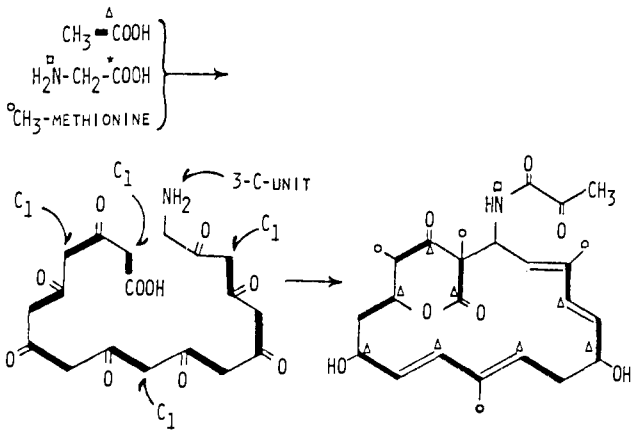
Scheme I



conducted with 90% enriched sodium [1-^{13}C]- and [1,2-^{13}C]acetate, [1-^{13}C]glycine, and [$^{13}CH_3$]methionine as well as with 90% enriched [^{15}N]glycine. These labeled precursors were added to shaking cultures of *Streptomyces sp.*[3] After fermentation, the labeled antibiotics were extracted into methylene chloride, acetylated with acetic anhydride in pyridine (48 h at 10 °C), and purified by preparative TLC using benzene–ethyl acetate (4:1). The lankacidin C diacetate (**2**) provided by this procedure was used for the ^{13}C NMR measurements.

The ^{13}C chemical shift assignments of natural-abundance lankacidin C diacetate shown in Table I were determined by off-resonance decoupling and by comparison with known carbon shift values of model compounds.[4] In addition, many of the previously established ^{1}H NMR shift assignments of the lankacidins[1] were used to determine many of the corresponding carbon shifts in selective proton decoupling experiments.

The labeling results summarized in Table I clearly establish that sodium [1-^{13}C]acetate enriches eight carbons—C-1, C-6, C-8, C-10, C-12, C-14, C-16, and C-18—of the macrolide ring. Incorporation of eight acetate units into the macrolide ring of **2** was corroborated by the antibiotic enriched with sodium [1,2-^{13}C]acetate, which showed eight pairs of carbon–carbon coupling signals as characteristic satellite signals flanking the center signal. Table I also lists the respective J values found.

Glycine was identified as the source of the C-3 amino group, since the ^{13}C NMR spectrum of **2** labeled by incorporation [1-^{13}C]glycine showed strong signal enhancement at only a single peak corresponding to the C-4 signal. A ^{15}N-enriched sample of lankacidin C diacetate prepared from feeding of [^{15}N]glycine indicated by mass spectrometry that a 20% excess ^{15}N was incorporated.[5] This result confirms that the N-C_3-C_4 grouping of the lankacidins is derived from glycine.

Use of [$^{13}CH_3$]methionine confirmed that the branching methyl groups C-19, C-20, C-21, and C-22 are derived through the acetate + C_1 pathway, since strong signal enhancement for only these four methyl carbons was observed. The absence of propionate participation in lankacidin biosynthesis was evident from the lack of any signal enhancement from a [1-^{13}C]propionate feeding experiment.

In contrast, the branching methyl groups of several other classes of macrolide antibiotics such as the 14-membered lactones in erythromycin[6] and picromycin[7] and the ansa macrolides rifamycin S,[8] streptrovaricin D,[9] and geldanamycin[10] have been established as coming from propionic acid units.

Scheme I shows the ^{13}C-label distribution established in our feeding studies. The formation of a linear polyketide chain is initiated by glycine incorporating eight acetic acid units, with methionine accounting for the four branching methyl groups in the positions indicated. A reasonable biogenetic route for the formation of the 17-membered carbocycle from the linear polyketide is through attack by the C-2 nucleophilic center on an electrophilic C-3 imine derivative of the glycine starter unit.

Only the origin of the three-carbon unit attached to the nitrogen remains unidentified in lankacidin biosynthesis; propionate and pyruvate are not incorporated. Further feeding experiments are required to establish its source.

