ISSN 1070-3632, Russian Journal of General Chemistry, 2016, Vol. 86, No. 12, pp. 2693–2695. © Pleiades Publishing, Ltd., 2016.
Original Russian Text © V.L. Gein, T.A. Silina, A.A. Cherepanov, A.P. Shishkin, B.Ya. Syropyatov, E.V. Voronina, L.I. Varkentin, 2016, published in Zhurnal Obshchei Khimii, 2016, Vol. 86, No. 12, pp. 2061–2063.

LETTERS TO THE EDITOR

Synthesis and Biological Activity of 1-Alkyl(heteryl)-5-oxopyrrolidine-3-carboxylic Acids

V. L. Gein*, T. A. Silina, A. A. Cherepanov, A. P. Shishkin, B. Ya. Syropyatov, E. V. Voronina, and L. I. Varkentin

Perm State Pharmaceutical Academy, Ministry of Healthcare of the Russian Federation, ul. Polevaya 2, Perm, 614990 Russia
**e-mail: geinvl48@mail.ru*

Received July 14, 2016

Keywords: 2-methylenebutanedioic (itaconic) acid, 1-alkyl(heteryl)-5-oxo-pyrrolidine-3-carboxylic acids, antimicrobial activity, anticoagulant activity

DOI: 10.1134/S1070363216120203

Biological activity of functional derivatives of pyrrolidine depends essentially on the degree of substitution of the heteroring and the nature of the substituents, in particular at the heteroatom. Substituted 5-oxopyrrolidinecarboxylic acids derivatives are promising compounds due to their availability and wide spectrum of biological activity [1–6]. In this regard, the synthesis of new derivatives of 5-oxopyrrolidine-3-carboxylic acid as well as studying their biological activity is of special interest.

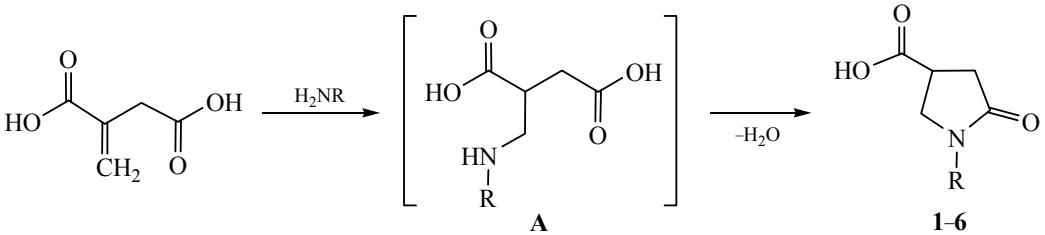
1-Alkyl(heteryl)-5-oxopyrrolidine-3-carboxylic acids were prepared by the method described previously in [1–5, 7]. Heating a mixture of aliphatic amine with an equimolar amount of itaconic acid without solvent until water liberation ceased afforded 1-alkyl-5-oxopyrrolidine-3-carboxylic acids **1**–**6**. In the case of heterocyclic amines acetic acid was used as a catalyst. 1-Heteryl-5-oxopyrrolidine-3-carboxylic acids **5** and **6** were isolated in good yields.

Apparently in the first stage, the addition of the amine to the double bond took place to form an intermediate **A**, which then underwent cyclization to provide the target compounds **1**–**6** (Scheme 1).

The resulting compounds **1**–**6** were colorless crystalline substances, soluble in polar organic solvents. Their structure and composition were confirmed by IR , NMR, and mass spectra, and elemental analysis data.

The IR spectra of compounds **1**–**6** contained the absorption bands of stretching vibrations of carboxyl OH (3150–3050 cm^{-1}) and C=O groups (1750–1730 cm^{-1}) as well as those pyrrolidin-5-one fragment (1720–1688 cm^{-1}). In the ^1H NMR spectra of compounds **1**–**6** there were the signals of oxopyrrolidine moiety and carboxy group (10.05–12.58 ppm). The peaks of molecular and fragment ions in the mass spectra of compounds **1**, **3**–**6** also confirmed the structure of compounds obtained.

