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1-Vinyl-3- and 1-Vinyl-5-pyrazolecarboxylic Acids. Synthesis and Anti-Burn Activity of Their Chitosan Salts

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Abstract—The synthesis of 1-vinyl-3- and 1-vinyl-5-pyrazolcarboxylic acids is developed and the anti burn activity of the chitosan salts of 1-vinyl-3(5)-carboxylic acids is studied.

Keywords: chitosan, vinylpyrazolecarboxylic acids, anti burn activity

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Pyrazolecarboxylic acids occupy an important place among functionally substituted pyrazoles. Some derivatives of pyrazolecarboxylic acids, like their amides, possess an expressed pharmacological activity [1]. 1-Carboxyethylpyrazoles were used as new curing and modifying agents for hot curing of epoxide resins [2]. Chitosan (natural polymer) salts with 1-vinylpyrazole-4-carboxylic acids were studied [3] and shown to possess very weak antimicrobial activity.

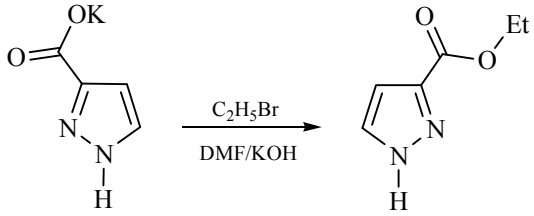
The search for anti burn coatings, which are biologically compatible with the organism, capable of resorption in the process of healing wounds, do not leave pathological traces and do not cause intoxication with metabolites, is one of most important problems of regenerative medicine. Taking into account that one of promising natural polymers used in the preparation of such biotransplants is chitosan and its modified derivatives [4], a synthesis of new modifications of chitosan and investigation of their biological activity is a very actual problem.

The aim of the present work was the development of methods of synthesis of 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **V, VI**, their use as modifiers of chitosan, and investigation of anti burn activity of the chitosan salts of 1-vinyl-3(5)-carboxylic acids.

Oxidation of technically available 3(5)-methylpyrazole [5] with potassium permanganate in aqueous medium is known to result in the formation of 3(5)-pyrazolecarboxylic acid (**I**) [5], which opens wide possibilities for preparation of new pyrazole derivatives.

For chemoselective vinylation of the nitrogen atom, the carbonyl group remaining intact in the molecule of 3(5)-pyrazole carboxylic acid (**I**), the method of introduction of protecting groups was suggested, which are stable in various reactions and can be easily removed [7].

Even the first experiments have shown that the alkyl group meets the mentioned conditions. The alkylation of potassium salt of 3(5)-pyrazole carboxylic acid (**Ia**) with ethyl bromide in dimethylformamide (DMF) gave ester **II** in 60% yield. No products of *N*-alkylation are formed under these conditions.

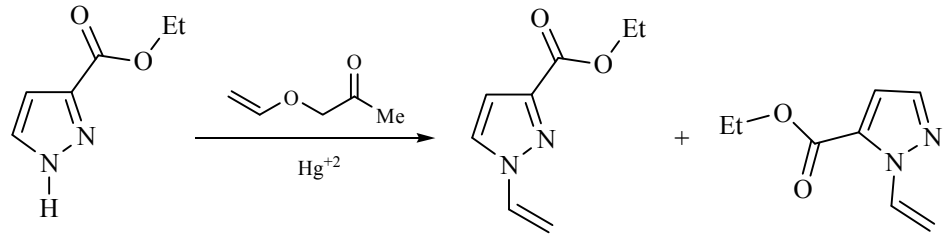


Ia **II**

Dioxane, benzene, or water cannot be used for alkylation of compound **I**. The use of phase transfer catalysts did not give any advantage, either.

