A second $NaBH_4$ reduction of the above material gave 8.46 g which was oxidized to yield 8.0 g of the alcohol-ketone mixture. A third reduction gave 8.0 g and oxidation gave 7.55 g. Of this mixture, 5.68 g was chromatographed on 450 g of activity IV neutral alumina using gradient elution by adding benzene to a reservoir of 2.5 l. of petroleum ether. Eluted in order were 0.5 g of the ketone **1**, 2.80 g of the α isomer, and 2.09 g of the β isomer.

The α isomer was recrystallized from ethanol to yield 1.31 g: mp 201–202°; ir max (Nujol) 3.02 μ (m). *Anal.* ($C_{20}H_{22}ClNO$) C, H, N, Cl.

The β isomer was recrystallized from ethanol to give 1.40 g: mp 215–216.5°; ir max (Nujol) 3.10 μ (m). *Anal.* ($C_{20}H_{22}ClNO$) C, H, N.

2-*p*-Chlorobenzylidene-3-quinuclidinone (4). A solution of 6.25 g (0.05 mol) of 3-quinuclidinone and 7.04 g (0.05 mol) of *p*-chlorobenzaldehyde in 15 ml of ethanol was treated with two pellets of KOH and refluxed for 4 hr. The yellow precipitate was collected, washed with ethanol, and dried to give 10.85 g (87.7%), mp 110–113°. A portion of this material, 1.50 g, was recrystallized from ethanol to give 1.03 g: mp 112.5–114.5°; ir max (Nujol) 5.85 (s) and 6.11 μ (s). *Anal.* ($C_{14}H_{14}ClNO$) C, H, N.

2-(4,4′-Dichlorobenzhydryl)-3-quinuclidinone (5). A Grignard reagent was prepared in the usual way from 5.23 g (0.0273 mol) of *p*-bromochlorobenzene and 0.66 g (0.0273 g-atom) of magnesium in 60 ml of ether and cooled with cold water. A solution of 4.50 g (0.0182 mol) of 2-*p*-chlorobenzylidene-3-quinuclidinone in 100 ml of benzene was added dropwise over 1 hr and the reaction mixture was stirred at ambient temperature overnight. After the addition of water the mixture was filtered through Celite, the salts being thoroughly washed with THF. Removal of the solvent *in vacuo* left a residue which was dissolved in CH_2Cl_2 and dried ($MgSO_4$). Solvent was removed *in vacuo* and the residue was recrystallized from ethanol to give 3.62 g (55.5%): mp 167.5–170°; ir max (Nujol) 5.81 μ. *Anal.* ($C_{20}H_{19}Cl_2NO$) C, H, N, Cl.

***cis*-2-(4,4′-Dichlorobenzhydryl)-3-quinuclidinol (6).** A solution of 13.12 g (0.0364 mol) of 2-(4,4′-dichlorobenzhydryl)-3-quinuclidinone and 20.0 g (0.098 mol) of aluminum isopropoxide in 300 ml of 2-propanol was heated in a flask bearing a short distillation column while nitrogen was passed into the solution. After 4.5 hr no acetone could be detected in the distillate with 2,4-DNP solution and the solvent was removed *in vacuo*. The residue was diluted with water, made alkaline with 50% sodium hydroxide, extracted with CH_2Cl_2, and dried ($MgSO_4$). Removal of solvent left 12.9 g of a white solid, mp 200–201°. Recrystallization from methanol gave 11.73 g: mp 201–202°; ir max (Nujol) 2.99 μ. *Anal.* ($C_{20}H_{21}Cl_2NO$) C, H, N, Cl.

***trans*-2-(4,4′-Dichlorobenzhydryl)-2-quinuclidinol (7).** 2-(4,4′-Dichlorobenzhydryl)-3-quinuclidinone, 6.0 g (0.0166 mol), was reduced with 1.9 g of $NaBH_4$ in the usual way in methanol-CH_2Cl_2 (1:1) to give 6.19 g of about an equal mixture of cis and trans alcohols. This product was refluxed with 2.46 g (0.051 mol) of NaH (50% in mineral oil) and 15.5 g (0.085 mol) of benzophenone in 100 ml of benzene for 7.5 hr and stirred overnight at room temperature. Excess NaH was destroyed by the cautious addition of ethanol and the solvent was removed *in vacuo*. The residue was treated with aqueous 2 *N* HCl and extracted with ether. The aqueous phase was then made alkaline with sodium hydroxide, extracted with CH_2Cl_2, washed, and dried ($MgSO_4$). Removal of solvent *in vacuo* gave 4.92 g of a solid which by tlc (alumina with ether) was a mixture of the trans alcohol **7** and ketone **5**. The two components were separated by chromatography using 120 g of neutral alumina. Elution with 50% benzene–petroleum ether gave 2.16 g of **5** and 0.50 g of a mixture of **5** and **7**. Elution with benzene and ether–benzene gave 1.85 g of **7**. The analytical specimen was prepared by recrystallization from cyclohexane and exhibited mp 220–221°; ir max (Nujol) 3.04 μ. *Anal.* ($C_{20}H_{21}Cl_2NO$) C, H, N, Cl.

References

(1) E. J. Warawa, N. J. Mueller, and R. Jules, *J. Med. Chem.*, **17**, 497 (1974) (paper 2).
(2) E. J. Warawa and J. A. Campbell, *J. Org. Chem.*, **39**, 3511 (1974).
(3) G. R. Clemo and E. Hogarth, *J. Chem. Soc.*, 1241 (1939).
(4) R. B. Woodward, N. L. Wender, and F. J. Brutschy, *J. Amer. Chem. Soc.*, **67**, 1425 (1945).
(5) C. A. Winter, E. A. Risley, and G. W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).
(6) S. Irwin, *Psychopharmacologia*, **13**, 222 (1968).

