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TEMPERATURE DERIVATIVES OF VISOCOSITY, DENSITY, AND REFRACTIVE INDEX FOR THE WATER–ETHANOL SYSTEM

Part VII.—The Temperature Derivatives of Refractive Index for Water and Very Dilute Aqueous Ethanol from 0.2% to 1.8% Ethanol

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The present communication gives the results of new experiments on temperature derivatives of refractive index with sodium D light for very dilute aqueous ethanol solutions from 0 to 2% ethanol, at concentration intervals of nearly 0.2% ethanol. All the measurements, including a set of experiments on pure water, have been duplicated with the Pulfrich and V-Block refractometers. While the temperatures of the minima are found to be essentially the same with the two refractometers, the amplitudes of about half the minima are 2 to 4 times larger with the Pulfrich instrument, and it is conjectured that this may be due to interfacial boundary effects.

The mean results are plotted as a synoptic concentration-temperature chart, showing the minima from 0% to 1.8% ethanol. It is found that, while the pattern is broadly the same as previously reported, the measurements at intervals of 0.2% ethanol concentration show up several time details, particularly at the very low ethanol concentrations, and provide evidence of branching of at least one minimum into two separate ones, as well as of rapid shifts in the region between 0 and 0.3% ethanol concentration.

Introduction

Following a preliminary experimental investigation of the temperature-derivative of refractive index of water, which showed the probable existence of a roughly sinusoidal component with a period of 4 deg C to 6 deg C, further measurements of the temperature-derivative of refractive index, dn/dT, were reported on a series of five aqueous ethanol solutions in the range 2.5-11% ethanol by Qureshi, Haider and Qurashi in Part I of the present series. All these results exhibited a sinusoidal component of dn/dT, which could be generally correlated with the corresponding jumps in flow activation energy, Eγ, already reported by Ahsanullah and Qurashi for aqueous solutions containing 2.5-30% ethanol by weight. Some refractometric measurements at closer concentration intervals of nearly 0.6% on dilute ethanol in the range 0.4-6% (by wt) using both the Pulfrich refractometer (n0 = 1.74) and the new V-block refractometer (n0 ± 1.51), have lately been reported in Part V of this series, and the correlation with the temperatures of the jumps in Eγ is fairly satisfactory above 2.8% ethanol, but certain discrepancies occur in the more dilute solutions. Figure 1 shows an enlarged plot of the concentration-temperature chart of the minima in (dn/dT) for concentrations below 2.5% ethanol, and it is seen that there is considerable ambiguity regarding the progressive changes in temperature versus concentration for these minima below 1.2%. Also, it was noted in a recent paper from this laboratory that the variations in this region do not always correspond with the changes in jumps of Eγ.

The present communication accordingly deals more specifically with differential refractometric work on such very dilute ethanol solutions, and gives the results of new experiments on the temperature-derivative of refractive index for some aqueous ethanol solutions containing from 0.2 to 1.8% ethanol by weight, using sodium D light and concentration intervals of approximately 0.2%.
SYNTHESSES AND MASS SPECTRAL STUDIES OF SOME BENZDIAZOLEs AND N-SKATYLTRIAZOLE

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Syntheses of N-skatylbenzimidazole (I), N-skatyl-2-methylbenzimidazole (II), N-skatyl-2-benzylbenzimidazole (III), N-skatyl-1,2,3-benzotriazole (IV) and N-(N-benzimidazolyl)ethyl 3-phenanthryl ketone (V) have been achieved through alkylation reaction of gramine. Their mass spectra have been studied by analysing metastable peaks and mass numbers.

In earlier communications we have described some alkylation reactions of Mannich bases.\(^1\,^{2}\,^{3}\,^{4}\)

In the present communication gramine and \(\beta\)-morpholinooethyl 3-phenanthryl ketone hydrochloride have been utilized as alkylating reagents for the syntheses of the corresponding \(\alpha\)-substituted benzimidazoles and a triazole, viz. N-skatyltriazole, for detailed mass spectral studies on the electron impact fragmentation of these types of compounds.

Gramine reacts with benzimidazole, 2-methylbenzimidazole, 2-benzylbenzimidazole, and \(1,2,3\)-benzotriazole in aqueous medium to give the corresponding \(\alpha\)-substituted benzimidazoles and \(\alpha\)-triazole when refluxed. Similarly, benzimidazole reacts with \(\beta\)-morpholinooethyl 3-phenanthryl ketone hydrochloride to give the corresponding \(\beta\)-(N-benzimidazolyl)-ethyl 3-phenanthryl ketone.

The mass spectrum (Fig. 1) of N-skatylbenzimidazole (I) showed a molecular ion peak at m/e 247\(^+\) (24%; a). This ion underwent fragmentation to lose benzimidazole moiety to give a very stable charged skatyl species at m/e 150\(^+\) (100%; m* 57; base peak). The molecular ion (a) also lost CH\(_3\)N to give rise to (b) at m/e 219\(^+\) (15%; m* 193.5). Yet a further fragmentation of (a) took place in the form of the loss of C\(_9\)H\(_7\)N with the transfer of one of its \(\gamma\)-protons to yield the benzimidazole ion (c) at m/e 118\(^+\) (45%; m* 56.4). The ion (c) gave rise to charged species at m/e 77\(^+\) (27%; m* 49.1; phenyl) and m/e 76\(^+\) (21%; m* 49; phenonium ion). The other significant fragmentation ions were at m/e 208\(^+\) (5%); 194\(^+\) (27%); 128\(^+\) (5%) 126\(^+\) (7%) and 102\(^+\) (19%).

The mass spectrum (Fig. 2) of N-skatyl-2-methylbenzimidazole (II) showed an intense molecular ion peak at m/e 261\(^+\) (95%). This molecular ion underwent further fragmentation to give very stable species of skatyl at m/e 150\(^+\) (100%; m* 66; base peak) as was observed in the previous case. The loss of one proton from this species gave rise to charged indolenine at m/e 129\(^+\) (30%). This molecular ion also lost the skatyl moiety (\(-\text{C}_9\text{H}_7\text{N}\)) with the migration of a \(\gamma\)-proton to form the 2-methylbenzimidazole molecular ion species m/e 132\(^+\) (78%; m* 66.7). Loss of the methyl group from the molecular ion also occurred to yield an ion at m/e 245\(^+\) (12%). This loss of methyl group also took place from the ion m/e 132\(^+\) to form an ionic moiety at m/e 117\(^+\) (10%) followed by the metastable peak at m/e 103. This charged ion again underwent further fragmentation to give charged phenyl and phenonium ions at m/e 77\(^+\) (27%) and 76\(^+\) (22%) respectively. The other important peaks were at m/e 245\(^+\), 233\(^+\), 219\(^+\), 190\(^+\), 165\(^+\), 164\(^+\), 143\(^+\), 133\(^+\), 131\(^+\), 103\(^+\) and 102\(^+\). The general fragmentation pattern of its mass spectrum is outlined in Chart 2.

The mass spectrum (Fig. 3) of N-skatyl-2-benzylbenzimidazole (III) gave a significant molecular ion at m/e 337\(^+\) which again gave rise to a similar stable charged skatyl moiety due to the cleavage of carbon and nitrogen bond at m/e 293\(^+\) (75%). This molecular ion underwent further fragmentation to give a charged benzyl species at m/e 261\(^+\) (27%). This charged ion again underwent further fragmentation to give charged species at m/e 246\(^+\) (27%); m* 193.5; base peak). The other significant fragmentation ions were at m/e 208\(^+\) (5%); 194\(^+\) (27%); 128\(^+\) (7%); 102\(^+\) (19%).

The mass spectrum (Fig. 4) of N-skatyl-2-benzylbenzimidazole (IV) is given in Chart 4. The general fragmentation pattern of its mass spectrum is outlined in Chart 4.

Chart 1.—Mass fragmentation pattern of N-skatylbenzimidazole.
POLAROGRAPHIC ANALYSIS OF COPPER BASE ALLOYS

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Two alloy systems Cu–Zn–Pb and Cu–Zn–Pb–Sn have been studied. Their polarography has been carried out in aqueous media using potassium chloride as the base electrolyte and normal calomel electrode (N.C.E.) as reference electrode. Since Sn (IV) forms a colloidal precipitate of Sn(OH)4 in warm aqueous medium, it was separated from the system by ion exchange method and determined polarographically in hydrochloric acid medium.

Since Heyrovsky's first polarographic measurements in 1921, polarography has become a useful instrumental technique for the analysis of a wide variety of materials. This technique is now well established in many analytical laboratories along with other modern physicochemical methods such as spectrophotometry and emission spectroscopy. The importance of the method has further been increased by the fact that several elements can be quantitatively determined in a single solution in a short time. The technique is also most versatile as it can be employed to macro and microanalytical problems and is ideal for the determination of trace metallic impurities.

Experimental

Leaded brasses, non-leaded brasses, leaded tin bronze, leaded high tin bronzes etc. are amongst the commonly used foundary alloys containing varying amounts of copper, lead, zinc and tin. Additions of other elements like nickel, aluminium, iron, manganese, silicon, phosphorus etc. are made to acquire special purpose alloys.

Copper gives reduction steps near to zero applied potential in most base electrolytes. Therefore, the current due to this reduction greatly restricts the determination of elements reducing at more negative potentials. Tin also reduces near to zero applied potential whereas lead and zinc have half wave potentials far removed from copper and tin. Separation of these elements is, therefore, essential. Whereas copper is usually removed by (i) forming copper cyanide complexes, (ii) precipitation, (iii) electrolytic deposition and (iv) precipitating other cations from the composite solution and determining copper separately, tin, lead and zinc are separated by conventional methods. The authors have employed ion exchange method for the separation of these cations, because it is not only quick but also quantitative.

(a) Ion Exchange Separation of Cu, Zn, Pb and Sn

A Pyrex glass column (20 × 1.5 cm) containing a strongly basic anion exchange resin (Amberlite IRA-400 Cl) was used for the separation of the above cations, using 2M HCl as the complexing medium. A mixture of 5 ml each of 5mM Cu++, Zn++, Pb++ and Sn(IV) was fed to the column at the rate of 1 ml/min. Copper was not adsorbed and passed through the column as such. It was collected in a 100-ml beaker. Pb++, Zn++ and Sn(IV) were held by the resin, wherefrom Pb++ and Zn++ were eluted with water and their combined solution preserved in another 100-ml beaker. Lastly, Sn(IV) was stripped off the column with 1M HNO3 and collected in another 100-ml beaker.

Apparatus and Reagents

A Cambridge pen writing polarograph, dropping mercury electrode capillary and thermostat, as supplied by the manufacturer, were used for recording the polarograms. The experiments were carried out in a cell as described by Vogel, with reference to a normal calomel electrode at 25°C. Dissolved air was removed from the solution by passing pure and dry nitrogen gas for about 10 min. The m value of the capillary was determined and found to be constant at 1.47 mg/sec at 25°C for over 6 months. The drop time was adjusted at 3.0 sec in KCl-supporting electrolyte for Cu, Pb and Zn and 3.0 sec in HCl-supporting electrolyte for Sn(IV) measured at the particular applied potential at which the diffusion current started.

Stock solutions of Cu, Pb, Zn and Sn (5.0 mm) were prepared in concd HCl from reagent quality metals. 0.1 M KCl supporting electrolyte and 0.2% Triton X-100 maximum suppressor stock solutions were prepared in distilled water. Potassium ferrocyanide, quinaladic acid, potassium chromate and sodium sulphide were used for qualitative indentification of metal cations during ion-exchange separations.
THE QUENCHING OF TRIPLET STATES OF ANTHRACENE IN SOLUTION BY ELECTRON ACCEPTOR COMPOUNDS

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Decay rates of triplet anthracene in solution containing different electron acceptor compounds have been measured and the bimolecular quenching constants have been evaluated. The second order rate constants for various energy acceptors in solution and at room temperature conform to the CT mechanism of Linschitz type. In cases where the difference between the triplet level of the energy donor and the CT state is within the range of singlet-triplet split in the energy donor molecule, the value obtained for \( k_q \) corresponds to unit encounter frequency but when the difference is large, the efficiency falls to the one described by Porter and Hoffman. The method and technique has been described elsewhere. The changes in the triplet decay rates observed upon flashing the photoreactive molecules in solution were studied at various concentrations of the electron donor compounds. In all cases a suitable filter solution was put in the outer jacket of the absorption cell so that the light was only absorbed by the energy donor compound. This was confirmed at each concentration by flashing the added compound alone in the reaction cell with filter solution in position. Strict checks were made on the absorption spectra of the separate components and that of the mixture to see if there was any indication of complex formation. Because of the strong quenching effect of oxygen on the triplet state, all the experiments were carried out with degassed solutions. It was noticed that repeated degassing resulted in some loss of the solvent which produced a change in the concentration of the solution. To prevent these changes in concentration, an extra 20 ml of the solvent was added into the reservoir bulb and before finally sealing off the solution, the liquid level was brought to a predetermined mark under room temperature conditions. When using electron acceptor compounds of high vapour pressure, for example, they were degassed in a special ampule fitted with a break-seal device, and then mixed with the degassed solution.

Materials.—In this kind of investigation purity of the solute as well as of the solvent is of prime importance. The methods used to purify the materials are described briefly as follows:

Anthracene (laboratory grade) was recrystallized twice with pure carbon tetrachloride, then sublimated twice under reduced pressure.

Nitrobenzene (A.R. grade) was twice distilled under reduced pressure.

