COMPOSITION OF THE OIL OF GREWIA ASIATICA (PHALSA) SEEDS

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Introduction

Grewia asiatica is a fruit bearing plant and is extensively cultivated in Indo-Pakistan sub-continent.[1] The plant bears fruit in early April which ripens in June. The fruit is a berry and is highly appreciated as a source of beverage for summer drinks. The kernel is enclosed in a tough covering. The seed contains 5% of the bright yellow coloured oil. This oil does not appear to have been studied so far. Investigations were therefore carried out on the nature of the oil by chemical and physical means which include spectroscopic and gas chromatographic studies.

Experimental

Recovery of Oil.—The fruit was separated into pulp and seed in an electric mincer. After thoroughly washing the seeds in water to remove any adhering pulp, they were dried, crushed in an iron pestle mortar and extracted with petroleum ether (40-60°) in a soxhlet apparatus. The solvent was removed after drying over anhydrous sodium sulphate.

Physico chemical characteristics were determined by the usual methods.[2,3] They are recorded below:

Percentage yield of the oil	=5%
Refractive index at 25°	=1.460
Specific gravity at 25°	=0.8832
Colour (lovibond)	=Y=20,R=3.6
Acid value	=3.13
Saponification value	=189.9
Iodine value	=132.74
Hydroxyl value	=Nil
Maleic anhydride value	=9.2
Thiocyanogen value	=51.57
Saturated acids	=19.8
Unsaponifiable matter	=2.8%

Resolution into Various Acid Fractions.—The oil was saponified with 0.5 N alcoholic KOH under reflux and in an inert atmosphere, for 5 hours. After removing the alcohol under reduced pressure from the soap solution, it was diluted with water and extracted with diethyl ether to remove unsaponifiable matter. The residual soap solution was acidified with sulphuric acid (4N) and extracted with diethyl ether to recover liberated acids.

The fatty acids were separated into fractions by Twitchells lead salt alcohol method, the solid acid fraction (mp. 52-54°) bearing 19.8% and liquid acids fraction 79.7%.

Examination of Saturated and Unsaturated Acids by Vapour Phase Chromatography (Griffin and George[5]).

Saturated Acids.—The methyl esters (31-33°) were injected into a column (pumice stone, 16-30 mesh, coated with 5% ° silicone elastomer E. 301), having column temp. 220°, bridge current 125 mA, nitrogen flow rate 1.25 litre/hr. column inlet pressure (20 cm.), column outlet pressure (3.5 cm.), chart speed 12″/hr. and record sensitivity × 1. The palmitic and stearic acids were detected and confirmed by the method of "addition of supposed constituents".[6] The percentage composition of acids, palmitic (42.75) and stearic (57.25) was calculated from their respective areas.

The identification of these acids (52-54° m.p.) and the proportion in which they are present were further confirmed by taking their melting points and mixed melting points. The latter was taken of a mixture of palmitic and stearic acid mixed in the same proportions as calculated from the chromatograms.

Unsaturated Acids.—The liquid acids were hydrogenated by Brown's method[7] in a Parr hydrogenator. Their methyl esters were analysed by vapour phase chromatography under similar conditions as described previously. A single peak was detected which was identified as that of stearic acid (68-69°). The presence of stearic acid was further confirmed by taking melting point and mixed melting point of the hydrogenated acid (68-69°).

Liquid acids were then analysed by injecting their methyl esters into a column (celite 30-80 mesh, coated with 10% glycol stearate) and oleic and linoleic acids were identified. The linoleic (82.72%) and oleic (17.28%) were calculated from their respective peak areas.

The unsaturated acids were further examined by preparing their bromo-derivatives[8] when only tetrabromostearic acid (m.p. 114-115°) was obtained.

Unsaponifiable Matter.—The unsaponifiable matter was crystallised from methanol when white leaflets (m.p. 139-140°) were obtained. The compound gives positive Libermann-Burchard test. Its acetate (m.p. 127-128°) and benzoate (m.p. 146-147°) were prepared by the usual methods. The melting and mixed melting points of the sterol and its derivatives with an authentic sample of β-sito sterol and its derivatives confirmed the presence of β-sito sterol.

