Acta Chemica Scandinavica B 30 (1976) 567—573

Synthesis of Some 4-Aminoalkyl-5-methyl-3-isoxazolols Structurally Related to Muscimol and γ-Aminobutyric Acid (GABA)

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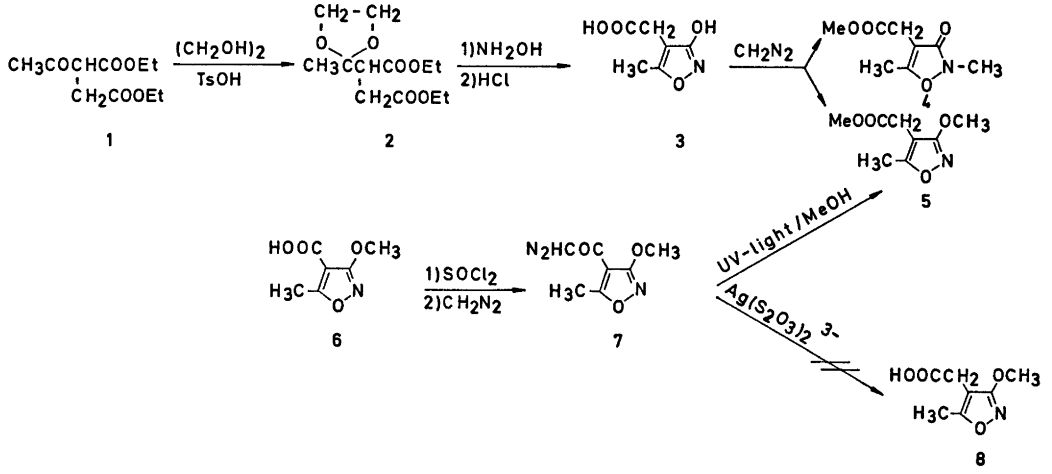
The synthesis of 4-(2-aminoethyl)-5-methyl-3-isoxazolol zwitterion (*16a*) and 4-(2-aminopropyl)-5-methyl-3-isoxazolol zwitterion (*16b*) which are structural analogues of 5-aminomethyl-3-isoxazolol (Muscimol) and of γ-aminobutyric acid (GABA) is described. The key reaction of the sequences leading to these compounds involves hydroxylamine treatment of the appropriately substituted five-membered cyclic enamides (*13a* and *b*). Furthermore the preparation of the β-alanine analogues 4-aminomethyl-5-methyl-3-isoxazolol zwitterion (*19*) and 4-(1-aminoethyl)-5-methyl-3-isoxazolol zwitterion (*20*) is described. The pK_A values of all four compounds have been determined.

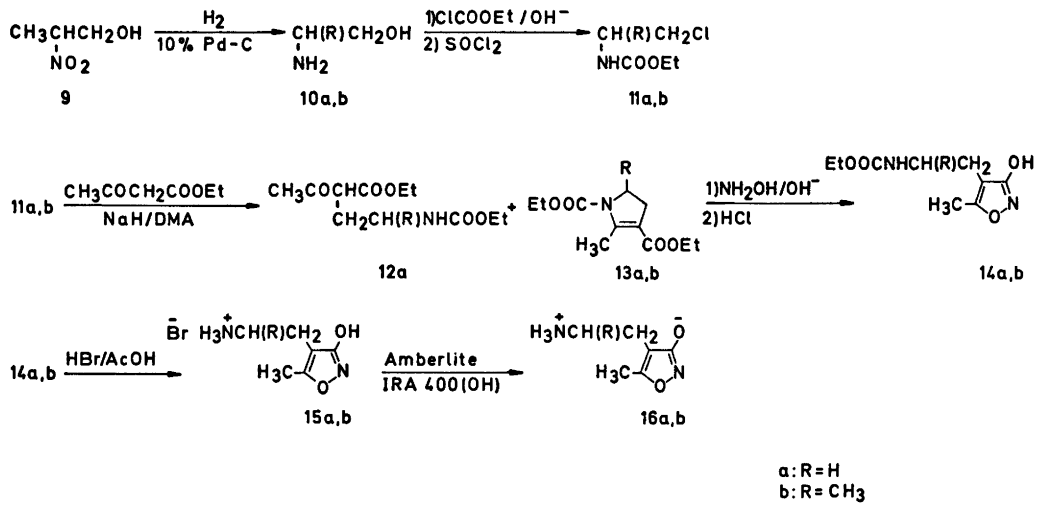
As part of the investigations of the biological properties of conformationally restricted analogues of γ-aminobutyric acid (GABA) structurally related to muscimol (5-aminomethyl-3-isoxazolol) [1,2] a series of bicyclic [3,4] and 5-aminoalkylsubstituted [5–7] 3-isoxazolols has been prepared. This paper presents the synthesis of some 3-isoxazololes with an aminoalkyl moiety in the 4-position.