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Copolymerization of acrylonitrile and allyl acetate with benzoyl peroxide as catalyst was carried out at 75±0.1°C. Monomer reactivity ratios were determined experimentally using Mayo and Lewis, mole ratio and mole fraction methods. The values of \( r_A \) and \( r_B \) measured as above were then compared with the values obtained by the modified Hammett equation. The results show that acrylonitrile enters in copolymerization fifty times more rapidly than allyl acetate. Some of the physical properties of the copolymer formed are also studied.

The behaviour of two monomers \( M_A \) and \( M_B \) in free radical type of copolymerization may be described by the following relation,

\[
\frac{d[M_A]}{d[M_B]} = \frac{[M_A]}{[M_B]} \times \frac{r_A [M_A] + [M_B]}{r_B [M_B] + [M_A]}
\]

where \([M_A]\) and \([M_B]\) represent the concentration of the unreacted monomers in the reaction mixture, \(d[M_A]/d[M_B]\) refers to the ratio of the two monomers in the increment of copolymer formed, \(r_A\) and \(r_B\) are reactivity ratios of the monomers. This equation is useful in measuring the factors which govern copolymerization reactions. In the differential form it is valid at any conversion for relating the instantaneously forming copolymers with instantaneous monomer compositions. On integrating equation 1, Mayo and Lewis\(^1\) obtained the following expression,

\[
\ln \frac{[M_B]}{[M_A]} = \frac{1}{p} \left( \ln \frac{[M_A]}{[M_B]} \right) - \frac{1-p}{p} \left( \ln \frac{[M_A]}{[M_B]} \right)_0 + \frac{1}{p} \ln \frac{[M_A]}{[M_B]} - \frac{1-p}{p} \ln \frac{[M_A]}{[M_B]}_0
\]

where \([M_A]_0\) and \([M_B]_0\) are the monomer concentration in feed, \(p=(1-r_A)/(1-r_B)\). Positive or negative values of \(p\) are arbitrary chosen by trial and error to find the corresponding values of \(r_A\) and \(r_B\). For any copolymerization reaction a plot of \(r_A\) versus \(r_B\) determined as above should give practically a straight line. Another reaction performed under identical conditions with different monomer feed will give a straight line plot of \(r_A\)-\(r_B\). At the point of intersection the corresponding values of \(r_A\) and \(r_B\) read from the graph should give fairly an accurate estimate of the reactivity ratios. Usually more than three experiments with different \([M_A]_0\) and \([M_B]_0\) are performed to estimate the magnitude of error involved in values of \(r_A\) and \(r_B\) determined by the intersection method.

Using equation\(^1\) Fineman and Ross\(^2\) derived the following rate equation to determine \(r_A\) and \(r_B\),

\[
\frac{F}{f} (f-1) = r_A F^2 - r_B
\]

where \(f\) is \(d[M_A]/d[M_B]\), \(F\) is monomer ratio, \([M_A]/[M_B]\). A plot of \(F/(f-1)/f\) against \(F^2/f\) is a straight line whose slope is \(r_A\) and intercept \(r_B\). This is called mole ratio equation.

The modified form of Fineman and Ross equation expressed in mole fractions of monomer is written as\(^3\)

\[
\frac{f_A}{(1-f_A)} F_A = r_B + \frac{f^2_A (F_A - 1)}{(1-f_A)^2 F_A} - r_A
\]

where \(f_A\) represents the mole fraction of monomer in feed, \(F_A\) the mole fraction of monomer in the increment of copolymer formed at the start of copolymerization.

The copolymerization of acrylonitrile with different monomers has already been studied by many workers. This paper describes the copolymerization of acrylonitrile and allyl acetate using benzoyl peroxide as initiator with a view to determine monomer reactivity ratios. To arrive at a better estimate of the reactivity ratio values comparative study of different methods is undertaken. Some of the physical properties of the copolymer formed are also reported.

**Experimental**

Procedure.—All copolymerization reactions were carried out in sealed Pyrex-glass tubes, 30 cm long and 20 cm internal diameter. 1.25 g acrylonitrile and 1.25 g allyl acetate were transferred through a long stem funnel into the reaction tube kept at 0°C. To this 0.025 g benzoyl peroxide was added and the tube sealed at the constriction. The reaction tube was then placed in a thermostatic bath whose temperature was maintained at 75±0.1°C. Several trial runs were carried, which indicated that after about 3 hr the entire amount of acrylonitrile polymerized.
CHEMICAL CONSTITUENTS OF JUTE – CORCHORUS CAPSULARIS AND CORCHORUS OLITORIUS

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A STOMACHICOLID METACERCARIA*

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The present communication of yasimin, the isolation and structure of a new metabolite, nidulin and mannitol, from the broth and mycelium and structures of two new metabolites of sparingly obtained from the ethereal s/zirin and exhaustively extracted in a Soxhlet apparatus mother mycelium was dried then the ether extract of the mycelium, through at medium enriched with carrot extract for 22 days with petroleum ether and finally with ether. The investigation gave the following nine metabolites shown the presence of three compounds. These perishable soluble yasimin mixed with two ethereal extract on standing yielded practically new products. Yasimin was separated through preparative TLC, fractional crystallisation and its mother preparative fraction afforded a semisolid material which was yasimin 162°C, while the other two were new entities not yet described. The work on the remaining metabolites is in progress and will be reported later.

Haiderin (IV).

Haiderin (m.p. 170°C) had the molecular formula C_{19}H_{18}O_{8}Cl by high resolution mass measurements. Its UV spectrum gave \( \lambda_{\text{max}} \) at 265 m\( \mu \) (e 10108) and a shoulder at 320 m\( \mu \) (e 1026). Its IR absorption bands were at 3401 cm\(^{-1}\) (OH), 1722 cm\(^{-1}\) (C=O) and two bands at 1610 cm\(^{-1}\) and 1590 cm\(^{-1}\) (benzene ring stretching). Both the UV and the IR absorption bands were the same as observed in the cases of yasimin, noridulin, and nidulin. Its mass spectrum showed a strong molecular ion peak at m/e 378 which lost 18 mass units, indicating the loss of one molecule of water, giving rise to an ionic...
In the previous communications, we have described the isolation and structures of yasimin and other metabolites of Aspergillus unguis.

In the present communication we are describing the biosynthesis of yasimin. Yasimin is a very attractive depsidone for biosynthetic studies, because of its origin from three different precursors using acetate–malonate, mevalonic acid and methionine. We have undertaken a preliminary study of the biosynthesis of ring A of yasimin, which could be derived from one acetate and three malonate units, due to the condensation and cyclisation of head-to-tail polyketide chain by aldol condensation, forming orsellinic acid moiety of yasimin. The origin of ring B could be attributed to two isoprene units, which will be tested by feeding (2-C\(^14\))-mevalonic acid. The C-methyl of the ring B was derived from methionine due to transmethylation at this position. It was already shown few years ago that ring B of nidulin was derived from isoleucine and the methyl group in the same ring was speculated to be derived from formaldehyde. The role of mevalonic acid for C-5 unit had been demonstrated in a wide variety of natural products such as terpenes, alkaloids and xanthones and as such it could be quite safe to expect that mevalonic acid will be incorporated in a high degree in the ring B of yasimin in comparison to isoleucine. Biosynthetic studies on the ring B are underway and will be reported later.

For biogenetic studies of ring A, a solution of sodium (1-C\(^14\))–acetate (0.6 m.c.) was fed to Aspergillus unguis after 3 days growth, on a modified Czapek–Dox medium enriched with carrot extract. After 22 days the mycelium was separated and worked up to yield pure yasimin, which was recrystallised to constant radioactivity (incorporation 0.9\%, 1.9\%; r.m.a. 2.6 x \(10^5\) and 5.3 x \(10^5\), respectively).

When yasimin (r.m.a. 2.6 x \(10^5\)) was hydrolysed and decarboxylated to decarboxyyasimin, the evolved carbon dioxide was assayed as barium carbonate (r.m.a. 0.34 x \(10^5\)). The ether linkage of decarboxyyasimin was cleaved with a mixture of hydroiodic and nitric acids yielding pure orcinol (r.m.a. 1.2 x \(10^5\), 3 x C\(^14\)). Orcinol was oxidised by Kuhn–Roth method and Schmidt reaction to obtain carbon dioxide, which was trapped as barium carbonate (r.m.a. 0.42 x \(10^5\), 1 x C\(^14\)) which is slightly higher than the one-third activity (0.4 x \(10^5\)) of orcinol. The remaining reaction mixture was reacted with 2,4-dinitrochlorobenzene, to get N-methyl-2,4-dinitroaniline which was inactive, indicating that the whole activity was in the carboxyl group.

These results were further verified by hydrolysing yasimin (incorporation 1.9\%, r.m.a. 5.3 x \(10^5\)) esterifying and breaking the ether linkage with nitric acid to get methyl orsellinate (r.m.a. 2.24 x \(10^5\), 4 x C\(^14\)). Kuhn–Roth oxidation of methyl orsellinate gave sodium acetate. This was further degraded by Schmidt reaction and the carbon dioxide gas evolved was trapped as barium carbonate (r.m.a. 0.6 x \(10^5\), 1 x C\(^14\)) which was again slightly higher than the exact one-fourth activity (r.m.a. 0.56 x \(10^5\)). As in the previous case, the remaining methylamine hydrochloride was once again found to be inactive. These results are summarised in Chart I.

The above results were supported by the degradation of yasimin derived from sodium (2-C\(^14\))-acetate (incorporation 1.2\%, r.m.a. 4.5 x \(10^5\)). On alkaline hydrolysis and decarboxylation with copper–bronzine in quinoline, followed by ether cleavage with a mixture of hydroiodic and nitric acids, yasimin yielded pure orcinol (r.m.a. 2.35 x \(10^5\), 4 x C\(^14\)). Kuhn–Roth oxidation and Schmidt degradation of the resulting sodium acetate gave barium carbonate which was found to be inactive but the N-methyl-2,4-dinitroaniline derivative of methylamine hydrochloride had again quite high activity (r.m.a. 0.77 x \(10^5\), 1 x C\(^14\)) against the exact one-fourth expected activity (r.m.a. 0.58 x \(10^5\), 1 x C\(^14\)).
STUDIES IN THE BIOCHEMISTRY OF MICROORGANISMS

Part XIV.—Biosynthesis of Amudol, a Metabolic Product of Penicillium martinsii Biourge

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The biogenesis of amudol has been examined by feeding sodium (1-C\textsuperscript{14})- and (2-C\textsuperscript{14})-acetates. It has been shown that amudol is formed by head-to-tail aldol condensation of one acetate and three malonate units.

In the earlier communications\textsuperscript{1-2} we have described the isolation and structures of amudol, amudane, and amujane—last three identified as griseofulvin, dehydrogriseofulvin and dihydrogriseofulvin—obtained as metabolites of the mold Penicillium martinsii.

The structure of amudol (I) brought out some very interesting features. The starting unit is oxidised to a primary alcoholic group, one of the hydroxyl group, which should have been located in position 3 on the polyketide chain is located at position 2 due to the phenomenon of hydroxylation-induced intramolecular migration,\textsuperscript{3} 'NIH shift' and lastly a chlorine atom is substituted in position 4. With these features available in the molecule of amudol it was considered desirable to study its biogenesis as a start leading to our projected studies of biogenesis in the cell-free systems.

Amudol is built up as a very simple molecule formed due to the head-to-tail condensation of one acetate and three malonate units. The present communication describes the biogenesis of amudol employing sodium (1-C\textsuperscript{14}), and also (2-C\textsuperscript{14})-acetate as precursors. Penicillium martinsii was grown on modified Czapek--Dox medium and pure amudol was obtained derived from (1-C\textsuperscript{14})-acetate and also from sodium (2-C\textsuperscript{14})-acetate separately. The incorporation was 1.45\% and 2.8\% respectively.

Amudol derived from sodium (1-C\textsuperscript{14})-acetate (r.m.a. 3.9 x 10\textsuperscript{5}) was oxidised and decarboxylated to give carbon dioxide, assayed as barium carbonate, which was almost inactive (r.m.a. 0.031 x 10\textsuperscript{5}). This small activity could be due to randomisation.

When amudol was nitrated\textsuperscript{4} and subjected to hypobromite degradation,\textsuperscript{5} it gave bromopercin, which was reduced and the resulting methylamine hydrochloride was assayed as its N-methyl-2,4-dinitroaniline derivative (r.m.a. 1.18 x 10\textsuperscript{5} for 1C\textsuperscript{18} against 1.30 x 10\textsuperscript{5}). This indicates that the lower activity was due to the benzene ring carbons, which were formed due to decarboxylation of malonate unit.

Dechlorination,\textsuperscript{4} reduction and Kuhn-Roth oxidation and Schmidt reaction of the resulting 2,5-dihydroxytoluene gave barium carbonate (r.m.a. 1.34 x 10\textsuperscript{5} for 1C\textsuperscript{18} against 1.30 x 10\textsuperscript{5}). This high activity was indicative of the starting unit of the molecule. The methylamine hydrochloride the second product in the Schmidt reaction, was assayed as N-methyl-2,4-dinitroaniline and was found to be inactive. All these results are outlined in Chart 1. Amudol derived from sodium (2-C\textsuperscript{14})-acetate (r.m.a. 6.27 x 10\textsuperscript{5}) when oxidised and decarboxylated to give carbon dioxide (assayed as barium carbonate) was found to be, as expected, active (r.m.a. 1.6 x 10\textsuperscript{5} against 1.5 x 10\textsuperscript{5}). This higher activity can be safely attributed to the starting unit of the molecule.

Similarly, amudol on nitration\textsuperscript{4} and hypobromite degradation,\textsuperscript{5} yielded bromopercin which was reduced and assayed as N-methyl-2,4-dinitroaniline, having slightly lower activity (r.m.a. 1.54 x 10\textsuperscript{5}; 1C\textsuperscript{18} against 1.56 x 10\textsuperscript{5}).

