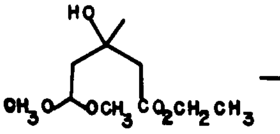

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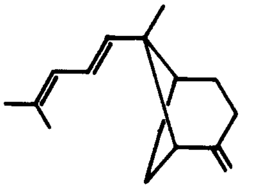

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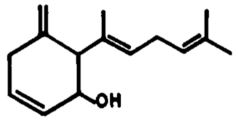

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$^3H/^{14}C$ ratio (1.58) indicated retention of $\frac{5}{6}$ of the tritium isotope.

In order to determine the positions of deuteration, the labeled ovalicin sample was analyzed directly by ^2H NMR. The resulting spectrum showed four signals: δ 4.21 (CDOCH$_3$, 1 D), 2.63 (CD$_2$CO, 1 D), 2.4 (CD$_2$CO, CD$_2$CH=, 2 D), and 2.09 (CD$_2$CH=, 1 D) (Figure 1).

The ^2H NMR results are consistent with the observed $^3H/^{14}C$ ratio and reconfirm previous experiments on the specific incorporation of mevalonate. Furthermore the retention of five of six deuteriums derived from C-5 of mevalonate sets strict boundary conditions on any mechanistic proposals for the oxidative cleavage of a bergamotene intermediate. Specifically such intermediates as the dehydrobergamotene (**4**), suggested by Birch,[17] or a monocyclic structure **5** related to carquejyl acetate[18] are ruled out, as is oxidation of the methylene bridge of bergamotene to a ketone.

The experiments described above illustrate the utility of ^2H NMR as a biosynthetic tool while providing important information bearing on the biosynthesis of ovalicin. Further biosynthetic applications of ^2H NMR will be reported in due course.[19,20]

References and Notes

(1) ^2H NMR has been used recently in a number of mechanistic studies: cyclohexadiene dimerizations,[3] the Diels-Alder dimerization of pentadiene,[4] the homoenolization of fenchone,[5] and the synthesis and rearrangement of benzvalene.[6] Except for the work of Stothers, these studies involved rather high levels of enrichment (>90%). Indirect detection of deuterium is possible. Broad-band deuterium decoupling of $^{13}CD_3$-enriched corrins has been used to demonstrate the absence of protium in any of the methionine-derived methyl groups.[7] A study of scytalone biosynthesis utilized $^{13}C-^2H$ coupling to assign the positions of deuterium enrichment.[8] There have been only two reports of the direct application of ^2H NMR to biosynthetic investigations. Sato has recently described a ^2H NMR study of griseofulvin biosynthesis[9] and Bycroft has detected deuterium in penicillin G derived from feeding of labeled cystine.[10]

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(14) Bergamotene has also been isolated from *Asperigillus fumigatus*, a fungus which produces fumagillin, a substance closely related to ovalicin; cf. S. Nozoe, H. Kobayashi, and N. Morisaki, *Tetrahedron Lett.*, 4625 (1976).

(15) ^1H NMR spectra (270 MHz) were obtained in CDCl$_3$ solutions with CHCl$_3$ as internal standard (δ 7.24) using a Bruker HX 270 operated in the FT mode, spectral width 3000 Hz, 16K. Reported coupling constants are ±1.0 Hz and chemical shifts are ±0.01 ppm.

(16) Proton decoupled ^2H NMR spectra were obtained at 41.44 MHz on degassed CHCl$_3$ solutions with CDCl$_3$ as internal standard (δ 7.24) in 10-mm sample tubes. A pulse angle of 90° was employed, spectral width 500 Hz, using 2K or 4K data points. Chemical shifts are ±0.02 ppm.

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(19) In a separate study of rosenonolactone biosynthesis we have recently obtained spectra with S/N = 30 in 4 hours (2K data points) on a 0.05-mmol sample enriched with 3% deuterium (D. E. Cane and P. N. Murthy, unpublished work). Such combinations of enrichment level and quantity of metabolite are well within the range usually achievable with microorganisms.

