Table II

$ArCH_2CONHN{=}CH$–(5-membered ring, O)–NO_2

Compd	Ar	Mp, °C	Yield, %	Formula[a]
12	C_6H_5S	130	89	$C_{13}H_{11}N_3O_4S$
13	$C_6H_5SO_2$	180	93	$C_{13}H_{11}N_3O_6S$
14	*m*-FC_6H_4S	185	96	$C_{13}H_{10}FN_3O_4S$
15	*m*-$FC_6H_4SO_2$	187	88	$C_{13}H_{10}FN_3O_6S$
16	*p*-$FC_6H_4SO_2$	180	93	$C_{13}H_{10}FN_3O_6S$
17	*o*-$ClC_6H_4SO_2$	215	95	$C_{13}H_{10}ClN_3O_6S$
18	*p*-$ClC_6H_4SO_2$	205–210	88	$C_{13}H_{10}ClN_3O_6S$
19	*p*-$CH_3OC_6H_4S$	155	82	$C_{14}H_{13}N_3O_5S$
20	*p*-$CH_3OC_6H_4SO_2$	190–210	85	$C_{14}H_{13}N_3O_7S$
21	*o*-$CF_3C_6H_4S$[b]	160	91	$C_{14}H_{10}F_3N_3O_4S$
22	*m*-$CF_3C_6H_4S$	175	88	$C_{14}H_{10}F_3N_3O_4S$
23	*m*-$CF_3C_6H_4SO_2$	186	93	$C_{14}H_{10}F_3N_3O_6S$
24	*m*-$NO_2C_6H_4S$	207	86	$C_{13}H_{10}N_4O_6S$
25	*m*-$NO_2C_6H_4SO_2$	236	88	$C_{13}H_{10}N_4O_8S$
26	*p*-$NO_2C_6H_4SO_2$	210	92	$C_{13}H_{10}N_4O_8S$

[a] See footnote *a* in Table I. [b] The corresponding hydrazide was prepared according to the reference 2.

Table III. Zones of Inhibition

	Av zone size, mm					
Compd	*S. a.*[a]	*S. e.*	*K. p.*	*S. f.*	*S. a.*[+]	*E. c.*
12	15.9[c]	17.8	11.4	11.3	15.2[c]	12.0
13	17.2	18.7	10.1	18.5	16.9	11.4
14	11.7	14.1			12.0	
15	15.4	20.2	9.9	16.6	15.7	10.8
16	14.5	18.7		16.1	13.9	9.8
17	16.3	19.4	9.6	16.0	16.6	10.9
18	16.1	19.4	9.7	15.3	17.0	9.7
19	10.0	14.0			10.7[c]	
20	15.1	18.0		17.8	16.8	9.6
21	16.8[c]	15.6	10.9[b]		18.8[c]	15.0
22	10.2	13.1			11.0	
23	15.4	18.4	9.4	12.0	15.8	
24	10.8	14.7	12.2[b]		13.3	
25	14.0	16.9		14.2	16.4	10.0
26	15.9	19.5		16.5	15.6	10.2
Furazolidone	21.2	25.3	20.6	13.9	22.4	23.4

[a] *S. a.* = *Staphylococcus aureus* ATCC 6538-p, *S. e.* = *Staphylococcus epidermidis* ATCC 12228, *K. p.* = *Klebsiella pneumoniae* ATCC 10031, *S. f.* = *Streptococcus faecalis* ATCC 8043, *S. a.*[+] = *Staphylococcus aureus* coagulase +, *E. c.* = *Excherichia coli.* [b] Inhibition zones were hazy. [c] Edges of inhibition zones were not sharp.

Experimental Section†

Arylthioacethydrazides and Arylsulfonylacethydrazides. To a soln of 0.01 mole of the appropriate ester in 15 ml of EtOH was added 0.011 mole of 99% $N_2H_4 \cdot H_2O$. The reaction mixt was stirred for 0.5 hr, then allowed to stand overnight. After cooling in an ice box, the cryst mass was filtered and recrystd from EtOH-H_2O (see Table I).

