phosphate (R_f 0.07) was liberated. Comparative chromatography with an authentic, biochemically derived sample of 2-deoxy-D-ribose 5-phosphate demonstrated that the component of R_f 0.13 was identical with this substance. The component of R_f 0.27 was not detectable either with periodate–benzidine spray or tetrazolium blue spray.[37] As the reaction progressed this component very slowly diminished while the proportion of 2-deoxy-D-ribose 5-phosphate increased. However, even after 2–3 days some of the former was still present. After 4 days a sample of the solution showed $[\alpha]^{20}D$ +19° (c 0.47) based on the theoretical yield of 2-deoxy-D-ribose 5-phosphate.

In one experiment, after the hydrolysis had progressed for 2 days, an aliquot of the solution was diluted with an equal volume of methanol and left for 4 hr. at room temperature. Chromatography then revealed a large increase in the quantity of the component of R_f 0.27.

A sample of the dimethyl acetal (11.1 μmoles/ml.) which had been hydrolyzed for 48 hr. as described above was assayed using deoxyribosephosphate aldolase.[15] D-Glyceraldehyde 3-phosphate (9.66 μmoles/ml.) and acetaldehyde (10.0 μmoles/ml.) were formed. A Dische diphenylamine test[15] indicated 10.6 μmoles/ml. of 2-deoxypentose.

Acknowledgments.—We are indebted to Mr. W. E. Pricer, Jr., of this Institute for enzymatic assays and authentic samples of 2-deoxy-D-ribose 5-phosphate. Analyses were carried out by the Institutes' Analytical Services Unit of this Laboratory under the direction of Dr. W. C. Alford.

BETHESDA 14, MD.

(37) W. J. Mader and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952).

[CONTRIBUTION FROM THE BIOCHEMICAL LABORATORY OF THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION]

The Synthesis by Plants of a New Disaccharide Containing 2-Deoxy-D-glucose[1]

BY G. A. BARBER

RECEIVED FEBRUARY 7, 1959

A new series of oligosaccharides containing 2-deoxy-D-glucose is synthesized when this monosaccharide is administered to plants. The predominant compound was isolated and crystallized. Evidence is presented that its structure is 6-*O*-(β-D-fructofuranosyl)-2-deoxy-D-glucose.

In an experiment designed to help to elucidate the mechanisms of flavonol glycosylation in plants, 2-deoxy-D-glucose was administered through the roots to buckwheat seedlings. Paper chromatography of an aqueous ethanol extract of the seedlings revealed the presence of several compounds with the mobilities of simple oligosaccharides which gave the Kiliani[2] reaction for deoxy sugars. The fastest moving of these seemed to be present in considerably higher concentration than the others as evidenced by the intensity of its reaction. The predominant compound was eluted from the chromatograms, hydrolyzed and again chromatographed. After hydrolysis, 2-deoxyglucose and fructose were the only compounds detected.

These results suggested that several oligosaccharides containing 2-deoxyglucose had been formed. Similar results were obtained with extracts of other plants to which the sugar had been administered. These included seedlings of corn, wheat, sorghum and cucumber, and the flower buds and leaves of corn, sorghum, tobacco and *Impatiens sultani*.

Procedures were developed for the isolation of milligram quantities of the predominant compound in crystalline form. It was found to be a disaccharide of 2-deoxyglucose and fructose, the most probable structure being 6-*O*-(β-D-fructofuranosyl)-2-deoxy-D-glucose.

Experimental

Materials.—3,5-Diaminobenzoic acid and 2-deoxy-D-glucose were purchased from the Aldrich Chemical Co. The sugar was recrystallized from a mixture of methanol and acetone before use as an analytical standard. Methyl-2-deoxy-α-D-glucopyranoside was synthesized by the method of Hughes, Overend and Stacey.[3] Whatman No. 1 chromatography paper was used unless otherwise indicated. Invertase was the "melibiase-free" product of the Nutritional Biochemical Corp. It did not hydrolyze maltose under the conditions of these experiments.

