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Synthesis of [2, 3-$^{13}C_2$-2,5-cyclohexadienyl] Ubiquinone 3[1]

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SUMMARY

Diethyl [2,3-$^{13}C_2$] succinate was prepared by the oxidative dimerization of ethyl lithio [2-^{13}C] acetate in the presence of $CuBr_2$. Its dienolate was generated with lithium diisopropylamide (LDA) and methylated with CH_3I to afford diethyl 2-methyl [2,3-$^{13}C_2$] succinate which by acid hydrolysis gave the corresponding labelled acid. Treatment of the latter with $SOCl_2$ provided 2-methyl [2,3-$^{13}C_2$] succinic anhydride. This anhydride upon treatment with Et_3N, $ZnCl_2$ and trimethylchlorosilane in acetonitrile afforded 2,5-bis (trimethylsilyloxy)-3-methyl [3,4 $^{13}C_2$] furan which was subjected to a Diels Alder reaction with trichloroethylene giving rise to 2,3-dichloro-5-methyl [5,6-$^{13}C_2$] 1,4-benzoquinone which, in turn, was transformed by a known procedure to [2,3-$^{13}C_2$ -2,5-cyclohexadienyl] ubiquinone 3.

Key words: Diethyl [2,3-$^{13}C_2$] succinate, 2-methyl [2,3-$^{13}C_2$] succinic acid, 2-methyl [2,3-$^{13}C_2$] succinic anhydride, 2,5-bis(trimethylsilyloxy)-3-methylfuran, Diels Alder reactions

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[1] •IUPAC Numbering: 5,6-dimethoxy-2-farnesyl-3-methyl [2,3-$^{13}C_2$]-1,4-benzoquinone (7)

••C.A. Nomenclature: [2, 3-$^{13}C_2$] 2,5-cyclohexadiene-1,4-dione, 2,3-dimethoxy-5-methyl-6-(3,7,11-trimethyl-2,6,10-dodecatrienyl)-, (*E*,*E*)- [C.A. Registry Number: 1173-76-8]

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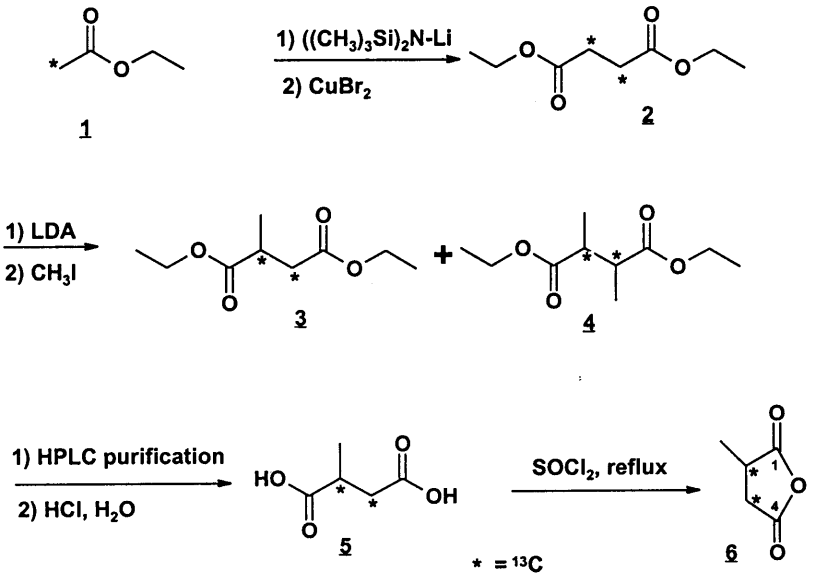
INTRODUCTION

Quinones labelled with stable isotopes are useful probes to study bacterial photosynthetic reaction centers by biophysical techniques such as FTIR (1), ENDOR (2), EPR (3) and NMR (4). Recently, using [1-^{13}C=O] and [4-^{13}C=O] quinones, FTIR showed that in the Q_A site only strong hydrogen bonding on 4-C=O occurs while in the Q_B site weak hydrogen bonding is present on both carbonyls (1, 1a). In order to improve our understanding of the interactions of the quinones with the protein surrounding, we developed a synthesis of labelled [2,3-$^{13}C_2$-2,5-cyclohexa dienyl] ubiquinone 3.