As an approach to the synthesis of 4-(2-aminoethyl)-5-methyl-3-isoxazolol (*16a*), the method of preparation of 3-isoxazololes *via* ethylene acetals of β-oxoesters [4,8,9] was extended to the preparation of 3-hydroxy-5-methylisoxazol-4-ylacetic acid (*3*). Reaction of the ethylene acetal *2* with hydroxylamine, however, gave *3* in a poor yield and since *5* could only be obtained in a 30 % yield by treatment of *3* with diazomethane further transformations in the planned reaction sequence were considered of limited value.

The synthesis of the 3-methoxyisoxazole *5* *via* the diazoketone *7*, which was readily obtained from the acid *6* [10] upon treatment with



Scheme 1.



Scheme 2.

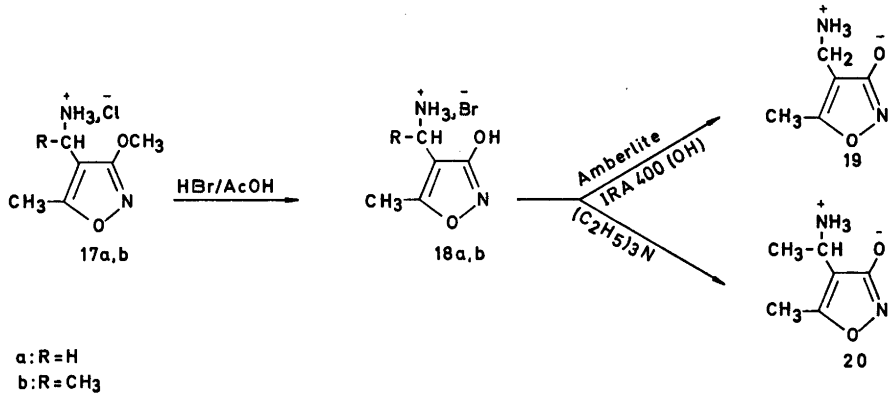
thionyl chloride followed by diazomethane, was then examined. A methanolic solution of the diazoketone *7* was irradiated with unfiltered UV-light to give *5* in a good yield. However, several experiments revealed the limit of the synthetic scale under the used conditions to be about 200 mg of *7*. Attempts to rearrange the diazoketone *7* to *8* using silver thiosulfate according to the method of Wiberg and Hutton [11] led to complex mixtures from which no compound was isolated.

Finally *16a* was successfully prepared by the sequence outlined in Scheme 2. Ethyl acetoacetate was alkylated by the urethane *11a* to give a mixture of two compounds. Column chromatography of the mixture gave the compound with the smaller R_F value in a pure state, whereas the other compound during this procedure was partly transformed into the compound with the smaller R_F value and could not be isolated in a pure state. Based on IR, UV, and ^{1}H NMR spectroscopy and supported by chemical evidence the compound with the smaller R_F value was shown to be the cyclic enamide *13a*. On the basis of spectroscopic and TLC examinations the other compound was assigned the β-oxoester structure *12a*. Treatment of the above-mentioned mixture of *12a* and *13a* in boiling toluene for 8 h using 4-toluenesulfonic acid as a catalyst completed the cyclization of *12a* into *13a* which was obtained in a good yield.

The cyclic enamide *13a* was treated with hydroxylamine to give a reasonable yield of the isoxazole *14a*. Investigation of the reaction mixture on TLC plates, however, revealed the presence of trace amounts of a compound which gave a violet spot on TLC using iron(III) chloride as a spraying reagent. The compound may be the corresponding 5-isoxazolone [3-methyl-4-(2-ethoxycarbonylaminoethyl)-5-isoxazolone] but several attempts to isolate the compound in a pure state were unsuccessful. The observed reaction course is remarkable as various β-oxoesters protected at the oxo group as benzyl-enamines, upon treatment with hydroxylamine exclusively yield 5-isoxazolones.[13]

In the sequence leading to the isoxazolol zwitterion *16b* the starting material 2-nitropropan-1-ol (*9*) was low-pressure hydrogenated using a 10 % Pd-C catalyst to give 2-aminopropan-1-ol (*10b*). The desired compound *16b* was obtained by a reaction sequence analogous to that described above including the cyclic enamide *13b*. All attempts to obtain *16b* in a crystalline state failed, and the compound could only be isolated as a glassy substance.

The synthesis of 4-aminomethyl-5-methyl-3-isoxazolol zwitterion (*19*) was achieved from 3-methoxy-4-aminomethyl-5-methylisoxazole hydrochloride (*17a*) prepared according to the directions of Bowden *et al.*[10] *17a* was readily cleaved by hydrogen bromide in glacial acetic



Scheme 3.

acid to give the 3-isoxazolol *18a* which by use of a strongly basic ion exchange resin gave the zwitterion *19*. This compound has previously been prepared by Bowden *et al.* by hydrolytic cleavage of 4-trifluoroacetamidomethyl-5-methyl-3-isoxazolol but was isolated as the 4-toluenesulfonate by these authors.[10]

A strong UV absorption at 281 nm and an absorption band at 1630 cm^{-1} in the IR spectra indicated the presence of conjugation in the compounds *13a* and *b*. This together with the ^1H NMR spectra and elemental analyses confirmed the cyclic enamide structure of these compounds.

