Stereoselective Disposition of the Geometric Isomers of a Novel Lipoxygenase Cyclo-oxygenase Inhibitor in Dog and Photochemical Interconversion of Its Isomers

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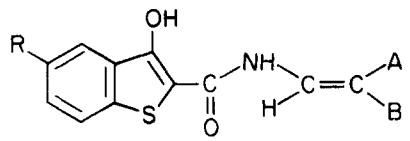
Abstract □ A sensitive (10 ng/mL) and specific high-performance liquid chromatographic (HPLC) assay, with electrochemical (EC) detection, for the geometric isomers of 3-hydroxy-*N*-(2-phenyl-2-(2-thienyl)ethenyl-5-(trifluoromethyl)benzo(*b*)thiophene-2-carboxamide in dog and human plasma has been developed. Both isomers strongly absorb light, leading to an efficient $E \Leftrightarrow Z$ photoisomerization. After iv administration of a single isomer (*Z*) to a dog, only the *Z* isomer was detected in plasma; no in vivo conversion to the *E* isomer was observed. However, when a mixture of the *E* and *Z* isomers (58.6:41.4) was administered in the same manner to the same dog, the *E*:*Z* ratio decreased significantly to 47.5:52.5 six hours after drug administration, indicating stereoselective disposition of the isomers. The elimination of the *E* isomer was found to be faster than that of the *Z* isomer.

Biochemical data have shown that *N*-(2,2-diarylethenyl)-3-hydroxybenzo(*b*)thiophene-2-carboxamides are a novel class of inhibitors of both the 5-lipoxygenase and cyclo-oxygenase pathways of arachidonic acid metabolism. A study of the structural requirements for the biological activity led to the selection of compound **1** as a clinical candidate: it is obtained synthetically as an eutectic mixture of its *E* (**2**) and *Z* (**3**) geometric isomers. Both the enamide substructure and the trifluoromethyl substituent have been found to be required for dual inhibitory activity in vivo.[1,2]

These studies have also indicated that **1** has a greater therapeutic index as an analgesic than indomethacin, ibuprofen, and piroxicam. As an anti-inflammatory agent, the therapeutic index of **1** was found to be greater than that of indomethacin and ibuprofen. Thus, **1** has been evaluated clinically as an agent possessing novel analgesic/anti-inflammatory activity. Human clinical studies were performed using the mixture of the *E* and *Z* isomers, and a nonstereoselective assay for **1** in human plasma and urine, based on HPLC with UV detection, was initially developed in our laboratories to support these studies. In order to assess the pharmacokinetic properties of the individual isomers, the development of a sensitive and stereoselective assay was necessary. It was found that **1**, its isomers, and several analogous compounds are all electrochemically (EC) active, allowing their determination using an HPLC method with EC detection. This paper describes the details of the stereoselective assays for the isomers of **1** in plasma based on the HPLC/EC methodology.

The transport of drugs in the blood and into target cells, storage of drugs, and their interaction with chiral living systems are often found to exhibit stereoselectivity. This is especially well documented for optical isomers.[3] The presence of a chiral center generally implies large differences for the enantiomers both in the activity and in their metabolic conversion and pharmacokinetic behavior.[4-7] These differences are equally likely for other types of stereoisomeric drugs,[8] among them asymmetrically substituted alkenes such as **2** and **3**. Similarly, as optical isomers, the geometric isomers may also produce distinct pharmacodynamic responses and may differ in their pharmacokinetic behavior. With the newly developed stereoselective HPLC/EC assay, pharmacokinetic properties of the individual isomers of **1** could be evaluated in dog. The studies on the potential interconversion of the isomers both in vivo and during sample preparation and dosing were also required to establish the validity of the analytical methodology employed and to support any conclusions about the in vivo disposition of the isomers. The results of all of these studies indicate that no in vivo conversion of the *Z* to *E* isomers takes place, and the disposition of the isomers is stereoselective with the *E* isomer (**2**) being eliminated faster than the *Z* isomer (**3**). Due to an efficient $E \Leftrightarrow Z$ photoisomerization, special precautions were necessary to avoid photochemical interconversion of the isomers during sample preparation and drug administration to animal subjects.



