ISSN 0018-019X

Erscheint 9mal jährlich - Paraît 9 fois par an

118. 7-Oxo-deacetamidocolchiceine and 7-Benzylimino-deacetamidocolchiceine: Two Novel Products from the Base Catalysed Reaction of (−)-*N*-Benzylidene-deacetylcolchiceine[1])

by **Maria A. Iorio**[2]) and **Arnold Brossi**[3])

Medicinal Chemistry, Laboratory of Chemistry, National Institute of Arthritis, Metabolism and Digestive Diseases, Bethesda, Maryland 20014, USA

and **J.V. Silverton**

Laboratory of Chemistry, National Heart, Lung, and Blood Institute
Bethesda, Maryland 20014, USA

Dedicated to Professor *Oskar Jeger* on his 60th anniversary

(23.I.78)

Zusammenfassung

Es wird gezeigt, dass bei der Racemisierung der aus natürlichem Colchicin leicht zugänglichen *Schiff*-Base **1**, mit Kaliumhydroxyd in Methanol, neben Benzaldehyd, Benzylamin und (±)-*N*-Desacetylcolchicinsäure (**4**), auch die beiden tricyclischen Verbindungen **2** oder **5** erhalten werden. Die Überführung des Ketimins **2** in das Diketon **5** gelingt leicht durch saure Hydrolyse. Letzteres kann mit Benzylamin unter den gewählten Racemisierungsbedingungen direkt in (±)-*N*-Desacetylcolchicinsäure (**4**) übergeführt werden. Ein Vergleich der NMR.-Spektren von **5** und seiner beiden Methyläther **6** und **7**, mit Substanzen der Colchicin-Reihe, gestattet die Identifizierung von **6** als 7-Oxo-desacetamidoisocolchicin und von **7** als 7-Oxo-desacetamidocolchicin, erlaubt jedoch keine eindeutige Zuordnung der beiden Sauerstoffunktionen im Ring C des Diketons **5**. Eine solche konnte jedoch durch eine Röntgenanalyse weiter präzisiert werden. Diese ergibt nämlich, dass das Diketon **5** ein durch Wasserstoffbrücken stabilisiertes Dimeres der Tautomeren **5a** (Ring C wie Isocolchicein) und **5b** (Ring C wie Colchicein) darstellt.

Treatment of the optically active *Schiff* base **1**, prepared in two steps from natural colchicine, with potassium hydroxide in refluxing methanol affords (±)-*N*-deacetylcolchiceine (**4**), an important intermediate in the synthesis of (±)-colchicine and (+)-colchicine [2] [3]. It can be assumed that the preparation of **4** from **1**

[1]) The nomenclature chosen derives from the terms colchicine and isocolchicine [1]. Thus compound **4** is named (±)-deacetamidocolchiceine, rather than (±)-trimethylcolchicinic acid, a term frequently encountered.

[2]) Visiting scientist on leave from the Istituto di Sanità, Roma, Italy.

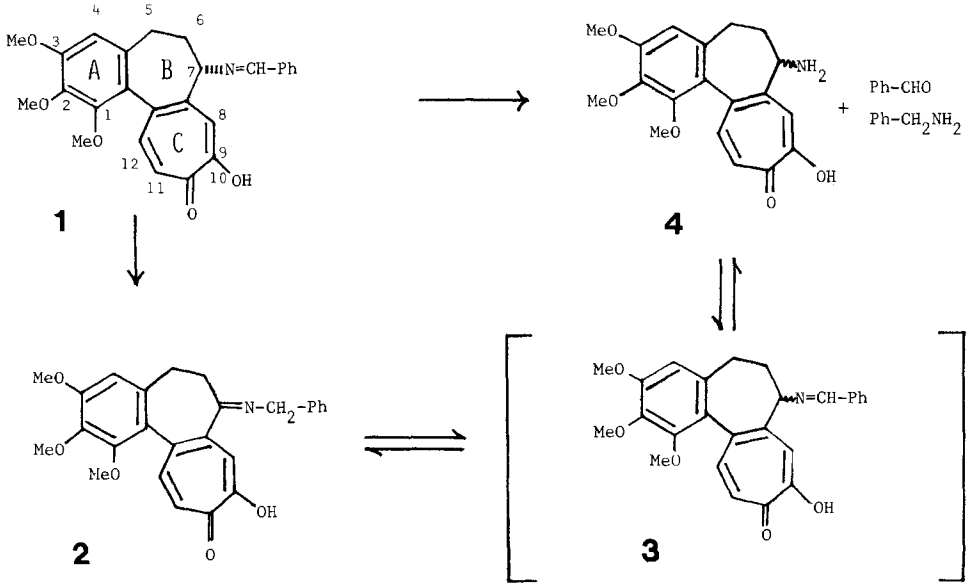
[3]) Author, to whom correspondence should be addressed.

