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LXXVIII.—*The Constituents of the Leaves and Stems of Daviesia latifolia.*

By Frederick Belding Power and Arthur Henry Salway.

Daviesia latifolia, R. Br. (Nat. Ord. *Leguminosae*), is a shrub which attains a height of about 60 to 90 cm. It is indigenous to Victoria, Australia, mostly south of the dividing range, where it is generally found on small mountain ridges, but is also said to occur in parts of Tasmania and New South Wales. In the districts where it grows it is known as the "Native Hop Bush," a designation which is supposed to have been given it on account of the bitter taste of the leaves, which resembles that of the hop, rather than to any similarity in appearance to the common hop plant (*Humulus Lupulus*, L.). An infusion of the leaves is considered to possess the properties of a tonic, diuretic, and hepatic stimulant, and is also said to be used as a remedy for hydatids and low fevers.

Some years ago attention was particularly directed to the abovementioned species of *Daviesia* by J. Bosisto (*Pharm. J.*, 1898, **60**, 187), who, by the simple method of evaporating an aqueous infusion of the leaves, had obtained a small amount of a bitter, crystalline substance. This was subsequently examined by Paul and Cownley (*loc. cit.*), who were led to conclude from the results of their examination that it was either of a glucosidal character or of the nature of a plant sugar, and considered that its persistent bitter taste, after recrystallisation from water, was in favour of its being a glucoside. Their observation that the substance appeared to be unchanged when precipitated from its solution in aqueous sodium hydroxide by acids could, however, not have been correct, as the present investigation has shown.

The crystalline, bitter substance contained in the leaves and stems of *Daviesia latifolia* has now been obtained in considerable quantity by the present authors. It is not properly a glucoside, but a dibenzoyl derivative of a new disaccharide, containing both a dextrose and a xylose complex, and possesses the empirical formula $C_{25}H_{28}O_{12},H_2O$. Such a compound has not hitherto been known to occur in nature, and, in accordance with its composition, it has been designated *dibenzoylglucoxylose*. The various other substances isolated in the course of the present investigation are enumerated at the end of this paper.

EXPERIMENTAL.

The plant under consideration was kindly brought to our notice by Dr. E. Gutheil, of Ballarat, Victoria, Australia, who also sent specimens of it. A large amount of material, consisting of the stems, with leaves, flowers, and fruit attached, was subsequently specially collected for us in Victoria during the latter part of the year 1912. After being carefully dried in the air it amounted to about 45 kilograms.

A small portion (10 grams) of the ground material was first tested for the presence of an alkaloid, but with a negative result.

Another portion (25 grams) of the ground material was successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 100°, were obtained:

Petroleum (b. p. 35—50°)	extracted	0·33 gram	= 1·32	per cent.
Ether	,,	0·85 ,,	= 3·40	,,
Chloroform	,,	0·75 ,,	= 3·00	,,
Ethyl acetate	,,	1·17 ,,	= 4·68	,,
Alcohol	,,	3·29 ,,	= 13·16	,,
	Total	6·39 grams	= 25·56	per cent.

For the purpose of a complete examination, 38·9 kilograms of the ground material were extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, a viscid, dark-coloured extract was obtained, amounting to 14·5 kilograms.

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

Two kilograms of the above-mentioned extract were mixed with water, and the mixture was distilled in a current of steam. On extracting the distillate with ether, about 1 gram of a pale yellow essential oil was obtained, which gradually deposited some colourless crystals on keeping. The latter were collected and recrystallised from water, when the substance separated in glistening leaflets, melting at 121°, and was identified as benzoic acid. In order to effect its complete removal from the essential oil, the latter was dissolved in ether, the ethereal liquid being then washed successively with aqueous sodium carbonate and water, dried, and the solvent removed. After this treatment the essential oil distilled between 200° and 220°, and was obtained as a pale yellow liquid, possessing a pleasant, aromatic odour. It was tested for the presence of furfuraldehyde, but with a negative result.

After the distillation of the extract with steam, as above described, there remained in the distillation flask a straw-coloured, aqueous liquid (*A*), and a quantity of a green, soft resin (*B*). These products, when cold, were separated by filtration, and the resin well washed with water, the washings being added to the main portion of the aqueous liquid.

