# Synthesis and Comparison of the Antiinflammatory Activity of Manoalide and Cacospongionolide B Analogues

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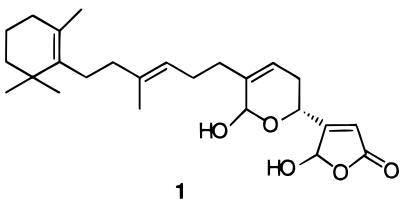
We have synthesized analogues of two naturally occurring antiinflammatory marine compounds, manoalide and cacospongionolide B, containing a pyranofuranone moiety which is considered the pharmacophoric group. The two compounds, and hence their analogues, differ in the presence or absence in the dihydropyran ring of an hemiacetal function which was considered essential to irreversibly inactivate phospholipase $A_2$ ($PLA_2$). The two series of compounds were tested for their inhibitory effects on secretory $PLA_2$ belonging to the groups I, II, and III, and the activities were found to be similar in both series, irrespective of the presence or absence of the additional hemiacetal function. In addition, the $PLA_2$ inhibitory activity increases with the increasing hydrophobic character of the side chain linked to the pyranofuranone moiety. The most active compounds, FCA and FMA, carry a farnesyl residue linked to the pyranofuranone substructure. The most potent $PLA_2$ inhibitor, FMA, was tested in the mouse carrageenan paw edema at the oral dose of 10 mg/kg and showed an activity similar to the reference antiinflammatory drug, indomethacin.

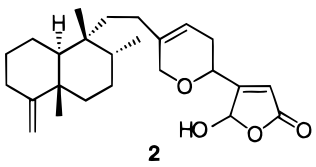
## Introduction

Marine invertebrates have proved to be a valuable source of antiinflammatory compounds which are potent inhibitors of phospholipase $A_2$ ($PLA_2$).[1]

Among these new antiinflammatory compounds, a class of biologically active sesterterpenes containing a pyranofuranone substructure has been isolated from soft sponges. These sesterterpenes can be grouped in two subclasses, exemplified by manoalide[2] (**1**) and cacospongionolide B[3] (**2**), differing from each other by the substitution in the dihydropyran ring.




**1**




**2**

Manoalide (**1**), which is by far the most studied compound, has been shown to irreversibly inhibit $PLA_2$ with the corresponding modification of a specific number of its lysine residues. Although the mechanism of inactivation has not been elucidated yet, differents models have been proposed mainly on the basis of structure−activity relationship studies.[4−10] All of the models propose that the rings of the pyranofuranone moiety open to generate α,β-unsaturated carbonyl compounds which could react in different ways with lysine residues of $PLA_2$. In particular, it was proposed that the aldehyde group arising from the hemiacetal function in the dihydropyran ring was essential to irreversibly inactivate $PLA_2$.[5,7,11,12]

On the other hand, cacospongionolide B (**2**), which lacks the hemiacetal function in the pyran ring, showed potent inhibitory activity on recombinant human synovial $PLA_2$ in vitro similar to that of manoalide, while a lower inhibitory activity was shown on other secretory enzymes such as bee venom $PLA_2$ and the enzyme present in exudates from zymosan-injected rat air pouch.[13,14]

We report here the synthesis of manoalide and cacospongionolide B analogues and the comparison of the $PLA_2$ inhibitory activity of both series in order to obtain additional information on structure−activity relationship, particularly (i) on the role of the hemiacetal function of the pyran ring and (ii) on the influence of the hydrophobic side chain in both series of compounds.

## Chemistry

We have recently reported[15] a new synthetic approach to pyranofuranones, which are considered the potentially pharmacophoric groups of these sesterterpenes. The procedure has been used for the synthesis of the methyl analogues of both manoalide and cacospongionolide B (MMA, **11a** and MCA, **12a**).

Since the synthetic sequence involves an alkylation step in which a side chain could be linked to the pyranofuranone moiety, the procedure is amenable to the synthesis of many analogues and to the synthesis

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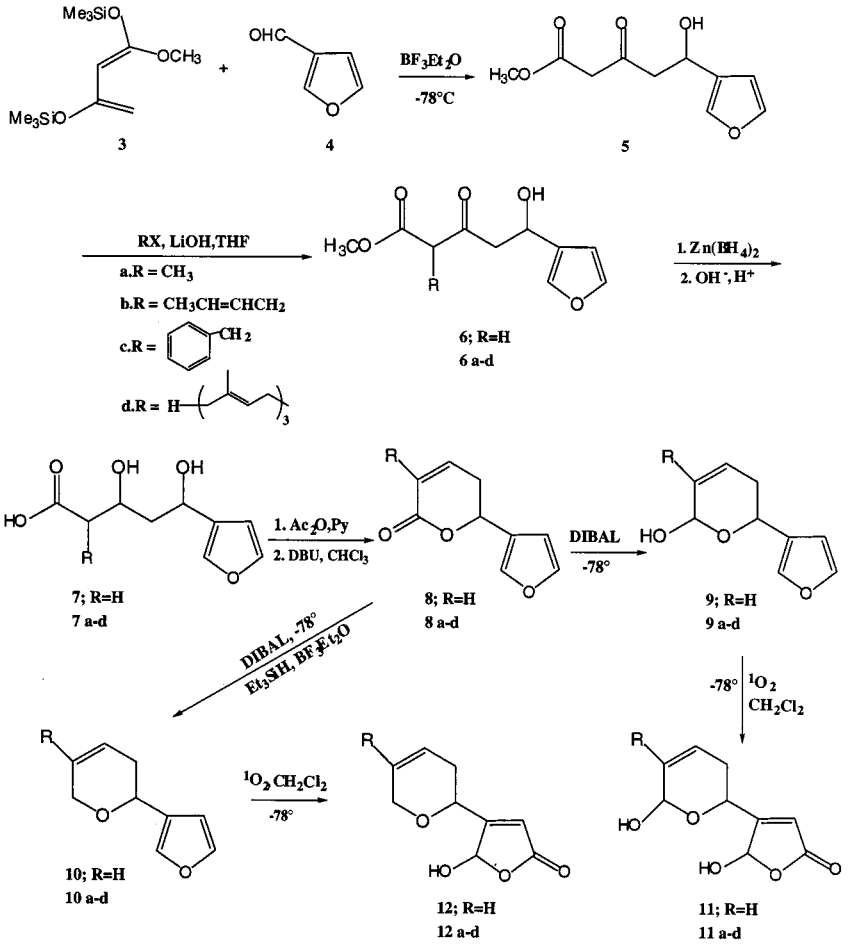
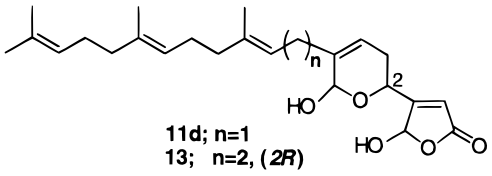
**Scheme 1**




of manoalide and cacospongionolide B. The synthetic sequence, for which we disclose here full experimental details, is reported in Scheme 1.

