isolated by filtration, washed with benzene, and dried in vacuo over P_2O_5 to yield 5.32 g (quantitative): mp 300–320 °C dec; NMR (Me_2SO-d_6) δ 5.4 (d, $^2J_{^{31}P\text{-}H}$ = 16 Hz, 2, CH_2), 7.1–9.2 (m, 21, aromatic, OH, NH_2).

Diethyl 5,8,10-Deaza-9,10-dehydrofolate (10). In an oven-dried three-necked flask equipped with stirrer, gas inlet tube, and Hg pressure seal was placed **9** (2.58 g, 5 mmol), diethyl 4-formylbenzoyl-L-glutamate,[9] and 50 mL of dry DMF. After flushing the reaction flask with N_2 and maintaining a positive pressure, EtONa (prepared from 0.245 g, 10.06 mmol, of Na in 17.5 mL of EtOH) was added dropwise over a period of 1 h. The reaction mixture was then stirred overnight at room temperature. The pH of the solution was adjusted to 6 with 1 N HCl and the DMF removed at 30 °C in vacuo. The resulting fluorescent yellow oil was dissolved in 100 mL of $CHCl_3$ and dried over Na_2SO_4, and the solvent was removed in vacuo. The residual oil was dried in vacuo overnight and the resulting solid recrystallized from EtOH–H_2O (1:1.3, 110 mL/g) to yield 2.0 g (81%): mp 225–230 °C dec; NMR (CF_3COOD) δ 1.4 (m, 6, CH_3), 2.7 (m, 4, CH_2CH_2), 4.4 (m, 4, $COCH_2$), 5.0 (m, 1, NCH), 6.7–8.8 (m, 9, aromatic olefin). Anal. ($C_{26}H_{28}N_4O_6$) C, H, N [a sample of analytical purity could only be obtained after purification by HPLC on a μ Bondapak-CN column using hexane–EtOAc–MeOH (5:1:0.15) as eluent].

Diethyl 5,8,10-Deazafolate (11). Once recrystallized **10** (1.65 g, 3.36 mmol) dissolved in 45 mL of DMF and 0.66 g of CH_3SO_3H was hydrogenated over PtO_2 (23 mg). Additional PtO_2 (3 × 30–35 mg) was added at intervals when uptake of H_2 ceased due to poisoning of catalyst. After shaking overnight, fresh catalyst (25 mg) was added whereupon no net uptake of H_2 was observed. TLC ($CHCl_3$–MeOH, 5.6:1) showed the reaction to be complete. The catalyst was filtered off and the DMF removed in vacuo. Water was added and the resulting solid filtered and dried in vacuo over P_2O_5. Recrystallization from EtOH gave 1.4 g (84%): mp 123–126 °C; NMR (CF_3COOD) δ 1.4 (m, 6, CH_3), 2.7 (m, 4, $CHCH_2CH_2$), 3.2 (br s, 4, $CH_2CH_2C_6H_4$), 4.4 (m, 4, CH_2), 5.0 (m, 1, CHNH), 7–8.6 (m, 7, aromatic). Anal. ($C_{26}H_{30}N_4O_6$·0.5H_2O·$CH_3CHOHCH_3$) C, H, N [a sample of analytical purity could only be obtained after purification by HPLC on a μ Bondapak-CN column using $CHCl_3$–2-propanol (15.5:1) as eluent].

5,8,10-Deazafolic Acid (2c). Compound **11** (0.61 g, 1.23 mmol) was stirred overnight in 40 mL of 0.2 N NaOH. After filtering the reaction mixture through Celite, the filtrate was acidified to pH 4 with 1 N HCl. The resulting product was isolated by centrifugation, washed with H_2O, acetone, and ether, and filtered to yield 0.36 g (64.4%) of **2c** as a cream solid: mp 236–240 °C dec; NMR (CF_3COOD) δ 2.8 (m, 4, $CHCH_2CH_2$), 3.2 (br s, 4, $CH_2CH_2C_6H_4$), 5.1 (m, 1, NCH), 7–8.8 (m, 7, aromatic). Anal. ($C_{22}H_{22}N_4O_6$·H_2O) C, H, N.

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Synthesis of Prostaglandin Synthetase Substrate Analogues. 2. (8*Z*,11*Z*,14*Z*)-15-Methyl-8,11,14-eicosatrienoic Acid

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The regioselective convergent synthesis of (8*Z*,11*Z*,14*Z*)-15-methyl-8,11,14-eicosatrienoic acid is described. This compound exhibited prostaglandin synthetase inhibitory activity, although it was not metabolized by the enzyme.

