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Study of the Reaction of 3-Formylrifamycin SV with Gaseous Ammonia and Acetone[‡]

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In the second stage of our study concerning the search for new antibacterial rifamycin antibiotics, reaction of the aldehydes 3-formylrifamycin SV (**1**) and 25-*O*-deacetyl-3-formylrifamycin SV with ammonia and acetone has been investigated. A new synthetic method for the preparation of a new group of rifamycin derivatives with a cyclic substituent at C-3 having a 4-piperidone structure, represented by compounds **6a**, **6b**, and **7a**, has been developed. The structures of the compounds and a reaction mechanism have been proposed on the basis of mass spectrometry results as well as 1D and 2D ^{1}H and ^{13}C NMR analysis. The results of the in vitro tests confirm the antituberculous activity of the synthesized compounds. Furthermore, **6a**, which is isolated in good yield, is a promising substrate for a new class of rifamycin derivatives.

Introduction

The purpose of the research carried out by our group is to find new semisynthetic rifamycins to complement the already known drug "arsenal" used against pathogenic mycobacteria like *M. tuberculosis*. New emerging resistance phenomena found with commercially available rifamycins like rifampicin, rifapentine and rifabutin call for new strategies and/or molecules. Rifamycins are characterized by a macrocyclic structure containing a naphthalenic chromophore encircled by a polyketide ansa chain (handle). Clinical testing and studies of structure–biological activity relationships have shown that the best activities are achieved if structural modifications are made at the positions C-3 and C-4 of the aromatic chromophore. The aim of this work is to replace the enamine fragment C-3–C=N found in rifampicin and rifapentine, which links the additional synthetic substituent to the rifamycin backbone, with another moiety like, for example, an amine C-3–*C*–N, an alkene C-3–*C*=*C* or an alkane C-3–*C*–*C*, which should display a higher resistance towards hydrolytic agents. Valuable substrates for this investigation, as in many semisynthetic rifamycins, are 3-formylrifamycin SV (**1**) and its 25-deacetyl derivative.

Numerous products of the reaction of **1** with ammonia derivatives are known. Rifampicin and rifapentine are hydrazone derivatives of **1**. Many products of the reaction of **1** with amines are known, but syntheses of rifamycin derivatives using ammonia are relatively scarce. Kump and Bickel, in the reaction of rifamycin S with liquid ammonia, obtained a product in which an =NH group was introduced in place of the carbonyl oxygen atom at C-1, whereas the reaction of rifamycin S with gaseous ammonia afforded the 3-aminorifamycin S derivative in low yield and selectivity.[1] Marsili et al. obtained 3-amino-4-deoxo-4-iminorifamycin S from the reaction of 3-aminorifamycin S with gaseous ammonia in THF.[2,3] From the condensation of this compound with various ketones, and especially with *N*-substituted 4-piperidones in the presence of reducing agents, a new, interesting group of spiropiperidylrifamycins was obtained. The formation of 4-deoxo-3,4-[2-spiro-(*N*-isobutylpiperidyl)]-(1*H*)-imidazo-(2,5-dihydro)rifamycin S (rifabutin), which has since become one of the most used rifamycin drugs, was the crowning achievement of their studies.[4–6]

In the reductive amination of **1** with ammonia and sodium cyanoborohydride in the presence of hydrogen chloride in methanol at room temperature, McCarthy et al. obtained a mixture of products from which they isolated *N*,15-didehydro-15-deoxo-3,15-epi[methano(imino)]rifamycin SV and 3-(aminomethyl)rifamycin SV.[7,8]

In the first part of our work, the products of the reaction of **1** with ammonia or primary alkylamines were characterized.[9] In the second part, presented in this contribution,

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we isolated and characterized the products of the reaction of **1** and of 25-*O*-deacetyl-3-formylrifamycin SV (as supplementary model substance) with ammonia and acetone.[10,11]

