CXCIII. STUDIES IN THE BIOCHEMISTRY OF MICRO-ORGANISMS.

XLVI. *i*-ERYTHRITOL, A METABOLIC PRODUCT OF *PENICILLIUM BREVI-COMPACTUM* DIERCKX AND *P. CYCLOPIUM* WESTLING.

BY ALBERT EDWARD OXFORD AND HAROLD RAISTRICK.

From the Division of Biochemistry, London School of Hygiene and Tropical Medicine, University of London.

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OF the many crystalline sugar alcohols occurring naturally in the plant kingdom, only mannitol has hitherto been isolated as a metabolic product of the lower fungi when grown on synthetic media. This alcohol is a tissue constituent of many, if not most, mould species and is sometimes present in large amounts in the metabolism solution if glucose or certain other simple sugars are supplied as the source of carbon. Sorbitol has only once, and dulcitol has never been isolated from the tissues of the higher fungi, but the tetrahydric alcohol *i*-erythritol ($CH_2OH.CHOH.CHOH.CH_2OH$) is of frequent occurrence in algae and lichens, and has also been isolated, together with mannitol [Zellner, 1910], from the spores of the corn smut *Ustilago maydis*, a micro-fungus parasitic on maize, *Zea Mays*. It might therefore be expected to occur among the metabolic products of moulds grown on synthetic media. The present paper describes the isolation of *i*-erythritol, in small yield, from the mycelia of two different, and not closely related species of *Penicillium*, *viz.*, *P. brevi-compactum* Dierckx and *P. cyclopium* Westling, both grown on synthetic media.

Since *i*-erythritol is very soluble in cold water (more soluble even than mannitol) it is reasonable to expect that when the mycelium contains a little *i*-erythritol, the metabolism solution may contain very much more, and in view of the difficulty of isolating *i*-erythritol from a dilute solution containing both glucose and mannitol, we cannot, at present, give any estimate of the maximum yield of this alcohol when the most suitable mould species is grown under optimum conditions. With each of the species named above, *i*-erythritol was found in the mycelium only when the metabolism solution still contained a considerable amount of unutilised glucose. The metabolism solution of *P. cyclopium*, grown on a glucose+tartrate medium, did however contain both mannitol and *i*-erythritol (the latter in very small amount) when all the glucose had been utilised and when the mycelium appeared to contain no *i*-erythritol at all. We have also shown, in the case of *P. cyclopium* at least, that *i*-erythritol is definitely a product of metabolism of glucose and that the presence of tartaric acid in the medium is not essential for its formation.

EXPERIMENTAL.

Isolation of i-*erythritol from the mycelium of* P. brevi-compactum *Dierckx.*

This experiment was a continuation of the work reported in Part XXXIV [Oxford and Raistrick, 1933] when a strain of *P. brevi-compactum* (L.S.H.T.M. Catalogue No. M 3 (1)) was grown on Raulin-Thom medium of the following composition: glucose, 75 g.; tartaric acid, 4 g.; ammonium tartrate, 4 g.;

diammonium hydrogen phosphate, 0·6 g.; K_2CO_3, 0·6 g.; $MgCO_3$, 0·4 g.; $(NH_4)_2SO_4$, 0·25 g.; $ZnSO_4, 7H_2O$, 0·07 g.; $FeSO_4, 7H_2O$, 0·07 g.; water to 1500 ml. 105 one-litre flasks, each containing 350 ml. of the above medium, were sterilised, sown and incubated at 24°, and 26 flasks were worked up after 8 days' incubation when about 50 % of the glucose had been metabolised. The resulting mycelium was dried (66 g.), powdered and extracted in a Soxhlet apparatus, first with light petroleum (B.P. 50–60°) for 2 days to remove fats and sterols and then with ether for 2 working days to remove mycophenolic acid. The extraction was then continued for 3 further working days with fresh ether, and at the end of this time it was found that some colourless material (0·2 g.) had separated from the extract, consisting of a number of visibly crystalline aggregates, mixed with a little amorphous material. The crystalline aggregates, separated by hand, had M.P. 90–100°, raised by crystallisation from alcohol-ether and then from alcohol alone to 116–120°, not changed by a further crystallisation from alcohol, from which the material separated in well-formed tetragonal crystals. It was readily soluble in water to give a neutral solution which gave no coloration with $FeCl_3$, and the Molisch and Millon reactions both gave negative results. The substance was obviously a polyhydric alcohol, but its M.P. was depressed by admixture with mannitol (M.P. 165°) or with sorbitol (M.P. 110°). A mixed M.P. with an authentic specimen of *i*-erythritol (M.P. 116–120°) showed no depression and the identity of the product with *i*-erythritol was confirmed by elementary analysis. (Found (Schoeller): C, 39·50, 39·50; H, 8·20, 8·14 %. $C_4H_{10}O_4$ requires C, 39·31; H, 8·25 %.[1])

It is evident that the statement in the literature that *i*-erythritol is quite insoluble in ether is incorrect. We find that 1 litre of boiling ether dissolves 25 mg. of the alcohol.

A similar procedure carried out on the mycelium of *P. brevi-compactum* (M 3 (1)), produced on the same medium after 11, 15, 22 and 56 days' incubation, yielded no *i*-erythritol in any case. Unfortunately the respective metabolism solutions, after having been worked up for phenolic substances, had been discarded long before the new product had been identified as *i*-erythritol.

