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THE FIRST COLCHICINE ANALOGUE WITH AN EIGHT MEMBERED B-RING. STRUCTURE, OPTICAL RESOLUTION AND INHIBITION OF MICROTUBULE ASSEMBLY[1]

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Abstract. Synthesis, crystal structure, enantiomeric resolution, and CD spectroscopy of the first colchicine derivative with an eight-membered B-ring lactam obtained via a Beckmann reaction, is described and only one of the stable atropisomeric enantiomers arrests microtubule assembly. © 1997 Elsevier Science Ltd.

The antimitotic alkaloid colchicine, from *Colchicum autumnale,* exerts its major biological effect by binding to tubulin, the basic subunit component of microtubules. This process leads to depolymerization of the microtubules with concomitant mitotic arrest. Colchicine and its derivatives have been extensively studied from both chemical and biological aspects.[2] We are interested in the structural requirements of colchicinoids for binding to tubulin, in particular the conformation around the pivot bond joining the A and C rings. The helicity around this bond is a*S* and the torsional angle is close to 54° in colchicine and active derivatives.[3-6] In an attempt to shed light on the stereochemical requirements for binding, we have been working towards syntheses of colchicine analogues with extended or contracted B-rings leading to systematic variations in the torsional angle between the A- and C-rings. One such approach via a Beckmann rearrangement is described here.

The *syn/anti* oximes, **2a** and **2b** were prepared according to the Scheme. The synthesis of compound **1** in three steps from colchicine has been described recently.[3b,7] The conditions for the Beckmann rearrangements were crucial. We found after several attempts that the use of polyphosphoric acid (PPA) at 65-70 °C for ca 20 h gave 55% **3** and 64% **4** in rather clean reactions. Higher temperatures generated degradation products and lower temperatures only gave recovered **2**. Other conditions led to tetracyclic isoxazole derivatives.[3b] The product from the reaction with **2a** had all the expected physical characteristics expected for the lactam **3** in contrast to the product from **2b**. Most strikingly, **4** was lacking the typical singlet at δ=6.5 for the H(4)-proton, had a ^{13}C NMR signal at δ=203.3 and an IR band at 1700 cm^{-1}.[8]

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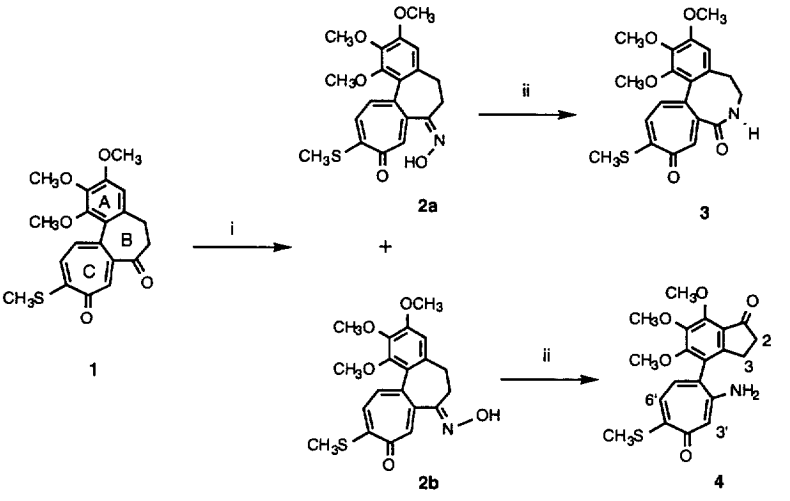

Scheme. *Reagents and reaction conditions (Compound 1 was prepared according to ref. 3b):* (i) $H_2NOH{\cdot}HCl$, Na_2CO_3 to pH=7, reflux 24 h, flash chromatography (silica, ethyl acetate/heptane), recryst. from ethanol ; (ii) Excess polyphosphoric acid, 65-70 °C, 20 h, flash chromatography (silica, ethyl acetate/heptane).

