was necessary. The lyophilized product was dissolved in acetone, and the solution was dried with $MgSO_4$ and evaporated. Attempts at crystallization were unsuccessful. The residue was repeatedly redissolved in acetone and recovered by evaporation, until it was only weakly positive to starch–iodide test paper. A final lyophilization afforded a grainy yellow solid (92% yield on a 15-g scale) that was negative to starch–iodide: mp softening 85–105° dec; R_f 0.45 (1:4) with no **14** at 0.20; pmr (D_2O) δ 4.87 (d, H-1, J = 3.5 Hz), 3.48 (s, OCH_3), 3.13 (s, NCH_3); no **14** at δ 2.70 (limit of detection < 2%). *Anal.* ($C_9H_{17}N_3O_7 \cdot 0.1CH_3COCH_3 \cdot 0.4H_2O$) C, H, N. The solid was stored at 25° for 16 months with no change. A solution in D_2O was unchanged after 1 week.

Methyl 6-Deoxy-6-(3-methyl-3-nitrosoureido)-α-D-glucopyranoside (18). Lyophilization afforded a yellow foamed glass that was crystallized (69%, 3.7 g) from hot *i*-PrOH: mp 102–104° dec (lit.[6] 106–107°); R_f 0.45 (1:4); pmr (D_2O) δ 4.78 (d, J = 3.0 Hz), 3.30 (s, OCH_3), 3.13 (s, NCH_3). *Anal.* ($C_9H_{17}N_3O_7$) C, H, N.

Methyl 2,3,6-Trideoxy-3-(3-methyl-3-nitrosoureido)-α-L-*lyxo*-hexopyranoside (21). In one 5-g run, renitrosation was necessary for complete conversion of **20**. Lyophilization produced a yellow gum that crystallized on trituration with ether (50% yield): mp 97–99°; R_f 0.70 (1:4); pmr δ 4.78 (rough t, H-1), 3.40 (s, OCH_3), 3.21 (s, NCH_3). Another sample in $CHCl_3$ solution was washed with H_2O, recovered by evaporation, and triturated (10% yield): mp 98–101°. *Anal.* ($C_9H_{17}N_3O_5$) C, H, N. A sample stored 8 months at 25° had decomposed.

Methyl 3-*N*-Carboxyamino-3-deoxy-α-D-altropyranoside γ-Lactam (22). When the reaction solution from nitrosation of **10** was neutralized with prewashed Dowex 2X-8 (CO_3), lyophilization afforded a white solid (63%). Recrystallization from *i*-PrOH yielded 33%: mp 124–128°; R_f 0.25 (1:4); ir 5.63, 5.83 (film from $CHCl_3$–MeOH), 5.7 μ (C=O); pmr (DMSO) δ 3.32 (s, OCH_3), no NCH_3. *Anal.* ($C_8H_{13}NO_6$) C, H, N.

N-(3-Amino-3-deoxy-α-D-ribofuranosyl)-*N*-methylamine *N*,*N*′-Cyclic Carbonate (23). A solution of 4.9 g (20 mmol) of **3** in 30 ml of 80% trifluoroacetic acid was concentrated after 3 hr at 25°. The residual white solid (4.1 g, mp 192–197°) was recrystallized from 95% EtOH to give 2.6 g (63% yield): mp 205–207°; R_f 0.2 (1:4); ir 6.0 μ (C=O); pmr (DMSO) δ 4.59 (q, tentatively assigned to H-1, J = 2.0 Hz, 3.8 Hz), 2.83 (s, NCH_3). *Anal.* ($C_7H_{12}N_2O_4$) C, H, N.

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References

(1) B. K. Bhuyan, T. J. Fraser, H. H. Buskirk, and G. L. Neil, *Cancer Chemother. Rep.* (*Part 1*), 56, 709 (1972), and leading references.
(2) P. S. Schein, *Cancer*, **30**, 1616 (1972), and leading references.
(3) J. H. Burchenal and S. K. Carter, *Cancer*, **30**, 1639 (1972).
(4) B. Bannister, *J. Antibiot.*, **25**, 377 (1972).
(5) T. Suami and T. Machinami, *Bull. Chem. Soc. Jap.*, 43, 3013 (1970).
(6) T. Machinami and T. Suami, *J. Chem. Soc. Jap.*, 46, 1013 (1973).
(7) E. J. Hessler and H. K. Jahnke, *J. Org. Chem.*, 35, 245 (1970).
(8) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.* (*Part 3*), 3 (no. 2, Sept 1972).
(9) J. M. Venditti, *Cancer Chemother. Rep.*, **2**, 35 (no. 1, Oct 1971).
(10) T. Suami and T. Machinami, *Bull. Chem. Soc. Jap.*, 43, 2953 (1970).
(11) H. S. Rosenkranz and H. S. Carr, *Cancer Res.*, 30, 112 (1970).
(12) A. Lev, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(13) C. Hansch, N. Smith, R. Engle, and H. Wood, *Cancer Chemother. Rep.* (*Part 1*), 56, 443 (1972).
(14) R. E. Schaub and M. J. Weiss, *J. Amer. Chem. Soc.*, 80, 4683 (1958).
(15) C. D. Anderson, L. Goodman, and B. R. Baker, *ibid.*, 80, 5247 (1958).
(16) A. N. Fujiwara, E. M. Acton, and L. Goodman, *J. Heterocycl. Chem.*, 7, 891 (1970).
(17) F. Cramer, H. Otterbach, and H. Springman, *Chem. Ber.*, 92, 384 (1959).
(18) F. Cramer, *Methods Carbohyd. Chem.*, **1**, 243 (1962).
(19) M. Furdik, S. Mikulasek, M. Livar, and S. Priehradny, *Chem. Zvesti*, 21, 427 (1967); *Chem. Abstr.*, 67, 116858y (1967).
(20) J. P. Marsh, C. W. Mosher, E. M. Acton, and L. Goodman, *Chem. Commun.*, 973 (1967).
(21) W. H. Myers and G. J. Robertson, *J. Amer. Chem. Soc.*, 65, 8 (1943).
(22) R. D. Guthrie and L. F. Johnson, *J. Chem. Soc.*, 4166 (1961).

Oximes of 3-Formylrifamycin SV. Synthesis, Antibacterial Activity, and Other Biological Properties

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The synthesis of the oximes of 3-formylrifamycin SV and the preparation of some of the O-substituted hydroxylamine intermediates are described. The chemical and physical characteristics, the antibacterial activity on wild-type and rifampicin-resistant strains, and other biological properties of the new derivatives are reported. Structure–activity relationships show that increasing the lipophilicity of the oxime substituent decreases the antibacterial activity both *in vitro* and in experimental infection, whereas inhibition of a rifampicin-resistant strain of *Staphylococcus aureus* and of several transcribing enzymes is increased.

