Scheme II. Model for the Prediction of Absolute Configuration of Sulfoxides in Asymmetric Oxidation of Sulfides

Chemicals. CH_2Cl_2 was purified by treatment with basic alumina, distilled over calcium hydride, and stored under nitrogen. TBHP solutions in CH_2Cl_2 were prepared according to ref 15 and stored over molecular sieves under nitrogen. TBHP solution in toluene was prepared according to ref 15c. Silica gel (Merck, Kieselgel 60) was used for column chromatography. (+)- and (−)-diethyl tartrate ((+)- or (−)-DET) was purchased from the Aldrich Co. Ti(O-*i*-Pr)$_4$ and Eu(hfc)$_3$ were obtained from Fluka. These reagents were used as such, and both were stored under anhydrous conditions. Sulfides were prepared according to the literature by alkylation of the corresponding thiols.

Asymmetric Oxidation. The procedure for sulfide oxidation is exemplified in the case of methyl *p*-tolyl sulfide. Ti(O-*i*-Pr)$_4$ (1.49 mL, 5 mmol) and (*R*,*R*)-DET (1.71 mL, 10 mmol) are dissolved at room temperature in 50 mL of CH_2Cl_2 under nitrogen. H_2O (5 mmol) is introduced through a spectrum via a microsyrynge. Stirring is maintained until the yellow solution becomes homogeneous (15–20 min) and sulfide (0.7 g, 5 mmol) is added. The solution is cooled to −20 °C or at the desired reaction temperature, and 5.5 mmol of a TBHP solution in CH_2Cl_2 (≃2 M) or toluene (≃3.6 M) are then introduced. After reaction, water (10 mol equiv) is added dropwise by a microsyringe to the solution at −20 °C. A strong stirring was maintained for 1 h at −20 °C and for one additional hour at room temperature. The white gel is filtered (a small amount of alumina added to the solution helps the filtration) and thoroughly washed with CH_2Cl_2. The filtrate is kept in the presence of NaOH (5%) and brine for 1 h and then separated. The organic phase is dried over Na_2SO_4 and concentrated to give the crude product, which does not contain sulfone. Chromatography (AcOEt, cyclohexane 1:1) on silica gel gives 0.70 g (90%) of methyl *p*-tolyl sulfoxide: $[\alpha]^{20}_D$ +131° (*c* 2, acetone) (lit.[6] data for enantiomerically pure (*R*)-sample: $[\alpha]^{20}_D$ +145.5° (acetone)). Enantiomeric excess was calculated to be 90% ee and confirmed by ^{1}H NMR (400 MHz) with a new chiral shift reagent.[27] The present procedure is an improvement of the one that was previously reported.[17]

For benzylic sulfoxides or sulfoxides bearing ester functions a modification of the workup is necessary. After the filtration the solution was washed only with brine. The experiments of Figure 3 were performed with use of 2 equiv of TBHP with respect to Ti(O-*i*-Pr)$_4$.

All the sulfoxides were isolated and characterized by the usual spectral methods. The careful drying of the sulfoxides is necessary before the measurement of optical rotation. The ee measurements were performed on freshly obtained samples. It is known that some sulfoxides can racemize slowly with time (e.g., for benzylic sulfoxides). We observed in the specific case of the methyl *p*-nitrophenyl sulfoxide a slow racemization with time. The oxidation on 40-mmol scale was done with 8.12 g of *p*-bromophenyl methyl sulfide at −21 °C for 24 h, following the same conditions as described above. The corresponding sulfoxide (6.66 g) was obtained in 76% yield and 80% optical purity.

Acknowledgment. We thank CNRS for its financial support. Two of us (P.P. and M.N.D.) acknowledge DGRST and IFP fellowships, respectively. We thank Professor K. B. Sharpless for useful discussions and communication of unpublished data.