5-Nitro-2-furfurylidene Arylthioacethydrazides and Arylsulfonylacethydrazides. To a soln of 0.01 mole of the appropriate hydrazide in 10 ml of EtOH, a hot soln of 0.01 mole of 5-nitro-2-furaldehyde in 10 ml of EtOH was added and the reaction mixt was warmed for 0.5 hr at 50–55°. After cooling, the reaction mixt was filtered and the residue was recrystd from EtOH (see Table II).

References

(1) N. Sharghi and I. Lalezari, *J. Chem. Eng. Data*, 8, 276 (1963).
(2) N. Sharghi and I. Lalezari, *ibid.*, 11, 612 (1966).

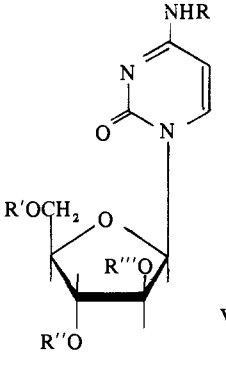
†Melting points were taken on a Kofler hot stage microscope. The ir spectra were determined with a Leitz Model III spectrograph (KBr). Nmr spectra were obtained on a Varian A60A instrument using Me_4Si as internal standard. Mass spectra were recorded on a Varian Mat 111 instrument.

Acyl Derivatives of 1-β-D-Arabinofuranosylcytosine†

John A. Montgomery* and H. Jeanette Thomas
Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205. *Received August 2, 1971*

1-β-D-Arabinofuranosylcytosine (I) is an effective anticancer agent against both experimental animal[1] and human[2] tumors, but it is rapidly deaminated in the human,[3] which may adversely affect its clinical utility. 1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)cytosine (II),[4] prepared in an effort to avoid this difficulty and enhance the oral activity of ara-C, was found to be somewhat less effective than the parent compound,[5] but a single dose of 1-(5-*O*-adamantoyl-β-D-arabinofuranosyl)cytosine (III),[6] which appears to be a repository agent, is almost as effective as ara-C on its optimal schedule (3 courses of multiple closely spaced doses with appropriate intervals for host recovery).[7] We desired to prepare tri-*O*-acyl derivatives of ara-C from higher aliphatic acids that might perform more effectively as "depot" agents, but selective O-acylation of ara-C could not be achieved. Treatment of adamantoyl chloride with ara-C in the presence of Et_3N gave only N^4-adamantoyl-1-β-D-arabinofuranosylcytosine (IV), which was also obtained by the reaction of ara-C with adamantanecarboxylic acid in the presence of dicyclohexylcarbodiimide or with adamantanecarboxylic anhydride in pyridine. Neil, *et al.*, did not selectively O-acylate ara-C either, but prepared a compound presumed to be the 5′-*O*,*N*-bisadamantoyl derivative (V), which was hydrolyzed by NaOH in aq MeOH in unspecified yield to the desired 1-(5-*O*-adamantoyl-β-D-arabinofuranosyl)-cytosine (III).[6] Since this method is not applicable to the preparation of tri-*O*-acyl derivatives because of the ease with which the 2′- and 3′-*O*-acyl group are saponified, N-deacylation of V by treatment with picric acid was attempted and was successful. Removal of the picric acid with ion-exchange resin then gave III, which was also prepared from V by treatment with hydrazine in pyridine.[8]

The reaction of ara-C with butyryl chloride gave a tributyryl derivative, but, unfortunately, its uv spectrum indicated that one of the butyryl groups was attached to the amino group—pmr spectroscopy was used to identify this compd as 1-(3,5-di-*O*-butyryl-β-D-arabinofuranosyl)-N^4-butyrylcytosine (VI), which was N-deacylated with picric acid to give 1-(3,5-di-*O*-butyryl-β-D-arabinofuranosyl)cytosine (VII). The desired tri-*O*-butyryl compd VIII was prepared by acylation of ara-C with butyric anhydride followed by N-deacylation of the tetrabutyryl compd IX with picric



I, R = R′ = R″ = R‴ = H
II, R = H; R′ = R″ = R‴ = CH_3CO–
III, R = R″ = R‴ = H; R′ = (1-adamantyl)CO–
IV, R′ = R″ = R‴ = H; R = (1-adamantyl)CO–
V, R″ = R‴ = H; R′ = R = (1-adamantyl)CO–
VI, R‴ = H; R = R′ = R″ = $CH_3(CH_2)_2CO$–
VII, R = R‴ = H; R′ = R″ = $CH_3(CH_2)_2CO$–
VIII, R = H; R′ = R″ = R‴ = $CH_3(CH_2)_2CO$–
IX, R = R′ = R″ = R‴ = $CH_3(CH_2)_2CO$–

†This work was supported by funds from the C. F. Kettering Foundation, and Chemotherapy, National Cancer Institute, National Institutes of Health, Contract Nos. PH43-64-51 and PH43-65-654.