The essential fatty acids, (8*Z*,11*Z*,14*Z*)-8,11,14-eicosatrienoic acid and arachidonic acid, are converted to the prostaglandins E_1 and E_2, respectively, by a multistage biosynthetic process catalyzed by the enzyme prostaglandin synthetase. As part of a program to prepare substrate analogues of these acids with potential prostaglandin synthetase inhibitory activity, we have synthesized (8*Z*,11*Z*,14*Z*)-15-methyl-8,11,14-eicosatrienoic acid (**1**).[1] This analogue was envisioned as a competitive, nonmetabolizable inhibitor, in which enzymatically catalyzed attack by oxygen at the C-15 position would be inhibited by the increased steric bulk of the methyl substituent.

The two intermediates selected for this 3 + 4 step convergent synthesis were (*Z*)-1-bromo-3-methyl-2-octene (**5**) and 11-chloro-1,4-undecadiyne (**7**), which were prepared from ethyl 2-octynoate (**2**) and 8-chloro-1-octyne (**6**), respectively.

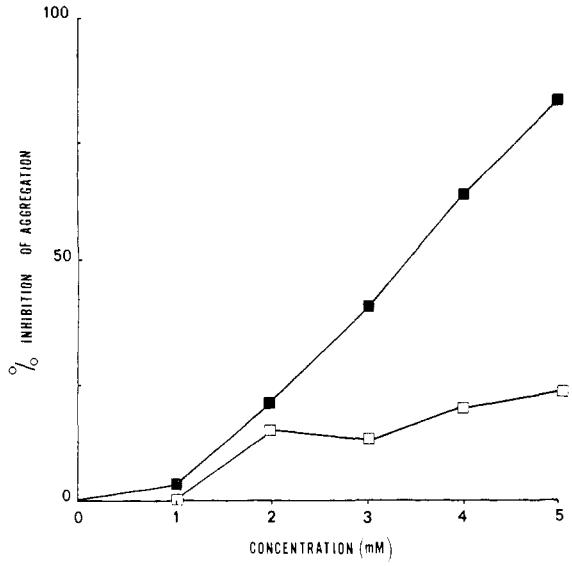
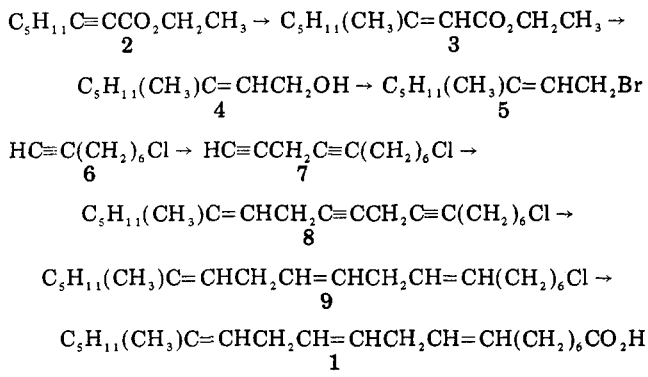


Figure 1. Representative experiment showing the inhibition of ADP-induced human platelet aggregation by the sodium salts of 1 (□) and ETA (■). See the Experimental Section for details.



Ethyl 2-octynoate (5 g) was transformed to ethyl (*Z*)-3-methyl-2-octenoate (**3**) by the low-temperature (−70 to −78 °C) addition of lithium dimethylcopper. The expected stereochemistry about the double bond of unsaturated ester **3** was verified by the NMR spectrum, which had one vinylic methyl group signal at 1.88 ppm.[2] Repetition of this reaction on a larger scale (20 g), in which careful temperature control was more difficult, produced a mixture having a *Z*:*E* ratio of 13:1. Subsequent transformations were performed on this mixture. Diisobutylaluminum hydride reduction (76% yield) of the ester followed by treatment with phosphorus tribromide–collidine (89% yield) afforded the allylic bromide **5**.[3]

The copper-catalyzed Grignard coupling of propargyl bromide with octyne **6** produced the other intermediate, 11-chloro-1,4-undecadiyne, in 60% yield. This labile diyne was immediately coupled with allylic bromide **5** to provide a 71% yield of (*Z*)-1-chloro-14-methylnonadeca-7,10-diyn-13-ene (**8**), the acetylenic bonds of which were hydrogenated in the presence of Lindlar catalyst. The (7*Z*,10*Z*,13*Z*)-triene **9** was obtained in 93% yield. The "*Z*" stereochemistry of the disubstituted double bonds was verified by the NMR coupling constants (J = 5–6 Hz), which are indicative of "*Z*" olefinic bonds,[4] and by the absence of the strong, distinctive infrared absorption at 935 cm^{-1} which is characteristic of an *E*-disubstituted olefin.[5] In addition, VPC, NMR, and mass spectral analyses indicated that this product was a mixture in which the 13*Z*:13*E* stereoisomer ratio was 13:1, indicating no isomerization had occurred during the Grignard coupling step. Using the entrainment method of Grignard formation followed by carbonation and chromatography, the triene was converted in excellent yield (73%) to the target compound **1**. This product contained 92% of the 14*Z* and 7% of the 14*E* isomers as determined by VPC analysis of their methyl esters. The overall yield for the sequence of reactions was 16%.

