Synthesis and 5-Lipoxygenase Inhibitory Activities of Eicosanoid Compounds

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Ten eicosanoid compounds (**3**, **6**, **9**, **11**, **12**, **15**, **18**, **21**, **23**, and **25**), methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-hydroxy-6,8,11,14-eicosatetraenoate (5-HETE, **10**), leukotriene A_4 (**26**), and (5*S*,6*E*,8*E*,10*E*,12*RS*,14*E*)-5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-diHETE, **27**) were prepared and their inhibitory activities against the 5-lipoxygenase from guinea pig polymorphonuclear leukocytes (PMNL) were tested. 5,6-Methanoleukotriene A_4 (**18**) was especially a potent and specific inhibitor of the 5-lipoxygenase without inhibiting the cyclooxygenase and the 12-lipoxygenase. Leukotriene A_4, 5-HETE, and 5,12-diHETE also have inhibitory activities against the 5-lipoxygenase at micromolar concentrations, which can regulate the formation of slow-reacting substance of anaphylaxis intracellulary.

The leukotrienes are potent mediators of hypersensitivity and inflammatory reactions and are composed of leukotrienes C_4 and D_4, which are the major functional components of the slow-reacting substance of anaphylaxis (SRS-A) and of the dihydroxy derivative leukotriene B_4, which is a chemotactic factor for neutrophiles and eosinophiles.[1] The leukotrienes are formed from arachidonic acid via two labile intermediates, 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and leukotriene A_4 (LTA_4).[1]

Although a number of specific inhibitors of cyclooxygenase, such as aspirin and indomethacin, have been reported, few inhibitors of lipoxygenase have been found. It is apparent that inhibitors of leukotriene biosynthesis could be of medical value.

We prepared ten eicosanoid compounds and tested their inhibitory activities against the 5-lipoxygenase from guinea pig polymorphonuclear leukocytes (PMNL). It was found that (7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-methano-7,9,11,14-eicosatetraenoic acid (5,6-methanoleukotriene A_4, **18**) was a potent and specific inhibitor of 5-lipoxygenase without inhibiting cyclooxygenase and 12-lipoxygenase.

We also tested the inhibitory activities of LTA_4 (**26**), 5-HETE (**10**), and 5,12-diHETE (**27**)[1] against 5-lipoxygenase. These endogenous compounds were found to have potent inhibitory activities at micromolar concentrations.

Chemistry. (5*Z*,8*Z*,11*Z*,14*Z*)-7,7-Ethano-5,8,11,14-eicosatetraenoic acid (7,7-ethanoarachidonic acid, **3**) was prepared as follows. 2,2-Ethano-1,3-propanediol (**1**) was converted to methyl (5*Z*)-8-hydroxy-7,7-ethano-5-octenoate (**2**) by the following sequence: (1) tetrahydropyranylation with dihydropyran (1 equiv) and *p*-TsOH in CH_2Cl_2; (2) oxidation with oxalyl chloride–Me_2SO[2] in CH_2Cl_2; (3) Wittig reaction with the ylide generated from [4-(carbohydroxy)-*n*-butyl]triphenylphosphonium bromide and *t*-BuOK in toluene; (4) esterification with CH_2N_2; (5) deprotection of the tetrahydropyranyl group with *p*-TsOH and MeOH. Compound **2** was transformed by the reaction with oxalyl chloride–Me_2SO[2] into methyl (5*Z*)-7-formyl-7,7-ethano-5-heptenoate, which was treated with the ylide A generated from [(4*Z*,7*Z*)-dodecadien-1-yl]triphenylphosphonium bromide[3] and *n*-BuLi in THF–HMPA at −78 °C, followed by hydrolysis to afford **3** as shown in Scheme I.

(8*Z*,11*Z*,14*Z*)-*cis*-5,6-Methano-8,11,14-eicosatrienoic acid (5,6-methanoarachidonic acid, **6**) was prepared as follows. Methyl (5*Z*)-8-hydroxy-5-octenoate (**4**) was converted to methyl 8-hydroxy-*cis*-5,6-methanooctanoate (**5**) by the following sequence: (1) acetylation with acetyl chloride and pyridine in CH_2Cl_2; (2) Simmons–Smith reaction[4] with CH_2I_2 and Zn–Cu couple in ether; (3) hydrolysis with LiOH in MeOH. Compound **5** was transformed by the reaction with oxalyl chloride–Me_2SO[2] into methyl 6-formyl-*cis*-5,6-methanohexanoate, which was treated with the ylide A in THF–HMPA at −78 °C, followed by hydrolysis to afford **6** as shown in Scheme I.

(6*E*,8*Z*,11*Z*,14*Z*)-5-(Hydroxymethyl)-6,8,11,14-eicosatetraenoic acid (**9**) was prepared in the same way as previously reported.[3] Methyl 5-[[(tetrahydropyranyl)oxy]methyl]-6-hydroxyhexanoate (**7**)[3] was transformed by the reaction with oxalyl chloride–Me_2SO[2] into methyl 5-[[(tetrahydropyranyl)oxy]methyl]-5-formylpentanoate, which was treated with 1-lithio-2-ethoxyethylene[5] in THF, followed by treatment with mesyl chloride and NEt_3 in CH_2Cl_2 to afford methyl (6*E*)-5-[[(tetrahydropyranyl)oxy]methyl]-7-formyl-6-heptenoate (**8**). Compound **8** was allowed to react with the ylide A in THF–HMPA at −78 °C, followed by methanolysis (*p*-TsOH in MeOH) and hydrolysis (NaOH–THF–H_2O) to afford **9** as shown in Scheme I.

Methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-oxo-6,8,11,14-eicosatetraenoate (**11**) was prepared by the oxidation of methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-hydroxy-6,8,11,14-eicosatetraenoate (5-HETE)[6] with activated MnO_2 in CH_2Cl_2 at room temperature in 77% yield (Scheme II).

Methyl (7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-epithio-7,9,11,14-eicosatetraenoate (5,6-epithioleukotriene A_4, **12**) was prepared according to Corey's method as shown in Scheme II.[7]

(7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-Methano-7,9,11,14-eicosatetraenoic acid (5,6-methanoleukotriene A_4, **18**)[3,8] was prepared in the same way as previously reported.[3] Methyl (5*E*)-7-hydroxy-5-heptenoate (**16**) was converted to methyl (7*E*,9*E*)-*trans*-5,6-methano-10-formyl-7,9-decadienoate (**17**) as follows: (1) Simmons–Smith reaction[4] with CH_2I_2/Zn–Cu in ether; (2) oxidation with oxalyl chloride–Me_2SO[2] in CH_2Cl_2; (3) treatment with 1-lithio-4-ethoxybutadiene[5] in THF followed by treatment with *p*-TsOH in H_2O–THF. Compound **17** was allowed to react with the ylide A in THF–HMPA at −78 °C to room temperature, followed by hydrolysis with 2 N NaOH in MeOH–THF, to afford **18** in 97% yield (Scheme III). (5*E*,7*E*,9*Z*,12*Z*)-*trans*-3,4-

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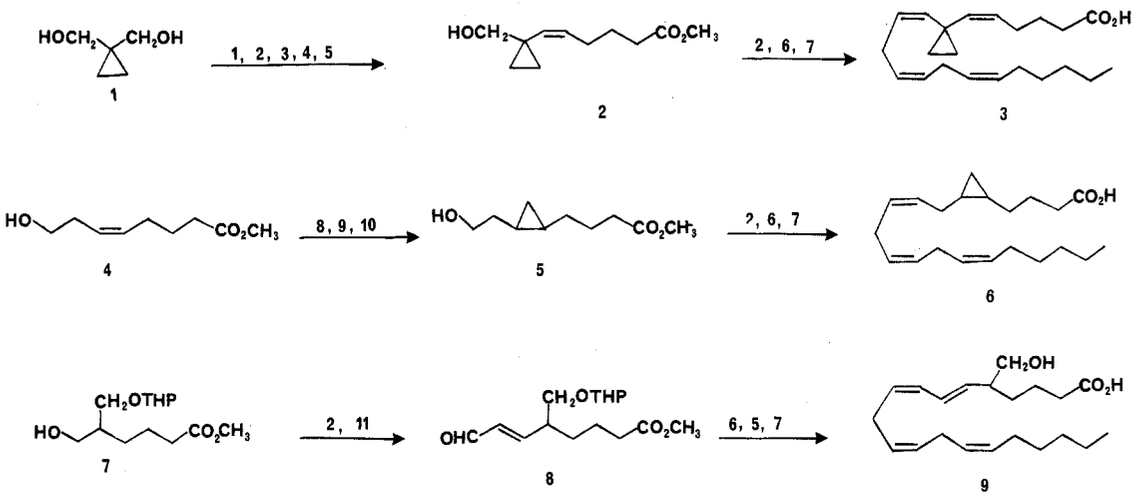
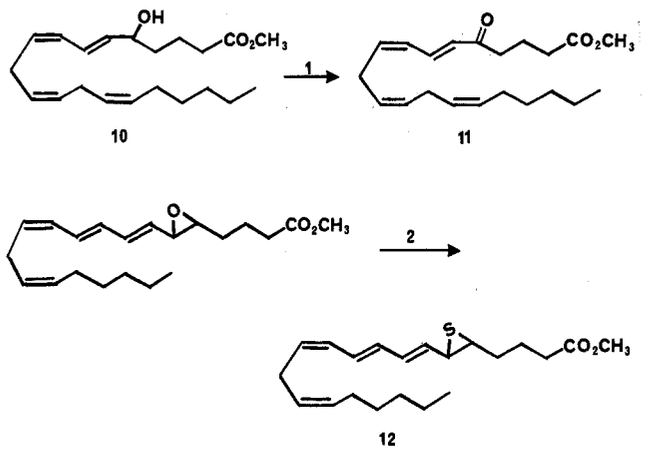
Scheme I[a]



