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Synthesis of Sodium Rifamycin S-3-Sulfonate and Its Reaction with Amines[1)]

MASAHIRO TAGUCHI,* TSUNEO KAWASHIMA, NORIO AIKAWA, and GORO TSUKAMOTO

Pharmaceuticals Research Center, Kanebo, Ltd., Tomobuchi-cho, Miyakojima-ku, Osaka 534, Japan

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Rifamycin S (**1**) was allowed to react with Na_2SO_3 under mild alkaline conditions to afford sodium rifamycin SV-3-sulfonate (**3**). Oxidation of **3** with MnO_2 gave sodium rifamycin S-3-sulfonate (**4a**). The reaction of **4a** with aromatic amines and aliphatic secondary amines led to 3-aminorifamycin derivatives **5a**—**g**. A novel cleavage reaction of the ansa-ring occurred to give compounds **7a**—**g**, when **4a** was allowed to react with aliphatic primary amines having at least one hydrogen atom at the α-position.

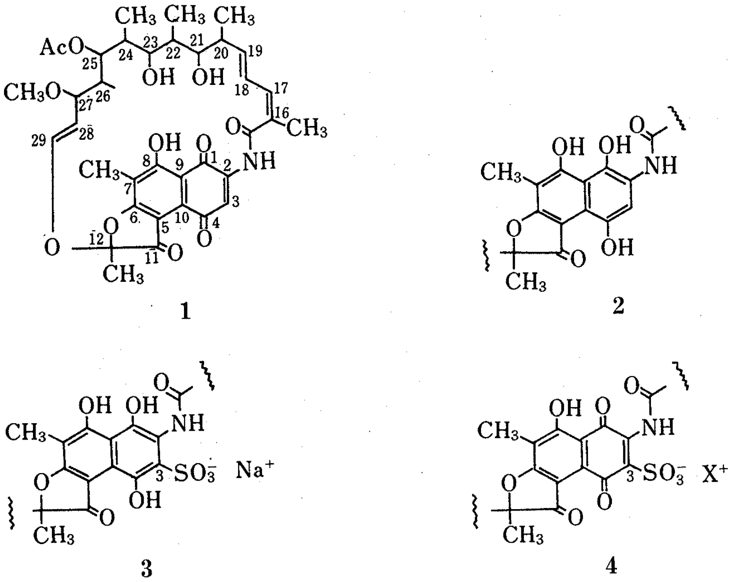
Keywords——rifamycin; 3-sulfonatorifamycin; 3-aminorifamycin; ansa-ring opening; sodium sulfite; sodium bisulfite

Among many 3-substituted rifamycins,[2)] 3-bromorifamycin S[2b)] is an important synthetic intermediate for various rifamycin derivatives,[2b,c,3)] because the bromine atom behaves as a good leaving group toward various nucleophiles.[4)] It is also known that the sulfonato group attached to naphthoquinones can behave as a good leaving group toward some nucleophiles.[4)] We synthesized sodium rifamycin S-3-sulfonate (**4a**), and investigated an alternative synthetic route *via* **4a** to 3-aminorifamycin derivatives,[5a)] which have high antibacterial activity.[5b)] We found a novel cleavage reaction of the ansa-ring of **4a** with aliphatic primary amines having at least one hydrogen atom at the α-position. This paper describes the synthesis of **4a** and its reaction with three types of amines (aromatic amines, aliphatic secondary amines, and aliphatic primary amines).

Synthesis of Sodium Rifamycin S-3-Sulfonate (4a)

It has been reported that 1,4-naphthoquinone reacts with $NaHSO_3$ to afford 1,4-naphthohydroquinone-2-sulfonate.[6)] In the reaction of rifamycin S (**1**) with $NaHSO_3$ in aq. dioxane it was found that only reduction of **1** occurred to afford rifamycin SV (**2**), and no addition product of the sulfonato group was obtained. However, under mild alkaline conditions, the addition reaction of the sulfonato group was found to occur as a main reaction.[7)] That is, rifamycin S (**1**) was allowed to react with Na_2SO_3 in an aq. $NaHCO_3$–dioxane mixture to afford sodium rifamycin SV-3-sulfonate (**3**) in 41% yield. This relatively low isolated yield is considered to be due to an inappropriate isolation procedure, because analytical thin-layer chromatography (TLC) showed that the formation of **3** proceeded almost quantitatively. In the infrared (IR) spectrum ($CDCl_3$) of **3**, the bands due to the sulfonato group were observed at 1222 and 1037 cm^{-1}. The pK_a value of **3** was 1.9 (measured by titration).

