Sensitivity of melphalan spray was evaluated by applying, respectively, 5-, 1-, and 0.5-μg samples of melphalan and ester derivatives on TLC plates, which were developed immediately and dried with cold air. Spraying of the plate with 5% 4-(*p*-nitrobenzyl)pyridine in 2-butanone, followed by heating at 110 °C for 5 min, and spraying with either 1 N NaOH or 50% Et_3N in acetone produced an intense but rapid-fading blue color. This spray can be used sequential to fluorescamine identification of primary amines but is not compatible with either of ninhydrin, chlorox–toluidine, or Pauly reagents. The detection limits (in micrograms of melphalan) for these sprays were 0.5 for fluorescamine, 1 for ninhydrin, and 5 for melphalan.

Bioassays for angiotensin II and analogues were performed on anesthetized rats (Zivic-Miller) and on uterine preprations (albino Wistar rats) suspended in de Jalon's solution (calcium 0.5 mM, pH 7.4) according to reported procedures,[48,49] with 1 g of contractile tension in the oxytocic assay equivalent to 3 cm on the recorder scale.

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Synthesis and Antibacterial Activity of Some Esters, Amides, and Hydrazides of 3-Carboxyrifamycin S. Relationship between Structure and Activity of Ansamycins

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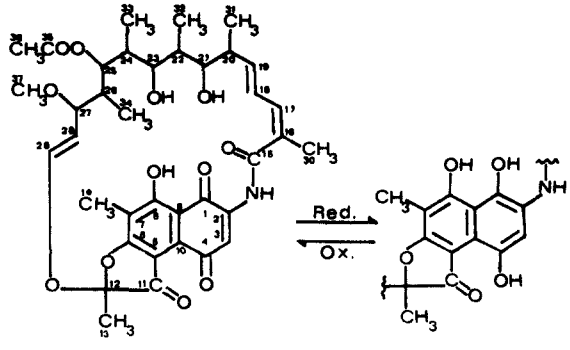
Esters, amides, and hydrazides of 3-carboxyrifamycin S were synthesized by oxidizing the cyanohydrin of 3-formylrifamycin SV to 3-(cyanocarbonyl)rifamycin S, followed by treatment with alcohols, amines and hydrazines. The in vitro microbiological activity of the derivatives was quite low, especially toward Gram-negative bacteria. This poor activity was not shown to be due to the inadequate inhibiting action on the bacterial DNA-dependent RNA polymerase but to the poor penetration of the compounds through the bacterial cell wall. The microbiological activity was correlated to the chemical properties of the substituent on C_3.

It is known that rifamycins S and SV (**1a,b**) form a very



rifamycin S (**1a**) rifamycin SV (**1b**)

stable complex with the bacterial DNA-dependent RNA polymerase (RNAP). The formation of this complex prevents the transcription process in bacterial cells.[1,2]

From structure–activity relationship studies carried out on rifamycins and related compounds,[3,4] the hypothesis was advanced that the essential requirements of the antibiotic to form the RNAP–antibiotic complex are the presence of at least a naphthalenic nucleus with a hydroxyl group on C_8 and an oxygen atom in C_1, either in the quinone or hydroquinone form, and two free hydroxyl groups on C_{21} and C_{23}. Moreover, the steric characteristics of the ansa chain must be such that the two hydroxyls on C_{21} and C_{23} can take a specific orientation with respect to the naphthalenic nucleus.[3]

To test this hypothesis, we tried to relate the differences of in vitro microbiological activity in a series of 3-substituted derivatives of rifamycin S and related compounds[5] to the influence on the ansa conformation of the steric characteristics of the substituents.

We suggest that the substituents introduced in position 3 may influence the ansa conformation and, consequently, the stability of the RNAP–antibiotic complex.

Dampier and Whitlock,[6] on the other hand, examined the relationship between the activity of a series of 3-substituted rifamycin S derivatives on RNAP isolated from *Escherichia coli* and the electronic nature of the substituents. They concluded that the activity is increased by electron-withdrawing substituents and decreased by electron-donating substituents. This conclusion is in agreement with the hypothesis that the formation of the complex between RNAP and the aromatic nucleus involves a donor–acceptor π complex.