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Lanthanide Effects on the Proton and Carbon-13 Relaxation Rates of Sarcosine. Evidence for Isostructural Amino Acid Complexes along the Lanthanide Series[1]

Sir:

Chemical shifts induced by lanthanide shift reagents should be useful in the determination of molecular structure and conformation in solution.[2,3] The trivalent lanthanide cations[4] or their EDTA chelates[5] can serve as shift reagents in aqueous solution and are applicable to the study of systems of biological

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Table I. Lanthanide Effected Longitudinal Relaxation Rates ($1/T_{1p}$, s^{-1}) and Distance Ratios for Sarcosine (1 M)

		Proton			Carbon-13				
Ln^{3+}	Concn, mM	CH_2	CH_3	r_{CH_3}/r_{CH_2}	CO_2^-	CH_2	CH_3	$r_{CH_2}/r_{CO_2^-}$	$r_{CH_3}/r_{CO_2^-}$
Pr	92.9	0.87	0.28	1.21	0.46	0.08	0.03	1.34	1.58
Nd	95.2	1.78	0.59	1.20	0.90	0.11	0.03	1.42	1.76
Gd	1.9	42.4	10.6	1.26	18.5	3.17	0.80	1.34	1.69
Tb	44.9	24.8	7.04	1.23	11.2	1.72	0.45	1.37	1.71
Dy	46.7	30.6	9.69	1.21	19.4	2.84	0.69	1.38	1.74
Ho	61.3	36.5	11.0	1.22	16.9	3.08	0.76	1.33	1.68
Er	46.6	20.9	7.00	1.20	8.85	1.66	0.41	1.32	1.67
Tm	57.0	11.3	4.30	1.17	7.32	1.04	0.34	1.38	1.67
Yb	83.2	2.60	0.92	1.19	1.18	0.24	0.06	1.30	1.64
Mean				1.21 ± 0.03				1.35 ± 0.04	1.68 ± 0.07
Mean: Pr–Tb				1.23 ± 0.03				1.37 ± 0.03	1.69 ± 0.06
Mean: Dy–Yb				1.20 ± 0.03				1.34 ± 0.03	1.68 ± 0.03

interest.[3,6] Imperative in most of the applications of the lanthanide shift method is the understanding of the shift mechanisms as well as of the structure of the complexes formed.[7] Chemical shifts in paramagnetic lanthanide complexes (relative to the diamagnetic state) are the sum of dipolar and contact contributions (cf. ref 1 and 7 and references cited therein). Only the dipolar shifts are geometrically related to molecular structure. Unfortunately a standard and reliable method for the separation of the two contributions has yet to be devised and structural interpretations involve numerous assumptions. The shifts induced by a series of lanthanides in the proton and carbon-13 spectra of the amino acid alanine have recently been interpreted in terms of two types of complexes.[8] For the light lanthanides (Pr^{3+}–Tb^{3+}) a structure involving monodentate coordination to the carboxylate is suggested, whereas with the heavier ions (Dy^{3+}–Yb^{3+}) the carboxyl group is bidentate.[9] Yet the complex dissociation constant is reported to be invariant along the series.[8] Since the free entropy change, resulting mainly from the dehydration of the cation, is known to be one of the important factors in the formation of lanthanide complexes,[10] the above conclusions, i.e., a structural change along the series that involves different ligand denticity coupled with invariant dissociation constant, seem to be contradictory, unless the entropy changes are almost entirely compensated by corresponding changes in enthalpy.

We propose the use of nuclear relaxation rates as an indicator of possible structural changes. The longitudinal relaxation rates in lanthanide complexes seem to be free from many of the complications encountered in the interpretations of chemical shifts and are almost entirely due to the dipolar interaction.[11] Thus the relaxation rates of different nuclei i and j, within the same complex relate to each other as $(r_j/r_i)^6$, where r_i is the distance of nucleus i from the central ion. Constancy of the intramolecular r_j/r_i ratio for different members of the lanthanide series should be a good criterion for the isostructurality of the complexes. Reported in this communication are the effects of paramagnetic trivalent lanthanides on the proton and carbon-13 longitudinal relaxation rates of the amino acid sarcosine (CH_3NHCH_2COOH) in D_2O. Measurements on molecular scale models constructed to conform with the structures given by Sherry and Pascual (cf. Figure 4 in ref 8) yield the following relative distances: $r_{CH_3}/r_{CH_2} = 1.23$, $r_{CH_2}/r_{CO_2^-} = 1.41$, $r_{CH_3}/r_{CO_2^-} = 1.75$ for bidentate coordination and $r_{CH_3}/r_{CH_2} = 1.64$, $r_{CH_2}/r_{CO_2^-} = 0.9$, $r_{CH_3}/r_{CO_2^-} = 1.48$ for monodentate coordination. The anticipated differences in the absolute values as well as in their trends should be sufficient to permit a meaningful interpretation of the relaxation data.

