A suspension of the crude Boc-methionylglycylphenylalanine amide (1.94 g) in dichloromethane (20 mL) was stirred at ambient temperature and TFA (3 mL) was added dropwise. The solution was stirred for 1 h, evaporated to dryness, and titurated with ether to give **L-methionylglycyl-L-phenylalanine amide** as a very hygroscopic white solid (0.65 g). The crude product was condensed with Boc-tryptophan (0.425 g) by the mixed-anhydride method described above, to give **18**, which was purified on a Harrison chromatotron using methylene chloride, ethanol, and ammonia (19:2:1) (0.174 g): mp 178–181 °C; $[\alpha]^{20}_D$ (MeOH) −10.16; ^{1}H NMR ($(CD_3)_2SO$) δ 1.3 (s, *tert*-butyl), 2.03 (s, SCH_3, Met), 3.7 (m, C-α-H, Gly), 4.45 (m, C-α-H, Phe), 4.30 (m, C-α-Met), 1.90 (m, C-β-H, Met), 2.45 (m, C-γ-H, Met), 2.8–3.2 (m, C-β-H for Trp, Phe), 7.55 (d, indole C-2-H). Anal. ($C_{32}H_{42}N_6O_6S\cdot0.5H_2O$) C, H, N.

***N*-(*tert*-Butyloxycarbonyl)-L-tryptophylglycyl-L-aspartyl-L-phenylalanine Amide (19).** Glycine (1.5 g) in aqueous sodium hydroxide solution (0.8 g in 2 mL of water) was condensed with Boc-Trp (3.04 g) by the mixed-anhydride method described above, to give Boc-trptophylglycine (1.06 g, 29%), which was used without further purification.

L-Aspartyl-L-phenylalanine amide hydrate (0.088 g) was condensed with crude Boc-trptophylglycine by the mixed-anhydride method described above, to give Boc-tryptophylglycylaspartylphenylalanine amide, which was recrystallized from ethyl acetate–methylene chloride as a white solid (0.035 g, 19%): mp 143–154 °C; $[\alpha]^{20}_D$ (MeOH) −24.74°; ^{1}H NMR ($(CD_3)_2SO$) δ 1.38 (s, *tert*-butyl), 3.78 (q, C-α-H-Gly), 4.05 (m, C-α-H-Asp), 4.39 (m, C-α-H-Phe), 2.4–3.4 (m, C-β-H for Trp, Asp, Phe), 6.9–7.4 (m, 9-H, Trp, Phe), 7.6 (d, indole C-2-H). Anal. ($C_{31}H_{38}N_6O_8\cdot1.5H_2O$) C, H, N.

Registry No. 1, 25126-32-3; **2**, 25679-24-7; **3**, 20988-64-1; **4**, 5534-95-2; **5**, 1947-37-1; **6**, 5934-92-9; **7**, 5241-71-4; **8**, 5241-58-7; **9**, 90736-02-0; **10**, 97533-28-3; 11, 33900-27-5; **12**, 63069-11-4; **13**, 106651-39-2; 14, 106651-40-5; **15**, 106651-41-6; **16**, 5235-21-2; **17**, 88463-50-7; **18**, 17338-87-3; **19**, 106651-42-7; Boc-Trp, 13139-14-5; *N*-carbobenzoxyglycyl-L-phenylalanine amide, 5513-69-9; glycyl-L-phenylalanine amide acetate, 13467-26-0; carbobenzoxy-β-alanyl-L-phenylalanine amide, 17471-89-5; β-alanyl-L-phenylalanine amide, 106757-00-0; *N*-(*tert*-butyloxycarbonyl)-γ-aminobutyric acid, 57294-38-9; *N*-(*tert*-butyloxycarbonyl)-(γ-aminobutyryl)phenylalanine amide, 106651-43-8; (γ-aminobutyryl)-L-phenylalanine amide, 106651-45-0; *N*-carbobenzoxy-(δ-aminovaleryl)-L-phenylalanine amide, 106651-46-1; (δ-aminovaleryl)-L-phenylalanine amide, 106651-48-3; carbobenzoxyglycylglycine, 2566-19-0; carbobenzoxyglycylglycyl-L-phenylalanine amide, 75501-76-7; glycylglycyl-L-phenylalanine amide diacetate, 106651-49-4; Boc-glycine, 4530-20-5; (*tert*-butyloxycarbonyl)-glycyl-L-phenylalanine amide, 33900-05-9; glycyl-L-phenylalanine amide (TFA salt), 97587-48-9; Boc-methionine, 2488-15-5; (*tert*-butyloxycarbonyl)-L-methionylglycyl-L-phenylalanine amide, 60058-46-0; L-methionylglycyl-L-phenylalanine amide, 106651-51-8; glycine, 56-40-6; Boc-tryptophylglycine, 25691-58-1; L-aspartyl-L-phenylalanine amide, 5241-71-4; cholecystokinin, 9011-97-6.

