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SYNTHESIS OF THE ENANTIOMERS OF 3-DEUTERIUM LABELLED LACTIC ACID

Michèle Sanière, Hassane El Hafa, Yves Le Merrer, Jean Claude Depezay.
Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques
(URA 400), Université René Descartes
45, rue des Saints-Pères, 75270 Paris Cedex 06, France.

Francis Rocchiccioli.
Service d'Endocrinologie (INSERM U 188), Hopital Saint Vincent de Paul
74, avenue Denfert Rochereau, 75674 Paris Cedex 14, France.

SUMMARY

Both R- and S- enantiomers of 3-monodeuterated lactic acid ([3-^{2}H]-2(R) and 2(S)-hydroxypropanoic acids) were synthesized by regiospecific nucleophilic opening of diastereoisomer epoxides derived from D-mannitol, with lithium aluminium deuteride. Spectroscopic analysis of the product shows a complete deutero-incorporation which makes [3-^{2}H]-lactic acid suitable as an internal standard using gas chromatography-mass spectroscopy.

Key words : [3-^{2}H]-lactic acid, deuterium-label, enantiomers, internal standard, gas chromatography-mass spectroscopy.

INTRODUCTION

Isotope tracer methods have been developed for studying lactate metabolism (1-4), particularly using lactic acid triply labelled with ^{13}C and gas chromatography-mass spectroscopy (3,4). In this paper we describe the synthesis of 3-monodeutero lactic acid of R or S configuration; this compound is suitable as an internal standard for determination of lactate from plasma since it has been labelled

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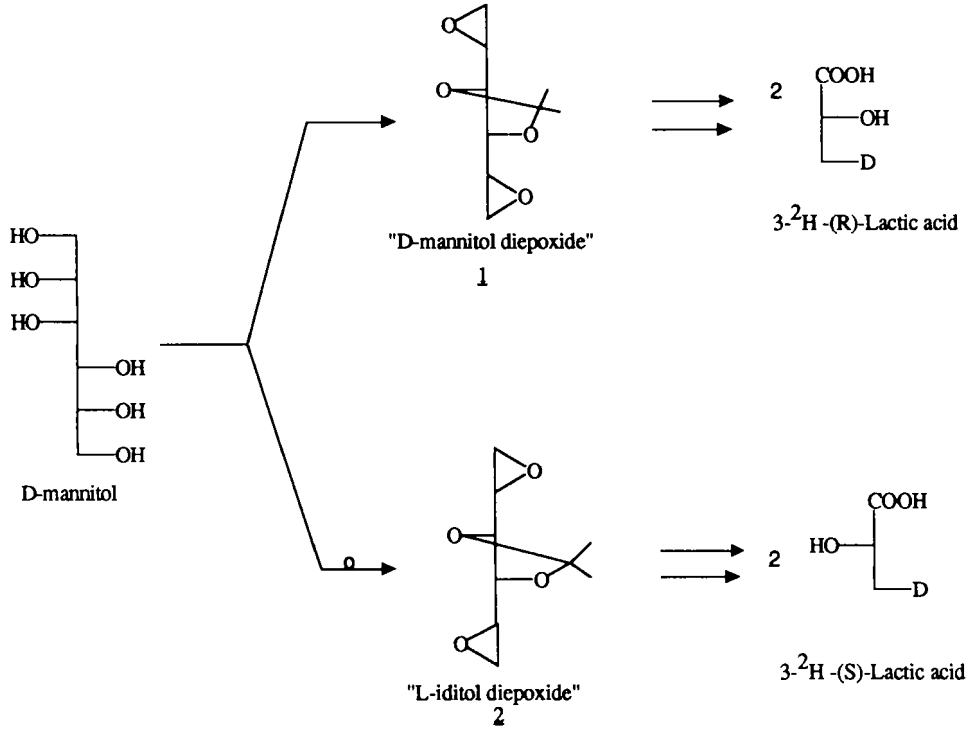
in a high isotopic enrichment and at a stable position of the molecule. The degree of labelling was checked by mass spectroscopic analysis and confirmed by ^{13}C NMR measurements.

