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, *HO*), 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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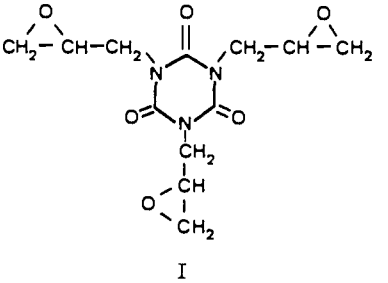
The crystal and molecular structures of α and β isomers of the antineoplastic alkylating agent 1,3,5-triglycidyl-*s*-triazinetrione (TGT) have been determined by X-ray diffraction. Although the isomers differ chemically only in the order of a carbon and an oxygen atom in one of the glycidyl epoxide rings, 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.



I

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

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Table I. Crystal Data

α-TGT	β-TGT
$C_{12}H_{15}N_3O_6$	$C_{12}H_{15}N_3O_6$
$M_r = 297$	$M_r = 297$
orthorhombic	rhombohedral
space group = $Pna2_1$	space group = R3
$a = 9.198$ (5) Å	$a = 8.964$ (5) Å
$b = 9.475$ (6) Å	$\alpha = 103.15$ (7)°
$c = 15.507$ (8) Å	
$V = 1351$ Å^3	$V = 652$ Å^3
$Z = 4$	$Z = 2$
asymmetric unit 1 molecule	asymmetric unit $^1/_3$ molecule
intensities with I's GE $3\sigma(I)$ = 981 (of 1193 collected)	intensities with I's GE $3\sigma(I)$ = 402 (of 757 collected)
range $0 < 2\theta < 130°$	range $0 < 2\theta < 130°$
$D_{calcd} = 1.46$ g cm^{-3}	$D_{calcd} = 1.51$ g cm^{-3}
μ(Cu Kα) = 10.3 cm^{-1}	μ(Cu Kα) = 10.6 cm^{-1}

tems;[2] both stereoisomers displayed high therapeutic indices, but α-TGT was superior in prolonging the lifespan of treated animals. α-TGT has also shown[3] some selectivity for cancer cells, low carcinogenic activity, and potency against tumors refractory to cyclophosphamide, indicating good clinical potential for the drug. A number of phase I trials[4-7] of α-TGT, however, have been disappointing, primarily due to thrombophlebitis at the injection site, a situation that could not be circumvented by dilution because of the poor solubility of the drug.

The striking antineoplastic activity of TGT has prompted us to elucidate the crystal and molecular structures of both stereoisomers,[8] in order to account for their differing physical properties, and to allow correlation of conformational features with biological efficacies. During the preparation of the crystals we discovered that the sample of α-TGT was not isomerically pure, but was in fact a mixture of both forms.

Experimental Section

Samples of α-TGT (NSC 296934) and β-TGT (NSC 296964) were obtained from the Drug Development Branch, National Cancer Institute. Crystals were grown in aqueous ethanol, and crystal data are given in Table I. Two crystal forms in approximately equal amounts crystallized together from the sample of α-TGT; one of the forms was shown by X-ray diffraction to be identical with the crystals of β-TGT. X-ray diffraction intensities were measured on an automated four-circle diffractometer using Cu Kα radiation and θ–2θ scan mode; the usual corrections were made and an empirical ϕ-sweep absorption correction was applied. The structures were solved with MULTAN 80.[9] Positional and anisotropic thermal parameters were refined by full-matrix least-squares methods. All hydrogen atoms were located on difference electron density maps; the five independent β-TGT hydrogen atoms and six of the 15 α-TGT hydrogens were included in the refinement with a fixed $B = 4.0$ Å^2. Convergence occurred at $R = 0.071$ for α and $R = 0.061$ for β. The atomic scattering factors used were as cited for non-hydrogen[10] and hydrogen[11] atoms. Final difference electron denisty maps for both isomers were essentially featureless, with maximum peak heights of ±0.4 electron. Fractional coordinates and equivalent isotropic temperatures factors of the non-hydrogen atoms are listed in Table II. Tables of anisotropic thermal parameters, hydrogen atom coordinates, and bond lengths and angles have been deposited as supplementary material.

