Oxygenation Reaction of Methyl Valproate with A Mono-oxygenase Model Reagent: Implication for Stereochemical Identification of the Mammalian Metabolites of Valproic Acid[1)]

Eiichi KOTANI, Tetsuya TAKEYA, Motohide YOSHIIKE, Atsuko WATANABE, and Seisho TOBINAGA*

Showa College of Pharmaceutical Sciences, Machida, Tokyo 194, Japan.
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Non-enzymic oxygenation reaction of methyl valproate (2) utilizing a simple model system for mono-oxygenases, $Fe(MeCN)_6^{2+}$–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[2)] in 1881, is widely used as a effective anticonvulsant.[3)] 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.*[4)] in 1963.

The metabolism of VPA in mammalian liver has been well studied,[5)] and the results indicate that there are several metabolic pathways[6)] 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-, ω-1 and ω-2 oxidation, and γ- and δ-dehydrogenations. Some of its metabolites are considered to contribute to its pharmacological actions and toxicity.[7)] Among its metabolites, **3a** appears to be the major causative agent of both liver damage and birth defects.[7a,8)]

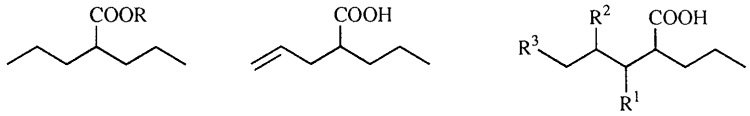
VPA is also known to be a substrate for the cytochrome P450s,[7a)] a family of ubiquitous heme proteins that function as mono-oxygenases. In general, the oxidation of saturated aliphatic hydrocarbons by the heme-containing cytochrome P450-dependent mono-oxygenases leads to hydroxylated products.[9)] In fact, three hydroxylated metabolites of **1**, *i.e.*, **3b**, **3c**, and **3d** are formed by liver microsomes from phenobarbital-treated rats,[10)] though their stereochemistry is uncertain.

We have examined the oxygenation reaction of various compounds with the reagent system $Fe(MeCN)_6^{2+}$–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.[1)] Herein, we report the oxygenation reaction of methyl valproate (**2**) with $Fe(MeCN)_6^{2+}$–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 $Fe(MeCN)_6^{2+}$–H_2O_2–Ac_2O in acetonitrile (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 $Fe(ClO_4)_2 \cdot 6H_2O$ and methyl valproate (**2**) in MeCN and Ac_2O (sufficient amounts of Ac_2O to remove water in 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 ^{1}H-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**



1: R=H; VPA
2: R=Me

3a; 4-ene-VPA

3b: R^1=OH, R^2=R^3=H; 3-OH-VPA
3c: R^1=R^3=H, R^2=OH; 4-OH-VPA
3d: R^1=R^2=H, R^3=OH; 5-OH-VPA

Chart 1

* To whom correspondence should be addressed.

