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Structural Analogues of Ibotenic Acid. Synthesis of (±)-α-Amino-3-hydroxy-5-methyl-4-isoxazoleacetic Acid and Derivatives Thereof

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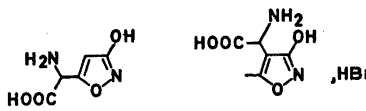
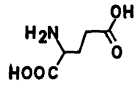
(±)-α-Amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid hydrobromide (*10*) and derivatives thereof are synthesized from 3-methoxy-5-methyl-4-isoxazolecarbaldehyde (*2*) prepared from 3-methoxy-5-methyl-4-isoxazolecarbonyl chloride. The aldehyde *2* was transformed into methyl α-hydroxy-3-methoxy-5-methyl-4-isoxazoleacetate (*4*) *via* the cyanohydrin acetate. Manganese(IV) oxide oxidation of *4* afforded methyl α-oxo-3-methoxy-5-methyl-4-isoxazoleacetate, the oxime of which was converted into methyl α-amino-3-methoxy-5-methyl-4-isoxazoleacetate (*7*). Selective cleavage of the ester and the ether groups of *7* gave α-amino-3-methoxy-5-methyl-4-isoxazoleacetic acid zwitterion (*8*) and methyl α-amino-3-hydroxy-5-methyl-4-isoxazoleacetate hydrobromide (*9*). The pK_A values of *10* have been determined.

Ibotenic acid, an amino acid isolated from *Amanita muscaria*,[1] has a powerful excitatory effect on central neurones, which is similar to the effects of the putative neurotransmitters glutamic and aspartic acid.[2,3] Ibotenic acid, which is a conformationally restrained analogue of glutamic acid (Scheme 1), may be a useful tool in the study of the transmitter function of glutamic acid.[2,3] This paper presents the synthesis of (±)-α-amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid hydrobromide (*10*) an analogue of ibotenic acid related to aspartic acid.

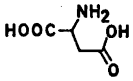


Ibotenic acid

10



Glutamic acid

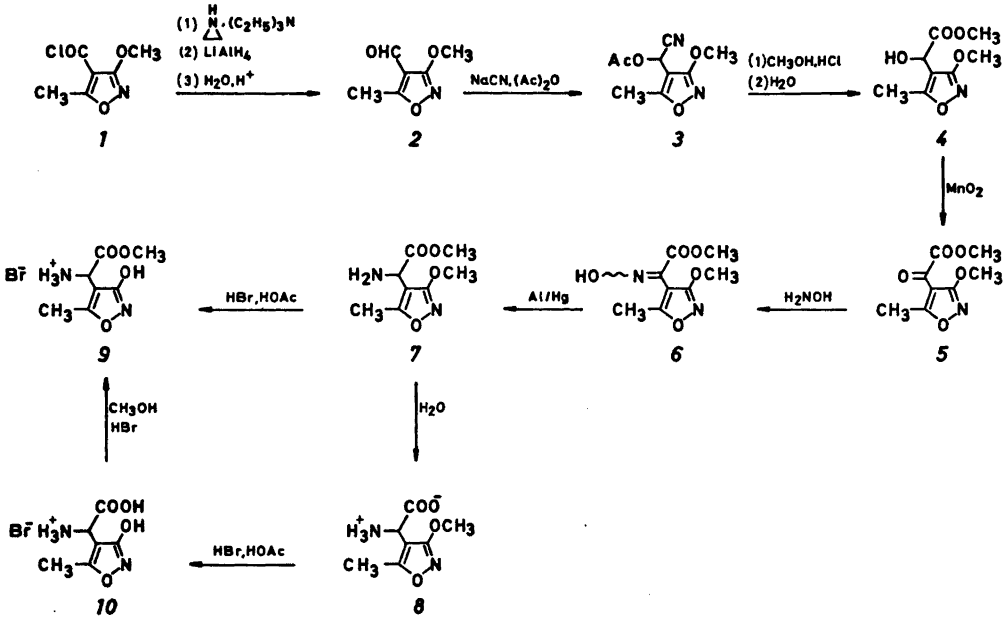


Aspartic acid

Scheme 1.

The aldehyde *2*, which is the key compound in the syntheses of *7* – *10*, was prepared from 3-methoxy-5-methyl-4-isoxazolecarbonyl chloride (*1*) (Scheme 2). Treatment of *2* with sodium cyanide and acetic anhydride converted it into the cyanohydrin acetate *3*, which was transformed into *4* by a Pinner synthesis followed by hydrolysis of the reaction product. Manganese(IV) oxide oxidation of *4* afforded the α-oxo ester *5* in almost quantitative yield. The oxime *6*, prepared from *5* by a conventional method was shown to be a mixture of the two stereoisomeric ketoximes. Aluminium amalgam reduction of *6* gave amine *7*, characterized as the hydrochloride. The ester group of *7* was hydrolyzed by boiling water, and selective cleavage of the ether group of *7* was accomplished by hydrogen bromide in glacial acetic acid. Demethylation of *8* afforded the aspartic acid analogue *10*. The relationships between the compounds *7*, *8*, *9*, and *10* were confirmed by esterification of *10* with methanol to give *9*.

