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# PREPARATION OF DI-(2-ETHYLHEXYL) ADIPATE AND SOME OF ITS METABOLITES LABELLED WITH DEUTERIUM

Richard B Moore

ICI plc, Central Toxicology Laboratory, Alderley Park, Macclesfield, SK10 4TJ, UK

## SUMMARY

The syntheses of deutero-labelled di-(2-ethylhexyl)adipate (DEHA), mono-(2-ethylhexyl) adipate (MEHA), 2-ethylhexan-1-ol, 2-ethylhexanoic acid, 2-ethyl-5-ketohexanoic acid, 3-ethyl-6-methyltetrahydro-2*H*-pyran-2-one and 2-ethylhexanedioic acid are described. All compounds were perdeutero-labelled in the ethyl group. The title compound, DEHA, was used in a human dosing study.

Key words: Deuterium Labelling, Di-(2-ethylhexyl) adipate, DEHA, metabolite, human dosing.

## INTRODUCTION

Plasticisers such as di-(2-ethylhexyl)adipate (DEHA) form an integral part of plastic films of the "cling film" type. These films are frequently used for wrapping food and plasticisers may to a certain extent, migrate into the food, particularly into fatty material. The maximum dietary daily intake of DEHA per capita has been estimated to be 8.2 mg[1].

Although DEHA has a very low acute toxicity in rodents, some concern has been expressed over an increased incidence of liver cancer in mice, but not rats, exposed to high concentrations of DEHA for a lifetime[2].

When considering the risk from DEHA for humans, the UK Ministry of Agriculture Fisheries and Food recently came to the conclusion that there is a 3000-fold or more margin between the estimated maximum human intake and the dose which caused cancer in mice. Because of this difference, the Ministry considered the possibility of risk to public health to be remote.[3]

Little is known about the fate of DEHA in the human body, hence the need for a study to provide definitive data on the disposition of DEHA in man. This will help to put the animal

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data into perspective and to derive a better understanding of the intake of DEHA by the general public. The results of this study have been reported elsewhere.[4]

To distinguish between DEHA administered for study purposes and DEHA derived from normal dietary intake, the compound was perdeutero-labelled in the ethyl group, as were a number of postulated metabolites. The ethyl group was chosen for two reasons. Firstly, this position is chemically stable and in rodents has been shown to be least prone to metabolism. Secondly, when identifying labelled materials by mass spectrometry, there is a large difference (10 mass units for the DEHA and 5 mass units for the metabolites) between dose and DEHA derived from diet.

Apart from DEHA (4) itself, the following compounds were synthesised with deuterium as potential human metabolites of DEHA from analogy with previous studies[5,6,7] in rodents: 2-[$^2H_5$-ethyl]hexanoic acid (2), 2-[$^2H_5$-ethyl]hexan-1-ol (3), mono-(2-[$^2H_5$-ethyl]hexyl) adipate (5), 2-[$^2H_5$-ethyl]-5-ketohexanoic acid (8), 3-[$^2H_5$-ethyl]-6-methyltetrahydro-2*H*-pyran-2-one (9) and 2-[$^2H_5$-ethyl]-hexanedioic acid (12).

The route to the labelled compounds is shown in Scheme 1.

## EXPERIMENTAL

Bromoethane-D5 was obtained from MSD Isotopes (99.2 atom % D). Other chemicals were the best commercially available. Solvents were redistilled before use and if necessary dried by literature methods.

