# Application of chemical P-450 model systems to studies on drug metabolism. Part X.[1] Novel hydroxylactonization of γ,δ- and β,γ-unsaturated carboxylic acids with an iron porphyrin–iodosylbenzene system

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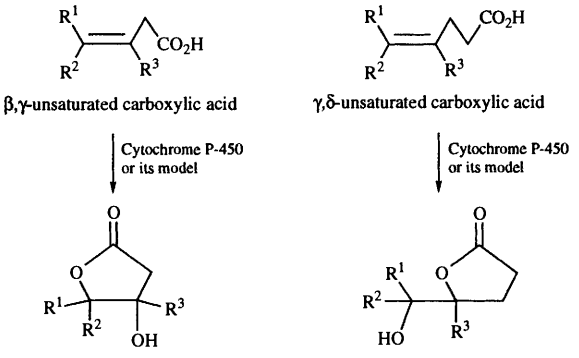
**The oxidative hydroxylactonization of γ,δ- and β,γ-unsaturated carboxylic acids by a chemical cytochrome P-450 model and rat liver microsomal systems has been investigated. In the chemical system using *meso*-tetrakis(2,6-dichlorophenyl)porphyrin iron chloride [Fe(TDClPP)Cl] with iodosylbenzene (PhIO), γ,δ-unsaturated carboxylic acids have been converted into δ-hydroxy-γ-lactones in high yield and with high stereoselectivity. As an example of a β,γ-unsaturated carboxylic acid, indomethacin has been converted into the corresponding β-hydroxy γ-lactone. Several experiments directed toward mechanistic elucidation of the lactonization exclude a mechanism occurring *via* an epoxide intermediate. The products have been used as standards to identify the metabolites in the microsomal oxidation. In the case of indomethacin, the γ-lactone form is detected as a metabolite in the rat liver microsomal system, in a yield of 1.33%; the yield is significantly decreased in the presence of 2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF-525A) and under a mixed CO–$O_2$ (4:1) atmosphere. Thus, these metabolites are considered to be formed by a cytochrome P-450-dependent reaction.**

Stereogenic and stereoselective multi-functionalization of unsaturated compounds is of great importance in the synthesis of target molecules having a number of asymmetric centres. Electrophilic heteroatom cyclizations have been powerful tools for the syntheses of complex molecules, including natural products, since two neighbouring chiral centres can be introduced selectively at the same time.[2] The introduced heteroatom groups such as halogeno, seleno and mercurio are then usually converted into other functional groups, for the preparation of the target. However, these conversions are not always successful in terms of yield or stereoselectivity. We have examined the application of chemical models of cytochrome P-450 to drug metabolism studies and organic synthesis for several years.[3] We have now found that γ,δ- and β,γ-unsaturated carboxylic acids afford δ-hydroxy-γ-lactones and β-hydroxy-γ-lactones, respectively, in one step with the iron porphyrin–iodosylbenzene (PhIO) system (Scheme 1). However, the sole example of a β,γ-unsaturated carboxylic acid was indomethacin. This type of lactonization has not previously been reported as far as we know. Further, we report here an apparently general metabolic reaction affording γ-lactone compounds from the corresponding γ,δ- and β,γ-unsaturated carboxylic acids, catalysed by cytochrome P-450.




**Scheme 1** Oxidative hydroxylactonization of γ,δ- and β,γ-unsaturated carboxylic acid

**Table 1** Hydroxylactonization of cyclohex-3-enecarboxylic acid by various chemical model systems

| Oxidizing system | Isolated yield (%) of **2a** |
|---|---|
| $Fe^{III}$(TPP)Cl–PhIO | ND |
| $Fe^{III}$(TDClPP)Cl–PhIO | 91 |
| $Mn^{III}$(TDClPP)Cl–PhIO | 68 |
| $Fe^{III}$(TDClPP)Cl–MCPBA | 44 |
| $Fe^{III}$(TDClPP)Cl–Pt colloid–$O_2$–$H_2$ | ND |
| $Fe^{III}$(TDClPP)Cl–AcOH–Zn–$O_2$ | ND |

