XXXII.—*The Constituents of the Bark of Prunus serotina. Isolation of* l-*Mandelonitrile Glucoside.*

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The bark of *Prunus serotina*, Ehrhart (*Prunus virginiana*, Miller), commonly known as "Wild Cherry Bark," has long been used medicinally, and is recognised by both the United States and British Pharmacopœias. As it yields benzaldehyde and hydrocyanic acid in contact with water, it was quite naturally supposed to contain amygdalin or an analogous substance, although the attempts which have hitherto been made to isolate the cyanogenetic compound were unsuccessful. It had also been observed some years ago (*Pharm. Rundschau*, New York, 1887, **5**, 205) that the bark contains a fluorescent principle, but the identity or composition of this substance was not ascertained.

From the above considerations, it will be seen that, up to the present time, very little has been known regarding the more important constituents of wild cherry bark, and it was therefore deemed of interest to subject it to a complete chemical examination. Such an investigation appeared particularly desirable in view of the fact that, within the past few years, three isomeric mandelonitrile glucosides, $C_{14}H_{17}O_6N$, have been isolated from natural sources, and that one of these, *l*-mandelonitrile glucoside, was obtained from a closely-related species of *Prunus*, namely, *P. Padus*, Linné. The designations of the three isomeric glucosides, and the sources from which they were obtained, are as follows: "sambunigrin," from the leaves of the common black elder (*Sambucus nigra*, Linné), by Bourquelot and Danjou (*Compt. rend.*, 1905, **141**, 598; *Arch. Pharm.*, 1907, **245**, 202, 474); "prulaurasin," from the leaves of the cherry-laurel (*Prunus Lauro-cerasus*, Linné), by Hérissey (*Compt. rend.*, 1905, **141**, 959; *Arch. Pharm.*, 1907, **245**, 463, 473); and "amygdonitrile glucoside," from the young twigs of *Cerasus Padus*, Delarb. (*Prunus Padus*, Linné), by Hérissey (*Arch. Pharm.*, 1907, **245**, 475, 641; compare also Caldwell and Courtauld Trans., 1907, **91**, 671).

With regard to the relationship of the three above-mentioned compounds, namely, "sambunigrin," "prulaurasin," and "amygdonitrile glucoside" (*l*-mandelonitrile glucoside), it may be noted that they represent the β-glucosides of dextro-, racemic, and lævo-mandelonitrile respectively. Although *l*-mandelonitrile glucoside, frequently designated as "Fischer's glucoside," had been obtained by E. Fischer (*Ber.*, 1895, **28**, 1508), by Caldwell and Courtauld (Trans., 1907, **91**, 670), and by Auld (Trans., 1908, **93**, 1276), by the partial hydrolysis

of amygdalin, its isolation by Hérissey (*loc. cit.*) was, until now, the only instance in which its occurrence in nature had been observed.

The present investigation of the bark of *Prunus serotina*, Ehrhart, has resulted in the isolation therefrom of *l*-mandelonitrile glucoside (m. p. 145—147°; $[\alpha]_D$ −29·6°), which has also been obtained in the form of its tetra-acetyl derivative (m. p. 136—137°; $[\alpha]_D$ −24°). This is, therefore, the second instance in which this glucoside has been observed to occur in nature. A summary of the results of the complete investigation of the bark, in the course of which a number of other substances have been isolated, is given at the end of this paper.

Experimental.

The material employed in the present investigation was obtained from the United States, and conformed in every respect to the description of the bark of *Prunus serotina*, Ehrhart, as recognised by the United States Pharmacopœia.

In order to determine the amount of hydrogen cyanide yielded by the bark, portions of 25 and 50 grams respectively of the finely-ground material were macerated with water for several days in a tightly-closed flask at a temperature of 20—25°. Steam was then passed through the mixture, and the distillate collected in a very dilute solution of sodium hydroxide. After the addition of a little sodium chloride, the product of distillation was titrated with a decinormal solution of silver nitrate. The amount of the latter solution required for 25 grams of bark was 3·4 c.c., and for 50 grams of bark, 7·0 c.c., thus corresponding to 0·0734 and 0·0756 per cent. of HCN respectively.

A portion of the bark was tested for the presence of an alkaloid, but with a negative result.

Fifty grams of the ground bark were successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 110°, were obtained:

Petroleum (b. p. 35—50°) extracted	0·33 gram	=	0·66	per cent.
Ether	2·47 ,,	=	4·94	,,
Chloroform	0·35 ,,	=	0·70	,,
Ethyl acetate	1·48 ,,	=	2·96	,,
Alcohol	3·53 ,,	=	7·06	,,
Total	8·16 grams	=	16·32	per cent.

For the purpose of a complete examination, a quantity (53·3 kilograms) of the ground bark was 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 14·09 kilograms.

Distillation of the Extract with Steam. Separation of Benzoic Acid and an Essential Oil.