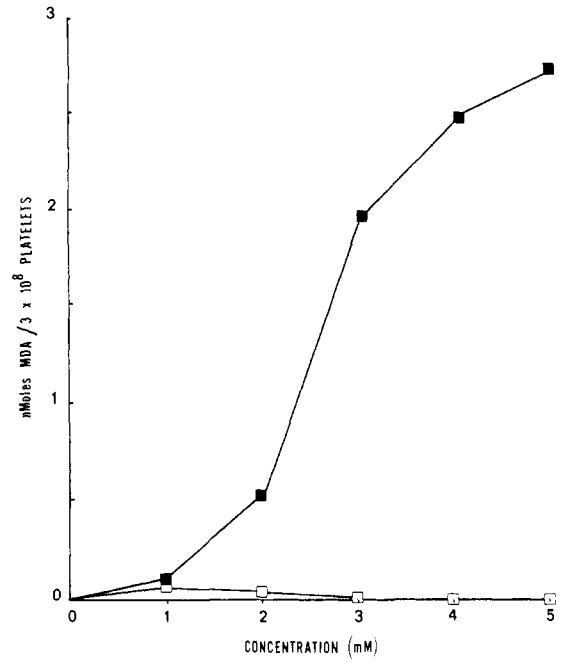


Figure 2. Representative experiment showing the formation of malondialdehyde (MDA) during ADP-induced platelet aggregation in the presence of the sodium salts of 1 (□) and ETA (■). See the Experimental Section for procedure.

The acid mixture was used without further purification for biological studies. Preliminary studies showed it to be a weak inhibitor of human platelet aggregation in response to adenosine diphosphate.[6] Our results indicate that the 15-methyl analogue **1** was a less effective inhibitor of aggregation than was (8*Z*,11*Z*,14*Z*)-8,11,14-eicosatrienoic acid (ETA) (Figure 1). However, unlike ETA, **1** was not a substrate for platelet prostaglandin synthetase (Figure 2). Evidently the presence of the 15-methyl group interfered with the oxidation of **1** to prostaglandin-type compounds in the platelet.

This analogue, with the 15-methyl substituent, showed no substrate activity when tested with the purified prostaglandin cyclooxygenase from seminal vesicles, but it was oxidized by soybean lipoxygenase. This result is surprising since both oxygenases remove the 13-L-hydrogen and insert oxygen at the 15 position. Even the insertion of an O_2 at the 11 position, the proposed first half of the cyclooxygenase reaction, did not occur. These results give further emphasis to the recognition that changes in the terminal portion of the acid chain can markedly reduce the ability of a polyunsaturated acid to serve as substrate to the cyclooxygenase (reviewed by Lands et al.[7]). The binding of the acid to the active site of cyclooxygenase evidenced by its K_I value at 2 μM was comparable to that reported for other related long-chain polyunsaturated acids[8] (2–15 μM). Thus the 15-methyl substituent left the affinity of the acid for the enzyme unimpared while preventing the cyclooxygenation reaction. Perhaps this type of substitution could be used to selectively inhibit cyclooxygenase-mediated oxygenations in tissues while allowing other lipoxygenase-like oxygenations to occur. The reversible, competitive nature of the inhibition by this acid appeared due to simple acyl chain adsorption to the active site; no irreversible inactivation occurred as seen for

some other substrate analogues.[9]

Experimental Section

Reactions were carried out under an argon atmosphere. Solvents were dried or distilled before use. Tetrahydrofuran (THF) was distilled under argon from lithium aluminum hydride. Boiling points were uncorrected. Evaporative distillations were done with a Büchi Kugelrohr apparatus. Vapor phase chromatograms were obtained with a Hewlett-Packard 4711A gas chromatograph equipped with a flame ionization detector. A 6 ft by 0.25 in. o.d., 3% OV-17, 3% OV-1, or 1% SE-30, high-performance Chromosorb W (AW-DMCS, 80–100 mesh) glass column was used. Helium was the carrier gas. Thin-layer chromatograms were run on Analtech analytical silica-gel plates. Infrared spectra were obtained with a Perkin-Elmer 137 spectrophotometer, and NMR spectra were obtained with a Varian A-60A NMR spectrometer using tetramethylsilane as an internal standard (δ 0) and solvents as specified. High-resolution mass spectral analyses were obtained by Dr. David Thomas, Department of Pharmaceutical Chemistry, SRI, on a CEC 21-110B high-resolution mass spectrometer, equipped with facilities for combination VPC/MS.