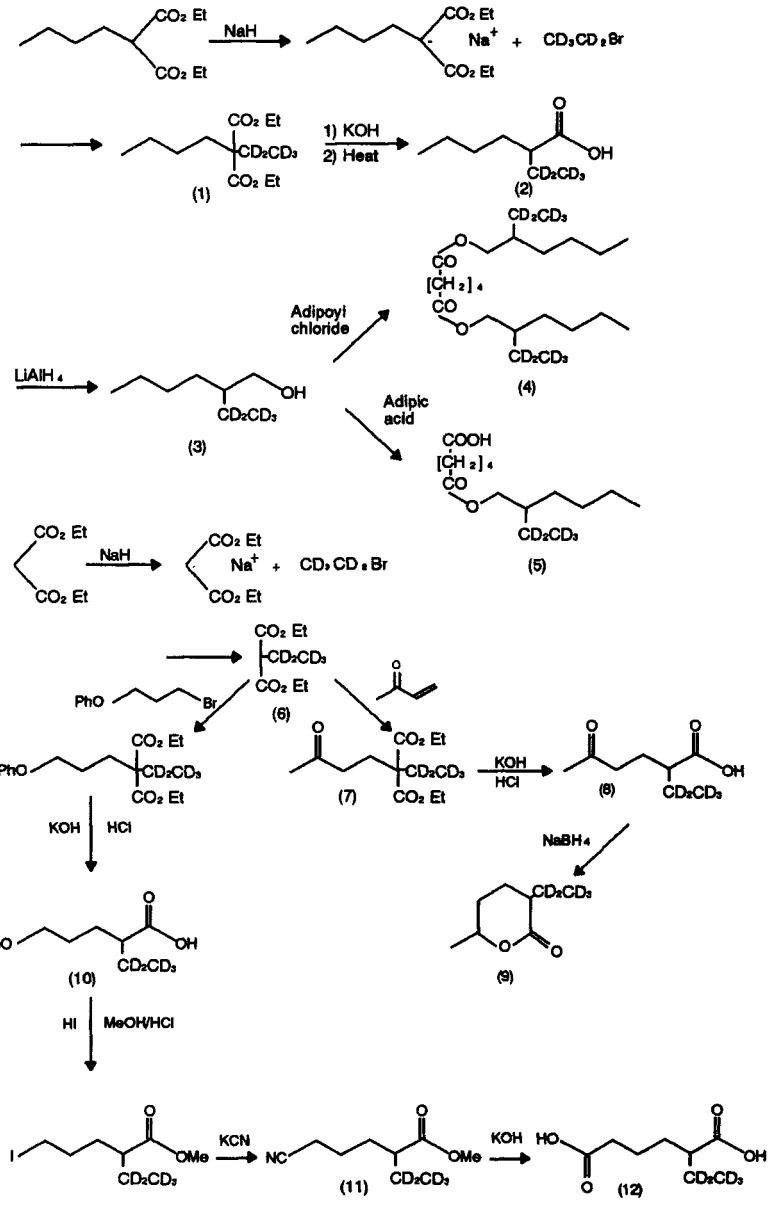
Infra-red spectra were recorded on a Perkin-Elmer 299B Spectrophotometer as a liquid film with NaCl plates. Mass spectra were recorded on a VG 70-70E mass spectrometer. $^1H$ and $^{13}C$ n.m.r were recorded on Brüker AM200 or AC250 spectrometers in $CDCl_3$ with $Me_4Si$ as an internal reference.

Gas liquid Chromatography was performed on a Hewlett Packard 5890 fitted with a methyl silicone 0.53 mm. x 10 m. capillary column. Thin layer chromatography was performed on Merck silica gel 60 plates. Preparative 'flash' column chromatography[8] was carried out using Merck silica gel 60 (230 to 400 mesh).

### Diethyl butyl-[$^2H_5$-ethyl]malonate (1)

A suspension of sodium hydride (5.06g of a 50% suspension in mineral oil, 0.106 mole) in dry THF (150ml) was stirred and raised to boiling. Diethyl butylmalonate (22.79g, 0.106 mole) was added dropwise and the mixture was boiled for a further 15 min. to allow complete hydrogen evolution. The mixture was then allowed to cool to 35°C and bromoethane-D5 (10g, 0.088 mole) was added dropwise. After stirring for 30 min. the temperature was raised to boiling point for a further 45 min. and allowed to cool. The reaction mixture was poured into water, partitioned with diethyl ether and the organic extracts were washed with aqueous NaCl solution, dried ($MgSO_4$) and evaporated to give impure 1. Diethyl butylmalonate was

removed from this material by boiling with vigorous stirring in a solution of KOH (11.2g, 0.2 mole) in water (50ml) for 2.5 hr. After cooling, the reaction mixture was extracted with diethyl ether and these extracts after drying and evaporation gave an oil which was subjected to short path distillation to give pure **1** (16.11g, 61%).




