Aminooxy Derivatives. IV. Antimicrobial Activity of Some O-Ethers of 4,6-Diamino-1,2-dihydro-1-hydroxy-2-substituted 1,3,5-Triazines

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Some new O-ethers of 4,6-diamino-1,2-dihydro-1-hydroxy-2-substituted 1,3,5-triazines have been prepared. The antibacterial properties of these compounds and of some previously prepared analogs are described. *In vitro* activity was observed against a wide range of bacteria; a number of compounds inhibited the growth of *Candida albicans*. Variation of substituents in the 1-position resulted in compounds with essentially predictable *in vitro* activity; variation of 2-substituents gave products showing variable activity. The microbiological properties of two of the most active compounds are described in detail.

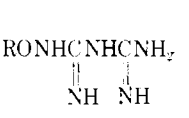
Some biological activities of a number of α-aminooxy acids and hydrazides, alkoxyamines, and alkoxy- and arylmethoxydiguanides have been described.[1] A brief résumé of aminooxy compounds of biological interest through 1959 was also given. Since this date, a considerable volume of work on the biological properties of aminooxy-containing compounds has appeared and more literature on the chemistry of substituted hydroxylamines has accumulated.

Ilvespää and Marxer[2] have made a comprehensive review of the literature on O-substituted oxyamines. The chemistry, pharmacology, and antibacterial activity of some oxyamines has been described by a number of workers[3]; apart from activity as antibacterials, results have been disappointing. Aminooxy analogs of some pharmacologically active amines prepared by Major and co-workers, and by others,[4] lacked the activity of the prototypes.

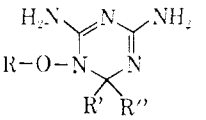
N-Oxyureas have been found to possess antitumor[5] and herbicidal[6] activities. A large amount of literature exists[7] on the inhibition of γ-aminobutyric acid transaminase by aminooxyacetic acid and homologs, and on the use of these compounds as anticonvulsants.

Hypocholesterolemic activity has been claimed for a number of benzyloxyamines[8] while the inhibition of dopamine β-oxidase by similar compounds has been described.[9]

Considerable *in vitro* bactericidal activity against gram-positive and gram-negative organisms was shown[1] by the alkloxydiguanides (I). The most active compound, decyloxydiguanide (I, R = $C_{10}H_{21}$) was moderately toxic to mice when administered intraperitoneally and did not protect mice infected intraperitoneally with *Streptococcus hemolyticus* group C. In a search for compounds of greater activity a number of cyclized derivatives of the diguanides was prepared. Brief results of the microbiological activities of some substituted N-oxydihydrotriazines (II) have been published.[10]

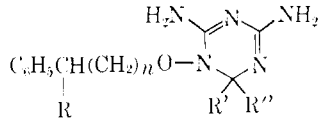


I

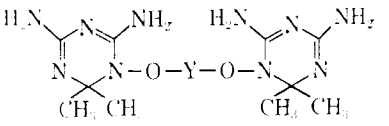


II

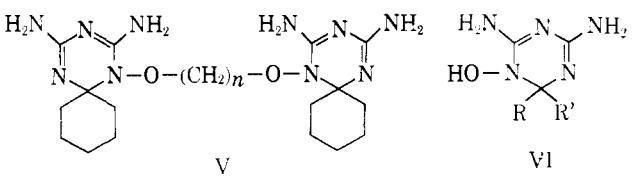
Chemistry.—The present communication describes the preparation of some new dihydrotriazine derivatives of types III, IV, and V and their microbiological properties, together with a more detailed account of the properties of the earlier compounds.



III



IV



V VI

All three types of compound were prepared by reaction of 4,6-diamino-1,2-dihydro-2,2-substituted 1-hydroxy-1,3,5-triazine (VI) (usually prepared *in situ*

(1) S. A. Price, P. Mamalis, D. McHale, and J. Green, *Brit. J. Pharmacol.*, **15**, 243 (1960).

(2) A. O. Ilvespää and A. Marxer, *Chimia*, **18**, 1 (1964).

(3) E. L. Schumann, R. V. Heinzelmann, M. E. Greig, and W. Veldkamp, *J. Med. Chem.*, **7**, 329 (1964); L. Paquette, *Tetrahedron Letters*, **No. 11**, 485 (1962); B. J. R. Nicolaus, G. Pagani, and E. Testa, *Helv. Chim. Acta*, **45**, 358 (1962), and several subsequent papers; A. F. McKay, D. L. Garmaise, G. Y. Paris, and S. Gelblum, *Can. J. Chem.*, **38**, 343 (1960); Belgian Patent 614,197 (1962); British Patent 892,593 (1962); L. Bauer and K. S. Suresh, *J. Org. Chem.*, **28**, 1604 (1963).

(4) R. T. Major, H. J. Hess, and C. A. Stone, *J. Med. Pharm. Chem.*, **1**, 381 (1959); R. T. Major and K. W. Ohly, *ibid.*, **4**, 51, 317 (1961); R. T. Major and F. Dürsch, *J. Org. Chem.*, **26**, 1867 (1961); F. Benington, R. D. Marin, and L. C. Clarke, *Nature*, **202**, 813 (1964); U. S. Patent 3,027,407 (1962).

(5) B. Stearns, K. A. Losee, and J. Bernstein, *J. Med. Chem.*, **6**, 201 (1963); British Patent 969,022; E. Boyland and R. Nery, *Nature*, **203**, 1379 (1964); W. Klötzer, *Monatsh.*, **95**, 265 (1964).

(6) British Patents 940,321 (1963), 950,254 (1964).

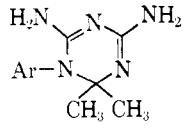
(7) J. P. Da Vanzo, M. E. Greig, and M. A. Cronin, *Am. J. Physiol.*, **205**, 833 (1961); A. Frank and K. Riedl, *Monatsh.*, **92**, 725 (1961); E. L. Schumann, L. A. Paquette, R. V. Heinzelman, D. P. Wallach, J. P. Da Vanzo, and M. E. Greig, *J. Med. Pharm. Chem.*, **5**, 464 (1962); C. K. Chai, E. Roberts, and R. L. Sidman, *Proc. Soc. Expil. Biol. Med.*, **109**, 491 (1962); Y. Knobler, Ch. Gilon, and M. Frankel, *Isarel J. Chem.*, **1**, 242 (1963); A. Richardson, *J. Med. Chem.*, **7**, 824 (1964); French Patent 1316M (1962).

(8) F. M. Berger and B. J. Ludwig (to Carter Products Inc.), Belgian Patent 612,879; A. O. Ilvespää and A. Marxer, *Helv. Chim. Acta*, **46**, 2009 (1963); *Gazz. chim. ital.*, **93**, 186 (1963).

(9) B. Nikodijevic, C. R. Creveling, and S. Udenfriend, *J. Pharm. Expil. Therap.*, **140**, 224 (1963); J. B. van Shoot, C. R. Creveling, T. Negatsu, and S. Udenfriend, *ibid.*, **141**, 74 (1963).

