Biological Effects of Modified Colchicines. 2. Evaluation of Catecholic Colchicines, Colchifolines, Colchicide, and Novel *N*-Acyl- and *N*-Aroyldeacetylcolchicines

Arnold Brossi,*,† Padam N. Sharma,† Louise Atwell,† Arthur E. Jacobson,† Maria A. Iorio,‡ Marisa Molinari,‡ and Colin F. Chignell§

Section on Medicinal Chemistry, Laboratory of Chemistry, National Institute of Arthritis, Diabetes and Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205, Laboratory of Pharmaceutical Chemistry, Istituto di Sanita, 00161 Rome, Italy, and Laboratory of Environmental Biophysics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709. *Received December 22, 1982*

A series of natural and synthetic colchicine derivatives was examined for their potency in the lymphocytic leukemia P388 screen in mice, for their toxicity in mice, and for their binding to microtubule protein. The natural alkaloids cornigerine and colchifoline and several N,O-substituted analogues of colchifoline were found to be as potent and as toxic as colchicine in the P388 screen with good affinity for tubulin. The 1,2-(methylenedioxy)-substituted isomer of cornigerine was considerably less potent in vivo than could have been anticipated from the in vitro tubulin binding data. Severral *N*-acyl and *N*-aroyl derivatives prepared from deacetylcolchicine showed high potency in the in vitro and in vivo screens. Colchicide was found to be highly potent in vivo, and *N*-carbethoxydeacetylcolchicine, a synthetic analogue of colchicine with a *N*-carbethoxy instead of an *N*-acetyl function, showed interesting biological properties.

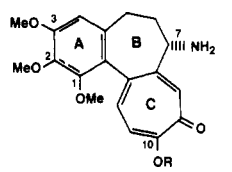
We reported in our first paper that tropolonic colchicines which showed weak binding to microtubule protein were essentially inactive in the lymphocytic leukemia P388 assay and that compounds showing low toxicity in mice always displayed significantly lower potency in the P388 screen.[1] Evaluation of the results obtained from the P388 assay, tubulin binding assay, and toxicity data appeared, then, to be a reasonable method for ascertaining the biological effects of colchicine derivatives. It was hoped that by appropriate modification of the colchicine structure a potent but much less toxic substitute for colchicine could ultimately be found.

Selective O-demethylation at C_1 of colchicine gave 1-demethylcolchicine, which was found to be practically inactive in vitro and in vivo.[1] O-Demethylation at C_3 of colchicine, on the other hand, afforded 3-demethylcolchicine, which was found remarkably active in vivo, although its *O*-glucoside, colchicoside, is practically inactive.[1] The substitution in the aromatic ring A has thus proven to be important both for tubulin binding and antimitotic effect[2,3] and this conclusion has been supported by the additional compounds examined herein.

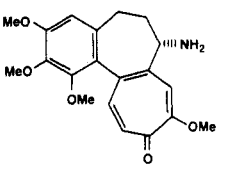
Modification of the *N*-acetamido side chain also significantly influenced antimitotic activity. Introduction of electron-withdrawing groups at the acetamido terminal increased potency,[4,5] whereas a change from an *N*-acyl to a *N*-aroyl group seemed to cause a decrease in potency.[5,6] In order to verify this point, which seemed very critical for our study, a series of novel *N*-acyldeacetylcolchicines was needed, and deacetylcholchiceine (**1**), its methyl ethers **2** and **3** (obtained by O-methylation of **1**) and demecolcine (**4**) were utilized for their preparation.

A third, interesting, group of compounds is represented by colchicide (**35**), a compound prepared by Rapoport et al. from thiocolchicine[7] and reported to be biologically active. Since the C_{10}-OMe group of colchicine was long considered to be essential for antimitotic activity and gave rise to the development of thiocolchicine and colchiceinamide, we considered a reevaluation of colchicide worthwhile.

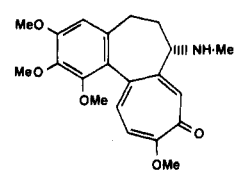
Chemistry. The first group of colchicine derivatives evaluated included the catechols **8** and **9**, prepared earlier



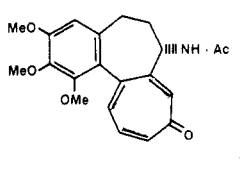
1: R = H
2: R = Me



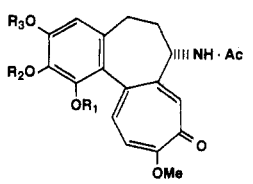
3



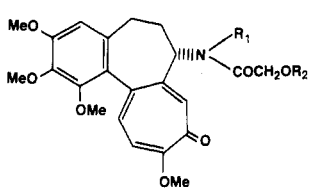
4



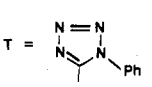
35 (Colchicide)



5 $R_1 = R_2 = R_3 = Me$
6 $R_1 = Me, R_2 + R_3 = -CH_2-$
7 $R_1 + R_2 = CH_2-, R_3 = Me$
8 $R_1 = R_2 = H, R_3 = Me$
9 $R_1 = Me, R_2 = R_3 = H$
10 $R_1 = R_3 = Me, R_2 = T$
11 $R_1 = R_2 = Me, R_3 = T$



