g. (23%), m.p. 200–203°. One recrystallization from alcohol gave pure material, m.p. 203–204°. A mixed melting point with an authentic sample prepared according to Hilbert[13] gave 202–204°. The ultraviolet absorption spectra were also similar (maximum at 274 mμ, minimum at 243 mμ).

A small sample of X was treated with methanolic hydrogen chloride according to Hilbert, and 1-β-D-glucopyranosyluracil[13] was isolated, m.p. 199–201°. A mixed melting point with authentic material[13] gave no depression.

Synthesis of Cytidine.—1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribose[16] (0.02 mole) was added to 250 ml. of anhydrous ether saturated with hydrogen chloride at 0°. After 4 days at 5–10° the solvent was removed *in vacuo* as previously described, and a benzene solution of the sirupy halogenose was added to an azeotropically-dried mixture of 7.5 g. of IX in hot xylene. After 5 minutes under reflux the stirred mixture clarified. After an additional 20 minutes the reaction was cooled, treated with petroleum ether, filtered and the precipitate dissolved in chloroform. The chloroform solution was treated in the usual manner, and upon removal of the solvent a sirup was obtained which was taken up in a minimum volume of warm ethyl acetate and treated with ether. Upon cooling overnight some unreacted 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose separated out (0.3 g., m.p. 126–129°) and was removed. In the filtrate a mass of crystals appeared which, upon filtration, gave 3.7 g. (XI) of white powdery material, m.p. 96–106° (to a viscous liquid). An additional 3.2 g. of lower melting material precipitated from the mother liquor (total yield of crude material 60%). Both fractions were combined and used directly for conversion to free nucleosides.

Crude XI (1.5 g.) was treated with 50 ml. of alcoholic ammonia in a sealed tube at 100° overnight. The contents were worked up in the usual manner (*vide supra*) and gave 520 mg. of cytidine (as the sulfate), 70%. Recrystallization from ethanol–water did not raise the melting point, 222–223°, and a mixed melting point with the synthetic material prepared *via* the *N*-acetylcytosine route or with material prepared from natural cytidine gave no depression.

Crude XI (0.4 g.) in 50 ml. of ethanol was treated with 2 ml. of sodium ethoxide (1 *N*) and the solution refluxed for 1 hr. The reaction was acidified with concentrated hydrochloric acid (0.5 ml.) and filtered from some sodium chloride. The acidic filtrate was warmed to reflux temperature for 10 minutes and then concentrated to a sirup which was taken into water and extracted with ether. The ether layer was discarded and the water layer was treated with charcoal and filtered. A spectral determination of the aqueous solution showed that uridine was present (no shift in the spectrum between *p*H 1 and 7, maximum at 262 mμ; in 0.1 *N* alkali, maximum at 263 mμ). When chromatographed in butanol–water (86/14) a major spot (similar in R_f to uridine) was obtained along with a minor component.

Polarimetric Investigations.—Optical rotations were determined with a polarimetric unit model D attachment[17] to the Beckman model DU spectrophotometer calibrated with standard sucrose solutions. For the determination of the rotations of the dialdehydes produced, solutions of known concentrations were treated in the polarimetric cell with excess sodium metaperiodate. Readings were taken at frequent intervals until a constant reading was attained. The specific rotations of the dialdehydes produced were based upon the original concentrations of the nucleoside solutions.

Metaperiodate Titration Studies.—Concentrations of nucleosides ranging between 0.0015 and 0.002 mmole per ml. were treated with excess sodium metaperiodate and titrated iodometrically according to the usual procedures.[18,19] The acidity produced was determined by the method of Jackson and Hudson.[20]

Acknowledgments.—The authors are indebted to Drs. A. Bendich and G. B. Brown for helpful discussions and continued interest.

(17) Standard Polarimeter Co., New York, N. Y.

(18) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).

(19) B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 592 (1944).

(20) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **61**, 1530 (1939).

NEW YORK 21, N. Y.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE U. S. VITAMIN CORPORATION]

Guanamine Diuretics

BY SEYMOUR L. SHAPIRO, VINCENT A. PARRINO, KARL GEIGER, SIDNEY KOBRIN AND LOUIS FREEDMAN

RECEIVED MARCH 28, 1957

A series of guanamines of the type I has been synthesized and evaluated as oral diuretics in rats. Diuretic activity is found to be critically dependent on structural characteristics of the group R. Certain of the variants of this series are the most active guanamine diuretics so far reported.

Although the diuretic activity of formoguanamine[1-4] (I, R = H) has been known for some time, it is only recently that structural variations with enhanced diuretic activity[5] have been characterized.

(1) (a) W. L. Lipschitz and E. Stokey, *J. Pharmacol.*, **83**, 235 (1945); (b) W. L. Lipschitz and Z. Hadidian, *ibid.*, **81**, 84 (1944) (diuretic studies in animals).

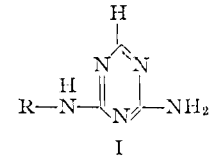
(2) (a) S. A. Freire, *Rev. brasil. biol.*, **8**, 1 (1948) [*C. A.*, **42**, 7876 (1948)]; (b) A. Turchetti, *Riforma med.*, **64**, 405 (1950) [*C. A.*, **44**, 10165 (1950)] (the mode of activity).

(3) (a) S. A. Freire, *Arquiv. biol.* (*São Paulo*), **31**, 141 (1947) [*C. A.*, **42**, 4672 (1948)]; (b) W. L. Lipschitz and E. Stokey, *J. Pharmacol. Exp. Therap.*, **92**, 131 (1948); (c) L. DeBellis, *Boll. soc. ital. biol. sper.*, **29**, 1224 (1953) [*C. A.*, **48**, 12306 (1954)] (use in humans).

(4) A. A. Kattus, E. V. Newman and J. Franklin, *Bull. Johns Hopkins Hosp.*, **89**, 1 (1951) [*C. A.*, **45**, 10401 (1951)] (use of acylated derivatives in human studies).

(5) (a) O. Chandler and G. Bulcsu, *Magyar Kém. Folyóirat*, **57**, 68 (1951) [*C. A.*, **46**, 4023 (1952)]; (b) Richter and Gedeon, *Vegyészeti Gyár Rt.*, (Hungarian Corp.), British Patent 676,024; (c) D. A. LeSher and F. E. Shideman, *J. Pharmacol. Exp. Therap.*, **116**, 38 (1956).

Maximum activity was noted in the compounds I, where R = phenyl, *p*-chlorophenyl, *p*-bromophenyl. Structures of this type have been reported[6] to yield remarkable results in cases of cardiac edema.



I

An additional variant,[7] claimed to yield diuretic activity, includes compounds of the type where R = H, and the hydrogen in the 6-position is replaced by a dialkylaminomethyl group.

(6) G. V. Austerweil, *Chemistry & Industry*, 372 (1952).

(7) V. Ettel and J. Nosek, *Chem. Listy*, **46**, 289 (1952) [*C. A.*, **47**, 4344 (1953)].

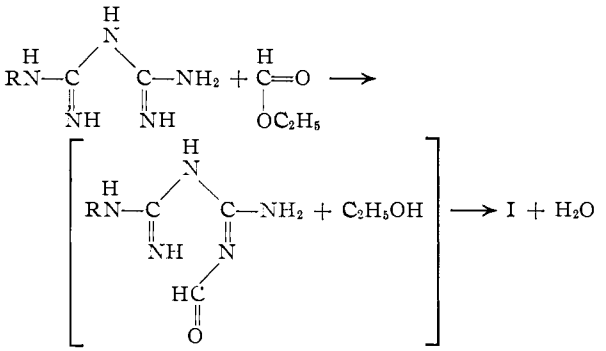
Our study was concerned with the locus of structural variation which would give maximal diuretic activity and minimal toxicity.

