INVESTIGATION OF THE CHARACTER OF SOME OF THE JUMPS IN ACTIVATION
ENERGY OF VISCOUS FLOW IN PURE LIQUIDS AND SOLUTIONS

Part I.—Some Measurements at Small Thermal Intervals on (a) Dilute Aqueous
Alcohol (11\%) and (b) Pure Water

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(Received October 20, 1964)

The occurrence of sharp jumps (of magnitude of the order of 5\%) in the activation energy of viscous flow $E_a$, for
several pure liquids and solutions is now well-established. A preliminary attempt has also been made to apply order-

disorder phenomena to estimate specific heat anomalies expected to be associated with any second-order transitions at these
jumps. Examination of temperature variations of (1) coefficient of dilatation and (2) index of refraction have supported
the existence of some type of changes occurring at the temperatures of these jumps, and the present communication gives
a further study of the nature of these jumps by remeasuring typical steps in 11\% dilute aqueous ethyl alcohol and in water
at the much closer intervals of 0.5 C. and 0.2 C., with improved experimental technique.

For 11\% aqueous alcohol, the measurements give temperature ranges of $0.19^\circ \pm 0.03^\circ$ C. for the jumps, which is
almost equal to the smallest temperature interval ($0.2^\circ$C.) used. Similar measurements for water give an
average of $0.16^\circ$C. for the widths of the jumps. The conclusion is drawn that these widths are entirely due to the
experimental interval and because $8T/T_c$ is less than 1/1000 in this case, therefore, for practical purposes, these jumps may
be considered as discontinuous changes in activation energy $E_a$. 
COMPARATIVE MEASUREMENTS OF THE TEMPERATURE DERIVATIVES OF VISCOSITY, DENSITY AND REFRACTIVE INDEX OF PURE LIQUIDS AND SOLUTIONS

Part II.—Dilatometric, Refractive Index and Flow Activation Energy Measurements on Benzene at Intervals of 1°C. to 2°C. in the Range of 10°C. to 50°C.

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(Received October 20, 1964)

In part I of this paper, some measurements on the coefficient of dilatation β of ethylene glycol in the range of 20°C. to 80°C. showed a roughly sinusoidal variation with peak-to-peak amplitude seven times the experimental error, and period 5°C. to 10°C. The majority of minima coincided within ±1°C. with sharp jumps already observed in E/R, and a similar correlation between activation energy of viscous flow E/R, refractive index and coefficient of dilatation was earlier observed in the case of water. To elucidate these phenomena, measurements on benzene, a non-hydroxylic liquid are now reported.

The values of β×10^4 for fairly regular cyclic behaviour, and maxima are observed at 15°C., 20°C., 25°C., 27°C., 31.5°C., 37°C., 40°C., 44°C., and 49°C., which coincide exactly in position with the previously found maxima in E/R. The peak-to-peak variation is 0.8 to 1 unit, which is about 15 times the standard deviation. A comparison is made with previous data on density reported by various workers, which shows evidence of 5 or 6 maxima. Measurements are also made for the temperature derivative of refractive index with NaD' and Cd green lines, and show reasonably cyclic variation.

It is found that the peaks based on refractive index occur at slightly higher temperatures than those for E/R. and β, the average temperature excess being 1.5°C., which is one quarter of a complete cycle (4°C.). This indicates that 
\[
\frac{dE}{dT}
\]
parallel either to the first derivative or to the integral of E/R and β. Further work on aqueous alcohol solutions is in hand.

Introduction

In part I of this series, some experiments were described on the measurement of coefficient of dilatation β of ethylene glycol at intervals of 1°C. to 2°C. in the range of 20°C. to 80°C., using a dilatometer with a long calibrated capillary tube. The mean of two sets of measurements of β had an accuracy of ±0.1×10^{-5}, which is considerably inferior (in absolute terms) to that obtained in the standard data on water below 20°C. Nevertheless, a plot of our mean data showed a number of roughly sinusoidal undulations, of peak-to-peak amplitude 7 times the experimental error, and periods of 5°C. to 10°C. The majority of the minima in this plot coincided within ±1°C. with the sharp jumps already observed in the activation energy Eₜ for flow of ethylene glycol by Ahsanullah and Qureshi. This lends further credence to a similar correlation pointed out in an earlier communication in the case of (i) the dilatation, (ii) Eₜ, and (iii) the refractive index of pure water by Ahsanullah and Qureshi.

While efforts are being made to improve the precision of the dilatometric measurements on ethylene glycol, it was considered desirable at this stage to make a parallel study of a non-hydroxylic liquid, and benzene was chosen for this purpose because of its relatively large coefficient of expansion (120×10^{-5}) and because preliminary measurements have already been made on the activation energy of flow, Eₜ in the range of 8°C. to 45°C., and had shown a sinusoidal behaviour, possibly going over into steps below 18°C.

