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Research Article

Stereochemical Characterization of the Diastereomers of the Amobarbital *N*-Glucosides Excreted in Human Urine

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The stereochemistry associated with the amobarbital *N*-glucoside diastereomers (**1a** and **1b**) that are excreted by humans in urine is unknown. Using X-ray crystallography, the absolute configuration of **1b** was determined to be S (C-5 position of the barbiturate ring). Following oral administration of amobarbital to Caucasians and Orientals, from 5 to 25% of the dose of amobarbital was excreted in the urine as **1b**. The other diastereomer, **1a**, accounted for less than 0.1 to 0.2% of the dose in four individuals, with none detected in nine individuals. The rate constants, $k_{f,1b}$, determined from the urinary excretion of **1b** were lower than those previously reported for unresolved amobarbital *N*-glucosides. However, based on the urinary excretion of **1b**, the rate constants, *K*, for elimination of amobarbital in Caucasians and Orientals were similar to those previously determined from the serum levels of amobarbital and the urinary excretion of unresolved amobarbital *N*-glucosides. In previous studies of the *N*-glucosylation of amobarbital, it is likely that a single *N*-glucose diastereomer, **1b**, was being observed.

KEY WORDS: diastereomers of 1-(β-D-glucopyranosyl)amobarbital; absolute configuration; X ray; human; urinary excretion; product enantioselectivity.

INTRODUCTION

The urinary excretion of amobarbital in humans is due primarily to two metabolites, 3′-hydroxyamobarbital and *N*-(1-β-D-glucopyranosyl)amobarbital (1,2). When D-glucose is coupled to either nitrogen of amobarbital, asymmetry is conferred at C_5 of the barbiturate ring and the *N*-glucose conjugates of amobarbital exist as two diastereomeric or epimeric metabolites (see Fig. 1). These diastereomers have been differentiated using HPLC and designated AMOA (**1a**) and AMOB (**1b**) based on their HPLC retention times (3). Following administration of amobarbital to humans, preferential excretion of only one diastereomer, **1b**, occurred in the urine (4). Since a product enantioselectivity has been observed for the excretion of these *N*-glucoside conjugates and amobarbital is the most thoroughly studied compound for this pathway in humans, it was of interest to determine their absolute configuration and investigate the relative importance of each diastereomer in the urinary disposition of amobarbital. In this study the absolute configuration of **1b** was determined using X-ray crystallography and the urinary excretion of the individual diastereomers was monitored using HPLC. Analysis of the urinary excretion rate of **1b** for both Caucasians and Orientals allowed comparison with prior studies in which the amobarbital *N*-glucoside diastereomers were not differentiated.

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MATERIALS AND METHODS

Supplies

5(*R*)-5-Ethyl-1-(1-β-D-glucopyranosyl)-5-(3-methylbutyl)-2,3,6-(1H,3H,5H)-pyrimidinetrione (**1a**) and 5(*S*)-5-ethyl-1-(1-β-D-glucopyranosyl)-5-(3-methyl-butyl)-2,3,6-(1H,3H,5H)-pyrimidinetrione (**1b**) were synthesized as previously described (4). The analytical equipment and supplies used for quantifying the amobarbital *N*-glucoside conjugates in urine have been reported (3).

Single-Crystal X-Ray Analysis of 1b

Crystallization of synthetic **1b** from water under slow evaporation conditions yielded plate-like rectangular crystals suitable for X-ray analysis. A crystal of approximate dimensions 0.02 × 0.25 × 0.30 mm was used for data collection on a Rigaku AFC5R diffractometer with graphite monochromatized CuKα radiation using an evacuated collimator and beam tunnel; the crystal-to-detector distance was 600 mm. Lattice parameters obtained from a least-squares refinement using the setting angles of 25 centered reflections in the range 39 to 40° Θ corresponded to a monoclinic cell of dimensions a = 7.7482(9) Å, b = 6.9816(6) Å, c = 20.967(2) Å, β = 91.317(8)°, and V = 1133.9(3) Å^3. Systematic absences condition, $o k 0$, $k \neq 2n$, coupled with statistical analysis of intensity distribution uniquely defined the space group $P2_1$ with Z = 2, D_x = 1.30 g/cm^3, absorption coeffi-