The infra-red spectra of the sterol and the authentic sample of β-sito sterol were also found to be identical. A yellow oil (0.45%) left after the crystallization of the sterol could not be identified.

Discussion

The vapour phase chromatography of the saturated and unsaturated acids showed the presence of palmitic and stearic, oleic and linoleic acids, respectively. The preparation of bromo-derivatives of the liquid acids gave no hexabromo stearic acid showing the absence of linolenic acid. Tetrabromo derivative (m.p. 114-115°) established the presence of linoleic acid. The rest of the liquid acids were taken as oleic acid.

The unsaturated acids when hydrogenated and analysed, were found to consist of stearic acid only. The further analysis of methyl esters of liquid acids by vapour phase chromatograph revealed the presence of oleic and linoleic acids only, thus confirming the findings from bromoderivatives.

The overall composition of the oil is as below:—

Palmitic acid	= 8.3%
Stearic acid	=11.0%
Oleic acid	=13.4%
Linoleic acid	=64.5%
Unsaponifiable	= 2.8%

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MICRO AND SEMIMICRO NON-DISTILLATION KJELDAHL METHOD WITH SEALED TUBE DIGESTIONS

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White and Long[1] modified the Kjeldahl method and concluded that digestion of an organic compound in a sealed tube avoides some of the uncertainties known to cause errors. These authors digested organic compounds with sulphuric acid in sealed tubes at 470°C. A considerable amount of mercuric oxide catalyst was used. In the case of nicotinic acid, which is ordinarily difficult to digest, the catalyst was unnecessary if sufficient digestion time (30 minutes) was allowed. They also suggested the use of sealed tube method when other constituents such as phosphorus were also to be determined.

Grunbaum et al.[2] applied the sealed tube digestion method to determine its range of application to various forms of nitrogen, some of which are

difficult to convert quantitatively to ammonia. The ring nitrogen compounds such as tryptophane and nicotinic acid which resist conversion of their nitrogen to ammonium sulphate were shown to give a recovery of 97.8 to 99% of nitrogen. It was further shown that sulphuric acid without catalyst, salt or oxidizing agent is capable of converting all the nitrogen of these compounds quantitatively to ammonia when the digestion is carried out in a sealed tube for two hours at a temperature of 420°C.

Baker[3] reported that good results could be obtained for nitro compounds if 50 mg. of thiosalicyclic acid or glucose were added to the digest in the sealed tube. According to Belcher, Bhasin and West,[4] nitrogen in nitro, azo and hydrazo compounds can be successfully determined by the sealed tube method on the sub-micro scales. They obtained excellent results when glucose was used as a reductant. They have, however, investigated only one hydrazine compound, penta fluorophenyl hydrazine chloride (nitrogen 11.94%).

The applicability of such an approach to a large number of organic compounds particularly the hydrazine compounds containing higher amounts of nitrogen has been further examined. In the course of these investigations the sealed tube digestion has been carried out on the micro and semimicro scales. Glucose has been used as a reductant for the compounds which require prereduction. After digestion, the modified Kjeldahl method was used, in which, the step of distillation has been eliminated.

Experimental

A. Reagents.—(1) Sodium hypochlorite solution: 0.05 N for semimicro and 0.02 N for micro determinations. (2) Arsenious oxide solution: 0.025 N for semimicro and 0.01 N for micro determinations. (3) Tartrazine indicator (aqueous), 0.05%. (4) Sulphuric acid (d 1.84)—A.R. (5) Sodium bicarbonate—A.R. solid. (6) Potassium bromide A.R. solid.

B. Apparatus.—(1) Digestion tubes: These were of pyrex glass tubing (11 mm. external diameter, 6 mm. internal diameter and 35 mm. length for micro digestion and 16 mm. external diameter and 13 mm.internal diameter and 55 mm. length for semimicro digestion) with hemi spherical seal at one end. (2) Heating system: The sealed tubes were heated in a Carius furnace which can accommodate four tubes. The heating chamber is insulated with glass wool and is heated by high grade wire element distributed all along the bottom and sides to ensure a uniform heating. There are four iron tubes in the furnace in which four sealed tubes can be placed in the horizontal position. A thermometer or a thermocouple can be placed in a pocket fitted at the top.

