[Chem. Pharm. Bull. 28(8)2309—2317(1980)]

Reaction of 3-Formylrifamycin SV with Formalin in Combination with Primary Alkylamines

Goro Tsukamoto, Masahiro Taguchi, and Norio Aikawa

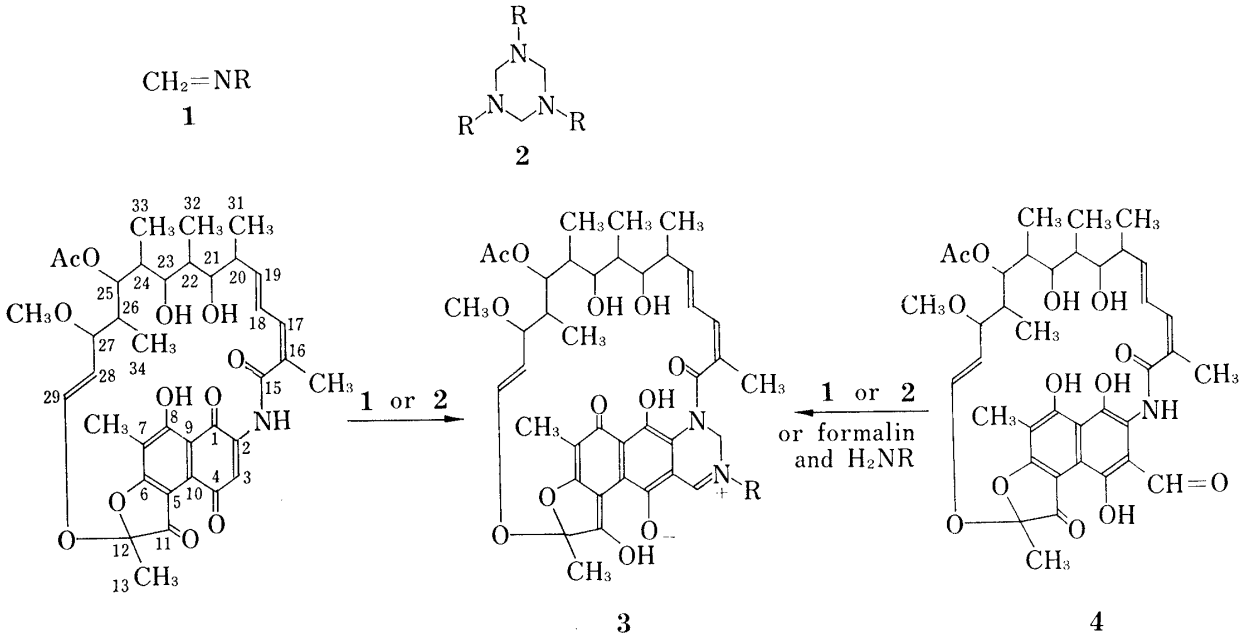
Pharmaceuticals Research Center, Kanebo, Ltd.[1]

(Received December 26, 1979)

3-Formylrifamycin SV (**4**) was found to react with formalin in combination with primary alkylamines to give 2,3-dihydropyrimido[4,5-*b*]rifamycin derivatives **3**. The iminium carbon of **3** was found to be reduced when primary alkylamines with at least one hydrogen atom at the α-position were used. The addition of an alcoholic OH group to the iminium carbon of **3** is also described.

Keywords——rifamycin; Schiff base; formalin; primary alkylamines; hydride transfer; stereoselectivity; pseudo base; UV

We recently reported that rifamycin S reacted with N-methylenealkylamines (**1**) or 1,3,5-trialkylhexahydro-*s*-triazines (**2**) to give 2,3-dihydropyrimido[4,5-*b*]rifamycin derivatives **3**,[2] which were identical with compounds obtained by Marsili and Pasqualucci,[3] who proposed that these compounds contained an oxazine ring. We also suggested that the earlier structure suggested by Maggi *et al.*[4] should be partly revised.[2b]



R = **a**: methyl, **b**: ethyl, **c**: *tert*-butyl, **d**: *n*-octyl, **e**: *sec*-octyl, **f**: *tert*-octyl, **g**: cyclohexyl, **h**: 1-adamantyl, **i**: 1,1-dimethyl-2-hydroxyethyl

Chart 1

1) Location: *Tomobuchi-cho, Miyakojima-ku, Osaka 534, Japan.*
2) *a*) G. Tsukamoto, N. Aikawa, T. Kawashima, M. Taguchi, and I. Utsumi, Japan. Kokai 147097 (1978) [*C.A.*, **90**, 204159*b* (1979)]; *b*) G. Tsukamoto, N. Aikawa, and M. Taguchi, *Chem. Lett.*, **1979**, 1313; *c*) G. Tsukamoto, N. Aikawa, and M. Taguchi, *Bull. Chem. Soc. Jpn.*, "in preparation."
3) L. Marsili and C. Pasqualucci, Ger. Offen. 2428387 (1975) [*C.A.*, **83**, 10188*v* (1975)].
4) N. Maggi, G.G. Gallo, and A. Vigevani, *Tetrahedron Lett.*, **1968**, 1763.

Here we report that 3-formylrifamycin SV[5] (**4**) also reacts with **1** or **2** or, more conveniently, with formalin in combination with primary alkylamines in pyridine, to give **3** in good yields. Moreover, formalin in combination with primary amines which have at least one hydrogen atom at the α-position reduces the iminium carbon of **3** to give 1,2,3,6-tetrahydropyrimido[4,5-*b*]rifamycin derivatives **6**, which are too unstable to be isolated.