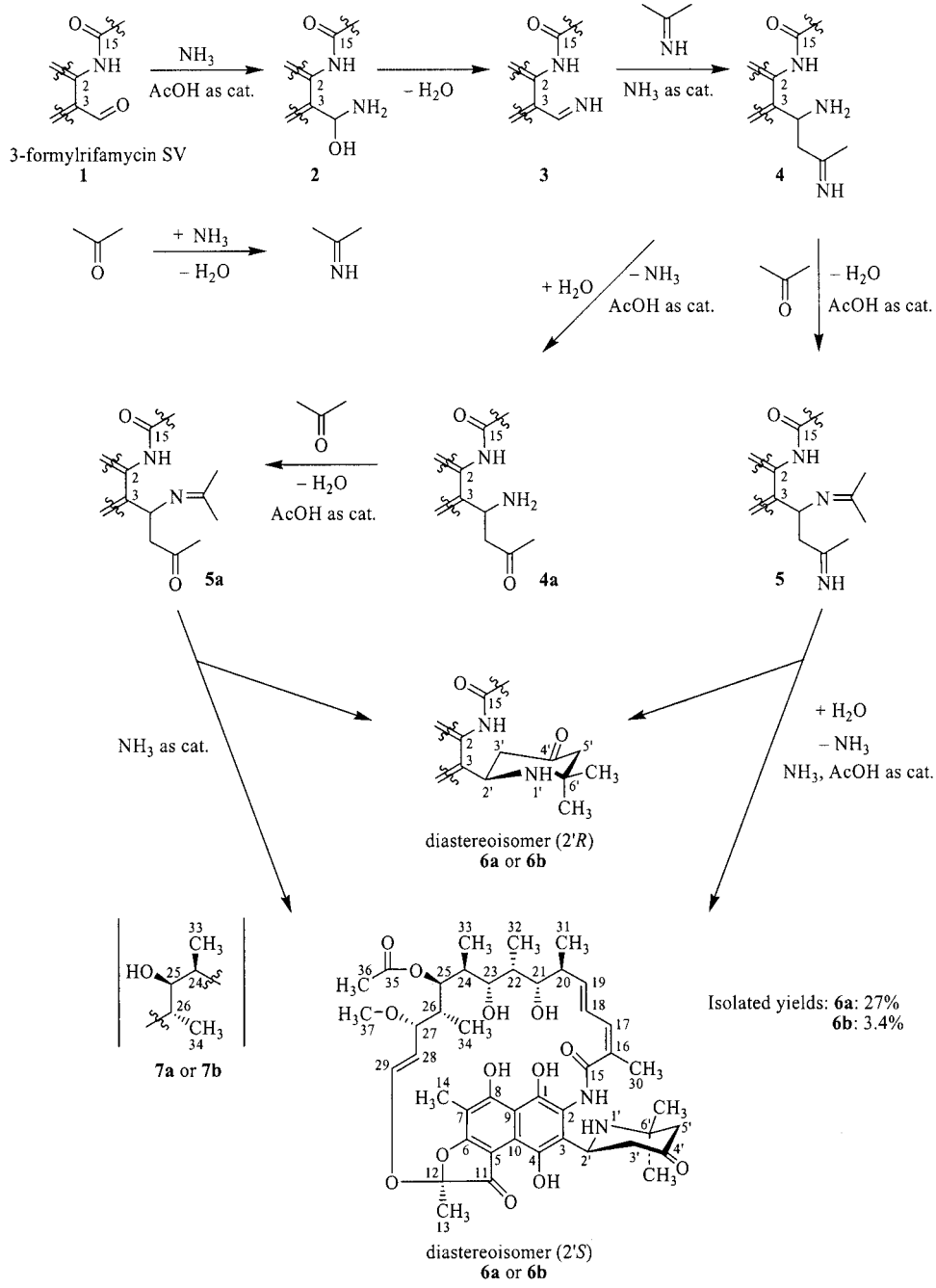
Results and Discussion

Synthesis and Purification

Saturation of a solution of **1** in acetone with gaseous ammonia in the presence of acetic acid gave a rapid reaction at temperatures above 10 °C and two orange products, **6a** and **6b**, were formed (Scheme 1).

TLC analysis showed two spots with a slight difference in retention coefficients [R_f(**6a**) > R_f(**6b**) in $CHCl_3$/MeOH (85:15)]. The **6a/6b** ratio increased with the reaction temperature. However, the selectivity of the reaction decreases significantly above 40 °C and other unidentified by-products are also generated. Interestingly, if the first stage of the reaction is conducted between 10 and 20 °C, the formation of a blue intermediary imine **3** with a higher retention factor [R_f(**3**) > R_f(**6a**)] is observed (Scheme 1).[9]

A mixture of **6a** and **6b** was isolated after completion of the reaction as an amorphous precipitate. The components were subsequently isolated and purified by different meth-



Scheme 1. Reaction of **1** with ammonia and acetone.

ods. Pure **6a** crystallized from the mixture of **6a** and **6b** in various solvents, such as acetone, chloroform and THF (isolated yield 27%). However, **6b** is considerably more soluble than **6a** and could only be isolated and purified by column chromatography (isolated yield 3.4%). The separation of **6b** from **6a** by column chromatography turned out to be far more complicated than expected, and even with the best eluent gradient we found ($CHCl_3$/MeOH, 85:15), the difference between the retention factors **6b** and **6a** was narrow, yielding very small amounts of **6b** in a pure form. No isomerisation of **6a** into **6b**, or conversely, was observed during "stability" studies run in different polar organic solvents (methanol, acetone, THF, DMSO) or in their mixtures with water at temperatures up to 50 °C. This reasonably excludes that these substances are conformers. The synthesis of **6a** and **6b** carried out under similar conditions

Table 1. ^{1}H and ^{13}C NMR spectroscopic data of **6a** in [D_6]DMSO.[a]

C atom	δ	H atom	δ	Mult.	$J_{H,H}$[*]	^{1}H,^{1}H COSY correlations	^{1}H,^{13}C HMBC correlations
		$N_{(amide)}$–H	9.07	s			C-1, C-2, C-3, C-15
C-1	149.2						
		1-OH	15.80	s			
C-2	118.6						
C-3	116.6						
C-4	144.7						
		4-OH	13.45	s			C-2, C-4, C-10, C-11[w]
C-5	97.4						
C-6	172.1						
C-7	101.0						
C-8	184.3						
		8-OH	9.81	br.		1′-H lr.	
C-9	114.5						
C-10	116.7						
C-11	185.3						
C-12	108.0						
C-13	21.1	3 13-H	1.66	s			C-11, C-12
C-14	7.2	3 14-H	1.90	s			C-6, C-8
C-15	168.1						
C-16	130.6						
C-17	133.0	17-H	6.28	d	(17,18) = 10.7	18-H, 19-H lr., 3 30-H lr.	
C-18	125.7	18-H	6.61	dd	(18,19) = 15.5	17-H, 19-H	
C-19	139.6	19-H	5.93	dd	(19,20) = 7.4	17-H lr., 18-H, 20-H	
C-20	39.6	20-H	2.25	m		19-H, 21-H, 3 31-H	
C-21	71.2	21-H	3.80	dd	(21,20) = 6.1	20-H, 21-OH	
		21-OH	4.96	[o]		21-H,	
C-22	37.6	22-H	1.38	m		23-H, 3 32-H	
C-23	76.5	23-H	2.85	m	(23,22) = 9.3	22-H, 23-OH	
		23-OH	4.24	d	(23,23-OH) ≈ 2	23-H	
C-24	33.0	24-H	1.72	m		3 33-H	
C-25	72.8	25-H	5.14	dd	(25,26) = 10.5	26-H	C-26, C-27, C-34, C-35
C-26	39.7	26-H	0.96	[o]		25-H, 3 34-H	C-26
C-27	76.2	27-H	3.34	[o]	(28,27) = 6.3	26-H, 28-H	
C-28	117.6	28-H	5.00	dd[o]	(28,29) = 12.5	27-H, 29-H	C-27
C-29	141.5	29-H	6.02	d		28-H	C-12, C-27, C-28
C-30	20.6	3 30-H	1.95	s		17-H lr.	C-15, C-16
C-31	17.4	3 31-H	0.95	d[o]	(31,20) = 6.9	20-H	C-19, C-20, C-21
C-32	9.0	3 32-H	0.37	d	(32,22) = 6.1	22-H	
C-33	12.4	3 33-H	0.91	d[o]	(33,24) = 7.0	24-H	C-23, C-24
C-34	8.3	3 34-H	–0.14	d	(34,26) = 6.8	26-H	C-25, C-26, C-27
C-35	169.5						
C-36	20.3	3 36-H	1.93	s			C-35
C-37	56.1	3 37-H	2.93	s			C-37
		1′-H	8.15	br.		8-OH lr.	
C-2′	50.2	2′-H	4.67	br.	(2′,1′) ≈ 8.0	3′-$H_{(a)}$	
C-3′	41.7	3′-$H_{(a)}$	3.56	dd	($3'_{(a)}$,2′) = 14.0	2′-H, 3′-$H_{(e)}$	
		3′-$H_{(e)}$	2.39	d[o]	($3'_{(e)}$,2′) < 2.0	3′-$H_{(a)}$	
C-4′	203.0				($3'_{(a)}$,$3'_{(e)}$) = 14.0		
C-5′	49.8	5′-$H_{(a)}$	3.12	d	($5'_{(a)}$,$5'_{(e)}$) = 15.3	5′-$H_{(e)}$	
		5′-$H_{(e)}$	2.39	d[o]		5′-$H_{(a)}$	
C-6′	57.9						
$C_{(a)}$–C-6′	22.7	3 $C_{(a)}$H–C-6′	1.22	s			C-5′, C-6′, $C_{(e)}$–C-6′
$C_{(e)}$–C-6′	27.0	3 $C_{(e)}$H–C-6′	1.51	s			C-5′, C-6′, $C_{(a)}$–C-6′

