JOURNAL OF BACTERIOLOGY, Mar. 1983, p. 1388–1393
0021-9193/83/031388-06$02.00/0

Vol. 153, No. 3

Isolation, Identification, and Synthesis of 2,3-Diamino-2,3-Dideoxyglucuronic Acid: a Component of *Propionibacterium acnes* Cell Wall Polysaccharide

C. S. CUMMINS[1]* AND R. H. WHITE[2]

Department of Anaerobic Microbiology[1] and Department of Biochemistry,[2] Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Received 18 October 1982/Accepted 27 December 1982

A previously undescribed component of the cell wall polysaccharide of *Propionibacterium acnes*, 2,3-diamino-2,3-dideoxyglucuronic acid, has been identified and synthesized. The component occurs to the extent of about 3 to 5% in the wall polysaccharides of *P. acnes* types I and II and in *Propionibacterium avidum* types I and II; it also appears to be present, but in much smaller amounts, in the cell wall of *Propionibacterium granulosum*.

Like most gram-positive organisms, *Propionibacterium acnes* has a cell wall which is composed largely of polysaccharide and peptidoglycan. In the case of *P. acnes* and the related species *Propionibacterium avidum* and *Propionibacterium granulosum*, the cell wall polysaccharide is known to contain some combination of the hexoses glucose, galactose, and mannose, together with the hexosamines glucosamine and galactosamine (7, 8, 17). However, the detailed structure has not been investigated, and since the polysaccharide is acidic but has negligible phosphate content (C. S. Cummins, unpublished data), there are obviously other components present.

In examining acid hydrolysates of the cell wall polysaccharide from *P. acnes* to determine the hexosamine composition, an unusual spot was observed on thin-layer chromatography plates, giving a bright blue color with the ninhydrin reagent. This material has been isolated and identified as 2,3-diamino-2,3-dideoxyglucuronic acid.

Unknown material from *P. acnes* polysaccharides giving blue spots with ninhydrin has been reported previously (1, 2). However, beyond establishing that the material was probably carbohydrate in nature, it was not identified further.

Acidic polysaccharides released into the medium by *P. acnes* during growth have been described by Dawes et al. (12) and Belsheim et al. (4), but neither group reported finding a constituent similar to the one described here.

MATERIALS AND METHODS

Strains of bacteria. *P. acnes* 0009 and 6637, *P. granulosum* 0507, and *P. avidum* 0575 and 0589 were from the culture collection of the Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg.

Growth of bacteria and preparation of cell walls. Bacteria were grown for 48 h under anaerobic conditions in a Trypticase (BBL Microbiology Systems)-yeast extract-glucose medium as described by Cummins and Johnson (10). Cultures were killed by heating at 56°C for 30 min, centrifuged, washed, and lyophilized. Cell walls were prepared as described by Cummins and Johnson (9). After thorough washing in distilled water, the cell walls were lyophilized and stored at −15°C.

Extraction of polysaccharide. Cell walls were extracted with 5% trichloroacetic acid to liberate polysaccharide: as an example, 200 mg of cell walls was extracted with 20 ml of 5% trichloroacetic acid for 30 min at 56°C with constant stirring. After centrifugation, the residual walls were extracted twice more under the same conditions. The three supernatants were pooled, 5 volumes of acetone and a few crystals of potassium acetate were added, and the polysaccharide was allowed to precipitate overnight at 4°C. The precipitate was recovered by centrifugation, redissolved in the minimal amount of distilled water, and dialyzed (8,000 to 10,000 molecular weight cutoff) against several changes of distilled water. The final solution from the dialysis sac was filtered through a 0.45-μm filter and lyophilized. The yield was generally 20 to 25% of the weight of the wall.