A quantity (4·5 kilograms) of the above-mentioned extract, representing about 17 kilograms of bark, was mixed with water, and steam passed through the mixture for several hours. The first portion of the distillate was tested for hydrogen cyanide, but with a negative result, thus affording evidence that no hydrolysis of the cyanogenetic glucoside had taken place in the process of distillation. The entire distillate, which amounted to about 8 litres, had an acid reaction, and contained some drops of oil floating on the surface. It was thoroughly extracted with ether, and the ethereal liquid shaken with a 5 per cent. solution of sodium carbonate. On acidifying the alkaline liquid, a substance separated in small, glistening plates, which, after recrystallisation from hot water, melted at 119—120°:

0·1665 gave 0·4210 CO_2 and 0·0785 H_2O. C = 68·9; H = 5·2.

$C_7H_6O_2$ requires C = 68·8; H = 4·9 per cent.

This substance was thus identified as benzoic acid, and the amount obtained was 3 grams.

The ethereal liquid, from which the benzoic acid had been extracted as above described, was washed, dried, and the ether removed, when a small amount (2·7 grams) of an essential oil was obtained. This oil had an agreeable, aromatic odour, quite distinct from that of benzaldehyde. It distilled between 100° and 120°/15 mm. as a pale yellow liquid, which, at 4°, deposited a very small quantity of a crystalline substance. The amount of essential oil was too small for its further examination.

Non-volatile Constituents of the Extract.

After the distillation of the extract with steam, as above described, there remained in the distillation flask a quantity of a green resin (A) and a dark-coloured, aqueous liquid. The latter was separated by filtration while still hot, and the green resin repeatedly treated with boiling water until nothing further was removed. The aqueous liquid and washings from the green resin were united, and allowed to stand for several days, when a quantity of a brown resin (B) slowly separated. This was removed from the liquid, and thoroughly washed with cold water, the washings being added to the aqueous liquid, which may be designated as (C).

Examination of the Green Resin (A).

This resin at the ordinary temperature was a dark green, waxy solid, and amounted to 180 grams. It was dissolved in alcohol and mixed with purified sawdust, the thoroughly dried mixture being then successively extracted in a Soxhlet apparatus with the following solvents: petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Green Resin.

This extract was a dark green, viscous liquid, and amounted to 80 grams. It was dissolved in ether, the ethereal solution being extracted with successive portions of aqueous sodium carbonate, and finally washed with water. The alkaline liquids and washings were united, acidified, and extracted with ether, when 27 grams of a viscid, oily liquid were obtained. On distilling this liquid under diminished pressure, it passed over between 215° and 270°/10 mm., and then became almost solid. It consisted of a mixture of fatty acids, which were examined in connexion with a similar product obtained from the non-acidic portion of the petroleum extract after its hydrolysis.

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

The ethereal liquid which had been extracted with sodium carbonate, as above described, was subsequently shaken with a solution of sodium hydroxide, which, however, removed nothing. The ether was then evaporated, when a quantity (40 grams) of an oily product was obtained. This was hydrolysed by heating with an alcoholic solution of potassium hydroxide, the alcohol 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 brown, crystalline product was obtained. This was distilled under diminished pressure, and then crystallised from a mixture of ethyl acetate and dilute alcohol, when it separated in fine, glistening, flat needles melting at 135—136°. The amount of this substance was 7 grams. After drying at 110°, it was analysed:

0·1342 gave 0·4100 CO_2 and 0·1474 H_2O. C = 83·3; H = 12·2.

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

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

0·2450, dissolved in 25 c.c. of chloroform, gave α_D −0°40′ in a 2-dcm. tube, whence $[\alpha]_D$ −34·0°.

The acetyl derivative, when crystallised from acetic anhydride, separated in needles melting at 118—119°.

0·2160, dissolved in 25 c.c. of chloroform, gave α_D −0°34′ in a 2-dcm. tube, whence $[\alpha]_D$ −32·8°.

The above-mentioned phytosterol was tested for the presence of stigmasterol, $C_{30}H_{50}O$, according to the method described by Windaus and Hauth (*Ber.*, 1907, **40**, 3681), and was found to be free from the latter compound. The melting point of its acetate would indicate that it is not identical with sitosterol (*Zeitsch. physiol. Chem.*, 1902, **34**, 461), but it agrees closely in its character with the phytosterol obtained from olive bark (Trans., 1908, **93**, 909).

Identification of the Fatty Acids.

The alkaline, aqueous solution of potassium salts from which the phytosterol had been removed by extraction with ether, as above described, was acidified, and again extracted with ether, the ethereal solution being 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 215° and 270°/10 mm. As these acids distilled within the same range of temperature as those previously obtained, which existed in the bark in a free state, for the purpose of their examination the two portions were mixed.

Twenty grams of the total mixed acids were converted into their lead salts, and the latter digested with ether, when a 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, when in each case they amounted to about 8 grams. The acids obtained from the soluble portion of lead salt were liquid, whilst those from the insoluble portion were solid.

The Liquid Acids.—These acids were fractionally distilled under diminished pressure, and the following fractions collected: (*a*) 214—216° (about 5 grams); (*b*) 216—230°/12 mm. (about 2 grams). They were analysed and their iodine values determined, with the following results:

(*a*) 0·1730 gave 0·4855 CO_2 and 0·1784 H_2O. C = 76·5; H = 11·4.
0·4190 absorbed 0·6312 iodine. Iodine value = 150·6.