[a] Abbreviations: (a) axial arrangement, (e) equatorial arrangement, lr. long range correlation, [o] this signal overlaps with another signal, [w] weak signal, [*] taken from ^{1}H NMR spectrum recorded at 500 MHz.

Table 2. ^{13}C and ^{1}H NMR spectroscopic data of **6b** in [D_6]DMSO.[a]

C atom	δ	H atom	δ	Mult.	$J_{H,H}$	$^{1}H,^{1}H$ COSY correlations	$^{1}H,^{13}C$ HMBC correlations
		$N_{(amide)}$–H	9.50	s			
C-1	149.1						
		1-OH	15.95	s			
C-2	118.5						
C-3	116.4						
C-4	144.3						
		4-OH	13.03	s			
C-5	97.4						
C-6	172.3						
C-7	101.0						
C-8	184.1						
		8-OH	8.70	d		1′-H	
C-9	114.4						
C-10	116.7						
C-11	185.1						
C-12	107.6						
C-13	21.1	3 13-H	1.65	s[o]			C-11, C-12
C-14	7.4	3 14-H	1.89	s[o]			C-6, C-8
C-15	169.8						
C-16	130.9						
C-17	132.7	17-H	6.21	d	(17,18) = 10.7	18-H, 30-H lr.	
C-18	127.6	18-H	6.96	dd	(18,19) = 15.5	17-H, 19-H	
C-19	138.2	19-H	5.75	dd	(19,20) = 7.2	18-H, 20-H	
C-20	40.7	20-H	2.23	m		19-H, 21-H, 3 31-H	
C-21	70.8	21-H	3.82	dd		20-H, 21-OH	
		21-OH	4.50	d		21-H	
C-22	37.6	22-H	1.40	m[o]		23-H, 3 32-H	
C-23	76.2	23-H	2.80	dd	(23,23-OH) = 5.0	22-H, 23-OH	
		23-OH	4.31	d		23-H	
C-24	34.0	24-H	1.63	m[o]		3 33-H	
C-25	73.4	25-H	5.08	dd[o]	(25,26) = 10.4	26-H	
C-26	40.1	26-H	0.90	[o]		25-H, 3 34-H	C-25
C-27	76.2	27-H	3.30	[o]	(27,28) = 7.5	28-H	C-25, C-34
C-28	119.5	28-H	5.15	dd[o]	(28,29) = 12.5	27-H, 29-H	
C-29	141.5	29-H	6.04	d		28-H	
C-30	19.7	3 30-H	1.92	s[o]		17-H lr.	
C-31	18.0	3 31-H	0.92	d[o]	(31,20) = 6.4	20-H	C-20, C-21
C-32	8.4	3 32-H	0.24	d	(32,22) = 6.4	22-H	C-22
C-33	14.2	3 33-H	0.83	d[o]	(33,24) = 6.4	24-H	C-23, C-24
C-34	8.8	3 34-H	0.16	d	(34,26) = 6.4	26-H	C-26, C-27
C-35	170.7						
C-36	20.7	3 H-36	1.99	s			C-35
C-37	55.8	3 H-37	2.94	s[o]			C-27
		1′-H	8.84	dd	(1′,2′) = 10.4	8-OH, 2′-H	
C-2′	50.6	2′-H	4.83	m	(2′,3′$_{(a)}$) = 13.2	1′-H, 3′-$H_{(a)}$, 3′-$H_{(e)}$	
C-3′	41.1	3′-$H_{(a)}$	3.65	dd	(2′,3′$_{(e)}$) ≈ 2.0	2′-H, 3′-$H_{(e)}$	
		3′-$H_{(e)}$	2.38	[o]	(3′$_{(a)}$,3′$_{(e)}$) = 13.2	2′-H, 3′-$H_{(a)}$	
C-4′	203.1						
C-5′	49.6	5′-$H_{(a)}$	2.93	[o]		5′-$H_{(e)}$	
		5′-$H_{(e)}$	2.42	[o]		5′-$H_{(a)}$	
C-6′	58.2						
$C_{(a)}$–C-6′	22.4	3 $C_{(a)}$H–C-6′	1.31	s			C-5′, C-6′, $C_{(e)}$–C-6′
$C_{(e)}$–C-6′	27.1	3 $C_{(e)}$H–C-6′	1.53	s			C-5′, C-6′, $C_{(a)}$–C-6′

[a] Abbreviations: (a) axial arrangement, (e) equatorial arrangement, lr. long-range correlation, [o] this signal overlaps with another signal.

using ammonium acetate instead of gaseous ammonia and acetic acid gave comparable yields.

