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SYNTHESIS OF (3-PYRIDYL)GLYOXYLIC ACID DERIVATIVES AND THEIR ANTIMICROBIAL PROPERTIES

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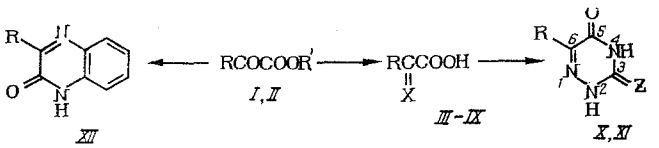
The synthesis and properties of alkyl and arylglyoxylic acids have been fairly widely investigated [2]. Compounds with different biological activity were detected [1, 8] among their transformation products. The preparation of several heteroarylglyoxylic acids has also been described [1], but their properties have practically not been investigated. It was interesting to carry out a comparative study of chemical and biological characteristics of these structurally related compounds, to determine the influence of the heteroaromatic and, in particular, the basic pyridine ring on the properties of the α-oxo-acids.

To solve the problem, we synthesized a representative of the heteroaromatic α-oxo-acids, (3-pyridyl)glyoxylic acid (I), and studied its chemical transformations. Acid I was prepared by oxidizing 3-ethylpyridine by $KMnO_4$ in an aqueous–alkaline medium and by the method described in [4]. Acid I is a fairly stable compound. Only when it is heated to melting point (170-180°C), decarboxylation is observed, with resinification of the products formed, possibly due to their polymerization. Acid I was esterified in a yield of 47% by heating twice with an ethanolic solution of HCl. When H_2SO_4 was used as the esterification catalyst, the yield of ethyl (3-pyridyl)glyoxylate (II) decreased to 18%. It was found that when ester II is boiled in an aqueous solution for 1 h, it hydrolyzes to acid I.

Acid I readily forms derivatives at the oxo group: hydrazone (III), oxime (IV), semicarbazone (V), thiosemicarbazone (VI), 4-phenylsemicarbazone (VII), 4-phenylthiosemicarbazone (VIII), phenylhydrazone (IX), While the reactions of the aliphatic and aromatic α-oxo-acids



I: R' = H; II: R' = Et; III: X = NNH_2; IV: X = NOH; V: X = $NNHCONH_2$; VI: X = $NNHCSNH_2$; VII: X = NNHCONHPh;; VIII X = NNHCSNHPh; IX:X = NNHPh; X:Z = O; XI:Z = S; I-XII: R = 3-pyridyl

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TABLE 1. Chemical Shifts of ^1H in Compounds I, III-XII (δ, ppm)

Compound	Pyridine ring				C_6H_5
	2	4	5	6	
I	9,03	8,36	7,64	8,77	—
III	8,62	7,95	7,46	8,48	—
IVa	8,35	7,70	7,47	8,44	—
IVs	8,60	7,91	7,42	8,43	—
V	8,73	8,07	7,43	8,48	—
VI	8,82	8,12	7,47	8,49	—
VIIa	8,40	7,78	7,47	8,46	7,05—7,55
VIIs	8,77	8,11	7,45	8,45	7,05—7,55
VIII	8,85	8,20	7,49	8,50	7,2—7,65
IXa	8,42	7,78	7,61	8,41	6,9—7,35
IXs	8,71	8,03	7,34	8,61	6,9—7,35
X	8,77	8,11	7,49	8,52	—
XI	8,82	8,16	7,52	8,54	—
XII	8,83	8,15	7,50	8,52	7,1—7,55

Note. The letter a or s after the number of the compounds indicates an anti- or syn-configuration. In all compounds the H_2 signal is doublet, the H_4 is multiplet, H_5 and H_6 are quartets, and the C_6H_5 signal is multiplet.

TABLE 2. Antimicrobial Activity of (3-Pyridyl)glyoxylic Acid Derivatives I, II, V-VIII, X, XI

Microorganisms	MIC, μg/ml							
	I	II	V	VI	VII	VIII	X	XI
Staph. aureus ATCC 25923	>250	>250	>250	>250	>250	62,5	>250	>250
Staph. pyogenes ATCC 12354	>250	>250	>250	>250	>250	62,5	>250	>250
E. coli ATCC 25922	>250	>250	>250	>250	>250	>250	>250	>250
Proteus vulgaris ATCC 6896	>250	>250	>250	>250	>250	>250	>250	>250
Ps. aeruginosa ATCC 27853	>250	>250	>250	>250	>250	>250	>250	>250
Microsporum canis*	>500	>500	>500	>500	>250	>250	>500	>500
Trichorphyton mentagraphytes var. gypseum*	>500	>500	>500	>500	>250	>250	>500	>500
Candida albicans*	>500	>500	>500	>500	>500	>500	>500	>500

*Clinical strains.