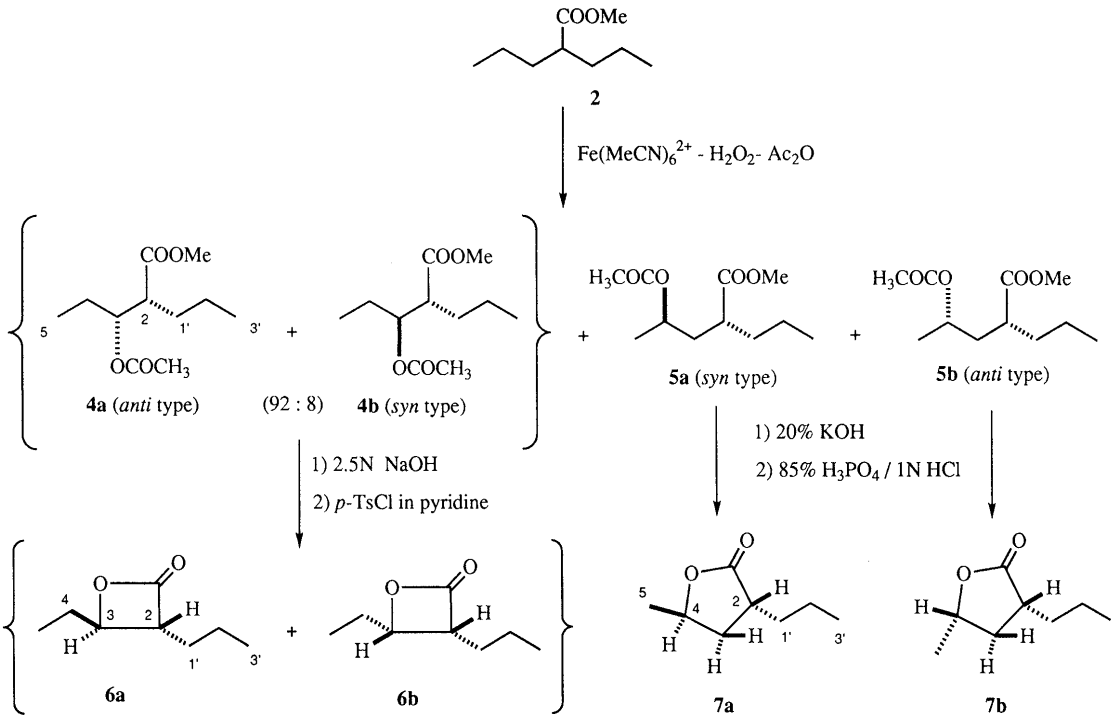


Chart 2

Table 1. Oxygenation Reaction of Methyl Valproate (**2**) with $Fe(MeCN)_6^{2+}$–H_2O_2–Ac_2O in MeCN

Run	Molar ratio			Total	Product (yield, %)			Recovery (%)
	2 :	Fe^{2+} :	H_2O_2		**4**[a]	**5a**	**5b**	
1	1	1.0	1.2	7.4	1.5	2.9	3.0	87.1
2	1	0.5	1.2	7.4	1.5	2.9	3.0	86.5
3	1	1.0	2.4	16.1	3.3	6.4	6.4	69.5
4	1	0.5	2.4	21.4	4.3	8.3	8.8	57.4
5	1	0.4	2.4	18.7	3.6	6.9	8.2	65.6
6	1	0.2	2.4	16.0	3.1	6.0	6.9	66.6
7	1	0.5	4.0	26.7	5.6[b]	10.8	10.3	38.7
8	1	0.5	8.1	26.5	5.6	10.7	10.2	47.1

a) A mixture of **4a** (*anti*) and **4b** (*syn*). *b*) The *anti*/*syn* ratio (=92 : 8) was determined by ^{1}H-NMR.

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 to be as shown in Chart 3. First, formation of **5a** may proceed as follows. The σ bond formation reaction of the Fe^{IV} 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 $Fe^{IV}(OH)(OAc)^{2+}$, a hypothetical active species[1a]) may take place with radical dehydration to yield the corresponding Fe^{IV} 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^{II} 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. Morever, the preferential stereoselective formation of **4a** in comparison with **4b** may also be due to thermodynamic stability differences between **2c**-2 and **2d**-2. Consequently, **4a** may result from the more favorable **2c**-2, in preference to **2d**-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

