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Synthesis of Analogues of GABA. II* 4-Alkyl-4-aminobut-2-enoic Acids and a New Synthesis of Some Vinyl α-Amino Acids

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Abstract

A series of 4-alkyl-4-aminobut-2-enoic acids (2)–(5) has been prepared as conformationally restricted analogues of GABA. The synthetic route which involved allylic bromination followed by displacement with ammonia also gave vinyl glycine analogues (7)–(9) as readily purified by-products of the reaction. The low biological activity *in vitro* against GABA uptake, binding and enzyme systems of (*E*)-2-aminocyclohexylideneacetic acid (4) and (*E*)-2-aminocyclopentylideneacetic acid (5) has been interpreted in terms of steric hindrance by the ring-forming methylene groups at the particular active sites concerned.

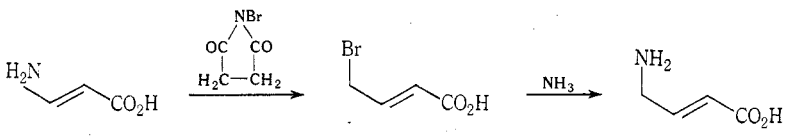
In connection with studies of the biological activity of unsaturated analogues of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (1),[1,2] further α,β-unsaturated analogues[3,4] were required with an alkyl substituent at the 4-position of GABA. The 4-methyl derivative, 4-aminopent-2-enoic acid (2), and the 4,4-dimethyl derivative, 4-amino-4-methylpent-2-enoic acid (3), were needed to complete a series for structure–activity studies. Two amino acids that could also be classed as 4-substituted α,β-unsaturated analogues, (*E*)-2-aminocyclohexylideneacetic acid (4) and (*E*)-2-aminocyclopentylideneacetic acid (5), were required in relation to the activity of isoguvacine (6) which acts as a potent GABA agonist at postsynaptic receptor sites.[5] Examination of Dreiding models of (4) and (5) showed that the expected conformation of the polar groups and the four carbon 'GABA backbone' of (5) but not of (4) matched that in isoguvacine (6). In (4) and (5) the $-(CH_2)_n-$ chain which forms the conformation-restricting ring system is on a different side of the four carbon 'GABA-backbone' compared to the ring-forming CH_2CH_2 group in isoguvacine (6). Compounds (4) and (5) were therefore considered valuable for investigating the steric effect of bulky substituents on various biological processes involving GABA.

A previous paper[3] detailed a convenient synthesis of 4-aminobut-2-enoic (4-aminocrotonic) acids from α,β-unsaturated acids, involving the action of *N*-bromosuccin-

* Part I, *Aust. J. Chem.*, 1978, **31**, 2283.

[1] Johnston, G. A. R., *Annu. Rev. Pharmacol.*, 1978, **18**, 269.
[2] Saclens, J. I., and Vinick, F. J., *Annu. Rep. Med. Chem.*, 1978, **13**, 31.
[3] Allan, R. D., and Twitchin, B., *Aust. J. Chem.*, 1978, **31**, 2283.
[4] Johnston, G. A. R., Curtis, D. R., Beart, P. M., Game, C. J. A., McCulloch, R. M., and Twitchin, B., *J. Neurochem.*, 1975, **24**, 157.
[5] Krogsgaard-Larsen, P., Johnston, G. A. R., Lodge, D., and Curtis, D. R., *Nature (London)*, 1977, **268**, 53.

imide followed by amination in liquid ammonia as shown in Scheme 1. The present paper describes the use of this procedure to synthesize the 4-substituted GABA analogues (2)–(5) and notes the formation of vinyl glycine analogues (β,γ-unsaturated α-amino acids) (7)–(9) as by-products in the preparation of all except (2). These easily isolated by-products could be classed as desirable since some β,γ-unsaturated α-amino acids possess biological activity,[6,7] and the synthesis of aliphatic and alicyclic derivatives has recently been the subject of several communications.[7–9]



Scheme 1

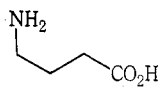
Synthesis of the Unsaturated Acids (10)–(13)

Of the α,β-unsaturated acids required for Scheme 1, pent-2-enoic acid (10) was commercially available, while 4-methylpent-2-enoic acid (11)[10] and cyclohexylideneacetic acid (12)[11] were readily prepared by literature methods. A slight increase in yield of (12) from 60 to 70% resulted from the use of *N*-methylpyrrolidone rather than dimethylformamide as solvent in the reaction of 2 equiv. of malonic acid with 1-morpholinocyclohex-1-ene.