Note. MIC) Minimal inhibiting concentration.

with semicarbazides proceed fairly completely in the presence of acid catalysts only, semicarbazones V and VII are formed from acid I in close to quantitative yields, in the absence of a catalyst.

The structure of compounds I, III-IX was confirmed by the ^1H NMR spectra, according to which compounds IV, VII, and IX exist in solutions in 0.5 N NaOH in the form of a mixture of syn- and anti-isomers* (Table 1). In the case of oxime IV, the signals of the isomers were assigned on the basis of the ^{13}C NMR spectrum of this compound, containing a ^{15}N atom in the NOH group. It is known that in ketoximes, the absolute value of the spin-spin coupling constant (SSCC) ($^2J_{^{13}C^{15}N}$) is higher for that one of the two carbon atoms geminal with respect ot the ^{15}N nitrogen atom, which is present at the anti-position to the OH group [5].

*In the present work, the syn-(anti)-isomer refers to the isomer with a syn-(anti) disposition of the carboxyl group and the residue at the nitrogen atom in the substituent attached to the C_3 atom of the pyridine ring.

Therefore, as the result of the difference in SSCC ($^2J_{^{13}COOH^{15}N}$) and $J^2J_{^{13}C^{15}N}$) for both the isomer predominating immediately after the dissolution of the sample (9 and ≥1 Hz, respectively) and for the minor isomer (≥1 and 8 Hz, respectively), it is possible to refer the first isomer to the anti- and the second to the syn configuration. Examination of the ^1H NMR spectra of a mixture of isomers of oxime IV reveals the following relationships between the chemical shifts of the $H_{(2)}$ and $H_{(4)}$ protons in syn- and anti-isomers:

$$^{anti}\delta H_{(2)} < \delta H_{(2)}{}^{syn} \quad (\Delta\delta = 0.25 \text{ ppm})$$
$$^{anti}\delta H_{(4)} < \delta H_{(4)}{}^{syn} \quad (\Delta\delta = 0.21 \text{ ppm})$$

These relationships in the case of geometrical isomers VII and IX indicate that the syn-isomer of compound VII and anti-isomer of IX predominate in solutions. A comparison of the chemical shifts of pyridine ring protons in the syn-anti isomers of compounds IV, VII, IX with similar values for compounds III, V, VI, and VIII shows that the latter compounds exist in the form of the syn-isomers.

When syn-semicarbazone (V) and syn-thiosemicarbazone VI were heated with aqueous solution of potassium carbonate, 3,5-dioxo-6-(3-pyridyl)-2,3,4,5-tetrahydro-1,2,4-triazine (X) and 5-oxo-6-(3-pyridyl)-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazine (XI) were obtained in yields of 55-65%. Cyclization of compound VI in an aqueous solution proceeds at room temperature, but at a lower yield (30%).

Acid I readily reacts with o-phenylenediamine to 2-oxo-3-(3-pyridyl)quinoxaline (XII) in the absence of an acid catalyst, which is usually used in similar reactions of aliphatic α-oxo-acids [3, 6]. The structure of compounds X-XII was confirmed by the ^1H NMR spectra. It is interesting to note that the values of the chemical shifts of $H_{(2)}$ and $H_{(4)}$ protons in these compounds differ markedly from $\delta H_{(2)}$ and $\delta H_{(4)}$ in the anti-isomers of IV, VII, IX, but are similar to the corresponding values for the remaining compounds existing in the syn-configuration. This serves as an independent confirmation of the correctness of the above assignment of the configurations of compounds III-IX, since, according to the character of steric interactions of a substituent at $C_{(3)}$ with the pyridine ring, namely the syn-isomers of derivatives III-IX should be considered as acyclic analogs of compounds X-XII.

