Stereospecific Metabolic Reduction of Ketones

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Abstract □ The stereospecificity of the metabolic reduction of arylalkylketones was investigated. The ketones, propiophenone (I), phenylacetone (III), and 1-phenyl-1,2-propanedione (V) were reduced *in vitro* and *in vivo* in rats and rabbits to the corresponding alcohols, 1-phenyl-1-propanol (II), 1-phenyl-2-propanol (IV), and 1-phenyl-1,2-propanediol (VIII), respectively. For the analysis, a capillary GLC method employing chiral derivatizing reagents for the resolution of these optically active alcohols was utilized. This study revealed that the metabolic reduction of each ketone produced the corresponding alcohol as a mixture of its enantiomers. With one exception, the mixtures obtained from all *in vivo* and *in vitro* reactions were shown to contain at least 70% of one isomer [*S*(−)-II, *S*(+)-IV, and *erythro*-VIII, respectively], with *in vitro* reduction showing the highest degree of stereospecificity (90–98%). The *in vivo* reduction of I by the rat was exceptional in that both optical isomers of II were recovered in equal proportions.

Keyphrases □ Ketones—stereospecific metabolic reduction, arylalkylketones, rats and rabbits □ Stereospecificity—metabolic reduction of arylalkylketones, rats and rabbits □ Metabolism—reduction of arylalkylketones, stereospecificity, rats and rabbits

Various studies on the biotransformation of xenobiotic ketones have established that ketone reduction is an important metabolic pathway in mammalian tissue (1–12). It has also been shown that when ketones are reduced metabolically, a significant product stereoselectively can be demonstrated to occur upon creation of the chiral alcohol (11–16). To determine the degree of stereoselectivity, a reliable method is required for the resolution of the stereoisomers. In many metabolic studies, the amount of product available for stereochemical analysis is often very small, making isolation and, hence, resolution procedures difficult.

As a continuation of earlier studies (2, 10), the metabolic reduction of three arylalkylketones and the efficient separation of the recovered stereoisomeric alcohols are described. Separation was accomplished by reacting the metabolites with optically active derivatizing reagents and resolving the resulting diastereoisomers by GLC. *In vivo* and *in vitro* metabolic reduction was studied in two species (the rat and the rabbit), and a comparison of the extent to which stereoselectivity occurs is provided.

EXPERIMENTAL

Compounds—Propiophenone (I), phenylacetone (III), and 1-phenyl-1,2-propanedione (V) were obtained commercially[1] and used as received. *RS*-1-Phenyl-1-propanol (II) and *RS*-1-phenyl-2-propanol (IV) were prepared by the asymmetric reduction of the corresponding arylalkylketone with the chiral sodium L-prolinate borane complex prepared according to a previously described procedure (17). Reduction of I gave a 72:28 ratio of the enantiomers of II [*S*(−)/*R*(+), respectively] as determined by polarimetric analysis[2]. Reduction of III gave a 60:40 ratio of the enantiomers of IV [*S*(+)/*R*(−), respectively]. The *erythro* and *threo* diastereoisomers of 1-phenyl-1,2-propanediol (VIII) were synthesized as previously reported (2).

Reagents—Trifluoroacetic anhydride (IX)[3] and *S*(+)-α-methylbenzyl isocyanate (X)[4] were used without further purification. The third derivatizing reagent *R*(−)menthyl chloroformate (XI) was prepared according to a previously described method (18). All solvents were reagent grade, redistilled prior to use.