When the preparation of cyclopentylideneacetic acid (13) was attempted by a similar route from 1-morpholinocyclopent-1-ene a good yield (63%) of cyclopent-2-enylacetic acid (14) with an endocyclic double bond resulted. Closer examination by n.m.r. spectroscopy showed that the required derivative with an exocyclic double bond (13) constituted 9% of the crude product. This was in marked contrast to the reaction forming the cyclohexyl derivative where the endocyclic double bond product (15) was not detected in the crude product.

Several studies[12,13] have reported the relative stabilities of exocyclic–endocyclic pairs such as (13)–(14) and (12)–(15). For the (12)–(15) system, equilibration of the unsaturated acids under acidic or autocatalysed conditions gave the same major product (endocyclic derivative) as basic equilibration of the unsaturated carboxylate anion or of the corresponding unsaturated ester.[12] The expected product if equilibration conditions were attained was therefore (15). For the cyclopentane system (13)–(14) equilibration of the unsaturated carboxylate anion gave the endocyclic derivative (14),[13] but basic equilibration of the unsaturated ethyl ester gave the exocyclic derivative corresponding to (13) as the major product.[12] The stabilities of the intermediates

[6] Rando, R., *Acc. Chem. Res.*, 1975, **8**, 281.
[7] Baldwin, J. E., Haber, S. B., Hoskins, C., and Kruse, L. I., *J. Org. Chem.*, 1977, **42**, 1239, and references cited therein.
[8] Suzuki, M., Nunami, K., and Yoneda, N., *J. Chem. Soc., Chem. Commun.*, 1978, 270.
[9] Chari, R. V. J., and Wemple, J., *Tetrahedron Lett.*, 1979, 111.
[10] Cambie, R. C., Hayward, R. C., Roberts, J. L., and Rutledge, P. S., *J. Chem. Soc., Perkin Trans. 1*, 1974, 1864.
[11] Prout, F. S., *J. Org. Chem.*, 1973, **38**, 399.
[12] Svata, V., and Prochazka, M., *Collect. Czech. Chem. Commun.*, 1975, **40**, 2315, and references cited therein.
[13] Goldberg, A. A., and Linstead, R. P., *J. Chem. Soc.*, 1928, 2343.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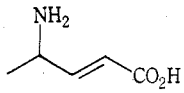
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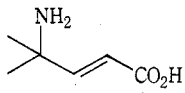
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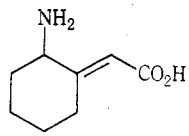
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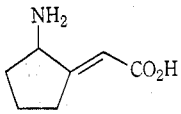
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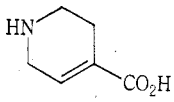
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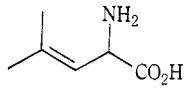
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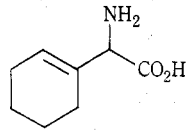
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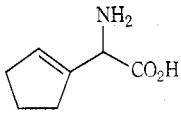
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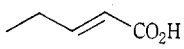
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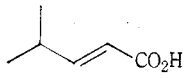
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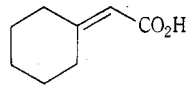
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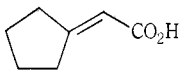
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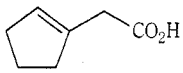
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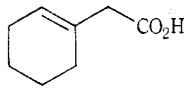
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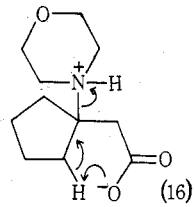
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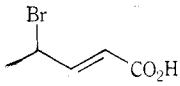
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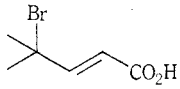
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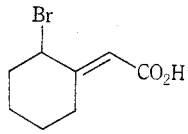
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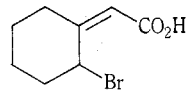
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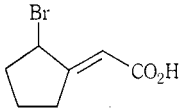
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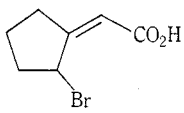
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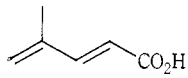
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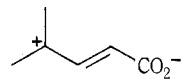
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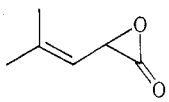
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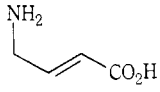
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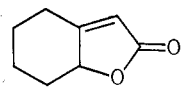
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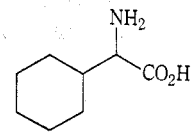

(28)

in the equilibration of (13)–(14) with acid or neutral conditions were expected to resemble those of the ester more closely than those of the carboxylate anion and so the exocyclic product (13) was expected under acid or neutral equilibration conditions.

