SYNTHESIS OF CARBON-14- AND TRITIUM-LABELLED 4-AMINO-3-(4-CHLOROPHENYL)-BUTYRIC ACID (BACLOFEN)

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SUMMARY

Two preparations of carbon-14 labelled 4-amino-3-(4-chlorophenyl)-butyric acid are described : First, the label is introduced in the 3-position of the butyric acid chain in nine steps, starting from $^{14}CO_2$. The total radiochemical yield is 30%, calculated from the first labelled intermediate, i.e. 4-chlorobenzoic acid. Second, the ^{14}C-atom is incorporated in the 4-position of the acid chain through three steps only, using $K^{14}CN$ as starting material with a yield of 39%.

Synthesis of the title compound labelled with tritium in the benzene nucleus is performed by catalytic bromine-tritium exchange, affording a product with a specific radioactivity of 9.38 Ci/mmol. The des-chloro compound which is obtained as a by-product, is separated by column chromatography.

Key Words : Carbon-14, Tritium, Selective Halogen-Tritium-Exchange, Baclofen

0362-4803/83/020213-19$01.90

Received July 23, 1982

INTRODUCTION

Baclofen is the active component in the muscle relaxant drug LIORESAL®. The compound has been designed with the idea to provide a centrally active substance having both, the structural features of the γ-amino butyric acid (GABA) and an additional lipophilic substituent, in order to facilitate the passage through the blood-brain-barrier (1).

Clinically, baclofen is widely used as an antispastic agent, particularly in patients suffering from multiple sclerosis and from spasticity of spinal or cerebral origin (2).

Recent studies have shown that baclofen possesses a stereospecific high binding affinity for a novel GABA receptor site in fragments prepared from crude synaptic membranes of the rat brain (3, 4).

In view of the recurrent interest for baclofen as a valuable tool in neurochemical research, in the present paper, we want to report on the synthesis of the carbon-14- and tritium-labelled drug.

DESIGN OF THE SYNTHESES AND DISCUSSION

Baclofen has been labelled with carbon-14 in the 3- and 4-position of the 4-amino-3-(4-chlorophenyl)-butyric acid chain, as well as with tritium in the benzene nucleus.

1. Carbon-14 labelling

1 A: 4-Amino-3-(4-chlorophenyl)-[3-^{14}C]butyric acid

The preparation carrying the carbon-14 in the 3-position of the butyric acid was tailored to provide a metabolically "stable" tracer for biological studies. In FIGURE 1 the reaction scheme for its preparation is shown. The label was introduced as carbon dioxide by a GRIGNARD reaction with 1-bromo-4-chlorobenzene. The 4-chlorobenzoic acid (II) was transformed to the acid chloride III, which upon ROSENMUND reduction with hydrogen yielded the 4-chlorobenzaldehyde (IV).

Subsequent condensation with ethyl acetoacetate and acid cleavage of compound V with conc. NaOH gave 3-(4-chlorophenyl)-glutaric acid (VI). The latter was treated with acetic acid anhydride providing the glutaric acid anhydride VII, which through the glutarimide VIII afforded the 3-(4-chlorophenyl)-glutaramic acid (IX). HOFMANN degradation, finally, with bromine in aqueous NaOH led to the 4-amino-3-(4-chlorophenyl)-butyric acid (X). The radiochemical yield, based on the 4-chlorobenzoic acid was 30%.

1 B: 4-Amino-3-(4-chlorophenyl)-[4-^{14}C]butyric acid

Biotransformation studies with baclofen in rat, dog and man (1) have revealed, that up to 85% of the dose is excreted in unchanged form. Furthermore, the major metabolite isolated is the deaminated 4-hydroxy-3-(4-chlorophenyl)-butyric acid, indicating that

