for use in studying the effect of a blocked 2-phenyl group on phototoxic potency. Steric hindrance of the phenyl group was attempted by substituting a methyl group in the 3 position of the quinoline nucleus, and by chlorine substitution in the 2 position of the phenyl ring. From the results in Table III (XXXV, XXXVI, XXXVII), it can be seen that steric hindrance of the phenyl group decreased the relative phototoxic potency. Further efforts in this direction are planned.

The Influence of the Carboxyl Group upon the Antibacterial Activity of Rifamycins[1]

N. MAGGI, S. FÜRESZ, AND P. SENSI

Research Laboratories, Lepetit S.p.A., Milan, Italy

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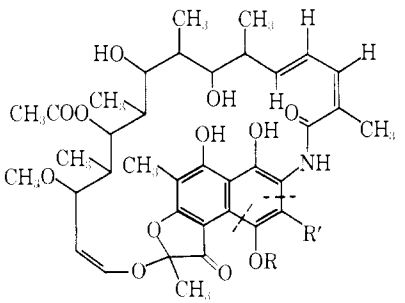
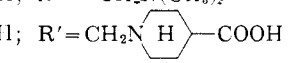
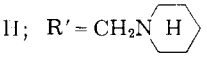
It has been demonstrated[2] that rifamycin B (I), one of the products of the metabolism of *Streptomyces mediterranei*,[3] is inactive *per se*. In aqueous media rifamycin B undergoes an oxidative and hydrolytic transformation to rifamycin S[4] which possesses high antibacterial activity. Rifamycin S is easily reduced to the hydroquinone form, rifamycin SV (II), which is now employed in the therapy of staphylococcal and other gram-positive infections, biliary tract infections, tuberculosis, and leprosy.[5] The structural difference between rifamycin B and rifamycin SV consists in the absence in the latter of the glycolic moiety bearing a free carboxyl group.[6]



I, R = CH_2COOH; R′ = H (rifamycin B)
II, R = H; R′ = H (rifamycin SV)
IIIa, R = H; R′ = $CH_2N(CH_3)CH_2COOH$
IIIb, R = H; R′ = $CH_2N(CH_3)_2$

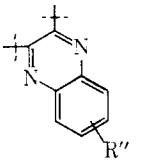
IVa, R = H; R′ = 

IVb, R = H; R′ = 

Va, R = H; R′ = $CH{=}NNHC_6H_4COOH$-*p*
Vb, R = H; R′ = $CH{=}NNHC_6H_5$
VIa, R = H; R′ = $CH{=}NN(CH_3)CH_2COOH$
VIb, R = H; R′ = $CH{=}NN(CH_3)_2$
VIIa, R = H; R′ = $CH{=}NN{=}CHC_6H_4COOH$-*p*
VIIb, R = H; R′ = $CH{=}NN{=}CHC_6H_5$

In a previous paper[7] we described the synthesis and properties of a class of derivatives of rifamycin B in which the carboxyl group was blocked (amides and hydrazides). These derivatives possessed *in vitro* antibacterial activity in most instances comparable to that of rifamycin SV.

Moreover, very recently[8] we pointed out the lowering of the antibacterial activity when the aromatic hydrogen in rifamycin SV is replaced by glutathionyl, carboxymethylthio, or β-carboxyethylthio residues. These findings lead us to infer that the presence of the carboxyl function in the rifamycin molecule could be responsible for the weakening of its antibacterial activity, and therefore we undertook the study of a number of semisynthetic rifamycins with a free carboxyl group, among some classes in which antimicrobial activity was demonstrated to be generally maintained. The products selected for this comparison include some Mannich derivatives of rifamycin SV (IIIa and IVa), hydrazones of 3-formylrifamycin SV (Va, VIa, and VIIa), and a carboxyrifazine (VIIIa). These derivatives were tested



VIIIa, R″ = COOH
VIIIb, R″ = H (rifazine)

for the *in vitro* antibacterial activity and compared with the analogous rifamycins without carboxyl group, already described (IIIb and IVb,[9] Vb, VIb, and VIIb,[10] VIIIb[11]).

Biological Results.—Table I summarizes the minimum inhibitory concentration of the carboxyrifamycins against some typical strains and compares it with that of the corresponding rifamycins without a carboxyl group. The resulting data clearly confirm a decrease of activity for the rifamycins investigated, when compared to the corresponding derivatives without the carboxyl group. The ratio of activity between derivatives with and without the carboxyl group presents large variations according to the type of derivatives taken into consideration and to the test microorganism. Particularly, *Staphylococcus aureus* is more sensitive to the structural variation here examined. In fact, the introduction of a carboxyl group induces a decrease of activity from 1:1000 (VIIIa:VIIIb) to 1:10 (IVa:IVb) against this strain.