In an attempt to explore fully the nature, the inter-relationship, and the physical basis of these sinusoidal variations and discontinuities, it was thought worthwhile to undertake coordinated measurements of co-efficient of dilatation, refractive index, and activation energy. This communication presents some measurements of this type on benzene.
STUDIES IN THE ALKALOIDS OF RAUWOLFIA CAFFRA SOND

Part I.—Isolation of Ajmalicine, Ajmaline, Raucaffrine* and Three New Alkaloids, Raucaffricine, Raucaffriline and Raucaffridine

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(Received September 17, 1964)

*Rauwolfia caffra* Sonder is known to be widely distributed in Central, East and South Africa. It has also been grown successfully under an experimental plantation project sponsored by the Pakistan Council of Scientific and Industrial Research in East Pakistan. The species is now considered to include *R. natalensis* Sond. Botanically *R. caffra* and *R. natalensis* Sond. are taken as conspecific and under the name *R. caffra* Sond.; *R. inebrians* K. Schum. is regarded as a synonym. Because of the widespread distribution of the species in Africa it seems likely that the synonymy should be even more extensive. *R. obliquirostris* Stapf and *R. gnetezii* Stapf presumably also belong here (under *R. caffra* Sond.) and *R. weitschichi* Stapf and *R. ochrovioloides* K. Schum are only doubtfully distinct.

No work on the chemical constituents of the plant seems to have been carried out till Rindl and Groenewood in 1932, working on the bark of *R. natalensis* (*R. caffra*), isolated two uncrytsallised alkaloidal fractions, one of which gave the blood-red colour reaction with nitric acid, characteristic of dihydroindole derivatives.

In the same year Koepfli reported the isolation of a crystalline alkaloid from the bark of *R. caffra* to which he assigned the molecular formula C_{20}H_{26}O_{3}N_{2}, on the basis of the analysis of the base and its halogen salts. This base was quite distinct from ajmaline, giving indications of a quarternary character, and was named rauwolfine. He further reported the isolation of two insufficiently characterized crystalline bases A and B, the former of which has been recorded as showing m.p. 294.95°C. (uncorr.).

* At the time this work was in hand, Kiang and Wan, J. Chem. Soc., 1396 (1960) had also been successful in isolation of raucaffrine, which they named as perakine, from another species of *Rauwolfia*, *R. perakensis*. The identity of the two bases has been established in the present work.

**This name should not be confused with the base isolated by van Itallie and Steenhauer (Arch. Pharm.; 1932, 270, 313), also named as rauwolfine, though it was obviously identical with ajmaline reported earlier (Siddiqui and Siddiqui, J. Indian Chem. Soc., 1931, 8, 667).

More recently Schuler and Warren have reported the isolation of ajmaline and reserpine from *R. natalensis* (*R. caffra*).

Following the studies in the isolation of alkaloidal complexes from the fresh undried roots of *R. serpentina* through a technique of dialysis with organic solvents, it was considered of interest to reinvestigate the constituents of *R. caffra* using freshly harvested, undried plant material. As a result of studies in the roots and root bark of the plant, obtained through air transport from an experimental plantation in East Pakistan, and also from South Africa, the following products have been isolated:—

(i) Raucaffrine
(Identified as perakine)
C_{21}H_{22}O_{3}N_{2}.

(ii) Raucaffridine
C_{21}H_{24}O_{3}N_{2}.

(iii) Ajmalicine
C_{21}H_{24}O_{3}N_{2} + \frac{1}{2}H_{2}O.

(iv) Ajmaline
C_{20}H_{26}O_{3}N_{2}.

(v) Raucaffricine
C_{26}H_{22}O_{8}N_{2}, \frac{1}{2}H_{2}O.

(vi) Raucaffriline
C_{21}H_{24}O_{3}N_{2}.

(vii) Serosterol
C_{30}H_{48}O_{2}.

(viii) Caffrosteryl
(An uncharacterised sterol)
C_{20}H_{34}O_{2}.

It may be noted that the base, Raucaffridine, was obtained only once in the first working of the sample from South Africa, and it was not possible for the present authors to isolate Koepfli’s rauwolfine from either of the two samples.

The procedure adopted by Siddiquis in the isolation of the various alkaloidal complexes of *R. serpentina* from its alcoholic dialysates were not found practicable for the isolation of the individual alkaloid in the present case as most of the alkaloidal products remained insoluble in amyl alcohol. In the case of *R. serpentina* most of the
STUDIES ON "SILAJIT" (ASPHALT)

Part I.—Composition of the Mineral and Proteinous Matter

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(Received August, 15 1964)

“Silajit” (asphalt) has been studied with respect to its proteinous and mineral matter. The quality as well as quantity of its amino acids is indicative of its animal origin.