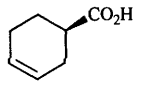
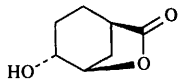
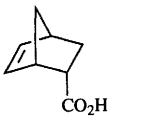
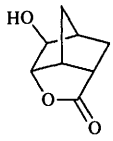
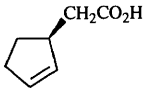
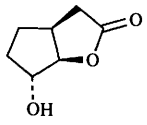
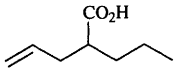
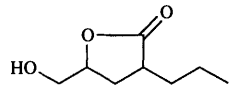
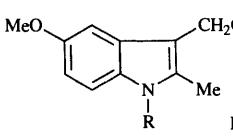
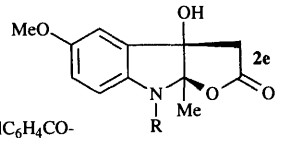
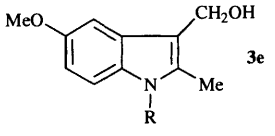
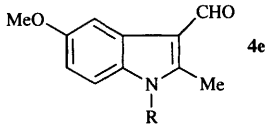
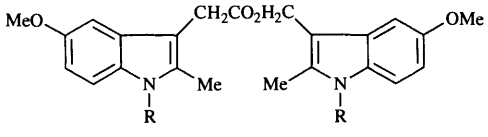
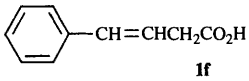
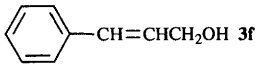
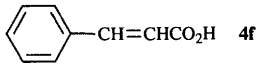
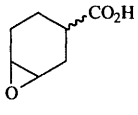
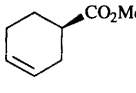
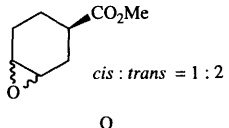
ND = not detected.

## Results

### Reactions with the chemical cytochrome P-450 model system

We have previously reported that the *meso*-tetrakis(pentafluorophenyl)porphyrin iron chloride [Fe(TPFPP)Cl]–PhIO system causes oxidative decarboxylation of carboxylic acids such as phenylacetic acid derivatives.[4] When Fe(TDClPP)Cl was used as a catalyst instead of Fe(TPFPP)Cl, indomethacin **1e** afforded not only decarboxylated products **3e**, **4e** and **5e**, but also an intramolecularly lactonized product **2e**.[5] Therefore, hydroxylactonization of cyclohexene-3-carboxylic acid by various metalloporphyrin–oxidant systems was examined (Table 1). Fe(TPP)Cl–PhIO and Fe(TDClPP)Cl–$O_2$–reductant systems[6] failed to oxidize **1a**. Since of those tested, the Fe(TDClPP)Cl–PhIO system was the most effective for the lactonization, we adopted it in this study. This novel hydroxylactonization also occurred in other γ,δ- and β,γ-unsaturated carboxylic acids exposed to the Fe(TDClPP)Cl–PhIO system (Table 2). γ,δ-Unsaturated carboxylic acids **1a–d** were efficiently oxidized to afford the corresponding δ-hydroxy-γ-lactone in good yield, by 2 equiv. of PhIO in the presence of Fe(TDClPP)Cl (0.5 mol %) at room temperature; the turnover number reached 180. In these reactions, no decarboxylation was observed. The introduction of the two O-functional groups onto the C=C double bond occurred in a specifically *trans* manner. On the other hand, β,γ-unsaturated