Similarly dechlorination,\textsuperscript{4} reduction and Kuhn-Roth oxidation followed by Schmidt reaction of the resulting 2,5-dihydroxytoluene gave barium carbonate (r.m.a. 0.00). The methylamine hydrochloride, the second product in the Schmidt reaction, was assayed as N-methyl-2,4-dinitroaniline (r.m.a. 1.59 x 10\textsuperscript{5} for 1C\textsuperscript{18} against...
A novel way has been employed to isolate the synthetic molecule due to the oxidation of the starting unit into benzyl alcohol, chlorination at position 4 in the benzene ring and the occurrence of the phenomenon of an "NIH" shift in this molecule.

The presence of all these interesting features in such a simple molecule, initiated us to synthesize the early possible precursors, in vitro, which led ultimately to its full synthesis. In the present paper the synthesis of amudol has been described, starting from a known compound: 2,5-dihydroxytoluene.

2,5-Dihydroxytoluene was acetylated with pyridine and acetic anhydride, yielding pure 2,5-diacetoxytoluene. The IR spectrum (thin film) showed absence of any hydroxyl group, but showed a strong absorption band at 1750 cm⁻¹ for acetyl group. It is interesting to observe that the benzenoid stretching decreased to 8%. The confirmation of the formation of diacetoxy derivative was further supported by its UV spectrum, which showed a λ_max at 266 μµ (ε 653.3) and 257 μµ (ε 618). The blue or red shift was not observed with a drop of base.

2,5-Diacetoxytoluene was chlorinated under controlled conditions. The resulting mixture of chlorinated compounds contained the desired 2,5-diacetoxy-4-chlorotoluene. Due to scarcity of the material the chlorinated compounds could not be separated at this stage. Their IR spectra showed strong absorption bands at 1750 cm⁻¹ (–OCOCH₃), 1600 cm⁻¹ and 1580 cm⁻¹ for benzenoid stretching. The chlorine absorption band appeared at 1000 cm⁻¹. The UV spectrum* showed λ_max at 265 μµ (ε 589.6) which was consistent with the known chloro-substituted benzenoid compounds, having acetyl groups as the other substituents on the benzene ring. This low absorption might be due to the electronegative chlorine.

The above mixture of chlorinated diacetoxy derivatives was oxidised with KMnO₄ in acetone which gave a very good yield of the chlorinated diacetoxybenzoic acids. Their IR spectrum (in chloroform) showed absorption bands between 3500 3100 cm⁻¹, 2900 2600 cm⁻¹ and then at 1700 cm⁻¹ for carboxylic group. Apart from these bands there was a strong absorption band at 1750 cm⁻¹ and 1740 cm⁻¹ for the acetyl and the ester groups respectively. The UV. Spectrum showed a strong absorption band at λ_max 268 μµ (ε 988.1). The somewhat low absorption value of the ester group was consistent with the already known example of methyl benzoate. The esters were reduced to benzylic alcohol with lithium aluminium hydride, yielding a mixture of three compounds, one of which was diacetoxyamudol. The quantity of the material so obtained was so small (15 mg) that isolation through preparative thin-layer chromatography was not attempted. Instead separation through radio-dilution method was tried. The cold mixture containing 2,5-diacetoxy-4-chlorobenzyl alcohol was dissolved in triethylamine, containing tritium oxide and heated (100°C) in a sealed tube for 2 hr. The tritiated mixture of products containing diacetoxyamudol was hydrolysed with alcoholic potassium hydroxide under nitrogen. To the worked up reaction product cold amudol (70 mg) was added. Since amudol is practically insoluble in benzene and since its dichloro and trichloro derivatives are insoluble, the mixture of 'hot' and 'cold' amudol with the other chlorinated products were dissolved in...
STUDIES IN THE BIOCHEMISTRY OF MICROORGANISMS

Part XVI.—Interconversion of Yasimin and Nornidulin, Metabolic Products of Aspergillus unguis

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The structural relationship of yasimin and nornidulin has been established through chlorination of yasimin into nornidulin and dechlorination of nornidulin to obtain yasimin.

In earlier communications we described the isolation, structure and biogenesis of the very interesting metabolite of Aspergillus unguis, yasimin. The structure of yasimin (I) was established as that of nornidulin (II) without its three chlorine atoms.

\[
\begin{align*}
(I) & \quad \begin{array}{c}
\text{HO} \\
\text{HC-CH}_3 \\
\text{C-CH}_3 \\
\text{Cl}
\end{array} \\
\text{HC-CH}_3 \\
\text{HO}
\end{align*}
\]

\[
\begin{align*}
(II) & \quad \begin{array}{c}
\text{HO} \\
\text{HC-CH}_3 \\
\text{C-CH}_3 \\
\text{Cl}
\end{array} \\
\text{HC-CH}_3 \\
\text{HO}
\end{align*}
\]

During a recent synthesis of amudol we carried out chlorination of the intermediate diacetyl derivative: 2,5-diacetoxytoluene successfully in presence of triethylamine as catalyst under irradiation with a 1000W lamp. It was felt that chlorination under similar conditions, if successful, could give nornidulin, the trichloro derivative of yasimin. This had to be under controlled conditions to reduce the possibility of chlorine substitution on the butyl side chain. Conversely, if nornidulin (II) could be dechlorinated it would give yasimin (I).

For obtaining nornidulin, a solution of yasimin in a mixture of chloroform and tetrahydrofuran containing triethylamine as catalyst was employed. The solution was saturated with chlorine gas in the cold and exposed to a 1000 W lamp. The chlorinated product was found to be a mixture of three products, one of which was nornidulin. It was separated through radiodilution method by the isolation of tritiated nornidulin (r.m.a. 3500) mixed with authentic sample of cold nornidulin.

For getting yasimin from nornidulin it was dechlorinated with the nickel-aluminium alloy in aqueous sodium hydroxide. This not only resulted in dechlorination, but also, as expected, in the opening of the ring B, yielding the hydroxy acid (III).

This was cyclised back by heating in alcohol containing a few drops of hydrochloric acid, to yield yasimin, confirmed through its UV and IR spectra, which were identical to that of the authentic specimen of the material.

Experimental

IR spectra were taken on a Perkin-Elmer 137 spectrophotometer and the UV spectra were measured in methanol on a Beckman D.K. 2. Radioactivity is described in relative molar activity. Petroleum ether employed had b.p. 65–85°C.

Chlorination of Yasimin (I).—Yasimin (20 mg) was dissolved in chlorine-saturated mixture of tetrahydrofuran (1 ml), chloroform (10 ml) and triethylamine (0.2 ml). The solution was exposed (4 hr) to the light of a 1000 W lamp. The product was taken up in ethyl acetate, washed with a few drops of dil HCl followed by water, dried and the solvent removed. The isolated material was transferred to a 6 in × 0.5 in (dia) tube and dissolved in triethylamine (0.5 ml). To this solution tritium oxide (1 drop, 5 curies) was added. The tube was sealed under vacuum and heated (2 hr) in a water bath. The product was taken up in ethyl alcohol and the solvent removed under reduced pressure. The residue was taken up in ethyl acetate, successively washed with 0.1N HCl and water and dried (Na₂SO₄). The solution, on removal of the solvent, gave the hot semi-solid
STUDIES ON THE SEED OIL OF ABRUS PRECATORIUS LINN

Part I.—Composition of Total Fatty Acids

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Oils of seven varieties of locally available *Abrus precatorius* seeds were examined and the fatty acid composition was determined qualitatively and quantitatively by GLC. As many as seventeen fatty acids have been identified in the oils of these seeds. IR spectra show no unusual features (conjugation, double or a, $\beta$-unsaturation). Although the pattern of fatty acids was found to be quite similar in all the seven varieties, the amounts of some constituent fatty acids differed slightly, especially in three principal varieties, namely white, black and scarlet.

*Abrus precatorius* Linn (Jequirity), locally known as *ratti* or *ghungchi*, is a plant of the natural order *Leguminosae* and suborder *Papilionaceae*. The plant is a perennial twiner with numerous stems and is a natural inhabitant of Indo-Pakistan subcontinent and other tropical countries.

The leaves, roots and seeds of *Abrus precatorius* are described as of medicinal value, an account of which has been given by Dymock. The seeds have been used externally against granular conjunctivitis and various forms of skin diseases, ulcers and affections of the hair, but the most interesting and important property of the seeds is that, when taken internally by women, they disturb the uterine functions and prevent conception.

The seeds of *Abrus precatorius* have attracted much attention on account of their toxic and agglutinating properties, and although the seed oil is reported to possess the antifertility activity, very little work has been carried out on the fatty acid composition of the oil. We have therefore, undertaken detailed gas chromatographic studies of the fatty acid composition of the oil of seven varieties of *Abrus precatorius* seeds available in the local market, and report their fatty acid composition in the present communication.

Materials and Methods

Seven varieties of *Abrus precatorius* seeds were obtained from the local market and graded as: (i) white seeds with no eye; (ii) yellowish seeds with brown eye; (iii) black seeds with no eye; (iv) dark brown seeds with black eye; (v) light brown seeds with black eye; (vi) scarlet seeds with black eye and (vii) vermilion shining scarlet seeds with black eye.

The seed coat was removed by coarse grinding and the kernel was ground into fine powder. The oil was extracted at room temperature with a mixture of chloroform–methanol (2:1 v/v), until all the oil was extracted. The solvent was distilled off, last traces being removed under vacuum.

Gas Liquid Chromatography.—Transesterification of the glycerides to their methyl esters, was carried out according to the procedure of Nelson. The esters were extracted with petroleum ether (b.p. 40°–60°C) and stored in screw-capped sample tubes in a refrigerator until analysed. The methyl esters were found to be suitable for chromatography without further purification.

The methyl esters were chromatographed on a Varian Aerograph model–600 gas chromatograph with a flame ionization detector. A stainless steel coiled column 5 ft 10 in long and 0.8 in outer diameter, containing 80 to 100 mesh Chromosorb W, coated with 20% DEGS (diethylene glycol succinate), was used for the regular packed column GLC. Nitrogen was used as carrier gas.

For all GLC determinations the gas flow was 25 ml/min; column temperature was 187°C; flash heater 250°C; and hydrogen flow rate 20 ml/min. The operating conditions were maintained constant throughout the analysis.

The identities of the individual fatty acids were achieved by co-chromatography with standard reference compounds; where standards were not available, the peaks were tentatively identified by comparing their relative retention times with known values or otherwise by plotting a log retention time curve against carbon number.
STUDIES ON THE SEED OIL OF ABRUS PRECATORIUS LINN

Part II.—Composition of the Lipid Classes

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The seed oils of white, scarlet and the black varieties of Abrus prectorius Linn were studied for their lipid composition with the help of TLC and GLC. Each oil was first separated by TLC into the respective lipid classes which were transesterified and analysed by GLC for their fatty acid composition. The weight percentages of TG in the white, scarlet and the black varieties were 39.2, 25.3 and 40.6% respectively. The lower percentage of TG in the scarlet variety is probably due to incomplete enzymic esterification of the DG, which is higher in this variety (13.5%). The black variety contained the highest amount of FFA (35.5%), showing the possibility of higher activity of lipolytic enzymes. In the individual lipid classes, palmitic and behenic acids were the major saturated fatty acids. Amongst the unsaturated acids, oleic and eicosenoic acids were most predominant, while linoleic was present in appreciable amounts. Linolenic acid was present in higher proportions in the DG, MG and SE fractions of scarlet and MG, SE and PL fractions of the black variety. Presence of odd numbered C15:0 and branched chain C18:0 acids in the oils could have originated from microorganisms, a number of which have already been reported in the seeds of Abrus precatorius Linn.

On the basis of the total fatty acid composition of Abrus precatorius Linn and in accordance with the classification of Roxburg it seems probable that there are three principal varieties of Abrus precatorius Linn,—white, scarlet and black—the other four varieties reported in the last communication are, in one way or the other, related to these three varieties. It was therefore thought of interest to study the various lipid classes of the white, scarlet and the black varieties in greater detail which is being discussed in the present investigation.

Materials and Methods

Oil was extracted from the powdered seeds with a mixture of chloroform—methanol (2:1 v/v) as described earlier. The separation of the various lipid classes was achieved by TLC on plates (20×20 cm) coated with silica gel G (Merck), 0.25 mm thick. The plates were activated at 110°C for 1 hr, and washed with methanol to remove any lipids or other impurities prior to the application of the sample.

Each oil sample (25 to 35 mg) was applied on the plates in the form of a band together with standard reference compounds, and developed with a mixture of ether, petroleum ether and acetic acid (20:80:1 v/v). After development, the plates were dried in air and the bands made visible with the help of iodine vapours (Fig. 1). The separated lipids were marked and scraped off the plates and quantitatively collected in screw-capped tubes for analysis.
STUDIES ON THE SEED OILS OF WITHANIA COAGULANS AND THEVETIA NERIFOLIA

Part I.—Fatty Acid Composition

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The seed oils of Withania coagulans Dunal and Thevetia nerifolia Juss on GLC analysis, have been shown to contain 16:0, 11.64 and 31.25; 18:0, 2.66 and 4.99; 20:0, 3.49 and 4.18; 18:1, 12.56 and 28.76 and 18:2, 69.65 and 29.25% respectively. Infrared spectra of the two oils indicated the absence of any unusual characteristics.

The seeds of the two commonly occurring plants viz. W. coagulans and T. nerifolia which belong to the Solanaceae and Apocynaceae families respectively were studied for their fatty acid composition.