(1) Mixture (2)/(3) (58.6/41.4)

(2) A=  , B=  , R=CF_3; E-isomer

(3) A=  , B=  , —"—; Z-isomer

(4) A=B=  , R=H

Experimental Section

Reagents—Acetonitrile, methanol, ethyl acetate, sodium hydroxide (10M solution), and standard buffer solutions (pH 4 and 7) were HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ). Tetrabutylammonium hydroxide (TBAH, 40% w/w, aqueous solution) and sodium phosphate monobasic (anhydrous) were purchased from Sigma Chemical Company (St. Louis, MO). The analytical standards of **1**, **2**, and **3**, and the internal standard *N*-(2,2-diphenylethenyl)-3-hydroxybenzo(*b*)thiophene-2-carboxamide (**4**), were obtained from Merck Sharp & Dohme Research Laboratories (Rahway, NJ). Blank human plasma was supplied by Sera-Tec Biologicals (North Brunswick, NJ). Standard solutions of **1**–**4** were prepared in various solvents (vide infra) and were stored at −20 °C when not in use.

Instrumentation—The Waters Associates 703 HPLC system,

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equipped with a 730 data module, a 720 system controller, a WISP 710B automatic injector, and a 6000A chromatographic pump (Waters-Millipore, Milford, MA) was used for all analyses. The detector was a Bioanalytical Systems (West Lafayette, IN) electrochemical detector equipped with a TL-8A flow cell with a single glassy carbon electrode, the RE-3 Ag/AgCl reference electrode, and an LC-4B amperometric controller. Also, a Kratos (Ramsey, NJ) variable wavelength UV absorbance detector (Spectroflow 733) was used in the initial stages of the assay development. The detector output signals were interfaced to a Hewlett-Packard Laboratory Automation System (HP 3357 LAS, Palo Alto, CA). The absorption spectra of 1 and its isomers were taken using a diode array spectrophotometer (Hewlett-Packard, model 8451). The cyclic voltammograms were obtained using the Bioanalytical Systems model CV-1B cyclic voltammograph, equipped with a model VC-2 cell, a working glassy carbon electrode, and an Ag/AgCl reference electrode.

Chromatographic Conditions—The mobile phase in the stereoselective assay (SS) was a mixture of acetonitrile and 0.005 M TBAH in a 0.0125 M NaH_2PO_4 (adjusted to pH 6.9 with 5M NaOH):methanol (35:40:25, v/v/v) solution, delivered at a flow rate of 1 mL/min. Two Chemcosorb 5-ODS-H C-18 analytical columns (15 × 0.46 cm, 5-μm spherical particle size; DyChrom, Sunnyvale, CA) were used in series. The potential applied to the working glassy carbon electrode of the EC cell was +0.60 V versus the standard Ag/AgCl electrode. The amperometric controller output was used at a sensitivity setting of 5 nA full scale and a time constant of 0.3 s.

In the initially developed nonstereoselective assay (NSS) for **1**, a Sepralyte C18 (5 μm, 4.6 × 250 mm) column, a mobile phase consisted of a mixture of methanol:2% acetic acid (85:15, v/v) that was delivered at a flow rate of 1 mL/min, and a UV detector set at 393 mm were utilized.

All mobile phase components were measured separately, mixed, and filtered through a 0.2-μm nylon 66 filter (Rainin Instruments, Woburn, MA). A Micro Guard Refill Cartridge ODS-10 (4 × 0.46 cm, BioRad Laboratories, Richmond, CA) was used as a guard column.

Standard Solutions—The stock standard solutions of **1** and **3** (1 mg/mL) were prepared in acetonitrile and mobile phase, respectively. The stability of **3** was significantly improved when mobile phase was substituted for acetonitrile. The reason for this better stability of **3** in the mobile phase (pH 6.9) than in neat acetonitrile is not known at present. Similar instability of **3** was noted in the other neat organic solvents, namely methanol and ethyl acetate. In the latter case, this is probably due to the presence of traces of acids which may have caused an acid-catalyzed $E \Leftrightarrow Z$ interconversion. The stock solutions of the internal standard (**4**, I.S.) were prepared in acetonitrile. Due to limited solubility, the concentration of the stock standard was 0.24 mg/mL. All of these findings were very important in choosing the mobile phase and the appropriate solvents for the liquid–liquid extraction of the isomers from biological matrices in the SS assay (vide infra), but were of no significance in the NSS assay.