A Novel Synthesis of Colchicide and Analogues from Thiocolchicine and Congeners: Reevaluation of Colchicide as a Potential Antitumor Agent

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Desulfurization of thiocolchicine with Raney nickel in a hydrogen atmosphere yielded tetrahydromethoxycolchicine (**2**), which was readily separated from unreacted thiocolchicine by chromatography and was smoothly oxidized to 10-demethoxycolchicine (colchicide) by Pd/C in refluxing toluene. Several analogues of colchicide were prepared from the corresponding thiocolchicines by this procedure. Treatment of colchicide with concentrated sulfuric acid yielded 2-demethylcolchicide. Colchicide and its analogues were found to be inactive in a tubulin-binding assay. Evidence is presented that colchicide prepared earlier from thiocolchicine with Raney nickel in aerial atmosphere was contamination with 1–2% thiocolchicine.

Methylthiocolchicide, commonly named thiocolchicine (**1**),[1-3] loses the methylthio group at C-10 when treated with Raney nickel catalyst in acetone under a hydrogen atmosphere, to afford tropone **3** named colchicide.[2-4] Reports that colchicide (**3**) binds well to tubulin in vitro[2-4] and showed, in addition, considerable activity in the P388 lymphocytic leukemia screen[4] prompted an extension of our earlier work to include a similar study of analogues of colchicide. In the course of these investigations, we found that the previously reported activity of **3** was attributable to a small amount of contamination by thiocolchicine (**1**). We now report results of such an investigation.

Chemistry

The procedure[2,4] that we previously used to remove the methylthio group in **1** to produce colchicide (**3**) with Raney nickel catalyst in an aerial atmosphere was not satisfactory in our hands, produced erratic results, and usually afforded besides **3** hydrogenated congeners of 10-demethoxycolchicine, which had to be removed by chromatography. Reductive desulfurization of thiocolchicine (**1**) over Raney nickel catalyst under a hydrogen atmosphere, on the other hand, afforded the well-known 10-demethoxy-8,10,11,12-tetrahydrocolchicine (**2**)[2,5,6] accompanied by another tetrahydro compound, probably the C-12,C-12a dehydro

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(1) To avoid the complicated *Chemical Abstracts* nomenclature for 10-demethoxycolchicine = colchicide = *N*-(5,6,7,9-tetrahydro-1,2,3-trimethoxy-9-oxobenzo[*a*]heptalen-7-yl)acetamide, we have named the novel analogues described here as colchicides, derived from the name colchicide given to tropone **3** and with the numbering indicated in its formula. In this way colchicides nicely correspond to their relatives of the colchicine family of compounds = colchicinoids. We prefer the name thiocolchicine over that of 10-thiomethylcolchicide, used by others in earlier communications.
(2) Rapoport, H.; Savigne, J. B. *J. Am. Chem. Soc.* **1955**, *77*, 667.
(3) Velluz, L.; Muller, G. *Bull. Soc. Chim. Fr.* **1955**, 194.
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(6) Muller, G.; Velluz, L. *Bull. Soc. Chim. Fr.* **1955**, 1452.