Pyrido[2,3-*d*]pyrimidine Antibacterial Agents. 3.[1] 8-Alkyl- and 8-Vinyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)pyrido[2,3-*d*]pyrimidine-6-carboxylic Acids and Their Derivatives

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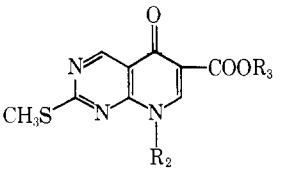
The preparation and antibacterial activity of a series of the title compounds (**21–73**) are described. These compounds were prepared from the 2-methylthio derivatives **2** and **3** *via* the 2-methylthio-8-substituted compounds **4–20**; compounds **4–20** easily underwent displacement reactions with a variety of piperazines to afford 2-(4-substituted or unsubstituted 1-piperazinyl) derivatives **21–56**, of which **21**, **22**, **27**, and **51** with unsubstituted piperazinyl group at position 2 are converted subsequently into **57–73** by alkylation, acylation, sulfonylation, or addition of isocyanates to the piperazine nitrogen. The hexahydro-1*H*-1,4-diazepinyl analog **74** was also prepared. The most active members in this series of compounds were found to be 8-ethyl- and 8-vinyl-5,8-dihydro-5-oxo-2-(1-piperazinyl)pyrido[2,3-*d*]pyrimidine-6-carboxylic acids (**22** and **51**), both of which are more active *in vitro* and *in vivo* against gram-negative bacteria, including *Pseudomonas aeruginosa*, than piromidic acid (**1**). Structure–activity relationships are discussed.

Our recent finding of piromidic acid (**1**)[1] (generic name of 8-ethyl-5,8-dihydro-5-oxo-2-pyrrolidinopyrido[2,3-*d*]pyrimidine-6-carboxylic acid), which possesses an excellent *in vitro* and *in vivo* activity[2] against staphylococci and gram-negative bacteria except *Pseudomonas aeruginosa*, prompted an extension of our study on the pyrido[2,3-*d*]pyrimidine in hopes of further enhancing the activity and broadening the antibacterial spectrum possessed by **1**. In view of the structure–activity relationship[1] that a secondary amino group at position 2 seemed to play an important role in enhancing the activity, we prepared a series of compounds having a piperazinyl group at position 2 as well as a variety of substituents at position 8 on 5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid.

Chemistry. Easily accessible compounds, 5,8-dihydro-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**2**) and its ethyl ester **3**,[3] served as starting materials in our project. Compounds **21–74** were prepared as shown in Scheme I by modifications of the procedures described previously.[1,3]

Compounds **2** and **3** were subjected to alkylation with an appropriate alkyl halide in dimethylformamide in the presence of potassium carbonate or sodium hydride to give the corresponding 8-alkyl derivatives **4–18**; acid **17** was

Table I. 8-Substituted 5,8-Dihydro-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids and Their Esters



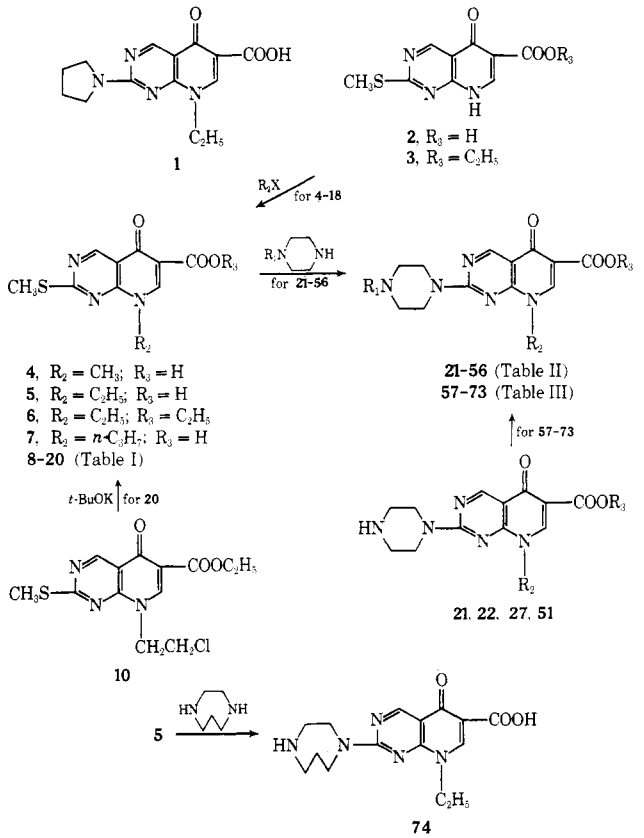
Compd	R_2	R_3	Procedure[a]	Reagent	Mp, °C	Recrystn solvent	Yield,[b] %	Formula[c]
8	CH_2CH_2OH	H	A	$BrCH_2CH_2OH$	221–224	DMF	62.5	$C_{11}H_{11}N_3O_4S$
9	CH_2CH_2OH	C_2H_5	B	$BrCH_2CH_2OH$	187–191	EtOH	61.5	$C_{13}H_{15}N_3O_4S$
10	CH_2CH_2Cl	C_2H_5	B	$BrCH_2CH_2Cl$	183–185	EtOH	60.2	$C_{13}H_{14}ClN_3O_3S$
11	$CH_2CH{=}CH_2$	H	A	$BrCH_2CH{=}CH_2$	198–200	DMF	73.5	$C_{12}H_{11}N_3O_3S$
12	$CH_2C{\equiv}CH$	H	A	$BrCH_2C{\equiv}CH$	207–210	EtOH	57.0	$C_{12}H_9N_3O_3S$
13	$CH_2C_6H_5$	H	A	$ClCH_2C_6H_5$	221–225	DMF	73.5	$C_{16}H_{13}N_3O_3S$
14	$CH_2C_6H_4$-*p*-OCH_3	H	A	$ClCH_2C_6H_4$-*p*-OCH_3	209–210	DMF	13.9	$C_{17}H_{15}N_3O_4S$
15	$CH_2C_6H_4$-*p*-Cl	H	A	$ClCH_2C_6H_4$-*p*-Cl	252–254	DMF	54.6	$C_{16}H_{12}ClN_3O_3S$
16	$CH_2C_6H_4$-*p*-NO_2	H	A	$ClCH_2C_6H_4$-*p*-NO_2	289–291	DMF	17.6	$C_{16}H_{12}N_4O_5S$
17	$CH_2CH_2C_6H_5$	H	D	*d*	267–269	DMF–EtOH	88.0	$C_{17}H_{15}N_3O_3S$
18	$CH_2CH_2C_6H_5$	C_2H_5	B	$BrCH_2CH_2C_6H_5$	122–124	EtOH	46.0	$C_{19}H_{19}N_3O_3S$
19	$CH{=}CH_2$	H	D	*d*	239–240	DMF	91.2	$C_{11}H_9N_3O_3S$
20	$CH{=}CH_2$	C_2H_5	C	*d*	164–166	EtOH	57.6	$C_{13}H_{13}N_3O_3S$

