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Phospholipids Chiral at Phosphorus. Synthesis and Stereospecificity of Phosphorothioate Analogues of Platelet-Activating Factor†

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ABSTRACT: R_P and S_P isomers of 1-*O*-hexadecyl-2-acetyl-3-thiophosphocholine (AGEPsC) have been synthesized. The activity of these isomers in platelet aggregation and serotonin secretion was compared with that of 1-*O*-hexadecyl-2-acetyl-3-phosphocholine (AGEPC). The results show that (S_P)-AGEPsC has the same activity as AGEPC within experimental error in both assays. The R_P isomer, however, is only 0.6–2% as active as AGEPC in platelet aggregation and serotonin release. The results suggest that the phosphate group of AGEPC is likely to be involved in the interactions with its receptor, at least in the events leading to platelet aggregation and secretion.

Platelet-activating factor (PAF)[1] is a naturally occurring simple phosphoglyceride with very potent biological activity (Snyder, 1986; Hanahan, 1986; Hanahan & Kumar, 1987). Its existence was first suggested by Henson (1970), Sirganian and Osler (1971), and Benveniste et al. (1972), but the exact chemical structure of the naturally occurring compound was not deduced until 10 years later (Hanahan et al., 1980). As shown in Figure 1, the structure of natural PAF is 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine (AGEPC), with the alkyl chain varying from C14:0 to C18:1.

Since its discovery, an enormous research effort has been focused on elucidating the biochemical nature of PAF. It is the first example of a phosphoglyceride with biological activity, apart from the familiar role as a structural entity in biological membranes. It is a potent mediator of many biological processes, having roles in antihypertensive activity, cardiovascular effects, bronchoconstriction, and immunopathological response [see reviews, Hanahan (1986) and Hanahan and Kumar (1987)]. To date, it is the most potent known activator of platelets, causing them to degranulate and aggregate at concentrations as low as 10^{-10} M in washed rabbit platelets.

The structural features important to the biological activity of this unique phosphoglyceride have been explored in depth by many laboratories, especially relative to substituents at the *sn*-1 and *sn*-2 positions. Consequently, only a few selective

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[1] Abbreviations: AGEPC, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine; AGEPsC, 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-thiophosphocholine; CDMP, chloro(*N*,*N*-diisopropylamino)methoxyphosphine; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPPsC, 1,2-dipalmitoyl-*sn*-glycero-3-thiophosphocholine; lyso-GEPC, 1-*O*-alkyl-2-lyso-*sn*-glycero-3-phosphocholine; lyso-GEPsC, 1-*O*-hexadecyl-2-lyso-*sn*-glycero-3-thiophosphocholine; PAF, platelet-activating factor; PLA2, phospholipase A_2; PLC, phospholipase C; TBAH, tetrabutylammonium hydroxide; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; EDTA, ethylenediaminetetraacetate.

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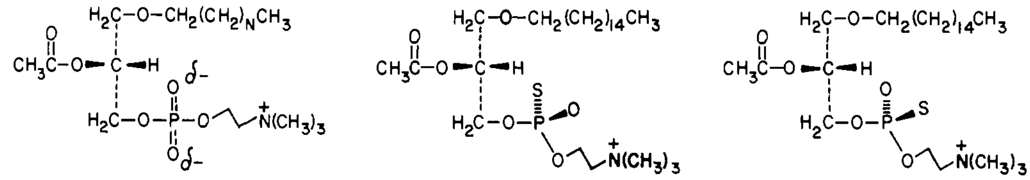

AGEPC (Rp)AGEPsC (Sp)AGEPsC

FIGURE 1: Structures of AGEPC, (R_P)-AGEPsC, and (S_P)-AGEPsC. Notice that for the natural PAF the alkyl chain of AGEPC varies from C14:0 to C18:1. However, the AGEPC used in the experiments of this paper was synthetic 1-*O*-hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine.

