CVIII. AN EXAMINATION OF THE METABOLIC PRODUCTS OF CERTAIN FUCOIDS.

II. MANNITOL AND MANNITAN.

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IN view of the acknowledged occurrence of the hexahydric alcohol mannitol among the Fucaceae repeated attempts were made to isolate this substance from the syrups obtained as described in the preceding paper [Haas and Hill, 1929] from *Pelvetia*, but without success; no trace of crystalline mannitol being obtained from alcoholic solutions by careful evaporation. It was therefore decided to make fresh aqueous, instead of alcoholic, extracts of *Pelvetia* as well as of *Laminaria digitata* and *Fucus serratus*; the two latter stood in marked contrast to *Pelvetia* since the isolation of crystalline specimens of mannitol from *Laminaria* and *Fucus* presented no difficulty whatever, whereas the final extract obtained from *Pelvetia* was extremely reluctant to crystallise.

The method employed for the isolation of mannitol and mannitol-containing syrups was the same in all experiments.

200 g. of dried and ground material were extracted with hot water in a double jacketed vessel for about 40 minutes and then pressed in a tincture press; the residue was once more extracted in the same way and the combined extracts were poured into an equal volume of absolute alcohol and set aside for 12 hours to allow the gelatinous precipitate to separate; the liquid was then strained through silk, and, after distilling off the alcohol, was evaporated to dryness over a water-bath. The product so obtained was a brown gummy mass rich in salts; it was transferred to a Soxhlet thimble suspended in a flask and extracted in the vapour of boiling alcohol for about 6 hours. The nature of the alcoholic extract so obtained varied with the different weeds. Thus the extract from *Fucus serratus* and *Ascophyllum nodosum* deposited white crystalline masses of mannitol and *Laminaria* extract likewise showed signs of crystallising, whereas the other weeds examined gave no crystals on cooling, but on evaporating off the alcohol yielded a highly viscous substance containing varying quantities of chlorophyll and other pigments. These were removed when necessary by washing with cold absolute alcohol. Since the syrups obtained by aqueous extraction of *Pelvetia* behaved just like the alcoholic extracts in refusing to yield crystalline mannitol, it was decided to attempt to isolate the mannitol, if present, in the form of its crystalline

tribenzal derivative. To this end the material, weighing about 3–5 g., was transferred to a small wide-mouthed bottle with not more than 1 cc. of water and then mixed with 6 cc. of 50 % sulphuric acid; after adding 3 cc. of benzaldehyde the whole was thoroughly shaken and left in a cool place for 3 days. The contents of the bottle were then diluted with water and the resulting solid product was washed first with water until free from acid and then with 65 % alcohol¹ to remove benzaldehyde; on crystallising from absolute alcohol it separated in white silky needles melting at about 204°–207° or higher. The melting-point of the tribenzal derivative of mannitol ($C_{27}H_{26}O_6$) is quoted in the literature as 207°, 213°–217° and 218°–222°; the variations are no doubt due to differences in the rate of heating.

A sample of such a product obtained from *Pelvetia canaliculata* collected in Aberdeen gave the following figures² on analysis:

0.0439 g. gave 0.1162 g. CO_2 , 0.0232 g. H_2O .

Found: C, 72.18 %; H, 5.92 %.

Calc. for $C_{27}H_{26}O_6$: C, 72.64 %; H, 5.83 %.

A product prepared as above from the syrup obtained from *Pelvetia canaliculata forma libera* collected at Blakeney Point proved to be a mixture from which it was possible to isolate by crystallisation some of the tribenzal derivative melting at 211°–215°, but from the mother-liquors was obtained a number of different fractions having much lower melting-points; these were not isolated in a state of purity but the impression was gained that the original syrup probably contained a mixture of a number of substances which would account for the difficulty of crystallisation persistently encountered in preparations from the form *libera*.

An estimation of the benzaldehyde contained in the tribenzal derivative melting at 211°–215° was carried out by hydrolysing 0.1666 g. for 3.5 minutes with 5 cc. of 2 % alcoholic hydrochloric acid and precipitating the resulting benzaldehyde by means of dinitrophenylhydrazine [Brady, 1926].

