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

Hypoglycemic Agents. I.[1] Chemical Properties of β-Phenethylbiguanide.[2] A New Hypoglycemic Agent[3]

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

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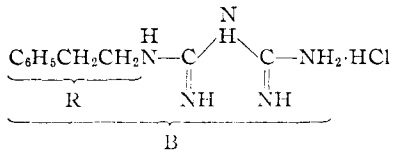
Some chemical properties of phenylethylbiguanide (I) are described. Hydrolyses indicate I to be stable in strongly acidic solutions, and to be degraded in hot alkaline solutions to β-phenethylguanidine, β-phenethylurea and β-phenethylamine. Attempted alkylation of I with alkyl halides yields instead the corresponding I hydrohalide salts. The amidinourea isosteres of I have been prepared (VIII and IX). A series of salts of I are described as well as a series of triazines obtained by the reaction of I with esters.

The hypoglycemic agent N^1-β-phenethylbiguanide hydrochloride (I)[4] has been extensively explored on a pharmacological[5] and clinical[6] level. In this paper some chemical properties of β-phenethylbiguanide[7] are reported. Conventionally, biguanide structures are written as shown for I. However, the biguanide molecule is more precisely represented as having a conjugated double bond system[8] stabilized in the form of a hydrogen-bonded intermolecular six-membered ring.[9,10] Biguanides are di-acid bases characterized by a strongly basic primary dissociation constant and a considerably weaker (by about 10 pK units) secondary dissociation constant. At physiological pH values the properties of a biguanide reflect the singly charged cation.[9]



I

This study has confirmed and expanded upon this description. The biguanide I formed readily isolable and stable di-acid salts (Table I, compounds 1, 3, 6, 7). The pH of a 0.1 M solution of the monohydrochloride is 6.7, and that of a 0.01 M solution of the dihydrochloride is 2.6.

Further characterization obtained by consideration of the ultraviolet absorption spectra is detailed in the equations

$$BH^+Cl^-(H_2O) \longrightarrow BH^+ + Cl^- \quad (1)$$

$$BH^+Cl^-(HCl) \longrightarrow BH_2^{++} + 2Cl^- \quad (2)$$

$$BH_2^{++}Cl_2^-(H_2O) \longrightarrow BH^+ + 2Cl^- + H^+ \quad (3)$$

$$B(H_2O) \longrightarrow BH^+ + OH^- \quad (4)$$

$$BH^+(NaOH) \longrightarrow B + H_2O \quad (5)$$

$$BH^+Cl^-(CH_3OH) \longrightarrow B(CH_3OH)H^+ + Cl^- \quad (6)$$

$$B(CH_3OH) \longrightarrow BH^+ + OCH_3^- \quad (7)$$

The high ϵ noted with BH^+Cl^- indicates that a conjugated double bond system exists. While Gage[9] contended that the spectral characteristics of the aryl biguanides were a function of resonance forms associated with the aniline moiety, the closely related spectra of phenylbiguanide hydrochloride and β-phenethylbiguanide (which cannot have aniline resonance) would reflect that the band noted for both structures is a function of double bond conjugation.

As the acidity of the solvent increases, the spectrum of BH^+Cl^- shows the ϵ to be considerably diminished, and finally in a medium 1×10^{-2} N with respect to hydrogen ion, only non-specific absorption is noted. Alternatively, the dihydrochloride in water shows the same absorption characteristics as BH^+Cl^-, indicating rapid hydrolysis of the doubly charged cation to the singly charged cation (equation 3).[11]

The spectrum of BH^+Cl^- in dilute alkali is identical with that observed in water, indicating that the singly charged cation persists (equation 4). However, as the basicity of the solvent is increased, the stronger base (OH^-) strips the proton from BH^+ and the resultant hypochromic effect defines the spectrum of B itself (equation 5).

In methanol a hyperchromic effect relative to the noted spectrum in water is obtained. From other experimental data below, it would appear that β-phenethylbiguanide in methanol is transformed as is shown in equation 7.

These considerations indicate that forms such as II contribute significantly to the structure of BH^+.

Other cyclic forms, as well as the open forms, both conjugated and unconjugated, in addition to a multiplicity of ionic forms may be written.

(1) Papers are in preparation describing over two hundred alkyl, arylalkyl, heteroarylalkyl, cycloalkyl and aryl biguanides which were examined for hypoglycemic activity.

(2) Presented in part at the New York Meeting, American Chemical Society, September, 1957.

(3) A number of clinical and pharmacological papers have appeared dealing with studies of the hydrochloride of this compound variously described as DBI and β-phenethylformamidinyliminourea.

(4) G. Ungar, L. Freedman and S. L. Shapiro, *Proc. Soc. Exp. Biol. Med.*, **95**, 190 (1957).

(5) (a) A. N. Wick, E. R. Larson and G. S. Serif, *J. Biol. Chem.*, **233**, 296 (1958); (b) R. H. Williams, J. M. Tyberghein, P. M. Hyde and R. L. Nielsen, *Metabolism*, **6**, 311 (1957); (c) S. S. Bergen, J. G. Hilton and W. S. Norton, *Proc. Soc. Exp. Biol. Med.*, **98**, 625 (1958).

(6) (a) J. Pomeranze, H. Fuiji and G. T. Mouratoff, *ibid.*, **95**, 193 (1957); (b) L. P. Krall and R. Camerini-Davalos, *ibid.*, **95**, 345 (1957); (c) R. H. Williams, D. C. Tanner and W. D. O'Dell, *Diabetes*, **7**, 87 (1958).

(7) The compound has been synthesized by methods previously described: (a) S. L. Shapiro, V. A. Parrino and L. Freedman, *J. Am. Pharm. Assoc., Sci. Ed.*, **46**, 689 (1957); (b) S. L. Shapiro, V. A. Parrino, K. Geiger, S. Kobrin and L. Freedman, THIS JOURNAL, **79**, 5064 (1957).