(*b*) 0·1890 gave 0·5360 CO_2 and 0·1930 H_2O. C = 77·3; H = 11·3.
0·2152 absorbed 0·3372 iodine. Iodine value = 156·7.

$C_{18}H_{34}O_2$ 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 „ „ = 181·4.

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

In order to obtain more definite information respecting the composition of the above mixture of unsaturated acids, the remaining portions of the two fractions (6 grams) were united, and oxidised in alkaline solution with potassium permanganate in the cold. On subsequently treating the mixture with a slight excess of sulphur dioxide, a white precipitate was produced, which was collected and digested with much ether. This removed about 0·2 gram of an acid melting at 128°, which was evidently dihydroxystearic acid, and its formation confirmed the presence of oleic acid in the mixture. The portion of the white precipitate which was not dissolved by the ether was almost completely soluble in hot water, from which, on cooling, a quantity (1·2 grams) of a crystalline substance separated, which melted at 158—160°:

0·0895 gave 0·2042 CO_2 and 0·0845 H_2O. C = 62·2; H = 10·5.
$C_{18}H_{36}O_6$ requires C = 62·1; H = 10·3 per cent.

This substance was a tetrahydroxystearic acid, and the amount obtained confirmed the previous indication of a predominating proportion of linolic acid in the original mixture.

The acid, aqueous liquid, which was separated by filtration from the above-mentioned white precipitate of oxidation products, was evaporated to dryness, and the residue extracted with alcohol. A very small amount (0·1 gram) of an acid melting at 168—170° was thus obtained. This was evidently a hexahydroxystearic acid (*iso*linusic acid), and its formation indicated the presence of a small proportion of *iso*linolenic acid, $C_{18}H_{30}O_2$, in the original mixture.

The Solid Acids.—These acids were fractionally crystallised from glacial acetic acid, but both the first and last fractions, (*a*) and (*b*), melted at about 56—58°, and possessed practically the same composition:

(*a*) 0·1320 gave 0·3670 CO_2 and 0·1520 H_2O. C = 75·8; H = 12·8.
(*b*) 0·1160 ,, 0·3225 CO_2 ,, 0·1325 H_2O. C = 75·8; H = 12·7.
$C_{16}H_{32}O_2$ requires C = 75·0; H = 12·5 per cent.
$C_{18}H_{36}O_2$,, C = 76·1; H = 12·7 ,,

As it was evident that no separation of the acids had been effected by crystallisation, a portion of the mixture was fractionally precipitated in the form of silver salts:

I. 0·1560 gave, on ignition, 0·0445 Ag. Ag = 28·5.
II. 0·1570 ,, ,, ,, 0·0450 Ag. Ag = 28·7.
III. 0·1700 ,, ,, ,, 0·0490 Ag. Ag = 28·8.
$C_{16}H_{31}O_2Ag$ requires Ag = 29·8 per cent.
$C_{18}H_{35}O_2Ag$,, Ag = 27·6 ,,

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 Ipuranol, $C_{23}H_{38}O_2(OH)_2$.

This extract was a brown, amorphous mass, and amounted to 50 grams. It was redissolved in about 1 litre of warm ether, and the ethereal solution allowed to stand for several days, when a very small amount (0·5 gram) of a grey precipitate was slowly deposited. This was collected and crystallised several times from a mixture of pyridine and dilute alcohol, when 0·2 gram of a substance was obtained which, under the microscope, was seen to consist of small rosettes of needles. It melted between 285° and 290° :

0·1178 gave 0·3125 CO_2 and 0·1130 H_2O. C = 72·3 ; H = 10·7.

$C_{23}H_{40}O_4$ requires C = 72·6 ; H = 10·5 per cent.

The substance yielded a colour reaction similar to that produced by the phytosterols. Thus, when dissolved in chloroform with a little acetic anhydride, and a drop of sulphuric acid subsequently added, a pink colour was produced, which rapidly changed to blue, and finally to green. When heated with acetic anhydride, an acetyl derivative was obtained which separated in leaflets melting at 160°.

The above results rendered it evident that the substance in question was ipuranol, a dihydric alcohol, which was first obtained in these laboratories from *Ipomoea purpurea*, Roth, and this has subsequently been isolated from nutmeg and from olive bark (*Amer. J. Pharm.*, 1908, **80**, 264, 576 ; Trans., 1908, **93**, 907).