Biosynthesis and Extraction.—The top segments of a number of mature tobacco plants (*Nicotiana tabacum*, var. Havana Seed) consisting of stem, two or three small leaves and several flower buds were cut from the plants. The base of each segment was immersed in a small vial containing 5 ml. of 0.05–0.1 *M* 2-deoxyglucose and allowed to absorb the solution in the diffuse light of the laboratory for about 48 hr. Water was added to maintain the volume as the solution was absorbed. The segments were ground in a Waring Blendor in a mixture of hot ethanol and 0.1 *M* potassium phosphate buffer, *p*H 7.0 (4:1 by volume), about 100 ml. being used per tobacco plant segment. The mixture was heated on a steam-bath for 5 minutes and filtered with the aid of Celite. The residue was heated again in the same volume of ethanol buffer, filtered and the two filtrates were combined. The extract was stirred with approximately 1 g. of activated carbon (Darco G-60), filtered and evaporated to a small volume *in vacuo* at 45°.

Isolation.—The concentrated extract from 3 plants was washed on to a 2.5 × 7 cm. carbon and Celite column prepared as described by Whistler and Durso.[4] The column was further washed with 500 ml. of water, and the compound was eluted with 500 ml. of 5% ethanol. To eliminate contaminating sucrose and traces of 2-deoxyglucose, the eluate was evaporated to a minimal volume and applied in a streak to washed Whatman 3 MM paper. The chromatograms were developed in a descending direction for three days with the organic phase of butanol, ethanol and water[2] (9:1:10 by volume). Under these conditions, 2-deoxyglucose usually ran off the chromatograms, while sucrose appeared 5 cm. or more below the new disaccharide. Marker strips cut from the chromatograms were sprayed with the Kiliani reagent to locate the compound. The appropriate section of paper was cut out and the substrate was eluted with water.

Crystallization.—The eluates were combined and evaporated to a small volume *in vacuo* at 35°. The concentrated solution was transferred to a conical centrifuge tube and evaporated to a sirup at room temperature under a stream of air. The sirup was dissolved in several ml. of absolute

(1) Partial support for this work by a grant from the National Science Foundation is acknowledged.

(2) S. Aronoff, "Techniques of Radiobiochemistry," The Iowa State College Press, Ames, Iowa, 1956, pp. 94–118.

(3) I. W. Hughes, W. G. Overend and M. Stacey, *J. Chem. Soc.*, 2846 (1949).

(4) R. L. Whistler and D. F. Durso THIS JOURNAL, **72**, 677 (1950).

ethanol and allowed to stand overnight in the refrigerator. A flocculent precipitate formed and was removed by centrifugation and discarded. The slightly yellow supernatant solution was decolorized with carbon, and the filtrate was evaporated to a small volume in a vacuum desiccator over concentrated sulfuric acid. More absolute ethanol was added, together with about 2 ml. of butanol, and the solution was again allowed to evaporate to a small volume in the desiccator. During this period small crystals formed. The stoppered tube was left for a few days in the refrigerator and the crystals were spun down, washed with a small amount of cold butanol and with ethyl ether and dried over sulfuric acid in a desiccator. Altogether, about 35 mg. of material was prepared in this fashion. The crystals were not chromatographically pure but contained traces of 2-deoxyglucose and another oligosaccharide. They began to sinter at 56° and were completely melted at 85°.

The yield of compound was low. Although the 2-deoxyglucose solution was readily taken up by the tobacco top segments, only 1 or 2% of the solute was converted to the disaccharide. Concurrent administration to the plant tissue of glucose, fructose or sucrose did not increase the extent of conversion.