N-Methylene-*tert*-octylamine (**1f**) was added to a solution of 3-formylrifamycin SV (**4**) in pyridine. After stirring at 40° for 2.3 hr, the reaction mixture was poured into 10% AcOH to provide a dark-blue colored precipitate, which, upon chromatography on silica gel, afforded a deep-blue colored compound in 88% yield. This compound was identical in terms of ultraviolet (UV), infrared (IR), and nuclear magnetic resonance (NMR) spectra with an authentic sample[2a,c] of the *tert*-octyl derivative **3f**.

In a similar manner, compound **4** was allowed to react with 1,3,5-trimethylhexahydro-*s*-triazine (**2a**) to afford the methyl derivative[2c,3] **3a** in 51% yield.

In the course of the above reactions, the formation of N-substituted 3-iminomethylrifamycin derivatives[6] (**5f** and **5a**) was clearly noted on analytical thin-layer chromatography (TLC). These observations led us to consider that 3-iminomethylrifamycin derivatives **5** might react with formalin to give 2,3-dihydropyrimido[4,5-*b*]rifamycin derivatives **3**. In fact, it was confirmed by analytical TLC that 3-(*tert*-octylimino)methylrifamycin SV (**5f**) reacted with formalin in pyridine to give **3f**.

Thus, in the synthesis of 2,3-dihydropyrimido[4,5-*b*]rifamycin derivatives **3** from 3-formylrifamycin SV (**4**), formalin and primary alkylamines can be used instead of **1** or **2**. In this case, excess formalin relative to amines must be employed, since the reaction of **3** with excess amounts of primary amines yields **5**,[7] especially in the presence of water. For example, an effective transformation of **3f** into **5f** by reaction with excess *tert*-octylamine in pyridine containing water was observed, while no transformation was observed when water was not added to the reaction solution.[8]

The results of this synthetic method for **3** are summarized in Table I. Compounds **3a**, **3c**—**3i** are very soluble in CH_3OH, $CHCl_3$, and AcOEt, but the ethyl derivative **3b** showed very low solubility in these solvents.

Table I. 2,3-Dihydropyrimido[4,5-*b*]rifamycin Derivatives **3** from 3-Formylrifamycin SV (**4**), Alkylamines, and Formalin

R	Conditions (°C)	Conditions (hr)	Yield (%)
a : methyl	42	3.5	68
b : ethyl	66	0.33	90
c : *tert*-butyl	42	3.0	69
d : *n*-octyl	r.t.	4.75	56
e : *sec*-octyl	r.t.	5.0	70
f : *tert*-octyl	43	4.5	83
g : cyclohexyl	42	3.0	85
h : 1-adamantyl	r.t.	2.75	90
i : 1,1-dimethyl-2-hydroxylethyl	45	3.0	78

5) *a*) N. Maggi and P. Sensi, Fr. Patent 1457435 (1966) [*C.A.*, **68**, 104836*x* (1968)]; *b*) N. Maggi and P. Sensi, *Farmaco. Ed. Sci.*, **22**, 316 (1967); *c*) A. Gianantonio, A. Fabrucci, S. Sacerdoti, and A. Soutzo, S. African Patent 6803308 (1969) [*C.A.*, **71**, 50008*v* (1969)].
6) Lepetit S.p.A., Neth. Appl. 6509961 (1966) [*C.A.*, 65, 5462 (1966)].
7) L. Marsili and C. Pasqualucci, Ger. Offen. 2433105 (1975) [*C.A.*, **82**, 156407*s* (1975)]; Gruppo Lepetit S.p.A., Japan. Kokai 87198 (1977) [*C.A.*, **88**, 190922*q* (1978)].
8) The effect of water on the conversion of **3** into **5** with amines will be described in a subsequent paper.

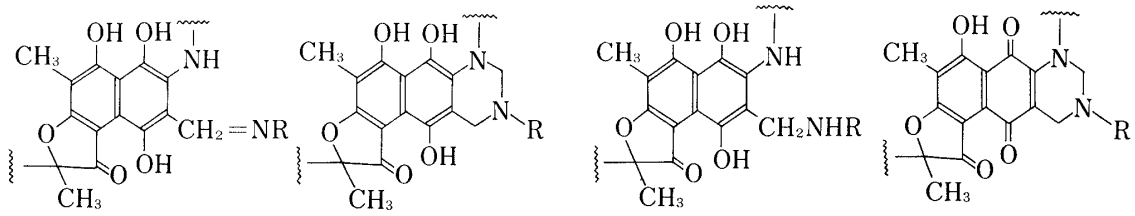
When aniline was used in this reaction instead of primary alkylamines, a green-colored compound was formed. Unfortunately, the green-colored compound decomposed partly during purification by preparative TLC on silica gel. However, the resulting products which contained the green-colored compound as a main component showed three singlet signals at δ 8.98, 15.56, and 16.42 ppm in the NMR spectrum ($CDCl_3$, TMS). Since such signals appeared in common with all 2,3-dihydropyrimido[4,5-*b*]rifamycin derivatives, the green-colored compound was presumed to be the 2,3-dihydropyrimido[4,5-*b*]rifamycin derivative **3** in which R is phenyl.

In the above reactions, yellow-colored compounds were formed as by-products in the case of primary amines which had at least one hydrogen atom at the α-position, *i.e.*, methylamine, *n*-octylamine, *sec*-octylamine, and cyclohexylamine. These yellow-colored compounds were transformed gradually into **3** and other compounds on silica-gel TLC plates. As a typical example, the structure of the yellow-colored compound formed in the synthesis of the methyl derivative **3a** was studied.

