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# SYNTHESES OF 3-*O*-SUBSTITUTED 3-*O*-DEMETHYLSPORARICINS A*

ISAMU WATANABE, KAZUHIRO KAMIYA, TAKASHI YAMAGUCHI, TOSHIHITO MORI,
*Tokyo Research Laboratories, Kowa Co., Ltd., Higashimurayama, Tokyo 189 (Japan)*
AND TSUTOMU TSUCHIYA
*Institute of Bioorganic Chemistry 1614 Ida, Nakahara-ku, Kawasaki-shi 211 (Japan)*

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ABSTRACT

The 3-*O*-methyl group of sporaricin A has been replaced by other alkyl groups to give 3-*O*-(3-amino-2-hydroxypropyl)- (**13**), 3-*O*-(4-amino-2-hydroxybutyl)- (**15**), and 3-*O*-(2,3-dihydroxypropyl)-sporaricin A (**14**). The 3-*O*-methyl group of sporaricin B was selectively removed under acidic conditions, and an allyl group was introduced onto the 3-hydroxyl group. The 3-*O*-allyl compound was 4-*N*-glycylated, the allyl group epoxidized, and the product treated with sodium azide or potassium cyanide, or hydrolyzed. The respective derivative obtained was then reduced, or deprotected, or both, to give **13**, **15**, and **14**. These compounds showed high antibacterial activities against bacteria resistant to sporaricin A.

## INTRODUCTION

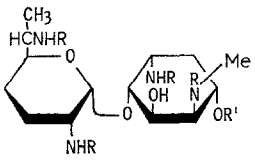
Sporaricins A and B are major components[1,2] isolated from the culture broth of *Saccharopolyspora hirsuta* subsp. *kobensis*[3]. They are pseudodisaccharides, and the structure of sporaricin B was determined[2] to be **1**, and that of sporaricin A, to be the 4-*N*-glycyl compound of sporaricin B. The amino sugar portion of sporaricins is, therefore, the 6-epi isomer[4] of purpurosamine B. Other, related, pseudodisaccharide antibiotics are the fortimicins[5], istamycins[6], sannamycins[7], and dactimicin[8]. Sporaricin A is highly active[9] against Gram-positive and -negative bacteria, including various aminoglycoside-resistant strains producing aminoglycoside 3′-phosphotransferases I and II [APH(3′)-I and APH(3′)-II], 6′-acetyltransferases [AAC(6′)], a 2′-acetyltransferase [AAC(2′)], and 3-acetyltransferases [AAC(3)], except for 3-acetyltransferase I [AAC(3)-I].

Chemical modifications of these kinds of pseudodisaccharide to improve their biological properties began after their discovery; attempted modifications were, for example, 3-*O*-demethylation[10,11], deoxygenation[12,13] at C-2 and -5, *N*-alkylation[14–16]

*Dedicated to Professor Sumio Umezawa on the occasion of his 73rd birthday and the 25th anniversary of the Microbial Chemistry Research Foundation.

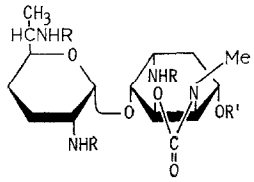
at C-4, -2′, and -6′, *N*-acylation[14,17,18] at C-4 and -2′, and epimerization[19,20] at C-2 and -6′.

We now describe another kind of modification, that is, replacement of the 3-*O*-methyl group of sporaricin A by certain other alkyl groups. The compounds prepared were 3-*O*-(3-amino-2-hydroxypropyl)- (**13**), 3-*O*-(4-amino-2-hydroxybutyl)- (**15**), and 3-*O*-(2,3-dihydroxypropyl)-3-*O*-demethylsporaricin A (**14**). These alkyl residues were chosen because it had been reported that amikacin[21] {1-*N*-[(*S*)-4-amino-2-hydroxybutanoyl]kanamycin A}, butikacin[22] {1-*N*-[(*S*)-4-amino-2-hydroxybutyl]kanamycin A}, and[23] 1-*N*-(1,3-dihydroxy-2-propyl)kanamycin B, all of them having the acyl or alkyl residue attached to the 1-amino group of kanamycins, showed strong antibacterial activity against bacteria resistant to kanamycins. Another point that should be mentioned is that C-1 in amikacin and other related compounds described (which bears *N*-acyl or *N*-alkyl groups) corresponds to C-3 of sporaricin. The C-3 atom of sporaricin A was, therefore, in terms of the antibacterial activity, considered to be the most suitable position to which to attach the side residues.

