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# ISOPRENOIDS OF *Gossypium* LEAVES AND THEIR EFFECT ON THE FUNCTIONAL ACTIVITY OF COTTON-SPROUT NUCLEI

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*A method of isolating the total isoprenoids and their separate components from cotton leaves was developed. Their effect on the level of protein biosynthesis by cotton-sprout nuclei was found to be greater in vivo than in vitro.* α*-Tocopherol was found to be the most active among them.*

**Key words:** isoprenoids, α-tocopherol, sterols, undecaprenol, phenylmethylsulfanilfluorite, cotton-sprout nuclei, protein synthesis.

Biologically active substances are widely used to increase the harvest of many agricultural crops. Among these, substances that affect biochemical processes, especially natural plant-growth stimulators that can affect plant cells and eventually regulate growth, development, and harvest size, are interesting. The mechanism of such regulation may be connected to protein synthesis in nuclei [1-4]. Thus, the action of hormones on DNA synthesis of cotton seeds was studied [5-8]. The incorporation of labeled amino acids into proteins synthesized in cell nuclei of isolated pea sprouts was investigated [9-10].

We previously studied the effect of various compounds on the rate of protein biosynthesis in cotton-sprout cell nuclei [11-13]. In particular, undecaprenol, the principal component of the total isoprenoids, increases the rate of nuclear protein synthesis by more than two times after preliminary soaking of cotton seeds in a 0.1% solution.

Our goal was to isolate the total isoprenoids and their principal components from cotton leaves and to determine the stimulators of nuclear protein synthesis among the plant substances for further study of their mechanism of action.

The study of certain secondary metabolites of cotton leaves of different genetic characteristics found that the isolated total extracts and polyisoprenoid derivatives increased the cotton productivity [14, 15]. Furthermore, the role of α-tocopherol in the regulation of growth and flowering has been described [16, 17].

The total isoprenoids were isolated by the literature method [18] from cotton leaves (L-249, L-4, L-446) collected during flowering. The principal components were polyisoprenoid alcohols, the content of which depended on the variety and type. However, they were similar to each other in types where the self-regulation mechanism was found. Among the polymeric homologous polyisoprenpoid alcohols of cotton, undecaprenol dominates. Therefore, its effect on biochemical processes of cotton nuclei was previously studied [13]. In continuation of these investigations, we studied the effect on these processes of other components in the total isoprenoids such as α-tocopherol and sitosterol (with small impurities of stigmasterol). Sitosterol and α-tocopherol were isolated from the total isoprenoids using column chromatography. We obtained polyprenol, total sterol, sitosterol, and α-tocopherol (mixed with its oxidized form epoxytocopherylquinone) fractions in yields of 65.2, 7.1, 5.6, and 1.5% of the total isoprenpoids, respectively.

Fractions were identified using TLC and standards. α-Tocopherol was purified from its oxidized form over a silica gel column. Pure α-tocopherol was isolated in 0.9% yield from the total isoprenoids.

We then studied the effect of α-tocopherol, the sitosterol fraction, and total isoprenoids on the synthesis of cotton-sprout nuclear proteins.

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TABLE 1. Effect of Isoprenoids and Their Separate Components on the Rate of Nuclear Protein Biosynthesis in Yulduz Cotton-Sprout Seeds

| Compound | Incubation time, min | Amount of sample used, µg/mL | Radioactivity of nuclear protein | |
|---|---|---|---|---|
| | | | imp./min·mg, M±m | % relative to control |
| Control | 15 | - | 250±25 | |
| | 30 | - | 283±66 | |
| | 45 | - | 266±28 | |
| | 60 | - | 262±66 | |
| | | In vitro | | |
| Σ-Isoprenoids | 15 | - | 302±19 | 120 |
| | 30 | 50 | 345±13 | 121 |
| | 45 | 100 | 278±80 | 104 |
| | 60 | 150 | 268±15 | 102 |
| α-Tocopherol | 15 | - | 306±19 | 122 |
| | 30 | 50 | 313±13 | 111 |
| | 45 | 100 | 262±46 | 98 |
| | 60 | 150 | 316±63 | 121 |
| Sitosterol | 15 | - | 239±15 | 95 |
| | 30 | 50 | 218±60 | 77 |
| | 45 | 100 | 233±80 | 87 |
| | 60 | 150 | 255±53 | 97 |
| | | In vivo | | |
| Σ-Isoprenoids | 15 | - | 444±33 | 177 |
| | 30 | - | 477±29 | 168 |
| | 45 | - | 509±31 | 197 |
| | 60 | - | 453±33 | 173 |
| α-Tocopherol | 15 | - | 505±40 | 202 |
| | 30 | - | 529±90 | 186 |
| | 45 | - | 671±57 | 252 |
| | 60 | - | 546±43 | 208 |
| Sitosterol | 15 | - | 478±40 | 191 |
| | 30 | - | 509±79 | 180 |
| | 45 | - | 599±64 | 225 |
| | 60 | - | 546±60 | 208 |

