oratories (Research Triangle Park, NC). The peptide antagonist CTP was synthesized as previously described (Pelton et al., 1986).[5] Somatostatin and DPDPE were purchases from Peninsula Laboratories (Belmont, CA). Naloxone hydrochloride and U50,488H were purchased from Sigma (St. Louis, MO) and Upjohn Diagnostics (Kalamazoo, MI), respectively. The δ receptor selective antagonist ICI 174,864 was purchased from Cambridge Research Biochemicals (Atlantic Beach, NY).

Data Analysis. All statistical calculations were carried out as described by Tallarida and Murray.[27] Tests for statistical significance included the analysis of variance, followed by a Student's *t* test for grouped data where significance was indicated.

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Acknowledgment. This research was supported by grants from the U.S. Public Health Service NS 19972 (V.J.H) and DK 36289 (V.J.H., H.I.Y., and T.F.B.).

Registry No. 1, 118377-52-9; 2, 118377-53-0; 3, 118377-55-2; 4, 118398-12-2; A, 33305-77-0; B, 55533-24-9; C, 55533-25-0; DPDPE, 88373-73-3; CTP, 103335-28-0; *p*-NO_2Phe, 949-99-5; BOC-Gly, 4530-20-5; BOC-D-Pen(4-MeBzl), 115962-34-0; BOC-Tyr(2,4-Cl_2-Bzl), 62630-98-2; H-Tyr-D-Pen-Gly-(*p*-NH_2)Phe-D-Pen-OH, 118377-51-8; BOC-Thr(Bzl), 15260-10-3; BOC-Pen(4-MeBzl), 104323-41-3; BOC-Lys(4'-ClZ), 33640-54-9; BOC-D-Trp, 5241-64-5; BOC-Cys(4-MeBzl), 61925-77-7; BOC-D-Phe, 18942-49-9; H-D-Phe-Cys-(*p*-NH_2)Phe-D-Trp-Lys-Thr-Pen-Thr-NH_2, 118377-54-1.

Synthesis and Evaluation of Iodinated Analogues of Diacylglycerols as Potential Probes for Protein Kinase C

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Analogues of diacylglycerol containing a 3-(3-amino-2,4,6-triiodophenyl)-2-ethylpropanoyl or 3-(3-amino-2,4,6-triiodophenyl)propanoyl group in the 2-position (**1a** and **1b**, respectively) were synthesized and shown to compete with [^{3}H]phorbol dibutyrate ([^{3}H]PDBu) for binding in a crude rat brain preparation. Phorbol diesters have been shown to bind specifically to protein kinase C and the PDBu receptor has been copurified with protein kinase C activity. The four diastereomers of **1a** (**1c–f**) were synthesized from chiral starting material and studied in the same assay. The affinities for the [^{3}H]PDBu binding site of **1a**, **1b**, and two isomers of **1a** with naturally occurring L configuration were comparable to that of 1-oleoyl-2-acetyl-*rac*-glycerol (OAG), but the D isomers of **1a** were essentially inactive. The chirality of the side chain did not influence the binding affinity. Activation of protein kinase C by **1a**, **1c**, and **1e** demonstrated the same stereochemical requirements, but none were as active as OAG. For the 1,3-isomers **2**, **2a**, and **2b**, the competitive binding studies gave different results. The racemic mixture and the D isomer, **2b**, were able to compete for binding, but the L isomer, **2a**, did not compete. These studies demonstrate that diacylglycerol binding to and activation of protein kinase C is stereospecific for the glycerol backbone, but not the side chain. Furthermore, the D-1,3-isomer must exist in a conformation such that the acyl and hydroxyl oxygens assume a spatial relationship similar to that in the L-1,2-isomers.

Diacylglycerols have long been known to serve as intermediates in lipid biosynthesis, but in the last few years another role has been elucidated. They are endogenous modulators of protein kinase C (PK-C) activity.[1,2] PK-C is involved in many biological processes, including cellular signal transduction and proliferation (for reviews, see ref 3–8). Activation of many cellular receptors causes phospholipase C to cleave phosphatidylinositol 4,5-bisphosphate into inositol 1,4,5-triphosphate (IP_3) and diacylglycerol. Diacylglycerol then forms a complex at the membrane with phosphatidylserine (PS), Ca^{2+}, and PK-C, thus activating the enzyme.[9] The activated kinase phosphorylates many proteins, but the mechanism by which this produces further biological responses is not fully understood.

The acyl groups in the majority of diacylglycerols produced in cells are stearate and arachidonate in the 1- and 2-positions, respectively.[10] A series of synthetic diacylglycerols with a variety of chain lengths and degrees of unsaturation are also able to bind to and activate PK-C.[1,2,9,11,12] There is a good correlation between their binding and activation potencies.[12] Other structure–activity studies indicate that the ester linkages and the free hydroxyl in the sn-3-position are required.[12] Also, only naturally occurring sn-1,2-isomers (L or *S* enantiomers), and not sn-2,3-isomers, can induce PK-C to phosphorylate histone.[13] The ability of 1,3-substituted diacylglycerols to activate PK-C is uncertain due to conflicting reports.[2,9,14,15]

PK-C is also activated by phorbol diesters, a class of potent tumor promoters.[16] Though these molecules have

