Biosynthesis of the Hypotensive Metabolite Oudenone by *Oudemansiella radicata*. 1. Intact Incorporation of a Tetraketide Chain Elongation Intermediate

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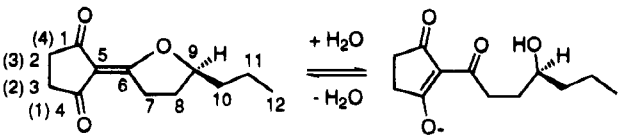
The biosynthesis of the fungal metabolite oudenone (**1**) was investigated in cultures of *Oudemansiella radicata*. Feeding experiments using ^{13}C- and ^{2}H-labeled precursors, as well as NMR analyses of the labeled metabolite, suggested a polyketide origin. The incorporation of six acetate units into the carbon skeleton of **1** was observed when cultures were fed the *N*-acetylcysteamine thioester derivative of ^{13}C-labeled acetate. Labeling of oudenone (**1**) from [1,4-$^{13}C_2$]succinate or L-[5-^{13}C]-glutamic acid was not observed, whereas the pattern of ^{13}C labeling from [2,3-$^{13}C_2$]succinate was identical to that observed with [1,2-$^{13}C_2$]acetate. The proposed advanced intermediate (5*S*)-5-hydroxyoctanoic acid (**2**) was synthesized as the deuterium labeled N-acetylcysteamine thioester derivative (*S*)-**19** and successfully incorporated into **1**. A biosynthetic scheme and cyclization mechanism, consistent with the experimental data, is proposed.

Introduction

Oudenone (**1**) (Scheme 1),[1] a metabolite of the fungus *Oudemansiella radicata*, is a strong inhibitor of catecholamine biosynthesis.[2] The biochemical mode of action of **1** is associated with inhibition of the enzymes phenylalanine and tyrosine hydroxylases. Kinetic studies using phenylalanine hydroxylase indicated that this inhibition is competitive with respect to the tetrahydropterin cofactor of the enzyme and noncompetitive with respect to phenylalanine and oxygen.[2a] The physiological effects of oudenone include the reduction of blood pressure, as demonstrated with spontaneously hypertensive test animals.[2b,3] Since the initial isolation and synthesis of oudenone,[1a] several synthetic methods for its preparation, and that of structural analogs, have been reported.[4]

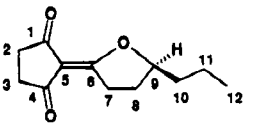
The structure of oudenone is unique among fungal metabolites (Scheme 1). In dry organic solvents, it is characterized by a tetrahydrofuran and 1,3-cyclopentadione moiety (**1a**). However, in aqueous solvents, structure **1a** is in dynamic equilibrium with the β-trione anion **1b**, *via* the simple addition or elimination of water (pK_a = 4.1). The biosynthetic origin and enzymatic reactions leading to the formation of oudenone are of interest, and they are under investigation in our laboratory. In this paper, we present results which establish that metabolite **1** is derived from six acetate units. In addition, the structure and absolute stereochemistry of an advanced chain elongation intermediate is described. On the basis of our results, a biosynthetic scheme and mechanism for the cyclization of a plausible open-chain hexaketide precursor of oudenone is proposed.

Scheme 1. Structural Features of Oudenone and pH-Dependence



UV_{max} = 284, pH < 3 (**1a**) UV_{max} = 246, pH > 7 (**1b**)

Table 1. ^{1}H and ^{13}C NMR Chemical Shift Assignments for Oudenone (1a) in $CDCl_3$



C	^{1}H (δ)	COSY	^{13}C (δ)	HETCOR
1			203.8	
2	2.55		34.6	H2
3	2.55		34.3	H3
4		200.5		
5		108.9		
6		184.4		
7	3.21 (m), 3.55 (ddd) (J = 20.4, 4.6 Hz)	H8a,b	33.4	H7a,b
8	1.77 (m), 2.29 (m)	H7a,b; H9	26.5	H8a,b
9	4.89 (m)	2H8; H10a,b	90.2	H9
10	1.65 (m), 1.87 (m)	H9, H11	36.4	H10a,b
11	1.45 (m)	H10a,b; H12	18.4	H11
12	0.95 (t, J = 7.3 Hz)	H11	13.5	H12

Results and Discussion

NMR Assignments. Although some of the NMR data of oudenone (**1**) were previously published,[1a,4b] the exact ^{1}H and ^{13}C chemical shift assignments were not reported. Most of the chemical shifts could be unequivocally assigned from the combined COSY and HETCOR NMR data (Table 1). The two carbonyl carbons were identified by observing the ^{13}C NMR spectrum of **1a** in the presence of tris[3-(heptafluoropropylhydroxymethylene)-(−)-camphorato]europium(III). A downfield shift of $\Delta\delta$ = 1.73 was observed for the C1 and C6 carbons. The shift observed for the C4 carbon was only $\Delta\delta$ = 0.63, suggest-

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(1) (a) Ohno, M.; Okamoto, M.; Kawabe, N. *J. Am. Chem. Soc.* **1971**, *93*, 1285. (b) Umezawa, H.; Takeuchi, T.; Iinuma, H.; Suzuki, K.; Ito, M.; Matsuzaki, M. *J. Antibiot.* **1970**, *23*, 514.

(2) (a) Koizumi, S.; Nagatsu, T.; Iinuma, H.; Ohno, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1982**, *35*, 458. (b) Nagatsu, T.; Mizutani, K.; Nagatsu, I; Umezawa, H.; Matsuzaki, M.; H.; Takeuchi, T. *Mol. Cell. Biochem.* **1973**, *1*, 107.

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Table 2. Relative ^{13}C Enrichment[a] and J_{C-C} Constants in the NMR of 1a after Incorporation of ^{13}C-Labeled Precursors (150 MHz, $CDCl_3$)

C	AcONa [1-^{13}C]	AcONa [1,2-$^{13}C_2$] $^1J_{C-C}$, Hz	Ac-NAC [1-^{13}C]	succinate [2,3-$^{13}C_2$] $^1J_{C-C}$, Hz	*n*-butyrate-NAC [1-^{13}C]
1	1.0	36.9	1.4	37.0	2.6
2	1.0	36.9	1.3	37.0	2.1
3	1.0	36.9	1.3	37.0	2.3
4	1.0	36.9	1.3	37.0	3.1
5	1.2	76.0	1.7	76.5	2.5
6	1.0	76.0	1.0	76.8	1.0
7	1.0	32.0	1.9	32.0	1.9
8	1.0	31.9	1.0	32.0	1.0
9	1.2	38.7	1.9	38.5	2.1
10	1.0	38.6	1.0	38.5	1.0
11	1.3	35.3	4.3	35.0	5.0
12	1.0	35.2	1.0	34.8	1.0

