63. Esters of 1-*O*-Demethylthiocolchicines: Formation of Isomers in Chloroform Solution

by **Peter Kerekes**[1]) and **Arnold Brossi***

Medicinal Chemistry Section, Laboratory of Chemistry, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205, U.S.A.,

and **Judith L. Flippen-Anderson**

Laboratory for the Structure of Matter, Naval Research Laboratory, Washington, D.C. 20375, U.S.A.,

and **Colin F. Chignell**

Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, U.S.A.

(20.XI.84)

1-*O*-Acetyl-1-*O*-demethylcolchicine, and acylated 1-*O*,2-*O*-didemethylthiocolchicines, in contrast to 2-*O*-acetyl-, 2-*O*,3-*O*-diacetyl- and 3-*O*-acetyl analogs, showed after standing in $CHCl_3$ solution significant changes in optical rotation, a duplication of ^{1}H-NMR signals, and the formation of new isomers on TLC. Solid-state X-ray diffraction of *O*-acetylated colchinoids and thio analogs, showed out of planar arrangements of the aromatic substituents, but the analysis could not help to explain the structures of the newly formed isomers in $CHCl_3$ solution.

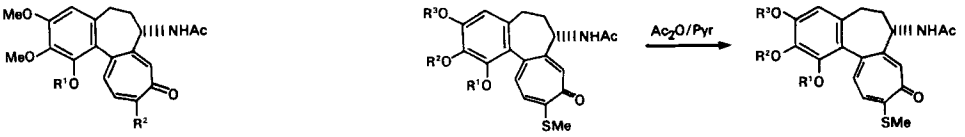
Introduction. – Colchicine, the major antimitotic alkaloid of the meadow saffron *Colchicum autumnale,* binds specifically and with high affinity to the tubulin dimer, the major protein subunit of microtubules [1]. 1-*O*-Demethylcolchicine (**1**) [2] binds substantially less to tubulin protein *in vitro* than colchicine, or its 2-*O*- and 3-*O*-demethyl congener, but binding was somewhat restored upon *O*-acetylation of **1** to **2** [1]. It is interesting to note that the rotational strength of **1** in $CHCl_3$ solution was considerably reduced after standing compared to that of colchicine, or its 2-*O*- and 3-*O*-demethyl congeners [1] [2].

It seemed interesting to study whether similar observations could be made in the isosteric series of 10-(methylthio)-10-demethoxycolchicine (**3**), which is easier amenable to chemical modification because of the increased stability of the tropolonic ring towards acids [3]. We hoped that such an investigation would substantiate the importance of aromatic substitution in ring A of colchinoids with regard to tubulin binding, and possibly gives clues on how inactivation of 1-*O*-demethylcolchicine in the tubulin-binding experiment could be explained.

We now report results of such a study, carried out with several phenolic thiocolchicines **4**–**8**, acetates **9**–**13** and the dibenzoate **14**, showing drastic changes in $CHCl_3$ solution in ^{1}H-NMR spectra, by optical rotations and chromatographic behavior, when the MeO group(s) was replaced by an AcO or BzO group(s) (**2**, **12**, **14**).

[1]) Visiting Scientist, Department of Chemistry, University of Debrecen, Debrecen 4010, Hungary. Passed away in Bethesda on August 1, 1984.

Scheme



1 R^1 = H, R^2 = OMe
2 R^1 = Ac, R^2 = OMe
3 R^1 = Me, R^2 = SMe

4 $R^1 = R^2$ = Me, R^3 = H
5 $R^1 = R^3$ = H, R^2 = Me
6 R^1 = Me, $R^2 = R^3$ = H
7 $R^1 = R^3$ = Me, R^2 = H
8 $R^1 = R^2$ = H, R^3 = Me

9 $R^1 = R^3$ = Ac, R^2 = Me
10 R^1 = Me, $R^2 = R^3$ = Ac
11 $R^1 = R^3$ = Me, R^2 = Ac
12 $R^1 = R^2$ = Ac, R^3 = Me
13 $R^1 = R^2$ = Me, R^3 = Ac
14 $R^1 = R^2$ = Bz, R^3 = Me

Chemistry. – Dihydroxy compounds **5** and **6** obtained from 10-demethoxy-3-*O*-demethyl-10-(methylthio)colchicine **4** [4] by treatment with conc. H_2SO_4 at 50–60°, were converted into acetates **9** and **10** by treatment with Ac_2O in pyridine. The novel 1,3-dihydroxy compound **5** was found to be a minor product formed in the H_2SO_4 reaction, and its structure was secured by spectral data. 2-Hydroxy compound **7** and 1,2-Dihydroxy compound **8** were obtained besides other, nonidentified products, by treating **3** with conc. H_2SO_4 [4] and converted similarly into acetates **11** and **12**. Included in the investigation were 3-*O*-acetyl-3-*O*-demethylcolchicine (**13**) and dibenzoate **14**, the latter obtained from **8** by treatment with benzoic anhydride in pyridine.

