Chemistry of Natural Compounds, Vol. 40, No. 1, 2004

DIGLYCYRRHIZATES, REGULATORS OF COTTON CELLULOSE FORMATION

A. A. Akhunov, Z. Golubenko, D. N. Dalimov, N. A. Abdurashidova, E. Ch. Mustakimova, F. A. Ibragimov, and G. O. Akbarova

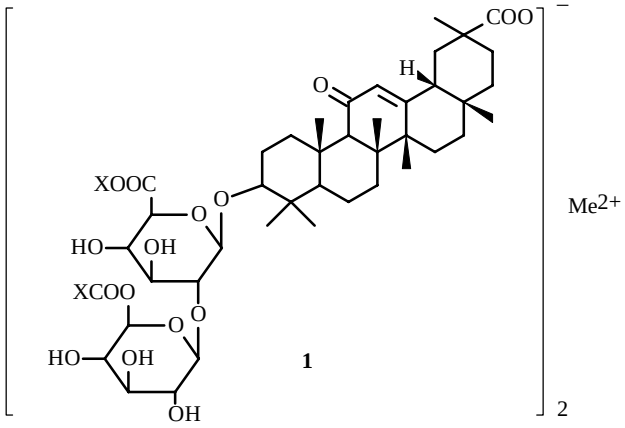
UDC 577.124.5:633.511

The influence of diglycyrrhizic acid derivatives on the activity of enzymes involved in cotton-fiber formation was investigated. Salts of diglycyrrhizic acid containing zinc, sodium, and cobalt were demonstrated to act as stimulants. It has been found that diglycyrrhizates can be used as cotton-fiber formation regulators.

Key words: diglycyrrhizates, cotton, fiber, proteins, glucansynthetase, peroxidase, cellulase.

Cotton is exceedingly responsive to the influence of various external effectors during vegetation, especially during formation of cellulose and ripening of fibers [1]. Physiological biochemical processes involving enzymes and the component composition of proteins change in the plant cells.

We previously reported [2] the synthesis of a series of diglycyrrhizates possessing high physiological activity and having the general formula **1**.



1

where Me: Mg, Ca, Mn, Fe, Co, Cu, Zn, Pb; X = H, Na, K

Glycyrrhizic acid (GC) is the core of these compounds and is the principal chemical component of licorice root (*Glycyrrhiza glabra* L.). The GC content in the root reaches 20-22% [3].

Therefore, it is important to study the action of the prepared diglycyrrhizates (DGC) on enzymes involved in the biosynthesis of cellulose fiber and the component composition of proteins from cotton genetic lines that differ in the degree of seed downiness.

The activities of the enzymes glucansynthetase, peroxidase, and cellulase in sprouts of the downy line L-489 changed differently. Glucansynthetase and peroxidase exhibited the highest activities in the presence of $ZnGC_2$ (380 and 300%, respectively) and Na_4CoGC_2 (100 and 300% greater than that of the control) whereas the activity of cellulase was 32 and 38% less than that of the control (Table 1).

A. S. Sadykov Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, 700143, fax (99871) 162 70 71, e-mail: root@ibc.edu.uz. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 58-61, January-February, 2004. Original article submitted July 21, 2003.

 0009-3130/04/4001-0066

TABLE 1. Dynamics of Enzyme Activities in 5-Day Cotton Sprouts of Downy Line L-489 and Bare-Seeded L-70 Influenced by Diglycyrrhizates