RESULTS AND DISCUSSION

Diastereoisomer diepoxides 1 and 2 , readily obtained from D-mannitol (in four and five steps respectively with 40% overall yield) following a previously described method (5), are respectively precursors of 3-deutero-(R)-lactic acid and (S)-enantiomer (scheme 1). Each molecule of D-mannitol leads via diepoxides without losing carbon, to two molecules of enantiomerically pure α-hydroxy acid.



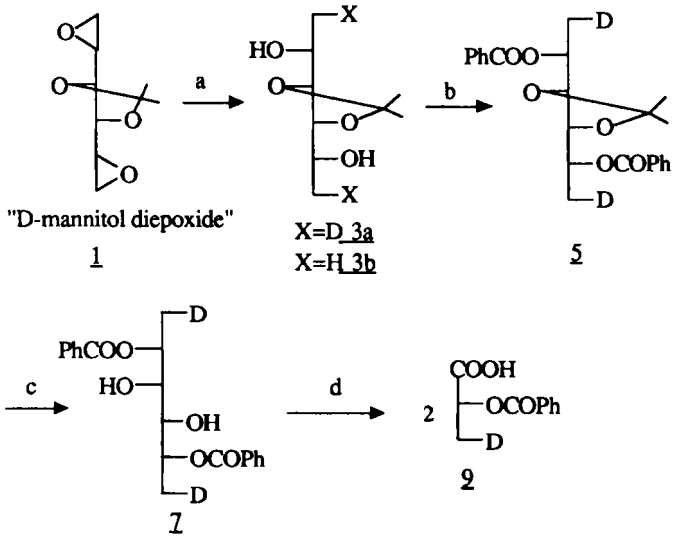
scheme 1

Regiospecific nucleophilic ring opening of the enantiomerically pure diepoxide 1 (scheme 2) by lithium aluminium deuteride (or hydride) in refluxing diethyl ether gave the dideutero diol 3a (or its hydrogenoanalogue 3b) in 80% yield after chromatography. The isotopic enrichment of 3a was quantitatively determined by analysis of the E.I. mass spectrum. The signal at m/z = 177 (fragment $[M-15]^+$) has a relative intensity of 12%, (m/z 59, relative intensity 100%) while signals at 176 and 175 belonging to $[M-15]^+$ fragment with deuterium enrichment of less than two, appear to be below 0.4%. The NMR spectra (^1H and ^{13}C) of dideutero 3a were compared with that of the unlabelled 3b. Both spectra differ only in the methyl part (CH_2D or CH_3). Dideuteriation causes replacement, on one hand of the doublet at 1.27 ppm (6H, J = 6.0 Hz) by a multiplet at 1.28 ppm (4H) in ^1H NMR (250 MHz), and on the other hand of the singlet (δ = 20.48 ppm) by a triplet (δ = 20.16 ppm, J_{C-D} = 18.95 Hz) in ^{13}C NMR. Furthermore a ^{13}C DEPT 135 study (distortionless enhancement by polarisation transfer) (6) shows a total dideuteration (absence of the singlet on the opposite side of the triplet). So with all these spectroscopic data, we believe that the isotopic enrichment of the dideuterated compound 3a was greater than 98%.