Registry No. 1, 623-13-2; (*R*)-**2**, 1519-39-7; $C_6H_5SCH_3$, 100-68-5; (*R*)-$C_6H_5S(O)CH_3$, 4850-71-9; *p*-$BrC_6H_4SCH_3$, 104-95-0; (*R*)-*p*-$BrC_6H_4S(O)CH_3$, 28227-62-5; *p*-$ClC_6H_4SCH_3$, 123-09-1; (*R*)-*p*-$ClC_6H_4S(O)CH_3$, 28227-63-6; *p*-$(MeO_2C)C_6H_4SCH_3$, 3795-79-7; (*R*)-*p*-$(MeO_2C)C_6H_4S(O)CH_3$, 93303-91-4; *o*-$(MeO_2C)C_6H_4SCH_3$, 3704-28-7; (*R*)-*o*-$(MeO_2C)C_6H_4S(O)CH_3$, 4850-73-1; *p*-$MeOC_6H_4SCH_3$, 1879-16-9; (*R*)-*p*-$MeOC_6H_4S(O)CH_3$, 93381-75-0; (*S*)-*p*-$MeOC_6H_4S(O)CH_3$, 93381-76-1; *o*-$MeOC_6H_4SCH_3$, 2388-73-0; (*R*)-*o*-$MeOC_6H_4S(O)CH_3$, 84413-74-1; *p*-$O_2NC_6H_4SCH_3$, 701-57-5; (*R*)-*p*-$O_2NC_6H_4S(O)CH_3$, 93222-06-1; *p*-$HOC_6H_4SCH_3$, 1073-72-9; (*R*)-*p*-$HOC_6H_4S(O)CH_3$, 93183-65-4; *p*-$(HOCH_2)C_6H_4SCH_3$, 3446-90-0; (*R*)-*p*-$(HOCH_2)C_6H_4S(O)CH_3$, 93183-64-3; *p*-$CH_3C_6H_4SCH_2CH_3$, 622-63-9; (*R*)-*p*-$CH_3C_6H_4S(O)CH_2CH_3$, 1519-40-0; *p*-$CH_3C_6H_4S(CH_2)_3CH_3$, 21784-96-3; (*R*)-*p*-$CH_3C_6H_4S(O)(CH_2)_3CH_3$, 20288-49-7; *p*-$CH_3C_6H_4SCH(CH_3)_2$, 14905-81-8; (*R*)-*p*-$CH_3C_6H_4S(O)CH(CH_3)_2$, 1517-74-4; *p*-$CH_3C_6H_4SCH_2C_6H_5$, 5023-60-9; (*R*)-*p*-$CH_3C_6H_4S(O)CH_2C_6H_5$, 4820-07-9; $CH_3(CH_2)_7SCH_3$, 3698-95-1; (−)-$CH_3(CH_2)_7S(O)CH_3$, 93183-63-2; $C_6H_5(CH_2)_3SCH_3$, 87231-07-0; (−)-$C_6H_5(CH_2)_3S(O)CH_3$, 93183-67-6; $(CH_3)_3CSCH_3$, 6163-64-0; (*R*)-$(CH_3)_3CS(O)CH_3$, 20580-80-7; $C_6H_5CH_2SCH_3$, 766-92-7; (*S*)-$C_6H_5CH_2S(O)CH_3$, 14090-81-4; *p*-$MeOC_6H_4CH_2SCH_3$, 5925-86-0; (−)-*p*-$MeOC_6H_4CH_2S(O)CH_3$, 93303-92-5; (±)-$CH_3CH_2CH(CH_3)SCH_3$, 64693-06-7; $CH_3CH_2CH(CH_3)S(O)CH_3$ (isomer 1), 93527-27-6; $CH_3CH_2CH(CH_3)S(O)CH_3$ (isomer 2), 93527-28-7; 2-(methylthio)-naphthalene, 7433-79-6; (*R*)-2-(methylsulfinyl)naphthalene, 18690-03-4; 2-(methylthio)pyridine, 18438-38-5; (*R*)-2-(methylsulfinyl)pyridine, 93183-62-1; 4-(methylthio)pyridine, 22581-72-2; (*R*)-2-(methylsulfinyl)pyridine, 93381-77-2; 2-(propylthio)naphthalene, 75052-54-9; (*R*)-2-(propylsulfinyl)naphthalene, 93381-78-3; (methylthio)cyclohexane, 7133-37-1; (−)-(methylsulfinyl)cyclohexane, 93183-66-5.

(26) Cooke, R. S.; Hammond, G. S. *J. Am. Chem. Soc.* **1970**, *92*, 2739.
(27) Deshmukh, M.; Dunach, E.; Jugé S.; Kagan, H. B. *Tetrahedron Lett.* **1984**, 3467.
(28) **Note Added in Proof**: A paper recently appeared (Di Furia, F.; Modena, G.; Seraglia, R. *Synthesis*, **1984**, 325) which describes asymmetric oxidation of four aryl alkyl sulfides (ee from 14% till 88%) by *t*-BuOOH and Ti(O-*i*-Pr)$_4$/(DET) (1:4).
The structure of the Sharpless reagent has just been established by X-ray cristallography. This analogue has a structure similar to that shown in Figure 3 where each tartramide acts as a bidentate diolate to a titanium and there is a double bridging between the two titaniums through the tartramide alkoxy groups (K. B. Sharpless, private communication).

Acutiphycin and 20,21-Didehydroacutiphycin, New Antineoplastic Agents from the Cyanophyte *Oscillatoria acutissima*

Joseph J. Barchi, Jr., Richard E. Moore,* and Gregory M. L. Patterson

Contribution from the Department of Chemistry, University of Hawaii, Honolulu, Hawaii 96822.
Received February 13, 1984

Abstract: The lipophilic extract of the freshwater blue-green alga *Oscillatoria acutissima* contains two novel macrolides, acutiphycin (**1**) and 20,21-didehydroacutiphycin (**2**), which exhibit cytotoxicity and antineoplastic activity. The structures and absolute stereochemistries of **1** and **2** have been determined by spectral studies and chemical degradation.

Blue-green algae, in particular those belonging to the Oscillatoriaceae, are potential sources of new antineoplastic agents.[1] The crude extract of *Oscillatoria acutissima*, for example, shows cytotoxicity against KB and NIH/3T3 cells and significant an-

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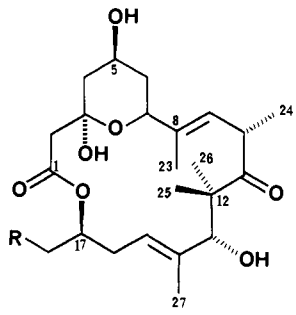
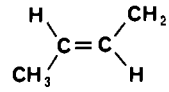
tineoplastic activity in vivo against murine Lewis lung carcinoma. We report here the isolation and structure elucidation of two novel macrolides, acutiphycin (**1**) and 20,21-didehydroacutiphycin (**2**),



1 R = $CH_3CH_2CH_2CH_2$

2 R = 

which account for the cytotoxicity[2a] and antitumor activity[2b] of this cyanophyte.

