Aust. J. Chem., 1981, **34**, 2641–5

Synthesis of Analogues of GABA. VII* (*Z*)- and (*E*)-4-Amino-3-(4-chlorophenyl)but-2-enoic Acids as Unsaturated Baclofen Derivatives

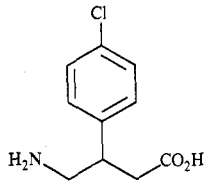
Robin D. Allan and Hue Tran

Department of Pharmacology, University of Sydney, N.S.W. 2006.

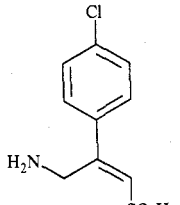
Abstract

(*Z*)- and (*E*)-4-Amino-3-(4-chlorophenyl)but-2-enoic acids have been synthesized from 4-chloroacetophenone as conformationally restricted analogues of baclofen. The corresponding unsaturated lactam (4) has been catalytically reduced and hydrolysed to baclofen to demonstrate the suitability of (4) as a precursor for radiolabelled baclofen of high specific activity.

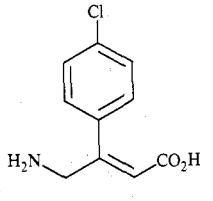
Baclofen [4-amino-3-(4-chlorophenyl)butanoic acid, β-*p*-chlorophenyl-γ-aminobutyric acid (1)] is a centrally acting skeletal muscle relaxant[1] for which the mechanism of action is not fully understood. Previous investigations[2–4] have shown that, despite the obvious structural relationship between baclofen and the inhibitory neurotransmitter γ-aminobutyric acid (GABA), baclofen appears not to act at bicuculline-sensitive postsynaptic receptors in the central nervous system. Activity at GABA receptors that are insensitive to the GABA antagonist bicuculline has previously been postulated for GABA analogues with certain folded conformations,[5] and recent



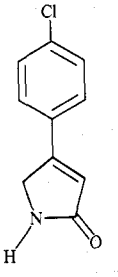
(1)



(2)



(3)



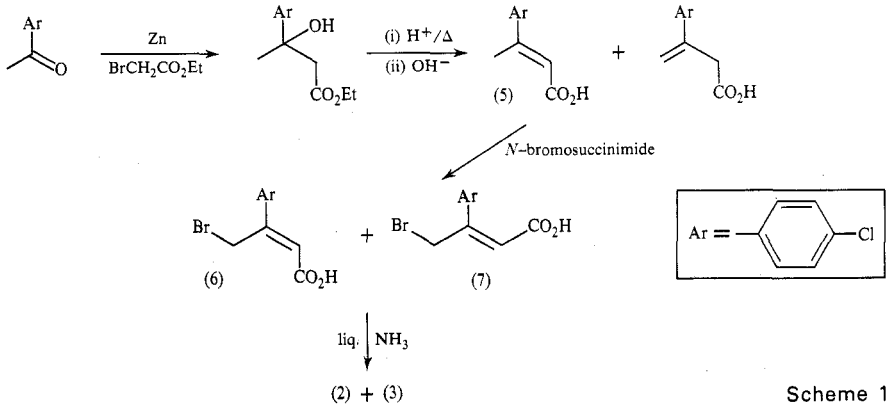
(4)

* Part VI, *Aust. J. Chem.*, 1981, **34**, 2231.

[1] Birkenmayer, W., in 'Spasticity—A Topical Survey' (Ed. W. Bein) p. 1 (Hans Huber: Vienna 1972).
[2] Waddington, J. L., and Cross, A. J., *J. Pharm. Pharmacol.*, 1979, **31**, 652.
[3] Johnston, G. A. R., Hailstone, M. H., and Freeman, C. G., *J. Pharm. Pharmacol.*, 1980, **32**, 230.
[4] Potashner, S. J., *J. Neurochem.*, 1979, **32**, 103.
[5] Johnston, G. A. R., in 'GABA in Nervous System Function' (Eds E. Roberts, T. N. Chase and D. B. Tower) p. 395 (Raven Press: New York 1976).

studies by Bowery and coworkers suggest that baclofen acts on a bicuculline-insensitive GABA receptor in both central and peripheral nervous tissue.[6,7]

This paper reports the synthesis of the *Z* and *E* isomers (2) and (3) of 4-amino-3-(4-chlorophenyl)but-2-enoic acid as conformationally restricted analogues of baclofen (1) for further structure–activity studies, and demonstrates the potential of the unsaturated lactam (4) for the preparation of radiolabelled baclofen.



