SYNTHESIS OF MALONDIALDEHYDE-1-^{2}H AND MALONDIALDEHYDE-1,3-$^2H_2$

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SUMMARY

Two synthetic routes for deuterium labelling of 1,1,3,3-tetraethoxypropane, the precursor of the ubiquitous natural compound malondialdehyde, are described. In one scheme, deuterium is incorporated in ethyl vinyl ether by metalation with t-butyllithium followed by quenching with ^2H_2O. The conversion of the deuterated ether to monodeuterated tetraethoxypropane, however, proceeds with an overall yield of only ~5%. As an alternative route, we have metalated the bis-1,3-propylene dithioacetal of malondialdehyde which undergoes excellent deuterium incorporation (92%). In addition, the latter method allows isotopic labelling of either one or two deuterium(s)/molecule. Hydrolysis of the deuterated dithioacetal to the corresponding tetraethoxypropane can be best effected by refluxing with H_gO(red)/$HgCl_2$/BF_3·etherate in the presence of $CH(OC_2H_5)_3/C_2H_5OH$. The overall yield of the method is 48%.

Key Words: Malondialdehyde-1,3-$^2H_2$;1,1,3,3-Tetraethoxypropane 1,3-$^2H_2$

INTRODUCTION

Nonspecific lipid peroxidation and prostaglandin biosynthesis generate significant amounts of malondialdehyde (MDA) in mammalian cells (1, 2). MDA is toxic (3) and mutagenic (4,5) and has been implicated in the aging process (6). Reports of these deleterious effects have prompted considerable interest in its chemical reactions with proteins and nucleic acids. Such studies require or are facilitated by the availability of isotopically labeled MDA. Carbon-14 or tritium-labeled material is essential for tracer studies, whereas deuterium-labeled MDA is useful for assignment of resonances in the NMR spectra of complex amino acid and nucleotide adducts. Although incorporation of deuterium at the α-position can be easily done by dissolving the compound in ^2H_2O, deuterium at this position is not stable and exchanges in protic solvents (7). The label should, therefore, be on

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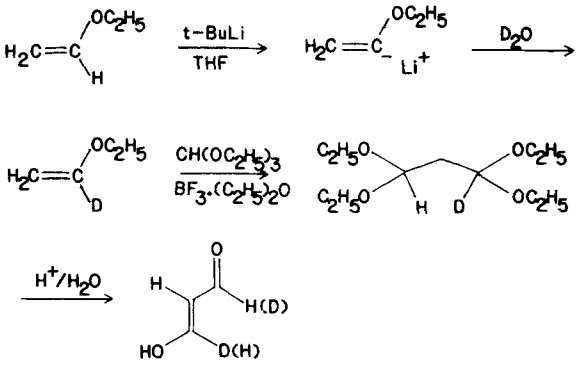
the aldehydic carbon(s). A synthesis of MDA-1,3-$^2H_2$ by reductive ozonolysis of methyl octadeuteroarachidonate was reported (8). This method, however, is not amenable for preparative scale-up and variable yields and the generation of a wide variety of side products severely limits its synthetic utility. We have designed two inexpensive and relatively easy synthetic routes for the preparation of MDA labeled with deuterium at position 1 and/or 3. Depending on the requirement, either MDA-1-^2H or MDA-1,3-$^2H_2$ can be generated. Furthermore, as a general strategy, these methods can be used to prepare tritium-labeled MDA by replacing the deuterium oxide with tritiated water.