Ethyl (*Z*)-3-Methyl-2-octenoate (3). To an ice-cooled solution of 58.3 mmol of CH_3Li (31.0 mL of 1.85 M ethereal CH_3Li, from which the ether had been removed at reduced pressure) in 100 mL of THF was added 5.64 g (29.2 mmol) of CuI. The deep brown solution that formed was stirred for 20 min and then cooled to an internal temperature of −70 to −78 °C (isopropyl alcohol–dry ice) while 5.0 g (29.7 mmol) of **2**[2] in 20 mL of THF was added over a period of 20 min. The reaction mixture was then stirred at −78 °C for 5 h. Next, 5 mL of ethanol, which had been cooled to −78 °C, was added with a precooled syringe to quench the reaction.[10] After the mixture was stirred for 5 min, it was poured into water and extracted with ether. The ether extracts were washed with water and saturated brine, dried (Na_2SO_4), and concentrated at reduced pressure to 6.4 g of yellow oil. Chromatography [180 g of Florisil, pentane, R_f = 0.06, 0.32 (**3**), 0.95] afforded 4.0 g (73%) of **3** as a colorless oil: VPC analysis, 3% OV-1, program, 100 °C (2 min), 16 °C/min, 200 °C (4 min), one peak, retention time of 6.8 min; IR (film) 1725 (C=O), 1660 (C=C), 1140, 1045, 850 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (t, J = 7 Hz, 3, OCH_2CH_3), 1.88 (d, J = 8 Hz, 3, $C{=}CCH_3$), 2.6 (m, 2, $C{=}CCH_2$), 4.13 (q, J = 7 Hz, 2, OCH_2CH_3), 5.63 (m, 1, C=CH); MS calcd for $C_{11}H_{20}O_2$ 184.1463, found 184.1462.

Repetition of this reaction on a 20-g scale produced an *E*:*Z* isomeric mixture: VPC analysis, 3% OV-17, program, 100 °C (4 min), 8 °C/min, 200 °C, retention times 3.3 [(*Z*)-**3**, 93%] and 4.4 min [(*E*)-**3**, 7%]; NMR ($CDCl_3$) δ 2.18 (d, J = ~1 Hz, "*E*" Me) and 1.88 (d, J = ~1 Hz, "*Z*" Me).

(*Z*)-3-Methyl-2-octen-1-ol (4). To a stirred ice-cooled solution of 3.0 g (16.3 mmol) of **3** in 10 mL of dry pentane was added 35 mL of cold 0.956 M diisobutylaluminum hydride (32.6 mmol) in hexane. Stirring was continued at ice-bath temperature for 45 min, and then 5.0 mL of ice water was added slowly. The reaction mixture soon set to a solid gel, which was diluted with ether, water, and enough 10% aqueous NaOH solution to solubilize most of the precipitate. The emulsion was broken by stirring. The aqueous layer was extracted with ether. The ether extract was washed with water, dried (Na_2SO_4), and filtered through Na_2SO_4–Celite. Concentration at reduced pressure gave 3.35 g of colorless oil: TLC (35% ether in pentane) R_f 0.05, 0.54 (**4**), 0.95. Chromatography (90 g of Florisil, 35% ether in pentane) afforded 1.75 g (76%) of **4**: colorless oil; IR (film) 3250 (OH), 1660 (C=C), 1000 cm^{-1} (C—O); NMR ($CDCl_3$) δ 1.73 (br s, 3, $C{=}CCH_3$), 2.1 (m, 2, $C{=}CCH_2$), 4.08 (d, J = 6 Hz, 2, $C{=}CCH_2O$), 5.4 (t, J = 6 Hz, 1, C=CH); MS calcd for $C_9H_{18}O$ 142.1358, found 142.1355.

(*Z*)-1-Bromo-3-methyl-2-octene (5). To 1.4 g (9.8 mmol) of **4**, 1.24 g (16.4 mmol) of dry LiBr, and 1.3 mL (9.8 mmol) of dry γ-collidine in 25 mL of anhydrous ether, which was cooled to −35 °C (acetonitrile–dry ice), was added with mechanical stirring 0.95 mL (10.0 mmol) of PBr_3 at such a rate that the reaction temperature did not rise (about 5 min). The reaction mixture became a white paste. The dry-ice bath was replaced by an ice bath, and stirring was continued for 2.5 h. The reaction mixture was then diluted with ice–water and pentane. The pentane extracts were washed with cold 10% HCl, saturated $NaHCO_3$, and saturated brine, dried ($MgSO_4$), and concentrated at reduced pressure to 2.0 g of an oil. Chromatography (60 g of Florisil, pentane) gave 1.78 g (89%) of **5**: colorless oil; IR (film) 1650 (C=C), 1195, 845 cm^{-1}; NMR ($CDCl_3$) δ 1.77 (s, 3, $C{=}CCH_3$), 4.0 (d, J = 8 Hz, 2, $C{=}CCH_2$), 5.50 (t, J = 6 Hz, 1, C=CH); MS calcd for $C_9H_{17}{}^{79}Br$ 204.0514, found 204.0529.

