

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 ( $Me_2SO-d_6$ )  $\delta$  5.4 (d,  $^2J_{3P-H} = 16$  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,<sup>9</sup> 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 ( $CF_3COOD$ )  $\delta$  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  $\mu$  Bondapak-CN column using hexane–EtOAc–MeOH (5:1:0.15) as eluent].

**Diethyl 5,8,10-Deazafofolate (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  $PtO_2$  ( $3 \times 30$ –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 ( $CF_3COOD$ )  $\delta$  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. ( $C_{26}H_{30}N_4O_6 \cdot 0.5H_2O \cdot CH_3CHOHCH_3$ ) C, H, N [a sample of analytical purity could only be obtained after purification by HPLC on a  $\mu$  Bondapak-CN column using  $CHCl_3$ –2-propanol (15.5:1) as eluent].

**5,8,10-Deazafofolic 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 ( $CF_3COOD$ )  $\delta$  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. ( $C_{22}H_{22}N_4O_6 \cdot H_2O$ ) C, H, N.

**Acknowledgment.** This work was supported by NIH Grant CA-14615.

## References and Notes

- (1) This research was presented in part at the 173rd National Meeting of the American Chemical Society, New Orleans, La., March 20–25, 1977, Abstracts, MEDI 73.
- (2) Y. H. Kim, Y. Gaumont, R. L. Kisliuk, and H. G. Mautner, *J. Med. Chem.*, **18**, 776 (1975).
- (3) M. G. Nair, P. T. Campbell, and C. M. Baugh, *J. Org. Chem.*, **40**, 1745 (1975).
- (4) M. G. Nair, P. T. Campbell, E. Braverman, and C. M. Baugh, *Tetrahedron Lett.*, **32**, 2745 (1975).
- (5) M. G. Nair and P. T. Campbell, *J. Med. Chem.*, **19**, 825 (1976).
- (6) R. F. Struck, Y. F. Shealy, and J. A. Montgomery, *J. Med. Chem.*, **14**, 693 (1971).
- (7) J. I. DeGraw, R. L. Kisliuk, Y. Gaumont, C. M. Baugh, and M. G. Nair, *J. Med. Chem.*, **17**, 552 (1974).
- (8) S. P. Acharya and J. B. Hynes, *J. Heterocycl. Chem.*, **12**, 1283 (1975).
- (9) J. B. Hynes and C. M. Garrett, *J. Med. Chem.*, **18**, 632 (1975).
- (10) O. D. Bird, J. W. Vaikus, and J. Clarke, *Mol. Pharmacol.*, **6**, 573 (1970).
- (11) R. W. McCuen and F. M. Sirotnak, *Biochem. Biophys. Acta*, **384**, 369 (1975).
- (12) S. C. Carlin, R. N. Rosenberg, L. VandeVenter, and M. Friedkin, *Mol. Pharmacol.*, **10**, 194 (1974).
- (13) J. Borsa and G. F. Whitmore, *Cancer Res.*, **29**, 737 (1969).
- (14) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
- (15) C. L. Krumdieck and C. M. Baugh, *Biochemistry*, **8**, 1568 (1969).
- (16) E. Campaigne and W. W. Meyer, *J. Org. Chem.*, **27**, 2835 (1962).
- (17) R. B. Baker, D. V. Santi, J. K. Coward, S. H. Shapiro, and J. H. Jordaan, *J. Heterocycl. Chem.*, **3**, 425 (1966).

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

Marcia I. Dawson,\* Margaret Reid,

Department of Bio-Organic Chemistry, Stanford Research Institute, Menlo Park, California 94025

Martin E. Hemler, and William E. M. Lands

Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48104.  
Received December 2, 1976

The regioselective convergent synthesis of (8Z,11Z,14Z)-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, (8Z,11Z,14Z)-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 (8Z,11Z,14Z)-15-methyl-8,11,14-eicosatrienoic acid (1).<sup>1</sup> 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.



