

# Rifamycins. XLI.<sup>1</sup> A New Class of Active Semisynthetic Rifamycins. N-Substituted Aminomethyl Derivatives of Rifamycin SV<sup>2</sup>

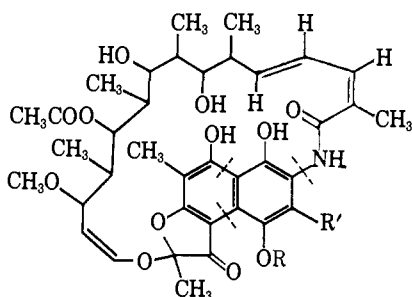
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A series of Mannich derivatives of rifamycin SV and their N-oxides have been synthesized. The preparation and the chemical and physical properties of these derivatives are described, and their *in vitro* and *in vivo* antibacterial activities and acute toxicities in mice are reported. Compared with rifamycin SV, some of these derivatives proved to be more active against experimental staphylococcal infections in mice, especially by oral administration.

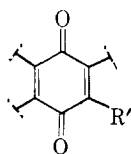
Recently<sup>3</sup> several new rifamycins obtained from rifamycin B (I), a metabolic product of *Streptomyces mediterranei*,<sup>4</sup> have been described. Rifamycin B is the starting material for the preparation of rifamycin SV (II) which is now widely employed clinically. In order to find new rifamycins with improved oral absorption and eventually also more activity against gram-negative bacteria than rifamycin SV (II), two new series of substitution products at position 3 of the latter were prepared. The products of type III were



I, R = CH<sub>2</sub>COOH; R' = H (rifamycin B)  
 II, R = H; R' = H (rifamycin SV)

III, R = H; R' = CH<sub>2</sub>N <  $\begin{matrix} R'' \\ R''' \end{matrix}$

IV, R = H; R' = CH<sub>2</sub>N <  $\begin{matrix} R'' \\ R''' \\ \text{O} \end{matrix}$



V, R' = H (rifamycin S)

VI, R' = CH<sub>2</sub>N <  $\begin{matrix} R'' \\ R''' \end{matrix}$

R'' and R''', see Table I

prepared by treating rifamycin S (V) with secondary amines in the presence of formaldehyde, followed by reduction of the reaction products (VI) with ascorbic acid. Although the Mannich reaction has been pre-

viously applied to quinones and hydroquinones,<sup>5</sup> in the case of the rifamycins only rifamycin S undergoes the Mannich reaction. Along with the N-substituted aminomethylrifamycins S (VI) a certain amount of rifamycin SV (II) and its Mannich derivatives (III) were observed at the end of the reaction due to the reducing property of the formaldehyde. Reduction of the mixture with ascorbic acid simplified the separation of the derivative III from rifamycin SV.

The Mannich derivatives of rifamycin SV are amphoteric; they are yellow-orange substances sparingly soluble in water at neutral pH. The elemental analyses of some of these derivatives differ slightly from the calculated values due to the difficulty in removing the solvent of crystallization. Nevertheless, their structure has been corroborated on the basis of chemical behavior and from physical data, such as n.m.r., infrared, ultraviolet, and visible spectra. Particularly indicative are the n.m.r. spectra which show the disappearance of the singlet at  $\delta$  7.8 present in the rifamycin S<sup>6</sup> and attributed to the only aromatic hydrogen. The ultraviolet and visible spectra are very similar to that of rifamycin SV with maxima at 220-225, 314-316, and 445-450 m $\mu$ . The polarographic analysis shows an oxidation wave with  $E_{1/2} = +0.05$  to  $+0.010$ , due to the hydroquinone system of rifamycin SV.

In Table I are given the yields, decomposition points, and microanalytical and spectrophotometric data of the N-disubstituted aminomethyl derivatives of rifamycin SV obtained according to three main reaction procedures A, B, and C (see Experimental Section).

Under appropriate conditions the Mannich derivatives of rifamycin SV can be oxidized by hydrogen peroxide to the corresponding N-oxides of the general formula IV. These compounds are acidic ( $pK \approx 5$ ) and their sodium salts are soluble in water. Reduction with sodium hydrosulfite reconverts them to the original aminomethylrifamycins (III). The analytical data of these N-oxides are reported in Table II. The *in vitro* activity of Mannich derivatives and their oxides, expressed as minimum inhibitory concentration against some strains of gram-positive and gram-negative bacteria as well as *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub>, is reported in Table III. They were tested also for *in vivo* activity in acute experimental staphylococcal infection in mice. These results are reported in Table IV.

