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On a New Type of Mucilaginous Material, Luteic Acid, Produced from Glucose by Penicillium luteum Zukal

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Part XIII.—*On a new type of mucilaginous material, Luteic acid, produced from Glucose by* Penicillium luteum Zukal.

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The *Penicillium luteum—P. purpurogenum* group of species of *Penicillium* described by Thom (1915), contains a number of species and strains, with a strain of *P. luteum* Zukal, at the one end of the series and *P. purpurogenum* Stoll, at the other end.

The strain of *P. luteum* Zukal, which occupies one end of this series, "produces ascospores freely in all the media used and conidia very sparingly. In the actively growing culture the dominant shades of colour are yellow with tardy appearance of red." *P. purpurogenum* Stoll, at the other end of the series, "produces only conidia, in which yellow shows transiently while red colours in mycelium and substratum are abundant." "The production of yellow in the surface growth at some period of colony development or under some cultural conditions is typical for the group. This may be dominant, transient, or almost lacking, yet it is not difficult to demonstrate in the organisms studied. Coincident with the change of colour in the surface or aerial growth we find at the *luteum* end of the series that yellow to orange shades predominate in the substratum. These slowly or but partially change to red as the colonies become old. In the forms producing conidia only, yellow or orange tones still appear in the young colony. The change to red is slow and only partial in some forms, but towards the *purpurogenum* end of the series the yellow colours are reduced to but transient appearances, replaced quickly and almost completely by red." (The quotations are from Thom's paper quoted above.)

The biochemical characteristics of the different species also vary considerably. Thus the acidity produced when the different species are grown on glucose solutions varies from negligible amounts at the *luteum* end of the series to extraordinarily high yields of acid—gluconic acid—by species at the *purpurogenum* end of the series. The strain *P. purpurogenum* var. *rubrisclerotium* Thom, No. 2670, mentioned by May, Herrick, Thom and Church (1927), gives yields of gluconic acid approximating 80 per cent. of the theoretical. The strains of *P. luteum*, giving low yields of acid, also give rise to extremely mucilaginous solutions, and the experiments described in this paper deal with the isolation and investigation of this mucilage.

The history of the culture of *P. luteum* Zukal, used in this work is as follows :—

P. luteum ZUKAL, Catalogue No. Ad. 30, obtained from the Centraalbureau voor Schimmelcultures at Baarn on 28th May, 1925.

Dr. CHARLES THOM, to whom this culture was sent for examination, wrote as follows : " No. Ad. 30 is apparently a member of the *P. luteum* group. In this case we also run up against a peculiar history, as you know. The original culture which I reported as *P. luteum*, No. 11, has been maintained for 20 years as an ascosporic form received from Prof. THAXTER, carried to Prof. WEHMER, labelled and agreed to by him as this species. The validity of this species is attacked by DERX and BIOURGE. Several times in the period during which I have kept it in culture it has separated into two lines, one of which is pale in colour, very little yellow either above or below and produces no ascospores. This form harmonises with Ad. 30 as I have received it. This strain would, in a way, satisfy DERX' contention that ascosporic forms in this series are produced by the conjugation of two strains which are separately non-ascosporic."

The following are the salient points of the carbon balance sheet prepared in metabolism experiment F 12 on Ad. 30 (see Part IV) :—

(1) Residual sugar : (*a*) By polarimeter, 0·275 per cent. ; (*b*) by SHAFFER and HARTMANN, 0·387 per cent. ; (*c*) by alkaline iodine, 0·454 per cent.
(2) Increase in acidity, 0·6 c.c. N/1 acid per 250 c.c. medium.
(3) Carbon precipitated as calcium salt by 80 per cent. alcohol, 0·477 gm.
(4) Carbon as CO_2 in solution, volatile neutral compounds, volatile acids and " carbon unaccounted for," were all negligible.
(5) Carbon as synthetic carbon in colloidal iron precipitate, 0·265 gm.

The indications from this balance sheet are that Ad. 30 forms a lævorotatory substance which is precipitated from strong alcohol as a calcium salt and which is precipitated, at any rate in part, by colloidal iron.