references on this topic are cited here. It has been shown that the ether linkage at the *sn*-1 position is required for biological activity. When replaced with an ester linkage, the activity, based on the concentration necessary to elicit aggregation of platelets or release of radiolabeled serotonin from platelets, was reduced 100-fold (Benveniste & Vargaftig, 1983). Naturally occurring AGEPC consists of a variety of chain lengths, from 14 to 18 carbons, and also an unsaturated chain, C18:1$\Delta^{cis9,10}$, with the C-16 chain most abundant (Pinckard et al., 1984; Weintraub et al., 1985). At the *sn*-2 position, a short chain ester is required for activity. Only the acetyl and propionyl derivatives are very active (Benveniste & Vargaftig, 1983). Ether analogues at the *sn*-2 position are less active than ester derivatives (Wykle et al., 1981). Lyso-GEPC (deacetylated PAF) was completely inactive. The unnatural enantiomer of AGEPC was suggested to be totally inactive by Wykle et al. (1981) but was shown to possess low activity (1000-fold less active) by others (Hanahan & Kumar, 1987).

Regarding the substituents at the *sn*-3 position, less information is available. Satouchi et al. (1981) showed that platelet activation is sensitive to the degree of methylation of the quaternary ammonium group of choline. The dimethylammonium analogue was about 2.5-fold less active than PAF, and the monomethylammonium derivative had about 20% of the activity of PAF. The ethanolamine analogue was less than 1000 times as active as PAF and had about the same activity as the phosphatidic acid and phosphorylethanol analogues. Tokumura et al. (1985) and Wissner et al. (1986) synthesized a variety of analogues varying the number of methylene bridges separating the phosphate and the trimethylammonium moieties. They found that as the bridge increased in length the bioactivity progressively decreased. Interestingly, the inhibitory activity was found to develop as the methylene bridge lengthened (Tokumura et al., 1985). Wissner et al. (1986) also synthesized an analogue with a 4-(trimethylammonio)butoxy group, instead of phosphocholine, that showed no bioactivity. They concluded that the phosphate group was essential for biological activity. A problem with this conclusion is that a CH_2CH_2 group is not a good analogue for a PO_3^{2-} group.

In order to probe the structural requirement of AGEPC in the phosphate headgroup and the possible involvement of the phosphate group in the interaction of AGEPC with receptors, we report synthesis and function of (R_P)- and (S_P)-AGEPsC (Figure 1). The rationale of our approach is that if the phosphate group is not required for activity or not involved in AGEPC–receptor interactions, sulfur substitution at the phosphate group and the phosphorus configuration should have very little effect on the biological activity of AGEPC. On the other hand, if (R_P)- and (S_P)-AGEPsC show activity different from each other and also from the natural AGEPC, it is most likely that the AGEPC–receptor interaction involves a stereospecific interaction between the phosphate group of AGEPC and the receptor via ionic interaction or hydrogen bonding. Such an approach has been used to conclude that the phosphate group of phospholipids is involved in the interaction of phospholipase A_2 (PLA2) from several different sources with substrates (Bruzik et al., 1983; Tsai et al., 1985) and that such an interaction is absent in the interaction of lecithin–cholesterol acyltransferase with its substrate (Rosario-Jansen et al., 1987).

MATERIALS AND METHODS

Materials. 2,3-Isopropylidene-*sn*-glycerol (**1**) ($[\alpha]^{20}_D$ = −14.2°) was purchased from Aldrich. 1-*O*-Hexadecyl-2-acetyl-*sn*-glycero-3-phosphocholine was purchased from Novabiochem AG (Läufelfingen, Switzerland). Bee venom PLA2 and PLC from *Bacillus cereus* were obtained from Boehringer Mannheim. Other biochemicals were purchased from Sigma. Other chemicals were of reagent grade.

Methods. Platelet aggregation and serotonin secretion assays were performed on washed rabbit platelets as described in Tokumura et al. (1985). Thin-layer chromatography was carried out as described previously (Bruzik et al., 1983). Solvent A consists of diethyl ether/hexane (2:1 v/v), and solvent B consists of $CHCl_3/CH_3OH/H_2O$ (66:33:4 v/v).

