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(*BRUCEA SUMATRANA*, ROXB.)

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The name Kô-sam, by which the seeds under investigation are known, appears to be of Chinese origin, although in the medical literature of China and Cochin China it is sometimes written Khô-sam. In the language of the latter country Khô-sam is stated to signify gentian. The botanical name of the plant producing the seeds is *Brucea sumatrana*, Roxb., a shrub growing about two mètres in height, belonging to the natural order of Simarubaceæ. An excellent botanical description of the genus *Brucea*, with illustrations of the flowers and fruits of the two more important species, *B. antidysenterica* and *B. sumatrana*, is given by Engler in 'Die natürlichen Pflanzenfamilien,' Theil III., Abtheil. 4, p. 220. Leipzig, 1896. In this work the following five species of *Brucea* are recorded, with indications of their respective habitats and uses:—(a)—*B. antidysenterica*, Lam., Abyssinia; *B. paniculata*, Lam., Tropical West Africa and Upper Guinea; *B. tenuifolia*, Engl., Usambara; *B. mollis*, Wall., Khasia. (b)—*B. sumatrana*, Roxb., from Farther India through the Indian Archipelago and Cochin China to Australia and the Philippines. "All the species are very bitter. The bark and the fruits of *B. antidysenterica* are used with success in Abyssinia for diarrhœa and

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fever. All the parts of *B. sumatrana* are esteemed in the East Indies as a stomachic tonic, and are also used for diarrhœa, intermittent fever, and worms."

A species of the botanically closely allied genus *Picrasma* is referred to in the 'Pharmacographia Indica,' Vol. 1, p. 287, as follows:—"*Picrasma quassioides*, Benn., is a small tree or large bush indigenous to the sub-tropical Himalaya and South China. It is recognised by the Indian Pharmacopœia under the name of *Brucea* (*Nima*) *quassioides*, and the bark, which is very bitter, has been recommended as a febrifuge." The wood of this plant has been assumed to contain quassin, but no complete chemical examination has as yet been made of it.

During the past few years Kô-sam seeds have been brought somewhat prominently to notice, on account of their reputed value as a remedy in dysentery. Dybowski, in some papers published in the *Revue des Cultures Coloniales*, Paris (Tome VI., January 5 and April 5, 1900), has particularly called attention to these seeds, and quotes Dr. Mougeot, of Saigon, as having discovered that five or six of the kernels, taken in the morning, crushed with crumb of bread, are a sovereign remedy for the most pernicious dysenteries of tropical countries. Dr. Mougeot has reported that out of 879 cases treated by him, 799 were completely cured in from three to six days, while fifty-seven of these cases required fifteen days. The popular interest in this remedy is shown by the following notice, which appeared in a recent issue of the *Rangoon Gazette*:—"The ravages which dysentery caused among Europeans in the Tropics before the disease was as well understood as at present were terrible, and now, although it is affirmed by many medical men that dysentery if taken in time is practically always amenable to treatment, and but seldom fatal even when for a short time neglected, yet the number of deaths caused by it, especially among children, is still very great. It is most certainly good news to hear that a new cure for it has been discovered in the seeds of the *Brucea sumatrana*, which are said to be as much a specific for it as quinine is for malaria."

The most extended notices that have thus far appeared relating to the botanical and chemical characters of Kô-sam seeds are contained in a series of papers published in the previously mentioned *Revue des Cultures Coloniales*, Paris, 1900, Nos. 47, 48, and 50, short abstracts of which have been given in the *Pharm. Journ.*, 1900, **64**, pp. 463, 687. The authors of the present paper have, nevertheless, deemed it desirable to consult the original publications, in order to ascertain the evidence upon which the statements relating to the chemical constituents of the seeds are based. As the publications referred to are, to a considerable extent, of a controversial nature, the following references to them are restricted to such details as have a direct bearing upon the results of our investigation, or which have seemed necessary for the more satisfactory elucidation of the subject.

Professors Heckel and Schlagdenhauffen (*loc. cit.*, No. 47, pp. 97-104) appear to have been the first to make a chemical examination of Kô-sam seeds. They state that from the close botanical relationship of *Brucea* with *Quassia amara* and *Quassia simaruba* they were at the beginning led to conclude that the active principle of the seeds would prove to be quassin —the bitterness being of the same nature—and that their chemical analysis had confirmed this supposition. The results of their investigation were given in the following order:—

(1) By extraction with carbon disulphide they obtained 57·14 per cent. of a fatty oil, having a yellow colour, a slightly bitter taste—attributed to a trace of quassin—and a density of 0·912.

(2) By subsequent abstraction with chloroform 0·483 per cent. of solid matter was obtained. On treating this with water a bitter principle was dissolved, leaving an insoluble substance of a resinous nature. The bitter principle is stated to have the characters of quassin, a conclusion which seems to depend upon their observation that when the aqueous solution was evaporated on a watch-glass to dryness with a drop of dilute hydrochloric acid, needle-shaped crystals were obtained, as in the case of quassin when tested under the same conditions. It was furthermore noted that by the action of concentrated sulphuric acid on these crystals and on pure quassin, identical results were obtained. As they subsequently remark that quassin suffers no change of colour in contact with concentrated sulphuric acid, the evidence of identity was of a purely negative character.

