

A second  $\text{NaBH}_4$  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  $\alpha$  isomer, and 2.09 g of the  $\beta$  isomer.

The  $\alpha$  isomer was recrystallized from ethanol to yield 1.31 g; mp 201–202°; ir max (Nujol) 3.02  $\mu$  (m). *Anal.* ( $\text{C}_{20}\text{H}_{22}\text{ClNO}$ ) C, H, N, Cl.

The  $\beta$  isomer was recrystallized from ethanol to give 1.40 g; mp 215–216.5°; ir max (Nujol) 3.10  $\mu$  (m). *Anal.* ( $\text{C}_{20}\text{H}_{22}\text{ClNO}$ ) 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  $\mu$  (s). *Anal.* ( $\text{C}_{14}\text{H}_{14}\text{ClNO}$ ) 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  $\text{CH}_2\text{Cl}_2$  and dried ( $\text{MgSO}_4$ ). 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  $\mu$ . *Anal.* ( $\text{C}_{20}\text{H}_{19}\text{Cl}_2\text{NO}$ ) 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  $\text{CH}_2\text{Cl}_2$ , and dried ( $\text{MgSO}_4$ ). 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  $\mu$ . *Anal.* ( $\text{C}_{20}\text{H}_{21}\text{Cl}_2\text{NO}$ ) 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  $\text{NaBH}_4$  in the usual way in methanol- $\text{CH}_2\text{Cl}_2$  (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  $\text{CH}_2\text{Cl}_2$ , washed, and dried ( $\text{MgSO}_4$ ). 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  $\mu$ . *Anal.* ( $\text{C}_{20}\text{H}_{21}\text{Cl}_2\text{NO}$ ) C, H, N, Cl.

## References

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## Pyrido[2,3-*d*]pyrimidine Antibacterial Agents. 3.<sup>1</sup> 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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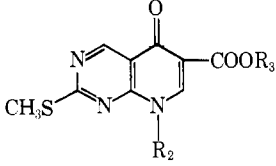
The preparation and antibacterial activity of a series of the title compounds (21–73) are described. These compounds were prepared from the 2-methylthio derivatives 2 and 3 *via* the 2-methylthio-8-substituted compounds 4–20; compounds 4–20 easily underwent displacement reactions with a variety of piperazines 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)<sup>1</sup> (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<sup>2</sup> 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<sup>1</sup> 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,<sup>3</sup> served as starting materials in our project. Compounds 21–74 were prepared as shown in Scheme I by modifications of the procedures described previously.<sup>1–3</sup>

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 <sub>2</sub>	R <sub>3</sub>	Pro- ce- dure <sup>a</sup>	Reagent	Mp, °C	Recrystn solvent	Yield, <sup>b</sup> %	Formula <sup>c</sup>
8	CH <sub>2</sub> CH <sub>2</sub> OH	H	A	BrCH <sub>2</sub> CH <sub>2</sub> OH	221–224	DMF	62.5	C <sub>11</sub> H <sub>11</sub> N <sub>3</sub> O <sub>4</sub> S
9	CH <sub>2</sub> CH <sub>2</sub> OH	C <sub>2</sub> H <sub>5</sub>	B	BrCH <sub>2</sub> CH <sub>2</sub> OH	187–191	EtOH	61.5	C <sub>13</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub> S
10	CH <sub>2</sub> CH <sub>2</sub> Cl	C <sub>2</sub> H <sub>5</sub>	B	BrCH <sub>2</sub> CH <sub>2</sub> Cl	183–185	EtOH	60.2	C <sub>13</sub> H <sub>14</sub> ClN <sub>3</sub> O <sub>3</sub> S
11	CH <sub>2</sub> CH=CH <sub>2</sub>	H	A	BrCH <sub>2</sub> CH=CH <sub>2</sub>	198–200	DMF	73.5	C <sub>12</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub> S
12	CH <sub>2</sub> C≡CH	H	A	BrCH <sub>2</sub> C≡CH	207–210	EtOH	57.0	C <sub>12</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub> S
13	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	A	ClCH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	221–225	DMF	73.5	C <sub>16</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S
14	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -OCH <sub>3</sub>	H	A	ClCH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -OCH <sub>3</sub>	209–210	DMF	13.9	C <sub>17</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub> S
15	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -Cl	H	A	ClCH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -Cl	252–254	DMF	54.6	C <sub>16</sub> H <sub>12</sub> ClN <sub>3</sub> O <sub>3</sub> S
16	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -NO <sub>2</sub>	H	A	ClCH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -NO <sub>2</sub>	289–291	DMF	17.6	C <sub>16</sub> H <sub>12</sub> N <sub>3</sub> O <sub>5</sub> S
17	CH <sub>2</sub> CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	D	<i>d</i>	267–269	DMF–EtOH	88.0	C <sub>17</sub> H <sub>15</sub> N <sub>3</sub> O <sub>3</sub> S
18	CH <sub>2</sub> CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	B	BrCH <sub>2</sub> CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	122–124	EtOH	46.0	C <sub>19</sub> H <sub>19</sub> N <sub>3</sub> O <sub>3</sub> S
19	CH=CH <sub>2</sub>	H	D	<i>d</i>	239–240	DMF	91.2	C <sub>11</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub> S
20	CH=CH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	C	<i>d</i>	164–166	EtOH	57.6	C <sub>13</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub> S