Spectral Methods. Routine ^1H, ^{13}C, and ^{31}P NMR analyses were performed on Bruker WP-200, AM-250, MSL-300, and AM-500 NMR spectrometers. Broad-band ^1H decoupling was used in ^{31}P and ^{13}C NMR. ^1H and ^{13}C chemical shifts were referenced to internal Me_4Si standard, while ^{31}P chemical shifts were referenced to external 85% H_3PO_4 (at 25 °C). Fast atom bombardment mass spectroscopy was performed on a VG 70-250S mass spectrometer.

Synthesis of **5** *from* **1**. The procedure of Baumann and Mangold (1964) was used for alkylation and deprotection of **1**. The mp of **2** was 63–65 °C after recrystallization from hexane (lit. 63–65 °C), and the yield of **2** was 87.5% (lit. 69%) from **1**: ^{13}C NMR δ 72.61, 71.96, 70.55, 64.43, and other high-field resonances from the palmityl chain.

Conversion of **2** to **3** with trityl chloride was carried out by the procedure of Chacko and Hanahan (1968). The product was identified by TLC (R_f = 0.28; hexane/ether, 8:2 v/v) and by ^1H NMR.

The reactions **3** → **4** → **5** were carried out as follows. One millimole (0.56 g) of **3** was dissolved in 30 mL of ethanol-free, anhydrous chloroform containing 0.12 g (1 mmol) of DMAP and 0.54 g (1.1 mmol) of palmitic anhydride. After stirring at room temperature overnight, the product **4** was purified by a silica gel column (eluent used was hexane/ether/glacial acetic acid, 8:2:0.2; TLC R_f = 0.75 in the same solvent system). The trityl group was then removed by treating **4** (in anhydrous CH_2Cl_2) with BF_3–MeOH as described by Hermetter and Paltauf (1981), except that the reagent was added in small aliquots. The product was purified by flash chromatography on a silica gel column using a stepwise elution gradient: 100 mL of light petroleum ether; 150 mL of light petroleum ether/diethyl ether (9:1); 120 mL of the same system (8:2); and 120 mL of the same system (9:3). The total chromatographic time was kept to less than 30 min to minimize isomerization of the product. The product (70% relative to **4**) was identified by TLC (R_f = 0.15, light petroleum ether/diethyl ether, 9:2) and by ^1H NMR (200 MHz, $CDCl_3$), as evidenced by a quintet at 5.0 ppm for the *sn*-2 methine protein and other resonances characteristic of the structure of **5**. The presence of ca. 15% β-isomer (1-*O*-hexadecyl-3-palmitoyl-*sn*-glycerol) was identified by TLC (R_f = 0.22 on the same system) and by a ^1H NMR resonance at 4.2 ppm assigned to the *sn*-3 CH_2 protons of the β-isomer. Since **1–5** are all known compounds, their spectral data are not described in detail. Some original spectra have been presented in Rosario-Jansen (1987).

Conversion of **5** *to* **6**. The alcohol **5** (1.2 mmol) was placed in a 100-mL round-bottom flask equipped with a magnetic stirrer, a septum port, and a vacuum adaptor and dried under vacuum (0.1 mmHg) overnight. Dry triethylamine (2.5 mmol) was distilled directly to the reaction vessel, followed by ethanol-free chloroform. CDMP (1.2 mmol) was added to the reaction vessel by gastight syringe via the septum port. The reaction was stirred for 30 min at room temperature in an air- and moisture-free environment. The reaction was deemed complete by TLC using diethyl ether/hexanes (2:1 v/v) as the developing solvent (solvent A) (R_f = 0.78). The solvent and triethylamine were evaporated under reduced pressure. The intermediate was not purified but used directly in the next step.

Choline tosylate (3.2 mmol) and 1*H*-tetrazole (3.7 mmol) dissolved in approximately 10 mL of tetrahydrofuran/acetonitrile (1:1 v/v) were added to the above intermediate by gastight syringe. The reaction was stirred for 30 min or until complete by TLC (R_f = 0, solvent A). The solvents were removed under reduced pressure prior to sulfurization.

Sulfurization was accomplished by dissolving the residue in dry, distilled toluene, followed by addition of excess sulfur (sublimed). The mixture was stirred overnight at room temperature, after which time the gummy mixture was washed twice with 1 M triethylamine bicarbonate buffer, pH 7.4. The organic layer was concentrated by rotary evaporation and rendered anhydrous by repeated evaporation from toluene.

