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SYNTHESIS OF DIACETYLENIC ANALOGUES OF LEUKOTRIENE A_4 (LTA_4) METHYL ESTER

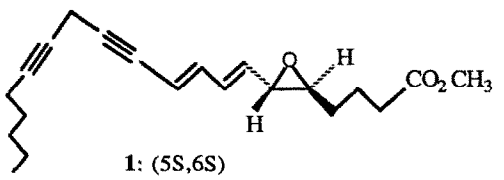
J.P. LELLOUCHE, J. DESCHAMPS, C. BOULLAIS, J.P. BEAUCOURT *

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

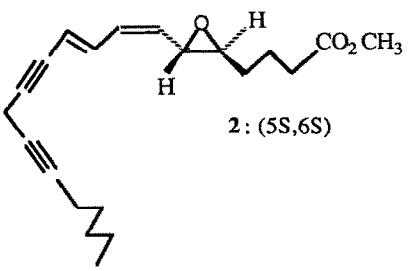
Summary

Methyl (5S,6S)-epoxy-11,14-eicosadiyne-7E,9E-dienoate **1** and its 7Z isomer **2** were prepared using Wittig methodology. **1** did not inhibit human neutrophil 5-lipoxygenase but rather stimulated LTB_4 formation.

Arachidonic acid [AA or 20:4 (n-6)] is a polyunsaturated 20 carbon acid. It is an important component of membrane phospholipids in animal cells. Various phospholipases can release AA, which is metabolized by 5-lipoxygenase to give peptidoleukotrienes [1] through LTA_4.
As a part of our studies on pharmacologically active lipids, we report here the syntheses of two diacetylenic analogues of LTA_4 methyl ester : methyl (5S,6S)-epoxy-11,14-eicosadiyne-7E,9E-dienoate **1** and its 7Z isomer **2** :

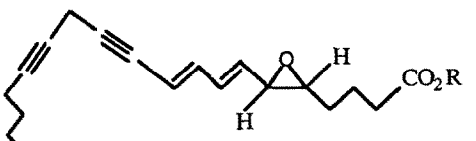


1: (5S,6S)



2: (5S,6S)

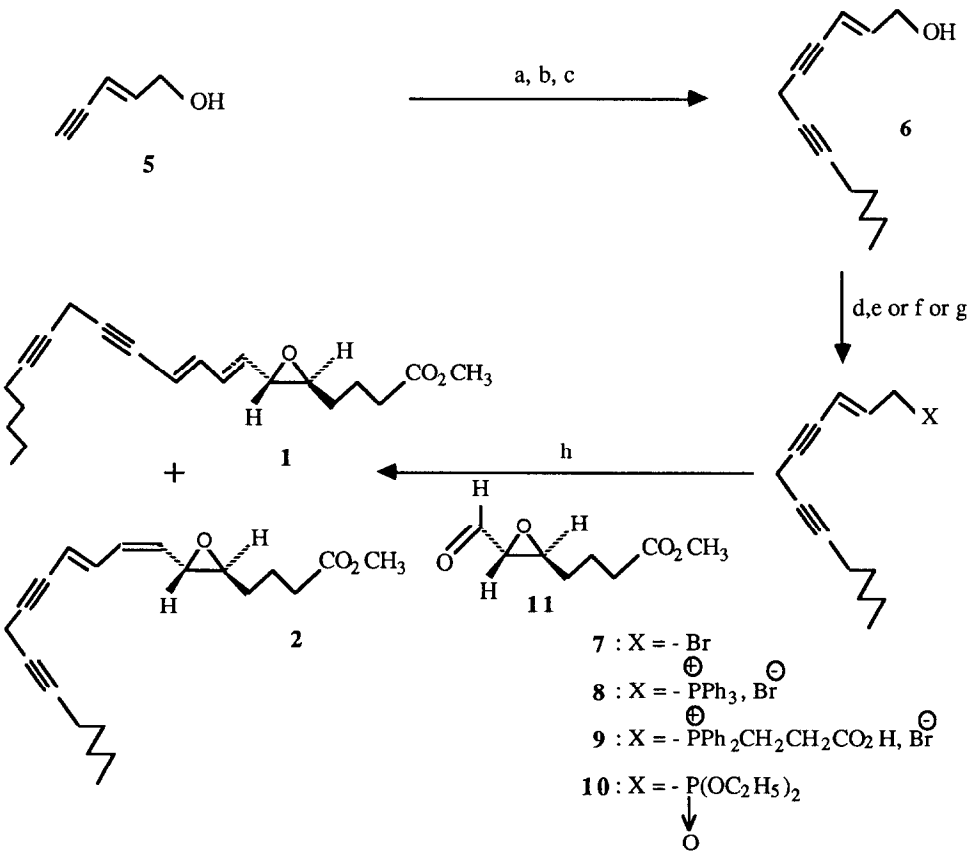
The synthesis of racemic epoxydiyne **4** via sulfonium ylid chemistry [2,3] has two major drawbacks :
- the time of reaction must not exceed 1 min at -25°C
- as **4** is a racemic, its ring opening by thiopeptides (glutathione, cysteinylglycine, cysteine) leads to diastereoisomeric diacetylenic peptidoleukotrienes which ought to be separable by HPLC. However, only the chromatographic resolution of the diastereomeric diacetylenic LTE_4 has been described [2].



