40. Revision of the Structure of 'Epoxycolchicine'

Arnold Brossi[1)2]), **Manfred Rösner**[2]), and **James V. Silverton**[3])

National Institutes of Health, Bethesda, Maryland 20205, USA

and **Maria A. Iorio**

Istituto Superiore di Sanità, 00161 Roma, Italy

and **Charles D. Hufford**

School of Pharmacy, University of Mississippi, University, Mississippi 38677, USA

(29.XI.79)

Revision der Struktur von «Epoxycolchicin»

Zusammenfassung

Die für natürliches «Epoxycolchicin» vorgeschlagene Struktur **2** muss aufgrund einer Kristall-Röntgenstrukturanalyse zu derjenigen des cyclischen Acetals **3** abgeändert werden. Es wird vorgeschlagen, den Namen «Epoxycolchicin» aus der Literatur zu streichen, da er irreführend ist.

Oxidation of colchicine (**1**) with aqueous sodium peroxide afforded in about 10% yield a product which was identical in every respect (see exper. part) with natural 'epoxycolchicine'[4]). The latter was recently isolated from *Colchicum latifolium* S.S. by *Šantavý et al.* [1] and structure **2** was proposed on the basis of physical, primarily NMR. data. It seemed unlikely that an α-epoxyketone of structure **2** would resist the alkaline reaction conditions during the oxidation of **1** with sodium peroxide, and the material prepared from colchicine (**1**) was therefore further investigated.

The ^{13}C-NMR. assignments of all the C-atoms in colchicine (**1**) and a number of its derivatives have recently been published [2–4]. A comparison of these data with those obtained for 'epoxycolchicine' *(Table 1)*, revealed that structure **2** was not compatible with its ^{13}C-NMR. spectrum, as shown by the appearance of four olefinic or aromatic C-atom signals assigned to C(4), C(8), C(11) and C(12) at 109.8, 128.8, 143.2 and 110.6 ppm as doublets and a signal for C(12a) at 65.8 ppm as a singlet. These data suggested that the proposed structure of 10,11-epoxycolchicine (**2**) is incorrect and that ring C should have a different arrangement of the extra oxygen function. It was therefore decided to determine the structure of

[1]) To whom correspondence should be addressed.

[2]) Medicinal Chemistry Section, Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases.

[3]) Laboratory of Chemistry, National Heart, Lung, and Blood Institute.

[4]) We thank Prof. Dr. *F. Šantavý* for a sample of this material.

0018-019X/80/2/0406-07$01.00/0

Table 1. *^{13}C-NMR. spectral data ($CDCl_3$) of 'epoxycolchicine'*

C-atom	1[a])	3[b])
C(1)	151.4 *s*	155.9 *s*
C(2)	142.2 *s*	142.1 *s*
C(3)	153.8 *s*	153.1 *s*
C(4)	107.9 *d*	109.8 *d*
C(5)	30.1 *t*	31.6 *t*
C(6)	36.6 *t*	36.4 *t*
C(7)	52.8 *d*	49.5 *d*
C(8)	130.7 *d*	128.8 *d*
C(9)	179.6 *s*	198.0 *s*
C(10)/C(9a)[c])	164.3 *s*	107.3 *s*
C(11)	113.1 *d*	143.2 *d*
C(12)	134.5 *s*	110.6 *d*
C(12b)	126.0 *s*	120.0 *s*
C(4a)	134.4 *s*	136.0 *s*
C(7a)	152.6 *s*	181.5 *s*
C(12a)	137.2 *s*	65.8 *s*
CH_3O–C(1)	61.3 *qa*[d])	60.5 *qa*[d])
CH_3O–C(2)	61.5 *qa*[d])	61.4 *qa*[d])
CH_3O–C(3)	56.3 *qa*[e])	55.9 *qa*
CH_3O–C(10)/CH_3O–C(9a)[c])	56.5 *qa*[e])	53.0 *qa*
CH_3CO	170.0 *s*	168.9 *s*
CH_3CO	22.7 *qa*	23.3 *qa*

[a]) The data and assignments are listed here only for comparison [2–4].

