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Synthesis and Properties of Mesoionic Pyrimido[1,2-*b*]pyridazine-2,4-diones and Mesoionic Pyridazino[2,3-*a*]-*s*-triazine-2,4-diones: Mesoionic Analogs Structurally Related to Fervenulin

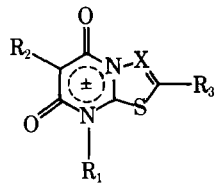
R. A. COBURN ˣ **and R. A. CARAPELLOTTI**

Abstract □ Derivatives of two new and unusual classes of heterocycles, possessing structural similarities to the broad spectrum antibiotic fervenulin, were synthesized and examined for *in vitro* antimicrobial activity. Only three of 17 mesoionic pyrimido[1,2-*b*]pyridazine-2,4-diones exhibited evidence of antimicrobial activity while seven of eight mesoionic pyridazino[2,3-*a*]-*s*-triazine-2,4-diones were active against one or more microorganisms. Susceptibility toward attack by nucleophiles of both mesoionic pyridazino[2,3-*a*]-*s*-triazine-2,4-diones and fervenulin was observed.

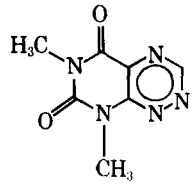
Keyphrases □ Pyrimido[1,2-*b*]pyridazine-2,4-diones—synthesized, *in vitro* antimicrobial activity screened □ Pyridazino[2,3-*a*]-*s*-triazine-2,4-diones—synthesized, *in vitro* antimicrobial activity screened □ Heterocycles—substituted pyrimido[1,2-*b*]pyridazines and pyridazino[2,3-*a*]-*s*-triazines synthesized, screened for antimicrobial activity □ Structure–activity relationships—substituted pyrimido[1,2-*b*]pyridazines and pyridazino[2,3-*a*]-*s*-triazines synthesized, antimicrobial activity screened □ Antimicrobial activity—substituted pyrimido[1,2-*b*]pyridazines and pyridazino[2,3-*a*]-*s*-triazines □ Mesoionic compounds—substituted pyrimido[1,2-*b*]-pyridazines and pyridazino[2,3-*a*]-*s*-triazines series synthesized, antimicrobial activity screened

The discovery of *in vitro* antibacterial activity of mesoionic thiazolo[3,2-*a*]pyrimidine-5,7-diones (I*a*, X = CH) and mesionic 1,3,4-thiadiazolo[3,2-*a*]pyrimidine-5,7-diones (I*b*, X = N) was reported recently (1). In particular, the most active I*b* compounds possess obvious structural similarities to the broad spectrum antibiotic fervenulin (II) (2, 3) (replacement of N=N by a sulfur atom). These findings prompted the examination of two other ring systems structurally similar to fervenulin, mesoionic pyrimido[1,2-*b*]pyridazine-2,4-diones[1] (III) and mesoionic pyridazino[2,3-*a*]-*s*-triazine-2,4-diones[2] (IV).

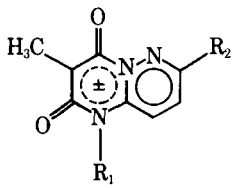
Reported here are the syntheses of a number of derivatives of mesoionic Structures III and IV and an examination of their chemical properties compared with those of fervenulin (II). These compounds were screened for *in vitro* antibacterial and antifungal activities and for *in vivo* antimalarial activity as part of an initial pharmacological investigation.



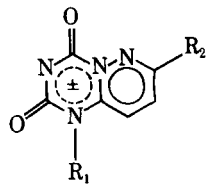
I*a*: X = CH
I*b*: X = N



II



III

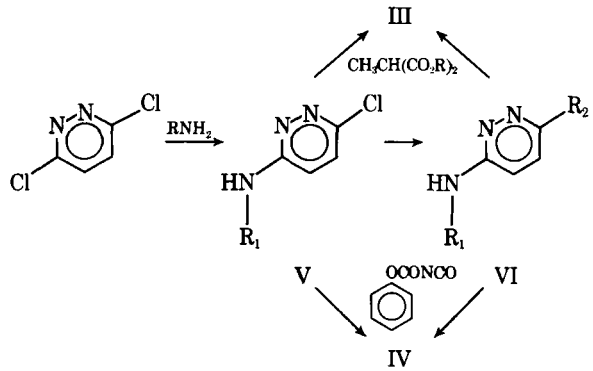


IV

CHEMISTRY

Compounds III*a*–III*q* (Table I) were prepared by the condensation of 3-aminopyridazines (secondary amines V and VI) with bis(2,4,6-trichlorophenyl) methylmalonate as previously described (1, 4), and IV*a*–IV*h* (Table II) were prepared by reaction of V and VI with phenoxycarbonyl isocyanate (Scheme I). The 3-(*N*-substituted amino)pyridazines (V) were prepared by the displacement of one chloro group of 3,6-dichloropyridazine with a primary alkyl-, aryl-, or aralkylamine. The chloro group of V could then be displaced to give VI with 6-substituents such as methoxy, morpholino, hydrogen, anilino, and *N*-methylpiperazyl (Table III).

