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THE CONSTITUENTS OF THE FRUIT
OF
ECBALLIUM ELATERIUM

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CCXVIII.—*The Constituents of the Fruit of Ecballium Elaterium.*

By Frederick Belding Power and Charles Watson Moore.

In a recent communication by the authors, entitled "Chemical Examination of Elaterium and the Characters of Elaterin" (*Pharm. J.*, 1909, [iv], **29**, 501), it was shown that the product known and officially recognised as "elaterin" is not homogeneous, but that it consists, to the extent of 60—80 per cent., of a crystalline substance which is completely devoid of the specific physiological action attributed to elaterin, and may be separated by a process of fractional crystallisation. This crystalline substance, which is much more highly lævorotatory and has a higher melting point than the crude elaterin, is accompanied in the latter by a compound of apparently the same percentage composition, but which possesses a very high degree of physiological activity and is dextrorotatory.

In view of the fact that the elaterium from which the above-mentioned "elaterin" is obtained represents only the material which is deposited from the juice of the fruit of *Ecballium Elaterium*, it appeared desirable that the entire fresh fruit should be subjected to a chemical examination in order to ascertain the nature of all its constituents. In conducting this investigation, special consideration has been given to the statement of Berg (*Bull. Soc. chim.*, 1897, [iii], **17**, 85) that elaterin does not pre-exist in the fruits of *Ecballium*, but that it is formed, after the expression of the juice, by the action of a ferment on an amorphous glucoside. The results which have now been obtained, with the deductions from them, are summarised at the end of this paper.

Experimental.

The material employed in this investigation consisted of the fresh, nearly ripe fruits of *Ecballium Elaterium*, A. Richard, which were very kindly supplied to us by Messrs. W. Ransom & Son, of Hitchin. They were collected during the latter part of August.

Separation of an Enzyme.

It has been indicated by Berg (*loc. cit.*) that the juice of the fruit of *Ecballium* contains an enzyme, or possibly a mixture of enzymes, which is capable of hydrolysing amygdalin, saccharose, and starch, and was designated by him as elaterase. In order to obtain this substance, the expressed juice from 3 kilograms of the fruit, amounting to about 900 c.c., was filtered, and mixed with 1500 c.c. of strong alcohol. A voluminous, light-coloured, amorphous precipitate was thus produced, which was collected, washed with a little alcohol, and dried, when it amounted to 1·5 grams. The product was readily soluble in cold water, and, although containing a large proportion of inorganic material, rapidly hydrolysed β-glucosides.

Extraction of the Fruit with Alcohol.

For the purpose of a complete examination, 27 kilograms of the fruit were employed.

With consideration of the statement by Berg (*loc. cit.*) that the elaterin contained in the fruit of *Ecballium* exists in the form of a glucoside which is readily hydrolysed by the enzyme present, the material was treated in such a manner at to preclude the possibility of any hydrolysis of its constituents taking place. Each fruit, therefore, was separately cut into several pieces, which were allowed to fall directly into strong alcohol, the total volume of the latter having been about 80 litres. After a short time, the mixture was heated to the boiling point, and kept at this temperature for about fifteen minutes. The whole was subsequently kept overnight, after which the marc was removed by filtration, the liquid thus obtained being then found to contain slightly over 75 per cent. by volume of alcohol.

The marc, separated by filtration as above described, was pressed, and the liquid thus obtained was added to the main portion of alcoholic extract. The seeds were then separated so far as possible from the pressed marc, and the latter completely extracted in a Soxhlet apparatus with hot alcohol, this extract being likewise added to that first obtained. The united liquids were then concentrated by distillation from a water-bath until alcohol ceased to pass over.

During the concentration of the alcoholic extract, as above described, there separated a quantity of green resin (A), which was collected on a filter and repeatedly treated with water until nothing further was removed. The filtrate and washings from this resin were united, and concentrated under diminished pressure to a volume of about 3 litres. During this operation a quantity of light brown, resinous material (B) separated, which was collected and thoroughly washed with cold water the washings being added to the aqueous liquid (C).

