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In Vitro Platelet-Activating Factor Receptor Binding Inhibitory Activity of Pinusolide Derivatives: A Structure–Activity Study

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Pinusolide, a labdane-type diterpene lactone isolated from *Biota orientalis*, was found to be a potent platelet-activating factor (PAF) receptor binding antagonist. To investigate the structure–activity relationship and find derivatives with improved pharmacological profiles, 17 pinusolide derivatives were prepared and tested for their ability to inhibit the PAF receptor binding. The results demonstrated that the carboxymethyl ester group at C-19, the integrity of the α,β -unsaturated butenolide ring, and the exocyclic olefinic function of pinusolide are all necessary for its maximum PAF receptor binding inhibitory activity. Among the derivatives, the 17-nor-8-oxo derivative **8** was found to be as potent as pinusolide. The results also suggested that several derivatives warrant further pharmaceutical and pharmacological studies due to their improved water solubility (**8** and **11**) and apparent lack of susceptibility to Michael-type nucleophilic addition (**13** and **18**).

Platelet-activating factor (PAF, 1-*O*-alkyl-2(*R*)-acetyl-glycerol-3-phosphorylcholine) is a potent phospholipid autacoid released by platelets, leukocytes, and other stimulated cells.¹ This factor exerts a wide range of biological activities such as platelet aggregation, bronchoconstriction, hypotension, and an increase in vascular permeability. Further, PAF has been reported to be involved in many pathological conditions including inflammation, allergy, anaphylaxis, endotoxin shock, and transplanted organ rejection.² It acts by specifically binding to a high-affinity G protein-coupled receptor, which was recently cloned.³ Therefore, PAF antagonists which block the specific binding to the receptor have been extensively sought, and a number of synthetic and natural antagonists with various chemical structures were found.^{1b,4} On the basis of these widely varying structural patterns of the PAF antagonists, a receptor model for PAF antagonist binding was postulated to be a multipolarized cylinder with a set of 'cache-oreilles' 10–12 Å apart and a hydrophobic pocket.⁵

In the course of the screening of novel PAF antagonists from medicinal plants, we have previously reported that pinusolide ((4*S*,5*R*,9*S*,10*R*)-8(17),13-labdadien-16,15-olid-19-oic acid methyl ester, **1**) possesses a potent PAF antagonistic activity.⁶ It is a labdane-type diterpenoid with an α,β -unsaturated butenolide ring. In this study, various derivatives of **1** were synthesized and tested for their ability to displace [³H]PAF-specific binding from rabbit washed platelets in order to study the structure–activity relationship.

Chemistry

A total of 18 pinusolide (**1**) derivatives were prepared for evaluation of their PAF receptor binding inhibitory activities through reduction (**3a,b**, **9**, **13**, **14**, **15**, **16**), oxidation (**3**, **6**, **7**, **8**, **10**, **11**, **12**, **17**), hydration (**5**), and methylation (**4**, **18**) of **1** or its derivatives. Pinusolidic acid (**2**) was isolated from the leaf extract of *Biota orientalis*.⁷

Reduction of **1** with [(C₂H₅)₂AlH₂]Na afforded the diol **3a** and triol **3b**.⁸ The major product, **3b**, was further oxidized by silver carbonate on Celite.⁹ In accordance with the literature,^{9a} two different lactones, **3** and **17**, were obtained with **17** being the major product (69%) resulting from the oxidation of the less hindered –C(15)–H₂OH group of **3b**. The –C(19)H₂OH group of **17** was further treated with Jones reagent and CH₂N₂ to yield **18**, which has a β -substituted lactone as compared to the α -substituted lactone in **1**.

Anti-Markovnikov hydration of the exocyclic double bond of **1** was effected by hydroboration with (CH₃)₂S·BH₃ followed by alkaline H₂O₂ oxidation to give **5**.¹⁰ The axial C-20 methyl group and bulky alkyl chain attached to C-9 impose a large degree of steric hindrance on the β -face of the C(8)–C(17) exocyclic double bond of **1**. The facts that hydroboration occurs with attack taking place from the less hindered face (α -face in the case of **1**) and that there are no other peaks around 2.5–4 ppm in ¹H NMR except a multiplet at 3.71 ppm strongly suggested that **5** contains an axial –CH₂OH, thus *S* configuration at C-8. Similarly, the configuration at C-8 of **6** and **7** was assumed to be *8R*, where oxidative attack by *m*-CPBA and OsO₄ occurred from the less hindered α -face.

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Extensive model studies by others indicated that equatorial substituents α to the flattened cyclic ketones such as **8** promote equatorial attack of hydride ("flattening rule") to give predominant formation of less stable axial alcohol.¹¹ On the basis of this and the chemical shift (δ 4.03) and coupling constant (bd, $J = 2$ Hz) of the H-8 proton, the configuration at C-8 of **9** was assigned to be *S*, where C-8 contains an equatorial proton and an axial hydroxyl group. On the other hand, since the Baeyer–Villiger reaction occurs with retention of stereochemistry at the migrating carbon center,¹² the configuration at C-9 of **10** was assigned to be *R*.

