

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF CHICAGO]

The Utilization of Various Sugars and their Derivatives by Bacteria

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Because the problem of fermentation and configuration has been studied in many cases with a very limited number of organisms and sugars, it was decided to reinvestigate this problem using a larger number of common organisms and as varied a number of sugars and their derivatives as could be obtained.

Experimental

Seventeen common pathogenic and non-pathogenic bacteria and two yeasts were used in this study. These organisms were obtained from the collection of the Department of Bacteriology and Parasitology through the courtesy of Dr. Koser. In certain cases several different strains of a given organism were used. In this particular study no differences between strains were noted, but with strains of some organisms, both qualitative and quantitative differences occur. They were inoculated on ordinary nutrient agar slants, kept at room temperature for forty-eight hours, and stored in the ice box. Transfers of old cultures to fresh agar slants were made every four to six weeks. At nine-month intervals, smears of each organism were made, stained, and examined under the microscope in order to detect any possible contamination.

The medium was made up according to the standard formula: 5 g. of peptone and 3 g. of beef extract in 1 liter of water were heated to boiling; the pH was adjusted to neutrality using thymol blue as an indicator. The mixture was further boiled for ten minutes and filtered through filter paper. Enough water was added to make 1 liter, and to this mixture was added a neutral solution (neutralized with 0.1 *N* sodium hydroxide) of the sugar or sugar derivative to be tested to make the concentration of the sugar 0.5%. Finally 8 ml. of brom cresol purple indicator was added (pH range is from 5.2 to 6.8). The medium was put into test-tubes in 7- to 10-ml. amounts, and a small inverted glass vial placed in each tube. The tubes were plugged with non-absorbent cotton.

Sterilization was carried out in an autoclave using 1 atm. of steam for ten minutes. A period of forty-eight to seventy-two hours always elapsed before the medium was used for an experiment in order to be sure there was no contamination or faulty sterilization.

In certain cases, the sugar solution was sterilized separately by passing the solution through a Seitz filter. The sterile sugar solution was then added directly to the tubes containing sterile medium. This procedure was followed whenever there was some doubt as to the stability of the particular compound at the sterilizing temperature of the autoclave. In no case, however, using this procedure were there obtained results different from those obtained when using the ordinary procedure.

Inoculation was performed by using a platinum wire to take a minute amount of a particular culture directly from the agar slant. The wire was then dipped into the tube

of medium. Aseptic precautions were observed throughout the inoculation process. The age of the culture was always from seven to ten days. The tubes were then incubated at 37°. The control in each experiment consisted of a non-inoculated tube of medium. In some cases a control was used consisting of tubes of ordinary nutrient broth (without the sugar) inoculated with each organism. In this fashion, acid production due to a spontaneous breakdown of the sugar or due to an abnormal bacterial breakdown of the constituents of the nutrient broth was ruled out.

The assumption was made that if an organism utilized the sugar or sugar derivative, then acid products would be formed; in certain cases gases might be liberated also. If acidic compounds were produced, the color of the indicator changed from purple to yellow. Gas production was detected by the amount of liquid displaced from the inverted vial.

Readings were taken once a day for five successive days and then every third day for a total period of two to three weeks. In the tables of results, 0 signifies no acid or gas; A, acid; AG, acid and gas.

If one excludes the results with glyceric aldehyde, then it is clearly apparent that it is the 5- and 6-carbon members which are fermented. Of the two, the pentose is less readily fermented than the hexose. The heptose and octose were not fermented by any organism. It is of great interest that three organisms fermented the nonose. Of the three, one was a yeast and this is in accord with early observations that yeasts will ferment 9-carbon sugars. This is the first observation as to the action of a large group of bacteria on a nonose, and indicates that the action of bacteria on sugars is quite different from that of yeasts.

The results with glyceric aldehyde may not be fully comparable with those from other sugars of the series inasmuch as the racemic mixture was used. However, several interesting points are brought up. All the organisms which fermented this sugar did so on the first day of incubation; also, after the third day of incubation, the media in every case returned to an alkaline pH, indicating that the acidic products formed in the first few days were further utilized by the organism. That the two yeasts did not ferment this sugar is somewhat of a surprise since Armstrong¹ states that yeasts will attack 3-carbon sugars.