O. acutissima was found in a freshwater pond in Manoa Valley, Oahu and grown in mass culture in the laboratory. The dichloromethane extract of the freeze-dried alga was subjected to gel filtration on Sephadex LH-20 with 1:1 dichloromethane/2-propanol followed by chromatography on Florisil with dichloromethane and HPLC on Partisil with 35% ethyl acetate and 1% ethanol in hexane to give acutiphycin (**1**) and 20,21-didehydroacutiphycin (**2**), both in 0.15% yield. After recrystallization from hexane–chloroform, **1** was obtained as a white solid with melting point 155–156 °C and $[\alpha]_D$ +107°; the melting point and $[\alpha]_D$ of **2** were virtually the same, 153–154 °C and +110°, respectively. Both compounds exhibited similar infrared spectra with intense carbonyl bands at 1715 cm^{-1}; compound **2**, however, showed an additional band at 960 cm^{-1} for a trans-1,2-disubstituted carbon–carbon double bond. In the positive ion fast atom bombardment (FAB) mass spectrum, **1** showed a $[MH-H_2O]^+$ ion at m/z 463 whereas **2** gave a MH^+ ion at m/z 479. The negative ion FAB spectrum of **1**, however, showed a $[M-H]^-$ ion at m/z 479. The molecular weights of **1** and **2** were therefore 480 and 478, respectively.

The ^1H and ^{13}C spectral data indicated that acutiphycin possessed six methyl groups, eight methylenes, seven methines, six quaternary carbons, and three exchangeable protons (Table I). Since the molecular weight of **1** was 480, we concluded that its elemental composition was probably $C_{27}H_{44}O_7$.

Three of the oxygens were in hydroxyl groups. Two of these OHs were secondary alcohol groups, since acutiphycin formed a diacetate which showed in its ^1H NMR spectrum two acetate signals and two methine signals that were shifted about 1 ppm downfield from those of **1**. The third OH appeared to be in a hemiketal functionality (which now accounted for a fourth oxygen) since this OH was connected to a quaternary carbon which had to have another oxygen on it to explain its carbon-13 chemical shift (δ 96.2). Since the proton signal for the hemiketal OH appeared as a doublet (J = 2.5 Hz), we suspected that we were looking at W-coupling and that the OH was trans to a proton on one of the carbons attached to the hemiketal carbon.[3] The

Table I. NMR Data for Acutiphycin (**1**) and 20,21-Didehydroacutiphycin (**2**)

^{13}C δ[a-c]		^1H δ[c,d]	^1H–^1H NOE
124.4 s	11		
170.9 s	1		
136.1 s	8[e]		
134.9 s	14[e]		
128.1 d	9	5.147 dq	
123.6 d	15	5.202 m	
96.2 s	3		
77.3 d	13	4.507 br s	
74.2 d	7	4.164 dd	9
73.8 d	17	4.893 dddd	
62.6 d	5	3.996 tt	6(e), 4(e)
53.0 s	12		
49.9 t	2	2.394 d	4(a)[f]
		2.278 d	
43.5 t	4(e)[f]	1.899 ddd	
	4(a)[f]	0.994 br dt	
41.3 d	10	3.856 dq	23, 13, 26
38.2 t	6(e)	1.537 ddt	
	6(a)	1.298 m	
34.4 t	16α	2.186 ddd	
	16β	1.97 m	
31.8 t (130.2 d)	20	1.16 m (5.37 m)	
31.0 t (31.6 t)	18	1.16 m (1.97 m)	
24.4 t (27.9 t)	19	1.16 m	
23.4 q	26	1.092 s	
22.0 t (124.9 d)	21	1.16 m (5.37 m)	
20.6 q	25	0.841 s	27, 13, 9
16.7 q	24	1.026 d	
13.9 q (17.7 q)	22	0.802 t (1.63 dm)	
12.9 q	27	1.431 t	16α
11.9 q	23	1.616 d	13, 10, 6(a)
	OH on C-3	5.362 d	

$J_{H,H}$ (Hz) for **1**:[g] 2.2′ = −14.6; 4.4′ = −11.8; 4(a),5 = 6(a),5 = 10.9; 4(e),5 = 6(e),5 = 4.6; 4(e),6(e) (W coupling) = 1.8; 6,6′ = −14.0; 6(a),7 = 11.7; 6(e),7 = 2.2; 9,10 = 11.0; 9,23 = 1.4; 10,24 = 6.8; 15,16β = 3.1, 15,16α = 10.6; 15,27 = 1.3; 16α,16β = −15.2; 16α,17 = 2.1; 16β,17 = 11.4; 16β,27 = 1.3; 17,18 = 4.5; 17,18′ = 8.1; 21,22 = 6.6. $J_{OH,H}$ (Hz)[h] for **1**: 3,4(a) = 2.5; 5.5 = 4.5; 13,13 = 4.1.

