Development Administration for support of this research. The 25-MHz ^{13}C NMR spectral studies were supported by National Science Foundation Grant No. CHE-76-05512 to the University of California at Berkeley. The technical assistance of Dr. S. Cooper and useful discussions with Dr. N. Edelstein are gratefully acknowledged.

References and Notes

(1) G. Wilkinson, F. A. Cotton, and J. M. Birmingham, *J. Inorg. Nucl. Chem.*, **2**, 95 (1956).
(2) M. F. Rettig, *J. Chem. Soc., Chem. Commun.*, 687 (1972).
(3) (a) M. E. Switzer, R. Wang, M. F. Rettig, and A. H. Maki, *J. Am. Chem. Soc.*, **96**, 7669 (1974); (b) J. H. Ammeter, R. Bucher, and N. Oswald, *ibid.*, 7883 (1974).
(4) R. S. Threlkel and J. E. Bercaw, *J. Organomet. Chem.*, **136**, 1 (1977).
(5) Anal. Calcd for $C_{20}H_{30}Mn$: C, 73.82; H, 9.29. Found: C, 73.96; H, 9.18.
(6) D. F. Evans, *J. Chem. Soc.*, 2003 (1959).
(7) The ^1H NMR spectrum of decamethylmanganocene exhibits a contact shifted methyl resonance (δ −4.7, ~200-Hz line width at 320 °C) with an isotropic shift of +6.7 ppm with respect to decamethylferrocene. In contrast, the methyl resonance of 1,1′-dimethylmanganocene exhibits a large isotropic shift of −70 ppm (1500-Hz line width) with respect to 1,1′-dimethylferrocene, in a system that represents an average of nearly equal population of the doublet and sextet state.[3a]
(8) J. C. Smart, J. L. Robbins, and D. Freyberg, unpublished results. A preliminary x-ray study indicates that the ring carbon to manganese distance in decamethylmanganocene is 2.13 Å. A gas phase electron diffraction study of 1,1′-dimethylmanganocene yielded two ring carbon to manganese distances: 2.42 Å for the sextet state and 2.14 Å for the doublet configuration. A. Almennigen, S. Samdal, and A. Haaland, *J. Chem. Soc., Chem. Commun.*, 14 (1977).
(9) E. O. Fischer and S. Breitschaft, *Chem. Ber.*, **99**, 2213 (1966).
(10) B. R. Francis, M. L. H. Green, T. Luong-thi, and G. A. Moser, *J. Chem. Soc., Dalton Trans.*, 1339 (1976).
(11) (a) W. E. Geiger, Jr., *J. Am. Chem. Soc.*, **96**, 2632 (1974); (b) J. D. L. Holloway, W. L. Bowden, and W. E. Geiger, Jr., *ibid.*, **99**, 7089 (1977).
(12) Ch. Elschenbroich and F. Gerson, *J. Am. Chem. Soc.*, **97**, 3556 (1975).
(13) Ch. Elschenbroich, F. Gerson, and F. Stohler, *J. Am. Chem. Soc.*, **95**, 6959 (1973).
(14) $E_{1/2}$ = −2.50 V (reduction); −0.56 V (oxidation) vs. SCE at a Pt electrode in dry, oxygen-free acetonitrile with Bu_4NBF_4 supporting electrolyte.
(15) (a) Anal. Calcd for $C_{20}H_{30}MnNa$: C, 68.95; H, 8.68. Found: C, 68.19; H, 8.72. (b) ^1H NMR: (60 MHz, THF-d_8) δ 1.87 (s). [^1H] ^{13}C NMR: (25 MHz, THF-d_8) δ 8.54, 72.4 relative to tetramethylsilane.
(16) J. L. Robbins, N. Edelstein, and J. C. Smart, manuscripts in preparation.

