+118° (c 1, MeOH); ir (KBr) 2950, 1540 (NH_3^+), 1782 (β-lactam), 1740, 1190 (ester), 810, 745, 695 cm^{-1} (phenyl); tlc (system V) R_f 0.47.

Benzyl Phenoxyacetamidobisnorpenicillanate (13a). A suspension of 12a (1.35 g, 3 mmol) in CH_2Cl_2 (120 ml) was neutralized with 1 equiv of Et_3N in CH_2Cl_2 and cooled (0°). Solutions of Et_3N (304 mg, 3.3 mmol) and phenoxyacetyl chloride (560 mg, 3.3 mmol) in CH_2Cl_2 (15 ml for each) were added gradually (in 1 hr) to the cooled solution of **12a**. After storage for 2 hr at 0° the reaction mixture was extracted successively with 0.05 *N* HCl, $NaHCO_3$ (5%), and H_2O. The organic layer was dried (Na_2SO_4) and evaporated and the residue was crystallized from Et_2O–petroleum ether, yielding **13a** (1.075 g, 87%): mp 113–114°; $[\alpha]^{25}D$ +145° (c 0.5, $CHCl_3$); mass spectrum M^+ 412; ir (KBr) 3370, 1685, 1520 (amide), 1796 (β-lactam), 1725, 1205 (ester), 745, 690 cm^{-1} (phenyl); nmr ($CDCl_3$) δ 3.45 (d, *J* = 5 Hz, H-2), 4.54 (s, OCH_2CO), 5.03 (5, *J* = 5 Hz, H-3), 5.22 (s, $OCH_2C_6H_5$), 5.38 (d, *J* = 4.5 Hz, H-5), 5.72 (dd, *J* = 4.5 and 9 Hz, H-6), 6.7–7.6 ppm (m, C_6H_5); tlc (system II) R_f 0.26.

Bisnorpenicillin V Potassium Salt (1a). A solution of **13a** (412 mg, 1 mmol) in EtOAc (30 ml) was hydrogenated over Pd/C (10%) (412 mg) for 5 hr at room temperature and at a pressure of 3 kg/cm^2. The catalyst was filtered off and washed with EtOAc. The combined filtrates were concentrated to 50 ml and H_2O (50 ml) was added. The cooled mixture was adjusted to pH 6.3 with KOH (0.2 *N*). Freeze-drying of the aqueous layer yielded the potassium salt of 1a (258 mg, 71.5%) which was crystallized from H_2O–Me_2CO: mp 175° dec; $[\alpha]^{25}D$ +185° (c 1, H_2O); ir (KBr) 3350 (NH), 1680, 1525 (amide), 1769 (β-lactam), 1610, 1405 (COO^-), 690, 750 cm^{-1} (phenyl); nmr (D_2O, DSSA) δ 3.40 (AB part of ABX pattern, J_{AX} = 4 Hz, J_{BX} = 6 Hz, J_{AB} = 11.5 Hz, H-2), 4.53 (s, $OCH_2C_6H_5$), 4.88 (X part of ABX pattern, J_{AX+BX} = 10 Hz, H-3), 5.37 (d, *J* = 4 Hz, H-5), 5.48 (d, *J* = 4 Hz, H-6), 6.7–7.5 ppm (m, C_6H_5); tlc (system III) R_f 0.66. *Anal.* ($C_{14}H_{13}N_2O_5SK$) C, H, N.

Determination of the Sensitivity to β-Lactamase. The rate of hydrolysis of 1a and 1b was determined at 30° and at pH 7 on 4-ml samples containing 12.5 μmol of penicillin and 13 units** of β-lactamase using the method described by Zyk.[16] Under these conditions the rate of hydrolysis was 17 μmol/hr for 1a and 19.5 μmol/hr for 1b.

Acknowledgments. We are indebted to the Belgian Fonds voor Wetenschappelijk Geneeskundig Onderzoek (FWGO) for financial support and to Dr. S. Toppet for determination of nmr spectra. Thanks are due to Professor G. Smets for providing these facilities. We also thank Dr. F. Compernolle and Dr. G. Janssen for mass spectral determinations and Professor H. Eyssen for the determination of the antibacterial activities. J. Hoogmartens is the recipient of a doctoral fellowship of the Instituut tot Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw.

