2-Acetamido-4-hydroxy-6-phenyl-5-pyrimidylpropionaldehyde Diethyl Acetal (XVII).—A solution of 5.0 g. (15.8 mmoles) of VI, 50 ml. of pyridine, and 15 ml. of acetic anhydride was heated in a bath at 85–90° for 1 hr. Solvent was removed by spin-evaporation *in vacuo*. The residue was dissolved in 50 ml. of warm toluene and again spin-evaporated *in vacuo*. The toluene treatment was repeated twice more to remove the last of the pyridine. Recrystallization from benzene gave 3.39 g. (60%) of product, m.p. 133–140°, that was suitable for further transformations. Two further recrystallizations from di-*n*-butyl ether gave white crystals (77% recovery), m.p. 142–144°; λ_{max}^{KBr} 3.12 (NH), 6.05, 6.15, 6.43 (C=O, aromatic double bonds, NH), 9.45 (ether C-O-C); 12.9 μ (phenyl).

Anal. Calcd. for $C_{19}H_{25}N_3O_4$: C, 63.6; H, 6.99; N, 11.7. Found: C, 63.7; H, 6.99; N, 11.6.

2-Acetamido-4-hydroxy-6-phenyl-5-pyrimidylpropionaldehyde (XVIII).—A stirred mixture of 1.00 g. (2.78) mmoles) of XVII and 50 ml. of water was refluxed for 1 hr. after solution took place, solution requiring 15 min. Spin-evaporation to dryness *in vacuo* left 0.747 g. (91%) of product, m.p. 148–152°. Recrystallization from benzene gave white crystals, m.p. 149–152°; yield, 0.689 g. (87%); λ_{max}^{KBr} 3.15 (NH), 5.89 (aldehyde C=O), 6.20 (broad) 6.47, 6.73 (amide C=O, aromatic double bonds, NH), 14.3 (phenyl), no acetal at 9.45 μ.

N-[1-(2-Amino-4-hydroxy-6-phenyl-5-pyrimidyl)-3-propyl]-*p*-aminobenzoyl-L-glutamic Acid (II).—A solution of 500 mg. (1.75 mmoles) of XVIII and 465 mg. (1.75 mmoles) of *p*-aminobenzoyl-L-glutamic acid in 10 ml. of N,N-dimethylformamide was stirred for 20 min., then diluted with 75 ml. of reagent methanol. After the addition of 1.0 g. of sodium borohydride over a period of about 10 min., the reaction mixture was magnetically stirred for 18 hr. To the mixture was added 25 ml. of 0.1 *N* sodium hydroxide, then the solution was spin-evaporated *in vacuo* to about 20 ml. and diluted with 50 ml. of water. The solution was acidified to pH 5 with 3 *N* hydrochloric acid. After being chilled, the mixture was filtered and the product washed with water. The crude material was dissolved in 15 ml. of 3 *N* hydrochloric acid and heated on a steam-bath for 15 min. to hydrolyze most of the contaminating intermediate "anil" that was present. The cooled solution was brought to pH 8 with 10% sodium hydroxide, and the solution was clarified by filtration. The filtrate was acidified to pH 5 with 3 *N* hydrochloric acid; the product was collected by centrifugation and washed successively with four 5-ml. portions each of water, ethanol, and dichloromethane; after being dried overnight *in vacuo*, the product weighed 0.31 g. (36%). The product was further purified by solution in hot N,N-dimethylformamide, addition of water to turbidity, and chilling; the recovery was 68%. For analysis the precipitation from N,N-dimethylformamide was repeated twice more. The compound retains traces of solvent tenaciously; acceptable combustion values were only obtained after drying the sample at 100° under high vacuum. The recovery of material was 60–75% in each reprecipitation. Ultraviolet data showed that the second and third reprecipitations gave material with constants essentially identical with the material obtained in the first reprecipitation from N,N-dimethylformamide. The constants were as follows: $\lambda_{max}^{pH\ 1}$ 226 (ϵ 21,900), 285 mμ (15,300); $\lambda_{max}^{pH\ 7}$ 220 (ϵ 26,900), 298 mμ (19,700); $\lambda_{max}^{pH\ 14}$ 294 mμ (ϵ 20,250); the Bratton-Marshall test showed[11] 2.6% "anil" was present.

Anal. Calcd. for $C_{25}H_{27}N_5O_6$: C, 60.9; H, 5.48; N, 14.2; O, 19.5. Found: C, 61.1; H, 5.46; N, 14.1; O, 19.6.

The Synthesis of Antineoplastic Agents. XXXII. N-Nitrosoureas.[1] I.

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A number of N-nitrosoureas have been synthesized and evaluated for activity against Leukemia L1210. The most active member of the series thus far evaluated, 1,3-bis(2-chloroethyl)-1-nitrosourea, is highly active in a number of other experimental animal tumor systems.

The reported ability of 1-methyl-3-nitro-1-nitrosoguanidine to increase the life span of mice implanted intraperitoneally with Leukemia L1210[2] prompted us to investigate the anticancer activity of the closely related compound, 1-methyl-1-nitrosourea.[3] Although this compound showed only borderline activity against Adenocarcinoma 755 and Sarcoma 180, it proved even more effective, in our hands, against Leukemia L1210 than 1-methyl-3-nitro-1-nitrosoguanidine, increasing the life span by a factor of 2. These significant results caused 1-methyl-1-nitrosourea to be selected, along with other compounds of known activity against L1210 such as amethopterin, for evaluation against L1210 implanted intracerebrally in mice.[5] Of the compounds evaluated in this test system prior to this study, only 1-methyl-1-nitrosourea has shown significant activity.[6] These results stimulated our interest in the preparation of a number of congeners of 1-methyl-1-nitrosourea[7] for screening against experimental animal neoplasms, particularly Leukemia L1210.

Chemistry.—Ureas and N-nitrosoureas of varied structure have been prepared in this continuing study. Table I summarizes the syntheses of those ureas that were used in the preparation of the previously undescribed N-nitrosoureas of Table II; in addition, Table I includes several new ureas, the attempted nitrosations of which have not led thus far to the isolation of pure N-nitroso derivatives. The synthetic procedures used in the preparation of the ureas are adapted from known methods, which are indicated in the footnotes to Table I. Examples of unusual variations of these procedures are described in the Experimental section.

Each of the tabulated nitrosations involved the use of a cold acidic medium and either aqueous sodium nitrite solution of variable concentration or solid sodium

(1) This work was supported by funds from the C. F. Kettering Foundation and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. SA-43-ph-1740. Part XXXI: C. Temple, Jr., C. L. Kussner, and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 866 (1962).

(2) Personal communication from Dr. Howard Bond of the Cancer Chemotherapy National Service Center. See also ref. 4.

(3) B. R. Baker and co-workers have investigated a large number of derivatives of 1-methyl-3-nitro-1-nitrosoguanidine itself.[4]

(4) W. A. Skinner, H. F. Gram, M. O. Greene, J. Greenberg, and B. R. Baker, *J. Med. Pharm. Chem.*, **2**, 299 (1960).

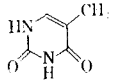
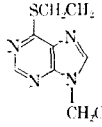
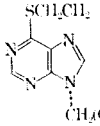
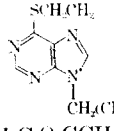
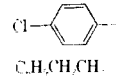
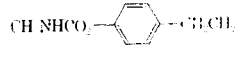
(5) A possible explanation of the failure of leukemias to respond to drug therapy is the sequestering of leukemic cells in the brain where they cannot be reached by drugs that fail to cross the so-called "blood-brain barrier." Compounds effective against experimental animal leukemias implanted in the brain might be of value in the treatment of the human disease.

(6) H. E. Skipper, F. M. Schabel, Jr., M. W. Trader, and J. R. Thomson, *Cancer Res.*, **21**, 1154 (1961).

(7) Degradation studies indicate that streptozotocin, a new broad spectrum antibiotic, contains an N-methyl-N-nitrosoamide or N-methyl-N-nitrosourea function.[8]

(8) E. R. Garrett, *J. Am. Pharm. Assoc. Sci. Ed.*, **49**, 767 (1960).

TABLE I
UREAS

R	R′	Method[a]	Recrystallization solvent	Yield,[b] %	M.p.,[c] °C.	ν (cm.⁻¹) in CO stretching region[d]	Formula	Analyses, % Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
A. Ureas, RNHCONHR′													
CF_3CH_2	H	A	Benzene	57–81	110	1660	$C_3H_5F_3N_2O$	25.35	25.49	3.55	3.63	19.74	20.00
$NCCH_2CH_2$	H	A	Ethanol	50	114	1670	$C_4H_7N_3O$	42.47	42.58	6.24	6.20	37.15	36.76
$C_6H_5CH_2CH_2$	H	B	Water	44	112[e]	1655	$C_9H_{12}N_2O$						
	H	A	Water	75	>250 dec.	1640, 1710	$C_6H_8N_4O_3$	39.13	39.25	4.38	4.67	30.43	29.98
	H	C	Water	30	>220 dec.	1660	$C_8H_{10}N_6OS$	40.32	40.23	4.23	4.23	13.45[f]	13.27[f]
	H	D		81	195	1660	$C_{15}H_{16}N_6OS$	54.87	54.56	4.91	4.99	25.60	25.55
	H	D		88	173	1665, 1650	$C_{12}H_{18}N_6OS$	48.97	48.95	6.17	6.27	28.56	28.79
	H	D	Water	45–100	105	1655	$C_{15}H_{24}N_6OS$	53.55	53.69	7.19	7.19	24.99	24.66
$H_5C_2O_2CCH_2$	CH_3	Ea	Benzene–hexane	50	80–82[g]	1640, 1760	$C_6H_{12}N_2O_3$	44.99	45.09	7.55	7.55	17.94	17.35
$ClCH_2CH_2$	CH_3	[h]	Benzene–pet. ether	38	97[i]	1630	$C_4H_9ClN_2O$						
CF_3CH_2	CH_3	Eb		84	130	1640	$C_4H_7F_3N_2O$	30.80	30.90	4.52	4.44	17.94	17.71
$NCCH_2CH_2$	CH_3	Eb		90	107	1630	$C_5H_9N_3O$	47.23	47.26	7.13	7.12	33.05	32.98
$CH_3CH_2CH_2CH_2$	CH_3	Eb	Water	61–100	72	1635	$C_6H_{14}N_2O$	55.35	55.63	10.84	10.97	21.52	21.58
$(CH_3)_3C$	CH_3	Eb	Benzene	87	145	1625	$C_6H_{14}N_2O$	55.35	55.60	10.84	11.03	21.52	21.92
$CH_3NHCO_2CH_2CH_2$	CH_3	F	Acetonitrile	70	140	1690, 1630	$C_6H_{13}N_3O_3$	41.13	41.31	7.48	7.47	23.99	23.71
$CH_3NHCOSCH_2CH_2$	CH_3	F	Acetonitrile	29–52	124	1660, 1620	$C_6H_{13}N_3O_2S$	37.69	37.87	6.85	7.21	21.98	22.07
C_6H_5	CH_3	Eb	Water	85	151[j]	1640	$C_8H_{10}N_2O$						
p-FC_6H_4	CH_3	Eb	Water	86	181	1640	$C_8H_9FN_2O$	57.13	57.05	5.41	5.39	16.67	16.69
p-ClC_6H_4	CH_3	Eb		70	207	1640	$C_8H_9ClN_2O$	52.04	52.19	4.91	4.91	15.18	15.04
p-HOC_6H_4	CH_3	Eb		90	151	1640	$C_8H_{10}N_2O_2$	57.82	57.79	6.07	6.11	16.86	16.76
p-$CH_3OC_6H_4$	CH_3	Eb		91	164[k]	1640	$C_9H_{12}N_2O_2$						
p-$(CH_3)_2NC_6H_4$	CH_3	Eb	Acetonitrile	53–78	210	1635	$C_{10}H_{15}N_3O$	62.15	62.39	7.82	7.92	21.75	21.60
p-$HOOCC_6H_4$	CH_3	Eb[l]	Water	35	>300[g]	1690, 1650	$C_9H_{10}N_2O_3$	55.66	55.73	5.19	5.26	14.43	14.43
p-$H_5C_2O_2CC_6H_4$	CH_3	Eb		67	178	1640, 1705	$C_{11}H_{14}N_2O_3$	59.45	59.68	6.35	6.45	12.60	12.60
p-$CH_3CONHC_6H_4$	CH_3	Ec[m]	Water	18–45	[n]	1655	$C_{10}H_{13}N_3O_2$	57.96	57.66	6.32	6.38	20.28	20.15
$C_6H_5CH_2$	CH_3	Eb	Water	87	97	1630	$C_9H_{12}N_2O$	65.83	65.53	7.37	7.63	17.06	16.78
	CH_3	Eb	Acetonitrile	89–93	165	1620	$C_9H_{11}ClN_2O$	54.37	54.55	5.58	5.52	14.10	13.71
$C_6H_5CH_2CH_2$	CH_3	Eb	Benzene–hexane	73	80	1620	$C_{10}H_{14}N_2O$	67.38	67.49	7.92	7.96	15.72	15.66
	CH_3	F	Acetonitrile	61–82	175	1710, 1635	$C_{12}H_{17}N_3O_3$	57.35	57.10	6.81	6.70	16.72	16.74