Results and Discussion

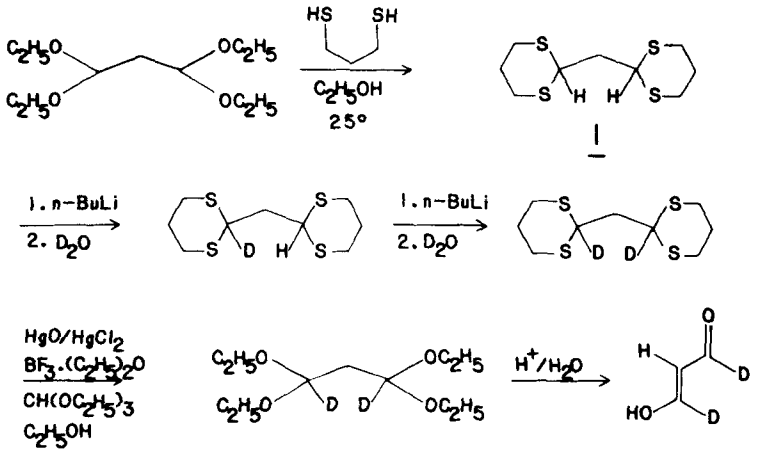
One synthetic route for the preparation is shown in Scheme I



Scheme I

Ethyl vinyl ether is efficiently metalated at the α-vinyl carbon by t-butyllithium in THF at low temperature. Quenching the mono-lithio anion with ^2H_2O generates the deuterated ether that is converted to mono-deuterated tetraethoxypropane by reaction with triethyl orthofomate in the presence of a catalytic amount of BF_3·etherate. The high volatility of ethyl vinyl ether and low conversion to the acetal limits the overall yield to ≈ 5%.

We therefore, devised an alternative procedure that makes use of the interconvertibility of tetraalkoxypropane with its bis-1,3-dithiane derivative 1



Scheme II

Preparation of 1 has been reported from 1,3-dithiane (9) but our procedure from tetraethoxypropane provides a much better yield (96 % vs. 62%). Metalation and deuterium incorporation also proceed with excellent conversion (92%). Integration of the methine resonances in the NMR spectra of 1 and its deuterated analog indicates incorporation of 1.2 deuteriums when 20% excess base is used. Increasing the proportion of n-butyllithium does not increase the percentage of isotope incorporated suggesting sluggishness of formation of the dianion. However, if recrystallized, deuterated 1 is further subjected to 20% excess base, the total incorporation is increased to nearly 2 deuteriums/molecule.

Direct hydrolysis of 1 to MDA proceeds in very low yield. It is, therefore, first converted to tetraethoxypropane be refluxing the H_gO(red)/ $HgCl_2/BF_3$·etherate in the presence of triethyl orthoformate and excess ethyl alcohol (10). The yield of the deuterated acetal by this method is 54% with an overall conversion of tetraethoxypropane to tetraethoxypropane-1,3-$^2H_2$ of 48%.

Hydrolysis of tetraethoxypropane to MDA is nearly quantitative in dilute solutions. Therefore, the yield of tetraethoxypropane usually determines the overall yield of the procedure.

Experimental

Bis(1,3-dithian-2-yl)-methane (1)

A solution of 2.2 g (10 mmol) tetraethoxypropane in 70 ml chloroform was stirred with 2.0 ml(20 mmol) 1,3-propane-dithiol for an hour at ambient temperature. Following addition of 2 ml BF_3·etherate stirring was continued for 15 hours. The solution was extracted twice with water, once with 7% KOH and then twice with water again. After drying over anhydrous $MgSO_4$, the excess solvent was evaporated to leave a white, crystalline compound. Methanol (25 ml) was added to the precipitate and the temperature was brought to boiling. Chloroform was added dropwise until complete solution had taken place and 0.25 g K_2CO_3 and 0.25 g Norit were carefully introduced into the boiling solution. Filtering and cooling gave 2.42 g (96%) 1. NMR ($CDCl_3$), δ 1.86(m)2H; 2.08(m)2H; 2.21 (t, 7.5 Hz)2H; 2.83(m)8H; 4.25(t, 7.5 Hz)2H.

