

hypophosphite for the conversion of D-glucuronolactone into L-ascorbic acid.

9. The presence of borate or Antabuse, each at mM-concentration, inhibits the conversion of D-glucuronolactone into L-ascorbic acid to about 50%.

10. The optimum conditions for the action of the enzyme system have been studied.

11. The biosynthesized ascorbic acid has been identified by paper chromatography and by a biological test with one guinea pig.

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#### REFERENCES

- Burns, J. J., Peyser, P. & Moltz, A. (1956). *Science*, **124**, 1148.
- Chatterjee, I. B., Ghosh, N. C., Ghosh, J. J. & Guha, B. C. (1957a). *Science*, **126**, 608.
- Chatterjee, I. B., Ghosh, N. C., Ghosh, J. J., Roy, R. N. & Guha, B. C. (1957b). *Sci. & Cult.* **23**, 50.
- Chatterjee, I. B., Ghosh, N. C., Ghosh, J. J. & Guha, B. C. (1958a). *Int. Symp. Enzyme Chem., Tokyo* (1957). (In the Press.)
- Chatterjee, I. B., Ghosh, N. C., Ghosh, J. J. & Guha, B. C. (1958b). *Sci. & Cult.* **23**, 382.
- Chen, Y. T., Isherwood, F. A. & Mapson, L. W. (1953). *Biochem. J.* **55**, 821.
- Colowick, S. P., Kaplan, N. O. & Ciotti, M. M. (1951). *J. biol. Chem.* **191**, 447.
- Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
- Grollman, A. P. & Lehninger, A. L. (1957). *Arch. Biochem. Biophys.* **69**, 458.
- Hassan, M. uL. & Lehninger, A. L. (1956). *J. biol. Chem.* **223**, 123.
- Horowitz, H. H., Doerschuk, A. P. & King, C. G. (1952). *J. biol. Chem.* **199**, 193.
- Horowitz, H. H. & King, C. G. (1953a). *J. biol. Chem.* **200**, 125.
- Horowitz, H. H. & King, C. G. (1953b). *J. biol. Chem.* **205**, 815.
- Isherwood, F. A., Chen, Y. T. & Mapson, L. W. (1954). *Biochem. J.* **56**, 1, 15.
- Jackel, S. S., Mosbach, E. H., Burns, J. J. & King, C. G. (1950). *J. biol. Chem.* **186**, 569.
- Longenecker, H. E., Fricke, H. H. & King, C. G. (1940). *J. biol. Chem.* **135**, 497.
- Longenecker, H. E., Musulin, R. R., Tully, R. H., & King, C. G. (1939). *J. biol. Chem.* **129**, 445.
- Mapson, L. W. (1943). *J. Soc. chem. Ind., Lond.*, **62**, 223.
- Mapson, L. W., Isherwood, F. A. & Chen, Y. T. (1954). *Biochem. J.* **56**, 21.
- Musulin, R. R., Tully, R. H., Longenecker, H. E. & King, C. G. (1939). *J. biol. Chem.* **129**, 437.
- Rabinowitz, J. L. & Sall, T. (1957). *Biochim. biophys. Acta*, **23**, 289.
- Roe, J. H. & Kuether, C. A. (1943). *J. biol. Chem.* **147**, 399.
- Roy, S. C., Roy, S. K. & Guha, B. C. (1946). *Nature, Lond.*, **158**, 238.
- Schneider, W. C. & Hogeboom, G. H. (1950). *J. biol. Chem.* **183**, 123.
- Stadtman, E. R., Novelli, G. D. & Lipmann, F. (1951). *J. biol. Chem.* **191**, 365.

## Identification of 6-O-Acetyl-D-Glucopyranose in *Bacillus megaterium* Cultures: Synthesis of 6-O-Acetyl-D-Glucopyranose and 6-O-Acetyl-D-Galactopyranose

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This paper deals with the characterization of the crystalline carbohydrate obtained from *Bacillus megaterium* (NCIB 8508) cultures (Duff & Webley, 1958; and Duff, Webley & Farmer, 1957).

Preliminary examination (including infrared analysis and paper chromatography) showed that this compound was an ester of equimolar proportions of glucose and acetic acid. One carbonyl group per molecule was found with assays made at pH values within a narrow range around neutrality to avoid removal of a possible labile acetyl group.

