Synthesis of Some Pyrazol-5-ols Related to Muscimol

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The syntheses of the zwitterions 3-aminomethylypyrazol-5-ol (5a), 3-(2-aminooethyl)pyrazol-5-ol (5b), 3-(1-aminooethyl)pyrazol-5-ol (5c), and 4,4-dimethyl-4,5,6,7-tetrahydropyrazolo[4,3-c]-pyridin-3-ol (7b) are described. 5a–c were prepared from the corresponding dihydrochlorides 4a–c by treatment with triethylamine. The 3-(2-aminooethyl)- and the 3-(1-aminooethyl)pyrazol-5-ol dihydrochloride (4b,c) were obtained by treatment of N-phthaloyl-β- and α-alaninmalonic ester (5b,c), respectively, with hydrazine hydrate in glacial acetic acid followed by refluxing of the formed 3-(N-phthaloylalanyl)-4-ethoxybenzoylpyrazol-5-ols 9b,c with 7 M hydrochloric acid. The 3-(2-aminooethyl)pyrazol-5-ol dihydrochloride (4b) reacted with acetone and triethylamine to give the 4,4-dimethyl-4,5,6,7-tetrahydropyrazolo[4,3-c]pyridin-3-ol (7b).

The syntheses of 3-[N-(o-carbazoylbenezolyl)-2-aminooethyl]pyrazol-5-ol hydrochloride (6b), N-phthaloyl-β-alanine hydrazide (10b), and the new tetracyclic ring system 4,5-dihydro-1-hydroxy-1H-pyrazolo[4',3':3,4]pyrido[2,1-a]isoindol-7-one (11b) are also described.

Muscimol (5-aminomethyl-3-isoxazolol), a constituent of Amanita muscaria, is a powerful GABA (γ-aminobutyric acid) agonist. Several muscimol analogues containing various aminoalkyl groups in position 4 or 5 of the 3-isoxazolol nucleus have been synthesized and tested biologically. As part of our interest in muscimol analogues in which the heterocyclic ring system has been changed, the zwitterions 3-aminomethylypyrazol-5-ol (azamuscimol) (5a), 3-(2-aminooethyl)pyrazol-5-ol (homozamuscimol) (5b), 3-(1-aminooethyl)pyrazol-5-ol (5c), and 4,4-dimethyl-4,5,6,7-tetrahydropyrazolo[4,3-c]pyridin-3-ol (7b) have now been prepared. Azamuscimol (5a) has a relatively weak depressant action on spinal neurons in cats (microelectrophoresis), and it has been shown to be an inhibitor of L-glutamate decarboxylase (GAD). Finally azamuscimol (5a) has been tested in vitro for antitubercular activity, but no antibacterial activity could be shown. The biological properties of the azamuscinol analogues 5b, 5c, and 7b will be examined in the near future.

Azamuscimol dihydrochloride (4a) was converted into the zwitterion 5a by treatment with triethylamine followed by addition of acetone. A new improved synthesis of ethyl (N-phthaloyl-β-alanyl)acetate (2b) was based on treatment of ethyl (N-phthaloyl-β-alanyl)acetate (1b) with concentrated aqueous ammonia in ethanol. Attempts to synthesize homoazamuscimol (5b) by the reaction sequence used for the preparation of azamuscimol (5a) (Scheme 1) failed, as 2b with hydrazine hydrate in ethanol at room temperature gave 11b (Scheme 2) as the main product. The tetracyclic ring system of 11b has not previously been described, but the corresponding tricyclic ring system without the pyrazolo ring is well-known, as 1b by treatment with base (pyridine) yielded 1-acetyl-3,4-dihydropyrido[2,1-a]isoindole-2,6-dione. Treatment of 2b with hydrazine hydrate in ethanol at 0–12 °C yielded a mixture of 3-(N-phthaloyl-2-aminooethyl)pyrazol-5-ol (3b) and unreacted 2b. Attempts to remove the phthaloyl group of 3b by treatment with hydrazine hydrate in boiling ethanol led to 6b, which was stable to boiling with 4 M hydrochloric acid for 3 h. Refluxing of 3b in 7 M hydrochloric acid for 3 h gave a complex reaction mixture containing unreacted 3b.

