Tetrahedron Letters,Vol.28,No.42,pp 4917-4920,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

BIOMIMETIC TOTAL SYNTHESIS OF COLNELEIC ACID AND ITS FUNCTION AS A LIPOXYGENASE INHIBITOR

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Summary: A biomimetic synthesis of colneleic acid (**2**) from 9*(S)*-hydroperoxy-10*(E)*,12*(Z)*-octadecadienoic acid (**1**) is reported. The lipoxygenase of potato which converts linoleic acid to **1** was found to be strongly inhibited by acid **2** (K_i = 8μM).

The lipoxygenase (LO) of potato *(Solanum tuberosum)* transforms the native polyunsaturated fatty acids, linoleic and linolenic acids, to a mixture of 9*(S)*- and 13*(S)*-hydroperoxides.[1] The release of free linoleic and linolenic acids, their lipoxygenation, and the subsequent conversion of the resulting hydroperoxides to other metabolites are all strongly stimulated by disruption of plant tissue. An enzyme preparation from potato homogenate transforms the 9*(S)*-hydroperoxide of linoleic acid (**1**) into the unusual divinyl ether colneleic acid (**2**), the physiological function of which is still unknown.[2] The potato system appears to be the first reported to catalyze this type of conversion,[3] although it is well known that various lipoxygenases can catalyze further conversions of the initially produced hydroperoxides. For example, soybean lipoxygenase catalyzes an allylic hydroperoxide → oxiranylcarbinol rearrangement which produces 13*(R)*-hydroxy-14*(R)*,15*(S)*-oxido-5,8,11*(Z)*-eicosatrienoic acid from 15*(S)*-hydroperoxy-5,8,11*(Z)*,13*(E)*-eicosatetraenoic acid (15-HPETE).[4] The biosynthesis of **2** from hydroperoxide **1**, which possibly involves a Baeyer-Villiger-like rearrangement (A→B→C), provides additional incentive for the investigation of such a pathway for the chemical synthesis of **2**. We report herein the successful realization of this biomimetic synthesis and the finding that colneleic acid is a very effective inhibitor of the potato lipoxygenase, a fact which suggests a possible role of **2** as a biological regulator.

Treatment of the methyl ester of **1**[5] with 2,6-dichlorobenzoic anhydride (3 equiv), 2,6-di-*t*-butyl-4-methylpyridine (3 equiv) and 4-dimethylaminopyridine (0.5 equiv) in methylene chloride at 23°C for 4 h, addition of a further 0.5 equiv of 4-dimethylaminopyridine and an additional reaction period of 6 h at 23°C, dilution with hexane and chromatography on silica gel gave the vinyl ether 2,6-dichlorobenzoate **3**[6] (62-73% yield) as a colorless oil. The use of a hindered base is critical since unhindered bases such as pyridine result in methyl 9-oxo-10*(E)*,12*(Z)*-octadecadienoate as a major product, formed by a simple secondary hydroperoxide benzoate → ketone elimination pathway. Acetic anhydride was not a satisfactory reagent since it produced lower yields (*ca.* 30%) of the required product than did 2,6-dichlorobenzoic anhydride.

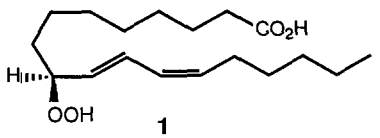
In addition to affording **3** in good yield, the dichlorobenzoic anhydride procedure detailed above, also gave colneleic acid methyl ester (**5**) in *ca.* 2.4% yield and the corresponding 8,9-*Z* isomer (**6**) in *ca.* 0.6% yield. Direct transformation of **3** to methyl colneleate was not possible under a variety of conditions including (1)

thermolysis in benzene or acetonitrile in the presence of bases such as 2,6-lutidine or CaH_2, (2) treatment with potassium hexamethyldisilazide or diethylaluminum diisopropylamide, (3) triethylaluminum (which cleanly replaced 2,6-dichlorobenzoyloxy by ethyl), or (4) trimethylsilyl triflate-diazabicycloundecene (DBU). The resistance of **3** to elimination indicates that methyl colneleate is probably formed in the above reaction by direct deprotonation of the oxocarbonium ion intermediate.