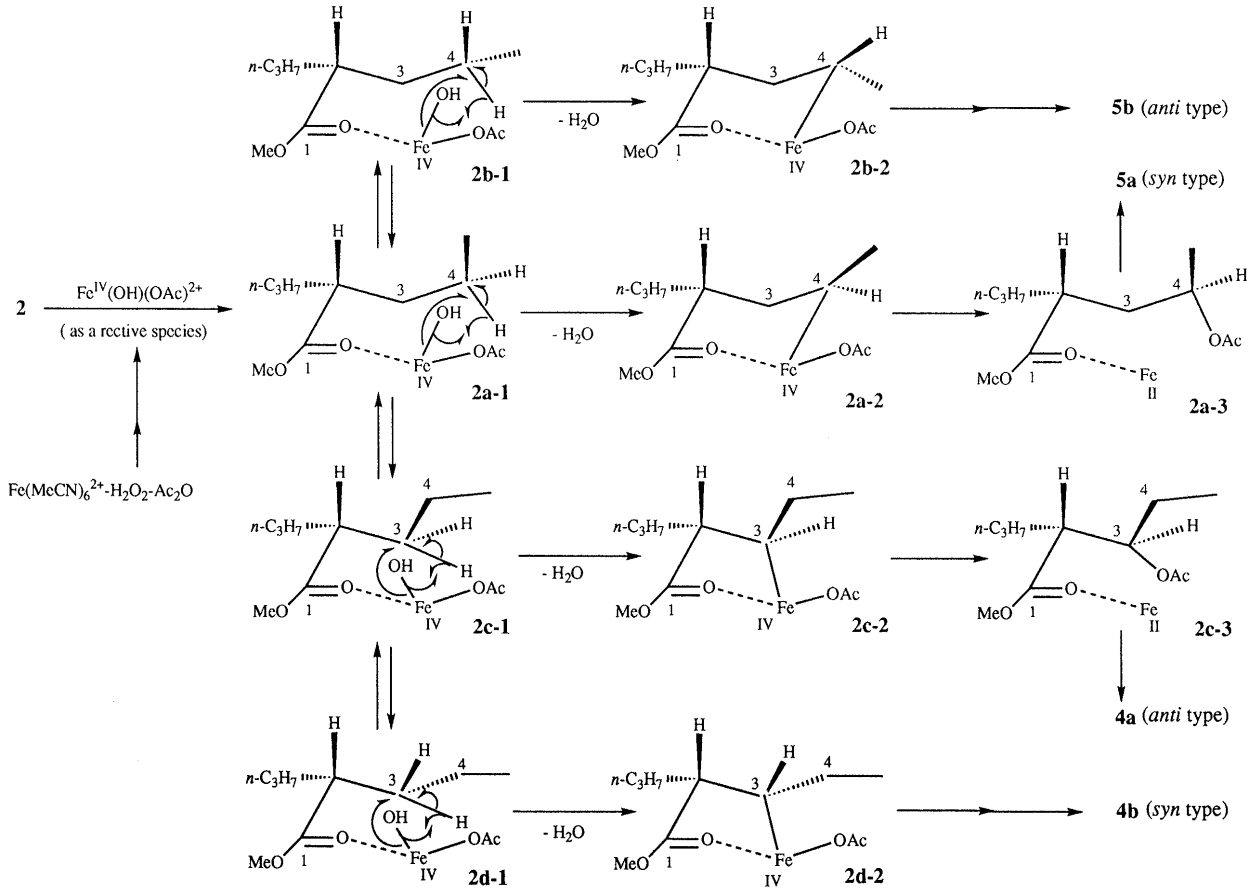


Chart 3. Proposed Mechanism for the Formations of **4a**, **4b**, **5a**, and **5b**

Table 2. Chemical Shifts (H-2, H-3α, H-3β, H-4) and Coupling Constants ($J_{2,3\alpha}$, $J_{2,3\beta}$, $J_{3\alpha,3\beta}$, $J_{3\alpha,4}$, $J_{3\beta,4}$, $J_{4,5}$) of Compounds **6a**, **7a**, and **7b** from ^{1}H-NMR Spectra at 500 MHz in $CDCl_3$[a]

Compd.	δ				J (Hz)					
	H-2	H-3α	H-3β	H-4	2,3α	2,3β	3α,3β	3α,4	3β,4	4,5
6a	3.19 (ddd)	4.18 (dt)	—	1.67—1.94 (m)	3.97	—	—	6.60	—	—
7a	2.61—2.67 (m)	2.08 (ddd)	2.02 (ddd)	4.68 (ddq)	7.32	9.15	12.97	7.02	5.19	6.41
7b	2.56—2.68 (m)	1.35—1.54[b] (m)	2.48 (ddd)	4.49 (ddq)	—[b]	8.55	12.51	10.38	5.50	6.10

a) Assignments based upon ^{1}H–^{13}C COSY and ^{1}H-NOE epemeriments. b) Overlapped with H-1′ and H-2′.

