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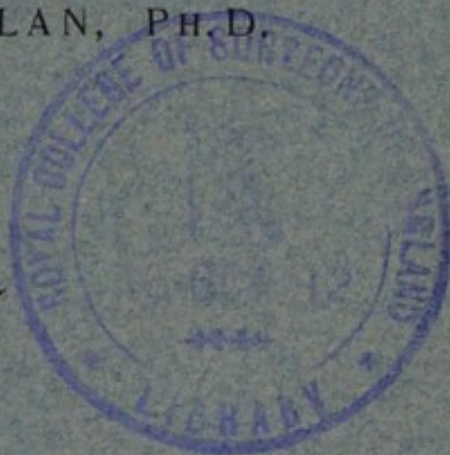
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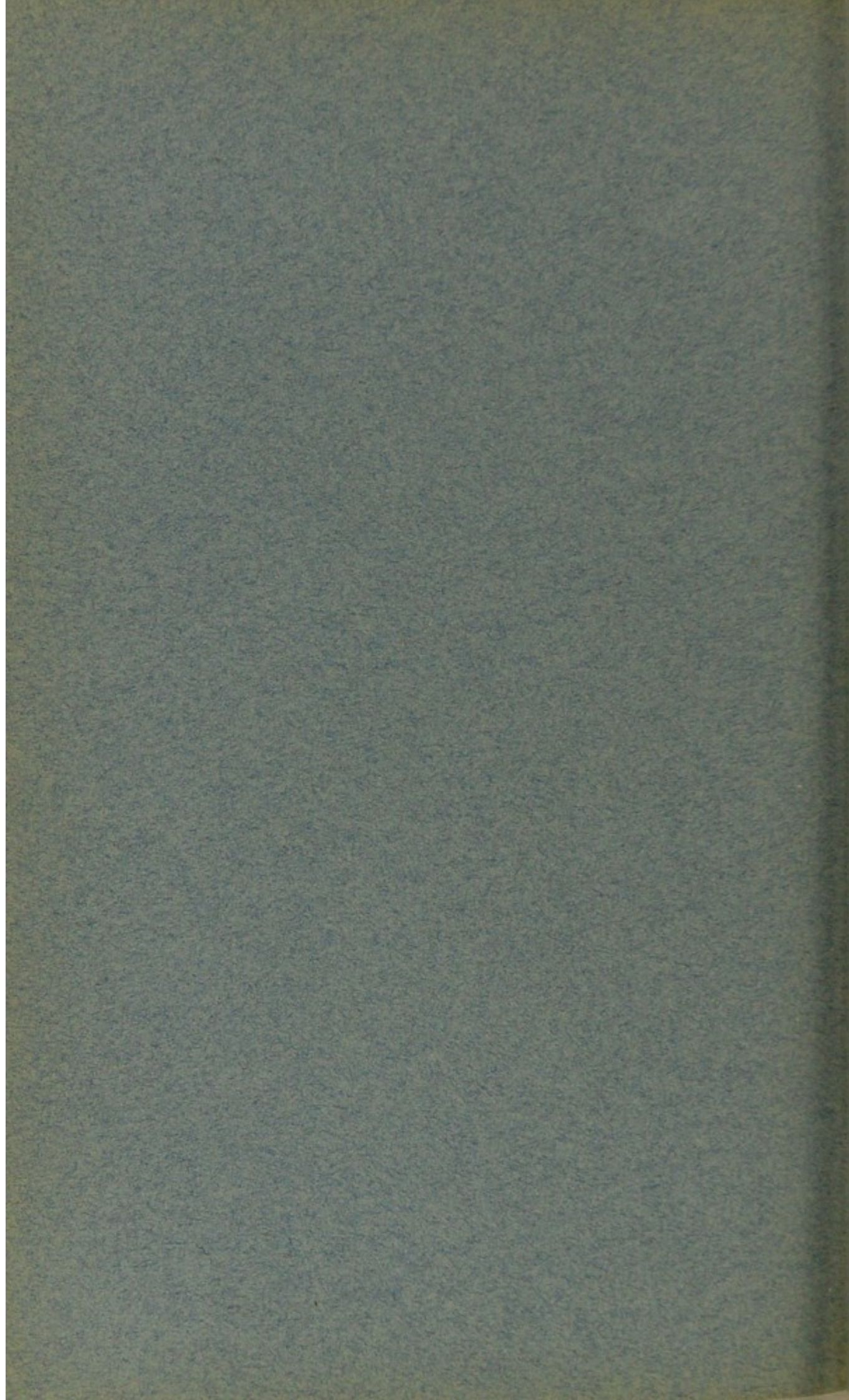
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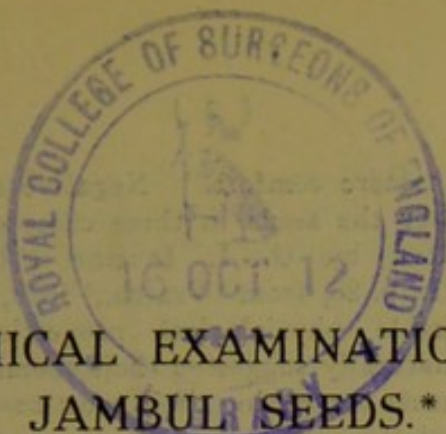
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OF
JAMBUL SEEDS