As a preliminary step in the isolation of the mucilaginous substance from cultures of Ad. 30 a number of test tube cultures, plugged with cotton wool, of this mould were made in 10 c.c. quantities of the usual CZAPEK-DOX solution containing 5 per cent. of glucose. These were incubated at 24° C. and tested periodically. The following general observations were made. The culture grew well at this temperature, giving rise to a white mycelium which showed very little sign of sporing. The metabolism solution became pale yellow in colour and very sticky in nature. It filtered quite clear, however, although very slowly. The sticky filtrate gave the following qualitative tests :—

(1) No coagulation on heating.
(2) Saturation with ammonium sulphate gave a sticky precipitate which coalesced and adhered to the sides of the tube.
(3) Addition of a large volume of alcohol gave rise to a white cloud which on shaking produced a white flocculent precipitate.

(4) Ferric chloride or basic lead acetate gave rise to heavy gelatinous precipitates.
(5) Biuret test negative.

These tests indicated that the substance was probably not protein in nature. Since the filtrate from the alcohol precipitate in test 3 and from the ferric chloride and basic lead precipitates in test 4 had lost all sign of stickiness, these precipitants seemed to offer methods of isolation. Comparison of alcohol and basic lead as precipitants indicated that alcohol was the more suitable. The work subsequently carried out will now be described under the following four main headings :—

(1) Preparation and general characteristics of crude material.
(2) Purification of crude material and properties of pure product, luteic acid.
(3) Acid hydrolysis of luteic acid and examination of hydrolytic products.
(4) Alkaline hydrolysis of luteic acid and examination of hydrolytic products. Preparation of luteose.

Section 1.—*Preparation and general characteristics of crude material.*

The large scale incubator described on p. 136 (Part VII), containing 60 litres of Czapek-Dox 5 per cent. glucose distributed in 12 trays, was sown on 29th July, 1927, with a spore suspension from 6 Roux bottle cultures on Czapek-Dox agar, 5 Roux bottle cultures on beer wort agar and 20 Czapek-Dox agar slopes in test tubes. The beer wort cultures provided very poor sporing growth, the suspension from these bottles containing mostly mycelium. Better growth and sporing was obtained in the Czapek-Dox agar cultures, although these were not very good. After mixing as usual with an aluminium stirrer the incubator was kept at room temperature. Aeration was started on 2nd August, 1927, and continued through the whole course of incubation. A sample from tray 8 was taken on 12th August, 1927 (after 14 days' incubation). The filtered solution contained 1·43 per cent. glucose by polarimeter and gave typical reactions with ferric chloride, basic lead acetate, alcohol and ammonium sulphate. Further samples were taken on 18th August, 1927, from trays 4 and 8.

They each gave quite good qualitative tests and the following figures for glucose :—

Sample.	Percentage Glucose by Polarimeter.	Percentage Glucose by Alkaline Iodine.	Difference.
Tray 4	0·165	0·615	0·450
Tray 8	0·098	0·547	0·449

Trays 7 to 12 were taken off on 18th August and trays 1 to 6 on the following day. As much liquid as possible was drained from the mycelium and filtered. The mycelium

itself, which was very thick and white and intensely slimy to the touch, was squeezed dry through muslin, filtered and the filtrate combined with the main filtrate. The combined filtrates were then evaporated in trays in a current of warm air to about 5 litres and an equal volume of alcohol added. By this means a large quantity of very sticky, toffee-like, precipitate was obtained. This was dissolved in water giving a very mucilaginous solution which was very difficult to filter. It was finally dealt with by diluting somewhat and filtering through a number of kieselguhr filters, giving a practically clear yellowish brown filtrate. To this was added a volume and a half of alcohol, giving rise to a white suspension which did not settle overnight. Tests on small quantities of this suspension showed that it was difficult to break up with alcohol alone, but that the addition of ether threw down the substance fairly completely. Hence one volume of ether was added with constant stirring to the alcohol-water suspension. The material separated as a pale yellowish brown mass similar to a soft toffee in appearance. This was spread in thin layers in dishes and dried in a vacuum desiccator. After drying the crude material was powdered and thus appeared as a pale yellowish brown amorphous solid. The yield of crude material was about 350 gm. (about 12 per cent.).