Scheme 1

We have shown in previous publications[8,9] that alkyl- and halogen-substituted 4-aminobut-2-enoic acids can be prepared from α,β-unsaturated carboxylic acids through allylic bromination followed by an amination reaction. The successful route to the required aromatic amino acids was based on this method and is illustrated in Scheme 1. A Reformatsky reaction on 4-chloroacetophenone gave the α,β-unsaturated acid (5) which was isolated by crystallization from a mixture with the corresponding β,γ-unsaturated acid. Allylic bromination of (5) gave a 12:1 mixture of monobrominated derivatives. The *Z* configuration as in (6) was assigned to the major product on the basis of the ^{1}H n.m.r. spectra where the γ-protons appear further downfield when compared with those in the minor product. In contrast to the amination of 3,4-dihalogenobut-2-enoic acids,[9] treatment of (6) with aqueous ammonia produced little amino acid product. However, liquid ammonia gave the required amino acid (2). Similarly a 1:1 mixture of (6) and (7) yielded a mixture of amino acids from which (3) could be isolated.

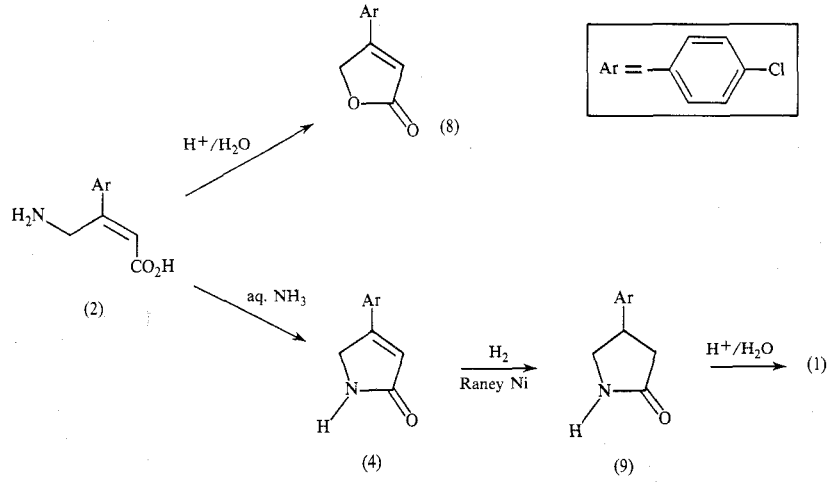
The assigned stereochemistry is supported by n.m.r. spectroscopy whereby the γ-protons are 0·4 ppm further downfield in the major product, consistent with a *cis* arrangement of the aminomethyl and carboxy groups as depicted in the *Z* isomer (2).[9] Furthermore the ^{13}C chemical shift differences between the non-aromatic carbons of (2) and (3), and in particular for C4 (as shown in Table 1), are consistent with the observed differences for *cis* and *trans* isomers of 4-amino-3-halogenobut-2-enoic acids.[9]

[6] Hill, D. R., and Bowery, N. G., *Nature (London)*, 1981, **290**, 149.
[7] Bowery, N. G., Doble, A., Hill, D. R., Hudson, A. L., Shaw, J. S., Turnbull, M. J., and Waddington, R., *Eur. J. Pharmacol.*, 1981, **71**, 53.
[8] Allan, R. D., and Twitchin, B., *Aust. J. Chem.*, 1978, **31**, 2283.
[9] Allan, R. D., Johnston, G. A. R., and Twitchin, B., *Aust. J. Chem.*, 1980, **33**, 1115.

Major difficulties in working with (2) and (3) were their low solubilities in aqueous solvents and their ready decompositions on heating to dissolve them in acidic or basic solution. As shown in Scheme 2, the unsaturated lactone (8) resulted from refluxing (2) in hydrochloric acid. Heating with 1 M ammonium hydroxide gave the unsaturated lactam (4) which was also formed on attempted preparation of the amino acid by treatment of the bromo acid (6) with aqueous ammonia.

