SHORT COMMUNICATIONS

CONSTITUENTS OF CASSIA ABSUS LINN

Isolation and Characterization of a Glycoside from the Seeds

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Cassia absus Linn has been mentioned in indigenous medicine as an agent useful for eye diseases.[1] Thus attention has been drawn from a long time to the study of its active constituents and interest was especially roused when the alkaloids chaksine and isochaksine were isolated from it.[2] In these laboratories studies have been continued on these two bases and in the course of these investigations it was found necessary to collect large amounts of the two alkaloids. In these experiments the seed kernels were extracted as usual first with petroleum ether and then by alcoholic hydrochloric acid. The solvent was recovered from the acid extract to give a pasty mass, which was extracted in aqueous solution with petroleum ether. This extract on standing gave a precipitate, which is the main subject of study in the present investigation. The precipitate has been identified as sitosterol-β-D-glucoside through its derivatives and by hydrolysis to the sterol and d-glucose.

The oil remaining after isolation of the glucoside has given β-sitosterol identified also in chaksu oil by Sen Gupta and Mosettig.[3]

Experimental

All melting points are uncorrected.

Isolation of Sitosterol-β-D-glucoside:—20 kg. of the seed kernels of *Cassia absus* Linn were crushed to 12 mesh and extracted exhaustively by steeping with petroleum ether (b.p. 60°–80°) at room temperature followed by similar extraction in the cold with alcoholic hydrochloric acid (0.3%). The solvent from the latter extract was removed in vacuo on the water-bath when a sticky brown residue (2.7 kg.) was obtained. It was taken up in water (1200 to 1800 ml.) and extracted exhaustively with petroleum ether. The last extract on standing for about 30 days deposited a yellowish powder which was filtered off and washed with methanol when a colourless mass (1.5 g.) m.p. 272°C., was obtained. It was almost insoluble in the common organic solvents but soluble in pyridine and dimethyl formamide. After four recrystallisations from the latter a colourless sample m.p. 293–294°C., $[\alpha]_D^{31}$ –47.6° (c, 0.5 in pyridine) was obtained {Lit.,[4] m.p. 293°C., $[\alpha]_D^{20}$ –43° (in pyridine)}. (Found: C, 72.03; H, 10.26. Calc. for $C_{35}H_{60}O_6$: C, 72.85; H, 10.49%).

The infrared spectrum indicated the presence of hydroxyl group or groups.

Preparation of the Acetyl Derivative.—To acetylate the hydroxyl groups 357 mg. of the substance and 8 ml. of acetic anhydride were refluxed for two hours. The clear solution was brought to dryness in vacuo on the water-bath, washed with water (8 ml.) and again similarly dried (438 mg.) After three recrystallisations from alcohol the colourless substance m.p. 166–168°C., $[\alpha]_D^{30}$ –24°, (c, 1 in chloroform) was obtained {Lit., m.p. 166–168°C.[4] $[\alpha]_D^{30}$ –24.5° (in chloroform.)[5]} It was readily soluble in chloroform, acetone and carbon tetrachloride, sparingly in cold methanol and ethanol. (Found: C, 69.80; H, 9.02; O, 21.57; Calc. for $C_{43}H_{68}O_{10}$: C, 69.36; H, 9.14; O, 21.51%).

Preparation of the Benzoyl Derivative.—To prepare the benzoate 100 mg. of the sterolglucoside was dissolved in 2.5 ml. of pyridine and benzoyl chloride (0.4 ml.) added. The mixture was then refluxed for one hour, poured into water (100 ml.) exhaustively extracted with ether. The ethereal layer was washed with sodium bicarbonate solution (5%), hydrochloric acid (1N), and water then dried over sodium sulphate and the solvent removed. The residue on repeated recrystallisation from alcohol, ethyl acetate/alcohol and ether successively furnished the pure product, m.p. 198°C., (Lit.[4], m.p. 198°C.).

Hydrolysis of Sitosterol - β-D.-glucoside.—The method followed was essentially that of Power and Solway[6] with minor modifications. 100 mg. of the sitosterol-β-D-glucoside was dissolved in 9 ml. of hot amyl alcohol and aqueous hydrochloric acid, (18%; 2 ml.) was added together with 0.5 ml. of ethanol to form a homogeneous liquid which was refluxed for two hours. The solvents were then removed in vacuo on the water-bath, and the dry residue exhaustively extracted with chloroform and water. The water extract was concentrated. An examination for optical activity revealed it to be dextro-rotatory. It also reduced Fehling's solution indicating the presence of reducing sugars.

The aqueous solution obtained above was mixed with phenylhydrazine (22 mg.), glacial acetic acid (1 drop) and sodium acetate (29 mg.) and the mixture heated on the boiling water-bath. The crude osazone obtained after washing with cold water was recrystallised from 50% alcohol to give yellow needles, m.p. 203–204°C. (Boch Block), mixed m.p. with an authentic sample of glucose phenylosazone prepared in this laboratory was undepressed.

The chloroform extract of the hydrolysis product of sitosterol-β-D-glucoside was dried and the solvent removed at 50°C. in vacuo. After three recrystallisations from pure alcohol the residue (82 mg.) furnished a colourless compound m.p. 134°C. (Lit.[7], m.p. 136–137°C.).

