plaining the low *in vivo* antileukemic activity of substituted hydroxyureas. The potential interrelationship between internal hydrogen bonding and conformational isomerization as well as the resultant effect on the pKa of hydroxyurea molecules and biological transport to the site of action is currently being studied. Initial *in vitro* tests on ribonucleotide reductase (mammalian) by analogs IV–VI indicate enzyme inhibition activity[7] at about 10 times the concentration required with hydroxyurea (I). Therefore, some property involving transport of these molecules to their site of action might be involved in their pharmacodynamics.

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NOTES

Rapid Determination of Dimethyl Polysiloxane by Proton Magnetic Resonance Spectroscopy

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Abstract □ A new procedure is described for the quantitative extraction and determination of dimethyl polysiloxane by ^{1}H-NMR spectroscopy, using tetrachloroethylene as the solvent and dioxane as the internal reference.

Keyphrases □ Dimethyl polysiloxane—coatings, NMR analysis □ NMR spectroscopy—analysis, dimethyl polysiloxane coatings □ Silicones—NMR analysis of dimethyl polysiloxane coatings

The increasing use of silicones in drug, cosmetic, and food industries has given rise to various chemical and physical methods for the identification of this material. Several methods allow quantitative determinations of siloxane. Gravimetric (1) or volumetric (2) assays, visible or UV determination after mineralization (3–7), potentiometric titration (8), and IR (3, 6, 9–12) or atomic absorption spectroscopy (5, 12–14) determination after extraction have been reported.

A simple assay by NMR spectroscopy of the dimethyl polysiloxane (dimethicone) coating on needles of syringes is presented. This new technique compares favorably with the usual IR method.

EXPERIMENTAL

Method—Commercially available dimethyl polysiloxanes (I) [range of viscosity 0.65–10^6 centistokes (cS)] are silicone polymers in which each silicon atom of the polysiloxane chain is bonded to two methyl groups.

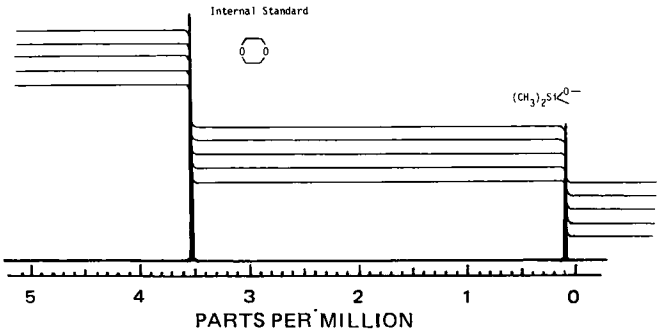

Figure 1—*Five traces of integral curve for analysis of dimethyl polysiloxane.*

Because of the presence of six equivalent hydrogen atoms per monomeric unit, I is a molecule of choice for quantitative estimations by NMR spectroscopy, showing a sharp singlet at δ 0.09 ppm (tetramethylsilane as reference). The area under a particular ^{1}H-NMR signal is directly proportional to the number of protons giving this signal and is easily obtained from the corresponding integral trace.

In a quantitative ^{1}H-NMR procedure (15), a particular integral value is related to a specific number of protons estimated by the inclusion of an internal standard in the solutions to be analyzed. Dioxane, at any suitable concentration (here at 0.4% v/v) in tetrachloroethylene, was chosen as a standard because it contains eight magnetically equivalent protons. Furthermore, tetrachloroethylene is a useful solvent both for the extraction of silicones and for ^{1}H-NMR spectroscopy.

Ratio values (R), defined as:

$$R = \frac{\text{mean value (mm) of integrals of } (CH_3)_2Si<^{O}- \text{ peak}}{\text{mean value (mm) of integrals of dioxane peak}} \quad \text{(Eq. 1)}$$

are obtained by five consecutive traces of the integral curve (Fig. 1) of the dioxane singlet at δ 3.53 ppm and the $(CH_3)_2Si<^{O}-$ singlet at δ 0.09 ppm.