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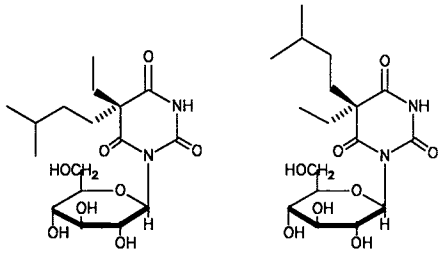

1a 1b

Fig. 1. The diastereomers of 1-(β-D-glucopyranosyl)amobarbital.

cient $\mu = 8.8\ cm^{-1}$, and $F(000) = 476$, at $T = 294$ K. Intensities in the range 2.5 to 60° Θ were collected using the ω-2Θ scan mode, scan width $(1.57 + 0.3\tan\Theta)°$, at a speed of 4°/min in omega. Weak reflections were rescanned (maximum, eight times) and the counts were accumulated to assure good counting statistics. Stationary background counts were recorded with a ratio of peak to background counting time of 2:1. Three standard reflections measured after every 100 reflections showed no significant variation in intensities over the time of data collection. Lorentz-polarization factor (*Lp*) and imperical absorption correction, based on azimuthal scans of several reflections, were applied. Of the 1842 unique reflections collected, 1799 with $I \geq 3\sigma(I)$ were considered observed, $\sigma^2(F_0^2) = [S^2(C + R^2B) + (pF_0^2)^2]/Lp$, where S = scan rate, C = total integrated peak count, R = ratio of scan time to background counting time, B = total background count, and $p = p$ factor (a correction factor for strong reflections). The structure was solved, and the nonhydrogen atoms were located via direct methods using MITHRIL (5) and DIRDIF (6). All calculations were performed on a micro Vax III using the TEXSAN crystallographic software package (7). The positions of the hydrogen atoms were located from a difference Fourier following a few cycles of full-matrix least-squares refinement minimizing: $\Sigma w[|F_0| - |F_c|]^2$, where $w = 4F_0^2/\sigma(F_0^2)$. The positions of the hydrogens attached to carbon atoms were then idealized by least-squares adjustment to the observed positions, but the coordinates of the noncarbon hydrogen atoms were not adjusted. All hydrogen atoms were excluded from refinement of nonhydrogen atoms with anisotropic temperature factors. Scattering factors were taken from International Tables of X-Ray Crystallography (8). The absolute configuration of the compound was established by reference to the known stereochemistry of the β-D-glucose moiety; the stereochemistry was subsequently confirmed by X-ray anomalous dispersion studies. Refinement converged at $R = 0.041$, $R_w = 0.066$, for 1799 reflections with $I \geq 3\sigma(I)$ and 270 parameters. Maximum parameter shift/esd (Δ/σ) was 0.04, and the highest peak and deepest trough on the final difference Fourier map were 0.25 and $-0.19\ e/Å^3$, respectively. Further crystallographic details appear in Table I. Final positional and equivalent isotropic thermal parameters, bond lengths and angles, anisotropic thermal parameters, and structure factors are deposited as supplementary material and are available from the authors.

Table I. Experimental Detail for the X-ray crystal structure of **1b**

Molecular formula	$C_{17}O_8N_2H_{28} \cdot 3H_2O$ (442.46)
Crystal dimensions (mm)	0.02 × 0.25 × 0.30
Lattice parameters	$a =$ 7.7482(9) Å
	$b =$ 6.9816(6) Å
	$c =$ 20.967(2) Å
	$\beta =$ 91.317(8) Å
	$V =$ 1133.9(3) Å^3
Space group	$P2_1$ ($Z = 2$)
D_{calc}	1.30 g/cm^3
$F_{(000)}$	476
$\mu_{(CuK\alpha)}$ ($\lambda = 1.54178$ Å)	8.83 cm^{-1}
Scan type	$\omega - 2\theta$
Scan rate	4°/min (in omega) (8 rescans)
$2\Theta_{max}$	120°
No. of reflections	Unique: 1842 Observed: 1799
Corrections	Lorentz-polarization Absorption (trans. factors: 0.87–1.00)
p factor	0.03
No. variables	270
Residuals: R, R_w	0.041, 0.066
Max shift/error in final cycle	0.04
Max., min. peak in final diff. map	+0.25, −0.19 e^-/Å^3

