PREPARATION OF β-ALANINE-3,3-D_2 and -2,2,3,3-D_4

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SUMMARY

β-Alanine-3,3-d_2 and -2,2,3,3-d_4 were prepared via a five-step procedure from ethyl cyanoacetate. The procedure involves reduction of the starting material with lithium aluminum deuteride, followed by benzoylation of the amino alcohol obtained, oxidation of the benzoylated compound, hydrolysis of benzoyl-β-alanine-3,3-d_2 with hydrochloric or deuterochloric acid, and then anion exchange of the hydrochloride or the deuterochloride. The isotopic purities of the d_2 and d_4 compounds were more than 98 and 97%, respectively.

Key Words: β-alanine-3,3-d_2, β-alanine-2,2,3,3-d_4, 3-benzoylamino-propionic-3,3-d_2 acid, 3-amino-1-propan-1,1,3,3-d_4-ol, ethyl cyanoacetate, lithium aluminum deuteride

Compounds labelled with stable isotopes are very important not only for the tracer technique, but also for the analysis of molecular vibrations. In the latter case their infrared and Raman spectra are necessary for the reliable assignment of observed bands and for the determination of force constants. Several deuterated compounds were prepared in our studies on vibrational spectra of β-lactams (1,2). 2-Azetidinone, the simplest β-lactam, is obtained by cyclization of

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β-alanine (1,3). Therefore, it is essential to synthesize the isotopic compounds of β-alanine. β-Alanine-2,2-d_2 is easily prepared by heating β-alanine in deuterochloric acid (1,4). However, the deuteration at the 3-position can not be accomplished by such a simple method.

The present paper deals with the preparation of the 3,3-di- and 2,2,3,3-tetradeuterated compounds of β-alanine. A number of methods for the preparation of β-alanine have been reported (5,6), since it is a biologically important substance as a constituent of coenzyme A, pantothenic acid and so on (7). The catalytic reduction of cyanoacetic acid or its esters (6) is applicable to the preparation of β-alanine-3,3-d_2. However, this method is not practical for the present purpose, because handling of deuterium gas is troublesome. Lithium aluminum hydride is very useful as a reducing reagent, and the necessary deuteride is commercially available. When this reagent is used, it is expected that cyanoacetate is reduced to 3-amino-1-propanol. If this amino alcohol is oxidized after benzoylation, benzoylated β-alanine will be obtained. The removal of the benzoyl group will give β-alanine. Billmans and Parker (8) obtained α-alanine from 2-amino-1-propanol by such a method.

$$\underset{[\ \mathrm{I}\ ]}{NC\text{-}CH_2\text{-}COOR} \xrightarrow{LiAlD_4} \underset{[\ \mathrm{II}\ ]}{H_2N\text{-}CD_2\text{-}CH_2\text{-}CD_2\text{-}OH} \xrightarrow{C_6H_5COCl} \underset{[\ \mathrm{III}\ ]}{C_6H_5CONH\text{-}CD_2\text{-}CH_2\text{-}CD_2\text{-}OH} \xrightarrow[NaOH]{KMnO_4} \underset{[\ \mathrm{IV}\ ]}{C_6H_5CONH\text{-}CD_2\text{-}CH_2\text{-}COOH}$$

$$[\ \mathrm{IV}\ ] \xrightarrow[2)\ \text{anion exchange}]{1)\ HCl} \underset{[\ \mathrm{V}\ ]}{H_2N\text{-}CD_2\text{-}CH_2\text{-}COOH}$$

$$[\ \mathrm{IV}\ ] \xrightarrow[2)\ \text{anion exchange}]{1)\ DCl} \underset{[\ \mathrm{VI}\ ]}{H_2N\text{-}CD_2\text{-}CD_2\text{-}COOH}$$

We have tried to prepare β-alanine-3,3-d_2 and -2,2,3,3-d_4 according to the above reaction scheme and obtained them in reasonable yields. The optimal reaction conditions were initially determined by using the unlabelled compounds. The isotopic purities were checked by NMR spectroscopy.