[a] Capital letters refer to procedures in the Experimental Section. [b] Yields are of purified product and are not maximal. [c] All compounds were analyzed for C, H, N, S, and, where present, Cl; analytical results were within ±0.4% of the theoretical values. [d] See the corresponding procedure in the Experimental Section.

prepared by subsequent hydrolysis of the ester **18** (Table I). Compounds **4–7** had been reported in the previous paper.[3] The requisite 8-vinyl derivative **20** was readily prepared by treating **10** with potassium *tert*-butoxide in a mixture of dimethyl sulfoxide and benzene and subsequently converted to the acid **19** by alkaline hydrolysis.

Nucleophilic displacement of the methylthio groups in compounds **4–20** by monosubstituted or unsubstituted piperazines occurred readily on simple heating to 110° in dimethyl sulfoxide, probably owing to the electron-attracting influence of the 5-oxo function, thus giving the corresponding compounds **21–36, 39–42, 45–47, 49–52, 55,** and **56** (Table II). Compounds **37** and **38** were prepared by treating **35** and **36**, respectively, with thionyl chloride. Treatment of **38** with sodium ethoxide in ethanol afforded the 8-vinyl derivative **53.** Compound **44** was obtained from the reaction of pyrrolidine with **43** which was prepared by chlorination of **41.** The acids **48** and **54** were routinely prepared from the esters **47** and **55**, respectively, by hydrolysis.

Scheme I



Substitution at the second nitrogen atom in the piperazine moiety of compounds **21, 22, 27,** and **51** with alkyl (including allyl, 2-propynyl, and benzyl), acyl, sulfonyl, and carbamoyl groups took place smoothly with the reagents indicated in Table III to give good yields of compounds **57–73;** compound **72** was prepared by subsequent hydrolysis of **73** (Table III).

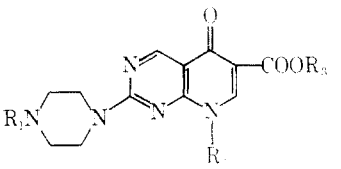
The hexahydro-1*H*-1,4-diazepinyl analog **74** was prepared by treating **5** with hexahydro-1*H*-1,4-diazepine in dimethyl sulfoxide.

The structures of all compounds were confirmed by their ir, uv, nmr, and mass spectra. The compounds **22, 33, 41, 45, 46, 49, 57,** and **58** appeared recently in the literature.[4]

Screening Results and Discussion. The results of the *in vitro* antibacterial screening for compounds **21–74,** excluding the esters which are essentially inactive, are compiled in Table IV. The data for piromidic acid (**1**) are included for comparison.

It is particularly noteworthy that replacement of the pyrrolidino group at position 2 of **1** by a piperazinyl group, giving **22,** causes an increase in the activity especially against *Pseudomonas aeruginosa* Tsuchijima, which is not significantly sensitive to **1.** Introduction of substituents to the piperazine nitrogen in **22,** however, resulted in a profound decrease in activity against *P. aeruginosa* (*e.g.*, **32, 33, 41–50,** and **57–67**). In most cases the activity against

Table II. 8-Substituted 2-(4-Substituted or unsubstituted 1-piperazinyl)-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids and Their Esters



