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A NEW SYNTHESIS, RESOLUTION AND *IN VITRO* ACTIVITIES OF (*R*)- AND (*S*)-β-PHENYL-GABA

R.D. Allan,[1*] M.C. Bates,[1] C.A. Drew,[1] R.K. Duke,[1] T.W. Hambley,[2] G.A.R. Johnston,[1] K.N. Mewett[1] and I. Spence[1]

Departments of Pharmacology[1] and Chemistry,[2] The University of Sydney, N.S.W. 2006, AUSTRALIA

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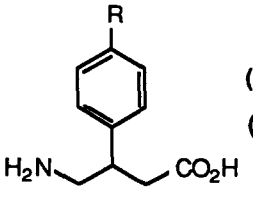
Abstract

β-Phenyl-GABA **(2)** was resolved by separation by crystallization and/or h.p.l.c. of the diastereoisomeric (*R*)-(-)-pantolactone esters of the N-phthalimido protected β-phenyl-GABA. The absolute stereochemical assignments obtained from chiroptical studies of the enantiomers **(8a)** and **(8b)** and an X-ray crystallographic study of the diastereoisomer **(7a)** were supported by the activities of the enantiomers **(8a)** and **(8b)** in binding and electrophysiological studies. Details of synthesis, binding, electrophysiological, chiroptical and X-ray crystallographic studies are reported.

Introduction

The γ-aminobutyric acid (GABA) analogues baclofen **(1)** (β-*p*-chloro-phenyl-γ-aminobutyric acid, 4-amino-3-(4-chlorophenyl)butanoic acid) and β-phenyl-GABA **(2)**, which differ only in that the former bears a *p*-chloro-substituent, are used clinically for different purposes. Baclofen is used as an antispastic agent, whilst β-phenyl-GABA is used as a mood elevator and tranquillizer.[1] Ong and Kerr[2] have shown differing actions of β-phenyl-GABA and baclofen on the isolated guinea pig ileum preparation, suggesting activation of different populations of receptors and this may explain the different therapeutic actions of these compounds.



(1) R = Cl

(2) R = H

While earlier studies were mainly concentrated on baclofen, recent literature indicated renewed interest in the biological activities of β-phenyl-GABA. These include anticonvulsant,[3] antiepileptic,[4] antistress,[5] antiamnesic and antihypoxic,[6] antihypertensive,[7] and analgesic activities.[8]

Literature observations suggested that the biological activities of baclofen resided in the *R*-enantiomer,[9-11] and that only one enantiomer of β-phenyl-GABA was

active as a central nervous system depressant.[12] In view of recurrent interest in the pharmacological activities of β-phenyl-GABA and in order to study the individual enantiomers in more detail, an alternative synthesis and resolution of β-phenyl-GABA which allows the potential for the preparation of resolved tritiated analogues of high specific activity was developed.

A resolution of baclofen by the chromatographic separation of diastereoisomeric amides formed by reaction of N-Boc protected racemic baclofen with α-methylbenzylamine has been reported but no experimental details were given.[9] β-Phenyl-GABA has been resolved by fractional crystallization of the cinchonidine salts[12] and α-methylbenzylamine salts[13] of the N-carbobenzyloxy protected racemate. In view of utilizing one of the synthetic intermediates for the preparation of resolved [^{3}H]-β-phenyl-GABA, the resolution by the separation of diastereoisomeric amides[9] is not suitable because isotopic dilution could result from exchange of amide hydrogen with tritium gas. Resolution based on formation of diastereoisomeric salts also has the disadvantage that the optical purity of diastereoisomeric salt relies mainly on chiroptical measurements. Chiroptical measurements of optical purity depend on analyte concentration, solvent and temperature and are, therefore, subject to inaccuracies especially when the rotation is small. Moreover, crystallization is crucial in this method and, in general, crystallization of the very small amount of compound which would be involved in tritiation is not practical and usually not feasible.

Racemic [^{3}H]-baclofen has been prepared by catalytic bromine/tritium exchange on 4-(3-bromo-4-chlorophenyl)pyrrolidone[14] and by catalytic hydrogenation using tritium of 4-(4-chlorophenyl)-1,5-dihydro-2*H*-pyrrol-2-one.[15] The latter method provided baclofen suitably labelled to high specific activity which was resolved by h.p.l.c. using a chiral mobile phase.[16] This method of resolution, however, led to a lower than expected recovery of radioactive product.[16]

This communication reports the utilization of readily available chiral hydroxylactone, (*R*)-(-)-2-hydroxy-3,3-dimethylbutyro-1,4-lactone, ((*R*)-(-)-pantolactone)[17] in the synthesis and resolution of β-phenyl-GABA via N-phthalimido protected diastereoisomeric esters and the suitability of the unsaturated intermediate as the precursor of radiolabelled β-phenyl-GABA of high specific activity.