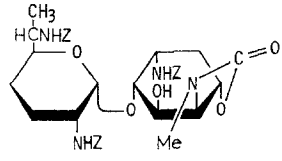


| | R | R′ |
|---|---|---|
| 1 | H | Me |
| 2 | H | H |
| 3 | Z | H |

Z = $OCOCH_2Ph$



| | R | R′ |
|---|---|---|
| 4 | Z | H |
| 5 | H | H |
| 6 | Z | $CH_2CH=CH_2$ |



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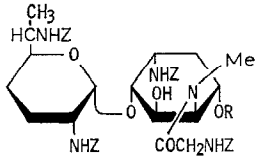
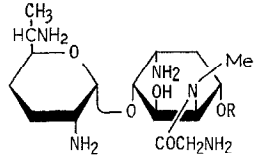


R

| | |
|---|---|
| 8 | $CH_2CH=CH_2$ |
| 9 | $CH_2CH\overset{O}{\frown}CH_2$ |
| 10 | $CH_2CH(OH)CH_2N_3$ |
| 11 | $CH_2CH(OH)CH_2CN$ |
| 12 | $CH_2CH(OH)CH_2OH$ |



R

| | |
|---|---|
| 13 | $CH_2CH(OH)CH_2NH_2$ |
| 14 | $CH_2CH(OH)CH_2OH$ |

## RESULTS AND DISCUSSION

In order to prepare the desired 3-*O*-alkyl compounds (**13–15**), selective removal of the 3-*O*-methyl group from sporaricin is an indispensable step. Martin *et al.*[10] prepared 3-*O*-demethylfortimicin B, in ~30% yield, by treating fortimicin B with

lithium and ethylamine. This radical-type procedure was applied[24] to the synthesis of 4″-*O*-demethylseldomycin factor 5. Watanabe *et al.*[25], however, succeeded in removing the 3-*O*-methyl group of sporaricin B under acidic conditions; treatment of sporaricin B (**1**) with 48% aqueous hydrobromic acid during 4 h at 90° gave 3-*O*-demethylsporaricin B (**2**) in ~60% yield without excessive cleavage of the glycosidic bond. This finding was somewhat surprising, because similar, or weaker, treatment of lividamine[26] [4-*O*-(2-amino-2,3-dideoxy-α-D-*ribo*-hexopyranosyl)-2-deoxystreptamine] gave products of cleavage at the glycosidic bond. In the present work, sporaricin B (**1**) was treated with concentrated hydriodic acid, to give **2** in 94% yield. The structure of **2** was confirmed by its $^{13}$C-n.m.r. spectrum (see Table I). The upfield shift[27] (9.94 p.p.m.) of the C-3 signal of **2** from the corresponding position of that of sporaricin B indicated that the *O*-methyl group on C-3 had been removed.

TABLE I

CHEMICAL SHIFTS OF $^{13}$C-N.M.R. SPECTRA OF SPORARICIN DERIVATIVES[a] AND SPORARICINS A AND B

| *Carbon atoms* | *Chemical shifts (p.p.m.)* | | | | | |
|---|---|---|---|---|---|---|
| | *Sporaricin B* | **2** | **13** | **15** | **14** | *Sporaricin A* |
| 1′ | 97.82 d | 97.78 d | 98.29 d | 98.10 d | 98.24 d | 98.38 d |
| 2′ | 50.57 d | 50.57 d | 50.69 d | 50.86 d | 50.71 d | 50.67 d |
| 3′ | 27.02 t | 26.96 t | 26.32 t | 26.19 t | 26.27 t | 26.34 t |
| 4′ | 27.29 t | 27.29 t | 27.34 t | 27.34 t | 27.31 t | 27.34 t |
| 5′ | 74.83 d | 74.78 d | 74.43 d | 73.92 d | 74.29 d | 74.52 d |
| 6′ | 50.57 d | 50.57 d | 50.49 d | 50.45 d | 50.47 d | 50.52 d |
| 7′ | 18.59 q | 18.54 q | 18.19 q | 17.85 q | 18.09 q | 18.29 q |
| 1 | 46.77 d | 46.87 d | 46.60 d | 46.60 d | 47.00 d | 46.58 d |
| 2 | 33.27 t | 37.53 t | 34.47 t | 34.42 t | 34.53 t | 33.88 t |
| | | | 34.54 t | 34.54 t | | |
| 3 | 78.43 d | 68.49 d | 72.27 d | 72.46 d | 72.27 d | 73.24 d |
| | | | 72.48 d | 72.63 d | 72.46 d | |
| 4 | 61.39 d | 62.77 d | 56.50 d | 56.50 d | 56.53 d | 56.63 d |
| 5 | 65.34 d | 65.43 d | 70.17 d | 70.10 d | 70.15 d | 70.12 d |
| 6 | 77.23 d | 77.31 d | 78.15 d | 77.93 d | 78.08 d | 78.26 d |
| N-$CH_3$ | 33.56 q | 33.58 q | 32.03 q | 32.13 q | 32.20 q | 31.82 q |
| Gly-$CH_2$ | | | 43.34 t | 43.34 t | 43.32 t | 43.31 t |
| Gly-CO | | | 176.43 s | 176.33 s | 176.40 s | 176.31 s |
| O-$CH_3$ | 56.95 q | | | | | 55.81 q |
| 1″ | | | 70.59 t | 69.10 t | 69.44 t | |
| | | | 70.88 t | 69.25 t | 69.71 t | |
| 2″ | | | 71.95 d | 72.19 d | 71.37 d | |
| | | | 72.14 d | 72.31 d | 71.56 d | |
| 3″ | | | 44.17 t | 33.93 t | 63.53 t | |
| 4″ | | | | 37.97 t | | |