The analogues were prepared by following the previously outlined procedure,[15] varying the alkyl halide in the alkylation step of the intermediate **5** (Scheme 1). When **5** was subjected to the subsequent reaction sequence without any alkylation step, the unsubstituted analogues **11** (MA = manoalide analogue) and **12** (CA = cacospongionolide B analogue) were obtained in 8.5% and 5% total yields. Conversely, the manoalide analogues **11a**−**d** and the cacospongionolide B analogues




**12a**−**d** were obtained in a 9.7, 3.2, 5.0, and 2.1% and 15.4, 1.2, 3.0, and 1.4% total yields, respectively, by using methyl iodide, crotyl bromide, benzyl bromide, and farnesyl bromide in the alkylation step. The selection of the readily available alkyl halides was dictated by the consideration of their high reactivity coupled with the need of alkyl chains of variable hydrophobicity. In particular, farnesyl bromide was selected because it allowed the preparation of the farnesyl manoalide analogue (FMA, **11d**) which is the lower homologue of thorectolide (**13**),[16] a naturally occurring sesterterpene belonging to the manoalide class of compounds for which no biological data have been reported.

As far as the synthetic sequence is concerned, it should be noted that the synthetic steps between compounds **5** and **8** were generally performed without isolation of the intermediates **6** and **7**, except for the isolation of **6d** in the course of the synthesis of FMA (**11d**) and FCA (**12d**). In addition, compounds **9** and **9a**−**d** were unstable. In particular **9** largely decomposed even if stored at −20 °C overnight, and therefore, it was reacted in the subsequent step immediately after its preparation.

## Pharmacological Results

**A. Enzyme Inhibitory Activity.** The two series of compounds were tested for their inhibitory effects on secretory $PLA_2$ belonging to the groups I (*Naja naja* venom and porcine pancreatic enzymes), II (human synovial recombinant enzyme), and III (bee venom enzyme). The results are reported in Table 1. The farnesyl analogues FMA (**11d**) and FCA (**12d**) were the most effective compounds and inhibited preferentially bee venom and human synovial $PLA_2$. The $IC_{50}$ of FCA was in the $\mu$M range, similar to that of manoalide, whereas FMA showed a higher potency.

Figures 1 and 2 illustrate the influence of FCA and FMA on the velocity of the enzymatic reaction as a function of enzyme concentration for bee venom $PLA_2$. The results were similar with both compounds. The regression line for FCA- or FMA-treated samples was

**Table 1.** Effect of Compounds on Secretory $PLA_2$ Activities[a]

| | *N. naja* venom % I (10 $\mu$M) | pancreas % I (10 $\mu$M) | human synovial % I (10 $\mu$M) | human synovial $IC_{50}$($\mu$M) | bee venom % I (10 $\mu$M) | bee venom $IC_{50}$($\mu$M) |
|---|---|---|---|---|---|---|
| MA (**11**) | 5.2 ± 2.0 | 1.0 ± 1.0 | 18.9 ± 3.1[b] | ND[d] | 0.7 ± 0.4 | ND |
| CA (**12**) | 10.1 ± 3.0 | 0.2 ± 0.2 | 42.3 ± 2.2[c] | ND | 9.3 ± 2.6 | ND |
| MMA (**11a**) | 3.0 ± 1.5 | 6.8 ± 1.6 | 3.2 ± 1.9 | ND | 14.9 ± 2.8 | ND |
| MCA (**12a**) | 1.4 ± 0.7 | 5.6 ± 1.3 | 0.1 ± 0.0 | ND | 8.0 ± 2.2 | ND |
| CMA(**11b**) | 1.8 ± 1.7 | 15.1 ± 2.7 | 21.2 ± 5.7 | ND | 24.8 ± 5.8 | ND |
| CCA (**12b**) | 7.1 ± 3.2 | 17.6 ± 1.9 | 24.8 ± 4.5 | ND | 40.1 ± 7.3[c] | ND |
| BMA (**11c**) | 7.1 ± 1.3 | 16.1 ± 2.2 | 11.9 ± 3.7 | ND | 16.1 ± 7.5 | ND |
| BCA (**12c**) | 7.6 ± 2.7 | 17.4 ± 2.1 | 15.5 ± 1.4 | ND | 30.3 ± 3.6 | ND |
| FMA (**11d**) | 33.6 ± 2.2[c] | 39.8 ± 1.0[c] | 74.6 ± 0.9[c] | 0.9 | 84.2 ± 2.4[c] | 0.5 |
| FCA (**12d**) | 17.7 ± 0.7[b] | 30.0 ± 2.0[c] | 63.1 ± 1.0[c] | 4.2 | 71.8 ± 3.6[c] | 1.7 |
| manoalide | 17.0 ± 1.7[b] | 32.3 ± 2.7[c] | 93.2 ± 0.2[c] | 3.9 | 62.5 ± 3.8[c] | 7.5 |

[a] Means ± SEM ($n$ = 6). [b] $P < 0.05$. [c] $P < 0.01$. [d] ND = not determined for those compounds which do not reach 50% inhibition at 10 $\mu$M.