The reduction of nitriles and esters with lithium aluminum hydride is well known (9), but no hydrogenation of cyanoacetic acid or its esters with this reagent has been reported in the literature except for the selective reduction of the ester with lithium aluminum hydride on silica gel (10), in which the ester group only is reduced to the alcohol. When ethyl or methyl cyanoacetate [I] was allowed to react with lithium aluminum hydride in dry ether, 3-amino-1-propanol was obtained in a 34-35% yield. The product was identified by comparison of its infrared spectrum with that of an authentic sample. Nitriles themselves are reduced to amines in 50-90% yields, and esters are converted to alcohols in yields usually more than 60% (9). The lower yield in the present work may be ascribed to the precipitation of an intermediate product which is highly insoluble owing to its bifunctionality (9). The corresponding deuterated amino alcohol [II] was obtained in a 42-47% yield.

The amino alcohol was benzoylated with benzoyl chloride in an aqueous solution of sodium hydroxide or a suspension of sodium carbonate in benzene. A derivative which was presumed to be the N,O-dibenzoylated compound, N-(3-benzoyloxypropyl)benzamide, was also formed, its content depending upon reaction conditions. Since the separation of the N-benzoylamino alcohol from the dibenzoyl compound is difficult, it is advisable to hydrolyze the ester moiety of the latter in the reaction mixture. Conditions for the hydrolysis were examined, and the following procedure was found to be suitable; after the benzoylation of the amino alcohol had been completed, the alkaline reaction mixture was heated at 60-65 °C. This procedure gave crude 3-benzoylamino-1-propanol or its 1,1,3,3-d_4 compound [III] in a 97-98% yield.

The oxidation of the alcohols with alkaline potassium permanganate gave crude 3-benzoylaminopropionic acid and its 3,3-d_2 compound [IV] in a 70-88% yield. For the undeuterated compound the identity was checked by infrared spectral comparison. A very small amount of benzamide (0.5%) was also obtained, showing that the cleavage of the N-C bond also occurred to a small extent.

These benzoylated β-alanines were quantitatively hydrolyzed by 20% hydrochloric acid to β-alanine and β-alanine-3,3-d_2 [V], respectively. The infrared spectrum of the β-alanine obtained was identical with that of an authentic sample, and the results of the elemental analysis were in good agreement with the calculated values. When 3-benzoylamino-propionic-3,3-d_2 acid [IV] was heated in 20% deuterochloric acid, β-alanine-2,2,3,3-d_4 [VI] was obtained.

These β-alanines were converted to 2-azetidinones (1,3) by using Mukaiyama's reagent (triphenylphosphine and di-2-pyridyl disulfide) (11). The azetidinone-N-sulfonates were also prepared.

EXPERIMENTAL

3-Amino-1-propan-1,1,3,3-d_4-ol [II]

In a 500ml three-necked flask provided with a teflon-sealed mechanical stirrer, a dropping funnel, and a thermometer, a solution of 9.00g of ethyl cyanoacetate [I] in 30ml of dry ether was added dropwise to a solution of 10.0g of lithium aluminum deuteride (Merck, an isotopic purity: min. 98%) in 400ml of dry ether with stirring at 2-5°C over a period of two hours and a half. Stirring was continued at the same temperature for an hour and then at room temperature for an hour. Water (20ml) was then added cautiously, one drop at a time. The contents were finely ground and filtered by suction. The cake was washed with five 30ml portions of ether, and then transferred to the flask and stirred with 150ml of ether. An ether-insoluble material was filtered out. It was furthermore heated under reflux in 200ml of ethyl alcohol, and the filtrate was evaporated under

reduced pressure. The residue was extracted repeatedly with ether (a total amount of 150ml). The combined etheral solutions were dried over anhydrous sodium sulfate. After evaporation of the solvent, the liquid product was distilled under reduced pressure to give 2.94g (46.7%) of II: bp 91-94°C/20mmHg. An isotopic purity of this compound was estimated to be over 98 % by NMR spectroscopy (a JEOL JNM-GX270 FT-NMR spectrometer was used).

