chloride, 824-98-6; *m*-methoxycinnamic acid, 6099-04-3; 3-(*m*-methoxyphenyl)propionic acid, 10516-71-9; 2-ethyl-5-methoxyindanone, 138090-26-3.

Supplementary Material Available: Atomic numbering schemes and tables of atomic coordinates, thermal parameters, bond lengths, and bond angles for compounds IIIa and 7; ^{1}H NMR spectra of compounds **2a**, **3c**, **6a–c**, **7**, **9**, **11–17**, IIIa,c,e,f, IVa; ^{13}C NMR spectra of compounds **6c**, **7**, and IIIb–f (40 pages). Ordering information is given on any current masthead page.

Biosynthesis of Purpactin A†

Hiroyuki Nishida,‡ Hiroshi Tomoda, Shigenobu Okuda, and Satoshi Ōmura*

Research Center for Biological Function, The Kitasato Institute, Minato-ku, Tokyo 108, Japan

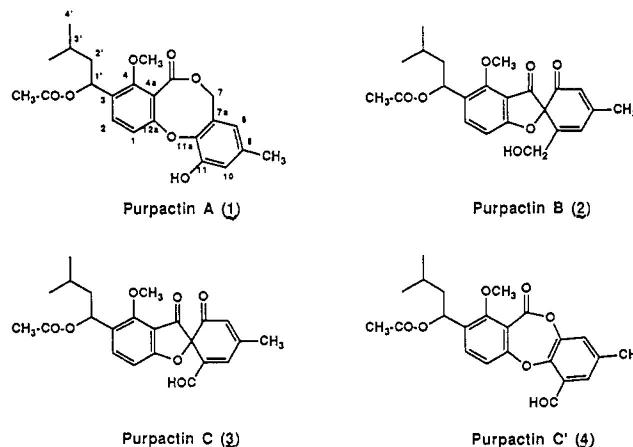
Received September 16, 1991

The biosynthetic origin of purpactin A (1) was studied by feeding sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates, D,L-[2-^{13}C]mevalonolactone, and L-[methyl-^{13}C]methionine to the producing organism *Penicillium purpurogenum* FO-608. ^{13}C NMR spectroscopy established that 1 is derived from one mevalonate, one methionine, and nine acetate units. In the biosynthetic scheme for 1 it is proposed that (1) the tricyclic skeleton of purpactin B (2) is produced first from a single octaketide chain condensed in a "head-to-tail" fashion, which involves decarboxylation of the "tail" carboxylic acid group to form the carbocyclic intermediate 5, an oxidative cleavage of the B ring of 5 to form benzophenone intermediates (rotation between 6a and 6b), and recyclization (phenol oxidative coupling), and then (2) a methyl residue from methionine, a C-5 unit from mevalonate, and an acetate are introduced into the isogrisan skeleton 7 to yield 2, and (3) finally 2 is nonenzymatically converted to 1.

Introduction

Purpactins A (1), B (2), and C (3), new acyl-CoA: cholesterol acyltransferase (ACAT) inhibitors, have been found in the cultured broth of *Penicillium purpurogenum* FO-608.[1,2] They showed ACAT inhibitory activity in both the enzyme assay using rat liver microsomes and the cell assay using J774 macrophages. It seems that purpactins A and C′ (4) (Chart I), having similar tricyclic skeletons, are nonenzymatically derived from purpactins B and C, respectively.[2] Purpactin A is structurally related to penicillide, which was reported as a root-growth stimulant by Sassa et al.,[3] but the biosynthetic study of penicillide has not been carried out in detail. Of particular interest are the decarboxylation, oxidative cleavage, and cyclization of a carbocyclic intermediate derived from a single octaketide chain and the conversion of 2 into 1. To clarify the biosynthetic pathway to 1, we obtained ^{13}C-enriched samples of 1 through feeding ^{13}C-precursors to the growing culture of *P. purpurogenum* FO-608.

