DEUTERIUM LABELLING OF THE GABA AGONIST THIOMUSCIMOL

Poul Jacobsen and Povl Krogsgaard-Larsen
Department of Chemistry BC, Royal Danish School of Pharmacy,
2, Universitetsparken, DK-2100 Copenhagen Ø, Denmark

SUMMARY

Deuterium labelled thiomuscimol (*6*) was synthesized *via* catalytic deuteration in strongly basic $C^2H_3O^2H$-^2H_2O (9:1) of 3-methoxy-4-bromo-5-(*N*-methoxycarbonylaminomethyl)isothiazole (*3*). The replacement of bromine by deuterium was accompanied by partial exchange of the side chain methylene protons for deuterium and by complete reesterification of the methoxycarbonyl group. Acid catalyzed deprotection of the reduction product 3-methoxy-4-[^{2}H]-5-(*N*-[^{2}H]methoxycarbonylamino[^{2}H]methyl)isothiazole (*5*) gave *6* with only minor loss of deuterium from the ring and side chain.

Key Words: Thiomuscimol, Muscimol, GABA, GABA Agonist, GABA Receptor, Deuterium

INTRODUCTION

The central inhibitory neurotransmitter 4-aminobutyric acid (GABA) is involved in the regulation of a variety of physiological processes and in the pathophysiology of certain neurological and psychiatric disorders.[1-3] These findings have focused the GABA receptors in neuropharmacological research and made studies of the multiplicity, distribution, and pharmacological characteristics of these receptors a very active research field.[4]

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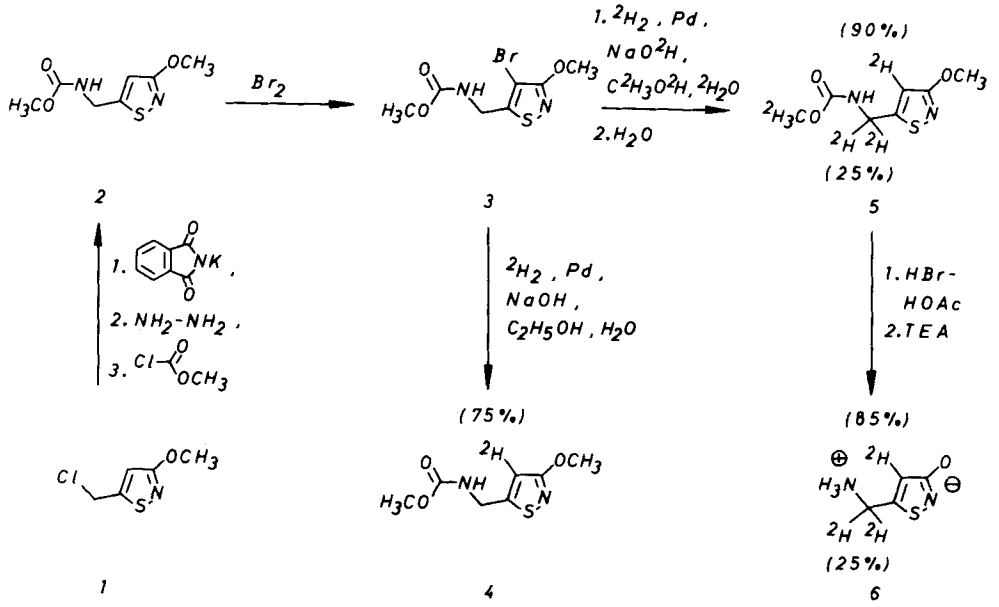
Figure 1. The structures of GABA, and the GABA agonists muscimol, thiomuscimol and THIP

Electrophysiological and in particular receptor binding studies have provided evidence of the existence of multiple types of GABA receptors.[4,5] Furthermore, the results of numerous binding studies support the view that the postsynaptic GABA receptors contain two or possibly three GABA recognition sites with different ligand affinities.[5-8] Both muscimol [9] and THIP (4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol) [10] are potent GABA agonists (Figure 1). However, while muscimol interacts with the GABA receptor sites in a manner very similar to that of GABA,[11,12] THIP selectively binds to the medium/low-affinity receptor sites.[8]. Thiomuscimol [13] (Figure 1) is approximately equipotent with muscimol as an agonist at GABA receptors on cat spinal neurones,[9] but it is more than two orders of magnitude weaker than muscimol after injection into certain regions of rat brains,[14,15] suggesting that thiomuscimol is a selective agonist at spinal GABA receptors.[15] In order to study this apparent selectivity of thiomuscimol in more detail we have now developed a procedure for deuterium labelling of thiomuscimol in preparation for tritiation of this compound.