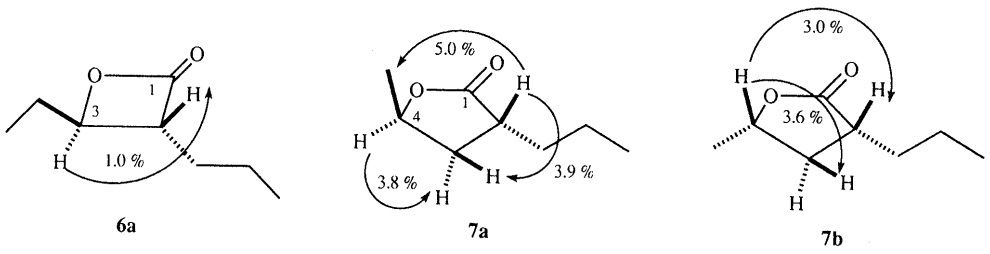


Fig. 1. Significant Enhancements of Signal Intensity in NOE Experiments on **6a**, 7a, and **7b**

determined by ^{1}H-NMR (see Experimental section). The stereochemistry of **6a** was established from the vicinal coupling constant between C_2-Hβ and C_3-Hα and a nuclear Overhauser effect (NOE) experiment. In the ^{1}H-NMR spectrum of **6a** in $CDCl_3$, the proton signal of C_3-Hα (δ 4.18) showed a small coupling constant ($J_{2,3}$ = 3.97 Hz) (Table 2). It was reported[12,13] that the NMR coupling constant $J_{2,3}$ between C_2-H and C_3-H is normally smaller for the *trans*-β-lactone than for the *cis*-β-lactone. No NOEs were observed between C_2-Hβ and C_3-Hα as shown in Fig. 1. Accordingly, **6a** was determined to have relative *trans*-configuration on the β-lactone ring. These