BY
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CHEMICAL EXAMINATION OF JAMBUL SEEDS.*

BY

FREDERICK B. POWER AND THOMAS CALLAN.

The so-called Jambul seeds are obtained from *Eugenia Jambolana*, Lam. (*Syzygium Jambolanum*, DC.), a tree belonging to the family of *Myrtaceæ*, and indigenous to the East Indies. The fruit of this tree, which appears to have been utilised for many centuries in its native country, is about the size and shape of an olive, of a purplish colour, and possesses an acidulous and astringent taste. Other parts of the tree, such as the leaves and bark, as well as the seeds, have likewise been employed medicinally on account of their astringent properties (compare 'Pharmacographia Indica,' Vol. II., p. 25).

Jambul seeds have been brought more prominently to notice in recent years on account of their suggested value in the treatment of diabetes. It was recorded, for example, by Binz (*Verhandl. d. Kong. für Innere Med.*, Wiesbaden, 1886) that when dogs were made diabetic by the administration of phloridzin, according to the method of von Mering, the exhibition of Jambul reduced the excretion of sugar to the extent of 50-90 per cent. Similar observations were made by Graeser (*Lancet*, November 2, 1889), and it is stated ('U.S. Dispensatory,' 18th Edition, p. 1651) that Dr. H. C. Wood has seen the sugar of the urine entirely disappear under the use of Jambul. On the other hand, Dr. Lenné (*Pharm. Centralh.*, October 26, 1899, p. 657, and *Proc. Amer. Pharm. Assoc.*, 1900, 48, 638) has observed that "although these seeds in the fresh state have been used with benefit by the natives of Java in cases of diabetes, they have no effect whatever in reducing the quantity of sugar; they simply appear to act as a palliative, enabling the patient to bear the burden of

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his illness with more comfort." Negative results from the administration of the seeds in three cases of diabetes were likewise reported by G. I. Iaveïne ('Pharmacographia Indica,' Vol. II., p. 28, from 'Vratch.,' 1889, p. 1029).

The remarkable physiological properties attributed to Jambul seeds naturally suggested their chemical examination, but the statements concerning their constituents are very discrepant. One of the earliest investigations of the subject was by Elborne (*Pharm. Journ.*, May 5, 1888, p. 921), who noted the presence of a trace of essential oil, small amounts of fat and resin, and 1.65 per cent. of gallic acid, but no substance to which the reputed action of the seed could be ascribed.

An examination of the seed was subsequently recorded by Charles Pottiez (*Ann. de Pharm.*, Louvain, 1899, 5, pp. 373-391 and 490-496). In this communication the author states to have found tannic acid ("jambo-tannic acid"), quercitol, cinnamic acid, and a resin, but does not mention the occurrence of gallic acid. The observations concerning quercitol and cinnamic acid do not appear, however, to have afforded sufficient evidence that these substances are constituents of Jambul seeds. With regard to quercitol, for example, it was noted (*loc. cit.*, p. 491) that 30 grammes of the powdered seed were extracted with cold water, the concentrated aqueous liquid treated with a little beer yeast, subsequently with basic lead acetate, and the filtered liquid, after the removal of the excess of lead, evaporated to a syrup, when, on cooling, a mass of rhomboidal crystals was obtained. These crystals were said to be insoluble in alcohol and in ether, and to possess "all the characters of quercitol," but no analysis, melting-point, or other information was recorded which would serve to confirm the accepted characters of this compound. The statement regarding the presence of cinnamic acid is equally unsatisfactory, since this was based on the separation of a small amount of crystalline substance from the resin, for which no characters distinctive of the respective acid were given. It was noted that "the micro-chemical study of the acid principle fully confirmed its nature."

