Journal of Natural Products
Vol. 51, No. 1, pp. 88-93, Jan-Feb 1988

NMR STUDIES OF COLCHICINE AND ITS PHOTOISOMERS, β- AND γ-LUMICOLCHICINES

DUANGDEUN MEKSURIYEN, LEE-JUIAN LIN, GEOFFREY A. CORDELL,

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

SIBABRATA MUKHOPADHYAY, and SUNIL K. BANERJEE

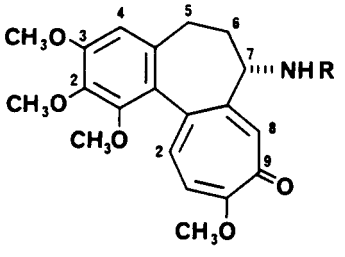
Department of Organic Chemistry, Regional Research Laboratory, Council on Scientific and Industrial Research, Jammu Tawi, India

ABSTRACT.—Unambiguous ^{1}H- and ^{13}C-nmr assignments of colchicine [**1**], β-lumicolchicine [**3**], and γ-lumicolchicine [**4**] have been obtained using a combination of 1D- and 2D-nmr spectroscopic techniques. The data indicate that γ-lumicolchicine [**4**] is a stereoisomer of β-lumicolchicine [**3**] and provide definitive evidence for their stereochemical assignments.

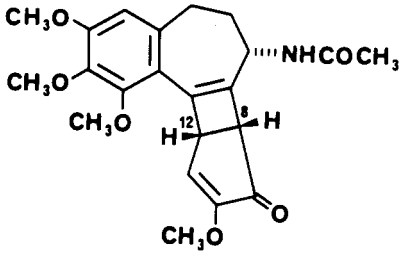
Colchicine [**1**], the principal alkaloid of *Colchicum autumnale* L., is found in at least ten other genera of the family Liliaceae (1). It is a highly active molecule biologically, possessing anti-inflammatory, antimitotic, and tumor-inhibiting activities (2). Several reviews of the chemistry and pharmacologic properties of **1** are available (3–8).

In attempting to apply Santavy's method (7) for the extraction of colchicine from *C. autumnale* to the tubers of *Gloriosa superba* L., a low yield was experienced in one of our laboratories (CSIR). Efforts then centered on the development of an alternative large-scale process for the extraction of *G. superba*. This process is based on the crystallization of the $CHCl_3$ extract of the H_2O-soluble fraction from EtOAc. The mother liquor still contains substantial quantities of **1**, which can be recovered through chromatography to afford pure **1**, together with *N*-formyl-*N*-desacetylcolchicine [**2**], β-lumicolchicine [**3**], and γ-lumicolchicine [**4**].

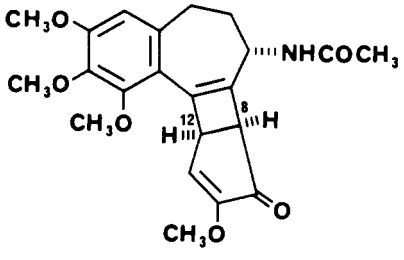
The latter two compounds were obtained initially as products of the uv irradiation of colchicine [**1**] (9–11) and were the first examples of photochemical electrocyclic reac-



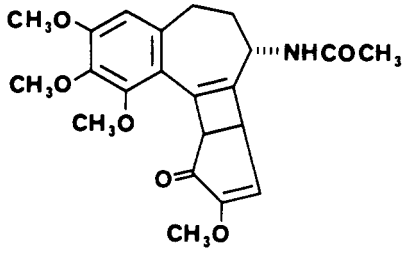
1 R=Ac
2 R=CHO



3



4



5

tions in the troponoids. They are also well-established natural products. Chapman and co-workers (12) established the structure and stereochemistry of β-lumicolchicine as **3** based on $NaBH_4$ reduction and the observed internal hydrogen bonding of the hydroxyl proton with the acetamido group. They inferred, based on the similarity of the uv, ir, and nmr spectra, that γ-lumicolchicine had the structure **4**, although structure **5** was not rigorously eliminated (12). Subsequently, Canonica and co-workers (13) deduced that **4** was, indeed, correct based on studies with the corresponding colchicine derivatives. However, substantive spectroscopic evidence for these structures and, in particular, verification of the structure of γ-lumicolchicine are lacking.

