KINETICS AND MECHANISM OF PERSULPHATE POLYMERIZATION OF ARCRYLONITRILE IN THE PRESENCE OF CHLORIDE IONS

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The kinetics and mechanism of polymerization of acrylonitrile were studied using potassium persulphate as an initiator. The parameters varied were the pH of the medium, and initiator and monomer concentrations. The effect of the chloride ions on the reaction rate was also investigated and it was observed that the chloride ions retarded the rate of polymerization. Further, the reaction was found to be second-order in order.

Introduction

During the past 20 years the kinetic studies of polyacrylonitrile have assumed a position of prime importance in the field of high polymers. The kinetics of acrylonitrile polymerization initiated by potassium persulphate, the kinetics of solution polymerization of acrylonitrile,5 and the photochemical initiation of polymerization of acrylonitrile by persulphate6 has been studied.

The present investigation was designed to observe the effect of pH, maintained by H2SO4 or HCl, on the rate of acrylonitrile polymerization initiated by potassium persulphate. The two mineral acids were selected with a view to determining the effect of sulphate or chloride ions under similar hydrogen ion concentrations. The choice of monomer was due to its great industrial importance in the preparation of synthetic fibres.

Experimental

Materials.—The following chemicals were used. Acrylonitrile (B.D.H.), potassium persulphate (E. Merck), sulphuric acid (B.D.H. Analar), hydrochloric acid (B.D.H. Analar), sodium chloride (May & Baker), sodium hydroxide (May & Baker).

Acrylonitrile was further purified by distillation under reduced pressure in order to remove inhibitor. The purity of the middle fraction was checked by measuring its physical constants (d25 0.8001, nD25 1.3885). These values agreed with those reported by Blout and Hokenstein.7

Procedure.—In order to investigate the kinetics and mechanism of polymerization three parameters, i.e. pH of the medium, initiator concentration and monomer concentration, were varied in turn keeping the other two constant. Further, the effect of chloride ions on the rate was studied. The rate was followed dilatometrically. Before degassing the reaction mixtures, the pH values were measured by means of a Cambridge pH-meter. The variation in pH was made with the addition of HCl and NaOH or H2SO4 and NaOH. The reaction mixture at the required pH value was filled in bulb B (Fig. 1) which was then attached to a high vacuum line. The solution was degassed by at least two cycles of freezing, pumping to 10^-5 mm and melting. While the solution was frozen in B, the stop-cock S1 was closed and the apparatus was detached from the vacuum line. Dilatometer was filled by inverting the bulb B and after closing stop-cock S2 it was detached at the point A from the bulb B.
ACID HYDROLYSIS OF PHENYL ACETAMIDE IN MIXED SOLVENT

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The kinetics of acid hydrolysis of phenyl acetamide has been studied in alcohol–water and dioxane–water in the ratio 50:50 at different temperatures (35–60°C). Since the acid hydrolysis is somewhat slow, high concentration of acid i.e. 3N HCl has been used in every experiment. In all cases the reaction was found to follow the first order kinetics. However, a break was noticed in the plot of log (a–x) vs. time giving two distinct straight lines. Probable mechanism of hydrolysis has been discussed. The energy of activation and the frequency factor were calculated in the two different solvent systems.

Though the acid and base hydrolysis of both aliphatic and aromatic amides has been investigated, the kinetics of hydrolysis of an amide with benzene ring substituted at α-position to carbonyl carbon atom has not been fully investigated. In the hydrolysis of phenyl acetamide in acid medium, Ali1 observed the phenomenon of maximum hydrolysis rate, as has been found in the case of other amides.2–5 Like other aliphatic amides, phenyl acetamide also follows first order rate equation. Ali indicated the existence of a break in the line of log (a-x) vs. time, suggesting a change in the mechanism at the later stage of hydrolysis.

In the present investigations the existence of a break when the hydrolysis reaction is carried out in mixed solvents, alcohol–water and dioxane–water, in the temperature range of 35° to 60°C has been established.

Experimental

Phenyl acetamide, prepared from phenylacetic acid, was repeatedly recrystallized to obtain a product with a m.p. of 157°C.

The hydrolysis reaction was carried out at 40°, 50° and 55°C in dioxane–water, and at 35° 45° and 60°C in alcohol–water in the ratio 50:50, keeping the acid concentration (3N HCl) and amide concentration (0.1M) constant.

Exactly 0.1M solution of phenyl acetamide was prepared. Measured quantity of the reaction mixture was then transferred to Pyrex test tubes. The attachment of the reaction tube to the holder and the immersion of the same in a constant temperature bath took about 20 minutes. The time required for filling process was maintained in all the experiments.

The reaction tube was removed at suitable intervals, and the contents analysed for ammonia. Sorensen’s formal titration6 method, with essential modifications, was used for the determination of ammonia. Formaldehyde reacts with ammonium salts in a neutral solution forming hexamethylenetetramine and liberating an equivalent amount of mineral acid which is then titrated with standard base.

6H₂CO+4NH₄Cl → 4HCl+6H₂O+N₄(CH₂)₆

Result and Discussion

The reaction was found to be of first order both from log (–dc/dt) vs. log c plots as well as from the linear plot of log (initial slope) against log c.

All the aliphatic amides so far studied2–3 have been shown to have first order hydrolysis rate. Our results is quite in harmony with them. All the aromatic amides have been found to follow second order law both in acid and base hydrolysis.7–8

Figures 1–4 show the log (a–x) vs. time plots in alcohol–water and dioxane–water at different temperatures.

There are breaks in the graphs, the experimental data falling clearly on two straight lines. The extent of hydrolysis at the point of break for different temperatures and in different solvents are given in Table 1.

The breaks occur only after 50% hydrolysis. Such a break has not been reported so far. Our observation can hardly be overlooked since the phenomenon occurs in every experiment, at different temperatures as well as in different solvents. The break in these plots suggests that there are possibly two mechanisms by which the amide hydrolysis takes place. Since similar behaviour is exhibited in both the solvent systems, the same mechanism occurs in both the cases.

The specific rate constant is calculated independently from the slope of both the straight lines. The rate constant of the first straight line is de-
KINETICS AND MECHANISM OF SULPHOXIDE OXIDATIONS

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Oxidation of diphenyl sulfoxide, \(p,p'\)-dimethyl-, \(p,p'\)-dimethoxy-, \(p,p'\)-dichloro-, and \(p,p'\)-dinitro-diphenyl sulfoxides with peroxybenzoic acid in toluene, acetone and methanol at 25°, 35° and 45°C (±0.5°) has been studied. Activation energies, frequency factors and entropies of activation for these oxidations were calculated. The differences in rate constants have been found to be approximately 55% dependent on differences in energy of activation and 45% on differences in entropy of activation. The rate constants have been observed to be inversely proportional to the dielectric constants of the solvents employed.

In an earlier study it was shown that the oxidation of sulfoxides to their corresponding sulfones follow second order kinetics. It was further pointed out there that electron donating groups in the \(p\)-position of the peroxybenzoic acid decreased the rate of oxidation while electron withdrawing groups increased it; reverse effects were shown to be exerted by these groups when present in the \(p\)-positions of the \(p,p'\)-disubstituted diphenyl sulfoxides.\(^1\) It was, therefore, concluded that in these oxidations there is a nucleophilic attack by the sulphur atom of the sulfoxide on the oxygen of the peroxy acid. This conclusion was contrary to the observation by Szmant, Harusberger and Krahe\(^2\) who reported that the oxidation of diphenyl sulfoxide by peroxybenzoic acid involved a nucleophilic attack of the latter on the sulphur atom of the former, although there was no evidence on this point.

In continuation of our previous work,\(^3\) therefore, the kinetics of the oxidation of diphenyl sulfoxide, \(p,p'\)-dichloro-, \(p,p'\)-dinitro-, \(p,p'\)-dimethyl- and \(p,p'\)-dimethoxy-diphenyl sulfoxides by peroxybenzoic acid have now been studied in various solvents and at different temperatures. These studies have been carried out with a view to obtaining a clearer insight into the mechanism of sulfoxide oxidations.