The procedure described by Rüttimann and Lorenz (5), which uses a key Diels Alder reaction between the dienol trimethylsilyloxy of the 2-methylsuccinic anhydride and trichloroethylene to afford the 2,3-dichloro-5-methyl-1,4-benzoquinone, is easy to adapt to incorporate the carbon-13 into the quinonic ring. Earlier we applied (6) this method to the preparation of singly ^{13}C-labelled ubiquinones at position 5 or 6 from the dienophile 2-bromo-1,1-dichloro [2-^{13}C] ethylene (6). Positions 1, 2, 3 or 4 were labelled starting from mono-^{13}C-labelled 2-methylsuccinic anhydride (1,7). Biological studies of the interactions in the neighbourhood of the methyl group and the side chain led us to label the 2-methylsuccinic anhydride rather than the trichloroethylene dienophile which otherwise would have been more easily prepared by chlorination of acetylene with $SbCl_5$ followed by a basic treatment (8).

Thus, the strategy for our synthesis was the preparation of 2-methyl [2,3-$^{13}C_2$] succinic anhydride **6** (scheme 1) which was used as its dienol trimethylsilyloxy **7** in a Diels Alder reaction with trichloroethylene to afford the labelled quinone **8**. The latter was transformed by the procedure of Rüttimann and Lorenz (5) to the title compound **12** (scheme 2).

2-Methyl [2,3-$^{13}C_2$] succinic anhydride 6 synthesis:

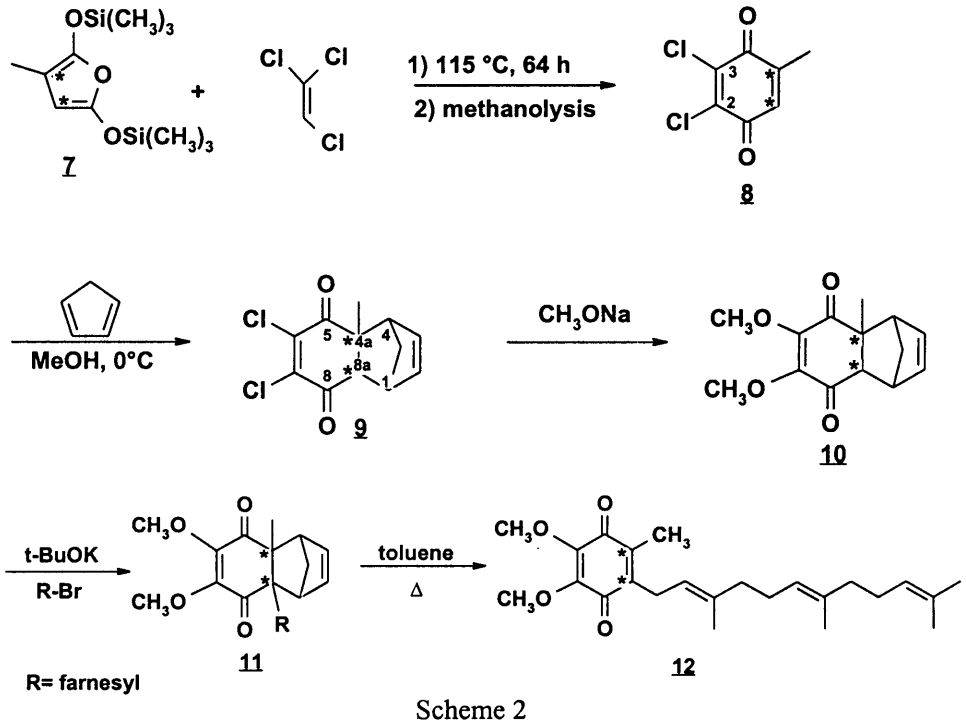


Scheme 1

Ethyl [2-^{13}C] acetate **1** reacted with lithium bis(trimethylsilyl)amide to form the lithio derivate (9) which was dimerized with copper (II) bromide (9a) to afford a mixture of diethyl [2,3 ^{13}C] succinate **2** and of bromo [2 ^{13}C] acetate (80/20 proportion). Some experiments showed that the presence of bromoacetate did not interfere with the yield in the next step. The dienolate of **2** was generated by

addition of LDA to this mixture, followed by methylation with CH_3I to afford a mixture of mono- and di-methylated esters **3** and **4** (10). These esters were separated by HPLC on a reversed phase column to obtain **3**. The acidic hydrolysis of **3** gave quantitatively the acid **5** which was converted by treatment with $SOCl_2$ to the anhydride **6** (overall yield: 7%).

