Notes

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-glyceryl-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 [^{3}H]PAF-specific binding from rabbit washed platelets in order to study the structure−activity relationship.

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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 [$(C_2H_5)_2AlH_2$]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)-H_2OH group of **3b**. The −C(19)H_2OH group of **17** was further treated with Jones reagent and CH_2N_2 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 $(CH_3)_2S{\cdot}BH_3$ followed by alkaline H_2O_2 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 ^{1}H NMR except a multiplet at 3.71 ppm strongly suggested that **5** contains an axial −CH_2OH, thus *S* configuration at C-8. Similarly, the configuration at C-8 of **6** and **7** was assumed to be 8*R*, where oxidative attack by *m*-CPBA and OsO_4 occurred from the less hindered α-face.

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Extensive model studies by others indicated that equitorial substituents α to the flattened cyclic ketones such as **8** promote equitorial 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 equitorial 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

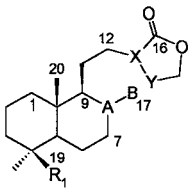
We have previously reported pinusolide (**1**) and pinusolidic acid (**2**) as PAF antagonists.[6,7] In this study, various derivatives of **1** were prepared, and their PAF receptor binding inhibitory activities were measured using rabbit platelets as the receptor source and [^{3}H]-PAF as a ligand. 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** ($IC_{50} = 110\ \mu M$) as compared to **18** ($IC_{50} = 6.0\ \mu M$). However, the binding affinity was much regained when the hydroxyl group was methylated as in **4** ($IC_{50} = 1.7\ \mu M$).

