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CHEMICAL EXAMINATION OF THE FRUIT OF BRUCEA ANTIDYSENTERICA, LAM.

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In a paper communicated to the British Pharmaceutical Conference in 1903, entitled "Chemical Examination of Kô-sam Seeds" (the fruit of *Brucea sumatrana*, Roxb.), it was noted in conclusion that "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 (compare Power and Lees, "Year-Book of Pharmacy," 1903, pp. 503–522; *Pharm. Journ.*, 1903, 71, pp. 183–189). In the previous communication reference was likewise made to a notice concerning this plant by Engler in "Die natürlichen Pflanzenfamilien." Theil III., Abtheil, 4, p. 220, Leipzig, 1896, where it is stated that "the bark and the fruits of *Brucea antidysenterica* are used with success in Abyssinia for diarrhœa and fever."[1]

It is now possible to record the results of a chemical examination of the Abyssinian species of *Brucea*, since a quantity of both the fruit and bark of the latter plant was placed at our disposal by Messrs. Burroughs, Wellcome and Co., of London. The material was obtained by them through the kind services of H.B.M. Minister, Lieut.-Colonel Sir J. L. Harrington, K.C.V.O., C.B., at Adis Ababa, Abyssinia, who refers to the plant, *Brucea antidysenterica*, under the name of "waginus," and states that it is chiefly found in the province of Gojam, although he had considerable difficulty in getting it brought to Adis Ababa. Dr. W. A. M. Wakeman, also of the British Legation at Adis Ababa, in sending a quantity of material, likewise noted that it was very difficult to obtain. He reported, furthermore, the following information: "The kernels of the dried seeds (crushed) are

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[1] Since the publication of the above-mentioned paper the anatomical characters of *Brucea sumatrana* and *B. antidysenterica* have been studied by R. Müller (*Pharm. Journ.* 1905, 74, p. 76, from *Zeitschr. d. Œsterr. Apoth. Vereins*, 1904, Nos. 29–36).

used occasionally by the natives of these parts where it grows, both externally and internally. Among some of its uses are those of a purgative, vermifuge, and alterative internally, while the local application to raw wounds is believed to encourage granulation and arrest putrefaction." To all the above gentlemen who have aided us in this investigation our acknowledgments are due.

EXPERIMENTAL.

The fruits of *Brucea antidysenterica*, as received by us, were considerably larger and more coarsely reticulate than those of *B. sumatrana* ("Kô-sam seeds"), and also much lighter in colour. As an indication of their relative weights it may be noted that about five of the former and thirty of the latter, respectively, weigh 1 Gm. The kernel of the seed possesses an intensely and persistently bitter taste.

As a preliminary experiment, a portion of the crushed fruit was extracted successively in a Soxhlet apparatus with the following solvents, and the resulting extracts finally dried in a water oven until of constant weight:—

Petroleum (b.p. 35–50° C.) . .	extracted	19·15	per cent.
Ether	,,	1·05	,,
Chloroform	,,	2·20	,,
Alcohol	,,	6·95	,,
Total . .		29·35	per cent.

For the complete examination of the constituents of the fruit, 2030 Gm. of the crushed material were extracted in a large Soxhlet apparatus, first with light petroleum (b.p. 35–50° C.), and subsequently with hot alcohol.

I. EXAMINATION OF THE PETROLEUM EXTRACT.

This extract was first heated on a water-bath and then kept for some time in a vacuum, in order to remove the solvent as completely as possible, when it amounted to 450 Gm., corresponding to 22·16 per cent. of the weight of the fruit. It had the characters of a fatty oil, and was of a brownish-yellow colour. Its constants were determined, with the following results:—Specific gravity 18°/18°C.=0·9025; iodine value=81·5; acid value=2·3; saponification value=185·4.

From these data it was evident that the fatty oil contained a large proportion of the glycerides of unsaturated acids, triolein, for example, having an iodine value of 86·2.