The standard solutions were further diluted to give a series of working standards with concentrations of 100, 10, and 1 μg/mL for **1** and **3**, and 25 and 10 μg/mL for **4**.

Due to the photochemical instability of the isomers *(vide infra)*, all manipulations with sample containing isomers (preparation, dilution of standards, etc.) have to be carried out in amber or aluminum foil-covered glassware and tubes, preferably in the dark room with only the red light on. Under these conditions, the stability of the individual isomers in the plasma samples and in the selected stock solutions were found to be adequate for the routine assay of the isomers.

The cyclic voltammogram of **1** was obtained using a 1×10^{-3} M solution of **1** in the mixture of ethanol and 0.2 M phosphate buffer (pH 7.5; 40:60, v/v).

Sample Preparation—Plasma (1 mL) and **4** (I.S., 20 μL of 10 μg/mL) were transferred to a polypropylene conical tube, and 1.2 mL of acetonitrile was added. The latter solvent was employed in the SS assay as both a deproteinizing and extracting medium. Several other extracting solvents were initially utilized, among them ethyl acetate, *t*-butyl ether, and a mixture of hexane and isoamyl alcohol. All of them were found to be inadequate due either to the instability of the isomers, poor recoveries, or extraction of impurities from plasma coeluting with compounds of interest or with the internal standard. Following vortexing (30 s) and centrifugation (10 min at 5000 rpm), an aliquot (750 μL) of the supernatant was mixed with 250 μL of the mixture methanol:water (1:1, v/v) to adjust the solvent strength to that of the mobile phase. After mixing, a 200-μL aliquot of this solution was directly injected into the HPLC/EC system. The amount injected was equivalent to only ~8% of the drug present in plasma. However, in order to avoid any interconversion of the isomers, no further pre-concentration or additional extraction steps to increase the amount of material available for injection on the column were attempted. These limitations were absent in the NSS assay where ethyl acetate (5 mL) extraction followed by solvent evaporation, reconstitution of the residue in the mobile phase (250 μL), and injection of the 40% (100 μL) of the material on column, were employed. Due to the high sensitivity and specificity of the EC detection, the very simple and straightforward sample preparation procedure described for the SS assay was adequate for achieving the required detection sensitivity of 10 ng for each isomer per milliliter of plasma, with no conversion of the isomers occurring during the workup.

Precision, Accuracy, Linearity, and Recovery—The standard curves for **1** and **3** were constructed by spiking human control plasma (1 mL) with known concentrations of **1** and **3** plus 200 ng of internal standard (**4**). This was done by spiking plasma with the appropriate volumes of the working standards and the I.S., and adjusting the volume of the added acetonitrile in each case to 0.2 mL. After the addition of 1 mL of acetonitrile (total acetonitrile volume equal to 1.2 mL), the assay procedure described above was followed. For **1**, the standard line was obtained from analysis of the combined areas of the two isomers **2** and **3**. The standard curves were constructed by plotting the drug concentration versus the drug peak areas or the ratio of drug to internal standard peak areas. Since generally better precision was obtained in the first case, the peak areas were employed for constructing calibration curves and calculation of the concentrations of the *E* and *Z* isomers. Unknown sample concentrations were calculated from the equation $y = mx + b$, as determined by the weighted linear regression analysis of the standard curve. The weight was set to equal the inverse of the variance at each concentration.

The precision of the method was determined by replicate analyses ($n = 6$) of human plasma at concentrations of 10 to 10,000 ng/mL. The coefficients of variation (CV) were always <10% for both **1** and **3**.

The detector response appeared to be linear in the wide range of concentrations of **1**, but a more reliable estimate could be obtained when two separate standard curves in the 10–250- (correlation coefficient $r = 0.9947$) and 250–10,000-ng/mL ($r = 0.9980$) ranges were employed. The reason for this better reliability was less bias caused by the extreme concentrations of the standard curve on the measured values, and was probably due to the limited dynamic range of the detector. For **3**, one standard curve in the range 10–1000 ng/mL ($r = 0.9991$) was employed.