Collection of Urine Samples

Eleven adult male volunteers were enrolled in the study after informed consent, medical history, and laboratory screening tests (free and conjugated bilirubin, γ-glutamyltransferase) were performed. Six subjects were Caucasian and five subjects were Oriental, with ages ranging from 24 to 51 years (mean, 29.5 years) and weights from 68.2 to 83.2 kg (mean, 72.5 kg). The subjects were instructed not to take any medication for 3 days before taking the drug and during the study. Alcohol consumption was not allowed 24 hr before and after taking the drug and was restricted during the remainder of the urine collection period. Five male nonsmoking Caucasians received a 200-mg oral dose of sodium amobarbital (806 μmol, capsule, Eli Lilly and Co., Indianapolis, IN) just prior to bedtime. The total urine was collected as individual samples at natural periods for 30 hr, followed by collection of morning urines for 3 to 5 additional days. At the 200-mg dose, the subjects encountered extensive sedation and disorientation the day following the dose. The study was continued at a lower dose, 100 mg amobarbital (442 μmol, acid form, tablet, Eli Lilly and Co.), with four nonsmoking Caucasian subjects (three of the same individuals) and five male nonsmoking Oriental subjects. In this part of the study, total urine was collected as individual samples at natural periods for approximately 55 hr, followed by collection of morning urines for 5 additional days. Morning urines consisted of samples collected between recorded urination prior to bedtime and first urination in the morning. The urine samples were refrigerated immediately upon collection, frozen within 48 hr, and kept at −20°C until assayed.

Analysis of 1a and 1b in Urine

The analytical method has been previously described (3). Basically, to prepare the samples for analysis, the inter-

nal standard (butalbital dissolved in methanol), was transferred to a screw-cap test tube (100 × 13 mm), evaporated to dryness, and reconstituted with 200 or 400 μl of urine. To the urine sample were added 1.0 ml of a saturated solution of ammonium sulfate and 3.0 ml of ethyl acetate. The solution was extracted using a rotary mixer for 30 min. After centrifugation, the ethyl acetate layer was transferred and dried with anhydrous sodium sulfate, after which 2.0 ml was transferred to a test tube and evaporated to dryness in a vortex evaporator at 30 mm Hg and room temperature. The residue was reconstituted with 200 μl of HPLC mobile phase regardless of whether 200 or 400 μl of urine was used, transferred to sample injection vials, and stored frozen until analyzed.

The samples were analyzed by HPLC using a C_{18} reversed-phase column as previously described (3). Each sample was assayed in duplicate. The HPLC assay was capable of differentiating both diastereomers of the amobarbital *N*-glucoside but did not permit quantitation of 3'-hydroxyamobarbital. The *N*-glucosides were labeled **1a** and **1b** based on the HPLC retention times, which were 14.2 and 15.9 min, respectively. The lower limit of detection for **1a**, **1b**, and amobarbital had been reported to be 2 nmol/ml, i.e., these analytes were detected at 2.0 nmol/ml but not at 1.0 nmol/ml following extraction of standards from 200 μl of urine. It was observed during these studies that a peak corresponding to **1a** was detected at levels of 1.4 nmol/ml provided that an interfering substance that elutes at 13.5 min was not present or was at low concentrations (Table IV). The percentage of the dose of **1a**, **1b**, and amobarbital in Table V was calculated by measuring the concentration of analyte in each urine sample, multiplying this value by the volume of urine, then summing these amounts and dividing by the dose of amobarbital. Complete (100%) absorption was assumed (1).

