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A New Simple Synthesis of 1,3-Dideuterated Malondialdehyde (3-Hydroxy[1,3-$^2H_2$]-2-propenal)

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SUMMARY

A synthetic route for obtaining 1-butoxy-1,3,3-triethoxy[1,3-$^2H_2$]propane a stable diacetal of the malondialdehyde, is described. The synthesis involves the condensation of deuterated butyl vinyl ether with deuterated triethyl orthoformate in the presence of montmorillonite clay K-10. The deuteration of butyl vinyl ether was accomplished via the intermediary formation of a stannane derivative which was quantitatively metallated by *n*-butyllithium and then treated with ^2H_2O. From the diacetal the 1,3-dideuterated malondialdehyde was obtained by a short treatment with acid ion exchange resin.

Key words: malondialdehyde; dideuterated malondialdehyde; 3-hydroxy[1,3-$^2H_2$]-2-propenal; 1-butoxy-1,3,3-triethoxypropane.

INTRODUCTION

Malondialdehyde (MDA) is the more abundant secondary oxidation product formed in lipid peroxidation (1), a process which is involved also in the pathogenesis of many human diseases (2-4). For this reason the determination of MDA in human blood or urine has received considerable attention and several useful methods have been introduced for its quantitative determination by means of TLC, HPLC, GLC (1) and GLC/MS (5), with or without derivatization. However no method using isotope dilution mass spectrometry and deuterated MDA as internal standard, has until now been reported.

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In the course of our work in the chemistry (6,7) and the biological effects (8-11) of carbonyls produced in lipid peroxidation we needed a method for the detection of MDA by means of GLC/MS using multiple selected ion monitoring and thus we decided to synthesise MDA sodium salt **1** deuterated at position 1 and 3, two positions non exchangeable in protic solvents (12, 13).

A literature search gave (14, 15) only a method amenable for the preparative scale-up obtention of 1,3-dideuterated MDA. The method (15) affords the dideuterated 1,1,3,3-tetraethoxypropane (TEP), a stable precursor of MDA through the sequence of reactions that involves: a) a two step metallation and quenching with ^2H_2O of the bis-1,3-propylene dithioacetal of MDA, b) a one pot regeneration and acetalisation of the aldehyde groups by refluxing the thioacetal with $HgO/HgCl_2/BF_3.Et_2O$ in the presence of triethyl orthoformate in ethanol.

The authors of this synthesis refer to some experiments devoted to obtain the same monodeuterated MDA precursor by reaction of deuterated ethyl vinyl ether with triethyl orthoformate in the presence of $BF_3.Et_2O$. However they obtained the product in very low total yields (ca. 5%), and explained the poor result as a consequence of the high volatility of ethyl vinyl ether and of a low conversion in the final condensation with triethyl orthoformate.

In this paper we report a convenient method which overcame the difficulties experienced by these authors by introducing three simple but decisive modifications which allowed us to obtain the dideuterated butoxytriethoxypropane **2** in 46 % total yield, an acetal which, by hydrolysis with acid ion exchange resin allows us to obtain the dihydrate sodium salt of 1,3-dideuterated MDA **1**.

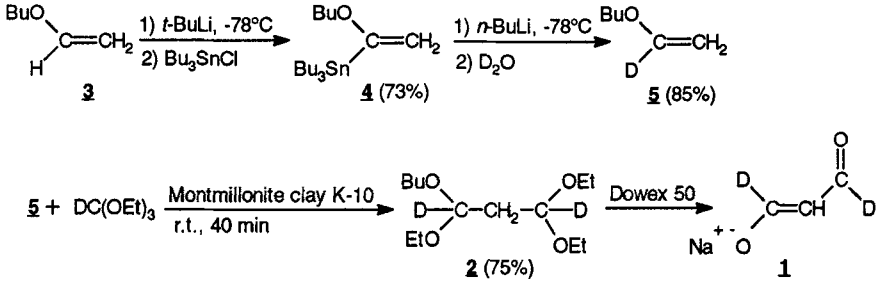
RESULTS AND DISCUSSION

The synthetic route for the preparation of deuterated MDA is reported in the scheme 1.

