230. The Structure Elucidation of Pseudothiocolchicine

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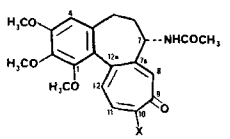
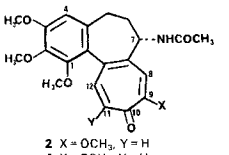
A colchicine derivative, previously isolated from the reaction of isocolchicine with sodium thiomethoxide in aqueous MeOH, was now identified as 11-(methylthio)isocolchicine on the basis of ^{1}H-, ^{13}C-NMR spectra and chemical correlation. This reaction represents the first example of abnormal nucleophilic displacement (telesubstitution) in the colchicine chemistry.

Colchicine (**1**) may be regarded as a prototype alkaloid occurring in members of the Liliaceae, especially in meadow saffron (*Colchicum autumnale*) and in the African climbing lily (*Gloriosa superba*). Compound **1** has powerful mitotic spindle inhibitory effect that cannot be utilized in the treatment of cancer because of high toxicity, however, is still being widely used for treatment of gout [1].



1 X = OCH_3
3 X = SCH_3
8 X = D
10 X = H



2 X = OCH_3, Y = H
4 X = SCH_3, Y = H
5 X = H, Y = SCH_3
6 X = H, Y = H
7 X = H, Y = D
9 X = D, Y = H

After a hiatus of over 20 years, there has been a great upsurge of interest in colchicine chemistry, mainly due to *Brossi* and coworkers, in order to separate important biological properties of these compounds from unwanted toxicity.

Over 30 years ago, *Velluz* and *Muller* [2] noted that the treatment of isocolchicine (**2**) with MeSNa in aq. MeOH yielded two new compounds: the major product (60%), named pseudothiocolchicine, which was not assigned a structure, and a minor compound (20%). Both compounds were proved to be isomers of thiocolchicine (**3**) as evidenced by elemental analysis, and the minor compound was reported to be isothiocolchicine (**4**). It was of interest for us to repeat the above work in order to identify '*Velluz*'s major compound' of undertermined structure.

Pseudothiocolchicine was obtained as pale yellow crystalline solid, m.p. 300°–302° (acetone), $[\alpha]_D^{25} = -28.4°$ ($c = 0.5$, $CHCl_3$). High-resolution MS analysis showed a parent ion at m/z 415.1460 indicating an elemental composition of $C_{22}H_{25}NO_5S$ and confirming that this compound is isomeric with **3** and **4**. The MS was comparable to that of other *Colchicum* alkaloids [3] in that the major fragmentations of the molecular ion involved competitive ejection of CO ($\rightarrow m/z$ 387) and $MeCONH_2$ ($\rightarrow m/z$ 356), as proven by B/E

linked-scan spectrum of ion m/z 415. The solution ($CHCl_3$) IR spectrum of pseudothiocolchicine had absorptions at 1678*s*, 1670*s*, and 1595*vs* cm^{-1}. This was different from the three-band pattern typically observed for other colchicine-related compounds in the 1700–1600 cm^{-1} region but was almost superimposable to that of isothiocolchicine (**4**).

The ^{1}H-NMR spectrum (200 MHz, (D_6)DMSO) exhibited, in addition to usual three-proton singlet at 1.87 ppm (NHAc), three singlets at 3.62, 3.84, and 3.88 ppm (OMe) and a one-proton singlet at 6.84 ppm (H–C(4)), a three-proton singlet at 2.32 ppm for MeS group, an *AB* system (J = 12.5) at 6.99/7.46 ppm, and a one-proton singlet at 7.13 ppm due to tropone protons[1]). Furthermore, the proton-decoupled ^{13}C-NMR ((D_6)DMSO) for **5** contained the requisite 22 lines and significant features included the appearance of signals at 132.1 (*d*), 132.6 (*d*), 132.9 (*d*), 138.1 (*s*), 141.8 (*s*), 154.0 (*s*) and 179.8 (*s*) that characterize tropone C-atoms.

