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DEUTERATION OF DL-4-HYDROXYPHENYLLACTIC ACID

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SUMMARY

The preparation of [D_2]-labelled DL-4-hydroxyphenyllactic acid by acid catalyzed hydrogen exchange at the aromatic ring is reported. Spectrometric analysis of the product shows that the deuteration is regiospecific and quantitative, which makes the deuterated compound suitable as an internal standard in the stable isotope dilution analysis of its naturally occurring analog in biological samples.

INTRODUCTION

Prenatal diagnosis of some inherited metabolic diseases is possible by direct chemical analysis of characteristic metabolites in amniotic fluid supernatant. This approach has the advantage of having results available within 2 - 3 days (1).

In tyrosinemia due to a deficiency of 4-hydroxyphenylpyruvic acid dioxygenase, 4-hydroxyphenyllactic acid (4-HPLA) is the major urinary metabolite (2,3). It is likely that 4-HPLA would be elevated in the amniotic fluid of a fetus affected with this disease.

The most sensitive and accurate approach to the detection of 4-

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HPLA would be to develop a stable isotope dilution technique, in which the stable isotopically labelled analog of 4-HPLA serves as an internal standard for quantification and as a carrier for the minute amounts of naturally occurring 4-HPLA through all steps of the analytical procedure.

A labelled analog of 4-HPLA was not commercially available and had to be synthesized. In this paper we describe the synthesis of [D_2]-4-HPLA by acid catalyzed hydrogen exchange at the aromatic ring of 4-HPLA.

For use as an internal standard, the compound should be labelled in a high isotopic enrichment and at a stable position of the molecule. The degree of labelling was checked by mass spectrometric analysis, and the position of the labels was confirmed by ^1H NMR measurements.

METHODS

DL-4-hydroxyphenyllactic acid was purchased from Sigma Chemical Company, St. Louis, U.S.A.. Deuterium chloride (20 wt. % solution in D_2O, > 99.96 atom % D), deuterium oxide (99.8 atom % D) and [D_3]-acetonitrile (99 atom % D) were obtained from Janssen Chimica, Belgium.

The deuteration of DL-4-HPLA was performed by heating the compound (50 mg, 0.2 mmole) in 9 % DCl/D_2O (2 ml) at 80 ^0C for 6 hours in a sealed tube. After cooling, the solution was saturated with NaCl and extracted with ethyl acetate (4 x 2.5 ml). The combined extracts were dried over $MgSO_4$, filtered and evaporated under a stream of nitrogen. The product was purified by recristallization from Et_2O.

Trimethylsilyl derivatives were prepared by adding pyridine (100 μl) and N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA, 100 μl) to an aliquot of the compounds and heating at 80 ^0C for 20

minutes. Samples of these solutions were directly used for gas chromatographic analysis.

GC analysis was performed on a Carlo Erba gas chromatograph operating at a constant temperature of 250 ^0C, using a CP sil 19 capillary column with flame ionization detection and helium as carrier gas. Mass spectra were determined on a Kratos MS 80 mass spectrometer in the E.I. mode and in the C.I. mode (NH_3 as reagent gas). ^1H NMR spectra of solutions of the compounds in CD_3CN were recorded on a Bruker WH 90 spectrometer with tetramethylsilane as internal reference; chemical shifts δ are given in ppm and coupling constants J in Hz.

RESULTS AND DISCUSSION

Although the preparation of a labelled version of 4-HPLA from commercially available labelled tyrosine should be possible by diazotation with nitrous acid and subsequent reaction of the formed diazo compound with hydroxylic ion (4), hydrogen exchange on 4-HPLA itself was envisaged to be the appropriate choice. The latter method has been successfully applied to the deuteration of compounds comparable to 4-HPLA (5) and is easy to perform.

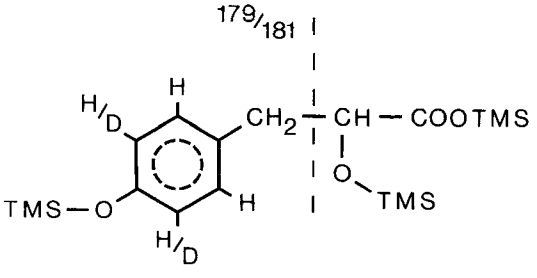
After the acid catalyzed deuteration of DL-4-HPLA by the above described procedure, the deuterated product was recovered in a quantitative yield, and its TMS derivative was gas chromatographically pure.

The identity of the labelled product was confirmed by comparing the E.I. mass spectrum of its TMS derivative with that of [D_0]-4-HPLA di-TMS. The most relevant fragments of the unlabelled and labelled compounds have been assigned and are listed in table 1.

Under the reaction conditions two hydrogen atoms appear to exchange for deuterium, affording [D_2]-4-HPLA. The isotopic enrichment of this product was quantitatively determined by analysis of the C.I. mass spectrum of its TMS derivative. In this

Table 1. E.I. mass spectral data (m/z) of $[D_0]$-4-HPLA and $[D_2]$-4-HPLA (relative intensities in parentheses).

$[D_0]$-4-HPLA	$[D_2]$-4-HPLA	fragment
398 (2%)	400 (2%)	$[M]^{+\cdot}$
383 (6%)	385 (5%)	$[M - CH_3]^+$
355 (6%)	357 (5%)	$[M - CH_3 - CO]^+$
308 (51%)	310 (42%)	$[M - HOTMS]^+$
179 (100%)	181 (100%)	$[M - TMSO-CH-COOTMS]^+$
147 (58%)	147 (55%)	$[(CH_3)_2-Si=O-Si-(CH_3)_3]^+$
73 (94%)	73 (96%)	$[Si-(CH_3)_3]^+$



spectrum the signal at m/z = 401 (fragment $[M + 1]^{+\cdot}$) has a relative intensity of 83 %, while signals at m/z = 400 and 399, belonging to $[M + 1]^{+\cdot}$ fragments with a deuterium enrichment of less than two, appear to be absent. This indicates that the exchange of two hydrogen atoms for deuterium is complete. No over-exchange , leading to a higher degree of deuterium incorporation, is seen.

Selected ion mass spectrometric analysis of the deuterated product revealed the amount of $[D_0]$-4-HPLA in $[D_2]$-4-HPLA to be 1 % (NH_3 - C.I., m/z = 399 resp. 401).

In order to determine which hydrogen atoms had been exchanged for deuterium, the ^1H NMR spectrum of $[D_2]$-4-HPLA was compared with that of the unlabelled 4-HPLA (6). Both spectra are similar in the lactic acid part of the molecular system, but differ in the phenolic part. Deuteration causes the complete disappearance of the signal at 6.78 ppm (d, J = 4.3 Hz, 2H, $H_3 + H_5$), and the substitution of the doublet at 7.14 ppm (J = 4.3 Hz, 2H, $H_2 + H_6$) by a singulet at 7.13 ppm (2H, $H_2 + H_6$). This elucidates the position of the deuterium atoms to be ortho to the hydroxylic

group at the ring, which is in accordance with the expected electron density distribution in the carbon ring system of comparable compounds (7).

It can be concluded that the described deuteration method results in the complete and regioselective exchange of two hydrogen atoms at the aromatic ring of 4-HPLA. The physical properties of the deuterated product make this compound suitable for use as an internal standard in the assay of its unlabelled analog in biological samples.

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