Oxygenation Reaction of Methyl Valproate with A Mono-oxygenase Model Reagent: Implication for Stereochemical Identification of the Mammalian Metabolites of Valproic Acid

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Non-enzymic oxygenation reaction of methyl valproate (2) utilizing a simple model system for mono-oxygenases, Fe(MeCN)_2^+ - H_2O_2 - Ac_2O in MeCN, was investigated in connection with stereochemical analyses of the mammalian metabolites of 1. This oxygenation reaction of methyl valproate (2) gave a 92:8 mixture of the anti-isomer 4a and the syn-isomer 4b, together with 5a, and 5b corresponding to the mammalian metabolites of 1. The stereochemistry of 4a, 5a, and 5b was elucidated by spectral analyses of the corresponding β-lactone 6a, γ-lactone 7a and 7b prepared from the oxygenation products. The asymmetric synthesis of (+)-7a was also achieved.

Key words: oxygenation; methyl valproate; iron-complex; model-enzyme; metabolite; asymmetric synthesis

Valproic acid (1; 2-n-propylpentanoic acid, VPA), first synthesized by Burton in 1881, is widely used as an effective anticonvulsant. It is a simple eight-carbon branched-chain fatty acid, whose esters were initially used as organic industrial solvents until the serendipitous discovery of its pharmacological properties by Meunier et al. in 1963.

The metabolism of VPA in mammalian liver has been well studied, and the results indicate that there are several metabolic pathways to various compounds such as 4-ene-VPA (3a), 3-OH-VPA (3b), 4-OH-VPA (3c), 5-OH-VPA (3d), etc. Further transformations include conjugation of VPA with glucuronic acid, β-oxidation, ω-1 and ω-2 oxidation, and γ- and δ-dehydrogenation. Some of its metabolites are considered to contribute to its pharmacological actions and toxicity. Among its metabolites, 3a appears to be the major causative agent of both liver damage and birth defects.

VPA is also known to be a substrate for the cytochrome P450s, a family of ubiquitous heme proteins that function as mono-oxygenases. In general, the oxidation of saturated aliphatic hydrocarbons by the heme-containing cytochrome P450-dependent mono-oxygenases leads to hydroxylated products. In fact, three hydroxylated metabolites of 1, i.e., 3b, 3c, and 3d are formed by liver microsomes from phenobarbital-treated rats, though their stereochemistry is uncertain.

We have examined the oxygenation reaction of various compounds with the reagent system Fe(MeCN)_2^+ - H_2O_2 - Ac_2O, a simple model system for mono-oxygenase, with a view to preparing the metabolites in large quantities for toxicological investigation and as a laboratory model for studying bio-oxygenation mechanisms. Herein, we report the oxygenation reaction of methyl valproate (2) with Fe(MeCN)_2^+ - H_2O_2 - Ac_2O, by non-enzymic methods, in order to obtain the information to assist in the stereochemical identification of metabolites of 1.

The non-enzymic oxygenation reaction of 2 was carried out utilizing Fe(MeCN)_2^+ - H_2O_2 - Ac_2O in acetonomitrile (MeCN) under various conditions according to the following procedure. A solution of 30% H_2O_2 in MeCN was added dropwise to a stirred solution of Fe(ClO_4)_2·6H_2O and methyl valproate (2) in MeCN and Ac_2O (sufficient amounts of Ac_2O to remove water from the iron salt and 30% H_2O_2), while the internal temperature was maintained at 25-35°C.

The above reaction afforded several oxygenation products, namely, a 92:8 mixture of 3-acetoxy esters (the anti-isomer 4a and the syn-isomer 4b) as well as 4-acetoxy esters (the syn-isomer 5a and the anti-isomer 5b) corresponding to mammalian metabolites of 1 such as 3b and 3c. The product yields varied with the reaction conditions used, as shown in Table 1. The ratio of 92:8 in the mixture of 4a and 4b was determined based on two acetyl singlet signals (δ 2.04, 2.06) in the 1H-NMR spectrum (run 7).