Assuming the conformational stabilization produced by the substituents at position 3 is due to a direct interaction between this substituent and the amide group on C_2 of the ansamycin,[5,7] we synthesized a series of esters, amides, and

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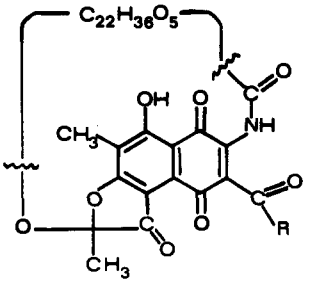
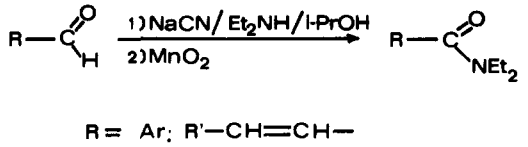
Table I. Antibacterial Activity of Some Derivatives of 3-Carboxyrifamycin S[a]



compd	R	formula	anal.	min inhibitory concn,[b] μg/mL A	B	C	D
5	OCH_3	$C_{39}H_{47}NO_{14}$	C, H, N	0.05	25–50	25–50	~50
6	$OCH_2CH(OH)CH_2(OH)$	$C_{41}H_{51}NO_{16}$	C, H, N	~0.5	>50	>50	>50
7	$c\text{-}N(CH_2CH_2)_2NCH_3$	$C_{43}H_{55}N_3O_{13}$	C, H, N	0.1	>50	>50	>50
8	$NHN(CH_3)_2$	$C_{40}H_{51}N_3O_{13}$	C, H, N	<0.005	>50	>50	>50
9	$N(CH_3)_2$	$C_{40}H_{50}N_2O_{13}$	C, H, N	0.025	>50	>50	>50
10	$N(CH_3)\text{-}c\text{-}N(CH_2CH_2)_2CH_2$	$C_{44}H_{57}N_3O_{13}$	C, H, N	~0.03	>50	>50	>50
11	$NH\text{-}C_6H_4\text{-}p\text{-}Br$	$C_{44}H_{49}BrN_2O_{13}$	C, H, N, Br	0.1	>50	>50	>50
rifamycin SV (1b)				<0.005	100	100	100
rifampicin				~0.025	25	6	12

[a] Melting points have not been included because the compounds decompose on heating. [b] A = *Straphylococcus aureus* 209 P (FDA); B = *Klebsiella pneumoniae* Ottaviani; C = *Escherichia coli* ML/35; D = *Salmonella paratyphi* B 0 248 K (Sclavo).

Scheme I

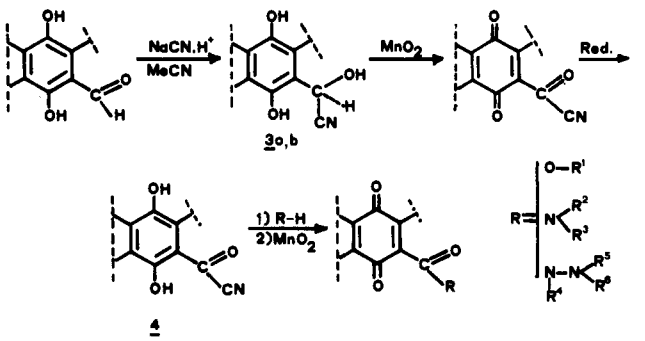


hydrazides of 3-carboxyrifamycin S. All these derivatives (5–11) have a group at C_3 which exerts a steric compression on the C_2 amide group and possesses strong electron-attracting properties.

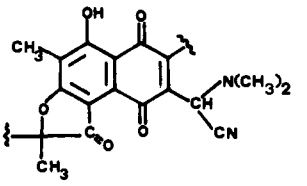
This paper describes the synthesis and the in vitro microbiological activity of the derivatives, and an evaluation of the results is attempted on the basis of their structure–activity relationship.

