PHENOLIC CONGENERS OF COLCHICINE: PREPARATION AND CHARACTERIZATION OF PHENOLIC AND CATECHOLIC ANALOGUES OF COLCHICINE, COLCHICEINE AND THIOCOLCHICINE

Anjum Muzaffar, Maria Chrzanowska, and Arnold Brossi*

Medicinal Chemistry Section, Laboratory of Analytical Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892, USA.

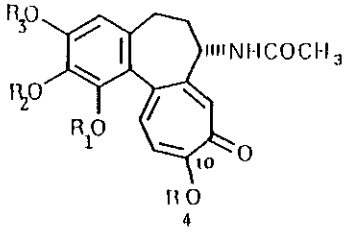
Abstract - Treatment of 2-demethylcolchicine (2) with sulfuric acid afforded 1,2-didemethylcolchicine (6) as the major, and 2,3-didemethylcolchicine (8) as the minor product. 2,3-Didemethylcolchicine (8) was prepared from 3-ethoxycarbonyl-3-demethylcolchicine by sulfuric acid treatment. Hydrolysis of catecholic compounds 6 and 8 with 0.1 N hydrochloric acid afforded colchiceines 13 and 14, respectively. Ester derivatives of 2-demethyl- and 3-demethylcolchicine, and of catechols 6 and 8 were prepared. A direct conversion of 3 into 3-demethylthiocolchicine (15) and conversion of 15 into ester derivatives is described. Cleavage of the ether groups of colchicine by boron tribromide is also reported.

The phenolic congeners of colchicine, 1-demethylcolchicine (1)[1], 2-demethylcolchicine (2)[1,2,3] and 3-demethylcolchicine (3)[3,4] are well-known and fully characterized. Our interest in these phenols is focussed primarily on 2 and 3, which both show antitumor activity[2,3], with 3 being less toxic than colchicine. Phenol 1, in contrast to 2 and 3, seems to favor a molecular conformation, which is not susceptible for binding to tubulin and is, therefore, not a very effective antimitotic agent. Demethylation of colchicine to 2 has been achieved with aluminum chloride in the presence of acetic anhydride.[1] An improvement in the yield of 2 was found when aluminum chloride was replaced by conc. sulfuric acid at 60°C.[2] This method usually afforded besides 2 catechols 6 and 8 which had to be removed by chromatography. A better procedure which affords 2 in about 40-45% yield is reported here. This phenol was further characterized as its ethyl carbonate ester 4, obtained by treatment of 2 with ethyl chloroformate in the presence of triethylamine.

Ethyl carbonate 5 was prepared in a similar manner from 3. Ethyl carbonate 4 was hydrolyzed with conc. sulfuric acid into 2 as seen by tlc analysis. Esters 4 and 5 are less polar than the parent phenols and can be analyzed on tlc.

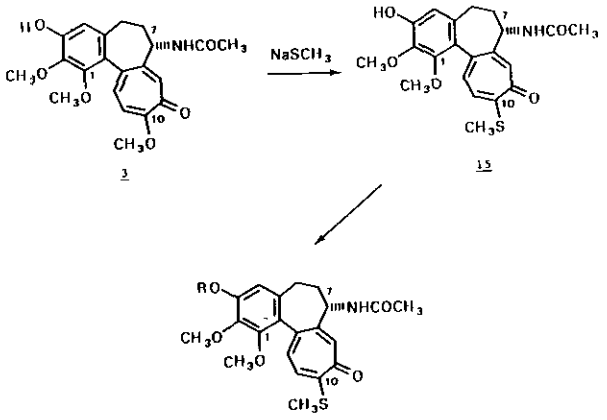
The catechols, 1,2-didemethylcolchicine (6) and 2,3-didemethylcolchicine (8), are less well-characterized and were obtained from colchicine with conc. sulfuric acid at 60-90°C.[5,6] Catechol 8 which possesses anti-inflammatory properties[7,8] was often obtained as a glue. This compound can be better prepared (in 25-30% yield) from the ethyl carbonate ester 5 by treatment with conc. sulfuric acid at 65°C and was obtained in crystalline form. These catechols have a high tendency to include solvents of crystallization (several prepartions showed some variation in melting points). The catechols were further converted into the corresponding bis-(ethyl carbonates) 7 and 9. 2,3-Didemethylcolchicine (8) was also characterized as its diacetate 10 and the diethoxyacetate 11. Treatment of 3 with 0.1N hydrochloric acid afforded the corresponding colchiceine 12 as a gum which showed the expected mass and uv spectra. Colchiceines 13 and 14 were prepared in a similar way from catechols 6 and 8, respectively.

