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Note

Synthesis of 5-deoxy-5-fluorosporaricin A

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In the accompanying paper[1], we reported syntheses of 3-fluoro-, 3-epi-3-fluoro-, and 3,3-difluoro-3-de(methoxy)sporaricin A, and showed that these compounds had lower acute-toxicities than that[2] of sporaricin A (**1**). This paper describes the synthesis of 5-deoxy-5-fluorosporaricin A (**8**), which was undertaken to determine the effect of substitution of the 5-hydroxyl group by fluorine on the antibacterial activity and toxicity.

The tetrakis(*N*-benzyloxycarbonyl) derivative (**3**), prepared by treating sporaricin B (**2**, ref. 2) with benzyl chloroformate, was treated with diethylaminosulfur trifluoride[3] as already described[1]. A monofluoro derivative (**4**) was formed in low yield (31%), together with another product (**9**, 39%) that was a 4,5-cyclic carbamate; compound **9** was, in turn, readily prepared from **3** by alkaline treatment. As fluoride ion is known to act as a base[4], the ions liberated during formation of **4** should abstract a proton from the 5-hydroxyl group of **3**, thus facilitating formation of the cyclic carbamate (**9**). Deprotection of **4** by catalytic hydrogenolysis gave 5-deoxy-5-fluorosporaricin B (**5**). The position and orientation of the fluorine atom introduced were determined from the ^{1}H-n.m.r. spectrum. As H-5 resonated downfield (δ 5.12) as a wide doublet (48 Hz = $^2J_{H,F}$) with small splittings, fluorination was concluded to have occurred at C-5. The large vicinal $^3J_{H-4,F}$ value (30 Hz) indicates[5] that H-4 and F-5 are *trans*-diaxially situated, and the zero magnitude of $^3J_{H-6,F}$ indicates that H-6 and F-5 are in gauche relationship, as dictated[5,6] by the anti-periplanar disposition of the two electronegative nuclei, namely fluorine (at C-5) and oxygen (at C-6). These results indicate that fluorine is axially disposed, that is, in the same orientation as that of the 5-hydroxyl group of sporaricin. This retention of configuration is somewhat surprising, because fluorination by this reagent is believed[7] to proceed via the intermediate (I) through the SN2 process (pathway *a*). In compound **4**, however, approach of the solvated fluoride ion to the intermediate (I) from the α-side will be somewhat hindered by the presence of comparatively bulky substituents at C-6, and this should facilitate the approach of the fluoride ion from the β-side to the carbocation intermediate (pathway *b*), or facilitate the SN1 reaction depicted in pathway *c*. However, further study will be necessary to clarify the mechanism.

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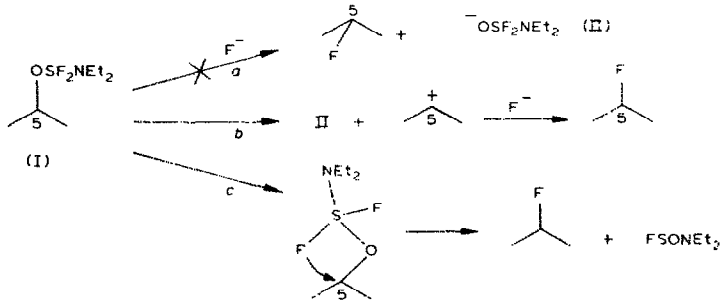
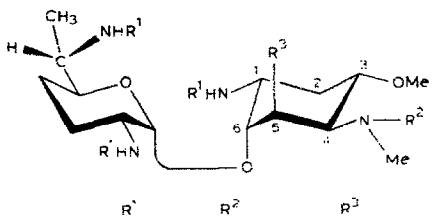
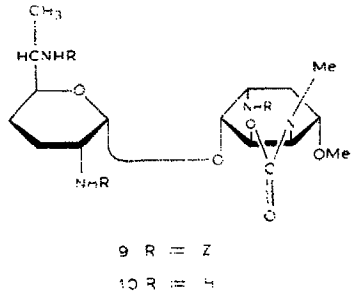


In order to attach the glycyl residue at the 4-methylamino group of **5**, the three amino groups at C-1, 2′, and 6′ were salicylidenated (to give **11**) according to the procedure of Martin *et al.*[8], and *N*-benzyloxycarbonylglycine was attached to the free methylamino group by the active ester method (to give **12**). After desalicylidenation to give **6**, the free amino groups were benzyloxycarbonylated affording **7**. Finally, catalytic hydrogenolysis gave the desired product (**8**). Its structure was confirmed by the ^{1}H-n.m.r. spectrum, in that the $^3J_{H\ 4,F}$ value was large (36 Hz). The ^{13}C-n.m.r. spectral data for **8** and sporaricin A are shown in Table I. No coupling ($^3J_{F,C}$) was observed between F-5 and C-1, and F-5 and C-3, indicating[9] that the fluorine at C-5 is axial.