Specimen	Protein		Glucansynthetase·10^{-6}		Peroxidase		Cellulase	
	mg/g	%	act. units	%	act. units	%	act. units	%
			L-489					
$CaGC_2$	330	110	0.7	29	0.116	323	65.2	65
K_4CaGC_2	320	106	2.3	92	0.133	266	95.1	95
Na_4CoGC_2	410	136	5.0	200	0.200	400	68.4	68
$ZnGC_2$	320	106	12.0	480	0.200	400	62.5	62
$GC_2(NH_3)_4$	285	95	3.0	120	0.016	32	70.35	70
Na_4MgGC_2	240	80	3.3	132	0.050	100	137.5	138
$MgGC_2$ ethylenediamine	215	72	2.5	100	0.050	100	123.8	124
$FeGC_2$	290	97	0.3	13	0.016	232	150	150
K_4CoGC_2	270	90	2.8	112	0.050	100	92.5	92
Na_4CuGC_2	270	90	2.5	100	0.037	82	141.3	141
Control	300	100	2.5	100	0.050	100	100	100
			L-70					
$CaGC_2$	395	90	0.16	32	0.030	62	102	70
K_4CaGC_2	390	89	0.47	94	0.041	90	142.9	98
Na_4CoGC_2	460	105	1.3	283	0.204	231	97.7	61
$ZnGC_2$	400	91	1.5	326	0.039	88	99.1	68
$GC_2(NH_3)_4$	370	84	0.64	128	0.045	100	119.6	82
Na_4MgGC_2	320	73	0.7	140	0.047	105	153.1	105
$MgGC_2$ ethylenediamine	350	80	0.52	103	0.045	100	163.3	112
$FeGC_2$	300	68	0.2	19	0.035	78	201.1	138
K_4CoGC_2	400	91	0.63	125	0.047	105	131.2	90
Na_4CuGC_2	360	82	0.49	98	0.037	82	167.7	115
Control	640	100	0.5	100	0.045	100	145.8	100

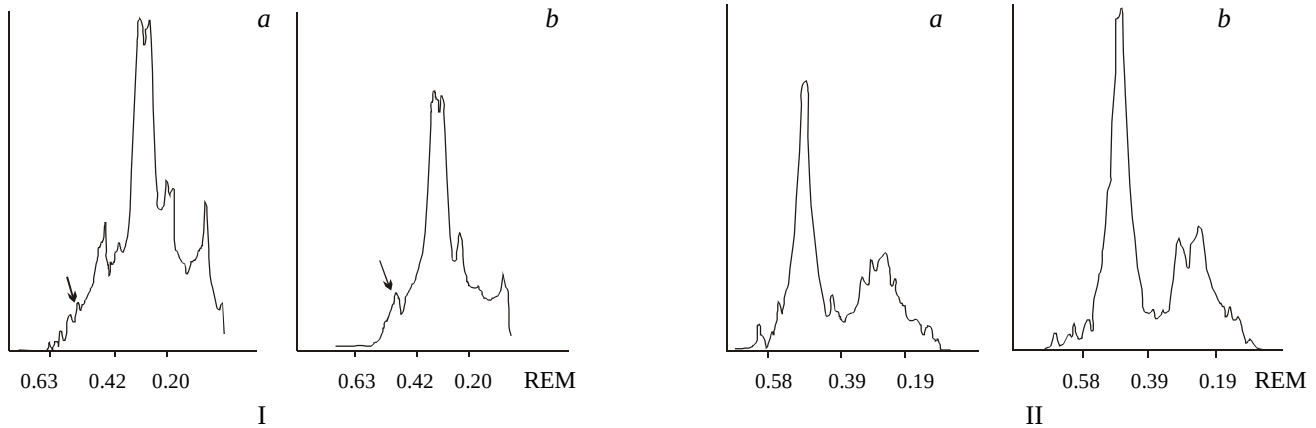


Fig. 1. Densitogram in PAAG (7.5%) of peroxidase isoforms from 5-day sprouts (I) and integument (II) of cotton lines L-489 (a) and L-70 (b).

Electrophoresis found that the relative electrophoretic mobility (REM) of peroxidase isozymes from the studied cotton specimens changed under the influence of the studied preparations. An additional isozyme with REM 0.47 appeared in the L-489 line with Na_4CoGC_2 (Fig. 1a, I) and in L-70 with REM 0.54 (Fig. 1b).

A change in protein biosynthesis is the response of a plant to the influence of any effectors. The study of the protein content and its component composition has shown that the studied preparations affect differently the enzyme activity in cotton.

