extraction with 3 ml. of 10% alkali and 5 ml. of water required no additional hydrosulfite and apparently removed little further hydroquinones. The neutral ethereal solution was shaken with salt solution, filtered through sodium sulfate, and evaporated. The residual, nearly colorless, solid weighed 106.5 mg. and melted at 100–102°. Crystallization from methanol–water gave 76 mg. of material, m.p. 102–103°, identified by the melting point (103–104°) of a mixture with the **2,3-dimethyl-1,4-quinone—2,3-dimethylbutadiene adduct.** For confirmation, the material was converted by acid isomerization, oxidation with nitrous acid and then with dichromate, and crystallization from methanol, into **2,3,6,7-tetramethyl-1,4-naphthoquinone**, m.p. 166–167°, mixed m.p. 166–167°.

The acidified solution of the aqueous alkaline extract was treated with 5 ml. of a solution 1 *N* in both ferric chloride and hydrochloric acid, and sodium chloride was added to the point of saturation. Extraction with ether gave a bright yellow oil that was treated with 1 ml. of acetic anhydride and 0.05 ml. of boron fluoride etherate and let stand at 25–27° for 2 hr. The still yellow solution was rinsed with a little ether into a 50-ml. distilling flask, 25 ml. of water was added and the mixture distilled. Ether came over first, followed by a yellow aqueous solution, and distillation was stopped when the solution remaining in the boiling flask was colorless. This residual solution was neutralized by addition of successive small portions of solid sodium bicarbonate, then salt was added until no more would dissolve, and the solution was extracted with ether. Evaporation of the dried solution gave 14.5 mg. of nearly colorless, crystalline product. This was dissolved in 1:1 ether–petroleum ether and the solution was clarified with Norit and then evaporated, with repeated addition of petroleum ether until diethyl ether was largely removed. After standing for some time at 5°, a small mass of prisms grew on the carborundum boiling stone, m.p. 102–103°; mixed m.p. with 2,5-dimethyl-1,3-triacetoxybenzene (m.p. 105–106°), 104–105°. The infrared spectrum was superposable on that of a synthetic sample.

The yellow aqueous steam distillate when suitably diluted showed an absorption maximum at 260 mμ (in water) with an extinction coefficient corresponding to 11.9 mg. of 2,3,5-trimethyl-1,4-quinone. The infrared spectrum corresponded exactly in all regions with that of synthetic quinone. The quinone was recovered by saturation with salt and ether extraction and treated with zinc dust, acetic anhydride and triethylamine for 10 min. at room temperature. The product, recovered after hydrolysis, washing with mineral acid and then bicarbonate, was taken up in ether and the solution was treated with petroleum ether and boiled down to the point of saturation. A white powder separated, m.p. 165–168°, and when recrystallized from benzene formed three small prisms, m.p. 166–168°; a mixture with **2,3,5-trimethylhydroquinone** melted at 166–169° and remelted at 165–168°. The material recovered from the mother liquors was heated with acetic anhydride and pyridine for 15 min. on the steam-bath and the neutral product crystallized from petroleum ether at 5°. It formed a small rosette of needles, m.p. 99°; a mixture with **2,3,5-trimethylhydroquinone diacetate** (m.p. 104–105°) melted at 100–102°.

The above procedure was worked out in trials on a synthetic mixture that, fortuitously, proved comparable in amount and proportions to the sample of gonyleptidine subsequently processed: 100 mg. of 2,3-dimethyl-1,4-quinone, 25 mg. each of 2,5-dimethyl-1,4-quinone and 2,3,5-trimethyl-1,4-quinone. Derivatives of each of the three components were isolated by the above procedure and identified after crystallization. The yields of uncrystallized products appear to afford a reliable indication of content and were as follows: 2,3-dimethyl-1,4-quinone—2,3-dimethylbutadiene, 150 mg. (94%); 2,5-dimethyl-1,3,4-triacetoxybenzene, 51.5 mg. (64%); 2,3,5-trimethylhydroquinone, 22 mg. (78%). On the assumption that the recovery was the same in the processing of gonyleptidine as in the processing of the synthetic mixture, the 115 mg. of gonyleptidine processed is estimated to have contained 71 mg. of 2,3-dimethyl-1,4-quinone, 11 mg. of 2,5-dimethyl-1,4-quinone and 15 mg. of 2,3,5-trimethyl-1,4-quinone.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, OREGON STATE COLLEGE]

The Synthesis of Certain O-Substituted Derivatives of Hydroxyguanidine and Hydroxybiguanide[1]

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RECEIVED SEPTEMBER 12, 1955

The syntheses of several oxygen (O) substituted alkyl derivatives of hydroxyguanidine and hydroxybiguanide which are of biological interest are described. These compounds were prepared from the corresponding α-alkylhydroxylamines using methylisothiourea sulfate to introduce the guanidine group and cyanoguanidine with cupric sulfate to produce the biguanides.

