ACETYLENIC ANALOGUES OF γ-AMINOBUTYRIC ACID

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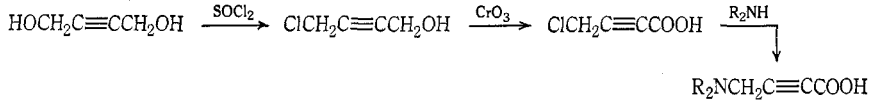
We report the preparation of 4-aminotetrolic acid (4-aminobut-2-ynoic acid) and some of its *N*-substituted derivatives as simple conformationally restricted analogues of the inhibitory synaptic transmitter, γ-aminobutyric acid (GABA). Little is known about the active conformation(s) of GABA that are important in its interactions with postsynaptic receptors, as structure–activity correlations are lacking with respect to GABA analogues of restricted conformation. To date, only one such analogue has been investigated in detail: muscimol, a psychoactive isoxazole isolated from the mushroom *Amanita muscaria*, has a powerful GABA-like inhibitory action on the firing of neurones in the central nervous system.[1]

The tetrolic acid derivatives were synthesized by direct nucleophilic attack of the appropriate amine on 4-chlorotetrolic acid,[2] which was prepared via 4-chlorobut-2-yn-1-ol[3] from the commercially available but-2-yne-1,4-diol, as outlined in Scheme 1. They were found to be stable, crystalline, high-melting zwitterionic compounds



Scheme 1

that are stronger acids than GABA. Their zwitterionic nature was particularly evident from the infrared spectra, which contained characteristic absorptions due to CO_2^- at 1600–1650 and 1325–1360 cm^{-1}, and N^+H at 2000–2300 cm^{-1}. Absorptions due to C≡C were found at 2220–2250 cm^{-1}.

Each of the *N*-substituted derivatives has been shown to inhibit the firing of central neurones.[4] 4-Aminotetrolic acid, the most potent inhibitor of this series, was less potent than GABA and, like that of GABA and muscimol, its inhibitory action was antagonized by the convulsant alkaloid bicuculline.[5] When injected into adult mice in doses up to 240 mg/kg, 4-aminotetrolic acid produced no apparent effects.

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Experimental

Infrared spectra were recorded in KBr disks using a Unicam SP200 spectrophotometer. N.m.r. spectra were recorded at 60 MHz in D_2O on a Perkin–Elmer R10 spectrometer and chemical shifts were measured on the δ-scale relative to sodium trimethylsilylpropanesulphonate as an internal standard. Mass spectra were measured at 70 eV using direct inlet systems on an A.E.I. MS902 (Research School of Chemistry, A.N.U.) or a Hitachi Perkin–Elmer RMU 6D spectrometer (Division of Applied Chemistry, CSIRO, Melbourne). Analyses were made by Dr J. E. Fildes and her staff at the Department of Medical Chemistry, A.N.U., and Mr D. Light of the same Department made the p*K* measurements by titration in aqueous solution at 20°.

4-Aminotetrolic Acid

Concentrated ammonia (45 ml, *d c.* 0·91) was added to a mixture of 4-chlorotetrolic acid (630 mg, b.p. 88–91°/7 mm) and ice (*c.* 10 g). The solution was allowed to stand at 0° for 1 hr, at room temperature for 1 hr, and at 4° for 3 days. The resulting yellow solution was taken to dryness in a vacuum, dissolved in the minimum quantity of water, and chromatographed on a column (3 by 10 cm) of Dowex 50W (H^+) ion-exchange resin (200–400 mesh). Elution with water (300 ml) removed unchanged 4-chlorotetrolic acid. The product was then eluted with aqueous pyridine (1M 200 ml),[6] the eluent evaporated to dryness in a vacuum, the residue dissolved in water and again evaporated to dryness to remove the last traces of pyridine. Recrystallization from ethanol–water after decolorization with charcoal gave colourless crystals (135 mg, 25% yield), m.p. above 300° (Found: C, 48·5; H, 5·1; N, 14·1. Calc. for $C_4H_5NO_2$: C, 48·6; H, 5·1; N, 14·1%). Mass spectrum: *m/e* 99 (M^+, 6%), 98 (11), 82 (M−OH, 5), 70 (M−N=CH_2, 7), and 54 (98−CO_2, 100%, base peak). p*K* values: 1·80±0·02, 8·34±0·02 (p*K* values for GABA measured under similar conditions:[7] 4·04, 10·71).