The actual product (14) was only expected in basic equilibrium conditions. Clearly both the practical results in these particular malonic-acid-buffered conditions were the reverse of those expected by thermodynamic predictions from other conditions. A possible explanation may lie both in the different reaction conditions and in the rate of attainment of equilibrium. The much greater rate of equilibration of the cyclopentane (13)–(14) system has previously been noted in basic conditions.[13] In the present case the relative ease of formation of an intermediate such as (16) and the corresponding cyclohexyl derivative may be an important factor. In addition, examination of Dreiding models indicates that elimination by an intramolecular mechanism as shown in (16) would involve very little conformational strain.

The published thermodynamic data on the corresponding esters[12] indicate that the required exocyclic unsaturated acid (13) would result from acid-catalysed isomerization. Initial attempts to isomerize the readily obtained (14) by refluxing in 2 M HCl for 5 h resulted in decarboxylation of 70% of the material, but n.m.r. showed a considerable proportion of (13) was formed. The required equilibrium was established at a lower temperature simply by dissolving (14) in trifluoroacetic acid. The predicted isomerization could be followed by n.m.r. spectroscopy and had a half-life of 2 days. The resultant solution contained 85% of (13) and 15% of (14). Purification of (13) was made easier by selective extraction of the stronger acid (14)[14] with a small amount of aqueous sodium bicarbonate. The required (13) was obtained from (14) in an isolated yield of 68%, and this route represents a most convenient and high yielding preparation of (13).

Table 1. Results of amination (Scheme 1)

α,β-Unsaturated acid	Brominated product	Yield of amino acids (%)[A]	$\alpha,\beta/\beta,\gamma$ ratio by n.m.r.	Product and yield (%)[B] α,β	β,γ
(10)	(17)	96	[C]	(2) 62	0
(11)	(18)	52	3 : 2	(3) —[D]	(7) 16
(12)	(19)+(20)[E]	19	2 : 3	(4) 3	(8) 9
(13)	(21)+(22)[F]	22	2 : 1	(5) 6	(9) 5

[A] Crude product isolated on a Dowex 50W (H^+) column. [B] Crystalline product. [C] All α,β. [D] Obtained pure by way of Scheme 2. [E] 4 : 5. [F] 2 : 1.

Synthesis of the Amino Acids

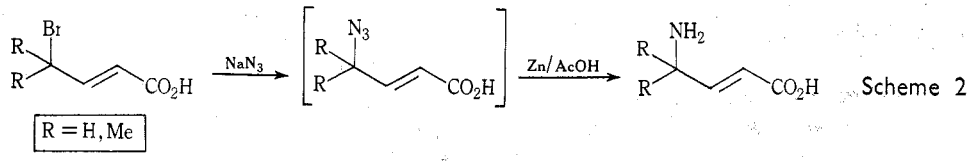
Allylic bromination of (10) and (11) as the first step in Scheme 1 gave (17) and (18) which were conveniently purified by crystallization. On the other hand bromination of the cyclic compounds (12) and (13) proceeded rapidly but gave, as expected by analogy with the corresponding ethyl ester of (12),[15] mixtures of *E* and *Z* isomers: a 4 : 5 mixture of (19) and (20) from (12) and a 2 : 1 mixture of (21) and (22) from (13). Separation of these bromo acids by crystallization was unsuccessful and the mixtures were used directly in the next step.

[14] Wheeler, O. H., and Lerner, I., *J. Am. Chem. Soc.*, 1956, **78**, 63.
[15] Schmid, H., and Karrer, P., *Helv. Chim. Acta*, 1948, **31**, 1067.

Table 1 summarizes the results of bromination and treatment of the intermediate bromo acids with liquid ammonia. Amination was complete in less than 2 h in all cases except for (17) when 12 h were necessary for complete reaction.

The possible product from allylic rearrangement of (17) during displacement, 2-aminopent-3-enoic acid, could not be detected in the n.m.r. spectrum of the crude reaction product. However, with the extra methyl group at the 4-position, (18) gave a 3 : 2 mixture of amino acids (3) and (7) together with some of the dehydrobrominated acid (23). As with the other product mixtures the β,γ-unsaturated α-amino acid was the least soluble amino acid formed and was readily isolated.