The slopes of standard curves for both **1** (as a sum of **2** and **3**) and **3** were the same, within experimental error, indicating that the EC response was the same for both isomers and that the relative peak area of the isomers can be used as a direct measure of the relative isomer content expressed in ng/mL.

The recovery of **1** and **3** from plasma was >95% at all concentrations.

Photochemical Interconversion of 2 and 3—Stock solutions (10 μg/mL) of **1**, **2**, or **3** in water (pH 9.3, adjusted with NaOH), a mixture of water and methanol (1:1, v/v, pH 9.3), or the mobile phase (SS assay), respectively, were placed in the Pyrex autosampler vials (1-mL capacity, 6 mm in diameter). Vials were placed on the laboratory bench and exposed to polychromatic laboratory room light (LRL). At various intervals, samples were analyzed directly by injecting 10-μL aliquots into the HPLC/EC system.

In Vivo Studies on the Conversion and Disposition of the Isomers of 1 in a Dog—The study of conversion of the *Z* to *E* isomer was performed in the following manner. To an adult beagle dog (8 kg), 7 mL of dosing solution containing 0.57 mg/mL of **3** in water (pH 9.3) was administered intravenously via the cephalic vein (0.5 mg/kg). Blood samples (~6 mL) were drawn up to 24 h post dose. Blood was collected in heparinized tubes covered with aluminum foil; plasma was isolated by centrifugation for 15 min at 5000 rpm. The content of the *E* (**2**) and *Z* (**3**) isomers in dog plasma was determined. The preparation of the infusion solution, its dosing, and blood sample collections were all performed using aluminum foil-covered tubes, glassware, and syringes.

The experimental protocol was the same in the study of the disposition of the isomers in a dog using the mixture of $E + Z$ isomers (**1**). Here, however, **3** was replaced by **1**, and the same dog was dosed

with 1.56 mg/kg of 1 containing 58.6 + 0.5% of 2 and 41.4 + 0.5% of 3. The content (ng/mL) of the *E* (2) and *Z* (3) isomers in dog plasma was determined here according to the SS assay procedure developed in human plasma. The latter procedure was found to be fully adequate for assaying 2 and 3 in dog plasma.

Results and Discussion

Stereoselective Assay of 1 and 3—After the initial clinical studies with 1, the development of a sensitive and specific assay for the geometric isomers 2 and 3 was necessary in order to study the pharmacokinetic properties of the individual isomers of 1. The originally developed NSS assay was based on HPLC with UV detection, and no separation of the isomers was possible. The retention time for 1 was 8.25 min. Also, interconversion of the isomers in ethyl acetate, used as an extracting medium, was observed. Therefore, a new approach was required to overcome all of the limitations of the NSS assay.

A baseline separation of 2 and 3 was achieved using the analytical columns and mobile phase described in the *Experimental Section*. Under the SS assay conditions utilized, the retention times for 2, 3, and 4 were 27.3, 29.7, and 11.5 min, respectively. However, when UV detection was attempted under these new conditions, the quantitation limit was only 100 ng/mL for each isomer. In order to improve assay sensitivity and be able to support future pharmacokinetic studies in human subjects, a more sensitive detection, other than UV, was desired.

Cyclic voltammetry measurements have indicated that both isomers of 1 are EC active, making it possible to develop the HPLC assay based on EC detection. Several analogues of 1 were also found to be EC active, among them 4. Since geometric isomerization is not possible for this compound, 4 was chosen as the I.S. for the SS assay. The low oxidation potential of 1 and its analogues (Figure 1) makes the HPLC/EC method especially suitable and specific since oxidation of many extractable plasma components is minimized.

The hydrodynamic voltammograms for 2 and 3 are shown in Figure 1. Since plasma extracts gave clean chromatograms at the potential of +0.6 V in the elution region of 2, 3, and 4, the EC flow cell was set at this potential to increase method selectivity without compromising sensitivity.

