Synthesis and stereochemical determination of diunsaturated valproic acid analogs including its major diunsaturated metabolite

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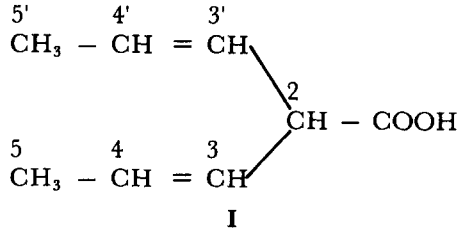
Abstract Valproic acid, an antiepileptic drug, is transformed into diunsaturated metabolites in humans. Synthesis of the geometric isomers of 2-(1'-propenyl)-2-pentenoic acid and 2-(1'-propenyl)-3-pentenoic acid was attempted using known procedures. The final product, a mixture of isomers, was converted into tert-butyldimethylsilyl or ethyl derivatives. Capillary gas-liquid chromatography-mass spectrometry analysis of the derivatives showed at least three isomeric dienoic acids from synthesized products. Argentation thin-layer chromatography was effective in resolving the isomeric mixture into a single isomer or mixture of two isomers. Thin-layer chromatography and gas-liquid chromatography retention data, photochemical isomerization studies, and nuclear magnetic resonance spectrometry were used to characterize the dienoic acids. By comparison of the retention times of the diunsaturated metabolites with synthesized reference compounds, the structure assigned to the major diunsaturated metabolite is 2-[(E)-1'-propenyl](E)-2-pentenoic acid. — **Acheampong, A., and F. S. Abbott.** Synthesis and stereochemical determination of diunsaturated valproic acid analogs including its major diunsaturated metabolite. *J. Lipid Res.* 1985. **26:** 1002-1008.

Supplementary key words valproic acid • diunsaturated acids • diunsaturated metabolites • isomers • capillary gas-liquid chromatography-mass spectrometry • nuclear magnetic resonance

Valproic acid (2-propylpentanoic acid, VPA), a major antiepileptic drug, is extensively metabolized in man. The metabolism of VPA is quite complex and studies on the metabolism of this drug have been the subject of several reviews (1, 2). Limited information is currently available on the structural identity of the diunsaturated metabolites of VPA (3-5), yet substantial increases in serum and urine levels of four diunsaturated metabolites were observed in a recent study (3) involving a patient with hepatic failure. Information on the pharmacological properties of these metabolites has been delayed due to their unavailability.

The dienoic metabolites with a terminal double bond, 2-(2-propenyl)-4-pentenoic acid and 2-propyl-(E)-2,4-pentadienoic acid, have been identified in man (3, 5). In a previous study (4), in which stable isotope techniques were used, we showed that the most prominent diunsaturated VPA metabolite does not possess a terminal double bond. Two possible structures, I or II, were proposed. The identity of the dienoic metabolites, not having a terminal double bond, has so far been deduced from chromatographic retention data (3, 5) and mass spectra data (3-5) without having precise data on the stereochemical configuration of the double bonds.

In this study, we report the synthesis of diunsaturated VPA isomers, I and II, using selective reagents and reaction procedures. The dienoic isomers have been subjected to argentation TLC and characterized by capillary GLC-MS and NMR. Synthesized dienoic acids were used to identify the major dienoic metabolite found in man.



I

3,3'-diene VPA

(3Z-3'E; 3Z-3'Z; 3E-3'E)

Abbreviations: VPA, 2-propylpentanoic acid; GLC-MS, gas-liquid chromatography-mass spectrometry; TLC, thin-layer chromatography; UV, ultraviolet; IR, infrared; NMR, nuclear magnetic resonance; t-BDMS, tert-butyldimethylsilyl; TMS, trimethylsilyl; (E), *trans*; (Z), *cis*; diene, diunsaturated; *p*-TsCl, *p*-toluenesulfonyl chloride; MsCl, methanesulfonyl chloride.