The methoxy goup was removed with trimethylamine. The dried residue was dissolved in anhydrous toluene. Trimethylamine was transferred to the reaction vessel, the reaction flask was tightly closed, and the mixture was stirred for 24 h. Trimethylamine and the solvents were removed, and the residue was chromatographed on silica gel by using solvent B ($CHCl_3/CH_3OH/H_2O$, 66:33:4 v/v) as the eluting solvent. Yield of pure product was 75%, based on the alcohol [TLC R_f = 0.6 (solvent B)].

The structure was confirmed by ^1H NMR. The presence of two diastereomers of **6** was evidenced by ^{31}P NMR (202.24 MHz, $CDCl_3$), which showed two signals of equal intensity at 56.20 and 56.27 ppm. The upper field signal (56.20 ppm) was assigned to the R_P isomer of **6** since in the reaction mixture of PLA2 hydrolysis (see next section) this signal was replaced by a signal at 57.83 ppm representing (R_P)-lyso-GEPsC (**7**). The presence of positional isomers of **6** was evidenced by two resonances of equal intensity at 55.57 and 55.77 ppm, which were unchanged in the reaction mixture of PLA2 hydrolysis.

Conversion of **6** *to* (R_P)-*AGEPsC* (**9**) *and* (S_P)-*AGEPsC* (**13**). The diastereomers of **6** were digested with bee venom PLA2, which is specific for the R_P isomer of thiophospholecithins. The thiophospholipid (106 mg) was dissolved in 3.7 mL of diethyl ether/chloroform (3:0.7 v/v) and added to 0.3–0.6 mg of PLA2 in 250 μL of 50 mM Tris buffer (10 mM calcium chloride, 1 mM Na_4EDTA, pH 7.2). The mixture was gently agitated at 30 °C for 4 h. The crude mixture was evaporated to dryness, redissolved in chloroform, and applied to a chromatographic column (silica gel). The products were eluted with solvent B. The first fraction contained unhydrolyzed S_P isomer **8** (R_f = 0.6, solvent B), contaminated with positional isomers. The second fraction contained (R_P)-lyso-GEPsC (**7**) (R_f = 0.3, solvent B). The S_P isomer **8** and (R_P)-lyso-GEPsC (**7**) were analyzed by ^{31}P NMR as described above.

The lyso compound **7** was dried in vacuo and added with 1 equiv of DMAP in dry chloroform. A slight excess of acetic anhydride was distilled directly to the flask containing **7** and DMAP. The reaction was followed by TLC (solvent B). The mixture was evaporated to dryness to remove solvent and excess acetic anhydride. The dried residue was extracted by the method of Bligh and Dyer (1959) and then treated with Rexyn I-300 (Fisher Scientific) to remove any traces of DMAP and acetate. The product, (R_P)-AGEPsC (**9**), was further purified by silica gel chromatography using solvent B: yield ~80%; R_f = 0.4 (solvent B). The corresponding R_f for AGEPC was 0.2.

The preparation of the S_P isomer was more difficult due to the presence of PLA2-resistant positional isomers. Compound **8** and the accompanying positional isomers (0.1 mmol) were dissolved in 10 mL of methanol containing 0.1 mmol of tetrabutylammonium hydroxide (TBAH). The solution was stirred at room temperature for 2 h or until complete on the basis of TLC (R_f = 0.3, solvent B). The mixture was extracted by the Bligh and Dyer technique (1959) before it was treated with Rexyn I-300 to remove TBAH. The resulting alcohols **10** and **11** were dried overnight under reduced pressure. The mixture was then dissolved in dry pyridine containing triphenylmethyl chloride (0.15 mmol). The solution was stirred over 24 h at room temperature. The β-isomer **11** became tritylated (R_f = 0.7, solvent B) due to the presence of a primary hydroxyl group. The (S_P)-lyso-GEPsC (**10**) was separated from the tritylated product by silica gel chromatography using solvent B. Acetylation of **10** as described above gave (S_P)-AGEPsC (**13**).