The pharmacokinetic parameters were determined from the urinary excretion rates of **1b** assuming that its formation was rate limiting (9,10). The following model was used:

$$\ln(\text{excretion rate } \mathbf{1b}) = \ln(k_{f,\mathbf{1b}} \cdot \text{dose}) - Kt \qquad (1)$$

The use of this model would appear to be valid since the oral absorption of amobarbital is rapid (11) and complete with 79–92% of the dose accounted for in the urine and 4–5% in the feces (1). In prior studies (12–14) the urinary excretion rate of amobarbital *N*-glucoside(s) was consistent with a one-compartment model and the rates of decline of the serum concentration of amobarbital paralleled the excretion rates of the *N*-glucosides in a first-order manner. It is assumed that the actual urinary excretion rate of **1b** is much greater than K, as has been shown for 3'-hydroxyamobarbital, a hydrophilic metabolite of amobarbital that is rapidly excreted in the urine (15). The parameter estimates for the slopes and ordinate intercepts were determined using the method outlined by Tang *et al.* (12) for the unresolved amobarbital *N*-glucosides. All of the data points were used starting with the second urine sample obtained the morning after taking the drug, i.e., after the absorption and distribution phase. The linear regression analysis used the general linear model (GLM) procedure (16). The elimination rate constant for amobarbital, K, was determined from the absolute value of the slope when plotting the natural logarithm of the urinary rate of excretion of **1b** versus time, using the midpoints of the urine collection periods for t. The formation rate constant for **1b**, $k_{f,\mathbf{1b}}$, was calculated from the ordinate intercept (17). The pooled data shown in Fig. 3 and in Table III were obtained by mathematically pooling the data into collection periods which ranged from 10.5 to 14 hr, starting with the second urine sample obtained the morning after taking the drug. The pooled data were analyzed by linear regression analysis comparable to the unpooled data. Analysis of variance with a model which includes different slopes for each race and different intercepts for each subject was performed using SAS PROC GLM. This statistical approach for comparing the residual variances, the slopes of the lines, and the ordinate intercepts was necessary since the urinary excretion rates differed from one individual to another and more data points were obtained for some individuals because of variations in urine flow.

RESULTS

Figure 2 shows an ORTEP (Oak Ridge Thermal Ellipsoid Program) plot of **1b** (most atoms have not been labelled for clarity). The sugar moiety is β-D-glucose and the barbiturate ring on the anomeric carbon is in an equatorial position. The barbiturate ring is almost planar, with the chiral carbon C5, out of the mean plane of the atoms in the ring by 0.13 Å. The absolute configuration at C5 is S. The molecule crystallized as a trihydrate, with three H_2O molecules involved in intra- and intermolecular hydrogen bonding networks. The intramolecular hydrogen bonds (see Fig. 2) involve the primary alcohol (O5-H) of the D-glucose and two water molecules: O5–O10 2.814(5) Å, O10-H · · · O5 169.9°, and O5–O11 2.693(4) Å, O5-H · · · O11 167.7°. The urea oxygen, O2 of the pyrimidinetrione, is H-bonded to the water oxygen O9; O9–O8 2.806(4) Å and O9-H · · · O8 142.3°. The

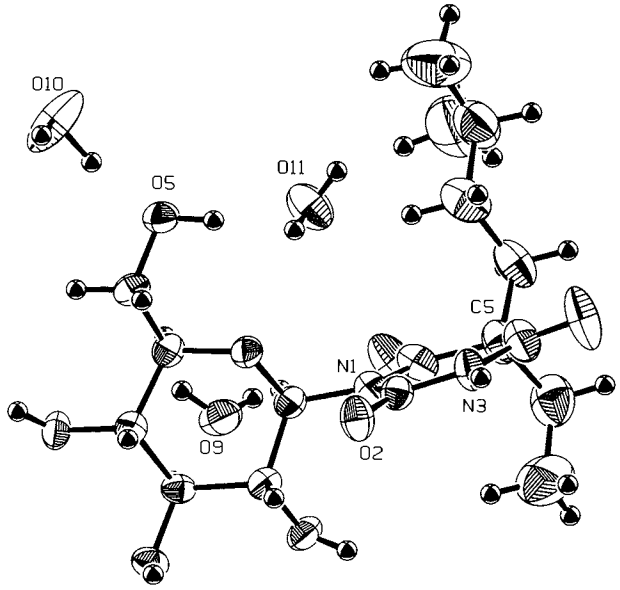

Fig. 2. ORTEP plot of **1b**. Only selected atoms have been labeled for clarity. Nonhydrogens are shown by using 50% probability thermal ellipsoids; hydrogen atoms are drawn with β set to 1 Å^2. The labels used in this figure do not necessarily correspond with the crystallographic numbering scheme provided in the supplementary material (available from the author).