Features of these reactions were as follows. i) The best total yield was obtained at the molar ratio of substrate (2): Fe^2+ : H_2O_2 = 1:0.5:4.0 (run 7). ii) Almost all the reactions under various conditions (runs 1—8) afforded a mixture of 4a and 4b, 5a, and 5b in similar ratios of ca. 1:2:2 (runs 1—8). iii) The oxygenation products 5a and 5b at the C-4 position were regioselectively obtained in preference to the oxygenation product (a mixture of 4a

Chart 1

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and 4b) at the C-3 position, and further there was no oxygenation product at the C-5 position. iv) The anti-isomer 4a was stereoselectively formed over the syn-isomer 4b.

The mechanism of formation of the oxygenation products 4a, 4b, 5a and 5b from 2 can be postulated as shown in Chart 3. First, formation of 5a may proceed as follows. The σ bond formation reaction of the Fe

V atom to the C4-position in compound 2a-1, in which iron is chelated to the carbonyl group of 2 (iron may be in the form of Fe

V(OH) (OAc)

V+, a hypothetical active species

(10) may take place with radical dehydroxylation to yield the corresponding Fe

V compound 2a-2 with the six-membered ring. This may exist in a chair conformation. Further, cleavage of the six-membered ring in 2a-2 may proceed with rearrangement of the acetoxy group to form the Fe

III compound 2a-3 with retention of configuration, and this may lead to 5a. Similarly, 5b, 4a, and 4b may be formed as shown in Chart 3.

The preferential regioselective formation of 5a and 5b in comparison with the mixture of 4a and 4b may be attributable to the thermodynamic stability differences between compounds with a six-membered ring, such as 2a-2 and 2b-2, and those with a five-membered ring, such as 2c-2 and 2d-2. Thus, 5a and 5b may be generated from 2a-2 and 2b-2, respectively. Moreover, the preferential stereoselective formation of 4a in comparison with 4b may also be due to thermodynamic stability differences between 2e-2 and 2f-2. Consequently, 4a may result from the more favorable 2e-2, in preference to 2f-2.

The stereochemistry of 4a, 5a, and 5b was assigned on the basis of spectral analyses of the corresponding β-lactone 6a, γ-lactone 7a and 7b, which were obtained by chemical transformation as follows. First, a mixture of 4a and 4b was transformed into the corresponding β-lactone, a 93 : 7 mixture of the trans-isomer 6a and the cis-isomer 6b by hydrolysis using alcoholic 20% KOH, followed by lactonization with p-toluenesulfonyl chloride (TsCl) in pyridine. 11) Compounds 6a and 6b could not be separated, but the ratio of ca. 93 : 7 in the mixture of 6a and 6b was
Table 2. Chemical Shifts (H-2, H-3α, H-3β, H-4) and Coupling Constants (J_{2,3α}, J_{2,3β}, J_{3α,3β}, J_{3α,4}, J_{3β,4}, J_{4,5}) of Compounds 6a, 7a, and 7b from ¹H-NMR Spectra at 500 MHz in CDCl₃ 

<table>
<thead>
<tr>
<th>Compd.</th>
<th>δ (Hz)</th>
<th>J (Hz)</th>
</tr>
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<tbody>
<tr>
<td>6a</td>
<td>3.19 (dd) 4.18 (dt)</td>
<td>1.67–1.94 (m)</td>
</tr>
<tr>
<td>7a</td>
<td>2.61–2.67 (m) 2.08 (dd)</td>
<td>2.02 (dd) 4.68 (dd)</td>
</tr>
<tr>
<td>7b</td>
<td>2.56–2.68 (m) 1.35–1.54 (m)</td>
<td>2.48 (dd) 4.49 (dd)</td>
</tr>
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a) Assignments based upon ¹H-¹³C COSY and ¹H-NOE experiments. b) Overlapped with H-1' and H-2'.

determined by ¹H-NMR (see Experimental section). The stereochemistry of 6a was established from the vicinal coupling constant between C₂-Hβ and C₃-Hβ and a nuclear Overhauser effect (NOE) experiment. In the ¹H-NMR spectrum of 6a in CDCl₃, the proton signal of C₃-Hβ (δ 4.18) showed a small coupling constant (J_{2,3} = 3.97 Hz) (Table 2). It was reported that the NMR coupling constant J_{2,3} between C₂-H and C₃-H is normally smaller for the trans-β-lactone than for the cis-β-lactone. No NOEs were observed between C₂-Hβ and C₃-Hβ as shown in Fig. 1. Accordingly, 6a was determined to have relative trans-configuration on the β-lactone ring. These
data indicated that 4a possesses relative anti-configuration between C-2 and C-3. A few proton signals of C3-48 and C2-47 (δ 3.59—3.64) of 6b appeared, but the other signals were hidden under those of 6a in the 1H-NMR spectrum of 6b. Further, the proton signal of C3-48 showed a large coupling constant (J2,3= 6.90 Hz). This suggests the presence of the cis-isomer 6b.