In contrast to mesoionic thiazolopyrimidinediones (I*a*), which readily acylate benzylamine (4), mesoionic pyrimidopyridazinediones

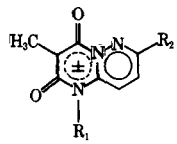


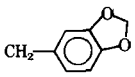
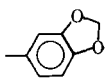
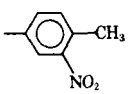
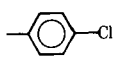
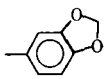
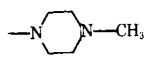
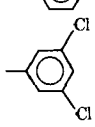
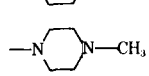
Scheme I

[1] *anhydro*-1-Substituted 2-hydroxypyrimido[1,2-*b*]pyridazinium-4-one hydroxide.

[2] *anhydro*-1-Substituted 2-hydroxypyridazino[2,3-*b*]-*s*-triazinium-4-one hydroxide.

Table I—Properties of Mesoionic Pyrimido[1,2-*b*]pyridazine-2,4-diones (III)



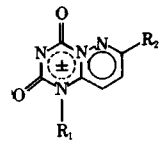
Compound	R_1	R_2	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
III*a*	CH_3	Cl	88	279–280°	$C_9H_8ClN_3O_2$	C 47.91 H 3.57 Cl 15.71 N 18.82	47.95 3.60 15.77 18.56
III*b*	$CH(CH_3)_2$	Cl	80	264–270°	$C_{11}H_{12}ClN_3O_2$	C 52.08 H 4.77 Cl 13.98 N 16.56	52.01 4.78 14.07 16.59
III*c*	$(CH_2)_3CH_3$	Cl	91	182–184°	$C_{12}H_{14}ClN_3O_2$	C 53.84 H 5.27 Cl 13.24 N 15.70	53.77 5.31 13.29 15.61
III*d*	$CH_2C_6H_4$	Cl	90	269–271°	$C_{15}H_{12}ClN_3O_2$	C 59.71 H 4.01 Cl 11.75 N 13.93	59.64 4.08 11.83 13.87
III*e*		Cl	69	250–251°	$C_{16}H_{12}ClN_3O_4$	C 55.58 H 3.50 Cl 10.25 N 12.15	55.53 3.54 10.20 12.08
III*f*	C_6H_5	Cl	79	255–260°	$C_{14}H_{10}ClN_3O_2$	C 58.45 H 3.50 Cl 12.32 N 14.61	58.73 3.66 12.12 14.50
III*g*		Cl	83	276–279°	$C_{15}H_{10}ClN_3O_4$	C 54.31 H 3.04 Cl 10.69 N 12.67	54.06 3.20 10.51 12.46
III*h*		Cl	91	293–295°	$C_{15}H_{11}ClN_4O_4$	C 51.96 H 3.20 Cl 10.23 N 16.16	51.82 3.24 10.27 16.17
III*i*	CH_3	OCH_3	95	265–267°	$C_{10}H_{11}N_3O_3$	C 54.30 H 5.01 N 19.00	54.46 5.08 18.95
III*j*	$CH_2C_6H_4$	OCH_3	87	240–243°	$C_{16}H_{15}N_3O_3$	C 64.64 H 5.09 N 14.13	64.58 5.14 14.03
III*k*	C_6H_5	OCH_3	93	310–312°	$C_{15}H_{13}N_3O_3$	C 63.60 H 4.63 N 14.83	63.36 4.68 14.95
III*l*	C_6H_5	H	97	314–315°	$C_{14}H_{11}N_3O_2$	C 66.40 H 4.38 N 16.59	66.57 4.39 16.63
III*m*	C_6H_5	NHC_6H_4	87	332–334°	$C_{20}H_{16}N_4O_2$	C 69.76 H 4.68 N 16.27	69.78 4.69 16.22
III*n*	$CH(CH_3)_2$	Morpholino	92	265–266°	$C_{15}H_{20}N_4O_3$	C 59.20 H 6.62 N 18.41	58.86 6.69 18.27
III*o*		Morpholino	90	294–296°	$C_{18}H_{17}ClN_4O_3$	C 57.99 H 4.60 Cl 9.51 N 15.03	57.63 4.62 9.37 14.87
III*p*			78	253–256°	$C_{20}H_{21}N_5O_4 \cdot 2H_2O$	C 55.68 H 5.84 N 16.23	55.72 5.88 16.20
III*q*			94	278–279°	$C_{19}H_{19}Cl_2N_5O_2$	C 54.30 H 4.56 Cl 16.87 N 16.66	54.23 4.60 16.91 16.66