[a] 75 MHz; in Me_2SO-d_6; solvent peak used as internal standard = 39.5. [b] ^1H–^{13}C connectivities determined by using the phase cycled 16 step heteronuclear chemical shift correlation map (CSCM) experiment. [c] Chemical shifts in parentheses for compound **2**. [d] 300 MHz; in 1:1 $CDCl_3$/benzene-d_6, residual benzene-d_5 peak used as internal standard = 7.15. [e] Assignments may be reversed. [f] Both protons on C-2 were irradiated simultaneously. [g] Coupling constants for **2** are essentially identical except $J_{20,21}$ = 15.2. [h] In Me_2SO-d_6, solvent peak used as internal standard = 2.52: δ 5.414 (d, OH on C-3), 5.070 (d, OH on C-13), 4.733 (d, OH on C-5). [i] (e) = equatorial and (a) = axial.

remaining three oxygens were in a ketone group and an ester functionality since there were signals at δ 214.4 and 170.9, respectively, in the ^{13}C NMR spectrum. The ester oxygen was apparently attached to a methine bearing two methylenes.

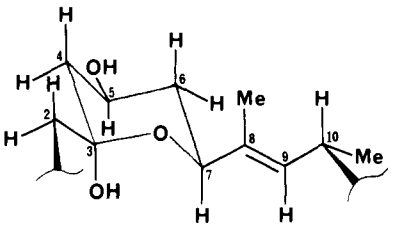
Three of the six quaternary carbons were now accounted for with the hemiketal, ketone, and ester groups. Two other quaternary carbons were located in two isolated trisubstituted ethenyl groups, both of which bore methyl groups and were *E* since the ^{13}C signals for the olefinic methyl groups were at high field, δ 11.9 and 12.9 respectively. Decoupling experiments established that one of the olefinic methines was attached to a methylene which in turn was connected to the methine bearing the ester oxygen. The sixth quaternary carbon was a saturated carbon which had to have two methyl groups on it since there were two three-proton singlets at δ 0.841 and 1.092.

The hemiketal group and one of the secondary alcohol groups were present in partial structure a on the basis of detailed NMR

(1) (a) Mynderse, J. S.; Moore, R. E.; Kashiwagi, M.; Norton, T. R. *Science (Washington, D.C.)* **1977**, *196*, 538. (b) Kashiwagi, M.; Mynderse, J. S.; Moore, R. E.; Norton, T. R. *J. Pharm. Sci.* **1980**, *69*, 735. (c) Moore, R. E. *Pure Appl. Chem.* **1982**, *54*, 1919.

(2) (a) For cytotoxicity against KB and NIH/3T3 cells, ED_{50} < 1 μg/mL for both **1** and **2**. (b) For activity against murine Lewis lung carcinoma, T/C = >150 at 50 mg/kg.

(3) A similar signal is seen in the ^1H NMR spectrum of aplysiatoxin where the one for the hemiketal OH on C-3 is a doublet (J = 2.0 Hz), showing W-coupling to an axial proton on C-4. Moore, R. E.; Blackman, A. J.; Cheuk, C. E.; Mynderse, J. S.; Matsumoto, G. K.; Clardy, J.; Woodard, R. W. Craig, J. C. *J. Org. Chem.* **1984**, *49*, 2484.

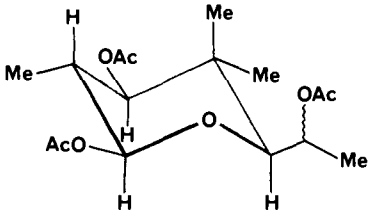


a

analysis. The chemical shifts and coupling constants of the protons on C-4, C-5, C-6, and C-7 and the W couplings between the axial OH on C-3 and the axial H on C-4 and between the equatorial protons on C-4 and C-6 indicated that C-3, C-4, C-5, C-6, and C-7 were in a tetrahydropyran ring. An isolated methylene group was attached equatorially to C-3 since one of the C-2 protons showed a positive NOE to the axial H on C-4. Furthermore, the quaternary carbon of one of the trisubstituted ethenyl groups was connected equatorially to C-7 (as shown in a) since strong positive NOEs were observed between the H on C-7 and the olefinic H on C-9 and between the 8-Me protons and the axial H on C-6.

Five of the six methyl carbons had now been accounted for. The remaining methyl group appeared to be in an *n*-alkyl substituent. An inventory of C, H, and O in the partial structures generated from the arguments above indicated that the *n*-alkyl group was a *n*-pentyl group which most likely was attached to the methine bearing the ester oxygen. The carbon-13 chemical shifts for this alkyl side chain matched fairly well with calculated values, viz., 13.9, 22.7, 32.7, and 26.0 for C-22, C-21, C-20, and C-19, respectively.[4]

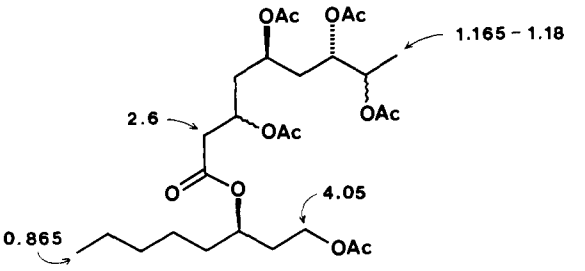
Chemical degradation allowed us to assemble a gross structure (**1**) for acutiphycin. Ozonolysis of **1** in dichloromethane at −78 °C and reduction of the resulting diozonide with a limited amount of sodium borohydride in ethanol produced a mixture of alcohols. Acetylation and HPLC on silica gave **3** as one of the major



3

products. Acid hydrolysis (0.5 N hCl, reflux 1 h) of the mixture of products containing the *n*-pentyl group (**4**), followed by acetylation of the hydrolyzate and HPLC, yielded **5** and **6**.

