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

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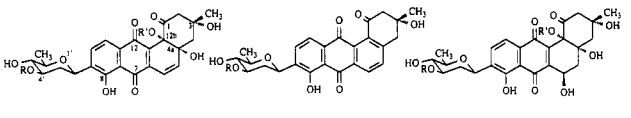
Received August 1, 1991 (Revised Manuscript Received November 5, 1991)

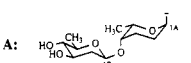
Experiments with $^{18}O_2$ and [1-^{13}C,$^{18}O_2$]acetate determined the biosynthetic origin of the oxygen atoms of aquayamycin (1), which is the aglycon moiety of the urdamycins A (2, kerriamycin B) and G (3, OM 4842) and the most frequently occurring aglycon among the angucyclines. The fact that 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]

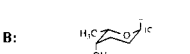
Aquayamycin (1) is an antibiotic, a cytostatic agent, and an enzyme inhibitor (e.g., tyrosyl hydroxylase, dopamine β-hydroxylase, and tryptophan 5-monooxygenase).[7-9] It is the most frequently occurring aglycon of biologically active angucycline antibiotics, most of which are enzyme inhibitors or cytostatics.[10] 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: R = R' = H
2: R = A, R' = B
3: R = R' = B
4: R = A
6: R = H
5: R = A, R' = B
7: R = R' = H



A:



B:

(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

Table I. ^{13}C NMR Data (CD_3OD, 125.7 MHz) of Urdamycin A (2) from the Feeding Experiments with [1-^{13}C,$^{18}O_2$]Acetate (A) and $^{18}O_2$ (B). δ/ppm Relative to Internal TMS

		upfield shifts (^{13}C–^{18}O)	
carbon	δ/ppm (^{13}C–^{16}O)	A Δδ/ppm	B Δδ/ppm
1	204.89	0.06	
3	72.37	0.03	
4a	82.80	0.03	
7	189.81	0.02	
8	158.74	0.02	
12	184.11		
12b	82.62		0.02
1C	95.64		0.03

In this paper are presented two experiments with ^{18}O-labeled precursors and an oxygenase inhibition experiment. The results provided some further insight into the biosynthesis of the important angucyclinone aquayamycin (1) as well as a completion of the biosynthetic pathway of the urdamycin family. The experiments are the first being reported regarding the biosynthetic origin of an angucycline group antibiotic and thus are of general interest for this group and other related tetracyclic decaketides.

Experimental Section

Fermentation with $^{18}O_2$. The fermentation of a 1-L culture of *Streptomyces fradiae* (strain Tü 2717) was carried out as described previously, but in a closed system fermentation apparatus (identical to the one of Vederas).[24] The fermentation was started with $^{16}O_2$; 20 h after the inoculation the fermentation atmosphere was replaced by an $^{18}O_2$-containing gas mixture (20% O_2, which was 50% enriched with ^{18}O, and 80% N_2). Within the following 28 h, 5 L of the ^{18}O-enriched oxygen was used. After 48 h, $^{16}O_2$ was used for the remaining 24 h. The 50% enriched $^{18}O_2$ was obtained from Cambridge Isotope Laboratories (CIL, Cambridge, MA).

Feeding with [1-^{13}C,$^{18}O_2$]Acetate. The [1-^{13}C,$^{18}O_2$]acetate was prepared from [1-^{13}C]acetate (99% ^{13}C) and $H_2{}^{18}O$ (97.3% ^{18}O);[25] both reagents were obtained from Isotec Inc. (Miamisburg, OH). The [1-^{13}C,$^{18}O_2$]acetate (99% ^{13}C, ca. 90% ^{18}O) (1 g) was fed in four portions to the growing culture of *S. fradiae* 24, 30, 36, and 42 h after inoculation.

Fermentation under Oxygen Deficiency. The fermentation was carried out in the same closed system apparatus as used for the $^{18}O_2$ experiment. The amount of oxygen was continuously decreased from 20% to 4% within 50 h and then kept at 4%.

Isolation Procedure. The isolation was performed as described before.[14,20] The yields were 60 mg of urdamycin A (2) and 50 mg of urdamycin B (4) in the $^{18}O_2$ experiment, 70 mg of 2 and 40 mg of 4 (feeding experiment with [1-^{13}C,$^{18}O_2$]acetate), and 120 mg of 4 (oxygen-deficiency experiment), respectively.

NMR Experiments. To detect the ^{18}O upfield shifts of the directly attached carbon atoms, the broadband decoupled ^{13}C NMR spectra of urdamycin A were recorded at 125.7 MHz. The ^{13}C-NMR spectrum of urdamycin A has been unequivocally assigned in context with the earlier biosynthetic studies.[20] The upfield shifts due to the ^{18}O were in the expected magnitude (Table I).