The IR, UV, and ^1H NMR data obtained from the 3-oxygenated isoxazole moieties of *3*, *5*, *7*, *14a,b*, *15a,b*, and from the 3-isoxazolone moiety of *4* are in accordance with the general findings described by Jacquier *et al.*[8] The spectroscopic data of the zwitterions *16a*, *19*, and *20* are in accordance with those published for other isoxazole zwitterions.[3-7] The IR absorption bands at 2100, 1620, and 1360 cm^{-1} in the spectrum of *7* are in accordance with the results published for various diazocarbonyl compounds by Yates *et al.*[14] Some IR, UV, and ^1H NMR data of the starting materials *6* and *17a,b* are given in the experimental part as no spectroscopic data of these compounds are available in the literature.

The pK_A values of *16a,b*, *19*, and *20* are given in the experimental part. The pK_A values of the compounds are in accordance with those published for other isoxazole zwitterions.[3-7]

EXPERIMENTAL

Unless otherwise stated the determination of melting points, the recording of IR, UV, and ^1H NMR spectra, and the performance of microanalyses were accomplished as described in a previous paper.[13] Thin layer and column chromatographic procedures were accomplished using silica gel GF_{254} plates (Merck) and silica gel, 0.05–0.20 mm (Merck), respectively. pH values were measured on a Radiometer pH meter 26. The pK_A values were determined according to the method described by Albert and Serjeant[15] as described in a previous paper.[5]

Diethyl acetosuccinate acetal (2). A solution of 54.0 g (0.25 mol) of diethyl acetosuccinate (*1*),[16] 25.0 g (0.40 mol) of ethylene glycol, and 1 g of 4-toluenesulfonic acid in 500 ml of benzene was refluxed for *ca.* 40 h using a Dean-Stark water separator. The solution was washed with two 200 ml portions of water, dried (K_2CO_3), and distilled to give 40.9 g (62 %) of *2* as a colourless oil, b.p. 155–158 °C/7 mmHg. An analytical sample was further purified by column chromatography using CH_2Cl_2 as an eluent. (Found C 55.30; H 7.84. Calc. for $C_{12}H_{20}O_6$: C 55.37; H 7.75). IR data (neat) cm^{-1}: 1735(s). ^1H NMR data ($CDCl_3$): δ 4.13 and 4.07 [2 × q (J = 7 Hz in both cases), 4 H, 2 × CH_2-CH_2-O]; 3.95 (s, 4 H, $O-CH_2-CH_2-O$); 3.3–3.0 (m, 1 H, $CH-CH_2$); 2.9–2.2 (m, 2H, $CH-CH_2$); 1.38 (s, 3 H, CH_3-C); 1.27 and 1.23 [2 × t (J = 7 Hz in both cases), 6 H, 2 × CH_3-CH_2-O].

3-Hydroxy-5-methylisoxazol-4-ylacetic acid (3). To a solution of 15.2 g (0.22 mol) of hydroxylamine hydrochloride in 400 ml of methanol was added 41.4 g (0.30 mol) of potassium carbonate. After stirring for 15 min 52.0 g (0.20 mol) of *2* was added and the suspension was refluxed for 12 h. The filtered reaction mixture was evaporated *in vacuo* to give an oil which was dissolved in 250 ml of water.

The aqueous solution was extracted with three 100 ml portions of methylene chloride which were discarded. To the aqueous phase was added 250 ml of concentrated hydrochloric acid and the solution was boiled for 1 h. The solution was extracted continuously for 2 h by ether-methylene chloride 4:1 to give 11.1 g of an oily product which was submitted to column chromatography (silica gel: 500 g; eluent: benzene-ethyl acetate-formic acid 10:20:0.3 to which increasing amounts of ethyl acetate were added) to yield 1.60 g (5.2 %) of yellowish crystals. An analytical sample was recrystallized from ethyl acetate to give colourless crystals, m.p. 150–152 °C. (Found: C 45.95; H 4.52; N 8.80. Calc. for $C_6H_7NO_4$: C 45.86; H 4.49; N 8.97). $\lambda_{max}(C_2H_5OH)$: 211 nm ($\varepsilon = 5.92 \times 10^3$). IR (KBr) cm^{-1}: 3600–2200(s); 1700(s); 1670(s); 1560–1520 (s, several bands). ^1H NMR (CD_3COCD_3): δ 9.68 (s, 2 H, O*H* and COO*H*); 3.33 (s, 2H, OC–C*H*$_2$–C); 2.27 (s, 3 H, C*H*$_3$–C=).