Physical Properties of *O*-Acetylated Demethylthiocolchicines. – A considerable change in optical rotation of 1-*O*-acetyl-1-*O*-demethylcolchicine (**2**), measured after standing in $CHCl_3$ solution, was reported [2]. This change was also found to occur in $CHCl_3$ solution of **12** and **14**, but not in thiocolchinoids having a CH_3O group at C(1) *(Table 1)*. The ^{1}H-NMR spectra of **2** and **12** were rather complex, showing a duplication

Table 1. *$[\alpha]_D$-Values of* O-*Acylated Demethylcolchicines and Demethylthiocolchicines in $CHCl_3$ Solution*[a])

Compounds	r.t. (25°)	After heating at 50°C[b])
1-*O*-Acetyl-1-*O*-demethylcolchicine (**2**)[c])	−185°	− 76°
1,2-Diacetyl-1,2-didemethylthiocolchicine (**12**)	−237°	− 30°
2-*O*,3-*O*-Diacetyl-10-demethoxy-2,3-didemethyl-10-(methylthio)colchicine (**10**)	−106°	− 97°
2-*O*-Acetyl-2-*O*-demethylthiocolchicine	−180°	−168°
Colchicine (**1**, R^1=CH_3)	−125°	−130°
10-Demethoxy-10-(methylthio)colchicine (**3**)	−220°	−218°
N-Deacetylcolchicine (**1**, R^1=CH_3,H instead of Ac)[c])	−161°	−154°
2-*O*,3-*O*-Dibenzoyl-10-demethoxy-2-*O*,3-*O*-didemethyl-10-(methylthio)colchicine (**14**)	−167°	+ 2°

[a]) Concentration of materials in $CHCl_3$ measured with a *Perkin-Elmer* Model *241 MC* polarimeter was 0.3%.
[b]) Some solvent may have evaporated during the heating, and slightly increased the concentration.
[c]) Obtained in crystalline form after crystallization from AcOEt, m.p. 125–127°.

of signals, reminescent to spectra observed with rotamers of *N*-formyltetrahydroisoquinolines [5]. The esters **12** and **14** studied in more detail behaved on TLC, after standing in $CHCl_3$ solution for 6 h, or after 2 h reflux, as a pair of rotamers. The newly formed and faster-moving isomers afforded after isolation from plates materials identical in every respect with starting materials. None of the compounds possessing a CH_3O group at C(1) showed a similar behavior. To secure more information regarding the structure of the newly formed isomers, acetate **2**, and diacetates **10** and **12**, which crystallized nicely after trituration with acetone, were subjected to single-crystal X-ray analysis. Many X-ray

studies of biologically active and less active colchinoids and thio analogs were carried out [6] [12], and we thought it interesting to compare the detailed data reported [12a–e] with those of compounds studied here.

Biological Evaluation. – Tubulin-binding affinity data of acetates described here were obtained by the method of *Zweig* and *Chignell* [7], measuring the inhibition of binding of tritiated colchicine (2.5 μM) to rat-brain tubulin in the presence of 25 μM amounts of compounds. *Table 2* shows that *O*-demethylation of thiocolchicine **3** to dihydroxy compounds **5**, **6**, and **8** was accompagnied with a considerable loss of binding potency.

Table 2. *Binding of Thiocolchicine Phenols and Acetates to Rat-Brain Tubulin Protein*

Compound		% Inhibition of ^{3}H-colchicine binding[a])
Monoacetate	**11**[b])	73
Monoacetate	**13**	79
Diphenol	**6**	66
Diphenol	**8**	27
Diphenol	**5**	40
Diacetate	**10**	57
Diacetate	**12**	53
Thiocolchicine	**3**	89
Colchicine	**1** (R^1=Me)	90

[a]) Percentage by which the binding of ^{3}H-colchicine (2.5 μM) to rat-brain tubulin is reduced in the presence of the thiocolchicine analogs (25 μM). Each value is the average of triplicate determinations. For details see [1].

[b]) See [7].

Acetylation of mono- or dihydroxy compounds prepared earlier [1] restored the binding affinity somewhat, more so with mono-acetates **11** and **13** than with diacetates **10** and **12**. The acetate **13** showed almost equal binding potency to that of 3-*O*-demethylthiocolchicine **4**, a compound which recently emerged by evaluation in several tumor assays as possibly superior and less toxic antimitotic agent than colchicine [8].

X-Ray Crystallographic Data for 2, 10, and 12. – Compound **2**: $C_{22}H_{25}NO_7\cdot$ (solvent), recrystallized from acetone, crystal size (0.07 × 0.20 × 0.35) mm, monoclinic, space group $C2$, a = 20.244(5) Å, b = 7.074(3) Å, c = 17.132(5) Å, β = 94.40(4)°, V = 2446.4(9) Å^3, z = 4, d_{calc} = 1.3 gcm^{-3}, μ = 8 cm^{-1} (*Fig. 1* and *Table 3*).

The 1820 independent reflections were measured out to a $2\theta_{max}$ = 116° with a computer controlled diffractometer (NICOLET R3M) at room temperature using CuK_α (λ = 1.5417 Å) radiation with a graphite monochromator on the incident beam. A partial structure found by applying direct methods in space group $C2$ was expanded into the full structure in space group $C1$, and then shifted along z to a proper origin in $C2$ [9] [10]. Full-matrix least-squares refinement (anisotropic for non-H-atoms; H-atoms isotropic riding on covalently bonded atoms, solvent atoms isotropic) using the 1584 reflections for which $|F_o| > 3\sigma|F_o|$ gave a final R factor of 5.1% (R_w = 5.7%). The goodness of fit parameter was 1.7.

Compound **10**: $C_{24}H_{25}NO_7S\cdot CH_3OH$, mol.wt. = 503.60, crystal size = (0.05 × 0.15 × 0.50) mm, orthorhombic, space group $P2_12_12_1$, a = 9.471(2) Å, b = 11.341(2) Å, c = 23.702(5) Å, V = 2546.0(7) Å^3, z = 4, d_{calc} = 1.31 gcm^{-3}, μ = 15.2 cm^{-1} (*Fig. 2* and *Table 4*).

The 2033 unique reflections were collected out to $2\theta_{max}$ = 116° under the same conditions used for **2**. The structure was solved by direct methods [9] [10]. Full-matrix least-squares (non-H-atoms anisotropic, H-atoms isotropic riding on covalently bonded atoms) refinement using the 1755 reflections for which $|F_o| > 3\sigma|F_o|$ gave a final R factor of 5.6% (R_w = 5.7). The goodness of fit parameter was 1.5.