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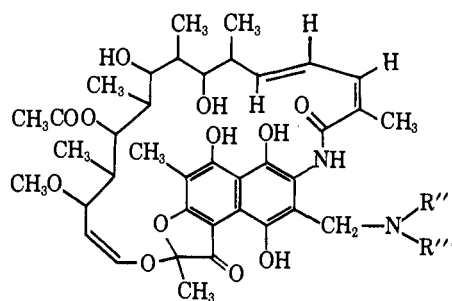
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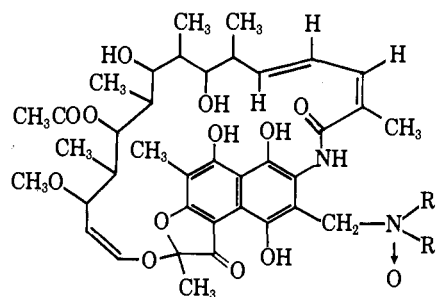
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TABLE I  
 N-SUBSTITUTED AMINOMETHYLRIFAMYCINS SV


Compd.	R''	R'''	Pro- cedure	Yield, %	Dec. pt., °C. <sup>a</sup>	Formula	Calcd., %			Found, %			Spectropho- tometric data <sup>b</sup>	
							C	H	N	C	H	N	$\lambda_{max}$ , m $\mu$	$\epsilon$
1	CH <sub>3</sub>	CH <sub>3</sub>	C	42	189	C <sub>40</sub> H <sub>84</sub> N <sub>2</sub> O <sub>12</sub>	63.64	7.21	3.71	62.88	7.55	3.53	314	18,070
2	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	B	72	186-188	C <sub>42</sub> H <sub>88</sub> N <sub>2</sub> O <sub>13</sub>	62.98	7.55	3.50	62.43	7.90	3.50	315	17,380
3	(CH <sub>2</sub> ) <sub>4</sub>		A	60	180-185	C <sub>42</sub> H <sub>86</sub> N <sub>2</sub> O <sub>12</sub>	64.60	6.97	3.59	63.82	7.50	3.70	314	17,810
4	(CH <sub>2</sub> ) <sub>5</sub>		A	45	190-192	C <sub>43</sub> H <sub>88</sub> N <sub>2</sub> O <sub>12</sub>	64.97	7.85	3.52	64.10	7.55	3.17	315	18,250
5	(CH <sub>2</sub> ) <sub>2</sub> O(CH <sub>2</sub> ) <sub>2</sub>		C	16 <sup>c</sup>	175-180	C <sub>42</sub> H <sub>86</sub> N <sub>2</sub> O <sub>13</sub>	63.30	7.08	3.52	63.48	7.28	3.60	316	20,070
6	$\begin{array}{c} \text{CH}_3 \\   \\ (\text{CH}_2)_2\text{N}(\text{CH}_2)_2 \end{array}$		C	5 <sup>c</sup>	220	C <sub>43</sub> H <sub>88</sub> N <sub>3</sub> O <sub>12</sub>	63.76	7.34	5.19	63.20	7.65	5.14	314	21,050
7	CH <sub>3</sub>	$\text{CH} < \begin{array}{c} (\text{CH}_2)_2 \\   \\ (\text{CH}_2)_2 \end{array} > \text{CH}_2$	A	47	202-205	C <sub>45</sub> H <sub>92</sub> N <sub>2</sub> O <sub>16</sub>	65.67	7.59	3.40	65.84	7.80	3.29	314	17,920
8	$\begin{array}{c} \text{CH}_2\text{OH} \quad \text{CH}_2\text{OH} \\   \quad   \\ \text{CH}(\text{CH}_2)_2\text{CH} \end{array}$		B	10 <sup>c</sup>	190-195	C <sub>44</sub> H <sub>89</sub> N <sub>2</sub> O <sub>14</sub>	62.84	7.19	3.33	62.03	7.60	3.37	314	17,890
9	(CH <sub>2</sub> ) <sub>4</sub> CH(CH <sub>2</sub> ) <sub>2</sub>		A	30 <sup>c</sup>	200-203	C <sub>44</sub> H <sub>89</sub> N <sub>2</sub> O <sub>12</sub>	65.33	7.48	3.46	64.84	7.80	3.39	314	17,680
10	$\begin{array}{c} \text{CH}_3 \\   \\ (\text{CH}_2)_2\text{CH}(\text{CH}_2)_2 \end{array}$		A	61	180	C <sub>44</sub> H <sub>89</sub> N <sub>2</sub> O <sub>12</sub>	65.33	7.48	3.46	64.87	7.65	3.44	315	17,190
11	$\begin{array}{c} \text{CH}_3 \quad \text{CH}_3 \\   \quad   \\ \text{CH}(\text{CH}_2)_2\text{CH} \end{array}$		A	11 <sup>c</sup>	200-205	C <sub>44</sub> H <sub>89</sub> N <sub>2</sub> O <sub>12</sub>	65.67	7.59	3.40	64.71	7.92	3.39	314	19,270
12	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> OH	A	5 <sup>c</sup>	170	C <sub>41</sub> H <sub>86</sub> N <sub>2</sub> O <sub>13</sub>	62.74	7.19	3.57	62.24	7.60	3.27	314	18,980
													447	14,430

