Conformational Isomerism and Its Relation to the Mutarotation of Isocolchicine

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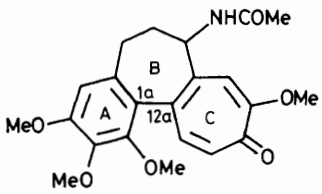
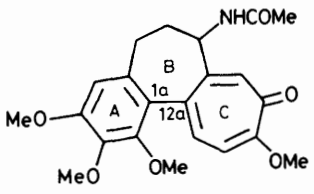
The structures of two atropisomers of isocolchicine that are present in mutarotated solutions have been determined utilizing 500 MHz ^{1}H n.m.r. data.

It is known that the optical rotation in chloroform solution of isocolchicine (**1**), but not colchicine (**2**), changes with time, although neither compound undergoes mutarotation in ethanol or other polar solvents.[1] Rapoport and Lavigne[2] suggested that mutarotation of (**1**) resulted from a hindered rotation between rings A and C which implies the existence of stable conformers in solution. Isocolchicine, which differs in structure from the powerful mitotic spindle inhibitor colchicine by the shifting of double bonds and the interchange of the keto and adjacent methoxy groups, shows little of the biological activity of colchicine (binding to tubulin, inhibiting mitosis, or relieving gout).[3] It has been suggested that conformational changes occur in (**2**) upon binding of the drug to tubulin.[4] Subtle differences in the shapes and stabilities of these isomers could have a bearing on their binding interactions.

Preliminary investigation of the isocolchicine mutarotation, using 200 MHz ^{1}H n.m.r. spectroscopy, indicated the presence of two conformers in a 10:1 ratio at room temperature. Because of the overlapping methylene resonances and the lack of separation of weak signals from the minor conformer it was necessary to obtain data at higher field



(**1**)



(**2**)

strength. All the couplings needed to establish the conformation of the major isomer and most of those of the minor isomer have now been derived from 500 MHz spectra. The presence of two conformations of ring B in (**1**), separated by a moderately high barrier, is consistent with these observations.

Examination of the ^{1}H n.m.r. spectrum of (**1**) in $CDCl_3$ (Tables 1 and 2) established that the major isomer has the same conformation in solution as in the crystalline state.[6] In this conformation (I), the dihedral angle between the planes defined by the A-ring and carbons C-7a, C-8, C-12a, and C-12 of the methoxytropone C-ring is *ca.* +53° (assuming a planar

Table 1. ^{1}H N.m.r. chemical shifts of isomeric isocolchicines (**1**).[a]

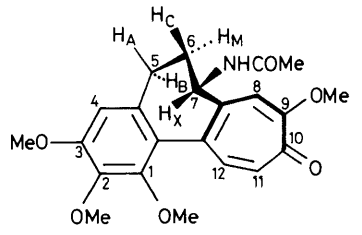
	4-H[b]	*pro*(*R*) 5-H (B)	*pro*(*S*) 5-H (A)	*pro*(*R*) 6-H (C)	*pro*(*S*) 6-H (M)	7-H (X)	8-H[b]	11-H	12-H	1-MeO	2-MeO	3-MeO[f]	9-MeO[f]	NHCO*Me*[g]	N-H
(I)	6.56	2.36(dt)	2.51(q)	2.30(tt)	2.02(dt)	4.63(p)	7.07[e]	7.15(d)	7.42(d)	3.67	3.93	3.90	3.96	2.06	6.33(d)[e]
(II)[c]	6.64	—	2.58(dt)[d]	2.70(dtd)	2.15(m)[d]	5.00(t)	6.86	7.16(d)	7.33(d)	3.67	3.94	3.91	4.01	1.63	5.03(d)

[a] Spectra obtained in $CDCl_3$ at 500 MHz. Sample concentration was 2.1×10^{-2} M. T = 23 °C. [b] Assigned according to ref. 5. [c] The A, B, C, and M labels for 5-H and 6-H of (I) are arbitrarily retained for (II). [d] These assignments are indefinite. [e] Concentration dependent (see Table 3). [f] Assigned by nuclear Overhauser effect (n.O.e.) difference measurements. [g] Another *N*-acetyl resonance, which was 10—15% as intense as the NHAc signal of (II), was observed at δ 1.70 due to an unknown conformer.

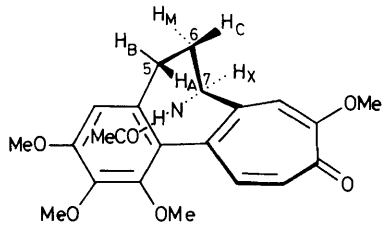
Table 2. Coupling constants (Hz) of isomeric isocolchicines (**1**).