Table 1. ^{13}**C n.m.r. data of (*Z*) amino acid (2) and (*E*) amino acid (3)**
In ppm downfield from tetramethylsilane in trifluoroacetic acid

Compound	Chemical shift (δ)				δ (*trans*) − δ (*cis*)			
	C1	C2	C3	C4	C1	C2	C3	C4
(2)	173·9	124·2	153·5	42·4	−1·4	−0·7	−1·5	7·0
(3)	172·5	123·5	152·0	49·4				



Scheme 2

Reduction of (4) with hydrogen over platinum or palladium catalysts led to mixtures of products for which ^{1}H n.m.r. and mass spectral evidence indicated dehalogenation, or reduction of the aromatic ring accompanied reduction in the lactam ring. However, hydrogenation with W-5 Raney nickel in ethanol successfully gave the saturated lactam (9) (Scheme 2) which was hydrolysed to baclofen (1). This confirms the potential of (4) as a substrate for reduction with tritium gas to generate radiolabelled baclofen. Such a radiolabelled compound with high specific activity would be extremely useful in neurochemical studies on baclofen and bicuculline-insensitive GABA receptors.

Experimental

^{1}H n m.r. spectra were measured at 60 MHz on a Perkin–Elmer R12 spectrometer or a Varian EM-360A spectrometer in either (D)chloroform or trifluoroacetic acid with tetramethylsilane as internal standard. ^{13}C n.m.r. spectra were recorded on a Jeol FX90Q instrument. Infrared spectra were recorded from Nujol mulls on a Perkin–Elmer 177 spectrophotometer. Melting points (uncorrected) were measured on a Reichert hot-stage apparatus. Microanalyses were determined by the Australian Microanalytical Service, Melbourne. Mass spectra were obtained on a Finnigan 2300E mass spectrometer.

Thin-layer chromatography (t.l.c.) on Merck Kieselgel 60 precoated t.l.c. plates in the solvent systems indicated gave the following R_F values (after visualization with ninhydrin). Butan-1-ol/acetic acid/water (4:1:1): (1) 0·53; (2) 0·54; (3) 0·49; (4) 0·77; (6) 0·84; (8) 0·83; (9) 0·68. Butan-1-ol/pyridine/water (2:1:1): (1) 0·57; (2) 0·64; (3) 0·54; (4) 0·80.

(E)-*3-(4-Chlorophenyl)but-2-enoic Acid* (*5*)

Addition of ethyl bromoacetate (234 g, 1·4 mol) to zinc (100 g, 1·5 mol) and 4-chloroacetophenone (155 g, 1·0 mol) in benzene (400 ml) at reflux temperature gave a complex which was hydrolysed with 2 M H_2SO_4 and yielded crude ethyl 3-(4-chlorophenyl)-3-hydroxybutanoate (240 g) which was used for the next step without purification.

A solution of ethyl 3-(4-chlorophenyl)-3-hydroxybutanoate (49 g, 0·2 mol) and *p*-toluenesulfonic acid (0·7 g) in toluene (100 ml) was refluxed with a Dean–Stark trap for 3 h. Toluene was removed at atmospheric pressure and the product distilled under vacuum to give a 2:1 mixture of α,β- and β,γ-unsaturated esters (41 g, 95%), b.p. 108–120°/0·5 mm. ^{1}H n.m.r. δ ($CDCl_3$) 6·1, q, H α; 2·5, d, H γ for α,β-unsaturated isomer. δ ($CDCl_3$) 5·4, s, 1H, HC=; 5·5, s, 1H, HC=; 3·5, s, 2H, H α for β,γ-unsaturated isomer.

The above ester mixture (37 g, 0·16 mol) was refluxed with 10% NaOH (190 ml) for 2 h. After impurities had been extracted with ether, the aqueous layer was acidified with 6 M HCl and extracted with ether. Washing with water, drying and solvent removal gave a crystalline 2:1 mixture (31 g, 95%) of the α,β-unsaturated acid (5) and the β,γ-unsaturated isomer [^{1}H n.m.r. δ ($CDCl_3$) 5·25, s, 1H, HC=; 5·5, s, 1H, HC=; 3·5, s, 2H, H α]. The *α,β-unsaturated acid* (5) crystallized from hexane/cyclohexane/ether (7 g, 22%), m.p. 138–139° (lit.[10] 134°). ν_{max} 3100–2500, 1680, 1620, 820 cm^{-1}. ^{1}H n.m.r. δ ($CDCl_3$) 11·6, br s, CO_2H; 7·4, s, ArH; 6·15, q, H α; 2·55, d, CH_3.