R = H : (±) **3**

R = CH_3 : (±) **4**

Both analogues **1** and **2**, and especially **1**, are particularly interesting because :
a) controlled Lindlar semi-hydrogenation of **1** with deuterium or tritium gas leads to 11,12,14,15-tetradeuterated or -tritiated LTA_4 methyl ester [11].
b) **1** and **2** are precursors in the syntheses of acetylenic analogues of peptidoleukotrienes.
On the other hand, racemic epoxydiyne **3** has been shown to inhibit 50 % of SRS-A activity at a concentration of 10 μM. The (5S,6R)-LTE_4 analogue shows 50 % SRS-A activity at 11 μM[2].
Thus, it seemed interesting to synthesize the chiral diacetylenic LTA_4-methyl ester analogues **1** and **2** according to the following scheme, in order to test their biological properties as well as those of the corresponding peptidoleukotrienes.



a : CH_2 = CH-OC_2H_5, toluenesulfonic acid, 0°C, 1 h ; b : C_2H_5MgBr, THF, 20°C ; CuBr, 20°C ; n-C_5H_{11}-C≡C-CH_2Br, 60°C, 45 min ; c : 0.5 N H_2SO_4, CH_3COCH_3, 3 h, 20°C ; d : PBr_3, 5 % pyridine in ether, 18 h, 20°C ; e : PPh_3, ether, 20°C, 48 h ; f : $PPh_2CH_2CH_2CO_2H$, ether, 20°C, 48 h ; g : $P(OC_2H_5)_3$, CH_3CN, 80°C, 7 h ; h : nBuLi : 1 eq for **8** and **10**, 2 eq for **9** ; anhydrous THF ; -80°C ; 30 min.

Diyne enol **6**, prepared from pent-4-yn-(2E)-en-1-ol **5** [4] according to ROSENBERGER et al.[3], was brominated to **7** [5] (63 % yield). When **7** was treated with triphenylphosphine, 3-diphenylphosphinoyl-propanoic acid [6] or triethyl phosphite, phosphonium salts **8** (85 %) and **9** (50 %) or phosphonate **10** (72 %) were formed [5,7]. A solution of one equivalent of chiral epoxyaldehyde **11** [8] in anhydrous THF was added dropwise to the ylid of **8**, **9** or **10** over the period of one hour at 0°C. The crude mixture of **1** and **2** obtained was purified by column chromatography on silica gel (hexane/ethyl acetate/triethylamine : 70/30/02). Final purification allowed the isolation of 7E-**1** and 7Z-**2** [9] (Yield from ylid of **8** : 40 % of **1/2** (21/79), from ylid of **9** : 44 % of **1/2** (54/46), from ylid of **10** : 25 % of **1/2** (81/19).

It must be noticed that the partially stabilized ylids react according to previously described results [10].

The lithio phosphonate formed from **10** is probably too nucleophilic towards the fragile epoxy bridge of **11**, which can explain the lower yield of **1/2** obtained (25 %), 7E-**1** being predominant. Better yields were obtained with the less nucleophilic phosphonium salts of **8** and **9**. The best results were obtained with the ylid of **9** (44 % of **1/2** : 54/46 with a slightly predominant 7E isomer formation) due to the following factors :

- acidic phosphine oxide formed during the coupling reaction is eliminated in the aqueous phase, which makes easier further HPLC purification of **1/2**.
- in our experimental conditions the lithio carboxylic group of the ylid of **9** is compatible with the epoxy bridge.

Biological evaluation of 1 .

Saponified **1** [12] did not inhibit soybean 15-lipoxygenase confirming the structural specificity of the enzyme for a -(1Z,3Z)-pentadiene unit.

The formation and assay of 5-HETE at 234 mμ, LTB_4, ω-hydroxy LTB_4 and ω-carboxy LTB_4 during the incubation of **1** with human neutrophils (10-30 mn incubation with 5 x 10^6 cells) showed that **1** did not inhibit human neutrophil 5-lipoxygenase [13]. It rather increased of about 30 % the amount of LTB_4 formed at concentrations of 1-10 μM [13]. The better bioavailability of endogeneous LTA_4 for LTA_4-hydrolase could account for this result. Since diacetylenic peptidoleukotrienes (DAPTS) are devoid of any contractile activity on guinea pig ileum [14], inhibition of glutathione-transferase by endogeneously biosynthetized DAPTS might be involved and explain our results as well as those reported by Rosenberger [2] on racemic (±) **3**.

On the other hand, **1** inhibited ω-hydrolase by 40 % at a concentration of 1μM and by 80 % at 10 μM [13].

REFERENCES AND NOTES

1 - S. HAMMARSTRÖM, R.C. MURPHY, B. SAMUELSSON, D.A. CLARK, C. MIOSKOWSKI, E.J. COREY, Biochim. Biophys. Res. Commun., 1979, 91, 1266 ; R.C. MURPHY, S. HAMMARSTRÖM, B. SAMUELSSON, Proc. Natl. Acad. Sci. USA, 1979, 76, 4275.