Characterization of AGEPsC was based on fast atom bombardment mass spectroscopy (m/z 540 $[MH]^+$, 498 $[MH - 42]^+$, and 200 due to the thiophosphocholine moiety), ^{31}P NMR (δ 56.7 in $CDCl_3$, 81.0 MHz), and ^1H NMR (500 MHz, CD_3OD, S_P isomer): δ 0.89 (t, J_{vic} = 6.9 Hz, terminal CH_3), 1.28 (bs, CH_2 of alkyl group), 1.59 (q, J_{vic} = 6.8 Hz, CH_2CH_2O), 2.06 (s, CH_3COO), 3.23 (s, NCH_3), 3.45 (m, tentatively CH_2CH_2O), 3.64 (t, J_{vic} = 4.7 Hz, tentatively CH_2N), 3.58 (dd, J_{gem} = 10.8 Hz, J_{vic} = 6.3 Hz, *sn*-1-CH_aH_b), 3.61 (dd, J_{gem} = 10.8 Hz, J_{vic} = 4.2 Hz, *sn*-1-CH_aH_b), 4.00 (ddd, J_{gem} = 11.1 Hz, J_{vic} = 5.9 Hz, $^3J_{HP}$ = 7.8 Hz, *sn*-3-CH_aH_b), 4.08 (ddd, J_{gem} = 11.1 Hz, J_{vic} = 4.4 Hz, $^3J_{HP}$ = 7.5 Hz, *sn*-3-CH_aH_b), 4.2–4.3 (m, CH_2CH_2N), 5.12 (m, *sn*-2-CH). The assignments were assisted by homonuclear decoupling and computer simulation.

Further Digestion of (R_P)-*AGEPsC with Phospholipase C.* The condition used was as described by Orr et al. (1982). At first we showed that 10 mg of AGEPC (from Avanti) was completely hydrolyzed by 4 units of PLC (*B. cereus*) in 24 h. Then 4 mg of (R_P)-AGEPsC was incubated with 100 units of PLC for 10 h under the same condition, followed by purification of the pure R_P isomer through a silica gel column (solvent system B). The sample was further purified by preparative TLC (solvent B) before being used in the activity assays.

Isolation of Platelets. Rabbit platelets were isolated and washed essentially as described by Shukla and Hanahan (1984). The final platelet pellet was resuspended in Tyrode–gelatin buffer, pH 6.5, to a concentration of 1.25 × 10^9 cells/mL. Unless otherwise stated, all reaction mixtures