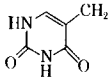
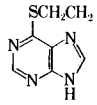
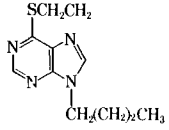
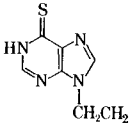
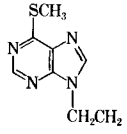
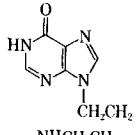
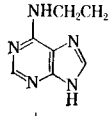
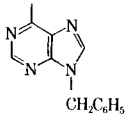
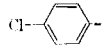
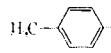
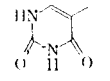
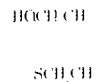
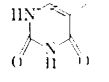
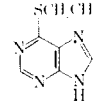
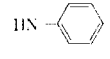
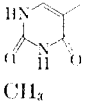
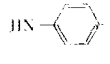
	CH_3	B	Ethanol	11	178	1650	$C_5H_9N_3OS$	37.73	37.77	5.70	5.87	20.15	20.17
	CH_3	Eb	Acetonitrile	57–82	141 dec.	1670	$C_7H_9N_3O$	55.61	55.33	6.00	5.85	27.80	27.80
	CH_3	Eb		60	190	1640	$C_{11}H_{11}N_3O$	65.67	65.65	5.51	5.71	20.88	20.98
	CH_3	B		49	>270	1660, 1640, 1715	$C_6H_8N_4O_3$	38.20[o]	38.53	4.54[o]	4.56	29.70[o]	29.85
	CH_3	Ed[p,m]	Water	63	245–246[g]	1670, 1750, 1630	$C_7H_{10}N_4O_3$	42.42	42.72	5.09	5.26	28.27	27.97
	CH_3	C	Ethanol	24	>230 dec.	1650	$C_9H_{12}N_6OS$	42.85[q]	42.98	4.80	4.91	33.32	32.92
	CH_3	D		72	141	1635	$C_{13}H_{20}N_6OS$	50.64	50.74	6.54	6.52	27.26	27.16
	CH_3	Ed[p,m]	Water	45	220 dec.	1660	$C_9H_{12}N_6OS$	42.28[o]	42.02	4.93[o]	5.19	32.74[o]	33.14
	CH_3	[h]	Acetonitrile	64	218	1635	$C_{10}H_{14}N_6OS$	45.11	45.39	5.30	5.51	31.57	31.69
	CH_3	Ed[p,m]		46	>260	1685, 1640	$C_9H_{12}N_6O_2$	45.76	45.96	5.12	5.10	35.58	35.80
	CH_3	[h]	Water	57	244	1625	$C_9H_{13}N_7O$	45.95	45.71	5.57	5.55	41.68	41.73
	CH_3	[r]	Methanol	68	230	1695	$C_{14}H_{14}N_6O$	59.56	59.57	5.00	5.04	29.77	29.97
$NCCH_2CH_2$	CH_2CH_2CN	Ee[s]				1640	$C_7H_{10}N_4O$						

TABLE I (*Continued*)

R	R'	Method[a]	Recrystallization solvent	Yield,[b] %	M.p.,[c] °C.	$\bar{\nu}$ (cm.$^{-1}$) in CO stretching region[d]	Formula	Analyses, %: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
A. Ureas, RNHCONHR' (*continued*)													
$NCCH_2CH_2$	$CH_2CO_2C_2H_5$	Eb		94	108	1645, 1735	$C_8H_{13}N_3O_3$	48.23	48.05	6.58	6.63	21.10	21.26
$H_5C_2O_2CCH_2$	$CH_2CO_2C_2H_5$	G		35	146–148[g,l]	1755, 1625	$C_9H_{16}N_2O_5$					12.06	12.20
$ClCH_2CH_2$	CH_2CH_2Cl	"	Ethanol	54	127[g]	1620	$C_5H_{10}Cl_2N_2O$						
CF_3CH_2	CH_2CF_3	G		33	157–158[g]	1650	$C_5H_6F_6N_2O$	27.90	27.59	2.25	2.53	12.55	12.18
$ClCH_2CH_2$	$CH_2CO_2C_2H_5$	H		60	106–107[g]	1625, 1750	$C_7H_{13}ClN_2O_3$	40.32	40.19	6.27	6.53	13.42	13.37
$ClCH_2CH_2$	C_6H_5	H[m]	Benzene	74–90	120–121[g,x]	1640	$C_9H_{11}ClN_2O$						
$ClCH_2CH_2$	*p*-C_6H_4Cl	H	Benzene	59	164–165[g,y]	1640	$C_9H_{10}Cl_2N_2O$						
$ClCH_2CH_2$	*p*-$C_6H_4OCH_3$	H		99	160[z]	1630	$C_{10}H_{13}ClN_2O_2$						
		B		93	250	1610	$C_{15}H_{14}Cl_2N_2O$	58.30	58.52	4.56	4.62	23.00[aa]	23.00[aa]
$C_6H_5CH_2CH_2$	$CH_2CH_2C_6H_5$	B	Benzene	75	140[bb]	1615	$C_{17}H_{18}N_2O$	76.08	76.24	7.51	7.53	10.44	10.44
	C_6H_5	Ia	Benzene–cyclohexane	48	108	1640	$C_{16}H_{18}N_2OS$	67.11	67.16	6.34	6.34	9.78	9.62
	$CH_2CO_2C_2H_5$	Ec[w]	Water	60	>300 dec.[g]	1670, 1690, 1715	$C_9H_{12}N_4O_5$	42.19	42.20	4.72	4.86	21.87	21.87
		B	Water	52	>250	1645, 1635, 1710	$C_7H_{10}N_4O_4$	39.25	39.47	4.71	4.64	26.19	25.99
	C_6H_5	Ib	Water	50	>220 dec.	1640	$C_{14}H_{14}N_6OS$	52.78[o]	52.58	4.60[o]	4.76	26.33[o]	26.21
B. Bisureas, R'NHCO-R-CONHR'													
NHNH	CH_3	Ja	Water	27	265[cc]	1665	$C_4H_{10}N_4O_2$						
$NH(CH_2)_2NH$	CH_3	Ja	Acetonitrile	85	232	1625	$C_6H_{14}N_4O_2$	41.37	41.52	8.10	8.27	32.17	32.28
$NH(CH_2)_3NH$	CH_3	Jb	Ethanol	75	210	1625	$C_7H_{16}N_4O_2$	44.66	44.64	8.57	8.32	29.77	29.72
$NH(CH_2)_4NH$	CH_3	K	Benzene–cyclohexane	25	239	1620	$C_8H_{18}N_4O_2$	47.50	47.26	8.97	8.30	27.70	27.61
$NH(CH_2)_5NH$	CH_3	K		35	207	1620	$C_9H_{20}N_4O_2$	40.99	50.06	9.32	9.25	25.91	25.93
$NH(CH_2)_5NH$	H	K		67	242	1660	$C_7H_{16}N_4O_2$	44.66	44.90	8.57	8.68		
$NH(CH_2)_5NH$	C_2H_5	L		18–29	192 dec.	1625	$C_{11}H_{24}N_4O_2$	54.07	54.29	9.90	9.92	22.93	22.81
$NHCH_2CH_2SSCH_2CH_2NH$	CH_3	Ja, K	Water	38, 41	155	1620	$C_8H_{18}N_4O_2S_2$	36.09	36.03	6.81	6.80	21.04	20.84
	CH_3	Jc	Ethanol	83	>250	1635	$C_8H_{16}N_4O_2$	47.98	47.90	8.05	7.45	27.98	27.66
	CH_3	Ja	Methanol	61	>250	1635	$C_{10}H_{14}N_4O_2$	54.04	54.16	6.35	6.09	25.21	25.16
$HN(CH_2)_3NH$		L		48	>200 dec.	1640, 1720	$C_{15}H_{16}N_8O_6$	41.05	41.06	4.24	4.33	29.46	28.90
	CH_3	Jd[m]		81	245 dec.	1665	$C_{10}H_{14}N_4O_4S$	41.37	41.53	8.10	8.27	32.17	32.28
C. Trisubstituted Ureas													
$CH_3NHCON(CH_3)_2$		Ma	[dd]	55	72–74[g,ee]	1640	$C_4H_{10}N_2O$						
$CH_3NHCON(CH_2CH_2OH)_2$		Mb		91	62–64[g]	1625	$C_6H_{14}N_2O_3$	44.43	44.25	8.70	8.61	17.26	16.97
$CH_3NHCON(CH_2CH_2CN)_2$		Mb		87	64–65[g]	1635	$C_8H_{12}N_4O$	53.32	53.02	6.71	6.61	31.09	30.82
$ClCH_2CH_2NHCON(CH_3)_2$		[ff]	Benzene–hexane	17	86	1635	$C_5H_{11}ClN_2O$	39.90	40.10	7.37	7.45	23.58[cc]	23.40[cc]

