SHORT COMMUNICATIONS

OSMIC ACID AS A COLORIMETRIC REAGENT FOR THE DETERMINATION OF ASCORBIC ACID

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Introduction

Ascorbic acid (Vitamin C) can be determined by (1) colorimetric methods, (2) titrimetric methods and (3) biological methods. In the colorimetric methods reagents most widely used include uranium nitrate,[1] phospho-tungstic acid,[2] 2,4-dinitrophenylhydrazine[3–5] and ferridipyridyl.[6]

The titrimetric methods are based on the application of reagents such as iodine, methylene blue,[7,8] ferricyanide,[9] 2,6-dichlorophenol-indophenol,[10] and N-bromsuccinimide.[11]

In the present paper, a colorimetric method is reported which is based on a colour reaction of ascorbic acid with osmic acid. The method is suitable for quick determination of vitamin C in pharmaceuticals and test solutions.

Experimental

Apparatus.—Lange photoelectric colorimeter Model IV.

Chemicals.—0.01 percent ascorbic acid solution prepared by dissolving A.R. grade ascorbic acid in water; 0.1 percent osmic acid solution prepared by dissolving the acid in water; buffer solution prepared by dissolving 3.4g. of KH_2PO_4 and 8.954g. of Na_2HPO_4 in 1 litre of distilled water. The solution maintains the pH at 7.0.

Procedure

Determination of Vitamin C in Test Solutions.—(A). A volume of the ascorbic acid solution containing not less than 300 µg of ascorbic acid was diluted to 12.5 ml. with distilled water. 10 ml. of the buffer solution, followed by 2.5 ml. of the osmic acid solution, were added to the diluted ascorbic acid solution. The colour was allowed to develop for 14 minutes and the solution was then diluted to 100 ml. Absorbance of the solution was measured in the colorimeter. The whole sequence of operations, from the time osmic acid was added till the measurement of absorbance, was completed in 15 minutes.

The absorbance was measured with different amounts of ascorbic acid and a standard curve was drawn between absorbance and concentration of ascorbic acid.

(B). The above procedure was repeated but dilution to 100 ml. was made just after the addition of osmic acid and the absorbance was recorded 15 minutes after the addition of osmic acid. The relationship between absorbance and concentration was found to be linear.

Determination of Vitamin C in Pharmaceutical Tablets.—Ten tablets were weighed and powdered. An accurately weighed quantity of the powder was dissolved in water and the volume made to 100 ml. The solution contains. 0.1 mg. of the powder in 1 ml. Vitamin C was determined in the sample using 3-5 ml. of the solution.

Results and Discussion

Ascorbic acid solutions of different strengths were prepared and their acid content determined by the proposed method (Table 1). It is observed (Table 2) that ascorbic acid can be reliably estimated by this method which is comparable with a titrimetric method based on N-bromsuccinimide.

The method is based on the selective colour reaction of ascorbic acid with osmic acid and the colour produced is measured expeditiously with a colorimeter. The colour formation of ascorbic acid with osmic acid is quite sensitive and this method has no stringent conditions attendant upon it. The method is workable for test solutions as well as for pharmaceuticals (Table 3). Many interferences have been checked which show that oxalates, starch, sucrose, lactose and glucose do not interfere. However, the application of this colour reaction still needs to be extended to the determination of the vitamin in fruits and vegetables.

TABLE 1.—COLORIMETRIC DETERMINATION OF ASCORBIC ACID (VITAMIN C) USING OSMIC ACID IN TEST SOLUTIONS.

Ascorbic acid present μg.	Ascorbic acid as determined by Procedure A μg.	Procedure B μg.	Error %
300	305	—	1.66
320	320	320	0.00
330	330	330	0.00
400	405	405	1.25
425	430	—	1.16
430	—	427.5	0.57
475	476.25	—	0.26
480	477	—	0.625
510	—	512.5	0.49
525	525	—	0.00
575	573	—	0.35
590	—	587.25	0.38
600	595	—	0.83

TABLE 2.—COMPARISON OF RESULTS BY OSMIC ACID AND N-BROMSUCCINIMIDE (N.B.S.).

Ascorbic acid present μg.	Found by osmic acid μg.	Error %	Found by N.B.S. μg.	Error %
300	305	1.66	306	2.00
320	320	0.00	316	1.25
400	405	1.25	395	1.25
425	430	1.16	422	0.7
475	477	0.42	474	0.21
525	525	0.00	516	1.71
550	550	0.00	544	1.1
575	577.5	0.44	562	1.9
600	598	0.33	593	1.1

TABLE 3.—ESTIMATION OF ASCORBIC ACID IN TABLETS BY 0.1% OSMIC ACID SOLUTION.

Sample	Ascorbic acid present μg.	Ascorbic acid determined μg.	Error %
Tablet	300	303.5	1.16
	350	354	1.14
	400	406	1.25

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DETERMINATION OF ANTIMONY IN THE PRESENCE OF BISMUTH*

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During a study of the electronic and magnetic properties of antimony-bismuth alloys, a method was required for rapid and accurate determination of antimony in the presence of bismuth. Conventional methods[1] usually require the antimony to be selectively separated first, while in some colorimetric methods, applicable only at trace concentrations, separation may not be necessary. In the rhodamine-B method[2] it is necessary that the bismuth concentration should be kept within the prescribed limits. The iodide method[3] permits simultaneous determination of antimony and bismuth but the accuracy is low (±2 percent). The methyl violet method[4] is selective for antimony but not so accurate. During titrimetric determination of antimony, the difficult step of separation may be avoided and analysis quickly performed if the interfering elements are selectively masked. For example, Cu(II) can be masked with citrates[5] during iodimetric titration of Sb(III).

