Alkylthiocolchicines and *N*-Deacetyl-alkylthiocolchicines and Their Antileukemic Activity

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Abstract □ A series of alkylthiocolchicines (methyl, ethyl, *n*-butyl, *n*-hexyl, *n*-octyl, and pinanyl) was prepared from colchicine by treatment with the appropriate alkyl mercaptan and *p*-toluenesulfonic acid. Some of these compounds (methyl-, ethyl-, and *n*-butylthiocolchicines) were deacetylated in good yields with 2 *N* hydrochloric acid in methanol. These compounds were tested for their antileukemic activity in an *in vitro* assay against L-1210 (mouse leukemia). Preliminary results showed that methylthiocolchicine is more active and the other alkylthiocolchicines are much less active than colchicine. *N*-Deacetyl-methylthiocolchicine is as active as colchicine.

Keyphrases □ Alkylthiocolchicines and *N*-deacetyl-alkylthiocolchicines—synthesized from colchicine, antileukemic activity □ Thiocolchicines, alkyl, and *N*-deacetyl-alkylthiocolchicines—synthesized from colchicine, antileukemic activity □ Colchicine derivatives—synthesis and antileukemic activity of alkylthiocolchicines and *N*-deacetyl-alkylthiocolchicines □ Antileukemic activity—synthesis and screening of alkylthiocolchicines and *N*-deacetyl-alkylthiocolchicines

The antitumor activity of colchicine, the major alkaloid of the autumn crocus, *Colchicum autumnale*, and the African climbing lily, *Gloriosa superba*, was first reported at the beginning of this century (1). The elucidation of its structure was finally completed from X-ray studies (2) and a number of total syntheses (3–8).

Colchicine is a mitotic poison, particularly in thymic, intestinal, and hematopoietic cells, which acts as a spindle poison and blocks the kinesis (9). Its effect on the mitotic spindle is thought to represent a special case of its effects on various organized, labile, fibrillar systems concerned with structure and movement (10, 11).

A study with ^{3}H-labeled colchicine showed that the drug is rapidly taken up by Ehrlich ascites and S-180 cells *in vitro* (12). The study did not establish a relationship between mitotic arrest and cytotoxicity but suggested that the latter probably arises, at least in part, from a degenerative process occurring in arrested cells. A separate investigation (13) showed that colchicine diminishes deoxycytidylate aminohydrolase activity in Ehrlich ascites cells and suggested that the drug's action on mitoses may be related to its action on this enzyme, thus regulating DNA synthesis. Numerous derivatives and analogs of colchicine have been examined for antimitotic activity, and this work has afforded a number of compounds for clinical study.

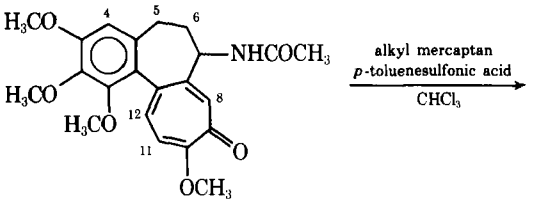
Recent reports on the muscle relaxant activity and antimitotic activity of 3-*O*-demethyl-3-*O*-β-D-glucopyranosyl-methylthiocolchicine prompted the preparation of compounds of this type for submission to the Cancer Chemotherapy National Center for evaluation in their intracranial antileukemic test system.

Table I—Antileukemic Activities of Alkylthiocolchicines and *N*-Deacetyl-alkylthiocolchicines Using the L-1210 (Mouse Leukemia) Tube Dilution *In Vitro* Assay

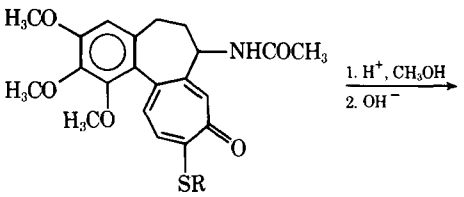
Compounds	Drug Activities, μg/ml	
	ID_{50}	ID_{90}
I (colchicine)	0.0077	0.011
II*a*	0.0018	0.0027
II*b*	0.019	0.027
II*c*	>0.1	>0.1
II*d*	>0.1	>0.1
II*e*	>0.1	>0.1
II*f*	>0.1	>0.1
III*a*	0.0070	0.011
III*b*	0.069	0.098
III*c*	>0.1	>0.1

