PREPARATIONS OF DEUTERIUM LABELLED GUVACINE AND ISOGUVACINE

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SUMMARY

The preparations of guvacine hydrochloride, in which a proton in position 2 (11) and protons in positions 2 and 5 (10) are selectively exchanged by deuterium, and of [2,6-^{2}H]isoguvacine hydrobromide (14a) are described. The deuterium labelled guvacine salts 10 and 11 were synthesized by triethylamine catalyzed exchange of hydrogen for deuterium in methyl N-nitroso-1,2,5,6-tetrahydropyridine-3-carboxylate (3) followed by denitrosation and hydrolysis. Treatment of N-nitroso-1,4,5,6-tetrahydropyridine-3-carboxylic acid with methanolic thionyl chloride gave 3,4,5,6-tetrahydropyridin-3-on ketoxime. Compound 14a was prepared by sodium borodeuteride reduction of 4-ethoxycarbonyl-1-methylpyridinium iodide (12) followed by N-demethylation and hydrolysis of the intermediate.

Key Words: Guvacine, Isoguvacine, Deuterium

INTRODUCTION

Isoguvacine is a very potent and specific γ-aminobutyric acid (GABA) receptor agonist,[1,2] whereas guvacine is a potent inhibitor af GABA uptake without any affinity for the GABA receptors (Figure 1).[2-4] Consequently isoguvacine and guvacine are important tools for studies of GABA mediated synaptic processes. This paper describes the syntheses of

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deuterium labelled guvacine and isoguvacine.

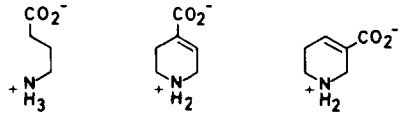
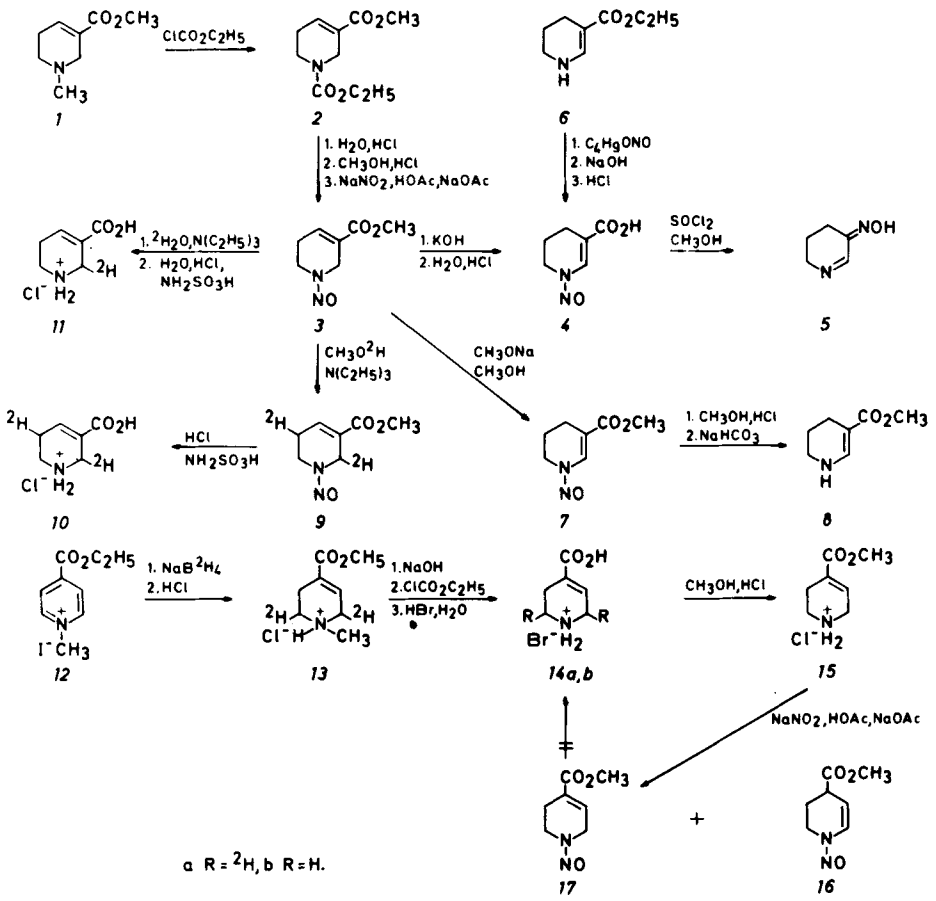


