Acknowledgment. This research was supported by the U.S. Army Research Office and the Texas Advanced Technology Research Program. We are indebted to Paul Fritz for his help in preparing the zeolites and Gordon Campbell for his assistance with the NMR instrumentation.

Registry No. I, 19252-53-0; $H_2C{=}CHCH_3$, 115-07-1.

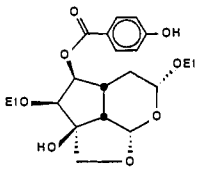
Total Synthesis of (-)-Specionin

Dennis P. Curran,*[1a] Patricia B. Jacobs, Richard L. Elliott, and Byeang Hyean Kim[1b]

Department of Chemistry, University of Pittsburgh Pittsburgh, Pennsylvania 15260

Received April 8, 1987

Specionin was isolated in 1983 by Nakanishi and Chang from the leaves of the *Catalpa speciosa* Warder tree.[2] This unusual iridoid has attracted interest because of its potent antifeedant activity against the Eastern spruce budworm, a common pest in North American forests. Isolated in only trace quantities, specionin was initially assigned structure **1** based on a detailed



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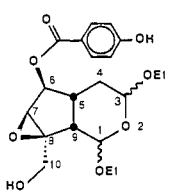
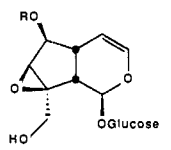


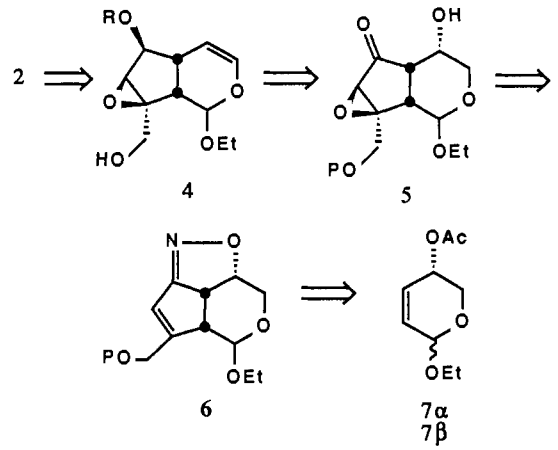
2a 1β, 3β
2b 1β, 3α
2c 1α, 3α
2d 1α, 3β



3a, R=H (catalpol)
b, R=COC_6H_4-p-(OH) (catalposide)

spectral analysis.[2] In 1985, a synthesis of compound **1** by Vandewalle and co-workers necessitated revision of the original proposal.[3a] Structure **2** was suggested, with the anomeric stereochemistry at C-1 and C-3 not absolutely certain. The common iridoid catalposide **3b** co-occurs with specionin, and it was indeed proposed that specionin might be an artifact of the ethanol extraction.[2] Structure **2** is consistent with this proposal. Very recently, Vandewalle has reported a racemic synthesis of the four possible anomers of specionin (**2a-d**) as well as the set of diastereomers epimeric at the epoxide-bearing carbons.[3b] With these diastereomers in hand, a detailed spectroscopic analysis permitted the assignment of specionin as the 1α,3β-isomer **2d**. We now report a stereoselective total synthesis of (-)-specionin which fully confirms the new structural assignment.

Scheme I



The key elements of a general approach to optically active iridoid aglucones are outlined in Scheme I.[4,5] One of our goals was the development of a method to introduce the enol acetal functionality embodied in the advanced intermediate **4**. Few methods exist for the introduction of this sensitive functional group,[6] which is present in many important iridoids such as catalpol (**3a**) and catalposide (**3b**). The precursor to **4** was envisioned to be β-hydroxy ketone **5**, which should be readily available by reduction of the corresponding Δ^2-isoxazoline **6**. In turn, **6** is prepared from **7**[7] by a variant of our recently developed Claisen/nitrile oxide sequence.[8]

While the anomeric stereochemistry of specionin was initially unclear, we felt that a trans disposition of the ethoxy groups was most likely (**2b** or **2d**).[9] Since our initial synthetic work directed at the structure **1** was performed by using 7β, we elected to prepare the 1β-isomers **2a** and **2b** with the advanced intermediates already in hand. Structures **2a** and **2b** were prepared by a sequence analogous to the one outlined in Scheme II.[10] Unfortunately, neither **2a** nor **2b** was identical with specionin; however, the synthesis did serve to confirm the structures of these two isomers in the Vandewalle mixture.[3b] This work convinced us that specionin must be **2d**. While acidic equilibration of **2a** and **2b** is conceivable, we elected to repeat the synthesis starting with 7α

(1) (a) Alfred P. Sloan Foundation Fellow, 1985-87; Camille and Henry Dreyfus Teacher Scholar, 1986-91; Merck Faculty Development Awardee, 1986-87. (b) Mellon Fellow, 1986-87, University of Pittsburgh.