involves aldimine-ketimine tautomerism (**1** to **2**) and equilibria between ketimine **2** and the racemic *Schiff* base **3**, before hydrolysis to (±)-*N*-deacetylcolchiceine (**4**) occurs.

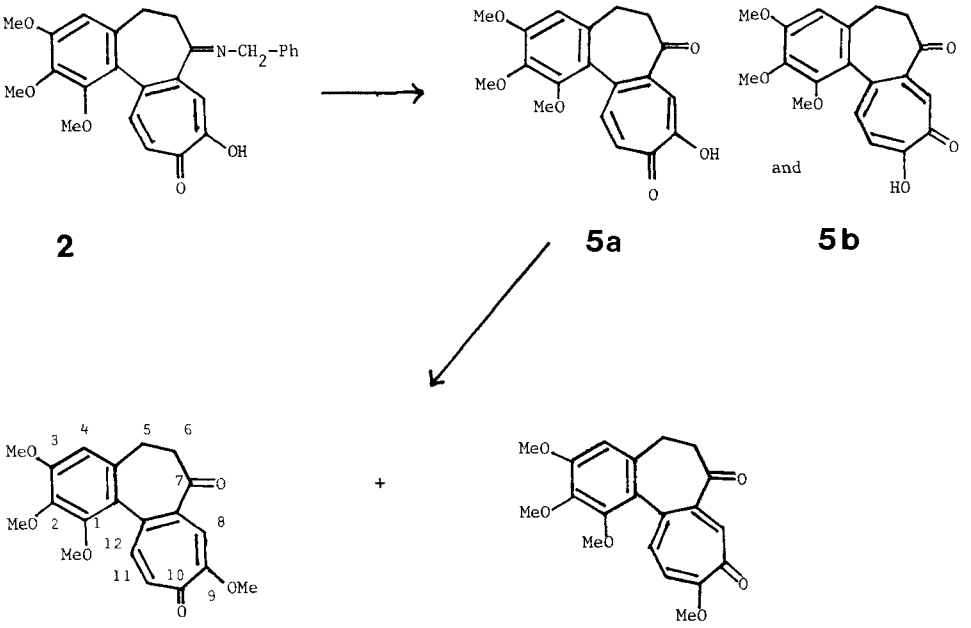
We now support this view with experimental data. Besides rac.-*N*-deacetylcolchiceine (**4**), and depending on the reaction conditions, two other optically inactive tricyclic compounds (**2** or **5**) were isolated. The complete racemization of the *Schiff* base **1** was necessary for a successful isolation of the various products formed. When *Schiff* base **1** was refluxed with a 7.5% solution of potassium hydroxide in methanol (see exper. part) the ether extracts of the acidified solution (pH 1) afforded, besides benzaldehyde, the diketone **5**, whereas the chloroform extract of the neutral solution (pH 5–7) contained (±)-*N*-deacetylcolchiceine (**4**) and benzylamine. Using higher concentrations of *Schiff* base **1** and 10% methanolic solutions of potassium hydroxide, we obtained by extraction with chloroform almost equal amounts of the optically inactive ketimine **2** and **4**. Whether the isolation of the ketimine **2**, instead of the diketone **5**, is the result of the considerable change in the reaction conditions or whether it is a consequence of variations in the isolation has not been determined. The latter possibility cannot be excluded, since the ketimine **2** is hydrolysed with acid to the diketone **5** and benzylamine. However the isolation of the ketimine **2** and the diketone **5** together with benzaldehyde and benzylamine, provides good evidence for the postulated sequence of reactions involving aldimine-ketimine tautomerism.