C. Procedure.—(i) Digestion: In the case of micro determination 5-10 mg. of the compound were digested with 1 ml. of concentrated sulphuric acid at 410-420°C. for 5-6 hours. For semimicro determination 20-50 mg. of the compound were digested with 3 ml. of concentrated sulphuric acid at 410-420°C. for 5-6 hours. The compounds containing oxidized type of nitrogen were digested along with glucose reductant. For such compounds 100 mg., and 350 mg. of glucose were used, respectively for micro and semimicro determinations. For sealing and opening of the tubes the method of Belcher et al.[5] was followed.(ii) Titration: The methods used for titration in both micro and semimicro determination are exactly the same which have already been reported.[6]

Discussion

After digestion, it is necessary to boil the contents of the tube to eliminate the traces of SO_2 and SO_3, otherwise the results are very high. A small quantity of sulphuric acid is left behind after digestion which can be neutralized by sodium bicarbonate. It is not necessary to use sodium hydroxide for neutralizing the acid.

The results of the compounds tested are reported in Tables 1 and 2.

TABLE 1.—MICRO DETERMINATION OF NITROGEN USING SEALED TUBE DIGESTION.

No	Compound	% of Nitrogen	
		Required	Found
1	Phenacetin ..	7.82	7.79, 7.77
2	8-Hydroxyquinoline ..	9.65	9.68, 9.71
3	m-Dinitrobenzene ..	16.67	16.66, 16.64
4	α-Nitroso-β-Naphthol ..	8.09	8.18, 8.13
5	S-Diphenylthiourea ..	12.26	12.22, 12.23
6	S-Benzoyl thiouronium ..	13.82	13.80, 13.78
7	Quinine Sulphate ..	7.16	7.05, 7.16
8	Azobenzene ..	15.38	15.29, 15.60
9	Hydrazine sulphate ..	21.54	14.43, 15.00
10	p-Nitrophenylhydrazine ..	27.43	20.72,18.14
11	2-4 Dinitrophenylhydrazine	28.27	25.80,24.86

TABLE 2.—SEMIMICRO DETERMINATION OF NITROGEN USING SEALED TUBE DIGESTION.

No.	Compound	% of Nitrogen	
		Required	Found
1	Phenacetin ..	7.82	7.78, 7.74
2	8-Hydroxyquinoline ..	9.65	9.65, 9.55
3	m-Dinitrobenzene ..	16.67	16.67, 16.60
4	α-Nitroso-β-Naphthol ..	8.09	8.19, 8.17
5	S-Diphenylthiourea ..	12.26	12.30, 12.36
6	S-Benzoyl thiouronium chloride ..	13.82	13.80, 13.84
7	Quinine sulphate ..	7.16	7.14, 7.20
8	Azobenzene ..	15.38	15.41, 15.65

Conclusion

The superiority of the sealed tube digestion method is that digestion can be completed without the use of any catalyst. In the sealed tube nothing can escape from or be absorbed by the digest. The sealed tube digestion has given better recoveries of nitrogen in large number of compounds on micro and semimicro scale. However, the recoveries of nitrogen in the case of hydrazine sulphate, p-nitro phenyl hydrazine and 2-4 dinitro phenyl hydrazine were not quantitative. Further investigations are required to make the method applicable to hydrazine compounds.

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ANTIMONY (III) CARBOHYDRATE COMPLEXES

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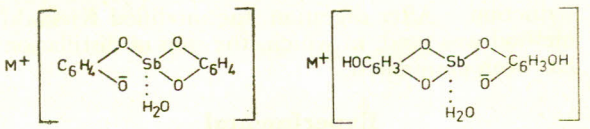
Introduction