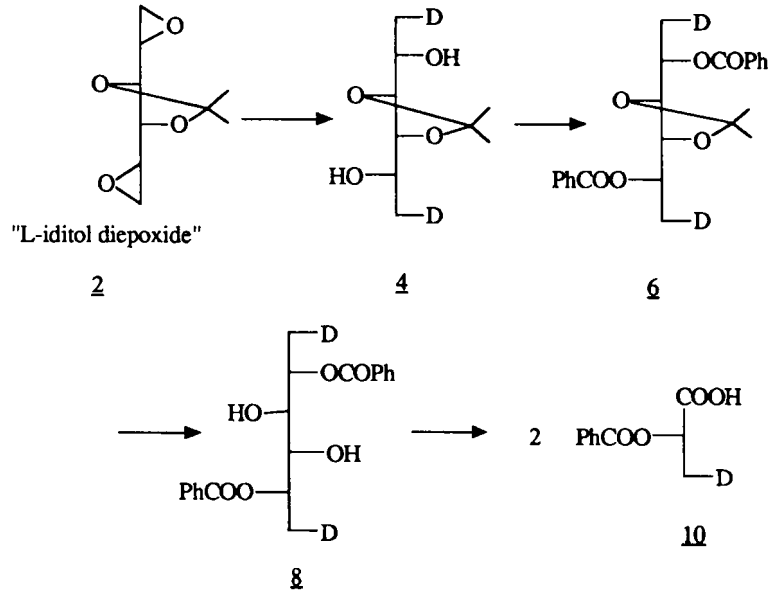
Benzoylation of the diol 3a produced the dibenzoate 5 with 70% yield after chromatography. Hydrolysis of the acetonide 5 with aqueous trifluoroacetic acid provided the diol 7. Finally the use of a mixture of chromium (VI) oxide-sodium peroxide (2.2eq.) in aqueous acetic acid allowed the one step conversion of the diol 7 into two molecules of the monodeuterated acid 9. After purification by column chromatography, the [3-^{2}H]-2(R)-benzoyloxy propanoic acid was isolated in 70% yield, $[\alpha]_D$ -23.0° (c 1.08, CH_2Cl_2)

From the diepoxide 2 , an identical reaction sequence (scheme 3) furnished the enantiomeric [3-^{2}H]-2(S)-benzoyloxy propanoic acid 10 in a similar overall yield (40% from 2).

Comparison by means of spectroscopic data (^1H and ^{13}C NMR, MS) showed that 10 was undistinguishable from its enantiomer 9, except for its optical rotation [10 : $[\alpha]_D$ + 22.3° (c 0.995, CH_2Cl_2)]. The enantiomeric purity of 9 and 10 has been confirmed by GC analysis on chiral glass capillary column after preparation of the amide derivatives by heating with isopropyl isocyanate (7) (retention times for isopropylamides of 9 and 10, respectively 20.6 min and 21.5 min at 165°C, Helium 1.5 bar).



scheme 2 : (a) $LiAlD_4$ or $LiAlH_4$, Et_2O; (b) PhCOCl, pyridine, CH_2Cl_2; (c) CF_3COOH, H_2O; (d) $NaIO_4$, CrO_3, CH_3COOH



scheme 3

In order to quantify the natural lactate in plasma, the benzoate 10 was subjected to alkaline hydrolysis and the resulting ([3-^{2}H]-2(S)-lactate acid) was transformed into its di-*t*-butyldimethylsilyl derivative (TBDMS) for utilisation as internal standard. This derivative was suitable for gas

chromatographic/mass spectroscopic analysis (8). The mass spectrum (E.I.) of 10 -di-TBDMS derivative presents fragments at m/z 234, m/z 262[M^+- 57] and m/z 304[M^+- 15] while natural lactate fragments are at m/z 233, m/z 261 and m/z 303.

In conclusion, we have described the first synthesis of each enantiomer of 3-deutero lactic acid, with complete and regiospecific incorporation of one deuterium atom at C-3. The deuterated product was suitable for use as an internal standard in determination of lactic acid in plasma.

EXPERIMENTAL

General data :

Diepoxides 1 and 2 were synthesized as previously described (5). Lithium aluminium deuteride (98 atom %D) was purchased from Janssen Chemica. All signals were expressed as ppm down field from tetramethylsilane used as an internal standard (δ-value). ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM 250 spectrometer. Mass spectra (MS) were recorded under Electronic Impact (EI) or Chemical Ionization (CI) conditions on a Riber 10-10 and a AEI MS 50 instruments. Infrared spectra were obtained with a Perkin-Elmer 783 spectrometer. Optical rotations were obtained with the indicated solvent and concentration in a 1 dm cell by using a Perkin-Elmer 241 polarimeter. All reactions were carried out under inert atmosphere of argon and were monitored by thin layer chromatography with Merck 60F-254 precoated silica (0.2mm) on glass. Flash chromatography (9) was performed with Merck Kieselgel 60 (230-400 mesh ASTM) silica. Analytical gas chromatography was performed on a chiral glass capillary column (chrompack XE 60 (S) -Val -(S) -α -phenylethylamide 25 m x 0.25 mm).