<sup>a</sup>Capital letters refer to procedures in the Experimental Section. <sup>b</sup>Yields are of purified product and are not maximal. <sup>c</sup>All compounds were analyzed for C, H, N, S, and, where present, Cl; analytical results were within  $\pm 0.4\%$  of the theoretical values. <sup>d</sup>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.<sup>3</sup> 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.

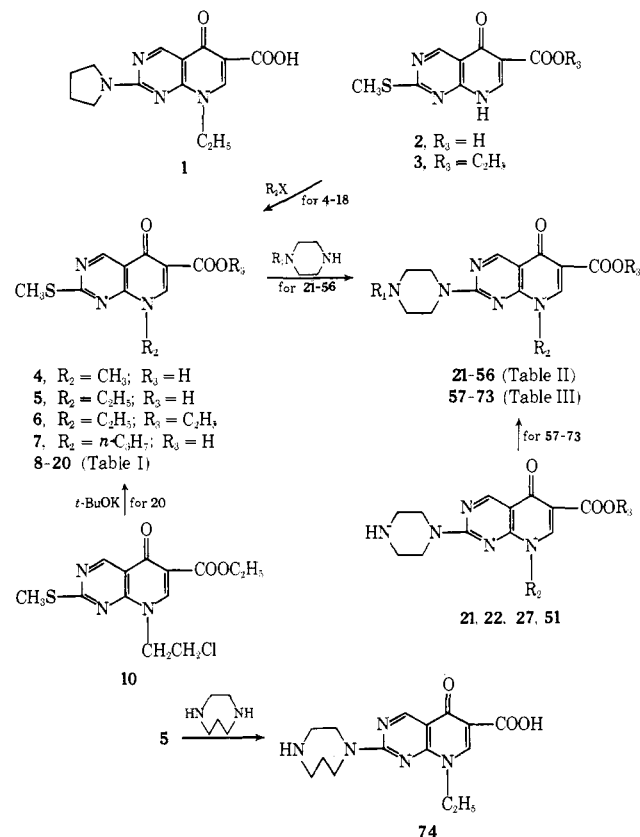
Substitution at the second nitrogen atom in the piperazine moiety of compounds 21, 22, 27, and 51 with alkyl (including allyl, 2-propynyl, and benzyl), acyl, sulfonyl, and carbamoyl groups took place smoothly with the reagents indicated in Table III to give good yields of compounds 57–73; compound 72 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.<sup>4</sup>

