IDENTIFICATION OF METABOLITES OF OCTAMETHYLCYCLOTETRAISILOXANE (D4) IN RAT URINE

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ABSTRACT:
Octamethylcyclotetrasiloxane (D4) is an industrial chemical of significant commercial importance. In this study, its major urinary metabolites were identified. The urine samples described here were collected from male and female Fischer rats (F-344) administered [14C]D4 i.v. The metabolite profile was obtained using an HPLC system equipped with a radioisotope detector. HPLC analysis was performed on a C18 column, using an acetonitrile/water mobile phase. The HPLC radiochromatogram revealed two major and at least five minor metabolites. The two major metabolites, constituting 75 to 85% of the total radioactivity, were identified as dimethyldiols [Me2Si(OH)2] and methylsilanetriol [MeSi(OH)3]. Formation of MeSi(OH)3 clearly established demethylation at the silicon-methyl bonds of D4. No parent D4 was present in urine. The minor metabolites identified were tetramethyldisiloxane-1,3-diol [Me2Si(OH)-O-Si(OH)Me2], hexamethytrisiloxane-1,5-diol [Me3Si(OH)-O-Si(OH)Me2-OSi(OH)Me2], trimethylsiloxane-1,3,3-triol [Me3Si(OH)2-O-Si(OH)Me2], dimethyldisiloxane-1,1,3,3-tetrol [Me3Si(OH)2-O-Si(OH)Me2], and dimethyldisiloxane-1,1,1,3,3-pentol [Si(OH)3-O-Si(OH)Me2]. The structural assignments were based on gas chromatography-mass spectrometry analysis of the tetrahydrofuran metabolite extracts, which were derivatized using bis(trimethylsiloxy)trifluoroacetamide, a trimethylsilylating agent. The structures were confirmed by synthesizing 14C-labeled standards and comparing their HPLC radiochromatograms with the corresponding components in the rat urine. GC-MS spectral comparisons of the trimethylsilylated derivatized standards and urinary components also were made to further confirm their identities. Finally, several of the urinary metabolites were fractionated using HPLC, and GC-MS comparisons were again made for positive structural identification. The pathways for metabolite formation are not yet understood, but a mechanistic hypothesis has been proposed to account for the various metabolites observed thus far.

Octamethylcyclotetrasiloxane (D4) is a colorless, volatile liquid with a vapor pressure of 1 mm at 25°C (Flanningam, 1986) and a water solubility of ~50 ppb (Varaprath et al., 1996). It is an industrial chemical of significant commercial importance with uses as an intermediate in the manufacturing of polydimethylsiloxane polymers for applications such as sealants, molded rubber products, fabric coatings, electrical components, and in personal care products such as antiperspirants, shampoos, and skin creams.

The growing use of D4 in commercial products and consumer applications prompted the Inter-Agency Testing Committee of the Environmental Protection Agency to include D4 on the 15th list of “Priority Chemicals” (Inter-Agency Testing Committee, 1988), recommending that D4 be considered for chemical and environmental fate/effects testing. As part of the chemical fate testing, several physical property determinations such as water solubility, octanol/water partition coefficient, and Henry’s Law constant (Hamelink et al., 1996) have been made. In addition, several biodegradation studies were undertaken: specifically, pharmacokinetic investigations of D4 distributions and absorption, and metabolism. The present study is concerned with the metabolism aspect of the pharmacokinetic investigations and the identification of essentially all major metabolites of D4 collected from rat urine after exposure to [14C]D4.

Materials and Methods

Instrumentation/Reagents. Radioactivity measurements were made using a liquid scintillation counter (model no. 2500 TR; Packard Instrument Co., Meriden, CT). HPLC analyses were performed with a Hewlett-Packard 1050 liquid chromatograph equipped with an HP Autosampler (model no. 79855A; Hewlett-Packard, Palo Alto, CA) and a Radiomatic detector (model no. 515 TR) from Packard. The detector was installed with a 500-μl liquid flow cell. HPLC conditions with the water/acetonitrile mobile phase were as follows: 100% water, 0 to 20 min; 100% water to 100% acetonitrile, 20 to 40 min; 100% acetonitrile, 40 to 60 min. A C18 Ultima column (4.6 × 250 cm and 5 μm; Alltech Associates, Inc., Deerfield, IL) was used as the stationary phase; Ultima-Flo M liquid scintillation cocktail was used in the flow cell. The ratio of column effluent to scintillation cocktail was 1:3. For samples that were not radiolabeled, an HPLC system (Hewlett-Packard) equipped with a refractive index detector (Hewlett-Packard) was used. HPLC fractions were collected using an automated fraction collector (model no. Fossy 200; Isco, Lincoln, NE).
coupled to HPLC systems. Acetonitrile was purchased from Fisher Scientific and deionized water was generated using Ultrapure Water Systems (Millipore). For liquid scintillation counting, the liquid scintillants Hionic-Fluor and Ultima-Gold obtained from Packard Instrument Co. were used.

