STEREOSELECTIVE SEMI-HYDROGENATION AND DEUTERATION OF A DIACETYLENIC PRECURSOR OF LEUKOTRIENE B_4 METHYL ESTER.

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SUMMARY

[6,7,14,15-$^2H_4$)-Leukotriene B_4 methyl ester was prepared by reduction with deuterium gas of a suitable precursor (deuterium incorporation > 90%). Several catalytic semi-hydrogenations were effected in order to determine the best conditions for the labeling step.

Key Words : Leukotriene B_4, deuterium labeling, semi-hydrogenation.

INTRODUCTION

Leukotriene B_4 (LTB_4), a 5-lipoxygenase metabolite of arachidonic acid, is the most potent chemotactic agent for macrophages and neutrophiles produced in man. Therefore it plays a major role in allergic, inflammatory and immunological states[1]. The availability of isotopically labeled forms of this mediator is of primary importance for defining its physiological role.

LTB_4 labeled with a stable isotope can be used as an internal standard for quantification by gas chromatography-mass spectrometry of LTB_4 in biological samples [2-4]. For this purpose, deuterium labeled analogues ([$^2H_4$]-LTB_4 and [$^2H_8$]-LTB_4) have already been prepared by chemical synthesis[4] or via biochemical synthesis[3,5]. The use of labeled LTB_4 as an internal standard for

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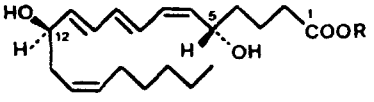
routine quantifications required a regular supply of this compound, which led us to develop a more convenient method for its preparation.

Recently[6], we described the total synthesis of a suitable precursor for labeling LTB_4, 12-O-benzoyl 6,7,14,15-tetradehydroleukotriene B_4 methyl ester 2a. We report herein the stereoselective semi-reduction of this diyne, by hydrogen or deuterium, leading to the corresponding LTB_4 or its tetradeuterated analogue 3a or 4a.



R = H, LTB_4

1, R = CH_3, LTB_4 methyl ester

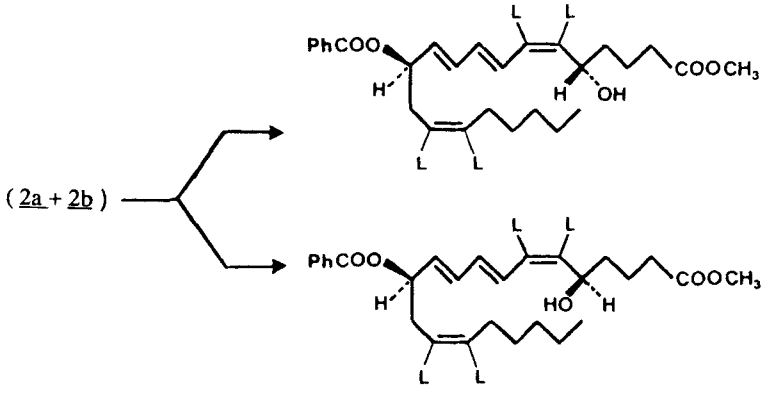
2a (5S,12R), X = H, Y = OH

2b (5R,12R), X = OH, Y = H

RESULTS AND DISCUSSION

Lindlar catalyst (lead-poisoned palladium on calcium carbonate) and palladium on barium sulfate have been frequently used for the stereoselective catalytic semi-hydrogenation of a triple bond into a Z-double bond. In the field of eicosanoid chemistry, only Lindlar catalyst (deactivated or not by organic bases) has been used[7].

In order to determine good conditions for the labeling step, we effected several catalytic hydrogenations (Table 1). The best results for this reduction were obtained with Lindlar or with palladium on barium sulfate catalysts (Table 1).



