gave a melting point and mixed melting point with authentic acetaldehyde 2,4-dinitrophenylhydrazone of 167–168°. The refluxing reaction mixture was allowed to distil slowly at atmospheric pressure into a receiver. The distillate (1830 ml.) was extracted with ether to give 1.2 g. of an oil upon evaporation. This was eventually combined with the main batch of oil obtained below.

The degraded material remaining in the pot was acidified by the dropwise addition, with stirring, of 400 ml. of concentrated hydrochloric acid. Overnight sweeping produced no additional solid in the derivatizing solution. The acidic ether was washed with water, and the washings brought to *p*H 7 and re-extracted. The combined ether layers were dried with Na_2SO_4 and evaporated to give an oil weighing 12.4 g. This oil was combined with that obtained from the extraction of the aqueous distillate above and distilled through a micro-still: cut 1, 1.5 g., b.p. 79–81° (20 mm.), $n^{25}D$ 1.4182; cut 2, 2.2 g., b.p. 81–86° (20 mm.), $n^{25}D$ 1.4172. Values obtained for authentic trimethylpyruvic acid under the same conditions are: b.p. 84–85° (20 mm.), $n^{26}D$ 1.4168. Samples of the fractions solidified when allowed to stand exposed to the atmosphere, as did trimethylpyruvic acid. When heated with 2,4-dinitrophenylhydrazine and sulfuric acid in ethanol, the fractions gave ethyl trimethylpyruvate 2,4-dinitrophenylhydrazone, identical with material obtained under the same conditions from authentic trimethylpyruvic acid: fine canary yellow needles (from alcohol–water), m.p. 163–164°, in agreement with the literature.[28] The distillation pot residue was a brown solid, which upon recrystallization from a 4:1 mixture of petroleum ether–ether was found to be trimethyllactic acid, m.p. and mixed m.p. 86–87°.

(2) **Bromination.** (a) **In Ethanol.**—Two grams (11.8 millimoles) of the same acid examined above was dissolved in 10 ml. of ethanol and monobrominated by the rapid addition of 23.3 ml. of 5% bromine in carbon tetrachloride (11.8 millimoles of Br_2). After 0.5 hour the discharge of the bromine color was almost complete. Solvent removal was accompanied by the evolution of hydrogen bromide, as detected by moist blue litmus paper. The oily residue was distilled: cut 1, 0.9 g., b.p. 78–81° (0.3 mm.); cut 2, 1.1 g., b.p. 81–83° (0.3 mm.). Both fractions solidified upon standing overnight. These were combined and recrystallized from 95% ethanol, m.p. 56–57°. The monobromide is insoluble in water, but dissolves with slight effervescence in 5% $NaHCO_3$ solution. *Anal.*[22] Calcd. for $C_9H_{13}O_3Br$: C, 43.39; H, 5.26; Br, 32.08. Found: C, 43.41; H, 5.17; Br, 32.33.

(b) **In Chloroform.**—One gram (5.9 millimoles) of the acid in 180 ml. of purified chloroform at ice temperature was treated rapidly with 1.0 g. (6.3 millimoles) of bromine in 10 ml. of chloroform. After two days at room temperature the color of the bromine was not discharged. Evaporation without heating gave a solid and a yellow liquid. The solid was sublimed (5 hours, 110°, 50 μ) to give m.p. and mixed m.p. with starting material of 185–187°. The liquid, upon distillation (b.p. 50° (80 μ)), gave four drops of an oil which solidified on standing. Recrystallization from ethanol gave crystals which did not depress the melting point of the monobromide obtained by bromination in ethanol.

(3) **Methylation.**—Slightly moist diazomethane in 50 ml. of ether from 42 millimoles of N-nitrosomethylurea[29] was added to a solution of 2.04 g. (12 millimoles) of the acid in a mixture of 50 ml. of ether and 8 ml. of absolute methanol. After standing for one hour the yellow color had disappeared. The solution was washed with 5% $NaHCO_3$ solution; the washings yielding no solid upon acidification. This was followed by further washing with 10% hydrochloric acid, then with water until the washings were neutral. The average yield of methylated acid from two identical runs was 48%. Distillation gave 1.5 g. of the methyl ether, b.p. 78.6–81.0° (0.16 mm.), $n^{25}D$ 1.4685. *Anal.*[20] Calcd. for $C_{10}H_{16}O_3$: C, 65.19; H, 8.76; OMe, 16.8. Found: C, 64.39; H, 8.96; OMe, 17.2. The product reverted to starting material after one month as shown by recrystallization and mixed m.p. determination.

For comparison with the above results, α-ethyltetronic acid was methylated. Diazomethane from 0.101 mole of N-nitrosomethylurea in 125 ml. of ether was poured into a cold solution of 4.0 g. (31.2 millimoles) of α-ethyltetronic acid[13] dissolved in a mixture of 50 ml. of ether and 5 ml. of methanol. The color of the diazomethane was discharged after 15 minutes. Extraction and washing as before, followed by distillation, gave 700 mg. (18%) of the methyl ether of α-ethyltetronic acid, b.p. 75–79° (60 μ), $n^{25}D$ 1.4833. *Anal.* Calcd. for $C_7H_{10}O_3$: OMe, 21.9. Found: OMe, 20.1. On standing, a solid was obtained which did not depress the melting point of the parent acid.