The crystal structure of **3** is shown in Fig. 1.[9] The crystals are racemates but all enantiomer sensitive information, including Fig. 1, is given for the atropisomer with S-biaryl configuration. There is an intermolecular hydrogen bond N1-H···O4 with an N···O distance 2.787(6) Å and an N-H···O angle of 167(6)°. The A and C-rings are fairly planar and the torsional angle between the least-squares planes A and C is 76.3°, some 20° larger than in most colchicinoid crystals. The parallel orientation of 1,2-dimethoxy groups observed in **3** is about as common as the antiparallel one in crystals of colchicinoids. According to molecular mechanics calculations [MM2(91)], which acceptably reproduce structures and energies of colchicinoids, the antiparallel conformation is favoured by ca 1 kcal/mol.[3]

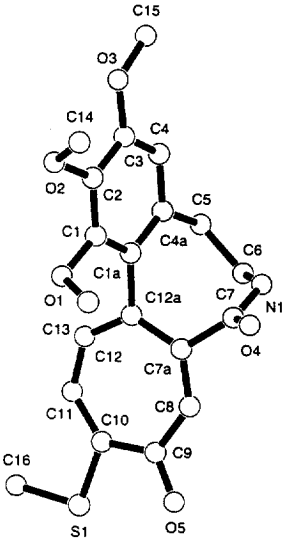

Figure 1. Molecular structure of **3** in the crystal. Hydrogen atoms are omitted.

^1H, ^{13}C NMR and 2-D NMR spectroscopy including COSY, NOESY and HETCOR experiments, as well as IR and MS, proposed structure **4** for the other isomer. In particular long-range HETCOR between protons in the five-membered ring and C(1a) was indicative. The amino protons showed long-range HETCOR crosspeaks to C(1') and C(3'), and a NOESY crosspeak between NH_2 and H(3'), verifying the position of the amino group. The tropone structure was further supported by strong NOESY crosspeaks between CH_3S and H(6'), a common feature for colchicines as the methylthio group points towards this proton, and long-range HETCOR (tuned for three bond couplings) crosspeaks from H(3') to two quaternary carbons [C(5') and C(1')]. The formation of **4** could be rationalized as acidolysis of the lactam followed by intramolecular Friedel-Crafts acylation of the activated A-ring. No lactam could be detected in this reaction.

Both **3** and **4** were resolved into atropisomeric enantiomers on a semipreparative scale by use of a microcrystalline triacetylcellulose column.[10] Fractions taken in the first and last parts of the eluate gave enantiomerically enriched lactams **3** [k(-)=1.45, k(+)=2.09, α=1.44], and indanone **4** [k(+)=2.59, k(-)=3.09, α=1.19], (signs at 436 nm). Further enrichment was achieved by recycling. The CD-spectra are shown in Fig. 2. The spectrum of **3** exhibits two bands at 355 and 285 nm, typical for thiocolchicine derivatives, and the signs of the Cotton effects indicate that the first eluted enantiomer has the same helicity as native colchicine.

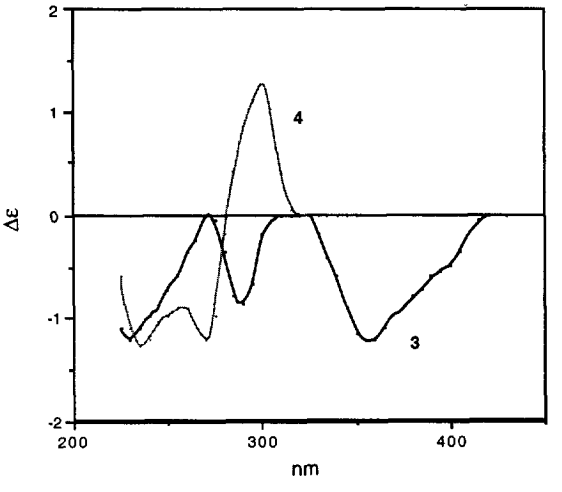

Figure 2. CD-spectra of the first eluted enantiomers (-)-**3** and (+)-**4** in ethanol.