Examination of the Aqueous Liquid (A).

The aqueous liquid was repeatedly extracted with ether, the combined ethereal extracts being dried, and the solvent removed. The residue, amounting to 25 grams, consisted of a pale green, crystalline solid, which was evidently a mixture of substances.

Isolation of Benzoic, Salicylic, and p-*Coumaric Acids.*

The above-mentioned crystalline solid was redissolved in ether, and the ethereal liquid shaken with aqueous ammonium carbonate until nothing further was removed. On acidifying the alkaline liquid, it yielded about 20 grams of a crystalline solid, which was found to consist largely of benzoic acid, together with small amounts of other acids. In order to separate these substances, the mixture was subjected to distillation in a current of steam. The distillate was then extracted with ether, and the ethereal liquid evaporated, when 7 grams of an acid were obtained, which crystallised from water in glistening leaflets, melting at 121°. This substance was identified as benzoic acid. (Found, C=68·6; H=5·0. Calc., C=68·9; H=4·9 per cent.)

After the removal of the benzoic acid by distillation in steam, as above described, there remained in the distillation flask a semi-solid residue of non-volatile acids. These were converted into their methyl esters by heating with methyl alcohol in the presence of dry hydrogen chloride. The excess of methyl alcohol was then removed, water added to the mixture, and the esters extracted with ether. The ethereal liquid was first washed with aqueous sodium carbonate to remove any unchanged acid, and then with aqueous sodium hydroxide. The latter extract, containing any phenolic ester originally present in the esterified mixture, was first warmed for a short time and then acidified, when 2 grams of a dark-coloured solid were precipitated. This was collected, dissolved in hot water, and the solution treated with a little animal charcoal. On cooling the filtered liquid, colourless needles were deposited, which melted at 154—155°. The substance gave a violet coloration with ferric chloride, and was identified as salicylic acid. (Found, C=60·9; H=4·5. Calc., C=60·9; H=4·3 per cent.)

The mother liquors remaining after the separation of the salicylic acid yielded, on concentration, a small amount of an acid which crystallised from water in needles, melting and decomposing at 210°. In alcoholic solution it gave with ferric chloride a golden-brown coloration. (Found, C=65·7; H=4·9. $C_9H_8O_3$ requires C=65·9; H=4·9 per cent.)

The substance is thus seen to possess the composition and properties of *p*-coumaric acid. Its identity was further confirmed by the fact that when mixed with a known specimen of the last-mentioned acid no depression of the melting point ensued.

Isolation of Fumaric Acid.

The ethereal solution of methyl esters, from which the esters of salicylic and *p*-coumaric acids had been removed by means of aqueous sodium hydroxide, as above described, was next washed with water, dried, and the solvent removed. The residual oil, amounting to about 8 grams, was subjected to fractional distillation, when two principal fractions were obtained, the first of which distilled at 194—198° (atmospheric pressure), and consisted of methyl benzoate, whilst the other fraction passed over at 190—200°/10 mm. as a viscid, yellow oil. The latter product was hydrolysed by heating with alcoholic potassium hydroxide, when a well-crystallised potassium salt was immediately deposited. This was collected, washed with alcohol, then dissolved in a little water, and hydrochloric acid added. The organic acid was then deposited as a colourless precipitate, which crystallised from water in stellar clusters of small needles, melting and decomposing at 290°.

The alkaline mother liquors from the above hydrolysis contained a mixture of acids, from which a further quantity of the acid, melting at 290°, as well as a little benzoic acid, was obtained.

The acid melting at 290° was proved by analysis (Found, $C=41·4$; $H=3·6$) and the determination of the molecular weight of the methyl ester (m. p. 103°; Found, M.W. = 143. $C_2H_2(CO_2Me)_2$ requires M.W. = 144) to be fumaric acid, which is stated to melt at 287°, and to yield a dimethyl ester melting at 102°. Its identity with fumaric acid was further confirmed by mixing a little of the above-mentioned methyl ester (m. p. 103°) with a known specimen of dimethyl fumarate, when no depression of the melting point was observed.