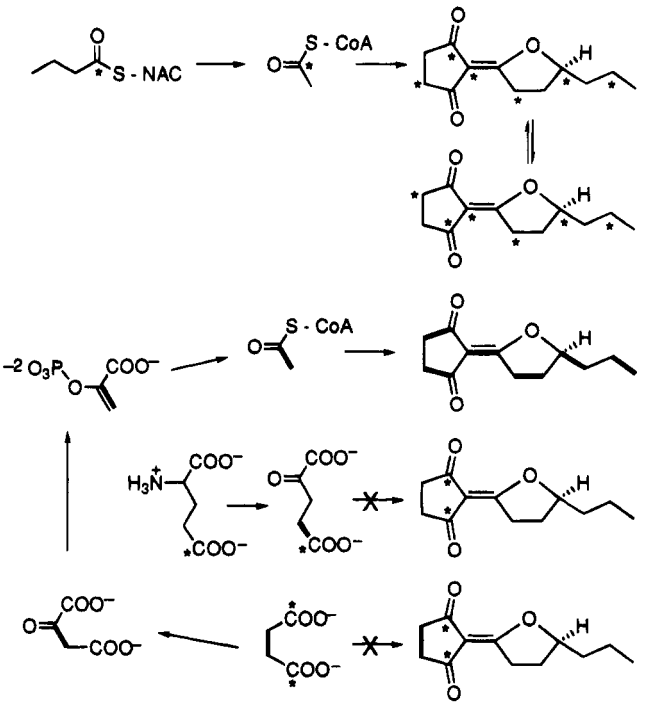
[a] Relative ^{13}C enrichments were measured from ^{13}C inverse gated NMR experiments (T_1 values measured to be ~1.5 to 12.5 s, D_1 set to 30 s). The values given are the ratios of relative intensity (area under the signal after deconvolution using Lorentzian fit) of ^{13}C resonance in enriched sample/relative intensity of the same ^{13}C resonance at natural abundance. ^{13}C enrichment of C12 is not expected from [1-^{13}C]acetate and so it was used as the standard.

ing a preferred coordination of the europium cation with two oxygens on one side of the molecule, as opposed to only one oxygen at the opposite side.

Biosynthesis of Oudenone (1): Incorporation of Primary Precursors. The production of oudenone in growing cultures of *O. radicata* was monitored by simple UV measurements. A shift in UV_{max} from 285 nm in acidic solution to 246 nm in neutral or basic solution is associated with the structural change of oudenone from **1a** to **1b**, respectively (Scheme 1).[1b] Therefore, the concentration of oudenone in growing cultures was estimated by measuring the ΔUV_{max} at 246 nm of 1 mL aliquots of the fermentation broth, diluted with a constant volume of pH 7.0 phosphate buffer and 0.1 N HCl.[5] A production curve was established for metabolite **1** by measuring the ΔUV_{max} every 12 h for a period of 30 days. The presence of **1** in the growing cultures was first observed after 8–9 days of incubation from the time of inoculation; it reached a maximum concentration at 16–19 days (ΔUV_{max} = 0.8–1.2), and it began to decrease after 20–21 days of incubation. Incorporation of stable isotopes into metabolite **1** was usually successful when labeled precursors were fed to the cultures shortly after the presence of oudenone could be detected (day 9–10, ΔUV_{max} = ~0.3). However, the addition of even small amounts of acetate or succinate to the growing cultures of *O. radicata*, caused a significant decrease in the amount of metabolite produced per unit volume of fermentation broth. Thus, isotopically labeled precursors had to be fed in very small amounts every 6–12 h, over a total period of 48 h, and cultures were harvested 12 h after the last feeding. The labeling and coupling patterns observed in the ^{13}C NMR spectra of **1**, after incorporation of [1-^{13}C]acetate and [1,2-$^{13}C_2$]acetate, clearly indicate that carbons C5–C12 are derived from four acetate units (Table 2). Incorporation of label from [1-^{13}C]acetate into the 1,3-cyclopentadione ring (carbons C1–C4) could not be observed, even after numerous feeding experiments.

(5) The UV absorption of oudenone (**1**) in pH 7.0 phosphate buffer, at 246 nm, is negligible. Thus, any absorption measured with the fermentation broth of *O. radicata*, in pH 7.0 phosphate buffer at 246 nm, was assumed to be due to components in the culture other than **1**.

Scheme 2 ^{13}C Incorporation from Primary Precursors into Oudenone (1a)



Initially, the observed labeling of the 1,3-cyclopentadione ring from [1,2-$^{13}C_2$]acetate was assumed to be due to the incorporation of labeled succinate, formed *in situ* through the Krebs cycle (Scheme 2). It was reasonable to speculate that the 1,3-cyclopentadione moiety of **1a** (or the 3-hydroxycyclopent-2-enone unit of **1b**) could be derived from succinate, or 5-aminolevulinic acid, as in the case of asukamycin[6] and reductiomycin.[6,7]

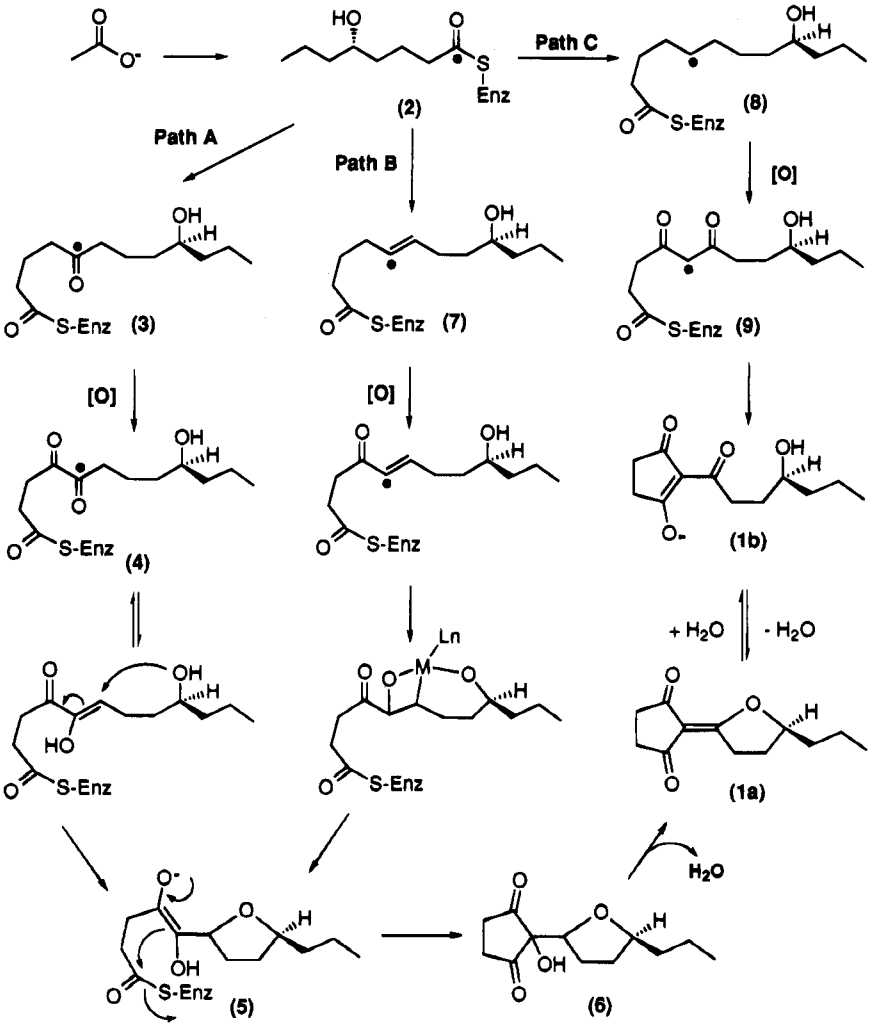
In order to test this hypothesis, a number of feeding experiments were carried out using sodium [1,4-$^{13}C_2$]-succinate, diethyl [1,4-$^{13}C_2$]succinate, and sodium [2,3-$^{13}C_2$]succinate. Incorporation of ^{13}C labels from sodium [1,4-$^{13}C_2$]succinate, or its diethyl ester, could not be detected, whereas the results obtained from the [2,3-$^{13}C_2$]-succinate experiment were identical to those observed with [1,2-$^{13}C_2$]acetate (Scheme 2, Table 2). Since [2,3-$^{13}C_2$]succinate could label phosphoenol pyruvate *via* oxaloacetate, the incorporation of the C2–C3 carbon unit from succinate to acetate, and subsequently to oudenone, would be expected (Scheme 2). Correlation through multiple-quantum coherence-INADEQUATE NMR data was consistent with the C–C coupling assignments made earlier, based on $^1J_{C-C}$ coupling constants (Table 2). Furthermore, the INADEQUATE NMR experiment did not reveal any coupling between C2 and C3 of oudenone, strongly suggesting that succinate is not a precursor in the biosynthesis of this metabolite.