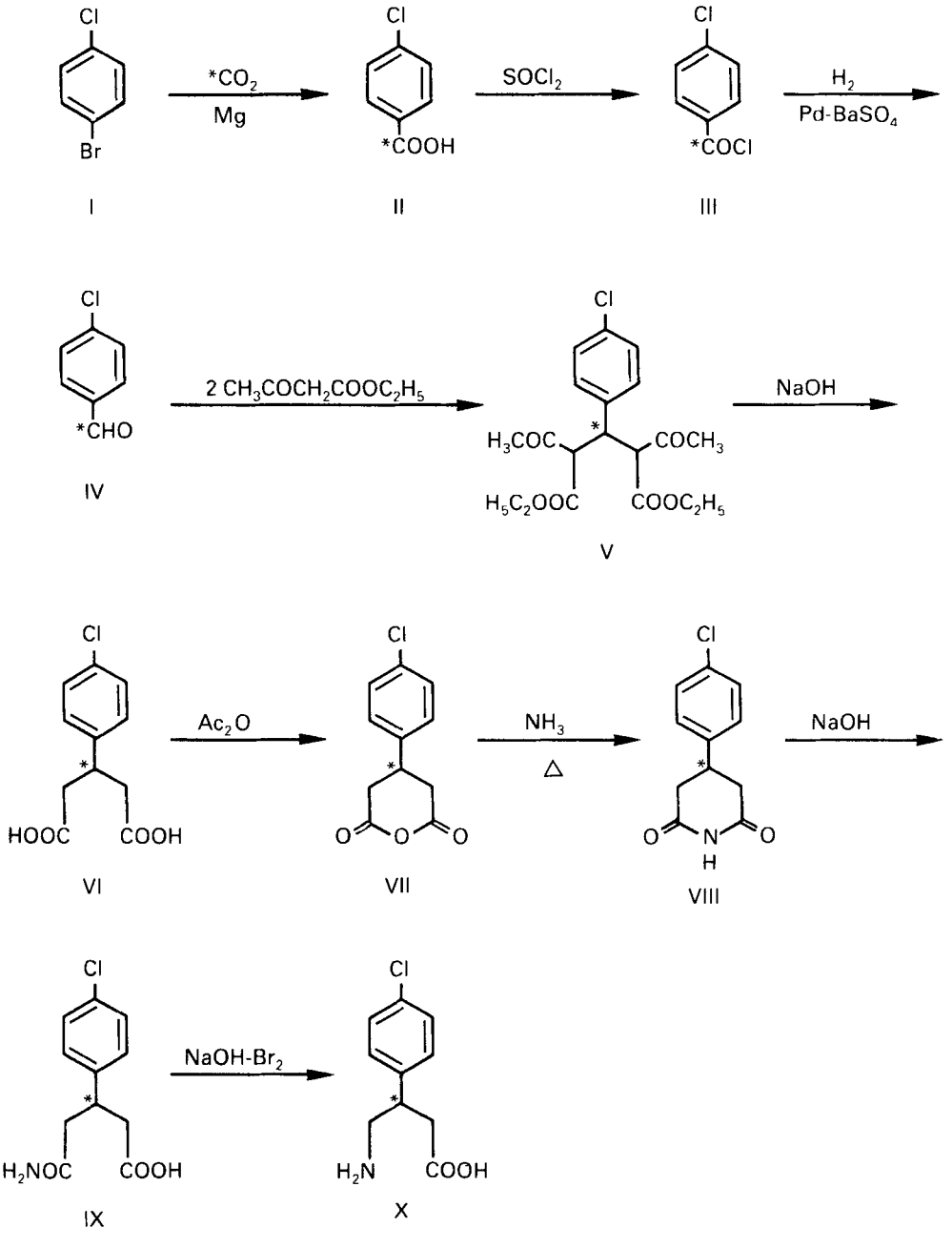

FIGURE 1: Synthesis of 4-amino-3-(4-chlorophenyl)-[3-^{14}C]butyric acid

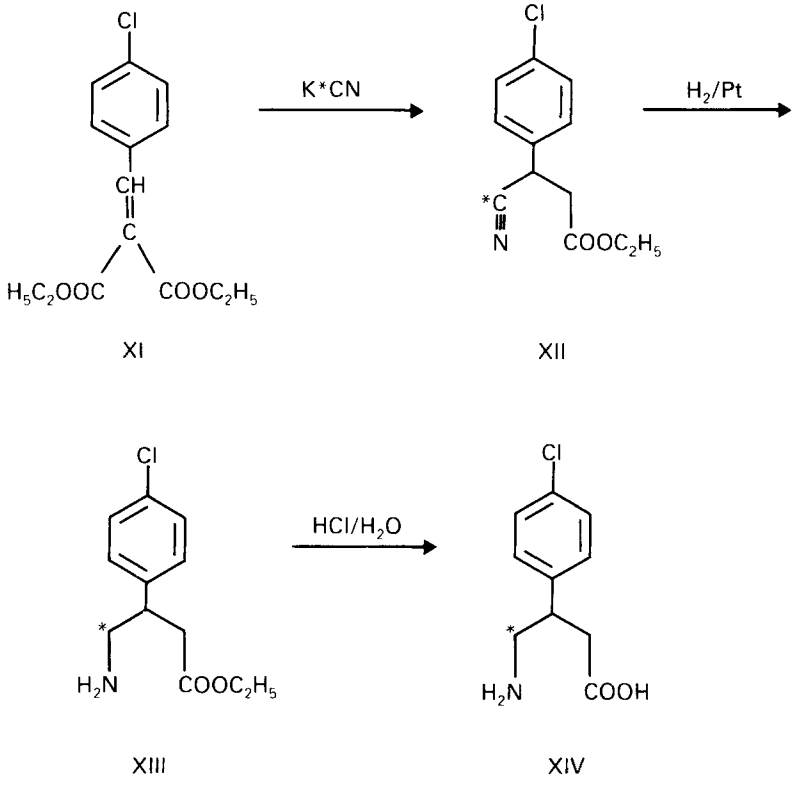

FIGURE 2: Synthesis of 4-amino-3-(4-chlorophenyl)-[4-^{14}C]butyric acid

metabolic breakdown of the carbon-skeleton of the drug - if it even does occur - is, quantitatively speaking, of minor importance. Therefore, for further biological experiments, we have performed as an alternative a simple and more convenient route for the preparation of the ^{14}C-labelled drug as outlined in FIGURE 2.

The 4-chlorobenzal-malonic acid diethyl ester (XI) was obtained by a KNOEVENAGEL condensation (5) using 4-chlorobenzaldehyde and

malonic acid diethyl ester. Addition of KCN to the double-bond of XI under heating in aqueous ethanol according to the method of BREDT and KALLEN (6) led to the cyano-propionic acid ethyl ester (XII). Conversion to the aminobutyric acid ester XIII by catalytic hydrogenation with platinum and hydrolysis easily afforded the title compound XIV. The overall radiochemical yield starting from $K^{14}CN$ was 39%.

2. Tritium labelling

For binding-site studies with baclofen high specific radioactivities are required which cannot be achieved by carbon-14 labelling. Here, tritium was the choice as a suitable radionuclide. The reaction pathway used for this purpose is displayed in FIGURE 3. Following the principle to incorporate the tritium in a late step of the reaction sequence - thus minimizing the handling with the highly radioactive material - the 4-(3-bromo-4-chloro-phenyl)-pyrrolidone-(2) (XVI) was prepared as intermediate for the catalytic bromine-tritium exchange reaction. Hydrolysis of the tritiated pyrrolidone XVIIb easily provided the desired product XIXb.

The next main problem, however, was to find reaction conditions under which a quantitative and, simultaneously, a selective bromine-tritium exchange could be accomplished with minimal loss of the neighbouring chlorine substituent.