On treatment with HIO_3 in aqueous acetone, **1** was converted to **11**. On the basis of results obtained with model compounds, this reaction was proposed to proceed via the 8-hydroxy-17-iodo intermediate that would be formed by electrophilic attack of I^+ ion, presumably donated by HOI-like species generated in situ by reduction of acetone by HIO_3 . Antielimination of a water molecule from the axial hydroxyl group on C-8 and axial H-9 and subsequent hydrolysis of the allylic iodine would yield **11**. Detailed accounts of this novel reaction will be reported elsewhere.¹³ Subsequent oxidation with PDC furnished the aldehyde derivative **12**.¹⁴

When **1** was reduced using Co(II) and NaBH_4 , the trisubstituted double bond in the lactone ring was selectively reduced to give **13**, while the disubstituted exocyclic double bond remained unreacted.¹⁵ The observed selectivity is most likely due to the electronic effect of the electron-withdrawing carbonyl function.

Results and Discussion

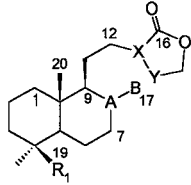
We have previously reported pinusolide (**1**) and pinusolidic acid (**2**) as PAF antagonists.^{6,7} In this study, various derivatives of **1** were prepared, and their PAF receptor binding inhibitory activities were measured using rabbit platelets as the receptor source and [^3H]-PAF as a ligand. CV 3988 (Takeda) was used as the reference compound throughout the study.¹⁷

IC_{50} values of derivatives were obtained, and the mean values of at least two independent measurements are shown in Table 1. The presence of a free acidic group (**2**) or a hydroxyl group (**3**) at C-19 significantly increased the IC_{50} values. This detrimental effect of a hydroxyl group at C-19 on the binding to the receptor was also observed with **17** ($\text{IC}_{50} = 110 \mu\text{M}$) as compared to **18** ($\text{IC}_{50} = 6.0 \mu\text{M}$). However, the binding affinity was much regained when the hydroxyl group was methylated as in **4** ($\text{IC}_{50} = 1.7 \mu\text{M}$).

Replacement of the α,β -unsaturated butenolide with a furan ring (**16**) (Table 2) resulted in a complete loss of the binding affinity, whereas β -substituted α,β -unsaturated butenolide derivative **18** was about 20 times less active than **1**, which has an α -substituted butenolide ring. The increased IC_{50} values of derivatives **3a**, **13**, and **15** indicated that the integrity of the butenolide structure is beneficial to the binding. However, since **13** and **15** are epimeric mixtures of unknown proportions at C-13, the exact potency of each epimer could not be determined.

Exocyclic olefinic bond, C(8)–17, in **1** was subjected to various reactions including hydration and oxidation (**5**–**12**). When the olefinic bond was oxidized to a glycol (**7**), it resulted in a complete loss in the binding affinity.

Table 1. Structure and in Vitro PAF Receptor Binding Inhibitory Activity of Pinusolide Derivatives







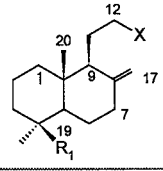





compd	X-Y	A-B	R ₁	IC ₅₀ (μM) (mean \pm S.D.)
CV 3988				7.8 \pm 1.0
1 (pinusolide)	>C=CH-	>C=CH ₂	-COOCH ₃	0.25 \pm 0.026
2 (pinusolidic acid)	>C=CH-	>C=CH ₂	-COOH	23 \pm 6.4
3	>C=CH-	>C=CH ₂	-CH ₂ OH	30 \pm 7.5
4	>C=CH-	>C=CH ₂	-CH ₂ OCH ₃	1.7 \pm 0.21
5	>C=CH-		-COOCH ₃	1.6 \pm 0.25
6	>C=CH-		-COOCH ₃	1.0 \pm 0.20
7	>C=CH-		-COOCH ₃	> 1000
8	>C=CH-	>C=O	-COOCH ₃	0.26 \pm 0.026
9	>C=CH-		-COOCH ₃	2.0 \pm 0.31
10	>C=CH-	-O-C=O	-COOCH ₃	8.0 \pm 1.5
11	>C=CH-	>C(9)=C(8)C(17)H ₂ OH	-COOCH ₃	4.0 \pm 0.81
12	>C=CH-	>C(9)=C(8)C(17)HO	-COOCH ₃	1.7 \pm 0.29
13	>CH-CH ₂ -	>C=CH ₂	-COOCH ₃	2.0 \pm 0.29
14	>CH-CH ₂ -	>CHCH ₃	-COOCH ₃	4.8 \pm 1.0
15	>CH-CH ₂ -	>C=O	-COOCH ₃	2.6 \pm 0.6

Table 2. Structure and in Vitro PAF Receptor Binding Inhibitory Activity of Pinusolide Derivatives Not Having the α -Substituted, 5-Membered Lactone Ring



compd	X	R ₁	IC ₅₀ (μM) (mean \pm S.D.)
16		-COOCH ₃	> 1000
3a		-COOCH ₃	12 \pm 3.1
3b		-CH ₂ OH	21 \pm 5.5
17		-CH ₂ OH	110 \pm 20
18		-COOCH ₃	6.0 \pm 1.7

Other modifications resulted in 4.0–32 times increases in the IC_{50} values, indicating the exocyclic olefinic function is preferable in binding to the PAF receptor. Interestingly, the ketonic derivative **8** showed a comparable inhibitory activity to the lead compound **1**.