<sup>a</sup> Melting points were indefinite. <sup>b</sup> Phosphate buffer pH 7.38. All derivatives show an absorption maximum at approximately 220 m $\mu$ . The values of  $\epsilon$  are not reported here. <sup>c</sup> No attempts were made to improve the yields.

 TABLE II  
 N-OXIDES OF N-SUBSTITUTED AMINOMETHYLRIFAMYCINS SV


Compd.	R	R'	Yield, %	Dec. pt., °C.	Formula	Calcd., %			Found, %			Spectrophotometric data <sup>a</sup>	
						C	H	N	C	H	N	$\lambda_{max}$ , m $\mu$	$\epsilon$
13	CH <sub>3</sub>	CH <sub>3</sub>	22 <sup>b</sup>	165	C <sub>40</sub> N <sub>54</sub> N <sub>2</sub> O <sub>13</sub>	62.33	7.05	3.63	61.47	7.30	3.66	316	16,740
14	(CH <sub>2</sub> ) <sub>4</sub>		36	180	C <sub>42</sub> H <sub>86</sub> N <sub>2</sub> O <sub>13</sub>	63.30	7.07	3.58	62.94	7.08	3.60	316	18,880
15	(CH <sub>2</sub> ) <sub>5</sub>		13 <sup>b</sup>	160	C <sub>42</sub> H <sub>86</sub> N <sub>2</sub> O <sub>14</sub>	63.73	7.21	3.23	62.82	7.24	3.40	316	18,400
16	CH <sub>2</sub> CH <sub>2</sub> OCH <sub>2</sub> CH <sub>2</sub>		10 <sup>b</sup>	170	C <sub>43</sub> H <sub>88</sub> N <sub>2</sub> O <sub>13</sub>	62.04	6.93	3.44	61.31	7.50	3.52	316	16,860
												450	11,620

<sup>a</sup> Phosphate buffer pH 7.38. <sup>b</sup> No attempts were made to improve the yields.

TABLE III  
 MINIMUM INHIBITORY CONCENTRATION<sup>a</sup> OF N-SUBSTITUTED AMINOMETHYLRIFAMYCINS SV AND THEIR N-OXIDES

Compl.	<i>Micrococcus aureus</i> ATCC 6538	<i>Streptococcus faecalis</i> ATCC 10541	<i>Streptococcus hemolyticus</i> C 203	<i>Bacillus subtilis</i> ATCC 6633	<i>Proteus vulgaris</i> ATCC 881	<i>Escherichia coli</i> ATCC 10536	<i>Klebsiella pneumoniae</i> ATCC 10031	<i>Pseudomonas aeruginosa</i> ATCC 10145	<i>Mycobacterium tuberculosis</i> H <sub>3</sub> R <sub>v</sub>
1	0.015	0.1	0.2	0.2	50	1	5	10	0.1
2	0.02	0.37	0.045	0.18	>100	50	25	100	0.18
3	0.05	0.5	0.15	0.5	100	2	5	20	0.05
4	0.05	0.5	0.5	0.2	>100	50	20	100	0.1
5	0.1	0.5	0.1	0.5	>100	20	50	100	0.1
6	0.02	0.2	0.5	0.5	100	20	50	50	0.2
7	0.02	0.2	0.5	0.1	50	10	20	50	1
8	1	10	0.05	50	>100	20	100	100	1
9	0.05	0.5	0.5	0.2	>100	10	20	100	0.5
10	0.05	1	0.5	0.2	>100	100	50	>100	1
11	0.01	0.3	0.75	0.2	>100	20	20	100	0.5
12	0.05	0.5	0.3	0.5	>100	2	10	10	0.05
13	0.002-0.2	0.1-5	0.05-5	10	>100	>100	100	>100	5
14	0.002	0.5	2	0.5	>100	50	>100	>100	5
15	0.005-0.01	0.1-1	0.05-2	0.5	>100	>100	>100	>100	2
16	0.01	0.5-2	1	0.2	>100	>100	>100	>100	2
Rifamycin SV <sup>b</sup>	0.005	0.5	0.0025	0.075	25	50	25	50	0.05

