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NMR Study of Amphetamines Using Europium Shift Reagents

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Abstract □ Amphetamine and certain of its methoxylated derivatives show a high degree of interaction with NMR shift reagents of the type tris(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione)europium(III). The shifts are not accompanied by appreciable line broadening, and both the aliphatic and aromatic protons can be resolved. The strong interaction between amine and shift reagent diminishes rapidly as the amine function is alkylated. For derivatives containing *ortho*-methoxyl groups, a weaker interaction with this functionality also becomes evident as the amine is alkylated. The stereospecificity of the shifting process was investigated by employing tris[3-(trifluoroacetyl)-*d*-camphorato]europium(III), a chiral shift reagent, with stereochemically pure enantiomers and known enantiomeric mixtures. Although certain (*R*)-enantiomers showed greater downfield C-methyl group shifts, these shift differences from the corresponding (*S*)-enantiomers were small and not well resolved.

Keyphrases □ Amphetamine and methoxylated derivatives—interaction with NMR europium shift reagents, stereospecificity of shifting process □ NMR spectroscopy—study of amphetamines and methoxylated derivatives, interaction with europium shift reagents, stereospecificity of shifting process □ Europium—NMR shift reagents, interaction with amphetamines and methoxylated derivatives

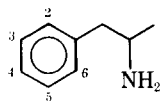
Methods for the use of lanthanide NMR shift reagents in spectral simplification and interpretation of molecules possessing a single functional group are well established (1–3). Their use with polyfunctional compounds and the techniques employing chiral shift reagents, however, are not as well characterized (4–6). Korver and Van Gorkom (7) referred to the scarcity of data defining the scope of application of chiral reagents in determining enantiomeric purity, while Armitage *et al.* (8) referred to the highly neglected problem of employing shift reagents in the determination of molecular configuration.

An interest in these analytical tools and a desire for an efficient assay of enantiomeric composition in certain amphetamine derivatives (9) were the bases for this investigation. The NMR spectra of amphetamine (I) and the derivatives II–VII were examined in the presence of increasing amounts of tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione)europium(III) (VIII). Certain enantiomers and enantiomeric mixtures of amphetamine, *p*-methoxyamphetamine, and 2,5-dimethoxyamphetamine were also studied with the chiral reagent tris[3-(trifluoroacetyl)-*d*-camphorato]europium(III) (IX).

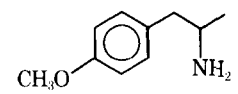
EXPERIMENTAL

Chemistry—The enantiomers of I–V were prepared by a modification of the method of Weinges and Graab (10) as described previously (9). The optical purity of these compounds is in the 96–99% range (9). Racemic samples were prepared by reaction of the appropriate benzaldehyde with nitroethane (11), followed by lithium aluminum hydride reduction of the resulting 1-phenyl-2-nitropropenes (12). Racemates were purified by distillation or by recrystallization of the hydrochloride salts.

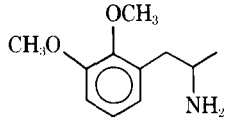
NMR Studies—Free amines were generated by first dissolving 100-μmole quantities of hydrochloride salts in 7 ml of water. These solutions were placed in separators, 5-ml portions of ether were added, and the aqueous phases were made basic with 0.5 ml of 3 *N* sodium hydroxide. After separation of the ether layers, the aqueous phases were extracted with a second 5-ml portion of ether. The



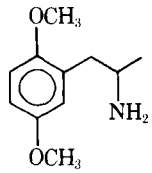
I: amphetamine



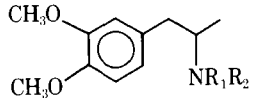
II: *p*-methoxyamphetamine



III: 2,3-dimethoxyamphetamine



IV: 2,5-dimethoxyamphetamine



V: 3,4-dimethoxyamphetamine, $R_1 = R_2 = H$

VI: 3,4-dimethoxy-*N*-methylamphetamine, $R_1 = CH_3$, $R_2 = H$

VII: 3,4-dimethoxy-*N*,*N*-dimethylamphetamine, $R_1 = R_2 = CH_3$

Table I—Lanthanide-Induced Shifts of Amphetamines[a]

