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Synthesis of Analogues of GABA. VI* Stereoisomers of *cis*-3-Aminocyclohexanecarboxylic Acid

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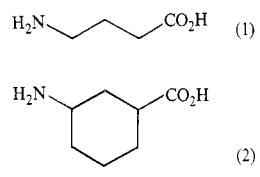
Abstract

The syntheses are described of (1*R*,3*S*)- and (1*S*,3*R*)-3-aminocyclohexanecarboxylic acids via unsaturated intermediates suitable for tritium labelling. The absolute stereochemistry was determined by an alternative synthesis of the (1*R*,3*S*) isomer from (*R*)-3-oxocyclohexanecarboxylic acid. The (1*S*,3*R*) isomer showed a similar potency to GABA as an inhibitor of the uptake of radioactive GABA by rat brain slices whereas the (1*R*,3*S*) isomer was at least 20 times less potent.

The importance of the inhibitory neurotransmitter γ-aminobutyric acid, GABA (1),[1,2] in certain neurological and psychiatric disorders has become generally accepted,[3] and its interaction in the mechanism of action of other pharmacologically active compounds such as benzodiazepines[4] and barbiturates[5] has recently been investigated. Structure–activity studies of conformationally restricted analogues show that GABA interacts with neuronal receptors, transport carriers and enzymes in certain active conformations.[6] The design of agents with selective actions upon particular processes in which GABA participates would be facilitated by a detailed understanding of the various active conformations of GABA which are involved.



At least two transport systems, which may be responsible for the inactivation of GABA after synaptic release, have been described in rat brain slices; a glial uptake system for which β-alanine is a selective substrate[7] and a neuronal uptake system

* Part V, *Aust. J. Chem.*, 1980, **33**, 1115.