Separation of 2,3-diamino-2,3-dideoxyglucuronic acid from other components of the polysaccharide. Polysaccharide (10 mg) was hydrolyzed in 4 ml of 6 N HCl for 18 h at 100°C, and the solution was filtered and evaporated to dryness under a heat lamp. The material was redissolved in 0.5 ml of distilled water and centrifuged to remove insoluble material, and the solution was applied to a 4.0- by 1.0-cm column of Dowex 50W-×12 (200 to 400 mesh) in H^+ form. The column was washed with 10 ml of distilled water, and the washings were discarded. A linear gradient of HCl from 0 to 2.0 N was applied to the column, and 2,3-

dideoxyglucuronic acid was eluted between 0.2 and 0.3 N HCl, shortly after glucosamine and galactosamine.

High-voltage electrophoresis. High-voltage electrophoresis was done in a Pherograph apparatus (type 64; Brinkmann Instruments Inc.). Whatman no. 1 paper was used, with the following buffers: pH 2.0, 150 ml of glacial acetic acid, 50 ml of formic acid, and distilled water to 1,000 ml; pH 3.5, 100 ml of glacial acetic acid, 10 ml of pyridine, and distilled water to 1,000 ml; pH 6.0, 100 ml of pyridine, 10 ml of glacial acetic acid, and distilled water to 1,000 ml. The normal run was for 1 h at about 2,000 V and 25 to 35 mA, depending on the buffer.

Thin-layer chromotography. (i) One dimensional. Avicel microcrystalline cellulose plates (20 by 20 cm; 250 μm thick; Analtec Inc., Newark, Del.) were used. The solvent system was pyridine-ethyl acetate-acetic acid-water (36:36:7:21 [vol/vol]). Separation was by ascending chromatography, with two successive ascents in the solvent; between ascents, the plates were dried for 15 min in a current of air. Spots were visualized by spraying the plates with a mixture of 2,4,6-trimethylpyridine (2 ml) and acetic acid (15 ml) in 0.1% ninhydrin in 95% ethanol (50 ml) and heating at 90°C for 3 min (6). Reducing substances were detected by the alkaline silver method of Trevelyan and Harrison (31).

(ii) Two dimensional. Two-dimensional chromatography was also done on Avicel 20- by 20-cm plates, with the following solvents: solvent A, 1-butanol-acetic acid-water (180:45:75 [vol/vol]); solvent B, phenol-water-NH_4OH (166 ml of 88% phenol, 34 ml of water, 1 ml of 30% NH_4OH). Each solvent was run up to about 1 cm from the top of the plate. After development in solvent A, the plate was thoroughly dried (1 h in a current of air) and then run in the other dimension in solvent B. The phenol solvent was removed by washing the plate twice in acetone in a shallow dish before visualizing the spots with the ninhydrin-collidine reagent. Crystals of KCN were added to the tank containing solvent B to prevent oxidation.

Preparation of *n*-butyl trifluoroacetate derivatives. A 0.1- to 1-mg amount of the sample to be derivatized was placed in 1 ml of 3 M HCl in *n*-butanol (Burdick and Jackson Laboratories, Inc.) and heated at 100°C for 1 h. Then, with the sample maintained at 90°C, the *n*-butanol was removed by a stream of nitrogen. The resulting residue was cooled, and 0.5 ml of an equal mixture of trifluoroacetic anhydride and methylene chloride was added. After sitting overnight at room temperature, the residue was concentrated to dryness and placed in 50 μl of the same solvent for gas chromatographic or gas chromatographic-mass spectrometric analysis.

Gas chromatography and gas chromatography-mass spectrometry. A Varian MAT 112 gas chromatograph-mass spectrometer modified with a single-stage glass separator was used for the work described here. Separations of the derivatives for either gas chromatographic or gas chromatographic-mass spectrometric analysis were performed with 6-ft by 1/8-in. (ca. 182.88- by 0.318-cm) glass columns packed with one of the following: 3% OV-17 on 100/120 Supelcoport, or 3% SP-2100 or 10% SP-2100 on 80/100 Supelcoport (Supelco, Inc.).

All gas chromatography was done by temperature programming. The *n*-butyl trifluoroacetyl derivatives were separated on the 10% SP-2100 column programmed from 160 to 300°C at 8°C per min and on the 3% OV-17 and 3% SP-2100 columns programmed from 100 to 300°C at 10°C per min. The trimethylsilyl (TMS) derivatives were separated on the 3% OV-17 column programmed from 200 to 300°C at 8°C per min.