Chemistry. In synthesizing the esters, amides, and hydrazides, we did not consider the possibility of obtaining them directly from 3-carboxyrifamycin S or from its acid chloride, because of their expected instability. Since 3-formylrifamycin SV was available,[8] we examined the possibility of using methods of synthesis that utilize aromatic aldehydes, to form amides, esters, and hydrazides, without going through the corresponding free carboxylic acid.[9–11] We rejected any methods that entailed very drastic reaction conditions and powerful oxidizing agents, to which the rifamycins are unstable, and attempted synthesis via the cyanohydrin[10] (see Scheme I), which allows the oxidation to be carried out under very mild conditions. According to this scheme, an aromatic aldehyde or an α,β-unsaturated compound is treated with sodium cyanide in the presence of amines in 2-propanol, and the mixture is then oxidized with manganese dioxide. When this reaction was attempted with 3-formylrifamycin SV in the presence of dimethylamine, it did not yield any of the expected amide but instead produced, as its main product,

Scheme II



a compound that was found to be aminonitrile 2, in the quinone form. This compound was also obtained from the cyanohydrin of 3-formylrifamycin SV by treatment with dimethylamine and oxidation with MnO_2.



2

To avoid the formation of the aminonitrile 2, we attempted to isolate the cyanohydrin, which was obtained by adding hydrocyanic acid to the 3-formylrifamycin SV. This could potentially be oxidized to ketonitrile under mild conditions. This route succeeded in yielding 3-(cyanocarbonyl)rifamycin S (Scheme II), from which 3-(cyanocarbonyl)rifamycin SV was produced by reduction with ascorbic acid.

The reaction was carried out by treating 3-formylrifamycin SV with sodium cyanide and isolating the cyanohydrin formed. The latter was crystallized from ethyl acetate and ethyl formate. The two epimeric forms of the cyanohydrin were isolated in this manner. On oxidation with manganese dioxide in acetonitrile, the cyanohydrin gave 3-(cyanocarbonyl)rifamycin S, which reacts with both amines and alcohols to give the corresponding amides and

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esters (see Table I). The intermediate 3-(cyanocarbonyl)rifamycin S decomposes readily from the moisture in the air. However, 3-(cyanocarbonyl)rifamycin SV is more stable in air and much less reactive. Thus, hydrazines, which rapidly reduce 3-(cyanocarbonyl)rifamycin S to 3-(cyanocarbonyl)rifamycin SV, react very slowly to give the hydrazides directly in the SV form.

Biological Results and Discussion

Table I shows the in vitro microbiological activities of one Gram-positive and three Gram-negative bacterial strains. The corresponding data for rifamycin SV and for rifampicin are also given for reference. The table shows that the derivatives display only slight or moderate activity against *Staphylococcus aureus* and have little or no activity against the three Gram-negative strains. On *Staphylococcus aureus* the two hydrazides tested are more active than either the esters or the amides. In particular, the *N,N*-dimethylhydrazide shows an activity comparable to that of rifampicin.

The fact that the microbiological activities are generally inferior to those expected may be attributable to a weak inhibiting action on the bacterial RNAP, in which case our hypothesis on the influence of the substituents, introduced in position 3, on the stability of the RNAP–antibiotic complex must be reconsidered. The other possibility may be related to poor penetration of the derivatives through the bacterial cell wall. We examined the first possibility by determining whether the low activity in vitro might be attributable to the stabilization of a conformation of the ansa chain that is different from that considered essential for interaction with the bacterial RNAP.

The ^{1}H NMR spectrum of the methyl ester of 3-carboxyrifamycin S in chloroform gave the following signals for the methyl groups on C_{20}, C_{22}, C_{24}, and C_{26}: δ 0.92, 1.02, 0.70, and 0.18, respectively. These chemical shifts are very close to those of rifampicin S and of other active derivatives of rifamycin S examined.[12] The chemical shifts of the methyl groups bound to the aliphatic bridge of the ansamycins are very sensitive to conformational variations of the ansa chain because of the shielding action exerted on them by the aromatic nucleus. Thus, the position of the signals of these groups in the ^{1}H NMR spectrum of the methyl ester of 3-carboxyrifamycin S is an indication that the conformation of the ansa segment between C_{20} and C_{26} is not appreciably different from that present in the active derivatives examined.

A further confirmation of the identity of the conformation of this ansa segment in the methyl ester of 3-carboxyrifamycin S and in rifampicin was obtained by Brufani and colleagues[13] by determining the structure of this ester by X-ray analysis. This structure showed that the conformation of the ansa chain along the tract comprising C_{21} and C_{23} is almost identical with that present in rifampicin[7] and in the *p*-iodoanilide of rifamycin B,[3] the two rifamycin derivatives that were examined by X-ray diffraction, both of which are very active in in vitro microbiological tests. Thus, the low activity of the derivatives of 3-carboxyrifamycin S cannot be attributed to a conformational variation of the ansa chain and is probably related to the electronic nature of the substituents introduced. The result is a probable negative influence upon the possibility of penetration of the derivatives through the bacterial cell wall.