In connection with the assumed presence of cinnamic acid, Pottiez (*loc. cit.*, p. 493) makes the further statement that it no doubt was this substance which Gerrard had met with in the seed, and which he called "jambosin," consequently that it possesses the formula $C_9H_8O_2$, and not $C_{10}H_{12}NO_2$, as given by the last mentioned author. This observation exhibits a remarkable misconception, inasmuch as the substance

for which Gerrard proposed the name "jambosin" was not an acid, nor was it obtained from Jambul seeds, but from the roots of a plant which has somewhat doubtfully been referred to *Myrtus Jambosa*, Linné (*Jambosa vulgaris*, DC.) (compare *Pharm. Journ.*, 1884 [3], 14, 717).

Another statement relating to Jambul seeds may briefly be considered. It has been claimed by Boersch that the active principle of these seeds is a crystalline glucoside, which was introduced into medicine under the names of "antimellin" or "djoëatin" (compare *Apoth. Zeit.*, 1899, p. 510; 1900, p. 92; 1901, p. 350). The substance was said to have been obtained in yellowish needles, melting at about 182° , possessing a sweetish-bitter taste, and apparently having the formula, $C_{13}H_{20}O_7$, but no analysis was recorded, nor has any further description of its characters been given. The clinical tests which have been made of this preparation by Hirschfeld (*Apoth. Zeit.*, 1901, p. 42) proved it to have no influence on the excretion of sugar in diabetes. A liquid preparation was subsequently issued under the above names, which appears to have consisted of a decoction of Jambul seeds (*Apoth. Zeit.*, 1900, p. 92), with possibly other substances dissolved therein (Hager's 'Handbuch,' 1902, Bd. II., p. 1010).

With consideration of the varying statements in the literature of this subject, as indicated above, it seemed desirable to submit Jambul seeds to a further and more complete examination, and the results are embodied in the present communication.

EXPERIMENTAL.

The material employed for this investigation consisted of a quantity of authentic Jambul seeds, which had been obtained directly from India.

PRELIMINARY TESTS.

A small quantity of the powdered seeds was boiled with water, and the mixture filtered. The cold, aqueous liquid gave an intense blue colouration with iodine solution (showing the presence of starch) and a bluish-black colouration with ferric chloride (indicating the presence of tannin).

Test for an Alkaloid.—Twenty grammes of the ground material were extracted with Prollius' fluid, and the liquid so obtained subsequently examined in the usual manner for the presence of an alkaloid. The results were perfectly negative.

Test for an Enzyme.—For this purpose 500 grammes of the ground material were mixed with about an equal weight of water, and the mixture kept at the ordinary temperature for twenty-four hours. To the expressed and filtered liquid twice its volume of alcohol was added, when a precipitate was produced, which, when dried in a vacuum over sulphuric acid, formed a dark-brown powder, and amounted to 3 grammes. This product, however, possessed none of the properties of an enzyme.

Extraction with Different Solvents.—Twenty-five grammes of the powdered seeds were successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:—

Petroleum (boiling point 35-50°) extracted	0.15 gramme =	0.6 per cent.
Ether.....	0.20 " =	0.8 "
Chloroform.....	0.15 " =	0.6 "
Ethy. Acetate	0.80 " =	3.2 "
Alcohol.....	1.70 " =	6.8 "
Total	3.0 grammes =	12.0 per cent.

EXTRACTION OF THE SEED WITH ALCOHOL.

For the purpose of a complete examination 53.72 kilogrammes of the ground seed were extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol the amount of concentrated extract was 17.66 kilogrammes.

DISTILLATION OF THE EXTRACT WITH STEAM.—SEPARATION OF AN ESSENTIAL OIL.

Two kilogrammes of the alcoholic extract (= 6084 grammes of seed) were distilled in a current of steam for several hours. The distillate, which contained a quantity of oil in suspension, was thoroughly extracted with ether, the ethereal liquid being then dried and the solvent removed. A small amount (3.6 grammes) of a pale yellow essential oil was thus obtained, which possessed an agreeable odour. It did not change in colour when kept for a prolonged period in a sealed tube, and gave no reaction for furfuraldehyde.