The ethereal extract of the original aqueous liquid having been shaken with ammonium carbonate for the removal of the acids, as above described, it was next extracted with aqueous sodium hydroxide. The last-mentioned alkaline extracts, on acidification, yielded about 2 grams of a green solid, which was found to consist almost entirely of benzoic acid. It is evident that the benzoic acid obtained in this manner could not have been present as such in the ethereal liquid, since the latter had already been exhaustively shaken with ammonium carbonate, and it therefore must have been in the form of a benzoyl derivative of a compound which was easily hydrolysed in contact with alkali hydroxides. In order to isolate the respective compound, the ethereal liquid was washed with water, dried, and the solvent removed, when about 2 grams of a green, viscid residue were obtained. This was heated with water, the hot mixture filtered to remove some oily, insoluble material, and the filtrate allowed to cool. On keeping the solution for some time, a crystalline substance was deposited, which, after further purification from water, melted at 147—148°. This substance readily yielded benzoic acid when treated with alkali hydroxides, and proved to be identical with the dibenzoylglucoxylose which was subsequently isolated in larger amount from the aqueous liquid, as described below.

Isolation of a Benzoyl Derivative of a New Disaccharide, Dibenzoylglucoxylose, $C_{11}H_{18}O_{10}(CO\cdot C_6H_5)_2,H_2O$.

The original aqueous liquid (*A*) which had been extracted with ether, as described above, was next repeatedly shaken with amyl alcohol. The combined amyl-alcoholic extracts were washed with a little water, and the solvent removed for the most part at 100° under diminished pressure, when a dark-coloured, syrupy residue was obtained. In order to remove the last traces of amyl alcohol, water was added, and the mixture concentrated to about half its volume under diminished pressure. The residual aqueous liquid then contained about 30 grams of a dark-coloured, viscid product, which in the course of several days became crystalline. The crystalline substance was collected, washed with dilute alcohol to remove some adhering viscid material, and then further purified by recrystallisation, first from alcohol and finally from water. It separated from the latter solvent in thin, colourless needles, which melted at 147—148°, contained water of crystallisation, and possessed an extremely bitter taste. The amount of this substance isolated from 2 kilograms of the alcoholic extract of the drug was about 20 grams, but on subsequently working up the remainder of the extract the total yield was 220 grams, thus representing about 0·56 per cent. of the weight of drug employed. A larger amount of the crystalline, bitter substance than here indicated was, however, actually present in the drug, inasmuch as some remained in the mother liquors from its crystallisation, and a considerable amount was also contained in the resin. The substance was analysed, with the following results:

1·1792, heated at 105°, lost 0·0393 H_2O. $H_2O = 3·3$.
0·1073 * gave 0·2254 CO_2 and 0·0525 H_2O. C = 57·3; H = 5·4.
0·1105 * „ 0·2338 CO_2 „ 0·0548 H_2O. C = 57·7; H = 5·5.
$C_{25}H_{28}O_{12},H_2O$ requires $H_2O = 3·3$ per cent.
$C_{25}H_{28}O_{12}$ requires C = 57·7; H = 5·4 per cent.

The molecular weight of the compound was determined by Barger's microscopic method.

0·4404,* in 10 c.c. alcohol, was between 0·09 and 0·10 mol., using benzil as the standard.

Mean, M.W. = 465. $C_{25}H_{28}O_{12}$ requires M.W. = 520.

From the above results it is evident that the crystalline, bitter substance possesses the empirical formula $C_{25}H_{28}O_{12},H_2O$. Its further examination has revealed the fact that it is a dibenzoyl derivative of a new disaccharide, $C_{11}H_{20}O_{10}$, the latter yielding on

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* Anhydrous substance.

hydrolysis 1 molecule of dextrose and 1 molecule of xylose. It is therefore proposed to designate the disaccharide as *glucoxylose*, and the original compound is consequently a *dibenzoylglucoxylose*. The properties of the latter compound, together with the reactions by means of which its identity has been established, will be fully described in a subsequent communication.

Isolation of a Quercetin Glucoside, $C_{27}H_{30}O_{16}$.

