DEUTERIUM LABELLING OF THE GABA AGONISTS THIP, PIPERIDINE-4-SULPHONIC ACID AND THE GABA UPTAKE INHIBITOR THPO

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SUMMARY

The preparations of deuterium labelled THIP ([7,7-^{2}H]4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol) (*7*) and THPO ([4,4,7-^{2}H]4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol) (*14*) are described. [7,7-^{2}H]-THIP (*7*) was synthesized by triethylamine catalyzed exchange of hydrogen for deuterium in 3-methoxy-6-nitroso-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine (*5*) using deuterium oxide, followed by acid catalyzed denitrosation and demethylation. A similar reaction sequence was employed for the preparation of [4,4,7-^{2}H]THPO (*14*), though the exchange reaction on the *N*-nitroso intermediate *12* required strongly basic conditions. Deuterium labelled P4S (piperidine-4-sulphonic acid) (*17*) was synthesized by catalytic deuteration of pyridine-4-sulphonic acid (*16*). The structures of the deuterated compounds *6*, *7*, *13*, *14*, and *17* were established using ^{1}H NMR spectroscopy, in the case of *17* supported by mass spectrometry.

Key Words: THIP, THPO, Piperidine-4-sulphonic acid, GABA Agonist, Deuterium

INTRODUCTION

Muscimol is a potent agonist at receptors for the central inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Figure 1).[1] However, muscimol also interacts with the neuronal and glial GABA uptake mechanisms,[1-3] and it is rapidly

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decomposed after peripheral administration to animals probably *via* a transamination reaction.[4,5] Thus, instability and lack of specificity limit the utility of muscimol as a tool for studies of GABA synaptic mechanisms *in vitro* and *in vivo*. The muscimol analogue THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol), on the other hand, is a specific GABA agonist,[1,6] and THPO (4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol) selectively inhibits glial GABA uptake without affecting significantly the GABA receptors.[7]

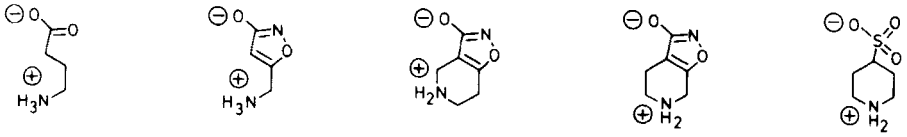
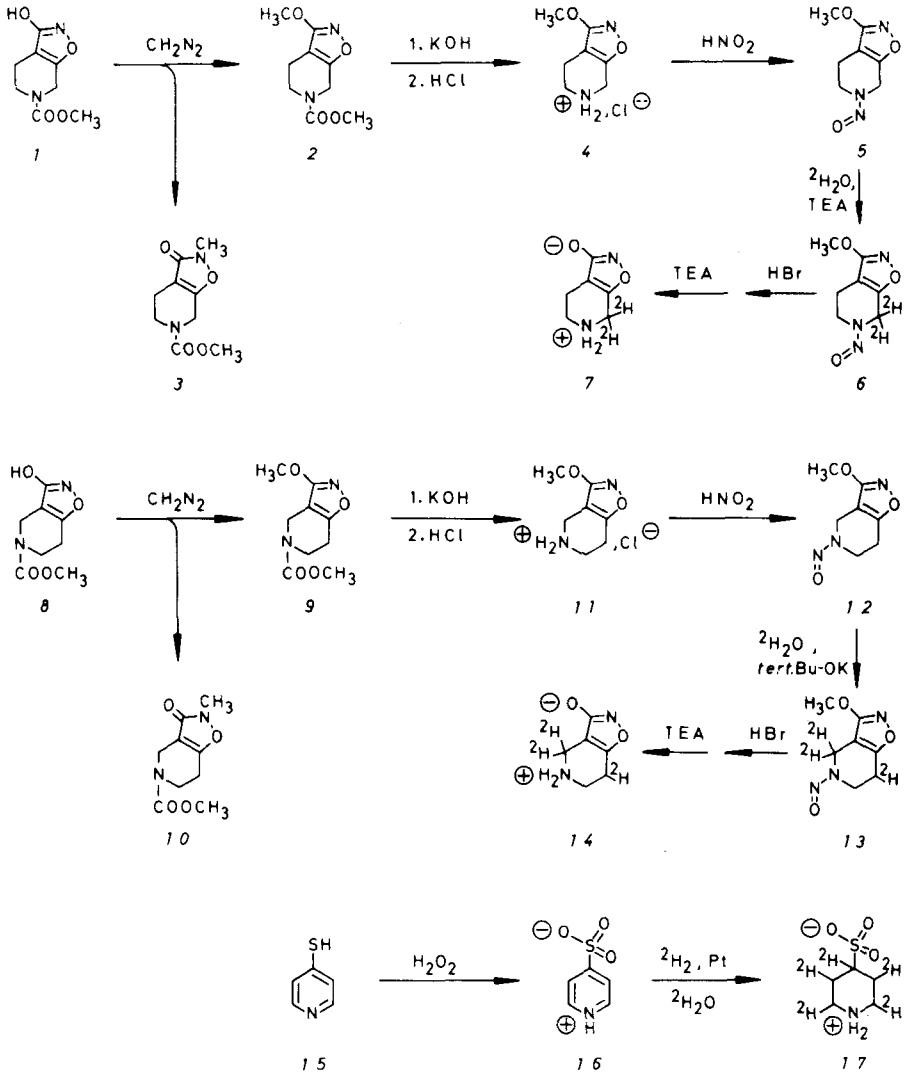


