Stereoselective Synthesis of the Diunsaturated Metabolites of Valproic Acid

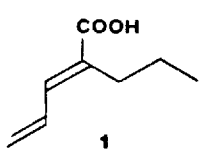
RONALD D. LEE, KELEM KASSAHUN, AND FRANK S. ABBOTT[x]

Received June 7, 1988, from the *Faculty of Pharmaceutical Sciences, 2146 East Mall, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1W5.* Accepted for publication December 21, 1988.

Abstract □ Two diene metabolites of valproic acid (VPA), (*E*)-2-*n*-propyl-2,4-pentadienoic acid (**1**) and (*E*)-2-(1′-propenyl)-(*E*)-2-pentenoic acid (**2**), were stereoselectively synthesized. Mesylate elimination in the final step to produce the unsaturation at position 2 was stereospecific for the (*E*)-configuration in the case of **2**. Gas chromatography-mass spectroscopy and NMR were used to confirm the configuration of each diene including the minor isomers, (*Z*)-2-*n*-propyl-2,4-pentadienoic acid (**9**) and (*Z*)-2-(1′-propenyl)-(*E*)-2-pentenoic acid (**18**). Analysis of the dienes, as PFB derivatives by negative chemical ionization GC-MS from a serum extract of a patient on VPA therapy, revealed the presence of four peaks that in order of elution correspond to **9**, **18**, **1**, and **2**.

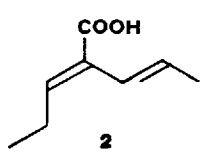
Valproic acid (VPA) is a widely used drug for the treatment of epilepsy.[1,2] Metabolism of VPA occurs mainly in the liver and several complex metabolic pathways have been presented.[3–5] Of recent interest are the diunsaturated metabolites of VPA, namely (*E*)-2-*n*-propyl-2,4-pentadienoic acid (**1**) and (*E*)-2-(1′-propenyl)-(*E*)-2-pentenoic acid (**2**). Of the five reported diene metabolites of VPA,[6] these two dienes (**1** and **2**) are most frequently found in patient serum and urine samples.[7] The metabolite **1** has been shown to be hepatotoxic in rats, while **2** was found to possess anticonvulsant activity in mice equipotent to the active (*E*)-2-*n*-propyl-2-pentenoic acid.[8,9]

In order to undertake metabolic and pharmacokinetic studies of **1** and **2**, methods of syntheses were required that would yield significant quantities of these dienes in a stereoselective manner. The synthesis of **1** as reported by Rettenmeier et al.[10] utilized a method that was stereoselective for **1** but gave low yields. A second approach to the synthesis of **1** proved not to be regiospecific with 2-(2′-propenyl)-2-pentenoic acid also being formed.[4] The synthesis of **2**, as originally reported from this laboratory, gave three isomers, but was selective for the (*E*,*E*)-isomer (**2**).[11] We report here further attempts at the stereoselective synthesis of **1** and **2** and attempts to determine whether the geometric isomers (*Z*)-2-*n*-propyl-2,4-pentadienoic acid (**9**) and (*Z*)-2-(1′-propenyl)-(*E*)-2-pentenoic acid (**18**) are also metabolites of VPA in humans.



1

(E)-2-n-Propyl-2,4-pentadienoic Acid



2

(E)-2-(1′-Propenyl)-E-2-pentenoic Acid

Experimental Section

Materials—Acrolein, *t*-butyldimethylsilylchloride (*t*-BDMS), *n*-butyllithium (1.6 M in hexane), 18-crown-6, diisopropylamine, 4-dimethylaminopyridine, methanesulfonyl chloride, 2-pentenone, potassium hydride (35% oil dispersion), propionaldehyde, triethylamine, and triphenylcarbenium tetrafluoroborate were purchased from Aldrich Chemical (Milwaukee, WI). Pentafluorobenzyl bromide was obtained from Alfa Products (Danvers, MA), *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) from Pierce Chemical (Rockford, IL), and hexamethylphosphoramide from Fisher Scientific (Fairlawn, NJ). All other reagents and solvents were of analytical grade.

Instrumentation—Proton NMR spectra were obtained on a Bruker WP-80, a Varian XL-300, or a Bruker WH-400 spectrometer in the Department of Chemistry, University of British Columbia. The solvent and internal standard used were deuterochloroform and tetramethylsilane, respectively.

Electron impact (EI) and negative chemical ionization (NCI) gas chromatography-mass spectrometry (GC-MS) of extracted patient serum samples and spiked control human serum samples were performed on a Hewlett-Packard 5987A instrument equipped with either a DB-1 fused silica capillary column (30 m × 0.32 mm i.d.; J & W Scientific, Folsom, CA) or an OV-1701 fused silica capillary column (25 m × 0.32 mm i.d.; Quadrex, New Haven, CT). Injections were performed in the splitless mode with a programmed oven temperature of 50 to 100 °C at 30 °C/min and 100 to 230 °C at 8 °C/min. The injection port temperature was 240 °C and the source temperature was 250 °C. The carrier gas used was helium with a head pressure of 10 psi. The EI and NCI spectra were recorded with the source pressure at 2×10^{-6} and 1 Torr, respectively. The reagent gas for NCI was methane.

