3. M. Zh. Zhurinov, B. A. Serikbaev, M. Ya. Fioshin, and Kh. A. Aslanov, Uzb. Khim. Zh., No. 3, 53 (1970).
4. R. Bergin, Acta Crystallogr., B27, 381 (1971).
5. R. A. Hearn, G. R. Freeman, and C. E. Bugg, J. Am. Chem. Soc., 95, 7150 (1973).
6. R. A. Hearn and C. E. Bugg, Acta Crystallogr., B28, 3662 (1972).
7. N. G. Bokii, Yu. T. Struchkov, A. E. Kalinin, V. G. Andrianov, and T. N. Sal'nikov, Crystal Chemistry [in Russian], VINITI, Moscow (1977), p. 56.
8. L. E. Sutton, Tables of Interatomic Distances and Configuration in Molecules and Ions, Special Publication No. 18, The Chemical Society, London (1965), p. S14s.
9. B. A. Frenz, Computing in Crystallography, Delft University Press, Delft, Holland (1978), p. 64.

C-10 DIPEPTIDE DERIVATIVES OF COLCHICINE

E. O. Esbolaev, N. A. Aitkhozhina, and L. A. Aleksandrova

UDC 547.944

The methyl esters of colchicidylglycyl-L-leucine, -L-phenylalanine, and -L-tryptophan and the ethyl esters of colchicidylglycylglycine, -L-isoleucine, -L-valine, -L-tyrosine, and -L-glutamic acid have been obtained by the condensation of colchicidylglycine and esters of amino acids. The structures of the compounds have been confirmed on the basis of their UV spectra and nuclear magnetic resonance spectra.

Colchicine is one of the most active compounds possessing cytotoxic activity. In spite of its high biological activity and its capacity for inhibiting the growth of many types of malignant cells, the use of colchicine in practical medicine is limited by its high toxicity. A broad spectrum of chemically modified analogs of colchicine has been obtained [1, 2]. Unfortunately, in the majority of cases, attempts to decrease its toxicity by changing its chemical structure have led to a considerable fall in, and sometimes to the loss of, biological activity [3]. The most effective of all known chemical derivatives of colchicine have proved to be thiocolchicine and colchicinamide [4, 5], which are less toxic than colchicine itself. Some amino acid derivatives of colchicine were obtained by V. V. Kiselev by the direct replacement of the C-10 methoxy group of the alkaloid by an amino group [6-8]. Colchicidylglycylglycine and colchicidylglycyl-D-valine have been synthesized similarly [9]. It has recently been shown that the introduction of amino acid residues into the structures of the alkaloids vinblastine [10] and ellipticine [11] leads to an increase in the efficacy of their action. The study of the chemotherapeutic properties of esters of the amino acid derivatives of vinblastine on the P388 and L1210 lines of tumor cells showed that what was important for the manifestation of activity was not only the nature of the amino acid residues but also the presence of the ester group [10]. The results of these investigations have created the prerequisites for the directed modification of certain low-molecular-mass biologically active compounds.

In the present paper we describe the synthesis of a number of C-10 dipeptide derivatives of colchicine. To obtain methyl and ethyl esters of colchicidylglycylamino acids we used cholicidylglycine (I) obtained by Kiselev's method [7] with some modifications. The structure of the compound obtained was confirmed by its UV and PMR spectra, TLC analysis, and electrophoretic mobility. In the UV spectra of (I) a shoulder appears at 408 nm on the band with its maximum at 354 nm due to the presence of a conjugated nitrogen atom in the structure of the molecule. When the solution under investigation was acidified, the

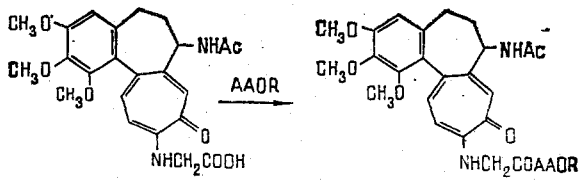
Institute of Molecular Biology and Biochemistry, Kazakh SSR Academy of Sciences, Alma-Ata. Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 91-95, January-February, 1989. Original article submitted March 10, 1988.