Isolation of β-Sitosterol.—After separation of sitosterol-β-D-glucoside the solvent was removed from the filtrate at 80°C. in vacuo when 160 g. of oil remained. 80 g. of this oil was saponified in the usual way. The unsaponifiable matter was exhaustively extracted with ether, the extract washed with water and dried. On removal of the solvent a residue (1.45 g.), m.p., 128–130°C., was obtained. After three recrystallisations of the residue from alcohol the partially purified sample melting in the range of 130–134°C., was obtained. A portion of this crystallisate (138 mg.) was further purified by chromatography through a column (12 cm. × 0.8 cm. dia.) containing 4 g. of Alumina (Woelm, almost neutral). The method followed was essentially that of Sen Gupta and Mosettig.[3] On elution (eluate collected in 20 ml. fractions) with a mixture of benzene, petroleum ether (10:90), the major portion of the substance (eluates nos. 3-10, containing 80.6 mg.) exhibiting a melting point of 134°C. was obtained. From the rest of the eluates (numbers 11–15) a crystalline residue, m.p. 137°C. was obtained. The former (fractions 3-10; 80.6 mg.) were mixed and rechromatographed on a similar column containing 4 g. of Alumina ("Woelm" almost neutral) of the same dsmensions to give 79.8 mg. of substance m.p. 136.5°C. On mixing this 79.8 mg. of the last chromatography with the fractions, m.p. 137°C. of the former and recrystallisation from ethanol a colourless crystallisate, m.p. 136.5°C. (Kofler block),$[\alpha]_D^{25}$ –39° (c, 1 in chloroform) was obtained.

{ Lit., m.p. 136-137°C.[7] $[\alpha]_D^{24}$ –36.7°C. (in chloroform)[8]. }

β-Sitosteryl Acetate.—100 mg. of the sterol, acetic anhydride (2 ml.) and pyridine (2 ml.) were refluxed for one hour. On cooling the mixture was poured into 200 ml. of ice-cold water when the impure sample was obtained as a precipitate m.p. 118–120°C. On recrystallisation from methanol, a colourless sample was obtained, m.p. 126–127°C.,

$[\alpha]_D^{25}$ –40° (c, 1 in chloroform). { (Lit.[7] m.p. 126–127°C., $[\alpha]_D^{31}$ –39.5° (in chloroform).}

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STRUCTURE OF ORGANOSELENOCYANATES

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Considerable interest seems to be centred on the structure of pseudohalogens like the cyanates and thiocyanates, but no information is so far available on the bonding of organoselenocyanates. The structure of a few inorganic compounds,

however, has been deduced from their I.R. spectra and suggested to be of the isoselenocyanate type.[1] A series of compounds has been prepared by us to study the type of bonding in organoselenocyanates, by recording their I.R. spectra.

By analogy with the thiocyanates, the selenocyanate group in the organoselenocyanate may either be disposed as a selenocyanate $R-Se-C \equiv N$ or it may have the isoselenocyanate structure $R-N=C=Se$. It is possible to distinguish the thiocyanates from the isothiocyanates since the former absorb at a higher frequency than the latter. The characteristic features of the spectra of these two types of compounds is that the thiocyanates have a sharp peak at 2130-2160 cm-[1] while the isothiocyanates have a strong band at 2040 to 2180 cm-[1] which can, in certain cases, be further resolved into a peak of weak intensity at 2000 cm-[1] and a strong band at 2100 cm-[1].[2] Furthermore, the first overtone of the bending frequency of the isothiocyanates has approximately the same intensity as that of the fundamentals, which is not the case for the thiocyanates.

Potassium selenocyanate has medium intensity peaks at 832 and 847 cm-[1]. The suggestion of an isoselenocyanate structure therefore seems to be justified on the above grounds. The following aromatic and aliphatic selenocyanates have been examined on a P.E. 237 spectrophotometer using rocksalt optics: (1) Phenyl selenocyanate. (2) Naphthyl selenocyanate. (3) o-methoxy phenyl selenocyanate. (4) p-tolyl selenocyanate. (5) p-chlorophenyl selenocyanate. (6) p-bromophenyl selenocyanate. (7) p-nitrophenyl selenocyanate. (8) 2-selenocyanato, 5 nitro toluene and (9) n-butyl selenocyanate.

Almost all of these compounds have a weak to medium intensity sharp peak at 2160 cm-[1] similar to the organothiocyanates.[3] Compounds in which the $2\nu_2$ overtone is observed have an absorption of very weak intensity at 830 cm-[1]. By analogy with the organothiocyanates, the compounds studied should have the selenocyanate structure $R-Se-C \equiv N$.

The reaction of organoselenocyanates with halogens gives the corresponding selenohalide easily which shows that the nitrile group occupies a terminal position in the compounds under study. This provides further support to the selenocyanate structure.