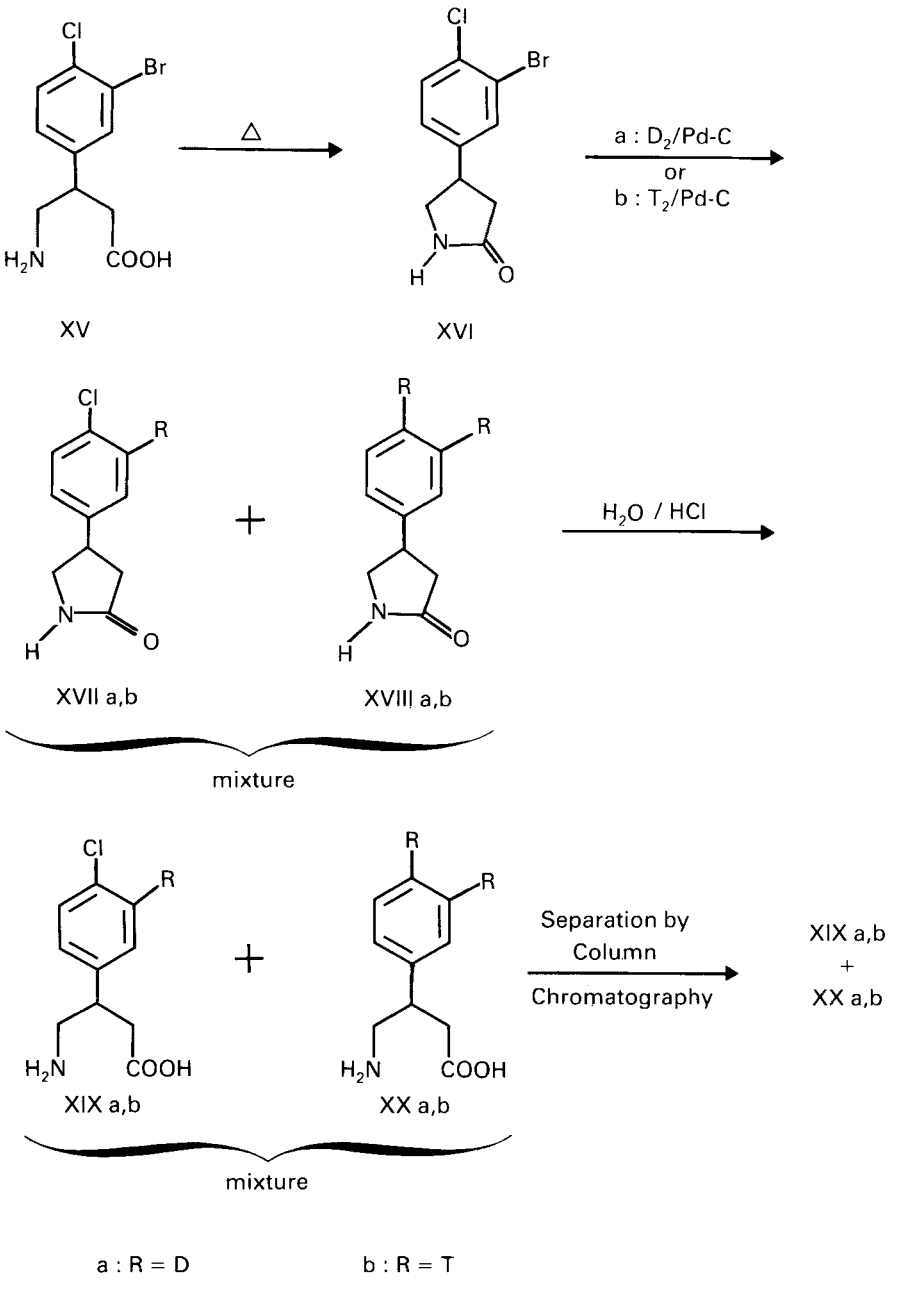

FIGURE 3 : Synthesis of 4-amino-3-(4-chloro-[3(N)-^{3}H]phenyl)-butyric acid

Numerous trial experiments using deuterium gas as the hydrogen tracer led to the most favourable reaction conditions as follows :

Treatment of XVI with deuterium gas in ethylene glycol monoethyl ether in the presence of palladium on coal and sodium acetate at room temperature yielded a mixture of XVIIa and XVIIIa. Separation of the products was achieved after hydrolysis in aqueous hydrogen chloride by column chromatography affording the two amino acids XIXa and XXa.

An analogous reduction with tritium gas instead of deuterium gas and subsequent workup gave rise to the tritiated baclofen XIXb, having a specific radioactivity of 9.38 Ci/mmol, after dilution with non-radioactive material. The des-chloro compound XXb, which was obtained as a by-product could be isolated upon column chromatography.

The product XIXb is stored in the solvent system used for the chromatography, i.e. sec. butanol - acetic acid - water (67:10:23), at the temperature of liquid N_2 in order to minimize the rate of decomposition.

EXPERIMENTAL

4-Chlorobenzoic acid chloride (III)

The solution containing 4.55 g (29.1 mmoles, 27.3 mCi) of 4-chlorobenzoic acid (II), obtained by standard procedures (7), and 35 ml

of $SOCl_2$ was refluxed for 5 hours and then evaporated to dryness under reduced pressure. In order to remove traces of remaining $SOCl_2$, the residue was dissolved in 5 ml of hexane and evaporated again. This procedure was repeated 3 times, always under careful exclusion of moisture, yielding 5.3 g of crude III.