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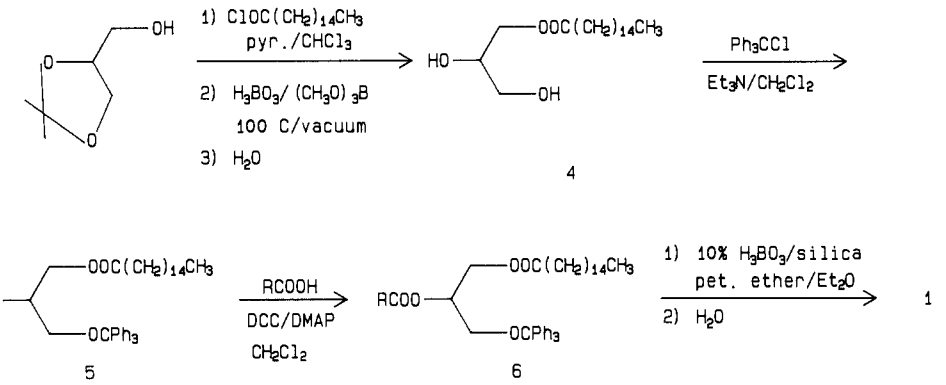
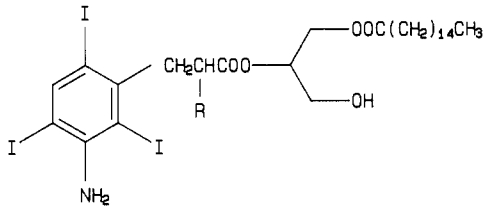
Scheme I



a complex diterpene ring system, they contain a hydroxyl and two acyl groups similar to those in diacylglycerols. They compete with diacylglycerols for binding to PK-C with about a thousandfold higher binding affinity than diacylglycerols.[17] In intact cells, phorbol diesters bypass phospholipase C activation and IP_3 production and bind to PK-C directly.[16] Like diacylglycerols, the phorbol diesters act by increasing the affinity of PK-C for Ca^{2+}, resulting in PK-C activation. This leads to numerous biological effects including tumor promotion, the mechanism of which remains to be elucidated.

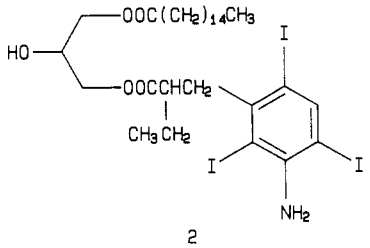
Despite the plethora of knowledge that has been acquired about PK-C in the last few years, much remains unknown. One of our interests has been the development of probes that can be utilized to study the enzyme. Diacylglycerol analogues that can be radioiodinated were of particular interest because of the ease of detection of radioiodine. These compounds can be used to determine the subcellular localization of PK-C and the tissue distribution within animals, as well as to further the knowledge of structure–activity relationships for binding and activation of PK-C.

For preliminary studies, the known imaging agent, 3-(3-amino-2,4,6-triiodophenyl)-2-ethylpropanoic acid (iopanoic acid), was incorporated into a diacylglycerol, **1a**.



1a, c–f R = CH_2CH_3

1b R = H



2

Iopanoic acid was chosen because it is commercially available and furnishes the iodine that can be readily exchanged with radioiodine.[18] The desethyl analogue, **1b**, which has an achiral iodinated side chain, was also synthesized. The four diastereomers of **1a**, **1c–f** (see Table I), were prepared separately to determine the influence of the chiral center in the acyl group upon binding to and activation of PK-C. Finally, because 1,2-diacylglycerols can undergo rearrangement to their 1,3-isomers, compound **2** and two of its diastereomers, **2a** and **2b**, were synthesized and evaluated for binding affinity.

Table I. Configurations of Chiral Centers in Diacylglycerol Analogues

compd	configuration	
	side chain	glycerol
1a	rac	rac
1b		rac
1c	*S*	L
1d	*R*	L
1e	*S*	D
1f	*R*	D
2	rac	rac
2a	*R*	L
2b	*R*	D

Chemistry

All 1,2-diacylglycerol analogues were synthesized by the steps illustrated in Scheme I. Isopropylidene glycerol was acylated with palmitoyl chloride. The product, **3**, could not be deprotected under normal acidic conditions because acid catalyzes migration of the acyl group to the secondary hydroxyl.[19] Deprotection was accomplished with the use of boric acid following the procedure of Jensen and Pitas.[20] The borate esters that were formed temporarily protected the products from acyl migration. The borate esters were readily hydrolyzed simply by washing with water, furnishing diol **4**. The primary hydroxyl of **4** was selectively protected by tritylation and the secondary hydroxyl was acylated with the acid of interest by a dicyclohexylcarbodiimide (DCC) coupling reaction to afford **6**. Detritylation of **6** was also carried out with boric acid to prevent acyl migration. In this case, Buchnea's procedure,[21] in which the acid was in a 10% mixture with silica gel, was utilized to simultaneously deprotect and purify the product.

Resolution of iopanoic acid was accomplished by the procedure of Pitre and Boveri.[22] Pitre[23] also determined the absolute configuration of the enantiomers. Resolution required the formation of the salts with (*R*)- and (*S*)-(α)-methylbenzylamine. Incomplete resolution was

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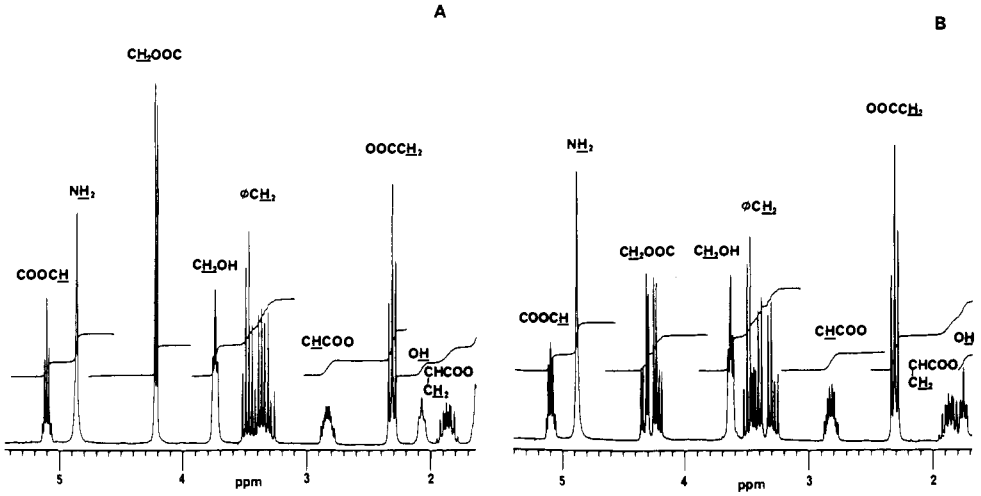