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



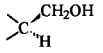
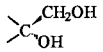
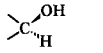
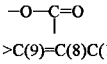
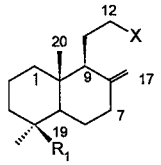
compd	X–Y	A–B	R_1	IC_{50} (μM) (mean ± S.D.)
CV 3988				7.8 ± 1.0
1 (pinusolide)	>C=CH–	>C=CH_2	–$COOCH_3$	0.25 ± 0.026
2 (pinusolidic acid)	>C=CH–	>C=CH_2	–COOH	23 ± 6.4
3	>C=CH–	>C=CH_2	–CH_2OH	30 ± 7.5
4	>C=CH–	>C=CH_2	–CH_2OCH_3	1.7 ± 0.21
5	>C=CH–		–$COOCH_3$	1.6 ± 0.25
6	>C=CH–		–$COOCH_3$	1.0 ± 0.20
7	>C=CH–		–$COOCH_3$	> 1000
8	>C=CH–	>C=O	–$COOCH_3$	0.26 ± 0.026
9	>C=CH–		–$COOCH_3$	2.0 ± 0.31
10	>C=CH–		–$COOCH_3$	8.0 ± 1.5
11	>C=CH–	>C(9)=C(8)C(17)H_2OH	–$COOCH_3$	4.0 ± 0.81
12	>C=CH–	>C(9)=C(8)C(17)HO	–$COOCH_3$	1.7 ± 0.29
13	>CH–CH_2–	>C=CH_2	–$COOCH_3$	2.0 ± 0.29
14	>CH–CH_2–	>CHCH_3	–$COOCH_3$	4.8 ± 1.0
15	>CH–CH_2–	>C=O	–$COOCH_3$	2.6 ± 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 ± S.D.)
16		–$COOCH_3$	> 1000
3a		–$COOCH_3$	12 ± 3.1
3b		–CH_2OH	21 ± 5.5
17		–CH_2OH	110 ± 20
18		–$COOCH_3$	6.0 ± 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 diethyldihydroaluminate (4.3 mL, 8.7 mmol) at room temperature under N_2, 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 Na_2SO_4, and chromatographed over a silica gel column ($CHCl_3$−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^+); ^{1}H NMR ($CDCl_3$) δ 0.51 (3H, s, −C(20)H_3), 1.19 (3H, s, −C(18)H_3), 3.62 (3H, s, −OCH_3), 4.16 (2H, d-like, J = 2.1 Hz, −C(16)H_2OH), 4.21 (2H, d, J = 6.8 Hz, =CHC(15)H_2OH), 4.55, 4.85 (each 1H, s, =C(17)H_2), 5.61 (1H, t-like, J = 6.8 Hz, =C(14)HCH$_2$OH). Anal. ($C_{21}H_{34}O_4$) C, H. **3b:** mp 93−7 °C; EIMS (*m*/*z*) 322 (M^+); ^{1}H NMR ($CDCl_3$) δ 0.65 (3H, s, −C(20)-H_3), 0.98 (3H, s, −C(18)H_3), 3.39, 3.75 (each 1H, d, J = 7.5 Hz, −C(19)H_2OH), 4.18 (2H, d, J = 2.1 Hz, −C(16)H_2OH), 4.21 (2H, d, J = 6.8 Hz, −C(15)H_2OH). Anal. ($C_{20}H_{34}O_3$) 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 Ag_2CO_3 (0.85 g, 3.1 mmol) and Celite (0.4 g) in benzene (7 mL) under dark and N_2 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 Na_2SO_4, and chromatographed over a silica gel column ($CHCl_3$−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^+)]; ^{1}H NMR ($CDCl_3$) δ 0.66 (3H, s, −C(20)H_3), 0.98 (3H, s, −C(18)H_3), 3.40, 3.75 (each 1H, d, J = 11 Hz, −CH_2OH), 4.58, 4.86 (each 1H, s, =C(17)H_2), 4.78 (2H, m, =CHC(15)H_2O-), 7.10 (1H, s-like, =C-(14)H-). Anal. ($C_{20}H_{30}O_3$) C, H. **17:** white crystals, mp 162−4 °C; EIMS (*m*/*z*) 318 (M^+), 300 ($M^+ - H_2O$), 287 ($M^+ - CH_2$-OH), 221 ($M^+ - C_5H_5O_2$); ^{1}H NMR ($CDCl_3$) 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)H_2), 4.71−4.72 (2H, m, =C(16)H_2O-), 5.85 (1H, s-like, =C(14)H-). Anal. ($C_{20}H_{30}O_3$) 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 $(C_2H_5)_2O{\cdot}BH_3$ (0.1 mL) and CH_2N_2 in ether (excess) at 0 °C. After 1 h of stirring, aliquots of NH_4OH were added to the reaction mixture, and the mixture was partitioned to H_2O (30 mL) and ether (20 mL). The ether layer was washed with brine, dried over Na_2SO_4, and chromatographed over a silica gel column (hexane−$CHCl_3$−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 ($M^+ - CH_2OCH_3$); ^{1}H NMR ($CDCl_3$) δ 3.09, 3.41 (each 1H, d, J = 9 Hz, −C(19)H_2OCH$_3$), 3.27 (3H, s, −C(19)H$_2$OCH_3), 4.57, 4.81 (each 1H, s, =C(17)H_2), 4.76−4.78 (2H, m, =CHC-(15)H_2O-), 7.09 (1H, t, J = 2.0 Hz, =C(14)H-). Anal. ($C_{21}H_{32}O_3$) C, H.

(8*S*)-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 $(CH_3)_2S{\cdot}BH_3$ (2.6 mL of 2.0 M in THF) at 0 °C under N_2. After 2 h 30% H_2O_2 (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 ($M^+ - H_2O$)]; ^{1}H NMR ($CDCl_3$) δ 3.63 (3H, s, −OCH_3), 3.71 (2H, m, −C(17)H_2OH), 4.79−4.80 (2H, m, =CHC(15)H_2O-), 7.13 (1H, s-like, =C(14)H-). Anal. ($C_{21}H_{32}O_5$) C, H.

(8*R*)-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 CH_2Cl_2 (3 mL) was stirred for 4 h under N_2. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and saturated $NaHCO_3$ solution (10 mL). The organic layer was washed with brine, dried over Na_2SO_4, 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^+)]; ^{1}H NMR ($CDCl_3$) 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, −OCH_3), 4.74−4.76 (2H, m, =CHC(15)H_2O-), 7.18 (1H, t, J = 1.6 Hz, =C(14)H-). Anal. ($C_{21}H_{30}O_5$) C, H.