**Experimental**

Absolute methanol, toluene and acetone used as solvents in these oxidations were purified according to the standard procedures.

Peroxybenzoic acid, diphenyl sulfoxide and \(p,p'\)-disubstituted diphenyl sulfoxides were prepared as described previously.\(^4\) The peroxybenzoic acid was prepared just before use in the kinetic runs, dissolved in the desired solvent and stored in a refrigerator (0°). The acid was iodometrically standardised and the solutions of different concentrations were then prepared by dilution. All the five sulfoxides were purified by crystallisation to constant melting points.

Kinetic measurements were carried out with solutions (50 ml) of each sulfoxide and peroxybenzoic acid in the desired solvent at 25°, 35° and 45°C (±0.5°). The temperature was thermostatically controlled and for each run a blank (containing only peroxybenzoic acid solution and no sulfoxide) experiment was also carried out to account for the loss of peroxybenzoic acid. The progress of oxidation was followed iodometrically at regular time intervals as described before.\(^5\)

**Discussion**

The rate data for the oxidation of diphenyl sulfoxide, \(p,p'\)-dimethyl-, \(p,p'\)-dimethoxy-, \(p,p'\)-dichloro-, and \(p,p'\)-dinitro-diphenyl sulfoxides with peroxybenzoic acid in toluene, acetone and methanol were obtained at three temperatures i.e., 25°, 35°, and 45°C (±0.5°). In all the cases, second order law was observed to be obeyed accurately. The activation energies and frequency factors were calculated from the variations of \(\log k\) with \(1/T\). The entropy of activation, \(\Delta S^{\circ}\), was calculated from the frequency factor \(\Lambda\) by means of the equation:\(^6\)

\[
\Lambda = e \left( \frac{k}{{h}} \right) \left( \exp \Delta S^{\circ} / R \right)
\]

The rate constant data alongwith \(E\), \(\ln \Lambda\) and \(\Delta S^{\circ}\) as obtained in these studies are given in Table 1. The experimental data employed in the determination of the order of these oxidations are recorded in Table 2. This data relate to all the five sulfoxides that have been studied in the three solvents at the three temperatures. Second order rate plots were excellent straight lines and some typical representatives are shown in Fig. 1; several representative Arrhenius plots are shown in Fig. 2. A plot of \(E\) versus \(T \Delta S^{\circ}\) for these oxidations in toluene, acetone and methanol at 25°
ACTION OF CARBON TETRACHLORIDE VAPOUR ON SULPHIDES

Part I.—$\text{Sb}_2\text{S}_3$ and CdS

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In the temperature range 200-500°C, carbon tetrachloride vapour reacts with $\text{Sb}_2\text{S}_3$ and CdS according as $2\text{Sb}_2\text{S}_3 + 3\text{CCl}_4 = 4 \text{SbCl}_3 + 3\text{CS}_2$ and $2 \text{CdS} + \text{CCl}_4 = 2 \text{CdCl}_2 + \text{CS}_2$. Formation of metal chlorides were quantitative; but that of CS$_2$ varied between 80.0-92% in the case of $\text{Sb}_2\text{S}_3$ and 84.5-97.9% in the case of CdS depending on temperature. The lower yield was found to be due to a side reaction (involving CS$_2$) that produces sulphur chlorides at lower temperatures up to 400°C. Above this temperature, the side reaction is minimum and only very small amounts of elementary sulphur were formed. No CS$_2$ was traced in the products. Optimum temperature was 450°C for both the reactions.

The usefulness of carbon tetrachloride as a chlorinating agent for inorganic compounds has been realised only recently, although some old references of a qualitative nature are available. Cambouilves$^1$ as far back as 1910 reported the action of carbon tetrachloride on various metal oxides, and indicated the temperatures at which the reaction started with different oxides. The information was of a qualitative nature, but the author suggested the formation of COCl$_2$. The recent trend of reducing metal chlorides by active metals has opened up the feasibility of chlorination of refractory oxides, particularly with carbon tetrachloride. Thus by the action of CCl$_4$ vapour, niobium and neptunium oxides have been effectively chlorinated. $^2, 3$ recently other oxides such as TiO$_2$, BeO, ZrO$_2$ and ThO$_2$, have also been converted to their corresponding chlorides by the action of CCl$_4$ at relatively lower temperatures. $^4$

The literature relating to the action of CCl$_4$ vapour on sulphides is still very meagre. Air-CCl$_4$ mixtures have been used to breakdown several sulphide minerals, the products being absorbed and oxidised in a suitable solvent. $^5$ Carbon tetrachloride reacts with iron pyrites at 320-400°C giving a mixture of FeCl$_3$, FeCl$_4$, FeS, S, C and CCl$_4, 2$ but with stibnite (apparently through mistake it was noted as stibine) at 300-320°C the reaction produces SbCl$_3$ and CS$_2$. Hydrogen sulphide reacts with CCl$_4$ at red heat to give thiocarbonyl chloride. $^7$ No information is available regarding the action of any other sulphide with CCl$_4$.

With gaseous chlorine $^8$ or even solid anhydrous ferric$^9$ or cupric $^{10}$ chlorides, antimony sulphide has been known to produce antimony trichloride. With CCl$_4$, although the formation of SbCl$_3$ may be anticipated, the fate of sulphur is not clearly understood and the formation of different carbon-sulphur compounds is probable.

Theoretical considerations suggest the possibility of formation of CS$_2$ with a number of metal sulphides at relatively low temperatures, and with H$_2$S at below 1500°C. This paper reports the results obtained with antimony and cadmium sulphides.

Thermodynamics

The thermodynamic feasibility of the reactions were estimated from the $\Delta G_T$ values for the formation of the different compounds calculated from the simple equation, $\Delta G_T = \Delta H_{298} - T\Delta S_{298}$. The values for $\text{Sb}_2\text{S}_3$ and SbCl$_3$ were taken from an earlier paper. $^{10}$ For the rest of the compounds, $\Delta H_{298}$ values were taken from Rossini et al. $^{11}$ For the different entities, S$^\circ$ was taken from Rossini et al., except for S$_2$(g) which was from Kubaschewski and Evans. $^{12}$ for ease of calculation, the values were rounded off to first decimal place. In the case of CdCl$_2$, the S$^\circ$ values quoted by the above two sources varied somewhat and 30.0 cal °C was chosen as a fair average.

The following data were used for calculation:

<table>
<thead>
<tr>
<th>Entities</th>
<th>CS$_2$</th>
<th>CCl$_4$</th>
<th>CdS</th>
<th>CdCl$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta H_{298}$</td>
<td>27.550</td>
<td>-25.500</td>
<td>-34.500</td>
<td>-93.000</td>
</tr>
<tr>
<td>$S^\circ_{298}$</td>
<td>35.8</td>
<td>74.0</td>
<td>17.0</td>
<td>30.0</td>
</tr>
</tbody>
</table>

The S$^\circ$ for C (graphite), S$_2$(g), Cl$_2$(g), and Cd(s) were taken to be 1.36, 54.4: 53.3 and 12.3 cal./°C respectively.

The following expressions were derived.

\[
\Delta G_T: \text{CS}_2 = 27.550 - 1.04 T
\]
\[
\Delta G_T: \text{CCl}_4 = -25.500 + 33.96 T
\]
\[
\Delta G_T: \text{CdS} = -34.500 + 22.50 T
\]
\[
\Delta G_T: \text{CdCl}_2 = -93.000 + 35.60 T
\]
RESERPINE ANALOGUES: SYNTHESIS OF DIBENZOQUINOLIZINE AND ISOQUINOLINE DERIVATIVES

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2,3,10,11-Tetramethoxy-12-carboxymethoxy-5,6,13,13a-tetrahydro-8H-dibenzo(a,g) quinolizine and two 5-carboxymethoxy-6,7-dimethoxyisoquinolinoine derivatives have been prepared for evaluation as reserpine analogues.