A purpose of this study was to find derivatives of **1**

that might exhibit improved pharmaceutical/pharmacological profiles, that is, longer biological half-life and improved oral activity. In line with this, derivatives **13**, **15**, and **18** received our attention, since these derivatives should not be susceptible to Michael-type nucleophilic addition at C-14, whereas **1** might form the Michael-type adduct with nucleophilic biomolecules resulting in short duration of in vivo activity.^{6b} A detailed study in this regard is being investigated.

Another approach to this end was to find derivatives that are less hydrophobic and have comparable inhibitory activity. It turned out that derivatives **8** and **11** whose log *P*(octanol/water) values are 0.86 and 0.26, respectively, were less hydrophobic than **1** (log *P*(octanol/water) = 3.12).

In conclusion, the carboxymethyl ester group at C-19, the integrity of the α,β -unsaturated butenolide ring, and the exocyclic olefinic function of **1** are all necessary for its maximum PAF receptor binding inhibitory activity. On the other hand, the results revealed that derivatives **8**, **11**, **13**, and **18** warrant further pharmaceutical and pharmacological studies due to their improved water solubility and/or apparent lack of susceptibility to Michael-type addition. Furthermore, the results obtained in this study will provide useful information for the interaction between the PAF receptor and its ligands.

Experimental Section

15,16-Dihydroxy-8(17),13-labdadien-19-oic Acid Methyl Ester (3a) and 8(17),13-Labdadiene-15,16,19-triol (3b). To a solution of **1** (1 g, 2.9 mmol) in ether (150 mL) was added dropwise sodium diethyldihydroaluminum (4.3 mL, 8.7 mmol) at room temperature under N₂, and the resulting gel-like precipitate was stirred for 2 h. The reaction mixture was digested by addition of 1 N HCl (30 mL) on ice. Following ether extraction, the organic layer was washed with brine, dried over Na₂SO₄, and chromatographed over a silica gel column (CHCl₃-MeOH, 30:1) to give **3a** (*R*_f = 0.2, 150 mg) and **3b** (*R*_f = 0.05, 300 mg). **3a**: colorless oil; EIMS (*m/z*) 350 (M⁺); ¹H NMR (CDCl₃) δ 0.51 (3H, s, -C(20)H₃), 1.19 (3H, s, -C(18)H₃), 3.62 (3H, s, -OCH₃), 4.16 (2H, d-like, *J* = 2.1 Hz, -C(16)H₂OH), 4.21 (2H, d, *J* = 6.8 Hz, =CHC(15)H₂OH), 4.55, 4.85 (each 1H, s, =C(17)H₂), 5.61 (1H, t-like, *J* = 6.8 Hz, =C(14)HCH₂OH). Anal. (C₂₁H₃₄O₄) C, H. **3b**: mp 93–7 °C; EIMS (*m/z*) 322 (M⁺); ¹H NMR (CDCl₃) δ 0.65 (3H, s, -C(20)-H₃), 0.98 (3H, s, -C(18)H₃), 3.39, 3.75 (each 1H, d, *J* = 7.5 Hz, -C(19)H₂OH), 4.18 (2H, d, *J* = 2.1 Hz, -C(16)H₂OH), 4.21 (2H, d, *J* = 6.8 Hz, -C(15)H₂OH). Anal. (C₂₀H₃₄O₃) C, H.

19-Hydroxy-8(17),13-labdadien-16,15-olide (3) and 19-Hydroxy-8(17),13-labdadien-15,16-olide (17). Compound **3b** (0.1 g, 0.31 mmol) was oxidized by refluxing for 1 h with Ag₂CO₃ (0.85 g, 3.1 mmol) and Celite (0.4 g) in benzene (7 mL) under dark and N₂ atmosphere. The reaction mixture was filtered to remove black silver and silver carbonate. Filter cake was washed with ether, and the organic layer was washed with brine, dried over Na₂SO₄, and chromatographed over a silica gel column (CHCl₃-benzene-MeOH, 1:1:0.5) to give **3** (*R*_f = 0.2, 20 mg) and **17** (*R*_f = 0.1, 45 mg). **3**: needles, mp 99–102 °C; HREIMS *m/z* 318.2193 [Δ -0.2 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 0.66 (3H, s, -C(20)H₃), 0.98 (3H, s, -C(18)H₃), 3.40, 3.75 (each 1H, d, *J* = 11 Hz, -CH₂OH), 4.58, 4.86 (each 1H, s, =C(17)H₂), 4.78 (2H, m, =CHC(15)H₂O-), 7.10 (1H, s-like, =C(14)H). Anal. (C₂₀H₃₀O₃) C, H. **17**: white crystals, mp 162–4 °C; EIMS (*m/z*) 318 (M⁺), 300 (M⁺ - H₂O), 287 (M⁺ - CH₂-OH), 221 (M⁺ - C₅H₅O₂); ¹H NMR (CDCl₃) δ 3.39 (1H, dd, *J* = 10.8, 4.5 Hz, -C(19)HHOH), 3.74 (1H, dd, *J* = 10.8, 5.8 Hz, -C(19)HHOH), 4.45, 4.87 (each 1H, s, =C(17)H₂), 4.71–4.72 (2H, m, =C(16)H₂O-), 5.85 (1H, s-like, =C(14)H). Anal. (C₂₀H₃₀O₃) C, H.