(1) E. F. Armstrong and K. F. Armstrong, "The Carbohydrates," Longmans, Green and Co., London, 1934, p. 225.

TABLE I

Organism	<i>d</i> -Glyceric aldehyde ^a	<i>d</i> -Arabinose ^b	<i>l</i> -Arabinose ^c	<i>d</i> -Ribose ^d	<i>l</i> -Ribose ^e	<i>d</i> -Lyxose ^f	<i>d</i> -Xylose ^g	<i>l</i> -Rhamnose ^h	<i>d</i> -Glucose	<i>d</i> -Mannose	<i>d</i> -Galactose
<i>B. megatherium</i>	A	0	A	A	0	0	A	0	A	AG	0
<i>Serratia marcescens</i>	AG	A	A	A	0	A	0	0	A	A	A
<i>Es. coli</i>	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
<i>A. aerogenes</i>	A	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
<i>Bact. friedländeri</i>	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG	AG
<i>Proteus vulgaris</i>	A	A	0	0	A	A	A	0	A	A	0
<i>S. aertrycke</i>	AG	A	AG	AG	A	AG	AG	AG	AG	AG	AG
<i>S. enteritidis</i>	A	A	AG	AG	A	A	AG	AG	AG	AG	A
<i>S. cholerae-suis</i>	A	A	0	AG	0	AG	AG	AG	AG	AG	AG
<i>S. paratyphi</i>	A	0	AG	AG	A	A	A	AG	AG	AG	AG
<i>S. Schottmülleri</i>	A	A	AG	AG	AG	AG	AG	AG	AG	AG	AG
<i>E. typhi</i>	A	0	0	A	0	0	0	0	A	A	0
<i>E. dysenteriae</i> , Flexner	A	0	A	A	A	0	0	0	A	A	0
<i>E. dysenteriae</i> , Sonne	A	A	A	A	A	A	A	A	A	A	A
<i>Sarcina lutea</i>	A	0	0	0	0	A	0	0	0	0	0
<i>Staph. aureus</i>	A	0	A	A	A	0	A	0	A	0	0
<i>Staph. albus</i>	A	0	A	A	A	A	A	0	A	A	A
<i>Sac. cerevisiae</i>	0	0	0	0	0	0	0	0	AG	AG	0
<i>Torula cremoris</i>	0	0	0	0	0	0	0	0	AG	AG	0

^a From Dr. J. W. E. Glattfeld (racemic mixture). ^b Prepared from calcium gluconate by the method of Hockett and Hudson, THIS JOURNAL, 56, 1632 (1934); m. p. 158-159°; $n_D^{20} 1.552 \pm 0.004$, $n_D^{25} 1.576 \pm 0.004$, cf. Wherry, THIS JOURNAL, 40, 1852 (1918). ^c Prepared from mesquite gum. ^d From Dr. P. A. Levene. ^e From Dr. Fred L. Humoller. ^f Purchased from Eastman Kodak Company. ^g Prepared from cottonseed hulls. ^h Prepared from lemon flavin, Walton, THIS JOURNAL, 43, 127 (1921), m. p. 94-95°; $n_D^{20} 1.525 \pm 0.003$, $n_D^{25} 1.531 \pm 0.003$. (*l*-Rhamnohexose prepared from *l*- α -rhamnohexonic acid [Fischer, *Ber.*, 23, 3105 (1890)], m. p., 178-179° [m. p., *l*- α -rhamnohexosazone, 197-198°] was not fermented by any organism.)

Kendall,² using twelve common bacteria, found that *dl*-glyceric aldehyde was not utilized by any of them. A possible explanation of this lack of agreement is that Kendall used as his indicator phenol red, whose lower pH limit is 7.0; his medium was originally at a pH of 7.2-7.4; the change from the original pH to 7.0 is not a very sharp one, and he may therefore have missed the production of acid. He also found that *Staph. aureus* did not ferment *d*-arabinose but *S. Schottmülleri*, *Es. coli*, and *A. aerogenes* did ferment this sugar. The results shown in Table I on *d*-arabinose and *d*- α -glucoheptose confirm the findings of Koser and Saunders,³ who used a large group of bacteria.

Comparing *l*-rhamnose and *l*- α -rhamnohexose one finds that the principle enunciated for the glucose series applies here also; the 7-carbon sugar is not utilized by the organism, no matter how readily the 6-carbon sugar may be.

