SYNTHESIS OF CARBON-14 AND DEUTERIUM LABELED VALPROIC ACIDS

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SUMMARY

Valproic acid and sodium valproate labeled with carbon-14 in the 2-position were synthesized. Deuterium labeled valproic acid was also synthesized and the extent of deuterium incorporation was determined by several analytical methods.

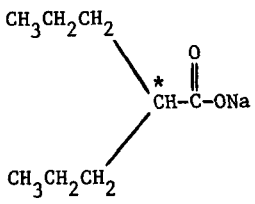
KEY WORDS: Carbon-14 labeling, Deuterium labeling, Infra-Red Spectrometry, ^{1}H-NMR, ^{13}C-NMR, Mass Spectrometry, Gas Liquid Chromatography, High Pressure Liquid Chromatography, Valproic Acid.

The anticonvulsive properties of sodium valproate (2-n-propyl-pentanoic acid) were first demonstrated by Meunier, *et al.*[1] Several other researchers have confirmed their findings, and the antiepileptic properties of this compound in both experimental and clinical epilepsy have been extensively reviewed.[2,3] Initial metabolism studies in rats were conducted with sodium valproate containing a ^{14}C-label in the carboxyl group or 1-position. It was found that some of the drug was decarboxylated *in vivo* and that approximately 15% of the ^{14}C-label was eliminated as $^{14}CO_2$.[4,5] In order to avoid the loss of label through respiration and to facilitate further biotrans-

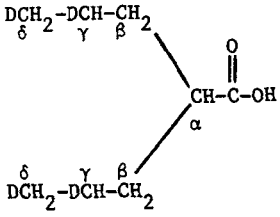
0362-4803/80/0517-0733$01.00

Received April 15, 1979
Revised July 12, 1979

formation studies, sodium valproate labeled with carbon-14 in the 2-position (I) and tetradeutero-valproic acid (II) were synthesized.