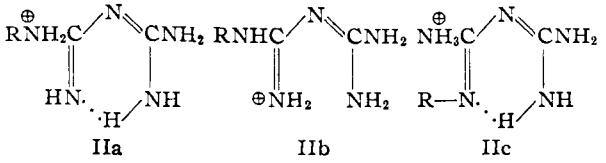
(8) W. D. Kumler, *J. Org. Chem.*, **20**, 700 (1955).

(9) J. C. Gage, *J. Chem. Soc.*, 221 (1949), has suggested such a structure for arylbiguanides.

(10) I. C. Kogon, THIS JOURNAL, **79**, 2253 (1957), has found evidence for similar structures in the closely related biurets.

(11) (a) F. H. Westheimer and O. T. Benfey, THIS JOURNAL, **78**, 5309 (1956); (b) C. A. Coulson, *Research*, **10**, 149 (1957); (c) C. Tanford, THIS JOURNAL, **79**, 5348 (1957); (d) D. H. McDaniel and H. C Brown, *Science*, **118**, 370 (1953); (e) W. H. T. Davison, *Chemistry & Industry*, 408 (1953), have rationalized the wide spread in the acid dissociation constants of certain dibasic acids on the basis of internal hydrogen bonded structures.

Considering the forms shown, the locus of the proton has important physiological consequences.[12] Many drugs owe their activity to a combination of lipophilic and hydrophilic substituents which control the locus of attack. In the form IIa, the only substantially lipophilic group, $C_6H_5CH_2CH_2^-$, is protonated, thus drastically reducing the lipophilic character of the entire molecule. In turn, forms IIb and IIc leave the RN– group in relatively non-polar form which would make the molecule more fat-soluble.



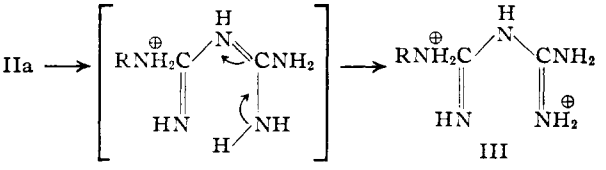
IIa IIb IIc

The electron-donating character of the β-phenethyl group should make its nitrogen the most susceptible to proton attack as in IIa. Alternatively, the RN– group as shown in IIc is the most basic site for attachment of a hydrogen bond. The fact that biguanides have been obtained with high hypoglycemic activity[1,2] wherein RHN– is substituted by $RN(CH_3)$– whose structure could not be defined by a form such as IIc, reflects that the favored form is IIa.

The acidic and basic hydrolyses of β-phenethylbiguanide were studied, particularly, to assess the hypoglycemic effect of the products of degradation.

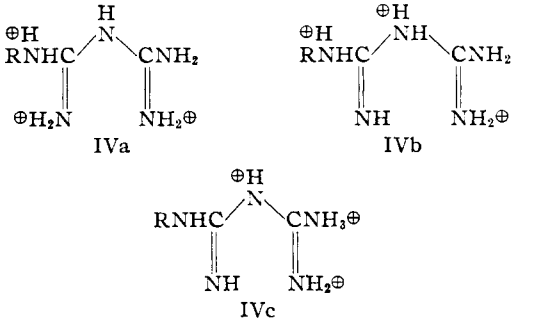
Aryl biguanides are converted to the corresponding 1-amidino-3-phenyl (and substituted phenyl) ureas[13] by boiling with aqueous hydrochloric acid solutions. β-Phenethylbiguanide proved to be surprisingly stable to acidic attack and was recovered unchanged, with isolation as the diacid salt after prolonged heating with 3 and 6 *N* hydrochloric acid, 50% sulfuric acid or polyphosphoric acid (Table I, compounds 1, 6, 7).

The ultraviolet absorption spectra had shown that as the acidity increases, only non-specific absorption is obtained, reflecting a loss of conjugation as the doubly charged cation BH_2^{++} is formed (equation 2). This transformation is shown by the form III (BH_2^{++}) which permits the widest charge separation. With these sites for



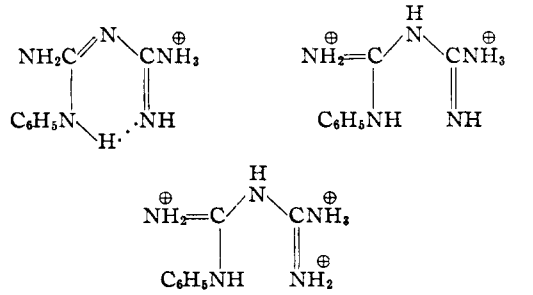
III

proton acceptance fulfilled, and since the molecule is completely stable as $BH_2^{++}Cl_2^=$, a third proton is necessary to satisfy the requirement for hydrolysis.[14] Addition of this third proton is associated with the form IV which proved to be invulnerable to attack of the water molecule at either of the carbon atoms in the molecule. The failure of the hydrolyses of form IV to 1-(N-β-phenethyl)-amidinourea also may be associated with addition of the elements of water, with subsequent reversal of this reaction, to the exclusion of elimination of ammonia.



IVa IVb IVc

In turn, the hydrolyses of the aryl biguanides could proceed through forms reflecting the lower basicity of aryl amines as compared to arylalkyl amines as shown for the mono-, di- and triprotonated phenylbiguanide molecule below (corresponding to forms IIc, III and IV above).



In the instance of the triprotonated phenylbiguanide, attack at the anilino-bearing carbon proceeds to give the 1-amidino-3-phenylurea.

At ambient temperatures β-phenethylbiguanide has been shown to be stable in aqueous alkali (see ultraviolet absorption spectra). However, at elevated temperatures, decomposition with formation of β-phenethylguanidine (V), β-phenethylurea (VI) and β-phenethylamine (VII) is noted when the free base (B), *per se* or with one equivalent of alkali[15] in water, are heated under reflux.