(20) This work was supported by the National Institutes of Health (GM 22172) and by a grant from the Merck Foundation. The Bruker HX 270 facility is supported by NIH Grant No. 1-P07-PR00798 from the Division of Research Resources.

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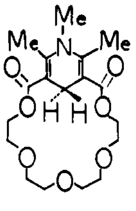
Unusual Stability Characteristics in Methanol of the Complexes of a New Pyridine-Substituted Cyclic Polyether-Ester Compound with Na^+, K^+, Ag^+, and Ba^{2+}—Comparison with Oxygen, Sulfur, and Nitrogen Analogues

Sir:

In two recent communications, van Bergen and Kellogg[1,2] report the synthesis of an unusual macrocycle **1** incorporating into the ring Hantzsch esters. These esters may be used as a "mimic" of NAD(P)H because of their hydride-donating properties. Their goal was to enhance hydride transfer by adding a crown ether ring onto the ester in hopes of holding metal cations, which are known to catalyze this transfer, in close proximity to the hydride donor. We report here the results of a calorimetric study of the cation binding properties of two ligands similar to **1**, namely **2** and **3**. Complexing data for other macrocyclic compounds **4–8** are also included to allow comparisons between these ester-containing systems and cyclic polyethers.

Like the Hantzsch ester crown, the macrocycles under consideration in this study differ from simple crown ethers in



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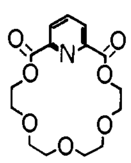

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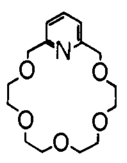

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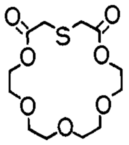

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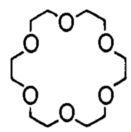

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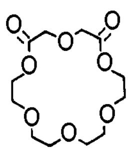

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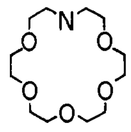

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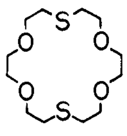

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Table I. Log K, ΔH (Kilocalories/Mole), and $T\Delta S$ (Kilocalories/Mole) Values for the Interaction of Several Macrocyclic Ligands with Metal Ions in Methanol at 25 °C and $\mu = 0.005$

Ligand	Value	Na^+	K^+	Ba^{2+}	Ag^+ (1:1)	Ag^+ (2:1)
2	Log K	4.29 ± 0.06	4.66 ± 0.02	4.34 ± 0.02	4.88 ± 0.05	
	ΔH	−6.19 ± 0.08	−9.3 ± 0.2	−6.03 ± 0.03	−7.83 ± 0.03	
	$T\Delta S$	−0.34	−2.9	−0.11	−1.17	
3	Log K	4.09 ± 0.02	5.35 ± 0.06	>6	>6	2.579 ± 0.003
	ΔH	−5.44 ± 0.05	−9.11 ± 0.11	−7.72 ± 0.18	−8.33 ± 0.08	−2.58 ± 0.22
	$T\Delta S$	0.14	−1.8	0.5	−0.1	0.94
4	Log K				3.05 ± 0.05	
	ΔH	*a*	*a*	*a*	−6.97 ± 0.08	
	$T\Delta S$				−2.81	
5	Log K	4.36[b]	6.05[b]	7.0[b]	4.58 ± 0.03	
	ΔH	−8.4[b]	−13.4[b]	−10.2[b]	−9.15 ± 0.11	
	$T\Delta S$	−2.4[b]	−5.2[b]	−0.7[b]	−2.90	
6	Log K	2.5[b]	2.79[b]	3.1[b]	2.50 ± 0.07	
	ΔH	−2.3[b]	−5.9[b]	−0.5[b]	−1.53 ± 0.12	
	$T\Delta S$	1.1[b]	−2.1[b]	3.7[b]	1.88	
7	Log K		3.90[c]			
8	Log K		1.15[c]			

[a] No measurable heat other than heat of dilution was found for the mixing of these cations and ligands. This result indicates that log K is small and/or the enthalpy change is near zero. [b] Reference 5. [c] Reference 3.

the presence of (a) a pyridine moiety and/or (b) ester moieties. The effect on cation binding of the introduction of these groups into the crown ether ring is well illustrated by the thermodynamic data of Table I for the reaction of ligands **2–6** with Na^+, K^+, Ba^{2+}, and Ag^+. The pyridine-containing ligands **2** and **3** have been found to possess rather unusual complexation characteristics differing significantly from those of their analogues, **5** and **6**, which do not contain the pyridine moiety.