Reaction of **3** with excess $Me_2AlSeMe$[7] in methylene chloride at -40°C for 30 min resulted in smooth conversion to the corresponding methylseleno ether **4** (87% yield after chromatography on silica gel using 20 : 1 hexane-ethyl acetate for elution).[8] Elimination of the methylseleno group from **4** was effected by oxidation to the selenoxide using 1.2 equiv of 2-benzenesulfonyl-3-phenyloxaziridine in methylene chloride containing 20 equiv of pyridine at 0°C for 15 min, addition of hexane and heating at 40°C for 30 min. Chromatography on silica gel using 20 : 1 hexane-ethyl acetate for elution furnished 79% of a mixture of methyl colneleate (**5**) and the corresponding 8,9-*Z* isomer **6** in a ratio of 1 : 1 (^{1}H NMR analysis). Separation of **5** and **6** was effected by preparative HPLC on silica gel (DuPont *Zorbax*-SIL, 0.2% THF in hexane, R_t of **5**, 14.4 min; R_t of **6**, 12.2 min).[9,10] A reference sample of methyl colneleate was prepared by incubation of the 15,000 g supernatant from potato homogenate at pH 7.5 with linoleic acid,[2] extraction, esterification and chromatography. This reference sample and synthetic **5** were identical by UV, IR, ^{1}H NMR and HPLC comparison. Saponification of **5** was accomplished by treatment with 2 : 1 dimethoxyethane-1M aqueous lithium hydroxide for 3 h at 23°C, acidification at 0°C to pH 4 and extraction with ether to give **2**. Because of the sensitivity of the vinyl ether unit, synthetic colneleic acid was stored by dissolving in ethanol and neutralizing with ammonia. Ethanolic solutions of the ammonium salt of 8,9-*Z*-isomer of colneleic acid were prepared in the same way. Concentrations of these acids were checked by UV measurement (ε 21,000 at 250 nm).

Attempts were also made to generate methyl colneleate (**5**) by solvolysis of derivatives of the oxiranylcarbinol **8**. Reaction of the methyl ester of hydroperoxide **1** with trifluoroacetic anhydride (3 equiv) and 2,6-lutidine (5 equiv) in methylene chloride at -78°C for 30 min produced the epoxy trifluoracetate **7** in 85% yield after extractive isolation and chromatography on silica gel.[11] Exposure of **7** to methanolic potassium carbonate at 0°C resulted in smooth conversion to the required oxiranylcarbinol **8**. Reaction of **8** with methanesulfonyl chloride and triethylamine in methylene chloride at -30°C gave the corresponding mesylate. Solvolysis of this mesylate in trifluoroethanol in the presence of calcium carbonate did not result in detectable amounts of methyl colneleate. In fact, the reaction products were found to retain the oxirane ring by ^{1}H NMR analysis. Although the fragmentation of oxiranylcarbinol mesylate was not observed in the present instance, examples of this type of reaction are known.[12]

Previous studies in these laboratories have demonstrated that arachidonic acid analogs in which a divinyl sulfide unit replaces a *Z,Z*-1,4-pentadiene unit can serve as inhibitors of LO enzymes.[13] For example, 13-thiaarachidonic acid (**9**) is an effective time-dependent irreversible inhibitor of soybean lipoxygenase.[13b] It was of interest, therefore, to test colneleic acid and its 8,9-*Z*-isomer as possible inhibitors of the lipoxygenation of linoleic acid by the potato lipoxygenase. The oxidation of linoleic acid to the 9-hydroperoxide **1** was followed by ultraviolet absorption; Lineweaver-Burk analysis showed K_M for this substrate at 23°C and pH 6.3 to be 41μM. K_i values were determined from rate runs in the presence of varying concentrations of **2** or the 8,9(*Z*)-isomer using Dixon analysis. Both **2** and the 8,9(*Z*)-isomer were found to be strong competitive inhibitors of the potato LO, the measured values of K_i at 23°C and pH 6.3 being 7.2μM for **2** and 8.7μM for the 8,9(*Z*)-isomer of **2**.[14]



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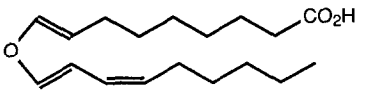

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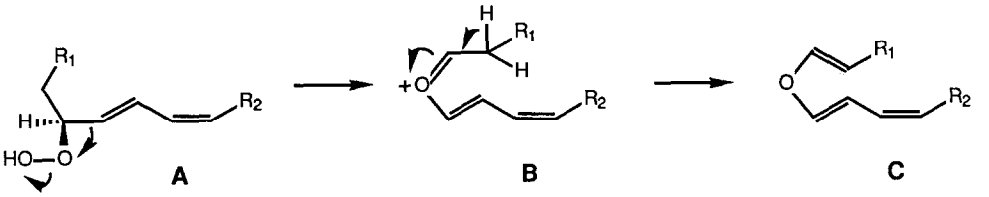