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Table I. Analytical and Physical Data of Other Tetrahydrocolchicines and Colchicides Prepared[a]

compd	yield, %	UV λ_{max} (EtOH) (log ϵ), nm	CIMS, m/e ($M^+ + 1$)	$[\alpha]^{20}_D$ (c, $CHCl_3$), deg	anal.
5	70	256 (4.07)	358		
6	68	231 (4.35), 328 (4.01)	354	−187 (0.2)	
6·HCl		231 (4.41), 328 (3.97)	354 (M^+ − HCl + 1)		$C_{20}H_{19}NO_5$·HCl·H_2O
14	75	256 (4.05)	402		
15	48	232 (4.17), 328 (3.77)	398		
16	84	234 (4.29), 329 (3.93)	356	−216 (0.34)	$C_{20}H_{21}NO_5$·0.5H_2O
19	74	256 (4.05)	402		
23	85	256 (4.03)	404		
24	16	231 (4.27), 328 (3.99)	400	−172° (0.1)	$C_{22}H_{25}NO_6$·0.5H_2O

[a] Colchicide 6·HCl (mp 147–148 °C) was a crystalline compound. All other colchicides described here were amorphous solids. Purity was checked by TLC (SiO_2), and the compounds were characterized by the data listed in the table.

Table II. Comparison of Tubulin-Binding Activity of Colchicides with 10-Methoxy and 10-Methylthio Analogues

colchicides	tubulin binding,[a] %	10-OMe	10-SMe
3 (colchicide hydrochloride)	13	90[b]	96[d]
11 (1-demethylcolchicide)	0	26	NT[f]
21 (2-demethylcolchicide)	26	50[b]	73[d]
16 (3-demethylcolchicide)	16	68[b]	84[d]
24 (*N*-(ethoxycarbonyl)deacetylcolchicine)	0	86[b]	93[d]
6 (10-demethoxycornigerine)	0	59[c]	91[e]
10 (1-acetyl-1-demethylcolchicine)	0	47[c]	NT

[a] Percentage by which the binding of [^{3}H]colchicine (2.5 μM) to tubulin from calf brain is reduced in the presence of the analogues at 25 μM. Easy assay is the average value of triple determinations. [b] *J. Med. Chem.* 1981, *24*, 257. [c] *Ibid.* 1983, *26*, 1365. [d] *Ibid.* 1985, *28*, 1204. [e] Personal communication from Dr. C. F. Chignell, Laboratory of Environmental Biophysics, NIH, Research Triangle Park, NC. [f] NT = not tested.

rahydro compound, probably the C-12,C-12a dehydro analogue, visible on TLC. Ketone **2** is readily oxidized by Pd/C catalyst in refluxing toluene under an argon atmosphere to colchicide (**3**) (Scheme I). The two-step process from **1** to **3** via **2**, affording colchicide hydrochloride in 62% yield, is in our opinion an improvement over the older process and seemed suitable for the preparation of analogues. Thiocornigerine (**4**), a new compound, prepared from 2,3-didemethylthiocolchicine[7] by partial synthesis with the methylenation applied earlier in the natural series of colchicine,[8] and known thiocolchicinoids **7**[9] and **12**,[10] used as acetates **8**[9] and **13**,[7] readily afforded ketones **5**, **9**, and **14**, respectively. All ketones were characterized by spectral data and showed the UV maximum at 256 nm in ethanol. Oxidation of **5**, **9**, and **14** with Pd/C in refluxing toluene afforded colchicides **6**, **10**, and **15** characterized by spectral data and showing the UV maximum at 328 nm in ethanol. Hydrolysis of acetates **10** and **15** with potassium carbonate in methanol afforded the phenols **11** and **16**, respectively, also fully characterized by spectral data. Although known acetate **18**[9] prepared from phenol **17**[10] gave tetrahydro ketone **19**, oxidation of the latter with Pd/C afforded a mixture of products from which colchicide **20** could only be obtained in trace amounts. Colchicide **21** could however be obtained in good yield by treating tropone **3** with concentrated sulfuric acid at 55–60 °C, a process in which selective O-demethylation occurs at the sterically most hindered methoxy group.[11,12] Tropone **3**, which proved to be stable at room temperature in concentrated sulfuric acid, also proved to be stable when refluxed in methanol in the presence of sodium methoxide.