Compound	—$NH_{2,1}$	—CH—	—CH_2—	—CH_3	Ar—$H_{2,6}$	Ar—$H_{3,4,5}$	2-O—CH_3	3-O—CH_3	4-O—CH_3	5-O—CH_3	NH—CH_3	N—$(CH_3)_2$
I	19.4	5.4	5.7	4.8	1.4	0.5	—	—	—	—	—	—
II	16.5	4.5	2.5	4.0	1.0	0.4	—	—	0.1	—	—	—
III	10.7	2.3	1.9	2.4	0.8	0.3	0.8	0.1	—	—	—	—
IV	13.4	3.2	2.0	3.7	1.0	0.3	0.5	—	—	0.2	—	—
V	14.1	4.1	2.3	3.6	1.0	0.4	—	0.2	0.1	—	—	—
VI	—	1.2	1.2	0.9	0.7	0.6	—	0.5	0.5	—	1.4	—
VII	—	0.2	0.2	0.1	0.2	0.2	—	0.2	0.2	—	—	0.2

[a] Data represent $\Delta\delta$ values for R/S = 0.2. Values increased in a linear fashion up to and including R/S = 0.5 for all compounds except I, where deviation from linearity was observed for R/S values >0.3.

Table II—IX-Induced Shifts of Racemic Amphetamines[a]

Compound	—NH_2	—CH—	—CH_2	—CH_3	Ar—$H_{2,6}$	Ar—$H_{3,4,5}$	2-O—CH_3	4-O—CH_3	5-O—CH_3
I[b]	12.3	2.5	1.7	1.9 2.0[c]	0.7	0.2	—	—	—
II[b]	8.5	1.7	1.3	1.4	0.5	0.2	—	0.1	—
IV[b]	9.4	2.1	1.4	1.5 1.6[c]	0.5	0.2	0.3	—	0.1

[a] Data represent $\Delta\delta$ for R/S = 0.2. Values increased in a linear fashion up to and including R/S = 0.5. [b] Both pure enantiomers and their racemic mixtures were examined. Data reported are for the racemic mixtures. In all cases, the pure enantiomer spectra were identical to those of their corresponding racemic mixtures, except that in the latter spectra separation of the —CH_3 resonances for each enantiomer of I and IV occurred, as indicated. Compound II did not exhibit such resolution even at R/S = 0.5. [c] (*R*)-Enantiomer.

ethereal extracts then were combined and evaporated *in vacuo*, and the residues were dissolved in 2.0 ml of benzene–ethanol (4:1) and again evaporated to dryness *in vacuo*. Each residue was dissolved in 0.5 ml of deuterochloroform (1% tetramethylsilane)[1].

Shift reagent solutions were prepared by dissolving 100 μmoles of VIII[2] or IX[3] in 5.0 ml of deuterochloroform (1% tetramethylsilane). Solutions were prepared immediately before use, and a new solution was prepared for each series of spectra obtained for a single amphetamine sample. NMR spectra were recorded on a 60-MHz spectrometer[4].

After an initial NMR spectrum was obtained without shift reagent, the deuterochloroform solutions were evaporated *in vacuo*, the residues were dissolved in 0.5 ml of the shift reagent solution, and the NMR spectra were recorded. This process was repeated with increasing increments of shift reagent. Alternatively, in instances where substrate volatility appeared to be a problem (*i.e.*, leading to losses of amphetamine), larger quantities of the free amine were isolated and separately weighed for admixture with shift reagent solutions.