Another phase of this study involved the preparation of the isosteres of BH^+Cl^-, the related 1-(N-β-phenethyl)-amidinourea (VIII) and 1-amidino-3-(β-phenethyl)-urea (IX). The isostere VIII was prepared by the acid hydrolysis of (β-phenethyl)-dicyandiamide following procedures described

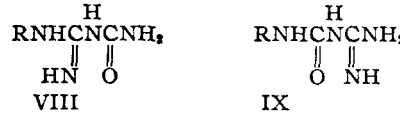
(12) The significance of such hydrogen bonded cyclic structures having profound influence on biological properties was suggested by F. H. S. Curd and F. L. Rose, *J. Chem. Soc.*, 729 (1946).

(13) (a) T. Urbanski, B. Skowronska-Serafinowa, H. Dabrowska and J. Jankowska, *Bull. acad. polon. sci. Classe III*, **1**, 74 (1953) [*C. A.*, **49**, 869f (1955)]; (b) T. Urbanski, B. Skowronska-Serafinowa and H. Dabrowska, *Rocsniki Chem.*, **29**, 450 (1955) [*C. A.*, **50**, 5548b (1956)]; (c) *ibid.*, **28**, 423 (1954) [*C. A.*, **50**, 205f (1956)]; (d) *Bull. acad. polon. sci. Classe III*, **2**, 453 (1954) [*C. A.*, **50**, 210h (1956)]; (e) F. H. S. Curd, D. G. Davey and D. N. Richardson, *J. Chem. Soc.*, 1732 (1949).

(14) J. T. Edward and S. C. R. Meacock, *J. Chem. Soc.*, 2000, 2007, 2009 (1957), have pointed out that amides are strong enough bases to be appreciably protonated in 3–6 *N* mineral acid. Below the concentration for maximum rate, the effect of increasing acid strength is chiefly to increase the concentration of the protonated intermediate; above the concentration for maximum rate, the effect is chiefly to decrease the concentration of the water. The rate-determining step is the attack of the water molecule on the conjugated acid of the amide, $RC(OH)=NH_2^+$.

(15) N. N. Crounse, *J. Org. Chem.*, **16**, 492 (1951), noted formation of isopropylamine, chloroaniline and ammonia in the alkaline (barium hydroxide) hydrolyses of chloroguanide.

in the literature,[16] while its isomer IX was obtained from reaction of guanidine with β-phenethyl isocyanate.[13e] An alternative preparation for IX



VIII IX

was through the diazotization[13e,17] of the biguanide which in the instance of the aryl biguanides has been proved to convert to the 1-amidino-3-arylureas,[17] and in the instance of N^1-aryl-N^5-alkylbiguanides to afford the 1-(N-alkylamidino)-3-arylureas.[13e] While Pellizzari[17] had shown that the N,N-pentamethylenebiguanide gave the 1-amidino-3-(N,N-pentamethylene)-urea, proof of the site of diazotization with a monosubstituted arylalkyl or alkylbiguanide was lacking. In view of the differential pattern of the acidic hydrolyses between aryl and aralkylbiguanides noted before, and the apparent preferential diazotization at the nitrogen closer to the aryl group in the mixed N^1-aryl-N^5-biguanides,[13e] a clear picture remained to be demonstrated.

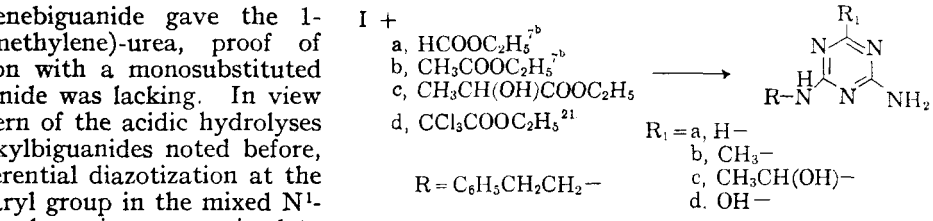
With β-phenethylbiguanide, diazotization has as yet failed to yield pure products. However, with an analogous biguanide it has been demonstrated that the same product, 1-amidino-3-*n*-butylurea is obtained by the diazotization of *n*-butylbiguanide nitrate, or by the reaction of guanidine with butyl isocyanate.

As this work developed,[1,2] it was recognized that in certain instances hypoglycemic effects obtained with selected N^1-substituted biguanides retained such activity when N^5 of the biguanide was substituted with a methyl group.[18] Accordingly, it was of interest to establish whether the free base, β-phenethylbiguanide, could be methylated by typical alkylating agents. Treatment with methyl iodide in methanol resulted instead in the isolation of the β-phenethylbiguanide hydroiodide. Similar reactions with methyl tosylate yielded the β-phenethylbiguanide toluenesulfonic acid salt, and the use of 1,6-dibromohexane afforded the β-phenethylbiguanide hydrobromide. Treatment under similar conditions with benzyl bromide afforded the β-phenethylbiguanide dihydrobromide, and benzyl methyl ether was isolated.

These reactions indicate that the biguanide in methanol solution, to some extent, exists as the biguanidinium methoxide (equation 7), and that the reaction taking place is nucleophilic displacement by methoxide of the halogen with formation of methyl ether and the salt of the biguanide.[19] It is not clear at this point how the dihydrobromide formed in the benzyl bromide reaction.

Frequently, a drug will derive its activity from a metabolic transformation product and a particularly striking example of this had been noted in the instance of the anti-malarial arylbiguanide, chloroguanide.[20] It was of interest, therefore, to establish whether β-phenethylbiguanide interacts with some metabolic intermediate to afford an active structure. *In vitro* experiments were conducted wherein the biguanide was allowed to react with esters including those yielding ions of significance in the carbohydrate cycle, to yield triazine derivatives which in turn were tested for hypoglycemic activity. These results are summarized in Scheme I.