Figure 1. The structures of GABA, muscimol, THPO, THIP and P4S

In spite of their zwitterionic structures (Figure 1) both THIP[8] and THPO[9] are capable of penetrating the blood-brain barrier, and neither compound affects the activity of the GABA-metabolizing enzyme GABA:2-oxoglutarate aminotransferase (GABA-T) *in vitro*.[1,10] Like THIP the structurally related amino acid piperidine-4-sulphonic acid (P4S) is a potent and specific agonist at GABA receptors in the mammalian central nervous system,[11] but in contrast to THIP, P4S is almost devoid of effect on GABA receptors in the invertebrate animal species so far studied.[12] The apparent utili-



Scheme 1.

ty of THIP, P4S, and THPO as tools for studies of GABA neurotransmission processes has prompted us to develop methods for labelling these compounds.

RESULTS AND DISCUSSION

The synthesis of 3-methoxy-6-nitroso-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine (*5*), the key intermediate in the reaction sequence for specific deuterium labelling of THIP is outlined in Scheme 1. Treatment of the starting material 6-methoxycarbonyl-THIP (*1*) with diazomethane gave a separable mixture of *2* and *3*, of which the former compound was converted into *5* *via* compound *4*. The C-7 protons of *5* were quantitatively replaced by deuterium under weakly basic aqueous conditions using triethylamine (TEA). Compound *6* was denitrosated and demethylated by treatment with hydrogen bromide and converted into [7,7-^{2}H]THIP (*7*) without significant loss of deuterium.

A similar reaction sequence was utilized for the preparation of labelled THPO (*14*) (Scheme 1). The exchange of the C-4 protons of *12* for deuterium atoms, however, required strongly basic conditions. This difference between the degree of activation of the C-7 protons in THIP and the protons in position 4 of THPO, both methylene groups being exposed to double activation by nitrogen and the isoxazole ring, is in agreement with the finding that the C-5 methyl group in 3-methoxy-4,5-dimethylisoxazole can be selectively lithiated.[13] Accordingly, the replacement of the C-4 protons in *12* by deuterium atoms under strongly basic conditions was accompanied by exchange of approximately one proton in position 7 (Scheme 1). Deuteration of *5* under the same conditions resulted in quantitative exchange of the C-7 protons, whereas the protons in position 4 were unaffected. Conversion of *13* into [4,4,7-^{2}H]THPO (*14*) was accomplished with-

out significant loss of deuterium atoms.

