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PARTIAL SYNTHESIS AND ANTITUBULIN ACTIVITY OF MINOR *COLCHICUM* ALKALOIDS: *N*-ACETOACETYL-DEACETYL-COLCHICINE AND 2-DEMETHYLSPECIOSINE (SPECIOCOLCHINE)

ANJUM MUZAFFAR, ARNOLD BROSSI,*

Section on Natural Products, Laboratory of Structural Biology, NIDDK

and ERNEST HAMEL

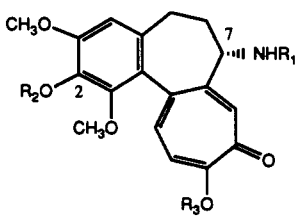
Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment NCI, National Institutes of Health, Bethesda, Maryland 20892

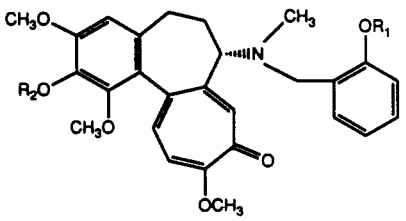
ABSTRACT.—Two minor *Colchicum* alkaloids, *N*-acetoacetyl-deacetylcolchicine [**1**] and 2-demethylspeciosine [**7**], were synthesized. The diacetate **8** of 2-demethylspeciosine was also prepared. The antitubulin activity of these compounds, in comparison to colchicine, was measured. *N*-Acetoacetyl-deacetylcolchicine [**1**] has in vitro activity similar to that of colchicine. Both 2-demethylspeciosine [**7**] and the diacetate **8** were considerably less potent inhibitors of tubulin polymerization.

N-Acetoacetyl-deacetylcolchicine [**1**] (1) and 2-demethylspeciosine [**7**], named speciocolchine (2), are minor *Colchicum* alkaloids. We have prepared **1** and **7** by partial synthesis from known colchicinoids (3) and report here the details of the synthesis together with the antitubulin activity of the synthetic compounds.

Synthesis of alkaloid **1**, which was isolated from *Colchicum autumnale* L. (Liliaceae) seeds (1), was accomplished from deacetylcolchicine [**2**] (4) with 2,4,6-trichlorophenyl acetoacetate in the presence of triethylamine to afford, after usual workup, acetoacetamide **1**. Hydrolysis of **1** with 20% H_2SO_4 afforded deacetylcolchiceine [**3**] (5). The structure of synthetic **1** is, with its synthesis and hydrolysis and the spectral data reported in the Experimental section, firmly established.

The comparison of synthetic with natural **1** on the basis of the data reported (1) reveals that the two compounds are most likely identical ([α]D, ms, uv, ^{1}H-nmr signals for methoxy and aromatic protons). The differences in the ir spectra (CO at 1715 cm^{-1} in synthetic versus 1765 cm^{-1} reported for natural), in the ^{1}H-nmr spectra (NH at δ 7.80 ppm for synthetic versus δ 7.31 ppm reported for natural), and in melting points (184° for synthetic versus 222–





1 R_1=$COCH_2COCH_3$, R_2=R_3=Me
2 R_1=H, R_2=R_3=Me
3 R_1=R_3=H, R_2=Me
4 R_1=R_3=Me, R_2=H
5 R_1=R_2=R_3=Me
9 R_1=Ac, R_2=H, R_3=Me

6 R_1=H, R_2=Me
7 R_1=R_2=H
8 R_1=R_2=Ac

223° reported for natural), cannot be explained at this moment because the natural alkaloid, which so far has been described only by the Olomouc group, is no longer available.[1]

Speciocolchicine [**7**] is a minor alkaloid present in *Colchicum ritchii* R. Br. (2), and its structure is firmly established by spectral data. The synthesis of **7** was achieved by reactions previously used in the synthesis of demecolcine [**4**] (6). *N*-Alkylation of 2-demethyldemecolcine [**5**] (7) with 2-bromomethylphenyl acetate (8) and treatment with 2 N NaOH in MeOH afforded **7**, which was purified by chromatography. The spectral data of **7** are in accordance with those reported for speciocolchine [**7**] (2)[2], and the diacetate **8**, prepared in the usual way, showed the correct mass of 547 $[M]^+$.

We examined the activity of the newly synthesized compounds as inhibitors of the polymerization of purified bovine brain tubulin as described in detail elsewhere (9). Compounds with strong inhibitory activity (IC_{50} values less than 5 μM) in this economical screening test have a high probability of being potent antimitotic cytotoxic agents.

In our previous study (10) IC_{50} values of 2.4 ± 0.1 (standard deviation) μM were obtained for both colchicine and demecolcine [**5**], and a value of 3.0 ± 0.2 μM was obtained for deacetylcolchicine [**2**]. These previously published values are included in Table 1. *N*-Acetoacetyl-deacetylcolchicine [**1**], whose synthesis is described here, was idential to colchicine and demecolcine as an inhibitor of tubulin polymerization (IC_{50}, 2.4 ± 0.2 μM), and thus this modification of the substituent at position C-7 has no significant effect on the drug–tubulin interaction. The relatively potent antitubulin activity of **1** demonstrated here suggests that this compound should be further investigated for therapeutic activity.