Sodium rifamycin S-3-sulfonate (**4a**) was obtained by oxidation of **3** with MnO_2 in aq. dioxane in 86% yield. In the IR spectrum ($CDCl_3$) of **4a** the bands due to the sulfonato group were observed at 1209 and 1042 cm^{-1}. The resolution of the nuclear magnetic resonance (NMR) spectrum ($CDCl_3$) of **4a** was very low, but the NMR spectrum of the tri-



a: $X^+ = Na^+$
b: X^+ = triethylammonium cation
c: X^+ = *tert*-butylammonium cation
d: X^+ = *tert*-octylammonium cation
e: X^+ = 1-adamantylammonium cation

Chart 1

ethylammonium salt **4b** was clear, and the singlet at δ 7.82 ppm due to the proton at the 3-position of **1** was absent. The pK_a value of **4a** was 5.3 (determined spectrophotometrically).

Reaction of 4a with Aromatic Amines and Aliphatic Secondary Amines

It is known that aniline reacts with sodium 1,4-naphthoquinone-2-sulfonate and ammonium 1,2-naphthoquinone-4-sulfonate to afford 2-anilino-1,4-naphthoquinone and 4-anilino-1,2-naphthoquinone, respectively.[6b,8] Sodium rifamycin S-3-sulfonate (**4a**) also reacted with aniline to afford a mixture of 3-anilinorifamycin S (**5a**), 3-anilinorifamycin SV (**6**), and sodium rifamycin SV-3-sulfonate (**3**). The formation of **6** and **3** may be due to the action of the bisulfite anion released during the course of the reaction. After treatment of the mixture with MnO_2, 3-anilinorifamycin S (**5a**) was obtained in 45% yield. Two other examples of the reaction of **4a** with aromatic amines are shown in Table I. The substitution reaction of the 3-sulfonato group of **4a** with aliphatic secondary amines also occurred to afford 3-aminorifamycin derivatives **5d**—**g**. The reaction conditions and the yields of **5** are summarized in Table I. In many cases MnO_2 was included in the reaction solution to prevent the reduction of **4a**.

a: NRR′ = anilino
b: NRR′ = (4-sulfamoylphenyl)amino
c: NRR′ = (4-carbamoylphenyl)amino
d: NRR′ = piperidino
e: NRR′ = morpholino
f: NRR′ = 4-methyl-1-piperazinyl
g: NRR′ = dimethylamino

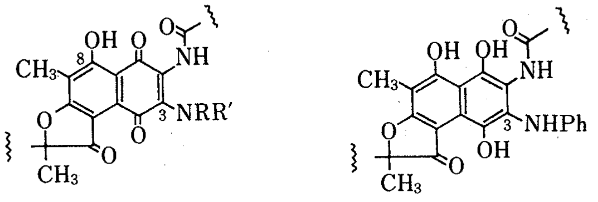


Chart 2

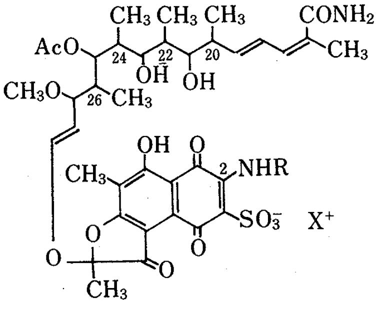
Reaction of 4a with Aliphatic Primary Amines

A novel cleavage reaction of the ansa-ring of **4a** was found, when **4a** was allowed to react with aliphatic primary amines having at least one hydrogen atom at the α-position, such as methylamine, cyclohexylamine, benzylamine *etc.* The reaction conditions and the yields of the cleavage products **7** are summarized in Table II. Compounds **7** were detected as a main product on analytical TLC. The relatively low isolated yields of **7** are presumably due to

Table I. Reaction of **4a** with Aromatic Amines and Aliphatic Secondary Amines

Amine	Molar ratio to **4a**	Reaction conditions °C	h	Solvent	Product	Yield (%)
Aniline[a]	11.3	r.t.	0.3	H_2O	**5a**	45
Sulfanilamide[b]	4.6	50—55	24	DMSO[c]	**5b**	15
4-Aminobenzamide[a]	11.0	50—55	3	DMSO	**5c**	41
Piperidine[b]	5.6	45—50	6	DMSO	**5d**	85
Morpholine[b]	5.4	45—50	3.5	DMSO	**5e**	81
4-Methylpiperazine[b]	4.0	r.t.	17.5	DMSO	**5f**	64
Dimethylamine[a]	3.0	45—50	2	DMSO–H_2O	**5g**	45

a) MnO_2 was used in the post-treatment. *b)* The reaction was carried out in the presence of MnO_2. *c)* Dimethylsulfoxide. r.t. = room temperature.