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It is known[6] that Bi (III) forms a strong complex with EDTA which remains undercomposed even in acid solutions. The EDTA complex of Sb (III) is, however, very weak.[7] It was further found that iodimetric titration of Sb (III) is not affected by the presence of EDTA. This suggested a method for selective masking of Bi (III) present, during titration of Sb (III) with iodine.

Method

Reagents.—Antimony potassium tartrate solution, 0.1 M; EDTA (di-sodium salt) solution, 0.1M; iodine solution, 0.01 N; Rochelle salt solution, saturated; sodium bicarbonate solution, saturated; sulphuric acid, concentrated; starch indicator, 1 percent freshly prepared; antimony metal, powder; bismuth metal, granular; copper metal, foil; lead metal, foil.

All these reagents, except EDTA, antimony metal and bismuth metal, were Anala R grade B.D.H. chemicals. The EDTA and antimony metal were reagent grade B.D.H. products while the bismuth metal was A.R. grade of Mallinckrodt Chemical Works, U.S.A.

The EDTA solution was standardised against pure mercury, while other solutions were standardised in the conventional way. The antimony metal was found to be 97.81 percent pure.

Procedure

For Antimony Potassium Tartarate in the Presence of EDTA.—Definite amount (~1.0 ml.) of the 0.1 M tartar emetic solution was measured out by means of a semi-micro burette and Rochelle salt solution (2 ml.) was added, followed by varying proportions of 0.1 M EDTA. The mixture was diluted with distilled water (50 ml.), made alkaline with sodium bicarbonate and titrated with iodine in the presence of starch (2 ml.). In the presence of a large excess of EDTA the blue colour of the solution at the end point tended to fade. Overtitration could be avoided by adding iodine to a point when the blue tinge was stable for thirty seconds.

For Mixtures of Antimony (Added as Tartar Emetic) and Bismuth—EDTA.—Bismuth metal (0.01—0.10 g.) was taken in a pyrex conical flask and tartar emetic solution (5 ml.) added. Concentrated sulphuric acid (4-6 ml.) was then added and the mixture was heated, gently until the tartarate was carbonised, and then strongly until all the bismuth had reacted and carbon was completely oxidised. The mixture was cooled to room temperature when sulphates of antimony and bismuth crystallised out. Rochelle salt solution (10 ml.) and distilled water (50 ml.) were added and the mixture was boiled for 5 minutes to remove sulphur dioxide completely. The solution was cooled again to room temperature. At this stage antimony was completely in solution but some bismuth sulphate remained undissolved. The EDTA solution (5 ml.) was added and the excess acid was neutralised with sodium bicarbonate and finally made alkaline; the bismuth sulphate was then completely dissolved. Starch (2 ml.) was added and titrated with 0.01 N iodine.

Final Method for Synthetic Mixtures of the Metals or Alloys.—Antimony (0.05 g.) and Bismuth (0.01 to 0.10 g.), or any convenient amount of the alloy containing the two metals in the above proportion was dissolved by heating with concentrated sulphuric acid (5-7 ml). It was cooled to room temperature and Rochelle salt solution (10 ml.) was added, followed by distilled water (50 ml.) The mixture was boiled for 5 minutes to remove sulphur dioxide completely. The rest of the procedure was the same as already described.

Results

Results given in Table 1 indicate that the presence of EDTA has practically no influence on the iodimetric titration of Sb(III) except at very high concentrations when some overtitration may occur. In Table 2, results for titration of Sb(III), derived from tartar emetic, in the presence of Bi(III)-EDTA under simulated conditions approaching that for metal mixtures or alloys, is given. It is found that Bi(III) when present in amounts 16 to 160 percent of Sb(III) does not influence the results for the latter which are accurate to $\pm$0.23 percent. Final results for analysis of synthetic mixtures of the metals or alloys are given in Table 3. It will be seen that for samples containing 0.05 g. Sb and 0.012 to 0.1147 g. Bi, Sb can be determined with an accuracy of $\pm$ 0.60 percent. With proper manipulation a sample can be easily analysed within 30 minutes.

Since estimation of Bi by EDTA is subject to interference by Sb[6], no attempts were made to analyse the solution (after iodine titration) for Bi by back titaration of excess EDTA. Separation of Bi by sulphide method should enable its determination.

It may be mentioned that the proposed method was also found suitable for determination of Sb

TABLE 1.—TITRATION OF ANTIMONY (III) WITH IODINE IN THE PRESENCE OF EDTA.

Antimony taken g.	0.1 M EDTA added ml.	Antimony found g.	Error %
0.01243	0.0	0.01243	0.00
0.01243	5.0	0.01243	0.00
0.01243	10.0	0.01237	−0.48
0.01243	15.0	0.01249	+0.48
0.01243	20.0	0.01255	+0.97

TABLE 2.—DETERMINATION OF ANTIMONY (III) IN THE PRESENCE OF BISMUTH (III)-EDTA.

Antimony taken g.	Bismuth taken g.	Antimony found g.	Error %
0.06039	0.0102	0.06039	0.00
0.06039	0.0249	0.06037	−0.12
0.06039	0.0511	0.06039	0.00
0.06039	0.0822	0.06053	+0.23
0.06039	0.1020	0.06039	0.00

TABLE 3.—DETERMINATION OF ANTIMONY IN SYNTHETIC MIXTURES AND ALLOYS WITH BISMUTH.

