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SYNTHESIS OF THE 7-CIS ISOMER OF THE NATURAL LEUKOTRIENE D_4

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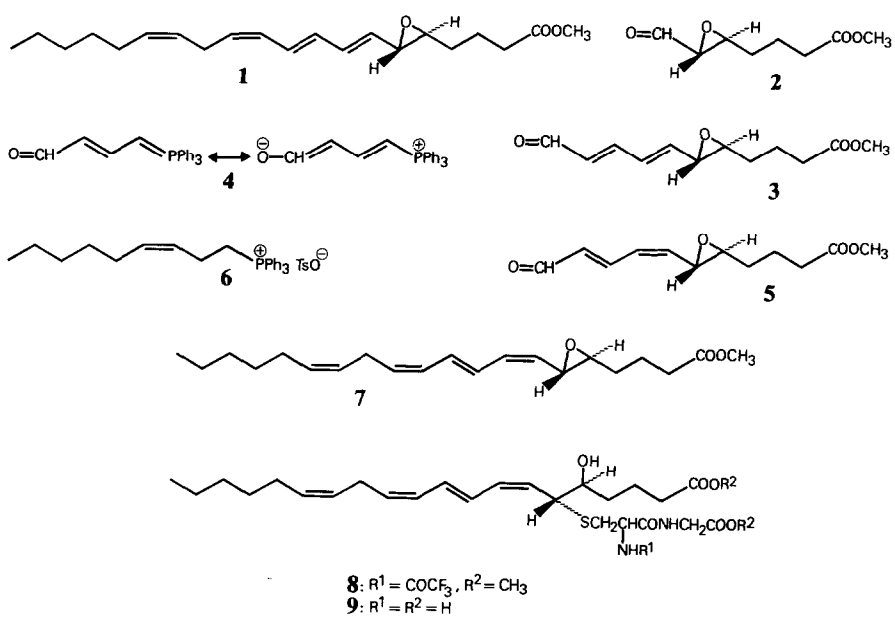
Summary: Methyl 5(S),6(S)-oxido-11-oxo-7-*cis*-9-*trans*-undecadienoate 5 was prepared and used for the synthesis of the novel 7-*cis*-LTD_4 (9) as well as, after isomerization to the *all-trans* dienal ester 3, for that of the natural leukotrienes.

In the pioneering synthesis by Corey et al.[1] of the optically active leukotriene A_4 methyl ester 1, the two *trans* double bonds of the conjugated triene system were introduced in a two-step extension of the epoxy aldehyde ester 2 to the diene homolog 3 using the four-carbon reagent (1-lithio-4-ethoxybutadiene) developed by Wollenberg [2].



In this letter we wish to report on an easy and efficient, two-step, conversion of 2 to 3 forming a practical alternative to the Wollenberg extension. In addition to the natural leukotrienes accessible from 3, a new intermediate (5) of our procedure made the synthesis of the unknown 7-*cis* stereoisomers of leukotriene A_4 methyl ester (7) and D_4 (9) possible.

Slow addition (over 1 h) at R.T. of a solution of the epoxy aldehyde ester 2[1] (2.89 mmol) in CH_2Cl_2 (15 ml) to triphenylphosphoranylidene-crotonaldehyde 4[3] (3.45 mmol) in the same solvent (15 ml) followed by stirring at R.T. for another 1.5 h and column chromatography (Merck silica gel, toluene: ethyl

acetate 4:1) of the crude product afforded an oily, about 4:1, mixture (^{1}H-NMR evidence[4]) of the novel *cis, trans* diene aldehyde ester 5 and of its *trans, trans* isomer 3 (total yield 91 %). Iodine (3 mg)-catalyzed isomerization of this mixture in CH_2Cl_2 (20 ml) resulted, after 2h at R.T., in a complete conversion to the crystalline *trans,trans* diene aldehyde 3 (92 % after chromatography on silica gel, toluene: ethyl acetate 4:1), m.p. 57.5-58.5°C (from Et_2O), $[\alpha]_D^{20}$ -31° ± 1° (c 1.98, $CHCl_3$).