RESULTS AND DISCUSSION

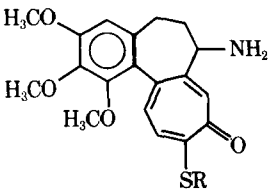
Methylthiocolchicine (II*a*) and ethylthiocolchicine (II*b*) were prepared by treating colchicine (I) with methyl and ethyl mercaptan, respectively, in the presence of *p*-toluenesulfonic acid as a cat-



I



II*a*: R = CH_3
II*b*: R = C_2H_5
II*c*: R = n-C_4H_9
II*d*: R = n-C_6H_{13}
II*e*: R = n-C_8H_{17}
II*f*: R = $C_{10}H_{17}$



III*a*: R = CH_3
III*b*: R = C_2H_5
III*c*: R = n-C_4H_9

Scheme I

alyst at room temperature for several days (14–16). However, after several preparations, it was found that the yield could be improved by using a large excess of mercaptan and by carrying out the reaction at a higher temperature in a shorter time. Under these reaction conditions, the ratio of IIa to I in the reaction mixture was as high as 20 to 1 according to UV spectroscopy.

In general, all reactions showed a gradual color change, indicating the progress of the reaction. TLC is a convenient method for following the reactions and for examining the purity of the products. The excess mercaptan can be removed by evaporation or by pouring the reaction mixture into petroleum ether (bp 30–60°). For the high boiling-point mercaptans, only the latter method affords satisfactory results.

A number of new alkylthiocolchicines, *n*-butyl-, *n*-hexyl-, *n*-octyl-, and pinanylthiocolchicines, were prepared. The yields in these cases were poor, ranging from 15 to 25% because the reactions were initially carried out at room temperature. In the case of *n*-butylthiocolchicine, the yield was improved to 45% by carrying out the reaction at 95° in a pressure tube for 4 hr. The reactions are given in Scheme I. *N*-Deacetyl-alkylthiocolchicines (III) can be obtained in good to excellent yields when alkylthiocolchicine (II) in methanol is refluxed with hydrochloric acid and basified with sodium hydroxide (16, 17).

All compounds of II and III can be purified by dry column chromatography on silica gel eluted with a solvent [ethanol–ethyl acetate (20:80, v/v)]. The R_f values in this system are 0.48, 0.75, and 0.45 for I, II, and III, respectively. However, some of the compounds could be purified satisfactorily by crystallization from suitable solvents as indicated in the *Experimental* section.

The compounds were tested[1] in an *in vitro* assay against L-1210 (mouse leukemia). The results (Table I) show that the antileukemic activity of methylthiocolchicine is greater than that of colchicine. In ethylthiocolchicine, the antileukemic activity is decreased from colchicine; in the other alkylthiocolchicines (where the alkyl group is large), the activity is decreased greatly. *N*-Deacetyl-methylthiocolchicine is as active as colchicine, while *N*-deacetyl-ethylthiocolchicine is much less active than colchicine.

EXPERIMENTAL

Commercial grade colchicine[2] was used without further purification. The melting points[3] reported are uncorrected. The IR spectra[4] of *n*-butylthiocolchicine (IIc) and *N*-deacetyl-butylthiocolchicine (IIIc) are reported here; the other spectra are practically identical with these two spectra in the region of 4000–750 cm^{-1}. The band intensities are abbreviated as follows: vs, very strong; s, strong; m, moderate; w, weak; and vw, very weak.

The UV spectra[5] were recorded using 95% ethanol as the solvent. The NMR spectra[6] were measured using tetramethylsilane as the internal standard and $CDCl_3$ as the solvent. The spectra are reported in the following order: chemical shift (δ), observed multiplicity, coupling constant, number of protons, and assignment. Signals other than for thioalkyl protons of the compounds except for IIc are omitted. They are similar in pattern as well as chemical shift to those of IIc.

Mass spectra[7] were recorded at 70 ev. Elemental analyses[8] and TLC[9] were carried out for each sample. Silica gel[10] (70–230 mesh) was used for dry column chromatography. The compounds were eluted with ethyl acetate–ethanol (80:20) and examined with UV light.