Introduction

“Silajit” or Momiae, a dark, sticky, bituminous substance is obtained in mountainous regions of Asia. In the Indo-Pakistan Sub-continent it is collected during the months of May to July, when the weather is very hot. The use of the drug is legendary in the East. Particularly it is an important drug of the Hindu and Muslim materia medica. It has been used for various diseases especially those of the genito-urinary system and against diabetes, gallstone, renal stone, anuria, anasarca tuberculosis, neurasthenia, eczema etc.1-3

From time to time attempts have been made to find out the chemical composition and physiological effects of the drug. Chopra et al.3 reported that the drug contains benzoic acid (18.58%), hippuric acid (6.13%), fatty acids (1.36%), resin and waxy matter (2.44%), gums (17.32%), albuminoids (16.15%) and mineral matter (34.95%).

The mineral matter has been shown to contain silica (4.6%), iron (6.51%), alumina (2.26%), lime (6.83%), magnesia (1.29%), potash (4.6%), sulphuric acid (0.54%), phosphoric acid (0.28%), chloride (0.2%) and nitrogen (5.64%). Detailed studies on the composition of the various organic fractions, however, have not so far been reported.

Therapeutically, the drug was shown to have antidiabetic action 4 and most of its medicinal effects were attributed to benzoic acid and benzoates which are present in the drug in a fairly large amount.

An important question which so far has remained unanswered in the investigations on this drug pertains to its origin. Some insight into this question can probably be gained by a thorough examination of its constituents. In the present studies, therefore, the drug has been investigated with respect to the constituents of the following fractions of the drug: (1) mineral matter, (2) proteinous matter (3) fatty acids (4) gums and (5) resin and waxy matter. This communication deals with the work on (1) mineral matter and (2) proteins. The other fractions would be dealt with in a subsequent report.

It is difficult to obtain silajit in a pure state as natural silajit contains clay, dust, etc., and the material available in the market is often adulterated with various foreign matter e.g., burnt sugar, coal-tar, pitch, etc. The silajit sample for the present investigations was especially obtained through the courtesy of Shabzada Hisamul Mulk, Governor of Drosh, Chitral State.

Experimental

Isolation of Proteins from Silajit

20 g. of silajit were extracted by hot percolation with 80 percent ethanol till the percolate was colourless. The alcohol was distilled out and the extract dried on a water bath and then under reduced pressure. The dried extract was dissolved in hot water, filtered and the filtrate shaken with chloroform, and then repeatedly with ethyl acetate to remove benzoic acid, hippuric acid and an oily substance. The aqueous solution was next heated on a water bath to remove traces of ethyl acetate, cooled and acidified with dilute hydrochloric acid, when a turbidity appeared at once. The turbid solution was repeatedly extracted with chloroform till the solution became clear. The solution was finally evaporated to dryness on a water bath. The nitrogen content of the residue obtained was 2.15 percent as determined by the Markham micro method.5

Acid Hydrolysis of the Proteins and Preparation of Hydrolysate

6-0.7 g. of the proteins were hydrolysed with 20 ml. of 6N hydrochloric acid for 24 hours. Temperature was adjusted to keep acid mixture just near boiling. The solution was filtered through sintered funnel and the residue washed with distilled water. The combined filtrate and
PREPARATION OF LINSEED FATTY ACIDS-BETULINOL ESTERS AND THEIR EVALUATION AS PROTECTIVE COATING VEHICLES

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(Received October 20, 1964)

This paper deals with a new series of esters prepared by condensation of linseed fatty acids with betulinol and extracts of Betula utilis (Bhojput) at 270-300°C. These compounds have longer induction period but ultimately become tack-free in a shorter period as compared with linseed oil. Esters prepared by partial reduction in acid value by extracts of Betula utilis and subsequent reaction with glycerol or pentaerythritol are the best in this series. The bodying characteristics and varnish-making qualities of these esters have also been studied. On the whole these esters are better than linseed oil for use in coating compositions.

Introduction

It is well known that the higher fatty acids can be readily esterified with alcohols by direct condensation at 180-200°C. This reaction has been made use of by re-esterifying the crude fatty acids of drying oils with polyhydric alcohols containing three or more hydroxyl groups. With the increase of complexity of alcohol, the rate of drying and the hardness of the film is correspondingly increased. For example, the glyceryl ester of linseed oil is better than raw oil in respect of having a shorter induction period and the pentaerythritol ester is superior in all respects. The use of meso-inositol, a cyclic hexahydric alcohol, containing only secondary hydroxy groups, is also reported to give a product which compares favourably with the pentaerythritol ester. Recent work carried out at these laboratories has shown that fatty acids can be directly esterified with betulinol or extracts of Betula utilis (Bhojput) as such but slightly higher temperatures are needed for bringing about the esterification. The present paper deals with the results of the studies carried out on preparation of linseed fatty acids-betulinol esters and their application in the field of protective coatings.