Separate experiments (Table 1) showed that little or no hydrolysis occurred at these pH values. From the above-mentioned evidence and because an acetylated phenylhydrazone and osazone were formed, it seemed unlikely that the hydroxyl groups on C<sub>(1)</sub> or C<sub>(2)</sub> were esterified. The failure to form a triphenylmethyl (trityl) ether strongly suggested that there was no free primary hydroxyl group. Helferich (1948) states that no case has been reported of a primary hydroxyl group unreactive towards trityl chloride with the conditions

used. The periodate oxidation of the ester was described previously (Duff *et al.* 1957; Duff, 1957); the failure to yield formaldehyde is almost certain proof that the primary alcoholic group is esterified.

An ester identical with that obtained from NCIB 8508 cultures can be prepared in the laboratory from glucose and 50% (v/v) acetic acid (Duff, 1957), but the process gives no information on the orientation of the acetyl group on the glucose molecule. It has now been possible to synthesize 6-*O*-acetyl-D-glucopyranose and the corresponding galactose ester via 6-*O*-acetyl-1:2:3:5-di-*O*-isopropylidene-D-glucopyranose and 6-*O*-acetyl-1:2:3:4-di-*O*-isopropylidene-D-galactopyranose respectively, thus confirming the structure suggested by analytical results (this paper and Duff, 1957). 3-*O*-Acetyl-D-glucose was also prepared, from 3-*O*-acetyl-1:2:5:6-di-*O*-isopropylidene-D-glucopyranose.

Aqueous oxalic acid (0.2*N*) was found best for preferentially removing the isopropylidene group from the above intermediates but dilute mineral acid in ethanol also served. The 6-*O*-acetyl-D-galactose obtained is shown to be identical with the ester from galactose and 50% (v/v) acetic acid (Duff, 1957).

#### METHODS AND RESULTS

*Melting points.* These were determined with the Kofler micro-heating stage (Shandon Scientific Co., London).

*Column chromatography.* A slurry of Whatman cellulose powder (standard grade) and water-saturated butanol was packed into columns (5 cm. × 85 cm.) in small portions with a closely fitting ram perforated with small holes. The mixture was applied as a syrup with water or butanol or both and the column eluted with water-saturated butanol. Fractions (about 5 ml.) were collected with an automatic machine (Hough, Jones & Wadman, 1950). The distribution of the sugars in the tubes was found by paper chromatography with a spray of aniline phthalate or benzidine-trichloroacetic acid. The tubes were grouped and the solvent was removed at 30° *in vacuo*. In some cases the syrupy acetylated sugar crystallized, in others it was necessary to treat with charcoal in aqueous solution before crystallization. The products were dried to constant weight *in vacuo*.

#### *Identification of the Bacillus megaterium metabolite as 6-O-acetyl-D-glucopyranose*

*Isolation.* The crystalline ester was obtained as described by Duff & Webley (1958). After five recrystallizations from water it had m.p. 133°;  $[\alpha]_D^{20} +42-48.2^\circ$  at equilibrium in water (*c*, 4.0) (Found: C, 43.1; H, 6.3; CH<sub>3</sub>·CO, 18.8. C<sub>3</sub>H<sub>14</sub>O<sub>7</sub> requires C, 43.2; H, 6.35; CH<sub>3</sub>·CO, 19.4%). The

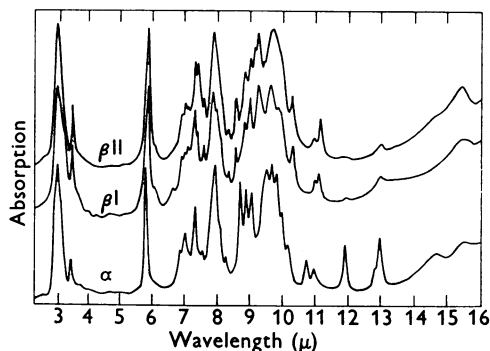


Fig. 1. Infrared spectra of different crystalline species of 6-*O*-acetyl-D-glucopyranose, isolated from *B. megaterium* cultures.

substance separated from water as aggregates of minute prismatic rods. It was soluble in ethanol, acetic acid, ethyl acetate, pyridine and acetone, and insoluble in light petroleum (b.p. 80–100°) and in benzene.