Figure 1. The structures of GABA, isoguvacine and guvacine

RESULTS AND DISCUSSION

Substitution of deuterium for hydrogen in positions 2 and 6 in a piperidine derivative has been accomplished *via* exchange of the α-protons in the corresponding N-nitroso derivative under strongly basic conditions. The labelled N-nitroso derivative is then denitrosated to give the desired amine.[5] However, treatment of 3, prepared from arecoline (1) by a new convenient synthesis, with ethanolic potassium hydroxide or sodium methanolate, gave the rearranged products 4 and 7, respectively (Scheme 1). Attempts to convert 4 into 7 by addition of thionyl chloride to a solution of 4 in methanol gave the ketoxime 5. The mechanism of the observed decarboxylative transnitrosation has not been investigated further. The structures of 4 and 7 were confirmed by synthesis of 4 from 6 and by conversion of 7 into the known compound 8.



Scheme 1.

Treatment of 3 with [O^{2}H]methanol in the presence of triethylamine led to an exchange of protons for deuterium in position 2 and to a smaller extent in position 5. Under these conditions the formation of 7 by double bond migration was shown only to proceed to a very small extent. If deuterium oxide was used as a medium instead of [O^{2}H]methanol only protons in position 2 were exchanged with deuterium. The mild conditions, at which these experiments are performed, together with the greater activation at the 2 than the 6 position might explain why no significant amount of deuterium is incorporated at the latter position. The deuterium labelled nitroso derivatives were converted into the desired hydrochlorides by treatment with hydrochloric acid with-

out significant loss of deuterium.

Attempts to incorporate deuterium into isoguvacine by a procedure analogous to that described for guvacine were unsuccessful. Nitrosation of 15 afforded a mixture of 16 and 17 in a poor yield. Furthermore attempts to convert 17 into 14b were abandoned, since exposure of 17 to hydrochloric acid led to a complex mixture, in which 14b could not be detected by TLC. In agreement with the lability of 3, compound 17 isomerized to give 16 by treatment with sodium methoxide.

[2,6-^{2}H]Isoguvacine hydrobromide (14a) was synthesized by sodium borodeuteride reduction of 12 and subsequent demethylation and hydrolysis of the intermediate 13 (Scheme 1). In agreement with the accepted mechanism for the reduction of quaternary pyridinium salts with borodeuteride,[6] one deuterium atom is incorporated in position 2 and one in position 6 of 13. Conversion of 13 into 14a proceeded without significant loss of deuterium.

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-Elmer grating infrared spectrophotometer model 457, a Perkin-Elmer ultraviolet visible spectrophotometer model 402, a JEOL JMN-C-60 HL (60 MHz) ^{1}H NMR instrument, and a Finnigan 3500 D mass spectrometer were used. Thin-layer chromatography (TLC) and column chromatography (CC) were accomplished by using silica gel F_{254} plates (Merck) and silica gel 0.05-0.200 mm (Merck), respectively.

1-Ethyl 3-methyl 1,2,5,6-tetrahydropyridine-1,3-dicarboxylate (2). Ethyl chloroformate (21.6 g; 200 mmol) was added dropwise to a stirred solution of arecoline (1) (15.5 g; 100 mmol) in 1,2-dichloroethane (1000 ml). After reflux for 3 h the mixture was cooled to room temperature and washed with 4 M hydrochloric acid (250 ml). Removal of the solvent and distillation gave an unexpected forerun of dimethyl adipate, followed by 2 (12.0 g; 56%), b.p. 120-130° C/0.16 kPa. Anal. $C_{10}H_{15}NO_4$: C, H, N. IR (film): 3000-2800 (several bands, m), 1710 (s), 1655 (m) cm^{-1}. ^1H NMR (CCl_4): δ 7.0-6.8 (1 H, m), 4.00 (q, J 7 Hz) and 4.00-3.8 (m) (a total of 4 H), 3.67 (3 H, s), 3.43 (2 H, t, J 6 Hz), 2.5-2.0 (2 H, m), 1.23 (3 H, t, J 7 Hz).