Methylthiocolchicine (IIa)—To a pressure bottle containing colchicine (20.0 g), *p*-toluenesulfonic acid monohydrate (4.0 g), chloroform (50 ml), and a magnetic bar cooled in an acetone–dry ice bath was added methyl mercaptan (110 g), also cooled previously in an acetone–dry ice bath. The pressure bottle was sealed and the reaction mixture was allowed to warm to room temperature with constant stirring. The pressure bottle was then heated in a bath at 70° for 3 hr with constant stirring. The bottle was allowed to cool to room temperature, cooled further in an acetone–dry ice bath, and then opened.

The excess methyl mercaptan was allowed to evaporate at 30–40° and was collected at acetone–dry ice temperature for reuse or was destroyed by passing through several traps filled with acidic potassium permanganate or potassium dichromate solution. The syrupy residue was dissolved in chloroform (200 ml) and washed several times with saturated sodium bicarbonate solution and finally with water. The chloroform layer was then dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The crude residue was crystallized from hot ethyl acetate (200 ml), giving 17.5 g of shiny yellow cubes. An additional 4.9 g of crude amorphous product, that could not be crystallized from ethyl acetate, was recovered from the mother liquor. The shiny crystals were crushed and dried at 65–70° under vacuum for several hours to yield 14.3 g (69%) of methylthiocolchicine, mp 189–190° [lit. (15) mp 192–194°]; UV (95% ethanol): λ_{max} 385 (log ϵ 4.25), 290 (4.06), and 257 (4.31) nm; NMR: 2.46 (s, 3, SCH_3).

Anal.—Calc. for $C_{22}H_{25}NO_5S$: C, 63.60; H, 6.07; N, 3.37; S, 7.72. Found: C, 63.46; H, 5.92; N, 3.37; S, 7.70.

Ethylthiocolchicine (IIb)—To the solution of colchicine (16.0 g) and *p*-toluenesulfonic acid (2.4 g) in chloroform (40 ml) in a pressure bottle was introduced ethyl mercaptan (50 ml). The mixture was then sealed and heated in a bath at 90–95° for 7 hr. After the evaporation of mercaptan, the reaction mixture was neutralized with sodium bicarbonate and extracted with chloroform. The chloroform extract was washed, dried, and then evaporated to dryness under reduced pressure at 45°. The residue was dissolved in hot ethyl acetate and gave ethylthiocolchicine, 9.92 g (58% yield), of pale-yellow cubic crystals, mp 204–205° [lit. (15) mp 207–208°]; UV: λ_{max} 386 (log ϵ 4.27), 290 (4.06), and 256 (4.32) nm; NMR: 1.45 (t, J = 7.3 Hz, 3, SCH_2—CH_3) and 2.95 (q, J = 7.3 Hz, 2, SCH_2—CH_3).

Anal.—Calc. for $C_{23}H_{27}NO_5S$: C, 64.31; H, 6.34; N, 3.26; S, 7.47. Found: C, 64.15; H, 6.48; N, 3.27; S, 7.58.

***n*-Butylthiocolchicine (IIc)**—The reaction was carried out and the product was isolated as described for IIb, yielding 45%, mp 121–124°; UV: λ_{max} 387 (log ϵ 4.25), 290 (4.03), and 257 (4.29) nm; IR (intensity, assignment): 3300 (s, amide NH stretching), 3058–2840 (s, CH stretching), 1670 (s, amide carbonyl stretching), 1616 (s, tropolone carbonyl stretching), 1546 (vs), 1493 (s), 1466 (s), 1433 (s), 1408 (s), 1372 (m), 1353 (s), 1326 (s), 1287 (m), 1239 (m), 1198 (m), 1156 (m), 1139 (s), 1098 (s), 1056 (w), 1022 (s), 993 (w), 979 (m), 954 (w), 923 (m), 903 (w), 843 (w), and 826 (vw) cm^{-1}; NMR: 0.99 [t, J = 7.0 Hz, 3, S—CH_2—$(CH_2)_2$—CH_3], 1.70 [m, 4, S—$CH_2(CH_2)_2$—CH_3], 1.99 (s, 3, $COCH_3$), 2.46 (broad s, 4, CH_2 of C-5 and C-6), 2.92 (t, J = 7.0 Hz, 2, S—CH_2—), 3.69, 3.92, 3.96 (three s, 3H each, OCH_3 of C_1, C_3, and C_2)[11], 4.69 (broad s, 1, H_7), 6.56 (s, 1, H_4), 7.20 (d, *AB* pattern, $J_{11,12}$ = 10.5 Hz, 1, H_{11}), 7.32 (d, 1, H_{12}), 7.52 (s, 1, H_8), and 8.38 (broad d, J = 7 Hz, 1, NH).