Various compounds possessing the basic structure of urea, thiourea, and/or guanidine have been shown to have biological activity. Canavanine, a close structural analog of arginine, recently has been shown to be a growth inhibitor of various bacterial species, and of both the Lee influenza and mouse encephalomyelitis viruses.[2] Other compounds such as paludrine or 1-(*p*-chlorophenyl)-5-isopropylbiguanide hydrochloride, 1-(*p*-chlorophenyl)-biguanide hydrochloride, L-arginine, various aldehyde semicarbazones, and guanylureas are of biological interest and possess the structural units listed above.

In conjunction with a study of the inhibitory properties of compounds of these types, the synthesis of certain O-substituted derivatives of hydroxyguanidine and hydroxybiguanides was undertaken. Many of these have not been prepared previously and such preparations are described in this paper.

The starting materials for these series of compounds were the corresponding alkoxyurethans and α-alkylhydroxylamines. All of these had previously been prepared by other workers,[3-4] with the exception of 3-methylbutoxyurethan (isoamoxyurethan) and α-3-methylbutylhydroxylamine (α-isoamylhydroxylamine). The method is, in general, the same for all of these compounds with the exception of α-methylhydroxylamine whose syn-

(1) These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and Oregon State College. Published with the approval of the Monographs Publication Committee, Oregon State College as Research Paper No. 284, School of Science, Department of Chemistry.

(2) K. S. Pilcher, K. F. Soike, V. H. Smith, F. Trosper and B. Folston, *Proc. Soc. Exp. Biol. Med.*, **88**, 79 (1955).

(3) C. H. Hecker, *Am. Chem. J.*, **50**, 445 (1913).

(4) (a) C. H. Andrews, H. King and J. Walker, *Proc. Roy. Soc.* (*London*), **B133**, 43–45 (1946); (b) A. T. Fuller and H. King, *J. Chem. Soc.*, 963 (1947).

thesis is described by Andrews, King and Walker.[4a] Briefly, the method consists of substituting the oxyurethan group for halogen in alkyl bromides or iodides by means of the potassium salt of hydroxyurethan[4b] followed by alkaline hydrolysis.

Since only a few alkoxy guanidines and alkylenedioxydiguanidines are described in the literature,[4b,5] several new analogs were prepared for the purposes of this study. These were prepared from the appropriate α-alkylhydroxylamine (free base) and methylisothiourea sulfate.

Although the reaction goes smoothly, the product is often contaminated with what appears to be largely unreacted methylisothiourea sulfate. However, by means of selective extraction and crystallization, the products can be purified. The yields ranged from 30–50% on the basis of the α-alkylhydroxylamine used.

A literature search revealed that only three alkoxybiguanides have been reported previously. Of these, two were synthesized by Fuller and King.[4b] Curd and Rose[6] prepared 1-(*p*-chlorophenyl)-5-methoxybiguanide by the method which was adapted to the work described here.

This method consists of heating at 70–80° one equivalent each of the α-alkylhydroxylamine (I), cyanoguanidine (II) and copper sulfate in aqueous solution. In each case, a pink to purplish-pink copper complex (III) is formed which is recovered from the reaction mixture. This complex is decomposed with hydrogen sulfide and the product obtained as the bisulfate salt (IV) after removal of cupric sulfide.

$$\underset{\text{I}}{RONH_2} + \underset{\text{II}}{NCNHC(=NH)NH_2} \xrightarrow{CuSO_4} \underset{\text{III}}{(RONHC(=NH)NHC(=NH)NH_2)_2CuSO_4}$$

$$\xrightarrow{H_2S} \xrightarrow{H_2SO_4} RON{-}C(=NH){-}NH{-}C(=NH){-}NH_2\cdot H_2SO_4 + CuS$$

The yields obtained were somewhat erratic and ranged from 10 to 30% over-all on the basis of the α-alkylhydroxylamine.