There are available in the literature several significantly different reports concerning the ^{13}C-nmr assignments of colchicine (14–21). These were made on the basis of ^{13}C-nmr shift theory, the multiplicities generated in the SFORD spectra, the intensities of the signals, and the comparison with structurally related compounds and selective single-frequency proton decoupling experiments. As a part of our efforts to assign the ^1H- and ^{13}C-nmr spectra of the photochemical isomers of colchicine, it became important to establish unambiguously the complete assignments of colchicine [**1**] through application of the nmr techniques, selective INEPT (22) and CSCM 1D (one-dimensional heteronuclear chemical shift via one-bond correlation) (23). These experiments, however, require that the ^1H-nmr spectrum be assigned. The ^1H-nmr analysis of colchicine has been described, but is incomplete for the protons in the cycloheptadiene ring (24–29) and the methoxyl protons (30). This led us to reestablish the proton assignments of colchicine.

The ^1H-nmr spectrum of colchicine was effectively attributed by an analysis of the homonuclear COSY and nOe difference spectra. A quadrupole broadened doublet at 8.64 ppm was assigned to a proton attached to acetamido nitrogen, which was coupled to H-7. A broad singlet at 7.69 ppm was assigned to H-8. An AB pattern ($J = 11$ Hz) at 7.39 and 6.93 ppm was assigned to the tropolone protons H-12 and H-11, respectively, the former being assigned on the evidence of a long-range coupling with H-8. The latter proton also gave evidence of long-range coupling with the methoxyl proton singlet at 4.03 ppm that could be assigned to 10-OCH_3. A singlet aromatic proton at 6.55 ppm for H-4 displayed long-range coupling with the methoxyl proton singlet at 3.92 ppm that could be assigned to 3-OCH_3 protons. A doublet of triplets at 4.66 ppm was assigned to H-7, based on the observed coupling with both the H-6 protons ($J = 11.8$, 6 and 5.8 Hz) and with the NH proton ($J = 6$ Hz). The former was further confirmed by decoupling difference spectroscopy; i.e., saturation of C-7 revealed the presence of two H-6 protons at 2.38 and 2.01 ppm. Attempts to assign the two sets of methylene protons at C-5 and C-6 using this technique and 2D-*J* resolved spectroscopy failed, principally due to the second-order spin effects. On the basis of the large coupling constant between H-7 and one of the H-6 protons this must be a *trans*-diaxial relationship, and, consequently, the acetamido group was equatorial (8,29).

Given the availability of the molecular conformation of a colchicine derivative, through single crystal X-ray crystallographic analysis (8,31), nOe difference spectra (32,33) were calculated to assign the four methoxy singlets unambiguously. Irradiation of the methoxy singlet at 4.03 ppm showed a nOe enhancement of H-11 (6.93 ppm, 5.6%), which confirmed that this signal represented the methoxyl group on the tropolone ring, i.e., 10-OCH_3. Irradiation of H-4 at 6.55 ppm showed a nOe enhancement of 3-OCH_3 (3.92 ppm, 1%). These two methoxyl group attributions were in agreement with the homonuclear COSY spectrum described above. Irradiation of H-12 (7.39 ppm) showed enhancement of H-11 (6.93 ppm, 7.1%) and the 1-OCH_3 (3.67 ppm, 0.4%). Comparison of our data with the literature values (27,30) indicates some

TABLE 1. ^{1}H-nmr Spectral Data of Colchicine [**1**], β-Lumicolchicine [**3**], and γ-Lumicolchicine [**4**].[a,b]