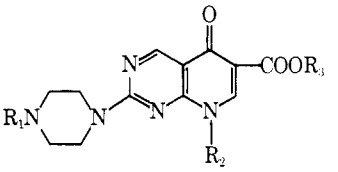
Compd	R_1	R_2	R_3	Procedure[a]	Mp, °C	Recrystn solvent	Yield,[b] %	Formula[c]
21	H	CH_3	H	E	294–295	DMF	87.5	$C_{13}H_{15}N_5O_3$
22[d]	H	C_2H_5	H	E	253–255[e]	DMF	72.5	$C_{14}H_{17}N_5O_3$
23[f]	H	*n*-C_3H_7	H	E	259–261	H_2O	52.7	$C_{15}H_{19}N_5O_3$
24[g]	H	CH_2CH_2OH	H	E	249–251	DMF	75.0	$C_{14}H_{17}N_5O_4$
25	H	$CH_2CH{=}CH_2$	H	E	253–255	DMF	39.5	$C_{15}H_{17}N_5O_3$
26	H	$CH_2C{\equiv}CH$	H	E	267–270 dec	DMF	15.5	$C_{15}H_{15}N_5O_3$
27[h]	H	$CH_2C_6H_5$	H	E	250–253	DMF	74.2	$C_{19}H_{19}N_5O_3$
28	H	$CH_2C_6H_4$-*p*-Cl	H	E	255–257	DMF	22.7	$C_{19}H_{18}ClN_5O_3$
29	H	$CH_2C_6H_4$-*p*-OCH_3	H	E	257–259	DMF	50.0	$C_{20}H_{21}N_5O_4$
30	H	$CH_2C_6H_4$-*p*-NO_2	H	E	273–276	DMF	22.7	$C_{19}H_{18}N_6O_5$
31	H	$CH_2CH_2C_6H_5$	H	E	240–246 dec	DMF	41.4	$C_{20}H_{21}N_5O_3$
32	CHO	C_2H_5	H	E	>300	DMF	61.4	$C_{15}H_{17}N_5O_4$
33	CH_3	C_2H_5	H	E	232–233[i]	EtOH	89.8	$C_{15}H_{19}N_5O_3$
34	CH_3	*n*-C_3H_7	H	E	254–257	DMF	57.3	$C_{16}H_{21}N_5O_3$
35	CH_3	CH_2CH_2OH	H	E	233–234	DMF	45.0	$C_{15}H_{19}N_5O_4$
36	CH_3	CH_2CH_2OH	C_2H_5	E	197–199	EtOH	72.6	$C_{17}H_{23}N_5O_4$
37	CH_3	CH_2CH_2Cl	H	F	226–228	DMF	10.0	$C_{15}H_{18}ClN_5O_3$
38	CH_3	CH_2CH_2Cl	C_2H_5	F	154–158	EtOAc	65.5	$C_{17}H_{22}ClN_5O_3$
39	CH_3	$CH_2CH{=}CH_2$	H	E	256–258	DMF	49.0	$C_{16}H_{19}N_5O_3$
40	CH_3	$CH_2C_6H_5$	H	E	251–255	DMF	53.0	$C_{20}H_{21}N_5O_3$
41	CH_2CH_2OH	C_2H_5	H	E	226–228[j]	EtOH	60.1	$C_{16}H_{21}N_5O_4$
42	$CH_2CH(OH)CH_3$	C_2H_5	H	E	214–216	EtOH	45.2	$C_{17}H_{23}N_5O_4$
43	CH_2CH_2Cl	C_2H_5	H	F	270–274 dec	*k*	33.0	$C_{16}H_{20}ClN_5O_3$
44	$CH_2CH_2N(CH_2)_4$	C_2H_5	H	G	251–257 dec	MeOH–H_2O	35.0	$C_{20}H_{28}N_6O_3\cdot 2HCl\cdot 0.5H_2O$
45	$CH_2C_6H_5$	C_2H_5	H	E	204–206[l]	EtOH	68.3	$C_{21}H_{23}N_5O_3$
46	$CH_2C_6H_4$-*p*-OCH_3	C_2H_5	H	E	198–199[m]	*n*-Hexane–$CHCl_3$	74.5	$C_{22}H_{25}N_5O_4$
47	$NHCOCH_3$	C_2H_5	H	E	>300	DMF	66.0	$C_{16}H_{20}N_6O_4$
48	NH_2	C_2H_5	H	H	249–250	*k*	68.0	$C_{14}H_{18}N_6O_3$
49	C_6H_5	C_2H_5	H	E	247–248[n]	Me_2CO–$CHCl_3$	48.4	$C_{20}H_{21}N_5O_3$
50	2-Pyridyl	C_2H_5	H	E	274–276	Me_2CO–$CHCl_3$	44.6	$C_{19}H_{20}N_6O_3$
51[o]	H	$CH{=}CH_2$	H	E	298–301 dec	EtOH–H_2O	60.0	$C_{14}H_{15}N_5O_3\cdot HCl$
52	H	$CH{=}CH_2$	C_2H_5	E	180–183	EtOAc	85.0	$C_{16}H_{19}N_5O_3$
53	CH_3	$CH{=}CH_2$	H	I	233–234	EtOH–$CHCl_3$	50.0	$C_{15}H_{17}N_5O_3$
54	$CH_2C_6H_5$	$CH{=}CH_2$	H	D	195–198	EtOH	40.6	$C_{21}H_{21}N_5O_3$
55	$CH_2C_6H_5$	$CH{=}CH_2$	C_2H_5	E	126–128	EtOAc–Et_2O	12.2	$C_{23}H_{25}N_5O_3$
56	C_6H_5	$CH{=}CH_2$	H	E	252–255 dec	DMF–EtOH	70.2	$C_{20}H_{19}N_5O_3$

[a–c] See footnotes in Table I. [d] HCl salt, mp >300°. *Anal.* ($C_{14}H_{17}N_5O_3\cdot HCl$) C, H, N, Cl. Trihydrate, mp 253–255°. *Anal.* ($C_{14}H_{17}N_5O_3\cdot 3H_2O$) C, H, N. On heating above 70° the trihydrate converts into the anhydride **22** with loss of $3H_2O$. [e] Lit.[4] 264° dec. [f] HCl salt, mp 294–295° dec. *Anal.* ($C_{15}H_{19}N_5O_3\cdot HCl$) C, H, N, Cl. [g] HCl salt, mp >300°. *Anal.* ($C_{14}H_{17}N_5O_4\cdot HCl$) C, H, N, Cl. [h] HCl salt, mp >300°. *Anal.* ($C_{19}H_{19}N_5O_3\cdot HCl$) C, H, N, Cl. [i] Lit.[4] 227°. [j] Lit.[4] 227°. [k] See the corresponding procedure in the Experimental Section. [l] Lit.[4] 207°. [m] Lit.[4a] 200°. [n] Lit.[4a] 249°. [o] Monohydrate, mp 153–159 and 203–207°. *Anal.* ($C_{14}H_{15}N_5O_3\cdot H_2O$) C, H, N.

both *Staphylococcus aureus* Terajima and *Escherichia coli* K-12 also was significantly decreased by the N-substitution. However, benzyl (**45**), *p*-methoxybenzyl (**46**), and 2-propynyl (**59**) groups caused the reverse effect on activity against *Staph. aureus*; *i.e.*, compounds **45, 46,** and **59** possess the activity superior to **22** or comparable to **1.** Compounds **33, 41, 43, 57,** and **58** exhibit the same *in vitro* activity against the bacteria, except *P. aeruginosa*, as that possessed by **22.** Replacement of piperazine in **22** by hexahydro-1*H*-1,4-diazepine giving **74** resulted in an appreciable decrease in the activity.