Rifampicin,[1] the well-known semisynthetic antibiotic of the rifamycin family orally effective against tuberculosis and other bacterial infections, has been extensively studied for its biological properties. Other semisynthetic rifamycin derivatives are, however, endowed with biological activities, in some cases different from that of rifampicin, and deserve further study. One class of these derivatives is that of the oximes of 3-formylrifamycin SV, some members of which have been synthesized by Sensi and coworkers in 1965.[2] Our attention on this class was aroused some years ago by the observation that the *O*-benzyloxime[2] (compound **40**, Table II), in contrast to rifampicin, appeared to inhibit RNA synthesis in chick embryo fibroblasts.[3] We then tested this compound for other biological properties and observed that at 20 μg/ml it was active *in vitro* against a *Staphylococcus aureus* strain resistant to 200 μg/ml of rifampicin. This prompted the synthesis of other derivatives of this series. In this paper we describe the synthesis of these compounds by condensation of 3-formylrifamycin SV[4] and the appropriate O-substituted hydroxylamines and the preparation of some of these intermediates and report the *in vitro* activity on several microbial strains and *in vivo* activity on *S. aureus* infections in mice. The biological properties of this class of rifamycins, observed in our and other laboratories, are discussed in relationship to their chemical structure.

Synthesis of the Intermediate O-Substituted Hydroxylamines. The O-substituted hydroxylamines used for the

Table I. O-Substituted Hydroxylamine Derivatives $RONH_2$

Compd	R	Formula[a]	Bp (mm), °C
57	$-CH(C_3H_7)C_3H_7$	$C_7H_{17}NO$	37–38 (0.1)
58	$-CH(C_2H_5)C_4H_9$	$C_7H_{17}NO$	36–39 (0.1)
59	$-CH(C_3H_7)C_4H_9$	$C_8H_{19}NO$	41–45 (0.1)
60	$-CH(C_2H_5)C_5H_{11}$	$C_8H_{19}NO$	37–40 (0.1)
61	$-CH(CH)_3C_5H_{11}$	$C_7H_{17}NO$	31–32 (0.5)
62	$-(CH_2)_6OH$	$C_6H_{15}NO_2$	84–85 (0.3)
63	$-CH_2-C_6H_3$-*o*,*p*-Cl_2	$C_7H_7Cl_2NO$	85–86 (0.2)
64	$-(CH_2)_4CH(Br)CH_2Br$	$C_6H_{13}Br_2NO$	*b*
65	$-(CH_2)_4CH{=}CH_2$	$C_6H_{13}NO$	70 (22)
66	$-(CH_2)_2$-c-NC_4H_8	$C_6H_{14}N_2O$	52–53 (0.1)
67	$-(CH_2)_2$-c-$N(CH_2CH_2)_2N-CH_3$	$C_7H_{17}N_3O$	80–83 (0.4)

[a] All compounds were analyzed for C, H, N, Cl, and Br. The analytical results were within ±0.4% of the theoretical values. [b] This compound decomposes on heating.

synthesis of the rifamycins can be divided in three groups: (1) *O*-alkyl, alkenyl, and aralkyl derivatives; (2) *O*-alkoxyalkyl and phenoxyalkyl derivatives; and (3) miscellaneous derivatives.

The hydroxylamines used for the synthesis of rifamycins **1–12, 14, 33, 40–44, 46, 48–50, 52, 54,** and **55** (Table II) were known products and were prepared according to published procedures (see, for instance, ref 5). The hydroxylamines of group 2, used for the synthesis of rifamycins **28–39,** and some of the hydroxylamines of group 1, used for the synthesis of rifamycins **20–23** and **25–27,** were kindly supplied by Drs. B. Cavalleri and A. Omodei-Salé of our laboratories; their syntheses will be reported elsewhere. In Table I are reported the other new O-substituted hydroxylamino derivatives; their syntheses have been performed according to a known method,[5] reaction of the suitable alkyl bromide with the K salt of the hydroxyurethane and alkaline hydrolysis of the intermediate *O*-alkylhydroxyurethane; details are reported in the Experimental Section.

Synthesis of 3-Formylrifamycin SV Oximes. The products have been synthesized by condensation of 3-formylrifamycin SV[4] with the suitable O-substituted hydroxylamine derivative in THF at room temperature. The reaction is complete within a few minutes and tlc of the reaction mixture shows the disappearance of the starting rifamycin, R_f 0.3 in the solvent system used (see Experimental Section), and the formation of the new product with higher R_f, varying between 0.4 and 0.6. The products could be crystallized from various solvents (generally methanol). The yields were high as is shown in Table II where the physicochemical characteristics of the compounds are reported.

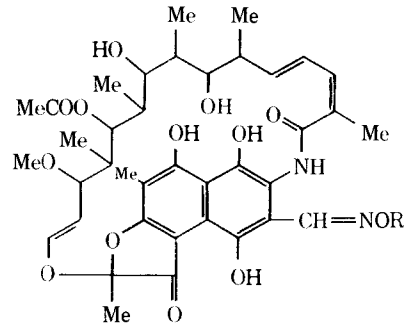
These rifamycins vary in color from yellow-orange to red; they are very soluble in common organic solvents but practically insoluble in water and in both acidic (0.1 *N* HCl) and alkaline media (saturated solution of $NaHCO_3$), with the exception of compounds **1–3** and of compounds carrying functionally substituted chains. The structures have been confirmed by physical data (pmr, uv, and visible spectra). In particular the pmr spectra were interpreted on the basis of the assignments made for 3-formylrifamycin SV,[4] the main variations being the shift of the signal at 10.59 ppm (attributed to the formyl proton) to 8.8 ppm and the appearance of the new signals due to the O-substituted hydroxylamine derivative residue. For activity comparison purposes, discussed later, the 16,17,18,19,28,29-hexahydro derivative of rifamycin SV 3-octyloxime, that is, the hexahydro derivative of compound **10,** was synthesized by condensing *N*-octylhydroxylamine with hexahydro-3-formylrifamycin SV. The preparation of the latter from hexahydrorifamycin SV[6] is reported in the Experimental Section.