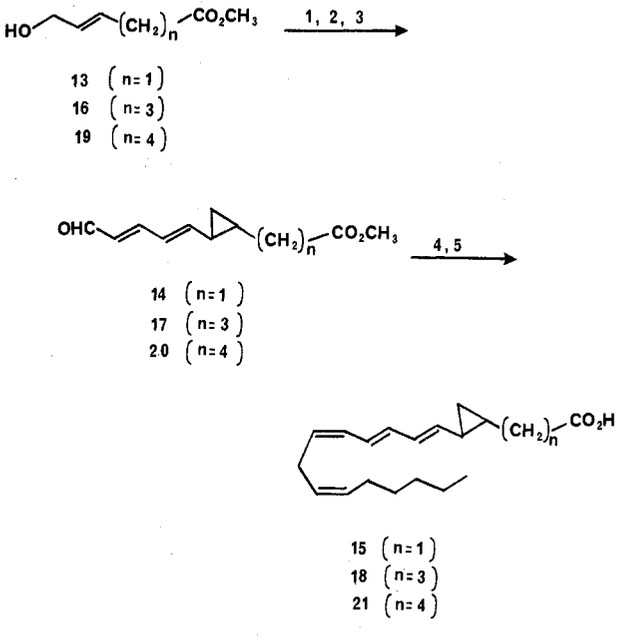
[a] 1, dihydropyran, *p*-TsOH, CH_2Cl_2. 2, $(COCl)_2$, Me_2SO, CH_2Cl_2; Et_3N. 3, $(Ph_3P(CH_2)_4COOH^+)$ Br^-, *t*-BuOK, toluene. 4, CH_2N_2. 5, *p*-TsOH, MeOH. 6, $Ph_3P{=}CHCH_2$-(*Z*)-$CH{=}CHCH_2$-(*Z*)-$CH{=}CHC_5H_{11}$. 7, NaOH. 8, CH_3COCl, pyridine, CH_2Cl_2. 9, CH_2I_2, Zn(Cu), ether. 10, LiOH, MeOH. 11, LiCH=CHOEt, THF; MsCl, Et_3N.

Scheme II[a]



[a] 1, MnO_2, CH_2Cl_2. 2, KSCN, $THF-H_2O$.

Scheme III[a]



[a] 1, CH_2I_2, Zn(Cu), ether. 2, Collins reagent, CH_2Cl_2. 3, LiCH=CHCH=CHOEt, THF; *p*-TsOH, $THF-H_2O$. 4, $Ph_3P{=}CHCH_2$-(*Z*)-$CH{=}CHC_5H_{11}$, THF-HMPA. 5, NaOH.

Table I. Inhibitory Activities against 5-Lipoxygenase

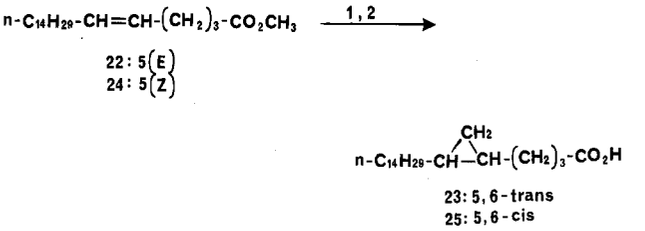
compd	inhibn, %, at 0.1 mM	IC_{50}, μM
3	34	
6	5	
9	47	100
11	93	20
12	60	65
15	84	28
18	98	3
21	86	20
23	49	100
25	1	
5-HETE		2
LTA (Li)	78	2
5,12-diHETE		2

Methano-5,7,9,12-octadecatetraenoic acid (**15**) and (8*E*,10*E*,12*Z*,15*Z*)-*trans*-6,7-methano-8,10,12,15-heneicosatetraenoic acid (**21**) were prepared in the same manner as shown in Scheme III.

Methyl (5*E*)- and (5*Z*)-eicosaenoates **22** and **24** were allowed to react with $CH_2I_2/Zn-Cu$[4] in ether, followed by hydrolysis with LiOH in H_2O–THF, to afford *trans*- and *cis*-5,6-methanoeicosanoic acid (**23** and **25**) (Scheme IV).

(6*E*,8*Z*,11*Z*,14*Z*)-5-Hydroxy-6,8,11,14-eicosatetraenoic acid (5-HETE, **10**),[6] (5*S*,7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-epoxy-7,9,11,14-eicosatetraenoic acid (leukotriene A_4, **26**),[9] and (5*S*,6*E*,8*E*,10*E*,12*RS*,14*E*)-5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (5,12-diHETE, **27**)[10] were prepared in the

Scheme IV[a]



[a] 1, CH_2I_2, Zn(Cu), ether. 2, LiOH, $THF-H_2O$.

(9) E. J. Corey, D. A. Clark, G. Goto, A. Marfat, and C. Mioskowski, *J. Am. Chem. Soc.*, **102**, 1436 (1980).

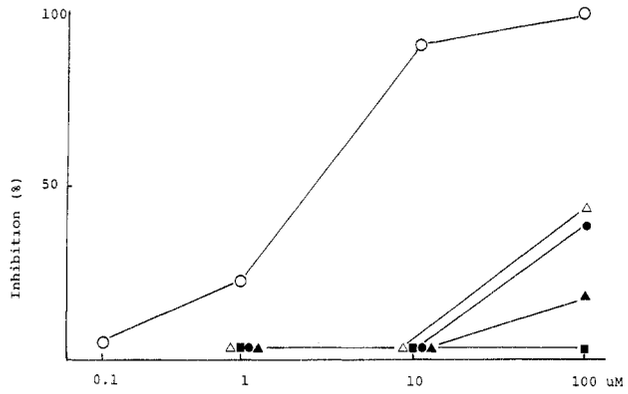


Figure 1. Inhibition of (○) 5-lipoxygenase, guinea pig leukocyte; (△) cyclooxygenase, guinea pig platelet; (■) cyclooxygenase, rabbit platelet; (●) 12-lipoxygenase, guinea pig platelet; and (▲) 12-lipoxygenase, rabbit platelet; by **18**. The values for inhibition are the mean of three to four experiments plus or minus SE, and their confidence limits are 95%. Inhibition of cyclooxygenase and 12-lipoxygenase was measured as described previously [T. Miyamoto, N. Ogino, S. Yamamoto, and O. Hayaishi, *J. Biol. Chem.*, **251**, 2629 (1976); G. A. Higgs, R. J. Flower, and J. R. Vane, *Biochem. Pharmacol.*, **28**, 1959 (1979)].

same way as previously reported.