12 $R_1 = R_2 = H$
13 $R_1 = Me, R_2 = H$
14 $R_1 = H, R_2 = Ac$
15 $R_1 = H, R_2 = Et$



in connection with our syntheis of cornigerine,[8] and the two *N*-phenyltetrazolyl ethers **10** and **11**, obtained from

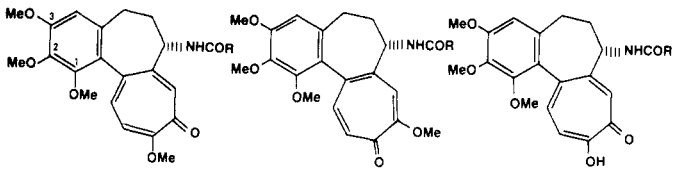
† NIADDK, NIH.
‡ Istituto di Santita.
§ NIEHS, NIH.

(1) M. Rösner, H. G. Capraro, A. E. Jacobson, L. Atwell, A. Brossi, M. A. Iorio, R. H. Sik, and C. F. Chignell, *J. Med. Chem.*, **24**, 257 (1981).
(2) M. H. Zweig and C. F. Chignell, *Biochem. Pharmacol.*, **22**, 2141 (1973).
(3) T. J. Fitzgerald, *Biochem. Pharmacol.*, **25**, 1383 (1976).
(4) H. Lettré, K. H. Donges, K. Barthold, and T. J. Fitzgerald, *Liebig's Ann. Chem.*, **758**, 185 (1972).
(5) F. F. Quinn and J. A. Beisler, *J. Med. Chem.*, **24**, 251 (1981).
(6) J. Leiter, V. Downing, J. L. Hartwell, and M. J. Shear, *J. Natl. Cancer Inst.*, **13**, 379 (1952).

their phenolic precursors[1] by O-alkylation with *N*-phenyltetrazolyl chloride. Both heterocyclic ethers were amorphous materials, but their chemical integrity was assured by MS and TLC analysis.

A second group included colchifoline (**12**), a minor contaminant of commercial colchicine,[9] and its derivatives **13–15**. The *N*-methyl derivative **13** was prepared by reacting the now readily available demecolcine (**4**)[10] with (trifluoroacetyl)glycolyl chloride, a method analogous to the synthesis of colchifoline from deacetylcolchicine.[11]

Other compounds of this group are represented by novel *N*-acyldeacetylcolchicines, *N*-aroyldeacetylcolchicines, and the ethyl carbamate **26**. The analogues **18**, **20**, **23**, and **26**



16	R = Ph			**17**	
18	R = CH_2Me	**19**			
20	R = CH_2CH_2Me	**21**			
22	R = C(Me)(Me)(Me)				
23	R = 3,4,5-trimethoxybenzoyl	**24**		**25**	
26	R = OEt	**27**		**28**	
29	R = CH_2-O-Ph				
30	R = CF_3, 2-OH				
31	R = CH=C(Me)-CH=CH-C(Me)=CH-CH=C(Me)-Me				
32	R = CH=CH-(2-OAc, 3,4-diOMe)				
33	R = CH=CH-(2-OH, 3,4-diOMe)				
12	R = CH_2OH	**34**			

were obtained from a mixture of deacetylcolchicine and deacetylisocolchicine[12] by N-acylation and separation of the isoisomers **19**, **21**, **24**, and **27** by chromatography over silica gel (method A). The two analogues **18** and **20** have been reported elsewhere,[13,14] but no experimental details were given. Mild acid hydrolysis of mixtures of **23** and **24**, and **26** with **27**, afforded the colchiceine analogues **25** and **28**, which could be recycled. The *N*-benzoyldeacetylcolchicine (**16**), evaluated earlier as a mixture of the two tautomeric ethers,[9] has now been prepared as the natural isomer, which on mild acid hydrolysis gave **17**. The analogues **16**, **20**, **22** and **29** were prepared from deacetylcolchicine, obtained from *N*-(trifluoroacetyl)deacetylcolchicine[10] and kept in the form of their *d*-tartrate salts[12] (method B).

The assignment of the normal and iso structures was based on ^1H NMR analysis[15] and the MS-fragmentation pattern, the iso compounds show more intense fragmentation peaks than the natural isomers,[9] and the well established behavior of the two isomers during chromatography, with the normal isomer moving faster on silica gel with $CHCl_3/MeOH/NH_4OH$ (90:9:1) than the iso isomer. Furthermore, the optical rotation of the iso forms were consistently more negative than the natural isomers in chloroform solution.

The partial ether cleavage of colchicine with concentrated sulfuric acid has now been applied to *N*-(trifluoroacetyl)deacetylcolchicine,[10] affording the 2-demethyl congener **30**, an interesting compound for further exploration of the 2-demethyl analogues of colchicine. The phenol **30** showed signals for C_1-OMe at δ 3.72, for C_3-OMe at 3.86, and for C_{10}-OMe at 4.00.

Colchicide (**35**), which lacks the C_{10}-OMe group of colchicine, was prepared from thiocolchicine by the reported procedure[7] and obtained in 46% yield, the byproduct being hexahydrodemethoxycolchicine. Colchicide, purified by chromatography and obtained as a glassy solid, showed one spot on TLC but had a considerably lower rotation than that reported in the literature.[7] This might be due to the presence of solvents orginating from the chromatography. The hydrochloride salt prepared for biological evaluation showed an unusually high optical rotation, $[\alpha]^{25}_D$ −456° ($CHCl_3$).