**One unit of β-lactamase is defined as the amount of enzyme that hydrolyzes 1 μmol of benzylpenicillin per hour at 30° and at pH 7.[15]

References

(1) R. B. Morin, B. G. Jackson, R. A. Mueller, E. R. Lavagnino, W. B. Scanlon, and S. L. Andrews, *J. Amer. Chem. Soc.*, **85**, 1896 (1963); **91**, 1401 (1969).
(2) D. H. R. Barton, D. G. T. Greig, G. Lucente, P. G. Sammes, M. V. Taylor, C. M. Cooper, G. Hewitt, and W. G. E. Underwood, *Chem. Commun.*, 1683 (1970); D. H. R. Barton, F. Comer, D. G. T. Greig, P. G. Sammes, C. M. Cooper, G. Hewitt, and W. G. E. Underwood, *J. Chem. Soc. C*, 3540 (1971).
(3) D. O. Spry, *J. Amer. Chem. Soc.*, **92**, 5006 (1970); *J. Org. Chem.*, **37**, 793 (1972).
(4) S. Kukolja and S. R. Lammert, *J. Amer. Chem. Soc.*, **94**, 7169 (1972).
(5) J. C. Sheehan, K. R. Henery-Logan, and D. A. Johnson, *J. Amer. Chem. Soc.*, **75**, 3292 (1953).
(6) H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., "The Chemistry of Penicillins," Princeton, N. J., 1949, p 908.
(7) J. C. Sheehan and K. R. Henery-Logan, *J. Amer. Chem. Soc.*, **79**, 1262 (1957); **81**, 3089 (1959).
(8) J. C. Sheehan and K. R. Henery-Logan, *J. Amer. Chem. Soc.*, **81**, 5838 (1959); **84**, 2983 (1962).
(9) J. C. Sheehan and D. A. Johnson, *J. Amer. Chem. Soc.*, **76**, 158 (1954).
(10) I. McMillan and R. J. Stoodley, *Chem. Commun.*, 11 (1968).
(11) J. C. Sheehan and J. A. Schneider, *J. Org. Chem.*, **31**, 1635 (1966).
(12) A. M. Felix, J. Unowsky, J. Bontempo, and R. I. Fryer, *J. Med. Chem.*, 11, 929 (1968).
(13) D. J. Tipper and J. L. Strominger, *Proc. Nat. Acad. Sci. U. S.*, **54**, 1133 (1965).
(14) J. C. Sheehan and D. R. Hoff, *J. Amer. Chem. Soc.*, **79**, 237 (1957).
(15) M. R. Pollock and A. M. Torriam, *C. R. Acad. Sci., Paris*, **237**, 276 (1953).
(16) N. Zyk, *Antimicrob. Ag. Chemother.*, 2, 356 (1972).

New Streptozotocin Analogs with Improved Antileukemic Activity†

Allan N. Fujiwara, Edward M. Acton,* and David W. Henry

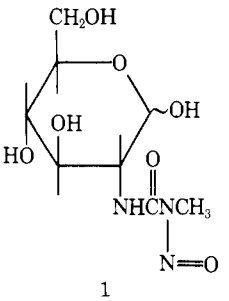
Life Sciences Division, Stanford Research Institute, Menlo Park, California 94025. *Received October 17, 1973*

The 3-methyl-3-nitrosoureido derivatives of the following amino sugars were prepared as analogs of streptozotocin with the anomeric carbon protected, by nitrosating the methylureas in water with N_2O_3: 3-amino-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose, methyl 3-amino-3-deoxy-β-D-xylopyranoside, methyl 3-amino-3-deoxy-α-D-altropyranoside, methyl 3-amino-3-deoxy-α-D-glucopyranoside, methyl 6-amino-6-deoxy-α-D-glucopyranoside, and methyl 3-amino-2,3,6-trideoxy-α-L-*lyxo*-hexopyranoside. Tests against murine leukemia L1210 show that the anticancer activity of streptozotocin not only was retained but was enhanced in most of these derivatives.