Figure 1. ^{1}H NMR spectra (270 MHz) of (A) 1-palmitoyl-2(*S*)-iopanoyl-*sn*-glycerol, **1c**, and (B) 1-palmitoyl-2(*R*)-iopanoyl-*sn*-glycerol, **1d**.

achieved by this procedure (with approximately 15% contamination with the opposite enantiomer in each), as determined by optical rotation of the acids and by NMR and HPLC analyses of the diastereomers formed by coupling these acids to the chiral glycerols. The four diastereomers (**1c–f**) were also synthesized by the method shown in Scheme I, using each enantiomer of iopanoic acid with the appropriate enantiomer of isopropylideneglycerol. Prior to detritylation, purification by HPLC was necessary to remove the impurity that resulted from incomplete resolution of iopanoic acid.

The 270-MHz NMR spectra of both the tritylated and detritylated diastereomers indicated differences in chemical shifts and splitting patterns. Mirror image pairs (i.e. 1-palmitoyl-2(*S*)-iopanoyl-3-*O*-trityl-*sn*-glycerol, **6c**, and 1-*O*-trityl-2(*R*)-iopanoyl-3-palmitoyl-*sn*-glycerol, **6f**) had the same characteristics but differed from the other pair. For example, the chemical shifts for the aromatic and amine protons on the iopanoate moiety differed slightly (see Experimental Section). Also, in the spectra of **6d** and **6e** and the detritylated diacylglycerols **1d** and **1e**, the methylene protons on the glycerol that are adjacent to the ester each appeared as a doublet of doublets because they are split by each other as well as by the neighboring methine (Figure 1B). In the spectra of **6c** and **6f**, on the other hand, these methylene protons appeared as a multiplet, and in **1c** and **1f** as simple doublets (Figure 1A). Similar patterns were seen for the methylene protons adjacent to the trityl ether. In **6d** and **6e**, each proton was a doublet of doublets, but in **6c** and **6f**, their shifts were so similar that they appeared as one doublet of doublets. In the spectra of all four diastereomers of the diacylglycerol, the methylene protons next to the hydroxyl were split into triplets, but chemical shifts were farther downfield in the spectra of **1c** and **1f** than **1d** and **1e**. The spectral variations between mirror image pairs indicate that the conformations of the two pairs are different.

1-Palmitoyl-3-iopanoyl-*rac*-glycerol (**2**) and its diastereomers, 1-palmitoyl-3(*R*)-iopanoyl-*sn*-glycerol (**2a**) and 3(*R*)-iopanoyl-1-palmitoyl-*sn*-glycerol (**2b**), were synthesized by acylation of 1-palmitoylglycerol (racemic or one enantiomer) with iopanoic acid or its *R* enantiomer. Purification by column chromatography and HPLC was required to remove the undesired isomers.

Biological Results and Discussion

Diacylglycerol analogues were assayed for their ability to compete with [^{3}H]phorbol dibutyrate ([^{3}H]PDBu) for binding to PK-C in a crude rat brain preparation by a modification of the procedure of Sharkey and Blumberg.[24] The analogues were compared to 1-oleoyl-2-acetyl-*rac*-glycerol (OAG), which is commonly used for studies of PK-C because it is active in intact cells as well as broken cell preparations.[12] Both **1a** and **1b** were comparable to OAG at concentrations of 1–100 μM in their abilities to compete for [^{3}H]PDBu binding (Figure 2A). The four diastereomers of **1a** were studied to determine if their binding affinities differ due to differences in conformation. The two diastereomers with the L configuration in the glycerol backbone, **1c** and **1d**, had similar displacement capacities to racemic **1a** (Figure 2B). We anticipated that one diastereomer would be approximately twice as active as the racemic mixture. However, this was not observed, possibly because the assay was carried out with a crude preparation containing many lipids and proteins that may have affected binding. As expected, the two D diacylglycerols, **1e** and **1f**, were only able to compete with [^{3}H]PDBu at the highest concentrations tested. These results indicate, for the first time, that the presence of a chiral center in the side chain does not influence binding to PK-C and confirmed that the stereochemistry of the glycerol backbone is essential. Also, the structure of the side chain, which in these molecules is much bulkier than those in previously studied diacylglycerols, had little effect on binding to PK-C.

The 1,3-substituted diacylglycerol, **2**, was also able to compete for binding but not to the same extent as its 1,2-isomer (Figure 3). Contrary to the results for the diastereomers of **1a**, the D-1,3-isomer was able to compete with [^{3}H]PDBu with about the same affinity as the racemic mixture, but the L-1,3-diacylglycerol did not compete significantly. This indicates that the relative position of the acyl and hydroxyl oxygens must be similar between the L-1,2- and D-1,3-diacylglycerol analogues. Molecular models of these compounds indicate that this stereochemical arrangement is possible.