Proton	Colchicine [**1**]	β-Lumicolchicine [**3**]	γ-Lumicolchicine [**4**]
4	6.55 s	6.50 s	6.48 s
5	2.54 m	2.77 dd (15.3, 8.7)	2.66 m
6	2.38 m 2.38 m 2.01 m	2.60 dd (15.3, 8.8) 2.00 m	1.96 m
7	4.66 dt (11.8, 6, 5.8)	4.82 m	4.66 dt (7.6, 6.5, 6.5)
8	7.69 s	3.61 dd (2.6, 1.8)	3.63 dd (2.8, 1.0)
11	6.93 d (11.0)	6.67 d (3.1)	6.62 d (3.8)
12	7.39 d (11.0)	4.11 dd (3.1, 2.6)	4.04 dd (3.8, 2.8)
14	1.96 s	2.06 s	2.02 s
1-OCH_3	3.67 s	3.98 s	3.96 s
2-OCH_3	3.95 s	3.88 s	3.87 s
3-OCH_3	3.92 s	3.86 s	3.86 s
10-OCH_3	4.03 s	3.68 s	3.69 s
NH	8.64 d (6.0)	6.17 d (7.2)	6.02 d (7.6)

[a]Determined at 360 MHz in $CDCl_3$, δ TMS=0 ppm.
[b]Data are expressed as δ H, multiplicity, (J, Hz).

changes in the attribution of the methoxy protons. Our proton assignments for colchicine are shown in Table 1.

With the proton assignments in hand, CSCM 1D (23) then permitted a detailed analysis of all hydrogen-bearing carbons. Magnetization transferred from the downfield ^{13}C satellite of H-12 afforded a positive resonance for C-12 at 135.8 ppm, and magnetization transferred from the upfield ^{13}C satellite of H-11 showed a negative resonance for C-11 at 113.1 ppm and a positive resonance for C-4 at 107.3 ppm. Similarly, irradiation of the downfield ^{13}C satellite of 10-OCH_3 positively enhanced the 10-OCH_3 at 56.5 ppm and to a lesser extent the 2-OCH_3 at 61.3 ppm. On the other hand, irradiation of the upfield ^{13}C satellite of 3-OCH_3 yielded negative enhancements for the 2-OCH_3 at 61.3 ppm and the 3-OCH_3 at 56.1 ppm. When the upfield ^{13}C satellite of H-6 was irradiated, two negative resonances for C-14 (22.6 ppm) and C-6 (36.1 ppm) were observed.

Given the known heteronuclear long-range coupling constants of colchicine [**1**] (20,21), the selective INEPT experiment (22) was performed for the assignment of the non-protonated carbons. Observation of the tropolone and aromatic ring carbons substituted by methoxyl groups was achieved with optimum values for the delays Δ_1 and Δ_2 for $J=4$ Hz. Selective irradiation of the methoxyl groups attached to C-1, C-3, and C-10 resulted in the assignment of these carbons with some intensity enhancement of C-2 due to partial saturation of the 2-OCH_3 resonance.

For the three-bond couplings of the quaternary aromatic and tropolone carbons, optimal values for the delays for $J=9$ Hz were determined. Irradiation of H-8 resulted in substantial enhancement of C-10, C-12a, and C-7, and to a lesser extent C-9 and C-7a. When H-12 was selectively irradiated, C-10, C-7a, and C-12b were enhanced as expected. The observed partial enhancement of C-12a was thought to be due to two-bond coupling to H-12, and the small negative enhancement of C-8 could possibly be due to residual selective population transfer from the upfield ^{13}C satellite of H-8. Finally, irradiation of H-4 caused the enhancement of C-2 and C-12b and to a lesser extent C-3 and C-4a due to two-bond coupling with H-4, as well as small enhancements of C-1 and C-12a due to four-bond coupling with H-4.

These complete ^{13}C data (Table 2) are in general agreement with those described previously (19,20) but with the ambiguities resolved.