Compound **12**: $C_{24}H_{25}NO_7S\cdot H_2O$, mol.wt. = 489.57, crystal size = (0.05 × 0.10 × 0.35) mm, orthorhombic, space group $P2_12_12_1$, a = 7.117(2) Å, b = 10.764(2) Å, c = 31.612(6) Å, V = 2421.7(8) Å^3, z = 4, d_{calc} = 1.34 gcm^{-3}, μ = 15.8 cm^{-1} (*Fig. 3* and *Table 5*).

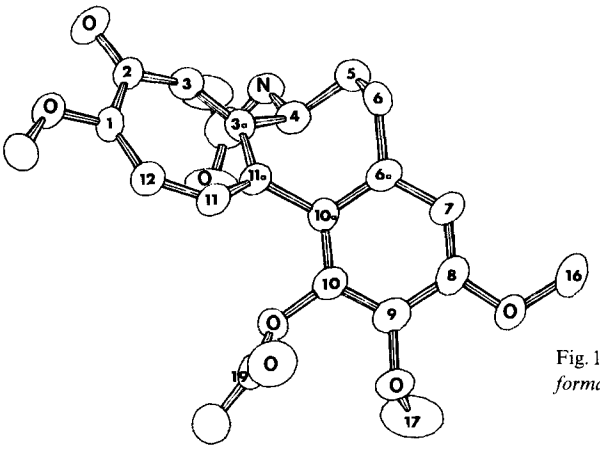


Fig. 1. *A diagram showing the structure and conformation of* **2**

Table 3. *Fractional Coordinates and* U_{eq} *Values for* **2**[a])

Atom	*x*	*y*	*z*	U_{eq}
O(1)	0.2547(1)	0.8110(5)	0.9196(2)	5.1(1)
O(2)	0.3784(2)	0.8596(7)	0.9235(2)	6.3(1)
O(4)	0.4666(2)	0.3595(6)	1.1437(2)	7.8(2)
O(8)	0.3512(2)	0.7794(6)	1.5055(2)	6.0(1)
O(9)	0.2741(2)	0.5243(6)	1.4245(2)	5.5(1)
O(10)	0.2771(2)	0.4874(6)	1.2680(2)	5.6(1)
O(10a)	0.1761(2)	0.6026(9)	1.2825(3)	9.3(2)
N(4)	0.5146(2)	0.6478(7)	1.1456(2)	5.1(2)
C(1)	0.2845(2)	0.8042(7)	0.9925(2)	4.3(2)
C(2)	0.3573(2)	0.8244(7)	0.9878(2)	4.2(2)
C(3)	0.4030(2)	0.7909(8)	1.0552(2)	4.2(2)
C(3a)	0.3974(2)	0.7660(8)	1.1326(3)	4.1(2)
C(4)	0.4620(2)	0.7320(8)	1.1855(3)	4.6(2)
C(5)	0.4861(2)	0.9178(9)	1.2243(3)	5.7(2)
C(6)	0.4297(3)	1.0285(9)	1.2590(3)	5.4(2)
C(6a)	0.3859(2)	0.8983(8)	1.3033(2)	4.5(2)
C(7)	0.3885(3)	0.9051(9)	1.3843(3)	5.1(2)
C(8)	0.3521(2)	0.7841(9)	1.4261(3)	4.9(2)
C(9)	0.3119(2)	0.6468(8)	1.3853(3)	4.5(2)
C(10)	0.3098(2)	0.6441(8)	1.3047(3)	4.5(2)
C(10a)	0.3443(2)	0.7695(8)	1.2617(2)	4.2(2)
C(11)	0.2748(2)	0.7795(8)	1.1377(3)	4.8(2)
C(11a)	0.3383(2)	0.7689(8)	1.1735(2)	4.1(2)
C(12)	0.2523(2)	0.7833(8)	1.0576(3)	4.6(2)
C(13)	0.1831(2)	0.7999(9)	0.9097(3)	5.3(2)
C(14)	0.5131(3)	0.4601(10)	1.1291(3)	6.2(2)
C(15)	0.5731(3)	0.3826(10)	1.0939(4)	7.7(3)
C(16)	0.3903(3)	0.9203(10)	1.5484(3)	7.0(2)
C(17)	0.3075(3)	0.3708(12)	1.4605(4)	9.6(3)
C(19)	0.2111(3)	0.4695(11)	1.2710(3)	7.0(3)
C(20)	0.1901(3)	0.2687(13)	1.2563(4)	9.3(3)
Solvent				
S1	0.0000	0.7304(12)	0.5000	
S2	−0.0566(11)	0.9412(25)	0.6398(9)	
S3	−0.0030(10)	0.9035(38)	0.5271(6)	
S4	0.0054(5)	0.9809(13)	0.6089(5)	
S5	0.0017(7)	1.0427(19)	0.5283(6)	

[a]) Standard deviations given in parentheses are based solely on least-squares results.