A novel approach to the dibenzo(a,g) quinolizine derivative has been made, the C ring being prepared first as the lactam and the latter cyclized.

Introduction

Apart from the analogues based on the intact reserpine skeleton and the simpler amine esters and amides of trimethoxybenzoic acid the more obvious portions of the reserpine molecule taken as models were the β-carboline and the indoletryptamine moieties. These compounds did not prove very useful as far as their reserpine-like properties were concerned. Thus the indoloquinolizine and the dibenzoquinolizine analogues, the latter of which may also be described as the despyrroloreserpine derivatives, have been examined of late as reserpine analogues. The dibenzoquinolizine nucleus, which occurs in the physiologically active berberine group of alkaloids, has been examined by several workers. Muller and Allais1 reported the synthesis of 10-methoxy-despyrroloreserpine (I, R = H). This compound and its 2α-iso derivative, corresponding to 2α-isoreserpine, were also reported by Protiva et al.2 Pelz et al.3 went further on to prepare the mescaline analogues of the above (I, R = MeO). According to preliminary pharmacological reports3 the mescaline derivatives exhibit activity similar to reserpine and isoreserpine. However, all these compounds may be presumed not to have been very effective. The fact that these compounds had the ring fully hydrogenated, coupled with the observations that some dibenzoquinolizine derivatives with the ring D aromatic, e.g., palmatine, have been described as hypotensive agents4 and there has been interest, of late, in other such derivatives as blood pressure lowering agents, e.g., canadine methocyanate5 and N-methylrhodanide,6 suggested to us that, keeping the ring D aromatic, the siting of a carbomethoxy group at C-16 of reserpine may result in compounds with enhanced reserpine-like properties. The despyrrolo compound IV was therefore prepared. It might be argued that for greater similarity in structure with reserpine it was preferable that the function at C-10 was the trimethoxybenzoyl ester, but from a consideration of the results of Logemann et al.,7 confirmed by Nogradi,8 who had shown that for the analogues having the reserpine skeleton with the ring E aromatic such esterification was contraindicated, the converse argument could likewise be put forward.

In the course of this synthesis a novel approach to the desired dibenzo(a,g)quinolizine derivative has been made, the ring C being prepared first as the lactam and the latter cyclized. The usual method for the preparation of the dibenzoquinolizine derivatives of the protoberberine type, to which the desired compound belongs, consists of condensing the benzylosiquinoline derivatives with formaldehyde. The synthesis of norcorydaline by Walker9 is a typical example. It can be schematically represented as below (scheme a). In this process rings A, B and D are formed first and the final condensation produces the C ring. Our method (b) consists of the condensation of homoveratrylamine with methyl 2-carboxymethoxy-3,4-dimethoxy-6-chloromethylphenyl-acetate (II) to give the lactam III which was cyclized with phosphorus oxychloride to the quaternary salt. Reduction with sodium borohydride furnished the final protoberberine derivative IV. The overall yield in this process was 90% which compares favourably with that in the usual method (ca. 25%), the calculations being based on the initial condensations to give the amide and the lactam. An additional advantage of the new process is that there is no scope of isomerides being formed.

Besides the compounds III and IV, the n-propyl analogue of the former has been prepared by us.
ISOLATION OF A NEW COMPOUND FROM LAVANDULA STOECHAS LINN

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A new crystalline substance, lavanol, C_{30}H_{46}O_{3}, m.p. 280-84°C, $[\alpha]_D^{33}+25^\circ$ (THF) has been isolated from Lavandula stoechas.

Lavandula stoechas Linn. (locally called ustukhud-dus) has been used as a drug in the indigenous system of medicine for the treatment of chest affection and to relieve biliiousness. It has been considered to be cephalic, deobstruent and carminative and used in chest affections. Muslim physicians considered it to be the broom for cleaning the brain and giving it strength.¹

Hahn et al. ³ isolated three sterols, besides camphor and a hydrocarbon nonacosane, from the petroleum ether extractive of the drug material. The sterols were reported to have m.p. 204-205°C, $[\alpha]^D +39.6^\circ$; m.p. 135-36°C $[\alpha]^D +23.5^\circ$; m.p. 268-70°C, $[\alpha]^D +67^\circ$.

Isolation of a smooth muscle relaxant principle identified as 7-methoxycoumarin has been reported.³ A new compound, designated as lavan ¹, different from the sterols reported by Hahn et al., has now been isolated from the ethanol extractive of the dried plant (stem) material of Lavandula stoechas Linn. Lavanol melts at 282-284°C, and has $[\alpha]_D^{33}+25^\circ$ (THF). It gives no absorption in the UV spectrum above 220 mμ. In the IR spectrum it absorbs at 3520, 3450, 1720 and 1700 i cm⁻¹ (KBr pellet) thus indicating the presence of an alcoholic function in addition to carbonyl groups. It analyses for C_{30}H_{46}O_{3}.

On acetylation it gives a mono-acetate, C_{32}H_{45}O_{4}, which in the IR spectrum gives peaks at 1740, 1720, 1700 i and 1275 cm⁻¹ thus showing the presence of an acetate group (1740 i cm⁻¹) in addition to the carbonyl functions present in the original molecule.

Lavanol on chromic acid oxidation yields a product which gives a positive Zimmerman reaction.

Experimental

Unless otherwise stated IR and UV spectra were taken in KBr pellet and ethanol respectively.

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STUDIES ON EUPHORBIA HELIOSCOPIA LINN

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One steroid with molecular formula \( \text{C}_{20}\text{H}_{30} \text{O} \), one triterpenoid with molecular formula \( \text{C}_{23}\text{H}_{42} \text{O}_{2} \) and a saturated aliphatic alcohol with molecular formula \( \text{C}_{19}\text{H}_{44} \text{O} \) have been isolated from \( E. \) helioscopia. The plant was also examined for the sugars present.

Introduction

\( Euphorbia \) helioscopia Linn., locally known as Ghandi Buti, is a very common plant available throughout the plains of West Pakistan. The roots are reported to be anthelmintic, whole plant cathartic, milky juice applied to eruptions, seeds with roasted pepper given in cholera, and oil from seeds used as a purgative.

Though other species of the plant have been studied by various workers very little work has been done on \( E. \) helioscopia and only the isolation of a saponin has been reported.

Our investigations gave three crystalline compounds: compound A, saturated aliphatic alcohol melting at 75-60\(^\circ\); compound B, a steroid melting at 133-4\(^\circ\); compound C, a triterpenoid melting at 183-4\(^\circ\).

The IR spectra of compound A gave a peak at 3450 cm\(^{-1}\) showing hydroxyl group. A saturated, straight chain alcohol with molecular formula \( \text{C}_{21}\text{H}_{44} \text{O} \) has been reported from the flowers of \( Forsythia \) intermedia\(^{14} \) which also formed acetate having the same m.p. as that of compound A. The compound was neither named by the previous workers nor detailed structure given. We propose the name "helioscopiol" for this alcohol. Due to non-availability of an authentic sample, the alcohol from \( F. \) intermedia direct proof of its identity with the compound could not be confirmed. Since it does not form an adduct with urea, it is in isoform\(^{15} \). Further work is in progress to determine the structure of the alcohol.

The IR spectra of compound B showed a peak at 3450 cm\(^{-1}\). The secondary nature of the group is indicated by the IR peak at 1360 cm\(^{-1}\). Its acetate gave IR peak at 1240 cm\(^{-1}\). This steroid may be \( \delta \)-dihydrofucosterol which has identical molecular formula, solubility, crystalline shape and m.p. The m.p. of acetates are also identical.\(^{16} \) In the absence of an authentic sample of \( \delta \)-dihydrofucosterol, the identity of the compound could not be directly confirmed.

