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SYNTHESIS OF THE REPEATING DISACCHARIDE UNIT OF THE GLYCAN MOIETY OF THE BACTERIAL CELL WALL PEPTIDOGLYCAN.

C. Merser and P. Sinaÿ

Laboratoire de Biochimie Structurale, U.E.R. de Sciences Fondamentales et Appliquées, 45045-Orléans, France.

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The rigidity of the bacterial cell wall is insured by a complex polymer called the peptidoglycan[1]. The glycan moiety of this polymer is composed of alternating units of 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine) and 2-acetamido-3-O-(D-1-carboxyethyl)-2-deoxy-D-glucose (N-acetylmuramic acid). The repeating disaccharide unit was isolated[2] after egg-white lysozyme solubilization of this polymer. Its structure has been the subject of controversy. A 1 $\overset{\beta}{\rightarrow}$ 6 linkage between N-acetylglucosamine and N-acetylmuramic acid, first ascribed on the basis of periodate experiments and color reactions[3], was then denied by a synthesis of the disaccharide 2-acetamido-2-deoxy-β-D-glucopyranosyl (1 → 6) N-acetylmuramic acid[2]. It is generally considered since that time that the repeating disaccharide has most probably structure 1, but a final evidence of the 1 → 4 nature of the glycosidic linkage is still lacking.

The purpose of this note is to report the first total synthesis of 8, a nicely crystalline derivative of 1 and to show its identity with the acetylated methyl ester of the natural product, so that structure 1 is now certain for the repeating disaccharide unit.

The secondary hydroxylic position on carbon atom 4 of an hexopyranose with a $^4C_1$ chair form conformation is particularly unreactive when the remaining hydroxylic groups are acylated[4]. This has considerably hampered the chemical synthesis of 1 → 4 disaccharides in satisfactory yields.

In the case of disaccharides where the reducing unit is a neutral sugar, the problem has been solved by using, as a precursor of this unit, a derivative with a non $^4C_1$ chair form conformation, either a $^1C_4$ chair form[5] or an acyclic structure[6]. In the case of disaccharides where the reducing unit is a N-acetylglucosamine moiety, an acyclic derivative has been first used by Heyns et al[7], then by others[8].

We have recently shown[9] that compound 2- a nicely crystalline derivative conveniently prepared in seven steps from starch [m.p. 118-119° , $[\alpha]_D^{20}$ - 39° (c 1, chloroform)] - is, after acetylation of the hydroxylic position and hydrogenolysis to remove the benzyl group, an attractive precursor for the synthesis of 1 → 4 disaccharides. We think it might be of general applicability for the synthesis of 1 → 4 (α or β) disaccharides bearing N-acetylglucosamine at the reducing unit, some of them being biologically important.

The versatility of this derivative is now illustrated with the synthesis of 8. 2-Acetamido-1,6-anhydro-4-O-benzyl-2-deoxy-β-D-glucopyranose[9] (2) gave, after condensation with L-2-chloropropionic acid[10] in dioxan and in the presence of sodium hydride, followed by esterification with diazomethane, the 2-acetamido-1,6-anhydro-4-O-benzyl-2-deoxy-3-O-(D-1-(methoxycarbonyl) ethyl)-β-D-glucopyranose[11] (3) [45% from 2, m.p. 92-93°, $[\alpha]_D^{20}$ - 31,5° (c 1, chloroform)]. After catalytic hydrogenation in methanol (Pd/C 10%, 24 h), the 2-acetamido-1,6-anhydro-2-deoxy-3-O-(D-1-(methoxycarbonyl)ethyl)-β-D-glucopyranose (4) was obtained [88%, m.p. 142-143°, $[\alpha]_D^{20}$ - 27°C (c 1, chloroform)]. Condensation of 4 with the 3,4,6-tri-O-acetyl-2-deoxy-2-diphenoxyphosphorylamino-α-D-glucopyranosyl bromide[12] (5)(4 h at reflux in dry benzene in the presence of mercuric cyanide) gave, after chromatographic purification (silica gel column) the protected disaccharide 6 [15%, m.p. 203-204°, $[\alpha]_D^{20}$ - 29° (c 1, chloroform)]. Molecular optical rotation and n.m.r. data are in favor of a β glycosidic linkage. Except in one case[7], the halogenosugar 5 has been reported to give mainly a β linkage using the conditions here selected[13]. Compound 7 was easily obtained by catalytic hydrogenation in acetic acid (Adams catalyst, 3 days at atmospheric pressure), followed by acetylation (acetic anhydride in pyridine). It is an amorphous derivative.

A key step for the feasibility of our synthesis is the possibility to selectively open the 1,6-anhydro ring without extensive damage of the internal glycosidic linkage. This was performed[5] by carefully controlled acetolysis, using the mixture acetic anhydride-acetic acid-sulfuric acid (70 : 30 : 2, v/v/v) at room temperature. A mixture of the anomeric acetates was obtained (74%, mainly α), the pure α anomeric form (8) being chromatographically purified [m.p. 237-238°, $[\alpha]_D^{20}$ + 38° (c 0,23, chloroform)]. After acetylation and esterification of the amorphous natural disaccharide 1, Sharon et al.[2] obtained a crystalline derivative [m.p. 235-236°, $[\alpha]_D^{22}$ + 40° (c 0,20, chloroform)]. Both compounds proved identical (mixed m.p., mass spectra). The mass spectra of our synthetic disaccharide 8 is clearly different from that of the analogous synthetic 1 → 6 disaccharide[2] (typical differences in intensity for the fragments $\frac{m}{e}$ 498, 546, 602, 662). Several other approaches[14] for the synthesis of 8 were tried, but proved unsuccessful.