Streptozotocin (1) is an antibiotic with antileukemic and diabetogenic activity in animals.[1] In human patients it has been used with success for the treatment of malignant insulinoma[2] and is being tested against the whole range of common tumor types.[3] Kidney damage is the most common and most severe of various toxic side effects, so that analogs and derivatives of 1 are of interest for either enhanced anticancer activity or reduced toxicity. Recently observed separation of antileukemic, diabetogenic, and antibacterial activities in a series of new streptozotocin isomers and analogs[1,4] emphasized the promise of further structural variations. Analogs blocked at the anomeric carbon of the sugar moiety are of interest for their greater ease and convenience in preparation, relative to free reducing sugars. The methyl glycosides[4,5] of 1 have been studied *in vitro*: the β anomer was just as active as 1, but the α anomer was twice as active.[4] The 6-

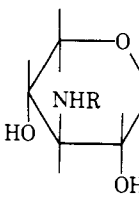
† This work was performed under the auspices of the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare, Contract No. NO1-CM-33742. The opinions expressed in this paper are those of the authors and not necessarily those of the NCI.

isomer of streptozotocin, as the α-methyl glycoside (**18**), was recently prepared and was active against Ehrlich ascites tumor in mice.[6] We have independently prepared **18** and five new methylnitrosoureido sugars with the anomeric carbon protected. Tests against leukemia L1210 in mice, a more predictive system for human tumors, show that activity of **1** was not only retained but actually enhanced in most of these analogs.

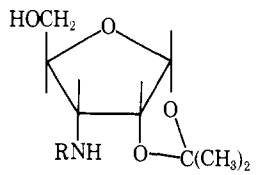


1

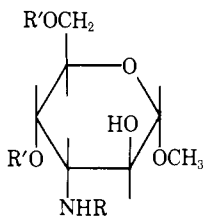
Chemistry. Various known aminodeoxy sugars, either as methyl glycosides (**2, 8, 12, 16, 19**) or a 1,2-*O*-isopropylidene derivative (**5**), were converted to the methylureas (**3, 6, 9, 13, 17, 20**) with methyl isocyanate. These (after removal of 4,6-*O*-benzylidene groups in the case of **9** and **13**) were nitrosated in aqueous solution with N_2O_3 as in the most direct synthesis of streptozotocin (**1**) itself.[7] From 1 to 2 molar equiv of N_2O_3 were used, so that after several hours, when nitrosation was generally complete, the solution still gave a positive test to starch–iodide paper. Work-up was by lyophilization. The only contaminants were a few per cent of starting material in some cases and adhering nitrous acid. Any starting material was removed by renitrosation to completion rather than attempts at separation. Excess nitrous acid was removed by crystallization of the product or by repeated evaporation of dried organic solutions and lyophilization. In the case of **7**, the reaction solution was conveniently neutralized with an ion exchange resin (CO_3^{2-}) prior to lyophilization. When this was tried in the work-up of **11** it caused facile cyclization to the adjacent *cis*-OH at C-4 to form the cyclic urethane **22** with loss of the elements of $CH_3NHN{=}O$. All the nitrosoureas (**4, 7, 11, 15, 18, 21**) were obtained as stable solids; **11** and **18** were crystallized from hot solvents. Only **15** could not be crystallized. All could be stored at 25° for 16–20 months without signs of decomposition, except **21**, which had decomposed after 8 months.



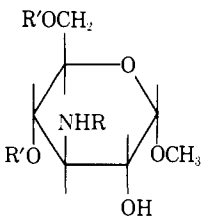
2, R = H
3, R = CONHCH$_3$
4, R = CON(NO)CH$_3$



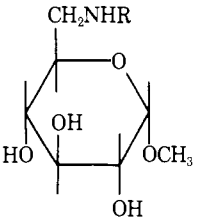
5, R = H
6, R = CONHCH$_3$
7, R = CON(NO)CH$_3$



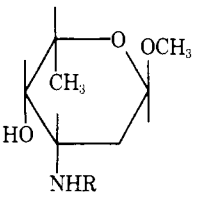
8, R = H
9, R = CONHCH$_3$
10, R = CONHCH$_3$
11, R = CON(NO)CH$_3$
} R′ + R′ = PhCH
} R′ = H



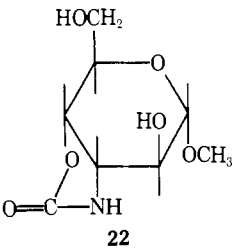
12, R = H
13, R = CONHCH$_3$
14, R = CONHCH$_3$
15, R = CON(NO)CH$_3$
} R′ + R′ = PhCH
} R′ = H



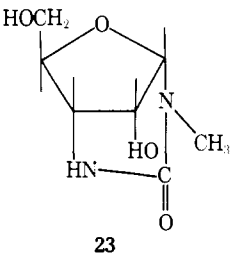
16, R = H
17, R = CONHCH$_3$
18, R = CON(NO)CH$_3$