(4) **Attempted Hydrogenation.**—No uptake of hydrogen gas was observed in 12 hours at atmospheric pressure when a solution of the acid in glacial acetic acid was shaken with Adams catalyst. Starting material was recovered.

(5) **Attempted Ozonolysis.**—A sample of the methyl ether of the acid was dissolved in ethyl acetate and treated with ozone from a discharge generator for four hours. Attempted hydrogenation of the product at atmospheric pressure using $Pd(OH)_2$ on $CaCO_3$ gave no discernible pressure drop after shaking for 10 hours. Starting material was reclaimed.

Acknowledgment.—G. H. D., Jr., wishes to acknowledge a grant-in-aid from the Hynson, Westcott, and Dunning Fund.

(28) P. Clarke, *Chemistry & Industry*, 1263 (1954).

(29) F. Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 166.

BALTIMORE 18, MD.

[CONTRIBUTION FROM THE ORGANIC RESEARCH LABORATORIES OF THE U. S. VITAMIN & PHARMACEUTICAL CORP.]

Hypoglycemic Agents. IV.[1–3] N^1,N^5-Alkyl- and Aralkylbiguanides

BY SEYMOUR L. SHAPIRO, VINCENT A. PARRINO AND LOUIS FREEDMAN

RECEIVED FEBRUARY 25, 1959

A series of N^1,N^5-substituted biguanides have been prepared by condensation of the amine hydrochloride, $R_1R_2NH\cdot HCl$, with the R_3R_4-substituted dicyandiamide. Reversal of the substituents on the initial reactants afforded the same biguanide. A greater bulk or number of substituents on the biguanide was associated with the formation of monopicrates rather than dipicrates and has been interpreted as restricting hydrogen bond formation and failure to afford the cyclic cation I. Hypoglycemic effects with these biguanides are noted, particularly when N^5-methyl or N^5-dimethyl substituents are introduced in physiologically active N^1-substituted biguanides.

In proposing the intramolecular hydrogen-bonded, six-membered ring structure (I)[1,3] for β-phenethylbiguanide and related N^1-substituted biguanides, several assumptions had been made.[4] With N^1,N^5-

(1) S. L. Shapiro, V. A. Parrino and L. Freedman, THIS JOURNAL, **81**, 2220 (1959).

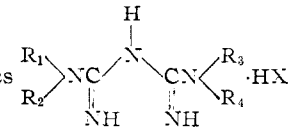
(2) S. L. Shapiro, V. A. Parrino, E. Rogow and L. Freedman, *ibid.*, **81**, 3725 (1959).

(3) S. L. Shapiro, V. A. Parrino and L. Freedman, *ibid.*, **81**, 3996 (1959).

(4) Assuming that within this series, peak hypoglycemic activity would be associated with similar molecular forms of the active species, the noted activity wherein R_1 and R_2 are hydrocarbon radicals requires that the N^2 of the biguanide be an imino nitrogen. Indications for a conjugated double bond system[1] complete the bond distribution pattern as shown for I, with the four hydrogen atoms at the N^4 and N^6