On the other hand, purification of the unrearranged product (3) by crystallization of the residues proved more difficult. The problem was finally overcome by reasoning that the rearrangement presumably occurs through the formation of intermediates such as (24) or more likely (25).[7] Ammonia, being basic, is likely to encourage α-lactone formation whereas a more nucleophilic and less basic nitrogen nucleophile such as the azide ion would effect the displacement under milder conditions.



Scheme 2

Treatment of (18) as in Scheme 2 (R = Me) with aqueous sodium azide gave a mixture of at least two compounds which were not isolated but reduced immediately to produce a 14% yield of amino acid. This material proved to be almost pure (3), uncontaminated by the rearranged product (7).

This reaction sequence was also applied to the unsubstituted but-2-enoic acid (Scheme 2, R = H) and gave *trans*-4-aminobut-2-enoic acid (26) in 32% overall yield. This procedure overcame crystallization difficulties associated with the formation of secondary amines as by-products of Scheme 1[3] and represents a most convenient method to prepare large quantities of (26).

Liquid ammonia amination of the mixture of (19) and (20) from cyclohexylideneacetic acid gave a mixture of (4) and the rearranged product (8) as the only major amino acids formed as detailed in Table 1. No n.m.r. signals expected from the *Z* amino acid which could be formed from (20) could be detected. The ether-soluble fraction of the product was shown by n.m.r. spectroscopy to contain unchanged (20) and the cyclized product (27)[15,16] in approximately equal proportions. Attempts to obtain the *Z* amino acid by the azide displacement (Scheme 2) on (20) were unsuccessful. The two amino acids (4) and (8) could be separated conveniently by crystallization albeit in low overall yield.

Similarly, from the mixture of 2-bromocyclopentylideneacetic acids (21) and (22), the amino acids (5) and (9) were isolated pure in low yields. The azide route (Scheme 2) was not successful in obtaining an improved yield of (5).

Confirmation of Assigned Structures

Examination of the n.m.r. spectra of the isomeric pairs (4) and (8), and (5) and (9), did not conclusively prove their structures. Further spectral and chemical evidence

[16] Epstein, W. W., and Sonntag, A. C., *Tetrahedron Lett.*, 1966, 791.

was therefore necessary to correlate them with (3) and (7) whose structures are obvious from n.m.r. data. The ultraviolet spectra for compounds (3), (4) and (5) (λ_{max} at 206, 206 and 210 nm respectively) were consistent with their conjugated chromophores while (7), (8) and (9) all had maxima at less than 200 nm as expected. Although the infrared spectra of these amino acids were mostly complex in the carbonyl region, the most intense bands for the conjugated derivatives (1560, 1550 and 1535 cm^{-1}) were at lower wavenumbers than their non-conjugated counterparts (1585, 1600 and 1585 cm^{-1} respectively). The mass spectra of all the amino acids with the exception of (3) had base peaks at $M^+ - 45$ corresponding to an initial loss of CO_2H. The non-conjugated (7), (8) and (9) all showed only low intensity (<3%) fragments above the base peak. The conjugated derivatives, consistent with the stronger non-allylic bonds to the carboxy groups, showed significant ions at higher *m/e* than $M - CO_2H$. (4) and (5) gave significant $M - H_2O$ ions (44 and 26%) while (2) gave its base peak at 114 ($M - CH_3$), consistent with a secondary methyl substituent β to the nitrogen and the double bond.

Final proof for the position of the amino group in the less soluble isomer came from the reduction of (8) to the known cyclohexylglycine (28).[17]

The *E* configuration of (4) and (5) follows from their n.m.r. spectra where the carbonyl group causes either one or two of the methylene protons *cis* to the carboxyl group to move downfield[11] and to be clearly separated from the envelope of the other methylene protons. The magnitude of this effect of the carboxyl group is quite large, as evidenced in the cyclohexyl series where the CHBr proton is shifted downfield in the *Z* isomer (20) (δ 6·48) when compared to the *E* isomer (19) (δ 4·87). If the product designated (4) possessed the *Z* configuration, it would be expected that the $CHNH_2$ resonance would be further downfield than δ 4·64 and would be the only ring proton separated from the CH_2 envelope. The result is consistent with the selective preparation by Scheme 1 of *E* amino acids from *E* and *Z* mixtures which has already been noted in the case of 4-amino-3-methylbut-2-enoic acid,[3] and agrees with chemical evidence whereby treatment of the 1 : 1 mixture of by-products (20) and (27) with sodium azide under basic conditions gave an increased quantity of the cyclic lactone (27).