SCHEME I. REACTION OF β-PHENETHYLBIGUANIDE WITH ESTERS



The reaction with ethyl lactate, in addition to yielding the required triazine, gave a compound with analyses reflecting loss of one mole of ethanol and one mole of ammonia. This has been tentatively assigned the structure 2-hydroxy-6-(1-hydroxyethyl)-4-(β-phenethyl)-amino-*s*-triazine.[22]

In compounds used pharmacologically, the intrinsic activity is at times desirably modified by the form, particularly the salt, in which it is used. The salt frequently can provide desirable physical characteristics requisite for formulatory work, but more important, may improve on the physiological activity of the compound.[23] This factor was extensively explored and the salts prepared are described in Table I.

An additional factor of interest was exploration of the chelation properties.[24] Preliminary work indicates formation of insoluble complexes with copper and that the biuret reagent described by Rosenthal and Cundiff[25] gives good color reactions (pink) with β-phenethylbiguanide and is useful in following the synthetic procedures.

(16) H. C. Carrington, A. F. Crowther and G. J. Stacey, *J. Chem. Soc.*, 1017 (1954).

(17) G. Pellizzari, *Gazz. chim. ital.*, **53**, 384 (1923).

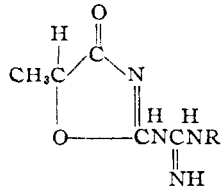
(18) Thus, N^1-benzyl-N^5-methylbiguanide and N^1-(β-phenethyl)-N^5-methylbiguanide were effective hypoglycemic agents (described in ref. 2).

(19) The reaction may be considered, in a sense, as a reversal of the demethylation of ethers by the salts of weak bases; see D. Klamann, *Monatsh. Chem.*, **84**, 814 (1953).

(20) A. F. Crowther and A. A. Levi, *Brit. J. Pharmacol.*, **8**, 93 (1953).

(21) S. L. Shapiro and C. G. Overberger, THIS JOURNAL, **76**, 97 (1954).

(22) An alternative structure being considered is



(23) (a) F. A. Alves, M. F. C. A. N. Graça and H. L. Baptista, *Nature*, **181**, 182 (1958); (b) M. A. Kaplan, H. L. Dickison, K. A. Hubel and F. H. Buckwalter, *Antibiotic Med. & Clin. Therapy*, **4**, 99 (1957); (c) W. D. Gray, R. T. Hill, R. Winne and R. W. Cunningham, *J. Pharmacol. Exp. Therap.*, **110**, 327 (1954); (d) J. F. Snell and R. Garkuscha, *Proc. Soc. Exp. Biol. Med.*, **98**, 148 (1958); (e) B. C. Bose, *et al.*, *Indian J. Med. Research*, **46**, 193 (1958) [*C. A.*, **52**, 13994b (1958)].

(24) S. P. Ghosh and A. K. Banerjee, *J. Indian Chem. Soc.*, **32**, 32 (1955), and previous papers have extensively explored reactions of this type with arylbiguanidies.

(25) H. L. Rosenthal and H. L. Cundiff, *Clin. Chem.*, **2**, 394 (1956).

The examination for hypoglycemic effect from the salts, the various degradation products, the isosteric amidino ureas and the derived triazines will be described later.[1]

Experimental[26]

N^1-β-Phenethylbiguanide Hydrochloride.—A mixture of 15.8 g. (0.1 mole) of β-phenethylamine hydrochloride and 8.4 g. (0.1 mole) of dicyandiamide was heated gradually, with stirring, in an oil-bath. The mixture began to melt at a bath temperature of 125° and was completely fluid at 130°. Further heating, over 20 minutes, to 145–150° initiated an exothermic reaction and the temperature of the fusion mixture (156°) exceeded the bath temperature by 6°. The bath was removed until the internal temperature reached 150° and heating was resumed at 150° for 1 hour. When cool, upon recrystallization (isopropyl alcohol) there was obtained 9.0 g. (37%) of product, m.p. 175–178°.

Anal. Calcd. for $C_{10}H_{16}ClN_5$: C, 49.7; H, 6.7; N, 29.0. Found: C, 49.7; H, 6.7; N, 29.4.

Salts of β-Phenethylbiguanide.—In the course of the investigation a variety of salts of β-phenethylbiguanide was prepared and these have been described in Table I. The tetraphenylboron salt was prepared following established procedures.[27] In general, the salts were prepared by reaction of equivalent quantities of the biguanide base and the acid using methanol or water as a solvent. In some instances the formed salt crystallized from the reaction mixture, although usually the solvent was evaporated and the residue of the formed salt recrystallized. A typical example is given below.

Saccharin Salt of N^1-β-Phenethylbiguanide (Compound 14, Table I).—An equimolar solution (0.02 mole) of saccharin and the biguanide in 25 ml. of methanol was evaporated to dryness. The residue (7.5 g.) was recrystallized from 100 ml. of acetonitrile. There was obtained 4.77 g. (62%) of product.