It is interesting to note here that all the selenocyanates prepared for this study were from the reaction with potassium selenocyanate which has an isoselenocyanate structure. This rearrangement is possibly due to the attack of the organic radical on selenium which occupies a terminal position in the potassium salt as in the following equation.

$$K^+ - N = C = Se + R^+ X^- \longrightarrow$$
$$R - Se - C = N + K^+ X^-$$
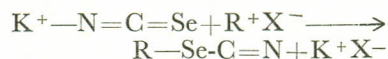
Further studies in this connection are in progress.

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MANGHOPIR SPRING WATERS

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Introduction

Some ten miles north of Karachi there are natural springs of hot and cold water. The locality is known by the name of a local saint "Mangho Pir". The temperature of hot water was found to be 48°C. and that of cold 32°C. taken in August. According to Pithawala[1] the hot water had the temperature of 126°F or 53°C. The waters are used for bathing and also for drinking. The patients who visit the locality are not only lepers and those suffering from skin diseases but also others with different types of ailments. Pithawala[1] states that "though leprosy is incurable the patients get much relief by the use of these waters". The popularity of the springs induced him to make this statement. Unfortunately the waters have not been analysed so far and apparantly Pithawala[2] never attempted any. His statement that "hot springs charged with sulphurated hydrogen is good for skin diseases" would leave the impression that the remark also applies to the waters of Manghopir but there is no indication to the effect. We found no sulphurated hydrogen in these waters. The problem was undertaken to discover the agency responsible for the improvement of skin diseases and thus for the popularity of these waters.

Experimental

pH. The samples were collected in polyethylene bottles from the channels. The water had no characteristic taste or smell. The pH value of the water was measured with a "Cambridge" pH meter. The hot water had a pH of 7.6 and cold water 7.2.

Total Solids.—One litre of the water was evaporated on water bath and the residue dried at 102°C. for four hours.

TABLE I.

Sample No.	Description	Collected in the month of	Total solids parts/million
I	Hot water	June	1283
2	,,	August	1361.2
I	Cold water	June	1843.5
2	,,	August	1736

The solid content of the cold water was higher than that of hot water and varied in composition from season to season.

Spectrography Analysis of the Total Solids.—The spectrographic analysis of the total solids indicated the presence of calcium, magnesium, sodium, potassium, silicon, iron, and copper (traces).

Chemical Analysis.—The water sample number (1) was analysed for calcium, magnesium, sodium, potassium, arsenic sulphate and chloride. The presence of arsenic was shown by Marsh's test. The arsenic was estimated quantitatively by the modified Gutzeit procedure of Lachele.[3] The water sample (1000 ml.) was evaporated to 200 ml. volume prior to the estimation of arsenic by this procedure. The method consists in treating the sample with ferrous sulphate, stannous chloride hydrochloric acid. Before connecting the apparatus with the disc containing part activated zinc pieces are added to the flask containg the water sample. The arsine which is evolved impinges upon the filter paper disc held between two ground glass female ball sockets. The coloured discs are then developed in cadmium iodide. The stain which is obtained is then compared with the standard stains produced in the similar manner. The results are shown in Table 2.

Discussion

Contrary to the popular belief no sulphurated hydrogen was found in the water samples.

TABLE 2.—PARTS PER MILLION.

No.	Description	CaO	MgO	Na$_2$O	K$_2$O	SO$_4''$	Cl'	As$_2$O$_3$
1.	1 cold water	58.7	75	651	18.5	415	1029	0.00125
2.	II hot water	52.9	64.8	545	29.9	258	479	0.003

While determining arsenic by Lachele[3] procedure some difficulties were experienced in getting a well defined and uniform stain. The use of hollow socket joint held with a strong clip gave well defined stains. It was also found that rate of nitrogen flow should be so adjusted that the water vapours were not carried to the disc.

Whatever curative power is attributed to these waters may be due to the extremely small amounts of arsenic present. However it will be interesting to study the changes in the composition of these waters with the season throughout the year.

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STUDIES OF THE CITRATE COMPLEX OF BARIUM

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Introduction

J. Schubert and J.W. Richter[1] have studied the dissociation constants of the citrate complexes of barium and strontium by the ion exchange method. According to them the values of dissociation constants increase with the rise in temperature and NH_4^+ ion concentration of the eluting solution. The stability constants as calculated from the values obtained by these authors at NH_4^+ ion concentration of 0.165M are found to be 2.0×10^2 at 25°C. & 1.25×10^2 at 37°C. for the barium citrate complex.

R.K. Patnaik and S. Pani have studied the citrate complexes of calcium[2] and strontium[3] by the pH titration method. According to these authors rare earth metals form stable 1:1 complexes with the citrate ligand. In the present work the method of Patnaik and Pani (loc. cit.) has been extended to the study of barium citrate complex.

Experimental

Reagents and Apparatus.—(i) Sodium hydroxide solution, 1.0M (ii) Citric acid solution, 0.05M (iii) Sodium citrate solution, 0.1M (iv) Sodium perchlorate solution, 0.05M (v) Barium perchlorate solution, 0.0525M. All the reagents except (iv) and (v) were of A.R. grade, while (iv) and (v) were prepared in the laboratory from the A.R. chemicals.

A Beckman pH meter with a glass and calomel electrode was used and solutions were stirred with a magnetic stirrer.

