Journal of Natural Products
Vol. 51, No. 6, pp. 1273-1276, Nov-Dec 1988

DEFINITIVE ^{1}H- AND ^{13}C-NMR ASSIGNMENTS OF ARTEMISININ (QINGHAOSU)

GÁBOR BLASKÓ,[1] GEOFFREY A. CORDELL,*

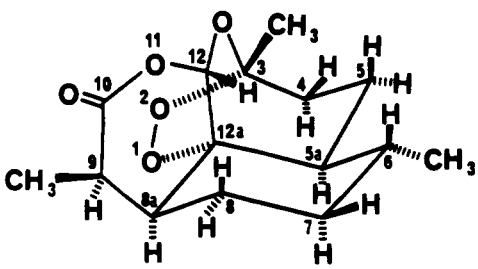
Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

and DAVID C. LANKIN

NMR Applications Laboratory, Varian Associates, 205 W. Touhy Avenue, Park Ridge, Illinois 60068

Artemisinin [**1**] (qinghaosu), isolated from *Artemisia annua* L. (Compositae), is a unique sesquiterpene lactone containing an endoperoxide bridge.[2] It is the principal constituent of the traditional Chinese herbal medicine Qinghao and is extremely active against chloroquine-resistant parasites in the treatment of cerebral malaria (2,3). The structure of **1** was determined by X-ray analysis (4), and a total synthesis has recently been developed (5). In 1985 three research groups independently reported (6–8) on the ^{13}C-nmr spectral analysis of artemisinin [**1**]; however, the three sets of assignments were almost entirely different from each other. In addition, the three papers utilized three different numbering systems for the molecule which has further confused the situation. Later, in 1987, a Chinese research group also reported (9) the ^{13}C-nmr assignment of **1** based on HETCOR and selective heteronuclear decoupling experiments. Their carbon assignments were similar to those reported by Zhongshan *et al.* (8). However, the proton assignments of **1** were deduced from the HETCOR experiment, and, thus, the chemical shift values were reported without the stereotopical assignments of nonequivalent protons.

Due to the great importance of artemisinin [**1**] and related compounds (10,11) as potential antimalarial drugs and the substantial interest concerning its chemistry and biosynthesis, we decided to resolve the existing controversy in the literature and provide rigorous and unambiguous assignments for the ^{1}H- and ^{13}C-nmr spectra of **1**. As we have done previously, we emphasize the substantial philosophical difference between proving ^{1}H- and ^{13}C-nmr assignments and reaching conclusions based on prior data.



1

Assignment of the ^{1}H-nmr spectrum of **1** was based on homonuclear COSY and two dimensional nOe (12) (NOESY) measurements. The chemical shift values of the three methyl groups, the 4-H,

[1]On leave from the Central Research Institute for Chemistry of the Hungarian Academy of Sciences, Budapest, Hungary.

[2]For a recent comprehensive review on artemisinin see Klayman (1).

5-H, 7-H, 8-H methylenes and the 5a-H, 6-H, 9-H, 12-H methine protons[3] were determined according to the corresponding geminal and vicinal coupling pattern apparent in the ^{1}H-^{1}H COSY spectrum, confirming the previous preliminary ^{1}H-nmr assignments (8,13,14). The NOESY spectrum (Figure 1) showed steric proximity, similar interactions were observed among 8a-H (δ 1.75, m), 5a-H (δ 1.37, m), and one of the 7-H protons (δ 1.08, m) that should be 7-H_α. NOe enhancement was also observed between 9-H (δ 3.90, qd) and 8a-H (δ 1.75, m) and between 4-H_α (δ 2.43, ddd) and 5-Hα (δ 2.01, m) permitting the complete and unambiguous assignment of the ^{1}H-nmr spectrum of **1** (Table 1). Substantial refinement of the ^{1}H-nmr assignments made previously (9) is now possible.