The fatty oil was then brought into a flask and a current of steam passed through it, but the distillate contained neither volatile acid nor essential oil. After separating the water from the fatty oil, the latter was mixed with alcohol and hydrolyzed by boiling for a short time with an alcoholic solution of potassium hydroxide (120 Gm). The greater portion of the alcohol was then removed, the strongly alkaline, semi-solid product of hydrolysis mixed intimately with clean sand, the mixture thoroughly dried on a water bath, and finally extracted in a Soxhlet apparatus with light petroleum.

Isolation of a Phytosterol, $C_{20}H_{34}O$.

The petroleum extract was of a bright yellow colour, and, after removing the solvent, a quantity (1.5 Gm.) of a yellow solid was obtained. This was dissolved in a mixture of ethyl acetate and alcohol containing a little water, when a substance separated in colourless, glistening plates, which, after several crystallizations, melted at 135–136° C. This substance, when dissolved in chloroform, gave on the addition of a little acetic anhydride and a drop of concentrated sulphuric acid the colour reaction characteristic of the phytosterols. It was analysed, with the following result :—

0·3168, when heated at 110° C., lost 0·0172 H_2O. $H_2O=5·4$.
0·1004 of anhydrous substance gave 0·3030 CO_2 and 0·1083 H_2O.

$$C=82·3 ;\ H=12·0$$

$$C_{20}H_{34}O \text{ requires } C=82·8 ;\ H=11·7 \text{ per cent.}$$

$$C_{20}H_{34}O,H_2O \text{ requires } H_2O=5·8 \text{ per cent.}$$

This substance is thus seen to be a *phytosterol*. It is apparently identical with the compound of the same empirical formula which had previously been isolated from the fatty oil of the fruit of *Brucea sumatrana* (*loc. cit.*). The mother liquors from the first crystallization of the phytosterol afforded, as is usually the case, a small quantity of a deep yellow, viscid liquid.

Acids Obtained by the Hydrolysis of the Fatty Oil.

Having removed the neutral constituents of the hydrolyzed oil, the residual mixture of potassium salts and sand was repeatedly extracted with hot water, and the aqueous, strongly alkaline solution acidified with sulphuric acid, when the liberated fatty acid separated on the surface of the liquid as a partially solid mass. The mixture was then distilled in steam, and a very small amount of volatile acid thus obtained, which, after conversion into a barium salt, afforded the reactions of *acetic* and *butyric acids.*

The acids remaining in the distilling flask were extracted with chloroform, the resulting solution washed with water, dried with calcium chloride, and the solvent removed, the last traces of chloroform being expelled by heating on a water bath under diminished pressure. About 250 Gm. of mixed fatty acids were thus obtained, the constants of which were determined with the following results :—Melting point 27–28° C. ; specific gravity 30°/30°C.=0·8980 ; iodine value=83·5 ; acid value=186·1 ; acetyl value=4·5.

From the iodine value, as determined by Hübl's method, it was concluded that the mixture consisted largely of unsaturated acids, oleic acid, for example, having an iodine value of 90·1. On the other hand, the acid value 186·1 indicated that only a small quantity of acids having a higher molecular weight than stearic and oleic acids were present, the acid values of the latter two acids being 197·5 and 198·9 respectively.

In order to separate the constituents of the mixed fatty acids, the solid portion, amounting to about 50 Gm., was first removed by filtration at the pump. It was fractionally crystallized from alcohol, when the first deposits melted at 60–62° C., but after four successive crystallizations melted constantly at 69·5° C. This was evidently a pure substance, and was analysed :—

0·1710 gave 0·4748 CO_2 and 0·2022 H_2O. C=75·7 ; H=13·1,
$C_{18}H_{36}O_2$ requires C=76·1 ; H=12·7 per cent.

This substance was thus identified as *stearic acid.*

The mother liquors resulting from the above fractionation contained a considerable quantity of an acid which, after repeated crystallization, separated in glistening leaflets melting at 57-60° C. Although this could not be obtained perfectly pure, it was analysed, with the following result :—

0·1681 gave 0·4636 CO_2 and 0·1898 H_2O. C=75·2 ; H=12·5.
$C_{16}H_{32}O_2$ requires C=75·0 ; H=12·5 per cent.