Procedure.—(a) Complex formation of barium with citrate ions was studied by titrating a known volume of citric acid solution, (i) alone, and (ii) in the presence of barium ions, against the sodium hydroxide solution. The results of titration are represented by the curves A and B of Fig. 1.

(b) The stability constant of barium citrate complex was determined as follows:—A known volume of sodium perchlorate solution was titrated (i) alone, and (ii) in the presence of barium ions, against the sodium citrate solution. The results of titration are shown in curves A and B of Fig. 2.

Discussion of the Results

Fig. 1.—Curve A represents the results of titration of a 200 ml. of a solution containing 0.01g. mole of citric acid, while curve B represents the results of titration of a solution containing 0.01g. mole, of citric acid+0.00105g. mole of barium perchlorate, against 1M sodium hydroxide solution.

Fig. 2.—Curve A represents the results of titration of a 100 ml. solution containing 0.005 g. mole of sodium perchlorate, while curve B represents the results of titration of a solution containing 0.005g. mole of sodium perchlorate+ 0.00105 g. mole of barium perchlorate.

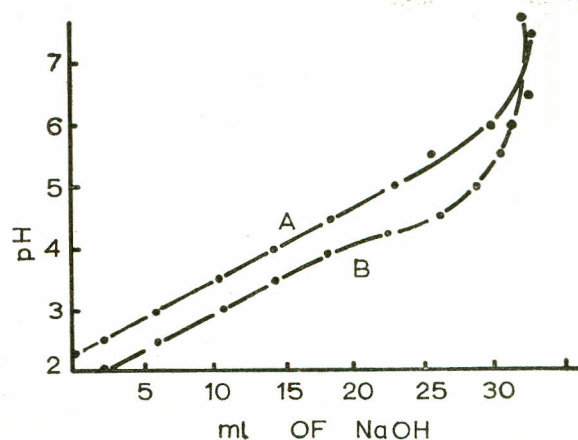Fig. 1 shows that pH of the system B, which contains barium, is lower than that of system A

Fig. 1.—Showing relationship between sodium hydroxide and pH.A, citric acid; B, citric acid + Ba $(ClO_4)_2$.
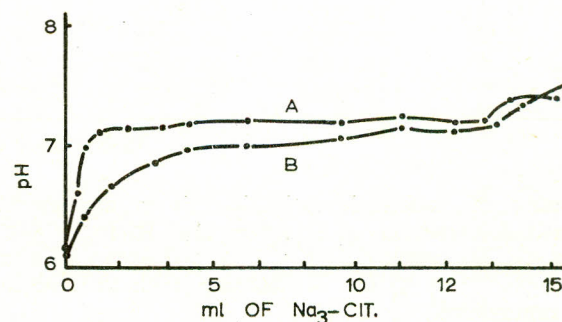

Fig. 2.—Showing relationship between Na_3-CIT and pH.A, $NaClO_4$; B, $NaClO_4$ + Ba $(ClO_4)_2$.

for the same volume of added sodium hydroxide solution, indicating the complex formation with barium. This is due to the liberation of free H^+ ions in the system B according to the reaction

$$Ba^{2+} \qquad + H_3 \cdot Cit \rightleftharpoons [BaCit^-] + 3H^+$$

In Fig. 2 also pH of the system B which contains barium is lower than that of the system A for the same volume of the added sodium citrate solution. This is due to the gradual removal of the citrate ions by complex formations from the system B, according to the reaction

$$Ba^2 + Na_3 \cdot Cit [Ba = Cit-] + 3 Na^+$$

Data from the Fig. 2 was used to calculate the stability constant K of the barium citrate complex according to the equation

$$K = \frac{[BaCit^-]}{[Ba^{2+}] \ [Cit^{3-}]}$$

The calculations are shown in the Table 1. The mean value of K for barium citrate complex in the pH range of 6.5 to 7.2 is found to be 2.16 × 10².

Conclusion

The mean value of stability constant (2.16 × 10² at 14°C. of barium citrate complex, found in the

References

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TABLE 1.

pH	$[Ct]_A \times 10^4$	$[Ct]_B \times 10^4$	$[BaCit^-] \times 10^4$	$[Ba(ClO_4)_2] \times 10^3$	$[Ba^{2+}] \times 10^3$	$K \times 10^{-2}$
6.50	1.5808	4.0667	2.4900	8.7144	8.4654	2.196
6.55	1.996	5.8000	3.8200	8.7000	8.3180	2.301
6.60	2.4938	7.6500	5.1700	8.6830	8.1660	2.537
6.65	3.3223	9.8601	6.5580	8.6637	8.0080	2.463
6.70	4.1494	12.4270	8.3100	8.6413	7.8103	2.564
6.75	5.2226	15.7626	10.5400	8.6126	7.5586	2.670
6.80	6.6225	19.7755	13.1530	8.5778	7.2625	2.733
6.85	8.2645	24.2316	15.9671	8.5380	6.9413	2.782
6.90	12.5407	30.8157	18.2750	8.5007	6.6725	2.184
7.00	20.2050	40.2450	20.0400	8.4170	6.4130	1.550
7.10	31.0080	52.1430	21.1350	8.3426	6.2291	1.100
7.20	43.2455	63.2455	20.0000	8.2680	6.2680	0.800

Mean value of K = 2.16 × 10²

course of present investigations is in agreement with the findings of Schubert and Richter (loc. cit.). The values of (K = 2.0 × 10² at 25°C. and 1.25 × 10² at 37°C.) K decrease with the rise in temperature.