Antibacterial Activity. Table III reports the *in vitro* antibacterial activity, the *in vivo* activity, and the acute toxicity of these rifamycins. The compounds are very active *in vitro* on gram-positive organisms (see Table III, columns 1, 3, and 4), although it can be noted that the activity decreases with the lengthening of the aliphatic chain (compounds **1–14, 33–36**) or with the addition of phenyl groups (compare compounds **2–4** with **40–43**). The presence of double or triple bonds in the chain does not affect significantly the activity (compare compounds **4** with **20** and **27; 6** with **21**); some variation is observed when OH groups are introduced (*cf.* **3** with **46** and **8** with **47**). As previously observed[7] for other rifamycin series the presence of a carboxyl group decreases dramatically the activity (see compounds **48–50**). Several compounds show an excellent activity on *Mycobacterium tuberculosis.* Substantially higher concentrations are needed for inhibition of gram-negative bacteria (see Table III, columns 5–8), but it is noteworthy that even *difficult* strains, like *Pseudomonas aeruginosa,* are sensitive to most compounds. Qualitatively, the same observations on activity–structure made for gram-positive organisms hold for *M. tuberculosis* and gram-negative bacteria; for the latter the range of activity appears narrower in part because MIC's higher than 100 μg/ml were not determined. All these observations indicate that the antibacterial activity of the compounds decreases linearly with the increase of lipophilicity of the substituent at position 3, the carboxyl compounds being the only exception. A quantitative analysis of these relationships with the Hansch approach has been made and published elsewhere.[8]

The mechanism of action of this series of compounds appears to be the same as that of rifampicin and the other rifamycins,[9] since representative compounds are shown, in a cell free system, to inhibit DNA-dependent RNA polymerase extracted from a sensitive strain. In the condition adopted (see Experimental Section) a concentration of 1 μg/ml resulted in a complete inhibition of the *E. coli* enzyme activity.

As mentioned in the introduction, a reason for the synthesis of these compounds was to test their activity on rifampicin-resistant strains. In Table III (column 2) the MIC's obtained on a *S. aureus* strain resistant to 200 μg/ml of rifampicin are reported. Similar activities have been observed with other *S. aureus* mutants, whereas rifampicin-resistant *E. coli* strains were found insensitive to high concentrations of the drugs. The activity against the resistant *S. aureus* strain increases with the length of the aliphatic chain and reaches the highest value when the chain is of 8 C atoms; for a longer chain the value decreases rapidly (see compounds **1–14**). Addition to the chain of phenyl groups increases considerably the activity (compounds **40–45**) whereas addition of nitrogen heterocycles results in inactive compounds (compounds **53–56**). The presence in the chain of double or triple bonds slight-

Table II. O-Substituted Oximes of 3-Formylrifamycin SV



Compd[a]	Lab code	R	Crystn solvent	Yield, %	Mp, °C[b]	Formula[c]	Spectrophotometric data[d] λ_{max}, nm	ϵ	λ_{max}, nm	ϵ
1	AF-O	H	EtOAc	70	190–193[e]	$C_{38}H_{48}N_2O_{13}$	323	20,300	468	13,300
2	AF-MO	CH_3	EtOAc–hexane	70	240–250[e]	$C_{39}H_{50}N_2O_{13}$	325	22,500	470	14,950
3	AF-EO	C_2H_5	EtOAc–hexane	70	214–215	$C_{40}H_{52}N_2O_{13}$	325	21,800	470	14,100
4	AF-08	n-C_3H_7	MeOH	85	181–183	$C_{41}H_{54}N_2O_{13}$	326	23,500	468	15,000
5	AF-09	i-C_3H_7	MeOH	80	229–231	$C_{41}H_{54}N_2O_{13}$	327	21,700	468	15,000
6	AF-010	n-C_4H_9	MeOH	75	220–222	$C_{42}H_{56}N_2O_{13}$	326	22,500	468	14,000
7	AF-012	n-C_5H_{11}	MeOH	80	200–202	$C_{43}H_{58}N_2O_{13}$	325	22,300	468	14,000
8	AF-024	n-C_6H_{13}	MeOH	60	137–140	$C_{44}H_{60}N_2O_{13}$	327	21,300	470	13,800
9	AF-022	n-C_7H_{15}	MeOH	60	134–140	$C_{45}H_{62}N_2O_{13}$	328	20,500	475	12,800
10	AF-013	n-C_8H_{17}	MeOH	70	188–190	$C_{46}H_{64}N_2O_{13}$	329	20,000	475	12,200
11	AF-021	n-C_9H_{19}	MeOH	60	177–181	$C_{47}H_{66}N_2O_{13}$	330	21,600	480	13,500
12	AF-023	n-$C_{10}H_{21}$	Hexane	50	97–101	$C_{48}H_{68}N_2O_{13}$	330	19,700	480	12,100
13	AF-025	n-$C_{11}H_{23}$	Hexane	50	170–173	$C_{49}H_{70}N_2O_{13}$	330	19,600	475	12,550
14	AF-018	n-$C_{12}H_{25}$	Hexane	60	95–104	$C_{50}H_{72}N_2O_{13}$	330	20,000	480	12,600
15	AF-026	CH-n-$(C_3H_7)_2$	MeOH	70	167–171	$C_{45}H_{62}N_2O_{13}$	326	19,700	470	12,700
16	AF-028	C_2H_5CH-n-C_4H_9	MeOH	60	195–210[e]	$C_{45}H_{62}N_2O_{13}$	327	22,700	472	14,000
17	AF-029	n-C_3H_7-CH-n-C_4H_9	MeOH	90	102–110[e]	$C_{46}H_{64}N_2O_{13}$	328	21,000	475	12,600
18	AF-032	CH_3CH-n-C_5H_{11}	MeOH	42	132–136	$C_{45}H_{62}N_2O_{13}$	328	22,400	475	13,900
19	AF-030	C_2H_5CH-n-C_5H_{11}	MeOH	40	100–110[e]	$C_{46}H_{64}N_2O_{13}$	328	17,600	475	12,700
20	AF-047	$CH_2CH{=}CH_2$	MeOH	90	170–173	$C_{41}H_{52}N_2O_{13}$	327	22,200	471	14,400
21	AF-046	$CH_2CH{=}CHCH_3$	MeOH	85	185–188	$C_{42}H_{54}N_2O_{13}$	327	22,000	470	14,400
22	AF-045	$CH_2C(CH_3){=}CH_2$	MeOH	70	160–162	$C_{42}H_{54}N_2O_{13}$	326	22,000	470	14,700
23	AF-044	$CH_2C(C_2H_5){=}CH_2$	MeOH	70	163–165	$C_{43}H_{56}N_2O_{13}$	327	22,700	472	15,000
24	AF-027	$(CH_2)_4CH{=}CH_2$	MeOH	55	174–175	$C_{44}H_{58}N_2O_{13}$	327	22,600	470	14,500
25	AF-049	Geranyl	MeOH	50	138–140	$C_{48}H_{64}N_2O_{13}$	330	21,200	480	13,200
26	AF-051	Farnesyl	Hexane	50	150–153	$C_{53}H_{72}N_2O_{13}$	330	22,100	480	13,200
27	AF-048	$CH_2C{\equiv}CH$	MeOH	90	192–194	$C_{41}H_{50}N_2O_{13}$	326	20,900	473	13,000
28	AF-053	$(CH_2)_2OCH_3$	MeOH	85	237–239	$C_{41}H_{54}N_2O_{14}$	327	21,700	472	11,800
29	AF-055	$(CH_2)_2OC_2H_5$	MeOH	80	225–227	$C_{42}H_{56}N_2O_{14}$	327	22,400	474	13,200
30	AF-034	$(CH_2)_2O$-i-C_3H_7	MeOH	85	189–191[e]	$C_{43}H_{56}N_2O_{14}$	328	21,900	472	13,850
31	AF-039	$(CH_2)_2C(OCH_3)HCH_3$	MeOH	82	210–212	$C_{43}H_{58}N_2O_{14}$	327	23,000	470	14,000
32	AF-054	$(CH_2)_2OC_4H_9$	MeOH	20	175–177	$C_{42}H_{60}N_2O_{14}$	325	21,600	470	14,100
33	AF-052	$(CH_2)_2OC_6H_5$	MeOH	85	137–140	$C_{46}H_{56}N_2O_{14}$	326	21,200	470	13,500
34	AF-035	$(CH_2)_4OC_6H_5$	Hexane	75	126–129	$C_{48}H_{60}N_2O_{14}$	327	22,400	475	14,400
35	AF-036	$(CH_2)_6OC_6H_5$	Hexane	35	138–142	$C_{50}H_{64}N_2O_{14}$	330	21,800	478	13,600