*Occurrence of  $\alpha$ - and  $\beta$ -anomers.* The infrared-absorption spectra of several crystalline samples of the acetylglucose from *B. megaterium* cultures were examined, over the range 2–16  $\mu$ , with the potassium bromide pressed-disk technique (Schiedt & Reinwein, 1952). The spectra were recorded on a Grubb Parsons double-beam infrared spectrometer equipped with sodium chloride prism. The spectra showed some differences due to variation in the proportion of three crystalline species, one an  $\alpha$ -anomer, and the others  $\beta$ -anomers ( $\beta$ I and  $\beta$ II) which probably differ only in their crystalline structures. The spectra of the purest available samples of these are given in Fig. 1. In most preparations one of the  $\beta$ -modifications predominated, giving typical  $\beta$ -glucopyranose bands in the 10.5–14  $\mu$  region (Barker, Bourne, Stephens & Whiffen, 1954) at 10.95 and 11.08  $\mu$  (913 and 903  $\text{cm}^{-1}$ ) for the  $\beta$ I form, and at 10.92 and 11.11  $\mu$  (916 and 900  $\text{cm}^{-1}$ ) for the  $\beta$ II form. The absorption spectrum of the  $\alpha$ -anomer showed typical  $\alpha$ -glucopyranose bands at 10.73, 10.96, 11.89, 12.82 and 12.96  $\mu$  (932, 912, 841, 780 and 771  $\text{cm}^{-1}$ ). The spectrum of the  $\alpha$ -anomer deviates widely from that of the two  $\beta$ -forms, which are clearly closely related although easily distinguished in the region 7–10  $\mu$ . In agreement with this interpretation, samples whose spectra indicated them to be nearly pure  $\beta$ -forms gave low initial rotations in water, about  $[\alpha]_D +32^\circ$ , whereas a sample of nearly pure  $\alpha$ -anomer (m.p. 148–150°, obtained by recrystallizing from acetic acid as described by Reeves, Coulson, Hernandez & Blouin, 1957), gave an initial rotation of  $[\alpha]_D^{14} +87.8^\circ$  in water (*c*, 1.3). Mixtures of the  $\alpha$ - and  $\beta$ -anomers gave inter-

mediate rotations. Spectra identical with the three forms of the natural acetylglucose were given by samples of the acetylglucose prepared from glucose and 50% (v/v) acetic acid (Duff, 1957). This supports the belief that the spectra correspond to three modifications of the same substance.

Absorption bands of the acetyl group (Bellamy, 1954) appear in all spectra. The band due to the C—O—C stretching vibration, at  $7.87\ \mu$  in the  $\beta$ -anomers and  $7.92\ \mu$  in the  $\alpha$ -anomer, is at lower wavelengths than the composite band given by more fully acetylated sugars, which appears near  $8.1\ \mu$  in the solid state (Kuhn, 1950), and at about  $8.2\ \mu$  in  $\text{CCl}_4$  solution, where it is associated with an inflexion or subsidiary maximum at  $8.0\ \mu$  (Isbell *et al.* 1957). Fully acetylated sugars absorb at  $7.3\ \mu$  in solution (Isbell *et al.* 1957) due to a deformation vibration of their methyl groups. There is a band at this wavelength in all three forms of 6-O-acetylglucose. The  $\beta$ II form has an equally strong band at  $7.41\ \mu$ , which probably also arises from this deformation vibration by splitting due to the action of crystal forces. The carbonyl-stretching vibration of the acetyl groups, at  $5.84\ \mu$  for the  $\beta$ -anomers and at  $5.77\ \mu$  for the  $\alpha$ -anomer, absorbs at longer wavelengths, owing to inter- or intramolecular hydrogen bonding, than does an un-bonded ester carbonyl vibration which generally absorbs near  $5.7\ \mu$ .

*Examination of products of acid hydrolysis.* The mixture obtained by hydrolysis ( $1.5N\text{-H}_2\text{SO}_4$  for 4 hr. at  $100^\circ$ ) was examined on the paper chromatogram after removal of acid with barium carbonate. The presence of glucose was indicated by its  $R_f$  value and was confirmed by epimerization with calcium hydroxide for 14 days and subsequent examination of the products by paper chromatography (Hough *et al.* 1950).

Glucosazone (m.p.  $208^\circ$  alone or mixed with authentic material) was obtained from another portion of the hydrolysate in a yield (49.6%) comparable with that obtained directly from an equivalent amount of glucose (46.5%).