Anal.—Calc. for $C_{25}H_{31}NO_5S$: C, 65.62; H, 6.83; N, 3.06; S, 7.01. Found: C, 65.33; H, 7.09; N, 2.93; S, 6.89.

***n*-Hexylthiocolchicine (IId)**—A mixture of colchicine (1.0 g), *p*-toluenesulfonic acid (0.20 g), chloroform (4 ml), and *n*-hexyl mercaptan (2 ml) was stirred in a closed container at room temperature for several weeks. The reaction mixture was poured slowly into petroleum ether (200 ml) with vigorous stirring, and a pale-yellow precipitate formed. The crude precipitate was dissolved in chloroform and purified by column chromatography. The product was crystallized from ethyl acetate–*n*-hexane, yielding 25%, mp 228–230°; UV: λ_{max} 387 (log ϵ 4.28), 290 (4.04), and 257 (4.31) nm; NMR: 0.91 [t, J = 6.5 Hz, 3, SCH_2—$(CH_2)_4$—CH_3], 1.20–1.78 [m, 8, SCH_2—$(CH_2)_4$—], and 2.90 (t, J = 6.5 Hz, 2, SCH_2—).

Anal.—Calc. for $C_{27}H_{35}NO_5S$: C, 66.78; H, 7.27; N, 2.88; S, 6.60. Found: C, 67.05; H, 7.53; N, 2.82; S, 6.71.

***n*-Octylthiocolchicine (IIe)**—The reaction was carried out and the product was isolated as described for IId. The product was obtained as amorphous powder in 20% yield; UV: λ_{max} 387 (log ϵ

[1] The Upjohn Co., Kalamazoo, Mich.
[2] S. B. Penick and Co., New York, N.Y.
[3] Thomas-Hoover Unimelt apparatus.
[4] Beckman IR-8 spectrophotometer.
[5] Cary-14 spectrophotometer.
[6] Varian A-60 spectrometer.
[7] LKB 9000 SN mass spectrometer.
[8] Midwest Microlab, Inc., Indianapolis, Ind.
[9] Eastman Chromagraph Sheet 6061 silica gel.
[10] EM Laboratories, Inc., Elmsford, N.Y.

[11] The examination of the NMR data on all alkylthiocolchicines confirmed that the peak at 4.06 (ppm, δ) is attributed to the methoxy group on tropolone ring of colchicine; the earlier assignment (Varian NMR No. 689) at 3.67 is wrong since this peak does not disappear when the tropolone methoxy group is replaced by an alkylthio group.

4.24), 290 (4.01), and 257 (4.28) nm; NMR: 0.89 [t, J = 6.5 Hz, 3, SCH_2—$(CH_2)_6$—CH_3], 1.34 [broad distorted singlet, 12, SCH_2—$(CH_2)_6$—], and 2.90 (t, J = 6.5 Hz, 2, SCH_2—).

Anal.—Calc. for $C_{29}H_{39}NO_5S$: C, 67.81; H, 7.65; N, 2.72; S, 6.24. Found: C, 67.61; H, 7.91; N, 2.52; S, 6.02.

Pinanylthiocolchicine (IIf)—The reaction was carried out and the product was isolated by the procedure described for II*d*, yielding 20%; NMR: 0.86 (s, 1), 1.10 (s, 3, CH_3), 1.25 (s, 3, CH_3), 1.47–2.76 (m, 15 but only 8 for pinanyl part, overlapped with $COCH_3$ and CH_2 of C-5 and C-6), and 2.95 (d, 2, SCH_2—).