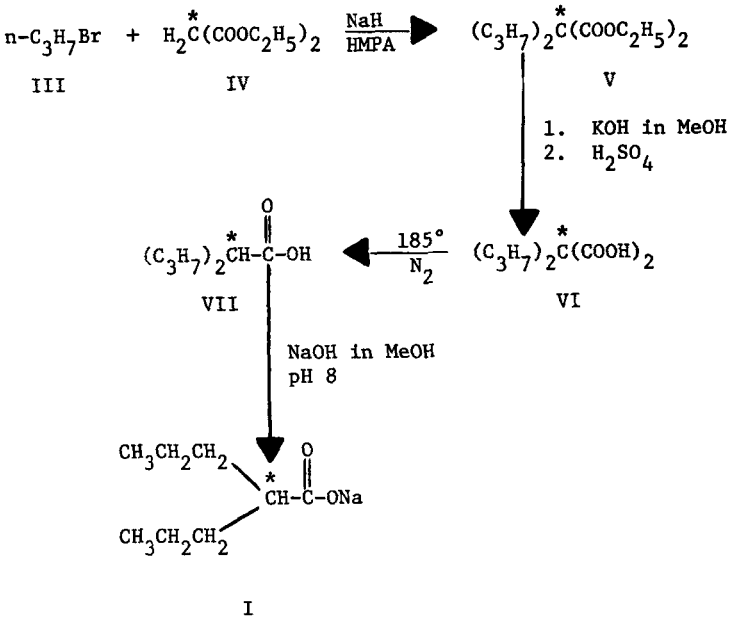
I



II

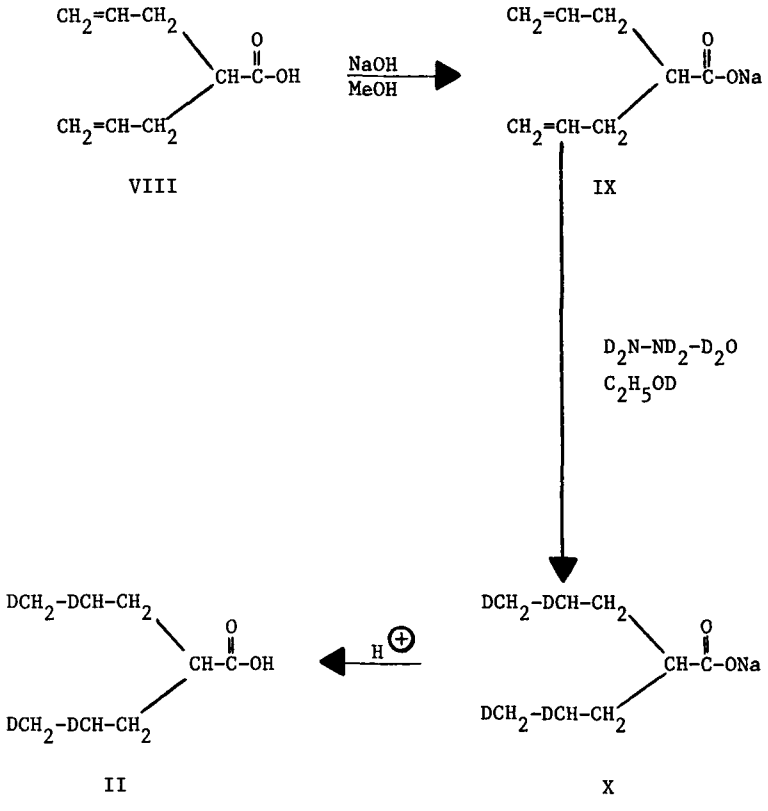
* Position of the carbon-14 label.

The carbon-14 labeled sodium salt of valproic acid, I, was synthesized according to the following scheme:



I

Of the many reaction schemes available for the synthesis of deuterium labeled valproic acid, II, the reaction of diallylacetic acid[6] with deuterium labeled hydrazine hydrate[7,8] was thought to offer the advantage of experimental simplicity in obtaining the desired product in moderate to reasonably high yield. Deuterium labeled valproic acid II, was therefore synthesized according to the following scheme:



VIII IX

II X

EXPERIMENTAL

Synthesis of Carbon-14 Labeled Sodium Salt of Valproic Acid (I):

To 15 ml of hexamethylphosphorictriamide (HMPA) was added 860 mg (10.5 mM) of 58.6% sodium hydride as a dispersion in mineral oil. The mixture was stirred under nitrogen until a dark color appeared. By means of a syringe, 400 mg (2.5 mM) of diethylmalonate-2-^{14}C (purchased from New England Nuclear, Boston, MA, its specific activity was 4.005 mCi/mMole) in 2 ml of HMPA was added with stirring and under nitrogen. When gas evolution was complete, 1200 mg (7.5 mM) of non-radioactive diethylmalonate in 3 ml of HMPA was added. The reaction mixture was stirred to 15-20 minutes under nitrogen, and then via a syringe 2.3 ml of purified n-propyl-bromide (25 mM) was added dropwise. The mixture was allowed to stir at room temperature for 6 hours, and then poured into ice water. The mixture was extracted with hexane and the extract was washed with water and dried over anhydrous magnesium sulfate. Analysis of the hexane solution by GLC, measuring both mass and the radioactive effluent, indicated that the dipropylmalonic ester (V) was greater than 98.5% pure. For gas liquid chromatography, a Barber-Colman series 5000 gas chromatograph equipped with a flame ionization detector and a glass column (8 ft x 4 mm) containing 5% Se-30 on Gas-Chrom Q (60-80 mesh, purchased from Applied Science Laboratories, State College, PA) was used. The carrier gas was nitrogen (flow rate 60 ml/min) and the temperature was 50° isothermal for 5 minutes and then programmed to 150° at 10°/min.

The hexane solution was evaporated leaving (V) as a colorless oil. To this, then was added a solution of 10 g of sodium hydroxide in 30 ml of methanol and 10 ml of water, and the mixture was stirred and refluxed for 6 hours. The hydrolysis mixture was diluted with water and cooled in an ice bath. To this alkaline solution was added, with stirring, 50 ml of 6N sulfuric acid, and the solution was extracted with ether. The ether extract was washed with water, dried over anhydrous magnesium sulfate, and evaporated to a colorless solid, i.e., malonic acid (VI). Thin-layer chromatography (TLC) in 4 different solvent systems indicated greater than 95% radiochemical purity. Approximately 9.7 mCi of the malonic acid (VI) was obtained.

The malonic acid (VI) was heated under nitrogen to 185-190° and kept at this temperature until evolution of CO_2 ceased. Valproic acid (VII)

remained as a lightly colored oil. This acid was then dissolved in methanol and titrated to pH 8 with a solution of sodium hydroxide in methanol. The cloudy solution was treated with activated charcoal and filtered through celite. The filtrate was evaporated in vacuo to a thick syrup. Upon further heating in vacuo solid began to form. The semi solid thus obtained was placed in a vacuum dessicator over P_2O_5 for several days. Approximately 8.07 mCi of I were thus obtained, which had a specific activity of 0.99 mCi/mMole.