In a second preparation of this material, carried out later, 280 gm. of pure product were obtained (about 9 per cent.).

As no methods of purification were at the time available, and as all attempts to crystallise the material failed, the experiments about to be described were carried out in order to obtain some information as to its general properties. (Note that all these experiments were carried out on the crude material prepared as just described.)

(1) *Ash determination.*—2·5016 gm. of the material dried *in vacuo* were ashed to constant weight. Weight of ash 0·2787 gm. equal to 11·14 per cent.

Qualitative tests showed that the ash consisted principally of magnesium carbonate and magnesium phosphate.

(2) *Matter volatile at* 100° C.—2·4131 gm. were dried to constant weight at 100° C. in air. The loss in weight was 0·2875 gm. equal to 11·92 per cent. This was apparently mostly alcohol.

(3) *Optical rotation of crude material.*—The crude material was strongly lævorotatory and gave values for $[\alpha]_{\text{Hg. green}}$ in 2 per cent. aqueous solution of — 24·9°, — 26·7°, — 27·7°, — 29·9°. These values were obtained at different times and the lack of agreement between them is probably due to the varying amount of volatile matter present.

(4) *Effect of enzymes.*—200 c.c. of a 1·996 per cent. solution of the crude material having a rotation of — 1·193° in a 20 cm. tube, corresponding to $[\alpha]_{\text{Hg. green}}$ of — 29·9°, were used for testing the effect of invertase and diastase. 50 c.c. of this solution were treated with 10 c.c. of commercial invertase solution (Difco brand). Another 50 c.c. of solution were treated with 0·1 gm. of commercial diastase (Pangestin Difco brand). A little toluene was added to each flask and these, together with blanks,

were incubated at 37° C. The effect of the enzymes was followed polarimetrically with the following results :—

Enzyme used.	Incubation Period.	°Rotation in 20 cm. Tube.	Initial °Rotation.
Invertase	3¾ hours	— 0·927	— 0·994
,, ...	22 hours	— 0·910	
,, ...	3 days	— 0·926	
Diastase	4¾ hours	— 1·202	— 1·193
,, ...	22½ hours	— 1·186	
,, ...	3 days	— 1·263	

It is evident from these results that the material was not hydrolysed by either diastase or invertase.

(5) *Hydrolysis with acid.*—The crude material gave very little, if any, reduction of BENEDICT'S solution. After boiling with N/1 H_2SO_4 for some time the resultant solution gave a copious reduction of BENEDICT'S solution. Hydrolysis had obviously taken place and the progress of hydrolysis was followed quantitatively as follows :—10 gm. of crude material were dissolved in the cold in 500 c.c. of N/1 H_2SO_4. The solution was immediately polarised in a 20 cm. tube and then heated on a boiling water bath, and samples taken at intervals and polarised with the following results :—

Time of Hydrolysis.	°Rotation in 20 cm. Tube.
Zero	— 0·997
15 mins.	— 0·380
30 mins.	— 0·187
1 hr.	+ 0·143
1½ hrs.	+ 0·480
2 hrs. 10 mins.	+ 0·956
3 hrs. 15 mins.	+ 1·297
4 hrs. 15 mins.	+ 1·475
5 hrs. 15 mins.	+ 1·568
7 hrs.	+ 1·590

(6) *Products of acid hydrolysis.*—A little of the above hydrolysis solution on heating with phenylhydrazine acetate and sodium acetate for about 1 hour gave a yellow osazone in small needle-shaped crystals similar in appearance to glucosazone. In order to identify this definitely as glucosazone 10 gm. of the crude material were hydrolysed with 500 c.c. of N/1 sulphuric acid on a boiling water-bath for five hours and neutralised with N/1 barium hydroxide. After filtration the solution was heated on a water-bath with 10 gm. of phenylhydrazine, 5·5 gm. of acetic acid and 10 gm. of sodium acetate.