The strong similarities of ^{1}H-NMR data of pseudothiocolchicine **5** and **4** (see *Exper. Part*), indicated that both contained 10-oxobenzo[*a*]heptalene system (isocolchicine series)[2]), but did not definitely establish the location of the MeS group in the former compound. This was further confirmed by desulfurization of pseudothiocolchicine with *Ra*/Ni under carefully controlled conditions to yield the hitherto unknown isocolchicide (**6**), identical to the one independently obtained from *Ra*/Ni desulfurization of **4**. The ^{13}C-NMR ((D_6)DMSO) of **6** generally parallels that of **4**, except that MeS signal is missing and C(9) appears at low field (137.2 or 139.4 ppm). A 200-MHz ^{1}H-NMR ($CDCl_3$) provided accurate NMR parameters and corroborated the assignment **6**: 6.84 (*dd*, J = 12.4, 3.2, H–C(11)); 6.97 (*dd*, J = 12.6, 3.2, H–C(9)); 7.18 (*d*, J = 12.4, H–C(12)); 7.35 (*d*, J = 12.6, H–C(8)).

The lack in ^{1}H-NMR of a four-bond H,H coupling ('W' path) across the tropone moiety was a reliable indicator for structure **5** for pseudothiocolchicine[3]). That this compound contained a MeS–C(11) group was definitely proven by its desulfurization in the presence of deuterated *Ra*/Ni [6] to produce [11-^{2}H]isocolchicide (**7**) with 72% specificity. In fact, the signal of H–C(11) at 6.84 ppm has been significantly reduced in intensity and the coupling constants to H–C(12) and H–C(9) have been largely eliminated. Moreover, a closer examination of the residual weak peaks for H–C(11) (*dd*) and for H–C(8) (*s*) suggests that they may be accounted for by the presence of non-deuterated compound (**6**, isocolchicide) and by a smaller contribution from [9,11-$^2H_2$]isocolchicide, respectively.

The reliability of this procedure was corroborated by the results obtained under similar conditions starting from **3** and **4**, which gave [10-^{2}H]colchicide (**8**) (> 95% specific) and [9-^{2}H]isocolchicide (**9**) (90% specific), respectively.

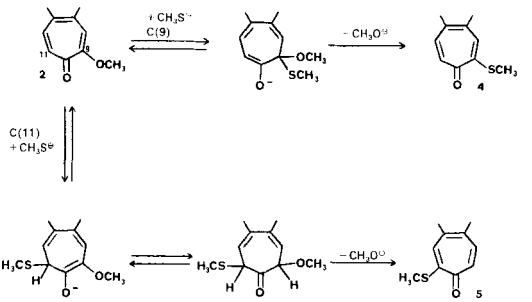
On the basis of the above mentioned results, electronic absorption spectra of **5** deserve some comments. The long-wavelength band at 330 nm is at first sight a surprising result, for we should expect, in principle, a bathochromic shift of 40–60 nm (*e.g.*, 325 nm in **6** *vs.*

[1]) In ^{1}H-NMR NOE difference (80 MHz, CD_2Cl_2) for **5**, the enhancement of signals resulting from irradiation at the frequency of the singlet at 2.25 ppm (MeS) was strong (35%) for the singlet at 7.26 ppm but was negligible for the *AB* system at 7.04/7.40 ppm (J = 12.4 Hz).

[2]) *Chemical Abstracts* name for isothiocolchicine (**4**): *(S)-N-[5,6,7,10-tetrahydro-1,2,3-trimethoxy-9-(methylthio)-10-oxobenzo[a]heptalen-7-yl]acetamide.*

[3]) These ^4J constants, whose magnitude are generally of the order of 2–3 Hz, have been observed previously for a variety of tropone derivatives [4]. ^{1}H-NMR of the known colchicide (**10**) [5] showed this long-range coupling (2.5 Hz) (see *Exper. Part*).

Scheme



363 nm in **4**; 327 nm in **10** *vs.* 383 nm in **3**) by the introduction of a MeS group adjacent to tropone carbonyl group. These evidences suggest that the free electron pair of the S-atom and tropone π system are not conjugated in the electronic ground state due to steric hindrance by the bulky MeO group at position 1.