Discussion

In this method, chirality of β-phenyl-GABA was generated by catalytic hydrogenation which allows the preparation using tritium gas of radiolabelled resolved material of high specific activity. The key intermediate **(6)**, an optically active ester of unsaturated β-phenyl-GABA in which the amino function is protected as a phthalimido group, was prepared from a suitably substituted α,β-unsaturated carboxylic acid as shown in Scheme 1. . The alternative and potentially easier method

of incorporating the phthalimido group before the pantolactone failed because the required phthalimido acid could not be prepared cleanly by hydrolysis of ethyl *E*-3-phenyl-4-phthalimidobut-2-enoate.

The N-phthalimido group was selected as the protecting group for the amine function because compounds possessing such groups are often crystalline and there is no active hydrogen to exchange with tritium gas.

Ethyl *E*-3-phenylbut-2-enoate, prepared from the condensation of acetophenone and triethylphosphonoacetate by a modified Wittig-Horner reaction,[18] gave, on alkaline hydrolysis, the corresponding acid **(3)**. Allylic bromination of the (*R*)-(-)-pantolactone ester **(4)** gave, after chromatographic purification, the corresponding monobrominated product **(5)** which reacted with potassium phthalimide in dimethylformamide yielding the unsaturated intermediate **(6)** in 80% yield. On catalytic hydrogenation, **(6)** afforded a mixture of diastereoisomeric esters **(7a)** and **(7b)**. The higher melting (m.p. 198.5-199.0 °C) diastereoisomer **(7a)**, the precursor of the biologically active enantiomer of β-phenyl-GABA, readily crystallized from ethyl acetate/cyclohexane. The lower melting (m.p. 59-60 °C) diastereoisomer **(7b)** could be crystallized from cyclohexane after removal of most of the remaining **(7a)**. Although there was no obvious difference between the Rf values of the diastereoismers, they were separable by h.p.l.c.

In all cases, ^{1}H n.m.r. of the crude products, **(3)**-**(6)** showed that the olefinic protons of the major products appeared further downfield when compared with those in the minor products, consistent with the cis arrangement of the olefinic proton and the phenyl group. Optical purity of the diastereoisomeric mixture of **(7a)** and **(7b)** during crystallization was conveniently assessed by ^{1}H n.m.r. ($CDCl_3$) from peak area ratios of methyl singlets as the ^{1}H n.m.r. spectrum of the pantolactone portion of the molecule was particularly simple, consisting of four singlets, readily observable in all cases. In the higher melting diastereoisomer **(7a)**, both methyl groups have the same chemical shifts and appeared as a singlet (δ 0.94) further downfield from those methyl singlets (δ 0.87 and 0.82) in the lower melting diastereoisomer **(7b)**. Downfield shifts of the α-methylene resonances (0.14 and 0.09 ppm) were also observed in **(7a)**. Although ^{13}C n.m.r. spectra of the diastereoisomers were not identical, there were no appreciable differences.

Attempts to selectively remove the ester portion of the diastereoisomer **(7a)** or **(7b)** led to the formation of a mixture of products as judged by t.l.c., a result consistent with our earlier unsuccessful attempts to selectively hydrolyse ethyl *Z*-3-phenyl-4-phthalimidobut-2-enoate. Both phthalimido and pantolactone ester groups were therefore cleaved with 6M HCl to give the required amino acid which was purified by ion exchange chromatography followed by crystallization.