Table II. Fractional Atomic Coordinates (×10^4) and Equivalent Isotropic Thermal Parameters (Å^2)

atom	x	y	z	B_{eq}
		α-TGT		
N1	5311 (8)	−1314 (7)	80 (0)	3.3 (4)
C2	4116 (12)	−418 (10)	28 (9)	3.6 (4)
N3	3593 (4)	75 (4)	836 (8)	2.9 (2)
C4	4122 (9)	−312 (9)	1595 (7)	2.8 (2)
N5	5281 (8)	−1213 (7)	1584 (4)	2.8 (2)
C6	5955 (6)	−1698 (6)	858 (9)	3.2 (4)
C7	5979 (12)	−1666 (12)	−759 (8)	3.6 (4)
C8	5319 (10)	−3007 (10)	−1112 (7)	4.3 (4)
C9	4312 (14)	−2978 (13)	−1862 (10)	6.5 (5)
O10	5812 (10)	−3424 (8)	−1935 (7)	6.1 (5)
C11	2301 (6)	989 (7)	813 (9)	4.3 (4)
C12	2691 (10)	2461 (9)	537 (7)	5.8 (5)
C13	3078 (15)	3524 (12)	1123 (10)	7.9 (6)
O14	1620 (6)	3489 (6)	790 (10)	7.4 (6)
C15	5973 (12)	−1757 (14)	2399 (9)	4.6 (4)
C16	5261 (15)	−3103 (12)	2726 (10)	6.7 (5)
C17	4419 (13)	−2942 (15)	3451 (9)	6.6 (5)
O18	5822 (10)	−3501 (9)	3539 (7)	6.4 (5)
O19	3596 (8)	−35 (8)	−632 (7)	4.5 (4)
O20	3569 (9)	44 (8)	2290 (8)	5.2 (4)
O21	7030 (5)	−2435 (5)	819 (7)	5.6 (4)
		β-TGT		
N1	514 (5)	1498 (5)	2438 (5)	4.1 (4)
C2	467 (8)	2488 (7)	1460 (6)	4.0 (4)
C3	−442 (7)	1590 (8)	3559 (7)	4.6 (4)
C4	429 (7)	2834 (7)	5128 (7)	5.1 (4)
C5	1687 (9)	4244 (9)	5368 (9)	6.2 (5)
O6	2046 (5)	2900 (5)	5824 (5)	6.3 (5)
O7	−406 (5)	3319 (5)	1440 (4)	5.6 (4)

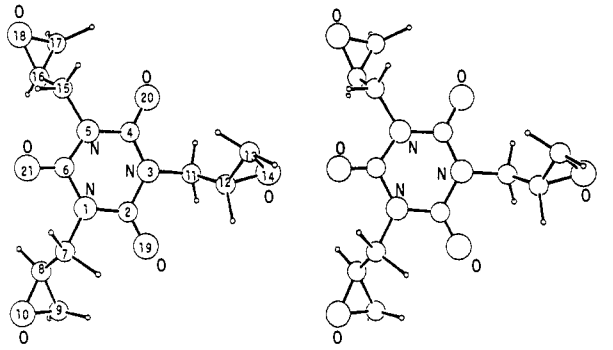

Figure 1. Molecular conformation of α-TGT and the atomic numbering scheme.

Results and Discussion

The structure determinations have confirmed that α-TGT and β-TGT are diastereomeric racemates with *R,R,S/S,S,R* and *R,R,R/S,S,S* configurations, respectively, at the epoxide groups in the two isomers. The molecular conformations in the crystals are shown in Figure 1 for α-TGT and Figure 2 for β-TGT. Although the two compounds differ in chemical structure only in the order of two (non-hydrogen) atoms (CH_2–O) in one epoxide group,

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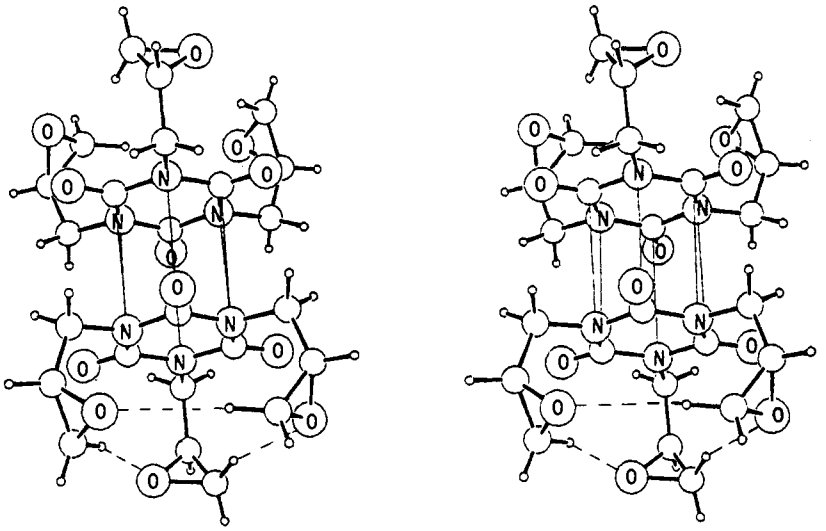

Figure 2. Stereoscopic view of the dimeric association of β-TGT.