(3) By subsequent extraction with alcohol 6·972 per cent. of solid matter was obtained. On treating this with water it was resolved into a soluble and an insoluble portion. The latter was partly composed of proteid matter, while the soluble portion also contained some nitrogenous principles of the same class, together with a certain quantity of glucose and saccharose. In the 6·972 parts of total residue they found 3·199 parts of albuminoid matter, the remainder being stated to consist of sugar and quassin, associated with another bitter principle and

saponin. They separated these principles by treating the powdered alcoholic extract with amyl alcohol. It is stated that on evaporating the latter liquid, and taking up the residue with water, the quassin is first dissolved, and some time afterwards, the second bitter principle. The first solution, which was very bitter, they evaporated to dryness, and then treated the residue with chlorine water by evaporating to dryness with the latter on a water-bath. No change of colour was observed under these conditions or when treated with concentrated sulphuric acid, and these negative characters were considered to appertain to quassin. Furthermore, as the residue was very bitter, and, under the previously mentioned conditions, formed needle-shaped crystals, they state that one may conclude, without fear of contradiction, that quassin is present. As to the other bitter principle, less readily soluble in water than quassin, it is said to have the property of becoming coloured blue when evaporated on a water-bath in the presence of a little chlorine water, and to be coloured violet by concentrated sulphuric acid. These reactions were compared with those afforded by saponin, and, although not agreeing completely, the presence of the latter was considered probable on account of the frothing of the liquid when shaken with water. Notwithstanding the above affirmations, it is remarked that, as the quantity of material operated upon was small, they give these results only as indications, and propose to repeat the experiments with one or two kilos of the seed. The amount of material actually used is nowhere stated.

(4) On finally extracting the seeds with water they obtained from the aqueous liquid a residue of gummy matter amounting to 20·5 per cent. The material left after extraction with the above solvents was found to contain an amount of insoluble albuminoid matter corresponding to 5·937 per cent. in the original seeds.

It will be seen from the results of the experiments of Heckel and Schlagdenhauffen, as above outlined, that they did not isolate any definite active principle from Kô-sam seeds, or even obtain it in such a state of purity as to permit of its satisfactory identification. In fact, they have not recorded in their paper a single analysis of an individual substance. It is, therefore, somewhat surprising that they should repeatedly and so positively affirm that the seeds in question contain quassin, as may be seen from the following sentences, which are literally quoted :—

" Comme on a pu le constater par l'analyse précédente, la graine de Kô-sam renferme comme principe dominant la quassine, ainsi qu'il était permis de le prévoir."

"En somme, le Kô-sam doit son action à la quassine, et il y a bel âge que l'emploi de ce principe actif est populaire en Europe par l'usage quotidien que l'on en fait dans le *Quassia amara*, si bien que le nouveau remède contre la dysenterie de M. Dybowski n'a rien de nouveau, les Abyssins l'employant de temps sans doute immémorial."

"Cette étude aura pour résultat de mettre les faits relatifs à ce pretendu nouveau medicament à leur veritable point scientifique, et, dès lors, les médecins coloniaux pourront poursuivre leurs expériences sur le Kô-sam en toute connaissance de cause."

The conclusions of Heckel and Schlagdenhauffen have elicited some comments from Dybowski (*loc. cit.*, No. 48, pp. 129-131), but, unfortunately, these are not altogether consistent, and, therefore, only tend to produce further confusion. Dybowski remarks that Schlagdenhauffen has made of Kô-sam a very complete analysis and a conscientious study, but that the conclusions Heckel draws therefrom are totally wanting in scientific precision. In the chemical portion of their paper they are said to err in two respects, which he will indicate. It appears to him, and an examination made by Bertrand likewise leads him to presume, that the fruits of *Brucea sumatrana* contain quassin. But to be able to make such an affirmation, and not give an *à priori* conclusion, it would have been better, in his opinion, to precisely characterise this substance by an elementary analysis, and by determining its crystalline form, melting point, solubility, etc. None of these data have been recorded, and the negative characters, together with the bitterness of the substance, to which Heckel appears to attach so much importance, and upon which he bases his conclusion, are considered inadequate and not to permit of any scientific deduction. Dybowski thinks that Schlagdenhauffen has overlooked the most interesting point in the study of Kô-sam, and that as he has mentioned the occurrence of "another bitter principle" he should have searched for this, the rest being of little importance. He then proceeds to state that Bertrand has isolated a glucoside having extremely active properties, and that it is the discovery of this substance, not the presence of quassin, which entitles Kô-sam to be regarded as a new remedy for dysentery.

A still later publication (*loc. cit.*, No. 50, pp. 196-201) embraces a conjoint study of Kô-sam, in which the botanical characters are considered by Dybowski, the chemical composition by Bertrand, and the physiological action by Phisalix. Although Bertrand, in his very brief communication, has indicated that he only gives the results of a preliminary investigation, his conclusion as to the glucosidal nature of the assumed active principle is not supported by any satisfactory experimental evidence of its correctness. After noting that the seeds contain 19·5 per cent. of fatty oil, he states that it is in the substances extracted by alcohol that one finds the active principle—kosamine, as he proposes to call it. This is said to possess extraordinary bitterness, and to be related to the glucosides, since it afforded a reducing sugar, probably glucose, when boiled with dilute acids, but the products of its hydrolysis were not determined.