Biological Activity

The compounds were tested by assaying (i) the inhibition of the high-affinity uptake of GABA into rat brain slices[18] at 5×10^{-4} M; (ii) the inhibition of sodium-independent binding of GABA to rat brain membranes[19] at 10^{-4} M, and (iii) the inhibition of the enzymes glutamate decarboxylase (GAD) and GABA-2-oxoglutarate aminotransferase (GABA-T)[4] from rat brain at 10^{-3} M.

The 4-methyl substituted GABA analogue (2) was only moderately active compared to the unsubstituted derivative (26),[20] inhibiting GABA uptake by 85 ± 1% at 5×10^{-4} M with a concentration for 50% inhibition (IC_{50}) of $83 \pm 10 \times 10^{-6}$ M and inhibited GABA binding by 67 ± 4% at 10^{-4} M. With the 4,4-dimethyl analogue (3), the increase in steric bulk due to the two methyl groups near the nitrogen atom resulted in a very

[17] Eisler, K., Rudinger, J., and Sorm, F., *Collect. Czech. Chem. Commun.*, 1966, **31**, 4563.
[18] Beart, P. M., Johnston, G. A. R., and Uhr, M. L., *J. Neurochem.*, 1972, **19**, 1855.
[19] Enna, S. J., and Snyder, S. H., *Brain Res.*, 1975, **100**, 81.
[20] Beart, P. M., Uhr, M. L., and Johnston, G. A. R., *J. Neurochem.*, 1972, **19**, 1849.

low GABA-like activity on all systems tested. GABA uptake and GAD were not significantly affected while inhibition of GABA binding was only $14 \pm 2\%$ at 10^{-4} M.

The cylcohexylideneacetic acid derivative (4) had no significant activity on GABA uptake, binding, GAD or GABA-T. The cyclopentylideneacetic acid derivative (5) was relatively inactive, with a $44 \pm 5\%$ inhibition of GABA uptake being the only significant effect on the four GABA processes investigated.

These results contrast with the potent activity of isoguvacine (6) on GABA binding,[5] and of (*E*)-4-aminobut-2-enoic acid (26) on uptake, binding and GABA-T.[4] Examination of Dreiding models of (6), (26), (4) and (5) suggests that, in (5) at least, the polar groups and the four-carbon 'GABA backbone' is capable of taking up the same conformation as in isoguvacine (6). This suggests that the major influence on the activity of (4) and (5) is the increase in steric bulk of the molecule due to the methylene groups of the cyclohexane and cyclopentane rings. The active sites for some GABA processes do accommodate analogues with cyclohexane ring systems, as evidenced by the activity of gabaculine [(−)-5-aminocyclohexa-1,3-diene-1-carboxylic acid] which is moderately active against GABA uptake and is a very potent inhibitor of the enzyme GABA-T.[21] However, the conformation of the cyclohexane ring in gabaculine is restricted by the two conjugated double bonds, and models show that these ring-forming methine groups are not as sterically demanding as the methylene groups of (4) or (5). It is concluded that bulky groups, attached to the GABA molecule in the positions defined by these methylene groups in (4) and (5), prevent interaction of GABA-like molecules with active sites concerned with uptake, binding and enzymic transformations of GABA.

Experimental

Proton magnetic resonance spectra were measured at 60 MHz on a Varian T60 spectrometer either in $CDCl_3$ or for amino acids in D_2O unless otherwise stated. Tetramethylsilane was used as internal standard in $CDCl_3$ and as an external standard in D_2O, and chemical shifts are recorded as δ values. Ultraviolet spectra were measured on a Varian Techtron 635 spectrophotometer, with water as solvent for amino acids and 95% ethanol for other compounds.

Infrared spectra were recorded from liquid films of Nujol mulls on a Unicam SP 200 spectrophotometer. Melting points (uncorrected) were measured on a Yanagimoto hot-stage apparatus. Microanalyses were determined by the A.N.U. Analytical Unit. Mass spectra were obtained on an AEI Ms-902 mass spectrometer at 70 eV.