Gas chromatography-mass spectrometry (GC-MS) was performed using a Hewlett-Packard 5890 Series II Gas Chromatograph, coupled with either an HP 5989A Mass Spectrometer or an HP 5970 Series Mass Selective Detector. Both GC-MS systems were equipped with HP 7673 GC/FSC (supercritical fluid chromatography) injectors and electronic pressure control units. Data analyses were performed using a Windows-based Chem Station. GC-MS conditions were as follows: MS source temperature, 200°C; MS quadrupole temperature, 100°C (for MS 5989); detector temperature, 280°C. Oven: initial temperature, 70°C; initial time, 3 min; heating rate, 20°C/min; final temperature, 210°C; injection port, 250°C. Inlet flow settings: Helium pressure, 15 kPa at 70°C; constant flow, ON; GC column, HP-5 (5% phenylmethylsilicone); column length, 30 m; column i.d., 25 mm; film thickness, 0.25 μm.

Samples or reagents were mixed with either a VWR vortex mixer (Scientific Industries Inc., Bohemia, NY) or a horizontal platform shaker (Eberbach Corporation, Ann Arbor, MI). [14 C]D4 used in animal exposures was custom synthesized at Wizard Laboratories (West Sacramento, CA) and randomly labeled. The specific activity of the [14 C]D4 was 2 mCi/mmol. Purity as determined by GC and HPLC was ~99%.

Sample Collection. Urine samples used in the metabolite investigation came from two studies. In one study, male and female F-344 rats (eight animals), CD-IF(344)/CrlBr, approximately 7 to 10 weeks of age were administered [14 C]D4 (Varaprath, 1999) as an emulsion i.v. via tail vein injection. The animals were purchased from Charles River Laboratories (Kingston, NY) and Charles River Canada Inc. (Constant, Quebec, Canada). Each animal received a nominal dose of 70 mg/kg D4. The [14 C]D4 emulsion, diluted with unlabeled D4 to achieve the desired radioactivity (58 μCi), was prepared in saline containing Emulphor EL-620L. Urine samples were collected at several time points (6, 12, 24, 48, and 72 h) after exposure. The 12-h samples from all animals were pooled and centrifuged before analysis.

In another study, four jugular vein-cannulated female F-344 rats (Charles River) were first induced with phenobarbital once a day for four consecutive days (80 mg/kg i.p.). They were then administered an emulsion (300 μl) of [14 C]D4 (70 mg/kg) i.v. via a jugular vein cannula. This procedure was carried out to obtain greater amounts of metabolites in urine. Urine samples were collected at 12, 24, 48, and 72 h after D4 administration. Again, 12-h samples from all animals were pooled and centrifuged to obtain a clear fluid. Urine samples were kept frozen at ~80°C until use. For the metabolite investigation in urine, i.v. was chosen because it allows for a direct delivery of the test material, by-passing any absorption barriers.

Concentration of samples (urine or HPLC fractions of the metabolites) was carried out using the Speed Vac system consisting of the following components: refrigerated condensation trap, model RT 100; chemical trap, model SC 120; rotary vacuum pump, model VP 100; Speed Vac concentrator, model SC 100). The system was purchased from Savant Instruments, Inc., (Farmingdale, NY).

Extraction of Metabolites. In metabolism studies it is desirable that the metabolites are completely extracted into an organic solvent for structural elucidation by GC-MS or other techniques. We have demonstrated in our previous work (Varaprath et al., 1998) that tetrahydrofuran (THF) is an extremely efficient solvent for extraction of urinary metabolites of D4. The extraction efficiency was estimated to be >95%, hence urine samples were subjected to THF extraction. It should be pointed out that although THF is completely miscible with water, it did form a separate layer in a urinary matrix due to the presence of dissolved salts. Aliquots (200–500 μl) of the clear urine samples were extracted repeatedly with THF (1 to 2 ml aliquots) until no significant amount of radioactivity was left in the urine residues. The combined extracts were treated with anhydrous MgSO4 to remove water, vortex mixed (4–5 min), and centrifuged (5 min) to obtain clear, dry THF extracts. The extracts were then concentrated to volumes of about 100- to 200-μl using either a rotary evaporator (5°C/20 mm Hg aspirator pressure) or by gently blowing nitrogen via Pasteur pipette along the sides of the container vials. It was shown by HPLC analysis that the extraction and concentration processes do not alter the metabolite profile or composition.