Synthesis of I, R = phenyl with substituents in position 6 such as CH_3, C_2H_5,[5a] CH_2CH_2–OCH_3, CH_2CH_2–O–C_2H_5, CH_2–CH_2–OH,[8] OH, CH_2Cl, $CHCl_2$[9] or acetylation of the amino group in the 4-position[10] or substituting an additional group in the R-bearing amino group (see Table I), indicated upon evaluation as diuretics that peak activities were restricted largely to variation within R, with the remaining positions in I being desirably unsubstituted.

The scope of the work was designed to include a systematic variation in R, where the groups were alkyl, isocyclic, cycloalkyl, arylalkyl and aryl, despite indications that the use of alkyl groups[5a] was associated with poor diuretic response.

Of the synthetic methods described[10–12] for preparation of the N-substituted guanamines, the most convenient appeared to be formylation (with ethyl formate or ethylene diformate) of the biguanide as shown in Scheme I.

SCHEME I



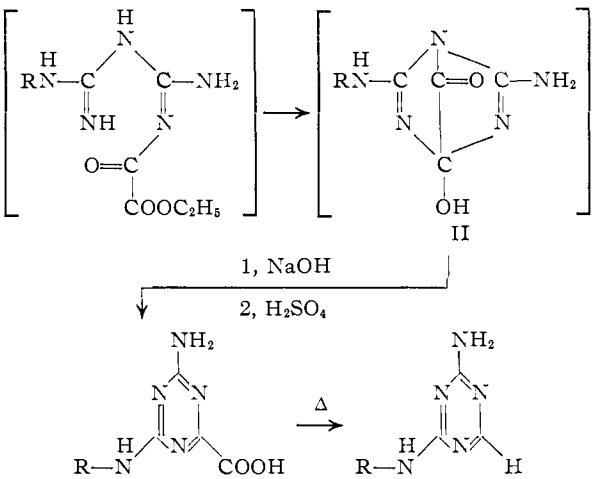
The preparation of the aryl biguanides proceeded without difficulty using the method of Curd and Rose.[13] The biguanides derived from N-methylaniline, N-ethylaniline and ethyl *p*-aminobenzoate were more conveniently prepared following the procedure of Jacobs and Jolles[14] using pyridine as a solvent.

The non-arylbiguanides were all prepared by fusion of intimate mixtures of the amine hydrochlorides with equimolar quantities of dicyandiamide at temperatures ranging from 140–160°[15] for about 1 hr.

Since purification of the biguanide was often difficult, the feasibility of proceeding directly from the alkylbiguanide in the unpurified fusion mixture to I, R = alkyl, was investigated. Parallel runs set up with cyclohexylamine hydrochloride following Scheme I wherein the N^1-cyclohexylbiguanide hydrochloride was isolated and purified or used unpurified in the fusion melt resulted in the identical product I, R = cyclohexyl, with the latter procedure affording superior yields based on cyclohexylamine hydrochloride.

The compound I (R = cyclohexyl) was also prepared following the procedure of Overberger and Shapiro.[10] Reaction of cyclohexylbiguanide with ethyl oxalate yielded the product II, which readily formed the 2-amino-4-cyclohexylamino-6-carboxy-*s*-triazine upon treatment with alkali and neutralization (Scheme II). Decarboxylation of this product at its melting point yielded I, R = cyclohexyl, identical with the product obtained above.

SCHEME II



The validity and utility of the synthetic approach wherein the biguanide is not isolated is further confirmed by comparisons of the melting point of our preparations and the following compounds prepared from the respective biguanides which have been reported in the literature: I, R equals *n*-butyl,[16] ethyl,[17] *n*-propyl,[18] allyl,[19] cyclopentyl,[20] benzyl,[21] *p*-chlorobenzyl[22] and α-phenylethyl.[23]

Further confirmation of structure was obtained from analysis, preparation of derivatives and consideration of the ultraviolet absorption spectra (see Experimental).

Pharmacology.—Selected guanamines in this series (compounds 11, 14, 20, 50) show marked diuretic activity (DA) at dosage levels lower than any previously reported compounds.

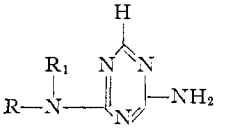
The DA is critically dependent on the structure of the group R, and definite relationships between structure and activity are evident.

In the *n*-alkyl group diuretic activity is obtained with R = ethyl through heptyl, with peak effects being noted with amyl and heptyl (compounds 2, 4, 7, 11, 18, 20). Compound 1, R = CH_3, did not

(8) C. G. Overberger and S. L. Shapiro, THIS JOURNAL, **76**, 1061 (1954).
(9) S. L. Shapiro and C. G. Overberger, *ibid.*, **76**, 97 (1954).
(10) C. G. Overberger and S. L. Shapiro, *ibid.*, **76**, 93 (1954).
(11) J. R. Geigy, Swiss Patent 252,530 [*C. A.*, **43**, 6246 (1949)].
(12) J. R. Geigy, Swiss Patent 254,538 [*C. A.*, **44**, 174 (1950)].
(13) F. H. S. Curd and F. L. Rose, British Patent 581,346 [*C. A.*, **41**, 3125 (1947)].
(14) B. R. Jacobs and Z. E. Jolles, British Patent 587,907 [*C. A.*, **42**, 214 (1948)].
(15) (a) K. Sugino and S. Idzumi, *J. Chem. Soc. Japan*, **65**, 265 (1944) [*C. A.*, **41**, 3762 (1947)]; (b) F. Bobeck, *Ann.*, **487**, 294 (1931); (c) F. H. Tendick and J. H. Burckhalter, THIS JOURNAL, **72**, 1862 (1950); (d) W. K. Detweiler and E. D. Amstutz, *ibid.*, **74**, 1483 (1952).
(16) J. R. Geigy, Swiss Patent 252,530 [*C. A.*, **43**, 6246 (1949)].
(17) J. R. Geigy, Swiss Patent 261,811 [*C. A.*, **44**, 4517 (1950)].
(18) J. R. Geigy, Swiss Patent 261,819.
(19) J. R. Geigy, Swiss Patent 261,812.
(20) J. R. Geigy, Swiss Patent 261,820.
(21) J. R. Geigy, Swiss Patent 261,813.
(22) J. R. Geigy, Swiss Patent 261,830.
(23) J. R. Geigy, Swiss Patent 261,814.