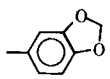
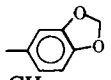
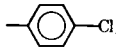
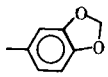
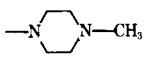
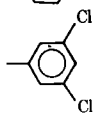
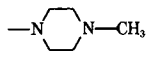
(III) were stable to nucleophilic attack by benzylamine in refluxing chloroform, ethanol, or acetonitrile for 7 days. However, mesoionic pyridazinotriazinedione (IV*c*) reacts readily with benzylamine in acetonitrile to form VII (Scheme II). Evidence in support of the structure assignment of VII follows from the reaction of V*h* with phenyl chloroformate to yield VIII, which reacted with the sodium salt of benzylurea to give a product with identical melting point, NMR spectrum, and R_f values to VII.

Compound IV*c* in aqueous acetonitrile reacts readily with water, affording the starting material V*h* (Scheme II). Only mesoionic pyridazinotriazinediones IV*a*–IV*c*, with chloro groups at their 7-positions, appear to be sensitive to nucleophilic attack by water. From the NMR spectra of IV*a*–IV*c* in moist dimethyl sulfoxide-d_6, it was estimated that these compounds have a half-life of about 30 min; under identical conditions, the NMR spectra of IV*d*–IV*h* showed no evidence of decomposition at 37° after several days.

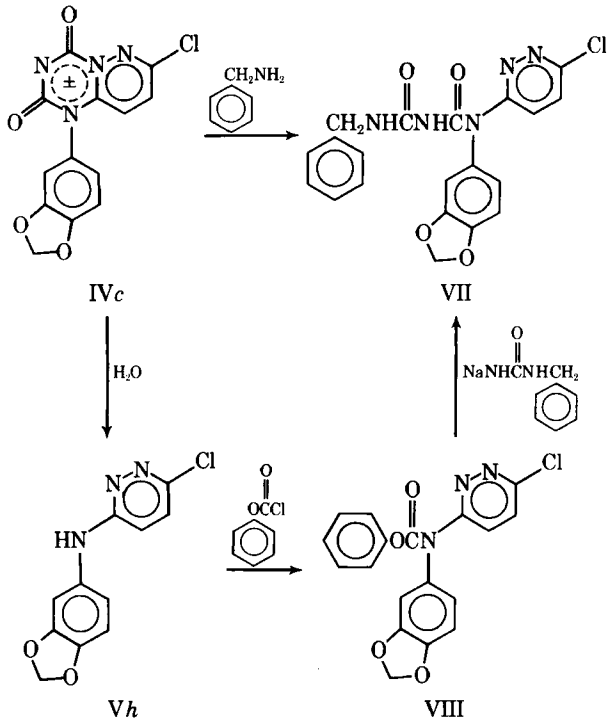
Little has been reported concerning the reactions of fervenulin with nucleophiles other than its susceptibility to degradation in aqueous alkali (5). Fervenulin (II) reacted with benzylamine in ethanol to afford the adduct IX (Scheme III). Evidence in support of the assign-

Table II—Properties of Mesoionic Pyridazino[2,3-*a*]-*s*-triazine-2,4-diones (IV)