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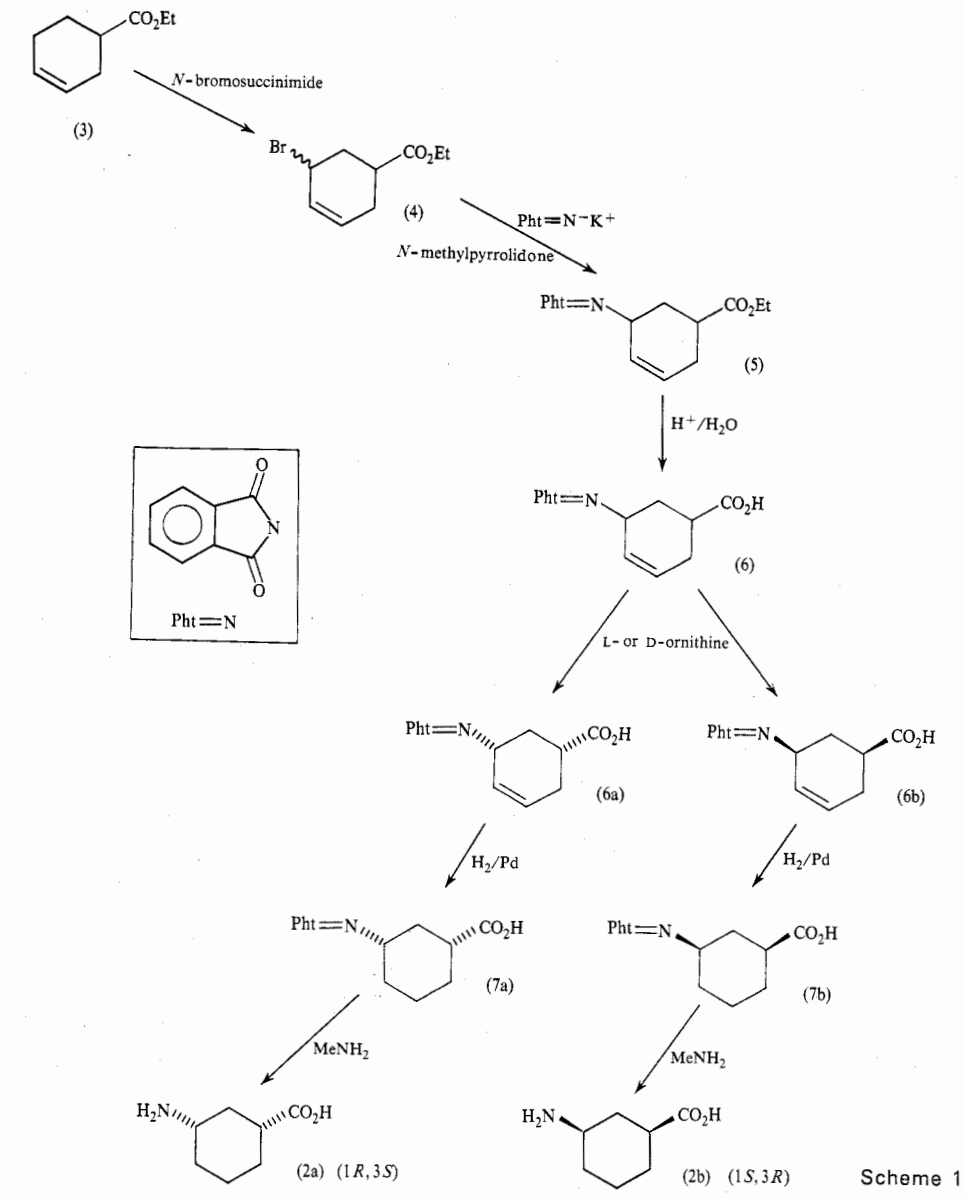
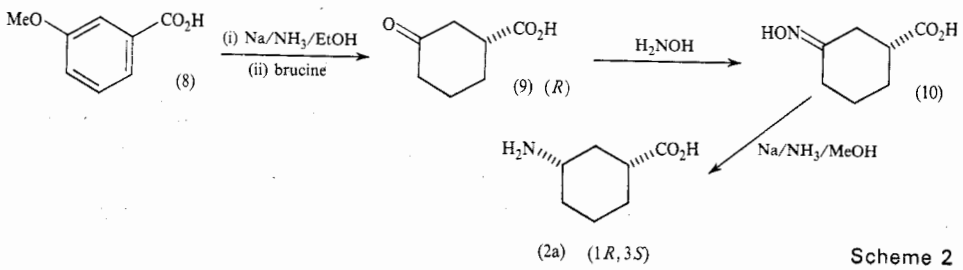


Scheme 1



Scheme 2

for which *cis*-3-aminocyclohexanecarboxylic acid (2) is a selective substrate.[8-10] The stereoselectivity shown by inhibitors of the GABA transport system has previously been studied for 3-aminocyclopentanecarboxylic acid,[11] (*E*)-4-aminopent-2-enoic acid[6,12] and a number of other non-carbocyclic uptake inhibitors.[13-15] This paper describes the preparation of the optical isomers of *cis*-3-aminocyclohexanecarboxylic acid and reports their activity on the uptake of GABA by rat brain slices.

cis-3-Aminocyclohexanecarboxylic acid (2) has previously been prepared by catalytic reduction of the corresponding aromatic acid.[16] An alternative synthetic pathway outlined in Scheme 1 was chosen to satisfy two considerations: the need to obtain both optical isomers for comparison and the desirability of using an intermediate suitable for conversion into radiolabelled (2).