Examination of the Green Resin (A).

This product was a dark green, soft mass, and amounted to 45·5 grams. It was dissolved in alcohol, and mixed with purified sawdust, the thoroughly-dried mixture being then successively extracted in a Soxhlet apparatus with light petroleum (b. p. 35—50°), ether, chloroform, and alcohol.

Petroleum Extract of the Green Resin.

This extract was a dark green, viscid liquid, and amounted to 38 grams. It was dissolved in ether, the ethereal liquid being shaken successively with solutions of sodium carbonate and sodium hydroxide, but nothing was thus removed. The ether was then evaporated, and the residue hydrolysed by heating with an alcoholic solution of potassium hydroxide, after which the alcohol was removed, water added, and the alkaline solution of potassium salts extracted with ether. The ethereal liquid was washed, dried, and the solvent removed, when a small quantity of a light brown, crystalline product was obtained. This was dissolved in about 150 c.c. of hot absolute alcohol, and the solution kept for some time, when a nearly colourless substance separated. The latter was collected, washed with cold alcohol, and then distilled under 15 mm. pressure. The distillate, which rapidly solidified, was crystallised from ethyl acetate, when small, colourless, glistening leaflets, melting at 68°, were obtained. This substance was a hydrocarbon, and appeared to be hentriacontane, $C_{31}H_{64}$, but the amount was too small for analysis.

Isolation of a Phytosterol, $C_{27}H_{46}O$.

The alcoholic solution from which the hydrocarbon had been removed by filtration, as above described, was concentrated to a small volume and diluted with water, when a quantity of crystalline substance separated. This was collected on a filter, and washed with a little ethyl acetate, after which it was distilled under diminished pressure. The distillate, which solidified on cooling, was crystallised from a mixture of ethyl acetate and dilute alcohol, when it formed colourless, glistening leaflets melting at 148°. The amount of substance so obtained was 0·7 gram:

0·2806, heated at 115°, lost 0·0134 H_2O. $H_2O = 4·8$.

0·1342 of anhydrous substance gave 0·4125 CO_2 and 0·1430 H_2O. $C = 83·8$; $H = 11·8$.

$C_{27}H_{46}O,H_2O$ requires $H_2O = 4·5$ per cent.

$C_{27}H_{46}O$ requires $C = 83·9$; $H = 11·9$ per cent.

The substance thus agrees in composition with a phytosterol, and it yielded the colour reactions of this class of compounds. A determination of its specific rotatory power gave the following result:

0·2055, made up to 20 c.c. with chloroform, gave $\alpha_D + 0°4'$ in a 2-dcm. tube, whence $[\alpha]_D + 3·2°$.

The acetyl derivative, when crystallised from acetic anhydride, separated in flat, glistening needles, melting at 155—157°.

The above-described phytosterol differs appreciably in some of its characters from the commonly-occurring substances of this class, as indicated, for example, by its higher melting point, its dextrorotation, and the fact that the acetyl derivative melts higher than the substance from which it was prepared.

The aqueous alkaline solution of potassium salts from which the hydrocarbon and phytosterol had been removed by extraction with ether, as above described, was acidified, and again extracted with ether. The ethereal liquid obtained in this way contained a small amount of a nearly colourless, very sparingly soluble substance in suspension. This was collected on a filter, washed with ether, and crystallised from pyridine, when it formed small, glistening plates, melting at 258—260°. It appears to be related to ipuranol, $C_{23}H_{38}O_2(OH)_2$, as it gives the characteristic colour reactions of this substance, but the amount obtained was insufficient for analysis (compare *Amer. J. Pharm.*, 1908, **80**, 264, 576; Trans., 1908, **93**, 907; this vol., p. 249).

Examination of the Fatty Acids.