3a, L = H

4a, L = D

3b, L = H

4b, L = D

These hydrogenations were performed on the more readily available 1 : 1 mixture of 2a and its corresponding C-5 epimer 2b[6]. Progress of the reaction was monitored by HPLC. After

hydrogenation for 4 hours on 5% $Pd/BaSO_4$ (Table 1, run 1), the chromatogram (Figure 1, profile 1) of the crude mixture, indicated that the diyne (peak D) had been converted, in 75% yield, into three main compounds which were purified by preparative HPLC :

- two major compounds (peaks B and A) having identical ^{1}H-NMR spectra corresponded to the expected polyenic diastereomers 3a and 3b, respectively the protected LTB_4 and its 5-epimer (*vide infra*). For each compound the Z configuration of the 6,7-double bond was confirmed by the value of the coupling constant : $J_{6-7} = 11$ Hz.

- an intermediate compound (peak C) was identified as the 14,15-didehydro protected LTB_4 (^{1}H-NMR : two triplets at δ 5.42 and 6.04 ppm were assigned to H-6 and H-7 respectively). The 6,7-triple bond of 2 thus appeared to be more reactive towards H_2 than the 14,15-triple bond.

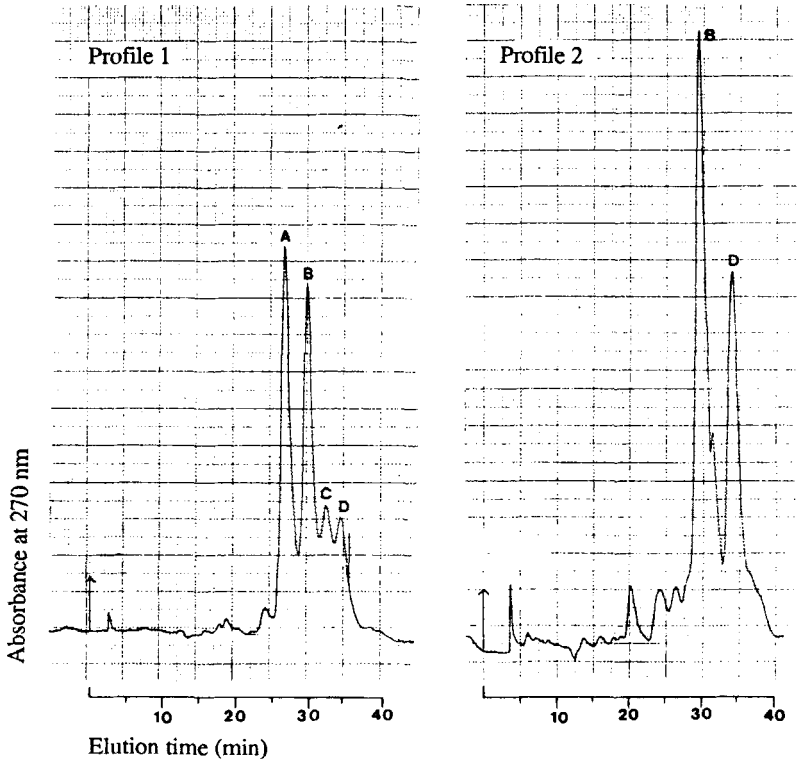


Figure 1 : Normal phase HPLC of crude hydrogenation products (run 1) of (2a+2b) (profile 1) and 2a (profile 2). Peaks : A = 12-O-benzoyl-5-epi LTB_4 methyl ester 3b, B = 12-O-benzoyl LTB_4 methyl ester 3a, C = 12-O-benzoyl-14,15-didehydro LTB_4 methyl ester, D = 12-O-benzoyl-6,7,14,15-tetradehydro LTB_4 methyl ester and its C-5 epimer (2a+2b).