**Table 2** Hydroxylactonization by the Fe(TDClPP)Cl–PhIO system

| Substrate | Product | Yield (%)[a] |
|---|---|---|
|  **1a** |  **2a** | 91 |
|  **1b** |  **2b** | 92 |
|  **1c** |  **2c** | 67 |
|  **1d** |  **2d** | 56 |
|  **1e** |  **2e** | 17 |
| R: *p*-ClC$_6$H$_4$CO- | | |
| |  **3e** | 7 |
| |  **4e** | 6 |
|  **5e** | | 22 |
|  **1f** |  **3f** | 32 |
| |  **4f** | 11 |
|  **1g** | No reaction | |
|  **1h** |  *cis* : *trans* = 1 : 2 | |
| $Me(CH_2)_7CH{=}CH(CH_2)_7CO_2H$ **1i** | $Me(CH_2)_7CH(O)CH(CH_2)_7CO_2H$ **3i** | 73 |

These reactions were carried out in dichloromethane at room temperature for 16 h under an argon atmosphere. Substrate, 1.0 mmol; Fe(TDClPP)Cl, 0.005 mmol; PhIO, 2.0 mmol; dichloromethane, 5 ml.
[a] Yields are based on substrate used.

**Table 3** Amount of γ-lactone obtained in the rat liver microsome system with indomethacin

| System | Amount of γ-lactone formed (pmol mg$^{-1}$ protein 60 min$^{-1}$)* |
|---|---|
| Complete | 60.0 ± 3.5 (1.33%) |
| −NADPH | ND[a] |
| Under CO:$O_2$ (4:1) | 12.9 ± 2.1 (0.32%)[b] |
| +SKF-525A | 6.6 ± 0.6 (0.15%)[b] |
| No microsome | ND |

* The data represent the mean ± SE of three experiments. The data in parentheses are % yields based on used indomethacin. [a] ND = Not detected. [b] This value was significantly different from the value in the complete system ($p < 0.01$).

carboxylic acids such as styrylacetic acid were mainly decarboxylated oxidatively under the same conditions, as described in the previous paper,[4] while indomethacin gave some **2e**, together with other products. The sole β,γ-unsaturated carboxylic acid used was indomethacin which was converted into the corresponding β-hydroxy lactone. Oxidative decarboxylation usually occurs preferentially over hydroxylactonization in the case of β,γ-unsaturated carboxylic acids. Oxidation of oleic acid, which is a η,θ-unsaturated carboxylic acid, only gave the corresponding epoxide whilst 3,4-epoxycyclohexanecarboxylic acid was inert under these reaction conditions.

### Metabolites in rat liver microsomal systems

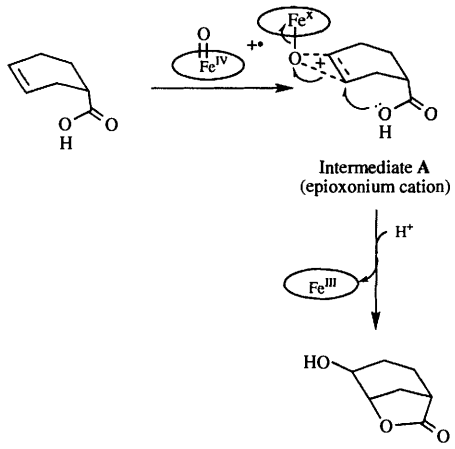
The metabolites in the liver microsomal systems, isolated and purified by TLC from the extract, were trimethylsilylated and their structures assigned by using GC–MS. The metabolites of cyclohex-3-enecarboxylic acid **1a**, bicyclo[2.2.1]hept-5-ene-2-carboxylic acid **1b**, cyclopent-2-enylacetic acid **1c**, 2-propylpent-4-enoic acid **1d** and indomethacin **1e** had mass spectral characteristics [retention time ($R_t$) of total ion chromatograms (TIC) and mass fragmentation patterns] similar to those of the authentic compounds. In the case of indomethacin, the spot on a 2-dimensional TLC plate of the γ-lactone **2e** was identified by comparison with an authentic sample. The amounts of γ-lactone **2e** produced by various microsomal systems are shown in Table 3. When indomethacin metabolism was carried out in the complete microsomal system, the amount of the γ-lactone formed was 60 pmol mg$^{-1}$ microsomal protein. In the absence of microsomes or NADPH, the γ-lactone was not detected. Incubation under an atmosphere of carbon monoxide and oxygen (80:20) resulted in *ca.* 80% inhibition of γ-lactone formation. Furthermore, the formation of γ-lactone was markedly inhibited by 2 mM SKF-525A (2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride), a specific inhibitor of cytochrome P-450.