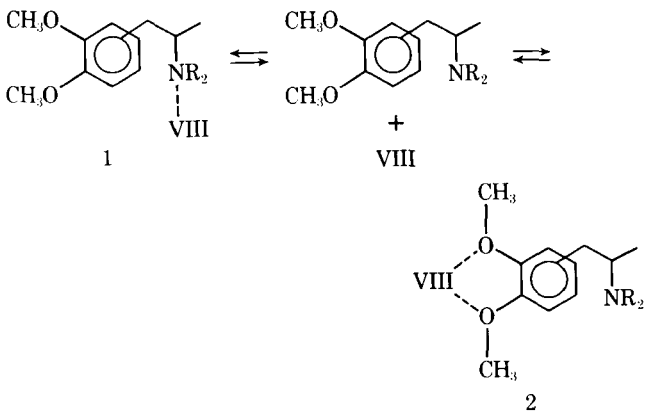
RESULTS AND DISCUSSION

The NMR spectral shifts of each proton signal accompanying incremental rises in shift reagent concentration were followed by plotting the proton signal location *versus* the molar ratio of shift reagent, R, to compound, S, at each increment (4). An R/S range of 0.0–0.5 was studied at four or more increments. Figure 1 illustrates the linear nature of the plots generated by this treatment.

Selected spectra from the amphetamine study are presented in Fig. 2 and are illustrative of the spectra for the methoxylated derivatives II–V as well. The utility of this method is demonstrated by the marked enhancement of resolution in the aliphatic spectral region and in the resolution of certain aromatic regions. Furthermore, in most instances, lanthanide-induced shifts were not accompanied by significant signal broadening up to R/S ratios of 0.5. With the secondary amine, VI, however, severe line broadening was inexplicably observed at R/S values above 0.17.

Table I presents the lanthanide-induced shift data for each compound obtained at similar R/S values and permits comparisons between compounds. Tabulation of the lanthanide-induced shifts data as their Demarco slopes (13–15) was not chosen because the polyfunctionality in many of these molecules offers different sites of interaction with the shift reagent (Scheme I), and a linearity beyond the actual R/S range studied should not be implied (4, 16–19). Similarly, tabulation as the Armitage bound chemical shifts seemed inappropriate (8, 20, 21).

From the decreasing —CH—C*H*$_3$ lanthanide-induced shifts in V–VII, it is apparent that the strong interaction between shift reagent and the amine function diminished rapidly as the amine was methylated (converted from primary to tertiary). This effect has



Scheme I—Possible sites of interaction between shift reagent and polyfunctional amphetamine derivatives

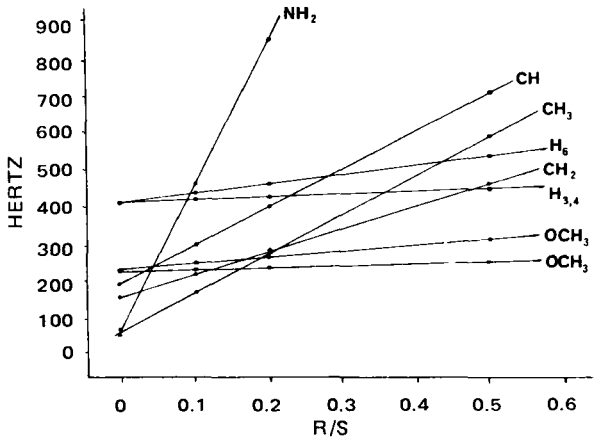


Figure 1—*Typical linear relationship observed between proton signal location (hertz) and R/S (actual data for IV).*

[1] Silanor-C, Merck Sharp & Dohme.
[2] Eu(fod)$_3$, Aldrich Chemical Co.
[3] Eu-OPT, Ventron Alfa Products.
[4] Varian T-60.