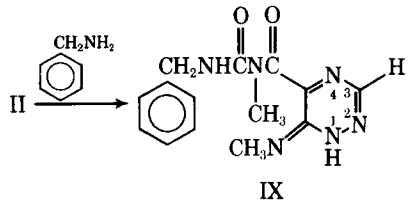


Compound	R_1	R_2	Yield, %	Melting Point	Formula	Analysis, % Calc.	Found
IV*a*	CH_3	Cl	79	215–217°	$C_7H_5ClN_4O_2$	C 39.55 H 2.37 Cl 16.68 N 26.35	39.50 2.49 16.78 26.31
IV*b*	$(CH_2)_3CH_3$	Cl	89	197–200°	$C_{10}H_{11}ClN_4O_2$	C 47.16 H 4.35 Cl 13.92 N 22.00	47.27 4.42 14.06 22.17
IV*c*		Cl	85	210–215°	$C_{13}H_7ClN_4O_4$	C 49.00 H 2.21 Cl 11.13 N 17.58	48.73 2.21 11.25 17.43
IV*d*		OCH_3	78	254–257°	$C_{14}H_{10}N_4O_5$	C 53.51 H 3.21 N 17.83	53.57 3.29 17.80
IV*e*	CH_3	Morpholino	94	277–280°	$C_{11}H_{13}N_5O_3$	C 50.19 H 4.98 N 26.60	50.21 5.00 26.63
IV*f*		Morpholino	85	284–286°	$C_{16}H_{14}ClN_5O_3$	C 53.42 H 3.92 Cl 9.85 N 19.47	53.48 3.94 9.88 19.54
IV*g*			83	212–217°	$C_{18}H_{18}N_6O_4 \cdot 2H_2O$	C 50.99 H 5.23 N 21.14	51.29 4.96 21.52
IV*h*			74	217–220°	$C_{17}H_{16}Cl_2N_6O_2$	C 50.14 H 3.96 Cl 17.41 N 20.64	50.17 4.00 17.37 20.68

ment of Structure IX is based upon NMR studies, IR spectrum, and elemental composition. Spin-decoupling studies carried out in dimethyl sulfoxide-d_6 showed the *N*-1 proton (δ 7.7), which undergoes rapid exchange in the presence of deuterium oxide, coupled to the C-3 proton (δ 10.8, J = 2 Hz) on the *as*-triazine nucleus. Both methyl groups appear as singlets at δ 2.7 and 2.9.



Scheme II



IX

Scheme III

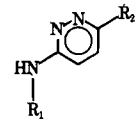
EXPERIMENTAL[3]

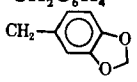
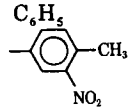
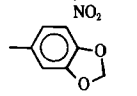
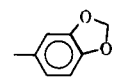
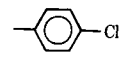
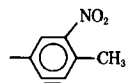
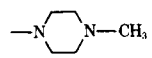
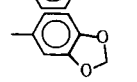
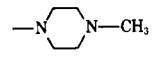
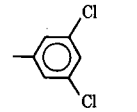
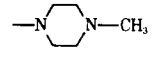
3- Chloro -6- (3,4- methylenedioxybenzylamino)pyridazine (V*e*)—3,6-Dichloropyridazine (4.9 g, 33 mmoles) and 3,4-(methylenedioxy)benzylamine (10 g, 66 mmoles) in ethanol (50 ml) were stirred for 30 min at room temperature and then refluxed for 7 hr. The solvent was evaporated *in vacuo*, and the residue was stirred with water (75 ml) and ice (50 g). The resulting white crystals were filtered, washed with water (3 × 75 ml), and air dried. Recrystallization from benzene yielded 4.5 g (52%) of V*e*, mp 139–142°; NMR (dimethyl sulfoxide-d_6): δ 4.5 (d, 2), 6.1 (s, 2), 6.9 (m, 3), and 7.4 (m, 2).

3-Chloro-6-(4-methyl-3-nitroanilino)pyridazine (V*g*)—3,6-Dichloropyridazine (15.0 g, 0.1 mole) and 4-methyl-3-nitroaniline (15.2 g, 0.1 mole) in ethanol (75 ml) were refluxed with stirring for 3 hr, and the solvent was evaporated *in vacuo*. The resulting residue was neutralized with an aqueous sodium carbonate solution (1.0 *M*), and the free base was filtered, washed with water (3 × 75 ml), and air dried. Recrystallization from methanol yielded 17.6 g (86%) of V*g*, mp 188–191°; NMR (dimethyl sulfoxide-d_6): δ 7.6 (m, 4), 8.7 (d, 1), and 10.0 (s, 1).

[3] Proton magnetic resonance (PMR) spectra were obtained on a Varian T-60 spectrometer, and chemical shifts are reported relative to tetramethylsilane. IR spectra were obtained on a Perkin-Elmer 237 grating spectrophotometer. Microanalyses were performed by Atlantic Microlab, Inc., Atlanta, Ga. All melting points were determined on a Mel-Temp melting-point apparatus and are uncorrected.

