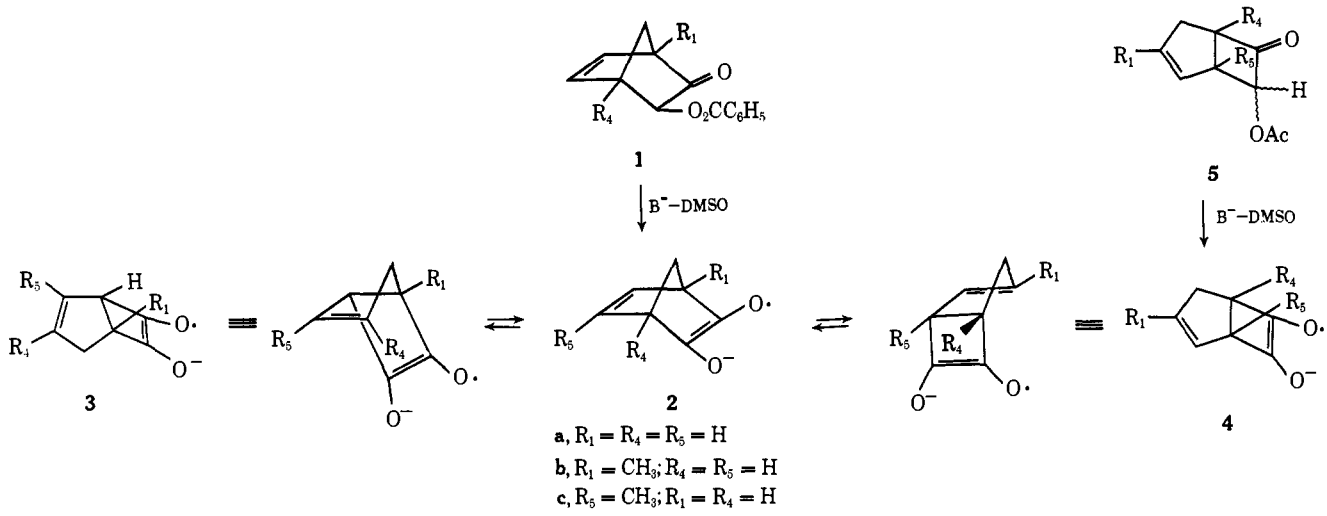
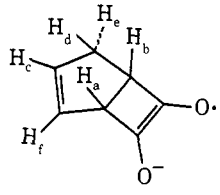
Chart I. Semidiones and Precursors





4a (enantiomer of **3a**)

$a_a^H = 12.3, a_b^H = 10.1, a_c^H = 2.0,$
$a_d^H = a_e^H = 0.5, a_f^H = 0.2$ G

Treatment of **1b** or **1b′** with base and DMSO in the flow system gave a single semidione whose esr spectrum indicated the presence of a single α-cyclobutane hydrogen, a^H = 13.1 G. Considerable fine structure was present in the spectrum but it has not been completely resolved. This result is consistent with the interpetation that the initially formed **2b** readily isomerized to **3b** in preference to **4b**. To further test this rationalization, and to explore the possibility of the reverse reaction (**3**, **4** → **2**), acetoxyketene was added to a mixture of 1- and 2-methylcyclopentadiene. Pure samples of **5b**[7,11] and **5c**[7,11] were isolated by glpc and converted to their semidiones by base and DMSO. Acetoxy ketone **5b** yielded only a single semidione whose spectrum consisted of a 13.1-G doublet with unresolved fine structure, identical with the spectrum observed from **1b** or **1b′**. This result strongly supports the conclusion that a rapid reversible isomerization between **2**, **3**, and **4** occurs. Apparently **3b** is more stable than **4b** because of nonbonded interactions.

Acetoxy ketone **5c** gave rise to a mixture of semidiones, assigned to **4c** (a^H = 1.75, 12.9 G) and **3c** (a^H = 9.75, 12.8 G) whose ratio depended on the flow rate with short reaction periods favoring the unrearranged **4c**.