Antimony taken g.	Bismuth taken g.	Antimony found g.	Error %
0.0499	0.0120	0.0497	−0.40
0.0500	0.0250	0.0503	+0.60
0.0496	0.0525	0.0496	0.00
0.0510	0.0791	0.0512	+0.39
0.0500	0.1147	0.0503	+0.60

in presence of Cu or Pb. In the former case, analysis of mixtures containing 0.05 g. Sb and 0.01 to 0.10 g. Cu gave results accurate to ± 0.70 percent. When Pb is used in place of Cu, results are more accurate (±0.50 percent), but in this case addition of even excess of EDTA fails to bring the $PbSO_4$ into complete solution. This however does not affect the titration.

Acknowledgement.—The author wishes to thank Professor M.H. Khundkar for his kind interest in the problem.

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STUDIES ON THE TENSILE CHARACTERISTICS OF BIBRIK WOOL

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Introduction

Study has been made on various samples of Bibrik wool fibres collected from different parts of Quetta, Baluchistan. The samples were tested, for diameter, elongation and strength; stress and tensile strength were determined. The tensile properties of true and medullated wool fibres have been compared with that of Kaghani, Hashtnagri, Harnai, Lohi and Waziri wool fibres in order to determine the values and suitability for apparel cloth or carpet manufacture.

Experimental

Each sample was cleaned, sorted into true and medullated types of fibres with the help of benzene test as given by Mumtaz Ahmad[1] in his work on Hashtnagri wool properties. The methods used for determination of breaking strength are the same as described in Harnai[2] wool fibres.

Results and Discussion

The mean values of all these properties are given in Table 1. Standard deviation and coefficient

of variation in diameter, breaking strength, stress and tensile strength are shown in Table 2. The relation between diameter and breaking strength of true and medullated wool fibres was studied and finally the graph has been plotted between them and a straight line is obtained in the case of medullated type of wool, while for true fibres the strength does not increase regularly with the increase of diameter, but at some points it also decreases with the increase of diameter.

The average type content in the 30 representative samples was 66.8 percent, true wool, 33.2 percent medullated. The variation in medullated fibres is from 3.2 percent to 37.2 while true fibres

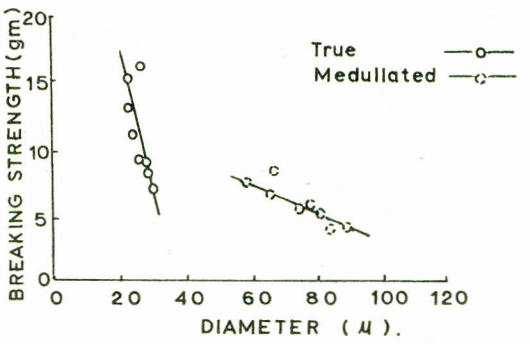

Fig. 1.—Relationship between breaking strength and diamater of true, medullated Bibrik wool fibers.

TABLE 1.—MEAN VALUES OF ALL THE TRUE, MEDULLATED WOOL FIBRES.

Name of Type	Diameter (μ)	Breaking strength: Single fibre g.	Breaking strength: in Bundle kg.	Elongation %	Stress mg./μ²	Tenacity g.	Tensile strength kg./cm²
True ..	26.7	6.6	0.95	24.2	12.2	1.3	1534
Heterotypical ..	—	—	—	—	—	—	—
Medullated ..	72.3	25.4	1.18	31.8	5.7	0.6	675

TABLE 2.—STANDARD DEVIATION AND COEFFICIENT OF VARIATION OF DIAMETER, BREAKING STRENGTH, STRESS, TENSILE STRENGTH OF TRUE, HETEROTYPICAL AND MEDULLATED WOOL FIBRES.

Type of fibres	Coefficient of Variation: Diameter μ	Coefficient of Variation: Tensile strength kg./cm²	Coefficient of Variation: Breaking strength g.	Coefficient of Variation: Stress mg./μ²	Standard Deviation: Diameter μ	Standard Deviation: Tensile strength kg./cm²	Standard Deviation: Breaking strength g.	Standard Deviation: Stress mg. 1/μ²
Mednllated	3.0	30.3	10.2	24.5	2.2	205	2.6	1.4
True	13.1	29.1	33.3	2.1	3.6	447	2.2	0.36

TABLE 3.—MEAN VALUE OF FIBRE DIAMETER.

Breed	True: Diameter μ	True: C. of V. %	Heterotypical: Diameter μ	Heterotypical: C. of V. %	Medullated: Diameter μ	Medullated: C. of V. %
Bibrik	26.7	13.1	—	—	72.3	3.0
Kaghani	29.2	7.5	40.0	10.2	57.0	18.9
Hashtnagri	25.2	11.9	40.5	9.3	51.8	12.5
Harnai	28.7	11.7	45.1	9.1	75.2	15.3
Lohi	20.8	10.9	39.8	16.8	66.8	16.1
Waziri	24.4	14.3	47.4	6.5	58.8	12.7

C. of V.=Coefficient of Variation.

is from 35.8 to 75.5 percent. The percentage variation shows that fineness of this breed is greater, while its coarser property is lesser.

The diameter of the medullated fibres ranges from 58.2μ to 88.8μ (mean 72.3μ) with the coefficient of variation and standard deviation 3 ± 2.2 (II) while for true fibres the diameter ranges from 22.6μ to 37.8μ (mean 26.7μ) with the coefficient of variation and standard deviation (13.1 ± 2.6) as shown in Table 2. The fine wool fibres having the diameter upto 20μ to 30 μ can be used for medium to low quality cloth of 24^s to 62^s spinning quality. The strength generally increases with the increase of diameter in the case of true fibres while it shows decreasing result in the case of

TABLE 4.—MEAN VALUE OF FIBRE STRENGTH.