36	AF-037	$(CH_2)_{10}OC_6H_5$	Hexane	20	108–112[e]	$C_{54}H_{72}N_2O_{14}$	331	22,500	481	12,500
37	AF-041	$(CH_2)_2O(CH_2)_2OCH_3$	MeOH	50	166–169[e]	$C_{43}H_{58}N_2O_{15}$	327	22,300	471	14,600
38	AF-042	$(CH_2)_2O(CH_2)_2OC_2H_5$	Hexane	80	115–118[e]	$C_{44}H_{60}N_2O_{15}$	327	22,000	471	14,700
39	AF-043	$(CH_2)_2O(CH_2)_2O$-*n*-C_4H_9	Hexane	80	142–145	$C_{46}H_{64}N_2O_{15}$	327	21,800	472	14,400
40	AF-BO	$CH_2C_6H_5$	MeOH	75	164–167	$C_{45}H_{54}N_2O_{13}$	325	23,800	465	15,500
41	AF-01	$(CH_2)_2C_6H_5$	EtOAc	67	130–134	$C_{46}H_{58}N_2O_{13}$	325	23,000	470	14,100
42	AF-015	$(CH_2)_3C_6H_5$	MeOH	70	160–161	$C_{47}H_{58}N_2O_{13}$	325	21,600	468	13,600
43	AF-05	$C_6H_5CHC_6H_5$	MeOH	60	145–150	$C_{51}H_{58}N_2O_{13}$	330	21,600	475	12,500
44	AF-017		MeOH	70	156–158	$C_{45}H_{53}BrN_2O_{13}$	328	22,400	475	13,200
45	AF-031	$CH_2C_6H_3$-*o*,*p*-Cl_2	MeOH	80	103–105	$C_{45}H_{52}Cl_2N_2O_{13}$	330	20,000	476	12,400
46	AF-03	$(CH_2)_2OH$	EtOAc	60	200–204[e]	$C_{40}H_{52}N_2O_{14}$	325	22,000	470	14,400
47	AF-033	$(CH_2)_6OH$	MeOH–H_2O	80	118–123	$C_{44}H_{60}N_2O_{14}$	327	21,500	471	14,500
48	AF-04	C(COOH)H-*i*-C_3H_7	Benzene	80	159–168[e]	$C_{43}H_{58}N_2O_{15}$	325	23,000	470	14,600
49	AF-019	C(COOH)HCH_2-*i*-C_3H_7	Chloroform	25	168–171	$C_{44}H_{58}N_2O_{15}$	325	22,100	470	13,400
50	AF-020	C(COOH)$HCH_2C_6H_5$	Chloroform	55	146–149	$C_{47}H_{58}N_2O_{15}$	326	21,800	470	14,000
51	AF-038	$(CH_2)_4C(Br)HCH_2Br$	MeOH	75	123–126[e]	$C_{44}H_{58}Br_2N_2O_{13}$	327	20,600	474	12,300
52	AF-050	C_6H_{11}	Acetone	60	235–237	$C_{44}H_{58}N_2O_{13}$	328	23,400	470	14,700
53	AF-PEO	$(CH_2)_2$-c-NC_4H_8	EtOH	40	162–168[e]	$C_{44}H_{59}N_3O_{13}$	327	21,000	472	14,000
54	AF-PIEO	$(CH_2)_2$-c-NC_5H_{10}	EtOH	50	>170[e]	$C_{45}H_{61}N_3O_{13}$	328	22,000	470	15,000
55	AF-MEO	$(CH_2)_2$-c-$N(CH_2CH_2)_2O$	MeOH	60	157–162[e]	$C_{44}H_{53}N_3O_{14}$	325	21,600	468	14,000
56	AF-MPEO	$(CH_2)_2$-c-$N(CH_2CH_2)_2NCH_3$	Acetone	40	187–188	$C_{45}H_{62}N_4O_{13}$	325	22,500	470	13,700

[a]Compounds **1**, **2**, **40**, and **55** have already been published. Their physicochemical characteristics are reported here for comparison. [b]All melting points were determined in open glass capillaries, using a Büchi apparatus, and are uncorrected. [c]All compounds were analyzed for C, H, N, Cl, and Br. The analytical results were within ±0.4% of the theoretical values. [d]In phosphate buffer, pH 7.38. [e]Decomposed.

Figure 1. Effect of rifamycins on DNA-dependent RNA polymerase from a rifampicin-resistant *E. coli* strain: O———O, rifampicin; ▲———▲, 3-formylrifamycin SV *O*-benzyloxime; ●———●, 3-formylrifamycin SV *O*-diphenylmethylmethyloxime. The dotted line indicates the inhibition by the same compounds of RNA polymerase extracted from a wild type *E. coli* strain.

ly decreases the activity (compare compounds **20**, **21**, and **24** with compounds **4**, **6**, and **8**); a stronger negative effect is provided by the hydroxyl group (compounds **46** and **47**) and more drastically by the carboxyl group (compounds **48–50**).

