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Structural Analogues of GABA. Synthesis of 5-Aminomethyl-3-isothiazolol (Thiomuscimol)

JYTTE LYKKEBERG and POVL KROGSGAARD-LARSEN

Royal Danish School of Pharmacy, Chemical Department BC, DK-2100 Copenhagen, Denmark

The synthesis of 5-aminomethyl-3-isothiazolol zwitterion (*12*), a thio analogue of muscimol (5-aminomethyl-3-isoxazolol), is described. Reaction of the starting material aminofumaramide (*1*) with excess of hydrogen sulfide gives dithiodisuccinamide (*2*) which is oxidized by bromine to give 3-hydroxyisothiazole-5-carboxamide (*3*). Conversion of *3* into the *O*-methyl derivative *5* by treatment with diazomethane, followed by reduction with diborane gives 3-methoxy-5-aminomethylisothiazole, the hydrochloride *9* of which is transformed into 5-aminomethyl-3-isothiazolol dihydrobromide (*10*). Treatment of *10* with ethanol gives the corresponding monohydrobromide *11* and conversion of *10* into 5-aminomethyl-3-isothiazolol zwitterion (*12*) is accomplished by treatment with triethylamine. The pK_A values of *12* are determined to 6.06 ± 0.03 and 8.85 ± 0.04.

Muscimol (5-aminomethyl-3-isoxazolol), a centrally active constituent of *Amanita muscaria*,[1,2] is a semirigid cyclic analogue of γ-aminobutyric acid (GABA).[3,4] Several muscimol analogues containing various aminoalkyl groups in position 4 or 5 of the 3-isoxazolol nucleus have been synthesized,[5–10] and a structure-activity correlation of this series of conformationally restricted GABA analogues has been made.[11] Other possible structural changes of muscimol are variations of the heterocyclic ring. This paper describes the synthesis of 5-aminomethyl-3-isothiazolol (thiomuscimol) (*12*) by the sequence outlined in Scheme 1. The biological properties of thiomuscimol (*12*) are being investigated.

Aminofumaramide (*1*) is treated with hydrogen sulfide in glacial acetic acid to give dithiodisuccinamide (*2*). A crude specimen of *2* is converted into 3-hydroxyisothiazole-5-carboxamide (*3*) by oxidation with bromine using a procedure analogous with that described by Goerdeler and Mittler.[12] Attempts to reduce the carboxamide group of *3* with diborane give very complex reaction mixtures from which no product can be isolated. The pronounced garlic smell of the mixture indicates degradation of the isothiazole ring of *3* under the conditions used, and protection of the hydroxy group of *3* prior to reduction is considered necessary.

Reactions of *3* with 4-toluenesulfonyl chloride and benzenesulfonyl chloride give *4a* and *4b*, respectively, in moderate yields and without contamination with the product containing an arylsulfonyl group in position 2 which is in agreement with the findings of Chan and Crow for similar reactions.[13] Reactions of *4a,b* with diborane, however, give complex reaction mixtures containing small amounts of ninhydrin-sensitive products, as revealed by

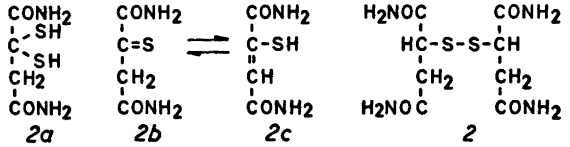


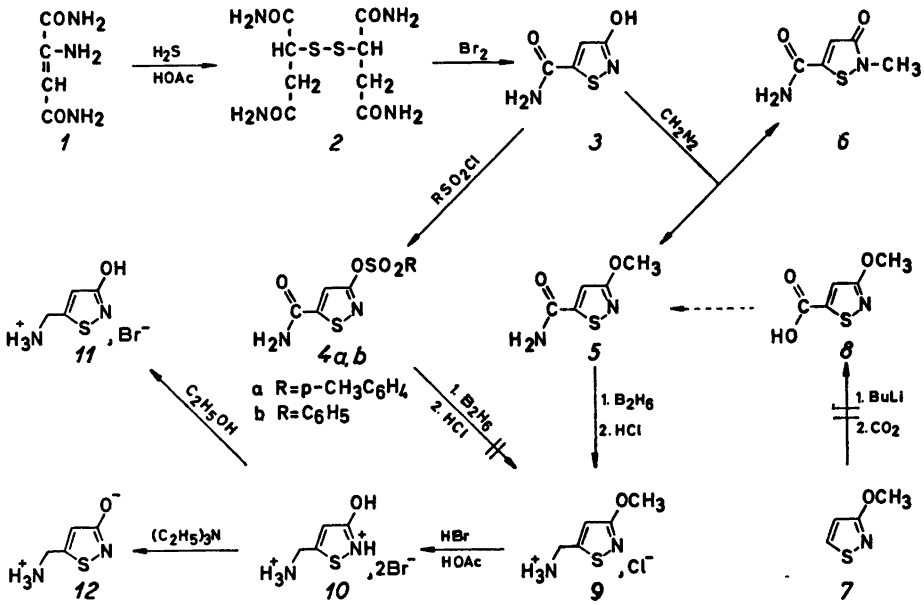
Fig. 1.