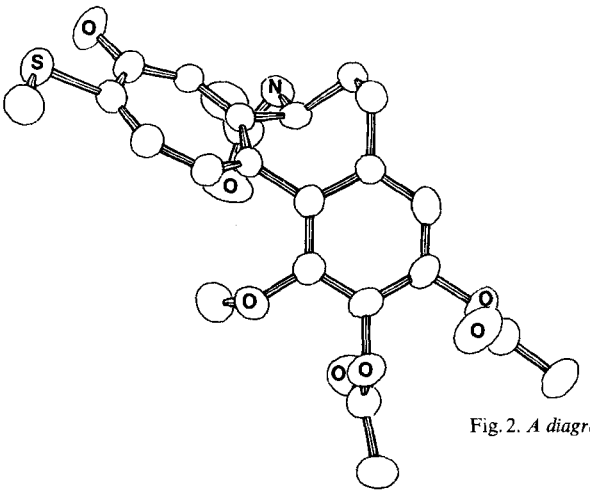


Fig. 2. *A diagram showing structure and configuration of* **10**

Table 4. *Fractional Coordinates and* U_{eq} *Values for* **10**[a])

Atom	x	y	z	U_{eq}
S(1)	0.8597(2)	−0.0856(1)	0.1827(1)	6.7(1)
N(4)	0.2648(4)	0.2289(4)	0.1311(2)	4.7(1)
O(2)	0.6554(5)	0.0598(4)	0.2195(1)	7.3(2)
O(4)	0.1682(5)	0.0476(4)	0.1314(2)	9.3(2)
O(8)	0.2066(4)	0.1433(3)	−0.1595(1)	6.1(1)
O(8a)	0.3786(5)	0.0815(5)	−0.2162(2)	9.1(2)
O(9)	0.2360(4)	−0.0823(3)	−0.1224(1)	6.0(1)
O(9a)	0.0458(4)	−0.0810(4)	−0.0661(2)	8.0(2)
O(10)	0.3816(5)	−0.1301(3)	−0.0245(1)	6.3(1)
C(1)	0.7342(5)	−0.0289(4)	0.1364(2)	4.7(2)
C(2)	0.6388(6)	0.0490(5)	0.1676(2)	5.3(2)
C(3)	0.5254(5)	0.1140(4)	0.1421(2)	4.3(2)
C(3a)	0.4712(5)	0.1203(4)	0.0893(2)	4.1(2)
C(4)	0.3444(5)	0.2022(4)	0.0805(2)	4.4(2)
C(5)	0.3918(5)	0.3163(4)	0.0513(2)	4.5(2)
C(6)	0.4857(6)	0.2944(5)	−0.0004(2)	5.2(2)
C(6a)	0.4272(5)	0.1940(4)	−0.0346(2)	4.2(2)
C(7)	0.3545(6)	0.2123(5)	−0.0846(2)	5.0(2)
C(8)	0.2903(5)	0.1212(5)	−0.1122(2)	4.9(2)
C(9)	0.3000(5)	0.0082(5)	−0.0913(2)	4.9(2)
C(10)	0.3726(6)	−0.0140(5)	−0.0427(2)	4.9(2)
C(10a)	0.4414(5)	0.0771(5)	−0.0137(2)	4.5(2)
C(11)	0.6318(6)	−0.0172(5)	0.0382(2)	5.0(2)
C(11a)	0.5184(5)	0.0565(4)	0.0404(2)	4.2(2)
C(12)	0.7280(5)	−0.0548(5)	0.0799(2)	5.0(2)
C(13)	0.9685(7)	−0.1722(5)	0.1390(3)	8.1(3)
C(14)	0.1791(6)	0.1478(5)	0.1529(2)	6.3(2)
C(15)	0.1030(8)	0.1792(6)	0.2055(3)	7.7(3)
C(16)	0.2607(6)	0.1164(5)	−0.2104(2)	5.9(2)
C(16a)	0.1552(8)	0.1324(6)	−0.2560(2)	8.5(3)
C(17)	0.1026(6)	−0.1130(5)	−0.1079(2)	5.7(2)
C(18)	0.0408(7)	−0.1941(5)	−0.1520(3)	7.9(3)
C(19)	0.2985(8)	−0.1585(6)	0.0249(3)	8.0(3)
Solvent				
C	0.1801(8)	0.5339(7)	0.1525(3)	9.6(3)
O	0.2862(4)	0.4656(3)	0.1757(2)	6.9(1)

[a]) Standard deviations given in parentheses are based solely on least-squares results.

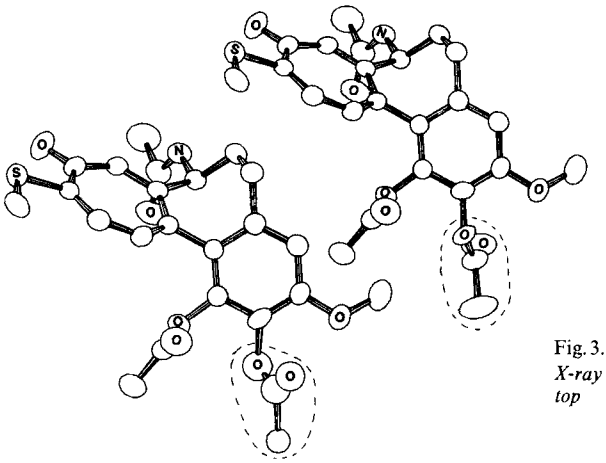


Fig. 3. *A diagram illustrating the results of the X-ray study on* **12**, *with the major isomer shown on top*

Table 5. *Fractional Coordinates and* U_{eq} *Values for* **12**[a])