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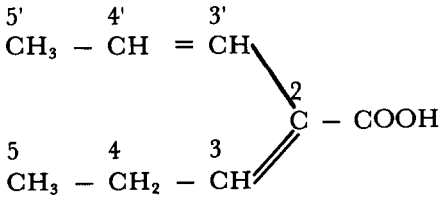

II

2,3'-diene VPA

(2Z-3'E; 2Z-3'Z; 2E-3'Z; 2E-3'E)

EXPERIMENTAL PROCEDURE

Materials

t-Butyldimethylsilyl chloride was from Applied Science Laboratories, Inc., State College, PA; n-butyllithium (1.6 M in hexane), diisopropylamine, and (E)-2-pentenoic acid were from Aldrich Chemical Co., Milwaukee, WI; trimethylanilinum hydroxide (0.2 M in methanol) was from Pierce Chemical Co., Rockford, IL; silver nitrate was from Nichol Chemical Co., Canada; and silica gel G was from E. Merck, Darmstadt, Germany. 2-Propyl-(E)-2,4-pentadienoic acid was a gift from Dr. T. A. Baillie (University of Washington, School of Pharmacy, Seattle, WA). Solvents and other reagents used were analytical grade.

Instrumentation

IR spectra were obtained using neat films on a Unicam SP 1000 spectrometer. ^{1}H-NMR spectra were taken, in $CDCl_3$ with tetramethylsilane as internal standard, on a Nicolet-Oxford-270 (270 MHz), Bruker WP-80 (80 MHz), or Bruker WH-400 (400 MHz) in the Department of Chemistry, University of British Columbia. Capillary GLC–MS analysis was performed on an HP 5987A instrument. A 12.5 × 0.2 mm i.d. fused silica column coated with cross-linked dimethylsilicone (Hewlett-Packard) was used. A Varian MAT-111 mass spectrometer coupled to an HP 5700A gas chromatograph was used to analyze mixtures of one or two isomers. A 1.8 m × 2 mm i.d. glass column was packed with 3% Dexsil 300 on 100–120 mesh Supelcoport (Supelco). Mass spectrometers were operated at ionization voltage of 70 eV. Capillary GLC–MS analysis of t-BDMS-derivatized acids was performed at oven temperatures from 50°C to 100°C at 30°C/min, then 100°C to 260°C at 8°C/min.

Synthesis of ethyl 2-(1'-hydroxypropyl)-3-pentenoate

The ester enolate of ethyl (E)-2-pentenoate (0.10 mol) was prepared at −78°C using lithium diisopropylamide (0.11 mol) in 120 ml of tetrahydrofuran and 19.7 g of hexamethylphosphoramide. Propionaldehyde (0.1 mol) was added dropwise to the solution and the reaction mixture was finally quenched with dilute HCl. The major product, ethyl 2-(1'-hydroxypropyl)-3-pentenoate, was isolated, bp 85°–90°C/0.25 mm (yield 48%). NMR (80 MHz): 0.95 (t, 3H), 1.25 (t, 3H), 1.55 (m, 2H), 1.75 (d, 3H), 2.55 (s, 1H), 3.40 (m, 1H), 3.80 (m, 1H), 4.2 (q, 2H), 5.3–5.9 (m, 2H). IR: 3400 cm^{-1} (O–H), 1735 cm^{-1} (C=O), 1635 cm^{-1} (C=C), 1175 cm^{-1} (C–O), 985 cm^{-1} (medium intensity, = CH, *trans*), 745 cm^{-1}.

(Z)-2-pentenoic acid was prepared according to Rappe and Adestrom (6), and the ethyl ester was synthesized using ethyl iodide, anhydrous potassium carbonate, and tetrahydrofuran as refluxing solvent. The ester enolate of ethyl (Z)-2-pentenoate (0.05 mol) was prepared at −78°C using lithium diisopropylamide (0.055 mol) in 60 ml of tetrahydrofuran and 9.8 g of hexamethylphosphoramide. Propionaldehyde (0.05 mol) was added dropwise to the solution and the reaction mixture was finally quenched with 10% HCl. The major product, ethyl 2-(1'-hydroxypropyl)-3-pentenoate, was isolated, bp 95°–100°C/1 mm (yield 50%). IR: 3400 cm^{-1} (O–H), 1735 cm^{-1} (C=O), 1640 cm^{-1} (C=C), 985 cm^{-1} (strong intensity, = CH, *trans*), 745 cm^{-1}.

Dehydration reactions of ethyl 2-(1'-hydroxypropyl)-3-pentenoate

Phosphorus pentoxide (36 mmol) was added to the unsaturated β-hydroxy ester (39 mmol, from ethyl (E)-2-pentenoate) in 60 ml of benzene and the mixture was refluxed for 4 hr. The diunsaturated esters isolated were saponified with dilute NaOH and subsequently acidified with dilute H_2SO_4 to give a mixture of seven isomeric dienoic acids, bp 105°–110°C/2.5 mm.