7

a: R = methyl, $X^+ = Na^+$
b: R = butyl, $X^+ = Na^+$
c: R = isobutyl, $X^+ = Na^+$
d: R = octyl, X^+ = octylammonium cation
e: R = cyclohexyl, X^+ = cyclohexylammonium cation
f: R = 2-adamantyl, X^+ = 2-adamantylammonium cation
g: R = benzyl, X^+ = benzylammonium cation

Chart 3

Table II. Reaction of **4a** with Aliphatic Primary Amines

Amine	Molar ratio to **4a**	Reaction conditions °C	h	Solvent	Product	Yield (%)
Methylamine	11.0	r.t.	19.5	DMSO	**7a**	34
Butylamine	11.0	r.t.	5.5	DMSO	**7b**	34
Isobutylamine	11.0	r.t.	17	DMSO	**7c**	40
Octylamine	6.4	r.t.	23	DMSO	**7d**	11
Cyclohexylamine	6.3	r.t.	23	DMSO	**7e**	22
2-Adamantylamine	5.0	50—55	24	Pyridine	**7f**	13
Benzylamine	15.4	r.t.	22	DMSO	**7g**	38

decomposition during the isolation procedure. The spectral data are shown in Table III. In the NMR spectra ($CDCl_3$) of the 3-aminorifamycin derivatives **5**, a doublet due to the 26-methyl group was observed at δ 0—0.3 and doublets due to the 20-, 22-, and 24-methyl groups at δ 0.5—1.1. The NMR spectra ($CDCl_3$) of **7** showed no signals at δ 0—0.3, and the signal due to the 26-methyl group overlapped the signals due to the 20-, 22-, and 24-methyl groups (δ 0.5—1.1). The downfield shift of the 26-methyl group signal has been related to the cleavage of the ansa-ring.[9,10] The IR spectra of **7** showed the ν_{NH} bands of the primary amide at 3500 and 3400 cm^{-1}, and the $\nu_{s\,SO_2}$ band of the sulfonato group at about 1030 cm^{-1}. These spectral data suggested the cleaved structure of **7**. In order to further confirm the cleaved structure of **7**, compound **7c** was degraded in an aq. AcOH solution as an example. The ansa-chain moiety **8** and the chromophore moiety **9** were obtained in 30% and 35% yields, respectively.

TABLE III. Physical Constants and Elemental Analyses of **7**

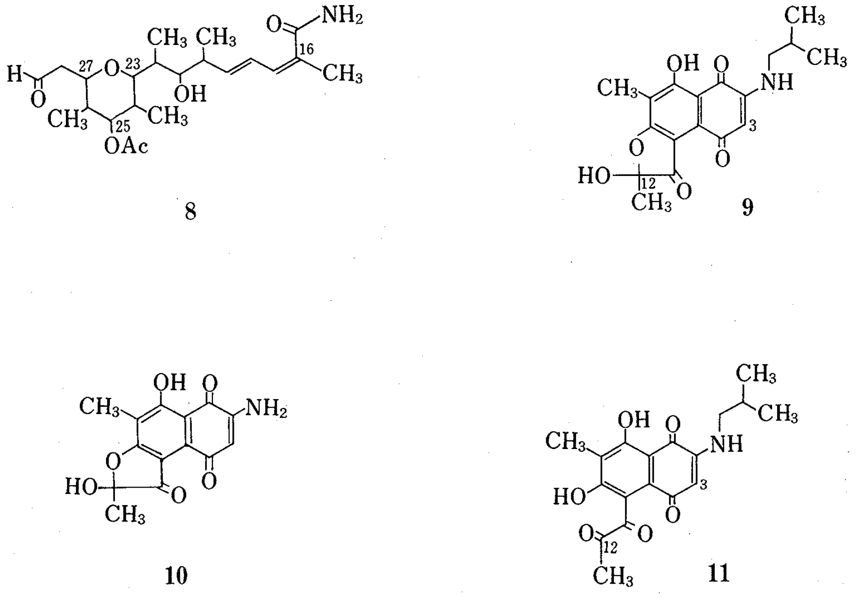
Compound	mp (°C)	IR ($CDCl_3$): cm^{-1}			UV $\lambda_{max}^{CH_3OH}$ nm (log ε)
		$\nu_{N\text{-}H\,(amide)}$	$\nu_{as\,SO_2}$	$\nu_{s\,SO_2}$	
7a	~156 (dec.)	3500 (sh), 3400	1220	1030	244 (4.54), 272 (4.34, sh), 321 (4.02)
7b	~143 (dec.)	3500 (sh), 3400	1220	1032	244 (4.58), 270 (4.35, sh), 321 (4.04)
7c	~150 (dec.)	3500 (sh), 3400	1220	1030	245 (4.60), 270 (4.36, sh), 322 (4.06)
7d	~91 (dec.)	3500 (sh), 3400	*a)*	1025	245 (4.60), 273 (4.37, sh), 325 (4.05)
7e	~135 (dec.)	3500 (sh), 3400	*a)*	1027	245 (4.62), 272 (4.38, sh), 327 (4.08)
7f	~160 (dec.)	3500 (sh), 3400	*a)*	1025	245 (4.57), 273 (4.36, sh), 326 (4.03)
7g	~121 (dec.)	3500 (sh), 3400	*a)*	1025	244 (4.58), 272 (4.34, sh), 321 (4.01)