Materials

Linseed Fatty Acids.—Prepared by saponification of raw oil followed by acidification; characteristics: acid value 185, iodine value 170.

Betulinol.—Prepared from the benzene extract of Betula utilis (local name: Bhojput) by double crystallisation; melting point 250-252°C.

Benzene Extract of Betula utilis.—A yellow powder; melting point 225-230°C.

Ester Gum.—Prepared by esterification of rosin with glycerol; characteristics: acid value 14-16; melting point 98-100°C.

Preparation of Esters

150 g. of linseed fatty acids and the amount of betulinol or the Betula utilis extract desired, were placed in a four-necked 500 mL flask equipped with a constant speed stirrer, an inlet for carbon dioxide and a distillation connection to assist in removal of water. The flask was heated by a heating mantle regulated by a variac, and during the reaction, a gentle current of dry carbon dioxide was passed over the surface of the reaction mixture. After the desired temperature had reached, samples were removed at regular intervals for examination of acid value to evaluate the progress of reaction.

As a guide in selecting the temperature best suited for the study, tests were made at three temperatures to determine the effect of temperature on the rate of esterification. The data for these tests obtained with pure betulinol and benzene and 40/60 petroleum ether extracts are presented in Fig. 1. In all cases, the acid number reached a constant value in about 90 to 120 minutes. The preferred temperature for carrying out the esterification was 270-280°C, as against 180-210°C required with other alcohols. It was also observed that, where extracts had been used for esterification, the acid value could not be reduced below 40. Use of excess quantity of these extracts did bring down the acid value but the film-forming properties of finished product was found to be poor. However, the use of glycerol or pentaerythritol to bring the acid value below the desired level of 20, gave satisfactory finished products.

Properties of Esters

The following properties of the esters were determined: acid value, colour value, viscosity, refractive index, density, saponification value and iodine value. The results are reported in Tables 1 and 2.
A MOULDING COMPOSITION FROM VEGETABLE-TANNED LEATHER SCRAP

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(Received August 3, 1964)

Tanned leather scrap was hydrolysed with sulphuric acid into a plastic mass which was dried and powdered. The powder was treated with formalin, blended with saw dust and other ingredients and then hot pressed or mixed with saw dust only, cold pressed, and later on, treated with formalin. The compositions obtained exhibit the requisites of urea-formaldehyde and phenol-formaldehyde resino us compositions.

Introduction

The raw material of hides and skins amounting to 16 million pieces per year, plays a vital role in the economy of Pakistan. Shoes, belts, saddlery, bags, wallets, sports, and fancy leather goods command a large sale both at home and foreign markets. Large quantities of vegetable-tanned leather scrap are available in Pakistan as a by-product from the leather consuming industries. The work reported here, therefore, is the result of an attempt to utilise this product which otherwise finds little application at present. Already it had been shown that the scrap can be used for the production of plywood adhesives. The scrap has now been converted into a moulding composition by a process which is simple and economical. Some of the earlier processes for the conversion of leather scrap into moulding compositions consist in general of the following steps:

1. The scrap, free from non-leather matter is first hydrolysed either with sulphuric acid or hydrochloric acid or ammonia. In another process the leather scrap is first treated with phenolic solution before digestion with an inorganic acid.

2. The hydrolysed leather is converted into a plastic mass by the addition of casein, zein and formaldehyde and also formaldehyde alone.

3. The plastic mass either as such or in combination with a filler is then moulded into articles of desired shape and dimensions.

The process outlined in this communication essentially follows on the pattern of the earlier processes. However, during its development, several simplifications and modifications have been introduced with a view to making it economical and widely applicable. One such modification is that the acid hydrolysis of the scrap is carried out under pressure. The other is that the hydrolysed scrap is first converted into powder form which can be transformed, if and when required, into a moulding composition by first adding saw dust to it and then moulding it in the cold state. The moulded material is finally treated with formalin. Alternatively, the leather powder is reacted with the aldehyde in hot water to yield a composition which in combination with a filler such as saw dust, and ester-gum, urea-formaldehyde and phenol-formaldehyde resins, can be hot-pressed into any shape.

For composition of low compressive strength, ester-gum constitutes an ideal additional plasticiser and binder whereas for materials of high compressive strength, saw dust, urea-formaldehyde and phenol-formaldehyde have been found most effective additives.

Materials and Methods

1. Leather scrap: it was supplied through the courtesy of local shoes manufacturers.
2. Sulphuric acid: commercial grade, d. 1.94.
3. Formalin: commercial grade.
4. Ester-gum: the gum was prepared from rosin and glycerine according to Chatfield and others.
5. Urea or phenol-formaldehyde: the plastics were made by the method of Redfarn and Allcot and Reffarn.
6. Saw dust: it was procured from Laboratories' workshop.