	Ether	26	85–86[gg]	1685	$C_3H_5ClN_2O$	40.41	40.60	6.10	6.24	18.86	18.58
	Et$_2$O	50–98	83–84[g]	1625	$C_7H_{13}N_3O$	53.48	53.29	9.62	9.74	26.73	26.62

[a] Typical procedures given in Experimental section: A, $RNH_2{\cdot}HCl + KNCO$ in H_2O; B, $RNH_2 + R'NHCON(NO)CH_3$ in H_2O (*cf.* ref. 10); C, RCl + $HSCH_2CH_2NHCONHR'$ in $HCON(CH_3)_2$ containing K_2CO_3; D, RSH + $ClCH_2CH_2NHCONHR'$ in $HCON(CH_3)_2$ containing K_2CO_3; E, $RNH_2 + CH_3NCO$ in (a) C_6H_6, (b) $CHCl_3$, (c) $HCON(CH_3)_2$, (d) H_2O, (e) Et_2O; F, amino alcohol, thiol, or phenol + $2CH_3NCO$ in $CHCl_3$ containing Et_3N; G, $2RNHCON(NO)CH_3 + H_2O \rightarrow RNHCONHR'$ (R′ = R); H, $R'NHCONH_2 + HCl$ in H_2O; I, $R'NHCONH_2 + ArSH$ in (a) C_6H_6, (b) $HCON(CH_3)_2$; J, diamine + $2\ CH_3NCO$ in (a) $CHCl_3$, (b) Et_2O, (c) H_2O, (d) $HCON(CH_3)_2$; K, diamine + $2CH_3N(NO)CONHR'$ in H_2O; L, $R'NH_2 + CH_3N(NO)CONH(CH_2)_nNHCON(NO)CH_3$ in H_2O; M, $CH_3NCO + R_2NH$ in (a) H_2O, (b) $CHCl_3$. [b] Based on pure compound isolated; a range indicates yields of pure and crude products having favorable melting point or spectral comparison. [c] Taken on Kofler Heizbank unless otherwise indicated. [d] Major bands, 1615–1750 cm.$^{-1}$, in order of intensity. [e] Lit. m.p. 113–114° [S. L. Shapiro, V. A. Parrino, and L. Freedman, *J. Am. Chem. Soc.*, **81**, 2220 (1959)]. [f] % S. [g] Capillary. [h] See Experimental section. [i] Lit. m.p. 95–96° [A. F. McKay, *Can. J. Chem.*, **31**, 284 (1953)]. [j] Lit.[10] m.p. 150.5–151.5°. [k] Lit. m.p. 164° [J. W. Boehmer, *Rec. trav. chim.*, **55**, 379 (1936)]. [l] Suspension treated with 8-fold excess CH_3NCO. [m] With Et_3N as catalyst. [n] Dec. >280° without melting. [o] As 0.25 hydrate. [p] Hydrochloride neutralized with NaOH. [q] % S: calcd., 12.71; found, 12.41. [r] From 9-benzyladenine and excess CH_3NCO in stainless steel bomb at 100° ove night [*cf.* G. Huber, *Angew. Chem.*, **69**, 642 (1957)]. [s] A. F. McKay, G. Y. Paris, and D. L. Garmaise, *J. Am. Chem. Soc.*, **80**, 6276 (1958). [t] Lit.[11c] m.p. 137°. [u] Method of ref. 25. [v] Lit.[25] m.p. 127°. [w] Y. Iwakura and A. Nabeya, *Nippon Kagaku Zasshi*, **77**, 773 (1956) (yield 42%). [x] Lit.[22] m p. 124°. [y] Lit. m.p. 175° [H. Najer, P. Chabrier, and R. Giudicelli, *Bull. soc. chim. France*, 352 (1959)]. [z] Lit. m.p. 158–161° [A. F. McKay, G. W. Hatton, and G. W. Taylor, *J. Am. Chem. Soc.*, **75**, 1120 (1953)]. [aa] % Cl. [bb] Lit. m.p. 138.5° [T. Curtius and H. Jordan, *J. prakt. Chem.*, [2] **64**, 308 (1901)]. [cc] Lit. m.p. 260° [C. Vogelsang, *Rec. trav. chim.*, **62**, 5 (1943)]. [dd] Dried by evaporation of EtOH solution and triturated in Et_2O. [ee] Lit. m.p. 74° [A. P. N. Franchimont, *Rec. trav. chim.*, **3**, 216 (1884)]. [ff] $(CH_3)_2NCOCl + HN\triangleleft$ in $CHCl_3$ (*cf.* ref. 25).

nitrite. Thus, these nitrosations can be roughly classified according to the medium used as designated in Table II, and typical procedures are described in the Experimental section. The variables were manipulated so that the N-nitrosoureas precipitated essentially pure; analytical samples were then obtained by appropriate washing and drying without recrystallization. Several simple 1-alkyl-1-nitrosoureas not included in Table II but screened against Leukemia L1210 have been described previously.[9]

All of the N-nitrosoureas described are characterizable solids, most of which appear to be stable when kept cold and dry. 3-(2-Cyanoethyl)-1-methyl-1-nitrosourea proved to be exceptionally unstable; separately prepared samples of this compound decomposed spontaneously, one within a few hours and the other within a few weeks, to a discolored semisolid mixture that showed a prominent NCO band in the infrared. Several ureas gave relatively unstable liquid nitroso derivatives, none of which was obtained pure and some of which decomposed during isolation (for example, 1-*t*-butyl-3-methyl-N-nitrosourea). The formation of oils from unsymmetrical 1,3-disubstituted ureas where solids were expected might in itself suggest nonselective nitrosation, but the analytically pure solids isolated have shown no evidence of random nitrosation.

Experience in this area indicates that in order to be nitrosated a urea nitrogen must possess a certain degree of nucleophilicity: for example, diethyl N,N′-carbonyldiglycinate resists nitrosation under the conditions employed, whereas 1,3-bis(2-chloroethyl)urea is readily nitrosated under similar conditions. Therefore, ethyl 5-(2-chloroethyl)hydantoate would be expected to give ethyl 5-(2-chloroethyl)-5-nitrosohydantoate (I) and not the 3-nitroso derivative. By such reasoning the structure of many of the compounds of Table II can be rationalized. Others, such as the 1,1′-polymethylenebis(3-methyl-3-nitrosoureas), require more rigorous proof. Thus, the interaction of 1,1′-pentamethylene-

$$ClCH_2CH_2N(NO)CONHCH_2CO_2C_2H_5$$

I

bis(3-methyl-3-nitrosourea) (II) and ethylamine in boiling water gave, as the only product isolated, 1,1′-pentamethylenebis(3-ethylurea) identical with an authentic sample prepared from 1,5-pentanediamine and ethyl isocyanate. This experiment was modeled after a

$$CH_3N(NO)CONHCH_2CH_2CH_2CH_2CH_2NHCON(NO)CH_3$$

II

published urea synthesis,[10] and the formation of the bisethylurea can be regarded as resulting from the addition of ethylamine to pentamethylene diisocyanate generated *in situ*. The alternative dinitroso structures would have given 1-ethyl-3-methylurea, 1-ethyl-3-(5-hydroxypentyl)urea, or a mixture of the two.

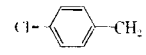
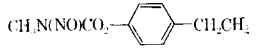
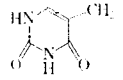
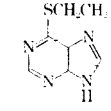
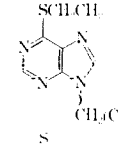
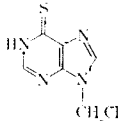
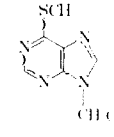
The formation of symmetrical 1,3-disubstituted ureas by the action of water on an isocyanate is well known.[11]

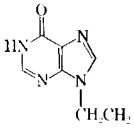
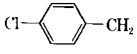
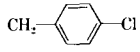
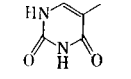
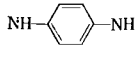
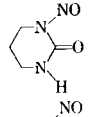
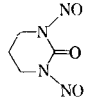
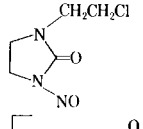
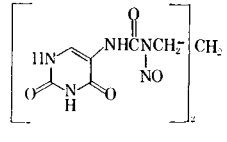
(9) (a) E. A. Werner, *J. Chem. Soc.*, **115**, 1093 (1919); (b) J. Marx and L. Marx-Moll, *Chem. Ber.*, **87**, 1499 (1954).

(10) J. L. Boivin and P. A. Boivin, *Can. J. Chem.* **29**, 478 (1951).

(11) (a) J. H. Saunders and R. J. Slocombe, *Chem. Rev.*, **43**, 203 (1948); (b) R. G. Arnold, J. A. Nelson, and J. J. Verbanc, *ibid.*, **57**, 47 (1957); (c) W. J. Humphlett and C. V. Wilson, *J. Org. Chem.*, **26**, 2507 (1961).