Identification of the Constituent Monosaccharides.—The monosaccharides in a hydrolyzed sample of the compound were identified by comparison of their chromatographic movements in three solvent systems to those of authentic monosaccharides. They were detected by spraying the papers with either α-naphthylamine, for reducing sugars, resorcinol for fructose, or the Kiliani reagent for deoxy sugars.[2] The three solvent systems used were: the organic phase of butanol, ethanol, water (9:1:10 by volume); ethyl acetate, pyridine, water (2:1:2 by volume); the organic phase of butanol, acetic acid, water (4:1:5 by volume).[2]

Proportionality of the Monosaccharides.—The molar proportion of each monosaccharide in the compound was determined by use of the resorcinol method for fructose[5] and the quinaldine assay for 2-deoxyglucose.[6] The quinaldine assay was modified to obtain greater sensitivity by heating the reaction mixtures in an autoclave at 120° for 15 minutes. Under these conditions, an equimolar quantity of fructose was found to increase the absorbancy by approximately 20%. The results were appropriately corrected. In both methods, the determinations are conducted in strongly acid solutions at elevated temperatures, and prior hydrolysis of the compound was unnecessary. This was confirmed for the fructose determination by comparing the absorbancy of solutions containing equimolar amounts of fructose or sucrose.

Hydrolysis.—The compound was hydrolyzed for 30 minutes in 0.1 N H_2SO_4 at 100°. Before chromatography, the acid was removed by neutralization with a cold saturated solution of $Ba(OH)_2$ and centrifugation. Hydrolysis of micromolar quantities by invertase was carried out at 35° for 1 hr. in 0.2 ml. of 0.05 M acetate buffer, pH 4.7, containing 0.4 mg. of the commercial invertase preparation. The extent of this hydrolysis was determined by the reduction of the Somogyi[7] reagent estimated colorimetrically by the method of Nelson.[8]

Bromine Oxidation.—Oxidation of the compound (2.0 mg.) with bromine and subsequent chromatography of the products were performed by the methods of Hudson and Isbell,[9] and Fitting and Putman.[10] Samples of maltose and 2-deoxyglucose were similarly oxidized for use as models.

Periodate Oxidation.—Because of the small amounts of material available, it was not practicable to select optimal conditions by construction of a rate curve for the course of the oxidation. It was found, however, that when the oxidation was conducted in an excess of 0.014 M sodium periodate at pH 3.4, as recommended by Bobbitt,[11] sucrose and methyl-2-deoxy-α-D-glucoside consume the theoretical number of moles of periodate in 2.25 hr. at room temperature in the dark. These reaction conditions were therefore chosen for oxidation of the disaccharide. The conditions were apparently not too severe since it was found, in one experiment, that oxidation for 24 hr. did not increase the consumption of periodate by the disaccharide. Disappearance of periodate was estimated by reduction of excess periodate with standard arsenite and back titration of the remaining arsenite with iodine.[12] A sample of about 2.0 mg. of the disaccharide was weighed accurately for each of these determinations. To correct for the water of hydration, the quantity used was calculated from an estimation of the fructose present; corrections were less than 10%.

The formic acid produced during the oxidation by periodate was estimated manometrically by the method of Perlin,[13] except that the reaction was carried out at pH 6.7 in an atmosphere of 5% CO_2 and 95% N_2 at 30°. Under these conditions, equilibrium was attained after about 18 hr.

Consumption of periodate was measured as described above and was found to agree with determinations on larger amounts of material under more nearly optimal conditions of oxidation.

Results and Discussion

The compound behaved as a disaccharide on carbon and on filter paper chromatograms. It was eluted from the carbon by 5% ethanol at about the same rate as sucrose. This was determined by chromatography on paper of aliquots of the fractions. Attempts to separate sucrose from the disaccharide by gradient elution with ethanol and water were unsuccessful. On paper, when developed with the organic phase of butanol, ethanol, water (9:1:10 by volume), the mobility of the disaccharide was between that of sucrose and glucose. With the other two solvent systems mentioned, it moved at about the same rate as glucose. Paper chromatography of the hydrolyzed compound showed the presence of two sugars, 2-deoxyglucose and fructose. Colorimetric determinations demonstrated that they were present in 1:1 molar proportions. The results of two analyses were as follows (the molecular weight of the disaccharide was taken as 326): exp. 1, 0.04 mg. (0.12 μmole) of the compound yielded 0.12 μmole of fructose and 0.10 μmole of 2-deoxyglucose; exp. 2, 0.11 mg. (0.34 μmole) of the compound yielded 0.30 μmole of fructose and 0.31 μmole of 2-deoxyglucose.