Replacement of the ethyl group at position 8 by different substituents, such as in compounds **21, 23–31, 34–40, 51, 53, 54, 56,** and **68–72,** caused generally a decrease in the *in vitro* antibacterial activities. Thus, lengthening or shortening of the carbon chain (**21, 23,** and **34**) and further substitution of the 8-ethyl group with such functional groups as OH (**24** and **35**), Cl (**37**), phenyl (**31**), and an unsaturated carbon (**25, 26,** and **39**) always resulted in a significant decrease in the activity compared with **22** and **33;** replacement of the ethyl group in **59** and **45,** both of which possessed good activity against *Staph. aureus*, by benzyl (**68** and **70,** respectively) reduced likewise the activity against this bacterium as well as the gram-negative bacteria. On the other hand, replacement of the ethyl group in **22** and **33** by benzyl resulted in a retention (**27**)

Table III. 8-Substituted 2-(4-Substituted 1-piperazinyl)-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids



Compd	R_1	R_2	R_3	Procedure[a]	Reagent	Mp, °C	Recrystn solvent	Yield,[b] %	Formula[c]
57	C_2H_5	C_2H_5	H	J	EtI	228–230[d]	$CHCl_3$–*n*-hexane	64.0	$C_{16}H_{21}N_5O_3$
58	$CH_2CH{=}CH_2$	C_2H_5	H	J	$CH_2{=}CHCH_2Br$	202–204[e]	$CHCl_3$–EtOH	10.6	$C_{17}H_{21}N_5O_3$
59	$CH_2C{\equiv}CH$	C_2H_5	H	J	$CH{\equiv}CCH_2Br$	254–257	$CHCl_3$–EtOH	64.5	$C_{17}H_{19}N_5O_3$
60	CH_2COOCH_3	C_2H_5	H	J	CH_3OOCCH_2Cl	231–233	$CHCl_3$–EtOH	73.0	$C_{17}H_{21}N_5O_5$
61	$COCH_3$	C_2H_5	H	K	Ac_2O	298–300	$CHCl_3$–C_6H_6	81.0	$C_{16}H_{19}N_5O_4$
62	$COCF_3$	C_2H_5	H	K	$(CF_3CO)_2O$	289–291 dec	DMF	98.5	$C_{16}H_{16}F_3N_5O_4$
63	$COOC_2H_5$	C_2H_5	H	J	$ClCOOC_2H_5$	>300	DMF	70.3	$C_{17}H_{21}N_5O_5$
64	SO_2CH_3	C_2H_5	H	J	CH_3SO_2Cl	>300	DMF	12.0	$C_{15}H_{19}N_5O_5S$
65	$SO_2C_6H_4$-*p*-CH_3	C_2H_5	H	J	TsCl	278–281	DMF	55.0	$C_{21}H_{23}N_5O_5S$
66	$CONHCH_3$	C_2H_5	H	L	CH_3NCO	253–256	DMF–EtOH	73.0	$C_{16}H_{20}N_6O_4$
67	$CSNHCH_3$	C_2H_5	H	L	CH_3NCS	230–233	$CHCl_3$–EtOH	86.2	$C_{16}H_{20}N_6O_3S$
68	$CH_2C{\equiv}CH$	$CH_2C_6H_5$	H	J	$CH{\equiv}CCH_2Br$	240–241	DMF	74.0	$C_{22}H_{21}N_5O_3$
69	$CH_2C_6H_5$	CH_3	H	J	$C_6H_5CH_2Cl$	291–294	DMF	68.8	$C_{20}H_{21}N_5O_3$
70[f]	$CH_2C_6H_5$	$CH_2C_6H_5$	H	J	$C_6H_5CH_2Cl$	219–221	DMF	80.0	$C_{26}H_{25}N_5O_3$
71	$CH_2C_6H_4$-*p*-OCH_3	$CH_2C_6H_5$	H	J	*p*-$CH_3OC_6H_4CH_2Cl$	236–237	DMF	95.6	$C_{27}H_{27}N_5O_4$
72	C_2H_5	$CH{=}CH_2$	H	D	*g*	216–218	EtOH	90.4	$C_{16}H_{19}N_5O_3$
73	C_2H_5	$CH{=}CH_2$	C_2H_5	J	EtOTs	134–135	EtOAc–Et_2O	88.7	$C_{18}H_{23}N_5O_3$

[a-c] See footnotes in Table I. [d] Lit.[4a] 229°. [e] Lit.[4a] 203°. [f] HCl salt, mp 230–250°. *Anal.* ($C_{26}H_{25}N_5O_3$·HCl) C, H, N, Cl. [g] See procedure D in the Experimental Section.

and an enhancement (**40**) of the activity against *P. aeruginosa*. Introduction of 8-benzyl groups with further substituents, such as *p*-Cl (**28**), *p*-MeO (**29**), and *p*-NO_2 (**30**), caused loss of the activity. Of particular interest, however, is the excellent effect of a vinyl group in position 8 that is evident by comparing the *in vitro* activity against *P. aeruginosa* of the 8-vinyl derivatives **51**, **53**, and **72** with that of the 8-ethyl derivatives **22**, **33**, and **57**, respectively.