It appears clear from these observations that the activity of the compounds on the resistant mutant is related to their structure in the opposite way to that of sensitive strains, increasing almost linearly with the lipophilicity of the substituent until a very high lipophilicity value is reached, after which time the activity sharply decreases (compounds **12–14**).

No definite conclusions could be reached about the mechanism by which the growth of rifampicin-resistant strains is prevented by the new derivatives. In all the cases so far examined mutation to rifampicin resistance has been shown to be due to an altered RNA polymerase, insensitive to the antibiotic action. Unfortunately we were unable to establish whether or not these new rifamycins active against resistant strains were also active against the resistant polymerase *in vitro* due to technical difficulties in obtaining a purified DNA-dependent RNA polymerase from *S. aureus*. Indirect evidence was sought by examining the effect of some of these compounds on the enzyme from *E. coli*.

In Figure 1 the *in vitro* inhibition of a RNA polymerase extracted from a rifampicin-resistant *E. coli* strain, shown by different concentrations of the diphenylmethyl and the benzyl derivatives (compounds **40** and **43**), is compared with the inhibition shown by rifampicin itself. Although the concentration of compound **43** needed to inhibit 50% of the enzymatic activity is substantially lower than that of rifampicin, in our opinion the difference is not sufficient to allow definite conclusions. Moreover, the activity of compound **40** appears similar to that of rifampicin. It can be noted here that low concentrations at this product stimulate the enzymatic activity, an effect reported also in respect of its activity on RNA instructed DNA polymerase of oncogenic viruses.[10] Further studies on one of the most active compounds, the octyl derivative (com-

Table III. Antibacterial Activity of Oximes of 3-Formylrifamycin SV

	Minimal inhibitory concentration, μg/ml									ED_{50}, mg/kg (*S. aureus* infections in mice)		LD_{50}, mg/kg iv (in mice)
Compd	*S. aureus* Tour	*S. aureus* Tour resistant to rifampicin	*S. hemolyticus* C 203	*Diplo. pneumoniae* U. C. 41	*P. vulgaris* ATCC 881	*E. coli* ATCC 10536	*Klebs. pneumoniae* ATCC 10031	*Pseud. aeruginosa* ATCC 10045	*M. tuberculosis* H 37 Rv ATCC 9360	os	sc	
1	0.02	>100	0.01	0.01	10	5	20	10	5	5.66	2.46	690.0
2	0.002	>100	0.01	0.01	2	5	10	10	0.05	0.53	0.87	152.0
3	0.005	100	0.01	0.005	2	5	20	10	0.1	0.87	0.76	154.0
4	0.005	50	0.05	0.02	10	5	20	20	0.05	2.46	1.87	94.5
5	0.005	50	0.05	0.02	10	5	20	20	0.05	1.87	1.41	93.5
6	0.01	20	0.1	0.02	10	5	20	20	0.1	5.28	4.60	73.2
7	0.01	10	0.2	0.02	10	10	20	50	1	5.28	4.29	82.9
8	0.05	5	0.5	0.02	20	10	50	20	0.5	12.8	8.57	
9	0.1	2	2	0.05	10	>100	>100	>100	2			86.0
10	0.1	1	0.5	0.02	>50	>50	>50	>50	>5	13	17.1	88.0
11	0.2	2	5	0.02	>100	>100	>100	>100	10	16	16	96.0
12	2	10	1	0.1	>100	>100	>100	>100	20	>16	>16	152.0
13	2	50	2	0.1	>100	>100	>100	>100	50	>16	>16	70.0
14	10	>100	10	0.2	>100	>100	>100	>100	>20	>16	>16	157.0
15	0.05	5	1	0.05	>100	20	>100	20	1	13	9.2	
16	0.1	5	1	0.5	>100	50	>100	>100	1	>16	>16	73.0
17	0.2	2	2	1	>100	>100	>100	>100	2	>16	>16	73.5
18	0.05	2	1	0.5	20	20	>100	>100	2			79.0
19	0.2	5	1	1	>100	>100	>100	>100	2	>16	>16	89.0
20	0.005	50	0.02	0.01	10	10	10	10	0.2	1.63	1.23	123
21	0.005	50	0.05	0.02	10	10	10	20	0.1	3.03	3.25	160
22	0.005	50	0.05	0.02	10	10	10	20	0.2	10	5.28	85
23	0.01	10	0.1	0.05	20	10	10	20	0.2	8.57	7.47	66.0
24	0.02	10	0.5	0.01	10	10	20	>100	0.5	11.3	9.85	77.5
25	0.5	2	2	1	>100	>100	>100	>100	5	30.8	35.4	80
26	5	>100	2	2	>100	>100	>100	>100	20			135.0
27	0.005	100	0.02	0.01	10	10	10	10	0.2	1.48	1.29	200
28	0.005	>100	0.01	0.01	10	10	20	20	0.1	0.93	0.76	330.0
29	0.005	>100	0.05	0.02	10	10	20	20	0.2	1.78	1.32	256.0
30	0.005	>100	0.05	0.02	10	5	20	20	0.1			212.0
31	0.005	>100	0.05	0.05	50	5	20	20	0.1			219.0
32	0.01	50	0.05	0.02	10	10	10	20	0.2	4.41	3.73	
33	0.005	20	0.1	0.05	10	10	10	20	0.2	8.0	6.96	110.0
34	0.01	5	0.2	0.1	5	20	20	20	0.5			139.0
35	0.05	2	1	0.5	>50	>50	>50	>50	2			96.5
36	5	100	5	1	>100	>100	>100	>100	10			162.0
37	0.005	>100	0.02	0.02	10	5	20	20	0.5			458.0
38	0.001	>100	0.02	0.02	10	10	50	20	0.5			370.0
39	0.01	50	0.05	0.02	50	10	20	50	0.5			190.0