(10) P. Mamalis, J. Green, D. J. Outred, and M. J. Rix, *J. Chem. Soc.*, 3915 (1962).

from the hydrochloride) with the requisite aralkyl halide or alkylene dihalide. Derivatives prepared included III in which n = 0 and R = alkyl; III in which R = H and n = 2–10; IV in which Y = alkylene, 1,4-diphenylyl, and 1,4-phenylene; and V in which n = 5–12 and R = H or methyl. The aralkylhalides were prepared by standard methods.



VII

An attempt to prepare 6-phenyl-1-hexanol by treatment of the Grignard reagent from 4-phenylbutyl bromide with ethylene oxide gave not the expected product but a mixture of butylbenzene and 4-phenyl-1-butanol. The required product was ultimately prepared from 2,3-dichlorotetrahydropyran and benzylmagnesium chloride, and the product was treated with sodium in ether and then catalytically hydrogenated.

Biological Activity.—The wide range of biological activity of the aryldihydrotriazines has been summarized.[11] We have noted a number of different types of activity with the analogous N-oxytriazines. The O-substituted N-oxydihydrotriazines (II, R′ = R″ = Me) displayed antibacterial activity *in vitro* which increased with increasing molecular weight of the O-substituent. In the alkyl-substituted series (II, R = alkyl; R′ = R″ = Me) this effect was clearly shown by a comparison of bacteriostatic activity against *Staphylococcus pyogenes* and *Escherichia coli* (Table I).

TABLE I

In Vitro MINIMUM INHIBITORY CONCENTRATION (MIC)[a] FIGURES FOR ALKYLOXYDIHYDROTRIAZINES (II, R′ = R″ = Me)

No.	R	*S. pyogenes*	*E. coli*	*C. albicans*	*Ps. aeruginosa*
177	C_2H_5	>600	>600	>60	600
1177	C_3H_7	300	>600	>60	600
167	C_4H_9	75	150	>60	>600
166	C_7H_{15}	4.7	19	>60	>600
164	C_8H_{17}	4.7	9.5	>60	75
191	C_9H_{19}	2.4	9.5	>60	150
162	$C_{10}H_{21}$	9.5	9.5	30	37
192	$C_{11}H_{23}$	2.4	4.7	15	37
173	$C_{12}H_{25}$	4.7	4.7	15	75
174	$C_{14}H_{29}$	4.7	19	15	600

[a] Concentration in γ/ml.; results after 24 hr.

The longer chain-length derivatives inhibited the growth of *Candida albicans* and *Pseudomonas aeruginosa*. The unsubstituted benzyloxytriazine (II, R = $PhCH_2$; R′ = R″ = Me) showed little *in vitro* activity but this could be modified by ring substitution. Substitution at positions 2 or 4 of the benzene ring (Table II) by halogen, nitro, or methoxycarbonyl gave derivatives moderately active against *S. pyogenes* and against *E. coli*, whereas carboxyl- (**447**) and sulfamyl-substituted (**511**) derivatives were inactive against *S. pyogenes;* **447** was also inactive against *E. coli*. The effect of the carboxyl group in reducing this type of activity is not unusual. A deactivating effect was also

(11) E. J. Modest in "Heterocyclic Compounds," Vol. VII, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1961, p. 717.

TABLE II

In Vitro ACTIVITY OF *ortho-* AND *para*-SUBSTITUTED BENZYLOXYDIHYDROTRIAZINES (II, R′ = R″ = Me)

		MIC, γ/ml.		
No.	R	*S. pyogenes*	*E. coli*	*Ps. aeruginosa*
189	Benzyl	75	300	>600
209	*p*-Chlorobenzyl	9.5	2.4	
210	*p*-Bromobenzyl	9.5	2.4	300
242	*p*-Nitrobenzyl	75	37.5	
425	*p*-Methoxycarbonylbenzyl	37	2.4	>600
511	*p*-Sulfamylbenzyl	>600	37	>600
316	*p*-Ethoxybenzyl	75	150	>600
464	*o*-Fluorobenzyl	37	9.5	>600
470	*o*-Chlorobenzyl	19	9.5	600
471	*o*-Bromobenzyl	4.7	1.2	300
255	*o*-Nitrobenzyl	19	4.7	
426	*o*-Methoxycarbonylbenzyl	19	9.5	>600
447	*o*-Carboxybenzyl	>600	>600	>600
410	*o*-Ethoxybenzyl	19	2.4	600

produced by insertion of a hydroxyl group at the end of the hydrocarbon chain of **162** giving II (R = $HO(CH_2)_{10}$; R′ = R″ = Me) (**609**). *In vitro* activity against *S. pyogenes* and *E. coli* was slightly reduced, while inhibitory action against *C. albicans* and *Ps. aeruginosa* was completely lost. Activity against *Trichomonas vaginalis in vitro* was similarly affected (**162**, MIC 62.5 γ/ml.; **609**, > 250 γ/ml.).

Replacement of the phenyl group in II (R = $PhCH_2$; R′ = R″ = Me) by larger aryl groups resulted in compounds very active *in vitro* against *S. pyogenes* and *E. coli* (Table III). There were also signs of activity against *Ps. aeruginosa* which were not shown by the aryl derivatives of Table II. This effect was not noted with the heterocyclic analogs and in some cases even resulted in lowered *in vitro* activity (*cf.* **377, 388,** and **428** with **202**) (Table III).

Results of the *in vitro* activities of a series of alkoxy-substituted benzyloxytriazines are given in Table IV. Most compounds showed appreciable activity; no definite correlation between chain length of the alkoxy group and activity was observable nor could differences in activity be assigned to position of substitution in the benzene ring. Irrespective of substituents, activity against *Ps. aeruginosa* was slight and *C. albicans* was sensitive only to the higher ethers.

It has already been shown[10] that the bacteriostatic activity against *S. pyogenes* and *E. coli* of the arylmethoxydihydrotriazines (II, R = aryl CH_2; R′ = R″ = Me) is greater than that of the aryldihydrotriazine analogs (VII, Ar = aryl). This increased activity was not observed using *S. hemolyticus*. Thus, both the 3,4- and the 3,5-dichlorophenyltriazines (VII, Ar = 3,4- and 3,5-$Cl_2C_6H_3$, **250** and **431**, respectively) were as active as the oxy analogs (**216** and **461**), the MIC being *ca.* 0.01 γ/ml. for all four compounds. In comparative subcutaneous tests of the systemic activity of compounds **431** and **461** in protecting mice infected with a lethal dose of *S. hemolyticus*, both compounds behaved similarly, our results with **431** confirming those of Fisher and Doub[12] with the same compound.

Many of the arylmethoxydihydrotriazines (II, R′ = R″ = Me), unlike the corresponding diguanides, showed *in vivo* activity when administered subcutane-

(12) M. W. Fisher and L. Doub, *Biochem. Pharmacol.*, **3**, 10 (1959).