Cyclopent-1-enylacetic Acid (14)

1-Morpholinocyclohex-1-ene (76·5 g, 0·5 mol) was added at room temperature to a solution of malonic acid (104 g, 1 mol) in *N*-methylpyrrolidone (250 ml) over 5 min. The mixture was heated slowly until gas evolution began (62°), and then kept at about 65° for 17 h. After cooling, the product was poured into a mixture for 6 M HCl (112 ml) and saturated sodium chloride solution (200 ml) and extracted with ethyl acetate (3 × 200 ml). The combined organic layers were washed with water (2 × 200 ml), then extracted with 10% sodium carbonate solution. The aqueous extract was acidified with concentrated HCl (160 ml) and extracted with diethyl ether (3 × 200 ml). This organic layer was washed with water (2 × 200 ml), saturated sodium chloride solution (200 ml) and dried (Na_2SO_4). Evaporation of the ether gave a crude yield of 49·2 g of (14) containing 9% of (13) by n.m.r. analysis. Crystallization from 60–80° light petroleum at −5° (2 crops) gave *cyclopent-1-enylacetic acid* (14) (39·8 g, 63%), m.p. 48–49° (lit.[13] 52°). ν_{max} 1705, 1400, 1025, 900, 710 cm^{-1}. N.m.r. δ 11·9, bs, CO_2H; 5·66, bs, =CH; 3·2, bs, CH_2CO_2H; 2·6–1·6, m, $(CH_2)_3$.

[21] Allan, R. D., Johnston, G. A. R., and Twitchin, B., *Neurosci. Lett.*, 1977, **4**, 51.

Isomerization to Cyclopentylideneacetic Acid (13)

Crystalline (14) (20 g, 160 mmol) was dissolved in trifluoroacetic acid (40 ml) and kept in a stoppered flask at room temperature (20°). The reaction was followed directly by n.m.r. analysis. After 1 week the mixture contained 75% of (13) and after 2 weeks 85% of (13). Further standing produced little detectable change. The trifluoroacetic acid was removed under vacuum, and the product was dissolved in ether (100 ml) and washed with water (4×100 ml). The solution was then washed successively with sodium bicarbonate portions (1·8 g, 20 mmol) in water (20 ml) until three neutral washings were obtained. After drying (Na_2SO_4), the ether was removed to give almost pure (13) (14·6 g). After decolorizing with charcoal in ethanol, crystallization from 60–80° light petroleum gave *cyclopentylideneacetic acid* (13) (13·6 g, 68%), m.p. 62·5–64° (lit.[13] 64°). N.m.r. δ 11·0, bs, CO_2H; 5·90, m, J 2 Hz, =CH; 3·0–2·3, m, 4H, CH_2C=; 2·0–1·5, m, 4H, CH_2CH_2. ν_{max} 1685, 1645, 1235, 930, 850, 680 cm^{-1}. λ_{max} 221 nm (ε 10600).

Bromination Procedure

A mixture of the α,β-unsaturated acid (20 mmol) and recrystallized *N*-bromosuccinimide (20 mmol) in carbon tetrachloride (40 ml) was magnetically stirred and refluxed until the suspension rose to the surface [24 h for (10), 30 min for (11), (12) and (13)]. The mixture was then cooled on ice, filtered and evaporated to an oil. Products from (10) and (11) were further purified by crystallization from 60–80° light petroleum/cyclohexane to give (17) (60%) and (18) (37%). Spectral data for the intermediate bromo acids are as follows:

(*17*).—N.m.r.: δ 7·24, dd, J 13, 6·5 Hz; 6·00, dd, J 13, 0·7 Hz; 4·75, 5 lines, J 7 Hz; 1·86, d, J 5·5 Hz. ν_{max} 1700, 1640, 1280, 930, 695 cm^{-1}.

(*18*).—N.m.r.: δ 7·34, d, J 16 Hz; 5·96, d, J 16 Hz; 1·94, s.

(*19*) *and* (*20*).—N.m.r. (19): δ 5·98, bs, =CH; 4·87, bs, CHBr; 3·1–2·6, bd, CH_2C=; 2·6–1·1, envelope. N.m.r. (20): δ 6·48, bs, CHBr; 5·70, bd, J 2 Hz, =CH. ν_{max} 1680s, 1640m, 1280, 950, 700 cm^{-1}.

(*21*) *and* (*22*).—N.m.r. (21): δ 6·15, m, =CH; 4·92, m, CHBr; 3·1–1·6, envelope, CH_2. N.m.r. (22): δ 5·8, m, CHBr; 5·15, m, =CH; 3·1–1·6, envelope, CH_2. ν_{max} 1690, 1650, 1240, 935, 860, 780 cm^{-1}.