TABLE I

GUANAMINES[a]



Compound	R	M.p., °C.[b,c]	Formula	Analyses, % Calcd. C	Calcd. H	Found[a] C	Found[a] H	Activity[d] Dose	Activity[d] Response
1	CH_3–	234–238	$C_4H_7N_5$	38.4	5.6	38.1	5.6	10	0
2	C_2H_5–[e]	195–197	$C_5H_9N_5$	43.2	6.5	43.1	6.4	3.5	2
3	CH_2=$CHCH_2$–[f]	148–149$_{c_1}$	$C_6H_9N_5$	47.7	6.0	47.5	5.9	8	4
4	n-C_3H_7–[g]	163–165	$C_6H_{11}N_5$	47.0	7.2	47.1	7.2	4	2
5	i-C_3H_7–	137–140	$C_6H_{11}N_5$	47.0	7.2	47.2	7.2	10	0
6	CH_2=$C(CH_3)CH_2$–	132–134$_{c_1}$	$C_7H_{11}N_5$	50.9	6.7	51.3	6.8	2.5	3
7	n-C_4H_9–[h]	140–143$_{c_1}$	$C_6H_{13}N_5$	50.3	7.8	49.9	8.0	3	3
8	i-C_4H_9–	142–147$_{c_1}$	$C_7H_{13}N_5$	50.3	7.8	50.3	7.7	3.5	4
9	sec-C_4H_9–	138–139$_{c_1}$	$C_7H_{13}N_5$	50.3	7.8	50.0	7.7	10	0
10	t-C_4H_9–	147–150$_{c_1}$	$C_7H_{13}N_5$	50.3	7.8	50.8	7.7	12	0
11	n-C_5H_{11}	115–118	$C_8H_{15}N_5$	53.0	8.3	53.1	8.3	1	4
12	$CH_3(CH_2)_2CHCH_3$–	136–138	$C_8H_{15}N_5$	53.0	8.3	53.4	8.3	20	0
13	$CH_3CH_2CHCH_3CH_2$–	123–124	$C_8H_{15}N_5$	53.0	8.3	52.9	8.4	5	3
14	$CH_3CHCH_3(CH_2)_2$–	125–127	$C_8H_{15}N_5$	53.0	8.3	53.1	8.3	2	4
15	$(CH_3)_3CCH_2$–	168–170$_{c_1}$	$C_8H_{15}N_5$	53.0	8.3	53.6	8.4	7	3
16	$CH_3CHCH_3CHCH_3$–	161–162	$C_8H_{15}N_5$	53.0	8.3	52.9	8.3	20	0
17	$CH_3CH_2CHC_2H_5$–	158–160	$C_8H_{15}N_5$	53.0	8.3	53.1	8.3	20	0
18	n-C_6H_{13}–	120–122	$C_9H_{17}N_5$	55.4	8.8	55.6	9.0	7.5	3
19	i-C_6H_{13}–	129–130	$C_9H_{17}N_5$	55.4	8.8	55.2	8.4	5	3
20	n-C_7H_{15}–	120–121	$C_{10}H_{19}N_5$	57.4	9.2	57.4	9.3	1	3
21	n-C_8H_{17}–	121–122	$C_{11}H_{21}N_5$	59.2	9.5	59.4	9.4	15	0
22	t-C_8H_{17}–	115–117	$C_{11}H_{21}N_5$	59.2	9.5	59.1	9.5	10	0
23	$C_4H_9CHC_2H_5CH_2$–	110–112$_{c_3}$	$C_{11}H_{21}N_5$	59.2	9.5	59.2	9.4	10	Sl.
24	n-C_9H_{19}–	106–110	$C_{12}H_{23}N_5$	60.7	9.8	61.2	9.6	15	0
25	n-$C_{10}H_{21}$–	104–106	$C_{13}H_{25}N_5$	62.1	10.0	61.9	9.8	15	0
26	n-$C_{12}H_{25}$–	110–113	$C_{15}H_{29}N_5$	64.5	10.5	64.2	10.3	15	0
27	n-$C_{14}H_{29}$–	97–101	$C_{17}H_{33}N_5$	66.4	10.8	66.0	10.7	15	0
28	n-$C_{16}H_{33}$–[i]	80–87	$C_{19}H_{37}N_5$	68.0	11.1	69.9	11.5	10	0
29	n-$C_{18}H_{37}$–	108–109	$C_{21}H_{41}N_5$	69.4	11.4	69.8	11.3	15	0
30	$CH_2BrCHBrCH_2$–[j]	115 d.$_{c_2}$	$C_6H_{14}Br_2ClN_5O_2$	18.7	3.7	18.6	3.8	10	Sl.
31	$CH_2OCH_3CH_2CH_2$–	113–117$_{c_1}$	$C_7H_{13}N_5O$	45.9	7.2	45.9	7.3	7.5	Sl.
32	CYP–[k,l]	161–162	$C_8H_{13}N_5$	53.6	7.3	54.2	7.6	4	3
33	$CYPCH_2$–	141–143	$C_9H_{15}N_5$	55.9	7.8	56.3	7.8	2	3
34	$CYPCH_2CH_2$–	148–149	$C_{10}H_{17}N_5$	57.9	8.3	58.1	8.4	3	3
35	CYHx–[m]	162–164	$C_9H_{15}N_5$	55.9	7.8	55.8	7.9	5	4
36	$CYHxCH_2$–	159–162	$C_{10}H_{17}N_5$	57.9	8.3	58.8	8.5	4	3
37	p-CH_3CYHx–	230–232	$C_{10}H_{17}N_5$	57.9	8.3	58.3	8.6	10	2
38	$CYHx(CH_2)_2$–	162–164	$C_{11}H_{19}N_5$	59.7	8.7	60.3	8.5	3	3
39	p-$CH_3CYHxCH_2$–	135–136	$C_{11}H_{19}N_5$	59.7	8.7	59.6	8.6	2	3
40	$CYHx(CH_2)_3$–	133–137	$C_{12}H_{21}N_5$	61.2	9.0	60.8	9.0	10	0
41	$CYHx(CH_2)_4$–	145–148	$C_{13}H_{23}N_5$	62.6	9.3	62.5	9.3	10	0
42	CYHp–[n]	142–144	$C_{10}H_{17}N_5$	57.9	8.3	57.8	8.1	10	4
43	d-Bornyl	164–166	$C_{13}H_{21}N_5$	63.1	8.6	63.1	8.6	5	Sl.
44	dl-Bornyl	172–175	$C_{13}H_{21}N_5$	63.1	8.6	62.7	8.6	10	0
45	dl-Fenchyl	78–80	$C_{13}H_{21}N_5$	63.1	8.6	62.8	8.4	5	Sl.
46	C_6H_5–CH_2–[o]	180–183$_{c_4}$	$C_{10}H_{11}N_5$	59.7	5.5	59.9	5.6	10	2
47	4-$ClC_6H_4CH_2$–[p]	198–201	$C_{10}H_{10}ClN_5$	51.0	4.2	51.3	4.5	2.5	4
48	$C_6H_5CHCH_3$–[q]	142–144$_{c_1}$	$C_{11}H_{13}N_5$	61.4	6.1	61.1	5.9	10	2
49	4-$ClC_6H_4CHCH_3$–	166–169	$C_{11}H_{12}ClN_5$	52.9	4.8	53.2	4.9	7	3
50	$C_6H_5(CH_2)_2$–	159–161$_{c_5}$	$C_{11}H_{13}N_5$	61.4	6.1	61.0	6.1	1	4
51	2-$ClC_6H_4(CH_2)_2$–	179–181	$C_{11}H_{12}ClN_5$	52.9	4.8	53.0	5.0	2	2
52	4-$ClC_6H_4(CH_2)_2$–	173–176	$C_{11}H_{12}ClN_5$	52.9	4.8	53.3	4.7	1	3
53	2,4-di$ClC_6H_3(CH_2)_2$–	195–198	$C_{11}H_{11}Cl_2N_5$	46.6	3.9	46.8	3.9	3	2
54	3,4-di$ClC_6H_3(CH_2)_2$–	184–186	$C_{11}H_{11}Cl_2N_5$	46.6	3.9	46.8	4.0	2	2
55	4-$BrC_6H_4(CH_2)_2$–	192–197	$C_{11}H_{12}BrN_5$	44.9	4.1	44.8	4.3	2	2T
56	3,4-di$CH_3OC_6H_3(CH_2)_2$–	155–156	$C_{13}H_{17}N_5O_2$	56.7	6.2	56.7	6.1	10	0
57	$C_6H_5(CH_2)_3$–	125–128	$C_{12}H_{15}N_5$	62.9	6.6	62.5	6.5	2.5	2
58	$C_6H_5(CH_2)_4$–	98–100	$C_{13}H_{17}N_5$	64.2	7.0	64.4	6.9	1.5	3

