1,1′-(1,32-Dotriacontanediyl)bis[2-acetyl-*sn*-glycero(3)phosphocholine]: A Long Persisting Agonist as a Potential Antihypertension Agent

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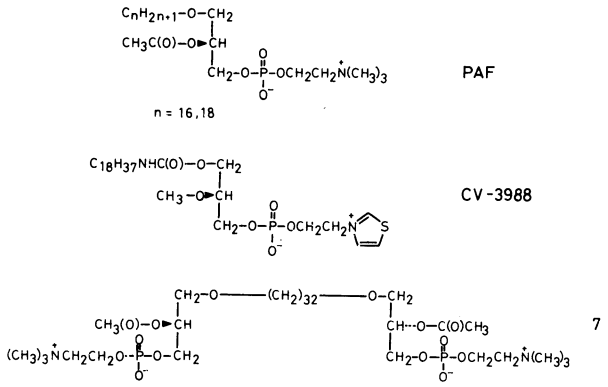
Synopsis. A dimer of platelet activating factor (PAF), 1,1′-(1,32-dotriacontanediyl)bis[2-acetyl-*sn*-glycero(3)phosphocholine] (**7**), was synthesized and examined for platelet aggregation and antihypertension activities. The compound **7** showed both activities with the maximum levels of 1/50—1/100-fold of those of PAF, but the antihypertension effect of **7** persisted much longer and appeared with time lag of 1—3 min. The unique expression of the activities was discussed in conjunction with the molecular structure.

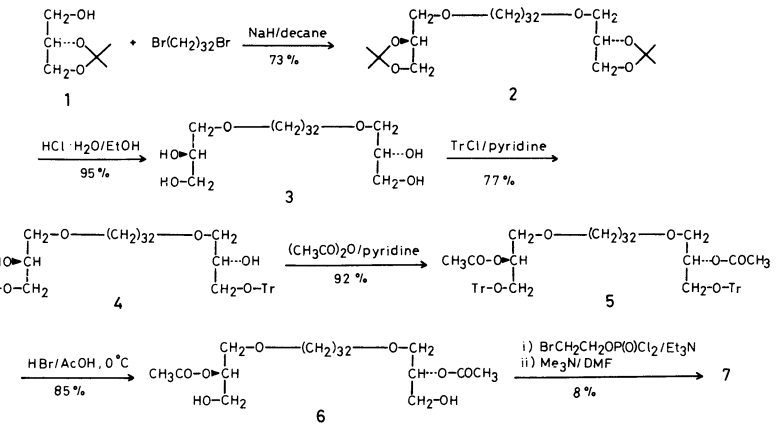
1-Alkyl-2-acetyl-*sn*-glycero(3)phosphocholine (alkyl: C_nH_{2n+1}, n=16 and 18) is released from IgE-sensitized basophiles. The lipids are potent platelet activating factor (PAF)[1,2] and serve as chemical mediators in physiological processes such as anaphylaxis and inflammation.[3] Various agonists have been synthesized to reveal the following structural features for the biological activity;[4] (a) a long chain-alkyl ether linkage at the *sn*-1-O atom,[5,6] (b) short chain acylation (C_{2-4}) at the *sn*-2-O atom,[7,8] and (c) a quaternary ammonium residue as small as $(CH_3)_3N^+$ of the phosphorylcholine moiety at the *sn*-3 position.[9,10] In this paper we wish to report preparation and biological activities (platelet activation and antihypertension) of a new agonist of PAF, 1,1′-(1,32-dotriacontanediyl)bis[2-acetyl-*sn*-glycero(3)phosphocholine] (**7**). The bis(glycerophospholipid) may be considered as a dimeric version of PAF.



Experimental

Synthesis. 2,3-Isopropylidene-*sn*-glycerol (**1**), after conversion into the sodium alkoxide, was heated with 1,32-dibromodotriacontane in decane at 140 °C. The resulting **2** was transformed via **3** and **4** into **5** by a series of usual reactions including acid catalyzed deisopropylidenation, tritylation and acetylation in an overall yield of 73%. Removal of the trityl groups of **5** required considerable experimentation since the acetyl group at the *sn*-2-O position tended to rearrange into the *sn*-3-O position.[11] A treatment of a benzene–ethyl acetate solution of **5** with 25% hydrogen bromide–acetic acid at 0 °C for 50 s was satisfactory to generate **6**, which was isolated by means of Sephadex LH-20 gel column chromatography. A subsequent treatment of **6** with 2-bromoethyl phosphorodichloridate in the presence of triethylamine at room temperature overnight and a reaction of the resulting phosphate with trimethylamine in *N*,*N*-dimethylformamide at 55 °C for 4 d provided **7**, which was isolated and purified by a combination of silica-gel and Sephadex LH-20 gel column chromatographies: 400 MHz ^{1}H NMR ($CDCl_3$–CD_3OD, 2:1 v/v; 7.26 ppm-singlet of $CHCl_3$ as an internal standard) δ 1.14 [coherent peak, 60H, $(CH_2)_{30}$], 2.45 (s, 6H, 2×*sn*-2-CH); IR (KBr) 1735 (C=O), 1240 and 1095 (P–O–C) cm^{-1}. Table 1 lists physical constants of **2**—**7**.