Streptococcus hemolyticus is less sensitive to these structural modifications; in some instances (IVa *vs.* IVb and VIIa *vs.* VIIb) there is no significant modification of activity. All the carboxyrifamycins are practically inactive against gram-negative bacteria and present only limited activity on *Mycobacterium tuberculosis;* on the contrary, the parent compounds show moderate activity on the former and are highly active on the latter.

(1) Rifamycins. XLIX.
(2) S. Füresz and M. T. Timbal, *Chemotherapia*, **7**, 200 (1963).
(3) P. Sensi, A. M. Greco, and R. Ballotta, *Antibiot. Ann.*, 262 (1959–1960).
(4) P. Sensi, R. Ballotta, A. M. Greco, and G. G. Gallo, *Farmaco, Ed. Sci.*, **16**, 165 (1961).
(5) N. Bergamini and G. Fowst, *Arzneimittel-Forsch.*, **15**, 951 (1965).
(6) W. Oppolzer, V. Prelog, and P. Sensi, *Experientia*, **20**, 336 (1964).
(7) P. Sensi, N. Maggi, R. Ballotta, S. Füresz, R. Pallanza, and V. Arioli, *J. Med. Chem.*, **7**, 596 (1964).
(8) N. Maggi and R. Pallanza, *Farmaco, Ed. Sci.*, **22**, 307 (1967).
(9) N. Maggi, V. Arioli, and P. Sensi, *J. Med. Chem.*, **8**, 790 (1965).
(10) N. Maggi, R. Pallanza, and P. Sensi, *Antimicrobial Agents Chemotherapy*, 770 (1965).
(11) G. G. Gallo, C. R. Pasqualucci, N. Maggi, R. Ballotta, and P. Sensi, *Farmaco, Ed. Sci.*, **21**, 68 (1966).

TABLE I

MINIMUM INHIBITORY CONCENTRATIONS[a]

Compd	*S. aureus* ATCC 6538	*S. hemolyticus* C 203	*S. faecalis* ATCC 10541	*P. vulgaris* ATCC 881	*E. coli* ATCC 10536	*K. pneumoniae* ATCC 10031	*P. aeruginosa* ATCC 10145	*M. tuberculosis* H 37 Rv
IIIa[b]	2	5	1	>100	>100	>100	>100	5
IIIb[c]	0.015	0.2	0.1	50	1	5	10	0.1
IVa	0.5	0.1	5	>100	>100	>100	>100	5
IVb[c]	0.05	0.5	0.5	100	50	20	100	0.1
Va	0.1	1	1	>100	>100	>100	>100	>5
Vb[c]	0.005	0.1	0.05	10	5	10	20	2
VIa	0.05	0.2	0.2	100	>100	>100	>100	>5
VIb[c]	0.001	0.02	0.01	5	5	10	20	0.05
VIIa	0.05	0.05	0.5	50	50	50	100	5
VIIb[c]	0.002	0.05	0.05	10	10	20	20	1
VIIIa	5	1	50	>100	>100	>100	>100	>5
VIIIb[c]	0.005	0.02	0.5	5	5	20	20	0.5

[a] In micrograms per milliliter. [b] As triethylammonium salt. [c] For synthesis and properties see ref 9–11.

Experimental Section[12]

Chemistry. 1.—Carboxylic acids belonging to the class of N,N-disubstituted aminomethylrifamycins (IIIa and IVa) were obtained directly by Mannich reaction from rifamycin S, formaldehyde, and the selected amino acid, following the previously described procedure (see ref 9, procedure A).

3-(N-Methyl-N-carboxymethyl)aminomethylrifamycin SV (IIIa).—The free acid showed a single spot in thin layer chromatography; ionization constants, $pK_a' \sim 1$,[13] $pK_a'' = 3.3$. IIIa was crystallized as the triethylammonium salt from THF (yield 16%); mp 160–180° dec; $\lambda_{max}^{pH\ 7.38}$ [mμ (ε)] 314 (18,100), 448 (13,770). *Anal.* ($C_{47}H_{69}N_3O_{14}$) H, N; C: calcd, 62.79; found, 62.18.

3-(4-Carboxypiperidino)methylrifamycin SV (IVa) was obtained, in 25% yield; mp 170–175° dec; $\lambda_{max}^{pH\ 7.38}$ [mμ (ε)] 314 (17,400), 450 (13,200); ionization constants, $pK_a' \sim 1$,[13] $pK_a'' = 5.4$. *Anal.* ($C_{44}H_{58}N_2O_{14}$) C, H, N.

2.—Va, VIa, and VIIa were synthesized from 3-formylrifamycin SV[14] and the corresponding hydrazino acids, according to the conventional procedures for aldohydrazones.

3-Formylrifamycin SV *p*-carboxyphenylhydrazone (Va) was obtained in 54% yield; mp 185° dec; $\lambda_{max}^{pH\ 7.38}$ [mμ (ε)] 365 (25,950), 488 (22,200); ionization constants, $pK_a' = 4.1$, $pK_a'' = 6.8$. *Anal.* ($C_{43}H_{53}N_3O_{14}$) C, H, N.