Table III

In Vitro Activity of Arylmethoxydihydrotriazines and Heterocyclic Dihydrotriazines (II, R′ = R″ = Me)

No.	R	MIC, γ/ml.		
		S. pyogenes	*E. coli*	*Ps. aeruginosa*
324	2-Phenanthrylmethyl	0.3	<0.03	
369	9-Anthrylmethyl	<0.6	<0.6	
373	9-Bromo-10-anthrylmethyl	<0.6	<0.6	150
377	8-Quinolylmethyl	19	9.5	
386	3-Pyrenylmethyl	<0.6	<0.6	37.5
388	2-Quinolylmethyl	9.5	19	
428	1,4-Benzodioxan-2-ylmethyl	19	2.4	>600
451	1-Methyl-4-naphthylmethyl	<0.6	<0.6	300
462	2-Methoxycarbonyl-5-furylmethyl	150	37	>600
508	2-Pyridylmethyl	300	300	>600
572	4-Diphenylylmethyl	2.4	19	75
580	4-(4′-Phenoxybenzyl)	4.7	19	75
Cf. 189	Benzyl	75	300	>600
202	1-Naphthylmethyl	0.6	0.075	300–600

Table IV

In Vitro Activity of Alkoxybenzyloxydihydrotriazines (II, R′ = R″ = Me)

No.	R	MIC, γ/ml.			
		S. pyogenes	*E. coli*	*Ps. aeruginosa*	*C. albicans*
376	4-Ethoxybenzyl	19	4.7	>600	
410	2-Ethoxybenzyl	19	2.4		
374	4-Propoxybenzyl	9.5	2.4		
429	2-Propoxybenzyl	2.4	37	600	150
375	4-Butoxybenzyl	9.5	1.2	300	
430	2-Butoxybenzyl	4.7	37	600	30
390	4-Pentyloxybenzyl	19	2.4	75–150	30
391	4-Isopentyloxybenzyl	19	<0.6	125	60
392	4-Hexyloxybenzyl	9.5	1.2	>600	30
458	2-Hexyloxybenzyl	<0.6	4.7	75	>240
393	4-Octyloxybenzyl	<0.6	1.2	>600	7.5
394	4-Decycloxybenzyl	<0.6	2.4	>600	15
453	3,4-Dimethoxybenzyl	37	9.5	>600	>240
454	3,5-Dimethoxybenzyl	19	<0.6	600	>240
460	2,6-Dimethoxybenzyl	9.5	75	600	
389	3,4-Methylenedioxybenzyl	75	9.5	>600	
435	3,4,5-Trimethoxybenzyl	9.5	4.7	600	

ously or orally to mice in doses of 100 mg./kg. Systemic activity was also shown by the decyloxy derivative (II, R = $C_{10}H_{21}$; R′ = R″ = Me) at a rather lower dosage; this result was rather unexpected from a surfactant type of compound. Some typical results against *S. hemolyticus* showing the reduced mortalities and increased survival times of treated mice are shown in Table V. All compounds showing appreciable *in vitro* activity were screened against this systemic mouse infection. Similarly, compounds active *in vitro* against *C. albicans* and *T. vaginalis* were examined in mice but none showed useful systemic activity against either organism or activity against enteric *C. albicans* infection.

Table V

In Vivo Activities of Some Arylmethoxydihydrotriazines[a] (II, R′ = R″ = Me) against *S. hemolyticus* Infections in Mice

No.	R	No. of mice injected	No. of survivors	Mean survival time of mice dying, days
202	1-Naphthylmethyl	6	3	4.7
208	2-Naphthylmethyl	6	3	3.3
239	1-Bromo-2-naphthylmethyl	6	4	5.5
275	4-*t*-Butylbenzyl	6	0	2.7
280	4-Ethylbenzyl	6	1	2.8
288	4-iso-Butylbenzyl	6	1	2.0
	Untreated control	12	0	1.4

[a] Administered subcutaneously in doses of 100 mg./kg.

Aryldihydrotriazines (VII) have been shown to be folic acid antagonists in systems utilizing the folic acid requiring microorganism *Streptococcus faecalis*.[13] We have observed that at least one of the arylmethoxytriazines (II, R = 1-naphthyl; R′ = R″ = Me, **202**) antagonized folic acid in a noncompetitive manner over a narrow range. It was therefore possible that the systemic activity of the oxytriazines might be of the type shown by the aryltriazines (VII) and by the diaminopyrimidines of Roth, *et al.*[14]

Fisher and Doub[12] demonstrated the synergistic effect of the triazine (VII, Ar = 3,5-$Cl_2C_6H_3$) with sulfamethoxypyridazine in acute and subacute streptococcal infections in mice. Their failure to demonstrate synergism *in vitro* may have been due to the presence of antisulfonamide substances in the medium used. Studies *in vitro* of the combined action of mixtures of **202** with sulfamethoxypyridazine have now been made against both *S. pyogenes* and *S. hemolyticus*. Synergistic inhibition was observed for both organisms.

(13) C. E. Foley, *Proc. Soc. Exptl. Biol. Med.*, **83**, 733, 740, 742 (1953).
(14) B. Roth, E. A. Falco, and G. H. Hitchings, *J. Med. Pharm. Chem.*, **5**, 1103 (1962).

TABLE VI
In Vitro ACTIVITY OF 2-SUBSTITUTED DIHYDROTRIAZINES (II)

No.	R	R'	R'	MIC, γ/ml. *S. pyogenes*	*E. coli*	*Ps. aeruginosa*	*C. albicans*	*T. vaginalis*
162	Decyl	Me	Me	9.5	9.5	2.4	10–20	62.5
534	Decyl		$(CH_2)_5$	<0.6	<0.6	>600	30	25
544	Decyl	CH_2CH_2	$CHMeCH_2CH_2$	<0.6	4.7	600	15	25
590	Decyl	H	$PhCH{=}CH$	1.2	4.7	75	15	12.5
164	Octyl	Me	Me	4.7	9.5		>60	
630	8-Hydroxyoctyl	CH_2CH_2	$CHMeCH_2CH_2$	2.4	2.4	150	240	250
202	1-Naphthylmethyl	Me	Me	0.6	0.075	300–600	>240	
596	1-Naphthylmethyl	H	Me	2.4	19		>240	>250
592	1-Naphthylmethyl	H	Et	2.4	19		>240	>250
588	1-Naphthylmethyl	H	$PhCH{=}CH_2$	2.4	19	150	>240	>250
535	1-Naphthylmethyl	H	*o*-$MeOC_6H_4$	9.5	9.5	300	>240	>250
494	1-Naphthylmethyl	CH_2CH_2	$CHMeCH_2CH_2$	9.5	<0.6		125	
510	1-Naphthylmethyl		$(CH_2)_5$	9.5	<0.6	300	125	300
216	3,4-Dichlorobenzyl	Me	Me	4.7	1.2	150		150
595	3,4-Dichlorobenzyl	H	Me	9.5	75		>250	
593	3,4-Dichlorobenzyl	H	Et	4.7	37		>250	
591	3,4-Dichlorobenzyl		$(CH_2)_5$	9.5	9.5	75	250	75
451	1-Methyl-4-naphthylmethyl	Me	Me	<0.6	<0.6	300		
533	1-Methyl-4-naphthylmethyl	CH_2CH_2	$CHMeCH_2CH_2$	2.4	<0.6	600	62.5	
532	1-Methyl-4-naphthylmethyl		$(CH_2)_5$	4.7	<0.6	300	62.5	