TABLE I

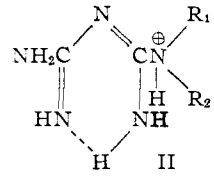
N^1,N^5-SUBSTITUTED BIGUANIDES



No.	R_1	R_2	R_3	R_4	HX	M.p.,[a] °C.	RS[b]	Formula	Analyses[c] Carbon, % Calcd.	Found	Hydrogen, % Calcd.	Found	Nitrogen, % Calcd.	Found
1	CH_3–	H	CH_3–	CH_3–	HNO_3	139–143	A	$C_6H_{14}N_6O_3$	29.1	29.1	6.8	6.8	40.8	40.3
2	CH_3–	H	CH_3–	CH_3–	2HPic.[d]	170–171	A	$C_{17}H_{19}N_{11}O_{14}$	33.9	33.7	3.2	3.6		
3	C_2H_5–	H	CH_3–	CH_3–	HCl	171–174	B	$C_6H_{16}ClN_5$	37.2	37.1	8.3	7.9		
4	C_2H_5–	H	CH_3–	CH_3–	2HPic.[d]	166–167	A	$C_{18}H_{21}N_{11}O_{14}$	35.1	34.9	3.4	3.3	25.0	25.0
5	n-C_3H_7–	H	CH_3–	CH_3–	HCl	166–167	B	$C_7H_{18}ClN_5$	40.5	40.2	8.8	8.9	33.7	33.8
6	i-C_3H_7–	H	CH_3–	CH_3–	HCl	202–203	B	$C_7H_{18}ClN_5$	40.5	41.3	8.8	8.8	33.7	34.2
7	i-C_3H_7–	H	CH_3–	CH_3–	2HPic.[d]	158–159	A	$C_{19}H_{23}N_{11}O_{14}$	36.2	36.5	3.7	3.7	24.5	24.1
8	C_3H_5–[e]	H	CH_3–	CH_3–	HCl	169–171	B	$C_7H_{16}ClN_5$	40.9	41.0	7.8	7.5	34.1	33.9
9	C_3H_5–[e]	H	CH_3–	CH_3–	2HPic.[d]	148–149	A	$C_{19}H_{21}N_{11}O_{14}$	36.4	36.7	3.4	3.6		
10	n-C_4H_9–	H	CH_3–	CH_3–	HCl	167–169	B	$C_8H_{20}ClN_5$	43.3	43.0	9.1	9.1	31.6	31.7
11	i-C_4H_9–	H	CH_3–	CH_3–	HCl	178–182	B	$C_8H_{20}ClN_5$	43.3	43.2	9.1	9.0	31.6	31.8
12	i-C_4H_9–	H	CH_3–	CH_3–	HPic.[d]	171–172	A	$C_{14}H_{22}N_8O_7$	40.6	41.0	5.4	5.7	27.0	27.1
13	n-C_5H_{11}–	H	CH_3–	CH_3–	HCl	157–159	B	$C_9H_{22}ClN_5$	45.9	46.1	9.4	9.2	29.7	29.8
14	i-C_5H_{11}–	H	CH_3–	CH_3–	HCl	169–170	C	$C_9H_{22}ClN_5$	45.9	46.1	9.4	9.3	29.7	29.7
15	i-C_5H_{11}–	H	i-C_5H_{11}–	H	HCl	183–184	B	$C_{12}H_{28}ClN_5$	51.9	51.6	10.2	9.9	25.2	24.9
16	i-C_5H_{11}–	H	i-C_5H_{11}–	H	HPic.[d]	159–160	D	$C_{18}H_{30}N_8O_7$	46.0	46.4	6.4	6.3		
17	$C_6H_5CH_2$–	H	CH_3–	H	HCl	145–150	E	$C_{10}H_{16}ClN_5$	49.7	49.9	6.7	6.6	29.0	28.9
18	$C_6H_5CH_2$–	H	i-C_5H_{11}–	H	HCl	182–184	A	$C_{14}H_{24}ClN_5$	56.5	56.6	8.1	8.2	23.5	24.0
19	$C_6H_5CH_2$–	H	CH_3–	CH_3–	HCl	181–184	F	$C_{11}H_{18}ClN_5$	51.7	51.9	7.1	7.4	27.4	27.2
20	$C_6H_5CH_2$–	H	$C_6H_5CH_2$–	H	HCl	196–197	G	$C_{16}H_{20}ClN_5$	60.5	60.4	6.3	6.3	22.0	21.8
21	$C_6H_5CH_2$–	H	$C_6H_5CH_2$–	H	HPic.[d]	162–163	A	$C_{22}H_{22}N_8O_7$	51.1	51.1	4.4	4.3	21.9	21.7
22	$C_6H_5CH_2$–	H	$C_6H_5CH_2$–	CH_3–	HCl	213–214	G	$C_{17}H_{22}ClN_5$	61.5	61.6	6.7	6.7	21.1	21.0
23	$C_6H_5CH_2$–	H	$C_6H_5CH_2$–	H	HCl	152–153	A	$C_{17}H_{22}ClN_5$	61.5	61.7	6.7	7.1	21.1	21.3
24	$C_6H_5CH_2$–	CH_3–	CH_3–	H	HCl	170–174	B	$C_{11}H_{20}ClN_5O$[f]	48.3	48.8	7.4	7.1	25.6	25.8
25	$C_6H_5CH_2$–	CH_3–	CH_3–	H	2HPic.[d]	125–126	G	$C_{23}H_{23}N_{11}O_{14}$	40.8	40.6	3.4	3.6	22.7	22.5
26	$C_6H_5CH_2$–	CH_3–	CH_3–	CH_3–	HCl	193–194	A	$C_{12}H_{30}ClN_5$	53.4	53.2	7.5	7.3	26.0	26.2
27	$C_6H_5CH_2$–	CH_3–	i-C_5H_{11}–	H	HCl	188–190	G	$C_{15}H_{26}ClN_5$	57.8	57.8	8.4	8.3	22.5	22.3
28	$C_6H_5CH_2$–	CH_3–	$C_6H_5CH_2$–	H	HCl	215–217	F	$C_{17}H_{22}ClN_5$	61.5	61.6	6.7	6.8	21.1	20.8
28a	$C_6H_5CH_2$–	CH_3–	$C_6H_5CH_2$–	H	HPic.[d]	147–148	A	$C_{23}H_{24}N_8O_7$	52.7	52.9	4.6	4.6		
29	$C_6H_5CH_2$–	CH_3–	$C_6H_5CH_2$–	CH_3–	HCl	207–209	G	$C_{18}H_{24}ClN_5$	62.5	62.7	7.0	7.0		
30	$C_6H_5CH_2$–	CH_3–	$C_6H_5CH_2$–	CH_3–	HPic.[d]	99–101	A	$C_{24}H_{28}N_8O_8$[f]					20.1	20.3
31	$C_6H_5CH_2$–	CH_3–	$C_6H_5CH_2CH_2$–	H	HCl	199–201	F	$C_{18}H_{24}ClN_5$	62.5	62.2	7.0	7.0	20.3	20.0
32	$C_6H_5CH_2$–	CH_3–	$C_6H_5CH_2CH_2$–	H	HPic.[d]	165–166	C	$C_{24}H_{26}N_8O_7$	53.5	53.6	4.9	4.9		
33	$C_6H_5CH_2CH_2$–	H	CH_3–	H	HCl	135–140	B	$C_{11}H_{18}ClN_5$					27.4	27.1
34	$C_6H_5CH_2CH_2$–	H	CH_3–	H	2HPic.[d]	174–175	F	$C_{23}H_{23}N_{11}O_{14}$	40.8	40.8	3.4	3.3		
35	$C_6H_5CH_2CH_2$–	H	CH_3–	CH_3–	HCl	201–203	F	$C_{12}H_{26}ClN_5$	53.4	53.4	7.5	7.6	26.0	26.4
36	$C_6H_5CH_2CH_2$–	H	$C_6H_5CH_2CH_2$–	H	HCl	178–180	F	$C_{18}H_{24}ClN_5$	62.5	62.3	7.0	7.0	20.2	19.8
37	$C_6H_5CH_2CH_2$–	H	$C_6H_5CH_2CH_2$–	H	HPic.[d]	148–149	G	$C_{24}H_{26}N_8O_7$	53.5	53.9	4.9	5.1	20.8	20.8