Scheme 1

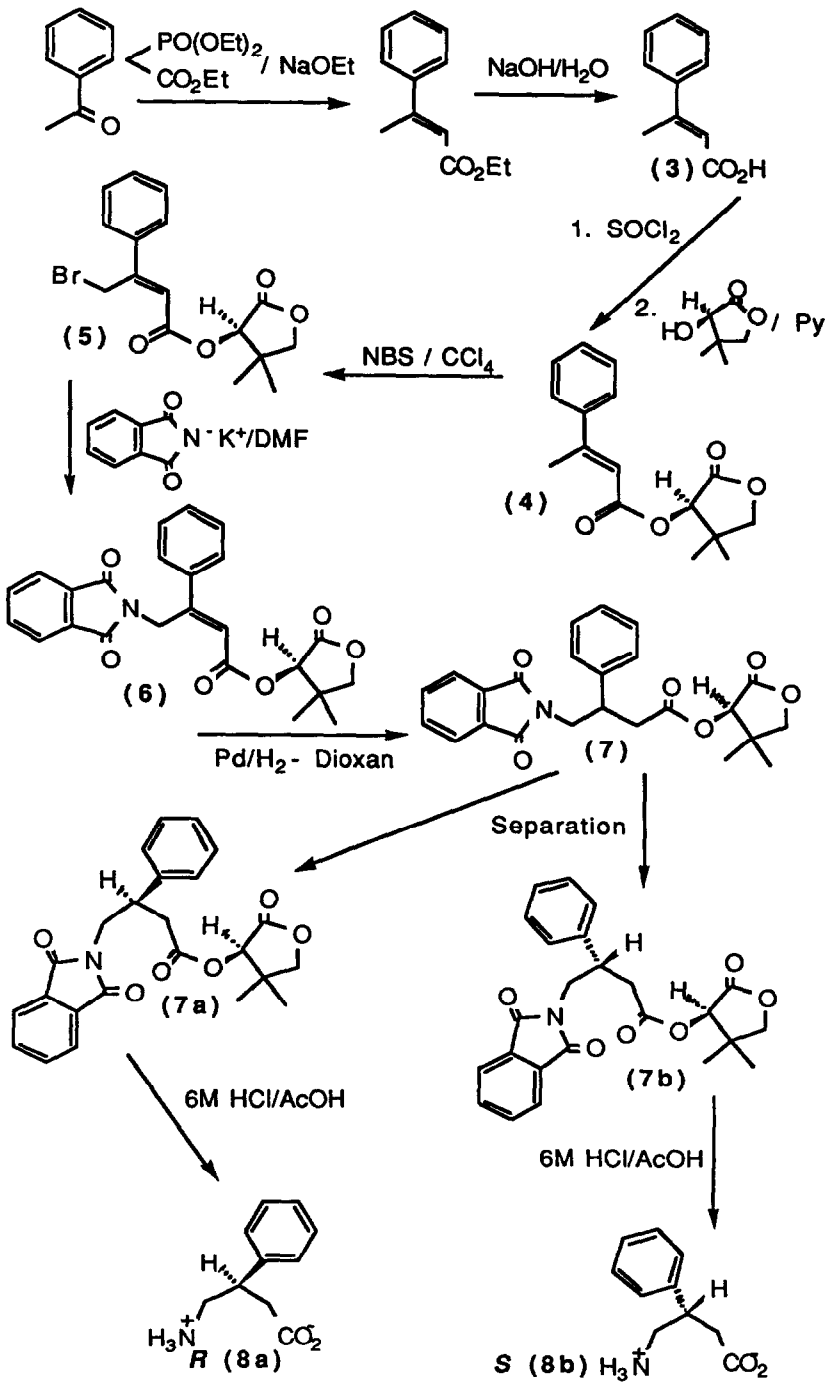

PO(OEt)2
CO2Et
/ NaOEt
NaOH/H2O
CO2Et
(3) CO2H
1. SOCl2
2.
/ Py
Br
(5)
NBS / CCl4
N⁻ K⁺/DMF
(4)
(6)
Pd/H2 - Dioxan
(7)
Separation
(7a)
(7b)
6M HCl/AcOH
6M HCl/AcOH
R (8a)
S (8b)

Table 1 summarizes the optical rotation values at the sodium D line of the enantiomeric amino acids, **(8a)** and **(8b)** at neutral and acidic pH. The results clearly show that the signs and magnitude of the optical rotations at the sodium D line change on protonation of the amino acids **(8a)** and **(8b)** and explains the inconsistency in the signs of the optical rotations of baclofen and β-phenyl-GABA reported[9-12,19] in the literature. Therefore, when referring to the optical rotations at the sodium D line for these compounds, the ionic state of the amino acids should be clearly defined.

Table 1

Compound	pH 1	pH 7
enantiomer **(8a)**	+2.3° (c=1.3, H_2O, conc. HCl)	-6.0° (c=2.5, H_2O)
enantiomer **(8b)**	-2.4° (c=1.3, H_2O, conc. HCl)	+6.3° (c=2.9, H_2O)

Binding and electrophysiological studies (see below) showed activity to reside mainly in the enantiomer **(8a)**. The optical rotations at the sodium D line of enantiomer **(8a)** are in agreement with those reported for the biologically active enantiomers of baclofen and β-phenyl-GABA (Table 2).

Table 2

Compound	$[\alpha]_D$
(-)-baclofen	-1.2° (c=1, H_2O)[9]
(-)-β-phenyl-GABA	-5.3° (c=1.1, AcOH)[12]
(-)-β-phenyl-GABA.HCl	+3.2° (c=5.4, MeOH)[12]

The biologically active enantiomer of baclofen has been shown to be the (-)-enantiomer[9,11] and the *R*-configuration was assigned to this enantiomer on the basis of X-ray crystallography.[20] Initially the *R*-configuration was assigned to the biologically inactive enantiomer of β-phenyl-GABA by its degradation to a compound of known absolute stereochemistry.[11] This assignment was later corrected to be of the *S*-configuration,[19] consistent with that of baclofen.[20] The conclusion drawn from the optical rotation at pH 1 and 7 is that our enantiomer **(8a)** has the *R*-configuration. However, because these optical rotation values at the sodium D line are small and the errors involved in the measurement are relatively large (believed to be about ± 0.5°), the assignment based on the optical rotation is not firm. The circular dichroic maxima for enantiomers **(8a)** and **(8b)** (Table 3) show that, contrary to the observations of the optical rotation at the sodium D line, the signs of the Cotton effect between 210-220 nm are not affected by the protonation of the amino acids. The sign of the Cotton effect due to $\pi-\pi^*$ transition in the CD spectra of β-phenyl-GABA is determined by asymmetric substitutions of the phenyl group, and since the amino and the carboxyl

groups are further away, they will exert only minor influence.[21] This is also the case for 2-substituted 1-phenylcyclohexanes.[22] The negative and positive Cotton effects at 217-218 nm were observed for 1*R* and 1*S* respectively, irrespective of amino or carboxyl substitutions at the 2-position on the cyclohexyl ring.[22] In addition, positive Cotton effects at 210-225 nm have also been observed in a series of (1*S*)-α-substituted ethylbenzenes.[23] At 211 nm, enantiomers **(8a)** and **(8b)** show negative and positive Cotton effects respectively, corresponding to *R*- and *S*-configurations, and this is consistent with the assignments from optical rotations.