TABLE II
N-NITROSOUREAS

R	R′	Nitrosation[a] Urea, mmoles	Medium: Acid, ml.	Medium: Water, ml.	Agent: $NaNO_2$, mmoles	Agent: Water, ml.	Yield, %	M.p.,[b] °C.	$\bar{\nu}$ (cm.$^{-1}$) in CO stretching region[c]	Formula	Analyses, %: Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found
					A. N-Nitrosoureas, $RNHCON(NO)R'$											
H	$CH_2CH_2C_6H_5$	3.1	Formic, 5	5	6.1	5	59	100 dec.	1745	$C_9H_{11}N_3O_2$	55.95	56.24	5.74	5.58	21.75	21.64
$ClCH_2CH_2$	CH_3	36	Formic, 10	100	90	35	74	65	1710, 1725	$C_4H_8ClN_3O_2$	29.03	28.91	4.87	4.99	25.40	25.22
CF_3CH_2	CH_3	6.4	Formic, 2	10	13	5	30	33–34[d]	1735	$C_4H_6F_3N_3O_2$	25.93	25.81	3.27	3.59	22.72	22.63
	CH_3	25	Formic, 100	0	75	15	79	115	1705	$C_9H_{10}ClN_3O_2$	47.55	47.75	4.43	4.42	18.45	18.09
$NCCH_2CH_2$	CH_3	4	Formic, 1	10	10	5	70	86 dec.	1725	$C_5H_8N_4O_2$	38.46	38.61	5.16	5.28	35.88	35.80
$C_6H_5CH_2CH_2$	CH_3	29	Formic, 40	50	60	20	84	58	1715	$C_{10}H_{13}N_3O_2$	57.96	58.02	6.32	6.15	20.28	20.27
$CH_3N(NO)CO_2CH_2CH_2$	CH_3	34	Formic, 50	0	171	30	66	70 dec.	1725, 1745	$C_6H_{11}N_5O_5$	30.90	31.22	4.75	4.91	30.04	29.86
C_6H_5	CH_3	45	Formic, 70	0	90	35	75	95 dec.	1720	$C_8H_9N_3O_2$	53.62	53.59	5.06	5.05	23.45	23.50
p-FC_6H_4	CH_3	44	Formic, 130	100	100	40	78	114 dec.	1740	$C_8H_8FN_3O_2$	48.71	48.66	4.09	4.13	21.28	21.16
p-ClC_6H_4	CH_3	27	Formic, 100	0	50	10	86	142 dec.	1730	$C_8H_8ClN_3O_2$	45.00	44.76	3.78	4.11	19.73	19.54
p-$CH_3OC_6H_4$	CH_3	28	Formic, 75	0	110	45	81	120 dec.	1715	$C_9H_{11}N_3O_3$	51.67	51.81	5.30	5.45	20.09	19.91
p-$HO_2CC_6H_4$	CH_3	21	Formic, 130	0	100	25	100	191 dec.	1680, 1740	$C_9H_9N_3O_4$	48.43	48.30	4.06	3.96	18.83	18.51
p-$H_5C_2O_2CC_6H_4$	CH_3	22.5	Formic, 80	10	50	20	92	120 dec.	1690, 1725	$C_{11}H_{13}N_3O_4$	52.58	52.65	5.22	5.39	16.73	16.78
	CH_3	24.5	Formic, 50	0	100	50	91	140 dec.	1710, 1750	$C_{12}H_{15}N_5O_5$	46.60	46.94	4.89	5.03	22.65	22.52
	CH_3	19	*N* HCl, 120	0	40	20	92	143 dec.	1725	$C_{11}H_{10}N_4O_2$	57.38	57.20	4.38	4.43	24.30	24.20
	CH_3	23	Formic, 550	0	57	40	83	215 dec.	1670, 1735	$C_6H_7N_5O_4$	33.81	33.86	3.31	3.32	32.86	32.80
	CH_3	2.5	Formic, 5	0	5	10	65	200 dec.	1680, 1715, 1750	$C_7H_9N_5O_4$	37.01	37.26	3.99	4.01	30.83	30.64
	CH_3	13	Acetic, 15	100	50	10	92	175 dec.	1715	$C_9H_{10}N_7O_2S$	38.43	38.23	3.94	4.03	34.87	34.59
	CH_3	30	*N* HCl, 200	0	70	15	81	100	1730	$C_{13}H_{19}N_7O_2S$	46.28	46.31	5.68	5.59	9.50	9.50
	CH_3	2	*N* HCl, 20	0	4	5	91	200 dec.	1720	$C_9H_{11}N_7O_2S$	38.43	38.16	3.94	4.14	34.87	34.78
	CH_3	1.9	*N* HCl, 20	0	4	5	90	165 dec.	1725	$C_{16}H_{15}N_7O_2S$	40.68	40.56	4.43	4.21	33.21	33.42

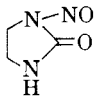
	CH_3	25	Formic, 50	0	60	10	57	200 dec.	1685, 1730	$C_9H_{11}N_7O_3$	40.75	40.83	4.18	4.14	36.97	36.92
	CH_3	12	*N* HCl, 45	0	25	20	88	193 dec.[d]	1650, 1715	$C_9H_{12}N_8O_2 \cdot HCl$	36.00[e]	35.89	4.37	4.69	37.36	37.39
$ClCH_2CH_2$	CH_2CH_2Cl	44	Formic, 50	0	100	60	71	30–32[d]	1725	$C_5H_9Cl_2N_3O_2$	28.06[f]	28.01	4.24	4.29	19.64	19.45
$H_5C_2O_2CCH_2$	CH_2CH_2Cl	2.4	Formic, 5	10	5	5	61	100–101[d]	1735, 1710	$C_7H_{12}ClN_3O_4$	35.39	35.46	5.09	5.03	17.66	17.78
C_6H_5	CH_2CH_2Cl	2.5	Formic, 17	10	6	5	61	75[d,g]	1725, 1695	$C_9H_{10}ClN_3O_2$	47.61	47.59	4.40	4.42	18.45	18.48
p-ClC_6H_4	CH_2CH_2Cl	2.2	Formic, 20	0	6	5	89	95	1730	$C_9H_9Cl_2N_3O_2$	41.26	41.22	3.47	3.49	27.10[h]	26.90[h]
p-$CH_3OC_6H_4$	CH_2CH_2Cl	2.2	Formic, 20	0	6	5	61	95 dec.	1705, 1735	$C_{10}H_{12}ClN_3O_2$	46.60	46.28	4.70	5.01	16.30	16.38
$NCCH_2CH_2$	CH_2CH_2CN	6	Formic, 20	40	12	20	60	110 dec.	1735	$C_7H_9N_5O_2$	43.07	43.13	4.65	4.86	35.89	36.25
		1.6	Formic, 30	0	30	0	93	140	1705	$C_{15}H_{13}Cl_2N_3O_2$	53.27	53.48	3.87	3.82	21.00	20.90
	CH_2CH_2OH	2.3	Formic, 20	0	6	3	56	170 dec.	1670, 1735	$C_7H_9N_5O_5$	34.57	34.72	3.73	3.64	28.80	28.64
B. N,N''-Dinitrosobisureas, R'N(NO)CO—R—CON(NO)R'																
NHNH	CH_3	40	Formic, 100	0	160	50	44	142 dec.	1710, 1750	$C_4H_8N_6O_4$	23.50	23.70	3.95	3.91	41.17	41.29
$NH(CH_2)_2NH$	CH_3	17	Acetic, 4.5	25	69	10	50	130 dec.	1705, 1740	$C_6H_{12}N_6O_4$	31.03	31.56	5.21	5.25	36.20	36.01
$NH(CH_2)_3NH$	CH_3	2.7	Acetic, 1	20	11	5	40	*ca.* 100 dec.[i]	1705	$C_7H_{14}N_6O_4$	34.14	34.16	5.73	5.80	34.13	34.39
$NH(CH_2)_4NH$	CH_3	61.4	Acetic, 68	1350	242	300	67	>110 dec.[i]	1715	$C_8H_{16}N_6O_4$	36.92	37.09	6.20	6.46	32.29	32.05
$NH(CH_2)_5NH$	CH_3	23	Formic, 50	40	50	10	79	110 dec.	1710	$C_9H_{18}N_6O_4$	39.41	39.31	6.62	6.62	30.78	30.78
$NH(CH_2)_2SS(CH_2)_2NH$	CH_3	10.5	Acetic, 28	110	45	10	84	*ca.* 100 dec.[i]	1700	$C_8H_{16}N_6O_4$	29.62	29.90	4.98	5.15	25.94	25.62
	CH_3	25	Acetic, 6	50	100	20	65	131 dec.	1705	$C_8H_{14}N_6O_4$	37.21	37.50	5.46	5.12	32.55	32.69
	CH_3	23	Formic, 100	0	113	30	95	>250 dec.	1710	$C_{10}H_{12}N_6O_4$	42.85	42.56	4.32	4.22	29.99	29.89
C. Miscellaneous N-Nitrosoureas																
		50	*N* HCl, 50	0	50	0	21	105 dec.	1700, 1725	$C_4H_7N_3O_2$	37.21	37.37	5.46	5.46	32.55	32.87
		50	6 *N* HCl, 50	0	300	0	76	133–135 dec.[d]	1750	$C_4H_6N_4O_3$	30.38	30.52	3.82	3.84	35.44	35.53
		6.7	*N* HCl, 7	0	7	5	73	75	1750	$C_5H_8ClN_3O_2$	33.81	33.97	4.53	4.53	19.90	20.00
		1.3	Formic, 50	0	26	0	61	190 dec.	1680, 1750	$C_{13}H_{14}N_{10}O_8$	35.52	35.63	3.21	3.17	31.86	31.67

[a] A solution or suspension of the urea in the medium indicated is treated cold (0–5°) with sodium nitrite either solid or in aqueous solution; after 0.5–2 hr. the precipitate is washed with water and dried *in vacuo;* several typical procedures are described in the Experimental section. [b] Taken on a Kofler Heizbank unless indicated otherwise; "dec." denotes relatively rapid decomposition before or during melting. [c] Major bands, 1650–1750 cm.$^{-1}$, in order of intensity. [d] Capillary. [e] Calcd. Cl, 11.80; found, 12.0. [f] Calcd. Cl, 33.14; found, 33.20. [g] Dec. 80°. [h] %Cl. [i] Indef.

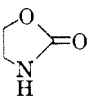
and therefore it is not surprising that isocyanates generated by the action of water on appropriate N-methyl-N-nitrosoureas also give ureas whether an amine is added or not. For example, 1,3-bis(2,2,2-trifluoroethyl)urea was formed according to the following over-all equation.

$$2CF_3CH_2NHCON(NO)CH_3 + H_2O \rightarrow (CF_3CH_2NH)_2CO + 2CH_3OH + 2N_2 + CO_2$$

When 1-methyl-1-nitroso-3-phenethylurea was allowed to decompose in boiling water in the absence of phenethylamine and in its presence, 1,3-bisphenethylurea was formed in yields of 77 and 84%, respectively. These results are compelling evidence for the structure of the nitroso compound, which might have been considered ambiguous in view of the facile nitrosation of phenethylurea. In the attempted preparation of 1,3-bis(2-hydroxyethyl)urea by the hydrolytic decomposition of 1-nitroso-2-imidazolidinone (III) in the presence of 2-aminoethanol, the only product isolated was 2-oxazolidinone (IV), whose infrared absorption spectrum is characterized by a CO band at 1735 cm.$^{-1}$; this is an unusual example of an N-nitrosourea decomposition in which both the hydroxyl and isocyanato functions are retained in the intermediate 2-hydroxyethyl isocyanate.



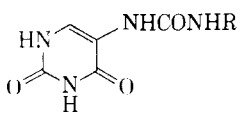
III



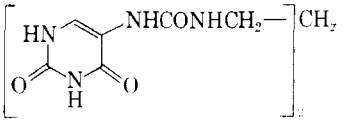
IV

During the course of preparation of N-nitrosoureas having heterocyclic carrier groups of biologic importance, some observations of interest were made in the preparation of intermediates.