TABLE I

SALTS OF β-PHENETHYLBIGUANIDE

No.[a]	Salt[b]	M.p.,[c,d] °C.	Analyses[e] Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
1	2HCl	214–216[d1]	43.2	43.6	6.2	6.1
2	HBr	153–155[d2]	[e1]			
3	2HBr	216–219[d2]	32.8	32.9	4.7	4.8
4	HI	136–138[d2]	36.1	36.4	4.8	5.3
5	HNO_3	156–158[d1]	44.8	44.3	6.0	5.7
6	$2HNO_3$	160–162[d3]	36.3	36.4	5.2	5.2
7	H_2SO_4	238–240[d4]	39.6	39.1	5.7	5.4
10	Tos[f]	146–147[d5]	54.1	54.2	6.1	6.3
14	Sac[g]	135–136[d5]	52.6	52.9	5 2	5.1
15	2Pic[h]	191–192[d5]	39.8	39.7	3.2	3.3
28	TPB[i]	157-159[d6]	77.9	77.5	6.7	7.1

[a] A number of additional salts which have been characterized have been omitted to save space. The complete table of salts prepared will be available upon request. The numbering of the compounds of the original table has been retained. Nitrogen analyses, also withheld to save space, are all satisfactory. [b] The acidic component forming the salt is listed. The formula of the derived salt is obtained by adding the elements of the acidic component to the molecular formula of β-phenethylbiguanide. [c] Melting points are not corrected. [d] Recrystallizing solvent: [d1] ethanol–hexane; [d2] isopropyl alcohol–hexane; [d3] ethanol; [d4] water; [d5] acetonitrile. [e] Analyses are by Weiler and Strauss, Oxford, England; [e1] Nitrogen: calcd./found, 24.5/24.8. [f] *p*-Toluenesulfonic acid. [g] Saccharin. [h] Picric acid. [i] Tetraphenylboron.

2-Amino-6-methyl-4-phenethylamino-*s*-triazine.—A solution of 0.1 mole of β-phenethylbiguanide in 100 ml. of methanol (prepared from 24.1 g. (0.1 mole) of the hydrochloride and 0.1 mole of sodium methoxide in methanol, and filtration of the formed sodium chloride) was cooled to −40° and treated with 13.2 g. (0.15 mole) of ethyl acetate. After standing 20 hours at 10°, the reaction mixture from which the product had separated was decanted into 300 ml. of water. Filtration yielded 18.7 g. (82%) which was recrystallized (acetonitrile). There was obtained 11.0 g. (48%) of product, m.p. 145–146°.

Anal. Calcd. for $C_{12}H_{15}N_5$: C, 62.9; H, 6.6; N, 30.6. Found: C, 62.8; H, 6.6; N, 30.8.

2-Amino-6-(1-hydroxyethyl)-4-(β-phenethyl)-amino-*s*-triazine.—A solution of 0.1 mole of β-phenethylbiguanide in 100 ml. of methanol was cooled to −40° and treated with 17.7 g. (0.1 mole) of ethyl lactate. After standing 20 hours at 10°, ammonia (odor) was noted and crystalline material, 1.9 g., had separated, m.p. 255–256°. Upon recrystallization (methyl Cellosolve), the m.p. was 258–259°. This was not the product, and its analyses reflected loss of 1.0 mole of ammonia and 1.0 mole of ethanol from the reactants. It has been formulated tentatively as 2-hydroxy-6-(1-hydroxyethyl)-4-(β-phenethyl)-amino-*s*-triazine.

Anal. Calcd. for $C_{13}H_{15}N_4O_2$: C, 60.0; H, 6.2; N, 21.5. Found: C, 59.8; H, 6.4; N, 20.8.

The picrate of this material, prepared from a methanolic solution by treatment with aqueous picric acid, melted at 184–187° (water).

Anal. Calcd. for $C_{19}H_{19}N_7O_8$: C, 48.1; H, 4.0; N, 20.7. Found: C, 48.3; H, 4.4; N, 20.8.

After separation of the product described above, the filtrate was decanted onto 300 g. of cracked ice. The resultant gum was separated, dried and triturated with dry ether. The residue as obtained was recrystallized (acetonitrile) and gave 2.46 g. (9.5%) of product, m.p. 107–111° dec.

Anal. Calcd. for $C_{13}H_{17}N_5O$: C, 60.2; H, 6.6; N, 27.0. Found: C, 60.3; H, 6.6; N, 26.9.

The picrate of the product prepared from a methanolic solution by treatment with aqueous picric acid melted at 185° (acetonitrile) dec.

Anal. Calcd. for $C_{19}H_{20}N_8O_8$: C, 46.7; H, 4.1; N, 23.0. Found: C, 46.6; H, 4.3; N, 23.1.

2-Amino-6-hydroxy-4-(β-phenethyl)-amino-*s*-triazine Hydrochloride.[21]—A solution of 0.1 mole of β-phenethylbiguanide in 100 ml. of methanol was treated with 19.2 g. (0.1 mole) of ethyl trichloroacetate. The exothermic reaction which resulted was accompanied by formation of a copious white precipitate. After 20 hours the precipitate (18.1 g.) was separated, dried and dissolved in 82 ml. of 3 *N* hydrochloric acid, and the solution filtered (carbon). On standing 20 hours, 15.1 g. (56%) of product was obtained, m.p. 227–238° dec.

Anal. Calcd. for $C_{11}H_{14}ClN_5O$: C, 49.4; H, 5.3; N, 26.2. Found: C, 49.1; H, 4.9; N, 26.4.

Reaction of β-Phenethylbiguanide with Benzyl Bromide.—A solution of 0.05 mole of β-phenethylbiguanide in 50 ml. of methanol was treated with 25.7 g. (0.15 mole) of benzyl bromide. After standing for 6 days at 20° the reaction mixture was decanted into 500 ml. of dry ether and 19.0 g. of crude product was obtained. Upon recrystallization (propyl alcohol), the product, 13.3 g. (72%), melted at 216–219° dec., and proved to be the dihydrobromide of β-phenethylbiguanide (compound 3, Table I).

In a similar run, after standing 14 days, the methanol was removed at atmospheric pressure and the residue extracted with four 25-ml. portions of ether. The ethereal extract was dried, the ether removed, the residue distilled and the portion boiling at 168–174° (6.7 g.) was collected (55% based on 0.1 mole of benzyl bromide [0.15 mole used]). This was benzyl methyl ether.

Anal. Calcd. for $C_8H_{10}O$: mol. wt., 122.2. Found: mol. wt., 126.0 (Rast).

A series of other similarly attempted alkylations resulted in these salts of β-phenethylbiguanide listed in Table I.