Treatment of colchicine with boron tribromide at room temperature gave a very polar compound as the major product. This was identified as the 1,2-didemethylcolchiceine (13) by comparison (tlc, uv, ir, ms) with a sample prepared directly from 6. Other products could not be identified.



1	$R_1=H, R_2=R_3=R_4=CH_3$	8	$R_1=R_4=CH_3, R_2=R_3=H$
2	$R_1=R_3=R_4=CH_3, R_2=H$	9	$R_1=R_4=CH_3, R_2=R_3=COOC_2H_5$
3	$R_1=R_2=R_4=CH_3, R_3=H$	10	$R_1=R_4=CH_3, R_2=R_3=COCH_3$
4	$R_1=R_3=R_4=CH_3, R_2=COOC_2H_5$	11	$R_1=R_4=CH_3, R_2=R_3=COCH_2OC_2H_5$
5	$R_1=R_2=R_4=CH_3, R_3=COOC_2H_5$	12	$R_1=R_2=CH_3, R_3=R_4=H$
6	$R_1=R_2=H, R_3=R_4=CH_3$	13	$R_1=R_2=R_4=H, R_3=CH_3$
7	$R_1=R_2=COOC_2H_5, R_3=R_4=CH_3$	14	$R_1=CH_3, R_2=R_3=R_4=H$

Sulfuric acid treatment of thiocolchicine and its analogs proceeds, in principal, parallel to the findings reported in colchicine series.[9,10] 3-Demethylthiocolchicine (15) which is a broad-spectrum antitumor agent and also effectively inhibits amyloid migration[11] was synthesized earlier from thiocolchicocide by cleaving the sugar moiety with 80% phosphoric acid. It can directly be obtained in 70% yield from 3 by reaction with sodium methanethiolate in water. Phenol 15 was further characterized as the acetate 16, the ethyl carbonate 17, the ethoxy acetate 18, the butyrate 19 and the amorphous benzoate 20 (See Scheme 1).



3

15

16 R = $COCH_3$
17 R = $COOC_2H_5$
18 R = $COCH_2OC_2H_5$
19 R = $COCH_2CH_2CH_3$
20 R = COC_6H_5

Scheme 1

EXPERIMENTAL

Melting points were taken on a Fischer-Johns apparatus and are uncorrected. The optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at temperature range 22-25°C. The uv spectra (λ max, ethanol) were measured on a Hewlett-Packard 8450 A uv/vis spectrophotometer. The ir spectra (ν max, KBr) were recorded on a Beckman IR 4230 instrument. Electron impact mass spectra were determined on a Finnigan 1015D spectrometer with a model 6000 data collection system. Thin layer chromatography plates (tlc) were purchased from Analtech, Inc, Newark, D. E. All the compounds reported here belong to the natural (-)-series of colchicinoids.

2-Demethylcolchicine (2).

Colchicine (1g, 2.5 mmol) was heated with conc. sulphuric acid (10 ml) at 45°C for 7h (reaction was monitored by tlc). The reaction mixture was cooled, poured on ice-cold isopropanol (45 ml), and then extracted with methylene chloride (450 ml). The organic layer was washed with sodium carbonate solution and brine, dried over anhydrous Na_2SO_4 and concentrated to give crude product (800 mg). Crystallization from chloroform yielded 2-demethylcolchicine (2) as yellow crystals (400 mg, 1.0 mmol, 42%): mp 199°C (lit. 190-200°C); $[\alpha]_D$ -134° (c=0.23, $CHCl_3$) [lit.[1] -137°(c=1.0, $CHCl_3$]; ms 385 (M+).

2-Ethoxycarbonyl-2-demethylcolchicine (4).

2-Demethylcolchicine (2) (220 mg, 0.50 mmol) was dissolved in methylene chloride (5 ml) and triethylamine (0.5 ml) and ethyl chloroformate (0.2 ml) in methylene chloride (1.5 ml) added dropwise under ice-cooling. A spontaneous color change (from yellow to colorless) was observed. The reaction mixture was stirred at room temperature for 1 h, then diluted with methylene chloride (20 ml), washed with 2N HCl (2 x 10 ml), water (1 x 10 ml), sodium bicarbonate solution (2 x 10 ml) and finally with brine. The organic extract was dried over anhydrous Na_2SO_4 and concentrated under vacuum to yield colorless amorphous material (200 mg). Crystallization from ethyl acetate gave white crystals (163 mg, 0.36 mmol, 72%): mp 220-222°C; $[\alpha]_D$ -178.5° (c=0.14, $CHCl_3$); uv 252, 345 nm; ms 457 (M+), 298 (100%).