**Table 2.** Inhibition of Bee Venom $PLA_2$ by FCA, FMA, Manoalide, and Cacospongionolide B over a Range of Preincubation Times and Irreversibility of Inhibition[a,d]

| compound | preincubation time, min | % inhibition for postincubation treatment: buffer | % inhibition for postincubation treatment: hydroxylamine | % irreversibility of inhibition |
|---|---|---|---|---|
| FCA | 5 | 58.5 ± 1.5 | 50.1 ± 2.1 | 85.6[b] |
| FCA | 15 | 58.7 ± 0.5 | 51.1 ± 2.5 | 87.1 |
| FCA | 60 | 65.3 ± 0.3 | 65.1 ± 0.3 | 99.7 |
| cacospongionolide B | 5 | 95.4 ± 2.5 | 81.9 ± 1.8 | 85.8 |
| cacospongionolide B | 15 | 95.4 ± 2.5 | 89.4 ± 2.8 | 93.7 |
| cacospongionolide B | 60 | 98.4 ± 1.3 | 99.3 ± 0.4 | 100.0 |
| FMA | 5 | 80.8 ± 2.5 | 39.1 ± 2.2 | 48.4[c] |
| FMA | 15 | 80.9 ± 1.9 | 56.9 ± 1.7 | 70.3[c] |
| FMA | 60 | 75.2 ± 1.2 | 71.5 ± 1.3 | 95.1 |
| manoalide | 5 | 42.4 ± 1.3 | 21.0 ± 3.2 | 49.5[c] |
| manoalide | 15 | 44.2 ± 2.4 | 28.0 ± 0.9 | 63.3[c] |
| manoalide | 60 | 47.3 ± 2.0 | 43.1 ± 1.8 | 91.1 |

[a] Means ± SEM ($n$ = 6). [b] $P < 0.05$ [c] $P < 0.01$. [d] Final concentration of FCA, FMA, manoalide, and cacospongionolide B at 10, 5, 10, and 50 $\mu$M, respectively.

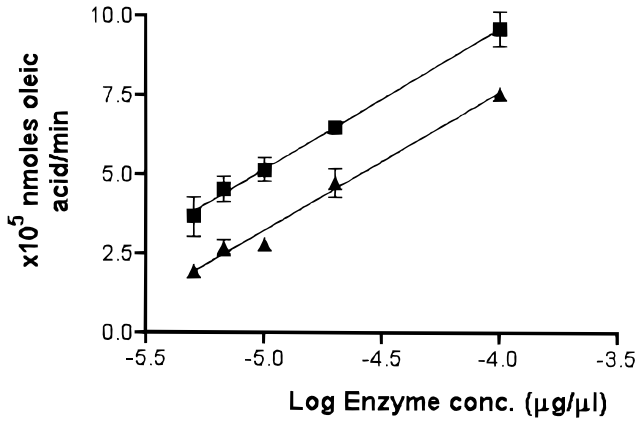



**Figure 1.** Effect of FCA (1 $\mu$M) on bee venom $PLA_2$ activity as a function of enzyme concentration. Results show means ± SEM for $n$ = 6: squares (enzyme control); triangles (enzyme + 1 $\mu$M FCA). Data for Figures 1 and 2 have been obtained using different pools of substrate.

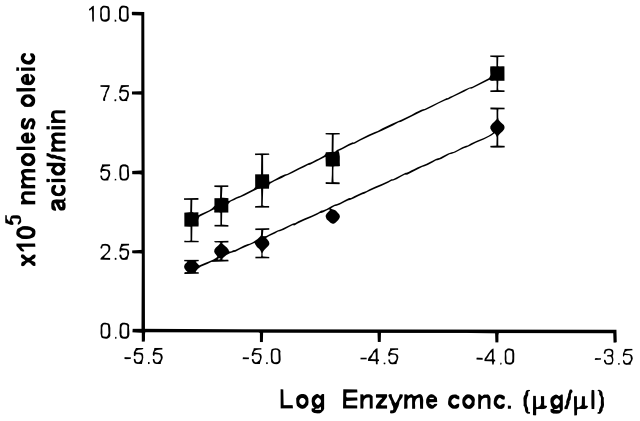



**Figure 2.** Effect of FMA (0.5 $\mu$M)) on bee venom $PLA_2$ activity as a function of enzyme concentration. Results show means ± SEM for $n$ = 6: Squares (enzyme control); circles (enzyme + 0.5 $\mu$M FMA).

shifted to the right of control values at a given velocity, without a significant difference in the slopes, which suggests an irreversible inhibition[17] as has been reported for manoalide.[18] Moreover, the reversibility of enzyme inhibition after treatment with hydroxylamine[9] was also tested. Table 2 shows the inhibition profile of bee venom $PLA_2$, preincubated with FCA and FMA and with manoalide and cacospongionolide B, after treatment with hydroxylamine. It can be observed that only in the early stages is there a partial recovery of enzyme activity, the reversibility being more effective for manoalide and its analogue FMA. On the other hand, this effect was not evidenced after 1 h of incubation of drug-treated enzyme solutions.

None of the compounds tested inhibited cytosolic $PLA_2$ activity from the human monocytic cell line U937 in a significant way (data not shown). Thus, the active compounds FCA and FMA showed a selectivity for secretory $PLA_2$.

**B. Antiinflammatory Activity.** The most potent $PLA_2$ inhibitor, FMA (**11d**), was tested in the mouse carrageenan paw edema at the oral dose of 10 mg/kg. An inhibitory effect on paw edema was observed 3 and 5 h after carrageenan administration, with percentages of inhibition of 42.9 ± 6.6 ($P < 0.01$) and 43.2 ± 7.5 ($P$

$< 0.01$), respectively. At the same dose, the reference antiinflammatory drug, indomethacin, inhibited edema by $40.0 \pm 5.8\%$ 3 h after carrageenan ($P < 0.01$) and by $42.3 \pm 4.1\%$ at the 5 h determination ($P < 0.01$).

## Discussion

Our results confirm that the pyranofuranone part interacts with $PLA_2$ enzymes and that the hydrophobic region of the molecule facilitates this interaction as previously noted.[8] Nevertheless, this moiety of the molecule can be either linear, as in FMA (**11d**) and FCA (**12d**), or include a cyclic part (manoalide and cacospongionolide B), but a certain degree of lipophilicity is necessary since analogues with a shorter lateral chain present a drastic reduction in activity.

Several reports on the structure−activity relationship of manoalide indicate that the most likely initial reaction between manoalide and $PLA_2$ is the formation of a Schiff base between a lysine residue and the aldehyde generated upon opening of the manoalide $\gamma$-lactone ring.[8−10] Hydroxylamine methodology[9] has been used to cleave Schiff bases, resulting in a partial recovery of the enzyme activity. In this respect, it should be noted that the presence of the second masked aldehyde group in the pyran ring was judged important for the complete inactivation of $PLA_2$ and for the irreversibility of the biological effect.[5,9] However, these conclusions were reached by comparing the bioactivity of manoalide with that of analogues in which the dihydropyran ring was opened, the nature of the interaction of the pyran ring with $PLA_2$ remaining undefined.[9] From our results (Table 2), it can be deduced that the recovery of enzyme activity after hydroxylamine treatment appears only in the early stages and is more prominent for those compounds which have two masked aldehyde groups such as manoalide and FMA. The differences observed within the values of the percentage of reversal inhibition with those previously reported for manoalide[9] can be strictly attributed to the experimental conditions used in the hydroxylamine methodology.