The structure of **3** was deduced by ^{1}H NMR analysis. The protons on C-1, C-2, and C-3 all appeared to be axial protons on a tetrahydropyran ring since large vicinal couplings were observed between the C-1 and C-2 hydrogens and between the C-2 and C-3 hydrogens. Furthermore, the proton on C-5 appeared to be in an axial environment, since appreciable positive NOEs were induced in the C-1 and C-3 proton signals when the C-5 proton was irradiated and conversely, in the C-5 proton signal when either the C-1 or C-3 proton was irradiated. We were surprised to find that **3** was a major product of this degradation. Obviously C-1 and C-6 in **3** had to have arisen from C-9 and C-14, respectively, in acutiphycin, but we did not expect C-9 in **1** to remain as a masked aldehyde functionality (in **7**) after borohydride reduction



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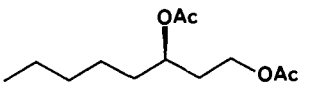

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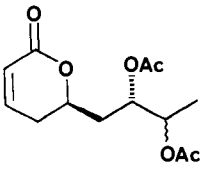

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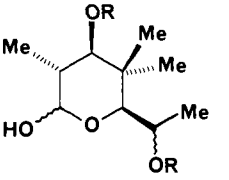
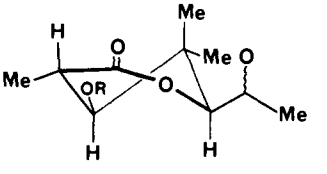


7 R = H

8 R = Ac

of acutiphycin diozonide. The keto group at C-11 in acutiphycin as well as the keto group generated at C-14 after ozonolysis, however, were reduced, but with a high degree of stereospecificity, to give secondary alcohol groups at C-3 and C-6 in **7**. The stereochemistry of C-6 in **3** and **7**, however, was not established.

The absolute stereochemistry of **3** was determined by a circular dichroism (CD) study of δ-lactone **9**, which was obtained by mild



9 R = Ac

10 R = H

acid hydrolysis of **3** to a 1:1 mixture of anomeric hemiacetals (**8**) followed by oxidation with manganese dioxide. The CD curve of **9** showed a negative Cotton effect at $[\theta]_{227}$ −2860, as did the corresponding diol **10**, $[\theta]_{224}$ −2570, from acid hydrolysis of **9**, indicating that the δ-lactone ring had the absolute stereochemistry shown in **9**.[5,6] The configurations at C-2 and C-5 on **9** were

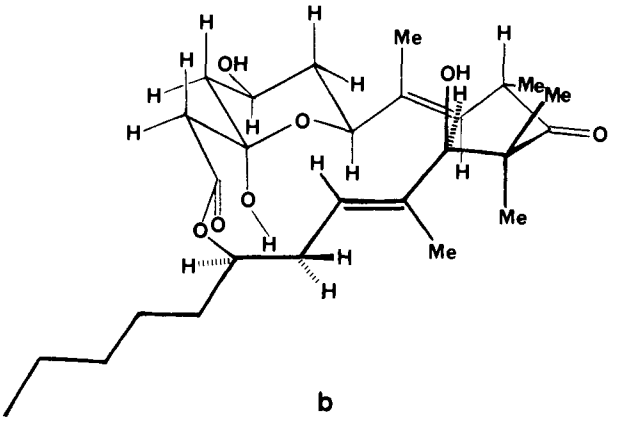
(4) Levy, G. C.; Nelson, G. L. Carbon-13 Nuclear Magnetic Resonance for Organic Chemists. Wiley-Interscience, New York, 1972.

(5) (a) Korver, O. *Tetrahedron* **1970**, *26*, 2391. (b) Cardellina, J. H., II; Moore, R. E.; Arnold, E. V.; Clardy, J. *J. Org. Chem.* **1979**, *44*, 4039. (c) Moore, R. E.; Bartolini, G.; Barchi, J.; Bothner-By, A. A.; Dadok, J.; Ford, J. *J. Am. Chem. Soc.* **1982**, *104*, 3776.

therefore *R* and *S*, respectively, and this meant that C-10 and C-13 in acutiphycin had to be *S* and *R*, respectively.

The absolute stereochemistry of **5**, $[\alpha]_D$ −18°, was solved by synthesis. (*Z*)-2-Octen-1-ol was converted by the Sharpless method to 2(*R*),3(*S*)-epoxy-1-octanol. The epoxide was then reduced with lithium aluminum hydride to a mixture of 1,2- and 1,3-diols. After destruction of the 1,2-diol in the mixture with periodate, (*S*)-1,3-octanediol, $[\alpha]_D$ + 15.2°, was obtained. The configuration of C-3 in **5** was therefore *R* which meant that C-17 in acutiphycin was *R*.