Chart I. Structures of Purpactins A, B, C, and C′



Experimental Section

Incorporation Experiments. The inoculum for the fermentation of *P. purparogenum* FO-608 was built up in two stages on a slant culture in a seed medium consisting of glucose (2%, w/v), yeast extract (0.2%), $MgSO_4 \cdot 7H_2O$ (0.05%), polypeptone (0.5%), KH_2PO_4 (0.1%), and agar (0.1%) for 2 days at 28 °C. The second-stage seed culture was used to inoculate a fermentation medium (100 mL in a 500-mL flask). The fermentation medium consisted of glycerol (2.5%, w/v), glucose (0.5%), peptone (0.5%), NaCl (0.2%), and agar (0.1%). At 24 h following inoculation, the labeled compounds were added in equal portions. Single-labeled acetates (sodium [1-^{13}C]- and [2-^{13}C]acetates, ISOTEC Inc., minimum 99 atom % ^{13}C) were included at a final concentration of 0.1%; double-labeled acetate (sodium [1,2-$^{13}C_2$]acetate, ISOTEC Inc., minimum 99 atom % ^{13}C) was added at a final concentration of 0.01%; and the labeled mevalonolactone and methionine (D,L-[2-^{13}C]mevalonolactone and L-[methyl-^{13}C]methionine, ISOTEC Inc., minimum 99 atom % ^{13}C) were both at 0.1%.

Isolation Procedure. Each fermentation broth was extracted twice with the same volume of EtOAc. The extracts were filtered through a phase separator (Whatman 1PS) and concentrated under reduced pressure to an oily residue. The residue was dissolved in CH_3CN and applied to an ODS column (YMC pack, 5-ODS, AM 324, 10 × 300 mm). The mobile phase consisted of 60% aqueous CH_3CN. The flow rate was maintained at 8 mL/min, and the column effluent was monitored by UV absorbance at 280 nm. The yields of the purified ^{13}C-labeled 1 were as follows: [1-^{13}C]acetate-labeled 1 (1 mg) from 200 mL of the fermentation broth, [2-^{13}C]acetate-labeled 1 (0.9 mg) from 200 mL of the fermentation broth, [1,2-$^{13}C_2$]acetate-labeled 1 (3.8 mg) from 500 mL of the fermentation broth, [2-^{13}C]mevalonolactone-labeled 1 (1 mg) from 200 mL of the fermentation broth,

† This manuscript is dedicated to the late Dr. Shigenobu Okuda.
‡ On leave from Pfizer Central Research, Nagoya, Pfizer Pharmaceuticals Inc., 5-2 Taketoyo, Aichi 470-23, Japan.

(1) Tomoda, H.; Nishida, H.; Masuma, R.; Cao, J.; Okuda, S.; Ōmura, S. *J. Antibiot.* **1990**, *44*, 136.
(2) Nishida, H.; Tomoda, H.; Cao, J.; Okuda, S.; Ōmura, S. *J. Antibiot.* **1990**, *44*, 144.
(3) Sassa, T.; Niwa, G.; Unno, H.; Ikeda, M.; Miura, Y. *Tetrahedron Lett.* **1974**, *45*, 3941.

0022-3263/92/1957-1271$03.00/0 © 1992 American Chemical Society

Table I. ^{13}C NMR Chemical Shifts,[a] Enrichment Ratio of Purpactin A Derived from ^{13}C-Single-Labeled Precursors, and J_{C-C} of [1,2-$^{13}C_2$]AcONa-Labeled Purpactin A

carbon	^{13}C chemical shift, ppm[a]	mult[b]	enrichment ratio[d] of 1 derived from: [1-^{13}C] AcONa[c]	[2-^{13}C] AcONa[c]	D,L-[2-^{13}C] MVL[c]	L-[Me-^{13}C] Met[c]	[1,2-$^{13}C_2$] AcONa[c] J_{C-C}, Hz
C-1	117.7	d	1.0	8.5	0.2[g]	0.8	58, 69
C-2	130.7	d	24.5	1.3	0.3	0.5	59, 58
C-3	134.3	s	<0.1	2.5	0.4	NE	70, 59
C-4	154.7	s	2.3	<0.1	NE	NE	70
C-4-OMe	62.7	q	<0.1	<0.1	NE	32.6	s[e]
C-4a[f]	119.8	s	NE	0.5	NE	NE	71
C-5	167.0	s	4.0	<0.1	NE	NE	71
C-7	68.9	t	<0.1	8.3	0.1	1.0	s[e]
C-7a	125.8	s	6.3	<0.1	0.2	NE	60
C-8	120.9	d	0.8	9.0	0.1	0.6	60
C-9	135.1	s	10.3	<0.1	0.5	NE	45
C-9-Me	20.9	q	0.5	5.0	NE	1.2	45
C-10	117.5	d	<0.1	9.3	0.1	0.8	s[e]
C-11	147.2	s	6.3	<0.1	0.5	NE	75
C-11a	141.2	s	<0.1	1.3	NE	NE	75
C-12a	151.5	s	4.0	<0.1	0.5	NE	69
C-1′	68.6	d	12.0	1.0	0.1	0.8	39
C-1′-CO	170.2	s	5.8	<0.1	NE	NE	59
C-1′-COMe	21.2	q	0.5	9.5	NE	NE	59
C-2′	45.3	t	0.4	7.8	0.8	1.2	39
C-3′	24.9	d	13.8	1.0	NE	0.9	35
C-3′-Me	21.9	q	0.8	6.5	1.0	1.0	35
C-4′	23.1	q	1.0	14.8	12.5	0.9	s[e]