4-Chlorobenzaldehyde (IV)

The above crude product (5.3 g, theor. amount 29.1 mmoles) was placed in a reaction flask equipped with a magnetic stirrer, with 50 ml of xylene, 0.35 g of 5% palladium on barium sulfate catalyst and 0.021 ml of "quinoline-sulfur" solution (8). This reaction mixture was stirred at 70 - 75°, while a weak stream of hydrogen was kept bubbling through the suspension until the formation of HCl ceased. After 28 hours the theoretical amount of HCl was released as monitored by titration of a 1 N NaOH solution in an adapter connected to the reaction flask. The catalyst was removed from the reaction mixture by filtration and the filtrate evaporated to dryness under vacuum, yielding 6.9 g of crude aldehyde IV.

2,2'-Diacetyl-3-(4-chlorophenyl)-glutaric acid diethyl ester (V)

The crude residue from the above reaction (6.9 g, containing a theor. amount of 29.1 mmoles) was dissolved in 4 ml ethanol and allowed to react overnight at room temperature with 7.6 g

(58.4 mmoles) of ethyl acetoacetate and 1.28 ml of piperidine. Upon addition of 50 ml of hexane and cooling to 0° the product precipitated as a crystalline solid. It was removed by filtration and washed with ethanol-hexane. Yield 7.25 g of compound V, m.p. 152-4°, 65% of the theor. amount based on the starting material 4-chlorobenzoic acid.

3-(4-Chlorophenyl)-glutaric acid (VI)

A mixture of 30 ml ethanol and 31 g of an aqueous NaOH solution (50%) was heated in an oil bath at 100°. During a time period of 3 hours, 7.25 g (18.9 mmoles) of V, dissolved in 50 ml of dioxane, were added dropwise to the hot solution, stirred with a magnetic stirrer. After heating of the reaction mixture for an additional hour at 100° and subsequent cooling to room temperature, the organic solvents were removed by distillation under reduced pressure. The remaining aqueous phase was carefully acidified by addition of 35 ml of conc. HCl at 0°, yielding precipitation of the product, which was collected on a glass sinter funnel and washed with water. After drying, 3.8 g (15.6 mmoles) of the crystalline glutaric acid VI were obtained, m.p. 161-3°.

3-(4-Chlorophenyl)-glutaric acid anhydride (VII)

The glutaric acid VI was dissolved in 20 ml of acetic acid anhydride and refluxed for 1 hour. Then, the reaction mixture was

concentrated under vacuum, the oily residue diluted with 5 ml of benzene and finally, hexane was added in small portions until crystallization began. After cooling to 0^{o}, the product was removed by filtration and washed with benzene-hexane. Thus, 3.4 g of the anhydride VII, m.p. 127-9^{o} were isolated.

3-(4-Chlorophenyl)-glutarimide (VIII)

Into the reaction flask containing 3.4 g (15.1 mmoles) of the solid compound VII cooled in an ice bath, 15 ml of conc. aqueous NH_3 were added drop by drop. The solution obtained was heated to 70^{o} within 30 minutes and then evaporated to dryness under reduced pressure. The flask containing the dry residue was immersed into a pre-heated oil bath at 185^{o} and kept at this temperature for 1 hour.

4-Amino-3-(4-chlorophenyl)-butyric acid (X)

The above crude residue, containing the theor. amount of 15.1 mmoles VIII, was dissolved in a solution of 0.7 g NaOH in 16 ml of water by stirring and warming to 50^{o}. To the glutaramic acid IX obtained, at a temperature of 10 - 15^{o}, an additional portion of 3.3 g of NaOH in 16 ml water was given. Subsequently, 1.35 ml of bromine (4.2 g, 26 mmoles) were added dropwise to the solution which was then allowed to warm up to room temperature and stirred again for 4 hours. Upon treatment with charcoal, concentration of

the solution to a volume of approximately 20 ml under reduced pressure and neutralization (pH 6.5 - 7.0) with conc. HCl - water (1:1), the free amino acid crystallized and was removed by filtration. Further purification was achieved by dissolution in 15 ml 1 N NaOH and re-precipitation by careful addition of 1 N HCl under stirring until the pH-range of 6.5 - 7.0 was reached. The isolated solid product was washed with water and dried at 0.01 mm Hg and 25°. 1.55 g (7.3 mmoles) of the pure product X were obtained, 48% of the theor. amount based on VII, having a specific activity of 4.4 μCi/mg.