5-Aminouracil responded well to reaction with 1,3-dimethyl-1-nitrosourea to give 1-methyl-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea (V) and with 1,1′-trimethylenebis[3-methyl-3-nitrosourea] to give the corresponding bisurea VI. The nitroso derivative of V, when allowed to react with 2-aminoethanol in water, afforded the hydroxyethylurea VII.

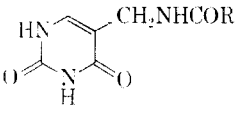


V, R=CH_3
VII, R=CH_2CH_2OH

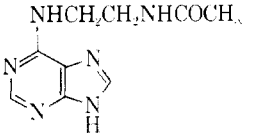


VI

In order to prepare the thymine analog IX in quantity, a new synthesis of the intermediate 5-(aminomethyl)uracil hydrochloride was developed. This involved amidomethylation of uracil with paraformaldehyde and acetonitrile in a mixture of acetic and sulfuric acids,[12] which gave yields up to 34% of N-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinylmethyl)acetamide (VIII). The hydrolysis of VIII in hydrochloric acid gave a good yield of a product that was identical with the 5-(aminomethyl)uracil hydrochloride prepared by a previously described procedure,[13] which in our hands was unadaptable to a large scale. A similar hydrolysis of N-(2-purin-6-ylaminoethyl)acetamide [N, from 6-chloropurine and N-(2-aminoethyl)acetamide] provided N^6-(2-aminoethyl)adenine as an uncharacterized hydrochloride, which was converted to the desired methylurea – a method which circumvents the mixture of products encountered in the previously described reaction of 6-chloropurine with ethylenediamine.[14]

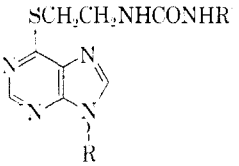


VIII, R = CH_3
IX, R = $NHCH_3$



X

1-Methyl-3-[2-(purin-6-ylthio)ethyl]urea (XI) was prepared in two ways, neither of which afforded good yields: (1) from 6-chloropurine and 1-(2-mercaptoethyl)-3-methylurea in N,N-dimethylformamide containing potassium carbonate and (2) from purine-6(1H)-thione and 1-(2-chloroethyl)-3-methylurea under the same conditions. In contrast, a good yield of 1-[2-(9-butyl-9H-purin-6-ylthio)ethyl]-3-methylurea (XII) was obtained from 9-butyl-9H-purine-6(1H)-thione. The preparation of the phenylurea XIII was accomplished by an unusual opening of 1-aziridinecarboxanilide by purine-6(1*H*)-thione in N,N-dimethylformamide.



XI, R = H, R′ = CH_3
XII, R = n-C_4H_9, R′ = CH_3
XIII, R = H, R′ = C_6H_5

The use of N-(2-aminoethyl)acetamide again provided useful protection of an amino group in the synthesis of 1-[2-(6-mercapto-9H-purin-9-yl)ethyl]-3-methylurea (XIX), its S-methyl derivative XX, and 1-[2-(6-hydroxy-9H-purin-9-yl)ethyl]-3-methylurea (XXI). The hydrochloric acid-catalyzed purine ring closure of N-{2-[(5-amino-6-chloro-4-pyrimidinyl)-amino]ethyl}acetamide (XIV) with ethyl orthoformate produced the key intermediate XV. The action of thiourea on XV, followed by mild basic hydrolysis, gave the acetamide XVI, which was hydrolyzed in hydrochloric acid to give 9-(2-aminoethyl)-9H-purine-6(1H)-thione dihydrochloride (XVII). Direct hydrolysis of XV by hydrochloric acid produced the hypoxanthine dihydrochloride XVIII. The reaction of neutralized aqueous solutions of these amine hydrochlorides with methyl isocyanate in the presence of triethylamine afforded the desired methylureas. Methylation of XIX afforded 1-methyl-3-{2-[6-(methylthio)-9H-purin-9-yl]ethyl}urea (XX).

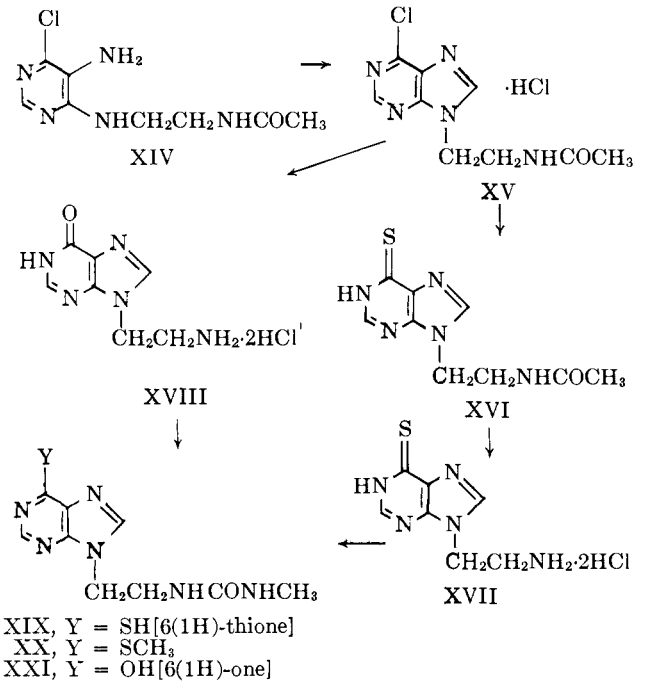
An intramolecular condensation of 1,3-bis(2-chloroethyl)urea was accomplished with sodium ethoxide in ethanol, and the product was identical with authentic 1-(2-chloroethyl)-2-imidazolidinone (XXII), which was prepared by a previously reported method.[15] The re-

(12) Cf. the amidomethylation of benzene derivatives [C. L. Paris and R. M. Christenson, *J. Org. Chem.*, **25**, 1888 (1960)].

(13) J. H. Burckhalter, R. J. Seiwald, and H. C. Scarborough, *J. Am. Chem. Soc.*, **82**, 991 (1960).

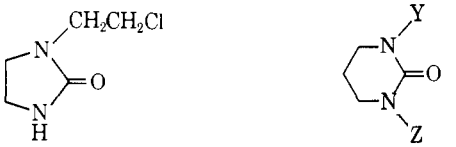
(14) H. Leuteritz and H. Ballweg, *Ann. Chem.*, **649**, 124 (1961).

(15) A. F. McKay, W. G. Hatton, and R. O. Brown, *J. Am. Chem. Soc.* **78**, 6144 (1956).



XIX, Y = SH[6(1H)-thione]
XX, Y = SCH_3
XXI, Y = OH[6(1H)-one]

ported nitrosations of 2-imidazolidinone[16] were extended to the preparation of 1-nitroso- and 1,3-dinitrosotetrahydro-2(1H)-pyrimidinones XXIII and XXIV.



Prominent infrared absorption bands in the carbonyl stretching region are recorded in Tables I and II for the ureas and the respective N-nitrosoureas. Comparisons reveal that nitrosation of urea functions always results in a shift toward higher wave numbers in the carbonyl region. The nitrosations of 2-imidazolidinone caused the most pronounced effect observed: 2-imidazolidinone itself absorbs at 1665 cm.$^{-1}$; the 1-nitroso derivative, at 1760 and 1730 cm.$^{-1}$; and the 1,3-dinitroso derivative, at 1795 cm.$^{-1}$

Experimental[17]

[(1,2,3,4-Tetrahydro-2,4-dioxo-5-pyrimidinyl)methyl]urea. Method A.—Potassium cyanate (183 mg., 2.26 mmoles) was added to a solution of 5-(aminomethyl)uracil hydrochloride (400 mg., 2.25 mmoles), and the resulting solution was refluxed for 1 hr. Removal of the solvent under reduced pressure left a white residue, which was twice recrystallized from water (10 ml.). The yield of the urea, dried at 100° *in vacuo*, was 308 mg. (75%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 262 (5.57) at pH 1, 262 (5.55) at pH 7, 286 (4.51) at pH 13.

1-Methyl-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)-urea (V). Method B.-A mixture of 5-aminouracil (4.4 g., 0.035 mole), 1,3-dimethyl-1-nitrosourea (4.5 g., 0.038 mole), and water (400 ml.) was heated under reflux for 2 hr. The solution was then concentrated under reduced pressure to 350 ml., treated hot with decolorizing carbon, and filtered. The cooled filtrate deposited a solid, which was suspended in 0.1 *N* hydrochloric acid (15 ml.) and stirred 15 min. The product was then washed with water and dried *in vacuo* over phosphorus pentoxide at 60° overnight; yield of V as a one-quarter hydrate, 2.5 g. (39%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 233 (sh) and 272 (5.84) at pH 1, 233 (sh) and 272 (5.85) at pH 7, 287 (7.13) at pH 13.

On a larger scale, this procedure gave a 49% yield.

1-(2-Hydroxyethyl)-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea (VII). Method B.—A mixture of 1-methyl-1-nitroso-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea (4.60 g., 0.0216 mole), 2-aminoethanol (1.27 ml., 0.0216 mole), and water (100 ml.) was heated under reflux for 2 hr. The reaction mixture diluted with water (1 l.) was heated to boiling, and a small amount of insoluble material was removed by filtration. The white solid deposited in the filtrate after two days at room temperature was washed with water and dried *in vacuo* at 100° overnight; yield of VII, 2.4 g. (52%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 273 (12.1) at pH 1, 273 (12.1) at pH 7, 286 (14.3) at pH 13.

1-Methyl-3-[2-(purin-6-ylthio)ethyl]urea (XI). Method C.—A solution of commercial 2-aminoethanethiol hydrochloride (13.0 g., 0.0116 mole) in methanol (90 ml.) was treated with a solution of sodium methoxide (5.5 g., 0.10 mole) in methanol (110 ml.), a slight excess of the hydrochloride being requisite. The solvent was removed under reduced pressure with care being taken not to volatilize the free base. The residue was extracted with two 50-ml. portions of warm chloroform, and the chloroform extract was filtered, cooled (0–5°), and treated with methyl isocyanate[18] (6.6 ml., 0.10 mole). After 15 min. at room temperature, the solvent was removed under reduced pressure leaving crude 1-(2-mercaptoethyl)-3-methylurea (13.0 g., 93%) as a pale yellow oil.