The comparison reported here (Table 1) of the activities of analogues having the same side chain and differing only in the presence or absence of the hemiacetal function in the dihydropyran ring shows that the hemiacetal function is not essential for $PLA_2$ inhibition nor for the irreversibilitry of enzyme inhibition. It may eventually increase the inhibitory potency as in the case of FCA and FMA. The comparison of the $PLA_2$ inhibitory profile of these analogues shows a certain selectivity for secretory $PLA_2$ enzymes belonging to groups II and III (CA, CCA, FMA and FCA). The interaction of the most active compounds (FCA and FMA) with the enzymatic protein appears to be irreversible.

Interestingly, the antiinflammatory activity of FMA after oral administration to mice is similar to that of indomethacin, suggesting that this class of compounds could be a model for the development of new antiinflammatory agents.

## Experimental Section

**Chemistry. General Procedures.** Optical rotations were measured at the sodium D line (589 nm) at room temperature with a JASCO DIP 1000 polarimeter. NMR spectra were recorded on a Bruker AM 250 (250.13 MHz for $^1H$ and 62.89 MHz for $^{13}C$) spectrometer. Chemicals shifts are given in ppm ($\delta$) scale; for the spectra in $CDCl_3$, the $CHCl_3$ signal was used as internal standard ($\delta$ 7.26 $^1H$, $\delta$ 77.0 $^{13}C$); for the spectra in $CD_3OD$, the MeOH signal was used as internal standard ($\delta$ 3.34 $^1H$, $\delta$ 49.0 $^{13}C$). $J$ values are given in Hz. Mass spectra were taken on a VG TRIO 2000 instrument. The molecular ion peak ($M^+$) is reported; when the molecular ion peak is undetectable, the first fragmentation ion peak is listed. All of the analogues reported here show an abundant $M^+ - 1$ ion. Column chromatographic separations were carried out using Silica gel 60 (70−230 mesh and 230−400 mesh, Merck). Hexane was dried by distillation from $P_2O_5$; *i*-$Pr_2NH$ from $CaH_2$; $Et_2O$, $CH_2Cl_2$, and toluene were freshly distilled from $CaH_2$ under an argon atmosphere from $CaH_2$. THF was distilled under argon and then from sodium benzophenone.

**Synthesis of Farnesyl Analogues FMA (11d) and FCA (12d). 1,3-Bis(trimethylsiloxy)-1-methoxybuta-1,3-diene (3).**[19] To a solution of diisopropylamine (0.9 mL, 6.39 mmol) in dry THF (22 mL) under argon was added at 0 °C 4.3 mL of *n*-BuLi (1.6 M in hexane). The reaction mixture was cooled to −78 °C, and methyl-3-trimethylsiloxybut-2-enoate (1.06 mL, 5.31 mmol) was added. The solution was stirred for 3 min and then quenched with trimethylchlorosilane (1.08 mL, 8.55 mmol). After 10 min, the solvent was removed under reduced pressure, and the residue was washed and filtered with cold dry hexane. The hexane was removed from the filtrate in vacuo to yield 1.24 g of **3** (90% yield).

$^1H$ NMR ($CDCl_3$) $\delta$ 4.48 (s, 1H), 4.15 (d, $J = 1.4$ Hz, 1H), 3.93 (d, $J = 1.4$ Hz, 1H), 3.55 (s, 3H), 0.21 (s, 9H), 0.23 (s, 9H).

**5-Furan-3-yl-5-hydroxy-3-oxo-pentanoic Acid Methyl Ester (5).** To a solution of **3** (2.5 g, 10 mmol) in 58 mL of dry $Et_2O$ under argon at −78 °C were added 3-furylaldehyde (1.44 g, 15 mmol) and $BF_3Et_2O$ (1.42 g, 10 mmol). The reaction mixture was stirred for 1 h in the dark, saturated $NaHCO_3$ was slowly added, and the mixture was allowed to warm to room temperature and extracted with $Et_2O$. The extract was dried ($MgSO_4$) and concentrated to give an oil, which was column chromatographed ($CHCl_3/Et_2O$, 96:4) to give **5** (1.7 g, 82%) as a pale yellow oil.

$^1H$ NMR ($CDCl_3$) $\delta$ 7.32 (s, 2H) and 6.33 (s, 1H) (furan protons), 5.07 (dd, $J = 3.9$, 8.3 Hz, 1H, H-5), 3.66 (s, 3H, $OCH_3$), 3.46 (s, 2H, H-2), 2.96 (dd, $J = 17.1$, 8.3 Hz, 1H, H-4a), 2.83 (dd, $J = 17.4$, 3.9 Hz, 1H, H-4b).

$^{13}C$ NMR ($CDCl_3$) $\delta$ 202.5, 167.2, 143.4, 139.0, 127.2, 108.3, 62.8, 52.5, 50.2, 49.5. Anal. C, H.

**2-(3-Furan-3-yl-3-hydroxy-propionyl)-5,9,13-trimethyl-tetradeca-4,8,12-trienoic Acid Methyl Ester (6d).** A mixture of **5** (0.71 g, 3.34 mmol), anhydrous THF (3 mL), and commercial LiOH monohydrate (0.14 g, 3.34 mmol) was stirred, in a round-bottom flask equipped with a reflux condenser, at about 55 °C for 15 min.[20] After the reaction mixture was cooled to 40 °C, farnesyl bromide (0.71 g, 5.01 mmol) was added, and the resulting mixture was stirred for 3 h at 40 °C. The reaction mixture was allowed to cool to room temperature, HCl 0.1 N was added until neutrality, and the mixture was extracted with $Et_2O$. The organic layer was dried over anhydrous $Na_2SO_4$ and evaporated under vacuum. Column chromatography ($CHCl_3/Et_2O$, 98:2) gave 529 mg of **6d** (70%) as a diastereomeric mixture ($^{13}C$ NMR analysis).

$^1H$ NMR ($CDCl_3$) $\delta$ 7.39 (s, 2H) and 6.37 (s, 1H) (furan protons), 5.14−5.02 (m, 4H, H-4, H-8, H-12, overlapped with the carbinol proton), 3.71 (s, 3H, $OCH_3$), 3.51 (t, $J = 7.4$ Hz, 1H, H-2), 2.97 (dd, $J = 8.8$, 15.7 Hz, 1H, H-2a propionyl moiety), 2.90 (dd, $J = 3.4$, 15.7 Hz, 1H, H-2b propionyl moiety), 2.5 (t, $J = 7.3$ Hz, 2H, H-3), 2.01 (m, 8H, H-6, H-7, H-10, H-11), 1.70 (s, 3H, $CH_3$), 1.65 (s, 3H, $CH_3$), 1.57 (s, 6H, $2 \times CH_3$).