Table I. Activity of the Butyryl Derivatives of Ara-C against Leukemia L1210[a]

No.	Name	Treatment: Dose, mg/kg[b]	Treatment: Schedule	Host wt. change $\Delta T/\Delta C$	Life-Span: T	Life-Span: C	Life-Span: % increase
VII	1-(3,5-Di-*O*-butyryl-β-D-arabinofuranosyl)-cytosine	400	qd 2-6	−2.9/+2.1			Toxic
		200	qd 2-6	−3.7/+2.1	15.2	8.9	70
		134	qd 2-6	−0.6/+2.0	13.8	8.5	62
		60	qd 2-6	+0.7/+2.0	15.6	8.5	83
VIII	1-(2,3,5-Tri-*O*-butyryl-β-D-arabinofuranosyl)-cytosine	450	qd 2-6	−0.7/+1.8	>25.0	9.1	>174[c]
		300	qd 2-6	−0.4/1.8	20.6	9.1	126
		200	qd 2-6	−0.2/2.0	19.4	8.5	128
		135	qd 2-6	+0.2/2.0	13.5	8.5	58
		80	qd 2-6	+0.7/2.0	13.0	8.5	52
		40	qd 2-6	−0.7/2.3	12.2	9.0	27
IX	1-(2,3,5-Tri-*O*-butyryl-β-D-arabinofuranosyl)-N^4-butyrylcytosine	600	Day 1 only	+0.4/+1.8	11.7	9.2	27
		400	Day 1 only	+1.0/+1.8	10.8	9.2	17
		266	Day 1 only	+1.3/+1.8	10.7	9.2	16
		177	Day 1 only	+1.2/+1.8	9.3	9.2	1

[a] 10^5 cells injected intraperitoneally. [b] Injected intraperitoneally. [c] Two 30-day survivors from six treated animals.

acid. This procedure was also used to prepare the tri-*O*-acetyl derivative II of ara-C and ^{3}H-labeled II for use in pharmacological studies.

Screening Data. Results of the evaluation of the butyryl derivatives of ara-C are given in Table I. The tetrabutyryl compd IX was essentially inactive when given as a single dose. Both the di-*O*-butyryl and the tri-*O*-butyryl compds (VII and VIII) were active on a chronic schedule, but the tri-*O*-butyryl compound VIII is clearly less toxic and more active; in fact, it gave two 30-day survivors at the highest dose tested. These results also show that the tri-*O*-butyryl compd VIII is superior to both ara-C and its tri-*O*-acetyl derivative II when they are given on a chronic schedule,[5] but it is inferior to both 1-(5-*O*-adamantoyl-β-D-arabinofuranosyl)cytosine[6] and to ara-C given on its optimal schedule[7] (see above), indicating that tri-*O*-acyl derivatives of longer chain length may be desirable for optimal activity.

Experimental Section

Uv spectra were detd in the solvents specified with a Cary Model 14 spectrophotometer, the ir spectra were detd in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer, and the pmr spectrum was detd in DMSO-d_6 with a Varian A60-A spectrophotometer. Melting points were taken on a Mel-Temp and are uncorrected.

1-(2,3,5-Tri-*O*-acetyl-β-D-arabinofuranosyl)cytosine (II). A soln of 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)-N^4-acetylcytosine[4] (411 mg, 1.00 mmole) and picric acid (411 mg, 1.80 mmoles) in MeOH (20 ml) was refluxed for 1 hr and then evapd to dryness *in vacuo*. A soln of the resulting yellow residue in 95% aq Me_2CO (25 ml) was stirred with enough Dowex 1-X8 (carbonate) ion-exchange resin to remove the picric acid and give a colorless soln. The soln was evapd to dryness and the resulting residue crystd from EtOAc–Et_2O. A soln of this cryst material in $CHCl_3$ (50 ml) was washed with H_2O (50 ml). The H_2O layer was then extd 6 times with $CHCl_3$ (50 ml each). The $CHCl_3$ exts were combined, dried ($MgSO_4$), and evapd to dryness. The residue, a white glass, crystd from EtOAc–Et_2O: yield, 200 mg (54%); mp 189–190°; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl 277 (13.2), pH 7, 233 (7.72), 269 (8.93), 0.1 *N* NaOH, 274 (9.80); $\bar{\nu}_{max}$ (cm^{-1}): 3445, 3320, 3120 (NH), 1735, 1655 (C=O), 1605, 1525 (C=C, C=N). These figures are in agreement with published data.[4]