Scheme 1.



R = $(CH_2)_7CH_3$ (**1**), $CH_2(CH_2)_{14}CH_3$ (**2**), cyclohexyl (**3**), *i*-Bu (**4**), 3-pyridyl (**5**), 2-benzothiazolyl (**6**).

Anticoagulant activity of 1-substituted 5-oxopyrrolidine-3-carboxylic acids **1–6**

Compound	Clotting time, s		Change in blood coagulation, %
	control	experiment	
1	37.90±3.47	37.70±1.50	0.5
2	27.01±1.13	27.20±1.51	–0.4
4	38.10±2.58	43.20±2.89	–13.4
6	64.30±1.79	83.00±3.39	–29.1
Heparin	29.9±0.48	36.6±1.82	–22.4
Etamsylate	28.9±1.11	24.5±0.94	15.2

Taking into account the fact that this class of compounds has not been studied in detail for the presence of any kind of biological activity, we carried preliminary testing pharmacological activity of the synthesized compounds.

Effects on blood coagulation system were studied *in vitro* (see the table). According to the data obtained 1-alkyl(heteryl)-5-oxopyrrolidine-3-carboxylic acids **4** and **6** show the direct anticoagulant action since they lengthen the time of citrated blood clotting, other compounds exhibit no pronounced effect on blood coagulation process.

Antibacterial activity of compounds **1–6** was determined with respect to the test cultures *Staphylococcus aureus* ATCC 6538-P and *Escherichia coli* ATCC 25922. According to the results, the compounds do not exhibit antibacterial activity.

General procedure for the synthesis of 1-alkyl-5-oxopyrrolidine-3-carboxylic acids 1-4. A mixture of 0.01 mol of 2-methylenebutanedioic acid and 0.01 mol of an aliphatic amine was heated at 170°C until water liberation ceased. After cooling, the reaction mixture was treated with ethanol. The precipitate was filtered off, dried, and recrystallized from ethanol.

1-Octyl-5-oxopyrrolidine-3-carboxylic acid (1). Yield 45%, mp 40–42°C. IR spectrum, ν, cm^{-1}: 1700 (C=O), 3100 (CO<u>OH</u>), 1740 (<u>CO</u>OH). ^{1}H NMR spectrum, δ, ppm: 11.14 s (1H, COOH), 3.61 m (2H, C^2H), 3.20 m (1H, C^3H), 2.54 m (2H, C^4H), 1.4 m [14H, $(CH_2)_7$], 0.91 t (3H, CH_3, J = 3.0 Hz). Mass spectrum, m/z (I_{rel}, %): 255 (15) $[M]^+$, 224 (7), 196 (22), 184 (20), 156 (100), 127 (42), 96 (12), 68 (18), 42 (16). Found, %: C 64.53; H 10.00; N 5.62. $C_{13}H_{23}NO_3$. Calculated, %: C 64.70; H 9.61; N 5.80.

1-Hexadecyl-5-oxopyrrolidine-3-carboxylic acid (2). Yield 56%, mp 79.5–80.5°C. IR spectrum, ν, cm^{-1}: 1705 (C=O), 3120 (CO<u>OH</u>), 1730 (<u>CO</u>OH). ^{1}H NMR spectrum, δ, ppm: 10.13 s (1H, COOH), 3.49 m (2H, C^2H), 3.16 m (1H, C^3H), 2.51 m (2H, C^4H), 0.86 t (3H, CH_3, J = 3.0 Hz), 1.24 m [30H, $(CH_2)_{15}$]. Found, %: C 71.25; H 11.01; N 3.84. $C_{21}H_{39}NO_3$. Calculated, %: C 71.34; H 11.12; N 3.96.

