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]

Acknowledgment.—We are indebted to Dr. R. C. Pasqualucci for spectrophotometric and potentiometric data and to Mr. A. Restelli for elemental microanalyses.

Bactericidal and Fungicidal Activity of Anthranilate Esters

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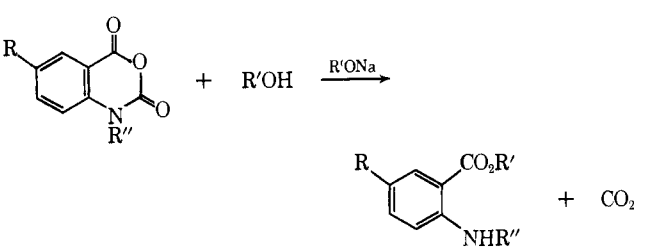
In view of the numerous reports which have appeared on the bactericidal and fungicidal activity of anthranilic acid[1–4] and its methyl ester,[5] we have synthesized a number of higher ester homologs and examined their activity in this area. A modification of the Staiger–Miller[6] method for ring opening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

(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).

(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).

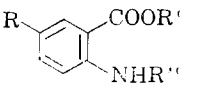
(3) G. Terui, T. Enatsu, and S. Tabata, *Hakko Kogaku Zasshi*, **39**, 724 (1961); *Chem. Abstr.*, **59**, 9111 (1963).

(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).

TABLE I
ANTHRANILATE ESTERS



No.	R	R'	R''	% yield	Bp (mm), °C	Formula[c]
I	H	$CH(CH_3)_2$	H	48	152–153 (16)	$C_{10}H_{13}NO_2$
II[a]	H	n-C_4H_9	H	66	173–175 (16)	$C_{11}H_{15}NO_2$
III[b]	H	$CH_2CH(CH_3)_2$	H	78	165–167 (16)	$C_{11}H_{15}NO_2$
IV	H	n-C_6H_{13}	H	64	194–195 (16)	$C_{13}H_{19}NO_2$
V	H	n-C_7H_{15}	H	74	203–204 (16)	$C_{14}H_{21}NO_2$
VI	H	n-$C_{10}H_{21}$	H	58	135–136 (16)	$C_{17}H_{27}NO_2$
VII	Cl	n-C_4H_7	H	63	195–196 (16)	$C_{11}H_{14}ClNO_2$
VIII	Cl	n-C_7H_{15}	H	78	234–235 (20)	$C_{14}H_{20}ClNO_2$
IX	Cl	$CH(CH_3)_2$	CH_3	55	Mp 69–70	$C_{11}H_{14}ClNO_2$

[a] Prepared as the HCl salt and the free base by ester interchange of methyl anthranilate: H. C. Brill, *J. Am. Chem. Soc.*, **43**, 1322 (1921). Brill reported bp 182° which could not be confirmed. [b] Also reported by J. Bredt and H. Hof, *Ber.*, **33**, 29 (1900), who reported bp 156–157° (13.5 mm). [c] All compounds were analyzed for N. Analytical data were within 0.25% of theoretical values.

anthranilates are essentially quantitative (with the exception of the isopropyl esters which experience a competitive formation of isopropyl N-*o*-carboxyphenylcarbamate),[6] the yields expressed in Table I are based on analytically pure material. All esters were purified by distillation except IX which was recrystallized from cyclohexane.

Biological Results.[7]—The anthranilates were screened at a concentration of 250 ppm mixed in melted Difco brain–heart infusion agar in which the test organisms were later cultured. The results are reported in Table II employing a visual rating of amount of colonial

TABLE II
TESTING RESULTS[a]

Compd	*Staphylococcus aureus*	*E. coli*	*Erwinia amylovora*	*Xanthomonas malvacearum*	*A. niger*
I	1	1	3	3	3
II	3	1	5	5	3
III	5	1	5	5	3
IV	1	1	5	1	1
V	1	1	1	1	1
VI	1	1	1	1	1
VII	5	1	1	1	1
VIII	1	1	1	1	1
IX	1	1	1	1	1

[a] See text for an explanation of data.

growth: 1 = no inhibition, 5 = complete inhibition. It is interesting that none of the esters inhibited *Escherichia coli* growth although anthranilic acid itself is active in this regard.[1,2] The three lower alkyl esters of anthranilic acid, *i.e.*, I, II, and III, showed a slight inhibition of *Aspergillus niger*, but the parent anthranilic acid is more active.[3] Secondary screening at reduced concentration levels, 60 and 15 ppm, demonstrated the esters to be of minimum effectiveness and of no commercial interest.

Experimental Section[8]

General Procedure.—For the formation of isopropyl and *n*-butyl esters the corresponding alcohols were employed as solvent and reactant. To 50–60 ml of the alcohol[9] in which a chip of freshly cut Na, approximately 0.1 g, had been dissolved, 0.02 mole of the required isatoic anhydride was added and the suspension was stirred at reflux. When the evolution of CO_2 had ceased and a clear solution resulted, the reaction medium was heated for another 0.5 hr and treated cautiously with 2–3 ml of 6 *N* HCl. The contents were filtered through $MgSO_4$, concentrated *in vacuo*, and distilled.

Esters of other alcohols were prepared by reaction of a 1:1 ratio of the alcohol, in which a chip of Na had been dissolved, and the isatoic anhydride in anhydrous dioxane. Typically, 0.02 mole of alcohol containing alkoxide, 0.02 mole of anhydride, and 35–50 ml of dioxane would be employed. The former procedure was utilized for isolation of product. All ir spectra were as expected.

(9) Because considerable foaming (CO_2) results on decomposition of isatoic anhydrides, it is desirable to employ a larger than normal reaction vessel.

The Preparation and Histomonastatic Activity of Some Haloethylimidazoles

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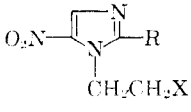
In a study of the synthesis and biological activity of nitroimidazoles we were led to investigate the haloethylimidazoles (I–III). These compounds were of interest because of the reported activity of the corre-



I, R = H; X = Cl
Ia, R = H; X = OH
II, R = CH_3; X = Cl
III, R = CH_3; X = Br
IIIa, R = CH_3; X = OH

sponding hydroxyethyl derivatives (Ia, IIIa[1]) against certain protozoans.[2–4] During the late stages of this investigation a report appeared which described the *in vitro* activity of I and II against *Trichomonas vagin-*

(1) Flagyl®.
(2) M. Boek, *Arzneimittel-Forsch.*, **11**, 587 (1961).
(3) R. Mandoul, R. Dargelos, and J. Millau, *Bull. Soc. Pathol. Exotique*, **54**, 12 (1961).
(4) D. Hoff and J. Bennett, U. S. Patent 3,107,201 (Oct 15, 1963).

(7) We are grateful to the Union Carbide Corp., Agricultural Research Station, Clayton, N. C., for providing the testing data.

(8) Combustion analyses were performed by V. B. F. in these laboratories. Infrared spectra were obtained neat on a Perkin-Elmer 257 spectrophotometer. Isatoic anhydride, 5-chloroisatoic anhydride, and N-methyl-5-chloroisatoic anhydride were provided as generous samples by Mannier Chemical Co., Toledo, Ohio.