In order to better understand the reaction and optimize its selectivity, we studied the reactivity of 25-*O*-deacetyl-3-formylrifamycin SV under the same experimental conditions. An analogous formation of two main products with very similar retention coefficients (TLC) was observed. A deacetyl analogue of **6a** (**7a**) was isolated from the reaction mixture and purified by crystallization from an acetone solution (Scheme 1). It was found to dissolve better than **6a** in other polar solvents, such as chloroform or THF. Separation of the deacetyl homologue of **6b** (**7b**) from the **7a**/**7b** reaction mixture was not possible neither by crystallization nor by column chromatography.

The structures of the obtained compounds **6a** (Table 1), **6b** (Table 2), and **7a** (Table 3) were proposed on the basis of their mass and 1D and 2D ^{1}H and ^{13}C NMR spectra.

Table 3. ^{13}C and ^{1}H NMR spectroscopic data of **7a** in [D_6]DMSO.

C atom	δ	H atom	δ	Mult.	$J_{H,H}$	$^{1}H,^{1}H$ COSY correlations	$^{1}H,^{13}C$ HMBC correlations
		$N_{(amide)}$–H	9.03	s			C-2, C-15
C-1	149.2						
		1-OH	15.80	s			C-16[w]
C-2	118.8						
C-3	116.4						
C-4	144.8						
		4-OH	13.44	s			C-4
C-5	97.3						
C-6	172.3						
C-7	100.8						
C-8	184.2						
		8-OH	9.82	d		1′-H lr.	
C-9	114.5						
C-10	116.6						
C-11	185.6						
C-12	107.8						
C-13	20.9	3 13-H	1.65	s			C-11, C-12, C-14
C-14	7.3	3 14-H	1.89	s			C-6, C-8, C-14
C-15	167.9						
C-16	130.5						
C-17	133.1	17-H	6.28	d	(17,18) = 11.0	18-H, 3 30-H lr.	
C-18	125.8	18-H	6.63	dd	(18,19) = 15.2	17-H, 19-H	
C-19	140.3	19-H	5.94	dd[o]	(19,20) = 6.6	18-H, 20-H	
C-20	40.1	20-H	2.24	m		19-H, 21-H, 3 31-H	
C-21	71.3	21-H	3.83	dd		20-H	
		21-OH	4. 90	d			
C-22	37.6	22-H	1.23	[o]		23-H, 3 31-H lr., 3 32-H	
C-23	76.4	23-H	3.15	[o]		22-H, 23-OH	
		23-OH	4.14	br.		23-H	
C-24	33.1	24-H	1.75	m		3 33-H	
C-25	68.0	25-H	3.66	dd	(25,26) = 10.0	26-H, 25-OH	
		25-OH	3.98	br		25-H	C-24
C-26	41.0	26-H	0.75	m		25-H, 3 34-H	
C-27	76.3	27-H	3.90	dd	(27,28) = 7.5	28-H	
C-28	119.1	28-H	5.05	dd		27-H, 29-H	
C-29	139.7	29-H	5.96	d[o]		28-H,	C-12, C-27, C-28
C-30	20.3	3 30-H	1.94	s		17-H lr.	C-15, C-16
C-31	17.5	3 31-H	0.96	d[o]	(31,20) = 6.3	20-H, 22-H lr.	C-19, C-20, C-21
C-32	8.0	3 32-H	0.23	d	(32,22) = 6.0	22-H	
C-33	12.7	3 33-H	0.93	d[o]	(33,24) = 6.6	24-H	C-23, C-24
C-34	8.2	3 34-H	–0.26	d	(34,26) = 6.3	26-H	C-25, C-26, C-27
C-35	–		–				
C-36	–	3 H-36	–				
C-37	56.5	3 H-37	3.06	s			C-27
		1′-H	8.16	m		2′-H, 8-OH lr.	
C-2′	50.2	2′-H	4.68	m	$(2',3'_{(a)})$ = 14.1	1′-H, 3′-$H_{(a)}$	
C-3′	41.6	3′-$H_{(a)}$	3.51	dd	$(3'_{(a)},3'_{(e)})$ = 14.1	2′-H, 3′-$H_{(e)}$	
		3′-$H_{(e)}$	2.38	d[o]		3′-$H_{(a)}$	
C-4′	203.0						
C-5′	49.7	5′-$H_{(a)}$	3.14	d[o]		5′-$H_{(e)}$	
		5′-$H_{(e)}$	2.36	d[o]		5′-$H_{(a)}$	
C-6′	57.8						
$C_{(a)}$–C-6′	22.7	3 $C_{(a)}$H–C-6′	1.21	s			C-5′, C-6′, $C_{(e)}$–C-6′
$C_{(e)}$–C-6′	26.9	3 $C_{(e)}$H–C-6′	1.51	s			C-4′, C-5′, C-6′, $C_{(a)}$–C-6′

[a] Abbreviations: (a): axial arrangement, (e): equatorial arrangement; lr.: long range correlation; [o] this signal overlaps with another signal; [w] weak signal.