After the original aqueous liquid had been extracted with amyl alcohol, as above described, it was concentrated somewhat under diminished pressure, and then treated with a slight excess of basic lead acetate solution, when an abundant, bright yellow precipitate was produced. The latter was collected, washed, suspended in water, decomposed by hydrogen sulphide, and the liquid filtered. The filtrate was concentrated under diminished pressure to a small volume, and then kept for some time, when a small amount (0·5 gram) of a crystalline, lemon-yellow solid slowly separated. This substance was purified by crystallisation from very dilute alcohol, and then obtained in thin needles, which sintered at about 180°, and gradually decomposed with effervescence as the temperature rose to 200°:

0·1066, heated at 130°, lost 0·0104 H_2O. $H_2O = 9·8$.
0·0944* gave 0·1823 CO_2 and 0·0430 H_2O. $C = 52·7$; $H = 5·1$.
$C_{27}H_{30}O_{16},4H_2O$ requires $H_2O = 10·6$ per cent.
$C_{27}H_{30}O_{16}$ requires $C = 53·1$; $H = 4·9$ per cent.

The above-described substance was only sparingly soluble in hot water, but readily soluble in alcohol. Its aqueous solution gave a bluish-black coloration with ferric chloride. In order to ascertain whether the substance was glucosidic, it was heated for some time in aqueous solution with dilute sulphuric acid, when a yellow precipitate was deposited. The latter, when collected and recrystallised from dilute alcohol, was obtained in yellow needles, melting and decomposing at 305—310°. It was readily soluble in alkalis, giving a deep yellow solution, and with ferric chloride it gave a bluish-black coloration. (Found, $C = 59·7$; $H = 3·6$. $C_{15}H_{10}O_7$ requires $C = 59·6$; $H = 3·3$ per cent.)

The composition and properties of this substance indicated it to be quercetin, and its identity with the latter was confirmed by the formation of an acetyl derivative, which crystallised in colourless needles, melting at 194—195°.

Since the aqueous liquid from the above-described hydrolysis readily reduced Fehling's solution, it is evident that the substance

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* Anhydrous substance.

hydrolysed was a quercetin glucoside. The composition of the latter renders it, moreover, probable that it was rutin, but the amount of substance available was too small to permit of the identification of the sugars.

The mother liquor remaining from the separation of the abovementioned glucoside was heated with dilute sulphuric acid, when a quantity of resinous material was deposited. Both this material and the aqueous, acid liquid from which it had separated were found to contain small quantities of benzoic acid and quercetin, thus indicating the presence in the original mother liquor of a small amount of the previously described dibenzoylglucoxylose, together with a quercetin glucoside.

The filtrate from the precipitate produced in the original aqueous liquid by the addition of basic lead acetate was deprived of lead by means of hydrogen sulphide, again filtered, and concentrated to the consistency of a syrup. The latter evidently contained a quantity of sugar, since it readily yielded *d*-phenylglucosazone (m. p. 210°). The syrup possessed a slightly bitter taste, but on keeping for a long time nothing crystalline separated from it. On heating with alkalis it developed ammonia, but no precipitate was produced by mercuric nitrate, nor could any definite basic substance be obtained by extracting the syrup with alcohol and subsequent treatment of the alcoholic solution.

Examination of the Resin (B).

The crude, resinous material which had been separated from the aqueous liquid (*A*), as previously described, was mixed with purified sawdust, and the thoroughly dried mixture extracted in a large Soxhlet apparatus with various solvents. The weights of the products obtained, dried at 100°, were as follows:

Petroleum (b. p. 35—50°)	extracted	309 grams.
Ether	,,	119 ,,
Chloroform	,,	19 ,,
Ethyl acetate	,,	12 ,,
Alcohol	,,	5 ,,
	Total	464 grams.

The above amount of resinous material, having been obtained from 2 kilograms of the alcoholic extract, is equivalent to about 8·6 per cent. of the weight of drug employed.

Petroleum Extract of the Resin.

This extract was a dark green, semi-solid, fatty mass, which possessed a bitter taste. It was dissolved in a large volume of ether, and the ethereal solution agitated with aqueous ammonium carbonate. The alkaline extract, on acidification, yielded a quantity (2 grams) of a crystalline solid, which was purified by recrystallisation from dilute alcohol. The substance was thus obtained in glistening leaflets, melting at 120°, and was identified as benzoic acid.