The GC-MS analyses of synthesized compounds were performed on a Hewlett-Packard 5700A gas chromatograph interfaced to a Varian MAT-111 mass spectrometer using a variable slit separator. The EI data were obtained with the source pressure at 5×10^{-5} Torr and an emission current of 300 μA at 70 eV. The following GC conditions were used: a column (1.8 m × 2 mm i.d.) packed with 3% Dexsil 300 on 100–120 mesh Supelcoport (Supelco, Bellefonte, PA); oven temperature was 50 to 270 °C at 8 °C/min; injection port temperature was 250 °C; carrier gas (helium) had a flow rate of 25 mL/min.

Chemistry—The syntheses of **1** and **2** are outlined in Schemes I and II, respectively.

Synthesis of 1 via the Elimination of the Mesyl Ester of Ethyl 2-*n*-Propyl-3-hydroxy-4-pentenoate (6)—Ethyl valerate (**4**) was prepared from valeric acid (**3**; 163.6 g, 1.6 mol) using excess ethanol (220.8 g, 4.8 mol) and 2 mL of concentrated H_2SO_4 refluxed in 500 mL of benzene for 48 h. The water generated was removed as a benzene azeotrope via a Dean-Stark apparatus. Isolation by fractional distillation yielded 129.7 g (62.3%) of **4**; bp 137–140 °C/760 mm (lit.[12] 145 °C/760 mm).

To a stirred solution of diisopropylamine (20.24 g, 0.2 mol) in 150 mL of dry THF at 0 °C, *n*-butyllithium (130 mL of 1.6 M in hexane, 0.2 mol) was added in a dropwise manner over a period of 15 min. The mixture was allowed to react for 15 min before being cooled to −78 °C. Compound **4** (26 g, 0.2 mol) in 10 mL of THF was slowly added in a dropwise manner over a 15-min period and the mixture was stirred for a further 20 min. Acrolein (11.2 g, 0.2 mol) in 10 mL of THF was added in a dropwise manner over a period of 15 min and the reaction was allowed to continue for a further 60 min before quenching with 15% HCl to bring the pH to 1. The aqueous phase was extracted three times with ether and the combined ethereal layers were washed with saturated $NaHCO_3$ followed by water. The organic fraction was then dried over anhydrous $MgSO_4$. Upon removal of the solvent by flash evaporation, fractional distillation afforded 23.6 g (63.4%) of ethyl

0022-3549/89/0800-0667$01.00/0

2-*n*-propyl-3-hydroxy-4-pentenoate (5); bp 74 °C/0.1 mm; GC-MS (EI) resulted in the following spectrum: *m/z* (%) 101(100), 73(80), 55(78), 130(32), 84(27), 141(7), 159(2), 169(2); ^{1}H NMR (80 MHz): 0.9 (t, 3H, CH_3—), 1.2 (t, 3H, —OCH_2—CH_3), 1.3–1.7 (m, 4H, CH_2—CH_2), 2.3–2.7 (m, 1H, CHCO), 2.4 (s, broad, 1H, OH), 4.2 (q, 2H, O—CH_2), 4.2–4.5 (t, 1H, CH—O), 5.1–5.4 (m, 2H, CH_2=), and 5.65–6.1 ppm (m, 1H, =CH).

An aliquot of 5 (7.4 g, 0.04 mol) and triethylamine (4 g, 0.04 mol) in 40 mL of dry dichloromethane were cooled to 0 °C. Methanesulfonyl chloride (4.89 g, 0.04 mol) cooled to 0 °C was added in a dropwise manner to the stirred mixture and allowed to react for 60 min. The reaction mixture was filtered by suction, the solvent was removed by flash evaporation, and an aliquot of the crude mesyl ester, 6, was analyzed by GC-MS. Two peaks corresponding to the diastereomers gave identical mass spectra; GC-MS (EI): *m/z* (%) 95(100), 67(65), 79(55), 168(45), 140(45), 123(30), 111(30). The crude mixture of 6 was reconstituted in 100 mL of dry THF and cooled to 0 °C, and potassium hydride (4.81 g, 0.12 mol) was added slowly. The reaction mixture was allowed to attain room temperature and was then stirred for 12 h. Excess potassium hydride was decomposed very cautiously at −78 °C with water and the aqueous fraction was extracted three times with ether. The combined organic fractions were dried over anhydrous Na_2SO_4 and the solvent was removed by flash evaporation. Fractional distillation of the residue afforded 1.5 g (22%) of a 3.5:1 (*E*:*Z*) isomeric mixture of ethyl 2-propyl-2,4-pentadienoate (7, 8); bp 70 °C/1 mm; GC-MS (EI) of 7: *m/z* (%) 95(100), 168(M^+,97), 67(80), 111(74), 123(74), 140(65), 79(40), 153(5); ^{1}H NMR (400 MHz) of the isomeric mixture: 0.92 (t, 3H, CH_3—), 1.31 (t, 3H, OCH_2—CH_3, (*E*)), 1.32 (t, 3H, OCH_2—CH_3, (*Z*)), 1.4–1.52 (m, 2H, —CH_2—CH_2), 2.29 (t, 2H, J = 7 Hz, CH_2—CH_2, (*Z*)), 2.4 (t, 2H, J = 7 Hz, CH_2—CH_2, (*E*)), 4.21 (q, 2H, O—CH_2, (*E*)), 4.31 (q, 2H, O—CH_2, (*Z*)), 5.31 (d, 1H, J_{BX} = 10 Hz, CH_2=, (*Z*)), 5.38 (d, 1H, J_{AX} = 16 Hz, CH_2=, (*Z*)), 5.44 (dd, 1H, J_{BX} = 10 Hz, J_{AB} = 2 Hz, CH_2=, (*E*)), 5.57 (dd, 1H, J_{AX} = 17 Hz, J_{AB} = 2 Hz, CH_2=, (*E*)), 6.34 (d, 1H, J = 12 Hz, —CH=C, (*Z*)), 6.68 (ddd, 1H, CH_2=*CH*, (*E*)), 7.17 (d, 1H, J = 12 Hz, —CH=C, (*E*)), and 7.14–7.25 ppm (m, 1H, CH_2=*CH*, (*Z*)).