The osazone was collected in four crops, giving a total yield of 12 gm. It was purified by precipitation from pyridine (see NEUBERG, 1899). The optical rotation of various fractions of the osazone in pyridine-alcohol solution according to NEUBERG's method was then determined. 0·2 gm. of dry osazone was dissolved in 4 c.c. of pure pyridine, 6 c.c. of absolute alcohol added and the solution polarised in a 10 cm. tube with the following results :—

Sample of Osazone.	°Rotation in 10 cm. Tube. Mercury Yellow Line.
1st crop	— 1·474
2nd crop	— 1·365
3rd crop	— 1·334
4th crop	— 1·485
Sample of pure glucosazone prepared from pure glucose ...	— 1·475

These results indicate that the only sugar formed by the hydrolysis of this material was probably glucose. Consideration of the yields of glucose obtained seemed to indicate that glucose was not the only product of hydrolysis and with a view to settling this point experiments were now undertaken to obtain as pure a sample of the material as possible. These are described under Section 2.

SECTION 2.—*Purification of crude material and properties of luteic acid.*

The analytical figures given on p. 258 show that the crude material contained an abnormal amount of ash. An attempt was made to reduce this to a minimum. It was hoped that, because of the mucilaginous nature of the crude material, it might be possible to dialyse away the mineral matter present. With this end in view collodion dialysing tubes were made in large boiling tubes. A 5 per cent. solution of the crude material was made and 150 c.c. quantities placed in the dialysing tubes, the open ends of which were closed by corks and made watertight with a layer of collodion. They were then dialysed against running tap water. Samples were taken out after two days' and four days' dialysis respectively and ash determinations carried out. The ash content was 8·36 per cent. in the first case and 7·20 per cent. in the second case. The dialysis was stopped after four days since microscopic examination showed that the solutions had become slightly infected. The material was recovered by evaporation and precipitation with alcohol. A quantity of this material was dried and ashed and a quantitative examination of the ash made. The ash amounted to 7·64 per cent. of the dry material, was practically free from phosphate, and consisted almost entirely of magnesium carbonate or oxide. It thus appeared probable that the *crude material*

was in reality a magnesium salt of an organic acid. Hence about 30 gm. of the crude material were dissolved in 500 c.c. of water acidified with 15 c.c. of concentrated hydrochloric acid. Complete solution was thus obtained and alcohol was now added to complete precipitation. The precipitate was considerably lighter in colour than that previously obtained and was not quite so sticky. It was redissolved in water and reprecipitated with alcohol, and a portion of this was dried in a vacuum desiccator and ashed. The percentage of ash was now only 1·14 per cent. The main bulk (23 gm.) was redissolved in 250 c.c. of water and acidified with 1 c.c. of concentrated hydrochloric acid and reprecipitated with alcohol. This time the material did not coagulate and form a uniform mass nearly so readily as it had done previously. It was again redissolved in 250 c.c. of water, the solution filtered and alcohol added. The addition of two volumes of alcohol, however, though giving rise to a very dense white suspension, did not produce an appreciable separation. Two volumes of ether were added, with constant stirring, and on allowing to stand a pure white precipitate settled out. This was filtered as quickly as possible on a Buchner funnel and washed with ether without ever being allowed to dry. It was finally drained and placed in a desiccator which was immediately evacuated. It dried quickly to a white mass, rather like starch, which could be quite easily ground to a fine white amorphous powder. This material constitutes the pure product on which all subsequent experiments were carried out, and to which we propose to give the name "luteic acid."

Properties of luteic acid.—Luteic acid is a fine white, somewhat hygroscopic and amorphous powder. Its aqueous solution is strongly acid to litmus, so that it seems evident that the crude material was in reality a magnesium salt of an acid which is now designated luteic acid. Luteic acid holds tenaciously small amounts of the solvents used in its purification, and for the subsequent analytical work all samples were dried to constant weight at 100° C. in a current of dry nitrogen. By this means no alteration in colour was produced, whereas drying at 100° C. in air gives rise to a yellowish product.

Ash determination.—0·5061 gm. gave 0·0040 gm. of ash equal to 0·79 per cent.

Equivalent of luteic acid.—0·5699 gm. of luteic acid was dissolved in water and titrated with N/10 sodium hydroxide to phenolphthalein. 13·11 c.c. of N/10 sodium hydroxide were needed for neutralisation. This corresponds to an equivalent of 434·7.