These data fit the models for the diacylglycerol/PK-C/Ca^{2+}/PS complex that have been proposed by others.[9,25] In these models, the side chains are projected into the lipid bilayer so their binding is nonspecific. The hydroxyl and acyl oxygens form a three-point attachment with Ca^{2+} and PK-C, which explains the stereochemical requirements for this part of the molecule.

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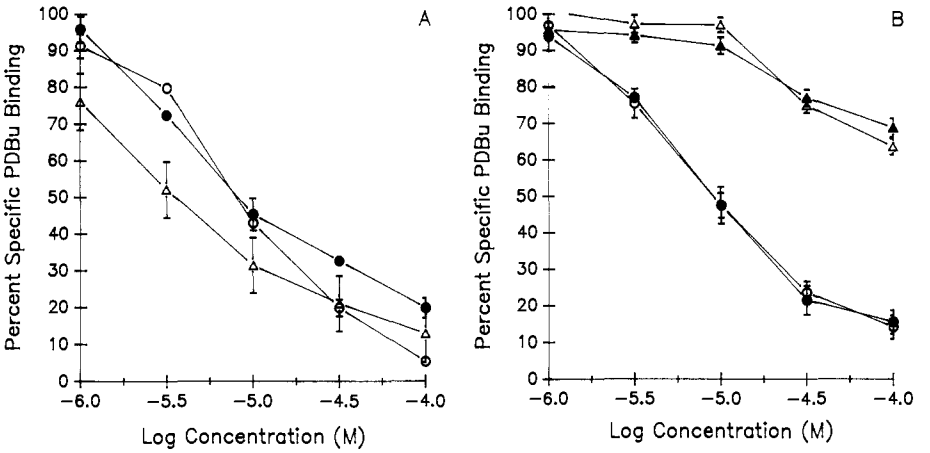

Figure 2. Competitive binding with [^{3}H]PDBu (20 nM) in a crude rat brain preparation. (A) 1-Palmitoyl-2-iopanoyl-*rac*-glycerol, **1a** (●); 1-palmitoyl-2-[3-(3-amino-2,4,6-triiodophenyl)propanoyl]-*rac*-glycerol, **1b** (△); and 1-oleoyl-2-acetyl-*rac*-glycerol (○). (B) 1-Palmitoyl-2(*S*)-iopanoyl-*sn*-glycerol, **1c** (○); 1-palmitoyl-2(*R*)-iopanoyl-*sn*-glycerol, **1d**, (●); 2(*S*)-iopanoyl-3-palmitoyl-*sn*-glycerol, **1e** (△); and 2(*R*)-iopanoyl-3-palmitoyl-*sn*-glycerol, **1f** (▲).

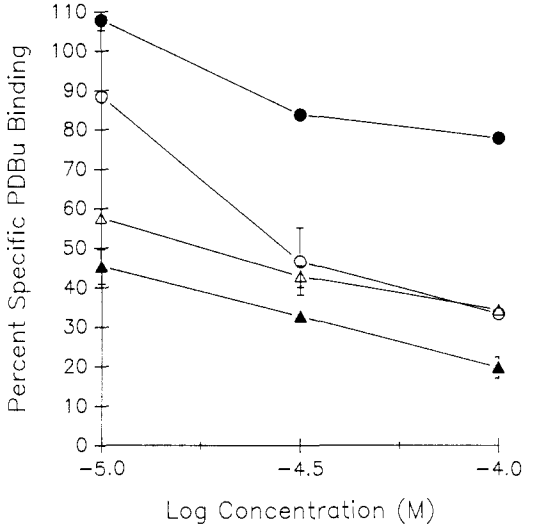

Figure 3. Competitive binding with [^{3}H]PDBu (20 nM) in a crude rat brain preparation. 1-Palmitoyl-2-iopanoyl-*rac*-glycerol, **1a** (▲); 1-palmitoyl-3-iopanoyl-*rac*-glycerol, **2** (○); 1-palmitoyl-3(*R*)-iopanoyl-*sn*-glycerol, **2a** (●); and 1(*R*)-iopanoyl-3-palmitoyl-*sn*-glycerol, **2b** (△).

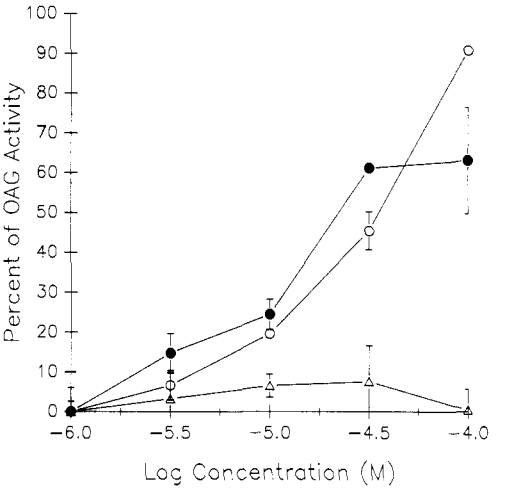

Figure 4. Activation of protein kinase C by diacylglycerol analogues. The amount of [^{32}P] transferred from [^{32}P]ATP to histone IIIs was determined in the presence of 1 μg/mL of PS and increasing concentrations of diacylglycerols. 1-Palmitoyl-2-iopanoyl-*rac*-glycerol, **1a** (○); 1-palmitoyl-2(*S*)-iopanoyl-*sn*-glycerol, 1c (●); and 2(*S*)-iopanoyl-3-palmitoyl-*sn*-glycerol, **1e** (△).