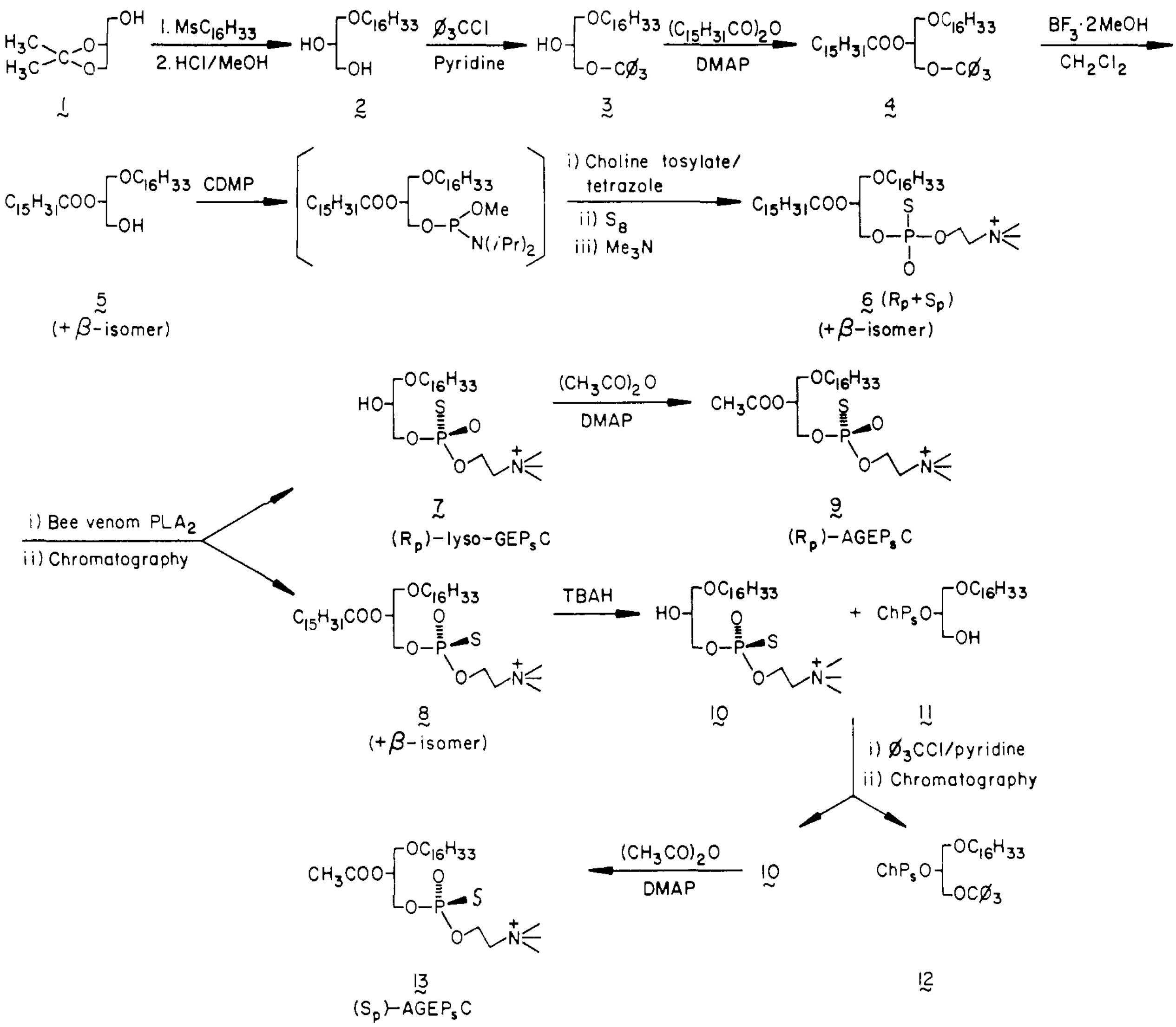

FIGURE 2: Synthetic scheme for (R_P)- and (S_P)-AGEPsC.

containing these platelets were incubated at 37 °C.

Aggregation Assay. The isolated washed rabbit platelets were suspended in Ca^{2+}-free Tydrode–gelatin buffer, pH 6.5 (1.25×10^9 cells/mL). One hundred microliters of the platelet suspension was transferred into tubes of the aggregometer and diluted 1:4 with Tyrode–gelatin buffer, pH 7.2, containing 1.33 mM Ca^{2+}. Subsequent to addition of the agonist, changes in the light transmittance were monitored by a Payton aggregation model dual channel (Payton Associates, Buffalo, NY).

Measurement of [^3H]Serotonin Secretion. Whole rabbit blood anticoagulated with 0.012 M trisodium citrate, 0.009 M citric acid, and 0.28% dextrose was incubated with [^{3}H]-serotonin (1 μCi/mL) for 15 min at 37 °C, and washed platelets were prepared as described above. Platelets (6.25×10^7 cells, 0.25 mL) in Tyrode–gelatin buffer, pH 7.2, containing 1 mM $CaCl_2$ were incubated with the agonist. The sample was mixed with 25 μL of ice-cold 1.5 M formaldehyde to stop the reaction and was centrifuged at 830*g* for 15 min at 4 °C. The radioactivity of the supernatant was measured by liquid scintillation counting. A 100% value for the [^{3}H]serotonin release was obtained by treatment of platelets with Triton X-100 (0.2%, final). Values of serotonin secretion were calculated according to the equation serotonin secretion (%) = [(stimulus-induced serotonin release − control serotonin release)/(total serotonin release − control serotonin release)] × 100. Each of the control (stimulus free) serotonin release values was below 5% of total serotonin release.