Compound	NMR ($CDCl_3$)		Formula	Analysis (%) Calcd (Found)			
	20-, 22-, 24-, and 26-C$\underline{H}_3$	2-N$\underline{H}$R		C	H	N	S
7a	*b)*		$C_{38}H_{48}N_2NaO_{15}S \cdot 2H_2O$	52.83 (53.27	6.07 6.30	3.24 3.28)	
7b	*b)*		$C_{41}H_{55}N_2NaO_{15}S \cdot 2H_2O$	54.30 (54.17	6.56 6.36	3.09 3.16)	
7c	*b)*		$C_{41}H_{55}N_2NaO_{15}S \cdot 2H_2O$	54.30 (54.14	6.56 6.52	3.09 3.07)	
7d	0.5—1.1	9.70	$C_{53}H_{83}N_3O_{15}S$	61.55 (61.19	8.09 8.11	4.06 3.98	3.10 3.11)
7e	0.5—1.2	9.70	$C_{49}H_{71}N_3O_{15}S$	60.41 (60.11	7.35 7.52	4.31 4.18	3.29 3.26)
7f	0.5—1.2	10.20	$C_{57}H_{79}N_3O_{15}S$	63.49 (63.01	7.38 7.56	3.90 3.93	2.97 3.04)
7g	0.5—1.1	9.94	$C_{51}H_{62}N_3O_{15}S$	61.93 (61.68	6.32 6.61	4.25 4.05	3.24 3.08)

dec., decomposition; sh, shoulder. *a)* Other bands are overlapped. *b)* The resolution of the spectrum was very low.

The mass spectrum of **8** showed a molecular ion peak at *m/e* 409. The absorption maximum at 244 nm in the ultraviolet (UV) spectrum (EtOH) of **8** supported the dienone structure. The IR spectrum ($CDCl_3$) of **8** showed the bands of the amide group (ν_{N-H}: 3523 and 3402; $\nu_{C=O}$: 1667 cm^{-1}), the formyl group (2737 cm^{-1}), and the acetyl group (1728 cm^{-1}). The NMR spectrum ($CDCl_3$) of **8** exhibited the formyl proton signal at δ 9.65 and the amide proton signal at δ 6.0. The signals due to the protons at the 23-, 25-, and 27-positions were observed at δ 3.26 (dd, $J=10$ and 2 Hz), δ 4.70 (dd, $J=11$ and 5 Hz) and δ 4.03 (ddd, $J=9$, 4, and 2 Hz), respectively. No signal due to the methoxy group was observed. The chemical shifts and the coupling constants of the protons at the 23-, 25-, and 27-positions of **8** were similar to those of the ansa-chain moiety reported by Oppolzer *et al.*[9,11]

The mass spectrum of **9** showed a one-proton adduct peak of the molecule at *m/e* 346, and a base peak at *m/e* 302 ($M^+ - C_3H_7$). The UV spectrum (EtOH) of **9** showed absorption



8 9

10 11

Chart 4

maxima at 232, 272, 312, and 357 nm, and was very similar to that of compound **10**, reported by Oppolzer *et al.*[9] The IR spectrum (KBr) of **9** showed bands due to the amino group (3290 cm^{-1}), the furanone group (1720 cm^{-1}), and the quinone group (1640 and 1605 cm^{-1}), and showed no band due to the sulfonato group. The elimination of the sulfonato group at the 3-position is presumably due to the acidic conditions. The NMR spectrum of **9** in DMSO-d_6 suggests that **9** is in equilibrium with the diketone form **11** (see Experimental). A similar equilibrium was reported in the case of **10**.[12]

In the reaction of **4a** with aliphatic primary amines having no hydrogen atom at the α-position, such as *tert*-butylamine, *tert*-octylamine, and 1-adamantylamine, neither the substitution reaction of the 3-sulfonato group nor the cleavage reaction of the ansa-ring occurred. The sodium salt of **4a** only changed into the corresponding alkylammonium salts **4c**—**e**. The unreactivity of these amines toward **4a** may be due to the steric hindrance of their alkyl substituents.

It is an interesting phenomenon that the reaction sites of **4a** with amines are different according to the type of amine involved, as described above, whereas 3-bromorifamycin S is attacked at the 3-position by amines regardless of the type of amine.[3a]

Experimental

All melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. UV spectra were recorded on a Shimadzu UV-210 spectrometer. IR spectra were obtained with a JASCO IRA-1 or a Shimadzu IR-440 spectrometer. NMR spectra were recorded on a JEOL PS-100 or a Hitachi R-24A spectrometer, using tetramethylsilane (TMS) as an internal reference. Mass spectra (MS) were measured with a Hitachi M-60 mass spectrometer. Analytical TLC was performed on Silica gel 60 F_{254} precoated plates (layer thickness 0.25 mm, E. Merck) with $CHCl_3$–CH_3OH (10 : 1 or 4 : 1) as the developing solvent. Preparative TLC was performed on Silica gel 60 F_{254} precoated plates (layer thickness 0.25, 0.5, or 2 mm, E. Merck) or Silica gel 60 precoated plates (layer thickness 2 mm, E. Merck). Column chromatography was carried out on Silica gel 60 (70—230 mesh, E. Merck). Titration was performed with a Hitachi-Horiba F-7 pH meter. Elemental analyses were performed at the Elemental Analysis Center of Kyoto University.

