

The temperature of the separator was 300°C, the temperature of the ion source 310°C, and the energy of the ionizing electrons 70eV.

As a result, we identified the  $C_{29}\Delta^5$  ( $M^+$  414) sterol  $\beta$ -sitosterol (I), the  $C_{29}\Delta^{5,22}$  ( $M^+$  412) sterol stigmasterol (II), the  $C_{28}\Delta^5$  ( $M^+$  400) sterol campesterol (III), and the  $C_{29}$  ( $M^+$  416) sterol 24-ethylcholestan-3-ol (IV). The sterol glycoside fraction consisted of a mixture of glycosides of sterols (I-IV). The isolation of sterols from a pentane extract of *S. collina*, including cholesterol and desmosterane has been reported previously [4]. Cholesterol and desmosterane were not detected in the material that we investigated.

The isolation of sterol glycosides from *S. collina* has not been reported previously.

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#### ENZYMATIC 5'-MONOPHOSPHORYLATION OF MODIFIED NUCLEOSIDES

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In the synthesis of nucleoside 5'-monophosphates frequent use is made of nucleoside phosphotransferases (NPTs), which catalyze the transfer of phosphate groups from molecules of organic monophosphate esters to the hydroxyls at the C-5' atoms of nucleosides. The sources of the enzymes are plants [1, 2] and bacteria [3, 4]. The latter source, in our view, is more promising because of the simplicity of production, since it is possible to use whole microbial cells as the enzyme preparation.

In order to expand the limits of use of NPTs in the production of modified nucleotides we have studied the possibility of synthesizing various 5'-mononucleotides modified in the carbohydrate moiety of the molecule with the aid of *Erwinia herbicola* 47/3 cells, which contain highly active NPTs. We selected the strain used earlier for the synthesis of AMP from adenosine (Ado) and p-nitrophenyl phosphate [5]. In this work we used Ado, 2'-deoxyadenosine (2'dAdo), guanosine (Guo), 2'-deoxyguanosine (2'dGuo), uridine (Urd), 2'-deoxyuridine (2'dUrd), cytidine (Cyd), 2'-deoxycytidine (2'dCyd), ribothymidine (Thd) and thymidine (2'dThd) from Fluka (Switzerland). The synthesis of 9-( $\beta$ -D-arabinofuranosyl)adenine (ara-Ade) has been described previously [6]. 2',3'-Dideoxythymidine (2',3'ddThd), 2',3'-dideoxy-2',3'-didehydrothymidine (2',3'dddeThd); 3'-azido-2',3'-dideoxythymidine (3'N<sub>3</sub>;2',3'ddThd); 1-( $\beta$ -D-arabinofuranosyl)thymine (ara-Thy), 9-( $\beta$ -D-xylopyranosyl)adenine (xylo-ade) and -guanine (xylo-Gua); 9-( $\beta$ -D-arabinofuranosyl)guanine (ara-Gua); 3'-amino-2',3'-dideoxyadenosine (2'NH<sub>2</sub>;2',3'ddAdo) and -thymidine (3'NH<sub>2</sub>;2',3'ddThd); 3'-fluoro-2',3'-dideoxyadenosine (2'F;2',3'ddAdo) and its  $\alpha$ -anomer ( $\alpha$ -3'S;2',3'ddGuo) and -thymidine (3'F;2',3'ddThd) were supplied by E. I. Kvasyuk, G. V. Zaitsova, and N. E. Pupeiko (Institute of Bioorganic Chemistry of the Academy of Sciences of the Belorussian SSR, Minsk). 2'-Amino-2'-deoxyuridine (2'NH<sub>2</sub>;2'dUrd), 2'-azido-2'-deoxyuridine (2'N<sub>3</sub>;2'dUrd), and 3'-amino-3'-deoxycytidine (3'NH<sub>2</sub>;3'dCyd) were obtained from Professor A. A. Kraevskii (Institute of Molecular Biology, Academy of Sciences of the USSR, Moscow).

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The preparation of the bacterial cells has been described in [5].

The reaction mixture contained 15 mM nucleoside, 90 mM p-nitrophenyl phosphate, 0.2 M Na acetate buffer (pH 4.5), and intact cells calculated by weight. The mixture was incubated at 50°C. The degree of conversion of the nucleosides into the 5'-monophosphates was determined by measuring spectrophotometrically the concentrations of substances in eluates from TLC plates, as described in [5]. The results of experiments in the phosphorylation of various nucleosides with the aid of Erw. herbicola 47/3 cells are given below:

Nucleoside	Degree of conversion, %	Nucleoside	Degree of conversion, %
Guo	80	Cyd	84
2'dGuo	73	2'dCyd	78
Ara-Gua	65	3'NH <sub>2</sub> ; 3'dCyd	70
Xylo-Gua	70	Ado	73
3'F; 2', 3'ddGuo	48	2'dAdo	65
α-3'F; 2', 3'ddGuo	60	Ara-Adc	40
Thd	79	Xylo-Adc	58
2'dThd	82	3'F; 2', 3'ddAdo	50
Ara-Thy	46	α-3'F; 2', 3'ddAdo	65
2', 3'ddThd	78	3'NH <sub>2</sub> ; 2'3'ddAdo	75
2', 3'dddeThd	81	Urd	78
3'F; 2', 3'ddThd	81	2'dUrd	78
3'NH <sub>2</sub> ; 2', 3'ddThd	73	2'NH <sub>2</sub> ; 2'dUrd	78
3'N <sub>3</sub> ; 2', 3'ddThd	75	2'N <sub>3</sub> ; 2'dUrd	65

It must be mentioned that the enzyme that we studied, just like plant NPTs [1] proved to be capable of phosphorylating nucleosides in the α-configuration. Interest is also presented by the fact that a thymidine analog - 2',3'dddeThd lacking hydroxyls at the C-2' and C-3' atom of the furanose ring and having a C-2'-C-3' double bond imparting conformational rigidity to the furanose ring - is also an effective substrate for the NPT studied.

In our view, the high specificity of the transformation of nucleosides into their 5'-monophosphates, together with the broad specificity of the enzyme, permit intact Erw. herbicola 47/3 cells to be regarded as a promising biocatalyst for obtaining various modified nucleoside 5'-monophosphates.

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