Toluenesulfonyl chloride–pyridine. Dehydration of the unsaturated β-hydroxy ester (33 mmol, from ethyl (E)-2-pentenoate) in 20 ml of pyridine with *p*-toluenesulfonyl chloride (45 mmol) was accomplished using the dehydration method of Eisner, Elvidge, and Linstead (7). The *p*-toluenesulfonate was refluxed with dilute NaOH and acidified to give an isolated product of four isomeric dienoic acids, bp 120°–128°/6 mm. NMR (270 MHz, $CDCl_3$) of mixture, 0.95–1.1 (t), 1.55 (d), 1.71 (dd), 1.85 (d), 2.16 (m), 2.35 (m), 2.55 (m), 4.05 (t), 5.65 (m), 5.73 (m), 5.86 (m), 5.95 (t), 6.03 (d), 6.14 (d), 6.83 (t), 7.00 (t) IR of mixture, 1690 cm^{-1} (C=O), 1633 cm^{-1} (C=C), 965 cm^{-1} (=CH, *trans*), 935 cm^{-1}, 735 cm^{-1}. NMR assay of the four dienoic acids mixture, using appropriate integrated areas, gave the ratio of isomers 3Z-3'Z, 2Z-3'E, 2E-3'Z, 2E-3'E as 12:14:56:18.

Methanesulfonyl chloride, KH. Dehydration of the unsaturated β-hydroxy ethyl ester (0.05 mol), derived from ethyl (E)-2-pentenoate, was carried out using methanesulfonyl chloride (0.06 mol), triethylamine (0.08 mol), and

KH (0.10 mol) according to the method of Kende and Toder (8). The product mixture, bp 75°-80°C/2 mm, contained three isomeric dienoates. IR: 1716 cm^{-1} (C=O), 1636 cm^{-1} (C=C), 968 cm^{-1} (=CH, *trans*), 756 cm^{-1}, 690 cm^{-1} (= CH, *cis*, more intense than = CH, *trans*).

Dehydration of the unsaturated β-hydroxy ethyl ester (0.02 mol), derived from ethyl (Z)-2-pentenoate, was carried out by treatment of the mesylate with KH (0.04 mol). The product mixture, bp 65°-70°C/0.1 mm, contained three isomeric dienoates. IR: 1733 cm^{-1} (C=O), 1716 cm^{-1} (C=O), 1655-1640 cm^{-1} (C=C), 985-965 cm^{-1} (= CH, trans), 745 cm^{-1}.

Argentation-TLC

Silica gel G was impregnated with $AgNO_3$ (20% w/w) and TLC plates were prepared (0.5 mm thick). A mixture of isomeric dienoates (20-40 mg) in $CHCl_3$ was applied across the plate (20 × 20 cm). Benzene-petroleum ether (bp 30°-60°C)-diethyl ether 80:20:5 was used as the mobile phase. R_f values of three bands were determined by charring bands with 50% H_2SO_4 and heating the plates at 200°C for 10 min. Uncharred bands were scraped off plates into centrifuge tubes and the ethyl esters were extracted with MeOH. Following filtration of methanolic solutions, the three fractions were reconstituted in $CDCl_3$ for GLC-MS and NMR analysis.

Derivatization

Acids were methylated by treatment with diazomethane generated according to the method of Levitt (9). t-BDMS derivatives of acids were formed as previously described (4). Ethyl esters of acids were formed using appropriate volumes of trimethylanilinum hydroxide solution and ethyl iodide as recommended (10).

In vivo metabolism — isolation procedure

Urine and serum samples obtained from a patient on VPA therapy were subjected to hydrolysis and extraction procedures as previously described (4).

Photochemical isomerization

A mixture of seven isomeric dienoic acids (1 g), derived from dehydration of β-hydroxy β'γ'-unsaturated ester with P_2O_5, was dissolved in 150 ml of hexane and added to a Pyrex well. 2-Propyl-(E)-2-pentenoic acid (2-ene VPA) was added as an internal standard. A quartz immersion well with a 450 W unfiltered Hanovia lamp was set into place and irradiation was carried out for 6 hr. Aliquots of the reaction mixture were removed periodically and the t-BDMS esters of photolyzed acids were analyzed by capillary GLC-MS.