19-Methoxy-8(17),13-labdadien-16,15-olide (4). To a solution of **3** (50 mg, 0.16 mmol) in ether (1 mL) were added (C₂H₅)₂O·BH₃ (0.1 mL) and CH₂N₂ in ether (excess) at 0 °C. After 1 h of stirring, aliquots of NH₄OH were added to the reaction mixture, and the mixture was partitioned to H₂O (30 mL) and ether (20 mL). The ether layer was washed with brine, dried over Na₂SO₄, and chromatographed over a silica gel column (hexane-CHCl₃-EtOAc, 4:10:0.1) to give **4** (*R*_f = 0.3, 10 mg): colorless crystal, mp 62–4 °C; EIMS (*m/z*) 332 (M⁺), 287 (M⁺ - CH₂OCH₃); ¹H NMR (CDCl₃) δ 3.09, 3.41 (each 1H, d, *J* = 9 Hz, -C(19)H₂OCH₃), 3.27 (3H, s, -C(19)H₂OCH₃), 4.57, 4.81 (each 1H, s, =C(17)H₂), 4.76–4.78 (2H, m, =CHC(15)H₂O-), 7.09 (1H, t, *J* = 2.0 Hz, =C(14)H). Anal. (C₂₁H₃₂O₃) C, H.

(8S)-17-Hydroxy-13-labden-16,15-olid-19-oic Acid Methyl Ester (5). To a stirred solution of **1** (30 mg, 0.087 mmol) in anhydrous THF (1 mL) was added (CH₃)₂S·BH₃ (2.6 mL of 2.0 M in THF) at 0 °C under N₂. After 2 h 30% H₂O₂ (10 mL) and 3 N NaOH (30 mL) were added to the reaction mixture, and the mixture was stirred for another 1 h at 50 °C. Following ether extraction, the organic layer was concentrated, and the residue was subjected to silica gel column chromatography (hexanes-EtOAc, 1:1) to give **5** (*R*_f = 0.2, 23 mg): needles, mp 88–90 °C; HREIMS *m/z* 346.2142 [Δ -0.2 mmu (M⁺ - H₂O)]; ¹H NMR (CDCl₃) δ 3.63 (3H, s, -OCH₃), 3.71 (2H, m, -C(17)H₂OH), 4.79–4.80 (2H, m, =CHC(15)H₂O-), 7.13 (1H, s-like, =C(14)H). Anal. (C₂₁H₃₂O₅) C, H.

(8R)-8,17-Epoxy-13-labden-16,15-olid-19-oic Acid Methyl Ester (6). A solution of **1** (0.15 g, 0.46 mmol) and *m*-CPBA (0.12 g, 0.92 mmol) in dry CH₂Cl₂ (3 mL) was stirred for 4 h under N₂. The reaction mixture was diluted with CH₂Cl₂ (20 mL) and saturated NaHCO₃ solution (10 mL). The organic layer was washed with brine, dried over Na₂SO₄, and chromatographed over a silica gel column (hexanes-EtOAc, 2:1) to give **6** (*R*_f = 0.2, 0.1 g): needles, mp 131–3 °C; HREIMS *m/z* 362.2092 [Δ -0.1 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 2.54 (1H, d, *J* = 4.2 Hz, -C(17)HH-O-), 2.72 (1H, dd, *J* = 1.8, 4.2 Hz, -C(17)HH-O-), 3.64 (3H, s, -OCH₃), 4.74–4.76 (2H, m, =CHC(15)H₂O-), 7.18 (1H, t, *J* = 1.6 Hz, =C(14)H). Anal. (C₂₁H₃₀O₅) C, H.

(8R)-8,17-Dihydroxy-13-labden-16,15-olid-19-oic Acid Methyl Ester (7). A solution of **1** (150 mg, 0.43 mmol) and OsO₄ (1.1 g, 0.43 mmol) in dioxane (11 mL) was stirred for 5 h under N₂. The reaction mixture was treated with saturated NaHSO₃, and the EtOAc extract was chromatographed over a silica gel column (CHCl₃-MeOH, 25:1) to give **7** (*R*_f = 0.3, 70 mg): needles, mp 121–2 °C; HREIMS *m/z* 362.2095 [Δ +0.2 mmu (M⁺ - H₂O)]; ¹H NMR (CDCl₃) δ 3.54 (1H, dd, *J* = 1.5, 11 Hz, -C(17)HHOH), 3.63 (3H, s, -OCH₃), 3.67 (1H, d, *J* = 11 Hz, -C(17)HHOH), 4.76–4.78 (2H, m, =CHC(15)H₂O-), 7.16 (1H, t, *J* = 1.8 Hz, =C(14)H). Anal. (C₂₁H₃₂O₆) C, H.

17-Nor-8-oxo-13-labden-16,15-olid-19-oic Acid Methyl Ester (8). To a stirred solution of **7** (0.14 g, 0.37 mmol) in MeOH (2.5 mL) was added NaIO₄ (0.2 g, 0.93 mmol) in 1 N H₂SO₄ (2 mL). After 2 h the reaction mixture was diluted with ether (15 mL), and the organic layer was washed with brine, dehydrated, and chromatographed over a silica gel column (CHCl₃-MeOH, 25:1) to give **8** (*R*_f = 0.3, 90 mg): needles, mp 131–2 °C; HREIMS *m/z* 348.1942 [Δ +0.5 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 3.62 (3H, s, -OCH₃), 4.76–4.77 (2H, m, =CHC(15)H₂O-), 7.14 (1H, t, *J* = 2.1 Hz, =C(14)H); ¹³C NMR (CDCl₃) δ 62.8 (C-9), 211.7 (C-8). Anal. (C₂₀H₂₈O₅) C, H.