Sodium Rifamycin SV-3-Sulfonate (3)——A 20 ml portion of water saturated with $NaHCO_3$ was added to a solution of 2.0 g of rifamycin S (**1**) in 20 ml of dioxane. To this mixture, a solution of 7.2 g of Na_2SO_3 in 40 ml of water was added. The mixture was stirred at room temperature for 2 h, then brine and NaCl were added, and the whole was extracted with acetone (200 ml × 2, 100 ml × 1). The extract was concentrated to about 100 ml *in vacuo*, then pH 4 disodium citrate–HCl buffer and NaCl were added to the concentrated solution, and the mixture was extracted with AcOEt. The extract was washed with brine, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The resulting residue was precipitated from benzene–AcOEt to afford 1.4 g of an orange-yellow powder. Recrystallization from hexane–AcOEt afforded 0.92 g (yield, 41%) of **3** as orange crystals. IR $\nu_{\max}^{CDCl_3}$ cm^{-1}: 1222 and 1037 (sulfonato group). UV $\lambda_{\max}^{\mathrm{pH\,2.15\ Na_2HPO_4-citric\ acid\ buffer}}$ nm (log ε): 314 (4.24), 451 (4.08). UV $\lambda_{\max}^{\mathrm{pH\,7.66\ Na_2HPO_4-citric\ acid\ buffer}}$ nm (log ε): 316 (4.23), 454 (4.14). pK_a 1.9 (measured by titration with 0.1 N NaOH in water). mp ~220 °C (dec.). *Anal.* Calcd for $C_{37}H_{46}NNaO_{15}S \cdot H_2O$: C, 54.34; H, 5.92; N, 1.71. Found: C, 54.34; H, 5.97; N, 1.65.

Sodium Rifamycin S-3-Sulfonate (4a)——A 600 mg portion of MnO_2 (Wako Pure Chemical Ind., Ltd.) was added to a solution of 300 mg of **3** in 10 ml of a 1 : 1 water–dioxane mixture. After being stirred at room temperature for 45 min, the reaction mixture was filtered to remove insoluble materials. AcOEt, pH 4 disodium citrate–HCl buffer, and NaCl were added to the filtrate. The whole was shaken, and the organic layer was separated, washed with brine, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The resulting residue was precipitated from hexane–AcOEt to afford 258 mg (yield, 86%) of **4a** as a yellow-brown powder. IR $\nu_{\max}^{CDCl_3}$ cm^{-1}: 1209 and 1042 (sulfonato group). UV $\lambda_{\max}^{\mathrm{pH\,3.66\ Na_2HPO_4-citric\ acid\ buffer}}$ nm (log ε): 281 (4.36), 340 (3.77, shoulder), 400 (3.72). UV $\lambda_{\max}^{\mathrm{pH\,7.66\ Na_2HPO_4-citric\ acid\ buffer}}$ nm (log ε): 260 (4.28, shoulder), 322 (4.37), 514 (3.63). pK_a 5.3 (determined spectrophotometrically from the absorbance at 514 nm in Na_2HPO_4–citric acid buffers). mp ~220 °C (dec.). *Anal.* Calcd for $C_{37}H_{44}NNaO_{15}S \cdot 3/2H_2O$: C, 53.88; H, 5.74; N, 1.70. Found: C, 53.92; H, 5.59; N, 1.70.

Triethylammonium Rifamycin S-3-Sulfonate (4b)——A solution of 70 mg of **4a** in 0.5 ml of CH_3OH was added to a solution of 1.0 g of triethylamine hydrochloride in 1 ml of water, and the reaction mixture was extracted with AcOEt. The extract was evaporated *in vacuo*, and the residue was dissolved in 0.75 ml of CH_3OH. This solution was added to a solution of 1.8 g of triethylamine hydrochloride in 1.8 ml of water. The reaction mixture was extracted with AcOEt, the extract was evaporated *in vacuo*, and the residue was dried over P_2O_5 *in vacuo*. The resulting residue was dissolved in AcOEt, and insoluble materials were filtered off. The filtrate was evaporated *in vacuo*, and the residue was precipitated from hexane–AcOEt to afford 70 mg (yield, 91%) of **4b** as a yellow-brown powder. NMR ($CDCl_3$) δ: 0.20 (3H, d, 26-CH_3), 0.67 (3H, d, 20-, 22-, or 24-CH_3), 0.92 (3H, d, 20-, 22-, or 24-CH_3), 1.05 (3H, d, 20-, 22-, or 24-CH_3), 1.40 (9H, t, methyl protons of triethylammonium cation), 1.73 (3H, s, 12-CH_3), 2.04 (3H, s, 25-OAc), 2.11 (3H, s, 16-CH_3), 2.28 (3H, s, 7-CH_3), 3.10 (3H, s, 27-OCH_3), 3.4 (overlaps other signals, methylene protons of the triethylammonium cation). mp ~160 °C (dec.). *Anal.* Calcd for $C_{43}H_{60}N_2O_{15}S \cdot 3/2H_2O$: C, 57.13; H, 7.02; N, 3.10; S, 3.55. Found: C, 57.17; H, 7.01; N, 2.89; S, 3.45.

