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Chemical Modification of the Amide Group of Rifamycin S[1)]

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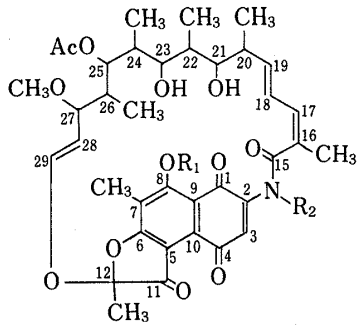
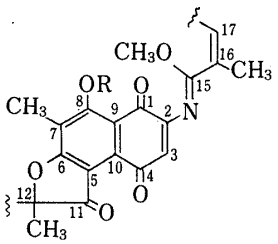
Methylation of rifamycin S (**1**) with MeI in CH_3CN or dimethylformamide (DMF) in the presence of Ag_2O afforded 8-*O*-methyl-*N*-methylrifamycin S (**3**). Demethylation of **3** with MgI_2·ether, followed by treatment with L-ascorbic acid and MnO_2, led to *N*-methylrifamycin S (**4**). Rifamycin S imino methylethers **5** and **6** gave the corresponding oxazolorifamycin derivatives **7** and **8** on reduction with L-ascorbic acid.

Keywords——rifamycin; *N*-methylrifamycin; oxazolorifamycin; rifamycin imino methylether; methyl iodide; magnesium iodide ether solvate

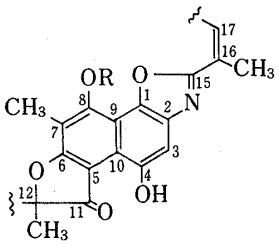
Many chemical modifications of the chromophore and the ansa-ring of rifamycin S (**1**) are known.[2,3)] With respect to the amide group of the ansa-ring, *O*-methylation to rifamycin S imino methylether (**6**) has been reported.[4)] Other modifications include cyclizations between the amide group and the chromophore.[3a,5)] This paper describes a modification of **1** to *N*-methylrifamycin S (**4**), providing another example of the chemical modification of the amide group.



1: $R_1=R_2=H$
2: $R_1=CH_3$, $R_2=H$
3: $R_1=R_2=CH_3$
4: $R_1=H$, $R_2=CH_3$



5: $R=CH_3$
6: $R=H$



7: $R=CH_3$
8: $R=H$

Chart 1

Oppolzer and Prelog obtained 8-*O*-methylrifamycin S (**2**) and **6** by the reaction of **1** with MeI in a $CHCl_3$–CH_3OH mixture in the presence of Ag_2O.[4)] When the reaction solvent was replaced by CH_3CN it was found that *N*-methylation of the amide group of **1** besides 8-*O*-methylation proceeded to afford 8-*O*-methyl-*N*-methylrifamycin S (**3**) in 58% yield. 8-*O*-Methylrifamycin S imino methylether (**5**) was also formed, but this compound was very sensitive to light, and was mostly changed into other compounds during the isolation procedure. The infrared (IR) and the nuclear magnetic resonance (NMR) spectra of **3** showed the tertiary amide $\nu_{C=O}$ at 1660 cm^{-1} and two new singlets at δ 3.34 (NCH_3) and δ 3.97 (8-

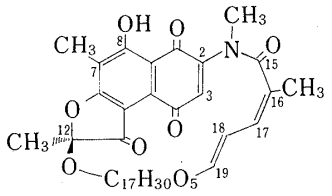
OCH_3), respectively. The IR spectrum of **5** showed the strong imino $\nu_{C=N}$ at 1633 cm^{-1}. Reduction of **5** with L-ascorbic acid in aqueous tetrahydrofuran (THF) gave the oxazolorifamycin derivative **7**. The IR and the NMR spectra of **7** showed the strong $\nu_{C=N}$ band of the oxazole ring[6] at 1668 cm^{-1} and a singlet at δ 3.99 (8-OCH_3), respectively. The structure of **7** was supported by the similar conversion of **6**[4] into **8**, which was identical with an authentic sample.[3a]

N-Methylation of the amide group of **1** in dimethylformamide (DMF) proceeded regiospecifically to afford **3** in 73% yield, and the formation of **5** was not observed on analytical thin layer chromatography (TLC) plates.

Demethylation of the 8-*O*-methyl group of **3** with MgI_2·ether, followed by treatment with L-ascrobic acid and MnO_2,[7,8] gave *N*-methylrifamycin S (**4**) in about 29% yield. The IR spectrum of **4** showed the amide $\nu_{C=O}$ at 1659 cm^{-1} and the δ_{O-H} of the 8-hydroxy group at 1416 cm^{-1}.[9]

The NMR spectra of rifamycins reflect the stereochemistry of the ansa-ring.[10] Judging from the close resemblance of the NMR spectra of **4** and **6** (see Experimental), the amide group of **4** seems to be present in the conformation **9**, in which the C(2)–N and the C(15)–C(16) bonds take a s-*syn* conformation, because the X-ray analysis of **6**[11] has shown that the imino group has the configuration **10** in which the C(2)–N and the C(15)–C(16) bonds are in a *cis* relationship.

