342. *Polysaccharides. Part XII. Acetolysis Products of Cellulose.*

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Divergent views have been held as to the nature and significance of the acetylated break-down product obtained by Hess and Friese (*Annalen*, 1926, **450**, 40, and later papers) as the result of subjecting cellulose to acetolysis under mild conditions. The substance was considered by Hess to be homogeneous and to have the structure of a disaccharide anhydride. Other workers (cf. Meyer and Mark, *Ber.*, 1928, **61**, 2432; Freudenberg, *Ber.*, 1929, **62**, 385) have doubted both the homogeneity and the molecular weight of the product and the view is widely held that " biosan acetate " is in reality a mixture of cellodextrin acetates of comparatively high molecular weight. During the course of the present work we have obtained strong evidence of the correctness of the latter view. Biosan acetate has a high molecular weight in camphor. It is not homogeneous and a study of its acetolysis products has shown that under mild conditions a mixture of cellodextrin acetates (molecular weight about 1600) is formed and some of the biosan remains unchanged. Under more drastic conditions a mixture of dextrin acetates was obtainable which was separated into fractions differing in m. p. and rotation and having molecular weights ranging continuously from approximately 640 to about 1300. These observations can be interpreted satisfactorily only if the supposed " biosan " is in reality a cellodextrin of high molecular weight (at least 2000). Further evidence pointing to a similar conclusion was provided by a study of methylated " biosan," which in camphor has a molecular weight greater than 2000.

Other products obtained by the acetolysis of cellulose concerning which a variety of opinions has been advanced are the " cello*iso*biose " and the trisaccharide " procellose " claimed respectively by Ost (Ost and Prosiegel, *Z. angew. Chem.*, 1920, **33**, 100; Ost and Knoth, *Cellulosechem.*, 1922, **3**, 25) and by Bertrand and Benoist (*Bull. Soc. chim.*, 1923, 1451; 1924, 58). The existence of cello*iso*biose as a disaccharide distinct from cellobiose has frequently been questioned and the fact that on acetylation the product is mainly cellobiose octa-acetate has been difficult to explain, more especially as the acetate of cello*iso*biose, which is formed only to a minute extent, cannot be converted into cellobiose octa-acetate. An unexpected solution of the problem emerges, however, from the results of our experiments on the acetolysis of cellulose under Bertrand's conditions. The " procellose " so obtained was acetylated and the acetyl derivative, which was readily isolated in good yield and was free from admixture with cellobiose octa-acetate, had properties similar to those recorded by Ost for the acetate of cello*iso*biose. It is difficult to differentiate with certainty between the octa-acetate of a disaccharide and the hendeca-acetate of a trisaccharide by analysis (see experimental section) or by ordinary methods of determining molecular weights (*e.g.*, raffinose acetate gives by Rast's method the value required by a disaccharide). Accordingly the acetate was deacetylated and methylated and examination of the fully methylated procellose revealed that it was in fact a trisaccharide. The rate of hydrolysis was similar to that of the methylated trisaccharide examined by Haworth, Hirst, and Thomas (J., 1931, 824) and it is highly probable that all three glucose units were of the pyranose type.

The present work provides strong support for the following general conclusions concerning the process of acetolysis. When this operation is conducted under mild conditions the product is a mixture of cellodextrins which contain comparatively long chains of glucose units linked as in cellobiose. Hess's cellobiosan is such a mixture. As the conditions of acetolysis become more drastic the average chain-length of the dextrins decreases and a stage of the degradation is reached which represents the optimum conditions for the formation of Bertrand's " procellose " which consists for the most part of a trisaccharide. Owing to grave experimental difficulties it is almost impossible to guarantee that acetolysis experiments performed apparently under identical conditions will give precisely the same type of mixture, and this accounts for the conflicting claims concerning the nature of " procellose " and " cello*iso*biose." It now seems reasonably certain that " cello*iso*biose " does not exist and that the material claimed as such is a mixture of cellobiose with a trisaccharide which would appear to be identical with Bertrand's " procellose."

EXPERIMENTAL.

