Phytochemistry, 1967, Vol. 6, pp. 309 to 312. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

LUTEIC ACID AND ISLANDIC ACID, COMPOSITION AND STRUCTURE

E. EBERT and M. H. ZENK

Botanical Institute, University of Munich, Munich, Germany

(*Received* 17 *June* 1966)

Abstract—The acidic polysaccharides of *Penicillium luteum* (luteic acid) and *P. islandicum* were isolated from the culture medium by precipitation with protamine. The molar ratio of glucose to malonic acid of luteic acid from different *P. luteum* strains varies greatly, but is strain specific. The acidic polysaccharide of *P. islandicum*, however, consists of glucose and malonic acid (molar ratio 1:1) and was named: islandic acid. The D-glucose units in islandic acid are linked through the β-1-6-position; the malonic acid is fixed in both polysaccharides to the alcoholic hydroxyl of C-3 of the glucose units as hemiester.

INTRODUCTION

LUTEIC acid, an extracellular acidic polysaccharide produced by *Penicillium luteum* is composed of glucose and malonic acid in a reported molar ratio of 2:1.[1,2] The β-D-glucose units are linked through the 1:6-positions[3]; the whole molecule of luteic acid consists of about 84 units. The malonic acid is attached to the polysaccharide as a hemiester; its position on the glucose units, however, has not yet been determined. *P. islandicum* is also reported to excrete a luteic acid-like polymer consisting of glucose and malonic acid (2:1) into the culture fluid.[4] We now give further information on the structure of luteic acid and the acidic polysaccharide of *P. islandicum*.

The position of the acid on the glucose polymer has been clarified by periodate oxidation and determination of the degradation products of the fully acylated polysaccharide. If malonic acid is bound to the second or fourth OH-group of the D-glucose units, 1 mole of IO_4^- should be consumed in each case. However, after reduction (KBH_4) and hydrolysis (H_2SO_4) of the resulting polyaldehyde and further reduction (KBH_4) of the cleavage products, one should obtain either 2 moles of glycerol in the case of a substitution at the C-2 hydroxyl group or, in the case of substitution at C-4, 1 mole erythritol and 1 mole ethylene glycol, per mole of D-glucose. If the malonic acid is bound to the 3-hydroxyl group, no IO_4^- would be consumed in the initial oxidation, and a hexitol would be formed during the subsequent degradation procedure mentioned above (or D-glucose prior to the second reduction).

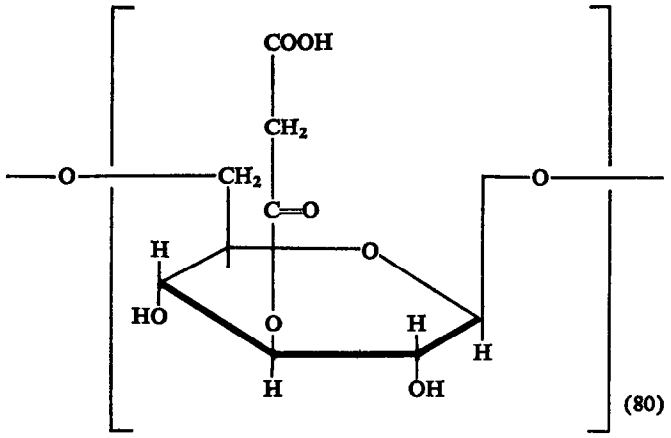
From the results given in Table 1 it is quite clear that in islandic acid the malonic acid is bound to the hydroxyl of C-3 of the glucose units. The small amount of periodate consumed by the esterified polymer might in part be accounted for by the oxidation of the reducing end-group. Islandic acid is therefore (I). For luteic acid our results with periodate oxidation show the same position of linkage between malonic acid and the carbohydrate component.

[1] H. RAISTRICK and M. L. RINTOUL, *Phil. Trans. Roy. Soc.* B220, 255 (1931).
[2] J. H. BIRKINSHAW and H. RAISTRICK, *Biochem. J.* 27, 370 (1933).
[3] C. G. ANDERSON, W. N. HAWORTH, H. RAISTRICK and M. STACEY, *Biochem. J.* 33, 272 (1939).
[4] J. BADDILEY, J. G. BUCHANAN and E. M. THAIN, *J. Chem. Soc.* 1944 (1953).