Finally the dihydrochloride 4b of homozamuscimol was prepared by treatment of 8b with hydrazine hydrate in glacial acetic acid followed by refluxing of the pyrazolol 9b.
with 7 M hydrochloric acid. Treatment of 8b with hydrazine hydrate in ethanol at ambient temperature yielded N-phthaloyl-β-alanine hydrazide (10b) (Scheme 2). Homoazamuscimol (5b) was prepared by treatment of the dihydrochloride 4b with triethylamine in ethanol. Attempts to convert 4b into 5b by treatment with triethylamine and acetone led to the tetrahydropyrazolo[4,3-c]pyridin-3-ol 7b, instead of the expected zwitterion 5b.

The 3-(1-aminooethyl)pyrazol-5-ol zwitterion (5c) could be prepared by the procedure used for homoazamuscimol (5b) using 8c as the starting material (Scheme 1). In an attempt to elevate the yield of 5c, 12 was used as starting material but the sequence outlined in

Scheme 1.

Scheme 2.

Scheme 1 also yielded 5c in a rather poor yield. Attempts to convert 5e into ethyl (N-phthaloyl-α-alanyl)acetate (2e) by refluxing of 5e with water or pyridine-water failed.

The structure determinations of the new compounds 4b, 5a-c, 7b, 6b, 5b-c, 7b and 10b were based on IR, UV, and 1H NMR spectroscopy and supported by elemental analyses. The structure of the new ring system of 11b was further confirmed by mass spectrometry. The described pyrazoles were assigned the pyrazolol structure rather than the pyrazolinone structure due to absence of infrared absorption at 1710–1700 cm⁻¹ and absence of ultraviolet absorption about 250 nm with an ε value of 1.8–2.5 × 10⁴ and about 310 nm with an ε value of 8.0 × 10⁴, all characteristics of pyrazolinones.¹⁶ The structure determination of the pyrazolols was supported by 1H NMR spectroscopy and by the finding that the pyrazolols formed coloured complexes with ferric chloride.¹³ Broad IR absorptions of 5a-c and 7b in the range 3600–1800 cm⁻¹ are in agreement with the presence of ammonium groups in these compounds, and consequently these compounds are zwitterions. The zwitterion 5c crystallized with 1 mol of water, the removal of which by heating was accompanied by destruction of the compound.

EXPERIMENTAL

Melting points are corrected and were determined with a hot stage microscope (Mikroskop-Heiztisch, 350 Ernst Leitz G.m.b.H., Wetzlar). Thin layer chromatography (TLC) was accomplished by using silica gel GF₂⁵₄ plates (Merck). The recording of IR (KBr technique), UV (methanol solutions), and ¹H NMR spectra, and the performance of microanalyses were accomplished as described in a previous paper.⁸ The mass spectrum was obtained with an AEII MS 902 mass spectrometer operating at 70 eV. pH values were measured on a Radiometer pH meter 26 and the pKₐ values were determined according to the method of Albert and Sorjeant.¹⁴

3-Aminomethylpyrazol-5-ol zwitterion (5a).

A solution of 1.86 g (10 mmol) of 4a in 3 ml of water was chilled in an ice bath and treated with 2.23 g (22 mmol) of triethylamine. The reaction mixture was diluted with 10 ml of acetone, stirred thoroughly, and filtered. Recrystallization (ethanol—water) gave 870 mg (66%) of 5a, H₂O, m.p. 220 °C (decomp.). Anal. C₇H₁₄N₂O₂H₂O: C, H, N. The water of crystallization could be removed upon heating in vacuo at 110 °C for 3 h. Anal. C₉H₈N₂O₄C: C, H, N. λₘₐₓ 240 nm (ε = 4.00 × 10⁴). IR data: 3600–2000 (s) (with submaxima at 3350, 3200, 3050, 2900, 2800, 2700, 2600, 2450, 2100), 1660 (m), 1620 (s), 1580 (s), 1550 (s), 1470 (s) cm⁻¹. ¹H NMR data (DMSO-d₆): δ 5.24 (s, 1 H, C=CH); 4.2 (broad signal from H₂O and NH protons); 3.52 (s, 2 H, N-CH₃); pKₐ values (H₂O, 21 °C): 1.85 ± 0.09, 6.15 ± 0.02, and 9.78 ± 0.02.