All mass spectra were recorded at 70 electron volts (eV) with an ion source temperature of 250°C. Isobutane was used as the reagent gas to obtain the chemical ionization mass spectra.

Preparation of 2,3-diamino-2,3-dideoxyglucuronic acid. A 100-mg amount of 2,3-diacetamido-2,3-dideoxy-D-glucose (U.S. Biochemical Corp.) was placed in 10 ml of 1.5 M HCl in CH_3OH and held at 60°C for 2 h. The methanol and HCl were then removed in a vacuum. The resulting methyl glucosides were dissolved in 5 ml of water and adjusted to pH 8 to 9 with a saturated sodium bicarbonate solution. A 200-mg amount of 5% platinum on carbon (Engelhard Industries, Newark, N.J.) was added, and the contents were oxygenated at 90°C by a fine stream of oxygen gas. The gas was added at such a rate that the catalyst was kept completely suspended in the medium. The reaction was conducted with a reflex condenser attached to the flask to reduce water loss. The pH of the solution, which drops during the reaction, was brought back to 8 to 9 by the addition of $NaHCO_3$. At the completion of the reaction, the catalyst was removed by filtration, and the resulting solution of methyl 2,3-diacetamido-2,3-dideoxyglucuronide was made 6 M in HCl by the addition of concentrated HCl. This material was then heated for 2 h at 110°C and dried in a vacuum. The residue, after solution in water, was purified by chromatography on a Dowex 50 H^+ column as described above. The fractions containing the desired product were dried in vacuo and crystallized from EtOH-H_2O mixtures.

RESULTS

Gas chromatography of the *n*-butyl trifluoroacetyl derivative of the unknown compound gave a different pattern of peaks depending on the chromatographic phase used for the separation. In each case, a major peak was the first to elute, followed by one or more smaller peaks. With the first peak assigned an intensity of 100, the minor peaks found with various phases were as follows: OV-17, one minor peak, intensity 49; 3% SP-2100, two minor peaks, intensities 33 and 14; 10% SP-2100, three minor peaks, intensities ~9, ~23, and 28. In every case, the smaller peaks appeared as a cluster of unresolved peaks which were well separated from the major peak. Gas chromatography-mass spectrometry of the peaks showed each of them to give almost identical mass spectra, indicating that they were isomers of a common compound. The generation of four peaks giving similar mass spectra from a single compound when using the *n*-butyl trifluoroacetyl derivatives is a general characteristic observed when assaying derivatives of aldoses or 2-amino-2-deoxyaldoses. The four peaks originate from the α and β anomers of the