Kosamine is described as being practically insoluble in most of the anhydrous solvents, such as petroleum ether, carbon disulphide, chloroform, &c., but to dissolve readily in water and in aqueous alcohol. It is not precipitated by either neutral or basic lead acetate, and it was by means of these properties that he was able to separate it from the various other substances accompanying it in the seeds, such as oil, resin, acids, &c. The subsequent remark, however, is somewhat significant, that he does not insist at present upon the chemical properties of kosamine, hoping to return to the subject when a sufficient quantity of the fruits shall have been obtained to enable him to complete the study. He also notes that he considered it more useful to determine the physiological properties of this active principle than to satisfy his curiosity as a chemist by reactions which are always destructive. Inasmuch as Kô-sam seeds have been observed by us to contain a considerable amount of a reducing sugar, it would be of some interest to learn how the so-called kosamine had been separated in a state of sufficient purity to even permit of its identification as a glucoside. No description, however, of this substance is given which would indicate that it was obtained in any other form than in solution, and it is recorded by Phisalix that he received it from Bertrand in this form for his physiological experiments.

The uncertainty respecting the individuality of the substance designated as kosamine naturally detracts from the importance and interest which the results of the study of its physiological action would otherwise possess, and it, therefore, does not seem necessary that the properties attributed to it should be considered here.

With this survey of the subject we proceed to give the results of our own investigation.

EXPERIMENTAL.

The material for this investigation was obtained through the kindness of Mr. H. N. Ridley, Director of the Botanic Gardens of the Straits Settlements, Singapore, who had specially procured a quantity of the seeds for Messrs. Burroughs, Wellcome and Co., of London. We had thus the assurance that the material was perfectly authentic.

The seeds are relatively small, the weight of thirty being about 1 Gm. When crushed, they develop a peculiar cheese-like odour, reminding of some of the fatty acids. The kernel of the seed possesses an intensely and persistently bitter taste. Before proceeding to a complete examination the following preliminary experiments were made :—

Test for Alkaloid.—10 Gm. of the powdered seeds were digested with Prollius' fluid and filtered. The filtrate left an oily residue, which, when treated with acidulated water, gave no reaction for alkaloid.

Extraction with various Solvents.—In order to ascertain the general character of the constituents, 50 Gm. of the seeds were extracted successively in a Soxhlet apparatus with the following liquids. After removing the solvents, the residual extracts were kept in a water-oven until of constant weight.

(1) Petroleum (b.p. 40—50°)	gave	10·88 Gm.	= 21·76	per cent.
(2) Ether	„	0·17 „	= 0·34	„
(3) Chloroform	„	0·65 „	= 1·30	„
(4) Alcohol	„	1·92 „	= 3·84	„
			27·24	„

The petroleum extract was a light yellow oil, very sparingly soluble in cold, but soluble in hot alcohol, and separating on cooling. The ether and chloroform extracts were resinous, and of a greenish-brown colour. When warmed with a little water and filtered, the solution from the ether extract was only faintly coloured by ferric chloride, while that from the chloroform extract gave a deep purple-brown colour. The alcoholic extract was dark brown, and of a resinous nature. When taken up with a little hot water and filtered, the aqueous liquid gave a deep olive-green colour with ferric chloride, was coloured intensely yellow by alkalis, and reduced Fehling's solution. When acidulated it gave reactions with the usual alkaloid reagents, which were evidently due to soluble proteid substances.

Test for an Enzyme.—100 Gm. of the ground seeds were macerated with water at the ordinary temperature for three days, and to the filtered liquid about three times its volume of alcohol was added. After standing a few hours, the flocculent precipitate was filtered off, washed with a little alcohol, and dried over sulphuric acid. The yield of product was 3 Gm. It was a grayish powder, which dissolved readily in water, forming a brown solution. The latter, when acidulated with acetic acid, was rendered slightly turbid by the usual proteid reagents. When a little crystallised amygdalin was brought into an aqueous solution of the substance, or into a mixture of the crushed seeds with water, the odour of benzaldehyde was rapidly developed. In contact with potassium myronate the odour of mustard oil could not be so positively recognised. Although the substance obtained by the above method was necessarily impure, it is evident that the seeds contain a hydrolytic enzyme.

Quantitative Determination of Tannin.—As most of the drugs employed in the treatment of dysentery contain more or less tannin, and as the presence of such a substance had been indicated by the preceding experiments, it seemed desirable to determine the amount contained in Kô-sam seeds. 20 Gm. of the powdered seeds were extracted with successive small portions of boiling water, and the cold, filtered liquid diluted to the volume of 250 C.c. 50 C.c. of this liquid when evaporated, and the residue dried in a water-oven till of constant weight, afforded 0·600 Gm. of extract. Another portion of the liquid was allowed to macerate with hide powder for two days. 50 C.c. of this filtered liquid, when evaporated and the residue dried, as before, till of constant weight, afforded 0·527 Gm. of extract. From these results the amount of substance absorbed by the hide powder, which is to be regarded as tannin, corresponds to 1·8 per cent. of the seeds. It was observed that, even after prolonged treatment with hide powder, the liquid still gave a greenish colour with ferric salts, but this was due, as will be seen later, to a substance differing essentially from tannin.

Separation of the Constituents of the Seeds.