Since C-10 in **1** was *S*, the absolute stereochemistry of the tetrahydropyran ring had to be 3*R*,5*S*,7*S*. The coupling between the C-9 and C-10 protons was 11.0 Hz, indicating that these two protons were anti to each other. The methyl group on C-8, the axial proton on C-6, and the C-10 H had to be eclipsed in the preferred conformation of **1**, since strong positive NOEs were seen in the axial C-6 proton and C-10 proton signals upon irradiation of the methyl group on C-8. The C-7 and C-9 protons also had to be eclipsed since a strong positive NOE was seen in the C-9 proton signal when the C-7 proton was irradiated (see *b*).



b

Additional NOE studies supported the proposed absolute stereochemistry of the tetrahydropyran ring and suggested b as the most probable conformation of the acutiphycin molecule in the solution state. Irradiation of the methyl group on C-8 produced a strong positive NOE on the C-13 proton, as did irradiation of the C-10 proton. Examination of a Dreiding model showed that the C-13 proton was oriented toward and close to both the 8-Me group and the C-10 proton. In the conformation depicted in b, one of the methyl groups on C-12 was close to the C-10 proton and the hydroxyl group on C-13 eclipsed the C-10 proton. This was concluded from the following data. Irradiation of the C-10 proton produced a significant NOE in the methyl group at δ 1.092. The C-10 proton signal showed a 0.5 ppm upfield shift when the OH group on C-13 in **1** was acetylated (from δ 3.928 for **1** to δ 3.450 for the diacetate in $CDCl_3$); none of the other proton signals changed more than ±0.1 ppm.

The CD spectrum of **6** showed a positive Cotton effect at 257 nm, [θ] +1150, indicating that C-5 in **6** was *R* and confirming the proposed absolute stereochemistry of the tetrahydropyran ring. (+)-Parasarbic acid, which has the same absolute configuration at C-5, shows $[\theta]_{262}$ +7430.[7,8]

The structure determination of 20,21-didehydroacutiphycin (**2**) was straightforward. Its ^1H and ^{13}C NMR spectra were essentially identical with those of **1**, except for the signals for the five-carbon side chain. The extra double bond, trans according to the IR spectrum, was located between C-20 and C-21. The coupling constant (*J* = 15.2 Hz) between the protons on C-20 and C-21 also indicated that the extra double bond was trans.

Experimental Section

Culture Conditions. *Oscillatoria acutissima* Kufferath, strain number B-1, was collected from a fresh, running water source in Manoa Valley on the island of Oahu, Hawaii. Clonal cultures were prepared by repeated subculture on solidified media.[9] The alga was cultured in 25-L bottles containing an inorganic medium, designated FM, in which the major salt concentrations corresponded to those in Fitzgerald's medium,[10] viz., sodium nitrate, 1.5 mM; dipotassium hydrogen phosphate, 0.06 mM; magnesium sulfate, 0.10 mM; calcium chloride, 0.25 mM; sodium carbonate, 0.19 mM; sodium metasilicate, 0.20 mM; MOPS (3-morpholinopropanesulfonic acid), 5.0 mM; minor elements solution,[11] 1 mL/L; trace elements solutions B_7 and C_{13},[10] 0.12 mL/L each. The pH of the medium was adjusted to 7.0 before sterilization. Cultures were illuminated continuously at an incident intensity of 330 μEinsteins m^{-2} s^{-1} from banks of cool-white fluorescent tubes and vigorously aerated with approximately 1% carbon dioxide in air at an incubation temperature of 24 ± 1 °C. After 3–4 weeks the alga was harvested by centrifugation. Yields of lyophilized cells typically ranged from 0.4 to 0.5 g/L of culture.

Isolation. Digestion of 275 g of freeze-dried *O. acutissima* with dichloromethane (2 L) yielded 9.2 g of crude extract. Gel filtration on a 114 × 8.5 cm column of Sephadex LH-20 with 1:1 dichloromethane/2-propanol afforded, after 3 L of eluant had passed, a fraction (482 mL) containing 3.04 g of material. Chromatography on Florisil (37 g, 31 cm × 2.5 cm column) using dichloromethane as the eluant gave 1.5 g of a crude mixture of **1** and **2**. HPLC on silica (Whatman Partisil M-9 column; 15-mg portions; 35% EtOAc, 1% EtOH in hexane) gave 300 mg of acutiphycin (**1**) and 320 mg of 20,21-didehydroacutiphycin (**2**). Crystallization from hexane–chloroform gave pure **1**, mp 155–156 °C and $[\alpha]_D^{25}$ +107° (CH_2Cl_2, *c* 1.0), and **2**, mp 153–154 °C and $[\alpha]_D^{25}$ +110° (MeOH, *c* 1.0).