The *N*-sinapinoyl compound **33**, prepared via its *O*-acetate **32**, and the *N*-retinoyl compound **31** were both prepared as potential UV-affinity labels, and for this reason they were only tested in vitro. Their UV absorption, however, can not be distinguished from that of colchicine and thus **33** and **31** were not useful as affinity labels, although **33** showed the expected bathochromic shift in alkaline solution.

Structure–Activity Relationships. It can be seen from Table I that a correlation exists between the in vivo potency of these compounds in the lymphocytic leukemia P388 screen of the National Cancer Institute (NCI) (see Experimental Section) and their toxicity in that screen. With few exceptions, molecular modifications that produced compounds with potencies equal to or less than colchicine resulted in correspondingly altered toxicities. Variations in structure that resulted in compounds having potencies similar to that of colchicine are embodied in the colchifolines **12** and **13** and the derivative **15** (Table I). The *N*-benzoyl analogue **16**, when tested earlier as a mixture of the natural and iso isomer,[6] was not found to be very potent in vivo. However, the pure isomer **16** is potent, almost comparable to colchicine in the P388 assay, as is the *N*-carbethoxy analogue **26**. In contrast, the 3,4,5-trimethoxybenzoyl analogue **23** is considerably less potent. N-Substituted compounds, which show binding to microtublue protein similar to colchicine, are the *N*-propionyl, *N*-butyryl, *N*-phenoxyacetyl, *N*-trifluoroacetyl, and the *N*-benzoyl derivatives.

The 10-demethoxycolchicine (**35**, colchicide) has suprising activity in vitro, since it has been generally accepted that the C_{10}-methoxy group of colchicine is essential for activity and is the place of interaction with biopolymers.

The colchifoline acetate **14**, which was only available in minute quantities, showed good affinity for microtubule protein. The isocolchicines **19** and **34** showed poor in vitro activity and were not investigated in vivo. The colchiceines **17**, **25**, and **28**, which belong to an inactive group of colchicine, were also not further evaluated. The phenyl-

(7) H. Rapoport and J. B. Lavigne, *J. Am. Chem. Soc.*, **77**, 667 (1955).
(8) M. Rösner, F. L. Hsu, and A. Brossi, *J. Org. Chem.*, **46**, 3686 (1981).
(9) M. A. Iorio, A. M. Farina, G. Cavina, L. Boniforti, and A. Brossi, *Heterocycles*, 14, 625 (1980).
(10) H. G. Capraro and A. Brossi, *Helv. Chim. Acta.*, **62**, 965 (1979).
(11) M. A. Iorio, M. Molinari, and A. Brossi, *Can. J. Chem.*, **59**, 283 (1981).
(12) R. F. Raffauf, A. L. Farren, and G. E. Ullyot, *J. Am. Chem. Soc.*, **75**, 5292 (1953).
(13) H. Lettré, *Angew. Chem.*, **63**, 421 (1951).
(14) H. Lettré in "Ergebnisse der Physiologie, Biologischem Chemie und Experimentellen Pharmakologie", Springer-Verlag, Berlin, 1950, pp 379–447.
(15) C. D. Hufford, H. G. Capraro, and A. Brossi, *Helv. Chim. Acta*, **63**, 50 (1980).

Table I. Potency in P388 Lymphocytic Leukemia Screen, Tubulin Binding, and Toxicity of Colchicine and Derivatives

no.	compound	potency,[a] μmol/kg	tubulin binding,[b] %	toxicity I[c]	toxicity II[d]
5	colchicine	0.4[e]	90	2.5	3.0 (2.5-4.0)
6	cornigerine	<10[f]	59	39.1	9.7 (7.0-13.1)
7	1,2-(methylenedioxy)-1,2-demethylcolchicine	inactive	48	104	>167[g]
8	1,2-didemethylcolchicine	inactive	0	>320	>450[h]
9	2,3-didemethylcolchicine	20[e]	18	54	>450[i]
10	2-*O*-(*N*-phenyltetrazolyl)-2-demethylcolchicine		52		
11	3-*O*-(*N*-phenyltetrazolyl)-3-demethylcolchicine		27		
12	colchifoline	0.7[e]	97	3.0	3.9 (2.9-5.1)
13	*N*-methylcolchifoline	0.6[e]	99	2.9	14.5 (11.2-18.9)
14	*O*-acetylcolchifoline		86		8.5 (6.6-11.2)
15	*O*-ethylcolchifoline	0.4	74	2.8	9.0 (6.5-12.6)
16	*N*-benzoyldeacetylcolchicine	1.0[e]	83	4.3	60.5 (43.2-85.0)
18	*N*-propionyldeacetylcolchicine		98		8.2 (4.8-14.0)
19	*N*-propionyldeacetylisocolchicine		10		
20	*N*-butyryldeacetylcolchicine	0.7[e]	98	5.8	5.8 (4.0-8.9)
22	*N*-pivaloyldeacetylcolchicine		65		9.3 (6.8-12.9)
23	*N*-(3,4,5-trimethoxybenzoyl)deacetylcolchicine	14.0[e]	46	72.6	>180[j]
26	*N*-carbethoxydeacetylcolchicine	0.7[e]	86	11.7, 1.5[k]	114 (88.3-147.1)
29	*N*-(phenoxyacetyl)deacetylcolchicine	1.3[e]	81	5.1	61.3 (45.0-83.5)
30	2-demethyl-*N*-(trifluoroacetyl)deacetylcolchicine	3.8[e]	96	23.5	32.5 (20.0-52.9)
31	retinoyldeacetylcolchicine		41		
32	*N*-(acetoxysinapinoyl)deacetylcolchicine		69		
33	*N*-inapinoyldeacetylcolchicine		69		
34	isocolchifoline		0		
35	colchicide	9.2[e]	87	54.2	168.9 (119.5-238.8)