As expected the values of the stability constants of the citrate complexes of the alkaline earth elements are found to decrease with the increase in the ionic size of elements.

Table 2 shows a comparison of the stability constant values of the citrate complexes of the alkaline earth metals.

TABLE 2.

Ions	Stability constant K	
	pH titration method	Ion exchange method
Ca^{2+}	2.66×10^3	—
Sr^{2+}	5.23×10^2	6.67×10^2, 5.0×10^2
Ba^{2+}	2.16×10^2	2.0×10^2, 1.25×10^2

TITRATION OF BERYLLIUM IN GLACIAL ACETIC ACID

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Introduction

Casey and Starke[1] have studied the titration of acetates of eighteen metals in glacial acetic acid potentiometrically using acetons perchloric acid as titrant. However, beryllium is not included in the list of metals studied by them. Estimation of beryllium in glacial acetic acid has not been so far reported in the literature. The present investigation was undertaken with a view to develop a method for the determination of beryllium in the non-aqueous medium.

Experimental

Apparatus.—A Cambridge pH-Meter equipped with a sleeve type calomel electrode and a glass electrode was used.

Reagents.—(1) Glacial acetic acid was purified by the method of Casey and Starke (loc. cit). (2) Basic beryllium acetate] BeO. 3Be (CH$_3$COO)$_2$], was prepared by heating beryllium carbonate with glacial acetic acid and sufficient acetic anhydride to react with any water present. The recrystallised sample (from glacial acetic acid) melted at 284°C., which is in agreement with the melting point of the pure substance reported in the literature. The sample was analysed for beryllium gravimetrically. Found, 8.86%; calculated 8.87%. (3) A standard solution of basic beryllium acetate was prepared in glacial acetic acid which contained 0.7290 mg. of Be per ml. Approx. 0.1N Acetous perchloric acid solution (B.D.H.) was used as titrant. This was standardized against 0.1N Sodium acetate solution, prepared from the Analytical grade reagent. Normality of HClO$_4$ solution = 0.098 N

1 ml of 0.098 N HClO$_4$ = 0.442 mg. Be.

Procedure.—No satisfactory end point could be obtained on titrating basic beryllium acetate directly with perchloric acid. Hence, the indirect method of titration was used. A measured excess of the standard perchloric acid was added to an aliquot containing 3-15 mg. of beryllium and the volume of perchloric acid consumed was determined by back titration with standard sodium acetate solution. The representative results of titrations are shown in Fig. 1. The end point was calculated by plotting the differential curve of $\Delta E/\Delta V$ against ΔV, the volume added near the end point. One such curve is shown in Fig. 2. As a check, the amount of beryllium was also determined gravimetrically.[2]

Results and Discussion

The results of beryllium determination for four different concentrations are given in Table 1.

TABLE 1.

Sr. No.	ml. of 0.098 N HClO$_4$ consumed	mg. of Be taken	mg. of Be found	
			titration method	Gravimetric method
1.	2.06	3.645	0.910	3.654
2.	4.12	7.290	1.821	7.292
3.	6.20	10.935	2.740	10.944
4.	8.25	14.580	3.646	14.606

The amount of Be found by titration in each case is 1/4th (25%) of the quantity actually taken.
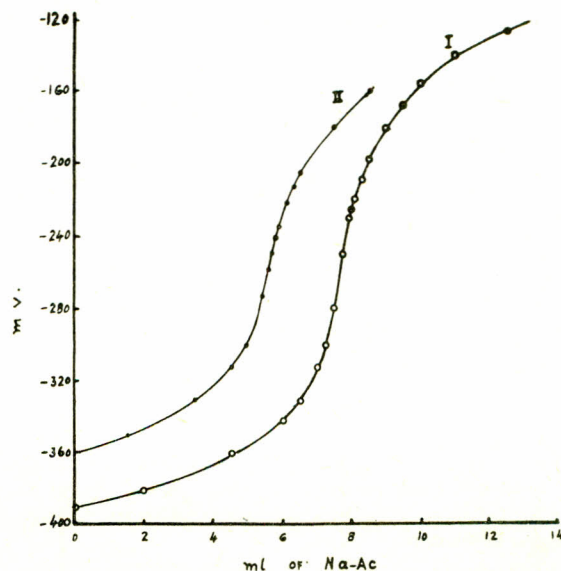