N-Methylrifamycin S (**4**) has much lower activities against microorganisms than **1**, as shown in Table I.



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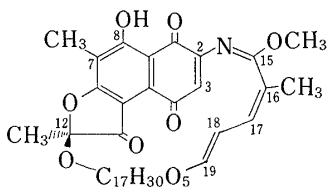

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Chart 2

TABLE I. Minimal Inhibitory Concentrations (MIC) of **1** and **4** against Microorganisms

Microorganism	MIC	
	1	**4**
Staphylococcus aureus FDA 209P JC-1	0.01	33
Staphylococcus aureus Terajima	0.01	100
Staphylococcus aureus MS-353	0.01	100
Staphylococcus aureus 209P	0.01	100
Staphylococcus aureus Smith IID 803	0.03	100
Staphylococcus epidermidis	0.03	>100
Micrococcus luteus ATCC 9341	0.01	>100
Micrococcus luteus R-1	>100	>100
Bacillus anthracis	0.1	>100
Bacillus subtilis ATCC 6633	0.1	>100
Bacillus cereus IAM 1029	1	>100
Escherichia coli NIHJ JC-2	>100	>100
Escherichia coli K-12 J53/R478 R-1	>100	>100

Experimental

Analytical and preparative TLC were performed on Silica gel 60 F_{254} pre-coated plates (0.25 mm, Merck) and Silica gel 60 pre-coated plates (2 mm, Merck), respectively. Column chromatography was carried out on Silica gel 60 (70—230 mesh, Merck). NMR spectra were recorded on a JEOL PS-100 spectrometer with tetramethylsilane (TMS) as an internal reference. IR spectra were obtained with a Shimadzu IR-440 spectrometer. Ultraviolet (UV) spectra were obtained with a Shimadzu UV-210A spectrometer. Melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. Elemental analyses were performed at the Elemental Analysis Center of Kyoto University. The minimal inhibitory concentrations were determined by the agar dilution method.

8-*O*-Methyl-*N*-methylrifamycin S (3) and the 8-*O*-Methyloxazolorifamycin Derivative 7——A mixture of 4.0 g of **1**, 20 ml of MeI, and 4.0 g of Ag_2O in 100 ml of CH_3CN was stirred at room temperature (r.t.) for 10 h. Insoluble materials were filtered off, and the filtrate was evaporated. The residue was column-chromatographed (2 : 1, hexane–AcOEt) to afford two yellow eluates. The first eluate was evaporated, and the residue was dissolved in THF. After treatment with excess L-ascorbic acid in aq. THF, the reaction mixture was extracted with AcOEt. The extract was washed with water and brine, dried over $MgSO_4$, and then evaporated. The residue was purified by preparative TLC (1 : 1, hexane–AcOEt), followed by precipitation from hexane–AcOEt, to afford 373 mg (yield, 9%) of **7** as an orange powder. The second eluate was evaporated, and the residue was precipitated from hexane–AcOEt to afford 2.42 g (yield, 58%) of **3** as a yellow powder. **3**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1735, 1670, 1660 (shoulder, amide $\nu_{C=O}$), 1630, 1610, 1589. NMR ($CDCl_3$) δ: 3.34 (s, NCH_3), 3.97 (s, 8-OCH_3). **7**: IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3520, 1710, 1668 (oxazole ring), 1633, 1605, 1538. NMR ($CDCl_3$) δ: −0.58 (3H, d, 26-CH_3), 0.56 (3H, d, 20-, 22-, or 24-CH_3), 0.87 (3H, d, 20-, 22-, or 24-CH_3), 1.15 (3H, d, 20-, 22-, or 24-CH_3), 1.85 (3H, s, 12-CH_3), 2.02 (3H, s, 25-OAc), 2.37 (3H, s, 16-CH_3), 2.53 (3H, s, 7-CH_3), 3.10 (3H, s, 27-OCH_3), 4.00 (3H, s, 8-OCH_3), 7.37 (1H, s, 3-H).

Compound **3** was also obtained in a higher yield as follows. A mixture of 4.0 g of **1**, 20 ml of MeI, and 4.0 g of Ag_2O in 100 ml of DMF was stirred at r.t. for 12.5 h. Insoluble materials were filtered off, and AcOEt was added to the filtrate. The solution was washed with brine to remove DMF, dried over $MgSO_4$, and then evaporated. The residue was purified by column chromatography (1 : 1, hexane–AcOEt), followed by precipitation from hexane–AcOEt, to afford 3.06 g (yield, 73%) of **3**.

