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THE CONSTITUENTS OF BRYONY ROOT

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XCVIII.—*The Constituents of Bryony Root.*

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Bryony root has been used medicinally from a very remote period on account of its cathartic properties, and was formerly recognised by several of the national Pharmacopoeias, but is now rarely employed. The plants yielding this root are *Bryonia alba*, Linné, and *Bryonia dioica*, Linné (Nat. Ord., *Cucurbitaceae*), which are botanically closely allied. They are indigenous to the greater part of Europe, but the last-named species is the only one of the genus commonly found in this country, and, therefore, is frequently designated as English bryony. The roots of the two species are generally considered to possess the same properties, and they appear to be indiscriminately collected, although it has been asserted by Petresco (*United States Dispensatory*, 18th edition, p. 279) that they differ appreciably in their physiological action.

Bryony root has been the subject of several investigations, chiefly for the purpose of determining the nature of its active constituent (compare Husemann, *Die Pflanzenstoffe*, second edition, p. 1349, and van Ryn, *Die Glykoside*, p. 463), but no complete chemical examination has hitherto been made of it. With the exception of the recorded presence of starch, gum, sugar, and fat, the information concerning the constituents of the root is, in fact, chiefly restricted to the statement that it contains an amorphous, bitter glucoside, designated as bryonin. This product, as obtained by Masson (*J. Pharm. Chim.*, 1893, [v], **27**, 300), formed pale yellow, amorphous laminæ or a white, amorphous powder, soluble in water and in alcohol, but insoluble in ether, and possessing a strongly bitter taste. It was stated to have the formula $C_{34}H_{48}O_9$, and, on heating with dilute sulphuric acid, to yield dextrose and an amorphous, yellow resin, designated as bryogenin, $C_{28}H_{38}O_4$. Another product obtained by Masson (*loc. cit.*), which was of a purely resinous nature, was termed bryoresin, and to this the formula $C_{37}H_{68}O_{18}$ was assigned.

A consideration of the method of preparation and characters of the so-called bryonin, as described in the literature, renders it apparent that it could not have represented a pure or homogeneous substance. Inasmuch as the present authors have recently made a complete examination of two other drugs obtained from cucurbitaceous plants, namely, elaterium (*Pharm. J.*, 1909, **83**, 501; Trans., 1909, **95**, 1985) and colocynth (Trans., 1910, **97**, 99), it was deemed of interest also to investigate the constituents of bryony root, especially as the latter is known to possess active purgative properties. The results of the present chemical investigation, and of the physiological tests, are summarised at the end of this paper.

Experimental.

The material employed for this investigation consisted of the roots of *Bryonia dioica*, Linné, which had been specially collected for us during the early part of October by Messrs. W. Ransom and Son, of Hitchin, under the personal superintendence of Mr. P. E. F. Perrédès, B.Sc., F.L.S. Our thanks are due to these gentlemen for the great care which they have exercised in this connexion.

The amount of fresh root collected was 107·5 kilograms, and this, after being sliced and dried, weighed 25·5 kilograms. The loss on drying was therefore equivalent to 76·3 per cent. of the original weight.

Separation of an Enzyme.

With consideration of the previously recorded statements (*loc. cit.*) that bryony root contains a glucoside, it was thought desirable to examine it for the presence of an enzyme. For this purpose one kilogram of the finely ground material was mixed with sufficient water to cover it, and the mixture kept for several hours, after which the aqueous liquid was expressed and filtered. To this liquid, in which the presence of starch was indicated, about twice its volume of alcohol was added, when a voluminous, light brown precipitate was produced. This was collected, washed with a little alcohol, and dried in a vacuum over sulphuric acid. It then amounted to 35 grams, or 3·5 per cent. of the weight of dried root employed. This product yielded the biuret reaction, and slowly hydrolysed both amygdalin and salicin, as also the glucosidic constituent of bryony root, which will subsequently be described.

Test for an Alkaloid.—Ten grams of the finely ground root were digested with Prollius' fluid, and the resulting liquid subjected to the usual tests for an alkaloid. The reactions thus obtained indicated the presence of a relatively small amount of such a substance.