NMR Studies

Generally, the assignments of the proton and carbon signals of **6a**, **6b**, and **7a** were based on 1D ^{1}H and ^{13}C NMR spectra, 2D $^{1}H,^{1}H$ COSY and $^{1}H,^{13}C$ HMQC correlation spectra (the latter were optimized for a single-bond coupling constant), and $^{1}H,^{13}C$ HMBC correlation spectra (optimized for long-range C–H coupling). In addition, DEPT 135 spectra as well as ^{1}H NMR spectroscopy after H→D exchange were used. The coupling constants obtained from the 1D ^{1}H NMR spectra were used to determine the configurations of the hydrogen atoms (equatorial or axial) at carbon atoms C-2′ and C-3′ in these rifamycin

derivatives. The signal assignments were also correlated with data from the literature for rifampicin and its derivatives,[12,13] rifamycin S and its derivatives,[14–16] and our own spectroscopic database.[9]

Determination of the Chemical Structure of New Compounds

Compound 6a

NMR Features of the Substituent at C-3

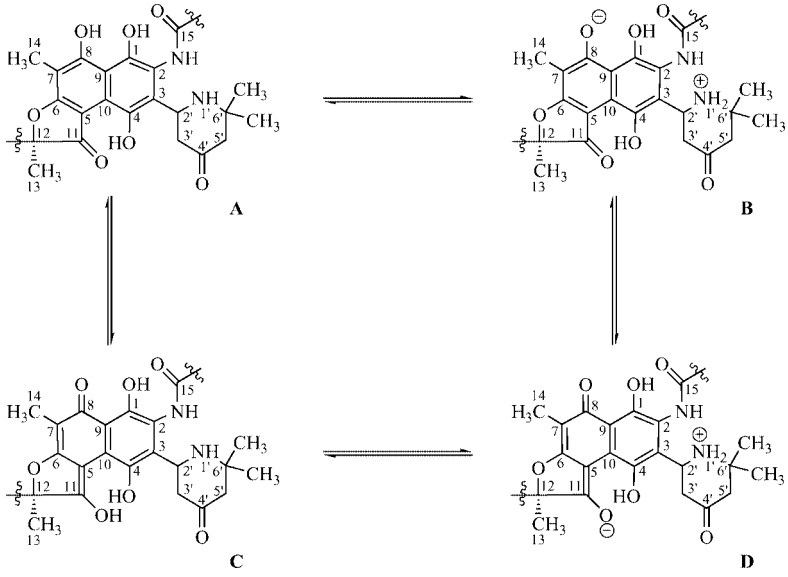
The signal at δ = 57.9 ppm in the ^{13}C NMR spectrum can be clearly attributed to C-6′. Two related signals for $C_{(a)}$–C-6′ and $C_{(e)}$–C-6′ are detected at δ = 22.7 and 27.0 ppm, respectively. In the ^{1}H NMR spectrum the proton signals of these methyl groups, which show the corresponding correlation signals in the ^{1}H,^{13}C HMQC spectrum and in the ^{1}H,^{13}C HMBC spectrum, are observed as singlets at δ = 1.22 and 1.51 ppm. A comparison of these ^{13}C NMR spectra with those reported in the literature for α,α-dimethyl derivatives of cyclohexane[17] allowed the following assignments to be made: δ = 22.7 ppm for $C_{(a)}H_3$–C-6′ and δ = 27.0 ppm for $C_{(e)}H_3$–C-6′. The signal at δ = 50.2 ppm in the ^{13}C NMR spectrum was assigned to C-2′. In the ^{1}H NMR spectrum the signal of 2′-H at δ = 4.67 ppm occurs as a broad multiplet [two doublets (dd) after H→D exchange]. The non-equivalency of the geminal protons at the adjacent carbon atom C-3′ is most likely the reason for this behavior. The 3′-H (a) and (e) protons give two separate groups of signals at δ = 3.56 (dd) and 2.39 (dd) ppm. Correlation signals between 2′-H and 3′-$H_{(a)}$ and 3′-$H_{(a)}$ and 3′-$H_{(e)}$ were found in the ^{1}H,^{1}H COSY spectrum.

The newly formed piperidine ring at C-3 probably takes the most stable chair conformation. The coupling constants in the ^{1}H NMR spectrum were used to determine the arrangement of the 2′-H bond with respect to the plane of this piperidine ring (axial or equatorial). According to the measured coupling constants [$^3J_{2'\text{-H},1'\text{-H}} \approx$ 8.0 Hz (disappearing after H→D exchange), $^3J_{3'\text{-H}_{(3.56)},2'\text{-H}}$ = 14.0 Hz, and $^3J_{3'\text{-H}_{(2.39)},2'\text{-H}} < 2.0$ Hz], it seems that both 3′-$H_{(3.56)}$ and 2′-H are axial protons, since only a coupling involving vicinal axial protons would have such a high coupling constant.[18,19] The remaining 3′-$H_{(2.39)}$ proton should therefore be an equatorial one.

In the ^{1}H NMR spectrum two signals at δ = 3.12 (d) and 2.39 (d) ppm were assigned to two magnetically inequivalent geminal protons bound to C-5′. A correlation between 5′-$H_{(a)}$ and 5′-$H_{(e)}$ was found in the ^{1}H,^{1}H COSY spectrum. In the DEPT 135 spectrum the negative signals of the C-3′ and C-5′ carbon atoms, characteristic of two methylene groups, appear at δ = 41.7 and 49.8 ppm. The correlations 3′-$H_{(a)}$/C-3′, 3′-$H_{(e)}$/C-3′, 5′-$H_{(a)}$/C-5′, and 5′-$H_{(e)}$/C-5′ were observed in the ^{1}H,^{13}C HMQC spectrum, and the correlations $C_{(a)}H_3$–C-6′/C-5′ and $C_{(e)}H_3$–C-6′/C-5′ were observed in the ^{1}H,^{13}C HMBC spectrum. The intense signal at δ = 203.0 ppm in the ^{13}C NMR spectrum was assigned to the carbonyl atom C-4′.[20]

Selected NMR Features of the Rifamycin Backbone

In the downfield part of the ^{1}H NMR spectrum (δ = 16.0–8.0 ppm) five singlets of five mobile protons can be found (all disappear after H→D exchange). The signal at δ = 13.45 ppm, which shows a long-range coupling with C-4 (δ = 144.7 ppm), belongs to the 4-OH group. In the ^{1}H,^{13}C HMBC spectrum the correlations 4-OH/C-2, 4-OH/C-10, and 4-OH/C-11 (weak signal) are also visible. The signal at δ = 9.07 ppm shows a long-range coupling with C-1 (δ = 149.2 ppm), C-2 (δ = 118.6 ppm), C-3 (δ = 116.6 ppm), and C-15 (δ = 168.1 ppm), consequently it was assigned as being due to the $N_{(amide)}$–H proton. The signal at δ = 15.80 ppm is characteristic of the 1-OH proton.[13] The remaining two signals (δ = 9.81 ppm) and (δ = 8.15 ppm) were attributed