Ethyl (N-phthaloyl-β-alanyl)acetate (2b). A solution of 13 g (40 mmol) of 2b in 60 ml of ethanol was treated with 4 ml of aqueous ammonia (g 0.88) during 10 min at 80 °C. After cooling to room temperature the precipitate was collected and washed with two 5 ml portions of ethanol. After drying 6.1 g (53%) of 2b was obtained, m.p. 90–91 °C (Ref. 11, m.p. 90 °C). λₘₐₓ 290 nm (ε = 1.80 × 10⁴), 241 nm (ε = 9.79 × 10³), 235 nm (ε = 1.38 × 10⁴), and 220 nm (ε = 3.63 × 10³). IR data: 3450 (m), 1770 (m), 1710 (s), 1440 (m) cm⁻¹. ¹H NMR data (DMSO-d₆): δ 7.87 (s, 4 aromatic protons); 4.10 (g, J = 7 Hz, 2 H, C–CH₂–OOC); 3.82 (t, J = 7 Hz, 2 H, N–CH₃–C); 3.63 (s, 2 H, CO–CH₃–COO); 2.98 (t, J = 7 Hz, 2 H, C–CH₃–CO); 1.71 (t, J = 7 Hz, 3 H, CH₃).

4,5-Dihydropyrazolo[4',3',4,3]-pyrido[2,1-a]isoindol-7-one (11b). To a suspension of 5.2 g (18 mmol) of 2b in 75 ml of ethanol was added 900 mg (18 mmol) of hydroazine hydrate. After stirring overnight at room temperature, the precipitate was collected and dried to give 1.2 g (28%) of 11b. An analytical sample was recrystallized in ethanol to give 11b as red needles, m.p. 284–285 °C. Anal. C₁₁H₁₀N₄O₂; C, H, N. λₘₐₓ 306 nm (ε = 1.61 × 10⁴) and 232 nm (ε = 2.18 × 10⁴). IR data: 3250 (m), 1710 (s), 1680 (s), 1620 (s), 1570 (m), 1470 (m), 1410 (m) cm⁻¹. ¹H NMR data (CF₃COOD): δ 9.1 (broad signal, 1 H, NH); 8.0 (s, 4 aromatic protons); 4.40 (t, 2 H, CH₃); 3.53 (t, 2 H, CH₃). MS [m/e (% rel. int.)]: 237 (100, M), 210 (32 [M–N₃H]), 182 (71. [M–CON–H]).¹⁸

3-[N-(Carbazoylbenzoyl)-2-aminoethyl]-pyrazol-5-ol hydrochloride (6b). To a suspension of 2.6 g (9 mmol) of 2b in 60 ml of ethanol was added 450 mg (9 mmol) of hydrazine hydrate. After stirring at 12 °C for 3 h the precipitate was collected and dried to give 1.4 g of a mixture of 3b and 2b (rendered probable by TLC). The crude mixture (1.4 g) and 300 mg (6 mmol) of hydrazine hydrate in 15 ml of ethanol was heated at reflux for 1 h. After cooling to room temperature the precipitate was collected and dissolved in 4 N hydrochloric acid. The solution was concentrated in vacuo and the crystalline residue recrystallized from methanol-ether to give 580 mg of 6b (20%). m.p. 285 °C (decomp.). Anal. C₁₆H₁₄N₄O₂ClC: C, H, N, Cl. λₘₐₓ 299 nm (ε = 6.73 × 10⁴), 249 nm (ε = 1.02 × 10⁴), and 210 nm (ε = 2.69 × 10⁴). IR data: 3700–1900 (s), 1600–1500 (s) (with

submaxima at 1655, 1635, 1630, 1620, 1610, 1545 (m), 1530 (s), 1480 (m), 1465 (s) cm\(^{-1}\). IR NMR data (DMSO-\(d_6\)): \(\delta\) 12.7 (s, 1 H, NH); 6.8 (6 H, ArH); 3.8 (m, aromatic protons); 5.8 (broad, 5 H, NH, OH, and C = CH); 2.9 (s, 4 H, 2 \times CH\(_2\)).