To identify the acidic portion of the ester, hydrolysis was normally carried out with *p*-toluenesulphonic acid as in the quantitative method (Duff, 1957). In one experiment the volatile acid was neutralized exactly with  $0.1N\text{-NaOH}$  and the solution evaporated to dryness. X-ray-powder photography of the residual solid showed that anhydrous sodium acetate was the only component. One spot only was obtained on a paper chromatogram prepared generally according to the method of Fewster & Hall (1951), and this was indistinguishable from the spot obtained by chromatography of sodium acetate. Duclaux numbers were determined generally as described by Morton (1938). The numbers, in this case based on the total

acid collected in the first 10 portions (10 ml. each) of distillate, when the volume of solution originally distilled was 110 ml., were: 6.0, 7.0; 14, 14; 21, 22; 29, 29; 37, 38; 47, 48; 58, 57; 69, 68; 85, 81 for authentic acetic acid and for a sample of the volatile acid from acetylglucose respectively. The lack of agreement in the last numbers is probably due to unavoidable differences in the rate of heating with the simple apparatus used. Apart from this there seems no doubt that the volatile acid is acetic acid.

The glucose content of the ester was determined after hydrolysis with  $1.5N\text{-H}_2\text{SO}_4$  as previously described. With these conditions about 2% of the sugar is destroyed (Pirt & Whelan, 1951) so that the proportion of sugar indicated by a copper-reduction method (79.7%) agrees with the theoretical value (81.5%).

*Reducing properties.* The material showed reducing properties on a paper chromatogram with the aniline phthalate and ammoniacal silver nitrate reagents (Partridge, 1946, 1949).

Reduction with sodium borohydride by the method of Lemieux & Bauer (1953), and examination of the products on the paper chromatogram revealed a component with  $R_f$  value 0.28 (i.e. approximately the same as glucose acetate). This substance reacted with the borax-phenol red reagent for polyols (Hockenfull, 1953) but did not reduce ammoniacal silver nitrate nor form a coloured spot with aniline phthalate.

*Determination of carbonyl by the Kiliani reaction.* The quantitative application of the Kiliani reaction described by Frampton, Foley, Smith & Malone (1951) was used. The nitrile was formed in buffered solution at pH 8.5 and 7.5. At this latter value the reaction is only about 40% complete but there is less loss of the acetyl group by hydrolysis (Table 1). The ammonia obtained by alkaline hydrolysis of the nitrile was estimated.

At pH 8.5 glucose and acetylglucose gave 0.96 and 1.0 mol. prop., and at pH 7.5, 0.36 and 0.44 mol. prop. of  $-\text{CHO}$  respectively.

At pH 8.5 galactose and 6-O-acetylgalactose (Duff, 1957) gave 1.0 and 1.0 mol. prop. of  $-\text{CHO}$  respectively. Xylose and acetylxylose (Duff, 1957) gave 1.0 and 0.80 mol. prop. of  $-\text{CHO}$  respectively.

*Determination of carbonyl by reaction with hydroxylamine hydrochloride.* In this case the proportion of carbonyl in glucose and in acetylglucose was determined at the slightly acid conditions (pH 5.2) required for condensation with hydroxylamine. The determination was carried out generally as described by Gladding & Purves (1943). These authors recommend that the hydrochloric acid liberated in the buffered solution ( $\text{NH}_2\text{OH}\cdot\text{HCl}-\text{NaOH}$ ) should be titrated with  $0.5N$ -alkali, but it was found possible to use  $0.13N$ -alkali with a

Table 1. *Hydrolysis of acetylglucose*

Acetylglucose (about 4 mg.) was dissolved in 0.1M-phosphate buffer or in acid (5 ml.) and the proportion of ester present determined by the colorimetric method initially and after a suitable period. The pH values were arranged to correspond with those used in the determination of carboxyl by the modified Kiliani reaction and with hydroxylamine, also with the preparation of the phenyl-osazone.

Medium	pH	Temp.	Time of hydrolysis (hr.)	Hydrolysis (%)
Buffer	8.5	37°	3	18
Buffer	7.5	37	3	0
Buffer	7.5	18	24	0
Buffer	5.2	100	1.5	13
Buffer	5.2	18	24	0
Buffer	3.1	100	0.3	0
N-H <sub>2</sub> SO <sub>4</sub>	—	28	4	50

corresponding increase in accuracy when the solution was continuously stirred and the end point (at pH 3.2) was indicated by a pH meter and glass electrode.