Because we had previously synthesized 2-demethylcolchicine [**9**] (10) and 2-demethyldemecolcine [**4**] (7), we evaluated both these compounds (for comparison to **7**, see below) as inhibitors of tubulin polymerization. Strikingly different results were obtained with the two demethylated derivatives. With 2-demethylcolchicine only a small loss of activity relative to colchicine occurred (IC_{50}, 3.7 ± 0.3 μM), while 2-demethyldemecolcine [**4**] was nearly noninhibitory (IC_{50}, 67 ± 7 μM).

TABLE 1. Inhibition of Tubulin Polymerization by Colchicinoids.[a]

Compound	IC_{50} (± SD) (μM)
Colchicine	2.4 (± 0.08)
2-Demethylcolchicine	3.7 (± 0.3)
N-Acetoacetyl-deacetylcolchicine [**1**]	2.4 (± 0.2)
Deacetylcolchicine [**2**]	3.0 (± 0.2)
2-Demethyldemecolcine [**4**]	67 (± 7)
Demecolcine [**5**]	2.4 (± 0.1)
Speciosine [**6**]	3.2 (± 0.4)
2-Demethylspeciosine [**7**]	19 (± 1)
2-Demethylspeciosine diacetate [**8**]	12 (± 0.3)

[a]A minimum of three independent determinations of IC_{50} values was performed with each agent, as described previously (9).

[1]Personal communication by Dr. V. Šimaneк, Medical Faculty, Institute of Medicinal Chemistry, Palacky University, Olomouc, Czechoslovakia 775 15.

[2]Personal communication by Prof. M. Shamma, Department of Chemistry, University of Pennsylvania, University Park, PA 16802. There was no natural material left for a tlc comparison with synthetic 2-demethylspeciosine [**7**].

Speciosine [**6**], previously reported (10) to be 70% as active as non-radiolabeled colchicine as an inhibitor of the binding of radiolabeled colchicine to tubulin, was found to have a similar small decrease relative to colchicine as an inhibitor of tubulin polymerization. The IC_{50} value obtained for speciosine [**6**] was 3.2 ± 0.4 μM. The effect of removing the methyl group at position C-2 in speciosine was intermediate between the analogous modifications of colchicine and demecolcine: 2-demethylspeciosine [**7**] had an IC_{50} value of 19 ± 1 μM, a considerable reduction relative to speciosine (cf. the case with colchicine), but the change was much less damaging than occurred in the demecolcine series. Acetylation of both hydroxyl groups of **7** to yield **8** resulted in a partial restoration of inhibitory activity (IC_{50} of **8**, 12 ± 0.3 μM) (11).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Fisher-Johns apparatus and are uncorrected. The optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at temperature range 22–25°. The uv spectra (λ max) were measured on a Hewlett-Packard 8450A UV/Vis spectrophotometer. The ir spectra were determined using a Beckman 4230 instrument. ^{1}H-nmr spectra were obtained on a JEOL FX-100 spectrometer using TMS as an internal reference. Ei mass spectra were determined on a Finnigan 1015D spectrometer with a model 6000 data collection system. Tlc plates (Si gel) were purchased from Analtech, Newark, Delaware. The solvent system for tlc analysis was $CHCl_3$-MeOH (9:1).

N-ACETOACETYL-DEACETYLCOLCHICINE [**1**].—To a solution of deacetylcolchicine (140 mg, 0.39 mmol) in pyridine (2 ml) was added 2,4,6-trichlorophenyl acetoacetate (550 mg, 1.95 mmol) under ice-cooling in the presence of N_2. The reaction mixture was stirred at 0–4° for 2 h. The reaction mixture was adjusted to pH 5 by the addition of 5% HCl and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with brine, dried over anhydrous Na_2SO_4, concentrated and chromatographed on Si gel, and eluted with $CHCl_3$-MeOH (99:1) as an amorphous material. Crystallization from EtOH afforded 90 mg (0.20 mmol, 52%) of an off-white powder; mp 183–184° [lit. (1) 222–223°]; $[\alpha]_D$ −198.7° ($c = 0.24$, $CHCl_3$) [lit. (1) $-194 \pm 4°$ ($c = 1.07$, $CHCl_3$)]; uv ($CHCl_3$) 243 and 349 nm; ir ($CHCl_3$) 1715 (CO), 1670 (CONH); ^{1}H nmr ($CDCl_3$, δ) 2.24 (3H, s, Ac), 3.42 (2H, s, $COCH_2CO$), 3.62 (3H, s, OMe), 3.88 (3H, s, OMe), 3.92 (3H, s, OMe), 3.97 (3H, s, OMe), 4.60 (1H, m, H-7), 6.52 (1H, s, ArH), 6.79 (1H, d, $J = 10.8$ Hz, ArH), 7.27 (1H, d, $J = 10.8$ Hz, ArH), 7.36 (1H, s, Ar-H), 7.80 (1H, bs, NH); ms *m/z* $[M]^+$ 441, 413, 391, 376, 357, 328, 312 (100%), 298, 297, 281. Hydrolysis of *N*-acetoacetyl-deacetylcolchicine (30 mg, 0.06 mmol) with 20% H_2SO_4 (1.5 ml) at 90° afforded deacetylcolchiceine (14 mg, 0.04 mmol, 60%); mp 154° ($CHCl_3$/MeOH); tlc comparison showed identity with an authentic sample.