	$J_{5A,5B}$	$J_{5A,6C}$	$J_{5A,6M}$	$J_{5B,6C}$	$J_{5B,6M}$	$J_{6C,6M}$	$J_{6C,7}$	$J_{6M,7}$	$J_{7,NH}$	$J_{11,12}$
(I)	−13.5[a]	6.5[a]	*ca.* 0[a]	13.0[a]	6.5[a]	−12.5[a]	6.5[a]	12.5[a]	7.0[b]	13.0[b]
(II)[b]	—	7.5	—	*ca.* 0	—	−14.5	7.5	*ca.* 0	7.5	13.0

[a] The experimental spectra of (I) at both 200 and 500 MHz were confirmed by simulation using these couplings. [b] Couplings determined by inspection.



(I)



(II)

c-ring). The B-ring proton resonances of (I) constitute an A, B, C, M, X five-spin system. Noteworthy features of the spectrum are the zero coupling between 5-H_A and 6-H_M (indicating an angle of *ca.* 90° between these protons) and the upfield shifts of 6-H and the 7-H resonances relative to those of the minor conformer (II).

An atropisomeric transformation, such as (I) ⇄ (II), changes the chirality of the pseudobiaryl moiety (C-1a, C-12a bond) from *R*, (I), to *S*, (II), [yielding a dihedral angle of *ca.* −53° between rings A and C in (II)] and places the C-7 acetamido group in the pseudoaxial position. This conformational change is accompanied (a) by an upfield shift of the N-H resonance owing to its placement below the shielding cone of ring A and its inaccessibility for dimer formation (*vide infra*) and (b) by a 0.4 p.p.m. downfield shift of 7-H upon its removal from the shielding cone of ring A. The signal arising from the *N*-acetyl group in (II) was shifted upfield, relative to that of (I) reflecting its proximity to the shielding cone of ring A. Further evidence for the ring flip about the C-1a, C-12a axis is provided by the change of $J_{5B,6C}$ from 13.0 Hz to *ca.* 0 Hz; these values are consistent with a change of angle between the two protons from *ca.* 180° to *ca.* 90° and would be predicted from the molecular models.

Table 3. Effect of concentration upon the chemical shifts of N-H and 8-H resonances of (I).[a]

Conc. (M)	δ N-H		δ 8-H	
	23 °C	90 °C	23 °C	90 °C
3.4×10^{-1}	8.01 (4.90)[b]	—	7.29	—
6.8×10^{-2}	6.54 (5.04)	5.99	7.02	6.97
6.8×10^{-3}	5.95 (5.03)	5.69	6.91	6.91
6.8×10^{-4}	5.89 (5.03)	—	6.90	—

[a] Spectra obtained in $CDCl_3$ at 200 MHz. [b] The chemical shifts in parentheses represent values for (II).

The 23.7 kcal/mol† activation energy reported[2] for the isocolchicine isomerization agrees with that observed[7] for biaryl systems that undergo atropisomerization. At temperatures below 20 °C, (II) was not detected in $CDCl_3$ solutions of (**1**) that had been kept for several hours. Equilibration of (**1**) at 23 °C in either $CDCl_3$ or [$^2H_2$]tetrachloroethane ([$^2H_2$]TCE) gave a 10 : 1 mixture of (I) and (II) concomitant with changes of optical rotation at 589 nm of 315.0 to 239.5° in $CHCl_3$ and 324.8 to 261.6° in [$^2H_2$]TCE. Equilibration of (**1**) in [$^2H_2$]TCE at temperatures ranging from 60 to 140 °C gave 12—22% of the minor conformer, (II). The two-fold difference in the amount of (II) formed at temperatures of 23 to 140 °C leads to $\Delta G°$ values for the (I) ⇄ (II) reaction of *ca.* 1.0—1.3 kcal/mol.

Concentration dependence of the chemical shift of the N-H, and to a lesser extent that of 8-H, indicates aggregation of the major isomer (I), whereas a five hundred-fold dilution of (II) had no appreciable effect on the chemical shift of the N-H (Table 3). Cryoscopic measurements on 4.0×10^{-2} M solutions of isocolchicine have shown the presence of dimers.[2] In a solution of similar concentration the N-H signal occurred at δ 6.54. The chemical shift of the N-H signal of (I) at 90 °C (6.8×10^{-3} M) (δ *ca.* 5.7) probably results from essentially monomeric (I) while the slightly downfield values (δ *ca.* 5.9—6.0) of the room temperature solutions of (I) (6.8×10^{-3}—6.8×10^{-4} M) indicate the presence of some dimers. It has been suggested that the failure of isocolchicine to mutarotate in polar solvents reflects a higher activation energy owing to an increased size of the acetamido group upon solvation.[2]

† 1 kcal = 4.18 kJ.

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