0.1666 g. gave 0.3056 g. hydrazone $\equiv$ 0.1157 g. benzaldehyde.

Found: benzaldehyde, 69.44 %.

Calc. for tribenzal mannitol: 71.30 %.

The evidence furnished by these figures may be taken as sufficient to establish beyond reasonable doubt the presence of mannitol in *Pelvetia*, short of actual isolation of the mannitol in crystalline form. In order, however, to place the matter on a still firmer basis an attempt was made to regenerate the mannitol from its tribenzal derivative; but here also difficulty was experienced in isolating the mannitol in crystalline form. Thus in a typical experiment 0.8 g. of the tribenzal derivative was boiled under a reflux condenser for 3.5 minutes with 10 cc. of 2 % alcoholic sulphuric acid. The resulting product was steam-distilled to remove benzaldehyde and then neutralised with baryta; the precipitate of barium sulphate was filtered off and the filtrate

¹ For a description of the contents of this solution see p. 1007.

² All combustions were performed by Mr F. Hall, Dyson Perrin's Laboratory, Oxford.

after evaporation to dryness gave a colourless syrup which on prolonged standing solidified. This substance, which was soluble in water, was shown to be mannitol by reconversion into the tribenzal derivative by means of benzaldehyde and sulphuric acid.

Mannitan. In the previous section has been described the evidence for the existence in the genus *Pelvetia* of mannitol; by the isolation, described below, of a compound which from analysis would appear to be a dibenzal derivative of mannitan, an anhydride of mannitol ($C_6H_{14}O_6 - H_2O = C_6H_{12}O_5$), it is concluded that the mannitol occurring in *Pelvetia* is accompanied by some mannitan. It must be admitted that such indirect evidence is unsatisfactory, more especially as the existing knowledge of the chemistry of the mannitans is very incomplete; they are imperfectly characterised substances of a gummy nature and the methods of their preparation would appear to lead to mixtures of two mono-anhydrides—the mannitans proper—with a dianhydride *isomannide* $C_6H_{10}O_4$ and possibly mannitol ether, $C_{12}H_{26}O_{11}$. There is moreover no evidence in the literature as to whether mannitans form benzal derivatives, but on theoretical grounds there appears to be no reason why they should not be capable of forming derivatives containing two benzal groups, of the composition $C_{20}H_{20}O_5$, produced according to the equation:

This matter will be referred to later in describing the attempts to synthesise such a compound, but in the meantime it is proposed to consider the evidence for the isolation from *Pelvetia* extracts of a presumed dibenzalmannitan by treatment of these extracts with benzaldehyde in the presence of sulphuric acid.

Dibenzalmannitan. In purifying the crude tribenzalmannitol prepared from either *Pelvetia canaliculata* collected in Aberdeen or in Blakeney or from *Pelvetia canaliculata forma libera* obtained from Blakeney, a second product was found. This substance, which appeared from its analysis to be the dibenzal derivative of a mannitan, was found either in the alcoholic washings of the crude tribenzal (see p. 1006) or in the mother-liquors from the crystallisation of this product. The new substance crystallises from dilute alcohol in felted masses of needles melting at 165° – 168° ; it is sparingly soluble in cold alcohol but readily soluble in ethyl acetate or chloroform and insoluble in light petroleum.

Samples prepared from *Pelvetia canaliculata* (Aberdeen and Blakeney respectively) gave the following figures:

0.0800 g.	gave 0.2058 g. CO_2 ,	0.0441 g. H_2O	(Aberdeen)
0.0713 g.	„	0.1835 g. CO_2 , 0.0379 g. H_2O	(Blakeney)
		C % H %	
Found:		70.15 6.12	(Aberdeen)
„		70.18 5.90	(Blakeney)
Calc. for $C_{20}H_{20}O_5$:		70.58 5.88	

A search for the presence of a similar material in the preparations of

tribenzalmannitol from *Laminaria digitata* and from *Fucus serratus* failed to reveal any. In order to meet the possible criticism that the dibenzalmannitan in question was an artifact, produced in the course of the reaction between mannitol and benzaldehyde, in the presence of 50 % sulphuric acid, a careful search was made for the presence of this substance in the mother-liquors of a preparation of tribenzalmannitol from pure mannitol, but none was found.