Upon hydrolysis the capacity of the disaccharide to reduce the Somogyi reagent was found to increase about 7 times. Since the reduction of the Somogyi reagent by uncombined 2-deoxyglucose was also found to be sluggish, this observation did not necessarily indicate that the disaccharide is non-reducing. In fact, further study showed that the carbonyl group of combined 2-deoxyglucose is free. Thus, after bromine oxidation of the disaccharide, paper chromatography showed that a slow-moving acidic compound had been produced. When the product of bromine oxidation was hydolyzed and chromatographed, another considerably more mobile acidic compound was observed. This had the same R_f value as the substance formed upon bromine oxidation of 2-deoxyglucose. This result was interpreted to mean that a bionic acid of the disaccharide had been formed by oxidation of what must be a free carbonyl group of 2-deoxyglucose. Further proof of this aspect of the structure of the compound was afforded by the mutarotation of the crystalline material. The $[\alpha]^{23}D$ (c 1.08 in

(5) J. H. Roe, *J. Biol. Chem.*, **107**, 13 (1934).
(6) F. B. Cramer and G. A. Neville, *J. Franklin Inst.*, **256**, 379 (1953).
(7) M. Somogyi, *J. Biol. Chem.*, **160**, 61 (1945).
(8) N. Nelson, *ibid.*, **153**, 375 (1944).
(9) C. S. Hudson and H. S. Isbell, THIS JOURNAL, **51**, 2225 (1929).
(10) C. Fitting and E. W. Putman, *J. Biol. Chem.*, **199**, 573 (1952).
(11) J. M. Bobbitt, *Advances in Carbohydrate Chem.*, **11**, 1 (1956).
(12) E. L. Jackson, *Org. Reactions*, **2**, 341 (1944).
(13) A. S. Perlin, THIS JOURNAL, **76**, 4101 (1954).