TABLE 1. PERIODATE OXIDATION AND DEGRADATION OF THE ESTERIFIED AND DEESTERIFIED POLYMERS (EACH 100 μM OF ANHYDRO-D-GLUCOSE UNITS)

	IO_4^- consumed (μM)	HCOOH formed (μM)	Degradation products
Islandic acid	7	0	D-Glucose*
Deesterified polymer	196	102	Glycerol, ethylene glycol

* Prior to the second reduction.



(I) ISLANDIC ACID.

RESULTS AND DISCUSSION

Acid hydrolysis of the purified acidic polysaccharides of *P. luteum* and *P. islandicum* showed that they contained only glucose and malonic acid. However, the ratio of glucose to malonic acid varied greatly (Fig. 1); the three different strains of *P. luteum* have a varying

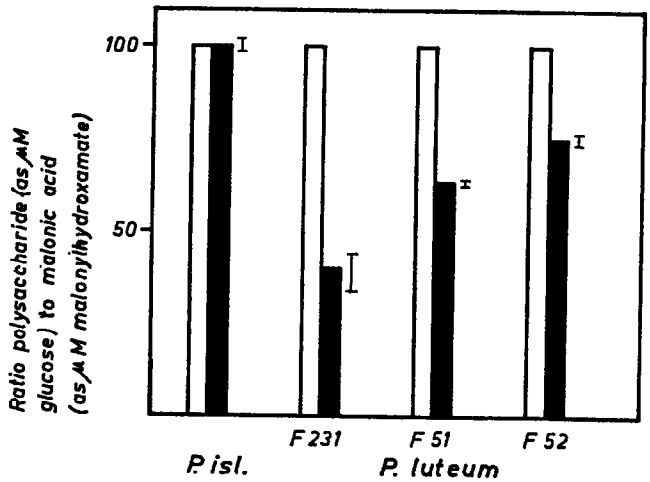


FIG. 1. COMPOSITION OF THE ACIDIC POLYSACCHARIDES OF *Penicillium islandicum* AND *Penicillium luteum* (□ GLUCOSE; ■ MALONIC ACID).

but strain specific ratio less than 1:1; but it was never found to be 2:1 as formerly[1,2] reported. We can state, therefore, that luteic acid cannot be regarded as a chemical entity of constant chemical composition; this is in agreement with the assumption of Birkinshaw and Raistrick.[2] On the other hand, *P. islandicum* produces a polysaccharide containing invariably 1 mole of malonic acid per mole of glucose; this acidic polysaccharide, therefore, must be regarded as having a constant chemical composition, and for this polymer we propose the name: islandic acid.

As indicated by an $[\alpha]_{6630}^{23}$ value of $-14°$, the β-D-glucose units in islandic acid are linked through the 1-6-positions, and this was confirmed both by permethylation studies[5] (main product, 2, 3, 4-tri-O-methyl-D-glucose) and by periodate oxidation of the deacylated polymer and determination of the cleavage fragments of the resultant polyaldehyde after reduction (KBH_4), hydrolysis (H_2SO_4) and further reduction (see Table 1 and Ref. 5). The i.r.-absorption maxima at 919 and 880 cm^{-1} are also indicative of a 1-6-β-linkage.[6] From the amount of reducing end-groups, as determined with the Nelson reagent,[7] the average chain length of the polymer is 80 ± 10 anhydro-D-glucose units. Islandic acid contains the malonic acid as a hemiester as indicated by the formation of malonylmonohydroxamate and the i.r. spectrum which shows strong absorption at 1740 cm^{-1} (ester bound carboxyl group) and 1600 cm^{-1} (carboxyl anion).[6]

With regard to the biosynthesis of islandic acid, we were able to demonstrate that $^{14}CO_2$ was incorporated into the malonic acid moiety of islandic acid to about 3 per cent, with a dilution factor of 9·5. The malonic acid fixed to the carbohydrate was degraded asymmetrically by thermal decarboxylation of islandic acid to "acetyl-islandic acid" and CO_2; the latter was derived from the carboxyl group of malonic acid distal to the ester linkage. This degradation showed that 100 per cent of the radioactivity of the malonic acid was found in the distal carboxyl group even after 30 hr of incubation with $^{14}CO_2$. This indicates that the biosynthesis of islandic acid most likely involves the transfer of the malonyl group of malonyl-CoA (formed by carboxylation of acetyl-CoA) to either free or nucleotide-bound glucose or even to the 1,6-polyglucan. The biosynthesis of islandic- and luteic acid has therefore to be regarded as a net CO_2-fixation mechanism in micro-organisms.