Longitudinal relaxation rates, $1/T_1$, were measured at a constant probe temperature of 27 °C and pH 4.8 ± 0.3 by the 180°–τ–90° pulse sequence at 270 and 67.89 MHz for protons and carbon-13, respectively, on a Bruker WH-270 spectrometer operating in the Fourier transform mode. The net paramagnetic contribution, $1/T_{1p}$, was obtained from the measured values by subtracting the relaxation rate of a similar solution containing the diamagnetic La^{3+} ion. The accuracy of these values is estimated to be ±10%. The experimental results along with the distance ratios are summarized in Table I. The Eu^{3+} ion is not included in the table since its effects were usually too small (of the order of the experimental error). The results show that different nuclei within the same complex experience different relaxation effects proving that conditions of rapid chemical exchange are fulfilled in the system. Note also that the effects experienced by the protons are much larger than those of carbon-13; e.g., the ratio for the methyl group is close to the $(\gamma_H/\gamma_c)^2$ ratio as expected for the dipolar interaction. All the distance ratios (calculated as the sixth root of the relaxation rate ratios) show remarkable constancy along the lanthanide series. Particular emphasis should be placed on the $r_{CH_2}/r_{CO_2^-}$ values which are the only ones free from possible complications due to internal rotations and conformational preferences within the sarcosine ligand.[12] Thus, according to the above criterion, the amino acid complexes are isostructural along the lanthanide series. Indication of the isostructurality of carboxylato complexes has previously been obtained from an analysis of the chemical shifts in the methoxyacetate–lanthanide system.[1] We note that the experimental distance ratios are in good agreement with the values anticipated for bidentate coordination (vide supra).

A number of possible sources for the different conclusions reached in other published work[8,9] may be suggested. These include (1) complications due to formation of higher than 1:1 complexes (e.g., 2:1 amino acid–lanthanide complexes have been observed[13]); (2) inadequate separation of dipolar and contact contributions (it has been shown that contact shifts in lanthanide complexes do not follow a predictable pattern[1,14]); (3) inadequacy of the axial model of dipolar shifts (deviations from this model have been reported[15]). Further experiments are needed in order to evaluate the contribution of each of these factors.

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Sequential Rearrangements via Bridged Carbocations

Sir:

Sequential rearrangements of alicyclic carbocations reveal structural and stereochemical preferences which were labeled "Memory Effects".[1] These phenomena were explained in terms of twisted ions separated by rotational barriers,[1] bridged ions (σ delocalization), and ion pairs.[2] Stereochemical control by ion pairing has been convincingly demonstrated,[2] but no unequivocal case of a sequential rearrangement via bridged ions appears to be known. We report here on sequential rearrangements involving phenonium ions.[3-5]

Scheme I

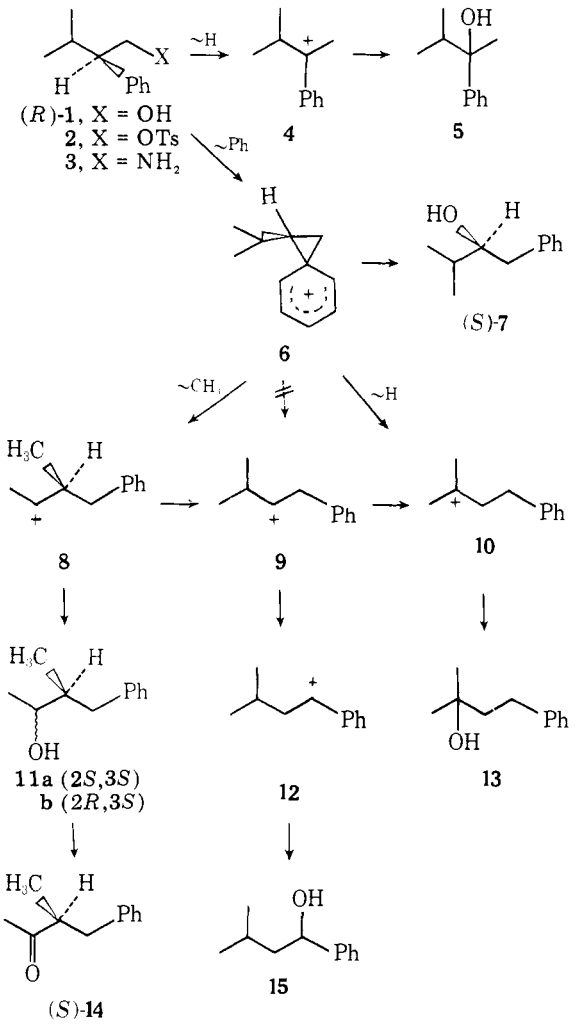
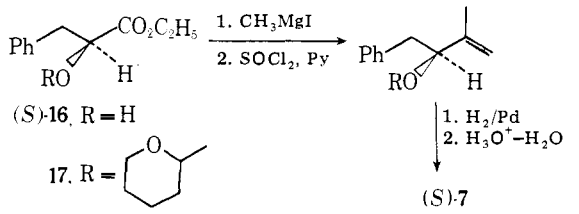