Deuterium labelled P4S (*17*) was synthesized by catalytic deuteration of a solution of pyridine-4-sulphonic acid (*16*), prepared from *15* as described earlier,[14] in deuterium oxide. Approximately the same degree of labelling of *17* was achieved by catalytic hydrogenation of *16* dissolved in deuterium oxide.

The structures of the deuterated compounds *6*, *7*, *13*, *14*, and *17* were established using ^{1}H NMR spectroscopy, in the case of *17* supported by mass spectrometry. The interpretation of the ^{1}H NMR spectra of *5*, *6*, *12*, and *13* was complicated by the fact that all of these compounds gave rise to two sets of resonance signals due to hindered rotation around the N-N bonds. While thin layer chromatography (TLC) of *5* and *6* revealed single spots, the two rotamers of both *12* and *13* could be detected as individual spots on TLC plates, probably reflecting higher rotational energy barriers of the N-N bonds in *12* and *13*.

The described conditions for deuteration of THIP and P4S have been utilized by New England Nuclear Corp., Boston, Massachusetts to prepare [^{3}H]THIP (25 Ci/mmol) and [^{3}H]P4S (35 Ci/mmol). The interactions of [^{3}H]THIP and [^{3}H]P4S with GABA receptors in rat[15] and bovine[16] brains, respectively, have been characterized.

EXPERIMENTAL

Melting points, determined in capillary tubes, are corrected. Elemental analyses were made by Mr. P. Hansen, Chemical Laboratory II, University of Copenhagen. A Perkin-El-

mer grating infrared spectrophotometer model 457 and a JEOL JMN-C-60 HL (60 MHz) ^{1}H NMR instrument were used. The field desorption mass spectrum of [^{2}H]P4S was obtained on a Varian MAT CH 5D instrument equipped with a combined EI/FI/FD ion source. The sample was introduced by the emitter dipping technique. The field ion emitter was a 10 µm tungsten wire activated in benzonitrile vapour. TLC and column chromatography (CC) were accomplished by using silica gel F_{254} plates (Merck) and silica gel 0.063-0.100 mm (Woelm), respectively. Columns were developed by stepwise gradient elution.

Methyl 3-methoxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-6-carboxylate (*2*). To a suspension of *1*[17] (9.90 g; 50 mmol) in ether (250 ml) was added with stirring an ether solution of diazomethane (*ca.* 3.15 g; *ca.* 75 mmol) prepared from *N*-methyl-*N*-nitroso-4-toluenesulphonamide (21.5 g; 100 mmol). After stirring for 2 h the remaining diazomethane was destroyed by addition of excess of formic acid. TLC [eluent: dichloromethane-ethyl acetate (3:1)] revealed the presence of two components in the evaporated reaction mixture (R_F = 0.47, R_F = 0.12). The two compounds were separated by CC [eluents: dichloromethane containing ethyl acetate (25-40%)]. The fractions containing *3* (R_F = 0.12) were discarded, and the fractions containing the component with R_F = 0.47 were concentrated to give *2* (3.84 g; 36%), m.p. 58-60 °C. Anal. $C_9H_{12}N_2O_4$: C, H, N. IR (KBr): 3000-2850 (several bands, m), 1705 (s), 1665 (m), 1430 (s) cm^{-1}. ^{1}H NMR (CCl_4): δ 4.40 (2 H, q, *J* 2 Hz), 3.94 (3 H, s), 3.65 (s) and 3.56 (t, *J* 6 Hz) (a total of 5 H), 2.35 (2 H, t).