It was found that complete transformation of **3a** into the yellow-colored compound occurred when **3a** was treated with 1,3,5-trimethylhexahydro-*s*-triazine (**2a**) in pyridine at 65° for 4 hr. Formalin in combination with aqueous methylamine also changed **3a** to the yellow-colored compound and other compounds. It was observed that the yellow-colored compound had the same *Rf* value and color on the analytical TLC plate as a compound which was formed by the reduction of **3a** with $NaBH_4$.

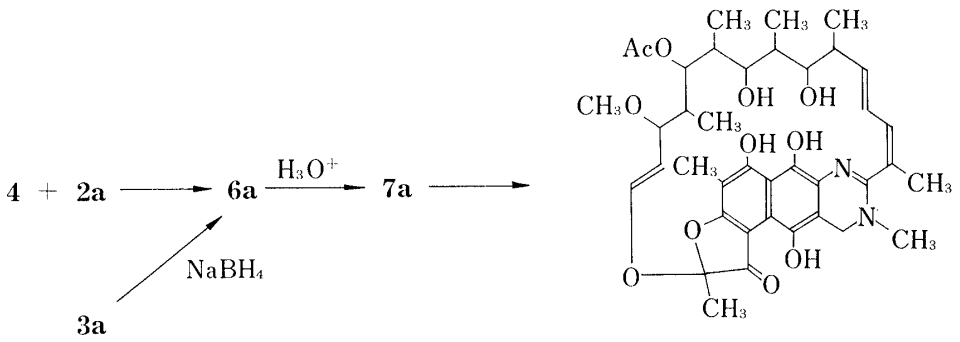
Judging from these observations, the yellow-colored compound was considered to be the 1,2,3,6-tetrahydropyrimido[4,5-*b*]rifamycin derivative **6a**. Unfortunately, compound **6a** was too unstable to be isolated, so its structure was determined as shown in Chart 3.

Compound **8** thus obtained was identical with an authentic sample.[9]



5 **6** **7** **9**

Chart 2



8

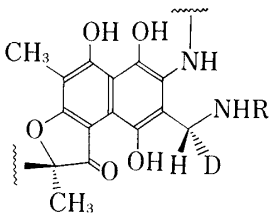
Chart 3

9) J.L. Moore and J.R. McCarthy, *Tetrahedron Lett.*, **1976**, 4541; J.R. McCarthy, J.L. Moore, and D.V. Wysong, *J. Med. Chem.*, **20**, 1272 (1977); Contrary to the report of McCarthy *et al.*, the addition of acid inhibited the transformation of **7a** into 8, as described in "Experimental." This inhibition was probably due to protonation at the nitrogen atom of the 3-(methylamino)methyl group of **7a**.

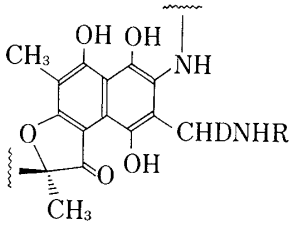
The conversion of **6** to **3** and other compounds on silica-gel TLC plates as described earlier can be interpreted in terms of the oxidation of **6** by air to the quinone form **9**, followed by isomerization to **3**, or by decomposition into other compounds. It will be reported[2c] that compounds **9** are intermediates in the reaction of rifamycin S with **1** or **2**.

The reduction of the iminium carbon of **3** was not caused by treatment with formalin only, primary amines only, the condensation products of formaldehyde with primary amines having no hydrogen atom at the α-position [such as N-methylene-*tert*-octylamine (**1f**) and 1,3,5-tri-*tert*-butylhexahydro-*s*-triazine (**2c**)], or formalin in combination with primary amines having no hydrogen atom at the α-position.

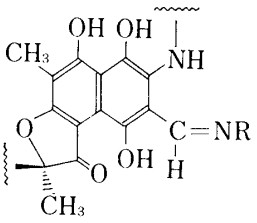
However, in combination with formalin, primary amines having at least one hydrogen atom at the α-position could reduce the iminium carbon of **3**. Thus, we considered that hydride transfer might take place from the α-position of primary amines to the iminium carbon of **3**. However, this proved to be incorrect, and hydride transfer from formaldehyde was demonstrated as follows: compound **4** was allowed to react with formalin-d_2 together with *n*-octylamine in pyridine at 72° for about 2 hr, followed by hydrolysis with dilute aqueous H_2SO_4 in AcOEt to give 3-(*n*-octylamino)methylrifamycin SV (**10d**) in which the 3-(*n*-octylamino)methyl group was deuterated stereoselectively. In the NMR spectrum of **10d** the lower of two doublet signals due to 3-C$\underline{H_2}$NH$(CH_2)_7CH_3$ at δ 4.20 and 3.76 ppm, which are observed in an authentic sample of **7d** and are coupled to each other (J=12 Hz), disappears completely, as shown in Fig. 1. This implies that the iminium carbon of **3d** is subjected to stereoselective reduction. The hydride attack was presumed to take place only from the α-face of **3** due to the steric hindrance of the ansa-ring. Similar stereoselective reduction was also recognized in the case of the reduction of **3d** with $NaBD_4$ in pyridine.