some other substrate analogues.<sup>9</sup>

## 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 ( $\delta$  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  $\text{CH}_3\text{Li}$  (31.0 mL of 1.85 M ethereal  $\text{CH}_3\text{Li}$ , from which the ether had been removed at reduced pressure) in 100 mL of THF was added 5.64 g (29.2 mmol) of  $\text{CuI}$ . The deep brown solution that formed was stirred for 20 min and then cooled to an internal temperature of  $-70$  to  $-78^\circ\text{C}$  (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  $-78^\circ\text{C}$  for 5 h. Next, 5 mL of ethanol, which had been cooled to  $-78^\circ\text{C}$ , was added with a precooled syringe to quench the reaction.<sup>10</sup> 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 ( $\text{Na}_2\text{SO}_4$ ), 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,  $100^\circ\text{C}$  (2 min),  $16^\circ\text{C}/\text{min}$ ,  $200^\circ\text{C}$  (4 min), one peak, retention time of 6.8 min; IR (film) 1725 ( $\text{C}=\text{O}$ ), 1660 ( $\text{C}=\text{C}$ ), 1140, 1045,  $850\text{ cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  1.27 (t,  $J = 7\text{ Hz}$ , 3,  $\text{OCH}_2\text{CH}_3$ ), 1.88 (d,  $J = 8\text{ Hz}$ , 3,  $\text{C}=\text{CCH}_3$ ), 2.6 (m, 2,  $\text{C}=\text{CCH}_2$ ), 4.13 (q,  $J = 7\text{ Hz}$ , 2,  $\text{OCH}_2\text{CH}_3$ ), 5.63 (m, 1,  $\text{C}=\text{CH}$ ); MS calcd for  $\text{C}_{11}\text{H}_{20}\text{O}_2$  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,  $100^\circ\text{C}$  (4 min),  $8^\circ\text{C}/\text{min}$ ,  $200^\circ\text{C}$ , retention times 3.3 [(Z)-3, 93%] and 4.4 min [(E)-3, 7%]; NMR ( $\text{CDCl}_3$ )  $\delta$  2.18 (d,  $J = \sim 1\text{ Hz}$ , "E" Me) and 1.88 (d,  $J = \sim 1\text{ Hz}$ , "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 ( $\text{Na}_2\text{SO}_4$ ), and filtered through  $\text{Na}_2\text{SO}_4$ -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 ( $\text{C}=\text{C}$ ),  $1000\text{ cm}^{-1}$  ( $\text{C}-\text{O}$ ); NMR ( $\text{CDCl}_3$ )  $\delta$  1.73 (br s, 3,  $\text{C}=\text{CCH}_3$ ), 2.1 (m, 2,  $\text{C}=\text{CCH}_2$ ), 4.08 (d,  $J = 6\text{ Hz}$ , 2,  $\text{C}=\text{CH}_2\text{O}$ ), 5.4 (t,  $J = 6\text{ Hz}$ , 1,  $\text{C}=\text{CH}$ ); MS calcd for  $\text{C}_9\text{H}_{18}\text{O}$  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  $\gamma$ -collidine in 25 mL of anhydrous ether, which was cooled to  $-35^\circ\text{C}$  (acetonitrile-dry ice), was added with mechanical stirring 0.95 mL (10.0 mmol) of  $\text{PBr}_3$  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  $\text{NaHCO}_3$ , and saturated brine, dried ( $\text{MgSO}_4$ ), 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 ( $\text{C}=\text{C}$ ), 1195,  $845\text{ cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  1.77 (s, 3,  $\text{C}=\text{CCH}_3$ ), 4.0 (d,  $J = 8\text{ Hz}$ , 2,  $\text{C}=\text{CH}_2$ ), 5.50 (t,  $J = 6\text{ Hz}$ , 1,  $\text{C}=\text{CH}$ ); MS calcd for  $\text{C}_9\text{H}_{17}^{79}\text{Br}$  204.0514, found 204.0529.