Atom	x	y	z	U_{eq}
S(1)	0.4226(2)	0.7004(1)	0.0454(1)	4.8(1)
N(4)	0.4299(2)	1.1871(3)	−0.0917(1)	4.0(1)
O(2)	0.3870(6)	0.9479(3)	0.0309(1)	6.2(1)
O(4)	0.1123(4)	1.1913(3)	−0.0950(1)	6.0(1)
O(8)	0.4026(7)	0.8010(4)	−0.2833(1)	9.7(2)
O(9)	0.1403(6)	0.6820(4)	−0.2375(1)	4.5(1)
O(9a)	−0.0113(8)	0.8527(4)	−0.2615(2)	8.0(2)
O(10)	0.0951(4)	0.7350(3)	−0.1539(1)	4.7(1)
O(10a)	0.1915(6)	0.5368(3)	−0.1533(1)	7.7(1)
C(1)	0.4143(5)	0.7624(3)	−0.0057(1)	3.5(1)
C(2)	0.4089(6)	0.8976(3)	−0.0040(1)	4.1(1)
C(3)	0.4305(6)	0.9756(3)	−0.0408(1)	3.7(1)
C(3a)	0.4313(5)	0.9544(3)	−0.0836(1)	3.4(1)
C(4)	0.4535(5)	1.0682(3)	−0.1128(1)	3.6(1)
C(5)	0.6475(6)	1.0707(4)	−0.1345(1)	4.1(1)
C(6)	0.7116(6)	0.9451(4)	−0.1521(1)	4.5(1)
C(6a)	0.5545(6)	0.8821(4)	−0.1753(1)	4.4(1)
C(7)	0.5555(7)	0.8764(5)	−0.2192(1)	5.7(1)
C(8)	0.4080(8)	0.8151(5)	−0.2407(1)	6.7(2)
C(9)	0.2665(7)	0.7609(5)	−0.2177(1)	5.9(2)
C(10)	0.2605(6)	0.7748(4)	−0.1738(1)	4.6(1)
C(10a)	0.4031(6)	0.8324(3)	−0.1519(1)	3.8(1)
C(11)	0.4061(6)	0.7247(3)	−0.0844(1)	4.1(1)
C(11a)	0.4092(6)	0.8372(3)	−0.1043(1)	3.6(1)
C(12)	0.4137(6)	0.6906(3)	−0.0414(1)	4.2(1)
C(13)	0.4421(7)	0.5364(4)	0.0370(2)	5.5(1)
C(14)	0.2613(7)	1.2399(4)	−0.0842(1)	4.7(1)
C(15)	0.2712(8)	1.3605(4)	−0.0606(2)	7.4(2)
C(16)	0.5049(9)	0.8868(6)	−0.3083(2)	8.0(2)
C(17)	0.0050(10)	0.7461(6)	−0.2607(2)	5.4(2)
C(18)	−0.1155(9)	0.6426(6)	−0.2831(2)	9.4(2)
C(19)	0.0681(8)	0.6112(4)	−0.1488(1)	5.5(1)
C(20)	−0.1307(8)	0.5825(5)	−0.1386(2)	6.9(2)
O(9D)[b])	0.0585(23)	0.7603(19)	−0.2364(4)	9.1(7)
O(9aD)[b])	0.2116(28)	0.6124(19)	−0.2633(6)	10.5(8)
C(17D)[b])	0.0757(45)	0.6707(26)	−0.2590(8)	7.9(10)
Water	0.2799(6)	0.1943(4)	0.0606(2)	9.8(2)

[a]) Standard deviations given in parentheses are based solely on the least-squares results.
[b]) Disordered atoms.

The 2595 independent reflections were measured out to a $2\theta_{max} = 130°$ using the same conditions as for **2**. The structure was solved by direct methods [9] [10] and refined by full-matrix least-squares procedures (with same conditions as for molecule **10**) on the 2270 reflections for which $|F_o| > 3\sigma|F_o$ giving a final R factor of 6.1% ($R_w = 6.5\%$). The goodness of fit parameter was 1.2. The SHELXTL system of programs [11] was used to perform all calculations except the molecular plots *(Figures 1–3)* which were done with program ORTEP [12]. Tables of H coordinates and bond lengths and angles have been deposited with the *Cambridge Crystallographic Data Centre*, University Chemical Lab., Cambridge, CB2 1EW, England.

Discussion of X-Ray Data (Numbering of C- and O-atoms shown in *Fig. 1*). – The results of the X-ray studies on **2**, **10**, and **12** are illustrated in *Figures 1–3*. The solid state conformations of these compounds are almost isostructural with that found for colchicine itself [13a] and for several other colchinoids [13a–e]. The only significant differences lie in the orientations of the various substituents on the aromatic six-membered ring. In **12**, the AcO moiety on C(6) was found to be disordered. Least-squares refinement indicated a ratio of ~ 3:1 for the two conformations. In the major component (*Fig. 3*, top) the AcO groups on C(9) and C(10) are rotated in opposite directions out of the plane formed by the six-membered ring and are approximately perpendicular to the ring plane (the C–C–O–C torsion angles are −77.6° (C(8)–C(9)–O(9)–C(17)) and 76.2° (C(9)–C(10)–O(10)–C(19)). In the lesser component (*Fig. 3*, bottom) both AcO groups are on the same side of the six-membered ring, however, the disordered Ac group is not rotated as far out of the plane (C(8)–C(9)–O(9)–C(17) torsion angle is only 47.7°) as it was in the major component. The normal conformation for a terminal MeO group on an aromatic ring would be coplanar with the ring [14], however, in **12** the MeO group on C(8) is rotated out of the ring plane by 24.0°. In **2** *(Fig. 1)*, the MeO group at C(8) is coplanar with six-membered ring (C(8)–C(9)–O(9)–C(17) = 2.4°). The adjacent MeO groups and the single AcO moiety are on opposite sides of the plane of the six-membered ring similar to the major component of **12**. The C–C–O–C torsion angles here are −78.6° for the MeO group (C(7)–C(8)–O(8)–C(17)) and 67.9° for the AcO moiety (C(9)–C(10)–O(10)–C(19)). In **10** *(Fig. 2)*; the MeO group cannot be coplanar with six-membered ring due to its proximity to the seven-membered ring and indeed is rotated out of the plane by 74.8°. As in **12**, the two adjacent AcO groups are approximately perpendicular to and rotated in opposite directions out of the plane of the six-membered ring (C(7)–C(8)–O(8)–C(16) = 105.0° and C(8)–C(9)–O(9)–O(17) = −94.0°). In all three molecules the RCH_3 group at C(1) is coplanar with the seven-membered tropolone ring.