Table 3

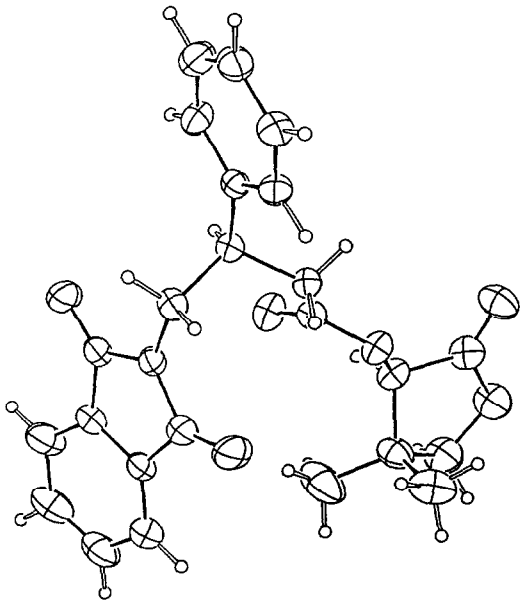
Compound	$\Delta\varepsilon_{211}$ ($M^{-1}cm^{-1}$) (95% EtOH)	$\Delta\varepsilon_{216}$ ($M^{-1}cm^{-1}$) (95% EtOH, at pH 1 (conc.HCl))
enantiomer **(8a)**	- 2.4	- 2.3
enantiomer **(8b)**	+ 2.4	+ 2.4

The *R*-configuration in **(8a)** was confirmed by an X-ray crystallographic study of **(7a)**†*. Single crystal X-ray methods, using the *R*-configuration of the pantolactone moiety as an internal reference (see experimental), established the *R*-configuration at the β-phenyl-GABA moiety in **(7a)**. An ORTEP plot of the diastereoisomer **(7a)** is shown in Figure 1.

In view of the interest in the stereospecificity and mechanism of action of ß-phenyl-GABA as a central nervous system depressant, the enantiomers were assayed for activity in two *in vitro* preparations. Figure 2 shows the results of inhibiting the binding of [^{3}H]-(-)-baclofen to rat cerebellar membranes by each enantiomer. Comparison of the IC_{50} values (the concentrations at which the specific binding of [^{3}H]-(-)-baclofen is reduced by 50%) of (-)-ß-phenyl-GABA (IC_{50} 4.1±0.4 μM (n=3)) and (+)-ß-phenyl-GABA (IC_{50} 699±56 μM (n=3)) shows that (-)-ß-phenyl-GABA is at least 100 fold more potent in this assay.

The enantiomers were also tested for their ability to depress the transmission of electrical signals in a central nervous system pathway using slices from the hippocampal region of the rat brain. Bath application of 1 μM (-)-ß-phenyl-GABA depressed physiological activity by 60% (n=5) (Figure 3). The electrophysiological assays were performed on hippocampal brain slices, recording extracellularly between the perforant path and CA3 cells. In contrast, the effect of the (+)-isomer at 1 μM was not significant (t-test $P>0.001$). A dose-response comparison between baclofen and (-)-ß-phenyl-GABA was performed. In order to depress the synaptic potential to an equal extent, a 100 fold greater concentration of (-)-ß-phenyl-GABA over racemic baclofen was necessary (n=5). All drug applications were of an accumulative dose-response form and completely reversible at final washout.

Figure 1



† The atomic co-ordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW. Any request should be accompanied by the full literature citation for this communication.

* Supplementary data available: observed and calculated structure factors. See Notice to Authors, *Tetrahedron* **40**(2), ii (1984).

Figure 2

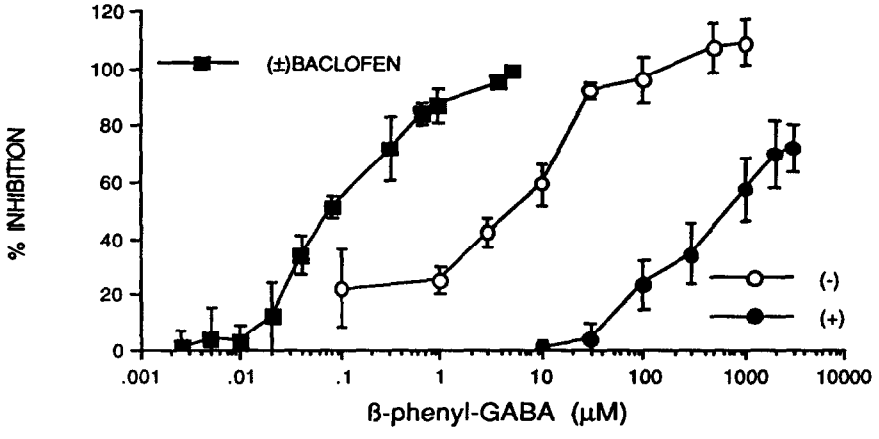


Figure 2. The activities of (-)-ß-phenyl-GABA (open circles) and (+)-ß-phenyl-GABA (shaded circles) at inhibiting the binding of [^{3}H]-(-)-baclofen to rat cerebellar membranes. See experimental section for details. The curve for baclofen (shaded squares) is shown for comparison.