(2) (a) Chang, C. C.; Nakanishi, K. *J. Chem. Soc., Chem. Commun.* **1983**, 605. (b) Chang, C. C. Ph.D. Thesis, Columbia University, 1985. We thank Professor Nakanishi for a copy of this thesis.

(3) (a) Van der Eycken, E.; Callant, P.; Vandewalle, M. *Tetrahedron Lett.* **1985**, *26*, 367. (b) Van der Eycken, E.; Van der Eycken, J.; Vandewalle, M. *J. Chem. Soc., Chem. Commun.* **1985**, 1719. Van der Eycken, E.; De Bruyn, A.; Van der Eycken, J.; Callant, P.; Vandewalle, M. *Tetrahedron* **1986**, *42*, 5385.

(4) (a) Most previous syntheses of iridoids can be classed into two strategies: oxidative cleavage of bicyclooctenes or [2 + 2] cycloaddition/retroaldol. For reviews, see: Paquette, L. A. *Top. Curr. Chem.* **1979**, *79*, 41. Thomas, A. F.; Bessiere, Y. In *The Total Synthesis of Natural Products*; ApSimon, J., Ed.; Wiley: New York, 1981; Vol. 4, pp 494-507. A new strategy based on Norrish I type cleavage of norbornanones has recently been developed by Vandewalle, see: ref 3. (b) For reviews on the structure and biological activity of iridoids, see: Bobbitt, J. M.; Segebarth, K. P. *Cyclopentanoid Terpene Derivatives*; Marcell Dekker: New York, 1969. El-Naggar, L. J.; Bell, J. L. *J. Nat. Products* **1980**, *42*, 649. Sticher, O. *New Natural Products and Plant Drugs with Pharmacological, Biological, and Therapeutic Activity*; Wagner, H., Wolff, P., Eds.; Springer-Verlag: New York, 1977; p 145.

(5) For recent developments in formation of the glycoside linkage, see: Tietze, L.-F.; Fischer, R. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 888. Battersby, A. R.; Westcott, N. D.; Glüsenkamp, K. H.; Tietze, L.-F. *Chem. Ber.* **1981**, *114*, 3439.

(6) Most past syntheses have been of iridoids possessing a carbomethoxy group at C-4. These compounds are more stable as evidenced by the ability to hydrolyze the glucoside without decomposition of the aglucone (see ref 4). For a recent preparation of the C-4 unsubstituted enol acetal by acetate pyrolysis, see: Trost, B. M.; Balkovec, J. M.; Mao, M. K.-T. *J. Am. Chem. Soc.* **1986**, *108*, 4974.

(7) The requisite starting materials, 7α and 7β, are readily available from D-xylal by Ferrier rearrangement. Fraser-Reid, B.; McLean, A.; Usherwood, E. W.; Yunker, M. *Can. J. Chem.* **1970**, *48*, 2877.

(8) Curran, D. P.; Jacobs, P. B. *Tetrahedron Lett.* **1985**, *26*, 2031.

(9) In a chair-like conformation, *cis*-**2a** should have both ethoxy groups equatorial-like and will profit from no anomeric effects. *cis*-**2c** should have both ethoxy groups axial-like and will suffer from unfavorable dipole interactions. See: Baker, R.; Brimble, M. A.; Robinson, J. A. *Tetrahedron Lett.* **1985**, *26*, 2115. For a detailed discussion of the stereochemistry and conformations of **2a-d**, see ref 3b.

(10) Jacobs, P. B., Ph.D. Thesis, University of Pittsburgh, 1986. Complete details of this work will be reported in a future full paper.