EXPERIMENTAL (CHEMICAL)

The ^1H NMR spectra of the compounds and the ^{13}C NMR spectrum of oxime IV were run on a XL-200 (Varian, Switzerland) spectrometer with a working frequency of 200 MHz for the ^1H nuclei and 50.3 MHz for ^{13}C nuclei. Solvent, 0.5 N NaOH; standard, dioxane (δ^1H = 3.74 ppm, $\delta^{13}C$ = 67.4 ppm).

(3-Pyridyl)glyoxylic acid (I) [4]. mp. 170-172°C (from water). IR spectrum, λ_{max}, cm^{-1}: 1605, 1640 (C=O), 3100, 3180 (OH), 1555, 1460 (C=C, C=N).

Ethyl(3-pyridyl)glyoxylate (II). A mixture of 0.95 g of (3-pyridyl)glyoxylic acid (I) and 10 ml of a 10% ethanolic solution of HCl is boiled for 4 h. The solution is evaporated *in vacuo* and the esterification process is repeated. To the residue ice water and a 15% solution of Na_2CO_3 are added, and the mixture is extracted by ether. The residue after the distillation of ether is distilled *in vacuo*. Yield, 0.44 g (39%) of ethyl ester II in the form of a mobile greenish liquid, bp 153-155°C (20 mm Hg). Found, %: C 60.18; H 5.31; N 8.03. $C_9H_9NO_3$. Calculated, %: C 60.33, H 5.06; N 7.82.

Hydrochloride —colorless crystals, mp 185-187°C (dec.). Found, %: C 49.09; H 4.88; Cl 15.45; N. 5.97. $C_9H_9NO_3 \cdot HCl$. Calculated, %: C 46.26; H 5.17; Cl 15.17; N 5.99.

Oxime of (3-Pyridyl)glyoxylic Acid (IV). A solution of 1.39 g (20 mmoles) of $H_2NOH \cdot HCl$ and 1.68 g (20 mmoles) of $NaHCO_3$ in 5 ml of water is added to a solution of 3.02 g (20 mmoles) of acid I in 10 ml of 50% aqueous ethanol, and the mixture is allowed to stand for 20 h at 4°C. The precipitate that separates is filtered, washed with water and ethanol. Yield, 3.05 g (92%). Colorless crystals, mp 191-192°C (from water). The compound is sparingly soluble in water, and in insoluble in ethanol, $CHCl_3$, and acetone. IR spectrum, λ_{max}, cm^{-1}: 1540, 1625, 1680 (C=O, C=N), 3100 (OH). ^{13}C NMR spectrum, δ, ppm, anti-isomer: 173.2 (9 Hz)* COOH; 153.9 C=NOH; 132.3 C_3; 138.8 C_4; 124.7 C_5; 149.3, 148.3 C_2, C_6; syn-isomer: 162.3 COOH; 155.5 C=NOH; 130.1 (8 Hz)* C_3; 135.1 C_4; 125.1 C_5; 146.7, 149.6:

*The absolute value of SSCC ($J_{^{13}C^{15}N}$) for compound containing ^{15}N in the NOH group is shown in brackets.

C_2, C_6. Found %: C 50.71; H 3.49; B 16.80; M^+ 166. $C_7H_6N_2O_3$. Calculated, %: C 50.61; H 3.64; N 16.89; M 166.

Phenylhydrazone of (3-Pyridyl)glyoxylic Acid (IX). A solution of 0.71 g (6.6 mmoles) of phenylhydrazine in 3 ml of 35% ethanol is added to a solution of 1 g (6.6 mmoles) of acid I in 2 ml of water. The solution is allowed to stand for 2 h at 20°C, and phenylhydrazone IX that separates is filtered with suction, and washed with water. Yield, 1.6 g (quantitative). Yellow crystals, mp 169-171°C. The compound is sparingly soluble in ethanol and, MeOH, insoluble in water, $CHCl_3$, and acetone. IR spectrum, λ_{max}, cm^{-1}: 1553, 1600 (C=N, C=O), 3290, 3285 (NH, OH). Found, %: C 65.03; H 4.5; N 17.61. $C_{13}H_{11}N_3O_2$. Calculated %: C 64.72; H 4.6; N 16.42.