After the preceding experiments a larger quantity of material was operated upon, as follows:—4 kilos of the finely-ground seeds were extracted in a percolator with cold alcohol, but as this removed but a small proportion of the fatty oil it was followed by light petroleum, which removed a large amount, and the extraction was then finally completed with alcohol. After distilling the petroleum and alcohol from these liquids, the residues were combined, a little water added, and steam passed through the mixture, in a distilling flask, in order to separate any volatile substances. The distillate had a strongly acid reaction. It was first shaken out with ether, and, after the removal of the latter, there remained a small amount (about 1·5 Gm.) of a brown, limpid liquid, which had the characteristic odour of the bruised seeds. On distillation, under ordinary pressure, it passed over between 100 and 190° as a faintly yellow liquid, having an odour suggestive of the ethyl esters of butyric and valeric acids. It was accordingly hydrolysed with an alcoholic solution of potassium hydroxide, steam distilled, and the distillate, which contained some oily drops, extracted with ether. After drying the latter liquid, and removing the ether, a very small amount of an oil was obtained, which had a somewhat aromatic odour, but which could not be further examined. The alkaline liquid remaining from the hydrolysis was acidulated with sulphuric acid and steam distilled. The clear acid distillate was treated with barium carbonate, filtered and evaporated, when a very small amount of a light yellow syrup was obtained, which afforded the reactions indicative of the barium salt of a butyric acid. The original aqueous distillate, which had been extracted with ether, was neutralised with barium carbonate. After filtering and evaporating, a syrupy liquid was obtained, which, on standing for some time, formed a crystalline mass. Its aqueous solution gave with silver nitrate a dense white precipitate which soon became black, owing to reduction, thus indicating the presence of formic acid, which was apparently associated with a very small amount of acetic acid. After separating the volatile substances, there remained in the distilling flask a mixture consisting of a dark coloured oily layer and a lower aqueous liquid. These were separated, the oily layer was diluted with light petroleum, filtered, and the petroleum removed by distillation.

(1) *Fatty Oil.*

The total amount of oil obtained was 803 Gm., or 20 per cent. of the original drug. It had a dark green colour, due to the presence of chlorophyll. Its density at 17° was 0·917. When a little of it was shaken with a mixture of nitric acid and water, and subsequently heated for a few minutes in a water-bath, it formed, after standing for a few hours, a soft, brownish-yellow mass. 725 Gm. of the oil were hydrolysed by boiling with 185 Gm. of potassium hydroxide, previously dissolved in alcohol. The alcohol was then distilled off, the residual strongly alkaline soap mixed with sand, dried, and extracted in a Soxhlet apparatus with light petroleum.

(*a*) *Neutral Constituents of the Oil.*—After the removal of the petroleum, a yellow, viscid oil was obtained, which had a peculiar odour. This was dissolved in hot glacial acetic acid, and, on cooling, a quantity of minute needle-shaped crystals was deposited. This crystalline substance was collected, washed with a little glacial acetic acid, and dried on a porous tile. It was then dissolved in boiling ethyl acetate, from which, on cooling, it separated in beautiful glistening leaflets, and when dried on a porous tile formed a lustrous mat. It melted at 67-68°. When again crystallised from ethyl acetate its melting point remained unchanged, and it was not affected by a further crystallisation from absolute alcohol.

0·1390 Gm. gave 0·4310 CO_2 and 0·1845 H_2O. C = 84·6; H = 14 7.

$C_{31}H_{64}$ requires C = 85·3; H = 14·7 per cent.

This substance was thus found to be a hydrocarbon. It was nearly insoluble in the usual organic solvents at the ordinary temperature, but more readily soluble in the hot liquids. When dissolved in dry ether it did not decolorise a solution of bromine in the same solvent. In all its properties it appears to be identical with the saturated hydrocarbon, hentriacontane, $C_{31}H_{64}$ (m.p. 68·1°), which has hitherto only been found in nature in beeswax. (Compare Beilstein's 'Handbuch der org. Chemie,' 1, p. 107.)

Although solid hydrocarbons, of both the aliphatic and the aromatic series, have been found in various essential oils (*Chem. Centralblatt*, 1902, II., p. 1117), comparatively few have been more directly isolated from plants, and these do not appear to have been very precisely identified. (Compare *Amer. Journ. Pharm.*, 1888, p. 321.)

The glacial acetic acid mother liquor from which the hydrocarbon was first obtained, deposited, on standing, a further amount of crystalline product, which was collected, dried, and recrystallised in the first instance from the first ethyl acetate mother liquor from the hydrocarbon, and finally from absolute alcohol. As thus obtained it melted between 70 and 85°, and, though obviously a mixture, it indicated some substance having a higher melting point than the hydrocarbon. The glacial acetic acid mother liquor, after standing for several days, afforded a still further amount of a white, crystalline product, which melted indefinitely between 110 and 130°.

The ethyl acetate mother liquor afforded, on evaporation to dryness, a crystalline residue, which melted like the above product between 110 and 130°. All the higher melting substance was then combined and recrystallised many times from absolute alcohol. The final product, representing the portion least soluble in alcohol, melted indefinitely between 112 and 129°. It was analysed.

0·1304 Gm. gave 0·3937 CO_2 and 0·1438 H_2O. C = 82·3 ; H = 12·2

This substance gave with chloroform, together with acetic anhydride and sulphuric acid, a colour reaction resembling that shown by the cholesterols and allied substances. The cholesterols, $C_{26}H_{44}O$, require, however, C = 83·9 and H = 11·8 per cent. The discrepancy in the figures could not be attributed to the presence of the hydrocarbon, which might seem likely from the melting point and a consideration of the very sparing solubility of the hydrocarbon in alcohol, since this requires 85·3 per cent. of carbon.

The several alcoholic mother liquors obtained in the course of separating the fraction melting at 112-129° were ultimately combined and concentrated to a very small volume. From this liquid there separated a fraction, exceeding in amount the preceding one, which melted at 130-131°. It was recrystallised from absolute alcohol without appreciably altering its melting point. On analysis:—

0·0412 Gm. gave 0·1240 CO_2 and 0·0456 H_2O. C = 82·1 ; H = 12·3.
0·1228 Gm. gave 0·3699 CO_2 and 0·1307 H_2O. C = 82·1 ; H = 11·8.