Capillary GLC-MS resolution of 2,3'-diene VPA and 2,4,-diene VPA

Samples of synthetic diene acids, 2-propyl-(E)-2,4-pentadienoic acid and 2-[(Z)-1'-propenyl]-(E)-2-pentenoic acid, or the urine extract from a patient were made with ethyl acetate and TMS-derivatives formed as previously described (4). Urine extracts were also spiked with synthetic diene acids prior to TMS derivatization. Capillary GLC-MS analysis of the TMS derivatives was performed using a 25 m × 0.3 mm i.d. SE 54 column at oven temperatures from 50° to 90°C at 30°C/min and then held at 90°C for 10 min.

RESULTS AND DISCUSSION

Stable isotope methodology and GLC-MS analysis of serum and urine samples from patients on VPA therapy have revealed that one of the major diene VPA metabolites has the structure I or II (4). In order to identify

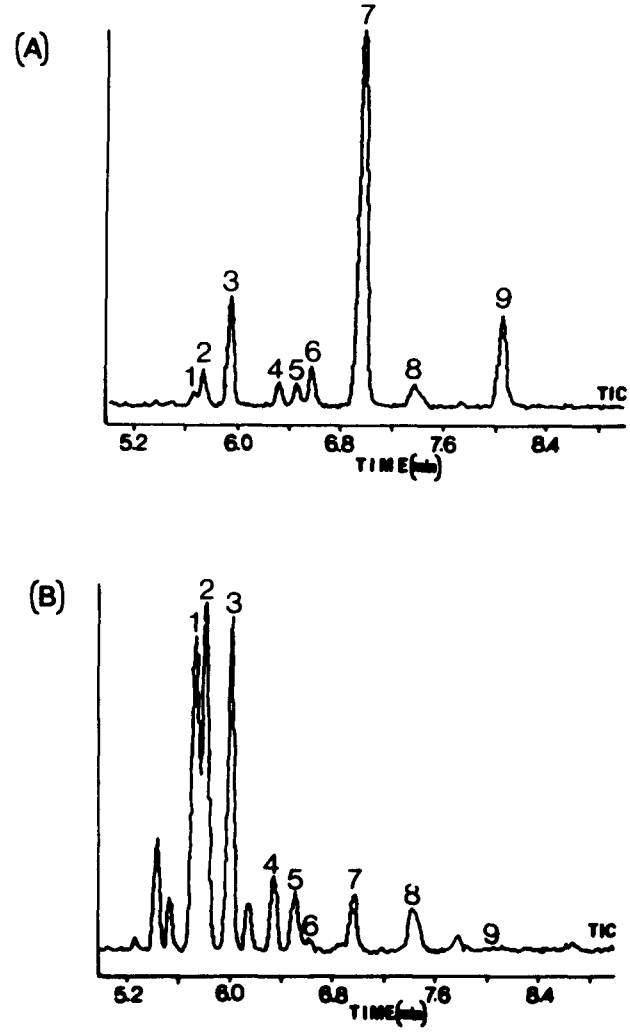

Fig. 1. Capillary GLC-MS separation of t-BDMS esters of isomeric diene-VPA. (A), before UV irradiation; (B), after 6 hr UV irradiation. Peak numbers correspond to: 1, (Z,E)-3,3'-diene; 2, (Z,E)-3,3'-diene; 3, (Z,Z)-3,3'-diene; 4, (E,E)-3,3'-diene; 5, (Z,E)-2,3'-diene; 6, (E)-2-ene VPA; 7, (E,Z)-2,3'-diene; 8, (Z,Z)-2,3'-diene; 9, (E,E)-2,3'-diene.