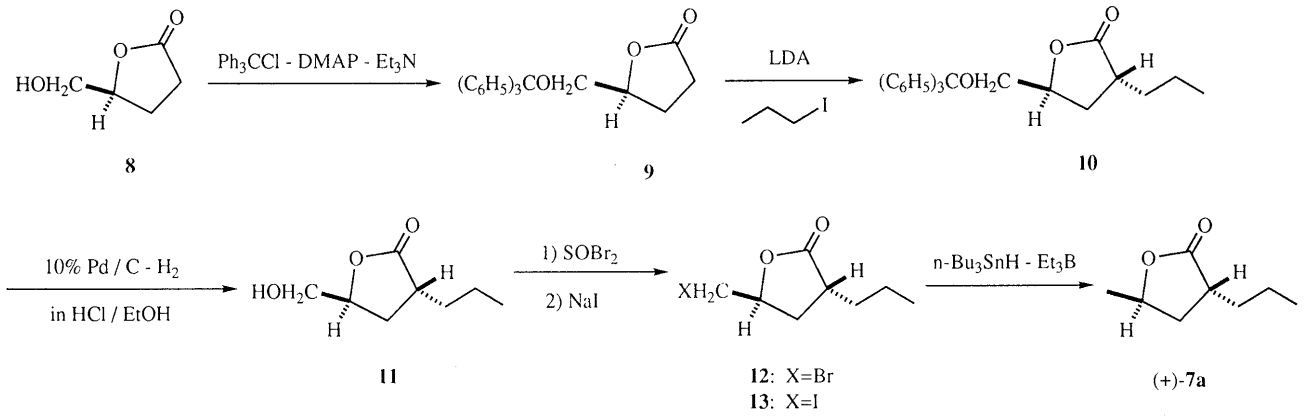


Chart 4

data indicated that **4a** possesses relative *anti*-configuration between C-2 and C-3. A few proton signals of C_3-H (δ 4.48) and C_2-H (δ 3.59—3.64) of **6b** appeared, but the other signals were hidden under those of **6a** in the ^{1}H-NMR spectrum of **6b**. Further, the proton signal of C_3-H showed a large coupling constant ($J_{2,3}=6.90$ 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% H_3PO_4 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 ^{1}H-NOEs were observed between C_2-Hβ and C_4-Me, C_3-Hα and C_4-Hα of **7a**. On the other hand, NOEs were also observed between C_4-Hβ and C_2-Hβ, C_4-Hβ and C_3-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 oxygenation product **5a**. The chiral synthon (+)-**8** was prepared from L-glutamic acid as described before.[14] Treatment of **8** with triphenylmethyl chloride (trityl chloride), dimethylaminopyridine (DMAP), and triethylamine at room temperature under an N_2 atmosphere gave the corresponding ether (+)-**9** in 69% yield. Lithiation of **9** with lithium diisopropylamide (LDA) and alkylation of the corresponding enolate with *n*-propyl iodide in the presence of hexamethyl phosphoric triamide (HMPA) in tetrahydrofuran (THF) at −78 °C afforded a crystalline product (+)-**10** in 56% yield.[15] Detritylation 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 ($SOBr_2$),[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 proceed with complete retention of configuration.[17] Subsequently, treatment of a solution of **13** and tributyltin hydride (*n*-Bu_3SnH)[18] in benzene with a catalytic amount of triethylborane (Et_3B) (1 M hexane solution) at 5—7 °C gave the desired γ-lactone (+)-**7a**, $[\alpha]_D^{19}+8.35°$ ($c=1.68$, 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 enzymic 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 ^{1}H- and ^{13}C-NMR spectra with JEOL JNM-EX90, JNM-GX270 and JNM-GSX500 spectrometers, with tetramethylsilane as an internal standard ($CDCl_3$ 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 F_{254} were used for column chromatography and thin-layer chromatography (TLC), respectively. Each organic extract was dried over Na_2SO_4. Preparative HPLC (high-performance liquid chromatography) was carried out with a JASCO HPLC system (pump, JASCO 880; RI-detector, JASCO 830) using a Silica-3301-N (Senshu Pac, 8ϕ × 300 mm i.d.) column.

Typical Procedure for Oxygenation of Methyl Valproate (2) with $Fe(MeCN)_6^{2+}$–H_2O_2–Ac_2O System A solution of 30% H_2O_2 (0.44 ml, 4 mmol) in MeCN (2 ml) was added dropwise to a solution of $Fe(ClO_4)_2\cdot 6H_2O$ (181 mg, 0.5 mmol), Ac_2O (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–CH_2Cl_2 (3 : 1, v/v). The organic layer was washed with saturated Na_2SO_3, saturated $NaHCO_3$, 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 (2*R**,3*R**)-methyl 3-acetoxy-2-propylpentanoate (**4a**) and (2*R**,3*S**)-methyl 3-acetoxy-2-propylpentanoate (**4b**) as a colorless oil. The second eluate (retention time 10.65 min) gave (2*R**,4*R**)-methyl 4-acetoxy-2-propylpentanoate (**5a**) as a colorless oil. The third eluate (retention time 11.13 min) gave (2*R**,4*S**)-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 ^{1}H-NMR (δ 2.04 and 2.06 for acetyl signals of **4a** and **4b**, respectively).

Data for **4a**: IR (oil) cm^{-1}: 1741, 1235. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.89 (3H, t, $J=7.32$ Hz, C_5-H or C3′-H), 0.91 (3H, t, $J=7.32$ Hz, C5-H or C3′-H), 1.21—1.76 (6H, m, C4-H, C1′-H, C2′-H), 2.04 (3H, s, OCOMe), 2.62—2.70 (1H, m, C2-H), 3.68 (3H, s, CO_2Me), 5.05 (3H, dt, $J=4.28$, 7.63 Hz, C3-H). ^{13}C-NMR ($CDCl_3$) δ: 9.2 (C5), 13.9 (C3′), 20.7 (C2′), 21.0 (OCO<u>Me</u>), 24.6 (C4), 30.3 (C1′), 48.9 (C2), 51.6 (CO_2Me),