11-Chloro-1,4-undecadiyne (7). The procedure of Eğe and co-workers[11] was adapted. The solvent was removed at reduced pressure from 21.5 mL of 3.2 M ethereal EtMgBr (68.8 mmol). The resulting white paste was dissolved in 40 mL of THF, and then 10.0 g (69.0 mmol) of freshly distilled 8-chloro-1-octyne[12] in 10 mL of THF was added. After foaming had ceased, the solution was immersed in a 60 °C oil bath, stirred for 50 min, and then cooled to room temperature. Cu_2Cl_2 (0.31 g, 3.1 mmol) was added, and heating at 60 °C was resumed for 20 min. The temperature of the stirred reaction mixture was maintained at 20–30 °C by intermittent ice-bath cooling, while 5.4 mL (68.9 mmol) of freshly distilled 3-bromo-1-propyne in 15 mL of THF was added over a period of several minutes. A yellow precipitate formed. The reaction mixture was stirred at room temperature for 1.0 h and then was poured into cold, almost-saturated aqueous NH_4Cl solution and extracted with ether. The combined extracts were washed with NH_4Cl solution and water, dried ($MgSO_4$), and concentrated at reduced pressure (below 50 °C) to give 18.5 g of an orange oil, which was submitted to short-path distillation under argon. 8-Chloro-1-octyne (2.41 g, 24%) was recovered at 31 °C (0.75 mm). The product (7.51 g, 60%) distilled at 78–90 °C (0.05 mm): very pale yellow oil; IR (film) 3150 (C≡CH), 1310 cm^{-1} ($C{\equiv}CCH_2C{\equiv}C$); NMR ($CDCl_3$) δ 2.05 (t, J = 2 Hz, 2, $CH_2CH_2C{\equiv}C$), 2.2 (m, 1, C≡CH), 3.13 (q, J = 2 Hz, 2, $C{\equiv}CCH_2C{\equiv}C$), 3.53 (t, J = 6 Hz, 2, CH_2Cl).

This skipped diyne was very unstable and yellowed immediately upon exposure to air. It was too unstable for high-resolution mass spectral analysis. Storage at −20 °C under argon minimized decomposition and polymerization. The pot residue from the distillation was distilled evaporatively at 78–80 °C (0.05 mm) to afford 0.44 g of a yellow oil, the NMR spectrum of which indicated a mixture containing 75% of the skipped diyne and 25% of an allene: NMR ($CDCl_3$) δ 4.7–5.1 (m, 0.75, $CH{=}C{=}CH_2$).

(*Z*)-1-Chloro-14-methylnonadeca-7,10-diyn-13-ene (8). A procedure of Eğe and co-workers[11] was adapted. Ether was removed at reduced pressure from 12.7 mL of 3.2 M ethereal EtMgBr (40.6 mmol). The white residue was dissolved in 32 mL of dry THF. Then 7.5 g (41.0 mmol) of freshly distilled 11-chloro-1,4-undecadiyne (**7**) in 16 mL of THF was added to the stirred solution at a slow rate so that the internal reaction temperature did not rise above 20 °C. Some ice cooling was necessary during the 5-min addition period. Foaming soon subsided. The solution was stirred at 20 °C for 45 min; then 0.212 g (2.1 mmol) of Cu_2Cl_2 was added, and stirring was continued for 20 min. Next, 8.43 g (41.1 mmol) of allylic bromide **5** in 16 mL of THF was added rapidly with intermittent ice-bath cooling to maintain the internal reaction temperature at 10–15 °C. A bright yellow precipitate soon formed. The reaction mixture was stirred at room temperature for 2.5 h, then poured into cold, almost-saturated NH_4Cl solution, and extracted with ether. The ether solution was washed with NH_4Cl solution and water. The ether extracts were dried ($MgSO_4$) and concentrated at reduced pressure in a water bath below 50 °C to afford 13.0 g of an orange oil that was evaporatively distilled. The 4.3 g of starting materials was collected at 58–90 °C (0.04 mm). The 8.8 g (71%) of **8** was collected at 160–180 °C (0.05 mm): very pale yellow oil; IR (film) 2100 (C≡C), 1660 (C=C), 1295 ($C{\equiv}CCH_2C{\equiv}C$), 725 cm^{-1}; NMR ($CDCl_3$) δ 1.67 (br s, 3, $C{=}CCH_3$), 1.8–2.1 (m, 4, $C{=}CCH_2CH_2$, $C{\equiv}CCH_2CH_2$), 2.7–2.9 (m, 2, $C{=}CCH_2C{\equiv}C$), 3.1 (m, 2, $C{\equiv}CCH_2C{\equiv}C$), 3.53 (t, J = 7 Hz, 2, CH_2Cl), 5.20 (t, J = 6 Hz, 1, C=CH); MS calcd for $C_{20}H_{31}{}^{35}Cl$ 306.2115, found 306.2130.