Metabolic Studies and Extractions—The *in vivo* study involved the oral administration of each substrate (I, III, or V) to male Wistar rats (250 g) and male New Zealand white rabbits (2 kg). The animals were kept in metabolic cages, and urine was collected over 48 hr and stored at 4° until examined. Bulked urine samples from each group of treated animals were divided into three equal portions. One portion was adjusted to pH 7.5 with solid sodium bicarbonate and extracted three times with equal volumes of a diethyl ether–methylene chloride mixture (3:2). A second portion was buffered to pH 7.0 with phosphate buffer and hydrolyzed for 36 hr at 37° after the addition of β-glucuronidase[5] (15,000 U/ml). The sample then was extracted as described above after adjusting the pH to 7.5 with sodium bicarbonate. The third portion was acidified to < pH 1 with concentrated hydrochloric acid and autoclaved at 125° and 15 psi for 45 min. After hydrolysis was complete, the pH was readjusted to 7.5 with 10% NaOH and the resulting solution was extracted as described above for portion 1.

For the *in vitro* studies, each substrate (I, III, or V) was incubated at 37° with 12,000×*g* liver homogenate supernatant (rat or rabbit) to which was added either a nicotinamide adenine dinucleotide phosphate (NADPH) or a nicotinamide adenine dinucleotide (NADH)-generating system (glucose-6-phosphate[6], magnesium chloride, and NADP[7] or NAD[8]). Extraction of metabolic products was carried out as with portion 1 of the *in vivo* study.

All extracts were concentrated and yielded yellowish oils, small aliquots of which were used for analysis by derivatization.

Derivatization—The *R*(−)menthyloxycarbonyl derivatives were prepared as follows: 100 μl of a standard XI solution (50 μmole/ml of toluene) was added to a small portion of the concentrated extract (20 μl) dissolved in 100 μl of pyridine. The mixture was allowed to react at room temperature for 30 min. After washing with water (1 ml), the organic phase was removed, dried over sodium sulfate, and concentrated under nitrogen to 5 μl, of which 0.2 μl was used for GLC.

N-(1-Phenylethyl)urethanes were prepared by adding 50 μl of a X solution (30 μmole/ml of toluene) to a portion (20 μl) of the concentrated extract dissolved in 100 μl of toluene. The mixture was tightly sealed[9] under a nitrogen atmosphere and kept at 120° for 2 hr. The reaction mixture was concentrated to 5 μl under a nitrogen stream, and a suitable portion was used for GLC.

Trifluoroacetyl derivatives of the metabolically produced alcohols were prepared by dissolving a small portion (20 μl) of the concentrated extract in 30 μl of ethyl acetate, to which 70 μl of IX was added. The reaction was allowed to proceed for 30 min at room temperature. The reaction mixture was evaporated under nitrogen and the residue obtained redissolved in 10 μl of cyclohexane, of which a 0.2-μl aliquot was injected.

The derivatized alcohol metabolites were identified by comparing their GLC retention times and mass spectra with those of authentic samples containing one optical enantiomer (or diastereoisomer) in known excess. Analyses were performed with a gas chromatograph[10] equipped with a flame ionization detector. The GLC conditions were: capillary column[11]

[1] Pfaltz and Bauer, Inc., Stamford, Conn.

[2] Optical rotation measurements were made using a Carl Zeiss Circular Polarimeter 0.01° equipped with a sodium D lamp (589.3 nm).

[3] Sigma Chemical Co., St. Louis, Mo.

[4] Aldrich Chemical Co., Inc., Milwaukee, Wis.

[5] Sigma Type H-1, Sigma Chemical Co., St. Louis, Mo.

[6] D-glucose-6-phosphate (Sigma grade, disodium salt) Sigma Chemical Co., St. Louis, Mo.

[7] Nicotinamide adenine dinucleotide phosphate, oxidized form (Sigma grade, monosodium salt), Sigma Chemical Co., St. Louis, Mo.

[8] β-Nicotinamide adenine dinucleotide, oxidized form (Grade III) Sigma Chemical Co., St. Louis, Mo.

[9] Reacti-vial (0.2 ml), Pierce Chemical Co., Rockford, Ill.

[10] Model 5710A, Hewlett-Packard.

[11] SE-30 (S.C.O.T.), Scientific Glass Engineering Pty, Ltd., (S.G.E. Inc.), Austin, Tex.