The formation of pseudothiocolchicine (**5**) presumably takes place *via* a conjugative addition-elimination mechanism as outlined in the *Scheme* and finds parallel in many studies, particularly those of *Pietra* and coworkers [7] on 2-halotropones.

Although heteronucleophiles are known to give ipsosubstitution on the troponoid MeO group in *Colchicum* alkaloids [8], the abnormal nucleophilic substitution (telesubstitution) appears to be unprecedented in literature and may well represent a novel and interesting variation in colchicinoids.

Experimental Part

General. M.p. (uncorrected) were determined with a *Büchi* apparatus. TLC and PLC plates were purchased from *Merck* and silica gel *60* (0.040–0.063 mm) was used for flash chromatography (FC) [9]. Solvent system used for TLC, PLC, and FC was $Me_2CO/CHCl_3$ 3:2. Optical rotations were measured by using a *Perkin-Elmer* model *241* polarimeter with the solvents specified. UV/VIS spectra [λ_{max} (log ε)] were recorded on a *Perkin-Elmer* spectrophotometer. CD spectra were measured on a *Jobin-Yvon Mark III*, with λ of extrema and of the zero passage λ_0 in nm (molar decadic circular dichroism [$\Delta\varepsilon$]). IR spectra were measured on a *Perkin-Elmer 681* spectrophotometer. ^{1}H-NMR spectra were determined by using a *Bruker WP-80* (80 MHz) and *Varian XL-200* (200 MHz) with TMS as internal reference (= 0 ppm) with *J* (Hz). ^{13}C-NMR spectra (25.2 MHz) were recorded on a *Varian XL-100*, TMS as internal reference (= 0 ppm); multiplicities from off-resonance decoupled spectrum. HR-MS, EI-MS and B/E linked-scan spectra were obtained by using a *VG 70-70 EQ* (70 eV).

(S)-N-[5,6,7,10-Tetrahydro-1,2,3-trimethoxy-11-(methylthio)-10-oxobenzo[a]heptalen-7-yl]acetamide (*Pseudothiocolchicine*; **5**). Compound **5** was prepared according to the procedure in [2]. M.p. 300°–302° (acetone) as pale yellow needles; $[\alpha]_D^{25} = -28.4°$ (c = 0.5, $CHCl_3$). IR ($CHCl_3$): 1678, 1670, 1595. UV/VIS (MeOH): 267 (4.13), 330 (4.37), 400 sh (3.95). CD (MeOH): 207 (+15.0), 220 (+8.4), 232 (+16.9), 247 (+7.12), 261 (+0.4), 285 (+1.54), 300 (+4.04), 382 (−16.9), 409 (+0.96); λ_0 at 310, 400. ^{1}H-NMR (200 MHz, (D_6)DMSO): 1.87 (*s*, $NCOCH_3$); 2.32 (*s*, CH_3S); 3.62 (*s*, CH_3O–C(1)); 3.84, 3.88 (2*s*, 2 CH_3O); 4.30 (*m*, H–C(7)); 6.84 (*s*, H–C(4)); 6.99/7.46 (*AB*, J = 12.5, H–C(9), H–C(8)); 7.13 (*s*, H–C(12)); 8.50 (*d*, J = 7.0, NH). ^{1}H-NMR (80 MHz, CD_2Cl_2): 1.89 (*s*, $NCOCH_3$); 2.25 (*s*, CH_3); 3.58 (*s*, CH_3O–C(1)); 3.81 (*s*, 2 CH_3O); 4.40 (*m*, H–C(7)); 6.40 (*d*, J = 6.4, NH); 6.56 (*s*, H–C(4)); 7.04/7.40 (*AB*, J = 12.4, H–C(9), H–C(8)); 7.26 (*s*, H–C(12)). ^{13}C-NMR ((D_6)DMSO): 150.3 (*s*, C(1)); 125.7 (*s*, C(19)); 140.5 (*s*, C(2)); 153.3 (*s*, C(3)); 107.0 (*d*, C(4)); 135.0 (*s*, C(4a)); 29.5 (*t*, C(5)); 36.8 (*t*, C(6)); 50.9 (*d*, C(7)); 141.8 (*s*, C(7a)); 132.6 (*d*); 132.9 (*d*); 138.1 (*s*, C(12a)); 179.8 (*s*, C(10)); 154.0 (*s*, C(11)); 132.1 (*d*, C(12)); 22.4 (*q*, CH_3CO); 14.1 (*q*, CH_3S). EI-MS: 415 (100), 387 (90), 356 (25), 328 (41), 297 (16), 266 (12), 152 (8), 83 (41). HR-MS: 415.1460 ($C_{22}H_{25}NO_5S$ requires 415.1453). Anal. calc. for $C_{22}H_{25}NO_5S$ (415.15): C 63.61, H 6.02, N 3.37; found: C 63.70, H 6.11, N 3.31.