Biological Assays. (i) Platelet Aggregation. A 9:1 volumetric mixture of male New Zealand white rabbit blood and 3.8% trisodium citrate was centrifuged at 1100 rpm for



10 min at room temperature. The upper layer, platelet-rich plasma (PRP), was collected. Then, the residual blood sample was centrifuged at 3000 rpm for 10 min at 4 °C to provide platelet-poor plasma (PPP). Platelet count in PRP was measured by Systemax Platelet Counter PL-100, and adjusted to 5×10^8 /ml by appropriate dilution with PPP. Platelet aggregation was initiated by adding 30 μl of **7** in a 0.025 M (1 M=1 mol dm^{-3}) tris-HCl buffer (pH 7) containing 0.13 M NaCl into 270 μl of PRP. The assay was conducted according to the turbidimetric procedure of Born and Cross.[13] In the experiment to know the effect of *rac*-3-[*N*-(octadecyl)carbamoyloxy]-2-methoxypropyl 2-(3-thiazolino)-ethyl phosphate (CV-3988)[12] on test compound-induced platelet aggregation, PRP was pretreated with 100 μM of CV-3988 for 3 min. Typical results are displayed in Fig. 1.

(ii) Antihypertension. Wister male rats (weight, 254—276 g) were anesthetized with sodium pentobarbitol (60 mg/kg, i.p.). Appropriate amounts of **7** dissolved in saline or PAF in 0.05 M tris-HCl–0.15 M NaCl buffer (pH 7) was then injected intravenously, and the blood pressure in jugular vein was measured for 15 min by the use of a pressure-transducer, a Nippon Hatsuden model RMP-6018. The results are shown in Fig. 2.

Results and Discussion

It was found that dimerization of PAF into **7** resulted in modifying the platelet aggregation and antihypertension activities in a unique manner; e.g., although the maximum levels of both activities of **7** were 1/50—1/100-fold of those of PAF (Fig. 1a and 1b; Fig. 2) and appeared to be dose-dependent in the examined range (10^{-1}—1 μM for platelet aggregation and 10—100 μg/kg of rat for antihypertension), the antihypertension effect was exhibited with time lag of

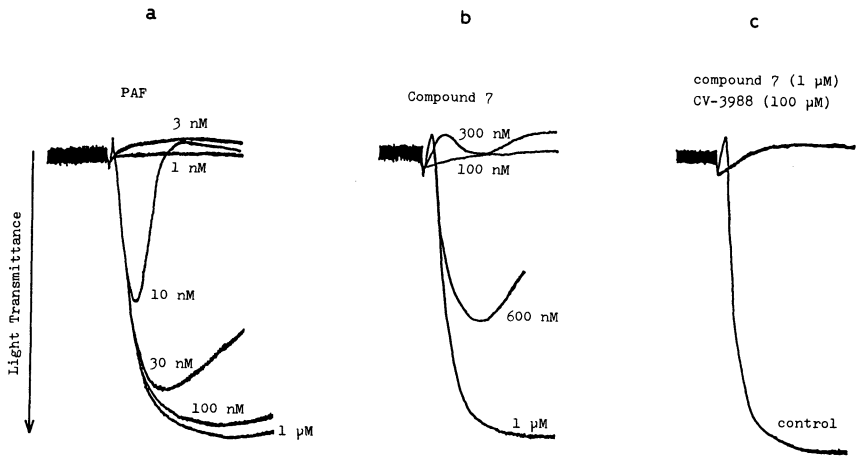


Fig. 1. Typical patterns of PAF- and compound **7**-induced rabbit platelet aggregation (a and b), and inhibitory effect (c) of CV-3988 on the compound **7**-induced aggregation.