40	0.01	20	0.01	0.01	2	10	10	0.5	>4	4	116.0		
41	0.02	5	0.1	0.02	10	10	20	0.2	4.92	4.92	76.5		
42	0.02	5	0.2	0.01	10	20	50	1	3.03	4.0	102.0		
43	0.1	1	0.5	0.05	>100	>100	>100	1	>16	>16	98.2		
44	0.01	2	0.5	0.01	10	10	20	0.5	>16	>16	90.0		
45	0.05	2	1	0.5	>100	>100	>100	>5	>16	>16	71.0		
46	0.05	>200	0.005	0.01	20	20	20	0.1					
47	0.01	100	0.02	0.05	20	20	20	0.5			232.0		
48	2	>200	0.5	0.5	>100	>100	>100	2					
49	0.1	>100	1	1	>100	>100	>100	>10	>16	>16			
50	0.2	>100	1	0.2	>100	>100	>100	5	>16	>16	510.0		
51	0.02	2	1	1	10	100	>100	0.5			122.0		
52	0.02	10	0.5	0.2	20	20	20	0.2	5.28	3.49	69.5		
53	0.05	>100	0.01	0.005	20	5	10	2	>4	>4	250.0		
54	0.05	>100	0.01	0.02	10	5	10	2	>4	>4	180		
55	0.02	>100	0.01	0.01	10	5	20	0.5	>4	>4			
56	0.1	>100	0.01	0.05	50	10	20	5	>8	>8	116		

pound **10**), have shown that its nonspecific binding to different proteins may be the reason for its inhibitory properties on rifampicin-insensitive RNA polymerase and other enzymes.[11]

Activity on Nonbacterial Systems. Representative compounds have been given together with rifamycins belonging to different series to other laboratories interested in assessing their activity on nonbacterial transcribing systems. Of special interest is the activity on RNA-instructed DNA polymerase (RIDP) of mouse Sarcoma virus, studied in M. Green's laboratory, which was found to be inhibited[10] to different extents by a concentration of 100 μg/ml of the alkyl-substituted compounds **6, 7,** and **10** and the aralkyl substituted **40** → **43.** Compounds **10** and **43** appeared the most active (complete inhibition at 20 μg/ml). This fact and the inactivity shown by compounds **53, 54, 48, 46** (bearing hydrophilic substituents), and **2–4** with short alkyl chains indicated that a lipophilic side chain was needed for this activity. A similar screening was contemporarily performed at N.C.I. by R. Gallo and coworkers[12] on RIDP from murine leukemia virus and on RIDP extracted from human leukemic leucocytes, and out of 200 rifamycins compounds **7, 10, 42,** and **43** were selected to be further evaluated together with six derivatives belonging to other series. As a part of the evaluation, these compounds were tested on DNA-instructed DNA polymerase from leukemic and normal blood cells and found active to about the same extent on both these enzymes.

A further indication of the rather general activity of rifamycin oximes with lipophylic side chains on transcribing enzymes was provided by the results obtained at the same time by Chambon, *et al.*, on DNA-instructed RNA polymerases A and B extracted from calf thymus.[13] His results demonstrate that compounds **6, 7, 10, 40,** and **43** are about as active on the mammalian enzyme as on the viral transcriptases, the activity increasing with the lipophilicity of the side chain. Another transcribing enzyme, the phage T2 transcriptase, was shown by Chamberlin[14] to be inhibited by compounds **10, 43, 42, 41,** and **40,** the concentration to obtain a 50% inhibition of activity varying from 45 μg/ml for compound **10** to 140 μg/ml for the least active compound **40.**

Most of the work of other laboratories was concentrated on the activity of diphenyl derivative **43** and the octyl derivative **10.** Inhibition of RNA polymerases from rat liver[15-17] and yeast[18,19] as well as inhibition of immunogenic RNA synthesis[20,21] and of ribonuclease H from rat liver[22] has been reported. It appears thus rather improbable that these most active compounds can be used as selective inhibitors of any transcribing enzyme, because they are about equally effective on most of them and because of their nonspecific binding to proteins. No direct evidence for the selective inhibition of one or the other macromolecular synthesis has been so far obtained for these compounds in intact cells systems like tissue cultures. A selective inhibition of Rous Sarcoma virus production in transformed chick fibroblast was, however, reported by Barlati and Vigier.[23] It is possible that some specificity could be demonstrated by some of the moderately active compounds; Di Mauro, for instance, has observed† a significant difference in inhibition of yeast polymerases Ib and II by compound **8.** To assess whether the structural features of the rifamycin molecule needed for inhibition of bacterial RNA polymerase were also necessary for activity on other transcribing enzymes, the activity on RIDP of Rausher MuLV of the hexahydro derivative of compound **10** was determined in comparison to that of compound **10**

† E. Di Mauro, personal communication.

itself. The two products were equally active on this enzyme, whereas the former is substantially less active on the bacterial system. From this and other evidence we concluded[24] that the structural requirements for activity on bacterial RNA polymerase differ from those reached for activity on reverse transcriptase.

In Vivo **Activity.** As an indication of *in vivo* activity the compounds were tested in *S. aureus* experimental infection in mice; the ED_{50} values obtained are reported in Table III, column 10. All the compounds carrying a lower alkyl, alkoxyalkyl, or alkenyl chain are remarkably effective both when subministered orally or subcutaneously. The efficacy appears proportional to the *in vitro* activity. The compounds most active on the rifampicin-resistant *S. aureus* mutant were tested on mice infected with this strain but no cure was observed even with the high dosages of 200 mg/kg. For some of the most active compounds the oral acute toxicity was determined in mice; in all cases the LD_{50} values were over 2000 mg/kg, a result that cannot be interpreted solely as due to lack of absorption because of the mentioned efficacy of the products in curing orally the experimental infections. The intravenous LD_{50} values are reported in Table III, last column. This type of subministration was chosen to eliminate as far as possible the differences in absorption between the products in order to obtain a parameter that could be compared with the activity of the compounds on mammalian enzymes. It has to be said, however, that the values reported are not completely reliable because to be administered some of the compounds had to be dissolved at very alkaline pH's and some alteration of the rifamycin molecule, like desacetylation,[25] may be occurred.

Concluding Remarks. Because of their antimicrobial activity, their efficacy in experimental infection, and relatively low toxicity, some of the compounds appear potentially useful therapeutic agents for bacterial infections, although none of them seems superior to rifampicin, the currently used rifamycin. However, compounds like **2**, **3**, and **28** which are five to ten times more active *in vitro* on *M. tuberculosis* than rifampicin could be taken into consideration as antitubercular agents.

Several properties of the compounds appear linked to the lipophilicity of the substituent of the oxime oxygen: decreased activity on most pathogenic strains, decreased efficacy in experimental infections, increased activity on rifampicin-resistant *S. aureus*, and increased activity on several transcribing enzymes. From the data reported in Table III it also appears that a lipophilic side chain confers some toxicity to the compounds although here the correlation is not so evident as for the other properties. The lack of specificity of their action leaves little hope for a therapeutic usefulness of the lipophylic members of this series of rifamycins.

Experimental Section

Chemistry. Pmr spectra were taken on a Varian D60 spectrometer at 60 MHz in $CDCl_3$ (TMS) (0.00 ppm); electronic absorption spectra were obtained on a Perkin-Elmer Model 4000A spectrometer; tlc was performed with silica gel HF_{254} (Merck).