RESULTS AND DISCUSSION

In continuation of our programme on labelling [16,17] and receptor binding studies [5,8,18] of GABA agonists we have now developed a procedure for labelling of thiomuscimol (Scheme 1).



Scheme 1.

Diprotected thiomuscimol (*2*) was prepared from 3-methoxy-5-chloromethylisothiazole (*1*) [19] in three steps involving a Gabriel synthesis and a conversion of the phthalimido intermediate into *2*. Low-pressure catalytic deuceration in ethanolic sodium hydroxide of *3*, prepared from *2* by treatment with neat bromine, afforded 3-methoxy-4-[^{2}H]-5-(*N*-methoxycarbonylaminomethyl)isoxazole (*4*) and the hydrogenated analogue (*2*) in a ratio of 3:1 as established by ^{1}H NMR spec-

troscopy. Catalytic deuteration of *3* in strongly basic $C^2H_3O^2H$-^2H_2O (9:1) resulted in an increased incorporation of deuterium. The ratio between C-4 labelled and C-4 unlabelled product was 9:1, and furthermore the methylene protons were partially (25%) exchanged for deuterium as indicated for compound *5* (Scheme 1). Acid catalyzed deprotection of *5* followed by treatment with triethylamine (TEA) to give deuterated thiomuscimol (*6*) was accomplished with only minor loss of deuterium from the ring and side chain.

EXPERIMENTAL

Melting points, determined in capillary tubes, are corrected. Analyses indicated by elemental symbols were within ±0.4% of the theoretical values and were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, DK-2750 Ballerup, Denmark. TLC and column chromatography (CC) were accomplished by using silica gel F_{254} plates (Merck) and silica gel, 0.063-0.100 (Woelm), respectively. A Perkin-Elmer grating infrared spectrophotometer (Model 247), a Perkin-Elmer ultraviolet-visible spectrophotometer (Model 402), and a Varian 360L (60 MHz) ^{1}H NMR instrument were used. ^{1}H NMR spectra were recorded using TMS as an internal standard.

3-Methoxy-5-(*N*-methoxycarbonylaminomethyl)isothiazole (*2*). A mixture of *1* [19] (2.5 g; 15 mmol) and potassium phthalimide (3.16 g; 17 mmol) in *N*,*N*-dimethylacetamide (40 ml) was stirred at 60 °C for 2 h. Stirring was continued at 25 °C for 24 h. Water (400 ml) was added, and the precipitate was washed with water (2 x 20 ml) and dried to

give 2.6 g (61%) of 3-methoxy-5-phthalimidomethylisothiazole. Without further purification this crude product (2.6 g; 9.5 mmol) was added a solution of hydrazine hydrate (1.08 g; 22 mmol) in EtOH (100 ml). The mixture was refluxed for 2 h and then evaporated *in vacuo*. Aqueous hydrochloric acid (35 ml; 2 M) was added, and the mixture was stirred at 50 °C for 10 min. Stirring was continued at room temperature for 24 h. The precipitate was filtered off and washed with water (2 x 15 ml). Upon adjustment of pH of the filtrate to 11 by addition of aqueous NaOH it was continuously extracted with ether-dichloromethane (4:1) for 3 h. The organic phase was dried (Na_2SO_4) and evaporated *in vacuo* to give 1.2 g of an oil. To a solution of this crude product in H_2O (25 ml) was added at 0 °C a solution of K_2CO_3 (3.0 g; 21 mmol) in H_2O (15 ml) and methyl chloroformate (4.0 g; 42 mmol). The mixture was stirred at 0 °C for 1 h and then at room temperature for 1 h followed by extraction with ethyl acetate (3 x 50 ml). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. CC [toluene-ethyl acetate (2:1)] gave pure *2* (1.39 g; 48% from *1*) as an oil. Anal. $C_7H_{10}N_2O_3S$: C, H, N, S. IR (film): 3300 (m), 2950 (w), 1700 (s), 1520 (s), 1460 (s), 1380 (s), 1250 (s) cm^{-1}. UV (methanol) 212 nm (log ε 3.80) and 255 nm (log ε 4.03). ^1H NMR (C^2HCl_3): δ 6.47 (1 H, s), 5.3 (1 H, broad signal), 4.55 (2 H, d), 3.97 (3 H, s), 3.70 (3 H, s).