After 18 hr. glucose (0.1402 g.) and acetylglucose (0.1671 g.) liberated acid corresponding to 1.11 and 1.15 mol.prop. of -CHO respectively. Galactose and 6-*O*-acetylgalactose (Duff, 1957) gave 0.95 and 0.95 mol.prop. of -CHO respectively. Xylose and acetylxylose (Duff, 1957) gave 0.95 and 1.1 mol.prop. of -CHO respectively.

*Formation of a phenylhydrazone.* Distilled phenylhydrazine (0.56 g., 1 mol.prop.) in methanol (10 ml.) was added to acetylglucose (1 g.) and the *phenylhydrazone* isolated in the usual way and recrystallized from methanol: m.p. 134–136°;  $[\alpha]_D^{20} - 13^\circ$  in water (c, 1.3) (Found: CH<sub>3</sub>·CO, 12.9; N, 8.60. C<sub>14</sub>H<sub>20</sub>O<sub>6</sub>N<sub>2</sub> requires CH<sub>3</sub>·CO, 13.8; N, 8.97 %).

*Formation of a phenyl-osazone.* When heated for 30 min. with a solution of phenylhydrazine acetate buffered at pH 5.8 only glucosazone was obtained (in small yield). The procedure of Levene, Meyer & Raymond (1931) gave about 25 % of the theoretical yield of an *osazone* (Found: CH<sub>3</sub>·CO, 9.9. C<sub>20</sub>H<sub>24</sub>O<sub>6</sub>N<sub>4</sub> requires CH<sub>3</sub>·CO, 10.8 %) with an infrared spectrum very similar to that of glucosazone but with an additional band at 5.79 μ corresponding to an acetyl carbonyl group.

*Attempted preparation of a trityl ether.* The method of Reynolds & Evans (1938) was used. Several experiments were performed with 1–3 g. amounts of ester or glucose and 1.1 mol.prop. of trityl chloride in dry pyridine followed by acetic anhydride. With glucose a reasonable yield of crystalline 1:2:3:4-tetra-*O*-acetyl-6-*O*-trityl-β-D-glucose was obtained but in no case was a trityl derivative obtained from acetylglucose.

#### *Synthesis of 6-O-acetyl-D-glucopyranose*

*6-O-Acetyl-1:2-isopropylidene glucose.* This was prepared by the method of Bell (1936) via the 1:2-isopropylidene-3:5-boric ester. Direct hydrolysis of the former substance with 0.5N-sulphuric acid was unsatisfactory in that the 6-*O*-acetyl-D-glucopyranose obtained (in poor yield) did not crystallize although it was chromatographically identical with the natural ester.

*6-O-Acetyl-1:2:3:5-di-O-isopropylidene glucose.* This was prepared from the mono-isopropylidene derivative (2.04 g.) by the method of Macphillamy & Elderfield (1939). The syrupy product (2.25 g.) was distilled twice in high vacuum (0.005 mm. Hg) at 140° and had  $[\alpha]_D^{18} + 36^\circ$  in CHCl<sub>3</sub> (c, 1.03) [Found: (CH<sub>3</sub>)<sub>2</sub>·C, 31.0; CH<sub>3</sub>·CO, 12.0. Calc. for C<sub>14</sub>H<sub>21</sub>O<sub>7</sub>, (CH<sub>3</sub>)<sub>2</sub>·C, 27.9; CH<sub>3</sub>·CO, 14.2 %]. Ohle & Varga (1929) recorded  $[\alpha]_D + 33^\circ$  for their product, obtained via 1:2:3:5-di-*O*-isopropylidene 6-*O*-*p*-toluenesulphonyl-D-glucose. Natural 6-*O*-acetyl-D-glucose (1 g.) was converted into the di-*O*-isopropylidene derivative (1.2 g.) similarly. The syrup obtained had  $[\alpha]_D^{18} + 29.0$  in CHCl<sub>3</sub> (c, 1.08). Its infrared spectrum (capillary film) differed from that of the synthetic material only in having additional absorption at 13.19 and 11.49 μ, presumably due to an impurity.