$^{13}C$ NMR ($CDCl_3$) $\delta$ 205.2, 169.5, 143.3, 139.0, 138.9, 135.2, 131.3, 127.2, 124.2, 123.7, 119.2, 108.4, 62.9 and 62.8 (1:1, diastereomeric carbinol carbons), 59.3, 52.4, 49.4, 39.6, 26.8, 26.75, 26.67, 26.4, 25.6, 17.6, 16.1, 15.9. Anal. C, H.

**6-Furan-3-yl-3-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-5,6-dihydro-pyran-2-one (8d).** A solution of $Zn(BH_4)_2$ in $Et_2O$ (56 mL, 0.2 M) was added to a solution of **6d** (1.14 g, 2.7 mmol) in anhydrous $Et_2O$ (40 mL), under $N_2$ at 0 °C.[21] After

the reaction mixture was stirred for 1 h at 0 °C, water was added, and stirring was continued for 30 min. The mixture was acidified with 0.1 N HCl and extracted with $Et_2O$. The ethereal extract was dried ($Na_2SO_4$) and, after evaporation of the solvent, the reduction product was dissolved in 0.1 N NaOH (150 mL) and stirred for 3 h at 40 °C. The solution was then allowed to cool to room temperature and acidified with 2 N $H_2SO_4$. Extraction with $Et_2O$, drying of the ethereal extract over anhydrous $Na_2SO_4$, and evaporation of the solvent afforded the crude hydroxyacid **7d** (862 mg).

To a solution of **7d** (862 mg) in $CH_2Cl_2$ (3 mL) were added, acetic anhydride (1.1 g) and pyridine (1.7 mL), and the solution was stirred overnight at room temperature. The reaction mixture was then diluted with $Et_2O$ (10 mL), and 1 N HCl (10 mL) was added. The ethereal layer was washed with saturated $NaHCO_3$ and brine. Drying ($Na_2SO_4$) and evaporation gave crude acetylated lactone (759 mg) which was dissolved in $CHCl_3$ (5 mL), and DBU (three drops) was added. The solution was allowed to stand at room temperature for 2 h and then acidified with 2 N HCl and extracted with $Et_2O$. The ethereal layer was washed with $H_2O$ and dried ($Na_2SO_4$). The solvent was removed under vacuum, and the product was chromatographed ($SiO_2$, $CHCl_3$) to afford **8d** (248 mg, 0.674 mmol, 35.7% from **6d**).

$^1$H NMR ($CDCl_3$) $\delta$ 7.47, 7.40, and 6.44 (s, 1H each, furan protons), 6.57 (m, 1H, H-4), 5.36 (dd, $J$ = 4.9, 10.8 Hz, 1H, H-6), 5.20−5.05 (m, 3H, H-2, H-6, H-10 of dodecatrienyl moiety), 3.02 (bs, 2H, H-1 of dodecatrienyl moiety), 2.59 (m, 2H, H-5), 2.07 (m, 8H, H-4, H-5, H-8, H-9 of dodecatrienyl moiety), 1.66 (s, 3H, $CH_3$ ), 1.61(s, 3H, $CH_3$), 1.59 (s, 6H, 2 × $CH_3$). Anal. C, H.

**6-Furan-3-yl-3-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-5,6-dihydro-2H-pyran-2-ol (9d).** To a toluene solution (0.3 M) of unsaturated lactone **8d** (248 mg, 0.674 mmol) cooled to −78 °C was added DIBAL (1 M in $CH_2Cl_2$, 0.7 mL, 0.674 mmol) dropwise.[22] The solution was stirred until TLC analysis judged the reaction to be complete. The mixture was then poured into a rapidly stirred mixture of ice (2.2 g) and acetic acid (0.7 mL), $CHCl_3$ (5.0 mL) was added, and the mixture was stirred vigorously for 10 min. After that time, another portion (9 mL) of $CHCl_3$ was added, and vigorous stirring continued until two distinct layers formed when the stirring was stopped (40 min). The mixture was extracted with $CHCl_3$, and the organic layer was washed with bicarbonate and brine and dried over anhydrous $Na_2SO_4$. After the solvent was removed under reduced pressure, the residue was purified by column chromatography ($SiO_2$). Elution with $CHCl_3$ afforded 102.2 mg (0.28 mmol) of **9d** (41%).

$^1$H NMR ($CDCl_3$) $\delta$ 7.47, 7.40 and 6.44 (s, 1H each, furan protons), 5.70 (m, 1H, H-4), 5.29 (d, $J$ = 1.1 Hz, 1H, H-2), 5.22−5.10 (m, 3H, H-2, H-6, H-10 of dodecatrienyl moiety), 4.97 (dd, $J$ = 3.8, 10.9 Hz, 1H, H-6), 2.80 (d, $J$ = 7.2 Hz, 2H, H-1 of dodecatrienyl moiety), 2.10 (m, 2H, H-5), 2.06 (m, 8H, H-4, H-5, H-8, H-9 of dodecatrienyl moiety), 1.68 (s, 3H, $CH_3$), 1.64 (s, 3H, $CH_3$), 1.60 (s, 6H, 2 × $CH_3$).

$^{13}$C NMR ($CDCl_3$) $\delta$ 143.2, 139.4, 137.7, 136.4, 135.2, 131.3, 126.5, 124.4, 124.0, 122.0, 120.6, 108.9, 91.8, 61.8, 39.7, 31.6, 31.3, 26.7, 26.5, 25.7, 17.7, 16.0. Anal. C, H.

**2-Furan-3-yl-5-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-3,6-dihydro-2H-pyran (10d).** A dry methylene chloride (0.9 mL) solution of the crude lactol (47.2 mg, 0.14 mmol) and triethylsilane[22] (24.4 mg, 0.21 mmol) was cooled under argon at −78 °C, $BF_3Et_2O$ (18 mL, 0.15 mmol) was added, and the solution was stirred until TLC indicated the disappearance of lactol and then quenched by the addition of saturated $NaHCO_3$ (1 mL). The mixture was warmed to room temperature with vigorous stirring and was extracted with $Et_2O$. The organic layer was washed with saturated $NaHCO_3$ and brine. The combined organic layers were dried over $Na_2SO_4$, and the solvent was evaporated, affording an oil which was purified by chromatography (silica gel, petroleum ether/ether, 99:1) yielding 11 mg of **10d** (48% yield).