Results and Discussion

Scheme I shows the three possible alternatives of a biosynthetic pathway leading to aquayamycin: two include urdamycinone B (6), and the third suggests urdamycinone F (7) as a central intermediate. The conversion of 6 into 1 requires either a dioxygenase for the direct cis introduction of the two adjacent angular oxygens at C-4a and C-12b or, alternatively, one monooxygenase, which would

Scheme I. Alternative Biosynthetic Pathways to Aquayamycin (1) ([O] = Oxygen from a Monooxygenase, O_2 = Oxygen from a Dioxygenase)

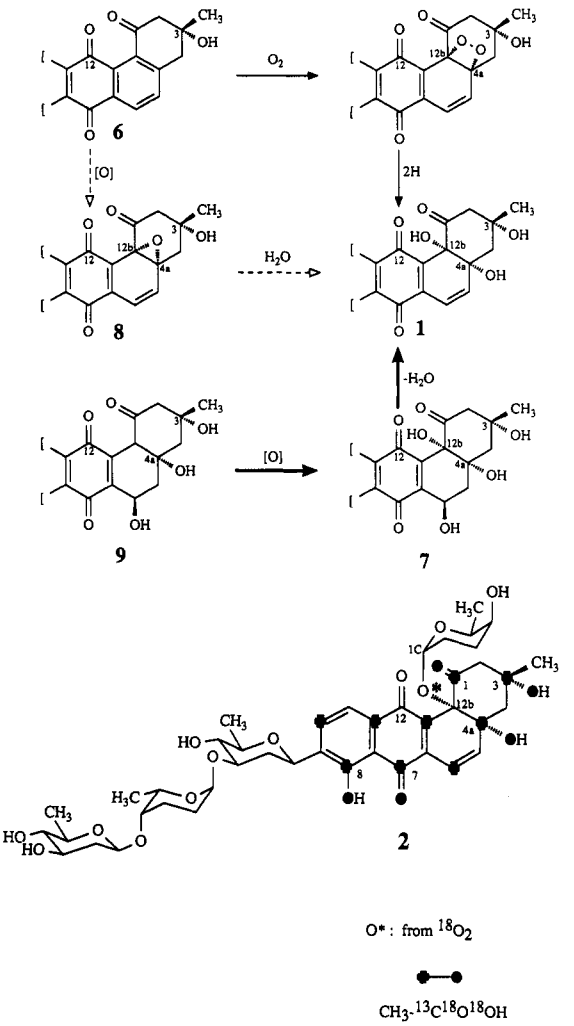
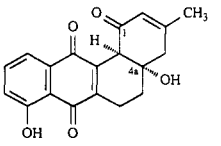


Figure 1. ^{18}O incorporation experiments on urdamycin A (2).

lead to the epoxide intermediate 8. The latter could be opened to 1 by an enzymatic cis attack of water. The third alternative retains the 4a-oxygen from the polyketide biosynthesis; i.e., 4a-O derives from acetate, and the other angular oxygen at C-12b has to be introduced by a monooxygenase. This alternative postulates an intermediate 9, which is similar to the recently described SF-2315 A[26] (10). Compound 9 can be further converted into urdamycinone F (7) before a dehydration leads to 1.

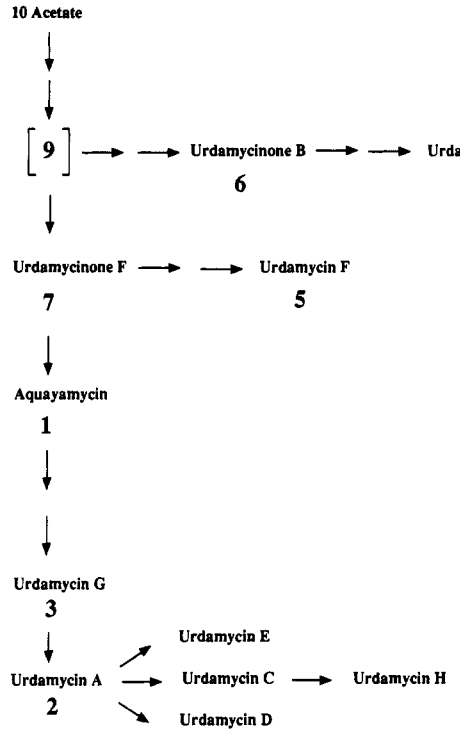


SF-2315 A (10, Stereochemistry is relative)