For the further determination of the characters of the essential oil a somewhat larger quantity was subsequently prepared. When distilled under diminished pressure (25 Mm.) it began to pass over at 118°, the greater portion distilling between 145° and 155°, and a final small portion at 155-170°. The oil possessed the following constants: $d_{20}^{20} = 0.9258$; $n_D^{20} = 1.51$ in 50 Mm. tube.

After the removal of the essential oil, as above described, there remained in the distillation flask a very dark-coloured aqueous liquid (A), together with a quantity of a soft resin (B). The resin was separated by filtration and thoroughly washed with hot water, the washings being added to the main portion of the aqueous liquid.

EXAMINATION OF THE AQUEOUS LIQUID (A).

The clear aqueous liquid was first repeatedly extracted with large quantities of ether, when it was observed that a crystalline substance was only slowly removed. In order to effect the removal of this substance as completely as possible, about twenty-five extractions with ether were necessary. The ethereal liquid had a bright green colour, which was evidently due to chlorophyll. After the removal of the solvent about 60 grammes of a green, crystalline product were obtained.

ISOLATION OF GALLIC ACID.

The above-mentioned crystalline product was first treated with hot chloroform, which removed only the chlorophyll. It was then found to be almost completely soluble in hot water, from which, on cooling, it separated in dark-brown needles. By repeated crystallisation from water, with the use of a little animal charcoal, the substance was finally obtained in almost colourless needles, which melted and decomposed at $241-243^{\circ}$, with evolution of gas. On analysis it gave the following result:—

0.4440, when heated at 100° , lost 0.0428 H_2O . $H_2O = 9.6$.
0.1367 of anhydrous substance gave 0.2480 CO_2 and 0.0458 H_2O .

$C = 49.5$; $H = 3.7$.

$C_7H_6O_5$, H_2O requires $H_2O = 9.6$ per cent.

$C_7H_6O_5$ requires $C = 49.4$; $H = 3.5$ per cent.

The substance was thus found to be gallic acid, with which it agreed in all its properties. Its identity was further confirmed by the preparation of the trimethyl derivative, which separated from water in colourless prisms, melting at $166-167^{\circ}$.

The aqueous mother-liquor remaining from the first crystallisation of the crude gallic acid was carefully examined, but it yielded only a further small amount of the same compound.

After extracting the aqueous liquid with ether as above described, it was shaken with chloroform, which, however, removed only traces of chlorophyll. The liquid was then repeatedly extracted with amyl alcohol, these extracts being

united and the solvent removed by distillation under diminished pressure. A highly coloured, viscous mass was thus obtained, which, after digesting with a little light petroleum, became solid, and when dried in a vacuum yielded 46 grammes of a chocolate-coloured powder. This product contained a large amount of tannin, since its dilute aqueous solution gave an intense bluish-black colouration with ferric chloride and an abundant precipitate with a solution of gelatin. The powder was further examined by extracting it in a Soxhlet apparatus with various solvents and heating the resulting extracts with 5 per cent. aqueous sulphuric acid. A small amount of gallic acid was thus obtained, but no sugar or other definite product was formed, and the material was therefore not glucosidic.

The aqueous liquid which had been completely extracted by the above-mentioned solvents was next treated with a slight excess of a solution of basic lead acetate. This produced an abundant brown precipitate, which was collected and thoroughly washed with water.

Basic Lead Acetate Precipitate.—This product was suspended in water, decomposed by hydrogen sulphide, the mixture filtered, and the dark-coloured filtrate concentrated under diminished pressure. The liquid, which gave a strong reaction for tannin, was kept for some weeks, when several grammes of a light brown solid were deposited. This was collected and treated with hot water, which, however, removed only small amounts of tannic and gallic acids. The undissolved portion was found to be soluble in hot pyridine, from which it separated in small needles, which did not melt at a temperature of 330° . A much larger amount of the same substance was subsequently obtained from the alcohol extract of the resin, and its composition and characters will be recorded in connection with the latter.