The mixture of 5a and 5b was hydrolyzed with alcoholic KOH followed by lactonization of the resulting β-hydroxy acids with 85% H3PO4 and 1 N HCl to give the corresponding trans-γ-lactone 7a and the cis-isomer 7b, respectively. Similar lactonization of the β-hydroxy acid prepared from the isolated compound 5a gave only 7a. The stereochemistry of 7a and 7b was established from NOE experiments. Clear 1H-NOEs were observed between C2-Hβ and C4-Me, C3-Hz and C4-Hz of 7a. On the other hand, NOEs were also observed between C2-Hβ and C2-Hβ, C3-Hβ and C2-Hβ of 7b. Accordingly, 7a and 7b were determined to have relative trans- and cis-configuration on the γ-lactone ring, respectively. These data showed that 5a and 5b have relative syn- and anti-configuration between C-2 and C-4, respectively.

Next, we investigated the asymmetric synthesis of the γ-lactone (+)-7a for direct comparison with (+)-7a prepared from the oxidation product 5a. The chiral synthon (+)-8 was prepared from t-glutamic acid as described before.14) Treatment of 8 with triphenylmethyl chloride (trityl chloride), dimethylaminopyridine (DMAP), and triethylamine at room temperature under an N2 atmosphere gave the corresponding ether (+)-9 in 69% yield. Lithiation of 9 with lithium disopropylamide (LDA) and alkylation of the corresponding enolate with n-propyl iodide in the presence of hexamethyl phosphoronic triamide (HMPA) in tetrahydrofuran (THF) at −78 °C afforded a crystalline product (+)-10 in 56% yield.15) Destrification of 10 with 10% palladium-charcoal (Pd/C) in ethanol (EtOH) containing concentrated HCl gave the hydroxy-γ-lactone (+)-11 in 85% yield. The bromination of 11 with thionyl bromide (SOBr2).16) followed by the halogen exchange reaction with sodium iodide (NaI), gave the corresponding iodo-γ-lactone (+)-13 in 49% yield from 11. This bromination is known to complete with retention of configuration.17) Subsequently, treatment of a solution of 13 and tributyltin hydride (n-Bu3SnH)18) in benzene with a catalytic amount of triethylborane (Et3B) (1 M hexane solution) at -5 to 7 °C gave the desired γ-lactone (+)-7a, [z]D25 +8.35° (c = 1.68, EtOH), in 91% yield without opening the lactone ring. All physical data for the asymmetrical synthetic product (+)-7a were identical with those of (+)-7a, except for the optical rotation.

These results may be useful in identifying the stereochemical features of the mammalian metabolites of 1. Investigations of the enzymatic oxygenation reaction of 1 utilizing rat liver S9 mix are in progress, aiming to elucidate the stereochemistry of the metabolites of 1 in rat.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were recorded with a JASCO IR-700 spectrometer, and 1H- and 13C-NMR spectra with JEOL JNM-EX90, JNM-GX270 and JNM-GSX500 spectrometers, with tetramethylsilane as an internal standard (CDCl3 solution). Mass spectra were recorded on a JEOL JMS-D300 spectrometer. Elemental analyses were done using a Yanaco CHN-MT-3 apparatus. Wako Silica gel C-200 (200 mesh) and Merck Kieselgel 60 F254 were used for column chromatography and thin-layer chromatography (TLC), respectively. Each organic extract was dried over Na2SO4. Preparative HPLC (high-performance liquid chromatography) was carried out with a JASCO HPLC system (pump, JASCO 880; RI-detector, JASCO 880) using a Silica-3301-N (Senshu Pac, 80×300 mm i.d.) column.