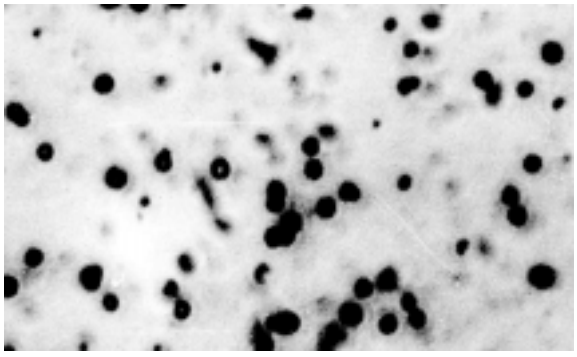

Fig. 1. Microscopic investigation of Yulduz cotton-seed-sprout nuclei (magnification 1.6×40).

Nuclei were isolated from 3-day Yulduz cotton-seed sprouts as before [11]. The purity and integrity of the nuclei were checked using a microscope. The nuclei were small and round (Fig. 1).

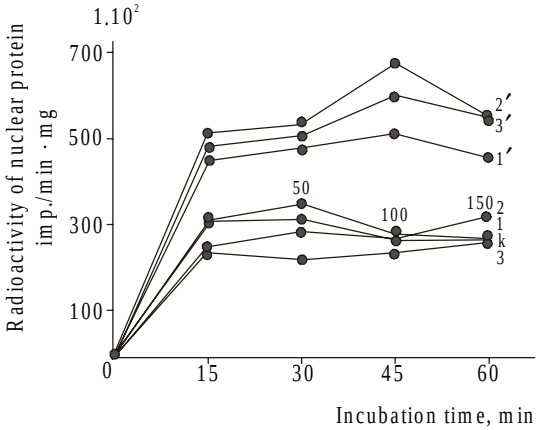


Fig. 2

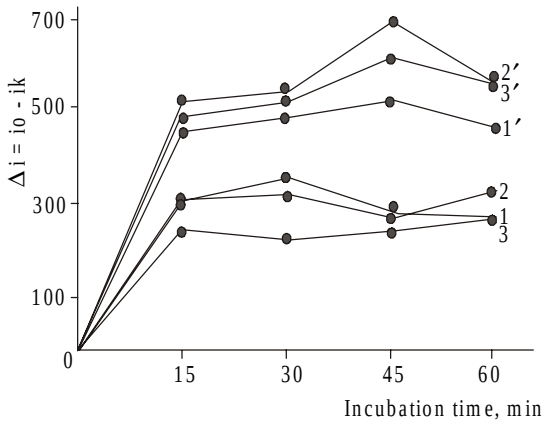


Fig. 3

Fig. 2. Effect of total isoprenoids and its separate components on rate of protein biosynthesis in nuclei of cotton sprouts. In vitro: control (k); Σ-isoprenoids (30 min, 50; 45 min, 100, 60 min, 150 μg/mL) (1); α-tocopherol (30 min, 50; 45 min; 100, 60 min, 150 μg/mL) (2); sitosterol (30 min, 50; 45 min, 100; 60 min, 150 μg/mL) (3). In vivo soaking of seeds in 0.1% solution for 2 h: Σ-isoprenoids (1′), α-tocopherol (2′), sitosterol (3′).

Fig. 3. Kinetics of differential radioactivity changes (relative to the control for total isoprenoids, α-tocopherol, and sitosterol) for protein biosynthesis in cotton-sprout nuclei. In vitro: control (k); Σ-isoprenoids (30 min, 50; 45 min, 100; 60 min, 150 μg/mL) (1); α-tocopherol (30 min, 50; 45 min, 100; 60 min, 150 μg/mL) (2); sitosterol (30 min, 50; 45 min, 100; 60 min, 150 μg/mL) (3). In vivo: Σ-isoprenoids (1′), α-tocopherol (2′), sitosterol (3′).

We also present data for the effect of other components and the total itself on these processes. The experiments were carried out as before [13].

We studied protein biosynthesis in isolated cotton-sprout nuclei. Protein synthesis was determined by incorporation of [$^{14}$C]-lysine into the synthesized protein. In the control, the rate of synthesis increased linearly for 15-30 min and then successively decreased. The process was nonlinear in 60 min. The incorporation of [$^{14}$C]-lysine was >50% over the first 30 min.

It was previously found using isolated rabbit-brain neuron nuclei that protein synthesis showed two limiting dependences on time. Low-molecular-weight proteins were formed during incubation for 30 min; high-molecular-weight ones, after that. Obviously protein formation in both cotton and animal [11] nuclei occurs with two limiting dependences on time [12].