Scheme 2. Tautomeric equilibrium found in [D$_6$]DMSO solution of compounds **6a**, **7a**, and **6b**.

to 8-OH and 1′-H, respectively. There is a correlation between these two protons in the ^{1}H,^{1}H COSY spectrum. The 8-OH/1′-H correlation, the downfield position of the 1′-H signal (δ = 8.15 ppm), and the small difference in the chemical shifts of the C-8 (δ = 184.3 ppm) and C-11 (δ = 185.3 ppm) signals probably result from the occurrence of a complex tautomeric equilibrium (Scheme 2) and predominance of the dipolar forms **B** and **D**.

Compound 6b

NMR Features of the Substituent at C-3

In parallel with **6a**, the signal for C-6′ was found at δ = 58.2 ppm and the two signals for $C_{(a)}H_3$–C-6′ and $C_{(e)}H_3$–C-6′ were observed at δ = 22.4 and 27.1 ppm, respectively. In the ^{1}H NMR spectrum, the signals of these methyl groups appear at δ = 1.31 and 1.53 ppm, respectively. Likewise, the signal assigned to C-2′ is found at δ = 50.6 ppm in the ^{13}C NMR spectrum. In the ^{1}H NMR spectrum, the signal of the related 2′-H occurs as a broad multiplet at δ = 4.83 ppm.

The signal at δ = 49.6 ppm was assigned to C-5′. The geminal protons 5′-$H_{(a)}$ and 5′-$H_{(e)}$ show two separate groups of signals in the ^{1}H NMR spectrum at $\delta \approx$ 2.93 and 2.42 ppm. The inequivalent geminal protons 3′-$H_{(a)}$ and 3′-$H_{(e)}$ yield two groups of signals, one at δ = 3.65 (dd) ppm and the second at δ = 2.38 (d) ppm, which overlap with the signals of 5′-$H_{(e)}$. The correlations 3′-$H_{(a)}$/2′-H, 3′-$H_{(e)}$/2′-H (weak signal), 3′-$H_{(a)}$/3′-$H_{(e)}$, and 5′-$H_{(a)}$/5′-$H_{(e)}$ were found in the ^{1}H,^{1}H COSY spectrum.

From the recorded coupling constants involving the different geminal and vicinal protons [10.4 Hz for $^3J_{1'\text{-H},2'\text{-H}}$ (this coupling disappears after H→D exchange), 13.2 Hz for $^3J_{2'\text{-H},3'\text{-H}_{(3.65)}}$, and about 2.0 Hz for $^3J_{2'\text{-H},3'\text{-H}_{(2.38)}}$] it seems that, as for **6a**, both 2′-H and 3′-$H_{(3.65)}$ are axial protons and that the rifamycin backbone is bound in an equatorial manner to the newly formed piperidine ring.

Selected NMR Features of the Rifamycin Backbone

In the ^{13}C NMR spectrum, the two signals assigned to C-8 and C-11 appear at δ = 184.1 and 185.1 ppm, in agreement with the respective ^{1}H,^{13}C HMBC correlations. In the downfield part of the ^{1}H NMR spectrum (δ = 16.0–8.0 ppm), five signals that disappear after H→D exchange are present. The signal at δ = 8.84 ppm was assigned to 1′-H. Interestingly, a correlation between 2′-H and 1′-$H_{(8.84)}$ was found in the ^{1}H,^{1}H COSY spectrum. In relation to this, the H→D exchange of this proton appears to be the slowest of the deuterium exchanges involving the hydroxy protons. This is typical for relatively slowly exchanging amine protons. The split signal at δ = 8.70 (d) ppm was assigned to the 8-OH proton. The reason for the splitting of the signal was found in the ^{1}H,^{1}H COSY spectrum, which displays a correlation involving 1′-H and 8-OH.

The observed 1′-H/8-OH correlation, the downfield position of the 1′-H proton signal (δ = 8.84 ppm), and the small difference in the chemical shifts of the C-8 (δ = 184.1 ppm) and C-11 (δ =185.1 ppm) signals, as in the case of **6a**, can be related to a rapid migration of the 8-OH proton and the coexistence in solution of equal quantities of tautomeric forms **A**–**D** (Scheme 2).

The remaining three low-field signals in the ^{1}H NMR spectrum (δ = 15.95, 13.03, and 9.50 ppm) were accordingly attributed to the 1-OH, 4-OH and $N_{(amide)}$–H protons (δ =15.80, 13.45, and 9.07 ppm in **6a**).

Compound 7a

We found that **7a** is a 25-*O*-deacetyl homologue of **6a** (Scheme 1) due to the similarity of the spectra recorded for **6a** and **7a** and also on the basis of mass spectrometric results. There are no signals for C-35 and C-36 in the ^{13}C NMR spectrum of **7a**, although they are present in the spectrum of **6a**. In the ^{1}H NMR spectrum, the broad signal of the 25-OH proton appears at δ = 3.98 ppm. Contrary to **6a**, a correlation between 1′-H and 2′-H was found in the ^{1}H,^{1}H COSY spectrum of **7a**, and in the ^{1}H,^{13}C HMBC spectrum a weak correlation between 1-OH and C-16 was observed.