Photochemical Interconversion of the *Z* and *E* Isomers of 1 in Liquid Phase—Both isomers of 1 strongly absorb light in the short wavelength region of the visible spectrum (Figure 2). The ε values of 17,800 and 12,700 $M^{-1}\,cm^{-1}$ in methanol at 400 nm were measured for 2 and 3, respectively. The absorption in the long wavelength region extends to 450 nm, creating the possibility for photochemical interconversion or other photochemical transformations of the isomers when samples are prepared and handled in Pyrex glass tubes. These tubes are partially transparent to the radiation with wavelengths >300 nm (Figure 2). Both isomers were found to be very sensitive to exposure to laboratory room light (LRL), as illustrated in Figure 3 and Table I.

A very efficient photochemical conversion of the thermodynamically less stable *Z* isomer (3) to the *E* isomer (2) takes place, leading to the photostationary state ratio (pss) in which the ratio of the isomers ($[E]_{pss}$:$[Z]_{pss}$, eq 1) is practically the same no matter which isomer or what mixture of isomers was initially used (Table I). As expected,[9,10] the *E* isomer was found to be photochemically more stable than the *Z* isomer.

$$\frac{[E]_{pss}}{[Z]_{pss}} = \frac{\varepsilon_Z \Phi_{Z \rightarrow E}}{\varepsilon_E \Phi_{E \rightarrow Z}} \qquad (1)$$

The pss ratio depends on the molar absorption coefficient of the isomers at the wavelength (λ) of excitation (ε_Z, ε_E) and quantum yields (Φ) of $Z \rightarrow E$ and $E \rightarrow Z$ photoisomerization. Since both ε and Φ depend on λ and on the solvent, and a polychromatic "light source" (LRL) was utilized in our studies, the pss ratio was not necessarily the same. In all cases, however, it was close to *E*:*Z* ≈ 80:20 (Table I).

The data in the last column of Table I also indicate that the total area of the *E* and *Z* peaks measured with EC detection is the same and independent of the actual ratio of the *E* and *Z* isomers in the mixture. This confirms the previous observation that the EC response is the same for both *E* and *Z* isomers and that, by measuring the relative peak heights or peak areas of the isomers, one can directly measure the relative content of both isomers expressed in ng/mL.

In Vivo Study with 3 on the Conversion of the *Z* to *E* Isomers of 1 in a Dog—Before any conclusions could be reached about the relative disposition of the isomers, the study of the conversion of the *Z* to *E* isomer in vivo was performed to determine if such isomer conversion takes place.

The contents (%) of the *Z* and *E* isomers before and at various time intervals after the injection of predominantly one isomer (97.0 ± 0.4% of 3 and 3.0 ± 0.4% of 2) to a male beagle dog are given in Table II. No conversion of the *Z* to the *E* isomer was observed in vivo. The relative disposition of the isomers cannot be reliably assessed from these data due to

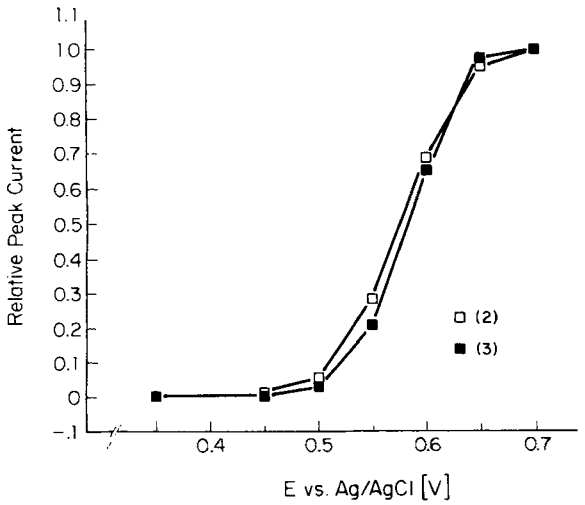

Figure 1—*Hydrodynamic voltammograms of* **2** *and* **3**. *Peak current responses, relative to peak current at +0.7 V, were plotted as a function of applied potential.*