Table III. Comparison of the in Vivo P388 Mouse Leukemia Test Data of Colchicide with That of Various 10-Substituted Analogues

compound	optimal dose[a]	T/C	MED[b] (T/C > 120)
colchicide hydrochloride	neg	neg	neg
colchicine	0.50	245	0.06
cornigerine	0.94	166	>0.40
thiocolchicine	0.18	193	0.05
10-benzylthiocolchicine	12.50	214	3.12
10-(methylamino)colchicine	0.32	166	0.04
10-(diethylamino)colchicine	3.12	216	0.39

[a] The dose levels are those that exhibit the highest T/C values [(days test animals live/days control animals live) × 100]. [b] Minimum effective dose (mg/kg) which produced a T/C greater than the threshold value of 125).

Reduction of known carbamate **22**,[3,13] with Raney nickel catalyst afforded tetrahydro ketone **23**, which on the basis of a qualitative TLC analysis was contaminated with an isomer. Oxidation of **23** with Pd/C in toluene afforded the desired colchicide **24** and also products that were not characterized.

Biological Evaluation

1. Binding to Tubulin Protein. The binding of the colchicide analogues to tubulin was determined by measuring their ability to displace [^{3}H]colchicine (**1**).[17] All compounds were initially tested at a 10-fold excess over the concentration of [^{3}H]colchicine.

2. Antitumor Testing. Colchicide hydrochloride (**3**) was evaluated against P388 leukemia, L1210 leukemia, B16 melanoma, M5076 sarcoma, and the L0X amelanotic me-

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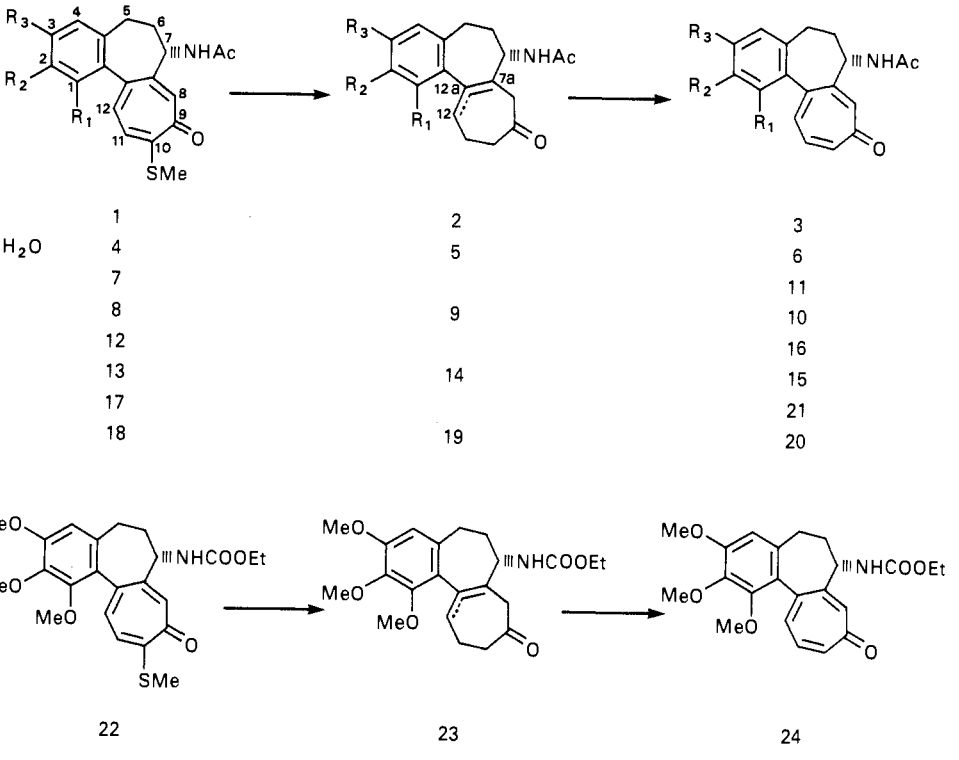
(14) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* 1978, *43*, 2723.
(15) Danieli, B.; Lesma, G.; Palmisano, G.; Riva, R. *Helv. Chim. Acta* 1985, *68*, 2173.
(16) The $[\alpha]^{20}_D$ in $CHCl_3$ changes from −244° to −135° over a period of 3 h.
(17) Zweig, M. H.; Chignell, C. F. *Biochem. Pharmacol.* 1973, *22*, 2142.