8-*O*-Methylrifamycin S Imino Methylether (5)——The first eluate, which was obtained by a procedure similar to that described for **7**, was evaporated and the residue was column-chromatographed twice (20 : 1, $CHCl_3$–acetone and then $CHCl_3$), then preparative TLC (10 : 1, $CHCl_3$–acetone) was performed. The following procedures were performed in the dark. The desired yellow band was collected, and extracted with AcOEt. Hexane was added to the extract, and the solution was concentrated to afford **5** as a yellow powder. Compound **5** was very sensitive to light. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500, 1733, 1673, 1633 ($\nu_{C=N}$), 1585.

The Oxazolorifamycin Derivative 8——A mixture of 100 mg of **6**[4] and 500 mg of L-ascorbic acid in 10 ml of CH_3OH was stirred at r.t. The color of the solution changed from yellow to reddish-orange. Excess citric acid and water were added, and the color of the solution gradually changed to yellow. The reaction mixture was extracted with AcOEt, and the extract was washed with brine, dried over $MgSO_4$, then evaporated. The residue was crystallized from aq. CH_3OH to afford 78 mg (yield, 81%) of **8** as yellow crystals; this product was identical with an authentic sample[3a] in terms of analytical TLC behavior (2 : 1, $CHCl_3$–acetone) and the NMR spectrum ($CDCl_3$).

***N*-Methylrifamycin S (4)**——Magnesium iodide ether solvate [prepared from 0.41 g of magnesium and 2.00 g of iodine in a mixed solvent of 8 ml of ether and 16 ml of benzene[12]] was added to a solution of 2.22 g of **3** in 80 ml of benzene. The mixture was stirred at r.t. for 4 h, then a solution of 5 g of L-ascorbic acid in aq. THF was added. The whole was stirred at r.t., then AcOEt was added and the reaction mixture was washed with 10% aq. L-ascorbic acid and brine, dried over $MgSO_4$, and evaporated. A mixture of the residue and 17.8 g of MnO_2 (70%, Wako Pure Chemical Ind., Ltd.) in 40 ml of $CHCl_3$ was stirred at r.t. for 3 h. Insoluble materials were filtered off, and the filtrate was evaporated. The residue was column-chromatographed (2 : 1, hexane–AcOEt and then AcOEt). Elution with hexane–AcOEt (2 : 1) afforded two yellow eluates. The first eluate was evaporated, and the residue was crystallized from hexane–AcOEt to afford 417 mg of **4** as orange crystals. From the second eluate, 509 mg (yield, 23%) of **3** was recovered. The AcOEt eluent afforded a violet eluate in which **4** was present in a salt form. This eluate was washed with dil. aq. citric acid and brine, dried over $MgSO_4$, and then evaporated. The residue was crystallized from hexane–AcOEt to afford 219 mg of **4** as orange crystals which were contaminated with a small amount of **3** (analytical TLC, 1 : 1, hexane–AcOEt). The yield of **4** was about 29%. **4**: mp ~180 °C (dec.). UV $\lambda_{max}^{pH\,7.0\ phosphate\ buffer}$ nm (log ε): 322 (4.27), 397 (3.57), 528 (3.47). IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3480, 1732, 1659 (shoulder, amide $\nu_{C=O}$), 1650, 1612, 1599, 1416 (δ_{O-H} of 8-OH). NMR ($CDCl_3$) δ: 0.60—1.14 (m, 20-, 22-, 24-, 26-CH_3), 1.70, (s, 12-CH_3), 2.06 (s, 16-CH_3), 2.10 (s, 25-OAc), 2.25 (s, 7-CH_3), 3.16 (s, 27-OCH_3), 3.37 (s, NCH_3), 4.95 (dd, J = 12, 10 Hz, 28-H), 5.15 (d, J = 3 Hz, 25-H), 5.34 (dd, J = 14, 9 Hz, 19-H), 5.70 (dd, J = 14, 11 Hz, 18-H), 5.86 (d, J = 12 Hz, 29-H), 6.00 (br d, J = 11 Hz, 17-H), 6.43 (s, 3-H), 12.95 (s, 8-OH). [**6**: NMR ($CDCl_3$) δ: 0.60—1.14 (m, 20-, 22-, 24-, 26-CH_3), 1.69 (s, 12-CH_3), 2.01 (s, 16-CH_3), 2.08 (s, 25-OAc), 2.24 (s, 7-CH_3), 3.15 (s, 27-OCH_3), 3.95 (s, 15-OCH_3), 4.94 (dd, J = 12, 10 Hz, 28-H), 5.16 (d, J = 3 Hz, 25-H), 5.35 (dd, J = 14, 9 Hz, 19-H), 5.64 (dd, J = 14, 11 Hz, 18-H), 5.86 (d, J = 12 Hz, 29-H), 5.90 (s, 3-H), 6.04 (br d, J = 11 Hz, 17-H), 13.21 (s, 8-OH).] *Anal.* Calcd for $C_{38}H_{47}NO_{12} \cdot 1/2H_2O$: C, 63.50; H, 6.73; N, 1.95. Found: C,

63.49; H, 6.76; N, 1.74.

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