Optical rotation.—An attempt was made to determine the optical rotation of luteic acid itself, but on account of the gelatinous nature of the solution this had to be abandoned and only an approximate value of the optical rotation of the sodium salt was obtained. 0·9942 gm. of the dried material was weighed out into a 500 c.c. measuring flask. The addition of about 100 c.c. of water produced a stiff gel. Addition of more water gave a very viscous liquid which did not seem to be a true solution as it was not homogeneous even after standing overnight. The calculated amount of N/10 sodium hydroxide for neutralisation was then added and the volume made up to 500 c.c. By this means a true solution was formed, of approximately 0·2 per cent. concentration, of

the sodium salt, but even this was still so viscous that centrifuging was necessary to remove bubbles before it could be polarised. The optical rotation in a 20 cm. tube with mercury green light was $-0{\cdot}187°$ corresponding to $[\alpha]_{\text{Hg. green}} = -47°$.

Action of Alkaline Iodine.—0·0718 gm. of luteic acid, dried to constant weight, was treated with alkaline iodine solution. After standing for two hours only 0·47 c.c. of N/10 iodine had been absorbed. It can therefore be concluded that the substance does not react with alkaline iodine and hence contains no free CHO groups. This conclusion is supported by the fact that the substance did not reduce BENEDICT'S solution, nor did it form an osazone with phenylhydrazine.

SECTION 3.—*Acid hydrolysis of luteic acid and examination of hydrolytic products.*

10 gm. of luteic acid were hydrolysed with 500 c.c. of N/1 sulphuric acid on a boiling water-bath for about nine hours. The sulphuric acid was removed quantitatively with pure barium hydroxide. The filtrate from the barium sulphate precipitate measured approximately one litre and was strongly acid in reaction. 10 c.c. of this solution required 4·49 c.c. of N/10 sodium hydroxide for neutralisation corresponding to approximately 45 c.c. of N/1 sodium hydroxide for the whole solution. Since 10 gm. of material would require only about 23 c.c. of N/1 sodium hydroxide for neutralisation before hydrolysis, it is evident that the acidity had been doubled during hydrolysis. The acid filtrate was now shaken with excess of pure precipitated calcium carbonate at a temperature of 50°—60° C. until the solution was permanently neutral in reaction. It was then filtered and the clear filtrate evaporated *in vacuo* at low temperature to about 50 c.c. During evaporation a quantity of white needle-shaped crystals separated (Fraction A). The filtrate from these was evaporated to 10 c.c. and 100 c.c. of pure methyl alcohol were added in portions, giving rise to a brownish flocculent precipitate (Fraction B). This was centrifuged off, washed with methyl alcohol and the methyl alcohol solution evaporated *in vacuo* to a sticky syrup which, on standing, set to a mass of yellowish brown crystals (Fraction C).

Treatment of Fraction A.

Weight = 1·5 gm.—Fraction A was recrystallised by dissolving in excess of water at about 50° C. and evaporating the solution *in vacuo*. This gave rise to a mass of white needles which were filtered off, washed and air dried. Filtrate and washings were combined with Fraction B. The calcium salt was only slightly soluble in cold water, moderately so in warm and appeared to separate in a different crystalline form from boiling water. It did not give any colour reaction with ferric chloride. A portion of the calcium salt which had been standing *in vacuo* over strong sulphuric acid was dried to constant weight at 110° C. It lost 6·99 per cent. on so drying.

0·1870 gm. of the material dried at 110° C. was heated to constant weight over a blow pipe. The weight of the residual calcium oxide, which was quite white in colour,

was 0·0707 gm., corresponding to 27·03 per cent. of calcium in the original calcium salt and a molecular weight of the free acid of 55 if monobasic, 110 if dibasic and 165 if tribasic.

Treatment of Fraction B.

Weight = 0·56 *gm.*—Fraction B was dissolved in about 100 c.c. of warm water giving a clear brownish solution. This solution was decolorised with a little blood charcoal, combined with the filtrate from Fraction A, and evaporated *in vacuo*. A further quantity of needle crystals was obtained. These were shown to be identical with the calcium salt in Fraction A and no other product was obtained from the mother-liquors.

Treatment of Fraction C.