Since rearrangements similar to **2** ⇄ **3** or **4** have not been recorded in the pyrolysis of norbornadiene,[12] it appears that the presence of the unpaired electron (added to the HOMO of the parent dione) is required. The rearrangement may be analogous to the photochemical conversion of norbornenones to bicyclo[3.2.0]hept-2-en-7-ones[13–15] and the photoisomerization of 1,4,4-trimethyl[3.2.0]hept-6-en-2-one to 4,4,6-trimethylbicyclo[3.2.0]hept-6-en-2-one[16] for which diradical intermediates have been postulated after promotion of an electron to π^* of the ketone function.[13] Facile scission of an α carbon–carbon bond in **2**, **3**, or **4** could also explain the short chemical lifetimes of the semidiones observed in this work. On the other hand a concerted 1,3-sigmatropic rearrangement of **2** ⇄ **3** or **4** is possible as in the thermal isomerization of bicyclo[3.2.0]hept-2-enes to norbornenes.[17] A one-step interconversion of **3** ⇄ **4** although possible does not seem likely.

(11) For **5b**, J_{12} = 3 Hz. For **5c**, J_{45} = 3.0, 6.5, J_{57} = 3.1 Hz. These coupling constants are in agreement with those reported for other bicyclo[3.2.0]hept-2-en-6-ones: W. F. Erman, *J. Amer. Chem. Soc.*, **89**, 3828 (1967); W. F. Erman, R. S. Treptow, P. Bakuzis, and E. Wenkert, *ibid.*, **93**, 657 (1971).

(12) W. G. Woods, *J. Org. Chem.*, **23**, 110 (1958); R. W. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **10**, 529 (1971).

(13) D. I. Schuster, M. Axelrod, and J. Auerbarh, *Tetrahedron Lett.*, 1911 (1963).

(14) D. E. Bays and R. C. Cookson, *J. Chem. Soc. B*, 226 (1967).

(15) This process appears to be reversible under certain conditions and may occur thermally; private communication from Dr. P. W. Schiess, University of Basel.

(16) G. Büchi and E. M. Burgess, *J. Amer. Chem. Soc.*, **82**, 4333 (1960).

(17) J. A. Berson, *Accounts Chem. Res.*, **1**, 152 (1968).

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Variation of Stoichiometry with Solvent in a Lanthanide Nuclear Magnetic Resonance Shift Reagent Complex[1]

Sir:

One reason for the considerable recent interest in lanthanide nmr chemical shift reagents is the proposed use of "bound" chemical shifts (Δ_B) in determination of molecular configuration, since molecular geometry is directly reflected in the Δ_B values. The first step in such analyses must be to obtain an accurate measure of Δ_B from concentration dependence of the induced shift, δ. It has recently been shown that Δ_B values may be obtained most easily and reliably from a plot of $[S]_0$

(1) Work supported by grants (to L. D. H. and A. G. M.) from the National Research Council of Canada and from the Research Committee of the University of British Columbia.

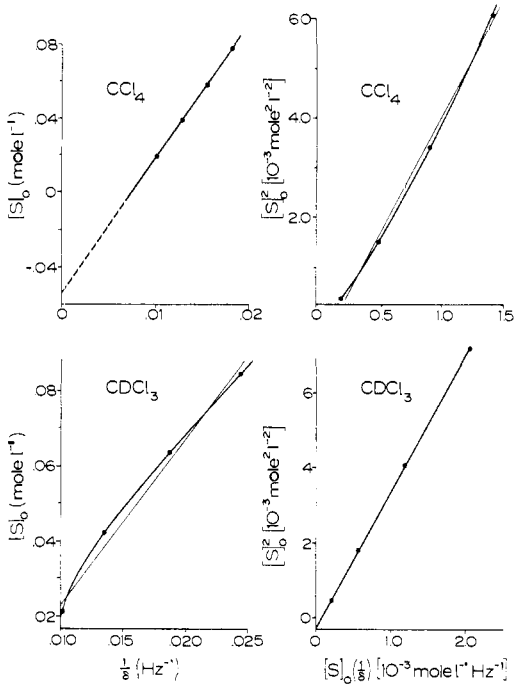

Figure 1. Determination of stoichiometry for the norcamphor–Eu(FOD)$_3$ complex from induced shift data for the H_4 proton of norcamphor. All experiments were performed at low $[L]_0/[S]_0$ ratios (*ca.* 0.05:1).