It is evident that this substance was *palmitic acid.*

The liquid acids, which were separated by filtration from the above-described solid portion, were considerable in amount, the weight being about 200 Gm. In order to effect a separation of the liquid acids they were converted into their methyl esters. For this purpose the oily liquid was dissolved in an excess of methyl alcohol, and, while being heated on a water-bath in a flask provided with a reflux condenser, a current of dry hydrogen chloride was passed through the solution for two hours. The greater part of the alcohol was then removed, water added, and the ester extracted with ether. The ethereal solution was shaken with an aqueous solution of sodium carbonate to remove any traces of unesterified fatty acids, then washed with water, dried, and the ether removed. The methyl esters were finally subjected to fractional distillation under a pressure of 13 Mm., when a number of fractions were collected having the characters below indicated.

Fractions.	Boiling Point.	Amount. Gm.	Spec. Gravity. 16°/16° C.	Iodine Value.
I.	Below 202° C./13 Mm.	6	—	—
II.	202° to 204°	41	0·8815	86·2
III.	204° to 206°	41	0·8792	93·1
IV.	206° to 208°	36	—	94·4
V.	208° to 210°	16	0·8788	94·3
VI.	210° to 220°	8	—	96·8
VII.	220° to 240°	5	—	98·8
VIII.	240° to 290°	7	—	106·8
IX.	Above 290°	5	—	98·2

The principal fractions (II., III., IV., and V.), which distilled within a very small range of temperature, were colourless mobile oils, and evidently consisted chiefly of a single substance. On comparing the iodine value of the various fractions with that of methyl oleate (85·9), it may be concluded that the four larger ones consisted chiefly of the latter compound, together with a small percentage of the ester of an acid possessing a higher degree of unsaturation, probably methyl linolate. The fractions II. and V. were analysed :—

Fraction II.—(202 to 204° C./13 Mm.).
0·1229 gave 0·3466 CO_2 and 0·1386 H_2O. C=76·9 ; H=12·5,
Fraction V.—(208 to 210° C./13 Mm.).
0·1448 gave 0·4086 CO_2 and 0·1572 H_2O. C=77·0 ; H=12·1,
Methyl oleate, $C_{19}H_{36}O_2$, requires C=77·0 ; H=12·2 per cent.
Methyl linolate, $C_{19}H_{34}O_2$, requires C=77·6 ; H=11·6 per cent.

Although the above data indicated that the fractions boiling from 202 to 210° C./13 Mm. consisted chiefly of methyl oleate, it was deemed of interest to ascertain what amount of the methyl esters of saturated acids they contained. For this purpose a portion of the mixed fractions (202 to 210° C./13 Mm.) was hydrolyzed by alcoholic potash, the acids liberated and extracted and then converted into their lead salts by precipitation with lead acetate in alcoholic solution. The lead salts were collected on a filter, washed with alcohol, and finally digested with ether. From the soluble portion, consisting of the salts of unsaturated acids, the acids were liberated, and their iodine value again determined by the Hübl method. This was found to be 98·4, a figure which is only slightly higher than that previously obtained. The evidence was thus afforded that the liquid acid contained in the fatty oil consisted chiefly of *oleic acid*, together with a small amount of an acid having a higher degree of unsaturation, which is probably *linolic acid*.

II.—EXAMINATION OF THE ALCOHOLIC EXTRACT.

The alcoholic extract, which was a thick, very dark coloured product, was brought into a flask with water and distilled in steam. The distillate contained a small amount of acid, which, after conversion into a barium salt, was found to consist of a mixture of *formic* and *butyric acids*.

After the steam distillation the contents of the distilling flask consisted of an aqueous liquid (A) containing a small amount of resinous substance (B) in suspension. These were separated by filtration and subsequently examined.