When again crystallised from absolute alcohol it melted at 130-132°. On analysis:—

0·1270 Gm. gave 0·3868 CO_2 and 0·1376 H_2O. C = 83·0 ; H = 12·0 ;
$C_{20}H_{34}O$ requires C = 82·8 ; H = 11·7 per cent.

A determination of its specific rotation, in chloroform, gave the following result:—

$\alpha_D = -0°54'$; c = 2·388 ; l = 1 dcm. Hence $[\alpha]_D^{23°} = -37·7°$.

When a small amount of the substance was dissolved in about 2 C.c. of chloroform, 20 drops of acetic anhydride added, and subsequently one drop of concentrated sulphuric acid introduced, a transient rose-pink colour was produced, changing successively to blue, green, and, on long standing, to brown.

Several substances corresponding to the formula $C_{20}H_{34}O$, which in their general character appear to be related to the cholesterols, have already been found in various plants, and designated respectively as quebrachol, cupreol, and cinchol or cinchocerotin. (Compare Beilstein's 'Handbuch der org. Chemie,' 3 edit., Band II., p. 1068.) It is therefore probable that, like the cholesterols, they constitute a distinct class of substances.

(*b*) *Acid Constituents of the Oil.*—The soap resulting from the hydrolysis of 725 Gm. of oil, and which, after being dried, had been thoroughly extracted with light petroleum for the removal of the neutral constituents, as previously described, was then dissolved in hot water. The aqueous solution was acidulated with hydrochloric acid, when the acids separated in the form of a semi-solid layer on the surface of the liquid. They were taken up with ether, the solution washed with water, dried with calcium chloride, and the ether removed. The residual oily liquid was then distilled under a pressure of 50 Mm. It commenced to distil at 240°, but only a few drops passed over up to 260°, between which point and 270° the remainder distilled as a light yellow oil. The most constant point during the distillation was between 262 and 265°, when but little remained in the distilling flask.

The iodine value of these mixed fatty acids, as determined by the Hübl method, was found to be 97·2. As oleic acid has a theoretical iodine value of 90·07, this result indicated the presence of a still more unsaturated acid, such as linolic acid, which has a theoretical iodine value of 181·43, especially as it was subsequently proved that some saturated acids were also present.

After standing for several hours the distilled oil formed a paste, owing to the separation of a quantity of crystals. This paste was then filtered with the aid of a pump, and the crystalline portion well pressed between folds of filter paper, in order to more completely free it from adhering oil. It was subsequently dissolved in warm 90 per cent. alcohol, and set aside to crystallise. The recrystallised acid was obtained from this alcoholic solution in three successive crops. The first crop was dissolved in light petroleum, and the solution soon deposited a quantity of beautiful glistening leaflets, melting at 68-69°. A solution of the acid in dry ether did not decolorise a solution of bromine in the same solvent. For analysis a portion was again crystallised from light petroleum, but without the melting point becoming changed.

0·1010 Gm. gave 0·2795 CO_2 and 0·1176 H_2O. C = 75·5; H = 12·9

0·1065 Gm. gave 0·2954 CO_2 and 0·1242 H_2O. C = 75·6; H = 12·9

$C_{18}H_{36}O_2$ requires C = 76·1; H = 12·7 per cent.

It is evident that this substance was stearic acid.

The alcoholic mother liquor from the first crop of crystals afforded a smaller second crop, which was recrystallised from 90 per cent. alcohol, when it melted at 55-56°. This was further treated in conjunction with a second batch of crystalline product which had separated from the oily mixture of acids after the above first filtration. The alcoholic filtrate from the second crop of crystals finally deposited a third crop. This was small in amount, handsomely crystalline, and melted at 53-54°. After recrystallisation from 90 per cent. alcohol it melted at 54°. On analysis:

0·1389 Gm. gave 0·3800 CO_2 and 0·1568 H_2O. C = 74·6 ; H = 12·5 ;

$C_{16}H_{32}O_2$ requires C = 75·0 ; H = 12·5 per cent.

This substance was undoubtedly palmitic acid.

Further evidence of this was afforded by an examination of the second batch of crystalline product from the original mixture of acids. This product was first recrystallised from 90 per cent. alcohol, when it melted at 53-54°. It was then combined with the before-mentioned second crop from alcohol, and the whole again recrystallised from 90 per cent. alcohol. It then melted at 57-59°, and distilled under 50 Mm. pressure between 240 and 255°.

A portion of the oily acid, from which the above-described crystalline acids had separated and had been removed from the liquid by filtration, was converted into a lead salt by heating with an excess of lead carbonate and a little water on a water-bath. The product was a homogeneous mass, which on cooling became hard. It was disintegrated and extracted with hot ether, the solution filtered, and shaken with an excess of dilute hydrochloric acid. The ethereal solution of the liberated fatty acid was then washed with water, dried with calcium chloride, and the ether removed. The residual light yellow liquid when distilled under a pressure of 50 Mm. passed over chiefly at 261-264° as a nearly colourless oil. On cooling slightly below the ordinary temperature, a small amount of colourless, crystalline leaflets separated out. The oily acid thus obtained was analysed with the following result:—

0·1177 Gm. gave 0·3269 CO_2 and 0·1265 H_2O. C = 75·7 ; H = 11·9.