[2,3-$^{13}C_2$-2,5-cyclohexadienyl] Ubiquinone 3 12 synthesis:



Scheme 2

2,5-Bis(trimethylsilyloxy)-3-methyl [3,4-$^{13}C_2$] furan **7** was obtained from the anhydride **6** by treatment with trimethylsilyl chloride/$ZnCl_2$/NEt_3 under the reaction conditions described in (11). It was then submitted to a Diels Alder reaction with trichloroethylene in the presence of 1,2-epoxybutane at 115°C for 64

h (5). Methanolysis of the reaction mixture gave the dichloroquinone **8** after purification by column chromatography (yield: 11%). The Diels Alder reaction of **8** with cyclopentadiene afforded the cycloadduct **9** (77 % yield). Substitution of the chlorine atoms of **9** by methoxy groups upon treatment with MeONa gave the dimethoxy derivative **10** in 49 % yield, after HPLC purification. Alkylation of **10** with freshly prepared farnesyl bromide gave **11** which afforded by a *retro* Diels Alder reaction [2,3-$^{13}C_2$-2,5-cyclohexadienyl] ubiquinone 3 **12** in 78% yield.

EXPERIMENTAL

General

Ethyl [2-^{13}C] acetate (isotopic enrichment: 98.8%) was obtained from *Isotec* France. HPLC analysis were performed on a *Merck* system (Darmstadt), and the HPLC purifications on a *Dupont* system with UV detection at 225 nm for esters and 254 nm for quinone derivatives. ^{1}H NMR spectra were recorded in $CDCl_3$ at 300 MHz and ^{13}C NMR spectra at 75 MHz on a *Bruker* AM 400. Gas chromatography (GC) analysis were performed on a *Varian* instrument with a FID detector on a *Chromasorb* DB17 column. All reactions were performed under nitrogen and solvents were dried on molecular sieves.

Diethyl [2,3-$^{13}C_2$] succinate 2:

To a THF solution of 1M lithium bis(trimethylsilyl)amide (22.7 mL, 22.7 mmol) cooled at - 78°C was added ethyl [2-^{13}C] acetate **1** (2 g, 22.4 mmol) and the mixture stirred for 15 min. $CuBr_2$ (5.1 g, 22.7 mmol) was added in one portion and

the reaction mixture was stirred for 15 min and allowed to warm to room temperature. 10% HCl (15 mL) and pentane (30 mL) were added and the organic layer was recovered and dried over $MgSO_4$. The organic extract was evaporated to give a residue which was purified on a short silicagel column with hexane: ethyl acetate: 9/1 as eluent to remove the copper salts. The solvents were evaporated to obtain 1.32 g of an oil made up of diethyl [2,3-$^{13}C_2$] succinate **2:** 80 and bromo [2-^{13}C] acetate: 20 (GC analysis). This mixture was used in the next step, without further purification. ^{1}H NMR: 1.17 (t, 2 CH_3-CH_2, J = 7 Hz), 2.53 (d, CH_2-C(2), CH_2-C(3), J(CH_2-C(2, 3),^{13}C) = 125 Hz), 4.06 (q, 2 CH_2-CH_3). ^{13}C NMR: 28.7 C(2), C(3). Isotopic enrichment: 98% (M.S. analysis).

Diethyl 2-methyl [2,3-$^{13}C_2$] succinate 3:

To a freshly prepared solution of LDA (10.8 mmol) in THF (20 ml) cooled at -78° C was slowly added a solution of the oil (1.32 g) obtained as described above in THF (20 mL) and stirred for 15 min. CH_3I (0.47 mL, 7.5 mmol) was added and the reaction mixture was stirred for 30 min. The reaction was quenched with 3M HCl (5 ml) and allowed to warm to room temperature. The evaporation of the reaction mixture gave 1.08 g of a mixture of esters 2: 3: 4 (25.7/65.6/8.7 proportions). The HPLC purification on a Stable Bond C18 column with CH_3CN: H_2O: 45/55 as eluent at a flow rate of 10 mL/min gave 0.16 g of **3** as a colorless oil (yield: 11%). ^{1}H NMR: 1.26 (m, 2.CH_3, J = 7 Hz), 2.34 (m, Ha-C(2), J(Ha-(C(2),^{13}C = 130 Hz), 2.66 (m, H-C(3), J(H-C(3), ^{13}C) = 130 Hz), 2.67 (m, Hb-C(2), J(Hb-C(2), ^{13}C) = 130 Hz). ^{13}C NMR: 35.5 (d, C(2), J = 36 Hz), 37.5 (d, C(3)).