Methyl 2,5-dimethyl-3-oxoisoxazolin-4-yl-acetate (4) and methyl 3-methoxy-5-methylisoxazol-4-ylacetate (5). To a solution of 750 mg (4.8 mmol) of 3-hydroxy-5-methylisoxazol-4-ylacetic acid (*3*) in 50 ml of ether was added with stirring a solution of *ca.* 0.5 g (*ca.* 15 mmol) of diazomethane (prepared from 4.30 g (20 mmol) of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide)[17] in 40 ml of ether. Stirring was continued for 2 h and the remaining diazomethane was destroyed by addition of excess of formic acid. The solution was evaporated to dryness *in vacuo* to give 950 mg of a colourless oil which was submitted to column chromatography (silica gel: 30 g; eluent: ether-petroleum ether 1:1). Distillation of the fractions containing *4* in a "Kugelrohr" at 1 mmHg (oven temp. 160 °C) gave 432 mg (49 %) of a colourless oil (Found: C 51.10; H 6.17; N 7.39. Calc. for $C_8H_{11}NO_4$: C 51.88; H 5.99; N 7.56). $\lambda_{max}(CH_3OH)$ 233 nm ($\varepsilon = 6.74 \times 10^3$). IR (neat) cm^{-1}: 3600–2700 (m); 1740(s); 1660(s). ^1H NMR ($CDCl_3$): δ 3.69 (s, 3 H, –COOC*H*$_3$); 3.47 (s, 3 H, N–C*H*$_3$); 3.27 (s, 2 H, =C–C*H*$_2$–C); 2.22 (s, 3 H, =C–C*H*$_3$). Distillation of the fractions containing *5* in a "Kugelrohr" at 1 mmHg (oven temp. 125 °C) gave 273 mg (30 %) of a colourless oil which solidified to colourless crystals, m.p. 41.5–42.5 °C. (Found: C 51.80; H 6.07; N 7.58. Calc. for $C_8H_{11}NO_4$: C 51.88; H 5.99; N 7.56). $\lambda_{max}(CH_3OH)$: 211 nm ($\varepsilon = 6.02 \times 10^3$). IR (KBr) cm^{-1}: 3700–3200 (m); 3050–2800(m); 1730(s); 1665(m); 1530(s); 1480(s). ^1H NMR ($CDCl_3$): δ 3.83 (s, 3 H, =C–OC*H*$_3$); 3.57 (s, 3 H, –COOC*H*$_3$); 3.15 (s, 2 H, =C–C*H*$_2$–C); 2.17 (s, 3 H, =C–C*H*$_3$).

3-Methoxy-5-methylisoxazole-4-carboxylic acid (6). *6* was prepared as described by Bowden *et al.*[10] to give crystals, m.p. 195–196 °C (Ref. 10: 195–197 °C). $\lambda_{max}(CH_3OH)$: 215 nm ($\varepsilon = 7.42 \times 10^3$). IR (KBr) cm^{-1}: 3600–2300(m); 1680(s); 1620(s); 1530(s); 1490(s). ^1H NMR ($CDCl_3$): δ 13.5–11 (broad band, 1 H, –COO*H*); 3.86 (s, 3 H, –OC*H*$_3$); 2.53 (s, 3 H, =C–C*H*$_3$).

3-Methoxy-4-diazoacetyl-5-methylisoxazole (7). A solution of 1.57 g (10 mmol) of 3-methoxy-5-methylisoxazole-4-carboxylic acid (*6*)[10] in 12 g (100 mmol) of thionyl chloride was refluxed for 40 min. Excess of thionyl chloride was removed *in vacuo* and the residual oil was dissolved in 25 ml of dry ether. The ethereal solution was added dropwise with stirring to a potassium hydroxide dried solution of *ca.* 1 g (*ca.* 24 mmol) of diazomethane (prepared from 7.17 g (33 mmol) of *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide)[17] in 75 ml of ether. Stirring was continued for 3 h and after addition of an excess of formic acid in order to destroy the remaining diazomethane the solution was evaporated to dryness *in vacuo* to give 1.79 g of crude product as yellow crystals. Recrystallization from ether-petroleum ether afforded 900 mg (50 %) of yellow crystals, m.p. 107.5–108.5 °C. (Found: C 46.35; H 4.02; N 23.10. Calc. for $C_7H_7N_3O_3$: C 46.41; H 3.90; N 23.20). $\lambda_{max}(CH_3OH)$: <210 nm; 246 nm ($\varepsilon = 9.07 \times 10^3$); 291 nm ($\varepsilon = 16.1 \times 10^3$). IR (neat) cm^{-1}: 3700–3200(w); 3120(w); 2950(w); 2110(s); 1620(s); 1595(s); 1520 (m); 1470(m); 1360(s). ^1H NMR ($CDCl_3$): δ 5.97 (s, 1 H, C*H*–N$_2$); 4.02 (s, 3 H, =C–OC*H*$_3$); 2.62 (s, 3 H, C*H*$_3$–C=).

Methyl 3-methoxy-5-methylisoxazol-4-ylacetate (5). A solution of 200 mg (0.11 mmol) of *7* in 50 ml of methanol was irradiated in a quartz-tube with unfiltered UV-light (Philips HPK 125 W, BA 15 D, Typ 57203B/00) for 2 h at *ca.* 10 °C. The solution was filtered and evaporated to dryness *in vacuo* and the residue was submitted to column chromatography (silica gel: 20 g; eluent: methylene chloride-ethyl acetate 3:1) to give 157 mg of crude product. Recrystallization from ether-petroleum ether (–70 °C) gave colourless crystals 87 mg (43 %) of which the IR-spectrum was identical with that of *5* prepared as described above.