3-Ethoxycarbonyl-3-demethylcolchicine (5).

To an ice-cold solution of 3-demethylcolchicine (3) (2 g, 5.19 mmol) in methylene chloride (10 ml) and triethylamine (1 ml) was added ethyl chloroformate (1.5 ml). The reaction mixture was stirred at room temperature for 1.5 h (for work-up procedure see preparation of 4). Fine white crystals were obtained on crystallization from ethyl acetate (1.6 g, 3.5 mmol, 67%): mp 250°C; $[\alpha]_D$ -261° (c=0.1, $CHCl_3$); uv 231, 341 nm; ir 1760 cm^{-1}; ms 457 (M+), 370, 298 (100%).

3-Demethylcolchiceine (12).

A solution of 3-demethylcolchicine (3) (100 mg, 0.25 mmol) in 0.25N hydrochloric acid (3 ml) was stirred at 80°C for 4 h. After evaporation of solvent in vacuum an amorphous material was obtained: $[\alpha]_D$ -313° (c=9.0, MeOH); uv 257, 351 and 377 nm (bands at 351 and 377 shifted to 358 and 407 on addition of 2 drops of 20% NaOH); ms 371 (M^+, 100%), 357, 343, 328.

1,2-Didemethylcolchicine (6).

A solution of 2-demethylcolchicine (2) (1.58 g, 4.1 mmol) in conc. sulphuric acid (5.3 ml) was heated at 85-90°C under argon for 3 h, the reaction mixture was poured on ice and the pH adjusted to 5 by the addition of 20% NaOH. Extraction with chloroform/methanol (3:1)

and crystallization from methanol yielded 1,2-didemethylcolchicine as yellow powder (370 mg, 0.99 mmol, 25%): mp 235°C (dec.); $[\alpha]_D$ -153° (c=0.34, DMF); uv 240, 357 nm; ms 371 (M+), 340. Several preparations showed variation in mp (235 to 253°C) and $[\alpha]_D$ (-153 to -157°) depending on the solvent used for crystallization and the drying time.

1,2-Diethoxycarbonyl-1,2-didemethylcolchicine (7).

1,2-Didemethylcolchicine (6) (85 mg, 0.23 mmol) was dissolved in methylene chloride (1 ml) and triethylamine (0.5 ml). Then ethyl chloroformate (0.2 ml) was added dropwise to the reaction flask on an ice-bath, the reaction mixture was stirred at room temperature for 1.5 h and worked up by the procedure described for the preparation of 4. Crystallization from ethyl acetate gave 7 as white powder (100 mg, 0.19 mmol, 83%): mp 260-262°C; $[\alpha]_D$ -134° (c=0.1, $CHCl_3$); uv 243 and 342 nm; ms 515 (M+), 471, 397, 310 (100%).

2,3-Didemethylcolchicine (8).

3-Ethoxycarbonyl-3-demethylcolchicine (5) (600 mg, 7.3 mmol) was stirred at 65°C with sulphuric acid (5 ml) for 5 h. Then the reaction mixture was cooled and 20% sodium carbonate solution was added to adjust the pH to 5. Extraction with 2:1 chloroform/isopropanol (4 x 200 ml) yielded a yellow solid (250 mg) which was crystallized from ethyl acetate to give (150 mg, 0.4 mmol, 30%) of 2,3-didemethylcolchicine (8): mp 199°C; $[\alpha]_D$ -232.4° (c=0.7, 1:1 $CHCl_3$/MeOH); melting point observed for several preparations varied from 193°-279°C and the specific optical rotation varied from -232 to -255°; uv 244 and 357 nm; ms 371 (M+), 357, 343, 312, 298, 280 (100%).

2,3-Diethoxycarbonyl-2,3-didemethylcolchicine (9).

