Chemistry of Natural Compounds, Vol. 34, No. 1, 1998

A NATURAL UNDECAPRENOL AND ITS INFLUENCE ON THE BIOCHEMICAL REACTIONS OF COTTON SEEDLING NUCLEI

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UDC 631.523

A method has been developed for isolating polyprenols from the leaves of cotton plants of lines L-463, L-501, and L-4. The influence of an undecaprenol on the level of biosynthesis of the proteins of the nuclei of cotton seedlings has been studied in vivo *and* in vitro. *It has been found that they double the level of biosynthesis nuclear proteins when the seeds are first wetted in a 0.1% solution.*

Polyprenols, which belong to the class of natural polyisoprenoids, are distributed in the green parts of many plants. They have the general formula $H\text{-}(CH_2\text{-}C(CH_3){=}CH\text{-}CH_2)_n\text{-}OH$, where the number of isoprenoid residues (n) varies from one plant family to another (for example, n = 11-13 for the cotton plant).

In the leaves of various species of trees polyprenol is found in the form of esters with acetic acid [1] and with higher fatty acids [2]. Plants of different families have different compositions of their polyprenols: in broad-leaved species of trees the number of isoprene units in the molecular chain ranges from 6 to 12 [3, 4] and in conifers from 10 to 20 [1, 5]. The amount of polyprenols varies according to the phase of development of the leaves, from 0.08% (in June) to 1.25% (in September).

The interest in polyprenols that has arisen in recent years is due mainly to the important role that they play as lipophilic precursors of sugars in the biosynthesis of bacterial polysaccharides and glycoproteins [7]. We have recently discovered a capacity of polyprenols for opening the Ca^{2+} channels of bilayer membranes [8].

The synthesis of polyprenols is a multistage process [4, 5]. The finding of new natural sources will open up prospects for the creation from them of drugs and other agents with a high penetrating capacity — for example, plant-protecting agents with improved membrane properties.

It must be mentioned that the biological function of endogenous polyprenols still remains unclear, although reviews have appeared both on their synthesis [9] and on their biological activity [10].

In the present paper we give results on the isolation of polyprenols from the leaves of cotton plants of lines L-463, L-501, and L-4, in which their level amounts to 1-3% of the air-dry mass, and also on the influence of undecaprenol on the biochemical reactions of cotton seedling nuclei.

Undecaprenol predominated in all the cotton plant lines (Table 1). The undecaprenol:dodecaprenol ratio was 2:1 for L-501 and L-4 and almost 3:1 for L-463.

From the leaves of an L-4 cotton plant we succeeded in isolating undecaprenol with a purity greater than 98%, as was confirmed by the results of physical methods of analysis. Its mass spectrum included the peaks of the molecular ion with m/z 766 ($C_{55}H_{90}O$) and fragments with m/z 748 ($M^+ - H_2O$) and 698 ($M^+ - 68$).

The following signals were observed in the PMR spectrum: two singlets at 1.62 and 1.54 ppm from *cis-* and *trans-*methyl groups in a ratio of 2:1, a multiplet in the 1.90-2.05 ppm region with its center at 1.96 ppm from the methylene groups of the isoprenoid chain, a doublet with its center at 3.96 ppm (J = 7.5 Hz) from the methylene groups of the terminal ${=}CH{-}CH_2{-}OH$ units, a broad singlet at 5.06 ppm from the olefinic protons of the internal units, and a triplet with its center

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 0009-3130/98/3401-0048$20.00 ©1998 Plenum Publishing Corporation

TABLE 1. Quantitative Levels of Polyprenols in the Total Extractives of Cotton Plant Leaves (mg/g)

Polyprenols	L-463	L-501	L-4
Undecaprenol	23.10	4.40	19.5
Dodecaprenol	8.80	2.20	8.7

TABLE 2. Influence of Undecaprenol on the Rate of Biosynthesis of Nuclear Proteins

Variant	Incubation, min	Amount of substances used, μg/ml	Radioactivity, pulses/min/mg of nuclear protein
Control	15	–	5210±287
	30	–	5683±277
	45	–	5703±771
	60	–	4734±723
Undecaprenol	15		4636±285
in vitro	30	50	4828±370
	45	100	6932±870
	60	150	4959±67
Undecaprenol	15		7922±628
in vivo	30	–	8506±211
	45	–	11486±480
	60	–	10037±381

at 5.33 (J = 7.5 Hz) from the olefinic protons of the terminal units. These results agree with the PMR spectrum of ficaprenol (n = 11) [4].