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Biosynthesis of the Nucleoside Skeleton of Polyoxins[1]

Sir:

Previous studies from our laboratory have shown that the 5-substituted pyrimidines of the polyoxin antibiotics[2] are derived from uracil and C-3 of serine. The biosynthesis of the 5-substituted uracils is not dependent on thymidylate synthetase.[3] The biosynthesis of the two side-chain amino acids was also studied in detail.[4-6] However, the biosynthesis of 5-amino-5-deoxy-D-allofuranoseuronic acid is not understood. This unique sugar amino acid is the common constituent of all the polyoxins. Previous experiments[3] have shown that ^{14}C-labeled glucose, ribose, and glycerol were incorporated into the uronic acid. However, with the exception of C-6′, the distribution of ^{14}C could not be determined because a practical carbon-to-carbon degradation method was not available. This difficulty has now been overcome by utilizing [1-^{13}C]glucose and ^{13}C NMR analysis. In addition, we have studied the biosynthesis of the nucleoside skeleton of the polyoxins in detail using ^{14}C-labeled compounds including [3-^{14}C]glycerate and [U-^{14}C]uridine. In this paper, we propose a novel biosynthetic pathway for the uronic acid moiety of **1** in Scheme I. This involves the condensation of uridine with phosphoenolpyruvate to afford octofuranuloseuronic acid as the intermediate. Subsequent oxidative elimination of the two terminal carbons would form the carbon skeleton of the nucleoside skeleton of the polyoxins (compound **1**, Scheme I).

Scheme I. Proposed biosynthetic pathway for the nucleoside skeleton of the polyoxins. Asterisk shows the ^{13}C enrichment from D-[1-^{13}C]glucose. Incorporation stage of the one-carbon unit into C-7 is not known.

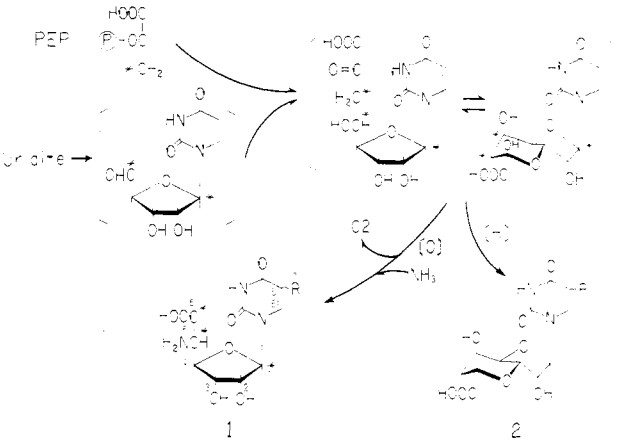

Table I. ^{13}C NMR[a] Chemical Shift Assignment and Enrichment of Carbons of Polyoxin C (**1**) Labeled by [1-^{13}C]Glucose

Carbon atom	Chemical shift,[b] ppm	Relative enrichment[c]
2	152.1	1.40[d]
4	165.4	0.94
5	114.6	0.90
6	140.9	1.05
7	57.2	1.00
1′	92.2	2.81
2′	73.1	1.23
3′	69.8	0.90
4′	81.5	1.00
5′	54.4	2.81
6′	169.2	3.23

[a] ^{13}C NMR spectra were taken on a JOEL FX-60 spectrometer. Solvent: D_2O–1 N HCl (1:1). [b] Chemical shift was expressed in parts per million relative to tetramethylsilane, calculated from dioxane, 67.4. [c] Relative enrichment was obtained by comparison of the integral curves of enriched and unenriched samples under the identical conditions. [d] This enrichment may be explained by the incorporation of [^{13}C]carbamoyl phosphate which is formed from D-[1-^{13}C]glucose through the hexose monophosphate oxidative pathway.