The free acids were obtained by stirring the isomeric mixture of 7 and 8 (100 mg, 0.6 mmol) in 10 mL of 1 M NaOH for 24 h at 25°C in a flask protected from light. The mixture was brought to a pH of 1 with 1 M HCl and extracted three times with ether. The combined ethereal fractions were dried over anhydrous Na_2SO_4 and the ether was removed by flash evaporation; GC-MS (EI) analysis of the *t*-BDMS ester derivative of 1: *m/z* (%) 197(100), 75(15), 123(15), 95(10), 67(7), 153(3), 155(2), 239(2);[7,10] GC-MS (EI) analysis of 9: 197(100), 75(45), 95(40), 155(20), 67(15), 123(10), 153(4), 239(3).

Synthesis of 7 by the Oxidation, via Allylic Hydride Abstraction, of 2-Propenyl propyl-*O*-ethyl-*O*-trimethylsilylketene acetal (11)—The synthesis of the starting material, ethyl 2-*n*-propyl-4-pentenoate (10), was based on the method of Rettenmeier et al.[10] To a solution of diisopropylamine (1.21 g, 12 mmol) in dry THF (10 mL) at 0 °C, *n*-butyllithium (7.5 mL of 1.6 M in hexane, 12 mmol) was added slowly and allowed to react for 15 min. The mixture was cooled to −78 °C and 10 (1.7 g, 10 mmol) in 3 mL of THF was added in a dropwise manner. After 60 min, chlorotrimethylsilane (1.85 g, 17 mmol) in 3 mL of THF was slowly added and the temperature was allowed to increase to 25 °C. After 60 min, the solvent was removed under reduced pressure using a vacuum pump. The crude 11 was then reconstituted in 10 mL of anhydrous dichloromethane. The mixture was slowly added to a solution of triphenylcarbenium tetrafluoroborate (4.95 g, 15 mmol) and collidine (1.45 g, 12 mmol) in dry dichloromethane (25 mL). After 30 min, the reaction was quenched with 30 mL of water and the aqueous phase was extracted three times with ether. The combined ether layers were consecutively washed with saturated $NaHCO_3$ and water, then dried over anhydrous Na_2SO_4. Upon removal of the solvent by flash evaporation, GC-MS (EI) analysis of the residue revealed poor yields of 7 and the presence of a significant amount of starting material 10.

Synthesis of 2 via Elimination of the Mesyl Ester of Ethyl (*E*)-2-(1′-hydroxypropyl)-3-pentenoate (15)—The (*Z*)-2-pentenoic acid (12) was synthesized using the Favorsky rearrangement of 1,3-dibromo-2-pentenone described briefly by Rappe and Adestrom[13] and in detail by Rappe.[14] The ethyl (*Z*)-2-pentenoate (13) was obtained by refluxing 12 (23.3 g, 0.23 mol) with ethyl iodide (71.76 g, 0.46 mol), potassium carbonate (23.84 g, 0.17 mol), and 18-crown-6 (3 g, 0.05 M) in 230 mL of dry THF for 6 h. The mixture was filtered by suction and fractional distillation afforded 17.1 g of 13 (yield 58%, bp 43–45 °C/10 mm); GC-MS (EI); *m/z* (%) 100(100), 83(95), 55(77), 29(65), 128(M^+, 30); ^{1}H NMR (80 MHz): 1.1 (t, 3H, CH_3—), 1.3 (t, 3H, —CH_3), 2.65 (q, 2H, CH_2), 4.2 (q, 2H, OCH_2), 5.7 (d, 1H, J = 10 Hz, CH=*CH*), and 6.2–6.5 ppm (dt, 1H, J_{cis} = 10 Hz, J_{gem} = 6 Hz, *HC*=CH).

To a stirred solution of diisopropylamine (11.13 g, 0.11 mol) in anhydrous THF (90 mL) at 0 °C, *n*-butyllithium (69 mL of 1.6 M in hexane, 0.11 mol) was added in a dropwise manner over a period of 15 min and allowed to react for 20 min. Upon cooling to −78 °C, hexamethylphosphoramide (19.57 g, 0.11 mol) was added in a dropwise manner to the mixture and stirred for 15 min. An aliquot of 13 (12.8 g, 0.1 mol) in 10 mL of THF was added in a dropwise manner. After 30 min, propionaldehyde (5.8 g, 0.1 mol) in 10 mL of THF was slowly introduced into the mixture and stirred for 30 min. The reaction was quenched with 15% HCl until a pH of 1 was attained. The aqueous portion was extracted three times with ether and the combined organic fractions were washed consecutively with saturated $NaHCO_3$ and water, and then dried over anhydrous Na_2SO_4. Removal of the solvent by flash evaporation and fractional distillation of the residue afforded 11.2 g (yield 60%) of ethyl (*E*)-2-(1′-hydroxypropyl)-3-pentenoate (14); bp 78 °C/0.22 mm (lit.[11] 95–100 °C/1 mm); GC-MS (EI): *m/z* (%) 29(100), 100(50), 55(49), 82(44), 128(40), 113(5), 141(2), 157(2); ^{1}H NMR (300 MHz): 0.95 (t, 3H, CH_3—CH_2), 1.3 (t, 3H, OCH_2—CH_3), 1.45 (m, 2H, CH_2), 1.7 (d, 3H, CH_3—CH=), 2.65 (broad s, 1H, OH), 3.0 (m, 1H, CH—C=O), 3.65–3.8 (m, 1H, CH—O), 4.15 (q, 2H, OCH_2—CH_3), 5.47 (m, 1H, *CH*=CH), and 5.63 ppm (m, 1H, =*CH*—CH).