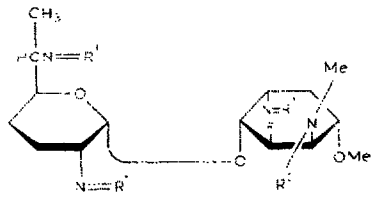


	R′	R²	R³
1	H	$COCH_2NH_2$	OH
2	H	H	OH
3	Z	Z	OH
4	Z	Z	F
5	H	H	F
6	H	$COCH_2NHZ$	F
7	Z	$COCH_2NHZ$	F
8	H	$COCH_2NH_2$	F
	Z:	$CO_2CH_2C_6H_5$	



9 R = Z

10 R = H



	R′	R″
11	CHC_6H_4OH (o)	H
12	CHC_6H_4OH (o)	$COCH_2NHZ$

The structure of the cyclic carbamate (**9**) described here was confirmed by i.r.- and n.m.r.-spectral studies of the de(benzyloxycarbonyl)ated derivative (**10**). The i.r. spectrum absorption at 1740 cm^{-1} of **10** showed the presence of a cyclic carbamate, and the ^{1}H-n.m.r. spectrum showed almost identical J values to those of the *O*-demethyl isomer[1] of **10**, namely, 4-*N*,5-*O*-carbonyl-3-de(*O*-methyl)sporaricin B (ref. 1).

5-Deoxy-5-fluorosporaricin A showed almost no antibacterial activity against common bacteria; this shows that the hydroxyl group at C-5 of sporaricin A plays an important role in determining antibacterial activity.

EXPERIMENTAL

General methods. — These were as in ref. 1

*1,4,2',6'-Tetrakis(*N*-benzyloxycarbonyl)-5-deoxy-5-fluorosporaricin B* (**4**) *and 1,2',6'-tris(*N*-benzyloxycarbonyl)-4-*N,5-O*-carbonylsporaricin B* (**9**). —

TABLE I

CHEMICAL SHIFTS OF ^{13}C-N M R SPECTRA PROTON DECOUPLED OF SPORARICIN A[a] AND 5-DEOXY-5-FLUOROSPORARICIN A[b] (**8**), AND $J_{C,F}$ VALUES OF **8**

Carbon atom	*Chemical shift (p.p.m.)*[c]			
	Fortunicin A[d]	*Sporaricin A*	**8**	$J_{C,F}$ *(Hz)*
1		47.41 d	47.14 d	
2		29.31 t	28.95 t	
3		71.91[e] d	71.34[f] d	
4		56.70 d	55.44	$^2J_{C-4,F}$ 18(d)
5		68.03 d	90.09	$^1J_{C-5,F}$ 179(d)
6		73.34 d	70.06	$^2J_{C-6,F}$ 26(d)
1'	95.4	92.75 d	93.22 d	
2'	51.7	51.90 d	51.75 d	
3'	21.6	21.35 t	21.04 t	
4'	26.3	26.35 t	26.17 t	
5'	70.9	71.04[e] d	71.29[f] d	
6'	49.4	49.72 d	49.56 d	
7'	15.0	15.32 q	15.16 q	
NCH_3		32.06 s	31.51 q	
			31.58 q	
Gly-CH_2		41.36 t	41.25 t	
Gly-CO		168.82 s	169.07 s	
O-CH_3		56.61 q	56.90 q	

[a]In D_2O at ~pD 2. [b]In D_2O at ~pD 3. [c]Measured from an internal reference of 1,4-dioxane, taken as +67.40 p.p.m., multiplicity signs are for the results obtained by off-resonance decoupling. [d]As the sulfate, see ref. 10. [e,f]May be interconverted

Sporaricin B base (**2**, 192 mg) was treated conventionally with benzyl chloroformate (800 mg) to give **3** as a solid, yield 450 mg (90%), $[\alpha]_D^{20}$ +46° (*c* 1, chloroform).

Anal. Calc. for $C_{47}H_{56}N_4O_{12}$: C, 64.96; H, 6.50; N, 6.45. Found: C, 64.72; H, 6.58; N, 6.16.