TABLE I (*Continued*)

Compound	R	M.p., °C.[b,c]	Formula	Analyses, % Calcd. C	Calcd. H	Found[a] C	Found[a] H	Activity[d] Dose	Activity[d] Response
59	$CH_3CH_2CHC_6H_5CH_2-$	137–138	$C_{13}H_{17}N_5$	64.2	7.0	64.2	7.4	2	4
60	$C_6H_5(CH_2)_5-$	115–118	$C_{14}H_{19}N_5$	65.3	7.4	65.0	7.6	4	3
61	$(C_6H_5)_2CH-$	196–197	$C_{16}H_{15}N_5$	69.3	5.5	69.3	5.6	15	0
62	$CH_2C_6H_5CHC_6H_5-$[i]	88–91[c6]	$C_{17}H_{17}N_5$	70.1	5.9	69.3	5.8	10	0
63	$(C_6H_5)_2CHCH_2-$	165–167	$C_{17}H_{17}N_5$	70.1	5.9	69.5	6.0	10	0
64	$CH_3CH(\beta N)-$[j,r]	164–166	$C_{14}H_{15}N_5$	67.9	5.7	66.2	5.8	10	Sl.
65	$(\alpha N)(CH_2)_2-$[s]	211–212	$C_{15}H_{15}N_5$	67.9	5.7	68.1	5.9	10	0
66	C_6H_5O-[t]	162–165	$C_8H_9N_5O$	50.3	4.7	50.5	4.8	7.5	0
67	C_6H_5-[u]	235[c8]						10	3
68	$4\text{-}ClC_6H_4-$	259[c8]	$C_9H_8ClN_5$	48.8	3.6	48.4	3.2	5	3T
69	$4\text{-}BrC_6H_4-$[v]	263–264[c6]						5	2T
70	$4\text{-}IC_6H_4-$	251–253 d.[c3]	$C_9H_8IN_5$	34.5	2.6	34.9	2.7	2.5	3T
71	$4\text{-}FC_6H_4-$	145–148	$C_9H_8FN_5$	52.9	3.9	52.9	4.0	5	3
72	$4\text{-}NH_2SO_2C_6H_4-$	265 d.[c3]	$C_9H_{10}N_6O_2S$	40.6	3.8	40.7	3.9	10	0
73	$4\text{-}C_2H_5COOC_6H_4-$	219–221	$C_{12}H_{13}N_5O_2$	55.6	5.1	55.7	4.8	10	0
74	$2\text{-}C_2H_5C_6H_4-$	194–196	$C_{11}H_{13}N_5$	61.4	6.1	61.6	6.2	2	3T
75	$-C_6H_4-CH_2-C_6H_4-$	217 d.[c9]	$C_{19}H_{18}N_{10}$	59.1	4.7	59.1	5.5	10	3
76	$\beta\text{-}N$[r]	233–238	$C_{13}H_{11}N_5$	65.8	4.7	65.9	4.7	5	4
77	3-Quin[w]	291–192 d.						20	2T
78	$n\text{-}C_4H_9$	118–120	$C_8H_{15}N_5$	53.0	8.3	52.6	8.3	20	0
79	$i\text{-}C_4H_9$	158–160	$C_8H_{15}N_5$	53.0	8.3	53.5	8.3	20	0
80	$n\text{-}C_5H_{11}$	120–122	$C_9H_{17}N_5$	55.4	8.8	55.9	8.7	20	0
81	$i\text{-}C_5H_{11}$	137–138	$C_9H_{17}N_5$	55.4	8.8	55.3	8.7	20	0
82	CYHx-	172–174	$C_{10}H_{17}N_5$	57.9	8.3	58.2	8.2	20	Sl.
83	CYHx-[x]	148–150	$C_{11}H_{19}N_5$	59.7	8.7	59.7	8.3	10	Sl.
84	C_6H_4-	185–187	$C_{10}H_{11}N_5$	59.7	5.5	59.5	5.9	10	Sl.
85	C_6H_4-[x]	178–180[c1]	$C_{11}H_{13}N_5$	61.4	6.1	61.6	6.1	5	Sl.
86	C_6H_4-[y]	185–186	$C_{16}H_{15}N_5$	69.3	5.5	68.8	5.4	10	0

[a] Analyses by Weiler & Strauss, Oxford, England. [b] Melting points are not corrected. [c] Yields are not reported since in the majority of the syntheses, efforts were not directed toward maximal yields. Yields of recrystallized product generally ranged between 20–40% based on amine hydrochloride. The recrystallizing solvent was acetonitrile unless otherwise indicated; c_1, benzene; c_2, acetone; c_3, methanol–water; c_4, propyl alcohol; c_5, ethyl acetate–hexane; c_6, acetone–hexane; c_7, isopropyl alcohol–ether; c_8, dioxane; c_9, propanol. [d] See end of Experimental section for detailed description of method of evaluation of diuretic response. [e] Ref. 17 reported m.p. 197.5°. [f] Ref. 19 reported m.p. 152.5°. [g] Ref. 18 reported m.p. 168°. [h] Ref. 16 reported m.p. 124°, then 144°. [i] Not obtained analytically pure. [j] Obtained as hydrochloride dihydrate. [k] Cyclopentyl. [l] Ref. 20 reported m.p. 164°. [m] Cyclohexyl. [n] Cycloheptyl. [o] Ref. 21 reported m.p. 182–182.5°. [p] Ref. 22 reported m.p. 200°. [q] Ref. 23 reported m.p. 138°. [r] β-N is β-naphthyl. [s] α-N is α-naphthyl. [t] Furfuryl. [u] Ref. 10 reported m.p. 234°. [v] Ref. 10 reported m.p. 260–261°. [w] 3-Quinolyl, analysis not acceptable; however, see picrate. [x] R_1 is ethyl. [y] R_1 is benzyl. [z] For compounds 1–77, R_1 = H; for compounds 78–86, R_1 = CH_3, unless otherwise indicated.

show activity, and R groups containing 8 or more carbon atoms were completely inactive (compounds 21–29).

In the branched alkyl groups, the same requisites for carbon content of the R chain obtain (compounds 22 and 23, inactive), and an additional factor is introduced. The presence of an alkyl group or more than one alkyl group on the α-carbon of R (the carbon joining the amino nitrogen) is associated with complete disappearance of activity. Thus, R = *n*-amyl (compound 11) is highly active, whereas R = 1-methylbutyl (compound 12) is completely inactive. This effect is manifest throughout as seen in the following comparisons: compound 7 *vs.* 9, 10; 11 *vs.* 12, 16, 17; 4 *vs.* 5.

Alkyl substituents attached at a position beyond the α-carbon of R do not interfere with DA, although compound 15, R = neopentyl, with two methyl groups on the β-carbon is considerably less active than R = *n*-amyl or *iso*-amyl. The data suggest that in R groups of the same carbon content, the isoalkyl is somewhat more effective tha n the *n*-alkyl (excluding isopropyl which bears a methyl group on the α-carbon), as comparison of the following compounds indicates: compound 8 *vs.* 7; 14 *vs.* 12; 19 *vs.* 18.

