SELECTIVE ETHER-CLEAVAGE OF THIOCOLCHICOSIDE AND THIOCOLCHICINE: CHARACTERIZATION OF 3- AND 2-DEMETHYLTHIOCOLCHICINE AND CATECHOLIC CONGENERS*

Padam N. Sharma and Arnold Brossi

Section on Medicinal Chemistry, Laboratory of Chemistry, National Institute of Arthritis, Diabetes, and Digestive, and Kidney Diseases, National Institutes of Health, Bldg. 4 Room 135 Bethesda, Maryland 20205, USA

ABSTRACT: Selective ether cleavage in the thiocolchicine series took a similar course as observed in the colchicine series. Thiocolchicoside (2) afforded with phosphoric acid, the known 3-demethylthiocolchicine (6), and thiocolchicine (1) gave with conc. sulfuric acid at 70°C, a mixture of the 2-demethylthiocolchicine (3) and the catechols 4 and 5.

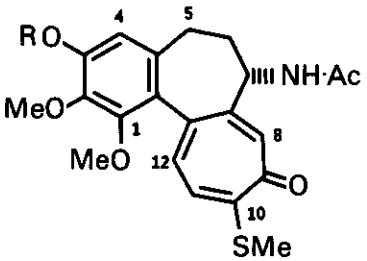
Microbial degradation of thiocolchicine (1) with a culture of Streptomyces griseus afforded as a major metabolite a monophenol of undetermined structure.[1] This phenol was not identical with 3-demethylthiocolchicine (6) prepared from thiocolchicoside (2)[2], suggesting that it may represent the unknown 2-demethyl analog 3. Selective ether cleavage in the colchicine series providing access to 3-demethyl- and 2-demethyl congeners has recently been reported,[3] suggesting that similar reactions might also succeed with the corresponding thiocolchicines. It was hoped that the unknown 2-demethylthiocolchicine (3) would become available by one of these methods. The results obtained in pursuing this objective shall now be reported: Treatment of thiocolchicoside (2) with 85% phosphoric acid at room temperature[3] afforded the known 3-demethylthiocolchicine (6) in 93% yield, demonstrating once more that this represents an excellent way to selectively cleave a glucosidic aromatic ether in the presence of methyl ether groups. Treatment of thiocolchicine (1) with conc. sulfuric acid at 70°C by methodology elaborated with colchicine[4] afforded after workup a mixture of products, containing besides starting material the 2-demethylthiocolchicine (3) and the catechols 4 and 5. The material moving fastest on silica gel was thiocolchicine (1), followed by 2-demethylthiocolchicine (3), identical in every respect with the microbial degradation product.[5] Phenol 3 was not identical with 3-demethylthiocolchicine (6) but also lacked a signal assigned to either 2-OMe or 3-OMe (Table). O-methylation of 3 with ethereal diazomethane afforded thiocolchicine (1).

*Dedicated to Dr. Ulrich Weiss at the occasion of his 75th birthday.

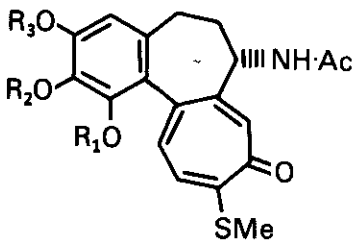
The slower moving materials, giving dark color reactions with $FeCl_3$ were isolated in amorphous form and turned out to represent the catechols 4 and 5, with 5 being the slower moving compound under these experimental conditions. The ^1H NMR data of 4 and 5 (Table) and the absence of the signals for 1-OMe and 2-OMe in 4 and 2-OMe and 3-OMe in 5, and both affording thiocolchicine (1) upon O-methylation with diazomethane, proved their catecholic structures.

It has now been demonstrated that the microbial metabolite obtained from thiocolchicine (1) with Streptomyces griseus more than 20 years ago is 2-demethylthiocolchicine, now readily available by the above mentioned procedure.

Both phenols, 3-demethylthiocolchicine (6, 85% T.B.) and 2-demethylthiocolchicine (3, 76% T.B.) bind similarly to rat brain microtubule protein as colchicine (Tubulin binding = T.B. = 90%)[6].



1: R = Me
2: R = Glucose



3: $R_1 = R_3 = Me$, $R_2 = H$
4: $R_1 = R_2 = H$, $R_3 = Me$
5: $R_1 = Me$, $R_2 = R_3 = H$
6: $R_1 = R_2 = Me$, $R_3 = H$

Table: ^1H NMR Data of Thiocolchicine and Derivatives[a]

Compounds	N-COMe	Chemical Shifts in δ Units[b] 1-OMe	2-OMe	3-OMe	10-SMe
1	1.84	3.52	3.78	3.83	2.40
3	1.84	3.45	-	3.80	2.40
4	1.84	-	-	3.80	2.40
5	1.84	3.45	-	-	2.40
6	1.84	3.52	3.79	-	2.44

a, in $DMSOd_6$; b, all singlets

EXPERIMENTAL

Melting points were taken on a Fisher-Johns apparatus and are uncorrected. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation of this laboratory. IR spectra were determined using Beckman 4230 instrument. ^{1}H NMR spectra were obtained on a JEOL FX-100 spectrometer with Me_4Si as an internal reference. Intermediate-range pH strips (pH 0-6 and 5-10) from Aldrich Chemical Company, Inc. Milwaukee, were used for pH determinations. Chemical ionization mass spectra (CIMS) were determined by using a Finnigan 1015D spectrometer with a Model 6000 data collection system. Thin-layer chromatography plates were purchased from Analtech, Inc., Newark, DE. Solvent systems used for TLC over silica gel were as follows: (A) CH_2Cl_2: MeOH (9.5:0.5); (B) CH_2Cl_2 : MeOH (9.3 : 0.7) (C) CH_2Cl_2 : MeOH (9.8 : 0.2).