Metalation and Deuterium Incorporation of 1

A solution of twice recrystallized 1 (1 g, 4 mmol) was dissolved in 15 ml dry tetrahydrofuran and stirred under argon at -30°. Butyllithium (3.1 ml of 1.6 M, 5 mmol) was slowly added to it and the mixture was stirred at -25° for 4 hours. An excess of ^2H_2O (1 ml, >99.9% ^2H) was added and the temperature was allowed to rise to 0°. After 30 min. stirring at this temperature, the solution was stirred for another 30 min at room temperature. An excess of water was added and the mixture was shaken with 1:1 methylene chloride:pentane. The organic phase was washed with water, dried (anhydrous K_2CO_3), and the solvent was evaporated. The deuterated compound was recrystallized from methanol-chloroform to yield 0.92 g (92%) of deuterated bis-dithiane. NMR ($CDCl_3$) δ 1.73(m)2H; 1.95(m)2H; 2.03(d, 7.5 Hz)2H; 2.7(m)8H; 4.10(t, 7.5 Hz)1H.

1,1,3,3-Tetraethoxypropane-1,3-^2H

In a 3-neck flask equipped with a reflux condenser deuterated 1 (0.25 g, 1 mmol) was dissolved in 4 ml dry tetrahydrofuran and triethyl orthoformate (0.8 g,4 mmol), BF_3·etherate (0.3 g, 2 mmol), HgO (0.8 g, 4 mmol) and absolute ethanol (40

ml) were sequentially added to it. Under a slow stream of argon with vigorous stirring, a solution of $HgCl_2$ (1.09 g, 4 mmol) in 10 ml dry tetrahydrofuran was added dropwise. The resultant mixture was refluxed for 10 hours under argon; it was then cooled, filtered, and poured into 150 ml absolute ethanol. Sodium hydroxide (1 N) was added dropwise until a yellow precipitate was formed. After filtration, the supernatant was extracted (three times) with 25 ml portions of ether. The ether layer was washed with water, dried (anhydrous K_2CO_3) and the ether was evaporated. A colorless oil remained which was distilled by short path distillation to separate unreacted triethyl orthoformate. The deuterated acetal (0.12 g, 54%) was obtained with an overall conversion of tetraethoxypropane to deuterated tetraethoxypropane of 48%. NMR (CDCl) δ 1.21 (t, 7.0 Hz)12H 1.95(d, 5.2 Hz)2H; 3.55(m)4H; 3.65(m)4H; 4.62(t, 5.2 Hz)1H.

Sodium Malondialdehydate-1-3-^2H

A portion of the 1,1,3,3,-tetraethoxypropane-1,3-^2H was converted to the corresponding sodium malondialdehydate by acid hydrolysis followed by basicification. NMR(D_2O) δ 5.31(d, 10.2 Hz)1H; 8.66(d, 10.2 Hz)1H.

References

(1) Bernheim, F., Bernheim, M.L.C. and Wilbur, K.M.-J. Biol. Chem., 174: 257(1948)

(2) Hamburg, M. and Samuelsson, B.- J. Biol. Chem., 242: 5344(1967)

(3) Crawford, D.L., Sinnhuber, R.O., Stout, F.M., Oldfield, J.E., and Kaufnes,J.-Toxic Appl. Pharmacol., 7: 826(1965)

(4) Mukai, F.H. and Goldstein, B.D.-Science, 191: 868(1976)

(5) Basu, A.K. and Marnett, L.J.-Carcinogenesis, 4: 331(1983)

(6) Mamkares, K.D.-Mech. Ageing Dev., 5: 171(1976)

(7) Marnett, L.J., Bienkowski, M.J., Raban, M. and Tuttle, M.A.- Anal. Biochem., 99: 458(1979)

(8) Hamberg, M., Niehaus, W.G. and Samuelsson, B.- Anal. Biochem., 22: 145(1968)

(9) Seebach, D., Jones, N.R. and Corey, E.J.-J. Org. Chem., 33: 300(1968)

(10) Vedejs, E. and Fuchs, P.L.-J. Org. Chem.-36: 366(1971)