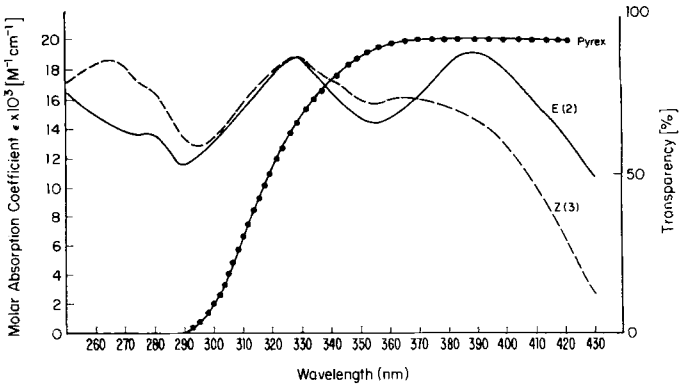

Figure 2—*Absorption spectra of* **2** *and* **3** *in methanol and transparency curve of Pyrex glass (2 mm thickness) in the near UV and visible region of the spectrum.*

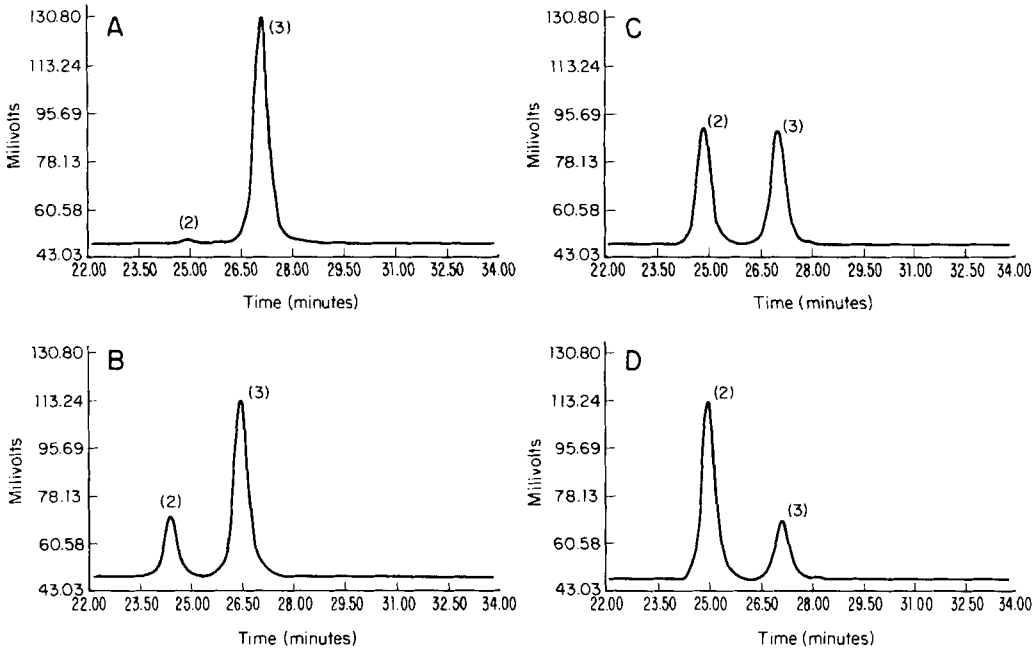

Figure 3—*Photochemical conversion of the Z* (**3**) *to E* (**2**) *isomer of* **1** *during exposure to polychromatic laboratory room light (for experimental conditions, see text). Figures* A, B, C, *and* D *represent the chromatograms after 0-, 20-, 60-, and 120-min exposures, respectively.*

Table I—Photochemical Interconversion of the *Z* (3) and *E* (2) Isomers of 1

Starting Compound	Time of Exposure to LRL, min	*E* Isomer (**2**), %	*Z* Isomer (**3**), %	Total Area of **1** (*Z* + *E*)[a]
Z Isomer (**3**)	0	2.0	98.0	1.00
	3	4.0	96.0	0.98
	7	13.0	87.0	0.98
	15	20.6	79.4	1.00
	20	24.5	75.5	1.01
	30	31.4	68.6	1.00
	60	48.5	51.5	0.99
	120	73.7	26.3	1.01
	150	76.3	23.7	0.98
E + *Z* Isomers (**1**)	0	58.7	41.7	1.00
	720	74.5	25.5	1.01
	840	77.4	22.6	1.02
E Isomer (**2**)	0	88.9	11.1	1.00
	720	83.9	16.1	0.99

[a] In arbitrary units, taken as 1.00 at time zero.