3-Methoxy-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridinium chloride (*4*). A solution of *2* (424 mg; 2 mmol) and potassium hydroxide (0.90 g; 16 mmol) in methanol (4 ml) was heated to 80 °C for 15 min. Upon addition of water (15 ml) the reaction mixture was extracted with chloroform (3 × 20 ml). After removal of the chloroform the crystalline residue was dissolved in ethyl acetate (2 ml), and upon addition of a solution of hydrogen chloride in ethyl acetate (5 ml; 2 M) 148 mg of crystals precipitated. Recrystallization (methanol-ethyl acetate) gave *4* (123 mg; 32%), m.p. 191 °C (decomp.). Anal. $C_7H_{11}ClN_2O_2$: C, H, Cl, N. IR (KBr): 3600-3300 (s), 3000-2300 [several bands, including 2930 (s) and 2760 (s)], 1665 (m), 1620 (m), 1575 (w), 1520 (s), 1490 (m), 1450 (w), 1435 (m), 1405 (m) cm^{-1}. ^1H NMR [D_2O (sodium 3-trimethylsilyl)propanesulphonate was used as an internal standard]: δ 4.41 (2 H, q, J 2 Hz), 4.00 (3 H, s), 3.56 (2 H, t, J 6 Hz), 2.76 (2 H, t, J 6 Hz).

3-Methoxy-6-nitroso-4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine (*5*). To a solution of *4* (0.48 g; 2.50 mmol) in acetate buffer (pH 4; 10 M; 25 ml) was added dropwise and with stirring a solution of sodium nitrite (3.45 g; 50 mmol) in water. The solution was heated to 90 °C for 30 min and then, after cooling to 25 °C, extracted with dichloromethane (3 × 10 ml). The dichloromethane was removed and the residue crystallized (toluene-light petroleum) to give *5* (374 mg; 81%), m.p. 97-99 °C. Anal. $C_7H_9N_3O_3$: C, H, N. IR (KBr): 3030-2860 (several bands, w), 1665 (m), 1520 (s), 1485 (m), 1445 (m), 1425 (s), 1410 (m) cm^{-1}. The ^1H NMR data ($CDCl_3$)

are consistent with *5* being a mixture (*ca.* 1:4) of two rotamers: δ 5.10 (0.4 H, q, *J* 2 Hz), 4.50 (1.6 H, s), 4.39 (1.6 Hz, t, *J* 6 Hz), 3.98 (t, *J* 6 Hz) and 3.90 (s) (a total of 3.4 H), 2.9-2.7 (1.6 H, m), 2.6-2.5 (0.4 H, m).

[7,7-^{2}H]3-Methoxy-6-nitroso-4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]pyridine (*6*). A solution of *5* (55 mg; 0.3 mmol), deuterium oxide (55 mg; 2.8 mmol), and triethylamine (TEA) (0.5 ml) in dioxane (0.5 ml) was heated in a sealed glass vessel to 60 °C for 26 h. The content of the vessel was concentrated and the residue crystallized (toluene-light petroleum) to give *6* (26 mg; 47%), m.p. 96-98 °C. TLC [toluene-ethyl acetate (1:1)] comparison with *5* corresponded. IR (KBr): 3030-2860 (several bands, w), 1665 (m), 1520 (s), 1485 (m), 1450 (m), 1420 (s), 1410 (m) cm^{-1}. The ^{1}H NMR data ($CDCl_3$) are consistent with *6* being a mixture (*ca.* 1:4) of two rotamers and with the presence of two deuterium atoms in position 7: δ 4.47 (1.6 H, t, *J* 6 Hz), 3.97 (t, *J* 6 Hz) and 3.94 (s) (a total of 3.4 H), 2.67 (1.6 H, t, *J* 6 Hz), 2.37 (0.4 H, t, *J* 6 Hz).

[7,7-^{2}H]4,5,6,7-Tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol ([7,7-^{2}H]THIP (*7*). Compound *6* (110 mg; 0.6 mmol), dissolved in a solution of hydrogen bromide in glacial acetic acid (43%; 1 ml), was heated to 90 °C for 3 min. The solution was concentrated and the residue dissolved in the same reagent (1 ml). After heating to 90 °C for 3 min the solution was concentrated and the crystalline residue dissolved in water (0.4 ml). After adjustment of pH of the solution to 6.5 by addition of a solution of TEA in ethanol (10 %;