Breed	True		Heterotypical		Medullated	
	Breaking strength g.	C. of V. %	Breaking strength g.	C. of V. %	Breaking strength g.	C. of V. %
Kaghani	16.4	9.1	21.6	9.4	29.9	7.7
Hashtnagri	8.3	20.5	20.2	19.3	31.3	7.7
Harnai	8.2	20.7	23.2	17.2	30.2	7.6
Lohi	7.4	34.3	10.6	37.2	19.0	24.3
Waziri	5.7	19.3	19.2	28.0	27.2	37.0
Bibrik	26.6	10.2	—	—	25.4	29.1

TABLE 5.—MEAN VALUE OF FIBRE ELONGATION.

Breed	True		Heterotypical		Medullated	
	Elongation %	C. of V. %	Elongation %	C. of V. %	Elongation %	C. of V. %
Bibrik	24.2	15.2	—	—	31.8	3.3
Kaghani	34.0	17.9	41.0	3.8	49.0	6.7
Hashtnagri	28.0	13.9	29.0	11.2	31.0	9.3
Harnai	31.0	14.8	26.0	31.1	28.1	19.2
Lohi	40.0	32.5	40.0	26.2	34.0	20.0
Waziri	35.1	42.1	39.05	9.2	39.5	42.6

TABLE 6.—PERCENTAGE COMPOSITION OF TRUE AND MEDULLATED, KAGHANI, HASHTNAGRI, HARNAI, LOHI AND WAZIRI WOOL FIBRES.

Breed	Type of Fibres as Percentage of Total Fibres.		
	True %	Heterotypical %	Medullated %
Kaghani	60	22	13
Hashtnagri	54	25	21
Harnai	55	27	18
Lohi	53	20	17
Waziri	33	49	17
Bibrik	66.8	—	33.2

medullated fibres. The coefficient of corelation found for true and medullated fibres, confirms the fact that this wool is fine and can be best suited for apparel cloth.

Comparing the tensile properties of Bibrik wool with other breeds i.e. Kaghani, Hashtnagri, Harnai, Lohi and Waziri, it was found that the values of diameter and elongation are the same, while the breaking strength differs widely (given in Table 4). It is concluded from the above comparison that the Bibrik wool is best suited in weaving woollen cloth. Furthermore, a soft wool having minimum elongation of 25 percent is desirable for weaving apparel cloth.

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PREPARATION AND BIOLOGICAL EVALUATION OF A PROTEIN ISOLATE FROM COMMERCIAL OIL-SEED CAKES

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Introduction

In earlier papers, Ali,[1] and Ali and Miller[2] have shown that average Pakistani diet is deficient in proteins and this can be remedied by increasing the protein supply from other sources such as oil-seed cakes, fish flour, leaf protein, etc. The oil-seed which is abundantly available in Pakistan, is cotton-seed, and a good deal of cotton-seed cake is produced in the country and is mainly used as a cattle feed. Attempts were, therefore, made to prepare an edible cotton-seed flour from cotton-seed cake. This was successfully produced[3] and its protein value could be increased to the level of animal proteins by proper supplementation.[4] The present investigations have been carried out with a view to preparing a protein isolate from commercial cotton-seed cake. Its net protein utilization (N.P.U.) value was also determined to evaluate protein quality, and compared with the N.P.U. value of the original material to ensure that there was no gross impairment of the protein quality by the processing technique.

Materials and Method

Cotton-seed cake (Lever Brothers, Raheemyar Khan) was ground to pass 100 mesh and defatted using petroleum ether (b.p. 60-90°C.); one kg. of the commercial cake yielded 560 g. of the defatted cake containing 40.6 percent protein.

The method for the isolation of protein is based on the technique adopted by K. Anantharaman *et al.*[5] from groundnut meal for isolating groundnut protein isolate. This essentially consists of extracting the protein by alkaline solution and precipitating the protein by acidifying the alkaline extract. After making a number of trials by varying the pH of the alkaline solution (A) and the pH of the acidified solution (B) to get the maximum yield of protein (Table 1), the following method was adopted.

TABLE 1.—YIELD OF COTTON-SEED PROTEIN ISOLATE UNDER VARYING CONDITIONS FROM 100 g. OF THE CAKE.

Product No.	pH of NaOH solution (A)	pH of acidified curd (B)	Yield of protein isolate, dry weight (g.)	% of protein in protein isolate
1	8	3	9.5	74.1
2	8	4	10.0	74.2
3	8	5	9.9	70.0
4	8	6	8.0	70.1
5	9	3	15.5	75.0
6	9	4	16.0	75.0
7	9	5	15.0	70.2
8	9	6	11.2	70.3
9	10	3	24.0	75.1
10	10	4	25.0	76.0
11	10	5	20.0	75.15
12	10	6	15.5	75.2
13	11	4	25.0	70.0

To 100 g. of cotton-seed flour 800 ml. of 0.1N sodium hydroxide was added. The pH was approximately 10. The mixture was shaken occasionally and kept overnight. A few drops of chloroform were added to prevent fermentation. The mixture was then filtered through a cloth bag and the residue was treated twice with 0.1N sodium hydroxide and filtered. It was repeatedly washed with water and sun-dried to serve as a cattle feed.

The filtrates from the above process were combined and acidified with 2N sulphuric acid with repeated shaking. When all the protein was coagulated and settled down, the proteins were separated by centrifuging and repeatedly washed with water until pH 6.5 was reached. It was treated with alcohol to remove water and finally dried in a vacuum oven at 50°C.

Loss of Protein in Processing.—The protein isolate was found free of gossypol when tested according to the methods of Podol Skaia,[6] and Royce, Harrison and Dean[7] as modified by Nazir *et al.*[3] In order to evaluate the recovery of protein from the original material, the fractions were weighed and nitrogen was estimated by micro-Kjeldahl method[8] and converted to protein by multiplying with 6.25. The results are given in Table 2 which show that about 11 percent protein is lost in processing.

