aS,7S-absolute configuration of natural (−)-colchicine and allocongeners

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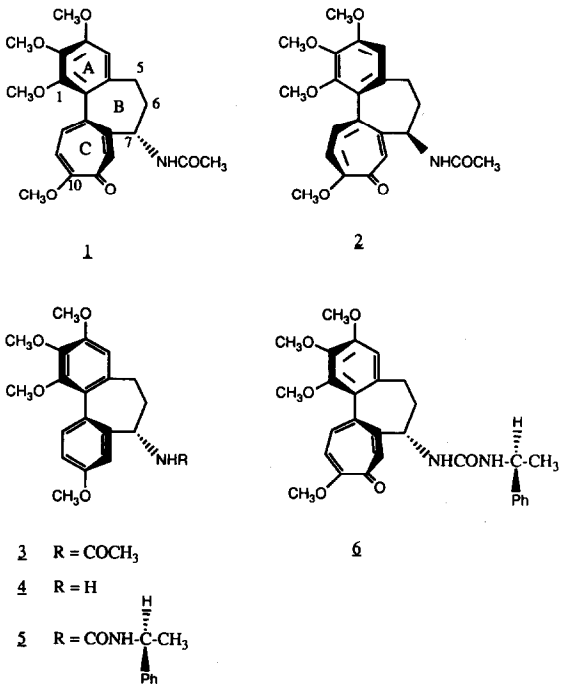
The aS,7S-absolute configuration of (−)-colchicine (**1**) and (−)-*N*-acetylcolchinol methyl ether (**3**, NCME) suggested on the basis of ^{1}H NMR data and negative Cotton effects at about 260 nm (EtOH) is firmly established by an X-ray analysis of urea **5**, a compound derived from **3**. Binding of these compounds to tubulin requires an aS-configuration of the biaryl system.

Colchicine; Allocolchicine; Tubulin binding

1. INTRODUCTION

Colchicine and several of its analogs, including compounds of the 10-thiomethyl ether series, are potent spindle toxins [1,2] with potential use in medicine [3]. (−)-*N*-Acetylcolchinol methyl ether (**3**) of the allo-series, obtained from colchicine by oxidation [4], also shows a high inhibitory effect on tubulin polymerization. All these compounds, in addition to the asymmetric carbon in C-7 position, are characterized by an axial asymmetry related to their phenyltropolonic or biaryl moiety. Consequently they may exist as an equilibrium or as isolated forms of two diastereoisomeric conformations, aS and aR ('a' means axial on the basis of rules established to assign the absolute configuration of optically active biphenyls [5]), of which only one is biologically active. It seemed important for this reason to base the proposed aS,7S-absolute configuration of natural (−)-colchicine (**1**) on firmer grounds [6]. The 7S-configuration of **1** was established by chemical degradation to an amino acid of known absolute configuration [7] and is supported by an X-ray analysis of an analog of the thio ether series measured in solid state [8]. Several X-ray structures reported for colchicine do not distinguish between aS,7S- and aR,7R-enantiomers [9,10]. The aR,7R-configuration of unnatural (+)-colchicine (**2**) was deduced from its ^{1}H NMR spectrum [3].

(−)-Colchicine (**1**) shows a negative Cotton effect at 260 nm (EtOH) in the CD spectrum, and (+)-colchicine (**2**) shows an opposite Cotton effect which originates from the skewed phenyl-tropolone systems [11,12]. Similar negative Cotton effects are reported for other colchicinoids, or analogs derived from natural colchicine, which suggests that they all have the aS-configuration [13,14]. The possible correlation of the optical properties and spectral data between (−)-colchicine (**1**) and (−)-*N*-acetylcolchinol methyl ether (**3**) was then studied. This would allow us to use the X-ray analysis of the urea (**5**) obtained from **3** to confirm the aS,7S-absolute configuration of (−)-colchicine.



1 2

3 R = $COCH_3$

4 R = H

5 R = $CONH-\overset{H}{\underset{Ph}{C}}-CH_3$

6

Fig.1. Compounds investigated.