Figure 3

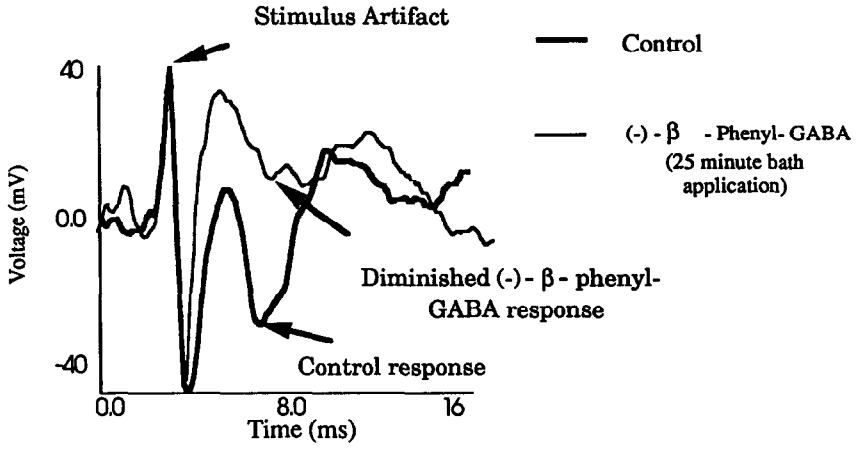


Figure 3. Extracellular recordings from control and bath perfused (25 minute application of (-)-ß-phenyl-GABA) hippocampal brain slices. The (-)-ß-phenyl-GABA treated slice has a depressed synaptic field potential seen at approximately 8.0 ms. Synaptic potentials shown above are the average of 5 sequential recordings.

Experimental

Melting points were determined on a Reichert hot stage apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 177 grating spectrophotometer. Optical rotations were record on a Perkin-Elmer 241 polarimeter. Circular dichroic spectra were recorded on a Jasco Model J-500C spectropolarimeter with a Jasco Model DP-500N Data Processor. ^1H and ^{13}C n.m.r. spectra were recorded at 89.6 MHz and 22.5 MHz respectively on a JEOL FX90Q NMR spectrometer. The peaks (^1H) and (^{13}C) of compounds measured in $CDCl_3$ are described in ppm downfield from tetramethylsilane (internal standard). The assignments of ^{13}C resonances were made from double resonances and gated decoupling techniques. Mass spectral data refer to chemical ionization using methane as reagent gas on a TSQ46 Finnigan/MAT spectrometer. Elemental analyses were carried out by the Australian Microanalytical Service and the results were within ±0.4% of the calculated values. T.l.c. was carried out with 0.25 mm silica gel F_{254} (E Merck) aluminium backed plate. Chromatographic separations were performed using short column vacuum chromatography[25] on Merck silica gel H (T.l.c. grade) unless stated otherwise. Light-petroleum refers to the fraction b.p. 65-70 °C.

E-3-Phenylbut-2-enoic acid (3)

M.p. 98-99 °C (Lit[26] m.p. 98.5 °C). ^1H n.m.r. ($CDCl_3$) δ 7.58-7.49, m, 5H, ArH; 6.18, q, $J_{2,4}$ 1.3 Hz, 1H, H-2; 2.65, d, $J_{4,2}$ 1.3 Hz, 3H, CH_3.

(*R*)-(-)-2-(3,3-Dimethylbutyro-1,4-lactonyl) *E*-3-phenylbut-2-enoate (4)

To acid **(3)** (32.4 g, 0.2 mole) was added thionyl chloride (25 ml) and the mixture was allowed to stand at room temperature overnight. The excess thionyl chloride was azeotropically removed with toluene (50 ml) under reduced pressure. The resulting acid chloride in dichloromethane (20 ml) was added dropwise to a well stirred solution of (*R*)-(-)-pantolactone (28.6 g, 0.22 mole) and pyridine (32.4 ml, 0.4 mole) in anhydrous dichloromethane (80 ml) at room temperature and the slightly basic mixture was stirred at room temperature overnight. The mixture was added to water and extracted with ethyl acetate. The organic fraction was washed in turn with 1M HCl saturated with NaCl, water, saturated bicarbonate, and water until neutral, and dried (Na_2SO_4). Removal of solvents under reduced pressure gave the crude product **(4)** as a dark brown oil. Purification by short column vacuum chromatography (light-petroleum/ethyl acetate : 4/1) gave a pale yellow viscous oil which crystallized from light-petroleum to give **(4)** as colourless needles, m.p. 75.5 °C, (37 g, 67%). Anal. Calcd. for $C_{16}H_{18}O_4$: C, 70.1; H, 6.6. Found: C, 70.0; H, 6.6%. ν_{max} (Nujol) 1748, 1723, 1625 cm^{-1}. *m/z*(%) 315(10), 303(25), 275(80), 145(100). ^1H n.m.r. ($CDCl_3$) δ 7.46-7.26, m, 5H, ArH; 6.26, q, $J_{2,4}$ 1.3 Hz, H-2; 5.48, s, 1H, H-2′; 4.08, br s, 2H, H-4′; 2.62, d, 3H, $J_{4,2}$ 1.3 Hz, CH_3; 1.25, s, 3H, 1.15, s, 3H, $C(CH_3)_2$. ^{13}C n.m.r. ($CDCl_3$) δ 172.8, C-1′, 165.1, C-1; 158.6, C-3; 141.7, ArC-1; 129.4, ArC-4; 128.6, ArC-3 and -5; 126.3, ArC-2 and -6; 115.3, C-2; 76.2, C-4′; 74.5, C-2′; 40.3, C-3′; 23.1, C-4; 20.0, 18.3, $C(CH_3)_2$.