 0009-3130/89/2501-0080 $12.50

intensity at 354 nm decreased and that at 378 nm increased, which indicates the presence of a heteroatom conjugated with an aromatic nucleus in the structure of the molecule.

The PMR spectrum of (I) lacked the signal at 4.06 ppm of the C-10 methoxy group of colchicine, and the signal of the methylene group of glycine appeared at 4.31 ppm. The electrophoretic mobility of this compound (E_f = +0.6 relative to picric acid) showed the presence of free carboxy group in the structure of the molecule.

The C-10 dipeptide derivatives of colchicine were obtained from the methyl and ethyl esters of the corresponding amino acids and (I) by the following scheme:



I II

where AAOR: a) GlyOEt, b) L-LeuOMe, c) IleOEt, d) L-ValOEt, e) L-TyrOEt, f) L-PheOMe, i) L-TrpOMe, j) L-GluOEt.

The condensations of (I) and the amino acid esters were carried out mainly by the mixed-anhydride method [12]. Because of the poor solubility of amino acid esters in chloroform, it was necessary first to select solvents for performing the reaction. The yield of the reaction depended substantially on the polarity of the solvent. The highest yield was observed when the reaction was performed in chloroform or in a mixture of chloroform and tetrahydrofuran. When the reaction was performed in a mixture of chloroform and dimethylformamide the yield was low (15-25%). The use of the carbodiimide method gave a complex mixture with a low yield of the final product.

After prolonged chromatography on alumina, the reaction products were chromatographically pure. The R_f values in TLC for all the derivatives of (I) differed appreciably from that of the initial compound (I) (Table 1).

No appreciable differences appeared in the UV spectra of the dipeptide derivatives of colchicine (IIa-j and I), which indicates the absence of a reaction disturbing the system of conjugated bonds of the alkaloid molecule.

In the PMR spectra of the dipeptide derivatives of colchicine (IIa-j), the signals connected with the basic nucleus of the alkaloid were similar to those of (I). The signals of the methoxy groups at C-1, C-2, and C-3 and of the acetyl group of the colchicine residue in (IIa-j) were observed, just as for (I), at 3.71, 3.99, 4.03, and 2.11 ppm, respectively. In addition to these signals, in the spectra of the methyl esters of the dipeptide derivatives (IIb, f, i) the signals of the ester methoxy group were observed at 3.61 and 3.81, depending on the nature of the amino acid. The signal of the ester ethoxy groups in compounds (IIa, c, d, e, and j) appeared at 4.18 ppm in the form of a quadruplet and at 1.31 ppm in the form of a triplet. The signals of the aromatic protons of the tryptophan, phenylalanine, and tyrosine residues were observed in the 6.70-7.60 ppm interval.

EXPERIMENTAL

Colchicine from Merck and glycine from Chemapol were used. The ethyl and methyl esters of amino acids were obtained in absolute ethanol and methanol, respectively, in the presence of thionyl chloride [12].

Chromatographic separation was performed on a column of alumina (neutral, Brockman grade II) (Reanal). The esters of the colchicidylglycylamino acids were eluted with a stepwise concentration gradient of ethanol in chloroform (from 0 to 10%). TLC analysis was conducted on Alugram plates with a fixed layer of silica gel (Macherey Nagel) in the following solvent mixtures: 1) butanol–acetic acid–water (4:1:1) and 2) chloroform–ethanol–ammonia (90:9:1). On TLC, the colchicine derivatives were detected from their UV absorption at 250 nm under a chemiscope. The presence of free amino acid esters was determined by treating the chromatogram with a 0.2% solution of ninhydrin in acetone.