A well stirred mixture of crude 1-(2-mercaptoethyl)-3-methylurea (13.0 g.), 6-chloropurine (13.8 g., 0.0895 mole), potassium carbonate (12.4 g., 0.0898 mole), and N,N-dimethylformamide (50 ml.) was heated at 80–90° for 2 hr. The reaction mixture was evaporated to dryness *in vacuo*, and the semisolid residue was extracted with three 50-ml. portions of hot ethanol. Concentration and cooling of the combined extracts produced two crops of crude XI, m.p. 230°, totaling 2.95 g. Additional product (3.4 g., m.p. 232°) was obtained from the mother liquor by evaporation to dryness *in vacuo* and precipitation of the residual solid from water solution (30 ml.) at pH 7. Recrystallization of the combined crops from ethanol and then from water afforded 5.33 g. (23.5%) of XI as a white solid (dried *in vacuo* at 100°); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 293 (13.9) at pH 1, 290 (16.1) at pH 7, 291 (14.3) at pH 13.

1-Methyl-1-nitroso-3-[2-(purin-6-ylthio)ethyl]urea.—A suspension of 1-methyl-3-[2-(purin-6-ylthio)ethyl]urea (XI) (3.2 g., 0.013 mole) in acetic acid (15 ml.) was diluted with water (100 ml.). The suspension was maintained at 0–5° by means of an ice bath and stirred, while an aqueous solution (10 ml.) of sodium nitrite (3.5 g., 0.051 mole) was added dropwise. Stirring was continued at 5° for 2 hr. The yellow product was washed with water (20 ml.) and dried *in vacuo;* yield, 3.3 g. (92%).

3-[2-(9-Butyl-9*H*-purin-6-ylthio)ethyl]-1-methylurea (XII). Method D.—A well stirred mixture of 9-butyl-9H-purine-6(1H)-thione[19] (10.0 g., 48.0 mmoles), potassium carbonate (6.9 g., 50 mmole), and N,N-dimethylformamide (70 ml.) was heated to 80°, and 1-(2-chloroethyl)-3-methylurea (6.84 g., 50.0 mmoles) was added in small portions. After 2 hr. at 80–90°, the mixture was cooled and diluted with water (250 ml.). The white solid that formed was washed with cold water and dried *in vacuo* at 100°; yield of XII, 10.6 g. (72%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 220 (11.5), 294 (16.8) at pH 1; 222 (12.0), 286 (18.3), 293 (18.3) at pH 7; 286 (18.3), 293 (18.3) at pH 13.

Ethyl 5-(2-Cyanoethyl)hydantoate. Method E.—Ethyl isocyanatoacetate[20] (5.0 ml., 0.043 mole) was slowly added to a cooled solution of 3-aminopropionitrile[21] (3.0 g., 0.043 mole) in

(16) (a) J. G. Michels, U. S. Patent 2,776,979 (1957); (b) A. F. McKay, W. R. R. Park, and S. J. Viron, *J. Am. Chem. Soc.*, **72**, 3659 (1950); (c) M. W. Kirkwood and G. F. Wright, *ibid.*, **76**, 1836 (1954).

(17) Melting points were determined on a Kofler Heizbank unless indicated otherwise; capillary melting points are uncorrected. Ultraviolet absorption spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer. Infrared absorption spectra were determined in pressed potassium bromide disks with a Perkin-Elmer Model 221 spectrophotometer. All N-nitrosoureas prepared were stored cold and dry.

(18) The Ott Chemical Co., Muskegon, Michigan.

(19) J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **80**, 409 (1958).

(20) Distillation Products Industries, Rochester 3, New York.

(21) S. R. Buc, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 93.

chloroform (100 ml.), and the solution was stirred at room temperature overnight. The solvent was evaporated under reduced pressure, and the resulting white crystalline residue was suspended in benzene, collected on a filter, and dried *in vacuo;* yield, 8.0 g. (94%).

***p*-[2-(3-Methylureido)ethyl]phenyl Methylcarbamate. Method F.**—Methyl isocyanate[18] (0.51 ml., 8.0 mmoles) was added to a cooled, stirred solution of tyramine (500 mg., 3.65 mmoles) in chloroform (20 ml.) containing triethylamine (5 ml.). Stirring was continued at 20° for 1 hr. and then at 35° for 1 hr. The white solid that formed was collected from the cooled reaction mixture and recrystallized from acetonitrile (30 ml.) as white needles; the yield of the vacuum-dried methylcarbamate was 564 mg. (61%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 264 (5.00) and 271 (4.27) in ethanol.

1,3-Bis(phenethyl)urea. Method G.—A stirred mixture of 1-methyl-1-nitroso-3-phenethylurea (1.0 g., 5.8 mmoles) and water (20 ml.) was heated under reflux for 1.5 hr. The precipitate that formed was collected at room temperature, washed with water, and air-dried; yield, 0.61 g. (77%).

The analytical sample was recrystallized from benzene as colorless plates, m.p. 140°.

Diethyl N,N'-Carbonyldiglycinate. Method G.—An aqueous solution (5 ml.) of sodium nitrite (1.7 g., 25 mmoles) was added dropwise to a stirred, cold (0–5°) solution of ethyl 5-methylhydantoate (1.00 g., 6.25 mmoles) in water (20 ml.) containing formic acid (1.5 ml.). After 30 min., the supernatant solution was decanted from the oily nitroso compound that had formed. The oil, washed with cold water (5 ml.), was suspended in water (10 ml.) and the mixture gradually heated. After 30 min. at 80° and 5 min. at 100°, the resultant solution was treated with Norit and filtered. The cooled filtrate deposited the carbonyldiglycinate as colorless needles, which were dried *in vacuo* at 76°; yield, 250 mg. (35%).

1-(2-Chloroethyl)-3-(*p*-methoxyphenyl)urea. Method H.—A solution of *p*-methoxyphenyl isocyanate[20] (11.5 g., 0.0767 mole) in chloroform (50 ml.) was added dropwise to a cold (0–5°), stirred solution of ethylenimine (4.0 ml., 0.077 mole) in the same solvent (150 ml.). Stirring was continued overnight at room temperature. Removal of the solvent under reduced pressure left a solid residue, which was recrystallized from benzene (100 ml.) to give 1-aziridinecarbox-*p*-anisidide (12.5 g., 85%) as white crystals, m.p. 117°. This product (10.0 g., 0.0522 mole) was added in portions to cold (0–5°), stirred, concentrated hydrochloric acid (80 ml.). Complete solution existed for only a few minutes, and the mixture was stirred for 1 hr. in the cold and diluted with water (50 ml.). The white urea that had formed was washed with water and dried *in vacuo;* yield, 11.9 g. (99%, 84% over-all).

1-Phenyl-3-[2-(purin-6-ylthio)ethyl]urea (XIII). Method I.—1-Aziridinecarboxanilide[22] (567 mg., 3.50 mmoles) was added in increments to a solution of purine-6(1H)-thione monohydrate (500 mg., 2.94 mmoles) in N,N-dimethylformamide (10 ml.) at 80°. After 1 hr. at 100°, the solution was cooled and diluted with water (65 ml.). The white precipitate was recrystallized from water (55 ml.) and dried *in vacuo* to give 540 mg. (58%) of XIII as a quarter hydrate; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 234 (18.0) and 294 (11.6) at pH 1, 230 (17.8) and 290 (13.3) at pH 7, 232 (22.3) and 291 (12.7) at pH 13.

1-(2-Chloroethyl)-3-methylurea.—Methylcarbamoyl chloride[18] (9.0 g., 0.097 mole) was added in small portions to a stirred, cold (5°) solution of ethylenimine (5.0 ml., 0.097 mole) in benzene (250 ml.). The solution was stirred at room temperature for 1 hr., then diluted with low-boiling petroleum ether (100 ml.), and cooled. The semisolid material that formed, from which the supernatant liquid was separated by decantation, was taken up in hot benzene (400 ml.) and the benzene solution concentrated to half volume. Dilution with low-boiling petroleum ether (60 ml.) afforded 1-(2-chloroethyl)-3-methylurea as white plates (5.0 g., 38%).

N-[(1,2,3,4-Tetrahydro-2,4-dioxo-5-pyrimidinyl)methyl]acetamide (VIII).—A suspension of powdered 95% paraformaldehyde (2.84 g., 0.090 mole) in glacial acetic acid (18.8 ml.) and concentrated sulfuric acid (4.85 ml.) was heated at 50° until complete solution resulted. To this solution, cooled to 35°, acetonitrile (4.66 ml., 0.090 mole) was added dropwise with stirring, the reaction temperature being kept between 35 and 45° by occasional external cooling. When exothermic reaction ceased, uracil (10.0 g., 0.089 mole) was added, and the mixture was heated at 85–90° for 5 hr. and then cooled. The thick reaction mass was thoroughly mixed with water (10 ml.) and the volatiles removed under reduced pressure. The residue was triturated in water (15 ml.) and the mixture carefully neutralized with an ice-cold solution of sodium hydroxide (6 g.) in water (15 ml.). The fine white solid that formed was collected and recrystallized from water (*ca.* 70 ml.) and dried *in vacuo* at 100°; yield of VIII, 5.56 g. (34%); m.p. > 260°. The ultraviolet absorption spectrum compared favorably with that of an analytically pure sample obtained by further recrystallization of a small sample from water; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 261 (7.87) at pH 1, 262 (8.00) at pH 7, and 285 (6.75) at pH 13.

Anal. Calcd. for $C_7H_9N_3O_3$: C, 45.90; H, 4.95; N, 22.94. Found: C, 45.79; H, 4.92; N, 22.76.

5-(Aminomethyl)uracil Hydrochloride.—A mixture of the acetamide VIII (2.5 g., 14 mmoles) and 6 *N* hydrochloric acid (40 ml.) was heated under reflux for 4 hr. The resulting solution was cooled and diluted with ethanol (280 ml.). The fine white solid that precipitated was washed with ethanol and dried *in vacuo;* yield, 1.7 g. (71%); m.p., *ca.* 250° dec.; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 260 (8.10) at pH 1, 260 (7.80) at pH 7, and 286 (5.90) at pH 13. Yields of the amine hydrochloride up to 78% and amounts up to 9 g. have been obtained by this procedure.

A small sample prepared by the procedure of Burckhalter, *et al.*,[13] gave the following λ_{max} in mμ ($\epsilon \times 10^{-3}$): 260 (7.90) at pH 1, 260 (7.87) at pH 7, and 286 (5.86) at pH 13; the reported[13] $\lambda_{max}^{H_2O}$ ($\epsilon \times 10^{-3}$) is 261.5 (6.30).

N-(2-Purin-6-ylaminoethyl)acetamide (X).—A solution of 6-chloropurine (10.0 g., 0.0650 mole) and N-(2-aminoethyl)acetamide (13.3 g., 0.130 mole) in 1-propanol (100 ml.) was refluxed for 6 hr. The pale yellow solid that precipitated when the reaction mixture was cooled was washed with 1-propanol and dried *in vacuo;* yield of X, 13.3 g. (93%); m.p. 238° dec.