Synthetic experiments. As already stated, the literature reveals no evidence of the existence of dibenzalmannitans, nor even of the ability of mannitans to react with benzaldehyde. It was therefore decided to attempt the preparation of mannitan with a view to preparing from it some of the dibenzalmannitan described above. According to Vignon [1874] a crystalline mannitan can be obtained in small quantity by treating mannitol with a quarter of its weight of water in sealed tubes for 2 hours at 280° or alternatively an amorphous one may be obtained by heating to 287°. He further states that a mannitan may be obtained by heating mannitol for 2 hours with half its weight of concentrated sulphuric acid at 120°–125° and that the same product may be produced by heating mannitol with a quarter of its weight of water at about 295° for 1.5 hours.

Our own experiments have shown that the temperature is indeed an important factor. Mannitol heated with a quarter its weight of water at about 290° for 1.5 hours is recovered almost unchanged, whereas if heated to about 300°, or a little over, it yields a product containing, in addition to unchanged mannitol, some substance capable of yielding an apparent dibenzal derivative of the composition $C_{20}H_{20}O_5$; but if heated to above 310° it yields a syrup from which no such product could be isolated. Heating with sulphuric acid at 120° likewise gave a syrup from which no solid derivative could be obtained by means of benzaldehyde and 50 % sulphuric acid.

In two experiments we found, after heating for 1.5 hours at 300°–308°, that on opening the tube a slight pressure had developed; the contents of the tube were dissolved in water, filtered and evaporated to dryness. There remained a semi-solid mass which when dissolved in 50 % sulphuric acid and shaken with benzaldehyde yielded after the usual treatment a large quantity of tribenzalmannitol, derived from unchanged mannitol, in addition to a small quantity of a substance which was readily soluble in alcohol and which after crystallisation from dilute methyl alcohol separates in stellar aggregates of glistening prisms melting at 113°–115°. These on analysis gave the following figures:

0.0739 g. gave 0.1906 g. CO_2 , 0.0395 g. H_2O .

Found: C, 70.33 %; H, 5.90 %.

Calc. for $C_{20}H_{20}O_5$: C, 70.58 %; H, 5.88 %.

It will be seen that this substance is identical in composition with the dibenzalmannitan obtained from *Pelvetia* extracts by benzaldehyde and sulphuric acid but, from its crystalline form and solubilities, it would appear to be isomeric with this substance.

On theoretical grounds it is possible to account for the existence of three isomeric mannitan containing an ethylene oxide ring,

and

only two of which, (A) and (B), however, should be capable of yielding dibenzal derivatives¹.

A consideration of the facts warrants the conclusion that a mannitan naturally occurs in *P. canaliculata* and its form *libera*. The habitat of these plants, especially of the form *libera*, is such that they are exposed to prolonged periods of drought and even desiccation, a point of some interest in that mannitan is artificially prepared from mannitol by methods of dehydration. How much mannitan is present in *Pelvetia* we are unable to say. The yield of the dibenzal derivative was always small, but this may be due to the fact that the reaction between mannitan and benzaldehyde is less easy to effect than that between mannitol and benzaldehyde in the conditions of our experiments.

It may be further remarked that the presence of mannitan, even in small quantity, might account for the reluctance to crystallise shown by the mannitol contained in the syrupy preparations made from *Pelvetia*.

SUMMARY.

1. The presence of mannitol in *Pelvetia canaliculata* and *P. canaliculata f. libera* has been established; its presence has also been confirmed in *Asco-phyllum nodosum*, *Fucus serratus* and *Laminaria digitata*.

2. The presence of mannitan in *Pelvetia canaliculata* and its form *libera*, which plants occupy the highest position in the emerging zone, is inferred by the isolation of a dibenzal derivative melting at 165°–166°.

3. An attempt to synthesise this dibenzal mannitan resulted in the production of an isomeric compound melting at 113°–115°.

4. The question of the existence of isomeric mannitans and their ability to form benzal derivatives is raised.

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¹ The possibility of the mannitans containing a propylene, butylene, amylene or hexylene oxide ring must be recognised. It has not been possible to determine the constitutional formulae of the two presumed dibenzal mannitans described in this paper.