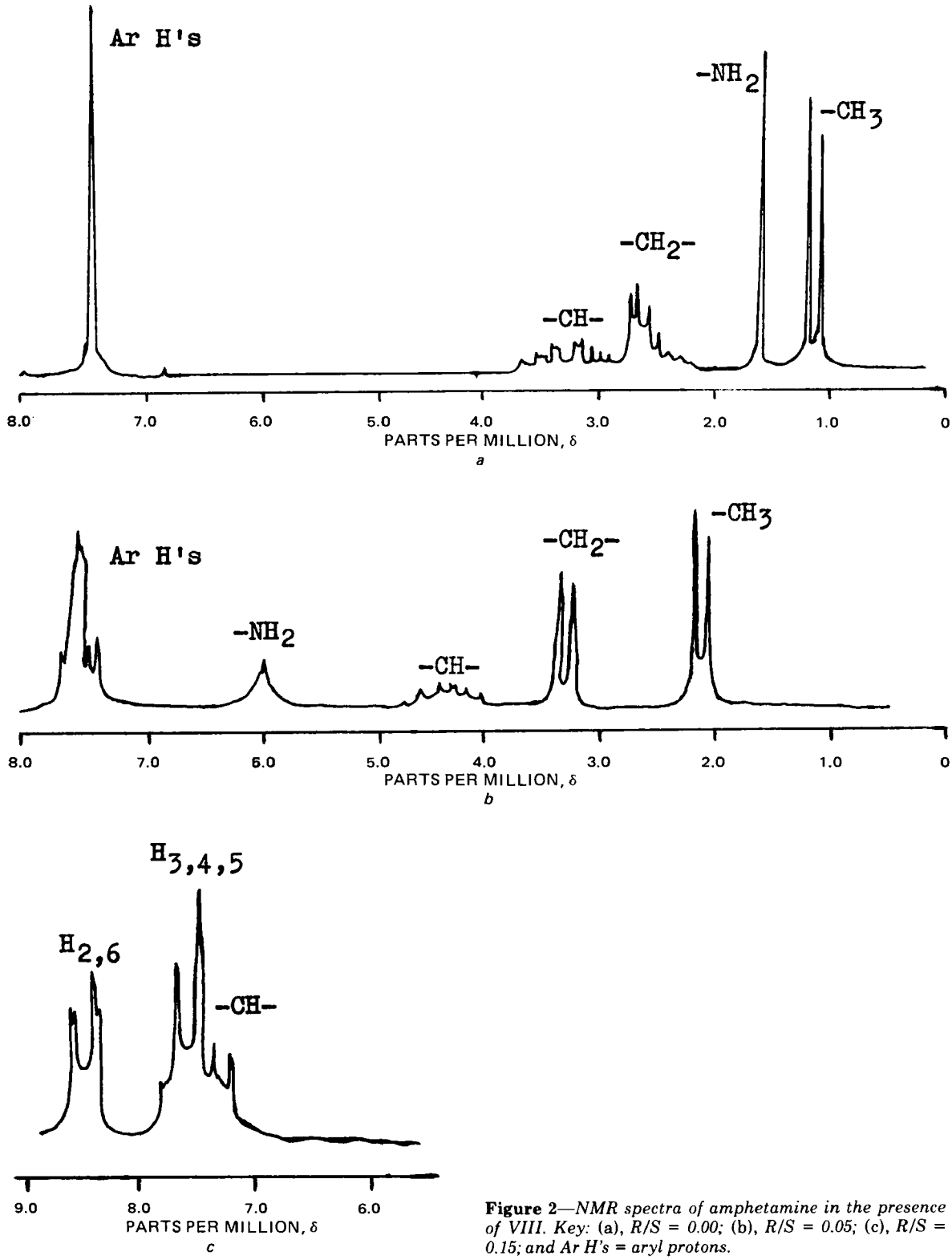


Figure 2—*NMR spectra of amphetamine in the presence of VIII. Key:* (a), *R/S = 0.00;* (b), *R/S = 0.05;* (c), *R/S = 0.15; and Ar H's = aryl protons.*

been attributed to an increase in steric hindrance for interaction with the nitrogen as it is alkylated (22). When comparing compounds V (primary amine) and VI (secondary amine), an increase in lanthanide-induced shifts of the methoxyl protons was observed, due perhaps to a shifting in equilibria (Scheme I) toward Structure 2 (or its equivalent). This phenomenon was somewhat curiously reversed with respect to the lanthanide-induced shifts of the methoxyl protons in Compounds VI and VII (tertiary amine). However, the lanthanide-induced shift of the *N*-methyl protons of VII was definitely decreased compared to that of V; and if shift reagent interaction occurred solely with the amine, then the lanthanide-induced shifts for the methoxyl protons in VII also should have been dramatically reduced compared to those in V.