[a]All samples were prepared as follows: an aqueous solution of the compound was passed through a column of CM Sephadex C-25 ($NH_4^+$), eluted with 0.5M aqueous ammonium hydroxide, and the eluate lyophilized.

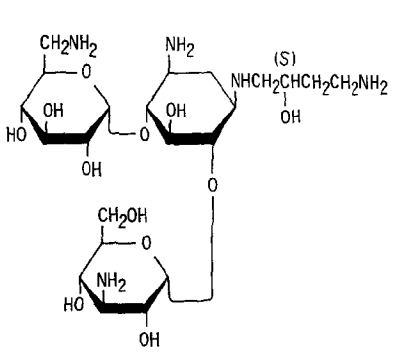
This unique behavior of sporaricin B may be ascribed to its structural features. In contrast to lividamine, the compound will assume (checked by a molecular model) a folded structure having the plane of the cyclohexane ring roughly at right angles to the plane of the pyranoid moiety; this will cause the glycosidic oxygen atom, bridging the two rings, to be isolated from the outside of the molecule, thereby preventing the approach of a hydrogen ion.

Treatment of **2** with benzyl chloroformate gave the tetrakis-*N*-(benzyloxycarbonyl) derivative (**3**) having free hydroxyl groups at C-3 and -5. Heating **3** in a basic medium gave rise to two compounds, in the ratio of ~4:1, that were separated by chromatography. The major (and slower-moving) product proved to be the 4,5-cyclic carbamate (**4**) having the 3-hydroxyl group free. The minor, faster-moving product was, therefore, assigned as the 3,4-cyclic carbamate (**7**). The structure of **4** was confirmed from the $^1$H-n.m.r. spectrum of the *N*-de(benzyloxycarbonyl) derivative (**5**) of **4**, downfield shifts being observed for the signals of H-4, -5, and $NCH_3$ in **5**.

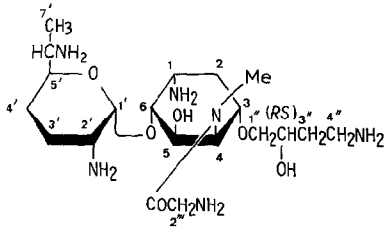
Several attempts to introduce, directly, the chosen alkyl groups at the 3-hydroxyl group of **4** failed, but then, an attempt was made to introduce a group later changeable to the desired alkyl groups. Among the groups tested, the allyl group was found suitable. Treatment of **4** with allyl bromide in *N*,*N*-dimethylformamide, in the presence of barium oxide and barium hydroxide, gave the 3-*O*-allyl compound (**6**) in a yield of 86%. The presence of the allyl group was confirmed by the $^1$H-n.m.r. spectrum of **6**. Selective cleavage of the 4,5-cyclic carbamate was then conducted by use of a limited amount of barium hydroxide in 1,4-dioxane–water, to give the amino alcohol, which, without purification, was condensed with the active ester prepared from *N*-(benzyloxycarbonyl)glycine and *N*-hydroxysuccinimide; the 3-*O*-allyl-4-*N*-[*N*-(benzyloxycarbonyl)glycyl] derivative (**8**) was obtained in 56% yield from **6**. Oxidation of **8** with *m*-chloroperoxybenzoic acid in chloroform gave the epoxy derivative (**9**) in 77% yield. The presence of the epoxy group was assumed from the products derived from **9** as described next.