$^1$H NMR ($CDCl_3$) $\delta$ 7.41, 7.39, and 6.42 (s, 1H each, furan protons), 5.57 (m, 1H, H-4), 5.18−5.09 (m, 3H, H-2, H-6, H-10 of dodecatrienyl moiety), 4.50 (dd, $J$ = 4.1, 9.6 Hz, 1H, H-2), 4.19 (d, $J$ = 15.6 Hz, 1H, H-6a), 4.10 (d, $J$ = 15.6 Hz, 1H, H-6b), 2.63 (d, $J$ = 7.1 Hz, 2H, H-1 of dodecatrienyl moiety), 2.30 (m, 2H, H-3), 2.07 (m, 8H, H-4, H-5, H-8, H-9 of dodecatrienyl moiety), 1.68 (s, 3H, $CH_3$), 1.60 (s, 9H, 3 × $CH_3$).

$^{13}$C NMR ($CDCl_3$) $\delta$ 143.3, 139.5, 124.6, 124.3, 120.9, 117.8, 68.9, 68.5, 39.9, 31.8, 31.6, 27.0, 26.7, 25.9, 18.0, 16.2. Anal. C, H.

**5-Hydroxy-4-{6-hydroxy-5-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-3,6-dihydro-2H-pyran-2-yl}-5H-furan-2-one (FMA, 11d).** To a solution of **9d** (22 mg, 0.064 mmol) in dry $CH_2Cl_2$ (6 mL) was added *N*,*N*-diisopropylethylamine (49 $\mu$L), and oxygen gas was bubbled through the solution for 10 min.[23] Thereafter, polymer-bound rose bengal catalyst (3.3 mg, 15% by weight) was added. The solution was stirred at −78 °C under an atmosphere of oxygen and irradiated with a 500-W tungsten incandescent lamp for 6 h. The reaction mixture was allowed to warm to room temperature, the photosensitizer was removed by filtration, and the $CH_2Cl_2$ solution was washed with aqueous $NaH_2PO_4$ buffer (1 M, pH = 4.3). The organic extracts were dried over anhydrous $Na_2SO_4$, and the solvent was evaporated in vacuo. The reaction mixture was purified by chromatography (silica gel, $CHCl_3$) to obtain the $\gamma$-hydroxybutenolide FMA (**11d**, 9 mg, 38% yield).

$^1$H NMR ($CDCl_3$) $\delta$ 6.14 (s, 1H, H-5), 6.07 (s, 1H, H-3), 5.68 (m, 1H, H-4 of pyran ring), 5.29 (s, 1H, H-6 of pyran ring), 5.19−5.10 (m, 3H, H-2, H-6, H-10 of dodecatrienyl moiety), 4.87 (dd, $J$ = 4.1, 9.8 Hz, 1H, H-2 of pyran ring), 2.79 (d, $J$ = 7.1 Hz, 2H, H-1 of dodecatrienyl moiety), 2.25 (m, 2H, H-3 of pyran ring), 2.09−2.02 (m, 8H, H-4, H-5, H-8, H-9 of dodecatrienyl moiety), 1.70−1.61 (s, 12H, 4 × $CH_3$).

$^{13}$C NMR ($CDCl_3$) $\delta$ pyranofuranone moiety 170.2 (C-2 of $\gamma$-hydroxybutenolide ring), 168.4 (C-4 of $\gamma$-hydroxybutenolide ring), 137.7 (C-5 of pyran ring), 120.6 (C-4 of pyran ring), 116.9 (C-3 of $\gamma$-hydroxybutenolide ring), 97.9 (C-5 of $\gamma$-hydroxybutenolide ring), 91.3 (C-6 of pyran ring), 62.9 (C-2 of pyran ring), 29.1 (C-3 of pyran ring), farnesyl moiety 138.1, 135.1, 131.3, 124.3, 123.9, 119.9, 39.6, 31.0, 26.7, 26.5, 25.7, 18.1, 16.0. MS $m/z$ 384 ($M^+ - H_2O$). Anal. C, H.

**5-Hydroxy-4-{5-(3,7,11-trimethyl-dodeca-2,6,10-trienyl)-3,6-dihydro-2H-pyran-2-yl}-5H-furan-2-one (FCA, 12d).** To a solution of **10d** (11 mg, 0.034 mmol) in dry $CH_2Cl_2$ (2.7 mL) was added *N*,*N*-diisopropylethylamine (26 $\mu$L, 4.3 equiv), and oxygen gas was bubbled through the solution for 10 min.[23] Thereafter, polymer-bound rose bengal catalyst was added (1.6 mg, 15% by weight). The solution was stirred at −78 °C under an atmosphere of oxygen and irradiated with a 500-watt tungsten incandescent lamp for 6 h. The reaction mixture was allowed to warm to room temperature, the photosensitizer was removed by filtration, and the $CH_2Cl_2$ solution was washed with aqueous $NaH_2PO_4$ buffer (1 M, pH = 4.3). The organic extracts were dried over anhydrous $Na_2SO_4$, and the solvent was evaporated under a vacuum. The reaction mixture was purified by chromatography (silica gel, $CHCl_3$) to obtain the $\gamma$-hydroxybutenolide FCA (**12d** 6.3 mg, 52% yield).

$^1$H NMR ($CDCl_3$) $\delta$ 6.14 (s, 1H, H-5), 6.05 (s, 1H, H-3), 5.55 (m, 1H, H-4 of pyran ring), 5.11−5.09 (m, 3H, H-2, H-6, H-10 of dodecatrienyl moiety), 4.41 (bt, H-2 of pyran ring), 4.15 (m, 2H, H-6 of pyran ring), 2.63 (d, $J$ = 7.1 Hz, 2H, H-1 of dodecatrienyl moiety), 2.28 (m, 2H, H-3 of pyran ring), 2.08−1.97 (m, 8H, H-4, H-5, H-8, H-9 of dodecatrienyl moiety), 1.68−1.60 (s, 12H, 4 × $CH_3$).