The ethereal liquid, from which the above-described substance had been separated, was washed, dried, and the solvent removed. A quantity (12 grams) of fatty acids was thus obtained, which, when distilled under diminished pressure, passed over between 230—235°/10 mm. Eight grams of the mixed acids were converted into their lead salts, and the latter digested with ether, when the larger portion was dissolved. Both the soluble and insoluble portions were decomposed by hydrochloric acid, and the regenerated fatty acids purified by distillation under diminished pressure. The soluble portion of the lead salts yielded 6 grams of liquid acids, whilst the insoluble portion gave 1·5 grams of solid acids.

The Liquid Acids.—These acids when distilled under diminished pressure passed over between 232° and 235°/15 mm.

An analysis and a determination of the iodine and neutralisation values gave the following results:

0·1366 gave 0·3860 CO_2 and 0·1370 H_2O. C = 77·1; H = 11·1.

0·4095 absorbed 0·8473 iodine. Iodine value = 206·9.

1·1120 neutralised 0·2211 KOH. Neutralisation value = 197·4.

$C_{18}H_{32}O_2$ requires C = 77·1; H = 11·4 per cent. Iodine value = 181·4;
Neutralisation value = 200·4.

$C_{18}H_{30}O_2$ requires C = 77·7; H = 10·8 per cent. Iodine value = 274·1;
Neutralisation value = 201·4.

These results indicated that the liquid acids consisted chiefly of a mixture of linolic and linolenic acids, the former predominating.

The Solid Acids.—These acids were crystallised from glacial acetic acid, but no pure substance could be isolated by this means, the product melting indefinitely between 54° and 58°.

An analysis and a determination of the neutralisation value gave the following results:

0·1432 gave 0·3940 CO_2 and 0·1631 H_2O. C = 75·1; H = 12·6.

0·7500 neutralised 0·1559 KOH. Neutralisation value = 207·9.

$C_{16}H_{32}O_2$ requires C = 75·0; H = 12·5 per cent. Neutralisation value = 219·1.

$C_{18}H_{36}O_2$ requires C = 76·1; H = 12·7 per cent. Neutralisation value = 197·7.

The above results indicated that the solid acids consisted of a mixture of palmitic and stearic acids in about equal proportions.

Ethereal Extract of the Green Resin. Isolation of "Elaterin."

This extract amounted to about 6 grams. A portion of it (about 2 grams) was readily soluble in ether, and consisted of a dark-coloured resin, whilst the remainder (4 grams) was a sparingly soluble, crystalline powder. From the resinous product nothing definite could be isolated, and as it yielded no sugar on boiling with a dilute solution of sulphuric acid in aqueous alcohol, it was not glucosidic.

The above-mentioned, crystalline powder was collected on a filter, washed with ether, and recrystallised from a small volume of absolute alcohol, when it was obtained in small, colourless, hexagonal prisms, melting and decomposing at 210—215°. On subjecting this product to fractional crystallisation from absolute alcohol, three principal fractions were obtained. Although a complete separation could not be effected by this means, owing to the limited quantity of material at disposal, the three fractions, nevertheless, exhibited very marked differences, as indicated especially by their solubilities, melting points, and optical rotatory power.

Fraction I.—This fraction formed small, colourless, hexagonal prisms, melting and decomposing at 230°. It was very sparingly soluble in alcohol, and amounted to 0·7 gram:

0·2554, made up to 20 c.c. with chloroform, gave α_D $-1^\circ 21'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-52 \cdot 4^\circ$.

Fraction II.—This fraction closely resembled fraction I in appearance. It was, however, more soluble in alcohol, and melted, with decomposition, at about 210°. It amounted to 1·5 grams.

0·2245, made up to 20 c.c. with chloroform, gave α_D $-0^\circ 48'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-35 \cdot 6^\circ$.

Fraction III.—This fraction crystallised in small, colourless plates, melting and decomposing at 189—190°. It was very soluble in alcohol, and amounted to only 0·25 gram:

0·2346, made up to 20 c.c. with chloroform, gave α_D $+0°24'$ in a 2-dcm. tube, whence $[\alpha]_D$ $+17·0°$.