1-Cyclohexyl-5-oxopyrrolidine-3-carboxylic (3). Yield 52%, mp 190–192°C. IR spectrum, ν, cm^{-1}: 1688 (C=O), 3150 (CO<u>OH</u>), 1710 (<u>CO</u>OH). ^{1}H NMR spectrum, δ, ppm: 11.07 s (1H, COOH), 4.10 m (2H, C^2H), 3.25 m (1H, C^3H), 2.60 m (11H, cyclohexyl), 2.54 m (2H, C^4H). Mass spectrum, m/z (I_{rel}, %): 225 (35) $[M]^+$, 196 (4), 182 (68), 166 (7), 144 (100), 127 (7), 115 (27), 84 (15), 68 (14), 55 (22). Found, %: C 62.58; H 8.14; N 6.59. $C_{11}H_{17}NO_3$. Calculated, %: C 62.54; H 8.11; N 6.63.

1-Isobutyl-5-oxopyrrolidine-3-carboxylic acid (4). Yield 44%, mp 77–78°C. IR spectrum, ν, cm^{-1}: 1720 (C=O), 3100 (CO<u>OH</u>), 1710 (<u>CO</u>OH). ^{1}H NMR spectrum, δ, ppm: 10.05 s (1H, COOH), 3.57 m (2H, C^2H), 3.17 m (1H, C^3H), 3.05 m [1H, $(CH_3)_2\underline{CH}CH_2$], 2.52 d and 2.43 d [2H, $(CH_3)_2CH\underline{CH_2}$, J = 4.0 Hz], 2.48 m (2H, C^4H), 0.83 d and 0.81 d [6H, $(CH_3)_2CHCH_2$, J = 4.0 Hz]. Mass spectrum, m/z (I_{rel}, %): 199 (18) $[M]^+$, 184 (16), 156 (100), 144 (6), 127 (50), 115 (4), 96 (12), 84 (9), 68 (22), 42 (24). Found, %: C 58.29; H 8.15; N 7.48. $C_9H_{15}NO_3$. Calculated, %: C 58.36; H 8.16; N 7.56.

General procedure for the synthesis of 1-heteryl-5-oxopyrrolidine-3-carboxylic acids 5 and 6. A mixture of 0.01 mol of 2-methylenebutanedioic acid, 0.01 mol of heterylamine and 1 mL of glacial acetic acid was heated at 170°C for 1 h until water liberation ceased. After cooling the reaction mixture was treated with ethanol. The precipitate was filtered off, dried, and recrystallized from ethanol.

5-Oxo-1-(pyridin-3-yl)pyrrolidine-3-carboxylic acid (5). Yield 68%, mp 180–183°C. IR spectrum, ν, cm^{-1}: 1710 (C=O), 3050 (CO<u>OH</u>), 1700 (<u>CO</u>OH). ^{1}H NMR spectrum, δ, ppm: 10.26 s (1H, COOH), 7.32 d and 7.72 d (4H, pyridyl, J = 5.0 Hz), 4.1 m (2H, C^2H), 3.29 m (1H, C^3H), 2.76 m (2H, C^4H). Mass spectrum, m/z (I_{rel}, %): 220 (30) $[M]^+$, 189 (2), 178 (2), 161 (5), 146 (2), 133 (7), 107 (100), 92 (5), 78 (12), 51 (6). Found, %: C 58.35; H 4.91; N 13.62. $C_{10}H_{10}N_2O_3$. Calculated, %: C 58.25; H 4.89; N 13.59.