Isolation of Myricyl Alcohol, $C_{30}H_{62}O$, *and Hentriacontane,* $C_{31}H_{64}$.

The ethereal solution which had been extracted with ammonium carbonate was evaporated, and the semi-solid residue dissolved in hot ethyl acetate. On cooling this solution, it deposited a considerable quantity (25 grams) of a solid, which was collected, the filtrate being set aside for subsequent examination. The solid material was fractionally crystallised from petroleum of high boiling point, the least soluble fraction (1 gram) thus obtained being then dissolved in hot ethyl acetate, and the solution decolorised by means of animal charcoal. The filtered liquid, on cooling, deposited thin, colourless needles, melting at 82—83°. (Found, C = 82·3; H = 14·2. $C_{30}H_{62}O$ requires C = 82·2; H = 14·2 per cent.)

This substance was thus identified as myricyl alcohol.

The mother liquors from the above-mentioned fractional crystallisation were united, the solvent was removed, and the residue then heated at 140° with an equal weight of phthalic anhydride. The product of the reaction was dissolved in ether, and the ethereal solution shaken with aqueous sodium carbonate, which effected the removal of the myricyl alcohol in the form of a sodium salt of its hydrogen phthalic ester. The ethereal liquid was then washed, dried, and the solvent removed, when about 20 grams of a solid were obtained, which crystallised from ethyl acetate in glistening leaflets, melting at 67—68°. (Found, C = 85·0; H = 14·5. $C_{31}H_{64}$ requires C = 85·3; H = 14·7 per cent.)

It is evident from these results that the substance is hentriacontane.

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

The ethyl acetate filtrate remaining after the removal of myricyl alcohol and hentriacontane, as described above, was evaporated, and the residue heated with an excess of alcoholic potassium hydroxide. The mixture was then freed from alcohol, water added, and the alkaline liquid extracted with ether. About 5 grams of a reddish-yellow solid were thus obtained, which was subjected to fractional crystallisation from alcohol. The least soluble fraction melted at 73—74°, and was evidently a mixture of myricyl alcohol and hentriacontane, whereas the most soluble fraction crystallised in thin leaflets, which, after further purification from ethyl acetate, melted at 135—136°. This substance gave the colour reactions of the phytosterols, and evidently belonged to that class of compounds.

0·0844 * gave 0·2594 CO_2 and 0·0898 H_2O. C=83·8; H=11·8.

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

Examination of the Fatty Acids.

The aqueous, alkaline liquid which had been extracted with ether for the removal of the unsaponifiable material, as above described, was acidified with dilute sulphuric acid, and again extracted with ether. This ethereal extract was filtered to remove a quantity (5 grams) of indefinite resinous material, the liquid being then washed, dried, and the solvent removed. The residual fatty acids were then converted into their methyl esters by heating with methyl alcohol in the presence of dry hydrogen chloride. The product thus obtained was distilled under diminished pressure, and three fractions were collected, which boiled respectively at 100—160°, 160—200°, and 200—210°/15 mm. The first fraction, which amounted to about 7 grams, was found to consist largely of methyl benzoate. The middle fraction (b. p. 160—200°/15 mm.) was very small in amount, the larger proportion of the methyl esters being contained in the fraction distilling at 200—210°/15 mm. As this contained some unsaturated esters, it was hydrolysed, the fatty acids thus obtained converted into their lead salts, and the latter treated with ether. The portion soluble in ether, when decomposed by hydrochloric acid, yielded 5 grams of liquid acids, whilst the insoluble portion gave about 3 grams of solid acids.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over between 225 and 235°/15 mm., the greater part distilling at 230—233°/15 mm. as a pale yellow oil. An analysis and a determination of the neutralisation and iodine values gave the following results:

0·0807 gave 0·2280 CO_2 and 0·0835 H_2O. C=77·1; H=11·5.

Neutralisation value=201; iodine value=173·2.

$C_{18}H_{32}O_2$ requires C=77·1; H=11·4 per cent. N.V.=200·4; I.V.=181·4.

It would appear from these results that the liquid acid consisted of nearly pure linolic acid.

The Solid Acids.—These acids, when crystallised from alcohol, separated in glistening leaflets, melting at 57—58°. The material was analysed and its neutralisation value determined:

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* Anhydrous substance.

