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C-10 AMINO ACID DERIVATIVES OF COLCHICINE

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The synthesis has been effected of C-10 N-acylated amino acid derivatives of colchicine by condensing (β-aminoethylamino)colchicide with N-acylated amino acids. The structures of the compounds obtained have been confirmed by their UV and PMR spectra and by thin-layer chromatography.

The modification of the chemical structure sof biologically active compounds permits their physiochemical and biological properties to be changed in a desired direction. Recently, in order to lower toxicity, ever-increasing use has been made of the method of conjugating known substances having a high antitumoral activity with such natural compounds as proteins, amino acids, and carbohydrates [1, 2]. As has been shown previously, on the condensation of deacetyl vinblastine with amino acid esters vinblastine derivatives possessing antitumoral activity and less toxic than the initial alkaloid are obtained [3]. Amino acid derivatives of ellipticine have also proved to be more effective than the initial alkaloid [4].

The study of the biological action of dipeptide derivatives of colchicine that we had synthesized previously on a culture of X63A 8-653 cells has shown that on the conjugation of colchicine with various amino acids their cytoxicity decreases [5, 6]. A dependence of the cytotoxicity on the nature of the amino acid residue is observed [6]. In view of this, the aim of the present work was to synthesize several N-acylamino acid derivatives of colchicine.

To obtain the amino acid derivatives of the alkaloid we first synthesized (β-aminoethylamino)colchicide by the nucleophilic replacement of the C-10 methoxy group of the alkaloid by an ethylenediamine group. This led to two compounds, with R_f 0.33 (system B) (IIa) and R_f 0.10 (system B) (IIb). Compounds (IIa) and (IIb) were isolated by chromatography on a column of ALugram silica gel (Macherey—Nagel) in a stepwise concentration gradient of ethanol in chloroform. The UV spectra of each compound, like the spectrum of colchicidylglycine and aminocolchicide, had adsorption maxima at 252, 355, and 408 nm (Table 1). Treatment of a chromatogram with a 0.2% alcoholic solution of ninhydrin revealed that compound (IIb) (E_f = −0.52 relative to picric acid) confirmed the presence of a free amino group in its structure. The NMR spectra of these compounds contained all the signals characteristic for the protons of colchicine apart from the C-10 methoxy group at 4.08 ppm. The results obtained permitted compound (IIa) to be identified as N,N'-dicolchicidylethylenediamine, and compound (IIb) as (β-aminoethylamino)colchicide. The N-actylated amino acid derivatives of colchicine were obtained by the scheme shown on the following page.

To study the influence of the nature of the amino acid residue on the biological properties of the alkaloid we used the following N-substituted amino acids (where F represents a formyl group and Ac an acetyl group); F-glycine (1), F-L-leucine (2), F-L-tryptophan (3),

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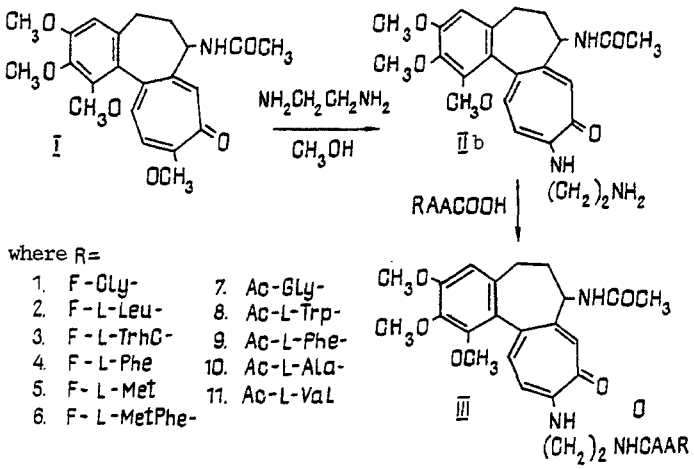
TABLE 1. UV Spectra and Results of Thin-Layer Chromatography

Compound	UV spectra, nm			TLC in system	
	m	max/lg ε	m	A	B
(I) Colchicine	245	352/4,22	408	0,66	0,8
(IIa) N,N'-Dicolchicidylethylenediamine	252	355	408		0,83
(IIb) (β-Aminoethylamino)colchicide(AEACol)	252	355/4,22	409		0,10
1. For-GlyAEACol	250	355/4,20	409	0,60	0,15
2. For-L-LeuAEACol	251	354/4,40	409	0,63	0,22
3. For-L-TrpAEACol	252	353/4,54	409	0,58	0,20
4. For-L-PheAEACol	251	354/4,49	408	0,65	0,18
5. For-MetAEACol	249	353,4,29	409	0,32	0,06
6. For-L-MetPheAEACol	247	352/4,25	408		0,10
7. Ac-GlyAEACol	253	353/4,25	409	0,54	0,17
8. Ac-L-TrpAEACol	253	354/4,51	409	0,54	0,15
9. Ac-L-PheAEACol	250	354/4,34	408	0,52	0,14
10. Ac-L-AlaAEACal	251	354/4,33	409	0,67	0,21
11. Ac-L-ValAEACol	251	354/4.41	409	0.43	0.07



F-L-phenylalanine (4), F-L-methionine (5), F-L-methionylphenylalanine (6), Ac-L-glycine (7), Ac-L-tryptophan (8), Ac-L-phenylalanine (9), Ac-L-alanine (10), and Ac-L-valine (11). The condensation of (β-aminoethylamino)colchicide with the carboxy groups of the N-acylated amino acids was achieved by the carbodiimide method in dimethylformamide [7].