The activation of PK-C by **1a** and the two enantiomers. **1c** and **1e**, was determined by measuring the transfer of [^{32}P]phosphate from [^{32}P]ATP to histone according to the procedure of Leach and Blumberg.[26] Because of the insolubility of these analogues in the aqueous media of the assay mixture, 0.1% DMSO was included in the diacylglycerol preparation. This concentration did not influence the activity of PK-C. In order to make comparison between experiments carried out with different enzyme preparations, the data are presented as the percent of activation of the standard OAG (10 μM). For all three compounds, the activity was much lower than that of OAG (Figure 4). As with the binding studies, the D diastereomer, **1e**, was essentially inactive. The L isomer, **1c**, on the other hand, was as active as the racemic mixture, **1a**.

The differences in experimental procedure between the two assays may explain the fact that the binding abilities of the analogues were similar to OAG, but PK-C activation by the analogues was much lower than that caused by OAG. The enzyme was more highly purified in the kinase assay. Therefore, the sensitivity of PK-C to the presence of the diacylglycerols may have been different between the binding and kinase assays. Another possible explanation is that these analogues are able to bind to PK-C but are unable to achieve the correct position in the enzyme–lipid complex to fully activate the enzyme. Further studies are required to determine if they are indeed partial agonists.

In conclusion, several iodinated diacylglycerol analogues that differ markedly in structure from the natural activators of PK-C were found to bind to PK-C and may prove to be useful tools for further studies of the enzyme, particularly when radioiodinated. Other iodinated analogues that more closely resemble natural diacylglycerols are presently under investigation.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Bruker WP270SY NMR instrument in $CDCl_3$ with TMS as internal standard. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Elemental analyses were performed by Midwest Microlabs, Indianapolis, IN, and are within ±0.4% of the calculated values. Iopanoic acid was obtained from Tokyo Kasei and other organic reagents from Aldrich Chemical Co. [^{3}H]PDBu (12–16 Ci/mmol) and [^{32}P]ATP were obtained from DuPont-New England Nuclear. Bovine brain phosphatidylserine,

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PDBu, and bovine γ-globulin were obtained from Sigma Chemical Co. Rats were obtained from Charles River Co.

HPLC Separation of Diastereomers of 6. A Whatman M20 Partisil 10 column was used with 20:1 (v/v) hexane/ethyl acetate as the mobile phase at a flow rate of 9 mL/min. Components were detected by their absorbance at 254 nm.

1-Palmitoyl-2-iopanoyl-3-*O*-trityl-*rac*-glycerol (6a). Anhydrous CH_2Cl_2 (80 mL) was added to a flask that contained 1-palmitoyl-3-*O*-trityl-*rac*-glycerol[20] (**5**, 5.00 g, 8.73 mmol), iopanoic acid (4.98 g, 8.72 mmol), and a catalytic amount of 4-(dimethylamino)pyridine (DMAP). DCC (1.81 g, 8.75 mmol) was added and the mixture stirred overnight at room temperature. After removal of the dicyclohexylurea by filtration, the filtrate was washed with 0.5 N HCl (2×), saturated $NaHCO_3$, water, and brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated. The crude oil (9.96 g) was divided in half and each portion purified by chromatography (120 g of silica gel, 8:1 hexanes/ethyl acetate). The pure fractions were combined, yielding **6a**: 5.88 g (60%). NMR and IR data were as expected. Anal. ($C_{49}H_{82}I_3NO_5$) C, H.

1-Palmitoyl-2-[3-(3-amino-2,4,6-triiodophenyl)-propanoyl]-3-*O*-trityl-*rac*-glycerol (6b). The reaction was carried out as described for **6a**, but with 3-(3-amino-2,4,6-triiodophenyl)propanoic acid (4.00 g, 7.37 mmol), 1-palmitoyl-3-*O*-trityl-*rac*-glycerol (4.22 g, 7.37 mmol), and DCC (1.53 g, 7.39 mmol) in CH_2Cl_2 (70 mL). Chromatography (180 g of silica gel, 7:1 hexanes/ethyl acetate) yielded pure **6b**: 6.22 g (77%); mp 58.5–60 °C. NMR and IR data were as expected. Anal. ($C_{47}H_{58}I_3NO_5$) C, H, I.

1-Palmitoyl-2-iopanoyl-*rac*-glycerol (1a). A column was prepared with 10% H_3BO_3 in silica gel[21] using petroleum ether as the solvent. The tritylated compound (**6a**, 0.9 g, 0.8 mmol) was applied to the top of the column, then eluted with petroleum ether (450 mL) until a yellow band spread throughout the column. Petroleum ether/diethyl ether (95:5) was used until the yellow color was gone (200 mL) and then 3:1 petroleum ether/diethyl ether was used (750 mL) to elute the borate ester of the diacylglycerol. The solvent was removed, the residue dissolved in $CHCl_3$, and the solution washed with water (3 × 35 mL). The $CHCl_3$ layer was dried ($MgSO_4$) and the solvent removed, leaving pure **1a**: 0.61 g (97%); mp 54–57 °C. NMR and IR data were as expected. Anal. ($C_{30}H_{48}I_3NO_5$) C, H, I.

1-Palmitoyl-2-[3-(3-amino-2,4,6-triiodophenyl)-propanoyl]-*rac*-glycerol (1b). Detritylation of **6b** was carried out as described for **6a**. The protected compound (**6b**, 1.50 g, 1.37 mmol) was loaded on the column and eluted, in order, with petroleum ether (800 mL), 95:5 petroleum ether/diethyl ether (200 mL), and 5:1 petroleum ether/diethyl ether (400 mL). Hydrolysis of the borate ester produced **1b**: 0.83 g (71%). NMR and IR data were as expected. Anal. ($C_{28}H_{44}I_3NO_5$) C, H, I.