2 - M. ROSENBERGER, Eur. Pat. Appl. EP 36,663/30 Sept. 1981 (CA : 96, 181124 u).

3 - M. ROSENBERGER, C. NEUKOM, J. Amer. Chem. Soc., 1980, 102, 5426.

4 - Manufactured by FARCHAN, Division Chempsampco, Inc.

5 - All intermediates had satisfactory ^{1}H-NMR, MS and UV data.

6 - H. DANIEL, M. LE CORRE, Tetrahedron Lett., 1987, 28, 1165. We thank M. LE CORRE for providing us with acidic phosphine.

7 - Phosphonium salts **8** and **9** are stable when stored at 0°C. Phosphonate **10** has to be stored in anhydrous THF solution at -80°C (2mg of **10** in 1 ml THF).

8 - J. ROKACH, R. ZAMBONI, C.K.LAU, Y. GUINDON, Tetrahedron Lett., 1981, 22, 2759.

9 -**1** UV (C_2H_5OH) : λmax_1 = 271 mμ (44500) ; λmax_2 = 283 mμ (35800). $[\alpha]^{20}$ = - 43° ($CHCl_3$; 1.14 g/100 ml) ; SM : m/e = 328.5 ($M^{+\cdot}$; 14.4 %) ; RMN-^{1}H (250 MHz ; $CDCl_3$; δ en ppm ; TMS standard) ; 0.90 [t, 3H (H_{20}), $J_{19,20}$ = 7.0 Hz] ; 1.32 to 1.80 [broad signal, 10 H (H_3 + H_4 + H_{17} + H_{18} + H_{19})] ; 2.15 [t, 2H (H_{16}), $J_{16,17}$ = 7.0 Hz] ; 2.38 [t, 2H (H_2), $J_{2,3}$ = 7.2 Hz] ; 2.87 [t, 1H (H_5), $J_{4,5}$ = 5.0 Hz] ; 3.13 [dd, 1H (H_6), $J_{5,6}$ = 2.0 Hz, $J_{6,7}$ = 8.0 Hz] ; 3.32 [s, 2H (H_{13})] ; 3.68 [s, 3H (-OCH_3)] ; 5.47 [dd, 1H (H_7), $J_{7,8}$ = 15.0 Hz] ; 5.63 [d, 1H (H_{10}), $J_{9,10}$ = 15.0 Hz] ; 6.40 [dd, 1H (H_8), $J_{8,9}$ = 11.0 Hz] ; 6.53 [dd, 1H (H_9)] ;

2 UV (C_2H_5OH) : λmax_1 = 271.5 mμ (32800) ; λmax_2 = 283.5 mμ (39900). $[\alpha]^{20}$ = - 8.5° ($CHCl_3$; 6.1 g/100 ml) ; SM : m/e = 328,5 ($M^{+\cdot}$; 19 %) ; RMN-^{1}H (250 MHz ; $CDCl_3$; δ en ppm ; TMS standard) ; 0.92 [t, 3H (H_{20}), $J_{19,20}$ = 7.0 Hz] ; 1.32 to 1.83 [broad signal, 10H (H_3 + H_4 + H_{17} + H_{18} + H_{19})] ; 2.17 [tt, 2H (H_{16}) , $J_{16,17}$ = 7.0 Hz, $J_{13,16}$ = 3.0 Hz] ; 2.40 [t, 2H (H_2), $J_{2,3}$ = 7.2 Hz] ; 2.88 [multiplet, 1H (H_5)] ; 3.33 [multiplet, 2H (H_{13})] ; 3.48 [dd, 1H (H_6), $J_{5,6}$ = 2.0 Hz, $J_{6,7}$ = 9,0 Hz] ; 3.70 [s, 3H (-OCH_3)] ; 5.10 [t, 1H (H_7), $J_{7,8}$ = 10.0 Hz] ; 5.67 [d, 1H (H_{10}) , $J_{9,10}$ = 16.0 Hz] ; 6.23 [t, 1H (H_8), $J_{8,9}$ = 10.0 Hz] ; 6.95 [dd, 1H (H_9)].

10 - Organophosphorus Reagents in Organic Synthesis, ed. J.L.G. CADOGAN, 1979, Academic Press, New-York.

11 - Partial reduction of **1** and **2** by deuterium gas will be described elsewhere (Tetrahedron Letters, following paper).

12 - F. FITZPATRICK, D. MORTON, M. WYNALDA, J. Biol. Chem., 1982, 257, 4680.

13 - We thank Pr. DELAFORGE (UA 400 - CNRS, Université R. DESCARTES, 45 rue des Saints-Pères, 75270 PARIS CEDEX O6) for these data.

14 - These results will be soon reported : J.P. LELLOUCHE, F. AUBERT, J.P. BEAUCOURT, E. RECHENCQ, G. NIEL, J.P. GIRARD, J.C. ROSSI, M. BOUCARD, Prostaglandins, in press.

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