(S)-N-[5,6,7,9-Tetrahydro-1,2,3-trimethoxy-9-(methylthio)-10-oxobenzo[a]heptalen-7-yl]acetamide (Isothiocolchicine; **4**) [2]. CD (MeOH): 206 (+37.4), 222 (+2.7), 234 (+7.0), 245 (+3.3), 267 (−3.3), 288 (−1.0), 307 (−4.2), 317 (−3.8), 360 (−10.9), 398 (−6.4); λ_0 at 256. IR ($CHCl_3$): 1650, 1600, 1548, 1490. UV/VIS (MeOH): 261 (4.35), 290 (4.11), 363 (4.29), 381 (4.28). ^{1}H-NMR (80 MHz, $CDCl_3$): 2.06 (*s*, $NCOCH_3$); 2.41 (*s*, CH_3S); 3.66 (*s*, CH_3O–C(1)); 3.88, 3.90 (2 CH_3O); 4.58 (*m*, H–C(7)); 6.51 (*s*, H–C(4)); 6.94, 7.39 (*AB*, *J* = 10.7, H–C(12), H–C(11)); 7.26 (*s*, H–C(8)). ^{13}C-NMR ($CDCl_3$): 150.1 (*s*, C(1)); 125.3 (*s*, C(19)); 140.6 (*s*, C(2)); 153.3 (*s*, C(3)); 107.6 (*d*, C(4)); 29.3 (*t*, C(5)); 37.8 (*t*, C(6)); 51.9 (*d*, C(7)); 144.4 (*s*, C(7a)); 124.0 (*d*, C(8)); 156.6 (*s*, C(9)); 180.9 (*s*, C(10)); 130.2 (*s*, C(11)); 140.5 (*d*, C(12)); 135.0 (*s*); 60.8, 60.6 (2*q*); 55.9 (*q*, CH_3O–C(3)); 168.7 (*s*, NHCO); 22.3 (*q*, $COCH_3$); 14.7 (*q*, CH_3S). EI-MS: 415 (40), 387 (23), 282 (100), 381 (66), 380 (15), 375 (50), 374 (16) 356 (10), 351 (18), 328 (46), 316 (55).