*6-O-Acetyl-D-glucopyranose.* 6-*O*-Acetyl-1:2:3:5-di-*O*-isopropylidene-D-glucose (0.4 g.) in ethanolic solution was treated with 10 ml. and then 20 ml. of 0.1N-H<sub>2</sub>SO<sub>4</sub> and left at room temperature for 3 hr. and then 6 hr. Examination by the paper chromatograms (benzidine-trichloroacetic acid spray) showed that the material had not been hydrolysed by these treatments. Accordingly the acid concentration was raised to 0.5N by the addition of 2N-acid. After 80 hr. at room temperature sulphuric acid was removed with barium carbonate and the supernatant solution evaporated to a syrup *in vacuo* at 25°. Chromatography on a cellulose column (40 cm. × 2.0 cm.) gave bulked fractions containing 14.6 and 36.4 mg. of a mixture (acetylglucose-glucose) and of acetylglucose respectively. The remaining fractions contained mono- and di-*O*-isopropylidene-6-*O*-acetylglucose. The fraction containing only acetylglucose crystallized and had  $[\alpha]_D^{14} + 47.6$  at equilibrium in water (c, 1.3) in good agreement with the value obtained for the natural ester. The infrared spectrum (Fig. 2) was virtually identical with that of the natural material, in the βI form (Fig. 1). The *R<sub>f</sub>* values of the natural and synthetic material were identical when run in four different solvent systems [butanol saturated with water, butanol-acetic acid-water (4:1:5, by vol.), phenol-ethanol-water (3:1:1, w/v/v), and ethyl acetate-acetic acid-water (3:2:3, by vol.)].

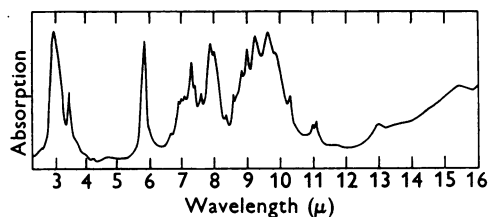


Fig. 2. Infrared spectrum of 6-O-acetyl-D-glucopyranose prepared from 6-O-acetyl-1:2:3:5-di-O-isopropylidene-D-glucose.

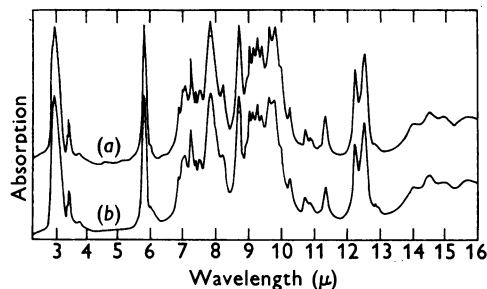


Fig. 3. Infrared spectra of 6-O-acetyl-D-galactopyranose: (a) prepared by acetylation of galactose with 50% acetic acid; (b) prepared from 6-O-acetyl-1:2:3:4-di-O-isopropylidene-D-galactose.

#### Synthesis of 6-O-acetyl-D-galactopyranose

Acetylation of diacetone galactose with sodium in dry ether followed by acetyl chloride (cf. Freudenberg & Smeykal, 1926) gave 6-O-acetyl-1:2:3:4-di-O-isopropylidene-D-galactose (Ohle & Berend, 1925). The isopropylidene groups from this (2.36 g.) were removed by heating at 55° with oxalic acid in 50% ethanol (0.2N, 1250 ml.) for 3 hr. (modified from the method of Levene & Tipson, 1936). Oxalic acid was removed with calcium carbonate and the filtrate evaporated *in vacuo* at 25° to a syrup. Paper chromatography showed that galactose, acetylgalactose and mono-isopropylidene acetylgalactose were the main components. Separation on a cellulose column gave a crystalline bulked fraction (0.34 g.) containing only 6-O-acetyl-D-galactose. The infrared spectrum did not clearly indicate a pyranose structure (Barker *et al.* 1954; Barker & Stephens, 1954), but such a structure seems likely from the method of preparation. The ester had m.p. 132–135° alone or mixed with material prepared by preferential acetylation of galactose with 50% (v/v) acetic acid (Duff, 1957), and the infrared spectra were virtually identical (Fig. 3). The  $R_f$  values on the paper chromatogram (0.25) were identical:  $[\alpha]_D^{25} + 66.9^\circ$  at equilibrium in water ( $c$ , 5.1) (Found:  $\text{CH}_3 \cdot \text{CO}$ , 19.3;  $\text{C}_8\text{H}_{14}\text{O}$ , requires  $\text{CH}_3 \cdot \text{CO}$ , 19.4%).