(A) *The Aqueous Liquid.*

The aqueous liquid was somewhat turbid, owing to the presence of finely-divided particles of resinous substance which could not be removed by filtration. On shaking it, however, with a little ether the resin was removed, and this was added to the larger portion previously obtained. The aqueous liquid, which was now perfectly clear, had a reddish-brown colour, a strongly acid reaction, and possessed an intensely bitter taste. With alkalies the liquid was rendered much deeper in colour, and with ferric chloride a dark brown colouration was produced. It readily reduced Fehling's solution, and gave a precipitate with tannic acid.

As it was shown in the investigation of the fruit of *Brucea sumatrana* (*loc. cit.*) that an intensely bitter principle could be extracted from the aqueous liquid corresponding to that above described by shaking with chloroform, the same treatment was adopted in the present instance. On repeatedly extracting the liquid with this solvent, however, only a very small amount of substance was removed. This possessed a bitter taste, but could only be obtained in the form of a dark brown varnish, differing in this respect from the corresponding bitter principle from the fruit of *Brucea sumatrana*. On dissolving this product in alcohol and allowing the solution to stand for a long time, a few needle-shaped crystals were deposited, which melted sharply at 108° C., but these did not possess a bitter taste, and the quantity was much too small for further examination.

The aqueous liquid, which had repeatedly been extracted with chloroform, still possessed a bitter taste, and showed the same behaviour towards reagents as above described. It was treated with a slight excess of basic lead acetate, when a copious yellow precipitate was produced. This was collected on a filter. and washed by the aid of a pump with a little water.

Basic Lead Acetate Precipitate. This was suspended in water, decomposed by hydrogen sulphide, and the mixture filtered. On concentrating the filtrate under diminished pressure a syrupy liquid was obtained, which gave an emerald-green colour with ferric chloride, but no crystalline substance separated from it, even after standing for a long time.

Filtrate from the Basic Lead Acetate Precipitate. This was treated with hydrogen sulphide for the removal of the lead, and the mixture filtered. The filtrate was concentrated under diminished pressure, when it formed a thick, dark brown syrup, which readily reduced Fehling's solution on heating. A portion of this syrup was treated with phenylhydrazine acetate, when it yielded an osazone, melting at 217° C., thus indicating the presence of a large amount of *glucose*.

With the endeavour to separate the bitter principle which still remained in the syrupy liquid, the latter was mixed with purified sawdust, the mixture thoroughly dried, and then successively extracted in a Soxhlet apparatus with ethyl acetate, alcohol, and water. Both the ethyl acetate and alcohol extracts were intensely bitter, but they formed only thick syrups, from which no solid substance could be separated. The final aqueous extract was almost completely devoid of bitterness, and evidently consisted chiefly of sugar.

(B) *The Resinous Substances.*

The total amount of resinous substance separated from the alcoholic extract, as previously described, was 21 Gm., thus representing about 1 per cent. of the weight of the fruit. It was dissolved in a little alcohol, the solution mixed with purified sawdust, and, after thoroughly drying the mixture, the latter was successively extracted in a Soxhlet apparatus with various solvents. The amounts of extract thus obtained, after drying in a water-oven, were as follows :—

Petroleum (b.p. 35–50° C.) .	extracted	6·2 Gm.	= 29·5 per cent.
Ether	,,	1·5 ,,	= 7·1 ,,
Chloroform . . .	,,	3·2 ,,	= 15·2 ,,
Ethyl Acetate . . .	,,	3·0 ,,	= 14·3 ,,
Alcohol	,,	6·9 ,,	= 32·9 ,,
Total . .		20·8 Gm.	= 99·0 per cent.

The petroleum extract formed a dark green, viscid mass. It was hydrolyzed with alcoholic potash, and, after the removal of the alcohol, water was added, and the alkaline liquid extracted with ether. The ethereal solution was washed, dried, evaporated, and the residue treated with alcohol, when a very small amount of a crystalline substance was obtained. This was recrystallized several times from ethyl acetate, when it separated in glistening plates, which melted sharply at 147° C., and afforded the colour reaction characteristic of the *phytosterols*. The alkaline liquid, from which this phytosterol had been abstracted by ether, was acidified with sulphuric acid, and again shaken with ether. This removed a dark green viscous substance, which apparently contained a small quantity of fatty acids.