The promising compounds in this series were tested against *Salmonella typhimurium* S-9 and *P. aeruginosa* No. 12 infections in mice on oral administration. The data for compounds **22**, **27**, **33**, **51**, **53**, **57**, **72**, and **1** are summarized in Table V. Compound **51** is the most effective for preventing the pseudomonal infection and its ED_{50} value is half of that of **22**. Against the *Salmonella* infection **22** is slightly more effective than **51**. Compounds **22** and **51** thus are superior to **1** regarding the experimental infections caused by the gram-negative bacteria in mice.

The *in vitro* and *in vivo* data demonstrate that unsubstituted piperazinyl at position 2 and ethyl and vinyl at position 8 are the most favorable substituents in this series for activity against the gram-negative bacteria, in particular, the *Pseudomonas* species. Compounds **22** and **51**, therefore, were selected for expanded chemotherapeutic studies. Data from that work will be published elsewhere.

Experimental Section

Melting points, determined on a Yanagimoto micro melting point apparatus, are uncorrected. The reactions were monitored routinely on tlc with Merck F 254 silica gel plates, which were generally developed with 10–15% MeOH in $CHCl_3$. Organic extracts were dried over anhydrous Na_2SO_4 or $MgSO_4$.

The majority of the required piperazines were purchased from commercial sources. 1-(2-Hydroxypropyl)piperazine[5] and 1-(*p*-methoxybenzyl)piperazine[6] were prepared according to the literature. 1-Acetamidopiperazine was obtained from 1-acetamido-4-benzylpiperazine[7] on hydrogenolysis in the usual manner and used without further purification. The syntheses of 5,8-dihydro-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic acid (**2**), its ethyl ester **3**, and 8-alkyl derivatives **4–7** had been described previously.[3]

8-Substituted 5,8-Dihydro-2-methylthio-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids and Their Esters (8–20). Procedure A. Alkylation of 2 (8 and 11–16, Table I). To a suspension of 2.37 g (10 mmol) of **2** in 70 ml of DMF were added successively a solution of 3.31 g (24 mmol) of K_2CO_3 in 18 ml of H_2O and 20–30 mmol of the appropriate alkylating agent listed in Table I. The mixture was heated to 85–90° for 3 hr with stirring and concentrated to dryness *in vacuo*. The residue was dissolved in *ca.* 20 ml of H_2O and the aqueous solution was acidified (pH 4–5) with AcOH to give a precipitate, which was collected and recrystallized from an appropriate solvent.

Procedure B. Alkylation of 3 (9, 10, and 18, Table I). To a stirred suspension of 2.65 g (10 mmol) of **3** in 40 ml of DMF was added carefully 0.52 g (14 mmol) of 65% NaH; an exothermic reaction set in and the temperature of the mixture rose to 40–60°. To the clear solution was added 20–30 mmol of the alkylating agent given in Table I. After being heated to 85–90° for 1–3 hr, the mixture was concentrated to dryness *in vacuo*, and the residue was taken up in $CHCl_3$. The solution was washed with H_2O and dried, and the $CHCl_3$ was evaporated to leave the crude product which was recrystallized from the appropriate solvent.

Procedure C. Elimination of HCl from 10 (20, Table I). To a stirred suspension of 11.4 g (34 mmol) of **10** in 170 ml of dry benzene was added a suspension of 7.75 g (70 mmol) of *t*-BuOK in 70 ml of anhydrous DMSO. The mixture was stirred at room temperature for 5 min and acidified (pH 1–2) with 1 *N* HCl, and 40 ml of H_2O was added. The resulting aqueous phase was separated and extracted twice with 80 ml of benzene. The extracts were combined with the original benzene phase, washed with H_2O, and dried. The benzene was evaporated *in vacuo* to give 9.1 g of the crude product, which was chromatographed on 72 g of silica gel (Mallinckrodt silicic acid) with $CHCl_3$ as eluent. The product obtained from the main fraction was recrystallized to give 5.83 g (57.6%) of **20** as colorless needles.

Procedure D. Hydrolysis (17 and 19, Table I). A mixture of 10 mmol of the ester **18** (3.6 g) or **20** (2.91 g) in 30 ml of aqueous 5% NaOH was heated to 85–90° for 10–15 min, cooled, and acidi-

Table IV. *In Vitro* Antibacterial Activity

Compd	Min inhibitory concn, μg/ml: *Staph. aureus* Terajima	*E. coli* K-12	*P. aeruginosa* Tsuchijima
21	>100	10	30
22	30	1	10
23	100	3	30
24	100	100	>100
25	100	10	30
26	>100	30	100
27	100	3	10
28	>100	10	>100
29	>100	>100	>100
30	>100	100	>100
31	>100	30	>100
32	100	10	>100
33	30	1	100
34	100	3	>100
35	100	10	>100
37	30	3	100
39	>100	3	100
40	100	3	30
41	30	1	>100
42	30	3	100
43	30	1	100
44	100	30	>100
45	10	3	100
46	10	3	30
47	100	30	>100
48	100	10	100
49	>100	>100	>100
50	>100	100	>100
51	>100	3	3
53	100	1	10
54	10	3	100
56	30	>100	>100
57	30	1	100
58	30	1	100
59	10	3	>100
60	100	100	>100
61	30	10	>100
62	30	3	30
63	>100	30	>100
64	100	10	>100
65	>100	>100	>100
66	>100	10	>100
67	30	10	>100
68	30	30	>100
69	30	3	100
70	>100	>100	>100
71	30	30	>100
72	100	1	30
74	100	10	30
1	10	1	100

fied (pH 5) with AcOH. The resulting precipitate was collected, washed with H_2O, and recrystallized.

8-Substituted 2-(4-Substituted or unsubstituted 1-piperazinyl)-5,8-dihydro-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acids and Their Esters (21–73). Compounds **54** and **72** were prepared from the esters **55** and **73**, respectively, according to procedure D described above.