The ethereal solution from which the ipuranol had been deposited, as above described, was shaken with several successive portions of aqueous sodium carbonate and finally washed with water. A quantity of a black, insoluble sodium compound was thus obtained, which, when acidified, yielded a quantity of resinous substance. As nothing definite could be directly separated from it, the substance was dissolved in alcohol, a little water added, and such an amount of sulphuric acid that the latter represented 5 per cent. of the mixture. The whole was then heated for several hours in a reflux apparatus, and the mixture subsequently distilled with steam, but no volatile product was obtained. There remained in the distillation flask a brown, aqueous liquid, together with a dark-coloured, resinous mass. The latter was collected, mixed with purified sawdust, and the thoroughly-dried mixture extracted with light petroleum. This removed about 10 grams of a viscid liquid, which, when distilled under diminished pressure, passed over between 210° and 220°/15 mm. as a clear, pale yellow oil,

amounting to about 5 grams. This oily liquid dissolved in warm alkali hydroxide, the solution becoming gelatinous on cooling. On allowing the oily liquid to stand for a few days, it deposited a very small amount of a saturated acid, which, after recrystallisation, melted at 50—55°. The liquid portion was unsaturated, and its analysis and determination of the iodine value gave the following results:

0·1273 gave 0·3565 CO_2 and 0·1390 H_2O. C = 76·4; H = 12·1.

0·1976 absorbed 0·1563 iodine. Iodine value = 79·1.

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

These results indicated that the oily liquid consisted to a large extent of oleic acid, which must have existed in the resin in the form of an ester other than a glyceride, although such an occurrence of oleic acid appears hitherto not to have been recorded.

The acid, aqueous liquid contained in the distillation flask, from which the above-described resinous material had been removed, was treated with baryta for the removal of the sulphuric acid. The filtered liquid, after concentration under diminished pressure, reduced Fehling's solution, and yielded a small amount of *d*-phenylglucosazone, melting at 206—208°. It was thus apparent that at least a portion of the above-described ethereal extract of the resin was glucosidic.

Chloroform, Ethyl Acetate, and Alcohol Extracts of the Green Resin. Isolation of β-Methylaesculetin.

These extracts all consisted of brown, resinous material, and amounted to 9, 16, and 20 grams respectively. As nothing of a definite nature could be isolated from them by the ordinary methods, they were united and heated for several hours in a reflux apparatus with dilute alcohol containing 5 per cent. of its weight of sulphuric acid. After the addition of water and the removal of the alcohol by distillation with steam, there remained in the distillation flask a brown, aqueous liquid, together with a quantity of resinous material. Nothing could be isolated from the resin, but on repeatedly extracting the acid, aqueous liquid with ether, a small quantity (0·3 gram) of a crystalline substance was obtained. This substance melted at 204°, and its solution in alkalis exhibited a fine blue fluorescence:

0·1272 gave 0·2920 CO_2 and 0·0484 H_2O. C = 62·6; H = 4·2.

$C_{10}H_8O_4$ requires C = 62·5; H = 4·2 per cent.

This substance was thus identified as β-methylæsculetin. Further quantities of it were subsequently obtained from the brown resin (B), and from the original aqueous liquid designated as (C), after hydrolysis with dilute sulphuric acid.

Examination of the Brown Resin (B).

This was a dark brown powder, and amounted to 171 grams. It was dissolved in alcohol, and mixed with purified sawdust, the thoroughly-dried mixture being then successively extracted in a Soxhlet apparatus with the following solvents: ether, chloroform, ethyl acetate, and alcohol.

Ethereal Extract of the Brown Resin.

This extract consisted of brown, resinous material, and amounted to only 15 grams. As nothing definite could be obtained from it by the usual methods or by extraction with alkalis, it was heated in a reflux apparatus with dilute alcohol containing 5 per cent. of its weight of sulphuric acid. After the addition of water, and the removal of the alcohol, a small amount of soft, resinous material was deposited. This was extracted with light petroleum, when a product was obtained which, after distillation under diminished pressure, yielded 5 grams of a liquid fatty acid. The latter was analysed, and its iodine value determined, with the following results:

0·1243 gave 0·3540 CO_2 and 0·1344 H_2O. C = 77·7; H = 12·0.

0·2030 absorbed 0·1829 iodine. Iodine value = 90·1.

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

This liquid acid evidently consisted for the most part of oleic acid, with a small amount of some substance of higher carbon content.

Chloroform Extract of the Brown Resin.

This was a light brown resin, which amounted to only 2 grams. It was redissolved in chloroform, and the solution extracted with several portions of aqueous sodium hydroxide. The combined alkaline liquids were acidified and extracted with chloroform, when about 0·1 gram of a crystalline substance was obtained. This substance melted at 204°, and was identified as β-methylæsculetin, which, in the present instance, was evidently contained in the bark in a free state and not in the form of its glucoside.

Ethyl Acetate and Alcohol Extracts of the Brown Resin.

Both these extracts consisted of brown, resinous material, and they amounted to about 15 grams and 140 grams respectively. As nothing definite could be obtained from them by the ordinary methods, they were united, and heated for several hours in a reflux apparatus with dilute alcohol containing 5 per cent. of its weight of sulphuric

acid. The mixture was then diluted with water, and the alcohol removed by distillation with steam, when there remained in the distillation flask a red, aqueous liquid and a quantity of finely-divided red resin. The latter, when collected and dried, amounted to 150 grams.

The above-mentioned red, aqueous liquid on extraction with ether yielded 0·4 gram of β-methylæsculetin. After this treatment the sulphuric acid was removed by means of baryta, and the filtered liquid concentrated under diminished pressure. It was found to contain sugar, since it readily reduced Fehling's solution and yielded *d*-phenylglucosazone, melting at 205—206°.