Frensdorff[3] showed that substitution of a nitrogen atom for one of the ether oxygens in the 18-crown-6 ring causes a considerable drop in the stability of the K^+ complexes (compare log K data for ligands **5** and **7** in Table I). However, introduction of a pyridine nitrogen into the 18-crown-6 ring to give **3** by a previously described synthesis[4] produces only a minor decrease in the stability of the complexes with Na^+, K^+, and probably Ba^{2+} with selectivity among these cations remaining essentially unaltered. The 1:1 Ag^+ complex of **3** is markedly more stable than the 18-crown-6–Ag^+ complex and is followed by the production of a fairly stable 2:1 complex as well. This latter observation results in a reversal of the selectivity order for bonding of $K^+ > Ag^+$ found in 18-crown-6 so that for the pyridine-containing ligand **3** Ag^+ is preferred. Frensdorff[3] noted this same reversal of $K^+ > Ag^+$ selectivity in water for **7**.

It is to be noted that the entropy term favors complexation by **3** over 18-crown-6 in every case while the enthalpy term works in opposition. This enthalpic destabilization of the complexes of **3** is much greater for the alkali metal cations than for Ag^+, accounting for the change in selectivity observed.

We have already noted[5] that a considerable drop both in cation complex stability and in cation selectivity is seen upon addition of two carbonyl groups to **5** to produce **6**. Insertion of a pyridine group between the two ester carbonyl oxygens of **6** to yield **2** results in an unexpected rise in log K (Table I) for complex formation over that found for the parent ligand **6**. As was the case with introduction of a pyridine moiety into the 18-crown-6 ring, this stability enhancement is in sharp contrast to the previously noted reduction in the stability of complexes of **7** where nitrogen has been substituted for oxygen in the 18-crown-6 ring. Furthermore, the destabilizing effect of the carbonyl groups has been largely offset especially in the case of the Na^+ and Ag^+ ions whose stability constants are brought back into the same range as those with 18-crown-6. However, the poor selectivity of the parent ligand **6** is maintained.

The thermodynamic origin for differences in selectivity between the macrocycles containing carbonyl oxygens and those which do not seems to vary. Comparing the two pyridine-containing ligands, **2** and **3**, in all cases the stability of complexes of the ligand without carbonyl groups is entropy favored with no systematic trend in ΔH, which varies little from the one ligand to the other. In the case of the all-oxygen ligands, **5** and **6**, the entropy term favors the complexes of the ligand with carbonyl groups while the enthalpy term for this ligand is by comparison very unfavorable.

The thermodynamic data in Table I show that the increased stability of complexes of **2** over that of complexes of the parent macrocycle **6** is due almost entirely to the enthalpy term in the case of the monovalent cations. However, a significant drop in entropy stabilization for the Ba^{2+} complex of **2** from that of **6** results in the reversal of the K^+-Ba^{2+} selectivity sequence between these two ligands. It is surprising for macrocycles to bind K^+ more strongly than Ba^{2+} as in the case of **2** inasmuch as these cations have nearly identical crystal radii. In fact, this selectivity sequence is one of the distinguishing features of valinomycin and certain other naturally occurring antibiotic macrocycles.[5] A few crown ethers and cryptands containing bulky hydrophobic groups duplicate this selectivity, as does another series of carbonyl-containing crowns recently synthesized by us.[6]