Derivatization. Being water soluble and polar, urinary metabolites of D4 were expected to have silanol structures with multiple hydroxy functionality. Although silanols such as dimethylosilanolated have been directly analyzed by GC-MS (Varaprath et al., 1995), silanols with >2 OH groups per Si do not survive the GC conditions and do not elute as such in GC. For this reason, the silanol functions of the metabolites were protected before GC-MS analysis using bis(trimethylsilyl)trifluoroacetamide (BSTFA), a trimethylsilylating agent. The reaction with BSTFA was quite rapid and took place under neutral conditions. The structural integrity of the silanol metabolites was expected to be preserved in such a mild environment. The dried and concentrated THF extracts were treated with equivalent amounts (v/v) of BSTFA purchased from Petrarch (now United Chemical Technologies, Inc., Bristol, PA), vortex-mixed for 2 to 5 min, and then shaken using a horizontal shaker for 2 h at ambient temperatures. BSTFA treatment was repeated as needed, if partial derivatization became apparent from GC-MS analyses. Derivatization with hexamethyldisiloxane (MM, 99.9% pure, obtained by spinning band distillation) was carried out in a similar way, except that a catalyst amount of hydrochloric acid also was added. This latter reagent was used to derivatize the metabolite dimethylosilanolated (containing single silicon species) only, because metabolites containing more than one silicon atom will undergo hydrolysis under acidic conditions.

Aqueous Silanol Functionality Test (ASFT; Mahone et al., 1983) of Urine Samples. A urine sample (500 μl) was placed in a 4-oz Teflon bottle with a screw cap, and 80 ml of 10% (v/v) hydrochloric acid was added. The bottle was tightly closed and shaken for 72 h using a wrist action mechanical shaker at ambient temperature. One milliliter of very high purity (99.9%) MM (11) was added to the bottle and shaking was continued for 48 h. The contents were then allowed to settle for about 10 min. First, the bottom aqueous phase was removed as completely as possible using a 100-ml syringe. The residual aqueous phase, along with the MM layer, was then collected into a 7-ml vial and centrifuged for 5 min. The clear MM layer was collected for analysis by GC-MS or HPLC.

[4 C]Methylsilanetriol [MeSi(OH)3, A]. This material was received from General Electric Company (GE). It was stored as an aqueous solution at a 650-ppm concentration. The radioactivity of the aqueous solution was determined to be 0.0071 mCi/ml. In HPLC, the material eluted at ~3.4 min.

[14 C]Dimethylosilanolated [MeSi(OH)2 or Monomerdiol, B]. One hundred milliliters of deionized water was placed in a clean 4-oz Teflon bottle and 15 μl of [14 C]dimethylsiloxane (neutral, 98.3% pure by GC, specific activity 2.4 mCi/mmol; custom synthesized at Wizard Laboratories) was added. The bottle was closed tightly with a Teflon screw cap and the contents were stirred using a magnetic stir bar for 2 h. Based on the expected quantitative transformation to dimethylosilanolated, the final concentration of the dimethylosilanolated was calculated to be ~100 ppm. In HPLC, this component eluted at ~13.4 min.

[14 C]Tetramethyldisiloxane-1,3-diol [HO-(Me2SiO)2-H or Dimerdiol, C]. Twenty microliters (neutral liquid) of [14 C]tetramethyldisiloxane (specific activity 2.4 mCi/mmol) was placed in a plastic centrifuge tube and 1 ml of deionized water was added. The tube was capped, vortex mixed for about 5 min, and set aside at room temperature. The sample was analyzed by HPLC under the usual conditions and the component eluted at ~31.7 min. At equilibrium, the dimerdiol (based on relative peak area of C-14 to the parent monomerdiol) accounted for about 22%.