[1,6 ^2H] -1,6-dideoxy -3,4-O-methylethylidene -D-mannitol 3a.

To a stirred suspension of lithium aluminium deuteride (100 mg, 2.4 mmol) in dry ether (4 mL) was added dropwise at room temperature the diepoxide 1a (372 mg, 2 mmol) in dry ether (6 mL). The mixture was refluxed for 1.5 hour, then H_2O (100 µL), 15% aqueous NaOH (100 µL) and H_2O (250 µL) were successively added to the cooled mixture. The precipitate was filtered ; the filtrate was dried ($MgSO_4$) and the solvent was removed under reduced pressure.

Flash chromatography of the residue (60 : 40 cyclohexane-ethylacetate , Rf 0.2) afforded 305 mg (80% yield) of 3a as a white crystalline solid m.p = 89°C; $[\alpha]_D$ - 8,7°(c 1.015, CH_2Cl_2), $[\alpha]_{365}$ - 43.6° ; IR (CH_2Cl_2) ν_{C-D} 2900 cm^{-1} ν_{OH} 3400 cm^{-1} ; ^1H NMR (250 MHz) δ 3.76 (m, 2H, H-2), 3.62(m, 2H, H-3), 1.35(s, 6H, CMe_2), 1.27(m, 4H, H-1); ^{13}C NMR; δ 108.72 ($\underline{C}Me_2$) ; 84.14 (C-2) ; 69.20 (C-3) ; 26.86 ($C\underline{Me}_2$) ; 20.19 (C-1,t, J_{C-D} = 18.95 Hz); MS[m/z,70ev] : 192 (M^+), 177 $(M-15)^+$.

1,6-dideoxy -3,4-O-methylethylidene -D-mannitol 3b

The workup of 3b was carried out under identical conditions as for 3a except $LiAlH_4$ was used instead of $LiAlD_4$. After flash chromatography 3b was obtained with 80% yield. m.p = 89°C ; $[\alpha]_D$ - 8.1°(c 1.015, CH_2Cl_2), $[\alpha]_{365}$ - 41.3°;^1H NMR (250 MHz) δ 3.74 (m, 2H, H-2), 3.51 (m, 2H, H-3), 1.34 (s, 6H, CMe_2), 1.28 (d, 6H, H-1, $J_{1,2}$ = 6Hz); ^{13}C NMR δ: 108.72 ($\underline{C}Me_2$), 84.17 (C-2), 69.24 (C-3), 26.85 ($C\underline{Me}_2$), 20.47 (C-1).

[1,6 ^2H] -dideoxy -3,4-O-methylethylidene -L-iditol 4

The workup of 4 was carried out under identical conditions as for 3a except L-Iditol diepoxide was used instead of D-mannitol diepoxide. After flash chromatography 4 was obtained with 80% yield. $[\alpha]_D$ + 27.4°(c 1.05, CH_2Cl_2), $[\alpha]_{365}$ + 70.8° ; IR (film) ν_{CD} 2190 cm^{-1} ν_{OH} 3400 cm^{-1};^1H NMR (250 MHz) δ 3.77(m, 2H, H-2), 3.67(m, 2H, H-3), 1.41(s, 6H, CMe_2), 1.20(m, 4H, H-1); ^{13}C NMR δ 109.40 ($\underline{C}Me_2$), 81.35 (C-2), 66.99 (C-3), 27.44 ($C\underline{Me}_2$), 19.88, (t,C-1, J_{C-D} = 18.90 Hz); MS (m/z 70 ev) 192 M^+ , 177 $(M-15)^+$.