0·0972 gave 0·2680 CO_2 and 0·1106 H_2O. C=75·2; H=12·6.
Neutralisation value=211.

$C_{18}H_{36}O_2$ requires C=76·1; H=12·7 per cent. N.V.=197·5.
$C_{16}H_{32}O_2$ „ C=75·0; H=12·5 „ „ N.V.=219·1.

The above results indicate the solid acids to have been a mixture of palmitic and stearic acids.

Ethereal Extract of the Resin.

This extract, when free from solvent, formed a dark green, viscid mass. It was digested with ether, when a portion of it (28 grams) was found to be sparingly soluble. The sparingly soluble material was collected, dissolved in a small quantity of alcohol, and the solution kept for some time, when a crystalline substance was slowly deposited. The latter, when recrystallised from water, separated in colourless needles, melting at 146—147°, and was found to be identical with the dibenzoylglucoxylose previously isolated from the original aqueous liquid (*A*). The alcoholic mother liquor remaining from the first crystallisation of the above-mentioned compound evidently still contained a considerable quantity of it, for, on heating with an alkali hydroxide, benzoic acid and a sugar were formed, and no other definite product could be isolated.

The portion of the ethereal extract of the resin which was readily soluble in ether was extracted successively with aqueous ammonium carbonate and sodium hydroxide. The first-mentioned alkali removed about 5 grams of benzoic acid, whilst the sodium hydroxide extract, on acidification, yielded a considerable quantity of dark-coloured, resinous material, from which nothing definite, except benzoic acid, could be isolated. After extraction with alkalis, the ethereal liquid was washed, dried, and the solvent removed. A residue was thus obtained, which, by treatment with petroleum of high boiling point, yielded a small quantity of myricyl alcohol, melting at 81—82°.

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

These extracts were small in amount, and formed dark-coloured, viscid products, which possessed a bitter taste. They were separately heated in dilute alcoholic solution with sulphuric acid, when each of them was found to yield both benzoic acid and a reducing sugar, thus indicating the presence of some of the previously-described dibenzoylglucoxylose. The last-mentioned compound, melting at 146—147°, was, moreover, directly isolated from the ethyl acetate extract of the resin by dissolving it in a little alcohol, and keeping the solution for some time. No other definite product could be obtained from these extracts.

Summary.

The material employed for this investigation consisted of the leaves and stems of *Daviesia latifolia*, R. Br. (Nat. Ord. *Leguminosae*), which had been specially collected for the purpose in Victoria, Australia.

An alcoholic extract of the material, when distilled with steam, yielded a small amount of a pale yellow essential oil, which possessed a pleasant, aromatic odour, and gradually deposited some crystals of benzoic acid.

From the portion of the alcoholic extract which was soluble in water the following definite compounds were isolated: (1) Benzoic, salicylic, *p*-coumaric, and fumaric acids; (2) a crystalline benzoyl derivative of a new disaccharide (*glucoxylose*), which possesses an extremely bitter taste. This bitter substance has the empirical formula $C_{25}H_{28}O_{12},H_2O$, melts at 147—148°, and has been designated *dibenzoylglucoxylose.* (3) A quercetin glucoside, $C_{27}H_{30}O_{16}$, which is probably identical with rutin. The aqueous liquid contained, furthermore, a quantity of sugar, which yielded *d*-phenylglucosazone (m. p. 210°).

The portion of the alcoholic extract which was insoluble in water, consisting chiefly of resinous material, amounted to about 8·6 per cent. of the weight of the drug. From the resinous material there were isolated: (1) Myricyl alcohol, $C_{30}H_{62}O$; (2) hentriacontane, $C_{31}H_{64}$; (3) a phytosterol, $C_{27}H_{46}O$; (4) a mixture of fatty acids, consisting of palmitic, stearic, and linolic acids. The resin also contained a considerable proportion of the above-mentioned dibenzoylglucoxylose, together with free benzoic acid.

This investigation has shown that the bitterness of the leaves of *Daviesia latifolia* is due to the crystalline substance which has been designated dibenzoylglucoxylose. The latter represents a type of compound which has not hitherto been observed to occur in nature, and its characters will be fully described in a subsequent communication.

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