3-Demethylthiocolchicine (6).

A solution of thiocolchicoside (2) (2.3 g, 3.96 mmol) in phosphoric acid (85-88%, 65 ml) after dissolving by heating, was stirred at room temperature for overnight. The TLC analysis showed the absence of 2 in the reaction mixture, (one small drop of reaction mixture was diluted with MeOH (0.5 ml) and applied to a TLC plate and run in solvent system A). The reaction mixture was poured on ice, adjusted to pH 5 by the addition of 15% aqueous NaOH solution, followed by several extractions with CH_2Cl_2 (4x25 ml). The combined organic layer were dried (Na_2SO_4) and evaporated to afford a residue, which was crystallized with acetone to afford 6 as a yellow solid (1.5 g, 93%), mp 316°C (lit.[2] 318°C); $[\alpha]_D^{25}$ = -251° (c 0.21, $CHCl_3$), (lit.[2] -249° (c 0.5, $CHCl_3$)); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440 (OH); CIMS: m/e 402 (M^+ +1); Anal. Calcd. for $C_{21}H_{23}NO_5S$: C, 62.82; H, 5.77; N, 3.48; S, 7.98. Found C, 63.09; H, 5.86; N, 3.26; S, 8.25%.

Demethylation of Thiocolchicine with conc. Sulfuric Acid:

A solution of thiocolchicine (1) (250 mg. 0.60 mmol) in conc. sulphuric acid (1.75 ml) was heated at 67-70°C (oil bath temperature) for overnight. The reaction mixture was poured on ice, diluted with water (2 ml) and the pH of the solution was adjusted to 5 by the addition of 2% aqueous NaOH solution and extracted with a mixture of $CHCl_3$/isopropanol (3:1, 4x5 ml). The combined organic layer was dried (Na_2SO_4) and evaporated to afford a residue showing several spots on TLC (solvent system B). The reaction mixture was purified by preparative layer chromatography (PLC) over silica gel to afford four almost uniform fractions. The fastest running fraction corresponded to thiocolchicine (1) (TLC, mp, IR, and CIMS). The residue obtained from the second fraction was rechromatographed (PLC) over silica gel by using solvent system C (double run) to afford 2-demethylthiocolchicine (3) as a pure yellow solid (50 mg, 21%), mp 189° (lit.[1] 190°C); $[\alpha]_D^{25}$ = -281° (c 0.48, MeOH), (lit.[1] -281° (MeOH)); IR ν_{max}^{KBr} cm^{-1}: 3410 (OH); CIMS: m/e 402 (M^+ +1); Anal. Calcd. for $C_{21}H_{23}NO_5S.1/2\ H_2O$: C, 61.44; H, 5.98; N, 3.41; S, 7.81. Found: C, 61.76; H, 6.32; N, 3.36; S, 7.87%.

The residue obtained from third fraction was further purified by chromatography (PLC) over silica gel (solvent system A), to afford 1,2-didemethylthiocolchicine (4) as a yellowish amorphous solid (20 mg, 8%), dark blue color with $FeCl_3$, mp 233°C; $[\alpha]_D^{25}$ = -209° (c 0.35, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH); CIMS: m/e 388 (M^+ +1).

The residue obtained from fourth fraction was also purified by further chromatography (PLC) over silica gel (solvent system A), to afford 2,3-didemethylthiocolchicine (5) as a yellowish solid (16 mg, 7%), dark blue color with $FeCl_3$, mp 185°C; $[\alpha]_D^{25}$ = -104° (c 0.2, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400 (OH); CIMS: m/e 388 (M^+ +1).

O-Methylations with Diazomethane:

2-Demethylthiocolhicine (3), 1,2-Didemethylthiocolchicine (4) and 2,3-Didemethylthiocolchicine (5) on methylation with excess ethereal solution of diazomethane afforded thiocolchicine (1), identical with a standard sample (mp, TLC, IR).

REFERENCES

1. L. Velluz and P. Bellet, C. R. Acad. Sci., 248, 3453 (1959).
2. L. Velluz and G. Muller, Bull. Soc. Chim., 755 (1954).
3. M. Rösner, H. G. Capraro, A. E. Jacobson, L. Atwell, and A. Brossi, J. Med. Chem., 24, 257 (1981).
4. M. Rösner, F. L. Hsu and A. Brossi, J. Org. Chem., 46, 3686 (1981).
5. The comparision was made by m.p., TLC, IR (KBr), and $[\alpha]_D$ in MeOH. We are grateful to Dr. P. Bellet from Roussel-Uclaf in Paris for providing us with substantial amounts of thiocolchicine and thiocolchicoside.
6. M. Iorio, A. Brossi and C. F. Chignell, Heterocycles, 9, 1 (1978).

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