Synthesis of Deuterium Labeled Valproic Acid (II):

To a solution of 70 gms (0.48 mole) of diallylacetic acid (VIII) in 250 ml of methanol, an equivalent amount of NaOH in methanol was added. The methanol solution was then evaporated to dryness in vacuo and dried at 60° in a vacuum oven for several days. The dry sodium salt of diallylacetic acid (IX) thus obtained was dissolved in 300 ml of hot deuterium labeled ethanol (C_2H_5OD) under anhydrous conditions. This solution was cooled to room temperature and 4.0 gms of anhydrous cupric sulfate and 200 gms (3.6 mole) of deuterium labeled hydrazine hydrate ($D_2N\text{-}ND_2\cdot D_2O$) (purchased from Merck & Co., St. Louis, MO) was added with stirring under an atmosphere of nitrogen. A dark brown color soon developed. The mixture was stirred vigorously while a stream of dry oxygen was bubbled gently into the mixture for approximately 12 hours. During this time, the reaction mixture was found to reflux due to the exothermic nature of the reaction. After completion of the reaction, the resulting dark colored mixture was evaporated in vacuo to a black, tarry material. This residue was then dissolved in hot water and made strongly acidic by addition of dilute H_2SO_4. The dark acidic aqueous solution was then extracted with chloroform and the organic layer was washed with H_2O and dried over anhydrous Na_2SO_4. It was then filtered and solvent from the clear filtrate was removed completely under vacuo. Gas liquid chromatography (GLC) of this residue using a Barber-Colman series 5000 gas chromatograph fitted with a flame ionization detector and 5% FFAP (Free Fatty Acid Phase, purchased from Applied Science Laboratories) column (glass, 8 ft x 4mm) indicated presence of approximately 15% of the unchanged diallylacetic acid. The conditions for gas liquid chromatography were: nitrogen as carrier gas with flow rate of 60 ml/min and temperature programmed to 110-210° at 10°/min.

The diallylacetic acid, however, could be removed by treating the mixture with bromine to form the brominated addition product and the desired saturated acid could then be easily isolated from the bromo derivative. Consequently,* the crude residue (amber colored oil) containing unchanged diallylacetic acid was dissolved in the minimum amount of CCl_4 and this solution was kept at 25°. Bromine was added to this solution dropwise with stirring until a faint yellow color persisted and the mixture was stirred for an additional hour. The CCl_4 solution was then washed with H_2O and dried over anhydrous Na_2SO_4. It was filtered and solvent from the clear filtrate was removed completely under vacuo leaving an oily residue. This oil was then subjected to vacuum distillation and the fraction boiling at 120-123°/18 mm was collected. This oil however, developed a pale yellow color on standing. Therefore, this oil was further purified by redistillation and a colorless fraction distilling at 102-104°/6 mm was collected. GLC (using Free Fatty Acid Phase column and identical conditions reported earlier) of this oil indicated absence of any impurity. In this way 31 gms (43.6% yield) of pure II was obtained. Unlabeled valproic acid and II exhibited identical boiling points (b.p. 227°) (reported b.p. 221-222°)[9] at atmospheric pressure as determined by differential thermal analysis. Methods of analysis used to establish chemical purity and identity of I, VII and II are given below:

DETERMINATION OF PURITY AND IDENTITY OF CARBON-14 LABELED VALPROIC ACID (VII) AND ITS SODIUM SALT (I)

Thin-Layer Chromatography:

Authentic unlabeled sodium valproate and I were applied to Brinkmann GF plates and developed in three different solvent systems. The plates were scanned with a Packard TLC plate scanner to determine radioactive peaks. The plates were then sprayed with $KMnO_4/H_2SO_4$ to visualize the spots. In each case one radioactive peak was observed which corresponded to the R_f of the authentic material. The R_f values and the solvents used are shown on the following page:

*A better way would have been to treat the mixture containing unreacted diallylacetic acid with additional amount of deuterium labeled hydrazine hydrate. However, due to the insufficient amount of labeled hydrazine hydrate on hand, the addition of bromine to the mixture was planned.

