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A Convenient and Inexpensive Synthesis of Labelled Methylmalonic and Propionic Acids: Application to the Synthesis of Methyl-d_3-Malonic and of Propionic-3,3,3-d_3 Acids.

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SUMMARY

A synthetic route for obtaining highly pure methylmalonic and propionic acids labelled at the methyl groups is validated by the preparation of methyl-d_3-malonic and propionic-3,3,3-d_3 acids. The synthesis involves the alkylation of the diethyl allylmalonate with iodomethane-d_3, deallylation of the resulting diethyl allylmethyl-d_3-malonate by treatment with (η^2-propene)Ti(O-*i*-Pr)$_2$ and hydrolysis. Decarboxylation of the obtained methyl-d_3-malonic acid affords propionic-3,3,3-d_3 acid.

Key words: methylmalonic acid, methyl-d_3-malonic acid, trideuteriomethylmalonic acid, propionic-3,3,3-d_3 acid.

INTRODUCTION

Increased concentrations of methylmalonic acid (MMA) in serum and in urine are believed to be important markers useful for the early diagnosis of vitamin B-12 deficiency (1a-c) and for the drug therapy monitoring in patients with methylmalonic acidemia (2,3). Accurate quantification of MMA

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in serum and urine (4) requires the use of gas chromatography-mass spectrometry (GC-MS) combined with the sensitive selected ion monitoring (SIM) and the dilution of the serum and urine samples with chemically and isotopically pure trideuteriomethyl malonic acid (M-d_3-MA) as internal standard. This compound is now commonly obtained by monomethylation of malonic esters and subsequent hydrolysis (5-6). However, since the dialkylation is a significant side reaction of malonic ester synthesis (7), its preparation is always accompanied by significant amounts of diethyl dimethylmalonate and of unchanged diethyl malonate which cannot be separated by distillation (8). The parallel formation of these homologues (9) is particularly damaging in the preparation of MMA labelled at the methyl group since it is responsible for the partial loss of expensive labelled iodomethanes used in the alkylation.

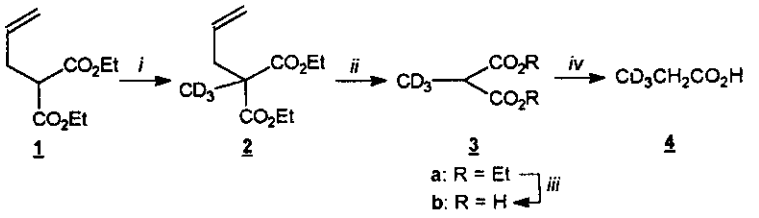
In this paper we report a convenient method which overcomes these difficulties and allows us to obtain M-d_3-MA (and in general MMA labelled at the methyl group) in pure form, satisfactory overall yield (61%) and in a relatively inexpensive way. Since MMA is easily decarboxylated to propionic acid (PA), the method reported here also represents an efficient economical route for obtaining propionic-3,3,3-d_3 acid (PA-d_3).

RESULTS AND DISCUSSION

The synthetic route for the labelling of the methyl group of MMA and of PA is exemplified by the preparations of M-d_3-MA (uncontaminated by the dimethylated homologue) and of PA-d_3 (Scheme). The synthesis exploits the elegant method recently reported by F. Sato et al. (10) for the protection and regeneration of one of the acidic hydrogen atoms of malonic ester.

According to F. Sato et al. (10), diethyl allylmalonate **1**, readily prepared from diethyl malonate and also commercially available, was used as a malonate ester with a protected acidic hydrogen atom. Its treatment with NaH and then iodomethane-d_3 in THF at room temperature provided the trideuteriomethyl compound **2** in quantitative yield. The obtained liquid compound was sufficiently pure (GLC) to be used in the following reaction without further purification.

Scheme



i: NaH, THF, r.t., 1 h; then CD_3I, r.t., 12 h; *ii*: Ti(O-*i*Pr)$_4$, *i*PrMgBr, -45°C, 1 h; *iii*: KOH, EtOH-H_2O, r.t., 1 h; *iv*: KOH, EtOH-H_2O, r.t., 1 h; then H_2SO_4, reflux, 1 h.