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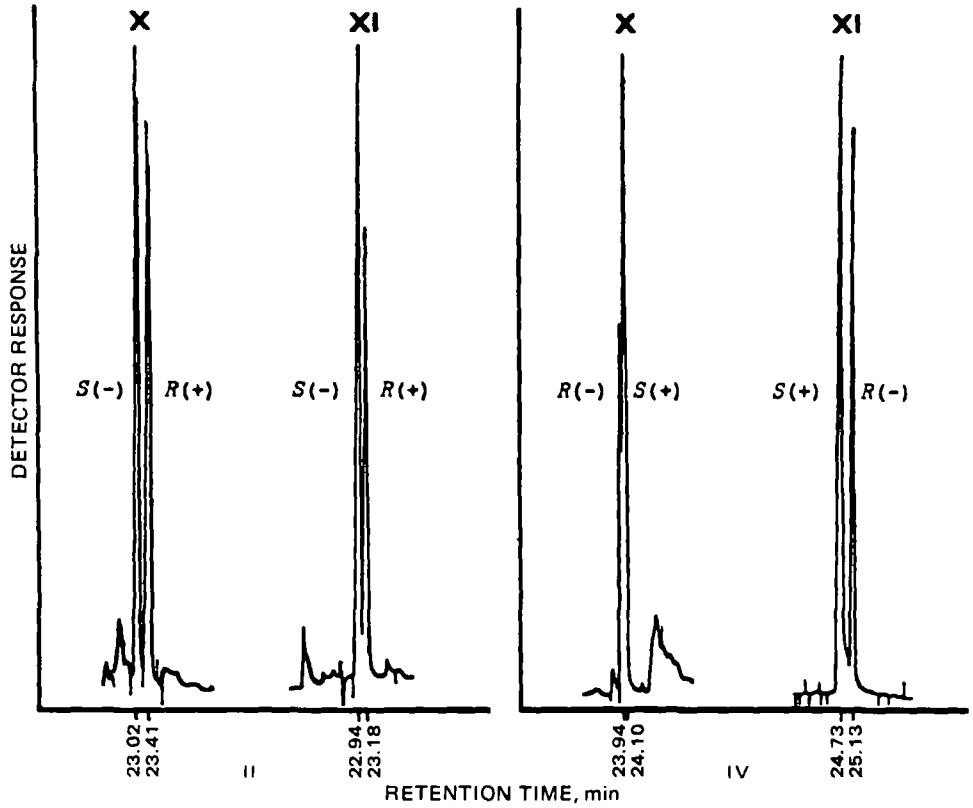


Figure 1—*Chromatograms (obtained by capillary GLC) of the diastereoisomeric pairs formed after reaction of alcohols with chiral derivatizing reagent. Key: alcohols: (II) 1-phenyl-1-propanol and (IV) 1-phenyl-2-propanol; reagents: (X)* R(−)*menthyl chloroformate and (XI)* S(+)-*α-methylbenzyl isocyanate.*

(52 m) with temperature programming from 150 to 250° (4°/min). The injection port and detector temperatures were 250 and 300°, respectively. The flow rate of the carrier gas, helium, was 3 ml/min (17 psi). Mass spectrometer[12] conditions were separator and ionization source temperatures, 180° and ionization energy, 70 eV. The GLC column and operating conditions were as described above.

RESULTS

In vivo and *in vitro* metabolism of I by the rat and and rabbit produced various metabolites (2, 11) including the racemate alcohol, *RS*-II. Similarly, racemic *RS*-IV was metabolically produced, together with other metabolites from III (1, 2, 10, 11). In this study the relative amounts of the enantiomeric alcohols were determined by reaction with the chiral derivatizing reagents, X or XI, and resolving the resulting diastereoisomeric derivatives by GLC.