Anal.—Calc. for $C_{31}H_{39}NO_5S$: C, 69.24; H, 7.31; N, 2.60; S, 5.96. Found: C, 69.05; H, 7.38; N, 2.58; S, 5.94.

***N*-Deacetyl-methylthiocolchicine (IIIa)**—To the clear solution of methylthiocolchicine (8.3 g) in methanol (120 ml) in a round-bottom flask was added 2 *N* hydrochloric acid (120 ml). The solution was refluxed for 40 hr, concentrated to 140 ml at 45° on a rotary evaporator, and then extracted with chloroform (5 × 120 ml). The water layer was stored, and the chloroform layer was washed with water (4 × 100 ml). The second portion of the water washings was concentrated to 30 ml and then allowed to evaporate slowly at room temperature to give yellowish green needles (2.54 g) of *N*-deacetyl-methylthiocolchicine hydrochloride (III*a*-HCl), mp 218–220°. The other three portions of the water washings were combined with the original water layer. The chloroform layer was evaporated to dryness and redissolved in hot ethyl acetate to give a mixture of III*a* and II*a* (0.64 g) in about a 3 to 2 ratio as determined by TLC.

The combined water layer was basified to pH 13 with 10 *N* sodium hydroxide and extracted with chloroform (5 × 120 ml). The combined chloroform extracts were washed with water and dried over anhydrous sodium sulfate. The clear solution was concentrated (40 ml), and then ether (240 ml) was added to give *N*-deacetyl-methylthiocolchicine (4.32 g) in crystalline form as pale-yellow shiny rectangular plates, mp 194–195° [lit. (17) mp 195°]. The combined yield of III*a* and III*a*-HCl was 89%.

For III*a*, the mass spectrum showed m/e (%) M^+ 373 (100), 345 (22), 328 (36), and 298 (42).

Anal.—Calc. for $C_{20}H_{23}NO_4S$: C, 64.32; H, 6.21; N, 3.75; S, 8.56. Found: C, 64.68; H, 6.53; N, 3.67; S, 8.86.

For III*a*-HCl, the mass spectrum showed m/e (%) M^+ 373 (355) and $H^{35}Cl$ 36 (100).

Anal.—Calc. for $C_{20}H_{23}NO_4S \cdot HCl$: C, 58.58; H, 5.90; Cl, 8.65. Found: C, 58.31; H, 5.90; Cl, 8.69.

***N*-Deacetyl-ethylthiocolchicine (III*b*)**—The reaction was carried out and the product was isolated by the procedure described for III*a*. The products III*b* and III*b*-HCl were crystallized from ethyl acetate and water, respectively, yielding 80%.

For III*b*, the melting point was 158–160° [lit. (17) mp 163°].

Anal.—Calc. for $C_{21}H_{25}NO_4S$: C, 65.09; H, 6.50; N, 3.62; S, 8.28. Found: C, 65.34; H, 6.21; N, 3.44; S, 8.14.

For III*b*-HCl, the melting point was 206–208°.

Anal.—Calc. for $C_{21}H_{25}NO_4 \cdot HCl$: Cl, 8.36. Found: Cl, 8.25.

***N*-Deacetyl-*n*-butylthiocolchicine (III*c*)**—The reaction was carried out and the product was isolated as described previously. The product was crystallized from ethyl acetate as yellow rectangular prisms, yielding 75%, mp 140–141°; IR: 3380 (m, NH_2 *anti-sym* stretching), 3320 (m, NH_2 *sym* stretching), 3050–2840 (s, CH stretching), 1615 (s, tropolone carbonyl stretching), 1560 (vs), 1493 (s), 1464 (s), 1435 (m), 1406 (s), 1376 (w), 1351 (s), 1321 (s), 1287 (w), 1235 (m), 1200 (s), 1147 (s), 1120 (w), 1092 (s), 1037 (w), 1017 (s), 1000 (m), 984 (m), 966 (m), 922 (s), 842 (w), 822 (vw), 796 (vw), and 755 (vw) cm^{-1}.

Anal.—Calc. for $C_{23}H_{29}NO_4S$: C, 66.47; H, 7.03; N, 3.37; S, 7.71. Found: C, 66.58; H, 7.12; N, 3.24; S, 7.52.

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