In order to convert the epoxy compound (**9**) into the 3-*O*-(3-amino-2-hydroxypropyl) compound (**13**), **9** was treated with sodium azide in *N*,*N*-dimethylformamide, to give the 3-azido-2-hydroxypropyl compound (**10**) as a stereoisomeric mixture. Likewise, in order to prepare the 3-*O*-(4-amino-2-hydroxybutyl) compound (**15**), **9** was treated with potassium cyanide in *N*,*N*-dimethylformamide, to give the 3-cyano-2-hydroxypropyl derivative (**11**), a precursor of **15**, as a stereoisomeric mixture. When **9** was treated in an acidic medium, a stereoisomeric mixture of the protected derivative (**12**) of the 3-*O*-(2,3-dihydroxypropyl) compound (**14**) was formed. Structural confirmations of **10**, **11**, and **12** were performed chemically and spectrometrically; in their infrared spectra, **10** and **11** gave absorption peaks for azido (2100 $cm^{-1}$) and cyano (2250 $cm^{-1}$) groups, respectively; respective treatment of **10** and **11** with trityl chloride in pyridine gave no trityl derivative, but **12** gave a trityl ether. This indicated that the free hydroxyl group on the side chain of **10**, and of **11**, was not on the terminal carbon atom.

Hydrogenation of the azido group and concomitant hydrogenolysis of the *N*-benzyloxycarbonyl groups of **10** in acetic acid with hydrogen and palladium black gave 3-*O*-(3-amino-2-hydroxypropyl)-3-*O*-demethylsporaricin A (**13**), as a stereoisomeric mixture. Hydrogenation of **11** with hydrogen and palladium black, followed by Raney nickel, gave a desired compound having a longer chain than **13**, namely, 3-*O*-(4-amino-2-hydroxybutyl)-3-*O*-demethylsporaricin A (**15**) as a stereoisomeric mixture. Similar deprotection of **12** gave 3-*O*-(2,3-dihydroxypropyl)sporaricin A (**14**).



Butirosin



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The $^{13}$C-n.m.r. data for compounds **13**, **14**, and **15** are listed in Table I. The chemical shifts of the skeleton carbon atoms of these compounds, certified by the off-resonance, decoupling method, are in good agreement with those[2] of sporaricin A. The resonances of C-2, -3, -1″, and -2″ were observed as twin signals having equal intensity. These results revealed that each of these 3-*O*-alkyl-3-*O*-demethylsporaricins A (**13**, **14**, and **15**) was a mixture epimeric at C-2″. Attempts to separate the epimers by thin-layer chromatography on silica gel, or by liquid chromatography using, a column of μBondapak $C_{18}$ were unsuccessful.

The antimicrobial activities of **13**, **14**, and **15**, compared with that of sporaricin A, were measured by an agar dilution method (see Table II). In conformity with our expectations, the synthetic 3-*O*-alkyl-3-*O*-demethylsporaricins A (**13**, **14**, and **15**) exhibited high antibacterial activities against *E. coli* JR 88 and *Pseudomonas aeruginosa* 99 producing the enzyme of AAC(3)-I, against which strains sporaricin A is inactive. Moreover, compounds **13** and **15** showed enhanced activity against *Pseudomonas aeruginosa* in comparison to that of sporaricin A.

## EXPERIMENTAL

*General methods.* — Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Chromatography was performed on silica gel (Kieselgel DC-Alufolien 60 $F_{254}$ E. Merck) for thin-layer chromatography, (Kieselgel 60 $PF_{254}$, E. Merck) for preparative thin-layer chromatography, and Wakogel C-200 for column chromatography. The spectrometric data were determined with the following in-