Weight = 8·48 *gm.*—This material was divided into two parts and treated as follows :—

(*a*) About 3 gm. were recrystallised from absolute methyl alcohol from which it separated in pure white prismatic needles having the following characteristics :—(1) Melting point 149° C. ; (2) 0·7573 gm. dried to constant weight at 110° C. was dissolved in 99·7 c.c. of water. Part of this, polarised as quickly as possible, gave a rotation of +3·56° in a 40 cm. tube. Another portion to which one drop of N/1 sodium hydroxide was added gave a *steady* rotation of +1·90° in a 40 cm. tube. These correspond to an initial $[\alpha]_{\text{Hg. green}} = +117{\cdot}2°$ and a final $[\alpha]_{\text{Hg. green}} = +62{\cdot}5°$; (3) an alkaline iodine estimation gave an estimated glucose content of 0·7479 per cent. on solution of (2).

(*b*) A portion was treated with phenylhydrazine and gave rise to an osazone which separated from the boiling solution in yellow needles having a melting point of 199° C. and a rotation in pyridine and absolute alcohol solution of — 1·52° in a 10 cm. tube, using the mercury green line. The observed value for pure glucosazone is — 1·51°.

All the above figures leave no doubt that Fraction C consisted entirely of glucose. The only products of hydrolysis were thus glucose and an acid, the calcium salt of which constituted Fractions A and B. This acid was identified as malonic acid by the following tests : 1·114 gm. of the air-dried calcium salt were treated with 0·8835 gm. of hydrated oxalic acid. The calcium salt was suspended in warm water and shaken with the oxalic acid for about an hour. The calcium oxalate was filtered off and the aqueous solution evaporated *in vacuo*. There was no separation until the volume had been reduced to about 1 to 2 c.c., when crystalline crusts began to separate round the edge of the liquid. The solution was dried down in a vacuum desiccator and the crude acid sublimed in a high vacuum. The acid readily sublimed at 110° — 120° C. forming a white sublimate consisting of prisms mostly combined in rosettes. It had the following properties :—

(*a*) It was very soluble in water, giving an intensely acid solution which did not reduce potassium permanganate in the cold, gave no colour reaction with ferric

chloride and no precipitate with 2:4-dinitrophenylhydrazine or with calcium acetate solution.

(*b*) It melted at 135° — 135·5° C. after softening at 133° C. Decomposition with gas evolution took place at the melting point. Admixture with a sublimed sample of synthetic malonic acid produced no lowering of the melting point.

(*c*) A few milligrammes heated with 1 c.c. of acetic anhydride gave a greenish yellow colour, showing also a greenish fluorescence which became more marked on the addition of 1 c.c. of glacial acetic acid. This test is held to be specific for malonic acid.

(*d*) 0·0349 gm. of sublimed material required 6·77 c.c. N/10 sodium hydroxide for neutralisation to phenolphthalein, corresponding to a combining weight of 51·6. Malonic acid has a combining weight of 52.

(*e*) The sample gave the following figures on combustion:—

Weight of Substance.	Weight of CO_2.	Weight of Water.	Percentage Carbon.	Percentage Hydrogen.
0·1532 gm.	0·1946	0·0559	34·63	4·08
0·1204 gm.	0·1516	0·0240	34·35	3·90
Theoretical for malonic acid $C_3H_4O_4$	—	—	34·60	3·88

Having shown that the only hydrolytic products were glucose and malonic acid it seemed desirable to obtain some information as to the relative proportions of these two compounds in the original molecule. The first step towards this was to estimate the amount of glucose produced on hydrolysis, and this was carried out as follows:— 1·0233 gm. of luteic acid dried in nitrogen to constant weight and corresponding to 1·0132 gm. of ash-free material were hydrolysed by heating on a water-bath with 50 c.c. of N/1 sulphuric acid for 8½ hours. At the end of that time the solution was made up accurately to 99·76 c.c. The glucose content of this solution was then estimated by:—

(*a*) *Polarimeter.*—Percentage of glucose in solution was 0·8395, corresponding to 82·7 gm. of glucose from 100 gm. of the original ash-free material.

(*b*) Wood-Ost *method.*—Percentage of glucose in solution was 0·846, corresponding to 83·3 gm. of glucose from 100 gm. of the original ash-free material.