(8*R*)-8,17-Dihydroxy-13-labden-16,15-olid-19-oic Acid Methyl Ester (7). A solution of **1** (150 mg, 0.43 mmol) and OsO_4 (1.1 g, 0.43 mmol) in dioxane (11 mL) was stirred for 5 h under N_2. The reaction mixture was treated with saturated $NaHSO_3$, and the EtOAc extract was chromatographed over a silica gel column ($CHCl_3$−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 ($M^+ - H_2O$)]; ^{1}H NMR ($CDCl_3$) δ 3.54 (1H, dd, J = 1.5, 11 Hz, −C(17)HHOH), 3.63 (3H, s, −OCH_3), 3.67 (1H, d, J = 11 Hz, −C(17)HHOH), 4.76−4.78 (2H, m, =CHC(15)H_2O-), 7.16 (1H, t, J = 1.8 Hz, =C(14)H-). Anal. ($C_{21}H_{32}O_6$) 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 $NaIO_4$ (0.2 g, 0.93 mmol) in 1 N H_2SO_4 (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 ($CHCl_3$−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^+)]; ^{1}H NMR ($CDCl_3$) δ 3.62 (3H, s, −OCH_3), 4.76−4.77 (2H, m, =CHC(15)-H_2O-), 7.14 (1H, t, J = 2.1 Hz, =C(14)H-); ^{13}C NMR ($CDCl_3$) δ 62.8 (C-9), 211.7 (C-8). Anal. ($C_{20}H_{28}O_5$) C, H.

(8*S*)-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 $NaBH_4$ (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 ($CHCl_3$−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^+)]; ^{1}H NMR ($CDCl_3$) δ 0.81 (3H, s, −C(20)H_3), 3.69 (3H, s, −OCH_3), 4.03 (1H, bd, J = 2.0 Hz, −C(8)H_{eq}OH), 4.76−4.79 (2H, m, =CHC(15)H_2O-), 7.14 (1H, t, J = 1.8 Hz, =C(14)H-). Anal. ($C_{20}H_{30}O_5$) C, H.

(9*R*)-8,9-Epoxy-17-nor-8-oxo-13-labden-16,15-olid-19-oic Acid Methyl Ester (10). To a stirred solution of **8** (52 mg, 0.15 mmol) and catalytic amounts of *p*-TsOH in $CHCl_3$ (1 mL) was added *m*-CPBA (37 mg, 0.30 mmol) in $CHCl_3$ (2 mL) under N_2. After 24 h at room temperature the reaction mixture was diluted with $CHCl_3$ (20 mL) and washed with saturated $NaHCO_3$. 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^+)]; ^{1}H NMR ($CDCl_3$) δ 0.77 (3H, s, −C(20)H_3), 3.66 (3H, s, −OCH_3), 4.01 (1H, dd, *J* = 2.7, 11.4 Hz, −C(9)*H*O-), 4.79−4.80 (2H, m, =CHC(15)H_2O-), 7.19 (1H, t, *J* = 1.8 Hz, =C(14)*H*-); ^{13}C NMR ($CDCl_3$) δ 86.7 (C-9), 175.7 (C-8). Anal. ($C_{20}H_{28}O_6$) C, H.

17-Hydroxy-8,13-labdadien-16,15-olid-19-oic Acid Methyl Ester (11). To a stirred solution of **1** (3 g, 8.6 mmol) in acetone (80 mL) was slowly added HIO_3 (1.52 g, 8.6 mmol) in H_2O (20 mL) at room temperature. After 3 h the reaction mixture was diluted with H_2O (150 mL). The ether extract was washed with brine, dried over Na_2SO_4, and chromatographed over a silica gel column ($CHCl_3$−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^+)]; ^{1}H NMR ($CDCl_3$) δ 0.84 (3H, s, −C(20)H_3), 3.67 (3H, s, −OCH_3), 4.11, 4.24 (each 1H, d, *J* = 11 Hz, −C(17)H_2OH), 4.83 (2H, m, =CHC(15)H_2O-), 7.19 (1H, s-like, =C(14)*H*-); ^{13}CMR ($CDCl_3$) δ 60.1 (C-17), 128.7 (C-9), 139.1 (C-8). Anal. ($C_{21}H_{30}O_5$) C, H.