ca. 0.8 ml) crude *7* (65 mg) precipitated. Recrystallization (water-ethanol) afforded *7* (34 mg; 40%), m.p. 240-242 °C (decomp.). TLC [butanol-glacial acetic acid-water (4:1:1)] comparison with authentic THIP[17] corresponded. IR (KBr): 3500-2200 (several bands, m-w), 1665 (m), 1645 (w), 1615 (w), 1505 (s), 1435 (s) cm^{-1}. The ^1H NMR data [D_2O (sodium 3-trimethylsilyl)propanesulphonate was used as an internal standard)] are consistent with the presence of two deuterium atoms in position 7: δ 3.50 (2 H, t, *J* 5 Hz), 2.66 (2 H, t, *J* 5 Hz).

Methyl 3-methoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridine-5-carboxylate (*9*). Compound *9* was synthesized as described above for *2* using *8*[18] (1.39 g; 7 mmol) as a starting material and diazomethane (*ca.* 0.42 g; *ca.* 10 mmol). TLC [eluent: toluene-ethyl acetate-acetic acid (49:49:2)] of the reaction mixture revealed the presence of two components (R_F = 0.41; R_F = 0.08). The two compounds were separated by CC [eluents: toluene containing ethyl acetate (40-60%)]. The fractions containing *10* (R_F = 0.08) were discarded, and the fractions containing the component with R_F = 0.41 were concentrated to give *9* (620 mg; 42%), m.p. 55-57 °C. Anal. $C_9H_{12}N_2O_4$: C, H, N. IR (KBr): 2995 (w), 1950 (m), 2860 (w), 1700 (s), 1675 (m), 1520 (s), 1490 (s), 1475 (w), 1440 (s) cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.30 (2 H, q, *J* 3 Hz), 4.00 (3 H, s), 3.95 (s) and 3.78 (t, *J* 6 Hz) (a total of 5 H), 2.74 (2 H, t, *J* 6 Hz).

3-Methoxy-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]pyridinium chloride (*11*). Compound *11* was synthesized as described

above for *4* using *9* (318 mg; 1.5 mmol) as a starting material and potassium hydroxide (1.12 g; 20 mmol) in methanol (5 ml). Recrystallization (methanol-ethyl acetate) of crude *11* (124 mg) gave *11* (75 mg; 32%), m.p. 195-197 °C (decomp.). Anal. $C_7H_{11}ClN_2O_2$: C, H, Cl, N. IR (KBr): 3600-3300 (m), 3150-2240 [several bands, including 2955 (s), 2900 (s), 2740 (s)], 1665 (m), 1575 (m), 1525 (s), 1490 (s), 1430 (w), 1420 (m) cm^{-1}. ^1H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulphonate was used as an internal standard)]: δ 4.16 (2 H, q, *J* 2 Hz), 3.99 (3 H, s), 3.63 (2 H, t, *J* 6 Hz), 3.2-3.9 (2 H, m).

3-Methoxy-5-nitroso-4,5,6,7-tetrahydroisoxazolo[4,5-*c*]-pyridine (*12*). Compound *12* was synthesized as described above for *5* using *11* (990 mg; 5.2 mmol) as a starting material, acetate buffer (pH 4; 10 M; 50 ml), and sodium nitrite (6.90 g; 100 mmol). Compound *12* (880 mg; 93%) was obtained as an oil. Anal. $C_7H_9N_3O_3$: C, H, N. TLC [eluent: toluene-ethyl acetate (4:1)] showed two spots (R_F = 0.30; R_F = 0.28), the component with R_F = 0.28 being dominant. Attempts to separate these two components by CC [eluents: toluene containing ethyl acetate (20-30%)] were unsuccessful; all fractions contained both components in the same ratio. IR (film): 3050-2800 (several bands, w), 1665 (m), 1520 (s), 1490 (m), 1450 (m), 1425 (s), 1410 (m) cm^{-1}. The ^1H NMR data (CCl_4) are consistent with *12* being a mixture (*ca.* 1:3) of two rotamers: δ 5.05 (0.5 H, q, *J* 2 Hz), 4.61 (1.5 H, t, *J* 6 Hz), 4.38 (1.5 H, q, *J* 2 Hz), 4.05 (t, *J* 6 Hz) and 3.93 (s) (a total of 3.5 H), 3.2-2.9 (1.5 H, m), 2.9-2.6 (0.5 H, m).