The structure determinations of the new compounds *2* – *10* are based on ^{1}H NMR, IR, and UV spectroscopic methods and supported by elemental analyses. The IR and ^{1}H NMR data obtained from the 3-oxygenated isoxazole



Scheme 2.

moieties are in accordance with those of related compounds.[4-6] The positions of the UV absorption maxima of *2–10* are in agreement with those of other 4,5-disubstituted 3-oxygenated isoxazoles.[4-6] The two stereoisomeric ketoximes *6* were separated by column chromatography (CC) and their spectroscopic data recorded, but no attempts were made to establish the stereochemistry of the two compounds.

The pK_A values of *14* (2.0 ± 0.1, 5.43 ± 0.03, 10.00 ± 0.04) are significantly different from those of ibotenic acid (3, 5.04, 8.16).[1] This difference may be explained by assuming the existence of an intramolecular hydrogen bond between the carboxylate and the 3-hydroxy groups of the zwitterion corresponding to *10* in agreement with the findings for a number of related carboxylic acids.[7]

EXPERIMENTAL

Unless otherwise stated the determination of melting points, the recording of IR, UV and ^{1}H NMR spectra, and the performance of microanalyses were performed as described in a previous paper.[8] TLC and CC were accomplished by using silica gel F_{254} plates (Merck) and silica gel, 0.05–0.200 mm (Merck), respectively. The pK_A values were determined as previously described.[9]

3-Methoxy-5-methyl-4-isoxazolecarbaldehyde (2). To a stirred and ice-cooled solution of aziridine (1.25 g; 29.1 mmol) and triethylamine (3.52 g; 32 mmol) in ether (100 ml) was slowly added a solution of 3-methoxy-5-methyl-4-isoxazolecarbonyl chloride (*1*)[10] (5.09 g; 29.1 mmol) in ether (200 ml). The mixture was stirred for 5 min and filtered. To the filtrate was added lithium aluminium hydride (1.5 g; 38 mmol) and the mixture was stirred for 2 h at 0 °C. Sulfuric acid (36 ml; 33 %) was added and the organic phase separated. The aqueous phase was extracted with three 30 ml portions of ether and the combined organic phases were dried (K_2CO_3) and evaporated *in vacuo* to give 3 g of colourless crystals. *2* (2.7 g; 66 %) was obtained by sublimation (bath temperature 60 °C, 2 kPa), m.p. 46–51 °C. Found: C 50.95; H 5.41; N 9.95. Calc. for $C_6H_7NO_3$: C 51.06; H 5.00; N 9.93. UV [methanol (log ε)]: 236 (3.81) nm. IR (film): 2950 (m), 1690 (s), 1610 (s), 1530 (s) cm^{-1}. ^{1}H NMR (CCl_4): δ 9.60 (1 H, s), 3.93 (3 H, s), 2.53 (3 H, s).

(±)-α-Acetoxy-3-methoxy-5-methyl-4-isoxazoleacetonitrile (3). To a solution of *2* (2.64 g; 18.7 mmol) in glacial acetic acid (9 ml) was added potassium cyanide (1.82 g; 28 mmol). After stirring for 40 min acetic anhydride (2.04 g; 20 mmol) was added and the mixture was heated to 50 °C for 1½ h. After cooling to room temperature the solution was added to a mixture of water (40 ml) and ether (50 ml).

The organic layer was isolated, washed with an aqueous solution of sodium carbonate (40 ml; 1 M), dried, and evaporated *in vacuo* to give 4 g of colourless crystals. Recrystallization afforded *3* (2.48 g; 63 %) as colourless needles, m.p. 78 – 79 °C (cyclohexane). Anal. $C_9H_{10}N_2O_4$: C, H, N. UV [methanol (log ε)]: 210 (3.77) nm. IR (KBr): 2950 (m), 1745 (s), 1650 (s), 1535 (s) cm^{-1}. ^1H NMR [CCl_4 – $CDCl_3$ (4:1)]: δ 6.10 (1 H, s), 3.97 (3 H, s), 2.50 (3 H, s), 2.13 (3 H, s).