3-Benzoylamino-1-propan-1,1,3,3-d_4-ol [III]

In a 200ml flask 5.61g of II was dissolved in 70ml of water and chilled with ice. After 7.0g of sodium hydroxide had been dissolved, 11.96g of benzoyl chloride was added dropwise to the vigorously stirred mixture at such a rate that the temperature did not rise above 5°C. The addition required about an hour. Stirring was continued for an hour below 5°C and for an additional hour at room temperature. The reaction mixture was then stirred at 60-65°C for an hour. The cooled mixture was extracted with chloroform (1×100ml and 6×50ml). The chloroform solution was dried over anhydrous sodium sulfate. Removal of the solvent gave 12.6g (97.3%) of III as an oily substance. For the unlabelled compound it has been reported that recrystallization from acetic acid ester gave crystals melting at 60-60.5°C (12). However, from the infrared spectrum this material (III) was satisfactory for the following reaction without purification.

3-Benzoylaminopropionic-3,3-d_2 acid [IV].

In a 300ml flask 12.62g of III was suspended in 150ml of water, and 0.7g of sodium hydroxide was dissolved. To the vigorously stirred mixture was added 18.87g of potassium permanganate at such a rate that the temperature did not rise above 35°C. Stirring was continued for eight hours longer. After the mixture had been warmed at 35-40°C for thirty minutes, the excess of permanganate was decomposed with sodium bisulfite.

The precipitate (manganese dioxide) was filtered out and washed repeatedly with warm water (a total amount of about 100ml). The combined filtrates were concentrated to about 60ml under reduced pressure, chilled, and acidified with hydrochloric acid. The crystallized material was filtered off, pressed to remove an oily substance, and dried in vacuo: yield 10.63g (79.1%). Recrystallization from water gave 6.81g of the pure product IV: mp 132.5°C. The mother liquor of the crude crystals was extracted with chloroform, and the extracts were evaporated to give an oily substance. A small additional amount (0.73g) of the pure carboxylic acid was obtained by reoxidation of this residue with potassium permanganate.

β-Alanine-3,3-d_2 [V]

In a 50ml flask were placed 3.12g of IV and 35ml of 20% hydrochloric acid, and the mixture was refluxed for ten hours. After it had been chilled in a refrigerator, the benzoic acid crystallized was filtered out and washed with a small amount of water. The combined filtrates were evaporated to dryness under reduced pressure. The β-alanine-3,3-d_2 hydrochloride obtained was dissolved in a small amount of water, and the solution was passed through an Amberlite IRA-45 column (about 60ml of the resin). About 250ml of the eluent was evaporated under reduced pressure, and the residue was dried in vacuo to give 1.41g (96.7%) of colorless crystals, which was recrystallized from 85% ethyl alcohol: 1.02g of the pure product V; mp 198.5-199°C (decomp). An isotopic purity was estimated to be minimum 98% by NMR spectroscopy.

β-Alanine-2,2,3,3-d_4 [VI]

In a 100ml flask 3.12g of IV was dissolved in 30ml of hot heavy water (CEA, an isotopic purity: min. 99.85%), and immediately the flask was placed in a desiccator. The solution was evaporated to dryness under reduced pressure at room temperature. The 3-benzoylaminopropionic acid-3,3,N,O-d_4 thus obtained was heated under reflux in 35ml of 20%

deuterochloric acid (Merck, an isotopic purity: min. 99%) for sixty hours. The reaction mixture was cooled. The benzoic acid crystallized was filtered out and washed with a small amount of heavy water. The combined filtrates were evaporated under reduced pressure to give β-alanine-2,2,3,3-d_4 deuterochloride. The deuterochloride was passed through an Amberlite IRA-45 column as described above. Evaporation of the water gave 1.46g (98.0%) of VI. It was recrystallized from 85% ethyl alcohol: 1.11g; mp 198.5-199°C (decomp). An isotopic purity was estimated to be more than 97% by NMR spectroscopy.

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