Table II—Content of the *Z* (3) and *E* (2) Isomers of 1 in Dog Plasma after Intravenous Injection of 3[a]

Time, h	Content, %	
	3 *Z* Isomer	**2** *E* Isomer
0	97.0[b]	3.0[b]
0.083	97.3	2.7
0.25	97.7	2.3
0.50	97.8	2.2
1	97.8	2.2
2	97.6	2.4
3	97.2	2.8
4	96.8	3.2
6	96.2	3.8
24	—[c]	—[c]

[a] Dose of 0.5 mg/kg. [b] This is the content (%) of the isomers in the infusion solution administered to the dog, which is found to be the same as in dog plasma spiked with the infusion solution; the content of **3** was 97.0 ± 0.4%, and was determined as an average of the content for the infusion solution immediately after preparation and after infusion. [c] The peak area of **2** was too small to measure.

the presence of predominantly one isomer in the infused mixture and large error of the *E*:*Z* ratio determinations.

Study of the Disposition of the *Z* and *E* Isomers of 1 in a Dog—After establishing that no in vivo interconversion of the *Z* to the *E* isomer is observed, similar studies were performed with the mixture of isomers (**1**) to determine if the disposition of the geometric isomers of **1** are stereoselective, and which, if any, of the isomers is eliminated faster.

The results of this study are summarized in Table III in which the content (%) of each of the isomers before and at various time intervals after drug injection, together with the concentrations of the isomers in dog plasma, are presented.

The data indicate that the *E*:*Z* ratio, after an initial small increase, decreases significantly from 58.6:41.4 to 47.5:52.5 six hours after drug administration. Assuming no in vivo interconversion of the *E* (**2**) to *Z* (**3**) isomers, a thermodynamically very unfavorable process, these results indicate a stereoselective disposition of the isomers in dog.

Pharmacokinetic Analysis—Plasma concentrations of the *E* and *Z* isomers following the bolus administration of 1.56 mg/kg of **1** to the beagle dog were each found to be adequately described by a biexponential function:

$$Cp = A \exp(-\alpha t) + B \exp(-\beta t) \qquad (2)$$

The best-fit parameters associated with the biexponential equation were obtained by weighted nonlinear regression, which is a modification of the Simplex optimization algorithm.[11] The weight was assumed to be proportional to the reciprocal of the observed plasma concentrations. The computed pharmacokinetic parameters are listed in Table IV. Comparisons of the observed and best-fit curves are given in Figure 4. These results show that the two isomers have comparable initial distribution volume. However, the *Z* iso-

Table III—Content of the *E* (2) and *Z* (3) Isomers of 1 in Dog Plasma after Intravenous Infusion of 1[a]

Time, h	2 *E* Isomer	3 *Z* Isomer	Ratio *Z*:*E*	Plasma Concentration, ng/mL	
				E Isomer	*Z* Isomer
0	58.6[b]	41.4[b]	0.706	—	—
0.083	60.2	39.8	0.661	7,650	5,050
0.25	59.2	40.8	0.689	5,960	4,110
0.50	58.4	41.6	0.712	4,740	3,370
1	56.0	44.0	0.786	3,320	2,610
2	53.0	47.0	0.887	2,010	1,780
3	51.2	48.8	0.953	1,140	1,080
4	49.5	51.5	1.040	774	790
5	47.7	52.3	1.096	555	607
6	47.5	52.5	1.105	396	439
24	—[c]	—[c]	—[c]	<10	—[c]

[a] Dose of 1.56 mg/kg. [b] The content of the individual isomers in the infusion solution and in the dog plasma spiked with this solution was repeatedly determined (18 determinations) and shown to be the same and unchanged after storage in the dark in the refrigerator. [c] The area under the *Z* (3) peak was too small to measure.