However, in one case the compound **2** was distilled and completely characterised. Its ^1H NMR spectrum differs from that of unlabelled isotopomer for the lack of the signal at δ 1.34 ppm due to the methyl group. Compound **2** was then treated with (η^2-propene)Ti(O-*i*-Pr)$_2$ readily generated *in situ* from 2 equiv of *i*-PrMgBr and 1 equiv. of Ti(O-*i*-Pr)$_4$ at –45°C (10). After a 1 h stirring the viscous mixture was poured into an ice cold solution of 1 M hydrochloric acid to afford in excellent yield (93% isolated) the trideuteriomethyl malonate **3a**. This compound shows the correct physicochemical properties and ^1H NMR spectrum which differs from that of the corresponding unlabelled compound for the lack of the signal at δ 1.34 ppm and for the simplification of the quartet at δ 3.35 ppm to an apparent singlet. Saponification of the diester **3a** afforded the M-d_3-MA **3b** as a crystalline white solid. Decarboxylation of **3b**, performed according to a common procedure afforded the PA-d_3.

In conclusion the method reported here represents a convenient and inexpensive route which allows one to prepare MMA and PA labelled at the methyl groups. Its utility appears particularly relevant when $^{11}CH_3I$, $^{13}CH_3I$ or $^{13}C^2H_3I$ must be used for the labelling, bearing in mind the very high cost of these alkylating agents. In addition, considering that methyl labelled methylmalonic acid is a useful precursor in various labelling syntheses (11), the method reported here for its preparation permits an easy optimisation.

EXPERIMENTAL

General.- Diethyl allylmalonate and iodomethane-d_3 (>99.5% deuterium labelled) were purchased from Aldrich. ^{1}H NMR spectra (500.13 MHz) were recorded in $CDCl_3$ at 303 K and were referenced to $CHCl_3$ at 7.24 ppm; signal multiplicity was designated according to the following abbreviations: s = singlet, br s = broad singlet, d = doublet, t = triplet. GLC analyses were carried out on a SPB-5 Supelco column (30 m x 0.32 mm; 0.25 μm film thickness); the column temperature was initially set at 100°C for 1 min, increased by 5°C/min until 150°C where it was kept for 2 min. TLC was carried out on silica gel 60 F_{254} microplates eluting with hexane-ethyl acetate (80:20; v/v) and the spots evidenced by exposure to iodine fumes. The progress of all the reactions and compound purity were monitored by GLC, TLC, ^{1}H NMR and elemental analysis. Usual work-up refers to extraction with ethyl acetate, washing with brine, drying over anhydrous sodium sulphate, filtration and removal of the solvent under reduced pressure.

Synthesis of diethyl allylmethyl-d_3-malonate **2**.- A solution of diethyl allylmalonate (2.0 g; 10.0 mmol) in anhydrous tetrahydrofuran (15 mL) was added dropwise to a suspension of NaH (0.25 g; 10.4 mmol) in THF (10 mL) at 0°C. The mixture was warmed up to 23°C and stirred for 1 h. At this time, CD_3I (1.70 g; 11.72 mmol; >99.5 atom % D) was added and the mixture was stirred overnight. The solvent was then evaporated under reduced pressure and the residue was diluted with ice cold water. Usual work-up afforded the diethyl allylmethyl-d_3-malonate **2** (1.74 g; Y = 80%) as a pale yellow liquid, sufficiently pure to be used in the following reaction. A purified sample was obtained by distillation under reduced pressure to afford a colourless liquid: bp 170-175°C (25 mmHg); IR (neat): ν_{max} 1730, 1640 cm^{-1}; ^{1}H NMR δ 5.66 (1H, ddt, J = 16.6, J = 10.0 and J = 7.2 Hz, CH_2=C<u>H</u>CH_2-), 5,10-5.00 (2H, AB part of an ABX system, C<u>H$_2$</u>=CHCH_2-) 4.14 (4H, q, J = 7.5 Hz, 2 x OC<u>H$_2$</u>CH_3) 2.57 (2H, d, J = 7.2 Hz, CH_2=CHC<u>H$_2$</u>), 1.20 ppm (6H, t, J = 7.5 Hz, 2 x OCH_2C<u>H$_3$</u>);

MS *m/z* 217 (M^+), 172 (M^+ - 45), 144 (M^+ - 73). The compound **2** was > 99.0% isotopically pure (^{1}H NMR and MS).