**11-Chloro-1,4-undecadiyne (7).** The procedure of Ege and co-workers<sup>11</sup> was adapted. The solvent was removed at reduced pressure from 21.5 mL of 3.2 M ethereal  $\text{EtMgBr}$  (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<sup>12</sup> in 10 mL of THF was added. After foaming had ceased, the solution was immersed in a  $60^\circ\text{C}$  oil bath, stirred for 50 min, and then cooled to room temperature.  $\text{Cu}_2\text{Cl}_2$  (0.31 g, 3.1 mmol) was added, and heating at  $60^\circ\text{C}$  was resumed for 20 min. The temperature of the stirred reaction mixture was maintained at  $20$ – $30^\circ\text{C}$  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  $\text{NH}_4\text{Cl}$  solution and extracted with ether. The combined extracts were washed with  $\text{NH}_4\text{Cl}$  solution and water, dried ( $\text{MgSO}_4$ ), and concentrated at reduced pressure (below  $50^\circ\text{C}$ ) 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  $31^\circ\text{C}$  (0.75 mm). The product (7.51 g, 60%) distilled at  $78$ – $90^\circ\text{C}$  (0.05 mm): very pale yellow oil; IR (film) 3150 ( $\text{C}=\text{CH}$ ),  $1310\text{ cm}^{-1}$  ( $\text{C}=\text{CCH}_2\text{C}=\text{C}$ ); NMR ( $\text{CDCl}_3$ )  $\delta$  2.05 (t,  $J = 2\text{ Hz}$ , 2,  $\text{CH}_2\text{CH}_2\text{C}=\text{C}$ ), 2.2 (m, 1,  $\text{C}=\text{CH}$ ), 3.13 (q,  $J = 2\text{ Hz}$ , 2,  $\text{C}=\text{CCH}_2\text{C}=\text{C}$ ), 3.53 (t,  $J = 6\text{ Hz}$ , 2,  $\text{CH}_2\text{Cl}$ ).

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  $-20^\circ\text{C}$  under argon minimized decomposition and polymerization. The pot residue from the distillation was distilled evaporatively at  $78$ – $80^\circ\text{C}$  (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 ( $\text{CDCl}_3$ )  $\delta$  4.7–5.1 (m, 0.75,  $\text{CH}=\text{C}=\text{CH}_2$ ).

**(Z)-1-Chloro-14-methylnonadeca-7,10-diyne-13-ene (8).** A procedure of Ege and co-workers<sup>11</sup> was adapted. Ether was removed at reduced pressure from 12.7 mL of 3.2 M ethereal  $\text{EtMgBr}$  (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  $20^\circ\text{C}$ . Some ice cooling was necessary during the 5-min addition period. Foaming soon subsided. The solution was stirred at  $20^\circ\text{C}$  for 45 min; then 0.212 g (2.1 mmol) of  $\text{Cu}_2\text{Cl}_2$  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  $10$ – $15^\circ\text{C}$ . A bright yellow precipitate soon formed. The reaction mixture was stirred at room temperature for 2.5 h, then poured into cold, almost-saturated  $\text{NH}_4\text{Cl}$  solution, and extracted with ether. The ether solution was washed with  $\text{NH}_4\text{Cl}$  solution and water. The ether extracts were dried ( $\text{MgSO}_4$ ) and concentrated at reduced pressure in a water bath below  $50^\circ\text{C}$  to afford 13.0 g of an orange oil that was evaporatively distilled. The 4.3 g of starting materials was collected at  $58$ – $90^\circ\text{C}$  (0.04 mm). The 8.8 g (71%) of 8 was collected at  $160$ – $180^\circ\text{C}$  (0.05 mm): very pale yellow oil; IR (film) 2100 ( $\text{C}=\text{C}$ ), 1660 ( $\text{C}=\text{C}$ ), 1295 ( $\text{C}=\text{CCH}_2\text{C}=\text{C}$ ),  $725\text{ cm}^{-1}$ ; NMR ( $\text{CDCl}_3$ )  $\delta$  1.67 (br s, 3,  $\text{C}=\text{CCH}_3$ ), 1.8–2.1 (m, 4,  $\text{C}=\text{CCH}_2\text{CH}_2$ ), 2.7–2.9 (m, 2,  $\text{C}=\text{CCH}_2\text{C}=\text{C}$ ), 3.1 (m, 2,  $\text{C}=\text{CCH}_2\text{C}=\text{C}$ ), 3.53 (t,  $J = 7\text{ Hz}$ , 2,  $\text{CH}_2\text{Cl}$ ), 5.20 (t,  $J = 6\text{ Hz}$ , 1,  $\text{C}=\text{CH}$ ); MS calcd for  $\text{C}_{20}\text{H}_{31}^{35}\text{Cl}$  306.2115, found 306.2130.