[b]) The assignment of the signal at 153.1 ppm to C(3) is based only on the presumption that its chemical shift would be unaffected by structural changes at C(12a) whereas the chemical shift of C(1) would be affected (*cf.* data of **1**).

[c]) Carbon atoms of colchicine (**1**) and **3**, respectively.

[d]) Assignments may be reversed.

[e]) Assignments may be reversed.

semisynthetic 'epoxycolchicine' by a single crystal X-ray analysis. For this purpose the colorless platelets of m.p. 251°, obtained by crystallization from ethyl acetate, were used. The X-ray analysis resulted in the structure of the cyclic acetal **3** for 'epoxycolchicine', and the ^{13}C-NMR. assignments are in excellent agreement with this structure. This follows from chemical shift theory, single-frequence off-resonance decoupling and comparison with colchicine and its derivatives [2–4]. The assignments for C(4), C(8), C(11) and C(12) were confirmed by selective single-frequency decoupling experiments.

X-ray analysis (see *Figure*). The crystal conformation, X-ray nomenclature and bond lengths of **3**, which resulted from the X-ray analysis, are shown as an ORTEP-drawing [5] in the *Figure*. It was assumed that the configuration of the N-atom was the same as that of colchicine and, given the small size of the crystals, it did not seem practical to attempt to measure the weak reflections necessary to determine the absolute configuration by the anomalous scattering of oxygen. There is probably no reason to suspect that the chemical reactions employed would change the configuration from that of natural colchicine.

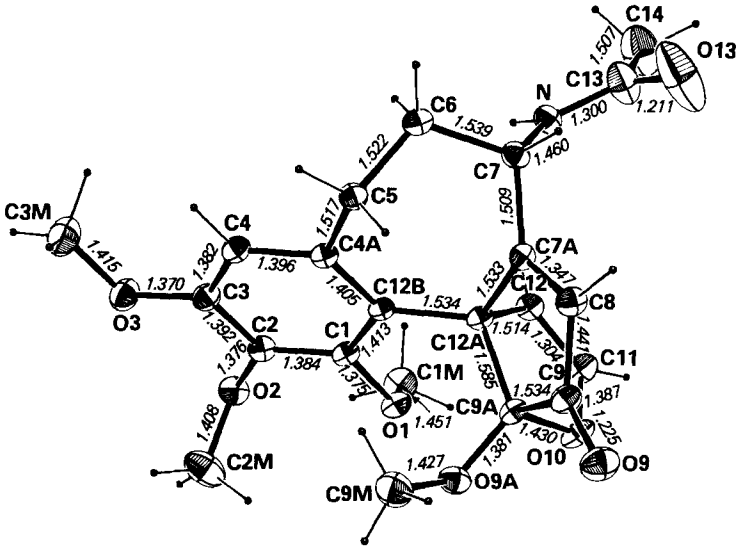
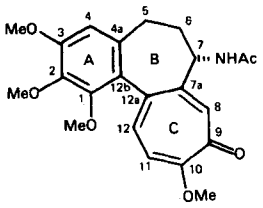


Figure. *The X-ray structure of* **3** *showing atomic numbering, bond lengths and apparent thermal ellipsoids (probability level 20%).* Hydrogen atoms are represented by arbitrary spheres. Esds of bond lengths are less than 0.005 Å, except in the acetamido side chain, where they are less than 0.006 Å.