Table III—Properties of 3-(*N*-Substituted Amino)pyridazines (V and VI)



Compound	R_1	R_2	Yield, %	Melting Point[a]	Formula	Analysis, % Calc.	Found
V*a*	CH_3	Cl	65	195–196°[b]	—[b]	—	—
V*b*	$CH(CH_3)_2$	Cl	88	103–105°	—[e]	—	—
V*c*	$(CH_2)_3CH_3$	Cl	69	99–101°[c]	—[e]	—	—
V*d*	$CH_2C_6H_4$	Cl	87	150–156°	—[f]	—	—
V*e*		Cl	52	139–142°[c]	$C_{12}H_{10}ClN_3O_2$	C 54.66 H 3.82 Cl 13.45 N 15.94	54.79 3.90 13.39 15.91
V*f*	C_6H_5	Cl	60	185–187°	—[f]	—	—
V*g*		Cl	86	188–191°	$C_{11}H_9ClN_4O_2$	C 49.92 H 3.43 Cl 13.40 N 21.17	49.92 3.49 13.34 21.09
V*h*		Cl	84	204–205°	$C_{11}H_8ClN_3O_2$	C 52.92 H 3.23 Cl 14.20 N 16.83	52.98 3.29 14.15 16.88
V*i*	$4\text{-}ClC_6H_4$	Cl	69	192–195°	—[f]	—	—
V*j*	$4\text{-}CH_3OC_6H_4$	Cl	62	145–147°	—[f]	—	—
VI*a*	CH_3	OCH_3	72	82–85°[c]	$C_6H_9N_3O$	C 51.79 H 6.52 N 30.20	51.75 6.44 29.98
VI*b*	$CH_2C_6H_4$	OCH_3	67	99–101°	—[f]	—	—
VI*c*	C_6H_5	OCH_3	60	112–115°	$C_{11}H_{11}N_3O$	C 65.66 H 5.51 N 20.88	65.43 5.47 20.69
VI*d*		OCH_3	90	135–137°[c]	$C_{12}H_{11}N_3O_3$	C 58.77 H 4.52 N 17.14	58.92 4.62 17.18
VI*e*	CH_3	Morpholino	84	195–197°[c]	$C_9H_{14}N_4O$	C 55.65 H 7.27 N 28.85	55.63 7.30 28.93
VI*f*	$CH(CH_3)_2$	Morpholino	81	135–137°[c]	$C_{11}H_{18}N_4O$	C 59.44 H 8.16 N 25.21	59.60 8.19 25.29
VI*g*		Morpholino	89	174–176°	$C_{14}H_{15}ClN_4O$	C 57.83 H 5.20 Cl 12.19 N 19.27	58.04 5.34 12.26 19.30
VI*h*			82	198–202°[c]	$C_{16}H_{20}N_6O_2$	C 58.52 H 6.14 N 25.59	58.73 6.22 25.65
VI*i*			88	181–182°[c]	$C_{16}H_{19}N_5O_2$	C 61.33 H 6.11	61.20 6.17
VI*j*			83	168–169°[c]	$C_{15}H_{17}Cl_2N_5$	C 53.27 H 5.07 Cl 20.96 N 20.71	53.01 5.15 20.84 20.57

[a] Unless otherwise indicated, the recrystallization solvent was absolute methanol. [b] Recrystallized from water. [c] Recrystallized from benzene. [d] Reference 6. [e] Reference 7. [f] Reference 8.

3-Chloro-6-(3,4-methylenedioxyanilino)pyridazine (V*h*)—This compound was prepared by the same method as V*g*, using 3,4-(methylenedioxy)aniline (13.7 g, 0.1 mole). After recrystallization from methanol, the yield was 21.0 g (84%) of V*h*, mp 204–205°; NMR (dimethyl sulfoxide-d_6): δ 6.0 (s, 2), 7.3 (m, 5), and 9.3 (s, 1).

3-(*N*-Substituted Amino)-6-methoxypyridazines (VI*a*–VI*d*)—The appropriate 3-(*N*-substituted amino)-6-chloropyridazine (100 mmoles) and sodium methoxide (0.6 g, 110 mmoles) in xylene (50 ml) were refluxed with stirring for 12 hr. The solvent was evaporated *in vacuo*, and the resulting residue was stirred in water (75 ml) for 15 min. The resulting dark-yellow crystals were filtered, washed with water (4 × 25 ml), and air dried. Recrystallization was from an appropriate solvent (Table III).

3-(*N*-Substituted Amino)-6-morpholinopyridazines (VI*e*–VI*g*)—The appropriate 3-(*N*-substituted amino)-6-chloropyridazine (10 mmoles) and morpholine (12 ml) were refluxed with stirring for 6 hr. The cooled reaction mixture was poured onto ice (30 g), producing pale-yellow crystals. Then the crystals were filtered, washed with water (3 × 25 ml), and air dried.