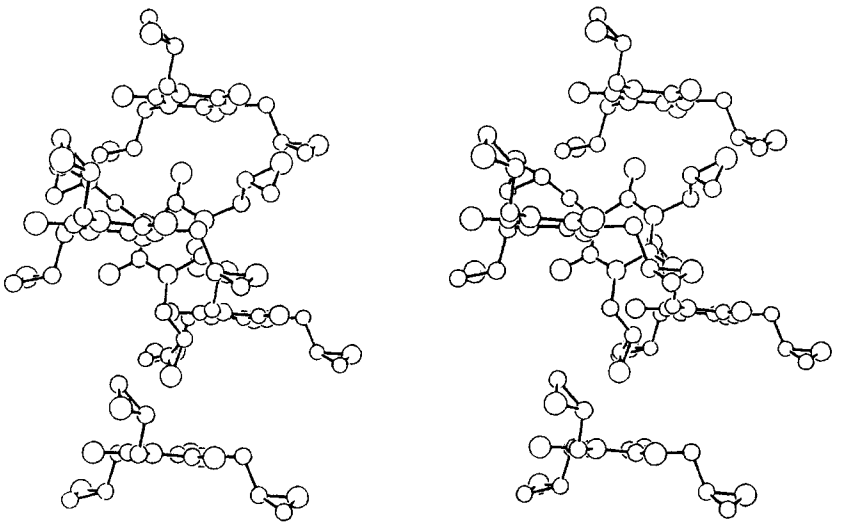

Figure 3. Stereoscopic view of the molecular packing of α-TGT.

their gross conformations are quite different: in α-TGT one of the glycidyl side chains is on the opposite side of the triazinetrione ring from the other two, while the β isomer has all three glycidyl chains on the same side of the central ring. Torsion angles describing the orientations of the side chains with respect to the rings are C2–N1–C7–C8 = −92°, C6–N5–C15–C16 = −92°, and C4–N3–C11–C12 = +108° for α-TGT, and corresponding angles in β-TGT = 92° (all three angles are equivalent due to molecular and crystal symmetry). In addition, the glycidyl groups adopt trans conformations in α-TGT (N–C–C–O torsion angles are +177°, −159°, +173°) and gauche conformations (N–C–C–O = −44°) in the β isomer. The triazinetrione ring is planar in both isomers.

The effect of the slight structural difference between α- and β-TGT on the crystal packing arrangement is very dramatic and allows us to readily explain the differences in physical properties of the two isomers. Figure 2 shows that β-TGT forms dimers in the solid state, with full overlapping of the triazinetrione rings, at a separation of 3.4 Å. Stacking of the π-electron systems in this way is possible because of the positioning of the three glycidyl chains on the same side of the ring. These strong dimeric associations are undoubtedly the major contributing factor to the higher melting point and lower solubility of the β isomer. The observed β-TGT conformation is further stabilized by interactions among the gauche-conformation glycidyl groups: inspection of Figure 2 reveals that a methylene hydrogen atom in each epoxide ring points at the oxygen of an adjacent epoxide in the same molecule. These are weak directed van der Waals contacts (H···O distance is 2.8 Å), but an accumulation of them, as here, has a significant effect (see below). The β-TGT dimers form columns in the crystal, held together by additional directed van der Waals interactions of the kind described above.

Although there is no a priori reason why the α isomer could not position its three glycidyl groups on the same side of the triazinetrione ring and form dimers similar to β-TGT, as shown in Figure 1 it adopts a different conformation, with extended trans positionings of the side chains, resulting in a less strongly bonded crystal structure. The apparent reason for this is that the interchange of positions of the carbon and oxygen atom in one epoxide ring prevents the regular series of methylene hydrogen···oxygen van der Waals interactions which stabilize the gauche configuration of the glycidyl chains, and without which even the stronger stacking interaction is insufficient for the dimer arrangement to be effected. Thus the greater solubility and lower melting point of the α isomer. The crystal packing arrangement of α-TGT is shown in Figure 3. Each molecule is sandwiched by two others whose triazinetrione planes are perpendicular to its plane, and the structure is stabilized by electronic interactions be-

tween π-electrons in the sandwiching planes and a carbonyl oxygen atom at one end of the molecule and an epoxide oxygen atom in the para-positioned glycidyl group. Distances between the carbonyl O21 and N3, C2, C4 of the sandwich plane are 2.88–3.11 Å, and between the epoxide O14 and the six atoms of the opposite plane range from 3.10 to 3.17 Å.