Diethyl 2-methyl-2-pyrroline-1,3-dicarboxylate (13a). To a suspension of 21.9 g (0.5 mol) of a 55 % dispersion of sodium hydride in mineral oil and 250 ml of DMA was added with stirring 65.0 g (0.5 mol) of ethyl acetoacetate. When the hydrogen evolution had ceased, 15 g of sodium iodide (dried at 140 °C for 24 h) and 83.5 g (0.55 mol) of *11a*[12] was added and the stirred solution was heated to 80 °C for 4 h. After cooling to room temperature 750 ml of water and 125 ml of hydrochloric acid (4 M) was added and the solution was extracted with three 200 ml portions of methylene chloride. The pooled organic phases were washed with 200 ml of water, dried ($MgSO_4$), and evaporated *in vacuo* to give an oil which was distilled at 144–146 °C/0.5 mmHg to give a colourless oil which solidified upon standing. TLC showed the distillate to consist of two compounds (spraying reagent DNP) of which the less polar compound was shown to be *13a* whereas the other compound according to the spectro-

scopic investigations is assumed to be the corresponding β-oxoester *12a*. IR (neat) cm^{-1}: 3700 – 3100(m); 3050 – 2800(m); 1740 – 1690(s, several bands); 1530(m). To complete the cyclization the mixture was dissolved in 400 ml of toluene and after addition of 1 g of *p*-toluenesulfonic acid the solution was refluxed for *ca.* 8 h using a Dean-Stark water separator, dried (K_2CO_3), and evaporated *in vacuo* to give an oil. Distillation at 126 – 135 °C/0.5 mmHg gave 73.7 g (66 %) of *13a* as a colourless oil which solidified upon standing. 0.5 g of *13a* was purified by column chromatography (silica gel: 25 g; eluent: benzene-ethyl acetate 4:1) to give crystals of *13a*. Recrystallization from methanol yielded colourless crystals, m.p. 56 – 57 °C. (Found: C 58.25; H 7.34; N 6.11. Calc. for $C_{11}H_{17}NO_4$: C 58.13; H 7.54; N 6.16). λ_{max}(C_2H_5OH): 281 nm ($\varepsilon = 19.5 \times 10^3$). IR data (KBr) cm^{-1}: 3600 – 3100(m); 3050 – 2800(s); 1740 – 1720(s); 1690(s); 1630(s). ^1H NMR ($CDCl_3$): δ 4.15 and 4.12 [2 × q ($J = 7$ Hz in both cases), 4 H, 2 × O – CH_2 – CH_3]; 4.0 – 3.5 (m, 2 H, CH_2 – C=C); 2.54 (s, 3 H, CH_3 – C=C); 1.27 and 1.25 [2 × t ($J = 7$ Hz in both cases), 6 H, 2 × O – CH_2 – CH_3].

*4-(2-Ethoxycarbonylaminoethyl)-5-methyl-3-isoxazolol (*14a*)*. To a solution of 2.1 g (30 mmol) of hydroxylamine hydrochloride in 50 ml of ethanol was added a solution of 6.3 g (*ca.* 90 mmol) of potassium hydroxide in 30 ml of ethanol. The mixture was stirred at room temperature for 5 min and cooled to −5 °C. To the cooled solution was added 3.7 g (15 mmol) of *13a* and after stirring for 5 min the reaction mixture was placed at 5 °C for 8 days. After evaporation to dryness *in vacuo* 15 ml of water and 15 ml of concentrated hydrochloric acid was added with cooling and stirring. The mixture was left at room temperature for 1 h and was subsequently extracted continuously for 1 h with ether-methylene chloride 4:1. The extract was dried ($MgSO_4$) and evaporated *in vacuo* to give 1.45 g of crude product which was purified by column chromatography (silica gel: 75 g; eluent: methylene chloride-ethyl acetate-formic acid 50:50:2) to give 700 mg of yellow crystals. Recrystallization from ether gave colourless crystals 373 mg (12 %) m.p. 138 – 139 °C. (Found: C 50.50; H 6.65; N 13.19. Calc. for $C_9H_{14}N_2O_2$: C 50.46; H 6.59; N 13.08). λ_{max}(C_2H_5OH): 213 nm ($\varepsilon = 5.76 \times 10^3$). IR (KBr) cm^{-1}: 3600 – 3200(s); 3200 – 2200(m); 1690(s); 1660(m); 1560 – 1520(s, several bands). ^1H NMR ($CDCl_3$-DMSO-d_6 6:1): δ 8.20 (s, *ca.* 1 H, O*H*); 5.9 – 5.5 (broadened signal, 1 H, N*H*); 4.00 [q ($J = 7$ Hz), 2 H, O – CH_2 – CH_3]; 3.5 – 3.0 (broadened q, 2 H, CH_2 – CH_2 – NH); 2.40 [t ($J = 6$ Hz), 2 H, =C – CH_2 – CH_2]; 2.19 (s, 3 H, CH_3 – C=); 1.18 [t ($J = 7$ Hz), 3 H, O – CH_2 – CH_3].