Comparing *d*-arabinose, *d*-glucose, and *d*-mannose, one readily sees that the hexoses are utilized by more organisms than is the pentose; but in the case of *d*-lyxose and *d*-galactose, the pentose is utilized by more organisms than is the hexose.

Cross and Tollens⁴ reported that *Saccharomyces*

(2) Kendall, Bly and Haner, *J. Infectious Diseases*, 23, 377 (1923).

(3) Koser and Saunders, *J. Bact.*, 26, 475 (1933).

(4) Cross and Tollens, *J. Landw.*, 59, 419 (1911).

did not ferment arabinose or rhamnose. Sohugen and Coolhaas⁵ found that *Sac. cerevisiae* would ferment galactose if there were a number of newly-formed cells present. Koser and Saunders³ obtained the same results as are here reported on *l*-rhamnose. Warren and Iredale⁶ noted that *S. Schottmülleri* would ferment rhamnose, the gas-producing power being destroyed after subculturing. Kendall² reported that *Staph. aureus*, *S. Schottmülleri*, *Es. coli*, and *A. aerogenes* fermented *d*-mannose. *B. megatherium* and *Sarcina lutea* did not ferment *d*-mannose, whereas *Sac. cerevisiae* and *Torula cremoris* did. He also reported that *Staph. aureus*, *S. Schottmülleri*, *Es. coli*, and *A. aerogenes* fermented *d*-galactose.

A comparison of six of the eight aldopentoses is shown in Table I. More organisms ferment *d*-ribose than any of the other pentoses. There are several interesting points to be observed: (1) *B. megatherium* ferments only the naturally-occurring pentoses; (2) *Proteus vulgaris*, while not fermenting the naturally-occurring forms, *d*-ribose and *l*-arabinose, does ferment their optical enantiomorphs; (3) *E. typhi* ferments only *d*-ribose, unlike the paratyphoids which readily fer-

(5) Sohugen and Coolhaas, *J. Bact.*, 9, 131 (1924).

(6) Warren and Iredale, *J. Hyg.*, 34, 203 (1934).

TABLE II

Organism	Sorbitol	<i>d</i> -Gluconic acid	Dulcitol	<i>d</i> -Galactonic acid	Mucic acid	Inositol ^a	Erythritol ^b	α -Methyl <i>d</i> -glucoside ^c	Glucosamine ^d	<i>d</i> - β -Glucos- nonose ^e
<i>B. megatherium</i>	0	A	0	0	0	0	0	0	0	0
<i>Serratia marcescens</i>	A	A	0	0	0	A	A	0	A	0
<i>Es. coli</i>	AG	AG	AG	AG	A	0	0	0	AG	0
<i>A. aerogenes</i>	AG	AG	0	0	AG	AG	0	AG	AG	0
<i>Bact. friedländeri</i>	AG	AG	AG	AG	AG	AG	0	AG	AG	0
<i>Proteus vulgaris</i>	0	AG	0	0	0	0	0	AG	0	A
<i>S. aertrycke</i>	AG	AG	AG	AG	AG	0	0	0	A	0
<i>S. enteritidis</i>	AG	A	AG	A	A	0	0	0	A	0
<i>S. cholerae-suis</i>	AG	AG	0	0	0	0	0	0	A	0
<i>S. paratyphi</i>	AG	AG	A	0	0	0	0	0	A	0
<i>S. Schottmülleri</i>	AG	AG	AG	AG	A	AG	0	0	A	0
<i>E. typhi</i>	A	A	0	0	0	0	0	0	0	0
<i>E. dysenteriae</i> , Flexner	A	A	0	A	0	0	0	A	0	0
<i>E. dysenteriae</i> , Sonne	0	A	0	A	A	0	0	0	0	0
<i>Sarcina lutea</i>	0	0	0	0	0	0	0	0	0	0
<i>Staph. aureus</i>	0	A	0	0	0	0	0	0	A	0
<i>Staph. albus</i>	A	A	A	0	A	A	A	A	A	A
<i>Sac. cerevisiae</i>	0	0	0	0	0	0	0	A	0	0
<i>Torula cremoris</i>	0	0	0	0	0	0	0	0	A	A

^a From Eastman Kodak Co. ^b From Dr. J. W. E. Glattfeld. ^c M. p. 166°. ^d Prepared from lobster shell. ^e $[\alpha]_D +12.7^\circ$. *d*- α -Glucoheptose (decomposed 190–206°), *d*- β -glucooctose (m. p. 120°), and *d*- β -glucosnonose (sirup) were prepared by the method of Hudson, Hartley, and Purves, THIS JOURNAL, 56, 1248 (1934). The heptose and octose were not fermented by any organism.

ment almost all the other pentoses; (4) *Sarcina lutea* ferments *d*-lyxose, but the two yeasts do not ferment any of the pentoses.