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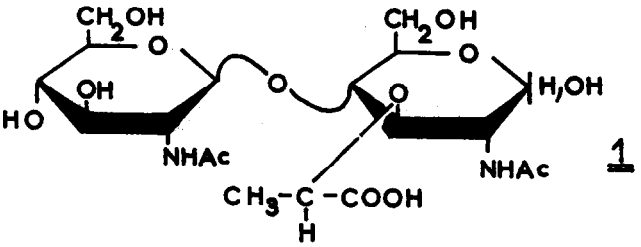

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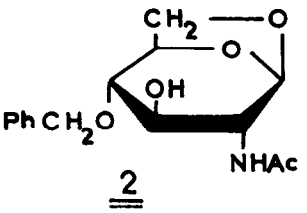

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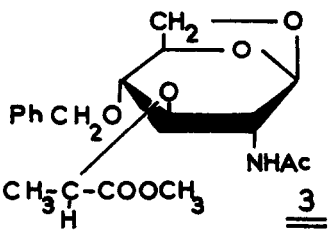

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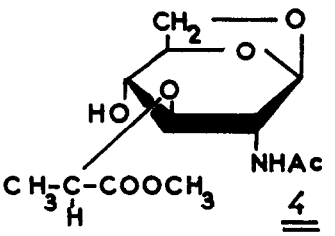

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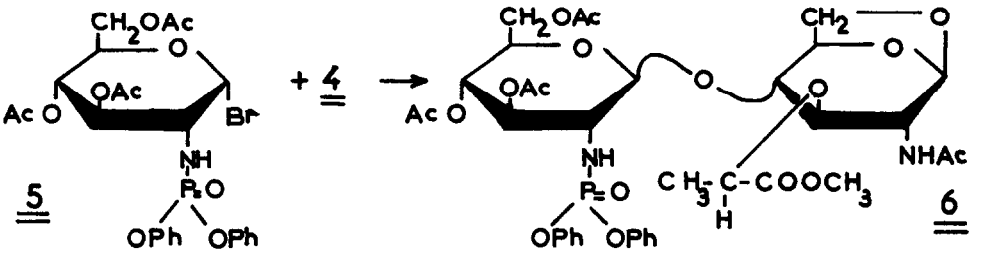

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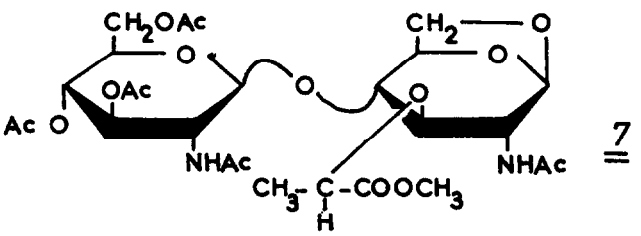

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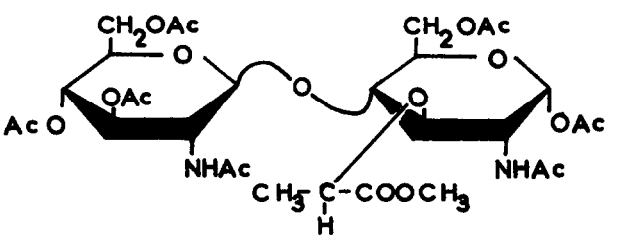

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NOTES AND REFERENCES.

1 M.R.J. SALTON, The Bacterial Cell Wall, Elsevier Publishing Corp., Amsterdam (1964).

2 N. SHARON, T. OSAWA, H.M. FLOWERS and R.W. JEANLOZ, J. Biol. Chem., 241 (1966) 223.

3 M.R.J. SALTON and J.M. GHUYSEN, Biochim. Biophys. Acta, 36 (1959) 552 ; 45 (1960) 355 ; H.R. PERKINS, Biochem. J. , 74 (1960) 182.

4 J.M. WILLIAMS and A.C. RICHARDSON, Tetrahedron, 23 (1967) 1369.

5 See for example : W.T. HASKINS, R.M. HANN and C.S. HUDSON, J. Am. Chem. Soc., 64 (1942) 1852 ; D. SHAPIRO, A.J. ACHER, Y. RABINSOHN and A. DIVER-HABER, J. Org. Chem., 36 (1971) 832 and references cited therein.

6 E.J.C. CURTIS and J.K.N. JONES, Can. J. Chem., 37 (1959) 358.

7 K. HEYNS, K. PROPP, R. HARRISON and H. PAULSEN, Ber., 100 (1967) 2655.

8 M.A.E. SHABAN and R.W. JEANLOZ, Carbohyd. Res., 20 (1971) 399 ; A. KLEMER and U. KRASKA, Tetrahedron Lett., 5 (1972) 431.

9 F. SCHMITT and P. SINAY, Carbohyd. Res., in press.

10 P. SINAY, M.D.A. HALFORD, M.S. CHOUDHARY, P.H. GROSS and R.W. JEANLOZ, J. Biol. Chem., 247 (1972) 391.

11 All the compounds described have satisfactory elementary analysis and i.r. and n.m.r. data in full agreement with the structure.

12 L. ZERVAS and S. KONSTAS, Ber., 93 (1960) 435.

13 See for example : D. BUNDLE and N. SHAW, Carbohyd. Res., 21 (1972) 211 ; R. IWAMOTO and Y. IMANAGA, Carbohyd. Res., 24 (1972) 133 ; Ref. 9.

14 J.P. COAT , J.M. PETIT and P. SINAY, Compt. Rend., 272 (1971) 1582.
C. MERSER and P. SINAY,Carbohydrate Discussion Group (1971), Royal Holloway College.