Scheme I



$R_1=R_2=R_3=OMe$	1	2	3
$R_1=OMe, R_2=R_3=O-CH_2O$	4	5	6
$R_1=OH, R_2=R_3=OMe$	7		11
$R_1=OAc, R_2=R_3=OMe$	8	9	10
$R_1=R_2=OMe, R_3=OH$	12		16
$R_1=R_2=OMe, R_3=OAc$	13	14	15
$R_1=R_3=OMe, R_2=OH$	17		21
$R_1=R_3=OMe, R_2=OAc$	18	19	20

22 23 24

lanoma in mice.[18] P388 leukemia, the tumor in which the original sample of **3** showed activity, was implanted intraperitoneally in mice. The compound was administered ip on a QD1 × 9 regiment. Antitumor activity is measured in terms of T/C (median survival time of treated animals/median survival time of untreated controls × 100). A compound is considered active if duplicate tests give T/C values equal to or greater than 120%.

3. Structure–Activity Relationships. The sample of colchicide hydrochloride that was prepared by the procedure previously reported[2,4] showed significant activity in P388 leukemia, giving T/C values of 233 and 250 at a dose of 20 mg/kg. Subsequent samples prepared by the same procedure failed to show any activity in P388 or any of the other tumors described above. A portion of the first sample was chromatographed by HPLC on a Porasil column with chloroform–methanol (98:2) as the mobile phase and it was found to be contaminated with 1.3% of thiocolchicine, the starting material. The optimal dose of thiocolchicine in P388 leukemia is about 0.18 mg/kg, producing a T/C value of about 200. The optimal dose of the first sample of **3** was 20 mg/kg and 1.4% contamination of this material with thiocolchicine would thus account for the previously reported activity. Samples of colchicide prepared by the method described in this paper have shown no trace of thiocolchicine and have shown no activity in antitumor assays.

The relative inactivity of the colchicides compared with their 10-methoxy and 10-methylthio analogues,[11,12] together with the well-known antimitotic activity of the 10-amino analogue, demonstrates the requirement for the presence of either an enol ether, a vinyl sulfide, or their nitrogen counterpart at the 10-position. This type of substitution is also essential for antitumor activity. The 10-thio and 10-amino analogues show appreciable antileukemic activity, and in fact, thiocolchicine is more potent than colchicine. Colchiceine (10-demethylcolchicine) is capable of tautomerization and has no biological activity, indicating the O, S, or N atoms at position 10 must be substituted in order to prevent tautomerization.

(18) National Cancer Institute, *In Vivo Cancer Models*, NIH Publication No. 84-2635, Feb 1984.