Preliminary Extraction of the Root.—Twenty-five grams of the ground material were extracted successively in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—50°) extracted	0·19 gram	= 0·76 per cent.	
Ether	,,	0·22 ,,	= 0·88 ,, ,,
Chloroform	,,	0·52 ,,	= 2·08 ,, ,,
Ethyl acetate	,,	0·80 ,,	= 3·20 ,, ,,
Alcohol	,,	2·70 ,,	= 10·80 ,, ,,
	Total 4·43 grams	= 17·72 per cent.	

For the purpose of a complete examination, 23·9 kilograms of the ground bryony root were completely extracted with hot alcohol. After the removal of the greater portion of the alcohol, a viscid, dark-coloured extract was obtained, amounting to 6·3 kilograms.

Distillation of the Extract with Steam. Separation of an Essential Oil.

A quantity (2 kilograms) of the above-mentioned extract, representing 7·6 kilograms of the ground root, was mixed with water, and steam passed through the mixture for some hours. The distillate, which amounted to 2·5 litres, contained some drops of oil floating on the surface. It was extracted with ether, the ethereal liquid being washed, dried, and the solvent removed, when a small

quantity of an essential oil was obtained. This was a pale yellow liquid, which possessed a characteristic odour. The amount of this oil was, however, too small to permit of its further investigation.

Non-volatile Constituents of the Extract.

After the distillation of the extract with steam, as above described, there remained in the distillation flask a dark-coloured, aqueous liquid (A) and a quantity of a brown resin (B). The latter was collected, and repeatedly washed with water until nothing further was removed, the washings being added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

Isolation of a Crystalline, Neutral Substance, $C_{20}H_{30}O_5$.

The aqueous liquid (A), which amounted to 5 litres, was repeatedly extracted with ether, the ethereal extracts being then united, washed with water, and concentrated to the volume of 500 c.c. The ethereal solution so obtained was extracted successively with dilute hydrochloric acid, aqueous ammonium carbonate, sodium carbonate, and potassium hydroxide, which, however, only removed small quantities of resinous products. The ethereal solution was accordingly washed with water, dried, and the solvent evaporated, when a quantity of a syrupy liquid remained, from which a crystalline substance slowly separated. The mixture was diluted with ether, and the crystalline material collected. It formed small, colourless needles, melting at about 220°, and amounted to 1·5 grams. After recrystallisation from alcohol, and then again from ether, it separated in glistening needles, melting and decomposing at 220—222°:

0·1358 gave 0·3407 CO_2 and 0·1090 H_2O. C = 68·4; H = 8·9.

After another crystallisation from ether it was again analysed:

0·1352 gave 0·3390 CO_2 and 0·1090 H_2O. C = 68·4; H = 8·9.

$C_{20}H_{30}O_5$ requires C = 68·6; H = 8·6 per cent.

The molecular weight of the substance was determined by the cryoscopic method:

0·3688, in 22·93 of acetic acid, gave $\Delta t - 0·199°$. M.W. = 315.

$C_{20}H_{30}O_5$ requires M.W. = 350.

$C_{16}H_{24}O_4$ „ M.W. = 280.

The *substance* thus appears to possess the formula $C_{20}H_{30}O_5$, although the formula $C_{16}H_{24}O_4$ is not excluded, and it is evidently a new compound.

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

0·3040, made up to 20 c.c. with chloroform, gave α_D +1°47′ in a 2-dcm. tube, whence $[\alpha]_D$ +58·6°.

The substance is moderately soluble in alcohol, but very sparingly so in ether, and practically insoluble in water. It contains no methoxyl group, and no crystalline acetyl or other derivative could be prepared from it.

Isolation of an Amorphous, Glucosidic Product.