[14 C]Hexamethytrisiloxane-1,5-diol [HO(SiMe2O)2-H or Trimerdiol, D]. One hundred microliters of the equilibrium mixture containing 22% [14 C]HO-SiMe2-O-SiMe2OH and 78% [14 C]MeSi(OH)2 was mixed with 200 μl of deionized water and 700 μl of Dow Corning (Midland, MI) Z-6329 Silane [Me2Si(OMe)2]. The contents were placed in a plastic centrifuge tube and vortex mixed at room temperature for 3 min and then shaken at 65°C for 24 h in a water bath. An HPLC radiochromatogram indicated a mixture containing dimer- and trimerdiol (70:30%, respectively). The trimerdiol eluted at 36.5 min.

[14 C]Trimethylsilanolated-1,3,5-triol [(HO)3Si-O-SiMe2O, E]. Method A. One hundred microliters of the 20,000-ppm aqueous solution of unlabeled Me2Si(OH)2 was mixed with 50 μl of a 650-ppm aqueous solution of [14 C]Me2Si(OH)2 (radioactivity 0.0071 mCi/ml). The ingredients were placed in a 1-ml-capacity glass vial and shaken at 50°C for 3 h. The HPLC radiochromatogram showed that the desired product eluted at 28.3 min. [Note: the 20,000 ppm aqueous solution of Me2Si(OH)2 in turn was prepared by hydrolyzing 600 μl of Me2Si(OMe)2 with 20 ml of deionized water in a high-density polyethylene bottle at room temperature over a 3-h period.]
Three hundred microliters of the 100-ppm $[^{14}C]$Me$_2$Si(OH)$_2$ aqueous solution was mixed with 300 μl of a 5000-ppm aqueous solution of unlabeled MeSi(OH)$_3$. The ingredients were placed in a 7-ml-capacity glass vial, then shaken at 45°C for 1 h. The HPLC radiochromatogram showed the product eluting at 28.2 min. [Note: the 5000-ppm aqueous solution of MeSi(OH)$_3$ in turn was prepared by hydrolyzing 757 μl of MeSi(OMe)$_3$ with 100 ml of deionized water in a 4-oz. Teflon bottle at room temperature overnight.]

$[^{14}C]$Dimethyldisiloxane-1,1,3,3-tetrol [(HO)$_2$SiMe-O-SiMe(OH)$_2$], C. One hundred microliters of a 650-ppm aqueous solution of $[^{14}C]$MeSi(OH)$_3$ (specific activity 0.0071 mCi/ml) was mixed with 200 μl of a 5000-ppm aqueous solution of unlabeled MeSi(OH)$_3$. The ingredients were shaken at 50°C for 3 h in a 7-ml-capacity glass vial. To increase the amount of the desired material, an additional 100 μl of 10,000-ppm aqueous unlabeled MeSi(OH)$_3$ was added and the mixture was heated at 50°C for 1.25 h. An HPLC radiochromatogram indicated that the desired product eluted at 7.5 min.

Results

Metabolite Profile. Of the various biological matrices, urine was chosen as the investigative medium because it is known to contain mostly metabolites. A metabolite profile showing the number and relative amounts of various biotransformation products generated from the parent material can be readily obtained if the metabolites are radiolabeled. Accordingly, rats were administered $[^{14}C]$labeled D$_4$ (Varaprath, 1999); urine samples were collected for analysis.

The HPLC radiochromatogram (Fig. 1) revealed two major metabolites (A and D) and at least five minor metabolites (B, C, E, F, and G). Combined, the two major components A and D constituted 75 to 85% of the total radioactivity in urine. The relative amounts of the various metabolites in rat urine collected from different experiments are shown in Table 1.

![Diagram of metabolites](image_url)

**Fig. 1.** Metabolites of D$_4$ in rat urine: Structural assignments to HPLC components.

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<thead>
<tr>
<th>Peak Label</th>
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<tbody>
<tr>
<td>A</td>
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<tr>
<td>B</td>
<td>Me$_2$Si(OH)-O-Si(OH)$_3$</td>
<td>8–10</td>
</tr>
<tr>
<td>C</td>
<td>MeSi(OH)$_2$-O-Si(OH)$_2$Me</td>
<td>2–6</td>
</tr>
<tr>
<td>D</td>
<td>Me$_2$Si(OH)$_2$</td>
<td>30–50</td>
</tr>
<tr>
<td>E</td>
<td>Me$_2$Si(OH)-O-Si(OH)$_2$Me</td>
<td>4–8</td>
</tr>
<tr>
<td>F</td>
<td>Me$_2$Si(OH)-O-Si(OH)Me$_2$</td>
<td>2–10</td>
</tr>
<tr>
<td>G</td>
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Identification of Major Metabolites (A): MeSi(OH)$_3$ and (D): Me$_2$Si(OH)$_2$. Structural assignments were made from GC-MS analyses of the trimethylsilylated THF extracts of the metabolites from urine. Tentative assignments of the structures were also made from HPLC analysis. HPLC retention time comparisons were made of the metabolites in urine with that of the synthetic $^{14}$C-labeled standards$^3$. Control urine samples collected from rats not exposed to $[^{14}C]$D$_4$ were also subjected to extraction, derivatization, and GC-MS analysis. Experiments with controls were especially critical in analyzing siloxanes to verify the absence of the potential metabolites in solvents and derivatization agents.