[a] Each sample was dissolved in $CDCl_3$. Chemical shifts are shown with reference to $CDCl_3$ as 77.0 ppm. [b] Multiplicities determined from DEPT spectrum. [c] AcONa = sodium acetate; MVL = mevalonolactone; Met = methionine. [d] Enrichment ratios were relative to the C-3′-Me signals ([1-^{13}C]acetate, [2-^{13}C]MVL and [Me-^{13}C]Met) as 1.0 and to the C-3′ signal ([2-^{13}C]acetate) as 1.0, respectively. [e] Signal was singlet, so the carbon had no coupling with others. [f] C-4a carbon was not detected under this condition. The chemical shift of this carbon was determined by a gated decoupling experiment. [g] Signals were not enriched.

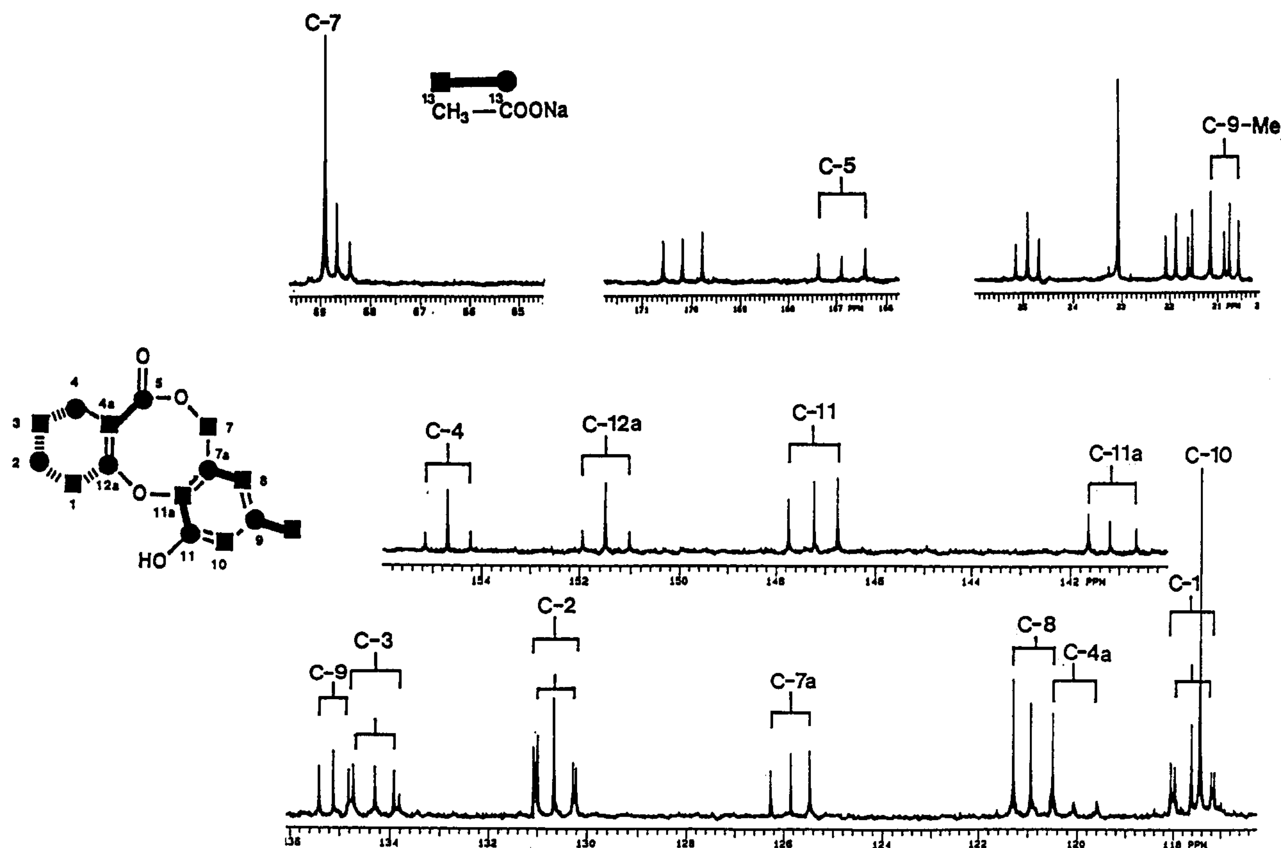


Figure 1. ^{13}C NMR spectrum of [1,2-$^{13}C_2$]acetate enriched purpactin A.

and [methyl-^{13}C]methionine-labeled 1 (1.5 mg) from 200 mL of the fermentation broth.

NMR Experiments. Proton-noise-decoupled spectra of sample enriched with [1,2-$^{13}C_2$]acetate were recorded at 75 MHz. A pulse width of 3–5 μs was used. The pulse delay time was 4.180 s.

Results

Sodium [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]acetates, D,L-[2-^{13}C]mevalonolactone, and L-[methyl-^{13}C]methionine were fed to cultures of *P. purpurogenum* FO-608 to establish

the biosynthetic origins of 1. The ^{13}C-enriched samples of 1 were isolated, and the positions of the ^{13}C-enriched carbon atoms were determined by ^{13}C NMR spectroscopy.