TLC, from which no product is isolated.

In another approach masking of the hydroxy group of *3* is accomplished by treatment with diazomethane, which in accordance with the findings for analogous reactions[13] gives a mixture of the *O*- and *N*-methyl derivatives *5* and *6*, respectively, and almost equal amounts are obtained. In an attempt to develop an alternative synthesis of *5* *via* 3-methoxyisothiazole-5-carboxylic acid (*8*), 3-methoxyisothiazole (*7*)[14] is treated with butyllithium followed by addition of carbon dioxide to the reaction mixture. However, *8* cannot be detected in the reaction mixture, which mainly consists of the starting material. Treatment of *5* with lithium aluminium hydride apparently results in a complete destruction of the compound. Finally *5* is reduced with diborane to give a reasonable yield of 3-methoxy-5-aminomethylisothiazole as the hydrochloride *9*. Compound *9* is converted into 5-aminomethyl-3-isothiazolol dihydrobromide (*10*) without purification. In an attempt to crystallize *10* from ethanol-ether the monohydrobromide *11* is obtained. Finally *10* is converted into 5-aminomethyl-3-isothiazolol zwitterion (*12*) by treatment with two equivalents of triethylamine.

A previous and rather inadequate description of the preparation of aminofumaramide (*1*)[15] did not include a structure determination of this compound. The IR, UV, and ^{1}H NMR spectra of *1* provide evidence of the depicted structure *1* of this compound. Reactions of enamines with hydrogen sulfide normally give *gem*-dithiols,[16] but the formations of thiones have been reported in some cases.[12,16] Thus, the structure of the product from the reaction between *1* and hydrogen sulfide was expected to be *2a* or *2b,c* (Fig. 1). These possible structures, however, can be ruled out by the absence of IR absorptions characteristic of thiol groups,[17] and by the absence of a strong thion stretching band in the range 1300 – 1200 cm^{-1} expected for *2b*.[12,18] Finally *2b* and *2c* can be excluded by the absence of UV absorption above 210 nm.[16] If *2b,c* is intermediarily formed, excess of hydrogen sulfide can convert *2b,c* into *2* in agreement with the findings of Bergmann *et al.*[19] This proposal is supported by IR spectroscopy and by elemental analysis, and finally the ^{1}H NMR spectrum exhibits a pattern characteristic of the ABX coupling system present in *2*.

Compound *3* shows absorptions in the range 3600 – 2400 cm^{-1} and at 1560 – 1540 cm^{-1},



Scheme 1.

characteristic of the 3-isothiazolol nucleus.[12] The compounds *5* and *6* can be clearly distinguished by spectroscopic methods. An absorption band of *6* at 1620 cm^{-1}, originating in the carbonyl group in position 3, is absent in the IR spectrum of *5*, and also in that of *3*. Furthermore the UV absorption maxima of *6* and *5* are in agreement with the general findings for *N*-substituted isothiazolin-3-ones and *O*-substituted 3-isothiazololes, respectively.[13,14] In the ^1H NMR spectra of *3* and *5* the protons in position 4 are very similar but they appear at a lower field than the corresponding proton in *6* in accordance with the general findings of Chan *et al.*[13,14] Absorptions in the IR spectra of *4a* and *4b* characteristic of sulfonyloxy groups (1360 and 1170 cm^{-1}) and the absence of absorptions at 1620 cm^{-1} compared with UV and ^1H NMR data provide evidence of *4a* and *4b* being 3-sulfonyloxyisothiazole rather than 2-sulfonylisothiazolin-3-one derivatives.