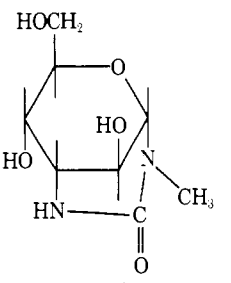
19, R = H
20, R = CONHCH$_3$
21, R = CON(NO)CH$_3$



22



23



24

An advantage of developing active compounds protected at C-1 was illustrated by side reactions encountered in some deblockings. Removal of the isopropylidene group from the intermediate urea **6** in 80% CF_3COOH afforded entirely the cyclized urea **23**, even at 0°. Similarly, if heating was applied in removal of the benzylidene group from **9**, or if CF_3COOH was used, there was some loss of the 1-OCH_3 and concomitant cyclization of the urea (to form **24**), according to pmr.

The various kinds of NCH_3 were nicely characterized by pmr spectra. For the methylureas, this signal was near δ 2.6 in DMSO-d_6 or at δ 2.62–2.78 in $CDCl_3$. Upon nitrosation, it was shifted to δ 3.2 in $CDCl_3$ or δ 3.13 in D_2O. In the 1,3-cyclic ureas (*e.g.*, **23** and **24**), the NCH_3 signal was near δ 2.8 in DMSO-d_6. Infrared carbonyl absorption, as expected, shifted from 6.05–6.13 μ in the methylureas to 5.78–5.90 μ on nitrosation. Distinct infrared bands for N=O at 6.7 μ were identifiable only in the spectra of **4** and **7**.

Biological Data. Preliminary evaluation of antitumor properties was done by Drug Research and Development, National Cancer Institute, according to its protocols.[8] Test results (T/C) for the six nitrosoureas against L1210

Table I. Test Results[a] *vs.* L1210 Murine Leukemia

Structure no.	NSC[b] no.	3 injections, saline, ip (qd, days 1, 5, 9) Dose, mg/kg	*T/C*, %	9 injections, saline, ip (qd, days 1–9) Dose, mg/kg	*T/C*, %
4	153365	400	162	400[c]	
		200	291	200	134
		150	167	100	224 (2 cures)
		100	160, 145, 136	75	186
		66	135, 138, 126	50	160
		44	126	33	135
		33	118	22	141
		27	112		
		16	108		
7	155692	400	107	400[c]	119
		200	100	300	105
		100	94	200	129, 198
				132	151
				100	124
11	156273	400	100		
		200	133	200	100
		150	105, 122	150	118, 136
		100	142, 158, 118	100	218, 103, 131
		66	100, 111	66	126, 137
				50	181
15	160466	400	214	400[c]	100
				200	177, 197
		200	166, 140	150	157
		100	130, 135	100	165, 145, 143, 151
		50	105, 127	66	137
		25	106	50	140, 137, 143
				44	124
				25	125, 128
				12.5	112
18	157724	400	150, 160	400	166
		300	145	300	173
		264	140	200	150, 170
		200	129, 132	132	144
		176	142	113	154
		132	120	100	118
		115	116	75	136
		100	115	50	131
		75	118	33	120
21	166643	500	100		
		400	132, 106		
		200	109		
		100	110		
1	85998	256	<125[d]	200[c]	103[e]
				100	111[e]
		32		50	127[e]
				25	125[e]

[a]The numerical results (*T/C*) are ratios of survival times of treated mice over control mice, expressed as per cent. A ratio ≥125 is a positive result denoting activity. [b]Accession number of the National Cancer Institute. [c]Toxic dose, animal deaths. [d]Data from ref 9. [e]These data are cited as representative. In otherwise identical tests, *T/C* at 50 mg/kg, the optimum dose, ranged from 100 to 148.

murine leukemia are listed in Table I. Streptozotocin (1) is active only on a daily treatment schedule, and just meets the minimum activity requirement in these tests for further development.[9] In the three-injection regimen compounds 4, 11, 15, and 18 displayed substantial activity, in contrast to streptozotocin which is without effect. In the nine-injection schedule all of the new drugs except 21 (not evaluated in this system) were active, including 7 which was not effective on the three-dose schedule. Compounds 15 and 18 are clearly at least equal to the parent antibiotic, and xylose derivative 4 appears definitely superior, effecting cures in two out of six mice at 100 mg/kg. Altrose derivative 11 yielded *T/C* values too erratic to allow a clear comparison with streptozotocin.