Experimental[7]

3-Methylbutoxyurethan (Isoamoxyurethan).—To a solution of 19.8 g. (0.30 mole) of 85% potassium hydroxide in 125 ml. of absolute alcohol was added 31.5 g. (0.30 mole) of hydroxyurethan[4b] and 59.4 g. (0.30 mole) of 1-iodo-3-methylbutane. After stirring to ensure a homogeneous solution, the reaction mixture was refluxed gently for 5–6 hours. Upon cooling, an equal volume of ether was added and the mixture set aside to allow potassium iodide to completely separate. The solid by-products were removed by filtration and the ether and alcohol were removed by evaporation. The crude product can be used without further purification for the next step. Distillation at reduced pressure gave a product boiling at 104–107° (4 mm.). The yield was 35–36 g. (68%).

Anal. Calcd. for $C_8H_{17}NO_3$: C, 54.8; H, 9.78. Found: C, 54.7; H, 9.85.

α-3-Methylbutylhydroxylamine (α-Isoamylhydroxylamine).—The crude product from the initial operation was refluxed in 100 ml. of water containing 50 g. of potassium hydroxide for 1–1.5 hours. After cooling, the hydrolysis mixture was extracted with three 50-ml. portions of ether. The ether extract was dried with anhydrous sodium sulfate and then distilled through a 24-cm. column packed with glass helixes. The free base distils at 130–132°. The yield was 10–15 g. (40–60%).

Anal. Calcd. for $C_5H_{13}NO$: C, 58.1; H, 12.7. Found: C, 57.9; H, 12.6.

α-3-Methylbutylhydroxylamine Hydrochloride (α-Isoamylhydroxylamine Hydrochloride).—The ether extract from above was extracted with 100 ml. of aqueous 3 *N* hydrochloric acid and the aqueous acidic layer separated and evaporated to dryness *in vacuo*. The crude hydrochloride salt was then dissolved in a hot mixture of one part of absolute alcohol and one part of ethyl acetate and filtered. On addition of excess (approximately 150 ml.) ethyl acetate to the filtrate and cooling, the hydrochloride crystallized out as white flakes. The crystals were thoroughly washed first with ethyl acetate and then with ether; yield 16 g. (38%), m.p. 115–116.5°.

Anal. Calcd. for $C_5H_{14}ClNO$: Cl, 25.4. Found: Cl, 25.0.

Propoxyguanidine Sulfate.—Methylisothiourea sulfate[8] (2.96 g., 0.0106 mole) in 30–40 ml. of water was heated to 80° and a solution of 1.60 g. (0.0213 mole) of α-propylhydroxylamine (free base) in 10 ml. of water was added slowly through a dropping funnel. Heating was continued at 80° for 2 hr. The solution was then refluxed for 1 hour to expel methyl sulfide. The solution was evaporated *in vacuo* and the residue boiled in 40 ml. of absolute alcohol for 2–3 minutes. After cooling, the insoluble portion (consisting of unreacted methylisothiourea sulfate as judged by m.p. and behavior) was removed by filtration and the filtrate evaporated *in vacuo*. The residue was dried over phosphorus pentoxide *in vacuo* and then made into a paste with a little absolute alcohol followed by addition of an equal volume of isopropyl alcohol. A semi-crystalline mass precipitated which after stirring and standing at room temperature for a few minutes completely solidified. This crude product, 2.37 g. (35%), m.p. 122.5–125°, was purified by treatment with norite in absolute alcohol followed by precipitation with isopropyl alcohol. The pure finely crystalline material melts at 123–125°.

Anal. Calcd. for $C_8H_{24}N_6O_6S$: N, 25.3; SO_4, 28.9. Found: N, 25.8; SO_4, 29.3.

Ethoxyguanidine Sulfate.—This material was prepared in the same manner as propoxyguanidine sulfate from 0.62 g. (0.0102 mole) of α-ethylhydroxylamine (free base) and 1.28 g. (0.046 mole) of methylisothiourea sulfate. After evaporation of the reaction mixture, the residue was extracted with methanol and filtered. This filtrate was evaporated to dryness and the residue made into a paste with absolute alcohol. The crude product solidified on triturating this paste to give 1.29 g. (46%), m.p. 110–120°, with some fusion at 53–62°, of the crude product. This was again dissolved in methanol and the above process repeated. After one to two recrystallizations from absolute alcohol, white plates were obtained which melted at 124–126°.