[a] Melting points are not corrected. [b] RS = recrystallizing solvent: A = ethanol–hexane, B = acetonitrile, C = ethanol, D = propanol–hexane, E = ethyl acetate, F = isopropyl alcohol, G = water. [c] Analyses by Weiler and Strauss, Oxford, England. [d] HPic. = picric acid. C_3H_5 = allyl. [f] Crystallizes as monohydrate.

unsymmetrically substituted biguanides, however, isomeric forms for I as typified by III and IV or



II

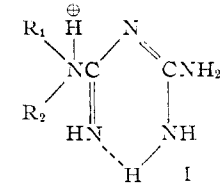
mixtures of these are possible. Inspection of molecular models of such compounds suggests steric inhibition of hydrogen bond formation.

In addition to examination of these factors, assessment of hypoglycemic effects with N^1,N^5-substituted biguanides was indicated, and the compounds prepared are described in Table I.

of the biguanide satisfying the site of high electron density at the imino nitrogen at N^2 to complete the sterically favorable six-membered hydrogen-bonded ring. The dominant protonated species was assigned with the H+ added to the R_1R_2-bearing nitrogen as a consequence of electron enrichment by the R groups.[5] The structure II having only the two hydrogens on N^4 available for hydrogen bonding is sterically less favorable.

(5) M. M. Tuckerman, J. R. Mayer and F. C. Nachod, THIS JOURNAL, **81**, 92 (1959).

Synthesis of the compounds was effected by fusion of the amine hydrochloride with the substituted dicyandiamide (see Table II) following methods



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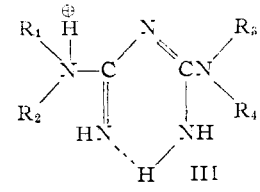
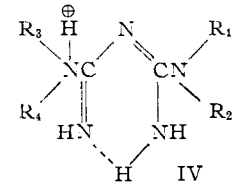


III



IV

previously described.[1,3] In three systems examined wherein the R_1R_2 groups were initially on the amine reactant and the R_3R_4 groups on the reactant dicyandiamide, when the substituent disposition was reversed, only a single compound

TABLE II

SUBSTITUTED DICYANDIAMIDES $\begin{matrix} R_1 \\ R_2 \end{matrix}\!\!>\!\!NCNHCH \parallel NH$

No.	R_1	R_2	M.p., °C.[a, b]	Formula	Analyses: Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
1	CH_3-	CH_3-	165–169	$C_4H_8N_4$	42.8	42.7	7.2	7.4	50.0	49.4
2	*i*-$C_5H_{11}-$	H	79–81[ba]	$C_7H_{14}N_4$	54.5	54.8	9.2	9.1	36.3	36.4
3[c]	$-(CH_2)_5-$		166–168[bb]	$C_7H_{12}N_4$	55.2	55.6	8.0	8.2	36.8	37.1
4[d]	$C_6H_5CH_2-$	H	101–104	$C_9H_{10}N_4$	62.1	61.8	5.8	5.7		
5	$C_6H_5CH_2-$	CH_3-	107–109	$C_{10}H_{12}N_4$	63.8	63.1	6.4	6.5	29.8	30.2
6	$C_6H_5CH_2CH_2-$	H	114–115	$C_{10}H_{12}N_4$	63.8	63.8	6.4	6.5	29.8	29.8

[a] Melting points are not corrected. [b] The recrystallizing solvent was ethyl acetate–hexane unless otherwise shown; [ba] benzene; [bb] xylene. [c] Ref. 28 reports m.p. 166–168°. [d] Ref. 28 reports m.p. 108–109°.

was isolated.[6] This would indicate that a particular structural form for I would predominate, and whichever synthetic combination is used equilibration proceeds to yield this form.