The IR spectra of compound C gave a peak at 1720 cm\(^{-1}\) showing the presence of carbonyl group, probably due to an ester. It could not, however, be hydrolysed. Further work is in progress to identify the compound.

Experimental

All the melting points were recorded by Gallenkamp electrothermal micro-melting point apparatus. Molecular weights and microanalyses were done by Dr. A. Bernhardt, 433, Mulheim Ruhr, West Germany. IR spectra were taken on Beckmann Spectrophotometer model IR-5.

Isolation of Compound A.—The plant was collected in the beginning of March from the Peshawar Valley and used fresh and also after air drying. The whole plant (1.25 kg) was percolated with 95\% ethanol 5 l.) four times and the extract reserved for further processing. The residue was soxhleted with petroleum ether (50-70\%) for 6 hr. The resulting extract was charcoaled, and the solvent removed completely. The residue was extracted with methanol and the extract concentrated on standing a solid deposited, m.p. 50-60\%. Recrystallisation from methanol gave a semi-crystalline product, m.p. 75-60\% (0.74\% yield on dry basis). (The alcohol from \( F. \) intermedia is reported to melt at 75-6\%^{14} \). Found C, 80.60; H, 14.23; O, 5.17\%. \( \text{C}_{21}\text{H}_{44} \text{O} \) requires C, 80.69; H, 14.19; O, 5.12\%.

The compound is soluble in hot petroleum ether, benzene, ether, chloroform, carbon tetrachloride, amyl alcohol, ethanol, methanol and ethyl acetate. It neither reduced Fehling's solution nor decolorised bromine in carbon tetrachloride.

The compound was acetylated by heating with acetic anhydride and fused sodium acetate. The acetate was crystallised from methanol as a semi-crystalline powder, m.p. 56-57\%. The acetate of the alcohol from \( F. \) intermedia is reported to melt at m.p. 56-7\%^{14} \).
STUDIES ON CARBOXYMETHYLCELLULOSE

Part IV.—Effect of Pressure Pretreatment

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By effecting the major part of the alkali treatment under pressure, better substitution may be obtained in carboxymethylation of jute cellulose. The time factor for pressure treatment is important and the optimum was found to be 4 hours. Thus, with impure jute, the highest value for degree of substitution obtained was 1.60.

Introduction

Carboxymethylation is usually carried out commercially with cotton lintres or quality wood pulp in aqueous medium. The products thus obtained are reported to possess a degree of substitution not exceeding 0.60, and largely used as a detergent aid. In this laboratory, a systematic study is being carried out on the process of carboxymethylation with particular reference to jute. It was first observed that in aqueous medium, carefully purified jute cellulose responded better towards carboxymethylation than cotton, but for a relatively high degree of substitution (ca. 1.05), an alkali concentration of 50-70% was required. It was further observed that with this degree of substitution the product developed an ability to form stable oil–water emulsions.

Later it was noted that by conducting the carboxymethylation in water–ethanol mixture, much better results were obtained with purified jute cellulose (D.S. of upto 1.30). This result was interesting, and a tentative mechanism of this reaction was also suggested. Subsequently, it was observed that better results (D.S. 1.48) could be obtained by starting impure jute (i.e. without prior removal of lignin) and this was explained by supposing that ethanol and chloroacetic acid, while causing the delignification, left the cellulose in a relatively more reactive state, which was probably responsible for the better carboxymethylation in the subsequent step.

During the course of the above studies it was thought that the soda cellulose formation plays a significant role in the overall reaction. In the present investigation therefore, the major part of the first step of alkali treatment was carried out under pressure. In order that the effect of such pressure treatment may be better understood, subsequent carboxymethylation was carried out with all the above conditions, viz. in aqueous medium with purified cellulose, in organic solvent medium with purified jute and in organic solvent medium with impure jute. It was noted that with pressure alkali treatment, better results were obtained in all cases and a maximum D.S. of 1.60 has been obtained with impure jute in single treatment.

Experimental

Jute fibre, after discarding the top-most and lowermost portions, was cut into sizes of about 1 in. It was then finally powdered in a shredder. The impure jute was used either as such or delignified.

For delignification, the powdered jute was first boiled for 20-30 min with 1% NaOH. After filtering and washing thoroughly with water, the main bulk of the adhering water was squeezed out and the moist powder treated with chlorine gas for about 1 hr when the colour changed to golden yellow. This was then washed twice with distilled water and then boiled with excess of 2% sodium sulphite solution and subsequently made alkaline and boiled for 5 min. It was then washed well on cloth filter. (If the cellulose thus obtained still retained some lignin, a second chlorination treatment was given.)

In the early experiments attempts were made to combine both the steps (conversion to alkali cellulose and carboxymethylation) and carry them simultaneously in the autoclave; but this was unsatisfactory. The product was somewhat charred and separation of the Na carboxymethylcellulose was difficult.

In the final procedure adopted, the impure or purified jute powder as described above, was steeped with the reagent for 1½ hr in the open and then for 4 hr in an autoclave. The autoclave was then allowed to cool down after which the carboxymethylation was carried out in the usual way as described in earlier papers. The degree of substitution was determined by the uranyl gravimetric method.

The product obtained from impure jute was of a golden yellow colour and contained a little lignin.
HIGHLY PURIFIED CELLULOSE FROM JUTE FIBRE

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Cellulose was prepared from jute-fibre (Corchorus olitorius) purer than any previously reported. A combination of steps was used for pure cellulose preparation, each of which is separately employed in various cellulose industries.

The purest pulp was exceptionally white and had a comparatively high viscosity. The xylose and 4-O-methyl-D-glucuronic acid contents were so low that it may be concluded that these impurities are not chemically combined with cellulose but are probably present as difficultly removable impurities or incrustation. The difficulty in removing the last traces of 4-O-methyl-D-glucuronic acid as well as xylose from the purest pulp supports the hypothesis that these two substances are chemically combined, but not with the cellulose.

**Introduction**

It is desirable to produce pulps which are as nearly pure cellulose as possible especially for the production of certain cellulose derivatives. Normal purifying techniques always leave a small quantity of hemicellulose with the cellulose.

The great difficulty of removing all the mannan and xylan by any means which does not disrupt the cellulose chains themselves has led to speculation that anhydromannose and anhydroxylose units might be an integral part of the wood cellulose molecule. The resistance of mannan and xylan to removal from wood pulp by such drastic reactions as nitration, xanthation, hydrolysis lent support to the chemical bond concept. Centola¹ was not able to separate completely the nitrates of other carbohydrates from cellulose nitrate and concluded that the non-glucose sugars to some extent form part of the molecular chains of cellulose. Das, Mitra and Warcham² isolated a glucose-xylose-arabinose compound from cotton alpha-cellulose and concluded that these sugars were present in the cellulose structure as a mixed crystal. Sarkar and co-workers³ have refuted the possible existence of mixed crystal in jute alpha-cellulose on the ground that xylan-free material can be prepared by treating isolated holocellulose with 9.5% caustic soda solution. More recent works⁴ have, however, failed to lend any support to the observation made by Sarkars and others. The presence of these non-cellulosic constituents according to the observation of Leech⁵, Rapson⁶ and Timell⁷ is due to the sorption and tenacious retention of these substances by alpha-cellulose. Support for this fact came from the work of Yllner and Enstrom.⁸

According to this theory hemicelluloses are believed to be relatively short chain compounds,
DIGESTIBILITY OF THE LEAF PROTEIN CONCENTRATES

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The enzymic digestion of leaf protein concentrates was carried out with trypsin, pepsin, torula yeast; and the enzymes present in the aqueous extracts of the berries of Withania coagulans and ox pancreas. The digestibility of the samples was determined after 3, 6, 9 and 24 hours.