The red, resinous material above described was only partly soluble in alcohol. The soluble portion was mixed with purified sawdust, and the thoroughly-dried mixture successively extracted in a Soxhlet apparatus with light petroleum and chloroform.

Petroleum Extract of the Red Resin.—This extract was very small in amount, and from it only a little phytosterol (m. p. 128—130°) was obtained.

Chloroform Extract of the Red Resin.—This extract was likewise small in amount. It was redissolved in chloroform, and the solution extracted with aqueous sodium hydroxide. On acidifying the alkaline solution, a product was obtained which, when recrystallised from alcohol, melted at 204°, and was identified as β-methylæsculetin. The amount of the latter substance obtained from this source was 1·3 grams, and it is probable that it was produced by the hydrolysis of some methylæsculin which had been occluded in the brown resin, for the aqueous liquid (C), subsequently to be described, yielded after hydrolysis a relatively large quantity of β-methylæsculetin, which undoubtedly was present in the form of its glucoside.

Fusion of the Insoluble Red Resin with Potassium Hydroxide.—As noted above, only a portion of the red, resinous material was soluble in alcohol. Of the insoluble portion, 25 grams were reduced to powder, and gradually introduced into 150 grams of potassium hydroxide in a state of fusion, the temperature of the mixture being maintained at 240—260° for a considerable time. After cooling, the mass was dissolved in water, the solution acidified with sulphuric acid, and distilled with steam. The distillate contained a quantity of volatile acid, which was converted into a barium salt, the latter amounting to 10 grams. On examining this salt, it was ascertained that the volatile acid consisted largely of acetic acid, with a relatively small proportion of formic and butyric acids.

After the removal of the volatile acids by distillation with steam, as above described, the contents of the distillation flask were extracted with ether. This removed about 5 grams of a crystalline

substance which, after repeated crystallisation from water, melted at 196—198°:

0·1252 gave 0·2510 CO_2 and 0·0470 H_2O. C = 54·7; H = 4·2.

$C_7H_6O_4$ requires C = 54·5; H = 3·9 per cent.

This substance was thus identified as protocatechuic acid.

Examination of the Aqueous Liquid (C).

This liquid, as already indicated, represented that portion of the original alcoholic extract of the bark which was soluble in cold water, and from which the previously-described resins (A) and (B) had been removed.

Isolation of Benzoic Acid.

The aqueous liquid was extracted many times with ether, the combined ethereal liquids being washed, dried, and the solvent removed. A quantity of a crystalline product was thus obtained, which was subjected to vigorous distillation with steam. The distillate had a distinctly acid reaction, and, on extraction with ether, yielded 9 grams of a crystalline substance melting at 120—121°:

0·1432 gave 0·3608 CO_2 and 0·0661 H_2O. C = 68·7; H = 5·1.

$C_7H_6O_2$ requires C = 68·8; H = 4·9 per cent.

This substance thus proved to be benzoic acid, and its identity was further confirmed by the formation of its methyl ester, boiling at 197—198°.

Isolation of Trimethylgallic Acid.

After completely removing the benzoic acid from the crystalline product by distillation with steam, as above described, the contents of the distillation flask were extracted with ether, and the ethereal liquid shaken with eight successive portions of a dilute solution of ammonium carbonate. The liquids obtained by the first three extractions with alkali gave, on acidification, a distinctly crystalline precipitate, and these were therefore united. The liquids obtained by the subsequent five extractions, when acidified, yielded a more or less amorphous precipitate, and this was separately examined, as described below.

The precipitate obtained by acidifying the liquids from the first three ammonium carbonate extractions was crystallised from ethyl acetate, when it yielded 10 grams of a crystalline compound melting at 165—169°. After several recrystallisations from the same solvent, the melting point remained constant at 167—169°:

0·1647 gave 0·3424 CO_2 and 0·0865 H_2O. C = 56·7; H = 5·8.

$C_{10}H_{12}O_5$ requires C = 56·6; H = 5·6 per cent.

This substance is evidently trimethylgallic acid, and its isolation is

of considerable interest from the fact that, so far as known to us, it is the first instance in which its occurrence in nature has been observed.

Further confirmation of the identity of the above substance was afforded by the preparation of some derivatives, a determination of the number of methoxyl groups, and its molecular weight. The methyl ester was prepared in the usual manner, and, as this can be volatilised with steam, it affords a ready method for the purification of the acid. When crystallised from water, it separated in long needles melting at 84°:

0·2098 gave 0·4485 CO_2 and 0·1190 H_2O. C = 58·3; H = 6·3.

$C_{11}H_{14}O_5$ requires C = 58·4; H = 6·2 per cent.

The acid was regenerated from this ester, and crystallised from ethyl acetate, when it melted at 167—169°, as before, and was again analysed:

0·1287 gave 0·2680 CO_2 and 0·0685 H_2O. C = 56·8; H = 5·9.

$C_{10}H_{12}O_5$ requires C = 56·6; H = 5·6 per cent.