Metabolite A. MeSi(OH)$_3$ (Methysilanetriol). The clue to the presence of this metabolite came from the application of an analytical procedure referred to as the ASFT (see Materials and Methods;
Varaprath et al., 1998). This is a novel method developed for the characterization of waterborne organosilicon substances. The technique is capable of analyzing waterborne organosilicon materials in the parts per million range. Stated briefly, in this method, all siloxane species are reduced to single silicon-containing species with -OH functionalities. Then in a subsequent step, the -OH functionalities are protected with trimethylsilyl groups to enable elution in GC or GC-MS for identification. Following this method, the urine sample was first subjected to acid digestion at ambient temperature with 10% (w/v) hydrochloric acid, during which the various metabolites present in urine underwent Si-O bond cleavage, producing three hydrolysis products, each containing a single silicon atom and two to four hydroxy groups (Fig. 2).

Depending on the extent of demethylation (i.e., loss of 0, 1, or 2 methyl groups on a silicon atom) that had occurred to the parent D₄ during biotransformation, three types of silanols were expected from the acid digestion step. The silanols were dimethylsilanediol, methylicosilanetriol, and silicic acid (structures 1, 2, and 3, respectively, in Fig. 2). In the subsequent step involving protection of the hydroxy functions by reaction with MM, the silanols were expected to generate the corresponding trimethylsilyl (TMS or Me₃Si-) derivatives (structures 4, 5, and 6, respectively). Products 4 and 5 [octamethyltrisiloxane (MDM) and methyltris(trimethylsiloxy)silane (M₃T)] were clearly observed in GC-MS analysis (Fig. 3). The presence of tetrakis(trimethylsiloxy)silane (M₄Q, structure 6) was seen at an approximately similar level in control samples and M₄Q therefore was not considered as the end product of the D₄ metabolite.

Both MDM and M₃T were also observed (at 48.1 and 53 min, respectively, as in the case of authentic standards) in the HPLC radiochromatogram of the ASFT (Fig. 4). M₄Q was not expected to be seen in HPLC because the precursor silicic acid contains no methyl groups and no radioactivity available for detection.

The most polar of the metabolite components in urine, eluting at 3.4 min in HPLC (A in Fig. 1), was suspected to be MeSi(OH)₃. To make a direct comparison, an aqueous solution of the ¹⁴C-labeled MeSi(OH)₃ was analyzed by HPLC. The HPLC retention time of 3.4 min for this standard confirmed its presence in urine.

The HPLC component in the test urine eluting around 3.4 min was collected from repeat injections. The fractions were combined, concentrated, extracted with THF, and then derivatized using BSTFA. An ion-extract chromatogram was generated from the GC-MS total ion data for the expected mass of the TMS derivative of the metabolite. MeSi(OH)₃, if present in urine, is expected to give the derivative M₃T with a mass (m/z) of 310. However, because methyl silicones lose methyl groups and seldom show the parent (M) ions, an ion-extract chromatogram was obtained for m/z 295 (M-15). Mass spectral fragmentation characteristics of the component eluting at 6.19 min matched with that of the standard sample of M₃T (i.e., major peaks at m/z 295, 207, and 73) confirming the presence of the metabolite MeSi(OH)₃ in urine.

It should be noted, however, that decamethyltetrasiloxane (MD₂M, an isomer of M₃T) also has an identical mass spectral fragmentation pattern and its retention time is also quite close to M₃T under the GC-MS conditions used. To distinguish the two and eliminate matrix-related variation in retention times, coelution experiments were performed. On spiking the urinary matrix in separate experiments with MD₂M and M₃T, it was found that the metabolite derivative did indeed coelute with the M₃T standard and not MD₂M. This result...
again clearly established the presence of M\textsubscript{1}T and the metabolite MeSi(OH)\textsubscript{3} in urine.