The therapeutic use of antimony compounds dates back to the Egyptian civilisation when naturally occurring antimony sulphide or stibnite was used as a prophylactic for certain eye diseases. The use of antimony compounds for the treatment of tropical diseases was initiated by the discovery of Plimmer and Thomson.[1] They found that tartar emetic (potassium antimony tartarate) was effective in curing trypanosomiasis. Later discoveries paved the way for the preparation of innumerable antimony compounds useful against diseases like visceral and cutaneous leischmaniasis, schistosomiasis, granuloma inguinale, trypanosomiasis and filariasis. Hence complexes with pyrocatechol,[2] ascorbic acid,[3] citric acid,[4] caffeic acid,[5] gallic acid[6] and other phenolic and poly hydroxy compounds have been reported by many workers. Yves Volmer and George Coettelman[7] obtained easily oxidisable, almost colourless crystalline complexes of structure:



(I) (II)

Benger Labs. Ltd.[8] have prepared a colloidal non-ionisable antimony complex with dextran forming a therapeutically effective drug useful in the treatment of leischmaniasis and schistosomiasis. They added low molecular weight dextran to a solution of antimony penta chloride in HCl (conc.), brought the pH to 5 by adding NaOH, heated to boiling, filtered and precipitated by ethyl alcohol. The product is water soluble.

Work on the preparation of various metal carbohydrate complexes [9-11] of possible therapeutic value has been in progress in this laboratory and results published. According to the scheme followed here, a hydroxide or hydrated oxide of a metal is prepared which is then washed free of electrolytes, and heated with the requisite amount of carbohydrate and sodium hydroxide in an electric oven until a complex is prepared. It is then

dissolved in water, and ampuled. In the present communication, sucrose, maltose and glucose colloidal complexes with antimony are being reported.

Preparation of Complex

1.2 g. of Sb_2O_3 (antimony trioxide) of B.D.H. quality was taken in a small beaker and to this were added 5 ml. of 15% NaOH and the paste thus formed was slightly warmed. It was then transferred to a porcelain dish with the help of distilled water and requisite amount of the carbohydrate added. After mixing well, the contents were heated in an electric oven at a fixed temperature until a complex was formed (tested by dissolving a small quantity in distilled water, a clear solution being obtained). Heating was then stopped, allowed to cool and then dissolved in 60 ml. of distilled water and centrifuged to remove the unreacted material. The various experimental details are summarised in the Tables 1, 2 and 3.

TABLE 1.—ANTIMONY SUCROSE COMPLEXES.

No.	Sb: Sugar in g.	Sb:NaOH in g.	Time in hours	Temperature in °C.	Percent metal	Stability on boiling	pH	Isoelect point	Density at 30° C.
1.	1:16	1:0.75	1—15	180	82	stable	4.25	1.60	1.049
2.	1:15	1:0.75	1—15	180	85	stable	4.45	1.65	1.045
3.	1:14	1:0.75	1—15	180	62	stable	4.50	1.60	1.041
4.	1:16	1:0.75	3—15	160	73	stable	5.75	2.10	1.047
5.	1:15	1:0.75	3—15	160	75	stable	5.50	1.85	1.042
6.	1:14	1:0.75	3—15	160	58	stable	6.10	1.75	1.042
7.	1:16	1:0.75	0—45	200	80	stable	4.70	1.80	1.050
8.	1:15	1:0.75	0—45	200	84	stable	4.50	1.80	1.051
9.	1:14	1:0:75	0—45	200	60	stable	5.35	1.90	1.048

TABLE 2.—ANTIMONY MALTOSE COMPLEXES.

No.	Sb: Maltose in g.	Sb: NaOH in g.	Time in hours	Temperature in °C.	Percent metal	Stability on boiling	pH	Isoelect point	Density at 30° C.
1.	1:16	1:0.75	1—0	180	75	stable	4.20	1.80	1.046
2.	1:15	1:0.75	1—0	180	82	stable	4.25	1.85	1.048
3.	1:14	1:0.75	1—0	180	63	stable	4.40	1.95	1.042
4.	1:16	1:0.75	1—25	160	68	stable	4.30	1.90	1.033
5.	1:15	1:0.75	1—25	160	72	stable	4.40	1.80	1.035
6.	1:14	1:0.75	1—25	160	54	stable	4.65	1.85	1.032

TABLE 3.—ANTIMONY GLUCOSE COMPLEXES.