The acetolysis of cellulose (cotton linters) was carried out exactly as described by Hess and his collaborators (*loc. cit.*) for the preparation and purification of cellobiosan acetate. Although care was taken to ensure exactly similar conditions during each expt., the products obtained differed from one another in certain respects and were not homogeneous. For instance, when a sample of material with m. p. 270—272° was dissolved in a mixture of equal vols. of $CHCl_3$ and MeOH and the solution was allowed to evaporate slowly, the first crop had m.p. 257—258°. A typical preparation of " cellobiosan acetate " had m. p. 268—270°, $[\alpha]_D^{20^\circ}$ $-$ 14·5° in $CHCl_3$ [Found : C, 49·2; H, 6·0; $CH_3{\cdot}CO_2H$, 61·8. Calc. for $C_{24}H_{32}O_{16}$: C, 50·0; H, 5·6; $CH_3{\cdot}CO_2H$, 62·5%).

" Cellobiosan acetate " dissolved readily in molten camphor, but caused no appreciable depression of the m. p. Hence the mol. wt. is probably high (above 3000).

Methylation of "Cellobiosan."—"Cellobiosan acetate" (10 g.), dissolved in acetone (250 c.c.), was methylated at 50—55° by Me_2SO_4 (60 c.c.) and 30% NaOH aq. (160 c.c.). This operation, which occupied 2 hrs., was performed in the usual way. The methylated product separated from the hot solution and was removed by filtration and washed several times with boiling H_2O. It was a white amorphous powder with properties similar to those ascribed by Hess to "methylated cellobiosan," m. p. 213—215°, $[\alpha]_D^{20^\circ}$ − 7·2° in C_6H_6 (*c*, 1·6) (Found : C, 52·9; H, 7·9; OMe, 44·6. Calc. for $C_{18}H_{32}O_{10}$: C, 52·9; H, 7·8; OMe, 45·6%). The apparent mol. wt. determined cryoscopically in camphor was 1600 (calc., 408). A sample of the methylated substance (0·8 g.) was heated at 180° for 30 mins. with four times its wt. of molten camphor. On removal of the camphor by extraction with Et_2O, there remained a colourless non-reducing solid (0·65 g.), m. p. 226—229°, $[\alpha]_D$ − 9·6° in C_6H_6 (*c*, 2·5). This had an apparent mol. wt. 2100. The treatment with molten camphor seemed to have removed a small amount of partly degraded material.

Acetolysis of "Cellobiosan."—"Cellobiosan acetate" (10 g.), dissolved in AcOH (30 c.c.), was heated at 105° for a few secs. with Ac_2O (50 c.c.) containing conc. H_2SO_4 (6·8 c.c.). When the colour began to darken, the mixture was poured into cold H_2O. The white ppt. was washed with H_2O until neutral and dried in a desiccator over soda-lime. Yield 6·4 g., m. p. indefinite, below 100°. $[\alpha]_D^{22^\circ}$ + 20° in $CHCl_3$ (*c*, 1·7). This material (6 g.) was sol. in hot EtOH, from which a portion separated on cooling. As the result of systematic fractionation from EtOH the following fractions were obtained.

	M. p.	$[\alpha]_D^{22^\circ}$ in $CHCl_3$.	Apparent mol. wt. in camphor.
Fraction I	184—185°	+28°	640
Fraction II	123—124	+12	920
Fraction III	139—140	+10	1300
Fraction IV	172—174	+ 8	1300

On another occasion the acetolysis of "cellobiosan acetate" (5 g.), dissolved in AcOH (30 c.c.), was carried out under milder conditions by the action of Ac_2O (25 c.c.) containing conc. H_2SO_4 (2 c.c.). The reaction was allowed to proceed for 30 secs. at 60°. The product (4·7 g., $[\alpha]_D$ − 11° in $CHCl_3$) was isolated as before and fractionated from EtOH. A considerable portion of it (3·5 g.) was only slightly sol. in this solvent and appeared to be unchanged "cellobiosan acetate," m. p. 257—259°, $[\alpha]_D$ − 14·7° in $CHCl_3$ (*c*, 1·5). There was obtained also a substance (0·4 g.), m. p. 164—165°, $[\alpha]_D$ − 8·6° in $CHCl_3$ (*c*, 2·7), which had an apparent mol. wt. 1600 in camphor.