D-[1-^{13}C]Glucose[7] (1.5 g, 90 at. %) was administered to a shaking culture of *Streptomyces cacaoi* var. *asoensis* 72 h after inoculation[8] (1.2 L of the medium in 20 500-mL flasks; medium composition, 3% glycerol[9], 1% glucose, 2% soybean meal; 4% dry yeast, 0.2% $NaNO_3$, 0.2% K_2HPO_4). After an additional 24-h incubation, the polyoxin complex was isolated from the culture filtrate.[10] Alkaline hydrolysis[2] of the complex afforded 10 mg of polyoxin C (**1**, R = CH_2OH). The ^{13}C NMR spectrum[11] of **1** showed significant enrichment of C-1′, C-5′, and C-6′ (Table I). The distribution of the label clearly indicates that this hexose derivative does not originate from the carbon skeleton of glucose nor is it formed by the condensation of two three-carbon units as suggested earlier.[3a] Instead, equal enrichment of C-1′ and C-5′ indicates that C-1′ ~ C-5′ originates from the ribose carbon skeleton. Known sugar metabolism supports the formation of [1,5-^{13}C]ribose from [1-^{13}C]glucose via the pentose phosphate cycle.[12,13] Taking into account the structure of octosyl acid[14] (**2**, R = COOH or CH_2OH), also produced by *S. cacaoi*, we considered the pos-

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Table II. Incorporation and Distribution of ^{14}C-Labeled Compounds into Polyoxin C (**1**)[a]

Compound added	Amount, μCi	sp act., Ci/mol	Polyoxin C isolated: sp act., mCi/mol	% distribution[b]: Uracil (C-2, -4, -5, -6)	C-7	C-6′
L-[*methyl*-^{14}C]Methionine	10	0.76	0.00			
DL-[3-^{14}C]Serine	38	1.5	0.35	18.7	83.5	1.7
[3-^{14}C]Pyruvate	50	2.03	0.11	83.0	4.1	9.5
DL-[3-^{14}C]Glycerate	16	0.24	0.029	62.8	7.0	30.7

[a] Feeding experiment was performed as described.[3] Labeled compounds were added 45 h after inoculation. The polyoxin complex was isolated after an additional 72-h incubation. [b] Chemical degradation procedure was described in ref 3a.

Table III. Incorporation and Distribution of [U-^{14}C]Uridine[a] into Polyoxin C (**1**) and the RNA Isolated from *S. cacaoi*

Compd isolated	sp act., mCi/mol	^{14}C % distribution: Base	Sugar[b]
Polyoxin C (**1**)	0.327[c]	74.0	26.0[d]
RNA-uridine	9.33	73.3	26.7
RNA-cytidine	2.43	68.7	31.3
RNA-adenosine	1.03	1.2	98.8
RNA-guanosine	1.03	2.1	97.9

[a] [U-^{14}C]Uridine (36 μCi, sp act. 513 Ci/mol) was added to the cultures (four 500-mL flasks). [b] (the specific activity of the nucleoside minus the specific activity of the base) ÷ (the specific activity of the nucleoside) × 100. [c] Carrier **1** (10 mg) was added to the alkaline hydrolysate of the polyoxin complex. Crystalline **1** was isolated by chromatography on charcoal followed by further purification by paper chromatography to a constant specific activity. [d] Percent distribution on C-6′ was found to be 1.7% by ninhydrin oxidation.

sibility that the condensation of ribose and a three-carbon unit would produce an octose carbon skeleton. Subsequent splitting off of the two terminal carbons might yield the sugar skeleton of **1**. Conversely, reduction at the ketal carbon (C-7′) would result in the formation of the anhydro ring structure of **2** (Scheme I). Higher incorporation of the label into C-6′ compared with C-1′ or C-5′ reflects the operation of the hexose monophosphate oxidative pathway, which yields unlabeled ribose from [1-^{13}C]glucose. The most probable candidate for this three-carbon unit would be phosphoenolpyruvate. [3-^{14}C]Glycerate[15] and [3-^{14}C]pyruvate were incorporated into **1** (Table II). However, the incorporation of ^{14}C into C-6′ by [3-^{14}C]glycerate was much higher than by [3-^{14}C]pyruvate. Moreover, pulse labeling with DL-[3-^{14}C]glycerate (administered 68 h after inoculation and isolated 1 h later) resulted in an 83% distribution of ^{14}C into C-6′. These data indicate that phosphoenolpyruvate is the direct three-carbon precursor. The low incorporation of ^{14}C into C-6′ from [3-^{14}C]pyruvate can be explained by the gluconeogenic formation of [3-^{14}C]phosphoenolpyruvate. The possibility of the addition of a one carbon unit to ribose can be eliminated for the following reasons: (i) L-[*methyl*-^{14}C]methionine was not incorporated into **1** (Table II), and (ii) only a small portion (1.7%) of the ^{14}C incorporated into **1** from [3-^{14}C]serine was found in C-6′ (Table II). If the ^{14}C had been incorporated via the one-carbon pool, the signal enrichment of C-7 and C-6′ in the [1-^{13}C]glucose experiment should be comparable.[16] The data show that there is no apparent enrichment of C-7 (Table I). These data strongly indicate that the origin of C-6′ is related directly to the glycolytic pathway and not to the one-carbon pool. A low incorporation of ^{14}C into C-6′ from [3-^{14}C]serine may be ascribed to the following pathway: serine → hydroxypyruvate → glycerate → phosphoenolpyruvate.