(7*Z*,10*Z*,13*Z*)-1-Chloro-14-methyl-7,10,13-nonadecatriene (9). To 4.3 g of Lindlar catalyst[13] in 500 mL of pentane was added 0.95 mL of redistilled quinoline. The magnetically stirred suspension was degassed three times with argon and three times with hydrogen and stirred under hydrogen for 5.0 min. Then a degassed solution of 3.74 g (12.2 mmol) of freshly distilled **8** in 50 mL of pentane was added, and stirring was continued for about 4 h until the theoretical amount of hydrogen had been absorbed

and the rate of hydrogen uptake had decreased.[13] The internal hydrogenation temperature was maintained at 15 °C by intermittent cooling. After the reaction mixture was degassed with argon, it was filtered through a sintered glass funnel (pentane rinse). The filtrate was washed with cold 5% HCl and water, dried ($MgSO_4$), and concentrated at reduced pressure in a water bath kept below 50 °C to afford 3.8 g of bright yellow oil, which was evaporatively distilled under argon at 180–200 °C (0.05 mm) to give 3.5 g (93%) of **9** as a very pale yellow oil: VPC analysis, 3% OV-1, 240 °C, two main peaks, retention times of 3.5 min (93.5%) and 3.8 min (6.1%); IR (film) 1550 (C=C), 1380, 1260 cm^{-1}; NMR ($CDCl_3$) δ 1.7 (br s, 3, C=CCH_3), 1.9–2.3 (m, 4, C=CCH_2CH_2), 2.7–3.0 (m, 4, C=CCH_2C=C), 3.53 (t, J = 6 Hz, 2, CH_2Cl), 5.13 (t, J = 7 Hz, 1, HC=CCH$_3$), 5.4 (2 t, J = 5 Hz, 4, HC=CH); MS calcd for $C_{20}H_{35}{}^{35}Cl$ 310.2403, found 310.2403.

(8Z,11Z,14Z)-15-Methyl-8,11,14-eicosatrienoic Acid (1). A procedure of Eğe and co-workers[11] was modified. To 0.399 g (0.016 g-atom) of Mg turnings and one crystal each of $HgCl_2$ and I_2 in 8 mL of dry THF was added 0.46 mL (0.6 mmol) of redistilled EtBr in 5 mL of THF. This mixture was stirred at reflux temperature for 1 h. Then 0.90 g (2.9 mmol) of **9** and 0.046 mL (0.6 mmol) of EtBr in 13 mL of THF were added over a period of 15 min to the dark-gray, refluxing solution. Heating at reflux and stirring were continued for 15 h; then the mixture was stirred at ambient temperature for an additional 4 h. Next, 0.66 mL (8.7 mmol) of EtBr in 2 mL of THF was added, and the mixture was heated at reflux for 30 min. Dry CO_2 was bubbled through the blackish brown reaction mixture, the internal temperature of which was maintained at 10–15 °C (ice bath). When the exotherm had ceased after about 5 min, the reaction mixture was heated at reflux for 30 min while CO_2 was bubbled through it. After cooling, the mixture was diluted with ether and cold 5% HCl. The aqueous layer was extracted with ether. The ether fraction was washed with water and brine, dried (Na_2SO_4), filtered through Celite, and concentrated to 1.0 g of a pale yellow oil. Chromatography (100 g, silica gel, 10% ethyl acetate in pentane, R_f 0.5) under argon afforded 0.67 g (73%) of colorless oil: IR (film) 2450–3100 (OH of CO_2H), 1740 (C=O of CO_2H), 935 cm^{-1} (OH of CO_2H); NMR ($CDCl_3$) δ 1.70 (s, 3, C=CCH$_3$), 5.13 (t, J = 7 Hz, 1, HC=CCH$_3$), 5.40 (2 AB quartets, J_{AB} = 5 Hz, 4, HC=CH), 8.1 (br s, 1, CO_2H); $n^{26}{}_D$ 1.4795; MS calcd for $C_{24}H_{44}O_2Si$ for trimethylsilyl ester 392.3110, found 392.3139.