#### Synthesis of 3-O-acetyl-D-glucose

3-O-Acetyl-1:2:5:6-di-O-isopropylidene-D-glucose (4.25 g., prepared generally according to Fischer & Noth, 1918) was hydrolysed as usual with 0.2N-oxalic acid (2000 ml.). After separation on a cellulose column (65 cm.  $\times$  2.6 cm.) a glass (0.37 g.) was obtained. This had  $[\alpha]_D^{25} + 46^\circ$  at equilibrium in water ( $c$ , 4.3). This gave a single spot with moderate 'trailing' when applied directly to the chromatogram ( $R_f$  0.33), but on keeping in water an isomerization occurred and two spots,  $R_f$  0.28 and 0.33, were obtained in approximately equal concentration. The former spot may correspond to 6-O-acetylglucose formed by transfer of an acetyl group via an ortho ester as recorded by Josephson (1929), who found that 3-O-acetyl-1:2-isopropylidene-D-glucose was converted into the 6-O-acetyl derivative when held in solution.

#### SUMMARY

1. The ester isolated from cultures of *Bacillus megaterium* (NCIB 8508) has been characterized as 6-O-acetyl-D-glucopyranose because of the following properties: (a) It has reducing properties and forms a phenylhydrazone. (b) It has 1 mol. prop. of -CHO (determinations were carried out on either side of pH 7.0 by different methods). (c) It forms a phenylosazone. (d) It does not form a trityl ether.
2. The hydrolysis of 6-O-acetyl-D-glucopyranose is described under various conditions of pH and temperature.
3. 6-O-Acetyl-D-glucopyranose and the corresponding galactose ester have been synthesized by methods designed to illustrate their structure, i.e. via the known di-O-isopropylidene ethers of the parent sugars.
4. The unstable syrupy 3-O-acetyl-D-glucose is similarly prepared.

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#### REFERENCES

- Barker, S. A., Bourne, E. J., Stephens, R. & Whiffen, D. H. (1954). *J. chem. Soc.* p. 3468.  
 Barker, S. A. & Stephens, R. (1954). *J. chem. Soc.* p. 4550.  
 Bell, D. J. (1936). *J. chem. Soc.* p. 859.  
 Bellamy, L. J. (1954). In *The Infrared Spectra of Complex Molecules*, pp. 22, 161. London: Methuen and Co. Ltd.  
 Duff, R. B. (1957). *J. chem. Soc.* p. 4730.  
 Duff, R. B. & Webley, D. M. (1958). *Biochem. J.* **70**, 520.  
 Duff, R. B., Webley, D. M. & Farmer, V. C. (1957). *Nature, Lond.*, **179**, 103.  
 Fewster, M. E. & Hall, D. A. (1951). *Nature, Lond.*, **168**, 78.

- Fischer, E. & Noth, H. (1918). *Ber. dtsh. chem. Ges.* **51**, 321.
- Frampton, V. L., Foley, L. P., Smith, L. C. & Malone, J. G. (1951). *Analyt. Chem.* **23**, 1244.
- Freudenberg, K. & Smeykal, K. (1926). *Ber. dtsh. chem. Ges.* **59**, 100.
- Gladding, E. K. & Purves, C. B. (1943). *Paper Tr. J.* **116** (no. 14), 26.
- Helferich, B. (1948). In *Advances in Carbohydrate Chemistry*, vol. 3, p. 79. New York: Academic Press Inc.
- Hockenhull, D. J. D. (1953). *Nature, Lond.*, **171**, 982.
- Hough, L., Jones, J. K. N. & Wadman, W. H. (1950). *J. chem. Soc.* p. 1702.
- Isbell, H. S., Smith, F. A., Creitz, E. C., Frush, H. L., Moyer, J. D. & Stewart, J. E. (1957). *J. Res. nat. Bur. Stand.* **59**, 41.
- Josephson, K. (1929). *Liebigs Ann.* **472**, 217.
- Kuhn, L. P. (1950). *Analyt. Chem.* **22**, 276.
- Lemieux, R. U. & Bauer, H. F. (1953). *Canad. J. Chem.* **31**, 814.
- Levene, P. A., Meyer, G. M. & Raymond, A. L. (1931). *J. biol. Chem.* **91**, 497.
- Levene, P. A. & Tipson, R. S. (1936). *J. biol. Chem.* **115**, 731.
- Macphillamy, H. B. & Elderfield, R. C. (1939). *J. org. Chem.* **4**, 150.
- Morton, A. A. (1938). In *Laboratory Technique in Organic Chemistry*, p. 131. International Chemical Series. New York: McGraw Hill.
- Ohle, H. & Berend, G. (1925). *Ber. dtsh. chem. Ges.* **58**, 2585.
- Ohle, H. & Varga, L. (1929). *Ber. dtsh. chem. Ges.* **62**, 2425.
- Partridge, S. M. (1946). *Nature, Lond.*, **158**, 270.
- Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
- Pirt, S. J. & Whelan, W. J. (1951). *J. Sci. Fd Agric.* **2**, 224.
- Reeves, R. E., Coulson, R. A., Hernandez, T. & Blouin, F. A. (1957). *J. Amer. chem. Soc.* **79**, 6041.
- Reynolds, D. D. & Evans, W. L. (1938). *J. Amer. chem. Soc.* **60**, 2559.
- Schiedt, U. & Reinwein, H. (1952). *Z. Naturf.* **7b**, 270.