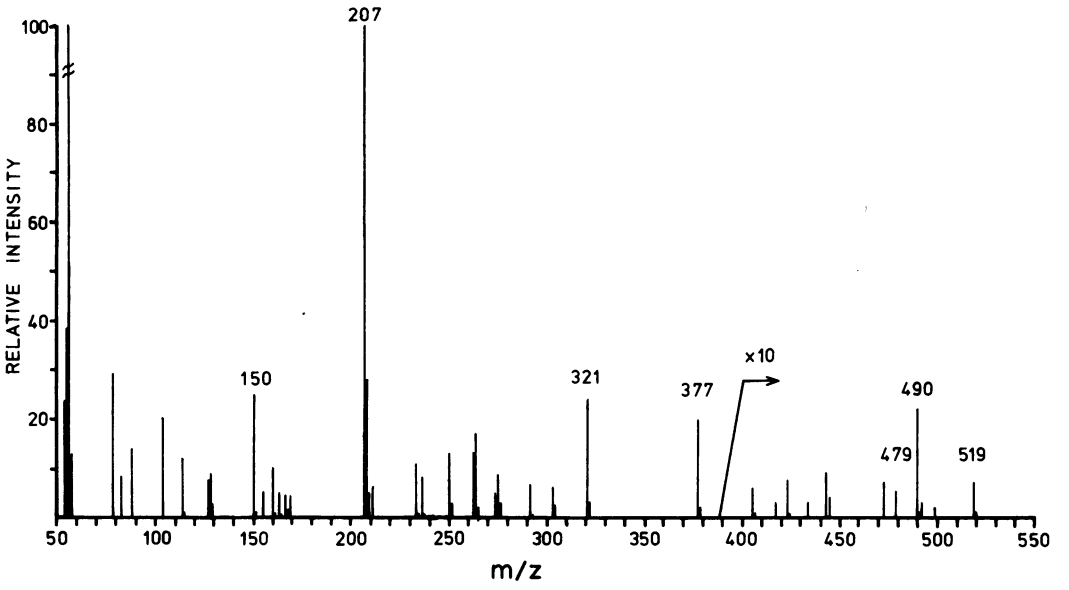

FIG. 1. Mass spectrum of the *n*-butyl trifluoroacetyl derivative of 2,3-diamino-2,3-dideoxy-D-glucuronic acid.

n-butyl furanoside and pyranoside isomers of the sugar. This is a general characteristic observed during the preparation of derivatives from equilibrium mixtures of free sugars (20, 30), glycosides (26), 2-amino-2-deoxyaldohexoses (18), and 2-amino-2-deoxyaldoglycosides (32). That the amount of *n*-butyl glycosides observed reflected an equilibrium mixture of glycosides was confirmed in that their relative intensities did not change on longer heating with the 3 M HCl in *n*-butanol. Both the intensities of these peaks and their retention times are definitive characteristics of a given sugar (30). As a general rule, the α-pyranoside derivatives have the shortest retention times, indicating that the major peak is an α-*n*-butyl pyranoside (30). The mass spectrum of this peak is shown in Fig. 1. The highest mass ion seen in the 70-eV spectra is at *m/z* 519. The mass spectrum, however, does not show any fragment ions which would indicate that this is the true molecular ion.

Assigning a molecular weight of *m/z* 592 to the derivative, however, would give the following fragment ions: $M^+ - C_4H_9O$, *m/z* 519; $M^+ - C_4H_9OOCH$, *m/z* 490; $M^+ - CF_3COO$, *m/z* 479; $M^+ - C_4H_9OOC - CF_3COOH$, *m/z* 377; and $M^+ - C_4H_9OOC - CF_3COO - C_4H_9$, *m/z* 321. To confirm this molecular weight, the chemical ionization spectra of the sample were obtained. They showed three major ions at MH^+, *m/z* 593; $MH^+ - C_4H_9OH$, *m/z* 519; and $MH^+ - (C_4H_9OH)_2$, *m/z* 445, confirming that the derivative has a molecular weight of 592. The fragments also show that the molecule contains two butyl groups, since a major ion at $MH^+ - (C_4H_9OH)_2$, *m/z* 445, was observed. This interpretation of the chemical ionization mass spectrum is supported by the CI mass spectrum of the *n*-butyl trifluoroacetyl derivative of glucosamine, which shows intense ions at MH^+, *m/z* 620, and $MH^+ - C_4H_9OH$, *m/z* 546, and no $MH^+ - (C_4H_9OH)_2$.

Since, based on its chromatographic characteristics and reaction with ninhydrin, the original molecule is known to contain amino groups, and since the derivative of the molecule has an even mass, we must conclude that the molecule has an even number of nitrogens. Assuming that the correct even number is two, and considering the observed molecular weight of the derivative and the chemical methods used in its preparation, there is only one logical structure which is consistent with the data, a diaminodideoxyhexuronic acid.

The position of the amino groups in the compound cannot be readily obtained from the mass spectral data of the derivative. However, gas chromatography of the derivative showed the presence of both furanoside and pyranoside forms of a sugar, which would strongly argue that the hydroxyl groups are in positions 4 and 5. If one of the amino groups had been in either position 4 or 5, this would have prevented formation of the furanoside or pyranoside isomer, respectively. This thus leaves only carbons 2 and 3 vacant for the two amino groups.