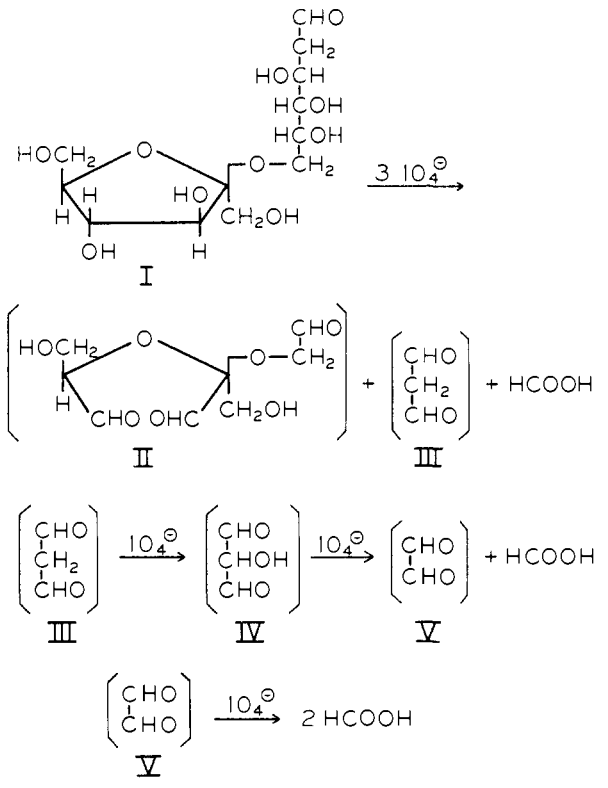


Fig. 1.—Postulated reactions of the disaccharide on oxidation with periodate. The disaccharide (I) reacts with 3 moles of periodate to give 1 mole of product (II) together with 1 mole of malonaldehyde (III) and 1 mole of formic acid. Malonaldehyde reduces another mole of periodate yielding 1 mole of tartronaldehyde (IV), which in turn consumes a mole of periodate and produces 1 mole of glyoxal (V) and 1 mole of formic acid. Finally, the glyoxal is cleaved to two moles of formic acid by 1 mole of periodate.

water) changed from +62° to +32.4° in less than three minutes, to +30.5° in 2.5 hr. and to +26.8° in 18 hr. By convention,[14] the direction of the change in rotation also suggests that the disaccharide had crystallized in the α form.

Hydrolysis was catalyzed readily by dilute acid or by the commercially purified invertase preparation. In Table I, the reactivities of sucrose and of the new disaccharide to hydrolysis by both catalysts are compared.

TABLE I

RELATIVE REACTIVITY OF SUCROSE AND THE NEW DISACCHARIDE TO HYDROLYSIS CATALYZED BY ACID AND BY INVERTASE

Reducing power measured by the methods of Somogyi and Nelson. Acid hydrolysis in 0.1 N H_2SO_4 at 100° for 30 min. Invertase hydrolysis in 0.2 ml. of 0.05 M acetate buffer, pH 4.7, with 0.4 mg. invertase.

	Absorbancy in a Coleman calorimeter with 525 mμ filter			
	Unhydrolyzed	Acid hydrolyzed	Invertase hydrolyzed	Ratio acid:inv.
Sucrose	0.045	0.560	0.473	0.84
Disaccharide	0.112	0.795	0.682	0.86

(14) C. S. Hudson, *Advances in Carbohydrate Chem.*, **3**, 1 (1948).

The D-configuration and furanose ring structure of fructose in the disaccharide, as well as the β-configuration about its anomeric carbon, were inferred from the ease of hydrolysis with invertase, since invertase has been shown to be highly specific for the β-D-fructofuranose structure.[15]

As shown in Table II, one mole of the disaccharide consumed about 6 moles of sodium periodate and produced about 4 moles of formic acid during the oxidation.

TABLE II

PERIODATE CONSUMPTION AND FORMIC ACID PRODUCTION

Exp. 1–2, Disaccharide incubated in 0.014 M sodium periodate at pH 3.4 at room temp. in the dark for 2.25 hr. Exp. 3–4, disaccharide incubated in 0.006 M sodium periodate, pH 6.7 at 30° in the dark for 18 hr.

Exp. no.	Disaccharide, μmoles	Moles periodate consumed/mole sugar	Moles formic acid formed/mole sugar
1	4.95	5.5	..
2	4.41	6.4	..
3	0.68	5.1	3.7
4	0.63	6.6	4.6

These results are consonant with the structure 6-*O*-(fructofuranosyl)-2-deoxyglucose. The rather large consumption of periodate presumably arises from the formation of a malonaldehyde intermediate which, itself, would be expected to consume three moles of periodate and to produce three moles of formic acid. According to the work of Huebner, *et al.*,[16] and Fleury and Courtois[17] as reported by Bobbitt,[11] the course of the periodate oxidation of the disaccharide should be as depicted in Fig. 1. Glycosylation at any carbon of 2-deoxyglucose other than number 5 or 6 would prohibit the formation of the malonaldehyde intermediate and thereby reduce sharply the consumption of periodate.

Some experimental evidence for the existence of a malonaldehyde intermediate has been acquired by use of the extremely sensitive thiobarbituric acid determination of Waravdekar and Saslaw.[18] Thiobarbituric acid, under the conditions employed, is reportedly highly specific for malonaldehyde. The new disaccharide is quite reactive with this reagent after brief oxidation with periodate as recommended by these authors.