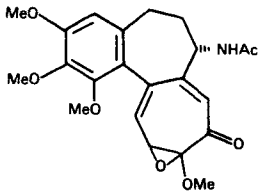
Table 2. *Bond angles for the heavier atoms* (Esds are given parenthetically)

O(1)–C(1)–C(2)	119.5 (2)	O(1)–C(1)–C(12B)	118.5 (2)
C(2)–C(1)–C(12B)	121.8 (3)	C(1)–O(1)–C(1M)	115.5 (3)
C(1)–C(2)–O(2)	120.3 (3)	C(1)–C(2)–C(3)	119.8 (3)
O(2)–C(2)–C(3)	119.9 (3)	C(2)–O(2)–C(2M)	114.1 (3)
C(2)–C(3)–O(3)	116.1 (2)	C(2)–C(3)–C(4)	119.4 (3)
O(3)–C(3)–C(4)	124.5 (3)	C(3)–O(3)–C(3M)	117.1 (3)
C(3)–C(4)–C(4A)	121.3 (3)	C(4)–C(4A)–C(5)	115.7 (3)
C(4)–C(4A)–C(12B)	120.2 (2)	C(5)–C(4A)–C(12B)	124.0 (2)
C(4A)–C(5)–C(6)	112.4 (2)	C(5)–C(6)–C(7)	113.5 (3)
C(6)–C(7)–C(7A)	111.7 (2)	C(6)–C(7)–N	110.2 (3)
C(7A)–C(7)–N	113.4 (2)	C(7)–C(7A)–C(8)	121.1 (2)
C(7)–C(7A)–C(12A)	125.5 (2)	C(8)–C(7A)–C(12A)	113.4 (2)
C(7A)–C(8)–C(9)	111.4 (3)	C(8)–C(9)–O(9)	128.9 (3)
C(8)–C(9)–C(9A)	107.9 (2)	O(9)–C(9)–C(9A)	123.2 (3)
C(9)–C(9A)–O(9A)	114.9 (2)	C(9)–C(9A)–O(10)	104.7 (2)
C(9)–C(9A)–C(12A)	105.5 (2)	O(9A)–C(9A)–O(10)	105.4 (2)
O(9A)–C(9A)–C(12A)	119.2 (2)	O(10)–C(9A)–C(12A)	106.0 (2)
C(9A)–O(9A)–C(9M)	116.1 (3)	C(9A)–O(10)–C(11)	108.0 (2)
O(10)–C(11)–C(12)	115.3 (3)	C(11)–C(12)–C(12A)	109.5 (3)
C(7A)–C(12A)–C(9A)	101.5 (2)	C(7A)–C(12A)–C(12)	106.8 (2)
C(7A)–C(12A)–C(12B)	115.8 (2)	C(9A)–C(12A)–C(12)	101.0 (2)
C(9A)–C(12A)–C(12B)	115.4 (2)	C(12)–C(12A)–C(12B)	114.5 (2)
C(1)–C(12B)–C(4A)	117.4 (2)	C(1)–C(12B)–C(12A)	118.4 (2)
C(4A)–C(12B)–C(12A)	124.3 (2)	C(7)–N–C(13)	124.0 (3)
N–C(13)–O(13)	122.2 (3)	N–C(13)–C(14)	117.5 (3)
O(13)–C(13)–C(14)	120.3 (4)		

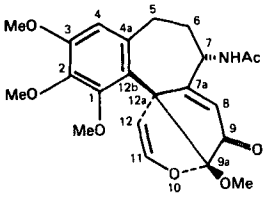
Scheme



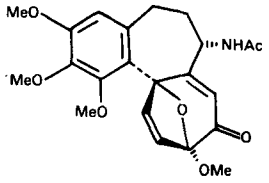
1
Colchicine



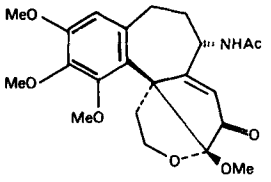
2
10,11-Epoxycolchicine



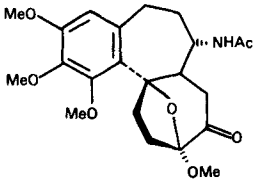
3
10,11-Oxy-10,12a-cyclo-10,11-secocolchicine



4
Oxycolchicine[5])



5
10,11-Oxy-10,12a-cyclo-10,11-seco-11,12-dihydro-colchicine



6
7a,8,11,12-Tetrahydro-oxycolchicine[5])

Bond lengths and angles *(Table 2)* appear reasonable and allow assignment of bond types without ambiguity. The fairly long bond C(12a)–C(9a), common to the two five-membered rings, is not unexpected since both atoms have four heavier atoms linked to them. The molecular packing appears logical and there are no strong interactions except for a fairly weak intermolecular hydrogen bond between N and O(9) along an axis of the primitive cell (***a***/2–***b***/2; length 3.022(3) Å).