Compound 14 (9.3 g, 0.05 mol) was added to triethylamine (8.1 g, 0.08 mol) in 40 mL of dry dichloromethane and the mixture was cooled to 0 °C. Methanesulfonyl chloride (6.87 g, 0.06 mol) at 0 °C was slowly added to the mixture and stirred for 60 min at 0 °C. The mixture was filtered by suction, the solvent was removed by flash evaporation, and the crude mesyl ester, 15, was analyzed by GC-MS. Two peaks corresponding to the diastereomers gave identical mass spectra; GC-MS (EI): *m/z* (%) 95(100), 67(55), 55(45), 123(30), 111(15), 139(15), 168(10), 153(5). The crude mixture of 15 was reconstituted in 150 mL of anhydrous THF and cooled to 0 °C, and potassium hydride (4.01 g, 0.1 mol) was cautiously added. The mixture was then brought to 25 °C and allowed to react for 12 h. Excess potassium hydride was carefully decomposed at −78 °C with water and the aqueous layer was extracted three times with ether. The combined organic fractions were consecutively washed with saturated $NaHCO_3$ and water, and then dried over anhydrous Na_2SO_4. The solvent was removed by flash evaporation and fractional distillation of the residue afforded 3.9 g [yield 46%, bp 70 °C/0.7 mm (lit.[11] 65–70 °C/0.1 mm)] of an isomeric mixture that by GC-MS was estimated to contain 81% of ethyl (*E*)-2-(1′-propenyl)-(*E*)-2-pentenoate (16) and 19% of ethyl (*Z*)-2-(1′-propenyl)-(*E*)-2-pentenoate (17); GC-MS (EI) of 16: *m/z* (%) 95(100), 168(M^+, 90), 79(64), 67(49), 123(46), 140(37), 111(30), 153(7)[11]; ^{1}H NMR (400 MHz): 1.0–1.1 (t, 3H, CH_3—CH_2), 1.32 (t, 3H, OCH_2—CH_3), 1.84 (d, J = 7 Hz, 3H, CH_3—CH=), 2.3 (m, 2H, CH_2—CH=), 4.2 (q, 2H, OCH_2—CH_3), 5.75 (trace, m, *CH*=CH), (*E*, *Z*)), 6.07 (m, 1H, *CH*=CH, (*E*, *E*)), 6.13 (d, J = 17 Hz, 1H, CH=*CH*, (*E*, *E*)), 6.55 (t, J = 7 Hz,1H,CH_2—*CH*=, (*E*, *E*)), and 6.8 ppm (trace, CH_2—*CH*=, (*E*,*Z*)).[11]

The free acids were obtained by stirring the mixture of 16 and 17 (2.7 g, 0.016 mol) in 25 mL of 2.2 M NaOH at 60 °C for 48 h. The mixture was extracted with 25 mL of hexane (discard) and the aqueous fraction was adjusted to a pH of 1 with 10% HCl. The acidic solution was extracted three times with ether and the combined ethereal portions were dried over anhydrous Na_2SO_4. Removal of the ether by flash evaporation and fractional distillation afforded 1.2 g (yield 52.1%, bp 84 °C/0.05 mm) of 2 and 18 with the (*E*, *E*)-isomer constituting ~94% of the mixture; ^{1}H NMR (400 MHz): 1.09 (t, J = 8 Hz, 3H, CH_3—CH_2), 1.57 (trace, d, CH_3—CH=, (*E*, *Z*)), 1.85 (d, J = 7 Hz, 3H, CH_3—CH=, (*E*, *E*)), 2.11 (trace, m, CH_2, (*E*, *Z*)), 2.34 (m, 2H, CH_2, (*E*, *E*)), 5.83 (trace, m, *CH*=CH, (*E*, *Z*)), 5.99 (trace, d, J = 11 Hz, CH=*CH*, (*E*, *Z*)), 6.02–6.11 (m, 1H, *CH*=CH, (*E*, *E*)), 6.15(d, J = 16 Hz, 1H, CH=*CH*, (*E*, *E*)), and 6.8 ppm (t, J = 8 Hz, 1H, CH_2—*CH*=, (*E*, *E*)); GC-MS (EI) analysis of the *t*-BDMS ester derivative of 2: *m/z* (%) 197(100), 75(35), 95(20), 123(10), 254(2), 139(2), 153(2), 167(2);[7] GC-MS (EI) analysis of the *t*-BDMS ester derivative of 18: 197(100), 75(20), 95(15), 123(10), 254(5), 139(2), 153(2), 167(2).