Compound *12* is the first example of a zwitterion in which a 3-isothiazolol nucleus constitutes the acidic moiety. The structure determinations of *12* and the corresponding mono- and dihydrobromide, *11* and *10* respectively, are based on UV, IR, and ^1H NMR spectroscopy supported by elemental analyses. Broad IR absorptions of *10*–*12* in the range 3600–2200 cm^{-1} are in agreement with the presence of ammonium groups in these compounds, and consequently *12* is a zwitterion. The UV data of *10*, *11*, and *12* are in agreement with the general findings of Chan *et al.*[14] The zwitterion *12* apparently crystallizes with a quarter of a mol of water, the removal of which by heating is accompanied by destruction of the compound.

EXPERIMENTAL

Melting points are corrected and were determined with a hot stage microscope (Mikroskop-Heiztisch, 350 Ernst Leitz, Wetzlar). 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.[20] pH values were measured on a Radiometer pH meter 26 and the pK_A values were determined according to the method of Albert and Serjeant [21] as described in a previous paper.[6]

Aminofumaramide (1). A mixture of 82.4 g (0.4 mol) of diethyl chlorofumarate [22] and aqueous ammonia (400 ml; ϱ 0.88) was stirred for 1 h at 0 °C. Stirring was continued for 18 h at 25 °C, and the precipitate was collected. After drying 45.2 g (88 %) of *1* were obtained; m.p. 188 °C (decomp.) [Ref. 15, m.p. 180 °C (decomp.)]. Recrystallization from water afforded *1* as colourless crystals, m.p. 198 °C (decomp.). λ_{max} 300 nm ($\varepsilon = 6.55 \times 10^3$). IR data ($cm^{-1}$): 3500–2900 (s), 1660–1610 (s), 1550 (s), 1420 (s). ^1H NMR data (DMSO-d_6): δ 7.7–6.7 (four broad signals, total 6 H, $2 \times CONH_2$ and NH_2); 5.05 (s, 1 H, $C=CH-C$).

Dithiodisuccinamide (2). A mixture of 5.2 g (0.04 mol) of *1* and 30 ml of glacial acetic acid was treated with excess of dry hydrogen sulfide during 1½ h at 80 °C. After cooling to room temperature the crystalline solid was filtered off and upon standing at 5 °C for 1 week the mother liquor was filtered to give an additional amount of crystalline product. The two products were combined, and after drying over P_2O_5 (12 h; 50 °C, 12 mmHg) 4.0 g (56 %) of crude *2* were obtained; m.p. 125 °C (decomp.). Recrystallization (water-ethanol-ether) of an analytical sample afforded *2* as colourless crystals, m.p. 176–177 °C (decomp.). (Found: C 32.56; H 4.65; N 18.97; S 19.48. Calc. for $C_8H_{14}N_4O_4S_2$: C 32.64; H 4.79; N 19.03; S 21.79). IR data (cm^{-1}): 3350 (s), 3180 (s), 2920 (w), 1660 (s), 1615 (s), 1410 (s). ^1H NMR data (D_2O): δ 4.62 (s, 8 H, DO*H*); 3.9–3.7 (t, 2 H, $2 \times C-CH-CH_2$); 2.8–2.5 (m, 4 H, $2 \times CH-CH_2-C$).

3-Hydroxyisothiazole-5-carboxamide (3). To a suspension of 2.9 g of crude *2* (10 mmol) in 100 ml of ethyl acetate were added dropwise 1 ml (*ca.* 10 mmol) of bromine in 15 ml of ethyl acetate. After stirring overnight at room temperature, the precipitate was collected and dried to give 2.8 g (97 %) of crude *3*. An analytical sample (300 mg) was taken up in a saturated aqueous solution of sodium hydrogen carbonate (7 ml). Upon extraction with ether (30 ml) the aqueous phase was adjusted to pH 6 with 4 M hydrochloric acid to give 170 mg of *3*, m.p. 220 °C (decomp.). Anal. $C_4H_4N_2O_2S$: C, H, N, S. λ_{max} 290 nm ($\varepsilon = 2.85 \times 10^3$) and 226 nm ($\varepsilon = 8.97 \times 10^3$). IR data ($cm^{-1}$): 3600–2400 (s) (with submaxima at 3320, 3150, 2750, 2675, 2600, 2500), 1660 (s), 1560 (m), 1460 (s), 1400 (m). ^1H NMR data (DMSO-d_6): δ 8.3 and 7.8 (two broad signals, total 2 H, $CONH_2$); 7.20 (s, 1 H, $C=CH-C$).