Filtrate from the Basic Lead Acetate Precipitate.—The filtrate from the lead compound, which had a pale yellow colour, was treated with hydrogen sulphide for the removal of the excess of lead, the mixture filtered, and this filtrate concentrated under diminished pressure to the consistency of a syrup. It contained a large quantity of sugar, since it readily reduced Fehling's solution and yielded *d*-phenyl-glucosazone, melting at 207° . The syrupy liquid, which was lævorotatory, deposited nothing on long keeping, and a portion of it was therefore treated with acetic anhydride, but no crystalline acetyl derivative of the sugar could be

obtained. Inasmuch as it has been stated (*loc. cit.*) that Jambul seeds contain quercitol, the acetylated syrup was specially examined for the presence of acetylquercitol, but no trace of such a compound could be detected. A portion of the syrup was heated with aqueous 5 per cent. sulphuric acid, but it yielded nothing definite by this treatment. When heated with a caustic alkali it developed ammonia, indicating the presence of protein substances.

EXAMINATION OF THE RESIN (B).

The soft, resinous material, which had been separated from the aqueous liquid, as previously described, was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture successively extracted in a Soxhlet apparatus with light petroleum, ether, chloroform, ethyl acetate, and alcohol.

PETROLEUM EXTRACT OF THE RESIN.

This extract, after the removal of the solvent, was a dark coloured, thick, oily mass, and amounted to 70.3 grammes. It was dissolved in ether, and the ethereal liquid extracted successively with dilute aqueous ammonium carbonate, sodium carbonate, and sodium hydroxide. The two alkaline carbonates removed only traces of amorphous material, while the caustic alkali extracted 35.3 grammes of an almost black resin, from which, however, nothing definite could be isolated.

After extracting the ethereal liquid with alkalies the ether was removed, and the fatty residue heated with an alcoholic solution of potassium hydroxide. To the alkaline mixture water was added, and the alcohol then removed by distillation in a current of steam. The alkaline, aqueous liquid was subsequently extracted with ether, the ethereal liquid being washed, dried, and the solvent evaporated, when a quantity (8.8 grammes) of a dark-coloured, semi-solid product was obtained. This was purified by distillation under diminished pressure, when a thick, yellow oil was obtained, which, on the addition of a little alcohol, deposited a very small amount of a colourless solid, melting at 78-80°. The solid substance apparently consisted of a mixture of an alcohol and a hydrocarbon, but the amount was too small to permit of their separation. After the removal of the solid substance there remained a viscid, oily product, which evidently contained traces of a phytosterol, since it gave the colour reactions of that class of compounds.

IDENTIFICATION OF THE FATTY ACIDS.

The alkaline liquid, after the removal of the unsaponifiable substances by extraction with ether, was acidified, and again extracted with ether. A quantity of fatty acids was thus obtained, which were purified by distillation under diminished pressure, and then amounted to 5.5 grammes. These acids were converted into their lead salts, and the latter digested with ether, when a portion dissolved. Both the insoluble and soluble portions were decomposed by hydrochloric acid, and the free fatty acids recovered.

The Liquid Acids.—These acids were purified by distillation under diminished pressure, and amounted to 4 grammes. An analysis and determination of the iodine value gave the following results:—

0.1214 gave 0.3408 CO_2 and 0.1268 H_2O . $\text{C} = 76.6$; $\text{H} = 11.6$.

0.2505 absorbed 0.3521 iodine. Iodine value = 140.6.

$\text{C}_{18}\text{H}_{34}\text{O}_2$ requires $\text{C} = 76.6$; $\text{H} = 12.1$ per cent.

Iodine value = 90.1.

$\text{C}_{18}\text{H}_{32}\text{O}_2$ requires $\text{C} = 77.1$; $\text{H} = 11.4$ per cent.

Iodine value = 181.4.

It may be concluded from these results that the liquid acids consist of a mixture of oleic and linolic acids, the latter preponderating.

The Solid Acids.—The portion of solid acid, after recrystallisation from glacial acetic acid, formed pearly leaflets, melting at $53-55^\circ$, and amounted to 1 gramme.

0.1258 gave 0.3493 CO_2 and 0.1426 H_2O . $\text{C} = 75.8$; $\text{H} = 12.6$.

$\text{C}_{16}\text{H}_{32}\text{O}_2$ requires $\text{C} = 75.0$; $\text{H} = 12.5$ per cent.

$\text{C}_{18}\text{H}_{36}\text{O}_2$ requires $\text{C} = 76.1$; $\text{H} = 12.7$ per cent.

The solid acids thus appear to consist of a mixture of palmitic and stearic acids, in approximately equal proportions.

ETHER AND CHLOROFORM EXTRACTS OF THE RESIN.