To determine if the biosynthesis proceeds at the nucleoside level or at the sugar level, a pulse-label experiment using [U-^{14}C]uridine was performed. [U-^{14}C]Uridine was added to growing cultures of *S. cacaoi* 45 h after inoculation and incubated for 1 h. The polyoxin complex and the RNA from the cell were isolated.[17] The distribution of ^{14}C between base and sugar of the uridine and cytidine isolated from the RNA was approximately the same as for **1**[18] (Table III). The specific activity of the uridine isolated from the RNA was considerably higher than that of the adenosine and guanosine. The data indicate that uridine was taken up intact without cleavage of the nucleoside bond. Therefore, uridine but not ribose is the direct precursor. As illustrated in Scheme I, uridine (or UMP) may first be oxidized to the 5′-aldehyde, which undergoes aldol condensation with phosphoenolpyruvate to give octofuranuloseuronic acid nucleoside. Subsequent elimination of two carbons (C-7′ and C-8′) followed by transamination would result in the formation of the nucleoside skeleton of the polyoxins. The isolation of octosyl acids[14] supports this pathway, since octosyl acid can be formed from the common intermediate by reduction at C-7′.[19]

There are a number of precedents of the same type of condensation in the biosynthesis of 2-keto-3-deoxyaldonic acids.[20] Formation of 3-deoxy-D-*arabino*-hepturosonate 7-phosphate, the precursor of the shikimate pathway, is a well-known example in which aldolase uses phosphoenolpyruvate as the substrate. The difference in the polyoxin complex is that the condensation occurs at the 5′ end of the nucleoside instead of at the anomeric carbon.

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References and Notes

(1) This paper constitutes a part of the series entitled "Biosynthesis of the Polyoxins, Nucleoside Peptide Antibiotics". For the preceding paper, see ref 6.
(2) K. Isono, K. Asahi, and S. Suzuki, *J. Am. Chem. Soc.*, **91**, 7490 (1969).
(3) (a) K. Isono and R. J. Suhadolnik, *Arch. Biochem. Biophys.*, **173**, 141 (1976); (b) S. Funayama and K. Isono, *Agric. Biol. Chem.*, **40**, 1039 (1976).
(4) K. Isono, S. Funayama, and R. J. Suhadolnik, *Biochemistry* **14**, 2992 (1975).
(5) S. Funayama and K. Isono, *Biochemistry*, **14**, 5568 (1975).
(6) S. Funayama and K. Isono, *Biochemistry*, **16**, 3121 (1977).
(7) D-[1-^{13}C]Glucose (90 at. %) was purchased from MSD, Canada.
(8) L-Aspartate (5×10^{-3} M) was added to the culture 15 min prior to the addition of D-[1-^{13}C]glucose.
(9) To minimize isotope dilution, glycerol was substituted for starch.
(10) K. Isono, J. Nagatsu, K. Kobonata, K. Sasaki, and S. Suzuki, *Agric. Biol. Chem.*, **31**, 190 (1967).
(11) Signal assignment was done based on an off-resonance spectrum as well as the reported data on nucleosides and amino acids, for example, the data given in (a) J. B. Stothers, "Carbon-13 NMR Spectroscopy", Academic Press, New York, N.Y., 1972, pp 468–482, and (b) H. H. Mansch and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **46**, 808 (1972).
(12) (a) S. Dagley and D. E. Nicholson in "An Introduction to Metabolic Pathways", Wiley, New York, N.Y., 1970, pp 125–127; (b) E. Racker in "The Enzymes", Vol. 5, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N.Y., 1961, pp 397–412.
(13) To verify the formation of [1,5-^{14}C]ribose from [1-^{14}C]glucose, D-[1-^{14}C]glucose was added to growing cultures of *S. cacaoi*. Adenosine was isolated from the RNA of the cells. Ribose was isolated by hydrolysis of adenosine with Dowex 50W(H^+). The ribose was oxidized with bromine to ribonic acid followed by periodate oxidation. Carbon 1 was isolated as CO_2 in hyamine and carbon 5 was isolated as formaldimedone. The distribution of ^{14}C was 41.5% on C-1 and 40.8% on C-5.
(14) K. Isono, P. F. Crain, and J. A. McCloskey, *J. Am. Chem. Soc.*, **97**, 943 (1976).