The possible origin of the C1–C4 moiety of oudenone from α-ketoglutarate, rather than succinate, was also considered. Biochemically, a head-to-head condensation of tetraketide **2** with the succinate portion of α-ketoglutarate *via* decarboxylation is analogous to the synthesis of acetolactate from pyruvate, catalyzed by a thiamine-

(6) Nakagawa, A.; Wu, T. S.; Keller, P. J.; Lee, J. P.; Omura, S.; Floss H. G. *J. Chem. Soc., Chem. Comm.*, **1985**, 519.

(7) (a) Cho, H.; Beale, J. M.; Graff, C.; Mocek, U.; Nakagawa, A.; Omura, S.; Floss, H. G. *J. Am. Chem. Soc.* **1993**, *115*, 12296. (b) Beale, J. M.; Lee, J. P.; Nakagawa, A.; Omura, S.; Floss, H. G. *J. Am. Chem. Soc.* **1986**, *108*, 331.

Scheme 3. Plausible Hexaketide Precursors and Their Cyclization to Oudenone (1)



dependent enzyme.[8] Recently, Grue-Sørensen and Spenser demonstrated a similar mechanism in the biosynthesis of the *Ephedra* alkaloids.[9] However, feeding experiments using L-[5-^{13}C]glutamic acid failed to to introduce any of the ^{13}C label into oudenone (**1**) (Scheme 2).

Due to the significant decrease in the production of metabolite **1**, subsequent to the addition of any labeled precursor to cultures of *O. radicata*, the amount of ^{13}C incorporation observed was usually very low. In an effort to overcome this problem, cultures were fed [1-^{13}C]acetate and [1-^{13}C]*n*-butyrate as the *N*-acetylcysteamine thioester derivatives (NAC), since NAC derivatives are better substrates for polyketide synthase enzymes than their corresponding carboxylic acids.[10,13] Significantly higher incorporation of labels was observed from both of these precursors (Table 2). The intact incorporation of butyrate was not detected,[11] due to the efficient *in vivo* degradation of small fatty acids by β-oxidation.[12] However, this experiment was pivotal in confirming the biosynthetic origin of the C1–C4 carbon unit of oudenone from two acetates (Scheme 2). Therefore, these results strongly suggest that oudenone (**1**) is derived from a hexaketide, formed from the head-to-tail condensation of six acetate units (Scheme 3, paths A, B, or C). The scrambling of ^{13}C label between C1 and C4 (Scheme 2), as well as C2 and C3, is due to the dynamic equilibrium between **1a** and **1b** (Scheme 1) in the fermentation broth of *O. radicata* (pH of fermentation medium = 4.5–5.5).

Synthesis and Incorporation of a Tetraketide Chain Elongation Intermediate. After the origin of the carbon skeleton of oudenone had been determined,

(8) Walsh, C. *Enzymatic Reaction Mechanisms;* W. H. Freeman and Co., San Francisco, 1979; pp 682–702.

(9) Grue-Sørensen, G.; Spenser, I. D. *J. Am. Chem. Soc.* **1994**, *116*, 6195.

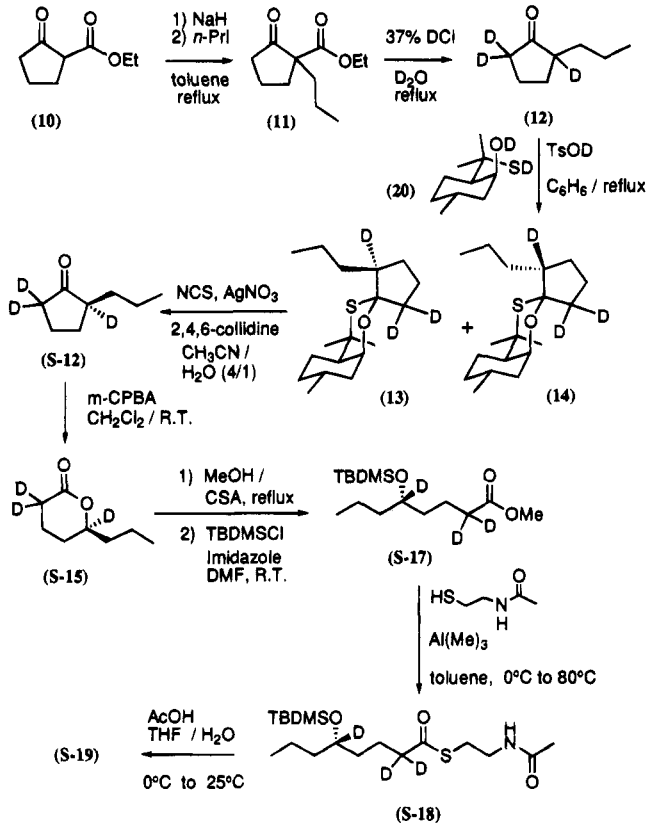
(10) *N*-Acetylcysteamine thioester (NAC) derivatives are believed to serve as mimics of the biologically relevant coenzyme A (CoA) or acyl carrier protein (ACP) thioesters involved in the biosynthesis of polyketides. (a) Rainwater, D. L.; Kolattukudy, P. E. *J. Biol. Chem.* **1985**, *260*, 616. NAC thioesters of chain elongation intermediates have been used previously for the intact incorporation of advanced precursors into polyketides. (b) Cane, D. E.; Lambalot, R. H.; Prabhakaran, P. C.; Ott, W. R. *J. Am. Chem. Soc.* **1993**, *115*, 522. (c) Cane, D. E.; Tan, W.; Ott, W. R. *J. Am. Chem. Soc.* **1993**, *115*, 527. (d) Sakuda, S.; Higashi, A.; Tanaka, S.; Nihira, T.; Yamada, Y. *J. Am. Chem. Soc.* **1992**, *114*, 663. (e) Li, Z.; Martin, F. M.; Vederas, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 1531. (f) Cane, D. E.; Prabhakaran, P. C.; Tan, W.; Ott, W. R. *Tetrahedron Lett.* **1991**, *32*, 5457. (g) Jacobs, A.; Staunton, J.; Sutkowski, A. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1113. (h) Staunton, J.; Sutkowski, A. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1110. (i) Yoshizawa, Y.; Li, Z.; Reese, P. B.; Vederas, J. C. *J. Am. Chem. Soc.* **1990**, *112*, 3212. (j) Cane, D. E.; Ott, W. R. *J. Am. Chem. Soc.* **1988**, *110*, 4840. (k) Yue, S.; Duncan, J. S.; Yamamoto, Y.; Hutchinson, C. R. *J. Am. Chem. Soc.* **1987**, *109*, 1253. (l) Cane, D. E.; Yang, C.-C. *J. Am. Chem. Soc.* **1987**, *109*, 1255.

(11) In this experiment, the mycelia of *O. radicata* cultures were transferred into high-glucose replacement media (ref 10i) before labeled sodium butyrate was added.

(12) (a) Thorpe, C. *Trends Biochem. Sci.* **1989**, *14*, 148. (b) Kunau, W.-H.; Buhne, S.; De La Graza, M.; Kionka, C.; Mateblowski, M.; Schultz-Borchard, U.; Thieringer, R. *Biochem. Soc. Trans.* **1988**, *16*, 418. (c) Miyakoshi, S.; Uchiyama, H.; Someya, T.; Satoh, T.; Tabuchi, T. *Agric. Biol. Chem.* **1987**, *51*, 2381.