$^{13}$C NMR ($CDCl_3$) $\delta$ pyranofuranone moiety 171.6 (C-2 of $\gamma$-hydroxybutenolide ring), 168.4 (C-4 of $\gamma$-hydroxybutenolide ring), 137.6 (C-5 of pyran ring), 120.2 (C-4 of pyran ring), 117.3 (C-3 of $\gamma$-hydroxybutenolide ring), 96.9 (C-5 of $\gamma$-hydroxybutenolide ring), 68.3 (C-6 of pyran ring), 62.9 (C-2 of pyran ring), 29.6 (C-3 of pyran ring), farnesyl moiety 135.2, 131.4, 124.3, 123.9, 120.2, 118.3, 39.6, 31.4, 26.7, 26.4, 25.7, 17.7, 16.0. MS $m/z$ 386 ($M^+$). Anal. C, H.

**Syntheses of the Other Analogues (MA, CA, CMA, CCA, BMA, BCA).** The syntheses of the other analogues have been performed as for FMA and FCA, giving rise to the following compounds.

**5-Hydroxy-4-(6-hydroxy-3,6-dihydro-2H-pyran-2-yl)-5H-furan-2-one (MA, 11):** $^1$H NMR ($CD_3OD$) $\delta$ 6.11 (s, 1H, H-5), 6.03−6.08 (m, 1H, H-4 of pyran ring), 5.99 (s, 1H, H-3), 5.82 (dd, 1H, $J$ = 2.1 Hz, 10.0 Hz, H-5 of pyran ring), 5.41 (d, 1H, $J$ = 2.5 Hz, H-6 of pyran ring), 4.87 (br m, 1H, H-2 of pyran ring), 2.23 (br m, 2H, H-3 of pyran ring).

$^{13}$C NMR ($CD_3OD$) $\delta$ 172.3 (C-2 of $\gamma$ -hydroxybutenolide ring), 170.8 (C-4 of $\gamma$ -hydroxybutenolide ring), 128.7 (C-4 of pyran ring), 126.8 (C-5 of pyran ring), 117.8 (C-3), 99.3 (C-5 of $\gamma$ -hydroxybutenolide ring), 96.5 (C-6 of pyran ring), 65.3 (C-2 of pyran ring), 30.0 (C-3 of pyran ring). MS $m/z$ 180 ($M^+ - H_2O$). Anal. C, H.

**5-Hydroxy-4-(3,6-dihydro-2H-pyran-2-yl)-5H-furan-2-one (CA, 12):** $^1$H NMR ($CDCl_3$) $\delta$ 6.17 (s, H-5), 6.06 (s, H-3), 5.75−5.84 (m, 2H, H-4 and H-5 of pyran ring), 4.42 (dd, $J$ = 5.0, 9.0 Hz H-2 of pyran ring), 4.28 (br s, 2H, H-6 of pyran ring), 2.23 (br m, H-3 of pyran ring).

$^{13}$C NMR $\delta$ ($CDCl_3$) $\delta$ 170.2 (C-2 of $\gamma$ -hydroxybutenolide ring), 168.3 (C-4 of $\gamma$ -hydroxybutenolide ring), 126.3 (C-4 of pyran ring), 122.8 (C-5 of pyran ring), 117.8 (C-3), 97.3 (C-5 of $\gamma$ -hydroxybutenolide ring), 69.3 (C-6 of pyran ring), 65.9 (C-2 of pyran ring), 29.7 (C-3 of pyran ring). MS $m/z$ 182 ($M^+$). Anal. C, H.

**4-(5-But-2-enyl-6-hydroxy-3,6-dihydro-2H-pyran-2-yl)-5-hydroxy-5H-furan-2-one (CMA, 11b):** $^1$H NMR ($CDCl_3$) $\delta$ 6.14 (s, 1H, H-5), 6.06 (s, 1H, H-3), 5.69 (m, 1H, H-4 of pyran ring), 5.50−5.40 (m, 2H, H-2, H-3 of butenyl moiety), 5.28 (s, 1H, H-6 of pyran ring), 4.85 (bd, H-2 of pyran ring), 2.76 (bs, 2H, H-1 of butenyl moiety), 2.26 (m, 2H, H-3 of pyran ring), 1.67 (d, $J$ = 6.0 Hz, 3H, $CH_3$ of butenyl moiety).

$^{13}$C NMR ($CDCl_3$) $\delta$ pyranofuranone moiety 171.6 (C-2 of $\gamma$-hydroxybutenolide ring), 168.4 (C-4 of $\gamma$-hydroxybutenolide ring), 136.7 (C-5 of pyran ring), 121.4 (C-4 of pyran ring), 117.5 (C-3 of $\gamma$-hydroxybutenolide ring), 98.0 (C-5 of $\gamma$-hydroxybutenolide ring), 91.1 (C-6 of pyran ring), 62.9 (C-2 of pyran ring), 30.0 (C-3 of pyran ring), side chain 127.9, 127.2, 36.2, 17.9. MS $m/z$ 251 ($M^+ - 1$). Anal. C, H.

**4-(5-But-2-enyl-3,6-dihydro-2H-pyran-2-yl)-5-hydroxy-5H-furan-2-one (CCA, 12b):** $^1$H NMR ($CDCl_3$) $\delta$ 6.14 (s, 1H, H-5), 6.05 (s, 1H, H-3), 5.57 (m, 1H, H-4 of pyran ring), 5.53−5.31 (m, 2H, H-2, H-3 of butenyl moiety), 4.42 (bs, 1H, H-2 of pyran ring), 4.15 (m, 2H, H-6 of pyran ring), 2.60 (d, $J$ = 6.0 Hz, 2H, H-1 of butenyl moiety), 2.28 (m, 2H, H-3 of pyran ring), 1.67 (d, $J$ = 6.0 Hz, 3H, $CH_3$ of butenyl moiety).

$^{13}$C NMR ($CDCl_3$) $\delta$ pyranofuranone moiety 171.6 (C-2 of $\gamma$-hydroxybutenolide ring), 168.4 (C-4 of $\gamma$-hydroxybutenolide ring), 136.7 (C-5 of pyran ring), 121.4 (C-4 of pyran ring), 117.5 (C-3 of $\gamma$-hydroxybutenolide ring), 98.0 (C-5 of $\gamma$-hydroxybutenolide ring), 68.1 (C-6 of pyran ring), 62.9 (C-2 of pyran ring), 30.3 (C-3 of pyran ring), side chain 127.9, 127.2, 36.2, 17.8. MS $m/z$ 236 ($M^+$). Anal. C, H.