## Discussion

Cyclohex-3-ene-1-carboxylic acid **1a** cyclized in the Fe(TDClPP)Cl–PhIO system to afford (1*S**,2*R**)-2-hydroxy-6-oxabicyclo[3.2.1]octan-6-one **2a** (91%). Compound **2a** is an important intermediate of FK-506, although it was obtained in only 30% yield by epoxidation of **1a** with MCPBA.[7] Substrate **1c** was converted into **2c** in which the relative configuration of the three neighbouring chiral centres was controlled. Each of compounds **1a**, **1b** and **1c** afforded a single stereoisomer **2a**, **2b** and **2c**. Thus, these cyclic substrates underwent stereospecific hydroxylactonization in moderate to high yield with the Fe(TDClPP)Cl–PhIO system. This reaction should be useful for stereo-controlled preparation of *trans-gem*-diols from γ,δ-unsaturated carboxylic acids.

One possibility for the mechanism of this new hydroxylactonization is that lactonized products are formed *via* the epoxides of the unsaturated carboxylic acids, although since 3,4-epoxycyclohexanecarboxylic acid **1g** was inert under these reaction

conditions, it being known to cyclize to afford **2a** only at high temperature,[7] this possibility is unlikely. Further, oxidation of the methyl ester of **1a** (**1h**) afforded its epoxides with low stereoselectivity (*cis*:*trans* = 1:2) which contrasts with and fails to explain the high yield and stereospecific conversion of **1a** into **2a**. In parallel with bromolactonization, for which formation of a three-membered bromonium cation intermediate has been proposed,[8] we propose that a three-membered epioxonium cation **A** is formed in the hydroxylactonization (see Scheme 2). A similar, but not identical, intermediate has




**Scheme 2** Proposed mechanism for oxidative hydroxylactonization in the iron porphyrin–iodosylbenzene system

been assumed to be formed by Ostovic and Bruice[9a] in their epoxidation catalysed by iron *meso*-tetrakis(2,6-dibromophenyl)porphyrin chloride. Traylor *et al.* also proposed a carbocation intermediate in the epoxidation of olefins in a metalloporphyrin-catalysed system.[9b] The high stereoselectivity in the reaction can be understood if the first step is a reversible step, and the second step proceeds irreversibly only when the 3-membered ring is *trans* to the carboxy group. Intermediate **A** is thought to be susceptible to nucleophilic attack by the carboxy group only when O=Fe$^{IV}$(Por)$^{+\bullet}$ or Ph–I$^{+}$–O–Fe$^{IV}$-(Por) attacks from the opposite side of the carboxy group, followed by the selective formation of **2a**. Fe(TDClPP)Cl was a much more effective catalyst for the hydroxylactonization than Fe(TPP)Cl, the former being resistant to the oxidative conditions, in contrast to the latter which readily decomposed. Therefore, the 2,6-dichlorophenyl groups are considered to contribute mainly to the protection of the porphyrin ring from an active species. In addition, it is also assumed that the electron-withdrawing substituents make the epioxonium intermediate more electrophilic, and promote the hydroxylactonization. The results obtained in the present study provide evidence for the formation of a cationic intermediate in the epoxidation of alkanes by high-valent oxo–metal porphyrin complex.