[a] Obtained by graphical estimation of the potency, which would give a T/C = 140. [b] Percentage by which binding is reduced by the presence of the inhibitor at 25 μM with [^{3}H]colchicine at 2.5 μM. Values are the average of triplicate assays. [c] Toxicity in the P388 NCI screen. Defined as that dose, in micromoles per kilogram, that caused an average weight loss >3 g in at least one out of the set of mice or the death of one or more of the mice in the set. Six mice were used at each of the dose levels. Deaths were noted on 5th day of a series of nine injections in 9 days (1 per day) of the compound. [d] Toxicity found after a single intramuscular injection, in micromoles per kilogram. The total number of deaths were counted after 7 days at various dose levels, and the LD_{50} was determined by probit analysis. Parenthesized numbers represent the 95% confidence interval. [e] Confirmed by a second P388 assay. [f] Estimated from two nontoxic dose levels of 19.6 and 9.8 μmol/kg, which gave T/C values of 161 and 155, respectively. [g] Twelve percent of mice were dead at 167 μmol/kg. [h] Ten percent of mice were dead at 450 μmol/kg. [i] No deaths occurred at highest dose level tested (450 μmol/kg). [j] Ten percent of mice were dead at 180 μmol/kg. [k] A second P388 assay showed markedly different toxicity. This compound is being further evaluated.

tetrazolyl ethers **10** and **11** and analogues of colchicine with unsaturated N-CO side chains were prepared as potential affinity labels and only tested in vitro.

A comparison between the 1,2-didemethyl compound **8** and its methylene ether **7** with the corresponding isomers **9** and **6** of the 2,3-didemethyl series in the P388 assay pointed again to the importance of the substitution at C-1 observed in the series of monophenols prepared earlier.[1] The C_1-OMe group in **6** is out of the plane of the aromatic ring, and its hydrogen bonding interaction is now being studied.[16] The very toxic compounds showed, with the exception of **16**, **26**, **29**, a reasonable correlation between the toxicities determined through single intramuscular injection in mice (toxicity II in Table I) and those found during the multiple-dose studies at NCI (toxicity I in Table I; see Experimental Section). A considerable difference was also found between the toxicities determined after multiple and single injection with compounds **9** and **35**. The compounds examined were almost always found to be more toxic in the multiple-dose study used in the NCI P388 assay than in the single-injection study. It is possible that the multiple doses used in the P388 assay at NCI tend to increase the toxic effect of the drug due to bioaccumulation, compared with the lower toxicity generally observed from a single dose where bioaccumulation would not be a problem. It can be observed from Table I that all the compounds found highly active in the P388 system did bind well to rat brain tubulin, confirming again that this assay is a valuable prescreen for colchicine-related compounds. Both catecholic colchicines **8** and **9**, the retinoyl derivative **31**, and the *N*-phenyltetrazolyl ethers **10** and **11** showed relatively poor binding and, thus, were not tested in vivo. Nevertheless, it is noteworthy that the *N*-acyl residue can be chemcially altered considerably before the tubulin binding disappears.

Colchicide, which lacks the 10-methoxy groups of colchicine, and two compounds with altered N-substituents, *N*-benzoyldeacetylcolchicine and *N*-carbethoxydeacetylcolchicine, all have somewhat different molecular structure than colchicine and are among the most interesting candidates discovered in this study and deserve further attention. *N*-Butyryl- and *N*-propionyldeacetylcolchicine were also found to be extremely potent, as noted earlier.[13,14]

Experimental Section

Melting points are uncorrected and were taken in a Büchi-Tottoli capillary apparatus and Fisher-Johns apparatus. Alumina Stratocrom ALF 254 (5 × 10 cm) and silica gel 60 F 254 (5 × 10 cm) were used for TLC, and silica gel 60 (0.04–0.06 mm) was used for column chromatography. The solvent system used for TLC, if not otherwise noted, was $CHCl_3$/MeOH/NH_4OH, 90:9:1. The analytical samples were dried in vacuo (10 mm) over P_2O_5 at 90–100 °C for 24 h. Microanalyses were performed in the Microanalytical Section of Istituto Superiore di Sanita (Rome) and the Laboratory of Chemistry, NIADDK, NIH, Bethesda, MD. Optical rotations were measured with a Perkin-Elmer Model 141 and 241 MC polarimeter in $CHCl_3$ and concentrations specified. UV spectra were measured in EtOH (otherwise stated) with a Hewlett Packard 8450 A spectrophotometer. Absorption maxima (λ_{max}) are reported in nanometers, followed by log ϵ values in

(16) A. Brossi, P. N. Sharma, and J. V. Silverton, in preparation.

parentheses. NMR spectra were obtained with a Varian 220 spectrometer with $(CH_3)_4Si$ as the internal standard. Electron-ionization mass spectra (EIMS) were obtained with a LKB 2091 mass spectrometer: equipped with a Digital PDP 11 data processing system, samples applied by direct inlet, and probe usually heated from 25 to 250 °C, 70 eV, source temperature 250 °C, acceleration potential 3500 V. Chemical-ionization mass spectra (CIMS) were determined by using a Finnigan 1015D spectrometer with a Model 6000 data collection system. Relative intensities are given in parentheses.