Physical data obtained by mass fragmentation and elemental analysis of the diketone **5** are in accord with the formula $C_{19}H_{18}O_6$, and the IR. and UV. spectra (exper. part) support a tropolonic chromophore in ring C. The latter, and the presence of an unchanged tricyclic carbon skeleton, were substantiated by

Scheme 1. *Functionalization of ring C is shown as in isocolchicine* (OMe instead of OH)



Scheme 2



methylation with diazomethane, which afforded isomeric methyl ethers **6** and **7**, separated by fractional crystallization. The higher melting isomer **7**, probably identical with a substance isolated during studies on the microbial degradation of colchicine[4]), was assigned the structure of 7-oxo-deacetamidocolchicine (**7**) based on the detailed analysis of its ^{1}H-NMR. spectrum (below). The lower melting isomer was 7-oxo-deacetamidoisocolchicine (**6**). Moreover, reaction of diketone **5** with benzylamine under equilibration conditions afforded (±)-*N*-deacetylcolchiceine (**4**) in about 20% yield.

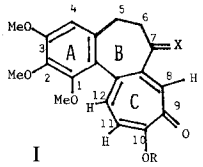
^{1}H-NMR. Discussion *(Table).* – The NMR. spectrum of **5** shows singlets for the aromatic methoxyl groups (δ 3.57, 3.87 and 3.89). The aromatic pattern of a tropolone system, as in colchicine and analogs, is shown by an *AB* quartet, assigned to the protons at C(11) and C(12) with coupling constants between 11–13 Hz. In the NMR. spectrum of *allo*-colchiceine[5]), an analog of colchiceine with a six-membered ring C (formula III, *Table* [4]), the protons at C(10) and C(11) give rise to an *AB* quartet with an *ortho*-coupling constant of 8 Hz. The lower field doublet is further split by *meta*-coupling with H–C(8). The arrangement of the oxygen

---

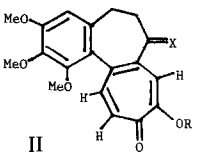
[4]) Personal communication from Dr. *Harry Wood*, Jr., National Cancer Institute, Bethesda, Maryland, USA. Unpublished report in a thesis of *Hj. Zeitler*, Technical University Munich, 1966.

[5]) Our material showed after sublimation the following data: m.p. 261°, $[\alpha]_D^{20} = -146.0°$ ($c = 0.98$, MeOH).

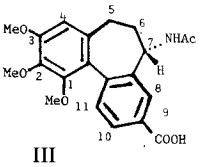
Table. *^{1}H-NMR. spectral data of 7-oxo-7-deacetamido-colchiceine and analogs*[a])



I



II



III

Compound	Structure	X	R	4-H	8-H	11-H	12-H
5	I or II	O	H	6.60 *s*	7.20 *s*	7.40 *d*	7.57 *d*
						$J = 12$	$J = 12$
7	I	O	Me	6.56 *s*	7.16 *s*	6.86 *d*	7.26 *d*
						$J = 11$	$J = 11$
Colchicine	I	H / NHAc	Me	6.57 *s*	7.67 *s*	6.93 *d*	7.40 *d*
						$J = 11$	$J = 11$
6	II	O	Me	6.60 *s*	6.66 *s*	7.36 *d*	7.23 *d*
						$J = 13$	$J = 13$
Isocolchicine	II	H / NHAc	Me	6.60 *s*	7.22 *s*	7.47 *d*	7.13 *d*
						$J = 12$	$J = 12$
Colchiceine	I or II	H / NHAc	H	6.60 *s*	7.66 *s*	7.65 *d*	7.39 *d*
						$J = 12$	$J = 12$
Colchinoic Acid	III			6.60 *s*	8.05 *s*[b])	8.00 *q*[c])	7.56 *d*[d])