Initially it was necessary to determine the GLC elution sequence of the mixture of diastereoisomers formed by the reaction of the racemate alcohols (II and IV) with the chiral reagents (X and XI). To accomplish this, authentic samples of II and IV were synthesized by a procedure which was known to produce mixtures of alcohols enriched in one of the enantiomers (17). Compound II was prepared by the asymmetric reduction of I with a sodium L-prolinate borane complex to yield a mixture of the enantiomers in which the *S*(−)-isomer predominated (see *Experimental*). A comparison of the magnitude of the optical rotation of the mixture with that of a pure sample of *S*(−)-1-phenyl-1-propanol permitted the calculation of the amounts of *S*(−)-II and *R*(+)-II in the synthetic product (Table I). Compound IV was prepared from III in a similar manner. The mixture of stereoisomers was strongly dextrorotatory, indicative of *S*(+)-IV being in enantiomeric excess. The calculated ratio of *S*(+)-IV/*R*(−)-IV is illustrated in Table I.

The mixture of stereoisomers (II and IV) were then reacted with the chiral reagents (X and XI) and the products analyzed by GLC (Fig. 1). The inability to detect any unreacted alcohols ensured complete derivatization. Peak areas were integrated and the ratio of isomers calculated. The values obtained were in close agreement with those obtained from polarimetric data (Table I), thus permitting peak identification.

The efficiency of peak resolution varied. A greater separation between *R*- and *S*-1-phenyl-2-propanol (IV) was obtained after derivatization with X then with XI (Fig. 1), which resulted in a more precise determination of optical purity (Table I). With *R*- and *S*-1-phenyl-1-propanol (II), better separation was obtained following derivatization with XI than with X. However, isomer ratios calculated from both chromatograms were virtually identical and in good agreement with those obtained polarimetrically (Table I).

Having demonstrated that the enantiomers of the racemate alcohols (II and IV) could be efficiently separated and quantitated as the corresponding diastereoisomers, it was possible to determine the extent to which stereoselectivity occurred during the metabolic reduction of arylalkylketones (I and III) and to assign absolute configuration to the metabolites.

When I was reduced *in vitro* (12,000×*g* liver homogenate) in rats or rabbits, 93–97% of the product alcohol (II) occurred as the *S*(−)-isomer, the remainder being the *R*(+) form (Table II). Species differences were minimal and nicotinamide adenine dinucleotide phosphate or nicotinamide adenine dinucleotide-generating systems were equally efficient. A structurally related alcohol, 1-phenyl-1-ethanol, produced by the reduction of acetophenone by an aromatic ketone reductase isolated from rabbit kidney, was similarly found to be predominantly (76%) in the *S*(−)

Table I—Stereochemical Analysis of Alcohols Obtained by Chemical Reduction of Arylalkylketones[a]

Method of Analysis	Alcohols[b] II	Alcohols[b] IV
Polarimetry	72/28[c]	60/40[d]
Derivatization/GLC		
R(−)-menthyl chloroformate	71/29	65/35
S(+)-*α*-methylbenzyl isocyanate	72/28	58/42

[a] A comparison of enantiomers detected by different analytical procedures. [b] Ratio of enantiomers: 1-phenyl-1-propanol (II), *S*(−)/*R*(+); 1-phenyl-2-propanol (IV), *S*(+)/*R*(−). [c] Based on $[\alpha]_D$ −32.50° (c 5.1, ethanol). R. H. Pickard and J. Kenyon, *J. Chem. Soc.*, **105**, 1115 (1914). [d] Based on $[\alpha]_D$ +16.10° (c 5.6, ethanol). *Ibid.*

[12] Hewlett-Packard model 5710 gas chromatograph coupled to a Hewlett-Packard quadrupole mass spectrometer model 5981A and a 5934A data system.