Recrystallization of a small sample from water with Norit treatment afforded white analytically pure X, m.p. 237° dec., which was dried *in vacuo* at 100°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 271 (15.9) at pH 1, 267 (17.0) at pH 7, 274 (16.5) at pH 13.

Anal. Calcd. for $C_9H_{12}N_6O$: C, 49.08; H, 5.49; N, 38.16. Found: C, 48.51; H, 5.73; N, 38.08.

1-Methyl-3-(2-purin-6-ylaminoethyl)urea.—A solution of the acetamide X (4.9 g., 0.022 mole) in 6 *N* hydrochloric acid (70 ml.) was refluxed for 5 hr. Evaporation of the solution to dryness *in vacuo* left a semisolid residue, which was triturated in cold ethanol (20 ml.). The remaining pale yellow solid was further washed with cold ethanol and dried *in vacuo* at 100°; weight of N^6-(2-aminoethyl)adenine as a crude hydrochloride, 5.1 g.; λ_{max} in mμ: 276 at pH 1, 266 at pH 7, and 274 at pH 13 (lit.[6] values: 275 at pH 1, 273.5 at pH 11).

Triethylamine (7.2 ml.) and then methyl isocyanate[18] (1.75 ml., 0.028 mole) in portions, were added to a cold (0–5°), stirred solution of the crude amine hydrochloride in water (60 ml.). After the mixture had been stirred for 30 min. at 10° and 3 hr. at room temperature, the precipitate (1.5 g.) was collected. Additional product (2.1 g.) was recovered by evaporating the filtrate to dryness *in vacuo*, neutralizing an aqueous solution of the residue, evaporating again to dryness, and triturating the residue in water. The combined crude products were recrystallized from water (50 ml.) with Norit treatment to give 3.0 g. (57%) of the methylurea as a vacuum-dried white powder, m.p. 244°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 274 (15.2) at pH 1, 267 (16.4) at pH 7, and 274 (16.3) at pH 13.

1-Methyl-1-nitroso-3-(2-purin-6-ylaminoethyl)urea Hydrochloride.—An aqueous solution (5 ml.) of sodium nitrite (345 mg., 5.00 mmoles) was added dropwise to a cold (0–5°), stirred solution of 1-methyl-3-(2-purin-6-ylaminoethyl)urea (500 mg., 2.33 mmoles) in *N* hydrochloric acid (10 ml.). Stirring was continued at 0–5° for 30 min., and the pale yellow precipitate that had formed was collected, washed with cold water (10 ml.), and dried *in vacuo;* yield of the nitrosourea as the hydrochloride, 570 mg. (92%).

Prepared under similar conditions, 1-methyl-1-nitroso-3-(8-quinolyl)urea precipitated as the free base.

N-2-[(5-Amino-6-chloro-4-pyrimidinyl)amino]ethylacetamide (XIV).—A solution of 5-amino-4,6-dichloropyrimidine[23] (5.0 g., 0.031 mole) and N-(2-aminoethyl)acetamide[24] (6.2 g., 0.061 mole) in 1-propanol (50 ml.) was refluxed for 6 hr. Removal of the

(22) S. Gabriel and R. Stelzner, *Ber.*, **28**, 2929 (1895).

(23) D. J. Brown, *J. Appl. Chem.* (London), **4**, 72 (1954).

(24) S. R. Aspinall, *J. Am. Chem. Soc.*, **63**, 852 (1941).

solvent under reduced pressure left a yellow semisolid, which was triturated in water (25 ml.). The resulting white solid was collected, washed with water, and dried *in vacuo* at 100°; yield of XIV, 3.6 g. (51%); m.p. 200°; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 303 (11.8) at pH 1, 263 (7.70) and 291 (8.65) at pH 7, and 263 (11.8) and 290 (8.55) at pH 13.

Anal. Calcd. for $C_8H_{12}ClN_5O$: C, 41.75; H, 5.26; N, 30.47. Found: C, 41.64; H, 5.48; N, 30.32.

N-[2-(6-Chloro-9H-purin-9-yl)ethyl]acetamide Hydrochloride (XV).—A mixture of XIV (3.5 g., 0.015 mole) and triethyl orthoformate (30 ml.) containing concentrated hydrochloric acid (3 ml.) was stirred for 20 hr. at room temperature. After the reaction mixture was cooled (5°), the white solid that had formed was collected and dried *in vacuo* at 78°; yield of XV, 3.0 g. (82%); m.p. > 110° (indefinite); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 264 (8.83) at pH 1, 264 (8.75) at pH 7, and 264 (8.70) at pH 13.

Anal. Calcd. for $C_9H_{10}ClN_5O \cdot HCl$: C, 39.17; H, 4.02; N, 25.36. Found: C, 39.45; H, 4.24; N, 25.52.

N-[2-(6-Mercapto-9H-purin-9-yl)ethyl]acetamide (XVI) Hemihydrate.—A solution of the hydrochloride XV (2.9 g., 0.011 mole) and thiourea (1.5 g., 0.020 mole) in 1-propanol (75 ml.) was heated under reflux for 6 hr. After the reaction mixture was chilled, the pale yellow solid that had formed was collected, washed with 1-propanol, and dried *in vacuo*. A solution of this crude isothiuronium salt in 5% aqueous sodium hydroxide, after being filtered, was neutralized with acetic acid. The white solid that precipitated was collected, washed with water, and dried *in vacuo* at 78°; yield of XVI as a hemihydrate, 0.59 g. (23%); m.p. >220° dec. (indefinite); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 223 (9.00) and 323 (21.3) at pH 1, 225 (9.57) and 320 (22.7) at pH 7, and 232 (14.3) and 310 (22.1) at pH 13.

Anal. Calcd. for $C_9H_{11}N_5OS \cdot 0.5H_2O$: C, 43.90; H, 4.93; N, 28.44. Found: C, 44.15; H, 5.10; N, 28.34.

This procedure carried out on a 0.5-mole scale gave a yield of about 68%.

9-(2-Aminoethyl)-9H-purine-6(1H)-thione Dihydrochloride (XVII).—A solution of the acetamide XVI · hemihydrate (400 mg., 1.67 mmoles) in 6 *N* hydrochloric acid (20 ml.) was refluxed for 2 hr. Dilution of the cooled reaction mixture with ethanol (20 ml.) and further cooling to 10° caused the precipitation of a pale yellow solid, which was collected, washed with ethanol, and dried *in vacuo* at 100°; yield of XVII, 395 mg. (83%); m.p. >270° dec.; λ_{max} in mμ ($\epsilon \times 10^{-3}$): 224 (8.75) and 322 (22.9) at pH 1, 226 (9.32) and 318 (21.3) at pH 7, and 232 (13.9) and 310 (21.9) at pH 13.

Anal. Calcd. for $C_7H_9N_5S \cdot 2HCl$: C, 31.35; H, 4.51; N, 26.14. Found: C, 31.60; H, 4.19; N, 26.10.

1-[2-(6-Mercapto-9H-purin-9-yl)ethyl]-3-methylurea (XIX).—Enough 0.1 *N* sodium hydroxide solution was added to the dihydrochloride XVII (400 mg., 1.5 mmoles) to give a solution of pH 6. To this solution, cooled in an ice bath, was added triethylamine (0.5 ml.) and then methyl isocyanate[18] (0.13 ml., 2.0 mmoles). This solution was stirred for 30 min. at room temperature and for 15 min. at 40°, then cooled and neutralized with *N* hydrochloric acid. The white solid that precipitated was recrystallized from water (5 ml.) and dried *in vacuo* at room temperature; yield of XIX as a one-quarter hydrate, 170 mg. (45%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 324 (21.2) at pH 1, 321 (23.3) at pH 7, and 232 (14.2) and 310 (22.2) at pH 13.

1-Methyl-3-{2-[6-(methylthio)-9H-purin-9-yl]ethyl}urea (XX).—Iodomethane (0.25 ml., 4.0 mmoles) was added to a well stirred suspension of the purine-6(1H)-thione XIX quarter hydrate (1.0 g., 3.9 mmoles) and potassium carbonate (0.55 g., 4.0 mmoles) in N,N-dimethylformamide (20 ml.). The mixture was heated at 60–70° for 2 hr., cooled, and diluted with water (50 ml.). The solution was neutralized with *N* hydrochloric acid and the solvent removed under reduced pressure. The white solid residue was triturated in cold water and recrystallized from acetonitrile (80 ml.). The yield of XX as white needles was 0.65 g. (64%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 220 (10.9), and 299 (16.2) at pH 1, 220 (11.4), 286 (17.7), and 293 (17.5) at pH 7, and 286 (17.6) and 293 (17.4) at pH 13.

1-[2-(6-Hydroxy-9H-purin-9-yl)ethyl]-3-methylurea (XXI).—A solution of the acetamide hydrochloride XV (850 mg., 3.1 mmoles) in 6 *N* hydrochloric acid (10 ml.) was heated under reflux for 1 hr. Excess hydrochloric acid was removed by evaporation *in vacuo*, and the semisolid residue was stirred in cold ethanol (10 ml.) for 20 min. The resultant white solid (the crude amine dihydrochloride XVIII) was collected, washed with ethanol, and dried *in vacuo*. A solution of this crude XVIII (730 mg.) in water (5 ml.) was neutralized with *N* sodium hydroxide solution, treated with triethylamine (0.8 ml.), cooled to 5°, and then treated with methyl isocyanate[18] (0.19 ml., 3.0 mmoles). The solution was stirred in the cold for 1 hr. and at room temperature overnight. Chilled and brought to pH 6 with *N* hydrochloric acid, the reaction solution deposited a small amount of impure product, which was removed by filtration. Overnight chilling of the filtrate produced additional precipitate, which was collected, washed with water, and dried *in vacuo* at 100°; yield of pure XXI, 270 mg. (37%); λ_{max} in mμ ($\epsilon \times 10^{-3}$): 250 (11.4) at pH 1, 248 (12.2) at pH 7, and 254 (12.9) at pH 13.

A larger amount of crude XVIII (0.08 mole) was converted to XXI in 46% yield.

1-(2-Chloroethyl)-2-imidazolidinone (XXII).—1,3-Bis(2-chloroethyl)urea[25] (5.0 g., 0.028 mole) was added to 50 ml. of ethanolic sodium ethoxide solution (from 0.63 g., 0.028 g.-atom, of sodium), and the resulting solution was heated under reflux for 1 hr. The sodium chloride that formed was removed by filtration. The filtrate was concentrated under reduced pressure to a semisolid residue, which was taken up in ether (20 ml.) and allowed to crystallize. The white needles that formed were dried in a stream of nitrogen; yield of XXII, 1.1 g. (26%).

