spectrum of racemic **25** is identical with that of (+)-**25** isolated from *Stoechospermum marginatum*.[1]

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Biosynthesis of Cationomycin: Direct and Indirect Incorporation of [^{13}C]Acetate and Application of Homoscalar Correlated 2-D ^{13}C NMR and Double Quantum Coherence

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Cationomycin is a polyether ionophore antibiotic produced by a rare actinomycete, *Actinomadura azurea*.[1,2] It is structurally unique, having an aromatic acyl side chain.[3] It binds selectively monovalent cations and is under development as a controlling agent for chicken coccidiosis because of its remarkable activity and relative low toxicity.[4] As part of the research directed toward chemical and biological modification of this interesting molecule, we report herein the biosynthesis of cationomycin, including the unambiguous assignment of the ^{13}C NMR of cationomycin labeled with [1,2-^{13}C]acetate by double quantum coherence[5] and homoscalar correlated 2-D ^{13}C NMR (COSY),[6] and a reasonable explanation for randomization of the [2-^{13}C]acetate.

An assignment of ^{13}C NMR of cationomycin[7] was based on that of structurally related laidlomycin,[8] INEPT ^{13}C NMR analysis,[9] and calculation with substituent parameters.[10] [1-^{13}C]Acetate, [1-^{13}C]propionate, [3-^{13}C]propionate, and [methyl-^{13}C]-L-methionine were incorporated as expected.[11] However,

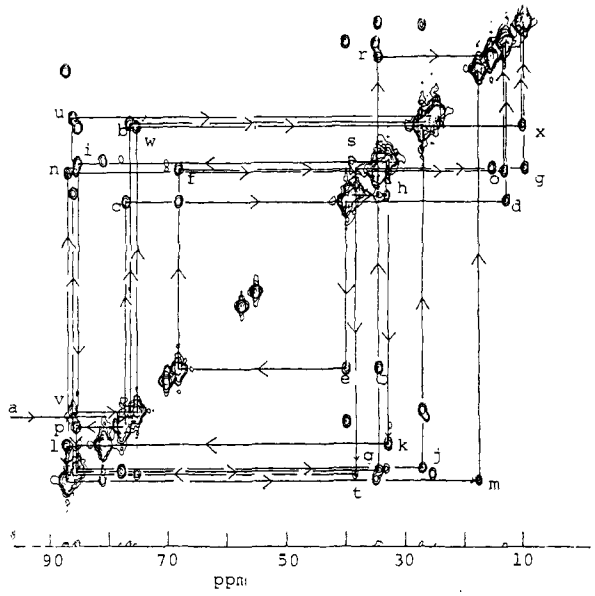
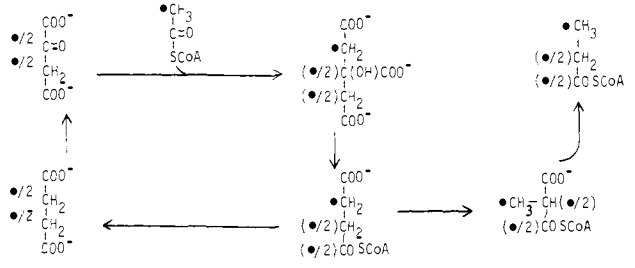


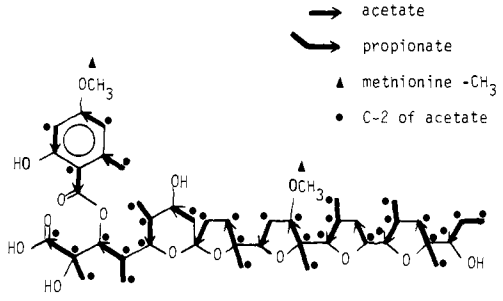
Figure 1. Homoscalar correlated 2-D ^{13}C NMR of cationomycin labeled with [2-^{13}C]acetate. The spectrum was obtained by COSY sequence[12] on ^{13}C nucleus with ^{1}H decoupling through the experiment at 100 MHz using Jeol GX 400 (acquisition time ca. 40 h, dimension of matrix 256 × 1024, dimension of transformation 512 × 1024, amount of the compound used ca. 20 mg). (a) Correlation of C-1 with C-2, (b) C-2 with 2-Me, (c) C-3 with C-4, (d) C-4 with 4-Me, (e) C-4 with C-5, (f) C-5 with C-6 (g) C-6 with 6-Me, (h) C-10 with C-11, (i) C-11 with C-12, (j) C-12 with 12-Me, (k) C-14 with C-15, (l) C-15 with C-16, (m) C-16 with 16-Me, (n) C-17 with C-18, (o) C-18 with 18-Me, (p) C-20 with C-21, (q) C-21 with C-22, (r) C-22 with 22-Me, (s) C-22 with C-23, (t) C-23 with C-24, (u) C-24 with 24-Me, (v) C-24 with C-25, (w) C-25 with C-26, (x) C-26 with C-27.