2,3-Didemethylcolchicine (8) (50 mg, 0.13 mmol) was dissolved in methylene chloride (2 ml) and triethylamine (0.5 ml). Reaction mixture was cooled in an ice bath and then ethyl chloroformate (0.2 ml) was added, the mixture was stirred at room temperature for 1.5 h and worked up by the procedure described for 4. Crystallization from ethyl acetate gave 2,3-diethoxycarbonyl-2,3-didemethylcolchicine (9) (30 mg, 0.06 mmol, 46%): mp 143°C; $[\alpha]_D$ -106° (c=0.2, $CHCl_3$); uv 249, 339 nm; ms 515 (M+), 456, 428, 397, 310 (100%).

2,3-Diacetyl-2,3-didemethylcolchicine (10).

2,3-Didemethylcolchicine (8) (83 mg, 0.22 mmol) was dissolved in pyridine (1 ml), acetic anhydride (0.5 ml) was added and the reaction mixture was allowed to stir at rt for 24 h. The pH of the reaction mixture was adjusted to pH 3 by the addition of 2N hydrochloric acid. The aqueous layer was extracted with chloroform (50 ml). The chloroform layer was washed with brine, dried over anhydrous Na_2SO_4 and evaporated to give brown residue. This was crystallized from ethyl acetate/ether to give 10 (58 mg, 0.12 mmol, 54.5%): mp

213-214°C; $[\alpha]_D$ -150° (c=0.1, $CHCl_3$); uv 232 and 340 nm; ir 1750 cm^{-1}; ms 455 (M+), 413, 371, 284 (100%).

2,3-Diethoxyacetyl-2,3-didemethylcolchicine (11).

To a solution of 2,3-didemethylcolchicine (8) (50 mg, 0.13 mmol) in methylene chloride (3 ml) and triethylamine (1 ml) was added ethoxyacetic anhydride (0.6 ml) under ice cooling. The reaction mixture was stirred at room temperature for 2.5 h, then diluted with methylene chloride (15 ml). The organic layer was washed with 20% HCl (1 x 10 ml), water (1 x 10 ml), saturated sodium carbonate solution (2 x 10 ml) and brine (1 x 15 ml), dried over anhydrous Na_2SO_4 and concentrated to give 11 as an amorphous material (30 mg, 0.06 mmol, 43%): $[\alpha]_D$ -102° (c=0.26, $CHCl_3$); ms 543, 515, 486, 471, 457, 443, 429, 398, 371, 284 (100%).

1,2-Didemethylcolchiceine (13).

1,2-Didemethylcolchicine (6) (147 mg, 0.4 mmol) was dissolved in acetic acid (2.5 ml) and 0.1 N hydrochloric acid (9.7 ml) and the mixture was heated at 125°C (oil-bath temperature) under argon for 2.5 h. Solvent was evaporated under high vacuum. Crystallization from hot water gave orange crystals: mp 179°C; $[\alpha]_D$ -322° (c=0.1, ethanol); uv 240 and 355 nm (band at 355 shifted to 400 on addition of 2 drops of 20% NaOH); ms 357 (M+), 343, 329, 315, 298 (100%).

2,3-Didemethylcolchiceine (14).

2,3-Didemethylcolchicine (8) (115 mg, 0.3 mmol) dissolved in acetic acid (2.0 ml) and 0.1 N hydrochloric acid (7.3 ml) was stirred at 120-125°C for 3 h. Solvent was evaporated under vacuum. Crystallization from acetone yielded brown crystals (34 mg 0.09 mmol, 32%): mp 226°C(dec.); uv 241 and 356 nm (band at 356 shifted to 402 on addition of 2 drops of 20% NaOH); ms 357 (M+).

3-Demethylthiocolchicine (15).

To a solution of 3-demethylcolchicine (3) (600 mg, 1.5 mmol) in water (6 ml) was added sodium methanethiolate (806 mg). The reaction mixture was stirred at room temperature for 24 h, diluted with 2% acetic acid solution (15 ml) and then extracted with chloroform (3x100 ml) to give yellow powder (530 mg). Crystallization from acetone yielded 15 (400 mg, 0.9 mmol, 67%) identical with that prepared from thiocolchicoside by treatment with 85% phosphoric acid: mp 310°C (lit.[11,14] 316, 318°C); $[\alpha]_D$ -259° (c=0.1, $CHCl_3$) [lit.[11,14] -251° (c=0.2, $CHCl_3$), -249° (c=0.5, $CHCl_3$)]; ms 401 (M+, 100%), 373, 314.

3-Acetyl-3-demethylthiocolchicine (16).