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Table 1

Chemical shifts (ppm) of H_7 and vicinal coupling constants $J_{7,6a}$ and $J_{7,6b}$ (Hz), of (7S)-colchicine (**1**) and (7S)-*N*-acetylcolchinol methyl ether (**3**) in $CDCl_3$ solution

	aS conformation			aR conformation			% aS
	δH_7	$J_{7,6a}$	$J_{7,6b}$	δH_7	$J_{7,6a}$	$J_{7,6b}$	
1	4.50	5.5	11.0	–	not measured	–	99
3	4.66	6.2	11.7	5.15	6.5	~0	75

2. MATERIALS, METHODS AND RESULTS

Natural (−)-colchicine (**1**) was purchased from Fluka Chemical Co. (Ronkonkoma, NY), and crystallized twice from ethyl acetate. Unnatural (+)-colchicine (**2**) [15], (−)-*N*-acetylcolchinol methyl ether (**3**) [4] and (−)-colchinol methyl ether hydrochloride (**4**·HCl) [16] were prepared by the published procedures. Urea **5** was prepared from amine **4** with R-(+)-1-(phenylethyl)isocyanate [17] in dichloromethane (mp 213–214°C, $[\alpha]_D$ − 38.4° (c = 0.15, $CHCl_3$)). Urea **6** (mp 160–162°C, $[\alpha]_D$ − 59° (c = 0.15, $CHCl_3$) similarly obtained from deacetylcholchicine did not afford crystals (MeOH/EtOAc) suitable for X-ray analysis.

2.1. *^1H NMR data*

The ^1H NMR spectra were measured on a Varian XL-300 spectrometer. The critical vicinal coupling constants ($J_{7,6a}$ and $J_{7,6b}$) of **1** and **3** in $CDCl_3$ solution are listed in table 1. The (7S)-*N*-acetylcolchinol methyl ether (**3**), exhibits an NMR spectrum consisting of two sets of signals in a 3:1 ratio, indicating the presence of two conformers. In the major conformer, the proton at C(7) shows vicinal coupling constants of $J_{7,6a}$ = 6.2 Hz and $J_{7,6b}$ = 11.7 Hz revealing dihedral angles between H_7,H_{6a} and H_7,H_{6b} of approximately 45° and 170°, respectively. These NMR data are consistent with a structure showing the acetamido group at C(7) in **3** to be equatorially oriented, with the biaryl moiety adopting an aS configuration. The minor component exhibits a spectrum with critical coupling constants of $J_{7,6a}$ = 6.5 Hz (table 1) and $J_{7,6b}$ ~ 0 Hz. These data reveal dihedral angles of approximately 35° and 85°, respectively, with the acetamido group in an axial orientation and the biaryl moiety in the aR configuration. The aS/aR equilibrium of **3** varies with solvent and temperature. For example, the aS conformer increases from 75% in $CDCl_3$ to 96% in acetone-d_6 as measured by NMR. As expected, an increase in optical rotation from −64.6° to −94.5° accompanied the solvent change. These measurements enable us to attribute a partial specific rotation of −71° to the biaryl system in compound **3**, and of −29.2° to the chiral carbon atom at C(7), respectively.

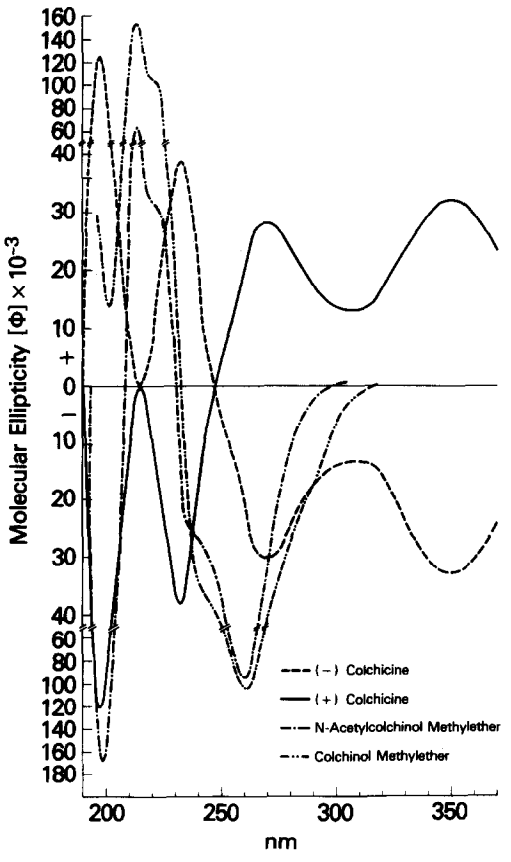

Fig.2. CD spectra of colchicine and its analogs in ethanol.