Amination of the Bromo Acids

The bromination product from the unsaturated acid (20 mmol) was dissolved in tetrahydrofuran (15 ml) and added dropwise to liquid ammonia (200 ml) over 3 min with vigorous magnetic stirring. After 2 h [12 h for (17)] the solvents were removed on a rotary evaporator. The crude product was dissolved in water (20 ml), acidified with 1 M HCl and extracted with diethyl ether (2×20 ml). The ether extract was dried (Na_2SO_4), the solvent removed and this fraction examined by n.m.r. spectroscopy.

(17) gave less than 1% ether-soluble product.

(18) gave a mixture containing (23) as the major constituent in approximately 5% yield. N.m.r. δ 9·1, s, CO_2H; 7·51, d, J 16 Hz, H_β; 5·92, d, J 16 Hz, H_α; 5·47, bs, 2H, $=CH_2$; 1·92, s, CH_3; is consistent with the literature[22] n.m.r. for (23).

(19) and (20) gave a 1 : 1 mixture of unchanged (20) and the cyclic lactone (27) in approximately 50% yield. N.m.r. (27): 5·7, 1H, s, =CH; 4·7, 1H, m, CHO; 3·0–1·2, 8H, complex, CH_2; is consistent with the literature n.m.r. spectrum.[10,16] ν_{max} (27) 2950, 1750, 1650, 1160, 1030, 905, 850, 720 cm^{-1}.

(21) and (22) gave about 50% yield of an oil with a significant n.m.r. signal at 5·88 and which was not further examined.

The acidified aqueous layer was adsorbed on a Dowex 50W (H^+) column (40 ml). The column was eluted with water until neutral and then the amino acid was removed with 1 M ammonium hydroxide. The ammonia wash was evaporated to dryness and the products were purified by crystallization. The β,γ-unsaturated α-amino acids (7), (8) and (9) crystallized from water while the GABA analogues (2), (4) and (5) crystallized from ethanol. The following pure products were obtained:

[22] Sundberg, R. J., Bukowick, P. A., and Holcombe, F. O., *J. Org. Chem.*, 1967, **32**, 2938.

(E)-*4-Aminopent-2-enoic acid* (2) (62%), m.p. 196–7°, n.m.r. and i.r. spectra identical with those of an authentic sample prepared from (±)-alanine.[3]

2-Amino-4-methylpent-3-enoic acid (7) (16%), m.p. >280° (Found: C, 55·9; H, 8·8; N, 10·8. $C_6H_{11}NO_2$ requires C, 55·8; H, 8·8; N, 10·8%). N.m.r. δ 5·69, bd, J 10 Hz, =CH; 4·93, d, J 10 Hz, CHN; 2·22, d, J 1 Hz, $(CH_3)_2C$=. ν_{max} 1650m, 1615m, 1585s, 1275, 845 cm^{-1}. λ_{max} less than 200 nm (ε_{210} 2900). Mass spectrum *m/e* 129 (M, 0·2%), 85 (8), 84 (100), 57 (7).

(E)-*2-Aminocyclohexylideneacetic acid* (4) (3%), m.p. 231–232° (Found: C, 61·8; H, 8·5; N, 8·7. $C_8H_{13}NO_2$ requires C, 61·9; H, 8·5; N, 9·0%). N.m.r. δ 6·22, s, =CH; 4·30, m, CHN; 3·40, 1H, bd, J 14 Hz, CHC=; 2·8–1·7, 7H, complex. ν_{max} 2170, 1650m, 1550s, 1365, 750 cm^{-1}. λ_{max} 206 nm (ε 6200). Mass spectrum *m/e* 155 (M, 1%), 137 (44), 110 (100), 93 (20), 56 (20).

α-*Aminocyclohex-1-enylacetic acid* (8) (9%), m.p. >280°, sublimes >240° (Found: C, 61·3; H, 8·4; N, 8·8. $C_8H_{13}NO_2$ requires C, 61·9; H, 8·5; N, 9·0%). N.m.r. (D_2O/DCl) δ 6·65, m, =CH; 5·10, s, CHN; 2·8–2·3, 4H, envelope; 2·3–1·9, 4H, envelope. ν_{max} 3150, 1600, 1380, 700 cm^{-1}. λ_{max} less than 200 nm, ε_{210} 2100. Mass spectrum *m/e* 156 (M+1, 1%), 140 (1), 138 (1), 110 (100), 93 (30), 57 (12).