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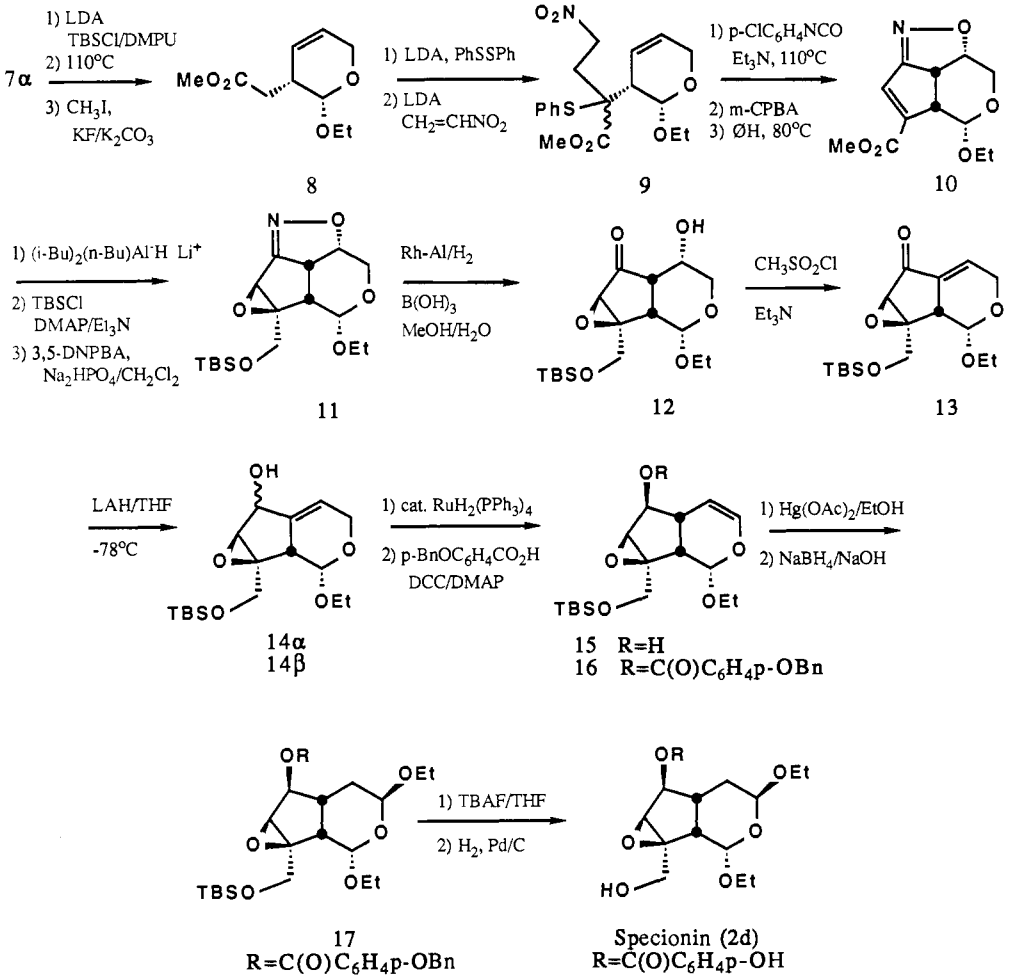
Scheme II



to provide rigorous proof of the structure of specionin.

The synthesis of specionin is outlined in Scheme II. Acetal 7α was subjected to the usual conditions for Ireland–Claisen rearrangement[11] to provide **8** in 75% yield after methylation. Standard sulfenylation (81%),[12] followed by conjugate addition of the resulting α-thio ester to freshly prepared nitroethylene,[13] provided **9** as a nearly equal mixture of diastereomers (85%). After nitrile oxide cycloaddition was accomplished (89%), the sulfide was oxidized, and sulfoxide elimination was effected by heating.[12] This provided a single tricyclic vinyl isoxazoline **10** in 54% yield. Selective reduction of the ester was accomplished with the "ate" complex derived from the addition of *n*-butyllithium to diisobutylaluminum hydride[14] to give an alcohol (42%). This was silylated with TBSCl (72%). Epoxidation of this allylic silyl ether from the convex face with buffered 3,5-dinitroperoxybenzoic acid[15] produced epoxy isoxazoline **11** as a single stereoisomer in 77% yield. The successful epoxidation of this relatively electron poor trisubstituted olefin relies on the very low reactivity of the Δ^2-isoxazoline ring toward oxidizing agents. The Δ^2-isoxazoline ring was smoothly cleaved by hydrogenolysis/hydrolysis with hydrogen gas and 5% rhodium on alumina in boric acid-doped aqueous methanol.[16] The β-hydroxy ketone **12** was isolated in 57% yield.