0·0957 Gm. gave 0·2644 CO_2 and 0·1032 H_2O. C = 75·3 ; H = 12·0.

Oleic acid, $C_{18}H_{34}O_2$, requires C = 76·6 ; H = 12·0 per cent.

Linolic acid, $C_{18}H_{32}O_2$, requires C = 77·1 ; H = 11·4 per cent.

A determination of the iodine value of this liquid acid, by Hübl's method, gave the figure 99·5, which is appreciably higher than the theoretical value for pure oleic acid, and the analytical figures are also not in agreement with the latter. As palmitic acid, $C_{16}H_{32}O_2$, requires C = 75·0 ; H = 12·5 per cent., the above results, together with a consideration of the action of nitrous acid, would seem to render it probable that the oily acid was composed of a mixture of oleic and linolic acids, together with some palmitic acid which had evidently not been completely separated by the process of purification through the lead salt.

(2) *Constituents of the Aqueous Liquid.*

It has been previously stated that after the distillation of the volatile substances, by steam, from the combined petroleum and alcoholic extracts of the seeds, there remained in the distilling flask an upper layer of fatty oil and a lower aqueous liquid, which were separated.

The aqueous liquid was turbid and did not afford a clear filtrate, owing apparently to a small amount of suspended resin. It was therefore shaken once with ether, which rendered it perfectly clear. The ether solution was distilled, and the residue therefrom, together with some resinous or other substances that had been obtained by filtering the petroleum solution of the fatty oil, was mixed with clean sand, dried and extracted in a Soxhlet apparatus, first with light petroleum, then with chloroform, and subsequently with alcohol. On finally extracting with water, nothing more was dissolved. The petroleum had extracted only a few grammes of dark-coloured, fatty matter. From the chloroform extract an appreciable amount of a dark-coloured substance was obtained, which was further treated in connection with a larger amount of substance subsequently obtained by extracting the above aqueous liquid with chloroform. The alcoholic extract was concentrated and poured into water, when a small quantity of resin was precipitated, which, when dried, formed a dark brown powder, and was not further examined.

The aqueous liquid, clarified by shaking with ether, had a reddish-yellow colour, an intensely bitter taste, and a strongly acid reaction. It gave a deep green colour with ferric chloride, an intense yellow colour with alkalis, a dense precipitate with tannic acid, and abundantly reduced Fehling's solution. It was shaken with six successive portions of chloroform, when finally nothing further was extracted. After the removal of the chloroform by distillation, a quantity of a dark brown, very viscid syrup was obtained. The latter, together with the residue obtained from the previously mentioned chloroform extract, was dissolved in hot alcohol, filtered, and the hot alcoholic liquid poured into a quantity of boiling water. This aqueous liquid was then rapidly filtered from some dark brown resin, evaporated to a small volume, mixed with prepared sawdust, and heated on a water-bath until quite dry. It was then extracted in a Soxhlet apparatus with dry ether. This slowly removed a substance which was only sparingly soluble, for it separated as a faintly green-coloured, granular crust on the bottom and sides of the flask whilst the extraction was in progress. The extraction was continued until nothing further was removed. The substance was collected, packed into a smaller Soxhlet apparatus, and again extracted with dry ether, when it was obtained in the same form as before, but much lighter in colour. The amount of this substance actually obtained was 6·5 Gm. For the purpose of comparison it will subsequently be referred to as " bitter principle (α)." It had the following characters:—

Bitter Principle (α).—It is a fine, granular powder of a greenish tinge, due to a trace of chlorophyll. Its taste is intensely and persistently bitter. Its melting-point is very indefinite, but below 100°. It contains no nitrogen. Numerous attempts were made to obtain this substance in a crystalline form, but without success. There was, therefore, no assurance of its being an individual substance. Nevertheless, merely for the purpose of a comparison with quassin, which Heckel and Schlagdenhauffen have so confidently stated to represent the chief bitter principle of Kô-sam seeds, it was analysed.

0·1373 Gm. gave 0·2950 CO_2 and 0·0863 H_2O. $C = 58·6$; $H = 7·0$
0·1364 Gm. gave 0·2932 CO_2 and 0·0838 H_2O. $C = 58·6$; $H = 6·8$.

The colour of its solution in chloroform did not permit of determining its optical rotation. It is very freely soluble in absolute alcohol and in chloroform, but very sparingly soluble in ether, even when warm. It is also very sparingly soluble in cold water, more readily on warming, and the solution becomes turbid on cooling, but the separated substance shows no tendency to crystallise. The aqueous solution slightly reduces Fehling's solution, and apparently to no greater extent after it has been heated with an acid. It is precipitated by tannic acid, and gives a deep brownish-black colour with ferric chloride.

The dry substance gives with concentrated sulphuric acid a brown colour, and about the same coloration with nitric acid. It dissolves, with a yellow colour, in a 10 per cent. solution of potassium hydroxide, and is re-precipitated on the addition of an acid. It is also soluble in a concentrated solution of sodium carbonate, forming a yellow solution.

Four grammes of the bitter principle were fused with 20 Gm. of potassium hydroxide, and the mixture kept at a temperature of 200°-220° until frothing ceased. The dark brown melt was taken up with water, acidulated with sulphuric acid, and steam distilled. The acid distillate was neutralised with barium carbonate, filtered, and evaporated, when it formed a light yellow syrup, which became crystalline. The solution of this salt reduced silver nitrate and mercuric chloride, and, when heated with a little alcohol and sulphuric acid, developed the odour of ethyl butyrate. The volatile products of the fusion, therefore, consisted chiefly of formic acid, with apparently a little of a butyric acid.