(*R*)-(-)-2-(3,3-Dimethylbutyro-1,4-lactonyl) *Z*-4-bromo-3-phenylbut-2-enoate (5)

To a solution of pantolactone ester **(4)** (16.7 g, 0.06 mole) in carbon tetrachloride (200 ml) was added N-bromosuccinimide (15 g, 0.08 mole) and dibenzoylperoxide (540 mg) and the mixture was refluxed with stirring for 6 h, cooled and succinimide filtered off. The filtrate was purified by short column chromatography (carbon tetrachloride/dichloromethane : 1/1 to dichloromethane) to give **(5)** as a very pale yellow viscous oil which solidified to give very pale yellow needles (17.8 g, 83%). *m/z*(%) 383(4), 381(4), 355(10), 353(10), 273(20), 225(100), 223(100). ^1H n.m.r. ($CDCl_3$) δ 7.62-7.18, m, 5H, ArH; 6.33, s, H-2; 5.51, s, H-2′; 5.08, 4.84, 2H, J_{AB} 9.5 Hz, CH_2Br; 4.09, br s, 2H, H-4′; 1.28, s, 3H, 1.18, s, 3H, $C(CH_3)_2$.

(*R*)-(-)-2-(3,3-Dimethylbutyro-1,4-lactonyl) *Z*-3-phenyl-4-phthalimidobut-2-enoate (6)

To a solution of the bromide **(5)** (5.4 g, 0.015 mole) in anhydrous dimethylformamide (50 ml) was added potassium phthalimide (3.7 g, 0.02 mole) and the mixture heated under stirring at 140 °C for 1 h. The mixture was added to water (50 ml) and extracted with ethyl acetate (3x150 ml). The extract was washed with water and dried (Na_2SO_4) then evaporated under reduced pressure to give a dark brown

oil. Purification by short column vacuum chromatography (light-petroleum/ethyl acetate : 3/1) gave a pink oil which was further purified by short column chromatography (toluene/ethyl acetate : 4/1) to give a faint yellow oil. Crystallization from ethyl acetate gave **(6)** as colourless needles, m.p. 143-144 °C, (5 g, 80%). Anal. Calcd. for $C_{24}H_{21}NO_6$: C, 68.7; H, 5.05; N, 3.3. Found: C, 68.4; H, 5.3; N, 3.7%. ν_{max} (Nujol) 1795 sh, 1775, 1705, 1630 cm^{-1}. *m/z*(%) 460(6), 448(14), 420(42), 290(100). ^1H n.m.r. ($CDCl_3$) δ 7.70-7.50, m, 4H, PhthArH; 7.50-7.10, m, 5H, ArH; 6.30, t, $J_{2,4}$ 1.3 Hz, H-2; 5.55, s, 1H, H-2′; 5.52, 5.37, J_{AB} 16.7 Hz, $J_{4,2}$ 1.3 Hz, NCH_2; 4.11, s, 2H, H-4′; 1.31, s, 3H, 1.20, s, 3H, $C(CH_3)_2$. ^{13}C n.m.r ($CDCl_3$) δ 172.5, C-1′; 167.6, PhthCO; 164.5, C-1; 156.7, C-3; 137.5, ArC-1; 133.9, PhthArC-3 and -6; 131.5, PhthArC-1 and -2; 129.4, ArC-4; 128.4, ArC-3 and -5; 126.9, ArC-2 and -6; 123.1, PhthArC-4 and -5; 119.0, C-2; 76.2, C-4′; 75.1, C-2′; 40.4, C-3′; 37.1, C-4; 23.1, 19.9, $C(CH_3)_2$.