TABLE 2. ^{13}C-nmr Spectral Data of Colchicine [**1**], β-Lumicolchicine [**3**], and γ-Lumicolchicine [**4**].[a]

Carbon	Colchicine [**1**]	β-Lumicolchicine [**3**]	γ-Lumicolchicine [**4**]
1	151.0	151.6	151.7
2	141.4	140.2	140.2
3	153.4	153.0	153.0
4	107.3	109.1	109.1
4a	134.3	138.7	138.8
5	29.8	32.5	32.2
6	36.1	31.3	30.8
7	52.9	51.3	49.1
7a	153.0	137.3	138.5
8	130.0	51.4	50.1
9	179.4	200.7	198.6
10	163.9	157.7	157.2
11	113.1	128.7	127.8
12	135.8	43.1	43.0
12a	137.1	145.0	146.6
12b	125.5	117.7	117.7
13	170.2	170.3	169.3
14	22.6	23.4	23.5
1-OCH_3	61.5	61.4	61.3
2-OCH_3	61.3	60.8	60.8
3-OCH_3	56.1	55.9	55.9
10-OCH_3	56.5	56.8	56.8

[a]Determined at 90.8 MHz in $CDCl_3$, δ TMS=0 ppm.

With the ^{1}H- and ^{13}C-nmr assignments of colchicine [**1**], assured, we turned our attention to the assignments of the photoisomers, β- and γ-lumicolchicines. The very close chemical shifts of similar functional groups posed some interesting problems in spectral assignment.

Some of the ^{1}H-nmr assignments of β-lumicolchicine have been reported previously (9,12). We have substantiated these and also achieved the assignments for the aromatic methoxyl groups through nOe difference spectroscopy. Irradiation of the singlet at 3.68 ppm produced a nOe enhancement (10.5%) in the doublet for H-11 at 6.67 ppm; the singlet therefore could be assigned to the 10-OCH_3. Irradiation of the singlet at 3.98 ppm produced enhancement of H-12 (4.11 ppm, 5.1%), H-11 (6.67 ppm, 2.6%), and H-8 (3.61 ppm, 2%), the indirect enhancement of the latter signal occurring due to the close proximity of H-8 and H-12, allowing their mutual cross-relaxation to transmit population disturbance to H-8. The resonance at 3.98 ppm must, therefore, be due to the 1-OCH_3 (3.86 ppm, 1.6%). Irradiation of H-4 (6.50 ppm) gave enhancement of 3-OCH_3 and H-5. The assignment of 3-OCH_3 was confirmed by the long-range coupling with H-4 in the COSY spectrum. The ^{1}H-nmr assignments for β-lumicolchicine [**4**] are shown in Table 1.

CSCM 1D techniques were used to achieve assignments for the protonated carbons. Thus, magnetization transferred from the downfield ^{13}C satellite of H-11 gave positive resonances for C-11 at 128.7 ppm and C-4 at 109.1 ppm and from the upfield ^{13}C satellites of H-7 and H-8 displayed negative resonances for C-7 at 51.3 ppm and C-8 at 51.4 ppm, respectively. Irradiation of the downfield satellite of 1-OCH_3 produced positive resonances for 1-OCH_3 at 61.4 ppm and for C-12 at 43.1 ppm, while irradiation of the upfield ^{13}C satellite of H-6 revealed negative resonances for C-6 at 31.3 ppm and for C-14 at 23.4 ppm.

A series of SINEPT experiments was used to assign the quaternary carbons. Irradia-

tion of the methoxyl protons on C-1, C-2, and C-10 gave a clear enhancement of the respective quaternary aromatic compounds. Irradiation of H-4 using 9 Hz for calculation of the delay resulted in the substantial enhancement of C-2, C-12b, and C-5 and to a lesser extent C-3, C-1, and C-11. The first two signals were enhanced due to two- and four-bond coupling to H-4, whereas the coupling for C-11 was enhanced due to residual spin population transfer (SPT) from the upfield ^{13}C satellite of H-11. Irradiation of H-11, using 6 Hz to establish the delay, gave significant enhancement to three signals (C-9, C-8, and C-12a) and lesser enhancement to C-10 and C-12. The complete ^{13}C assignments of β-lumicolchicine [**3**] are shown in Table 2.

The ^{1}H and ^{13}C assignments for γ-lumicolchicine [**4**] were made with the same series described above and are shown in Tables 1 and 2, respectively. In this instance, the nOe difference experiment did not permit the assignment of the 1-OCH_3 at 3.96 ppm, and, consequently, a 2D-nOe spectrum was obtained.