The possibility exists that it is not the presence of the nitrogen donor which is responsible for the unexpectedly high stability of complexes of **2**, but rather the presence of the aromatic ring, which might alter the effect of the carbonyl groups on the complexation. If indeed the effect is conformational in origin, this factor will be important to the understanding of Hantzsch ester crown complexation with metal ions. To check this hypothesis, we have obtained additional structural information concerning **2** and **6** and their K^+ complexes from infrared spectra using KBr wafers. The spectrum of **6** shows the two carbonyl groups to be in different environments, the carbonyl stretching frequencies being 1715 and 1730 cm^{-1}. However, upon complexation with K^+, we observe a single carbonyl stretch at 1740 cm^{-1}. From this finding we conclude that (i) since the carbonyl stretch has been shifted to a higher frequency, the complex probably does not involve the carbonyl oxygen atoms, and (ii) the molecule is highly organized around the central K^+. We have completed x-ray structure determinations of **6** and its K^+ complex[7] and find the results to be consistent with the infrared spectral findings that carbonyl oxygens do not participate in bonding of the cation. The spectra of **2** and its K^+ complex do not show the marked change in the infrared spectra noted for **6**. This observation may well result from the pyridine moiety imparting organization and rigidity to the ring both in the uncomplexed and complexed forms. Combining these results with the unusual stability effects recorded for ligand **3**, we conclude that it is participation of the pyridine group in complexation which is responsible for the behavior of ligand **2** rather than conformational factors.

The replacement of an oxygen atom in **6** by sulfur produces **4** which shows no heat of reaction with Na^+, K^+, or Ba^{2+}. The large size of the sulfur may force the sulfur atom out of the ring, as in the case of sulfur-substituted 18-crown-6.[8] The observation that **4** complexes alkali metal ions even more poorly than 1,10-dithia-18-crown-6, **8**, suggests that the steric consideration mentioned, namely orientation of the sulfur atom away from the ring, if present in **4**, does not enhance carbonyl participation in complexation. If this conclusion is extended to the case of the pyridine-containing ligand, it lends even greater support to the idea that the pyridine nitrogen, not the ester carbonyls, is responsible for its unusual behavior.

The log K, ΔH, and $T\Delta S$ values were obtained by a calorimetric titration procedure which has been described previously.[9] Ligand **2** was standardized by calorimetric titration with K^+ and found to be 98.2% pure. It was not possible to standardize **4** by this direct procedure since the log K value for formation of the Ag^+ complex was not sufficiently high to produce an end point. Variation of a ligand purity factor in the curve fitting computer routines used for data analysis indicated that **4** is greater than 98% pure.

To prepare **2**, 2,6-pyridinedicarboxylic acid chloride (20.4 g, 0.1 mol) and 19.4 g (0.1 mol) of tetraethylene glycol, both in 200 mL of a 50:50 mixture of tetrahydrofuran and benzene, were slowly added while stirred to 1 L of benzene at 50 °C. The resulting reaction mixture was stirred for 48 h. After cooling, the mixture was filtered and the solvents were removed under vacuum. The resulting light viscous oil was repeatedly extracted with hot hexane to yield 25.4 g (77%) of a white solid: mp 83.5–85 °C; IR 1730 cm^{-1} (C=O); NMR δ 3.85 (s, 8 H, OCH_2CH_2O), 3.95 (m, 4 H, $COOCH_2CH_2O$), 4.63 (m, 4 H, $COOCH_2$), and 8.0–8.5 (m, 3 H, aromatic H). Anal. Calcd for $C_{15}H_{19}NO_7$: C, 55.38; H, 5.88, N, 4.31; mol wt, 325.32. Found: C, 55.46; H, 5.96; N, 4.47; mol wt, 329.