The structure of the new disaccharide that best fits the available evidence is 6-*O*-(β-D-fructofuranosyl)-2-deoxy-D-glucose. More rigorous characterization must await the accumulation of larger quantities of material.

It has been found recently that a dialyzed homogenate of the leaves of *Impatiens sultani* catalyzes the formation of oligosaccharides containing 2-deoxyglucose when allowed to react with 2-deoxyglucose and sucrose. This would suggest that these compounds are synthesized in one of the transglycosylase reactions common to plants and microörganisms.[19] In the course of studying

(15) M. Adams, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **65**, 1369 (1943).

(16) C. F. Huebner, S. R. Ames and E. C. Bubl, *ibid.*, **68**, 1621 (1946).

(17) P. F. Fleury and J. E. Courtois, *Compt. rend.*, **223**, 633 (1946).

(18) V. S. Waravdekar and L. D. Saslaw, *Biochim. et Biophys. Acta*, **24**, 439 (1957).

(19) J. Edelman, *Advances in Enzymol.*, **17**, 189 (1956).

these reactions in cell-free systems, it is hoped that methods can be developed for a more rapid and efficient production of this whole series of oligosaccharides.

Acknowledgments.—I wish to thank Professor J. Fruton, Department of Biochemistry, Yale University, for the use of the polarimeter and Dr. G. Taborsky for making the measurements. Thanks are also due to Dr. W. S. McNutt and Dr. I. Zelitch for helpful advice and to Dr. H. B. Vickery for assistance with the manuscript.

NEW HAVEN, CONN.

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

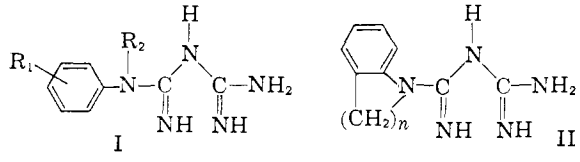
Hypoglycemic Agents. II.[1-3] Arylbiguanides

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

RECEIVED DECEMBER 19, 1958

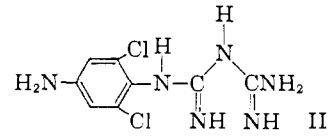
Arylbiguanides of the type *I* have failed to yield compounds with outstanding hypoglycemic activity. The ultraviolet absorption spectra are interpreted to indicate "acetanilide resonance" or "biguanide resonance" depending on the steric factors in the aryl ring of I. Arylbiguanides are characterized as fairly stable to basic but vulnerable to acidic hydrolysis, in contrast to aralkylbiguanides.

In continuation of our study of hypoglycemic biguanides[1-3] a series of arylbiguanides of the types I and II have been prepared[4,5] and examined for



I II

hypoglycemic activity, ultraviolet absorption characteristics and hydrolytic stability. Most of the arylbiguanides (Table I) were prepared by the aqueous method of Curd and Rose,[6] although in several instances (compounds 1 and 2) pyridine[7] was employed as the solvent. In a few cases, the product was preferably isolated as the nitrate or the free base (see Table I). With *p*-aminosalicylic acid as the reactant amine, decarboxylation[8] occurred to yield *m*-hydroxyphenylbiguanide. The product from the monohydrochloride of 2,6-dichloro - *p* - phenylenediamine was assigned the structure III.[9]



III

Many of the biguanides were characterized as the dipicrates, although a monopicrate[2,10] of the 2,6-dimethylphenylbiguanide was obtained by controlling the quantity of aqueous picric acid used.

The hydrolytic stability of the arylbiguanides in aqueous systems contrasted with observations under comparable conditions with β-phenethylbiguanide.[2] Phenylbiguanide proved to be fairly stable to basic hydrolysis, while treatment of (*m*-chlorophenyl)-biguanide with 3 *N* hydrochloric acid yielded 1-amidino-3-(*m*-chlorophenyl)-urea[11] and *m*-chloroaniline.