remaining water oxygens and their hydrogens are involved in intermolecular H bonding with the secondary hydroxyl groups of the glucose unit and the keto oxygen, O_2 of the pyrimidinetrione unit (not shown).

The parameters, K and $k_{f,1b}$, obtained for each of the subjects in this study are shown in Table II. In some individuals, considerable fluctuation in the urinary excretion rate was observed during the collection periods. This can be minimized by mathematically pooling the data into larger collection periods, as illustrated in Fig. 3. This effectively smoothed the data and improved the values of the correlation coefficients. Figure 3 shows representative plots of rate of excretion of **1b** versus time for subject 3 at both the 100-mg and the 200-mg doses. The slopes and ordinate intercepts obtained when using either natural collection periods or mathematically pooled samples (10.5 to 14 hr) were comparable (legend to Fig. 3). Since pooled data treatment had been used in other studies for the analysis of the unresolved amobarbital *N*-glucosides, the analysis of the results obtained upon pooling these data is also included in Table III.

The values obtained for the slope and intercept for the combined data in each experimental group (Oriental, 100 mg; $K_{average}$ = 0.0317, Y-INT = 7.68: Caucasian, 100 mg; $K_{average}$ = 0.0183, Y-INT = 7.12: Caucasian, 200 mg; $K_{average}$ = 0.0278, Y-INT = 7.78) are similar to the mean values listed in Table III. The F tests from the analysis of variance indicated that there is a significant difference (P = 0.0001) in the slopes obtained for the Caucasians at the 100-mg dose compared to either the Caucasians at the 200-mg dose or the Orientals at the 100-mg dose. The wide variation in the individual subject values obtained for the ordinate

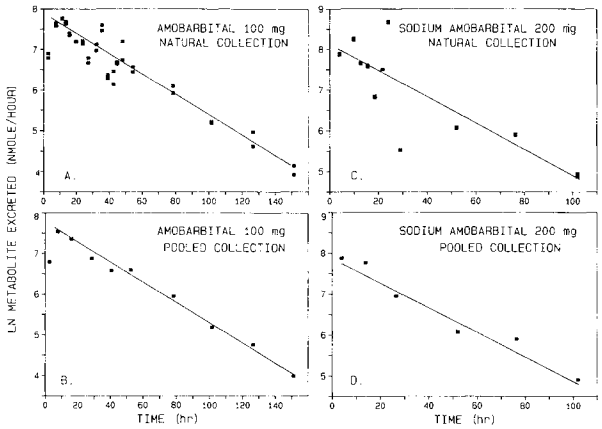

Fig. 3. Urinary excretion rate of **1b** in subject 3 after oral dosing with amobarbital. (A) K = 0.0233 ± 0.0015, Y-INT (ordinate intercept) = 7.78, r^2 = 0.880. (B) K = 0.0244 ± 0.0010, Y-INT = 7.80, r^2 = 0.989. (C) K = 0.0325 ± 0.0058, Y-INT = 8.05, r^2 = 0.558. (D) K = 0.0292 ± 0.0025, Y-INT = 7.88, r^2 = 0.937.