Fig. 1.—Curve I; 5 ml. Be - Acetate + 10 ml. HClO$_4$ Vs Na - Acetate. Curve ; 10 ml. Be - Acetate + 10 ml. HClO$_4$ Vs Na - Acetate
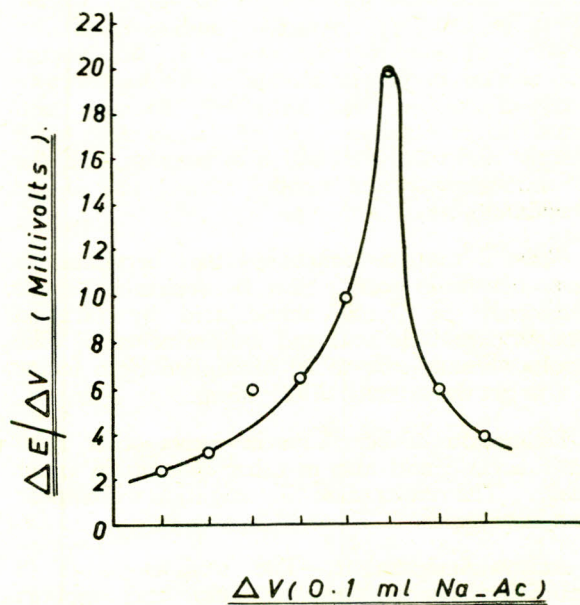

Fig. 2.—Differential curve drawn from the titration curve I.

The values obtained are fairly constant over a four-fold increase in concentration, indicating a stoichiometric reaction between basic beryllium acetate and perchloric acid. A possible explanation of this could be the breaking up of the complex basic acetate by interaction with the perchloric acid yielding beryllium perchlorate, beryllium acetate and water.

BeO. $3Be\ (CH_3COO)_2 + 2HClO_4 = Be\ (ClO_4)_2$
$+ 3Be\ (CH_3COO)_2 + H_2O$
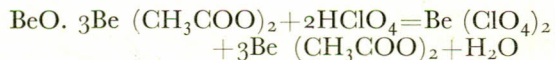
This would involve the consumption of one half equivalent of perchloric acid per gram atom of beryllium, or 1 g. equivalent of Be = 1/4 equivalent of perchloric acid. This would give a result of 25% of the theoretical, not far from the values obtained.

The probability of complex formation of the type $[Be_4(OH)\ (CH_3COO)_6]\ CH_3COO$, as has been observed by Casey and Starke[1] in the case of acetates of iron, aluminium and chromium is ruled out in this case because it would yield the perchlorate of the complex beryllium cation involving the consumption of only 1/8th equivalent of perchloric acid per gram equivalent of beryllium. This would correspond to a result of 12.5% of the theoretical value, which is against the experimental values obtained.

Moreover, the proposed mechanism of reaction shows that the reaction of perchloric acid takes place only with the basic portion (BeO) of the acetate and that the normal beryllium acetate $[Be\ (CH_3\ COO)_2]$ remains undissociated in acetic acid and does not react. In this respect the normal beryllium acetate resembles the acetates of copper, tin, antimony, bismuth and uranium rather than those of the alkaline earth metals[1] and this behaviour is in keeping with the electronegativity and smaller atomic size of the beryllium atom.

Thus it may be concluded that beryllium as basic beryllium acetate may be determined quite accurately in glacial acetic acid by titration against perchloric acid and sodium acetate. The results obtained are to be multiplied by a factor of 4 to get the amount of beryllium.

Estimation of beryllium in forms other than basic acetate and also in other solvents is under study. The results of this investigation will appear in a later paper.

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PROTEOLYTIC DECOMPOSITION OF FISH MUSCLE PROTEINS UNDER DIFFERENT CONDITIONS

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Introduction

Tyrosine value generally indicates the degree of proteolytic decomposition and represents not only the free tyrosine but also trytophane, and compounds which are already present in the tissues and are generated in larger quantities during spoilage. Previous workers attributed the softening of fish flesh to the effect on tissues that occurs due to the influence of bacteria from air or their enzymes. The spoilage of fish during storage under ordinary conditions at room temperature by measurement of iodine absorption, titratable acidity values[1] and autodehydrogenase activities[2] of several species of fish, were studied by Qudrat-i-Khuda et al. who showed that there was notable change in tyrosine value during storage at room temperature. After the fish is caught from the sea, it usually goes through two stages before reaching the consumers. The first stage is between fishing and reaching the commercial markets and the other between the commercial markets and consumers. At these two stages, fish is mixed with crushed ice to retard spoilage. In this investigation the effect of cold storage on the tyrosine value of local fish muscle has been studied and the results are presented in this paper.

Materials and Methods

Experiments had been done on the two Nile fish species Karmout (*Clarias angiullaris*) and Schall (*Synodents schall*). These two species were chosen in preference to other Egyptian fishes because they could be easily obtained very fresh (alive).

Extraction of the Muscle for Tyrosine Determination.— 3-5 grams of the fish muscle were weighed, minced in a clean mortar with a suitable portion of quartz and, then exhaustive extraction was carried out by 2 N. sulphuric acid solution, the mixture centrifuged and the supernatant liquid transferred quantitatively to a volumetric flask (50 cc). The residue was extracted twice again with sulphuric acid (2 N.) in a similar manner and the total extract made up to 50 cc for tyrosine determination.