2-*O*-(*N*-Phenyltetrazolyl)-2-demethylcolchicine (10). A mixture of 2-demethylcolchicine, (600 mg, 1.55 mmol), 5-chloro-1-phenyl-1*H*-tetrazole (330 mg, 1.83 mmol), finely ground K_2CO_3 (330 mg, 2.39 mmol), and DMF (15 mL) was stirred for 48 h until the starting material disappeared according to TLC. After the addition of 50 mL of water, the material was extracted with CH_2Cl_2 (4 × 50 mL), and the combined organic extracts were washed with brine, dried (Na_2SO_4), and evaporated to dryness to give 1 g of a slightly yellowish residue. The product behaves on TLC as a single entity. The material needed for the biological experiments was obtained by flash column chromatography over silica gel and eluted with CH_2Cl_2/MeOH (30:1) to afford a residue, which was triturated with cyclohexane to afford a pure compound (550 mg, 67%): CIMS, *m/e* 529 ($M^+ + 1$); UV λ_{max} 233 nm (log ϵ 2.83), 345 (2.58).

3-*O*-(*N*-Phenyltetrazolyl)-3-demethylcolchicine (11) was prepared in a manner similar to **10**, from 3-demethylcolchicine, and was obtained as a yellowish powder, which was pure on TLC: CIMS, *m/e* 529 ($M^+ + 1$); UV λ_{max} 231 nm (log ϵ 4.05), 339 (3.87).

***N*-(Hydroxyacetyl)demecolcine (13).** To a solution of demecolcine (**4**; 0.8 g, 2.24 mmol) in 20 mL of CH_2Cl_2 and 0.7 mL of pyridine, coooled in an ice bath, was carefully added, under stirring and anhydrous conditions, 0.43 mL (0.64 g, 3.36 mmol) of (trifluoroacetyl)glycolyl chloride. The mixture was stirred for 1 h (TLC showed the absence of **4** and the appearance of a new spot running slightly slower than demecolcine). The organic layer was washed with H_2O (2 × 5 mL), dried (Na_2SO_4), and concentrated to leave a residue, which was chromatographed through a column of silica gel and eluted with $CHCl_3$/MeOH/NH_4OH (97.0:2.5:0.5) to give pure compound **13** (540 mg): mp 208–209 °C; $[\alpha]^{22}_D$ −223° (*c* 1.06, $CHCl_3$). Anal. ($C_{23}H_{27}NO_7$) C, H, N.

***O*-Acetylcolchifoline (14).** To a solution of colchifoline (**12**; 200 mg, 0.48 mmol) dissolved in 20 mL of dry benzene were added acetic anhydride (1 mL) and pyridine (1 mL), and the mixture was refluxed for 1 h until the TLC, on silica gel, showed the absence of colchifoline in the reaction mixture. The solvent was removed in vacuo, and the residue was diluted with ice–H_2O and ammonia and extracted with CH_2Cl_2. The organic phase was dried (Na_2SO_4) and evaporated to give a frothy yellow solid (180 mg, 82%): mp 120–124 °C; EIMS, *m/e* 457 (M^+).

***O*-Ethylcolchifoline (15).** To a solution of deacetylcolchicine (400 mg, 1.12 mmol) in CH_2Cl_2 (10 mL) and (2.26 mL) pyridine (0.4 mL) was added freshly distilled ethoxyacetic anhydride at ice-bath temperature under stirring. After the solution was left standing at room temperature overnight, the solvent and the pyridine were evaporated, and the residue was taken into $CHCl_3$, washed with brine, dried over Na_2SO_4, and passed through a column of 20 g of silica gel. Elution with $CHCl_3$/MeOH/NH_4OH (80:20:2) afforded an amorphous product (640 mg) showing two spots on TLC originating from a small contamination with deacetylcolchicine and the isomeric ether: EIMS, *m/e* 443 (M^+).

Preparation of *N*-Acyldeacetylcolchicine Derivatives. Method A. From a Mixture of Deacetylcolchicine and Deacetylisocholchicine. The procedure adopted is as follows for compounds **18** and **19**.