Hydrolysis of Leather Scrap.—The scrap was cut into small pieces and washed with water to remove the adhering dirt. The pieces were then digested under 20 psig, pressure for 30 minutes in an autoclave with dilute sulphuric acid (1.4% V/V) in the ratio of 7:15 respectively. The resultant hydrolysed mass was washed with water to remove excess sulphuric acid and most of the tannins. It was then dried, sieved, powdered and sieved for further processing.
PREPARATION AND BIOCHEMICAL ASSAY OF PHARMACEUTICAL ENZYMES
FROM PANCREAS (A SLAUGHTER HOUSE WASTE)

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(Received June 29, 1964)

Four different methods were tried for the extraction of pancreatin from the pancreas of ox and buffalo (a slaughter house waste). Activity units of the enzyme constituents i.e. amylase, lipase and trypsin were determined in the various pancreatin powder preparations.

It was observed that the acetone precipitation method was most suitable. The product thus obtained exhibited maximum enzymatic activity and the method also proved fairly economical.

Introduction

All the enzymes, associated with the living cells of plants or animals, are proteinous in nature and defined as heat labile, biological catalysts possessing a specific power of catalysing reaction without being destroyed in the process or becoming part of the final product. In the last 30 years a great deal of work has been done in which classification, investigation, and mode of action of many enzymes have been performed. Since 1864, when pepsin was included in British Pharmacopoeia, the importance of enzymes in pharmacy and medicine has steadily increased. But there is still a good deal to learn about the medicinal aspects of enzymes, because of the 700 classified enzymes known to-day, relatively a few are important in medicine.

Pancreas of ox and buffalo which is a slaughter house waste in Pakistan is of great importance due to the presence of some important enzymes in its juice. Pancreas secretes internally Insulin and externally pancreatin. Biochemical assay shows that pancreatin consists of amylase, lipase and trypsin as the major constituents. These enzymes do not exist as a whole but are secreted as zymogen—in the duodenum in which pancreas secretion is discharged. Pancreatin in alkaline or neutral media, digests proteins, converts starch in to sugar and saponifies fats. It is used to check the destructive action of free hydrochloric acid in stomach. Medicinally it is administered by mouth to treat the pancreatic deficiency pancreatitis, fibrocystic disease of pancreas, aids in digestion and absorption of food of all types. It is also used in preparing predigested peptonized food for patients, desizing of textiles and leather hating. Trypsin (in capsule, dry powder or in ointments) is used to clean wounds, ulcers, abscesses, to remove eschars from second degree burns.

The aim of the present work is to utilize the pancreas, a slaughter house waste, for the production of pancreatin, the importance of which in medicine, has been discussed.

Experimental

Preparation of Pancreatin.—Fresh ox pancreas were freed from adhering fat and external membrane. They were stored in deep freeze. Frozen pieces were dipped in liquid air and ground into a fine powder in a pestle mortar. The powder thus obtained was macerated with 15% alcohol and distilled water separately.

Maceration with 15% Alcohol.—The pancreas powder (837 g.) was macerated in 15 percent alcohol (1500 ml.) for three days and stirred twice a day for an hour at 0°C. The liquid extracted was squeezed, filtered and centrifuged at 5000 rpm at 0°C. to remove insoluble matter. The resulting liquid (1720 ml.) is a weak solution of enzymes mostly in the form of unactivated precursors together with soluble proteins and other unwanted material. The precipitation of enzymes is induced by the addition of high concentration of salts such as MgSO₄, NH₄Cl and MgCl₂. All the procedures were conducted at temperature near 0°C, and repeated several times for complete precipitation. The precipitate was filtered and redissolved in 15 percent alcohol. After a number of precipitations the purified enzymes obtained were freeze-dried in order to have a minimum loss in potency and a final product with very low moisture content. The data in respect of the yields of pancreatin with concentrated solutions of magnesium sulphate, magnesium chloride and ammonium chloride is given in Table 1.

Acetone Precipitation Method.—Frozen pancreas pieces were powdered in the same way as in Method No. 1. The powder (279 g.) was macerated in 80 ml. of distilled water and kept continuously stirred for 6 hours at 0°C. Then it was squeezed through a cheese cloth. The extract was filtered and centrifuged at 15000 rpm.
BIOCHEMICAL AND NUTRITIONAL STUDIES ON EAST PAKISTAN RICE AND RICE PRODUCTS

Part III.—Differential Titratable Acidity and Auto-esterase Activity of Raw and Parboiled Rice and Effect on their Storage

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(Received June 29, 1964)

The effect of parboiling treatment on the titratable acidity and on the activity of esterase group enzymes was investigated on sixteen varieties of rice. The results show that in the resting stage the parboiled rice contains comparatively higher titratable acidity than raw rice but the incubation of these samples for 5 hours at 37°C. caused greater percent increase in the case of raw rice than in parboiled one. On storage the increase of acidity was less in parboiled samples than in the raw ones. Incubation of the stored samples caused similar higher percentage increase in the acidity of raw rice than of the parboiled ones. The significance of the results has been discussed in the light of the apparent relationship between the lower esterase activity of parboiled rice and paddy and their longer storage life.