To verify this second possibility, the inhibiting action of the methyl ester of 3-carboxyrifamycin S on RNAP isolated from *E. coli* was determined. The derivative was found to be highly active, with an inhibition of the RNAP that approximated that of rifampicin and of other active derivatives tested.[13] The high activity of the methyl ester of 3-carboxyrifamycin S on the isolated RNAP is a further confirmation of the importance of the conformational factor and the electronic effect of the substituents, as suggested by Dampier and Whitlock,[6] for the inhibition of the enzyme. However, the low activity on the bacterial cells should probably be attributed to penetration problems and not to bacterial inactivation. In fact, it should be remembered that no cases of resistance due to inactivation of the antibiotic have ever been found among rifamycins.[14]

Thus, we attempted to relate the probable poor penetration of the derivatives of 3-carboxyrifamycin S to changes in the physicochemical characteristics of the compound produced by introducing a strongly electron-withdrawing group into position 3 of the naphthoquinone nucleus. In particular, we examined the possibility that the low penetration might be attributable to an increased acidity of the phenolic hydroxyl on C_8. As is known, the presence of acidic groups hinders the passive transport of molecules through bacterial cell walls, especially those of Gram-negative bacteria.

The pK_a of the methyl ester of 3-carboxyrifamycin S in water, measured in water/methylcellosolve solutions and calculated by extrapolation to 100% water, is 4.69. This is appreciably below the value (5.03) found by the same method for rifamycin S. Also, 3-nitrorifamycin S (previously described in the German Application 2908-855) was found to be active on the isolated RNAP,[13] to have a MIC of 1.25 μg/mL on *Staphylococcus aureus*, and to have a pK_a of 4.35. The introduction of the nitro group into position 3 thus produced effects on the isolated RNAP and in vitro similar to those of the carboxy derivatives.

The hypothesis that the low antibacterial activity of the derivatives of 3-carboxyrifamycin S is connected with the acidity of the phenolic hydroxyl on C_8 is also confirmed by the appreciably greater activity on *Staphylococcus aureus* of the hydrazides in comparison with esters or amides. These results are in accordance with the lower electron-attracting power of the hydrazide groups compared to the ester and amide groups.

A systematic examination of the in vitro activity of rifamycin S and SV derivatives with various substituents in position 3 of the aromatic nucleus on various Gram-positive and Gram-negative bacterial strains as a function of their pK_a is currently under way in our laboratories. The aim is to check whether there is any correlation between the in vitro activities and the acidity of the phenolic hydroxyl on C_8.

Experimental Section

Electronic spectra were recorded with a double-beam Perkin-Elmer 550 spectrophotometer, and the absorbances at different wavelengths were obtained by digital reading. Infrared spectra were obtained by means of a Perkin-Elmer 257 grating spectrometer. ^{1}H and ^{13}C NMR were run on a Varian FT 80A in $CDCl_3$ using Me_4Si as reference signal. TLCs were performed on silica gel 60 F_{254} plates, layer thickness 0.2 mm (Merck); the R_f values were indicated as: R_f (F) = R_f of a compound referred to the R_f of 3-formylrifamycin SV; R_f (S) = R_f of a compound referred to the R_f of rifamycin S. Preparative chromatography was carried out on silica gel 60 F_{254} precoated plates (thickness

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2 mm, Merck), and column chromatography was carried out on silica gel 60, 70–230 ASTM (Merck). Manganese dioxide used as oxidizing material was according to the Rosenkranz method.[15]

Synthesis of 3-[(*N*,*N*-Dimethylamino)cyanomethyl]rifamycin S (2). Method A. NaCN (0.300 g, 6 mmol) was suspended in a solution of dimethylamine (0.450 g, 10 mmol) in isopropyl alcohol (30 mL). To the resulting stirred mixture was added 0.725 g (1 mmol) of 3-formylrifamycin SV at room temperature, and after 30 min, the suspension was oxidized with MnO_2 (3.8 g). The reaction was followed by TLC (chloroform/methanol, 30:1), and after 2 h, a violet spot with R_f (S) 0.81 was observed. At this time, the mixture was filtered and to the filtrate was added chloroform (200 mL). The organic layer was washed with 10% aqueous citric acid (2 × 50 mL) and with water, dried (Na_2SO_4), and evaporated. The residue was purified by preparative chromatography (chloroform/methanol, 40:2): yield 0.370 g (47.5%).