vs. $(1/\delta)$ when $n = 1$, or from a plot of $[S]_0^2$ *vs.* $[S]_0 \cdot (1/\delta)$ when $n = 2$ for the scheme[2,3]

$$L + nS \rightleftarrows LS_n \qquad (1)$$

where all experiments are conducted at low $[L]_0/[S]_0$ ratios to ensure the applicability of the one-step binding model (1). It is vital to establish the stoichiometry of the complex, in order to decide on the most reasonable orientation of the electronic *g*-tensor principal axis relative to the bound organic substrate—this orientation is the starting point of attempts to fit calculated to observed Δ_B values to obtain molecular geometry. In this communication, we report on the norcamphor–Eu(FOD)$_3$ system,[4] for which the S:L stoichiometry changes from 1:1 to 2:1 on change of solvent from CCl_4 to $CDCl_3$; implications of this result concerning geometry of the complex are discussed.

When binding of substrate to lanthanide is strong, as with the binding of alcohols or amines to Eu(FOD)$_3$,[3] then extraction of stoichiometry is possible only from experiments conducted at large $[L]_0/[S]_0$ ratios, and such studies will be complicated by the presence of any intermediate equilibria in eq 1. On the other hand, the binding of ketones is considerably weaker, as seen in Table I, so that stoichiometry of the norcamphor–Eu(FOD)$_3$ complex may be obtained from the simpler treatment possible at low $[L]_0/[S]_0$ ratios.[3] In the upper left plot of Figure 1, the data from the CCl_4 solvent are plotted in terms of variables which will produce a straight-line graph when the binding is 1:1, and the fit in this case is excellent—as a check, the same data are plotted (upper right of the figure) in terms of variables which lead to a straight-line plot when the binding is 2:1, and the marked curvature of this graph is evidence that 2:1 binding is *not* present in CCl_4 solvent. In contrast, the same analysis applied to shift data obtained in $CDCl_3$ solvent is shown in the two bottom plots of the figure; in this case, the data may be fitted only by the assumption of 2:1 binding. It is thus evident that for this particular ketone, the stoichiometry of the complex changes from 1:1 to 2:1 upon change of solvent from CCl_4 to $CDCl_3$.

Table I. Bound Chemical Shifts (Δ_B) and Binding Constants (K_B) for the Association of Norcamphor with Eu(FOD)$_3$ Shift Reagent in Two Different Solvents

	H_1		H_3-*exo*		H_3-*endo*		H_4	
Solvent	Δ_B	K_B	Δ_B	K_B	Δ_B	K_B	Δ_B	K_B
CCl_4	21.4[a]	23.7[b]	23.5	22.3[b]	24.1	22.7[b]	7.8	22.3[b]
$CDCl_3$	9.1	>1000[c]	9.7	>1000[c]	10.1	>1000[c]	3.4	>1000[c]

[a] All Δ_B values in parts per million, to ±10%. [b] K_B in l. mol^{-1}, to ±10%. [c] K_B in l.^{2} mol^{-2}. K_B values in the two solvents differ by a factor of [S] because of the different stoichiometry in the two solvents. Typical values of [S] were about 0.05 *M* in the present experiments, so that the binding constant changes somewhat on change of solvent.