Methyl iodide afforded the hydriodic acid salt (compound 4).

Methyl tosylate yielded the *p*-toluenesulfonic acid salt (compound 10).

(26) Descriptive data shown in the table are not reproduced in the Experimental section.

(27) (a) W. E. Scott, H. M. Doukas and P. S. Schaffer, *J. Am. Pharm. Assoc., Sci. Ed.*, **45**, 568 (1956); (b) F. E. Crane, Jr., *Anal. Chem.*, **28**, 1794 (1956); (c) A. J. Barnard, Jr., *Chemist-Analyst*, **45**, 110 (1956).

1,6-Dibromohexane gave the hydrobromic acid salt (compound 2).

Reaction of β-Phenethylbiguanide with Mineral Acids. Hydrochloric Acid.—A solution of 0.05 mole of β-phenethylbiguanide hydrochloride in 100 ml. of 3 *N* hydrochloric acid was heated under reflux for 1 hour. The reaction mixture was evaporated to dryness (18 mm.) and the residue, 13.3 g. (95%), recrystallized from ethanol and ethanol–hexane. There was obtained 9.0 g. (65%) of the dihydrochloride (compound 1, Table I). The identity was confirmed by conversion to the dipicrate (compound 15, Table I) and the dinitrate (compound 6, Table II).

A similar run, substituting 6 *N* hydrochloric acid, resulted in recovery of 8.95 g. (64%) as the recrystallized β-phenethylbiguanide dihydrochloride.

Polyphosphoric Acid.—A slurry of 24.1 g. (0.1 mole) of β-phenethylbiguanide hydrochloride in 25 g. of polyphosphoric acid was heated for 3 hours in an oil-bath maintained at 110°. No signs of decomposition or discoloration were noted. The cooled reaction mixture was dissolved in 160 ml. of water, carbon added and the solution filtered and treated with 51.0 g. of sodium nitrate. On standing, 21.3 g. (76%) of the crude dinitric acid salt was obtained, m.p. 144° dec. This was purified by solution in water and precipitation with sodium nitrate followed by recrystallization from ethanol to yield the pure dinitrate, m.p. 160–162° (compound 6, Table I). The identity was confirmed by conversion to the dipicrate (compound 15, Table I).

50% Sulfuric Acid.—A slurry of 24.1 g. (0.1 mole) of β-phenethylbiguanide hydrochloride in 30 ml. of 50% sulfuric acid (1:1) rapidly dissolved to yield a light yellow solution. The reaction mixture was heated for 3 hours in an oil-bath maintained at 110°. The cooled reaction mixture was diluted with 86 ml. of water and after a few minutes copious crystallization occurred. After standing 20 hours at 10°, 20.0 g. (66%) was separated, m.p. 213°. Recrystallization (water) raised the m.p. to 238–240°. The product proved to be the sulfuric acid salt (compound 7, Table I).

Alkaline Hydrolyses of β-Phenethylbiguanide.—These studies included reactions involving an excess of alkali as well as aqueous hydrolysis of the free base.

Isolation of β-Phenethylurea and β-Phenethylamine.—A solution of 12.1 g. (0.05 mole) of β-phenethylbiguanide hydrochloride in 100 ml. of water was treated with 10 ml. of 40% sodium hydroxide (0.1 mole) and heated under reflux for 2 hours. The reaction mixture was cooled and stored at 10° for 20 hours. The crystalline precipitate (2.8 g.) which formed was separated and recrystallized from 6 ml. of water. There was obtained 1.75 g., m.p. 110–111°. This product did not depress the melting point of authentic β-phenethylurea,[28] m.p. 113–114°, mixed m.p. 111–112°. The filtrate was extracted with four 40-ml. portions of ether. The ethereal extracts were combined, dried (sodium sulfate), filtered, the ether removed and the residue of 3.08 g. was fractionally distilled. The portion (0.72 g.) distilling at 60–66° (6 mm.) was collected and by conversion to the picrate, m.p. 168–170°, was established as β-phenethylamine.

Anal. Calcd. for $C_{14}H_{14}N_4O_7$: C, 48.0; H, 4.0; N, 16.0. Found: C, 48.3; H, 3.9; N, 16.4.

There was no depression of melting point upon mixture with authentic picrate of β-phenethylamine.

β-Phenethylurea.—This product was preferably prepared following the procedure detailed by Milionis and Adams[29] for 1,1-diallylurea. To a mixture of 17.6 g. (0.145 mole) of β-phenethylamine in 15 ml. of water and 15 ml. of 12 *N* hydrochloric acid at 30°, there was added slowly (over 10 minutes) 12.5 g. (0.155 mole) of finely powdered potassium cyanate. After all of the cyanate had been added, a copious precipitate (19.3 g., m.p. 96–103°) formed which was separated and washed with 25 ml. of water. On recrystallization (water) there was obtained 12.4 g. (52%), m.p. 112–113°. The product formed a picrate, m.p. 113–115° (water).

Anal. Calcd. for $C_{15}H_{15}N_5O_8$: C, 45.8; H, 3.8; N, 17.8. Found: C, 46.0; H, 3.8; N, 17.7.

Isolation of β-Phenethylguanidine.—A solution of 12.1 g. (0.05 mole) of β-phenethylbiguanide hydrochloride in 100 ml. of water was treated with 8.2 ml. (0.05 mole) of 6.08 *N* sodium hydroxide and heated under reflux for 2 hours, cooled and stored at 10° for 3 days. The crystalline precipitate (β-phenethylurea) which formed was separated, 4.85 g. (59%), m.p. 109–111°. The filtrate, 110 ml., was neutralized (methyl red) with 5.8 cc. of 3 *N* hydrochloric acid and treated with 14.7 g. of sodium nitrate. After standing 2 hours, 1.42 g. (13%) of product which proved to be β-phenethylguanidine nitrate, was obtained, m.p. 130–133°. On recrystallization from water containing excess sodium nitrate, the m.p. was raised to 133–135°.[30]

Anal. Calcd. for $C_9H_{14}N_4O_3$: C, 47.8; H, 6.2; N, 24.2. Found: C, 47.8; H, 6.1; N, 24.3.