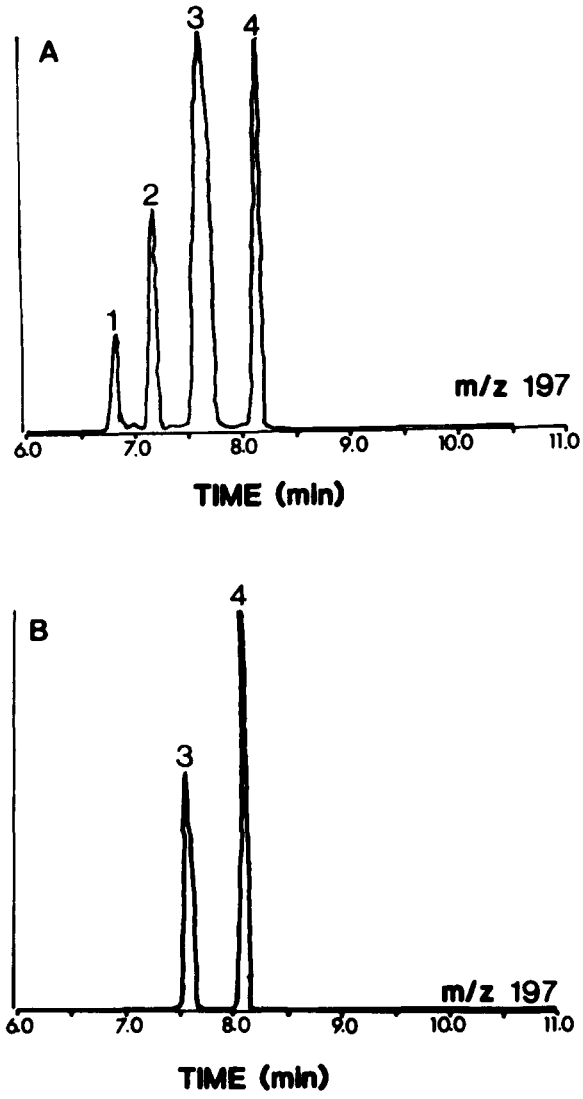

Fig. 2. Mass chromatograms of t-BDMS esters of A) the four-diene-VPA isomeric mixture prepared using p-TsCl; B) diene-VPA metabolites in urine extract. Peak 1, (Z,Z)-3,3'-diene; 2, (Z,E)-2,3'-diene; 3, (E,Z)-2,3'-diene; 4, (E,E)-2,3'-diene.

these metabolites, stereoselective synthesis of the two diene acids, I and II, was attempted by preparing stereospecifically β-hydroxy-β',γ'-unsaturated esters which were dehydrated by various agents.

Synthesis

In this study, NMR and IR analysis have shown that under kinetically controlled reaction conditions, propionaldehyde adds regiospecifically at the α-position of α,β-unsaturated ester enolates to give ethyl 2-(1'-hydroxypropyl)3-pentenoate as the major product. Addition of aldehydes to enolates of α,β-unsaturated esters is reported to occur at either the α- or γ-carbon of the esters (8, 11, 12). NMR and IR analysis appear to indicate that ethyl 2-(1'-hydroxypropyl)-(Z)-3-pentenoate predominates over the (E)-isomer when ethyl (E)-2-pentenoate is the starting material while ethyl 2-(1'-hydroxypropyl)-(E)-3-pentenoate is the major isomer using ethyl (Z)-2-pentenoate. These results agree with the reported stereochemical course of this reaction (8, 13). Phosphorus pentoxide dehydration of ethyl 2-(1'-hydroxypropyl)-3-pentenoate, derived from ethyl (E)-2-pentenoate, gave the seven possible isomeric acids of structures I and II. **Fig. 1** shows the total ion chromatogram of the t-BDMS esters of this mixture.

Photochemical isomerization

Photochemical isomerization of the unsaturated acids provided an indirect method of determining the positional