(8S)-17-Nor-8-hydroxy-13-labden-16,15-olid-19-oic Acid Methyl Ester (9). To a stirred solution of **8** (30 mg, 0.086 mmol) in MeOH (2 mL) was added NaBH₄ (0.96 g, 25 mmol). After 15 h the reaction mixture was digested by addition of 1 N HCl (2 mL) and extracted with EtOAc. The EtOAc layer was washed with brine, dehydrated, and chromatographed over a silica gel column (CHCl₃-MeOH, 15:1) to give **9** (*R*_f = 0.3, 27 mg): rodlike crystals, mp 120–2 °C; HREIMS *m/z* 350.2092 [Δ -0.1 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 0.81 (3H, s, -C(20)H₃), 3.69 (3H, s, -OCH₃), 4.03 (1H, bd, *J* = 2.0 Hz, -C(8)H_{eq}OH), 4.76–4.79 (2H, m, =CHC(15)H₂O-), 7.14 (1H, t, *J* = 1.8 Hz, =C(14)H). Anal. (C₂₀H₃₀O₅) C, H.

(9R)-8,9-Epoxy-17-nor-8-oxo-13-labden-16,15-olid-19-oidic Acid Methyl Ester (10). To a stirred solution of **8** (52 mg, 0.15 mmol) and catalytic amounts of *p*-TsOH in CHCl₃ (1 mL) was added *m*-CPBA (37 mg, 0.30 mmol) in CHCl₃ (2 mL) under N₂. After 24 h at room temperature the reaction mixture was diluted with CHCl₃ (20 mL) and washed with saturated NaHCO₃. The organic layer was dehydrated and chromatographed over a silica gel column (hexanes–EtOAc, 1:1) to give **10** (*R*_f = 0.3, 50 mg): needles, mp 110 °C; HREIMS *m/z* 364.1890 [Δ +0.4 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 0.77 (3H, s, –C(20)H₃), 3.66 (3H, s, –OCH₃), 4.01 (1H, dd, *J* = 2.7, 11.4 Hz, –C(9)HO–), 4.79–4.80 (2H, m, =CHC(15)H₂O–), 7.19 (1H, t, *J* = 1.8 Hz, =C(14)H); ¹³C NMR (CDCl₃) δ 86.7 (C-9), 175.7 (C-8). Anal. (C₂₀H₂₈O₆) C, H.

17-Hydroxy-8,13-labdadien-16,15-olid-19-oidic Acid Methyl Ester (11). To a stirred solution of **1** (3 g, 8.6 mmol) in acetone (80 mL) was slowly added HIO₃ (1.52 g, 8.6 mmol) in H₂O (20 mL) at room temperature. After 3 h the reaction mixture was diluted with H₂O (150 mL). The ether extract was washed with brine, dried over Na₂SO₄, and chromatographed over a silica gel column (CHCl₃–MeOH, 100:1) to give **11** (*R*_f = 0.2, 2.5 g): white crystals, mp 134–6 °C; HREIMS *m/z* 362.2090 [Δ –0.3 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 0.84 (3H, s, –C(20)H₃), 3.67 (3H, s, –OCH₃), 4.11, 4.24 (each 1H, d, *J* = 11 Hz, –C(17)H₂OH), 4.83 (2H, m, =CHC(15)H₂O–), 7.19 (1H, s-like, =C(14)H); ¹³CMR (CDCl₃) δ 60.1 (C-17), 128.7 (C-9), 139.1 (C-8). Anal. (C₂₁H₃₀O₅) C, H.

17-Oxo-8,13-labdadien-16,15-olid-19-oidic Acid Methyl Ester (12). To a stirred solution of **11** (30 mg, 0.082 mmol) in CH₂Cl₂ (2 mL) was added PDC (31 mg, 0.082 mmol) at room temperature. After 1 h the reaction mixture was diluted with saturated Na₂SO₃ (2 mL). The CH₂Cl₂ extract was washed with brine, dried over Na₂SO₄, and chromatographed over a silica gel column (hexanes–EtOAc, 2:1) to give **12** (*R*_f = 0.3, 24 mg): white crystals, mp 141–3 °C; HREIMS *m/z* 360.1937 [Δ +0.0 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 0.91 (3H, s, –C(20)H₃), 3.65 (3H, s, –OCH₃), 4.80–4.81 (2H, m, =CHC(15)H₂O–), 7.19 (1H, s-like, =C(14)H), 10.15 (1H, s, –C(17)HO); ¹³CMR (CDCl₃) δ 130.1, 130.2 (C-8, C-13), 161.4 (C-9), 189.9 (C-17). Anal. (C₂₁H₂₈O₅) C, H.