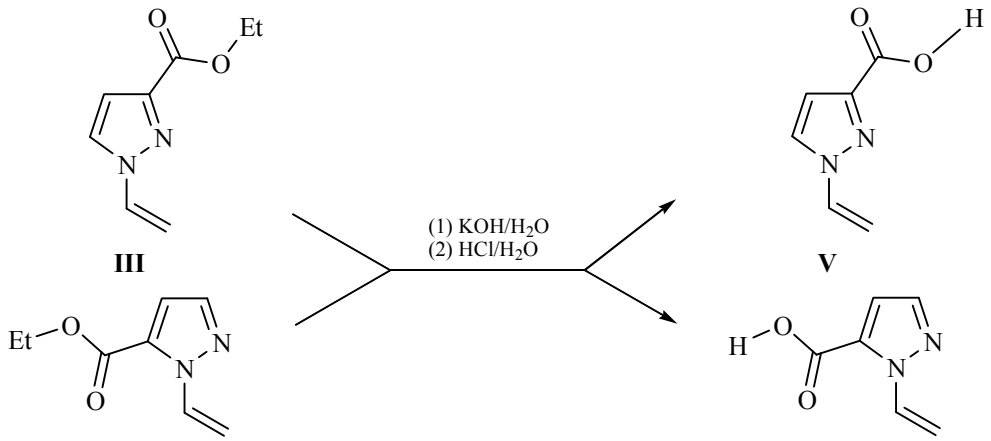
Transvinylation of the esters of 3(5)-pyrazole carboxylic acid (**II**) with vinyl acetate in the presence of catalytic amounts of mercury(II) sulfate gave a mixture of isomeric esters of 1-vinyl-3- and 1-vinyl-5-pyrazole carboxylic acids **III, IV** in total yield of 80% and the ratio of isomers 3 : 2, respectively [8]. The isomers were separated by conventional distillation. In most

Scheme 1.



II **III** **IV**

Scheme 2.



III **V**

IV **VI**

cases the substituted pyrazoles formed by alkylation at nitrogen form difficultly separable mixtures [9] (Scheme 1).

The ease of separation of the isomers in the case of esters of 1-vinylpyrazole carboxylic acid **III, IV** is due to the existence of the intramolecular hydrogen bond between the α-proton of the vinyl group and the carbonyl oxygen atom (CH···O=C) in ester **IV** lacking in its isomer **III**. In the ^{1}H NMR spectrum the signal of the α-proton in **IV** is shifted downfield and appears at 8.03 ppm [10]. In compound **III**, the α-proton is not deshielded by the hydrogen bond and resonates upfield, at 7.01 ppm.

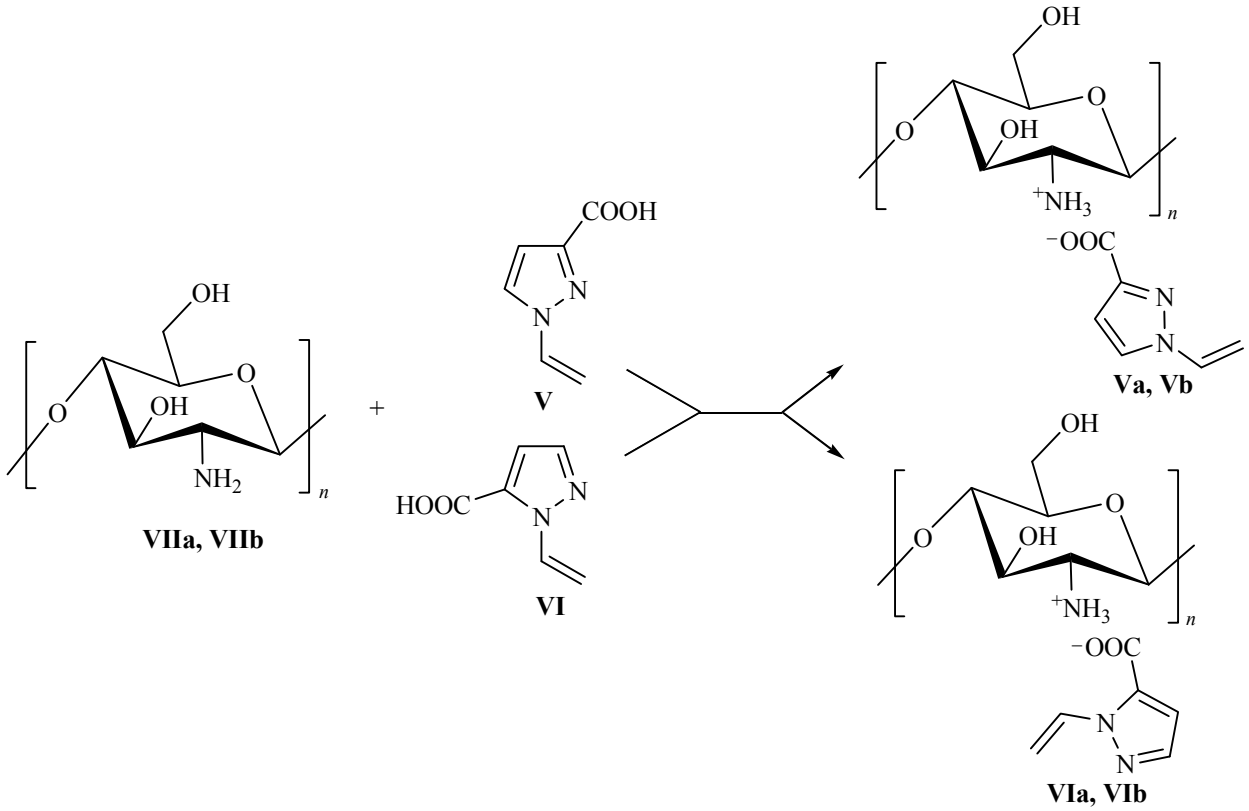
The hydrolysis of esters of 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids with aqueous potassium hydroxide affords the corresponding salts and, after acidification, 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **V, VI** (Scheme 2).