The fruit of W. coagulans has been used in the Indo-Pakistan subcontinent for quite a long time for making cheese. Due to religious bias cheese made with animal rennet is generally not acceptable to the people of this region. Therefore, attempts have been made in these Laboratories for the commercial preparation of a vegetable rennet. While making the rennet, it has been found that seeds which go waste. In order to economise this enzymatic preparation and in view of the importance of vegetable oils it was considered worthwhile to study the nature and composition of the oil from this source.

The seeds of Thevetia nerifolia, which is commonly cultivated as an ornamental shrub in the gardens in plains, were also studied for their fatty acid composition. The seeds of this plant have been used for suicidal and homicidal purposes and as an abortifacient by women in Bengal and the neighbouring provinces. The oil has also been used externally in skin disease. De Vry Tijdschr has obtained from the kernel of the seeds 57% of an oil with a density of 0.9148 at 25°C. Jean Gattefosse has reported 58% yield of the oil. In the present investigation 55% yield of the oil has been recorded. Whereas the oil has been used as a purgative, alcoholic extracts of the seed have been reported as effective contact insecticides.

Experimental and Results

Material.—The berries of W. coagulans were purchased from the local market. They were soaked in water for ½ hr and the swollen berries were then crushed by hand and filtered through muslin cloth. The seeds were washed several times with water to free them from adhering matter, dried and ground into a coarse powder. The seeds of T. nerifolia were handpicked from a plant in a local garden in December and the outer coat was removed mechanically. Thekernel was crushed and used for oil extraction.

Extraction of Oil.—The coarsely ground seeds of W. coagulans and T. nerifolia (200 g and 60 g respectively) were extracted separately with petroleum ether (b.p. 60–80°C) using a Soxhlet extractor till no oil was being extracted. The extracts were dried over anhydrous sodium sulphate and then filtered. The solvent was removed under reduced pressure and the residue was dried at 75–80°C for ½ hr and weighed (30 g; 33 g; i.e. 15% and 55% respectively).

Transmethylation of Oils.—The transmethylation was carried out by the method of Hammond and Lundberg with some modification. The oils were weighed (0.0372 g and 0.0296 g respectively) and applied separately in the form of a band using petroleum ether (b.p. 40–60°C) as solvent on activated silica thin layer glass plates. The plates were prepared by spreading 30 g silica gel for TLC in 60 ml H₂O for five plates, and activated for ½ hr at 80°C. The bands were scraped off and transferred to two different transmethylation tubes with Teflon stoppers. Methanolic sulphuric acid (20%, 1 ml) was added in each tube which were placed in an oven at 80°C for 2 hr. The tubes were taken out and allowed to cool to room temperature. Petroleum ether Analar (b.p. 40–60°C, 2–3 ml) was added to each tube followed by 1 ml water. The contents of each tube were shaken vigorously to extract the transmethylated
INCORPORATION OF IAA TO PROTEINS AND RNA IN VITRO*

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The incorporation of IAA-1-14C and IAA-2-14C into buffer-soluble protein and RNA fractions of Taraxacum roots was studied in vitro. It was observed that a small proportion of the total auxin supplied was incorporated into protein and RNA. The incorporation of IAA-1-14C increased linearly from 2 to 4 hr.

In the past attempts have been made to explore the physiological significant of bound auxins, auxin-complexes and auxin precursors in plant tissues.\textsuperscript{1-6} Siegel and Galston\textsuperscript{7} observed the coupling of IAA with pea root proteins in vivo and in vitro. Galston\textsuperscript{8} found that the binding of IAA to the proteins of pea roots occurs only under conditions of active aerobic respiration.

The present work was designed to investigate the auxin (IAA) binding capacity of proteins and nucleic acid (RNA) in the crude homogenate of Taraxacum root in vitro.

Material and Methods

*This work was carried out at the Department of Botany, University of Sheffield, and is based on part of a thesis accepted for the degree of Ph. D.

Taraxacum officinale Web. roots were extracted with 0.02M phosphate buffer, pH 7.4, and centrifuged in the cold (5°C) at 4000×g for 20 min. The supernatant solution, containing the buffer-soluble protein and RNA, was used for the study. The auxin used was IAA-1-14C (specific activity = 183 μCi/mg) and IAA-2-14C (specific activity = 16.1 μCi/mg), obtained from the Radiochemical Centre, Amersham, England.

IAA was added to 4 ml of the buffer-soluble extract (containing 1.8 mg of total protein and 0.61 mg of total RNA) to give a final concentration of 10⁻⁵M. The tubes were kept in beakers covered with black-polythene sheets to prevent IAA destruction by light. These mixtures were incubated for 1-4 hr on a rotary shaker at 25°C in the dark. 5 ml of 10% trichloroacetic acid (T.C.A.) was then added to stop the reaction and the tubes were left at 5°C for 1 hr, after which protein was separated by centrifugation at 4000×g for 20 min. The precipitates were washed once with 5% TCA and three times with 20 ml of 95% ethanol. The supernatant solutions were pooled and the pH adjusted to 5 with 1N NaOH. A few drops of 10% NaCl were added and the solutions were allowed to stand overnight at 5°C. The RNA was then centrifuged down at 10,000×g for 20 min.\textsuperscript{9}

Protein and RNA precipitates so obtained were washed with ether twice, to ensure that no IAA remained. The precipitates were dissolved separately in 2 ml of 0.5N NaOH and digested at 37°C for 4 hr. Their radioactivity was measured using a liquid-scintillation counter (I.D.L. type 6012), 0.2 ml aliquots of the solution being mixed with 3.0 ml of the liquid scintillator NE-220, (obtained from Nuclear Enterprises (G.B.) Limited, Edinburgh) in 10 ml glass bottles. Each sample was counted for 400 sec.

Results and Discussion

Results of IAA incorporation into buffer-soluble proteins and RNA after 4 hr of incubation are presented in Table 1. It is clear from these results that a small proportion of IAA is incorporated into the protein and RNA fractions. An average of 1.36% IAA-2-14C and 1.45% IAA-1-14C of the total counts added was incorporated into buffer-soluble proteins while the percentage of total radioactivity in the buffer-soluble RNA was 0.742 in IAA-2-14C and 0.644 in IAA-1-14C treatments.

Kinetics of IAA-1-14C incorporation into buffer-soluble proteins and RNA after 1, 2, 3 and 4 hr of incubation at 25°C are presented in Table 2 and Fig. 1. From these results it appears that a small proportion of the total auxin supplied is incorporated into protein and RNA after 1 hr and the rates of incorporation increases linearly from 2 to 4 hr (Fig. 1) in both the cases. The greater increase of IAA incorporation into protein and RNA from 1-2 hr might suggest the availability of more binding sites for IAA into these molecules than at later stages. The mechanism of IAA incorporation into protein and RNA fractions was not studied.

It is not clear from these results whether IAA or some metabolite of IAA was incorporated into...
NEMATICIDAL PROPERTIES OF DIFFERENT AROMATIC FRACTIONS OF PETROLEUM

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Six aromatic fractions of petroleum (one of them chlorinated) were tested for their nematicidal properties against Helicotylenchus sp. The toxicity of these fractions was compared with that of Nemagon. The results indicate that Fraction 400N has got more nematicidal properties than the remaining fractions as it showed 100% mortality of Helicotylenchus sp. at a concentration of 30 ppm after 24 hr of treatment.

Although Needham\textsuperscript{1} discovered Anguina tritici causing ear-cockle disease of wheat in as early as 1743 and Butler\textsuperscript{2} carried out excellent work on the “Ufra” disease of rice caused by Ditylenchus angustus, it was only recently that the importance of plant parasitic nematodes as agents of destruction of crops was realised. It has been estimated that nematodes cause heavy losses annually which is evidenced by the fact that almost all the crops—rice, cotton, jute, sugarcane, tea, tobacco, fruit trees, vegetables and ornamental plants are attacked by nematodes. Banana and papaya (among the fruit trees) and tomato, okra, brinjal, cauliflower, potato and turnip etc. (among the vegetables) grown in the southern west Pakistan are being severely damaged due to the nematode attacks. In this connection the records of Timm,\textsuperscript{4,5,6} Brown,\textsuperscript{7} Kafi\textsuperscript{8} and Akhtar\textsuperscript{9} are worth mentioning.

Six aromatic fractions of petroleum were tested for their nematicidal properties. Helicotylenchus sp. which parasitises banana and other crops was used as test nematode.

Materials and Methods

Bioassays were carried out for testing the nematicidal properties of these substances. One per cent solution of each substance was prepared in different solvents from which solutions of the strength of 10, 20 and 30 ppm were prepared as testing media for the nematodes.

Nematodes belonging to the genus Helicotylenchus were isolated from the soil around the banana roots. Isolation was done by modified Baermann technique in which about 100 ml of soil was spread over a tissue paper placed on a sieve which was already kept in an enamal tray. Water was poured into the tray till the sieve base was just awash and the tray was placed in a cool place.

After 12 hr water was transferred to the flask and left for about 2 hr and was then decanted. Mature Helicotylenchus specimens were isolated under binoculars. Identification was done after Thorne.\textsuperscript{10}

One hundred mature nematodes were taken for each treatment of different solutions. Three replicates were kept and the experiment was repeated three times. Per cent mortalities of Helicotylenchus sp. were counted after 24 hr of treatment and were corrected by using Abbott's formula. For comparison Nemagon was used.

<table>
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<th>Substance</th>
<th>Strength ppm</th>
<th>% mortality after 24 hr</th>
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<tr>
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<td>Fraction 400N</td>
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TOXICITY OF SOME INSECTICIDES TO THE RED COTTON BUG, DYSDERCUS KOENIGI (FAB)

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(Received October 24, 1969)

The LD₅₀ values and relative toxicity of five organophosphorus insecticides, Azodrin, Bidrin, Birlane, and Dimecron were determined by topical application to third instar nymph and adult of red cotton bug, Dysdercus koenigi (Fab.). Azodrin and bidrin were found to be most toxic to the nymphs and adults, respectively. The nymphs were comparatively more susceptible to the insecticides tested.

Comparatively very little toxicological work has been done on the red cotton bug, Dysdercus koenigi (Fab.). Insecticidal control of this pest, however, has been reported by several workers. Trehan et al. determined the LD₅₀ values of several selected insecticides and concluded that gamma-BHC, endrin, parathion and aldrin were more toxic than DDT. Lal mentioned that alphamethyl acetic acid (NAA)—a plant growth regulator, increased the toxicity of malathion to red cotton bug.

Considerable research has been reported on the toxicities of chemicals on different species of cotton insects. However, it is not safe to assume that chemicals effective in controlling other insects will also control red cotton bug. The work reported herein, though based on laboratory evaluation will help field researchers to select a suitable insecticide for control of the red cotton bug.

Methods and Materials

The red cotton bug was first collected from the field and then was transferred in the laboratory to rear on the leaves of okra, Hibiscus esculentus Linn. Only the adults and third instar nymphs were used in the test. Five organophosphorus insecticides, azodrin, bidrin, birlane, diazinon and dimecron were used. Desired working concentrations were prepared by adding requisite quantity of acetone. Four dosage levels were used for each insecticide. One hundred each of the third instar nymphs and adults were selected at random, weighed collectively, and mean weight per individual calculated. This weight was used as a basis for calculation of insecticide dosages. One μl of the insecticide-acetone solution was applied on the tip of the abdomen of each insect with the help of a micro-syringe. Following treatment the insects were transferred to glass jars containing fresh okra leaves. Ten insects were used in each treatment and the treatments were replicated at least four times. An acetone-treated control was maintained in each test. All experiments were done under the same conditions of temperature and humidity (29 ± 1°C and 75 ± 5% R.H.). Mortality counts were made 24 hr after the treatment. The moribund insects were also counted as dead. The data were subjected to probit analysis. The median lethal dose (LD₅₀) was determined from the LD-P line and fiducial limits were set at the 95% level of probability.

Results and Discussion

The results for the contact toxicity of the insecticides tested against the nymphs and adults of the cotton bug are summarized in Tables 1 and 2, respectively. For a comparison of the toxicities, the LD₅₀ values and regression equations for each of the insecticides were determined.

It appears that the relative toxicities of the compounds against the nymphs and adults varied considerably. In case of the nymph, the toxicity was in the order: azodrin > bidrin > dimecron > dimecron > birlane after 24 hr of treatment, while in the adult it was bidrin > dimecron > azodrin > dimecron > birlane. The values of relative toxicity of the different insecticides have been calculated by taking dimecron as unity. In all cases the probit analyses of the percentage mortality gave χ² values which are not indicative of significant heterogeneity.

The results show that the insect was susceptible to all the test insecticides. Birlane, however, showed least toxicity to both the nymph and the adult. Of the two life stages, the nymph was significantly more susceptible than the adult. Furr and Calhoon found several insecticides more toxic to third instar than the fourth instar larvae of fall armyworm. McPherson et al. applied DDT, BHC-DDT-sulphur mixture and endrin.
A NEW SPECIES OF LEPTUS FROM PAKISTAN (ACARINA: ERYTH RAEIDAE)

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(Received September 2, 1969)

A new species of mite belonging to genus Leptus latrellii, 1795 (=Achorolophus Berlese, 1891) has been described from Karachi out of a collection of 60 female specimens. Morphological description has been given and the species has been compared with the closely related species, Leptus vilosus Berlese, 1910.

**Leptus karachiensis** sp. nov. (Fig 1-7)

Dimension: Body length, excluding gnathosoma, 1.124 mm; width 0.794 mm (means of 10 specimens).

Dorsum: Dark red, oval mite with numerous rod-like lightly pigmented setose 0.022 mm long setae. Crista stout, 0.453 mm long, extending up to the level of coxae III; anterior sensillary area with a pair of finely setose, 0.08 mm long sensillary setae and 8 stout densely setose nonsensillary setae; posterior sensillary area pear shaped, with a pair of finely setose sensillary setae 0.112 mm long. A pair of well-developed lenslike eyes on either side above the anterior half of the crista; each eye 0.125 mm away from the crista.

