SYNTHESIS OF TETRADEUTERATED LTA₄ METHYL ESTER

J.P. LELLOUCHE, F. AUBERT, J.P. BEAUCOURT *

Service des Molécules Marquées, CEN Saclay, 91191 GIF-SUR-YVETTE, FRANCE.

Summary

Progress in GC/MS coupling in the field of lipids prompted us to prepare deuterated leukotrienes as stable standard compounds. Thus, chiral [11,12,14,15]-tetradeuterated LTA₄ methyl ester 4b was synthesized by reduction of the appropriate diyne precursor and will lead to the corresponding tetradeuterated peptidoleukotrienes.

The complex metabolism of arachidonic acid $^{(1)}$ (AA or 20:4 n-6) implies high performance analysis and identification methods of isolated metabolites. Among such methods as HPLC, RIA or EIA, GC/MS coupling offers the following advantages :

1. chromatographic retention time and mass fragmentometry are characteristic of a compound.

2. amounts of 10 pg to 1 ng can be assayed by this method.

However, GC/MS coupling implies the preparation of standards labelled with stable isotopes such as deuterium or carbon 13.

In our previous work $^{(2)}$, we described the synthesis of two diacetylenic analogues 1 and 2 of LTA₄ methyl ester. We describe here the partial reduction of 1 and 2 by hydrogen or deuterium gas leading to the corresponding LTA₄ methyl ester or its tetradeuterated species as well as their 7Z isomers. Both (11,12) and (14,15) triple bonds of 1 and 2 were simultaneously semi-hydrogenated in this reaction.

Semi-hydrogenation of polyacetylenic compounds is a difficult reaction which yield varies with the stability and reactivity of the substrates ⁽³⁾. Generally, yields increase when Lindlar catalysts are deactivated by organic bases such as pyridine or quinoleine.

Both (5S,6S) diyne epoxydes 1 and 2 have been semi-hydrogenated on Lindlar catalyst ⁽⁴⁾ in anhydrous 0.01 % pyridine in hexane ⁽⁵⁾. After filtration (MILLIPORE LS type, Teflon, 5 μ), the raw product is purified by preparative HPLC (semi-preparative LICHROSORB Si 60 Column, 7 m μ , MERCK; hex/AcOEt/Et₃N : 100/1/1; 5 ml/min). LTA₄ methyl ester 4, its 7Z isomer 7 ^(6,7) as well as intermediates 5, 6, 8 and 9 ⁽⁶⁾ were obtained in various yields (see table).

Remarks : (see table)

- Hexane must contain exactly 0.01 % pyridine in volume. When hexane is used alone (experiments 1 and 2), the yield decreases. An excess of pyridine (0.02 % to 0.05 % in volume) inhibits hydrogen or deuterium absorption.

- Both stereoisomers 1 and 2 have approximately the same reactivity towards H_2 and D_2 (experiments 4,5,7,8).

- By HPLC analyses, it has been shown that both triple bonds in (11,12) and (14,15) positions have the same reactivity during semi-hydrogenation of 1 and 2 (the ratio 5/6 and 8/9 is constant as assayed by UV at 278.5 mµ).

- In experiment 3, the 7a solution contains 45 % of unreacted epoxydiyne 2. Thus, in spite of the yield (43 %), the experimental conditions used in this run cannot be applied in synthesis.

- The lack of mass (4+5+6 versus 1 and 7+8+9 versus 2) is ascribed to non characterized over-reduced compounds and decomposition of isolated epoxydes during the chromatographic purification.



(I): H₂ or D₂, Lindlar catalyst, $0.0 1^0/_0$ pyridine in anhydrous hexane, 20°C, 1 bar.