Experimental Section

Melting points were determined on a Fisher-Johns apparatus and are corrected. UV spectra were obtained with a Hewlett-Packard 8450A spectrophotometer. IR spectra were recorded on a Beckman IR 4230 spectrometer. NMR spectra were obtained with a Varian HR-220 or a JEOL FX-100 spectrometer with Me_4Si as an internal standard. CIMS spectra were recorded on a Finnigan 1015D spectrometer with a Model 6000 data-collection system. Optical rotations were measured on a Perkin-Elmer 241MC polarimeter. Elemental analyses were performed by Atlantic Microlab, Inc. Flash chromatography refers to the procedure of Still et al.[14] SiO_2, Merck, grade 60, 230–400 mesh, was used. Raney nickel was purchased from Aldrich.

8,10,11,12-Tetrahydro-10-demethoxycolchicine (2). To a mixture of 8.14 g (19.6 mmol) of thiocolchicine (1) in 250 mL of acetone was added 150 g of wet Raney nickel catalyst, washed three times with 50 mL of acetone. After being stirred for 20–30 min under a hydrogen atmosphere, the solution, which turned from yellow to slightly yellow, was filtered through a short pad of Celite. Evaporation followed by flash chromatography ($CHCl_3$–MeOH, 100:6) of the filtrate gave 6.05 g (82%) of a mixture **2** and its C-12,C-12a isomer as a slightly yellow foam: UV (EtOH) λ_{max} nm (log ϵ) 256 (4.10); CIMS, m/e 374 ($M^+ + 1$).[2]

Colchicide (3). A mixture of 2.0 g (5.36 mmol) of **2** and 4 g of 10% Pd/C catalyst in 100 mL of toluene was refluxed overnight under argon. The product, obtained after removal of the catalyst by filtration, followed by evaporation of the solvent, was purified by flash chromatography ($CHCl_3$–MeOH, 100:5) to yield 1.5 g (76%) of **3** as a brownish foam. The hydrochloride salt was obtained according to the original procedure[2] as long yellow needles: mp 128–129 °C (lit.[2] mp 119–121 °C, lit.[4] mp 124–126 °C); $[\alpha]^{20}_D$ −486° (*c* 0.23, $CHCl_3$) (lit.[4] $[\alpha]^{18}_D$ −456° (*c* 0.5, $CHCl_3$), $[\alpha]^{20}_D$ −253 (EtOH) (lit.[2] $[\alpha]^{20}_D$ −260° (EtOH); UV (EtOH) λ_{max} nm (log ϵ) 231 (4.23), 328 (3.83). For additional data, see ref 15.

Thiocornigerine (4). A mixture of 900 mg (2.32 mmol) of 2,3-didemethylcolchicine, 3.3 g of anhydrous K_2CO_3, 17 mL of 1-methyl-2-pyrrolidone, and 3.3 mL of bromochloromethane was heated at 70 °C oil bath temperature under an argon atmosphere for 20 h. The reaction mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2 (4 × 20 mL). The combined organic

extracts were washed with H_2O (2 × 5 mL), dried over Na_2SO_4, and concentrated to afford a yellow solid residue, still containing pyrrolidone, which was removed under high vacuum. The product was chromatographed on 10 g of SiO_2 (first with CH_2Cl_2 to remove an impurity and then with CH_2Cl_2–MeOH, 9:1) to afford a yellow material, which after trituration with ether gave 410 mg (44%) of thiocornigerine (**4**) as a canary-yellow solid: mp 140 °C; $[\alpha]^{20}_D$ −155° (*c* 0.65, $CHCl_3$); NMR ($CDCl_3$) δ 1.85–2.45 (m, 4 H, 2 H_5 and 2 H_6), 2.06 (s, 3 H, NHAc), 2.50 (s, 3 H, SCH_3), 3.81 (s, 3 H, OCH_3), 4.70 (m, 1 H, H_7), 6.02 (s, 4 H, OCH_2O), 6.48 (s, 1 H, H_4), 7.18–7.46 (m, 3 H, H_8, H_{11}, H_{12}); CIMS, *m/e* 388 (M^+ + 1). Anal. ($C_{20}H_{21}NO_5S$) C, H, N, S.