The data indicate that β-lumicolchicine [**3**] and γ-lumicolchicine [**4**] are stereoisomers at the C-8,12 ring junction, as had been suspected previously (12). Additional experiments provided the respective stereochemistry of these two compounds. Thus, the existence of a nOe among the 1-OCH_3, H-12, and H-11 in β-lumicolchicine [**3**] and the absence of these enhancements on irradiation of 1-OCH_3 in γ-lumicolchicine [**4**] indicated that in **3** these protons must be proximate. In β-lumicolchicine [**3**] irradiation of H-7 led to nOe enhancement of the NH (2.0%), H-6 (1.3%), H-5β (0.8%), and, most importantly, H-8 (2.7%). On the other hand, irradiation of the same signal in **4** enhanced the NH (2.0%), H-6 (1.1%), and H-5β (0.5%) but not H-8. In retrospect, the deshielding of C-6, C-7, C-8, and C-9 indicates that these carbons are somewhat closer in **3** than they are in **4**. Consequently, in **3** H-7 and H-8 are both β, whereas in γ-lumicolchicine [**4**] these protons are *trans* to each other.

EXPERIMENTAL

Colchicine used in this study was purchased from Sigma Chemical Co., St. Louis, Missouri. Approximately 100 mg of colchicine was dissolved in 0.5 ml of $CDCl_3$, containing 0.1% TMS as an internal standard. The solution was transferred to 5-mm nmr tubes and used for all nmr studies.

The ^{1}H-nmr spectra were obtained with Nicolet NMC 360 (360 MHz) and Varian XL-300 (300 MHz) spectrometers. All ^{13}C-nmr spectra were measured at 90.8 MHz using a Nicolet NMC 360 spectrometer. Data are reported in ppm downfield from TMS.

Homonuclear COSY and 2D-*J* resolved spectra were recorded at 1K with a Varian XL 300 spectrometer. Standard Varian pulse programs were used. NOESY spectra were recorded at 1K with a Nicolet NMC 360 spectrometer (34). NOe and decoupling difference spectroscopy were measured on the Nicolet NMC 360 spectrometer. Both nOe and control spectra were acquired by interleaving simultaneously under identical conditions apart from the frequency of irradiation time; the power level and time of irradiation were the same in both spectra (32). Subsaturating power levels were selected to achieve adequate frequency selectivity, and a presaturation time following a 90° pulse was set at 20 sec to ensure complete recovery of the equilibrium magnetization for all nuclei of interest. The delay time was 100 μsec to ensure appropriate frequency switching. Data sets of 16K covering a spectral width of 2000 Hz were acquired, and a 2.0 Hz line broadening was applied to the data prior to Fourier transformation. The samples for nOe experiments were degassed using a repeated freeze-pump-thaw cycle and then closed under N_2 (subaseal).

The one-dimensional heteronuclear ^{1}H-^{13}C shift correlation (CSCM 1D) and selective INEPT experiments were performed on a Nicolet NMC 360 spectrometer. Data sets of 16K covering a spectral width of 10,000 Hz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% C_6D_6 (ir $J = 6.7$ Hz) in a 5-mm nmr tube (35). The radio frequency field strength of the soft proton pulse was on the order of 25 Hz for the performance of CSCM 1D (23) and selective INEPT (22) experiments.

ACKNOWLEDGMENTS

This work was supported, in part, by grant CA-20164 from the Division of Cancer Treatment, National Cancer Institute, Bethesda, Maryland. We thank the Research Resources Center, University of Illinois at Chicago, for the provision and maintenance of high-field nmr spectroscopic facilities. Part of this work was carried out when one of us (L.-J.L.) was the recipient of Dorothea H. Fleming Graduate Student Fellowship.