75.2 (C3), 170.4 (OCOMe), 173.7 (C1). CI-MS *m/z*: 217 $(M+1)^+$. **5a**: IR (oil) cm^{-1}: 1736, 1242. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.90 (3H, t, $J=7.01$ Hz, C3'-H), 1.21 (3H, d, $J=6.41$ Hz, C5-H), 1.26—1.92 (6H, m, C3-H, C1'-H, C2'-H), 2.02 (3H, s, –OCOMe), 2.46—2.55 (1H, m, C2-H), 3.67 (3H, s, $-CO_2Me$), 4.81—4.91 (1H, m, C4-H). ^{13}C-NMR ($CDCl_3$) δ: 13.9 (C3'), 20.3 (C5), 20.5 (C2'), 21.2 (COMe), 35.0 (C1'), 38.4 (C3), 41.5 (C2), 51.5 (CO_2Me), 69.1 (C4), 170.6 (OCOMe), 176.3 (C1). CI-MS *m/z*: 217 $(M+1)^+$. **5b**: IR (oil) cm^{-1}: 1735, 1240. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.89 (3H, t, $J=7.33$ Hz, C3'-H), 1.22 (3H, d, $J=6.10$ Hz, C5-H), 1.24—1.65 (6H, m, C3-H, C1'-H, C2'-H), 2.01 (3H, s, –OCOMe), 2.35—2.46 (1H, m, C2-H), 3.68 (3H, s, $-CO_2$Me), 4.85—4.97 (1H, m, C4-H). ^{13}C-NMR ($CDCl_3$) δ: 13.9 (C3'), 20.2 (C5), 20.4 (C2'), 21.2 (COMe), 34.7 (C1'), 38.3 (C3), 41.4 (C2), 51.5 (CO_2Me), 69.5 (C4), 170.6 (OCOMe), 176.4 (C1). CI-MS *m/z*: 217 $(M+1)^+$.

Lactonization of a Mixture of 4a and 4b A mixture of **4a** and **4b** (120 mg, 0.56 mmol) was added to ethanol (3 ml) and aqueous sodium hydroxide (2.5 N, 3.5 ml); the mixture was stirred at 80 °C for 3 h and evaporated under reduced pressure. The residue was dissolved in water and the solution was washed with ether. The aqueous solution was acidified and extracted with ether, then the ethereal solution was washed with water, dried, and concentrated. Anhydrous pyridine (3.2 ml) and *p*-toluenesulfonyl chloride (191 mg, 1 mmol) were added to the residue at 0—5 °C and the resulting mixture was stirred for 30 min, then placed in a refrigerator overnight. The reaction mixture was poured into ice–water and the whole was extracted with several volumes of ether. The combined ethereal layers were washed with saturated $NaHCO_3$ and H_2O, and then dried and concentrated. The residue was subjected to silica gel column chromatography (AcOEt : hexane = 1 : 10, v/v as an eluant). The eluate gave 30 mg (38%) of a 93 : 7 mixture of (2*R**,3*R**)-3-ethyl-2-propyl-3-propanolide (**6a**) and (2*R**,3*S**)-3-ethyl-2-propyl-3-propanolide (**6b**) as a colorless oil. The ratio of 93 : 7 was determined by ^{1}H-NMR (δ 4.18 and 4.48 for C3-H signals of **6a** and **6b**, respectively). Data for **6a**: IR (oil) cm^{-1}: 1819. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.96 (3H, t, $J=7.32$ Hz, C5-H), 1.01 (3H, t, $J=7.32$ Hz, C3'-H), 1.40—1.48 (2H, m, C2'-H), 1.67—1.94 (4H, m, C1'-H, C4-H), 3.19 (1H, ddd, $J=9.00$, 6.71, 3.97 Hz, C2-H), 4.18 (1H, dt, $J=6.50$, 3.97 Hz, C3-H). ^{13}C-NMR ($CDCl_3$) δ: 9.1 (C3'), 13.9 (C5), 20.4 (C2'), 27.5 (C4), 30.0 (C1'), 55.6 (C2), 79.2 (C3), 171.6 (C1). CI-MS *m/z*: 143 $(M+1)^+$.