1-Demethyl-1-acetylthiocolchicine (8) (according to a modified procedure of Bladé-Font[9]). To a cold (0–5 °C) solution of 5 g (12.05 mmol) of thiocolchicine (**1**) in 50 mL of CH_2Cl_2 and 15 mL of acetyl chloride was added 5 mL of $SnCl_4$, and the mixture stirred at room temperature. After 24 h a substantial amount of thiocolchicine was still present in the reaction mixture, and addition of $SnCl_4$ and prolonged reaction time had no influence on the ratio of starting material and product. The reaction mixture was cooled and cold water was added slowly. After extraction with CH_2Cl_2 (3×), the CH_2Cl_2 layer was washed with saturated $NaHCO_3$ solution (2×), dried, and evaporated to give 5.2 g of a yellow solid, which was directly hydrolyzed to the mixture of thiocolchicine (**1**) and phenol **7** by stirring for 30 min at room temperature in a mixture of 20 mL of MeOH and 50 mL of 1 N NaOH. The MeOH was evaporated and the residue extracted extensively with Et_2O to get all the thiocolchicine (**1**) out. The water phase was acidified (pH 4) with 2 N HCl, and the yellow precipitate filtered, washed with H_2O, and dried overnight to give 1.32 g (66% based on recovered **1**) of **7**. Acetylation with 6 mL of acetic anhydride and 12 mL of pyridine gave 1.28 g (88%) of **8** as yellow crystals, mp 218–219 °C (from ethyl acetate, lit.[9] mp 210 °C then 220 °C).

1-Demethyl-1-acetyl-8,10,11,12-tetrahydro-10-demethoxycolchicine (9) and 1-Demethyl-1-acetylcolchicide (10). A mixture of 1 g (2.26 mmol) of **8** in 100 mL of acetone was treated with Raney nickel catalyst as described earlier (see preparation of **2**). After 1 h, starting material could not be detected on TLC, but two new spots corresponding to compounds **9** and **10** were observed. Flash chromatography ($CHCl_3$–MeOH, 100:7) gave 460 mg of **9** as the faster moving compound and 280 mg of **10**.

9: light yellow foam; UV (EtOH) λ_{max} nm (log ε) 256 (4.04); CIMS, *m/e* 402 (M^+ + 1).

10: yellow crystals from MeOH–ether, mp 163–164 °C; $[\alpha]^{20}_D$ −235° (*c* 0.26, MeOH);[16] UV (EtOH) λ_{max} nm (log ε) 232 (4.18), 328 (3.78); IR (Nujol) 1765, 1670, 1605, 1590 cm^{-1}; NMR (CD_3OD) δ 1.87 and 2.19 (m, 2 H, H_6), 1.98 (s, 3 H, NHAc), 2.18 (s, 3 H, OAc), 2.46 and 2.70 (m, 2 H, H_5), 3.81 and 3.93 (s, 6 H, OCH_3), 4.44 (m, 1 H, H_7), 6.90 (s, 1 H, H_4), 7.05 (m, 2 H, H_{10}, H_{12}), 7.18 (d, *J* = 2.5 Hz, 1 H, H_8), 7.40 (dd, J_1 = 11.5, J_2 = 9.5 Hz, 1 H, H_{11}), 8.61 (d, *J* = 7 Hz, 1 H, NH); CIMS, *m/e* 398 (M^+ + 1). Anal. ($C_{22}H_{23}NO_6 \cdot 0.75H_2O$) C, H, N.

Refluxing **9** in toluene with Pd/C catalyst (see preparation of **3**) gave a compound in 72% yield, which was in all respects identical with **10**.