The original aqueous liquid (A) which had been extracted by means of ether, as above described, was thoroughly extracted with successive portions of amyl alcohol. These extracts were united, washed with water, and concentrated to a volume of 1·5 litres, when, on cooling, a considerable quantity (70 grams) of a light brown, amorphous product separated. This was collected, washed first with a little dry amyl alcohol, then with ethyl acetate, and subsequently extracted with the latter solvent in a Soxhlet apparatus. A relatively small portion of the product was thus removed, and formed, when dry, a yellowish-brown, amorphous powder. This was readily soluble in alcohol and in water, but all attempts to obtain it in a crystalline condition were unsuccessful. It possessed a bitter taste, and its aqueous solution gave a dense precipitate with tannic acid, but no coloration with ferric chloride. When heated with dilute sulphuric acid it was rapidly hydrolysed, with the production of a brown resin and a sugar, which yielded *d*-phenylglucosazone, melting at 208—210°. Its hydrolysis was also slowly effected by the enzyme contained in the root, although emulsin appeared to have little or no action on it. In view of the glucosidic character of the product, a portion of it was heated with acetic anhydride in the presence of a little *d*-camphorsulphonic acid. A vigorous reaction ensued, but no crystalline acetyl derivative could be obtained.

Isolation of an Amorphous Alkaloidal Principle.

The amyl-alcoholic mother liquors remaining from the separation of the above-described glucosidic product were diluted with amyl alcohol, and repeatedly shaken with dilute hydrochloric acid. The acid extracts were united, made alkaline with ammonia, and extracted with amyl alcohol, when a small quantity of material was removed, which was weakly basic, and responded to the usual alkaloid reagents. This product formed a brownish-yellow, intensely bitter, amorphous powder, which was soluble in water and in

alcohol, but almost insoluble in ether or chloroform. Its aqueous solution gives an abundant precipitate with tannic acid. When heated with hydrochloric acid, it was rapidly decomposed, with the formation of ammonia, and the latter was also evolved on heating the substance with alkali hydroxides. The alkaloidal principle appears to be incapable of forming any crystalline salt.

The original aqueous liquid, after being extracted with amyl alcohol as above described, was treated with a slight excess of a solution of basic lead acetate. A copious brown precipitate was thus produced, which, however, when decomposed by hydrogen sulphide, yielded nothing definite. The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the lead, and the filtered liquid concentrated to the consistency of a thin syrup. This contained a considerable quantity of sugar, since it readily yielded *d*-phenylglucosazone, melting at 208—210°.

Examination of the Resin (B).

The resin was a dark brown, viscid product, and amounted to 160 grams, being thus equivalent to about 2 per cent. of the weight of dried root employed. It was dissolved in alcohol, mixed with purified sawdust, and the dried mixture extracted successively in a Soxhlet apparatus with light petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

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

The petroleum extract was a viscid liquid, and amounted to 99 grams. It was dissolved in ether, and the ethereal solution shaken with aqueous potassium carbonate, which, however, only removed traces of fatty acids. The ether was accordingly evaporated, and the residue hydrolysed by boiling for some time with an alcoholic solution of potassium hydroxide. The alcohol was then evaporated, water added, and the alkaline aqueous liquid extracted with ether, the ethereal liquid being dried and the solvent removed, when a crystalline residue was obtained. This was recrystallised from a mixture of dilute alcohol and ethyl acetate, when it formed glistening plates, melting at 137°. The amount of this substance was 2·5 grams:

0·7532, on heating at 110°, lost 0·0354 H_2O. $H_2O = 4·7$.

0·1468 * gave 0·4490 CO_2 and 0·1590 H_2O. $C = 83·4$; $H = 12·0$.

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

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

* Anhydrous substance.

The substance thus agrees in composition with a phytosterol, and it yielded the colour reactions of that class of compounds. It was found to be optically inactive, as was also the case with the phytosterol obtained by the present authors from colocynth, although the two compounds are not identical (compare Trans., 1910, **98**, 105). The acetyl derivative, when crystallised from acetic anhydride, separated in glistening plates, melting at 155—157°.

Isolation of a New Dihydric Alcohol, Bryonol, $C_{22}H_{34}O_2(OH)_2$.