The fact that the compound failed to form a 3-6 lactone during the treatment with acidified *n*-butanol provides additional evidence for an amino group at C-3. Compounds with a free

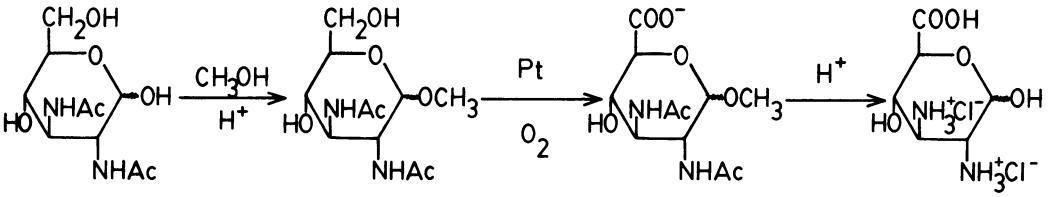

FIG. 2. Outline of the chemical synthesis of 2,3-diamino-2,3-dideoxy-D-glucuronic acid.

hydroxyl group at C-3, such as glucuronic acid, readily form a 3-6 lactone on acid treatment. This would be completely blocked by an amino group at C-3 in the same stereochemistry as the glucose C-3 hydroxyl group, since the amino group will not produce a cyclic lactam under the acidic conditions.

Considering the above information, the recent identification of 2,3-diamino-2,3-dideoxy-D-glucose in *Rhodopseudomonas* spp. (19, 27), and the identification of 2-amino-2-deoxyglucuronic acid (14, 15, 25, 28, 33), 2-amino-2-deoxymannuronic acid (24), and 2-amino-2-deoxygalacturonic acid (5, 21) in bacterial polysaccharides, it was concluded that the compound was 2,3-diamino-2,3-dideoxy-D-glucuronic acid.

To confirm the structure proposed for the unknown compound, it was compared to a synthetic sample of known structure prepared as outlined in Fig. 2. The synthesis began with 2,3-diacetamido-2,3-dideoxy-D-glucose, which is converted into a mixture of the α and β methyl glycosides by reaction with 1.5 M HCl in anhydrous methanol. (The progress of this reaction and the subsequent reaction steps were followed by gas chromatography of the TMS derivatives of the sugars on the 6-ft by 1/8-in. 3% OV-17 column.) The conversion of the sugar to the methyl glycosides was required to prevent oxidation of the C-1 carbon in subsequent steps. The TMS derivative of the starting material gave two peaks, with the first and most intense peak representing the $(TMS)_3$ derivative of the α isomer and the second peak representing the tri-TMS derivative of the β isomer. During the formation of the methyl glycoside, two new peaks appeared representing the α and β methyl glycosides. The mass spectra of these peaks showed both to have a $M^+ - 15$ ion at *m/z* 405, indicating that they represented the $(TMS)_2$ derivative of the glycosides. Catalytic oxidation of the glycoside mixture by oxygen in the presence of platinum on carbon was next used for the oxidation of the primary hydroxyl groups of the methyl glucosides. This is a very well characterized reaction and has been used extensively for the oxidation of the C-6 position in sugars (16). Several examples are known for the direct oxidation of both alkyl (3, 22) and aryl glycosides (23) to glucuronides.

The product of this reaction produced a single gas chromatographic peak with $M^+ - 15$ at *m/z* 434, which is that expected for the methyl TMS derivative of 2,3-diacetamido-2,3-dideoxy-D-glucuronic acid. This product had a longer retention time than either the starting materials or the methyl glycosides and presumably contains both the α and β anomers. Acid hydrolysis of the oxidized material then gave 2,3-diamino-2,3-dideoxy-D-glucuronic acid, which was purified by column chromatography on Dowex 50.

Apart from the identity of the mass spectra of the natural and synthetic materials, they both gave the same distinctive blue color with the ninhydrin-collidine reagent on thin-layer chromatography plates and ran to identical positions in one-dimensional and two-dimensional chromatography. The R_f values of 2,3-diamino-2,3-dideoxyglucuronic acid and some other amino sugars and amino acids are given in Table 1.