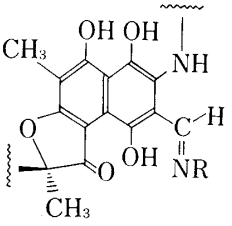
10



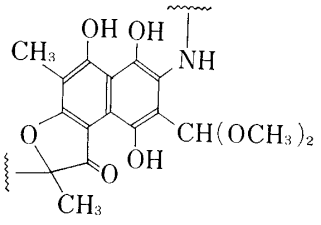
11



12



13

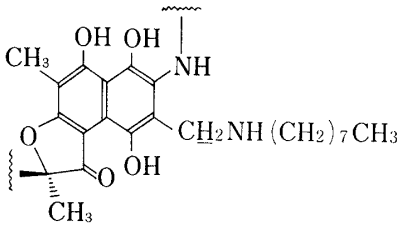


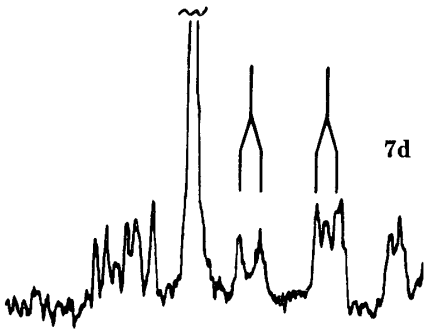
14

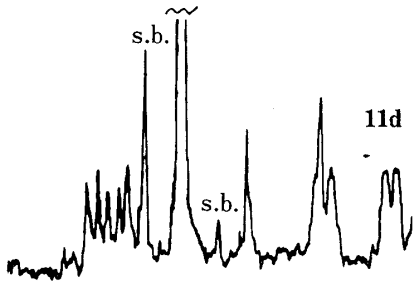
Chart 4

The NMR spectrum of **11d**, which was obtained by reductive amination[9] of **4** with *n*-octylamine and $NaBD_4$, showed two singlet signals at δ 4.20 and 3.76 ppm, as shown in Fig. 1. This apparent non-stereoselective reductive amination was probably due to the presence of species such as **12d** and **13d**.

Formalin in combination with primary amines having no hydrogen atom at the α-position, or the condensation products of formaldehyde with primary amines having no hydrogen atom at the α-position, could not reduce the iminium carbon of **3** as described earlier. This is







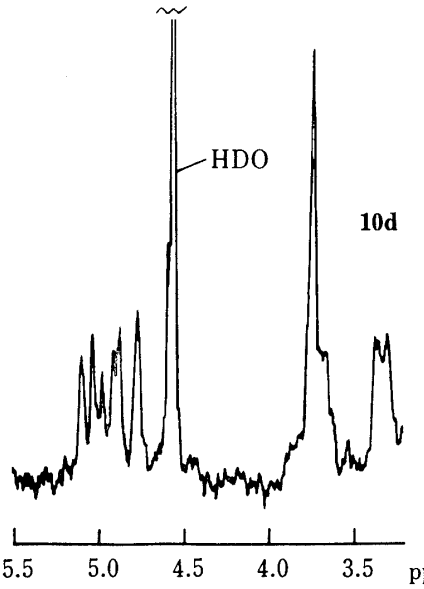


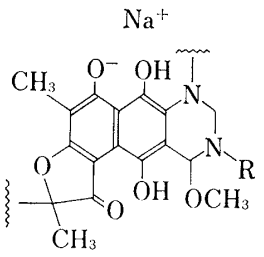
Fig. 1. NMR Spectra of **7d**, **10d**, and **11d** in $CDCl_3$ after Treatment with D_2O.

s.b.: side band.

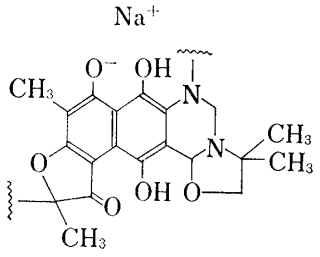
TABLE II. The Degree of Conversion of 2,3-Dihydropyrimido-[4,5-*b*]rifamycin Derivatives **3** into the Adducts **15** in CH_3OH Saturated with $NaHCO_3$ at Room Temperature

R	Content of 3[a] (%)	Content of **15** (%)
a : methyl	0	100
b : ethyl	4	96
c : *tert*-butyl	73	27
d : *n*-octyl	6	94
e : *sec*-octyl	50	50
f : *tert*-octyl	97	3
g : cyclohexyl	55	45
h : 1-adamantyl	90	10

a) The content of **3** was calculated from the absorbance at 620 nm in the UV spectra. About 0.5 mg of **3** was dissolved in 10 ml of CH_3OH saturated with $NaHCO_3$.



15



16

Chart 5

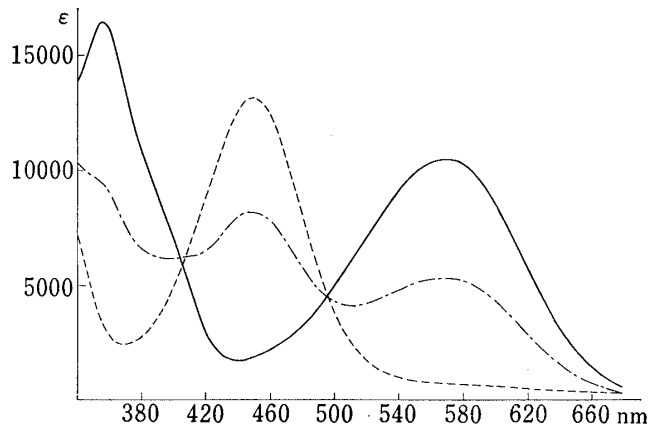


Fig. 2. The UV Spectra of **3i** in Na_2HPO_4-Citric Acid Buffers

——: pH 3.82, ——: pH 6.23, ----: pH 8.04.

probably due to the steric hindrance of the nitrogen substituents of reducing species such as 1,3,5-trisubstituted hexahydro-*s*-triazines.[10]

The maximum absorption at 591 nm (log ε=3.95) in the UV spectrum of **3a** in CH_3OH disappeared completely in CH_3OH saturated with $NaHCO_3$, and a new absorption appeared at 454 nm (log ε=4.13). It seems likely that **3a** was converted to the adduct **15a** in CH_3OH saturated with $NaHCO_3$, because 3-formylrifamycin SV dimethylacetal[5b] (**14**) had maximum absorption at 450 nm (log ε=4.15) in phosphate buffer at pH 7.38.