The refined position of O(13) shows a moderately close approach to C(14) in a molecule related by the crystallographic twofold axis (3.120 Å). However, the ends of the 50% probability ellipsoid for O(13) are sufficiently distant to produce better contact distances and a two-site refinement might be possible for this atom. No attempts at such refinement have been carried out since elucidation of the disorder is irrelevant for chemical purposes and two-site refinement of C(13) and C(14) would not be allowed by the resolution of the data (*ca.* 0.5 Å). All other molecular contacts correspond to *van der Waals* distances.

---

[5]) Note by the editors: The IUPAC nomenclature of 'oxycolchicine' is 10,12a-epoxycolchicine.

The conformation of the seven-membered ring can be described as a somewhat distorted twist-boat, following *Bucourt* [6], rather than the boat conformations observed in colchiceine [7] and 7-oxo-7-deacetamido-colchiceine [8]. The five-membered rings could both be described as very flattened half chairs (the largest torsion angle observed is −6.1 degrees), in contrast to the usual situation in cyclopentene rings where the envelope conformation is adopted [6].

Conclusions. Based on the above data, 'epoxycolchicine' has the structure **3** of a cyclic acetal with two fused five-membered rings attached to ring B. Since 'epoxycolchicine' does not contain the proposed epoxide ring, its name should be changed to 10,11-oxy-10,12a-cyclo-10,11-secocolchicine (**3**), isomeric to the known 'oxycolchicine'[5]) (**4**), prepared from colchicine with chromic acid [9].

Compound **3** has a strong negative $[\alpha]_D$ in chloroform solution, suggesting that the chiral center at C(7) of **1** is not affected during the oxidation with sodium peroxide. Formula **3** therefore represents the absolute structure of this alkaloid. Oxycolchicine (**4**), on the other hand, has a strong positive $[\alpha]_D$ in chloroform solution [9b]. In our opinion this finding can be best explained by the two newly formed dihydrofuran units in **3** and **4** having a quasi-enantiomeric arrangement, and formula **4** should therefore most likely represent the absolute structure of synthetic oxycolchicine. Molecular model inspection indicates that the C(7a), C(8)-double bond is less hindered in **4** than in **3**. The formation of a dihydroderivative **5** in the catalytic hydrogenation of **3** in methanol over Pd/C catalyst, and a tetrahydroderivative **6** from **4** under the same conditions[6]), are in accord with this analysis (see exper. part).

Experimental Part

General Remarks. Melting points (m.p.) are uncorrected. UV. spectra: in ethanol, λ_{max} in nm, ε in parentheses. IR. spectra: in $CHCl_3$, data in cm^{-1}. Mass spectra (MS.): *m/z*, electron impact, 70 eV. ^{1}H-NMR. spectra: 100 MHz, in $CDCl_3$, internal standard TMS ($\delta = 0$ ppm). ^{13}C-NMR. spectra: *Jeol*-FX 60, 15.03 MHz, 45° pulse angle, 10 second repetition. TLC.: SiO_2 GF or Al_2O_3 GF, *Analtech*, Newark, DE.