Compound **4** was prepared by slowly dripping 19.0 g (0.102 mol) thiodiglycolyl dichloride and 19.7 g (0.102 mol) tetraethylene glycol, each diluted to 150 mL with benzene, from separate addition funnels into 1 L of rapidly stirring benzene at 50 °C. After the mixture was stirred for 68 h at 50 °C, the benzene was removed under reduced pressure and the crude product was distilled: 6.29 g (20%); bp 181 °C (0.4 mm); mp 41–43 °C. The product was recrystallized from chloroform-ether yielding long white needles, mp 43.5–44.5 °C. Compound **4** exhibited the following spectra: IR 1740 cm^{-1} (C=O); NMR δ 3.54 (s, 4 H, $COCH_2S$), 3.71 (s, 8 H, OCH_2CH_2O), 3.77 (m, 4 H, $COOCH_2CH_2O$), 4.36 (m, 4 H, $COOCH_2$). Anal. Calcd for $C_{12}H_{20}O_7S$: C, 46.74; H, 6.54; S, 10.40; mol wt, 308.35. Found: C, 46.95; H, 6.62; S, 10.48; mol wt, 306.

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^{31}P Chemical Shift Anisotropy in Solid Nucleic Acids

Sir:

So far, no report seems to have appeared on direct measurements of ^{31}P NMR chemical shift tensors of nucleic acids in solid state, although some efforts have been made in solutions.[1-3] In the present study, principal values of ^{31}P chemical shift tensors of nucleoside monophosphates (both in salt form and in free acid form), a cyclic mononucleotide, homopolyribonucleotides, tRNA, and DNA (and DL-α-dipalmitoyllecithin for comparison) were determined in their powder-line states by a combined use of cross-polarization technique and proton-dipolar decoupling.[4]

The experiments were done at 290 K on a home-built spectrometer[5] operating at 24.112 MHz for ^{31}P and 59.526 MHz for ^{1}H. The Hartman-Hahn condition was satisfied with rotating fields of 50 G for ^{31}P and 20 G for ^{1}H. In order to shorten the recycle time, the proton magnetization was brought back to the direction of a static field by a 90° pulse immediately after decoupling.

Table I summarizes the chemical shift parameters relative

Table I. Principal Values of the Chemical Shift Tensor for ^{31}P in Some Nucleic Acids[a]

Ester	Nucleic acid	σ_{11}	σ_{22}	σ_{33}	$\overline{\sigma}$[b]	σ_{soln}[c]
Monoester (salt)	5′-AMP(Na_2)	−78	29	29	−7	−4.1[d]
	5′-GMP(Na_2)	−63	25	25	−4	−4.1[d]
	5′-UMP(Na_2)	−67	27	27	−4	−4.0[d]
	3′-UMP(Na_2)	−66	27	27	−4	−4.1[d]
Monoester (free acid)	5′AMP	−77	−8	83	−1	−0.3[e]
	3′-AMP	−71	−11	90	3	
	3′-CMP	−67	−11	72	−2	
	5′-dCMP	−77	−1	87	3	
Diester	poly A(K)	−89	−24	110	−1	1.0[f]
	poly G(K)	−83	−24	112	2	1.1[f]
	poly C(K)	−92	−25	114	−1	1.2[f]
	poly U(K)	−85	−24	112	1	0.7[f]
	$tRNA^{phe}_{yeast}$	−86	−30	107	−3	0~2[g]
	DNA(salmon)(Na)	−83	−23	110	2	~1[h]
	3′,5′-cAMP	−88	−38	124	−1	1.6[e]
	DPL[i]	−103	−35	131	−2	

[a] All values are in parts per million relative to 85% H_3PO_4, taking upfield as positive. Principal values are determined by differentiation of powder spectra and believed to be accurate to ±5 ppm. [b] $\overline{\sigma} = (\sigma_{11} + \sigma_{22} + \sigma_{33})/3$. [c] Chemical shift values in aqueous solutions. [d] Values at pH 8.0.[13] [e] Values at pH 3.0.[13] [f] Reference 14. [g] Main peak only.[1] [h] Reference 15. [i] DL-α-dipalmitoyllecithin (DPL). The principal values of DPL were already reported by other authors. Our results are in good agreement with those reported by Griffin,[16] and a little different from those by Kohler and Klein.[17]