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Fig. 1.—*Leptus karachiensis* n. sp. (dorsum of female). Fig. 2.—Dorsal seta. Fig. 3.—*Leptus karachiensis* n. sp. (venter of female). Fig. 4.—Crista. Fig. 5.—Pedipalp. Fig. 6.—Tarsus I. Fig. 7.—Tarsus IV.
ANDRABIA, NEW GENUS AND A. KASHMIRESIS, NEW SPECIES (TYPHLOCYBINAE: CICADELLIDAE) ON THE PLANT TEMBER (ZANTHOXYLUM ALATUM) IN NORTHERN AREAS OF WEST PAKISTAN

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(Received October 16, 1969; revised February 19, 1970)

The genus Andrabia, new genus includes a single species. A. kashmireshis, new species a pest of wild plant tember, Zanthoxylum alatum in northern hill areas of West Pakistan, particularly in the valleys of Azad Kashmir and Abbottabad. The genus is remotely related to a few erythroneurine genera described by Ghauri\textsuperscript{1} and Mahmood\textsuperscript{2} from Philippines Islands, but can be easily distinguished on the basis of characters of pygofer and male plate.

During field visits to the northern hill areas of West Pakistan for the study of leafhopper fauna, extensive stippling type of damage on the upper surface of the leaves of wild plant, tember (Zanthoxylum alatum) was noticed. These marks usually indicate the presence of typhlocybine leafhoppers, and are the areas of leaf rendered dead by the feeding punctures of these leafhoppers. The leafhopper responsible for the damage was collected and studied by the present worker. It is presently being described as a new genus and a new species. The method and technique of study was similar to that followed by Ahmed\textsuperscript{3,4}.

**Andrabia, New Genus**

Type of the genus Andrabia kashmireshis, new species.—Hindwings with venation typically erythroneurine. Forewings with first, second and third apical cells successively shorter in length; first apical cell with base oblique; second apical cell elongate, narrower than either adjoining apical cell, with sides subparallel; third apical cell broadening towards apex; outer apical cell short, not reaching wing apex, with or without a few adventitious anteapical cells along costal margin; costal plaque present.

Male genitalia with male plate, in lateral view, situated in middle, with short macrosetae, a few on lateral margin in basal half, a row in distal half on lateral and mesal margins, continuous at apex; in ventral aspect with four macrosetae arranged obliquely in basal half; pygofer with posterior part of disc narrowed, with a fringe of microsetae on posterior margin, a few macrosetae posterosdorsally, processes lacking. Style somewhat similar to that of Thaia Ghauri, with cephalic part 2/3 in length of caudal part, with a narrow ‘flange’ on lateral margin, in middle of caudal part, apex curved dorsad, with a spiny extreme tip. Connective V-shaped, arms stout, wide, with a median cephalic lobe. Aedeagus with preatrial part directed dorsad, shaft bent caudad at 90\(^\circ\) to preatrium, with two pairs of preatrial processes, one pair, much slender arising laterodorsally, and the second pair arising laterally, both near base of preatrium.

The genus has been named after Mr. S.D. Andrabi, Director of Agriculture, Azad Kashmir Government.

**Discussion**

Andrabia, new genus has been included in the tribe Erythroneurini on the basis of fusion of vannal veins in the hindwings. This character according to Young\textsuperscript{5} has been observed either in the tribe Erythroneurini or some genera of tribe Dikraneurini in the family Cicadellidae. The absence of submarginal vein at wing apex however clearly differentiates Andrabia, new genus from any Dikraneurine genus.

The second important character of the tribe Erythroneurini is the presence of a preapical lobe, and an apical extension on style. Andrabia, new genus does not have any of these characters developed typically. The so called ‘flange’ in this case has neither the shape of a lobe, nor is it preapical in position. It is developed as a narrow lateral projection, somewhere in the middle of the caudal part of style. Moreover the apical extension, is neither truncated at apex, nor forms ‘heel’ for the development of a second apical extension. It is rather narrowed to a spiny tip.

Mahmood\textsuperscript{2} and Mahmood and Ahmed\textsuperscript{6} observed that the oriental Erythroneurini show much more diversification in the characters of style than that reported by Young\textsuperscript{5} for American species of the tribe. Mahmood\textsuperscript{2} described a number of new genera i.e. Thilus, Hardiana, and Makilingana, and Ghauri\textsuperscript{1} described Thaia, which all have style shape, appreaching to the style shape of Andrabia, new genus to various degrees. All of Mahmood’s
Prof. G.B. Poulton's classical work on *Colours of Animals* explains how most insects have a protective coloration. A typical instance can be provided by the homopterous insect, *Cicadella viridis*. It is coloured green as it feeds on weeds of the same colour. The insect is widely distributed in Europe particularly in Denmark and Italy. Practically any locality, where weeds are growing in a wet meadow, would offer some specimens of this insect. In places it has been found in such abundance as though nature had meant to cultivate it in a farm. Thus there is no scarcity of material and the findings described here can be easily confirmed.

Poulton explains that the gorgeous and iridescent colours of many butterflies and beetles are due to the physical phenomenon of interference. This has been further explained in a relatively modern work of Prochnow. In such cases no pigment accounts for coloration. But it is quite conceivable that an underlying pigment can be superposed by interference, when the colour would appear somewhat different to the pigment itself. Probably this explains how the wings of the female *C. viridis* are bright green, as the name suggests, while those of the male have a bluish metallic sheen. Now the majority of insects do possess actual pigments. But their study started only when biochemistry developed into an independent branch of chemistry. Although dyes, like the cochineal, were studied when chemists were anxious to synthesize dyestuffs, pioneering work on insect pigments started with the isolation of a pterin from the wings of the cabbage-white-butterfly by Prof. Hopkins of Cambridge. By now pterins represent a group of important biological substances which includes folic acid. Work is also being continued at Cambridge, on colours of aphids, which was initiated by Lord Todd.

Considering the fact that the chemistry of insect pigments had hardly begun, their genesis has hitherto remained untouched. However, instead of its appearing a formidable problem, in some cases at least, there is a clue promising to facilitate research even on insect colours themselves. The symbiotic bacteria of two spittle-insects, *Aphrophora salicis* and *A. spumaria*, produce pigments similar in appearance to 'yellow ochre', and 'burnt-sienna', which is reddish. The pigments of the symbiotic bacteria match with the colours of their respective insect hosts. Thus it would be easier to isolate the bacterial pigment after growing the germ as much as one desires, study the chemical constitution, and identify the same with the pigment of the insect body.

A problem becomes the more interesting as it proves to be many sided. Such happened to be the case with *Cicadella viridis*. The wings of the female, are yellowish green, while its body colour is lemon yellow; both are seen in Fig. 1 where the insect is shown in profile. Its head, in front, has a touch of orange, and traces of this colour are also present elsewhere on the body. The abdomen is seen best from underneath; Fig. 2 shows it as not being uniform in colour, there being traces of orange, as also of an olive-green colour. The abdomen of the male is clearly more orange, as also its head and legs. Of the two, the female is lighter, in all the shades of colours, thus representing 'the fair sex'. This reveals that the oxidation-reduction system differs in the two sexes. It may be stated in advance that the male is more orange due to the pigment being β-carotene, and the female paler because carotene has been transformed into vitamin A, which may be looked upon here as 'leuco-carotene'.

Within the abdomen, on either side of it, the insect carries tumours in its fatty tissue. The germs are bacteria so that the tumour should be designated bacteriotype, and not mycetome, which would imply that the contents represent fungal mycelia. A smear from the bacteriotype, however, would show not only the causal germ, but also cellular debris, resulting from the attack of the parasite up on host tissue. Figure 3 shows a smear of bacteriotype with large protoplasmic residues, P, staining blue with Giemsa, and possessing vacuoles, V. A cell is seen with a nucleus, N, being large enough to remind that giant-cell formation is a common feature in comparative pathology, representing tissue response to an invading germ. There are two bacteria, one long and isolated, R, which produces a red colony,
THE LENGTH-WEIGHT RELATIONSHIP AND CONDITION OF TRICHIURUS SAVALA
GU\'V. AND VAL.

A.K.M. BASHIRULLAH and M.A. KADER

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(Received October 20, 1969; revised March 10, 1970)

The method of analysing the length weight relationship of fish is reviewed. The total length—weight relationship of Trichiurus savala Guv. and Val. is expressed by \( \log W = 0.0331 + 0.0935099 \log L \) for male and \( \log W = -1.95865 + 2.19460 \log L \) for female subgroups. The regression of body weight on total length are calculated to be \( Y = -15.68 + 1.3 X \) and \( Y = 33.8 + 0.191 Y \). The values of coefficient correlation, \( r \) is found to be 0.498.

The mean values of condition, \( K \) and relative condition factor, \( Kn \) in the length—weight relationship of \( T. savala \) are 0.052 and 0.1203 for male and 0.055 and 0.66 for female subgroups.

Trichiurus savala, commonly known as ribbon-fish, is one of the important demersal fishes of the Bay of Bengal. It is popularly known as 'Talwar' in Karachi, Sind and Makran9 and 'Churee mach' in Chittagong and Cox's Bazar. \( T. savala \) is abundant in the inshore waters of the Indian seas and the Bay of Bengal.10,11 The fishing centres in the Bay of Bengal are the waters surrounding Sonadia, Moiskals, Kutubdia, St. Martin's, Dubla islands, and the coast of Teknaf, Cox's Bazar and Chittagong. \( T. savala \) and \( T. haumela \) constitute the ribbon-fish fishery in the Bay of Bengal. The ribbon-fish is very delicious eaten fresh but never reaches beyond the local consumers. Most of the fishes are sun-dried and exported to the neighbouring countries. Not much work has been done in Pakistan on \( T. savala \), although it is an economically important fish. The present paper is a part of the studies on the biology of \( T. savala \).

There is a particular relationship between the length and weight of fish if there is no significant change in form and specific gravity of the species. For an ideal fish, \( n=3 \) in \( W=aL^n \) and this has been observed by Allen\(^{11} \) and Ricker.\(^{10} \) The cube relationship is hardly expected as most of the species do change their shape. The authors\(^{4,6,7} \) found \( n>3 \) which is indicative that the fish weight increases as the cube of the length. It is assumed in the present investigation that \( n<3 \) as it appears from the peculiar ribbon-like shape of \( T. savala \), where weight increases very little with considerable increase in length.

Individual variations from the general length—weight relationship have been frequently studied under the term 'condition'.\(^{4,6,12,13} \) The changes in condition have usually been analysed by means of a 'condition factor' or 'coefficient of condition'. This is calculated as a ratio between the observed weight and that expected from observed length.

Materials and Methods

Materials.—Trichiurus savala were collected bi-monthly from fisherman's catch at the fish landing terminals at Cox's Bazar during the period June 1967 to April 1968. The fishes were preserved in 5\(^{\circ} \) Formalin and then shipped to the Fisheries Laboratory, Department of Zoology, Dacca University.

Length was measured in mm and the body weight in g.

Methods.—The body of the fish is continually changing in its proportions during its life time and the simple cube law expression \( (K=W/L^3 \) where \( W=weight \) and \( L=length \) does not hold throughout the life of the fish. It has been found that the length—weight relationship of most fish can adequately be described by a formula,\(^{11} \)

\[
W = e^{\cdot n} \tag{1}
\]

where \( e \) is a constant and \( n \) an exponent. This formula has been followed in these studies.

The exponential form of relationship in formula (1) can be expressed in the logarithmic form, as

\[
\log W = \log e + n \log L \tag{2}
\]

The values of \( e \) and \( n \) have been calculated for total length-body weight relationship using the log-log relationship in formula (2). The values of \( \log e \) and \( n \) in formula (2) were calculated by using the mathematical relationship.\(^{11} \) This relationship has been used simply by plotting the log of the weight against the log of the length for both male and female fish.

The calculated weight for the corresponding observed mean length were obtained by using the formula (2).
Two species of the sub-family Pilumninae are reared in the laboratory. First zoea of *Pilumnus vespertilio* (Fabricius) has been described by Aikawa\(^1\) and the first zoea of *Pilumnus longicornis* (Hilgendorf) by Prasad and Tampi.\(^4\) However the description of the second and third zoeal stages of *Pilumnus vespertilio* (Fabricius) is new to science.

Materials and Method

Collections of ovigerous female crabs were made from Manora Island and Native Jetty, Karachi during 1964–65. Rearing methods were used as described by Costlow and Bookhout.\(^2\) *Artemia* nauplii were fed to the larvae. Temperature and salinity were maintained at 28°C and 35 p.p.t. respectively during the rearing. Larvae were preserved in 5% Formalin for further study, and lactic acid used as clearing agent.\(^3\) Drawings were made with the help of camera lucida. Measurements were made with the help of an ocular micrometer. Total length was measured from the base of the rostrum to the tip of the telson.

Moulting Periods

The eggs of *Pilumnus vespertilio* (Fabricius) hatched into prezoea which moulted after four hours into the first zoea. First zoea moulted after four days into the second zoeal stage which remained active for five days and moulted into the third zoeal stage. The third zoeae were not active and remained at the bottom and died after 6 hr and none were reared beyond this stage. The eggs of *Pilumnus longicornis* (Hilgendorf) hatched into the first zoea which died after 2 days.

Descriptions

1. *Pilumnus vespertilio* (Fabricius)

Prezoea: It is 2741\(\mu\) in length; spines of the cephalothorax are not fully developed; rudimentary dorsal, rostral and lateral spines are however present; buds of the thoracic appendages have appeared.

