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Isolation of atropisomers in both the isocolchicide and colchicide series of alkaloids and determination of their chiroptical properties

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Abstract

The isolation and dichroic behavior of atropisomeric colchicinoids is described here for the first time. The cases range from stable to rapidly interconverting atropisomeric couples. © 2000 Elsevier Science Ltd. All rights reserved.

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Natural (–)-colchicine is known to bind to tubulin, disrupting the microtubule assembly and thus becoming an important tool in cancer research. Certain aspects of the binding of (–)-colchicine to tubulin have not yet found a satisfactory rationalization, however. Thus, while it is widely agreed that tubulin discriminates the helicity of the atropisomer, the basis for assuming that the (*aR*,7*S*)-**1**[1] conformer of (–)-colchicine is required for binding rests on a comparison with (*aS*,7*R*)-**1**, where both the helicity of the atropisomer and the configuration at C7 have been inverted.[2]

Isocolchicine, which also fails to bind to tubulin, interestingly shows mutarotation in non-polar solvents,[3] a phenomenon never encountered with either colchicine or any other colchicide (=demethoxycolchicine)[4] derivative. Imaginatively, five decades ago mutarotation of isocolchicine was attributed to the existence of atropisomers, i.e. to hindered rotation around the bond that interconnects the benzene and cycloheptatrienone rings.[3] Thirty years had to elapse until the (*aR*,7*S*)-isocolchicine ((*aR*,7*S*)-**2**) and (*aS*,7*S*)-isocolchicine ((*aS*,7*S*)-**2**) conformers were detected by ^{1}H NMR in $CDCl_3$ solution,[4] while the corresponding conformers for colchicine have always defied observation.

Interest in semisynthetic colchicinoids of low general toxicity that may retain antimitotic activity has led us to isolate for the first time (*aS*,7*S*) and (*aR*,7*S*) conformers in both the above classes of compounds, without the introduction of any anchoring C7-derivatization that alters

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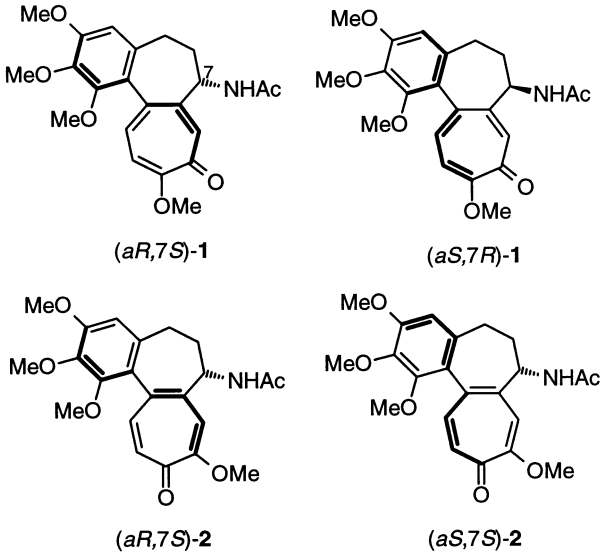
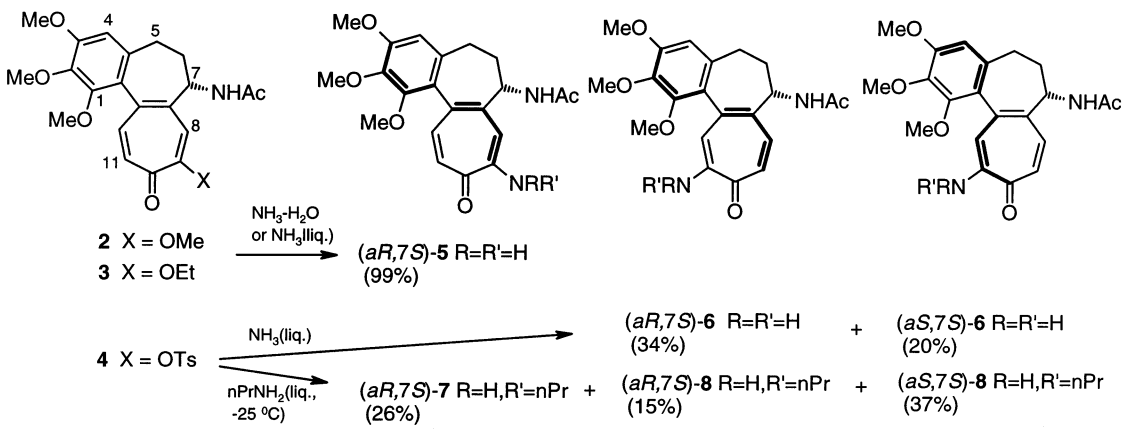