**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

**Scheme I**

**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 <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Pro- ce- dure <sup>c</sup>	Mp, °C	Recrystn solvent	Yield, <sup>b</sup> %	Formula <sup>c</sup>
21	H	CH <sub>3</sub>	H	E	294–295	DMF	87.5	C <sub>13</sub> H <sub>15</sub> N <sub>5</sub> O <sub>3</sub>
22 <sup>d</sup>	H	C <sub>2</sub> H <sub>5</sub>	H	E	253–255 <sup>n</sup>	DMF	72.5	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O <sub>3</sub>
23 <sup>f</sup>	H	<i>n</i> -C <sub>3</sub> H <sub>7</sub>	H	E	259–261	H <sub>2</sub> O	52.7	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
24 <sup>e</sup>	H	CH <sub>2</sub> CH <sub>2</sub> OH	H	E	249–251	DMF	75.0	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O <sub>4</sub>
25	H	CH <sub>2</sub> CH=CH <sub>2</sub>	H	E	253–255	DMF	39.5	C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>3</sub>
26	H	CH <sub>2</sub> C≡CH	H	E	267–270 dec	DMF	15.5	C <sub>15</sub> H <sub>15</sub> N <sub>5</sub> O <sub>3</sub>
27 <sup>h</sup>	H	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	E	250–253	DMF	74.2	C <sub>19</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
28	H	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -Cl	H	E	255–257	DMF	22.7	C <sub>19</sub> H <sub>18</sub> ClN <sub>5</sub> O <sub>3</sub>
29	H	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -OCH <sub>3</sub>	H	E	257–259	DMF	50.0	C <sub>20</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>
30	H	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -NO <sub>2</sub>	H	E	273–276	DMF	22.7	C <sub>19</sub> H <sub>18</sub> N <sub>5</sub> O <sub>5</sub>
31	H	CH <sub>2</sub> CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	E	240–246 dec	DMF	41.4	C <sub>20</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
32	CHO	C <sub>2</sub> H <sub>5</sub>	H	E	>300	DMF	61.4	C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>4</sub>
33	CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	E	232–233 <sup>i</sup>	EtOH	89.8	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
34	CH <sub>3</sub>	<i>n</i> -C <sub>7</sub> H <sub>7</sub>	H	E	254–257	DMF	57.3	C <sub>16</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
35	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> OH	H	E	233–234	DMF	45.0	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O <sub>4</sub>
36	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> OH	C <sub>2</sub> H <sub>5</sub>	E	197–199	EtOH	72.6	C <sub>17</sub> H <sub>23</sub> N <sub>5</sub> O <sub>4</sub>
37	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> Cl	H	F	226–228	DMF	10.0	C <sub>15</sub> H <sub>18</sub> ClN <sub>5</sub> O <sub>3</sub>
38	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub> Cl	C <sub>2</sub> H <sub>5</sub>	F	154–158	EtOAc	65.5	C <sub>17</sub> H <sub>22</sub> ClN <sub>5</sub> O <sub>3</sub>