<sup>a</sup> Minimum inhibitory concentration, in  $\gamma$ /ml., is the lowest concentration of antibiotic that prevents visible growth after 18 hr. of incubation for gram-positive and gram-negative bacteria; after 7 days of incubation for *M. tuberculosis*. <sup>b</sup> For comparison.

 TABLE IV  
 In Vivo ACTIVITY<sup>a</sup> OF N-SUBSTITUTED AMINOMETHYLRIFAMYCINS SV AND THEIR N-OXIDES

Compl.	ED <sub>50</sub> , mg./kg. (range)	
	P.o.	S.c.
1	13.9 (15.9-12.2)	2 (2.28-1.75)
2	12.1 (13.6-10.8)	6.4 (6.76-5.39)
3	16 (19.3-13.3)	4.3 (4.80-3.83)
4	7.5 (8.90-6.26)	4 (4.78-3.35)
5	9.8 (10.8-8.98)	9.2 (10.6-7.96)
6	>16	6.5 (7.13-5.92)
7	10.6 (12.1-9.17)	7.5 (8.36-6.67)
8	...	...
9	12.1 (13.6-10.8)	4.9 (5.59-4.34)
10	7 (7.74-6.27)	5.3 (5.92-4.70)
11	10.6 (11.9-9.36)	5.7 (6.45-4.96)
12	>16	<8
13	>16	~16
14	>16	>16
15	~16	~12
16	~16	~12
Rifamycin SV <sup>b</sup>	260	10.7

<sup>a</sup> Activity against staphylococcal infections in mice. <sup>b</sup> For comparison.

### Experimental Section

**Chemistry.**—The Mannich derivatives were synthesized following three main procedures. Method A consisted of treating the rifamycin with an excess of the amine and aqueous formaldehyde in tetrahydrofuran at room temperature, followed by separation of the desired product from unreacted rifamycin and other by-products by column chromatography. Procedure B differed from A in that the unreacted rifamycin and other by-products were separated by extraction and the pure product crystallized from various solvents. In procedure C rifamycin was submitted to more severe reaction conditions, *i.e.*, at the boiling point of the solvent, with excess of reagents, purification and isolation being accomplished by column chromatography.

The amines used were available from commercial sources; only 2,5-dihydroxymethylpyrrolidine was synthesized according to the procedure of Cignarella.<sup>7</sup>

The derivatives obtained were tested by thin layer chromatography, on silica gel plates, following Stahl's technique,<sup>8</sup>

using acetone as eluent ( $R_f$  varying from 0.5-0.8); they were checked spectrophotometrically for their purity, reading absorption values after dissolution in phosphate buffer at pH 7.48.

The N-oxides of the Mannich derivatives of rifamycin SV listed in Table II were synthesized according to a general procedure. Any attempt to obtain an oxide from 3-diethylaminomethylrifamycin SV (Table I, 2) failed: the substance was recovered unchanged at the end of the reaction. The N-oxides, obtained in crystalline form were examined by thin layer chromatography on silical gel; their  $R_f$  in acetone varied from 0.1-0.3, sharply different from those of the starting materials.

**3-Pyrrolidinomethylrifamycin SV (Table I, 3). Procedure A.**—To a stirred solution of 7 g. (0.01 mole) of rifamycin S in 50 ml. of tetrahydrofuran was added cautiously 1.7 ml. (0.02 mole) of pyrrolidine and 2.3 ml. (0.03 mole) of aqueous formaldehyde (40%), at room temperature. The solution was kept overnight at the same temperature and then concentrated under reduced pressure to 15 ml. The concentrate was poured into 35 ml. of a 10% aqueous solution of ascorbic acid, stirred, and cooled at 5-10°. After 10 min. the slurry was extracted twice with 50 ml. of ethyl acetate. The combined extracts were dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated to about 10 ml. The concentrate was placed on a column of 150 g. of silica gel (Merek, 0.2-0.5 mm.), pre-washed with ethyl acetate then eluted with ethyl acetate. The first eluate (200 ml.) was discarded then the further eluate (about 400 ml.) was collected, concentrated to a small volume, and poured under agitation into a beaker containing 10 vol. of hexane. The precipitated product was collected and dried (4.2 g.). The amorphous yellow-orange product gave a single spot ( $R_f \approx 0.7$ ) on thin layer chromatography. The analytical data obtained from a sample crystallized from ethanol-water were in accordance with the calculated values.