2-Methyl [2,3-$^{13}C_2$] succinic acid 5:

A 7.5% aqueous HCl solution (2 mL) was added to **3** (0.14 g, 0.8 mmol). A few drops of ethanol were added until complete dissolution. The reaction mixture was refluxed for 18h and evaporated to dryness to give 0.1 g (quantitative yield) of **5** as a solid. The crude product was directly used in the next step. ^1H NMR (D_2O): 1.42, 1.44 (2 s, 2 CH_3-CH_2), 2.79 (dd, Ha-C(3)), 2.92 (dd, Hb-C(3)), 3.11 (m, H-C(2)). ^{13}C NMR: 16.6 CH_3, 36.2 (d, C(2), J(C(2), ^{13}C) = 36 Hz), 37.7 (d, C(3), J(C(3), ^{13}C) = 36 Hz).

2-Methyl [2,3-$^{13}C_2$] succinic anhydride 6:

To 0.1 g of **5** (0.75 mmol) was added $SOCl_2$ (1 mL). The mixture was refluxed for 2h and evaporated to dryness to give 88 mg (quantitative yield) of **6** which was directly used in the next step.

2,5-Bis(trimethylsilyloxy)-3-methyl [3,4-$^{13}C_2$] furan 7:

This compound was prepared as described in ref.(11). A fine suspension of $ZnCl_2$ (30 mg, 0.22 mmol) in NEt_3 (0.35 mL) was stirred for 1h at 20°C and added to a solution of **5** (88 mg, 0.77 mmol) in CH_3CN (1.6 mL) followed after 5 minutes by the dropwise addition of TMS-Cl (0.28 mL, 2.2 mmol) and stirred overnight. After filtration, the filtrate was evaporated to give an oil which was dissolved in diethyl ether (20 mL). The solution was filtered and evaporated. The resulting oil was dissolved in cold hexane (20 mL), filtered and evaporated, 0.12 g of **7** was obtained as a yellow oil, which was used in the next step, without further purification.

2,3-Dichloro-5-methyl [5,6-$^{13}C_2$] 1,4-benzoquinone 8:

The following procedure was adapted from that described in ref (5). A sealed glass tube containing [3,4-$^{13}C_2$] 2,5-bis(trimethylsilyloxy)-3-methylfuran 7 (0.12 g, 0.46 mmol) trichloroethylene (0.25 mL, 2.78 mmol) and 1,2 epoxybutane (0.02 mL, 0.23 mmol) was heated at 115°C for 64 h. The reaction mixture was treated with methanol (10 ml) at 45°C for 10 min and evaporated *in vacuo*. The residue was purified on a silicagel column with hexane:ethyl acetate: 9/1 as eluent to give 30 mg of the quinone **8** (yield: 33%). ^{1}H NMR: 2.15 (m, CH_3), 6.78 (m, H-C(6), J(H-C(6), ^{13}C = 130 Hz). ^{13}C NMR: 130.9 (d, C(5), J(C(5), ^{13}C) = 63 Hz), 143.7 (d, C(6), J(C(6), ^{13}C) = 63 Hz).

(1α,4α,4aβ,8aβ)-6,7-Dichloro-1,4,4a,8a-tetrahydro-4a-methyl-1,4-methano [4a,6a-$^{13}C_2$] naphtalene-5,8-dione 9: (see ref. (5))

To a solution of **8** (25 mg, 0.128 mmol) in methanol (10 mL) cooled at 0°C was added freshly distilled cyclopentadiene (0.2 ml, 2.4 mmol). The reaction mixture was stirred for 30 min at 0°C then overnight at room temperature. The solvent was evaporated *in vacuo* and the oil was purified by HPLC on a silicagel column with hexane:ethyl acetate: 97.5/2.5 as eluent to give 25.4 mg of **9** (yield: 77%). ^{1}H NMR: 1.56 (s, CH_3-C(4a)), 1.61, 1.72 (m, CH_2), 3.03 (m, H-C(8a), J(H-(C8a),^{13}C(8) = 135 Hz), 3.18, 3.51 (2 br.s, H-C(1), H-C(4)), 6.03, 6.15 (dd, J(H-C(2), H-C(3) = 6.3, H-C(2), H-C(3)); ^{13}C NMR: 134.7 (d, C(4a)), J(C(4a), ^{13}C(8a) = 39 Hz), 137.9 (d, C(8a)). J(C(8a), ^{13}C(4a)) = 39 Hz.

