[4] in the assignments for C-5, C-6 and C-2', C-3'. The correctness of the assignments as listed in Table 1 was established unambiguously by examination of the proton-coupled spectrum of **2** and by conducting selective proton decouplings. In the proton-coupled spectrum the signal at δ_C

Table 1

^{13}C nmr Assignments for **1**, **2**, **3**, **4**, and **5**

Carbon Number	**1**[a]	**3**	**4**	**5**	**2**
2	144.3(d)	144.7(d)	143.8(d)	144.3(d)	151.8(d)
3	121.8(d)	121.5(d)	122.6(d)	124.4d	99.2(d)
4	134.8(d)	129.6(d)	142.6(s)	143.3(s)	149.6(s)[b]
4a	130.0(s)	124.6(s)	129.7(s)	124.4(s)	117.7(s)
5	91.9(d)	134.0(s)	88.2d	133.9(s)[b]	122.2(d)
6	159.6(s)	150.1(s)	159.4(s)	151.2(s)	124.7(d)
7	96.9(d)	94.7(d)	96.2(d)	94.3(d)	134.6(s)
8	145.2(s)	142.1(s)	145.7(s)	142.2(s)	128.3(d)
8a	135.5(s)	131.5(s)	134.9(s)	133.5(s)[b]	149.4(s)[b]
1'	48.1d	48.3(d)	48.1(d)	48.2(d)	48.4(d)
2'	34.1t	34.3(t)	34.1(t)	34.2(t)	34.4(t)
3'	29.3t	30.3(t)	30.2(t)	29.8(t)	23.9(t)
4'	41.7t	42.2(t)	42.1(t)	42.0(t)	52.6(t)
5'	20.5q	20.7(q)	20.5(q)	20.7(q)	20.0(q)
OCH_3(C-6)	55.2q	57.0(q)	55.1(q)	56.9(q)	---
OCH_3(C-5)	---	61.2(q)	---	61.3(q)	---
CH_3(C-4)	---	---	19.0q	22.9(q)	---
$\underline{CH_2}CH_3$	---	---	---	---	46.8(t)[c]
$CH_2\underline{CH_3}$	---	---	---	---	11.7(q)[c]

[a]The data for **1** have been previously published[2] and are listed here for comparison only.

[b]Assignments interchangeable within the same column.

[c]Double intensity signals.

122.2 (C-5) appears as a sharp doublet ($^1J_{C-H}$ = 159.1 Hz) while the signal at δ_C 124.7 (C-6) appears as a dd ($^1J_{C-H}$ = 168.9 Hz, $^3J_{C-H}$ = 5.9 Hz). These coupling patterns are consistent with three bond coupling patterns well established for **1**.[2,3] The

assignments for C-2' and C-3' are also reversed from the previous data based on the relationship to 1[3] and also from the protonation data reported for 1[3] and 2[5].

The assignments of all the carbons except C-4 and C-4a for 4-methylprimaquine (4) were readily established by comparison with those of primaquine (1), and examination of the proton-coupled spectrum. By conducting a long-range selective proton decoupling experiment, C-4 and C-4a could be differentiated. Irradiation at δ_H 2.5 (CH_3 at C-4) at low decoupling power clearly showed the signal at δ_C 129.7 (C-4a) as a sharp doublet (three bond coupling to H-3 only) while the signal at δ_C 142.6 sharpened considerably to a dd (three bond couplings to H-2 and H-5).

The addition of a methoxyl group at C-5 as is the case for 5 causes some shifting of the aromatic carbon signals but again these assignments (Table 1) could be confirmed by comparison with 4 and by examining the proton-coupled spectrum of 5. Again, C-4 and C-4a could be distinguished by utilizing the long-range selective decoupling experiment. Irradiation at δ_H 2.8 (CH_3 at C-4) showed the signal at δ_C 124.4 (C-4a) as a doublet (three bond coupling to H-3) and signal at δ_C 143.3 (C-4) as a doublet (three bond coupling to H-2).

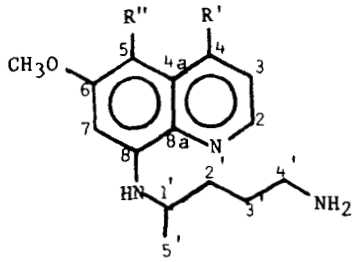
The ^{13}C-nmr assignments (Table 1) for 5-methoxyprimaquine (3) were also established by comparison with 1 and confirmation established from proton-coupled data and long-range selective proton decouplings. The proton-coupled data showed the signal at δ_C 150.1 (C-6) as a quartet ($^3J_{C-H}$ = 3.0), δ_C 142.1 (C-8)

as a sharp singlet (no three bond couplings), δ_C 134.0 and δ_C 131.5 as complex multiplets, and δ_C 124.6 (C-4a) as a doublet ($^3J_{C-H}$ = 6.8 Hz). Assignments for C-5 and C-8a were established by irradiation at δ_H 3.9 (OCH_3) at low decoupling power where the signal at δ_C 134.0 sharpened considerably as the result of loss of coupling to the methoxyl protons; the signal at δ_C 131.5 (C-8a) was unaffected.