This implies, in general, that a second point of interaction in this region of the molecule (2 in Scheme I) is enhanced by a shifting in equilibria as the interaction with the amine (1 in Scheme I) decreases. Similar preferential shift reagent interactions between two functionalities on opposite ends of molecules were reported previously (23). Taylor and Walters (24) found that when the primary amine of a phosphorus-containing compound was methylated (primary to secondary), interaction with shift reagent switched from the amine to the phosphorus atom.

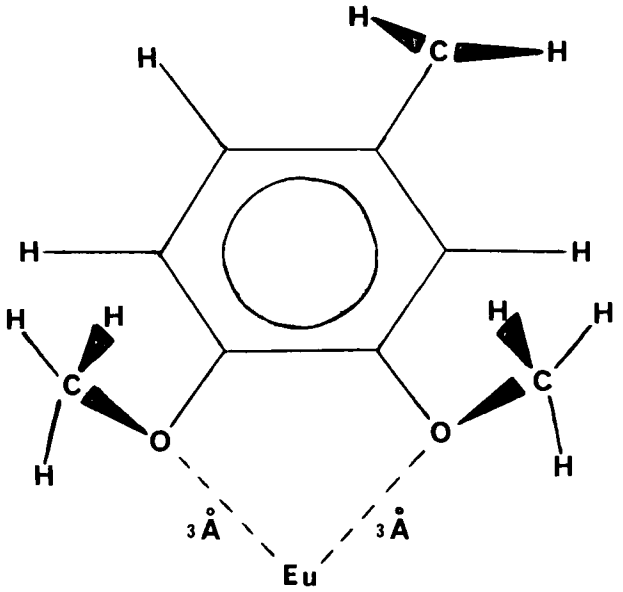


Figure 3—*Conformationally semirigid portion of the VII–europium bidentate complex model as it was positioned on the dipolar map of Wing* et al. (38).

Shift reagents have been found to interact with aromatic methoxyl groups in preference to aliphatic ethers (25), carbonyl groups (26), and pyrimidine nitrogens (27), but this interaction is generally regarded as being rather weak. However, when two methoxyl groups are present in an *ortho* relationship, it has been suggested that a bidentate interaction of the shift reagent can occur and that this type of interaction is considerably stronger (28). Lanthanide ions can show coordination numbers of three to 10 or 12 (29), and octavalent coordination, required for bidentate interaction, is reminiscent of the europium shift reagent form originally employed by Hinckley (30). The following structural types also have been implicated in bidentate interactions with NMR shift reagents: alicyclic diamines (31), furanone aliphatic ethers (32), aliphatic polyethers (33–35), and a tridentate interaction with alditols (36).

To define the nature of the shift reagent interaction occurring in VII, attempts were made to determine the geometry of the VIII–VII complex. Application of the McConnel–Robertson (37) equation and its logarithmic form (18) were attempted first. As is commonly done (38), the angular dependency term was ignored. In addition, only the molecular fragment embodying the methoxyl, aromatic, and methylene protons was examined. This portion of the molecule (Fig. 3) can be considered conformationally semirigid in each europium complex and should most readily lend itself to analysis.

The assumption that a europium atom location, midway between two functional groups, indicates a bidentate interaction may be erroneous for two reasons. First, a monodentate interaction, to an equal extent (through competitive equilibria) with each functional group, could also give an average McConnel–Robertson location midway between the two functionalities. An averaged europium atom location between two hydroxyl groups was demonstrated by Cockerill and Rackham (39). Second, bidentate interaction does not require that the europium atom be symmetrically located between the two functions, because the extent of interaction with each function will be determined by the unique electronic and steric properties of that function.