[a]) Chemical shifts (δ) in $CDCl_3$ (TMS internal standard) at 100 MHz.
[b]) *Meta*-coupling is not evident in this solvent for this proton, but a new split is observed with spectrum run in CD_3OD.
[c]) 10-H of III partially overlaped by 8-H peak.
[d]) 11-H of III.

functions at C(9) and C(10) of ring C in compound **5** cannot be clarified, even by comparison of its spectrum with that of its methyl ethers **6** and **7** and that of colchiceine *(Table)*. In **7** the proton at C(11) is shifted upfield relative to the corresponding signal in **5**, owing to the shielding effect of the adjacent methoxy group at C(10). In **6** it is the H–C(8) which receives the same shielding effect from the methoxy group at C(9) and appears therefore at higher field than the corresponding signal in **7**. The same effects influence the chemical shifts of these protons in colchicine and isocolchicine (see *Table*). It is not possible to say that **5** is a single tautomer nor that the oxygen functions in ring C are as in isocolchicine or colchicine.

X-Ray analysis *(Fig.)*. – Further evidence regarding this interesting point was obtained by single crystal X-ray analysis using established techniques. This shows that the crystal of the diketone **5** is composed of an approximately equimolar random mixture of the two-dimensionally very similar isomeric molecules **5a** and **5b** of the isocolchicine and colchicine series, in agreement with the chemical evidence. Apart from the tropolone ring, all bond lengths and angles are essentially as expected. Except for the C(9)–C(10) bond (1.443 Å), all lengths in the tropolone ring are approximately equal to that in benzene and the two tropolonoid C–O bond lengths are nearly equal (see Experimental part). It is not profitable to discuss the actual values in view of the probable disorder.

There are two close approaches, across a center of symmetry, of atoms in different molecules; O(9)–O′(9), 2.896 Å and O(9)–O′(10), 3.080 Å. All other intermolecular contacts correspond to *Van der Waals* distances. The molecules seem to be packed together as dimers through H-bonding. Analysis of the interatomic distances suggests two possible H-bonding schemes in the crystal: i) with hydroxy groups at C(10) in the two molecules: colchinoid – colchinoid or ii) with one hydroxy group at C(9) and the other at C(10) in the associated molecule: isocolchinoid – colchinoid or *vice versa*. The situation with both hydroxy groups at C(9): isocolchinoid – isocolchinoid leads to hydrogen atoms being within 1.75 Å of each other and appears improbable. If the two possible schemes are of equal probability, the crystal would not be a 1:1 but a 3 colchinoid:2 isocolchinoid mixture and this might explain the fact that the C(9)–O(9) and C(10)–O(10) distances are not exactly equal, being 1.285 and 1.311 Å respectively. Suggestively, the observed values are essentially what one would obtain by a 3:2 weighting of the distances given in a recent accurate tropolone ether structure [5], *viz.* 1.284 and 1.309 Å respectively (the actual mean C–O distances reported are 1.2335 and 1.3595 Å). The molecular conformation and thermal ellipsoids [6] are shown in the *Figure.*

The formation of 7-oxo-deacetamidoisocolchicine (**6**) and 7-oxo-deacetamidocolchicine (**7**) upon methylation provides sound chemical evidence for the presence of a tropolonic structure in **5**, comparable to that of colchiceine [7]. Its *in situ* conversion into (±)-*N*-deacetylcolchiceine (**4**) with benzylamine under the conditions for the racemization of **1**, is not tailored to optimal yields but constitutes indirectly a novel partial synthesis of (±)-colchicine.

Single crystal X-ray crystallography suggests that diketone **5** is an H-bonded dimer of **5a** and **5b**. The similarity in the properties of diketone **5** and colchiceine [7] suggest that also colchiceine might be an H-bonded dimer rather than a single tautomer.