In Table II is shown a comparison of the fermentation of different stages of oxidation of glucose and galactose. It is clear that the aldehyde is fermented by the greatest number of organisms. The acid and alcohol are fermented by about the same number of organisms, which is less than the number for the aldehyde. In the case of glucose and galactose, one may also conclude that if the alcohol and/or aldonic acid are fermented, then the corresponding aldehyde will also be fermented, but the converse does not always hold. From the data presented it appears that the terminal group of the molecule is of considerable importance; oxidation of the primary alcohol group to a carboxyl group results in the molecule not being utilized by any organism (in the case of glucuronic acid); however, substitution of a carboxyl group for both the aldehyde and alcohol groups, as in the case of mucic acid, results in the molecule being as readily fermented as the aldonic acid.

It should be pointed out that mucic acid can be very useful to bacteriologists for differentiating between closely related organisms. It is fermented by *Es. coli* but not by *E. typhi*; by *S. Schottmülleri* (paratyphoid B) but not by *S. paratyphi* (paratyphoid A); by *E. dysenteriae*, Sonne,

but not by *E. dysenteriae*, Flexner; and by *Staph. albus* but not by *Staph. aureus*. In addition, it can be used to differentiate between three organisms in the *Salmonella* group, *S. aertrycke* yielding acid and gas, *S. enteritidis* producing only acid, and *S. cholerae-suis* not fermenting the compound.

Kendall² reported that *Staph. aureus*, *P. vulgaris*, and *E. dysenteriae*, Sonne, did not ferment sorbitol; whereas, *E. dysenteriae*, Flexner, *E. typhi*, *S. paratyphi*, *S. Schottmülleri*, and *Es. coli* did ferment sorbitol. He also reported that none of these organisms fermented either dulcitol or mucic acid. In 1930, Kendall and Gross⁷ reported that *A. aerogenes*, *Es. coli*, *P. vulgaris*, and *S. Schottmülleri* fermented ammonium glucuronate, whereas *Staph. aureus* did not. As mentioned previously, Kendall in his work used phenol red as an indicator. The change from the pH of the medium (7.2–7.4) to the acid pH limit of the indicator (7.0) probably does not give sufficient color change to enable one to observe carefully whether there is or is not acid. In addition, the ammonium salt of glucuronic acid was used whereas in the present study the sodium salt was used; it is possible that what Kendall observed was not a utilization of the entire compound but a utilization of the ammonia, leaving behind the untouched acid.

(7) Kendall and Gross, *J. Infectious Diseases*, 47, 249 (1930).

Poe, Field, and Witt⁸ studied the fermentation of sorbitol by ten species of the *Escherichia* and five species of the *Aerobacter* group. They report that as a whole the *Aerobacter* group showed greater total acidity than the *Escherichia* group. Dozois, Hachtel, Carr, and Krantz⁹ report that *Aerobacter* failed to attack dulcitol though *Es. coli* did ferment this alcohol. Interesting was their observation that none of their organisms attacked the anhydride, dulcitan.

Kendall² observed that *Staph. aureus* did not ferment gluconic or galactonic acid; *E. dysenteriae*, Flexner, *E. dysenteriae*, Sonne, *E. typhi*, *S. paracyphi*, *S. Schottmülleri*, *Es. coli*, and *P. vulgaris* fermented both gluconic and galactonic acids. He also concluded that the acid is not fermented if the corresponding aldehyde is not fermented.

l- α -Rhamnohexonic acid (m. p., phenylhydrazide, 208–9°), *d*- α -glucoheptonic acid (m. p., phenylhydrazide, 173°), *d*- α -glucooctonic acid (m. p., phenylhydrazide, 174°), and *d*- β -glucononic acid (m. p., phenylhydrazide, 194–5°) were also studied but no organism was found able to ferment any of these acids.