Resolution of Iopanoic Acid. The procedure of Pitre and Boveri[22] was utilized to afford 2.87 g (24%) of (*R*)-(−)-iopanoic acid [mp 155.5–158 °C, $[\alpha]_D = -4.1 \pm 0.1°$ (*c* 2, EtOH) (lit. mp 162–163 °C, $[\alpha]_D = -5.2 \pm 0.1°$)] and 2.47 g (21%) of (*S*)-(+)-iopanoic acid [mp 158–160 °C, $[\alpha]_D = +3.4 \pm 0.3°$ (*c* 2, EtOH) (lit. mp 162 °C $[\alpha]_D = +5.1 \pm 0.1°$)].

1-Palmitoyl-2(*S*)-iopanoyl-3-*O*-trityl-*sn*-glycerol (6c). A DCC coupling reaction was utilized as described previously with 1-palmitoyl-3-*O*-trityl-*sn*-glycerol (0.30 g, 0.53 mmol), (*S*)-iopanoic acid (0.30 g, 0.53 mmol), and DCC (0.12 g, 0.58 mmol) in CH_2Cl_2 (4.8 mL). Column chromatography (75 g silica gel, 10:1 hexanes/ethyl acetate) afforded 0.55 g (93%) of **6c**. Removal of the isomer that resulted from incomplete resolution of iopanoic acid required preparative HPLC: retention time, approximately 1.9 h; 270-MHz NMR δ 8.07 (s, 1 H, Ar *H* on iop.), 7.20–7.45 (m, 15 H, Ar *H* on trityl), 5.28–5.37 (quint, 1 H, COOC*H*), 4.84 (s, 2 H, NH_2), 4.18–4.32 (m, 2 H, CH_2OOC), 3.30–3.37 (m, 2 H, PhCH_2), 3.26–3.28 (dd, 2 H, CH_2OC), 2.80–2.90 (m, 1 H, C*H*COO), 2.18–2.23 (t, 2 H, OOCCH_2), 1.8–1.9 (m, 1 H, CH_2CHCOO), 1.49–1.55 (m, 3 H, CH_2CHCOO and OOCCH$_2$CH_2), 1.21–1.32 (br s, 24 H, (CH_2)$_{12}$), 0.84–0.98 (m, 6 H, CH_3).

1-Palmitoyl-2(*R*)-iopanoyl-3-*O*-trityl-*sn*-glycerol (6d). The above procedure was repeated with (*R*)-iopanoic acid, producing 0.46 g (77%) of **6d**. The retention time on HPLC was approximately 1.4 h; 270-MHz NMR δ 7.99 (s, 1 H, Ar *H* on iop.), 7.21–7.44 (m, 15 H, Ar *H* on trityl), 5.29–5.39 (quint, 1 H, COOC*H*), 4.73 (s, 2 H, NH_2), 4.35–4.41 (dd, 1 H) and 4.17–4.25 (dd, 1 H) (CH_2OOC), 3.28–3.37 (m, 2 H, PhCH_2), 3.20–3.25 (dd, 1 H) and 3.09–3.15 (dd, 1 H) (CH_2OC), 2.72–2.84 (m, 1 H, C*H*COO), 2.18–2.23 (t, 2 H, OOCCH_2), 1.8–1.9 (m, 1 H, CH_2CHCOO), 1.48–1.56 (m, 3 H, CH_2CHCOO and OOCCH$_2$CH_2), 1.21–1.32 (br s, 24 H, (CH_2)$_{12}$), 0.84–0.91 (m, 6 H, CH_3).

1-*O*-Trityl-2(*S*)-iopanoyl-3-palmitoyl-*sn*-glycerol (6e). This was prepared in the same manner as **6c** with 1-*O*-trityl-3-palmitoyl-*sn*-glycerol (0.25 g, 0.44 mmol), (*S*)-iopanoic acid (0.25 g, 0.44 mmol), and DCC (0.10 g, 0.48 mmol) in CH_2Cl_2 (4 mL). The yield was 0.43 g (86%) of **6e**. The retention time and NMR data were identical with those of 1-palmitoyl-2(*R*)-iopanoyl-3-*O*-trityl-*sn*-glycerol (**6d**).

1-*O*-Trityl-2(*R*)-iopanoyl-3-palmitoyl-*sn*-glycerol (6f). Conditions were identical with those for **6e**. The reaction yielded 0.38 g (73%) of product **6f** with retention time and NMR data identical with those for **6c**.

1-Palmitoyl-2(*S*)-iopanoyl-*sn*-glycerol (1c). The precursor (**6c**, 0.30 g, 0.27 mmol) was detritylated with 10% H_3BO_3 on silica gel as described previously, yielding 0.18 g (75%) of product: mp 61–62 °C; 270-MHz NMR δ 8.08 (s, 1 H, Ar *H*), 5.07–5.11 (quint, 1 H, COOC*H*), 4.86 (s, 2 H, NH_2), 4.20–4.22 (d, 2 H, CH_2OOC), 3.72–3.78 (t, 2 H, CH_2OH), 3.39–3.47 (dd, 1 H) and 3.23–3.37 (dd, 1 H) (PhCH_2), 2.78–2.90 (m, 1 H, C*H*COO), 2.28–2.34 (t, 2 H, OOCCH_2), 2.07 (t, 1 H, O*H*), 1.8–1.9 (m, 1 H, CH_2CHCOO), 1.55–1.63 (m, 3 H, CH_2CHCOO and OOCCH$_2$CH_2), 1.25 (br s, 24 H, (CH_2)$_{12}$), 0.85–0.94 (m, 6 H, CH_3).