Preparation of "Procellose."—The acetolysis of cellulose (filter-paper) was carried out by the method of Bertrand and Benoist (*loc. cit.*) and the directions of these authors for the isolation of procellose from the acetolysis product were followed exactly up to the final stage when the solution of procellose in 85% EtOH was left to crystallise. After several months the amount of cryst. material was negligible. The alc. solution was then concentrated to small vol. and the free sugar precipitated as a syrup by the addition of acetone. The sugar hardened on trituration with acetone and was finally obtained as a white powder, which was washed with acetone and ether and dried in a vac.

desiccator. It was strongly reducing and very hygroscopic. The yield was 57 g. from 3500 g. of cellulose.

The acetyl derivative was prepared by heating the sugar (1 part) with Ac_2O (7 parts) containing anhyd. NaOAc (2 parts) for 30 mins. at 100°. The solution was poured into ice-water and the white powder which separated was washed until free from acid (yield, 1·4 parts). The acetate was purified by fractionation from aq. EtOH : a white powder was obtained in characteristic microscopic globules. It did not appear to be cryst. and attempts to induce crystn. from EtOH, MeOH, C_6H_6, EtOAc, AcOH, dioxan, $CHCl_3$, and mixtures of these solvents were unsuccessful. The m. p., specific rotation, and apparent mol. wt. remained unchanged after the substance had been precipitated 15 times in succession from aq. EtOH. M. p. about 120°, with softening from 109°; $[\alpha]_D^{19^\circ}$ + 5·0° in C_6H_6 (c, 3·8). Apparent mol. wt. 690 (cryoscopic in camphor) [Found: C, 49·8; H, 6·2. Calc. for $C_{28}H_{38}O_{19}$ (disaccharide): C, 49·5; H, 5·6%. Calc. for $C_{40}H_{54}O_{27}$ (trisaccharide): C, 49·7; H, 5·6%].

When heated for 45 mins. at 100° with Ac_2O (50 c.c.) containing a little $ZnCl_2$ (cf. Hudson and Johnson, *J. Amer. Chem. Soc.*, 1915, **37**, 1276), the acetate (3·0 g.) was transformed into an amorphous white powder (2·5 g.) which, after purification by pptn. from aq. EtOH, had m. p. 128—130°, and $[\alpha]_D^{19^\circ}$ + 18° in $CHCl_3$ (c, 3·6).

Simultaneous Deacetylation and Methylation of the Procellose Acetate.—The acetate (10·4 g.), dissolved in acetone (100 c.c.), was treated in the usual way with Me_2SO_4 (90 c.c.) and 30% NaOH aq. (230 c.c.). After removal of the acetone by evaporation, the mixture was heated for an hr. at 100°, and cooled, and the product (5 g.) extracted with $CHCl_3$. After three methylations by Ag_2O and MeI a glassy solid (4·8 g.) was obtained. This was distilled under diminished press., giving an extremely viscid syrup (A), b. p. about 300° (bath temperature)/0·04 mm., $[\alpha]_D^{17^\circ}$ + 27° in $CHCl_3$ (c, 0·9) [Found: C, 53·1; H, 8·4; OMe, 49·9. Calc. for $C_{20}H_{38}O_{11}$ (disaccharide): C, 52·8; H, 8·4; OMe, 54·6%. Calc. for $C_{29}H_{54}O_{16}$ (trisaccharide): C, 52·9; H, 8·3; OMe, 51·8%].

Hydrolysis of the Methylated Product.—The syrup (A) (1·73 g.) was hydrolysed by 5% HCl aq. at 95—100° in about 2 hrs., the course of the reaction being followed polarimetrically. $[\alpha]_D^{20^\circ}$ + 36° (after 30 mins. at 95°); + 66° (60 mins.); + 75° (90 mins.); + 78° (120 mins., const. value). The acid was neutralised with $BaCO_3$ and the clear filtered solution was extracted with $CHCl_3$ (8 × 30 c.c.). The $CHCl_3$ left on evaporation a colourless syrup (0·55 g.) which rapidly crystallised. Recrystn. from light petroleum (b. p. 40—60°) gave tetramethyl glucopyranose, m. p. 85—87°, alone or when mixed with an authentic sample of m. p. 87—88°.

The neutral aq. solution was evaporated to dryness, and the solid so obtained was extracted with boiling $CHCl_3$. Removal of the solvent left a colourless syrup (0·6 g.) which rapidly crystallised and on recrystn. from dry Et_2O gave 2 : 3 : 6-trimethyl glucopyranose, m. p. and mixed m. p. with an authentic sample 106—108°.

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