All the data from the feeding experiments are summarized in Table I. Clearly the 1′-acetoxy moiety of the side chain of 1 was derived from acetate, the 3′-methylbutyl moiety was from mevalonate, and the 4-methoxymethyl moiety was from the methyl residue of L-methionine. Note that [2-^{13}C]mevalonolactone was incorporated into 3′-methylbutyl moiety very efficiently and with high [^{13}C]-enrichment of the C-4′ position (Table I). The carbon of the tricyclic skeleton (9-methyl-11-hydroxy-5*H*,7*H*-dibenzo[*b*,*g*]-1,5-dioxocin-5-one) of 1 was also determined. In the respective sodium [1-^{13}C]- and [2-^{13}C]acetate incorporation experiments, the seven carbons (C-2, C-4, C-5, C-7a, C-9, C-11, and C-12a) and the eight carbons (C-1, C-3, C-4a, C-7, C-8, C-9-Me, C-10, and C-11a) were enriched (Table I). This indicates that the tricyclic skeleton of 1 is derived from an octaketide chain. In a similar experiment with sodium [1,2-$^{13}C_2$]acetate (Figure 1), there were three kinds of signals, doublet, double–doublet, and singlet carbons. From the coupling constants measured (Table I), six pairs of doublet signals (C-1 to C-12a, C-3 to C-4, C-4a to C-5, C-7a to C-8, C-9 to C-9-Me, and C-11 to C-11a), three pairs of signals made up each of two doublets (C-1 to C-2 and C-12a, C-2 to C-1 and C-3, and C-3 to C-2 and C-4), and two uncoupled signals (C-7 and C-10) were observed.

Discussion

If the distribution of the ^{13}C-enriched carbons obtained from these experiments is explained, it may be possible to propose the biosynthetic pathway of purpactin A (1).

As previously reported,[2] 1 is produced via purpactin B (2) and the conversion of 2 to 1 proceeds nonenzymatically. Therefore, it is possible to propose that the tricyclic skeleton (spirobenzofuran-1,2′-cyclohexa-3′,5′-diene-2′,3-dione (7)) of 2 is synthesized first, then the side chain is introduced to afford 2, and finally 2 is converted to 1.

