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BRIEF COMMUNICATIONS

A STUDY OF POLYPLOID ACTIVITY IN SOME COLCHICINE DERIVATIVES

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Polyploidy plays the main role in the origination of cultivated plants. The most widely used agent for producing polyploids is colchicine, under the action of which it is possible to obtain autopolyploids of almost any plant [1, 2]. Investigations are being made with the aim of finding new colchicine derivatives having a higher capacity for causing polyploidy, while breeders are placing particular value on lowering the toxicity of these substances.

In a study of the cytotoxic action of a number of colchicine derivatives on cells of *Allium cepa* L. (Table 1), we found substances causing various degrees of polyploidy of the plant cells (group I). Together with these, we identified substances with a capacity for inhibiting mitosis without the appearance of polyploidy and with the subsequent death of the cells, i.e., substances possessing a considerable antitumoral activity (group II) [3]. These compounds proved less toxic and more active than colchicine: the substances of the first group as agents causing polyploidy, and those of the second group as antitumoral agents [3].

It has been shown that the introduction into the colchicine molecule of such alkylating groupings widely known in the chemistry of antitumoral compounds [4] as aziridine or chloroethylamine as synthons enhancing the antibacterial effect of the initial carriers leads to the appearance of more considerable polyploidizing and antitumoral activities than in colchicine.

In the present paper we give the results of an investigation of a group of substances promoting polyploidy. These are a series of colchicine derivatives with various substituents in positions C-7 and C-10. Primary screening has been made of 10-hydroxyethylamino-10-demethoxy-N-deacetylcolchicine (**2**), 10-hydroxyethylamino-N-methyl-N-deacetyl-10-demethoxycolchicine (**1**) and of 10-(β-chloroethylamino)-10-demethoxycolchicine (**3**), 10-ethyleneamino-10-demethoxycolchicine (**4**), and 10-amino-7-(2-hydroxypropylamino)-7-deacetamido-10-demethoxycolchicine (**5**) from radicles of the onion *Allium cepa*, after which investigations were continued on seeds of the white mulberry *Morus alba*.

The cytological investigations on onion radicles were performed after their immersion in solutions of the compounds taken in concentrations of 0.01, 0.05, and 0.10%. Aqueous solution of colchicine at the same concentrations and water were used as controls. In a study of the action of the colchicine derivatives on the mulberry seeds, in each variant we treated and sowed 100 seeds of a mulberry of the hybrid combination "Zimostoikaya × 7-67" ($2n = 28$). The time of treatment was 24 h.

The results of the cytological experiment on onion radicles were evaluated by a four-point scheme, and the action of the substances on the mulberry seeds from the total number of seedlings produced by 100 seeds and the number of polyploid plants among them. As follows from Table 1, all the substances apart from (**1**) led to the appearance of a larger number of mulberry polyploids than colchicine.

Only in a low concentration did substance (**1**), with a low cytological evaluation (1 point), give polyploid plants — two out of the 10 seedlings obtained, which indicates a pronounced cytotoxic action for it. The action of compound (**2**) in a low concentration was somewhat superior to that of colchicine, but a lower germinating capacity of the seeds showed its high cytotoxicity. Substance (**3**) shows the greatest effect in a concentration of 0.05%. In this case the yield of the polyploids is in 2 times above then colchicine is used. However the activity of (**2**) is falling when the concentration of it is increasing. In

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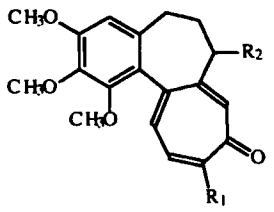
TABLE 1. Cytotoxic Evaluation and Polyploid Activity of Colchicine Derivatives