Abdomen: A pair of thick lateral knobs on the second segment and a pair of hooks on the third segment; telson fork depth is more than its body length (235 and 220\(\mu\) respectively) sharp projecting points of the prong are finely serrated; a long spine emerge on the lateral side of the prong.

Antennule: (264\(\mu\)) bears four aesthetes.

Antenna: Protopodite (294\(\mu\)) is much longer than the rostral spine of the cephalothorax; bears teeth on distal spine; exopodite bears four setae.

Mandible: bears three large and several small teeth.

Maxillule: bears six plumose setae on the coxal endites; five setose spines on the basal endite; six plumose setae on the terminal and one on the basal segment of the endopodite.

Maxilla: bears eight (5+3) plumose setae on coxal endites; seven (4+3) setae on the basal endites; eight (3+5) setae on the endopodite; four thick plumose setae on the margin of the scaphognathite which terminates as a thick plumose seta.

First maxilliped: has no seta on the basis; four setae emerge on the exopodite; setation on the five segmented endopodite is still enclosed.

Second maxilliped: has no seta on the basis; four setae emerge on the exopodite; three segmented endopodite has imperfectly developed setation.

First zoea: (Fig. 1–3): It is 2867\(\mu\) in length; the dorsal spine is more than seven times as long as the rostral spine of the cephalothorax (Fig. 3g) (514 and 72\(\mu\) respectively); a pair of small lateral spines present on the cephalothorax (Fig. 1a) there are blunt teeth on the dorsal spine; postero-
EFFECT OF THE COOLING PATTERN AND FREEZING DURING RIGOR ON THE QUALITY OF MUTTON

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(Received February 2, 1970)

It is now well established that the solubility of muscle protein fractions, particularly that of myofibrillar proteins, has a positive influence on the tenderness and associated quality characteristics of meat. Hamm and Deathrage,1 Hegerty and Bratzler, and Pearson2 reported significant relationships between water holding capacity, tenderness and the solubility of beef muscle proteins. Ushborne, Kemp and Moody,3 and Topel, Merkel and Wismer-Pedersen4 showed a significant correlation between muscle protein solubility and the juiciness, firmness, overall eating satisfaction and ease of P.S.E. (pale, soft and exudative) muscle development in pig muscle. Summarizing various studies on poultry muscle, Donnelly, Rongey and Bursuk5 reported that protein composition alters with the desirable functional properties of the meat during rigor. In the present study also, the muscle proteins solubility was used as a measure of ovine meat quality.

Animal carcase does not cool uniformly due to the different position of the muscles with respect to the (chill room) environment. This gives rise to slow cooling rate for deep muscles and fast cooling rate for surface muscles. In the present study the slow and fast cooling regimes for ovine muscle were deduced from the cooling data of the longissimus dorsi (4th thoracic) and triceps muscles respectively of a flock of 14.6 sheep of various breeds. Solubility and associated determinations were made from 8 additional sheep of unknown history.

Immediately after slaughter, a portion of minced longissimus dorsi muscle was allowed to go into rigor according to the cooling regimes shown in Table 1. Another portion quick frozen in a solid CO₂ and ethanol mixture 20 min after slaughter, was also thawed according to slow and fast cooling regimes. The solubility of the protein fractions was determined by the method of Hill.6 The pH was measured with a radiometer pH meter using a glass electrode. The standardization of the pH meter was done at room temperature (23°C) against a phosphate buffer of pH 6.86. The sarcomere length was an average of 25 measurements recorded from five different muscle photographs taken by a phase contrast microscope. Expressible juice ratio was determined with the filter paper press technique reported by Briskey, Hoekstra, Phillips and Crummer.7

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<td>30°C</td>
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<td>0.5</td>
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<td>20°C</td>
<td>1.5</td>
<td>2.0</td>
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<td>4.0</td>
<td>10.0</td>
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</tr>
<tr>
<td>5°C</td>
<td></td>
<td>5°C until ultimate pH was reached.</td>
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</table>

The two cooling regimes did not affect the amount of soluble myofibrillar, soluble sarcoplasmic, W.H.C., time to reach ultimate pH, % moisture and % fat of the ovine muscle at the time of its ultimate pH. The characteristics which showed the influence of cooling regimes were the sarcomere length of the muscle and the ultimate pH value. It was interesting to note that if the muscle was frozen immediately post mortem, the influence of cooling regimes on sarcomere length and ultimate pH value disappeared (Table 2). The data suggests that as long as the tissue is undergoing chemical changes of rigor mortis, the slow and fast cooling muscles should not differ in their eating quality based on protein solubility, particularly when the freeze treatment is rendered beforehand. Another interesting feature which came to light was that the common assumption of the more relaxed the muscle the higher the solubility of myofibrillar proteins does not hold good if the muscles reached a certain contraction state under different cooling regimes.

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CHEMICAL CONSTITUENTS OF JUTE – CORCHORUS CAPSULARIS AND
CORCHORUS OLITORIUS

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A STOMACHICOLID METACERCARIA*

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Technology Section


EFFECT OF CHELATING AGENTS ON THE BIOSYNTHESIS OF RIBOFLAVIN
BY CANDIDA GUILLIERMONDI

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The effect of chelating agents on riboflavin production, cell dry weight and glucose consumption by C. guilliermondii in shake-flask cultures was studied. The complexing agents tested were: ethylenediamine-tetraacetic acid (EDTA), diaminocyclohexane-N,N'-tetraacetic acid (CDTA), diethylenetriaminepentaacetic acid (DTPA), and nitrilotriacetic acid (NTA). The amount of chelating agents added to the medium was 2.02 to 18.36 mm.

Of all the chelating agents, EDTA gave highest yield of riboflavin and their stimulatory effect was in the decreasing order of EDTA, CDTA and DTPA. NTA at all levels, however, did not stimulate riboflavin production.

The stability constants of the metal chelates and their structural formulae were important factors in stimulating riboflavin formation.

The selected species of Candida, such as C. guilliermondii and C. flareri produce riboflavin. Tanner et al.12 have reported that riboflavin formation by Candida species is very sensitive to iron concentration, the tolerance limit of iron being 0.005-0.01 µg/ml. Media containing organic nutrients, therefore, need to be freed of excess iron. The conventional methods use ion-exchange resins3 and treatment with a chloroform solution of 2-hydroxyquinoline.4 These methods are exacting, laborious and subject to failure by contamination of the medium from dirt and walls of the vessels. Choudhary and Pirt5-7 have reported a new and as yet little used method based on adding metal-chelating agents to the medium so as to decrease the concentration of free metal ions to the desired values. The significance of the method is that the metal complexes act as a 'metal buffer' which reversibly dissociates so as to replace free metal ions as they are consumed by a growing organism, or to combine with metal ions added to the system. For wider aspects of the subject, the symposium in Federation Proceedings8 should be consulted. The metal-complexing agents are available in large number and their physiological effects have been little investigated and need to be better known if metal buffers are to be used more widely. The purpose of the present study was to investigate the effect of metal chelates as metal buffer on the biosynthesis of riboflavin by Candida guilliermondii in shake flasks.

The theory of metal-complexing agents, which is analogous to that of hydrogen-ion buffers has been described by Chaberek et al.9 The dissociation of a simple metal complex MX is represented by

\[ MX^{2-n} \rightleftharpoons M^{+2} + X^{-n} \]

where \( M^{-2} \) is the metal ion and \( X^{-n} \) is amount of chelating agent of valency \( n \). The stability constant \( K \) is given by

\[ K = \frac{(MX^{2-n})}{(M^{+2})(X^{-n})} \]

where the brackets indicate molar concentrations. The stability constant is used as a measure of the degree of chelation. Values of the stability constants for metals and chelating agents used in the work are given in Table 1.

Table 1.—Log 'K' Values (Stability Constants) of Metal Chelates.*

<table>
<thead>
<tr>
<th>Metal</th>
<th>NTA</th>
<th>EDTA</th>
<th>CDTA†</th>
<th>DTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe³⁺</td>
<td>15.87</td>
<td>25.1</td>
<td>31.0</td>
<td>28.6</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>12.68</td>
<td>18.8</td>
<td>21.3</td>
<td>21.1</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>10.45</td>
<td>16.5</td>
<td>18.67</td>
<td>18.3</td>
</tr>
<tr>
<td>Fe⁴⁺</td>
<td>8.84</td>
<td>13.33</td>
<td>16.5</td>
<td></td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>7.44</td>
<td>14.04</td>
<td>16.78</td>
<td></td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>7.00</td>
<td>8.69</td>
<td>10.32</td>
<td></td>
</tr>
</tbody>
</table>

*All values from Bjerrum et al.10 except † from Simonet al.11 ‡ Not known.

Methods

Organism.—The yeast strain of Candida guilliermondii ATCC-9058 was used throughout present investigation. It was maintained on agar medium containing (g/l): glucose 40; agar 20; KH₂PO₄ 0.5; MgSO₄.7H₂O 0.5; (NH₄)₂SO₄ 2.0; asparagine 2.0 and biotin 1.0 mg/l.

Inoculum Preparation.—The yeast, after growing on agar medium at 30° for 3 days was inoculated.
NUTRITIONAL REQUIREMENTS OF STREPTOMYCES ROSEOCHROMOGENUS FOR THE PRODUCTION OF CYCLOSERINE

Part II. Effect of Phosphorus, Magnesium and Trace Metals

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(Received November 4, 1969; revised January 19, 1970)

The nutritional requirements of Streptomyces roseochromogenus NRRL-B2036 for cycloserine synthesis in shake flasks were studied. Single variable and factorial experiments were conducted to determine the effects of nitrogen, potassium, magnesium, iron, manganese and zinc on the production of cycloserine. The medium which gave best result was (g/l): starch 50, urea 4.8, MgSO₄·7H₂O 5.0, K₂HPO₄ 5.0, FeSO₄·7H₂O 0.02, ZnSO₄ 0.02, and MnSO₄ 0.01. The yield of cycloserine was improved by increasing zinc or K₂HPO₄. Interactions were found between urea and MgSO₄·7H₂O, K₂HPO₄ and MgSO₄·7H₂O, urea and zinc, manganese and zinc and iron and manganese.

In continuation of the work reported earlier, on the effect of carbon and nitrogen sources on the synthesis of cycloserine, a study was made to determine the optimum concentration of phosphorus, magnesium and trace metals such as iron, zinc and manganese. Literature dealing with mineral metabolism of Actinomycetes was reviewed by Thornberry² and Thornberry and Anderson³, Temple,⁴ Chaloupka,⁵ Saunders and Sylvester,⁶ and Johnstone and Wakesman⁷ studied the effect of potassium, magnesium, iron, zinc and manganese on antibiotic production other than cycloserine.

In the present work, the influence of the various constituents of media were investigated on the production of cycloserine with single variable and with a factorial design with multiple variables.

Materials and Methods

Organism.—The strain of Streptomyces roseochromogenus NRRL B-2036 was used in the present study. The culture was maintained on the agar medium consisting of (g/l): glucose 10, agar 20, beef extract 1.0, yeast extract 1.0, casein hydrolysate 2.0. The cultures were grown at 30°C for 10 days.

Inoculum Preparation.—Vegetative inoculum was used in the present investigation. The composition of the inoculum medium was (g/l): glucose 10.0, beef extract 1.0, yeast extract 1.0, casein hydrolysate 2.0. The inoculum medium 25 ml in 300-ml conical flask was inoculated with a loop of mycelium from the agar slant. It was incubated at 30°C for 48 hr.

Results

Single Variable Experiments with Trace Metals.—The effect of each of the trace metals such as iron, zinc and manganese on cycloserine formation by S. roseochromogenus was studied in the basal medium (Figs. 1–3). The concentration of one of the trace metals was changed while the other trace metals were held at the concentration of the fer-
STUDIES ON METHODS OF CITRIC ACID FERMENTATION FROM MOLASSES BY ASPERGILLUS NIGER

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(Received December 30, 1969; revised April 2, 1970)

A study of the effect of different concentrations of sugar and added inorganic nutrients and of different pH values of the fermentation medium on the citric acid production from cane molasses was made. The use of molasses in final concentration of 12.5–15.0% sugar was found best. The initial pH ranging from 3.5 to 6.0 in the molasses solution was found suitable for citric acid production. The concentration of added inorganic salts should not exceed 4.0 g NaNO₃, 1.0 g KH₂PO₄, 0.23 g MgSO₄·H₂O, 0.02 g FeCl₃, 0.0012 g ZnSO₄ and 0.0012 g MnCl₂·H₂O in the fermentation of local cane molasses. At higher concentration of salts fungal growth increased and the citric acid production decreased. As a source of nitrogen, peptone was inferior to sodium nitrate and potassium nitrate whose effect appears to be the same in the citric acid production.

Citic acid was first isolated and crystallised from lemon juice by Scheele. The acid was commercially produced from citrus fruits chiefly in Italy. Wehmer found that the citric acid was a fermentation product of molds. Thom and Currie, Currie, and Doelger and Presscott showed that the citric acid could be produced in bulk from fermentation by Aspergillus niger. Now-a-days the citric acid comes almost wholly from the fermentation industries of U.S.A., U.K., Germany, France and Japan. A large number of patents have been taken out in the field but the actual methods at present in use in different countries have not been made public in their entirety, mainly because of the trade secrecy.

The (1) organisms, (2) inorganic salt requirements, (3) pH of the medium, (4) temperature, (5) sugar concentration, (6) volume of the solution, (7) air supply, (8) incubation period and (9) surface or submerged culture methods are the main considerations of a method of industrial production of citric acid by fermentation.

Suitable conditions required for surface culture method have been studied from the beginning by many workers. In general, highest yields of citric acid have been obtained in the fermentation medium adjusted to suitable pH with metal concentrations deficient for optimal growth of the fungus.

