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Synthesis of leukotrienes labelled with deuterium: [11,12,14,15-$^{2}H_4$]-LTA_4 -LTC_4, -LTD_4 and -LTE_4

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SUMMARY

Semi-hydrogenation by D_2 gas on Lindlar catalyst of an acetylenic precursor led to [11,12,14,15-$^{2}H_4$]-LTA_4 methyl ester 1a. Nucleophilic opening of the epoxide ring by amino thioacids afforded after saponification the corresponding deuterated peptidoleukotrienes LTC_4, LTD_4 and LTE_4.

Key Words: Semi-hydrogenation, deuterated leukotrienes, peptidoleukotrienes, deuterium.

INTRODUCTION

Leukotrienes are lipoxygenase derivated metabolites of arachidonic acid [1,2]. Their involvement in numerous disease states such as asthma, allergic diseases or inflammation implies high performance analytical techniques in order to detect and identify leukotrienes and their metabolites. Among these techniques, GC-MS coupling offers many advantages but requires stable isotope labelled standards (^{2}H or ^{13}C). Moreover, there must be a mass difference of at least 3 between the leukotriene and its labelled counterpart. As far as we know, deuterated leukotrienes have not yet been described in the literature.

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We describe here the synthesis of:

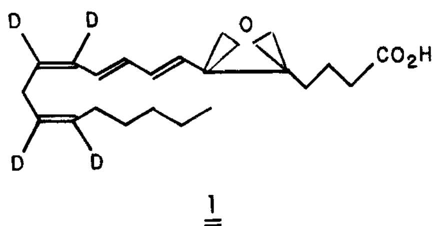
1 : (11, 12, 14, 15-$^{2}H_4$) - LTA_4 methyl ester

2 : (11, 12, 14, 15-$^{2}H_4$) - LTC_4

3 : (11, 12, 14, 15-$^{2}H_4$) - LTD_4

4 : (11, 12, 14, 15-$^{2}H_4$) - LTE_4

which led us to optimize the semi-hydrogenation of their diacetylenic precursor 5 [3].



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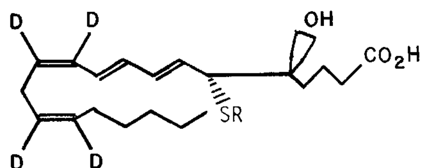