[1,6 ^2H] -2,5- dibenzoyl -1,6-dideoxy -3,4-O-methylethylidene D-mannitol 5

To a stirred solution of 3a (279 mg, 1.45 mmol) in CH_2Cl_2 (3.2 mL) and pyridine (1.2 mL 14.5 mmol) was added dropwise benzoylchloride (425 µL, 3.65 mmol). After stiring 30 min at 0°C and 3 hours at room temperature, the reaction mixture was acidified with aqueous HCl (1N) until pH1 and was extracted with ether (5 x 10 mL). The combined ether layers were washed with brine, dried ($MgSO_4$), and the solvent was removed under reduced pressure. Flash chromatography of the residue (95:5 toluene - ethyl acetate, Rf 0.3) afforded 400 mg (80% yield) of 5. m.p = 63°C ; IR (CH_2Cl_2) ν_{C-D} 2190 cm^{-1} $\nu_{C=O}$ 1720 cm^{-1} ; $[\alpha]_D$ - 21.7°(c 1.15, CH_2Cl_2), $[\alpha]_{365}$ - 87°; ^1H NMR (250 MHz) δ 8.0,7.50,7.40(m, 10H, Ph), 5.31(ddd, 2H, H-2, J = 6.5,1.5 Hz), 4.19(m, 2H, H-3) 1.48-1.36(m, 10H, H-1, CMe_2); ^{13}C NMR δ 165.72 (Ph-$\underline{C}OO$), 132.98, 130.12, 129.66, 128.32 (Ph), 110.81 ($\underline{C}Me_2$), 80.59 (C-2), 71.32 (C-3), 27.69 ($C\underline{Me}_2$), 15.89, (t,C-1, J_{C-D} 19.47 Hz). Anal. $C_{23}H_{24}D_2O_6$ Calcd : C 68.99, (H+ $\frac{D}{2}$) 6.54; Found C 68.84, (H + $\frac{D}{2}$) 6.62.

[1,6 ^2H] -2,5- dibenzoyl-1,6 -dideoxy -3,4-O-methylethylidene -L-iditol 6

The workup of 6 was carried out under identical conditions as for 5

Flash chromatography of the crude product (95:5 toluene - ethylacetate, Rf 0.31) afforded 6 (70% yield). m.p = 57°C ; $[\alpha]_D$ + 39.5°(c 1.065, CH_2Cl_2), $[\alpha]_{365}$ +130.3°; ^1H NMR (250 MHz) δ 8.05, 7.55, 7.4(3m, 10H, Ph), 5.33(t, 2H, H-2, $J_{1,2}$ = 6.5 Hz), 4.05(s, 2H, H-3), 1.48(s, 6H, CMe_2) , 1.40(d, 4H,H-1,$J_{1,2}$ = 6,5 Hz); ^{13}C NMR δ 165.89 (Ph$\underline{C}OO$) ;133.04, 130.11, 129.68, 128.37 (Ph) ;109.81 ($\underline{C}Me_2$) ;78.90 (C-2) ;69.20 (C-3) ; 27.22 ($C\underline{Me}_2$) ; 16.68 (C-1,t, J_{C-D} = 19.77 Hz);

MS(70eV m/z %) 385 ([M-15]$^+$, 85) , 250 (30), 192 (15), 156 (15), 105 (100), 77 (30); Anal. $C_{23}H_{24}D_2O_6$. Calcd : C 68.99, (H + $\frac{D}{2}$) 6.54; Found C 69.16, (H + $\frac{D}{2}$) 6.62.

[1,6 ^{2}H] -2,5- dibenzoyl -1,6 -dideoxy -D-mannitol 7

The acetonide 5 (440 mg, 1.1 mmol) in 90% aqueous trifluoroacetic acid (10 mL) was stirred 1 hour at 0°C. The reaction mixture was then diluted with CH_2Cl_2 (20 mL) and water (60 mL), and extracted with ether (5 x 20 mL). The combined organic extracts were washed with $NaHCO_3$ aqueous solution (3%) until pH 7, dried ($MgSO_4$) and the solvent was removed under reduced pressure. Flash chromatography of the residue (50:50 cyclohexane - ethylacetate, Rf 0.37) afforded 260 mg (72% yield) of 7. ^{1}H NMR (250 MHz) δ 8.05, 7.55, 7.40(3m, 10H, Ph); 5.2(m, 2H, H-2); 3.65(d, 2H, H-3, $J_{2,3}$=7Hz); 1.45(d, 4H, H-1, $J_{1,2}$=6.5Hz).