The degree of conversion of **3** to **15** in CH_3OH saturated with $NaHCO_3$ varied with the substituent R. The results are shown in Table II. In the case of the *tert*-butyl derivative **3c**, 27% of **3c** was converted to **15c** in CH_3OH saturated with $NaHCO_3$, while the 1,1-dimethyl-2-hydroxyethyl derivative **3i** was converted completely to the intramolecular ring-closed species **16** under the same conditions.

As the ring-closed species **16** was stable compared with **15**, attempts were made to isolate the 25-O-desacetyl derivative of **16** by treating the 25-O-desacetyl derivative of **3i** with $NaHCO_3$ in CH_3OH. The 25-O-desacetyl derivative of **16** was obtained as a yellow solid which was slightly green-colored due to contamination with a small amount of the 25-O-desacetyl derivative of **3i**. This solid displayed a maximum absorption in the UV spectrum at 450 nm in CH_3OH which was similar to that which appeared in the solution of **3** in CH_3OH saturated with $NaHCO_3$. The NMR spectrum of this solid in DMSO-d_6 showed no signal at δ 9.02 ppm, corresponding to the iminium proton of the 25-O-desacetyl derivative of **3i**. This solid was insoluble in $CHCl_3$. Generally, metal salts of rifamycin SV derivatives are insoluble in $CHCl_3$. Consequently, this solid may be the sodium salt. On treatment with acid, this solid and species **15** in CH_3OH saturated with $NaHCO_3$ reverted to **3**.

These observations support the structure shown in Chart 5 for species which are formed by the dissolution of **3** in CH_3OH saturated with $NaHCO_3$. (It is not clear which phenolic hydroxyl group dissociates, so the structure of **15** in which the 8-hydroxyl group dissociates is tentative.)[11]

As shown in the UV spectra (Fig. 2) of **3i** in Na_2HPO_4–citric acid buffers, compound **3i** is in equilibrium with its pseudo base **16** at pH about 6. The pK_a determined from the UV spectral data was 6.16. Similar phenomena were observed by Schneider and Müller in a solution of 2,3,5,6-tetrahydro-10b*H*-oxazolo[2,3-*a*]isoquinoline in CH_3OH, where the N,O-acetal form is preferred to iminium salts.[12]

The contents of **15** in CH_3OH saturated with $NaHCO_3$, as shown in Table II, increase in this order: tertiary<secondary<primary carbon atom at the α-position in the substituent R of **3**. This implies that the degree of addition of CH_3OH to the iminium carbon of **3** depends on the steric hindrance of the substituent R of **3**. A similar trend was also observed in the reduction of the iminium carbon of **3d**, **3e**, or **3f** with 1,3,5-trimethylhexahydro-*s*-triazine (**2a**) in pyridine at 65°. Namely, **3d** was most easily reduced, while **3f** was not reduced. In all these cases, the formation of **6a** was noted. The formation of **6a** is presumably due to the reduction of **3a**, which is considered to be produced by exchange reactions of **3d**, **3e**, and **3f** with **2a**.

These observations suggest that the effects of the substituent R of **3** on the reduction of the iminium carbon are similar to those of the substituent of primary amines, used in combination with formalin as described earlier, on their reducing properties. Further work is necessary in order to elucidate these interesting phenomena.

10) H. Möhrle and V. Scharf, *Pharmazie*, **33**, 784 (1978).

11) P. Ferrari and G.G. Gallo, *Farmaco, Ed. Sci.*, **30**, 676 (1975); In this paper, there is an example of dissociation of an 8-hydroxyl group.

12) W. Schneider and B. Müller, *Arch. Pharm.* (Weinheim, Ger.), **295**, 571 (1962).

Experimental

Analytical thin-layer chromatography was performed on silica gel 60 F_{254} pre-coated plates (layer thickness 0.25 mm, E. Merck), using $CHCl_3$–CH_3OH (10: 1) as a developing solvent. Preparative thin-layer chromatography was performed on silica gel 60 F_{254} pre-coated plates (layer thickness 0.5 mm, E. Merck) or silica gel 60 pre-coated plates (layer thickness 2 mm, E. Merck). Column chromatography was carried out on silica gel 60 (70—230 mesh, E. Merck), or Wakogel C-200 (Wako Pure Chemical Ind., Ltd.), or on Wakogel C-200 buffered with phosphate buffer at pH 8.

NMR spectra were recorded on a JEOL PS-100 spectrometer in $CDCl_3$ or DMSO-d_6 solution (with TMS as an internal reference); chemical shifts are reported as values in ppm relative to TMS.

IR spectra were obtained with a Hitachi Perkin-Elmer model 225 spectrometer in $CHCl_3$ solution or in KBr disks.

UV spectra were obtained with a Shimadzu UV-210A spectrometer. CH_3OH solution saturated with $NaHCO_3$ was prepared by adding 4 g of $NaHCO_3$ (special grade, Wako Pure Chemical Ind., Ltd.) to 400 ml of CH_3OH (special grade, Wako Pure Chemical Ind., Ltd.). The suspension was shaken at room temperature for 5 min, and excess $NaHCO_3$ was removed by filtration. The filtrate was used for UV spectroscopy.