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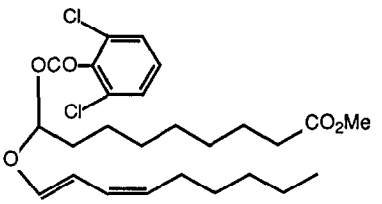

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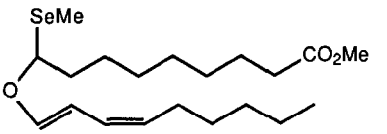

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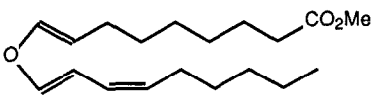
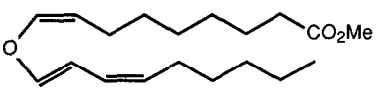
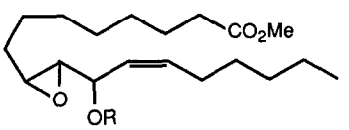


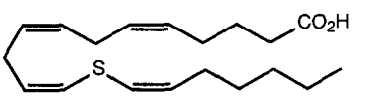
5



6



7 R = $COCF_3$
8 R = H



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2. (a) T. Galliard and D. R. Phillips, *Biochem. J.*, **129**, 743 (1972); (b) T. Galliard, D. R. Phillips, and D. J. Frost, *Chem. Phys. Lipids*, **11**, 173 (1973); (c) T. Galliard, D. A. Wardale, and J. A. Matthew, *Biochem. J.*, **138**, 23 (1974); (d) T. Galliard and J. A. Matthew, *Biochem. Biophys. Acta*, **398**, 1 (1975); (e) T. Galliard, D. R. Phillips, and J. A. Matthew, *ibid.*, **409**, 157 (1975); (f) L. Crombie, D. O. Morgan, and E. H. Smith, *J. Chem. Soc. Chem. Commun.*, **1987**, 502; (g) L. Crombie and D. O. Morgan, *ibid.*, **1987**, 503.
3. In addition the potato LO is of considerable interest because it transforms arachidonic acid into 5*(S)*-HPETE the predecessor of the leukotrienes [E. J. Corey, J. O. Albright, A. E. Barton, and S. Hashimoto, *J. Am. Chem. Soc.*, **102**, 1435 (1980)] and further (in the presence of O_2) into leukotriene A_4 [T. Shimizu, O. Rädmark, and B. Samuelsson, *Proc. Natl. Acad. Sci. USA*, **81**, 689 (1984); T. Shimizu, T. Izumi, Y. Seyama, K. Tadokoro, O. Rädmark, B. Samuelsson, *Proc. Natl. Acad. Sci. USA*, **83**, 4175 (1986)].
4. See (a) E. J. Corey and M. M. Mehrotra, *Tetrahedron Letters*, **24**, 4921 (1983); (b) E. J. Corey, M. M. Mehrotra and J. R. Cashman, *Tetrahedron Letters*, **24**, 4917 (1983); (c) refs. cited in 4a.
5. Obtained by aerobic incubation of linoleic acid with purified[1] potato 5-LO enzyme, esterification with ethereal diazomethane and chromatography on silica gel using 4 : 1 hexane-ethyl acetate for elution.
6. Thin layer chromatography (TLC): (SiO_2, 4 : 1 hexane/ethyl acetate) R_f = 0.35; ^1H NMR ($CDCl_3$, 300MHz): δ 7.30-7.37 (m, 3H, aromatic), 7.64 (d, 1H, J=12.0Hz, H-11), 6.32 (t, 1H, J=6.5Hz, H-9), 6.08 (dt, 1H, J=0.8, 11.3Hz, H-12), 5.84 (tt, 1H, J=1.2, 11.1Hz, H-13), 5.29 (dt, 1H, J=10.8, 7.6Hz, H-14), 3.67 (s, 3H, OMe), 2.30 (t, 2H, J=7.5Hz, H-2), 2.08 (q, 2H, J=7.4Hz, H-15), 1.87-1.94 (m, 2H, H-8), 1.61 (5et, 2H, J=7.2Hz, H-3), 1.50 (5et, 2H, J=7.5Hz, H-16), 1.2-1.45 (m, 12H), 0.88 (t, 3H, J=6.8Hz, H-19); IR (neat, cm^{-1}): ν_{max} 2928, 2855, 1739, 1435, 1268, 1169, 1146, 909; MS (DCI): m/e 499 (M^+), 309 (M^+ - dichlorobenzoate), 191 (dichlorobenzoic acid), 173 (dichlorobenzoyl + H), 155.