TABLE 2. Effect of $CoCl_2$ on Enzymes Involved in Fiber Formation

Specimen	Glucansynthetase·10^{-6}		Peroxidase		Cellulase	
	act. units	%	act. units	%	act. units	%
Control	2.5	100	0.050	100	120	100
10^{-3} M	3.4	136	0.062	123	82	68
10^{-4} M	2.2	88	0.050	100	96	80
10^{-6} M	2.0	80	0.041	82	108	90

TABLE 3. Enzyme Activities in Fruit Elements of Cotton Genetic Lines Treated with Diglycyrrhizates

Specimen	Total protein		Glucansynthetase·10^{-6}		Peroxidase		Cellulase	
	act. units	%	act. units	%	act. units	%	act. units	%
L-489, fiber control	200	100	11	100	0.016	100	5.9	100
L-489, integument control	500	100	5.3	100	0.016	100	20	100
L-489+Na_4CoGC_2 fiber	381	191	14	127	0.116	725	0	0
L-489+Na_4CoGC_2 integument	431	87	32	604	0.033	205	49.2	246
L-489+$ZnGC_2$ fiber	200	100	7.8	71	0.016	100	0	0
L-489+$ZnGC_2$ integument	600	120	27	509	0.016	100	20	100
L-70, integument control	700	100	2.6	100	0.016	100	16.6	100
L-70+Na_4CoGC_2 integument	441	63	1.7	39	0.016	100	30.4	183
L-70+$ZnGC_2$ integument	361	52	3.9	150	0.033	206	21.9	132

DGC, which increased the activities of glucansynthetase and peroxidase, also increased the content of soluble proteins. The component composition of cotton-sprout proteins also changed. The protein composition of L-489 showed de novo polypeptides of molecular weight 38, 56, and 88 kDa; L-70, 56 and 88 kDa.

Then, the effect of DGC salts was studied in the bare-seeded line L-70, for which the enzyme activities are less than in L-489 according to earlier investigations [4].

The salts Na_4CoGC_2 and $ZnGC_2$ increased the sprouting of seeds from this line under laboratory conditions by 10 and 7%, respectively. According to glucansynthetase activity, cellulose accumulated more vigorously in varieties with added DGC: Na_4CoGC_2 increased cellulose accumulation by 183%; $ZnGC_2$, by 226% more than the control (Table 1). Na_4CoGC_2 also had a positive effect on peroxidase, 131%, but a suppressive effect on cellulase, a decrease of 39%.

According to the literature [5], cellulose synthesis in cotton fiber increased under the influence of divalent ions such as Ca^{2+} and Mg^{2+} whereas K^+ inhibited its formation. In our experiments, glucansynthetase and peroxidase activities increased markedly in the presence of Co salts. We investigated the effect of $CoCl_2$ on the studied enzymes for comparison and found that a stimulating effect appeared at a concentration exceeding greatly (10^{-3} M) the concentration of the Co DGC salts (Table 2).

The activities of glucansynthetase and peroxidase increased by 36 and 23%, respectively, whereas that of cellulase decreased by 32%.

The significant difference in the stimulating concentrations of $CoCl_2$ and Co DGC salts leads to the conclusion that DGC has a specific effect that is due to the molecular structure.

A study of the influence of DGC on the development of fruit elements of downy L-489 and bare-seeded L-70 cotton lines was especially interesting. For this, plants were sprayed with DGC solutions (10^{-4} M, Na_4CoGC_2 and $ZnGC_2$) during flowering.

These investigations showed that cellulose accumulation in the fiber and integument of L-489 increased under the influence of the Na—Co salt (Table 3). $ZnGC_2$ suppressed the activities of glucansynthetase and cellulase in fiber whereas the peroxidase activity remained at the control level.

A study of the peroxidase isospectrum in integument of L-489 as affected by DGC is consistent with the presence of an isoform with REM 0.55 (Fig. 1a, I). The electrophoretic mobility of the isoforms increased insignificantly in the isospectrum of fiber.

Cellulose biosynthesis increased markedly in bare-seeded line L-70 under the influence of $ZnGC_2$. The enzyme activities increased by 50-106%.

It was found that an isoform with REM 0.63 appeared for L-70 integument in the peroxidase spectrum under the influence of DGC (Fig. 1b, II).