TABLE 1. UV Spectra and Results of TLC Analysis

Compound	Yield, %	TLC in system 1	TLC in system 2	λ_{max}, nm/log ε		
Colchicine [13]		0,66	0,78	245/4,77	352/4.22	408/4,21
I		0,61	0,00	253/4,52	354/4,40	
IIa	56	0,68	0,33	252/4,53	354/4,36	408/4,19
b	39	0,65	0,40	251/4.43	353/4,24	408/4 12
c	45	0,68	0,37	249/4,55	355/4,32	406/4.23
d	41	0,72	0,40	251/4.48	355/4,29	407/4.08
e	52	0,71	0,34	251/4,48	354/4,36	408/4.09
f	48	0.73	0,43	252/4,45	355/4,28	407/4,03
i	56	0,69	0,43	254/4.67	356/4.45	407/4,21
j	68	0,74	0,57	251/4,54	354/4,42	406/4.15

TABLE 2. Parameters of the PMR Spectra of C-10 Dipeptide Derivatives of Colchicine, δ, ppm

Compound	$-COCH_3$	C-1, OCH_3	C-2, OCH_3	C-3, OCH_3	C-10, OCH_3	Aromatic protons	CH_3-CH_2-O- Ester	CH_3-CH_2O- Ester	CH_3O- Ester
Colchicine	2,16 s	3,75 s	4,02 s	4,04 s	4,15 s	6,50–7,58	—	—	—
	2,16 s	3,71 s	4,02 s	4,04 s	—	6,49–7,50	—	—	—
IIa	2,09 s	3,74 s	3,99 s	4,03 s	—	6,50–7,58	1,36 t	4,22 q	
b	2,10 s	3,71 s	3,99 s	4,03 s	—	6,48–7,56	—	—	3,61 s
e	2,09 s	3,72 s	4,00 s	4,04 s	—	6,49–7,65	1,42 t	4,21 q	—
f	2,11 s	3,71 s	3,99 s	4,03 s	—	6,46–7,52	—	—	3,81 s
i	2,11 s	3,76 s	3,99 s	4,03 s	—	6,50–7,58	—	—	3,40 s
j	2.12 s	3,71 s	3,99 s	4,03 s	—	6,59–7,60	1,33 t	4,19 q	—

The electrophoretic analysis of the compounds obtained was carried out on FN-16 paper in 0.02 M NH_4HCO_3 (pH 7.5) and in a 6% solution of acetic acid, pH 2.5 (800 V, 22 V/cm) with the use of picric acid as marker.

UV spectra were taken in ethanol on a Specord M-40 spectrometer. Nuclear magnetic resonance spectra (in CD_3OD and $CDCl_3$) were taken on a Varian XL-100 spectrometer using hexamethyldisilazane as internal standard.

Colchicidylglycine (I). A solution of 800 mg of colchicine in 1 ml of ethanol was treated with 1.5 g of glycine and 2 ml of 10 N NaOH solution. The reaction mixture was heated for 5 h and was then left overnight at room temperature. During the reaction, the color of the mixture changed from light yellow to dark brown. The course of the reaction was monitored by TLC [2]. After the end of the reaction, the solvent was distilled off in vacuum, the residue was treated with 2 ml of water, and the colchicine was extracted with chloroform. Then the aqueous phase was acidified by the addition of 1 M hydrochloric acid until the appearance of a yellow resinous precipitate (pH 6.5) and was extracted with chloroform until the yellow color had disappeared from the aqueous phase. The organic phase was washed several times with water and was dried over sodium sulfate, and it was then filtered and the chloroform was distilled off in vacuum. The dry residue was triturated in dry ether and was washed several times and dried in vacuum. This gave 620 mg of a yellow amorphous substance.