The limited number of unsaturated alkyl groups studied does not permit characterization of the effect of the double bond too definitely, but comparison of R = allyl and propyl, and methallyl and isobutyl (compound 3 *vs.* 4; 6 *vs.* 8) indicates that the double bond does not interfere with DA.

Substituents other than alkyl such as Br (compound 30) and CH_3O (compound 31) on otherwise active chains considerably depressed activity.

The cycloalkyl and cycloalkylalkyl compounds also show a dependence on the bulk of R. Compounds where R exceeds eight carbon atoms were inactive (compounds 40, 41, 43–45). The cycloalkyls containing 5, 6 and 7 carbons (compounds 32, 35, 42) were all extremely active. Introduction of methylene chains between the cycloalkyl group and the amino nitrogen retained DA (compounds 33, 34, 36, 39) until the carbon content of R exceeded 8 atoms (compounds 40, 41). A comparison between cyclohexylmethyl *vs.* *p*-methylcyclohexyl and cyclohexylethyl *vs.* *p*-methylcyclohexylmethyl (compounds 36 *vs.* 37; 38 *vs.* 39) does not

permit unequivocal assessment of the effect of alkyl groups on the cycloalkyl rings.

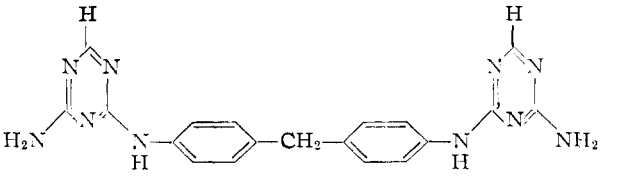
The arylalkyl series is also characterized by specific structural requirements. While the phenylalkyls show many active compounds, use of R components containing 2 phenyl groups (compounds 61–63) or aryl such as furfuryl (compound 66) or naphthyl groups (compounds 64, 65) is associated with loss of DA. As the phenylmethylene group is lengthened, it is observed that even numbers of methylene groups (compounds, 50, 58 *vs.* 46, 57, 60) are more effective than those with odd numbers of methylene groups. The phenyl group augments the DA associated with a particular alkyl group (compound 46 *vs.* 1; 50 *vs.* 2; 57 *vs.* 4; 58 *vs.* 7) until the 5-membered alkylene chain is reached (compound 11 *vs.* 60). The phenyl group need not be confined to the ω-position (compounds 58, 59). Methyl groups on the α-carbon atom in this category, while not having the marked effect observed in the alkyl series, considerably diminish the DA of the corresponding unbranched guanamines (compound 48 *vs.* 50; 49 *vs.* 52). While halogenation of the phenyl ring had desirable effects in many instances and was quite extensively studied (compounds 47, 49, 52–55), noted oral toxicity at even the very low doses used in testing (compounds 55, 68–70), made this approach unattractive. In contrast, the oral LD_{50} in mg./kg. rate of some of the other compounds in this series were compound 11, 290; compound 14, 1050; compound 35, 870. Methoxylation of the phenyl ring (compound 56) destroyed DA of the active nucleus of compound 50.

Another structural characteristic defining DA is the need for the hydrogen atom on the R-bearing nitrogen atom. Replacement of the hydrogen by alkyl invariably destroyed DA in otherwise active structures (compound 78 *vs.* 7; 79 *vs.* 8; 80 *vs.* 11; 81 *vs.* 14; 82, 83 *vs.* 35; 84–86 *vs.* 67).

Certain aromatic derivatives were explored to supplement the series of Clauder and Bulscu.[5a] These workers had shown the electron-donor groups such as alkoxy, alkyl and dialkylamino on the phenyl ring depress the DA of the unsubstituted phenyl ring. While halogen (Br, Cl) on the ring promotes the DA, our findings reflected that these substituents greatly enhanced toxicity (compounds 68, 69) as did iodine (compound 70). In contrast, R = *p*-fluorophenyl (compound 71) was more active than R = phenyl and not any more toxic. This finding is not unexpected since in many structural categories, fluorine or hydrogen has parallel pharmacologic effects.[24] The inhibition attributed to electron-donor groups[5a] suggested exploration of negative groups, but use of sulfonamido (compound 72) and carbethoxy (compound 73) as substituents on the phenyl ring destroyed DA.

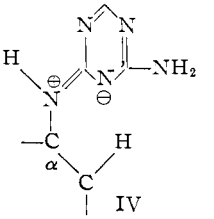
Poor DA had been reported with R = α-naphthyl,[5a] but the structurally allied R = *o*-ethylphenyl (compound 74) showed pronounced DA as well as toxicity similar to the halo substituents. In contrast, the less sterically hindered compound 76, R = β-naphthyl, had excellent DA. This suggested investigation of the structurally allied R = 3-quinolinyl (compound 77) which proved to be toxic.

Interestingly, the *p,p'*-methylenedianiline derivative III (compound 75) showed activity equivalent to R = phenyl (compound 67).



III

This survey has served to restrict the structural requirements for DA to I. However, the broad structural range of active structures permissive within R makes it likely that the locus for the physiological binding of I does not depend too critically on R but rather is associated with the *s*-triazine ring. In turn, the function of R might be considered as that of promotion of a desirable solubility relationship for aligning the active portion of the molecule, as has been described for the barbiturates.[25] If R contributes in this nonspecific fashion, it is surprising that such small changes in R will destroy activity. The depressant effect on DA of α-alkyl substituents may be associated with such resonance forms[26] as IV which could lock the triazine ring in an inactive form.



IV

Inspection of the ultraviolet absorption spectra (Table IV) shows no difference whatever between the spectra of compounds which are effective diuretics and compounds which are completely inactive. However, Braude[27] has pointed out that ultraviolet light absorption is not too sensitive an index for steric effects, requiring energy differences of about 2 kcal./mole for wave length displacements of 5 mμ, in contrast to reaction rates (in this case, the chemistry of the diuretic response) where energy differences of 0.1 kcal./mole are detectable.

It is relevant to consideration of IV[28] to point

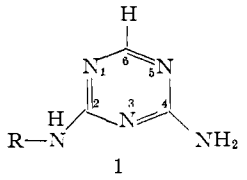
(24) H. L. Friedman, American Chemical Society, New York Meeting, September, 1954, p. 23N.

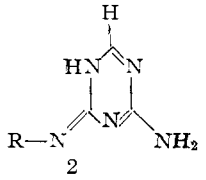
(25) A. Burger, "Medicinal Chemistry," Vol. I, Interscience Publishers, Inc., New York, N. Y., 1951, p. 122.

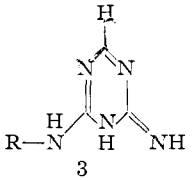
(26) E. W. Hughes, THIS JOURNAL, **63**, 1737 (1941).

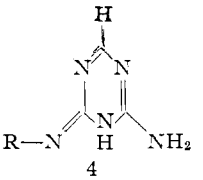
(27) E. A. Braude and F. Sondheimer, *J. Chem. Soc.*, 3754 (1956).