Changes in the spectra of soluble proteins can be seen from the electrophoretic analysis of the component composition of integument and fiber proteins. Integument of L-489 has polypeptides of molecular weight 21, 40, and 67 kDa; L-70, 22, 36, 37, and 52 kDa; fiber affected by the preparations, 37, 60, and 70 kDa.

Tables 1-3 indicate that Na, Co, and Zn salts of DGC in general have positive effects on cotton development in the initial stage and during fruit ripening. The observed increase of cellulose synthesis was determined by the change of glucansynthetase activity and the rate of redox processes involving peroxidase in addition to hydrolytic reactions involving cellulase activity. Ions of Co, Zn, and Ca increase membrane permeability, as a result of which overall metabolic cell processes accelerate [6]. This confirms also the increase of enzyme activities.

The investigation of protein component composition is consistent with changes in the overall plant-cell metabolism. However, it can be assumed that the properties of the downy and bare-seeded lines are retained under the influence of the above preparations.

Thus, DGC salts containing Na, Co, and Zn can be used as cotton sprouting and fiber-formation regulators.

EXPERIMENTAL

Cotton genetic lines L-70 (bare-seeded) and L-489 (downy seeds) were investigated.

Salts of DGC $CaGC_2$, K_4CaGC_2, Na_4CoGC_2, $ZnGC_2$, $FeGC_2(NH_3)_4$, Na_4MgGC_2, $MgGC_2$ (ethylenediamine), $FeGC_2$, K_4CoGC_2, and Na_4CuGC_2 were prepared as before [4].

We used 5-7-day sprouts as the earliest stage of plant ontogenesis in the experiments. Fruit elements were collected from 20-day specimens for fiber-development dynamics.

Cotton seeds were cleaned with conc. H_2SO_4 and quickly washed under a stream of cold water. The effect of DGC on the sprouting and morphological and biochemical properties of sprouts was investigated by storing cleaned seeds for 1 d in 10^{-6}-10^{-7} M solutions of DGC salts. Swelled seeds were placed in paper cartons and grown for 5-6 d at constant temperature (27°C). The effect of DGC salts on fiber development was determined by spraying plants with solutions (10^{-4} M) during flowering.

A homogenate containing enzyme preparations from sprouts and cotton fiber was isolated by grinding material in liquid nitrogen and adding buffers separately for each enzyme.

Glucansynthetase activity was determined as before [4].

Peroxidase activity was found by the Boyarkin spectrophotometric method [7].

Protein component composition was found electrophoretically in a PAAG gel gradient (from 10 to 15%) in the presence of sodium dodecylsulfate according to King and Laemmli [8]. Bovine serum albumin (BSA), chymotrypsinogen, and cytochrome C were used as markers for determining molecular weights. Protein content was determined by the Lowry method [9].

Cellulase activity was determined relative to a colored insoluble substrate [4].

REFERENCES

1. A. M. Reinbol′d, *Agrokhimiya*, No. 5, 116 (1986).
2. Yu. I. Isaev, D. N. Dalimov, and I. R. Askarov, *Khim. Prir. Soedin.*, Spec. Ed., 71 (1998).
3. I. A. Murav′ev and V. S. Solov′ev, *Progress in the Study and Use of Licorice in the USSR* [in Russian], Nauka, Leningrad (1986), p. 4.

4. A. A. Akhunov, Z. Golubenko, N. A. Abdurashidova, E. Ch. Mustakimova, F. A. Ibragimov, and G. O. Akbarova, *Khim. Prir. Soedin.*, 317 (2001).
5. N. C. Carpita and D. P. Delmer, *Plant Physiol.*, **66**, 911 (1980).
6. A. I. Gagel′gans and B. A. Tashmukhamedov, *Biological Membranes and Membrane-Active Compounds* [in Russian], B. A. Tashmukhamedov, ed., Fan, Tashkent (1985), p. 206.
7. A. N. Boyarkin, *Biokhimiya*, **16**, 352 (1951).
8. J. King and U. K. Laemmli, *J. Mol. Biol.*, **62**, 465 (1971).
9. O. H. Lowry, N. G. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).