Bruce¹⁰ has reported a detailed study of the relationship between molecular structure and the ability of organisms to grow in solutions of organic acids. His study is not strictly comparable to the present work because he used fatty acids and was only interested in whether or not the organisms would grow. However, his conclusions are of interest. They were: (1) the *cis* isomer has greater ability to support growth than the *trans* form; (2) among the lower normal fatty acids (one to four carbon atoms) growth is more abundant in media containing acids with even numbers of carbon atoms than in those with odd numbers but substitution of an hydroxyl group for hydrogen in these acids causes a reversal of this relationship; (3) the amino acids show the same relationship as the hydroxy acids; (4) growth diminishes as the molecular weight of the acid increases; substitution of a phenyl group diminishes the ability of the substance to support growth.

In addition to the compounds reported in Tables I and II, the following compounds were studied: α -methyl-*d*-mannoside, glucose ethyl mer-

captal, and 1,2-isopropylidene-3,5-benzylidene-glucuronic acid. They were completely negative. Gulonic acid, 1,2-isopropylidene-glucose and 1,2-5,6-*dl*-isopropylidene-glucose were fermented by only one organism, *Staph. albus*. However, even in tubes where there was no fermentation there was adequate growth of the organisms, showing that the sugar derivatives had no toxic action and that the organisms were able to grow on the food material supplied by the peptone and beef extract.

The following points should be noted: (1) glycoside formation decreases greatly the availability of the compound for the organisms (it has been shown previously³ that β -methyl-*l*-arabinoside and β -methyl-*d*-xyloside are not fermented by any of these organisms); (2) a modified acetal formation, with sulfur in place of oxygen, results in a compound that is not attacked by any organism; (3) substitution of an amino group on the second carbon atom does not markedly affect the ease of fermentation of the sugar; (4) substitution in the first and second carbon atoms decreases the ability of organisms to ferment glucose.

Hees and Tropp¹¹ reported that benzylthio-glucoside and glucose ethyl mercaptal were fermented by *A. aerogenes* but not by *Es. coli*. Kendall and Gross⁷ report the following results: (1) *S. Schottmülleri* and *Es. coli* fermented glucosamine, but *Staph. aureus* and *P. vulgaris* gave doubtful results; (2) *P. vulgaris* fermented α -methyl-*d*-glucoside but *Staph. aureus*, *S. Schottmülleri*, *Es. coli*, and *A. aerogenes* did not; (3) *A. aerogenes* and *Es. coli* did not ferment diacetone-glucose, whereas *Staph. aureus*, *S. Schottmülleri* and *P. vulgaris* did ferment this glucose derivative.

Koser and Saunders³ studying a group of organisms containing all those used in the present study obtained identical results with α -methyl-*d*-glucoside, glucose ethyl mercaptal and glucosamine except for the following: *Staph. albus* did not ferment the glucoside; *B. megatherium*, *E. typhi*, and *E. dysenteriae*, Sonne, attacked glucosamine, but *Torula cremoris* did not. An explanation for the latter discrepancy may lie in the fact that their control tube of glucosamine became acid after four to five days, thereby obscuring their results.

Tittsler and Sandholzer¹² studied the fermen-

(8) Poe, Field, and Witt, *U. Colo. Bull. Stud.*, Nos. 2 and 3, 165 (1933).

(9) Dozois et al., *J. Bact.*, **30**, 189 (1935); **32**, 499 (1936).

(10) Bruce, *This Journal*, **57**, 1495 (1935).

(11) Hees and Tropp, *Zentr. Bakt. Parasitenk. I Abt.*, **100**, 273 (1926).

(12) Tittsler and Sandholzer, *J. Bact.*, **29**, 363 (1935).

tation of α -methyl-*D*-glucoside by members of the *Escherichia-Aerobacter* group and reported that all the *Aerobacter* and one-third of the intermediate strains attacked the glucoside though none of the *Escherichia* showed any sign of fermenting this compound. Their results are essentially in accord with earlier findings of Koser and Saunders.¹³ Wedum and Walker¹⁴ found that *Torula* and "yeast" did not ferment glucosamine; *staphylococcus*, *Es. coli*, *A. aerogenes*, *S. paratyphi*, *S. Schottmülleri*, *S. enteritidis*, and *E. dysenteriae*, Flexner, did utilize glucosamine. They state that among sixty-one strains of bacteria there were no exceptions to the rule that bacteria which fermented glucose also fermented glucosamine.