Compound			Cytotoxic activity on *Allium cepa*	Concentration, %	Number of seedlings of *Morus alba* from 100 seeds		2n	Literature
	R_1	R_2			total	of which polyploid		
1	$-NHCH_2CH_2OH$	$-NHCH_3$	+	0.01	10	2	28/56	5
				0.05	0	0		
				0.1	0	0		
2	"	$-NH_2$	++	0.01	12	9	28/56	6
				0.05	11	3	56	
				0.1	2	0		
3	$-NHCH_2CH_2Cl$	$-NHCOCH_3$	++	0.01	21	14	28/56	7
				0.05	14	5	28/56	
				0.1	19	7	28/56	
4	$-N(CH_2)_2$	$-NHCOCH_3$	+++	0.01	40	10	56	7
				0.05	24	14	56	
				0.1	18	9	28/56	
5	$-NH_2$	$-NHCH_2CHOH$	++++	0.01	36	18	28/56	8
		|		0.05	43	27	56/112	
		CH_3		0.1	12	8	28/56	
6	Colchicine-	Control-	++	0.01	18	7	28/56	
	OCH_3	$NHCOCH_3$		0.05	14	4	28/56	
				0.1	0	0		
	Water-	Control			82	0		

a concentration of 0.01%, the ethyleneamino derivative (**4**) had a favorable influence on the germinating capacity of the seeds, but when the concentration was increased to 0.05% the germinating capacity fell, although 60% of the seedlings obtained were polyploid.

The greatest effect was revealed by substance (**5**) in a concentration of 0.05% — of the 43 plants obtained, 27 (63%) were polyploid, and among them there were not only tetra- but also octaploids ($2n = 112$). Substance (**5**) was awarded 4 points in a cytological analysis using onion radicles. It considerably shortened the chromosomes and was almost nontoxic. After treatment for 24 h with substance (**5**) the growth of the cells was prolonged and a large number of dividing cells was observed at the metaphase (two-chromatid) stage. It must be mentioned that this compound is 50 times less toxic than colchicine.

Using aminodeacylated colchicine derivatives and colchicine derivatives alkylated in the C-7 group as examples in the study of the kinetics and thermodynamics of their binding with tubulin by the tryptophan fluorescence quenching method, it has been shown [9, 10] that the interactions of these derivatives with tubulin are energetically favorable. The results obtained confirmed that the *B*-ring section of aminocolchicines interacts with the dimeric tubulin molecule, which explains the more pronounced polyploid activity of compound (**5**) with a N-β-hydroxypropyl group in the C-7 position.

REFERENCES

1. A. I. Atabekova and E.I. Ustinova, *Plant Cytology* [in Russian], Koloc, Moscow (1971), p. 132.
2. L. P. Breslavets, *Polyploidy in Nature and Experiment* [in Russian], Moscow (1963), p. 364.
3. Z. M. Enikeeva, O. Yu. Balenkov, and I. N. Dimant, *Proceedings of the Ist Conference of Oncologists of the Republic of Uzbekistan with International Participation* [in Russian], Tashkent, Vol. 4 (1994) p. 17.
4. W. C. Ross, *Biological Alkylating Agents*, Butterworths, London (1962) [Russian translation, Meditsina, Moscow (1964), p. 260].
5. A. I. Begisheva, Z. M. Enikeeva, Zh. A. Goloshchapova, O. S. Otroshchenko, and T. I. Tuzhilkova, *USSR Inventors' Certificate* No. 926904 (1982).
6. Z. M. Enikeeva, A. I. Begisheva, A. S. Sadykov, Zh. A. Goloshchapova, T. N. Tuzhilkova, R. A. Alimov, N. K. Muratkhodzhaev, and T. M. Fuzailova, *USSR Inventors' Certificate* No. 10447130 (1983).
7. Z. M. Enikeeva, *Khim. Prir. Soedin.*, No. 6, 782 (1998).
8. Z. M. Enikeeva, A. I. Begisheva, K. S. Ogurtsov, and F. G. Gatin, *USSR Inventors' Certificate* No. 1329122 (1987).
9. C. Dumortier, J. L. Potenziano, S. Bane, and Y. Engelboghs, *Eur. J. Biochem.*, **249**, No. 1, 265 (1997).
10. G. Chakrabarti, S. Sengupta, and B. Bhattacharrya, *J. Biol. Chem.*, **271**, No. 6, 2897 (1996).