Leaf proteins showed high rate of digestion with trypsin, pepsin and the enzymes present in the aqueous extract of ox pancreas. The enzymes present in torula yeast and the berries of Withania coagulans showed poor proteolytic activity.

Introduction

Grass, is the largest single crop in the world. The changes in the flavour and texture that the animals bring about in the proteins present in the leaves of grass is very much liked by most of the people. This advantage is, however, gained at the expense of heavy losses because the overall efficiency of animals when turning plant material into human food is usually greater than that of many foodstuffs. This indicates that plant leaves may be an important source of food for human consumption. However, amino acid composition alone may not give a true picture as digestibility may effect the nutritive value of LPC. The chemical decomposition during acidic or alkaline digestion and the loss of certain essential amino acids make these methods unreliable. Enzymic methods of digestion have least effect on the composition of the final products. The action of proteolytic enzymes on pure proteins and on proteins in combination with complex materials as they occur in nature have been studied by various workers but little has been accomplished on the action of proteolytic enzymes on leaf proteins. However, a notable contribution has been recently made by Akeson and Stahmann. This work was undertaken to study and compare the action of various proteolytic enzymes of plant and animal origin on LPC.

Experimental

Extraction of Leaf Proteins.—Young, leaves of Shatalla (Trifolium resupinatum), Rawan (Lathyrus aphaca) and from a mixed crop of sarson (Brassica campestris), Shatalla, methi (Trigonella foenum-graceum) and Jai (Avena sativa) were stripped from tough stems. The proteins were extracted by the procedure used by Crook and Holden. Leaves were separated minced in a domestic electric mincing machine and the pulp squeezed through a fine cloth. A second extract was made by adding a suitable amount of water to the residue; remincing and squeezing again. Proteins were precipitated by heating the juice to 80°C. The coagulum was transferred to long cloth bags and excess of water was removed by hand-pressing. The protein cake thus obtained was repeatedly washed with water and hand-pressed till the liquid issuing out was clear. The protein samples thus obtained were placed in polythene bags and stored in a freezer at -10°C.

Determination of Dry Matter and Nitrogen Content.—Dry matter (DM) was determined by heating the homogenised protein sample at 100°C for 40 hr. Nitrogen content determinations were made by micro-Kjeldahl procedure using copper-selenium catalyst. Proteinous nitrogen (PN) and non-proteinous nitrogen (NPN) in the samples was estimated as trichloroacetic acid (TCA)—insoluble and TCA-soluble N respectively. The protein contents of the samples were calculated as protein N x 6.26.

Determination of Digestibility.—Trypsin (B.D.H.), pepsin (Merck), torula yeast (Candida utilis), the enzymes present in the aqueous extracts of the berries of Withania coagulans and ox pancreas were used for the determination of the digestibility of leaf-proteins.

One g of the homogenised LPC sample was suspended in 20 ml of the buffer solution in 50-ml flasks. The samples, after addition of suitable concentrations of the enzymes, were incubated at an optimum temperature and were removed after 3, 6, 9 and 24 hr. The proteins present in 2 ml of the suspension were precipitated by the addition of 2 ml 26% TCA and were separated by centrifuging at 2500 x g for 15 min. The N-content of the supernatent (NPN) was determined. The
EFFECT OF FERROCYANIDE ON THE PRODUCTION OF CITRIC ACID FROM CANE MOLASSES BY ASPERGILLUS NIGER

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(Received January 31, 1966; revised May 13, 1967)

The citric acid fermentation of cane molasses by Aspergillus niger was studied. Addition of ferrocyanide (600 ppm) greatly increased the citric acid yield (60 g/l). The important factors in the production of citric acid are ferrocyanide concentration, morphology of the mould growth and initial pH of the medium. The insoluble complexes of ferrocyanide with heavy metals acted as metal buffers in the fermentation media which made the metal ions available at concentration suitable for maximum citric acid production. The concentration of free ferrocyanide was slightly affected during fermentation. Clarification of the molasses media showed no significant effect on citric acid production.

Introduction

The mineral contents of the molasses particularly trace metals such as iron, zinc, copper and manganese present a critical problem in submerged citric acid fermentation by Aspergillus niger. Potassium ferrocyanide forms insoluble complexes with heavy metals and thus it has been generally used to reduce the trace metal concentrations of fermentation media which increases the citric acid production. In addition to the removal of heavy metals, ferrocyanide has direct toxic effect on the mould metabolism as reported by Martin.

The present investigations describe the effect of ferrocyanide on citric acid production by Aspergillus niger from cane molasses, obtained from sugar factories in West Pakistan. The effect of free ferrocyanide and insoluble complexes in the citric acid fermentation has also been studied.

Methods

Mould Strains.—The strains of Aspergillus niger used were; wis. 72-4, locally isolated WRL 14, WRL 50 and WRL 51.

Inoculum Preparation.—Spore inoculum was used in the present study. A simple synthetic agar medium—containing (g/l): sucrose, 150; agar, 20; NH₄NO₃, 2.5; KH₂PO₄, 1.0; MgSO₄·7H₂O, 0.25; and trace metals (mg/l): Fe+++ (FeCl₃) 2.20; Cu++ (CuSO₄), 0.48 and Zn++ (ZnSO₄) 3.80—was used. Inoculum medium was autoclaved at 121° for 15 min. 15 ml of agar medium was transferred in a cotton wool-plugged bottle and the cultures were incubated at 30°. Spores from 5 to 7 day old cultures were wetted with 5 ml of 0.05% solution of sodium laurel sulphate. The plate was washed with sterile distilled water. The combined washings were made up to 50 ml and shaken with glass beads to break up clumps of spores. 25 ml of fermentation medium in 300 ml flask was inoculated with 1 ml of spore inoculum.

Fermentation Medium.—Cane molasses obtained from Mardan, Charsadda, Rahwali and Lyallpur sugar factories in West Pakistan were used for fermentation. Sugar contents of the molasses were 60, 52, 47 and 43% respectively. For medium preparation, the molasses were diluted to 15% sugar concentration with tap water. The molasses solution was adjusted to pH 6.0 and sterilized at 121° for 15 min. Potassium ferrocyanide solution was sterilized by membrane filter.

Conditions of Cultivations.—The culture temperature was 30°. All fermentations were carried in shake flasks. The rotary shaker was placed in a room at 28±2°. The flasks were rotated at 200 rev/min with 1½ in. amplitude throw.

Molasses: Clarification.—The molasses solution, after adding 35 ml. of 1 N H₂SO₄ per litre, was boiled for ½ hr, cooled, neutralized after cooling with lime water and left to stand overnight. The clear supernatant liquid was used for fermentation.

Analytical Methods.—Mycelial dry weight was determined by filtering 25 ml of culture through weighed Whatman paper No. 41, and washed 3-4 times with tap water. The mycelium was dried at 105° overnight before weighing. Citric acid was estimated colorimetrically by the method of Marrier and Boulet and sugars by ferrocyanide reduction method, a modification of Fujita and Watake. Free ferrocyanide was estimated by the method of Marrier and Clark.

Results

Selection of Strain.—Unclarified Rahwali molasses medium was employed for citric acid fermentation. Ferrocyanide addition was made at the
A STUDY OF THE EFFECTS OF HISTAMINE, FOLIC ACID, ACRIFLAVINE AND INDOLEACETIC ACID ON THE MITOTIC ACTIVITY OF EMBRYONIC CHICK HEART FIBROBLASTS GROWN IN VITRO

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In order to study the effects of chemicals on mitosis, four chemicals, acriflavine, folic acid, histamine and indoleacetic acid were selected. The effects of these chemicals on mitotic activity were determined by observation, by counting the phases and using the formula devised by Wilson and Leduc.

Mitotic activity = number of mitotically active cells
area of section x nuclear density of section

The results indicated that the highest mitotic activity was in the explants which contained 50 μg/ml of indoleacetic acid. All concentrations of acriflavine were strong mitotic inhibitors. Folic acid and histamine were mitotic stimulants at 50 μg/ml concentration. But 10 μg/ml and 100 μg/ml concentration had no effect at all.