The acid residue from the steam distillation was saturated with ammonium sulphate and shaken out several times with ether. The ethereal solution was washed, dried, and the ether removed. The residue was a light brown varnish, from which nothing crystalline could be obtained. Its aqueous solution gave a brownish-black coloration with ferric chloride.

In order to compare the characters of the above-described bitter principle (a) with those of quassin, which, as recorded in chemical literature, are somewhat conflicting, a specimen of "Crystallised Quassin" was procured. It was perfectly white and crystalline, but was recrystallised from hot absolute alcohol, from which it separated on cooling in fine white glistening needles. It then had the following characters:—

It melted sharply at 252-253°. On analysis:

0·0645 Gm. gave 0·1584 CO_2 and 0·0424 H_2O. $C = 67·0$; $H = 7·3$
0·1173 Gm. gave 0·2889 CO_2 and 0·0773 H_2O. $C = 67·2$; $H = 7·3$

Various formulæ have been assigned to the substances designated as quassin, but the above figures would agree best for the formula $C_{28}H_{36}O_8$, which requires $C = 67·2$; $H = 7·2$ per cent.

A determination of the specific rotation, in chloroform, gave the following result:—

$a_D = +0°56'$; $c = 2·8$; $l = 1$ dcm. Hence $[a]_D^{27°} = +33·3°$

It was only moderately soluble in hot absolute alcohol, and, on cooling, separated almost immediately in fine, glistening needles. It was very sparingly soluble in cold, more readily in boiling water, from which, on cooling, it separated in small, glistening needles. It did not reduce Fehling's solution, nor did it give any colour with ferric chloride. It was slowly soluble in a cold 10 per cent. solution of potassium hydroxide, but without resinification, which has been stated to take place in the case of quassin.

The dry substance afforded with concentrated sulphuric acid a bright emerald-green colour, soon changing to yellow. Concentrated nitric acid causes no change of colour.

In Beilstein's 'Handbuch der org. Chemie,' 3rd edit., III., p. 646, the characters ascribed to quassin are in some respects essentially different from the above, as will be seen from the following description there recorded. "Fine needles. Melting point, 210-211°. Very readily soluble in alcohol, acetic acid, and chloroform, but sparingly soluble in ether. In chloroform $[\alpha]_D = +37{\cdot}8°$. Soluble in free alkalis, but not in alkali carbonates, and is resinified by alkalis. Its aqueous solution reduces Fehling's solution, but is not coloured by ferric chloride.

In Schmidt's *Pharm. Chemie*, 3rd edit., II., p. 1,516, it is stated regarding quassin that "concentrated sulphuric acid dissolves it without colour, and on the addition of a little sugar a red coloration is produced. When fused with potassium hydroxide, it affords protocatechuic and acetic acids."

The results of the above comparative experiments render it evident that the bitter principle (*a*) isolated by us from Kô-sam seeds differs in many important respects from quassin, and, with reference to the method of purification employed, it cannot be considered at all probable that it contains the latter.

The aqueous liquid, from which the above-described bitter principle (*a*) had been extracted by shaking several times with chloroform, was then further examined. It still possessed a strongly bitter taste. On the addition of basic lead acetate a dense, yellow precipitate was obtained, which was separated with the aid of a pump, and washed with a little water. This precipitate and the filtrate therefrom were then separately treated as follows :—

(A) *Basic Lead Acetate Precipitate.*—This was suspended in water, decomposed by hydrogen sulphide, and the liquid filtered. The filtrate had a bright yellow colour, and gave a deep green coloration with ferric chloride. When concentrated, it formed a reddish-yellow syrup, which, after standing for two weeks, showed no sign of crystallisation. It was then mixed with prepared sawdust, thoroughly dried, and extracted successively in a Soxhlet apparatus with ether, chloroform, ethyl acetate, and absolute alcohol. The ethereal extract afforded a very slight, crystalline residue, the alcoholic solution of which gave a deep emerald-green colour with ferric chloride. The residue from the chloroform extract was also very slight, but amorphous, and gave a similar coloration with ferric chloride. The extractions with ethyl acetate and with alcohol afforded rather darker coloured liquids, from which nothing crystalline separated. After distilling off the respective solvents, the residues from the latter two extracts were mixed, and, suspecting the presence of some substance of the quercetin type, the whole was dissolved in warm alcohol, to which a concentrated solution of 20 Gm. of potassium acetate in hot alcohol was subsequently added. (Compare *Journ. Chem. Soc.*, 1899, **75**, p. 433.) As this afforded nothing crystalline the alcohol was removed, the residue taken up with water, and the aqueous solution, after being saturated with ammonium sulphate, shaken out many times with ether. The ethereal liquid, which had a bright yellow colour, was washed once with water, dried with calcium chloride, and the ether removed. The residue gave a bright green colour with ferric chloride and a deep yellow colour with alkalis, but afforded nothing crystalline; on the addition of water only a tarry substance was obtained.