3-Anilinorifamycin S (5a)——Aniline (88 mg) was added to a solution of 67 mg of **4a** in 1.5 ml of water. After being stirred at room temperature for 20 min, the reaction mixture was extracted with AcOEt under acidic conditions (dil. aq. H_2SO_4 solution). The extract (about 10 ml) was washed with brine, and dried over $MgSO_4$. Next, 212 mg of MnO_2 was added, and the reaction mixture was stirred at room temperature for 15 min, then filtered to remove insoluble materials. The filtrate was evaporated to dryness *in vacuo*, and the residue was chromatographed on silica gel thin-layer plates with $CHCl_3$–CH_3OH (10 : 1) as a developing solvent. The desired band was collected, and extracted with CH_3OH. AcOEt was added to the extract, and the mixture was washed with dil. aq. H_2SO_4 solution and brine, then dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The resulting residue was precipitated from hexane–$CHCl_3$ to afford 30 mg (yield, 45%) of **5a** as a dark-green powder, which was identical with an authentic sample[13] in terms of analytical TLC behavior. IR $\nu_{\max}^{CDCl_3}$ cm^{-1}: 3465, 3335, 1731, 1677, 1634, 1611. NMR ($CDCl_3$) δ: 0.20 (3H, d, 26-CH_3), 6.8—7.5 (5H, m, aromatic protons of the anilino group), 7.64 (1H, s, amide proton or amino proton), 8.00 (1H, s, amide proton or amino proton), 13.44 (s, 8-OH).

3-(4-Sulfamoylphenyl)aminorifamycin S (5b)——Sulfanilide (2.00 g) and MnO_2 (1.00 g) was added to a solution of 2.00 g of **4a** in 20 ml of DMSO. The mixture was stirred at 50—55 °C for 25 h, then insoluble materials were filtered off. The filtrate was washed with dil. aq. H_2SO_4 solution and water, then dried over $MgSO_4$, and evaporated to dryness *in vacuo* to give 1.09 g of a black solid. The solid was purified by column chromatography (50 : 1 $CHCl_3$–CH_3OH) followed by precipitation from ether–alcohol to afford 0.33 g (yield, 15%) of **5b** as a black powder. mp ~200 °C (dec.). UV $\lambda_{\max}^{CH_3OH}$ nm (log ε): 222 (4.56), 270 (4.53), 329 (4.32), 552 (3.54). IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3460, 3350, 1725, 1630, 1600, 1160. NMR ($CDCl_3$) δ: 0.24 (3H, d, 26-CH_3), 5.02 (2H, s, SO_2NH_2), 6.88 (2H, d, aromatic protons of the (4-sulfamoylphenyl)amino group), 7.76 (2H, d, aromatic protons of the (4-sulfamoylphenyl)amino group), 8.10 (1H, s, amide proton or amino proton), 8.30 (1H, s, amide proton or amino proton), 12.93 (s, 8-OH). *Anal.* Calcd for $C_{43}H_{51}N_3O_{14}S \cdot 3/2H_2O$: C, 57.84; H, 6.10; N, 4.71. Found: C, 57.80; H, 5.90; N, 4.59.

3-(4-Carbamoylphenyl)aminorifamycin S (5c)——This compound was obtained as a black powder by a procedure similar to that described for **5a**. mp ~188 °C (dec.). UV $\lambda_{\max}^{CH_3OH}$ nm (log ε): 222 (4.58), 272 (4.52), 332 (4.34), 558 (3.56). IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3460, 3400, 1725, 1675, 1635, 1610. NMR ($CDCl_3$) δ: 0.21 (3H, d, 26-CH_3), 5.78 (2H, br s, $CONH_2$), 6.84 (2H, d, aromatic protons of the (4-carbamoylphenyl)amino group), 7.65 (2H, d, aromatic protons of the (4-carbamoylphenyl)amino group), 7.88 (1H, s, amide proton or amino proton), 8.16 (1H, s, amide proton or amino proton), 13.05 (s, 8-OH). *Anal.* Calcd for $C_{44}H_{51}N_3O_{13} \cdot H_2O$: C, 62.32; H, 6.30; N, 4.96. Found: C, 62.39; H, 6.04; N, 4.72.

Compounds 5d—g——These compounds were obtained by procedures similar to those described for **5a** and **5b**, and were identical with authentic samples[13] in terms of analytical TLC behavior. Physical constants are as follows.