7. A. P. Kozikowski and A. Ames, *J. Org. Chem.*, **43**, 2735 (1978).
8. TLC: (SiO_2, 4 : 1 hexane-ethyl acetate R_f = 0.5; ^1H NMR ($CDCl_3$, 300MHz): δ 6.54 (d, 1H, J=11.8Hz, H-11), 5.98 (t, 1H, J=11.6Hz, H-12), 5.86 (tt, 1H, J=1.2, 10.5Hz, H-13), 5.26 (dt, 1H, J=12.4, 7.5Hz, H-14), 5.03 (dd, 1H, J=5.8, 7.5Hz, H-9), 3.65 (s, 3H, OMe), 2.30 (t, 2H, J=7.4Hz, H-2), 2.08 (q, 2H, J=7.4Hz, H-15), 2.00 (s, 3H, SeMe), 1.83-2.02 (m, 2H, H-8), 1.61 (5et, 2H, J=7.2Hz, H-3), 1.41 (m, 2H, H-16), 1.2-1.39 (m, 12H), 0.88 (t, 3H, J=6.8Hz, H-19); IR (neat, cm^{-1}): ν_{max} 2927, 2854, 1739, 1653, 1609, 1160; MS (DCI): m/e 403 (M^+), 309 (M^+ - SeMe), 265 (M^+ - O-CH=CH-CH=CH-C_5H_{11}).
9. Found for **5**: ^1H NMR ($CDCl_3$, 500MHz): δ 6.51 (d, 1H, J=12.0Hz, H-11), 6.26 (d, 1H, J=12.2Hz, H-9), 5.99 (dt, 1H, J=0.8, 11.8Hz, H-12), 5.85 (tt, 1H, J=11.1, 0.8Hz, H-13), 5.29 (dt, 1H, J=10.7, 7.5Hz, H-14), 5.13 (dt, 1H, J=12.2, 7.5Hz, H-8), 3.67 (s, 3H, OMe), 2.30 (t, 2H, J=7.5Hz, H-2), 2.10 (q, 2H, 7.4Hz, H-15), 1.95 (q, 2H, J=7.0Hz, H-7), 1.62 (5et, 2H, J=7.2Hz, H-3), 1.33-1.4 (m, 4H, H-6, 16), 1.25-1.33 (m, 8H), 0.88 (t, 3H, J=6.8Hz, H-19); IR (neat, cm^{-1}): ν_{max} 2928, 2855, 1738, 1651, 1611, 1171, 915; UV (hexane): λ_{max} 249 nm; MS (EI): m/e 308 (M^+), 251 (M^+ - C_4H_9), 165, 151, 137, 123, 109; HRMS: m/e calcd for $C_{19}H_{32}O_3$ 308.23513, found 308.23367.
10. Found for **6**: ^1H NMR ($CDCl_3$, 500MHz): δ 6.54 (d, 1H, J=12.0Hz, H-11), 6.16 (dt, 1H, J=6.2, 1.3Hz, H-9), 5.99 (t, 1H, J=12.0Hz, H-12), 5.84 (tt, 1H, J=11.3, 1Hz, H-13), 5.28 (dt, 1H, J=10.8, 7.5Hz, H-14), 4.60 (dt, 1H, J=6.2, 7.5Hz, H-8), 3.66 (s, 3H, OMe), 2.30 (t, 2H, J=7.5Hz, H-2), 2.07-2.14 (m, 4H, H-7, 15), 1.60 (5et, 2H, J=7.5Hz, H-3), 1.22-1.40 (m, 8H), 0.88 (t, 3H, J=6.8Hz, H-19); IR (neat, cm^{-1}): ν_{max} 2928, 2855, 1740, 1647, 1608, 1167; UV (hexane): λ_{max} 249 nm; MS (EI): m/e 308 (M^+), 251 (M^+ - C_4H_9), 165, 151, 137, 123, 109; HRMS: m/e calcd for $C_{19}H_{32}O_3$ 308.23513, found 308.23431.
11. See E. J. Corey, W.-g. Su, and M. M. Mehrotra, *Tetrahedron Letters*, **25**, 5123 (1984) for method.
12. See, G. R. Clark, *Tetrahedron Letters*, **25**, 2839 (1984).
13. (a) E. J. Corey, J. R. Cashman, T. M. Eckrich, and D. R. Corey, *J. Am. Chem. Soc.*, **107**, 713 (1985); (b) E. J. Corey, M. d'Alarcao, and S. P. T. Matsuda, *Tetrahedron Letters*, **27**, 3585 (1986).

14. This research was assisted financially by grants from the National Science Foundation and the National Institutes of Health.

(Received in USA 20 July 1987)