Skeleton 7 is suggested to be derived from the hypothetical benzophenone intermediates **6a** and **6b** and is formed via decarboxylation of a single octaketide chain and oxidative cleavage at the B ring of a carbocyclic intermediate, an anthraquinone, or anthrone form 5 (Figure 2). The isolated signals of C-10 and C-7 carbons of 1 originally derived from the C-2 position of acetate could be explained as a result of the decarboxylation and oxidative cleavage reactions, respectively. Similar decarboxylation and oxidative cleavage were observed in the biosynthesis of varieconxanthone,[4] shamixanthone,[5] and tajixanthone.[6] In general, the biosynthesis of the fungal anthraquinones is from a single octaketide chain and almost all the anthraquinones, except cinnalutein,[7] cinnarubin,[8] and endocricin,[9] are decarboxylated at the "tail" carboxylic acid. This decarboxylation may occur before the aromatic ring formation as there are no neutral anthraquinones formed from anthraquinone acids.[10] The three pairs of double–

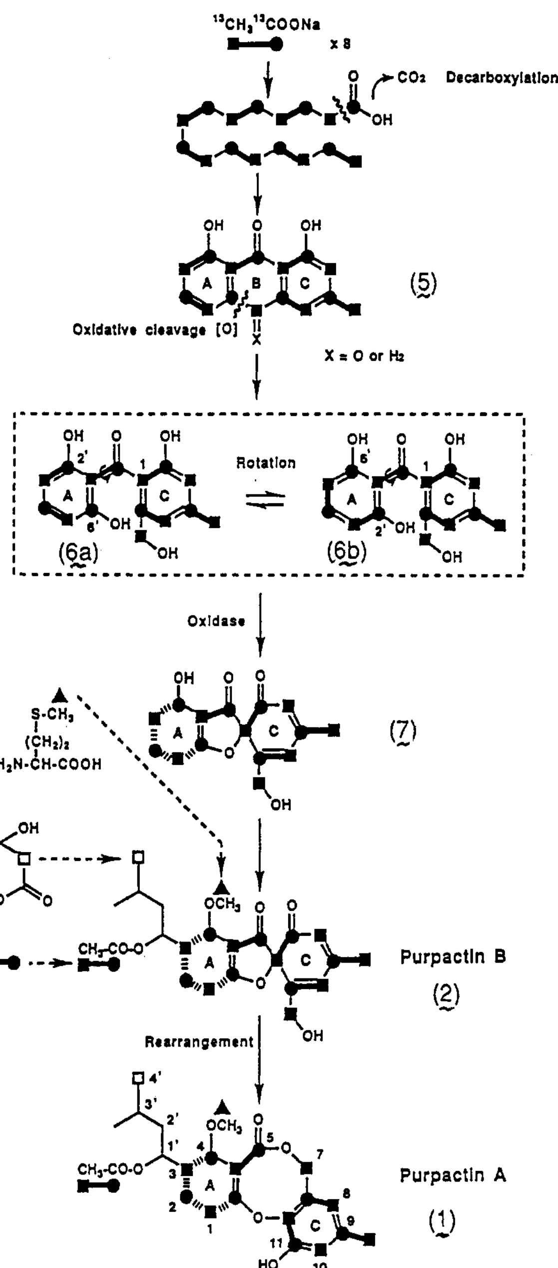


Figure 2. Proposed biosynthetic pathway of purpactins A and B.

doublet carbons in the [1,2-$^{13}C_2$]acetate feeding experiment could be explained as a result of the rotation. The phloroglucinol moiety (A ring) at the step of the benzophenone intermediate formation rotates between **6a** and **6b** (Figure 2). Furthermore, an equal distribution between **6a** and **6b** was also demonstrated since the relative intensities of the C-4 and C-12a satellites were only half of those of the remaining nuclei derived from intact acetate units (Figure 1). An analogous rotation has been reported from the study of the biosynthesis of griseofulvin.[11] And then, an oxidation, phenol oxidative coupling (cyclization between positions C-1 and C-2′ or C-1 and C-6′ of 3) is catalyzed by enzymes similar to sulochrin[12] and dihydrogeodin[13] oxidase to give the tricyclic skeleton of 7.