Lactonization of the Mixture of 5a and 5b The reaction mixture obtained by oxygenation of **2** was worked up according to the typical procedure described above. To the resulting mixture (778 mg) of **5a** and **5b** was added a solution of 20% aqueous KOH (20 ml) in methanol (10 ml), and the whole was stirred at 60 °C (internal temperature) on an oil-bath for 2 h. The reaction mixture was washed with ether. The aqueous solution was acidified and extracted with ether–CH_2Cl_2 (3 : 1, v/v). The organic layer was dried and concentrated to give a yellow oil. Then 85% H_3PO_4 (16 ml) and 1 N HCl (16 ml) were added to this oil and the whole was stirred at 50 °C (internal temperature) for 3 h. The reaction mixture was diluted with saturated NaCl and extracted with ether. The ethereal solution was dried and concentrated. The residue was subjected to preparative HPLC with hexane–AcOEt (10 : 1, v/v). The first eluate gave 35.6 mg of (2*R**,4*R**)-4-methyl-2-propyl-4-butanolide (**7a**) as a colorless oil. The second eluate gave 33.1 mg of (2*R**,4*S**)-4-methyl-2-propyl-4-butanolide (**7b**) as a colorless oil.

7a: IR (oil) cm^{-1}: 1770. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.95 (3H, t, $J=7.32$ Hz, C3'-H), 1.37 (3H, d, $J=6.41$ Hz, C5-H), 1.39—1.50 (3H, m, C1'-H, C2'-H), 1.76—1.85 (1H, m, C1'-H), 2.02 (1H, ddd, $J=12.97$, 9.15, 5.19 Hz, C3-Hβ), 2.08 (1H, ddd, $J=12.97$, 7.32, 7.02 Hz, C3-Hα), 2.61—2.67 (1H, m, C2-Hβ), 4.68 (1H, ddq, $J=7.02$, 6.41, 5.19 Hz, C4-Hα). ^{13}C-NMR ($CDCl_3$) 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*: 143 $(M+1)^+$.

7b: IR (oil) cm^{-1}: 1771. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.95 (3H, t, $J=7.32$ Hz, C3'-H), 1.42 (3H, d, $J=6.10$ Hz, C5-H), 1.35—1.54 (4H, m, C3-Hα, C1'-H, and C2'-H), 1.76—1.93 (1H, m, C1'-H), 2.48 (1H, ddd, $J=11.60$, 8.55, 5.50 Hz, C3-Hβ), 2.56—2.68 (1H, m, C2-Hβ), 4.49 (1H, ddq, $J=10.38$, 6.10, 5.50 Hz, C4-Hβ). ^{13}C-NMR ($CDCl_3$) δ: 13.8 (C3'), 20.5 (C5), 20.9 (C2'), 32.4 (C1'), 36.9 (C3), 41.2 (C2), 75.0 (C4), 179.1 (C1). CI-MS *m/z*: 143 $(M+1)^+$.

(+)-(*S*)-4-[(Trityloxy)methyl]-4-butanolide (9) The alcohol-lactone (**8**) (9.75 g, 84 mmol), triphenylmethyl chloride (25.8 g, 92.4 mmol), DMAP (0.41 g, 3.36 mmol), and triethylamine (16.2 g, 159 mmol) were allowed to react overnight at room temperature under an N_2 atmosphere. The reaction mixture was poured into ice–water and extracted with $CHCl_3$. The organic layer was washed with saturated NH_4Cl and H_2O, 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). $[\alpha]_D^{21}$ +25.22° ($c=1.69$, EtOH). IR (KBr) cm^{-1}: 1763. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.94—2.36 (2H, m, C3-H), 2.48—2.72 (2H, m, C2-H), 3.14 (1H, dd, $J=10.55$, 4.39 Hz, C5-H), 3.43 (1H, dd, $J=10.55$, 3.52 Hz, C5-H), 4.58—4.71 (1H, m, C4-H), 7.23—7.50 (15H, m, C5-OPh$_3$). *Anal.* Calcd for $C_{24}H_{22}O_3$: C, 80.42; H, 6.19. Found: C, 80.45; H, 6.18. CI-MS *m/z*: 359 $(M+1)^+$.