Wittig condensation (in THF - hexamethylphosphoric triamide,-78°C) of the dienal ester 3 with a phosphorane prepared *in situ* (BuLi) from the crystalline phosphonium tosylate 6[5] (1.1 equiv.) gave, after work-up and purification as described by Corey[1], LTA_4 methyl ester 1 (88 %), m.p. 28-32°C, λ_{max}^{MeOH} 270,278, 290 nm (ε 43 900, 56 700, 43 100)[6,7]. Using the two-step procedure of Corey et al.[1], leukotriene D_4 (potassium salt), λ_{max}^{EtOH} 271, 280.5, 290 nm(ε 39 200, 48 600, 38 400)[6], homogeneous on reversed-phase HPLC[13] (MeCN: H_2O : AcOH = 650 : 350 : 1, adjusted to pH 5.6 with NH_4OH) and of comparable biological activity with that of the natural LTD_4[8,9], was prepared from the latter compound.

On the other hand, the *all-trans* dienal ester 3 could be partially separated from the above-mentioned 1:4 mixture with 5 by low-temperature (-25°C) crystallization from a concentrated Et_2O solution; chromatography (silica gel, toluene : ethyl acetate 4:1) of the mother liquor residue afforded then the *cis, trans* isomer 5 (64-70% from 2) as a yellowish oil, λ_{max}^{EtOH} 275 nm, according to high-resolution ^{1}H-NMR[4] still containing about 10% of 3. Removal of the residual 3 proved difficult, however, thanks to a clean HPLC separation in the last step of the synthesis, pure 7-*cis*-LTD_4 could ultimately be prepared from this 90 % pure 5.

Reaction of 5 (THF-hexamethylphosphoric triamide,-78°C) with 1-triphenylphosphoranylidene-3-*cis*-nonene, liberated, as before in the preparation of LTA_4 methyl ester 1, from the phosphonium tosylate 6[5] (1.1 equiv.), gave, after 20 min. at -78°C, quenching with phosphate buffer, pH 8.0, extraction with Et_2O and, finally,rapid chromatography on basic alumina (Woelm, act. V; hexane with 0.5% Et_3N), the methyl ester of 7-*cis*-LTA_4 7 (73%) as a colorless oil containing about 10% of LTA_4 methyl ester 1 as the only contaminant (^{1}H-NMR evidence).[10]

Opening of the epoxide ring of 7 with N-trifluoroacetyl - L-cysteinylglycine[11] (2 equiv.) and triethylamine (4 equiv.) in MeOH was accomplished in 3 h at R.T. and afforded, after RP plate chromatography[12] (MeCN:H_2O = 2:1) (91% yield) and additional RP HPLC purification[13] (MeCN:H_2O:MeOH = 6.5:2.5:1), the dimethyl ester of N-trifluoroacetyl-7-*cis*-leukotriene D_4 8 (stereochemically 90% pure)[14] (50-55%). Its deprotection with 0.13 M K_2CO_3 (5 equiv.) in MeOH-H_2O (1:3) (40 h, R.T.) and RP plate chromatography[12] (MeCN:H_2O = 2:1) resulted in a 9:1 mixture (56%) of 7-*cis*-LTD_4 9 and of LTD_4 (as K-salts); the two components were separated by RP-HPLC[13] in a MeOH-THF-H_2O (48:12:40) system containing 0.04% AcOH and adjusted to pH 5.4 with aqueous ammonia (retention times of 9 and LTD_4, at R.T. and flow-rate of 0.8 ml/min., being 19.6 and 22.1 min., resp.).

Pure 7-*cis*-LTD_4 9, λ_{max}^{EtOH} 271.5, 281, 290.5 nm ($\varepsilon_{281} \sim 50000$)[15], displayed con-

tractile activity on isolated guinea pig ileum and induced bronchoconstriction in anesthetized guinea pig, its activity in both cases being about 1/10 of that of LTD_4[16].

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References and Notes

1) E.J.Corey, D.A.Clark, G.Goto, A.Marfat, Ch.Mioskowski, B.Samuelsson and S. Hammarström, J.Am.Chem.Soc. 102, 1436 (1980).