Elemental analysis were performed at the Elemental Analysis Center of Kyoto University.

The *tert*-Octyl Derivative 3f from 3-Formylrifamycin SV (4) and N-Methylene-*tert*-octylamine (1f)——A solution of 134 mg of 3-formylrifamycin SV (**4**) in 3 ml of pyridine was treated with 73 mg of N-methylene-*tert*-octylamine (**1f**). After stirring at 40° for 2.3 hr the solution was poured into cold 10% AcOH to give a dark-blue precipitate. The precipitate was separated from the solution by filtration, washed with 10% AcOH and water, and dried over P_2O_5 *in vacuo*. Upon column chromatography (buffered Wakogel C-200, 100: 1 $CHCl_3$–CH_3OH) the resulting precipitate afforded 138 mg (88%) of **3f** as a deep-blue solid, which was identical with an authentic sample.[2a,2c]

The Methyl Derivative 3a from 3-Formylrifamycin SV (4) and 1,3,5-Trimethylhexahydro-*s*-triazine (2a)——1,3,5-Trimethylhexahydro-*s*-triazine (**2a**) (68 mg) was added to a solution of 367 mg of 3-formylrifamycin SV (**4**) in 3 ml of pyridine. After stirring at 40° for 2 hr the solution was poured into cold 10% AcOH to give a slightly reddish dark-blue precipitate. The precipitate was separated from the solution by filtration, washed with 10% AcOH and water, and dried over P_2O_5 *in vacuo*. Upon column chromatography (buffered Wakogel C-200, 80: 3—20: 1 $CHCl_3$–CH_3OH) the resulting precipitate afforded 193 mg (51%) of **3a** as a deep-blue solid, which was identical with an authentic sample of **3a** prepared as described below.

2,3-Dihydropyrimido[4,5-*b*]rifamycin Derivatives 3 from 3-Formylrifamycin SV (4), Primary Alkylamines, and Formalin——As a typical run, the preparation of the cyclohexyl derivative **3g** is described below.

A solution of 698 mg of 3-formylrifamycin SV (**4**) in 10 ml of pyridine was treated with 402 mg of formalin, followed by the addition of 238 mg of cyclohexylamine. After stirring at 42° for 3 hr the solution was poured into cold 10% AcOH to give a dark-blue precipitate. The precipitate was separated from the solution by filtration, washed with 10% AcOH and water, and dried over P_2O_5 *in vacuo*. Upon column chromatography (buffered Wakogel C-200, 50: 1 $CHCl_3$–CH_3OH) the resulting precipitate afforded 670 mg (85%) of **3g** as a deep-blue solid. UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 227 (4.54), 276 (4.31), 312 (4.20), 361 (4.27), 595 (4.04). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1715, 1660, 1604. NMR ($CDCl_3$) δ: 8.84 (1H, s, iminium proton), 14.86 (s, 1-OH or 11-OH), 16.46 (s, 1-OH or 11-OH). *Anal.* Calcd for $C_{45}H_{58}N_2O_{12}\cdot 3/4H_2O$: C, 64.93; H, 7.20; N, 3.36. Found: C, 65.10; H, 7.11; N, 3.20.

Physical constants of other derivatives are as follows. **3a**: UV $\lambda_{max}^{CH_3CN}$ nm (log ε): 226 (4.55), 280 (4.26), 311 (4.20), 360 (4.19), 602 (3.99). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1710, 1650, 1619. NMR ($CDCl_3$) δ: 8.65 (1H, s, iminium proton), 14.72 (s, 1-OH or 11-OH), 16.43 (broad s, 1-OH or 11-OH). *Anal.* Calcd for $C_{40}H_{50}N_2O_{12}\cdot H_2O$: C, 62.49; H, 6.82; N, 3.64. Found: C, 62.51; H, 6.62; N, 3.46. **3b**: UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 226 (4.55), 276 (4.29), 311 (4.18), 360 (4.20), 592 (3.99). IR ν_{max}^{KBr} cm^{-1}: 3460, 1714, 1650, 1612. NMR (DMSO-d_6) δ: 9.54 (1H, s, iminium proton), 14.28 (s, 1-OH or 11-OH), 16.11 (broad s, 1-OH or 11-OH). *Anal.* Calcd for $C_{41}H_{52}N_2O_{12}\cdot 2H_2O$: C, 61.48; H, 7.05; N, 3.50. Found: C, 61.52; H, 6.82; N, 3.30. **3d**: UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 227 (4.54), 277 (4.29), 310 (4.19), 360 (4.23), 595 (4.00). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1714, 1655, 1612. NMR ($CDCl_3$) δ: 8.64 (1H, s, iminium proton), 14.73 (s, 1-OH or 11-OH), 16.43 (s, 1-OH or 11-OH). *Anal.* Calcd for $C_{47}H_{64}N_2O_{12}$: C, 66.49; H, 7.60; N, 3.30. Found: C, 66.29; H, 7.55; N, 3.23. **3e**: UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 226 (4.54), 276 (4.32), 314 (4.17, shoulder), 361 (4.26), 596 (4.02). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3460, 1720, 1655, 1598. NMR ($CDCl_3$) δ: 8.85 (1H, s, iminium proton), 14.99 and 15.03 (s, 1-OH or 11-OH; these two singlet signals should be due to diastereomers resulting from the asymmetric center of the *n*-octyl group), 16.44 (s, 1-OH or 11-OH). *Anal.* Calcd for $C_{47}H_{64}N_2O_{12}\cdot 1/2H_2O$: C, 65.79; H, 7.64; N, 3.27. Found: C, 65.73; H, 7.56; N, 3.11. **3i**: UV $\lambda_{max}^{pH\ 4.02\ citrate\ buffer}$ nm (log ε): 233 (4.46), 274 (4.29), 311 (4.18), 356 (4.21), 571 (4.02). UV $\lambda_{max}^{CH_3OH\ saturated\ with\ NaHCO_3}$ nm (log ε): 226 (4.63), 314 (4.30), 449 (4.14). IR ν_{max}^{KBr} cm^{-1}: 3440, 1715, 1656, 1593. NMR (DMSO-d_6) δ: 9.02 (1H, s, iminium proton), 14.34 (s, 1-OH or 11-OH), 16.12 (broad, 1-OH or 11-OH). *Anal.* Calcd for $C_{43}H_{56}N_2O_{13}\cdot H_2O$: C, 62.45; H, 7.07; N, 3.39. Found: C, 62.50; H, 7.00; N, 3.41. The 25-O-desacetyl derivative of **3i** was obtained from 25-O-desacetyl-3-formylrifamycin SV. UV $\lambda_{max}^{pH\ 4.02\ citrate\ buffer}$ nm (log ε): 234 (4.46), 272 (4.30), 309 (4.17), 356 (4.20), 568 (4.00). UV $\lambda_{max}^{CH_3OH\ saturated\ with\ NaHCO_3}$ nm (log ε): 226