Introduction

In the previous communication by Qudrat-i-Khuda, De and Debnath, it was shown that during parboiling of paddy the amylase of the rice grains is inactivated to a great extent resulting in the production of less quantity of reducing sugar in the parboiled rice in the resting stage and under incubation with water, buffer etc. for a considerable period. This was considered as one of the essential factors contributing to longer storage life of the parboiled rice and paddy.

In consideration of the above finding it was hoped that other enzymes like esterases etc. which lead to the production of titratable acids and rancidity in the raw rice causing festation by organism, mouldy taste and bad appearance as reported by Kik and Williams, Hunter et al., and Houston and others, may be severely inactivated by parboiling treatment. With this possibility in view the present work has been undertaken and the results of titratable acidity values of rice as affected by parboiling are presented in this communication. The effect of storage on the above values of raw and parboiled rice are also presented so as to correlate the parboiling treatment with the storage life of rice.

Experimental

Sixteen varieties of paddy were collected in the month of February 1961, and unmilled raw and parboiled rice samples were prepared from these varieties according to the traditional technique detailed in the previous communication.

The rice samples, ground to 100 mesh powder, were then used in all the determinations detailed in the following:

Estimation of Titratable Acidity of Raw and Parboiled Samples.—5 g. of the powder were thoroughly shaken with 100 ml. distilled water for 2 hours with a 5 ml. of 1:1 alcohol and acetone mixture, previously neutralised with phenolphthalein, so as to inactivate the esterases and other enzymes and thus to prevent formation of acids during the period of shaking. The extracts were then titrated against 0.01 N-NaOH and the acidity expressed in terms of ml. of NaOH required.

The general method of determining the titratable acidity by extraction with boiling water, which easily destroys the enzymic activity, was avoided for the reason that by such hot extraction non-enzymatic hydrolysis could also occur which would yield results that might not correlate with the values obtained by incubation of same samples at 37°C, as employed for assessment of autoesterase activity detailed in the following.

Evaluation of Auto-esterase Activity.—The titratable acids as mentioned above represent the inorganic acids mainly phosphoric acid, organic acid produced by the breakdown of starch through Embden Meyerhof and Kреб's cycles, and the fatty acids (mostly the short chain water dispersible ones). The enzymes involved in the process mainly belong to esterase groups like phosphatase (acid), hexose phosphatases, phytase and lipase. In the present investigation the measurement of the above group of esterases has been effected by determination of the titratable acidity after incubation of the samples at 37°C. Since the activity of the enzymes was not determined by measurement of the water dispersable titratable acidity after incubation of the exogenous substrates but only by allowing the tissue enzymes to
PECTIC ENZYME OF PENICILLIUM FREQUENTANS INVOLVED IN THE RETTING OF JUTE

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(Received December 11, 1963)

Penicillium frequentans secreted a pectic enzyme in the culture media with or without pectin. Of the different substrates, pectin, sodium-pectate, de-esterified pectin, starch and albumin tested, the enzyme was active only on the substrates, pectin, sodium-pectate and de-esterified pectin. The activity was optimum at pH 3.2 and 5.0 and at a temperature of 23°C, but it deacreased with dilution and was not inhibited on dialysis. The enzyme macerated both potato discs and jute barks and retted jute stems. It has been identified as a mixture of pectin-polygalacturonase (PG) and pectin-methylase-cerase (PME). The mechanism of jute retting in terms of hydrolysis by the above enzyme has been discussed.

Introduction

Loosening of jute fibre in jute stems (Corchorus capsularis and C. olitorius) is accomplished by a process known as "retting". During this process the fibres bundles from the cortex and wood are separated, and a partial digestion of the cementing material (mostly of pectic nature) between the fibres in the bundles is effected. According to Kertesz,¹ Mitscherlich first attributed these changes to the pectic enzymes produced by microorganisms, but comparatively little is known about these enzymes. He further added that while there are many organisms capable of attacking pectin and performing retting, only a few have been used for producing enzymes, which are capable of retting in the absence of living organisms. Katagari and Makaha compared the pectin decomposing enzymes produced by retting bacteria and found that crude preparations showed remarkable specificity in their actions towards various fibre plants. Baruah and Baruah² reported that an enzyme mixture named, Hiparol, secreted by Thielaviopsis paradoxus (De Seynes) V. Hohn were more active than bacterial enzymes in the retting of jute and coconut husk fibres. Kertesz³ suggested that a thorough reconsideration of the role, as well as the use, of pectolytic enzyme in the retting of plant fibres would be most desirable.