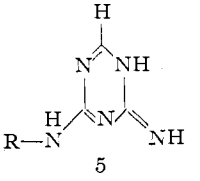
(28) (a) M. M. Winbury, *J. Pharmacol. Exp. Therap.*, **105**, 326 (1952); (b) M. M. Winbury, D. L. Cook and W. E. Hambourger, *ibid.*, **111**, 395 (1954); (c) M. M. Winbury, D. L. Cook and W. E. Hambourger, *Arch. intern. pharmacodynamie*, **97**, 125 (1954). In contrast to the undesirable effects noted in this series with the isopropyl and *sec*-butyl groups, Winbury, *et al.*, ascribe superior ganglionic blocking activity to quaternary ammonium derivatives containing secondary alkyl groups such as isopropyl and *sec*-butyl. It is suggested "that α-methyl branching may improve blocking action by restricting rotation of the alkyl groups about the quaternary nitrogen. This would increase the statistical possibility of the cation having the molecular configuration (bond angle, distance) required for adsorption on the receptor site."

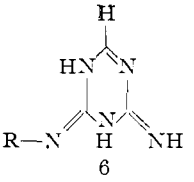

1

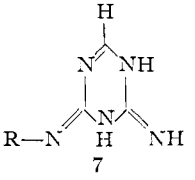

2


3


4


5


6


7

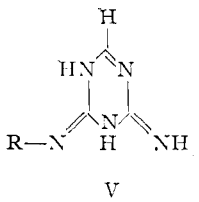
out that the hydrogen of carbon-2 of the ring compounds, cyclic and aryl, would not be spatially equivalent to the hydrogen on an α-methyl group.

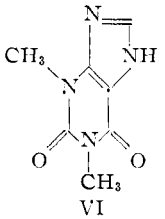
While the evidence at hand does not justify an extensive discussion, certain considerations for the active form for I are suggested by the formulas.

The basicity[29] of I is reduced relative to formoguanamine by the introduction of alkyl or aryl groups on the amino nitrogen, perhaps due to the reduction in symmetry and the change in resonance. This effect is augmented by the replacement of alkyl groups for hydrogen in the 6-position.

In view of the weakly basic character of I, it is likely that its physiological effect is manifest from reaction as the free base, which is a resonance hybrid of a number of non-equivalent forms[30] in the planar triazine system.[31]

In the case of formoguanamine I, R = H, structures 2 and 5, 3 and 4 and 6 and 7 are equivalent. Since hyperconjugative as well as steric effects[32,33] associated with alkyl at the 6-position would depress the population of forms of type 2, 5, 6 and 7, the lack of DA in such structures and alternatively, activity in I, can be ascribed to existence of these forms. The need for hydrogen on the R-bearing amino group for DA eliminates form 5. The capacity of a *single* methyl group on the α-carbon atom to render otherwise active structures inactive would reflect that this methyl group in any of its alternative conformations would provide steric resistance. Thus while steric interference with the hydrogen on N in position 1 is possible in form 2, no such hindrance is indicated when the methyl group has the alternative conformation and is oriented toward the ring nitrogen of position 3. A similar argument holds for form 7. These factors would argue that only form 6 would be rendered inactive by one methyl substituent on the α-carbon of R and point to form 6 (V) as the active structure. Interestingly, V bears a formal resemblance to theophylline (VI).


V


VI

Experimental[34]

Materials.—Most of the amines used in this work were obtained from commercial sources. Those not commercially available were prepared as follows: α-Phenylpropylamine, cyclopentylamine and β-cyclohexylethylamine were prepared from the appropriate halide by a modified Gabriel reaction.[35] Aromatic aldehydes were converted to the nitrostyrenes[36] which were reduced with lithium aluminum hydride[36] to yield the following β-arylsubstituted ethylamines: *p*-chlorophenyl, *o*-chlorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl and *p*-bromophenyl.

Reduction of amides with lithium aluminum hydride[37] yielded β-cyclopentylethylamine, cyclohexylpropylamine, cyclohexylbutylamine, β,β-diphenylethylamine, 4-phenylbutylamine, β-(α-naphthyl)-ethylamine and 2-phenylbutylamine. Reduction of the corresponding oxime[37] yielded 4-methylcyclohexylamine. By means of the Hofmann reaction,[38] cyclopentylacetamide, cyclohexylacetamide, 4-methylcyclohexylacetamide and 3-methylvaleramide were converted to cyclopentylmethylamine, cyclohexylmethylamine, 4-methylcyclohexylmethylamine and 2-methylbutylamine, respectively. The mixed (bornyl and neobornyl) *d*-bornyl- and *dl*-bornylamines were made from *d*-camphor and *dl*-camphor and fenchylamine from fenchone.[39] Neopentylamine was prepared from *t*-butylacetamide.[40] By means of the Leuckart reaction,[41] the respective methyl ketones were converted to α-*p*-chlorophenylethylamine and α-(β-naphthyl)-ethylamine. Desoxybenzoin was converted to 1,2-diphenylethylamine.[42]

Cyclohexylbiguanide Hydrochloride.—A finely ground mixture of 27 g. (0.2 mole) of cyclohexylamine hydrochloride and 16.8 g. (0.2 mole) of dicyandiamide was heated at 150–160° for 30 minutes whereupon an exothermic reaction occurred and the fusion mixture crystallized. Cooling and solution of the reaction mixture in methanol, upon standing, yielded 20 g. (43.5%) of crystals, m.p. 200–210°. The product, recrystallized from water, melted at 225–227°.

Anal. Calcd. for $C_8H_{18}N_5Cl$: C, 43.7; H, 8.3; N, 31.9. Found: C, 43.4; H, 8.21; N, 32.2.

2-Amino-4-cyclohexylamino-*s*-triazine. I, R = Cyclohexyl. a. From Cyclohexylbiguanide Hydrochloride.—A solution of 2.3 g. (0.1 mole) of sodium in 80 ml. of methanol was prepared and 22 g. (0.1 mole) of cyclohexylbiguanide hydrochloride added, followed by the addition of 7.4 g. (0.1 mole) of ethyl formate. The reaction mixture was maintained at room temperature for 18 hr. and then treated with 50 ml. of water. On standing several days, 10 g. (52%) of product crystallized, melting at 157–163°. Recrystallized from acetonitrile, the product melted at 162–164°.

I, R = Cyclohexyl. b. From Cyclohexylamine Hydrochloride.—Fusion of 0.2 mole each of cyclohexylamine hydrochloride and dicyandiamide was made as above. The

(29) J. R. Dudley, THIS JOURNAL, **73**, 3007 (1951).
(30) I. M. Klotz and T. Askounis, *ibid.*, **69**, 801 (1947).
(31) P. J. Wheatley, *Acta Cryst.*, **8**, 224 (1955).
(32) I. M. Klotz and T. Askounis, THIS JOURNAL, **76**, 4625 (1954).
(33) C. G. Overberger and S. I. Shapiro, *ibid.*, **76**, 1855 (1954).
(34) Descriptive data shown in tables are not reproduced in Experimental section.
(35) J. C. Sheehan and W. A. Bolhofer, THIS JOURNAL, **72**, 2786 (1950).
(36) D. E. Worrall, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 413.
(37) W. G. Brown, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 469.
(38) E. S. Wallis and J. F. Lane, ref. 37, Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 267.
(39) A. W. Ingersoll, J. H. Brown, C. K. Kim, W. D. Beauchamp and G. Jennings, THIS JOURNAL, **58**, 1808 (1936).
(40) F. C. Whitmore and A. H. Homeyer, *ibid.*, **54**, 3435 (1932).
(41) A. W. Ingersoll, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 503.
(42) I. A. Kaye and C. L. Parris, THIS JOURNAL, **74**, 1566 (1952); see footnote 23 of this reference.

cooled reaction mixture was dissolved in 100 ml. of methanol and filtered. The filtrate was added to a solution prepared from 3.45 g. (0.15 mole) of sodium in 100 ml. of methanol. Ethyl formate (14.0 g., 0.19 mole), was added and the reaction mixture maintained at room temperature overnight. Dilution with an equal volume of water and standing 48 hr. yielded 17.5 g. (45.5%) of product. On recrystallization from acetonitrile, a mixed melting point of products obtained by the two reactions was undepressed.