Synthesis of the Rifamycin Oximes. All the products listed in Table II were prepared according the procedure below exemplified for compound **10**.

3-Formylrifamycin SV *O*-*n*-Octyloxime (10). To a suspension of 7.25 g (0.01 mol) of 3-formylrifamycin SV in 100 ml of THF was added 1.6 g (0.011 mol) of *O*-*n*-octylhydroxylamine at room temperature. After 15 min tlc ($CHCl_3$-MeOH, 9:1) showed only the yellow spot of the new rifamycin derivative (R_f 0.5). The solution was concentrated to dryness. The residue was dissolved in 200 ml of EtOAc and washed twice with 30 ml of dilute (10%) HCl, to remove the excess *O*-*n*-octylhydroxylamine, and then twice with 30 ml of water. The organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was dissolved in MeOH and the solution concentrated to about 30 ml. The product crystallized out and, after chilling (2 hr), was collected, washed with cold MeOH, and dried (8 g). The pmr spectrum shows, together with the common signals of rifamycins, the presence of new signals: $-(CH_2)_7CH_3$ (t, 0.9), $-CH_2$ (m, 1.35, 12 H), $OCH_2(CH_2)_6$ (t, 4, 2 H), $-CH{=}N$ (s, 8.8, 1 H).

Synthesis of O-Substituted Hydroxylamines. The hydroxylamine derivatives used for the preparation of rifamycins have been prepared (except compound **64**; Table I) with the following standard synthetic procedure.

***O*-Hex-5-enylhydroxylamine (65).** Hydroxyurethane (0.1 mol) and 6-bromo-1-hexene (0.1 mol) were added, at 0°, to absolute EtOH (60 ml) containing dissolved KOH (0.1 mol), and the reaction mixture was boiled for 6 hr. After removal of KBr by filtration, the alcohol was distilled off under reduced pressure. The residue oil was dissolved in water (50 ml) containing NaOH (20 g) and was refluxed for 8 hr. The oily layer which separated was taken up in ether, and after distillation under vacuum 0.065 mol of *O*-hex-5-enyl hydroxylamine was obtained: bp 70° (22 mm). The pmr spectrum shows the following signals: $O(CH_2)_2CH_2CH_2$- (m, 1-2, 4 H), OCH_2-CH_2- (m, 2.10, 2 H), OCH_2- (t, 3.67, 2 H), $-CH{=}CH_2$ (m, 4.8-6.2, 3 H), ONH_2 (broad signal, 5.45, 2 H), which disappears after exchange with D_2O.

***O*-5,6-Dibromo-*n*-hexylhydroxylamine (64).** Bromine (0.011 mol) was added, at room temperature, during 15 min to a stirred solution of 0.01 mol of the above compound (65) in CCl_4 (50 ml). The solution was washed twice with 15 ml of a dilute (10%) solution of Na_2CO_3 to remove the excess of bromine and dried (Na_2SO_4), and the solvent was removed under vacuum. It has been impossible to determine the boiling point of this compound, because it decomposes on heating. Pmr of the crude material shows the disappearance of the signals of the $-CH{=}CH_2$ group.

3-Formylhexahydrorifamycin SV. To a solution of 1.1 g (0.0157 mol) of hexahydrorifamycin S[6] in 200 ml of THF were added 8.3 ml (0.078 mol) of *tert*-butylamine, 5 ml a 40% formaldehyde solution, and 8.3 g of MnO_2 and the mixture was heated at 55° during 7 hr, with stirring. After removal of MnO_2 by filtration, to the mixture was added a solution of 11 g of ascorbic acid in 100 ml of H_2SO_4 (8%) and the mixture allowed to stand to room temperature with stirring. After 3 hr, the reaction mixture was diluted with cold water (300 ml) and extracted with EtOAc (2 × 300 ml). The organic layer was washed with water (3 × 100 ml), dried (Na_2SO_4), and concentrated to dryness. The residue was dissolved in 10 ml of $CHCl_3$, placed on a column of 550 g of silica gel, prewashed with $CHCl_3$, and then eluted with a mixture of $CHCl_3$-MeOH (98:2). The first eluate (about 800 ml) was discarded; then the further eluate (about 2000 ml) was collected and concentrated to dryness. The residue was dissolved in 150 ml of hexane and concentrated under reduced pressure to about 30 ml. The product crystallized out and, after chilling (2 hr), was collected and dried (2.5 g). The pmr spectrum shows, together with the common signals of hexahydrorifamycin S, the disappearance of the signal of the proton in position 3 of the aromatic ring (δ 7.80) and the presence of the signal of the formyl proton at δ 10.65; mp 126-133°; ultraviolet spectrum in phosphate buffer (pH 7.38) λ_{max} 329 mμ (ε 11,600), 496 (11,000). *Anal.* ($C_{38}H_{53}NO_{13}$) C, H, N.

3-Formylhexahydrorifamycin SV *O*-*n*-Octyloxime. To a solution of 730 mg (0.001 mol) of 3-formylhexahydrorifamycin SV in 20 ml of THF was added 160 mg (0.0011 mol) of *O*-*n*-octylhydroxylamine at room temperature. After 15 min tlc ($CHCl_3$-MeOH, 9:1) showed only the yellow-orange spot of the new derivative (R_f 0.7). The solution was concentrated to dryness and, after usual treatment, the product crystallized out from heptane (700 mg). The pmr spectrum shows, together with the common signals of hexahydrorifamycin, the presence of new signals: $-(CH_2)_7CH_3$ (t, 0.9), $-CH_2$ (m, 1.3, 12 H), OCH_2- (t, 4.2, 2 H), $-CH{=}N$ (s, 8.8); mp 85-92°; ultraviolet spectrum in phosphate buffer (pH 7.38) λ_{max} 330 mμ (ε 18,000), 477 (10,700). *Anal.* ($C_{46}H_{70}N_2O_{13}$) C, H, N.

Biological Tests. Antimicrobial Activity. *In vitro* antibacterial activity was tested in liquid media by the serial dilution method. The results are expressed as minimum inhibitory concentrations (MIC) determined as previously described.[26]

The *in vivo* activity was tested in acute experimental infections in mice as previously described[27] and the data are expressed as ED_{50}.

Acute Toxicity. The acute toxicity of the derivatives was determined in mice. The compounds were administered by intravenous route to the animals; these were observed for 168 hr and deaths were recorded. The Litchfield and Wilcoxon[28] method was used to calculate the LD_{50} values.