Synthesis of 16 by the Oxidation, via Allylic Hydride Abstraction, of 1-Propenyl propyl-*O*-ethyl-*O*-trimethylsilylketene acetal

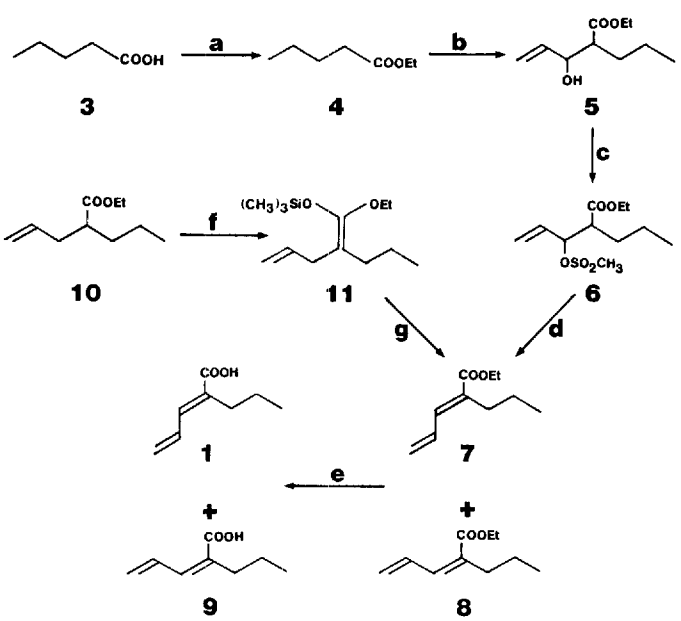
(20)—Ethyl (*E*)-2-*n*-propyl-3-pentenoate (19) was synthesized according to the procedure of Acheampong.[15] The experimental methods to produce the acetal, 20, and the oxidation by allylic hydride abstraction to give 16, were identical to those described for the synthesis of 7 from 10; GC-MS (EI) of crude 20: *m/z* (%) 73(100), 95(80), 124(50), 169(11), 213(11), 242(M^+11), 141(10). Following the hydride abstraction step, GC-MS (EI) of the product revealed two isomers, 16 and 17, plus starting material 19. Fractional distillation failed to separate the products from the starting material.

Sample Preparation—A serum sample (1 mL) from an epileptic patient on valproic acid (VPA) therapy and two control serum samples, to which had been added either 1 and 9 or 2 and 18 (final concentrations of 15 μg/mL), were extracted in duplicate according to the procedures of Abbott et al.[7] The pH was adjusted to 2 with 3 M HCl and the sample was extracted twice with 1 mL of ethyl acetate by gentle mechanical rotation. The combined organic fractions were dried over anhydrous Na_2SO_4 and the volume was reduced to 200 μL with nitrogen. The three sets of extracted samples were derivatized with either MSTFA,[7] *t*-BDMS,[7] or pentafluorobenzyl bromide,[16] and 1 μL was injected for GC-MS analysis by either EI or NCI.

Results and Discussion

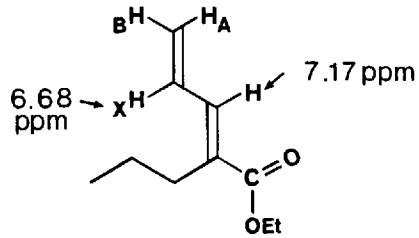
Synthesis of the Dienes by the Stereoselective Elimination of the Corresponding Mesyl Esters—The synthesis of 1 via this route is outlined in Scheme I. The facile synthesis and high yields of the hydroxy ester 5 make this approach the most feasible in spite of disappointingly low yields in the final elimination step. Nevertheless, the 22% yield of esters 7 and 8 from the potassium hydride elimination of the mesyl ester exceeds reported yields of 7%.[10] The *E*-to-*Z* isomer ratio (78% of 7, 22% of 8) also compares favorably with the reported 90% isomeric purity of the (*E*)-isomer 1 obtained following hydrolysis of the ester.[10] While not done in this instance, further separation of the two isomers could be achieved by preparative argentation-TLC. This methodology was originally used to separate the isomers of 2-(1′-propenyl)-2-pentenoic acid.[11]

The predominance of the (*E*)-isomer 7 is consistent with the stereospecific elimination of mesylates by KH claimed by Kende and Toder[17] for the synthesis of (*Z*)-2-(1′-propenyl)-(*E*)-2-hexadecenoic acid. However, the significant yield obtained for the (*Z*)-isomer 8 suggests that in the formation of the 2,4-conjugated diene ester, elimination of mesylate was not stereospecific. Alternatively, partial isomerization of 7 to 8 may have occurred during workup. The absence of any other diene isomers in the product confirmed the regiospecificity of the reaction. Conjugative elimination is perhaps a factor in the regiospecificity, since attempts to produce the monounsaturated ethyl 2-*n*-propyl-2-pentenoate via mesylate elimination were unsuccessful.

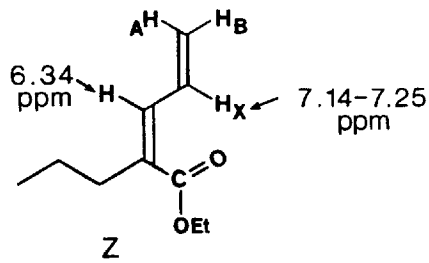


Scheme I—Synthesis of 2-*n*-propyl-2,4-pentadienoic acid by mesylate elimination and hydride abstraction. (a) EtOH, H_2SO_4, benzene, Δ; (b) $[(CH_3)_2CH]_2NH$, *n*-BuLi, CH_2CHCHO, THF, −78 °C; (c) $(C_2H_5)_3N$, CH_3SO_2Cl, CH_2Cl_2, 0 °C; (d) KH, THF, room temperature; (e) 1 M NaOH, 1 M HCl; (f) $[(CH_3)_2CH]_2NH$, *n*-BuLi, $(CH_3)_3SiCl$, THF, −78 °C; (g) collidine, triphenylcarbenium fluoroborate, CH_2Cl_2, room temperature.