3-(*N*-Substituted Amino)-6-*N*-(4-methylpiperazyl)pyridazines (VI*h*–VI*j*)—The appropriate 3-(*N*-substituted amino)-6-chloropyridazine (20 mmoles) and *N*-methylpiperazine (20 ml) were refluxed with stirring for 6 hr. The excess *N*-methylpiperazine was removed *in vacuo*, and the resulting residue was stirred with water (25 ml) and ice (25 g). The resulting pale-yellow crystals were filtered, washed with water, and air dried.

Mesoionic Pyrimido[1,2-*b*]pyridazine-2,4-diones (III*a*–III*q*)—An intimate mixture of equal molar quantities of the appropriate (*N*-substituted amino)pyridazine (V or VI) and bis(2,4,6-trichlorophenyl) methylmalonate was heated on an oil bath (160°) under a slow stream of nitrogen until a clear melt was obtained (7–10 min). The cooled oil was triturated with ether, leading to the crystallization of III. Then the crystals were filtered, air dried, and recrystallized from acetonitrile.

Table IV—Zones of Inhibition[a]

Compound	*E. coli*	*Ent. cloacae*	*K. pneumoniae*	*Sal. typhimurium*	*S. marcescens*	*Staph. aureus*	*Staph. epidermidis*	*C. albicans*
IV*a*	—	—	—	—	—	—	—	11
IV*b*	—	—	11	—	12	—	—	—
IV*c*	—	—	—	—	—	—	—	—
IV*d*	—	—	—	—	—	8	—	—
IV*e*	—	—	—	—	10	—	—	—
IV*f*	—	—	—	—	12	—	—	—
IV*g*	—	—	—	—	—	8	10	—
IV*h*	—	12	8	—	12	18	13	10
Nystatin	20	22	22	18	16	18	19	15
Furazolidone	19	21	17	18	—	22	14	—

[a] Diameter (millimeters) of zones of inhibition.

Mesoionic Pyridazino[2,3-*a*]-*s*-triazine-2,4-diones (IV*a*–IV*h*)—To the appropriate (*N*-substituted amino)pyridazine (V or VI) (4.0 mmoles) in dry acetonitrile (20 ml) was added phenoxycarbonyl isocyanate (9) (1.0 g, 6.0 mmoles), and the reaction mixture was allowed to stir at room temperature for 2 hr with the exclusion of moisture. The precipitate was filtered, washed with ether, and air dried. Purification of IV was accomplished by stirring in refluxing dry acetonitrile (10–20 ml) for 20–30 min. The recrystallized mixture was filtered while still warm and dried *in vacuo*, affording only minor loss of product.

Reaction of IV*c* with Water—To IV*c* (500 mg, 1.6 mmoles) in acetonitrile (30 ml) was added water (10 ml) (Scheme II), and the reaction mixture was stirred at room temperature for 30 min and refluxed for 30 min. The reaction mixture was evaporated *in vacuo*, and the resulting residue was extracted with chloroform. The chloroform extract was dried (anhydrous sodium sulfate), filtered, and evaporated *in vacuo*, yielding a residue which was crystallized with ether–benzene (1:10). The crystalline material was chromatographed on a silica gel (Woelm) column. Elution with benzene–ethyl acetate and recrystallization from benzene yielded 135 mg (34%) of V*h*, mp 208–211° dec.

Anal.—Calc. for $C_{11}H_8ClN_3O_2$: C, 52.92; H, 3.23; Cl, 14.20; N, 16.83. Found: C, 53.19; H, 3.30; Cl, 14.32; N, 17.07.

Phenyl *N*-(3,4-Methylenedioxyphenyl)-*N*-[3-(6-chloropyridazyl)]carbamate (VIII)—Compound V*h* (500 mg, 2.0 mmoles), phenyl chloroformate (3 ml), and sodium carbonate (250 mg, 2.0 mmoles) (Scheme II) were refluxed for 30 min. The reaction mixture was filtered, and the filtrate was treated with water (5 ml) and ethyl acetate (5 ml), leading to the precipitation of white crystals. Then the crystals were filtered, washed with ether (10 ml), and air dried. Recrystallization from methanol yielded 500 mg (68%) of VIII, mp 176–182°; NMR (dimethyl sulfoxide-d_6): δ 6.2 (s, 2), 7.1 (m, 3), 7.4 (m, 5), and 8.2 (q, 2).