Semicarbazone of (3-Pyridyl)glyoxylic Acid (V). A solution of 1.47 g (13 mmoles) of semicarbazide hydrochloride and 1.1 g (13 mmoles) of $NaHCO_3$ in 4 ml of water is added to a solution of 2 g (13 mmoles) of acid I in 4 ml of water; a white precipitate begins to separate immediately. The reaction mixture is allowed to stand for 4 h at 20°C, the precipitate is filtered, washed with water and ethanol. Yield 2.65 g (96%). Colorless crystals, mp 219-220°C (dec). The compound is sparingly soluble in water and insoluble in organic solvents. IR spectrum, λ_{max}, cm^{-1}: 1680, 1730 (C=O), 3300-3500 (NH, OH). Found, %: C 42.18; H 4.51; N 25.15. $C_8H_8N_4O_4 \cdot H_2O$. Calculated, %: C 42.47; H 4.46; N 24.76.

4-Phenylsemicarbazone of (3-Pyridyl)glyoxylic Acid (VII). A solution of 1 g (6.6 mmoles) of 4-phenylsemicarbazide in 45 ml of ethanol is added to a solution of 1 g (6.6 mmoles) of acid I in 7 ml of 70% aqueous ethanol, and the mixture is allowed to stand for 10 h at 20°C. The precipitate is filtered, washed with water and ethanol. Yield 1.7 g (92%). Colorless crystals, mp 208-209°C (dec). The compound is sparingly soluble in water, and insoluble in ethanol, MeOH, $CHCl_3$, and acetone. IR spectrum, λ_{max}, cm^{-1}: 1594, 1620, 1668 (C=O, C=N), 3080, 3295, 3520 (NH, OH). Found, %: C 59.06; H 4.20; N 19.80. $C_{14}H_{12}N_4O_3$. Calculated, %: C 59.15; H 4.25; N 19.71.

Thiosemicarbazone of (3-Pyridyl)glyoxylic Acid (VI). A solution of 1 g (6.6 mmoles) of acid I in 10 ml of water is mixed with 0.6 g (6.6 mmoles) of thiosemicarbazide in 15 ml of water, and a light-yellow precipitate separates immediately. After 4 h, the precipitate is filtered, and washed with water and ethanol. Thus, 1.4 g (95%) of thiosemicarbazone VI are obtained. The compound is dissolved in 62.2 ml of 1 N NaOH and the solution is acidified by 62.2 ml of 1 N HCl. The precipitate is filtered, and washed with water. Yield, 1.25 g (85%). Light yellow crystals, mp 252-255°C. The compound is practically insoluble in water and in organic solvents. IR spectrum, λ_{max}, cm^{-1}: 1580, 1618, 1630, 1660 (C=N, C=O), 3060, 3092, 3190, 3285, 3375 (OH, NH). Found, %: C 42.93; H 3.56; N 25.06; M^+ 224. $C_8H_8N_4O_2$. Calculated, %: C 42.85; H 3.60; N 24.98. M 224.

4-Phenylthiosemicarbazone of (3-Pyridyl)glyoxylic Acid (VIII). A solution of 2.21 g (13 mmoles) of 4-phenylthiosemicarbazide in 45 ml of ethanol, heated to 90°C, is added to a solution of 2 g (13 mmoles) of acid I in 5 ml of water. The precipitate that separates after 20 h of standing at 20°C is filtered, washed with water and ethanol. Yield, 3.45 g (87%). Yellow crystals, mp 207-208°C. The compound is insoluble in water and usual organic solvents. When made alkaline by an equivalent amount of 1 N NaOH, a colorless Na salt is formed, which is also insoluble in water. By treatment of a suspension of the Na salt in water by 1 N HCl, the initial yellow acid is regenerated again. Found, %: C 55.81; H 3.77; N 18.71; $C_{14}H_{12}N_4O_2$. Calculated, %: C 55.99; H 4.03; N 18.65.

3,5-Dioxo-6-(3-Pyridyl)-2,3,4,5-tetrahydro-1,2,4-triazine (X). A 0.7 g portion (3.1 mmoles) of semicarbazone V is added to a solution of 1.36 g of potassium carbonate in 10 ml of water, and the mixture is boiled for 9 h. The solution is filtered and acidified by 1 N HCl to pH 6.0-7.0. The precipitate is filtered, and washed with water and ethanol. Yield, 0.35 g (55%). Colorless crystals, mp 344-345°C (dec., from DMFA). The compound is soluble in DMFA, sparingly soluble in acetone, and insoluble in water, ethanol, MeOH, $CHCl_3$. IR spectrum, λ_{max}, cm^{-1}: 1597, 1650, 1705 (C=N, C=O), 3120, 3220 (NH). Found, %: C 50.47; H 3.10; N 29.50; M^+ 190. $C_8H_6N_4O_2$. Calculated, %: C 50.53; H 3.18; N 29.46; M 190.