The amount of malonic acid produced on hydrolysis has not been specifically determined, but may be deduced approximately from the following consideration. On p. 262 it is noted that the hydrolytic products from 10 gm. of purified material required 45 c.c. of N/1 sodium hydroxide for neutralisation. This corresponds to a yield of 23·4 gm. of malonic acid from 100 gm. of material.

SECTION 4.—*Alkaline hydrolysis of luteic acid and examination of hydrolytic products. Preparation of luteose.*

10 gm. of luteic acid were hydrolysed by boiling for $1\frac{1}{2}$ hours under reflux with 300 c.c. of N/4 barium hydroxide. During the hydrolysis there was a copious separation of barium malonate which, at the end of the hydrolysis, was filtered off. The hydrolysis mixture was made up to 500 c.c., 25 c.c. of concentrated HCl added with constant shaking, and then 550 c.c. of 96 per cent. alcohol. There was a copious separation of a pure white amorphous material. The mixture was left at 0° C. and the precipitate filtered off later. It was then redissolved in 150–200 c.c. of hot water, cooled, 2 c.c. of concentrated HCl added, and then an equal volume of alcohol. The resultant precipitate was filtered, washed and again dissolved in hot water from which it was precipitated by the addition of an equal volume of alcohol, but in this case no acid was added. The precipitated material, to which we propose to give the name "luteose," was filtered off, washed with alcohol and ether and dried in a desiccator.

Luteose was shown to be a complex polysaccharide and had the following properties: It consisted of a pure white, light amorphous powder, which is appreciably soluble in cold water, and readily soluble in hot water giving a clear colourless solution which on cooling becomes opalescent, and in moderately strong solutions is very viscous. The aqueous solution gives no colour with iodine.

The following estimations were carried out on samples of luteose dried to constant weight *in vacuo*.

1. *Ash.*—0·1815 gm. gave 0·0003 gm. ash corresponding to 0·16 per cent.

2. *Acidity.*—Luteose is definitely neutral in reaction, since with 0·2351 gm. a single drop of N/10 sodium hydroxide was sufficient to render the solution alkaline to phenolphthalein.

3. *Optical Rotation.*—Luteose is markedly lævorotatory. Because of the fact that its aqueous solution is slightly opalescent in the cold the optical rotation was determined in a jacketed tube at 90° C. 0·5248 gm. was dissolved in 50 c.c. of water and polarised in a 20 cm. tube at 90° C. The average of a large number of readings obtained was — 0·886° for the mercury yellow light corresponding to $[\alpha]^{90^\circ\,C.}_{Hg.\ yellow} = -42{\cdot}2^\circ$, and — 0·974° for the mercury green light corresponding to $[\alpha]^{90^\circ\,C.}_{Hg.\ green} = -46{\cdot}4^\circ$.

4. *Hydrolysis by boiling dilute acid.*—0·4819 gm. of luteose was hydrolysed by boiling for six hours with 10·03 c.c. of N/1 H_2SO_4. The hydrolysis mixture was cooled and titrated with N/1 NaOH of which 10·10 c.c. were required, thus indicating that no acid was formed during the hydrolysis. The mixture was made up to 49·92 c.c., filtered, and the glucose content estimated :—

(*a*) By polarimeter = 0·967 per cent.
(*b*) By WOOD-OST method = 0·964 per cent.

Since 0·4819 gm. dissolved in 49·92 c.c. corresponds to 0·965 per cent. only small amounts of any hydrolytic product other than glucose can have been formed. This

was finally proved by converting the hydrolysis product into the osazone by treatment with phenylhydrazine and polarising the solution of the osazone in pyridine and ethyl alcohol. 0·2 gm. of the osazone (M. Pt. 205° C.) dissolved in a mixture of 4 c.c. of pyridine and 10 c.c. of ethyl alcohol gave a rotation of — 1·35° (Observed value for pure glucosazone = — 1·50°.)