The *silver* salt was obtained as a crystalline precipitate by the addition of silver nitrate to a solution of the ammonium salt. It was washed with a little cold water, dried in a vacuum over sulphuric acid, and analysed:

0·5070 gave, on ignition, 0·1710 Ag. Ag = 33·7.

$C_{10}H_{11}O_5Ag$ requires Ag = 33·8 per cent.

A determination of the number of methoxyl groups in the acid by Perkin's modification of the Zeisel method gave the following result:

0·1316 gave 0·4370 AgI. OMe = 43·8.

$C_7H_3O_2(OMe)_3$ requires OMe = 43·9 per cent.

The molecular weight of the acid was determined by the cryoscopic method in acetic acid solution:

0·2965 in 22·2415 of acetic acid gave Δt – 0·254°. M.W. = 201.

$C_{10}H_{12}O_5$ requires M.W. = 212.

Isolation of p-*Coumaric Acid.*

After the removal of the trimethylgallic acid from the ethereal liquid by the first three extractions of the latter with aqueous ammonium carbonate, as above described, the subsequent extractions with this alkali gave, when acidified, about 8 grams of a product melting at 210—213° with evolution of carbon dioxide. This was recrystallised from hot water, from which it separated in small, colourless needles melting, as before, at 210—213°:

0·1580 gave 0·3810 CO_2 and 0·0690 H_2O. C = 65·8; H = 4·9.

$C_9H_8O_3$ requires C = 65·9; H = 4·9 per cent.

This substance was thus identified as *p*-coumaric acid. Further confirmation of its identity was obtained by methylating a portion of the acid, when the methyl derivative, $CH_3 \cdot O \cdot C_6H_4 \cdot C_2H_2 \cdot CO_2H$ (m. p. 170°), and its methyl ester, $CH_3 \cdot O \cdot C_6H_4 \cdot C_2H_2 \cdot CO_2 \cdot CH_3$ (m. p. 88—90°), were obtained.

The aqueous liquid, from which the three above-mentioned acids had been obtained by extraction with ether, was concentrated under diminished pressure to a volume of about 6 litres, and treated with a solution of basic lead acetate. This produced a voluminous, yellow precipitate, which was collected, washed, and then suspended in water and decomposed by hydrogen sulphide. On filtering the mixture, a liquid was obtained which gave a bluish-black coloration with ferric chloride, and evidently contained a quantity of tannin, but nothing crystalline could be separated from it.

The filtrate from the basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the excess of lead, and the filtered liquid concentrated under diminished pressure to a volume of about 1·5 litres. This liquid, on cooling, formed a gelatinous mass, which again became fluid on warming or on dilution with water or alcohol. The nature of the substance to which the gelatinisation of the liquid was due could not be ascertained. The liquid contained a cyanogenetic glucoside, together with a sugar which yielded *d*-phenylglucosazone, melting at 208—210°.

With the object of isolating the cyanogenetic compound from the above-mentioned aqueous liquid, the latter was divided into two equal portions. One of these portions, amounting to 800 grams, was mixed with purified sawdust, and the thoroughly-dried mixture extracted for eight days in a Soxhlet apparatus with dry ethyl acetate. As the cyanogenetic compound could not be separated from the resulting extract, the latter was mixed with a small quantity of sawdust, and extracted for three days with boiling chloroform, but this treatment removed only traces of the glucoside. The chloroform extract was therefore washed with a little water, then shaken with a dilute solution of sodium hydroxide to remove any acidic or phenolic substances, and, finally, the solvent evaporated. A very small amount (about 0·01 gram) of a crystalline substance was thus obtained, which, after recrystallisation from a mixture of chloroform and ether, melted sharply at 240—241°, but could not be further examined.

In order to ascertain the amount of cyanogenetic glucoside in the aqueous liquid, 25 grams of the remaining portion of the latter were treated with emulsin, when 0·0783 gram of hydrogen cyanide was evolved, which would correspond to 0·8555 gram of a compound having the molecular weight of mandelonitrile glucoside. The amount

of such a glucoside in the total aqueous liquid (1600 grams), representing 4·5 kilograms of the original alcoholic extract or 17 kilograms of bark, would thus have been about 55 grams, or 0·32 per cent. of the weight of the bark.

Although the above attempts to isolate the cyanogenetic compound from the aqueous liquid were unsuccessful, it was subsequently obtained from the original alcoholic extract of the bark by a special method, which will be described below.

Isolation of β-Methylaesculetin.

A quantity (100 grams) of the above-mentioned aqueous liquid was diluted with water to the measure of about 500 c.c., then 25 grams of sulphuric acid added, and the mixture boiled for several hours. Steam was subsequently passed through the mixture in order to remove any volatile products formed by the hydrolysis. The contents of the distillation flask were then filtered, the acid, aqueous liquid being extracted first with ether, and subsequently with chloroform. After the removal of the respective solvents, residues were obtained which were separately dissolved in about 10 c.c. of water. On keeping these solutions overnight, they deposited small quantities of a substance which, when crystallised from alcohol, melted constantly at 204°, and was identified as β-methylæsculetin. The amount of this substance thus obtained was 0·4 gram, which would correspond to 6·4 grams from the total aqueous liquid, the latter representing about 17 kilograms of bark. As the β-methylæsculetin was obtained from this liquid only after hydrolysis, it is highly probable that it existed in the form of its glucoside, methylæsculin, and the corresponding amount of the latter compound would be about 12 grams, or 0·07 per cent. of the weight of the bark.