In **2**, there is an area of the cell which is occupied by disordered solvent molecules. The diffraction peaks can be fit by a mixture of H_2O and acetone molecules but the exact amount of each moiety could not be precisely determined. The solvent molecules can form H-bonds among themselves but do not appear to form H-bonds to the acetate molecule. There is one intermolecular acetate...acetate H-bond in which the NH group acts as the donor and the carbonyl O-atom at C(2) is the acceptor (N...O = 2.96 Å). In **10**, there is one intermolecular H-bond. The hydroxy O-atom in the solvent molecule (MeOH) acts as the donor to the carbonyl O-atom on the (C(2)) (O...O = 2.76 Å). In **12**, the solvent molecule (H_2O) acts as an acceptor in a H-bond with the NH group (N...O = 2.97 Å) and as a donor to both the carbonyl O-atom at C(2) (O...O = 2.92 Å) and to the carbonyl O-atom in the acetylamine group (O...O = 2.88 Å). The carbonyl O-atom at C(2) acts as an acceptor in all three compounds.

The changes in [α] values and the complexity of ^{1}H-NMR signals of 1-*O*-acylated 1-*O*-demethylcolchicine and thio congeners, but particularly the formation of new isomers seen in $CHCl_3$ solution, seem to arise from the introduction of an acyl group at the OH function of 1-*O*-demethylcolchinoids. It seems prudent to assume that the 1-*O*-acylated species exist in $CHCl_3$ solution largely as two different conformers, where the bulky 1-acyloxy group on the aromatic ring, the *N*-acetyl side chain, and the tropolonic carbonyl are being held together differently, possibly with the help of solvent molecules. Whether only one of the two 1-acyloxy isomers is involved in the interaction with tubulin protein has not been elucidated. Colchicine conformers in solution can derive by different arrangements of the aromatic substituents, as seen in the solid state by X-ray diffraction, or by rotamerism of the *N*-acetyl group, atropisomerism by hindered rotation around the phenyl-tropolone axis, or a combination of these possibilities with the assistance of solvent molecules. This problem, which may be relevant to colchicine's binding to tubulin protein is, therefore, a rather complex one requiring further study.

Experimental Part[2])

General. Melting points (m.p.) (corrected) were determined with a *Fisher-Johns* apparatus. TLC plates were purchased from *Analtech, Inc.*, Newark, DE and silica gel *60* (0.040–0.063 mm) was used for column chromatography (CC). Solvent systems used for TLC over silica gel and for CC were as follows: (A) $CHCl_3$/MeOH 97:3, (B) $CHCl_3$/MeOH 93:7; (C) $CHCl_3$/MeOH 90:10. Optical rotations were measured by using a *Perkin-Elmer* Model *241 MC* polarimeter with the solvents and concentrations specified. IR spectra were recorded on a *Beckman IR 4230* spectrometer. ^{1}H-NMR spectra were determined by using a *Jeol JNM-FX 100* spectrometer with TMS as internal reference. CI-MS were obtained by using a *Finnigan 1015D* spectrometer with a Model *6000* data-collection system.

*Demethylation of 10-Demethoxy-3-*O*-demethyl-10-(methylthio)colchicine* (**4**). A soln. of 1.0 g (2.5 mmol) of **4** in conc. H_2SO_4 (7.0 ml) was immersed in a preheated oil bath (85–86°) and stirred at this temp. for 20 min. The resulting deep red soln. was diluted with ice and the pH of the soln. was adjusted to 5 with 15% NaOH. The orange-yellow precipitate was extracted with $CHCl_3$/MeOH (3:1, 8 × 40 ml), the org. layer washed with brine (2 × 20 ml), dried (Na_2SO_4) and evaporated to give 0.88 g of crude product showing two major spots on TLC (A). The mixture was separated by CC on silica gel (A). The faster running fraction yielded 19.5 mg (2%) of *10-demethoxy-1-*O*,3-*O*-didemethyl-10-(methylthio)colchicine* (**5**). M.p. 235–237° (MeOH); $[\alpha]_D^{25} = -335.8°$ (c = 0.055, MeOH). IR (KBr): 3320 (OH), 1650 and 1610 (C=O). ^{1}H-NMR ((D$_6$)DMSO): 1.83 (*s*, NCOMe); 2.36 (*s*, SMe); 3.70 (*s*, 2-OMe); 4.34 (*m*, H–C(7)); 6.21 (*s*, 1H, ArH); 6.98 (*s*, 1H, ArH); 7.20 (*s*, 2H, ArH); 8.44 (*d*, J = 8, NH); 8.88 (*s*, OH); 9.48 (*s*, OH). MS: 388 ($M^+ + 1$).

The second fraction gave after recrystallization from acetone 433 mg (44.8%) of *10-demethoxy-2-*O*,3-*O*-didemethyl-10-(methylthio)colchicine* (**6**). M.p. of the pure compound 199–201°; $[\alpha]_D^{25} = -347.8°$ (c = 0.27, MeOH). IR (KBr): 3300 (OH), 1650 and 1600 (C=O). ^{1}H-NMR ((D$_6$)DMSO): 1.80 (*s*, NCOMe); 2.36 (*s*, SMe); 3.41 (*s*, 1-OMe); 4.32 (*m*, H–C(7)); 6.40 (*s*, 1H, ArH); 6.96 (*s*, 1H, ArH), 8.52 (br. *s*, OH and NH); 9.18 (*s*, OH). MS: 388 ($M^+ + 1$).