Procedure E. Substitution with Piperazines (Table II). A mixture containing 10 mmol of 2-methylthio derivative (**4–20**), 20–40 mmol of the appropriate piperazine, and 50–80 ml of DMSO was heated to 90–110° for 1.5–3 hr with stirring; during the reaction MeSH gas was evolved. The solid that separated on cooling (in some cases, after evaporation of the solvent followed by trituration of the residue with H_2O) was collected and recrystallized from the appropriate solvent.

Table V. Effect on Systemic Infections in Mice on Oral Administration

Compd	ED_{50}, mg/kg: *P. aeruginosa* No. 12	*S. typhimurium* S-9
22	103	12.0
27	>200	67.7
33	200	3.9
51	50	17.7
53	141	5.3
57	200	<20.0
72	164	5.4
1	>200	30.7

Procedure F. Chlorination (37, 38, and 43, Tabel II). To a stirred solution of 8.0 g (22.2 mmol) of **36** in 80 ml of $CHCl_3$ was added 6 ml of $SOCl_2$. After being stirred for 30 min at room temperature, the mixture was heated to reflux for 2 hr. The solvent and the excess of $SOCl_2$ were evaporated *in vacuo*, and 10 ml of H_2O was added. The resulting mixture was neutralized with aqueous, saturated $NaHCO_3$ and extracted three times with 30 ml of $CHCl_3$. After the extracts had been washed with H_2O and dried, the $CHCl_3$ was evaporated to leave the crude product, which was recrystallized to give 5.5 g (65.5%) of **38** as colorless plates.

Compounds **37** and **43** were prepared from **35** and **41**, respectively, in a similar manner as described above. However, isolation and purification of **43**, because of its poor solubility, were carried out as follows. After the reaction had been complete, the mixture was concentrated to dryness *in vacuo*. Neutralization of the residue with aqueous $NaHCO_3$ gave the crude product, which was collected, washed with H_2O, dissolved in aqueous 30% AcOH, and filtered (charcoal). The filtrate was neutralized with aqueous 5% NaOH to give the product which was reprecipitated twice from 30% AcOH with 5% NaOH in the same way as described above to afford **43** as colorless prisms.

Procedure G. Amination (44, Table II). A mixture containing 366 mg (1 mmol) of **43**, 284 mg (4 mmol) of pyrrolidine, 150 mg (1.5 mmol) of Et_3N, 10 ml of DMF, and 3 ml of MeOH was heated to 100° for 1.5 hr and then concentrated to dryness *in vacuo*. Ethanolic 10% HCl was added to the residue, giving the HCl salt which was recrystallized to give 185 mg (39.1%) of **44** as colorless powder.

Procedure H. Hydrolysis (48, Table II). A mixture of 1.5 g (4.17 mmol) of **47** in 20 ml of aqueous 10% HCl was heated to 90° for 1 hr. The solid that separated on cooling was collected by filtration and dissolved in 10 ml of H_2O. The solution was filtered (charcoal) and the filtrate was neutralized with aqueous 5% NaOH to yield a precipitate, which was collected and washed successively with H_2O and EtOH. Because recrystallization from DMF, $CHCl_3$-EtOH, or pyridine caused a change in a color of the product, purification of the crude product was carried out by reprecipitation from aqueous 10% HCl with aqueous 5% NaOH. A crop of 0.90 g (68.0%) of **48** as colorless scales was obtained.

Procedure I. Elimination of HCl from 38 (53, Table II). To a solution prepared from 1.0 g (27 mmol) of 65% NaH and 30 ml of EtOH was added 1.5 g (3.95 mmol) of **38**. The mixture was heated to reflux for 2 hr and, after addition of 20 ml of H_2O, for an additional 1 hr, and then concentrated to dryness *in vacuo*. The residue was dissolved in 20 ml of H_2O. The aqueous solution was neutralized with dilute AcOH and extracted with $CHCl_3$. After the extract had been washed with H_2O and dried, the $CHCl_3$ was removed to leave the crude product which was recrystallized to give 0.63 g (50%) of **53** as colorless needles.

Procedure J. Alkylation and Sulfonylation (Table III). To a stirred suspension of 10 mmol of **21** (2.89 g), **22** (3.0 g), or **27** (3.65 g) were added successively 1.5 g (15 mmol) of Et_3N and 11–20 mmol of the alkyl or sulfonyl halide given in Table III. The mixture was heated to 85–90° for 1–3 hr and then concentrated to dryness *in vacuo*. The residue was triturated with EtOH or H_2O to give the crystalline product, which was collected and recrystallized from the appropriate solvent.

Procedure K. Acylation (61 and 62, Table III). A mixture

containing 10 g (33 mmol) of **22**, 60 ml of Ac_2O, and 1.0 ml of concentrated H_2SO_4 was heated to 90° for 2 hr. After evaporation of the excess Ac_2O the residue was triturated with cold H_2O. The resulting solid was collected by filtration, washed with H_2O, and recrystallized to give 9.22 g (81.0%) of **61** as colorless fine prisms.

Compound **62** was prepared in a similar fashion as described above by using $(CF_3CO)_2O$ without H_2SO_4.

Procedure L. Reactions with Isocyanate and Isothiocyanate (66 and 67, Table III). To a stirred solution of 1.5 g (4.95 mmol) of **22** in 200 ml of 1,2-dichloroethane was added 0.42 g (7.43 mmol) of methyl isocyanate. The mixture was allowed to react for 30 min under ice cooling and then for an additional 1 hr at room temperature. The solvent was evaporated to leave the crude product, which was recrystallized to give 1.3 g (73%) of **66** as colorless prisms.

Compound **67** was prepared in the same manner by using methyl isothiocyanate.