**3-Diethylaminomethylrifamycin SV (Table I, 2). Procedure B.**—Rifamycin S (7 g., 0.01 mole) was dissolved into 50 ml. of tetrahydrofuran at room temperature. To the stirred solution was added 2.1 ml. (0.02 mole) of diethylamine, followed by 2.3 ml. (0.03 mole) of aqueous formaldehyde (40%). The solution was kept at room temperature for 24 hr., then poured into 50 ml. of a 10% aqueous solution of ascorbic acid, stirred, and cooled at 5-10°. After dilution with 300 ml. of water, the whole was extracted with 350 ml. of ethyl acetate. The organic extract was concentrated under reduced pressure to approximately 35 ml. The product crystallized out readily and, after chilling (2-3 hr.), was collected, washed with ethyl acetate, and dried (5.05 g.). The crystalline, orange product was homogeneous in thin layer chromatography ( $R_f \approx 0.65$ ) as above. A sample recrystallized

(7) G. Cignarella and G. G. Nathansohn, *Gazz. chim. ital.*, **90**, 1495 (1960).

(8) E. Stahl, "Dünnschicht Chromatographie," Springer-Verlag, Berlin, 1962.

from ethyl acetate gave analytical results in agreement with the calculated values.

**3-Morpholinomethylrifamycin SV (Table I, 5). Procedure C.**—To a stirred solution of 7 g. (0.01 mole) of rifamycin S in 50 ml. of tetrahydrofuran 1.75 ml. (0.02 mole) of morpholine and 2.3 ml. (0.03 mole) of formaldehyde (40%) were added at room temperature. The solution was then refluxed for 14 hr. and concentrated to a small volume. The concentrate was poured, with stirring, into 35 ml. of a 10% solution of ascorbic acid at 5–10°. After 10 min. the crude product was extracted with ethyl acetate, concentrated to 10 ml., and passed through a silica gel column (as in A) with ethyl acetate as eluent. The first 500 ml. of eluate was discarded, then the subsequent 600 ml. containing the pure product was collected and concentrated to a small volume under reduced pressure. The concentrate was poured with stirring into 10 vol. of hexane. The yellow-orange precipitate was filtered, washed with hexane, and dried (1.1 g.). It was chromatographically homogeneous ( $R_f \approx 0.7$ , in the same system as A and B) and was analytically pure.

**3-Pyrrolidinomethylrifamycin SV N-Oxide (Table II, 14).**—Twenty grams (0.025 mole) of 3-pyrrolidinomethylrifamycin SV (Table I, 3) was dissolved in 130 ml. of ethyl acetate. The solution was cooled at 0–5° and then, with stirring, 2.38 ml. of H<sub>2</sub>O<sub>2</sub> (35%) (0.025 mole) was added followed by 3.48 ml. (0.025 mole) of triethylamine. The solution was kept at 4–5° for 24 hr., after which two extractions with 130 ml. of phosphate buffer, pH 7.3, were made. The combined aqueous extracts were washed with ethyl acetate. The solution was then acidified with 10 g. of ascorbic acid and re-extracted with ethyl acetate. The organic extract was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under vacuum to 10–15 ml. After cooling overnight at 0–5°, a yellow product crystallized out. It was collected, washed with ethyl acetate, and dried under vacuum at 40° (6 g.). The product so obtained showed a single spot in thin layer chromatography and its analytical data were in agreement with the calculated values.

**3-Pyrrolidinomethylrifamycin SV (Table I, 3) from Its N-Oxide (Table II, 14).**—Three grams (0.0038 mole) of 3-pyrrolidinomethylrifamycin SV was dissolved in 260 ml. of ethanol, and under cooling and stirring a solution of 1.3 g. (0.0076 mole) of sodium hydrosulfite in 130 ml. of water was added drop by drop. After 10 min. the reaction solution was poured into 1300 ml. of water, then extracted with ethyl acetate. The organic extract, washed with water once, was concentrated under vacuum to 100 ml. and poured into 1000 ml. of hexane. The yellow-orange precipitate was collected, washed with hexane, and dried under vacuum at 40° (2.0 g., 66.6%). The product, crystallized once from 1:1 ethanol-water, showed analytical data (thin layer chromatography, n.m.r. and ultraviolet spectra) identical with those of 3.