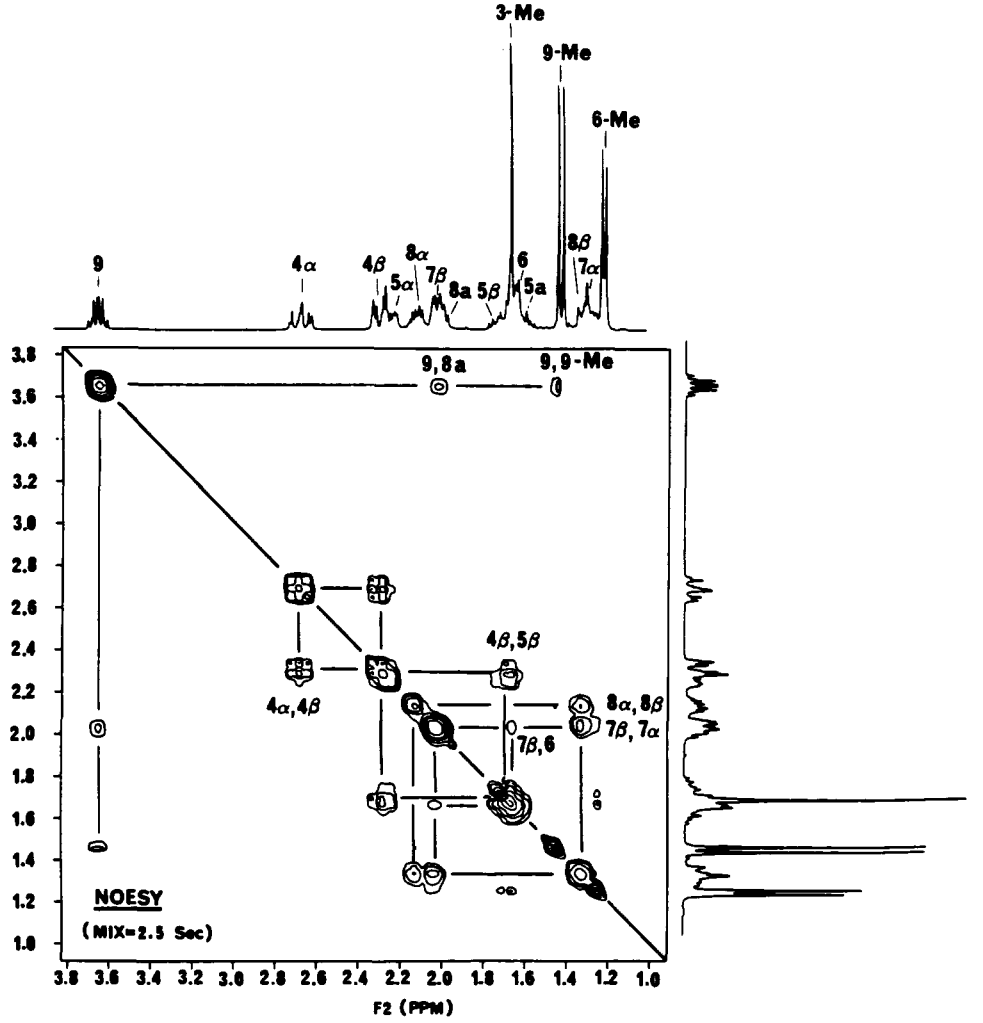


FIGURE 1. NOESY Spectra of Artemisinin [1].

The ^{13}C-nmr chemical shifts of artemisinin [**1**] were assigned through a series of APT, CSCM 1D (15), selective

[3]The numbering system used for artemisinin [**1**] is that recommended by IUPAC.

TABLE 1. ^{1}H- and ^{13}C-NMR Assignments of Artemisinin [**1**].[a]

Proton	**1**[b]	Carbon	**1**
4α	2.43 (ddd, 14.7, 13.6, 4.2)		
4β	2.05 (ddd, 14.7, 5.2, 3.4)	3	105.22
5α	2.01 (m, 14.5, 5.5)	4	35.77
5β	1.47 (m, 14.5, 5.5)	5	24.79
5a-(α)	1.37 (m, 11.5, 7.0)	5a	49.90
6α	1.42 (m, 13.0, 6.4)	6	37.42
7α	1.08 (m, 13.5)	7	33.45
7β	1.79 (m, 13.5)	8	23.32
8α	1.87 (m, 14.0)	8a	44.80
8β	1.12 (m, 14.0)	9	32.78
8a-(α)	1.75 (m, 13.5, 5.5)	10	171.92
9	3.40 (qd, 7.2, 5.4)	12	93.62
12	5.87 (s)	12a	79.38
3-Me	1.44 (s)	3-Me	25.10
6-Me	0.99 (d, 6.4)	6-Me	19.74
9-Me	1.21 (d, 7.2)	9-Me	12.47

[a]Recorded in $CDCl_3$. Chemical shift values are reported in δ values downfield from internal TMS.

[b]Multiplicity and coupling constants (Hz) are shown in parentheses. For overlapped multiplets not every coupling constant could be identified, and in some cases only the half-height of the signal (in Hz) could be determined.