(B) *Filtrate from Basic Lead Acetate Precipitate.*—After the removal of the lead by hydrogen sulphide, and filtering, a yellow liquid was obtained, which, when concentrated, formed a reddish-yellow syrup. It was allowed to stand for two weeks, but nothing crystalline separated. It gave no precipitate with tannic acid, as the original aqueous liquid did before the extraction of the bitter principle with chloroform, and it was only very slightly coloured by ferric chloride. It still possessed, however, an intensely bitter taste, and abundantly reduced Fehling's solution. It was therefore mixed with prepared sawdust, thoroughly dried, and, as in the case of the lead acetate precipitate, extracted successively in a Soxhlet apparatus with ether, chloroform, ethyl acetate, and absolute alcohol. Both the ether and chloroform extracts afforded only very slight residues. The ethyl acetate and alcohol extracts were dark in colour, and afforded only syrupy residues, which contained an abundance of a reducing sugar, whose osazone melted at 204-205°.

Although it was to be presumed that if any quassin were contained in the seeds it would have been completely removed by the method employed in the isolation of bitter principle (α), yet as Heckel and Schlagdenhauffen have indicated in their paper (*loc. cit.*) that they separated what they assumed to be quassin by extracting the alcoholic extract of the seeds with amyl alcohol, it was thought desirable to subject the above-mentioned, very bitter, ethyl acetate and alcoholic extracts to a similar treatment. The residues from these two extracts were therefore mixed, dissolved in water, and the liquid shaken out many times with hot amyl alcohol. This afforded a dark coloured liquid, which was washed with water, and the amyl alcohol removed by distillation under diminished pressure. A considerable amount of a dark coloured, amorphous extract was thus obtained, which possessed a very bitter taste. An attempt was made to purify it by dissolving in warm ethyl alcohol, mixing the solution with prepared sawdust, and, after thoroughly drying, extracting successively in a Soxhlet apparatus with ether, chloroform, and absolute alcohol. All these extracts afforded amorphous residues, which were dark in colour, possessed a bitter taste, and reduced Fehling's solution.

These experiments afforded ample evidence that the bitter principle (β) contained in the aqueous solution, after the removal by chloroform of bitter principle (a), is essentially different from the latter, and also that it is not quassin.

The powdered seeds, which in the beginning had been thoroughly extracted with light petroleum and with alcohol, were dried, and a portion finally extracted with hot water. This afforded a very dark coloured liquid, which was concentrated and mixed with alcohol, when a considerable amount of gummy matter was precipitated. The filtered liquid, after the removal of the alcohol, was mixed with prepared sawdust dried, and extracted in a Soxhlet apparatus with strong alcoho , but it yielded nothing of interest.

Summary and Conclusions.

The results of this investigation may be briefly summarised as follows:—

Kô-sam seeds contain no alkaloid. The amount of tannin found was 1·8 per cent. They contain a small amount of a hydrolytic enzyme. The combined alcoholic and petroleum extracts of the seeds afforded the following substances: A small amount of an inconstantly boiling mixture of esters, probably of a butyric acid, and having the odour of the crushed seeds; also a very small amount of free formic acid.

A fatty oil, in an amount equivalent to 20 per cent. of the seeds, consisting chiefly of the glycerides of oleic, linolic stearic, and palmitic acids, associated with a saturated hydrocarbon, hentriacontane, $C_{31}H_{64}$, m.p. 67°-68°, and a crystalline substance, $C_{20}H_{34}O$. The latter melts at 130°-133°, and has $[\alpha]_D^{23°} = -37{\cdot}7°$; it is allied to the cholesterols, and agrees in composition with quebrachol, cupreol and cinchol. (Compare Beilstein's 'Handbuch der org. Chemie,' 3rd edit., ii., p. 1068.)

Two Bitter Principles.—One of these (α) was completely extracted by chloroform from an aqueous solution of them which also contained a quantity of a reducing sugar and a ver small amount of some substance which caused the solution to give a deep green colour with ferric chloride, but which was not isolated. Bitter principle (α), which was thus soluble in chloroform, was subsequently obtained from ether, in which it is only sparingly soluble, as a light coloured amorphous powder. Bitter principle (β), which was insoluble in chloroform under the conditions mentioned, could only be obtained as a brown extract. It was definitely shown that neither of these bitter principles can be regarded as quassin.

The results of this investigation, therefore, do not enable us to confirm the statement of Heckel and Schlagdenhauffen (*loc. cit.*) that Kô-sam seeds contain quassin, of which they have in fact presented no satisfactory evidence, nor do they afford any justification of the statement of Bertrand (*loc. cit.*) respecting the glucosidal nature of a bitter principle which he has termed "kosamine." It is to be noted, moreover, that Bertrand has given no indication of having actually isolated any definite substance to which such a name could properly be attached. Although mentioning its insolubility in certain anhydrous solvents, and that it is not precipitated by either neutral or basic lead acetate, by means of which it is stated that it may be separated from such substances as oil, resin, acids, etc., there is still no evidence that the principle to which he attributes the activity of the seeds was obtained in any more definite form than an aqueous solution, which must also have contained a considerable quantity of sugar.

A correct conclusion respecting the active principle of Kô-sam seeds could apparently only be formed when some definite constituent of them, such as the bitter principle (α), isolated by us, is tested clinically with reference to its particular value in the treatment of dysentery. It is more probable that the therapeutic value of the drug depends upon the combined action of its constituents.

In this connection, it would seem desirable to ascertain whether other parts of this plant, such as the bark, may not be as efficient as the seeds, since the latter are somewhat difficult to obtain in quantity. It would also be of interest to determine, by a comparative examination, the constituents of the closely-allied Abyssinian plant, *Brucea antidysenterica*, which, on account of the properties indicated by its name, is highly esteemed in its native country.

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