The aqueous mother liquors of the two successive precipitations were combined and mixed with a solution of 2.0 g of non-radioactive X in NaOH. By analogous isolation and re-precipitation a second batch of 1.6 g of pure X was obtained, having a specific radioactivity of 0.80 μCi/mg.

Taking both batches together, the total radiochemical yield was 8.1 mCi, or 30% calculated from 4-chlorobenzoic acid (II).

The radiochemical purity was checked by TLC on silica gel with n-butanol - acetic acid - water (67:10:23), paper electrophoresis (buffer pH 2.8, 220 V, 3 hrs) and inverse isotope dilution analysis, and according to these criteria, was found to be greater than 99%.

3-Cyano-3-(4-chlorophenyl)-propionic acid ethyl ester (XII)

Into the solution of 603 mg of KCN (8.7 mmoles, 49.6 mCi) dissolved in 1 ml water, 2.32 g (8.2 mmoles) of 4-chlorobenzal-malonic acid diethyl ester (5) (XI) in 22 ml of ethanol were added. The mixture was heated under an atmosphere of N_2 for 16 hours at 70°. After cooling to 0°, the precipitated carbonate was removed by filtration, the clear solution was acidified with 1.6 ml of 4 N HCl and evaporated under vacuum to dryness. The residue was dissolved in diethyl ether and washed with water. From the organic layer, after drying with Na_2SO_4, evaporation and distillation at 0.05 mm Hg and 140°, 1.63 g of a crude oily product XII were obtained. Analysis by GC revealed a chemical purity of 91%, indicating an actual yield of 1.48 g (6.25 mmoles) or 71.8% based on the applied KCN.

4-Amino-3-(4-chlorophenyl)-butyric acid ethyl ester (XIII)

The above crude product (6.25 mmoles) was dissolved in 11 ml of ethanol, 60 mg of platinum oxide and 0.75 ml of 10 N HCl were added and the resulting mixture was hydrogenated at room temperature and atmospheric pressure. Within 7 hours the theoretical volume of hydrogen was absorbed. The catalyst was removed, the filtrate evaporated under reduced pressure and the residue washed with 30 ml of diethyl ether, yielding 1.46 g of crude hydrochloride of XIII.

4-Amino-3-(4-chlorophenyl)-butyric acid (XIV)

Hydrolysis of the above product, 1.46 g (5.25 mmoles), was accomplished by refluxing in 17 ml of 5 N HCl for 16 hours. The solution was cooled to room temperature, the solvent removed by lyophilization and the residue crystallized from ethanol-diethyl ether to give 1.1 g of the hydrochloride of XIV.

For purification and isolation of the free amino acid, the product was re-precipitated twice : First the product was dissolved in water and precipitated by neutralization with aqueous NaOH. Subsequently the procedure was repeated inversely by dissolution in NaOH and careful addition of HCl. The product thus obtained was washed with water and dried under vacuum yielding 526 mg (2.46 mmoles) of pure XIV with a specific radioactivity of 26.0 µCi/mg.

By addition of 700 mg of non-radioactive hydrochloride of XIV to the above ethanol-diethyl ether mother liquor and analogous recrystallization and re-precipitation another batch of 572 mg product having the specific radioacitivity of 10.1 µCi/mg was obtained.

Consequently, the total radiochemical yield accomplished was 19.5 mCi, or 39% based on the starting KCN.

Analysis of the product was performed by TLC on silica gel (solvent systems : Methanol - chloroform - acetic acid, 80:15:5, and n-butanol - water - acetic acid, 67:23:10),

mass spectrometry, inverse isotope dilution analysis and paper electrophoresis (phthalate buffer pH 3.0, 220 V, 2.5 hours).

Through these methods, the identity and a chemical and radiochemical purity greater than 99% were established.

4-(3-Bromo-4-chlorophenyl)-pyrrolidone-(2) (XVI)

The 4-amino-3-(3-bromo-4-chlorophenyl)-butyric acid (XV) was prepared by condensation of 3-bromo-4-chlorobenzaldehyde (9) with malonic acid diethyl ester to the corresponding benzal-malonic acid diethyl ester, addition of KCN, catalytic hydrogenation and subsequent hydrolysis in analogy to the reaction path outlined above (Figure 2).