The effect of the total isoprenoids, α-tocopherol, and sitosterol on the rate of protein synthesis in isolated nuclei was studied at various concentrations (50, 100, and 150 μg/mL) in vitro and in vivo.

Figure 2 shows the effects of the compounds on protein biosynthesis in cotton-sprout nuclei both in vitro and in vivo after soaking cotton seeds in solutions of the studied compounds.

Figure 2 indicates that the rate of formation of the first limiting protein in the control extends mainly up to 45 min incubation. However, the maximal formation is observed within 30 min after incubation. Then, the rate of protein synthesis stabilizes.

The data show that the studied substances at the indicated concentrations stimulate protein synthesis in the range 11-20%. Sitosterol, in contrast with the total isoprenoids and α-tocopherol, slightly suppresses the process, i.e., protein synthesis is suppressed by 23% at 50 μg/mL and by 3% at 150 μg/mL. Comparison of these results and previous ones for the effect of undecaprenol shows that the total isoprenoids and their separate components at a dose of 50 μg/mL affect the first limiting protein (i.e., synthesis of low-molecular-weight proteins) whereas undecaprenol at a concentration of 100 μg/mL more significantly stimulates the synthesis of high-molecular-weight proteins, i.e., by 42% compared with other isoprenoids [13].

Thus, the substances isolated by us affect insignificantly in vitro protein synthesis in isolated cotton nuclei.

Then we investigated the system in vivo using cotton seeds that were preliminarily soaked in 0.1% solutions of the total isoprenoids, α-tocopherol, and sitosterol.

Protein synthesis increased by almost two times after 15 min of incubation (Fig. 2) and then proceeded linearly up to

the 30-min mark. From the 30-min mark protein synthesis increased and then decreased at the 45-min mark. All three substances (total isoprenoids, α-tocopherol, and sitosterol) stimulated the process during 60 min of incubation by almost two times (173, 208, and 208%, respectively) compared with the control (Table 1).

Protein formation under the influence of total isoprenoids, α-tocopherol, and sitosterol is maximal compared with the control after 45 min of incubation (197, 252, 225%, respectively). Among these, α-tocopherol is the most active (252%). The effect of the studied substances on the formation of high-molecular-weight proteins is more noticeable (Fig. 2). However, the question of the functional role of the proteins synthesized in the nuclei remains unanswered.

The results indicate that these natural substances stimulate protein synthesis after soaking seeds in vivo by more than two times compared with the in vitro system. We obtained analogous data [13] using undecaprenol to stimulate the synthesis of nuclear proteins. A comparison of the influence of these three groups of substances on protein-synthesizing activity shows that the total isoprenoids and undecaprenol in identical concentrations have about equal effects. The observations are more clearly illustrated by plotting the differential changes of [$^{14}$C]-lysine incorporation into the nuclear protein (Fig. 3).

Figure 3 shows that the total isoprenoids and the separate components α-tocopherol and sitosterol that are isolated from it stimulate significantly one of the key biochemical processes, synthesis of specific nuclear proteins. The total isoprenoids and their separate components, like undecaprenol, intensify protein formation in cell nuclei. It can be assumed that this process is related to the activation of cytosolic components of both protein and nonprotein nature.

## EXPERIMENTAL

**Isolation of Total Isoprenoids.** Dried (room temperature, 100 g) and ground cotton leaves were treated successively with EtOH (3 L) and hexane (benzene, 750 mL). The extracted raw material was removed by filtration. The filtrate was treated with aqueous KOH (90 mL, 50%), shaken at room temperature for 45 min, and diluted with an equal volume of water. The hexane layer was separated, washed with water until the washings were neutral (pH 7), and dried over anhydrous $Na_2SO_4$. The solvent was removed to afford total isoprenoids (11.2 g).

**Isolation of Tocopherol and Sterols.** The total isoprenoids (10 g) were separated over a column (150×30 cm) packed with talc (60 g) by elution with hexane and then hexane:chloroform of increasing polarity to give 50 fractions that were identified by TLC. Analogous fractions were combined. Fractions 32-36 contained isoprenols; 38-41, α-tocopherols; 46-49, sterols; 47, sitosterol (traces of stigmasterol). The α-tocopherol fraction was separated again over a column packed with silica gel (particle size 0.06-0.10 mm) by elution with hexane and then hexane: chloroform (50:1, 10:1, 5:1, 2:1, 1:1, 1:2). α-Tocopherol was isolated using hexane:chloroform (1:2) and was identified by comparison with a standard and qualitative reactions with Emmery—Engel reagent.

Yulduz cotton seeds were used to investigate the effects of the isoprenoids and their separate components.