	Solvent	R_f
1.	n-BuOH sat. with 10% NH_4OH solution	0.44
2.	$CHCl_3$-MeOH-glacial HOAc (80-20-2)	0.84
3.	$CHCl_3$-MeOH (50-50)	0.78

Samples of I were spotted on Analtech GF plates and developed in solvents 1 and 3. The silica gel was sequentially scraped from the plates into vials containing 1 ml of 50% dimethylformamide-water and then suspended in Insta-gel; the radioactivity was determined by scintillation counting. Based on the distribution of radioactivity along the length of the chromatographic plate, it could be shown that I was about 99.4% radiochemically pure.

Mass Spectral Analysis:

The mass spectra of VII and the authentic unlabeled valproic acid were in agreement. In the mass spectrum of the radiolabeled material, the carbon-14 label was observed at m/e 104 and m/e 75 corresponding to the carbon-12 ions at m/e 102 and m/e 73 for the two most abundant fragments of the unlabeled material.

Proton NMR:

The proton-nmr spectra (60 MHz) of I and the authentic unlabeled sodium valproate were identical. These data are given in Table 1.

Table 1. ^1H NMR Results for Sodium Valproate and I

Group	Chemical Shift (ppm*)	Multiplicity	Relative Integral
δ-CH_3	0.88	complex	14
β,γ-CH_2-	1.1-1.7	complex	
α-CH-	2.22	multiplet	1

* Measured in ppm downfield from the internal standard. Spectra were recorded on D_2O solutions on a Varian Associates' T-60 instrument.

DETERMINATION OF PURITY AND IDENTITY OF DEUTERIUM LABELED VALPROIC ACID (II)

High Pressure Liquid Chromatography:

Purity of II and its identity with valproic acid was determined using high pressure liquid chromatography (HPLC). The HPLC system contained a μ-Bondapak/C_{18} (4 mm x 30 cm) column and a refractive index detector. The solvent system was CH_3CN-H_2O-HOAc (glacial) (35:64:1; pH 3.0) with a flow rate of 1 ml/min at 1900 p.s.i. Under these conditions, II and valproic acid showed identical retention values. No other peaks were observed in the chromatograms.

Carbon-13 NMR:

Carbon-13 is known to couple magnetically with protons but singlet peaks for ^{13}C are obtained by proton noise decoupling. When a deuterium atom is attached to ^{13}C, coupling occurs and is observed in the CMR spectrum as a triplet whose center is shifted upfield (diamagnetic) relative to the nonlabeled ^{13}C peak. High resolution (25.2 MHz) CMR data of valproic acid and II are recorded in Table 2.

Table 2. ^{13}C NMR Comparison of Valproic Acid and II

Carbon Atom	Valproic Acid (ppm)	II (ppm)
α	45.6 (singlet)	45.5 (singlet)
β	35.2 (singlet)	35.2 (singlet)
γ	21.1 (singlet)	20.7 (triplet, J_{CD}=19.2 Hz)
δ	14.2 (singlet)	13.8 (triplet, J_{CD}=19.2 Hz)

The ^{13}C NMR spectral data of II indicated deuterium labeling on the γ and δ carbon atoms without significant labeling on the α and β carbon atoms.

Proton NMR:

A comparison of the high resolution proton NMR (100 MHz) data for valproic acid and II is shown in Table 3.

Table 3. ^{1}H NMR Comparison of Valproic Acid and II

	Valproic Acid			II		
Protons	-CH	$-(CH_2CH_2)_2$	$-(CH_3)_2$	-CH	$-(CHDCH_2)_2$	$-(CH_2D)_2$
Chemical Shift (ppm)	2.2-2.5	1.1-1.8	0.8-1.1	2.2-2.5	1.1-1.8	0.8-1.1
No. of Protons (Theory)	1	8	6	1	6	4
No. of Protons (Observed)	0.85	8.1	6.0	0.89	6.1	4.1
Integral (mm)	9	86	64	12.5	85	57

The carboxyl acid proton in these compounds appeared downfield (between 10 and 10.7 δ). This proton is readily exchangeable and would not be labeled with deuterium under the conditions used for the synthesis of II. The proton NMR spectral data indicate therefore, that II is labeled in the positions indicated and that there is insignificant, if any, deuterium on either α or β carbon-atoms in this molecule.