(4.62), 314 (4.28), 450 (4.12). IR ν_{max}^{KBr} cm^{-1}: 3440, 1656, 1593. NMR (DMSO-d_6) δ: 9.02 (1H, s, iminium proton), 14.23 (s, 1-OH or 11-OH), 16.11 (broad, 1-OH or 11-OH). *Anal.* Calcd for $C_{41}H_{54}N_2O_{12}\cdot H_2O$: C, 62.74; H, 7.19; N, 3.57. Found: C, 62.72; H, 6.99; N, 3.57.

Compounds **3c**, **3f**, and **3h** were identical with authentic samples.[2,3]

N,15-Didehydro-15-deoxo-3,15-epi(methano(methylimino)rifamycin SV (8) from 3-Formylrifamycin SV (4) and 1,3,5-Trimethylhexahydro-*s*-triazine (2a)——1,3,5-Trimethylhexahydro-*s*-triazine (**2a**) (161 mg) was added to a solution of 320 mg of 3-formylrifamycin SV (**4**) in 4 ml of pyridine. After stirring at 63—65° for 6 hr the reaction mixture was poured into 60 ml of cold 10% AcOH and extracted with AcOEt, then 40 ml of cold 0.5% H_2SO_4 was added to about 40 ml of the extract. After stirring at room temperature for 2.5 hr the layer of AcOEt was separated, washed with brine, dried over $MgSO_4$, and evaporated to dryness *in vacuo* to afford a dark-brown solid which had a slight odor of AcOH. Upon column chromatography (silica gel 60, 50: 1—24: 1 $CHCl_3$–CH_3OH) the resulting solid afforded 168 mg of a brown solid. The resulting brown solid was dissolved in a small volume of AcOEt-*n*-hexane and allowed to stand in a refrigerator to afford 61 mg of orange crystals which consisted of 3-(methylamino)methylrifamycin SV (**7a**) contaminated with a small amount of N,15-didehydro-15-deoxo-3,15-epi(methano(methylimino))rifamycin SV (**8**). The orange crystals were separated from the solution by filtration. The filtrate, which contained **7a** and **8** (about 1: 1), was evaporated to dryness to afford 77 mg of a dark-brown solid consisting of **8** as the main component. Upon preparative TLC (silica gel 60, 10: 1 $CHCl_3$–CH_3OH) the resulting dark-brown solid afforded 43 mg of **8**, which was identical with an authentic sample prepared as described below.

Synthesis of N,15-Didehydro-15-deoxo-3,15-epi(methano(methylimino))rifamycin SV (8) as an Authentic Sample[9]——A 500 mg portion of 40% aqueous methylamine solution was added to a solution of 149 mg of 3-formylrifamycin SV (**4**) in a mixed solvent of 6 ml of 2-propanol and 4 ml of $CHCl_3$. After stirring at room temperature for 10 min a small amount of $NaBH_4$ was added to the reaction solution cooled with ice-cold water. After stirring for 15 min at the same temperature, 4 ml of acetone was added to the reaction solution, then the reaction solution was brought to room temperature. The reaction solution was acidified with 2% H_2SO_4 and the products were extracted with $CHCl_3$. The extract, which contained **7a** as a main component, was washed with brine, and dried over $MgSO_4$. A small amount of ascorbic acid or AcOH was added to the resulting extract, but the transformation of **7a** into **8**, which was reported by McCarthy *et al.*[9] was not observed. The solution was then washed with aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$, and allowed to stand at room temperature for 3 days. The transformation of **7a** to **8** was noted. The solution was evaporated to dryness *in vacuo* to afford 114 mg of an orange-brown solid. Upon preparative TLC (silica gel 60, 10: 1 $CHCl_3$–CH_3OH) the resulting solid afforded 65 mg of 8 as an orange-brown solid. The UV spectrum of the solid in CH_3OH was in good agreement with the data reported by McCarthy *et al.*[9]