Expe- riment	1 or 2 mg	Solvent	Catalyst ⁽⁴⁾ (mg)	$\begin{array}{c} H_2 \text{ or } D_2 \\ \% \end{array}$	Yield (b) 4 or 7: mg (%)	Unreacted 1 or 2 : mg	(5+6) ^(d) or (8+9): mg
1	2 : 20	Hexane 5 ml	2	H ₂ :100	7a: 5.4 (27)	2 : 0.4	(8a +9 a) : 1.7
2	2 : 20	11	2	H ₂ :80	7a: 3.6 (23)	2:6.6	(8a +9a) : 2.6
3	2 : 21	0.01 % pyridine in hexane 5 ml	2	H ₂ :40	7a: 3.9 (43)	2:8.5	(8a +9a) : 2.5
4	2 :19.7	"	2	H ₂ :85	7a : 6.1 (36)	2 : 2.2	(8a +9a) : 2.7
5	2 : 22.2	"	2	D ₂ :85	(c) 7 b: 6.3 (32)	2 : 2.3	(8b + 9b): 3.2
6	2 : 22	11	2	D ₂ :85	(c) 7 b : 7.7 (40)	-	(8b + 9b): 3.0
7	1:19.8	11	2	H ₂ :85	4a : 5.5 (32)	1 : 0.4	(5a +6a) : 2.5
8	1:19.3	н	2	D ₂ :85	(c) 4b : 5.5 (33)	1 : 0.7	(5b + 6b) : 2.6

Catalytic semi-hydrogenation of diyne epoxydes 1 and 2

a) The absorption of hydrogen or deuterium was measured by a TOEPPLER pump ⁽⁹⁾ (20°C, 1 bar). Except in experiment 1, hydrogen or deuterium absorption was stopped before reaching stoechiometric amount. Percentage given corresponds to ratio V/V_{th} (V = volume of H₂ or D₂ absorbed; V_{th} = theoretical stoechiometric volume).

b) Yields of 4 and 7 were calculated according to the amount of H_2 or D_2 absorbed, without taking into consideration the possible recycling of 5, 6, 8, 9 and unreacted 1 and 2.

c) 4b and 7b had an isotopic enrichment superior to 98 % by ¹H-NMR (300 MHz; CDCl₃). Deuterium was detected only in positions 11, 12, 14 and 15.

d) Ratios of 5/6 and 8/9 : approximately 1/1.

CONCLUSION

Semi-hydrogenation of epoxy diynes 1 and 2 by H_2 or D_2 led to LTA₄ methyl ester or [11,12,14,15⁻²H₄]- LTA₄ methyl ester and their 7Z isomers, precursors of peptidoleukotrienes which syntheses are in progress.

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4. Lindlar Catalyst (FLUKA n° 62145 Batch 2160 14-782). Reproducible results are obtained with such a commercial catalyst.

5. Pyridine stabilizes reaction products, especially 4 and 7 which are less stable in acidic medium than their corresponding diacetylenic precursors 1 and 2.

6. All compounds were characterized by MS, UV, ¹H-NMR (300 MHz) and 2D-NMR (300 MHz) when the coupling constant J could not be determined by 1D-NMR (chemical shifts homonuclear correlations and J resolved homonuclear correlations). Analyses of **4a** and **7a** are identical to previously published results (8).