Allylic bromination of ethyl cyclohex-3-ene-1-carboxylate (3) with *N*-bromosuccinimide gave the unsaturated bromo ester (4). Reaction of this crude product with potassium phthalimide in *N*-methylpyrrolidone gave ethyl 5-phthalimidocyclohex-3-ene-1-carboxylate as a mixture of isomers from which the major product, the *cis* isomer (5), could be purified by crystallization. The key intermediate (6) with an acid functionality as a handle for resolution and a double bond suitable for radiolabelling with tritium gas was formed by acid hydrolysis of (5). L- and D-Ornithine formed crystalline salts with (6) which yielded the resolved phthalimido acids (6a) and (6b) respectively. Catalytic reduction to (7a) and (7b) followed by removal of the phthaloyl protecting group with methylamine[17] produced the two enantiomers (2a) and (2b) with $[\alpha]_D^{20}$ $-5\cdot0°$ and $+4\cdot5°$ respectively. The *cis* stereochemistry of (2a) and (2b) was confirmed by comparison of infrared and ^{1}H n.m.r. spectra with those of authentic *cis*- and *trans*-3-aminocyclohexanecarboxylic acids.[18] This proved the *cis* stereochemistry as depicted for the intermediates (6) and (7).

The absolute stereochemistry of the amino acids (2a,b) was determined by an alternative synthesis of (2a) illustrated in Scheme 2. Birch reduction of (8) gave 3-oxocyclohexanecarboxylic acid which was resolved with brucine[19] to the (1*R*) isomer (9), the rotation ($[\alpha]_D^{20}$ $-17\cdot6°$) of which was more than twice that previously reported ($[\alpha]_D^{20}$ $-8\cdot2°$). Attempts to obtain the other isomer from the mother liquids were unsuccessful. Metal/ammonia reduction of the (1*R*) hydroxyimino acid (10) yielded a mixture from which (2a) ($[\alpha]_D^{20}$ $-4\cdot5°$) could be crystallized. This product must have the (1*R*,3*S*) configuration shown in (2a) if the stereochemistry at the

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carboxyl group is retained. The correspondence of the rotations of (2a) from two independent synthetic routes is consistent with a high optical purity of these compounds.

When tested on the inhibition of GABA uptake into rat brain slices,[20] the IC_{50} value [the concentration producing 50% inhibition of (^{3}H)GABA uptake] for the (1*S*,3*R*) isomer (2b) was 25 ± 2 μM compared to greater than 500 μM for the (1*R*,3*S*) isomer (2a). Isomer (2b) thus showed similar potency to GABA in inhibiting (^{3}H)GABA uptake.

The synthesis described in this paper offers a route to radioactive (1*S*,3*R*)-3-aminocyclohexanecarboxylic acid of high specific activity through reduction of (6b) with tritium, and such a radiolabelled compound may be a useful, highly selective agent with which to investigate the neuronal transport of GABA.

Experimental

Melting points were determined on a Yanagimoto hot-stage apparatus and are uncorrected. Microanalyses were determined by the Australian National University Analytical Servicing Unit. Infrared spectra were determined as Nujol mulls or liquid films on a Unicam SP200 instrument. ^{1}H n.m.r. spectra were measured on a Varian T60 spectrometer and are recorded in δ values relative to $SiMe_4$ as internal standard in $CDCl_3$ or as external standard in D_2O. Optical rotations were determined on a Perkin–Elmer 241 MC polarimeter. Cyclohex-3-enecarboxylic acid and 3-methoxybenzoic acid were purchased from Fluka AG.

Ethyl cis-*5-Phthalimidocyclohex-3-ene-1-carboxylate* (*5*)

Ethyl cyclohex-3-ene-1-carboxylate (3) (15·4 g, 0·1 mol) (obtained from the corresponding acid with ethanol and hydrogen chloride), *N*-bromosuccinimide (19·6 g, 0·11 mol) and azobisisobutyronitrile (150 mg) were heated under reflux in carbon tetrachloride (90 ml) for 1 h when the suspended solid had risen to the surface. The mixture was cooled on ice, filtered and the solvent removed under vacuum to give the crude bromo ester (4) (23·5 g) as an oil. ν_{max}: 1730, 1175, 1030, 780, 725 cm^{-1}. ^{1}H n.m.r. ($CDCl_3$): δ 6·0–5·6, m, 2× olefinic CH; 5·0–4·7, m, CHBr; 4·1, q, OCH_2; 3·3–1·9, complex, ring CH_2 and $CHCO_2$; 1·2, t, CH_3.