DISCUSSION

The presence of the unknown component eventually identified as 2,3-diamino-2,3-dideoxyglucuronic acid was originally noticed because of the presence of a bright blue spot in thin-layer chromatograms of acid hydrolysates of a cell wall polysaccharide from a *P. acnes* strain. These chromatograms were sprayed with a solu-

TABLE 1. R_f value of 2,3-diaminoglucuronic acid in comparison with that of other components of polysaccharide and peptidoglycan[a]

Substance	R_f relative to glucosamine
Muramic acid	1.14
Glucosamine	1.0
Galactosamine	0.87
Alanine	0.74
2,3-Diamino-2,3-dideoxyglucuronic acid (natural and synthetic)	0.74
Glutamic acid	0.43
Glycine	0.43
Aspartic acid	0.24
Lysine	0.21
Diaminopimelic acid	0.09

[a] Conditions: 20- by 20-cm Avicel cellulose plate; solvent, ethyl acetate-pyridine-acetic acid-water; two ascents, each to within 3 cm of top of plate.

tion of 0.1% ninhydrin in 95% acetone and heated at 90 to 100°C for about 3 min. In typical form, the unknown spot was bright sky blue in color, resembling that given by aspartic acid under favorable conditions.

There was, in fact, an element of serendipity attached to the original discovery of the substance, since the bright blue color was subsequently found to be difficult to elicit under the original conditions. However, it can regularly be produced by using the ninhydrin-acetic acid-collidine mixture described by Cowgill and Pardee (6). This was the reagent used by Adlam and Reid (1) when they described an apparently similar spot in their hydrolysates. In fact, Adlam and Reid described two unknown components, both giving a distinctive blue color, but we have only been able to detect one such spot in our material. Our original detection of the blue color may have been due to the presence of residual acetic acid and pyridine from the solvent in the cellulose layer of the plate.

The material was weakly reducing in comparison with the strength of its reaction with ninhydrin, and it gave only a faint spot in thin-layer chromatography by the alkaline silver technique. On high-voltage electrophoresis on paper (ca. 50 V/cm), the substance moved toward the cathode at pH 2.0, 3.5, and 6.0, indicating that it carried a net positive charge under these conditions. The R_f value was about 1.05, taking glucosamine as 1.00. It was noticed that the material gave a very diffuse spot after high-voltage electrophoresis at pH 2.0, although at the other pH values the spot was as compact as those given by the standard amino sugars.

Suitable methods for the estimation of the 2,3-diaminouronic acid have yet to be worked out; it appears to give little or no color with the carbazole reagent used for uronic acid estimation or with the *p*-dimethylaminobenzaldehyde reagent used for amino sugars. As judged by the strength of reaction with ninhydrin when compared with known amounts of glucosamine on thin-layer chromatography plates, it would appear to be present to the extent of 3 to 5% of the polysaccharide, but exact measurements will have to await the isolation of larger amounts of pure material.

The 2,3-diaminohexuronic acid component was originally detected in hydrolysates of a cell wall polysaccharide extracted from a strain of *P. acnes* serotype I (VPI 0009). Preliminary examinations of cell wall polysaccharides from other strains of skin propionibacteria indicate that the diaminohexuronic acid is also present in strains of *P. acnes* serotype II (VPI 6637) and in strains of both serotypes of *P. avidum* (VPI 0575 and 0589). It also appears to be present, but in much smaller amounts, in the polysaccharide from *P. granulosum* (VPI 0507). This is of interest because *P. avidum* strains show about 50% DNA homology with *P. acnes*, whereas strains of *P. granulosum* show only about 12 to 15% (17). Since *P. granulosum* is obviously much more distantly related to *P. acnes* than is *P. avidum*, it might be expected that the structure of its polysaccharide would be substantially different.

Although quite a wide variety of amino sugars have been described from bacterial polysaccharides (see, e.g., references 13 and 29), among them 2-amino-2-deoxyglucuronic acid (25), it does not seem that a diaminohexuronic acid has been described previously. The presence in the polysaccharide of *P. acnes* of a substance carrying acidic groups would explain satisfactorily the negative charge of the material. However, too little is known as yet about the general structure of these polysaccharides to determine what role, if any, the diaminohexuronic acid might play in the immunological reactions of the polysaccharide or in the reticulo-stimulatory properties shown by some strains of *P. acnes* (e.g., references 1 and 11).