Method B. To a suspension of compound **3b** (1.50 g, 2 mmol) in isopropyl alcohol (30 mL) was added dimethylamine (0.90 g, 20 mmol), and the mixture was stirred at room temperature for 3 h. MnO_2 (6.5 g) was added, and the suspension was stirred for 2 h. At this time, the mixture was treated as in the method A: yield 0.690 g (44.35%); UV (methanol) λ_{max} 272 nm (log ϵ 4.30), 330 (4.26), 550 (3.60); ^{1}H NMR δ 2.83 [s, 6 H, $N(CH_3)_2$]. Anal. ($C_{41}H_{51}N_3O_{12}$) C, H; N: calcd, 5.40; found, 4.87.

Synthesis of 3-(Cyanohydroxymethyl)rifamycin SV (3a and 3b). To a suspension of 0.200 g (4 mmol) of sodium cyanide in 30 mL of anhydrous acetonitrile was added gradually 1 g (1.38 mmol) of 3-formylrifamycin SV and 2 drops of glacial acetic acid. The mixture was stirred at room temperature, and the reaction was followed by TLC (chloroform/methanol/acetic acid, 88:12:1). After 3 h, two yellow spots with R_f (F) 0.4 and 0.6 were observed. At this time, 20 mL of 10% aqueous citric acid was added, and the solution was washed with chloroform. The organic layer was washed three times with water, dried (Na_2SO_4) and stripped. The residue was dissolved by heating in ethyl formate, and on cooling, 0.463 g (yield 45%) of 3-(cyanohydroxymethyl)rifamycin SV (**3a**) crystallized, corresponding to the product with R_f (F) 0.4. The ethyl formate mother liquor was brought to dryness, and the residue was crystallized from ethyl acetate to yield 0.360 g (yield 35%) of 3-(cyanohydroxymethyl)rifamycin SV (**3b**) with R_f (F) 0.6: UV (methanol), **3a**, λ_{max} 228 nm (log ϵ 4.59), 314 (4.27), 447 (4.16); **3b**, 228 nm (log ϵ 4.58), 314 (4.20), 447 (4.11). IR (chloroform), **3a**, 2225 (vw) cm^{-1}; **3b**, 2230 (vw) cm^{-1}. ^{1}H NMR, **3a**, δ 4.75–5.25 (br s, 1 H, OH), 6.35 [s, 1 H, CH(OH,CN)]; **3b**, δ 5.0–5.25 (br s, 1 H, OH), 6.37 [d, 1 H, J = 4 Hz, CH(OH,CN)]. ^{13}C NMR, **3a**, δ 55.12 [CH(OH,CN)], 121.74 (C≡N); **3b**, δ 54.96 [CH(OH,CN)], 122.11 (C≡N). Anal. ($C_{39}H_{48}N_2O_{13}$) C, H, N.

Synthesis of 3-(Cyanocarbonyl)rifamycin SV (4). To a solution of 0.5 g (0.66 mmol) of **3a** or **3b** in 80 mL of anhydrous acetonitrile was added 1.5 g of manganese dioxide. The mixture was stirred at room temperature for 40 min and the manganese dioxide was filtered off. The filtrate, containing a light violet product which had on TLC R_f (S) 0.6 (chloroform/methanol, 30:1), was treated with a 20-mL solution of L(+)-ascorbic acid saturated acetonitrile. The mixture was stirred for 20 min; a brick-red product with R_f (F) 0.34 (chloroform/methanol, 43:7) was visible by TLC. To the solution concentrated under vacuum was added 200 mL of chloroform, and the organic layer was washed twice with water (40 mL), dried, and brought to dryness. The residue was purified on a silica gel column (12 × 1.5 cm; eluent: chloroform:methanol, 43:7): yield 80%; UV (acetonitrile) λ_{max} 218 nm (log ϵ 4.48), 232 (4.44), 312 (4.08), 477 (3.93); IR (chloroform) 2250 (vw), 1760 (m), 1720 (s), 1700 (s), 1685 (w) cm^{-1}; ^{13}C NMR δ 135.75 (CN), 159.46 (COCN). Anal. ($C_{39}H_{46}N_2O_{13}$) C, H, N.

