SYNTHESIS OF 4-AMINO-3-p-CHLOROPHENYL-BUTYRIC ACID-2,2,4,4-$^{2}H_{4}$ (BACLOFEN)

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SUMMARY

The synthesis of the title compound is described. It involves base catalyzed substitution of deuterium for hydrogen in 3-p--chlorophenylglutaric acid which is subsequently transformed to baclofen. The overall yield is 80%.

INTRODUCTION AND DISCUSSION

In order to develop a mass fragmentographic method for the determination of 4-amino-3-p-chlorophenylbutyric acid (baclofen) in body fluids the deuterium labelled compound was required as an internal standard. The most easily available alternative, baclofen dideuteriated in the 2 position, was not suitable due to the interference from compounds in biological samples at the mass number of the standard. However, the mass number two units higher was found to be much more free from interference. Accordingly, the title compound was synthesized as illustrated in scheme 1, which is essentially the procedure of Keberle and Faigle (1). Since we have not found this synthesis described in the literature, we have included full experimental details.

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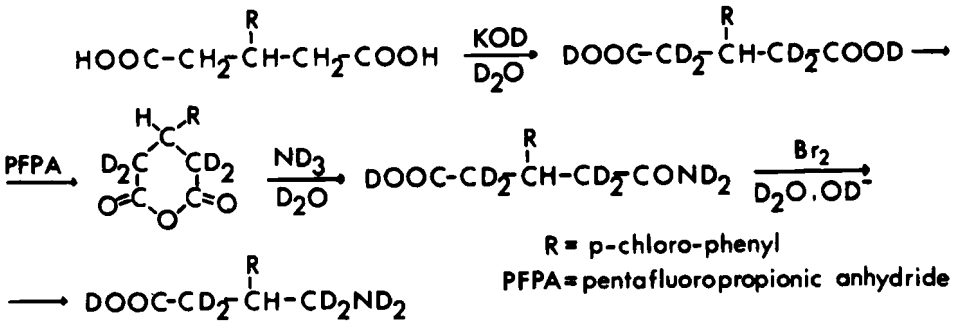

SCHEME 1 Synthesis of baclofen-D_4.

The labelling of 3-p-chlorophenylglutaric acid was performed as described by Atkinson et al. (2). Attempts to achieve exchange at 100°C resulted in very incomplete labelling. This indicates that there is little risk of back-exchange with this standard, even in strongly basic medium.

When the labelled acid was treated with acetic anhydride in order to prepare the anhydride, the label disappeared completely. Further experiments showed this to be due to trace amounts of base present in the starting material, catalyzing the exchange of hydrogen between the different anhydrides present in the reaction mixture.

In fact, this exchange could be used for the preparation of deuteriated acids as is indicated in the experimental section. This procedure seems to be convenient for the preparation of certain deuteriated acids in small amounts. The high cost of the reagent excludes the method from large scale preparations.

In principle, deuteriated reagents should not be needed after

the labelling step. The use of these here was merely a safety measure for which the price is not prohibitively high.

The partial mass spectra of baclofen and baclofen-D_4 as the pentafluoropropylester pentafluoropropionamide are shown in Fig. 1.

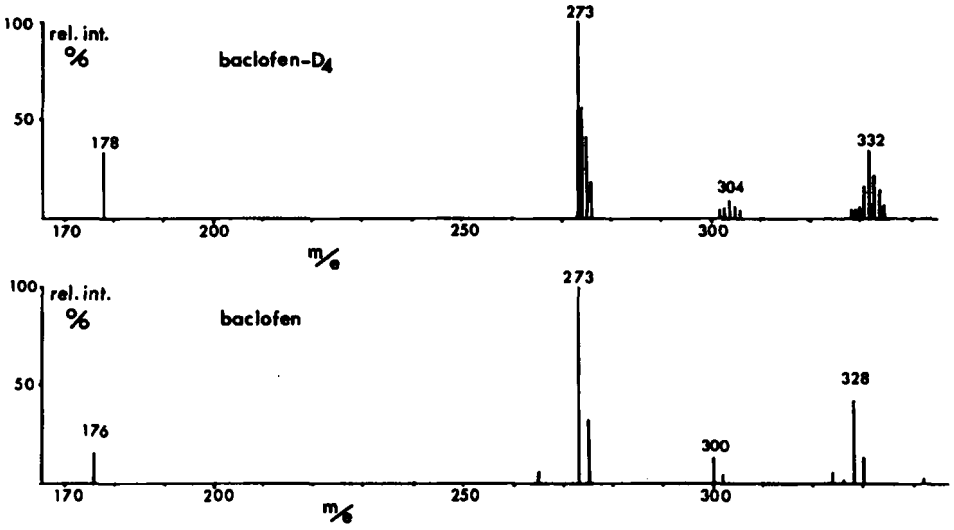

FIG. 1 Mass spectra of baclofen-D_4 and baclofen. The molecular ion is not discernible.

As the molecular ion (at 491 for the non-labelled compound) is not discernible, the degree of labelling has to be judged from fragment ions. It can be seen from the spectrum that four deuterium atoms are incorporated in a relatively good yield. The presence of a substantial amount of the ion of mass 328 is probably due to some impurity. A proposed fragmentation pattern is shown in Fig. 2. The structure of the ion of mass 273 is only tentative and is by no means proved.