8-Ethyl-5,8-dihydro-2-[1-(hexahydro-1*H*-1,4-diazepinyl)]-5-oxopyrido[2,3-*d*]pyrimidine-6-carboxylic Acid (74). To a solution of 3.0 g (30 mmol) of hexahydro-1*H*-1,4-diazepine in 30 ml of DMF which had been maintained at 90° was added in portions 3.0 g (11.3 mmol) of **5**. The mixture was heated to 110° for 2 hr and then concentrated to dryness *in vacuo*. The residue was dissolved in 10% AcOH and filtered (charcoal). The filtrate was neutralized with aqueous, saturated $NaHCO_3$ and kept in a refrigerator overnight. The crystals that separated were collected and recrystallized from aqueous 50% EtOH to give 1.2 g (33.4%) of **74** as colorless powder, mp 244–246°, which is slightly hygroscopic. *Anal.* ($C_{15}H_{19}N_5O_3 \cdot \frac{1}{3}H_2O$) C, H, N.

Microbiological Methods. *In vitro* antibacterial tests were carried out by the broth-dilution method using nutrient broth[2] for *Staph. aureus* Terajima, *E. coli* K-12, and *P. aeruginosa* Tsuchijima.

In vivo antiacterial evaluation for *S. typhimurium* S-9 infection in mice was carried out according to the method of Shimizu, *et al.*[2] The data for *P. aeruginosa* No. 12 infection was obtained in a similar procedure. Groups of ten male mice (ddy strain, 18–20 g) were infected intraperitoneally with *P. aeruginosa* (10–20 LD_{50}) suspended in nutrient broth with 4% mucin. The test compounds were suspended in 0.2% sodium carboxymethylcellulose and administered orally at 0 and 6 hr postinfection. Survival rates were determined after 1 week.

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References

(1) S. Minami, T. Shono, and J. Matsumoto, *Chem. Pharm. Bull.*, **19**, 1426 (1971) (paper 2).
(2) M. Shimizu, S. Nakamura, and Y. Takase, *Antimicrob. Agents Chemother.*, **1970**, 117 (1971).
(3) S. Minami, T. Shono, and J. Matsumoto, *Chem. Pharm. Bull.*, **19**, 1482 (1971).
(4) (a) M. Pesson, Belgian Patent 802860 (1974); *Chem. Abstr.*, in press; (b) M. Pesson, P. de Lajudie, M. Antoine, S. Chabassier, D. Richer, and P. Girard, *C. R. Acad. Sci., Paris, Ser. C*, **278**, 1169 (1974).
(5) L. J. Kitchen and C. B. Pollard, *J. Org. Chem.*, **8**, 338 (1943).
(6) Y. Ikeda, Y. Nitta, and K. Yamada, *Yakugaku Zasshi*, **89**, 669 (1969).
(7) E. A. Conroy and R. P. Parker, U. S. Patent 2,663,707 (1953); *Chem. Abstr.*, **49**, 4731 (1955).

Purinylhydantoins. Facile Conversion of the Naturally Occurring *N*-(Purin-6-ylcarbamoyl)-L-amino Acids into 3-Purin-6-ylhydantoins and 3-Cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins†

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The naturally occurring *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT, **1b**), *N*-(purin-6-ylcarbamoyl)glycine (PCG, **1a**), and some of their analogs were converted into novel purine derivatives, the purinylhydantoins. The PCT and PCG underwent intramolecular cyclization in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) to give the 3-purin-6-ylhydantoins (**2a–c**). The same hydantoins were also obtained when the PCT and PCG were allowed to react through the mixed anhydride formed from cyclohexyl isocyanate or ethyl chloroformate. 1,3-Dicyclohexyl-1-[*N*-(purin-6-ylcarbamoyl)aminoacyl]ureas **3a** and **3c**, by-products obtained from the DCC reaction, were rapidly converted in aqueous NaOH to another type of purinylhydantoins, the 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins **4a** and **4b**. Compound **4a** when heated in base underwent hydrolysis of the hydantoin ring giving biuret *N*-(cyclohexylcarbamoyl)-*N*-(purin-6-ylcarbamoyl)glycine (**5a**) and *N*-(purin-6-ylcarbamoyl)glycine cyclohexylamide (**6a**). The characterization of these hydantoins was carried out by uv, nmr, and mass spectrometry. The 3-purin-6-ylhydantoins and 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins showed growth inhibitory activity in the cultured leukemic cells, while the parent amino acid compounds were inactive.

The chemical reactions of the naturally occurring 6-ureidopurines, *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT, **1b**, Scheme I) and *N*-(purin-6-ylcarbamoyl)glycine (PCG, **1a**),[1,2] and their analogs have been of interest to us from two standpoints. Firstly, the 6-ureidopurines derived from amines showed very good cytokinin activity[3,4] as well as a growth inhibitory activity in human leukemic myeloblast cell line (RPMI 6410); however, the analogs derived from the amino acids were devoid of these activities.[2] Thus the compounds of the latter type with the masked carboxyl groups should be of biological interest. Secondly, since the *N*-(purin-6-ylcarbamoyl)-L-threonine is an anticodon adjacent base in tRNA's which respond to the codons beginning with A, reactions of PCT could be very useful in the modification of these tRNA's. This paper describes a facile conversion of naturally occurring *N*-(purin-6-ylcarbamoyl)-L-threonine (PCT, **1b**) and *N*-(purin-6-ylcarbamoyl)glycine (**1a**) into the novel purine derivatives, the 3-purin-6-ylhydantoins **2**. It also discusses the conversion of *N*-(purin-6-ylcarbamoyl)aminoacylureas **3** into another type of hydantoins, 3-cyclohexyl-1-(purin-6-ylcarbamoyl)hydantoins **4**. Structure determination, chemical properties, and biological activities of these hydantoins and ureas are also described.

The usual method of preparing hydantoins by heating the *N*-carbamoylamino acids and their esters in concentrated HCl[5,6] failed in the case of PCG and PCT. The reaction of 6-ureidopurine with glyoxal in acidic medium[7] also failed to give the desired hydantoin **2a**. These condi-

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