1-Palmitoyl-2(*R*)-iopanoyl-*sn*-glycerol (1d). Deprotection of **6d** (0.31 g, 0.27 mmol) afforded 0.20 g (89%) of the diacylglycerol: mp 62–63 °C; 270-MHz NMR δ 8.09 (s, 1 H, Ar *H*), 5.07–5.11 (quint, 1 H, C*H*COO), 4.87 (s, 2 H, NH_2), 4.29–4.38 (dd, 1 H) and 4.20–4.25 (dd, 1 H) (CH_2OOC), 3.61–3.65 (t, 2 H, CH_2OH), 3.38–3.40 (dd, 1 H) and 3.30–3.32 (dd, 1 H) (PhCH_2), 2.78–2.89 (m, 1 H, C*H*COO), 2.27–2.33 (t, 2 H, OOCCH_2), 1.75 (t, 1 H, O*H*), 1.8–1.9 (m, 1 H, CH_2CHCOO), 1.55–1.63 (m, 3 H, CH_2CHCOO and OOCCH$_2$CH_2), 1.25 (br s, 24 H, (CH_2)$_{12}$), 0.86–0.96 (m, 6 H, CH_3).

2(*S*)-Iopanoyl-3-palmitoyl-*sn*-glycerol (1e). The trityl group was removed from **6e** (0.35 g, 0.31 mmol), leaving 0.17 g (61%) of **1e**: mp 64–66 °C; NMR data were identical with those for **1d**.

2(*R*)-Iopanoyl-3-palmitoyl-*sn*-glycerol (1f). Detritylation of **6f** (0.22 g, 0.20 mmol) yielded 0.16 g (88%) of **1f**: mp 63–65 °C; NMR data were identical with those for **1c**.

1-Palmitoyl-3-iopanoyl-*rac*-glycerol (2). 1-Palmitoyl-*rac*-glycerol (**4**, 0.75 g, 2.27 mmol), iopanoic acid (1.30 g, 2.27 mmol), a catalytic amount of DMAP, and CH_2Cl_2 (25 mL) were combined in a flame-dried flask. DCC (0.52 g, 2.50 mmol) was added and the mixture stirred at room temperature overnight. The DCU was removed by filtration and the filtrate washed with 1 N HCl (2×), saturated $NaHCO_3$, water, and brine. The organic phase was dried ($MgSO_4$) and evaporated, leaving a mixture of products (2.14 g). Chromatography with gradient elution (150 g of silica gel, 10:1, 8:1, 6:1, 4:1 hexanes/ethyl acetate) yielded a fraction enhanced in the 1,3-isomer (**2**, 0.98 g, 49%). Further purification (90 g of silica gel, 3:1:1 hexanes/ethyl acetate/$CHCl_3$) produced a small pure fraction of **2**. Removal of the last traces of the undesired isomer required HPLC (mobile phase, 20% hexane in $CHCl_3$; flow rate, 0.7 mL/min; retention time, 33 min). **2**: 270-MHz NMR δ 8.08 (s, 1 H, Ar *H*), 4.85 (s, 2 H, NH_2), 4.01–4.18 (m, 5 H, CH_2COO and C*H*OH), 3.37–3.45 (dd, 1 H) and 3.20–3.31 (dd, 1 H) (CH_2Ph), 2.75–2.90 (m, 1 H, C*H*COO), 2.32–2.38 (t, 2 H, OOCCH_2), 2.27–2.29 (d, 1 H, *H*O), 1.79–1.91 (m, 1 H, CH_2CHCOO), 1.56–1.63 (m, 3 H, CH_2CHCOO and CH_2CH$_2$COO), 1.25 (br s, 24 H, (CH_2)$_{12}$), 0.85–0.96 (m, 6 H, CH_3). Anal. ($C_{30}H_{48}I_3NO_5$) C, H, I.

1-Palmitoyl-3(*R*)-iopanoyl-*sn*-glycerol (2a). This was synthesized as described for the racemic compound for 1-palmitoyl-*sn*-glycerol (187.4 mg, 0.57 mmol), (*R*)-iopanoic acid (323.7 mg, 0.57 mmol), DCC (129.6 mg, 0.63 mmol), and a catalytic amount of DMAP in CH_2Cl_2 (6.3 mL). Purification by chromatography (60 g of silica gel, 6:1 hexanes/ethyl acetate) yielded 101.7 mg of **2a** (20%). HPLC, as described above, was used to further purify the product. **2a**: 270-MHz NMR δ 8.07 (s, 1 H, Ar *H*), 4.84 (s, 2 H, NH_2), 3.99–4.16 (m, 5 H, CH_2OOC and HOC*H*), 3.30–3.44 (dd, 1 H) and 3.22–3.30 (dd, 1 H) (CH_2Ph), 2.80 (m, 1 H, C*H*COO), 2.30–2.36 (m, 3 H, *H*O and OOCCH_2), 1.8–1.9 (m,

1 H, CH_2CHCOO), 1.58–1.65 (m, 3 H, CH_2CHCOO and $OOCCH_2CH_2$), 1.24 (br s, 24 H, $(CH_2)_{12}$), 0.85–0.95 (m, 6 H, CH_3).