Acute toxicity among the new drugs appears to be somewhat lower than that found for streptozotocin. None of the drugs assayed on the nine-injection regimen caused toxic deaths among test mice at 200 mg/kg, a level where streptozotocin begins to show this effect. At 400 mg/kg 4 and 7 did cause one toxic death out of six animals, and three out of ten for 15. Compound 18 at this level was nontoxic, however.

Although this series of compounds is too small to allow a meaningful correlation of structure with activity, the results complement and extend earlier findings. It is clear from the accumulating studies that the nature of the sugar carrying the *N*-methyl-*N*-nitrosourea moiety is not critical. Thus, the present investigation embraces two pentoses and three hexoses with the ureido substituent at the 3 or 6 position, and antitumor activity is present in all but daunosamine derivative 21. Bhuyan, *et al.*,[1] demonstrated that the nitrosourea side chain conferred activity upon galacto- and glucopyranosides when in the 1 position and also established that an open-chain sugar derivative (1-amino-1-deoxy-D-glucitol) provided an active carrier for the *N*-methyl-*N*-nitrosourea moiety. These investigators also found that complete acetylation of active analogs of streptozotocin was not detrimental to antitumor efficacy. Our work confirms the findings of Bannister[4] and of Suami and Machinami[5] that methyl glycosides are ac-

ceptable or advantageous structural features for streptozotocin analogs. Suami and Machinami[10] also recently noted that analogs of streptozotocin based on the aminocyclitol inosamine were active in the Ehrlich ascites and HeLa carcinoma experimental tumor systems.

Insofar as results from the various studies may be compared, there do not seem to be large differences in potency among streptozotocin analogs. This, coupled with the substantial structural variations that are compatible with activity, suggests that the sugar moiety is functioning as a comparatively nonspecific hydrophilic carrier for the *N*-methyl-*N*-nitrosourea group. The carrier role must also be associated with other functions, however, as it has been demonstrated that streptozotocin differs substantially from *N*-methyl-*N*-nitrosourea in its biological and metabolic properties.[11]

Because of the possible transport function of the sugar, we obtained partition coefficient data for the compounds prepared in this study.‡ Log *P* values for compounds **4**, **11**, **15**, and **18** are −1.08, −0.82, −1.57, and −1.45, respectively. That of streptozotocin is −1.45.[12] Unfortunately, values for the least active compounds of the group (**7** and **21**) could not be obtained. It is of interest that all partition values for the new compounds are within 0.6 log units of the parent and all compounds are roughly of equivalent activity. The least active compounds, although partition coefficients were not obtained, would be expected to have appreciably higher lipid affinity than the other drugs. The calculated[12] log *P* value of **7** is −0.46 and that of **21** is +0.61. It is of interest that the optimum log *P* value for a series of antitumor *N*-(2-haloethyl)-*N*-nitroso-*N'*-alkylureas derived from a synthetic lead was −0.6.[13] Higher log *P* values were associated with decreased activity in that study.

Experimental Section

Melting points were observed on a Fisher-Johns hot stage and are uncorrected. Infrared spectra were determined routinely in Nujol mull (solids) or as a liquid film. Pmr spectra were determined on a Varian A-60A spectrometer in $CDCl_3$ solution with Me_4Si as internal reference (δ = 0.0), unless otherwise designated in DMSO (dimethyl sulfoxide-d_6, internal Me_4Si) or D_2O (external Me_4Si). Signals are designated as s (singlet), doublet (d), triplet (t). Integrated peak ratios were as expected from the structure assignments. Thin-layer chromatography was done with silica gel HF (E. Merck) on 5 × 20 cm glass plates in MeOH–C_6H_6 (solvent ratios are given in parentheses following the R_f's), unless the solvent is otherwise designated. Organic solutions were commonly dried over $MgSO_4$, and evaporations were carried out *in vacuo*.

Methyl 3-Deoxy-3-(3-methylureido)-β-D-xylopyranoside (3). A solution of 6.4 g (40 mmol) of methyl 3-amino-3-deoxy-β-D-xylopyranoside[14,15] (**2**) in 36 ml of H_2O was cooled to 0° and treated with 2.6 ml (44 mmol) of CH_3NCO in three portions. After 1 hr at 0° the solution was allowed to warm, clarified by filtration, and evaporated. Recrystallization of the residue from EtOH–EtOAc (2:3) afforded 5.2 g (59%): mp 184–185.5°; R_f 0.8 (1:1); pmr (DMSO) δ 4.13 (d, H-1, J = 6.5 Hz), 3.38 (s, OCH_3), 2.58 (d, NCH_3, J = 5.0 Hz, collapsed on D_2O exchange). *Anal.* ($C_8H_{16}N_2O_5$) C, H, N.