(13) (a) Katz, L.; Donadio, S. *Annu. Rev. Microbiol.* **1993**, *47*, 875. (b) Hopwood, D. A.; Sherman, D. H. *Annu. Rev. Genet.* **1990**, *24*, 37.

Scheme 4. Synthesis of (5*S*)-[2-$^2H_2$,5-^2H]-5-Hydroxyoctanoyl NAC Thioester



we began to explore the mechanism of cyclization as well as the structure of the "precyclization" intermediate. Extensive investigations into the biosynthesis of macrolides, anthracyclines, tetracyclines, polyethers, and other polyketides have established that these metabolites share a common mechanism of biosynthesis. Biosynthetic and molecular genetics studies have demonstrated that polyketide synthases (PKSs) catalyze the synthesis of oligoketides from primary precursors *via* repeated decarboxylative Claisen condensations, similar to the fatty acid synthases (FASs) mechanism.[13] Genetically engineered microorganisms and selectively modified PKS genes have been designed to further explore the biosynthesis of polyketides and to produce novel metabolites.[14] Unlike fatty acids, most polyketides have complex structures with one or more ring moieties. At present, little is known about the role of polyketide cyclase enzymes; they are presumed to catalyze the formation of five- or six-membered rings from open-chain oligomers. These enzymes play a key role in determining the structural variations among different metabolites derived from the same open-chain oligoketide. Recently, Shen and Hutchinson reported the isolation and characterization of the first polyketide cyclase enzyme, tetracenomycin F2 cyclase, involved in the biosynthesis of tetracenomycin C.[15]

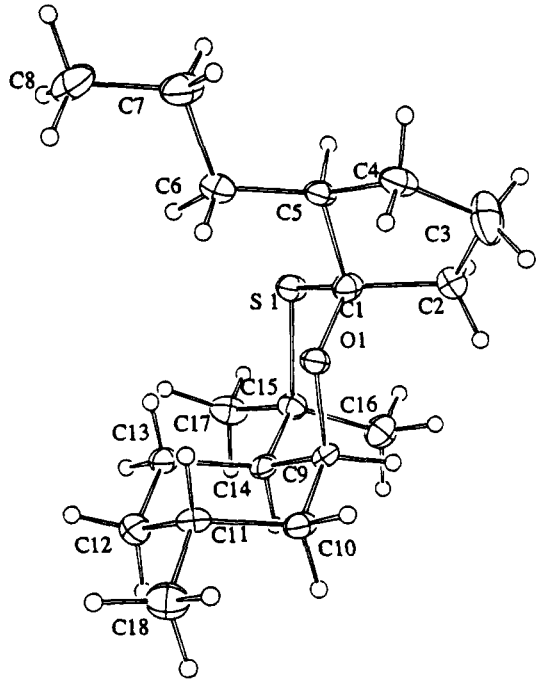


Figure 1. ORTEP Diagram of Oxathiane **13**.

In order to further explore the the biosynthesis of oudenone (**1**) and some of the molecular recognition elements between advanced substrates and the oudenone PKS catalytic domains, the synthesis and incorporation of (5*S*)-[2-$^2H_2$,5-^2H]-5-hydroxyoctanoate (**2**), believed to be a PKS chain elongation intermediate (Scheme 3), was investigated. Commercially available ethyl-2-oxocyclopentanecarboxylate (**10**) was first alkylated with *n*-propyl iodide at the α carbon (**11**) and then hydrolyzed and decarboxylated with DCl in D_2O to give racemic ^2H-labeled *n*-propylcyclopentanone (**12**) (Scheme 4).[16] Asymmetric acetalization of **12** with the chiral hydroxy thiol **20**[17,18] under standard conditions (catalytic amounts of *p*-toluenesulfonic acid,[18] benzene, reflux) afforded the diastereomeric mixture of oxathianes **13** and **14** in 2:1 ratio.[19] Optically pure **13** and **14** were isolated by flash column chromatography. The absolute stereochemistry of the major diastereomer **13** was confirmed by X-ray crystallography (Figure 1). Hydrolysis of oxathiane **13** with NCS and $AgNO_3$,[20] in the presence of a large excess of 2,4,6-collidine in aqueous CH_3CN at 0 °C, led to the isolation of the (2*S*)-(+)-[2-^2H, 5-$^2H_2$]-2-propylcyclopentanone ((*S*)-**12**). Determination of the enantiomeric purity of (*S*)-**12** by analysis of its NMR data in the presence of chiral complexing reagents, or chiral lanthanide shift reagents, was unsuccessful. However, after its hydrolysis in aqueous CH_3CN and subsequent Baeyer–Villiger oxidation of the product (*S*)-**12** to the (3*S*)-(−)-[3-^2H,6-$^2H_2$]-3-propyl-δ-valerolactone ((*S*)-**15**), an insignificant amount of deuterium exchange was observed at

(14) Reviews: (a) Tsoi, C. J.; Khosla, C. *Chem. Biol.* **1995**, *2*, 355. (b) Rohr, J. *Angew. Chem., Int. Ed. Engl.* **1995**, 34, 881. Articles: (c) McDaniel, R.; Ebert-Khosla, S.; Hopwood, D. A.; Khosla, C. *Nature* **1995**, *375*, 549. (d) Cortes, J.; Wiesmann, K. E. H.; Roberts, G. A.; Brown, M. J. B.; Staunton, J.; Leadlay, P. F. *Science* **1995**, *268*, 1487. (e) Fu, H.; Ebert-Khosla, S.; Hopwood, D. A.; Khosla, C. *J. Am. Chem. Soc.* **1994**, *116*, 4166. (f) Shen, B.; Hutchinson, C. R. *Science* **1993**, *262*, 1535. (g) McDaniel, R.; Ebert-Khosla, S.; Hopwood, D. A.; Khosla, C. *Science* **1993**, *262*, 1546. (h) Donadio, S.; Staver, M. J.; McAlpine, J. B.; Swanson, S. J.; Katz, L. *Science* **1991**, *252*, 675. (i) Caffrey, P.; Green, B.; Packman, L. C.; Rawlings, B. J.; Staunton, J.; Leadlay, P. F. *Eur. J. Biochem.* **1991**, *195*, 823. (j) Cortes, J.; Haydock, S. F.; Roberts, G. A.; Bevitt, D. J.; Leadlay, P. F. *Nature* **1990**, *348*, 176.

(15) Shen, B.; Hutchinson, C. R. *Biochemistry* **1993**, *32*, 11149.

(16) Rojo, J. A.; Perkins, E. G. *Lipids* **1989**, *24*, 467.

(17) The hydroxy thiol **20** was prepared from (+)-pulegone following Eliel's original procedure. (a) Eliel, E. L.; Lynch, J. E.; Kume, F.; Frye, S. V. *Org. Synth.* **1987**, *65*, 215. (b) Eliel, E. L.; Lynch, J. E. *Tetrahedron Lett.* **1981**, *22*, 2855.

(18) Protons were exchanged with deuterium from D_2O, in order to avoid any loss of deuterium label at the chiral center in the 2-propylcyclopentanone moiety of **13** and **14**.

(19) Nishida, M.; Nakaoka, K.; Ono, S.; Yonemitsu, O.; Nishida, A.; Kawahara, N.; Takayanagi, H. *J. Org. Chem.* **1993**, *58*, 5870.

