THE DIGESTION OF PECTIN AND METHYLATED GLUCOSES BY VARIOUS ORGANISMS*

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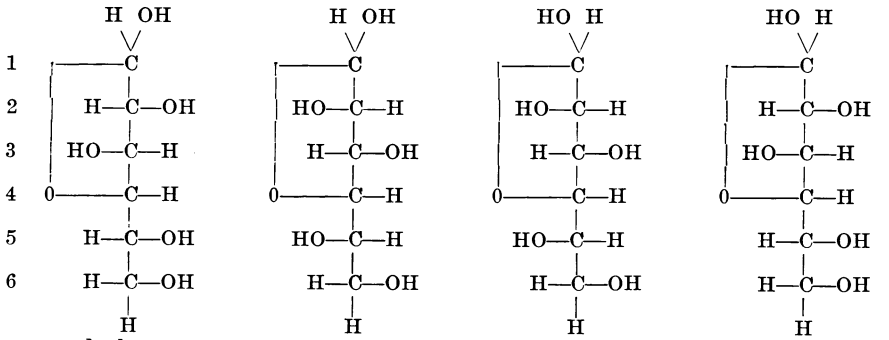
Introduction

This preliminary paper is the result of work started with the object of determining the effect of introduced methyl groups upon the digestion of carbohydrates by microorganisms. At the same time, it was hoped that methylated glucoses and pectin might offer a means of distinguishing between *Bact. aertrycke, Bact. schotmülleri,* and other closely related forms. These two organisms, up to the present time, are not distinguishable on the basis of morphological, cultural, or physiological characteristics. It was also thought that possibly a relation might exist between the digestion of pectin and the methylated glucoses.

The only paper in the literature recording the digestion of methylated hexoses by organisms is that of IRVINE and HOGG (**4**) who record a few experiments made with 3-monomethyl glucose. It was found by these authors that living top yeast and macerated extract, prepared from dried Munich bottom yeast, had no action upon the sugar. They also found that, of the seven species of bacteria tried, *Bacterium coli commune, Bact. lactis aerogenes, Bact. proteus, Bact. paratyphosum, Bact. cloacae, Bact. typhosum,* and *Staphylococcus pyogenes aureus,* all of which are glucose fermenters, only *Bact. cloacae* Jordan digested the methylated glucose with the production of acid and gas.

Methylated glucoses

Glucose may have the following structures[1] depending upon the spacial configuration of the molecule:



* Reported at the meeting of the Iowa Academy of Sciences, April, 1926.

[1] The recent paper of HIRST, Jour. Chem. Soc., London **129**: 350. 1926, indicates that the oxide linkage of glucose should be amylene oxidic <1, 5> instead of butylene oxidic <1, 4> as shown.

It is seen that we might theoretically have methoxy groups attached to carbons one to six on each of the configurations given. Furthermore, we might have all possible combinations of two to five methyl groups for each configuration, and if we include the so-called γ-sugars (compounds in which the oxidic linkage is believed to be other than butylene oxidic <1, 4>) the list is greatly increased.

A simple calculation will show, then, that we have theoretically possible the following methylated glucoses: 20 monomethyl glucoses (including the methyl glucosides), 56 dimethyl glucoses, 76 trimethyl glucoses, 52 tetramethyl glucoses, and 20 pentamethyl glucoses, a total of 224 different methylated glucoses, not including the gamma sugars, since each glucose derivative can occur as the α- or β-modification and each of these as dextro- or levo-glucose.

A careful search of the literature revealed that only a very few of these theoretically possible sugars have been prepared. These are tabulated below, the doubtful ones being indicated by a question mark.

TABLE I

SUMMARY OF METHYLATED GLUCOSES DESCRIBED IN THE LITERATURE

NUMBER OF METHYL GROUPS				
1	2	3	4	5
α–d–1 β–d–1 α–d–2 (?) α–d–3 β–d–3 α–d–6 (?)	α–d–1, 3 α–d–2, 3 β–d–2, 3 α–d–2, 5 (?)	α–d–1, 2, 3, α–d–1, 2, 5, (?) α–d–2, 3, 5, α–d–2, 3, 6, α–d–3, 5, 6, α–d–2, 5, 6, (?)	α–d–1, 2, 3, 5 β–d–1, 2, 3, 5 α–d–1, 2, 3, 6 β–d–1, 2, 3, 6 α–d–1, 4, 5, 6 (?) β–d–2, 3, 5, 6 α–d–2, 3, 5, 6 α–d–3, 4, 5, 6	α–d–1, 2, 3 5, 6 β–d–1, 2, 3 5, 6

Materials and methods

The media used in these investigations were of two kinds: One containing peptone, and the other containing no peptone, and hence no carbon except the carbon of the pectin or the carbon of the methylated glucoses. They were made up as follows:

MEDIUM WITH PEPTONE

2 gms. bacto-peptone
1 gm. K_2HPO_4
10 cc. 1 per cent. Andrades indicator
3 gms. pectin or sugar
1000 cc. distilled water

MEDIUM WITHOUT PEPTONE

2 gms. ammonium chloride
2 gms. K_2HPO_4
10 cc. 1 per cent. Andrades indicator
3 gms. pectin or sugar
1000 cc. distilled water

The media were adjusted to a P_H of 7.0 to 7.2 and tubed. In all of the work, twenty-four hour glucose-phosphate agar slants were used for inoculation of the culture media. Inoculations in every case were made as heavy as possible, care being especially taken with the medium containing no peptone to see that the inoculations were positive. The tubes, after inoculation, were incubated 24 to 98 hours at 37° C., excepting those organisms whose optimum was 25° to 27° C., and any acid, gas or gumminess recorded each day.