LITERATURE CITED

1. G.A. Cordell, "Introduction to Alkaloids—A Biogenetic Approach," Wiley-Interscience, New York, 1981.
2. S.L. Wallace, *Am. J. Med.*, **30**, 439 (1961).
3. J.W. Cook and J.D. London, *Alkaloids (N.Y.)*, **2**, 261 (1952).
4. W.C. Wildman, *Alkaloids (N.Y.)*, **6**, 247 (1960).
5. K.R. Fell and D.R. Ramsden, *Lloydia*, **30**, 123 (1967).
6. W.C. Wildman, in: "Chemistry of the Alkaloids." Ed. by S.W. Pelletier, Van Nostrand Reinhold, New York, NY, 1970, p. 199.
7. F. Santavy, *Acta Univ. Palack. Olou.*, **90**, 15 (1979).
8. H.G. Capraro and A. Brossi, *Alkaloids (N.Y.)*, **23**, 1 (1984).
9. R. Grewe and W. Wulf, *Chem. Ber.*, **84**, 621 (1951).
10. F. Santavy, *Biol. Listy*, **31**, 246 (1950).
11. E.J. Forbes, *J. Chem. Soc.*, 3864 (1955).
12. O.L. Chapman, H.G. Smith, and R.W. King, *J. Am. Chem. Soc.*, **85**, 803 (1963).
13. L. Canonica, B. Danieli, P. Manito, and G. Russo, *Tetrahedron Lett.*, 607 (1969).
14. A.R. Battersby, P.W. Sheldrake, and J.A. Milner, *Tetrahedron Lett.*, 3315 (1974).
15. S.P. Singh, S.S. Pamar, V.I. Stenberg, and S.A. Farnum, *Spectrosc. Lett.*, **10**, 1001 (1977).
16. C.D. Hufford, C.C. Collins, and A.M. Clark, *J. Pharm. Sci.*, **68**, 1239 (1979).
17. P. Sedmera, H. Potesilova, V. Malichova, V. Preininger, and F. Santavy, *Heterocycles*, **12**, 337 (1979).
18. A. Blade-Font, R. Muller, J. Elguero, R. Faure, and E.J. Vincent, *Chem. Lett.*, 233 (1979).
19. J. Elguero, R.N. Muller, A. Blade-Font, R. Faure, and E.J. Vincent, *Bull. Soc. Chim. Belg.*, **89**, 193 (1980).
20. B. Danieli, G. Palmisano, and G. Severini Ricca, *Gazz. Chim. Ital.*, **110**, 351 (1980).
21. C.D. Hufford, H.-G. Caprano, and A. Brossi, *Helv. Chim. Acta*, **63**, 50 (1980).
22. A. Bax, *J. Magn. Reson.*, **57**, 314 (1984).
23. S.K. Sarkar and A. Bax, *J. Magn. Reson.*, **62**, 109 (1985).
24. G.L. Buchanan, A.L. Porte, and J.K. Sutherland, *Chem. Ind. (London)*, 859 (1962).
25. A.D. Cross, F. Santavy, and B. Trivedi, *Collect. Czech. Chem. Commun.*, **28**, 3402 (1963).
26. A.D. Cross, A. El-Hamidi, J. Hrbek, Jr., and F. Santavy, *Collect. Czech. Chem. Commun.*, **29**, 1187 (1964).
27. V. Delaroff and P. Rathle, *Bull. Soc. Chim. Fr.*, 1621 (1965).
28. A.D. Cross, A. El-Hamidi, L. Pijewska, and F. Santavy, *Collect. Czech. Chem. Commun.*, **31**, 374 (1966).
29. V.V. Kiselv and M.E. Perel'son, *Khim. Prir. Soedin.*, 535 (1974).
30. H. Yamamoto, A. Hara, S. Inokawa, and T. Nozoe, *Bull. Chem. Soc. Jpn.*, **56**, 3106 (1983).
31. M.A. Iorio, A. Brossi, and J.V. Silverton, *Helv. Chim. Acta*, **61**, 1213 (1978).
32. L.D. Hall and J.K.M. Sanders, *J. Am. Chem. Soc.*, **102**, 5703 (1980).
33. D. Neuhaus, R.N. Sheppard, and I.R.C. Bick, *J. Am. Chem. Soc.*, **105**, 5996 (1983).
34. R.E. Hurd, *Relax. Times*, **4**, 6 (1984).
35. A. Bax, *J. Magn. Reson.*, **52**, 76 (1983).

Received 8 June 1987