1-Demethylcolchicide (11). A mixture of 80 mg (0.2 mmol) of **10**, 240 mg of anhydrous K_2CO_3, and 5 mL of MeOH was stirred at room temperature under argon for 2 h. After filtration, 50 mL of $CHCl_3$ was added, together with 2 N HCl, until the pH reached 5. The H_2O phase was extracted once with $CHCl_3$, and the combined organic layer was washed with brine, dried over $MgSO_4$, and evaporated to give 70 mg of a yellow solid. Crystallization from EtOH–ether gave 60 mg (84%) of **11** as slightly yellow crystals: mp 213–214 °C; $[\alpha]^{20}_D$ −261° (*c* 0.3, MeOH); UV (EtOH) λ_{max} nm (log ε) 234 (4.47), 329 (4.01); IR (Nujol) 3350, 1665, 1610, 1590 cm^{-1}; NMR (CD_3OD) δ 1.87 and 2.17 (m, 2 H, H_6), 1.98 (s, 3 H, NHAc), 2.37 and 2.59 (m, 2 H, H_5), 3.82 and 3.89 (s, 6 H, OCH_3), 4.47 (m, 1 H, H_7), 6.48 (m, 1 H, H_4), 7.06 (m, 2 H, H_{10}, H_{12}), 7.21 (d, *J* = 3 Hz, 1 H, H_8), 7.43 (dd, J_1 = 12, J_2 = 9 Hz, 1 H, H_{11}); CIMS, *m/e* 356 (M^+ + 1). Anal. ($C_{20}H_{21}NO_5 \cdot 1.25H_2O$) C, H, N.

2-Demethylcolchicide (21). A mixture of 100 mg (0.27 mmol) of colchicide (**3**) in 2 mL of concentrated H_2SO_4 was stirred at 55–60 °C (oil bath temperature). After 4 h the reaction mixture was poured on ice and the pH adjusted to 5 with 10% NaOH. Extraction with $CHCl_3$–MeOH (4:1) (3×), drying over $MgSO_4$, and evaporation gave a yellow solid, which was purified by flash chromatography ($CHCl_3$–MeOH, 100:8) to give 80 mg (83%) of a yellow amorphous solid: $[\alpha]^{20}_D$ −268° (*c* 0.11, $CHCl_3$); UV (EtOH) λ_{max} nm (log ε) 234 (4.44), 330 (3.99); IR ($CHCl_3$) 3580, 3490, 3320, 1690, 1645, 1575 cm^{-1}; NMR ($CDCl_3$) δ 1.79 and 2.19 (m, 2 H, H_6), 1.99 (s, 3 H, NHAc), 2.49 (m, 2 H, H_5), 3.67 and 3.93 (s, 6 H, OCH_3), 4.59 (m, 1 H, H_7), 6.51 (s, 1 H, H_4), 7.04–7.27 (m, 4 H, H_8, H_{10}, H_{11}, H_{12}); CIMS, *m/e* 356 (M^+ + 1). Anal. ($C_{20}H_{21}NO_5 \cdot 0.5CH_3OH$) C, H, N.

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Structure–Activity Studies of Antagonists of Luteinizing Hormone-Releasing Hormone with Emphasis on the Amino-Terminal Region[1]

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The structure–activity relationship of the hydrophobic amino terminal region of the antagonist [N-Ac-D-Nal^1,D-pClPhe^2,D-Trp^3,D-Arg^6,Phe^7,D-Ala^{10}]-LH-RH has been investigated by the incorporation of a variety of amino acids with emphasis on positions 1, 2, and 3. The analogues were prepared by routine solid-phase peptide synthesis. All purifications were performed in two stages: gel permeation chromatography followed by preparative, reversed-phase, high-performance chromatography. The analogues were assayed in a standard rat antiovulatory assay using a 40% propane-1,2-diol–saline vehicle. A simplified antagonist was developed that allowed the removal of the custom-synthesized D-pClPhe and the labile D-Trp while retaining antiovulatory potency. The compound [N-Ac-D-Nal^1,D-$Phe^{2,3}$,D-Arg^6,Phe^7,D-Ala^{10}]-LH-RH caused a 56% blockade of ovulation at the 500-ng dose and is approximately equipotent with the parent analogue in this system.

Since the discovery of the postulated luteinizing hormone-releasing hormone (LH-RH), a decapeptide with the sequence Glp-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, extensive structure–activity studies have produced both

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