2 : R = L-γ-glutamyl-L-cysteinylglycine

3 : R = L-cysteinylglycine

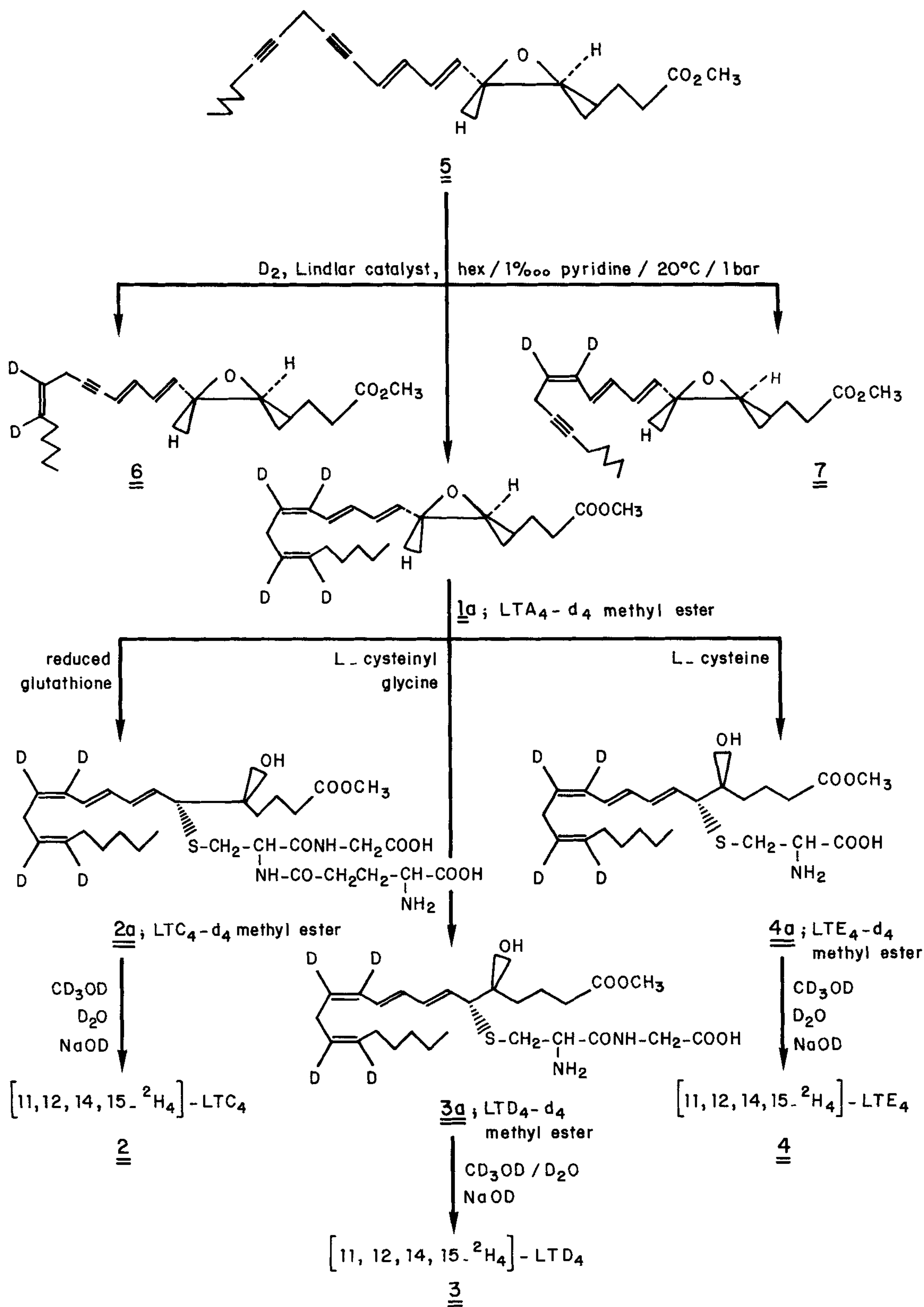
4 : R = L-cysteine

DISCUSSION

Semi-reduction of diacetylenic compounds is rather tedious and yields vary according to different substrates [4]. Usually, commercial Lindlar catalysts are de-activated by organic bases such as pyridine or quinoline. In particular, pyridine has a double action: it poisons the catalyst, thus avoiding the total reduction of the triple bond and on the other hand it stabilizes the reaction products, which, in our case, are more fragile in acidic medium than the diacetylenic precursor 5.

Thus, 5 was semi-hydrogenated by deuterium gas on Lindlar catalyst in anhydrous hexane containing 1% pyridine under one bar pressure and at room temperature to afford 1a. The volume of D_2 gas used was checked by a TOEPPLER pump [5]. The best yields of 1a (26%) were obtained when 85% of the theoretical amount of deuterium necessary for semi-hydrogenation of both triple bonds were used. In these conditions, the amount of by-products 6 + 7 did not exceed 3%.

Reaction scheme



The presence of 1% pyridine in hexane increases the yield (26% instead of 20% without pyridine). However, deuterium absorption is completely inhibited in presence of 2 to 5 % pyridine in hexane.

Compound 1a, as well as 6 and 7, were purified by preparative HPLC. As the free acid 1 is unstable, it was stored as its methyl ester 1a which may be saponified just prior to use.

LTA_4-d_4 methyl ester 1a was then reacted with the appropriate unprotected aminoacid thiol in 4% triethylamine methanol, which induced ring opening of the (5S,6S) epoxide. Thus, L-γ-glutamyl-L-cysteinyl glycine, L-cysteinylglycine and L-glycine afforded respectively the methyl esters of deuterated peptido-leukotrienes LTC_4 2a, LTD_4 3a and LTE_4 4a (72%, 80% and 30% yields). These compounds were purified by high-performance liquid chromatography.

Opening of epoxide 1a on the most nucleophilic C_6 position was checked by ^1H-NMR and UV-spectrophotometry (absence of allyl carbinol proton =C=CHOH at 4 ppm and λ_{max}^{MeOH} = 280 nm). These results were in agreement with previous studies [6].

Saponification of 2a, 3a and 4a by CD_3OD/NaOD led to the desired LTC_4-d_4 2, LTD_4-d_4 3, and LTE_4-d_4 4 with respective yields of 82%, 58% and 25%. These were purified by high-performance liquid chromatography.

Compounds 1, 2, 3 and 4 were checked for purity by HPLC (comparison with authentic samples), ^1H-NMR. Their biological activity evaluation is under way. Chemical and stereochemical purities were superior to 98%. Isotopic enrichment was at least 98%.

EXPERIMENTAL SECTION

General

HPLC analyses were performed on a Waters system (pump 6000 A and UV detector n° 481).