The results of these initial studies gave poor fits in both cases. This finding can be attributed either to contact contributions to the shifting process (40) or, more likely, to a neglect of the angular dependency term (41). The latter possibility is particularly appealing since a bidentate complex would restrict the rotational freedom about the europium–substrate single bonds. This effect of a bidentate interaction is extremely important, because such rotation about the symmetry axis in solution (42) gives effective axial symmetry. Such solution symmetry has been offered as one explanation for the success of the McConnel–Robertson equation in light of the growing X-ray evidence that implies that it should not work due to the lack of axial symmetry in many solid-state complexes (43–46).

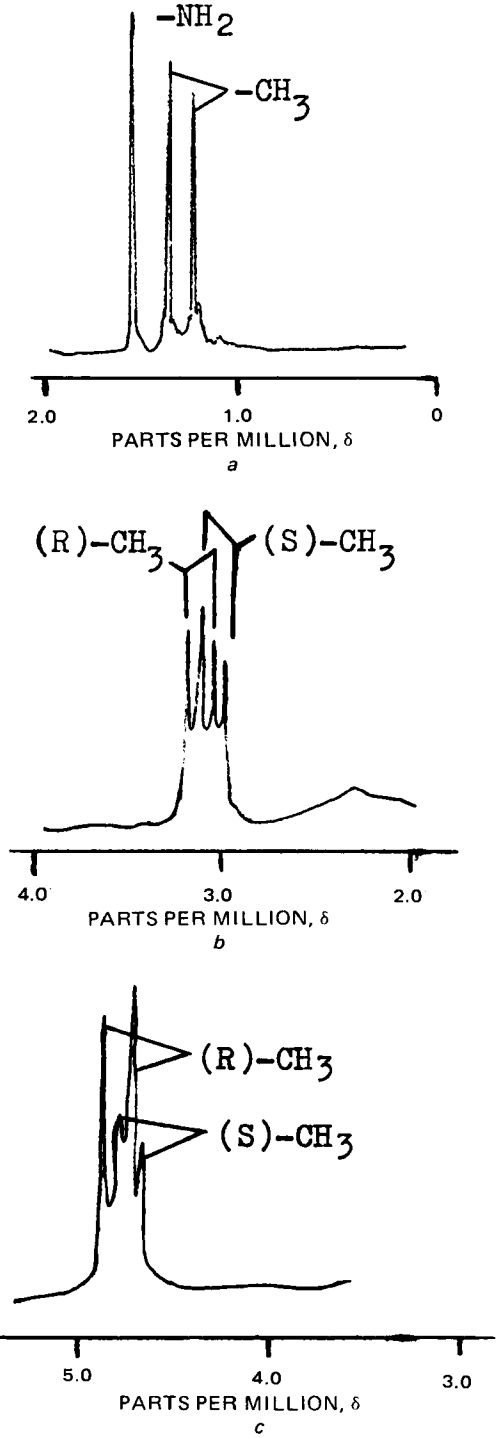


Figure 4—*NMR selectivity of the diastereotopic interaction of IX with enantiomeric mixtures of amphetamine. Key:* (a), (±)-*I, R/S = 0;* (b), (±)-*I, R/S = 0.25; and* (c), (±)-*I spiked with* (R)-*enantiomer.*

The graphical approach described by Wing *et al.* (38) was also employed to determine the europium atom location. This method accounts for the angular dependency term in the shifting process. Figure 3 illustrates the graphical positioning[5] of the semirigid por-

[5] The molecular fragment shown in Fig. 3 was superimposed on the dipolar map supplied by Wing *et al.* (38). Proper alignment of the europium and ligand oxygen atoms permitted assignment of coordinates, which predicted the relative lanthanide-induced shifts of the depicted protons.