The three fractions gave on analysis the following results:

I. 0·1607 gave 0·4056 CO_2 and 0·1180 H_2O. C = 68·9; H = 8·1.
II. 0·1468 „ 0·3710 CO_2 „ 0·1114 H_2O. C = 68·9; H = 8·4.
III. 0·1355 „ 0·3406 CO_2 „ 0·1005 H_2O. C = 68·6; H = 8·2.
$C_{20}H_{28}O_5$ requires C = 68·9; H = 8·0 per cent.
$C_{24}H_{34}O_6$ „ C = 68·9; H = 8·0 „
$C_{28}H_{38}O_7$ „ C = 69·1; H = 7·8 „

From these results it is evident that the above-described, sparingly soluble, crystalline powder corresponds to the product known as "elaterin," and which is recognised under this title by the British and United States Pharmacopœias. The formulæ which have been assigned to elaterin by various investigators are as follows: $C_{20}H_{28}O_5$, Zwenger (*Annalen*, 1842, **43**, 460); $C_{24}H_{34}O_6$, Hemmelmayr (*Ber.*, 1906, **39**, 3380); $C_{28}H_{38}O_7$, Berg (*Bull. Soc. chim.*, 1897, [iii], **17**, 85; 1906, [iii], **35**, 435). It has, however, quite recently been shown by the present authors (*Pharm. J.*, 1909, [iv], **29**, 501) that the product known as elaterin is not homogeneous, as has hitherto been assumed, but that it consists of a mixture of substances which, although possessing apparently the same percentage composition, differ essentially in their physical properties, especially in their optical rotatory power, as also in their physiological action. The above-noted results of the present investigation therefore afford further confirmation of the previous conclusions.

The final alcoholic mother liquors obtained during the separation of the above-described elaterin fractions yielded, on dilution with water, a small amount of a colourless substance, which crystallised in small needles and melted and decomposed at 168—170°:

0·2098, heated at 115°, lost 0·0070 H_2O. H_2O = 3·3.

0·1318 of anhydrous substance gave 0·3264 CO_2 and 0·0980 H_2O. C = 67·5; H = 8·2.

A determination of its specific rotatory power gave the following result:

0·2942 of anhydrous substance, made up to 20 c.c. with chloroform, gave α_D $+0°38'$ in a 2-dcm. tube, whence $[\alpha]_D$ $+21·5°$.

These results would indicate that the above-described substance consisted to a considerable extent of the dextrorotatory constituent of elaterin, but the amount of material available was so small that it was impossible to obtain any evidence of its purity.

Inasmuch as we have shown that the product known as elaterin consists of two substances of apparently the same percentage composition, one of which is lævorotatory and devoid of physiological activity, whilst the other is dextrorotatory and possesses an intensely purgative action, it seems desirable that the respective substances should be designated by distinctive names. It is therefore proposed to designate the chief constituent of crude elaterin, which is lævorotatory, as α-elaterin, and the dextrorotatory, physiologically active constituent, as β-elaterin.

Chloroform and Alcohol Extracts of the Green Resin.

Both of these extracts consisted of brown, resinous material, and they amounted to 0·3 and 0·5 gram respectively. As nothing definite could be isolated from them, they were united, and boiled for some hours with a dilute solution of sulphuric acid in aqueous alcohol. No sugar was formed by this treatment, and the extracts, therefore, were evidently not glucosidic.

Examination of the Brown Resin (B).

This was a light brown powder, and amounted to 6·5 grams. It was dissolved in alcohol, and mixed with purified sawdust, the thoroughly dried mixture being then successively extracted in a Soxhlet apparatus with ether, chloroform, and alcohol. The ether removed about 5·5 grams of material, from which, with the exception of small amounts of elaterin, nothing definite could be isolated. The chloroform and alcohol extracts amounted to only 0·2 and 0·5 gram respectively, and consisted of brown resins. Nothing of a glucosidic nature was contained in any of these extracts.