[1,6 ^{2}H] -2,5-dibenzoyl -1,6 -dideoxy- L-iditol 8

The workup of 8 was carried out under identical conditions as for 7 to yield the product. $[\alpha]_D$ + 41.9° (c 0.1, CH_2Cl_2) ; $[\alpha]_{365}$ + 155.5° ; m.p = 99°C ; IR (CH_2Cl_2) : ν_{C-D} 2190 cm^{-1} $\nu_{C=O}$ 1700 cm^{-1} ν_{OH} 3500 cm^{-1}; ^{1}H NMR (250 MHz) δ 8.05, 7.55, 7.4(3m, 10H, Ph), 5.33(m, 2H, H-2), 3.73(d, 2H, H-3,$J_{2,3}$=5 Hz), 1.40(d br, 4H, H-1,$J_{1,2}$=6,5 Hz) ; ^{13}C NMR δ 166.4 (PhCOO); 138.1, 129.6, 128.4 (Ph); 73.5, 72.7 (C-2,3) ; 16.33 (C-1,t, J_{C-D}=19.86Hz).

[3-^{2}H]-2(R)-benzoyloxy-propanoic acid 9

To the mixture of chromium (VI) trioxide (146 mg, 1.46 mmol) and sodium periodate (316 mg, 1.46 mmol) in aqueous acetic acid (80%) was added dropwise at room temperature a solution of 7 (263 mg, 0.73 mmol) in aqueous acetic acid 80% (8 mL). After stirring during 4.5 hours the reaction mixture was poured into water-ether (15 mL - 15 mL) , acidified with aqueous HCl (1N) until pH1 and extracted with ether (5 x 25 mL). The combined ether layers were washed with brine, dried ($MgSO_4$), and the solvent was removed under reduced pressure.

Flash chromatography of the residue (80:19:1 methylene chloride - methanol - 33% aqueous ammonia, Rf 0.17) afforded 200 mg (70% yield) of 9 ; m.p = 80°C ; $[\alpha]_D$ - 23.0° (c 1.08, CH_2Cl_2), $[\alpha]_{365}$ - 133°; IR (CH_2Cl_2): ν_{C-D} 2300 cm^{-1} ν_{COOH} 2500-3300.; ^{1}H NMR (250 MHz) δ 8.5, 7.55, 7.4(3m, 5H, Ph), 5.34(t, 1H, H-2,$J_{1,2}$=7Hz), 1.65(d, 2H, H-1,$J_{1,2}$=7Hz); ^{13}C NMR : 176.9 (COOH), 165.9 (PhCOO), 133.4, 129.8, 129.1, 128.4 (Ph), 68.5 (C-2), 16.6 (C-1,t, J_{C-D}=19.99Hz); MS (70 ev m/z %) 195 [M$^+$, (55)], 151 (15), 105 (100), 77 (60), 51 (35).GC analysis on chiral column of the isopropylamide of 9 prepared according to (7a):Chrompax XE-60-(S)-valine-(S)-α-phenylethylamide 25m x 0.25 mm, 165°C, Helium 1.5 bar, t = 20.6 min; enantiomeric purity >98%. Anal $C_{19}H_9O_4D$ Calcd : C 61.53 , (H +$\frac{D}{2}$) 5.16. Found C : 60.97 (H + $\frac{D}{2}$) 5.31 .

[3-^{2}H]-2(S)-benzoyloxy-propanoic acid 10

The workup of 10 was carried out under identical conditions as for 9

Spectroscopic data of 10 were undistinguishable from that of its enantiomer 9 except its optical rotation. $[\alpha]_D$ + 22.3°(c 0.995, CH_2Cl_2), $[\alpha]_{365}$ + 127°. GC analysis on chiral column of the isopropylamide of 10 according to (7a) : (same conditions as for 9) t = 21.5 min.; enantiomeric purity >98%.

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