The stage was now set for introduction of the enol acetal. Mesylation of **12** was accompanied by direct elimination to produce enone **13** (70%). Reduction of **13** with lithium aluminum hydride in THF at −78 °C produced a 3/1 mixture of allylic alcohols **14**β/**14**α in 93% yield. These stereoisomers were not easily separated. Direct exposure of the mixture to a catalytic amount of tetrakis(triphenylphosphine)ruthenium dihydride in ethanol[17] induced smooth olefin migration to provide a 3/1 mixture of readily separable enol acetals. The major, crystalline β-epimer **15** (a silylated derivative of the α-ethyl aglucone of catalpol, **3a**) was isolated in 64% yield. Standard DCC-mediated coupling of **15** with (*p*-benzyloxy)benzoic acid provided **16** (61%). Introduction of the 3β-ethoxy group was smoothly accomplished by a standard oxymercuration/demercuration sequence.[18] Compound **17**, a protected derivative of specionin, was isolated as a single stereoisomer in 70% yield. No evidence for the formation of even trace amounts of the 1α,3α isomer was obtained. It was clear that **17** possessed the same stereochemistry as specionin from the similarities in the ^{1}H NMR spectra of the two compounds.

(11) (a) Ireland, R. E.; Mueller, R. H; Willard, A. K. *J. Am. Chem. Soc.* **1976**, *98*, 2868. (b) Ireland, R. E.; Thaisrivongs, S.; Vanier, N.; Wilcox, C. S. *J. Org. Chem.* **1980**, *45*, 48.

(12) Trost, B. M.; Salzmann, T. N.; Hiroi, K. *J. Am. Chem. Soc.* **1976**, *98*, 4887.

(13) Ranganathan, D.; Rao, C. B.; Ranganathan, S.; Mehrotra, A. K.; Iyengar, R. *J. Org. Chem.* **1980**, *45*, 1185. Miyashita, M.; Yamaguchi, R.; Yoshikoshi, A. *J. Org. Chem.* **1984**, *49*, 2857.

(14) Kim, S.; Ahn, K. H. *J. Org. Chem.* **1984**, *49*, 1717. Trost, B. M.; Jungheim, L. N. *J. Am. Chem. Soc.* **1980**, *102*, 7910. Kovacs, G.; Galambos, G.; Juvancz, Z. *Synthesis* **1977**, 171.

(15) Rastetter, W. H.; Richard, T. J.; Lewis, M. D. *J. Org. Chem.* **1978**, *43*, 3163.

(16) The more commonly used catalysts such as Raney nickel, palladium on carbon, or platinum oxide resulted in decomposition. Curran, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 5826.

(17) Takahashi, M.; Suzuki, H.; Moro-Oka, Y.; Ikawa, T. *Chem. Lett.* **1981**, 1435. Felföldi, K.; Bartók, M. *J. Organomet. Chem.* **1985**, *297*, C-37.

(18) Larock, R. C. *Solvomercuration/Demercuration Reactions in Organic Synthesis*; Springer-Verlag: Berlin, 1986. Assuming that the rate-limiting step in the oxymercuration is attack of the ethanol, β-attack should be favored since α-attack would develop dipolar repulsions between the incoming C-3 ethoxy group and the axial-like C-1 ethoxy group. The C-1β ethoxy isomer shows no stereoselectivity in the oxymercuration since its ethoxy group is equatorially disposed. This, and other stereochemical points, will be more fully addressed in a full paper.

Indeed, after desilylation with TBAF and palladium-catalyzed hydrogenolytic cleavage of the benzyl group, specionin (**2d**) was isolated in 90% yield.[19]

In summary, specionin has been prepared in about 18 steps from 7α. Six of the seven stereogenic centers have been introduced with complete stereocontrol; only in the case of introduction of the C-6 hydroxyl group is a mixture obtained in which the desired isomer predominates (3/1). The synthesis serves to confirm the structural assignment of specionin which has recently been made by Vandewalle and co-workers. More importantly, it should provide a general entry into iridoid aglucones of the catalpol type, and it is easily envisioned that many other related molecules could be prepared with the highly oxygenated intermediates already in hand.[20]

Acknowledgment. We thank the National Institutes of Health (GM-31678) for funding of this work. We also thank Professor M. Vandewalle for a friendly exchange of samples, spectra, and unpublished results.