2-DEMETHYLSPECIOSINE [**7**].—A mixture of 2-demethyldemecolcine (50 mg, 0.14 mmol) in MeCN (4 ml), 2-bromoethylphenyl acetate (0.5 ml), and K_2CO_3 (108 mg) was stirred under N_2 at room temperature for 2 days (tlc monitoring). The reaction mixture was filtered, and the filtrate was chromatographed on Si gel and eluted successively with $CHCl_3$ and $CHCl_3$-MeOH (98:2) to afford the acetate. The acetate was dissolved in MeOH (3 ml); 2 N NaOH (0.3 ml) was added and the solution stirred at room temperature for 2.5 h. The solvent was evaporated; the residue was dissolved in H_2O and acidified with 5% HOAc to pH 5; and the product was extracted with $CHCl_3$. The organic layer was concentrated and chromatographed on Si gel using $CHCl_3$ and then $CHCl_3$-MeOH (96:4) to afford 2-demethylspeciosine as an amorphous powder (38 mg, 0.08 mmol, 59%): $[\alpha]_D$ −33° ($c = 0.16$, $CHCl_3$) [lit. (2) −37° ($c = 0.13$, $CHCl_3$)]; uv (MeOH) 255, 350 nm; ir ($CHCl_3$) 3530 (OH), 1610, 1585, 1560 cm^{-1}; ^{1}H-nmr ($CDCl_3$, δ) 2.22 (3H, s, N-Me), 3.15 (1H, m, H-7), 3.53 (3H, s, OMe), 3.95 (3H, s, OMe), 3.98 (3H, s, OMe), 5.62 (1H, bs, OH), 6.51 (1H, s, H-4), 6.76 (1H, d, $J = 10.4$ Hz, H-11), 7.27 (1H, d, $J = 10.4$ Hz, H-12), 7.61 (1H, s, H-8); ms *m/z* $[M]^+$ 463, 357, 328, 298, 267, 193 (100%). The ^{1}H-nmr spectrum is practically superimposable with that reported earlier (2).

2-DEMETHYLSPECIOSINE DIACETATE [**8**].—2-Demethylspeciosine (15 mg, 0.03 mmol), was stirred overnight at room temperature with pyridine (2 ml) and Ac_2O (1 ml). Solvent was evaporated, H_2O was added, and the product was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 5% HCl and brine, dried over anhydrous Na_2SO_4, and concentrated to give an amorphous gum (13 mg, 0.02 mmol, 75%): $[\alpha]_D$ −19.9° ($c = 0.4$, $CHCl_3$); ms *m/z* $[M]^+$ 547, 532, 504, 424, 398, 370 (100%).

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LITERATURE CITED

1. F. Šantavý, P. Sedmera, J. Vokoun, S. Dvarackova, and V. Šimánek, *Collect. Czech. Chem. Commun.*, **48**, 2989 (1983).
2. A.J. Freyer, M.H. Abu Zarga, S. Firdous, H. Guinaudeau, and M. Shamma, *J. Nat. Prod.*, **50**, 684 (1987).
3. H.G. Capraro and A. Brossi, "The Alkaloids," Academic Press, New York. Vol. 23, part 1, 1984
4. R.F. Raffauf, A.L. Farren, and G.E. Ullyot, *J. Am. Chem. Soc.*, **75**, 5292 (1953).
5. H. Fernholtz, *Angew. Chem.*, **65**, 319 (1953).
6. H.G. Capraro and A. Brossi,, *Helv Chim. Acta.*, **62**, 965 (1979).
7. R. Dumont, A. Brossi, and J.V. Silverton, *J. Org. Chem.*, **51**, 2515 (1986).
8. D.L. Fields, J.B. Miller, and D.D. Reynolds, *J. Org. Chem.*, **29**, 2640 (1964).
9. A. Muzaffar, A. Brossi, C.M. Lin, and E. Hamel, *J. Med. Chem.*, **33**, 567 (1990).
10. M. Rösner, H.G. Capraro, A.E. Jacobson, L. Atwell, A. Brossi, M.A. Iorio, T.H. Williams, R.H. Sik, and C.F. Chignell, *J. Med. Chem.*, **24**, 257 (1981).
11. M. Cernoch, J. Malinsky, O. Telupilova, and F. Šantavý, *Arch. Int. Pharmacodyn. Therap.*, **99**, 141 (1954).

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