Salts **Va, Vb, VIa, VIb** of 1-vinyl-3- and 1-vinyl-5-pyrazole carboxylic acids with chitosans **VIIa, VIIb** were obtained by the earlier described general procedure [11] (Scheme 3).

The reaction proceeds smoothly at refluxing chitosan **VIIa, VIIb** and the corresponding pyrazolecarboxylic acid **V, VI** in water in the course of 1–3 min; after filtration and removal of insoluble residues the solution was dried by pouring to form thin films whose elasticity increases with the growing amount of chitosan.

In the IR spectra of the chitosan salts of 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **Va, Vb, VIa, VIb** the absorption of the pyrazol ring at 1510 cm^{-1} and the vinyl group (C=C) at 1640 cm^{-1} is observed, as well as the stretching vibrations of the C–H groups of chitosan at 2878 cm^{-1} and OH groups at 3450–3050 cm^{-1}. Unlike the pattern of IR spectra of the starting 1-vinyl-3- and 1-vinyl-5-pyrazole carboxylic acids **V, VI**, the intensity of the vinyl group is several times reduced. The same is true of the absorption of the pyrazole ring but not of the N^+H_3, OH, CH vibrations of the chitosan motif. Interestingly, the bending vibrations of the primary amino group are practically lacking, which is indicative of the formation of the chitosan salts with 1-vinylpyrazole carboxylic acids **V, VI**. In the ^{1}H NMR spectra of

Scheme 3.



compounds **Va, Vb, VIa, VIb** the signals of protons of the vinyl group at 4.94, 5.71 ppm and 4.90, 5.79 ppm (=$\underline{CH_2}$) and 7.2, 8.05 ppm (=CH) are retained, as well as the signals of the pyrazole ring protons at 6.73, 6.85 ppm and 7.52, 7.94 ppm. In a stronger field, at 3.5–4.0 ppm, the resonance of the CH groups (H^1, H^2, H^3, H^4, H^5, H^6 and $H^{6'}$) of chitosan is observed.

It was noted that practically all functional properties of chitosan depend on its molecular parameters, mainly on the mass (degree of polymerization) [12, 13]. From this point of view, introduction of 1-vinylpyrazole carboxylic acids in the molecule of chitosan is not a simple substitution because polymerization of the vinyl groups can change the molecular mass of chitosan and thus control its functional properties. However, polymerization of salts **Va, Vb, VIa, VIb** under the used conditions (H_2O, T = 20–50°C, $Na_2S_2O_7$) failed, apparently, because of hindering the polymerization process by macromolecules of chitosan.

Anti burn activity of 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **V, VI**, low-molecular (**VIIa**) and high-molecular (**VIIb**) chitosan, as well as the salts of chitosan with 1-vinyl-3- and 1-vinyl-5-pyrazole carboxylic acids **Va, Vb, VIa, VIb** were investigated. The dynamics of the regeneration process was evaluated on the basis of objective data: macrofotographic and planimetric methods of measuring variation of the wound surface as well as morphological analysis of histological and cytological skin cuts of the control and experimental animals.

These studies have shown that 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **V, VI** do not show anti burn activity compared to chitosan **VIIa, VIIb** or to the control group. However, salts **Vb, VIb** of high-molecular chitosan with 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **V, VI** show notable anti burn activity against experimental thermal 2nd degree burns as compared with both the control group and 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **V, VI** or high-molecular chitosan **VIIb**.