TABLE II

MINIMAL INHIBITORY CONCENTRATIONS ($\mu$g/mL) OF SPORARICIN DERIVATIVES (**13**, **15**, AND **14**) AND SPORARICIN A

| *Test organism* | **13** | **15** | **14** | *Sporaricin A* |
|---|---|---|---|---|
| *Staphylococcus aureus* 209P | 0.39 | 0.39 | 0.39 | 0.39 |
| *Bacillus anthracis* | ≤0.1 | ≤0.1 | ≤0.1 | 0.2 |
| *cereus* | 0.78 | 1.56 | 3.13 | 1.56 |
| *subtilis* ATCC 6633 | 0.2 | ≤0.1 | 0.2 | 0.2 |
| *Streptococcus faecalis* | 50 | 50 | 100 | 25 |
| *Escherichia coli* NIHJ | 3.13 | 3.13 | 3.13 | 3.13 |
| K-12 ML1410 | 3.13 | 3.13 | 6.25 | 3.13 |
| R81[a] | 3.13 | 3.13 | 6.25 | 3.13 |
| R-82[b] | 3.13 | 3.13 | 6.25 | 3.13 |
| R-101[c] | 3.13 | 3.13 | 6.25 | 3.13 |
| R-176[d] | 6.25 | 6.25 | 6.25 | 3.13 |
| JR88[e] | 6.25 | 3.13 | 6.25 | >100 |
| *Proteus vulgaris* OX-19 | 3.13 | 3.13 | 3.13 | 1.56 |
| *Klebsiella pneumoniae* PCI 602 | 1.56 | 1.56 | 0.78 | 1.56 |
| *Pseudomonas aeruginosa* No. 12 | 0.39 | 0.39 | 1.56 | 0.39 |
| No. 99[e] | 6.25 | 6.25 | 25 | >100 |
| TI-13[a] | 3.13 | 3.13 | 12.5 | 6.25 |
| $A_3$ | 1.56 | 3.13 | 6.25 | 6.25 |
| K-11 | 3.13 | 6.25 | 12.5 | 12.5 |
| TK-157[b] | 1.56 | 3.13 | 12.5 | 6.25 |
| GN315[f] | 3.13 | 6.25 | 12.5 | 12.5 |
| *Proteus inconstans* GN1554[g] | 6.25 | 6.25 | 6.25 | 6.25 |
| *Serratia marcescens* | 1.56 | 1.56 | 3.13 | 3.13 |

[a]Resistance mechanism: APH(3′)-I. [b]APH(3′)-II. [c]AAD(2″). [d]AAC(3)-II. [e]AAC(3)-I. [f]AAC(6′)-IV. [g]AAC(2′).

struments. Infrared spectra: JASCO model IRA-I spectrometer; mass spectra: JEOL model JMS-D-300; n.m.r. spectra: Bruker WM 250, JEOL model JNM-FX-200, and JNM-MH-100 spectrometers. Chemical shifts for $^1$H-n.m.r. spectra determined in $CDCl_3$ were measured relative to internal tetramethylsilane, and, in $D_2O$, relative to external tetramethylsilane.

*3-O-Demethylsporaricin B* (**2**). — A solution of sporaricin B hydroiodide (**1**, 538 mg) in 56% hydriodic acid was heated for 7 h at 70° in a sealed tube. Evaporation gave a syrup that, after neutralization of the acid with aqueous ammonia, was chromatographed on a column of CM-Sephadex C-25 ($NH_4^+$) resin (12 mL) with 0.2→0.5M aqueous ammonia (concentration, gradually changed). From the ninhydrin-positive fractions, solid **2** was obtained as the base, 180 mg (94%), $[\alpha]_D^{25}$ +140° (*c* 2, water); $^1$H-n.m.r. ($D_2O$): $\delta$ 1.54 (d, 3 H, *J* 6.5 Hz, $CCH_3$), 2.88 (s, 3 H, $NCH_3$), 3.01 (dd, 1 H, $J_{3,4}$ 10.0, $J_{4,5}$ 3.5 Hz, H-4), 4.37 (dd, 1 H, $J_{5,6}$ 3.5, $J_{1,6}$ 3.0 Hz, H-6), 4.85 (t, 1 H, *J* 3.5 Hz, H-5), and 5.46 (d, 1 H, *J* 3.3 Hz, H-1′); *m/z* 319.2343 $(M + H)^+$; Calc. for $C_{14}H_{31}N_4O_4$: 319.2343.

*1,4,2',6'-Tetrakis*-N-(*benzyloxycarbonyl*)-*3*-O-*demethylsporaricin B* (**3**). — To an ice-cold mixture of **2** (1 g, as the base) and anhydrous sodium carbonate (1.3 g) in 1:4 water–methanol (50 mL) was added benzyl chloroformate (2.6 mL) under stirring, and stirring was continued for 3 h. Evaporation gave a residue that was extracted with chloroform, and the extract was washed with water, dried ($Na_2SO_4$), and evaporated to a syrup that was chromatographed on a column of silica gel with 197:3 chloroform–methanol, to give solid **3**; 2.6 g (97%). Recrystallization from benzene gave needles, m.p. 153–154°, $[\alpha]_D^{25}$ +44° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 1705 $cm^{-1}$ (NHCOO-); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 1.04 (d, 3 H, *J* 6.5 Hz, $CCH_3$) and 3.04 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{46}H_{54}N_4O_{12}$: C, 64.62; H, 6.37; N, 6.55. Found: C, 64.53; H, 6.46; N, 6.38.