TABLE VII
In Vitro ACTIVITY OF BISTRIAZINYL DERIVATIVES

No.	Compd.	MIC, γ/ml. *S. pyogenes*	*E. coli*	*Ps. aeruginosa*	*C. albicans*
395	IV, Y = *p*-$CH_2C_6H_4CH_2$	19	2.4	>600	
551	IV, Y = $(CH_2)_5$	150	>600	>600	
702	IV, Y = $(CH_2)_6$	37	37	300	>60
717	IV, Y = $(CH_2)_8$	9.5	19	75	60–240
523	IV, Y = $(CH_2)_{10}$	1.2	<0.6	9.5	30
654	IV, Y = $(CH_2)_{12}$	2.4	4.7	37	30
701	V, R = H; *n* = 6	9.5	2.4	600	>60
703	V, R = CH_3; *n* = 6	4.7	2.4	300	>60
713	V, R = H; *n* = 8	2.4	1.2	300	30
576	VI, R = CH_3; *n* = 8	1.2	7.5	62.5	19
628	V, R = H; *n* = 10	<0.6	2.4	150	60
632	V, R = CH_3; *n* = 10	<0.6	2.4	150	30
552	V, R = H; *n* = 12	<0.6	2.4	75	1.9
553	V, R = CH_3; *n* = 12	1.2	2.4	300	1.9
574	IV, Y = *p*-$CH_2C_6H_4C_6H_4CH_2$-*p*	2.4	<0.6	37	60
583	IV, Y = *p*-$CH_2C_6H_4OC_6H_4CH_2$-*p*	<0.6	2.4	37	60

In attempts to obtain compounds showing greater activity against *Ps. aeruginosa*, the effect of different substituents at position 2 of the triazine ring was investigated. Little effect on the *in vitro* activities of triazines (II) was observed (Table VI) although the spirocyclohexyl derivatives **534** and **544** were considerably less active against *P. aeruginosa* than the dimethyl or styryl derivatives **162** and **590**. Replacement of the 2,2-dimethyl group by monomethyl reduced activity against *E. coli;* activity was essentially unchanged by replacement of dimethyl with spirocyclohexyl.

Compounds in which two triazine molecules were attached to the ends of a hydrocarbon chain (V and IV, Y = alkylene) and to *p,p'*-substituted diphenyls or diphenyl ether showed increasing broad spectrum activity with increasing chain length and a maximum activity with a chain of 10 carbon atoms. Activity against *Ps. aeruginosa* is often greatly reduced by the presence of a 2-spirocyclohexyl group (*cf.* **523** with **628**) but compare the anomalous **576** with **717**. Results are collected in Table VII.

Further Studies on the Properties of the 1-Decyloxytriazine (162).—Of the compounds listed in Table I, the 1-decycloxy derivative was selected for further study. Activities *in vitro* against a range of bacteria, fungi, and dermatophytes are given in Table VIII.

When mice infected intraperitoneally with *S. hemolyticus* group C (Pion strain, Wellcome Culture Collection No. 4) were given a single subcutaneous dose of 10 mg./kg., survival time was prolonged in comparison with an infected control group. By the oral route, a single dose of 10 mg./kg. failed to protect similarly infected mice, and doses of 100 mg./kg. and 1 g./kg. were toxic. This compound, therefore, shows systemic antibacterial activity together with toxicity. The therapeutic index is therefore low.

When tested by the technique of Calman and Murray[15] with *S. pyogenes*, the concentration of **162** bacter-

(15) R. M. Calman and J. Murray, *Brit. Med. J.*, 200 (1956).

TABLE VIII

In Vitro ACTIVITY OF **202** AND **162** IN BROTH AND IN THE PRESENCE OF 10% HORSE SERUM

Microorganism	202, γ/ml. MIC (Broth)	202, γ/ml. MLC[a] (Serum)	162, γ/ml. MIC (Broth)	162, γ/ml. MLC[a] (Serum)
S. hemolyticus Gp.A WO 63	0.003	0.015		
S. hemolyticus Gp.B WO 69	0.003	0.03		
S. hemolyticus Gp.C WO 71	0.003	0.003		
S. hemolyticus WO 61			0.15–0.6	0.3–1.2
S. faecalis WO 73	0.003	0.003	9.5	9.5
S. pyogenes WO 79	1.0	4.0	9.5	9.5
S. pyogenes 4163			0.6	0.6
S. pyogenes Oxford	0.5	1.0		
Corynebacterium sp. WO 81	0.25	0.5		
Bacillus subtilis ATCC 6633	0.0007	0.0015	<0.15	0.15–1.2
Shigella sonnei WO 84	1.0	4.0		
E. coli WO 95	4.0	>4.0	1.25	5
E. coli 8196	0.03	0.125		
E. coli WO 96	4.0	>4.0		
Proteus morganii WO 92	7.8			
P. vulgaris WO 91	1.95			
P. vulgaris WO 90	7.8			
P. mirabilis WO 94	3.9			
P. mirabilis WO 93	15.6		37	37
Ps. aeruginosa WO 83	300–600		2.4	4.7
Klebsiella sp.	0.97–1.95			
Salmonella enteritidis WO 87	1.95		19	19
Salm. anatum WO 86	3.9			
Salm. typhimurium WO 88	2.0–4.0	>4.0	19	19
C. albicans	>150		7–15	
Trichophyton mentagrophytes			4.8	

[a] Minimum lethal concentration.

icidal in 2.5 min. at room temperature was 1200 γ/ml. as compared with that of chlorhexidine (600 γ/ml.); concentrations bactericidal after 48 hr. at 37° were 9.5 and 2.4 γ/ml. Compound **162** appears to be almost as rapidly bactericidal as chlorhexidine but unlike the latter is readily absorbed after oral and parenteral administration.

Further Studies on the Properties of the 1-Naphthylmethoxytriazine (202).—A wide range of *in vitro* activity was shown by this compound and results are given in Table VIII. Contact time tests showed it to be slower acting than the 1-decyloxytriazine against *S. pyogenes*.

Subcutaneous administration to mice in doses of 100 mg./kg. delayed deaths from intraperitoneally induced infections with *S. hemolyticus*. Administered orally, the dose required to protect infected mice was variable. Because of its acute toxicity (LD_{50} oral 960 mg./kg., i.p. 90 mg./kg.), systemic use was not considered.

When administered in the food at a level of 50 p.p.m., it was found to be toxic to chickens from 1 day old. This chronic toxicity was shown to be attributable to the antifolic acid effect of the compound which could be antagonized by the addition of as little as 1 p.p.m. of folic acid to the diet. Antagonism between **202** and folic acid was demonstrated with the folic acid requiring organism *S. faecalis* and shown to be of a noncompetitive nature. The compound was effective against experimentally induced *Eimeria tenella* infections in chickens and this anticoccidial activity was not reversed by the addition of 1 p.p.m. of folic acid to the diet. Similar effects have been observed with pyrimethamine by Joyner.[16]

(16) L. P. Joyner, *Res. Vet. Sci.*, **1**, 2 (1960).