It is of some importance that *i*-inositol is attacked by several organisms, inasmuch as this compound is not, strictly speaking, a carbohydrate or carbohydrate derivative. The small number of organisms that ferment erythritol and gulonic acid would lead one to surmise that both erythrose and gulose would not be fermented by many organisms.

Poe, Field, and Witt⁸ report that members of the *Aerobacter* group fermented *i*-inositol, but that members of the *Escherichia* group did not.

A comparison between fermentation of sugars in an ordinary nutrient culture medium and a synthetic medium with the addition of growth factor¹⁵ was made.

The results of the fermentation in both types of medium are very similar except that in some cases there was no gas produced in the synthetic medium though there was gas production in the ordinary nutrient culture broth. Bronfenbrenner and Schlesinger¹⁶ made a study of this point using *Es. coli* and found that the amount of gas produced varied directly with the concentration of the peptone in the medium and inversely with the amount of buffer salts present. The difference in gas production in the two media is probably due to the lack of peptone in the synthetic medium. In certain cases there was no fermentation in the synthetic medium though there was

some in the other medium. The explanation for this probably lies in the fact that in those instances growth was not as vigorous in the synthetic as in the regular medium. The important point to be stressed is that in no case was there fermentation in the synthetic medium if there was no fermentation in the nutrient medium.

Summary

1. The fermentation of the following sugars and their derivatives by bacteria and yeasts has been studied for the first time: *D*- α -glucoheptose, *D*- α -glucoheptonic acid, *D*- β -glucooctose, *D*- β -glucooctonic acid, *D*- β -glucononose, *D*- β -glucononic acid, *L*- α -rhamnohexose, *L*- α -rhamnohexonic acid, *D*-ribose, *L*-ribose, *D*-lyxose, 1,2- β -diisopropylidene-glucose, 1,2-isopropylidene-3,5-benzylidene-glucose, *D*-erythritol, and *D*-gulonic acid.

2. Fermentation studies on other sugars and their derivatives have been extended.

3. The 5- and 6-carbon sugars are most easily attacked by most organisms, the pentoses being attacked by fewer organisms than the hexoses except in the case of *D*-lyxose and *D*-galactose. The 7- and 8-carbon sugars studied were not attacked by any of the nineteen organisms used. The nonose was fermented by only three organisms, one of which was a yeast. *L*- α -Rhamnohexose was not fermented. In no case was there fermentation of the aldonic acid of a sugar containing seven or more carbon atoms.

4. In a comparison between the optical enantiomorphs of ribose and arabinose, it was found that the naturally-occurring form of each sugar was fermented by more organisms than the synthetic form. However, in general the organisms did not distinguish between the *D*- and *L*-forms.

5. Substitution of a carboxyl group for the carbonyl group decreases to a small extent the number of organisms which can utilize the compound. Usually if a sugar acid is fermented by an organism then the corresponding sugar will also be fermented.

6. Substitution of a primary alcohol group for the carbonyl group decreases to a small extent the number of organisms which will ferment the compound. If the alcohol is fermented, then the corresponding sugar will also be fermented.

7. Mucic acid was utilized by many organisms and can be used to distinguish between many closely related bacteria.

(13) Koser and Saunders, *J. Bact.* **24**, 267 (1932).

(14) Wedum and Walker, *J. Infectious Diseases*, **57**, 160 (1935).

(15) Koser and Saunders, *ibid.*, **56**, 305 (1935). The synthetic medium was prepared according to the following formula: Na_2HPO_4 , 1.4 g., KH_2PO_4 , 1.0 g., NaCl , 2.0 g., MgSO_4 , 0.1 g., cystine, 0.1 g., *L*-asparagine, 3.0 g., tryptophane, 0.2 g., and water to make one liter. To this is added 20 cc. of a growth-factor preparation containing 0.11% total solids and no ash.

(16) Bronfenbrenner and Schlesinger, *Proc. Soc. Exptl. Biol. Med.*, **16**, 44 (1918); see also Hajna, *J. Bact.*, **33**, 339 (1937).

8. Glycoside formation decreases to a great extent the number of organisms that will attack a sugar.

9. Substitution of an amino group on the second carbon atom of glucose does not appreciably affect the fermentability of the sugar.