HPLC purifications were obtained with a Waters system (pump 590 and UV detector n°481).

NMR spectra were recorded on a Bruker AC 300 (300 MHz) equipped with an Aspect 3000 calculator (TMS internal standard).

FAB mass spectra were recorded on a NERMAG spectrometer.

UV specta were recorded on a spectrophotometer Beckman 5230.

[11, 12, 14, 15-$^2H_4$]-Leukotriene A_4 methyl ester **1a**

17.5 mg of **5** (52.6 µmoles) in 5 ml anhydrous hexane containing 1% pyridine were reduced with D_2 gas on 2 mg Lindlar catalyst under stirring at atmospheric pressure. The reaction was stopped after absorption of 85 % of the theoretical deuterium gas volume (2.3 cm^3). After filtration on 0.5 µm Millipore filter and evaporation, the product was purified by HPLC on a semi-preparative Merck Si 60 Lichrosorb column eluted by hexane/ethyl acetate/triethylamine 100:1:1 at a flow rate of 5 ml/min (UV max at 280 nm). There was obtained 4.6 mg of **1a** (26% yield).

^{1}H-NMR(CD_3OD, δ in ppm)

0.86(t,3H,H20,J19-20 = 7.0 Hz); 1.30(multiplet,6H,H17+H18+H19); 1.61(multiplet,2H,H4) ; 1.79 (multiplet,2H,H3) ; 2.05(t,2H,H16,J16-17 = 6.9 Hz); 2.38(t,2H,H2,J2-3=7.5 Hz); 2.85(t,1H,H5,4-5=5.5 Hz); 2.90(s,2H,H13); 3.13(dd,1H,H6,J6-7 = 7.8 Hz, J6-5 = 2.1 Hz); 3.65(s,3H,-CO_2CH_3); 5.36(dd,1H, H7,J7-6 = 7.8 Hz, J7-8 = 15.0 Hz); 6.18(dd,1H,H9,J9-8 = 10.8 Hz, J9-10 = 15.4 Hz); 6.46(dd,1H,H8,J8-7 = 15.3 Hz, J8-9 = 10.8 Hz); 6.54(d,1H,H10,J10-9 = 15.9 Hz).

H.P.L.C. (analysis): silicagel column Lichrosorb Merck, eluent hexane/ethyl-acetate/triethylamine 100:1:1, U.V. detection at 280 nm, flow-rate: 2 ml/min,retention time:6 min 50.

U.V.(methanol) $\lambda_{max\ 1}$ = 280 nm (36213); $\lambda_{max\ 2}$ = 278 nm (48657) ; $\lambda_{max\ 3}$ = 290.5 nm (38888)

$[\alpha]_D^{20}$ = - 28°4 (c = 0.4583 g/100 ml in hexane)

General procedure for the preparation of deuterated peptidoleukotrienes methyl esters **2a**, **3a** and **4a**

To 4.78 mg (0.014 mmole) of **1a** in 7 ml CD_3OD containing 4% Et_3N was added 0.085 mmole (6 equiv) of the appropriate amino acid: glutathione (26 mg), L-cysteinylglycine monohydrate (16 mg) or L-cysteine (13 mg). The

reaction mixture was stirred for 22 hours at 20°C under N_2 atmosphere. After evaporation to dryness, the residue was taken up in 1 ml of methanol, the solution was filtered on Sep Pak C 18 and evaporated. The residue was purified by HPLC on a Merck RP 18 Lichrosorb column (7 µm), eluted with methanol/water/acetic acid 75:25:0.1 and buffered with ammonia to pH 5.6 (flow rate: 5 ml/min; UV max at 280 nm). The product was then freeze-dried, checked and analyzed.

[11,12,14,15-$^2H_4$]-Leukotriene C_4 methyl ester 2a

^{1}H-NMR(CD_3OD, δ in ppm)

5.60(dd,1H,H7,J7-6 = 10.1 Hz, J7-8 = 14.1 Hz); 6.13-6.27(dd dd,2H,H8+H9); 6.54(d,1H,H10,J10-9 = 14.2 Hz).

H.P.L.C.(analysis): on reverse phase column Lichrosorb RP 18 Merck, eluent: methanol/water/acetic acid 75:25:0.1 buffered with ammonia to pH 5.6, U.V. detection at 280 nm, flow rate: 1 ml/min, retention time: 8 min.

U.V.(methanol): $\lambda_{max\ 1}$ = 270 nm; $\lambda_{max\ 2}$ = 280 nm ; $\lambda_{max\ 3}$ = 290 nm.