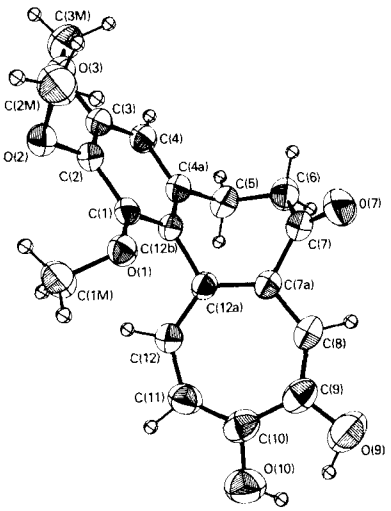


Fig. *The X-ray structure of the diketone mixture* **5a,b** *showing atomic numbering and apparent thermal ellipsoids (probability level 50%).* Hydrogen atoms are represented by arbitrary spheres.

Experimental Part

General Remarks. – Melting points (m.p.) are uncorrected. UV. spectra: λ_{max} in nm, ε in parentheses. IR. spectra: in $CHCl_3$ unless specified otherwise, data in cm^{-1}. ^1H-NMR. spectra: 100 MHz in $CDCl_3$, internal standard TMS ($\delta = 0$ ppm), J = coupling constant in Hz; abbreviations: s = singlet, d = doublet, br. = broad, sh. = shoulder. Mass spectra (MS.) *m/e*, electron impact 70 eV (relative intensity). TLC.: SiO_2 GF, *Analtech*, Newark, DE.

7-Oxo-deacetamidocolchiceine (**5**) *and (±)-*N*-deacetylcolchiceine* (**4**) *from (−)-*N*-benzylidene-deacetylcolchiceine* (**1**). A solution of the optically active *Schiff* base **1** (5.0 g) in 150 ml methanol containing 7.5% KOH was stirred at reflux for 96 h. After evaporating the yellow solution to dryness at 30° under vacuum, the residue was dissolved in 150 ml water, acidified with aqueous HCl-solution to pH 1 and extracted after 1 h with 4 × 50 ml ether. The ether extracts afforded, after concentration to ¼ of the original volume under vacuum and filtration, 1.2 g yellow needles of the diketone **5**, m.p. 154° (from methanol) unchanged after 2 crystallizations from CH_2Cl_2/hexane and high vacuum sublimation. – $[\alpha]_D^{20} = 0°$ ($CHCl_3$). – UV. (propanol-2): 248 (25,900) and 358 (17,200). – IR. ($CHCl_3$): 3250 (br., OH) and 1718 (CO). – MS.: 342 (M^+, 100), 314 ($M^+ - CO$, 29).

$C_{19}H_{18}O_6$ (342.23) Calc. C 66.66 H 5.30% Found C 66.96 H 5.39%

The mother liquor from the recrystallization of **5** contained benzaldehyde. The acidic aqueous solution was cooled in an ice-bath, adjusted with 20% aqueous NaOH to pH 5–6 and the precipitate was extracted with $CHCl_3$. Further addition of 20% NaOH-solution to the aqueous solution to pH 7 was followed by a second extraction with $CHCl_3$[6]). The $CHCl_3$ extracts afforded after drying (Na_2SO_4) and evaporation, 3.0 g of a residue which was dissolved in a minimum of hot methanol. After standing overnight, 1.3 g of compound **4** was isolated, m.p. 244°. $[\alpha]_D^{20} = 0°$ ($CHCl_3$/MeOH 4:1). – UV. (MeOH): 230 (30,600), 239 (29,700), 248 (sh; 28,600), 351 (17,400), 363 (sh; 16,000), in good agreement with data reported [2]. The mother liquors contained benzylamine, besides **4** and diketone **5**. – TLC.: (SiO_2, $CHCl_3$/MeOH/aq. ammonia 80:18:2, I_2). – GC.: (3% SE 30, 100–120 mesh gaschrom Q, silanized, 65°, glass col. 1.83 m).