(19) Our synthetic specionin was identical in all respects with a sample kindly provided by Professor Nakanishi via Professor Vandewalle. The optical rotation of specionin has apparently not been reported. We find that natural specionin exhibits an $[\alpha]^{25}_D = -30.7$, *c* 0.08, $CHCl_3$. Our synthetic specionin: $[\alpha]^{25}_D = -29.5$, *c* 0.30, $CHCl_3$; ^{13}C NMR ($CDCl_3$) δ 15.1, 15.3, 29.1, 33.1, 40.3, 60.6, 61.2, 63.1, 63.9, 66.6, 79.0, 93.8, 96.1, 115.3, 122.2, 132.2, 160.4, 166.6; (CD_3OD) δ 15.5, 15.6, 30.3, 34.2, 41.2, 61.2, 61.4, 64.0, 64.8, 67.3, 80.7, 94.9, 97.8, 116.2, 122.0, 133.0, 163.8, 168.3.

(20) All intermediates described in Scheme II have been fully characterized. A forthcoming full paper will describe in detail the synthesis of specionin as well as the preparation of isomers **2a** and **2b** and the synthesis of ethyl catalpol.

Kinamycin Biosynthesis. Derivation by Excision of an Acetate Unit from a Single-Chain Decaketide Intermediate

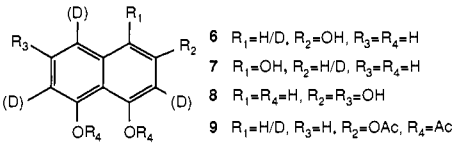
Pamela J. Seaton and Steven J. Gould*[1]

Department of Chemistry, Oregon State University Corvallis, Oregon 97331

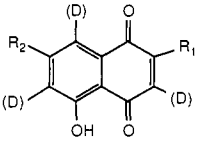
Received March 16, 1987

We have previously reported[2,3] on the incorporation of a variety of labeled acetates during the biosynthesis of the kinamycin antibiotics,[4-7] produced by *Streptomyces murayamaensis* ATCC 21414. Incorporation of sodium [1,2-$^{13}C_2$]acetate, **1**, established the polyketide nature of the kinamycin skeleton, as shown in Scheme I for kinamycin D, **2**. The labeling pattern was consistent with condensation of two nonsymmetrical intermediates, **3** and **4**, to give a tetracyclic benz[*b*]carbazole **5**. We now report unsuccessful efforts to support this hypothesis, the structure of a newly characterized metabolite of the same organism, and direct proof that this is a key intermediate in kinamycin biosynthesis from a single decaketide precursor.

Polyhydroxynapthalenes **6**,[8,9] **7**,[10,11] and **8**[12] as well as the acetate[13] **9** of **6** and naphthoquinones **10**,[14] **11**,[15] and **12**,[16,17] were



6 R_1=H/D, R_2=OH, R_3=R_4=H
7 R_1=OH, R_2=H/D, R_3=R_4=H
8 R_1=R_4=H, R_2=R_3=OH
9 R_1=H/D, R_3=H, R_2=OAc, R_4=Ac



10 R_1=H/D, R_2=H
11 R_1=OH, R_2=H
12 R_1=R_2=OH

synthesized to test as potential biosynthetic intermediates with isotope dilution experiments involving feedings with sodium [2-^{14}C]acetate. Five of these, **6**, **7**, and **9**-**11**, were also synthesized with deuterium labels to be used for direct feeding experiments.

Each of the deuteriated compounds was fed in multiple pulses to cultures[18] of *S. murayamaensis*, and the derived samples of **2** were analyzed by ^2H NMR.[19] Juglone, **10**, was toxic to the organism. In no case was deuterium incorporation into **2** observed.[20] In the isotope dilution experiments, no radioisotope was trapped by any of the test compounds.

Concomitant with these studies, we further investigated the structures of other colored metabolites of this organism. A crude fraction containing four of these as well as kinamycins and murayaquinone[21] was isolated from 7 L of a 26-h production broth.[22] These were separated by chromatography on Silicar CC-4[23] and elution with CH_2Cl_2. A green metabolite was eluted first and was recrystallized from CH_2Cl_2/hexane to afford dark green needles (26 mg), which proved to be a known compound, **13**,[24] previously

(1) Career Development Awardee of the National Cancer Institute (Grant CA-00880), 1979-1984.

(2) Sato, Y.; Gould, S. J. *Tetrahedron Lett.* **1985**, *26*, 4023.

(3) Sato, Y.; Gould, S. J. *J. Am. Chem. Soc.* **1986**, *108*, 4625.

(4) Ito, S.; Matsuya, T.; Omura, S.; Otani, M.; Nakagawa, A.; Iwai, Y.; Ohtani, M.; Hata, T. *J. Antibiot.* **1970**, *23*, 315.