The use of indoleacetic acid, a plant growth hormone, augmented the growth of chick heart fibroblasts. The similarity of effects of indoleacetic acid on plant and animal cells, may be due to the similar mechanism of nuclear division.

Introduction

Harrison's tissue culture technique has been used extensively in the study of the effects of various chemicals on mitosis, mitotic activity and physiology of the cells.

Blumenthal reported on the influences of cyanides on developing Arbacia punctulate embryos. He placed the eggs in the cyanide solutions of various concentrations at various stages of development and found that the eggs continue mitotic activity at all concentrations.

Creech studied the action of methylcholanthrenecholeic acid, acenaphthenecholeic acid, dibenzanthracenecholeic acid, and phenanthrenecholeic acid on the rate of mitosis of connective tissue fibroblasts, which were taken from embryonic mice. He added the chemicals to the tissue cultures in various concentrations ranging from 0.1 mg/ml to 0.001 mg/ml.

As a result of these experiments, Creech reported an increase of 2% in the mitotic count from the cultures which contained the lowest amount of acenaphthenecholeic acid and methylcholanthrenecholeic acid.

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Introduction

Harrison first observed the development of nerve fibers that were grown in hanging drop preparations. Since that time considerable progress has been made in the development of this technique and various others have been developed.

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As a result of these experiments, Creech reported an increase of 2% in the mitotic count from the cultures which contained the lowest amount of acenaphthenecholeic acid and methylcholanthrenecholeic acid. He further reported that dibenzanthrenecholeic acid caused an increase in the mitotic count by 7.9%, while phenanthrenecholeic acid reduced the count by 34.9% at 0.1 mg/ml concentration. Ten-fold increase in concentration caused a retardation of mitosis.

Woodard and Estes studied the effects of 1.0 mg/ml colchicine solution on mitosis in the neural tube of chick embryos.

The results reported by the investigators, showed that the percentage of all phases except metaphase, eventually declined (prophase from 2.8% to 1.1%, anaphase from 0.2% to 0.0% and telophase from 4.6% to 0.0%). In metaphase it increased 2.4% to 20.5%. This rise in the number of metaphase stages was accounted for by the accumulation of nuclei in this phase. In their conclusion they indicated that colchicine at this concentration i.e.1 mg/ml was not mitotic stimulant but produced a metaphase block.

In the study of the action of drugs on mesenchymal tissue of chick embryos, Lettre added ammonium carbonate, adenosine, adenosine triphosphate, pyocyanine, acriflavine, colchicine, and adenaline solutions to the culture media in various concentrations.

The study showed that ammonium carbonate, adenosine, and adenosine triphosphate have produced vacuolization of cytoplasm, and pyocyanine increased uptake of oxygen by the cells and inhibited cell division. While the acriflavine produced well-known phenomenon of sticking and clumping of chromosomes during mitosis.
STUDIES ON FUNGITOXICITY OF A COPPER BASED COMPOUND S-3

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Fungitoxicity studies of a new copper-based compound, S-3, were carried out in comparison with another imported copper compound against some plant pathogenic fungi. It was found that the compound S-3 was more effective in controlling the growth of Helminthosporium anomalous than Colloidal copper but was equally effective against Fusarium solani.

The effectiveness of S-3 against Fusarium dimerum and Alternaria tenuis was found to be less as compared to Colloidal copper. This may be due to more resistant nature of these two fungi towards compound S-3.

The present studies, show that S-3 may be recommended for further exploitation as a commercial fungicide.

Introduction

Certain cations particularly copper, mercury, and zinc are known for a long time for their toxic action against most fungi. The use of ordinary inorganic salts of these metals is limited due to their ability of making solution with water. Metallic soaps of fatty and synthetic acids being insoluble in water have a definite advantage in this regard. Copper naphthenate has been reported to have the property of preserving cellulosic fabrics and wood. Copper oleate has particularly been mentioned in the literature for its fungicidal and insecticidal activity. The availability of oleic acid in Pakistan for making copper oleate is almost nil because of lack of proper facilities. The copper soap of castor oil, referred to as S-3 here, the chief constituent of which is ricinoleic acid (85-90%), has been tested for its fungicidal activity. Other known substances were also studied for comparison in test experiments. The soap was made by the well-known process of precipitation. Triethanolamine soap of castor fatty acids was used to emulsify the fungicide.

Experimental

Preliminary screening of S-3 was carried out using the poisoned food technique with solid media as a base. Synthetic nutrient medium, Czapek's Dox agar, was used throughout these experiments.

The different constituents of the medium, including KH₂PO₄, were dissolved and 240.0, 237.0, 228.0, 216.0 and 192.0 ml of the solution were poured into 500 ml conical flasks. To each flask 4.8 g of powdered agar was added. One flask with 240 ml solution was kept as control. The flasks were autoclaved at 15 lb pressure for 15 minutes, and then allowed to cool to 45°C. 3.0, 6.0, 12.0, 24.0 and 48.0 ml of the 8% emulsifiable concentrate of compound S-3 were added to each flask to obtain concentrations of 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6% S-3 as recommended in Reference 7. Two flasks containing 1 and 2% of the emulsifier, used with compound S-3, were also taken. The flasks were shaken to mix the compound and the medium thoroughly. The medium was poured into 12 sterilized petri plates of 9-cm diameter and allowed to solidify. Out of 12 petri plates of each concentration, 3 were inoculated with 4 mm discs cut from the advancing edges of a 4-day old culture of Alternaria tenuis grown on potato dextrose agar and another 3 with Fusarium solani isolated from infected potatoes. Of the remaining 6 plates, 3 were inoculated with Helminthosporium anomalous and the last 3 with Fusarium dimerum. Controls were inoculated simultaneously and all the petri dishes were incubated at room temperature.

A similar set of experiment was carried out with colloidal copper to compare with compound S-3. These experiments were repeated about five times. Diameters of fungus colonies were measured after 48, 96, 120 and 144 hr.

Results

The diameter of each colony was measured along the two lines crossing each other at the centre. The observations for all the concentrations of the fungicides and the various fungi, in triplicate, were thus taken and the mean values with standard deviations calculated.

Inhibition percentage was calculated by subtracting the growth obtained in various concentrations of a fungicide from that of controls.
FUNGAL INFECTIONS AND INFESTATIONS OF VEGETABLES FROM KARACHI MARKET

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A survey, to study the various fungi present on vegetables sold in Karachi markets, was conducted. Potatoes, tomato, spinach, garlic, onion, peas, chillies, ginger and methi etc., collected from six different and far apart vegetable markets, were found to be infected or infested with Aspergillus niger, Alternaria sp., Fusarium solani, Cercospora heticola and Rhizopus nigricans etc. Most prevalent among the fungi isolated were Aspergillus niger and Alternaria sp.

Introduction

Investigations in the last few decades have shown that potatoes, tomatoes, peas, beans, carrots, radish, turnip and beets were found to be infected with rots, blights, canker, wart, crown gall, grey mold, leaf spot and mildews. Hafeez and Hasan have prepared a chart for general information of agriculturists describing diseases of vegetables. In the present study, therefore, a more specific and detailed survey of various fungi present on the vegetables sold in the Karachi markets has been made in order to ascertain the losses caused by them and also to suggest some possible control. Another aim of this study was to acquire acquaintance with the flora of local vegetables and to use these isolates as test organisms for carrying out fungicidal evaluations of indigenous materials.

Materials and Methods

Samples of vegetables from six different places, Nazimabad, Jail Road, Landhi, Jamshed Road, Ranchore Lines and Empress Market, were collected at different times of the year. Each sample was kept in a precleaned polyethylene bag to avoid any contamination.