All the compounds obtained were isolated by preparative thin-layer chromatography. Their structures were confirmed by their UV and NMR spectra and by TLC analysis (Tables 1 and 2) and their electrophoretic mobilities. The UV spectra of compounds (III, I-II) were identical with the spectra of (IIa) and (IIb) (Table 1). The PMR spectrum of compound (III, I-II contained all the signals characteristic for colchicine, namely: at 2.16 ppm for the acetyl group of the alkaloid, and at 3.71-3.75, 3.96-4.02, and 4.02-4-04 ppm – for the C-1, C-2, and C-3 methoxy groups, respectively. In each of the spectra of compounds (III, 8-11), in addition to the signals mentioned, singlet signals were observed at 1.96 and 2.10 ppm, which are characteristic for the N-acetyl group in an amino acid resiue. The signal of the formyl proton in compounds (III, 2-4) was observed in the weak-field region at 8.29-9.6 ppm. The signals of the aromatic proton of the tryptophan, phenylalanine, and tyrosine residues were observed in the 6.50-7.70 ppm interval, and in compounds (III, 3, 4, 8) in the 6.50-8.28 ppm interval (Table 2).

EXPERIMENTAL

Colchicine obtained form the Batumi Pharmaceutical factory was used. TLC was conducted on Alugram plates with a fixed layer of silica gel (Macherey–Nagel, Germany) in the following solvent mixtures: A) n-butanol–acetic acid–water (4:1:1); and B) chloroform–ethanol–ammonia (90:9:1). The electrophoretic analysis of the compounds obtained was conducted on FN-16 paper in 0.02 M NH_4HCO_3 (pH 7.5) and in acetic acid solution, pH 2.5 (800 V, 22 V/cm) using picric acid as marker. UV spectra were recorded in ethanol on a Specord M-40 spectrophotometer, and PMR spectra (in $CDCl_3$ for compounds (I), (IIb), and (III, 2, 3, 4a, 8, and 11), and CD_3OD for (IIa)) on a Varian XL-100 instrument (USA) using hexamethyldisilazane as internal standard.

TABLE 2. Parameters of the PMR Spectra of Colchicine and its Derivatives

Compound (solvent)	$COCH_3$	C-1, OCH_3	C-2, OCH_3	C-3, OCH_3	Aromatic protons	N-acetyl	N-formyl
(I) Colchicine ($CDCl_3$)	2,16 (3H, s)	3,75 (3H, s)	4,02 (3H, s)	4,04 (3H, s)	6,50—7,58		
(II) · a (CD_3OD)	2,16 (3H, s)	3,71 (3H, s)	4,02 (3H, s)	4,04 (3H, s)	6,81 - 7,59		
(III) · 2 ($CDCl_3$)	2,16 (3H, s)	3,72 (3H, s)	4,00 (3H, s)	4,04 (3H, s)	6,60—7,53		8,29 (1H, s)
3 ($CDCl_3$)	2,16 (3H, s)	3,73 (3H, s)	4,00 (3H, s)	4,04 (3H, s)	6,60—7,86		8,87 (1H, s)
4 ($CDCl_3$)	2,16 (3H, s)	3,72 (3H, s)	3,99 (3H, s)	4,04 (3H, s)	6,60—7,66		9,67 (1H, s)
8 ($CDCl_3$)	2,16 (3H, s)	3,73 (3H, s)	3,96 (3H, s)	4,02 (3H, s)	6,60—8,28	1,96 (3H, s)	
11 ($CDCl_3$)	2,16 (3H, s)	3,72 (3H, s)	3,99 (3H, s)	4,04 (3H, s)	6,60—7,86	2,10 (3H, s)	

(N-β-Aminoethylamino)colchicie (IIb). A solution of 800 mg (2 mmole) of colchicine in 3 ml of ethanol was treated with 0.650 ml (10 mmole) of freshly distilled ethylenediamine. The mixture was boiled in a sealed tube [sic] for 20 min and was left at room temperature for 15-18 h. Then the tube was opened, and the ethanol was distilled off in vacuum. The dry residue was dissolved in 1 ml of chloroform and was deposited on a column (1.6 × 20 cm) containing Macherey–Nagel silica gel. The unchanged colchicine was eluted with chloroform. The reaction products were eluted in a 0-10% gradient of ethanol in chloroform. Substance (IIb) (R_f 0.33, B) was eluted with chloroform–ethanol (9:1), and substance (IIa) (R_f 0.10, B) with chloroform–ethanol (7:3). The solvent was distilled off in vacuum. In each case, the residue was triturated under dry ether and was dried in vacuum at 40°C. Both substances were light yellow in color and were readily soluble in chloroform, alcohol, and dimethylformamide, and sparingly soluble in water.

Condensation of (β-Aminoethylamino)colchicide with N-Acylated Amino Acids. A solution of 0.2 mmole of a N-acylated amino acid in 1 ml of dimethylformamide was treated with 30 mg (1.5 mmole) of dicylohexycarbodiimide and 44 mg (0.1 mmole) of (β-aminoethylamino)colchicide (AEAC) (IIb). The mixture was stirred at 0°C for 2 h and was then left at room temperature for 15-18 h. When the reaction was complete, 0.05 ml of 1 M HCL was added. After an hour, the precipitate was filtered off and was washed with chloroform. The solvent was distilled off in vacuum. The dry residue was washed with ether and was dissolved in chloroform. Compounds (III, I-II) were purified by two-dimensional thin layer chromatography on Macherey–Nagel plates with a non-fixed layer of silica gel (20 × 20 × 0.3 cm) in systems B and A, successively. The band with the substance was removed from the plate and extracted with ethanol. The ethanol was distilled off in vacuum, the residue was distilled in chloroform, and the solution was dried over anhydrous Na_2SO_4. After the chloroform had been distilled off, the dry residue was triturated in ether and was dried in vacuum at 40°C. All the compounds obtained were chromatographically pure and were readily soluble in ethanol and dimethylformamide.

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