Acetylation of mono- and dihydroxy compounds was carried out in a pyridine/Ac_2O mixture at r.t. After workup the crude acetyl derivatives were crystallized from acetone/Et_2O or AcOEt.

*2-*O*-Acetyl-10-demethoxy-2-*O*-demethyl-10-(methylthio)colchicine* (**11**). Acetylation of 160 mg (0.4 mmol) *10-demethoxy-2-*O*-demethyl-10-(methylthio)colchicine* (**7**) afforded 140 mg **11** (79.2%). M.p. 186–188° (acetone/Et_2O); $[\alpha]_D^{21} = -185°$ (c = 0.42, $CHCl_3$). IR ($CHCl_3$): 3455 and 3300 (CONH), 1762, 1670 and 1603 (C=O).

---

[2]) The compounds described crystallize with H_2O or solvents of crystallization, which are difficult to remove by standard methods. Routine elemental analyses were, therefore, omitted. The purity of each compound was secured by TLC, its structure verified by spectroscopic methods, and the compounds further characterized by optical rotation.

^{1}H-NMR ($CDCl_3$): 1.96 (*s*, NCOMe); 2.36 (*s*, OCOMe); 2.40 (*s*, SMe); 3.56 (*s*, 1-OMe); 3.84 (*s*, 3-OMe); 4.64 (*m*, H–C(7)); 6.56 (*s*, 1H, ArH); 7.00 (*d*, *J* = 9, 1H, ArH); 7.28 (*d*, *J* = 9, 1H, ArH); 7.39 (*s*, 1H, ArH); 7.73 (*d*, *J* = 7, NH). MS: 444 ($M^+ + 1$).

3-O-Acetyl-10-demethoxy-3-O-demethyl-10-(methylthio)colchicine (**13**). Prepared from **4** (160 mg, 0.4 mmol) affording **13** (107 mg, 60.5%). M.p. 160–162° (acetone/Et_2O); $[\alpha]_D^{21} = -133.7°$ (*c* = 0.46, $CHCl_3$). IR ($CHCl_3$): 3445, 3330 (CONH), 1763, 1670 and 1603 (C=O). ^{1}H-NMR ($CDCl_3$): 1.96 (*s*, NCOMe); 2.32 (*s*, OCOMe); 2.41 (*s*, SMe); 3.64 (*s*, 1-OMe); 3.91 (*s*, 2-OMe); 4.60 (*m*, H–C(7)); 6.64 (*s*, 1H, ArH); 7.04 (*d*, *J* = 10, 1H, ArH); 7.28 (*d*, *J* = 10, 1H, ArH); 7.38 (*s*, 1H, ArH); 7.64 (*d*, *J* = 6, NH). MS: 444 ($M^+ + 1$).

1-O,3-O-Diacetyl-10-demethoxy-1-O,3-O-didemethyl-10-(methylthio)colchicine (**9**). Prepared from **5** (10.5 mg, 0.027 mmol) affording **9** (12 mg, 94.5%). M.p. 220–222° (dec., acetone/Et_2O); $[\alpha]_D^{23} = -197.5°$ (*c* = 0.08, $CHCl_3$). IR ($CHCl_3$): 3460, 3300 (CONH), 1778, 1683 and 1608 (C=O). ^{1}H-NMR ($CDCl_3$): 1.96 (*s*, NCOMe); 2.20 (*s*, OCOMe); 2.34 (*s*, OCOMe); 2.40 (*s*, SMe); 3.83 (*s*, 2-OMe), 4.66, 4.94 (2*m*, H–C(7)); 6.83 (*s*, 1H, ArH); 6.88–7.28 (*m*, 4H, 3 ArH and NH). MS: 472 ($M^+ + 1$).

2-O,3-O-Diacetyl-10-demethoxy-2-O,3-O-didemethyl-10-(methylthio)colchicine (**10**). Prepared from **6** (116.2 mg, 0.3 mmol) affording **10** (80 mg, 56.5%). M.p. 166–168° (MeOH); $[\alpha]_D^{25} = -106.6°$ (*c* = 0.416, $CHCl_3$). IR ($CHCl_3$): 3460, 3300 (CONH), 1778, 1718, 1680 and 1612 (C=O). ^{1}H-NMR ($CDCl_3$): 1.97 (*s*, NCOMe); 2.28 (*s*, OCOMe); 2.32 (*s*, OCOMe); 2.40 (*s*, SMe); 3.57 (*s*, 1-OMe), 4.60 (*m*, H–C(7)); 6.80 (*s*, 1H, ArH); 7.02–7.32 (*m*, 4H, 3 ArH and NH). MS: 472 ($M^+ + 1$).

1-O,2-O-Diacetyl-10-demethoxy-1-O,2-O-didemethyl-10-(methylthio)colchicine (**12**). Obtained from *10-demethoxy-1-O,2-O-didemethyl-10-(methylthio)colchicine* (**8**)[3] (116.2 mg, 0.3 mmol) affording **12** (107 mg, 75.6%). M.p. 255–257° (dec., acetone/Et_2O); $[\alpha]_D^{25} = -237.1°$ (*c* = 0.54, $CHCl_3$), changing to −50.3 after 7 h. IR ($CHCl_3$): 3422, 3380 (CONH), 1760, 1678 and 1610 (C=O). ^{1}H-NMR ($CDCl_3$): 1.60 and 1.66 (2*s*, NCOMe); 2.11 and 2.13 (2*s*, OCOMe); 2.28 and 2.30 (2*s*, OCOMe); 2.38 (*s*, SMe); 3.84 and 3.87 (2*s*, 3-OMe), 4.68 and 4.94 (2*m*, H–C(7)); 6.34 and 6.52 (2*d*, *J* = 8, NH); 6.68 and 6.74 (2*s*, 1H, ArH); 6.84 (*d*, *J* = 9, 1H, ArH); 7.04 (*d*, *J* = 9, 1H, ArH); 7.16 and 7.23 (2*s*, 1H, ArH). MS. 472 ($M^+ + 1$).