This investigation, was undertaken to: (1) study the nature of the jute retting enzyme of a fungus, Penicillium frequentans, which retted jute stems in moist conditions in a short time and was isolated and identified in this laboratory, and (2) ascertain the feasibility of utilizing these enzymatic preparations under controlled conditions of retting.

Materials and Methods

The fungus identified as Penicillium frequentans was isolated from a decaying filter paper. The culture filtrate was prepared by growing the fungus for 2-4 days, on an autoclaved liquid medium, containing glucose 3%, peptone 0.5%, NH₄NO₃ 0.16%, K₂HPO₄ 0.34%, and MgSO₄ 7H₂O 0.19% and then filtering the culture, as reported by Gupta. This culture filtrate was designated as the pectic enzyme preparation.

The enzymic activity of this preparation was determined by measuring the viscosity fall of different substrates, albumin, starch and apple pectin. To 90 ml. of each of 1% solution of starch, 10% (v/v) solution of albumin and 0.5% solution of pectin, 10 ml. of the enzyme preparation were added and allowed to stand for one hour. The viscosity fall was measured with Ostwald’s viscometer (16 seconds). For determining the relationship of enzyme production with dry weight of mycelia, the fungus was grown in the medium adjusted to different pH values, its dry weight was determined and the enzymic activity of the culture filtrate was measured viscometrically, using 0.5% pectin as substrate. For studying the effect of pectin in the medium on the production of enzyme, 1% pectin was incorporated in the medium and the enzyme activity was measured viscometrically.

The effect of pH on the enzymic activity was studied in two ways: (1) the mixture of culture filtrate and the substrate (0.5% pectin) in the ratio of 1:9 was adjusted to different pH values and buffered with acetate and phosphate, (2) the enzymic activity was determined by measuring the released reducing sugar from the same pectin solution, using Stiles, Peterson and Fred's method.

For determining the effect of temperature, the enzyme substrate mixture (10:90) was incubated at different temperatures for 20 minutes and then the released reducing sugar was measured.

The effect of enzyme preparation on the hydrolysis of pectin, sodium pectate and acid de-esterified pectin was studied by allowing the enzyme substrate mixture (10:90) for 20 minutes at 30°C, and then measuring the hydrolysis with viscometer and
THE STUDY OF THE ALIMENTARY TRACT OF SCHISTOCERCA GREGARIA (FORSKAL) (ORTHOPTERA : ACRIDIDAE)

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(Received August 5, 1963)

The average length of gut in normal position was 44.8 and 51.8 mm, in male and female Desert locust, respectively. Histologically the alimentary tract was surrounded by peritoneum, muscular layers and epithelium throughout its length. The development of muscular and cuticular layers was found to be maximum in the fore-gut. Longitudinal muscles in mid-gut were outside the circular muscles. The mid-gut and the hepatic caeca were the only part of the gut having columnar ciliated cells. The peritrophic membrane was non-cuticular ring.

Histologically hind-gut resembled the fore-gut except that the six longitudinal muscles were external to the circular muscles.

Introduction

The vast damages done by the attacks of the Desert locust, Schistocerca gregaria (Forskål) on different parts of the world do not need any introduction. They are voracious eaters and consume lot of green vegetation. In order to understand their nature of tremendous food consumption, research work on their feeding habits in relation to the physiology of the digestive system was undertaken. Before starting work on the physiological aspect of any organ it is important to have a complete understanding about its structure. Chauvin did some work on the anatomy of the digestive system of Desert locust, in which he studied the pigmentation of cells in the crop, gizzard and rectum. He paid little attention to the structure of alimentary tract, except reporting that it consisted of a typical structure of an Acridine constituting epithelium, muscular layers and intima. Hence it was found necessary to investigate the external and internal structure of different parts of gut before undertaking the work on physiology.

Material and Method

The locusts were kept in wooden cages of size 40" x 27" x 24", which were fitted with two electric bulbs of 200 watts each, at the top of the cage to maintain 33±5°C temperature. They were fed on cabbage leaves, but during nymphal stages they were also given artificial diet consisting of glucose, yeast and bran in the ratio of 2:1:2. The preserved as well as fresh specimens of locust were employed to study the anatomy. For histological work fresh four-day old adult locusts were used. The locusts were starved before cutting the sections, as sand particles in the gut were a hinderence in getting good sections. Preservation was done in 70% alcohol. The alimentary canal was fixed in Bouin's fixative after being freed from tracheae and Malpighian tubules. Tissues were embedded in paraffin at 50°C, melting point. The sections were cut at 3 to 4 microns and were stained with haematoxylin and eosin.