RESULTS

Synthesis of (R_P)- and (S_P)-AGEPsC. The synthetic procedures are outlined in Figure 2. The steps **1** → **2** → **3** were performed according to the method of Baumann and Mangold (1964) and Chacko and Hanahan (1968), respectively. In the following step, a palmitoyl group was introduced to the *sn*-2 position (**4**) followed by detritylation to give 1-palmityl-2-palmitoyl-*sn*-glycerol (**5**). Compound **5** from deprotection with BF_3–CH_3OH was accompanied by ca. 20% of the β-isomer, where the 2-palmitoyl group migrated to the 3-position. Introduction of the thiophosphocholine group according to the method of Bruzik et al. (1986) via a highly specific phosphorylating agent ClP(OMe)N(*i*-Pr)$_2$ (abbreviated as CDMP) gave (R_P + S_P)-1-palmityl-2-palmitoyl-*sn*-glycero-3-thiophosphocholine (**6**). Digestion by phospholipase A_2 from bee venom (Bruzik et al., 1983) gave (R_P)-lyso-GEPsC (**7**), which was then acetylated to (R_P)-AGEPsC (**9**). The S_P isomer **8** and the accompanying β-isomer recovered from the PLA2 reaction were then hydrolyzed by a weak base to give (S_P)-lyso-GEPsC (**10**) and the corresponding β-isomer **11**. Separation of **10** and **11** was achieved by subjecting the mixture to tritylation. Since only the primary alcohol in **11** was tritylated (to give **12**), the lyso-(S_P)-GEPsC (**10**) was recovered from chromatography and acetylated to give (S_P)-AGEPsC

Table I: Summary of Biological Activity

compound	concn (M) for 50% platelet aggregation	concn (M) for 50% serotonin secretion
AGEPC	1.3×10^{-10}	1.5×10^{-10}
(R_P)-AGEPsC	1.5×10^{-8}	0.75×10^{-8}
	$(2 \times 10^{-8})^a$	$(2.0 \times 10^{-8})^a$
(S_P)-AGEPsC	1.25×10^{-10}	2.3×10^{-10}

[a] Obtained from a separate experiment, after exhaustive digestion of the R_P isomer by phospholipase C, as described in the text.

(**13**). As noted under Materials and Methods, the R_f value of AGEPsC is substantially higher than that of AGEPC, which is in agreement with the difference between DPPsC and DPPC (Bruzik et al., 1983).

The above procedures gave (R_P)- and (S_P)-AGEPsC with minimal amounts of positional isomers. This was accomplished partially due to the use of a long-chain ester group in **4–6**. When the acetyl group was used instead, complete and random isomerization was observed in **5**. The long-chain analogue **6** was also a better substrate for PLA2 relative to acetyl analogues.

Activity of (R_P)- *and* (S_P)-*AGEPsC toward Platelet Activation and Serotonin Release.* Table I shows the result of the assays of rabbit platelet aggregation induced by the natural AGEPC and (R_P)- and (S_P)-AGEPsC. It is clear that (S_P)-AGEPsC is a potent agonist with almost identical activity compared to AGEPC (molarity for 50% aggregation is 1.25×10^{-10} M). The R_P isomer of AGEPsC, however, requires ca. 1.5×10^{-8} M to induce 50% aggregation. Thus, (R_P)-AGEPsC is only 0.83% as active as the S_P isomer.

The result of serotonin release assays are also shown in Table I. Again (S_P)-AGEPsC shows comparable activity relative to the natural AGEPC. (R_P)-AGEPsC, however, is only 3% as active as the S_P isomer or 2% as active as AGEPC. Despite the lower activity of the R_P isomer, the dose–response curve of both R_P and S_P isomers of AGEPsC have shapes similar to that of AGEPC.