The extracts obtained by the other above-mentioned solvents were all amorphous, and, although carefully examined, no crystalline substance could be separated from them.

SUMMARY AND CONCLUSIONS.

In summarizing the results of this investigation it will be seen that the more important constituents of the fruit of *Brucea antidysenterica*, Lam., are the following :—

1. A *fatty oil*, amounting to 22·16 per cent. of the weight of the fruit. This oil, on hydrolysis, yielded chiefly *oleic acid*, together with a small amount of an acid having a higher degree of unsaturation, which is probably *linolic acid*; considerable amounts of *palmitic* and *stearic acids*, and a very small quantity of *acetic* and *butyric acids*. It afforded, furthermore, a small amount of a *phytosterol*, $C_{20}H_{34}O, H_2O$ (m.p., 135-136°C.), whilst another phytosterol, melting at 147° C., was obtained from the petroleum extract of the resins.

2. A small amount of free volatile acids, consisting of a mixture of *formic* and *butyric acids*.

3. A quantity of *resinous substances*, corresponding to about 1 per cent. of the weight of the fruit. From these, with the exception of a small amount of the above-mentioned phytosterol, nothing crystalline could be isolated.

4. A *bitter principle*, or possibly a mixture of such principles, which could only be obtained in an amorphous form.

5. A considerable quantity of amorphous yellow *colouring matter*.

6. A large amount of a sugar, which yielded an osazone melting at 217°C., and was therefore evidently *glucose*.

The constituents of the fruit of *Brucea antidysenterica*, Lam., are thus found to be very similar in character to those of the fruit of *Brucea sumatrana*, Roxb ("Kô-sam seeds"), and it may consequently be assumed that the two species possess similar medicinal properties. The bitter principles appear, however, to be contained in relatively larger amount in the fruit of *Brucea sumatrana* than in that of the Abyssinian species, and in view of the difficulty experienced in collecting the fruit of the latter it is not probable that it will acquire a very extended use.

It may finally be noted that the *Pharmacopoea Nederlandica* (*Editio Quarta*, 1905) has given official recognition to the fruit of *Brucea sumatrana*, Roxb., which is described under the title of "Fructus Bruceæ," and it is there stated that in the Dutch East Indies this is known, among other names, as "bidji makasar" (*grana macasaria*), and "tambara maridja."

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1. A fatty oil, amounting to 22·46 per cent. of the weight of the fruit, which, on hydrolysis, yielded chiefly oleic acid, together with a small amount of an acid having a higher degree of unsaturation, which is probably linolic acid, considerable amounts of palmitic and stearic acids, and a very small quantity of cerotic and butyric acids. It afforded, furthermore, a small amount of a phytosterol, $C_{27}H_{46}O$ (m.p., 135-136° C.), whilst another phytosterol, melting at 147° C., was obtained from the petroleum extract of the resins.

2. A small amount of free volatile acids, consisting of a mixture of formic and butyric acids.

3. A quantity of resinous substance, corresponding to about 4 per cent. of the weight of the fruit. From these, with the exception of a small amount of the above-mentioned phytosterol, nothing crystalline could be isolated.

4. A bitter principle, or possibly a mixture of such principles, which could only be obtained in an amorphous form.

5. A considerable quantity of amorphous yellow colouring matter.

6. A large amount of a sugar, which yielded an osazone melting at 217° C., and was therefore evidently glucose.

The constituents of the fruit of *Brucea antidysenterica*, Lam., are thus found to be very similar in character to those of the fruit of *Brucea sumatrana*, Roxb. ("Kosam seeds"), and it may consequently be assumed that the two species possess similar medicinal properties. The bitter principles appear, however, to be contained in relatively larger amount in the fruit of *Brucea sumatrana* than in that of the Abyssinian species, and in view of the difficulty experienced in collecting the fruit of the latter it is not probable that it will acquire a very extended use.

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