***N*-Propionyldeacetylcolchicine (18) and *N*-Propionyldeacetylisocolchicine (19).** To a crude mixture of **2** and **3**, prepared from deacetylcholchiceine[12] (2 g, 5.60 mmol) dissolved in dry benzene (50 mL) and pyridine (3 mL), was added propionic anhydride (3 mL) in one portion, and the solution was heated under stirring at 55–60 °C for 4 h [TLC: Al_2O_3, $CHCl_3$/MeOH (97.5:2.5)]. The solvent was removed in vacuo, and the residue, diluted with ice–H_2O and ammonia, was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and evaporated to afford an oil. The oily residue was chromatographed on a column (2.5 × 80 cm) of silica gel packed with $CHCl_3$ and eluted with $CHCl_3$/MeOH/NH_4OH (97.0:2.5:0.5). The more readily eluted *N*-proionyldeacetylcolchincine (**18**) was crystallized from EtOAc/Et_2O (300 mg, 13% from deacetylcolchiceine): mp 155–157 °C (with gas evolution, shrinks at 139 °C); $[\alpha]^{21}_D$ −129° (*c* 1.11, $CHCl_3$); EIMS, *m/e* 413 (M^+). Anal. ($C_{23}H_{27}NO_6$) C, H, N.

The second eluted component, *N*-propionyldeacetylisocolchicine (**19**), crystallized from EtOAc–Et_2O (450 mg, 19% from deacetylcolchiceine): mp 205–207 °C; $[\alpha]^{22}_D$ −257° (*c* 0.92, $CHCl_3$); EIMS, *m/e* 413 (M^+). Anal. ($C_{23}H_{27}NO_6$) C, H, N.

Other derivatives listed below were similarly prepared by method A. The yield of the individual isomers based on deacetylcolchiceine varied between 10 and 20%.

***N*-Butyryldeacetylcolchicine (20) and *N*-Butyryldeacetylisocolchicine (21) (with Butyryl Chloride).** Compound **20** was obtained as an amorphous yellow powder after trituration with Et_2O: mp 129–133 °C; $[\alpha]^{21}_D$ −157° (*c* 1.08, $CHCl_3$); EIMS, *m/e* 427 (M^+). Anal. ($C_{24}H_{29}NO_6 \cdot {}^1/_2H_2O$), C, H, N. Compound **21**: light yellow solid; mp 127–131 °C (shrinks at 110 °C); $[\alpha]^{26}_D$ −254° (*c* 1.05, $CHCl_3$); EIMS, *m/e* 427 (M^+). Anal. ($C_{24}H_{29}NO_6$) C, H, N.

***N*-(3,4,5-Trimethoxybenzoyl)deacetylcolchicine (23) and *N*-(3,4,5-Trimethoxybenzoyl)deacetylisocolchicine (24) (with 3,4,5-Trimethoxybenzoyl Chloride).** Compound **23**: amorphous yellow powder; mp 166–169 °C (shrinks before melting); $[\alpha]^{20}_D$ −16° (*c* 0.97, $CHCl_3$); EIMS, *m/e* 551 (M^+). Anal. ($C_{30}H_{33}NO_9 \cdot {}^1/_2H_2O$), C, H, N. Compound **24**: crystallized from EtOAc–MeOH; mp 274–275 °C; $[\alpha]^{20}_D$ −92° (*c* 0.95, $CHCl_3$); EIMS, *m/e* 551 (M^+). Anal. ($C_{30}H_{33}NO_9$) C, H, N.

***N*-Carbethoxydeacetylcolchicine (26) and *N*-Carbethoxydeacetylisocolchicine (27) (with Ethyl Chloroformate and Triethylamine).** Compound **26**: crystallized from EtOAc–MeOH; mp 209–211 °C; $[\alpha]^{20}_D$ −173° (*c* 1.0, $CHCl_3$); EIMS, *m/e* 429 (M^+). Anal. ($C_{23}H_{27}NO_7$) C, H, N. Compound **27**: crystallized from EtOAc; mp 202–203 °C; $[\alpha]^{20}_D$ −249° (*c* 0.96, $CHCl_3$); EIMS, *m/*E 429 (M^+). Anal. ($C_{23}H_{27}NO_7$) C, H, N.

Method B. From Deacetylcolchicine. *N*-Pivaloyldeacetylcolchicine (22). A solution of *N*-deacetylcolchicine (500 mg, 1.40 mmol) in dry benzene (30 mL), pyridine (1 mL), and trimethylacetic anhydride (1 mL) was stirred at 55–60 °C. Acylation was complete after 7–8 h (TLC). The mixture was concentrated in vacuo, and the residue was diluted with H_2O and ammonia and extracted with CH_2Cl_2. The oranic layer was dried (Na_2SO_4) and evaporated to give a yellow solid, which was purified through column chromatography [Al_2O_3 activity II, with $CHCl_3$/MeOH (99.5:0.5) as eluent] to give *N*-pivaloyldeacetylcolchicine (**22**) as an amorphous powder (350 mg, 57%): mp 139–143 °C (with gas evolution); $[\alpha]^{21}_D$ −168° (*c* 0.94 $CHCl_3$); EIMS, *m/*E 441 (M^+). Anal. ($C_{25}H_{31}NO_6$) C, H, N.

Other derivatives prepared by method B are listed below. The yield of the desired compounds varied between 50 and 70% based on deacetylcolchicine.

***N*-Benzoyldeacetylcolchicine (16)**: triturated with Et_2O to afford a pure solid compound; mp 260 °C; $[\alpha]^{26}_D$ −47° (*c* 0.96, $CHCl_3$); EIMS, *m/e* 461 (M^+); CIMS, *m/e* 462 ($M^+ + 1$). Anal. ($C_{27}H_{27}NO_6$) C, H, N.

***N*-Butyryldeacetylcolchicine (20)**: mp 129–133 °C; all the data (IR and MS) were identical with the material obtained earlier by method A.