Colour Reagent for Colorimetric Determination.—The technique of measurement of the tyrosine values by the phenol reagent of Folin and Ciocalteu[5] which has been adopted by Bradley and Bailey[6], Beathy and Collins[7] Tarr and Bailey[8] Wood *et al* Sigurdsson *et al*[10] and Tarr[11] for the assessment of fish spoilage, was used as colouring reagent in this investigation.

Colorimetric Technique for Tyrosine Determination.— Five ml. of the phenol reagent were added to five ml. of the fish muscle extract, mixed well and 25 ml. of saturated sodium carbonate solution was added. This mixture was left for half an hour and then filtered. Tyrosine value was determined in the clear filtrate by standard solution.

Results

The work was carried out on samples put in crushed ice and samples put at room temperature (30°C.) to show the variation of the tyrosine value related to fish species and temperature. Moisture and nitrogen content were also determined. These experiments were repeated many times on the two different kinds of fish, and other fishes of the same species and weight, and the results are reported in Tables 1 and 2.

TABLE 1.—TYROSINE VALUES FOR KARMOUT AND SCHALL KEPT AT ROOM TEMPERATURE DEGREE (30°C).

| Time after death (hours) | Karmout | | | | Schall | | | |
	Moisture %	Crude *protein* % by wet weight	Tyrosine value mg. per 100 g. flesh	Increase in T.V. mg. per 100 g. dry matter	Moisture %	Crude *protein* % by wet weight	Tyrosine value mg. per 100 g. flesh	Increase in T.V. mg. per 100 g. dry matter
0	79.81	16.46	25	—	81.12	16.61	14	—
3	79.53	16.65	26	4	80.73	16.92	18	19
6	79.06	17.03	28	10	80.25	17.31	20	27
9	78.67	17.33	32	27	79.81	17.65	23	39
12	78.13	17.51	46	87	79.32	18.06	37	104
15	77.72	18.03	61	150	78.64	18.64	53	173
24	77.16	18.47	70	183	78.06	19.14	65	222

T.V.=Tyrosine Value

TABLE 2.—TYROSINE VALUES FOR KARMOUT AND SCHALL MIXED WITH CRUSHED ICE.

Time after death hours	Karmout				Schall			
	Moisture %	Crude *protein* % by wet weight	Tyrosine value mg. per 100 g. flesh	increase in T.V. mg. per 100 g. dry matter	Moisture %	Crude *protein* % by wet weight	Tyrosine value mg. per 100 g. flesh	Increase in T.V. mg. per 100 g. dry matter
0	79.68	16.60	32	6	81.31	16.46	26	—
12	80.03	16.26	39	38	81.90	15.90	34	78
24	81.60	14.97	48	104	82.42	15.42	52	156
36	81.45	15.07	59	161	82.16	15.60	63	214
48	82.15	14.48	64	201	82.61	15.18	67	246

T.V.=Tyrosine Value

The data indicates that: (1) Tyrosine value of the fresh fish differed according to the fish species, moreover it differed from one fish to another of the same fish species. (2) Tyrosine value of Karmout preserved at room temperature and in crushed ice increased relatively slowly than did Schall in preserved under the same conditions. (3) The increase in tyrosine value of Karmout preserved crushed ice reached 104 mg. per 100 dry matter after 24 hours while it reached 183 after 24 hours when it was preserved at room temperature (30°C.). (4) Experiments on Schall showed that the increase in tyrosine value was 156 mg. per 100 g. dry matter in cold storage after 24 hours. However the increase was 222 mg. when it was left at room temperature degree. (5) Spoilage in Schall fish was faster than in Karmout fish. (6) Generally, cold storage delays spoilage but it does not prevent it.

Conclusion

Proteolytic decomposition of fish muscle protein under different condition was studied by determining the tyrosine value. Results indicated that crushed ice did not prevent spoilage but retarded it.

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METHOD OF DETECTION OF PIRICULARIA ORYZAE AND HELMINTHOSPORIUM ORYZAE, ON MALT AGAR*

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The rice blast disease caused by *Piricularia oryzae* results in serious losses.[1] Infected seeds germinate poorly and a proportion of the seed is

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wasted. The seed infection may occur before, during, and after the flowering period and the kernel may be infected without any symptoms being visible on the glumes. In case of severe infection the kernels fail to develop and only the empty glumes are left, and there is little difference in weight between obviously diseased kernels and those of healthy appearance in the inoculated lots or the controls.[2,3] Furthermore the inoculated seeds are found on microscopic examination to contain the hyphae of *P. oryzae* in the tissue of the embryo, endosperm, bran layers and glumes and conidia are detected between the glume and their kernels.[2]

Orton[5] listed the rice blast fungus as seed-borne and mentioned the work of Tisdale[1] who isolated the fungus from the seed on blotting paper. However, he pointed out that it was not usually considered a seed-borne parasite, because Nishikado and Mayake[2] had failed to control *P. oryzae* by the hot water treatment. The problem of isolating the fungus on malt from the seed has been as old as the fungus itself.[4] A number of experiments on the samples from the South of France were carried out on different media, in the laboratory.