Synthesis of 3-Carbomethoxyrifamycin S (5). Compound **4** (0.750 g, 1 mmol) in 20 mL of chloroform was oxidized with 1.4 g of MnO_2 at room temperature. After 10 min the manganese dioxide was filtered off and 50 mL of anhydrous methanol was added. The mixture was stirred at 50 °C for 15 min and stripped, and the residue was crystallized from acetonitrile. Compound **5** was obtained as a yellow product (changing to violet on exposure to air), which on TLC has R_f (S) 0.83 (chloroform/methanol, 22:1): yield 0.6 g (80%); UV (methanol) λ_{max} 218 nm (log ϵ 4.56), 268 (4.34), 318 (4.25), 395 (3.66), 535 (2.78); IR (chloroform) 1735 (s), 1715 (s), 1660 (m), 1640 (m), 1630 (s), 1600 (s), cm^{-1}; ^{1}H NMR δ 3.94 (s, 3 H, $COOCH_3$).

Synthesis of 3-(1-Glyceroxycarbonyl)rifamycin S (6). To a solution of 0.750 g (1 mmol) of **4** in 20 mL of chloroform was added 1.5 g of MnO_2 and the suspension was stirred for 10 min at room temperature. The MnO_2 was filtered off and to the filtrate was added 10 mL of glycerin α,β-isopropylidene ether. After 2 h, the reaction was stopped, the solution was concentrated to a small volume, 100 mL of ethyl acetate was added, and the organic phase was repeatedly washed with water, dried, and stripped. The residue was purified by preparative chromatography. The compound was dissolved in 25 mL of dioxane and treated with 15 mL of 20% sulfuric acid. The acetonide protection of the glyceryl groups was hydrolyzed in 4 h. Ethyl acetate (100 mL) was added to the mixture, and the organic layer was washed with water, dried, and stripped. The residue was purified by preparative chromatography (chloroform/methanol, 22.5:2.5): R_f (S) 0.62; yield 40%; IR (chloroform) 1740 (s), 1710 (s), 1660 (w), 1640 (m), 1625 (s), 1600 (s) cm^{-1}; ^{1}H NMR δ 4.6–4.8 (m, 4 H, CH_2OH and 2 OH), 4.4–4.9 (m, 3 H, CH_2O and CHOH).

Synthesis of 3-[(*N*-Methyl-*N'*-piperazinyl)carbonyl]rifamycin S (7). To 2.0 g (2.65 mmol) of 3-(cyanocarbonyl)rifamycin SV (**4**), dissolved in 100 mL of anhydrous dioxane, was added 0.6 mL (5.3 mmol) of *N*-methylpiperazine and the reaction was stirred at room temperature. After 30 min, 3 g of MnO_2 was added. After 15 min, the manganese dioxide was filtered off, and the organic phase was concentrated under vacuum to one-third of the volume, diluted with 100 mL of chloroform, and washed several times with water. The mixture was dried and stripped. The residue was crystallized from acetonitrile. **7** was a violet product (yellow in chloroform) with R_f (S) 0.62 (TLC, chloroform/methanol, 23:2): yield 1 g (46%); UV (methanol) λ_{max} 272 nm (log ϵ 4.35), 312 (4.22), 400 (3.59), 530 (3.41); IR (chloroform) 1740 (s), 1710 (s), 1655 (s), 1630 (s), 1595 (s) cm^{-1}; ^{1}H NMR δ 2.42 (s, 3 H, NCH_3), 2.5–2.8 [m, 4 H, $N(CH_2)_2$], 3.2–3.7 [m, 4 H, $N(CH_2)_2$].