Allylic Bromination of (E)-*3-(4-Chlorophenyl)but-2-enoic Acid* (*5*)

The α,β-unsaturated acid (5) (2 g, 10 mmol) was refluxed with *N*-bromosuccinimide (1·9 g, 10·5 mmol) in carbon tetrachloride (50 ml) for 48 h, and the hot solution filtered. After cooling, the crystalline bromo acid (6) (1·36 g, 50%) was filtered off. The filtrate was washed with water, dried and evaporated to dryness to give a crude crystalline 1:1 mixture of (6) and (7) (0·24 g). ^{1}H n.m.r. for the *E* isomer (7): δ ($CDCl_3$) 7·6–7·2, m, ArH; 6·6, s, H α; 4·4, s, CH_2Br.

Recrystallization for chloroform/methanol gave pure (Z) *bromo acid* (6) (1·0 g, 37%), m.p. 170–175° (Found: C, 43·5; H, 2·8. $C_{10}H_8BrClO_2$ required C, 43·6; H, 2·9%). ν_{max} 1685, 1620, 835 cm^{-1}. ^{1}H n.m.r. δ ($CDCl_3$) 7·5–7·3, m, ArH; 6·2, s, H α; 4·9, s, CH_2Br.

(Z)-*4-Amino-3-(4-chlorophenyl)but-2-enoic Acid* (*2*)

Crude bromo acid (6) (6 g) was dissolved in tetrahydrofuran (15 ml) and added with stirring over 2 min to liquid ammonia (200 ml). The solvent was removed under vacuum after 3 h. After acidification with 1 M HCl and extraction with ether (2 × 50 ml), the solvent was removed from the aqueous layer and the crude product dissolved in trifluoroacetic acid (3 ml). On removal of excess trifluoroacetic acid under vacuum, the product was adsorbed on Dowex 50W (H^+) ion-exchange resin (40 ml), washed with water and eluted with 1 M pyridine. The pyridine solution was concentrated under vacuum to deposit crystals of the (Z) *amino acid* (2) (0·7 g, 16%), m.p. 185–193° (dec.) (Found: C, 56·5; H, 4·6; N, 6·8. $C_{10}H_9ClNO_2$ requires C, 56·8; H, 4·8; N, 6·6%). ν_{max} 2650, 1630, 1610, 820 cm^{-1}. ^{1}H n.m.r. δ (CF_3CO_2H) 7·55, ArH; 6·7, s, H α; 4·7, q, *J* 6 Hz, $CH_2NH_3^+$. Mass spectrum (methane chemical ionization) *m/e* 214 (6%), 212 (M+1, 16), 196 (33), 194 (100).

The trifluoroacetic acid *salt* of (2) was obtained by dissolving (2) in trifluoroacetic acid and adding ethyl acetate to crystallize, m.p. 190–195° (dec.) (Found: C, 44·0; H, 3·3; N, 4·4. $C_{12}H_{10}ClF_3NO_4$ requires C, 44·4; H, 3·4; N, 4·3%).

(E)-*4-Amino-3-(4-chlorophenyl)but-2-enoic Acid* (*3*)

Combined residues from the crystallization of (6) (3·5 g) containing an approximately 1:1 mixture of (6) and (7) was aminated by the same procedure as above. Crystallization was monitored by t.l.c. in butan-1-ol/pyridine/water 2:1:1, and recrystallization, after adsorption of appropriate

[10] Koelsch, C. F., and Boekelheide, V., *J. Am. Chem. Soc.*, 1944, **66**, 412.

fractions on Dowex 50W (H^+) ion-exchange resin and elution, gave two crops of (E) *amino acid* (3) (95 mg, 4%), m.p. 212–214° (dec.) (Found: C, 56·5; H, 4·9; N, 6·9. $C_{10}H_9ClNO_2$ requires C, 56·8; H, 4·8; N, 6·6%). ν_{max} 2630, 1660, 1580, 825 cm^{-1}. ^1H n.m.r. δ (CF_3CO_2H) 7·6–7·1, m, ArH; 6·4, s, =CH; 4·3, q, J 6 Hz, $CH_2NH_3^+$. Mass spectrum (methane chemical ionization) *m/e* 214 (33%), 212 (M+1, 100), 196 (30), 194 (90).