It was noted that the N^1,N^5-substituted biguanides having N^5-benzyl-N^5-methyl substitution were much more readily isolated than N^5-methylbiguanides. Accordingly, the potential of debenzylation[8] by hydrogenolysis was explored, and N^1-benzyl-N^1-methylbiguanide was successfully reduced to N^1-methylbiguanide, isolated as the dihydrochloride. However, with the more complex biguanide, compound 26, the hydrogenolysis failed, as it did with N^1,N^5-dibenzylbiguanide.[9]

For compounds with particular group bulk or disposition, steric hindrance[10] to hydrogen bond formation (I) was possible and is suggested by the results below.

Throughout this work many of the biguanides have been characterized as picric acid derivatives. As a rule, the only product isolated was the dipicrate.[11] In the present series (Table I) many of the compounds yielded monopicrates, particularly as the bulk of the substituent increased on N^5 (compounds 16, 21, 28, 30, 32, 38), sugggesting a structural influence. While the biguanides are diacid bases, it has been shown[1] for the dihydrochloride, that the second mole of hydrogen chloride is not retained, as a consequence of the occupation of the second basic site by hydrogen bond formation. It would then be unlikely that formation of the dipicrate *vs.* the monopicrate would be a function of a locus of the second proton,[12] or a function of atomic distances[13] between nitrogen atoms. Our experimental pattern is just the reverse of that of Knott and Breckenridge[14] in that with increasing bulk of the substituents, monopicrates are obtained, and the majority of the structures which reflected less steric hindrance yielded dipicrates insoluble in water.[15]

The observations suggest that the formation of the dipicrate involves addition of one mole of picric acid in salt formation, and that the second mole of picric acid adds to the hydrogen bonded cation I as a molecular complex.[16,17] Formation of the monopicrate serves as an indicator for structures with steric inhibition of hydrogen bond formation as shown for I.[18]

(6) Reaction of the amine hydrochloride with the dicyandiamide proceeds through attack at the cyano group as is shown in Scheme I and could conceivably yield the isomers shown.

SCHEME I

$$R_1R_2NH\cdot HCl + NCNHC(=NH)NR_3R_4 \longrightarrow III$$

$$R_3R_4NH\cdot HCl + NCNHC(=NH)NR_1H_2 \longrightarrow IV$$

Recently,[7] isomeric forms for triguanides have been implied and described.

(7) K. N. Nandi and M. A. Phillips, *Chemistry & Industry*, 719 (1958).

(8) W. H. Hartung and R. Siminoff, "Organic Reactions," Vol. VII, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 263.

(9) Debenzylations of tertiary amine groups proceed far more readily than secondary amines, *ibid.*, p. 276.

(10) (a) W. F. Forbes and J. F. Templeton, *Can. J. Chem.*, **36**, 180 (1958); (b) S. Searles, M. Tamres and G. M. Barlow, THIS JOURNAL, **75**, 71 (1953); (c) W. S. Frye, *J. Chem. Phys.*, **21**, 2 (1954); (d) A. H. Blatt, *J. Org. Chem.*, **20**, 591 (1955); (e) G. J. Brealy and M. Kasha, THIS JOURNAL, **77**, 4462 (1955); (f) K. Nakamoto, M. Margoshes and R. E. Rundle, *ibid.*, **77**, 6840 (1955); (g) C. G. Cannon, *Spectrochim. Acta*, **10**, 341 (1958); (h) E. W. Gill and E. D. Morgan, *Nature*, **183**, 248 (1959).

(11) In one instance,[2] N^1-benzyl-N^1-phenylbiguanide afforded a monopicrate. The bulky character of the substituent groups suggested a steric influence, and it was established that (2,6-dimethylphenyl)-biguanide would afford a monopicrate by controlling the quantity of picric acid used[2] although the dipicrate formed under the usual conditions. In contrast, using aqueous picric acid with β-phenethylbiguanide[1] only the dipicrate was isolated.

(12) T. R. Harkins and H. Freiser, THIS JOURNAL, **77**, 1374 (1955), discuss compounds which possess two basic nitrogen atoms that combine with only one proton in basic solution.

(13) R. A. Abramovitch, *J. Chem. Soc.*, 3839 (1954).

(14) R. F. Knott and J. G. Breckenridge, *Can. J. Chem.*, **32**, 512 (1954), in an investigation of 2,2′-bipyridyl analogs obtained only monopicrates with their compounds, suggesting formation of a hydrogen bond between nitrogen atoms by the first proton which was sufficiently strong to hold the molecule in *cis* configuration. Alternatively, when they employed substituents which afforded sufficient steric repulsion to such hydrogen bond formation, the two ring systems were free to rotate about the connecting bond and presumably assumed a coplanar *trans* configuration and afforded dipicrates.