the natural conformational tendency of the system and imports new dichroic bands which obtrude chiroptical observations of the colchicinoid moiety.[5] The synthesis of amino derivatives using neat amines or ammonia as nucleophiles, which also furnished the thus far elusive C8-substituted colchicides, were keys to the discoveries. This furnished the first CD spectra for pure conformers in these series, allowing us to disentangle the contribution of the helicity versus the sp^3 chiral center.

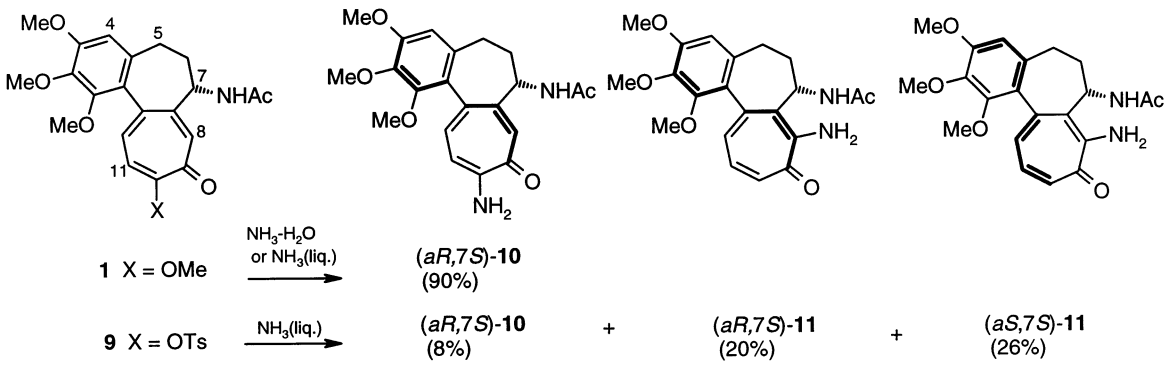
Ammonia, either as an aqueous solution or as a pure liquid, and (7*S*)-isocolchicine (**2**) or the ethoxy analog (**3**) gave (*aR*,7*S*)-9-aminoisocolchicide ((*aR*,7*S*)-**5**) in high yield (Scheme 1). In contrast, liquid ammonia and (7*S*)-9-tosyloxycolchicide (**4**) gave two isolatable conformers (*aR*,7*S*)-**6** and (*aS*,7*S*)-**6** of substitution only at C11. *n*-Propylamine and **4** gave the same pattern of reactivity, except for also furnishing the product of *ipso* substitution ((*aR*,7*S*)-**7**) and for a slightly faster change in solution of the (*aS*) form ((*aS*,7*S*)-**8**) to the (*aR*) form ((*aR*,7*S*)-**8**), which required a quicker isolation procedure (Scheme 1). While the regioisomeric pattern of



Scheme 1.

reactivity of **2**–**4** was expected from the chemistry of cycloheptatrienone analogs,[6] clean isolation of conformers in the isocolchicide series has no precedents. In the solid state (*aR*,7*S*)-**6** proved indefinitely stable. In solution (*aS*,7*S*)-**6** showed a tendency to give the (*aR*) form. Kinetics in DMSO proved to be first order, with $t_{1/2}$ 55 h at 25°C, $\Delta H^{\ddagger}$ 23.1 Kcal mol^{-1}, $\Delta S^{\ddagger}$ −5.6 e.u. Under the same conditions the transformation of (*aS*,7*S*)-**8** into (*aR*,7*S*)-**8** proved to be slightly faster, $t_{1/2} = 36.7$ h.