*4-(2-Aminoethyl)-5-methyl-3-isoxazolol hydrobromide (*15a*)*. A solution of 400 mg (1.87 mmol) of *14a* in glacial acetic acid (5 ml) containing 43 % of hydrogen bromide was refluxed for 90 min. After reflux for 60 min an additional amount of 3 ml of glacial acetic acid containing 43 % of hydrogen bromide was added. After cooling to room temperature the solution was evaporated to dryness *in vacuo* to give brownish crystals. Recrystallization from methanol-ether gave 343 mg (77 %) of colourless crystals, m.p. 205 – 206 °C (decomp.). (Found: C 32.45; H 5.01; Br 35.58; N 12.63. Calc. for $C_6H_{11}BrN_2O_2$: C 32.31; H 4.97; Br 35.82; N 12.56). λ_{max}(CH_3OH): < 210 nm. IR (KBr) cm^{-1}: 3600 – 2200(s); 1660(s); 1595(m); 1575(m); 1540 – 1520(s, several bands); 1500(s); 1370(w). ^1H NMR (DMSO-d_6): δ 8.3 – 7.6 (broad signal, 3 H, NH_3^+); 3.2 – 2.3 (broad signal, 4 H, CH_2 – CH_2 – NH_3^+); 2.25 (s, 3 H, CH_3 – C=). The OH-proton could not be detected.

*4-(2-Aminoethyl)-5-methyl-3-isoxazolol zwitterion (*16a*)*. A solution of 1.80 g (8.1 mmol) of *15a* in water (12 ml) was passed through a column containing an ion exchange resin [Amberlite IRA 400 (OH), 40 ml] using acetic acid (1 M) as an eluent. Recrystallization from water-ethanol gave 339 mg (30 %) of colourless crystals, m.p. 159.5 – 161.5 °C (decomp.). (Found: C 50.85; H 7.21; N 19.98. Calc. for $C_6H_{10}N_2O_2$: C 50.69; H 7.09; N 19.71). λ_{max} (CH_3OH): 213 nm ($\varepsilon = 5.83 \times 10^3$). IR (KBr) cm^{-1}: 3600 – 2200(s); 2090(w); 1655(s); 1570 – 1550(s, several bands); 1500 – 1460(s, several bands). pK_A-Values (H_2O, 17 °C): 5.12 ± 0.05, 10.42 ± 0.06. ^1H NMR {D_2O [sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard]}: δ 4.79 (s, *ca.* 4 H, DO*H*); 3.10 [t ($J = 6$ Hz), 2 H, CH_2 – NH_3^+]; 2.55 [t ($J = 6$ Hz), 2 H, CH_2 – CH_2 – C=]; 2.15 (s, 3 H, CH_3 – C=).

*2-Aminopropan-1-ol (*10b*)* was prepared by low pressure (3 atm.) hydrogenation of an ethanolic solution of 2-nitropropan-1-ol (*9*) in a PARR hydrogenation apparatus using 500 mg of a 10 % Pd-C catalyst and 500 ml of ethanol per 0.5 mol of starting material. Yield 70 %, b.p. 71 – 74 °C/11 mmHg. (Ref. 18: b.p. 80 – 86 °C/20 mmHg. Ref. 19: b.p. 76 – 78 °C/15 mmHg).

*1-Chloro-2-ethoxycarbonylaminopropane (*11b*)* was prepared *via* 1-hydroxy-2-(ethoxycarbonylamino)propane as described by Najer *et al.*[20] using *10b* as starting material.

*Diethyl 2,5-dimethyl-2-pyrroline-1,3-dicarboxylate (*13b*)*. To a suspension of 17.9 g (0.41 mol) of a 55 % dispersion of sodium hydride in mineral oil, and 200 ml of DMA was added with stirring 53.0 g (0.41 mol) of ethyl acetoacetate. When the hydrogen evolution had ceased, 61.5 g (0.41 mol) of sodium iodide (dried at 140 °C for 24 h) and 66.2 g (0.45 mol) of *11b* was added and the solution was stirred for 3 h at 100 °C. After cooling to room temperature the crude product was isolated as described for *13a*. Treatment with *p*-toluenesulfonic acid in toluene under reflux for 20 h and isolation as described for *13a* gave 33.7 g of crude product as a brownish oil which was

used without further purification for the preparation of *14b*. An analytical sample of the crude product was purified by column chromatography (silica gel: 25 g; eluent: methylene chloride) to give a colourless oil which was distilled in a "Kugelrohr" at 0.5 mmHg (oven temperature 160 °C) to give pure *13b* as an oil. (Found: C 59.65; H 7.85; N 5.72. Calc. for $C_{12}H_{19}NO_4$: C 59.73; H 7.94; N 5.81). λ_{max} (CH_3OH): 281 nm($\varepsilon = 17.2 \times 10^3$). IR (neat) cm^{-1}: 3700 – 3100(w); 3050 – 2800(s); 1725(s); 1690(s); 1630(s). ^1H NMR (CCl_4): δ 4.07 and 4.00 [2 × q ($J = 7$ Hz in both cases), 4 H, $2 \times O-CH_2-CH_3$]; 4.5 – 3.8 (m, 1 H, $N-CH-CH_3$); 3.2 – 1.9 (m, 2 H, $=C-CH_2-CH$); 2.44 (t, 3 H, $CH_3-C=$); 1.6 – 1.0 (m, 9 H, CH_3-CH and $2 \times O-CH_2C-CH_3$).