Bond distances and angles within the TGT molecules, although not specifiable with high precision because of the limited X-ray data, fall within normal ranges of values.

As stated earlier, crystals of both isomers of TGT were obtained together from solvent evaporation of a solution of what was supposedly pure α-TGT. We measured melting points of the "α-TGT" sample and of crystals of the two isomers in order to correlate the substances with previously reported characterizations. Melting points were as follows: mixture 105–108 °C, α 104–106 °C, β 148–152 °C.

Conclusions

The crystal and molecular structure determinations of α- and β-TGT have revealed the basis for their differing physical properties. Ring stacking dimeric association, facillitated by regular interactions between glycidyl groups that are possible only when their configurations are *R,R,R/S,S,S*, is the reason for the much lower solubility and higher melting point of the β isomer. Differences in biological properties between α- and β-TGT, e.g. greater ability of α-TGT to prolong lifespan in treated animals, are probably also attributable solely to differences in solubility, as the only distinguishing feature between *R,R,S/S,R,R* and *R,R,R/S,S,S* configurations is the propensity of the latter to form intra- and intermolecular associations.

The melting point of the mixed sample of TGT is the same as that of the α isomer, leading one to question whether some of the clinical trials were conducted with TGT mixtures, rather than pure α-TGT as reported. If so, the major clinical problem, thrombophlebitis due to poor drug solubility, may be somewhat alleviated by the use of α-TGT only.

Because of the significant antineoplastic activity of TGT, it would be highly desirable to obtain biologically active derivatives with much greater water solubilities. In this respect it is noteworthy that no report of resolving either TGT racemate into its enantiomers has yet appeared. Analysis of the TGT crystal structures suggests that enantiomers of β-TGT should be able to adopt packing arrangements similar to the racemate, but the situation for α-TGT enantiomers could possibly be different, and therefore perhaps lead to increased solubility. Additionally, the TGT structure determinations suggest that modifications to the triazinetrione ring should be attempted, to reduce electronic π-character and hence reduce intermolecular attractions of the kinds observed in both isomers and increase solubility. One such modified compound, triglycidylurazol, has recently been undergoing clinical studies.[12-14]

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Registry No. α-TGT, 59653-74-6; β-TGT, 59653-73-5.

Supplementary Material Available: Figure showing bond lengths and angles and tables listing anisotropic thermal parameters and hydrogen atom coordinates for α-TGT and β-TGT (5 pages). Ordering information is given on any current masthead page.

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Effects of Steroid D-Ring Modification on Suicide Inactivation and Competitive Inhibition of Aromatase by Analogues of Androsta-1,4-diene-3,17-dione

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Analogues of androsta-1,4-diene-3,17-dione (**3a**) in which the D ring is modified were prepared and tested as suicide inactivators and competitive inhibitors of human placental aromatase. As long as the five-membered ring is intact, modifications of the D ring such as reduction or removal of the carbonyl group or conversion to a γ-butyrolactone cause a <6-fold decrease in affinity for and rate of inactivation of aromatase, compared to **3a**. Thus, an oxygen atom at C-17 is not required for binding of these inhibitors to aromatase, suggesting that hydrogen bonding to the D-ring oxygen does not play a major role in binding. Opening the D ring converts the cyclopentane ring to an alkyl chain and causes a >300-fold decrease in affinity; this can be partially reversed by shortening the chain length. These results are consistent with a model in which the free chain of the opened D ring adopts conformations that sterically interfere with binding of the inhibitor to the enzyme. These findings may have practical applications in drug design, by allowing the preparation of 17-deoxo analogues that have high affinity for aromatase but that are not subject to reduction of the 17-carbonyl group, which is a major mode of metabolism of **3a**.

Estrogens are linked to a number of human diseases, including carcinoma of the breast; the ability to decrease the physiologic activity of estrogens is therefore an important clinical goal. Owing to the wide anatomic distribution of aromatase,[1-3] the enzyme that biosynthesizes estrogens from androgens (Scheme I, **1a** → **1b** → **1c** → **2**), a pharmacologic approach is the only practical way to block

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