In the IR spectrum there were absorption bands of stretching vibrations at (cm^{-1}) 3333 (ν OH), 2962 and 2926 (ν CH in CH_3 and CH_2), and 1666 (ν C=C of an isoprene residue).

Since it is known that polyprenols possess the capacity for opening Ca^{2+} channels and are membrane-active substances [8], it appeared of interest to study the influence of undecaprenol on the biochemical reactions of the nuclei of cotton plant seedlings — i.e., to investigate its action on the rate of synthesis of protein in eukaryotic cell nuclei.

The action of undecaprenol was studied in two variants: 1) the soaking of the seeds in a 0.1% solution of undecaprenol for 2 h (*in vivo*); and 2) direct addition to the incubation medium to determine its influence on the kinetics of the nuclear synthesis of protein (*in vitro*) (Table 2).

In view of its poor solubility in water, for the performance of the experiments the undecaprenol was first dissolved in a small amount of alcohol, and water was then added to give the necessary concentration.

An advantage of the *in vitro* method consists in the fact that, in the first place, only a small amount of substance is necessary for the investigation, and, in the second place, it is possible to determine the activity of a given compound rapidly from its effect on the rate of biosynthesis of proteins by the cell nuclei.

The influence of undecaprenol on the rate of formation of protein in the nuclei was investigated in various concentrations (50, 100, and 150 μg/ml). To study protein biosynthesis in isolated nuclei we used labeled amino acids. The nuclei were incubated at 37°C. Protein synthesis was determined from the inclusion of [^{14}C]lysine.

The inclusion of [^{14}C]lysine in the nuclear proteins rose over 45 min, the radioactivity of the total percentage of proteins synthesized amounting to more than 90% [sic]. For 30-45 min there was a nonlinear rise in the rate of synthesis and then its level fell (Fig. 1).

The isolated cotton plant nuclei continued to synthesize proteins including the [^{14}C]amino acid in their molecules for 45 min. The inclusion of [^{14}C]lysine in the cotton plant nucleus, as in animal nuclei [11], shows the existence of a two-threshold dependence of its intensity on the time. It is possible that in the nuclei of cotton plant cells two proteins undergo time-dependent synthesis, just as in the case of animal nuclei.

The results obtained show that in the control variant the rate of formation of protein gradually increased during 45 min of incubation and then no longer affected the formation of protein [sic]. This pattern was obtained both on the addition of undecaprenol to the incubation medium and when the seeds were soaked in a 0.1% solution of it.

The kinetics of the synthesis of protein in the nuclei of cotton seedlings functioned nonlinearly for 45 min, when the maximum formation of protein took place. Neither a further increase in the time of incubation nor a rise in the concentration

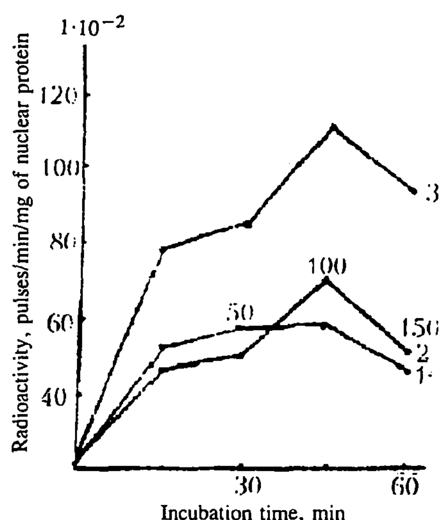

Fig. 1. Influence of undecaprenol on the rate of synthesis of nuclear proteins on incubation for 60 min: *1*) control; 2) *in vitro* experiment; 30 min, 50 μg; 45 min, 100 μg; 60 min, 150 μg; *3*) soaking of the seeds.

of undecaprenol raised the rate of formation of nuclear protein. In the wetted seeds, protein synthesis increased almost twofold as compared with the control during 45 min (Table 2). Consequently, in the main, undecaprenol acts on the nuclear synthesis of protein *in vivo*.

The influence of undecaprenol on protein-synthesizing activity was revealed qualitatively in a graphical illustration of the dynamics of the inclusion of [^{14}C]lysine in the nuclear protein as compared with the control (see Fig. 1).