(20) Corey, E. J.; Erickson, B. W. *J. Org. Chem.* **1971**, *36*, 3553.

the stereogenic center (<2%), indicating that (*S*)-**15** had formed in high enantiomeric purity (Scheme 4).[21]

Attempts to react (*S*)-**15** with *N*-acetylcysteamine (NAC) as the the thallous salt,[22] the trimethylsilyl sulfide derivative in the presence of $AlCl_3$,[23] or the free thiol in the presence of $Al(CH_3)_3$[24] did not give any of to the desired NAC-thioester (*S*)-**19**; only starting material was recovered. As an alternative, the lactone (*S*)-**15** was first converted to the methyl ester **16** and the hydroxyl group was protected with TBDMSCl to give (*S*)-**17** in order to prevent relactonization during the synthesis of the thioester (*S*)-**19** (Scheme 4). The methyl ester (*S*)-**17** was subsequently reacted with NAC in the presence of $Al(CH_3)_3$,[24] from 0 to 80 °C, to obtain thioester (*S*)-**18** in good yield. Hydrolysis of the silyl ether protecting group was carried out in a mixture of AcOH, THF, and H_2O (2:1:1), giving a quantitative yield of the free alcohol (*S*)-**19**. Determination of the optical purity (% ee) of (*S*)-**19** by analysis of its NMR data in the presence of chiral lanthanide shift reagents and by chiral HPLC was unsuccessful.[19] However, ^1H and ^2H NMR analysis of the final product ((*S*)-**19**), as well as all intermediate compounds (Scheme 4), indicated that the loss of deuterium label at the stereogenic center (C5) was <2%, strongly suggesting that (*S*)-**19** had been synthesized in high enantiomeric purity.

Pulse feeding of (*S*)-**19** to cultures of *O. radicata*, in the presence of the β-oxidase inhibitor 3-(tetradecylthio)-propanoic acid,[10e] gave oudenone (**1a**) with a deuterium label at C9 as predicted (Figure 2). The percent incorporation of (*S*)-**19** into **1a** was 0.86%, determined by ^2H NMR from the relative intensities of the deuterium signals between labeled **1a** and the internal solvent $CHCl_3$ (natural abundance).

Thus, the ability of (5*S*)-5-hydroxyoctanoyl NAC thioester (*S*)-**19** to serve as a substrate in the biosynthesis of oudenone was established in the whole-cell system of *O. radicata* cultures. Furthermore, the incorporation of **2** into the tetrahydrofuran moiety of **1a** was achieved without change in the absolute stereochemistry of the chiral center. The origin of the 1,3-cyclopentadione moiety of **1a** was shown to be from two acetate units, condensed in the usual head-to-tail chain elongation mechanism of polyketide biosynthesis. On the basis of these results, a number of PKS hexaketide products can be proposed as the open-chain precursor to oudenone. We have speculated on the structure and cyclization mechanism for three such plausible open-chain hexaketides (Scheme 3). Cyclization of the α-diketone **4** to oudenone (**1a**) would be analogous to the "polyepoxide cascade" model postulated by Cane, Celmer, and Westley for the construction of tetrahydrofurans in polyether antibiotics such as monensin A (Scheme 3, path A).[25] Alternatively, the "oxidative polycyclization" model, proposed more recently by Townsend, Basak, and McDonald,[26] for the biosynthesis of these tetrahydrofurans was also considered (Scheme 3, path B). However, it seems less likely that the carbonyl moiety at C5 will be reduced and eliminated, only to be reoxidized at a later point in the biosynthetic scheme (Scheme 3, path B). The third plausible open-chain precursor, 9-hydroxydodecanoic acid (**8**), will have be oxidized twice before it can cyclize by simple aldol-type condensation (Scheme 3, path C). We believe that the α-diketone **4** is the most likely precursor; it is reasonable to assume that oxidation at C4 of the open-chain hexaketide **3** occurs before cyclization. If the α-diketone **4** is the cyclase substrate, a favorable mechanism could involve an intramolecular Michael addition, followed by a Claisen type of intramolecular condensation, and dehydration of intermediate **6** to give oudenone (**1a**). This cyclization mechanism could plausibly be catalyzed by pyridoxal phosphate and is analogous to that proposed by Floss and co-workers for the formation of the 2-amino-3-hydroxycyclopent-2-enone moiety in the biosynthesis of reductiomycin.[6a] In the absence of the C4 carbonyl, nucleophilic attack of the C9 hydroxyl on the C6 enolate carbon would be very unfavorable.

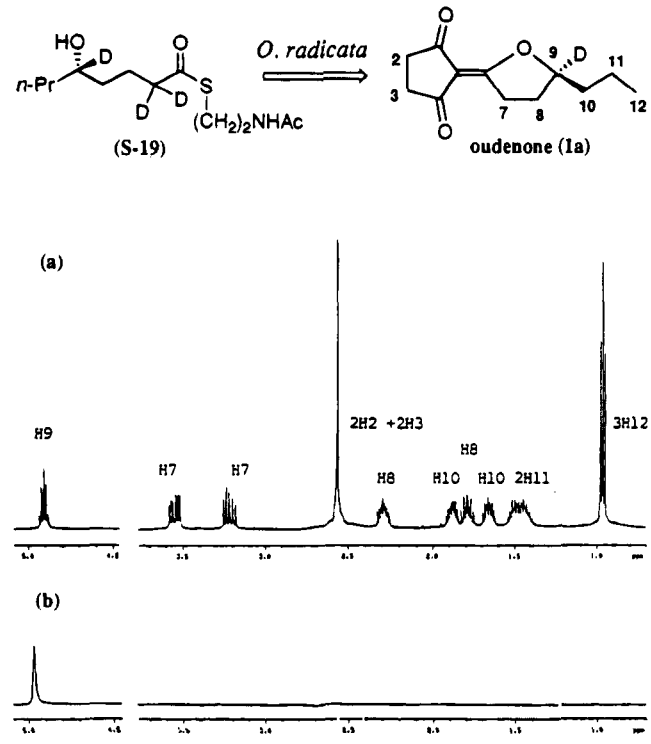


Figure 2. (a) 500 MHz 1H NMR spectrum of oudenone (**1a**) in $CDCl_3$. (b) 76.7 MHz ^2H NMR spectrum of **1a** in $CHCl_3$ derived from the intact incorporation of (5*S*)-[2-$^2H_2$,5-^2H]-5-hydroxyoctanoate.

Conclusions. The results presented in this study provide strong evidence for the pathway and mechanism of cyclization proposed in the biosynthesis of oudenone (Scheme 3, path A). The successful incorporation of tetraketide **2** into oudenone (**1**) is consistant with previous studies showing the intact incorporation of advanced intermediates into the chain-elongation mechanism of polyketide biosynthesis.[9b-1,27] Further evidence for or against the plausible precursors **4**, **7**, and **9**, as well as the catalytic mechanism of the cyclase enzyme, is being sought in experiments currently in progress in our laboratory.

(21) Determination of %ee by ^2H NMR analysis in the presence of Eu(hfc)$_3$ was unsuccessful, most likely due to the broadness of the deuterium signal. The extent of racemization was estimated from the amount of ^2H exchange, calculated from the relative intensity of the signals in the ^1H NMR spectrum of (*S*)-**15**.