2) R.H.Wollenberg, Tetrahedron Letters, 717 (1978).

3) The phosphorane 4 was prepared essentially according to M.J.Berenguer, J. Castells, R.M.Galard and M.Moreno-Manas, Tetrahedron Letters, 495 (1971). Reaction of γ-bromocrotonaldehyde [M.J.Berenguer, J.Castells, J.Fernandez and R.M.Galard, Tetrahedron Letters, 493 (1971)] (0.09 mole) with Ph_3P (0.1 mole) in acetone (60 ml) at R.T. for 16 h. gave a pale yellow crystalline precipitate of the phosphonium bromide, m.p. 174-176°C (71%) from which 4 was liberated in CH_2Cl_2 with an excess of cold 1 N NaOH. Trituration of the crude CH_2Cl_2 extract with cold acetone afforded deep-red crystals of 4, m.p. 163-165°C (lit. :"unstable crystals", m.p. 153-155°C) (81%), stable even on prolonged storage at R.T.

4) ^{1}H-NMR data on 5 (360 Mc, $CDCl_3$, δ in p.p.m.): 1.6-1.9 [2H(3)+2H(4)], 2.41 [2H(2)], 2.93 [H(5), $J_{5,6}$= 2Hz], 3.56 [H(6), $J_{6,7}$= 8Hz], 3.68 [OCH_3], 5.59 [H(7), $J_{7,8}$ = 11 Hz], 6.21 [H(10), $J_{9,10}$=15, $J_{10,11}$= 8 Hz], 6.46 [H(8), $J_{8,9}$ = 12 Hz], 7.60 [H(9)], 9.65 [H(11)]. For ^{1}H-NMR data on the *all-trans* isomer 3, see reference 1.

5) Phosphonium tosylate 6, m.p. 80-83°C (CH_2Cl_2-Et_2O), was prepared by heating at 90°C (argon) 3-*cis*-nonene-1-yl-*p*-toluenesulfonate[1] (0.1 mole) and Ph_3P (0.1 mole) in acetonitrile (40 ml) for 48 h. and crystallization of the crude product from the above-mentioned two solvents ; white crystals thus obtained contain about 0.5 mole CH_2Cl_2 and 0.25 mole Et_2O as solvents of crystallization.

6) For reproduceable ε values, rigorous exclusion of air in the preparation of the highly dilute UV samples was found essential.

7) Further spectral characterization of 1: IR (CH_2Cl_2): 3000, 2950, 2920, 2855, 1730, 1460-1420, 1200, 1173, 1110, 1000, 968, 891, 860 cm^{-1}. -^{1}H-NMR (360 Mc, $CDCl_3$, δ in p.p.m.): 0.90 [3H(20)], 2.07 [2H(16), $J_{15,16}$= 7.5 Hz], 2.39 [2H(2)], 2.86 [H(5), $J_{5,6}$= 2 Hz], 2.95 [2H(13), $J_{12,13}$= $J_{13,14}$= 7.5 Hz], 3.14 [H(6), $J_{6,7}$= 7.5 Hz], 3.68 [OCH_3], 5.3 - 5.5 [H(7) + H(12) + H(14) + H(15)], 6.01 [H(11), $J_{10,11}$= $J_{11,12}$= 11 Hz], 6.19 [H(9), $J_{8,9}$= 11, $J_{9,10}$= 15 Hz] , 6.47 [H(8), $J_{7,8}$= 15 Hz], 6.55 [H(10)].

8) R.A.Lewis, K.F.Austen, J.M.Drazen, D.A.Clark, A.Marfat and E.J.Corey, Proc. Natl.Acad.Sci. USA 77, 3710 (1980).

9) E.J.Corey, D.A.Clark, A.Marfat and G.Goto, Tetrahedron Letters, 3143 (1980).