4-(2-Ethoxycarbonylaminopropyl)-5-methyl-3-isoxazolol (14b) was prepared as described above for *14a*. 7.23 g (30 mmol) of crude *12b* gave 4.55 g of crude product which was submitted to column chromatography [silica gel: 200 g; eluent: methylene chloride-ethyl acetate-formic acid (60:40:1)] to give 2.9 g of product. Recrystallization from ethyl acetate-benzene gave 820 mg (14 %) of colourless crystals, m.p. 170 – 170.5 °C. (Found: C 52.50; H 6.97; N 12.11. Calc. for $C_{10}H_{16}N_2O_4$: C 52.62; H 7.07; N 12.27). λ_{max}(CH_3OH): 216 nm ($\varepsilon = 6.70 \times 10^3$). IR (KBr) cm^{-1}: 3340(m); 3200 – 2200(m); 1680(s); 1660(m); 1560 – 1520(s, several bands). ^1H NMR ($CDCl_3$ – DMSO-d_6 5:3): δ 7.2 – 6.5 (broad s, *ca.* 1 H, O*H*); 6.5 – 6.0 (broadened signal, 1 H, N*H*); 3.95 [q ($J = 7$ Hz), 2 H, $O-CH_2-CH_3$]; 4.2 – 3.3 (m, 1 H, $-CH-NH$); 2.35 [d ($J = 7$ Hz), 2 H, $=C-CH_2-CH$]; 2.20 (s, 3 H, $CH_3-C=$); 1.18 and 1.05 [2 × t ($J = 7$ Hz in both cases), 6 H, $2 \times O-CH_2-CH_3$].

4-(2-Aminopropyl)-5-methyl-3-isoxazolol hydrobromide (15b). A solution of 250 mg (1.1 mmol) of *14b* was treated with glacial acetic acid containing 43 % of hydrogen bromide as described for the preparation of the compound *15a* to give an oil which crystallized from methanol-ether to give 219 mg (85 %) of *15b* as colourless crystals, m.p. 198 – 199 °C (decomp.). (Found: C 35.25; H 5.78; Br 33.70; N 11.82. Calc. for $C_7H_{13}BrN_2O_2$: C 35.46; H 5.52; Br 33.71; N 11.82). λ_{max}(CH_3OH): 211 nm ($\varepsilon = 5.88 \times 10^3$). IR (KBr) cm^{-1}: 3600 – 3300(w); 3300 – 2300(s); 2050 – 1900(w); 1660(m); 1595(m); 1530(s); 1500(s). ^1H NMR {D_2O [sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard]}: δ 4.90 (s, *ca.* 4 H, DO*H*); 3.8 – 3.2 (m, 1 H, $CH-CH_3$); 2.60 [d ($J = 6$ Hz), 2 H, $=C-CH_2-CH$]; 2.30 (s, 3 H, $CH_3-C=$); 1.28 [d ($J = 6$ Hz), 3 H, CH_3-CH].

3-Methoxy-4-aminomethyl-5-methylisoxazole hydrochloride (17a) was prepared as described by Bowden *et al.*[10] to give crystals, m.p. 222 – 223 °C (decomp.). Ref. 10: m.p. 229 °C (decomp.). λ_{max}(CH_3OH): 210 nm ($\varepsilon = 5.93 \times 10^3$). IR (KBr) cm^{-1}: 3600 – 3300(m); 3300 – 2500(s); 1655(m); 1525(s); 1480(s). ^1H NMR (DMSO-d_6): δ 9.0 – 8.2 (broadened s, 3 H, NH_3^+); 3.87 (s, 3 H, OCH_3); 3.66 (s, 2 H, $CH_2-NH_3^+$); 2.42 (s, 3 H, $=C-CH_3$).

4-(2-Aminopropyl)-5-methyl-3-isoxazolol zwitterion (16b). pK_A Values (H_2O, 21 °C): 4.87 ± 0.05, 10.06 ± 0.06.

4-Aminomethyl-5-methyl-3-isoxazolol hydrobromide (18a) was prepared as described above for *15a* using 200 mg (1.12 mmol) of *17a* as starting material. The crude product obtained as reddish yellow crystals was recrystallized from methanol-ether to give 125 mg (53 %) of colourless crystals, m.p. 209 – 209.5 °C (decomp.). (Found: C 28.90; H 4.46; Br 38.10; N 13.57. Calc. for $C_5H_9BrN_2O_2$: C 28.72; H 4.34; Br 38.23; N 13.40). λ_{max}(CH_3OH): < 210 nm. IR (KBr) cm^{-1}: 3600 – 3300(m); 3300 – 2300(s); 1655(s); 1585(m); 1560 – 1500(s, several bands). ^1H NMR (DMSO-d_6): δ 8.6 – 7.4 (broad signal, 4 H, O*H* and NH_3^+); 4.0 – 3.4 (broadened s, 2 H, $CH_2-NH_3^+$); 2.35 (s, 3 H, $=C-CH_3$).