The pectin, a very pure form, was obtained from the Research Laboratories of the California Fruit Growers Exchange, and was used as received. It contained, as reported by them, aluminium oxide as an impurity. It was tested for reducing sugar which was found to be absent.

The method followed for the preparation of the 3-monomethyl glucose was essentially that of IRVINE and HOGG (**4**). The reactions involved are:

$$\alpha\text{-d-glucose} \xrightarrow[\text{1 per cent. HCl gas}]{\text{acetone}} \text{diacetone glucose} \xrightarrow[\text{Reference (2)}]{\text{sodium}} \text{Na salt}$$

$$\text{of diacetone glucose} \xrightarrow[\text{iodide}]{\text{methyl}} \text{3-monomethyl diacetone glucose}$$

$$\xrightarrow[\text{acid}]{\text{dilute}} \underset{\text{3-monomethyl glucose (I).}}{\text{CHOH.CHOH.CHOCH}_3\text{.CH.CHOH.CH}_2\text{OH}}$$

The 3-monomethyl glucose thus secured is readily soluble in water, less so in methyl alcohol, and is very sparingly soluble in other organic solvents. It has a melting point of 157–158° C., and a rotation in water of +55.5° (final). It exhibits mutarotation. It was purified by recrystallization from methyl alcohol.

The preparation of 1, 2, 3, 5-tetramethyl glucose and 1, 2, 3, 5, 6-pentamethyl glucose may be described together, since they are formed in the same methylation process. The method of HAWORTH (**3**) was used. The reaction occurs as follows:

$$\alpha\text{-d-glucose} \xrightarrow[\substack{\text{.25 per cent. HCl}\\ \text{Reference (1)}}]{\text{CH}_3\text{OH}} \alpha\text{-methyl glucoside} \xrightarrow[\text{NaOH}]{\text{dimethyl sulfate}}$$

$$\underset{\text{pentamethyl glucose (II)}}{\text{CHOCH}_3\text{.CHOCH}_3\text{.CHOCH}_3\text{.CH.CHOCH}_3\text{.CH}_2\text{OCH}_3}$$

and

$$\underset{\text{tetramethyl glucose (III)}}{CHOCH_3.CHOCH_3.CHOCH_3.CH.CHOCH_3.CH_2OH \quad (O)}$$

The methylated glucoses thus secured are syrups. They were repeatedly fractionated for purification. They did not reduce Fehling's solution.

The tetramethyl glucose (III) is soluble in water, chloroform, methyl iodide and in common organic solvents. It has a refractive index number equal to 1.4583 and $D\ \frac{20}{4}$ equal to 1.158. It does not show mutarotation.

The pentamethyl glucose (II) is soluble in alcohol, water, acetone, ether, and methyl iodide. Index of refraction, n_D is equal to 1.4454. It has the following rotations: +147.4° in water and +153.9° in alcohol. It does not show mutarotation.

Description of organisms

The organisms of group I belong to the colon-typhoid group and were mainly of fecal origin. Those of group II were obtained from activated sludge from creamery wastes, and group III are organisms from miscellaneous sources. The figures in parentheses indicate the number of strains used. All cultures which attacked pectin and the methylated glucoses were examined morphologically and culturally to determine their position in the classification of the colon-typhoid group as given by WELDIN and LEVINE (5).

GROUP I

Bacterium coli (4), *Bact. paragrünthali* (2), *Bact. communior* (1), *Bact. coscoroba* (2), *Bact. grünthali* (5), *Bact. neapolitanum* (1), *Bact. pseudocoloides* (1), *Bact. schafferi* (4), *Bact. vesiculosum* (2), *Bact. aerogenes* (9), *Bact. cloacae* (7), *Bact. levans* (3), *Bact. oxytocum* (13), *Bact. vulgaris* (6), *Bact. abortivoequinum* (2), *Bact. aertrycke* (1), *Bact. paratyphi* (5), *Bact. flexneri* (6), *Bact. schotmülleri* (4), *Bact. pullorum* (5), *Bact. morgani* (2), *Bact. enteritidis* (3), *Bact. suipestifer* (3), *Bact. ambiguum* (1), *Bact. typhi murium* (1), *Bact. dispar* (1), *Bact. typhi* (2), *Bact. alkalescens* (1), *Bact. sanguinarium* (2), *Bact. anatum* (1), *Bact. pfaffii* (1), *Bact. jeffersonii* (1), *Bact. rettgeri* (1), *Bact. shigae* (1), *Bact. rhinoscleromatis* (1), *Bact. viscosum* (1), *Bact. viscosum aerogenes* (1).