Esters of C-10-Colchicidylglycylamino Acids (IIa-j). A solution of 20 mg (0.05 mmole) of (I) in 200 μl of dry chloroform was treated with 5.4 μl (0.06 mmole) of triethylamine. The resulting mixture was cooled to −20°C with dry ice in acetone. Then, with this temperature being maintained, and with stirring, 50 μl of a 1 M solution of isobutyl chloroformate (0.05 mole) in dry chloroform was added dropwise. The reaction mixture was stirred at −10°C for 15 min. Then 200 μl of a solution of the amino acid ester in tetrahydrofuran containing 0.1 mmole of the amino acid ester and 0.1 mmole of triethylamine was added dropwise to the resulting mixture.

The reaction mixture obtained was stirred at −5°C for another 30 min and then at room temperature for an hour. After the end of the reaction, the solvent was distilled off from

the reaction mixture and the residue was dissolved in the minimum volume of chloroform and was deposited on a column of alumina (0.9 × 10 cm). The column was washed with chloroform, and then the substance was eluted in a concentration gradient (from 0 to 10%) of ethanol in chloroform. The fractions containing the colchicine derivatives were combined, and the chloroform was distilled off. A dry oily residue was obtained which was then triturated in dry ether, washed several times with ether, and dried in vacuum at 35°C. The light yellow compounds obtained were chromatographically pure and contained none of the starting materials. They were readily soluble in chloroform, ethanol, and ethyl acetate.

Tables 1 and 2 give the results of TLC analysis and the UV spectra and nuclear magnetic resonance spectra of the following dipeptide derivatives of colchicine obtained by the above-described procedure: the ethyl esters of colchicidylglycylglycine (IIa), of colchicidylglycyl-L-isoleucine (IIc), of colchicidylglycyl-L-valine (IId), of colchicidylglycyl-L-tyrosine (IIe), and of colchicidylglycyl-L-glutamic acid (IIj) and the methyl esters of colchicidylglycidyl-L-leucine (IIb), of colchicidylglycyl-L-phenylalanine (IIf), and of colchicidylglycyl-L-tryptophan (IIi).

SUMMARY

The synthesis of a number of C-10 dipeptide derivatives of colchicine has been effected by the condensation of colchicidylglycine with amino acid esters by the mixed-anhydride method. The structures of the compounds obtained have been confirmed by UV and PMR spectroscopy and by thin-layer chromatography.

LITERATURE CITED

1. H. G. Capraro and A. Brossi, in: The Alkaloids, Academic Press, New York, Vol. 23 (ed. A. Brossi) (1984), p. 1.
2. M. K. Yusupov and A. S. Sadykov, Khim. Prir. Soedin., 3 (1978).
3. M. Rosner, H. G. Capraro, A. E. Jacobson, L. Atwell, and A. Brossi, J. Med. Chem., 24, 257 (1981).
4. P. Kereks, P. N. Sharma, A. Brossi, C. F. Cignell, and F. R. Quinn, J. Med. Chem., 28, 1204 (1985).
5. V. V. Kiselev, Khim. Prir. Soedin., 3 (1977).
6. V. V. Kiselev and N. L. Serova, Zh. Obshch. Khim., 36, No. 33 (1966).
7. V. V. Kiselev, Zh. Obshch. Khim., 31, 334 (1961).
8. V. V. Kiselev, Zh. Obshch. Khim., 34, 68 (1964).
9. V. V. Kiselev, Zh. Obshch. Khim., 41, 464 (1971).
10. S. P. Kandukuri, R. Bhushana, M.-P. M. Collard, J. P. C. Dejunghe, G. Atassi, J. A. Hannart, and A. Trouet, J. Med. Chem., 28, 1079 (1985).
11. A. K. Larsen, J. Paoletti, J. Beledhradek, and J. Paoletti, Jr., Cancer Res., 46, 5236 (1986).
12. J. P. Greenstein and M. Winits, Chemistry of the Amino Acids, Wiley, New York (1961).
13. The Merck Index, Merck, Rahway, N.J., eighth edition (1968), p. 278.