39	CH <sub>3</sub>	CH <sub>2</sub> CH=CH <sub>2</sub>	H	E	256–258	DMF	49.0	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
40	CH <sub>3</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	E	251–255	DMF	53.0	C <sub>20</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
41	CH <sub>2</sub> CH <sub>2</sub> OH	C <sub>2</sub> H <sub>5</sub>	H	E	226–228 <sup>j</sup>	EtOH	60.1	C <sub>16</sub> H <sub>21</sub> N <sub>5</sub> O <sub>4</sub>
42	CH <sub>2</sub> CH(OH)CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	E	214–216	EtOH	45.2	C <sub>17</sub> H <sub>23</sub> N <sub>5</sub> O <sub>4</sub>
43	CH <sub>2</sub> CH <sub>2</sub> Cl	C <sub>2</sub> H <sub>5</sub>	H	F	270–274 dec	<i>l</i>	33.0	C <sub>16</sub> H <sub>20</sub> ClN <sub>5</sub> O <sub>3</sub>
44	CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>2</sub> ) <sub>4</sub>	C <sub>2</sub> H <sub>5</sub>	H	G	251–257 dec	MeOH–H <sub>2</sub> O	35.0	C <sub>20</sub> H <sub>28</sub> N <sub>6</sub> O <sub>3</sub> ·2HCl· 0.5H <sub>2</sub> O
45	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	H	E	204–206 <sup>l</sup>	EtOH	68.3	C <sub>21</sub> H <sub>23</sub> N <sub>5</sub> O <sub>3</sub>
46	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -OCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	E	198–199 <sup>m</sup>	<i>n</i> -Hexane–CHCl <sub>3</sub>	74.5	C <sub>22</sub> H <sub>25</sub> N <sub>5</sub> O <sub>4</sub>
47	NHCOCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	E	>300	DMF	66.0	C <sub>16</sub> H <sub>20</sub> N <sub>6</sub> O <sub>3</sub>
48	NH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	H	H	249–250	<i>l</i>	68.0	C <sub>14</sub> H <sub>18</sub> N <sub>6</sub> O <sub>3</sub>
49	C <sub>6</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	H	E	247–248 <sup>n</sup>	Me <sub>2</sub> CO–CHCl <sub>3</sub>	48.4	C <sub>20</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
50	2-Pyridyl	C <sub>2</sub> H <sub>5</sub>	H	E	274–276	Me <sub>2</sub> CO–CHCl <sub>3</sub>	44.6	C <sub>19</sub> H <sub>20</sub> N <sub>6</sub> O <sub>3</sub>
51 <sup>o</sup>	H	CH=CH <sub>2</sub>	H	E	298–301 dec	EtOH–H <sub>2</sub> O	60.0	C <sub>14</sub> H <sub>15</sub> N <sub>5</sub> O <sub>3</sub> ·HCl
52	H	CH=CH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	E	180–183	EtOAc	85.0	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
53	CH <sub>3</sub>	CH=CH <sub>2</sub>	H	I	233–234	EtOH–CHCl <sub>3</sub>	50.0	C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>3</sub>
54	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH=CH <sub>2</sub>	H	D	195–198	EtOH	40.6	C <sub>21</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
55	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH=CH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	E	126–128	EtOAc–Et <sub>2</sub> O	12.2	C <sub>23</sub> H <sub>25</sub> N <sub>5</sub> O <sub>3</sub>
56	C <sub>6</sub> H <sub>5</sub>	CH=CH <sub>2</sub>	H	E	252–255 dec	DMF–EtOH	70.2	C <sub>20</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>