Experimental[17]

Alcohols were prepared from benzaldehyde and Grignard reagents, and their properties agreed largely with the literature values. The following were prepared: 1-phenyl-1-propanol,[18] b.p. 92–94° (8 mm.), $n^{24}D$ 1.5194; 1-phenyl-1-pentanol,[19] b.p. 114–118° (8 mm.), $n^{24}D$ 1.5093; 1-phenyl-1-heptanol,[20] b.p. 144–148° (12 mm.), $n^{20}D$ 1.5040; 1-phenyl-1-nonanol,[21] b.p. 168–173°, $n^{24}D$ 1.4940; 1-phenyl-1-undecanol,[22] b.p. 155° (0.4 mm.), $n^{22}D$ 1.4878, solidified on cooling.

4-Phenyl-1-butanol.—A Grignard reagent was prepared from 14.0 g. of magnesium turnings and 89 g. of phenethyl bromide in 200 ml. of dry ether. To the cooled (−10°) stirred mixture was added a solution of 31.5 g. of ethylene oxide in 250 ml. of ether over 1.5 hr., and the mixture refluxed 2 hr. The ether was replaced by 500 ml. of dry benzene and refluxed for 45 min., cooled, and decomposed with ice and 30% sulfuric acid. Extraction with ether and evaporation of the solvent gave an oil which on distillation gave two main fractions: (a) b.p. up to 74° (0.35 mm.), $n^{19}D$ 1.5070 (26.0 g.); and (b) b.p. 90–96° (0.35 mm.), $n^{19}D$ 1.5225 (42.5 g.). Redistillation of (a) gave a fraction, b.p. 47–48° (11 mm.), $n^{21}D$ 1.4890, which was substantially pure ethylene bromohydrin (by analysis, refractive index, and infrared spectrum). Distillation of (b) gave 4-phenyl-1-butanol, b.p. 88–90° (0.25 mm.), $n^{20}D$ 1.5224, a figure differing considerably from that in the literature.[23]

Anal. Calcd. for $C_{10}H_{14}O$: C, 80.01; H, 9.34. Found: C, 79.90; H, 9.34.

(17) Melting points are uncorrected. The purity of previously described materials was checked by infrared spectra and by gas phase chromatography.

(18) H. Davies and F. S. Kipping, *J. Chem. Soc.*, **99**, 298 (1911), give b.p. 106–108° (18 mm.).

(19) R. O. Roblin, D. Davidson, and M. T. Bogert, *J. Am. Chem. Soc.*, **57**, 155 (1935), give b.p. 137° (21 mm.).

(20) U. Calaticchi, *Atti reale accad. Lincei*, [5] **19**, 601 (1910).

(21) J. Harman and C. S. Marvel, *J. Am. Chem. Soc.*, **54**, 2519 (1932), give b.p. 124–129° (3 mm.), $n^{20}D$ 1.4966.

(22) F. L. Breusch and M. Oguze, *Chem. Ber.*, **87**, 1225 (1954), give m.p. 33–34°.

(23) S. P. Lagerev, *Tr. Uzbeksk. Gos. Univ.*, **6**, 71 (1936), gives b.p. 140° (14 mm.), $n^{16}D$ 1.4310.

TABLE IX
HYDROBROMIDES OF DIHYDROTRIAZINES (III)

No.	R	R'	R''	n	M.p., °C.	% carbon Calcd.	% carbon Found	% hydrogen Calcd.	% hydrogen Found	% nitrogen Calcd.	% nitrogen Found
...	CH_3	$(CH_2)_5$		0	238–239[a]	51.50	51.68	6.57	6.83	17.69	17.74
746	C_2H_5	$CH_2CH_2CH(CH_3)CH_2CH_2$		0	219–220[b]	52.68	52.43	6.84	6.79	17.11	16.83
751	C_2H_5	CH_3	CH_3	0	193–195[c]	47.21	46.97	6.18	6.01	19.68	19.39
819	C_4H_9	CH_3	CH_3	0	195–197[d]	50.00	50.23	6.78	6.95	18.25	18.42
749	C_4H_9	$CH_2CH_2CH(CH_3)CH_2CH_2$		0	215–217[b]	54.86	54.82	7.37	7.17	15.98	15.82
817	C_6H_{13}	$(CH_2)_5$		0	194[a]	55.74	55.82	7.58	7.77	15.50	15.39
818	C_7H_{13}	CH_3	CH_3	0	198–199[d]	52.45	52.70	7.28	7.39	17.00	17.26
825	C_8H_{17}	CH_3	CH_3	0	229–230[e]	54.56	54.44	7.73	7.97	15.91	15.80
810	C_8H_{17}	$CH_2CH_2CH(CH_3)CH_2CH_2$		0	208–210[f]	58.38	58.43	8.18	8.28	14.18	14.01
822	$C_{10}H_{21}$	$(CH_2)_5$		0	195–197[f]	59.00	59.30	8.28	8.43	13.76	13.65
790	H	$CH_2CH_2CH(CH_3)CH_2CH_2$		1	257[f]	51.50	51.26	6.57	6.35	17.67	17.39
757	H	$CH_2CH_2CH(CH_3)CH_2CH_2$		2	226–227[f]	52.76	53.07	6.84	6.60	17.07	17.14
756	H	CH_3	CH_3	3	180[d]	48.65	48.57	6.53	6.46	18.91	18.65
753	H	$CH_2CH_2CH(CH_3)CH_2CH_2$		3	223–224.5[f]	53.79	53.76	7.07	6.84	16.54	16.67
	H	CH_3	CH_3	5	188–190[f]	51.22	51.07	7.08	7.13	17.57	17.68
	H	$(CH_2)_5$		5	214–215[f]	54.80	55.05	7.31	7.21	15.97	16.17
	H	CH_3	CH_3[g]	7	177[b]	52.28	52.32	5.96	5.79	19.50	19.21
	H	$(CH_2)_5$		7	210–212[f]	56.65	56.81	7.72	7.40	15.03	15.11

[a] Recrystallized from water containing a few drops of 48% HBr. [b] Recrystallized from aqueous ethanol. [c] Recrystallized from ethanol. [d] Recrystallized from 2-propanol. [e] Recrystallized from 2-propanol–ether. [f] Recrystallized from aqueous ethanol containing a few drops of 48% HBr. [g] Picrate, prepared by treatment of the crude hydrobromide with aqueous lithium picrate.

6-Phenylhexan-1-ol was prepared by the method of Crombie and Harper[24] from 2,3-dichlorotetrahydropyran and benzylmagnesium chloride; this product had b.p. 148–154° (8 mm.), $n^{22}D$ 1.5162.[25]

Bromides were prepared by treatment of the alcohols with hydrogen bromide in benzene at room temperature: 1-phenyl-1-bromopropane,[26] b.p. 87–90° (8 mm.), $n^{22}D$ 1.5510; 1-phenyl-1-bromopentane,[27] b.p. 115–117° (8 mm.), $n^{23}D$ 1.5355.

1-Phenyl-1-bromoheptane had b.p. 94° (0.25 mm.), $n^{23}D$ 1.5230.