Isomerization of **12**.

Compound **12** (13 mg) was dissolved in $CHCl_3$ (2 ml) and refluxed for 3 h. TLC (C) indicated besides starting material and additional new spot with higher R_f value. The soln. was evaporated in vacuum and the residue applied to CC on silica gel. The mixture was separated using solvent system C. The first fraction (3 mg) gave the new isomer, contaminated with a small amount of starting material. It was crystallized from acetone/Et_2O. The crystals obtained were identical with starting material (TLC, m.p.). A second fraction (8.6 mg) was identical with starting material (TLC, m.p.). The second fraction was dissolved in $CHCl_3$ (2 ml) and stirred overnight at 50° (bath temp.). TLC indicated the presence of two compounds in a nearly 1:1 ratio. After evaporation and crystallization from acetone/Et_2O, the substance obtained was identical in every respect with starting material. The same behavior was shown by compounds **2**, **9** and **14**, but not by compounds **1**, **3**, **10**, **11**, and **13**.

1-O,2-O-Dibenzoyl-10-demethoxy-1-O,2-O-didemethyl-10-(methylthio)colchicine (**14**). This compound was prepared from **8** (116.2 mg, 0.3 mmol) and benzoic anhydride (226 mg, 1 mmol) in 2.5 ml pyridine: **14** (140 mg, 78.3%). M.p. 183–185° (acetone/Et_2O); $[\alpha]_D^{26} = -167.1°$ (*c* = 0.76, $CHCl_3$), changing to +2.3° after 4 h. IR ($CHCl_3$): 3460, 3380 (CONH), 1750, 1685 and 1618 (C=O). ^{1}H-NMR ($CDCl_3$): 1.84 and 2.0 (*s*, NCOMe); 2.21 (*s*, SMe); 3.86 and 3.88 (*s*, 3-OMe); 4.76 and 4.92 (*m*, H–C(7)); 6.64–8.08 (*m*, 15H, 14 ArH and NH). MS 596 ($M^+ + 1$).

Equilibration of **14** in $CHCl_3$ soln. afforded, as in the case of **12**, a 1:1 mixture of isomers. Isolation of the faster moving isomer afforded a material which was identical by α_D, m.p. and TLC with the original sample.

We are grateful to Dr. *P. Bellet* from *Roussel-Uclaf* in Paris for providing us with substantial amounts of thiocolchicine and thiocolchicoside. We also thank Dr. *H. Yeh* and Dr. *N. Whittaker* from our Laboratory for recording the NMR and mass spectra.

[3]) Catechol **8** [4] was crystallized from acetone and showed m.p. 238–240°; $[\alpha]_D^{25} = -328°$ (*c* = 0.4, MeOH).

REFERENCES

[1] M. Rösner, H.-G. Capraro, A. E., Jacobson, L. Atwell, A. Brossi, M. A. Iorio, T. H. Williams, R. H. Sik, C. F. Chignell, *J. Med. Chem.* **1981,** *24,* 257.
[2] A. Bladé-Font, *Afinidad* **1979,** *36,* 329.
[3] L. Velluz, G. Muller, *Bull. Soc. Chim. Fr.* **1954,** 1072.
[4] P. N. Sharma, A. Brossi, *Heterocycles* **1983,** *20,* 1587.
[5] P. Buchs, K. C. Rice, A. Brossi, J. V. Silverton, R. Potenzone, Jr., *J. Org. Chem.* **1982,** *47,* 4134.
[6] H. G. Capraro, A. Brossi, 'Tropolonic Colchicine Alkaloids', in 'The Alkaloids', Ed. A. Brossi, Academic Press, New York 1984, Vol. XXIII, pp. 1–70.
[7] M. H. Zweig, C. F. Chignell, *Biochem. Pharmacol.* **1973,** *22,* 2142.
[8] P. Kerekes, P. N. Sharma, A. Brossi, C. F. Chignell, F. F. Quinn, *J. Med. Chem.*, in press (1985).
[9] J. Karle, I. L. Karle, *Acta Crystallogr.* **1966,** *21,* 849.
[10] J. Karle, *Acta Crystallogr., Sect. B* **1968,** *24,* 182.
[11] G. M. Sheldrick, 'SHELXTL', Minicomputer Programs for Structure Determination, University of Gottingen, 1980.
[12] C. K. Johnson, ORTEP, Report ORNL-3794. Oak Ridge National Laboratory, Tennessee, 1965.
[13] a) L. Lessinger, T. N. Margulis, *Acta Crystallogr., Sect. B* **1978,** *34,* 578; b) L. Lessinger, T. N. Margulis, *ibid.* **1978,** *34,* 1556; c) J. V. Silverton, *ibid.* **1979,** *35,* 2800; d) C. Miravitelles, C. Solans, A. Blade-Font, G. Germain, J. DeClereq, *ibid.* **1982,** *28,* 1782; e) P. N. Sharma, A. Brossi, J. V. Silverton, *ibid.*, in press.
[14] I. L. Karle, 'Elucidation of Structural Formula, Configuration, and Conformation of Alkaloids by X-Ray Diffraction', in 'The Alkaloids', Ed. A. Brossi, Academic Press, New York, 1984, Vol. XXII, pp. 49–83.