5d: IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1}: 3460, 3380, 1730, 1710, 1675, 1615. NMR ($CDCl_3$) δ: 0.20 (3H, d, 26-CH_3), 1.50—2.00 (6H, m, piperidine ring), 3.28—3.64 (4H, m, piperidine ring). **5e**: IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1}: 3470, 3380, 1735, 1720, 1680, 1625. NMR ($CDCl_3$) δ: 0.15 (3H, d, 26-CH_3), 3.2—4.1 (8H, m, morpholine ring). **5f**: IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1}: 3460, 3390, 1730, 1720, 1680, 1620. NMR ($CDCl_3$) δ: 0.15 (3H, d, 26-CH_3), 2.30 (3H, s, N–CH_3). **5g**: IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1}: 3460, 3390, 1725, 1715, 1680, 1610. NMR ($CDCl_3$) δ: 0.20 (3H, d, 26-CH_3), 3.05 (9H, s, $N(CH_3)_2$ and OCH_3).

Compounds 7a and 7b——These compounds were obtained from **4a** and 40% aq. methylamine solution or butylamine by a procedure similar to that described for **7c**. The reaction conditions and the physical constants are given in Tables II and III, respectively.

Compound 7c——A 1.0 g portion of isobutylamine was added to a solution of 1.0 g of **4a** in 20 ml of DMSO. After being stirred at room temperature for 17 h, the reaction mixture was poured into AcOEt, and washed with dil. aq. H_2SO_4 solution and brine, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The resulting residue was chromatographed twice on silica gel columns with $CHCl_3$–CH_3OH (10 : 1 and 4 : 1) as eluents. The desired fractions were collected, and evaporated to dryness *in vacuo*. The resulting residue was dissolved in CH_3OH, and this solution was poured into brine to afford a reddish-brown precipitate. The resulting precipitate was extracted with AcOEt under acidic conditions (dil. aq. H_2SO_4 solution), and the extract was washed with brine, dried over Na_2SO_4, and evaporated to dryness *in vacuo*. The resulting residue was precipitated from hexane–$CHCl_3$ to afford 454 mg (yield, 40%) of **7c** as a reddish-brown powder. Physical constants are shown in Table III.

Compound 7d——A 1.0 g portion of octylamine was added to a solution of 1.0 g of **4a** in DMSO. After being stirred at room temperature for 23 h, the reaction mixture was poured into AcOEt, and washed with dil. aq. H_2SO_4 solution and brine, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The resulting residue was chromatographed on a silica gel column with $CHCl_3$–CH_3OH (20 : 1) as an eluent. The desired fractions were collected, and evaporated to dryness *in vacuo*. The resulting residue was chromatographed on silica gel thin layer plates with $CHCl_3$–CH_3OH (7 : 1) as a developing solvent. The desired band was collected, and extracted with $CHCl_3$–CH_3OH (12 : 1), then the extract was evaporated *in vacuo*. The resulting residue was dissolved in AcOEt, and excess octylamine was added to this solution. The mixture was washed with dil. aq. H_2SO_4 solution and water, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. The resulting residue was precipitated from hexane–AcOEt to afford 132 mg (yield, 11%) of **7d** as a brown powder. Physical constants are given in Table III.

Compounds 7e—g——These compounds were obtained from **4a** and cyclohexylamine, 2-adamantylamine, or benzylamine by a procedure similar to that described for **7d**. The reaction conditions and the physical constants are listed in Tables II and III, respectively.