1-Methyl-1-nitroso-3-phenethylurea.—A solution of 1-methyl-3-phenethylurea (5.1 g., 0.029 mole) in formic acid (40 ml.) was diluted with water (50 ml.) and chilled (0–5°) in an ice bath. An aqueous solution (20 ml.) of sodium nitrite (4.0 g., 0.057 mole) was added dropwise with stirring. After 30 min. in the cold, the pale yellow nitrosourea that had formed was washed with cold water (20 ml.) and dried *in vacuo;* yield 5.0 g. (84%).

1,3-Bis(2-chloroethyl)-1-nitrosourea.—A solution of sodium nitrite (6.9 g., 0.10 mole) in water (60 ml.) was added dropwise to a cold (0–5°), stirred solution of 1,3-bis(2-chloroethyl)urea[25] (8.0 g., 0.044 mole) in formic acid (50 ml.). The reaction mixture was stirred further at 0° until the pale yellow oil that had formed solidified. The nitrosourea was collected and washed quickly with cold water (2 × 10 ml.), and dried *in vacuo;* yield 6.7 g. (71%).

1,1'-Trimethylenebis[1-nitroso-3-(1,2,3,4-tetrahyro-2,4-dioxo-5-pyrimidinyl]urea.—Solid sodium nitrite (1.8 g., 26 mmoles) was added in small increments to a well stirred, cold (0–5°) suspension of the bisurea VI (500 mg., 1.31 mmoles) in 98–100% formic acid (50 ml.). Stirring was continued at 0–5° for 2 hr. The pale yellow dinitroso derivative that had formed was washed with water and dried *in vacuo;* yield 250 mg. (61%).

1,3-Dimethyl-1-nitroso-2-thiourea.[26]—Cold 3.7 *N* sulfuric acid (30 ml.) was added dropwise over a period of 1 hr. to an ice-cold, well stirred solution of 1,3-dimethyl-2-thiourea (11.5 g., 0.111 mole) and sodium nitrite (7.95 g., 0.115 mole) in water (100 ml.), and stirring was continued for 30 min. at 0–5°. The yellow precipitate was washed with cold water (50 ml.) and dried in a stream of nitrogen; the yield of nitroso compound, m.p. 44–45° (capillary), was 100%. One recrystallization of a small sample from hexane gave the analytical sample as yellow plates, melting point unchanged.

Anal. Calcd. for $C_3H_7N_3OS$: C, 27.05; H, 5.30; S, 24.08. Found: C, 26.97; H, 5.30; S, 24.19.

Screening Results[28]

The compounds that have been prepared and evaluated for antileukemic activity can be grouped conveniently according to structure into seven classes that give rise to the divisions of Table III. In Table III A are listed analogs in which the methyl group of 1-methyl-1-nitrosourea has been replaced by other alkyl

(25) H. Bestian, *Ann. Chem.*, **566**, 210 (1950).

(26) This compound has been described previously without analysis.[27]

(27) (a) M. Freund and E. Asbrand, *Ann.* **285**, 166 (1895); (b) R. Singh, *J. Indian Chem. Soc.*, **31**, 355 (1954).

(28) The cytotoxicity determinations[29] and the life span experiments with Leukemia L1210[6] were carried out by the protocols set up by the Cancer Chemotherapy National Service Center. The Leukemia L1210 data was plotted and the dosage-response plots interpreted by published procedures.[30]

(29) Cancer Chemotherapy National Service Center, *Cancer Chemotherapy Rept.*, **1**, 63 (1959).

(30) H. E. Skipper and L. H. Schmidt, *ibid.*, **17**, 1 (1962).

TABLE III

RELATIONSHIP OF ANTILEUKEMIC ACTIVITY OF N-NITROSOUREAS TO THEIR STRUCTURE

Test System: Leukemia L1210. R: i.p., qd 1 to death (or 30 days)

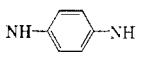
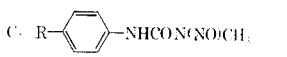
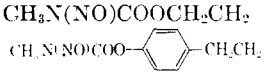
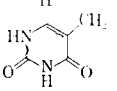
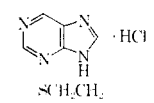
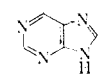
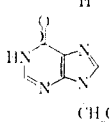
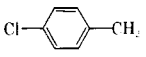
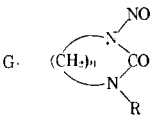
R	Cytotoxicity index, μg./ml.[a]	Minimum effective dose, mg./kg./day[b]	Optimal dose, mg./kg./day[c]	Therapeutic ratio, OD/MED[d]	Maximum effectiveness, % ILS at OD[e]
		A. $RN(NO)CONH_2$			
CH_3	57	9	12	<2	109 (7)[f]
C_2H_5	30		50		34
$ClCH_2CH_2$[g]		0.4	0.9	2.5	131
$CH_3(CH_2)_2$	58		100		30
$CH_2{=}CHCH_2$	>100		100		32
$CH_3(CH_2)_3$	18		200		29
$C_6H_5CH_2$	86				Inactive
$C_6H_5CH_2CH_2$	36				Inactive
		B. $CH_3N(NO)CORCON(NO)CH_3$			
NHNH	7.4	14	20	<2	52 (2)[f]
$NH(CH_2)_2NH$	1.8		<25		>35
$NH(CH_2)_3NH$	28	50	100	2	100
$NH(CH_2)_4NH$	35	100	200	2	63
$NH(CH_2)_5NH$	32	120	150	<2	90
	24	20	30	<2	62
	31				Inactive
$NH(CH_2)_2SS(CH_2)_2NH$	31	40	40	<2	40
		C. 			
H	24		50		22
F	10		50		29
Cl	38		50		28
CH_3O	32		50		33
C_2H_5OOC	36	20	50	2.5	67
HOOC	28	12	40	3.5	103
		D. $RNHCON(NO)CH_3$			
CH_3	18	50	75	<2	61
$ClCH_2CH_2$	2.8	135	150	<2	46
$CH_3N(NO)COOCH_2CH_2$	19		9		24
	11		25		24
	50				Inactive
$C_6H_5CH_2CH_2$	100				Inactive
	29		25		24
	>100	5	40	8	129 (2)
	100		65		33
	52		>400		>26
	37	100	160	<2	113 (2)
	43		100		28
		E. $RNHCON(NO)CH_2CH_2Cl$			
$ClCH_2CH_2$	3.1	1.7	8	5	184 (7)[f]
$C_2H_5OOCCH_2$	9		>100		>26
C_6H_5	3.1	4.8	12	2.5	89 (2)[f]

TABLE III (*Continued*)

R	Cytotoxicity index, μg./ml.[a]	Minimum effective dose, mg./kg./day[b]	Optimal dose, mg./kg./day[c]	Therapeutic ratio, OD/MED[d]	Maximum effectiveness, % ILS at OD[e]
F. RNHCON(NO)R					
$NCCH_2CH_2$	12	50	75	<2	53
$HOOCCH_2CH_2CH_2$[h]	>100				Inactive
	41				Inactive

G. 

R	n					
H	2	32	6.8	18	2.5	71 (2)[f]
NO	2	6		6		24
H	3	>100	29	50	<2	81
NO	3	5.5				Inactive

[a] The concentration necessary to inhibit the growth of KB cells to 50% of control growth determined from semilog plots of concentration *vs.* the ratio of the growth of treated cells to the growth of control cells. [b] The minimum dose that will increase the life span of leukemic mice 40% (ILS_{40}) determined from dose–response plots. [c] The dose at which the maximum increase in life span occurs (OD) determined from dose–response plots. [d] The ratio of the optimal dose (OD) to the minimum effective dose (MED). [e] Average per cent increase in life span of treated animals over control animals [100 $(T/C - 1)$] at the optimal dose. [f] Number of points from which the % ILS at the OD was determined. [g] See ref. 31. [h] Prepared according to A. F. McKay, P. Claire, and E. J. Tarlton, Can. Patent 562,952 (1958).

or aralkyl groups. With the single exception of 1-(2-chloroethyl)-1-nitrosourea,[31] the activity of the other 1-substituted 1-nitrosoureas was significantly lower than that of 1-methyl-1-nitrosourea.

This initial finding led us to turn our attention primarily toward congeners of 1-methyl-1-nitrosourea substituted on N-3. These compounds can be grouped into the bis symmetrical structures listed in Table III B and the variety of structures containing one methylnitrosoureido moiety listed in Table III (C and D).

In contrast to the 1-substituted 1-nitrosoureas of Table III A, a number of the compounds containing the methylnitrosoureido moiety show a degree of activity equal to or greater than that of 1-methyl-1-nitrosourea. Of the aliphatic bis structures, 1,1′-trimethylenebis[3-methyl-3-nitrosourea] appears to produce the greatest increase in life span, although 1,1′-pentamethylenebis[3-methyl-3-nitrosourea] is apparently almost as effective. The effectiveness of these aliphatic bismethylnitrosoureas is reminiscent of the activity of tetramethylene bis(methanesulfonate) (Myleran)[32] and certain bis(2-chloroethylamino)alkane congeners of nitrogen mustard.[33] The *p*-phenylenediamine derivative is also quite active, but the piperazine[34,35] derivative is not.[36]

Among the phenylureas in Table III C only the *p*-aminobenzoic acid derivative and its ethyl ester showed significant activity. It is of interest that, although 1,3-dimethyl-1-nitrosourea shows good activity (Table III D), its thio analog is inactive and highly toxic. Of the other 1-methyl-1-nitroso-3-substituted ureas, two heterocycles, 1-methyl-1-nitroso-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea and 1-methyl-1-nitroso-3-[2-(purin-6-ylthio)ethyl]urea, are quite active. Note that the homolog of 1-methyl-1-nitroso-3-(1,2,3,4-tetrahydro-2,4-dioxo-5-pyrimidinyl)urea, which is a derivative, of thymine instead of uracil, is only slightly active.

Although the mononitroso derivatives of the cyclic ureas, 2-imidazolidinone and tetrahydro-2(1H)-pyrimidinone, are quite active, the dinitroso derivatives are more toxic and inactive (see Table III G).

The most active compound studied thus far, 1,3-bis(2-chloroethyl)nitrosourea, which does not contain a methyl group but is related to 1-(2-chloroethyl)-1-nitrosourea[31] (Table III E), is quite active against a number of mouse, rat, and hamster tumors as well as other animal leukemias.[38] The compound can be administered i.p., subcutaneously, or orally with equal efficacy. Its activity against intracerebrally implanted L1210 leukemia, which is the subject of another report,[37] was the basis for the initiation of clinical trials.[39] Extremely hazardous delayed toxicity from chronic administration of 1,3-bis(2-chloroethyl)nitrosourea to dogs and monkeys has been observed.[39] Because of these latter observations work is continuing on the synthesis and evaluation of other N-nitrosoureas in an effort to dissociate toxicity from antileukemic activity.

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