To a cold (−15°) solution of the solid in dichloromethane (6.5 mL) was added diethylaminosulfur trifluoride (0.45 mL) and the solution was kept for 15 min in the cold, and then for 30 min at room temperature. After addition of water (0.1 mL) followed by stirring for 30 min, dichloromethane (50 mL) was added, and the solution was washed with aqueous sodium hydrogencarbonate (saturated) and water, dried (anhydrous sodium sulfate), and evaporated. T.l.c. (1:1 benzene–ethyl acetate) of the residue showed spots at R_F 0.38 (**9**), 0.72 (trace, **3**), 0.85 (**4**), and 0.95 (trace). Separation of the products on a column of silica gel with benzene–ethyl acetate (6:1 processed to 1:1) gave as solids **4** (142 mg, 31%), recovered **3** (60 mg, 13%), and **9** (155 mg, 39%).

Compound **4** had $[\alpha]_D^{20}$ +57° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.94 (s, 3 H, NCH_3) and 3.37 (s, 3 H, OCH_3).

Anal. Calc. for $C_{47}H_{55}FN_4O_{11}$: C, 64.81; H, 6.37; N, 6.43; F, 2.18. Found: C, 64.99; H, 6.57; N, 6.35; F, 2.32.

Compound **9** had $[\alpha]_D^{20}$ +12° (*c* 1, chloroform); ν_{max}^{KBr} 1755 and 1710 cm^{-1} (cyclic carbamate); ^{1}H-n.m.r. ($CDCl_3$): δ 2.84 (s, NCH_3) and 3.37 (s, OCH_3).

Anal. Calc. for $C_{40}H_{48}N_4O_{11}$: C, 63.15; H, 6.36; N, 7.36. Found: C, 63.36; H, 6.39; N, 7.11.

Compound **9** was also prepared by refluxing overnight a mixture of **3** (800 mg) and sodium hydrogencarbonate (800 mg) in 1:1 aqueous methanol (40 mL), followed by chromatography (silica gel; 5:2→1:1 chloroform–ethyl acetate), to give 588 mg (84%) of **9**.

5-Deoxy-5-fluorosporaricin B (**5**). — A solution of **4** (405 mg) in 0.2M methanolic hydrochloric acid (20 mL) was hydrogenated in the presence of 10% palladium-on-charcoal for 4 h under hydrogen at 1 atm pressure. Filtration followed by evaporation of the filtrate gave a residue that was charged onto a column of Dowex 50W (NH_4^+ form) which, after washing thoroughly with water, was developed with M aqueous ammonia. The eluate was evaporated to give the carbonate of **5** as a solid, yield 146 mg (79%), $[\alpha]_D^{20}$ +132° (*c* 1, methanol); ^{1}H-n.m.r. (20% ND_3 in D_2O): δ 1.02 (d, 3 H, CCH_3), 1.39 (dq, 1 H, *J* 5, 12, 12, 12 Hz, H-4'a), 1.51 (q, 1 H, H-2a), 1.6–1.85 (m, 3 H, H-3'a,3'e,4'e), 2.18 (dt, 1 H, H-2e), 2.37 (s, 3 H, NCH_3), 2.63 (ddd, 1 H, H-4), 2.7–2.85 (m, 2 H, H-2',6'), 3.10 (dq, 1 H, H-1), 3.41 (s, 3 H, OCH_3), ~3.4 (m, 1 H, H-3), 3.53 (m, 1 H, H-5'), 4.06 (narrow m, 1 H, H-6), 4.92 (d, 1 H, H-1'), and 5.12 (ddd, 1 H, H-5); $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ 12, $J_{1,2e} = J_{2e,3}$ ~4, $J_{3,4}$ 10, $J_{4,5} = J_{5,6}$ ~2, $J_{1,6}$ 3.5, $J_{1',2'}$ 3.5, $J_{5,F}$ 48, $J_{4,F}$ 30, and $J_{6,F}$ ~0 Hz.

Anal. Calc. for $C_{15}H_{31}FN_4O_3 \cdot H_2CO_3$: C, 48.47; H, 8.39; N, 14.13; F, 4.79. Found: C, 48.41; H, 7.83; N, 13.42; F, 4.30.