In a similar manner, 128 mg (40%) of ^{3}H-labeled II was prepd from ^{3}H-labeled ara-C.

1-(5-*O*-Adamantoyl-β-D-arabinofuranosyl)cytosine (III). A. A soln of 1-(5-*O*-adamantoyl-β-D-arabinofuranosyl)-N^4-adamantoylcytosine (6.80 g, 11.5 mmoles) and hydrazine hydrate (3.0 ml, 48.0 mmoles) in pyridine–AcOH (90 ml of 4:1 v/v) was left for 48 hr at room temp and poured into H_2O (1200 ml). The ppt that immediately formed was collected by filtration after cooling several hours. This solid was triturated first with several portions of Et_2O and then $CHCl_3$. A soln of the insol material in MeOH (300 ml) was evapd to 100 ml. The solid was recrystd from EtOH: yield, 974 mg (21%); mp 299–301° dec.

The analytical sample was obtd from a previous run by recrystn from MeOH. It was dried for 20 hr at 100° (0.07 mm) over P_2O_5: mp 299–300° dec [lit.[7] 300–301°]; λ_{max} nm ($\epsilon \times 10^{-3}$), 0.1 *N* HCl, 280 (13.4); pH 7, 271 (9.35), 0.1 *N* NaOH (unstable); $\bar{\nu}_{max}$ (cm^{-1}): 3460 (OH), 3340, 3270, 3230 (NH), 2905, 2850 (CH), 1695, 1655 (C=O), 1635, 1605, 1525 (C=C, C=N). *Anal.* ($C_{20}H_{27}N_3O_6$) C, H, N.

B. A soln of 1-(5-*O*-adamantoyl-β-D-arabinofuranosyl)-N^4-adamantoylcytosine (638 mg, 10.8 mmoles) and picric acid (638 mg, 27.9 mmoles) in MeOH (25 ml) was refluxed for 5 hr, dild with H_2O (5 ml), and stirred with Dowex 1-X8 (carbonate) ion-exchange resin until colorless. The resin was then removed by filtration and washed several times with hot MeOH. The combined filtrate and wash were evapd to dryness *in vacuo*. The residue obtd was triturated with two 20-ml portions of acetone. The acetone-insol material then crystd from MeOH as a white solid: yield 200 mg (51%); mp 298–299° dec.

1-β-D-Arabinofuranosyl-N^4-adamantoylcytosine (IV). A suspension of 1-β-D-arabinofuranosylcytosine hydrochloride (527 mg, 1.00 mmole) and Et_3N (405 mg, 4.00 mmoles) in anhyd dioxane (40 ml) was stirred for 15 min. A soln of adamantoyl chloride (397 mg, 2.00 mmoles) in dioxane (10 ml) was added to the suspension over a 30-min period. The reaction mixt was stirred at room temp for 20 hr, dild with H_2O (2 ml), stirred for 30 min, and evapd to dryness *in vacuo* below 35°. The residue crystd from MeOH–EtOAc: yield 205 mg (22%); mp 234–236° dec with sintering at 130°. The analytical sample was obtd by recrystn from MeOH. It was dried at 100° (0.07 mm) over P_2O_5: λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 241 (9.48), 311 (15.7), pH 7, 248 (15.9), 299 (9.18), 0.1 *N*, NaOH (unstable); $\bar{\nu}_{max}$ (cm^{-1}): 3465 (broad) (OH, NH), 2900, 2850 (CH), 1705, 1640 (C=O), 1550, 1480 (C=C, C=N). *Anal.* ($C_{20}H_{27}N_3O_6 \cdot H_2O$) C, H, N.