(15) A single exception in the compounds studied was isolation of the monopicrate of N^1-isobutyl-N^5,N^5-dimethylbiguanide (compound 12). Under the usual experimental conditions, the dipicrate of this compound was apparently soluble in aqueous picric acid medium, and on addition of excess biguanide, the reaction mixture afforded the precipitate of the monopicrate.

(16) R. D. Kross and V. A. Fassel, THIS JOURNAL, **79**, 38 (1957).

(17) Some measure of the difference in the mode of linkage in dipicrates of biguanides is to be inferred from T. Callan and N. Strafford, *J. Soc. Chem. Ind. (London)*, **43**, 1 (1924), who observed that with the dipicrate of *o*-tolylbiguanide, the second molecule of picric acid is only loosely combined and loses some picric acid on recrystallization from alcohol. It should be pointed out that many of our dipicrates were stable upon recrystallization from organic solvents, including alcohol.

(18) Although none of the biguanides derived from the compounds characterized as monopicrates (except compound 11) afforded practicable hypoglycemic activity, the structure–activity relationships would suggest that the sum of the carbon content of the substituents was too high for effective hypoglycemia.

Pharmacology.—The compounds were evaluated for hypoglycemic activity by methods previously described[3] and results are noted in Table III.

TABLE III

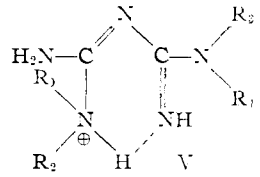
HYPOGLYCEMIC ACTIVITY OF N^1,N^5-SUBSTITUTED BIGUANIDES[a,b]

4+	3+	2+	1+	0
10	1, 11	5	15	3, 20, 29
17	6, 19	35	24	13, 22, 36
	8, 33		26	14, 23
			31	18, 27

[a] The compounds were evaluated subcutaneously in guinea pigs at test levels corresponding to $^1/_5$ to $^1/_{10}$ of the $LD_{min.}$ (minimum lethal dose subcutaneous in mice). Numbers correspond with compound numbers in Table I and compounds are classified according to hypoglycemic response in terms of percentage reduction of blood sugar from the normal blood sugar of the animal; 0 = less than 10% reduction; 1+ = 10–20% reduction; 2+ = 21–35% reduction; 3+ = 36–60% reduction and 4+ = over 60% reduction. [b] The following compounds were evaluated orally: 1, 0; 10, 2+; 17, 3+.

The data indicate that substantial hypoglycemic activity may be attained with methyl or dimethyl substitution in the N^5-position in biguanides having active N^1-substituents. The use of larger substituents in the N^5-position in similar systems is associated with considerable diminution or disappearance of activity. Insofar as it has been explored, the active N^1,N^5- substituted biguanides are orally absorbed less effectively than the corresponding N^1-substituted biguanides.

The fact that no practicable hypoglycemia is noted with tetrasubstituted compounds would discount the significance of such structures as V.



The substituted dicyandiamides showed little or no hypoglycemic effect with the exception of β-phenethyldicyandiamide which was slightly active.

Certain of the other previously described derivatives[1] of phenethylbiguanide including β-phenethylamidinourea, N-amidino-N^1-β-phenethylurea, β-phenethylurea, β-phenethylamine, 2-amino-4-β-phenethylamino-*s*-triazine, 2-amino-4-β-phenethylamino-6-methyl-*s*-triazine and 2-amino-4-β-phenethylamino-6-(α-hydroxyethyl)-*s*-triazine were without significant hypoglycemic activity. Pharmacological studies of the *in vitro* hydrolysis product, β-phenethylguanidine, have been described in detail[19] and show a complicated pattern involving hyper- and hypoglycemia.

Among the arylbiguanides, the most active compound was (2,4,6-trimethylphenyl)-biguanide which showed 4+ hypoglycemia at 50 mg./kg. (sc.) ($LD_{min.}$ 200 mg./kg.), although it proved to be inactive when tested *per os* at 80 mg./kg. Other arylbiguanides which showed 2+ activity were (2-methyl-4-chlorophenyl)-biguanide, (2-methyl-5-chlorophenyl)-biguanide and N^1-ethyl-N^1-(2-methylphenyl)-biguanide. It is of interest that these biguanides all manifested "biguanide" type resonance[2] in contrast to the "acetanilide" type resonance obtained with many of the other arylbiguanides.

The designation of the hydrogen-bonded[20] cyclic[21] cation[22] I (protonated at the R_1R_2-bearing nitrogen[1,23] in equilibrium with the dibasic cation[1,24] at the *p*H of gastric juice and stomach contents) as the physiologically active form of these biguanide oral[25] hypoglycemic agents may be of considerable importance in pharmaoclogical studies on the mode of action of these compounds. Such studies, particularly with phenethylbiguanide (DBI), are being pursued in our laboratories[26] and elsewhere.

Experimental[27]

N^1-Benzyl-N^1-methyldicyandiamide (Table II, Compound 5).—The dicyandiamides were prepared following the procedure of Redmon and Nagy[28] and were typified by the preparation below.