A solution of the crude product from above (9·65 g, 41 mmol) in *N*-methylpyrrolidone (10 ml) was added dropwise over 40 min to a suspension of potassium phthalimide (8·44 g, 46 mmol) in *N*-methylpyrrolidone (50 ml) heated in an oil bath at 80°. After heating for a further 2·5 h the solution was cooled and added to a mixture of ethyl acetate (150 ml) and ice-cold water (500 ml). The organic layer was washed with water (200 ml), ice-cold 1 M NaOH (50 ml) and again with water (150 ml). Drying (Na_2SO_4) and evaporation of the solvent yielded a crystallizing oil (7 g, 57%) which was recrystallized from carbon tetrachloride/60–80° light petroleum to give the *phthalimido ester* (5), m.p. 99–102° (Found: C, 68·1; H, 5·6; N, 4·7. $C_{17}H_{17}NO_4$ requires C, 68·2; H, 5·7; N, 4·7%). ν_{max}: 1720, 1700, 860, 710 cm^{-1}. ^{1}H n.m.r. ($CDCl_3$): δ 7·8, arom. CH; 5·95, 1H, m, =CH; 5·60, 1H, br d, *J* 10 Hz, =CH; 5·0, m, CHN; 4·18, q, OCH_2; 2·6–2·0, 5H, m; 1·25, t, CH_3.

cis-*5-Phthalimidocyclohex-3-ene-1-carboxylic Acid* (*6*)

Phthalimido ester (5) (2·3 g) was magnetically stirred with a mixture of dioxan (45 ml), conc. hydrochloric acid (5 ml) and water (10 ml), and refluxed for 6 h. The solvent was removed under vacuum and the resultant solid rubbed with acetone and filtered to give the *phthalimido acid* (6) (1·5 g, 72%), m.p. 203–205° (Found: C, 66·1; H, 4·9; N, 4·9. $C_{15}H_{13}NO_4$ requires C, 66·4; H, 4·8; N, 5·2%). ν_{max}: 1765, 1730, 1680, 1160, 1100, 860, 710 cm^{-1}. ^{1}H n.m.r. (0·5 M NaOD): δ 8·2–7·9, 4H, m, arom. H; 6·5, 1H, br d, *J* 11 Hz, =CH; 6·1, 1H, br d, *J* 11 Hz, =CH; 5·0, obscured CHN; 3·3–1·9, 5H, complex.

[20] Iversen, L. L., and Neal, M. J., *J. Neurochem.*, 1978, **15**, 1141.

Resolution of cis-*5-Phthalimidocyclohex-3-ene-1-carboxylic Acid* (*6*)

L-Ornithine was prepared from the hydrochloride salt (1·1 g, 6·5 mmol) by absorbing the salt on a column of Dowex 50W (H^+) ion-exchange resin (8 ml), washing with water and eluting with 1 M ammonium hydroxide. Removal of the solvent under vacuum gave an oil which was taken up in water (5 ml). The solution was used to dissolve the phthalimido acid (6) (1·76 g, 6·5 mmol) with gentle warming, and after the addition of methanol (3 ml) the solution was set aside to crystallize. The resultant crop (2·0 g) was recrystallized three times by dissolving in 2 vol. of water and adding 1·5 vol. of methanol. Recovery each time was about 60% and the final crop (545 mg) of the L-ornithine salt of (6a) had $[\alpha]_D^{20}$ +42°, $[\alpha]_{578}^{20}$ +44° (*c*, 1 in H_2O). The ornithine salt was dissolved in water (3 ml) and acidified with 6 M HCl to precipitate the unsaturated phthalimido acid (*6a*) (330 mg), $[\alpha]_D^{20}$ +47·5°, $[\alpha]_{578}^{20}$ +49·5° (*c*, 1 in MeOH).