SCHEME 1

**2-[$^2H_5$-Ethyl]hexanoic acid (2)**

Diethyl butyl-[$^2H_5$-ethyl]malonate **1** (11.67g, 0.047 mole) was dissolved in methanol (120ml) and a solution of NaOH (40g, 1.0 mole) in water (40ml) was added. The mixture was vigorously stirred and heated to reflux for 3 hr. The homogeneous solution was evaporated to half volume, 6M HCl (200ml) was added carefully, and extraction with diethyl ether gave a white crystalline solid. This solid was heated at 165°C for 1 hr. when carbon dioxide evolution had ceased and the resulting oil was purified by short path distillation to give **2** (6.91g, 98%).

**2-[$^2H_5$-Ethyl]hexan-1-ol (3)**

2-[$^2H_5$-Ethyl]hexanoic acid **2** (7.84g, 0.053 mole) was dissolved in dry THF (25ml) and added dropwise to a suspension of lithium aluminium hydride (4.0g, 0.105 mole) in dry THF (50ml) over a period of 30 min. The mixture was boiled for 1 hr. and allowed to cool. Water (4ml) was added followed by 15% aqueous NaOH solution (4ml) followed by more water (12ml). The resulting granular slurry was filtered and washed repeatedly with diethyl ether. Similarly, the filtrate was partitioned with diethyl ether and the combined organic extracts were washed with aqueous NaCl solution, dried ($MgSO_4$) and evaporated to give an oil, that gave pure **3** after short path distillation (5.0g, 70%).

**Di-(2-[$^2H_5$-ethyl]hexyl) adipate (4)**

Adipoyl chloride (3.39g, 0.019 mole) was added dropwise over a period of 20 min. to a solution of 2-[$^2H_5$-ethyl]hexan-1-ol **3** (5.0g, 0.037 mole) in hexane (80ml) and dry pyridine (10ml). The mixture was left stirring overnight and then poured into aqueous sodium bicarbonate solution (100ml). After equilibration, the aqueous layer was further extracted with diethyl ether and the combined organic extracts were washed with 2M HCl and aqueous NaCl solution, dried ($MgSO_4$) and evaporated to give an oil. Chromatography on silica gel eluting with hexane/ethyl acetate (9:1) gave a pure colourless oil which after short path distillation gave **4** (5.1g, 36%) which was pure by gc and tlc.
$^1$H-NMR δ 0.87 (t, 6H), 1.25 (s, 12H), 1.52 (t, 2H), 1.65 (m, 4H), 2.31 (6t, 4H), 3.96 (d, 4H).
$^{13}$C-NMR δ 9.8 (m), 13.9, 22.7 (m) 22.9, 24.5, 28.9, 30.7, 34.0, 35.5, 66.8, 173.4.
EI-MS (m/z) 381 (M+1, 8), 246 (17), 129 (100), 117 (38), 75 (39).

**Mono-(2-[$^2H_5$-ethyl]hexyl) adipate (5)**

Adipic acid (1.38g, 0.009 mole) was heated to its melting point and 2-[$^2H_5$-ethyl]hexan-1-ol (1.28g, 0.009 mole) was added dropwise over 30 min. The mixture was then heated for a

further 4 hr. After cooling, the reaction mixture was taken up in saturated aqueous sodium bicarbonate solution (20ml) and extracted with diethyl ether. The aqueous residue was acidified with concentrated HCl and re-extracted with diethyl ether. These extracts were dried ($MgSO_4$), and evaporated to give an oil which was purified by chromatography on silica gel, eluting with chloroform/methanol (19:1). After chromatography, the material was further purified by short path distillation to give **5** (1.43g, 57%).
$^1$H-NMR δ 0.88 (t, 3H), 1.28 (s, 6H), 1.57 (m, 1H), 1.69 (m, 4H), 2.38 (m, 4H), 3.99 (d, 2H).
CI-MS (m/z) 264 (M+1, 18), 246 (19), 147 (17), 129 (100).