No.	Sb: Glucose in g.	Sb:NaOH in g.	Time in hours	Temperature in °C.	Percent metal	Stability on boiling	pH	Isoelect point	Density at 30° C.
1.	1:16	1:0.75	0—50	180	70	stable	4.20	1.90	1.033
2.	1:15	1:0.75	0—50	180	89	stable	4.50	1.85	1.036
3.	1:14	1:0.75	0—50	180	67	stable	4.59	1.95	1.027
4.	1:16	1:0.75	1—15	160	62	stable	4.60	2.25	1.028
5.	1:15	1:0.75	1—15	160	78	stable	4.35	1.95	1.025
6.	1:14	1:0.75	1—15	160	58	stable	4.50	1.50	1.021

Results

1. For 1.2 g. of antimony (III) trioxide (equivalent to 1 g. of elemental antimony) 0.75 g. of NaOH was required to give stable complex. 2. Best ratio of antimony (III) with sucrose, maltose and glucose was 1: 15 expressed in g. 3. Best temperature for complex formation in case of glucose and maltose was 180°C. With sucrose good results were obtained at 180°C. and 200°C. 4. Iso-electric points and densities refer to 1% solution of the complex at 30°C. 5. The toxicity tests were performed according to Probit's method, using male white rats, the time of observation being 24 hours and route of administration intraperitonial. The details are summarised below:

Sb (III) Glucose Complex

24 hrs. LD_{50}^{WR}=25 mg./per kg. body weight. i.p.
24 hrs. LD_{100}^{WR}=50 mg./per kg. body weight. i.p.

Sb (III) Sucrose Complex

24 hrs. LD_{50}^{WR}=20 mg./per kg. body weight. i.p.
24 hrs. LD_{100}^{WR}=40 mg./per kg. body weight. i.p.

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HISTOCHEMICAL LOCLAIZATION OF POLYSACCHARIDES IN THE MID-GUT OF DESERT LOCUST, SCHISTOCERCA GREGARIA (FORSKAL)

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Introduction

Chemical methods have been used for a long time for finding the content of the insect tissues but they are not helpful in locating the actual site of origin of the substance. Therefore, histochemical methods were developed to identify the substances at the site of origin and have been used for more than 20 years ever since. Nakamura[1] localized alkaline phosphatases in the silk gland of *Bombyx*. In the present studies periodic acid Schiff's Reaction[2] has been used for histochemical localization of polysaccharides in the mid-gut of the desert locust. In this reaction periodic acid is employed as an oxidising agent. This breaks the C-C bond of the various carbohydrate structures, converting them into dialdehydes. These aldehyde groups, in combination with Schiff's reagent, produce a red dye. The intensity of the red colour variesa ccording to the number of aldehydes produced and the amount of polysaccharides material present in the living tissues.

Materials and Methods

Freshly chilled locusts were quickly dissected out after snipping their legs, wings and head. Longitudinal cuts were made from thorax down to the abdominal segments on the dorsal and lateral sides. The guts were quickly taken out and were preserved in 70% alcohol which had been previously cooled at 3°C. After chilling the guts for thirty minutes in alcohol, the mid-guts were cut out into 5 m.m. pieces and placed into chilled formalin-alcohol which was made by mixing one part of 40% formalin with nine parts of 70% alcohol. The mid-gut pieces were kept in this fixative for six hours after which they were transferred to the grades of alcohol ranging from 70% to 100%. They were kept for six hours in each grade. After dehydration, the mid-gut pieces were immersed in a solution of 0.5% celloidin for twenty-four hours after which they were drained rapidly and immersed in the chloroform for twenty minutes. Two washings of chloroform were given. Then the pieces were transferred and kept in melted paraffin wax for an hour and a

half They were embedded under vacuum in the paraffin wax of 56°C. m.p. and the paraffin blocks of the pieces were made. The sections were cut at 6μ fixed to the prepared albuminised slides and left for drying overnight. Next day the sections were dewaxed in xylol for thirty minutes, washed with absolute alcohol, flooded with 95% alcohol for three minutes and after draining of the alcohol the slides were dipped in 0.5% celloidin for five minutes. Then the excess of celloidin was shaken off and the celloidin film was hardened by keeping the section on slides in 80% alcohol for ten minutes. The slides were then stained for polysaccharides by the Periodic Acid Schiff's Reaction.