## Metabolism of 6-O-Acetyl-D-Glucopyranose and other Monoacetyl-sugars by Strains of *Bacillus megaterium* and other Soil Organisms

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The discovery that 6-O-acetyl-D-glucopyranose is a major metabolite of a cobalamin-producing strain of *Bacillus megaterium* (NCIB 8508) has been reported (Duff, Webley & Farmer, 1957). The present paper describes the production of the ester by growing and resting cells of this strain (and others) and the occurrence of various enzyme systems in cell-free extracts, including a specific 6-O-acetyl-D-glucose deacetylase.

The production of an acetylated sugar by an organism that synthesizes cobalamin is of particular interest in that the vitamin itself contains acetyl and ribose residues. Cobalamin production has been reported in 10 of the 14 strains of *B. megaterium* used in this work (Garibaldi, Ijichi, Snell & Lewis, 1953), but only three produced acetylglucose. The strain NCIB 8508, however, produced the greatest amount of both metabolites. Acetylglucose is a major product of this strain, as up to 25% of the glucose used appears as the ester in growth experiments.

This work represents the first isolation of an acetylated monosaccharide from natural sources although acetylated polysaccharides have been found in nature (e.g. in the gum from *Sterculia setigera*: Hirst, Hough & Jones, 1949).

### MATERIALS AND METHODS

*Organisms.* The cobalamin-producing strain of *B. megaterium* mainly used was obtained from the National Collection of Industrial Bacteria (NCIB 8508). A full description of its characteristics is given by Garibaldi *et al.* (1953). It is referred to by these workers as *B. megaterium* NRRL B-938. Also tested were two other strains of *B. megaterium* from the NCIB (2605 and 7581), and 11 from Dr N. R. Smith's collection (NRS strains) obtained by courtesy of Dr Ruth Gordon, Institute of Microbiology, Rutgers University, U.S.A.

The cultures were maintained as spores formed after growth on nutrient-agar slopes. These stocks were kept at 2° and cultures were restarted from them when required by subculture on to glucose-nutrient-agar slopes or plates.

Unless otherwise stated, the strain numbers in the text refer to the NCIB strains of *B. megaterium*.

*Large-scale growth experiments.* These were carried out on a shaking machine (Webley & Duff, 1955) in a constant temperature room at 25° in 1 l. Pyrex culture flasks (no. 1410), each containing 200 ml. of liquid medium. The composition of the medium used, unless otherwise stated, was as follows: glucose, 50 g.; KH<sub>2</sub>PO<sub>4</sub>, 0.5 g.; K<sub>2</sub>HPO<sub>4</sub>, 0.5 g.; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.5 g.; KCl, 0.2 g.; FeSO<sub>4</sub>, 0.02 g.; CoCl<sub>2</sub>·6H<sub>2</sub>O, 0.012 g.; (NH<sub>4</sub>)HPO<sub>4</sub>, 1 g.; yeast extract (Difco), 2 g.; CaCO<sub>3</sub>, 10 g.; 1 l. of water. The CaCO<sub>3</sub> was sterilized separately in the culture flasks with 5 ml. of water. The mineral salts + yeast extract and the glucose