Table II. Parameters Derived for Urinary Excretion of **1b** in Caucasian and Oriental Male Subjects Using Unpooled Data[a]

Subject No.	Race[b]	Dose (mg)[c]	n[d]	R^2[e]	K (hr^{-1})[f]	SE of K	Y − INT[f]	SE of Y − INT	$k_{f,1b}$ (hr^{-1})	% dose[g]
2	C	100	34	0.893	0.0168	0.0010	7.40	0.065	0.00369	21.9
3	C	100	36	0.880	0.0233	0.0015	7.78	0.093	0.00542	23.2
4	C	100	24	0.847	0.0185	0.0017	6.27	0.080	0.00119	6.4
11	C	100	39	0.924	0.0178	0.0008	6.94	0.050	0.00234	13.2
		Mean			0.0191		7.10		0.00316	16.2
		SD (n − 1)			0.0029		0.65		0.00182	7.9
5	O	100	20	0.897	0.0411	0.0033	8.21	0.172	0.00828	20.1
6	O	100	20	0.540	0.0118	0.0026	7.18	0.123	0.00298	25.3
8	O	100	20	0.806	0.0256	0.0030	6.70	0.234	0.00184	7.2
9	O	100	20	0.861	0.0352	0.0033	7.40	0.260	0.00369	10.5
10	O	100	26	0.956	0.0314	0.0014	8.22	0.097	0.00841	26.8
		Mean			0.0290		7.54		0.00504	18.0
		SD (n − 1)			0.0112		0.66		0.00309	8.8
1	C	200	15	0.857	0.0220	0.0025	7.42	0.163	0.00208	9.4
2	C	200	20	0.914	0.0301	0.0022	8.34	0.131	0.00522	17.3
3	C	200	27	0.558	0.0325	0.0058	8.05	0.245	0.00389	12.0
4	C	200	20	0.857	0.0268	0.0026	7.04	0.153	0.00142	5.3
7	C	200	22	0.900	0.0281	0.0021	7.96	0.153	0.00354	12.6
		Mean			0.0279		7.76		0.00323	11.3
		SD (n − 1)			0.0039		0.52		0.00151	4.4

[a] Model: ln(**1b** excretion rate) = $-Kt$ + ln($k_{f,1b}$ · dose).
[b] C, Caucasian; O, Oriental.
[c] 100 mg = 441,950 nmol amobarbital, acid form; 200 mg = 805,575 nmol sodium amobarbital.
[d] Number of data points per subject.
[e] Correlation coefficient.
[f] Parameter estimates of slope ($-K$) and ordinate intercept (Y − INT), along with the standard error (SE) of the estimates as determined by SAS PROC GLM.
[g] ($k_{f,1b}/K$) · 100 = % of dose excreted as **1b**.

Table III. A Summary of Elimination Constants for Amobarbital and Amobarbital *N*-Glucosides

Study	Subject group	n^a	Dose (mg)	Mean K (hr^{-1})	SD of K	$k_{f,1b}$ (hr^{-1})	SD of $k_{f,1b}$	Mean % dose excreted as 1b[b]
I	O-NS-M[c]	5	100	0.0290	0.0112	0.00504	0.00309	17.4
	Pooled[d]			0.0277	0.0086	0.00500	0.00288	18.1
I	C-NS-M[e]	4	100	0.0191	0.0029	0.00316	0.00182	16.5
	Pooled			0.0186	0.0036	0.00288	0.00171	15.5
I	C-NS-M	5	200	0.0279	0.0039	0.00323	0.00151	11.6
	Pooled			0.0271	0.0033	0.00314	0.00155	11.6
II[f]	O-NS-M	6	120	0.0248	0.0041	0.0087	0.0012	35.1
II[f]	C-NS-M	13	120	0.0263	0.0045	0.0057	—	21.7
III[g]	?[f]	8	120	0.0221	0.0050	0.00703	0.00318	31.8
III[f,g]	?	8	120	0.0240	0.0061	—	—	—

[a] Number of subjects.
[b] (Mean $k_{f,1b}$)/(mean K) · 100.
[c] Oriental, nonsmoking male subjects.
[d] Pooled refers to the parameters derived from mathematically pooling data within subjects to smooth variation due to urine flow as described in text.
[e] Caucasian, nonsmoking male subjects.
[f] Ref. 13; usual dose, 120 mg sodium amobarbital; total urine pooled into 12-hr batches for 96–120 hr.
[g] Ref. 12; dose, 120 mg sodium amobarbital; total urine pooled into 12-hr batches for 120 hr.

intercepts resulted in no significant difference ($P = 0.4995$) when comparing the values obtained for the average ordinate intercept for each experimental group.