7-Benzylimino-deacetamidocolchiceine (**2**) *and* (**4**) *from* Schiff *base* (**1**). A solution of the *Schiff* base **1** (5.8 g) in 100 ml methanol containing 10% KOH was refluxed for 96 h. The yellow solution was concentrated at 30° under vacuum and worked up as described for the isolation of **5**. All operations involving addition of HCl- and neutralization with 20% NaOH-solution were performed with solutions cooled in an ice-bath. The ether extracts contained largely benzaldehyde. The combined $CHCl_3$ extracts gave a residue which was dissolved in a minimal amount of hot methanol. Upon cooling 1.9 g of crude **4** was obtained which afforded 1.2 g of pure material after crystallization from methanol/$CHCl_3$. Compound **4** was identical with the sample above. The mother liquors on further standing gave a second compound which, after filtration and recrystallization from methanol/CH_2Cl_2, gave 1.6 g of the ketimine **2**, m.p. 167–168°. – $[\alpha]_D^{20} = 0°$ (MeOH). – UV. (EtOH): 247 (36,400), 356 (26,000), 370 (sh., 21,700), 408 (sh., 8700). – IR.: 3220 (br., OH), 1650 (C=N). – MS.: 431 (M^+).

$C_{26}H_{25}NO_5$ (431.47) Calc. C 72.37 H 5.84 N 3.25% Found C 72.11 H 6.00 N 2.95%

In other experiments the ketimine **2** obtained had m.p. 155°. This modification was converted into the higher melting form (167–168°) by recrystallization from ethanol/CH_2Cl_2 after seeding with a sample of m.p. 167–168°[7]).

Hydrolysis of the ketimine **2** *to the diketone* **5**. The ketimine **2**, m.p. 167–168°, (150 mg) was refluxed in 5 ml 10% aqueous HCl-solution for 1 h. The solution was made alkaline after cooling, and benzylamine (GC.) was extracted with ether. Extractions of the aqueous solutions adjusted to pH 5 with $CHCl_3$ afforded a residue which crystallized from methanol (86 mg). This material was identical with the diketone **5** (m.p., mixed m.p., TLC., IR. ($CHCl_3$)).

---

[6]) The extraction with $CHCl_3$ at 2 different pH values is important for avoiding the formation of untractable emulsions.

[7]) The NMR. and IR. spectra of the 2 ketimine forms are superimposable in solution.

Synthesis of (±)-N-deacetylcolchiceine (**4**) *from the diketone* **5**. The diketone **5** (0.58 mmol, 200 mg) and benzylamine (0.58 mmol, 62.6 mg) were refluxed in 6 ml 7.5% methanolic KOH-solution for 96 h. After evaporation to dryness and the usual work-up, the ether extracts contained benzaldehyde and starting material, whereas the $CHCl_3$ extracts contained benzylamine and **4**. The latter was obtained after evaporation and recrystallization from methanol (38 mg). The material is identical with a sample of **4** prepared by the other route.

7-Oxo-deacetamidoisocolchicine (**6**) *and 7-oxo-deacetamidocolchicine* (**7**) *from the diketone* **5**. The solution of the diketone **5** (300 mg) in 10 ml CH_2Cl_2 was treated at 0° with 10 ml of an ethereal solution of diazomethane (*ca.* 0.5 mol) under stirring for 1 h. Two new compounds could be detected by TLC. ($CHCl_3$/MeOH, 9:1). After evaporation and crystallization from methanol/CH_2Cl_2 the higher melting isomer (**7**) separated, m.p. 229–230°. – UV. (MeOH): 242 (28,800) and 346 (15,700). – IR.: 1720 (C=O). – MS.: 356 (M^+).

$C_{20}H_{20}O_6$ (356.36) Calc. C 67.40 H 5.66% Found C 67.06 H 5.84%

From the mother liquor, after careful crystallization from ethyl acetate/methanol, the second isomer **6** was obtained, m.p. 190–192°. – UV. (MeOH): 245 (29,600) and 342 (15,700). – IR.: 1715 (C=O). – MS.: 357 ($M^+ + 1$). For an interpretation of the NMR. spectra of **6** and **7**, see *Table*.

$C_{20}H_{20}O_6$ (356.36) Calc. C 67.40 H 5.66% Found C 67.51 H 5.56%

X-Ray crystallographic analysis. – 1. *Crystallographic data.* – $C_{19}H_{18}O_6$, Mol.Wt. = 342.32. Triclinic, $a = 7.424$ (1), $b = 10.584$ (1), $c = 10.910$ (1) Å, $\alpha = 91.10$ (1), $\beta = 99.77$ (1), $\gamma = 97.08$ (1)°, U = 837.7 Å^3, Z = 2. Space group $P\bar{1}$ (C_i^1), Dx = 1.308. 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 3282 unique reflections, 2395 of which had $Io > \sigma(Io)$. *Lorentz* and polarisation corrections were applied but not absorption corrections (prior to data collection, the crystal was ground to an ellipsoid approximately $0.2 \times 0.2 \times 0.3$ mm^3). There was no indication of significant radiation damage during data collection.