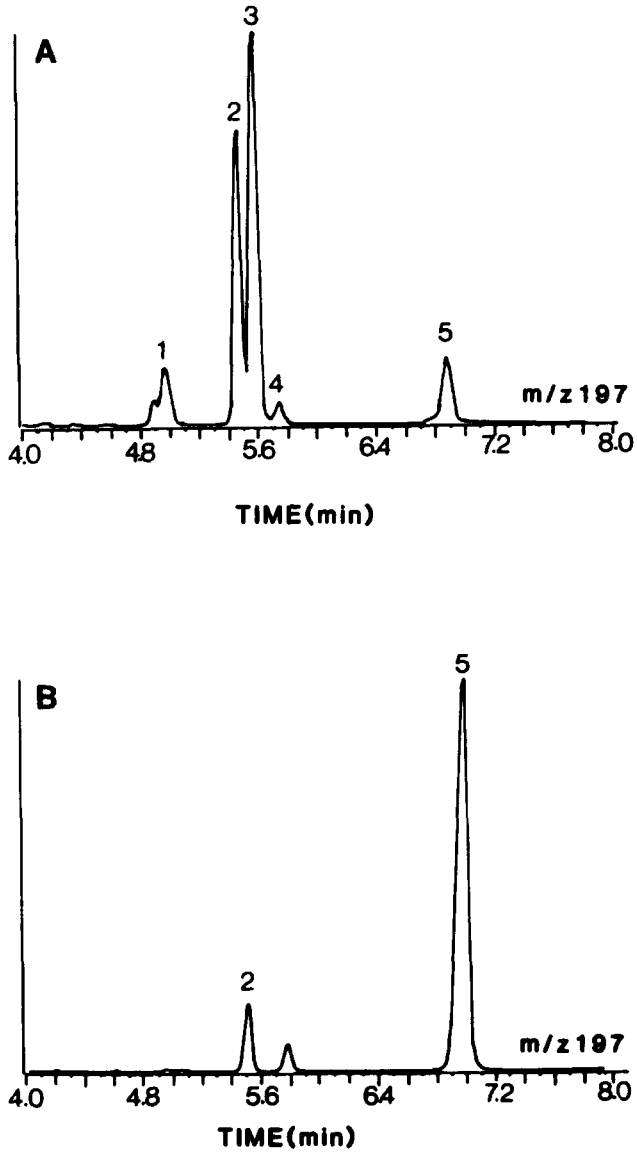

Fig. 3. Capillary GLC–MS separation of TMS derivatives of A) synthesized 2,3'-diene VPA and 2,4-diene VPA; B) diene-VPA metabolites in urine extract. Peak 1, (Z)-2,4-diene VPA; 2, (E)-2,4-diene VPA; 3, (E,Z)-2,3'-diene VPA; 4, (Z,E)-2,3'-diene VPA; 5, (E,E)-2,3'-diene VPA.

TABLE 1. Composition and chromatographic data of a mixture of synthesized isomeric dienoates

Diene VPA Ethyl Ester	Product-Ratio with Reactant, Ethyl 2-Pentenoate[a]		TLC	t_R[c] (min)	Mass Spectrum (70 ev) m/z (Relative Intensity)
	E	Z	R_f[b]		
3Z-3'Z		0.44	0.42–0.49 (Band Ia)	9.37	95 (100%), 168 (7%), 139 (2%), 122 (1%)
2Z-3'E	0.07		0.53–0.62 (Band IIIb)	10.0	95 (100%), 168 (50%), 140 (41%), 122 (24%)
2E-3'Z	0.71	0.08	0.45–0.53 (Band IIb)	10.5	95 (100%), 168 (57%), 140 (41%), 122 (28%), 153 (4%)
2E-3'E	0.22	0.48	0.53–0.62 (Band IIIa,b)	11.1	95 (100%), 168 (54%), 140 (35%), 122 (32%), 153 (4%)

[a]Ratio of isomers in synthetic product mixture before TLC using either ethyl (Z) or (E)-2-pentenoate as reactant.
[b]Band in which isomer is concentrated.
[c]GLC-MS retention time of isomeric peaks analyzed with 3% Dexsil 300 column (1.8 m × 2 mm i.d.), helium, 25 ml/min. Column temperature of 50°C to 280°C at rate of 8°C/min.

isomers. *Cis* and *trans* α,β-unsaturated acids have been reported to isomerize photochemically to *cis* and/or *trans* β,γ-unsaturated acids (14, 15). UV irradiation of the seven-isomeric-acid mixture resulted in a striking build-up of four peaks (peaks 1, 2, 3, 4) which were assigned to β,γ-β',γ'-diunsaturated acids. Peaks 1 and 2 can be described as geometric enantiomers since the double bonds are identically substituted and thus only partially resolved on the capillary column. The peaks whose height decreased with UV irradiation (peaks 7, 9) were described as *trans* α,β-diunsaturated acids. The peaks whose height did not show any significant change (peaks 5, 8) were described as *cis* α,β-diunsaturated esters, since under the conditions of photoisomerization they can be formed from the corresponding *trans* α,β-isomers and in turn isomerize to the β,γ-isomers (14, 15).