Results and Discussion

We tested the inhibitory activities of eicosanoid compounds, 5-HETE, LTA_4, and 5,12-diHETE against the 5-lipoxygenase of guinea pig PMNL. Compound **3** (7,7-ethanoarachidonic acid) exhibited a weak inhibitory activity, but **6** (5,6-methanoarachidonic acid) was inactive. Compounds **11** and **9**, derivatives of 5-HETE and 5-HPETE, respectively, had IC_{50} values of 20 and 100 μM. Compounds **12** (5,6-epithio-LTA_4) and **18** (5,6-methano-LTA_4), in which the oxygen atom of LTA_4 was replaced by the sulfur atom and the methylene moiety, were each active with IC_{50} values of 65 and 3 μM. Compound **18** selectively inhibited the 5-lipoxygenase activity without inhibiting the cyclooxygenase and the 12-lipoxygenase of guinea pig and rabbit platelet as shown in Figure 1. Both **21** (homo analogue of **18**) and **15** (dinor analogue of **18**) showed the losses of activity relative to that of **18**. These findings showed that the distance between the carboxylic group and the cyclopropane moiety was critical for the maximum activity. It is noteworthy that **23**, the perhydro derivative of **18** showed a more potent inhibitory activity compared to that of **25**, the cis isomer of **23**. The trans geometry of the cyclopropane moiety in **18** and **23** is important to the inhibitory activity, in agreement with the epoxide moiety of LTA_4 having the trans geometry. It was found that LTA_4, 5-HETE, and 5,12-diHETE inhibited the 5-lipoxygenase activity at micromolar concentrations (IC_{50} = 2 μM). This finding suggests that these 5-lipoxygenase products might regulate the formation of the 5-lipoxygenase products (leukotrienes C_4, D_4, and B_4 and 5-HETE) intracellularly.[11]

Experimental Section

^{1}H NMR spectra were taken on a Varian XL-100 or -200 or on a JEOL PMX-60 spectrometer in $CDCl_3$ or CCl_4. Chemical shifts are reported as parts per million relative to Me_4Si as an internal standard. IR spectra were recorded on a Hitachi Model EPI-G2 or 260-30. Mass spectra were obtained on a JMS-01SG double-focusing spectrometer. Ultraviolet spectra were determined on a Hitachi 124 type double-beam spectrometer.

For TLC analysis throughout this work, Merck TLC plates (Kieselgel 60 F_{254}, precoated, layer thickness 0.2 mm) were used. Column chromatography was carried out on silica gel (Merck, particle size 0.063–0.20 mm). All chromatography solvents were distilled prior to use.

All reactions were conducted under an atmosphere of dry argon. All solvents for the reaction were distilled before use. Ether and THF were distilled from sodium benzophenone ketyl; CH_2Cl_2, benzene, and Et_3N were distilled from CaH_2.

3-[(Tetrahydropyranyl)oxy]-2,2-ethano-1-propanol. To a solution of **1** (683 mg, 6.7 mmol) and *p*-TsOH–H_2O (2 mg) in CH_2Cl_2 (20 mL) cooled in an ice–water bath was added dropwise dihydropyran (0.61 mL, 6.7 mmol). After being stirred for 30 min at 0 °C, the mixture was allowed to warm up to room temperature, and further stirring was continued for 30 min. The reaction was quenched by the addition of Et_3N (0.1 mL), and the solvent was removed in vacuo. The residue was purified by column chromatography on silica gel with cyclohexane–ethyl acetate (1:1) to give the title compound (550 mg, 44% yield): R_f 0.55 (EtOAc–cyclohexane, 1:1); MS, m/z 187 (M^+ + 1), 186 (M^+), 185 (M^+ – 1), 168, 155, 113, 101, 85; IR (film) ν 3400, 1437, 1353, 1200, 1137, 1120, 1025, 903, 870, 813 cm^{-1}; NMR ($CDCl_3$) δ 4.60 (1 H, m), 3.74 (1 H, d, J = 10 Hz), 3.62 (1 H, d, J = 11 Hz), 3.47 (1 H, d, J = 11 Hz), 3.38 (1 H, d, J = 10 Hz), 4.02–3.24 (2 H, m), 0.51 (4 H, br s); MS ($C_{10}H_{18}O_3$) calcd, m/z 186.12558; found, m/z 186.12473.

3-[(Tetrahydropyranyl)oxy]-2,2-ethano-1-propanal. To a solution of oxalyl chloride (0.49 mL, 5.60 mmol) in CH_2Cl_2 (10 mL) was added a solution of dimethyl sulfoxide (0.79 mL, 11.2 mmol) in CH_2Cl_2 (1 mL) in 2 min at –70 °C. Violent gas evolution was observed. The solution was stirred at –70 °C for 10 min, and a solution of 3-[(tetrahydropyranyl)oxy]-2,2-ethano-1-propanol (520 mg, 2.8 mmol) in CH_2Cl_2 (4 mL) was added dropwise over a period of 2 min. The mixture was stirred at –78 °C for 15 min. After Et_3N (3.10 mL, 22.4 mmol) was added at –78 °C, the cooling bath was removed, and the mixture was stirred for 30 min. A mixture of ether (20 mL) and water (20 mL) was added, and further stirring was continued for 5 min. The aqueous layer was separated and extracted with ether. The combined organic layers were washed successively with 0.5 M HCl, water, and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was column chromatographed on silica gel with cyclohexane–ethyl acetate (85:15) to afford the title compound (443 mg, 84% yield): R_f 0.48 (EtOAc–cyclohexane, 1:1); MS, m/z 184 (M^+), 166, 155, 149, 101, 85; IR (film) ν 1710 cm^{-1}; NMR ($CDCl_3$) δ 9.06 (1 H, s), 4.60 (1 H, m), 3.83 (1 H, d, J = 11 Hz), 3.69 (1 H, d, J = 11 Hz), 1.22 (1 H, m), 1.07 (1 H, m); MS ($C_{10}H_{16}O_3$) calcd, m/z 184.10994; found, m/z 184.10931.

Methyl (5*Z*)-8-[(Tetrahydropyranyl)oxy]-7,7-ethano-5-octenoate. To a stirred suspension of (4-carboxybutyl)triphenylphosphonium bromide (1.975 g, 4.46 mmol) in toluene (15 mL) was added *t*-BuOK (1.10 g, 9.80 mol). The mixture was heated at 90 °C for 40 min with stirring and then cooled to room temperature. To this mixture was added dropwise a solution of 3-[(tetrahydropyranyl)oxy]-2,2-ethano-1-propanal (410 mg, 2.23 mmol) in toluene (8 mL). The mixture was stirred at room temperature for 30 min and then at 46 °C for 30 min; it was then allowed to cool to 0 °C. After the addition of water, 1 M HCl was added slowly until pH 3 was reached. The aqueous layer was separated and extracted with ether. The combined organic layers were washed with water, followed by saturated brine, dried over Na_2SO_4, and concentrated in vacuo to a volume of 50 mL. This solution was treated with an excess of diazomethane at 0 °C and concentrated in vacuo. The residue was purified by column chromatography on silica gel with cyclohexane–ethyl acetate (85:15) to provide the title compound (660 mg, 99% yield): R_f 0.58 (EtOAc–cyclohexane, 1:1); IR (film) ν 1738, 1435, 1027 cm^{-1}; NMR ($CDCl_3$) δ 5.51 (1 H, d, J = 10 Hz), 5.22 (1 H, dt, J = 10 and 6 Hz), 4.50 (1 H, m), 3.60 (3 H, s), 0.60 (4 H, m); MS ($C_{16}H_{26}O_4$) calcd, m/z 282.18310; found, m/z 282.18228.

Methyl (5*Z*)-8-Hydroxy-7,7-ethano-5-octenoate. A mixture of methyl (5*Z*)-8-[(tetrahydropyranyl)oxy]-7,7-ethano-5-octenoate (640 mg), MeOH (5 mL), and *p*-TsOH (3 mg) was stirred at room temperature for 1 h. The reaction was quenched by addition of Et_3N (0.2 mL). Concentration in vacuo gave the crude oil, which was purified by column chromatography on silica gel (cyclo-

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(11) J. Y. Vanderhoek, R. W. Bryant, and J. M. Bailey, *J. Biol. Chem.*, **255**, 10064 (1980).

hexane–ethyl acetate, 2:1) to furnish the title compound **2** (373 mg, 85% yield): R_f 0.63 (EtOAc–cyclohexane, 1:1); MS, m/z 198 (M^+), 180, 168; IR (film) ν 3430, 1740, 1220 cm^{-1}; NMR δ 5.53 (1 H, d, J = 10.5 Hz), 5.37 (1 H, dt, J = 10.5 and 6 Hz), 3.62 (3 H, s), 3.33 (2 H, br s), 2.10–2.40 (4 H, m), 1.55–1.85 (2 H, m), 0.59 (2 H, m), 0.56 (2 H, m); MS ($C_{11}H_{18}O_3$) calcd, m/z 198.12558; found, m/e 198.12745.