Acetylation of acutiphycin. A solution of 100 μL of acetic anhydride, 100 mL of triethylamine, and a trace of 4-(dimethylamino)pyridine[13] in 1 mL of dichloromethane was cooled to 0 °C under argon and a solution of **1** in 1 mL of CH_2Cl_2 was added. The reaction was monitored by thin-layer chromatography. Two new higher R_f spots appeared (0.65 and 0.73, 1:1 EtOAc/hexane) initially, but after 2.5 h TLC analysis showed only one spot at R_f 0.73. Water was added, and the organic layer was washed successively with 5% HCl, saturated sodium bicarbonate solution, water, and saturated sodium chloride solution, dried over sodium sulfate, and evaporated to give 6.4 mg of an oil. HPLC purification on a silica column (30% EtOAc, 0.5% EtOH in hexane) afforded essentially one product which proved to be acutiphycin 5,13-diacetate by NMR analysis: ^1H NMR ($CDCl_3$) δ 2.09 (s, OAc), 2.023 (s, OAc), 5.270 (tt, H on C-5) 5.504 (br s, H on C-13). The ^1H NMR spectrum of acutiphycin in $CDCl_3$ showed signals at δ 4.261 and 4.627 for the C-5 and C-13 protons, respectively.

Ozonolysis of 1. A solution of 21 mg of acutiphycin in 4 mL of dichloromethane was cooled to −78 °C and treated with ozone. When all of the starting material had reacted (TLC analysis) nitrogen was bubbled through the solution for 10 min to expel excess ozone. The mixture was allowed to warm to 0 °C and a solution of sodium borohydride (23 mg) in 0.5 mL EtOH was added dropwise. This mixture was stirred for 45 min and the excess hydride was decomposed with 2 mL of 1 M potassium phosphate buffer (pH 7). The organic solvents were evaporated, and the residue was dried in vacuo. The solid was treated with acetic anhydride/pyridine (1:1) overnight at room temperature. After evaporation of the reagents and standard workup, 28 mg of an oil was recovered. TLC analysis of this crude mixture (1:1 EtOAc/hexane) showed the presence of both high and low R_f material (0.7 and 0.2, respectively). Separation by chromatography on a short silica column (Bond Elut, Analytichem International) gave the faster moving material, eluted with dichloromethane, and the slower moving material, eluted with ethyl acetate. The high R_f fraction was purified by HPLC on Partisil (20% EtOAc in hexane) to yield 9.1 mg of compound **3**: ^1H NMR ($CDCl_3$) δ 5.330 (d, *J* = 9.2 Hz, H on C-1), 5.069 (quintet, H on C-6), 4.583 (d, *J* = 11.2 Hz, H on C-3), 3.286 (d, *J* = 5.5 Hz, H on C-5), 2.112 (s, OAc), 2.099 (s, OAc), 2.002 (s, OAc), 1.880 (m, H on C-2), 1.217 (d, *J* = 6.4 Hz, Me on C-6), 0.957 (s, equatorial Me on C-4), 0.829

(6) The presence of acetoxyl groups on the δ-lactone does not affect the sign of the Cotton effect in the CD curve; e.g., compare the CD of δ-lactone **17** in ref 5c, $[\theta]_{224}$ +2300 (EtOH), and the corresponding diacetate, $[\theta]_{224}$ +3900 (EtOH).

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(s, axial Me on C-4), 0.790 (d, J = 6.6 Hz, Me on C-2); ^{1}H (irr) → ^{1}H (positive NOE): 5.330 → 4.583, 3.286; 5.069 → 3.286, 1.217, 0.957, 0.829; 4.583 → 5.330, 0.790; 3.286 → 5.330, 5.069, 4.583, 1.217; 1.217 → 5.069, 3.286; 0.957 → 5.069, 4.583, 3.286; 0.829 → 5.069, 1.880, 1.217; 0.790 → 5.330, 4.583, 1.880.

The slower moving material was purified in a similar manner (1:1 EtOAc–hexane) to give two fractions, each one containing the compounds of gross structure **4**. No attempt was made to fully characterize each isomer; instead the mixture was refluxed for 1 h with 0.5 N HCl. After the solution had cooled, the water was removed (in vacuo) and the hydrolyzate was acetylated with Ac_2O/pyridine (2:1) overnight at room temperature. After removal of the reagents and standard workup, a crude oil was obtained which after HPLC on a silica column (30% EtOAc in hexane) afforded 1 mg of diacetate **5** and 1 mg of lactone **6**. Compound **5** had the following properties: $[\alpha]^{25}_D$ −18° (CH_2Cl_2, c 0.5); ^{1}H NMR ($CDCl_3$) δ 4.958 (m, H on C-3), 4.070 (t, 2 H on C-1), 2.024 (s, OAc on C-1 and C-3), 1.765–1.955 (m, 2 H on C-2), 1.53 (br m, 2 H on C-4), 1.26 (br m, 6 H on C-5, C-6, and C-7), 0.862 (t, 3 H on C-8). Compound **6** had the following properties: CD (MeOH) $[\theta]_{257}$ = +1150; ^{1}H NMR ($CDCl_3$) δ 6.850 (m, H on C-3), 6.016 (dt, 9.8 and 1.8 Hz, H on C-2), 5.14 (m, C-7 H and C-8 H), 4.459 (m, H on C-5), 2.36 (m, 2 H on C-4), 2.044 (s, OAc), 2.031 (s, OAc), 1.90 (m, 2 H on C-6), 1.213 (d, J = 6.7 Hz, 3 H on C-9).