The picrate of β-phenethylguanidine was prepared and melted at 174–175° (water).

Anal. Calcd. for $C_{15}H_{16}N_6O_7$: C, 45.9; H, 4.1. Found: C, 45.9; H, 4.3.

β-Phenethyl isocyanate was prepared following the general directions of Allen and Bell.[31] From 84.0 g. (0.5 mole) of hydrocinnamyl chloride there was obtained 60.0 g. (82%) of ester, b.p. 84–85° (3.2 mm.), $n^{20}D$ 1.5240.

Anal. Calcd. for C_9H_9NO: C, 73.5; H, 6.2; N, 9.5. Found: C, 73.4; H, 6.1; N, 9.5.

1-Amidino-3-(β-phenethyl)-urea Nitrate.—Acetone, 50 ml., was treated with 1.15 g. (0.05 g. atom) of sodium. After the sodium had dissolved, the cooled reaction mixture was treated with 4.5 g. (0.05 mole) of guanidine hydrochloride followed by 7.5 g. (0.05 mole) of β-phenethyl isocyanate. The mixture was heated under reflux for 3 hours. When cool, 30 ml. of water was added and the reaction mixture neutralized with 3 *N* hydrochloric acid. Ether, 60 ml., was added, the mixture shaken and the aqueous phase separated and treated with 10.0 g. of sodium nitrate. The formed precipitate, 1.65 g., was separated, dried and recrystallized (acetonitrile). There was obtained 0.52 g. (4%), m.p. 161–163°.

Anal. Calcd. for $C_{10}H_{15}N_5O_4$: C, 44.6; H, 5.6; N, 26.0. Found: C, 43.8; H, 5.5; N, 25.9.

The picrate melted at 238–241° (propyl alcohol–hexane).

Anal. Calcd. for $C_{16}H_{17}N_7O_8$: C, 44.1; H, 3.9. Found: C, 44.4; H, 4.1.

1-Amidino-3-(*n*-butyl)-urea Nitrate (from Butyl Isocyanate).—A solution of 9.0 g. (0.1 mole) of guanidine hydrochloride in 15 ml. of water was cooled and treated with 4 ml. of 6 *N* sodium hydroxide followed by 5.0 g. (0.05 mole) of *n*-butyl isocyanate. An additional 4.7 ml. of 6 *N* sodium hydroxide (total 0.05 mole) was added dropwise over 20 minutes with continued cooling and stirring. When the reaction temperature reached 15° a mild exothermic reaction occurred. After standing 2 hours, the reaction was neutralized (methyl red) with 19 ml. of 3 *N* hydrochloric acid, with noted vigorous effervescence of gas. The filtrate was treated with 40 ml. of saturated sodium nitrate solution and the formed precipitate was filtered, dried and recrystallized from water. There was obtained 1.83 g. (17%) of product, m.p. 127–130°.[32]

Anal. Calcd. for $C_6H_{15}N_5O_4$: C, 32.6; H, 6.8. Found: C, 32.2; H, 6.1.

The picrate melted at 211–213° (acetonitrile).

Anal. Calcd. for $C_{12}H_{17}N_7O_8$: C, 37.2; H, 4.4; N, 25.3. Found: C, 37.0; H, 4.4; N, 25.1.

1-Amidino-3-(*n*-butyl)-urea Nitrate (from Diazotization of N^1-*n*-Butylbiguanide).—A solution of 11.0 g. (0.05 mole) of *n*-butylbiguanide nitrate in 50 ml. of 3 *N* hydrochloric acid and 20 ml. of water was cooled to 15° and treated over 10 minutes with a solution of 5.0 g. of sodium nitrite in 10 ml. of water. After stirring 1 hour, the formed precipitate was separated, dried and recrystallized (acetonitrile). There was obtained 0.85 g. (8%) of product, m.p. 126–131°; mixed m.p. was undepressed with product above, 125–130°. The picrate melted at 215–218° (acetonitrile); mixed m.p. was undepressed with product above, 212–215°.

N^1-β-Phenethyldicyandiamide.—A mixture of 73.8 g. (0.5 mole) of β-phenethylamine hydrochloride, 55.0 g. (0.5

(28) J. S. Buck, THIS JOURNAL, **56**, 1607 (1934).

(29) J. P. Milionis and P. Adams, U. S. Patent 2,734,083 (Feb. 7, 1956) [*C. A.*, **50**, 13085g (1956)].

(30) F. L. Scott, D. G. O'Donovan and J. Reilly, THIS JOURNAL, **75**, 4053 (1953), report melting points of the nitric acid salt and the picric acid salt of this guanidine as 135–137° and 176°, respectively.

(31) C. F. H. Allen and A. Bell, *Org. Syntheses*, **24**, 94 (1944).

(32) E. Junod, *Helv. Chim. Acta*, **35**, 1667 (1952), reported m.p. 136–137° for the nitrate, and m.p. 211–212° for the picrate.

mole) of sodium dicyanamide, 500 ml. of butyl alcohol and 40 ml. of water was heated under reflux for 7.5 hours. The formed sodium chloride was separated and the filtrate concentrated to a thick sirup under vacuum (50 mm.). Upon treatment with water the product granulated to a white solid which was dried and recrystallized (ethyl acetate). There was obtained 78.5 g. (83%), m.p. 114–115°.

Anal. Calcd. for $C_{10}H_{12}N_4$: C, 63.8; H, 6.4; N, 29.8. Found: C, 63.8; H, 6.5; N, 29.8.