EXPERIMENTAL

The organisms used were *P. luteum* Zukal strains F51, F52 and F231 kindly provided by Dr. Freeman; *P. islandicum* Sopp was obtained from the culture collection in Baarn. The fungi were grown on Czapek–Dox[2] medium containing 5% glucose. After 8 days of growth the culture medium was separated from the mycelium by filtration and the polysaccharides isolated. On addition of "Cetavlon" (cetyltrimethyl ammonium bromide) to the filtrate an isoluble cetyltrimethyl ammonium salt of the acidic polysaccharide was precipitated.[8] This complex was dissolved in 2 M NaCl and 10 vol. ethanol:ether (10:1) added. The precipitated polysaccharide was washed free from Cetavlon by additional washing with ethanol and dried *in vacuo*. The isolation of the acidic polysaccharides was quantitatively achieved, however, by a convenient novel method. First the total polysaccharide content of the culture fluid was precipitated with alcohol.[1,2] The dried polysaccharides were made up to a 1% aqueous

[5] E. EBERT and M. H. ZENK, *Arch. Mikrobiol.* **54**, 276 (1966).
[6] D. GLICK, *Methods of Biochemical Analysis*, Vol. 3, p. 213. Interscience, New York (1960).
[7] S. P. COLOWICK and N. O. KAPLAN, *Methods in Enzymology*, Vol. 3, p. 73. Academic Press, New York (1957).
[8] P. F. LLOYD, G. PON and M. STACEY, *Chem. Ind. (London)* 172 (1956).

solution, and 0·4 vol. of a 2% solution of protamine (Eli Lilly Comp., Indianapolis, U.S.A.) were added. After leaving at 4° overnight the acidic polysaccharides formed a water-insoluble protamine complex which was centrifuged off, washed with water and dissolved in 1 M NaCl. The protamine was precipitated with 3 N trichloroacetic acid, centrifuged off, and the clear supernatant dialysed for 18 hr against 15 vol. of deionized water. The acidic polysaccharide was then precipitated by ethanol and dried to a powder. This procedure, in contrast to the Cetavlon treatment, gave a quantitative yield of the acidic polysaccharides. About 6% of the glucose from the medium was converted to the acidic polysaccharides. The glucose content of the polysaccharide was determined with the anthrone reagent;[9] the amount of ester-bound malonic acid with the hydroxylamine method[10] at pH 11. The only ferric positive product of the action of alkaline hydroxylamine on the malonic acid containing polysaccharides was malonylmonohydroxamate as shown by R_f values on paper chromatograms in three different solvents: butanol–propionic acid–H_2O (750:352:498) R_f 0·37;[11] *n*-propanol–NH_3–H_2O (6:3:1) R_f 0·23[12] and isopropanol:HCOOH:H_2O (8:1:1) R_f 0·51,[13] as compared with those of an authentic sample. The experimental details of the permethylation and periodate oxidation studies have been given earlier.[5]

Acknowledgements—Our thanks are due to Dr. G. G. Freeman, ICI, Stevenston, for providing us with his strains of *P. luteum*, to Dr. W. Tanner for his aid in preparing the manuscript and the "Deutsche Forschungsgemeinschaft" for financial support.

[9] D. L. MORRIS, *Science* **107**, 245 (1948).
[10] F. LIPMANN and L. C. TUTTLE, *J. Biol. Chem.* **159**, 21 (1945).
[11] H. KAUSS, *Biochem. Biophys. Res. Commun.* **18**, 170 (1965).
[12] P. HIRSCH, *Arch. Mikrobiol.* **46**, 53 (1963).
[13] G. C. SCHMIDT, C. FISCHER and J. M. MCOWEN, *J. Pharm. Sci.* **52**, 468 (1963).