1-(5-*O*-Adamantoyl-β-D-arabinofuranosyl)-N^4-adamantoylcytosine (V). To a soln of 1-β-D-arabinofuranosylcytosine hydrochloride (4.28 g, 16.28 mmoles) in pyridine (300 ml) was added, with stirring and cooling, a soln of adamantoyl chloride (6.48 g, 32.56 mmoles) in 20 ml of C_6H_6 over a 30-min period. After another 15 min in the cold, the soln was left for 20 hr at room temp, dild with H_2O (16 ml), stirred for 30 min, and evapd to dryness at less than 45°. A soln of the residue in $CHCl_3$ (150 ml) was washed 3 times with satd $NaHCO_3$ soln (100 ml), 2 times with H_2O (100 ml), dried ($MgSO_4$), and evapd to dryness *in vacuo*. The residue was triturated several times with petr ether and then pptd as a gel from EtOAc (30 ml). A white solid was obtd: yield 8.15 g (85%); mp 185° dec. The analytical sample was obtd from a previous run. It was dried at 78° (0.07 mm) over P_2O_5 for 20 hr: mp 185° dec; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 247 (11.4), 307 (11.2); $\bar{\nu}_{max}$ (cm^{-1}): 3400 (broad) (OH), 3410 (NH), 2900, 2850 (CH), 1725 (sh), 1710, 1650 (C=O), 1620, 1550, 1480 (C=C, C=N). *Anal.* ($C_{31}H_{41}N_3O_7 \cdot 1.4H_2O$) C, H, N.

1-(3,5-Di-*O*-butyryl-β-D-arabinofuranosyl)-N^4-butyrylcytosine (VI). To a soln of 527 mg (2.00 mmoles) of 1-β-D-arabinofuranosylcytosine hydrochloride in pyridine (25 ml) was added, with cooling and stirring, a soln of butyryl chloride (852 mg, 8.00 mmoles) in C_6H_6 (5 ml) over a 30-min period. The reaction soln was left in the cold another 15 min. Then, after 20 hr at room temp, it was

dild with H_2O (4 ml), stirred for 30 min at room temp, and evapd to dryness at less than 45°. A soln of the residue in $CHCl_3$ (50 ml) was washed 3 times with satd $NaHCO_3$ soln (50 ml), 2 times with H_2O (50 ml), dried ($MgSO_4$), and evapd to dryness *in vacuo*. The residue crystd from EtOAc: yield 335 mg (37%). The analytical sample was obtd by recrystn from EtOAc. It was dried at 100° (0.07 mm) over P_2O_5 for 20 hr: mp 203–204°; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 242 (9.80), 308 (14.2), pH 7, 248 (16.2), 297 (9.40), 0.1 *N* NaOH, 275 (9.74), 303 (sh) (4.35); $\bar{\nu}_{max}$ (cm^{-1}): 3400 (broad) (OH), 3320 (NH), 2965, 2930, 2875 (CH), 1730, 1720, 1655 (C=O), 1605, 1580, 1480 (C=C, C=N); δ in ppm: 0.9 (m, CH_3), 1.6 and 2.4 (m, CH_2 of butyryl), 4.3 (m, $C_2'H$, $C_4'H$, and $C_5'H_2$), 5.0 (t, $C_3'H$), 6.0 and 6.1 (overlapping d, $C_2'OH$ and $C_1'H$), 7.2 and 7.9 (AB pair, C_5H and C_6H), 10.8 (s, NH). These assignments were verified by spin decoupling. *Anal.* ($C_{21}H_{31}N_3O_8$) C, H, N.

1-(3,5-Di-*O*-butyryl-β-D-arabinofuranosyl)cytosine (VII). A soln of 1-(3,5-di-*O*-butyryl-β-D-arabinofuranosyl)-N^4-butyrylcytosine (1.18 g, 2.51 mmoles) and picric acid (1.18 g, 5.10 mmoles) in MeOH (100 ml) was refluxed for 1 hr and stirred with enough Dowex 1-X8 (carbonate) ion-exchange resin to give a colorless soln. Evapn of the soln to dryness gave a white glass. A $CHCl_3$ soln of the glass was washed with H_2O, dried ($MgSO_4$), and evapd to dryness *in vacuo*. The residue crystd from EtOAc: yield 640 mg (66%); mp 166–167°; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 212 (9.55), 278 (13.2); pH 7, 230 (sh) (7.83), 270 (9.18); 0.1 *N* NaOH, 230 (sh) (7.83), 273 (9.53); $\bar{\nu}_{max}$ (cm^{-1}): 3420, 3345, 3230, 3115 (NH), 2965, 2935, 2905, 2875 (CH), 1735, 1660, 1640 (C=O), 1620, 1605, 1525, 1485 (C=C, C=N). *Anal.* ($C_{17}H_{25}N_3O_7$) C, H, N.