Citic acid which has many industrial uses is now wholly obtained by imports from abroad. The manufacture of citric acid can stop import and as well as solve the problem of the economic utilization of our molasses which are the chief raw materials for citric acid industries. Here the concentration of sugar and added inorganic nutrients, and initial pH value of the fermentation medium made from local cane molasses have been studied in order to find out optimum condi-

Method

The determination of citric acid followed the technique of Taylor as modified by Hughes and Solomos. Sugars were determined according to the method of Somogyi as described by Hockenhull and Herbert.

Preparation of Inoculum.—The organisms were sporulated on the slanted medium of Waksman and Fred in the test tube. Ten ml of sterilized distilled water was added to the tube, shaken and poured in the flask containing 90 ml sterilized water. The spore suspension was added to the fermentation medium.

The fermentation was done during this experimental work according to the surface culture technique. The flasks after inoculation of spores were left undisturbed in the laboratory at room temperature which ranged from 24.0 to 31.5°C depending on the season of the year. After the surface of the fermentation medium was covered by fungal mat, the cotton plug was removed from the flask. No extra air was supplied. Results of four experiments done under each condition were close and the average results are shown in Tables 1-5.

Results and Discussion

Isolation of Organisms.—The organism was isolated from the atmosphere in the laboratories of the Department of Botany and from the soil and
EFFECT OF METAL IONS ON THE PRODUCTION OF ACETIC ACID BY ACETOBACTER ACETI

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The metal ions requirement of Acetobacter aceti (Strain NRC-722) for acetic acid production were undertaken. All the metals were stimulatory at their optimum concentrations which varied from metal to metal. 3.7% acetic acid was produced in the presence of each of iron and calcium at a concentration of 10⁻³ M and 10⁻¹ M respectively, as against 0.6% in the absence of these metals. The optimum level of magnesium, manganese and copper, for maximum production of acetic acid was 10⁻³ M. The yield was further raised to 6.5% when all the metals were present at their optimum concentration.

Studies dealing with the nutritional requirements of acetic acid bacteria have revealed the importance of certain minerals on their growth. Fe, Mg, Mn and Na are the essential constituents from glucose and ethanol by a species of Acetobacter. The present investigation describes the effect of five different metals on the metabolic steps leading to the fermentation of acetic acid by the bacteria. The present investigation describes the effect of five different metals on the metabolic steps leading to the fermentation of acetic acid from glucose and ethanol by a species of Acetobacter aceti.

**Experimental**

**Source of Culture.**—The culture of Acetobacter aceti NRC-722 was obtained from National Research Council, Ottawa, Canada.

**Medium for Maintaining Stock Culture.**—The culture was maintained on agar medium containing 1% glucose, 0.5% yeast extract, 0.25% calcium carbonate and 2.5% agar and was subcultured fortnightly.

**Basal Medium.**—Glucose 2.0 g, ethanol 4.0 ml (v/v), casein hydrolysate 0.5 g, pantothenic acid 100 mg, nicotinic acid 40 mg, p-aminobenzoic acid 40 mg, anurine hydrochloride 100 mg, metal ions, variable concentration, double-distilled water to make 100 ml, pH 6.2.

**Preparation of Inocula.**—Slants having luxurient cell growth after 48 hr of incubation were washed with sterile double-distilled water. The cell suspension was homogenised with glass beads and adjusted to 0.3 optical density at 660 μm.

**Methods**

In the typical experiments all inorganic salts were omitted. The control in our experiments contained EDTA 10⁻⁴ M to bind the indigenous metal ions in media if any. The salts of metal ions were added separately to the medium in different concentrations. All the chemicals were of analytical grade (E. Merck). Solutions of glucose, casein hydrolysate, EDTA and salts of metal ions were sterilized separately at 15 psi for 15 min. Ethanol and vitamin solutions were sterilized by passing through Seitz filter.

The basal medium (19.8 ml) was dispensed aseptically into 100-ml capacity sterilized Erlenmeyer flasks and inoculated with 0.2 ml of the inocula. The flasks were incubated at 30 ± 0.5°C on an incubator-cum-shaker having 88 strokes/min. 1 ml broth was taken aseptically, for estimation of acidity, after 24-hr interval. The controls were run simultaneously.

Total acidity and the percentage of acetic acid was determined by titrimetric method (A.O.A.C.) Vitamin-free casein hydrolysate was prepared by the method of Barton-Wright. The optical density of the inocula was adjusted with Unicamp Spectrophotometer SP-600.

**Results**

The effect of different concentration of the metal ions, separately, on the accumulation of acetic acid by A. aceti are summarised in Tables 1-5. Figure 1 shows the combined effect of all the metal ions, at their respective optimum concentration on the fermentation of acetic acid.

**Discussion**

It is evident from the results (Tables 1-5) that the metals employed in the study were stimulatory for the enzymic reactions, leading to the fermentation of acetic acid. Less acetic acid was always formed wherever EDTA was added in the culture media to bind indigenous and free metal ions.
PRODUCTION OF YEAST CELLS FROM HYDROCARBONS

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(Received December 30, 1969; revised March 14, 1970)

Seventeen strains of yeasts were propagated on media containing kerosene, n-hexadecane and Sui gas (a natural gas containing 95% methane), as the sole source of carbon. Kerosene inhibited growth of all the microorganisms, whereas n-hexadecane supported it. 5 strains utilised Sui gas, but the growth was less on n-hexadecane. Addition of 0.2% peptone to the medium stimulated the growth. A locally isolated yeast strain No. Y3/IRL gave a maximum (78.9%) conversion of n-hexadecane into cellular mass.

Utilization of hydrocarbons by microorganisms has been studied during the last decade. A large number of bacteria, yeasts and molds, capable of assimilating hydrocarbons, have been reported by various workers.

Yeast propagation on hydrocarbon was first reported by Champagnat et al. in 1963. Miller et al. isolated a strain of Candida intermedia, capable of utilising hydrocarbons, from soil. Later, these workers observed that a mixed culture of Candida intermedia and Candida lipolytica produced better cell yields on gas-oil fraction. Takahashi et al. screened a large number of typical and unknown yeast strains for utilisation of hydrocarbons. Dostalek et al. studied biomass production and deparaffination of gas oil by Candida lipolytica.

Yeast has been found to be more efficient hydrocarbon converter than bacteria. It is also easier to harvest yeast cells from a medium.

Utilisation of kerosene, n-hexadecane and Sui gas (a natural gas containing 95% methane) by various strains of yeasts is reported in this paper.

Experimental

Organisms

Seventeen strains of yeasts belonging to different type culture collections were employed during these investigations.

Media

The following are the two media employed:-

Medium A.—Solution 1: Ammonium nitrate 3.0 g, potassium dihydrogen phosphate 2.5 g, magnesium sulfate 7 Aq. 1.0 g, boron 0.01 ppm, copper 0.01 ppm, iodine 0.1 ppm; iron 0.05 ppm, and zinc 0.07 ppm, distilled water 900 ml.

Solution 2: Inositol 0.20 mg, thiamine HCl 0.04 mg, riboflavin 0.20 mg, pyridine HCl 0.40 mg, nicotinic acid 0.2 mg, p-aminobenzoic acid 0.2 mg, calcium pantothenate 0.2 mg, and biotin 0.2 mg prepared the stock solution containing the desired concentration in 65 ml distilled water.

Medium B.—Ammonium sulfate 3.0 g, potassium dihydrogen phosphate 2.5 g, magnesium sulfate 7 Aq. 1.0 g; distilled water 1 liter.

Medium A was used for liquid hydrocarbons, while Medium B was employed for Sui gas.

Solution 1 (18.0 ml) was taken in Erlenmeyer flask of 50-ml capacity and sterilised at 10 lb/in² for 15 min. Solution 2 was sterilized by passing through Seitz membrane filters. 1.3 ml of this solution containing the desired concentrations of the vitamins was transferred aseptically into the flasks. The pH was adjusted to 4.5. 0.2-ml portions of n-hexadecane, kerosene, sterilised by filtration, were aseptically added into each flask.

Medium B (19.5 ml) was transferred into 300-ml towers, and sterilised at 10 lb/in² for 15 min. H₂S and mercaptans present in Sui gas were eliminated by passing over a column of activated charcoal. The gas was sterilised by passing through glass wool plug and distilled water.

The fermentation was carried out in Erlenmeyer flasks of 300-ml capacity. These flasks were shaken on a rotary shaker (125 rev/min) and were maintained at a temperature of 30 ± 0.5°C.

Preparation of Inoculum

The inoculum was prepared in a 300-ml Erlenmeyer flask to which 50 ml of the medium A, along with 1 ml n-hexadecane was added. The flask was incubated at 30°C for 5 days on a rotary shaker. The cells were harvested in a tared centrifuge tube, resuspended in saline water and finally given two washings with sterile distilled water. 0.5 ml of the suspension containing 10–20mg of the cells (on wet basis) was used as inoculum.
AN ASSAY OF SEVIN* FORMULATIONS USING MICRO STEAM DISTILLATION AND ITS APPLICATION TO STUDY OF INSECTICIDE DISTRIBUTION IN GRANULES

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(Received December 26, 1969)

Experimental

Assay of Sevin

The method is based on the steam distillation of the methylamine formed by the alkaline hydrolysis of Sevin. The methylamine is absorbed in boric acid and titrated with 0.01N hydrochloric acid using a mixed indicator (methyl red-bromocresol green).

Reagent

Boric Acid-Mixed Indicator Solution.—For absorption of methylamine, 20 ml 0.1% ethanolic bromocresol green was mixed with 4 ml methyl red and added to 500 ml 2% boric acid solution in water.

Apparatus

Microdistillation assembly—Kjeldahl type—used in these tests was similar to the Quickfit-Quartz Assembly Cat. No. 21/100 MC consisting essentially of a 100-ml kjeldahl flask, a distillation head with steam inlet and a Leibig condenser (Fig. 1). Details are given in the diagram, although similar equipment of a different design could be used if tests were made to ensure complete distillation of the amine and that no alkali is splashed over.

Procedure

Approximately 4 mg Sevin was introduced into the distillation apparatus. Two methods were used depending upon the physical state of the sample. Solid samples were weighed directly into the distillation kjeldahl flask which was then fixed to the head of the apparatus. Liquid samples were pipetted into the flask through the ground joint in the head, used later in the procedure to introduce steam. It was then rinsed with about 1 ml water. Subsequent operations were the same whenever the form of Sevin sample, and were as follows. Sodium hydroxide 40% (2.5 ml) was added through the joint in the head (as for liquid samples) followed by a rinse with about 1 ml water. Steam was then passed through

*Carbaryl (1-naphthyl-N-methyl carbamate)
†From Rothamstead Experimental Station, Harpenden, U.K. on secondment to the Department of Plant Protection, Karachi under U.K. Colombo Plan arrangements from September 1967-68.
SPECTROPHOTOMETRIC DETERMINATION OF PETKOLIN FROM PLANT SURFACES

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Spectrophotometric determination of residues of Petkolin was done on the cotton leaves, according to the method described by Avens et al.12 after certain modifications. Different concentrations and different volumes of the Petkolin emulsion were used for spraying and the amount of residue was calculated on the basis of colorimetric estimations by using Fat dye as an agent. It was found that the residue of Petkolin persisted for 5-7 days in all the replicates and patterns.

Although much work has been done on different aspects of Petkolin1 by various workers2-8 no work has been done on its residual effect. During the residual studies of Petkolin on crop it was found desirable to develop a chemical method for determining its traces quantitatively on plants. Various methods9-12 were tried but the method described by Avens et al.12 for the determination of mineral oil deposits on plants, with certain modifications, was found most suitable for the analysis of Petkolin (Chlorinated Petrol).

Materials and Methods

Preparation of Dyed Petkolin.—One hundred millilitres of dyed Petkolin was prepared by dissolving 4 mg of Fat Red dye per ml. This mixture was heated at a low temperature (40°C) with intermittent shaking for 1 hr. Thus the deeply red coloured Petkolin was obtained and it was allowed to stand overnight at room temperature (23°C). Next day it was again heated for 1 hr and filtered through a sintered glass filter. This filtered, coloured Petkolin was taken as stock solution. From this stock solution 40% emulsifiable concentrate of coloured Petkolin was prepared and then the emulsion of 0.5, 1.0, 2.0 and 3.0% for spraying on plants.

Application and Extraction Method.—The experiments were designed in two different sets. In the first set 1 ml emulsions of coloured Petkolin per leaf, of different concentration, ranging from 0.5 to 3.0%, were sprayed on the upper surface of the leaves of cotton plant (in field) by the help of De-Vilbiss atomizer. In the second set, 1.2, 3 ml per leaf of 1% emulsion of coloured Petkolin were sprayed. The cotton plant leaves of approximately equal size (average area 16.5 in2) were used during the experiment. Each concentration was sprayed on five leaves separately and each set of experiments was replicated thrice. After spraying coloured Petkolin the samples were randomly taken at 24, 48, 72, 120, 144 and 168 hr. interval for residue analysis. The leaves were stored in a deep-freeze chamber until the time of extraction. The extraction was done in 100 ml petroleum ether by using Soxhlet apparatus, for 2 hr. The extract was subsequently removed in an Erlenmeyer flask and was made up to 250 ml by adding further quantity of petroleum ether.

Colour Measurement.—The transmission measurements were taken by the help of Beckman Model DB 620 Spectrophotometer with 5 ml cell size. Petroleum ether was used as reference during colorimetric determination. The wavelengths were varied from 410 to 580 mp to find the optimum wavelength at which maximum absorption takes place. This was found to be 520 mp in the case of Fat Red dye as against 515 mp described by Avens et al12 for oil red ‘O’ dye. Therefore, all the determinations were done at 520 mp wavelength. Transmittancy value graph (Fig. 1) for measuring the quantity of residues colorimetrically was prepared by dissolving different quantities of Fat Red dye in petroleum ether.