3. *Structure Analysis.* The structure was solved using MULTAN [8], with some difficulty. Standard approaches invariably led to maps consisting of a hexagonal net of peaks. Finally, using considerations implicit in the work of *Laing* [9] and *Thiessen & Busing* [10], a set of origin-defining planes was obtained and used with variables consisting of the phases of 5 planes all of whose indices were even. There was a uniquely defined best solution whose E-map showed all the heavier atoms.

Standard refinement techniques [11], with weighting following *Peterson & Levy* [12] and scattering factors as given in XRAY72 [11], allowed recognition of all hydrogen atoms except for that attached to the tropolone hydroxyl oxygen atom. The model was refined to an R-factor of 0.039 (based on observed reflections) with isotropic thermal parameters for hydrogen atoms and anisotropic thermal parameters for all others.

A difference map, calculated at this point in the refinement, showed a broad peak (over 1 Å wide) whose centroid was approximately equidistant from the 2 tropolone oxygen atoms. The bonds, despite the very respectable esd.s of less than 0.003 Å, did not show the alternation of lengths in the tropolone ring observed by *Derry & Hamor* [13]. The anisotropic motion of the atoms of the tropolone ring also had maximal directions in the plane of the ring which seemed rather unlikely physically.

For the final cycles of refinement, 2 half hydrogen atoms were inserted at the edges of the broad peak in the difference map. The distance between the half atoms

was about 1 Å and the resolution of the data (maximum $\sin\theta/\lambda = 0.62$ Å^{-1}) was sufficient to allow refinement. The new model refined to an R-factor of 0.038. Tables of observed and calculated structure factors, atomic parameters, and molecular dimensions may be obtained from the authors.

We thank Dr. *H. Yeh*, Microanalytical Services and Instrumentation Section, Laboratory of Chemistry, NIH, Bethesda, Maryland for helpful discussions regarding the NMR. interpretations and Dr. *J. Minamikawa* from our laboratory for technical assistance. Generous gifts of colchicine were provided by Dr. *W.E. Scott*, Hoffmann-La Roche Inc., Nutley, New Jersey (USA) and are gratefully acknowledged.

REFERENCES

[1] *W.C. Wildman* in Manske's 'Alkaloids', Vol. VI, Academic Press (New York and London 1960) and *W.C. Wildman & B.A. Pursey*, ibid. Vol. XI (1968). *F. Šantavý & T. Reichstein*, Helv. *40*, 1606 (1950).
[2] *H. Corrodi & E. Hardegger*, Helv. *40*, 193 (1957).
[3] *A. Bladé-Font*, Tetrahedron Letters *1977*, 2977. A very elegant and high yield conversion of natural colchicine into (±)-colchicine is described.
[4] *F. Šantavý*, Helv. *31*, 821 (1948).
[5] *J.V. Silverton, C. Kabuto, K.T. Buck & M.P. Cava*, J. Amer. chem. Soc. *99*, 6708 (1977).
[6] *C.K. Johnson*, ORTEP, Oak Ridge National Laboratory Report ORNL-3794 (1965).
[7] *R.M. Horowitz & G.E. Ullyot*, J. Amer. chem. Soc. *74*, 587 (1952).
[8] *P. Main, M.M. Woolfson & G. Germain*, MULTAN, a computer program for the automatic solution of crystal structures, Universities of York and Louvain (1971).
[9] *M. Laing*, Acta crystallogr. *A 32*, 673 (1976).
[10] *W. Thiessen & W.R. Busing*, Acta crystallogr. *A 30*, 814 (1973).
[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).
[13] *J.E. Derry & T.A. Hamor*, J. chem. Soc. Perkin II *1972*, 694.