Table I. Products of the Solvolysis of **2** (Water–Dioxane, 4:1, 80 °C) and of the Deamination of **3** (0.03 M in $HClO_4$, pH 3.5, 25 °C)

	Alkenes	5	7	11a	11b	13	15	1
(*R*)-**2**	12.1	1.3	52.9	7.7	9.9	9.2		3.2
(*R*)-**3**	2.5	17.5	47.6	2.5	3.1	6.7	12.8	3.7
[2-^{2}H]-**3**	4.3	13.4	48.6	2.1	3.5	6.9	13.5	5.6

Scheme II



The tosylate **2** of (−)-(*R*)-3-methyl-2-phenyl-1-butanol[6] (**1**) was solvolyzed in aqueous dioxane at 80 °C in the presence of 2,6-lutidine (Scheme I, Table I). The major product, 3-methyl-1-phenyl-2-butanol (**7**), arises by a 1,2-phenyl shift. **7**, isolated from the solvolysis products by GLC, $[\alpha]^{24}_D$ −51.3° (*c* 1.95, CCl_4), was optically pure (±3%) as estimated by NMR in the presence of a chiral shift reagent[7] and by GLC of its *N*-trifluoroacetylprolyl ester.[9] The absolute configuration of (−)-**7** was established as *S* by correlation with (*S*)-2-hydroxy-3-phenylpropionic acid[10] as shown in Scheme **II**.[11] The complete inversion of configuration associated with the phenyl shift suggests formation of **7** from the phenonium ion **6** rather than from the open 3-methyl-1-phenyl-2-butyl cation **9**.

2-Methyl-4-phenyl-2-butanol (**13**), the product of a sequential Ph,H shift, was formed in the solvolysis of **2**, but 3-methyl-1-phenyl-1-butanol (**15**) was not obtained. The absence of **15** constitutes additional evidence against the open cation **9** which would be expected to undergo 1,2-H shifts to give **10** and **12** with comparable case. When **9** was generated by nitrous acid deamination of 1-benzyl-2-methylpropylamine, **13** and **15** were produced in a 2.3:1 ratio.

3-Methyl-4-phenyl-2-butanol (**11**) originates from **2** by a sequential Ph,CH_3 shift. The mixture of diastereoisomers **11a,b** was oxidized to give (+)-(*S*)-3-methyl-4-phenyl-2-butanone (**14**) of 83% optical purity. The configuration of **14** has been established by correlation with 2-methyl-3-phenylpropionic acid.[12] As some racemization was likely to occur during the oxidation of **11**, the enantiomeric purities of **11a** (95 ±3%) and **11b** (95 ± 3%) were determined by GLC of their (*S*)-2-acetoxypropionates.[13] The stereochemistry of the **2** → **11** transformation (displacement of phenyl by methyl with almost complete inversion) indicates that **11** arises via phenonium ion **6**, methyl acting as an internal nuclophile.[14]

The nitrous acid deamination[16] of **3** was studied for comparison with **2**. Again, **7**, $[\alpha]^{24}_D$ −52.0° (*c* 2.57, CCl_4), **11a** (enantiomeric purity by GLC, 98 ± 2%), and **11b** (enantiomeric purity, 99 ± 1%) were produced with complete inversion. The most significant feature distinguishing deamination from solvolysis is the formation of substantial quantities of **15**. The reaction path leading to **15** was explored with the aid of [2-^{2}H]-**3** (Scheme III). The label was completely recovered in fragment A (*m/e* 107) of **15**, establishing an isopropyl shift as the exclusive source of **15**. A sequential Ph,H shift involving the open cation **9** would have placed the label at C-2 of **15**.

$$Ph{=}{=}CH{=}{=}OH^+$$
A

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