Table 1 : Heterogeneous catalytic reduction of (2a + 2b)[a]

Run	Catalyst (c)	Solvent	Pre-reduction time (h)	Poison	Reduction time (h)	HPLC analysis %[b] (2a+2b)	HPLC analysis %[b] (3a+3b)
1	5% $Pd/BaSO_4$ (50 to 100%)	EtOAc	2	-	4	15	65 to 80
2	5% $Pd/BaSO_4$ (100%)	MeOH	2	-	1	0	0[d]
3	10%$Pd/BaSO_4$ (78%)	EtOAc	2	Quinoline[e]	0.5	0	0[d]
4	10%$Pd/BaSO_4$[f] (30%)	EtOAc	0.5	Quinoline[e]	1.5	20	70
5	Lindlar (200%)	EtOAc	-	-	3	15	70
6	Lindlar (40 then 70%)	EtOAc	-	-	20 then 5	20	70

(a) Every run was attempted with 3mg ($6.6.10^{-3}$ mmol) of substrate at room temperature and atmospheric pressure.
(b) Percentage relative to total area of peaks A, B, C and D.
(c) Catalyst/substrate ratios in weight.
(d) Only non characterized over-reduced compounds were formed.
(e) Run 3 : 32.10^{-3} mmol, run 4 : 11.10^{-3} mmol ($\approx 13.10^{-3}$ mmol of quinoline/mg of catalyst) ; 0.5h before precursor was added.
(f) A 10% dispersion of this catalyst in Na_2SO_4 was used so as to avoid weighing small quantities.

were recorded on a Bruker AM (250 MHz). Mass spectra (MS) were recorded on a Finnigan 4690 quadripole spectrometer under Chemical Ionization (CI) conditions.

HPLC conditions

The rate of hydrogenation was monitored by HPLC (Waters, μ Porasil 3.9 mm x 30 cm, eluent 80:20:0.1 hexane-ethylacetate-triethylamine, flow rate 1 mL/min, detection 270 nm). Retention time : unreacted product (2a+2b) : 33.7 min, protected LTB_4 3a : 29.5 min, protected 5-epi-LTB_4 3b : 26.5 min.

12-O-benzoyl-LTB_4 methyl ester 3a and its 5-epimer 3b :

A mixture of 5% $Pd/BaSO_4$ (5 mg) in ethylacetate (750 μl) was pretreated with hydrogen at room temperature for 2h (the catalyst turned black). Then the diyne mixture (2a+2b) (7 mg, 15.10^{-3} mmol) in ethylacetate (250 μl) was introduced via syringe and hydrogenated for 4h. After filtration on a 5 μm Millipore filter and concentration, the product was purified by HPLC on a semi-preparative column (Waters, μ Porasil 7.8 mm x 30 cm, eluent 80:20:0.1 hexane-ethylacetate-triethylamine, flow rate 1.6 mL/min), to give 3b (1.0 mg, 15%) and 3a (1.0 mg, 15%). 3a : ^{1}H-NMR ($CDCl_3$):δ 0.85 ppm (t,3H,H-20,J_{19-20}=7Hz); 1.20-1.75(m,10H,H-3,4,17,18,19); 2.04(m,2H,H-16); 2.34 (m,2H,H-2); 2.53(m,2H,H-13); 3.65(s,3H,OCH_3); 4.56(m,1H,H-5); 5.32-5.53(m,3H,H-6,14,15) 5.56(q,1H,H-12,J_{11-12}=6.5Hz,J_{12-13}=6.5Hz); 5.79(dd,1H,H-11,J_{10-11}=15Hz,J_{11-12}=6.5Hz); 6.05 (t,1H,H-7,J_{6-7}=11Hz,J_{7-8}=11Hz); 6.20(dd,1H,H-9,J_{8-9}=14.5Hz,J_{9-10}=10.5Hz); 6.37(dd,1H, H-10,J_{9-10}=10.5Hz,J_{10-11}=15Hz); 6.49(dd,1H,H-8,J_{7-8}=11Hz,J_{8-9}=14.5Hz). MS.(CI, ammonia) : m/z 472[M^++18], 350[M^++18-C_6H_5COOH]. 3b : ^{1}H-NMR spectrum as 3a.