Silicone is quantitatively estimated by comparing R values of solutions of unknown concentration in I with R values of standard solutions.

This new method and the usual IR assay are applied to I of different viscosities (20, 50, 200, 300, and 1000 cS). Silicones having a mean number of dimethyl siloxane units from 10 to about 350 are readily measured with these techniques.

Apparatus—NMR spectra were obtained on a 60-Hz spectrometer[1], and IR spectra were obtained on a double-beam spectrophotometer[2].

Reagents—Tetrachloroethylene, dioxane, and methylene chloride were spectroscopic grade solvents. Compounds I were commercially available silicones[3].

Procedures—*^{1}H-NMR Analysis*—Standard solutions were prepared with weighed samples of I of different viscosities. Linear regression of R, y, with concentration, x, was obtained with a range of 0–6.0 mg/ml from standard curves. The simple regression line of y on x calculated by the method of least squares was $y = 0.24x - 0.04$. A coefficient of correlation of 0.996 for more than 100 samples ascertained its significance. The regression passed through the origin, and the standard deviation of the intercept was 0.09.

IR Analysis—Control analyses were made by the IR method (9, 12), using the >Si—O— stretching band at 1090 cm^{-1} in methylene chloride solution. The calibration line related absorbance values with the concentration in the 0–2000-μg/ml range; the coefficient of correlation was 0.944.

Assay—*^{1}H-NMR Spectroscopy*—Metal needles, cut from 10 syringes pretreated with silicone, were collected in a flat-bottom flask of 3 ml and extracted by stirring for 5 min at room temperature with 1.0 ml of tetrachloroethylene–dioxane (99.6:0.4 v/v). NMR spectroscopy curves were made on 0.5-ml samples of the solution.

[1] Perkin-Elmer R 12.

[2] Beckman IR-4.

[3] The 360 medical fluids (20 and 200 cS) were supplied by Dow Corning Corp.; M 1028/50, 300, and 1000 cS were supplied by U.C.B., Belgium; and Baysilon M 300 (300 cS) was from Bayer, Germany.

Table I—Milligrams of the Silicone Recovered per Syringe

Assay	Silicone, mg	
	NMR Method	IR Method
1	0.148	0.154
2	0.151	0.154
3	0.152	0.148
4	0.150	0.152
5	0.150	0.147
6	0.149	0.153
7	0.150	0.150
8	0.151	0.152
Mean value	0.150	0.151
SD	1.25×10^{-3}	3.08×10^{-3}

Table II—Milligrams of the Silicone Recovered per Syringe as a Function of Concentration of the Lubrication Liquid Assay by NMR

Silicone in Lubrication Liquid, %	Silicone per Syringe, mg
20	0.15
10	0.05
7	0.04

Table III—Recovery of I

Lubrication Liquid[a], mg	Assay by NMR Method		Recovery, %
	R Values	Silicone, mg	
750.2	0.670	147.5	98.3
907.5	0.754	183.0	100.8
861.2	0.711	167.5	97.3
915.0	0.808	180.0	98.4
		Mean value	98.7

[a] Silicone (20%) in chloroform.

IR Spectroscopy—Needles, precoated with silicone, were stirred for 5 min at room temperature in methylene chloride (1.0 ml). The absorbance of the peak at 1090 cm^{-1} was compared with the standard curve.

RESULTS

Table I shows the data obtained by the two methods with eight groups of 10 syringes from the same batch. These needles were pretreated with a 20% (v/v) chloroform solution of I (360 medical fluid, 350 cS) as the lubrication liquid. The assays on batches treated with the lubrication liquid containing different concentrations of silicone are listed in Table II.

Recovery experiments (Table III) showed that 98.7% of I was recovered after a single extraction. Recovery experiments were done by coating sheets of glass with various weighed quantities of a 20% (w/v) chloroform solution of silicone. After evaporation of the chloroform, glass sheets were extracted with 50 ml of tetrachloroethylene–dioxane (99.6:0.4 v/v) and assayed by NMR.