(E)-*2-Aminocyclopentylideneacetic acid* (5) (6%), m.p. 180–181° (Found: C, 59·6; H, 7·8; N, 9·7. $C_7H_{11}NO_2$ requires C, 59·5; H, 7·9; N, 9·9%). N.m.r. δ 6·52, q, J 2 Hz, =CH; 4·64, m, CHN; 3·4–3·05, 2H, m; 2·9–1·9, 4H, m. ν_{max} 2150, 1635m, 1565m, 1535s, 1315, 885, 730, 680 cm^{-1}. λ_{max} 210 nm (ε 8800). Mass spectrum *m/e* 141 (M, 2%), 123 (26), 113 (14), 96 (100), 95 (30), 79 (10), 67 (14).

α-*Aminocyclopent-1-enylacetic acid* (9) (5%), m.p. 235–240 (dec.) (lit.[8] 232–234°) (Found: C, 59·2; H, 7·9; N, 9·7. $C_7H_{11}NO_2$ requires C, 59·5; H, 7·9; N, 9·9%). N.m.r. (D_2O, DCl) δ 6·59, bs; 5·2–5·4, obscured; 3·2–2·7, 4H, m; 2·7–2·2, 2H, m. N.m.r. (D_2O/NaOD) δ 6·20, bs, =CH; 4·50, s, CHN; 3·1–2·6, 4H, m; 2·6–2·2, 2H, m. ν_{max} 1655m, 1620m, 1585s, 1520, 1120, 680 cm^{-1}. λ_{max} less than 200 nm, ε_{210} 2800. Mass spectrum *m/e* 141 (M, 0·5%), 96 (100), 79 (18).

Preparation of (3) and (26) by Azide Displacement (Scheme 2)

To a magnetically stirred solution of 4-bromo-4-methylpent-2-enoic acid (18) (4·0 g, 21 mmol) in tetrahydrofuran (25 ml) was added sodium azide (4·04 g, 63 mmol) in water (10 ml). The temperature immediately rose to 30°. After 2 min, zinc dust (4·0 g) was added and the temperature maintained with ice cooling at 30–40°. Further zinc (4·0 g) was added over 30 min until gas evolution ceased. The mixture was filtered and the solvent removed on a rotary evaporator. The amino acid product was isolated by absorbing the product on Dowex 50W (H^+) (150 ml), and after washing with water, the amino acid was removed with 1 M ammonium hydroxide. Evaporation to dryness gave 400 mg (14%) which was shown by n.m.r. to be about 95% pure with an impurity at δ 2·38. Crystallization from ethanol gave an analytical sample (143 mg) of *4-amino-4-methylpent-2-enoic acid* (3), m.p. >280°, sublimes >240° (Found: C, 55·6; H, 8·8; N, 10·5. $C_6H_{11}NO_2$ requires C, 55·8; H, 8·6; N, 10·8%). N.m.r. δ 7·02, d, J 16 Hz; 6·37, d, J 16 Hz; 1·93, s. ν_{max} 2600, 2170, 1650m, 1630m, 1560s, 970, 700 cm^{-1}. λ_{max} 206 nm (ε 5000). Mass spectrum *m/e* 129 (M, 3%), 114 (100), 96 (12), 78 (20), 58 (17).

Similarly treatment of the crude product of bromination of crotonic acid (2·15 g, 25 mmol) with sodium azide followed by zinc reduction gave, after precipitation with ethanol, (E)-*4-aminobut-2-enoic acid* (26) (800 mg, 32%), m.p. 162–167° (lit.[4] 165–168°), with an n.m.r. spectrum identical to that of an authentic sample.

Reduction of (8) to Cyclohexylglycine (28)

A suspension of (8) and Adams catalyst (75 mg) in 1 M HCl (925 ml) was shaken at atmospheric pressure under hydrogen until the theoretical quantity was absorbed (30 min). The solution was filtered and the solvent removed under vacuum. The hydrochloride salt was suspended in water (20 ml) and the pH adjusted to 6 with 1 M ammonium hydroxide. After 30 min the precipitate formed was filtered off and recrystallized from acetic acid (40 ml) to give *cyclohexylglycine* (28) (387 mg, 51%), m.p. >290°, sublimes 260° (Found: C, 60·8; H, 9·5; N, 8·7. $C_8H_{15}NO_2$ requires C, 61·1; H, 9·6; N, 8·9%). N.m.r. (D_2O/DCl) δ 4·58, d, J 5 Hz, CHN; 2·8–1·4, m, consistent with the reported n.m.r. spectrum.[23] ν_{max} 1600, 1310, 1125, 680 cm^{-1}.

[23] Warren, R. J., Zarembo, J. E., Staiger, D. B., and Post, A., *J. Pharm. Sci.*, 1976, **65**, 738.

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