Anal.—Calc. for $C_{18}H_{12}ClN_3O_4$: C, 58.47; H, 3.27; Cl, 9.59; N, 11.36. Found: C, 58.35; H, 3.28; Cl, 9.51; N, 11.26.

Reaction of Fervenulin (II) with Benzylamine—Fervenulin (II) (100 mg, 0.52 mmole) and benzylamine (2 ml) in ethanol (15 ml) (Scheme III) were refluxed for 70 hr with stirring. The solvent and excess benzylamine were evaporated *in vacuo*, and the resulting residue was crystallized from ether (10 ml), filtered, and air dried. Recrystallization from chloroform–ether yielded 145 mg (94%) of IX, mp 224–226°; NMR (dimethyl sulfoxide-d_6): δ 4.3 (d, 2), 7.3 (s, 5), 7.7 (d, 1), and 10.8 (d, 1).

Anal.—Calc. for $C_{14}H_{16}N_6O_2$: C, 55.99; H, 5.37; N, 27.98. Found: C, 55.88; H, 5.39; N, 27.93.

1-(3,4- Methylenedioxyphenyl) -1- [3-(6-chloropyridazyl)]-5-benzylbiuret (VII)—*Method A: Reaction of IV*c *with Benzylamine*—To IV*c* (200 mg, 0.63 mmole) in dry acetonitrile (10 ml) was added benzylamine (0.1 ml) (Scheme II), and the mixture was allowed to stir at room temperature for 90 min. The reaction mixture was stirred with charcoal and filtered, and the filtrate was evaporated *in vacuo*. The resulting residue was crystallized from ether, producing tan crystals which were filtered and air dried. Recrystallization from benzene yielded 240 mg (90%) of VII, mp 169–170° dec.; NMR (dimethyl sulfoxide-d_6): δ 4.4 (d, 2), 6.2 (s, 2), 7.0 (m, 3), 7.4 (s, 5), and 7.7 (q, 2).

Anal.—Calc. for $C_{20}H_{16}ClN_5O_4$: C, 56.41; H, 3.79; Cl, 8.33; N, 16.45. Found: C, 56.64; H, 3.86; Cl, 8.23; N, 16.32.

Method B—To a suspension of sodium hydride (57% oil dispersion; 150 mg, 6.25 mmoles) in benzene (10 ml) was added benzylurea (150 mg, 1.0 mmole). The reaction mixture was stirred at room temperature for 1 hr, followed by the addition of VIII (250 mg, 0.7 mmole) (Scheme II). Stirring was continued at room temperature for 2.5 hr, and then the reaction mixture was washed with portions of water until neutral. The benzene layer was dried (anhydrous sodium sulfate), filtered, and evaporated *in vacuo*. The resulting yellow crystals were recrystallized from benzene, yielding 125 mg (42%) of VII, mp 167–169° (identical NMR spectra and R_f values as product prepared by reaction of IV*c* with benzylamine).

Antimicrobial Testing Procedure—The following organsms were employed: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 23355), *Salmonella typhimurium* (ATCC 14028), *Serratia marcescens* (ATCC 8100), *Staphylococcus epidermidis* (ATCC 12228), *Candida albicans* (ATCC 10231), *Staphylococcus aureus* (ATCC 25923), and *Klebsiella pneumoniae* (ATCC 23357). A lawn was prepared on trypticase[4] soy agar plates, using 1 ml of a 24-hr growth of the test organism in trypticase soy broth. Paper disks (6 mm), impregnated with 1 mg of the test compounds, were placed on the agar and incubated for 24 hr at 37°. Included on each plate were disks[4] impregnated with the commercially available antimicrobial agents nystatin and furazolidone[5].

RESULTS AND DISCUSSION

Only three of 17 III derivatives, III*a*, III*g*, and III*h*, showed any evidence of inhibition in this assay. They exhibited activity against *S. marcescens*, *Staph. aureus*, *Staph. epidermidis*, or *C. albicans*. This activity is in contrast to the more widespread activity (seven of eight) of the mesoionic pyridazino[2,3-*a*]-*s*-triazine-2,4-diones (IV*a*–IV*h*, Table IV) and the previously reported thiazolo- and 1,3,4-thiadiazolo[3,2-*a*]pyrimidinediones (I*a* and I*b*) (1). No significant blood schizonticidal antimalarial activity against *Plasmodium berghei*-infected mice was found[6] for III*a*, III*b*, III*d*, III*k*, III*n*, III*o*, IV*c*, and IV*f*.