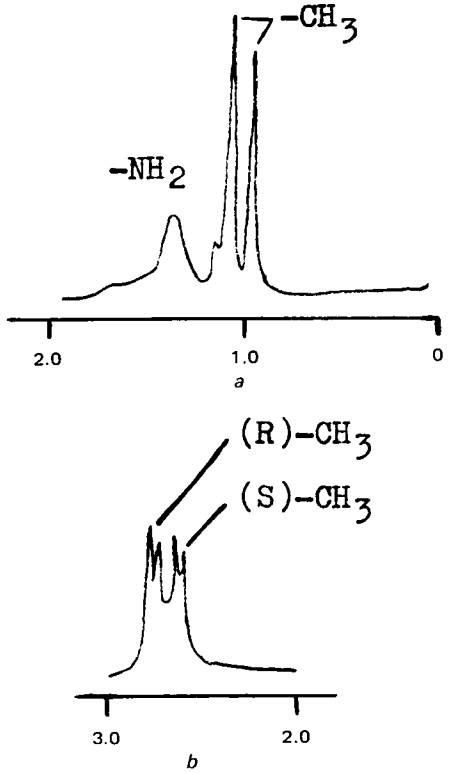


Figure 5—*NMR selectivity of the diastereotopic interaction of IX with enantiomeric mixtures of IV. Key:* (a), (±)-*IV, R/S = 0; and* (b), (±)-*IV, R/S = 0.22.*

tion of VII, which successfully predicted the observed lanthanide-induced shifts for the methoxyl, aromatic, and methylene protons. The europium atom–oxygen distance of 3 Å is in accord with the literature (4). However, the bonding picture implied by the graphical method indicates that interaction of the europium with the two methoxyl groups occurs *via* a single *d*-orbital of the europium atom. Such an event is questionable, and this method probably is subject to the same limitations as the mathematical approach when dealing with polyfunctional molecules capable of interacting with a shift reagent through various functional groups and/or in a bidentate fashion.

The interactions of IX with racemates and resolved enantiomers of I, II, and IV also were investigated. As indicated in Table II, these interactions are similar in magnitude to those observed with the VIII reagent (Table I). There were no dramatic spectral differences between enantiomers, but subtle lanthanide-induced shift preferences for the (*R*)-enantiomer methyl groups of I and IV were seen (Figs. 4 and 5). This assignment was based on (*R*)-spiked racemic samples in which the correspondingly larger peak moved the farthest downfield. Preferential shifts of (*R*)-enantiomers have been observed for related structures (47, 48), and this study indicates that the technique can be successfully applied to certain amphetamines.

However, a lack of enantiomeric discrimination obtained with (*R*)-*p*-methoxyamphetamine indicates that the method may not be useful for all compounds within this class. It was originally thought that IX might be useful in optical purity studies with amphetamines and/or similar systems. Results from these investigations indicate that its utility may be limited to only certain structures, although work with higher resolution spectrometers (100 MHz or greater) might provide sufficient separation of component C-methyl signals to study enantiomeric enrichment.

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ˣ To whom inquiries should be directed.

Intestinal Secretion of Erythromycin Base

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Abstract □ Erythromycin fluxes into rabbit midjejunal segments were studied. When erythromycin was infused into the jugular vein of anesthetized rabbits, the antibiotic was secreted into the segments at a rate of 0.0136 ± 0.0023 mg/min. Preloading of the segments with five and 20 times the plasma concentration did not diminish this secretion. Protein binding of the antibiotic within the lumen could not explain this secretion, since both ultrafiltration and chromatography of luminal solutions indicated that the biological activity was free erythromycin. Moreover, the transmural potential across the intestinal mucosa is not likely to be the principal driving force, since greater than 80 mv would be required to sustain the observed secretion against an imposed 20-fold concentration difference between blood and lumen. The best explanation for the intestinal secretion of erythromycin appears to be an active transport pathway capable of concentrating erythromycin in the lumen. It is not clear what endogenous substances are transported by this pathway.

