

CIRCULAR DICHROIC SPECTRA AND IONIZATION PROCESSES OF SOME
TROPOLONIC ALKALOIDS

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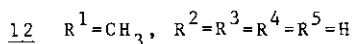
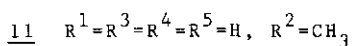
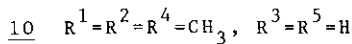
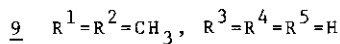
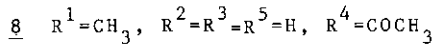
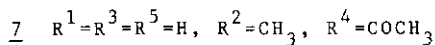
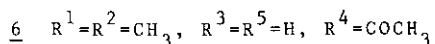
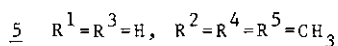
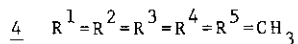
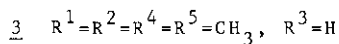
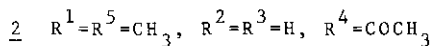
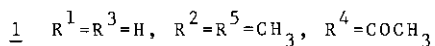
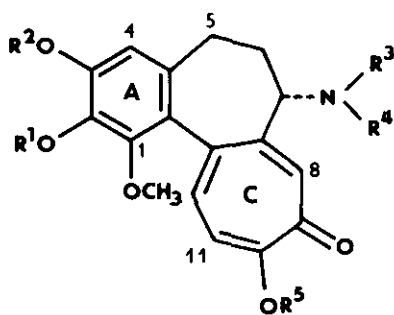
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Abstract - Some tropolonic alkaloids have been investigated by circular dichroic (CD) spectroscopy in dependence on pH. Their pK_a values in water-ethanol mixture (1:1) are given. Molar circular dichroism values ($\Delta\epsilon$) are reported for all the measured alkaloids. The pH-depending changes in CD spectra are observed in demethylcolchicines and colchiceines. In the former they are associated with the ionization of phenolic groups in aromatic ring A, and in the latter with conformational changes of tropolonic ring C due to ionization of hydroxyl group at C-10.

Tropolonic colchicine alkaloids occur only in the plants of the family Liliaceae.¹ They are usually classified as neutral and phenolic compounds (with N-acetoamido function at C-7 and methoxyl or hydroxyl substituents on ring A) or basic compounds (with amino function at C-7).² Colchiceines are keto enols with a 10-hydroxyl instead of a 10-methoxyl substituent. All naturally occurring tropolonic alkaloids possess one chiral center at C-7, and its absolute configuration is S.³ The conformation of colchicine was studied in the crystal by X-ray crystallography⁴ and established in solution by ¹H and ¹³C NMR spectroscopy.⁵ Colchicine and its congeners inhibit cell processes depending

on microtubule function.⁶ Recently, it has been suggested that colchicine binds to tubulin in a skewed conformation arrived at by twisting the trisubstituted ring A around the phenyl-tropolone axis.⁷ The substituents on ring A and at C-7, together with carbonyl group at C-9, were proved to be important for biological activity of tropolonic alkaloids.¹



In our previous work we discussed the chiroptical properties of tropolonic alkaloids in relation to stereochemistry.⁸ Six dichroic bands (A-F) can be distinguished in their CD spectra. The most characteristic features are the negative Cotton effects (CE) in the 360-260 nm region. In the CD spectrum of colchicine, the first negative CE at 351 nm appears to result from $\pi \rightarrow \pi^*$ transition of methoxytropolone system, the second one at 274 nm arises from electric dipole coupling of the transition moments associated with 1L_b transitions of biaryl system.⁸ In the present work we have studied the effect of ionization on the CD spectra and stereochemistry of some tropolonic alkaloids.

The ionization constants of alkaloids 1-12 are given in Table 1.⁹ Table 2 presents the wavelength parameters and molar circular dichroism values for

Table 1. The pK_a Values in Water-Ethanol (1:1) and Protonation Sites for Studied Alkaloids

Alkaloid	pK_a	Protonation Sites
2-Demethylcolchicine (<u>1</u>)	10.50 ± 0.05	O-2
3-Demethylcolchicine (<u>2</u>)	9.65 ± 0.03	O-3
Demecolcine (<u>3</u>)	5.72 ± 0.06	N-7
N-Methyldemecolcine (<u>4</u>)	5.25 ± 0.03	N-7
2-Demethyldemecolcine (<u>5</u>)	5.70 ± 0.06 10.34 ± 0.03	N-7 O-2
Colchicine (<u>6</u>)	7.18 ± 0.06	O-10
2-Demethylcolchicine (<u>7</u>)	7.12 ± 0.04 10.30 ± 0.07	O-10 O-2
3-Demethylcolchicine (<u>8</u>)	7.10 ± 0.03 10.16 ± 0.06	O-10 O-3
N-Deacetylcolchicine (<u>9</u>)	5.49 ± 0.04 7.37 ± 0.03	N-7 O-10
Demecolcine (<u>10</u>)	5.38 ± 0.06 7.90 ± 0.02	N-7 O-10
2-Demethyl-N-deacetylcolchicine (<u>11</u>)	6.03 ± 0.05 10.26 ± 0.07	N-7 and O-10 O-2
3-Demethyl-N-deacetylcolchicine (<u>12</u>)	5.93 ± 0.04 10.06 ± 0.05	N-7 and O-10 O-3