GLC elution order

Additional support for a tentative assignment of configuration of the dienoic acid peaks in Fig. 1 was obtained from the GLC retention data. Shorter retention times for β,γ-unsaturated esters compared with α,β-unsaturated esters, as well as shorter retention times for the *cis*-isomer of a given position compared to the *trans*-isomer on non-polar columns, have been frequently observed (16, 17). The two peaks before peak 1 in Fig. 1B, which were confirmed as 3-ene VPA and *cis* 2-ene VPA, were produced by photochemical isomerization of *trans* 2-ene VPA (peak 6) added as an internal standard. Based on retention times, the two major diunsaturated metabolites in the urine and serum of patients would have a *trans* configuration at the α,β double bond since they correspond to peaks 7 and 9 (Fig. 1).

Fig. 2A shows the mass chromatograms of the M^+-57 ion of the t-BDMS esters of the four-diene-VPA isomeric mixture obtained from dehydration of the unsaturated β-hydroxy ester with *p*-toluenesulfonyl chloride. The peaks 3 and 4 were due to *trans* α,β-β',γ'-diunsaturated acids (see synthesis for NMR analysis) and had identical retention times to those of the diunsaturated VPA metabolites (Fig. 2B), when chromatograms of the t-BDMS derivatives were obtained either on a 12.5-m dimethylsilicone column or a 25-m SE 54 column.

However, Kochen et al. (3) had reported that one of the diene VPA metabolites had the same retention time as 2-propyl-(E)-2,4-pentadienoic acid when TMS-derivatives were formed. Granneman et al. (5) have recently postulated that there could be two diene metabolites of VPA with the 2,3'-diene structure and they identified another diene metabolite as 2-propyl-(E)-2,4-pentadienoic acid. With synthesized 2-propyl-(E)-2,4-pentadienoic acid available as a gift, we decided to investigate further the relative retention times of the 2,4-diene VPA, three isomers of 2,3'-diene VPA (see Fig. 2A), and the two major diene VPA metabolites. Separation of the dienes was accomplished using a 25-m SE 54 column (**Fig. 3**) by forming TMS derivatives instead of t-BDMS derivatives. One of the two major diene VPA metabolites and 2-propyl-(E)-2,4-pentadienoic acid had identical retention times that were different from that of 2-[(Z)-1'-propenyl]-(E)-2-pentenoic acid (peak 3 in Fig. 3A). The diene VPA metabolite, which dominates in urine (peak 5 in Fig. 3B), had the exact retention time as 2-[(E)-1'-propenyl]-(E)-2-pentenoic acid. The small peak in Fig. 3B had a relative retention time similar to that of 2-[(E)-1'-propenyl]-(Z)-2-pentenoic acid (peak 4 in Fig.

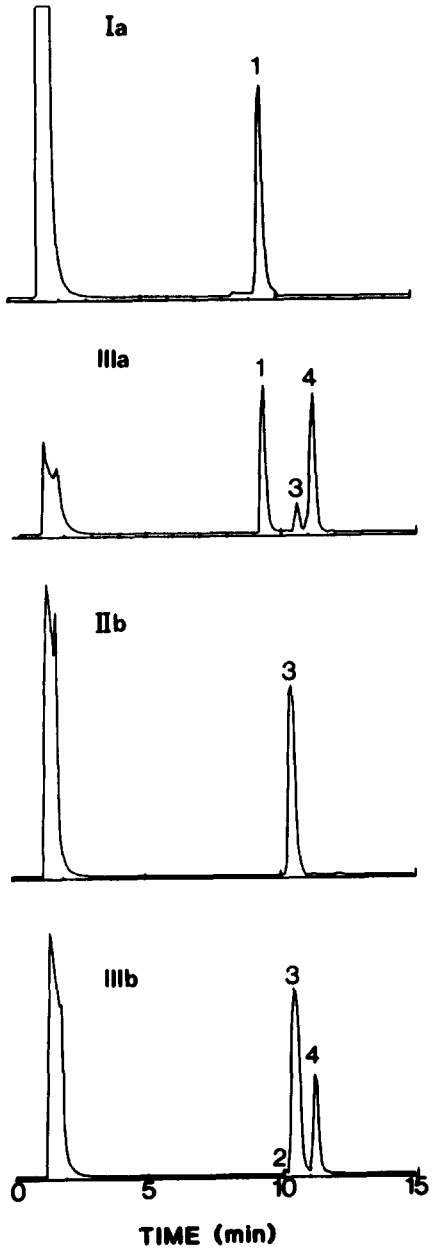

Fig. 4. GLC–MS analysis of dienoates eluted from TLC plates. Total ion chromatograms I, II, III correspond to ethyl esters in bands I, II, III, respectively; a and b refer to esters synthesized from (Z)- and (E)-2-pentenoate, respectively. Peak 1, (Z,Z)-3,3'-diene; 2, (Z,E)-2,3'-diene; 3, (E,Z)-2,3'-diene; 4, (E,E)-2,3'-diene.