The configuration of the diene isomers 7 and 8 can be assigned based on the stereoselectivity of the elimination reaction to give the (*E*)-isomer[10,17] and on NMR evidence. The expected planarity of the conjugated 2,4-diene VPA esters provide for significant shielding and deshielding effects of the carbonyl group on neighboring vinylic protons (see structure).



Ethyl (E-2-n-Propyl-2,4-pentadienoate) (7)

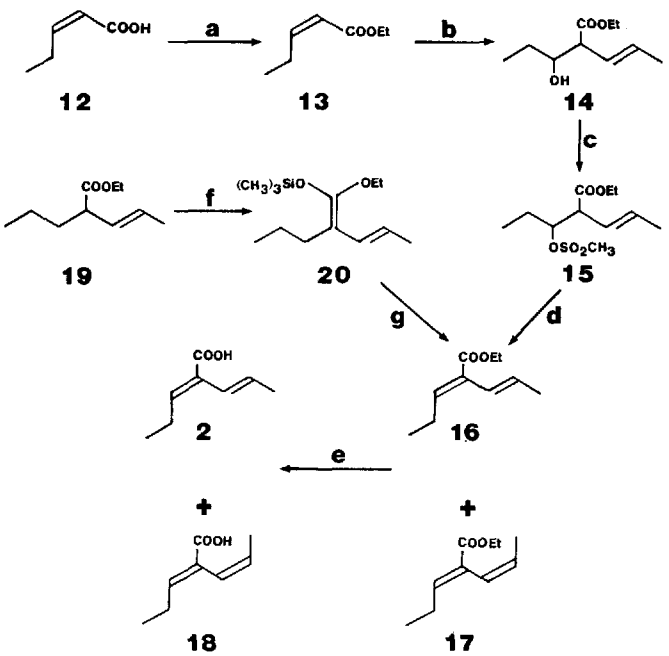


Ethyl (Z)-2-n-propyl-2,4-pentadienoate (8)

For example, the proton at C_3 in the major (*E*)-isomer 7 is highly deshielded and appears at 7.17 ppm. In the (*Z*)-isomer 8, a marked upfield shift of the C_3 proton to 6.34 ppm occurs that is consistent with reduced deshielding by the carbonyl. Similar effects are observed for the C_4 proton H_X. In the (*E*)-isomer, H_X occurs at 6.68 ppm and is shifted downfield to 7.14–7.25 ppm in the (*Z*)-isomer as a consequence of increased deshielding.

The free acid 1, obtained by alkaline hydrolysis of 7, appeared to be quite reactive because the isolated clear product soon became yellowish. The GC-MS analysis of the *t*-BDMS ester derivative of the yellowish product revealed the presence of several unidentified peaks appearing at longer retention times. These peaks, which were initially absent, appear to be polymers of 1. A structurally similar compound, 2,4-pentadienoic acid, is known to polymerize in aqueous solution.[18]

The synthesis of 2 by the elimination of the mesyl ester outlined in Scheme II was briefly described by Acheampong and Abbott[11] and was based on the method of Kende and Toder[17] for the synthesis of 2,3′-diene esters. The elimination reaction on 15 primarily gave the (*E*,*E*)-isomer 16 with minor amounts of the (*E*,*Z*)-isomer 17. In contrast to the results obtained for the synthesis of 7, elimination of the mesylate group of 15 gave exclusively the (*E*)-configuration at position 2. In the NMR spectra, the chemical shift values for the proton at C_3 in 16 and 17 are 6.55 and 6.8 ppm, respectively. These downfield values again correspond to the deshielding effects of the carbonyl group. The presence of the (*E*,*Z*)-isomer 17 was



Scheme II—Synthesis of 2-(1′-propenyl)-2-pentenoic acid by mesylate elimination and hydride abstraction. (a) EtI, K_2CO_3, 18-crown-6, THF, Δ; (b) $[(CH_3)_2CH]_2NH$, *n*-BuLi, HMPA, CH_3CH_2CHO, THF, −78 °C; (c) $(C_2H_5)_3N$, CH_3SO_2Cl, CH_2Cl_2, 0 °C; (d) KH, THF, room temperature; (e) 2.2 M NaOH, 10% HCl; (f) $[(CH_3)_2CH]_2NH$, *n*-BuLi, $(CH_3)_3SiCl$, THF, −78 °C; (g) collidine, triphenylcarbenium fluoroborate, CH_2Cl_2, room temperature.

thought to result from isomerization of the C_3,-double bond, perhaps during the mesylation or elimination steps, because the NMR spectrum of **14** was exclusively that of the (*E*)-isomer. In contrast to previous reports,[11] (*E*)-2-(1′-propenyl)-(*Z*)-2-pentenoic acid was not present since the C_3 proton that occurs at 5.92 ppm was absent. This was also confirmed by synthesizing this diene isomer and comparing GC-MS and NMR data.

Upon hydrolysis of the esters **16** and **17**, the isolated acids were found to consist of 94% **2** and 6% **18** based on GC-MS analysis of the *t*-BDMS derivatives. The overall yield of **2** was greatly enhanced by improving the synthesis of the starting ester **13**. On storing the product at −20 °C for several months, a white milky precipitate forms. The clear supernatant was 98% of **2** upon GC-MS analysis.