[6,7,14,15-$^2H_4$]-12-O-benzoyl-LTB_4 methyl ester 4a :

This compound was prepared and purified in the same conditions as 3a and 3b.

4a : ^{1}H-NMR($CDCl_3$):δ 0.87 ppm (t,3H,H-20,J_{19-20}=7Hz); 1.20-1.75(m,10H,H-3,4,17,18,19); 2.03(m,2H,H-16); 2.32(m,2H,H-2); 2.51(m,2H,H-13); 3.64(s,3H,OCH_3); 4.56(br.t,1H,H-5, J_{4-5}=6.5Hz); 5.56(q,1H,H-12,J_{11-12}=6.5Hz,J_{12-13}=6.5Hz); 5.79(dd,1H,H-11,J_{10-11}=15Hz, J_{11-12}=6.5Hz); 6.20(dd,1H,H-9,J_{8-9}=14.5Hz,J_{9-10}=10.5Hz); 6.37(dd,1H,H-10,J_{9-10}=10.5Hz, J_{10-11}=15Hz); 6.49(d,1H,H-8,J_{8-9}=14.5Hz). MS(CI, ammonia) : m/z : 476[M^++18,100%], 354[M^++18-C_6H_5COOH,92%].

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Furthermore when enantiomerically pure diyne 2a was hydrogenated peaks B and D only were observed (Figure 1, profile 2)

Removal of compound B benzoyl protecting group with K_2CO_3/MeOH generated a diol which was identified as LTB_4 methyl ester 1 by correlation with an authentic sample in two different HPLC systems (normal and reverse phases)[6].

Consequently we have established unequivocally that the reduction of the diastereomeric diyne mixture (2a + 2b) affords two compounds 3a and 3b which were separated by HPLC : protected LTB_4 3a had the higher retention time (peak B) and protected 5-epi-LTB_4 3b correspond to peak A.

The experimental data tested are shown in Table 1 :

- with 5% Pd/$BaSO_4$, in methanol (run 2) instead of ethyl acetate (run 1), only over-reduced products were obtained (compounds of lower polarity).

- with a similar quantity of 10% Pd/$BaSO_4$ in ethyl acetate the hydrogenation was complete even when the catalyst was poisoned by quinoline (run 3). However semi-reduction occurred with a 30% catalyst/substrate ratio (run 4).

- with Lindlar catalyst, either a large excess (200%) must be used or the catalyst is added in two batches (runs 5 and 6).

In the same way reduction with deuterium gas of (2a + 2b) was carried out according to the experimental conditions of run 1 to give tetradeuterated compound 4a and 4b in a similar yield. In the ^{1}H-NMR spectrum of 4a the signals at δ 5.3-5.5 (m, H-6, H-14 and H-15), 6.04 (t, H-7) observed for the non-labeled 3a completely disappeared , and the signal at 6.49 for H-8 (dd) was reduced to a doublet. The mass spectrum (CI, ammonia) confirms the tetradeuterated structure of 2a : m/z = 476 [M^++18], 354 [M^++18-C_6H_5COOH] (ions are shifted by 4 mass units). The percentage of deuterium incorporation (not optimized), determined by MS on the [M^++18] ion was 90%.

Thus LTB_4 methyl ester and [6,7,14,15-$^2H_4$]LTB_4 methyl ester were readily obtained by semi reduction of a suitable diyne precursor.

EXPERIMENTAL

General data

Diynes 2a and (2a+2b) were synthesized as previously described[6]. Lindlar catalyst and 10% Pd/$BaSO_4$ were purchased from Fluka, 5% Pd/$BaSO_4$ from Aldrich. Deuterium gas (isotopic purity > 99%) was obtained from Union Carbide. Quinoline was distilled before use. Reductions with hydrogen and deuterium gas were performed in a classical hydrogenation apparatus. ^{1}H-NMR spectra

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