Scheme I. Pathway for Propionate from [2-^{13}C]Acetate through the Krebs Cycle[a]



[a] Parentheses show the labeling pattern for the second cycle.

Scheme II. Biogenesis of Cationomycin



feeding of [2-^{13}C]acetate resulted in considerable randomization. In the ^{13}C NMR spectrum of cationomycin labeled with [1,2-^{13}C]acetate, the application of double quantum coherence and homoscalar 2-D ^{13}C NMR revealed eight pairs of ^{13}C–^{13}C coupling, $J_{1'\text{-}CO,1'}$ (= 76 Hz), $J_{2',3'}$ (= 70.8 Hz), $J_{4',5'}$ (= 65.8 Hz), $J_{6',6'\text{-Me}}$ (= 42.7 Hz), $J_{7,8}$ (= 37.8 Hz), $J_{9,10}$ (= 41.5 Hz), $J_{13,14}$ (= 36.6 Hz), and $J_{19,20}$ (= 36.6 Hz).

In the case of [2-^{13}C]acetate, the carbons that should be derived from C-1, C-2, and C-3 of propionate were also enriched. Homoscalar correlated 2-D ^{13}C NMR and double quantum coherence

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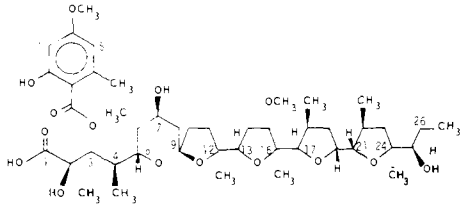
(6) Though 2-D INADEQUATE experiments have generally been applied to assignment of double-labeled compounds,[5] satisfactory data were obtained by homoscalar correlated 2-D ^{13}C NMR (COSY) experiment in this case.
(7) The numbering was conventionally adopted as follows:



(8) Seto, H.; Ōtake, N. *Heterocycles* **1982**, *17*, 555.
(9) (a) Morris, G. A.; Freeman, R. *J. Am. Chem. Soc.* **1979**, *101* 760. (b) Doddrell, D. M.; Pegg, D. T. *J. Am. Chem. Soc.* **1980**, *102*, 6388. INEPT ^{13}C NMR spectra were obtained at 25 MHz using Jeol FX 100.
(10) Wehrli, F. W.; Wirthlin, T. "Interpretation of Carbon-13 NMR Spectra"; Heyden: London, 1976.
(11) The experiment was done by feeding ^{13}C-labeled compounds (100–400 mg, 90% enriched) in two portions at 36 and 48 h after inoculation to a shaking culture of *A. azurea* in an organic medium (350 mL). After a total 144-h fermentation, cationomycin was isolated as described before,[1] average yield ca. 20 mg.
(12) Bax, A.; Freeman, R. *J. Magn. Reson.* **1981**, *42*, 164.

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experiments revealed the correlation among the labeled carbons (Figure 1) and the reasonable values of coupling constants (data not shown). The indirect incorporation of [2-^{13}C]acetate can be reasonably explained by the multiple passages of [2-^{13}C]acetate through the Krebs cycle followed by the methyl malonate–propionate shunt via succinate. For the first cycle, only C-3 of propionate derived from succinate would be labeled. However, for the second cycle, C-1, C-2, and C-3 of propionate derived from succinate should be labeled in the enrichment ratio of 0.5:0.5:1. This ratio would reach 1:1:1 by the multiple passages (Scheme I). From the above results, it has been concluded that cationomycin is biosynthesized from 8 mol of acetate, 9 mol of propionate, and 2 mol of methionine methyl as shown in Scheme II.

In order to study in more detail the biosynthetic pathway of cationomycin, appropriately labeled preformed side chain acids were used for the feeding experiments. However, both [OCH_3-^{13}C]-4-methoxy-6-methylsalicylate[13] and [CH_3-^{3}H]orsellinate[14] were not incorporated at all. It appears that the preformed acids are not activated in vivo.

Registry No. Cationomycin, 80394-65-6; acetic acid, 64-19-7; propionic acid, 79-09-4.

(13) The compound was prepared from orsellinic acid ([methyl-^{13}C]methyl iodide (90% enriched), KOH/Me_2SO, 30 min at room temperature). Satisfactory analytical data have been obtained for the compound.

(14) Labeled by Tritium Labelling Services of Amersham International Limited, Buckinghamshire, England HP7 9LL. The specific activity was 0.408 Ci/mol.