the studied compounds in different ionization states.¹⁰ Neutral forms of 2-demethylcolchicine (1) and 3-demethylcolchicine (2) give similar CD curves (Table 2). Ionization of hydroxyl groups is accompanied with the shift of the longest wavelength CE from 345-350 to 380-405 nm (Fig. 1). In 2-demethyl derivative 1, the ionization of the phenolic group influences only slightly the position of CE at 350 nm. In the UV spectra, the difference between the ionized and nonionized forms manifests only in 3-demethyl derivative 2 by bathochromic shift of the longest absorption band

Table 2. Dichroic Bands of Tropolonic Alkaloids in Different Ionization States in Water-Ethanol (1:1)

Alkaloid	pH	CD, maxima: λ nm ($\Delta\epsilon$)					
		A	B	C	D	E	F
<u>1</u>	4.0 ^b	380(-2.5) ^a	350(-6.3)	282(-6.0) ^a 266(-6.5)	250(0.6) ^a	234(11.0)	
	12.2 ^c	404(-2.8)	345(-5.6)	288(-7.1)		240(15.0)	219(-4.2)
<u>2</u>	4.0 ^b		348(-7.9)	273(-6.3)	254(-0.6)	230(10.0)	212(-0.4)
	12.2 ^c	387(-5.9)		312(-1.8)	258(-8.8)	230(7.8)	213(10.0)
<u>3</u>	7.8 ^b		342(-6.8)	280(-5.8)	250(8.6) ^a	232(22.5)	211(-4.6)
	4.0 ^e	390(1.0)	360(-6.2) ^a 340(-8.3)	280(-6.0)		232(26.0)	
<u>4</u>	7.8 ^b		346(-6.2)	282(-4.5)	254(4.4) ^a	233(24.0)	212(-10.0)
	4.0 ^e	393(1.3)	344(-5.9)	277(-4.6)	262(-3.0) ^a	232(18.0)	212(-1.4)
<u>5</u>	7.8 ^b		350(-5.6)	286(-4.1)	248(9.8) ^a	233(22.3)	208(-6.7)
	4.0 ^e		342(-5.6)	280(-3.6) ^a 265(-3.8)		232(22.3)	208(-1.2)
	12.2 ^c	404(-3.1)	343(-4.9)	282(-6.9)		240(27.0)	208(-11.0)
<u>6</u>	4.0 ^b		340(-11.6)	279(-4.6)	251(5.6) ^a	235(12.0)	216(-3.2)
	12.2 ^d	402(5.2)	376(-9.6) ^a 346(-16.4)	279(-5.2) ^a	265(-11.6)	237(-6.0)	
<u>7</u>	4.0 ^b		340(-11.3)	274(-3.2)	253(3.9) ^a	239(12.6)	221(-8.1)
	12.2 ^{c, d}	409(3.2)	377(-7.7) ^a 349(-12.9)	283(-2.9) 270(4.8)	260(3.5)	235(-17.7)	

<u>9</u>	4.0 ^e		342(-10.7)	281(-3.4)		234(18.8)
	6.5 ^{d, e}	406(3.8)	364(-9.4)	276(-4.4)		235(19.2)
			347(-11.8)	268(-3.8) ^a		
<u>10</u>	12.2 ^{c, d}	403(4.8)	372(-3.3)	276(-5.8) ^a		239(16.1)
			342(-12.3)	267(-7.7)		
	4.0 ^e		343(-9.8)	284(-3.0)	256(7.2) ^a	233(16.3)
<u>11</u>	7.8 ^d	405(5.4)	362(-7.0) ^a	277(-5.4) ^a		243(14.4)
			347(-11.7)	268(-6.8)		
<u>11</u>	4.0 ^e		345(-9.8)	280(-2.4)		236(18.6)
	7.8 ^d	406(4.2)	367(-7.8)	284(-2.4)	264(3.0) ^a	236(15.7)
			348(-9.8)			
<u>12</u>	12.2 ^{c, d}	409(0.4)	368(-7.6) ^a	284(-2.7)		249(14.2)
			348(-10.3)			
	4.0 ^e		343(-9.7)	284(-2.9)	252(8.5) ^a	227(16.9)
<u>12</u>	7.8 ^d	405(4.1)	364(-8.2)	279(-3.3)	264(-1.0) ^a	234(13.5)
			348(-10.5)			
<u>12</u>	12.2 ^{c, d}		390(-6.4) ^a	280(-0.8)	264(2.8) ^a	239(10.0)
			359(-10.5)			

^a Shoulder. ^b Neutral form. ^c Ionization of phenolic hydroxyl group. ^d Ionization of tropolonic hydroxyl group. ^e Ionization of nitrogen atom.