(15) Prepared by diazotization of DL-[3-^{14}C]serine: E. Fischer and W. A. Jacobs, *Chem. Ber.*, **40**, 1068 (1907).
(16) It was shown that [3-^{14}C]serine is an efficient one-carbon precursor for C-7.[3]
(17) (a) G. Schmidt and S. J. Thannhauser, *J. Biol. Chem.*, **161**, 83 (1945); (b) I. R. Lehman, M. J. Bessman, E. S. Simms, and A. Kornberg, *J. Biol. Chem.*, **233**, 163 (1958).
(18) The earlier experiment[3] using growing cells showed that ^{14}C from [U-^{14}C]uridine was incorporated into the pyrimidine base of the polyoxins but apparently not into the sugar moiety. It may be that [^{14}C]phosphoribosyl pyrophosphate (PRPP) formed by the equilibrium, UMP ↔ uracil + PRPP, was diluted predominantly with endogenous PRPP during the long period of incubation.
(19) ^{13}C NMR analysis of ^{13}C-enriched octosyl acid was not possible because the yield under these fermentation conditions was too low. Experiments are currently underway to obtain a mutant which produces octosyl acid in larger yields.
(20) W. A. Wood in "The Enzymes", Vol. 7, P. D. Boyer, Ed., Academic Press, New York, N.Y., 1972, pp 281–302.

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Bis(pentamethylcyclopentadienyl)actinide Chemistry: Properties of Stable Thorium and Uranium Dialkyls and Hydrides

Sir:

Currently two major goals in organoactinide chemistry[1] are to design ways of manipulating coordinative unsaturation for optimum chemical reactivity and to develop meaningful chemical comparisons between organoactinide reaction patterns and those of transition metal organometallics. In regard to the former point, one of our aims has been to explore the chemistry of species with fewer *pentahaptocyclopentadienyl* ligands (e.g., $U[R(C_5H_4)_2]X_2$,[2] $U(C_5H_5)_2X_2$[3] (X = functional group)) than in the more saturated $M(C_5H_5)_3X$ (M = Th, U; X = functional group) derivatives.[4] In this communication we report initial results on new bis(pentamethylcyclopentadienyl) derivatives of thorium and uranium. These compounds represent some of the most chemically reactive and versatile organoactinides prepared to date. Furthermore, in regard to the second point above, they provide a direct chemical comparison to an analogous series of transition metal (Ti, Zr) organometallics.[5] Among the new compounds we discuss here are the most thermally stable actinide polyalkyls prepared to date and their facile reaction with hydrogen. We report the first organoactinide hydrides, which demonstrate the existence of isolable compounds with actinide–hydride bonds.[6]