Keyphrases □ Erythromycin—intestinal secretion, rabbit midjejunal segments □ Intestinal secretion—erythromycin, rabbit midjejunal segments □ Antibiotics—erythromycin, intestinal secretion, rabbits

In humans, oral administration of erythromycin free base produces low blood levels of antibiotic. For example, a 250-mg tablet of erythromycin free base produces average peak blood levels of 0.12–0.54 μg/ml (1, 2). In comparison, similarly administered propionyl erythromycin produces peak blood levels of 1.11–1.92 μg/ml.

The lower blood levels of erythromycin base have been attributed to a number of factors including: (*a*) a deleterious effect of ingested food on absorption of the base (3), (*b*) acid degradation in the stomach (4), (*c*) the volume of distribution following absorption (5), (*d*) biliary secretion (6–8), and (*e*) a combination of low absorption rate with high renal excretion rate (9).

Preliminary studies in this laboratory confirmed the observation (10) that a fraction of intravenously administered erythromycin base appeared in the intestinal lumens of rats with extracorporal bile fistulas. Since biliary excretion was clearly eliminated as a source of intestinal secretion, these studies suggested the direct secretion of erythromycin across the intestinal mucosa. Such a pathway of secretion might be important not only as a new route of elimination but also as an influence on the oral administration of erythromycin.

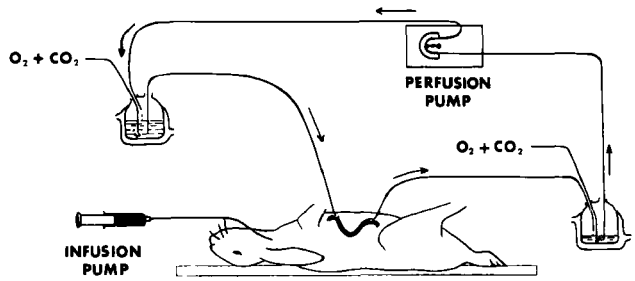

Figure 1—*Schematic representation of perfusion circuit through rabbit midjejunum.*

EXPERIMENTAL

Surgical Preparation—Male New Zealand white rabbits, 3.2–3.7 kg, were anesthetized with 50 mg/kg iv of secobarbital[1]. A 50-cm segment of midjejunum, measured 75 cm proximal to the ileocecal valve, was cannulated both proximally and distally. The segment did not include the bile duct. Krebs–Ringer bicarbonate buffer, saturated with 95% oxygen–5% carbon dioxide at 40°, was initially washed through the segment. This buffer had the following composition: Na^+, 0.143 *M*; K^+, 0.006 *M*; Ca^{+2}, 0.003 *M*; Mg^{+2}, 0.001 *M*; Cl^-, 0.128 *M*; PO_4^{-2}, 0.001 *M*; SO_4^{-2}, 0.001 *M*; and HCO_3^-, 0.025 *M*.

After rinsing with buffer, air was injected to displace excess solution. One hundred milliliters of buffer was then circulated through the segment between two water-jacketed reservoirs maintained at 40° (Fig. 1). Inflow pressure was maintained below 20 cm of water to prevent damage to the mucosa. Both reservoirs were initially bubbled with 95% oxygen–5% carbon dioxide. As the experiment progressed, the pH of the perfusate tended to increase but was maintained at a constant value of 7.4 by increasing the proportion of carbon dioxide in the gas mixture.

A polyethylene cannula in the left external jugular vein allowed vascular infusion of erythromycin. Blood samples were withdrawn through a second cannula placed in the left common carotid artery.

Perfusion Experiments—A constant concentration of erythromycin in the plasma (1 μg/ml) was maintained by a bolus injection of 3 mg into the jugular cannula followed by infusion at a rate of 5 mg/hr. The appearance of erythromycin in the intestinal segment was then followed by sampling at 20-min intervals. Carotid blood samples were taken concomitantly. The perfusate and carotid plasma were assayed by the disk-plate microbiological assay tech-

[1] Seconal, Eli Lilly and Co.