7. 4b

 $\begin{array}{l} {\rm RMN-^{1}H} \ (300 \ {\rm MHz}, \ {\rm CDCl}_{3}, \ \delta \ in \ ppm): 0.90 \ (t, \ 3H, \ H_{20}, \ J_{19,20} = 7.0 \ Hz) \ ; \ 1.20-1.40 \ (broad signal, \ 6H, \ H_{17} + H_{18} + H_{19}) \ ; \ 1.65 \ (multiplet, \ 2H, \ H_{4}) \ ; \ 1.80 \ (multiplet, \ 2H, \ H_{3}) \ ; \ 2.05 \ (t, \ 2H, \ H_{16}, \ J_{16,17} = 6.9 \ Hz) \ ; \ 2.40 \ (t, \ 2H, \ H_{2}, \ J_{2,3} = 7.5 \ Hz) \ ; \ 2.85 \ (t, \ 1H, \ H_{5}, \ J_{4,5} = 5.5 \ Hz) \ ; \ 2.90 \ (s, \ 2H, \ H_{13}) \ ; \ 3.15 \ (dd, \ 1H, \ H_{6}, \ J_{5,6} = 2.1 \ Hz, \ J_{6,7} = 7.9 \ Hz), \ 3.65 \ (s, \ 3H, \ -CO_2CH_3) \ ; \ 5.40 \ (dd, \ 1H, \ H_{7}, \ J_{7,8} = 15.1 \ Hz) \ ; \ 6.20 \ (dd, \ 1H, \ H_{9}, \ J_{8,9} = 10.8 \ Hz, \ J_{9,10} = 14.8 \ Hz) \ ; \ 6.45 \ (dd, \ 1H, \ H_{8}) \ ; \ 6.55 \ (d, \ 1H, \ H_{10}). \end{array}$

SM : m/e = 336 (M^{+.}); $[\alpha]^{20}$ = -28.4 ° (0.46 g in 100 ml hexane); UV (hexane) : $\lambda \max_1 = 268.0 \text{ m}\mu$ (41300); $\lambda \max_2 = 278.5 \text{ m}\mu$ (51900); $\lambda \max_3 = 290.5 \text{ m}\mu$ (38700).

7 b

 $\begin{array}{l} {\rm RMN}^{-1}{\rm H} \ (300 \ {\rm MHz}, \ {\rm CDCl}_3, \ \delta \ {\rm in} \ {\rm ppm}): 0.90 \ (t, \ 3H, \ {\rm H}_{20}, \ {\rm J}_{19,20} = 7.0 \ {\rm Hz}) \ ; \ 1.20\text{-}1.40 \ (broad signal, \ 6H, \ {\rm H}_{17} + \ {\rm H}_{18} + \ {\rm H}_{19}): 1.55\text{-}1.90 \ (broad signal, \ 4H, \ {\rm H}_3 + \ {\rm H}_4): 2.05 \ (t, \ 2H, \ {\rm H}_{16}, \ {\rm J}_{15,16} = 6.9 \ {\rm Hz}): 2.40 \ (t, \ 2H, \ {\rm H}_2, \ {\rm J}_{2,3} = 7.5 \ {\rm Hz}): 2.88 \ (dt, \ 1H, \ {\rm H}_5, \ {\rm J}_{4,5} = 4.9 \ {\rm Hz}, \ {\rm J}_{5,6} = 1.8 \ {\rm Hz}): 2.95 \ (s, \ 2H, \ {\rm H}_{13}): 3.50 \ (dd, \ 1H, \ {\rm H}_6, \ {\rm J}_{6,7} = 8.5 \ {\rm Hz}): 3.80 \ (s, \ 3H, \ -{\rm CO}_2{\rm CH}_3): 5.05 \ (dd, \ 1H, \ {\rm H}_7, \ {\rm J}_{7,8} = 11.0 \ {\rm Hz}): 6.30 \ ({\rm multiplet}, \ 1H, \ {\rm H}_8, \ {\rm J}_{8,9} = 9.2 \ {\rm Hz}): 6.60 \ ({\rm multiplet}, \ 2H, \ {\rm H}_9 + \ {\rm H}_{10}, \ {\rm J}_{9,10} = 14.7 \ {\rm Hz}). \end{array}$

SM : m/e = 336 (M^{+.}); $[\alpha]^{20}$ = -44.1 ° (0.38 g in 100 ml hexane); UV (hexane) : $\lambda \max_1 = 268.5 \text{ m}\mu$ (39300); $\lambda \max_2 = 278.5 \text{ m}\mu$ (50600); $\lambda \max_3 = 290.0 \text{ m}\mu$ (37500).

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