1(*R*)-Iopanoyl-3-palmitoyl-*sn*-glycerol (2b). A DCC coupling reaction was carried out with 3-palmitoyl-*sn*-glycerol (200.0 mg, 0.61 mmol), (*R*)-iopanoic acid (345.5 mg, 0.61 mmol), DCC (139.4 mg, 0.67 mol), and a catalytic amount of DMAP in CH_2Cl_2 (6.7 mL). Chromatography (60 g of silica gel, 6:1 hexanes/ethyl acetate) afforded 213 mg of **2b** (40%), which was further purified by HPLC as described above: 270-MHz NMR δ 8.08 (s, 1 H, Ar *H*), 4.85 (s, 2 H, NH_2), 4.08–4.21 (m, 4 H, CH_2OOC), 4.01–4.01 (quint, 1 H, HOC*H*), 3.37–3.45 (dd, 1 H) and 3.21–3.32 (dd, 1 H) (CH_2Ph), 2.80 (m, 1 H, $CHCOO$), 2.32–2.38 (m, 2 H, $OOCCH_2$), 2.27–2.29 (d, 1 H, *H*O), 1.8–1.9 (m, 1 H, CH_2CHCOO), 1.58–1.66 (m, 3 H, CH_2CHCOO and $OOCCH_2CH_2$), 1.24 (br s, 24 H, $(CH_2)_{12}$), 0.85–0.96 (m, 6 H, CH_3).

PDBu Receptor Assay. A modification as previously described[27] of the method of Sharkey and Blumberg[24] was used to assess analogue affinity for PDBu receptor binding. Briefly, rat brain cytosol was prepared from whole Sprague–Dawley rat brains by homogenization in 50 mM Tris-HCl (pH 7.4) with Brinkman polytron. The homogenate was centrifuged at 100000*g* for 60 min. The resulting supernatant was divided into aliquots and stored at −70 °C until used. Protein concentration was determined by the method of Bradford.[28] Reaction mixture with total volume of 250 μL contained 0.02 μM [^{3}H]PDBu, rat brain cytosol (200 μg/mL protein), 0.05 M Tris-HCl (pH 7.4), 4 mM $CaCl_2$, 100 μg/mL PS, and 2 mg/mL bovine γ-globulin. Nonspecific binding was determined by measuring binding in parallel reactions containing 12 μM PDBu. Diacylglycerols were prepared by sonicating with PS in water twice for 15 s at a setting of 3–4 on the Bronson Cell Disruptor 200. The reaction tubes were incubated at 37 °C for 30 min and then put on ice for 5 min. Polyethylene glycol (PEG) was added to give a final concentration of 15%, the tubes vortexed, left on ice for 15 min, and spun in a microfuge for 5–10 min. The supernate was removed, and the pellets were washed with 400 μL of 15% PEG, cut from the centrifuge tubes, dried, and radioactivity determined by liquid scintillation spectroscopy using a Beckman L581000 counter. All experiments were conducted in duplicate or triplicate.

Protein Kinase C Activation Assay. The enzyme was prepared as described by Leach and Blumberg.[26] Following DEAE chromatography, the peak fractions were made 1 M in ammonium sulfate and applied to a phenylsepharose column (0.75 × 30 cm) and eluted with an ammonium sulfate gradient (1.0–0 M). The fractions containing activity were pooled and stored at −20 °C in the presence of 10% glycerol and 0.01% Triton X-100. Activity was determined by measuring the transfer of ^{32}P from [^{32}P]ATP to histone IIIs. The reaction mixture had a total volume of 50 μL and contained 40 mM Tris-HCl (pH 7.4), 15 mM magnesium acetate, 50 μM [^{32}P]ATP (225-325 dpm/pmol), 75 μg of histone IIIs, and 11 μg of enzyme. The diacylglycerols were sonicated with PS and DMSO for 20 s in Tris-HCl buffer such that the final concentrations in the reactions were 1 μg/mL PS and 0.1% DMSO. The reactions were carried out in 1.5 mL tubes for 7 min at 30 °C. The tubes were placed on ice immediately after incubation and 25 μL from each spotted on 2 × 2 cm^2 phosphocellular paper. This was washed five times with water and once with acetone, dried, and counted.

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Crystallographic Resolution and Crystal and Molecular Structures of Stereoisomers of 1,3,5-Triglycidyl-*s*-triazinetrione

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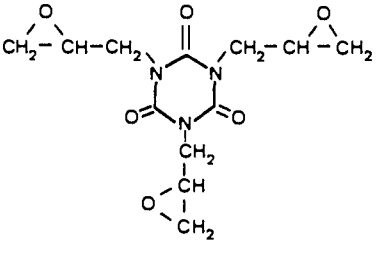
The crystal and molecular structures of α and β isomers of the antineoplastic alkylating agent 1,3,5-triglycidyl-*s*-triazinetrione (TGT) have been determined by X-ray diffraction. Although the isomers differ chemically only in the order of a carbon and an oxygen atom in one of the glycidyl epoxide rings, the molecular conformations and crystal packing arrangements are very different. The different physical and biological properties of the two stereoisomers can be explained on the basis of the structures. The sample of α-TGT was found to be a mixture of α and β forms, and it is suggested that use of pure α-TGT may lead to better therapeutic results.

Alkylating agents comprise one of the most useful classes of anticancer drugs, and much attention has been devoted to the search for new agents with better therapeutic potentials. The alkylating properties of epoxy groups are well known and the antitumor activities of bifunctional epoxides have been described. Only recently, however, have compounds with larger numbers of epoxy functions been investigated as anticancer agents; one of the first of these is the triepoxide 1,3,5-triglycidyl-*s*-triazinetrione (TGT). Originally synthesized by Budnowski,[1] TGT (I) can be resolved into two stereoisomers, α-TGT and β-TGT, possessing *R,R,S*/*S,S,R* and *R,R,R*/*S,S,S* configurations, respectively, in the epoxide groups. Despite having only this one small structural difference, the two forms differ significantly in their physical properties, α-TGT having a lower melting point (105 vs 156 °C)[1] and a much higher water solubility (1.01 vs 0.053% at 20 °C)[2] than β-TGT.



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The anitumor properties of α- and β-TGT have been investigated in various transplantable mouse tumor sys-

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