*1,2',6'-Tris*-N-(*benzyloxycarbonyl*)-*3*-O,*4*-N-*carbonyl-3*-O-*demethylsporaricin B* (**7**) and *1',2',6'-tris*-N-(*benzyloxycarbonyl*)-*4*-N,*5*-O-*carbonyl-3*-O-*demethylsporaricin B* (**4**). — To a solution of **3** (100 mg) in 1,4-dioxane (2.0 mL) was added 0.1M aqueous barium hydroxide (1.5 mL), and the mixture was stirred for 1 h at 60°, t.l.c. with 19:1 chloroform–methanol then showed two marked spots, of **4** ($R_F$ 0.44) and **7** ($R_F$ 0.54). Introduction of an excess of carbon dioxide, followed by evaporation, gave a residue that was extracted with chloroform, and the extract washed with water, dried ($Na_2SO_4$), and evaporated. The syrupy residue was subjected to preparative, thin-layer chromatography on silica gel with 10:1 chloroform–methanol, to give solid **4**, 68 mg (78%), and solid **7**, 13 mg (15%).

Compound **4**: $[\alpha]_D^{23}$ +33° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 1760 $cm^{-1}$ (4,5-cyclic carbamate); $^1$H-n.m.r. ($CDCl_3$): $\delta$ 1.07 (d, 3 H, *J* 6.0 Hz, $CCH_3$) and 2.87 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{39}H_{46}N_4O_{11}$: C, 62.72; H, 6.21; N, 7.50. Found: C, 62.63; H, 6.26; N, 7.35.

Compound **7**: $[\alpha]_D^{23}$ +60° (*c* 0.7, chloroform); $\nu_{max}^{CHCl_3}$ 1755 $cm^{-1}$ (3,4-cyclic carbamate); $^1$H-n.m.r. ($CDCl_3$); $\delta$ 1.03 (d, 3 H, *J* 6 Hz, $CCH_3$) and 2.57 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{39}H_{46}N_4O_{11}$: C, 62.72; H, 6.21; N, 7.50. Found: C, 62.92; H, 6.41; N, 7.27.

*4*-N,*5*-O-*Carbonyl-3*-O-*demethylsporaricin B* (**5**). — A solution of **4** (200 mg) in acetic acid (3.5 mL) was hydrogenolyzed with hydrogen at atmospheric pressure in the presence of palladium black. After filtration, and dilution of the filtrate with water (400 mL), the acid was neutralized with aqueous ammonia, and the solution was placed on a column of CM-Sephadex C-25 ($NH_4^+$) resin (20 mL), and eluted with 0.1→0.5M aqueous ammonia. The fractions containing **5** were lyophilized, to give solid **5**, 73 mg (79%), $[\alpha]_D^{23}$ +86° (*c* 4, water); $\nu_{max}^{KBr}$ 1745 $cm^{-1}$ (cyclic carbamate); $^1$H-n.m.r. ($D_2O$): $\delta$ 1.56 (d, 3 H, *J* 6.7 Hz, $CCH_3$), 3.51 (s, 3 H, $NCH_3$), 4.32 (dd, 1 H, $J_{4,5}$ 7.8, $J_{3,4}$ 7.3 Hz, H-4), 5.44 (dd, 1 H, $J_{4,5}$ 7.8, $J_{5,6}$ 5.3 Hz, H-5), and 5.54 (d, 1 H, *J* 3.7 Hz, H-1'); *m/z* 345.2138 $(M + H)^+$; Calc. for $C_{15}H_{29}N_4O_5$: 345.2138.

*3*-O-*Allyl-1,2',6'-tris*-N-(*benzyloxycarbonyl*)-*4*-N,*5*-O-*carbonyl-3*-O-*demethyl*-

*sporaricin B* (**6**). — To a solution of **4** (1 g) in dry *N*,*N*-dimethylformamide (20 mL) were added allyl bromide (4 mL), barium oxide (2 g), and barium hydroxide monohydrate (400 mg), and the mixture was stirred in the dark for 21 h at room temperature. After addition of chloroform (150 mL), the suspension was filtered, and the filtrate was evaporated under diminished pressure to a syrup that was chromatographed on a column of silica gel with 199:1 chloroform–methanol, to give solid **6**, 899 mg (86%); $[\alpha]_D^{20}$ +40° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 1760 and 1710 $cm^{-1}$; $^1$H-n.m.r. ($CDCl_3$) (at 250 MHz): $\delta$ 1.08 (d, 3 H, *J* 7.0 Hz, $CCH_3$), 2.84 (s, 3 H, $NCH_3$), 3.92 (q, 1 H, $J_{1''A,1''B}$ 12, $J_{1''A,2''} = J_{1''B,2''}$ = 6 Hz, H-1″A), 4.09 (q, 1 H, H-1″B), 5.22 (d with small splittings, 1 H, $J_{2'',3''A(cis)} \sim 10$ Hz, H-3″A), 5.27 (d with small splittings, 1 H, $J_{2'',3''B(trans)} \sim 17$ Hz, H-3″B), and 5.85 (o, 1 H, H-2″). These assignments were confirmed by decoupling experiments.