3-(N-Phthaloyl-2-aminoethyl)-4-ethoxy carbonylpyrazol-5-ol (9b). A mixture of 1.8 g (5 mmol) of 8b\(^{13}\) and 250 \(\mu\)l (5 mmol) of hydrazine hydrate in 8 ml of glacial acetic acid was refluxed for 1 h. The solution was evaporated in vacuo and upon recrystallization (ethanol) of the residue 0.9 g (55\% of 9b) was obtained, m.p. 175 - 177\(^\circ\)C. Anal. \(C_7H_8N_2O_4\): C, H, N. \(\lambda_{max}\) 263 nm (\(\varepsilon = 6.12 \times 10^4\)), 240 nm (\(\varepsilon = 3.13 \times 10^4\)), and 220 nm (\(\varepsilon = 4.67 \times 10^4\)). IR data: 3250 (m), 1770 (s), 1710 (s), 1590 (s), 1530 (m), 1470 (m), 1440 (m) cm\(^{-1}\). \(\Delta\) NMR data (DMSO-\(d_6\)): \(\delta\) 9 - 12 (broadened singal, 2 H, NH and OH); 7.83 (s, 4 aromatic protons); 4.05 (q, \(J = 7\) Hz, 2 H, C-CH\(_2\)-OCO); 3.85 (t, \(J = 6\) Hz, 2 H, C-CH\(_2\)-OCO); 3.00 (m, \(J = 7\) Hz, 2 H, C-CH\(_2\)-OCO); 2.12 (s, \(J = 7\) Hz, 3 H, CH\(_3\)).

3-(2-Aminomethyl)pyrazol-5-ol dihydrochloride (4b). A mixture of 3.7 g (11 mmol) of 9b and 90 ml of 7 M hydrochloric acid was refluxed for 2 h. After cooling to room temperature the precipitate was removed and the solution was concentrated in vacuo. The crystalline residue was recrystallized from methanol-ether to give 1.2 g (55\% of 4b, m.p. 185 - 188\(^\circ\)C (decomp.). Anal. \(C_7H_8N_2O_4Cl_2\): C, H, N, Cl. \(\lambda_{max}\) 246 nm (\(\varepsilon = 1.73 \times 10^4\)), and 218 nm (\(\varepsilon = 5.49 \times 10^4\)). IR data: 3600 - 2100 (s) with submaxima at 3350 and 3150), 1625 (s), 1570 (m), 1640 (m), 1470 (s) cm\(^{-1}\). \(\Delta\) NMR data (DMSO-\(d_6\)): \(\delta\) 13.37 (s, 3 H, NH and OH, exchangeable with D\(_2\)O); 8.40 (s, 3 H, NH); 5.87 (s, 1 H, C = CH); 3.97 (m, 2 H, NH); 1.8 (m, 4 aromatic protons). 1H NMR data (DMSO-\(d_6\)): \(\delta\) 9.14.4 (broad signal, 2 H, NH and OH); 7.32 (s, 4 aromatic protons); 5.60 (q, \(J = 7\) Hz, 1 H, N-CH\(_2\)-C); 4.03 (q, \(J = 7\) Hz, 2 H, C-CH\(_2\)-OCO); 1.75 (d, \(J = 7\) Hz, 3 H, CH\(_3\)-C-N); 1.08 (t, \(J = 7\) Hz, 3 H, CH\(_3\)-C-OCO).