A mixture of 79.3 g. (0.5 mole) of benzylmethylamine hydrochloride, 55 g. (0.5 mole) of sodium dicyanamide in 500 ml. of 1-butanol and 40 ml. of water was stirred and heated under reflux for 7 hours. The formed sodium chloride was separated and the filtrate concentrated to dryness. The residue was granulated under 200 ml. of water and filtered, yielding 87.0 g. (93%) of product.

The biguanides described in Table I were prepared by the same general procedure and typical examples are given below.

N^1,N^5-Dibenzyl,N^1-methylbiguanide Hydrochloride (Table I, Compound 28).—A mixture of 16.2 g. (0.093 mole) of benzyldicyandiamide (Table II, compound 4) and 11.4 g. (0.093 mole) of N-methylbenzylamine hydrochloride was heated gradually with stirring in an oil-bath. The mixture began to melt at 55° and fused completely at 110° (bath 116°). Heating was continued over 30 minutes with gradual rise of bath temperature to 149°. The cooled fusion product was recrystallized from water and yielded 18.0 g. (58%).

The same compound was obtained (no depression in mixed m.p.) by treating benzylamine hydrochloride with N-methylbenzyldicyandiamide (Table II, compound 5). In a similar fashion it was established that reaction of β-phenethyldicyandiamide and benzylamine hydrochloride, or reaction of benzyldicyandiamide with β-phenethylamine hydrochloride, afford the same compound, N^1-benzyl-N^5-(β-phenethyl)-biguanide hydrochloride (Table I, compound 23).

(19) G. Kroneberg and K. Stoepel, *Arzneimittel-Forsch.*, **8**, 470 (1958).

(20) Other publications involving consideration of hydrogen bond formation in biological systems; (a) L. Pauling and R. B. Corey, *Arch. Biochem. Biophys.*, **65**, 164 (1956); (b) W. Traub, *Science*, **129**, 210 (1959); (c) E. R. Garrett, THIS JOURNAL, **80**, 4049 (1958); (d) I. M. Klotz and J. Ayers, *ibid.*, **79**, 4078 (1957).

(21) In the anti-malarial arylbiguanide, the existence of a cyclic form of the molecule has been implied to have biological significance.[1]

(22) The fact that the metabolically active form is an ion is of interest in view of B. D. Davis, *Arch. Biochem. Biophys.*, **78**, 497 (1958), who states "a survey of metabolic reactions discloses that all known low molecular weight and water-soluble biosynthetic intermediates possess groups that are essentially completely ionized at neutral *p*H."

(23) Protonation at this site should substantially reduce fat solubility; W. D. Dettbarn, I. B. Wilson and D. Nachmansohn, *Science*, **128**, 1275 (1958).

(24) Recent work has indicated that basic drugs are concentrated in the gastric juice; P. A. Shore, B. B. Brodie and C. A. M. Hogben, *J. Pharmacol. Exp. Therap.*, **119**, 361 (1957).

(25) While the hypoglycemic effect was noted in a broader structural range, the additional requisite of oral effectiveness has restricted the structural scope of active compounds largely to those wherein R_1 is C_4–C_8 alkyl and ω-C_6–C_8 aralkyl.

(26) G. Ungar, S. Psychoyos and H. Hall, *Metabolism*, in press (1959).

(27) Descriptive data shown in the tables are not herein reproduced.

(28) B. C. Redmon and D. E. Nagy, U. S. Patent 2,455,807 (Dec. 7, 1948).

The alternative synthetic paths also yielded the same compound in the preparation of N^1-benzyl-N^1-methyl-N^5-(β-phenethyl)-biguanide hydrochloride (Table I, compound 31).

Methylbiguanide Dihydrochloride (by Catalytic Debenzylation of N^1-Benzyl-N^1-methylbiguanide Hydrochloride).—A solution of 12.0 g. (0.05 mole) of N^1-benzyl-N^1-methylbiguanide hydrochloride[8] in 120 ml. of ethanol and 50 ml. of water was treated with a suspension of palladium-on-carbon (from 1.0 g. of palladium chloride in 35 ml. of water and 0.5 ml. of 3 *N* hydrochloric acid, and 10.0 g. of carbon (Darco)) and hydrogenated at 45° under 50 lb. hydrogen pressure for 22 hours in the Parr hydrogenator. At this point two equivalents of hydrogen was absorbed. The catalyst was removed and the filtrate (strong toluene odor) was evaporated to dryness. The residue of 6.45 g. was leached with 900 ml. of acetonitrile and the insoluble portion of 5.65 g. was recrystallized (ethanol) to yield 3.23 g. of a mixture of mono- and dihydrochloride of the product. One gram of this mixture was dissolved in 20 ml. of methanol and 2.5 ml. of 3 *N* hydrochloric acid and was evaporated to dryness. The residue recrystallized (ethanol–hexane) gave 0.92 g. of product which melted at 225° dec.

Anal. Calcd. for $C_8H_{11}Cl_2N_5$: C, 19.2; H, 5.9; N, 37.2. Found: C, 19.2; H, 5.9; N, 36.5.

The dipicrate melted at 198–199°.