(+)-(2*R*,4*S*)-2-Propyl-4-[(trityloxy)methyl]-4-butanolide (10) A solution of HMPA (2.78 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 NH_4Cl (30 ml). The mixture was extracted with ether (50 ml × 3). The organic solution was washed with H_2O, 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). $[\alpha]_D^{20}$ +21.57° ($c=1.69$, EtOH). IR (KBr) cm^{-1}: 1763. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.93 (3H, d, $J=6.32$ Hz, C3'-H), 1.26—1.49 (3H, m, C1'-H, C2'-H), 1.79—1.96 (2H, m, C3-H), 2.10—2.20 (1H, m, C1'-H), 2.73—2.84 (1H, m, C2-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 (1H, m, C4-H), 7.12—7.44 (15H, m, C5-OPh$_3$). *Anal.* Calcd for $C_{27}H_{28}O_3$: C, 80.96; H, 7.05. Found: C, 80.99; H, 7.04. CI-MS *m/z*: 401 $(M+1)^+$.

(+)-(2*R*,4*S*)-4-(Hydroxymethyl)-2-propyl-4-butanolide (11) Compound **10** (400 mg, 1.0 mmol) was hydrogenated in the presence of 10% Pd/C (60 mg) in concentrated HCl (1.5 ml) and EtOH (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. $[\alpha]_D^{20}$ +14.69° ($c=1.63$, EtOH). IR (oil) cm^{-1}: 3412, 1761. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.95 (3H, t, $J=6.70$ Hz, C3'-H), 1.35—1.52 (3H, m, C1'-H, C2'-H), 1.74—1.88 (2H, m, C3-H), 2.45—2.56 (1H, m, C1'-H), 2.67—2.79 (1H, m, C2-H), 3.61—3.67 (1H, m, C5-H), 3.82—3.92 (1H, m, C5-H), 4.57—4.64 (1H, m, C4-H). CI-MS *m/z*: 159 $(M+1)^+$.

(+)-(2*R*,4*S*)-4-(Iodomethyl)-2-propyl-4-butanolide (13) Thionyl 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 stirred for 1 h at 60 °C, then chloroform (200 ml) was added. The resulting chloroform solution was washed with H_2O (100 ml × 3) and saturated $NaHCO_3$ (20 ml), then dried and concentrated to give (+)-(2*R*,4*S*)-4-(bromomethyl)-2-propyl-4-butanolide (**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. $[\alpha]_D^{20}$ +2.81° ($c=1.61$, EtOH). IR (oil) cm^{-1}: 1770. ^{1}H-NMR (270 MHz, $CDCl_3$) δ: 0.96 (3H, t, $J=7.32$ Hz, C3'-H), 1.36—1.56 (3H, m, C1'-H, C2'-H), 1.74—1.89 (2H, m, C1'-H), 2.09—2.30 (2H, m, C3-H), 2.68—2.79 (1H, m, C2-H), 3.27 (1H, dd, $J=10.38$, 7.33 Hz, C5-H), 3.39 (1H, dd, $J=10.38$, 4.58 Hz, C5-H), 4.54—4.63 (1H, m, C4-H). ^{13}C-NMR ($CDCl_3$) δ: 7.4 (C5), 13.8 (C3'), 20.4 (C2'), 33.1 (C1'), 33.5 (C3), 39.2 (C2), 76.8 (C4), 178.6 (C1). CI-MS *m/z*: 268 $(M+1)^+$, 269 $(M+2)^+$.

(+)-(2*R*,4*R*)-4-Methyl-2-propyl-4-butanolide (7a) Tributyltin hydride (*n*-Bu_3SnH; 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. Triethylborane (Et_3B; 1.0 M hexane solution, 40 μl, 0.04 mmol) was added and the resulting mixture was stirred at 0 °C for 3 h. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (4 ml). Potassium fluoride (KF; 130 mg, 2.25 mmol) and H_2O (1 ml) were added to the above CH_2Cl_2 solution and the whole was vigorously stirred at room temperature for 5 h. The precipitated tributyltin fluoride was removed by filtration and washed with CH_2Cl_2. The combined filtrate was passed through a short column of anhydrous Na_2SO_4 (10 g). The eluate was concentrated and the residual oil was purified by silica gel column chromatography (AcOEt : hexane = 1 : 5, v/v as an eluant) to give 95 mg (91%) of (+)-**7a** as a colorless oil. $[\alpha]_D^{19}$ +8.35° ($c=1.68$, EtOH). IR (oil) cm^{-1}: 1770.

CI-MS m/z: 143 $(M+1)^+$.

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