It may be questioned whether the observed activity of the R_P isomer is due to trace contamination of the S_P isomer. Although both isomers were pure on the basis of ^{31}P NMR, the limit of detection was 1–2% under our experimental conditions. We therefore subjected the R_P isomer to exhaustive digestion by phospholipase C. Phospholipase C has been shown to specifically hydrolyze the S_P isomer of DPPsC (Bruzik et al., 1983; Jiang et al., 1985) and is expected to hydrolyze the trace (S_P)-AGEPsC under our experimental conditions. The resulting R_P isomer showed similar activity (Table I, data in parentheses) compared to the sample before such a treatment. Thus, our data suggest that the true activity of (R_P)-AGEPsC is ca. 10^{-2} relative to that of AGEPC in both aggregation and secretion assays.[2]

Discussion

Synthesis. Our procedure for obtaining the sulfur analogues of AGEPC can be adapted for the synthesis of natural AGEPC. Instead of oxidizing the tervalent phosphorus intermediate with sulfur, it can be oxidized with peroxides (Bruzik et al., 1986). After the long-chain PAF analogue is obtained, the ester can be easily cleaved with base and reacylated with acetic anhydride to give AGEPC. The use of a long-chain ester as a protective group, along with slow addition of boron trifluoride to remove the trityl group, reduces the occurrence of positional isomers. Alternative synthetic procedures for saturated and unsaturated AGEPC have been summarized by Hanahan and Kumar (1987).

During the course of this work, a Russian group also reported synthesis of phosphorothioate analogues of AGEPC (Gordeev et al., 1986). However, they started from racemic 1-*O*-octadecylglycerol and had not considered the new chiral center formed at phosphorus. Their spectral characterization was also incomplete and questionable (^{31}P δ 39.5 in $CDCl_3$ was significantly different from our data), and no biological study had been reported.

AGEPC–Receptor Recognition. Since the S_P isomer of AGEPsC behaves almost indistinguishably from the natural AGEPC, while the R_P isomer is less active by a factor of ca. 10^2, the results suggest that the phosphate group is likely to be involved in the AGEPC–receptor interaction.

In the case of PLA2 from bee venom, the S_P isomer of DPPsC (the phosphorothioate analogue of DPPC) is less reactive than the R_P isomer by a factor of ca. 1700. Detailed kinetic studies on the metal ion dependence of stereospecificity allowed us to conclude that the *pro-S* oxygen of DPPC is coordinated to the cofactor Ca^{2+} in the active site of PLA2, which is an essential feature of enzyme–substrate recognition in the catalysis of PLA2 (Tsai et al., 1985). It is unclear whether the AGEPC–receptor recognition also involves a divalent metal ion as in the case of PLA2. Although extracellular Ca^{2+} is required for the initiation of aggregation and secretion (Hanahan & Kumar, 1987), it is not required for binding of AGEPC to the platelet, since AGEPC has been shown to desensitize rabbit platelet in the absence of Ca^{2+} (Tokumura et al., 1985). Receptor binding studies also showed that addition of Ca^{2+} increased the nonspecific binding of AGEPC without affecting the specific binding (Homma et al., 1987). Other possible explanations for the observed stereospecificity in the binding of AGEPsC are an ionic interaction between the phosphate group and a positively charged amino acid side chain or simply a hydrogen-bonding interaction.

Conclusion. Our stereochemical studies suggest that the phosphate group of AGEPC is likely to be involved in the interaction with its receptor, at least in the events leading to platelet aggregation and secretion. Such a stereochemical probe could be used to probe the mechanism of other events induced by PAF. Furthermore, since the biosynthesis and metabolism of AGEPC are likely to involve phospholipases (Hanahan, 1986; Snyder et al., 1985; O'Flaherty & Wykle, 1983) and since phospholipases A_2, C, and D are all sensitive to the phosphorus configuration of chiral thiophospholipids (Bruzik et al., 1983; Jiang et al., 1985), it should be possible to probe some biosynthetic and metabolic problems of AGEPC by use of appropriate chiral thiophospholipids.

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[2] Preliminary data on platelet aggregation assayed in Tsai's laboratory, presented at the 10th International Conference on Phosphorus Chemistry (Rosario-Jansen et al., 1987), showed that (R_P)-AGEPsC was less active than AGEPC by only a factor of 10. The data presented in this paper were obtained in Hanahan's laboratory. The quantitative discrepancy in the preliminary data could be attributed to lack of experience in such assays in Tsai's laboratory and/or use of samples with lower isomeric purity.

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