The vapor-phase chromatogram (3% OV-17, 240 °C) of the methyl ester, which was prepared by diazomethane treatment of the acid, had two main peaks at 5.5 (92% of the peak area) and 6 min (7% of the area) and three minor peaks at 4.1, 4.5, and 7 min. The vapor-phase chromatogram (3% SE-30, 230 °C) of the trimethylsilyl ester had the same pattern. VPC–MS analyses indicated that the compound corresponding to the first peak (0.2% of the area) had an m/e equal to 378, suggesting material lacking the C-15 methyl group. The second (0.2% of the area), third (92%), and fourth (7%) peaks possessed m/e values of 392. These compounds were 8,11,14-olefinic bond isomers. The fifth peak (0.6% of the area) had an m/e of 406 and a fragmentation pattern indicating the presence of a methyl group at C-14. This 14,15-dimethyl impurity may arise by methyl iodide alkylation of the anion formed by addition of lithium dimethylcopper to ethyl 2-octynoate. Methyl iodide is found as an impurity in commercial methyllithium.

Platelet Aggregation. Venous blood (54 mL) from healthy human volunteers, who had not taken aspirin for at least 1 week, was collected in tubes containing 6 mL of 3.8% trisodium citrate. Approximately 13 mL of platelet-rich plasma (PRP) was collected by centrifuging the blood at 200g for 20 min at room temperature. The platelet content of the PRP was determined manually (Unopette test 5855, Becton-Dickinson) and ranged from 4 to 6 × 10^8 cells/mL. Approximately 13 mL of platelet-poor plasma (PPP) was collected by recentrifuging the blood at 1200g for 20 min.

Platelet aggregation was studied using a Chrono-Log platelet aggregometer equipped with a differentiator. A 0.5-mL sample of PRP, which was stirred continuously in a siliconized cuvette at 37 °C, was used for each determination. Aggregation was initiated by the addition of 0.01 mL of 1 mM ADP in 0.9% NaCl and was monitored for 2–3 min until maximum aggregation had occurred, which, in the absence of inhibitors, was 65–75% with PPP as the reference blank. Test compounds were converted to their sodium salts, which were prepared under argon as 50 mM solutions in 0.1 M Na_2CO_3 and stored on ice. These solutions (0.01–0.05 mL) were added to the stirred PRP 2 min before the addition of ADP.

Malondialdehyde (MDA) Assay. The determination of the amount of MDA formed during platelet aggregation has been described by Silver and co-workers.[14] MDA formation is used as an indication of prostaglandin endoperoxide production. A 0.12 M thiobarbiturate solution was freshly prepared by dissolving thiobarbituric acid (Eastman) in 0.26 M Tris (Sigma) and adjusting the pH to 7.0 with concentrated HCl. After the extent of aggregation had been measured, as described above, the contents of each cuvette were maintained at 37 °C for an additional 27 min without stirring. The contents were then decanted into 0.5 mL of 20% Cl_3CCO_2H in 0.6 M HCl (w/v), mixed well, and centrifuged at 1300g for 20 min at room temperature. A 0.65-mL portion of the clear, debris-free supernatant was mixed with 0.13 mL of thiobarbiturate solution and heated in a boiling water bath for 15 min. After the solution had cooled to room temperature, its absorption at 533 nm was measured in a Gilford 250 spectrophotometer. The MDA formed by the platelets was determined by the difference in absorption of an extract of PRP and an identically treated extract of PPP. The recovery of MDA, which was generally 75–80%, was determined by adding known amounts of MDA to PRP and treating as described above.

Enzyme Assays. The 15-methyleicosatrienoic acid was dissolved as a stock solution (9 mM) in heptane and, immediately prior to assay, 1-mL aliquots were evaporated to dryness under nitrogen gas and resuspended in 0.1 M Tris–chloride buffer (pH 8.5) containing 0.67 mM phenol. Arachidonic acid and other fatty acids used as controls for the assays were handled in a similar manner. The cyclooxygenase preparation, isolated as described earlier,[15] was supplemented with 0.5 μM hematin in the reaction mixtures for optimal activity. Oxygen consumption catalyzed by the cyclooxygenase was measured in 3-mL chambers using oxygen electrodes with a Yellow Springs Instrument Co. oxygen monitor. Compound 1 was not oxidized by the cyclooxygenase system whereas arachidonate was fully oxidized. On the other hand, compound 1 was fully oxidized when soy lipoxygenase was used in place of the cyclooxygenase. Compound 1 reduced the rate of cyclooxygenation of arachidonic acid in a competitive manner characterized by a K_I value of 2 μM. The K_I value was determined (based on eight assays) by the graphical method of Dixon.[16] The degree of inhibition was also constant during progressively longer periods of preincubation (up to 12 min), and no evidence for irreversible enzyme inactivation was obtained.