Examination of the Aqueous Liquid (C).

This liquid, as already indicated, represented that portion of the original alcoholic extract of the *Ecballium* fruits which was soluble in cold water, and from which the previously-described resins (A) and (B) had been separated. It was extracted many times with ether, the combined ethereal liquids being washed, dried, and the solvent removed, but only a small amount of an indefinite, amorphous product was thus obtained.

The aqueous liquid, after extraction with ether, was shaken with successive portions of amyl alcohol, the combined extracts being washed, and the solvent removed by distillation under diminished pressure. A quantity (about 15 grams) of resinous material was thus obtained, which was only sparingly soluble in water. As nothing definite could be isolated from it, it was boiled for some hours with dilute alcohol containing 3 per cent. of its weight of sulphuric acid. The chief product of this operation was, however, entirely resinous, and, as no sugar was formed, it was evident that the material contained nothing glucosidic.

After extraction with amyl alcohol, as above described, the aqueous liquid was concentrated, and treated with a large volume of strong alcohol. This precipitated a quantity of inorganic material, consisting chiefly of potassium chloride and sulphate, together with a sugar which yielded *d*-phenylglucosazone, melting at 216°. The clear liquid was then decanted and the alcohol removed, when a small quantity of a syrup was obtained. This was dissolved in a small quantity of water and kept for several days, but nothing separated. The solution was then diluted with water, and, after adding 5 per cent. of its weight of sulphuric acid, the whole was boiled for several hours. The only products resulting from this treatment that could be identified were a little formic acid and furfuraldehyde.

Summary.

The results of the present investigation of the fruit of *Ecballium Elaterium* may be summarised as follows:

In accordance with the previous observation of Berg (*Bull. Soc. chim.*, 1897, [iii], **17**, 85), the fruit was found to contain a small amount of an enzyme which is capable of hydrolysing β-glucosides.

The liquid obtained by the extraction of 27 kilograms of the fresh fruit with alcohol yielded, on concentration, a quantity (45·5 grams) of a green resin, and, by the further evaporation of the liquid, 6·5 grams of brown, resinous material were separated. The green resin, when extracted with various solvents, yielded a very small amount of a hydrocarbon (m. p. 68°), which was probably hentriacontane, $C_{31}H_{64}$; a phytosterol, $C_{27}H_{46}O$ (m. p. 148°; $[\alpha]_D$ $+3{\cdot}2°$); a substance melting at 258—260°, which appears to be related to ipuranol, $C_{23}H_{38}O_2(OH)_2$; a mixture of fatty acids; and a product corresponding to the so-called "elaterin," the characters of which have previously been elucidated by the authors (*Pharm. J.*, 1909, [iv], **29**, 501). It is now proposed to designate the lævorotatory constituent of crude elaterin as α-elaterin, and the dextrorotatory, physiologically active constituent as β-elaterin. From the brown, resinous material, with the exception of small amounts of elaterin, nothing definite could be isolated. The portion of the alcoholic extract of the fruit which was soluble in water contained a considerable amount of inorganic salts, consisting chiefly of potassium chloride and sulphate, and a sugar which yielded *d*-phenylglucosazone (m. p. 216°).

No evidence could be obtained of the presence of a glucoside of elaterin, which has been stated by Berg (*loc. cit.*) to be contained in the juice of the fresh fruit, nor could any other glucosidic substance be detected. It was, on the other hand, quite definitely ascertained that the so-called "elaterin" exists in the fruit as such and not in a combined state. The various amorphous products which were described many years ago by Walz (*N. Jahrb. Pharm.*, 1859, **11**, 21, 178) under the names of prophetin, ecbalin or elateric acid, hydroelaterin, and elateride were evidently indefinite mixtures, and are therefore entitled to no further consideration in chemical literature.

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