Taken together, the proposed biosynthetic pathway of

(4) Chexal, K. K.; Holker, J. S. E.; Simpson, T. J.; Young, K. *J. Chem. Soc., Perkin Trans. 1* **1974**, 543.
(5) Chexal, K. K.; Fouweather, C.; Holker, J. S. E.; Simpson, T. J.; Young, K. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1584.
(6) Kamal, A.; Husain, S. A.; Qureshi, A. A. *Pak. J. Sci. Ind. Res.* **1971**, *14*, 90.
(7) Reininger, W.; Steglich, W.; Moser, M. *Z. Naturforsch. Teil B* **1972**, *27*, 1009.
(8) Steglich, W.; Reininger, W. *Chem. Ber.* **1972**, *105*, 2922.
(9) Wheeler, M. M.; Wheeler, D. M. S.; Peterson, G. W. *Phytochemistry* **1975**, *14*, 288.
(10) Steglich, W.; Arnold, R.; Losel, W.; Reininger, W. *J. Chem. Soc., Chem. Commun.* **1972**, 102.
(11) Sato, Y.; Oda, T. *Tetrahedron Lett.* **1976**, *44*, 3971.
(12) Nordlov, H.; Gatenbeck, S. *Arch. Microbiol.* **1982**, *131*, 208.
(13) Fujii, I.; Iijima, H.; Tsukita, S.; Ebizuka, Y.; Sankawa, U. *J. Biochem.* **1987**, *101*, 11.

purpactin A (1) is summarized in Figure 2. Thus, the biosynthesis of purpactin A is unique and purpactin B is the first isogrisan compound derived from the single octaketide chain.

Acknowledgment. We thank Ms. N. Sato for running the ^{13}C NMR spectra. This work was supported in part by a grant from the Ministry of Education, Science and Culture of Japan.

Biosynthetic Origin of the Oxygen Atoms of Aquayamycin: Aspects for the Biosynthesis of the Urdamycin Family and for Aquayamycin-Containing Angucycline Antibiotics in General

Györgyi Udvarnoki, Thomas Henkel, Reinhard Machinek, and Jürgen Rohr*

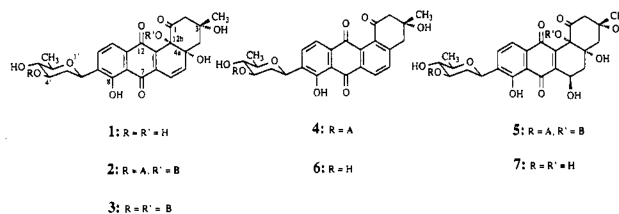
Institut für Organische Chemie der Universität, Tammannstr. 2, D-3400 Göttingen, Germany

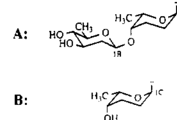
Received August 1, 1991 (Revised Manuscript Received November 5, 1991)

Experiments with $^{18}O_2$ and [1-^{13}C,$^{18}O_2$]acetate determined the biosynthetic origin of the oxygen atoms of aquayamycin (1), which is the aglycon moiety of the urdamycins A (2, kerriamycin B) and G (3, OM 4842) and the most frequently occurring aglycon among the angucyclines. The fact that 4a-O derives from acetate and 12b-O from molecular oxygen leads to the postulation of a biosynthetic scheme, in which a hypothetical tetracyclic compound 9 similar to SF-2315 A (10) and urdamycinone F (7) are key intermediates. Oxygen deficiency experiments with the urdamycin producer *Streptomyces fradiae* (strain Tü 2717) resulted in the production of only urdamycin B (4), when the oxygen level was reduced gradiently from 20% to 5% during the fermentation process. This result is in conformance with the postulated biosynthesis scheme; i.e., suppression of the 12b-monooxygenase leads to a shunt pathway with 4 being the final product.

Experiments with ^{18}O-containing precursors are more and more utilized in biosynthetic studies since the methods for the NMR analysis of the ^{18}O-labeled compounds were established.[1-5] The results afford additional insight into the biosynthetic pathways, possible intermediates, and mechanistic aspects. In addition, the inhibition of oxygenases with, e.g., P-450, inhibitors is being used as an approach to obtain such biosynthetic intermediates.[6]