**Metabolite D, Me\textsubscript{2}Si(OH)\textsubscript{2} (Monomerdiol).** Significant amounts of MDM in the ASFT (Figs. 3 and 4) of rat urine containing D\textsubscript{4} metabolites strongly suggested precursors of the structure HO-(Me\textsubscript{2}SiO)\textsubscript{x}-H, among others. Of these, the presence of HO-(Me\textsubscript{2}SiO)-H (dimethylsilanediol or monomerdiol) was confirmed by HPLC radiochromatogram comparison with an authentic sample of \textsuperscript{14}C-labeled material. The HPLC retention time (13.2 min) of the synthetic standard was identical with that of a peak found in urine. The standard \textsuperscript{14}C-labeled Me\textsubscript{2}Si(OH)\textsubscript{2} was synthesized by hydrolyzing \textsuperscript{14}C-Me\textsubscript{2}Si(OMe)\textsubscript{2} (Varaprath, 1999) under neutral conditions with deionized water, and its formation was confirmed by direct GC-MS (electron impact ionization/selected ion monitoring mode) analysis (m/z 77, M-15) on a 10-ppm dilute solution in THF (Varaprath et al., 1995).

The component in urine eluting at 13.2 min in HPLC (Fig. 1) was then collected in the usual manner from multiple injections. The fractions were combined and concentrated. The concentrate was analyzed by GC-MS after derivatization carried out with both MM and BSTFA. However, a much cleaner spectrum was obtained in this case by using MM as the derivatization agent (not shown). Comparison of the GC-MS retention time (4.5 min) and mass spectrum (m/z 221, 103, and 73) of the metabolite with those of the standard clearly indicated the presence of MDM and thus the metabolite Me\textsubscript{2}Si(OH)\textsubscript{2} in urine.

**Identification of Minor Metabolites:**

**Metabolites B: (OH)\textsubscript{3}Si-O-Si(OH)Me\textsubscript{2} and C: MeSi(OH)\textsubscript{2}-O-Si(OH)\textsubscript{2}Me.** The HPLC retention times of B and C lie between those of A (3OH functions per Si) and D (2OH functions per Si), but much closer to A than D. The very low retention times of these metabolites would indicate the prevalence of OH functions, with a lesser number of methyl groups per silicon atom. An ion-extract chromatogram of the BSTFA-derivatized THF extract of the test urine sample showed the presence of three components (retention times: 8.98, 9.09, and 9.18 min), each with a mass m/z of 443. The mass fragmentation patterns were quite similar, which suggested that they are structural isomers. The component with the retention time 9.09 min was determined to be tetradecamethylpentasiloxane (an impurity in BSTFA) by comparison to a standard (obtained by fractionation of a mixture containing linear siloxanes). Several structural possibilities existed for the other two components that pertain to mass 443. However, taking into consideration both the HPLC and GC-MS data, the following structures were tentatively assigned for the trimethylsilyl (TMS) derivatives eluting at 8.98 and 9.18 min, respectively: Me\textsubscript{2}Si(OTMS)-O-Si(OTMS)\textsubscript{3} and MeSi(OTMS)\textsubscript{2}-O-Si(OTMS)Me. These structures indicated the presence of corresponding silanols Me\textsubscript{2}Si(OH)-O-Si(OH)\textsubscript{3} and MeSi(OH)\textsubscript{2}-O-Si(OH)\textsubscript{2}Me as metabolites in urine. Because the structure with the silicic acid backbone [that is, Me\textsubscript{2}Si(OH)-O-Si(OH)\textsubscript{3}] was expected to be more polar, this was assigned to component B in the HPLC radiochromatogram, and the other structure [MeSi(OH)\textsubscript{2}-O-Si(OH)\textsubscript{2}Me] to C.

An authentic \textsuperscript{14}C-labeled sample of MeSi(OH)\textsubscript{2}-O-Si(OH)\textsubscript{2}Me was synthesized by condensation of \textsuperscript{14}C-MeSi(OH)\textsubscript{3} with unlabeled Me-Si(OH)\textsubscript{3}. The latter was added to increase the concentration of the reactant and facilitate the equilibration to dimerization. The reaction was also conducted at a slightly elevated reaction temperature (40–50°C) to rapidly attain equilibrium. GC-MS of the TMS derivative from this synthetic material extracted into THF was found to match both in retention time and mass spectral characteristics to the metabolite component eluting at 9.18 min (m/z = 443, 355, 281, 221, 147, and 73). Also, this synthetic \textsuperscript{14}C-MeSi(OH)\textsubscript{2}-O-Si(OH)\textsubscript{2}Me had the same HPLC retention time as the component C present in urine samples.