Anal. Calcd. for $C_{13}H_{19}Br$: C, 61.17; H, 7.46; Br, 31.88. Found: C, 61.32; H, 7.32; Br, 31.99.

1-Phenyl-1-bromononane had b.p. 108° (0.25 mm.), $n^{23}D$ 1.5165.

Anal. Calcd. for $C_{15}H_{23}Br$: C, 63.69; H, 8.19; Br, 28.26. Found: C, 63.51; H, 8.40; Br, 28.05.

1-Phenyl-1-bromoundecane had b.p. 132° (0.25 mm.), $n^{24}D$ 1.4990. Analysis showed this compound to be impure, but it gave a good yield of triazine **822**.

4-Phenyl-1-bromobutane was prepared in the usual way; this compound had b.p. 76–80° (0.25 mm.), $n^{21}D$ 1.5390; lit.[23] b.p. 130° (10 mm.), $n^{16}D$ 1.598. Analysis showed this compound to be impure. Pure triazines (**753** and **756**) were, however, prepared from this material.

6-Phenyl-1-bromohexane was prepared from the carbinol with hot 48% hydrobromic acid; the bromide[25] had b.p. 149–154° (8 mm.), $n^{22}D$ 1.5305.

Anal. Calcd. for $C_{12}H_{17}Br$: Br, 33.26. Found: Br, 33.15.

4-Chloro-3-nitrobenzyl Bromide.—Five grams of 4-chloro-3-nitrotoluene, heated under reflux in 30 ml. of CCl_4 and illuminated with a 500-w. lamp, was treated with 3.2 g. of bromine in 10 ml. of CCl_4 over 30 min. Evaporation and distillation of the residue gave 1.5 g. of unchanged material and 3.35 g. of product, b.p. 116–119° (0.25 mm.), $n^{23}D$ 1.6130.

Anal. Calcd. for $C_7H_5BrClNO_2$: C, 33.55; H, 1.99; N, 5.58. Found: C, 33.71; H, 2.16; N, 5.23.

4-Chloro-3-nitrobenzyl benzhydroxamate was prepared from the benzyl bromide and sodium benzhydroxamate[10]; the product had m.p. 108–109° [ethyl acetate–petroleum ether (b.p. 60–80°)], yield 50%.

Anal. Calcd. for $C_{14}H_{11}ClN_2O_4$: C, 54.80; H, 3.59; N, 9.15. Found: C, 55.00; H, 3.38; N, 9.34.

4-Chloro-3-nitrobenzyloxyamine.—A mixture of 17.6 g. of 4-chloro-3-nitrobenzyl benzhydroxamate, 12.5 ml. of concentrated HCl and 75 ml. of methanol was refluxed for 2 hr., the methanol was removed, and the residue was partitioned between water and petroleum ether (b.p. 60–80°). A small amount of insoluble material at this point was identified as diphenylurea, m.p. 243–245°. The aqueous layer was basified with 4 *N* NaOH and extracted with ether. Evaporation of the ether and distillation of the residual oil gave 10.1 g. of product, b.p. 128–131° (0.25 mm.), $n^{23}D$ 1.5800.

Anal. Calcd. for $C_7H_7ClN_2O_3$: C, 41.53; H, 3.46; N, 13.85. Found: C, 41.45; H, 3.65; N, 13.58.

1-(4-Chloro-3-nitrobenzyloxy)-4,6-diamino-1,2-dihydro-1,3,5-triazine-2-spiro[4-methylcyclohexane] Hydrobromide.—A solution of 7.8 g. of 4,6-diamino-1,2-dihydro-1-hydroxy-1,3,5-triazine-2-spiro[4-methylcyclohexane] hydrochloride in 50 ml. of methanol was treated with 1.26 g. of NaOH in a little water. Solvents were removed under reduced pressure, and the residue was dried by azeotropic distillation with benzene. A mixture of the dried residue, 8.0 g. of 4-chloro-3-nitrobenzyl bromide, and 60 ml. of dimethylformamide was stirred at 100° for 10 min. and cooled, and the inorganic material was filtered off. After removal of the solvent, the residual solid (10.35 g.) was stirred with cold water and the insoluble material was crystallized from aqueous ethanol giving 6.45 g. of white needles, m.p. 218–220°.

Anal. Calcd. for $C_{16}H_{22}BrClN_6O_3$: C, 41.68; H, 4.81; N, 18.18. Found: C, 41.89; H, 4.96; N, 17.91.

Other triazines prepared by a similar procedure are given in Tables IX, X, and XI.

4,6-Diamino-1,2-dihydro-1-(8-hydroxyoctyloxy)-1,3,5-triazine-2-spiro[4-methylcyclohexane] Hydrobromide.—A suspension of the base from 6.54 g. of 4,6-diamino-1,2-dihydro-1-hydroxy-1,3,5-triazine-2-spiro[4-methylcyclohexane] hydrochloride suspended in 50 ml. of dimethylformamide was treated with 3.6 g. of octamethylene dibromide and stirred on the steam bath for 20 min. After cooling, inorganic material was filtered off, and the filtrate was evaporated. Trituration of the residue with acetone afforded 10.4 g. of crude material which, after several crystallizations from aqueous ethanol, gave 2.0 g. of colorless needles, m.p. 186–190°, which by analysis appeared to be the title compound and not the expected symmetrical 1,8-bis(triazinyloxy)octane.

Anal. Calcd. for $C_{17}H_{34}BrN_5O_2$: C, 48.71; H, 8.13; N, 16.66. Found: C, 48.90; H, 8.29; N, 16.78.

Treatment of the crystallization liquor with aqueous lithium picrate failed to give a crystalline picrate.

Attempts to treat the 1-hydroxytriazine base with 1-bromooctan-8-ol failed to give crystalline products.

4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-(10-hydroxydecyloxy)-1,3,5-triazine Hydrochloride.—A suspension of the base from 8.2 g. of 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-hydroxy-1,3,5-triazine hydrochloride in 60 ml. of dimethylformamide was stirred on the steam bath with 10.69 g. of 1-bromodecanol for 30

(24) L. Crombie and S. H. Harper, *J. Chem. Soc.*, 1707 (1950).

(25) J. von Braun, *Ber.*, **44**, 2876 (1911), gives b.p. 166–168° (13 mm.).

(26) V. Grignard and K. Ono, *Bull soc. chim. France*, [4] **39**, 1593 (1926), give b.p. 112–114° (15 mm.), $n^{19}D$ 1.5517.

(27) J. B. Conant and A. H. Blatt, *J. Am. Chem. Soc.*, **50**, 555 (1928), give b.p. 120–130° (10 mm.).

