ISOLATION OF GOITRIN FROM RAPE AND AN INVESTIGATION OF ITS PMR SPECTRUM

M. Yu. Kornilov, A. V. Turov,
M. V. Mel'nik, and G. T. Dem'yanchuk

UDC 547:582.683.2

In order to expand the variety of sources of plant fodder protein, wide use has recently been made of rape and wild cabbage. In view of this, the study and monitoring of antinutritional substances in feedstuffs from these crops (seeds, meal, cake) are necessary. The greatest danger for animals is presented by goitrin (5-vinyloxazolidin-2-thione) (I) which possesses pronounced goitrogenic properties [1]. It is formed by the enzymatic hydrolysis of progoitrin glucosinolate (2-hydroxybut-3-enyl glucosinolate).

Goitrin has previously [2] been isolated from the roots and seeds of rape by its transfer from a hydrolyzed mixture into an aqueous solution of alkali followed by neutralization and extraction with ether and purification in several processes. However, the goitrin obtained by this method had inadequate purity and yield. We have suggested a simpler and more effective method for isolating the goitrin — the chromatographic separation of the mixture of products from the hydrolysis of the glucosinolates of the defatted flour of rape seed on a column of activated silica gel (with diethyl ether as the eluent), which enables adequate amounts of high-purity goitrin to be obtained. The fractions containing (I) we selected on the basis of their UV spectra (λ_{max}^{ether} 248, log ε 4.23). Rechromatography of the fractions collected yielded a pure sample of (I), C_5H_7ONS, mp 50°C (ether). IR spectrum: λ_{max}^{film}, cm^{-1}: 3390 (N-H), 1620 (—CH=CH_2), 1200 (C=S), 1075 (—C—O—C—), 1145 (—C—N—), 650, 620 (β-ring).

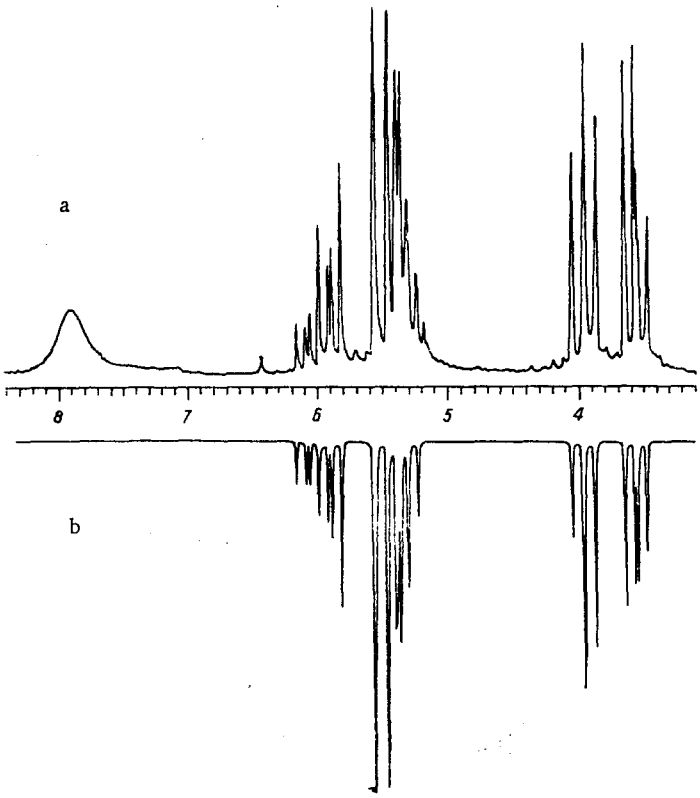
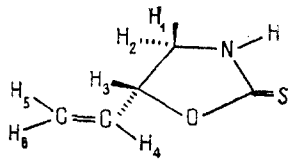


Fig. 1. Fragments of the experimental (a) and calculated (b) PMR spectra of goitrin (the signal of the NH proton is not given in spectrum (b)).

Ivano-Frankovo Scientific-Research Station of Cruciferous Crops. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 769-771, September-October, 1987. Original article submitted April 7, 1987.

0009-3130/87/2305-0645$12.50

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.



I

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.

LITERATURE CITED

1. E. B. Astwood, J. Pharmacol. Exp. Ther., 78, 79 (1943).
2. E. B. Astwood, M. A. Greer, and M. G. Ettlinger, J. Biol. Chem., 181, 121 (1949).

IMMOBILIZATION OF MODIFIED HEPARIN ON A COLLAGEN FILM

T. I. Velichko, N. N. Anikeeva,
N. V. Fedoseeva, and G. S. Katrukha

UDC 547.962.9:615.33

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-

M. V. Lomonosov Moscow State University. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 771-772, September-October, 1987. Original article submitted May 12, 1987.

 0009-3130/87/2305-0646$12.50