ACKNOWLEDGMENTS

We acknowledge the valuable assistance of Cacia Shabdach, Steve Stimpson, and Paul Jackson in various stages of this investigation. We thank Kim Harich for the gas chromatographic-mass spectrometric analysis.

The work was funded by Public Health Service grant CA 24729 from the National Cancer Institute and by the Commonwealth of Virginia.

LITERATURE CITED

1. **Adlam, C., and D. E. Reid.** 1978. Comparative studies on the cell wall composition of some anaerobic coryneforms of varying lympho-reticular stimulatory activity, p. 115–120. *In* A. H. Griffiths and R. H. Regamey (ed.), Developments in biological standardization. S. Karger, Basel.
2. **Adlam C., D. E. Reid, A. J. Marshall, and P. Torkington.** 1979. Chemical and biological properties of a purified lympho-reticular stimulating fraction of *Corynebacterium parvum*. (*Propionibacterium acnes*, type 1). J. Med. Microbiol. 12:429–440.
3. **Barker, S. A., E. J. Bourne, and M. Stacey.** 1951. The synthesis of uronic acids. Chem. Ind. 1951:970.
4. **Belsheim, J., A. Dalen, H. Gnarpe, L. Hellgren, O.-J. Iversen, and J. Vincent.** 1979. The effect of acidic polysaccharides and prostaglandin-like substances isolated from *Propionibacterium acnes* on granulocyte chemotaxis. Experientia 35:1587.
5. **Clark, W. R., J. McLaughlin, and M. E. Webster.** 1958. An aminohexuronic acid as the principal hydrolytic component of the VI antigen. J. Biol. Chem. 230:81–89.
6. **Cowgill, R. W., and A. B. Pardee.** 1957. Determination of the structure of a peptide, p. 52, *In* Experiments in biochemical research techniques. Wiley, New York.
7. **Cummins, C. S.** 1975. Identification of *Propionibacterium acnes* and related organisms by precipitin tests with trichloroacetic acid extracts. J. Clin. Microbiol. 2:104–110.
8. **Cummins, C. S.** 1980. Serology of propionibacteria, p. 205–221. *In* D. W. Lambe, R. J. Genco, and K. J. Mayberry-Carson (ed.), Anaerobic bacteria, selected topics. Plenum Publishing Corp., New York.
9. **Cummins, C. S., and J. L. Johnson.** 1971. Taxonomy of

the clostridia: wall composition and DNA homologies in *Clostridium butyricum* and other butyric acid-producing clostridia. J. Gen. Microbiol. **80**:433–442.