Methyl (5*Z*)-7-Formyl-7,7-ethano-5-heptenoate. In the same way as described in the preparation of 3-[(tetrahydropyranyl)-oxy]-2,2-ethano-1-propanol, **2** (100 mg, 0.5 mmol) was converted to the corresponding aldehyde (95 mg, 95%) with oxalyl chloride (0.09 mL, 1.0 mmol), dimethyl sulfoxide (0.14 mL, 2.0 mmol), and Et_3N (0.55 mL, 4 mmol) in CH_2Cl_2 (5 mL), followed by column chromatography on silica gel (cyclohexane–ethyl acetate, 85:15): R_f 0.36 (EtOAc–cyclohexane, 1:2); MS m/z 196 (M^+), 178, 165; IR (film) ν 1735, 1710, 898 cm^{-1}; NMR ($CDCl_3$) δ 9.05 (1 H, s), 5.70 (1 H, dt, J = 6.5 and 10.5 Hz), 5.48 (1 H, d, J = 10.5 Hz), 3.60 (3 H, s), 2.36 (2 H, t, J = 6.5 Hz), 2.07 (2 H, t, J = 6.5 Hz), 1.55–1.85 (2 H, m), 1.25–1.45 (2 H, m), 0.90–1.10 (2 H, m); MS ($C_{11}H_{16}O_3$) calcd, m/z 196.10994; found, m/z 196.10875.

Methyl (5*Z*,8*Z*,11*Z*,14*Z*)-7,7-Ethano-5,8,11,14-eicosatetraenoate. To a solution of [(4*Z*,7*Z*)-dodecadien-1-yl]triphenylphosphonium bromide (152 mg, 0.3 mmol) in THF–HMPA (10:1, 3.3 mL) cooled to −78 °C was added dropwise 1.5 M *n*-BuLi (0.2 mL, 0.3 mmol) over a period of 3 min. The resulting orange solution was stirred at −78 °C for 40 min, and a solution of methyl (5*Z*)-7-formyl-7,7-ethano-5-heptenoate (56 mg, 0.285 mmol) in THF was added. The color of the solution changed from orange to yellow. The mixture was stirred at −78 °C for 1 h. A mixture of *n*-hexane (10 mL), ether (10 mL), and water (15 mL) was added, and the resulting mixture was allowed to warm up to room temperature. The organic layer was washed with water and then with saturated brine, dried over Na_2SO_4, and concentrated in vacuo under an atmosphere of argon. The residue was purified by column chromatography on silica gel (*n*-hexane–ether, 95:5) to afford the title compound (92 mg, 92% yield): R_f 0.30 (*n*-hexane–ether, 9:1); MS, m/z 344 (M^+), 328, 316, 315, 290; IR (film) ν 1740, 1435, 1020 cm^{-1}; NMR ($CDCl_3$) δ 5.10–5.60 (8 H, m), 3.65 (3 H, s), 3.01 (2 H, br t, J = 6 Hz), 2.84 (2 H, br t, J = 5 Hz), 2.44–1.89 (6 H, m), 1.60–1.40 (2 H, m), 0.92 (3 H, t, J = 6 Hz), 0.76 (4 H, br s); MS ($C_{23}H_{36}O_2$) calcd, m/z 344.27151; found, m/z 344.27107.

(5*Z*,8*Z*,11*Z*,14*Z*)-7,7-Ethano-5,8,11,14-eicosatetraenoic Acid (3). To a solution of methyl (5*Z*,8*Z*,11*Z*,14*Z*)-7,7-ethano-5,8,11,14-eicosatetraenoate (20 mg) in methanol (0.7 mL) and tetrahydrofuran (0.5 mL) cooled in an ice–water bath was added 2 M NaOH (0.2 mL). The mixture was stirred at room temperature for 3 h. Acidification (pH 3) by addition of AcOH, followed by concentration in vacuo left the crude oil, which was subjected to column chromatography on silica gel (*n*-hexane–ethyl acetate, 8:2) to furnish the title compound **3** (18 mg, 95% yield: R_f 0.21 (*n*-hexane–EtOAc, 7:3); MS, m/z 330 (M^+), 302, 301, 276, 259, 245, 243, 233, 232, 231, 229, 219, 205, 192, 191, 179, 175, 173, 159; IR (film) ν 3600–3200, 1710, 1420, 964 cm^{-1}; NMR δ 5.10–5.55 (8 H, m), 2.95 (2 H, br t, J = 6 Hz), 2.80 (2 H, br t, J = 5 Hz), 2.47–1.95 (6 H, m), 1.55–1.85 (2 H, m), 0.88 (3 H, t, J = 6 Hz), 0.73 (4 H, br s); MS ($C_{22}H_{34}O_2$) calcd, m/z 330.25587; found, m/z 330.25611.

Methyl (5*Z*)-8-Acetoxy-5-octenoate. Acetyl chloride (295 mg, 3.3 mmol) was added dropwise to a solution of **4** (570 mg, 3.3 mmol) in CH_2Cl_2 (5 mL) and pyridine (0.4 mL) at 0 °C. During this operation, a white solid appeared. Stirring was continued at 0 °C for 10 min, and then at room temperature for 30 min. A mixture of ethyl acetate (5 mL), *n*-hexane (5 mL), and water (5 mL) was added and the resulting mixture was stirred for 5 min. Washing of the organic layer with water followed by saturated brine, drying on Na_2SO_4, and removing the solvents left the crude oil, which was subjected to column chromatography on silica gel (*n*-hexane–ethyl acetate, 7:3) to afford the title compound (682 mg, 96% yield): R_f 0.44 (*n*-hexane–EtOAc, 7:3); MS, m/z 214 (M^+), 183, 154, 123, 122, 81; IR (film) ν 1735, 1430, 1030 cm^{-1}; NMR ($CDCl_3$) δ 5.38 (2 H, m), 4.01 (2 H, t, J = 6 Hz), 3.63 (3 H, s), 2.02 (3 H, s); MS ($C_{11}H_{18}$)$_4$) calcd, m/z 214.12050; found, m/z 214.11898.

Methyl 8-Acetoxy-*cis*-5,6-methanooctanoate. Iodine (2 mg) was added to a suspension of zinc–copper couple (1.96 g) in ether (2 mL). After the mixture was heated to 50 °C and the color of iodine disappeared, methylene iodide (0.8 mL, 10 mmol) was added. Stirring was continued at 50 °C for 1 h. The color of the solution changed from colorless to black. A solution of methyl (5*Z*)-8-acetoxy-5-octanoate (436 mg, 2.0 mmol) in ether (3 mL) was added, and the mixture was stirred at 50 °C for 2.5 h. Then methylene iodide (0.8 mL, 10 mmol) was added, and stirring was continued at 50 °C for 5 h. The mixture was allowed to cool to room temperature and treated with aqueous saturated NH_4Cl. During this operation, gas evolved violently. The product was extracted with ether. The combined ethereal extracts were washed with aqueous saturated NH_4Cl and then with saturated brine, dried over Na_2SO_4, and concentrated in vacuo. Purification of the residue by column chromatography on 10% $AgNO_3$-impregnated silica gel (*n*-hexane–ethyl acetate, 9:1) afforded the title compound (350 mg, 77% yield): R_f 0.46 (*n*-hexane–EtOAc, 7:3); MS m/z 228 (M^+), 197, 168, 136, 94; IR (film) ν 3050, 2990, 2850, 1735, 1035 cm^{-1}; NMR ($CDCl_3$) δ 4.03 (2 H, t, J = 7 Hz), 2.27 (2 H, t, J = 6 Hz), 1.97 (3 H, s), 0.30–1.00 (3 H, m), 0.15 (1 H, m); MS ($C_{12}H_{20}O_4$) calcd, m/z 228.13615; found, m/z 228.13785.

Methyl 8-Hydroxy-*cis*-5,6-methanooctanoate (5). A mixture of methyl 8-acethoxy-*cis*-5,6-methanooctanoate (340 mg, 1.49 mmol), LiOH (3 mg, 0.15 mmol), and MeOH (2 mL) was stirred at room temperature for 22 h. AcOH was added slowly until pH 3 was reached. Then the solvent was evaporated in vacuo, and the crystal product was directly subjected to column chromatography on silica gel (*n*-hexane–ethyl acetate, 1:1): R_f 0.13 (*n*-hexane–EtOAc, 7:3); MS, m/z 186 (M^+), 168, 155, 136, 94; IR (film) ν 3370, 3050, 175, 1060 cm^{-1}; NMR ($CDCl_3$) δ 3.65 (2 H, t, J = 7 Hz), 3.61 (3 H, s), 2.31 (2 H, t, J = 7 Hz), 1.91–1.10 (6 H, m), 0.50–0.90 (3 H, m), 0.24 (1 H, m); MS ($C_{10}H_{18}O_3$) calcd, m/z 186.12558; found, m/z 186.12342.