Elemental analysis, calculated for $C_{11}D_3H_{15}O_4$: C, 60.81; H + D, 9.74. Found: C, 61.1; H, 9.8%.

Synthesis of diethyl methyl-d_3-malonate **3a**.- To a solution of diethyl allylmethyl-d_3-malonate (1.05 g; 4.8 mmol) in diethyl ether (5.0 mL) and titanium(IV) isopropoxide (2.85 mL, 9.7 mmol), cooled at -45°C, isopropyl magnesium chloride was added dropwise (9.7 mL of a 2 M solution in diethyl ether) and the mixture stirred for 1 h at -45°C. At this time, the brown mixture was poured into an ice cold aqueous solution of 1 M hydrochloric acid (15 mL) and worked-up to afford the diethyl methyl-d_3-malonate **3a** (0.80 g; Y = 93%): colourless oil (bp 95-96°C at 16 mmHg); IR (neat) ν_{max} 1730, 1640 cm^{-1}; ^{1}H NMR δ 4.12 (4H, q, J = 7.0 Hz, 2 x $OC\underline{H}_2CH_3$) 3.35 (1H, br s, $CD_3C\underline{H}$), 1.19 ppm (6H, t, J = 7.0 Hz, 2 x $OCH_2C\underline{H}_3$); MS *m/z* (relative intensity) 177 (M^+, 7), 174 (0.04), 150 (17), 147 (0.04), 133 (6), 132 (100), 131 (12), 129 (0.04), 106 (3), 105 (34), 104 (16), 103 (2), 87 (1), 86 (11%) (identical to that described in reference 12). The compound **3a** was > 99.0% isotopically pure (^{1}H NMR and MS).

Elemental analysis, calculated for $C_8D_3H_{11}O_4$: C, 54.22; H + D, 9.67. Found: C, 54.3; H, 9.6%.

Synthesis of methyl-d_3-malonic acid **3b**.- The diethyl methyl-d_3-malonate **3a** (1.0 g; 5.6 mmol), dissolved in ethanol (10 mL) was added dropwise to a solution of aqueous potassium hydroxyde (17.6 mmol; 4 mL of a 4.4 M solution) and the mixture stirred for 1 h. At this time, the solution was concentrated under reduced pressure in order to remove the ethanol, cooled at 0°C and neutralised with acidic ion-exchange resin (Dowex 50 x 8-200). The mixture was then filtered and lyophilised to afford the methyl-d_3-malonic acid **3b** (0.56 g; Yield = 82%): mp 133-134°C (from diisopropyl ether-hexane); IR (nujol) ν_{max} 3500-2500, 1700 cm^{-1}; ^{1}H NMR δ 3.35 ppm (1H, br s, $CD_3C\underline{H}$); MS *m/z* (relative intensity) 121 (19), 104 (59), 94 (3), 77 (100), 74 (2), 59 (70), 57 (55), 56 (6%). The compound was > 99.0% isotopically pure (^{1}H NMR and MS).

Elemental analysis, calculated for $C_4D_3H_3O_4$: C, 39.67; H + D, 7.49. Found: C, 39.5; H, 7.6%.

Synthesis of propionic-3,3,3-d_3 acid **4**.- The diethyl methyl-d_3-malonate **3a** (1.00 g; 5.6 mmol), dissolved in ethanol (1.5 mL) was added dropwise to a solution of aqueous potassium hydroxyde (17.6 mmol; 4 mL of a 4.4 M solution) and the mixture stirred for 1 h. The solution was then concentrated under a flow of argon in order to remove the ethanol, cooled at 0°C and cautiously acidified with aqueous sulphuric acid (4 M). The solution was refluxed for 1 h, cooled and continuously extracted with diethyl ether to afford the propionic-3,3,3-d_3 acid **4** (0.36 g; Y = 83%): bp 140-141°C; IR (neat) ν_{max} 3500-2500 1720 cm^{-1}; ^1H NMR δ 2.36 ppm (2H, br s, CD_3CH_2COOH); MS *m/z* (relative intensity) 77 (M^+, 100%);. The compound was > 99.0% isotopically pure (^1H NMR and MS).

Elemental analysis, calculated for $C_3D_3H_3O_2$: C, 46.74; H + D, 11.76. Found: C, 46.5; H, 11.6%.

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