On boiling the β-methylæsculetin for about ten minutes with acetic anhydride, its *acetyl* derivative was formed. This compound is only sparingly soluble in cold acetic anhydride, from which it separates in colourless leaflets, melting at 176—177°:

0·1140 gave 0·2564 CO_2 and 0·0460 H_2O. C = 61·3; H = 4·5.

$C_{10}H_7O_4(CO{\cdot}CH_3)$ requires C = 61·5; H = 4·3 per cent.

In extracting the β-methylæsculetin from the hydrolysed aqueous liquid by means of ether, as above described, it was observed that the ethereal liquid also contained a small amount of an acidic substance which was readily soluble in water. A further quantity of this substance was obtained, and its identification effected, as described below.

Isolation of l-*Mandelic Acid.*

A quantity (400 grams) of the previously-mentioned aqueous liquid was diluted with water to the measure of 1 litre, 50 grams of sulphuric acid added, and the mixture boiled for six hours. It was then subjected to distillation with steam, when the distillate was found to contain small amounts of furfuraldehyde, benzaldehyde, and benzoic acid. The aqueous, acid liquid remaining in the distillation flask, after being allowed to cool, was filtered, and extracted twelve times with ether. The ethereal liquid from the first extraction was discarded as being likely to contain a little benzoic acid. All the subsequent extractions were united, and the solvent removed, when a residue was obtained which was mixed with 50 c.c. of water. On allowing the liquid to stand for some time, a quantity of β-methylæsculetin was deposited, which was removed by filtration, and the strongly acid, aqueous filtrate extracted five times with ether. The ethereal liquid was then repeatedly extracted with small quantities of a dilute solution of ammonium carbonate until the aqueous liquids just began to show an alkaline reaction. The combined ammonium carbonate extracts were acidified with hydrochloric acid and extracted with ether, when about 3 grams of a crystalline substance were obtained, melting at 128—130°. After recrystallisation from benzene, it melted at 130—133°:

0·1238 gave 0·2880 CO_2 and 0·0624 H_2O. C = 63·4; H = 5·6.

The substance was again crystallised from benzene, when it melted at 132—133°, and was again analysed:

0·1276 gave 0·2965 CO_2 and 0·0635 H_2O. C = 63·4; H = 5·5.

$C_8H_8O_3$ requires C = 63·2; H = 5·3 per cent.

0·3710, dissolved in 20 c.c. of water, gave in a 2-dcm. tube α_D − 5°44′, whence $[\alpha]_D$ − 154·5°.

The substance was thus identified as *l*-mandelic acid, and its isolation from the aqueous liquid, after hydrolysis, afforded evidence of the presence of either amygdalin or *l*-mandelonitrile glucoside.

Isolation of l-*Mandelonitrile Glucoside.*

One kilogram of the original alcoholic extract, representing about 3·8 kilograms of the bark, was mixed with 2 litres of water, and the volatile constituents were removed by distillation with steam. The contents of the distillation flask were then filtered while hot from the previously-described green resin, the latter being thoroughly washed with water, and the washings added to the filtrate. After allowing the combined aqueous filtrate and washings to stand for

several weeks, a quantity of the previously-described brown resin was deposited, from which the clear liquid was decanted. This liquid, being large in amount, was divided into four portions, each of which was concentrated as far as possible under diminished pressure. To each portion 200 c.c. of alcohol were then added, and, after complete solution, the thin syrups were again concentrated under diminished pressure, this operation being repeated until the mass became too viscid for further concentration. Each portion was then dissolved in 200 c.c. of absolute alcohol, and to the thin syrups so obtained 1 litre of boiling, dry ethyl acetate was added in each case. After standing for several hours, the liquids were decanted from the precipitated syrup, concentrated to about 100 c.c., and, while still hot, 500 c.c. of boiling, dry ethyl acetate added to each portion. They were again allowed to stand for several hours, when some syrupy material was deposited, from which the clear ethyl acetate liquids were decanted. These were then united, concentrated to the measure of 500 c.c., and, after cooling, 500 c.c. of dry ether added. The clear liquid was then separated, the solvent completely removed, and the residue dissolved in about 300 c.c. of cold water. This aqueous liquid was shaken with small successive portions of ether in order to remove the acids which were known to be present, after which it was treated with a solution of normal lead acetate until no further precipitate was produced. The yellow precipitate was removed by filtration with the aid of the pump, and the aqueous filtrate concentrated on the water-bath under diminished pressure to about 30—40 c.c. A mixture of 300 c.c. of alcohol and 300 c.c. of ethyl acetate was then added, and the whole allowed to stand overnight. The clear liquid, decanted from a small amount of a yellow precipitate, was concentrated to a small volume, the residual product being then dissolved in water and treated with hydrogen sulphide for the removal of the lead. After filtration, the aqueous liquid was concentrated under diminished pressure to the measure of about 50 c.c., when an excess of calcium carbonate was added to neutralise the free acetic acid, and the mass then extracted with alcohol. This alcoholic extract was evaporated to dryness on a water-bath under diminished pressure, and the residue dissolved in ethyl acetate, when, after concentrating the solution to about 20 c.c., the glucoside slowly crystallised in small, colourless needles. It was recrystallised from ethyl acetate, when two crops of crystals were obtained. The first fraction of crystals, which amounted to 0·6 gram, melted initially at 144—146°, and, after further crystallisation, at 145—147°. The second fraction of crystals, which was very small in amount (about 0·1 gram), was somewhat less pure, and melted at 135—140°. Both these fractions, when treated with emulsin, yielded hydrogen