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NMR Determination of Enantiomers of 7-Chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo[3,2-*b*][1,3]benzoxazin-9-one Using Chiral Shift Reagent, Tris[3-(heptafluorobutyryl)-*d*-camphorato]europium(III)

PHILIP REISBERG [x], IAN A. BRENNER, and JEROME I. BODIN

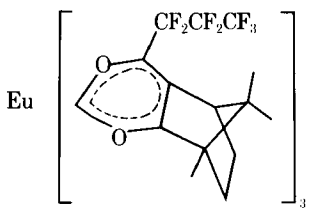
Abstract □ A simple NMR method was developed for the determination of the enantiomers of 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo[3,2-*b*][1,3]benzoxazin-9-one. Chiral shift reagent, tris[3-(heptafluorobutyryl)-*d*-camphorato]europium(III), causes the doublet assigned to the protons of the 2-methyl group, which normally appears at about 1.5 ppm, to split into two pairs of doublets and to shift downfield to about 2.0–3.5 ppm. The downfield pair of doublets represents the two enantiomers present in one racemate, designated as the β-form, while the upfield pair represents the enantiomers of the racemate designated as the α-form. From the integration of the area under the doublets, the relative concentration of all four enantiomers was determined.

Keyphrases □ NMR spectroscopy—determination, enantiomers of 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo[3,2-*b*][1,3]-benzoxazin-9-one using europium chiral shift reagent □ Europium—chiral shift reagent, use in NMR determination of enantiomers of substituted isoxazolo[3,2-*b*][1,3]benzoxazin-9-one □ Enantiomers—substituted isoxazolo[3,2-*b*][1,3]benzoxazin-9-one, NMR determination using europium chiral shift reagent □ Chiral shift reagent, europium—use in NMR determination of enantiomers of substituted isoxazolo[3,2-*b*][1,3]benzoxazin-9-one

The use of deuterated tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedionato)europium(III) shift reagent to determine the ratio of the diastereoisomers of 7-chloro-3,3a-dihydro-2-methyl-2*H*,9*H*-isoxazolo[3,2-*b*][1,3]benzoxazin-9-one[1] (I) was reported previously (1). An extension of this method to determine the enantiomers of each isomeric pair by use of the chiral shift reagent tris[3-(heptafluorobutyryl)-*d*-camphorato]europium(III) (II) has been successfully accomplished.

DISCUSSION

The use of lanthanide shift reagents in NMR spectroscopy in the last few years has been of great help in elucidating the structures of many organic compounds (2). With the introduction of the new optically active shift reagents (3), a new dimension was added to this approach. The chiral shift reagent can discriminate between enantiotropic groups and can separate peaks arising from the corresponding protons of a racemate. When the reagent is added to a chiral sample, pseudocontact shift differences are observed in the NMR spectrum.



II

The successful determination of the optical purity of different types of compounds has been reported (4, 5). Chiral shift reagents also have been used to distinguish *meso-* from *dextro-* or *levo-*diastereoisomers of dimethyl 2,3-diaminosuccinate (6). As with the nonchiral lanthanide shift reagents, the europium chiral complexes produced a downfield shift while the praseodymium chiral complexes did the reverse.

Since I has two optically active centers, it can exist as four different stereoisomers. In this work the presence of the four stereoisomers was investigated by use of the chiral shift reagent.

EXPERIMENTAL

Apparatus—Spectra were obtained at 60 MHz using an analytical spectrophotometer[2]. A sweep time of 250 sec and a sweep width of 500 Hz were used, unless otherwise indicated; the δ-scale was used throughout. Tetramethylsilane (III), 1% (v/v) in deuterated chloroform[3], was used as the internal reference from which chemical shifts were measured.

Reagents and Chemicals—Compound I, α- and β-forms, was used (1). For the chiral shift reagent, a 0.3 *M* solution of II was

[1] Previously referred to in the literature as W-2395.

[2] Varian T-60.

[3] Norell Chemical Co., Landing, N.J.