17-Oxo-8,13-labdadien-16,15-olid-19-oic Acid Methyl Ester (12). To a stirred solution of **11** (30 mg, 0.082 mmol) in CH_2Cl_2 (2 mL) was added PDC (31 mg, 0.082 mmol) at room temperature. After 1 h the reaction mixture was diluted with saturated Na_2SO_3 (2 mL). The CH_2Cl_2 extract was washed with brine, dried over Na_2SO_4, 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^+)]; ^{1}H NMR ($CDCl_3$) δ 0.91 (3H, s, −C(20)-H_3), 3.65 (3H, s, −OCH_3), 4.80−4.81 (2H, m, =CHC(15)H_2O-), 7.19 (1H, s-like, =C(14)*H*-), 10.15 (1H, s, −C(17)*H*O); ^{13}CMR ($CDCl_3$) δ 130.1, 130.2 (C-8, C-13), 161.4 (C-9), 189.9 (C-17). Anal. ($C_{21}H_{28}O_5$) C, H.

8(17)-Labden-16,15-olid-19-oic Acid Methyl Ester (13). To an ice-cold solution of **1** (20 mg, 0.057 mmol) and $CoCl_2$·-$6H_2O$ (14 mg, 0.059 mmol) in dry EtOH (2 mL) was added $NaBH_4$ (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^+)]; ^{1}H NMR ($CDCl_3$) δ 2.45 (1H, m, −C(13)-*H*COO-), 3.62 (3H, s, −OCH_3), 4.32 (2H, m, −CH_2C(15)H_2O-), 4.53, 4.88 (each 1H, s-like, =C(17)H_2); ^{13}CMR ($CDCl_3$) 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. ($C_{21}H_{32}O_4$) C, H.

Labdan-16,15-olid-19-oic 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 H_2 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^+)]; ^{1}H NMR ($CDCl_3$) δ 0.64 (3H, s, −C(17)H_3), 2.42 (1H, m, −C(13)*H*COO-), 3.60 (3H, s, −OCH_3), 4.17, 4.33 (each 1H, m, −C(15)H_2O-); ^{13}CMR ($CDCl_3$) 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. ($C_{21}H_{34}O_4$) C, H.

17-Nor-8-oxo-labdan-16,15-olid-19-oic 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 $HCOONH_4$ (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^+)]; ^{1}H NMR ($CDCl_3$) δ 0.53 (3H, s, −CH_3), 2.42 (1H, m, −C(13)*H*COO-), 3.63 (3H, s, −OCH_3), 4.19, 4.34 (each 1H, m, −C(15)H_2O-); ^{13}C NMR ($CDCl_3$) 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. ($C_{20}H_{30}O_5$) C, H.

15,16-Epoxy-8(17),14,16-labdatrien-19-oic 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% H_2SO_4, 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^+ − $COOCH_3$), 81 ($C_5H_5O^+$); ^{1}H NMR ($CDCl_3$) δ 3.61 (3H, s, −OCH_3), 4.57, 4.89 (each 1H, s-like, =C(17)H_2), 6.25 (1H, d, *J* = 1.6 Hz, =C(14)*H*-), 7.19 (1H, s, =C(16)*H*O-), 7.35 (1H, d, *J* = 1.6 Hz, =C(15)*H*O-). Anal. ($C_{21}H_{30}O_3$) C, H.

8(17),13-Labdadien-15,16-olid-19-oic 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 Na_2SO_3 solution (5 mL), and the ether extract was concentrated. The crude reaction product thus obtained was further treated with CH_2N_2 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 Na_2SO_4, 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^+)]; ^{1}H NMR ($CDCl_3$) δ 3.62 (3H, s, −OCH_3), 4.46, 4.91 (each 1H, s, =C(17)H_2), 4.71−4.72 (2H, m, =C(16)H_2O-), 5.85 (1H, s-like, =C(14)*H*-); ^{13}C NMR ($CDCl_3$) 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. ($C_{21}H_{30}O_4$) C, H.

In Vitro PAF Receptor Binding Assay. The PAF receptor binding assay using washed rabbit platelets and [^{3}H]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 (2×10^8 cells/mL), 25 μL of [^{3}H]PAF (0.6 nM, 60 000 dpm) with or without unlabeled PAF (500-fold excess over [^{3}H]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 [^{3}H]PAF in the absence and presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. In a set of experiments, [^{3}H]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 IC_{50} value of a sample was defined as the final concentration of the sample required to block 50% of the specific [^{3}H]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 ^{13}C NMR spectral data (3 pages). Ordering information is given on any current masthead page.

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