At the time of collection, the characteristic disease symptoms of fungal infection if any were noted. The samples were examined directly under dissecting microscope to observe the type of infections. Infected portions were implanted on solidified Czapek-Dox agar medium, with an acidic pH to eliminate bacteria. Some of the pieces were implanted after sterilizing the surfaces with mercuric chloride (1:1000) solution for 1/2, 1 and 2 minutes, although use of 70% alcohol has also been suggested by some workers.

For excising and implanting the diseased materials of vegetables, the scissors, scalpels, needles, and forceps were sterilized by dipping in methylated spirit and flaming several times. Usually three pieces from each specimen were inoculated in one petri plate and the growth observed after 24 hours of incubation at 28°C. Control plates (without any vegetable pieces) were also used simultaneously.

Results and Discussion

Potato (Solanum tuberosum L.).—Samples of infected potatoes, sometimes having some apparent injury, were collected from all the places visited (Fig. 1.). Characteristic white colonies of Fusarium sp., appeared in all petri plates inoculated with diseased pieces of tomato. Branched and septate mycelium along with typical sickle or crescent shaped and hyaline macroconidia as well as microconidia were observed. Fusarium is very notorious in attacking potatoes.

Tomato (Lycopersicum esculentum L.).—Like potato, tomato is also attacked by a number of pathogenic fungi. Three different fungi were isolated from the surface of the collected specimens. The fungi, Aspergillus niger, Rhizopus nigricans and Alternaria sp., were found on the surfaces as forming a definite greenish patch somewhat round in shape and at lower-half portion of the fruit. R. nigricans causes a soft rot, a ripe fruit-rot and is prevalent in many other types of plants. It penetrates through wounds and the decay is very rapid at room temperature. This disease chiefly occurs in storage and transit but may also occur occasionally in the field.

Spinach (Spinacia oleracea L.).—It is one of the most commonly consumed vegetable, very sensi...
QUANTITATIVE DETERMINATION OF ACID AND ALKALINE PHOSPHATASE IN DIFFERENT PARTS OF THE ALIMENTARY CANAL OF DESERT LOCUST, SCHISTOCERCA GREGARIA (FORSKAL)*

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Biochemical estimation of phosphomonoesterases was done in different parts of the alimentary canal. Quantitative determination of phosphatases showed strong acid phosphatase activity in Malpighian tubules, posterior-midgut, fore-hindgut, caecum and anterior-midgut in decreasing order. Moderate activity was noticed in hind-hindgut, midhindgut, salivary glands and hind-foregut. Low activity was found in fore-foregut and mid-foregut.

Strong alkaline phosphatase activity was found in caecum, anterior-midgut and salivary glands. Moderate activity was found in hind-foregut and fore-foregut, while low in posterior midgut, mid-foregut, fore-foregut, hind-hindgut, mid-hindgut and Malpighian tubules.

Phosphomonoesterases, have been determined biochemically and localized histochemically in higher animals and plants. Most of the workers have histochemically localized these enzymes in the alimentary canal and other tissues. Phosphatases have been studied histochemically in normal organs and tissues, the salivary glands of Drosophila melanogaster, insect tissue, the gut of blow-fly larva, Musca domestica (L.), salivary glands of the large milkweed bug, and the stable fly. Glycerophosphate as substrate, strong acid phosphatase activity has been reported in the midgut of silkworm. Acid phosphatase activity has been studied in the digestive tract of milkweed bug, Oncopeltus fasciatus (Dallas). High concentration of alkaline phosphatase has been found in the intestine of silkworm, Bombyx mori (L.) by using the method of References 13 and 14.

Material and Methods

Different methods have been used by different authors for the determination of phosphatases. During the present investigation the methods of Bessey et al. and Andersch and Szczypinski as modified by Ashrafi and Fisk have been employed with some modifications.

1. REAGENTS

(a) Substrate.—0.0143 M stock solution of di-sodium p-nitrophenyl phosphate was prepared by dissolving 100 milligrams of sigma 104-R substrate (trade name of p-nitrophenyl phosphate) in 25 ml twice distilled demineralized cold water, and was stored in freezing chamber at -15°C. This colorless solution represented a molarity of 0.00143 M (final) when used during experiment.

(b) Enzyme Source.—Adult locust (28-day old), fed on 5% glucose solution, 1 day prior to the experiment, were selected for enzyme study. Locust was dissected in cold water and the alimentary canal was transferred to a test tube containing 5 ml chilled twice distilled demineralized water. It was grounded for exactly 3 minutes in “Teflon Pyres” tissue grinder. The homogenate was filtered through a 2 mm. thick glass fibre layer in a Gooch crucible under moderate suction and the filtrate was collected in the microfilter tube placed in an ice tray. The filtrate was diluted to 10 ml by adding 5 ml of cold twice distilled demineralizer water. This represented 1/10th part of the alimentary canal per ml.

(c) Colorimetric Standard.—A 0.01 M stock solution of p-nitrophenol was prepared by dissolving 0.1391 g of p-nitrophenol (spectrophotometric grade) in 100.0 ml of cold twice distilled demineralized water. This was diluted in the ratio of 1:200, to prepare working standard solution of 0.00005 M, yellow in color.

(d) Acid Buffer Solution.—500 ml of 0.09 M citric acid solution, 180 ml of 1 N NaOH solution and 120 ml of 0.1 N HCl were mixed to prepare the
A MELTING POINT METHOD FOR WAXES

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A new technique based on the variation of physical state under the influence of surface tension near the transition point has been evolved for the determination of melting point of waxes. An apparatus employed in this technique has been devised and successfully operated. Using this technique two methods have been developed, one of moderate accuracy of the order ±1.5 C and the other of considerably improved accuracy. The latter method employs graphical extrapolation. Results of the melting point determination for three waxes are given and compared with those obtained by a conventional method.

Introduction

Waxes in general are complex mixtures of various compounds having melting points scattered over a wide range of temperature; consequently the “melting point of wax” does not carry the same connotation as accorded to that of a pure compound or to that of a relatively simple mixture of substances.

In the process of melting, in addition to the phenomenon of “premelting”, waxes undergo a number of changes in the physical state, manifested by the phenomenon of softening. The process of softening of a wax begins with the melting of the lowest-melting point constituent followed by others in the order of ascending melting points. The relative abundance of the constituents of a given specimen of a wax determines its physical characteristics at any given temperature.

The methods for the determination of melting point of waxes in use are rather unsatisfactory because these methods do not provide any precise information about the transitional state of the wax under examination. At best they yield values corresponding to the arbitrary definitions of melting points which are invariably given with these methods.2–6

On close examination these definitions are found to have no theoretical support. For example, the definitions of melting point given with “ball and ring” method or Pohl’s method are only a means of providing a convenient index for waxes.

On account of the complex nature of the wax composition, it is difficult to give a theoretical definition of melting point of waxes. However, some physical property should be used as a criterion for melting point of waxes to indicate the behaviour of the state of wax at the melting point. The melting point obtained would then provide a better index in comparison to those obtained by the conventional methods.

Methods developed on the basis of viscosity as the physical criterion have been tested by Marshall.1 In the case of the crystalline paraffins and the plastic microcrystalline waxes there is no pronounced rise in viscosity as the solidification point of the wax is approached from either the rising temperature or the falling temperature side. In case of the hard microcrystalline waxes complications are introduced due to pronounced hysteresis in viscosity on the rising temperature curve. The viscosities immediately above the melting point are very high in comparison to the “falling temperature” values of the viscosity in this temperature range.

On account of these drawbacks in the viscometric method of melting point it is necessary to explore the use of other physical properties for a dependable criterion for melting point of waxes. The present work describes a method based on the effect of surface tension. The actual measurement of the surface tension is not, however, required to arrive at the melting point. It is the difference of behaviour of physical states of the wax under the influence of surface tension that is used to determine the melting point.

Liquids show a characteristic tendency to acquire a minimum surface area under their own surface tension almost instantaneously, whereas in solids the effect of this tendency is delayed or offset due to the forces responsible for their rigid form. In waxes an intermediate effect is observed over a considerable range of temperature.