Typical Procedure for Oxidation of Methyl Valproate (2) with Fe(NeCN)6-H2O-AcO-O System A solution of 30% H2O2 (0.44 ml, 4mmol) in MeCN (2 ml) was added dropwise to a solution of Fe(NeCN)6·2H2O (181 mg, 0.55 mmol), AcO-O (1.2 ml, 11.7 mmol) and methyl valproate (2) (158 mg, 1 mmol) in MeCN (4 ml), while the internal temperature was maintained at 25–35 °C, under vigorous stirring. The reaction mixture was poured into ice water and extracted with ether–CH2Cl2 (3:1, 1 v/v). The organic layer was washed with saturated Na2SO4, saturated NaHCO3, and brine, and then dried. Concentration afforded a yellow oil. This oil was subjected to silica gel column chromatography. The first eluate with hexane–AcOEt (10:1, v/v) gave methyl valproate (2). The second eluate with hexane–AcOEt (10:1, v/v) gave a mixture of 4a, 4b, 5a and 5b. The mixture was further subjected to preparative HPLC with hexane–AcOEt (10:1, v/v). The first eluate (retention time 10.02 min) gave a 92:8 mixture of (2R,3R*)-methyl 3-acetoxy-2-propylpentanoate (4a) and (2R,3S*)-methyl 3-acetoxy-2-propylpentanoate (4b) as a colorless oil. The second eluate (retention time 10.65 min) gave (2R,4R*)-methyl 4-acetoxy-2-propylpentanoate (5a) as a colorless oil. The third eluate (retention time 11.13 min) gave (2R,4S*)-methyl 4-acetoxy-2-propylpentanoate (5b) as a colorless oil. The yields are listed in Table 1. The ratio of 92:8 was determined by 1H-NMR (δ 2.04 and 2.06 for acetyl signals of 4a and 4b, respectively).
The reaction mixture was poured into ice-water and extracted with CHCl₃. The organic layer was washed with saturated NaHCO₃ and H₂O, then dried and concentrated. The residue was recrystallized from ethanol to yield 20.7 g (68.9%) of 9 as colorless crystals, mp 149—150°C (ethanol). [α]D²⁰ +25.22° (c = 1.69, EtOH). IR (KBr) cm⁻¹: 1763. 1H-NMR (90 MHz, CDCl₃) δ: 1.94—2.36 (2H, m, C-H), 2.48—2.72 (2H, m, C-H), 3.14 (1H, dd, J = 10.55, 3.95 Hz, C-H), 3.43 (1H, dd, J = 10.55, 3.52 Hz, C-H), 4.58—4.71 (1H, m, C-H), 7.23—7.50 (15H, m, C5-OPh). Anal. Calcd for C₂₂H₂₀O₄: C, 78.42; H, 6.19. Found: C, 78.45; H, 6.19.

(2R,4S)-(2-Propyl-4-[(tritylmethyl)oxy]-4-butanolide) (10) A solution of HMPA (2.7 ml, 16 mmol) and 9 (2.86 g, 8.0 mmol) in anhydrous THF (32 ml) was added to a solution of LDA (8.0 ml, 16 mmol) in anhydrous THF (32 ml). The mixture was stirred at −78°C for 30 min, then propyl iodide (1.55 ml, 16 mmol) was added. The whole was stirred at −78°C for 2.5 h and then the reaction was quenched with saturated aqueous NH₄Cl (30 ml). The mixture was extracted with ether (50 ml × 3). The organic solution was washed with H₂O, then dried and concentrated. The residue was purified by silica gel column chromatography. The eluate with AcOEt-hexane (1:10, v/v) gave 1.79 g (56.1%) of 10 as colorless prisms, mp 91—93°C (ethanol). [α]D²⁰ +21.57° (c = 1.69, EtOH). IR (KBr) cm⁻¹: 1763. 1H-NMR (270 MHz, CDCl₃) δ: 0.93 (3H, d, J = 6.32 Hz, C5-H), 1.52—1.74 (8H, m, C-H), 2.10—2.22 (10H, m, C-H), 2.73—2.84 (14H, m, C3-H), 3.12 (1H, dd, J = 10.37, 4.27 Hz, C5-H), 3.40 (1H, dd, J = 10.37, 3.67 Hz, C5-H), 4.52—4.59 (14H, m, C4-H), 7.12—7.44 (15H, m, C5-OPh). Anal. Calcd for C₂₂H₂₂O₃: C, 80.96; H, 7.05. Found: C, 80.99; H, 7.04.