1-(2,3,5-Tri-*O*-butyryl-β-D-arabinofuranosyl)cytosine (VIII). A soln of 1-(2,3,5-tri-*O*-butyryl-β-D-arabinofuranosyl)-N^4-butyrylcytosine (4.84 g, 9.25 mmoles) and picric acid (4.84 g, 11.1 mmoles) in MeOH (200 ml) was refluxed for 1 hr and evapd to dryness *in vacuo*. A soln of the yellow residue in 95% aq Me_2CO (100 ml) was stirred with enough Dowex 1-X8 (carbonate) ion-exchange resin to give a colorless soln. Evapn of the soln to dryness gave a syrup that crystd from Et_2O. A soln of the cryst product in $CHCl_3$ (100 ml) was washed with 0.1 *N* H_2SO_4 (100 ml), satd $NaHCO_3$ soln (100 ml), and then H_2O (100 ml), dried ($MgSO_4$), and evapd to dryness *in vacuo*. The residue crystd from Et_2O: yield, 3.20 g (76%); mp 127–129°. The analytical sample was obtd from a previous run by recrystn from Et_2O and dried at 78° (0.07 mm) over P_2O_5 for 8 hr: mp 126–127°; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 277 (13.2); pH 7, 233 (7.67), 269 (8.88); 0.1 *N* NaOH, 274 (10.0); $\bar{\nu}_{max}$ (cm^{-1}): 3445, 3320, 3265, 3125 (NH), 2965, 2935, 2875 (CH), 1760, 1735, 1655 (C=O), 1605, 1525, 1495, 1475 (C=C, C=N). *Anal.* ($C_{21}H_{31}N_3O_8$) C, H, N.

1-(2,3,5-Tri-*O*-butyryl-β-D-arabinofuranosyl)-N^4-butyrylcytosine (IX). A soln of 1-β-D-arabinofuranosylcytosine hydrochloride (3.00 g, 10.8 mmoles) in pyridine (300 ml) contg butyric anhydride (7.86 ml, 47.7 mmoles) was heated at 80–85° for 2 hr. Another 1.86 ml of butyric anhydride was added and heating continued for 1 hr. The soln was then evapd to 60 ml and poured into ice water (300 ml). The resulting mixt was extd 3 times with $CHCl_3$ (200 ml). The $CHCl_3$ ext was extd 2 times with satd $NaHCO_3$ soln (300 ml), then H_2O (300 ml), dried ($MgSO_4$), and evapd to dryness *in vacuo*. Crystn of the residue from Et_2O–petr ether gave a white solid: yield 4.85 g (86%); mp 91–93°. The analytical sample was obtd from a previous run by recrystn from Et_2O–petr ether. It was dried at 78° (0.07 mm) over P_2O_5 for 20 hr: mp 91–93°; λ_{max} nm ($\epsilon \times 10^{-3}$): 0.1 *N* HCl, 248 (12.3), 301 (8.85); pH 7, 249 (15.9), 297 (8.26); 0.1 *N* NaOH, 274 (9.73), 305 (sh) (3.51); $\bar{\nu}_{max}$ (cm^{-1}): 3230 (NH), 2965, 2930, 2875 (CH), 1730, 1665 (C=O), 1610, 1550, 1480 (C=C, C=N). *Anal.* ($C_{25}H_{37}N_3O_9$) C, H, N.

Acknowledgments. The authors are indebted to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectra determinations reported herein, to Mrs. Martha Thorpe for her interpretation of the pmr data, and to Dr. W. R. Laster for the leukemia L1210 screening data.