In the viscometric method, the viscosity of wax is determined at different temperatures in its softening range and a plot of viscosity against temperature is obtained. The melting point of the wax is indicated by a pronounced change in the slope of the curve. However, in the present method the time required for retraction of a thread of the wax drawn at different temperatures is determined and a plot of the time against temperature is obtained. The melting point
STUDIES ON RESINS FROM MAKERWAL COAL

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Makerwal coal is known to contain a high percentage of resins which are recoverable by solvent extraction. Alternative methods of recovery reported earlier 3,5 show a wide degree of variation in the yield of resins and the quality of coal residue. An attempt has been made to evaluate these methods, by studying separately the behaviour of original coal, solvent extracted resins and the coal residue from solvent extraction, on superheated steam treatment and carbonisation. It was found that on superheated steam treatment at 300-330°C these resins were only partially recoverable while on carbonisation extensive cracking of the resins was observed. The experimental data from the present work and critical review of the earlier work in this direction has clarified some of the hitherto reported anomalies of the behaviour of Makerwal coal.

Introduction

Makerwal coal of the Trans-Indus area is known to contain a substantial amount of solvent-extractable resins. However, the yield and the nature of the extracted resins to some extent depends upon the nature of the solvent used. The most convenient solvents so far reported are n-propanol, benzene, or a 70:30 benzene-alcohol mixture. Powdered Makerwal coal and other coals have been treated3,4 with the superheated steam for the recovery of these resins in a depolymerised and chemically modified form with the simultaneous partial desulphurisation of coals below the sub-carbonisation temperature up to 330°C. Further work on superheated steam treatment of powdered Makerwal coal and other West Pakistan coals in the temperature range of 300-600°C had shown5 that in the course of 6-hr treatment at 300°C only 2.9% of condensable volatile products, termed as "tar oil," were obtained. Moreover, the quality of coal residue from this treatment as shown by the yield and proximate analysis differed considerably from the one reported earlier.3,4 The anomalous behaviour of Makerwal coal in the superheated steam treatment has also been mentioned by other workers,6 who have attempted to explain this behaviour through the study of the chemical composition of ash without any conclusion. These differences and anomalies in the results of superheated steam treatment of powdered Makerwal coal necessitated the study of the behaviour of solvent extractable resins from Makerwal coal.

Experimental

200 g of Makerwal coal ground to pass 70-mesh B.S. sieve was exhaustively extracted using benzene-ethanol mixture 7:3 v/v as solvent in the Soxhlet extractor. The extracted material was recovered by distilling the solvent mixture and its last traces were removed by drying under vacuum. The material thus obtained was a dark brown powder.

The coal residue left after solvent extraction was made free from solvent by vacuum drying. The results are given in Table 1.

The proximate analyses, and Gray-King carbonisation assays at 600°C of original Makerwal coal, coal residue from solvent extraction and solvent extracted resins were carried out by following the standard procedures.

Superheated steam treatment of 15-20 g Makerwal coal, coal residue from solvent extraction and solvent extracted resins was carried out separately at 300° and 330°C in a standard quartz tube used in the Gray-King assay. To hold the material in position a loose asbestos plug was introduced. The tube was placed in an electrically heated Gray-King tube furnace with precise temperature recording arrangement. The superheated steam from a boiler was passed to a gas-heated metallic superheater with temperature recording arrangement. The superheated steam (about 200 g/hr.) was then introduced through a perforated tube into the coal bed ensuring an intimate contact of steam with the material to be treated. The superheated steam at (320°C) was introduced into the retort heated to 150°C the temperature of the furnace was gradually raised (5°C/min) and then maintained at 300 ± 5°C until no more material distilled over with the steam (about 1.5 to 2.0 hr). In a separate experiment the original coal residue from solvent extraction was first treated at 300°C as before and then the temperature of the retort was raised to 330 ± 5°C and further quantity of distillable material was collected. The superheated steam treatment was discontinued when no more material distilled over with the steam (about 1 to 1.5 hr). The results are shown in Table 2.

Results and Discussion

By the solvent extraction of original Makerwal coal (B.S.-70 mesh) it was found that the major
RELATIONSHIPS OF MEDULLATION TO FINENESS AND TOUGHNESS OF WOOL FIBRES FROM A FLEECE OF BIBRIK WOOL

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A sample of Bibrik fleece was sorted into three fibre types: true, heterotype and kemp. The sorting resulted in a ratio of 1.9 of secondary to primary fibres in the fleece. Percentage medullation (P) in each heterotypical fibre was estimated by a new method employing a specially graduated lameter. Relationships of P to root mean square radius \( \sqrt{\sigma_f} \) and toughness (T) in each fibre were obtained from data on 60 fibres. A significant positive correlation between P and \( \sqrt{\sigma_f} \) and a significant negative correlation between P and T were revealed in the case of heterotypical fibres. In the 40 kempy fibres studied, the coefficient of correlation between P and \( \sqrt{\sigma_f} \) was not significant but that between P and T was highly significant. As expected, the kemp fibres had much more P and lower T than the heterotypical fibres.

Comparison of the average values of P in sample of the heterotypical fibres indicated that median rather than mean is more representative of class average owing to skewness of the distributions of parameters such as the radii of fibres and medulla.

Introduction

Medullation of wool is generally governed by the genetic influence but is partially modified by environmental interaction, in particular, exposure after shearing. Medullated wool is mainly used in carpet and tweed industries. As fibre strength plays an important role in these industries, it is desirable to assess the relationship between medullation and strength as applicable to commercial usage. The strength characteristics in this study are represented by toughness, the product of breaking stress and extension of a fibre, which is an index of durability of the finished product. The objectives of the study were defined as follows:

1. Estimation of the percentage numbers of true, heterotypical and kemp fibres in a Bibrik fleece.


3. Comparison of the various methods of estimating percentage medullation.

4. Investigation of relationships of percentage medullation to fibre fineness and toughness.

The manufacturing potential of raw wool is largely determined by such characteristics as fineness, length, strength, colour and medullation. In addition to the difference in strength, for example, medullated fibres differ from non-medullated fibres in dye uptake. Furthermore, any relationship between medullation fineness and strength, if established, will assist the industry in identifying the fibres.

Experimental

Sampling and Sorting.—A fleece of Bibrik was spread uniformly and divided into 40 equal areas. A handful of fibres picked up from each area was halved and one half was rejected randomly. This operation was repeated a few times to obtain a sub-sample of 0.06 g corresponding to each of the 40 sub-areas. All the 40 sub-samples thus drawn were examined in benzene and sorted into three main classes, namely, true, heterotype, and kemp. This classification has been adopted from Von Bergen but, for the purposes of the present study the term heterotypical was defined to include partially and fully medullated fibres. Likewise, all fully medullated fibres showing signs of opaqueness and/or brittleness were classified as kemp, so that any additional effects due to these factors could be eliminated from observations on the heterotypical class. This resulted into a higher percentage of fibres classed as kemp than would be expected; but was in line with the aim of the work to investigate the relationship between medullation and toughness rather than to emphasize the exact distribution of the various types of fibres. Fibres in each class were counted. Out of 2125 heterotypical fibres, 600 were drawn at random for the estimation of medulla and mechanical properties. Similarly, 500 kempy fibres were drawn from a total of 8732. The unequal proportion of fibres selected for the study was due to the fact that kempy fibres were more uniform than the heterotypical with respect to their medullation distribution.

Measurement of Medulla and Mechanical Properties.—All measurements were carried out at about 65%.
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THE HEALTHY JUICE EFFECT IN VIRUS TRANSMISSION*

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SYNTHESIS OF 3-METHYL-4-BENZYLIDENE ISOXAZOLONE AND 4,4-BENZAL-BIS-(3-METHYL-ISOXAZOLONE-5)

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