Table II—Stereochemical Analysis of Alcohols Obtained from the Metabolic Reduction of Arylalkylketones

Substrate	Methodology: System	Cofactor[a]	Pretreatment[b]	Metabolic Alcohol	Ratio of Enantiomers of Metabolic Alcohols[c] Species: Rat	Rabbit
I	*In vitro*	NADPH	N/P	II	96/4	93/7
		NADH	N/P		97/3	94/6
	In vivo	—	N/P		57/43	91/9
		—	Enzyme		52/48	89/11
		—	Acid		42/58	65/35
III	*In vitro*	NADPH	N/P	IV	91/9	94/6
		NADH	N/P		90/10	92/8
	In vivo	—	N/P		81/19	87/13
		—	Enzyme		80/20	88/12
		—	Acid		72/28	79/21

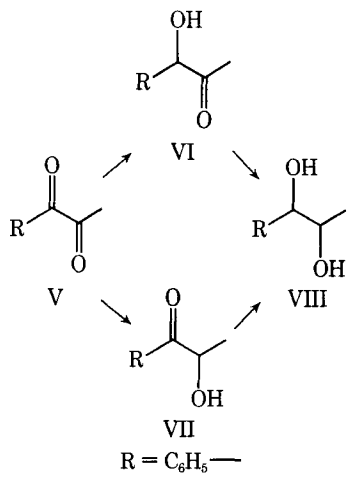
[a] Generating system; glucose-6-phosphate, magnesium chloride, and $NADP^+$ or NAD^+. [b] Hydrolytic pretreatment of biological sample: N/P, no pretreatment; enzyme, β-glucuronidase (15,000 U/ml, incubated 36 hr at 37°); acid, pH < 1 (autoclaved 40 min at 125° and 15 psi). [c] Ratio of enantiomers (as diastereoisomeric derivatives); 1-phenyl-1-propanol [S(−)/R(+)-II] as R(−)menthyl chloroformate derivative, 1-phenyl-2-propanol [S(+)/R(−)-IV] as S(+)-α-methylbenzyl isocyanate derivative.

form (12). *In vitro* reduction of phenylacetone (III) in rats and rabbits paralleled what was observed with the substrate I. The alcohol, IV, was recovered mainly as the S(+)-enantiomer (90–94%), regardless of the species and cofactor (Table II).

In vivo reduction of I by rabbits gave results comparable to those observed in *in vitro* studies. Approximately 90% of the alcohol recovered from urine was determined to be S(−)-1-phenyl-1-propanol [S(−)-II]. An earlier study has shown that after administration of propiophenone to rabbits, II was recovered entirely as the S(−)-glucuronide conjugate (11). In contrast to what occurred in rabbits *in vivo*, reduction of I in rats resulted in the recovery of a markedly higher proportion of the R-alcohol [R(+)-II]. In this instance, stereoselective reduction was less evident (Table II). Both enzymatic and acidic hydrolysis of the urine collected in the *in vivo* studies resulted in an increase in the relative amounts of R(+)-II isolated.

In vivo reduction of III to its corresponding alcohol (IV) resulted in the preferential formation of the S(+)-IV isomer, regardless of species (Table II). The ratio of enantiomers [S(+)/R(−)] formed by rat and rabbit were 81:19 and 87:13, respectively, corresponding to approximately twice the amount of R(−)-IV observed *in vitro*. In contrast, an earlier study (11) reported that when III was reduced in the rabbit, no trace of R(−)-1-phenyl-2-propanol was found. The S(+)-IV/R(−)-IV ratio was again influenced by the method used to hydrolyze urine (Table II).

In vivo and *in vitro* metabolism of V was also investigated in rats and rabbits. Reduction yielded three metabolites of major interest for this study: 1-hydroxy-1-phenyl-2-propanone (VI), 2-hydroxy-1-phenyl-1-propanone (VII), and 1-phenyl-1,2-propanediol (VIII), which was obtained as a mixture of its diastereoisomers (*erythro*- and *threo*-VIII) (Scheme I). Neither of the intermediate ketol metabolites (VI or VII) could be completely derivatized with either chiral reagent (X or XI) and, therefore, the optical purities of these metabolites could not be established. Similarly, the four-component mixture of optical isomers of the diol, VIII (1*S*2*R*- and 1*R*2*S*-*erythro*, 1*S*2*S*- and 1*R*2*R*-*threo*) could not be separated using the chiral reagents, but it was possible to separate the diastereoisomers as their trifluoroacetyl derivatives.