3-(*n*-Octylamino)methylrifamycin SV (7d)——A 300 mg portion of *n*-octylamine was added to a solution of 333 mg of 3-formylrifamycin SV (**4**) in 10 ml of $CHCl_3$. After stirring at room temperature for 5 min, a small amount of $NaBH_4$ was added to the reaction solution cooled with ice-cold water. After stirring for 15 min at the same temperature, 6 ml of acetone was added to the reaction solution, then the reaction solution was brought to room temperature. The reaction solution was acidified with 1% H_2SO_4 and the products were extracted with $CHCl_3$. The extract was washed with brine and dried over $MgSO_4$, followed by evaporation to dryness to afford an orange-brown solid. Upon column chromatography (silica gel 60, 30: 1 $CHCl_3$–CH_3OH; Wakogel C-200, 200: 1—50: 1 $CHCl_3$–CH_3OH) the resulting solid afforded an orange solid. Recrystallization from *n*-hexane–AcOEt gave **7d** (35 mg) as orange crystals. The NMR spectrum of **7d** is shown in Fig. 1. *Anal.* Calcd for $C_{46}H_{66}N_2O_{12}\cdot 1/2H_2O$: C, 65.15; H, 7.96; N, 3.30. Found: C, 65.12; H, 8.09: N, 3.10.

Deuterated 3-(*n*-Octylamino)methylrifamycin SV (11d)——A solution of 58 mg of 3-formylrifamycin SV (**4**) in 2 ml of pyridine was treated with 111 mg of *n*-octylamine. After stirring at room temperature for 5 min, a small amount of $NaBD_4$ (E. Merck) was added to the reaction solution cooled with ice-cold water. The reaction solution, after stirring at the same temperature for 5 min, was acidified with cold 5% H_2SO_4 and the products were extracted with 10 ml of AcOEt. The extract was washed with brine, dried over $MgSO_4$, and evaporated to dryness *in vacuo* to afford 68 mg of an orange solid. The resulting solid was purified by preparative TLC (silica gel 60 F_{254}, 30: 1 $CHCl_3$–CH_3OH), followed by treatment with 10% ascorbic acid, to afford 36 mg of **11d** as an orange-brown solid. The NMR spectrum of **11d** is shown in Fig. 1.

Stereoselectively Deuterated 3-(*n*-Octylamino)methylrifamycin SV (10d) from the *n*-Octyl Derivative 3d and $NaBD_4$——A small amount of $NaBD_4$ was added to a solution of 30 mg of the *n*-octyl derivative **3d** in 1 ml of pyridine cooled with ice-cold water. After stirring at the same temperature for 4 min, 4 ml of AcOEt was added. The resulting solution was added to 30 ml of cold 5% H_2SO_4, followed by the addition of 16 ml of AcOEt. After stirring at room temperature for 1.5 hr the layer of AcOEt was separated, washed with cold 5% H_2SO_4 and brine, dried over $MgSO_4$ and evaporated to dryness *in vacuo* to afford 31 mg of an orange solid. The resulting solid was purified by preparative TLC (silica gel 60 F_{254}, 30: 1 $CHCl_3$–CH_3OH), followed by treatment with 10% ascorbic acid to afford 24 mg of an orange-brown solid, which was identical with **10d** obtained as described below.

Stereoselectively Deuterated 3-(*n*-Octylamino)methylrifamycin SV (10d) from 3-Formylrifamycin SV (4),

***n*-Octylamine, and Formalin-d_2**——A solution of 49 mg of 3-formylrifamycin SV (**4**) in 1 ml of pyridine was treated with 459 mg of formalin-d_2 (E. Merck) dissolved in 0.2 ml of pyridine, followed by the addition of 427 mg of *n*-octylamine dissolved in 0.1 ml of pyridine. After stirring at 72° for 2.25 hr the reaction solution was cooled with ice-cold water, followed by the addition of 20 ml of AcOEt. The resulting solution was added to 30 ml of cold 5% H_2SO_4, followed by the addition of 10 ml of AcOEt. After stirring at room temperature for 1.5 hr the layer of AcOEt was separated, washed with brine and added to 30 ml of 1% H_2SO_4. After stirring at room temperature for 20 min, the layer of AcOEt was separated and washed with brine, dried over $MgSO_4$ and evaporated *in vacuo* to dryness to afford 103 mg of a brown oily solid. The resulting oily solid was chromatographed (Wakogel C-200, 50: 1 $CHCl_3$–CH_3OH) to afford 25 mg of a solid. The resulting solid was further purified by preparative TLC (silica gel 60 F_{254}, 20: 1 $CHCl_3$–CH_3OH), followed by treatment with 10% ascorbic acid to afford 16 mg of **10d** as a greenish brown solid, contaminated with a very small amount of a deep-blue compound (detected by analytical TLC). The NMR spectrum is shown in Fig. 1.

Isolation of the 25-O-Desacetyl Derivative of 16——$NaHCO_3$ (500 mg) was added to a solution of 64 mg of the 25-O-desacetyl derivative of **3i** in 20 ml of CH_3OH. After shaking for 10 min excess $NaHCO_3$ was filtered off. The filtrate was evaporated to dryness *in vacuo*. The resulting residue was dissolved in acetone and insoluble materials were filtered off. The filtrate was concentrated *in vacuo* and was added dropwise to *n*-hexane with stirring to afford 43 mg of a slightly green-colored yellow precipitate. UV $\lambda_{max}^{CH_3OH}$ nm ($E_{1\,cm}^{1\%}$): 226 (499), 315 (225), 450 (145), 600 (11, shoulder). The absorption at 600 nm was due to contamination with a small amount of the 25-O-desacetyl derivative of **3i**.

Acknowledgement The authors are grateful to Professor S. Oae of the University of Tsukuba for valuable advice and discussions. Thanks are also due to Dr. I. Utsumi, Director of this Research Center, for his encouragement.