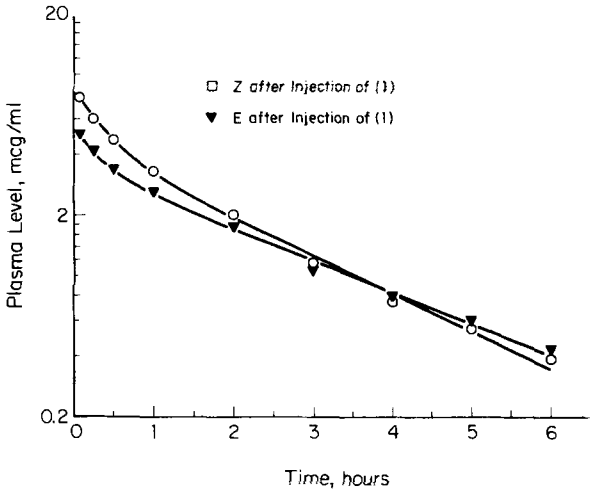

Figure 4—*The semilog plot illustrating the dependence between the concentration (ng/mL) of the isomers of* **1** *in dog plasma and time after drug injection.*

Table IV—Pharmacokinetic Parameters following Intravenous Administration of a Mixture of the *E* and *Z* Isomers (1) to a Beagle Dog

Parameter	Z Isomer	E Isomer
Dose, mg/kg	0.646	0.914
A, ng/mL	2.061	3.972
B, ng/mL	3.545	4.487
α, h^{-1}	3.203	2.569
β, h^{-1}	0.363	0.426
Half-life (0.693/β), h	1.91	1.63
Total [AUC] (A/α + B/β), μg h/mL	10.4	12.1
Initial distribution volume [Dose/(A + B)]	115	108
Total clearance (Dose/[AUC]), L/h/kg	62	76

mer is eliminated slightly more slowly than the *E* isomer, indicating stereoselective disposition kinetics of the two isomers.

In conclusion, the plasma levels of the photochemically labile geometric isomers of 1 can be determined using the newly developed stereoselective assay procedure that is based on HPLC with EC detection. This assay procedure requires special handling of the plasma samples during sample preparation and drug administration to human and animal subjects. The disposition of the isomers in a dog is stereoselective, leading to a faster elimination of the *E* isomer (2).

References and Notes

1. Allison, D. L.; Anderson, R. L.; Bach, R. J.; Bailey, P. J.; Baker, R. K.; Barker, P. L.; Bonney, R. J.; Dallob, A. L.; Derks, M.; Dougherty, H. W.; Egan, R. W.; Gallagher, T. F.; Goldenberg, M. M.; Ham, E. A.; Hand, K. M.; Hopple, S. L.; Humes, J. L.; Lanza, T. J.; Luell, S.; Meurer, R.; Miller, D.; Moore, V. L.; Rosa, R.; Rupprecht, K. M.; Tischler, A. N.; Witzel, B. E. *Abstracts*, 193rd National Meeting of the American Chemical Society, Denver, CO; American Chemical Society: Washington, DC, April 1987; Abstract MEDI 041.
2. Goldenberg, M. M.; Buntinx, A.; DeSchepper, P.; Gresele, R.; Vermylen, J., submitted for publication in *Clin. Pharmacol. Ther.*
3. Simonyi, M.; Fitos, I.; Visay, J. *Trends Pharmacol. Sci.* **1986**, *7(3)*, 112.
4. Testa, B. *Trends. Pharmacol. Sci.* **1986**, *7(2)*, 60.
5. Drayer, D. E. D. *Clin. Pharmacol. Ther.* **1986**, *40(2)*, 125.
6. Ariens, E. J. *Trends Pharmacol. Sci.* **1986**, *7(5)*, 200.
7. Walle, T.; Walle, U. K. *Trends Pharmacol. Sci.* **1986**, *7(4)*, 155.
8. Lehmann, F. P. A. *Trends Pharmacol. Sci.* **1986**, *7(7)*, 281.
9. Cowan, D. O.; Drisko, R. L. *Elements of Organic Photochemistry*; Plenum: New York, 1976; p 367.
10. Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry*; Plenum: New York, 1980; Part A, p 484.
11. Nelder, J. A.; Mead, R. *Comput. J.* **1965**, *7*, 308.