<sup>a–c</sup>See footnotes in Table I. <sup>d</sup>HCl salt, mp >300°. *Anal.* (C<sub>14</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>·HCl) C, H, N, Cl. Trihydrate, mp 253–255°. *Anal.* (C<sub>14</sub>H<sub>17</sub>N<sub>5</sub>O<sub>3</sub>·3H<sub>2</sub>O) C, H, N. On heating above 70° the trihydrate converts into the anhydride 22 with loss of 3H<sub>2</sub>O. <sup>e</sup>Lit.<sup>4</sup> 264° dec. <sup>f</sup>HCl salt, mp 294–295° dec. *Anal.* (C<sub>15</sub>H<sub>19</sub>N<sub>5</sub>O<sub>3</sub>·HCl) C, H, N, Cl. <sup>g</sup>HCl salt, mp >300°. *Anal.* (C<sub>14</sub>H<sub>17</sub>N<sub>5</sub>O<sub>4</sub>·HCl) C, H, N, Cl. <sup>h</sup>HCl salt, mp >300°. *Anal.* (C<sub>19</sub>H<sub>19</sub>N<sub>5</sub>O<sub>3</sub>·HCl) C, H, N, Cl. <sup>i</sup>Lit.<sup>4</sup> 227°. <sup>j</sup>Lit.<sup>4</sup> 227°. <sup>k</sup>See the corresponding procedure in the Experimental Section. <sup>l</sup>Lit.<sup>4</sup> 207°. <sup>m</sup>Lit.<sup>4a</sup> 200°. <sup>n</sup>Lit.<sup>4a</sup> 249°. <sup>o</sup>Monohydrate, mp 153–159 and 203–207°. *Anal.* (C<sub>14</sub>H<sub>15</sub>N<sub>5</sub>O<sub>3</sub>·H<sub>2</sub>O) 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 <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Pro- ce- dure <sup>a</sup>	Reagent	Mp, °C	Recrystn solvent	Yield, <sup>b</sup> %	Formula <sup>c</sup>
57	C <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	H	J	EtI	228–230 <sup>d</sup>	CHCl <sub>3</sub> - <i>n</i> -hexane	64.0	C <sub>16</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
58	CH <sub>2</sub> CH=CH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	H	J	CH <sub>2</sub> =CHCH <sub>2</sub> Br	202–204 <sup>e</sup>	CHCl <sub>3</sub> -EtOH	10.6	C <sub>17</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
59	CH <sub>2</sub> C≡CH	C <sub>2</sub> H <sub>5</sub>	H	J	CH≡CCH <sub>2</sub> Br	254–257	CHCl <sub>3</sub> -EtOH	64.5	C <sub>17</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
60	CH <sub>2</sub> COOCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	J	CH <sub>3</sub> OOCCH <sub>2</sub> Cl	231–233	CHCl <sub>3</sub> -EtOH	73.0	C <sub>17</sub> H <sub>21</sub> N <sub>5</sub> O <sub>5</sub>
61	COCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	K	Ac <sub>2</sub> O	298–300	CHCl <sub>3</sub> -C <sub>6</sub> H <sub>6</sub>	81.0	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> O <sub>4</sub>
62	COCF <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	K	(CF <sub>3</sub> CO) <sub>2</sub> O	289–291 dec	DMF	98.5	C <sub>16</sub> H <sub>16</sub> F <sub>3</sub> N <sub>5</sub> O <sub>4</sub>
63	COOC <sub>2</sub> H <sub>5</sub>	C <sub>2</sub> H <sub>5</sub>	H	J	ClCOOC <sub>2</sub> H <sub>5</sub>	>300	DMF	70.3	C <sub>17</sub> H <sub>21</sub> N <sub>5</sub> O <sub>5</sub>
64	SO <sub>2</sub> CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	J	CH <sub>3</sub> SO <sub>2</sub> Cl	>300	DMF	12.0	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O <sub>5</sub> S
65	SO <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> -CH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	J	TsCl	278–281	DMF	55.0	C <sub>21</sub> H <sub>23</sub> N <sub>5</sub> O <sub>5</sub> S
66	CONHCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	L	CH <sub>3</sub> NCO	253–256	DMF-EtOH	73.0	C <sub>16</sub> H <sub>20</sub> N <sub>6</sub> O <sub>4</sub>
67	CSNHCH <sub>3</sub>	C <sub>2</sub> H <sub>5</sub>	H	L	CH <sub>3</sub> NCS	230–233	CHCl <sub>3</sub> -EtOH	86.2	C <sub>16</sub> H <sub>20</sub> N <sub>6</sub> O <sub>3</sub> S
68	CH <sub>2</sub> C≡CH	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	J	CH≡CCH <sub>2</sub> Br	240–241	DMF	74.0	C <sub>22</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
69	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>3</sub>	H	J	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl	291–294	DMF	68.8	C <sub>20</sub> H <sub>21</sub> N <sub>5</sub> O <sub>3</sub>
70 <sup>f</sup>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	J	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl	219–221	DMF	80.0	C <sub>26</sub> H <sub>25</sub> N <sub>5</sub> O <sub>3</sub>
71	CH <sub>2</sub> C <sub>6</sub> H <sub>4</sub> - <i>p</i> - OCH <sub>3</sub>	CH <sub>2</sub> C <sub>6</sub> H <sub>5</sub>	H	J	<i>p</i> -CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub> CH <sub>2</sub> Cl	236–237	DMF	95.6	C <sub>27</sub> H <sub>27</sub> N <sub>5</sub> O <sub>4</sub>
72	C <sub>2</sub> H <sub>5</sub>	CH=CH <sub>2</sub>	H	D	<i>g</i>	216–218	EtOH	90.4	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> O <sub>3</sub>
73	C <sub>2</sub> H <sub>5</sub>	CH=CH <sub>2</sub>	C <sub>2</sub> H <sub>5</sub>	J	EtOTs	134–135	EtOAc-Et <sub>2</sub> O	88.7	C <sub>18</sub> H <sub>23</sub> N <sub>5</sub> O <sub>3</sub>