3-(4-Toluenesulfonyloxy)isothiazole-5-carboxamide (4a). To a solution of 290 mg (2 mmol) of crude *3* in 2 ml of dry pyridine were added 380 mg (2 mmol) of 4-toluenesulfonyl chloride in 2 ml of dry pyridine over a period of 15 min with stirring. Upon standing for 4 h the solution was concentrated *in vacuo* to 1 ml, and upon addition of 50 ml of water 200 mg of TLC-pure *4a* precipitated. The aqueous layer was extracted with ether (30 ml) to give further

70 mg of *4a*. Total yield of crude *4a:* 45 %. An analytical sample was recrystallized (ethanol) to give *4a*, m.p. 123–125 °C. Anal. $C_{11}H_{10}N_2O_4S_2$: C, H, N, S. λ_{max} 262 nm ($\varepsilon = 7.75 \times 10^3$) and 230 nm ($\varepsilon = 27.8 \times 10^3$). IR data ($cm^{-1}$): 3440 (m), 3180 (m), 1680 (s), 1540 (m), 1380 (s), 1360 (s), 1170 (s). ^1H NMR data (DMSO-d_6): δ 8.40 and 8.00 (two broad signals, total 2 H, $CONH_2$); 7.9–7.3 (m, 5 H, C_6H_4 and $C=CH-C$); 2.40 (s, 3 H, CH_3).

3-Benzenesulfonyloxyisothiazole-5-carboxamide (4b). *4b* was prepared as described above for *4a* using 290 mg (2 mmol) of crude *3* and 353 mg (2 mmol) of benzenesulfonyl chloride. Total yield of crude *4b*: 40 %. An analytical sample was recrystallized (ethanol-water) to give *4b*, m.p. 143–145 °C. (Found: C 42.42; H 2.96; N 9.85; S 21.80. Calc. for $C_{10}H_8N_2O_4S_2$: C 42.25; H 2.84; N 9.86; S 22.56). λ_{max} 262 nm ($\varepsilon = 8.20 \times 10^3$) and 222 nm ($\varepsilon = 24.8 \times 10^3$). The IR spectrum of *4b* was almost identical with that described above for *4a*. ^1H NMR data (DMSO-d_6): δ 8.6–7.5 (m, 8 H, $CONH_2$, $C=CH-C$ and C_6H_5).

2-Methyl-3-oxoisothiazoline-5-carboxamide (6) and 3-methoxyisothiazole-5-carboxamide (5). To a solution of 15 g (0.10 mol) of crude *3* in 1000 ml of ether was added with stirring a solution of *ca.* 6 g (0.15 mol) of diazomethane [prepared from 43 g (0.20 mol) of *N*-methyl-*N*-nitroso-4-toluenesulfonamide] in 400 ml of ether. Stirring was continued for 18 h and the remaining diazomethane was destroyed by addition of glacial acetic acid (2.5 ml). 5.9 g (36 %) of crude *6* was filtered off and recrystallized (water) to give 4.3 g of pure *6*, m.p. 215 °C (decomp.). Anal. $C_5H_6N_2O_2S$: C, H, N, S. λ_{max} 318 nm ($\varepsilon = 3.63 \times 10^3$) and 229 nm ($\varepsilon = 8.43 \times 10^3$). IR data ($cm^{-1}$): 3300 (m), 3130 (m), 1690 (s), 1670 (s), 1620 (s), 1555 (m), 1400 (m). ^1H NMR data (DMSO-d_6): δ 8.40 and 8.05 (two broad signals, total 2 H, $CONH_2$); 6.82 (s, 1 H, $C=CH-C$); 3.21 (s, 3 H, $N-CH_3$).

The ethereal filtrate was dried ($MgSO_4$) and evaporated *in vacuo* to give 6.5 g (40 %) of crude *5*, 5.5 g of which were purified by column chromatography [silica gel: 275 g; eluent: methylene chloride-ethyl acetate (4:1)] to give 4.0 g of *5*, m.p. 167–169 °C. Anal. $C_5H_6N_2O_2S$: C, H, N, S. λ_{max} 286 nm ($\varepsilon = 3.15 \times 10^3$) and 224 nm ($\varepsilon = 9.51 \times 10^3$). IR data ($cm^{-1}$): 3360 (m), 3150 (m), 1710 (s), 1690 (s), 1560 (m), 1475 (s), 1390 (s). ^1H NMR data (DMSO-d_6): δ 8.25 and 7.85 (two broad signals, total 2 H, $CONH_2$); 7.29 (s, 1 H, $C=CH-C$); 3.92 (s, 3 H, $O-CH_3$). 1.0 g of crude *5* was purified by sublimation *in vacuo* (12 mmHg) at 140 °C to give 0.7 g of product. Recrystallization from ethyl acetate gave 0.5 g of colourless crystals, m.p. 166–169 °C.