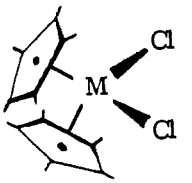
The reaction of pentamethylcyclopentadienide Grignard reagent (prepared by metalating pentamethylcyclopentadiene[7] with isopropylmagnesium chloride in refluxing toluene) with thorium and uranium tetrachlorides produces, after filtration and removal of the solvent, crystalline bis(pentamethylcyclopentadienyl)actinide dichlorides in 70–90% yield.

$$2(CH_3)_5C_5^- + MCl_4 \xrightarrow[100\ °C]{\text{toluene}} M[(CH_3)_5C_5]_2Cl_2 + 2Cl^- \quad (1)$$

1a, M = Th (colorless needles)

b, M = U (red-orange needles)

These air-sensitive new complexes were characterized by IR and ^{1}H NMR spectroscopy and by elemental analysis.[8a] Cryoscopic molecular weight measurements in benzene show **1b** to be monomeric; **1a** is insufficiently soluble for such determinations.[9] For **1b** we propose the monomeric solution structure shown; the solid-state structure of **1a** and **1b** must await diffraction studies. The lack of association in



1b

solution and the relatively low uranium coordination number in the present case stands in contrast to the associated $U[R(C_5H_4)_2]X_2$ species.[2]

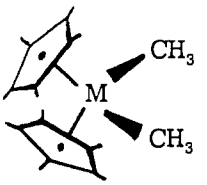
In diethyl ether solution, the bis(pentamethylcyclopentadienyl) dichlorides can be alkylated with methyllithium according to eq 2.

$$M[(CH_3)_5C_5]_2Cl_2 + 2LiCH_3 \xrightarrow[-78\ °C]{(C_2H_5)_2O} M[(CH_3)_5C_5]_2(CH_3)_2 + 2LiCl \quad (2)$$

2a, M = Th (colorless needles)

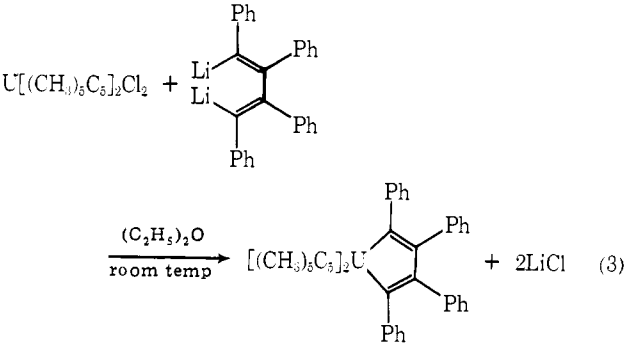
b, M = U (orange needles)

The products were isolated in 65–70% yield by evaporation of the ether and recrystallization from toluene. The air-sensitive new dialkyls were characterized by the same techniques as the dichlorides.[8b] We find them to be monomeric in benzene[8b] and propose the structure shown. Unlike previously reported actinide polyalkyls,[1,2,10] **2a** and **2b** possess very high thermal



2

stability. In toluene solution at 100 °C, **2a** has a half-life of ~1 week, while **2b** has a half-life of ~16 h. Of thermal stability comparable with that of **2b** is the uranium metallocycle prepared via the route of eq 3 using 1,4-dilithiotetraphenylbuta-



(3)

diene.[11] This product, after crystallization from toluene, was characterized by the chemical and physical methods described above.[8c]

The reaction of **1** and **2** in toluene yields monoalkylated derivatives

$$M[(CH_3)_5C_5]_2Cl_2 + M[(CH_3)_5C_5]_2(CH_3)_2 \xrightarrow[\text{room}]{\text{toluene}} 2M[(CH_3)_5C_5]_2(Cl)CH_3 \quad (4)$$

3a, M = Th (colorless needles)

b, M = U (orange-red needles)

with eq 4 lying 90–95% to the right as determined by ^{1}H NMR spectroscopy. Interestingly, molecular weight measurements in benzene[8d] show **3b** to be monomeric, while **3a** is dimeric. The

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