**Biological Tests. In Vitro Activity.**—The antimicrobial activity of these new rifamycins was assayed by determining the minimum inhibitory concentrations (MIC) against gram-positive and gram-negative bacteria using the serial dilution technique in nutrient broth. The bacterial inoculum for each strain was standardized by turbidimetric assay in order to have comparative values for the different derivatives. The MIC was the lowest concentration of antibiotic which prevented visible growth after an 18-hr. incubation at 37°. A comparative study of these derivatives was extended also to their antituberculous activity. The inoculum consisted of 0.5–1% of a 7–9 day culture of *Mycobacterium tuberculosis* H<sub>37</sub>R<sub>v</sub> grown in Dubos medium; the tests were carried out in Kirschner medium and observations were made after 7 days of incubation at 37°.

**In Vivo Activity.**—The therapeutic activity of the Mannich derivatives of rifamycin SV and their oxides was tested in acute experimental staphylococcal infections in mice. The bacterial suspension contained 5% hog gastric mucin. The animals were infected by administering 0.5 ml. of the suspension intraperitoneally; ten mice for each dose of antibiotics were employed. Treatment was started 30 min. after infection and was given in two daily administrations (morning and evening) for 3 days. The ED<sub>50</sub> expressed as mg./kg. was calculated according to the method of Spaerman-Kärber,<sup>9</sup> 7 days after infection; the untreated controls died within 48 hr.

## Discussion

The substitution of the only aromatic hydrogen in the molecule of rifamycin SV with N-substituted amino-methyl groups (or their N-oxides) does not suppress the antibacterial activity. In comparison with rifamycin SV, there are some changes in the activity against gram-positive, gram-negative and mycobacteria, but from these changes a correlation among the type of the substituents and activity is hardly possible. For the gram-positive bacteria a certain decrease of activity of the Mannich derivatives in comparison with rifamycin SV is particularly evident in the case of *Staphylococcus aureus*, *Streptococcus hemolyticus*, and *Bacillus subtilis*, but not for *Streptococcus faecalis*. The activity of the Mannich derivatives against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* is of the same order as that of rifamycin SV and in most instances a little superior against *E. coli*. As a general consideration, the ratio of the activity against gram-negative to that against gram-positive bacteria is more favorable for these derivatives than for rifamycin SV. The activity against *M. tuberculosis* is generally decreased in comparison with rifamycin SV.

The N-oxides present, in each case, a lower activity than that of rifamycin SV. It is interesting to note that the reported activities in some cases show a certain range. This fact no doubt is attributable to the high instability of these derivatives and consequently to a partial and variable degradation during the incubation time. The low activity against *M. tuberculosis* is perhaps a consequence of the long incubation period necessary for this microorganism.

The *in vivo* data in Table IV deserve some comments. As reported in a previous paper,<sup>3</sup> our preliminary screening of new rifamycins includes the test of oral and subcutaneous activity on the staphylococcal infection in mice. The ratio ED<sub>50</sub> *p.o.*/ED<sub>50</sub> *s.c.* is supposed to give some information on the potential use of new rifamycins by the oral route. Among the Mannich derivatives, most of them show a very good *in vivo* activity by subcutaneous administration. Some of them show, in our experimental conditions, a ratio ED<sub>50</sub> *p.o.*/ED<sub>50</sub> *s.c.* of from 1–2 which is very favorable in comparison with rifamycin SV. The solubility in water of the Mannich derivatives of rifamycin SV is very low because of their amphoteric nature, while their lipid solubility is high. The results obtained with these derivatives fit well with the hypothesis<sup>3</sup> that the ratio of lipid solubility to water solubility has to be considered of importance for the gastrointestinal absorption of new rifamycins. The Mannich derivatives with a low ratio ED<sub>50</sub> *p.o.*/ED<sub>50</sub> *s.c.* were selected for further investigations on the oral absorption and biliary elimination. These studies are now in progress. The N-oxides show an *in vivo* activity lower than that of the Mannich derivatives and were not further investigated.

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