The alkaline liquid, which had been extracted with ether as above described, was acidified, and again extracted with ether. The ethereal extracts were united, after which a quantity of an almost colourless, sparingly soluble substance which accompanied them was separated by filtration. This substance was crystallised, first from a mixture of pyridine and ethyl acetate, and then from glacial acetic acid, when it was obtained in small, colourless plates, melting and decomposing at 210—212°. The quantity so obtained was about 0·8 gram:

0·1324 gave 0·3508 CO_2 and 0·1210 H_2O. C = 72·3; H = 10·1.
$C_{22}H_{36}O_4$ requires C = 72·4; H = 9·9 per cent.

This substance, when dissolved in chloroform with a little acetic anhydride, gave, on the addition of a few drops of concentrated sulphuric acid, a series of colour reactions similar to those produced by the dihydric alcohol ipurganol, $C_{21}H_{32}O_2(OH)_2$ (Power and Rogerson, *J. Amer. Chem. Soc.*, 1910, **32**, 89), and it appears, in fact, to be the next higher homologue of the latter. Like ipurganol, it dissolves in concentrated sulphuric acid with a yellow colour, the solution showing a green fluorescence.

No substance possessing the formula of that above described appears to have hitherto been recorded. Being, therefore, a new compound, it is proposed to designate it *bryonol*, with reference to the generic name of the plant from which it has been isolated.

Diacetylbryonol, $C_{22}H_{34}O_4(CO{\cdot}CH_3)_2$.—This was obtained by heating bryonol with acetic anhydride. It crystallises from alcohol in long needles, melting at 152°:

0·1184 gave 0·3000 CO_2 and 0·0960 H_2O. C = 69·1; H = 9·0.
$C_{26}H_{40}O_6$ requires C = 69·6; H = 8·9 per cent.

From the above results it is evident that bryonol belongs to a group of dihydric alcohols which are represented by the general formula $C_nH_{2n-8}O_4$. The known members of this group, all of which have been isolated in these laboratories, now comprise the following compounds: ipurganol, $C_{21}H_{32}O_2(OH)_2$ (*loc. cit.*); *bryonol*,

$C_{22}H_{34}O_2(OH)_2$; grindelol, $C_{23}H_{36}O_2(OH)_2$ (*Proc. Amer. Pharm. Assoc.*, 1907, **55**, 342); and cucurbitol, $C_{24}H_{38}O_2(OH)_2$ (*J. Amer. Chem. Soc.*, 1910, **32**, 367).

Examination of the Fatty Acids.

The ethereal liquid, from which the bryonol had been separated by filtration, as above described, was washed, dried, and the solvent removed, when a quantity (20 grams) of fatty acids was obtained, which, when distilled under diminished pressure, passed over between 230° and 260°/15 mm. The mixed acids were converted into their lead salts, and the latter digested with ether, when a portion 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 lead salts yielded 11 grams of liquid acids, whilst the insoluble portion gave 8·5 grams of solid acids.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over between 220° and 230°/15 mm. An analysis and a determination of the iodine value gave the following results:

0·1373 gave 0·3865 CO_2 and 0·1390 H_2O. C = 76·8; H = 11·2.

0·3195 absorbed 0·5410 iodine. Iodine value = 170.

$C_{18}H_{34}O_2$ requires C = 76·6; H = 12·1 per cent. Iodine value = 90·1.

$C_{18}H_{32}O_2$ „ C = 77·1; H = 11·4 „ „ Iodine value = 181·4.

It thus appears that the liquid acids consisted of a mixture of oleic and linolic acids, the latter predominating.

The Solid Acids.—These acids melted at 55—57°, and on analysis gave the following result:

0·1436 gave 0·3982 CO_2 and 0·1620 H_2O. C = 75·6; H = 12·6.

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

$C_{18}H_{36}O_2$ „ C = 76·1; H = 12·7 „ „

From this result it is evident that the solid acids consisted of a mixture of palmitic and stearic acids, and apparently in about equal proportions.

Ether, Chloroform, Ethyl Acetate, and Alcohol Extracts of the Resin.