The ultraviolet absorption data (Table II) show that compared to I, R_2 = H, hypsochromic shifts are obtained with R_2 as methyl and ethyl, with the latter contributing a hyperchromic effect[12] (see compound 2 *vs. e;* 5 *vs.* 3; 8 *vs.* 6).

Many spectral characteristics similar to those of the acetanilides,[13,14] are noted although certain differences exist. Thus the effect of a single *o*-substituent is not nearly so marked with arylbiguanides, and while 2,6-disubstituted-acetanilides would not show any specific absorption[15] the 2,6-

(1) Presented in part at the Meeting of the American Chemical Society, New York, N. Y., September, 1957.

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

(3) S. L. Shapiro, V. A. Parrino and L. Freedman, *ibid.*, **81**, 3728 (1959).

(4) Structural variation of the substituted phenyl group as dimethyl, ethyl, halo and methoxyphenyl was indicated by analogy with congeners of such compounds having high hypoglycemic activity in the aralkylbiguanide series.[1-3].

(5) The evaluation of *p*-aminophenylbiguanide (compound 21, Table I) was suggested by the work of C. E. Braun, *J. Biol. Chem.*, **89**, 97 (1930), who reported hypoglycemic effects in rabbits with an *impure* preparation of *p*-aminophenylguanidine hydroiodide. Pure preparations of this salt or of other salts were found to be ineffective by T. B. Parks and C. E. Braun, *ibid.*, **91**, 629 (1931). Although the mode of processing (C. E. Braun, THIS JOURNAL, **54**, 1511 (1932)), would reflect an authentic preparation of the desired guanidine, a possibility existed that the impure preparations which showed hypoglycemic action may have been converted in part to the *p*-aminophenylbiguanide, which in turn might have been the active product. Thus, for example, C. E. Braun, *ibid.*, **55**, 1280 (1933), isolated *p*-tolylbiguanide hydrochloride in the preparation of *p*-tolylguanidine.

(6) F. H. S. Curd and F. L. Rose, British Patent 581,346 [*C. A.*, **41**, 3125 (1947)].

(7) B. R. Jacobs and Z. E. Jolles, British Patent 587,907 [*C. A.*, **42**, 214 (1948)].

(8) For instability of *p*-aminosalicylic acid in aqueous system, see C. Ghilmatti, *Farm. sci. e tec.* (*Pavia*), **3**, 652 (1948) [*C. A.*, **43**, 2973b (1949)].

(9) Other syntheses in this series indicate that 2,6-substituents on the reactant aniline do not critically restrict biguanide formation. Assuming that the proton would be attached at the more basic amino group in position 1 (steric inhibition of resonance), the resultant biguanide would have the structure III.

(10) T. Callan and N. Strafford, *J. Soc. Chem. Ind.* (*London*), **43**, 1 (1924), found that while *o*-tolylbiguanide forms a dipicrate, only one mole is firmly bound.

(11) See ref. 13 of ref. 2 for pertinent data on this hydrolysis.

(12) J. C. Gage, *J. Chem. Soc.*, 221 (1949).

(13) H. E. Ungnade, THIS JOURNAL, **76**, 5133 (1954).

(14) The spectral data for acetanilides corresponding to the phenyl substitution shown in Table I, compound number, λ_{max} $\epsilon \times 10^{-3}$ are reproduced from ref. 13: *e*, 242, 14.4; 3, 230, 6.28; 6, 245, 14.0; 9, 245, 14.85; 12, 240, 10.4; 13, 245, 14.9; 14, 249, 17.8; 15, 234, 7.6; 16, 246, 14.0; 17, 252, 18.7; 18, 246, 13.6; 24, 245, 11.7.

(15) B. M. Wepster, "Progress in Stereochemistry," Vol. 2, Academic Press, Inc., New York, N. Y., 1958, pp. 115–120.