The synthesis of Me\textsubscript{2}Si(OH)-O-Si(OH)\textsubscript{3} was attempted by the reaction of an aqueous solution of silicic acid [generated from the hydrolysis of Si(OMe)\textsubscript{4}] with \textsuperscript{14}C-Me\textsubscript{2}Si(OH)\textsubscript{2}. Only trace amounts of the desired product could be obtained but the HPLC retention time of the synthetic material matched that of metabolite B, and the mass spectral fragmentation patterns of their TMS derivatives were identical and nearly identical with that of the TMS derivative of metabolite C (see above).

**Minor Metabolites, F: HO-(Me\textsubscript{3}SiO)\textsubscript{2}-H (Tetramethyldisiloxane-1,3-diol or Dimerdiol) and G: HO-(Me\textsubscript{3}SiO)\textsubscript{3}-H (Hexamethytrisiloxane-1,5-diol or Trimerdiol).** As noted earlier, significant amounts of MDM in the ASFT (Figs. 3 and 4) of rat urine containing...
D₄ metabolites indicated the presence of several structures, including the type HO-(Me₅SiO)₃-H. Of these, the presence of HO-(Me₅SiO)₂-H (D) has already been clearly demonstrated. The presence of HO-(Me₅SiO)₂-H and HO-(Me₅SiO)₃-H as metabolites of D₄ in urine was also established (as in the case of D), by generating authentic ¹⁴C-labeled materials and making HPLC comparisons.

A simple way to generate these materials has been found. It is known that at concentrations of just 2% in aqueous solutions, dimethylsilanediol undergoes a self-condensation reaction to form the dimmer (Varaprath et al., 1995). We have taken advantage of this ready dimerization tendency by hydrolyzing [¹⁴C]Me₅Si(OMe)₂ (20 μl) with 1 ml of deionized water to achieve a 2% (v/v) solution of [¹⁴C]Me₅Si(OH)₂, promoting self-condensation. To accelerate the equilibration reaction to form the dimerdiol, the reaction mixture was then gently shaken at a slightly elevated temperature (40–50°C). The progress of the reaction was monitored by HPLC analysis.

By increasing the concentration of the reaction mixture through addition of Me₅Si(OH)₃ (labeled or unlabeled) and continuing the heating, additional condensation to form the trimerdiol (G) was achieved. From the HPLC analysis, the retention times for the dimerdiol and trimerdiol standards generated were determined to be 31.7 and 36.5 min. The two components in the test urine that eluted at essentially identical retention times were therefore assigned the corresponding structures. GC-MS analysis of their TMS derivatives showed masses mlc: 295 (M-15), 207, 103, and 73 and 369 (M-15), 281, 147, and 73, respectively, as expected for the products MD₃M and dodecamethylpentasiloxane. The retention times 6.51 and 8.08 min, respectively, and mass spectral patterns of these TMS derivatives also matched commercially available standards.

Metabolite E: Me(OH)₂Si-O-Si(OH)Me₂. The HPLC retention time of metabolite E lies between that of dimethylsilanediol (D) and tetramethyldisiloxane-1,3-diol (dimerdiol, F), but much closer to the latter. Therefore, the metabolite must have an intermediate polarity, suggesting the possibility of an oxidized dimerdiol structure, [Me(OH)₂Si-O-Si(OH)Me₂], for this metabolite, containing three OH functionalities per molecule.

To conclusively prove the structure, the [¹⁴C]-labeled standard was synthesized by two different routes. In one, [¹⁴C]Me₅Si(OH)₃ was allowed to react with unlabeled Me₅Si(OH)₂, and in the other, unlabeled Me₅Si(OH)₂ was allowed to react with [¹⁴C]Me₅Si(OH)₃. The resulting products from both of the routes had similar retention times in HPLC: 28.3 and 28.2 min, respectively, and they matched the component present in urine. GC-MS analyses of the TMS derivative from the synthetic material also showed similarities in retention time (7.93 min) and mass spectral characteristics (mlc = 369, 281, 147, and 73) to the component present in the urine.