***N*-(Phenoxyacetyl)deacetylcolchicine (29)**: crystallized from 2-propanol–Et_2O; Mp 235 °C; $[\alpha]^{26}_D$ −91° (*c* 1.11, $CHCl_3$); EIMS, *m/e* 491 (M^+); CIMS, *m/e* 492 ($M^+ + 1$). Anal. ($C_{28}H_{29}NO_7$) C, H, N.

Acetate of *N*-sinapinoyldeacetylcolchicine (32) (with acetoxysinapinoyl chloride[17]): obtained on trituration with Et_2O (2 mL) to afford pure solid; mp 168 °C; $[\alpha]^{26}_D$ +96° (*c* 0.60, $CHCl_3$); UV λ_{max} 231 nm (log ϵ 2.08), 296 (4.07), 344 (3.93); EIMS, *m/e* 605 (M^+); CIMS, *m/e* 606 ($M^+ + 1$). Anal. ($C_{33}H_{35}NO_{10}$) C, H, N.

***N*-Sinapinoyldeacetylcolchicine (33).** To a solution of **32** (100 mg, 0.16 mmol) in acetone (5 mL) was added dropwise concentrated ammonia solution (4.5 mL), and the reaction mixture was stirred at room temperature for 2 h, until TLC showed the absence of starting material in the reaction mixture. Acetone was

(17) C. D. Hurd, *J. Am. Chem. Soc.*, **71**, 1016 (1949).

removed at high vacuum at room temperature to leave the aqueous layer. It was diluted with water (10 mL) and extracted with CH_2Cl_2 (4 × 5 mL). The combined organic layer was washed with 10% aqueous $NaHCO_3$ solution (3 × 10 mL), dried (Na_2SO_4), and concentrated at 40 °C under reduced pressure to leave a solid residue, which was taken into Et_2O (2 mL) and triturated to afford a pure (**33**; 85 mg, 94%): mp 160 °C; $[\alpha]^{26}_D$ +98° (*c* 0.40, $CHCl_3$); UV λ_{max} 242 nm (log ϵ 3.44), 334 (3.40); UV (EtOH + NaOH) λ_{max} 247 nm (log ϵ 3.78), 376 (3.79); EIMS, *m/e* 563 (M^+). Anal. ($C_{31}H_{33}NO_9$) C, H, N.

***N*-Retinoyldeacetylcolchicine (31).** A solution of deacetylcolchicine (**2**; 100 mg, 0.28 mmol), retinoic acid (100 mg, 0.33 mmol), dicyclohexylcarbodiimide (DCC; 100 mg, 0.48 mmol),and 4-pyrrolidinopyridine (10 mg, catalytic amounts) in dry CH_2Cl_2 (3 mL) was stirred overnight at room temperature. The reaction mixture was filtered, and the filtrate was washed with H_2O (3 × 3 mL), 10% aqueous $NaHCO_3$ solution (3 × 3 mL), 0.1 N HCl (3 × 3 mL), and H_2O (3 × 3 mL), dried (Na_2SO_4), and evaporated to leave a residue, which was taken into Et_2O. The solid was filtered off, and the filtrate was concentrated to afford an oily residue, which was crystallized from a mixture of Et_2O–petroleum ether to afford a yellow crystalline solid (116 mg, 65%): mp 149 °C; $[\alpha]^{21}_D$ +16° (*c*, 0.65, $CHCl_3$); UV λ_{max} 243 nm (log ϵ 3.81), 350 (4.15); EIMS, *m/e* 639 (M^+); CIMS, *m/e* 640 (M^+). Anal. ($C_{40}H_{49}NO_6$) C, H, N.

Hydrolysis of *N*-Acyldeacetylcolchicine Derivatives. The general procedure followed is illustrated with the preparation of **25**.

***N*-(Trimethoxybenzoyl)deacetylcolchiceine (25).** A mixture of **23** and **24** (600 mg) dissolved in 25 mL of MeOH and 25 mL of 0.5 N HCl was heated under stirring (oil bath, 100–110 °C) until TLC indicated that almost all of the starting material had disappeared (ca. 10 h). It was kept overnight in a refrigerator, and the precipitate was collected and then washed thoroughly with cold H_2O, and the solid was crystallized with MeOH–H_2O to afford **25** (460 mg, 78%): mp 147 °C (shrinks at 137 °C); $[\alpha]^{20}_D$ −92° (*c* 0.95, $CHCl_3$); EIMS, *m/e* 537 (M^+). Anal. ($C_{29}H_{31}NO_9$) C, H, N.

Other *N*-acyldeacetylcolchicine derivaties were hydrolyzed in the same way, and the colchiceine derivatives were obtained in 60 to 80% yield and characterized as follows.

***N*-Benzoyldeacetylcolchiceine (17)**: crystallized from MeOH; mp 250 °C; $[\alpha]^{26}_D$ −149° (*c* 0.20, $CHCl_3$); EIMS, *m/e* 447 (M^+). Anal. ($C_{26}H_{25}NO_6$) C, H, N.

***N*-Carbethoxydeacetylcolchiceine (28)**: mp 172–173 °C; $[\alpha]^{20}_D$ −240° (*c* 1.0, $CHCl_3$); EIMS, *m/e* 415 (M^+). Anal. ($C_{22}H_{25}NO_7$) C, H, N.