Table 1. Physicochemical Results[a]

Compound	Purification	Yield %	Mp/°C	$[\alpha]_D^{25}$	Silica-gel TLC, R_f	C and H analysis FAB mass spectrum
2	Silica-gel column[b]	73	69—72	−6.9° (*c* 1.3, chloroform)	0.37[h]	*m/z* 711 (M+1) (Calcd for $C_{44}H_{86}O_6$, 710).
3	Recrystal. (chloroform)	95	112—117	−2.8° (*c* 1.5, chloroform)	0.35[i]	Calcd for $C_{38}H_{78}O_6$: C, 72.33; H, 12.46%. Found: C, 71.90; H, 12.53%.
4	Silica-gel column[c]	77	g)	+2.6° (*c* 1.25, chloroform	0.39[j]	*m/z* 1115 (M+1) (Calcd for $C_{76}H_{106}O_6$, 1114).
5	Silica-gel column[f]	92	g)	+11.7° (*c* 1.7, chloroform	0.61[j]	*m/z* 1199 (M+1) (Calcd for $C_{80}H_{110}O_8$, 1198).
6	Sephadex LH-20 gel column[d]	85	77—79	−3.5° (*c* 3.5, chloroform)	0.23[k]	*m/z* 715 (M+1) (Calcd for $C_{42}H_{82}O_8$, 714).
7	Silica-gel column,[e] then Sephadex LH-20	8	241—244	−3° (*c* 0.3, chloroform–methanol, 2:1 v/v)	0.40[l]	*m/z* 1045 (M+1) (Calcd for $C_{52}H_{106}O_{14}N_2P_2$, 1044)

a) Silica-gel column support, Merck 7734, 70—230 mesh; Silica gel TLC, precoated sheet, Merck Art. 5735; matrix used in the FAB mass spectra, 3-mercapto-1,2-propanediol-glycerol, 1:1 v/v. b) Hexane–ethyl acetate–triethylamine in gradually changing from 70:4:1 to 40:4:1 v/v. c) Hexane–ethyl acetate in gradually changing from 13:1 to 3:1 v/v. d) Chloroform–methanol, 2:1 v/v. e) Chloroform–acetone–methanol–concd ammonia in gradually changing from 8:6:6:1 to 8:6:6:3 v/v. f) Hexane–ethyl acetate, 7:1 v/v. g) A compound to melt at room temperature. h) Hexane–ethyl acetate, 5:1 v/v. i) Chloroform–methanol, 8:1 v/v. j) Hexane–ethyl acetate, 3:1 v/v. k) Chloroform–ethyl acetate, 1:1 v/v. l) Chloroform–acetone–methanol–concd ammonia, 4:2:6:3 v/v.

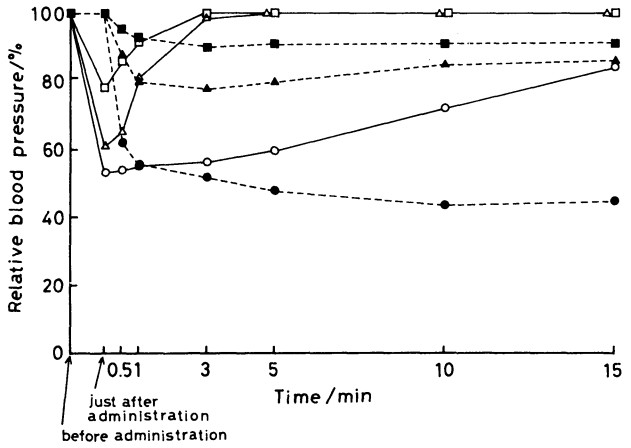


Fig. 2. Time course of antihypertension effects of compound **7**, 100 μg/kg (●), 30 μg/kg (▲), 10 μg/kg (■), PAF, 1.0 μg/kg (○), 0.3 μg/kg (△), and 0.1 μg/kg (□) in rats. Each point represents the mean of three animals.

1—3 min and seemed to persist for a period much longer than PAF (Fig. 2). By contrast, the effect of PAF was transient and became inactive rapidly within a few minutes after administration. The integrated biological activity over the effective time, though examined only qualitatively, may thus be comparabale with or surpass that of PAF. A mechanism of the PAF-effect has not been understood well.[12,14-16] The compound **7**, however, was seemed to act on platelets in a manner similar to PAF because (i) **7** resembled with PAF in aggregation pattern and (ii) the activity of **7** was inhibited competitively by an antagonist of PAF, CV-3988[14] (typical aggregations: Fig. 1a—c). The decreased magnitudes in the biological activities of **7** may be attributed to conformational or steric effect of the 1,32-dotriacontanediyl residue and/or the increased polarity due to two phosphorylcholine residues. Such a structural modulation would result in slow metabolism leading to long duration of the activities, and the time lag as well, which might be ascribed to slow accommodation of **7** to PAF-receptors on platelets.

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