These extracts amounted to 37 grammes and 0.4 gramme respectively. They were dark-coloured, amorphous products, and nothing definite could be isolated from them.

ETHYL ACETATE EXTRACT OF THE RESIN.

This extract, after the removal of the solvent, was a dark-coloured resinous product, amounting to 5 grammes. It was repeatedly digested with small amounts of cold alcohol, when most of the resinous material dissolved, leaving 0.7 gramme of a greenish, amorphous substance. The latter

was found to be identical with the substance isolated from the aqueous liquid (A) by treatment with basic lead acetate, and, as indicated in that connection, was obtained in much larger amount from the alcohol extract of the resin. Nothing definite could be obtained from the above-mentioned resinous material, even after heating with 5 per cent. sulphuric acid in aqueous alcohol.

ALCOHOL EXTRACT OF THE RESIN.

This extract was a dark-coloured solid, and amounted to 33 grammes. It was repeatedly digested with small amounts of cold alcohol, which removed a quantity of highly-coloured resinous material, leaving a very sparingly soluble, brown, amorphous substance, amounting to about 18 grammes.

The resinous material was heated with 5 per cent. sulphuric acid in aqueous alcohol, but it yielded nothing definite. After the removal of the sulphuric acid the resulting aqueous liquid reduced Fehling's solution, but no osazone could be prepared from it, and the material was evidently not glucosidic.

ISOLATION OF A NEW PHENOLIC SUBSTANCE, JAMBULOL, $C_{16}H_8O_4(OH)_5$.

The above-mentioned, very sparingly soluble portion of the alcohol extract of the resin was first boiled for several hours with alcohol, which removed a further small amount of amorphous colouring matter. The residue was then dissolved in boiling pyridine, when a very dark-coloured solution was obtained, from which, on cooling, a substance separated in well-formed, dark-coloured needles. These crystals were collected, and found to contain pyridine of crystallisation, for on exposure to the air for a short time, or immediately on covering them with alcohol, they were converted into a light-brown powder, which, however, retained traces of pyridine very tenaciously.

For the purpose of analysis a portion of the substance was recrystallised five times from pyridine, then treated with boiling alcohol to remove traces of the solvent, and finally dried at 125°.

0.1736 gave 0.3552 CO_2 and 0.0349 H_2O . $C = 55.8$; $H = 2.2$.
 $C_{16}H_8O_9$ requires $C = 55.8$; $H = 2.3$ per cent.

The substance is very sparingly soluble in a dilute solution of sodium carbonate, but dissolves readily in dilute aqueous potassium hydroxide, forming an intensely yellow liquid. On acidifying the alkaline solution the original substance is

precipitated. In order to prove its homogeneity, the substance was again crystallised twice from pyridine, then dissolved in a cold, dilute solution of potassium hydroxide, the liquid filtered, acidified, and the resulting precipitate treated with boiling water for the removal of any impurity that might be soluble in the latter. The substance was then again analysed.

0.1804 gave 0.3672 CO_2 and 0.0380 H_2O . $\text{C} = 55.5$; $\text{H} = 2.3$.
 $\text{C}_{16}\text{H}_8\text{O}_9$ requires $\text{C} = 55.8$; $\text{H} = 2.3$ per cent.

From the above results it is evident that the substance has the empirical composition $\text{C}_{16}\text{H}_8\text{O}_9$, although possibly possessing double the molecular weight indicated by this formula. On account of its very sparing solubility this determination could not be accomplished. As the compound has not heretofore been described, it is proposed to designate it *Jambulol*, with reference to the source from which it has been obtained and its phenolic properties.

Jambulol is insoluble, or nearly so, in all the usual organic solvents, with the exception of pyridine. As already noted, it separates from its solution in the latter in brown crystals which contain solvent of crystallisation. When anhydrous, it forms a brown powder which does not melt even at 340° . It dissolves in concentrated sulphuric acid, giving a yellow solution. When a trace of the substance is gently heated with dilute nitric acid a fugitive deep red colour is produced. It contains no methoxyl group.

Penta-acetyljambulol, $\text{C}_{16}\text{H}_3\text{O}_9(\text{CO}\cdot\text{CH}_3)_5$.—When jambulol is boiled with acetic anhydride it readily yields an acetyl derivative which is very sparingly soluble in the acid anhydride, but dissolves readily in boiling nitrobenzene. From the latter, on cooling, it separates in pale brown leaflets, which melt and decompose at about 335° .