It is interesting to speculate on the nature of binding in the complex which might lead to such behavior. One intriguing (but not the only) possibility is that the actual stoichiometry in CCl_4 solvent is 2:2, with both ketones acting as bridging monodentate ligands (*i.e.*, with both ketones situated between the two lanthanides). On this point, X-ray analysis[5] has shown that Pr(DPM)$_3$ (which is isomorphous with Eu(DPM)$_3$)[4] exists as a dimer in the solid; two ligand oxygens bridge the complex so that each metal atom is surrounded by seven oxygen atoms. There is some nmr evidence[6] that some Eu(DPM)$_3$ persists as the dimer even in CCl_4 and $CDCl_3$ solutions. In another dimer, $Pr_2(FOD)_6(H_2O)_2$, bridging involves not only two ligand oxygens but also one molecule of water,[7] so that each metal atom is now surrounded by eight oxygen atoms; the remaining water molecule is hydrogen bonded to ligand side chains. Very recently, there has been a direct observation of 2:1 binding of dimethyl sulfoxide to tris(1,1,1,-2,2,3,3-heptafluoro-7,7-[$^2H_6$]dimethyl[$^2H_3$]octane-4,6-dionato)europium because in that system separate nmr signals could be observed (and thus the areas measured) due to the slow exchange of substrate on and off the complex.[8] In view of these independent observations, it seems quite possible that other Lewis bases might be expected to bridge $M_2(FOD)_2$ dimers. Proceeding along these lines, a change to the more polar $CDCl_3$ solvent would be expected to cleave the dimer, so that an additional norcamphor molecule could occupy the now-vacant coordination site. In support of this view, it may be noted from the shift data in Table I that the Δ_B values of CCl_4 are about twice as large as those in $CDCl_3$.

In conclusion, we have observed a definite solvent

(2) I. Armitage, G. Dunsmore, L. D. Hall, and A. G. Marshall, *Chem. Commun.*, 1281 (1971).

(3) I. Armitage, G. Dunsmore, L. D. Hall, and A. G. Marshall, *Can. J. Chem.*, 50, 2119 (1972).

(4) Abbreviations used: Eu(FOD)$_3$ for tris(2,2-dimethyl-6,6,-7,7,8,8,8-heptafluoro-3,5-octanedionato)europium(III); Eu(DPM)$_3$ for tris(dipivalomethanato)europium(III).

(5) C. S. Erasmus and J. C. A. Boeyens, *Acta Crystallogr., Sect. B*, 26, 1843 (1970).

(6) M. K. Archer, D. S. Fell, and R. W. Jotham, *Inorg. Nucl. Chem. Lett.*, 7, 1135 (1971).

(7) J. P. R. deVilliers and J. C. A. Boeyens, as reported by R. E. Rondeau and R. E. Sievers, *J. Amer. Chem. Soc.*, 93, 1522 (1971).

(8) D. F. Evans and M. Wyatt, *J. Chem Soc., Chem. Commun.*, 2167 (1972).

dependence in the stoichiometry of the norcamphor–Eu(FOD)$_3$ complex; this dependence may well be associated with the presence of dimeric solution species; and if the effect is at all general, it indicates that any attempts to deduce molecular conformation from Δ_B values using lanthanide shift reagents should be approached with extreme caution pending establishment of stoichiometry of the complex involved.

(9) F. J. Nicholson Scholar; H. R. MacMillan Family Fellowship, 1971–1972.
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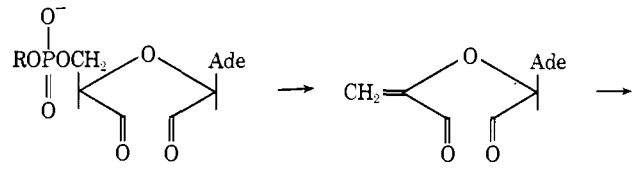
The Sequence Analysis of Polyribonucleotides by Stepwise Chemical Degradation. A Method for the Introduction of Radioactive Label into Nucleoside Fragments after Cleavage

Sir:

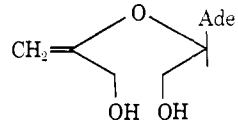
In 1953 Whitfeld and Markham[1] and Brown, *et al.*,[2] suggested that a method for the sequence analysis of polyribonucleotides might be perfected by studying the successive chemical removal of nucleotides from their 3′ terminals. The procedure would involve (i) the periodate oxidation of the terminal cis glycol group of the polynucleotide chain, (ii) the removal of the terminal nucleoside by a base-catalyzed β-elimination reaction, and (iii) the removal of the terminal phosphate group so formed by phosphatase, leaving the polynucleotide chain in a condition suitable for a second cycle of degradation. Since that time the method has been studied in many laboratories and, in all cases, primary amines have been used to catalyze the elimination reaction and, in most cases, the reaction has been carried out in the presence of the excess periodate carried over from the oxidation step. Under these conditions the product arising from the oxidized terminal nucleoside is the purine or pyrimidine base.