Results and Discussion

The external morphology of the alimentary canal was found to have all the characteristic features of Acridine, Fig.1. It was a large cylindrical tube of epithelium surrounded by muscular coat. In normal position the average length of gut in male was about 44.8 mm, while in female it was about 51.8 mm. It was slightly longer than the body length. The diameter of the tract was variable depending upon the gut contents. Pharynx, oesophagus and colon were of minimum diameter. It was clearly divisible into fore-gut, mid-gut and hind-gut. The main salivary ducts united after entering the head. Similar findings were reported by Albrecht in the grasshopper, Nomadacris septemfasciata (servi). Mid-gut was shorter than fore and hind-gut. The anterior lobes of caeca were larger than the posterior lobes. The combined length of both the lobes was a little smaller than that of the mid-gut (Table 1). Hodge has described the caeca of the same length as that of the mid-gut of Melanoplus differentialis.

The diameter of the caeca was less than half of the mid-gut. The data for the average measurement of the different regions of the alimentary canal are given in Table 1.

Fore-gut.—The fore-gut extending from the posterior end of the cibarium, approximately reached up to the first abdominal segment, Fig. 2. The cephalic region of the fore-gut was supported by
COMPARATIVE STUDY OF THE ZOEAE OF THE SAND CRABS, PHILYRA CORALLICOLA (ALCOCK), PHILYRA GLOBOSA (FABRICIUS) AND DESCRIPTION OF FIRST ZOEA OF LEUCOSIA PUBESCENS (MIERS) (DECAPODA: CRUSTACEA)

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(Received August 3, 1964)

The paper deals with the comparative study of first zoal stages of three crabs of subtribe Oxyystomatinae found in Karachi. Zoecae of Philyra corallicola and Leucosia pubescens were obtained by rearing the crabs in the laboratory. Description of zoa of P. globosa was taken from Chhapgar for comparison. Measurements of zoae were taken which indicate that zoae of P. globosa is larger than zoae of P. corallicola. Significant structural differences are also recorded.

Introduction

The first zoal stage of every species of crabs can be distinguished by its specific individual characters. It can be obtained by having the eggs hatched in the laboratory. Once the identification is established it can be further collected from the plankton for obtaining the rest of the zoal stages by molting. In view of this the authors have made comparative study of first zoae of the three different species of subtribe oxyystomatinae found on Karachi sandy shore and offshore waters. There is a profound transformation at metamorphosis in crabs and the prezoea, zoa and megalopa stages are quite distinct from each other. The fundamental structural peculiarities possessed by the first zoae are retained without much change throughout the subsequent zoal stages. This makes the identification of the first zoae of any species a useful starting point to envisage the possibilities of later development.

No study of this type has so far been reported from Karachi but in India, Mc Cann, Menon, Naidu, Chacko, Prasad and Tampi have worked on the life histories of some species of crabs and the study of zoa was made. This paper includes the complete description of zoa of Leucosia pubescens and P. corallicola.

Materials and Method

Collection was made from Sandspit, Hawkes Bay, Manora Island, and Korangi Creek, Karachi, and berried female were brought to the laboratory in sea water. Rearing experiments were done during summer of 1962, in the laboratory of the Marine Fisheries Department. Crabs were kept in glass aquaria with sea water. They were fed on bits of shrimps, mussel, and fish. The eggs, dark brown in colour, were found to be well developed when captured. They did not hatch until after four days. Pre-zoae were obtained soon after the hatching but they were not markedly different from each other and hence left out of comparison. The first zoae of P. corallicola and L. pubescens lived for 80 hours, and were then preserved. Drawings were made with the help of camera lucida. Measurements were taken with the help of micrometer eye piece. Description of zoa of P. globosa is based on the study of its life history by Chhapgar.

Description of First Zoea of P. corallicola.—

The first zoal larva (Plate 1, Fig. 1) is characterized in this species by the presence of a long spine directed vertically upward on the thorax, and by the spine-like rostrum directed vertically downward. The larva swims with the help of first and second maxillipeds. It has a short angular carapace and a slender, segmented abdomen. The eyes are strongly pigmented. There are well-developed masses of pigment on the dorsal side of the abdominal segments. The first maxilla (Fig. 7) consists of a short, narrow and bilobed exopodite. The inner lobe bears 8 setae and outer one bears 3. The one-lobed endopodite bears 5 setae. First and second maxillipeds (Figs. 4 and 5) are large and with exopodites equipped with terminal long feather-like bristles. The first maxilliped (Fig. 5) consists of a short base with an exopodite of two segments bearing four long plumose setae and an endopodite of five segments each of which bears one seta except the apical segment which bears 4 large terminal and one small proximal setae. There are 6 plumose setae on basipodite on its inner side. The second...
SHORT COMMUNICATION

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(Received August 26, 1964)

NON-SPECIFIC INHIBITION OF THE RAT UTERUS BY HEPARIN

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(Received May 29, 1964)

STUDIES ON SHARK LIVER OIL

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