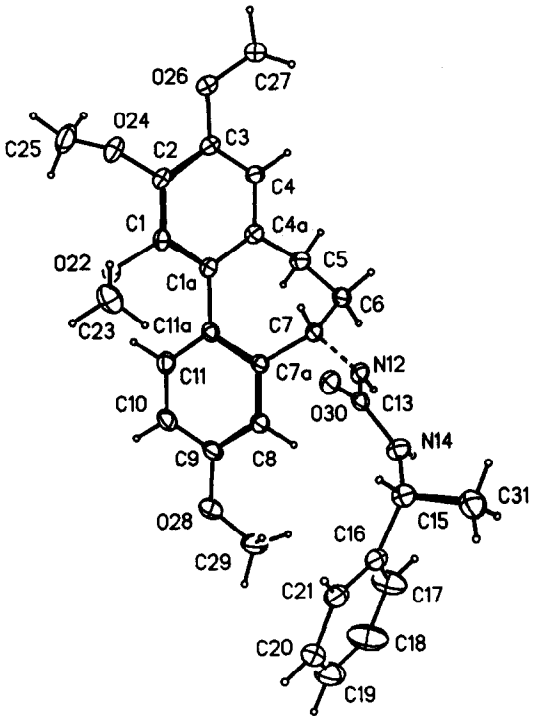

Fig.3. X-ray analysis of urea (**5**). Themal ellipsoid plot from experimentally determined coordinates.

The presence of aS and aR conformers and their interconversion has been reported in colchicinoids [3] and in (−)-androbiphenyline [18]. The NMR spectrum of natural (7S)-colchicine (**1**), however, shows that it exists predominantly as an aS conformer (>99%) in both acetone-d_6 and $CDCl_3$. Unnatural (7R)-colchicine (**2**), with its acetamido group also in equatorial orientation, on the other hand, adopts the aR conformation.

2.2. *CD spectral analysis*

The CD spectra were recorded (if not otherwise stated) in ethanol at normal temperature on a Jasco model J-500A recording spectropolarimeter, equipped with a model DP-500N data processor and an IBM BC-XT computer. The results are summarized in fig.2.

The CD spectrum of (−)-colchicine (**1**) is, as expected, a mirror image of that of (+)-colchicine (**2**). As reported in the literature for **1** [14], they exhibit up to seven CD bands (including inflections and shoulders) between 400 and 190 nm, the probable origin of which was discussed. The CD spectrum of (−)-colchicine (**1**) was also measured

in chloroform. In this solvent the spectral pattern is similar to the one in ethanol and only minor shifts in the position and in the intensity of the CD were observed due to the differences in the solvent polarities. The CD-spectra of compounds **3**, **4**, and **5** of the allo-series are analogous and exhibit multiple Cotton effcts, including shoulders and inflections, between 350 and 190 nm. In all 3 cases, there is a strong negative CD-band at about 260 nm, in agreement with the indicated aS-configuration of the biphenyl chromophore. The molecular ellipticities when measured for urea **5** in EtOH were as follows: 760 (292 nm), −95.000 (261 nm), − 30.000 (240 nm), −32.000 (237 nm).

2.3. *X-ray analysis of 1-phenylethylurea* **5**

Compound **5** crystallized in the trigonal space group R3, with cell dimensions $a = b = 22.702(2)$, $c = 13.322(2)$ A. Vol. = 5946.0(1.3) A3, Z = 9, $d_{calc} = 1.201$ g/cm^3, λ(Cu Kα) = 1.54184 A, μ = 0.66 mm^{-1}, T = 295 K. The final R values are R = 0.033 and wR = 0.038 for the 1907 independent observed reflections. The goodness-of-fit parameter was 1.55 and the final difference map was featureless. Bond distances and angles are all within the normal range. The results of the X-ray analysis are shown in fig.3 and confirm the aS,7S,15R-absolute configuration of urea **5**. The mole ratio of water to **5** was 0.05:1. Tables of coordinates and bond distances and angles have been deposited with the Crystallographic Data Centre, Cambridge University, University Chemical Lab., Cambridge, England.

3. CONCLUSIONS

Natural (−)-colchicine (**1**) and derived (−)-*N*-acetylcolchinol methyl ether (−)-NCME (**3**) show negative Cotton effects at 260 nm (MeOH) which marks them as aS-configurated biaryls. This is supported for (−)-NCME (**3**) of the allo-series with an X-ray analysis of urea **5** which shows that it has the aS,7S,15R-configuration. All biologically active colchicinoids and compounds derived from colchicine including allo-compounds show strong negative specific rotations, suggesting that the aS-configuration of the biaryl system is a requirement for their interaction with the protein [6,19].

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