Conversion of 3 to Lactone 9. To a solution of 5 mg of compound **3** in 2 mL of CH_2Cl_2 was added a catalytic amount of p-toluenesulfonic acid monohydrate. After 6 h at room temperature the mixture was diluted with CH_2Cl_2 and poured over ice-cold saturated sodium bicarbonate solution. The organic layer was washed with water and saturated sodium chloride solution, dried, and evaporated to give 3 mg of a 1:1 mixture of the α and β hemiacetals (**8**). This material was treated without purification with excess MnO_2 (20 mg) in CH_2Cl_2 at room temperature. After 12 h the mixture was filtered through a small column of silica gel (1 g). Elution with ethyl acetate afforded 2.1 mg of an oil which was purified by HPLC on a silica column (35% EtOAc, 1% EtOH in hexane) to give 1.5 mg (overall yield 36%) of lactone **9**: CD (MeOH) $[\theta]_{227}$ = −2860; ^{1}H NMR ($CDCl_3$) δ 5.203 (qd, J = 6.3 and 3.9 Hz, H on C-6), 4.834 (d, J = 10.5 Hz, axial H on C-3), 4.082 (d, J = 3.9 Hz, axial H on C-5), 2.573 (dq, J = 10.5 and 6.8 Hz, axial H on C-2), 2.143 (s, OAc) 2.058 (OAc), 1.333 (d, J = 6.3 Hz, Me on C-6), 1.261 (d, J = 6.8, Me on C-2), 0.993 (s, Me on C-4), 0.989 (s, Me on C-4). Hydrolysis of **9** with 0.5 N HCl (reflux) gave the corresponding diol **10**; CD (MeOH) $[\theta]_{224}$ = −2160; ^{1}H NMR ($CDCl_3$) δ 4.024 (br qd, J = 6.3 and 4.3 Hz, H on C-6), 3.311 (br d, J = 10.2 Hz, H on C-3).

Synthesis of (*S*)-(+)-1,3-Diacetoxyoctane. (*Z*)-2-Octen-1-ol (950 mg) was epoxidized by the Sharpless method[14] employing unnatural (−)-diethyl tartrate as the chiral pool. After 48 h at −20 °C, standard workup and HPLC on silica (30% EtOAc in hexane) gave 2(*R*),3(*S*)-epoxy-1-octanol. The epoxide (33 mg) was treated with lithium aluminum hydride (19 mg) in tetrahydrofuran (2.5 mL).[15] After 20 min at 0 °C, water (0.5 mL) was added followed by 6–8 drops of 2% H_2SO_4. This mixture was filtered and evaporated to dryness. The residue was taken up in ethanol–water (1:3) and treated with sodium metaperiodate (23 mg) in water (1 mL) at 0 °C. After 2.5 h excess $NaBH_4$ was added followed by 1 M potassium phosphate (pH 7) buffer. The mixture was stirred until it was homogeneous (1 h). The ethanol was evaporated, and the remaining aqueous mixture was lyophilized. The dried residue was treated with Ac_2O/pyridine (2:1) overnight at room temperature. After standard workup and HPLC on Partisil (20% EtOAc in hexane), (*S*)-1,3-diacetoxyoctane was obtained, $[\alpha]^{25}_D$ + 15.2° (CH_2Cl_2, c 1.7).

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Registry No. 1, 93279-26-6; **1** diacetate, 93279-28-8; **2**, 93279-27-7; (*S*)-**5**, 93222-25-4; (*R*)-**5**, 93222-26-5; **6**, 93222-27-6; **9**, 93222-28-7; **10**, 93222-29-8; (*Z*)-2-octen-1-ol, 26001-58-1; (−)-diethyltartrate, 13811-71-7.

Supplementary Material Available: 300-MHz ^{1}H NMR spectra of **1** and **2** in $CDCl_3$ (2 pages). Ordering information is given on any current masthead page.

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Preparation of Furanoradialene by the Flash Vacuum Pyrolysis of Diesters of 3,4-Bis(hydroxymethyl)-2,5-dimethylfuran[1]

Walter S. Trahanovsky* and Timothy J. Cassady

Contribution from Ames Laboratory and the Department of Chemistry, Iowa State University, Ames, Iowa 50011. *Received September 19, 1983. Revised Manuscript Received July 2, 1984*

Abstract: Pyrolysis of 3,4-bis(acetoxymethyl)-2,5-dimethylfuran (**8**) gives a 12% yield of a [4 + 2] dimer (**10**) of furanoradialene (**4**). A similar pyrolysis of 3,4-bis(benzoyloxymethyl)-2,5-dimethylfuran (**9**) gives **10** in 69% yield. Low-temperature ^{1}H and ^{13}C NMR spectral studies show that **4** is the primary pyrolysis product from **8** and **9**, which forms **10** upon warming. Pyrolysis of 3,4-bis[acetoxydideuteriomethyl]-2,5-dimethylfuran (**8**-d_4) gives [4 + 2] dimer **10**-d_8 via the intermediacy of **4**-d_4. A concerted mechanism is proposed for the conversion of furanoradialene (**4**) to **10**.

Recently we reported that the flash vacuum pyrolysis (FVP) of (2-methyl-3-furyl)methyl benzoate (**1**) gives good yields of 2,3-dimethylene-2,3-dihydrofuran (**2**).[3] Compound **2** is an air-sensitive material which readily undergoes dimerization, forming the head-to-head [4 + 4] dimer (**3**). Although **2** has been previously postulated as an intermediate in the liquid-phase pyrolysis

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