(*R*)-(-)-2-(3,3-Dimethylbutyro-1,4-lactonyl) 3-phenyl-4-phthalimido-butanoate (7a) and (7b)

To 10% palladium on charcoal (21 mg) was added a solution of the unsaturated intermediate **(6)** (210 mg, 0.5 mmole) in dioxan (30 ml) and the mixture hydrogenated for 5 days at atmospheric pressure and room temperature. The catalyst was filtered off and the filtrate evaporated under reduced pressure to give a colourless oil which was purified by short column vacuum chromatography (light-petroleum/ethyl acetate : 2/1) to give a mixture of diastereoisomers as a glassy viscous oil (200 mg, 95%). Crystallization from ether/light-petroleum gave the higher melting diastereoisomer **(7a)** as colourless solid which was almost pure as judged by its ^1H n.m.r. spectrum. Recrystallization from ethyl acetate/cyclohexane gave **(7a)** as colourless plates; m.p. 198.5-199.0 °C; $[\alpha]_D$ +31.3° (c=5, $CHCl_3$). $\Delta\varepsilon_{245}$ (EtOH) -2.1, $\Delta\varepsilon_{221}$ +25.1 $M^{-1}cm^{-1}$. ^1H n.m.r. spectrum showed no contamination from the lower melting diastereoismer **(7b)**. Anal. Calcd. for $C_{24}H_{23}NO_6$: C, 68.4; H, 5.5; N, 3.3. Found: C, 68.1; H, 5.4; N, 3.3%. λ_{max}(EtOH) nm(ε) 294.6(2070), 245sh(8400), 221.1(44000). ν_{max} (Nujol) 1780, 1773, 1740, 1722 cm^{-1}. *m/z*(%) 462(2), 450(17), 422(100), 332(5), 320(5), 292(77), 274(7). ^1H n.m.r. ($CDCl_3$) δ 7.88-7.54, m, 4H, PhthArH; 7.27, br s, 5H, ArH; 5.21, s, H-2′; 4.04-3.52, m, 3.93, s, 3.87, s, 5H, H-3, H-4′ and NCH2; 3.01, br s, 1H, 2.93, br s, 1H, H-2; 0.94, s, 6H, $C(CH_3)_2$. ^{13}C n.m.r. ($CDCl_3$) δ 172.1, C-1′; 170.3, C-1; 168.1, PhthCO; 139.9, ArC-1; 134.0, PhthC-3 and -6; 131.8, PhthC-1 and -2; 128.7, ArC-3 and -5; 127.5, ArC-2 and -6; 123.4, ArC-4; 123.2, PhthC-4 and -5; 76.1, C-4′; 75.0, C-2′; 43.3, C-4; 40.5, C-3; 40.0, C-3′; 37.7, C-2; 22.5, 19.6, $C(CH_3)_2$.

Removal of most of the remaining **(7a)** by repeated fractional crystallization from cyclohexane gave a colourless oil which contained about 90-95% of the lower melting diastereoisomer **(7b)** as judged from its ^1H n.m.r. spectrum. Crystallization from cyclohexane gave the lower melting diastereoisomer **(7b)** as colourless needles. Alternatively, the residue could be separated on preparative h.p.l.c. (silica gel, light-petroleum/ethyl acetate : 4/1); the lower melting diastereoisomer **(7b)**, being less

polar, was eluted first as an oil which slowly crystallized. Recrystallization from cyclohexane afforded colourless needles; m.p. 59-60 °C. $[\alpha]_D$ -20.7° (c=4, $CHCl_3$). $\Delta\varepsilon_{242}$ (Cyclohexane) +1.1, $\Delta\varepsilon_{219}$ -24.5; $\Delta\varepsilon_{247}$ (EtOH) +0.2, $\Delta\varepsilon_{220}$ -37.5 $M^{-1}cm^{-1}$. ^1H n.m.r. spectra of samples obtained from both methods of purification showed no contamination from the higher melting diastereoisomer **(7a)**. Anal. Calcd. for $C_{24}H_{23}NO_6$: C, 68.4; H, 5.5; N, 3.3. Found: C, 68.2; H, 5.8; N, 3.0%. λ_{max} (EtOH) nm(ε) 294.5(2100), 245sh(7900), 223.8(41000). ν_{max} (Nujol) 1805, 1775, 1745, 1710 cm^{-1}. *m/z*(%) 462(2), 450(12), 422(100), 332(5), 320(5), 292(78), 274(7). ^1H n.m.r. ($CDCl_3$) δ 7.88-7.54, m, 4H, PhthArH; 7.28, br s, 5H, ArH; 5.21, s, H-2′; 4.03-3.51, m, 3.92, s, 3.89, s, 5H, H-3, H-4′ and NCH_2; 2.92, br s, 1H, 2.84, br s, 1H, H-2; 0.87, s, 3H, 0.82, s, 3H, $C(CH_3)_2$. ^{13}C n.m.r. ($CDCl_3$) δ 172.2, C-1′; 170.5, C-1; 168.0, PhthCO; 139.7, ArC-1; 134.0, PhthArC-3 and -6; 131.8, PhthArC-1 and -2; 128.7, ArC-3 and -5; 127.8, ArC-2 and -6; 127.5, ArC-4; 127.4, PhthArC-4 and -5; 76.1, C-4′; 75.0, C-2′; 42.9, C-4; 41.3, C-3; 40.0, C-3′; 38.2, C-2; 22.5, 19.6, $C(CH_3)_2$.