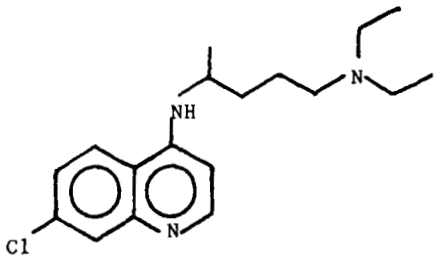
The pKa values for primaquine (1) have been determined by ^{13}C nmr shift experiments (10.39 and 3.20).[3] The addition of a methoxyl group to the nucleus appeared to have little effect on the pKa values. Using a procedure as outlined previously[3] the pKa values for 3 were determined as 10.20 and 3.09 (primary amine and quinoline, respectively).

EXPERIMENTAL

The ^{13}C nmr data were obtained at 15.03 MHz on a JEOL FX60 FT-NMR spectrometer using TMS as internal standard and $CDCl_3$ as solvent. Singlefrequency off-resonance decoupling was used to confirm multiplicity assignments. The spectra were obtained using a 45° pulse, 5 sec. repetition and 8,192 datum points. The proton-coupled data was obtained using the gated decoupling technique (decoupler off during data acquisition). The long-range selective proton decouplings were conducted by centering the decoupler on the appropriate proton resonances and conducting the decoupling (single frequency) at a power level of $\gamma H_2/2\pi$= 200 Hz. This technique was used extensively to aid in the assignments for colchicine.[9]



1, R' = R" = H
3, R' = H; R" = OCH_3
4, R' = CH_3; R" = H
5, R' = CH_3; R" = OCH_3



2

The pKa determination for **3** was conducted essentially as described previously.[3] The water acetonitrile system was used and a linear regression analysis was used for each determination. The pKa values in pure water were determined by extrapolation of the pKa values for each solvent composition.

Primaquine and chloroquine samples were obtained commercially.

ACKNOWLEDGEMENT

This investigation received the financial support of the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases. We also gratefully acknowledge Walter Reed Army Institute of Research for the sample of 4-methylprimaquine, Dr. R. C. Gupta, University of Mississippi for providing 5-methoxyprimaquine, and Dr. Tom Goodwin, Hendrix College, Arkansas for providing 5-methoxy-4-methylprimaquine.

REFERENCES

1. Baker, J.K., Bedford, J.A. Clark, A.M. and McChesney, J.D. Metabolism and Distribution of Primaquine in Monkeys. Pharm. Res. 1984; 2:98.

2. Clark, A.M., Hufford, C.D. and McChesney, J.D. Primaquine: Metabolism by Microorganisms and ^{13}C-NMR Assignments. Antimicrob. Agents Chemother. 1981; 19:337.

3. Hufford, C.D., McChesney, J.D. and Baker, J.K. Assignments of Dissociation Constants of Primaquine by ^{13}C-NMR Spectroscopy. J. Heterocylc. Chem. 1983; 20:273.

4. Singh, S.P. Parmu, S.S. and Stenberg, V.I. Carbon-13 Nuclear Magnetic Resonance Spectra of Potent Antimalarials: Primaquine and Chloroquine. J. Heterocylc. Chem. 1978; 15:9.

5. Griggs, B.G., Wilson, W.D. and Boykin, D.W. Assignments of the Carbonl3 Chemical Shifts of Quinacrine, Chloroquine and Related Compounds in D_2O Solution. Org. Mag. Res. 1978; 11:81.

6. Allahyari, R., Strother, A., Fraser, I.M. and Verbiscar, A.J. Synthesis of Certain Hydroxy Analogues of the Antimalarial Drug Primaquine and Their In Vitro Methemeglobin-Producing and Glutathione-Depleting Activity in Human Erythrocytes. J. Med. Chem. 1984; 27:407.

7. LaMontagne, M.P., Markovac, A. and Menke, J.R. Antimalarials 10. Synthesis of 4-Substituted Primaquine Analogues as Candidate Antimalarials. J. Med. Chem. 1977; 20:1122.

8. LaMontagne, M.P., Markovac, A. and Khan, M.S. Antimalarials 13. 5-Alkoxy Anlogues of 4-Methylprimaquine. J. Med. Chem. 1982; 25:964.

9. Hufford, C.D., Collins, C.C. and Clark, A.M. Microbial Transformations and ^{13}C-NMR Analysis of Colchicine. J. Pharm. Sci. 1979; 68:1239.

Date Received: 12/05/85
Date Accepted: 01/07/86