4-Aminomethyl-5-methyl-3-isoxazolol zwitterion (19). A solution of 330 mg (1.58 mmol) of *18a* in water (2 ml) was passed through a column containing an ion exchange resin [Amberlite IRA 400, (OH), 5 ml] using acetic acid (1 M) as an eluent. Recrystallization of the crude product (methanol-ether) gave 102 mg (50 %) as colourless crystals, m.p. 163.5 – 165 °C (decomp.). (Found: C 43.00; H 6.56; N 19.90. Calc. for $C_5H_8N_2O_2 \cdot 2/3H_2O$: C 42.85; H 6.71; N 19.99). [Found after drying of *19* over P_2O_5 (24 h; 75 °C; 0.1 mmHg): C 44.95; H 6.76; N 20.91. Calc. for $C_5H_8N_2O_2 \cdot 1/3H_2O$: C 44.77; H 6.51; N 20.89]. λ_{max}(CH_3OH): < 210 nm. IR (KBr) cm^{-1}: 3600 – 3300(m); 3300 – 2300(s); 2180(m); 1655(m); 1520 – 1480(s, several bands). pK_A Values (H_2O, 21 °C): 4.74 ± 0.05, 9.95 ± 0.03. ^1H NMR data [D_2O (sodium 3-(trimethylsilyl)-propanesulfonate was used as an internal standard)]: δ 4.73 (s, 4 H, DO*H*); 4.1 – 3.6 (broad signal, 2 H, $CH_2-NH_3^+$); 2.5 – 2.0 (broad signal, 3 H, $CH_3-C=$).

3-Methoxy-4-(1-aminoethyl)-5-methylisoxazole hydrochloride (17b) was prepared as described by Bowden *et al.*[10] to give crystals, m.p. 199.5 – 200.5 °C (decomp.). (Ref. 10: m.p. 203 °C). λ_{max}(CH_3OH): 212 nm ($\varepsilon = 4.57 \times 10^3$). IR (KBr) cm^{-1}: 3600 – 3300(w); 3300 – 2400(s); 2050 – 1900(w); 1640(s); 1610 – 1580(m, several bands); 1535(s); 1505(s); 1470(s). ^1H NMR (DMSO-d_6): δ 9.0 – 8.1 (broadened s, 3 H, NH_3^+); 3.87 (s, 3 H, OCH_3); 4.5 – 4.0 (m, 1 H, $CH_3-CH-NH_3^+$); 2.40 (s, 3 H, $=C-CH_3$); 1.48 [d ($J = 6$ Hz), 3 H, CH_3-CH].

4-(1-Aminoethyl)-5-methyl-3-isoxazolol hydrobromide (18b) was prepared as described above for *15a* using 500 mg (2.6 mmol) of *17b* as starting material. The crude oily product was crystallized from methanol-ether to give 432 mg (75 %) of colourless crystals, m.p. 193 – 195 °C (decomp.). (Found: C 32.25; H 5.03; Br 36.05; N 12.71. Calc. for $C_6H_{11}BrN_2O_2$: C 32.30; H 4.97; Br 35.82; N 12.56). λ_{max} (CH_3OH): 211 nm ($\varepsilon = 5.10 \times 10^3$). IR (KBr)

cm^{-1}: 3600–3300(m); 3300–2300(s); 1660–1640(s); 1540(s); 1495(s). ^1H NMR (DMSO-d_6): δ 8.7–7.3 (broad signal, 3 H, NH_3^+); 4.5–3.7 (m, 1 H, $CH_3-CH-NH_3^+$); 2.33 (s, 3 H, $CH_3-C=$); 1.47 [d ($J=6$ Hz), 3 H, CH_3-CH]. The OH proton could not be detected.

4-(1-Aminoethyl)-5-methyl-3-isoxazolol zwitterion (20). To a solution of 39 mg (0.17 mmol) of *18b* in 2 ml of ethanol was added 50 µl (5 mmol) of triethylamine at 40 °C, and the reaction mixture was kept at room temperature to complete crystallization. 19 mg (80 %) of colourless crystals were obtained. Recrystallization from water-ethanol-ether gave crystals, m.p. 204.5–205 °C (decomp.). (Found: C 50.40; H 7.27; N 19.92. Calc. for $C_6H_{10}N_2O_2$: C 50.69; H 7.09; N 19.71). $\lambda_{max}(CH_3OH)$: <210 nm. IR (KBr) cm^{-1}: 3600–3300(m); 3300–2300(m); 2200(m); 1640(m); 1520–1490(s). pK_A Values (H_2O, 20 °C): 4.74±0.03, 9.73±0.03. ^1H NMR {D_2O [sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard]}: δ 4.75 (s, 3 H, DO*H*); 4.28 [q ($J=7$ Hz), 1 H, CH_3-CH]; 2.23 (s, 3 H, $CH_3-C=$); 1.58 [d ($J=7$ Hz), 3 H, CH_3-CH].

Thanks are due to the Danish Medical Research Council for supporting this work.

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Received October 27, 1975.