Anal. Calcd. for $C_{15}H_{15}N_{11}O_{14}$: C, 31.5; H, 2.7. Found: C, 31.0; H, 2.9.

Similar attempted debenzylations with compounds 20 and 26 of Table I were unsuccessful and afforded only recovery of the initial reactant.

TABLE IV

SPECTRA OF BIGUANIDES[a]

No.[b]	λ_{max}, mμ	$\epsilon \times 10^{-3}$
17	236	16.5
19	236	14.6
20	237	17.8
24	238	15.5
26	240	17.9
28	240	17.4
29	244	18.9

[a] The spectra were determined in water in the Beckman DK recording spectrophotometer, using 1-cm. cells. [b] The compounds correspond to compound numbers in Table I.

Ultraviolet Absorption Spectra.—The spectra of some of the compounds have been described in Table IV. Relative to the spectrum of β-phenethylbiguanide hydrochloride,[1] it is noted that increasing substitution in the N^1,N^5-substituted biguanides is associated with bathochromic and hyperchromic effects.

Acknowledgment.—The authors are grateful to Dr. G. Ungar for the results of the hypoglycemic tests reported herein, and to M. Blitz for the ultraviolet absorption spectra.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XX. Diazoacetyl Analogs of Chlorambucil

BY W. A. SKINNER, HELEN F. GRAM, CAROL W. MOSHER AND B. R. BAKER

RECEIVED FEBRUARY 12, 1959

3-(*p*-Diazoacetylphenyl)-propionic acid (VI) was synthesized from methyl hydrocinnamate (I) *via* the key intermediate, 3-(*p*-glycylphenyl)-propionic acid hydrochloride (V). Attempts to synthesize 3-(*p*-diazoacetamidophenyl)-propionic acid (XV) by two different routes failed on the last step. However, the corresponding methyl ester XVI was synthesized from 3-(*p*-aminophenyl)-propionic acid (VII) *via* methyl 3-(*p*-glycylaminophenyl)-propionate hydrochloride (XIV).

Interest in diazo derivatives of amino acids has been stimulated by the discoveries that azaserine[2] (O-diazoacetyl-L-serine) and DON[3] (6-diazo-5-oxo-L-norleucine) show activity in inhibiting the growth of the Crocker Sarcoma-180 tumor.

Sarcolysine (3-{*p*-[bis-(2-chloroethyl)-amino]-phenyl}-DL-alanine, "phenylalanine mustard")[4] has been shown to be one of the more active nitrogen mustard compounds. In addition, Luck[5] has found that the two next higher homologs of phenylalanine mustard are also effective on the Cloudman malignant melanoma (S-91).

Everett, *et al.*,[6] found that a series of *p*-[bis-(2-chloroethyl)-amino]-phenylcarboxylic acids inhibit the growth of the transplanted Walker rat Sarcoma-256, the most active compound being the butyric acid derivative (chlorambucil). The methyl and ethyl esters were also active.

The hypotheis has been proposed[7] that azaserine, sarcolysine and chlorambucil might be considered members of a broad class of anticancer agents consisting of metabolites bearing an alkylating group that function by irreversible inhibition of the corresponding enzymes. This hypothesis suggests that 3-phenylpropionic acid or 4-phenylbutyric acid be considered as carrier (metabolite) groups for the diazoalkyl grouping characteristic of azaserine. This paper describes the synthesis of two such compounds, namely, 3-(*p*-diazoacetylphenyl)-propionic acid (VI) and methyl 3-(*p*-diazoacetamidophenyl)-propionate (XVI).

3 - (*p* - Diazoacetylphenyl) - propionic acid (VI) was synthesized in five steps from methyl hydrocinnamate (I). A Friedel-Crafts reaction of acetyl

(1) This program is under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper in this series *cf.* C. D. Anderson, L. Goodman and B. R. Baker, paper XIX of this series, THIS JOURNAL, **81**, 3967 (1959).

(2) C. C. Stock, D. A. Clarke, H. C. Reilly, S. M. Buckley and C. P. Rhoads, *Nature*, **173**, 71 (1954).

(3) D. A. Clarke, H. C. Reilly and C. C. Stock, Abstracts of Papers, 129th Meeting, American Chemical Society, Dallas, Texas, April, 1956, p. 12-M; H. A. DeWald and A. M. Moore, THIS JOURNAL, **80**, 3941 (1958).

(4) F. Bergel, V. C. E. Burnop and J. A. Stock, *J. Chem. Soc.*, 1223 (1955); F. Bergel and J. A. Stock, *ibid.*, 2409 (1954); L. F. Larinov, A. S. Khokhlov, E. N. Shkodinskaia, O. S. Vasina, V. I. Trusheikina and M. A. Novikova, *Lancet*, **269**, 169 (1955).

(5) J. M. Luck, *Cancer Res.*, **17**, 1071 (1957); H. E. Smith and J. M. Luck, *J. Org. Chem.*, **23**, 837 (1958).

(6) J. L. Everett, J. J. Roberts and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

(7) H. F. Gram, Carol W. Mosher and B. R. Baker, paper XVIII of this series, THIS JOURNAL, **81**, 3103 (1959).