3-Formylrifamycin SV N-methyl-N-carboxymethylhydrazone (VIa) was obtained in 45% yield; mp 190° dec; $\lambda_{max}^{pH\ 7.38}$ [mμ (ε)] 236 (31,800), 340 (26,700), 478 (16,300); ionization constants, $pK_a' = 5.0$, $pK_a'' = 6.1$. *Anal.* ($C_{41}H_{53}N_3O_{14}$) H, N; C: calcd, 60.65; found, 60.02.

3-Formylrifamycin SV *p*-carboxybenzylidenehydrazone (VIIa) was obtained in 30% yield; mp 198° dec; $\lambda_{max}^{pH\ 7.38}$ [mμ (ε)] 313 (35,000), 500 (13,500); ionization constants, $pK_a' = 4.2$, $pK_a'' = 6.6$. *Anal.* ($C_{46}H_{53}N_3O_{14}$) H, N; C: calcd, 63.36; found, 62.85.

3. 6′- (or 7′-) Carboxyrifazine (VIIIa) was prepared analogously to VIIIb.[11] Its isolation required countercurrent separation of the reaction products (*n*-BuOH–phosphate buffer pH 6.5); yield 6–7%; mp 180° dec; $\lambda_{max}^{pH\ 7.38}$ [mμ (ε)] 247 (41,700), 345 (24,600), 537 (5700); ionization constants, $pK_a' = 3.3$, $pK_a'' = 5.6$. *Anal.* ($C_{43}H_{49}N_3O_{13}$) H, N; C: calcd, 63.17; found, 62.52.

Biological Tests.—The antimicrobial activity for all the derivatives was assayed by determining the minimum inhibitory concentrations (MIC) using the procedure already described in previous papers.[7–9]

(12) The products were checked for purity by thin layer chromatography. Melting points are uncorrected. Uv spectra were recorded in phosphate buffer pH 7.38 with a Perkin-Elmer Model 4000 A spectrometer. pK_a values, unless otherwise specified, were performed by potentiometric techniques (solvent, 30% aqueous methanol), pK_a' referring to the first acid ionization (*peri*-dihydroxy group) and pK_a'' to the second one (carboxyl group). Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values.

(13) Determined spectrophotometrically in MeOH–H_2O solution (3:1).

(14) N. Maggi, G. C. Gallo, and P. Sensi, *Farmaco, Ed. Sci.*, **22**, 316 (1967).

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Bactericidal and Fungicidal Activity of Anthranilate Esters

NED D. HEINDEL, THOMAS F. LEMKE, SALLY M. LEMKE, AND VELMER B. FISH

Chandler Laboratory of Chemistry, Lehigh University, Bethlehem, Pennsylvania

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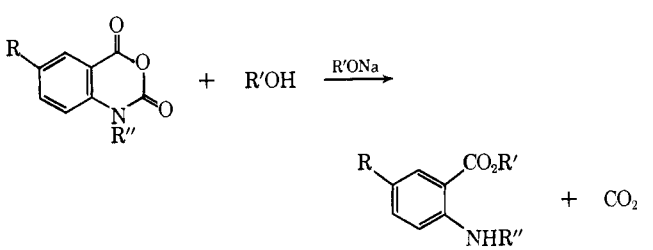
In view of the numerous reports which have appeared on the bactericidal and fungicidal activity of anthranilic acid[1–4] and its methyl ester,[5] we have synthesized a number of higher ester homologs and examined their activity in this area. A modification of the Staiger–Miller[6] method for ring opening of isatoic anhydrides to the anthranilates was employed.

Since anthraniloylanthranilic acid appears as an undesired by-product when traces of water are contained in the alcohol which serves as the reactant–solvent, we have utilized anhydrous alcohols and a trace of their corresponding alkoxides as ring-opening nucleophiles (instead of NaOH as originally suggested).[6] Although the conversions of the isatoic anhydrides to the



R = H or Cl; R″ = H or CH_3

(1) B. E. Volcani, S. Sicher, E. D. Bergmann, and H. Bendas, *J. Biol. Chem.*, **207**, 411 (1954).

(2) Y. Raoul, J. Chopin, and A. Ayrault, *Compt. Rend.*, **224**, 1309 (1947).

(3) G. Terui, T. Enatsu, and S. Tabata, *Hakko Kogaku Zasshi*, **39**, 724 (1961); *Chem. Abstr.*, **59**, 9111 (1963).

(4) A. R. Penfold and R. Grant, *J. Proc. Roy. Soc. N. S. Wales*, **58**, 117 (1924).

(5) J. C. Maruzella and E. Bramnick, *Soap. Perfumery Cosmetics*, **34**, 743 (1961).

(6) R. P. Staiger and E. B. Miller, *J. Org. Chem.*, **24**, 1214 (1959).