The synthesis was performed using commercially available butyl vinyl ether **3** in place of the highly volatile ethyl vinyl ether. Since its direct labelling by means of *tert*-butyllithium (*t*-BuLi) metallation (16) followed by quenching with ^2H_2O, showed always only a partial incorporation of deuterium (the best result, obtained using 2 molar equivalent of *t*-BuLi, showed a 75 % incorporation of deuterium), we decided to quench the metallated vinyl ether by tributyltin chloride. In this way the (1-butoxyvinyl)tributyltin **4** was obtained as an oil which could be easily purified by distillation. The stannane derivative was completely deuterated in highly repetitive yields by treatment with *n*-butyllithium (*n*-BuLi) in THF at -78 °C and quenching with ^2H_2O to give the deuterated compound **5**. The ^1H NMR spectrum of **5** differs from that of unlabelled butyl vinyl ether

for the lack of the signal at 6.43 ppm and for the modification of double doublets at 4.14 and 3.93 ppm in a triple doublets in which the H,H coupling constants (14.5 and 6.5 Hz respectively) are substituted by those of H,D (2.2 and ca. 1 Hz).

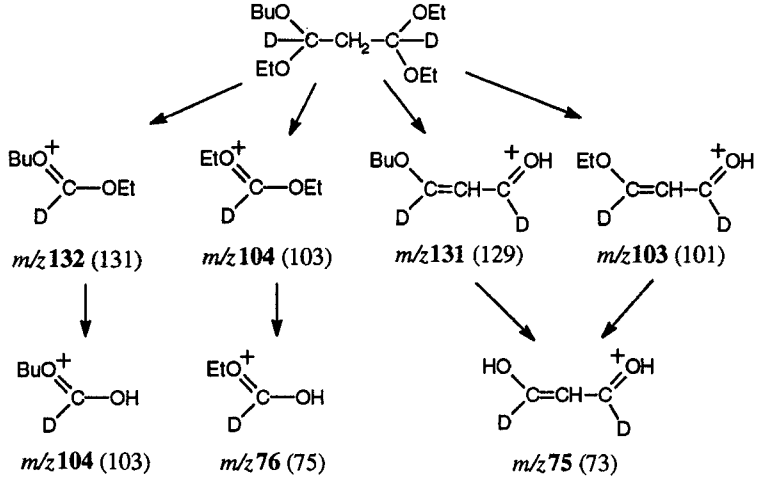
SCHEME 1



The successive condensation of deuterated butyl vinyl ether **5** with deuterated triethyl orthoformate (17) was performed in good yield using as effective catalyst the montmorillonite clay K-10 which was used because of its efficiency in the condensation of acetals with vinyl ethers (18).

The deuterated diacetal **2** was obtained in pure form and in 75 % yield by distillation. Its ^1H NMR spectrum differs from that of the corresponding unlabelled compound, obtained by using non-deuterated reagents, for the lack of two triplets (J = 6.0 Hz) at 4.52 and 4.51 ppm indicative of the

SCHEME 2



The valued in parentheses refer to the fragmentation of the non deuterated compound

protons at position 1 and 3 and for the simplification of the double doublet at 1.83 ppm (6.0 and 6.0 Hz) in an apparent singlet at 1.83 ppm.

The comparative study of mass spectra of **2** and of non deuterated analogue allows a complete assignment of the fragmentation peaks observed (Scheme 2).

The hydrolysis of **2** in dilute solution parallels that of TEP (19) and allows us to obtain the sodium salt of 1,3-dideuterated MDA **1** with the same yield.

In conclusion the method here reported represents an efficient alternative route for obtaining 1,3-dideuterated malondialdehyde **1**.