4-(4-Chlorophenyl)furan-2(5H)-one (8)

The (*Z*) amino acid (2) (50 mg) was refluxed in 1 M HCl (40 ml) for 10 h. After cooling, the crystalline material was filtered off, and more product recovered from the filtrate by chloroform extraction. The combined fractions were recrystallized from chloroform/cyclohexane to give *unsaturated lactone* (8) (44 mg, 80%), m.p. 178–179° (Found: C, 61·5; H, 3·6. $C_{10}H_7ClO_2$ requires C, 61·2; H, 3·6%). ν_{max} 1740, 1580, 820 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$) 7·1–7·6, m, ArH; 6·3, t, =CH; 5·15, d, CH_2O. Mass spectrum (methane chemical ionization): *m/e* 197 (33%), 195 (M+1, 100).

4-(4-Chlorophenyl)-1,5-dihydro-2H-pyrrol-2-one (4)

The (*Z*) amino acid (2) (1·0 g) was stirred in 1 M ammonium hydroxide for 1 h at 70°. On cooling the product crystallized and was filtered to give the *unsaturated lactam* (4) (0·60 g, 65%), m.p. 208–210° (Found: C, 61·7; H, 4·2; N, 7·2. $C_{10}H_8ClNO$ requires C, 62·0; H, 4·2; N, 7·2%). ν_{max} 3200, 1680, 1640, 825 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$, CD_3OD) 7·4, s, ArH; 6·3, s, =CH; 4·3, s, CH_2N.

Hydrogenation of Unsaturated Lactam (4)

The unsaturated lactam (4) (200 mg, 1 mmol) in absolute ethanol (45 ml) was shaken at room temperature for 16 h with freshly prepared Raney nickel W-5 catalyst (0·75 ml) and 4 atmospheres of hydrogen. The mixture was filtered, the filtrate evaporated and the product recrystallized from chloroform/ether/hexane to give the saturated lactam (9) (130 mg, 59%), m.p. 124–125° (lit.[11] 119°). ν_{max} 3420, 3190, 1670, 830 cm^{-1}. ^1H n.m.r. δ ($CDCl_3$) 7·3, s, 4H, ArH; 6·4, br s, NH; 3·9–3·2, m, 3H, $CHCH_2N$; 2·6, m, 2H, $CH_2C{=}O$; 1·8, s (exchanges with D_2O), 2H, H_2O. This product was identical (n.m.r., i.r., m.p. and t.l.c.) with a sample of (9) prepared by dehydrating an authentic sample of baclofen by a published procedure.[11] Both samples gained one molar proportion of water of crystallization during recrystallization from ether mixtures.

Hydrolysis of (9) to Baclofen (1)

The saturated lactam (9) (60 mg, 0·3 mmol) was refluxed for 2 h in 6 M HCl (25 ml). After being washed with ether (2 × 25 ml), the aqueous solution was evaporated under vacuum and the product adsorbed on Dowex 50W (H^+) ion-exchange resin (5 ml). After washing with water, elution with 1 M pyridine and concentration under vacuum, crystalline baclofen (1) was obtained (32·5 mg, 54%), m.p. 203–205°. ^1H n.m.r. δ (DCl, D_2O, external tetramethylsilane) 7·95, s, ArH; 3·85, m, 3H, NCH_2CH; 3·35, m, 2H, CH_2CO_2H. Identical (m.p., t.l.c., and n.m.r. and i.r. spectra) with authentic baclofen.

Acknowledgments

We thank the Department of Pharmacy, University of Sydney, for n.m.r. and mass spectra, the Department of Organic Chemistry, University of Sydney, for the use of hydrogenation equipment, and Ciba–Geigy Aust. Ltd for supplying an authentic sample of baclofen. Support of this work by a National Health and Medical Research Council Grant is gratefully acknowledged.

Manuscript received 26 August 1981

[11] Bladé-Font, A., *Tetrahedron Lett.*, 1980, **21**, 2443.