TABLE X
BISTRIAZINYLALKANES IV AND V

No.	Type	R	n	Y	Salt	M.p., °C.	% carbon Calcd.	% carbon Found	% hydrogen Calcd.	% hydrogen Found	% nitrogen Calcd.	% nitrogen Found
551	IV	..	..	$(CH_2)_5$	2HBr	239–241[a,b]	33.13	33.26	5.88	6.00	25.74	25.43
702	IV	..	..	$(CH_2)_6$	2HBr	266[c]	41.37	41.23	6.58	6.51	21.96	22.22
717	IV	..	..	$(CH_2)_8$	2HBr	223–226[d,c]	36.93	37.02	6.52	6.56	23.86	23.63
..	IV	..	..	$(CH_2)_8$	Dipicrate	237–239[f]	40.78	40.95	4.78	4.90	25.38	25.31
714	IV	..	.	$(CH_2)_9$	2HBr	212–214[g]	38.10	38.34	6.66	6.77	23.35	23.28
523	IV	..	..	$(CH_2)_{10}$	2HBr	216–218[c]	39.18	39.27	6.87	7.02	22.78	22.94
654	IV	..	..	$(CH_2)_{12}$	2HBr	209–211[c]	41.09	40.80	7.17	7.15		
577	IV	..	..	$(CH_2)_{12}$	Disaccharinate	182–184[g]	50.80	50.91	6.87	6.87	19.75	19.92
658	IV	..	..	$(CH_2)_{12}$	Disaccharinate	115–118[h]	50.80	50.91	6.87	6.68	19.75	19.88
701	V	H	6	...	2HBr	250–252[i]	41.37	41.52	6.58	6.39	22.03	22.21
703	V	CH_3	6	...	2HBr	252–253[c]	43.21	43.15	6.91	6.75	21.10	21.14
713	V	H	8	...	2HBr[j]	250–252[c]	43.21	42.94	6.91	6.90	21.10	21.24
576	V	CH_3	8	...	2HBr[k]	209–212[c]	45.02	45.30	7.24	7.40	20.18	19.95
585	V	H	9	...	2HBr	245[d]	44.00	44.18	7.04	7.07	20.55	20.61
575	V	CH_3	9	...	2HBr	218–220[m]	45.72	46.01	7.38	7.59	19.82	19.79
628	V	H	10	...	2HBr	254–255[d]	45.02	45.13	7.24	7.04	20.18	19.88
632	V	CH_3	10	...	2HBr	237–239[n]	46.51	46.73	7.48	7.18	19.38	19.16
552	V	H	12	...	2HBr	231–234[o]	46.51	46.74	7.48	7.60	19.38	19.24
553	V	CH_3	12	...	2HBr	241–243[d]	48.03	48.09	7.78	7.59	18.63	18.86

[a] Crystallized from aqueous ethanol containing a few drops of 48% HBr. [b] Further purification was not attempted. [c] Crystallized from ethanol–ether. [d] Prepared by cyclization of the dignanide with acetone. [e] Crystallized from aqueous ethanol–ether. [f] Crystallized from acetic acid. [g] Crystallized from ethanol–ethyl acetate. [h] Prepared subsequently to **577**, this compound also appeared to be a disaccharinate with identical *in vitro* and *in vivo* antibacterial properties. [i] Obtained by drying the hemihydrate, m.p. 236–238°. [j] *Anal.* Calcd. for $C_{22}H_{42}Br_2N_{10}O_2$: C, 40.79; H, 6.70; N, 21.72. Found: C, 40.72; H, 6.44; N, 21.97. [k] Hemihydrate. [m] For an experiment in which a different product was obtained, see text.

TABLE XI
DIHYDROTRIAZINES (II)

No.		R'	R''	Salt	M.p., °C.	% carbon Calcd.	% carbon Found	% hydrogen Calcd.	% hydrogen Found	% nitrogen Calcd.	% nitrogen Found
760	$C_{10}H_{21}$	$(CH_2)_6$		HCl	214–216[a]	58.81	58.93	9.81	9.84	18.08	18.13
	$C_{11}H_{23}$	CH_3	CH_3	HBr	195–197[b]	48.94	48.80	8.73	8.80	17.91	17.83
	$C_{14}H_{29}$	CH_3	CH_3	HBr	195–196[b]	52.53	52.68	9.20	9.14	16.14	16.29
	$C_6H_5CH{=}CHCH_2$	CH_3	CH_3	HBr	217–218[c]	47.48	47.22	5.65	5.54	19.80	19.77
	$C_6H_5CH{=}CHCH_2$	$(CH_2)_5$		HBr	227–228[d]	51.84	51.99	6.08	6.17	17.83	18.04
	$C_6H_5CH{=}CHCH_2$	$CH_2CH_2CH(CH_3)CH_2CH_2$		HBr	228–229[d]	52.90	53.14	6.37	6.37	17.22	17.39
	1-$C_{10}H_7CH_2$[e]	H	C_6H_5	HCl	210–212[f]	62.98	63.07	5.24	5.19	18.35	18.48

[a] Crystallized from ethanol containing hydrogen chloride. [b] Crystallized from dilute aqueous HBr. [c] Crystallized from ethanol. [d] Crystallized from aqueous ethanol containing HBr. [e] 1-Naphthylmethyl. [f] Crystallized from ethanol–petroleum ether (b.p. 60–80°).

min. The cooled mixture was filtered, and the filtrate was evaporated under reduced pressure. The low-melting solid remaining after trituration with acetone and ether was dissolved in methanol and treated with aqueous lithium picrate giving a crude picrate (7.2 g.), m.p. 168–172°. Several crystallizations from ethanol gave the triazine picrate (4.4 g.), m.p. 180–182°.

Anal. Calcd. for $C_{21}H_{34}N_8O_9$: C, 46.43; H, 6.25; N, 20.64. Found: C, 46.57; H, 6.35; N, 20.55.

Treatment of the picrate (4.4 g.) with dilute HCl and ether gave the triazine hydrochloride (2.6 g.), m.p. 140–142°.

Anal. Calcd. for $C_{15}H_{32}ClN_5O_2$: C, 51.46; H, 9.16; N, 20.01. Found: C, 51.28; H, 9.30; N, 20.23.

Microbiological Methods. (1) *In Vitro* **Tests. A. General Methods.**—Minimum inhibitory concentrations (MIC) for *S. pyogenes*, *E. coli*, and *Ps. aeruginosa* were determined by inoculating doubling dilutions of compounds in concentrations of 600–0.6 γ/ml. in 0.5 ml. of Lemco broth with 1 drop of a 10^{-5} dilution of logarithmic phase culture (*S. pyogenes* and *E. coli*) or 0.2×10^{-1} dilution (*Ps. aeruginosa*). Turbidity readings were recorded after 18 hr. of incubation at 37°. For tests against *C. albicans* compounds were diluted in 0.5-ml. vol. of peptone–water and inoculated with 1 drop of an overnight broth culture; minimum inhibitory concentrations were read after 24 hr. of incubation at 37°. Trichomonacidal concentrations were determined by inoculating 10 ml. of liver-infusion broth containing dilutions of compounds with 0.1 ml. of a 48-hr. culture of *T. vaginalis* and examining microscopically for motile trichomonads after 72 hr. of incubation at 37°.

B. Tests for Combined Action of Sulfamethoxypyridazine and 202 against *S. pyogenes* and *S. hemolyticus*, Group C.—Both compounds were added to 0.5-ml. vol. of Jewell and Pearmain's medium[28] containing 1% horse blood singly and in combinations over a range of concentrations and inoculated with 1 drop of a 10^{-3} dilution of logarithmic phase culture. Turbidity readings after 24 hr. of incubation indicated that the minimum inhibitory concentration of the compounds in combination was lower than that of either tested singly.