Synthesis of 6a and 6b: Mechanistic Proposal

The chain of reactions starts with the condensation of 3-formylrifamycin SV (**1**) with gaseous ammonia catalyzed by acetic acid, which is followed by dehydration of the intermediary α-amino alcohol **2** to the blue imine **3** observed by TLC (Scheme 1).[9] Simultaneously, 2-propano imine is formed from the reaction of acetone with ammonia.[21] Then, a succession of rapid transformations involving a series of intermediates is most likely to happen. Imine **3** condenses with 2-propano imine to form β-amino imine **4**, which reacts subsequently with a second molecule of acetone to form β-imino imine **5**. The cyclization and hydrolysis of **5** follows to afford a mixture of diastereoisomers **6a** and **6b**. Alternatively, imine **4** could first react with water and then the thus-formed intermediate **4a** would react with acetone to form the β-imino ketone **5a**, which, after cyclization, would afford **6a** and **6b**. The water necessary for this hydrolysis would be generated during the formation of **3**, 2-propano imine, and **5**.

Somewhat surprisingly an aldol reaction[22] involving **1** and acetone does not seem to take place. According to the classic aldol reaction mechanism, one could expect the successive formation of the corresponding β-hydroxy ketone intermediate and, after rapid elimination of water, the α,β-unsaturated ketone, which is a rifamycin SV derivative. However, this compound [3-(3-oxo-1-butenyl)rifamycin SV], which has been isolated and characterized by us independently,[23] is not found in the reaction mixture. Thus, the formation of a cyclic piperidyl substituent takes place with the participation of four molecules: one molecule of **1**, one of ammonia, and two of acetone. The surprisingly short reaction time (it can be measured in seconds) of this multistage synthesis demonstrates the unusually high reactivity of the intermediates formed, as well as the efficiency of the acid/base catalysis.

Initial Studies on the in vitro Antibacterial Properties of 6a, 6b, and 7a

These novel rifamycin derivatives were tested for tuberculostatic activity against different strains of *Mycobacterium tuberculosis* in vitro. The following strains were used: (i) standard strains (M. tbc $H_{37}Rv$ and M. tbc bovis); (ii) some wild strains isolated from patients with tuberculosis and sensitive to rifampicin (RMP) and rifabutin (RBT) (M. tbc 234, M. tbc 243); and (iii) some wild strains isolated from patients and resistant to RMP and RBT (M. tbc 220, M. tbc 52). They were also tested against different types of MOTT (mycobacteria other than tuberculosis) that are sensitive or resistant to RMP and RBT (*M. kansasii*, *M. scrofulaceum*, *M. avium intracellulare*, and *M. fortuitum*). The concentrations studied were 8, 4, 2, 1, 0.5, 0.25, and 0.12 $\mu g\,mL^{-1}$. The microbiological screening confirmed significant activity of compounds **6a**, **6b**, and **7a** (MIC in the range of 1–8 $\mu g\,mL^{-1}$). The strains resistant to RMP and RBT were resistant to all three compounds at concentrations lower than 8 $\mu g\,mL^{-1}$.

The substituent located at C-3 of the naphthalenic unit of the new rifamycin derivatives contains a carbonyl group which seems to have a significant influence on the lowering of their antituberculous activity. This narrow activity is probably a result of the high polarity of the functional group, which induces a lower diffusion of the molecule through the mycobacterium's cell membrane.[24–26]

Conclusions

A new group of rifamycin derivatives (**6a**, **6b**, and **7a**) with a cyclic substituent at C-3 having a 4-piperidone structure has been prepared from the reaction of 3-formylrifamycin SV (**1**) or 25-*O*-deacetyl-3-formylrifamycin SV with ammonia and acetone.[10] This reaction proceeds in a unique and unexpected manner.

Spectroscopic observations show that **6a** and **6b** coexist as diastereoisomers that differ in the spatial location of the substituents at the stereogenic center C-2′ (Scheme 1). In DMSO, these compounds are probably present as a mixture of tautomers with a predominance of the dipolar forms **B** and **D** (Scheme 2). This equilibrium probably causes a similarity of the molecular environment of carbon atoms C-8 and C-11 in **6a** and **6b**, which leads to only a small difference for the chemical shifts of these atoms in the ^{13}C NMR spectra (see Tables 1 and 2). Similar phenomena regarding the reaction products of **1** with primary alkylamines or ammonia were observed and described in our previous publication.[9]

The synthesized compounds show weaker antimycobacterial activity than the known drugs rifampicin and rifabutin. Compound **6a** is of potential importance as a new starting compound for the preparation of new rifamycin derivatives.

Experimental Section

General: The commercial-grade reagents 25-*O*-deacetyl-3-formylrifamycin SV, 3-formylrifamycin SV (**1**), gaseous ammonia, acetone, and acetic acid, were used without further purification. TLC was performed on Al-precoated 0.2-mm silica gel 60 F_{254} plates, with $CHCl_3$/MeOH (85:15) as eluent. Preparative column chromatography was performed on silica gel 60 (0.040–0.063 mm), with $CHCl_3$/MeOH (85:15) as the mobile phase. Solvents for chromatography are listed as volume/volume ratios. ^1H NMR spectra were measured at either 200 or 400 MHz, and ^{13}C NMR spectra at either 50 or 100 MHz, as indicated. The samples were dissolved in [D_6]-DMSO. Chemical shifts are reported in ppm relative to TMS as internal standard. Coupling constants are reported in Hz. In order to unequivocally attribute some coupling constants and chemical shifts of **6a**, 1D ^1H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded. Mass spectra and high-resolution mass spectra were recorded using the ESI technique. Accurate masses are reported for the molecular ion [M + 1] or a suitable fragment ion. The samples were dissolved in CH_3OH. Optical rotations were obtained with a digital polarimeter. IR spectra were recorded using KBr pellets. The new rifamycin derivatives have no definite melting points; they do not melt up to 300 °C and slowly decompose at temperatures over 160 °C. This behavior, which is a common phenomenon in the chemistry of natural and semisynthetic rifamycins, has been observed many times during our studies and has also been noted in the literature.[27,28] Biological tests: tuberculostatic properties of the compounds were studied in Youmans liquid medium containing OADC. Rifampicin (RMP) and rifabutin (RBT) were used as reference drugs. Attempts to perform an X-ray analysis on **6a** failed. Small ill-shapen lamellas were obtained from THF and chloroform. The crystals isolated from acetone were large, but crushed into thin plates during the diffraction measurement.