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Experimental Section

General. NMR spectra were obtained at 20–22 °C. ^1H, ^2H, and ^{13}C NMR chemical shifts are given in ppm and are referenced to the internal deuterated solvent (^2H NMR was recorded in $CHCl_3$). All reactions were run under a nitrogen atmosphere using oven-dried syringes and glassware when appropriate. THF was distilled from Na/benzophenone, CH_2Cl_2 was distilled from P_2O_5, MeOH was distilled from Mg turnings, and toluene and benzene were distilled from $CaCl_2$. Reagents and solvents were purchased from Aldrich Chemical Co. and VWR Scientific of Canada, respectively. Isotopically labeled reagents were purchased from Cambridge Isotope Laboratories. Stock cultures of *O. radicata* ATCC 20295 were maintained on PDY (ATCC medium No. 337) plus 1.5% agar in slant tubes at 4 °C. All culture media and glassware were autoclaved prior to use, and all biological manipulations were conducted under a Labconco sterile hood. *O. radicata* cultures were grown at 26.5 °C and 140 rpm, in a New Brunswick Scientific shaker. A Sorvall RC-5B refrigerated centrifuge, equipped with a GSA rotor, was used for all transfers of *O. radicata* mycelium to replacement medium. Flash column chromatography was carried out on Merck Kieselgel 60, 230–400 mesh, no. 9385 silica gel. Reversed-phase flash column chromatography was carried out on silica gel reacted with *n*-octadecyltrichlorosilane, following previously reported procedures.[28]

Fermentation of *Oudemansiella radicata* ATCC 20295 for Precursor Feeding Experiments. Autoclaved medium (2 g of glucose, 5 g of Avicel microcrystalline cellulose, 0.5 g of yeast extract, 100 mL of nanopure H_2O, in a 500 mL flask) was inoculated with 10–15 small scrapings of mycelia from a PDY agar plate of *O. radicata*. The culture was grown in an incubater shaker at 26.5 °C and 140 rpm for 15–18 days. Oudenone (**1**) production was usually observed after 9–10 days of growth, and a maximum concentration was reached approximately 6–8 days later. For feeding experiments involving primary precursors (acetate, succinate, glutamic acid), the labeled substrates were dissolved in nanopure H_2O (10–20 mg in 1 mL), and the solution was sterilized by filtration through a sterile Millipore 0.22 μm filter (Millex-GS) and administered using pulse feeding techniques, over a period of 48 h, starting as soon as the presence of oudenone could be detected by UV. The cultures were harvested 12 h after the last feeding, and the isotopically labeled oudenone was isolated. For feeding experiments involving the NAC derivatives, or the diethyl derivative of succinate, the substrates were dissolved in absolute ethanol, and the same protocol for feeding and harvesting was followed. In addition, for experiments with the NAC derivative of butyrate, the mycelia of the original production cultures were transferred into an autoclaved 250 mL centrifuge tube and centrifuged at 10 000 rpm (Sorvall GSA rotor) for 15 min. The mycelia were resuspended in replacement medium (10 g of glucose, 0.3 g of KH_2PO_4, 0.1 g of $MgSO_4 \cdot H_2O$, 100 mL of nanopure H_2O), transferred into a 500 mL flask and reincubated on a shaker at 26.5 °C and 140 rpm. The ^2H-labeled substrate (*S*)-**19** (32 mg) was dissolved in absolute ethanol (1.3 mL) and administered in 100 μL aliquots every 6–12 h. At the same time, the β-oxidase inhibitor 3-(tetradecylthio)propanoic acid (186 mg dissolved in absolute ethanol 2.8 mL) was administered in 30 μL aliquots (every 6–12 h) in order to suppress degradation of (*S*)-**19**.

UV Assays for Monitoring Oudenone (1) Production. Fermentation broth aliquots (3 mL) were withdrawn from an actively growing culture every 12–24 h. The sample was filtered and diluted to 10 mL with distilled H_2O. A sample (1.00 mL) was subsequently added to a solution of pH = 7.0 phosphate buffer (4.00 mL) and to a solution of 0.1 N HCl (4.00 mL). The UV_{max} of oudenone is at 246 nm in phosphate buffer (pH = 7.0) , whereas it shifts to 284 nm in acid (0.1 N HCl).[5] Thus, the amount of oudenone present in the fermentation broth was estimated by measuring the difference in UV absorbance between the phosphate buffer and HCl solutions of the broth at 246 nm.

Isolation and Purification of Oudenone (1). The fermentation broth (100 mL) was filtered through cheesecloth and then extracted with *n*-butanol (3 × 100 mL). Evaporation of the butanol under high vacuum gave a light brown syrup which was redissolved in EtOAc (10 mL) and extracted with H_2O (pH = 7.0, 3 × 10 mL). The pH of the aqueous layer was adjusted to 3.0 with 1.0 N HCl and extracted with EtOAc (3 × 10 mL). The EtOAc layer was dried with anhydrous $MgSO_4$, filtered, and evaporated to dryness to give 10–20 mg of crude oudenone as a yellow oil. Pure oudenone (~5–10 mg) was isolated after reversed flash column chromatography on C18 silica.[28] The column was eluted with a linear gradient from 0.1% AcOH/99.9% H_2O to 0.1% AcOH/49.9% H_2O/50% MeOH. The elution of oudenone from the column was monitored by UV.

2-Propylcyclopentanone (12). Sodium hydride (2.52 g, 105.6 mmol) was suspended in dry toluene (300 mL) and cooled in an ice bath under N_2. Ethyl 2-oxocyclopentanecarboxylate (15 g, 96 mmol) dissolved in 50 mL of toluene was added dropwise *via* a syringe, over a period of 20 min. The reaction mixture was allowed to warm to rt, and it was stirred for an additional 15 min. Propyl iodide (32.60 g, 192 mmol) was added, and the mixture was refluxed for 48 h. The reaction mixture was then cooled to rt, quenched with H_2O (~1 mL), and extracted with 200 mL of H_2O. The aqueous layer was further extracted with EtOAc (2 × 100 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated to give compound **11** as a yellow oil (22 g, ~100%). Compound **11** was nearly pure by NMR and TLC; thus, it was used in the synthesis of **12** without further purification. TLC [silica, hexanes:EtOAc (10:1)]: $R_f = 0.22$. ^1H NMR ($CDCl_3$, 270 MHz) δ: 0.90 (t, J = 7.3 Hz, 3H), 1.25 (t, J = 7.3 Hz, 3H), 1.08–1.42 (m, 2H), 1.48–1.63 (dt, 1H), 1.8–2.2 (m, 4H), 2.25–2.58 (m, 3H), 4.10–4.21 (q, J = 7.3 Hz, 2H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 214.7, 170.8, 61.0, 60.3, 37.7, 35.8, 32.5, 19.4, 18.0, 14.2, 13.9.

Concentrated HCl (37%, 100 mL) was added to crude **11** (22 g), and the mixture was allowed to reflux for 24 h. The reaction mixture was cooled, extracted with Et_2O (2 × 100 mL), washed with saturated $NaHCO_3$ (2 × 100 mL) and brine (100 mL), dried over $MgSO_4$, and concentrated to give **12** as a crude oil. After vacuum distillation, pure **12** was obtained in an 82% overall yield (9.9 g). TLC [silica, hexanes:EtOAc (4:1)]: R_f = 0.71. ^1H NMR ($CDCl_3$, 270 MHz) δ: 0.80 (t, J = 7.3 Hz, 3H), 1.08–2.30 (5m, 11H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 221.5, 48.7, 37.9, 31.7, 29.4, 20.55, 20.52, 13.8.

[2-^{2}H,5-^{2}H$_2$]-2-Propylcyclopentanone (12). The deuterium-labeled **12** was obtained *via* hydrolysis and decarboxylation of **11** in 37% DCl in D_2O following the same procedure as for the unlabeled compound. ^1H NMR ($CDCl_3$, 270 MHz) δ: 0.87 (t, J = 7.3 Hz, 3H), 1.1–1.5 (m, 4H), ~1.6–1.8 (m, 2H), ~1.9–2.0 (m, 1H), 2.15–2.25 (m, 1H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 221.9, 48.4 (t), 37.5 (m), 31.7, 29.4, 20.7, 20.5, 14.0. ^2H NMR ($CDCl_3$, 76.7 MHz) δ: 2.03–2.08 ($^2H_2$) 2.26 (^2H). MS [(NH_3) Cl, direct inlet, 58 °C] *m/z* (rel intens, assignment): 147 [73.6, $(M + NH_4)^+$ of **12** with three deuterium atoms], 146 [24.2, $(M + NH_4)^+$ of **12** with two deuterium atoms], 145 [2.2, $(M + NH_4)^+$ of **12** with 1 deuterium atom].