Methyl 7-Formyl-*cis*-5,6-methanoheptanoate. In the same way as described in the preparation of 3-[(tetrahydropyranyl)-oxy]-2,2-ethano-1-propanal (**5**; 191 mg, 1.03 mmol) was oxidized to the corresponding aldehyde (180 mg, 95%) with oxalyl chloride (0.17 mL, 2 mmol), dimethyl sulfoxide (0.28 mL, 4 mmol), and NEt_3 (1.11 mL, 8 mmol) in CH_2Cl_2 (7 mL), followed by column chromatography on silica gel (*n*-hexane–ethyl acetate, 4:1): R_f 0.34 (*n*-hexane–EtOAc, 7:3); MS, m/z 184 (M^+), 155, 152, 140, 67; IR (film) ν 3050, 1730, 1430, 1250, 1167 cm^{-1}; NMR ($CDCl_3$) δ 9.78 (1 H, t, J = 4 Hz), 3.64 (3 H, s), 2.20–2.50 (4 H, m), 1.70 (2 H, m), 0.60–1.50 (5 H, m), 0.13 (1 H, m); MS ($C_{10}H_{16}O_3$) calcd, m/e 184.10994; found, m/e 184.10778.

Methyl (8*Z*,11*Z*,14*Z*)-*cis*-5,6-Methano-8,11,14-eicosatrienoate. To a solution of [(3*Z*,5*Z*)-dodecadien-1-yl]triphenylphosphonium bromide (230 mg, 0.45 mmol) in THF–HMPA (10:1, 5.5 mL) was added at −78 °C a solution of *n*-BuLi (1.5 M, 0.27 mL, 0.41 mmol) in hexane. The solution turned orange-red. Stirring was continued at −78 °C for 50 min. A solution of methyl 7-formyl-*cis*-5,6-methanoheptanoate (50 mg, 0.27 mmol) in THF (2 mL) was added. After stirring at −78 °C for 1 h, a mixture of *n*-hexane (10 mL) and water (5 mL) was added. The mixture was allowed to warm up to room temperature, washed with water and saturated brine, dried over Na_2SO_4, and concentrated in vacuo. Column chromatography of the residue on silica gel (*n*-hexane–ether, 95:5) gave the title compound (86 mg, 96% yield); R_f 0.38 (*n*-hexane–ether, 9:1); MS, m/z 332 (M^+), 301, 278, 275, 232; IR (film) ν 3050, 3010, 2950, 1740, 1453, 1430, 1020 cm^{-1}; NMR ($CDCl_3$) δ 5.20–5.60 (6 H, m), 3.65 (3 H, s), 2.79 (4 H, m), 2.35 (2 H, t, J = 7.5 Hz), 2.04 (4 H, m), 0.87 (3 H, t), 0.90–0.50 (3 H, m), 0.23 (1 H, m); MS ($C_{22}H_{36}O_2$) calcd, m/z 332.27151; found, m/z 332.27235.

(8*Z*,11*Z*,14*Z*)-*cis*-5,6-Methano-8,11,14-eicosatrienoic Acid (6). Methyl (8*Z*,11*Z*,14*Z*)-*cis*-5,6-methano-8,11,14-eicosatrienoate (69 mg, 0.21 mmol) was treated with 1 M NaOH (0.5 mL, 1 mmol) in THF–MeOH (1:1, 2 mL) at room temperature for 3 h. After concentration in vacuo, acetic acid was added until pH 3 was reached. The mixture was diluted with water (5 mL) and extracted with ether. The extracts were washed with water and then saturated brine, dried over Na_2SO_4, and concentrated in vacuo. Purification by column chromatography on silica gel (*n*-hexane–ethyl acetate, 4:1) provided the title compound **6** (66 mg, 99% yield): R_f 0.23 (*n*-hexane–EtOAc, 7:3); MS m/z 318 (M^+) 264, 261, 247, 233, 231, 220, 207, 194; IR (film) ν 3600–2400, 3050, 2950, 2850, 1710, 1020, 938 cm^{-1}; NMR ($CDCl_3$) δ 5.20–5.60 (6 H, m),

2.79 (4 H, m), 2.40 (2 H, t, J = 7.5 Hz), 2.02 (4 H, m), 1.80 (8 H, m), 0.88 (3 H, t), 0.80–0.40 (3 H, m), 0.20 (1 H, m); MS ($C_{21}H_{34}O_2$) calcd, m/z 318.25587; found, m/z 318.25478.

Methyl 5-[[(tetrahydropyranyl)oxy]methyl]-5-formylpentanoate (7)[3] (1.38 g, 5.3 mmol) was oxidized to the corresponding aldehyde (1.46 g, 90% yield) with oxalyl chloride (0.90 mL, 9.0 mmol), dimethyl sulfoxide (1.17 mL, 16.4 mmol), and NEt_3 (5.57 mL, 40.3 mmol) in CH_2Cl_2 (8 mL), as described in the preparation of 2,2-ethano-3-[(tetrahydropyranyl)oxy]-1-propanol: R_f 0.45 (CH_2Cl–ether, 5:1); MS, m/z 258, 240, 226, 173, 157, 85; IR (film) ν 2950, 2870, 1735, 1165 cm^{-1}; NMR ($CDCl_3$) δ 9.61 (1 H, d, J = 3 Hz), 4.53 (1 H, br), 3.60 (3 H, s), 4.33–3.45 (4 H,m), 2.87–2.14 (3 H, m); MS ($C_{13}H_{22}O_5$) calcd, m/z 258.14671; found, m/z 258.14826.

Methyl (6*E*)-5-[[(Tetrahydropyranyl)oxy]methyl]-7-formyl-6-heptenoate (8). n-BuLi (1.5 M, 0.14 mL, 0.21 mmol) was added dropwise to a cold (−78 °C) solution of 1-ethoxy-2-(tri-n-butylstannyl)ethylene (86 mg, 0.24 mmol) in THF (1 mL) while the temperature was maintained below −70 °C. After stirring at −78 °C for 1 h, a solution of methyl 5-[[(tetrahydropyranyl)-oxy]methyl]-5-formylpentanoate (52 mg, 0.20 mmol) in THF (1 mL) was added dropwise. The mixture was stirred for an additional 10 min and then poured onto a saturated $NaHCO_3$ solution (20 mL). The product was extracted with ether. The ethereal extracts were washed with water and then saturated brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by column chromatography on silica gel (CH_2Cl_2–ether, 10:1) afforded methyl 8-ethoxy-6-hydroxy-5-[[(tetrahydropyranyl)oxy]methyl]-7-octenoate (34 mg, 52%): MS, m/z 330, 312, 284, 259, 245, 85. During the measurement of the NMR spectrum, the compound was changed to the α,β-unsaturated aldehyde 8. Mesyl chloride (10.8 μL, 0.14 mmol) was added to a stirred solution of methyl 8-ethoxy-6-hydroxy-5-[[(tetrahydropyranyl)oxy]methyl]-7-octenoate (34 mg, 0.103 mmol) and triethylamine (20.6 μL, 0.192 mmol) in CH_2Cl_2 (1 mL) at −20 °C. Stirring was continued at −20 °C for 1.5 h and then at 0 °C for 10 min. The mixture was poured onto an aqueous $NaHCO_3$ solution, and the product was extracted with ether. Washing with saturated brine, drying over $MgSO_4$, and concentrating in vacuo gave the crude oil, which was column chromatographed on silica gel (CH_2Cl_2–ether, 10:1) to afford pure 8 (13 mg, 45% yield): R_f 0.40 (CH_2Cl_2–ether, 5:1); MS, m/z 284 (M^+), 266, 254, 240, 199, 85; IR (film) ν 2940, 2870, 1735, 1635 cm^{-1}; NMR ($CDCl_3$) δ 9.55 (1 H, d, J = 7.5 Hz), 6.77 (1 H, dd, J = 15.5 and 8.0 Hz), 6.17 (1 H, ddd, J = 15.5, 7.5, and 0.5 Hz), 4.70–4.45 (1 H, m), 3.67 (3 H, s), 4.10–3.25 (4 H, m), 2.85–2.45 (1 H, m), 2.45–2.10 (2 H, t, J = 6 Hz); MS ($C_{15}H_{24}O_5$) calcd, m/z 284.16236; found, m/z 284.16362.

Methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-[[(Tetrahydropyranyl)oxy]-methyl]-6,8,11,14-eicosatetraenoate. To a solution of [(3*Z*,6*Z*)-dodecadien-1-yl]triphenylphosphonium bromide (274 mg, 0.54 mmol) in THF (5 mL) was added n-BuLi (1.5 M in hexane, 0.25 mL, 3.0 mmol) dropwise. After the resulting orange solution was stirred at −78 °C for 15 min, hexamethylphosphoric triamide (0.52 mL, 3.0 mmol) was added, and stirring was continued at −78 °C for 5 min. To this ylide solution was added dropwise a solution of 8 (70 mg, 0.246 mmol) in THF (3 mL). The mixture was stirred at −78 °C for 30 min, warmed up to room temperature, and poured onto aqueous $NaHCO_3$ solution. The product was extracted with ether, and the extracts were washed with saturated brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (ether–hexane, 1:5) to give the title compound (72 mg, 68% yield): R_f 0.50 (n-hexane–ether, 1:1); MS, m/e 432 (M^+), 401, 330, 317, 250, 150, 85; IR (film) ν 3010, 2940, 2870, 1835, 1440, 985, 910 cm^{-1}; NMR ($CDCl_3$) δ 6.40 (1 H, dd, J = 14 and 10 Hz), 5.95 (1 H, t, J = 10 Hz), 5.70–5.00 (6 H, m), 4.80–4.40 (1 H, br), 3.70 (3 H, s), 4.20–3.20 (4 H, m), 3.20–2.70 (4 H, m), 2.00–1.10 (16 H, m), 0.85 (3 H, t); MS ($C_{27}H_{44}O_4$) calcd, m/z 432.32394; found, m/z 432.32301.

Methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-(Hydroxymethyl)-6,8,11,14-eicosatetraenoate. Methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-[[(tetrahydropyranyl)oxy]methyl]-6,8,11,14-eicosatetraeoate (70 mg, 0.16 mmol) was treated with p-toluenesulfonic acid (2 mg) in methanol (5 mL) at room temperature for 30 min. The mixture was poured into aqueous $NaHCO_3$ (15 mL), and the product was extracted with ether. Washing of the ethereal extracts with brine and the removing the solvents left the crude oil, which was purified by column chromatography on silica gel (ether–hexane, 1:1) to furnish the alcohol (53 mg, 95% yield): R_f 0.20 (n-hexane–ether, 1:1); MS, m/z 348 (M^+), 318, 317, 304, 276, 150; IR (film) ν 3400, 3010, 1735, 990, 960 cm^{-1}; NMR ($CDCl_3$) δ 6.40 (1 H, dd, J = 14 and 10 Hz), 5.90 (1 H, t, J = 10 Hz), 5.71–5.00 (6 H, m), 3.65 (3 H, s), 3.91–3.33 (2 H, m), 3.22–2.68 (4 H, m), 2.63–1.95 (5 H, m), 1.80 (1 H, s), 0.85 (3 H, t); MS ($C_{22}H_{36}O_3$) calcd, m/z 348.26643; found, m/z 348.26754.

(6*E*,8*Z*,11*Z*,14*Z*)-5-(Hydroxymethyl)-6,8,11,14-eicosatetraenoic Acid (9). A mixture of methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-(hydroxymethyl)-6,8,11,14-eicosatetraenoate (49 mg, 0.14 mmol), 2 M NaOH (0.42 mL, 0.84 mmol), and methanol–THF (1 mL, 1:1) was stirred at 25 °C for 17 h. The pH was adjusted to ~4 by addition of 1 M HCl, and the product was extracted with ether. The extracts were washed with water and then with brine, dried over $MgSO_4$, and concentrated in vacuo. Column chromatography on silica gel (ether–ethyl acetate, 10:1) afforded the title compound (38 mg, 81% yield): R_f 0.34 (ether); MS, m/z 334 (M^+), 316, 304, 303, 262, 245; IR (film) ν 3350, 3010, 1705, 985, 950 cm^{-1}; NMR ($CDCl_3$) δ 6.45 (1 H, dd, J = 15.0 and 10.5 Hz), 5.98 (1 H, t, J = 15.0 and 10.5 Hz), 5.70 (2 H, br), 5.60–5.00 (6 H, m), 3.60 (1 H, dd, J = 5.5 and 10.5 Hz), 3.50 (1 H, dd, J = 7.0 and 10.5 Hz), 3.15–2.55 (4 H, m), 2.50–2.20 (2 H, t), 2.3–1.85 (3 H, m), 0.85 (3 H, t); UV (EtOH) λ_{max} 236 nm (ϵ 28 000); MS ($C_{21}H_{34}O_3$) calcd, m/z 334.25078; found, m/z 334.24918.

Methyl (6*E*,8*Z*,11*Z*,14*Z*)-5-Oxo-6,8,11,14-eicosatetraenoate (11). To a solution of **10** (135 mg, 0, 404 mmol) in CH_2Cl_2 (7 mL) was added manganese dioxide (1.05 g, 12 mmol) in one portion. The mixture was stirred violently at room temperature for 18 h. After further addition of manganese dioxide (500 mg), the stirring was continued for 6 h. The mixture was filtered through a pad of silica gel. Washing with ethyl acetate and evaporating the filtrate afforded a crude oil, which was purified by column chromatography on silica gel (ethyl acetate–cyclohexane, 1:10) to furnish the title compound 11 (103 mg, 77% yield): R_f 0.40 (EtOAc–cyclohexane, 4:1); MS, m/z 332, 301, 278, 235, 231, 207; IR (film) ν 1735, 1690, 1665, 1620, 1430 cm^{-1}; NMR ($CDCl_3$) δ 7.35 (1 H, dd, J = 11.0 and 15.5 Hz), 5.70–6.29 (3 H, m), 5.40 (4 H, m), 3.67 (3 H, s), 3.10 (1 H, m), 2.83 (1 H, m), 2.65 (2 H, t, J = 7.0 Hz), 2.39 (2 H, t, J = 7.0 Hz), 2.20–1.75 (4 H, m), 0.89 (3 H, m); UV (MeOH) λ_{max} 278.5 nm (ϵ 27 000); MS ($C_{21}H_{32}O_3$) calcd, m/z 332.23513; found, m/z 332.23246.

Methyl (7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-epithio-7,9,11,14-eicosatetraenoate (12). A mixture of methyl (7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-epoxy-7,9,11,14-eicosatetraenoate[12] (150 mg, 0.45 mmol), MeOH (2.2 mL), and water (1.1 mL) was stirred at room temperature for 24 h. After the addition of an aqueous succinic acid buffer solution (pH 4.0, 70 mL), the product was extracted with ethyl acetate. The extracts were washed with water and then saturated brine and dried over $MgSO_4$. Since the ester function was hydrolyzed to the acid group during the reaction, a few drops of a solution of diazomethane in ether was added. The solvents were evaporated, and the residue was subjected to column chromatography on silica gel (ether–n-hexane, 1:10) to provide the title compound **12** (62 mg, 40% yield): R_f 0.50 (ether–n-hexane, 1:2); MS, m/z 316, 285, 206, 131, 117, 105, 91; IR (film) ν 1735 cm^{-1}; NMR ($CDCl_3$) δ 6.70–5.90 (4 H, m), 5.60–5.20 (4 H, m), 3.67 (3 H, s), 3.26 (1 H, dd, J = 9.0 and 5.5 Hz), 3.05–2.70 (3 H, m), 0.90 (3 H, m); MS ($C_{21}H_{32}O_2$) calcd, m/z 316.24022; found, m/z 316.24262.

Methyl 7-Hydroxy-*trans*-5,6-methanoheptanoate. A mixture of zinc–copper couple (1.37 g, 21 mgatom), iodine (3 mg), methylene iodide (0.85 mL, 10.5 mmol), and ether (10 mL) was heated at reflux for 30 min. A solution of methyl (5*E*)-7-hydroxy-5-heptenoate (1.09 g, 7.0 mmol) in ether (10 mL) was added. Heating at reflux was continued for 1 h, and then methylene iodide (0.30 mL, 3.5 mmol) was added every 1 h three times. The mixture was stirred at reflux for an additional 2.5 h. After cooling to room temperature, the mixture was filtered, and the solid was washed with ether. The filtrate was washed

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successively with aqueous NH_4Cl, aqueous $NaHCO_3$, and saturated brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was column chromatographed on silica gel (ether–*n*-hexane, 1:1) to give the title compound (10.4 g, 80% yield): R_f 0.40 (ether); MS, *m/z* 172, 155, 144, 140, 131, 129, 123; IR (film) ν 3400, 3070, 1740, 1440, 1035 cm^{-1}; NMR ($CDCl_3$) δ 3.67 (3 H, s), 3.44 (2 H, d, J = 7.0 Hz), 2.36 (2 H, t), 2.03 (1 H, br s), 1.95–1.50 (2 H, m), 1.50–1.10 (2 H, m), 1.00–0.50 (2 H, m), 0.50–0.15 (2 H, m); MS ($C_9H_{16}O_3$) calcd, *m/z* 172.10994; found, *m/z* 172.11125.