I, R = Cyclohexyl. c. From Reaction Product of Cyclohexylbiguanide and Ethyl Oxalate. Preparation of II.—Molar quantities of cyclohexylamine hydrochloride and dicyandiamide were fused as above. The cooled reaction mixture was suspended in 200 ml. of isopropyl alcohol and a solution prepared from 23 g. (1.0 mole) of sodium in 200 ml. of methanol and 500 ml. of isopropyl alcohol added. After standing 48 hr., the precipitated sodium chloride was filtered off and the filtrate treated with 162 g. (1.1 moles) of diethyl oxalate. After a mild exothermic reaction, precipitation of white crystals of II ensued, and the reaction mixture was maintained at 10° overnight and filtered yielding 108.1 g. (45.5%) of product, m.p. 209° dec. On recrystallization from acetonitrile, the m.p. was 211° dec.

Anal. Calcd. for $C_{10}H_{15}N_5O_2$: C, 50.6; H, 6.4; N, 29.5. Found: C, 50.7; H, 6.7; N, 28.6.

The exact structure of this product as is the case of the analogous product from phenylbiguanide[10] is not known, and one of several structural possibilities has been indicated in the Discussion. For convenience it will be designated as II.

2-Amino-4-cyclohexylamino-6-carboxy-*s*-triazine.—A suspension of 9.48 g. (0.04 mole) of II in 150 ml. of water was treated with one equivalent of 1.0 *N* sodium hydroxide. After gentle warming (50°) almost all of the solid dissolved and the solution was filtered. Addition of an equivalent of 1 *N* sulfuric acid resulted in formation of a white precipitate which was separated, resuspended and vigorously stirred in 200 ml. of water and filtered. Air-drying yielded 7.4 g. (74%) of product which melted with decomposition at 205–209°.

The product was heated in the Abderhalden pistol under boiling toluene for 2 hr. and indicated 9.3% H_2O; calcd. for 1.5 H_2O, 9.75%. The dried product melted at 204–207° dec.

Anal. Calcd. for $C_{10}H_{15}N_5O_2$: C, 50.6; H, 6.4; N, 29.5. Found: C, 50.1; H, 6.7; N, 30.4.

Thermal Decarboxylation of 2-Amino-4-cyclohexylamino-6-carboxy-*s*-triazine.—The hydrated acid (2.0 g., 0.008 mole) was heated in an oil-bath at 210° for 10 minutes at which point evolution of carbon dioxide had ceased. The molten residue of 2-amino-4-cyclohexylamino-*s*-triazine crystallized on cooling, yielding 1.37 g. (88.9%) of product, m.p. 157–160°; recrystallized from acetonitrile, m.p. 157–158°; mixed m.p. with I, R = cyclohexyl, undepressed.

The identity of the decarboxylation product was further confirmed by preparation of the picrate, m.p. 201–202°; mixed m.p. with picrate of I, R = cyclohexyl, undepressed. Data for this picrate and some of the other picrates prepared are shown in Table II.

TABLE II[a]

ANALYTICAL DATA ON PICRATES

R	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Found C	Found H
Butyl	192–195	39.4	4.1	39.2	4.3
Isobutyl	203–204	39.4	4.1	39.3	4.0
Cyclohexyl	205–207	42.6	4.3	43.1	4.2
β-Phenethyl	181–183	45.9	3.6	45.8	3.6
n-Amyl	148	41.0	4.4	41.2	4.6
Isoamyl	185	41.0	4.4	40.5	4.6
3-Quinolyl	269 d.	46.3	2.8	46.3	3.0

[a] Empirical formula by adding elements of picric acid to formulas of free bases.

Monoacetate of I, R = Cyclohexyl.—A solution of 3.0 g. (0.0155 mole) of I, R = cyclohexyl, in 75 ml. of acetic anhydride was refluxed for 3 hr. On cooling, 1.8 g. (50%) of product precipitated, m.p. 205°. After recrystallization from propanol the product melted at 206–207°.

Anal. Calcd. for $C_{11}H_{17}N_5O$: C, 56.2; H, 7.3. Found: C, 55.5; H, 7.2.

Monopropionate of I, R = Cyclohexyl.—This product was prepared as above, substituting propionic anhydride, in 33% yield. The product melted at 197–198° (acetonitrile).

Anal. Calcd. for $C_{12}H_{19}N_5O$: C, 57.8; H, 7.7; N, 28.0. Found: C, 58.0; H, 7.7; N, 27.8.

2-Amino-4-(β-phenylethyl)-amino-*s*-triazine Hydrochloride.—A mixture of 15.8 g. (0.1 mole) of β-phenylethylamine hydrochloride and 8.4 g. (0.1 mole) of dicyandiamide was heated and stirred in an oil-bath maintained at 148–150°. The reaction mixture upon reaching 130° was completely fluid, and with continued stirring an exothermic reaction resulted, the temperature of the reaction mixture rising to 156°. Heating was continued for 1 hr. The cooled reaction mixture was then treated with a solution of 60 ml. of methanol containing 5.4 g. (0.1 mole) of sodium methoxide, 4.2 g. (0.07 mole) of methyl formate was added and the reaction mixture was allowed to stand for 24 hr. The formed I, R = β-phenylethyl, was converted to its hydrochloride by addition of 1:1 methanol–hydrochloric acid to *p*H 2.5. The solvents were removed by distillation and the residue (19.6 g.) dissolved in 294 ml. of water. Addition of sodium chloride (20 g./100 ml. of reaction mixture) precipitated the hydrochloride of I, R = β-phenylethyl. The product was washed with saturated sodium chloride, filtered, dried and recrystallized from isopropyl alcohol (Table III). There was obtained 10.27 g. (42.4%) of product.

TABLE III[a]

ANALYTICAL DATA ON HYDROCHLORIDES

R	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Found C	Found H
Cyclohexyl	212–214	47.2	7.0	47.1	6.8
β-Phenylethyl	185–186	52.5	5.6	52.3	5.5
n-Amyl	208–210	44.2	7.4	44.4	7.3
Isoamyl	203–205	44.2	7.4	44.0	7.5

[a] Empirical formula by addition of HCl to formulas of free bases.

The hydrochlorides of some of the other products were isolated by conventional procedures and recrystallized from isopropyl alcohol. The hydrochlorides crystallized as the monohydrochlorides and were readily water-soluble and non-hygroscopic. Some of these are described in Table III.

Diacetate of I, R = β-Phenylethyl.—To a mixture of 20 ml. of acetic anhydride and 4 drops of pyridine was added 2.15 g. (0.01 mole) of 2-amino-4-β-phenylethyl-amino-*s*-triazine, and the reaction was refluxed for 2 hr. On cooling, 2.7 g. (90%) of the diacetate crystallized. Upon recrystallization from acetonitrile, it melted at 150–151°.

Anal. Calcd. for $C_{15}H_{17}N_5O_2$: C, 60.2; H, 5.7; N, 23.4. Found: C, 60.4; H, 5.7; N, 23.2.