3-Deoxy-1,2-*O*-isopropylidene-3-(3-methylureido)-α-D-ribofuranose (6) was similarly obtained from 3-amino-3-deoxy-1,2-*O*-isopropylidene-α-D-ribofuranose[16] (**5**) in 64% yield: mp 162–164°; R_f 0.4 (1:1); pmr δ 5.88 (d, H-1), 4.66 (t, H-2, $J_{1,2} = J_{2,3}$ = 3.7 Hz), 2.78 (d, NCH_3, J = 5.0 Hz, collapsed on D_2O exchange), 1.55 (s, CMe_2), 1.37 (s, CMe_2). *Anal.* ($C_{10}H_{18}N_2O_5$) C, H, N. It could also be recrystallized from *i*-PrOH.

Methyl 6-Deoxy-6-(3-methylureido)-α-D-glucopyranoside (16). Methyl 6-azido-6-deoxy-α-D-glucopyranoside[17,18] (8 g) in 100 ml of 95% EtOH was reduced with 0.3 g of Pd black and 4 ml of hydrazine, added in several portions, with swirling and warming for 2 hr. Filtration and evaporation afforded the residual **6**-amine **16**: R_f 0.2 in MeOH; pmr (DMSO) δ 4.58 (d, H-1, J = 3.2 Hz), 3.30 (s, OCH_3). The urea **13**, obtained as for **3**, was contaminated with 1,6-dimethylbiurea [from reaction between unremoved hydrazine and methyl isocyanate, mp 250–254° (lit.[19] mp 257–259°)] and was purified by column chromatography and recrystallization (three crops, 64% yield) from *i*-PrOH: mp 174–176° (lit.[6] 176–177°); R_f 0.8 (1:1); pmr (DMSO) δ 4.59 (d, H-1, J = 3.2 Hz), 3.30 (s, OCH_3), 2.58 (d, NCH_3, J = 4.5 Hz, collapsed on D_2O exchange). *Anal.* ($C_9H_{18}N_2O_6$) C, H, N.

Methyl 2,3,6-Trideoxy-3-(3-methylureido)-α-L-*lyxo*-hexopyranoside (20). The parent amine, methyl daunosaminide[20] (**19**, in H_2O, 13 ml/g), yielded 61% of **20**, mp 191–192°, recrystallized from hot H_2O: R_f 0.4 (1:4); pmr (D_2O) δ 4.87 (rough t, H-1), 3.44 (s, OCH_3), 2.76 (s, NCH_3), 1.25 (d, CCH_3). *Anal.* ($C_9H_{18}N_2O_4$) C, H, N.

Methyl 4,6-*O*-Benzylidene-3-deoxy-3-(3-methylureido)-α-D-altropyranoside (9). The parent amine[21] **8** in tetrahydrofuran (THF)–H_2O (5:3) yielded **9** as a foamed glass (93%): R_f 0.7 (1:1); pmr δ 5.52 (s, PhCH), 4.63 (s, H-1), 3.35 (s, OCH_3), 2.62 (m, NCH_3, collapsed to s on D_2O exchange). *Anal.* ($C_{16}H_{22}N_2O_6$) C, H, N.

Methyl 4,6-*O*-Benzylidene-3-deoxy-3-(3-methylureido)-α-D-glucopyranoside (13). The parent amine[22] **12**, also in THF–H_2O (10:3), almost immediately yielded a precipitate (76%), mp 277–287°, after trituration with $CHCl_3$: R_f 0.45 (1:4). *Anal.* ($C_{16}H_{22}N_2O_6$) C, H, N.

Methyl 3-Deoxy-3-(3-methylureido)-α-D-altropyranoside (10). A solution of 7.1 g (21 mmol) of **9** in 150 ml of THF–H_2O (3:7) was stirred with about 20 g of prewashed 50–100 mesh Dowex 50X-8 (H) strongly acidic ion exchange resin at 25° for 5.5 hr. The resin was removed and the filtrate concentrated (high vacuum) to a 4.6 g (88%) of a foamed glass: R_f 0.25 (1:4); pmr (DMSO) δ 4.47 (d, H-1, J = 2.0 Hz), 3.32 (s, OCH_3), 2.58 (s, NCH_3). *Anal.* ($C_9H_{18}N_2O_6$) C, H, N.