3-(1-Aminopropyl)-pyrazol-5-ol dihydrochloride (5a). A solution of 500 mg (1.5 mmol) of 9c and 12 ml of 7 M hydrochloric acid was refluxed for 2 h. After cooling to room temperature the precipitate was removed and the solution was concentrated in vacuo to give 200 mg (1 mmol) of 5c as an oil. The oily product was dissolved in 5 ml of ethanol and treated with 280 \(\mu\)l (2 mmol) of triethylamine to give 500 mg of crude product. Recrystallization (methanol-ether) gave 380 mg (68\% of 7b, 6H, H; m.p. 200 \(^\circ\)C (decomp.). Anal. \(C_7H_8N_2O_4Cl_2\): C, H, N. The water of crystallization could be removed upon heating in vacuo at 120 \(^\circ\)C for 18 h. Anal. \(C_7H_8N_2O_4\): C, H, N. \(\lambda_{max}\) 245 nm (\(\varepsilon = 4.52 \times 10^4\)). IR data: 3900 - 2000 (s) with submaxima at 1320, 1300, 1280, 1250, 1200, 1150 (s), 1580 (s), 1530 (s), 1490 (s), 1430 (s) cm\(^{-1}\). \(\Delta\) H NMR data (DMSO-\(d_6\)): \(\delta\) 5.5 (broad signal, 5 H, H\(_2\)O and 3 NH, exchangeable with D\(_2\)O); 2.85 (t, \(J = 7\) Hz, 3 H, CH\(_2\)); 2.32 (t, \(J = 7\) Hz, 2 H, CH\(_2\)); 1.22 (s, 6 H, 2 \times CH\(_3\)).

N-Phthaloyl-\(\beta\)-alanine hydrazide (10b). 10b was synthesized as described above for 11b using 1.8 g (5 mmol) of 8b\(^{13}\) and 250 \(\mu\)l (5 mmol) of hydrazine hydrate. After stirring for 5 min 0.9 g (77\% of 10b) was obtained. An analytical sample was recrystallized (ethanol) to give 10b, m.p. 174 - 176 \(^\circ\)C. Anal. \(C_7H_8N_2O_4\): C, H, N. \(\lambda_{max}\) 293 nm (\(\varepsilon = 1.67 \times 10^4\)), 241 nm (\(\varepsilon = 1.06 \times 10^4\)), 232 nm (\(\varepsilon = 1.59 \times 10^4\)), and 220 nm (\(\varepsilon = 4.01 \times 10^4\)). IR data: 3300 (m), 1770 (m), 1710 (s), 1640 (s), 1610 (m), 1530 (m), 1440 (m) cm\(^{-1}\). \(\Delta\) H NMR data (DMSO-\(d_6\)): \(\delta\) 7.85 (s, 4 aromatic protons); 5.1 (broad signal, 3 H, NH, exchangeable with D\(_2\)O); 3.75 (t, \(J = 7\) Hz, 2 H, C-CH\(_2\)-CO).
2500, 2100), 1660 - 1490 (s) (with submaxima at 1640, 1565, 1550, 1540), 1490 - 1420 (s) (with submaxima at 1460, 1450, 1440) cm\(^{-1}\).

1H NMR data (DMSO-d\(_6\)): δ 7.9 (broad signal, 6 H, 4 NH and H\(_2\)O); 5.3 (s, 1 H, C=CH); 4.0 (broad signal, 1 H, CH); 1.4 (broad signal, 3 H, CH\(_3\)).

(b) A solution of 1.3 g (5 mmol) of 12\(\alpha\) in 20 ml of ethanol was treated with 250 μl (5 mmol) of hydrazine hydrate for 10 min. The solution was concentrated in vacuo and the oily residue was dissolved in 20 ml of 2 M hydrochloric acid. The acid solution was evaporated in vacuo to give 450 mg (2.3 mmol) of 4e as an oil. The oily product was dissolved in 10 ml of ethanol and treated with 550 μl (4 mmol) of triethylamine. The precipitate was collected, dried and upon recrystallization (water-ethanol) 70 mg (16 %) of 5e.H\(_2\)O was obtained.

**Biological test.** Azamuscimol (5a) was tested *in vitro* on Löwenstein-Jensen medium against strains of *M. tuberculosis* (No. E 10883/45 and 1921/41) as described by Engbæk *et al.* Azamuscimol (5a) had no influence on the bacteria growth at concentrations where isoniazid caused complete inhibition.

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