Synthesis of the Dienes by the Oxidation of an Alkyl *O*-Ethyl-*O*-trimethylsilylketene Acetal—This approach to the synthesis of **1** and **2**, as described in Schemes I and II, respectively, was based on the method reported by Jung et al.[19] for converting a ketone to an enone via hydride abstraction with triphenylcarbenium tetrafluoroborate. This oxidation was also applied to synthesize α,β-unsaturated esters. Yields up to 50% were reported.[19] The synthesis of **7** by hydride abstraction of **11** with triphenylcarbenium tetrafluoroborate gave two geometric isomers along with a significant amount of starting material **10**. Similar results were obtained when this method was applied to the synthesis of **2**. Up to 60% of the product mixture contained **19**. The added difficulty of separating products from starting material limits this synthetic approach to the diene metabolites. Synthesis of **7** and **16** by an unequivocal route helped verify the structures of these dienes.

Diunsaturated Metabolites of Valproic Acid in a Patient Serum Sample—Two methods of GC-MS analysis were used to study the primary geometric diene isomers of VPA metabolites in the serum of patients, namely EI and NCI. Mass spectra of the TMS and *t*-BDMS derivatives of the diene metabolites **1** and **2** were identical to the synthesized standards. The GC-MS data including the significant ions for these diene derivatives have previously been reported.[4,5,6,10,11,20] The mass spectra of the pentafluorobenzyl derivatives of **1** and **2**, shown in Figure 1, also matched the synthesized diene standards. Because of the similarity of the metabolite spectra and other diene metabolites,[6] the identification of the dienes was approached by selected ion monitoring using three derivatives on two columns of differing polarity. Under EI conditions, a single selective ion at *m/z* 197 was monitored. This ion, $(M-15)^+$ for TMS and $(M-57)^+$ for *t*-BDMS, represents loss of a methyl group or a tertiary butyl group, respectively. When negative CI conditions for the pentafluorobenzyl derivatives were used, a single selective ion at *m/z* 139 corresponding to $(M-181)^-$ or loss of the pentafluorobenzyl group was monitored.

The chromatographic conditions typical for the analysis of VPA metabolites on a semipolar OV-1701 column[7] failed to resolve the TMS derivatives of **18** and **1**. Similar results were found when the TMS derivatives were run on a DB-1 column (Figure 2). Although not shown, the *t*-BDMS derivatives of **18** and **1** also failed to resolve on either column. The PFB derivatives gave good resolution of all four diene isomers on a DB-1 column (Figure 3) and on the more polar OV-1701 column. These results appear to confirm that the minor diene isomers, **18** and **9**, are metabolites of VPA found in human serum.

In summary, the mesylate elimination reaction is a stere-

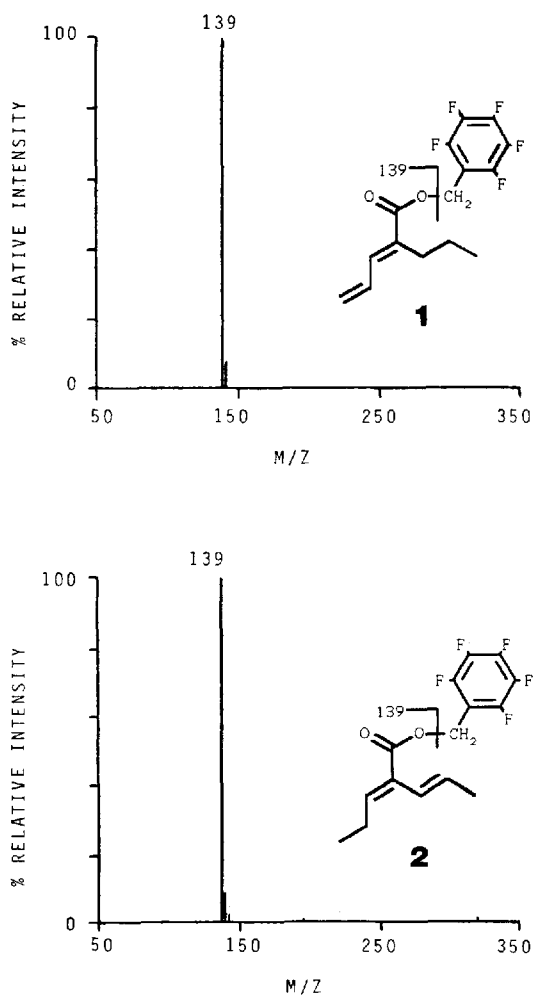


Figure 1—Negative chemical ionization mass spectra of the pentafluorobenzyl esters of **1** and **2**.

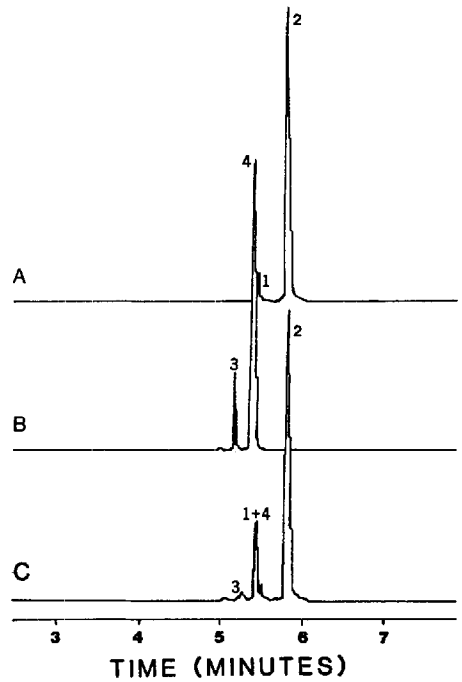


Figure 2—Mass chromatograms at *m/z* 197 of TMS derivatives of (*A*) peak 1 (**18**) and peak 2 (**2**) in control human serum; (*B*) peak 3 (**9**) and peak 4 (**1**) in control human serum; (*C*) serum sample of a patient on VPA therapy containing peaks 1 to 4, on a DB-1 column.