EXPERIMENTAL

General.- ^{1}H- and ^{13}C- NMR spectra were recorded on a Bruker AM-500 instrument and are reported in δ units relative to $CHCl_3$ fixed at 7.24 ppm or to H_2O fixed at 4.54 ppm for the ^{1}H spectra and relative to $CDCl_3$ fixed at 77.00 ppm or to [$^{2}H_6$]dimethylsulfoxide (DMSO-d_6) fixed at 39.50 ppm for the ^{13}C spectra. Mass spectra were determined on a Varian 112 S mass spectrometer by direct inlet. Usual work-up refers to washing the organic layer with water, drying it over Na_2SO_4, and evaporating the solvent under reduced pressure.

(1-Butoxyvinyl)tributyltin **4**.- *tert*-Butyllithium (200 ml of a 1.7 M solution in pentane; 0.34 mol) was added dropwise, under argon, to a solution of butoxyethene **3** (88 ml, 0.68 mol) in dry THF (400 ml) cooled to -78 °C. The temperature was carefully maintained under -65 °C during the addition which causes the formation of a yellow precipitate. Then the cooling bath was removed and the solution was stirred at -10 °C for 10 min (the yellow precipitate disappeared at ca. -30 °C). At this time the solution was recooled to -78 °C and tributyltin chloride (92 ml, 0.34 mol) dissolved in dry THF (200 ml) was slowly added maintaining the temperature under -60 °C. The resulting colourless solution was stirred at -78 °C for 1 h and at room temperature for 30 min. The organic solvent was then evaporated under reduced pressure and the oil residue was dissolved in ethyl acetate (300 ml). Usual work-up afforded an oil which was distilled at 100-110 °C (0.01 mmHg) to afford (1-butoxyvinyl)tributyltin **4** (96 g, 73 % yield referred to *t*-BuLi), a colourless liquid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.67 (1H, d, J = 2.1 Hz, =CH-$\underline{H}_a$), 4.03 (1H, d, J = 2.1 Hz, =CH-$\underline{H}_b$), 3.63 ppm (2H, t, J = 6.5 Hz, OC$\underline{H}_2$-); MS (only fragments related to more abundant tin isotope are reported) *m/z* (relative intensity) 333 (27), 235 (41), 277 (30), 179 (100), 121 (59).

1-Butoxy-[1-^{2}H]ethene **5**.- *n*-Butyllithium (41.9 ml of a 1.6 M solution in hexane; 67.0 mmol) was slowly added to a solution of the stannane derivative **4** (20 g, 61 mmol) in dry THF (50 ml) at -78 °C under argon. The resulting mixture was stirred at -78 °C for 2 h and then was quenched with a solution of ^2H_2O (1.3 ml, 72.0 mmol; 99.96 % of ^2H) in dry THF (5 ml) at -78 °C. The mixture was then stirred at -78 °C for 2 h and at room temperature for 30 min.

The mixture was fractionated by an efficient distillation apparatus and the fraction boiling at 90-110 °C was collected to give the liquid compound **5** (5.2 g, 85 % yield) in nearly pure form. A sample, purified by successive distillation, showed: ^1H NMR (500 MHz, $CDCl_3$) δ 4.14 (1H, dt, J_{HH} = 2.1 and J_{HD} = 2.2 Hz, =CH-*H*$_a$), 3.93 (1H, dt, J_{HH} = 2.1 and J_{HD} ≈ 1 Hz, =CH-*H*$_b$), 3.65 (2H, t, J = 7.5 Hz, OC*H*$_2$-), 1.61 (2H, tt, J = 7.5 and 7.5 Hz, OCH_2-C*H*$_2$-), 1.39 (2H, tq, J = 7.5 and 7.5 Hz, -C*H*$_2$-CH_3), 3.65 ppm (3H, t, J = 7.5 Hz, -C*H*$_3$); MS *m/z* (relative intensity) 74 (61), 59 (100). The compound was 99,7 % isotopically pure (MS and ^1H NMR).