(*R*)-4-Amino-3-phenylbutanoic acid (8a)

A solution of the higher melting diastereoisomer **(7a)** (837 mg, 2 mmole) in glacial acetic acid (12 ml) and 6 M HCl (24 ml) was heated under reflux for 10 h. The mixture was evaporated to dryness under reduced pressure, then resuspended in water (20 ml). The solid was filtered off and the filtrate purified on an ion-exchange column (Dowex, AG50). The required product **(8a)** eluted with 1 M pyridine and the solution was evaporated to dryness under reduced pressure to give a straw coloured solid. This was further purified by gel filtration on Sephadex G10 to give a colourless solid. Crystallization from ethanol/water gave the required amino acid as a colourless crystalline solid; m.p. 193-194 °C subl. (lit[12] m.p. 149-152 °C decomp.), (319 mg, 90%). Anal. Calcd. for $C_{10}H_{13}NO_2$: C, 67.0; H, 7.3; N, 7.8. Found: C, 66.7; H, 7.7; N, 8.2%. λ_{max}(EtOH) nm(ε) 258.3(240), 206.7(8200). *m/z*(%) 220(10), 208(10), 191(11), 180(100), 163(28), 162(23); ν_{max} (Nujol) 3350, 2175, 1645 cm^{-1}; ^1H n.m.r. (1 M NaOD/D_2O; dioxan, δ 3.74) δ 7.52-7.16, m, 5H, ArH; 3.24-2.66, m, 1H, 2.83, br s, 2H, 2.82-2.24, m, 2H, H-3, NCH_2 and $CH_2CO_2^-$. ^{13}C n.m.r. (1 M NaOD/D_2O; dioxan, δ 67.66) δ 182.5, C-1; 143.9, ArC-1; 129.8, ArC-3 and -5; 129.0, ArC-2 and -6; 127.8, ArC-4; 47.7, C-4; 46.9, C-3; 43.4, C-2.

(*S*)-4-Amino-3-phenylbutanoic acid (8b)

This amino acid **(8b)** was prepared from the lower melting diastereoisomer **(7b)** using the same method as that for the *R*-enantiomer **(8a)**. (*S*)-β-Phenyl-GABA crystallized as colourless needles, m.p. 194-196 °C subl. (lit[12] m.p.154-156 °C decomp.). Anal. Calcd. for $C_{10}H_{13}NO_2$: C, 67.0; H, 7.3; N, 7.8. Found: C, 66.6; H, 7.6; N, 7.9%. Ultraviolet, infrared, ^1H and ^{13}C n.m.r., and mass spectra were identical to those of (*R*)-β-phenylGABA **(8a)**.

X-ray Crystallography

Crystals of diastereoisomer **(7a)** are orthorhombic, space group $C222_1$, a=13.211(3), b=16.471(4), c=19.903(9) Å, V=4331 Å^3, Z=8. Data were collected on an Enraf-Nonius CAD4-F diffractometer with MoKα radiation and were reduced using the Enraf-Nonius SDP package.[27] The structure was solved by direct methods using SHELXS-86[28] and refined by full matrix least-squares methods using SHELX-76.[29] Final R on 1565 F ($F>5\sigma(F)$) was 3.40%, R_w=3.90%, w=1.72/($\sigma^2(F_o)$+ 0.00030F_o^2), all shifts less than 0.01σ, largest peaks in a final difference map less than 0.12eÅ^{-3}. Figure was drawn using ORTEP.[30]

Biological Studies

Binding Methods

The binding assays were carried out as described by Drew *et al.*[31] except that the cerebella were frozen before preparation and isoguvacine was omitted from the buffer. [^{3}H]-(-)-Baclofen (4-7 nM) bound to the rat cerebellar membranes in Tris-HCl buffer (containing 2.5 mM calcium chloride) in a saturable manner (33±2% specific binding, (n=6)), non-specific binding being defined as the amount of radioactive ligand bound in the presence of 100 µM unlabelled (±)-baclofen.

The protein content of the membrane preparations (300-400 µg per assay tube) were determined by the method of Lowry *et al.*[32] using bovine serum albumin (Sigma) as standard.

All results are given as mean±s.e.m. of at least three separate experiments (each in quadruplicate s.e.m. ≤ 6%). IC_{50} values are the weight mean value of three separate experiments using at least five concentrations of the compound, each in quadruplicate.

[^{3}H]-(-)-Baclofen (38.9 Ci/mmol) was obtained from New England Nuclear and unlabelled (±)-baclofen from Ciba-Geigy.

Electrophysiological Techniques

Extracellular recordings techniques were carried out as described in Koerner *et al.*[33] except that field potentials were recorded in the CA3 cell layer in response to stimulation of the perforant path. Stimuli were delivered once every 4 s and 5 sequential responses were averaged. The bath was perfused with (-)-ß-phenyl-GABA for 30 minutes with individual and averaged recordings taken every 5 minutes.

Potentials were digitised (1 sample every 10 µs) and displayed on both an NEC Powermate™ computer fitted with an RC-ELECTRONICS™ data acquisition program and an Apple Macintosh SE fitted with a MacLab™ system.

Measurements were made of the peak-to-peak amplitude of the orthodromically activated population spike and these results entered into the Statview 512+™ programme for statistical analysis.

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