Determination of Net Protein Utilization.—The cotton-seed flour and the cotton-seed protein isolate were mixed in a semi-synthetic diet so that the protein content was 10 percent. The composition of the diets/kg. is shown in Table 3.

The N.P.U. at 10 percent protein level was determined according to the method of Miller and Bender,[9] using male albino rats, weighing 35-40 g. for a period of ten days. The results are given below:

	N.P.U. %		
	Exp. 1	Exp. 2	Mean
Cotton-seed flour	41.6	44.4	43.0
Cotton-seed protein isolate	39.0	41.17	40.1

Discussion

Use of cotton-seed flour as source of protein in developing countries has been limited because(1) it contains gossypol which needs to be completely removed before it can be used as human food and (2) commercial cotton-seed cake may contain dust particles and other impurities due to unhygienic storage conditions.

These disadvantages have been largely overcome in the protein isolate described in this paper. Further it contains a higher concentration of protein (76 percent), is free from gossypol and other impurities and can therefore be considered suitable as a protein supplement in Pakistani dietary.

During the course of processing, only 11.1 percent protein is lost and the residue left after extracting protein can still be used as cattlefeed. The N.P.U. determination has shown that the protein value of the isolate was only slightly lower than that of the original material which shows that not much damage to protein has been caused during the course of processing.

Acknowledgement.—The authors are grateful to Dr. Karimullah, Director, West Regional Laboratories, for providing necessary facilities to work on this problem.

TABLE 2.

	Wt. of protein concentrate (g.)	Protein %	Total Protein (g.)
Cottonseed flour (A)	100.0	40.6	40.6
Protein isolate (B)	25.0	76.0	19.0
Residue left after extracting with 0.1N NaOH (C)	61.0	17.5	10.5

Protein left in aqueous phase=A-(B+C)=40.6-(19.0+10.5) =11.1 g. Loss of protein, 11.1%

TABLE 3.—COMPOSITION OF DIETS.

Diet	Protein Isolate (g.)	Maize Starch (g.)	Fat. (Star Vanaspati) (g.)	Formula I 10 containing vitamins and minerals
Non-protein	—	500.0	150.0	350.0
Cotton-seed flour	250.0	250.0	150.0	350.0
Cotton-seed protein isolate	133.3	366.7	150.0	350.0

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A NOVEL REARRANGEMENT REACTION

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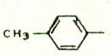
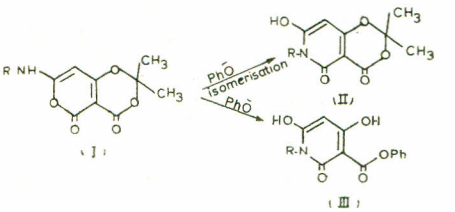
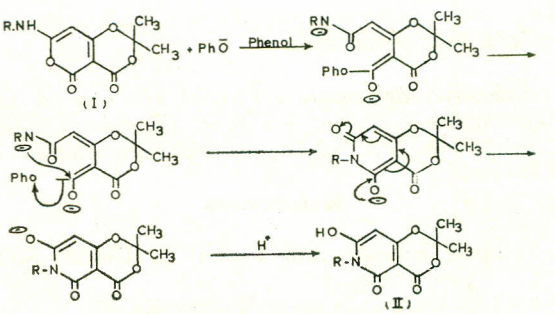
Formation of ethyl-1,2-dihydro-4,6-dihydroxy-1-phenyl-2-oxo-pyridine-3-carboxylate [1] (VI), from 7-anilino-2, 2-dimethyl-4,5-dioxopyrano (4,3-d)-1, 3-dioxin under the influence of sodium ethoxide has already been reported and generality[2] of the reaction has been confirmed. Treatment of the compounds represented by formula(I) with phenoxide in phenol did not give analogous products i.e. no trace of phenylester pyridones (III) were found; instead these rearranged to form new dicyclic products (II) listed in the following along with their ultra-violet light absorption data:

No.	Amino pyrano-1,3-dioxins[4] (I) R	Product II	M. P.	U.V. light absorption (in ethanol 95%) max	log
1.		$C_{15}H_{13}NO_5$	214°	316	4.69
2.		$C_{16}H_{15}NO_5$	188°	316	4.57
3.		$C_{15}H_{12}ClNO_5$	184°	312	4.68
4.		$C_{15}H_{12}BrNO_5$	207°	313	4.39
5.		$C_{19}H_{16}NO_5$	194°	313	4.49

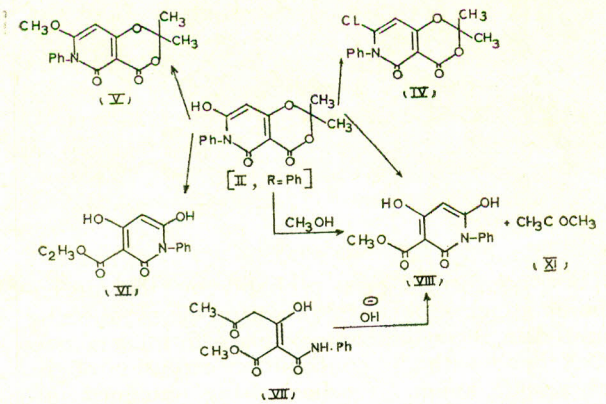
These new dicyclic compounds have a characteristic absorption in the range λ_{max} 316-312 mμ and so these resemble chloropyrano-1,3-dioxins [3] which have the characteristic absorption in the same region. These are phenolic in character and produce effervescence with aqueous sodium bicarbonate. These reactions of phenoxide on the compounds (I) can be written as follows:



These are formed most probably by the nucleophilic attack of phenoxide anion on the pyrane ring and then recyclisation took place forming a new C-N bond with the concomitant explusion of the phenoxide anion. 1,3-dioxin ring remained unchanged. The proposed mechanism is as follows:



In agreement with the above structure (II) assigned to these new products, the compounds $C_{15}H_{13}NO_5$ (II, R=Ph) reacted with phosphorus oxychloride and yielded a chloro-product $C_{15}H_{12}ClNO_4$ (IV) m.p. 163° U.V. λ_{max} 328mμ (log ε , 4.01), I.R. ν (in Nujol) 1751 cm-1 (C=o,4) 1664 cm-1 (C=o,5), evidently formed by the removal of hydroxyl group at position 7 by chlorine, and consequently it was neutral in character. Treatment of (II, R=Ph) with diazomethane, gave another neutral product $C_{16}H_{15}NO_5$ (V) m.p. 183°, U.V. λ_{max} 300mμ (log ε, 4.03), λ_{max} 276mμ (log ε , 4.30), I.R. ν 1724 cm-1 (C=o,4), 1672 cm-1 (C=o,5). Further reaction of (II, R=Ph) with sodium ethoxide in ethanol and sodium methoxide in methanol yielded ethylester pyridone (VI) and methylester pyridone (VIII), respectively and were identified by authentic samples prepared by standard method.[1] The structure (II) for the new products was confirmed when the compound (II, R=Ph) reacted with methanol alone, forming methylester pyridone (VIII) and acetone (XI) which was identified as its 2,4:dinitrophenylhydrazone. The above degradative reactions are depicted as follows:



The conversion of (I) into (II) represents new hetrocyclic rearrangement and is general in nature, This work has been completed and shall be reported in a later publication

Acknowledgement.—Thanks are due to Dr. Salimuzzaman Siddiqui, F.R.S., Director, Central Laboratories, P.C.S.I.R., for his keen interest in this work.

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A NOTE ON THE NATURE OF BONDING IN LEATHER

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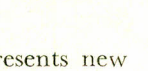
An extensive amount of work has been done to elucidate the nature of bonding in leather. The main theories in this connection are the Adsorption theory, the Residual valence theory, the Salt formation theory and the Coordination theory. Among these the Coordination theory is placed on a better footing and explains the mechanism of tannage reasonably well.[1] This communication is concerned with the explanation of the mechanism of tannage in terms of coordination polymer formation.

Coordination polymers have been worked out only recently[2] and are substances in which a metal atom is linked with poly-functional ligands. Polymers so far known are substances which have carbon, hydrogen, nitrogen and oxygen as the building units. Coordination polymers on the other hand are compounds which have an inorganic backbone, or substances in which an inorganic element is imbedded in an organic casing or a shell. Collagen presents just this type of picture and it is possible to explain the various properties of leather, and thus place the coordination theory on a much stronger footing.

Collagen is a polyfunctional organic ligand having crosslinks such as  as in the coordinate valence bond, which are mainly hydrogen bonds. It has in addition salt like linkages between the basic and the acidic groups of the adjacent polypeptide chains which impart zwitterionic properties to the collagen. As early as 1929 it was suggested by Meyer that the role of the tanning agent is to introduce crosslinking in the protein lattice. The most suitable tanning agents are also those which are themselves polyfunctional in nature. Thus polyphenols or tannins are able to introduce crosslinks among the polypeptide chains and so are the mineral tanning agents which are metal ions having empty d-orbitals, or formaldehyde which has a polymeric structure itself. The formation of crosslinks through the tanning agents allows the collagen portion of the molecule to approach greater stability than would otherwise be the case. That this stability is attained through the tanning process is indicated by the rise in the shrinkage temperature. In the case of coordination polymers also the introduction of crosslinks by metal ions gives additional stability to the organic portion of the molecule.

Crosslinking through coordination brings about order in the molecular lattice, and affords stability to the collagen through hydrogen bonding. Since hydrogen is being shared by the polypepide chains it accounts for a high molecular weight of 55,000 for the native collagen, and treatment with formaldehyde results in the formation of subunits with a molecular weight of 11,000,[3] apparently due to the formation of a crosslink which inhibits chain growth as is the case with coordination polymers.[4]

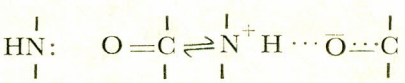
Tannage possibly involves two steps: (1) The acidic condition in which tannage occurs makes the carboxyl group available for coordination, and

(2) the zwitterionic nature of the pelt releases a charge on the amino groups. The former provides coordination sites and the latter along with the hydroxyl and other proton donor groups acts as the catenating group providing a strong backbone. Catenation through hydrogen may be taken as hydrogen bond formation. The role of catenating groups is just as important as that of the coordinating groups for the formation of coordination polymers, since their absence reduces the uptake of chromium as in acetylation of the amino and hydroxyl groups.

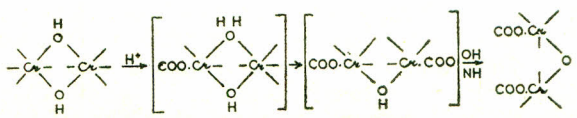
The various mineral tanning agents are fixed in different ways but on the whole involve the same mechanism viz. introduction of crosslinks. The fixation of polymetaphosphate and silica seems to be similar. Fixation of silica is only possible when it exists in sol form i.e. in a polymeric condition, prior to tannage. A polymer is able to present itself in the form of a polyfunctional group which may then introduce crosslinks in various ways.