Results and Discussion

The results obtained are shown in Fig. 1. Some parts of the mid-gut epithelium showed strong red stain, while the other parts have taken the moderate red stain. The areas with strong red stain showed the presence of the following polysaccharides: glycogen; glycerophosphate; arabinose; chitin; ribose; serine. The parts which took up the moderate stain showed the presence of the following: glucuronic acid; galactose; glucose; glucose-1- phosphate; maltose.

After confirming the presence of polysaccharides in the epithelial cells of the mid-gut, it was decided to locate the presence of glycogen by using the Bauer Feulgen's method.

The Bauer Feulgen's method[3] consists of chromium trioxide, 4% aqueous solution; 0.5% Basic Fuchsin aqueous solution and potassium metabisulphite, 0.5% aqueous solution. After twenty minutes the slides were gently agitated for about two minutes in potassium metabisulphite solution three times and were later washed in running water for ten minutes. Due to the action of chromic acid intense reddish violet dye[4] was produced in the epithelial cells of the mid-gut, which indicated the presence of glycogen in the protoplasm of the cells. (Fig. 2).

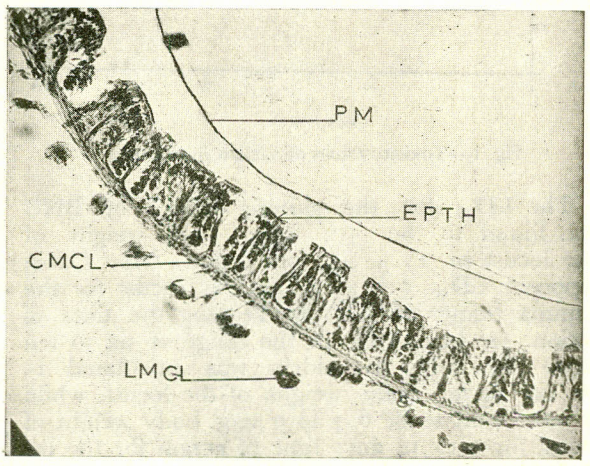

Fig. 1.—Epithelial cells of the mid-gut showing the presence of polysaccharides in the protoplasm; PM—Paritrophic membrane; EPTH, Epilhelium; CMCL, Circular muscles; LMCL, Longitudinal muscles, (Photo-micrograph).

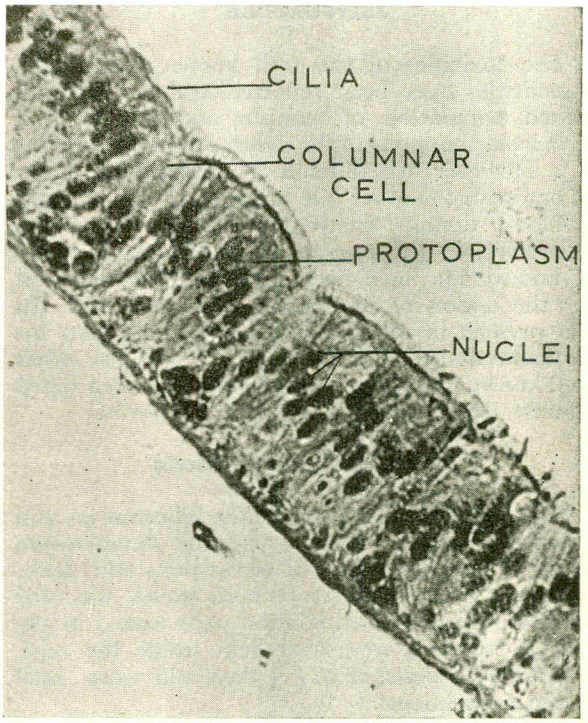

Fig. 2.—Magnified cross-section of mid-gut; dark areas localize the presence of glycogen in the epithelial cells.

The above findings show the presence of polysaccharides in the mid-gut of desert locust and the localization of glycogen in the protoplasm of the epithelial cells.

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