δ-Hydroxy-γ-lactone-type products were previously reported in the metabolism of γ,δ-unsaturated carboxylic acids (for example, 2-propylpent-4-enoic acid[10]) and they have been considered to be formed *via* the epoxide *in vivo*. However, it seems to be possible from the analogy with the results of the model system that epoxides are not intermediates in the formation of these metabolites.

It seems likely that oxidative γ-lactonization of γ,δ- and β,γ-unsaturated carboxylic acids generally occurs as a pathway of hepatic metabolism *in vivo*. In the absence of NADPH, the γ-lactone was not formed in the microsomal oxidation of indomethacin. This result indicates that the hydroxylactonization is NADPH-dependent. Carbon monoxide strongly inhibited γ-lactone formation. Flavin-monooxygenase is not responsible for this lactonization, since flavin-catalysed reactions are unaffected by CO. Formation of γ-lactone by rat microsomes was strongly inhibited by SKF-525A, a selective inhibitor of cytochrome P-450. Each of these results provides evidence for the involvement of cytochrome P-450 in the metabolism.

In conclusion, we have found that γ,δ-unsaturated carboxylic acids are efficiently cyclized to give δ-hydroxylated γ-butyrolactone derivatives upon the oxidation with PhIO catalysed by Fe(TDClPP)Cl. Indomethacin, which is a β,γ-unsaturated carboxylic acid, also lactonized in the same manner. This hydroxylactonization proceeded with high stereoselectivity (*trans* addition). This high selectivity requires the involvement of an intermediate other than a simple epoxide. The lactonization is thought to have potential for synthesis. Microsomes also lactonized these unsaturated carboxylic acids and this metabolism was cytochrome P-450-dependent.

## Experimental

### Materials and methods

[$^{14}$C]Indomethacin was obtained from NEN Research Products. The specific radioactivity was 3.91 MBq mg$^{-1}$ and the radiochemical purity was <98.2% by TLC. This compound was purified prior to use by HPLC. Unlabelled indomethacin was purchased from Sigma Company. SKF-525A was a generous gift from Smith Kline and Fujisawa. Cyclohex-3-ene-1-carboxylic acid, cyclopent-2-enylacetic acid and *trans*-styrylacetic acid were purchased from Aldrich Chemical Company Inc. Bicyclo[2.2.1]hept-5-ene-2-carboxylic acid was purchased from Lancaster. 2-Propylpent-4-enoic acid was synthesized according to Cregge *et al.*[11] *trans*-Cinnamic acid and cinnamyl alcohol were purchased from Kanto Chemical Co., Inc. *meso*-Tetrakis(2,6-dichlorophenyl)porphyrin iron chloride [Fe(TDClPP)Cl] was prepared according to the literature, and the UV–VIS spectrum was consistent with the literature values.[12] Iodosylbenzene (PhIO) was purchased from Tokyo Kasei Chemical Co. For TLC separation, Kieselgel 60F$_{254}$ (E. Merck, Darmstadt, F.R.G.), 0.25 mm thickness, was used. In the case of indomethacin metabolites, TLC plates were developed first with EtOAc–hexane (1:1, v/v), then with $CHCl_3$–$Me_2CO$–HOAc (10:2:0.1, v/v). A JEOL JMS-D300 mass spectrometer equipped with a JGC-20K gas chromatograph and a JMA-2000S data analysis system was used for the measurement of indomethacin metabolite. The glass column (2 mm diameter × 1 m) was packed with 3% silicone OV-61 on Gaschrom Q, 80–100 mesh (Gasukuro Kogyo, Tokyo, Japan). The injection port was heated at 280 °C and the column temperature was 250–300 °C. Helium was used as a carrier gas, with a column head pressure of 1.0 kg cm$^{-2}$. The ion source temperature, ionization potential and ionization current were set at 220 °C, 70 eV and 300 μA, respectively. A Hitachi M-2000 mass spectrometer equipped with a Hitachi G-3000 gas chromatograph was used to compare each substrate metabolite with an authentic sample. The column was a fused silica capillary, 20 m × 0.32 mm DB-1 (J&W Scientific, Rancho Cordova, CA), operated at 60–280 °C. Helium was used as a carrier gas, with a column head pressure of 0.5 kg cm$^{-2}$. The ionization potential and ionization current were set at 70 eV and 300 μA, respectively. Before the measurement, the authentic compound and metabolite were trimethylsilylated with *N*,*O*-bis(trimethylsilyl)acetamide–pyridine–trimethylchlorosilane (5:4:1, v/v). For measurement of radioactivity, a Beckman liquid scintillation counter (model 3801) was employed. Counting efficiency was determined by automatic external standardization. JEOL JNM-EX400 and GSX-400 (400 MHz) FT nuclear magnetic resonance spectrometers were used to measure $^{1}$H and $^{13}$C NMR spectra. The sample was dissolved in $CDCl_3$, and analysed with $Me_4Si$ as an internal standard. A Perkin-Elmer 204 elemental analyser was used for elemental analysis. IR spectra were recorded with a JASCO