Desulfurization of **5** *with* Ra/*Ni to (S)-N-[5,6,7,10-Tetrahydro-1,2,3-trimethoxy-10-oxobenzo[a]heptalen-7-yl]acetamide (Isocolchicide;* **6**). A mixture of **5** (270 mg, 0.65 mmol) and *W-2 Ra*/Ni (*ca.* 3 g; previously deactivated by refluxing with acetone for 10 h) in acetone (40 ml) was stirred overnight at r.t. The colourless residue obtained after removal of the catalyst by filtration followed by evaporation of the solvent *i.v.* was chromatographed (FC). The major product **6** was obtained as a colourless foam in 35% yield. IR ($CHCl_3$): 1670, 1620, 1590, 1560. UV/VIS (MeOH): 231 (4.41), 325 (4.01). ^{1}H-NMR (200 MHz, $CDCl_3$): 1.86 (*s*, $NCOCH_3$); 3.64 (*s*, CH_3O–C(1)); 3.82, 3.88 (2*s*, 2 CH_3O); 4.24 (*m*, H–C(7)); 6,83 (*s*, C(4)); 6.84 (*dd*, *J* = 12.4, 3.2, H–C(11)); 6.97 (*dd*, *J* = 12.6, 3.2, H–C(9)); 7.18 (*d*, *J* = 12.4, H–C(12)); 7.35 (*d*, *J* = 12.6, H–C(8)). ^{13}C-NMR ((D_6)DMSO): 150.0 (*s*, C(1)); 125.0 (*s*, C(1a)); 139.6 (*s*, C(2)); 153.4 (*s*, C(3)); 107.8 (*d*, C(4)); 29.2 (*t*, C(5)); 37.4 (*t*, C(6)); 51.1 (*d*, C(7)); 146.1 (*s*, C(7a)); 133.5 (*d*, C(8)); 137.2 (*d*); 185.5 (*s*, C(10)); 139.4 (*d*); 140.7 (*d*, C(12)); 135.0 (2*s*, C(12a), C(4a)); 60.8, 60.6 (2*q*); 55.8 (*q*, CH_3O–C(3)); 168.8 (*s*, CONH); 22.3 (*q*, CH_3CO). HR-MS: 369.1583 ($C_{21}H_{23}NO_5$ requires: 369.1576). This compound was identical in all respects with that produced by desulfurization with *Ra*/Ni of **4**.

Desulfurization of **5** *with Deuterated* Ra/*Ni*. Deuterated *Ra*/Ni was prepared as described by *Wu* and coworkers [6]. To *Ra*/Ni-d (*ca.* 500 mg) in a 10-ml round-bottomed flask was added **5** (50 mg, 0.12 mmol) in MeOD (3 ml) and the mixture was stirred at r.t. After 2 h, the solvent was removed by pipet and the catalyst was washed with MeOD (2 ml, 2×). The solns. were combined, filtered through a *Celite* pad and evaporated *i.v.* PLC of the residue afforded a colourless foam (12 mg; single spot in TLC) which appeared to be a 2.6:2:1 mixture (checked by 200-MHz ^{1}H-NMR) of **7**, **6**, and [9,11-$^2H_2$]isocolchicide, resp.

(S)-N-(5,6,7,9-Tetrahydro-1,2,3-trimethoxy-9-oxobenzo[a]heptalen-7-yl)acetamide (Colchicide; **10**). It was prepared according to [5] by desulfurization of **3**. IR ($CHCl_3$): 1670, 1620, 1590, 1540. UV/VIS (MeOH): 235 (4.32), 327 (4.00). ^{1}H-NMR (200 MHz, (D_6)DMSO): 1.85 (*s*, $NCOCH_3$); 3.57 (*s*, CH_3O–C(1)); 3.79, 3.84 (2*s*, 2 CH_3O); 4.29 (*m*, H–C(7)); 6.79 (*s*, H–C(4)); 6.91 (br. *dd*, *J* = 11.1, 2.5, H–C(10)); 7.03 (*d*, *J* = 2.5, H–C(8)); 7.04 (*d*, *J* = 8.9, H–C(12)); 7.32 (*dd*, *J* = 11.1, 8.9, H–C(11)); 8.60 (*d*, *J* = 7.3, NH). ^{13}C-NMR ((D_6)DMSO): 151.1 (*s*); 125.4 (*s*, C(1a)); 141.7 (*s*, C(2)); 158.3 (*s*, C(3)); 107.4 (*d*, C(4)); 133.9 (*s*, C(4a)); 29.9 (*t*, C(5)); 36.0 (*t*, C(6)); 52.4 (*d*, C(7)); 152.5 (*s*); 136.4 (*d*); 187.3 (*s*, C(9)); 144.3 (*d*, C(10)); 136.7 (*d*); 135.2 (*d*, C(12)); 140.2 (*s*, C(12a)); 61.6, 61.2 (2*q*); 56.1 (*q*, CH_3O–C(3)); 169.6 (*s*, CON); 22.9 (*q*, CH_3CO). EI-MS: 369 (33), 341 (38), 310 (11), 298 (12), 282 (92), 268 (54), 251 (42), 118 (100).

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