0.1506 gave 0.3108 CO_2 and 0.0430 H_2O . $\text{C} = 56.3$; $\text{H} = 3.2$.
 $\text{C}_{16}\text{H}_3\text{O}_9(\text{CO}\cdot\text{CH}_3)_5$ requires $\text{C} = 56.3$; $\text{H} = 3.2$ per cent.

It is evident from this result that the above-described compound contains five acetyl groups. An attempt was made to confirm this by heating the compound with acids and weighing the residual hydrolytic product, but owing to the insolubility of both the acetyl derivate and the parent substance concordant results could not be obtained. Thus, on boiling the compound for six hours with concentrated hydrochloric acid, the loss in weight was 19.2 per cent., whereas on heating at 100° for eight hours with sulphuric acid of about 80 per

cent. strength the loss was 36.3 per cent. The calculated decrease in weight by the elimination of five acetyl groups is 37.9 per cent.

Pentabenzoyljambulol, $C_{16}H_{13}O_9(CO \cdot C_6H_5)_5$.—This derivative was readily obtained by adding to a solution of the jambulol in hot pyridine an excess of benzoyl chloride, when, on cooling, the benzoyl derivative separates in small, colourless plates. After recrystallisation from pyridine the compound begins to soften at 328° , and melts, without decomposition, at 333° .

0.1670 gave 0.4334 CO_2 and 0.0500 H_2O . $C = 70.8$; $H = 3.3$.
 $C_{16}H_{13}O_9(CO \cdot C_6H_5)_5$ requires $C = 70.8$; $H = 3.2$ per cent.

The analysis of this compound has afforded further evidence of the presence of five hydroxyl groups in jambulol. Attempts were made to prepare a pentamethyl derivative, but the product obtained was insoluble in all the ordinary solvents, and, therefore, could not be purified.

SUMMARY AND CONCLUSIONS.

The results of the present investigation of Jambul seeds, from *Eugenia Jambolana*, Lam. (*Syzygium Jambolanum*, DC.), may briefly be summarised as follows:—

A preliminary examination of the seed showed them to contain neither an alkaloid nor an enzyme, but an abundance of starch and tannin.

An alcoholic extract of the seed, when distilled in a current of steam, yielded a small amount of a pale yellow essential oil, which possessed the following constants:—

$d_{20^\circ/20^\circ} = 0.9258$; $n_D^{20} = 1.51$ in a 50 Mm. tube.

The portion of the alcoholic extract which was soluble in water contained considerable amounts of tannic and gallic acids, together with a sugar, which yielded *d*-phenylglucosazone (m.p. 207°), and a small amount of a phenolic substance, which was obtained in much larger quantity from the resin, as noted below.

The portion of the alcoholic extract which was insoluble in water consisted of a soft resin, amounting to about 2.4 per cent. of the weight of the seed. From this material, by its successive extraction with various solvents, the following products were obtained:—A mixture of fatty acids, consisting of palmitic, stearic, oleic, and linolic acids; a very small amount of a solid (m.p. $78-80^\circ$), which, apparently, was a mixture of an alcohol and hydrocarbon; and a trace of a phytosterol. The

most interesting constituent of the resin, however, is a new phenolic substance possessing the empirical formula $C_{16}H_8O_9$, which has been designated *Jambulol*. This substance is a light-brown powder, which is insoluble, or nearly so, in the usual organic solvents, but separates from its solution in pyridine in brown needles containing solvent of crystallisation. The following crystalline derivatives of the substance have been prepared:—*Penta-acetyljambulol*, $C_{16}H_3O_9(CO \cdot CH_3)_5$, which forms pale brown leaflets, melting and decomposing at about 335° , and *pentabenzoyljambulol*, $C_{16}H_3O_9(CO \cdot C_6H_5)_5$, which was obtained in small, colourless plates, melting, without decomposition, at 333° .

The statement of Pottiez (*Ann. de Pharm.*, 1899, 5, 491-493) that Jambul seeds contain quercitol and cinnamic acid could not be confirmed, for there was no evidence of the presence of either of these substances in the seeds which have now been examined.

With regard to the very indefinite product which was brought to notice some years ago by Boersch under the name of "Antimellin" (*Apoth. Zeit.*, 1899, p. 510), and was said to be a glucoside, it need only be stated that no substance of a glucosidic nature could be found by us in Jambul seeds.