(−)-10,11-Oxy-10,12a-cyclo-10,11-secocolchicine (**3**) *from colchicine* (**1**). To a solution of 0.4 g (1 mmol) of colchicine (**1**) in 40 ml of water were added 0.1 g of sodium peroxide (1.3 mmol). The orange-red solution was stirred at room temp. and monitored by TLC. (SiO_2, CH_2Cl_2/MeOH 95:5, or Al_2O_3, $CHCl_3$/MeOH 97.5:2.5) until **1** could no longer be detected (6–24 h). The alkaline aqueous solution was extracted several times with CH_2Cl_2. The organic extracts afforded, after drying (Na_2SO_4) and evaporation, a glassy residue that was crystallized from a minimal amount of hot 2-propanol. Recrystallization from ethanol gave 40 mg of compound **3** as colorless crystals, m.p. 251° (decomp.). Crystallization could also be achieved from ethyl acetate. $[\alpha]_D^{22} = -211°$ ($c = 1$, $CHCl_3$). – UV.: 211 (56700), 231 (sh. (21000). – IR.: 1712, 1678 (C=O). – ^{1}H-NMR. (olefinic and aromatic part only): 6.39 (H–C(4)); 6.18 (H–C(8)); 6.40 (*d*, $J = 3$, H–C(11)); 5.08 (*d*, $J = 3$, H–C(12)). – MS.: 415 (M^+).

$C_{22}H_{25}NO_7$ (415.44) Calc. C 63.60 H 6.07 N

---

[6]) TLC. analysis (Al_2O_3, CH_2Cl_2/MeOH 99:1) of this material showed two closely running spots. The formation of diastereomers during the catalytic reduction can not be ruled out.

All given data of semisynthetic **3** correspond to the previously reported data [1] of natural material. In a side-by-side comparison of both samples, identical TLC., mass spectra and ^{1}H-NMR. spectra (100 MHz, $CDCl_3$) were obtained, and in a similar comparison both materials were found to be identical by mixed m.p. and optical rotation[7]).

10,11-Oxy-10,12a-cyclo-10,11-seco-11,12-dihydrocolchicine (**5**) from **3**. A solution of 150 mg of **3** in 10 ml of methanol was hydrogenated at atmospheric pressure over 10 mg of 5% Pd/C for 24 h at room temp. The solution was evaporated after filtration of the catalyst, and the residue submitted to column chromatography (d = 18 mm, l = 100 mm) on alumina (activity grade III, neutral). Elution with CH_2Cl_2 and evaporation of the solvent gave an oil which crystallized on addition of diethyl ether, yielding **5** as colorless crystals, m.p. 211°. $[\alpha]_D^{20} = -115°$ ($c = 0.45$, $CHCl_3$). – UV.: 212.5 (47000), 230 sh. (20600). – IR.: 1716, 1674 (C=O). – ^{1}H-NMR. (olefinic and aromatic part only): 6.40 (H–C(4)); 5.94 (H–C(8)). – MS.: 417 (M^+).

7a,8,11,12-Tetrahydrooxycolchicine (**6**) *from* **4**. Hydrogenation and chromatography were carried out under the same conditions as described above. Elution with CH_2Cl_2/MeOH 99.5:0.5 and evaporation of the solvents afforded an oil which crystallized on addition of petroleum ether, yielding **6** as colorless crystals, m.p. 192–194°. $[\alpha]_D^{20} = +6.2°$ ($c = 0.60$, $CHCl_3$). – UV.: 208 (50300), 227.5 sh. (13100). – IR.: 1737, 1667 (C=O). – ^{1}H-NMR. (olefinic and aromatic part only): 6.36 (H–C(4)). – MS.: 419 (M^+).

X-ray crystallographic analysis of **3**. – 1. *Crystallographic data.* $C_{22}H_{25}NO_7$, mol. wt. = 415.44. Monoclinic, $a = 12.756(2)$ Å, $b = 8.333(1)$ Å, $c = 19.623(2)$ Å, $\beta = 92.75(1)°$, $U = 2083.44$ Å^3, $Z = 4$. Space group $C2$ (C_2^3), $d = 1.324$ g cm^{-3}. Cell dimensions were obtained by least squares analysis using reflections measured at $\pm\theta$ on a diffractometer (Cu$K\alpha$ radiation, $\lambda = 1.5418$ Å).