DS-701G spectrophotometer. Melting points were determined on a Yanagimoto micromelting point apparatus, without correction.

**Reaction in the chemical model system**

**(A) Hydroxylactonization of cyclohex-3-enecarboxylic acid.** A mixture of Fe(TDClPP)Cl (5.3 mg, 5 μmol), cyclohex-3-enecarboxylic acid **1a** (252 mg, 1 mmol), iodosylbenzene (440 mg, 2 mmol) and $CH_2Cl_2$ (10 ml) was stirred for 20 h at room temperature under an argon atmosphere. Excess of PhIO was filtered off and the resulting mixture was separated by silica gel column chromatography to give 3,4-dihydroxycyclohexanecarboxylic acid-γ-lactone **2a**, 276 mg, 91%; mp 130–132 °C (from $CH_2Cl_2$–hexane); $\delta_H$(400 MHz, $CDCl_3$–$Me_4Si$) 1.53 (1 H, m), 1.75 (1 H, m), 2.08 (1 H, br s), 1.88 (1 H, m), 2.24 (1 H, m), 2.29 (1 H, dd, *J* 2.8, 18.4), 2.84 (1 H, dd, *J* 10.4, 18.8), 3.27 (1 H, m), 4.34 (1 H, m) and 4.74 (1 H, d, *J* 6.8); $\delta_C$ 22.6 (t), 27.2 (t), 31.1 (t), 38.4 (d), 65.0 (d), 79.1 (t) and 178.9 (s); $\nu_{max}$(KBr)/$cm^{-1}$ 3420 (OH), 2960, 2930, 2890 (CH) and 1755 (C=O); *m/z* 142 [$M^+$]; (Found: C, 59.39; H, 7.17. Calc. for $C_7H_{10}O_3$: C, 59.17; H, 7.04%); $R_t$ [GC–MS (trimethylsilylated derivative)] 7.02 min; *m/z* 214 [$M^+$], 199, 171, 158, 129, 116, 101 and 73.

**(B) Hydroxylactonization of bicyclo[2.2.1]hept-5-ene-2-carboxylic acid.** The reaction mixture with bicyclo[2.2.1]hept-5-ene-2-carboxylic acid **1b** was worked up according to the previously outlined procedure to give 5,6-dihydroxybicyclo[2.2.1]-octane-2-carboxylic acid-γ-lactone **2b** (92%); mp 157–159 °C (lit.,[13] 160 °C, recryst. from $CH_2Cl_2$–light petroleum); $\delta_H$(400 MHz, $CDCl_3$–$Me_4Si$) 1.59 (1 H, m), 1.64 (1 H, m), 1.98 (1 H, dd, *J* 6.8, 11.2), 2.02 (1 H, dd, *J* 4.4, 10.0), 2.14 (1 H, dd, *J* 1.6, 11.2), 2.41 (1 H, dd, *J* 1.2, 2.4), 2.52 (1 H, dd, *J* 4.4, 11.2), 3.17 (1 H, dd, *J* 1.6, 4.8), 3.76 (1 H, s) and 4.43 (1 H, d, *J* 4.8); $\delta_C$ 31.6 (t), 33.9 (t), 38.2 (d), 43.7 (d), 44.9 (d), 78.0 (d), 87.0 (d) and 180.0 (s); $\nu_{max}$(KBr)/$cm^{-1}$ 3320 (OH), 2970, 2920, 2880 (CH) and 1775 (C=O); *m/z* 154 [$M^+$]; $R_t$ [GC–MS (trimethylsilylated derivative)] 7.68 min; *m/z* 211 [$M^+ - 15$], 183, 129 and 73.