Synthesis of 3-[(*N*,*N*-Dimethyl-*N'*-hydrazino)carbonyl]rifamycin S (8). *N*,*N*-Dimethylhydrazine (2 mL, 25 mmol) was added portionwise to 2 g (2.65 mmol) of **4** dissolved in 100 mL of anhydrous dioxane. The solution was stirred at room temperature until the color turned yellow. MnO_2 (3 g) was added to the mixture and stirred. After 20 min, the manganese dioxide was filtered off and chloroform (100 mL) was added. The organic phase was repeatedly washed with water, dried, and stripped. The residue was purified on a silica gel column (eluent: chloroform/methanol/acetic acid, 22.5:2.5:0.25). The brown-violet fractions containing a product with R_f (S) 0.65 (TLC eluent: chloroform/methanol/acetic acid, 22.5:2.5:0.25) were combined and evaporated to one-third of the volume. Chloroform (100 mL) was added to the concentrated liquor, and the solution was washed with water, dried, and stripped. The residue was precipitated from *n*-hexane: yield 42%; UV (methanol) λ_{max} 270 nm (log ϵ 4.35), 310 (4.31), 400 (3.54), 530 (3.44); IR (chloroform) 1730 (s), 1700 (s), 1640 (s), 1630 (s), 1595 (s) cm^{-1}; ^{1}H NMR δ 2.90 (s, 3 H, NCH_3), 3.00 (s, 3 H, NCH_3).

Synthesis of 3-[(*N*,*N*-Dimethylamino)carbonyl]rifamycin S (9). A saturated solution of dimethylamine in tetrahydrofuran (5 mL) was added to 0.750 g (1 mmol) of **4** dissolved in 20 mL of anhydrous dioxane. The reaction was stirred in a closed vessel at 40 °C for 20 min. Then the manganese dioxide was filtered off and 100 mL of chloroform was added. The solution was repeatedly washed with water, dried, and stripped. The residue was purified by preparative chromatography (chloroform/methanol, 23:2): 0.500 g (yield 73%) of compound **9** was obtained; TLC (chloroform/methanol, 23:2) R_f (S) 0.80; UV (methanol) λ_{max} 213 nm (log ϵ 4.57), 272 (4.37), 308 (4.22), 400 (3.62), 530 (2.61); IR (KBr) 1740 (s), 1710 (s), 1670 (m), 1660 (m), 1635 (s), 1600 (s) cm^{-1}; ^{1}H NMR δ 3.00 (s, 3 H, NCH_3), 3.07 (s, 3 H, NCH_3).

Synthesis of 3-[[*N*-(*N'*-Piperidinyl)-*N*-methylamino]carbonyl]rifamycin S (10). *N*-(Methylamino)piperidine (0.50 g, 4.38 mmol) was added to a solution of 0.750 g (1 mmol) of **4** in 20 mL of anhydrous dioxane. The reaction was left at room temperature for 48 h, 1.5 g of MnO_2 was added, and the suspension was stirred for 20 min. The manganese dioxide was filtered off,

(15) O. Mancera, G. Resenkranz, and F. Sandkeimer, *J. Chem. Soc.*, 2189 (1953).

and the filtrate was stripped. The residue was purified by preparative chromatography (chloroform/methanol, 23:2): 0.260 g of compound **10** was obtained (yield 31%); TLC (chloroform/methanol, 24:1) R_f (S) 0.67; IR (chloroform) 1735 (m), 1720 (s), 1700 (s), 1655 (m), 1625 (s), 1595 (s) cm^{-1}.

Synthesis of 3-[(*p*-Bromoanilino)carbonyl]rifamycin S (11). *p*-Bromoaniline (0.400 g, 2.3 mmol) was added to a solution of 1.0 g (1.3 mmol) of **4** in 25 mL of anhydrous dioxane. The reaction was carried out at room temperature, being stirred for 2 h. MnO_2 (2 g) was then added, and 20 min later, the manganese dioxide was removed by filtration. Chloroform (100 mL) was added, and the organic phase was repeatedly washed first with 0.01 N hydrochloric acid and later with water. The dried solution was stripped. The residue was purified by preparative chromatography (chloroform/methanol, 22.5:2.5), R_f (S) 0.9. The light brown product was precipitated from chloroform with hexane: yield 0.400 g (34%); IR (chloroform) 1740 (s), 1710 (s), 1685 (m), 1635 (s), 1605 (s), 1575 (w) cm^{-1}; ^1H NMR δ 7.5-7.8 (m, 4 H, aromatic), 9.75 (br s, 1 H, CONH).

Biological Test. Antimicrobial Activity. MIC values were determined in a liquid medium, by means of the serial dilution method in test tubes. The medium employed was brain-heart infusion (BHI, Difco). The inoculum size was always 10^6 cells/mL. The MIC was defined as the lowest antibiotic concentration that prevented a visible growth after 24 h of incubation at 35 °C.