8(17)-Labden-16,15-olid-19-oidic Acid Methyl Ester (13). To an ice-cold solution of **1** (20 mg, 0.057 mmol) and CoCl₂·6H₂O (14 mg, 0.059 mmol) in dry EtOH (2 mL) was added NaBH₄ (4.3 mg, 0.11 mmol). After 3 h the reaction mixture was treated with 3 N HCl (3 mL). The ether extract was concentrated, and the residue was chromatographed over a silica gel column (hexanes–EtOAc, 7:1) to give **13** (*R*_f = 0.2, 15 mg): C(13)-epimeric mixture; HREIMS *m/z* 348.2302 [Δ +0.2 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 2.45 (1H, m, –C(13)-HCOO–), 3.62 (3H, s, –OCH₃), 4.32 (2H, m, –CH₂C(15)H₂O–), 4.53, 4.88 (each 1H, s-like, =C(17)H₂); ¹³CMR (CDCl₃) δ 29.4 (29.6) (pair, C-14), 39.4 (C-13), 66.40 (66.45) (pair, C-15), 177.9 (C-19), 179.7 (C-16). Anal. (C₂₁H₃₂O₄) C, H.

Labdan-16,15-olid-19-oidic Acid Methyl Ester (14). **1** (0.35 g, 1 mmol) was catalytically hydrogenated by stirring 30 min in 3 mL of dry THF with 0.32 g of 10% Pd/C under H₂ gas. The reaction mixture was filtered and worked up to obtain **14** (0.35 g): needles, C(13)-epimeric mixture, mp 65–7 °C; HREIMS *m/z* 350.2457 [Δ +0.0 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 0.64 (3H, s, –C(17)H₃), 2.42 (1H, m, –C(13)HCOO–), 3.60 (3H, s, –OCH₃), 4.17, 4.33 (each 1H, m, –C(15)H₂O–); ¹³CMR (CDCl₃) δ 14.1, 14.7 (14.8) (C-20, C-17), 29.17 (29.21) (pair, C-14), 39.2 (C-13), 52.0, 53.0 (C-8, C-9), 66.4 (66.5) (pair, C-15), 178.2 (C-19), 179.6, (179.8) (pair, C-16). Anal. (C₂₁H₃₄O₄) C, H.

17-Nor-8-oxo-labdan-16,15-olid-19-oidic Acid Methyl Ester (15). To a solution of **8** (0.10 g, 0.29 mmol) in MeOH (2.5 mL) were added 10% Pd/C (0.1 g) and HCOONH₄ (0.18 g, 2.9 mmol), and the reaction mixture was stirred for 30 min at room temperature. The reaction product was partitioned to ether and chromatographed over a silica gel column (hexanes–EtOAc, 4:1) to give **15** (*R*_f = 0.2, 70 mg): needles, C(13)-epimeric mixture, mp 97–9 °C; HREIMS *m/z* 350.2095 [Δ +0.2 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 0.53 (3H, s, –CH₃), 2.42 (1H,

m, –C(13)HCOO–), 3.63 (3H, s, –OCH₃), 4.19, 4.34 (each 1H, m, –C(15)H₂O–); ¹³C NMR (CDCl₃) δ 30.5 (C-14), 63.2 (C-9), 66.6 (C-15), 177.3 (C-19), 179.8 (C-16), 211.6 (C-8). Anal. (C₂₀H₃₀O₅) C, H.

15,16-Epoxy-8(17),14,16-labdatrien-19-oidic Acid Methyl Ester (16). To a solution of **1** (17 mg, 0.049 mmol) in dry THF (2 mL) was added DIBALH (0.05 mmol) in hexane at –30 °C. After 5 h the reaction mixture was digested by addition of 1 mL of 10% H₂SO₄, and the EtOAc extract was chromatographed over a silica gel column (hexanes–EtOAc, 3:1) to give **16** (*R*_f = 0.2, 10 mg): solids; EIMS (*m/z*) 330 (M⁺), 271 (M⁺ – COOCH₃), 81 (C₅H₅O⁺); ¹H NMR (CDCl₃) δ 3.61 (3H, s, –OCH₃), 4.57, 4.89 (each 1H, s-like, =C(17)H₂), 6.25 (1H, d, *J* = 1.6 Hz, =C(14)H), 7.19 (1H, s, =C(16)HO–), 7.35 (1H, d, *J* = 1.6 Hz, =C(15)HO–). Anal. (C₂₁H₃₀O₃) C, H.

8(17),13-Labdadien-15,16-olid-19-oidic Acid Methyl Ester (18). To a solution of **17** (0.10 g, 0.31 mmol) in acetone (4 mL) was added Jones reagent (1.25 mmol) at room temperature. After 6 h the reaction was quenched by addition of saturated Na₂SO₃ solution (5 mL), and the ether extract was concentrated. The crude reaction product thus obtained was further treated with CH₂N₂ in ether for 1 h at 0 °C. Following addition of glacial acetic acid (0.1 mL) to stop the reaction, the ether extract was washed with brine, dried over Na₂SO₄, and chromatographed over a silica gel column (hexanes–EtOAc, 5:1) to give **18** (*R*_f = 0.3, 40 mg): colorless oil; HREIMS *m/z* 346.2143 [Δ –0.1 mmu (M⁺)]; ¹H NMR (CDCl₃) δ 3.62 (3H, s, –OCH₃), 4.46, 4.91 (each 1H, s, =C(17)H₂), 4.71–4.72 (2H, m, =C(16)H₂O–), 5.85 (1H, s-like, =C(14)H); ¹³C NMR (CDCl₃) δ 73.2 (C-16), 106.8 (C-17), 115.4 (C-14), 147.6 (C-8), 171.2 (C-13), 174.4 (C-15), 177.9 (C-19). Anal. (C₂₁H₃₀O₄) C, H.