3-Methoxy-5-aminomethylisothiazole hydrochloride (9). To a solution of 1.1 g (7.0 mmol) of 3-methoxyisothiazole-5-carboxamide (*5*) in 100 ml of ice-cooled dry tetrahydrofuran was added diborane, externally generated from 0.72 g (19 mmol) of sodium borohydride in diglyme (60 ml) and 4.3 g (30 mmol) of boron trifluoride etherate in diglyme (40 ml).[23] The mixture was refluxed for 18 h, and after cooling to 25 °C followed by the addition of 4 M hydrochloric acid (15 ml), the tetrahydrofuran was removed *in vacuo*. A 50 % aqueous solution of potassium hydroxide (5 ml) was carefully added to the ice-cooled liquid residue, and the solution was extracted with three 40 ml portions of ether. The combined ether phases were dried and evaporated *in vacuo* to give 0.65 g of an oil. The oily product was dissolved in ethanol (4 ml) and upon addition of an ethanolic solution of hydrogen chloride prepared from ethanol (6 ml) and acetyl chloride (1.3 ml) followed by addition of ether (100 ml), 0.36 g (29 %) of crude *9* precipitated, m.p. 175 °C (decomp.). IR data (cm^{-1}): 3600–2400 (s) (with submaxima at 3425, 2950, 2850), 1950 (m), 1560 (s), 1470 (s), 1390 (s). ^1H NMR data (DMSO-d_6): δ 9.1–8.3 (broad signal, 3 H, NH_3^+, exchangeable with D_2O); 6.97 (s, 1 H, $C=CH-C$); 4.15 (broad signal, 2 H, $NH_3^+-CH_2$); 3.87 (s, 3 H, OCH_3).

5-Aminomethyl-3-isothiazolol dihydrobromide (10). A mixture of 120 mg (0.67 mmol) of crude *9* and 3 ml of glacial acetic acid containing 43 % of hydrogen bromide was heated at 90 °C for a total of 1 h. After reflux for 30 min an additional amount of 2 ml of glacial acetic acid containing 43 % of hydrogen bromide was added. After cooling to room temperature the crystalline solid was filtered off, and after drying over potassium hydroxide (18 h; 40 °C, 12 mmHg) 180 mg (87 %) of *10* were obtained, m.p. 160–163 °C (decomp.). Anal. $C_4H_8Br_2N_2OS$: C, H, Br, N, S. IR data (cm^{-1}): 3500–2300 (m) (with submaxima at 3410, 3120, 2970, 2560, 2470), 1580 (s), 1490 (m). The UV and ^1H NMR (DMSO-d_6) spectra of *10* were identical with those of *11* described below.

5-Aminomethyl-3-isothiazolol hydrobromide (11). Attempts to recrystallize 170 mg of *10* from ethanol-ether afforded 68 mg (55 %) of *11*, m.p. 165–167 °C (decomp.). Anal. $C_4H_7BrN_2OS$: C, H, Br, N, S. λ_{max} 262 nm ($\varepsilon = 4.47 \times 10^3$). IR data ($cm^{-1}$): 3600–2300 (s) (with submaxima at 3425, 3000), 1980 (m), 1620 (s), 1590 (s), 1560 (s), 1480–1450 (s). ^1H NMR data (DMSO-d_6): δ 8.8–8.2 (broad signal, 3 H, NH_3^+, exchangeable with D_2O); 6.82 (s, 1 H, $C=CH-C$); 4.25 [q (*J* 2.5 Hz), 2 H, $NH_3^+-CH_2$].

5-Aminomethyl-3-isothiazolol zwitterion (12). To a solution of 146 mg (0.50 mmol) of *10* in 500 µl of water were added 140 µl (1.0 mmol) of triethylamine to give 60 mg of *12* as a precipitate. Recrystallization (ethanol-water) gave 23 mg (34 %) of *12*, $\frac{1}{4}H_2O$, m.p. 140 °C (decomp.). Anal. $C_4H_6N_2OS, \frac{1}{4}H_2O$: C, H, N, S. λ_{max} 264 nm ($\varepsilon = 4.82 \times 10^3$). IR data ($cm^{-1}$): 3600–3200 (m), 3100–1800 (s) (with sub-

maxima at 2800, 2600, 2200), 1655 (m), 1555 (s). pK_A-values (H_2O, 21 °C): 6.06 ± 0.03, 8.85 ± 0.04.

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