(2R,4S)-(4-(Hydroxymethyl)-2-propyl-4-butanolidine) (11) Compound 10 (400 mg, 1.8 mmol) was hydrolyzed in the presence of 10% Pd/C (60 mg) in concentrated HCl (1.5 ml) and EOH (40 ml) at room temperature for 12 h. The catalyst was removed, and the filtrate was concentrated. The residue was subjected to silica gel chromatography. The eluate with AcOEt-hexane (1:4, v/v) gave 135 mg (85.1%) of 11, as a colorless oil. [α]D²⁰ +14.69° (c = 1.63, EtOH). IR (oil) cm⁻¹: 3412, 1761. 1H-NMR (270 MHz, CDCl₃) δ: 0.95 (3H, t, J = 6.70 Hz, C5-H), 1.51—1.54 (8H, m, C-H), 2.83—2.97 (10H, m, C3-H), 4.59—4.62 (14H, m, C4-H), 7.12—7.50 (15H, m, C5-OPh). Anal. Calcd for C₂₂H₂₂O₃: C, 78.47; H, 6.19.

(2R,4S)-(4-(Iodomethyl)-2-propyl-4-butanolidine) (12) Thiouyl bromide (1.4 g, 6.6 mmol) was added to a mixture of 11 (950 mg, 6.0 mmol) and anhydrous pyridine (530 mg, 6.7 mmol) at 40—50°C. The mixture was refluxed for 1 h. The reaction mixture was cooled to 0°C, quenched with water, and the resulting colorforming solution was washed with H₂O (100 ml × 3) and saturated NaHCO₃ (20 ml), then dried and concentrated to give (2R,4S)-4-(bromomethyl)-2-propyl-4-butanolidine (12) as an oil. Anhydrous acetone (20 ml) and NaI (2.5 g) were added to the oil obtained above and the whole was refluxed for 12 h. The reaction mixture was cooled and the resulting white precipitate was removed by filtration. The filtrate was evaporated under vacuum. The residue was subjected to silica gel column chromatography (AcOEt-hexane = 1:5, v/v as an eluant). The eluate gave 780 mg of 13, in 48.8% yield from 11, as a colorless oil. [α]D²⁰ +2.81° (c = 1.61, EtOH). IR (oil) cm⁻¹: 1770. 1H-NMR (270 MHz, CDCl₃) δ: 0.95 (3H, t, J = 7.32 Hz, C5-H), 1.55 (5H, m, C-H, C3-H, C7-H), 2.06—2.15 (4H, m, C3-H, C5-H, C7-H), 2.61—2.67 (1H, m, C2-H), 4.68 (1H, ddq, J = 7.02, 6.41, 5.19 Hz, C4-Ha), 1.36—1.65 (8H, m, C-H). 13C-NMR (CDCl₃) δ: 13.8 (C3), 20.6 (C5), 21.3 (C2), 32.8 (C1), 35.1 (C3), 39.1 (C2), 76.4 (C4), 179.5 (C1). CI-MS m/z: 268 (M⁺+1), 269 (M⁺+2).

(2R,4R)-4-Methyl-2-propyl-4-butanolidine (7a) Tributyltin hydride (n-B₃SnH₃, 172 mg, 0.52 mmol) was added to a solution of 13 (105 mg, 1.0 mmol) in benzene (4 ml) at 5—7°C. Triethyloborane (Et₃B, 1.0 mm hexane solution, 40 μl, 0.04 mmol) was added and the resulting mixture was stirred at 0°C for 3 h. After removal of 13-C(NMR), the residue was dissolved in CH₂Cl₂ (4 ml). Potassium fluoride (KF, 130 mg, 2.25 mmol) and H₂O (1 ml) were added to the above CH₂Cl₂ solution and the whole was vigorously stirred at room temperature for 5 h. The precipitated tributyltin fluoride was removed by filtration with washed with CH₂Cl₂. The combined filtrate was passed through a short column of anhydrous Na₂SO₄ (10 g). The eluate was concentrated and the final oil was purified by silica gel column chromatography (AcOEt-hexane = 1:5, v/v as an eluant) to give 95 mg (91%) of (2R,4R)-4-Methyl-2-propyl-4-butanolidine (7a) as a colorless oil. [α]D²⁰ +8.35° (c = 1.68, EtOH). IR (oil) cm⁻¹: 1770.
References