1-Butoxy-1,3,3-triethoxy-[1,3-$^2H_2$]propane **2**.- To a solution of **5** (5.0 g, 49.5 mmol) and triethoxy[$^2H_1$]methane (17) (7.4 g, 49.5 mmol; 98.7 % of ^2H) in diethylether (30 ml) was added montmorillonite clay K-10 (80 mg). The resulting mixture was stirred at room temperature for 2.5 h, then it was filtered on a pad of Celite, washing three times with diethylether (5 ml each). The solvent was removed under reduced pressure and the residue was fractionated with an efficient distillation apparatus. The fraction boiling at 135-145 °C and 15 mmHg was collected to give compound **2** (9.3 g, 75% yield) as a colourless liquid: ^1H NMR (500 MHz, $CDCl_3$) δ 3.58-3.30 (16H, overlapping, 8 x O-C*H*$_2$-), 1.83 (2H, s, D-C-C*H*$_2$-C-D), 1.45 (2H, tt, J = 7.5 and J = 7.5 Hz, OCH_2-C*H*$_2$-), 1.28 (2H, tq, J = 7.5 and J = 7.5 Hz, -CH_2-C*H*$_2$-CH_3), 1.09 (9H, t, J = 7.5 Hz, 3 x OCH_2-C*H*$_3$), 0.81 ppm (3H, t, J = 7.5 Hz, $OCH_2CH_2CH_2$C*H*$_3$); ^{13}C NMR (125.7 MHz, $CDCl_3$, proton off resonance decoupling) δ 99.61 (t, J ^{13}C-^2H = 24.0 Hz, D-*C*-CH_2-), 99.56 (t, J ^{13}C-^2H = 24.0 Hz, -CH_2-*C*-D), 65.10 ($OCH_2CH_2CH_2CH_3$), 60.74 (OCH_2-CH_3), 37.59 (D-C-*C*H$_2$-C-D), 31.75 ($OCH_2CH_2CH_2CH_3$), 19.13 ($OCH_2CH_2CH_2CH_3$), 14.98 (OCH_2-*C*H$_3$), 13.49 ppm ($OCH_2CH_2CH_2CH_3$); MS *m/z* (relative intensity) 132 (36), 131(10), 104 (100), 103 (24), 76 (68), 75 (32), 57 (13). The compound was 97.8 % isotopically pure (^1H NMR and MS).

3-Hydroxy[1,3-$^2H_2$]-2-propenal (sodium salt of malondialdehyde) **1**.- The procedure reported by L. J. Marnett (19) for the hydrolysis of TEP was used.

A suspension of diacetal **2** (1.0 g, 4.0 mmol) and Dowex 50 ion exchange resin (5.0 g) in H_2O

(10 ml) was shaken at room temperature for 1 h. Then the resin was filtered and the solution carefully titrated to pH 7 with 5 M, 1 M and 0.1 M aqueous solution of sodium hydroxide in the sequence. The neutral solution was extracted three times with dichloromethane and lyophilised. The solid residue was redissolved in H_2O (1 ml) and purified as reported (19) to afford the dihydrate sodium salt **1** (0.2 g, 38 % yield): ^1H NMR (500 MHz, D_2O) δ 5.12 ppm (singlet), this unique signal, after a period of several minutes began to become weaker; ^{13}C NMR (125.7 MHz, DMSO-d_6, proton off resonance decoupling) δ 189.08 (t, $J\ ^{13}C\text{-}^2H$ = 27.7 Hz, C-1 and C-3), 108.36 ppm (C-2). The compound was 97.8 % isotopically pure (^1H NMR and MS).

Elemental analysis, calculated for $C_3D_2HO_2Na\ .\ 2H_2O$: C, 27.28; H + D, 6.85: Found: C, 27.75; H, 6.68.

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