Scheme I

As observed with ketones I and III, *in vitro* reduction of V resulted in the formation of one predominant isomer of the diol metabolite (VIII) (Table III). Between 94 and 98% of VIII was recovered as the *erythro*-diastereoisomer. Only marginal variation in the measured *erythro–threo* ratio of VIII was observed, regardless of the source of the liver preparation or the nature of the cofactor utilized.

In vivo reduction of V similarly produced *erythro*-VIII as the major diastereoisomer, regardless of species. Ratios of the isomers isolated (*erythro–threo*-VIII) were 75:25 and 86:14, recovered from rats and rabbits, respectively. Hydrolysis of urine with either β-glucuronidase or acid caused no change in the ratio (Table III).

DISCUSSION

Conventional methods for determining the optical purity of metabolites have generally relied on isolating sufficient quantities of product for polarimetric analysis (11, 19–22). This presents a problem when the amount of product available is too small for accurate measurement of optical rotation. The use of chiral reagents and subsequent analysis of diastereoisomeric products by GLC (23–27) offers a sensitive alternative for the quantification of stereoisomeric metabolites. This analytical procedure provided the data summarized in Table II, which reveal that the metabolic reduction of I and III demonstrates a high degree of product stereoselectivity. The metabolites, II and IV, respectively, were recovered predominately as S-isomers in all studies with one exception: *In vivo* reduction of I by rats provided anomalous data.

In quantitative terms, the *in vitro* reduction of arylalkylketones has been reported to be significantly dependent on both the species and cofactor (2–4, 6–8, 10, 12), but the present study indicates that enantioselective preference is virtually unaffected by these variables.

The ratios of isomers isolated from the *in vivo* studies did vary, however, depending on the nature of the hydrolytic treatment of the urine samples prior to extraction and derivatization of the alcohol metabolites. A noticeable increase in the amount of R(+)-II recovered, relative to that of S(−)-II, was observed in both species after enzymatic hydrolysis (Table II). Since it has already been demonstrated that both enantiomers of II undergo glucuronide conjugation in rabbits (11), this observation suggests

Table III—Stereochemical Analysis of 1-Phenyl-1,2-propanediol Obtained from the Metabolic Reduction of 1-Phenyl-1,2-propanedione

Methodology: System	Cofactor[a]	Pretreatment[b]	Ratio of Diastereoisomers of VIII[c] Species: Rat	Rabbit
In vitro	NADPH	N/P	97/3	97/3
	NADH	N/P	94/6	98/2
In vivo	—	N/P	75/25	86/14
	—	Enzyme	74/26	87/13
	—	Acid	72/28	85/15

[a] Generating system; glucose-6-phosphate, magnesium chloride, and $NADP^+$ or NAD^+. [b] Hydrolytic pretreatment of biological sample: N/P, no pretreatment; enzyme, β-glucuronidase (15,000 U/ml, incubated 36 hr at 37°); acid, pH < 1 (autoclaved 40 min at 125° and 15 psi). [c] Ratio of diastereoisomers, *erythro–threo*-1-phenyl-1,2-propanediol (VIII) as the trifluoroacetylated derivative.