(7*S*)-Colchicine (**1**) and ammonia also gave the expected[6] *ipso* substitution product ((*aR*,7*S*)-**10**), while 10-tosyloxycolchicide (**9**) and liquid ammonia quite unexpectedly gave, besides (a*R*,7*S*)-**10**, two isolatable conformers (*aR*,7*S*)-**11** and (*aS*,7*S*)-**11** from substitution at C8, a position that had always resisted substitution in all previous attempts with a variety of nucleophiles and colchicinoids[7] (Scheme 2). Unlike **6** and **8**, where the (*aS*) form in solution changes irreversibly to the (*aR*) form, the (*aR*) and (*aS*) forms of **11** showed a solvent-dependent equilibrium, $K_{R/S} = 1.4$ in EtOH and <0.08 in $CHCl_3$ (equilibration between (*aS*,7*S*)-**11** and (*aR*,7*S*)-**11** in EtOH at 25°C is attained in ca. 6 h). The relative proportions of the conformers isolated as semicrystalline materials proved to be the same as in the solution from which they were obtained by evaporation. On re-dissolution of the semicrystalline material, the proportion of the two conformers which is characteristic of the solvent used was taken again. Remarkably, the non-hydroxylic solvent stabilizes the (*aS*) helicity of **11** that involves an encumbered pseudoaxial position for the acetylamino group.[1]



Scheme 2.

With both forms (*aR*,7*S*) and (*aS*,7*S*) of the **6**, **8**, and **11**[8] conformers at hand, we are now in a position to describe unambiguously for the first time the dichroic spectra of conformers in both the colchicide and isocolchicide series of alkaloids. Ethanol was used as solvent in order to extend the measurements toward the far UV region, and the temperature was lowered to 0°C to slow down the interconversions of the conformers (Fig. 1). The (*aS*,7*S*)-**6** and (*aR*,7*S*)-**6** conformers are characterized by substantially specularly-related CD spectra with a series of bands (Fig. 1A, curves a and b, respectively) that correspond to those observed in the UV absorption spectra. In the approximation of independent contributions, this suggests a negligible contribution (curve c) by the asymmetric carbon (C7) to the CD spectrum in the accessible spectral region. The specular relationship of spectra implies a specular helicity of the two conformers. The same conclusions are arrived at on examining the CD spectra for the 11-*n*-propylamino analogs (Fig. 1B, where the (*aR*) spectrum (curve b) was directly obtained from pure stable (*aR*,7*S*)-**8**, as obtained by HPLC, whereas the (*aS*) spectrum (curve a) for

(*aS*,7*S*)-**8** was calculated from an 3:2 (*aS*,7*S*)/(*aR*,7*S*) mixture **8**, as determined by ^{1}H NMR. The difference spectrum (curve c) represents the modest contribution by the chiral carbon.