In a nucleus there are many cooperative systems and therefore the effect of activators leads to a change in the activity of nuclei. The most probable systems affected are the DNA–protein system and the modification, glycosylation, phosphorylation, and other, reactions, and also the biosynthesis of proteins.

It may be assumed that undecaprenol exerts a fairly considerable stimulating influence on one of the key biochemical processes — the synthesis of specific nuclear proteins — by activating cytosolic components of both protein and nonprotein natures, as well as components of the multienzyme system.

Thus, on the basis of the results obtained, it may be concluded that undecaprenol intensifies the formation of protein in cell nuclei when the seeds are soaked in a 0.1% solution.

EXPERIMENTAL

IR spectra were taken on a Perkin-Elmer System 2000 FT-IR instrument, and mass spectra on a MKh 1310 instrument with direct injection of the sample at an evaporator temperature of 150°C, an ionizing voltage of 50 V, and a collector current of 40 μA. PMR spectra were obtained on a Tesla BS-567 A, 100 MHz, instrument, δ-scale, internal standard HMDS. TLC was conducted on Silufol UV-254 plates in the solvent system hexane–chloroform (1:2) with revelation by a 1% solution of vanillin in concentrated sulfuric acid or with iodine vapor. Undecaprenol had R_f 0.24.

The quantitative levels of polyprenols in the leaves of cotton plants of the L-463 and L-501 lines were determined by the method of multipeak monitoring [12], and in the L-4 line on a MS 25 RF chromato-mass spectrometer (Kratos, United Kingdom) with a DS 90 data-processing system [13].

Isolation of the Polyprenols. Air-dry leaves of L-463, L-501, and L-4 cotton plants (10 g each) were extracted successively with 300 ml of 96% ethanol (3 × 100 ml) and then with 300 ml of benzene. To each extract were added 90 ml of a 50% aqueous solution of KOH and 200 mg of pyrogallol and the mixtures were shaken at room temperature for an hour. Then they were diluted with water, and the benzene layer was separated off and was washed with water to neutrality and dried with anhydrous Na_2SO_4. The organic layer was concentrated in a rotary evaporator. Further purification of the prenols was

carried out on KSK silica gel with elution first by hexane and then by a hexane-ethyl acetate system with gradually increasing polarity. The yield of polyprenols amounted to 1%, and that of undecaprenol to 0.7% of the air-dry weight of the plant material.

To investigate the biochemical reaction of undecaprenol, we used cotton plant seeds of the Yulduz variety. Seedlings were obtained by soaking the cotton plant seeds in a 0.1% solution of undecaprenol at 30°C for 3 days. The nuclei were isolated at 0-4°C in medium I, containing 0.25 M sucrose, 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mM PMSF, and 10 mM tris-HCl buffer with pH 7.58. The homogenate was filtered through a layer of calico. The residue was again homogenized and filtered, and the filtrate was centrifuged at 600 rpm for 5 min and then the supernatant at 3500-4000 rpm for 15 min. The residue of nuclei was suspended in solution II (0.1 M sucrose, 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mM PMSF, 10 mM tris-HCl buffer with pH 7.58) and was stratified in a Beckman ultracentrifuge (USA) with a SW-27 rotor at 24,000 rpm for 60 min. The deposit of nuclei was washed again with solution II and, after checking under the microscope, was used for the subsequent experiments.

Protein synthesis was monitored from the inclusion of [^{14}C]ysine [14] in the protein synthesized by the nuclei. The synthesis of protein in isolated nuclei of cotton plant seedlings was carried out in a shaking water bath at 37°C for 60 min. For this we used 0.5 ml of a nuclear suspension [0.25 M sucrose, 0.003 M $CaCl_2$, 0.003 M $MgCl_2$,0.02 M tris-HCl, pH 7.0, 0.1 ml of DL/[^{14}C]lysine with a sp. act. of 1 μCi/mmole (100,000 pulses/min)], and undecaprenol in concentrations of 50, 100, and 150 μg/ml. Incubation was stopped by the addition of 2 ml of 10% trichloroacetic acid to the reaction mixture. The mixture was left in the cold for 30 min and was then centrifuged at 3000 rpm for 10 min. The deposit was subsequently washed with 5% TCA and once with 96% ethanol on a membrane millipore filter with a pore diameter of 1.5 μm (Sympor), and dried in the air. The radioactivity of the product was determined in 10 ml of ZhS-9 scintillation liquid on a Beckman DS-230 counter, and the protein content by Lowry's method [15].

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