Methyl 3-Deoxy-3-(3-methylureido)-α-D-glucopyranoside (14). A suspension of **13** was treated as for **10**. After 15 hr, the resultant clear solution was concentrated to remove THF, extracted with CH_2Cl_2 to remove benzaldehyde, and evaporated. The residue was crystallized from *i*-PrOH (50% yield): mp 171–172°; R_f 0.20 (1:4); pmr (DMSO) δ 4.63 (d, H-1, J = 3.2 Hz), 3.35 (s, OCH_3), 2.60 (s, NCH_3). *Anal.* ($C_9H_{18}N_2O_6$) C, H, N.

Methyl 3-Deoxy-3-(3-methyl-3-nitrosoureido)-β-D-xylopyranoside (4). A solution of 4.4 g (20 mmol) of the urea **3** in 35 ml of H_2O at 0° was treated with 2.0 ml (38 mmol) of liquefied N_2O_3 (Matheson, nitrogen trioxide). After 4 hr at 0°, the solution (pH 2, still positive to starch–iodide test paper) was lyophilized. The sticky residue was dissolved in 40 ml of CH_2Cl_2 and the solution was dried over $MgSO_4$ and filtered. Addition of 9 ml of ether to the filtrate and chilling at 0° overnight produced 3.1 g (62%) of yellow crystals: mp 123–124° dec; R_f 0.4 (1:4); pmr δ 4.68 (d, H-1, $J_{1,2}$ = 3.5 Hz), 3.51 (s, OCH_3), 3.20 (s, NCH_3). *Anal.* ($C_8H_{15}N_3O_6$) C, H, N. On a 30-g scale, a second lyophilization of the initial product afforded a crystalline, analytically pure monohydrate (95% yield), mp 112–112.5°, otherwise identical with the anhydrous sample. A sample was stored at 25° for 20 months without any change.

3-Deoxy-1,2-*O*-isopropylidene-3-(3-methyl-3-nitrosoureido)-α-D-ribofuranose (7). The reaction solution from 4.2 g of **6** was neutralized to pH 6 with Dowex 2X-8 (CO_3) ion exchange resin. Lyophilization then afforded a hygroscopic gum (95% yield): R_f 0.6 (1:4); pmr δ 5.97 (d, H-1, J = 3.6 Hz), 3.22 (s, NCH_3), 1.57 (s, CMe_2), and 1.38 (s, CMe_2). *Anal.* ($C_{10}H_{17}N_3O_6 \cdot 0.25H_2O$) C, H, N. A solution in CH_2Cl_2 was dried and evaporated, and the residue crystallized on trituration with ether–petroleum ether: mp 89–90° The crystallization sample was stored at 25° for 19 months without any change.

Methyl 3-Deoxy-3-(3-methyl-3-nitrosoureido)-α-D-altropyranoside (11). Without prior neutralization of the reaction solution from **10**, lyophilization gave a crystalline solid that was further dried *in vacuo* at 40° for 6 hr (4.5 g, 91% yield): mp 116–117°; R_f 0.45 (1:4); pmr (D_2O) 3.42 (s, OCH_3), 3.13 (s, NCH_3). *Anal.* ($C_9H_{17}N_2O_3$) C, H, N. Recrystallization from hot 95% EtOH raised the melting point to 117.5–118°. The solid was stored at 25° for 18 months without any change. The D_2O solution for pmr was stored for 2 weeks with no change.

Methyl 3-Deoxy-3-(3-methyl-3-nitrosoureido)-α-D-glucopyranoside (15). The lyophilization residue was a hygroscopic foamed glass that retained N_2O_3 after repeated lyophilization. Despite that, in each of three runs, the product contained several per cent of unreacted **14**, so that a second treatment with N_2O_3

‡ We are grateful to Dr. Robert R. Engle of Drug Research and Development, NCI, and Dr. W. J. Haggerty of Midwest Research Institute, Kansas City, for these data.

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.

Acknowledgment. The authors are indebted to Miss Elizabeth M. McCarthy for the elemental analyses.

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

Renato Cricchio,* Giancarlo Lancini, Giovanni Tamborini, and Piero Sensi

Research Laboratories, Gruppo Lepetit S.p.A., via Durando, 38, 20158 Milano, Italy. *August 3, 1973*

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