**Diethyl [$^2H_5$-ethyl]malonate (6)**

Diethyl malonate (15.44g, 0.096 mole) was added dropwise to a stirred suspension of sodium hydride (4.63g of a 50% suspension in mineral oil, 0.096 mole) in boiling dry THF (150ml). The mixture was boiled for a further 30 min. and then cooled to 30°C. [$^2H_5$]Bromoethane (10g, 0.0877 mole) was added dropwise and on completion of addition, the solution was raised to boiling for 2hr. After cooling the mixture was poured into water (200ml) and partitioned with diethyl ether. The combined organic extracts were washed with aqueous NaCl solution, dried ($MgSO_4$), and evaporated to an oil which was distilled at reduced pressure (69°C @ 1 torr) to give **6** (12.15g, 72%).

**Diethyl 3-ketobutyl-[$^2H_5$-ethyl]malonate (7)**

Diethyl [$^2H_5$-ethyl]malonate **6** (6.48g, 0.034 mole) was added dropwise to a suspension of sodium hydride (0.33g of a 50% suspension in mineral oil, 0.007 mole) in dry THF (50ml), and the stirred mixture was brought to boiling. Methyl vinyl ketone (3.52g, 0.05 mole) was added dropwise to the boiling solution which was then allowed to cool and stir overnight. The mixture was poured into water (100ml) and partitioned with diethyl ether. The combined organic extracts were washed with aqueous NaCl solution, dried ($MgSO_4$), and evaporated to an oil which was distilled at reduced pressure (130°C @ 1.5 torr) to give **7** (4.35g, 50%).

**2-[$^2H_5$-Ethyl]-5-ketohexanoic acid (8)**

Diethyl 3-ketobutyl-[$^2H_5$-ethyl]malonate **7** (9.47g, 0.036 mole) was boiled with a solution of KOH (9.0g, 0.161 mole) in water (10ml) for 4 hr. The solution was allowed to cool, poured into water (50ml) and acidified to pH 1. After extraction with diethyl ether, the evaporated extract was boiled overnight in a solution of concentrated sulphuric acid (4ml) in water (20ml). After cooling, the reaction mixture was extracted with diethyl ether and the combined extracts were dried ($MgSO_4$) and evaporated to give an oil, which was subjected to short path distillation to give pure **8** (3.17g, 54%).
$^1$H-NMR δ 1.83 (dt, 2H, J=7, J=7 Hz), 2.15 (s, 3H), 2.30 (t, 1H), 2.47 (t, 2H).
EI-MS (m/z) 164 (M+1, 3), 146 (25), 117 (26), 75 (31), 43 (100).

**3-[$^2H_5$-Ethyl]-6-methyltetrahydro-2*H*-pyran-2-one (9)**

2-[$^2H_5$-ethyl]-5-ketohexanoic acid **8** (2.60g, 0.016 mole) was dissolved in ethanol (50ml.) and sodium borohydride (0.30g, 0.008 mole) was added to the ice-cold stirred solution. After 1 hr. a further, similar sample of sodium borohydride was added and the mixture was allowed to stir and warm to room temperature overnight. A final sample of sodium borohydride of the same weight was added and after 1 hr., the reaction mixture was evaporated. The residue was taken up in ether saturated with HCl (30ml) and stirred at room temperature for 2 hr. The solvent was evaporated and the residue was suspended in water (50ml) and extracted with diethyl ether. Evaporation of the organic extracts gave an oil which was chromatographed on a column of silica gel eluting with hexane/ethyl acetate (4:1) to give pure **9** as a mixture of diastereoisomers (0.54g, 23%).
EI-MS (m/z) 148 (M+1, 3), 115 (100), 75 (70), 61 (87), 57 (35), 41 (42).
Compound **9** can be converted into the sodium salt of the hydroxy acid by reaction with sodium hydroxide solution in the normal manner. Problems associated with analysing what is essentially an equilibrium mixture between **9** and its free acid in biological samples will be described elsewhere[9].