Discussion

Organosilane and siloxane compounds are known to undergo metabolic transformation. The metabolic fates of several silicon-containing compounds have been reported. Fessenden and Ahlfors (1967) examined urinary metabolites of siliccarbamates, viz, bis(hydroxymethyl)dimesitylsilane dicarboxamidate (CH₃₂Si(CH₂ONH₂)₂), (hydroxymethyl)-dimethyl-n-propylsilanecarbamate CH₃CH₂CH₂Si(CH₃)₂CH₂ONH₂, and bis(hydroxymethyl)-di-n-propylsilanedicarboxamidate (CH₃CH₂CH₂Si(CH₃)₂CH₂ONH₂)₂ after oral administration to rats. The formation of oxidative dealkylation (silanols) and oxidative hydroxylation products was observed.

In dealing with any metabolism work involving silicones, it is important to be cognizant of potential problems. The procedures normally used in identification of organic metabolites may not be directly applicable to silicones due to significant differences in chemical properties between the two. Organic metabolites can often tolerate harsh workup procedures. However, in the case of silicones, milder workup procedures should be used. Acids and bases must be avoided, because rearrangements, self-condensation, and isomerization are common occurrences under these conditions. These phenomena are especially pronounced if the metabolites contain hydroxy functions. The extreme affinity of silicon for oxygen is the cause of several of the rearrangement reactions. Functional groups such as -OH, -COOH, and -CH₂OH are commonly seen in organic drug metabolites. If such functionalities are formed from siloxane metabolism, they can potentially undergo rearrangement with migration of the Si atom from carbon to oxygen (Brook and Gilman, 1955; Brook and Iachia, 1961). In addition, background levels of silicone species in solvents used for extraction, or reagents used for metabolite derivatization, may also contribute to artifacts (Fessenden and Hartman, 1970).

In conclusion, the major metabolites of octamethycyclotetrasiloxane (D₄) in rat urine have been identified. The methods used were quite specific, and minimize ambiguities in the structural assignments. Simple procedures were developed to synthesize ¹⁴C-labeled metabolite standards, which enabled direct HPLC comparisons with urinary components. This methodology greatly facilitated structural identification. The process also used the optimum solvent (THF) to extract the metabolites (essentially quantitatively) and a mild derivatization agent (BSTFA) to permit identification using GC-MS. The metabolites identified have also clearly established that some demethylation occurs at the silicon-methyl bond.

Mechanistic Pathways for Metabolite Formation. The mechanistic pathways for the formation of the various metabolites are not clearly understood. However, possible pathways for their generation are speculated and presented in Fig. 5, where it is suggested that intermediate 2 can be the precursor to most of the metabolites A to G. It is known that such an intermediate is generated in the atmospheric oxidation of D₄ (Sommerlade et al., 1993). However, to our knowl-
edge, there is no precedent for direct oxidation of a Si-CH\textsubscript{3} group to an Si-OH function in drug metabolism. We therefore propose the formation of an intermediate Si-CH\textsubscript{2}OH group (structure \textit{8}), which is well preceded to rearrange to Si-OH (\textit{10}): The latter can hydrolyze easily to methanol and Si-OH. The sequence Si-CH\textsubscript{2}OH \rightarrow Si-CHO \rightarrow Si-OH resulting from continued oxidation and hydrolysis reactions is also plausible.

We were unable to detect the intermediate \textit{9} in test urine. It may be that this species is quite unstable and underwent a rapid ring-opening reaction with water to generate the intermediate \textit{10}. Cleavage of Si-O bonds in \textit{10} at different sites by hydrolysis can then account for the metabolites \textit{A, D, E, and G}. The metabolites \textit{D, E, and G} could also have resulted directly from either \textit{7} or \textit{8} by a series of hydrolysis reactions. The other metabolites \textit{B, C, and E} can be formed in a variety of ways, as shown in Fig. 5, including self- and cross-condensation or via oxidation and hydrolysis reactions of the initially formed metabolites. At every stage, oxidation and hydrolysis reactions could potentially occur. Oxidation must be catalyzed by an enzyme (McKim et al., 1998), whereas hydrolysis may or may not be enzyme-mediated. It is not necessary that metabolites containing fewer silicon atoms than the parent D\textsubscript{4} [such as Me\textsubscript{2}Si(OH)\textsubscript{2}] be formed only by sequential hydrolysis of higher homologs. A one-step mechanism involving simultaneous cleavage of two Si-O bonds can also be envisioned to form Me\textsubscript{2}Si(OH)\textsubscript{2}.

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References