INEPT (16) and HETCOR experiments. One-bond polarization transfer experiments (CSCM 1D) and the HETCOR spectrum confirmed all the methyl group (δ 12.47, 19.74, and 25.10 for C-9, C-6, and C-3 methyl groups, respectively) and methylene group signals (δ 23.32, 24.79, 33.45, and 35.77 for C-8, C-5, C-7, and C-4, respectively). The double cross peaks for the methylene carbons further confirmed the assignment of the 4-H, 5-H, 7-H, and 8-H proton pairs in the ^{1}H-nmr spectrum. Due to the lack of separation in the proton spectrum of 5a-H, 6-H, and 8a-H absorptions from other signals, CSCM 1D and HETCOR experiments permitted only the unambiguous assignment of the C-9 methine carbon at δ 32.78. Selective INEPT irradiation of 9-Me enhanced the carbon signals at δ 171.92 and 44.80, thereby establishing the assignment of C-12 and C-8a, respectively. Irradiation of the 6-Me using ^3J values of 2.5, 4.0, or 6.0 Hz, however, enhanced the C-7 methylene carbon (δ 33.45) and two methine carbons at δ 37.42 and 49.90, which did not allow for the unambiguous assignment of C-5a and C-6. The latter were distinguished when the well-resolved 4-Hα was irradiated resulting in enhancements at δ 49.90 (C-5a) and δ 25.10 (3-Me). Assignment of C-5a was further confirmed when 12-H was irradiated, and the enhancements of δ 49.90 (C-5a), 105.22 (C-3) and 171.54 (C-10) were observed. In this way all of the carbon-13 resonances of artemisinin [**1**] were proven (Table 1), and the data reported by Zhongshan *et al.* (8) and Huang *et al.* (9) were confirmed. Revisions are necessary for the carbon-13 assignments appearing in Acton and Klayman (6) and El-Feraly *et al.* (7).

EXPERIMENTAL

^{1}H-nmr spectra were determined on a Varian XL-300 or a Varian VXR-300 spectrometer operating at 300 MHz. Standard Varian pulse programs were used for the ^{1}H-^{1}H COSY and NOESY spectra. The HETCOR spectrum was obtained on the Varian XL-300 spectrometer operating at 300 MHz for ^{1}H and 75.4 MHz for ^{13}C. APT spectrum was recorded on the x-axis, ^{1}H-nmr spectrum on the y-axis. ^{13}C-nmr measurements, CSCM 1D, and selective INEPT

spectra were obtained on a Nicolet NMC-360 spectrometer operating at 90.8 MHz.

ACKNOWLEDGMENTS

This work was supported, in part, by a grant from the Tsumura Juntendo, Inc., Research Foundation, New York, NY. The authors thank the Research Resources Center, University of Illinois at Chicago, for the provision of nmr spectroscopic facilities.

LITERATURE CITED

1. D.L. Klayman, *Science*, **228**, 1049 (1985).
2. L.J. Bruce-Chwatt, *Brit. Med. J.*, **284**, 767 (1982).
3. X.-O. Luo and C.-C. Shen, *Med. Res. Rev.*, **7**, 29 (1987).
4. J.-M. Liu, M.-Y. Wu, J.-F. Fan, Y.-Y. Tu, Z.-H. Wu, Y.-L. Wu, and W.-S. Chou, *Acta Chim. Sin.*, **37**, 129 (1979).
5. G. Schmid and W. Hofheim, *J. Am. Chem. Soc.*, **105**, 624 (1983).
6. N. Acton and D.L. Klayman, *Planta Med.*, **441**, (1985).
7. F.S. El-Feraly, M.M. El-Sherei, C.D. Hufford, E.M. Croom, and T.J. Mahier, *Spectrosc. Lett.*, **18**, 843 (1985).
8. W. Zhongshan, T.T. Nakashima, K.R. Kopecky, and J. Molina, *Can. J. Chem.*, **63**, 3070 (1985).
9. J.-J. Huang, K.M. Nicholls, C.-H. Cheng, and Y. Wang, *Acta Chim. Sin.*, **45**, 305 (1987).
10. X.D. Luo, J.C.Y. Herman, A. Brossi, J.L. Flippen-Anderson, and R. Gilardi, *Helv. Chim. Acta*, **67**, 1515 (1984).
11. Y. Li, P.L. Xu, Y.X. Chen, L.Q. Li, Y.Z. Gai, D.S. Wang, and Y.P. Zhena, *Yaoxue Xuebao*, **16**, 429 (1981).
12. D.J. States, R.A. Haberkorn, and D.J. Ruben, *J. Magn. Reson.*, **48**, 286 (1982).
13. Qinghaosu Research Group, *Kexuetongbao*, **22**, 142 (1977).
14. B. Wang and F. Yin, *Zhongcaoyao*, **97**, 101 (1984).
15. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
16. S.K. Sarkar and A. Bax, *J. Magn. Reson.*, **62**, 109 (1985).

Received 16 May 1988