Seeds were soaked for 2 h in 0.1% solutions of the total isoprenoids, α-tocopherol, and sterol. Then they were washed and kept for three days at 28°C (thermostat). Nuclei from sprouts were isolated at 0-4°C in medium I, which contained saccharose (0.25 M), $MgCl_2$ (10 mM), NaCl (50 mM), PMSF (phenylmethylsulfanylfluorite, 0.1 mM), tris-HCl (pH 7.58 buffer, 10 mM) and layered in a saccharose gradient (2.2 M) in a Beckman untracentrifuge (USA) with an SW-27 rotor for 60 min at 24,000 rpm. The precipitated nuclei were washed again with medium II and were used after checking with a microscope. The purity and integrity of the cotton-sprout nuclei were checked using a microscope before using them for protein synthesis and [$^{14}$C]-lysine incorporation. The effects of the total isoprenoids and its separate components such as α-tocopherol and sitosterol were studied both in vitro (direct injection into the incubation medium) and in vivo (soaking seeds for 2 h in a 0.1% solution of the studied substance).

**Preparation of Solutions of Substances.** The total isoprenoids and their separate components (up to 500 mg) were dissolved in EtOH (2 mL) and diluted with distilled water to the desired concentration (0.1%).

**Protein synthesis** was monitored by [$^{14}$C]-lysine incorporation [11] into the nuclear protein. Protein biosynthesis in isolated nuclei of cotton-seed sprouts was carried out for 60 min at 37°C on a rocking water bath. We used 0.5 mL of a nuclear suspension (0.25 M saccharose, 0.003 M $MgCl_2$, 0.02 M tris-HCl, pH 7.0) and [$^{14}$C]-lysine (dl, 0.1 mL, specific activity 1 μCi/mM, 100,000 imp./min), total isoprenoids, α-tocopherol, and sitosterol at concentrations of 50, 100, and 150 μg/mL. The incubation was stopped by addition into the reaction mixture of trichloroacetic acid (TCAC, 2 mL, 10%). The mixture was cooled (30 min) and centrifuged for 10 min at 3000 rpm. The precipitate was washed successively on a millipore membrane

(Sympor, 1.5 mm diameter) with TCAC (5%) and EtOH (96%) and dried in air. The radioactivity of the product was determined in scintillation cocktail ZhS-8 (10 mL) on a Beckman DS-230 counter; protein content, by the Lowry method [19].

**REFERENCES**


1. W. R. Allen, *Trends Biochem. Sci.*, **3**, No. 10, 225 (1978).
2. J. A. Loild, *Trends Biochem. Sci.*, **3**, No. 10, 228 (1978).
3. K. S. Matinyan and S. R. Umanskii, *Biokhimiya*, **43**, 111 (1978).
4. O. Kh. Saitmuratova, Author's Abstract of a Doctoral Dissertation in Biological Sciences (1991).
5. L. G. Nikell, ed., *Plant Growth Regulating Chemicals*, CRC Press, Boca Raton, Fla. (1983).
6. V. I. Kefeli, *Prikl. Biokhim. Mikrobiol.*, **17**, 115 (1981).
7. T. A. Babaev, D. B. Gulyamov, R. Sattyev, et al., *Uzb. Biol. Zh.*, No. 4, 6 (1978).
8. T. A. Babaev, R. Sattyev, Kh. M. Karimova, and E. R. Yusupova, *Uzb. Biol. Zh.*, No. 2, 4 (1987).
9. M. L. Birnstil and B. B. Hyde, *J. Cell Biol.*, **18**, No. 1, 41 (1963).
10. S. M. Durmishidze and D. I. Dzhokhadze, *Dokl. Akad. Nauk SSSR*, **242**, No. 2, 453 (1988).
11. O. Kh. Saitmuratova and O. G. Nemtsova, *Dokl. Akad. Nauk Resp. Uzb.*, No. 12, 43 (1988).
12. O. Kh. Saitmuratova, *Uzb. Biol. Zh.*, No. 5, 3 (1991).
13. A. M. Rashkes, O. Kh. Saitmuratova, N. K. Khidyrova, V. B. Leontʹev, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 65 (1998).
14. N. K. Khidyrova, N. I. Borovinskaya, N. N. Gazaeva, and A. M. Rashkes, in: Abstracts of Papers of a Regional Scientific-Practical Conference, Fergana (1993), 18.
15. N. I. Borovinskaya, N. K. Khidyrova, N. N. Gazaeva, and A. M. Rashkes, in: Abstracts of a Regional Scientific-Practical Conference, Fergana (1993), 19.
16. T. Basynski, *Naturwissenschaften*, **54**, No. 13, 339 (1967).
17. Jpn. Pat. No. 41,827, 18N515P (1973).
18. N. K. Khidyrova, N. M. Mamatkulova, M. M. Kiktev, and Kh. M. Shakhidoyatov, *Khim. Prir. Soedin.*, 478 (2000).
19. O. H. Lowry, N. J. Rosebrough, A. U. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).