RNA Polymerase Inhibition Test. DNA-dependent RNA polymerase from a rifampicin-sensitive and a rifampicin-resistant *E. coli* strain was purified by ammonium sulfate fractionation and DEAE cellulose chromatography, according to the procedure of Burgess.[29] Polymerase activity was assayed by determining the incorporation of [^{14}C]AMP into acid insoluble material using highly polymerized calf thymus DNA as a template. The assay conditions were as reported by Burgess,[29] the concentration of the enzyme in our tests being about 8 units per milliliter of final assay mixture.

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References

(1) N. Maggi, C. R. Pasqualucci, R. Ballotta, and P. Sensi, *Chemotherapia*, 11, 285 (1966).
(2) N. Maggi, R. Pallanza, and P. Sensi, *Antimicrob. Ag. Chemother.*, 765 (1965).
(3) L. Thiry and G. C. Lancini, *Nature (London)*, **227**, 1048 (1970).
(4) N. Maggi, G. G. Gallo, and P. Sensi, *Farmaco, Ed. Sci.*, **22**, 316 (1967).
(5) E. L. Schumann, R. V. Heinzelman, M. E. Greig, and W. Veldkamp, *J. Med. Chem.*, **7**, 329 (1964), and references cited therein.
(6) W. Oppolzer, Konstitution der Rifamycine, Promotion Arbeit E.D.T.H., Zurich, 1963.
(7) N. Maggi, S. Furesz, and P. Sensi, *J. Med. Chem.*, 11, 368 (1968).
(8) G. Pelizza, G. C. Lancini, G. C. Allievi, and G. G. Gallo, *Farmaco, Ed. Sci.*, **28**, 298 (1973).
(9) G. C. Lancini and G. Sartori, *Experientia*, **24**, 1105 (1968).
(10) C. Gurgo, R. Ray, and M. Green, *J. Nat. Cancer Inst.*, **49**, 61 (1972).
(11) S. Riva, A. Fietta, and L. G. Silvestri, *Biochem. Biophys. Res. Commun.*, **49**, 1263 (1972).
(12) S. S. Yang, F. M. Herrera, R. G. Smith, M. S. Reitz, G. C. Lancini, R. C. Ting, and R. C. Gallo, *J. Nat. Cancer Inst.*, **49**, 7 (1972).
(13) M. Meilhac, Z. Tysper, and P. Chambon, *Eur. J. Biochem.*, **28**, 291 (1972).
(14) M. J. Chamberlin and J. Ring, *Biochem. Biophys. Res. Commun.*, **49**, 4 (1972).
(15) T. Onishi and M. Muramatsu, *Biochem. J.*, **128**, 1361 (1972).
(16) P. Juhasz, B. J. Benecke, and K. H. Seifart, *FEBS Lett.*, **27**, 30 (1972).
(17) H. W. Butterworth, R. F. Cox, and C. Chesterton, *Eur. J. Biochem.*, **23**, 229 (1971).
(18) R. Adman, L. D. Schultz, and B. D. Hall, *Proc. Nat. Acad. Sci. U. S.*, **69**, 1702 (1972).
(19) E. Di Mauro, C. P. Hollenberg, and B. D. Hall, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2818 (1972).
(20) H. Saito and S. Mitsuhushi, *J. Antibiot.*, **25**, 477 (1972).
(21) S. Kurashige and S. Mitsuhashi, *J. Antibiot.*, **25**, 619 (1972).
(22) C. E. Sekeris and W. Roewekamp, *FEBS Lett.*, **23**, 34 (1972).
(23) S. Barlati and P. Vigier, *FEBS Lett.*, **24**, 343 (1972).
(24) S. Barlati, A. Brega, R. Cricchio, G. C. Lancini, and L. G. Silvestri, *Biochem. Biophys. Res. Commun.*, submitted for publication.
(25) N. Maggi, A. Vigevani, and R. Pallanza, *Experientia*, **24**, 209 (1968).
(26) P. Sensi, N. Maggi, R. Ballotta, S. Furesz, R. Pallanza, and V. Arioli, *J. Med. Chem.*, **7**, 596 (1964).
(27) V. Arioli, R. Pallanza, S. Furesz, and G. Carniti, *Arzneim.-Forsch.*, **17**, 523 (1967).
(28) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
(29) R. R. Burgess, *J. Biol. Chem.*, **244**, 6160 (1969).

Synthesis, Chemistry, and Biological Activity of 5-Thiocyanatopyrimidine Nucleosides as Potential Masked Thiols

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The reaction of chlorothiocyanogen (ClSCN) with various derivatives of uracil was investigated as a potential route to 5-mercaptouracils. Reaction of 1,3-dimethyluracil, 1-(β-D-ribofuranosyl)uracil, 1-(2′-deoxy-β-D-ribofuranosyl)uracil, 1-(β-D-arabinofuranosyl)uracil, 1-(2′,3′,5′-tri-*O*-chloroacetyl-β-D-ribofuranosyl)uracil, and 1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)uracil with ClSCN in acetic acid gave the corresponding 5-thiocyanato derivatives in fair to excellent yields. These 5-thiocyanatopyrimidine nucleosides can be reduced by dithiothreitol, sodium dithionite–2-mercaptoethanol, or glutathione to the biologically active 5-mercaptopyrimidine nucleosides. The intermediate 5-thiocyanatopyrimidine nucleosides themselves show significant biological activity, probably as the result of *in vivo* reduction.

The chemical modification of preformed nucleosides has the advantage of convenience for the preparation of substituted purine and pyrimidine nucleoside analogs. Such an approach is more direct than the laborious condensation of a base with an appropriately protected sugar. A representative example is the direct fluorination of protected pyrimidine nucleosides by trifluoromethyl hypofluorite.[1,2]

5-Mercaptouridine (**1**) and 5-mercapto-2′-deoxyuridine (**2**), previously synthesized by a modification of the Hilbert–Johnson reaction,[3-5] both show significant antibacterial and antitumor properties.[6,7] Alternate approaches to **1** and **2** have included the addition of methyl hypobromite to uracil derivatives followed by reaction with sodium disulfide.[8] The latter procedure is suitable for the thiolation of polynucleotides.[9,10] Because of the success of the use of chlorothiocyanogen[11,12] (ClSCN) in the preparation of sulfur analogs of dopamine and norepinephrine, and because of our continuing interest in modified polynucleotides,[13-16] we have investigated the thiocyanation of pyrimidine nucleosides as a route to 5-mercaptopyrimidine nucleosides. (For a preliminary account of a portion of this work, see ref 17.)

Chemistry. Exploratory experiments with 1,3-dimethyluracil (**3**) revealed that the 2,4-dioxopyrimidine ring readily reacted with chlorothiocyanogen to give a moder-

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