(1α,4α,4aβ,8aβ)-6,7-Dimethoxy-4a-methyl-1,4-methano [4a,8a-$^{13}C_2$] naphtalene-5,8-dione 10:

To a cooled solution of **9** (21 mg, 0.081 mmol) in toluene (3 ml) was added a solution of 5.4 M NaOMe (0.085 mL, 0.459 mmol). It was stirred for 30 min at 5°C and for 2h at 20°C. The reaction mixture was made neutral with acetic acid and diluted with water (5 mL) then extracted with diethyl ether (3x10 ml). The combined organic extracts were washed with water (10 mL) and dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by HPLC on a silicagel column with hexane:ethyl acetate: 9/1 as eluent to give 9.9 mg of **10** (yield: 49%). ^1H NMR: 1.46 (m, CH_3, J(H-C(4a), ^{13}C) = 4 Hz), 1.52, 1.58 (dd, CH_2), 2.80 (m, J(H-C(8a), ^{13}C(4a) = 135 Hz), 3.06, 3.4 (2 br.s, H-C(1), H-C(4)), 3.90, 3.92 (2s, 2 CH_3O), 5.99, 6.14 (dd, H-C(2), H-C(3)). ^{13}C NMR: 52.4 (d, C(4a), J(C(4a), ^{13}C(8a) = 39 Hz), 56.8 (d, C(8a), J(C(8a), ^{13}C(4a) = 39 Hz).

(1α,4α,4aβ,8aβ)-(all-E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl-1,4,4a,8a-tetrahydro-6,7-dimethoxy-4a-methyl-1,4-methano [4a,8a-$^{13}C_2$] naphtalene-5,8-dione 11: (see ref. (5))

t-BuOK (10 mg, 0.088 mmol) was partially dissolved in a solution of *t*-BuOH/toluene (125 µl, 4/1) at 0°C and added dropwise to a solution of **10** (9.9 mg, 0.039 mmol) in *t*-BuOH/toluene (125 µl, 4/1). At the end of the addition, freshly prepared farnesyl bromide (58 mg, 0.2 mmol) in *t*-BuOH/toluene (125 µl, 4/1) was added rapidly. After stirring for 20 min at 0°C, 2 ml of water was added and the mixture extracted with hexane (3x5 ml). The organic extracts were combined and evaporated to give an orange oil which was purified by HPLC on

silicagel with hexane:ethyl acetate: 9/1 as eluent and gave 5.5 mg of **11** (yield: 31%). ^{1}H NMR: 1.49 (m, CH_3-C(4a), J(H-C(4), ^{13}C) = 3Hz), 1.55 (s, CH_3-C(12')), 1.57 (br.s, CH_3-C(11')), CH_3-C(3')), 1.65 (s, CH_3-C(7')), 1.9-2.03 (m, 4 allyl-CH_2), 2.3, 2.8 (m, Ha-C(1'), Hb-C(1')), 2.99, 3.07 (br.s, H-C(1), H-C(4)), 3.87, 3.89 (2s, 2 CH_3O), 5.06 (m, 3 allyl-H), 6.04 (br.s, H-C(2), H-C(3)); ^{13}C NMR: 55.7 (d, C(4a), J(C(4a), ^{13}C(8a) = 40 Hz), 59.2 (d, C(8a), J(C(8a), ^{13}C(4a) = 40 Hz).

2-(all-E)-3,7,11-Trimethyl-2,6,10-dodecatrienyl-5,6-dimethoxy-3-methyl [2,3-$^{13}C_2$]-2,5-cyclohexadien-1,4-dione: ([2,3-$^{13}C_2$ -2,5-cyclohexadienyl] Ubiquinone 3): 12:

Compound **11** (5 mg, 0.01 mmol) was dissolved in toluene (5 ml). The solution was refluxed for 20 min. The solvent was evaporated to give an orange oil which was purified by HPLC on silicagel with hexane:ethyl acetate: 9/1 as eluent to give 3.6 mg of **12** (yield: 86%). ^{1}H NMR: 1.56 (s, CH_3-C(12')), 1.57 (s, CH_3-C(11')), 1.65 (s, CH_3-C(7')), 1.72 (s, CH_3-C(3')), 1.95-2.06 (m, 4 allyl-CH_2), 2.0 (s, CH_3-C(3)), 3.16 (dd, CH_2-C(1'), J(CH_2-C(1'), ^{13}C(6)) = 11 Hz), 3.93, 3.98 (2s, 2 CH_3O), 4.95 (t, H-C(2')), 5.05 (m, H-C(5'), H-C(8')); ^{13}C NMR : 138.5 (d, C(3), J(C(3), ^{13}C(2) = 66 Hz), 141.4 (d, C(2), J(C(2), ^{13}C(3) = 66 Hz); Isotopic enrichment: 98%.

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