[4,4,7-^{2}H]3-Methoxy-5-nitroso-4,5,6,7-tetrahydro[4,5-*c*]-pyridine (*13*). A solution of *12* (73 mg; 0.4 mmol), potassium *tert*-butoxide (45 mg; 0.4 mmol), and deuterium oxide (250 mg; 14 mmol) in dioxane (2 ml) was heated in a sealed glass vessel to 100 °C for 20 h. The content of the vessel was concentrated. The residue, dissolved in water (10 ml), was extracted with chloroform (3 × 10 ml). The combined chloroform phases were washed with water (10 ml), dried ($MgSO_4$), and concentrated to give *13* (69 mg; 93%) as an oil. TLC [eluent: toluene-ethyl acetate (4:1)] comparison with *12* corresponded. IR (film): 3100-2800 (several bands, w), 1665 (m), 1520 (s), 1485 (m), 1450 (m), 1430 (s), 1405 (m) cm^{-1}. The ^{1}H NMR data ($CDCl_3$) are consistent with *13* being a mixture (*ca.* 1:3) of two rotamers and with the presence of two deuterium atoms in position 4 and one in position 7: δ 4.7-4.6 (1.5 H, m), 4.2-4.1 (m) and 4.00 (s) (a total of 3.5 H), 3.2-2.9 (0.75 H, m), 2.9-2.6 (0.25 H, m).

[4,4,7-^{2}H]4,5,6,7-Tetrahydroisoxazolo[4,5-*c*]pyridin-3-ol ([4,4,7-^{2}H]THPO) (*14*). Compound *14* was synthesized as described above for *7* using *13* (174 mg; 0.94 mmol) and two 1 ml portions of a solution of hydrogen bromide in glacial acetic acid (43%). Recrystallization (water-ethanol) of crude *14* (99 mg) afforded *14* (80 mg; 60%), m.p. 253-255 °C (decomp.). TLC [butanol-glacial acetic acid-water (4:1:1)] comparison with authentic THPO[18] corresponded. IR (KBr): 3600-2100 (several bands, m-w), 1665 (m), 1610 (w), 1490 (s), 1465 (s), 1420 (s) cm^{-1}. The ^{1}H NMR data [D_2O containing an amount of hydrogen bromide equivalent with the amount of *14* (sodium 3-(trimethylsilyl)propanesulphonate was

used as an internal standard)] are consistent with the presence of two deuterium atoms in position 4 and one in position 7: δ 3.63 (2 H, t, *J* 4 Hz), 3.2-2.9 (1 H, m).

[^{2}H]Piperidine-4-sulphonic acid ([^{2}H]P4S) (*17*). A solution of *16* (100 mg; 0.63 mmol) in deuterium oxide (5 ml) was deuterated (*ca.* 300 kPa) for 18 h using platinum(IV) oxide (75 mg) as a catalyst. After filtration the solution was concentrated and the residue recrystallized (water-ethanol) to give *17* (64 mg; *ca.* 60%), m.p. > 300 °C. TLC [eluent: butanol-glacial acetic acid-water (4:1:1)] comparison with authentic P4S[11] corresponded. IR (KBr): 3600-3300 (s), 3050-2950 (s), 2950-2400 (several bands, m-w), 1665 (m), 1635 (s), 1465 (m) cm^{-1}. ^{1}H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulphonate was used as an internal standard)]: δ 3.7-3.5 (m), 3.2-2.8 (m), and 2.1-1.7 (m) (ratio *ca.* 4:2:5). Field desorption mass spectral data (% rel. int.): 175(15), 174(46), 173(88), 172(100), 171(92), 170(50), 169(25). These data indicate that the main component of *17* contains six deuterium atoms.

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