(2) *In Vivo* **Tests.**—Compounds showing activity *in vitro* were tested against appropriate infections in mice by the following methods.

A. Protection against Systemic *S. hemolyticus* Infection in Mice.—A four-stage screening procedure (Table XII) was used,

TABLE XII

Stage	Route	Dose × 100, mg./kg.
1	Subcutaneous	1
2	Subcutaneous	3 (during 7 hr.)
3	Oral (mice previously starved for 4 hr.)	1
4	Oral (mice previously starved for 4 hr.)	10

in each stage of which 8 mice were dosed with the compound and 4 of these were, in addition, infected with a lethal dose of *S.*

[28] P. Jewell and G. E. G. Pearmain, *J. Clin. Path.*, **7**, 308 (1954).

hemolyticus, group C (Pion strain B.W., C.N.4.). Thus both the mouse toxicity of the compound and its effect upon the course of infection could be studied simultaneously. Compounds were suspended in 5% gum acacia and given in a dose of 0.5 ml. immediately after intraperitoneal infection with 0.2 ml. of a 10^{-3} dilution of 6-hr. blood broth culture of the streptococcus.

B. Evaluation of Compounds against *Candida albicans* Infection in Mice. (i)—A technique described by Lindh,[29] in which an infection of the gastrointestinal tract of mice was produced by administering a diluted fluid Sabouraud medium culture of *C. albicans* in lieu of drinking water. The compound under investigation was administered, at previously determined nontoxic doses in the food, and quantitative estimations of *C. albicans* were made from fecal pellets. Only active compounds, such as the control drug nystatin, which are not appreciably absorbed from the gastrointestinal tract, effectively suppressed this infection. Compounds potentially suitable for topical application may be revealed by this method.

(ii)—To study activity against systemic *C. albicans* infection, mice were injected intravenously with a culture of *C. albicans* of standard density and dosed subcutaneously with 50 mg./kg. of the compound under investigation, the initial dose being given 2 hr. after infection and subsequent doses 24 and 48 hr. later. Unprotected mice usually died within 21 days due to systemic spread of infection from primary kidney lesions. Amphotericin in 3 doses of 12.5 mg./kg. protected the majority of mice. Details of this technique were kindly supplied by Mr. L. J. Hale, Boots Pure Drug Co. Ltd., Nottingham, England.

C. Activity against *Trichomonas vaginalis* Infection in Mice.—The literature describing attempts to induce trichomonas infection in laboratory animals and the experimental chemotherapy of such infection has been comprehensively reviewed by Ryley and Stacey.[30] The following technique was selected because in infection caused by *T. vaginalis*, topically active drugs have been largely superseded by those that are active after oral administration.

Mice, in groups of 10, were injected subcutaneously with approximately 2×10^6 *T. vaginalis* in 0.5 ml. of liver-infusion medium and immediately given a single oral dose of 100 mg./kg. of compound; similar doses were given on each of the following 4 days. The mice were killed 7 days after infection and examined for trichomonal subcutaneous lesions. The majority of mice given doses of metronidazole (12.5 mg./kg.) on this schedule were free from lesions.

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(29) H. F. Lindh, *Antibiot. Chemotherapy*, **9**, 226 (1959).

(30) J. F. Ryley and G. J. Stacey, *Parasitology*, **53**, 303 (1963).

New Sulfonamides

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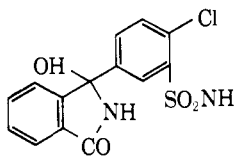
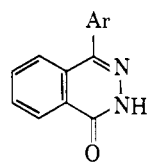
The preparation is described of several 4-aryl-1(2H)-phthalazinones and 3-aryl-3,4-dihydro-(2H)-1,2,4-benzothiadiazine 1,1-dioxides. The compounds were inactive in diuretic tests.

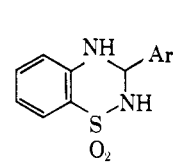
The isoindoline derivative I (chlorthalidone),[1] although developed from the disulfonamide carbonic anhydrase inhibitors, was shown to have a similar electrolytic excretion pattern to the thiazides.[2,3] It differs structurally from the thiazides in that the heterocycle is attached to the benzene ring bearing the sulfonamido and halogen groups by a single bond to a quaternary carbon atom and is thus, unlike the thiazides, nonplanar. In order to find whether other acidic heterocyclic ring structures could replace the isoindoline ring of I, the compounds II and III (Ar = 4′-Cl-3′-$H_2NSO_2C_6H_3$) and related structures were prepared for testing as diuretics (see Tables I and II).



I



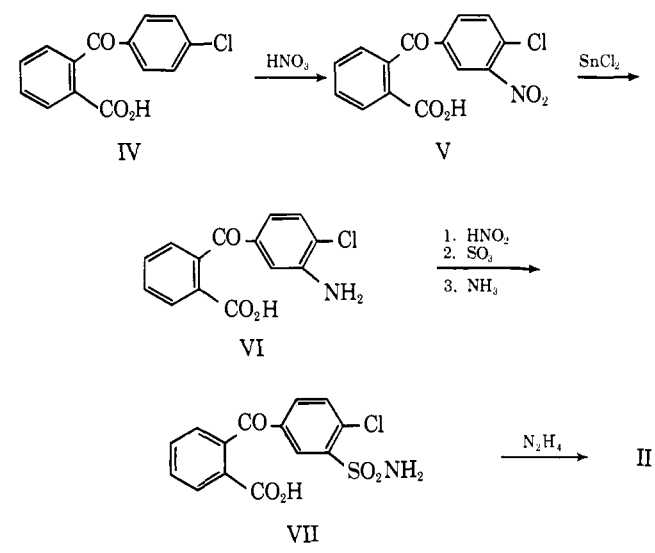
II



III

The phthalazinone II was prepared from 4′-chlorobenzophenone-2-carboxylic acid by the route shown in Chart I.

CHART I



4′-Chloro-3′-nitrobenzophenone-2-carboxylic acid (V) was first obtained[4,5] by nitration of 4′-chlorobenzophenone-2-carboxylic acid (IV) in a sulfuric–nitric acid mixture. In our hands this procedure led to a mixture of dinitro compounds. Nitration of IV with fuming nitric acid at 90° gave a mixture of mono and dinitro compounds,

(1) W. Graf, E. Girod, E. Schmid, and W. G. Stoll, *Helv. Chim. Acta*, **42**, 1085 (1959).

(2) E. G. Stenger, H. Wirz, and R. Pulver, *Schweiz. Med. Wochschr.*, **89**, 1126, 1130 (1959).

(3) R. Veyrat, E. F. Arnold, and A. Duckert, *ibid.*, **89**, 1133 (1959).

(4) Basler Chem. Fabrik., German Patent 148,110 (1903); *Chem. Zentr.*, I, 328 (1904).

(5) W. Bradley and H. E. Nurster, *J. Chem. Soc.*, 2180 (1951).