While no antimicrobial mechanism of action could be established for mesoionic Structures I*a* and I*b* and series III and IV, an apparent correlation exists between the reactivity of the test compounds toward nucleophiles and their *in vitro* antibacterial activity. Thus, little activity was found among compounds in series III, which are stable to benzylamine, while a number of derivatives of IV, which readily acylate amines, display activity. This finding lends support to the hypothesis (11) that these fused-ring mesoionic compounds may act as acylating agents *in vivo*.

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Prodrug Approaches to Enhancement of Physicochemical Properties of Drugs IV: Novel Epinephrine Prodrug

ANWAR HUSSAIN [x] and J. E. TRUELOVE

Abstract □ The synthesis and characterization of a prodrug that appears to overcome the problem of inefficient absorption of epinephrine through the lipoidal membranes of the eye are described. The enzymatic rate of regeneration of epinephrine from the prodrug was determined using a rabbit eye homogenate, rabbit plasma, and human plasma. The prodrug had no activity of its own when tested against a guinea pig smooth muscle preparation. Upon enzymatic regeneration of epinephrine from the prodrug, however, the reaction mixture exhibited α-adrenergic activity equivalent to that of epinephrine when tested in the same preparation.

Keyphrases □ Prodrugs—epinephrine prodrug synthesized, screened for adrenergic activity, rabbit eye homogenate and plasma, human plasma □ Epinephrine prodrug—synthesized, screened for adrenergic activity, rabbit eye homogenate and plasma, human plasma □ Adrenergic activity—epinephrine prodrug, screened in rabbit eye homogenate and plasma, human plasma

Previous reports in this series (1–3) presented examples of prodrug approaches that may be used to modify the physicochemical properties of drug molecules to reduce the gastric irritation of aspirin or to improve the dissolution characteristics of highly water-insoluble compounds such as allopurinol and phenytoin. This report presents an example demonstrating the utility of the prodrug approach in improving the efficiency of absorption of a highly polar molecule through lipoidal membranes.

BACKGROUND

Although epinephrine has been used for many years in eye drops for the management and treatment of glaucoma and in inhalant preparations for the treatment of bronchial asthma, problems arise with its use. One major problem is the occurrence of undesirable side effects, both ocular and systemic. McClure (4) recently listed some side effects resulting from topical applications of epinephrine.

In the treatment of glaucoma, relatively concentrated epinephrine solutions are instilled directly into the eye. However, because it is highly polar, little drug is absorbed. The remainder of the solution reaches the general circulation through the tear ducts, exerting its undesirable systemic side effects (5). Since many glaucomatous patients are over 40 years of age, some may have cardiac or circulatory disorders which could be aggravated by systemically absorbed epinephrine. Therefore, an improved form of epinephrine that would be effective at low concentrations seemed desirable.

The fundamental problem with epinephrine is its inefficient transport across lipoidal barriers due to its high polarity and low lipid solubility. It was felt that the transient blocking of the phenolic hydroxy groups would enhance the lipophilicity of epinephrine and significantly facilitate its absorption through the lipoidal membranes of the eye.

The synthesis of a novel prodrug of epinephrine (6), which has been shown to be approximately 100 times more effective clinically than epinephrine itself in the management of glaucoma (4), is reported here. Furthermore, the prodrug has been shown to be about 100–400 times weaker than epinephrine in affecting the cardiovascular systems of dogs and cats (4).

A successful epinephrine prodrug should be more lipophilic than epinephrine, possess adequate water solubility, regenerate epinephrine at a reasonable rate, and be stable enough to be formulated into conventional dosage forms. Furthermore, the blocking groups, upon cleavage, should have no toxicity of their own.

The prodrug, 3,4-dipivaloyloxy-α-(methylaminomethyl)benzyl alcohol perchlorate salt (I) (Scheme I) was a suitable candidate. Since the general pharmacology, toxicology, and clinical evaluation of the prodrug have already been reported (4), this article is concerned with the synthesis and *in vitro* enzymatic hydrolysis of the drug in a rabbit eye homogenate, rabbit plasma, and human plasma.

EXPERIMENTAL

Synthesis[1] of I—Fifty grams (0.27 mole) of α-chloro-3′,4′-dihydroxyacetophenone[2] (II) was dissolved in 200 ml of methanol. (Slight warming may be necessary to complete the solution.) Then 100 ml of

[1] The general synthetic procedure was presented in U.S. pat. 3,809,714.
[2] Obtained from a commercial source or synthesized by the reaction of pyrocatechol and chloroacetyl chloride in refluxing benzene.