(*1*R,*3*S)- *and* (*1*S,*3*R)-*3-Phthalimidocyclohexanecarboxylic Acids* (*7a*) *and* (*7b*)

The acid (6a) (320 mg) was dissolved in methanol (35 ml) and shaken under hydrogen with 10% palladium on carbon (60 mg) at atmospheric pressure for 2 h. The solution was then filtered and evaporated to give the phthalimido acid (7a) (310 mg). ν_{max}: 1760w, 1705, 1090, 1015, 910 cm^{-1}. ^1H n.m.r. ($CDCl_3/CD_3SOCD_3$): δ 7·8, 4H, arom. CH; 4·2, 1H, m, CHN; 2·7–1·0, 9H, complex. $[\alpha]_D^{20}$ −7·0°, $[\alpha]_{578}^{20}$ −6·5° (*c*, 1 in MeOH).

Similarly reduction of (6b) (200 mg) gave (7b) (200 mg), with a ^1H n.m.r. spectrum identical with that of (7a). $[\alpha]_D^{20}$ +5°, $[\alpha]_{578}^{20}$ +5° (*c*, 1 in MeOH).

(*1*R,*3*S)- *and* (*1*S,*3*R)-*3-Aminocyclohexanecarboxylic Acids* (*2a*) *and* (*2b*)

The phthalimido acid (7a) (310 mg) was dissolved in 30% methylamine in water (5 ml) and allowed to stand at room temperature for 4 days. After removal of the solvent under vacuum the product was absorbed on a column of Dowex 50W (H^+) ion-exchange resin (4 ml) and washed with water. Elution with 1 M pyridine and evaporation to dryness produced the crude amino acid (108 mg, 73%) which was crystallized from water/ethanol to give the (*1*R,*3*S) *amino acid* (2a) (37 mg), m.p. 240° with solidification and remelting at 265–280° (dec.) (Found: C, 58·5; H, 9·1; N, 9·7. $C_7H_{13}NO_2$ requires C, 58·7; H, 9·2; N, 9·8%). Infrared and ^1H n.m.r. spectra were identical with those of an authentic sample of (1*RS*,3*SR*)-3-aminocyclohexanecarboxylic acid. $[\alpha]_D^{20}$ −5·0°, $[\alpha]_{578}^{20}$ −5·0° (*c*, 1 in H_2O).

Similar treatment of (7b) with methylamine yielded crude (2b) (80 mg) which on crystallization gave the (*1*S,*3*R) *amino acid* (2b) (39 mg), m.p. 240° with solidification and remelting at 270–280° (dec.) (Found: C, 58·5; H, 9·1; N, 9·9. $C_7H_{13}NO_2$ requires C, 58·7; H, 9·2; N, 9·8%). Infrared and ^1H n.m.r. spectra were identical with those of (2a). $[\alpha]_D^{20}$ +4·5°, $[\alpha]_{578}^{20}$ +5·0° (*c*, 1 in H_2O).

(R)-*3-Oxocyclohexanecarboxylic Acid* (*9*)

(*RS*)-3-Oxocyclohexanecarboxylic acid was prepared by a method based on that of Birch *et al.*[21] but was isolated by extraction because of unpredictable losses during distillation of the reaction product. To a magnetically stirred solution of 3-methoxybenzoic acid (50 g), in liquid ammonia (1000 ml) and ethanol (150 ml), sodium (29 g) was added over 2 h. After a further hour ammonium chloride (80 g) was added and the solvent evaporated. The product was dissolved in water, acidified with concentrated hydrochloric acid (5 ml) and heated to boiling for 2 min. The solvent was once again removed and the residue taken up in a minimum amount of water and extracted with chloroform (5 × 100 ml). Evaporation of the chloroform gave a viscous oil which was crystallized by adding a small amount of ethyl acetate and by standing at 5°. Evaporation of the aqueous layer and extraction of the resultant solid with chloroform gave further product. The yield of crystalline (*RS*) keto acid suitable for resolution was 29·5 g (63%). ^1H n.m.r. ($CDCl_3$): δ 10·7, 1H, COOH; 3·1–1·7, 9H, complex.