References and Notes

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(Acylaryloxy)acetic Acid Diuretics. 1. (2-Alkyl- and 2,2-Dialkyl-1-oxo-5-indanyloxy)acetic Acids

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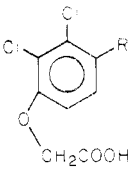
The discovery of the (acryloylaryloxy)acetic acids as a new class of potent diuretics prompted the investigation of related bicyclic compounds. Annelated analogues of the parent series, the (2-alkyl- and 2,2-dialkyl-1-oxo-5-indanyloxy)acetic acids, were the subject of this study. Those compounds, unlike the monocyclic parent compound, lacked the double bond adjacent to the carbonyl group. More importantly, they possessed both saluretic and uricosuric properties. The optimal single 2-substituents for maximal saluretic and uricosuric activity were determined. In general, better activity was observed when a second 2-alkyl substituent (especially methyl) was present in the molecule. Replacement of the carboxy substituent by 5-tetrazolyl generally resulted in a reduction in activity.

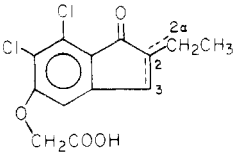
The mercurial phenoxyacetic acid diuretics, merbaphen and mersalyl, served as models for the design, and ultimately led to the discovery, of the potent (acryloylaryloxy)acetic acid diuretics,[1] the best known of these loop diuretics being ethacrynic acid[2] (**1a**). Recently four series of (vinylaryloxy)acetic acids, including those types illustrated by **1b–e**, have been described.[3-6] Each of these types of compounds mimics the mercurials, eliciting marked



1a, R = $-COC(=CH_2)C_2H_5$
b, R = $-CH=C(COCH_3)_2$
c, R = $-CH=C(CH_3)COCH_3$
d, R = $-CH=C(CH_3)NO_2$
e, R = $-CH=CR^1R^2$
f, R = $-COCH(CH_3)C_2H_5$

saluresis in dogs but not in rats. They react with compounds containing sulfhydryl groups in a manner similar to that observed with mercurial diuretics. They differ notably from the mercurials in two respects. Compounds like **1a** show no difference in saluresis under conditions of acidosis or alkalosis, whereas mercurials are ineffective under conditions of alkalosis and are potentiated by acidosis.[7] Mercurials generally produce little change in uric acid excretion while **1a** causes uric acid retention which may result in hyperuricemia.[8,9] In addition, while the compounds of type **1a–e** did react with compounds containing sulfhydryl groups, there was poor correlation between either the rate or extent of this reaction and diuretic activity. Although this lack of correlation may be attributable to the fact that absorption and distribution phenomena as well as metabolic and deactivation reactions are encountered when diuretic activity is measured, it appears that the role of sulfhydryl binding is of secondary importance in the mechanism of action of diuretics of type **1a–e**.

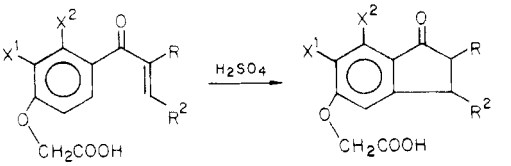
Following the observation that ethacrynic acid underwent intramolecular cyclialkylation to **2a** upon treatment with sulfuric acid, our initial efforts were directed toward the introduction of a double bond in the molecule (**2b,c**).



2a, no double bond
b, 2,3 double bond
c, 2,2α double bond

(The chemistry and biological activity of compounds of type **2b,c** will be reported in a subsequent paper.) It soon became apparent from biological data obtained in chimpanzees that the saturated compounds of type **2a** were also diuretic and had either no effect on serum urate or were frankly uricosuric. It has been shown in our laboratories that dihydroethacrynic acid (**1f**) exhibits weak but significant saluretic and diuretic activity and also is uricosuric in chimpanzees.[10] Thus the mechanism of action of **1f** apparently mimics compounds of type **2a** rather than **1a**.

Chemistry. Most of the (1-oxo-2-monoalkyl-5-indanyloxy)acetic acids (**4**) were prepared by the cyclialkylation of the correspondingly substituted (acryloylphenoxy)acetic acids (**3**)[11] in concentrated H_2SO_4 according to the following reaction and these products are listed in Table I.



3 4

Attempts to prepare the 2,2-dialkyl-substituted compounds **12** by direct alkylation of the methyl esters of the corresponding 2-alkyl-substituted compounds **4** were unsa-