The following methods were found to be unsuitable for the preparation of 4-aminotetrolic acid by amination of 4-chlorotetrolic aci.d (1) Decomposition of the hexamine adduct under acidic conditions.[8] (2) Stirring 4-chlorotetrolic acid with concentrated aqueous ammonia at room temperature for 2 days. (3) Employing ammonium carbonate and concentrated aqueous ammonia as the aminating agent. (4) A small-scale amination with liquid ammonia gave traces of a product having the mass spectrum of 4-aminotetrolic acid, but attempts to scale up this procedure resulted in the formation of intensely coloured products.

4-Morpholinotetrolic Acid

This was prepared from aqueous morpholine (1·2 g in 10 ml water) and a mixture of 4-chlorotetrolic acid (460 mg) and ice (*c.* 10 g). After 2 hr at 0° and 20 hr at room temperature, the mixture was worked up as before and recrystallized from ether–ethanol–water to yield colourless crystals (280 mg, 43%), m.p. above 300° (Found: C, 56·7; H, 6·6; N, 8·3. Calc. for $C_8H_{11}NO_3$: C, 56·8; H, 6·6; N, 8·3%). Mass spectrum *m/e* 169 (M^+, 2%), 148 (7), 125 (59), 124 (18), 95 (8), 86 (24), 82 (6), 80 (12), 68 (29), 67 (33), 66 (15), 56 (16), 55 (9), 54 (10), 53 (10), 44 (100%, base peak). N.m.r. spectrum: δ 4·19 (2H, singlet), 4·01 (4H, triplet, *J* 5 Hz), and 3·45 (4H, triplet, *J* 5 Hz). p*K* values: 1·79±0·02, 5·57±0·05.

4-Piperazinotetrolic Acid

This was prepared from aqueous piperazine (2·41 g in 10 ml water) and 4-chlorotetrolic acid (920 mg) in ice, as described above (1 hr at 0°, 22 hr at room temperature), as colourless crystals (610 mg, 46%) from ether–ethanol–water, melting partly above 220° (Found: C, 57·1; H, 6·9; N, 16·6. Calc. for $C_8H_{12}N_2O_2$: C, 57·1; H, 7·2; N, 16·7%). Mass spectrum: *m/e* 168 (M^+, 24%), 140 (5), 126 (28), 124 (54), 109 (15), 96 (9), 95 (8), 94 (15), 85 (12), 83 (19), 82 (100%,

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base peak), 56 (39). N.m.r. spectrum: δ 3·54 (2H, singlet), 3·30 (4H, multiplet), and 2·93 (4H, multiplet). pK values: 2·78±0·004, 9·28±0·04 (only two could be measured).

4-Piperidinotetrolic Acid

This was prepared from aqueous piperidine (2·63 g in 10 ml water) and 4-chlorotetrolic acid (880 mg) as above (2 hr at 0°, 20 hr at room temperature). Recrystallization from methanol–ether gave colourless crystals (650 mg, 53%), m.p. above 300° (Found: C, 64·9; H, 7·8; N, 8·6. Calc. for $C_8H_{13}NO_2$: C, 64·7; H, 7·8; N, 8·4%). Mass spectrum: m/e 167 (M^+, 2%), 166 (4), 123 (40), 122 (100%, base peak), 94 (15), 84 (12), 83 (16), 82 (17), 81 (23), 80 (11), 68 (10), 67 (14), 55 (23), 54 (11), 44 (67). N.m.r. spectrum: δ 4·08 (2H, singlet), 3·35 (4H, multiplet), and 1·78 (6H, multiplet). pK values: 1·78±0·04, 8·03±0·03.

4-Pyrrolidinotetrolic Acid

When aqueous pyrrolidine (1·04 g in 10 ml water) was added to a mixture of 4-chlorotetrolic acid (500 mg) and ice (*c.* 10 g) there was an immediate reaction with the evolution of white fumes. After 15 min the resulting pale yellow solution was worked up in the usual way. Recrystallization from methanol–ether gave colourless crystals (325 mg, 50%), m.p. above 300° (Found: C, 62·8; H, 7·1; N, 9·1. Calc. for $C_8H_{11}NO_2$: C, 62·7; H, 7·2; N, 9·1%). If the reaction mixture was allowed to stand a further 15 min at 0°, a deep yellow solution was produced from which the desired product could be isolated in only 20% yield. Mass spectrum: m/e 153 (M^+, 3%), 152 (5), 109 (47), 108 (100%, base peak), 81 (20), 80 (10), 70 (14), 68 (13), 66 (10), 55 (8), 44 (57). N.m.r. spectrum: δ 4·14 (2H, singlet), 3·42 (4H, triplet, J 7 Hz), and 2·07 (4H, quintet, J 4 Hz). pK values: 1·76±0·05, 8·25±0·02.

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