Infra-Red Spectral Analysis:

Valproic acid exhibited the following infra-red absorption frequencies: a very strong absorbance at 1700 cm^{-1} for the carbonyl stretching vibration of $-\overset{O}{\overset{\|}{C}}-OH$ group, an absorbance at 2960 cm^{-1} for $-CH_3$ groups, the absorbancies at 2870 cm^{-1} for $-CH_2-$ and $-CH_3$ and 2930 cm^{-1} for $-CH_2-CH_2-$ group. In the infra-red spectrum of II, a strong absorbance at 2170 cm^{-1} was observed. This is characteristic of the -CD bond. Also, the absorbance at 2960 cm^{-1} for $-CH_3$ group was greatly reduced, thus indicating that contribution due to $-CH_3$ group was affected. Also, 2870 cm^{-1} absorbance was found to shift to 2850 cm^{-1}. Although the infra-red spectral data cannot be considered to be definitive as to the amount and specificity of labeling, it, nonetheless, served as an indication of the desired distribution of deuterium in the molecule.

Mass Spectral Analysis:

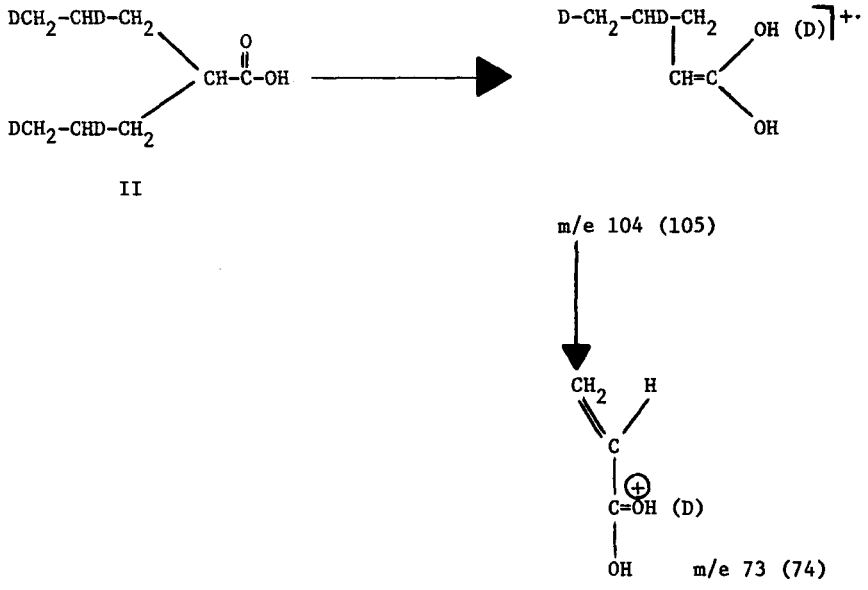
High resolution (electron impact) mass spectral data on valproic acid and II are reported in Table 4.

Table 4. Mass and Isotopic Abundance Results for Valproic Acid and II

Valproic Acid			II		
Mass	% Intensity	Empirical Formula	Mass	% Intensity	Empirical Formula
73.0283	100.00	$C_3H_5O_2$	73.0286	84.33	$C_3H_5O_2$
74.0330	4.55	$*C_3H_5O_2$	74.0348	74.21	$*C_3H_4DO_2$
102.0678	95.20	$C_5H_{10}O_2$	102.0649	3.20	$C_5H_6D_2O_2$
103.0715	5.92	$*C_5H_{10}O_2$	103.0724	24.70	$*C_5H_7D_2O_2$
104.0642	0.66	-	104.0798	100.00	$C_5H_8D_2O_2$
-	-	-	105.0857	73.13	$C_5H_7D_3O_2$

* Includes carbon-13 isotope.

Sample behavior, fragmentation patterns, and rearrangement products involving either hydrogen or deuterium transfer appeared essentially identical for both compounds. The fragmentation pattern for II is represented in the scheme shown below:



II

These results and the other spectral evidence clearly support the desired incorporation of deuterium in II.

ACKNOWLEDGMENTS

The authors thank Dr. R. Egan and Ms. R. Stanaszek for ^{13}C-NMR and ^{1}N-NMR spectral analysis, Ms. S. Mueller for mass spectrometry, Mr. W. Washburn for infra-red spectrometry, Dr. R. Hasbrouck for assistance in high pressure liquid chromatography, and Dr. T. Marriott for his comments and suggestions. The authors also thank Ms. Marcia Grissom for help in the preparation of this manuscript.

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