**2-[$^2H_5$-Ethyl]-5-phenoxypentanoic acid (10)**

Diethyl [$^2H_5$-ethyl]malonate **6** (6.15g, 0.032 mole) was added dropwise to a stirred solution of sodium hydride (1.62g of a 50% dispersion in mineral oil, 0.034 mole) in dry THF. The mixture was stirred at room temperature for 30 min. and phenoxypropyl bromide (7.23g, 0.034 mole) was added slowly. The mixture was then boiled for 2 hr. and allowed to stand at room temperature overnight. The reaction mixture was poured into water (150ml) and equilibrated with ethyl acetate. The organic extracts were dried ($MgSO_4$), evaporated and distilled at reduced pressure (170°C @ 1.4 torr) to give diethyl(3-phenoxypropyl)-[$^2H_5$-ethyl]malonate (5.26g). This material dissolved in methanol (60ml) and was boiled for 2 hr. with a solution of NaOH (20g, 0.5 mole) in water (20ml). Evaporation of the reaction mixture gave a paste which was taken up in water (100ml) acidified to pH 2 and extracted with ethyl acetate. The organic extracts were dried and evaporated to give a solid which was heated to 210°C until no further carbon dioxide was evolved. On cooling the reaction mixture became solid **10** (3.56g, 49%) which was used without further purification.

**Methyl [$^2H_5$-ethyl]-5-cyanopentanoate (11)**

2-[$^2H_5$-ethyl]-5-phenoxypentanoic acid **10** (3.56g, 0.016 mole) was heated to boiling with 57% hydriodic acid (25ml) for 6 hr., then allowed to stand at room temperature overnight. The reaction mixture was poured into water (150ml) and extracted with ethyl acetate. The combined ethyl acetate extracts were washed with sodium metabisulphite solution and water,

dried ($MgSO_4$), evaporated and chromatographed on a column of silica gel eluting with hexane/ethyl acetate (19:1) to give pure methyl [$^2H_5$-ethyl]-5-iodopentanoate (1.56g, 0.006 mole). This material was dissolved in dimethyl sulphoxide (20ml) and heated at 35°C with potassium cyanide (0.41g, 0.007 mole) for 3 hr. then stirred at room temperature overnight. The reaction mixture was poured into water (200ml) and extracted with diethyl ether. The combined organic extracts were washed with aqueous NaCl solution, dried ($MgSO_4$) and evaporated to give an oil which was chromatographed on a column of silica gel eluting with hexane/ethyl acetate (3:1) to give pure **11** (0.93g, 36%).

**2-[$^2H_5$-Ethyl]-hexanedioic acid (12)**

Methyl [$^2H_5$-ethyl]-5-cyanopentanoate **11** (0.93g, 0.006 mole ) was boiled with a 35% aqueous solution of sodium hydroxide for 16 hr. when it was found that no more ammonia was evolved. The reaction mixture was poured into water (50ml) and the pH was adjusted to 2 with concentrated hydrochloric acid. The mixture was extracted with ethyl acetate and the combined extracts were dried ($MgSO_4$), evaporated and the residue was subjected to short path distillation to give pure **12** (0.82g, 86%).
$^1$H-NMR δ 1.6 (m, 4H), 2.3 (m, 3H), 11.2 (s, 2H).
$^{13}$C-NMR δ 10.3 (m), 22.3, 24.0 (m), 30.7, 33.8, 46.4, 179.9, 182.6.
-veFAB-MS (m/z) 178 (M-1, 100), 134 (80), 116 (12).

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