The (*RS*) keto acid (29·5 g) was dissolved in ethanol (80 ml) and added to brucine (82·6 g) in ethanol (300 ml). The salt crystallized after reduction of the total volume to 300 ml under vacuum. Two recrystallizations gave the brucine salt (40 g) of the (*R*) isomer (9). $[\alpha]_D^{20}$ −23·3° (*c*, 1·5 in H_2O) {lit.[19] $[\alpha]_D^{32}$ −23·26° (*c*, 0·98 in H_2O)}. A solution of this brucine salt of (9) (33 g) in water

[21] Birch, A. J., Hextall, P., and Sternhell, S., *Aust. J. Chem.*, 1954, **7**, 256.

(150 ml) and 1 M HCl (70 ml) was evaporated to dryness and extracted with diethyl ether (2 × 100 ml) to give an oil (4 g). Further keto acid was recovered by suspending the remaining solid in ethanol, filtering, evaporating the ethanol and dissolving in ether. Combination of both ether-soluble oils gave the (*R*) keto acid (9) as a slowly crystallizing oil (6·85 g, 73%), m.p. 68–71°, with a ^{1}H n.m.r. spectrum identical to that of the unresolved material. $[\alpha]_D^{20}$ −17·6°, $[\alpha]_{578}^{20}$ −18·4°, $[\alpha]_{546}^{20}$ −20·6° (*c*, 4 in MeOH) {lit.[19] $[\alpha]_D^{24}$ −8·2° (*c*, 3·01 in MeOH)}.

(R)-*3-Hydroxyiminocyclohexanecarboxylic Acid* (*10*)

The (*R*) keto acid (9) (2·0 g) in water (10 ml) was mixed with a solution of hydroxylamine hydrochloride (2·0 g) and sodium acetate (6 g) in water (20 ml) and allowed to stand at room temperature overnight. Filtration gave the (*R*) hydroxyimino acid (10) (500 mg, 23%), m.p. 142–145°. ν_{max}: 3500, 1690, 1650, 1285, 970, 925 cm^{-1}. $[\alpha]_D^{20}$ −61°, $[\alpha]_{578}^{20}$ −63° (*c*, 1 in H_2O).

(*1*R,*3*S)-*3-Aminocyclohexanecarboxylic Acid* (*2a*)

To a stirred solution of the (*R*) hydroxyimino acid (10) (500 mg) in liquid ammonia (40 ml) and methanol (10 ml) was added sodium (1 g) in small pieces over 5 min at which time the blue colour persisted for several minutes. After 15 min, ammonium chloride (2·5 g) was added and the solvent removed under vacuum. The amino acid product was isolated by absorption on a Dowex 50W (H^+) ion-exchange column (100 ml), by washing with water and by elution with 1 M ammonium hydroxide. The crude product after evaporation (340 mg) crystallized from water/ethanol, and this crop (160 mg) was recrystallized from water/methanol to give (1*R*,3*S*) amino acid (2a) (54 mg, 12%), m.p. 240–270° (dec.) (Found: C, 58·4; H, 9·1; N, 9·7. $C_7H_{13}NO_2$ requires C, 58·7; H, 9·2; N, 9·8%). Infrared and ^{1}H n.m.r. spectra were identical with those of the racemic material. $[\alpha]_D^{20}$ −4·5°, $[\alpha]_{578}^{20}$ −4·5° (*c*, 1 in H_2O). Gas chromatography of the *N*-trifluoroacetyl methyl ester derivative of the 160-mg crop showed less than 3% contamination by the corresponding *trans* isomer.

Inhibition of GABA *Uptake into Rat Brain Slices*

This was measured by the procedure of Iversen and Neal,[20] (^{3}H)GABA being used.

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