3-Methoxy-4-bromo-5-(*N*-methoxycarbonylaminomethyl)isothiazole (*3*). A mixture of *2* (1.0 g; 4.9 mmol) and bromine (20 ml) was stirred at room temperature for 24 h. Oxygen was passed through the reaction mixture to remove excess bromine.

A saturated solution of $NaHCO_3$ (40 ml) was added, and the mixture was extracted with chloroform (2 x 50 ml). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. Recrystallization (ether-light petroleum) gave *3* (1.05 g; 76%). M.p. 83.0-84.0 °C. Anal. $C_7H_9N_2O_3SBr$: C, H, N, S, Br. IR (KBr): 3250 (m), 2950 (w), 1680 (s), 1530 (s), 1480 (m), 1380 (s), 1290 (s) cm^{-1}. UV (methanol): 214 nm (log ε 3.70) and 264 nm (log ε 3.72). ^1H NMR (C^2HCl_3): δ 5.4 (1 H, broad signal). 4.55 (2 H, d), 4.10 (3 H, s), 3.72 (3 H, s).

3-Methoxy-4-[^{2}H]-5-(*N*-methoxycarbonylaminomethyl)isothiazole (*4*). To a solution of *3* (200 mg; 0.71 mmol) in EtOH (40 ml) was added aqueous 2 M NaOH (800 µl; 1.6 mmol). The solution was deuterated at low pressure using Pd/C (5%; 70 mg) for 4 h. After filtration and evaporation *in vacuo* of the solution the crude product was mixed with H_2O (25 ml) and extracted with ether (2 x 25 ml). The organic phase was dried (Na_2SO_4) and evaporated *in vacuo*. CC [toluene-ethyl acetate (2:1)] gave *4* (105 mg; 75%). TLC [toluene-ethyl acetate (2:1)] revealed that *4* and *2* had identical R_F values (0.42). ^1H NMR (C^2HCl_3): δ 6.49 (0.25 H, s), 5.5 (1 H, broad signal), 4.55 (2 H, d), 3.97 (3 H, s), 3.70 (3 H, s).

3-Methoxy-4-[^{2}H]-5-(*N*-[^{2}H]methoxycarbonylamino[^{2}H]methyl)-isothiazole (*5*). To a solution of *3* (150 mg; 0.53 mmol) in $C^2H_3O^2H$ (1 ml) was added 7 M NaO^2H in ^2H_2O (150 µl; 1.1 mmol). The solution was deuterated at low pressure using Pd/C (5%; 30 mg) for 20 h. After filtration and evaporation *in vacuo* of the solution the crude product was mixed with H_2O (5 ml) and extracted with ether (2 x 10 ml). The organic phase was

dried (Na_2SO_4) and evaporated *in vacuo*. CC [toluene-ethyl acetate (2:1)] gave *5* (70 mg; 70%). TLC [toluene-ethyl acetate (2:1)] revealed that *5* and *2* had identical R_F values (0.42). ^1H NMR (C^2HCl_3): δ 6.49 (0.1 H, s), 5.5 (1 H, broad signal), 4.55 (1.5 H, d), 3.97 (3 H, s).

4-[^{2}H]-5-[^{2}H]methylamino-3-isothiazolol (thiomuscimol) (*6*). A mixture of *5* (55 mg; 0.27 mmol) and a solution of HBr in glacial acetic acid (1.5 ml; 33%) was heated at 90 °C for 20 min. Upon addition of an additional portion of the reagent (1.5 ml; 33%) heating was continued for 20 min. After cooling to room temperature the crystalline solid was filtered off, washed with glacial acetic acid (3 x 1 ml) and dried over KOH to give *6*, dihydrobromide (64 mg; 84%), identified by comparison of the ^1H NMR data with those of an authentic sample of thiomuscimol dihydrobromide.[13] ^1H NMR [$(C^2H_3)_2SO$]: δ 9.63 (2 H, broad s), 8.6 (3 H, broad signal), 6.90 (0.15 H, s), 4.3 (1.5 H, m).

To a solution of crude *6*, dihydrobromide (64 mg; 0.22 mmol) in EtOH (2 ml) was added triethylamine (TEA) (60 µl; 0.44 mmol). After 2 h at room temperature the crystalline solid was filtered off and washed with EtOH (1 ml) to give *6* (24 mg; 69% from *5*). TLC [butanol-acetic acid-water (4:1:1)] revealed that *6* and an authentic sample of thiomuscimol [13] had identical R_F values (0.28).

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