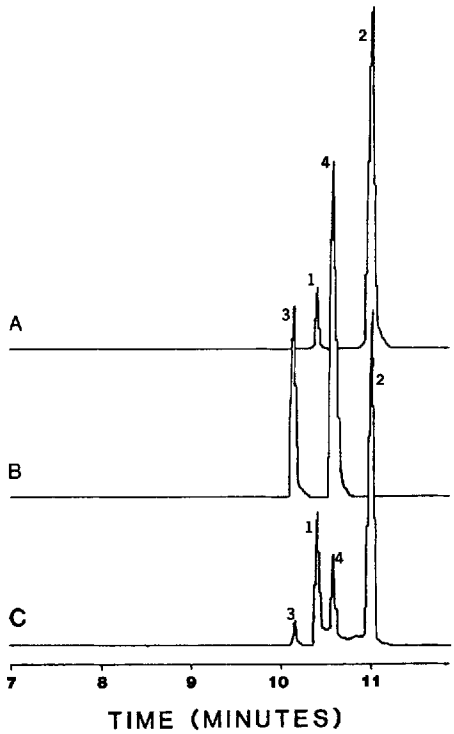


Figure 3—Mass chromatograms at *m/z* 139 of PFB derivatives of (*A*) peak 1 (**18**) and peak 2 (**2**) in control human serum; (*B*) peak 3 (**9**) and peak 4 (**1**) in control human serum; (*C*) serum sample of a patient on VPA therapy containing peaks 1 to 4, on a DB-1 column.

oselective method for obtaining significant quantities of two important diene metabolites of VPA. The NMR analysis of the isomers confirmed the stereoselectivity of the reaction. Four isomers of the two dienes were found as metabolites in humans.

References and Notes

1. Dulac, O.; Steru, D.; Rey, E.; Perret, A.; Arthuis, M. *Brain Dev.* **1986**, *8*, 47–52.
2. Olmos-Garcia de Alba, G.; Valdez, J. M. *Clin. Electroencephalogr.* **1986**, *17*, 14–20.
3. Granneman, G. R.; Wang, S. I.; Machinist, J. M.; Kesterson, J. W. *Xenobiotica* **1984**, *14*, 375–387.
4. Rettenmeier, A. W.; Gordon, W. P.; Prickett, K. S.; Levy, R. H.; Lockard, J. S.; Thummel, K. E.; Baillie, T. A. *Drug Metab. Dispos.* **1986**, *14*, 443–453.
5. Abbott, F. S.; Kassam, J.; Orr, J. M.; Farrell, K. *Clin. Pharmacol. Ther.* **1986**, *40*, 94–100.
6. Kochen, W.; Sprunck, H. P.; Tauscher, B.; Klemens, M. *J. Clin. Chem. Clin. Biochem.* **1984**, *22*, 309–317.
7. Abbott, F. S.; Kassam, J.; Acheampong, A.; Ferguson, S.; Panesar, S.; Burton, R.; Farrell, K.; Orr, J. *J. Chromatogr.* **1986**, *375*, 285–298.
8. Kesterson, J. W.; Granneman, G. R.; Machinist, J. M. *Hepatology* **1984**, *4*, 1143–1152.
9. Abbott, F. S.; Acheampong, A. A. *Neuropharmacology* **1988**, *27*, 287–294.
10. Rettenmeier, A. W.; Prickett, K. S.; Gordon, W. P.; Bjorge, S. M.; Chang, S-L.; Levy, R. H.; Baillie, T. A. *Drug Metab. Dispos.* **1985**, *13*, 81–96.
11. Acheampong, A.; Abbott, F. S. *J. Lipid Res.* **1985**, *26*, 1002–1008.
12. *CRC Handbook of Chemistry and Physics*, 52nd Ed.; Weast, R. C., Ed.; Chemical Rubber: Cleveland, OH, 1971–1972; p C-411.
13. Rappe, C.; Adestrom, R. *Acta Chem. Scand.* **1965**, *19*, 383–389.
14. Rappe, C. *Org. Syntheses* **1979**, *53*, 123–127.
15. Acheampong, A. A., M.Sc. Thesis, University of British Columbia, Vancouver, B.C., 1982.
16. Kassahun, K.; Burton, R.; Abbott, F. S., submitted for publication in *Biomed. Mass Spectrom.*
17. Kende, A. S.; Toder, B. H. *J. Org. Chem.* **1982**, *47*, 163–167.
18. Bando, Y.; Minoura, Y. *J. Polym. Sci.* **1976**, *14*, 1195–1203.
19. Jung, M. E.; Pan, Y-G. *J. Org. Chem.* **1977**, *42*, 3961–3963.
20. Abbott, F. S.; Lee, R. D.; Acheampong, A. A. *Abstracts*, 134th Annual Meeting and Exhibit, Academy of Pharmaceutical Research and Science, Chicago, IL, March 1987; American Pharmaceutical Association: Washington, DC, 1987; Abstract P-33.

Acknowledgments

The authors would like to thank Dr. J. G. Slatter for his helpful suggestions. This work was supported by a grant from the Medical Research Council of Canada. Taken in part from the M.Sc. thesis of Ronald D. Lee.