N-Nitrosoguvacoline (3). A solution of 2 (9.8 g; 48 mmol) in 6 M hydrochloric acid (80 ml) was refluxed for 6 h. Upon cooling guvacine hydrochloride (6.0 g; 80%) precipitated. A refluxed suspension of guvacine hydrochloride (4.0 g; 25 mmol) in methanol (40 ml) was saturated with hydrogen chloride until a solution was obtained (3 h). The solution was concentrated, and the residue extracted with chloroform (100 ml). Removal of the chloroform and recrystallization (acetonitril) of the residue afforded guvacoline hydrochloride (3.0 g; 70%), m.p. 121-122° C (decomp.). (Ref. 7: 121-122° C). A stirred solution of guvacoline hydrochloride (2.02 g; 11.4 mmol) in pH 4, acetate buffer (10 M; 120 ml) was maintained at 95° C and treated dropwise for 1 h with sodium nitrite (15.0 g; 217 mmol) in water (30 ml). The reaction mixture was extracted with methylene chloride (120 ml). The methylene chloride extract was washed with two 60 ml portions of 1 M sodium carbonate, and

the methylene chloride evaporated. The oily residue was distilled to give 3 (1.56 g; 80%), b.p. 108° C/53 Pa (Ref. 8: 137-138° C/0.53 kPa). The mass spectrum of 3 was in agreement with the published data.[8] UV [methanol (log ε)]: 215 (3.95), 243 (sh, 3.65) nm. IR (film): 1720 (s), 1660 (m), 1430 (s) cm^{-1}. The ^1H NMR data (CCl_4) of 3 are consistent with a mixture (ca. 3:7) of two rotamers (cf. Ref. 9): δ 7.1-6.9 (2 H, m), 4.83 (0.6 H, q), 4.4-4.0 (3.1 H, m), 3.8-3.5 (m), 3.73 (s), and 3.70 (s) (a total of 3.3 H), 2.8-2.0 (2 H, m).

N-Nitroso-1,4,5,6-tetrahydropyridine-3-carboxylic acid (4). Method A. A solution of 3 (0.83 g; 4.9 mmol) in an ethanolic 0.6 M potassium hydroxide (80 ml) was refluxed for 1 h. The solvent was removed, and the solid residue was dissolved in water (2 ml). After acidification (4 M hydrochloric acid, 11 ml) the solution was extracted with two 10 ml portions of ether. The ether extract was concentrated to give a residue (0.84 g). Recrystallization [benzene-cyclohexane (1:1)] afforded 4 (266 mg; 35%); m.p. 121-123° C. Anal. $C_6H_8N_2O_3$: C, H, N. UV [methanol (log ε)]: 292 (4.03) nm. IR (KBr): 3100-2400 (s), 1690 (s), 1640 (m), 1480 (s), 1420 (m) cm^{-1}. ^1H-NMR ($CDCl_3$): δ 11.8 (1 H, s), 8.87 (1 H, t, J 2 Hz), 3.63 (2 H, t, J 6 Hz), 2.47 (2 H, t), 2.1-1.5 (2 H, m).

Method B. A solution of 6[10] (310 mg; 20 mmol) and butyl nitrite (226 mg; 2.2 mmol) in glacial acetic acid (5 ml) was stirred for 10 min. The solvent was removed and the residue dissolved in an 0.75 M ethanolic potassium hydroxide. After reflux for 10 min the reaction mixture was worked up as described above for method A to give 165 mg (53%) of crystals, the IR spectrum of which was identical with that of 4 prepared according to method A.

3,4,5,6-Tetrahydropyridin-3-on ketoxime (5). To a solution of 4 (156 mg; 1 mmol) in methanol (5 ml) was added thionyl chloride (0.5 ml), and the mixture was stirred for 30 min. After evaporation of the solvent the residue was dissolved in 1 M sodium carbonate (1 ml), and the solution extracted with three 7 ml portions of ether. The combined ether phases were dried and concentrated *in vacuo* to give 5 (60 mg; 54%), m.p. 144-146° C (water). Anal. $C_5H_8N_2O$: C, H, N. UV [methanol (log ε)]: 230 (4.10) nm. IR (KBr): 3200-2100 (s), 1615 (s). ^{1}H NMR ($CDCl_3$): δ 8.00 (1 H, t), 3.8-3.5 (2 H, m), 2.57 (2 H, t, J 6 Hz), 2.0-1.4 (2 H, m). MS [IP 70 eV; m/e (% rel. int.)]: 112 (10, M), 95 (10, [M-OH]), 68 (20), 41 (100).

Methyl N-nitroso-1,4,5,6-tetrahydropyridine-3-carboxylate (7). Compound 3 (340 mg; 2.0 mmol) was dissolved in a 0.67 M methanolic solution of sodium methanolate (3 ml) and the mixture was refluxed for 25 min. The reaction mixture was poured into a saturated aqueous sodium bicarbonate solution (20 ml), extracted with two 20 ml portions of ether, and the ether extracts were concentrated to give 270 mg of crystals, which was recrystallized (cyclohexane) to give 7 (193 mg; 57%), m.p. 65-66° C. Anal. $C_7H_{10}N_2O_3$: C, H, N. UV [methanol (log ε)]: 292 (3.99) nm. IR (KBr): 1740 (s), 1680 (s), 1500 (s), 1460 (s) cm^{-1}. ^{1}H NMR (CCl_4): δ 8.73 (1 H, t, J 2 Hz), 3.83 (s) and 3.5-3.4 (t) (a total of 5 H), 2.49 (2 H, t), 2.0-1.7 (2 H, m).