Following the 100-mg dose the initial morning concentrations of **1b** ranged from 1.6 to 159 nmol/ml and, during the remainder of the first day, ranged from 2.1 to 93.8 nmol/ml (average, 20.3 ± 17.5). After 125 hr, the concentration of **1b** in the urine was present at concentrations approaching the lower limit of detection or was not detected. Metabolite **1a** was observed infrequently during the first 30 hr after dosing and, when detected, was present at concentrations around the lower limit of detection. Metabolite **1a** was observed in two Caucasians at the 200-mg dose and in two Orientals at the 100-mg dose. In the individuals where **1a** was detected, it was detected only in occasional samples (Table IV). Based on these individual samples it would appear that the relative ratio of **1a** to **1b** could be high. However, in 17 samples collected during the first 56 hr, the concentration of **1b** ranged from 30.0 to 159 nmol/ml, yet no **1a** was detected. No correlation could be made concerning the ratio of **1a** to **1b** formed and/or excreted in the urine. Only small amounts of amobarbital were excreted unchanged in the urine. The 1–2% of amobarbital excreted in the urine (Table V) was consistent with previous reports (1,18–20). The percentages of the amobarbital dose actually quantified in the urine as **1a**, **1b**, and amobarbital are shown in Table V.

Table IV. Detection of 1a

Subject No.	Race	Dose (mg)	MPTTIME[a] (hr)	[1a], nmol/ml	[1b], nmol/ml
3	C	200	76.3	4.9	15.2
4	C	200	18.8	2.4	20.7
			27.2	2.9	16.3
5	O	100	41.3	1.5	7.9
6	O	100	29.4	1.4	29.9

[a] Midpoint of collection time.

A summary of the elimination constants obtained for amobarbital and **1b** in this study or amobarbital *N*-glucoside(s) in previous studies (12,13) is shown in Table III. Although the number of subjects is small, the values obtained for K in Caucasians at the 100- and 200-mg doses suggest that K increases as the dose increases (21). Consequently, further statistical comparison of the means obtained from the various studies at different doses was not appropriate with the data available.

DISCUSSION

Conjugation of a glycoside to a barbiturate nitrogen may

Table V. Urinary Excretion of Amobarbital Detected as Amobarbital and Amobarbital *N*-Glucosides

Subject No.	Race	Dose (mg)	% dose detected as			Total % dose detected
			1a	1b	Amobarbital	
2	C	100	ND[a]	15.0	1.2	16.2
3	C	100	ND	15.5	1.0	16.5
4	C	100	ND	4.4	2.1	6.5
11	C	100	ND	4.3	2.8	7.1
5	O	100	<0.1	12.8	ND	12.9
6	O	100	<0.1	15.7	0.2	16.0
8	O	100	ND	6.9	0.3	7.2
9	O	100	ND	6.8	0.7	7.5
10	O	100	ND	22.9	ND	22.9
1	C	200	ND	8.1	0.5	8.6
2	C	200	ND	10.8	1.8	12.7
3	C	200	<0.1	8.8	0.7	9.6
4	C	200	<0.2	3.1	1.0	4.2
7	C	200	ND	9.5	1.2	10.7

[a] Not detected.

confer chirality to the aglycone and diastereomers would be formed. Spectroscopic methods are not currently available for predicting the absolute configuration associated with these chiral conjugates, especially the barbiturate *N*-β-D-glucoside conjugates. By obtaining the crystal structure of one of the amobarbital *N*-glucoside diastereomers, the absolute configuration at C-5 of the barbiturate ring could be determined in addition to providing conformational information for further molecular modeling studies (22). The molecular structure of **1b** determined from X-ray crystallography showed unambiguously that the absolute configuration at the C-5 position of the barbiturate ring is S. Therefore, the absolute configuration at C-5 for **1a** must be R. Based on this report and previous studies (3,4), the absolute configuration of the major amobarbital *N*-glucoside conjugate excreted in human urine is *S*. In a structurally related system, the major phenobarbital *N*-glucoside conjugate excreted in human urine was the *S* diastereomer (C-5 of the barbiturate ring) (23). Based on the two barbiturates characterized to date, when the glucose and barbiturate ring are superimposed, there is overlap of the large substituent (phenyl and isoamyl) and small substituent (ethyl) for the major barbiturate *N*-glucoside diastereomer excreted in the urine. This is consistent with a common metabolic pathway for formation of the *N*-glucoside conjugates of both barbiturates.