Thermochemically Based Strategies for C–H Activation on Saturated Hydrocarbon Molecules. Ring-Opening Reactions of a Thoracyclobutane with Tetramethylsilane and Methane

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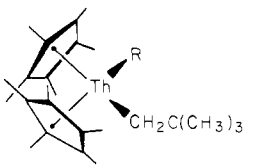
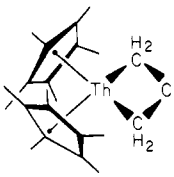
Devising effective strategies for the selective, metal-mediated, homogeneous activation of C–H bonds on exogenous saturated hydrocarbon molecules currently represents a great challenge.[1,2] Transition-metal systems involving oxidative addition/reductive elimination sequences have received the greatest attention,[1-3] although recent results with a trivalent organolanthanide[4] suggest "heterolytic" C–H activation pathways may also be widely applicable. We recently reported an actinide-centered cyclometalation reaction in which extrusion of a saturated hydrocarbon molecule occurs (eq 1, R = neopentyl) to yield thoracyclobutane



50 °C, cyclohexane (R = neopentyl, closed container)

I, R = neopentyl



+ RH (1)

2

2.[5] Thermochemical data[6] (D(Th–R))[7] indicate that unfavorable nonbonded interactions[5b,8] and/or intrinsically weak Th–neopentyl bonds in **1** partly compensate for the nonnegligible metallacyclic ring strain energy in **2** (ca. 15 kcal/mol[6]). As a consequence, the endothermicity of eq 1 (ca. +7 kcal/mol) is sufficiently small for R = neopentyl to be outweighed by entropic ($T\Delta S$)[9,10] factors; thus, $\Delta G < 0$. These observations raise the fascinating question as to whether, for judiciously selected D(Th–R) and D(R–H), eq 1 can be rendered sufficiently endothermic (eq 2)[11] that $\Delta G >$

$$\Delta H_1 \approx D(\text{Th–R}) + D(\text{neopentyl–H}) - D(\text{Th–CH}_2(\mathbf{2})) - D(\text{R–H}) \quad (2)$$

0, i.e., that complex **2** will stoichiometrically activate saturated hydrocarbons.[12] Taking tetramethylsilane and methane as examples, we confirm this hypothesis and report that these substrates are readily activated by a tetravalent actinide complex according to the reverse of eq 1.

Complex **2** was synthesized and purified as described previously.[5a] Due to the extreme sensitivity of the subject compounds, reactions were carried out in sealed NMR tubes under scrupulously anaerobic and anhydrous conditions in the dark. From steric considerations and since D(Th–secondary alkyl) is likely to be less than D(Th–primary alkyl),[6,13a] cyclohexane-d_{12} was chosen as the solvent.[13b] The reaction of **2** with tetramethylsilane, as monitored by 270-MHz ^{1}H NMR, is shown in Figure 1. Disappearance of signals due to **2** is accompanied by the appearance of a new thorium complex, **3**, formulated as shown in eq 3 on the basis of ^{1}H and ^{13}C NMR as well as comparison with an authentic sample.[14a,b] The very high solubility of **3** has so far limited isolated

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(4) (a) Watson, P. L. *J. Chem. Soc., Chem. Commun.* **1983**, 276-277; (b) *J. Am. Chem. Soc.* **1983**, *105*, 6491-6493.

(5) (a) Bruno, J. W.; Marks, T. J.; Day, V. W. *J. Am. Chem. Soc.* **1982**, *104*, 7357-7360; (b) *J. Organomet. Chem.* **1983**, *150*, 237-246. (c) Bruno, J. W.; Smith, G. M.; Marks, T. J., manuscript in preparation.

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(7) D = Bond disruption enthalpy, as defined in: Pilcher, G.; Skinner, H. A. In "The Chemistry of the Metal-Carbon Bond"; Hartley, F. R., Patai, S., Eds.; Wiley: New York, 1982; pp 43-90.

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(11) D(Th–CH_2(**2**)) in eq 2 refers to the metallocycle-opening bond disruption enthalpy in **2**.

(12) Complex **2** has previously been shown to activate arenes[5a]—usually[1-3] a far less demanding transformation.

(13) (a) For example, Cp'_2Th isopropyl compounds rapidly and quantitatively isomerize to the *n*-propyl analogues.[13c] (b) Thermolysis of **2** in cyclohexane-d_{12} and subsequent GC/MS analysis of the hydrolysis products gives no evidence of C–D activation.[13c] (c) Bruno, J. W. Ph.D. Thesis, Northwestern University, Evanstown, IL, 1982.

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