An attempt at partial saponification to obtain the monoacetate was unsuccessful. Saponification in methanolic hydrochloric acid yielded the free base, confirmed by mixed melting point. Recent clarification of the acetylation pattern in amino heterocyclic structures would indicate that the diacetate[43] reflects monoacetylation of each amino group.

2-Amino-4-(2,3-dibromopropyl)-amino-*s*-triazine.—To a solution of 1.51 g. (0.01 mole) of 2-amino-4-allylamino-*s*-triazine in 140 ml. of hot benzene was added 1.6 g. (0.01 mole) of bromine in 10 ml. of benzene with shaking. After standing at 10° for several hours, 2.6 g. of a yellow precipitate separated. When recrystallization was not successful, the product was dissolved in hydrochloric acid and the solution filtered and concentrated to a gummy solid in the desiccator. Purification was achieved by solution in isopropyl alcohol and precipitation with anhydrous ether yielded the hygroscopic product in 1.2 g. yield (31.3%).

***p*-Carbethoxyphenylbiguanide.**—A mixture of 19 g. (0.1 mole) of ethyl *p*-aminobenzoate hydrochloride and 8.4 g. (0.1 mole) of dicyandiamide was added to 35 ml. of pyridine and the reaction mixture refluxed for 3 hr. Upon cooling, a dense crystalline precipitate formed which after rinsing

(43) A. P. Phillips and J. Mentha, THIS JOURNAL, **76**, 6200 (1954).

with acetone, afforded 16.9 g. (59.5%) of the biguanide hydrochloride.

One gram of the hydrochloride in 15 ml. of warm (75°) solution containing an equivalent of sodium hydroxide on cooling yielded 0.6 g. (69%) of the biguanide base, which after recrystallization from acetonitrile melted at 172–173° dec.

Anal. Calcd. for $C_{11}H_{15}N_5O_2$: C, 53.0; H, 6.1. Found: C, 52.4; H, 5.9.

N'-(3-Quinolyl)-biguanide.—A solution of 2.9 g. (0.02 mole) of 3-aminoquinoline, 1.7 g. (0.02 mole) of dicyandiamide, 1.8 ml. of hydrochloric acid and 8 ml. of propanol was refluxed for 1.5 hr. and cooled. The yellow solid of the biguanide hydrochloride which precipitated, 4 g. (73%), was recrystallized from methanol as faint yellow crystals, m.p. 212–215°.[44]

Anal. Calcd. for $C_{11}H_{13}N_5Cl\cdot 0.5H_2O$: C, 48.2; H, 5.1. Found: C, 47.8; H, 5.9.

The free base prepared from an aqueous solution of 2.65 g. (0.01 mole) in 15 ml. of water and treated with 0.44 g. (0.011 mole) of sodium hydroxide precipitated as a tan solid (2.2 g.), recrystallized from acetonitrile, m.p. 187° dec.

Anal. Calcd. for $C_{11}H_{12}N_5$: C, 57.9; H, 5.3; N, 36.8. Found: C, 58.0; H, 5.3; N, 37.2.

The dipicrate melted at 240° (dec., H_2O).

Anal. Calcd. for $C_{23}H_{18}N_{12}O_{14}$: C, 40.2; H, 2.6; N, 24.5. Found: C, 40.1; H, 2.5; N, 24.2.

Ultraviolet Absorption Spectra.—The spectra of a small series of the compounds were determined in methanol with a model DU Beckman spectrophotometer using 1-cm. cells (Table IV).

TABLE IV

ULTRAVIOLET ABSORPTION SPECTRA OF N-SUBSTITUTED GUANAMINES (I)

R	λ_{max}, mμ	$\epsilon \times 10^{-3}$	λ_{min}, mμ	$\epsilon \times 10^{-3}$
Methyl	263	3.45	246	2.23
Ethyl	263	3.76	246	2.51
Propyl	263	3.78	247	2.50
Isopropyl	264	3.80	246–247	2.50
Butyl	263	3.80	249	2.90
Isobutyl	263–264	3.80	247	2.63
sec-Butyl	264	3.88	247	2.55
t-Butyl	262	3.65	247	2.63
Hexyl	265	3.95	247	2.58
Octyl	265	3.60	246–248	2.40
Tetradecyl	264	3.30	249	2.28
Benzyl	264	4.00	247	2.85
α-Phenethyl	263	4.35	248–249	3.18
β-Phenethyl	263	4.13	248	2.96
3,4-Dimethoxy-β-phenethyl	267–275	5.50	250	3.45
p-Chlorobenzyl	264	4.60	248	3.35
Cyclohexyl	264	3.50	248	2.46

(44) For preparative methods for other biguanides containing the quinoline nucleus, see (a) N. L. Drake and R. J. Kray, THIS JOURNAL, **76**, 1320 (1954); (b) R. Roger, *J. Chem. Soc.*, 1665 (1949); (c) J. M. Gulland and P. E. Macey, *ibid.*, 1257 (1949).

Consideration of these spectra in terms of reported values for substituted aminotriazines[33] shows a bathochromic effect and a slight hyperchromic effect relative to formoguanamine when one of the amino hydrogens of this compound is substituted by alkyl groups (Table IV). The virtual identity of these spectra in methanol would also support the identity in the assigned structures.

Pharmacological Evaluation as Diuretics.—The compounds were evaluated orally in rats following the method of Lipschitz.[45] Twelve rats were used for each dosage level tested. The data in Table I reflect an arbitrary description of diuretic response, 0–4 ranging from a response not significantly different from control (O activity response) to a response considerably more than control or clearing all or more of the administered saline as urine within 5 hr. (4 activity response). The dosage level shown in Table I was the lowest which gave a significant diuretic response. The letter T shown after the activity value in Table I indicates that at this dosage level death occurred in the test animals within a 7-day post-test interval. Typical experimental results are shown in Table V wherein % diuretic activity (volume of urine collected divided by saline administered × 100) is reported *vs.* hours post drug administration as compared to similarly saline-primed rats not given any drug. A more extensive report of the pharmacological work will be reported elsewhere.

TABLE V

% DIURETIC ACTIVITY AS FUNCTION OF TIME

Dose level, mg./kg.	% diuretic excretion (hours) 1	2	3	4	5
Compound 14					
2	24.4	42.0	64.0	82.3	110.2
1	20.6	34.0	49.8	64.6	79.8
0	2.9	7.5	12.1	16.5	22.9
Compound 50					
1	20.6	41.3	66.6	88.1	100.2
0	5.3	6.9	11.4	15.8	23.3
Compound 48					
10	20.4	42.2	55.2	70.8	79.3
0	1.0	16.1	23.5	33.1	37.2
Compound 22					
10	2.2	8.1	16.8	19.9	25.1
0	2.9	9.7	14.9	18.6	24.8

A variant of the screening technique used above with rats has been recently evaluated[46] as reflecting a response obtained with normal humans but not necessarily reflecting an indication of the relative diuretic potency of the drug in humans with congestive failure.

Acknowledgment.—The authors are indebted to Dr. G. Ungar and his staff for the pharmacological screening of some of the compounds.

YONKERS 1, NEW YORK

(45) W. L. Lipschitz, Z. Hadidian and A. Kerpscar, *J. Pharmacol. Exp. Therap.*, **79**, 97 (1943).

(46) J. D. McColl, J. M. Parker and J. K. W. Ferguson, *Can. J. Biochem. Physiol.*, **34**, 903 (1956).