In the case of chrome tannage only 1 percent of Cr_2O_3 is sufficient to bring about crosslinks for the stability of the collagen. The rest of the chrome compound is utilized in the formation of further subunits, possibly having lower molecular weights. The mechanism may be represented in the following form.

The collagen lattice should be considered as a system of π electrons arising out of the C=O linkages in the peptide chain. The lone pair on nitrogen and oxygen cause high repulsions and since the bond pair: bond pair repulsion is much lower than a lone pair: lone pair repulsion,[5] the stereochemical distribution for least electrostatic repulsion will cause a puckering of the peptide chain. It is the stereochemical distribution of this type that makes the various functional groups of the collagen available for reaction. The hydrogen in the peptide chains will interact with the π electrons of the carbonyl or keto-imide linkages and produce hydrogen bonds. Mobile π electrons usually give rise to unusal stability and their interaction with lone pair electrons results in the establishment of a charge on the atoms concerned e.g. the interaction of the lone pair on nitrogen will result in the following type of structures:



A hydrogen bond is therefore easily formed. NHO bridges as indicated here are quite common in proteins. Hydrogen bonds are also formed by other groups such as hydroxy, amide, amino and carboxy. The reaction may be started by the coordination of the carboxyl groups. Since every sixth residue in collagen has a carboxyl group, the uptake of chromium to stabilise this group is small. Under the acidic conditions of the pelt only this group can take part in the formation of a coordinate bond. The proton involved in the hydrogen bond is transferred to the Ol-bridge and severes the chrome-Ol bridge giving an open chain structure. Another chromium atom is then ready to be coordinated. The protons play a vital role and are responsible for carrying out the reaction to completion.



Since water exists in the lattice of collagen in an ice-like structure[6] and in helical form the water liberated may be acting as a career, and thus the aquated complex may be getting distributed throughout the lattice of the protein.

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SCOPE FOR GROWING AUTUMN POTATOES UNDER ORDINARY CONDITIONS IN THE PESHAWAR VALLEY

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Introduction

Potato is a heavy feeder (i.e. more plant food is needed) crop and requires a high state of soil fertility and plenty of water. Therefore, the main source of impediment in the way of its progress is the non-availability of organic manures and regular water supply in rural areas. Besides

this, it is thought that early frost in Peshawar Valley hinders the cultivation of the autumn crop of potatoes. In order to clear up these two points, the author has planned to carry out research, to see whether these objections have any scientific support.

For this, two dozes of organic manures in combination with two dozes of artificial fertilizers were tried. The behaviour of these treatments on the success of crop is discussed in this paper.

As the problem in view is of a special nature, not much information is available in the literature. Most of the work has been done under favourable conditions and it has been estimated that artificial fertilizers, whether alone or in combination with organic manure enhanced the yield of potatoes. Few references are made here.

Bushnell[1] found that the yields of potatoes are low sometimes due to the unsuitable physical condition of the field for crops. Fertilizers, both stable and artificial, can raise the yield by improving the physical condition and increasing the plant food material of the soil.

Daines and Mortain[2] found that the nitrogen supplied from a combination of organic and inorganic (ammonium sulphate) produced higher yields of potatoes. Nicholes and Catlow[3] concluded that the nitrogen deficiency was the main factor limiting the total yield in potatoes. Low yields are also associated with the bulky use of organic manures. Larson[4] has shown in his experiments that the generous supply of dung increases the yield of potatoes considerably, but has no influence on the effect of nitrogen or phosphorus. Zobel, Lorenz and Underhill[5] concluded from their experiments, where they used two dozes (that is, 80 lbs. and 160 lbs. of nitrogen on potatoes) that both the dozes gave significantly higher yield than control.

Materials and Method

The experiment was laid out at the Agricultural Expansion Farm, Agricultural College, University of Peshawar, during 1961-62 and 1962-63, in a split plot design system, with 1/160th of an acre as net size of plot in six replications. The following treatments were tried, which were arranged at random, as shown by the sketch plan in Tables 1 and 2.

TABLE 1.—SKETCH PLAN SHOWING THE LAYOUT OF THE EXPERIMENT FOR THE YEAR 1961-62.

	M_1		M_2		M_2		M_1			
R_1	N_1	N_2	N_1	N_2	N_2	N_2	N_1	N_2	R_2	
	1	2	3	4	1	2	3	4		
	M_2		M_1		M_2		M_1			
R_3	N_2	N_1	N_1	N_2	N_2	N_1	N_1	N_2	R_4	
	1	2	3	4	1	2	3	4		
	M_1		M_2		M_1		M_1			
R_5	N_1	N_2	N_2	N_1	N	N_2	N_2	N_1	R_6	
	1	2	3	4	1	2	3	4		

TABLE 2.—SKETCH PLAN SHOWING THE LAYOUT OF THE EXPERIMENT DURING 1962-63.

	M_1		M_2		M_1		M_2			
R_1	N_2	N_1	N_1	N_2	N_1	N_2	N_2	N_1	R_2	
	1	2	3	4	1	2	3	4		
	M_2		M_1		M_1		M_2			
R_3	N_1	N_2	N_1	N_2	N_2	N_1	N^2	N_2	R_4	
	1	2	3	4	1	2	3	4		
	M_1		M_2		M_2		M_1			
R_5	N_2	N_1	N_2	N_1	N_1	N_2	N_1	N_2	R_6	
	1	2	3	4	1	2	3	4		

Treatments.—(a) Organic manure (Farmyard Manure) (i) M_1=No manure. (ii)M_2=12 cart-loads of farmyard manure per acre.

(b) Artificial fertilizer (ammonium sulphate). (i) N_1=100 lbs. N in the form of ammonium sulphate per acre. (ii) N_2=150 lbs. N in the form of ammonium sulphate per acre.