[11,12,14,15-$^2H_4$]-Leukotriene D_4 methyl ester 3a

^{1}H-NMR(DMSO, δ in ppm)

5.58(dd,1H,H7,J7-8 = 14.3 Hz, J7-6 = 10.4 Hz); 6.05-6.22(dd dd,2H,H8+H9); 6.46(d,1H,H10,J10-9 = 14.4 Hz).

H.P.L.C.(analysis): same conditions as 2a, retention time: 7 min 50.

U.V.(methanol): $\lambda_{max\ 1}$ = 270 nm; $\lambda_{max\ 2}$ = 280 nm ; $\lambda_{max\ 3}$ = 290 nm.

[11,12,14,15-$^2H_4$]-Leukotriene E_4 methyl ester 4a

^{1}H-NMR(CD_3OD, δ in ppm): 5.63 (dd,1H,H7,J7-8 = 14.3 Hz, J7-6 = 10 Hz); 6.13-6.33 (dd dd,2H,H8+H9); 6.55 (d,1H,H10,J10-9 = 14.2 Hz).

H.P.L.C.(analysis): same column, eluent: methanol/water/acetic acid 80:20:0.1 buffered with ammonia to pH 5.6, flow rate: 1 ml/min, U.V. detection at 280 nm, retention time: 14 min.

U.V.(methanol): $\lambda_{max\ 1}$ = 269 nm; $\lambda_{max\ 2}$ = 278 nm ; $\lambda_{max\ 3}$ = 290 nm.

General procedure for the preparation of deuterated peptidoleukotrienes 2a, 3a and 4a

0.0023 mmole of methyl ester 2a, 3a or 4a was added to 1.16 ml of 0.1 N NaOD at 0°C over one hour. The reaction was monitored by analytical HPLC (Prolabo S5 ODS_2 column eluted by methanol/water/acetic acid 75:25:0.1 and buffered with ammonia to pH 5.6). After neutralisation to pH:7 by 1N acetic acid, the reaction mixture was evaporated to dryness, taken up with 1 ml of methanol, filtered on Sep Pak C 18 and once more evaporated. The residue was purified by HPLC as before (flow rate: 2.5 ml/min, UV detection at 280 nm). The solution was neutralized to pH 7 by ammonia, concentrated, diluted with degassed water and finally hydrolyzed.

[11,12,14,15-$^2H_4$]-Leukotriene C_4 2

^{1}H-NMR(CD_3OD, δ in ppm)
5.60(dd,1H,H7,J7-6 = 10.1 Hz, J7-8 = 14.1 Hz); 6.20(dd dd,2H,H9+H8);
6.54(d,1H,H10,J10-9 = 14.2 Hz).
H.P.L.C.(analysis): on reverse phase column S5 W ODS 2 Prolabo, eluent: methanol/water/acetic acid 75:25:0.1 buffered with ammonia at pH 5.6, flow rate: 1 ml/min, U.V. detection at 280 nm, retention time: 9 min20.
U.V.(methanol): $\lambda_{max\ 1}$ = 270 nm; $\lambda_{max\ 2}$ = 279.5 nm ; $\lambda_{max\ 3}$ = 290 nm.

[11,12,14,15-$^2H_4$]-Leukotriene D_4 3

^{1}H-NMR(CD_3OD, δ in ppm): 5.63 (dd,1H,H7,J7-6 = 10.4 Hz, J7-8 = 14.2 Hz);
6.12- 6.30 (dd dd,2H,H8+H9); 6.53 (d,1H,H10,J10-9 = 14.3 Hz).
H.P.L.C.(analysis): same conditions as before, retention time: 10 min.
U.V.(methanol): $\lambda_{max\ 1}$ = 270 nm; $\lambda_{max\ 2}$ = 279 nm ; $\lambda_{max\ 3}$ = 290 nm.

[11,12,14,15-$^2H_4$]-Leukotriene E_4 4

^{1}H-NMR(CD_3OD, δ in ppm): 5.63(dd,1H,H7,J7-6 = 10 Hz, J7-8 = 14.3 Hz);
6.09-6.22(dd dd,2H,H8+H9) ; 6.55 (d,1H,H10,J10-9 = 14.2 Hz).
H.P.L.C.(analysis): same conditions as before, retention time: 10 min50.
U.V.(methanol): $\lambda_{max\ 1}$ = 270 nm; $\lambda_{max\ 2}$ = 279 nm ; $\lambda_{max\ 3}$ = 290 nm.

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