Methyl (±)-α-hydroxy-3-methoxy-5-methyl-4-isoxazoleacetate (4). To ice-cooled methanol (6 ml) saturated with dry hydrogen chloride *3* (1.26 g; 6.00 mmol) was added, and the mixture was left at 4 °C for 16 h with occasional stirring during the first hour. The solution was evaporated *in vacuo* to give 1.53 g of a crystalline residue, which was dissolved in iced water (4 ml). The solution was extracted with six 6 ml portions of ether. The combined organic phases were evaporated *in vacuo* and the residue distilled to give *4* (0.91 g; 76 %) as a colourless oil, b.p. 100 – 102 °C/67 Pa. Anal. $C_8H_{11}NO_5$: C, H, N. UV [methanol (log ε)]: 212 (3.82) nm. IR (film): 3600 – 3100 (s), 2950 (m), 1750 (s), 1650 (s), 1530 (s) cm^{-1}. ^1H NMR (CCl_4): δ 4.80 (1 H, d, *J* 5 Hz), 3.87 (3 H, s), 3.70 (3 H, s), 3.53 (1 H, d, *J* 5 Hz), 2.30 (3 H, s).

Methyl (±)-α-oxo-3-methoxy-5-methyl-4-isoxazoleacetate (5). To a solution of *4* (0.70 g; 3.5 mmol) in ether (80 ml) was added manganese(IV) oxide (3.04 g; 35 mmol). The mixture was stirred at room temperature for 3 h and filtered. The filtrate was evaporated *in vacuo* to give *5* (0.69 g; 99 %) as a cololuress oil, b.p. 88 – 92 °C/0.1 kPa. Anal. $C_8H_9NO_5$: C, H, N. UV [methanol (log ε)]: 245 (3.93) nm. IR (film): 2950 (m), 1745 (s), 1685 (s), 1680 (s), 1600 (s), 1525 (s) cm^{-1}. ^1H NMR (CCl_4): δ 3.92 (3 H, s), 3.77 (3 H, s), 2.58 (3 H, s).

Methyl α-hydroxyimino-3-methoxy-5-methyl-4-isoxazoleacetate (6). A solution of *5* (2.02 g; 10.3 mmol), hydroxylammonium chloride (790 mg; 11.3 mmol), and sodium acetate trihydrate (1.52 g; 11.3 mmol) in aqueous methanol (90 ml; 50 %) was refluxed for 6 h. The solution was concentrated to 30 ml *in vacuo*, and the residue extracted with ether (30 ml). The organic phase was concentrated *in vacuo* to give *6* (1.85 g; 84 %) as colourless crystals, in which two compounds could be detected by TLC [eluent: benzene – ethyl acetate (2:1); $R_F = 0.35$ and $R_F = 0.21$, respectively]. The two compounds were shown to be the two stereoisomeric ketoximes. *6* (625 mg) was submitted to CC [silica gel: 63 g; eluent: benzene – ethyl acetate (7:3)] to give the oxime having the greater R_F value (222 mg) as colourless crystals, m.p. 136 – 142 °C (water). Anal. $C_8H_{10}N_2O_5$: C, H, N. UV [methanol (log ε)]: < 210, 241 (sh, 3.70) nm. IR (KBr): 3600 – 2700 (s), 1750 (s), 1640 (s), 1615 (s), 1540 (s) cm^{-1}. ^1H NMR (DMSO-d_6): δ 12.07 (1 H, s), 3.87 (3 H, s), 3.77 (3 H, s), 2.43 (3 H, s). An amount of 241 mg of the oxime having the lower R_F value was isolated, m.p. 163 – 163.5 °C (water). Anal. $C_8H_{10}N_2O_5$: C, H, N. UV [methanol (log ε)]: 212 (4.04), 243 (sh, 3.62) nm. IR (KBr): 3600 – 2800 (s), 1730 (s), 1650 (m), 1620 (s) cm^{-1}. ^1H NMR (DMSO-d_6): δ 13.00 (1 H, s), 3.83 (3 H, s), 3.73 (3 H, s), 2.23 (3 H, s).

Methyl (±)-α-amino-3-methoxy-5-methyl-4-isoxazoleacetate (7). To a solution of *6* (1.85 g; 8.65 mmol) in methanol – water (1:1) (60 ml) was added aluminium amalgam prepared by treatment of aluminium strips (3.04 g; 112 mmol) with an aqueous mercury(II) chloride solution (120 ml; 5 %) for 30 s followed by washing with ethanol. After stirring for 18 h at room temperature the mixture was filtered, concentrated to *ca.* 20 ml, and extracted with ether – methylene chloride (5:2) in a Kutscher-Steudel apparatus. The organic phase was evaporated *in vacuo* to give an oil, ball-tube distillation of which at 0.1 kPa (oven temperature 185 °C) afforded *7* (1.13 g; 65 %) as a colourless oil. ^1H NMR (CCl_4): δ 4.20 (1 H, s), 3.90 (3 H, s), 3.65 (3 H, s), 2.30 (3 H, s), 1.80 (2 H, s).