In Vitro PAF Receptor Binding Assay. The PAF receptor binding assay using washed rabbit platelets and [³H]PAF was carried out according to the method of Valone with some modification.¹⁶ The reaction mixture consisted of 200 μL of washed rabbit platelet suspension (2 × 10⁸ cells/mL), 25 μL of [³H]PAF (0.6 nM, 60 000 dpm) with or without unlabeled PAF (500-fold excess over [³H]PAF), and 25 μL of sample in 2% DMSO. After 1-h incubation at room temperature, the free and bound ligands were separated by filtration using Whatman GF/C glass fiber filters. The difference between total radioactivities of bound [³H]PAF in the absence and presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. In a set of experiments, [³H]PAF was incubated with different concentrations of sample, and the inhibitory effect of the sample on the specific binding is expressed as percent inhibition of the control. The IC₅₀ value of a sample was defined as the final concentration of the sample required to block 50% of the specific [³H]PAF binding to rabbit platelet receptors, and the mean values obtained from at least duplicate determinations (*n* = 2–4) are given together with standard deviations.

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Supporting Information Available: IR, EIMS, and ¹³C NMR spectral data (3 pages). Ordering information is given on any current masthead page.

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Supporting Information

IR (KBr), EIMS (70 eV, relative intensity %) and ^{13}C MR spectra of compounds.

- 3a:** EIMS m/z 350 (M^+ , 0.84), 332 ($\text{M}^+ - \text{H}_2\text{O}$, 9.6), 235 (18.5), 189 (32.3), 161 (35.7), 121 (100).
- 3b:** EIMS m/z 322 (M^+ , 0.62), 304 ($\text{M}^+ - \text{H}_2\text{O}$, 9.2)
- 3:** IR 3420, 1752; EIMS m/z 318 (M^+ , 11.4), 300 ($\text{M}^+ - \text{H}_2\text{O}$, 18.9), 287 ($\text{M}^+ - \text{CH}_2\text{OH}$, 100), 217 ($\text{M}^+ - \text{H}_2\text{O} - \text{C}_4\text{H}_3\text{O}$, 30.5), 205 (45.2), 191 (22.6), 121 (40.5), 109 (40.2).
- 4:** EIMS m/z 332 (M^+ , 6.4), 300 ($\text{M}^+ - \text{CH}_3\text{OH}$, 47.7), 287 ($\text{M}^+ - \text{C}_2\text{H}_5\text{O}$, 100), 217 (36.6), 205 (64.5), 121 (74.2), 109 (84.5).
- 5:** IR 3520 ($-\text{OH}$), 1760, 1720; EIMS m/z 364 (M^+ , 1.0), 346 ($\text{M}^+ - \text{H}_2\text{O}$, 11.3), 334 (63.1), 286 (100), 121 (81.2), 109 (58.5).
- 6:** EIMS m/z , 362 (M^+ , 1.4), 302 ($\text{M}^+ - \text{HCOOCH}_3$, 3.4), 285 (11.1), 265 (77.1), 173 (47.1), 121 (100), 109 (34.3).
- 7:** IR 3544 ($-\text{OH}$), 1752, 1720; EIMS m/z 362 ($\text{M}^+ - \text{H}_2\text{O}$, 22.4), 349 ($\text{M}^+ - \text{CH}_2\text{OH}$, 29.1), 302 ($\text{M}^+ - \text{H}_2\text{O} - \text{HCOOCH}_3$, 4.9), 289 ($\text{M}^+ - \text{CH}_2\text{OH} - \text{HCOOCH}_3$, 9.7), 271 ($\text{M}^+ - \text{CH}_2\text{OH} - \text{H}_2\text{O} - \text{HCOOCH}_3$, 90.2), 121 (78.6), 109 (100); ^{13}C MR (CDCl_3) δ 13.2 (C-20), 18.9 (C-2), 21.3 (C-11), 22.7 (C-12), 28.3 (C-6), 28.5 (C-18), 37.6, 39.2, 39.6 (C-1, C-3, C-7, C-10), 43.7 (C-4), 51.2 (C-21), 56.3 (C-5), 59.3 (C-9), 62.5 (C-17), 70.3 (C-15), 74.9 (C-8), 135.1 (C-13), 144.6 (C-14), 174.8 (C-16), 177.2 (C-19).
- 8:** EIMS m/z 348 (M^+ , 4.1), 289 ($\text{M}^+ - \text{COOCH}_3$, 4.4), 273 (10.4), 251 (32.2), 223 (100), 173 (11.2), 121 (38.0), 109 (10.8); IR 1753, 1713; ^{13}C MR (CDCl_3) δ 13.2 (C-20), 19.7 (C-2), 20.2 (C-11), 25.0 (C-12), 25.7 (C-6), 28.8 (C-18), 38.0 (C-3),

