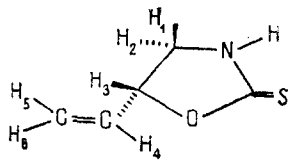
To confirm the structure of goitrin, its PMR spectrum was recorded (Fig. 1). A broad singlet of the N—H proton was located in the weakest field (7.9 ppm). The protons of the methylene group present in the oxazolidine ring gave signals with their centers at 3.54 and 3.94 ppm. A multiplet of the remaining four protons was located in the 5.2-6.2 ppm region.



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To analyze the observed PMR spectrum we used the PANIC program. In the light of the numbering of the protons given above, the calculated spectral parameters were as follows:

a) Chemical shifts (ppm from TMS): H_1 −3.55; H_2 −394; H_3 −5.34; H_4 −5.97; H_5 − 5.49; H_6 −542; N—H −7.9.

b) Spin—spin coupling constants (SSCCs) (Hz): $^2JH_1H_2$ = 9.5; $^3JH_1H_3$ - 7.8; $^3JH_2H_3$ = 8.5; $^3JH_3H_4$ = 7.3; $^3JH_4H_5$ = 17.2; $^3JH_4H_6$ = 10.4. The remaining SSCCs were equal to zero.

The spectrum was taken on a WP-100SY spectrometer with a working frequency of 100 MHz (solvent: $CDCl_3$). The calculations were carried out by the PANIC program (Bruker) on an ASPECT-200 computer.

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IMMOBILIZATION OF MODIFIED HEPARIN ON A COLLAGEN FILM

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Continuing investigations on the covalent immobilization of heparin on a collagen film, we came to the conclusion that it was necessary to introduce additional amino groups into the heparin molecule. With this aim, by the method of Finlay et al. [1], we have obtained p-aminophenethylheparin, containing about 10 μmole of p-aminophenethyl groups per 1 g of heparin. For comparison, we immobilized heparin and p-aminophenethylheparin on collagen by the three methods shown in the scheme.

Immobilization by method (I) was effected with the aid of a water-soluble carbodiimide after the alkaline activation of the collagen [2], as a result of which the amount of reactive carboxy groups in the protein increased through the partial breakdown of the microstructure of the fibrils in the surface layer of the film. In method II, we used the azide method of forming a peptide bond after the conversion of the free carboxy groups in the collagen into azide groups [3]. In method III, the collagen was first treated with the trifunctional reagent cyanuryl chloride (CC) and then, after the replacement of the second chlorine atom by an aniline residue, it was condensed with a modified or free heparin [4].

The conditions for performing the immobilization reactions and the degree of immobilization of the heparin are shown in the scheme. The amount of attached heparin was calculated from the results of amino acid analysis. These results convincingly showed that the modification of heparin by the introduction of additional amino groups into its molecule substantially increased the degree of immobilization of the heparin on the collagen. Of the three methods of immobilization considered in this work, the best results were given by the carbodiimide method in which 1-cyclohexyl-3-[2-(N-methylmorpholinio)ethyl]carbodiimide tolu-

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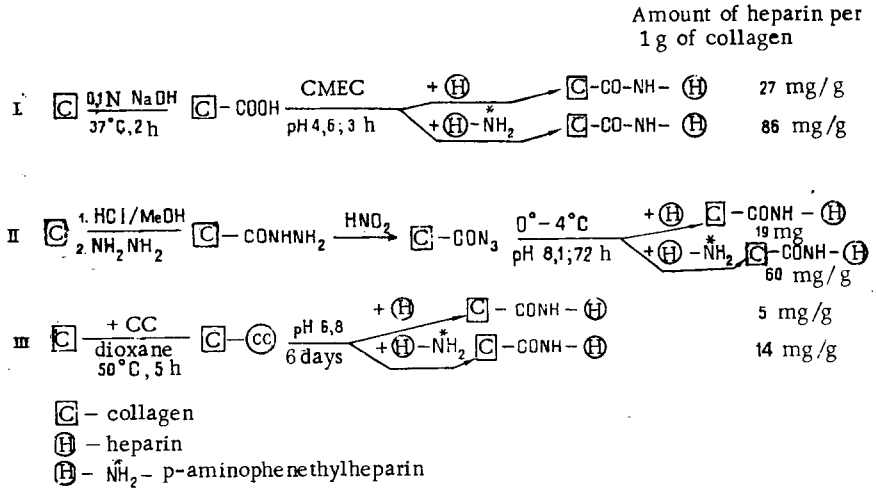

Methods of immobilizing heparin and p-aminophenethylheparin on a collagen film

ene-4-sulfonate (CMEC) was used: by this method it was possible to add 86 mg of p-aminophenethylheparin to 1 g of collagen.

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INFLUENCE OF COUMARINS ON THE BIOSYNTHESIS OF MELANIN BY THE FUNGUS *Verticillium dahliae*

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UDC 577.15/17:582.89: 576.809.8:632.428

Up to the present time, in the fight against some diseases of agricultural crops, such as piriculariosis of rice, synthetic preparation (tricyclazole, pyroquilon, etc.) are used the mechanism of the action of which is based on the inhibition of the biosynthesis of melanin in the pathogen. Of natural ecologically harmless substances a capacity for blocking the pentaketide pathway for the biosynthesis of melanin has been established only for coumarin (the lactone of cis-ortho-hydroxycinnamic acid) [1]. In the present communication we give information on the influence of some coumarin derivatives on the melaninogenesis of the fungus *Verticillium dahliae* Kleb.

An isolate of *V. dahliae* KhL-1,3 from the collection of fungi of the Division of General Genetics of the Cotton Plant of the Tadzhikstan SSR Acddemy of Sciences was cultivated on an agarized Czapek-Dox medium in Petri dishes in the dark at 24-25°C for 7-10 days. All the compounds in this investigation were supplied by V. M. Malikov and E. Kh. Batirov of the coumarin and terpenoid chemistry laboratory in the Institute of the Chemistry of Plant Substances of the Uzbek SSR Academy of Sciences. As a standard we used tricyclazole — a known inhibitor of the pentaketide pathway for the biosynthesis of melanin by fungi [1]. The substance was added to the cultivation medium as described previously [2].

The capacity of the substances investigated for blocking the melaninogenesis of the fungus *V. dahliae* was judged from the disappearance of the black coloration of the micro-

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