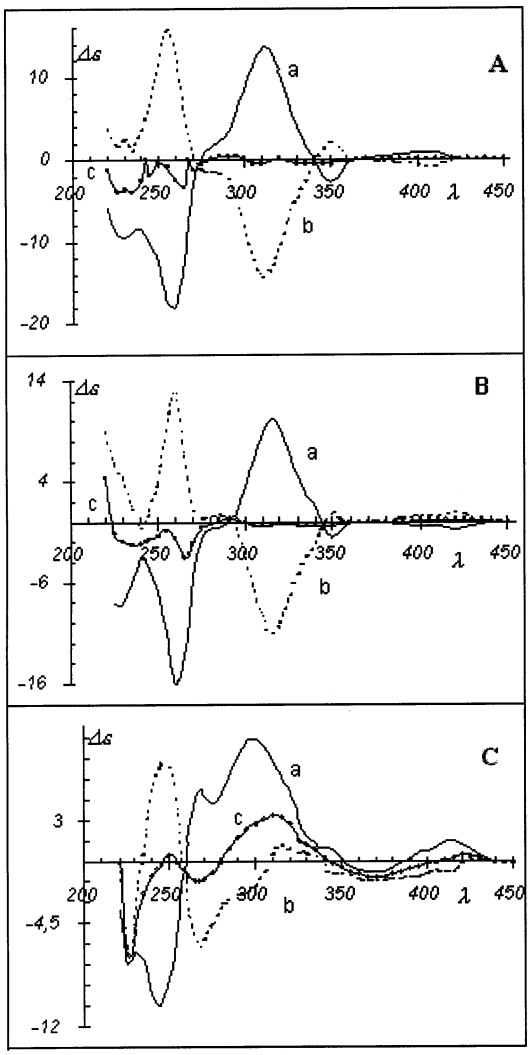


Figure 1. (**A**) Circular dichroism spectra of (*aS*,*7S*)-**6** (curve a) and (*aR*,*7S*)-**6** (curve b) in EtOH, difference spectrum (curve c). (**B**) Circular dichroism spectra of (*aS*,*7S*)-**8** (curve a) and (*aR*,*7S*)-**8** (curve b) in EtOH, difference spectrum (curve c). (**C**) Circular dichroism spectra of (*aS*,*7S*)-**11** (curve a) and (*aR*,*7S*)-**11** (curve b) in EtOH, circular dichroism spectrum of the equilibrium mixture (curve c). $\Delta\varepsilon$ (° L mol^{-1} cm), λ (nm)

In contrast, the CD spectra of (*aS*,7*S*)-**11** (Fig. 1C, curve a) and (*aR*,7*S*)-**11** (curve b)[9] are not in a specular relationship. Assuming from the above experience a modest contribution only by the chiral carbon C7, these observations may be rationalized by different dihedral angles between the planes of the benzene and cycloheptatrienone rings in the two atropisomers. Stabilization of (*aS*,7*S*)-**11** in the non protic solvent suggests the intervention of intramolecular H-bonding, which is indicated by MMX molecular mechanics calculations[10] to occur between an amino proton (or ammonium proton in the tropylium oxide canonical form) and the acetyl-

amino carbonyl group in the least strained (*aS*) conformer, while no useful distance for H-bonding resulted from calculations for the (*aR*) least strained conformers. In the MMX simulation, the H-bonding for the (*aS*) conformer induced a ca. 10^0 larger dihedral angle between the benzene and cycloheptatrienone rings, while lowering the strain energy with respect to the (*aR*) conformer. In all other cases the said dihedral angle was calculated to be the same for the two conformers, in line with the experimental observations.

This work offers biochemists new tools to fully verify with compounds of (7*S*) configuration bearing the natural acetylamino group the helicity requirements for binding to tubulin.

Acknowledgements

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8. All new compounds (**6**, **8**, and **11**) were fully characterized by HR-MS, ^{1}H and ^{13}C NMR, and UV spectra. Their (*aS*) and (*aR*) conformers were distinguished by δ_H values and *J* coupling pattern for H-7,[4,5] as follows (in $CDCl_3$): δ (^{1}H)/$J_{7,NH}$/$J_{7,pro(R)\text{-}6}$/$J_{7,pro(S)\text{-}6}$ 5.03/7.0/7.1/0, 4.60/6.1/5.3/12.2, 5.01/7.0/7.1/0, 4.59/6.2/5.3/12.0, 5.67/9.2/8.8/0, and 4.70/8.0/6.0/12 for (*aS*,7*S*)-**6**, (*aR*,7*S*)-**6**, (*aS*,7*S*)-**8**, (*aR*,7*S*)-**8**, (a*S*,7*S*)-**11**, and (*aR*,7*S*)-**11**, respectively. It should be noticed that (a*S*) conformers are characterized by $J_{7,pro(S)\text{-}6}$ ca. 0. In agreement, molecular modeling[10] for the (a*S*) conformers showed that the dihedral angle between H7 and *pro*(*S*)-H6 is close to 90°.
9. The CD spectrum of conformer (a*S*,7*S*)-**11** (Fig. 1C, line a) was measured directly from the isolate from $CDCl_3$ solution. In contrast, the CD spectrum of conformer (a*R*,7*S*)-**11** (line b) was calculated from spectrum a and c, where the latter corresponds to the final equilibrium mixture composed of 42% (*aS*,7*S*)-**11** and 58% (*aR*,7*S*)-**11**.
10. Molecular mechanics calculations were performed by the MMX force field as implemented into PCMODEL V 7.0 by Serena Software, Bloomington, Indiana.