(5) Hata, S.; Omura, S.; Iwai, Y.; Nakagawa, A.; Otani, M. *J. Antibiot.* **1971**, *24*, 353.

(6) Omura, S.; Nakagawa, A.; Yamada, H.; Hata, T.; Furusaki, A.; Watanabe, T. *Chem. Pharm. Bull.* **1973**, *21*, 931.

(7) Sato, Y.; Geckle, M.; Gould, S. J. *Tetrahedron Lett.* **1985**, *26*, 4019.

(8) For the natural occurrence of **6** and **11**, see: (a) Stipanovic, R. D.; Bell, A. A. *Mycologia* **1977**, *69*, 164, (b) Tokousbalides, M. C.; Sisler, H. D. *Pestic. Biochem. Physiol.* **1979**, *11*, 64.

(9) Synthesis of **6** followed the procedure described in the following: Cameron, D. W.; Feutrill, G. I.; Pannan, L. J. H. *Aust. J. Chem.* **1980**, *33*, 2531. Deuterium was introduced by direct exchange of **6** in D_2O/OD^-.

(10) For the natural occurrence of **7** and **10**, see: Mueller, W. H.; Leistner, E. *Phytochemistry* **1978**, *17*, 1735.

(11) **7** was synthesized from juglone (**10**)[15] as described in the following: Thomson, R. H. *J. Chem. Soc.* **1950**, 1737.

(12) Biosynthesis of **2** must proceed through nonsymmetrical intermediates.[3] **8** was synthesized as a negative control for isotope dilution experiments as described by the following: Bycroft, B. W.; Cashyap, M. M.; Leung, T. K. *J. Chem. Soc., Chem. Commun.* **1974**, 443.

(13) Acetate **9** was obtained by reaction of **6** with Ac_2O/pyridine. Spectral properties were identical with those reported by the following: Findlay, J. A.; Kwan, D. *Can. J. Chem.* **1973**, *51*, 1617.

(14) Synthesis of **10** from 1,5-dihydroxynaphthalene is described by the following: Wakumutsu, T.; Nishi, T.; Ohnuma, T.; Ban, Y. *Synth. Commun.* **1984**, *14*, 1617. Deuterio-1,5-dihydroxynaphthalene (obtained by exchange in D_2O/OD^-) was used to generate deuterio-**10**.

(15) **11** was synthesized from juglone as described in the following: Thomson, R. H. *J. Org. Chem.* **1951**, *16*, 1082.

(16) For natural occurrence of **12**, see: Sankawa, U.; Shimada, H.; Sato, T.; Kinoshita, T. *Tetrahedron Lett.* **1977**, 483.

(17) **12** was synthesized from **8** as described in the following: Baker, P. M.; Bycroft, B. W. *J. Chem. Soc., Chem. Commun.* **1968**, 71.

(18) Production cultures (200 mL; 2% glycerol, 0.1% K_2HPO_4, 0.1% asparagine, 0.04% $MgSO_4$, 0.01% $FeSO_4$) were innoculated with 10 mL of a 48-h seed culture[3] of *S. murayamaensis*.

(19) ^2H NMR were taken on a Bruker AM 400 spectrometer at 61.4 MHz by using a 5-mm probe with broad band proton decoupling.

(20) **6** and **7** were partially oxidized to the quinones; **9** was recovered unchanged, and symmetrical tetrol **8** was totally oxidized to flaviolin (**12**) by production cultures. Neither **8** nor **12** are metabolites of *S. murayamaensis*.

(21) Sato, Y.; Kohnert, R.; Gould, S. J. *Tetrahedron Lett.* **1986**, *27*, 143.

(22) Production broth (7 L) (3% glycerol, 0.13% $(NH_4)_2SO_4$, 0.1% K_2HPO_4, 0.04% $MgSO_4$, 0.01% $FeSO_4$, and 0.2% $CaCO_3$) in a 14-L vessel (New Brunswick Scientific Microferm stirred fermentor) was innoculated with 200 mL of a 48-h seed culture and incubated at 25-26 °C, 300 rpm, 10 L/min aeration. Upon acidification of the 26-h broth, the mycelia floated to the surface allowing for the broth to be siphoned off, and the remaining cell suspension was sonicated. The broth and cell suspension were combined, extracted with toluene, and concentrated to a brown residue (930 mg) which contained the green, purple, and yellow pigments as well as the kinamycins and murayaquinone.

(23) Silicar CC-4 was obtained from Mallinkrodt (7086).

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