Formation of the lactam XVI was easily accomplished by repeated sublimation of 500 mg (1.7 mmoles) XV in a bulb tube with 4 bulbs at 250° and 0.1 - 0.5 mm Hg. The sublimation was repeated 3 times in the same tube, yielding 429 mg of crude product, which upon crystallization from benzene gave 392 mg of pure XVI as colourless crystals, m.p. 129-32°.

Tritiation of XVI yielding a mixture of XVIIb and XVIIIb

The bromine-tritium exchange was carried out in a round bottom flask attached to a vacuum manifold having a total volume of

75 ml. In the reaction flask, the mixture of 275 mg (1.0 mmole) pyrrolidone XVI, 100 mg sodium acetate (1.2 mmoles), 60 mg Pd-C 10% and 10 ml of freshly distilled ethylene glycol monoethyl ether was frozen with liquid N_2, the system was evacuated and filled with approximately 190 Ci of tritium gas, released from an uranium trap. The exchange reaction was effected by magnetic stirring at room temperature and monitored with a pressure gauge. Within 24 minutes the pressure dropped from 798 to 518 mm Hg, indicating the absorption of 1.2 mmoles tritium. The remaining tritium gas was removed by re-adsorption on uranium, the manifold flushed with N_2 and the reaction flask removed from the manifold. The catalyst was separated from the reaction mixture by filtration, the filter rinsed with a total of 10 ml ethylene glycol monoethyl ether and the combined clear solution lyophilized to dryness.

In order to remove the labile tritium, the residue was dissolved and lyophilized again 3 times with portions of 10 ml methanol each. To the product thus obtained, 10 ml of ethyl acetate and 2 ml of water were added. In a separatory funnel the two phases were separated, the aqueous layer extracted with 30 ml of ethyl acetate and the organic layers washed with 30 ml of water. Liquid scintillation counting of the ethyl acetate solution, containing a mixture of compounds XVIIb and XVIIIb, indicated a total radioactivity of 29 Ci.

Hydrolysis of the mixture containing XVIIb and XVIIIb and separation of the two products

The above ethyl acetate solution was divided into two equal parts. One half was lyophilized, sealed together with 3 ml of 6 N HCl into an ampoule with a magnetic stirrer and heated in an oil bath at 100° for 2.5 hours. The resulting dark solution was evaporated under vacuum, the residue dissolved in 1 ml of sec. butanol - acetic acid - water (67:10:23) and eluted from a column packed with 35 g of silica gel 60, using the same solvent system. Fractions of 1 ml each were collected. Analysis of the fractions was performed by reversed-phase TLC using silanized silica gel and acetate buffer pH 4 - acetone (98:2), a system which allows separation of the two products XIXb and XXb. Thus, the following preparations were obtained :

Fractions 31 - 37 :	3.2 Ci	XIXb
Fractions 38 - 41 :	0.49 Ci	mixture XIXb + XXb
Fractions 42 - 47 :	2.4 Ci	XXb

4-Amino-3-(4-chloro-[3(N)-^{3}H]phenyl)-butyric acid (XIX)

The combined fractions 31 - 37 (3.2 Ci) were shown to have a radiochemical purity of 90% by TLC. For further purification, 30 mg of non-radioactive baclofen were added, the solution was lyophilized and the column chromatography repeated as described above.

Ultimately, a solution of 25 ml sec. butanol - acetic acid - water (67:10:23) was prepared containing 46.3 mg baclofen, as determined by UV-spectrometry at 263 nm. The total radioactivity was 2.03 Ci and the radiochemical purity 98%, based on TLC and inverse isotope dilution analysis. From these data, a specific radioactivity of 9.38 Ci/mmol or 43.9 mCi/mg can be calculated.

The product is stored in the solvent system used for the column chromatography. Upon storage at room temperature, a loss of the radiochemical purity of 5% per month was observed. The rate of decomposition is considerably decreased at lower temperature.

Repurification of the product easily can be achieved by column chromatography.

4-Amino-3-[3,4(N)-^{3}H]phenyl-butyric acid (XXb)

The above fractions 42 - 47 containing 2.4 Ci were diluted with the same solvent system to a total volume of 25 ml. Analysis by TLC revealed the presence of the des-chloro product of baclofen (XXb) with a radiochemical purity of 98%.

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