GROUP II

Bacillus albolactus (2), *B. cereus* (4), *B. mesentericus* (1), *B. panis* (2), *B. megatherium* (1), *B. aterrimus* (1), *B. niger* (1), *Pseudomonas myxo-*

genes (1), *Pseud. fluorescens* (1), *Pseud. ovalis* (2), *Pseud. pavonacea* (3), *Flavobacterium ovalis* (2), *Flav. acetylicum* (2), *Flav. suaveolens* (3), *Flav. zettnowi* (1), *Flav. deciduosum* (1), *Serratia rubida* (2), *Ser. rubrica* (2), *Ser. amyloruber* (1), *Bact. metalkalescens* (1), *Rhodococcus coralinus* (1).

GROUP III

Saccharomyces ellipsoideus (2), *Sac. cereviseae* (1), *Torula rosea* (1), *B. subtilis* (2), *B. sphaericus* (1), *B. mesentericus* (1), *Staphylococcus aureus* (2), *Staph. albus* (3), *Ser. marcescens* (3), *B. fusiformis* (1), *Pasteurella cholera gallivarum* (1), *B. aceto-ethylicus* (2), *Pseud. cyanogenes* (3), *Pseud. pyocyanea* (2), *Pseud. fluorescens* (1), *Sac. pombe* (1), *Past. suiseptica* (1), *Sarcina lutea* (1), *Monilia variabilis* (1), *Micrococcus luteus* (1), *B. mesentericus fuscus* (1), *Bact. abortus* (1), *Micr. flavescens* (1).

Results

The cultures which attacked the pectin with the production of acid and gas are the following, the numbers in parentheses being the laboratory numbers of the cultures: Seven out of thirteen strains of *Bact. oxytocum* (305, 369, 499, 139, 261, 269, 270); four out of nine strains of *Bact. aerogenes* (117, 80, 256, 257); two out of two strains of *Bact. viscosum aerogenes* (248, 298); and two out of two strains of *Bact. aceto-ethylicus* (172, 173). Garden soil and manure produced acid and no gas, and garden soil produced gumminess in both media. *Bact. schafferi* (103) produced gumminess in peptone media. All other cultures of Groups 1 to 3 failed to attack pectin with the production of acid, gas or gumminess. There was no difference between the two media, *i.e.*, no culture produced acid or gas in the synthetic non-peptone medium which did not produce it in the peptone medium, and vice versa.

A larger variety of cultures produced acid and gas from the 3-monomethyl glucose. They were: Six out of seven strains of *Bact. cloacae* (233, 235, 236, 264, 136, 263); one out of three strains of *Bact. levans* (238); one out of fourteen strains of *Bact. oxytocum* (499); one strain of *Bact. pseudocoloides* (486); and one out of nine strains of *Bact. aerogenes* (268). Manure gave acid and gas, but garden soil gave acid and no gas.

The tetramethyl and pentamethyl glucoses were not digested by any of the cultures of Groups 1, 2 or 3 with the production of acid and gas. The test with manure was indefinite, a slight acidity being produced with a bubble of gas.

Discussion

From a consideration of the cultures used and the cultures digesting pectin with the production of acid and gas, it is evident that only those

organisms commonly occurring in the soil are capable of attacking pectin with the production of acid and gas. None of the intestinal forms, classified in the sub-genus *Escherichia* of the genus *Bacterium,* produced acid or gas from the pectin. It was to be expected that organisms digesting pectin would be found in the soil, since the rotting of wood and plant tissue, containing pectin bodies, takes place readily in the soil.

Again, it is evident that only those organisms occurring commonly in the soil are capable of attacking 3-monomethyl-glucose with the production of acid and gas. It was expected that very few organisms, if any, would be able to touch the tetramethyl and pentamethyl glucoses. In these two sugars the hydroxyl groups are protected by methyl groups, and this parallels the cellulose molecule in which hydroxyl groups are presumably bound in ether linkages. It seems, therefore, that those organisms of the colon-typhoid group, which are vigorous fermenters of glucose, are unable to attack methylated glucoses when the number of methyl groups becomes large.

It is interesting to speculate just how the number and position of the methyl groups in the sugar molecule affects the digesting ability of the organisms. It is hoped to make these speculations the basis of a later investigation on methylated hexoses.

Conclusions

1. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by those members of the colon-typhoid group commonly found in feces.

2. The organisms attacking pectin and 3-monomethyl glucose with the production of acid and gas were those generally associated with the soil.

3. Pectin and the methylated glucoses tried were not digested with the production of acid or gas by organisms isolated from the activated sludge of creamery wastes.

4. All of the members of the colon-typhoid group tested were incapable of digesting 1, 2, 3, 5-tetramethyl glucose and 1, 2, 3, 5, 6-pentamethyl glucose.

5. *Bact. schotmülleri, Bact. aertrycke* and other closely related forms can not be differentiated on the basis of the digestion of pectin and the methylated glucoses used.

6. It is suggested that an agar medium containing only 3-monomethyl glucose may be useful for the isolation of *Bact. cloacae.*

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