10) The following data were obtained on 7 as described in the text: R_f(Et_3N-pretreated silica gel plate, hexane: Et_2O 1:1): 0.49. - λ_{max}^{MeOH} 270, 278.5, 290. - IR (CH_2Cl_2): 3000, 2940, 2910, 2850, 1727, 1460-1410, 1200, 1167, 1105, 990, 940, 880, 860 cm^{-1}. - ^{1}H-NMR (360 Mc, $CDCl_3$, δ in p.p.m.): 0.89 [3H(20)], 2.06 [2H(16)],2.39 [2H(2)], 2.88 [H(5), $J_{5,6}$= 2 Hz], 2.96 [2H(13), $J_{12,13}$ = $J_{13,14}$= 8 Hz] , 3.50 [H(6), $J_{6,7}$= 8.5 Hz], 5.06 [H(7), $J_{7,8}$= 10.5 Hz], 5.35 [H(14), $J_{14,15}$= 10.5 Hz], 5.43 [H(15), $J_{15,16}$= 7 Hz], 5.50 [H(12), $J_{11,12}$= 10.5 Hz], 6.09 [H(11), $J_{10,11}$= 10.5 Hz], 6.31 [H(8), $J_{8,9}$= 11 Hz], 6.6 [H(9) + H(10)]. Several characteristic peaks suggest the presence of ∿10% of LTA_4 methyl ester 1.

11) The protected dipeptide was prepared according to Corey et al.[9]. The corresponding cystine derivative was purchased from BACHEM AG, Bubendorf, Switzerland.

12) Preparative plates Opti-UP C_{12}, ANTECH AG, Bennwil, Switzerland.

13) Nucleosil C_{18}, 10μ, of Macherey-Nagel, Düren, W.Germany, was used as column material.

14) Characteristic data on 8: R_f(Et_3N-pretreated silica gel plate, toluene: ethyl acetate 1:1) : 0.33. - IR(CH_2Cl_2): 3370 (broad), 2950, 2920, 2850, 1750-1720, 1680, 1520 (broad), 1435, 1210, 1167, 995 cm^{-1}. - λ_{max}^{MeOH} 272, 281.5, 291.5. - ^{1}H-NMR (360 Mc, $CDCl_3$, δ in p.p.m.): 0.90 [3H(20)], 2.08 [2H(16), $J_{15,16}$= = 7 Hz], 2.37 [2H(2)], 2.79 + 2.95 [2H($CH_{2(cys)}$)], 2.95 [2H(13), $J_{12,13}$= $J_{13,14}$= 8 Hz], 3.67 and 3.78 [2 x OCH_3], ∿3.8 [H(5)], ∿4.0-4.1 [2H($CH_{2(gly)}$)], 4.04 [H(6), $J_{5,6}$= 3.5, $J_{6,7}$= 10.5 Hz], 4.58 [$CH_{(cys)}$], 5.32 [H(14), $J_{14,15}$ = 11 Hz], 5.43 [H(15)], 5.49 [H(7), $J_{7,8}$= 11 Hz], 5.52 [H(12), $J_{11,12}$ = 11 Hz], 6.10 [H(11), $J_{10,11}$= 11 Hz], 6.38 [H(8), $J_{8,9}$= 11 Hz], 6.52 [H(9), $J_{9,10}$= 14 Hz], 6.67 [H(10)], 6.90 [$NH_{(gly)}$], 7.47 [$NH_{(cys)}$]. Several additional, small, peaks suggest the presence of ∿10% of the 7-*trans* isomer of 8 (*i.e.* protected LTD_4).

15) ^{1}H-NMR spectrum on 9 (360 Mc, CD_3OD, δ in p.p.m.): 0.90 [3H(20)], 2.10 [2H (16)], 2.95 [2H(13)], 5.3 - 5.5 [H(7) + H(12) + H(14) + H(15)], 6.13 [H(11), $J_{10,11}$= $J_{11,12}$= 10.5 Hz], 6.32 [H(8), $J_{7,8}$= $J_{8,9}$= 10.5 Hz], 6.57 [H(9), $J_{9,10}$ = 15 Hz], 6.65 [H(10)].

16) R.Menassé and U.Niederhauser, biological results to be published elsewhere.

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