Degradation of 7c——A 1 ml portion of water was added to a solution of 299 mg of **7c** in 6 ml of AcOH. The mixture was stirred at room temperature for 18 h, and the resulting precipitate was collected by filtration. The precipitate was washed with AcOH and AcOEt to give 30 mg (yield, 25%) of **9** as brown crystals. The filtrate and the washings were combined, diluted with water, and extracted with AcOEt. The extract was washed with water, aq. $NaHCO_3$ solution, and brine, then dried over Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel thin-layer plates (three developments with 10 : 1 benzene–CH_3OH), and the desired band detected under a UV lamp (2537 Å) was collected, and extracted with AcOEt. The extract was evaporated *in vacuo* to afford 43 mg (yield, 30%) of **8** as a colorless glass. From the above washings with aq. $NaHCO_3$ solution, 12 mg (yield, 10%) of **9** was also obtained. **8**: MS *m/e*: 409 (M^+). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ε): 244 (4.19). IR $\nu_{\text{max}}^{\text{CDCl}_3}$ cm^{-1}: 3523, 3402, 2737, 1728, 1667. NMR ($CDCl_3$) δ: 1.97 (3H, d, J=1.5 Hz, 16-CH_3), 2.11 (3H, s, 25-OAc), 3.26 (1H, dd, J=10, 2 Hz, 23-H), 4.03 (1H, ddd, J=9, 4, 2 Hz, 27-H), 4.70 (1H, dd, J=11, 5 Hz, 25-H), 6.0 (br, $CONH_2$), 9.65 (1H, t, J=1 Hz, CHO). *Anal.* Calcd for $C_{22}H_{35}NO_6$: c, 64.52; H, 8.61; N, 3.42. Found: C, 62.84; H, 8.70; N, 3.32. **9**: mp ~178 °C (dec.). MS *m/e*: 346 ($M+H^+$), 302 ($M^+-C_3H_7$). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ε): 232 (4.44), 272 (4.30), 312 (3.98), 357 (4.08). IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}: 3290, 1720, 1640, 1605. NMR (DMSO-d_6) δ: 0.90 (6H, d, methyl protons of the isobutyl group), 1.49 (s, 12-CH_3 of **9**), 1.96 (m, 1H, methine proton of the isobutyl group), 2.10 (s, 3H, 7-CH_3), 2.43 (s, 12-CH_3 of **11**), 3.0 (m, 2H, methylene protons of the isobutyl group), 5.49 (s, 3-H of **11**), 5.56 (s, 3-H of **9**). Other signals which disappeared after treatment with D_2O were seen at δ 7.50 (t-like), 7.81 (s), 8.05 (t-like), 12.06 (s), and 12.76 (s). The ratio of **9** to **11** was about 3/2 based on the integral values of the signals due to the 12-methyl groups of **9** and **11**. *Anal.* Calcd for $C_{18}H_{19}NO_6$: C, 62.60; H, 5.55; N, 4.06. Found: C, 62.16; H, 5.52; N, 4.29.

***tert*-Butylammonium Rifamycin S-3-Sulfonate (4c)**——By a procedure similar to that described for **4d**, 0.18 g (yield, 56%) of **4c** was obtained as a brown powder from 0.30 g of **4a** and 0.08 g of *tert*-butylamine. mp ~177 °C (dec.). UV $\lambda_{\text{max}}^{\text{CH}_3\text{OH}}$ nm (log ε): 215 (4.49), 277 (4.42). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1}: 3460, 1720, 1670, 1640, 1590, 1255, 1195, 1040. NMR ($CDCl_3$) δ: 1.42 (9H, s, methyl protons of *tert*-butylammonium cation). *Anal.* Calcd for $C_{41}H_{56}N_2O_{15}S \cdot H_2O$: C, 56.80; H, 6.74; N, 3.23; S, 3.70. Found: C, 56.53; H, 6.99; N, 3.17; S, 3.52.

***tert*-Octylammonium Rifamycin S-3-Sulfonate (4d)**——A solution of 0.50 g of **4a** and 0.50 g of *tert*-octylamine in 3 ml of DMSO was heated at 50—55 °C for 4 h. The reaction solution was poured into 30 ml of 10% aq. citric acid solution, and the reaction mixture was extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and evaporated to dryness *in vacuo*. Upon column chromatography (100 : 3, $CHCl_3$–CH_3OH) followed by precipitation from hexane—ether, the resulting residue afforded 0.15 g (yield, 26%) of **4d** as a yellow-brown powder. mp ~165 °C (dec.). UV $\lambda_{\text{max}}^{\text{CH}_3\text{OH}}$ nm (log ε): 213 (4.47), 276 (4.40). IR $\nu_{\text{max}}^{\text{CHCl}_3}$ cm^{-1}: 3460, 1720, 1670, 1640, 1590, 1255, 1195, 1040. NMR ($CDCl_3$) δ: 1.13 (9H, s, methyl protons of the *tert*-octylammonium cation), 1.51 (6H, s, methyl protons of the *tert*-octylammonium cation), 1.81 (2H, s, methylene protons of the *tert*-octylammonium cation). *Anal.* Calcd for $C_{45}H_{64}N_2O_{15}S \cdot H_2O$: C, 58.55; H, 7.21; N, 3.03; S, 3.47. Found: C, 58.05; H, 7.09; N, 2.77; S, 3.26.

1-Adamantylammonium Rifamycin S-3-Sulfonate (4e)——By a procedure similar to that described for **4d**, 0.89 g (yield, 63%) of **4e** was obtained as a brown powder from 1.20 g of **4a** and 2.06 g of 1-adamantylamine. mp ~204 °C (dec.). UV $\lambda_{\max}^{CH_3OH}$ nm (log ε): 212 (4.50), 276 (4.38). IR $\nu_{\max}^{CHCl_3}$ cm^{-1}: 3460, 1720, 1670, 1640, 1590, 1255, 1040. NMR ($CDCl_3$) δ: 1.60—2.40 (methylene and methine protons of the adamantylammonium cation). *Anal.* Calcd for $C_{47}H_{62}N_2O_{15}S \cdot H_2O$: C, 59.73; H, 6.83; N, 2.96; S, 3.39. Found: C, 59.57; H, 6.96; N, 2.73; S, 3.36.

References and Notes

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