2. *Data collection.* Intensities were measured with a computer-controlled diffractometer (*Nonius* CAD-4) using graphite-monochromatised Cu$K\alpha$ radiation. There were 2122 unique reflections, 1982 of which had $Io > \sigma(Io)$. *Lorentz-* and polarisation-corrections were applied but not absorption-corrections. There was no indication of significant radiation damage during data collection. The crystal used for data collection had dimensions $0.35 \times 0.15 \times 0.04$ mm and was the largest one which could be found.

3. *Structure analysis.* The structure was solved using MULTAN 78 [10]. The only point of note was that the 'magic integer' technique incorporated in the program made the solution feasible since nine variable phases appeared needed. The corresponding E-map showed all the heavier atoms of the ring system and the first atoms of each side chain. This model was refined using the programs of the XRAY72 [11] system with weights following *Peterson & Levy* [12]. Evidence for all missing atoms was found and atomic labels were readily assigned on the bases of bond lengths and thermal parameters. The oxygen and methyl C-atoms of the acetamido side chain have very anisotropic thermal parameters and orientational disorder is suspected. Density attributable to all expected H-atoms was found in difference maps. Anisotropic thermal parameters were used for heavier atoms and isotropic parameters for H-atoms. The temperature factors for methyl H-atoms were assigned values slightly larger than the isotropic factors for their attached C-atoms but the values were not refined. Given the possible disorder of the acetamido chain, the parameters of the H-atoms of the acetamido methyl C-atom, C(14), were not refined although apparent evidence for their presence was seen in difference maps. The structure was refined in two groups, alternately holding all parameters for one group constant and refining the other group. The final R-factor of 4.3% is a little high by the standards of this laboratory but was not unexpected, given that the small volume of the crystal would have entailed measurement times considerably longer than the maximum time (3 min) actually employed, were our normal theoretical intensity precision (3%) to be attained.

Tables of observed and calculated structure factors and atomic parameters may be obtained from the authors.

---

[7]) Personal communication by Prof. *Šantavý*.

REFERENCES

[1] *H. Potěšilová, L. Dolejš, P. Sedmera & F. Šantavý,* Coll. Czechoslov. chem. Commun. *42*, 1571 (1977).
[2] *A. Bladé-Font, R. Muller, J. Elhuero, R. Faure & E.-J. Vincent,* Chemistry Letters *1979*, 233.
[3] *C. D. Hufford, C. C. Collins & A. M. Clark,* J. pharm. Sci. *68*, 1239 (1979).
[4] *C. D. Hufford, H.-G. Capraro & A. Brossi,* Helv. *63*. 50 (1980).
[5] *C. K. Johnson,* ORTEP, Oak Ridge National Laboratory Report ORNL-3794 (1965).
[6] *R. Bucourt,* 'Topics in Stereochemistry', Vol. 8, ed. E.L. Eliel & N.L. Allinger, John Wiley, New York 1974.
[7] *J. V. Silverton,* Acta crystallogr. *B35*, 1979, in press.
[8] *M.A. Iorio, A. Brossi & J. V. Silverton,* Helv. *61*, 1213 (1978).
[9] a) *G.L. Buchanan, A. McKillop, A.L. Porte & J.K. Sutherland,* Tetrahedron *20*, 1449 (1964); b) *F. Šantavý,* Chem. Listy *46*, 488 (1952).
[10] *P. Main, S.E. Hull, L. Lessinger, G. Germain, J.-P. Declercq & M.M. Woolfson,* MULTAN78, a system of computer programs for the automatic solution of crystal structures from X-ray diffraction data, Universities of York and Louvain 1978.
[11] *J.M. Stewart, G.J. Kruger, H.L. Ammon, C. Dickinson & S.R. Hall,* XRAY72, technical report TR-192, Computer Center, University of Maryland 1972.
[12] *S. W. Peterson & H.A. Levy,* Acta crystallogr. *10*, 70 (1957).