These extracts amounted to 25, 15, 2, and 15 grams respectively. They were dark-coloured resins, and, with the exception of about 0·2 gram of the previously-described crystalline, neutral substance, which was isolated from the ethereal extract, nothing definite could be isolated from them.

Summary.

The material employed for the present investigation consisted of the roots of *Bryonia dioica*, Linné, which had been specially collected for the purpose.

The roots were found to contain an enzyme, which was obtained in the form of a light brown powder. This product slowly hydrolysed the glucosidic constituent of the root, and also effected the hydrolysis of amygdalin and salicin.

An alcoholic extract of the dried roots, when distilled in a current of steam, yielded a small amount of a pale yellow essential oil, which possessed a characteristic odour. From the portion of the extract which was soluble in water there were isolated: (i) a small amount of a colourless, crystalline, neutral *substance* (m. p. 220—222°), which appears to possess the formula $C_{20}H_{30}O_5$, and has $[\alpha]_D$ +58·6°; (ii) an amorphous, glucosidic product, having a brown colour and a bitter taste, which, when hydrolysed by heating with dilute sulphuric acid or by the enzyme contained in the root, yielded a brown resin and a sugar, from which *d*-phenylglucosazone (m. p. 208—210°) was prepared; (iii) an amorphous, alkaloidal principle, possessing a brownish-yellow colour and an intensely bitter taste, but which was very weakly basic, and appeared to be incapable of forming any crystalline salt. The aqueous liquid contained, furthermore, a quantity of sugar, which yielded *d*-phenylglucosazone (m. p. 208—210°).

The portion of extract which was insoluble in water consisted of a dark brown, viscid resin, amounting to about 2 per cent. of the weight of dried root employed. From this material the following compounds were isolated: (i) a phytosterol, $C_{27}H_{46}O$ (m. p. 137°), which was optically inactive; (ii) a new dihydric alcohol, *bryonol*, $C_{22}H_{34}O_2(OH)_2$, melting at 210—212°, and yielding a *diacetyl* derivative, melting at 152°. Bryonol evidently belongs to a group of dihydric alcohols possessing the general formula $C_nH_{2n-8}O_4$, which comprises the following additional compounds: ipurganol, $C_{21}H_{32}O_2(OH)_2$, grindelol, $C_{23}H_{36}O_2(OH)_2$, and cucurbitol, $C_{24}H_{38}O_2(OH)_2$; (iii) a mixture of fatty acids, consisting of oleic, linolic, palmitic, and stearic acids.

Inasmuch as both the above-mentioned glucosidic product and the alkaloidal principle, as well as the aqueous liquid from which they had been removed, were abundantly precipitated by tannic acid, it follows that the preparations obtained by previous investigators by means of this reagent, which were regarded as a glucoside and designated "bryonin," must have consisted of complex mixtures, the constituents of which, moreover, were not entirely glucosidic.

The various chemical formulæ that have been assigned to these amorphous compounds are accordingly in the highest degree fallacious.

In order to ascertain the source of activity of the root, a number of products obtained in the course of the present investigation were kindly tested for us by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories, to whom our best thanks may here be expressed. All the experiments were conducted with small dogs.

The crystalline, neutral substance, $C_{20}H_{30}O_5$, and the glucosidic product, in amounts of 0·1 gram, had no effect. The alkaloidal principle, in the same dose, produced slight purgation. The portion of the alcoholic extract which was soluble in water, and from which the above-mentioned products had previously been removed by successive extraction with ether and amyl alcohol, had no appreciable effect in amounts corresponding to about 4 grams of the dried root. The resinous material of the root, as well as the products obtained by its successive extraction with light petroleum, ether, chloroform, and ethyl acetate, produced marked purgation in doses of 1 gram, whereas the final alcohol extract of the resin had practically no effect.

From the above results it is obvious that the activity of bryony root cannot be attributed to a single definite principle, and it would appear that its purgative property resides chiefly in the resinous and alkaloidal constituents. The assumption of previous investigators that the active principle is a glucoside, has thus been shown to be incorrect.

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