- 39.6 (C-1), 43.1, 43.7, 44.4 (C-4, C-7, C-10), 51.5 (C-21), 55.0 (C-5), 62.8 (C-9), 70.3 (C-15), 134.6 (C-13), 144.7 (C-14), 174.7 (C-16), 177.4 (C-19), 211.7 (C-8).
- 9:** EIMS m/z 350 (M^+ , 4.5), 335 ($M^+ - CH_3$, 100), 275 ($M^+ - CH_3 - HCOOCH_3$, 45.0), 237 (37.2), 175 (21.5), 121 (75.2), 109 (68.7).
- 10:** IR 3522, 1753, 1716; EIMS m/z 364 (M^+ , 4.4), 267 ($M^+ - C_5H_5O_2$, 6.0), 224 (44.3), 207 ($M^+ - HCOOCH_3 - C_5H_5O_2$, 7.2), 196 (80.2), 164 (45.0), 121 (45.0), 109 (100); ^{13}C MR ($CDCl_3$) δ 11.6 (C-20), 19.2 (C-2), 21.3 (C-11), 22.6 (C-12), 27.1 (C-6), 28.9 (C-18), 34.4 (C-3), 36.3 (C-7), 38.0 (C-1), 40.8 (C-10), 45.0 (C-4), 51.6 (C-21), 59.3 (C-5), 70.3 (C-15), 86.7 (C-9), 133.6 (C-13), 145.9 (C-14), 174.6 (C-16), 175.7 (C-8), 177.1 (C-19).
- 11:** IR 3542 (sharp, intramolecular H-bonded allylic OH), 1761 (ester, C=O), 1694 (lactone, intramolecular H-bonded); EIMS m/z 362 (M^+ , 6.0), 344 ($M^+ - H_2O$, 1.5), 312 (4.5), 285 (15.5), 269 (24.8), 187 (36.5), 173 (100), 131 (23.1), 123 (39.0), 105 (39.9).
- 12:** IR 2748 (aldehyde CH), 1759, 1711 (aldehyde C=O), 1657; EIMS m/z 360 (M^+ , 39.5), 332 ($M^+ - C=O$, 5.1), 301 (22.8), 263 (58.8), 249 (100), 203 (29.7), 189 (85.6), 121 (28.7), 105 (55.3); ^{13}C MR ($CDCl_3$) δ 14.4 (C-20), 16.0 (C-2), 16.3 (C-11), 21.3 (C-12), 22.0 (C-6), 25.1 (C-18), 26.4 (C-3), 32.4 (C-7), 34.2 (C-1), 38.3, (C-10), 40.7 (C-4), 48.1 (C-21), 49.5 (C-5), 67.1 (C-15), 130.1, 130.2 (C-8, C-13) 141.9 (C-14), 161.4 (C-9), 170.8 (C-16), 174.6 (C-19), 189.8 (C-17).
- 13:** IR 1773, 1719; EIMS m/z 348 (M^+ , 20), 288 ($M^+ - HCOOCH_3$, 99), 273 (33), 181 (42.3), 149 (41.6), 121 (100), 109 (34.4); ^{13}C MR ($CDCl_3$) δ 12.4 (C-20), 19.8 (C-2), 21.3, 21.5 (C-11, C-12), 26.1, 28.5 (28.7) (C-6, C-18), 29.4 (29.6) (pair, C-14), 38.1 (C-3), 38.6 (C-7), 39.07 (39.13) (pair, C-1), 39.4 (C-13), 40.2 (40.3) (pair, C-

- 10), 44.2 (C-4), 51.1 (C-21), 55.7, 56.2 (56.3) (C-9, C-5), 66.40 (66.45) (pair, C-15), 106.5 (106.7) (pair, C-17), 147.8 (147.9) (pair, C-8), 177.9 (C-19), 179.7 (C-16).
- 14:** IR 1767, 1711; EIMS *m/z*, 350 (M^+ , 1.5), 318 (8.4), 290 (80.0), 123 (100), 109 (34.4)
- 15:** EIMS *m/z* 350 (M^+ , 78.1), 335 ($M^+ - CH_3$, 100), 275 ($M^+ - CH_3 - HCOOCH_3$, 76.0), 223 (99.1), 163 (47.3), 121 (89.2); ^{13}C MR ($CDCl_3$) δ 12.9 (C-20), 19.6 (C-2), 20.5 (C-11), 25.5, 28.67, 28.71, 30.5, 37.9, 39.5, 39.7, 43.0, 43.5, 44.3, 51.3 (C-21), 54.9 (C-5), 63.2 (C-9), 66.6 (C-15), 177.3 (C-19), 179.8 (C-16), 211.6 (C-8).
- 16:** EIMS (*m/z*) 330 (M^+ , 48), 271 ($M^+ - COOCH_3$, 14.7), 189 (38.5), 121 (100), 81 ($C_5H_5O^+$, 100);
- 17:** IR 3493, 1730; EIMS *m/z* 318 (M^+ , 15.2), 300 ($M^+ - H_2O$, 10.6), 287 ($M^+ - CH_2OH$, 31.4), 221 ($M^+ - C_2H_2O_2$, 42.1), 203 (42.2), 123 (80.0), 98 (100).
- 18:** EIMS *m/z* 346 (M^+ , 32.3), 314 (8.8), 286 (35.2), 249 ($M^+ - C_3H_5O_2$, 48.9), 189 (71.7), 121 (100), 98 (78.0); ^{13}C NMR ($CDCl_3$) δ 12.6 (C-20), 19.9 (C-2), 21.5 (C-11), 26.3 (C-12), 27.5 (C-6), 28.9 (C-18), 38.2 (C-3), 38.7 (C-7), 39.3 (C-1), 40.4 (C-10), 44.3 (C-4), 51.3 (C-21), 55.5 (C-9), 56.3 (C-5), 73.2 (C-16), 106.8 (C-17), 115.4 (C-14), 147.6 (C-8), 171.2 (C-13), 174.4 (C-15), 177.9 (C-19).