2-Demethyl-*N*-(trifluoroacetyl)deacetylcolchicine (30). A solution of *N*-(trifluoroacetyl)deacetylcolchicine (1.35 g, 3.07 mmol) in concentrated H_2SO_4 (5 mL) was stirred for 3 h at 60–70 °C bath temperature. The reaction mixture was cooled, ice was added, and the pH was adjusted to 11.0 with 2 N NaOH and extracted several times with CH_2Cl_3/2-propanol (1:1) to afford an organic residue. This material was dissolved in CH_2Cl_2, the insoluble material was filtered off, and the residue obtained on evaporation of the filtrate was passed through a column of silica gel, with $CHCl_2$/MeOH (95:5) as eluent. The solid obtained was crystallized from a mixture of CH_2Cl_2/hexane to afford yellow crystals (325 mg, 24%): mp 270 °C (softens at 170 °C); EIMS, *m/e* 439 (M^+). Anal. ($C_{21}H_{20}NF_3O_6$) C, H, N.

Colchicide (35). To a solution of of thiocholchicine (430 mg, 1.03 mmol) in dry acetone (80 mL), under a nitrogen atmosphere, was added 5 g of wet Raney nickel catalyst (washed with 3 × 10 mL of acetone), and the mixture was stirred at room temperature. The reaction was monitored by TLC [silica gel, $CHCl_3$/MeOH (95:5)]. After 2 h, the second portion of catalyst (5 g) was added, and stirring was continued for 15 h. TLC showed a main spot corresponding to colchicide (R_f 0.30) and minor spots corresponding to thiocolchicine (R_f 0.42) and hexahydrodemethoxycolchicine (R_f 0.13).[7] The catalyst was filtered over a pad of Celite, and the red solution was evaporated. The residue was chromatographed on a column (40 × 2 cm) of silica gel and eluted with $CHCl_3$/MeOH (97.5:2.5) to afford a glassy solid (176 mg, 46%): $[\alpha]^{24}_D$ −166° (*c* 0.53, $CHCl_3$) [lit.[7] $[\alpha]^{27}_D$ −196° (*c* 0.47, $CHCl_3$)]. Anal. ($C_{21}H_{23}O_5N\cdot{}^1/_2H_2O$) C, H, N. The hydrochloride salt was crystallized from EtOAc–MeOH: mp 124–126 °C (lit.[7] 119–121 °C); $[\alpha]^{18}_D$ −456° (*c* 0.50, $CHCl_3$); EIMS, *m/e* 369 (M^+); ^1H NMR ($CDCl_3$) δ 2.00 (s, 3 H, COMe), 2.2–2.6 (m, 4 H, 2 CH_2), 3.69 (s, 3 H, C_1 OMe), 3.92 and 3.90 (2 s, each 3 H, C_2 OMe and C_3 OMe), 4.68 (m, 1 H, C_7 H), 6.58 (s, 1 H, C_4 H) 7.40 (s, 1 H, C_8 H), 7.20–7.60 (m, 3 H, C_{10} H, C_{11} H, C_{12} H), 7.66 (d, NH, exchanges on addition of D_2O).

Acknowledgment. The NCI data listed in Table I are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. We thank Dr. P. Bellet from Roussel Uclaf, Paris, who has supported our program for many years with appreciable amounts of thiocolchicine, needed for the preparation of colchicide. We also thank Dr. Jan Wolff from the NIADDK, for giving us advise and useful criticism.

Registry No. **2**, 3476-50-4; **3**, 47477-04-3; **4**, 477-30-5; **5**, 64-86-8; **6**, 6877-25-4; **7**, 78249-43-1; **8**, 78231-83-1; **9**, 57866-21-4; **10**, 86436-31-9; **11**, 86436-32-0; **12**, 74515-40-5; **13**, 86436-33-1; **14**, 86436-34-2; **15**, 86436-35-3; **16**, 63989-75-3; **17**, 14686-58-9; **18**, 86495-10-5; **19**, 86455-81-4; **20**, 86436-36-4; **21**, 86436-37-5; **22**, 86436-38-6; **23**, 86436-39-7; **24**, 86436-40-0; **25**, 86436-41-1; **26**, 86436-42-2; **27**, 86436-43-3; **28**, 86436-44-4; **29**, 86436-45-5; **30**, 86436-46-6; **31**, 86436-47-7; **32**, 86436-48-8; **33**, 86436-49-9; **34**, 78517-63-2; **35**, 518-15-0; **35**·HCl, 86436-50-2; 2-demethylcolchicine, 7336-36-9; 3-demethylcolchicine, 7336-33-6; 5-chloro-2-phenyl-1*H*-tetrazole, 14210-25-4; (trifluoroacetyl)glycolyl chloride, 78517-62-1; ethoxyacetic anhydride, 14521-87-0; propionic anhydride, 123-62-6; butyryl chloride, 141-75-3; 3,4,5-trimethoxybenzoyl chloride, 4521-61-3; ethyl chloroformate, 541-41-3; trimethylacetic anhydride, 1538-75-6; acetoxysinapinoyl chloride, 39657-48-2; retinoic acid, 302-79-4; *N*-(trifluoroacetyl)deacetylcolchicine, 26195-65-3; thiocolchicine, 2730-71-4.