*Anal.* Calc. for $C_{42}H_{50}N_4O_{11}$: C, 64.11; H, 6.40; N, 7.12. Found: C, 64.22; H, 6.44; N, 6.91.

*3-O-Allyl-1,2′6′,2‴,-tetrakis-N-(benzyloxycarbonyl)-3-O-demethylsporacin A* (**8**). — To a solution of **6** (899 mg) in 1,4-dioxane (18 mL) was added 0.34M aqueous barium hydroxide (14 mL), and the mixture was stirred for 18 h at 60°. After introduction of an excess of carbon dioxide, the suspension was filtered, and the filtrate was evaporated to dryness. To a solution of the residue in dry 1,4-dioxane (23 mL) were added *N*-hydroxysuccinimide ester (637 mg) of *N*-(benzyloxycarbonyl)glycine and triethylamine (500 mg), and the mixture was heated for 2 h at 90°, cooled, and evaporated to a residue that was extracted with chloroform. The extract was washed with water, dried ($Na_2SO_4$), and evaporated to a syrup that was subjected to preparative, thin-layer chromatography on silica gel with 15:1 chloroform–methanol, and the zone containing **8** was collected to give a solid, 612 mg (56%); $[\alpha]_D^{24}$ +35° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 1705 and 1635 $cm^{-1}$; $^1$H-n.m.r. ($CDCl_3$); $\delta$ 1.06 (d, 3 H, *J* 6.5 Hz, $CCH_3$) and 2.90 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{51}H_{61}N_5O_{13}$: C, 64.34; H, 6.46; N, 7.36. Found: C, 64.67; H, 6.75; N, 7.01.

*1,2′,6′,2‴-Tetrakis-N-(benzyloxycarbonyl)-3-O-demethyl-3-O-(2,3-epoxypropyl)-sporaricin A* (**9**). — To a solution of **8** (287 mg) in chloroform (3.0 mL) was added *m*-chloroperoxybenzoic acid (315 mg), and the mixture was stirred in the dark for 16 h at room temperature. After dilution with chloroform (20 mL), the solution was successively washed with 5M aqueous sodium hydroxide and water, dried ($Na_2SO_4$), and evaporated. The syrupy residue was chromatographed on a preparative, thin-layer plate of silica gel with 15:1 chloroform–methanol, to give solid **9**, 226 mg (77%); $[\alpha]_D^{25}$ +38° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 1705 and 1640 $cm^{-1}$; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 1.08 (d, 3 H, *J* 6.0 Hz, $CCH_3$) and 2.96 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{51}H_{61}N_5O_{14}$: C, 63.28; H, 6.35; N, 7.23. Found: C, 63.18; H, 6.46; N, 7.03.

*3-O-(3-Azido-2-hydroxypropyl)-1,2′,6′,2‴-tetrakis-N-(benzyloxycarbonyl)-3-O-demethylsporaricin A* (**10**). — To a solution of **9** (293 mg) in dry *N*,*N*-dimethylformamide (16 mL) was added sodium azide (197 mg), and the mixture was stirred for

16 h at 70°. The usual processing gave a crude product that was purified by preparative, thin-layer chromatography on silica gel with 7:1 chloroform–methanol, to give solid **10**, 243 mg (79%); $[\alpha]_D^{23}$ +30° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 2100 ($N_3$), 1705, and 1635 $cm^{-1}$; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 1.08 (d, 3 H, *J* 7.0 Hz, $CCH_3$) and 2.94 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{51}H_{62}N_8O_{14}$: C, 60.58; H, 6.18; N, 11.08. Found: C, 60.85; H, 6.32; N, 10.56.

*3-O-(3-Amino-2-hydroxypropyl)-3-O-demethylsporaricin A* (**13**). — Compound **10** (243 mg) was hydrogenolyzed as described for **5**, to give solid **13**, 41 mg (38%); $[\alpha]_D^{22}$ +106° (*c* 1, water); $\nu_{max}^{KBr}$ 1630 $cm^{-1}$ (Amide I); $^1$H-n.m.r. ($D_2O$): $\delta$ 1.54 (d, 3 H, *J* 6.5 Hz, $CCH_3$), 3.55 (s, 3 H, $NCH_3$), 4.03 (s, 2 H, gly-$CH_2$), and 5.45 (d, 1 H, *J* 3.5 Hz, H-1′); *m/z* 449.3104 $(M + H)^+$; Calc. for $C_{19}H_{41}N_6O_6$: 449.3088.