([N-β-Phenethyl]-amidino)-urea Nitrate Monohydrate.—A mixture of 4.7 g. (0.025 mole) of β-phenethyldicyan diamide in 12 ml. of isopropyl alcohol and 4 ml. of hydrochloric acid was heated under reflux for 2.5 hours. The hot solution was poured into 70 ml. of water, filtered (carbon), neutralized (methyl red) with 40% sodium hydroxide and filtered (carbon). After addition of 25.0 g. of sodium nitrate and storage at 10° for 6 hours, 4.4 g. (61%) of product was obtained, m.p. 155–158°; recrystallized (ethanol–hexane) m.p., 162–164°.

Anal. Calcd. for $C_{10}H_{15}N_5O_4 \cdot H_2O$: C, 42.0; H, 6.0; N, 24.4. Found: C, 41.9; H, 5.9; N, 25.1.

The picrate melted at 195–197° (ethanol–hexane).

Anal. Calcd. for $C_{16}H_{17}N_7O_8$: C, 44.2; H, 3.9; N, 22.5. Found: C, 44.1; H, 3.9; N, 22.9.

Ultraviolet Absorption Spectra.—The absorption spectra were determined in a DK-1 Beckman recording spectrophotometer using 1-cm. cells. The pertinent data are recorded for the compounds examined solvent, λ_{max} mμ, ε × 10^{-3}.

β-Phenethylbiguanide hydrochloride:water, 233, 14.5; 1 × 10^{-3} *N* HCl, 233, 11.2; 1 × 10^{-2} *N* HCl, non-specific absorption; 1 × 10^{-4} *N* NaOH, 233, 14.5; 1 × 10^{-1} *N* NaOH, 232, 12.7; 1 *N* NaOH, 225–228 (plateau), 12.0; methanol, 234, 17.7.

β-Phenethylbiguanide dihydrochloride:water, 233, 14.3.

Phenylbiguanide hydrochloride:water, 242, 14.6; 5 × 10^{-3} *N* HCl, 242, 11.8; 1 × 10^{-2} *N* NaOH, 223–230 (plateau), 12.4.

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YONKERS 1, N. Y.

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Oxidative Cleavage of Amides. A Method for Selective Chemical Degradation of Peptides[1,2]

BY E. J. COREY AND L. F. HAEFELE

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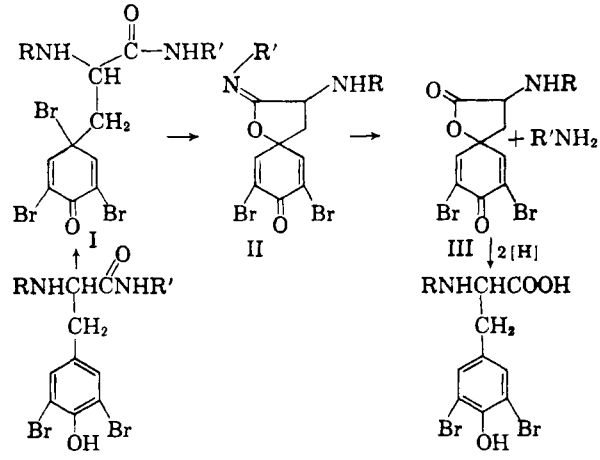
It has been demonstrated that the carbonyl–nitrogen fission of tyrosine amides by bromine, first observed by du Vigneaud and co-workers with oxytocin and vasopressin, occurs with simple tyrosine amide and phloretamide derivatives as well. The cleavage reaction has been shown to be an oxidative process in which the phenolic ring is converted to a dienone system, and not simple hydrolysis. The studies indicate that the reaction is general for this type of amide and is useful for the selective chemical degradation of peptides.

Du Vigneaud and co-workers, in the course of their important studies on the structures of oxytocin and vasopressin, made the interesting discovery that treatment with aqueous bromine causes cleavage of these polypeptides selectively at the amide linkage between the carbonyl of tyrosine and the nitrogen of the attached amino acid unit.[3–6] Thus in the case of oxytocin fission occurs between tyrosine and isoleucine units and in the case of vasopressin between tyrosine and phenylalanine units. A striking feature of this cleavage is the speed with which it occurs even under very mild conditions. In a typical experiment cleavage was effected by treatment of the polypeptide with bromine in aqueous methanol containing 0.1 *N* hydrogen chloride at −10 to −15° for one hour. Under these conditions the degree of simple acid-catalyzed hydrolysis is negligible.

It was also reported by du Vigneaud and Ressler[6] that with a solution of 1 *N* hydrogen bromide or glacial acetic acid containing bromine, cleavage did not occur although the tyrosine unit underwent dibromination to a 3,5-dibromotyrosyl residue. The cleavage reaction could also be prevented by prior conversion of the phenolic hydroxyl in the tyrosine unit to an ether function.

All these phenomena can be interpreted reasonably in terms of the intermediacy of the species I, II and III, which implies that the fission observed by du Vigneaud and co-workers might be a general and useful reaction. The intermediate I is closely analogous to the perbromophenols, *e.g.*, phenol tetrabromide, and the remaining steps have ample precedence.[7] The intermediate II might also be formed directly without the intervention of I by a concerted reaction. If a mech-



(1) Taken from the Ph.D. thesis of L. Haefele, University of Illinois, July, 1958.

(2) This work was generously supported by the Alfred P. Sloan Foundation.

(3) J. M. Mueller, J. G. Pierce and V. du Vigneaud, *J. Biol. Chem.*, **204**, 857 (1953).

(4) C. Ressler, S. Tripett and V. du Vigneaud, *ibid.*, **204**, 861 (1953).

(5) E. A. Popenoe and V. du Vigneaud, *ibid.*, **205**, 133 (1953).

(6) C. Ressler and V. du Vigneaud, *ibid.*, **211**, 809 (1954).

(7) See, for example, F. L. Scott, R. E. Glick and S. Winstein, *Experientia*, **13**, 183 (1957).