The spectrum of **4** was totally different and the absolute configuration was not established. Racemization of **3** in benzene in a sealed tube at 110 °C gave a rate constant of $6.9 \cdot 10^{-5}$ s^{-1} corresponding to a rotational barrier of 30.4 kcal mol^{-1}, assuming $k_{rot} = 0.5\ k_{rac}$.

In vitro assembly of bovine tubulin and microtubule associated proteins to microtubules was inhibited by (-)-**3** but not by (+)-**3** (Fig. 3) nor by any of the enantiomers of **4** in reasonable concentrations (not shown). The very weak effect of (+)-**3** corresponds to the expected result of the contamination by 18 % of the active enantiomer. The concentration of (-)-**3** needed to inhibit microtubule assembly is ca 10 times higher than for colchicine. As **3** is not only highly twisted around the pivot bond but also a comparetively rigid molecule, the tubulin binding site must be able to accomodate such species.

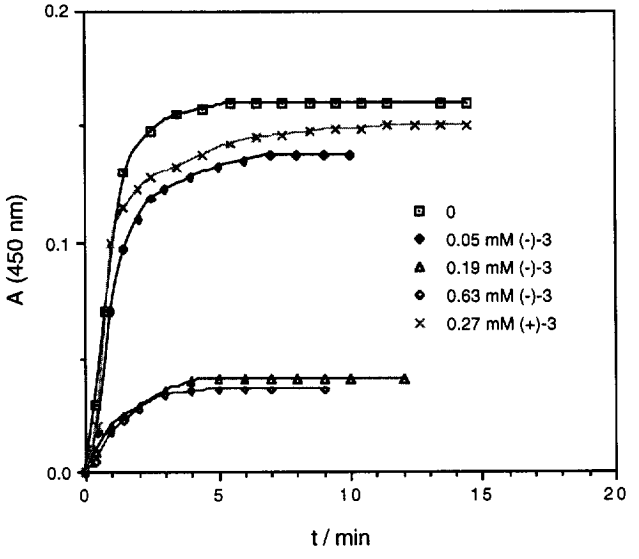

Figure 3. Effect of the enantiomers of **3** on the turbidity time course of the assembly of microtubule protein at 37 °C. All samples contained 2.5 mg/mL bovine tubulin (including 20% MAPs). The drug concentrations are indicated in the figure and ee was ≥ 96% for (-)-**3** and 64% for (+)-**3**.[11]

In summary, we report the synthesis and stereochemical properties of the first colchicinoid with an expanded B-ring and demonstrate that highly twisted, conformationally rigid colchicinoids retain their biological effect provided that they possess the same helicity as colchicine. Further studies on the biological properties of **3** and of other B-ring modified colchicine derivatives are in progress.

Acknowledgment: We thank the Swedish Natural Science Research Council for financial support.

Notes and References

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8. The compounds had the following physical properties **3**: mp 209-212 °C; $[\alpha]^{25}_{D}$ = +1010 (c $3.07 \cdot 10^{-3}$, EtOH); IR (KBr) ν [cm^{-1}]: 3450, 3220, 3100, 2940, 1670, 1630, 1605, 1560, 1495; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.09 (1H, s), 7.07 (1H, d, *J* =10 Hz), 7.06 (1H, d, *J* =10 Hz), 6.46 (1H, s), 5.80 (1H, t, *J* =6.6 Hz), 3.90 (3H, s), 3.88 (3H, s), 3.66 (3H, s), 3.56-3.47 (1H, m), 3.37-3.28 (1H, m), 2.92-2.84 (1H, m), 2.83-2.74 (1H, m), 2.46 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 182.9, 172.7, 160.7, 154.3, 152.0, 147.0, 142.0, 136.0, 135.8, 132.0, 131.1, 127.8, 126.6, 109.6, 61.7, 61.6, 56.5, 40.2, 33.6, 15.7; UV (EtOH) 360 (4.12), 261 (4.26) and 204 (4.62); HRMS: $C_{20}H_{21}NO_5S$ calcd for 387.1140 found 387.1143. **4**: m.p. 214-216 °C; IR (KBr)ν [cm^{-1}]: 3420, 3340, 3200, 2910, 1700, 1630, 1570, 1520 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (1H, d, *J* =10.0 Hz), 6.61 (1H, d, *J* =10.2 Hz), 6.58 (1H, s), 4.46 (2H, s, br), 4.10 (3H, s), 3.91 (3H, s), 3.90 (3H, s), 2.87-2.72 (2H, m), 2.66-2.61 (2H, m), 2.35 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.3, 180.1, 160.5, 157.7, 153.2, 153.1, 151.3, 145.3, 133.6, 129.6, 125.7, 125.4, 121.4, 113.8, 62.7, 62.0, 61.9, 37.3, 24.7, 15.6; UV (EtOH) 370 (4.29), 292 (4.59) and 212 (3.88); HRMS: $C_{20}H_{21}NO_5S$ calcd for 387.1140 found 387.1137.