*1,2′,6′,2‴-Tetrakis-*N*-(benzyloxycarbonyl)-3-O-(3-cyano-2-hydroxypropyl)-3-O-demethylsporaricin A* (**11**). — A mixture of **9** (120 mg) and potassium cyanide (80 mg) in dry *N,N*-dimethylformamide (3.6 mL) was stirred for 6 h at 70°. Evaporation *in vacuo* gave a residue that was extracted with chloroform. The extract was washed with water, dried ($Na_2SO_4$), and evaporated to a syrup that was chromatographed on a preparative thin-layer of silica gel with 10:1 chloroform–methanol, to give solid **11**, 98 mg (80%); $[\alpha]_D^{25}$ +39° (*c* 1, chloroform); $\nu_{max}^{KBr}$ 2250 (CN), 1700, and 1635 $cm^{-1}$; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 1.08 (d, 3 H, *J* 6.5 Hz, $CCH_3$), 2.48 (d, 2 H, *J* 7.0 Hz, $CH_2CN$), and 2.92 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{52}H_{62}N_6O_{14}$: C, 62.76; H, 6.28; N, 8.45. Found: C, 62.71; H, 6.39; N, 8.09.

*3-O-(4-Amino-2-hydroxybutyl)-3-O-demethylsporaricin A* (**15**). — A solution of **11** (98 mg) in acetic acid (2 mL) was hydrogenolyzed with hydrogen under atmospheric pressure in the presence of palladium black for 4 h. The catalyst was removed by filtration, and the filtrate was hydrogenated with hydrogen at 60 lb.in.$^{-2}$ in the presence of Raney nickel for 8 h. Processing as described for the preparation of **5** gave solid **15**, 19 mg (42%); $[\alpha]_D^{25}$ +132° (*c* 0.37, water); $\nu_{max}^{KBr}$ 1625 $cm^{-1}$; $^1$H-n.m.r. ($D_2O$): $\delta$ 1.52 (d, 3 H, *J* 7 Hz, $CCH_3$), 3.56 (s, 3 H, $NCH_3$), 4.05 (s, 2 H, gly-$CH_2$), and 5.46 (d, 1 H, *J* 3.5 Hz, H-1′); *m/z* 463.3234 $(M + H)^+$; Calc. for $C_{20}H_{43}N_6O_6$: 463.3242.

*1,2′,6′,2‴-Tetrakis-*N*-(benzyloxycarbonyl)-3-O-demethyl-3-O-(2,3-dihydroxypropyl)sporaricin A* (**12**). — To a solution of **9** (152 mg) in 67% aqueous acetone (5.8 mL) was added concentrated sulfuric acid (100 mg) dropwise, with stirring, at room temperature. After 4 h, the acid was neutralized with 4M aqueous sodium hydroxide. Evaporation of the mixture gave a residue that was extracted with chloroform. The crude product obtained on evaporating the extract was subjected to preparative, thin-layer chromatography on silica gel with 10:1 chloroform–methanol, to give solid **12**, 122 mg (79%); $[\alpha]_D^{23}$ +36° (*c* 1, chloroform); $\nu_{max}^{CHCl_3}$ 1705 and 1635 $cm^{-1}$; $^1$H-n.m.r. ($CDCl_3$): $\delta$ 1.06 (d, 3 H, *J* 6 Hz, $CCH_3$) and 2.91 (s, 3 H, $NCH_3$).

*Anal.* Calc. for $C_{51}H_{63}N_5O_{15}$: C, 62.12; H, 6.44; N, 7.10. Found: C, 62.28; H, 6.66; N, 6.84.

*3-O-Demethyl-3-O-(2,3-dihydroxypropyl)sporaricin A* (**14**). — A solution of **12** (81 mg) in acetic acid (2 mL) was treated as described for **5**, to give solid **14**, 21 mg (57%); $[\alpha]_D^{22}$ +109° (*c* 1, water); $\nu_{max}^{KBr}$ 1630 $cm^{-1}$; $^1$H-n.m.r. ($D_2O$): $\delta$ 1.52 (d, 3 H, *J* 7 Hz, $CCH_3$), 3.52 (s, 3 H, $NCH_3$), 4.01 (s, 2 H, gly-$CH_2$), and 5.39 (d, 1 H, *J* 3.5 Hz, H-1′): *m/z* 450.2930 $(M + H)^+$; Calc. for $C_{19}H_{40}N_5O_7$: 450.2928.

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