Note, that high-molecular chitosan **VIIb** as compared to low-molecular chitosan **VIIa** possesses a more emphasized anti burn activity. The same is true for the salts of chitosan with 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylic acids **Vb, VIb**. The anti burn

activity of compounds **Vb, VIb** are practically the same.

EXPERIMENTAL

IR spectra were obtained on a Nexus Thermo Nicolet Corporation spectrometer. ^{1}H NMR spectra were registered on a Varian Mercury instrument (300 MHz) in DMSO-d_6–CCl_4 or D_2O–CF_3COOH. Commercial chitosan (Sigma Aldrich) with medium- or low-viscosity molecular mass was used.

Ethyl 3(5)-pyrazolecarboxylate (II). To a mixture of 11.2 g (0.1 mol) of 3(5)-pyrazolecarboxylic acid (**I**) and 5.6 g (0.1 mol) of potassium hydroxide in 100 mL of DMF 16.4 g (0.15 mol) of ethyl bromide was added dropwise at 40°C. After completion of the addition, the reaction mixture was stirred at this temperature for 12 h, cooled, filtered, the solvent was removed at a reduced pressure, to the residue the solution of sodium carbonate was added, and the crystals formed were filtered to obtain 8.6 g (60%) of compound **II**, mp 162-163°C, IR spectrum, ν, cm^{-1}: 1530 (ring), 1720 (C=O), 3260 (NH). ^{1}H NMR (DMSO-d_6), δ, ppm: 1.42 t (3H, –$\underline{CH_2}$–$\underline{CH_3}$, *J* 7.2 Hz), 4.20 q (2H, –$\underline{CH_2}$–$\underline{CH_3}$, *J* 7.2 Hz), 6.78 d (1H, 4-H, *J* 1.9 Hz), 7.50 d [1H, 3(5)-H, *J* 1.9 Hz], 12.0 br.s (1H, NH). Found, %: C 51.05; H 5.94; N 20.14. $C_6H_8N_2O_2$. Calculated, %: C 51.43; H 5.71; N 20.00.

Ethyl 1-vinyl-3- and 1-vinyl-5-pyrazolecarboxylates (III, IV). To 50 mL of vinyl acetate 1.0 g of mercury(II) sulfate and 14 g (0.1 mol) of ethyl 3(5)-pyrazolecarboxylate (**II**) was added, heated at reflux for 1 h, cooled, filtered, vinyl acetate was removed at a reduced pressure, the residue was washed with 2 N solution of sodium carbonate, extracted with chloroform, dried over $MgSO_4$, chloroform was removed, the residue was distilled in a vacuum.

Ethyl 1-vinyl-5-pyrazolecarboxylate (IV). Yield 3.95 g (23.5%), bp 75°C (1 mmHg), n_D^{20} 1.5112, d_4^{20} 1.0620. IR spectrum, ν, cm^{-1}: 1540 (ring), 1640 (C=C), 1720 (C=O). ^{1}H NMR (DMSO-d_6), δ, ppm: 1.40 t (3H, –$\underline{CH_2}$–$\underline{CH_3}$, *J* 7.2 Hz), 4.22 q (2H, –$\underline{CH_2}$–CH_3, *J* 7.2 Hz), 4.95 d.d (1H, CH=$\underline{CH_2}$, *J* 15.6 and 1.3 Hz), 5.85 d.d (1H, CH=$\underline{CH_2}$, *J* 8.7 and 1.3 Hz), 6.80 d (1H, 4-H, *J* 1.9 Hz), 7.95 d (1H, 3-H, *J* 1.9 Hz), 8.03 d.d (1H, $\underline{CH}$=CH_2, *J* 15.6 and 8.7). Found, %: C 58.08; H 6.49; N 16.30. $C_8H_{10}N_2O_2$. Calculated, %: C 57.82; H 6.07; N 16.86.