9. *Crystallography.* $C_{20}H_{21}NO_5S$, M = 387.45. A yellow (001) crystal plate, elongated along [100], was mounted on a Huber diffractometer/Rigaku RU-H2R rotating anode generator with graphite-monochromated MoKα X-radiation. Crystal size 0.30 x 0.12 x 0.05 mm, monoclinic, space group $P2_1/a$, a = 7.740(2), b = 20.264(4), c = 12.535(3) Å, β = 94.78(3)°, U = 1959.2(6) $Å^3$, Z = 4, D_c = 1.313 g cm^{-3}, $F(000)$ = 816, μ = 0.195 mm^{-1}. A hemisphere of data, 6202 reflections, was collected with the ω-$2\theta_{max}$ technique and $2\theta_{max}$ = 50° at 25 °C.

Some selected bond lengths (Å) and bond angles(°): S1-C10 1.741(6), S1-C16 1.787(6), O1-C1 1.377(6), O1-C13 1.401(8), O2-C2 1.377(6), O2-C14 1.413(7), O3-C3 1.374(6), O3-C15 1.421(7), O4-C7 1.227(7), O5-C9 1.226(7), C1a-C1 1.399(7), C1a-C4a 1.404(7), C1a-C12a 1.494(7), C6-N1 1.450(7), N1-C7 1.330(7), C7a-C7 1.514(8), C7a-C8 1.356(7), C7a-C12a 1.430(8), C8-C9 1.439(7), C9-C10 1.465(8), C10-C11 1.366(7), C11-C12 1.428(8), C12a-C12 1.370(8), C10-S1-C16 104.0(3), C4a-C5-C6 115.9(3), C1-O1-C13 114.5(5), C5-C6-N1 115.1(5), C6-N1-C7 124.9(6), C2-O2-C14 116.3(5), C3-O3-C15 118.3(5), C8-C7a-C12a 129.9(5), N1-C7-C7a 115.3(5), C7a-C8-C9 133.7(6), C8-C9-C10 122.2(6), C9-C10-C11 127.7(5), C10-C11-C12 130.5(6), C7a-C12a-C12 123.4(5), C11-C12-C12a 131.6(6).

The structure was solved by direct methods (SIR-92), and refined on F using the *teXsan* programme package. All non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were included at calculated positions with fixed isotropic thermal parameters, except for the amino hydrogen, which was refined isotropically. R = 0.053, wR = 0.056, S = 1.19 for 2017 observed data, I>3 $\sigma(I)$. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to The Director, CCDC,12 Union Road, Cambridge CB2 1EZ UK (fax: Int. Code +(1223) 336-033; e-mail: deposit@chemcrys.cam.ac.uk). *Cambridge Structural Database,* Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, England.

10. Equipment according to: Isaksson, R.; Roschester, J. *J. Org. Chem.,* 1985, **50,** 2519-2521. The eluate was analyzed by both UV (254 nm) and polarimeter (436 nm).

11. Preparation of bovine brain tubulin and microtubule associated proteins and turbidity measurements were performed according to: de Pereda, J. M.; Wallin, M.; Billger, M.; Andreu, J. M. *Cell Motil. Cytoskeleton,* 1995, **30,** 153-163.

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