5. *Combustion results.*—The following results were obtained on combustion (SCHOELLER, Berlin), on a sample dried to constant weight *in vacuo* over P_2O_5 at 50° C.:—

Weight of substance analysed.	Weight of carbon dioxide.	Weight of water.	Percentage C.	Percentage H.
3·993 mgm. (0·015 mgm. residue)	mgm. 6·345	mgm. 2·31	43·51	6·50
4·374 mgm. (0·022 mgm. residue)	6·950	2·54	43·56	6.53

We desire to express our thanks to Prof. T. M. LOWRY and Mr. MORTON for the observation that luteic acid on hydrolysis with dilute barium hydroxide gives rise to the acid-free polysaccharide, luteose, described above.

Discussion of results obtained.

The following results are of significance in attempting to obtain some idea of the constitution of luteic acid:—

(*a*) It is an acid having a combining weight of 434·7 (p. 261).

(*b*) It does not react with alkaline iodine, does not reduce BENEDICT'S solution, nor form an osazone with phenylhydrazine (p. 262). These observations show that the material contains no free CHO groups.

(*c*) It is lævorotatory (p. 261).

(*d*) It is not hydrolysed either by diastase or invertase.

(*e*) It is hydrolysed by acids giving glucose and malonic acid as the only products of hydrolysis (pp. 262–264).

(*f*) The original acidity of the material is doubled on acid hydrolysis (p. 262). This indicates that for every free COOH group present in the original material another COOH group is freed during hydrolysis.

(*g*) On acid hydrolysis 100 gm. of luteic acid give rise to about 83 gm. of glucose and 23·4 gm. of malonic acid (p. 264). A compound of two molecules of glucose condensed with one molecule of malonic acid with the loss of two molecules of water would, by hydrolysis of 100 gm., give rise to 84·1 gm. of glucose and 24·3 gm. of malonic acid.

(*h*) On alkaline hydrolysis with dilute barium hydroxide, luteic acid gives rise to malonic acid and a complex polysaccharide, luteose, which is neutral in reaction and which is lævorotatory (p. 265).

Bearing all the above results in mind it seems probable that luteic acid is a complex compound, each molecule of which is built up of a number of similar units. Each unit in its turn is a condensation product of two molecules of glucose with one molecule of malonic acid, with the loss of two molecules of water, in which one COOH group is free while the other is in combination, and in which the two CHO groups are linked in such a way as to destroy their aldehydic properties. Such a substance would have a combining weight of 428, its acidity would double on hydrolysis, and 100 gm. would give rise to 84·1 gm. of glucose and 24·3 gm. of malonic acid.

Since alkaline hydrolysis liberates all the malonic acid and produces a new polyglucose which has obviously a high molecular weight it appears that luteic acid is a complex malonyl polyglucose, perhaps somewhat similar in structure to acetyl cellulose or nitrocellulose.

Natural polysaccharides of this type, which give rise on hydrolysis to a simple acid in addition to glucose, are very rare. Curiously enough the only other well-authenticated example is also of micro-biological origin. The American workers HEIDELBERGER and GOEBEL (1927) have recently shown that in the metabolism solution of young cultures of *Pneumococcus* there is present a substance which they designated the "soluble specific substance," which has the remarkable property of being precipitated from solution by an anti-pneumococcal serum prepared from the same type of *Pneumococcus*. The same substance was also found in the blood of animals infected with *Pneumococcus*. This "soluble specific substance," which is very similar in physical properties to luteic acid gives on hydrolysis glucose and glucuronic acid. Thus the main chemical difference between these two substances is in the nature of the acids produced by hydrolysis.

The natural gums, *e.g.*, gum arabic, gum tragacanth, etc., are, of course, of a somewhat similar chemical nature, since the work of O'SULLIVAN has shown that they are complex acids built up by the condensation of pentoses and hexoses with an apparently complex acid, the nature of which has not been worked out.

Summary.

Penicillium luteum ZUKAL (non-ascosporic strain) produces as the result of its growth on glucose a mucilaginous material, luteic acid, the nature and properties of which have been investigated. This substance, the salts of which give rise to very viscous solutions, is a colloidal material of high molecular weight built up of unusual polysaccharide units, each of which is a product arising from the condensation, by the loss of two molecules of water, of two molecules of glucose with one molecule of malonic acid in such a way as to leave only one carboxyl group of the latter free. On alkaline hydrolysis luteic acid gives rise to a neutral, lævorotatory poly-glucose, for which the name luteose is proposed.