Methyl (±)-α-amino-3-methoxy-5-methyl-4-isoxazoleacetate hydrochloride. To *7* (95 mg; 0.48 mmol) was added methanol saturated with dry hydrogen chloride (0.5 ml). The mixture was evaporated *in vacuo* and the residue recrystallized (2-propanol) to give the hydrochloride (61 mg; 55 %) as colourless crystals, m.p. 194 – 198 °C (decomp.). Anal. $C_8H_{13}ClN_2O_4$: C, H, Cl, N. UV (methanol): < 210 nm. IR (KBr): 3200 – 2500 (s), 1755 (s), 1650 (s), 1580 (s), 1530 (s) cm^{-1}. ^1H NMR (DMSO-d_6): δ 9.2 – 8.8 (3 H, broad signal), 5.23 (1 H, s), 3.87 (3 H, s), 3.73 (3 H, s), 2.43 (3 H, s).

(±)-α-Amino-3-methoxy-5-methyl-4-isoxazoleacetic acid zwitterion (8). A solution of *7* (530 mg; 2.65 mmol) in water (4 ml) was refluxed for 16 h. The mixture was concentrated *in vacuo*, and the residue recrystallized (water – methanol) to give *8* (165 mg; 33 %) as colourless crystals, m.p. 212 – 214 °C (decomp.). Anal. $C_7H_{10}N_2O_4$: C, H, N. UV (methanol): < 210 nm. IR (KBr): 3220 (m), 3100 – 2300 (s), 1640 (s), 1560 (s), 1520 (s) cm^{-1}. ^1H NMR {D_2O – CF_3COOH (50:3), [sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard]}: δ 5.17 (1 H, s), 4.95 (8 H, s), 3.97 (3 H, s), 2.43 (3 H, s).

Methyl (±)-α-amino-3-hydroxy-5-methyl-4-isoxazoleacetate hydrobromide (9). A solution of *7* (52 mg; 0.26 mmol) in a solution of hydrogen bromide in glacial acetic acid (1 ml; 43 %) was refluxed for 2 min. A further amount of 1 ml of the above-mentioned reagent was added and the solution was refluxed for a further 3 min. Upon evaporation *in vacuo* the residue was recrystallized (2-propanol – ether) to give *9* (22 mg; 32 %) as slightly coloured crystals, m.p. 177 – 178 °C. Found: C 30.70; H 4.31; N 10.30. Calc. for $C_7H_{11}BrN_2O_4$: C 31.48; H 4.15; N 10.49. UV (methanol): < 210 nm. IR (KBr):

3300–2300 (s), 1760 (s), 1650 (m), 1550 (m), 1505 (s) cm^{-1}. ^1H NMR [D_2O (acetonitrile, the δ value of which was defined as 2.02, was used as an internal standard)]: δ 5.25 (1 H, s), 4.75 (6 H, s), 3.84 (3 H, s), 2.35 (3 H, s).

(±)-α-Amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid hydrobromide (10). *10* was synthesized as described for *9* using *8* (186 mg; 1 mmol) as a starting material and two 2 ml portions of the reagent. *10* (200 mg; 79 %) was obtained as colourless crystals, m.p. 212–213 °C (decomp.) (2-propanol–ether). Anal. $C_6H_9BrN_2O_4$: C, H, Br, N. UV (methanol): < 210 nm. IR (KBr): 3600–2300 (s), 1755 (s), 1650 (m), 1605 (m), 1580 (m), 1540–1500 (several s bands) cm^{-1}. ^1H NMR [D_2O–DMSO-d_6–CF_3COOH (3:1:1) (acetonitrile, the δ value of which was defined as 2.02, was used as an internal standard)]: δ 5.12 (1 H, s), 2.37 (3 H, s). pK_A values (H_2O, 26 °C): 2.0 ± 0.1, 5.43 ± 0.3, 10.00 ± 0.04.

Methyl (±)-α-amino-3-hydroxy-5-methyl-4-isoxazoleacetate hydrobromide (9). To a mixture of *10* (86 mg; 0.40 mmol) and methanol (1 ml) was added a solution of hydrogen bromide in glacial acetic acid (0.2 ml; 43 %). The solution obtained was refluxed for 20 h and concentrated *in vacuo*. The residue was recrystallized to give *9* (52 mg; 50 %) as colourless crystals, the IR spectrum of which was identical with that of *9* prepared from *7*.

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