**(C) Hydroxylactonization of cyclopent-2-enylacetic acid.** The reaction mixture with cyclopent-2-enylacetic acid **1c** was worked up according to the previously outlined procedure, giving 2,3-dihydroxycyclopentaneacetic acid-γ-lactone **2c** as an oil (67%); $\delta_H$(400 MHz, $CDCl_3$–$Me_4Si$) 1.59 (1 H, m), 1.65 (1 H, m), 1.75 (1 H, m), 2.11 (1 H, m), 2.29 (1 H, d, *J* 11.6), 2.35 (1 H, br s), 2.50 (1 H, m), 3.89 (1 H, s), 4.55 (1 H, t, *J* 5.2) and 5.23 (1 H, d, *J* 3.9); $\delta_C$ 30.8 (t), 31.5 (t), 35.6 (t), 36.1 (d), 76.3 (d), 90.2 (d) and 177.6 (s); $\nu_{max}$(KBr)/$cm^{-1}$ 3400 (OH), 3040, 2960, 2880 (CH) and 1772 (C=O) [Found: *m/z* (HRMS) 142.0629. Calc. for $C_7H_{10}O_3$: 142.0630]; $R_t$ [GC–MS (trimethylsilylated derivative)] 7.15 min; *m/z* 199 [$M^+ - 15$], 181, 171, 155, 129, 101 and 73.

**(D) Hydroxylactonization of 2-propylpent-4-enoic acid.** The reaction mixture with 2-propylpent-4-enoic acid **1d** was worked up according to the previously outlined procedure to give 4,5-dihydroxy-2-propylpentanoic acid-γ-lactone **2d** as an oil (56%); diastereomeric ratio 81 : 19; $\delta_H$(400 MHz, $CDCl_3$–$Me_4Si$) 0.95 (3 H, t, *J* 7.2), 1.38–1.47 (3 H, m), 1.81 (1 H, m), 1.89 (1 H, m), 2.22 (1 H, br s), 2.33 (1 H, m), 2.66 (1 H, m), 3.63 (1 H, dd, *J* 5.6, 12.0), 3.92 (1 H, dd, *J* 3.2, 12.8), 4.50 (0.81 H, m) and 4.6 (0.19 H, m); $\delta_C$ 13.8 (q), 20.5 (t), 29.7 (t), 32.5 (t), 40.4 (d), 63.8 (t), 78.6 (d) and 178.6 (s); $\nu_{max}$(neat)/$cm^{-1}$ 3400 (OH) and 1765 (C=O) [Found (HRMS): 158.0890. Calc. for $C_8H_{14}O_3$: 158.0943]; $R_t$ [GC–MS (trimethylsilylated derivative)] 7.36 min; *m/z* 215 [$M^+ - 15$], 158, 129, 116, 103 and 73.