Oxathianes 13 and 14. The deuterium-labeled, racemic **12** (756 mg, 5.9 mmol) dissolved in dry benzene (20 mL) was added to a solution of hydroxythiol **20** (1.10 g, 5.8 mmol) in dry benzene (60 mL). A catalytic amount of *p*-toluenesulfonic acid (~10 mg) was added, the flask was fitted with a Dean–Stark trap, and the reaction mixture was refluxed for 24 h. [In order to avoid loss of deuterium label, both the hydroxy thiol **20** and *p*-toluenesulfonic acid were dissolved in an organic solvent and extracted with D_2O, to exchange ^1H for ^2H, before they were used to carry out this reaction.] The benzene was removed, and the crude product was dissolved in Et_2O (100 mL), washed with a saturated, aqueous $NaHCO_3$ (2 × 100 mL), and brine (100 mL). The ether layer was dried over $MgSO_4$ and concentrated to give the diastereometic mixture of oxathiane **13** and **14** in ~2:1 ratio (1.58 g, 91% total yield). Flash

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column chromatography using a linear solvent gradient from pure hexanes to 0.25% EtOAc in hexanes permitted the isolation of the desired diastereomer **13** in ~60% yield (1.06 g). Compound **13** was crystallized from pentane; its structure, including absolute stereochemistry, was confirmed by X-ray crystallography.

Major product **13**. Mp: 34.5–35.0 °C. TLC (silica gel, hexanes): $R_f = 0.48$. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.80 (d, J = 5.9 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H), ~0.9–1.0 (m, 2H), 1.09 (s, 3H), ~1.2–1.4 (m, 4H), ~1.5–1.7 (m, 1H), 1.50 (s, 3H), ~1.7–1.9 (m, 9H), 4.01 (br s, 1H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 14.4, 21.6, 21.7, 22.3, 22.4, 25.9, 28.4, 29.5, 30.5, 32.2, 34.8, 40.5 (m), 41.8, 43.7, 44.1, 51.4 (t), 65.6, 89.8. MS [(NH_3) Cl, direct inlet, 100 °C] *m/z* (rel intens, assignment): 300 and 299 [13.0 and 27.0, $(M + H)^+$ of **13** with 3 and 2 ^{2}H, respectively], 265 and 264 [6.9, $(M - H_2S)^+$ with 3 and 2 ^{2}H, respectively], 226 and 225 [8.8 and 17.3, $(M - (CH3)_2CS)^+$ with 3 and 2 ^{2}H, respectively], 171 [62.0, (M + H − *n*-propylcyclopentanone with 3 ^{2}H)$^+$], 138 [59.6, $(171 - SH)^+$], 137 [100.0, $(171 - H_2S)^+$]. The pure minor product **14** was isolated as a colorless oil. TLC (silica gel, hexanes): $R_f = 0.39$. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.80 (d, J = 5.9 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H), 1.1 (s, 3H), 1.6 (s, 3H), ~0.8–2.0 (m, 16H), 4.01 (br s, 1H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 14.5, 20.5, 21.6, 21.8, 22.3, 25.7, 27.4, 30.3, 32.2, 32.5, 34.8, 38.5 (m), 41.8, 43.9, 51.5 (t), 67.9, 93.1.

(2*S*)-(+)-[2-^{2}H,5-^{2}H$_2$]-2-Propylcyclopentanone (12). Compound **13** (920 mg, 3.09 mmol) was dissolved in a 4:1 mixture of CH_3CN:THF and then quickly added into a vigorously stirred, ice-cold solution of 80% aqueous CH_3CN containing *N*-chlorosuccinimide (2.52 g, 18.8 mmol), $AgNO_3$ (3.36 g, 19.78 mmol), and 2,4,6-collidine (4.56 g, 37.7 mmol). The mixture was allowed to stir in an icebath for 30 min followed by the successive addition of saturated Na_2SO_3 and brine (3 mL of each). A 1:1 mixture of hexanes:CH_2Cl_2 (60 mL) was added. The mixture was filtered, and the filter cake was washed thoroughly with hexanes:CH_2Cl_2 (1:1). The organic layer was dried over anhydrous $MgSO_4$ and concentrated to give (*S*)-(+)-**12** as a crude oil. Purification by flash column chromatography using Et_2O:petroleum ether (1:25) as the eluting solvent, followed by vaccum distillation, gave the pure product in ~50% yield (198 mg). $[\alpha]_D$: +103 (*c* 0.312, THF). The ^{1}H and ^{13}C NMR data were identical to that for the racemic compound.

(3*S*)-(−)-[3-^{2}H,6-^{2}H$_2$]-3-Propyl-δ-valerolactone (15). *m*-Chloroperbenzoic acid (1.7 g, 9.85 mmol) was added to a solution of (*S*)-**12** (1.2 g, 9.52 mmol) in CH_2Cl_2 (40 mL). The reaction mixture was allowed to stir at rt for 24 h. It was then filtered, washed with saturated $NaHCO_3$ (3 × 30 mL), dried over $MgSO_4$, and concentrated to give a crude oil. After vacuum distillation, lactone (*S*)-**15** was isolated as a colorless oil, in 85% yield (1.2 g). TLC [silica, EtOAc:hexanes (1:4)]: $R_f = 0.22$. $[\alpha]_D$: −63.4° (*c* 3.38, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.9 (t, J = 7.3 Hz, 3H), 1.3–1.9 (m, 8H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 13.7, 18.0, 18.1, 27.5, 27.8 (m), 37.6, 79.7 (t), 171.9. ^{2}H NMR ($CDCl_3$, 46.1 MHz) δ: 2.28 and 2.42 (^{2}H6), 4.17 (s, ^{2}H3). MS [(NH_3) Cl, direct inlet, 100 °C], *m/z* (rel intens, assignment): 163 [6.0, $(M + NH_4)^+$ of **15** with three deuterium atoms], 146 [100.0, $(M + H)^+$ of **15** with three deuterium atoms], 145 [32.4, $(M + H)^+$ of **15** with two deuterium atoms], 144 [5.0, $(M + H)^+$ of **15** with one deuterium atom], 128 [10.4, $(M + H - H_2O)^+$ of **15** with three deuterium atoms], 120 [52.0, $(M + NH_4 - n$-propyl group$)^+$ of **15** with three deuterium atoms], 119 [10.8, $(M + NH_4 - n$-propyl group$)^+$ of **15** with two deuterium atoms].

Nonlabeled 3-Propyl-δ-valerolactone (**15**). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.9 (t, J = 7.3 Hz, 3H), 1.3–1.9 (m, 8H), 2.3–2.6 (2 m, 2H, 2H6), 4.3 (m, 1H, H3). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 13.6, 17.9, 18.2, 27.5, 29.2, 37.7, 80.1, 171.7.