References

(1) J. S. Evans, E. A. Musser, G. D. Mengel, K. R. Forsblad, and J. H. Hunter, *Proc. Soc. Expl. Biol. Med.*, 106, 350 (1961).
(2) R. D. Carey and R. R. Ellison, *Clin. Res.*, 13, 337 (1965).
(3) G. W. Camiener and C. G. Smith, *Biochem. Pharmacol.*, 14, 1405 (1965).
(4) A. P. Martinez, W. W. Lee, and L. Goodman, *J. Med. Chem.*, 9, 268 (1966).
(5) J. Venditti, *Proc. Chemother. Conf. Ara-C: Develop. Appl.*, 1969, 10 (1970).
(6) G. L. Neil, P. F. Wiley, R. C. Manak, and T. E. Moxley, *Cancer Res.*, 30, 1047 (1970).
(7) H. E. Skipper, F. M. Schabel, Jr., and W. S. Wilcox, *Cancer Chemother. Rep.*, 51, 125 (1967).
(8) R. L. Letsinger, P. S. Miller, and G. W. Grams, *Tetrahedron Lett.*, 2621 (1968).

Antibacterial Activity of *o*-Amino-*N*-hydroxybenzenesulfonamides†

Ned D. Heindel,* C. C. Ho Ko, Richard B. Birrer, and Joseph R. Merkel

Department of Chemistry, Lehigh University, Bethlehem, Pennsylvania 18015. *Received July 9, 1971*

Several reports in the chemical literature have demonstrated the potentiating effect of an NOH moiety on the antibacterial properties of an arylsulfonamide. For example, *N*-hydroxybenzenesulfonamide is more potent against *Mycobacterium tuberculosis* than is benzenesulfonamide[1] and several N^4-acyl-N^1-hydroxybenzenesulfonamides are more effective against β-hemolytic streptococci in mice than is sulfanilamide itself.[2] Furthermore, the SO_2NHOH grouping is well known as a chelator of metal ions[3,4] a property which might be expected to be reflected in enhanced antibacterial effects.[5] However, N^1-hydroxysulfanilamide appears less active than the nor-OH counterpart *in vitro* but of equivalent activity *in vivo* by virtue of a metabolic conversion of sulfanilamide.[6] Tests against *Escherichia coli*[7] and other microorganisms[8] have shown that *o*- and *m*-aminobenzenesulfonamides, lacking the NOH on the sulfonamide group, do not inhibit bacterial growth.

Thus, our observation of significant antibacterial potency in several *o*-amino-*N*-hydroxysulfonamides, appears as a striking example of the activity promoting effects of an NOH function. We have prepared these materials (**1**, **2**, **4**, **5**, **6**) through the intermediacy of the *o*-aminobenzenesulfonyl chlorides and their subsequent reaction with hydroxylamines (H_2NOR) to yield both *N*-OH and *N*-OMe systems. Employing a cyclization method previously applied to ortho-substituted carboxamides and dimethyl acetylenedicarboxylate,[9,10] the 1,2,4-benzothiadiazine (**7**) was prepared. Methylation of this heterocyclic with aq Me_2SO_4 yielded **3**. By a technique described by Wei, *et al.*[11] for condensation of aldehydes with *o*-amino-*N*-hydroxybenzenesulfonamides, *p*-nitrobenzaldehyde and the 2-amino-4,5-dichloro-*N*-hydroxybenzenesulfonamide (**1**) gave the 1,2,4-benzothiadiazine (**8**) in 90% yield.

Biological Activity. Compounds were applied to penicillin assay disks (Schleicher and Schuell Co., 12 mm diam) as either solution or suspension in 95% EtOH to achieve a concn of 4 mg/disk of test substance. The disks were then air-dried and placed on the surface of brain heart infusion agar medium (Difco) which had been seeded with the test organism. The assay plates were incubated at 37° and inhibition zones were measured after 24 and 48 hr (see Table I).

A free *o*-NH_2 and an SO_2NHOR appear to be essential for inhibitory activity since neither the heterocyclics, **7** and **8**, nor the *N*,*N*-Me_2N analog, **3**, displayed any measurable

†Supported by Grant 1 RO1 13562 from the National Institute of Mental Health. Taken in part from the Ph.D. thesis of C. C. H. K. (1971) and the B. A. Honors report of R. B. B. (1971).