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Synthesis and Antibacterial Activity of 1-(Arylamino)-1*H*-pyrroles and 4-(1*H*-Pyrrol-1-ylimino)-2,5-cyclohexadienes

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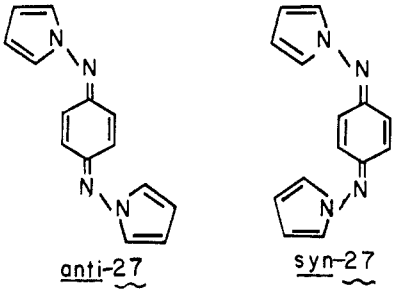
The syntheses of 1-(arylamino)-1*H*-pyrroles and 4-(1*H*-pyrrol-1-ylimino)-2,5-cyclohexadienes are described. Several of these compounds express in vitro antibacterial activity or can be metabolized to show in vitro antibacterial activity, and a few examples have shown efficacy against tuberculosis in mice. One compound, *N,N'*-(2,5-cyclohexadiene-1,4-diylidene)bis-1*H*-pyrrol-1-amine, is completely effective at 6.25 mg/kg against *Mycobacterium tuberculosis* H37Rv.

The efficacy of the experimental antitubercular agent *N,N'*-(2,5-cyclohexadiene-1,4-diylidene)bis-1*H*-pyrrol-1-amine (azarole, *anti*-**27**) has been reported to be due to its



stimulation of cell-mediated immunity.[1] This unique compound is one of a series of structurally related 1-(arylamino)-1*H*-pyrrol-1-amines and 4-(1*H*-pyrrol-1-ylimino)-2,5-cyclohexadienes that were investigated for in vitro antibacterial activity and in vivo antitubercular activity. The purpose of this paper is to describe the synthesis of this series of compounds and to relate some of the structural requirements necessary for their antibacterial effects.

Chemistry. The compounds described in Tables I and II were prepared utilizing the procedures outlined in Scheme I.

1-(Arylamino)-1*H*-pyrroles **3-13** were prepared by a sequence of reactions starting with the condensation of benzoic acid 1-arylhydrazides[2-4] [e.g., **1** (R = Bz; X = 4-OCH_2Ph)] and 2,5-diethoxytetrahydrofuran or 2,5-hexanedione in hot glacial acetic acid, followed by alkaline hydrolysis of the intermediate benzamides, and then catalytic hydrogenolysis of the benzyl ethers where appropriate. In a similar manner, **14** was prepared by the catalytic debenzylation of the reaction product of 1-methyl-1-[4-(phenylmethoxy)phenyl]hydrazine (**2**) and 2,5-diethoxytetrahydrofuran.

The synthesis of **16** was accomplished by basic H_2O_2 oxidation, followed by catalytic debenzylation of **15**, which was the reaction product of **4** and oxalyl chloride.

Monoacetylated products **17** or **31** resulted when **8** or **30** were reacted with excess acetic anhydride in pyridine. Similarly, **8** was treated with either 1 or 2 equiv of 4-methylbenzoyl chloride to give **19** or **20**, respectively. Diacetylated **18** was produced when **17** was combined with acetic anhydride using sodium hydride as base.

The 4-(1*H*-pyrrol-1-ylimino)-2,5-cyclohexadienes **21-24** and compound **32** were obtained by mild oxidation of **8**, **12**, **13**, **5**, and **30**, respectively, with yellow HgO or Ag_2O. Other cyclohexadienes, **25-28**, were directly prepared by the trifluoroacetic acid catalyzed reaction of 1*H*-pyrrol-1-amine[5] (**36**) with the appropriate benzoquinone. Separation of **27** into anti and syn isomers was accomplished by fractional crystallization. The 100-MHz NMR spectrum of *anti*-**27** showed quinone signals at 7.38 and 7.12 ppm with ortho coupling ($J \simeq 10$ Hz), and *syn*-**27** showed quinone signals at 7.35 and 7.09 ppm with no ortho coupling. These NMR results are similar to those reported for *anti*- and *syn-N,N'*-(2,4-cyclohexadien-1,4-diylidene)-bis(2,6-diethylaniline).[6]

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