3A), a by-product in the synthesis of 2-[(E)-1'-propenyl]-(Z)-2-pentenoic acid (see also Fig. 2A). This diene has been suggested to be another VPA metabolite (5), however this has not been confirmed by use of the reference compound.

Argentation-TLC

The combination of methanesulfonyl chloride and KH in the dehydration of the β-hydroxy β',γ'-unsaturated ester gave the minimum number of isomeric diene acids. This result agrees with the stereoselective nature of these reagents as reported by Kende and Toder (8). **Table 1** shows the identity, proportion, and R_f values of the isomers in the three bands following argentation TLC. The proportion of isomers in the mixture of dienoates, before and after argentation TLC, was determined by NMR. **Fig. 4** shows the profile of components in the three bands following argentation TLC and GLC–MS analysis. The order of elution of isomeric dienoates and the mobility of the isomers on the TLC plate (Table 1) agree with the chromatographic properties of the stereoisomers. *Trans-trans* diunsaturated esters are reported to be less polar (higher R_f) than their corresponding *cis-cis, trans-cis,* or *cis-trans* isomers (17, 18). Moreover, conjugated dienoates have been reported to have higher R_f values than their nonconjugated congeners using similar eluents (17, 18).

Mass spectra of the four dienoic acid ethyl esters show the intense $M^{\cdot+}$, m/z 168, of 2,3'-dienes compared to the 3,3'-dienes (Table 1). Diene ethyl esters from the TLC bands could be isolated either as a single isomer or as a mixture of isomers (Fig. 4). Products were characterized by NMR spectroscopy and **Table 2** summarizes the NMR data for the dienoate isomers. The chemical shift values assigned the olefinic protons, β-vinyl proton, methylene and methyl protons adjacent to the double bonds are characteristic of the stereochemistry of dienoate isomers (19, 20).

CONCLUSION

The two diunsaturated VPA metabolites most frequently found in patient samples have been assigned the chemical structures 2-propyl-(E)-2, 4-pentadienoic acid (minor metabolite) and 2-[(E)-1'-propenyl]-(E)-2-pentenoic acid (major metabolite). The stereochemical course of the synthetic reaction, using ethyl (E)-2-pentenoate, produces a mixture of three isomers with (E,Z)-2,3′ diene VPA as the major component, while the reactant ethyl (Z)-2-pentenoate affords (E,E)-2,3'-diene VPA as the major product. A combination of various techniques, direct and indirect, have proved useful in identifying the metabolites. The significance of this study is that evaluation of the anticonvulsant and toxic properties as well as pharmacokinetic behavior of the diene VPA isomers can be undertaken with due regard to their importance as metabolites of

TABLE 2. NMR (400 MHz) data for diene VPA ethyl esters

Dienoate	(Multiplicity) CH_3	CH_3-C=	CH_2	CH	H(3')[a]	H(4')[a]	H(3)[a]
2E-3'E	1.04(t)	1.84(d)	2.31(m)		6.17(d) J = 16Hz	6.08(dq) J = 16Hz	6.59(t)
2E-3'Z	1.04(t)	1.54(dd)	2.11(m)		6.01(d) J = 11.4Hz	5.79(dq) J = 11.4Hz	6.79(t)
2Z-3'E	1.04(t)	1.70(d)	2.44(m)		[b]	[b]	5.92(t)
3Z-3'Z		1.66(dd) 1.69(d)		3.5(t)	5.5-5.6(m)	5.6-5.7(dq)	

[a]Position of hydrogen in the branched-carboxylic acid ester.
[b]Resonance peaks were very weak due to small amounts of the isomer obtained.

VPA. The stereochemical configuration of the double bonds will provide useful information as to the metabolic origin of these diene metabolites.

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