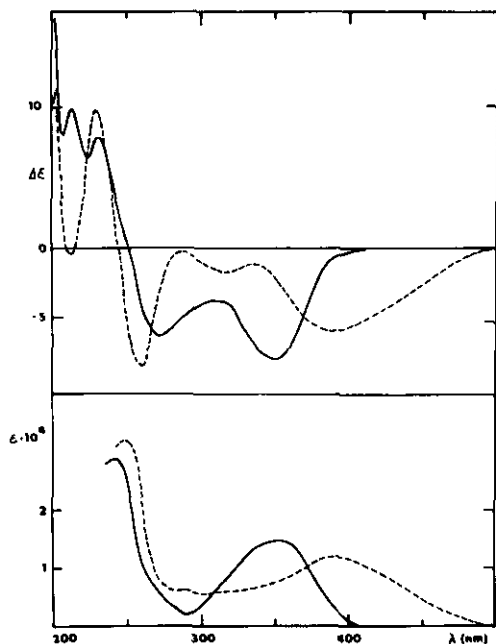


Fig. 1. CD and UV spectra of 3-demethylcolchicine (2) in the mixture water-ethanol (1:1) at pH 4 (—) and pH 12.2 (---)

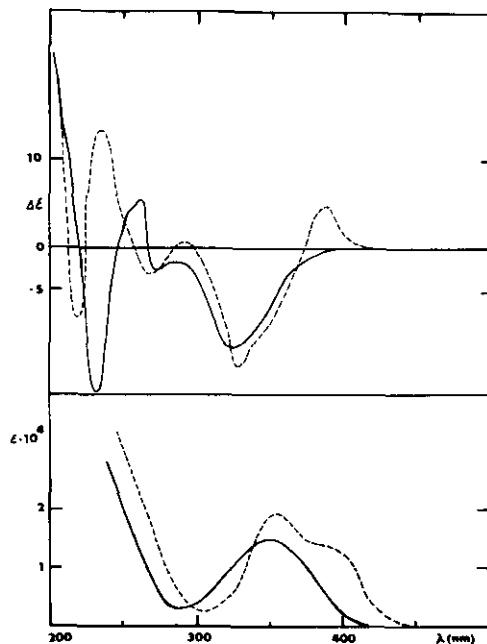


Fig. 2. CD and UV spectra of 2-demethylcolchicine (7) in the mixture water-ethanol (1:1) at pH 4 (—) and pH 12.2 (---)

by 38 nm (Fig. 1). Ionization of amino group at C-7 in N-deacetyl derivatives 3-5 is not associated with significant changes in CD and UV spectra (Table 2). Ionization of the hydroxyl group at C-10 (tropolone ring C) in colchicine (6) and its derivative 7 (Fig. 2) causes hyperchromic and bathochromic shifts of the longest wavelength absorption band in the UV spectra. In the CD spectra, the CEs associated with the short wavelength absorption bands reverse their signs after ionization, and a new positive CE appears at 410-400 nm. These phenomena can be interpreted as a change in conformation of tropolone ring C due to negatively charged oxygen atom or keto-enol tautomerism. In colchicine derivatives with amino or N-methylamino functions at C-7 (9-12), only a new positive CE at 405 nm appears after ionization of 10-hydroxyl group (Table 2). The change of sign of the intensive CE in the 240-235 nm region is characteristic

only for colchiceines with N-acetamido function (6 and 7).

This work demonstrates that ionization of phenolic groups leads to shifts of CEs associated with the longest wavelength absorption band. Ionization of tropolonic hydroxyl group induces a conformational change of tropolonic ring C accompanied with significant alteration of the CD spectra. The pH-depending changes in CD spectra of tropolonic alkaloids are more discernible than in UV spectra. Therefore, the CD spectroscopy is suitable for differentiation between colchicine and colchiceine derivatives.

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9. The ionization constants were measured spectrophotometrically. The pH values of the samples were adapted with citrate, triethanolamine, glycine buffers, different concentrations of perchloric acid, and sodium hydroxide, resp. All absorbance and pH measurements were made at 25°C and ionic strength 0.1. The pH values were measured with a glass and a silver/silver chloride electrode filled with 0.1 mol/l NaCl in water-ethanol (1:1).
10. CD spectra were measured at 25°C on Jobin-Yvon Mark V Dichrographe equipped with Apple II microcomputer and with a thermostated cuvette compartment using quartz cells of 0.1-1.0 cm pathlength.

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