(5*S*)-(−)-[2-^{2}H$_2$,5-^{2}H]-5-Hydroxyloctanoyl Methyl Ester (16). Lactone **15** (876 mg), freshly distilled methanol (15 mL), and a catalytic amount of (±)-10-camphorsulfonic acid (10 mg) were added to a dry flask (25 mL). The reaction mixture was refluxed for 12 h under N_2, cooled to rt, and partitioned between Et_2O (20 mL) and saturated $NaHCO_3$ solution (10 mL). The aqueous layer was extracted further with Et_2O (3 × 10 mL), and the organic layers were combined, dried ($MgSO_4$), and concentrated to give crude **16**. Flash column chromatography using 25% EtOAc in hexanes gave pure **16** as a colorless oil (1.036 g, 96% yield). TLC [silica, EtOAc: hexanes (2:3)]: $R_f = 0.5$. $[\alpha]_D$: −1.6° (*c* 2.94, THF). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.87 (t, J = 6.6 MHz, 3H), 1.2–1.8 (m, 8H), 3.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 67.5 MHz): δ 13.9, 18.6, 20.7, 33.4 (m), 36.4, 39.3, 51.3, 70.3 (t), 174.1. Nonlabeled 5-Hydroxyoctanoate Methyl Ester (16). ^{1}H NMR ($CDCl_3$, 270 MHz) δ 0.89 (t, J = 6.6 Hz, 3H), 1.2–1.9 (m, 8H), 2.38 (t, J = 7.3 Hz, 2H), 3.65 (m, 1H), 3.61 (s, 3H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 14.6, 19.3, 21.5, 34.2, 37.0, 39.9, 51.6, 70.8, 172.9.

(5*S*)-[2-^{2}H$_2$,5-^{2}H]-5-[(*tert*-Butyldimethylsilyl)oxy]-octanoate Methyl Ester (17). *tert*-Butyldimethylsilyl chloride (525 mg, 3.48 mmol) and imidazole (564 mg, 8.28 mmol) were dissolved in dry DMF (1 mL). Methyl ester **16** (145 mg, 828 mmol) was added, and the reaction mixture was stirred under N_2 at rt for 48 h. The reaction was subsequently quenched with the addition of Et_2O (10 mL) and brine (5 mL). The aqueous layer was further extracted with Et_2O (2 × 10 mL), and the ether layer was dried ($MgSO_4$) and concentrated to give the crude **17**. Flash column chromatography using 5% EtOAc in hexanes as the eluting solvent led to the isolation of **17** as a colorless oil (206 mg, 86% yield). TLC [silica, EtOAc: hexanes (1:20)]: $R_f = 0.46$. $[\alpha]_D$ −0.46° (*c* 3.9, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.01 (s, 6H), 0.86 (s, 9H), 0.86 (t, J = 5.3 Hz, 3H), 1.2–1.7 (m, 8H), 3.63 (s, 3H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: −4.5, 14.3, 18.1, 18.5, 20.8, 26.0, 34.2 (m), 36.4, 39.3, 51.4, 71.6 (t), 174.1.

Nonlabeled Compound **17**. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.01 (s, 6H), 0.86 (s, 9H), 0.86 (t, J = 5.3 Hz, 3H), 1.2–1.7 (m, 8H), 2.28 (t, J = 7.3 Hz, 2H), 3.63 (m, 1H), 3.63 (s, 3H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: −4.5, 14.3, 18.1, 18.5, 20.8, 26.0, 34.2, 36.4, 39.3, 51.4, 71.6, 174.1.

(5*S*)-[2-^{2}H$_2$,5-^{2}H]-5-[(*tert*-Butyldimethylsilyl)oxy]-octanoyl NAC Thioester 18. Dry benzene (2.5 mL) and trimethylaluminum (1.7 mL of a 2 M solution in toluene, 3.4 mmol) were mixed and cooled in an ice bath under N_2. A solution of *N*-acetylcysteamine (407 mg, 3.42 mmol) in dry benzene (1 mL) was added, and the reaction mixture was allowed to warm to rt while being stirred for 15 min. A solution of compound **18** (200 mg, 0.680 mmol) in dry benzene (1 mL) was added, and the mixture was stirred at rt for 24 h. The reaction was quenched with the addition of Et_2O (20 mL) and KH_2PO_4 buffer (pH = 7, 20 mL). The aqueous layer was further extracted with ether (3 × 20 mL), and the combined organic layer was washed with brine (20 mL), dried ($MgSO_4$), and concentrated to give a yellow oil. The crude product was purified by flash column chromatography (60% EtOAc in hexanes) to give pure **18** in 63% yield (162 mg). TLC [silica, EtOAc:MeOH (10:1)]: $R_f = 0.59$. $[\alpha]_D$: −0.406° (*c* 2.96, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.02 (s, 6H), 0.86 (s, 9H), 1.2–1.5 (m, 6H), 1.6–1.8 (m, 2H), 1.94 (s, 3H), 2.98 (t, J = 6.3 Hz, 2H), 3.41 (dt, $J_1 = J_2$ = 6.3 Hz, 2H), 5.85 (br, 1H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: −4.5, 14.2, 18.0, 18.4, 21.4, 23.1, 25.8, 28.4, 36.0, 39.2, 39.7, 170.2, 199.9. MS [(NH_3) Cl, direct inlet, 240 °C], *m/z* (rel intens, assignment): 321 and 320 [55.1 and 15.5 $(M + H^+ - NHAc)^+$ of **18** with 3 and 2 ^{2}H, respectively], 176 [71.1, $(M + H^+ - TBDMSi^2H - CH_2CH_2NHAc)^+$ of **18** with 3 ^{2}H], 86 [100.0, $(CH_2CH_2NHAc)^+$]. Nonlabeled Compound **18**. ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.02 (s, 6H), 0.86 (s, 9H), 1.2–1.5 (m, 6H), 1.6–1.8 (m, 2H), 1.94 (s, 3H), 2.55 (t, J = 7.5 Hz, 2H) 2.98 (t, J = 6.3 Hz, 2H), 3.41 (dt, $J_1 = J_2$ = 6.3 Hz, 2H), 3.63 (m, 1H), 5.85 (br, 1H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: −4.5, 14.2, 18.0, 18.4, 21.4, 23.1, 25.8, 28.4, 36.0, 39.2, 39.7, 44.2, 71.5, 170.2, 199.9.

(5*S*)-[2-^{2}H$_2$,5-^{2}H]-5-Hydroxyloctanoyl NAC Thioester 19. Acetic acid (0.5 mL) and H_2O (0.25 mL) were mixed and cooled in an ice bath. A THF solution (0.25 mL) of the silyl ether **18** (13.7 mg, 0.0365 mmol) was added, and the reaction was allowed to stir at 0 °C for 5 min and at then rt for 1 h. The reaction mixture was then neutralized with aqueous $NaHCO_3$ and extracted with EtOAc (3 × 10 mL). The combined EtOAc layers were dried ($MgSO_4$) and concentrated to yield the final product **19** as an oil (12 mg, ~100% yield). TLC [silica, EtOAc]: $R_f = 0.16$. $[\alpha]_D$: −1.86° (*c* 1.72, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 270 MHz) δ: 0.89 (m, 3H), 1.22–2.95 (m, 8H), 1.96 (s, 3H), 2.99 (t, J = 6.3 Hz, 2H), 3.41 (dt, $J_1 = J_2$ =

6.3 Hz, 2H), 5.86 (br, 1H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 14.0, 18.8, 21.8, 23.2, 28.6, 36.4, 39.6, 39.7, 43.9 (m, intensity too weak to be observed with a small amount of sample), 71.1 (t), 170.3, 200.1. MS data of **19** was almost identical to that of lactone **15**; thioester **19** was found to be fairly unstable even at rt. Nonlabeled Compound **19**. ^1H NMR ($CDCl_3$, 270 MHz) δ: 0.89 (m, 3H), 1.22–2.95 (m, 8H), 1.96 (s, 3H), 2.58 (t, J = 7.3 Hz, 2H), 2.99 (t, J = 6.3 Hz, 2H), 3.41 (dt, $J_1 = J_2$ = 6.3 Hz, 2H), 3.58 (m, 1H), 5.86 (br, 1H). ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ: 14.0, 18.8, 21.8, 23.2, 28.6, 36.4, 39.6, 39.7, 43.9, 71.1, 170.3, 200.1.

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Supporting Information Available: ^1H, ^{13}C, and ^2H NMR spectra of oudenone (**1**) and all synthetic compounds (55 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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