Anal. Calcd. for $C_6H_{20}N_6O_6S$: N, 27.6; SO_4, 31.6. Found: N, 26.3; SO_4, 32.0.

Isopropoxyguanidine Sulfate.—This guanidine derivative was made in the same manner as *n*-propoxyguanidine sulfate from 1.76 g. (0.0235 mole) of α-isopropylhydroxylamine (free base) and 3.26 g. (0.0117 mole) of methylisothiourea sulfate. The crude product, 2.59 g. (35%), m.p. 162–175°, was purified by treatment with norite and two recrystallizations from absolute alcohol. The final crystalline product melts at 176–179°.

Anal. Calcd. for $C_8H_{24}N_6O_6S$: N, 25.3; SO_4, 28.9. Found: N, 25.3; SO_4, 29.0.

3-Methylbutoxyguanidine Nitrate (Isoamoxyguanidine Nitrate).—To 50 ml. of water–95% ethyl alcohol (1:1) were added 3.47 g. (0.0125 mole) of methylisothiourea sulfate and 2.60 g. (0.025 mole) of α-isoamylhydroxylamine (free base) and the resulting solution refluxed for three hours. The solution was then evaporated *in vacuo* and the residue dissolved as completely as possible in 50 ml. of ether–absolute alcohol (1:1). After standing overnight, a small amount (0.70 g.) of methylisothiourea sulfate was removed by filtration. After removal of the alcohol and ether, 4.0 g. of ammonium nitrate in 25 ml. of water was added to the sirupy residue. Upon cooling in a refrigerator overnight, crude 3-methylbutoxyguanidine nitrate, 3.67 g. (70%), in

(5) E. Borek and H. T. Clarke, *J. Biol. Chem.*, **125**, 479 (1938).

(6) F. H. S. Curd and F. L. Rose, *J. Chem. Soc.* 729 (1946).

(7) All melting points were run on a Fisher melting point block.

(8) F. Arndt, *Ber.*, **54**, 2236 (1921).

the form of slightly yellow needles deposited. Further treatment with norite and crystallization from water gave white needles melting at 92–94° dec.

Anal. Calcd. for $C_6H_{16}N_4O_4$: C, 34.6; H, 7.74. Found: C, 34.6; H, 7.82.

Attempts to isolate this guanidine derivative as the sulfate salt were unsuccessful.

Ethoxybiguanide Hydrogen Sulfate.—To 2.28 g. (0.027 mole) of cyanoguanidine (recrystallized practical grade, m.p. 206–208°) and 3.13 g. (0.0125 mole) of copper sulfate pentahydrate were added 2.44 g. (0.0250 mole) of α-ethylhydroxylamine hydrochloride, 50 ml. of water and 20 ml. of 1.25 *N* sodium hydroxide.

This mixture was heated with stirring for nine hours at approximately 70–80°. Water (50 ml.) was added to the hot reaction mixture and the purple-pink copper–biguanide complex collected by filtering. This copper complex was suspended in 125 ml. of hot water and decomposed with hydrogen sulfide. The precipitated copper sulfide was removed and the filtrate evaporated *in vacuo* to near dryness. To the residue, in the following order, were added 50 ml. of methanol–ether (1:1), 4 ml. of 6 *N* sulfuric acid and 50 ml. of ether. The resultant solution was chilled which gave a white crystalline solid, 1.14–2.42 g. (19–40%), m.p. 146.5–8.0°. Two grams were purified by dissolving in 4–5 ml. of warm water and precipitating with 200 ml. of methanol–ether (1:1) and 150 ml. of ether yielding feathery white crystals, m.p. 147.5–149.0°.

Anal. Calcd. for $C_4H_{13}N_5O_5S$: N, 28.8; SO_4, 39.5. Found: N, 29.1; SO_4, 39.5.