**4-(5-Benzyl-6-hydroxy-3,6-dihydro-2H-pyran-2-yl)-5-hydroxy-5H-furan-2-one (BMA, 11c):** $^1$H NMR ($CDCl_3$) $\delta$ 7.31−7.16 (m, 5H, phenyl), 6.11 (s, 1H, H-5), 6.02 (s, 1H, H-3), 5.61 (m, 1H, H-4 of pyran ring), 5.18 (s, 1H, H-6 of pyran ring), 4.86 (bd, 1H, H-2 of pyran ring), 3.41 (ABq, 2H, $J$ = 15.5 Hz, $ArCH_2$), 2.24 (m, 2H, H-3 of pyran ring).

$^{13}$C NMR ($CDCl_3$) $\delta$ pyranofuranone moiety 171.6 (C-2 of $\gamma$-hydroxybutenolide ring), 168.4 (C-4 of $\gamma$-hydroxybutenolide ring), 137.1 (C-5 of pyran ring), 122.6 (C-4 of pyran ring), 117.5 (C-3 of $\gamma$-hydroxybutenolide ring), 98.1 (C-5 of $\gamma$-hydroxybutenolide ring), 90.7 (C-6 of pyran ring), 62.8 (C-2 of pyran ring), 28.9 (C-3 of pyran ring), side chain 138.2, 129.1, 128.5, 126.4, 38.1. MS $m/z$ 270 ($M^+ - H_2O$). Anal. C, H.

**4-(5-Benzyl-3,6-dihydro-2H-pyran-2-yl)-5-hydroxy-5H-furan-2-one (BCA, 12c):** $^1$H NMR ($CDCl_3$) $\delta$ 7.33−7.15 (m, 5H, phenyl), 6.15 (s, 1H, H-5), 6.03 (s, 1H, H-3), 5.58 (m, 1H, H-4 of pyran ring), 4.41 (bt, 1H, H-2 of pyran ring), 4.12 (ABq, 2H, $J$ = 11.8 Hz, H-6 of pyran ring), 3.26 (ABq, 2H, $J$ = 15.5 Hz, $ArCH_2$), 2.30 (m, 2H, H-3 of pyran ring).

$^{13}$C NMR ($CDCl_3$) $\delta$ pyranofuranone moiety 171.0 (C-2 of $\gamma$-hydroxybutenolide ring), 167.8 (C-4 of $\gamma$ -hydroxybutenolide ring), 136.6 (C-5 of pyran ring), 118.5 (C-4 of pyran ring), 117.6 (C-3 of $\gamma$ -hydroxybutenolide ring), 97.7 (C-5 of $\gamma$ -hydroxybutenolide ring), 69.4 (C-2 of pyran ring), 68.0 (C-6 of pyran ring), 29.1 (C-3 of pyran ring), side chain 138.2, 129.1, 128.5, 126.4, 39.7. MS $m/z$ 272 ($M^+$). Anal. C, H.

**Biological Assays. Assay of Secretory $PLA_2$.** This activity was assayed by using a modification of the method of Franson et al.[24] *N. naja* venom, bee venom, and porcine pancreatic and human recombinant synovial enzymes were diluted in 10 $\mu$L of 100 mM Tris-HCl, 1 mM $CaCl_2$ buffer, pH 7.5. Enzymes were preincubated at 37 °C for 5 min with 2.5 $\mu$L of test compound solution or its vehicle in a final volume of 250 $\mu$L. Incubation proceeded for 15 min in the presence of 10 $\mu$L of autoclaved oleate-labeled membranes and was terminated by addition of 100 $\mu$L ice-cold solution of 0.25% BSA in saline to a final concentration of 0.07% w/v. After centrifugation at 2500$g$ for 10 min at 4 °C, the radioactivity in the supernatants was determined by liquid scintillation counting. Results were expressed as percentages of inhibition, and $IC_{50}$ values were determined by plotting log concentration versus inhibition values in the range from 10 to 90% inhibition. Inhibition/reactivation studies with hydroxylamine hydrochloride[9] were performed by incubating bee venom $PLA_2$ at 41 °C with FCA, FMA, manoalide, and cacospongionolide B or with methanol (control) in Tris buffer (100 mM Tris-HCl, 1 mM $CaCl_2$ buffer, pH 7.5) over a range of different preincubation times, using those inhibitory concentrations of drugs which produce submaximal (90−95%) effect in order to appreciate recoveries. The control and drug-treated enzyme solutions were then diluted 2-fold with Tris buffer or with hydroxylamine hydrochloride in Tris buffer (final concentration of hydroxylamine 50 mM, pH 7.5). The solutions were incubated for 2 h at 41 °C, and then the $PLA_2$ activity was measured (final concentration of FCA, FMA, manoalide, and cacospongionolide B under assay conditions was 10, 5, 10, and 50 $\mu$M, respectively).

**Assay of Cytosolic $PLA_2$.** Cytosolic $PLA_2$ activity was measured as the release of radiolabeled arachidonic acid according to the method of Clark et al.[25] using cytosolic fractions of human monocytic U937 cells as the source of enzyme and 1-palmitoyl-2-[$^{14}$C] arachidonyl-*sn*-glycero-3-phosphocholine (57.0 mCi/mmol, $2 \times 10^6$ cpm) as the substrate. Test compounds were dissolved in methanol and added to the reaction mixture just before the addition of the enzyme solution. The final concentration of methanol in the reaction mixture was less than 1%, which showed no effect on the enzyme activity. The reaction was stopped after a 60 min incubation period at 37 °C by adding with 0.5 mL of isopropyl alcohol/heptane/0.5 M $H_2SO_4$ (10:5:1). Heptane (0.7 mL) and water (0.2 mL) were then added, and the solution was vigorously mixed for 15 s. The heptane phase was mixed with 100 mg of silica gel 60 (Merck, 70−230 mesh) and centrifuged, and the radioactivity in each supernatant was measured.[26]

**Carrageenan Edema**. Swelling was induced following a modification of the technique of Sugishita et al.[27] Female Swiss mice (20−25 g) were fasted for 12 h with free access to water. Drugs or vehicle (ethanol, Tween 80, distilled water: 5:5:90, v/v/v) was administered orally (0.5 mL) 1 h before injection of carrageenan (0.05 mL; 3% w/v in saline) into the subplantar area of the right hind paws of the animals in groups of six. The volumes of injected and contralateral paws were measured at 1, 3, and 5 h after induction of edema by using a plethysmometer (Ugo Basile, Comerio, Italy). The volume of edema was calculated for each animal as the difference between the carrageenan-injected and contralateral paw volume. The antiinflammatory effect was expressed as the percentage of inhibition compared with that of the control group.

**Statistical Analysis.** The level of statistical significance was determined by analysis of variance (ANOVA) followed by Dunnett's $t$-test for multiple comparisons.

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