1-(Benzo[*d*]thiazol-2-yl)-5-oxopyrrolidine-3-carboxylic acid (6). Yield 83%, mp 225–227°C. IR spectrum, ν, cm^{-1}: 1740 (C=O), 3050 (CO<u>O</u>H), 1720 (<u>CO</u>OH). ^{1}H NMR spectrum, δ, ppm: 12.58 s (1H, COOH), 7.32 d, 7.44 d, 7.79 d and 7.98 d (4H, benzothiazolyl, J = 1.0 Hz), 4.32 m (2H, C^2H), 3.54 m (1H, C^3H), 2.48 m (2H, C^4H). Mass spectrum, m/z (I_{rel}, %): 276 (78) $[M]^+$, 245 (8), 217 (35), 189 (42), 163 (100), 150 (9), 136 (18), 108 (12), 69 (6), 55 (5). Found, %: C 55.02; H 3.88; N 10.71; S 12.19. $C_{12}H_{10}N_2O_3S$. Calculated, %: C 54.95; H 3.84; N 10.68; S 12.23.

^{1}H NMR spectra (DMSO-d_6) were recorded on a Bruker AM-300 (300 MHz) instrument, internal reference TMS. IR spectra were obtained on a Specord M-80 spectrometer from mulls in mineral oil. Mass spectra were taken on an Agilent 7820 gas chromatograph equipped with a mass selective detector with the use of a HP-5MS capillary column (30 × 0.25 mm) at an energy of ionizing electrons of 70 eV. 1-Substituted 5-oxopyrrolidine-3-carboxylic acids were analyzed by gas chromatography-mass spectrometry in the form of methyl esters obtained by reacting the corresponding acids with iodomethane. Elemental analysis was performed on a Perkin Elmer 2400 analyzer. Melting points were measured on a PTP (M) instrument.

Antibacterial activity was determined with respect to the test cultures *Staphylococcus aureus* ATCC 6538-P and *Escherichia coli* ATCC 25922 by two-fold serial dilutions in a liquid medium with a bacterial load of 250 thousand microbial units per 1 mL of a solution [8]. Minimum inhibitory concentration (MIC) was evaluated by the absence of bacterial growth. The last tube with growth retardation (a clear solution) corresponds to MIC of the test compound against this strain. Chloramine B and furacilin were used as comparative compounds.

Anticoagulant activity was examined with use of a Minilab 701 coagulometer. Citrated (3.8%) blood (9 : 1) was used. Effect of the test compounds on blood clotting was studied in the same concentration of 1.0 mg/mL using a solution of heparin (1.0 U/mL) as a reference for anticoagulant activity or etamsylate solution (1.0 mg/mL) as a reference for haemostatic activity.

REFERENCES

1. Beresnevicius, Z.I. and Viliunas, V., *Chem. Heterocycl. Compd*., 2000, vol. 36, no. 7, p. 818. doi 10.1007/BF02256916
2. Voskiene, A. and Mickevicius, V., *Chem. Heterocycl. Compd*., 2007, vol. 43, no. 11, p. 1379. doi 10.1007/S10593-007-0213-8
3. Kolobov, A.V., Ovchinnikov, K.L., Danilova, A.S., and Kofanov, E.R., *Izv. Vuzov, Ser. Khim. i Khim. Tekhnol.*, 2006, vol. 43, no. 3, p. 3.
4. Mickevicius, V. and Patupaite, A., *Chem. Heterocycl. Compd*., 2000, vol. 36, p. 837. doi 10.1007/BF02256919
5. Mickevicius, V., Beresnevicius, I.G., Mickevicius, M., and Sapijanskaite, B., *Chem. Heterocycl. Compd*., 2005, vol. 41, no. 7, p. 932. doi 10.1007/S10593-005-0251-z
6. Kharchenko, Y.V., Detistov, O.S., and Orlov, V.D., *Chem. Heterocycl. Compd*., 2008, vol. 44, p. 600. doi 10.1007/s10593-008-0080-y
7. *Syntezy organicheskikh preparatov* (Syntheses of Organic Compounds), Moscow: Inostrannaya Literatura, 1949, no. 2, p. 82.
8. Pershin, G.N., *Metody eksperimental'noi khimioterapii* (Experimental Chemotherapy Methods), Moscow: Meditsinskaya Literatura, 1971.