**(7Z,10Z,13Z)-1-Chloro-14-methyl-7,10,13-nonadecatriene (9).** To 4.3 g of Lindlar catalyst<sup>13</sup> 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.<sup>13</sup> 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<sub>4</sub>), 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<sup>-1</sup>; NMR (CDCl<sub>3</sub>)  $\delta$  1.7 (br s, 3, C=CCH<sub>3</sub>), 1.9–2.3 (m, 4, C=CCH<sub>2</sub>CH<sub>2</sub>), 2.7–3.0 (m, 4, C=CCH<sub>2</sub>C=C), 3.53 (t, *J* = 6 Hz, 2, CH<sub>2</sub>Cl), 5.13 (t, *J* = 7 Hz, 1, HC=CCH<sub>3</sub>), 5.4 (2 t, *J* = 5 Hz, 4, HC=CH); MS calcd for C<sub>20</sub>H<sub>35</sub><sup>35</sup>Cl 310.2403, found 310.2403.

**(8Z,11Z,14Z)-15-Methyl-8,11,14-eicosatrienoic Acid (1).** A procedure of Ege and co-workers<sup>11</sup> was modified. To 0.399 g (0.016 g-atom) of Mg turnings and one crystal each of HgCl<sub>2</sub> and I<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub>SO<sub>4</sub>), 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<sub>f</sub>* 0.5) under argon afforded 0.67 g (73%) of colorless oil: IR (film) 2450–3100 (OH of CO<sub>2</sub>H), 1740 (C=O of CO<sub>2</sub>H), 935 cm<sup>-1</sup> (OH of CO<sub>2</sub>H); NMR (CDCl<sub>3</sub>)  $\delta$  1.70 (s, 3, C=CCH<sub>3</sub>), 5.13 (t, *J* = 7 Hz, 1, HC=CCH<sub>3</sub>), 5.40 (2 AB quartets, *J<sub>AB</sub>* = 5 Hz, 4, HC=CH), 8.1 (br s, 1, CO<sub>2</sub>H); *n*<sub>D</sub><sup>26</sup> 1.4795; MS calcd for C<sub>24</sub>H<sub>44</sub>O<sub>2</sub>Si 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 methylolithium.

**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<sup>5</sup> 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<sub>2</sub>CO<sub>3</sub> 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.<sup>14</sup> 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<sub>3</sub>CCO<sub>2</sub>H 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,<sup>15</sup> was supplemented with 0.5  $\mu$ M 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 lipoygenase 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<sub>i</sub>* value of 2  $\mu$ M. The *K<sub>i</sub>* value was determined (based on eight assays) by the graphical method of Dixon.<sup>16</sup> 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

- (1) (a) (8Z,11Z)-15-Methyl-8,11,14-eicosatrienoic acid has been prepared by P. F. Beal, S. Gunther, J. E. Pike, and W. P. Schneider (Upjohn Co.), S. African Patent 68 03 390 (Nov 27, 1968). (5Z,8Z,11Z,14E)-15-Methyl-5,8,11,14-eicosatetraenoic acid has been prepared by R. I. Fryer, N. W. Gilman, and B. C. Holland, *J. Org. Chem.*, **40**, 348 (1975). (b) For the preceding paper in this series, please see M. Dawson and M. Vasser, *J. Org. Chem.*, **42**, 2783 (1977). (c) Support of the synthetic portion of this work by the National Institutes of Child Health and Human Development, Contract No. N01-HD-4-2832, is gratefully acknowledged.
- (2) S. B. Bowlus and J. A. Katzenellenbogen, *Tetrahedron Lett.*, 1277 (1973).
- (3) Configurational assignments were confirmed by NMR vinylic methyl proton resonances. Chemical shift data for vinylic methyl groups in similarly functionalized trisubstituted olefins have been reported: see R. B. Bates and D. M. Gale, *J. Am. Chem. Soc.*, **82**, 5749 (1960); K. Ogura, T. Nishino, T. Koyama, and S. Seto, *ibid.*, **92**, 6036 (1970); J. H. Babler, *J. Org. Chem.*, **41**, 1262 (1976).
- (4) R. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds", Wiley, New York, N.Y., 1963, p 87.
- (5) L. J. Bellamy, "The Infrared Spectra of Complex Molecules", Wiley, New York, N.Y., 1975, p 38.
- (6) In a similar experiment, Dr. M. J. Silver, Department of Medicine, Cardeza Foundation for Hematologic Research,

Thomas Jefferson University, Philadelphia, Pa., has also shown that 1 inhibited human platelet aggregation in response to ADP. We thank Dr. Silver for doing this study.