10. Gulonic acid, erythritol, and 1,2-isopropylidene-glucose were not fermented by most of the organisms.

11. Glucose ethyl mercaptal, 1,2-5,6-diisopropylidene-glucose, 1,2-isopropylidene-3,5-benzylidene-glucose, and glucuronic acid were not fermented by any organism.

12. In general, any departure from the original structure of a naturally-occurring sugar renders the sugar less likely to be fermented by microorganisms.

CHICAGO, ILLINOIS

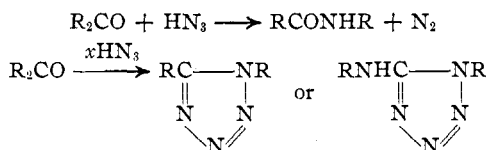
RECEIVED AUGUST 27, 1937

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

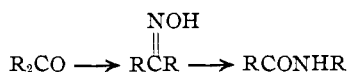
The Reaction of Hydrazoic Acid with Benzil¹

BY M. A. SPIELMAN AND F. L. AUSTIN

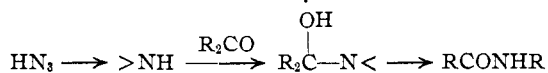
K. F. Schmidt^{2,3,4} has published several papers on the reaction between hydrazoic acid and ketones in the presence of sulfuric acid or similar reagents. With one equivalent of hydrazoic acid the product is an amide. Two or more equivalents of hydrazoic acid lead to tetrazoles, alkylaminotetrazoles, or both.



In his first paper Schmidt formulates the reaction as first yielding an oxime which then undergoes the Beckmann rearrangement.



Later papers make no mention of the hypothesis but give the impression that the essential factor is the imino diradical $>NH$ which adds to the carbonyl group to form a univalent nitrogen derivative of a type once in favor as an intermediate in several molecular rearrangements. In a footnote³ the idea is attributed to Stollé.



Although the Schmidt reaction has been used in a few instances⁵ it has never been extensively

(1) This work was supported in part by the Wisconsin Alumni Research Foundation.

(2) Schmidt, *Acta Acad. Aboensis Mat. et Phys.*, **2** (1924); *C. A.* **19**, 3248 (1925).

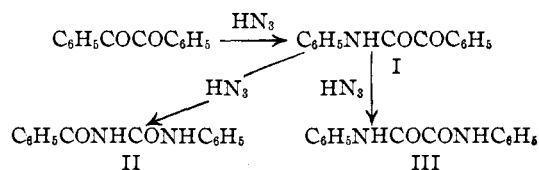
(3) Schmidt, *Ber.*, **57**, 704 (1924).

(4) Schmidt, *Friedl.*, **15**, 221, 333 (1926); **16**, 2862 (1928); German Patents 427,858, 439,041, 479,016.

(5) Ruzicka Goldberg, Hürbin and Boeckenoogen, *Helv. Chim. Acta*, **16**, 1323 (1933); v. Braun and Heymons, *Ber.*, **63**, 502 (1930).

studied, and the experiments here described were undertaken with the idea of expanding our knowledge of the field. No α -diketone has been investigated, and benzil was selected as an example of this type of compound. The present paper reports its reaction with hydrazoic acid.

Benzil and hydrazoic acid, in the presence of sulfuric acid at 0–10°, give as the principal product 30–60% of the theoretical yield of benzoylphenylurea, II. The first fission undoubtedly takes place unsymmetrically, as phenylglyoxanilide, I, is the only ketonic intermediate possible.



A second Schmidt reaction, resulting in bond rupture between the two carbonyl groups, would then lead to the main product, II. There is some evidence for this view in the fact that oxanilide, III, which would be formed by insertion of an imino group between the carbonyl and phenyl group, is also found in small amounts. The relative yield of products indicates that in the second step division between the two carbonyls is the more rapid reaction.

The oxime which by means of the Beckmann rearrangement produces benzoylphenyl urea is benzil γ -dioxime,⁶ but this compound is stable to hydrazoic-sulfuric acid, and the Beckmann rearrangement as a factor is thus clearly eliminated. The preformation of imino radicals is also improbable because of the fact that hydrazoic

(6) Beckmann and Köster, *Ann.*, **274**, 19 (1893).