Each lot of grain was shaken in sterile water, and after decantation and centrifugation, the residue was examined under microscope. It was noted that the two genera of Mucoraceae, Rhizopus sp. and Mucor sp. appeared frequently and therefore it was essential to disinfect the seeds with sodium hypochlorite tetra 1%, for five minutes, for the complete elimination of the above two species. The method used is well known as 'Ulster method', the media being 2% malt agar, incubated at 28°C. The results given in Table 1 were obtained from several varieties of rice which had been analysed previously by the method of dilution and show the absence of the colonies of Piricularia on the malt agar at 28°C., in spite of the fact that the spores had already been detected.

Methods of Isolation

(a) *Effect of Temperature.*—It was not possible to change the temperature in favour of Piricularia against the competition offered by the Saprophytes. On the contrary, Helminthosporium presented no difficulty at 28°C.

(b) *Surface Sterilization of the Seeds.*—Many fungicides were used in the experiments, the powdering of grains, at different concentrations and duration, but to no effect. Mercuric biochloride was found to be very effective in the elimination of *Epicoccum* sp.

TABLE 1.—FUNGI FOUND ON DIFFERENT VARIETIES OF RICE.

Varieties		Alternaria	Epicoccum	Helminthos	Percentage of Colonies Fusarium
Americano	..	80	15	0	0
Rinaldo-Berasi	..	85	10	0	5
Maratelli	..	85	0	0	0
Stirpe-136	..	90	6	4	0
Cammargue	..	95	5	0	0
Montpellier	..	90	10	0	0

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(c) *Addition of Selective Fungicides in the Media*—.A selected number of the fungicides used are given below:- Penta chloronitro benzene 30% (P.C.N.B.). Captane. Streptomycine. Rose bengal. These fungicides were added to malt agar solution, in different concentrations, before sterilization. The fungicide 30% Pentan chloronitro benzene was found to be the best for the slow growth of *Alternaria* at a concentration of 1 part per 1000 of malt agar, but on the other hand, it retarded the growth of *Piricularia* and *Helminthosporium*.

Method of Isolation of H. oryzae and P. oryzae

From the stock, 100 kernels were taken for each test, their surfaces sterilized by 50-60% alcohol and then soaked for the interval (variable) in a 1 to 1,000 solution of mercuric chloride. The kernels were later washed five times successively by sterilized water, dried and placed on malt agar. These were incubated for seven days at room temperature (18°-22°C.) in sterile moist chambers. The results are recorded in Table 2.

Table 2 shows the method described favours growth of *Piricularia* and *Helminthosporium*. It is of utmost importance to reduce the number of the colonies of bacteria, and this could be brought about by carrying out the experiment under sterile condition.

The kernels were taken, their surfaces sterilized by 50% alcohol for 3 minutes and soaked for 10 minutes in 1 to 1,000 solution of mercuric chloride, they were then washed by 95% alcohol and five times by sterile water, wrapped in sterile filter paper and placed in dessicator. They were dried under vaccum for 3 hours. After drying they were transferred in petri dishes, on malt agar media, according to 'Ulster method'. These were placed in (sterile) moist chambers for seven days at room temperature (about 1-20°C.). They

TABLE 2.—ACTION OF THE SUPERFICIAL DISINFECTION ON THE FUNGI ISOLATED FROM THE KERNELS.

Methods of Disinfection		Minutes	*P. oryzae*	*H. oryzae*	Percentage of Colonies	
					Alternaria	*Epicoccum*
1. Alcohol 50%	..	3	2	33	30	35
HgCl$_2$	..	6				
2. Alcohol 50%	..	3	4	44	22	30
HgCl$_2$	..	7				
3. Alcohol 50%	..	3	13	13	27	47
HgCl$_2$	..	10				
4. Alcohol 60%	..	5	2	35	33	35
HgCl$_2$	..	10				

TABLE 3.—THE RELATIVE FREQUENCIES OF FUNGI ISOLATED FROM THE RICE GRAINS, AFTER SURFACE STERILIZATION, AND INCUBATED IN THE OPEN AIR OR IN THE HUMID CHAMBER.

Method of Disinfection		Incubation	*P. oryzae*	*H. oryzae*	*Alternaria*	*Epicoccum*
1. NaOCl 1% min; Grains non-rinsed	..	air	0	0	80	20
2. HgCl$_2$ 1% min;	..	air	13	43	30	14
5 rinses	..	Humid Chamber	17	35	32	16
3. HgCl$_2$ 1% 5 min;		air	2	20	30	28
5 rinses	..	Humid Chamber	8	20	41	31
4. Plain	..	air	0	21	25	54
Non-disinfected	..	Humid Chamber	0	15	34	51

were then transferred under light at 18°-20°C. for sporulation, for about 48 hours.

The colonies of brownish, black, Piricularia, were found to have submerged mycelia with a greenish black centre with a lighter outer rim. Within two days the brownish black colour disappears giving place to dark green.

Conclusions

The isolation of the parasites in the presence of the saprophytes presents a great difficulty. However it has been observed that the media of oatmeal agar is of great help for the development and growth of *Helminthosporium*.

Until more information is available in respect of the infection of *Piricularia* in different parts of the rice kernel, it would be inadvisable to state whether the disease caused by *Piricularia oryzae* is seed-borne or not.

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