Aquayamycin (1) is an antibiotic, a cytostatic agent, and an enzyme inhibitor (e.g., tyrosyl hydroxylase, dopamine β-hydroxylase, and tryptophan 5-monooxygenase).[7-9] It is the most frequently occurring aglycon of biologically active angucycline antibiotics, most of which are enzyme inhibitors or cytostatics.[10] More recently, representatives of this type of antibiotic were also found as platelet-aggregation inhibitors.[11-13] The urdamycin family[14-18] represents a unique collection of angucycline antibiotics with a high variety in the aglycon moieties. Two of the urdamycins, namely the main component, urdamycin A (2, identical with kerriamycin B[19]), as well as its immediate biosynthetic precursor urdamycin G (3, identical with OM-4842[11]), contain 1 as the aglycon moiety. Further biosynthetic interconversion reactions of 2 into other urdamycins (C, D, E, and H) are described elsewhere; however, the urdamycins B (4) and F (5) could not be placed in a biosynthesis scheme.[20-23]





(1) Vederas, J. C. *Can. J. Chem.* **1982**, *60*, 1637–1642.
(2) Vederas, J. C. *Nat. Prod. Rep.* **1987**, *4*, 277–337.
(3) Holker, J. S. E.; Kaneda, M.; Ramer, S. E.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1987**, 1099–1100.
(4) Hutchinson, C. R.; Sherman, M. M.; Vederas, J. C.; Nakashima, T. T. *J. Am. Chem. Soc.* **1981**, *103*, 5953–5956.
(5) Tsou, H. R.; Ahmed, Z. H.; Fiala, R. R.; Bullock, M. W.; Carter, G. T.; Goodman, J. J.; Borders, D. B. *J. Antibiot.* **1989**, *42*, 398–406.
(6) Oikawa, H.; Ichihara, A.; Sakamura, S. *J. Chem. Soc., Chem. Commun.* **1988**, 600–602.
(7) Sezaki, M.; Hara, T.; Ayukawa, S.; Takeuchi, T.; Okami, Y.; Hamada, M.; Nagatsu, T.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 91–97.
(8) Hayaishi, O.; Okuno, S.; Fujisawa, H. *Biochem. Biophys. Res. Commun.* **1970**, *39*, 643–650.
(9) Sezaki, M.; Kondo, S.; Maeda, K.; Umezawa, H.; Ohno, K. *Tetrahedron* **1970**, *26*, 5171–5190.
(10) Thiericke, R.; Rohr, J. *Nat. Prod. Rep.*, in press.
(11) Ōmura, S.; Nakagawa, A.; Fukamachi, N.; Miura, S.; Takahashi, Y.; Komiyama, K.; Kobayashi, B. *J. Antibiot.* **1988**, *41*, 812–813.
(12) Kawashima, A.; Yoshimura, Y.; Gotō, J.; Nakaike, S.; Mizutani, T.; Hanada, K.; Omura, S. *J. Antibiot.* **1988**, *41*, 1913–1914.
(13) Kawashima, A.; Kishimura, Y.; Tamai, M.; Hanada, K. *Chem. Pharm. Bull.* **1989**, *37*, 3429–3431.
(14) Drautz, H.; Zähner, H.; Rohr, J.; Zeeck, A. *J. Antibiot.* **1986**, *39*, 1657–1669.
(15) Rohr, J.; Zeeck, A. *J. Antibiot.* **1987**, *40*, 459–467.
(16) Rohr, J.; Zeeck, A.; Floss, H. G. *J. Antibiot.* **1988**, *41*, 126–129.
(17) Henkel, T.; Ciesiolka, T.; Rohr, J.; Zeeck, A. *J. Antibiot.* **1989**, *42*, 299–311.
(18) Rohr, J. *J. Antibiot.* **1989**, *42*, 1482–1488.
(19) Hayakawa, Y.; Adachi, K.; Iwakiri, T.; Imamura, K.; Furihata, K.; Seto, H.; Ōtake, N. *Agric. Biol. Chem.* **1987**, *51*, 1397–1405.
(20) Rohr, J.; Beale, J. M.; Floss, H. G. *J. Antibiot.* **1989**, *42*, 1151–1157.
(21) Rohr, J. *J. Chem. Soc., Chem. Commun.* **1989**, 492–493.
(22) Rohr, J. *J. Chem. Soc., Chem. Commun.* **1990**, 113–114.
(23) Rohr, J. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1051–1053.

0022-3263/92/1957-1274$03.00/0 © 1992 American Chemical Society