The three alternatives could be distinguished via the ^{18}O-labeling experiments. As depicted in Figure 1, the oxygens at C-1, C-3, C-4a, C-7, and C-8 derive from acetate and the one at C-12b from molecular oxygen (see also Table I). The oxygen at C-12 should also derive from molecular oxygen but may be introduced via a monooxygenase during an earlier stage of the biosynthesis

(24) Vederas, J. C. In *Mycotoxins and Phytotoxins*; Steyn, P. S., Vleggaar, R., Eds.; Elsevier Science: Amsterdam, 1986; pp 97–108.
(25) Boyer, P. D.; Koeppe, O. J., Luchsinger, W. W. *J. Am. Chem. Soc.* **1956**, *78*, 356–357.
(26) Sasaki, T.; Gomi, S.; Sezaki, M.; Takeuchi, Y.; Kodama, Y.; Kawamura, K. *J. Antibiot.* **1988**, *41*, 843–848.

Scheme II. Biosynthetic Pathway of the Urdamycins



(within the first 20 h of the fermentation) before the $^{18}O_2$ level—in our experiment—was high enough to label this position.

The results are only in agreement with the third alternative of Scheme I and thus allow a completion of the biosynthesis scheme of the urdamycins as depicted in Scheme II. Whether **7** is indeed an intermediate cannot be proven further due to the minimal production of urdamycin F (**5**) by *Streptomyces fradiae* (average amount: 0.05 mg/L).[14]

The experiment in which the molecular oxygen was decreased to 4% yielded only urdamycin B (**4**). This is also in accordance with Scheme II, if the 12b-monooxygenase is supressed due to the oxygen deficiency. Dehydration and glycosylation reactions represent the shunt pathway leading to **4**. A local oxygen deficiency during the fermentation process may be in general the reason for the urdamycin B (**4**) production.

There are some related polyketide metabolites having angular oxygens. For the tetracenomycins[27-30] and the tetracyclines[31,32] a biosynthetic pathway via an aromatic intermediate is favored or proven, respectively. However, an alternative pathway of the tetracenomycins with one angular oxygen deriving from acetate was also discussed.[33] The only proven example having an angular oxygen deriving from acetate is viridicatumtoxin.[34]

Aquayamycin (**1**) is now the second such example, and the first angucyclinone, in which the biosynthetic origin of the angular oxygens was investigated. Even though a novel type of pathway for such an angucyclinone was recently described,[35] the biosynthetic pathway postulations outlined in the Schemes I (alternative with the bold arrows) and II (parts) may have general meaning for most of the biosyntheses of angucyclinones with angular oxygens.[10]

Acknowledgment. The work was financially supported by the Deutsche Forschungsgemeinschaft, the Fonds der Chemischen Industrie (Grant No. 635050) and the NATO (Grant No. 0368/88), and by the Hoechst AG (Frankfurt/Main, Germany) with chemicals. Prof. Dr. John C. Vederas (University of Alberta, Edmonton, Alberta, Canada) is thanked for his advice in context with the construction of the closed system fermentation apparatus and the $^{18}O_2$ experiment.

(27) Motamedi, H.; Wendt-Pienkowski, E.; Hutchinson, C. R. *J. Bacteriol.* **1986**, *167*, 575–580.
(28) Yue, S.; Motamedi, H.; Wendt-Pienkowski, E.; Hutchinson, C. R. *J. Bacteriol.* **1986**, *167*, 581–586.
(29) Rohr, J.; Eick, S.; Zeeck, A.; Reuschenbach, P.; Zähner, H.; Fiedler, H. P. *J. Antibiot.* **1988**, *41*, 1066–1073.
(30) Hutchinson, C. R. Presented at the 22nd National Medicinal Chemistry Symposium, Austin, TX, July 29–Aug 2, 1989.
(31) Thomas, R.; Williams, D. J. *J. Chem. Soc., Chem. Commun.* **1985**, 802–803.
(32) Mitscher, L. A.; Swayze, J. K.; Högberg, T.; Khanna, I.; Raghav Rao, G. S. *J. Antibiot.* **1983**, *36*, 1405–1407.
(33) Anderson, M. G.; Khoo, C. L.-Y.; Rickards, R. W. *J. Antibiot.* **1989**, *42*, 641–643.
(34) De Jesus, A. E.; Hull, W. E.; Steyn, P. S.; Van Heerden, F. R., Vleggaar, R. *J. Chem. Soc., Chem. Commun.* **1982**, 902–904.
(35) Gould, S. J.; Halley, K. A. *J. Am. Chem. Soc.* **1991**, *113*, 5092–5903.