*1,2',6'-Tris(*N*-benzyloxycarbonyl)-4-*N*-(*N*-benzyloxycarbonylglycyl)-5-deoxy-5-fluorosporaricin B* (**7**). — A solution of **5** (180 mg) and salicylaldehyde (720 mg) in methanol (18 mL) was boiled under reflux for 1 h and then evaporated to a syrup, a benzene solution of which was repeatedly evaporated with several additions of benzene (to remove remaining salicylaldehyde) to give **11** as a syrup (405 mg); ^{1}H-n.m.r. ($CDCl_3$): δ 8.17, 8.38, and 8.53 (each s, 1 H, three N=CH). To a solution of the syrup (**11**) in oxolane (9 mL) were added the *N*-hydroxysuccinimide ester (360 mg) of *N*-benzyloxycarbonylglycine and triethylamine (0.3 mL), and the mixture was heated overnight at 37°. Evaporation gave a residue (mainly **12**) which was extracted with chloroform. The organic solution was shaken with 0.2M aqueous hydrochloric acid, and the aqueous layer was washed thoroughly with chloroform, and then evaporated to give crude **6** as a solid, yield 372 mg. To a solution of the solid in methanol (18 mL) were added benzyl chloroformate (750 mg) and anhydrous sodium carbonate (0.9 g), and the mixture was stirred for 1 h at room temperature. Conventional processing gave crude **7** as a solid, which was chromatographed on a column of silica gel (first with benzene and then with 3:1→2:1 benzene–ethyl acetate) to give solid **7**, yield 224 mg (53%), $[\alpha]_D^{20}$ +38° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.83 (s, 3 H, NCH_3), 3.32 (s, 3 H, OCH_3), and 4.55 (d with small splittings of J ~4 Hz, H-5); $J_{5,F}$ 48 Hz.

Anal. Calc. for $C_{49}H_{58}FN_5O_{12}$: C, 63.42; H, 6.30; N, 7.55; F, 2.05. Found: C, 63.13; H, 6.49; N, 7.54; F, 2.27.

5-Deoxy-5-fluorosporaricin A (**8**). — A solution of **7** (40 mg) in 0.2 M methanolic hydrochloric acid (7 mL) was hydrogenated as described for **5**. The product obtained was chromatographed on a column of CM-Sephadex C-25 with 0→0.3M aqueous ammonia. The ninhydrin-positive fractions were freeze-dried and, after neutralization of the resulting solid with 0.2M methanolic hydrogen-chloride, the solution was evaporated to give the solid hydrochloride salt of **8**, yield 22 mg (95%), $[\alpha]_D^{20}$ +69° (*c* 1, methanol); *m/z* 391.2580 (M^+); 392.2645 [$(M + H)^+$], calc. for $C_{17}H_{35}FN_5O_4$: 392.2670; ^{1}H-n.m.r. (D_2O, pD 3): δ 1.36 (d, 3 H, CCH_3), 1.65 (m, 1 H, H-4'a), 1.96 (q, 1 H, H-2ax), 2.05–2.2 (m, 3 H, H-3'e,3'a,4'e), 2.67 (dt, 1 H, H-2e), 3.09 (s, 3 H, NCH_3), ~3.45 (1 H, H-6), 3.49 (s, 3 H, OCH_3), 3.65 (m, 1 H, H-2'), 3.85–4.0 (m, 2 H, H-1,5'), 4.13 (ABq, J 16.5 Hz, $COCH_2NH_2$), 4.15 (dt?, 1 H, H-3), 4.51 (narrow m, 1 H, H-6), 4.63 (dd, with small splittings, 1 H, H-4), 5.31 (d with small quartets, 1 H, H-5), and 5.50 (d, 1 H, H-1'); $J_{1,2a} = J_{2a,2e} = J_{2a,3}$ 12, $J_{1,2e} = J_{2e,3}$ 4, $J_{3,4}$ 11, $J_{4,5}$ ~1.5, $J_{5,6}$ ~3, $J_{1,6}$ <2, $J_{4,F}$ 36, $J_{5,F}$ 45, and $J_{6,F}$ ~0 Hz.

*4-*N*,5-O-Carbonylsporaricin B* (**10**). — A solution of **9** (80 mg) in acetic acid (4 mL) was hydrogenated and the product was isolated as described for **5** to give **10** as the solid carbonate, yield 36 mg (81%), $[\alpha]_D^{20}$ +105° (*c* 1, water); ν_{max}^{KBr} 1740 cm^{-1}; ^{1}H-n.m.r. (20% ND_3 in D_2O): δ 1.02 (d, 3 H, CCH_3), 2.17 (dt, 1 H, H-2e), 2.94 (s, 3 H, NCH_3), 3.17 (dt, 1 H, H-1), 3.43 (s, 3 H, OCH_3), 3.69 (m, 1 H, H-3), 3.77 (t, 1 H, H-4), 4.03 (t, 1 H, H-6), 4.85 (dd, 1 H, H-5), and 4.97 (d, 1 H, H-1); $J_{1,6} = J_{1,2e}$ 4.5, $J_{1,2a}$ 8.5, $J_{2e,3}$ ~4, $J_{2a,2e}$ 13.5, $J_{3,4} = J_{4,5}$ 7.5, $J_{5,6}$ 5 Hz.

Anal. Calc. for $C_{16}H_{30}N_4O_5 \cdot H_2CO_3$: C, 48.56; H, 7.67; N, 13.33. Found: C, 48.81; H, 7.43; N, 13.08.

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