5-oxo-6-(3-pyridyl)-3-thioxo-2,3,4,5-tetrahydro-1,2,4-triazine (XI). A 0.5 g portion (2.2 mmoles) of thiosemicarbazone VI is mixed with a solution of 1 g of potassium carbonate in 8 ml of water, and the solution obtained is boiled for 8 h. The reaction mixture is diluted with 2 ml of water, decolorized by charcoal, and treated with 1 N HCl to pH 6.0-7.0.

The precipitate that separates is filtered, and washed with water and ethanol. Yield, 0.3 g (65%). Yellow crystals, mp 319-320°C (dec., from DMFA). The compound is sparingly soluble in acetone, MeOH, and insoluble in water, ethanol, and $CHCl_3$. IR spectrum, λ_{max}, cm^{-1}: 1618, 1700 (C=N, C=O), 3080 (NH). Found, %: C 46.54; H 3.07; N 27.20; S 15.29; M^+ 206. $C_8H_6N_4OS$. Calculated, %: C 46.50; H 2.93; N 27.16; S 15.50; M 206.

2-Oxo-3-(3-Pyridyl)-1,2-dihydroquinoxaline (XII). A solution of 0.5 g (3.3 moles) of acid I in 3 ml of water is added to a solution of 0.36 g (3.3 mmoles) of o-phenylenediamine in 3 ml of 95% ethanol. After mixing the solution, immediately a rose color appears, which rapidly deepens, and after 5 min, an abundant precipitate separates, while the temperature of the reaction mixture increases to 40°C. The mixture is allowed to stand for 20 h at 20°C, the precipitate is filtered and washed with water. Yield, 0.5 g (68%). Light-yellow crystals, mp 238-240°C (from 50% ethanol). IR spectrum, λ_{max}, cm^{-1}: 1593, 1610, 1660 (C=N, C=O), 3100 (NH). Found, %: C 69.24; H 4.04; N 19.19. $C_{13}H_9N_3O$. Calculated, %: C 69.95; H 4.06; N 18.82.

EXPERIMENTAL (MICROBIOLOGICAL)

The antibacterial and antifungal activity of compounds I-XI was studied by the method of double serial dilutions in liquid culture media. The concentration of the compounds preliminarily dissolved in DMFA was from 500 to 1 μg/ml. The antibacterial activity was determined in a Hottinger bullion containing 120 mg % of aminic nitrogen, while the antifungal properties were determined in Saburo bullion with the addition of 2% glucose and 2% maltose. The activity of the compounds was studied with respect to reference strains of Gram-positive (*Staphylococcus aureus* ATCC 25923, Staphylococcus pyogenes ATCC 12354) and Gram-negative bacteria (*Escherichia coli* , ATCC 25922, *Proteus vulgaris* ATCC 6896, *Pseudomonas aeruginosa* ATCC 27853), clinical strains of fungi-dermatophytes (*Microsporum canis* and *Trichophyton mentagraphtes* var. *gypseum*), and yeast-like fungi (*Candida albicans*). The optical density of a suspension of 24-h old agar cultures were determined with reference to a standard of 5 turbidity units, followed by dilution with a sterile physiological solution to a final concentration of $1\text{-}2\cdot10^5$ COU/ml for bacteria and about $2\cdot10^6$ COU/ml for fungi. The bacteria were incubated for 18-20 h at 35-37°C, fungi-dermatophytes for 5-6 days, and the yeast-like fungi for 20-24 h at 25-28°C.

The activity was taken into account visually according to the last test tube in which no visible growth of the microorganisms was evident. The minimal inhibiting concentrations were determined in micrograms per ml.

Our study showed the compounds IV-VI, X, XI have no antibacterial and anti-fungal activity. Compounds VII and VIII show a weak activity with respect to dermatophytes and medium activity with respect to gram-positive bacteria. A certain increase in the antimicrobial properties is possibly due to the introduction of the phenyl group into the semi(thiosemi)carbazone part of the molecule.

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