Synthesis of 3-(6,6-Dimethyl-4-oxo-2-piperidyl)rifamycin SV (6a and 6b): 3-Formylrifamycin SV (**1**; 5.8 g, 8 mmol) was dissolved in a mixture of acetone (46.0 g, 0.8 mol) and acetic acid (2.4 g, 40 mmol). The reaction mixture was stirred at 30–36 °C and then saturated with gaseous ammonia for 3 min. The stirring was continued at this temperature for a further 10 min and a color change of the reaction mixture from dark red to olive-green was observed. The complete conversion of aldehyde **1** was controlled by TLC. The mixture was concentrated to about 25 mL (occasionally, **6a** crystallized during this operation), then diluted with 75 mL of chloroform and washed twice with 160 mL of water, the pH of the aqueous phase being adjusted to ca. 7.0 with 5% sulfuric acid. The remaining organic phase was dried with anhydrous sodium sulfate and, after separation of the drying agent, concentrated to ca. 25 mL. After crystallization (5 °C, 16 h) the orange solid was filtered off, washed with cold chloroform (5 mL, 10 °C), and dried under vacuum at 50 °C. The crude product was recrystallized from acetone to yield 1.8 g (27% yield) of the pure crystalline orange product **6a**. Chloroform filtrates were concentrated to dryness to yield 3.0 g of an amorphous solid containing compound **6b** as the main product; 1 g of this solid was then purified by column chromatography. Selected fractions of the eluate were washed twice with water, dried with anhydrous sodium sulfate, filtered, and then concentrated to about one-fifth of the initial volume, followed by sixfold dilution with *n*-hexane to obtain 0.075 g (3.4% yield) of the solid amorphous orange product **6b** after filtration and drying under vacuum. **6a:** See Table 1 for ^{13}C and ^1H NMR spectroscopic data. MS (ESI): *m*/*z* (%) = 823.4 (100.0) $[M+H]^+$, 845.4 (43.0) $[M+Na]^+$. HRMS calcd. for $[M+H]^+$ $C_{44}H_{59}N_2O_{13}$ 823.4012; found 823.4048 (error: 4.4 ppm). $[\alpha]_D^{27.6} = +280.0 \pm 20.0$ (*c* = 0.1, dioxane). IR: $\tilde{\nu}$ = 3402 (br.), 2971, 2938, 1728, 1646, 1620, 1560 cm^{-1}. **6b:** See Table 2 for ^{13}C and ^1H NMR spectroscopic data. MS (ESI): *m*/*z* (%) = 823.4 (100.0) $[M+H]^+$, 845.4 (31.2) $[M+Na]^+$, 855.4 (55.0) $[M+H+MeOH]^+$. HRMS calcd. for

Table 4. Antimycobacterial activity (MIC[a] in $\mu g\,mL^{-1}$) of compounds **6a**, **6b**, and **7a** with reference to rifampicin (RMP) and rifabutin (RBT).[b]

No.	Compound	M. tbc H37Rv	M. tbc bovis	*M. kansasii*	*M. scrofulaceum*	*M. avium intracellulare*	*M. fortuitum*	M. tbc 234 sensitive	M. tbc 243 sensitive	M. tbc 220 resistant	M. tbc 52 resistant
1	**6a**	2	2	8	> 8	> 8	> 8	2	2	> 8	8
2	**6b**	2	2	> 8	> 8	> 8	> 8	1	1	> 8	8
3	**7a**	8	8	8	> 8	> 8	> 8	4	8	> 8	> 8
4	RMP	1	2	> 8	2	> 8	> 8	1	1	> 8	> 8
5	RBT	0.5	8	1	1	2	8	0.5	0.5	> 8	8

[a] Minimum inhibitory concentrations. [b] The samples were dissolved in propylene glycol. MICs were determined in a Youmans liquid medium with 10% addition of serum. Strain growth was checked after 3 d for the quick-growing strain (*M. fortuitum*) and after 21 d for slow-growing strains (all the other ones).

$[M+H]^+$ $C_{44}H_{59}N_2O_{13}$ 823.4012; found 823.4025 (error: 1.6 ppm). IR: $\tilde{\nu}$ = 3400 (br.), 2960, 2930, 1720, 1710, 1654, 1648, 1638 cm^{-1}.

Synthesis of 25-*O*-Deacetyl-3-(6,6-dimethyl-4-oxo-2-piperidyl)rifamycin SV (7a): 25-*O*-Deacetyl-3-formylrifamycin SV (3.0 g, ca. 4 mmol) was dissolved in a mixture of 40 mL of 2-propanol, 9.2 g (160 mmol) of acetone, and 1.2 g (20 mmol) of acetic acid. The reaction mixture was stirred at 35–40 °C and then saturated with gaseous ammonia for 2 min. Stirring was continued at this temperature for a further 10 min until the starting aldehyde was completely converted [TLC; $CHCl_3$/MeOH (85:15)]. The reaction mixture was then concentrated under vacuum to about 20 mL, diluted with 50 mL of chloroform, and washed twice with 75 mL of water; the pH of the aqueous phase was adjusted to ca. 7.0 with 5% sulfuric acid. The remaining organic phase was dried with anhydrous sodium sulfate, and, after separation of the drying agent, concentrated to dryness to give 3.2 g of crude **7a**. Crystallization from 30 mL of acetone gave 1.1 g (31.2% yield) of pure crystalline **7a**. See Table 3 for ^{13}C and ^1H NMR spectroscopic data. MS (ESI): *m/z* (%) = 803.4 (100.0) $[M+Na]^+$, 781.4 (9.0) $[M+H]^+$, 749.4 (42.0) $[M+H-MeOH]^+$. HRMS calcd. for $[M+Na]^+$ $C_{42}H_{56}N_2NaO_{12}$ 803.3725; found 803.3748 (error: 2.3 ppm). IR: $\tilde{\nu}$ = 3404 (br.), 2972, 2937, 1728, 1646, 1620, 1560 cm^{-1}.

Biological Tests: See Table 4.

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