To a solution of 15 (100 mg, 0.249 mmol) in pyridine (1 ml) was added acetic anhydride (0.5 ml) and the mixture was stirred at room temperature for 18 h. After work-up by the

procedure described for 10 105 mg of crude extract was obtained which was crystallized from ethyl acetate/ether to give fine yellow crystals of 16 (56 mg, 0.126 mmol, 50%): mp 182°C; $[\alpha]_D$ -188° (c=0.1, $CHCl_3$); ms 443 (M+), 410, 373, 314 (100%).

3-Ethoxycarbonyl-3-demethylthiocolchicine (17).

Compound 15 (100 mg, 0.25 mmol) was dissolved in methylene chloride (3 ml) and triethylamine (0.5 ml) and ethyl chloroformate (0.5 ml) was added under cooling. The reaction mixture was allowed to stir at rt for 2.5 h and then diluted with methylene chloride (20 ml). The organic layer was washed with 2N HCl, saturated sodium carbonate solution and brine to give 150 mg of the crude extract. Crystallized from ethyl acetate to give 17 as yellow crystals (105 mg, 0.22 mmol, 85%): mp 249°C; $[\alpha]_D$ -128° (c=0.1, $CHCl_3$); uv 259, 363, 380 nm; ms 473 (M+), 440, 229 (100%).

3-Ethoxyacetyl-3-demethylthiocolchicine (18).

To an ice-cold solution of 15 (100 mg, 0.249 mmol) in methylene chloride (2 ml) and triethylamine (0.5 ml) was added ethoxyacetic anhydride (0.2 ml). The reaction mixture was stirred at rt for 2 h and worked up by the procedure as described for 11. Crystallization from ethyl acetate afforded 18 as yellow crystals (60 mg, 0.12 mmol, 49.5%): mp 247°C; $[\alpha]_D$ -115.5° (c=0.1, $CHCl_3$); ms 487 (M+,100%), 454, 428, 401, 373, 314.

3-Butyryl-3-demethylthiocolchicine (19).

A solution of 3-demethylthiocolchicine (15) (100 mg, 0.249 mmol) in pyridine (1 ml) was stirred at room temperature for 2.5 h with butyric anhydride (0.1 ml, 0.50 mmol). Solvent was evaporated under vacuum, the residue was dissolved in chloroform. The chloroform layer was washed with 5% HCl (2x10ml), water (1x10ml), sodium carbonate solution (1x15ml) and brine (1x15ml), dried over anhydrous Na_2SO_4 and concentrated to give an amorphous material (163 mg). Crystallization from ethyl acetate yielded yellow crystals (70 mg, 0.148 mmol, 60%) mp 232°C; $[\alpha]_D$ -138.8° (c=0.11, $CHCl_3$); ms 471 (M+), 438, 401, 373, 314 (100%).

3-Benzoyl-3-demethylthiocolchicine (20).

3-Demethylthiocolchicine (15) (100 mg, 0.249 mmol) was stirred with pyridine (2 ml) and benzoyl chloride (0.2 ml, 1.4 mmol) for 1.5 h and worked up by the procedure described for 18 to yield 240 mg of an oily substance. This was subjected to flash chromatography on silica gel using chloroform/methanol (99:1) as an eluant to give 20 as an amorphous material (40 mg, 0.08 mmol, 32%): $[\alpha]_D$ -111°(c=0.8, $CHCl_3$); ms 505 (M+), 472, 458, 446, 418, 401, 373, 314.

Cleavage of Colchicine by Boron Tribromide.

To a solution of colchicine (1g, 2.5 mmol) in methylene chloride (10 ml) was added boron tribromide (5 ml) on an ice-bath. After 5 min reaction mixture was stirred at room tem-

perature for 1.5h. Solvent was evaporated, the residue was dissolved in methanol (50 ml) and refluxed for 1 h. Methanol was evaporated, the residue was basified with sodium carbonate solution to pH 5 and then extracted with ethyl acetate (4x125 ml). The organic layer was dried over anhydrous Na_2SO_4, concentrated and crystallized from hot water to give 12 as orange crystals (400 mg, 1.1 mmol, 43%): mp 181°C; $[\alpha]_D$ -351° (c=0.1, ethanol); uv 241, 355 nm (band at 355 shifted to 400 on addition of 2 drops of 20% NaOH); ms 357 (M+).

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Received, 15th August, 1988