In earlier studies on a method for RNA sequence analysis carried out in this laboratory,[3] primary amines in the presence of excess periodate were also used for the elimination step. More recently, however, in the adaptation of these procedures to an automated solid support system[4] for the sequence analysis of fragments from large ribonucleic acids,[5] the oxidation step is physically separated from the elimination step and the latter proceeds in the absence of periodate. Under these conditions, the primary amine reacts with the oxidized glycol group and the terminal nucleoside is obtained as a morpholine derivative which is subsequently converted to the base by heat, prior to spectrophotometric analysis. The present work was directed toward increasing the sensitivity of RNA sequence analysis by developing a method by which radioactive label could be introduced into the nucleoside fragment after the elimination reaction. To this end we now show that, if the excess periodate is removed after the oxidation of an oligonucleotide and if the elimination reaction is carried out in the absence of a primary amine, a product is formed which contains all the carbon atoms of the original terminal nucleoside. The product is presumably an unsaturated dialdehyde of the type II which can be reduced with sodium borohydride to a stable derivative III, thus providing a means by which tritium can be introduced into the released fragment.



Ia, R = H
Ib, R = guanosine-3′

II



III

The β-elimination reaction has been studied with two model compounds, adenosine 5′-phosphate and guanylyl-(3′–5′)-adenosine (GpA). Adenosine 5′-phosphate (5 g, 14 mmol) was dissolved in water (50 ml) and brought to pH 8.4 with aqueous NaOH. A solution of sodium periodate (2.93 g, 14 mmol) in water (60 ml) was added and the mixture kept at 25° for 5 min. The solution was then heated at 45° for 3 hr and then cooled to 0°. Sodium borohydride (0.52 g, 14 mmol) was added and the mixture was kept at 4° for 12 hr. The solution was applied to a column (4 × 20 cm) of Dowex 1-X8 (100–200 mesh, acetate form) ion-exchange resin and the product was washed through with water. The eluate was concentrated *in vacuo* to about 10 ml and kept at 4°, yielding the white crystalline product III (2.61 g, 76%) which was recrystallized from water: mp 176–177°; uv λ_{max} 259 nm (ϵ 14,000), at pH 7; nmr (DMSO-d_6) δ 8.27 (s, 1), 8.20 (s, 1), 6.23 (t, 1), 4.27 (d, 2); mass spectrum (65 eV) *m/e* 251 (M^+), 194, 178, 164, 134.

Anal. Calcd for $C_{10}H_{13}N_5O_3$: C, 47.80; H, 5.22; N, 27.87. Found: C, 47.66; H, 5.25; N, 27.66.

A study of the rate of β elimination at 45° of the oxidation product of adenosine phosphate (Ia) was carried out in water, pH 8.4, and also in 0.4 *M* *N*,*N*,*N*′,*N*′-tetramethylglycinamide hydrochloride which had been titrated to pH 8.4 at 25° with NaOH. Samples of the reactions were withdrawn at various times, reduced with excess sodium borohydride, and chromatographed on Whatman No. 3 MM paper in the solvent system isopropyl alcohol (70 ml)–concentrated ammonia (10 ml)–water (20 ml). The amounts of the product III (R_f 0.75) and the reduced derivative of Ia (R_f 0.11) in each sample were determined spectrophotometrically after elution from the paper. Small amounts (<5%) of adenine (R_f 0.49) were also formed in these reactions. The degradation of Ia was quantitative after 3 hr in the

(1) P. R. Whitfeld and R. Markham, *Nature (London)*, **171**, 1151 (1953).
(2) D. M. Brown, M. Fried, and A. R. Todd, *Chem. Ind. (London)*, 352 (1953).
(3) H. L. Weith and P. T. Gilham, *J. Amer. Chem. Soc.*, **89**, 5473 (1967); H. L. Weith and P. T. Gilham, *Science*, **166**, 1004 (1969).
(4) H. L. Weith and P. T. Gilham, 160th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1970, Abstract BIOL 18.
(5) M. Rosenberg, G. T. Asteriadis, H. L. Weith, and P. T. Gilham, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **30**, 1101 (1971).