Methyl 1,4,5,6-tetrahydropyridine-3-carboxylate (8). A solution of 7 (170 mg; 1 mmol) and acetyl chloride (0.3 ml; *ca.* 4 mmol) in methanol (3 ml) was refluxed for 45 min and concentrated. The residue was dissolved in a saturated aqueous sodium bicarbonate solution (5 ml), and the solution was extract-

ed with two 5 ml portions of ether. The combined ether phases were concentrated to give an oil, kugelrohr distillation of which (oven temperature 200° C; 0.13 kPa) afforded a colourless oil (61 mg; 43%) having IR and ^{1}H NMR spectroscopic data similar to those published for 8.[11]

[^{2}H]-N-Nitrosoguvacoline (9). A sealed glass vessel containing a solution of 3 (340 mg; 2.0 mmol) and triethylamine (0.3 ml) in [O^{2}H]methanol was heated to 55° C for 62 h. The content of the vessel was concentrated to give 351 mg of an oil. TLC [eluent: toluene-ethyl acetate (3:1)] comparisons with 3 corresponded except for a small impurity of 7. MS [IP 70 eV; m/e (% rel. int.)]: 170 (9), 171 (47), 172 (100), 173 (94), 174 (56). Mol % deuterium incorporation: 3, nondeuterated, 15, monodeuterated, 33, dideuterated, 31, trideuterated, 18, tetradeuterated.

[^{2}H]Guvacine hydrochloride (10). A solution of 9 (170 mg; 1 mmol) and sulfamic acid (97 mg; 1 mmol) in 6 M hydrochloric acid (2 ml) was refluxed for 90 min. Upon cooling a precipitate of 10 (105 mg; 64%) was formed. TLC [eluent: butanol-methanol-ammonia 25% (6:3:1)] comparisons with authentic guvacine corresponded. MS [IP 70 eV; m/e (% rel. int.)]: 127 (20), 128 (57), 129 (100), 130 (87), 131 (40). Mol % deuterium incorporation: 7, nondeuterated, 19, monodeuterated, 33, dideuterated, 29, trideuterated, 13, tetradeuterated. ^{1}H NMR data [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 7.2-7.0 (1 H, m), 4.0-3.8 (0.5 H, broad signal), 3.5-3.2 (2 H, m), 2.8-2.4 (1 H, m).

[^{2}H]Guvacine hydrochloride (11). A sealed glass vessel containing 3 (85 mg; 0.5 mmol), triethylamine (50 µl), and deuterium oxide (0.5 ml) was heated to 80° C for 64 h. To the content of the vessel was added sulfamic acid (50 mg; 0.5 mmol) and 6 M hydrochloric acid (1 ml), and the mixture was refluxed for 45 min. Upon cooling 11 (40 mg; 50%) precipitated. TLC [eluent: butanol-methanol-ammonia 25% (6:3:1)] comparison with guvacine corresponded. ^{1}H NMR [D_2O sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 7.2-7.0 (1 H, m), 4.0-3.8 (1.1 H, m), 3.33 (2 H, t), 2.8-2.3 (2 H, m).

[2,6-^{2}H]Ethyl 1-methyl-1,2,5,6-tetrahydropyridine-4-carboxylate hydrochloride (13). To a stirred solution of 4-ethoxycarbonyl-1-methylpyridinium iodide [12] (12) (733 mg; 2.50 mmol) in methanol (25 ml) was at 0° C portionwise added sodium borodeuteride (115 mg; 2.75 mmol) during a period of 10 min. Stirring was continued for 150 min, during which period the temperature was allowed to rise to 25° C. The solvent was removed, the residue was dissolved in water, and the solution was extracted with three 25 ml portions of ether. The combined ether extracts were concentrated to give 429 mg of an oil, which was dissolved in 2 M hydrochloric acid (5 ml). The solution was concentrated and the residue crystallized from ethanol-ether (1:2) to give 405 mg (78%) of 13. TLC [eluent: butanol-glacial acetic acid-water (4:1:1)] comparisons with a nondeuterated sample [2] corresponded. IR (KBr): 3600-3300 (m), 3000-2900 (m), 2700-2300 (s), 1700 (s), 1660 (s) cm^{-1}. ^{1}H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 7.0-6.9 (1 H, m), 4.33 (2 H, q, *J* 7 Hz), 4.0-3.2 (2 H, m), 3.05 (3 H, s), 2.9-2.6 (2 H, m), 1.33 (3 H, t, *J* 7 Hz). MS

(IP 70 eV) showed a molecular ion at 171.