- (7) W. E. M. Lands, M. Hemler, and C. G. Crawford in "Biochemistry and Metabolism of Polyunsaturated Acids", R. T. Holman, Ed., American Oil Chemists Society Press, Champaign, Ill., 1977.
- (8) W. E. M. Lands, P. R. LeTellier, L. H. Rome, and J. Y. Vanderhoek, *Adv. Biosci.*, **9**, 15 (1973).
- (9) J. Y. Vanderhoek and W. E. M. Lands, *Biochim. Biophys. Acta*, **296**, 374 (1973).
- (10) E. J. Corey and J. A. Katzenellenbogen, *J. Am. Chem. Soc.*, **91**, 1851 (1969); J. B. Siddall, M. Biskup, and J. H. Fried, *ibid.*, **91**, 1853 (1969).
- (11) S. N. Ege, R. Wolovsky, and W. J. Gensler, *J. Am. Chem. Soc.*, **83**, 3080 (1961).
- (12) R. A. Raphael and F. Sondheimer, *J. Chem. Soc.*, 2100 (1950).
- (13) H. Lindlar, *Helv. Chim. Acta*, **35**, 446 (1952).
- (14) J. B. Smith, C. M. Ingeman, and M. J. Silver, *J. Lab. Clin. Med.*, **88**, 167 (1976).
- (15) M. Hemler, W. E. M. Lands, and W. L. Smith, *J. Biol. Chem.*, **251**, 5575 (1976).
- (16) M. Dixon, *Biochem. J.*, **55**, 171 (1953).

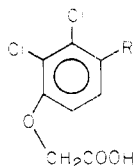
## (Acylaryloxy)acetic Acid Diuretics. 1. (2-Alkyl- and 2,2-Dialkyl-1-oxo-5-indanyloxy)acetic Acids

Otto W. Woltersdorf, Jr.,\* S. Jane deSolms, Everett M. Schultz, and Edward J. Cragoe, Jr.

Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486. Received May 17, 1977

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.

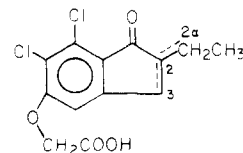
The mercurial phenoxyacetic acid diuretics, merbaphen and mersalyl, served as models for the design, and ultimately led to the discovery, of the potent (acryloylaryloxy)acetic acid diuretics,<sup>1</sup> the best known of these loop diuretics being ethacrynic acid<sup>2</sup> (1a). Recently four series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



- 1a, R = -COC(=CH<sub>2</sub>)C<sub>2</sub>H<sub>5</sub>  
 b, R = -CH=C(COCH<sub>3</sub>)<sub>2</sub>  
 c, R = -CH=C(CH<sub>3</sub>)COCH<sub>3</sub>  
 d, R = -CH=C(CH<sub>3</sub>)NO<sub>2</sub>  
 e, R = -CH=CR<sup>1</sup>R<sup>2</sup>  
 f, R = -COCH(CH<sub>3</sub>)C<sub>2</sub>H<sub>5</sub>

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.<sup>7</sup> Mercurials generally produce little change in uric acid excretion while 1a causes uric acid retention which may result in hyperuricemia.<sup>8,9</sup> 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.

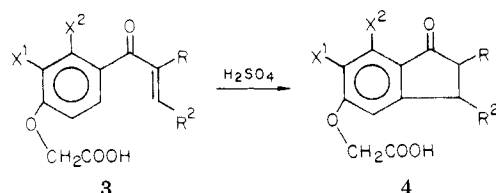
Following the observation that ethacrynic acid underwent intramolecular cyclization to 2a upon treatment with sulfuric acid, our initial efforts were directed toward the introduction of a double bond in the molecule (2b,c).



- 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.<sup>10</sup> 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 cyclization of the correspondingly substituted (acryloylphenoxy)acetic acids (3)<sup>11</sup> in concentrated H<sub>2</sub>SO<sub>4</sub> according to the following reaction and these products are listed in Table I.



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-