A considerable amount of mycelium of another strain of *P. brevi-compactum* (L.S.H.T.M. Catalogue No. P. 75), grown on the same medium until all or nearly all of the glucose had been metabolised, was similarly worked up, but no trace of *i*-erythritol was found. This mycelium did, however, contain mannitol, a fact previously noted by Alsberg and Black [1913] in their biochemical study of another strain of *P. brevi-compactum* freshly isolated from mouldy Italian maize.

Isolation of i-*erythritol from the mycelium of* P. cyclopium *Westling.*

Thom [1930] places this organism among the "Fasciculata" group of *Penicillium* species. Morphologically it is not related to the "brevi-compactum" group.

In our first experiments on the metabolism of *P. cyclopium*, the results of which will form the subject of a later communication, 45 flasks, each containing 350 ml. of Raulin-Thom medium (for composition see p. 1599), were sterilised, sown with a spore suspension of *P. cyclopium* (purchased from Baarn, August 1931, L.S.H.T.M. Catalogue No. P. 123), and incubated for 16 days at 24°, at the end of which time the metabolism solution contained only 1 % of glucose. The mycelium was separated, dried and powdered (214 g.). It was first defatted by extraction for 2 working days with light petroleum (B.P. 50–60°) in a Soxhlet apparatus, then extracted with ether for about 14 working days until no more colourless

[1] The analyst also reported that the substance contained about 20 % alkoxyl as determined by the Zeisel method. Erythritol, reduced by HI, yields *sec*butyl iodide, B.P. 119°, which would be partly distilled over under the conditions of the estimation.

solid separated from the extract. This product was purified by crystallisation from alcohol, after which it was treated with water (10 ml.) and a little insoluble amorphous material removed by filtration. The aqueous filtrate was evaporated to dryness in a vacuum desiccator over solid KOH, to yield fine, large crystals of *i*-erythritol (1·5 g.), M.P. 116–120°, not depressed by admixture with authentic *i*-erythritol, M.P. 116–120°. The crystals so obtained were recrystallised from hot dioxan, as coarse needles, M.P. 116–120°. (Found on this specimen (Schoeller): C, 39·76; H, 8·34 %. $C_4H_{10}O_4$ requires C, 39·31; H, 8·25 %.)

0·2877 g. in 11·0 g. H_2O depressed the F.P. by 0·422°; hence mol. wt., 115. $C_4H_{10}O_4$ requires mol. wt., 122. A 3 % aqueous solution of the substance was optically quite inactive.

Acetyl derivative. A solution of the mould product, M.P. 116–120° (0·1 g.) in an excess of acetic anhydride containing a few drops of pyridine was boiled for a minute, kept overnight at 34° and then evaporated to dryness in a vacuum desiccator over solid KOH. The product, crystallised from light petroleum (B.P. 80–100°), formed small prisms, M.P. 83–85° alone or mixed with authentic tetra-acetyl-*i*-erythritol, M.P. 83–85°, similarly prepared. (Found, on acetyl derivative of mould product (Schoeller): C, 50·01, 49·89; H, 6·24, 6·08 %. $C_{12}H_{18}O_8$ requires C, 49·64; H, 6·25 %.)

A little *i*-erythritol and some mannitol were also isolated from the metabolism solution of *P. cyclopium* when grown on Raulin-Thom medium at 24° for 24 days, at which time all the glucose had been metabolised.

Production of i-*erythritol by* P. cyclopium *on a medium containing glucose as sole source of carbon.*

In the experiments so far described in this paper, the medium used has always contained tartaric acid in addition to glucose. It was obviously of interest to determine whether the 4-carbon alcohol *i*-erythritol is to be regarded as a metabolic product of the 4-carbon acid, tartaric acid, or whether it may be regarded as a metabolic product of glucose. To this end ten one-litre conical flasks, each containing 350 ml. of Czapek-Dox medium of the following composition: glucose, 50 g.; $NaNO_3$, 2 g.; KH_2PO_4, 1 g.; $MgSO_4$, $7H_2O$, 0·5 g.; KCl, 0·5 g.; $FeSO_4$, $7H_2O$, 0·01 g.; water to 1000 ml., were sterilised, sown with a spore suspension of *P. cyclopium*, and incubated at 24° for 39 days, *i.e.*, until all the glucose had been metabolised. The dried mycelium (32 g.) when exhaustively extracted with ether (after first defatting with light petroleum) yielded a little colourless material, part of which was soluble in water, the aqueous solution yielding 0·14 g. of almost pure *i*-erythritol, M.P. 110–118°, raised by crystallisation from alcohol-ether to 116–120°, alone or mixed with authentic *i*-erythritol.

SUMMARY.

i-Erythritol has been isolated in small yield from the metabolism of glucose by *Penicillium brevi-compactum* Dierckx and *Penicillium cyclopium* Westling. It occurs both in the mycelium and in the metabolism solution. It is present in the mycelium in the largest amounts in the earlier stages of growth.

REFERENCES.

Alsberg and Black (1913). *U.S. Department of Agriculture, Bureau of Plant Industry, Bulletin* No. 270.
Oxford and Raistrick (1933). *Biochem. J.* **27**, 1473.
Thom (1930). The Penicillia. (Baillière, Tindall and Cox, London.)
Zellner (1910). *Monatsh. Chem.* **31**, 617.