Blank or check values were determined by extracting samples of unsprayed material and making the measurement as in the case of sprayed samples. Values from check material were then deducted from those obtained from the sprayed material to give the net Petkolin present.

Calculation of the Quantity of Petkolin Deposits.—The amount of Petkolin deposited is calculated from the equation given below:

\[ Q = K \log \frac{100}{\% T} \]

where \( Q \) is the quantity of Petkolin per 50 ml sample; \( \% T \) is the per cent transmittance measured by the spectrophotometer; \( K \) is a constant, which is characteristic of dyed Petkolin being determined; \( K \), is calculated for dyed Petkolin used in the following manner.
EVALUATION OF KEROCIL—A CHLORINATED MINERAL OIL AS PRIMARY PLASTICIZER

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(Received October 29, 1969; revised February 2, 1970)

Kerocil, a name given to a chlorinated mineral oil (C₁₂₋C₁₇) has been found to be a good primary plasticizer for polyvinyl chloride (PVC). The process of its manufacture together with a detailed study of its plasticizing properties on polyvinyl chloride has been carried out. The results of this study compare favourably with the other well-known primary plasticizer namely the diethyl phthalate (DOP).

Plasticizers as the name implies introduce the various plasticizing properties namely the flowability, elastic modulus and the melt viscosity in the resins. The plasticized resins find their application in shoe, clothings, water-proof materials, artificial leather, toys and numerous other industries. At present its major use is with the PVC as a plasticizing agent.

In 1870, Job and Hyatt were the first to discover that camphor could plasticize nitrocellulose. Later the introduction of triphenyl phosphate in 1912 followed by tricresyl phosphate did help to alleviate the situation. During and after the World War II, the rapidly growing vinyl industry has come out to be the largest consumer of the plasticizers.

Plasticizers have been generally classified as the primary and the secondary plasticizers. Primary plasticizers are those which do not exude or spew from a plasticized composition after flexing or on long storage, under accelerated aging tests. Those which have limited compatibility are termed as secondary-plasticizers. Thus broadly speaking a primary plasticizer is completely compatible with resins, fillers, pigments and is sufficiently permanent i.e. it retains the "plasticizing quality" throughout its useful life. It is still difficult to classify a plasticizer, some may be called primary while the other a secondary e.g. Di-isodcycyl phthalate is primary for PVC and secondary for PVA, thus showing that there is no hard and fast rule to demarcate one from the other.

A plasticizer in the form of chlorinated hydrocarbons based on the indigenous raw materials namely the petroleum hydrocarbons and the chlorine gas has been prepared in these laboratories. The plasticizer, which has been patented under the name of Kerocil, compares favourably with the imported plasticizer as shown in the present study.

Experimental

Production of Kerocil

Petroleum hydrocarbons (C₁₂₋C₁₇) boiling in the range of 150–250°C, is cooled to 20°C in a vertical glass or glass-lined column filled with cooling coils. Chlorine gas is injected at a rate varying between 0.2 to 1 lb/hr per litre of the hydrocarbons. The chlorination is continued till the density of the chlorinated hydrocarbons rises to 1.20–1.25 g/cc.

The resulting chlorinated hydrocarbons is scrubbed free of the acid and dried over anhydrous, sodium-sulphate.

Physical Testing of Kerocil

Experiments were carried out on casted PVC sheets using DOP and Kerocils (hereafter denoted as Kerocil-I and II). Kerocil I has the density of 1.25 and chlorine contents 45% while Kerocil II has the density of 1.3 and chlorine contents of 50–60%.) as plasticizers, with the following compositions: PVC 100 parts; Plasticizer 80 parts; Fillers 15 parts; Pigment 1 part; Stabilizer 1 part.

The ingredients were thoroughly mixed and left for 24 hr. The casted sheets were cured at 165°C and tested for hardness, tensile strength, effect of solvents and the volatility.

(a) Compatibility Test.—Compatibility as Boyen views it, is the determined quantity of the plasticizer that can be added to polymer before phase separation occurs.

It has been found that the Kerocils are compatible with PVC up to 50% without exudation.

(b) Shore Hardness Tests.—Plasticized sheets are normally low in hardness. The hardness of the
STUDIES ON THE PRODUCTION OF COMPOSITE COPPER LEAD POWDER AND BEARING SLEEVES

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The paper describes a new technique for the production of copper-coated lead powder and its subsequent use for making bearing sleeves.

The investigations outline the advantages of fluoborate over acetate process for the preparation of Cu-Pb powders and relate also how the different Cu-Pb composition and sintering temperature bring about variations in physical properties of the bearing sleeves.

The bearing materials mainly required for internal combustion engines consist essentially of a steel backing with a lining of copper-lead alloy. The lining serves to impart anti-frictional properties, a fairly good proportion of softness and an adequate load-carrying capacity. The bearings are produced by casting the alloy on steel or by sintering process. The inherent difficulties involved in the casting process are that of poor wettability between steel and copper-lead; segregating tendency of lead and therefore local variations in composition.

The sintering process which involves preparation of Copper-Coated lead powders and sintering of the powder on the steel has been found to be more efficient and eliminate the difficulties encountered in the casting method (Figs. 1 and 2).

The purpose of this paper is to work out the details for the preparation of different compositions of Cu–Pb powders and to study the effect of sintering temperature and other conditions responsible for marked variations in physical properties of the bearing material.

A reference regarding the preparation of composite copper-lead powder by acetate process\(^1\) is available. In this process, fine lead powder is introduced into copper acetate solution whereby precipitation of copper films on the particles takes place.

Mantell\(^2\) initiated development work on the preparation of composite metal powders through an aqueous acetate electrolyte, but the details are lacking. Some details of depositing and refining of lead from acetate,\(^3\) fluosilicate,\(^4\) fluoborate,\(^5\) cyanide\(^6\) electrolyte are available and so also of copper from cyanide,\(^7\) acid copper sulphate,\(^8\) fluoborate,\(^9\) Kochler,\(^10\) Drouilly and Fisher\(^11\) have described the methods of preparation of copper powder by the electrolysis of acid copper sulphate solution but the operating conditions vary very greatly.

Before trying to prepare composite copper-lead powder electrolytically, from a single bath, the general conditions for making copper powder and for depositing lead have been kept in view. The paper also compares the advantages of fluoborate over acetate process.

**Experimental**

Acetate Process.—Copper and lead acetate solutions were electrolysed between 60:40 cast copper–lead anodes and nickel cathode. The conditions for the preparation of 60:40 copper-coated–lead powder by this process were investigated.

Electrolyte: Copper 15.0 g/l; Lead 3.0 g/l; Free acetic acid 250 g/l.

Current: Cathode current density 125 asf; Anode current density 100 asf; Voltage 12.5 V.

Yield: Total 7.0 g/hr; G/KW/hr 224; Current efficiency 20%.

Sieve Analyses of the Powder (B.S.S.):

-\(+200\): 15%\(^\dagger\); \(-200+325\): 80%\(^\dagger\); \(-325\): 5%\(^\dagger\).
FELTING POTENTIAL OF PAKISTANI WOOL

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The feltability of Pakistani wools has been investigated and an attempt has been made to examine correlations between this parameter and natural variations in the different wool characteristics. Product of number of crimps/inch and fibre diameter has been found to be an important factor, affecting the relative felting behaviour of various wools.

Most Pakistani wools are coarse and the bulk is exported for carpet manufacture. Some studies have already been made on their characteristics. Preliminary investigations have revealed that most of these wools possess good felting properties. In fact, coarse felts for floor coverings are made from these wools by hand in some parts of the country. It is, therefore, desirable to study their felting behaviour in order to assess their suitability for different end uses.

Although most of the studies have revealed the scale structure of the fibre surface as the fundamental characteristic that imparts felting ability to wool, the differences in the felting behaviour of different wools, however, are largely determined by other factors, especially the variations in crimp parameters with the dimensional characteristics of fibres playing a minor role. In continuation of these studies it is worthwhile to examine Pakistani wools so as to establish the factors affecting their relative felting capacity.

An examination of feltability is of considerable importance for other reasons; high felting wool is keenly sought by the manufacturers of nonwoven commercial felts. Besides tightly structured fabrics are obtained by milling (felting) after weaving because the limitations of textile machinery do not warrant the production of such garments by weaving alone. Poor felting wool is highly valuable in the production of suiting and hosiery so that these products do not lose their shape in spite of frequent washing while in service.

Materials and Methods

The wool samples representing 13 breeds were collected from different regions of Pakistan. The samples were cleaned as usual and carefully hand-carded in order to obtain thoroughly randomized fibre assemblies. A well-known method which consists of three dimensionally shaking a fixed quantity of wool (1g) in a bottle containing a felting medium for a given time (60 min), was employed. The diameter of the resultant ball gives an indication of the feltability of the wool, lower diameters signifying higher felting and vice versa. In our case 3 g of wool was used for each test in an endeavour to obtain more representative results and to accentuate the felting differences. After preliminary experiments, the testing conditions were fixed at 120 ml of 0.1 N HCl at room temperature as the felting medium which was contained in a wide neck screw cap cylindrical bottle 7.57 cm long, 6.72 cm internal diameter. The bottle was agitated for 60 min at 180 rev/min on a three dimensional shaker (Gallenkamp). At least 2 balls were produced from each wool and the diameter was measured in three mutually perpendicular directions with a vernier calliper.

Staple length was determined by employing a foot-rule and fibre diameter was measured with the help of a projection microscope (lanameter) at a magnification of ×500. Frictional coefficients were measured in 0.1 N HCl as reported in a previous publication. Two-inch lengths of five randomly selected staples were cut from the root side. One hundred fibres were withdrawn (20 from each staple) at random and number of crimps/in was determined for each breed. A histogram (Fig. 1) shows the frequency of number crimps/in for Kail breed and indicates the type of

![Histogram showing frequency of number crimps/in for Kail breed.](image)
PLY EFFECT ON YARN SHRINKAGE DUE TO RELAXATION AND FELTING

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The relaxation shrinkage of worsted ply yarns increases with the single's ply number, twist factor and tex (yarn mass per unit length), but ply twist is unlikely to be a significant factor. Besides, the ply number has accounted for 99.9% variation of the relaxation. However, the yarn felting shrinkage usually decreases with the increase of ply number, especially, the even number. The general effect of ply number may correspond closely to that of twist factor rather than tex. Eventually, in harmony with observations on the worsted ply, a large difference of felting rates has been noted between 1- and 2-ply woollen yarns of 4 widely different wools.

Relaxation shrinkage also known as the crimp recovery of wool fibres, occurs in the recently manufactured yarns and fabrics when they are left unstrained, especially in the wet state, due to the release of processing strains and hygral expansion. It may survive the finishing treatments to pose the problems of dimensional change during end-uses of wool fabrics. Furthermore, the rigid requirements of garment-making behoove the weavers to produce definite fabric width. As a result, the relaxation shrinkage of various manufacturing lines of a raw wool is usually determined by independent trials of considerable cost. An a priori knowledge of the relaxation process is likely to reduce the number of these expensive trials, particularly, in the Pakistani mills where the Merino wool tops are imported for apparel manufacture.

In addition to yarn geometry, the relaxation shrinkage may depend upon fibre length, diameter and crimp, probably, because the processing operations are normally adjusted according to fibre geometry. Although yarn relaxation may account for the major bulk of fabric relaxation shrinkage, the latter depends additionally on the weaving or knitting stress which, in turn, is highly influenced by yarn geometry such as tex, twist factor and perhaps, ply number. These variables have, therefore, been studied by processing the same wool in order to minimise variations due to the differences of fibre attributes.

On the other hand, yarn felting shrinkage is mainly dependent on twist factor and tex. Whilst the twist shows highly significant negative correlation with yarn and fabric felting rates, the tex effect observed on fabric felting is likely to be complicated by its association with fabric cover factor and the number of fibres in the yarn cross-section. Moreover, literature does not appear to report any systematic study of ply number even though plying is frequently used in yarn manufacture and always it increases tex. Hence, a study of the effect of ply number on felting shrinkage seems desirable both as an end in itself and as a means of clarifying the following divergencies.

Early works generally demonstrated a higher felting rate of the worsted than that of the woollen fabrics produced from the same wool. This is because other studies indicated greater fibre decrimping in the worsted than that in the woollen manufacture and the resulting uncrimping may accentuate felting rate. But later works showed a considerably higher felting rate of the single's woollen cloth than that of the comparable worsted fabric of 2-ply, and the reversal could not be cogently explained in terms of the reported differences of fibre and/or fabric attributes between them. This paradox, however, can be reconciled by assuming an effect of ply number which, therefore, deserves a thorough examination in juxtaposition with other significant parameters.

With a view to evade possible variations due to fabric structures, however, the present analysis has been restricted to the yarns. Although Mercer pointed out that yarn linear shrinkage was more closely related to the typical felting behaviour of a wool than the change of fabric area or thickness, the point of view needs to be treated with certain reservations. Nevertheless, a knowledge of ply felting rates may be useful for quality control in some wet finishing operations such as back-washing, dyeing and shrink-proofing which are often convenient to execute at the yarn stage.

Experimental

A Merino wool of fibre fineness 20.4 µ and length 9.0 cm was processed almost identically in the shortened Bradford system and spun in a Magnum Spinning Frame in which the doubling and draft were adjusted to produce singles of nominal tex/twist factor—90/2.5, 90/2.0, 90/1.7,