Methyl 6-Formyl-*trans*-5,6-methanohexanoate. To a solution of pyridine (4.8 mL, 60 mmol) in methylene chloride (50 mL) was added portionwise anhydrous chromic acid (3.0 g, 30 mmol). The red-black solution was stirred at room temperature for 15 min. After addition of Celite (no. 545, 15 g), methyl 7-hydroxy-*trans*-5,6-methanoheptanoate (524 mg, 3.0 mmol) was added at 0 °C. Stirring was continued at 0 °C for 10 min, and allyl alcohol (2.0 mL, 30 mmol) was added to quench the reaction. After the solution was stirred at 0 °C for 10 min, sodium bisulfate (20 g, 0.12 mmol) was added, and further stirring was continued at room temperature for 5 min. The mixture was filtered through a pad of $MgSO_4$. The filtrate was concentrated in vacuo. The residue was dissolved in ether. The insoluble materials were removed by filtration. The ethereal solution was concentrated in vacuo to give the crude oil, which was purified by column chromatography on silica gel (ether–*n*-hexane, 1:2) to afford the title compound (459 mg, 90% yield): R_f 0.50 (ether); MS, *m/z* 171 (M + 1), 170 (M^+), 152, 142, 139, 110; IR (film) ν 3000, 1740, 1710 cm^{-1}; NMR ($CDCl_3$) δ 9.5 (1 H, d, J = 5 Hz), 3.67 (3 H, s), 2.36 (2 H, t, J = 7 Hz), 2.00–1.15 (7 H, m), 1.10–0.80 (1 H, m); MS ($C_5H_{14}O_3$) calcd, *m/z* 170.09429; found, *m/z* 170.09321.

Methyl (7*E*,9*E*)-5,6-Methano-10-formyl-7,9-decadienoate. To a solution of 1-(tri-*n*-butylstannyl)-4-ethoxybutadiene (542 mg, 1.4 mmol) in THF (5 mL) was added dropwise *n*-BuLi (1.45 M, in hexane, 0.9 mL, 1.3 mmol). Stirring was continued at −78 °C for 1 h and then at −40 °C for 15 min. The mixture was cooled again to −78 °C, and a solution of methyl 6-formyl-*trans*-5,6-methanohexanoate (188 mg, 1.1 mmol) in THF (5 mL) was added. After the mixture was stirred at −78 °C for 1 h, it was poured onto aqueous $NaHCO_3$ (10 mL), and the product was extracted with ether. Washing with brine, drying over $MgSO_4$, and concentrating in vacuo afforded the crude oil, which was dissolved in methanol (5 mL) and cooled to −30 °C. $NaBH_4$ (84 mg, 2.2 mmol) was added, and the mixture was stirred at −30 °C for 15 min. The reaction was quenched by the addition of acetic acid (0.15 mL). The product was extracted with ether, and the extracts were washed with aqueous $NaHCO_3$ and then saturated brine, dried over $MgSO_4$, and concentrated in vacuo. To a solution of the residue in THF–water (10:1, 5 mL) was added *p*-toluenesulfonic acid (2 mg). The mixture was stirred at room temperature for 15 min, diluted with ether (20 mL), washed with aqueous $NaHCO_3$ and then saturated brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (CH_2Cl_2–ether, 20:1) to give the title compound **17** (167 mg, 68%): R_f 0.40 (ether–*n*-hexane, 2:1); MS, *m/z* 222 (M^+), 191, 190, 178, 173; IR (film) ν 3000, 1740, 1680, 1630, 900 cm^{-1}; NMR ($CDCl_3$) δ 9.50 (1 H, d, J = 8 Hz), 7.04 (1 H, dd, J = 10.5 and 15 Hz), 6.36 (1 H, dd, J = 10.5 and 15 Hz), 6.04 (1 H, dd, J = 8 and 15 Hz), 5.80 (1 H, dd, J = 9.5 and 15 Hz), 3.76 (3 H, s), 2.35 (2 H, t), 2.00–1.55 (2 H, m), 1.55–0.60 (6 H, m); MS ($C_{13}H_{18}O_3$) calcd, *m/z* 222.12558; found, *m/z* 222.12639.

Methyl (7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-Methano-7,9,11,14-eicosatetraenoate. To a solution of [(3*Z*)-nonen-1-yl]triphenylphosphonium iodide (565 mg, 1.10 mmol) in THF (10 mL) was added dropwise *n*-BuLi (1.45 M in hexane, 0.76 mL, 1.10 mmol) at −78 °C. After the mixture was stirred at −78 °C for 15 min, hexamethylphosphoramide (1.91 mL, 11.0 mmol) was added, and stirring was continued for 15 min. A solution of **17** (203 mg, 0.91 mmol) in THF (5 mL) was added dropwise. The mixture was stirred at −78 °C for 30 min, warmed up to room temperature over a period of 1 h, and poured into aqueous $NaHCO_3$. The product was extracted with ether, and the extracts were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (ether–*n*-hexane, 1:20) to afford the title compound (211 mg, 70% yield): R_f 0.18 (ether–*n*-hexane, 1:20); MS, *m/z* 330, 316, 299, 273; IR (film) ν 3010, 1740, 990, 960 cm^{-1}; NMR ($CDCl_3$) δ 6.50–5.70 (4 H, m), 5.70–5.00 (4 H, m), 3.65 (3 H, s), 2.92 (2 H, t, J = 6.5 Hz), 2.33 (2 H, t, J = 7.5 Hz), 2.20–1.90 (2 H, m), 1.90–1.50 (2 H, m), 0.88 (3 H, t), 1.05–0.40 (3 H, m); MS ($C_{22}H_{34}O_2$) calcd, *m/z* 330.25586; found, *m/z* 330.25327.

(7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-Methano-7,9,11,14-eicosatetraenoic Acid (18). A mixture of methyl (7*E*,9*E*,11*Z*,14*Z*)-*trans*-5,6-methano-7,9,11,14-eicosatetraenoate (211 mg, 0.64 mmol), 2 M KOH (0.64 mL, 1.28 mmol), THF (3 mL), and methanol (5 mL) was stirred at room temperature for 24 h. The pH was adjusted to 4 by addition of 1 M HCl, and the product was extracted with ether. The extracts were washed with water and then brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane–ether, 5:1) to provide the title compound **18** (197 mg, 97% yield): R_f 0.25 (ether–*n*-hexane, 1:1), MS, *m/z* 316, 255, 245, 231, 229; IR (film) ν 3020, 1715, 990, 960 cm^{-1}; NMR ($CDCl_3$) δ 9.5 (1 H, br), 6.60–5.80 (4 H, m), 5.60–5.00 (4 H, m), 3.05–2.70 (2 H, m), 2.38 (2 H, t, J = 7.5 Hz), 2.25–1.95 (2 H, m), 1.95–1.55 (2 H, m), 1.00–0.40 (6 H, m); UV (MeOH) λ_{max} 273 (ϵ 42 000), 283 (50 000), 294 (39 000); MS ($C_{21}H_{32}O$) calcd, *m/z* 316.24022; found, *m/z* 316.24227.

(8*E*,10*E*,12*Z*,15*Z*)-*trans*-6,7-Methano-8,10,12,15-heneicosatetraenoic acid (21) and (5*E*,7*E*,9*Z*,12*Z*)-*trans*-3,4-methano-5,7,9,12-octadecatetraenoic acid (15) were synthesized in the same way as described above, starting with methyl (6*E*)-8-hydroxy-6-octenoate (**19**) and methyl (3*E*)-5-hydroxy-3-pentenoate (**13**), respectively. **21**: R_f 0.26 (*n*-hexane–ether, 1:1); MS, *m/z* 330, 219, 91, 79, 67; IR (film) ν 2600, 1708, 1630, 987 cm^{-1}; NMR ($CDCl_3$) δ 6.65–5.90 (4 H, m), 5.68–5.10 (4 H, m), 3.00 (2 H, m), 2.39 (2 H, m), 2.12 (2 H, m), 1.12–0.49 (6 H, m); UV (EtOH) λ_{max} 273 (ϵ 42 000), 283 (50 000), 294 (40 000); MS ($C_{22}H_{34}O_2$) calcd, *m/z* 330.25587; found, *m/z* 330.25591. **15**: R_f 0.18 (ether–*n*-hexane, 1:1); MS, *m/z* 288, 178, 117, 105, 91, 79; IR (film) ν 3010, 1710, 990, 960 cm^{-1}; NMR ($CDCl_3$) δ 6.60–5.80 (4 H, m), 5.60–5.00 (4 H, m), 2.92 (2 H, m) 2.33 (2 H, dd), 2.07 (2 H, m), 0.89 (3 H, m), 0.72 (2 H, m); UV (EtOH) λ_{max} 273 (ϵ 42 000), 283 (50 000), 294 (40 000); MS ($C_{19}H_{28}O_2$) calcd, *m/z* 288.20892; found, *m/z* 288.21033.