Propoxybiguanide Hydrogen Sulfate.—Prepared essentially in the same manner as ethoxybiguanide hydrogen sulfate from 2.79 g. (0.0250 mole) of α-propylhydroxylamine hydrochloride. Since the copper complex is soluble in hot water, this intermediate was obtained after cooling the reaction mixture in a refrigerator overnight with no further dilution with water. The product, consisting of white rosettes, 1.02–1.38 g. (12–16%), m.p. 135–136°, was purified by dissolving in 4 ml. of water and precipitating with 50 ml. of 1:1 methanol–ether and 50 ml. of ether to give a product melting at 136.0–136.5°.

Anal. Calcd. for $C_5H_{15}N_5O_5S$: N, 27.3; SO_4, 37.4. Found: N, 27.3; SO_4, 37.8.

Isopropoxybiguanide Hydrogen Sulfate.—Prepared in the same manner as the *n*-propoxybiguanide derivative from 2.79 g. (0.0250 mole) of α-isopropylhydroxylamine hydrochloride. The product consisted of white needles, 1.38–1.71 g. (21–27%), m.p. 136–138°. The purified material melted at 138–140°.

Anal. Calcd. for $C_5H_{15}N_5O_5S$: N, 27.3; SO_4, 37.4. Found: N, 27.2; SO_4, 37.4.

Methoxybiguanide Hydrogen Sulfate.—This compound was prepared from 2.30 g. (0.027 mole) of α-methylhydroxylamine hydrochloride, 2.28 g. (0.027 mole) of cyanoguanidine, 3.13 g. (0.0125 mole) of copper sulfate pentahydrate and 22 ml. of 1.25 *N* sodium hydroxide in the same manner as ethoxybiguanide hydrogen sulfate. After completion of the reaction (7–8 hr.), the reaction mixture was chilled for several hours in a refrigerator without any dilution with water in order to remove the pink copper–biguanide complex which in this case was quite soluble in water. The crude product consists of small gray or white crystals, 0.4–1.5 g. (6–24%), which melt at various temperatures but usually in the range 75–90° and sometimes as high as 140–150°. It is difficult to purify the material because of its tendency to sirup-out of solutions instead of crystallizing. However, enough was obtained for analysis by treating it in the same manner as for propoxybiguanide hydrogen sulfate and then recrystallizing from isopropyl alcohol containing about 4% water; obtained fine white crystals, m.p. 157–158°.

Anal. Calcd. for $C_5H_{11}N_5O_5S$: N, 30.6; SO_4, 41.8. Found: N, 30.4; SO_4, 41.7.

3-Methylbutoxybiguanide Hydrogen Sulfate (Isoamoxybiguanide Hydrogen Sulfate).—This compound was prepared essentially in the same manner as the previous biguanide derivatives from 3.49 g. (0.0250 mole) of α-(3-methylbutyl)-hydroxylamine hydrochloride. However, some modifications in the procedure were followed here. The reaction was run for approximately 20 hr. The copper complex along with a green solid[9] of unknown composition was deposited during the reaction and collected after cooling to room temperature. No 6 *N* sulfuric acid was added to the evaporation residue with the methanol and ether.

The crystalline white product, 0.44–0.71 g. (6–10%), m.p. 124–129°, was purified by making a paste of it with a few drops of water and then dissolving this in a small amount of methanol–ether (1:1). To this solution was added an equal volume of ether followed by chilling to give the pure crystalline material, m.p. 131.5–132.5°. Usually a small second crop is obtained by addition of more ether.

Anal. Calcd. for $C_7H_{19}N_5O_5S$: N, 24.6; SO_4, 33.7. Found: N, 24.3; SO_4, 34.2.

Butoxybiguanide Hydrogen Sulfate.—Prepared in the same manner as 3-methylbutoxybiguanide hydrogen sulfate from 3.09 g. (0.0250 mole) of α-butylhydroxylamine hydrochloride. The white crystalline solid, 0.50–0.74 g. (8–11%), m.p. 117–119°, after purification in the same manner as the 3-methylbutoxy analog gave a product melting at 119–121°. A small amount of this sulfate salt was converted into the nitrate salt with ammonium nitrate. The recrystallized nitrate salt melts at 115–115.5°. Fuller and King[4b] report the melting point as 116–117°.

Anal. Calcd. for $C_6H_{17}N_5O_5S$: N, 25.8. Found: N, 25.9.

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(9) Note: The green solid mentioned above which accompanied the copper–biguanide complex occurs in some of the other biguanidine preparations but in much smaller amounts. In any event, its presence does not appear to affect the isolation and purification of these compounds.