<sup>a</sup>c-See footnotes in Table I. <sup>d</sup>Lit.<sup>4a</sup> 229°. <sup>e</sup>Lit.<sup>4a</sup> 203°. <sup>f</sup>HCl salt, mp 230–250°. Anal. (C<sub>26</sub>H<sub>25</sub>N<sub>5</sub>O<sub>3</sub>·HCl) C, H, N, Cl. <sup>g</sup>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<sub>2</sub> (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<sub>50</sub> 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<sub>3</sub>. Organic extracts were dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> or MgSO<sub>4</sub>.

The majority of the required piperazines were purchased from commercial sources. 1-(2-Hydroxypropyl)piperazine<sup>5</sup> and 1-(*p*-methoxybenzyl)piperazine<sup>6</sup> were prepared according to the literature. 1-Acetamidopiperazine was obtained from 1-acetamido-4-

benzylpiperazine<sup>7</sup> 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.<sup>3</sup>

**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<sub>2</sub>CO<sub>3</sub> in 18 ml of H<sub>2</sub>O 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<sub>2</sub>O 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<sub>3</sub>. The solution was washed with H<sub>2</sub>O and dried, and the CHCl<sub>3</sub> 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<sub>2</sub>O 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<sub>2</sub>O, 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<sub>3</sub> 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, $\mu\text{g/ml}$		
	<i>Staph. aureus</i>	<i>E. coli</i> K-12	<i>P. aeruginosa</i>
	Terajima		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<sub>2</sub>O, 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

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

Compd	ED <sub>50</sub> , mg/kg	
	<i>P. aeruginosa</i>	<i>S. typhimurium</i>
	No. 12	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

cooling (in some cases, after evaporation of the solvent followed by trituration of the residue with H<sub>2</sub>O) was collected and recrystallized from the appropriate solvent.

Procedure F. Chlorination (37, 38, and 43, Table II). To a stirred solution of 8.0 g (22.2 mmol) of 36 in 80 ml of CHCl<sub>3</sub> was added 6 ml of SOCl<sub>2</sub>. 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<sub>2</sub> were evaporated *in vacuo*, and 10 ml of H<sub>2</sub>O was added. The resulting mixture was neutralized with aqueous, saturated NaHCO<sub>3</sub> and extracted three times with 30 ml of CHCl<sub>3</sub>. After the extracts had been washed with H<sub>2</sub>O and dried, the CHCl<sub>3</sub> 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<sub>3</sub> gave the crude product, which was collected, washed with H<sub>2</sub>O, 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<sub>3</sub>N, 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<sub>2</sub>O. 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<sub>2</sub>O and EtOH. Because recrystallization from DMF, CHCl<sub>3</sub>-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<sub>2</sub>O, for an additional 1 hr, and then concentrated to dryness *in vacuo*. The residue was dissolved in 20 ml of H<sub>2</sub>O. The aqueous solution was neutralized with dilute AcOH and extracted with CHCl<sub>3</sub>. After the extract had been washed with H<sub>2</sub>O and dried, the CHCl<sub>3</sub> 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<sub>3</sub>N 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<sub>2</sub>O 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  $\text{Ac}_2\text{O}$ , and 1.0 ml of concentrated  $\text{H}_2\text{SO}_4$  was heated to  $90^\circ$  for 2 hr. After evaporation of the excess  $\text{Ac}_2\text{O}$  the residue was triturated with cold  $\text{H}_2\text{O}$ . The resulting solid was collected by filtration, washed with  $\text{H}_2\text{O}$ , 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  $(\text{CF}_3\text{CO})_2\text{O}$  without  $\text{H}_2\text{SO}_4$ .

**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-1H-1,4-diazepinyl)]-5-oxopyrido[2,3-d]pyrimidine-6-carboxylic Acid (74).** To a solution of 3.0 g (30 mmol) of hexahydro-1H-1,4-diazepine in 30 ml of DMF which had been maintained at  $90^\circ$  was added in portions 3.0 g (11.3 mmol) of **5**. The mixture was heated to  $110^\circ$  for 2 hr and then concentrated to dryness *in vacuo*. The residue was dissolved in 10%  $\text{AcOH}$  and filtered (charcoal). The filtrate was neutralized with aqueous, saturated  $\text{NaHCO}_3$  and kept in a refrigerator overnight. The crystals that separated were collected and recrystallized from aqueous 50%  $\text{EtOH}$  to give 1.2 g (33.4%) of **74** as colorless powder, mp  $244\text{--}246^\circ$ , which is slightly hygroscopic. *Anal.* ( $\text{C}_{15}\text{H}_{19}\text{N}_5\text{O}_3 \cdot \frac{1}{3}\text{H}_2\text{O}$ ) C, H, N.

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

*In vivo* antiacetal evaluation for *S. typhimurium* S-9 infection in mice was carried out according to the method of Shimizu, *et al.*<sup>2</sup> 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  $\text{LD}_{50}$ ) 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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## 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  $\text{NaOH}$  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**),<sup>1,2</sup> and their analogs have been of interest to us from two standpoints. Firstly, the 6-ureidopurines derived from amines showed very good cytokinin activity<sup>3,4</sup> 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.<sup>2</sup> 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  $\text{HCl}$ <sup>5,6</sup> failed in the case of PCG and PCT. The reaction of 6-ureidopurine with glyoxal in acidic medium<sup>7</sup> also failed to give the desired hydantoin **2a**. These condi-

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