The urinary excretion rate of amobarbital *N*-glucoside(s) has been shown to parallel the rate of decline of the serum concentration of amobarbital in a first order relationship (13). The development of a "shortcut" method (12) established that total urine collection was not necessary to estimate the overall rate constant, K, and the rate constant for amobarbital *N*-glucoside(s) excretion, $k_{N\text{-Glu}}$ (referred to as $k_{f,1b}$ in this paper). As shown in Table III, comparison of the results for K obtained in this study with those previously reported for K and $k_{N\text{-Glu}}$, similar values for K were observed for all groups except the Caucasians at the 100-mg dose. The K obtained for this group was significantly lower than that obtained for Caucasians at the 200-mg dose or the Orientals at the 100-mg dose. These observations suggest that at higher doses of amobarbital, the kinetics for amobarbital elimination may deviate from linearity (24). However, additional dose–response studies would be needed to confirm this observation.

Previous studies (12–14,25,26) of amobarbital *N*-glucoside excretion have indicated a systematic difference between Orientals and Caucasians. These previous studies had used a GC-MS procedure (on-column methylation and pyrolytic cleavage of the nitrogen glucose bond followed by quantification of *N*-methylamobarbital) that did not differentiate the amobarbital diastereomers (25). To determine if the difference previously reported between Orientals and Caucasians might be attributable to differential formation of **1a** and **1b**, the urinary excretion of these metabolites was monitored in both races. The percentage of amobarbital that was excreted in the urine as **1b** over 7 days (approximately 4–5 half-lives) ranged from 5.3 to 23.2% in the Caucasians and 7.2 to 26.8% in the Orientals when calculated using $k_{f,1b}/K$ (Table II). Therefore, the difference observed between Orientals and Caucasians was not associated with a product enantioselectivity for urinary excretion of the amobarbital *N*-glucosides.

The urinary excretion parameters obtained for **1b** were comparable to that described for the unresolved amobarbital *N*-glucosides. However, for the three Caucasians that had taken both doses, the percentage excreted as **1b** was greater at the lower dose. Although this sampling is very small, this was unexpected. The variation obtained for $k_{f,1b}$ was too great to give any significance to the differences in the mean values obtained for each group. However, the values obtained for $k_{f,1b}$ appear to be lower than those obtained for $k_{N\text{-Glu}}$ in previous studies. This is especially noticeable when comparing the average percentage of the dose excreted as **1b** in this study to the percentage of the dose excreted as amobarbital *N*-glucoside in earlier studies (Table III). There is almost a twofold difference in these results. It is possible that the change in dose may contribute to part of this difference; however, the limited ability to detect **1a** would not account for the remaining difference. Since the HPLC assay is more structure specific than the GC/MS assay, it is possible that the analytical method used in the prior studies may have been detecting and quantitating an additional metabolite(s) as amobarbital *N*-glucoside.

CONCLUSION

Although studies of the *N*-glucosylation pathway are very limited, amobarbital is the most thoroughly studied compound for this pathway in humans. This study has shown that the *S* diastereomer (C-5 of the barbiturate ring) is the major amobarbital *N*-glucoside conjugate excreted in human urine. In the subjects studied the differences in formation of the *N*-glucosides observed between Orientals and Caucasians were not due to the formation of different diastereomeric products. The urinary excretion of the single diastereomer of the amobarbital *N*-glucoside occurs with a formation rate constant comparable to, although somewhat lower than, those previously reported. Further studies of this unusual metabolic pathway are under way to determine if chirality on the C-5 side chain (i.e., pentobarbital) will affect the enantioselective formation and/or excretion of the diastereomers of the barbiturate *N*-glucoside conjugates.

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