Ethyl 1-vinyl-3-pyrazolecarboxylate (III). Yield 9.3 g (56%), bp 106°C (1 mmHg), n_D^{20} 1.5220, d_4^{20} 1.1427. IR spectrum, ν, cm^{-1}: 1530 (ring), 1640 (C=C), 1720 (C=O). ^{1}H NMR (DMSO-d_6), δ, ppm: 1.40 t (3H, –$\underline{CH_2}$–$\underline{CH_3}$, *J* 7.2 Hz), 4.24 q (2H, –$\underline{CH_2}$–CH_3, *J* 7.2 Hz), 4.95 d.d (1H, CH=$\underline{CH_2}$, *J* 15.6 and 1.3 Hz), 5.95 d.d (1H, CH=$\underline{CH_2}$, *J* 8.7 and 1.3 Hz), 6.78 d (1H, 4-H, *J* 2.2 Hz), 7.50 d (1H, 5-H, *J* 2.2 Hz), 7.01 d.d (1H, $\underline{CH}$=CH_2, *J* 15.6 and 8.7 Hz). Found, %: C 57.34; H 5.81; N 16.39. $C_8H_{10}N_2O_2$. Calculated, %: C 57.82; H 6.07; N 16.86.

1-Vinyl-3-pyrazolecarboxylic acid (V). The mixture of 8.3 g (0.05 mol) of compound **III**, 4 g (0.1 mol) NaOH, and 20 mL of water was stirred for 2 h at room temperature, washed with ether, water layer was neutralized with HCl, the formed crystals were filtered and dried to obtain 3.1 g (45%) of compound **V**, mp 89°C (benzene). IR spectrum, ν, cm^{-1}: 1530 (ring), 1645 (C=C), 1720 (C=O). ^{1}H NMR (DMSO-d_6), δ, ppm: 4.94 d.d (1H, CH=$\underline{CH_2}$, *J* 8.8 and 1.2 Hz), 5.71 d.d (1H, CH=$\underline{CH_2}$, *J* 15.7 and 1.2 Hz), 6.73 d (1H, 4-H, *J* 2.4 Hz), 7.20 d.d (1H, $\underline{CH}$=$\underline{CH_2}$, *J* 15.7 and 8.9 Hz), 7.94 d (1H, 5-H, *J* 2.4 Hz), 12.20 br.s (1H, COOH). Found, %: C 52.49; H 4.14; N 20.44. $C_6H_6N_2O_2$. Calculated, %: C 52.17; H 4.34; N 20.28.

1-Vinyl-5-pyrazole carboxylic acid (VI). Prepared as above from 8.3 g (0.05 mol) of ethyl ester of 1-vinyl-5-pyrazole carboxylic acid (**IV**). Yield 3.3 g (48%), mp 85°C (benzene). IR spectrum, ν, cm^{-1}: 1520 (ring), 1645 (C=C), 1720 (C=O). ^{1}H NMR (DMSO-d_6), δ, ppm: 4.90 d.d (1H, CH=$\underline{CH_2}$, *J* 8.8 and 1.2 Hz), 5.79 d.d (1H, CH=$\underline{CH_2}$, *J* 15.4 and 1.2 Hz), 6.85 d (1H, 4-H, *J* 1.9 Hz), 7.52 d (1H, 3-H, *J* 1.9 Hz), 8.05 d.d (1H, $\underline{CH}$=$\underline{CH_2}$, *J* 15.4 and 8.8 Hz), 12.70 br.s (1H, COOH). Found, %: C 52.39; H 4.14; N 20.44. $C_6H_6N_2O_2$. Calculated, %: C 52.17; H 4.34; N 20.28.

Salts of chitosan with 1-vinyl-3-pyrazolecarboxylic acid and 1-vinyl-5-pyrazolecarboxylic acid (Va, Vb, VIa, VIb). 1.38 g (0.01 mol) of 1-vinyl-3- or 1-vinyl-5-pyrazolecarboxylic acid **V, VI** and 0.0125 mol of chitosan **VIIa, VIIb** (degree of deacetylation 75–85%) was refluxed in 50 mL of distilled water for 1–3 min to complete dissolution of chitosan with subsequent filtration of the obtained solution.

Polymer films were prepared from the solutions of the corresponding salts **Va, Vb, VIa, VIb** by pouring in polyethylene dishes of 5–10 cm diameter and drying at room temperature. All samples were used as 5% aqueous solutions of 50 mL volume. For this, 5% aqueous solutions of compounds **V, VI, Va, Vb, VIa, VIb** were prepared. For dissolution of chitosan **VIIa,**

VIIb and 1-vinylpyrazolecarboxylic acid **V, VI** 1% HCl was used.

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