No basal doze of organic manure was applied to any plot. Farmyard manure, according to treatments, was applied about one month before sowing the crop and thoroughly mixed in the plots. Ammonium sulphate was applied before the earthing up of the crop. Only one weeding and one earthing up could be applied due to unavoidable circumstances. In all, five waterings were applied at the intervals of 12 to 15 days.

Due to the late arrival of the seed, the crop could not be sown in the first week of September during both years, according to our schedule. It was sown on 29th and 21st September, during 1961-62 and 1962-63, respectively. The crop was not attacked by any disease or insects during both the periods but was badly affected by frost (which started from the third week of November and continued for more than 2 months at a stretch during 1962-63 only).

Ultimash variety was selected for the experiment because it is the latest high-yielding variety with best cooking qualities. The seed was applied by the Agriculture Department, West Pakistan, Lahore, from Sialkot and Lyallpur respectively.

Experimental

The results, thus obtained were statistically analysed and are put forth in Table 3. The data of actual yield per plot and per acre are given in Table 4.

Table 3 supplies the following results: (1) The dozes of organic manures were non-significant during 1961-62. But during 1962-63, M_1 (that is, no manure) gave a significantly higher yield at 1% level as compared to M_2, that is, 12 cart loads of farmyard manure per acre. (2) The dozes of artificial fertilizers were statistically at par with each other during both the years. (3) Interactions

TABLE 3.—ANALYSIS OF THE VARIANCE OF TUBER YIELDS (MAUNDS PER ACRE) OBTAINED FROM THE FACTORIAL EXPERIMENT ARRANGED TO STUDY THE EFFECT OF ORGANIC AND INORGANIC MANURES ON THE YIELD OF POTATOES (TUBERS) DURING 1961-62 AND 1962-63.

Due to	1961-62					1962-63			
	D.F.	S.S.	M.S.	F	Remarks	S.S.	M.S.	F.	Remarks
Blocks	5	574.58	114.91	—	—	13481.48	2696.29	—	—
Manure (Organic) ..	1	158.62	158.62	—	—	3026.26	3026.26	68.3	00
Error I	5	1794.98	358.99	—	—	221.51	44.30	—	
Nitrogen (Artificial fertilizer)	1	10.40	10.40	—	—	164.01	164.01	0.74	N.S.
Manure x Nitrogen ..	1	43.53	43.53	—	—	175.94	175.94	0.79	N.S.
Error II	10	789.37	78.93	—	—	2205.47	2205.47	—	—
Total: ..		3371.48	765.38			19274.67	18312.27		

C.D. at 1% level

Manures = 2.71 mds. per acre
Nitrogen = 4.28 „ „ „
Manure
Nitrogen = 6.06 „ „ „

TABLE 4.—ACTUAL YIELD OF POTATOES (TUBERS) PER PLOT IN SEERS AND CHATTANKS PER ACRE IN MAUNDS AND SEERS.

Replication	Yield per plot in seers and chattank								Yield per acre in maunds and seers							
	1961-62				1962-63				1961-62				1962-63			
	M1-N	M1-N2	M2-N1	M2-N2	M1-N1	M1-N2	M2-N1	M2-N2	M1-N1	M1-N2	M2-N1	M2-N2	M1-N1	M1-N2	M2-N1	M2-N2
R1	26-4	37-7	49-14	40-6	22-12	26-2½	15-15	22-1½	105-0	149-30	199-20	161-20	91-0	104-25	63-30	88-15
R2	30-3	25-4	38-12	37-12	8-9½	6-3	8-12	12-½	120-30	101-0	155-0	151-8	34-15	24-30	35-0	48-5
R3	39-6	26-9	53-13	42-0	17-1	13-5	28-15	17-3½	157-20	106-10	215-10	168-0	68-10	53-10	115-30	68-35
R4	47-15	54-2	38-6	21-1	11-8	13-15	8-7	4-1	191-30	216-20	153-20	84-10	46-0	55-30	33-30	16-10
R5	39-6	31-8	69-9	57-2	19-3	22-9½	27-7½	27-2	157-20	126-0	278-10	228-20	76-30	90-15	109-35	108-20
R6	26-4	35-7	34-2	56-9	14-7½	6-15½	16-9	13-15	105-0	141-30	136-20	226-10	57-35	27-35	66-10	55-30
Means	34-14⅜	34-11½	47-6⅜	42-9⅜	15-12	14-13½	17-3½	16-11½	139-23⅜	140-8⅓	189-26⅜	169-36⅜	62-15	59-17½	70-29⅙	60-39⅙

(M×N) were also non-significant statistically during both the years.

Discussions and Conclusion

The experiment was arranged with the view to assess, whether the autumn potatoes can be raised in the Peshawar valley under the prevalent conditions in rural areas, where the availability of organic manure and water supply is a problem, and to confirm whether the artificial fertilizer can be a substitute for organic manures or not or if the yield of the crop is hindered in any way by early frost. As the study was of a peculiar nature, interesting and encouraging results have been obtained.

1. In heavy soils, with the normal interval of irrigation (12 to 15 days) without organic manures, supplemented with 100 pounds of nitrogen (5 to 6 maunds) per acre in the form of ammonium sulphate, normal yield of autumn potatoes can be produced, which is quite clear from Table 4.

2. If the frost starts from 15th to 20th November and continues for long, it does hinder the normal growth and the yield of autumn potatoes, provided the crop is sown after 20th September, which can be seen from Table 4. In the author's opinion, if the sowing is completed within the first week of September, this problem can be solved, but it requires further investigations.

The heavy frosts of November and December, 1962, would probably severely damage even early planted potatoes, but as stated above, the matter requires further study for confirmation.

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