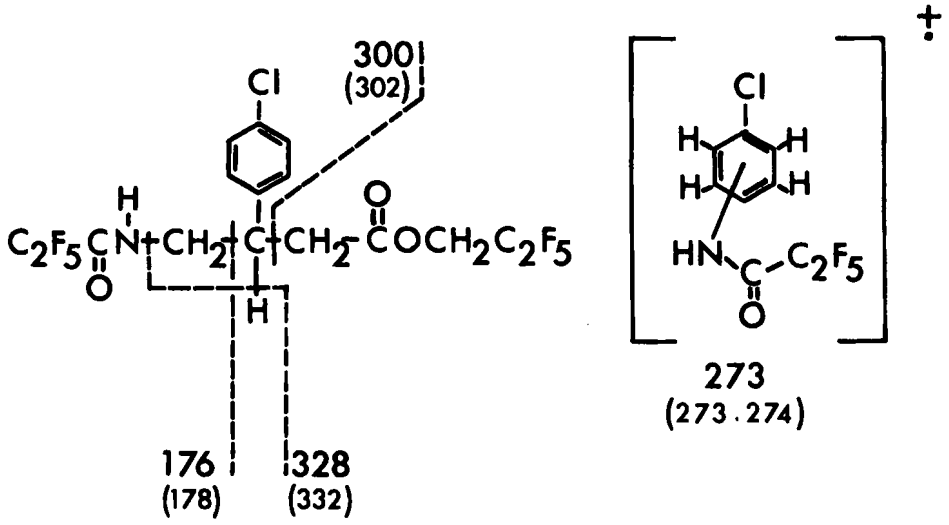

FIG. 2 Fragmentation of baclofen. The m/e values for the corresponding fragments of the labelled compounds are shown within parenthesis. M^+=491 (495).

EXPERIMENTAL

General

Concentrations were performed in a rotary evaporator at a bath temperature of 40-60°C. Melting points were obtained on a Reichert melting point microscope and are not corrected. Mass spectra were obtained on a Finnigan 3200 mass spectrometer, with EI ionization, at an electron energy of 50 eV. The deuteriated reagents were obtained from commercial sources and contained more than 99 atom % deuterium.

3-p-chlorophenylglutaric acid-2,2,4,4-$^2H_4$ (I)

3-p-chlorophenylglutaric acid (3) (3.00 g) was dissolved in D_2O (10 ml) containing KOD (2.80 g). The mixture was transferred to a steel autoclave and kept at 150°C for 24 hours. The solvent was removed and fresh D_2O (10 ml) was added. The mixture was kept at 150°C for 48 hours after which time it was acidified

with 4 M HCl. The resulting precipitate was collected by filtration. Yield 2.74 g. Mp 165-7°C (Lit. (3) 166-8°C, from H_2O). The material was used as such without further purification.

3-p-chlorophenylglutaric anhydride-2,2,4,4-$^2H_4$

I (0.5 g) was refluxed with pentafluoropropionic anhydride (5 ml) for 3 hours. The mixture was concentrated and used immediately in the next step. The procedure originally described (1) could not be used as all the label disappeared at reflux in acetic anhydride. Mp. of non-labelled material (crude product) 120-130°C.

Mass spectral data: m/e (%) 231 (2), 230 (6), 229 (9), 228 (14), 227 (7), 143 (17), 142 (39), 141 (56), 140 (100), 139 (29), compared with the same fragments for non-labelled material 226 (6), 224 (18), (M^+) 140 (32) 138 (100).

Labelling with acetic anhydride-$^2H_6$

3-p-chlorophenylglutaric acid, palmitic acid or stearic acid (5 mg), potassium carbonate (1 mg) and acetic anhydride-D_6 (100 µl) were kept in a sealed tube at 150°C for 12 hours. The excess of anhydride was evaporated and, in the case of palmitic and stearic acid, water was added which was extracted with chloroform. Mass spectrometric analysis showed the complete incorporation of 4 deuterium atoms in 3-p-chlorophenylglutaric anhydride and 2 in palmitic or stearic acid.

4-carboxybutyramide-2,2,4,4-$^2H_4$ (III)

II (all material from the previous step) was dissolved in ND_3/D_2O

(20%, 3 ml) with shaking. After 1 hour the mixture was evaporated to dryness. 2 M HCl was added (6 ml) and the solution was extracted with ethyl acetate. The ethyl acetate phase was concentrated and used as such in the following operation. Yield 0.44 g. The material was chromatographically pure as judged from TLC (SiO_2; EtOAc-MeOH-H_2O, 80:15:5). Mp of non--labelled material 171-2°C.

4-amino-3-p-chlorophenylbutyric acid-2,2,4,4-$^2H_4$ (IV)

III from the previous step was dissolved in 4 M NaOH in D_2O (5 ml). To this solution 1 M BrO^- in D_2O (2.5 ml) was added in one portion. The hypobromite solution was prepared by dissolving Br_2 (0.26 ml) in 4 M NaOH in D_2O)5 ml). The mixture was kept at 60°C for 1 hour. The pH was adjusted to 5.5 with 4 M HCl. After cooling, the precipitate was collected by filtration. Yield 0.39 g. Mp 200-5°C (Mp. of authentic material 200-3°C). Neither Mp. was sharp but showed some decomposition from about 170°C. The material chromatographed as one spot at TLC on silica gel, using the system BuOH-AcOH-H_2O 4:1:1.

The material was also analysed by GC-MS, as its pentafluoropropylester, pentafluoropropionamide derivative. It contained only minor volatile impurities and could be used as such for the mass fragmentographic procedure, which is described elsewhere (4).

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