[2,6-^{2}H]-1,2,3,6-Tetrahydropyridine-4-carboxylic acid hydrobromide (14a). An iced solution of 13 (405 mg; 1.9 mmol) in 1 M sodium hydroxide (10 ml) was extracted with five 10 ml portions of methylene chloride. The combined organic phases were concentrated. The residue was dissolved in 10 ml 1,2-dichloroethane, and after addition of ethyl chloroformate (2.0 ml; ca. 15 mmol) the solution was refluxed for 20 h. The residue obtained after removal of the dichloroethane was dissolved in an aqueous solution of hydrogen bromide (2 ml; 48%) and the solution was refluxed for 2½ h. The reaction mixture was evaporated and the residue was recrystallized [water-2-propanol-ether (1:4:2)] to give 14a (92 mg; 23%). TLC [eluent: butanol-glacial acetic acid-water (4:1:1)] comparisons with a nondeuterated sample [2] corresponded. ^{1}H NMR [D_2O (sodium 3-(trimethylsilyl)propanesulfonate was used as an internal standard)]: δ 7.0-6.9 (1 H, m), 4.2-3.8 (1 H, m), 3.6-3.3 (1 H, m), 2.8-2.4 (2 H, m). MS (IP 70 eV) showed a molecular ion at 129.

Methyl 1,2,3,6-tetrahydropyridine-4-carboxylate hydrochloride (15). A solution of isoguvacine hydrobromide [2] (6.24 g; 30 mmol) in methanol (50 ml) was saturated with dry hydrogen chloride and heated to 40° C for 30 min. The solution was stirred at room temperature for 21 h, concentrated, and the residue recrystallized (acetonitrile) to give 15 (3.86 g; 73%), m.p. 159-161° C (decomp.). Anal. $C_7H_{12}ClNO_2$: C, H, Cl, N. IR (KBr): 3050-2300 (several bands, s), 1705 (s), 1655 (s) cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 10.2 - 9.9 (2 H, broad signal), 7.0-6.8 (1 H,

m), 4.0-3.7 (m) and 3.73 (s) (a total of 5 H), 3.5-3.1 (2 H, t), 2.9-2.4 (2 H, m).

Methyl N-nitroso-1,2,3,4-tetrahydropyridine-4-carboxylate (16) and methyl N-nitroso-1,2,3,6-tetrahydropyridine-4-carboxylate (17). A stirred solution of 15 (336 mg; 1.9 mmol) in pH 4, acetate buffer (10 M; 20 ml) was maintained at 95° C and treated dropwise for 1 h with sodium nitrite (2.5 g; 36 mmol) in water (5 ml). The reaction mixture was extracted with methylene chloride (30 ml). The methylene chloride extract was washed with two 20 ml portions of 1 M sodium carbonate. The combined organic extracts were concentrated and the residue was distilled in a kugelrohr (oven temperature 200° C, 0.13 kPa) to give 100 mg of an oil, which by ^{1}H NMR spectroscopy and by TLC analysis [eluent: methylene chloride-ethyl acetate (11:1)] was shown mainly to consist of a 1:1 mixture of 16 and 17 having R_F = 0.53 and R_F = 0.39, respectively. The two compounds were separated by CC [eluent: methylene chloride-ethyl acetate (98:2)] to give 16 as an oil. Anal. $C_7H_{10}N_2O_3$: C, H, N. IR (CCl_4): 2950 (m), 1645 (s), 1460 (s) cm^{-1}. ^{1}H NMR (CCl_4): δ 7.80 (1 H, double d, J 2 Hz and 9 Hz), 5.17 (1 H, double d, J 5 Hz and 9 Hz), 3.8-3.4 (m) and 3.63 (s) (a total of 5 H), 3.3-3.0 (1 H, m), 2.2-1.8 (2 H, m). Compound 17 was obtained as an oil. Found: C 48.90; H 5.82; N 16.10. Calc. for $C_7H_{10}N_2O_3$: C 49.40; H 5.92; N 16.46. IR (CCl_4): 2950 (m), 1625 (s), 1460 (m) cm^{-1}. ^{1}H NMR (CCl_4): δ 7.0-6.7 (1 H, m), 4.5-4.0 (3 H, m), 3.9-3.8 (m) and 3.73 (s) (a total of 4 H), 2.9-2.6 (2 H, m).

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