**(E) Hydroxylactonization of indomethacin.** The reaction mixture with indomethacin **1e** was worked up according to the previously outlined procedure, giving 8-(4-chlorobenzoyl)-2,3,8,8a-tetrahydro-3a-hydroxy-5-methoxy-8a-methyl-2-oxo-3a*H*-furo[2,3-*b*]indole **2e** (17%), *N*-(chlorobenzoyl)-3-hydroxymethyl-5-methoxyindole **3e** (7%), *N*-(chlorobenzoyl)-3-formyl-5-methoxyindole **4e** (5.6%) and the ester form **5e** (22%).[4]

Compound **2e**: mp 226–228 °C (decomp.) (from EtOH); $\delta_H$(400 MHz, $CDCl_3$–$Me_4Si$) 1.56 (3 H, s), 2.79 (1 H, br s), 3.12 (1 H, d, *J* 18.0), 3.19 (1 H, d, *J* 18.0), 3.84 (3 H, s), 6.91 (1 H, dd, *J* 2.6, 8.9), 7.01 (1 H, d, *J* 2.6), 7.42 (1 H, d, *J* 8.9), 7.44 (2 H, d, *J* 8.4) and 7.59 (2 H, d, *J* 8.4); $\nu_{max}$(KBr)/$cm^{-1}$ 3390 (OH), 1797 (C=O) and 1634 (C=O); *m/z* 373 [$M^+$] (Found: C, 60.99; H, 4.29; N, 3.70. Calc. for $C_{19}H_{16}NO_5Cl$: C, 61.05; H, 4.31; N, 3.75%); $R_t$ [GC–MS (trimethylsilylated derivative)] 9.45 min; *m/z* 445 [$M^+ - 15$], 139, 111 and 73. Results for compounds **3e**, **4e** and **5e** were given in an earlier report.[4]

**(F) Decarboxylation of *trans*-styrylacetic acid.** The reaction mixture with *trans*-styrylacetic acid **1f** was worked up according to the previously outlined procedure to give cinnamyl alcohol **3f** (32%) and cinnamic acid **4f** (17%).

**Preparation of microsomes**

Rats (7 weeks of age, *ca.* 220 g) received intraperitoneal injections of 60 mg $kg^{-1}$ sodium phenobarbital (Wako Pure Chemical Industries Co., Ltd) once daily for 3 days and then were starved overnight and killed by exsanguination from the abdominal aorta. The livers were removed immediately, and perfused with ice-cold 1.15% aq. KCl. Microsomes were prepared according to the method of Sato and Omura.[14] The protein concentration of microsomal preparation was determined by the method of Lowry *et al.*[15] with bovine serum albumin as a standard.

**Incubation conditions of microsomal systems and TLC autoradiograms**

The standard incubation mixture contained Cofactor-I (Oriental Yeast Co., Ltd.), 8.6 mM $MgCl_2$, 17.0 mM KCl, 2.6 mM G-6-P, 2.0 mM NADPH, 2.2 mM NADH, 42.1 mM $Na_2HPO_4$ and 10.3 mM $NaH_2PO_4$ (final concentrations), 20 units of glucose-6-phosphate dehydrogenase, 6 mg of microsomal protein and 100 mM phosphate buffer (pH 7.4). The final volume of each incubation mixture was 2 ml. The reaction was initiated by addition of 500 nmol of substrate ([$^{14}$C]-indomethacin; 27 nmol) in 50 μl of EtOH and run at 37 °C for 60 min. The non-NADPH incubation medium was prepared independently by the elimination of NADPH and NADH from the complete medium. For the inhibition study, SKF-525A was added directly to the system at a final concentration of 2 mM. The incubation mixture was immediately extracted with EtOAc. The organic solution was evaporated under reduced pressure and the resulting residue was dissolved in MeOH. The MeOH solution was spotted onto a TLC plate and subjected to 2-dimensional development. The plate was sprayed with Enhancer (NEN) and wrapped with Lumirrormembrane (6 μm, Mitsubishi Rayon) before being placed on X-ray film (Fuji Medical RX type) to obtain the autoradiogram. Each region having $^{14}$C-activity, visualized as a dark spot on the autoradiogram, was scraped from the plate, suspended in 1 ml of MeOH and mixed with 10 ml of scintillator (ACS-II, Amersham) for counting.

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