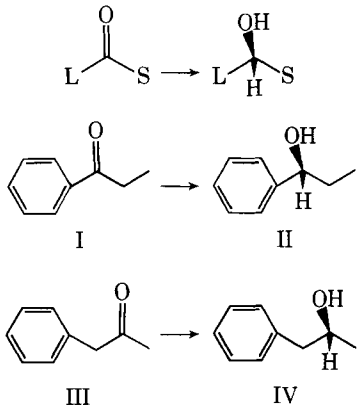
that a greater proportion of the $R(+)$-isomer is conjugated relative to the $S(-)$ form. Only minor changes were detected in the $S(+)/R(-)$ ratio of 1-phenyl-2-propanol (IV) after hydrolysis with β-glucuronidase, when compared with the ratio observed with no pretreatment of the urine samples.

The much higher levels of both $R(+)$-II and $R(-)$-IV measured after acidic hydrolysis possibly could be due to a more efficient cleavage of the conjugate with acid treatment than with β-glucuronidase. However, a more plausible explanation is that racemization of isomers occurred during the harsh treatment created by conditions of low pH and elevated temperature (19). This would account for the difficulty in obtaining reproducible results after acidic hydrolysis.

The absence of any noticeable change in the ratio of diastereoisomers of VIII suggest that both the *erythro* and *threo* isomers are conjugated to the same extent. However, it is perhaps more likely that because of the high polarity of the diols, neither compound (*erythro*-VIII nor *threo*-VIII) undergoes conjugation before being excreted in the urine.

A strong correlation between *in vivo* and *in vitro* results in the rabbit has been demonstrated in the present study with both substrates (Table II). A similar correlation was observed between *in vivo* and *in vitro* reduction of III in the rat, but not when I was the substrate (Table II). In the latter instance, enantioselectivity was clearly demonstrated in *in vitro* studies, but *in vivo* reduction of I appeared to be nonstereoselective. This anomalous situation may be the result of further preferential metabolism of $S(-)$-1-phenyl-1-propanol *in vivo* in the rat. Related studies support this suggestion. It has been established (28) that when 1-phenyl-1-ethanol was administered to rats as the $R(+)$-isomer, it underwent extensive conjugation and was excreted largely as the glucuronide, whereas the $S(-)$-isomer was further oxidized to $S(-)$-mandelic acid. If the metabolite, II, behaved similarly *in vivo* in the rat, it would account for the lower percentage recovery of $S(-)$-II compared with that obtained with the *in vitro* study. In addition, the increased recovery of $R(+)$-II after enzymatic treatment of the urine sample could be rationalized by liberation of the $R(+)$-isomer from the glucuronide conjugate.

The enantioselectivity of the reduction of the arylalkylketones (I and III) can be rationalized, based on a formula established previously (29) and confirmed by others (22, 30–33). This formula essentially states that if the ketone is positioned so the large substituent is placed to the left, reduction mainly occurs such that the resultant hydroxyl group rises above the plane of the molecule. Compounds I and III, therefore, could be predicted to be preferentially reduced to alcohols with the *S*-configuration (Scheme II: L, large substituent; S, small substituent). By applying Prelog's rule, it is possible to predict the stereochemistry of the diol (VIII), assuming that reduction proceeded *via* the ketoalcohol intermediates (VI and VII) and that the pattern of preferred stereoselectivity remains constant. Consequently, VIII should occur predominantly as the 1*R*2*S*-diastereoisomer. Although it was not possible to resolve the 1*R*2*S*- and 1*S*2*R*-*erythro* isomers, this prediction was accurate in as much as VIII recovered from the reduction of the diketone *in vitro* by both rat and rabbit was mainly (94–98%) the *erythro* form, the remainder being *threo*-VIII (Table III). Compound VIII recovered from *in vivo* studies



Scheme II

was also predominantly the *erythro* diastereoisomer, although a greater amount of the *threo*-compound was present compared to what was obtained from *in vitro* studies (Table III). However, caution should be exercised when attempting to explain this apparent decrease in stereoselectivity, since additional factors such as further metabolism and differences in distribution and elimination may contribute to the overall variation in recovery of diastereoisomers. In this instance there is the additional possibility that racemization resulting from interconversion of the ketol intermediates (VI ⇄ VII) could have also occurred (2, 23).

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