10. **Cummins, C. S., and J. L. Johnson.** 1981. The genus *Propionibacterium*, p. 1894–1902. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. Springer-Verlag, New York.
11. **Cummins, C. S., S. A. Stimpson, R. L. Tuttle, and C. J. Weck.** 1981. Observations on the nature of the determinants in anaerobic coryneforms which produce splenomegaly. J. Reticuloendoth. Soc. **29**:1–13.
12. **Dawes, J., J. J. Tuach, and W. H. McBride.** 1974. Properties of an antigenic polysaccharide from *Corynebacterium parvum*. J. Bacteriol. **120**:24–30.
13. **Eveleigh, D.** 1980. Microbial monosaccharides and polysaccharides, p. 61–74. *In* A. I. Laskin and H. A. Lechevalier (ed.), Handbook of microbiology, vol. IV, 2nd ed. CRC Press, Boca Raton, Fla.
14. **Hanessian, S., and T. H. Haskell.** 1964. Structural studies on staphylococcal polysaccharide antigen. J. Biol. Chem. **239**:2758–2764.
15. **Haskell, T. H., and S. Hanessian.** 1963. 2-Amino-2-deoxy-D-glucuronic acid, the main constituent of SPA (Staphylococcal polysaccharide antigen). Nature (London) **199**:1075–1076.
16. **Heyns, K., and H. Paulsen.** 1962. Selective catalytic oxidation of carbohydrates employing platinum catalysts. Adv. Carbohydr. Chem. **17**:169–221.
17. **Johnson, J. L., and C. S. Cummins.** 1972. Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical propionibacteria and strains of *Arachnia propionica*. J. Bacteriol. **109**:1047–1066.
18. **Kärkkäinen, J., and R. Vihko.** 1969. Characterization of 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, and related compounds, as their trimethylsilyl derivatives by gas-liquid chromatography-mass spectrometry. Carbohydr. Res. **10**:113–120.
19. **Keilich, G., J. Roppel, and H. Mayer.** 1976. Characterization of a diaminohexose (2,3-diamino-2,3-dideoxy-D-glucose) from *Rhodopseudomonas viridis* lipopolysaccharides by circular dichroism. Carbohydr. Res. **51**:129–134.
20. **König, W., H. Bauer, W. Voeltu, and E. Bayer.** 1973. Gaschromatographic und Massenspektrometric trifluoracetylierten Kohlenhydrate. Chem. Ber. **106**:1905–1919.
21. **Liau, D., M. A. Melly, and J. H. Hash.** 1974. Surface polysaccharide from *Staphylococcus aureus* M that contains taurine, D-aminogalacturonic acid, and D-fucosamine. J. Bacteriol. **119**:913–922.
22. **Marsh, C. A.** 1952. The synthesis of conjugated hexuronic acids. J. Chem. Soc. **1952**:1578–1582.
23. **Marsh, C. A., and G. A. Levvy.** 1958. The synthesis of aryl glycosiduronic acids. Biochem. J. **68**:617–621.
24. **Mayer, H.** 1969. D-Mannosaminuronsäure-baustein des K7-antigens von *Escherichia coli*. Eur. J. Biochem. **8**:139–145.
25. **Perkins, H. R.** 1963. A polymer containing glucose and aminohexuronic acid isolated from the cell walls of *Micrococcus lysodeikticus*. Biochem. J. **86**:475–483.
26. **Reinhold, V. N.** 1972. Gas-liquid chromatographic analysis of constituent carbohydrates in glycoproteins. Methods Enzymol. **25**:244–249.
27. **Roppel, J., H. Mayer, and J. Wickessn.** 1975. Identification of a 2,3-diamino-2,3-dideoxyhexose in a lipid A component of lipopolysaccharides of *Rhodopseudomonas viridis* and *Rhodopseudomonas palustris*. Carbohydr. Res. **40**:31–40.
28. **Sandford, P. A., P. R. Watson, and A. R. Veanes.** 1973. An extracellular microbial polysaccharide composed of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucuronic acid: radiochemical and gas-chromatographic analysis of the products of methanolysis. Carbohydr. Res. **29**:153–164.
29. **Sharon, N.** 1965. Distribution of amino sugars in microorganisms, plants and invertebrates, p. 2–44. *In* R. W. Jeanloz and E. A. Balazs (ed.), The amino sugars, vol. IIA. Academic Press, Inc., New York.
30. **Sweeley, C. C., W. W. Wells, and R. Bentley.** 1963. Gas chromatography of carbohydrates. Methods Enzymol. **8**:95–108.
31. **Trevelyan, W. E., and J. S. Harrison.** 1952. Studies on yeast metabolism. I. Fractionation and microdetermination of cell carbohydrates. Biochem. J. **50**:299–303.
32. **Ueno, T., N. Kurihara, and M. Nakajima.** 1967. Synthetic studies of carbohydrate antibiotics. Part III. Gas-liquid chromatography of trifluoroacetyl derivatives of carbohydrates including cyclitols. Agric. Biol. Chem. **31**:1189–1194.
33. **Williamson, A. R., and S. Zamenhof.** 1963. The type-specific substance of *Hemophilus influenzae*, type d: the natural occurrence of glucosamine uronic acid. J. Biol. Chem. **238**:2255–2258.