Methyl *cis*-5,6-Methanoeicosanoate. A mixture of methylene iodide (0.2 mL, 2.46 mmol), zinc–copper couple (400 mg), iodine (1 mg), and ether (0.5 mL) was stirred and heated at reflux for 30 min. A solution of methyl (15*Z*)-eicosenoate (**22**; 25 mg, 0.077 mmol) in ether (3 mL) was added dropwise. The mixture was heated at reflux for 18 h and cooled to room temperature. Aqueous NH_4Cl (2 mL) was added, and the product was extracted with ether. The extracts were washed with water and then saturated brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by column chromatography on 15% $AgNO_3$-impregnated silica gel (*n*-hexane) to give the title compound (25 mg, 95% yield): R_f 0.53 (benzene–*n*-hexane, 1:1); MS *m/z* 338 (M^+), 306; IR (film) ν 1740 cm^{-1}; NMR ($CDCl_4$) δ 3.53 (3 H, s), 2.42 (2 H, t, J = 6 Hz), 1.00–0.00 (7 H, m); MS ($C_{22}H_{42}O_2$) calcd, *m/z* 338.56046; found, *m/z* 338.56132.

***cis*-5,6-Methanoeicosanoic Acid (23).** A mixture of methyl *cis*-5,6-methanoeicosanoate (8 mg, 0.024 mmol), LiOH (10 mg), THF (1 mL), and water (0.4 mL) was stirred at 40 °C for 5 h. The pH was adjusted to 3 by addition of 2 M HCl, and the product was extracted with ether. The extracts were dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (CH_2Cl_2–ethyl acetate, 9:1) to afford the title compound **23** (7.5 mg, 94% yield): R_f 0.50 (EtOAc–cyclohexane, 2:3); MS, *m/z* 324 (M^+) 306, 183; IR (film) ν 3500–2500, 1710 cm^{-1}; NMR ($CDCl_3$) δ 2.42 (2 H, t, J = 6.5 Hz), 0.89 (3 H, m), 0.75–0.00 (4 H, m): MS ($C_{21}H_{40}O_2$) calcd, *m/z* 324.30281; found, *m/z* 324.30401.

***trans*-5,6-Methanoeicosanoic acid (25)** was prepared in the same way as described for *cis*-5,6-methanoeicosanoic acid (37 mg, 81% yield): R_f 0.49 (EtOAc–cyclohexane, 2:3); MS, *m/z* 324 306; IR (film) ν 3050, 1700 cm^{-1}; NMR (CCl_4) δ 2.44 (2 H, t, J = 6.5 Hz), 0.99 (3 H, t), 0.30–0.00 (4 H, m); MS ($C_{21}H_{40}O_2$) calcd, *m/z* 324.30281; found, *m/z* 324.30455.

Biological Measurement. Preparation of Enzyme. The preparation of polymorphonuclear leukocytes (PMNL) was mostly based on the method previously reported by Sbarra and Karnovsky.[10] To four guinea pigs (350–500 g), a sterile 2% casein solution was injected intraperitoneally in one-tenth volume of body

weight. After 14-16 h, the animals were killed by bleeding from the carotid artery. Peritoneal exudate was collected, and the cavity was washed twice each with 30 mL of physiological saline containing 15 mM potassium phosphate buffer at pH 7.4, 1 mM EDTA, and 15 μM indomethacin. The combined exudate was filtered through four layers of cheesecloth, followed by centrifugation at 500g for 3 min. Cell pellets were suspended in 0.2% sodium chloride solution for 30 s for lysis of contaminating red cells. After centrifugation at 200g for 5 min, the cells were suspended in 50 mM potassium phosphate at pH 7.4 containing 1 mM EDTA and 15 μM indomethacin. The cell suspension (2×10^8 cells/mL) was sonicated at 20 Hz for 30 s, and the sonicate was centrifuged at 105000g for 60 min. The cytosol fraction was concentrated to one-fourth of the original volume by a Diaflo membrane (XM-50). The concentrate (PMNL cytosol) was used as an enzyme throughout this work.

Assay of 5-Lipoxygenase. The standard reaction mixture (0.1 mL) contained 0.1 M potassium phosphate at pH 7.4, 1 mM $CaCl_2$, and enzyme. After preincubation of enzyme for 5 min at 30 °C, [1-^{14}C]arachidonic acid [200000 cpm (5 nmol^{-1} (5 μL of ethanol)$^{-1}$] was added, and the reaction was performed at 30 °C for 5 min. The reaction was terminated by the addition of 0.3 mL of a mixture of ethyl ether/methanol/0.2 M citric acid (30:4:1) precooled at 0-4 °C. The organic layer (50 μL) was applied to a precoated 60 F_{254} glass plate. Arachidonic acid, 5-HETE, and 5,12-diHETE were also placed as a reference. Thin-layer chromatography was carried out with a solvent system of ethyl ether/petroleum ether/acetic acid (85:15:0.1) at 4 °C (R_f values: 15-HETE, 0.32; 5-HETE, 0.23; 5,12-diHETE and LTB_4, 0.03). The measurement of the radioactivity on the silica gel plate was performed as described previously.[12] The PMNL cytosol transformed arachidonic acid to 5-HETE, LTB_4, and 5,12-diHETE. These reaction products were identified by high-performance liquid chromatography and gas chromatography-mass spectrometry. RP-HPLC was performed on a Nucleosil C_{18} (4.6 × 250 mm, 5-μm particles, purchased from Macherey-Nagel Co., Düuran, Germany); solvent MeOH/H_2O/AcOH (75:35:0.01); flow rate 0.9 mL/min, pressure 250 kg/cm^2; monitered by absorbance at 232 or 280 nm. The retention volumes are as follows: 5-(*S*),12(*S*)-diHETE, 2.75; 5(*S*),12(*R*)-diHETE, 3.00; LTB_4, 3.19; 15-HETE, 5.84; 12-HETE, 6.45; 5-HETE, 8.06.

The data of GC-MS spectrometry are in agreement with the published spectra.[14]

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Novel 17α-Chloro-17β-sulfinyl Steroids as Specific Inhibitors of Sebaceous Gland Activity: Potential Antiacne Agents[1]

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The preparation and antisebaceous gland activities of a series of 17α-chloro-17β-sulfinyl steroids are described. They were obtained from the corresponding 17α-sulfides by chlorination and oxidation with iodobenzene dichloride in aqueous pyridine at −40 °C. A single-crystal X-ray structure determination of 17α-chloro-17β-(benzylsulfinyl)-1,4-androstadiene-3,11-dione (**4**) established the absolute configuration at sulfur to be *R*. From an analysis of their CD spectra, some of the other α-chloro sulfoxides were also assigned the same absolute stereochemistry at sulfur. Inhibition of sebaceous gland activity, after topical application of the test compounds, was determined in hamsters and found to reach a maximum with **4**. The 17β-sulfone and 17α-sulfide corresponding to **4** were less potent. Subcutaneous administration of **4** produced no antiandrogenic effects in either hamsters or rats.

Acne vulgaris is a chronic condition involving the pilosebaceous unit, characterized fundamentally by the presence of comedones and secondarily by inflammatory papules, pustules, or cysts. Although the disease has many contributing factors, it has become generally accepted that abnormal sebum production by the sebaceous gland is a major contributor to the etiology of acne.[2-5] Since acne generally appears at puberty, a time when great hormonal changes occur, the possibility that acne is related to hormonal activity was raised[2] as long ago as 1937. Indeed, it was subsequently found that exogenous administration of androgens induced acne in both males and females.[4] Furthermore, both of the potent antiandrogens cyproterone acetate[6,7] and 17α-methyl-*B*-nortestosterone **23**[6,8] were shown to inhibit sebum production in man and were also effective against acne. Side effects,[9] due to their antiandrogenic effects on other tissues, have precluded their clinical use in the control of acne, however.

It is clear then that a close correlation exists between sebaceous gland inhibition and the amelioration of acne. Furthermore, it is apparent that such inhibition can be accomplished by blocking the action of androgens. We therefore set out to identify a compound that would inhibit

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