cyanide, benzaldehyde, and glucose. The first fraction was analysed, with the following result :

0·1392 gave 0·2904 CO_2 and 0·0750 H_2O. C = 56·9 ; H = 6·0.

$C_{14}H_{17}O_6N$ requires C = 56·9 ; H = 5·8 per cent.

0·3605, dissolved in 20 c.c. of water, gave in a 2-dcm. tube α_D − 1°4′, whence $[\alpha]_D$ − 29·6°.

The above-described cyanogenetic compound was thus identified as *l*-mandelonitrile glucoside.

A determination of the specific rotatory power of the small fraction of glucoside melting at 135—140° gave the following result :

0·0956, dissolved in 20 c.c. of water, gave in a 2-dcm. tube α_D − 0°14′, whence $[\alpha]_D$ − 24·4°.

Tetra-acetyl-l-mandelonitrile Glucoside.—In the preliminary attempts to obtain the cyanogenetic glucoside from wild cherry bark a method was employed which, although not resulting in the isolation of the glucoside itself, led to the preparation of its acetyl derivative. This method varied from that above described for the isolation of the glucoside at the stage when the yellow precipitate produced by normal lead acetate was obtained. After the removal of this precipitate by filtration, the filtrate was treated with hydrogen sulphide for the removal of the lead, again filtered, and the liquid concentrated to about 200 c.c. It was then repeatedly extracted with hot amyl alcohol, the temperature of the mixture being kept at about 80°. By this means the glucoside was separated from the last traces of sugar, after which the solvent was removed by distillation in a rapid current of steam. The aqueous liquid contained in the distillation flask was then concentrated to the consistency of a thick syrup, the latter boiled for an hour with twenty times its weight of acetic anhydride, and the product poured into water. After allowing the mixture to stand for some hours, it was extracted with ether, the ethereal liquid being subsequently shaken with a 5 per cent. solution of sodium hydroxide in order to remove small amounts of acidic substances, and afterwards washed with water. On concentrating the ethereal liquid to a small bulk and allowing it to stand, the acetyl derivative of the glucoside separated in long needles. It was twice crystallised from alcohol, when it melted at 136—137°. The amount of this derivative obtained from 1 kilogram of the alcoholic extract, representing about 3·8 kilograms of the bark, was 0·6 gram :

0·1458 gave 0·3025 CO_2 and 0·0740 H_2O. C = 56·6 ; H = 5·6.

$C_{22}H_{25}O_{10}N$ requires C = 57·0 ; H = 5·4 per cent.

0·3470, dissolved in 20 c.c. of ethyl acetate, gave in a 2-dcm. tube α_D − 0°50′, whence $[\alpha]_D$ − 24·0°.

The *l*-mandelonitrile glucoside is accompanied in the wild cherry

bark by an enzyme, which may be obtained by adding to a cold aqueous infusion of the bark a large volume of strong alcohol. The precipitate thus produced is a light-coloured, amorphous powder, readily soluble in cold water. It contains a large proportion of inorganic material, but readily hydrolyses β-glucosides.

Summary.

The results of this investigation may be summarised as follows :

The material employed, consisting of the air-dried bark of *Prunus serotina*, Ehrhart, yielded on maceration with water an amount of hydrogen cyanide corresponding to about 0·075 per cent. of its weight. It contains a relatively small amount of a cyanogenetic glucoside, which has been shown to be *l*-mandelonitrile glucoside, $C_{14}H_{17}O_6N$ (m. p. 145—147° ; $[\alpha]_D$ − 29·6°), and the latter has also been obtained in the form of its tetra-acetyl derivative (m. p. 136—137° ; $[\alpha]_D$ − 24·0°). An enzyme which hydrolyses β-glucosides is also present in the bark.

An alcoholic extract of the bark, when distilled with steam, yielded small amounts of benzoic acid and an essential oil, but no hydrogen cyanide. The essential oil distilled between 100° and 120°/15 mm., and possessed an odour quite distinct from that of benzaldehyde. The non-volatile constituents of the bark, as obtained after treating the alcoholic extract with steam, consisted of a green resin, insoluble in either hot or cold water; a brown resin, readily soluble in the hot aqueous liquid, but which was slowly deposited on standing ; and material which remained dissolved in the cold aqueous liquid. The green resin, amounting to about 1 per cent. of the weight of the bark, yielded a phytosterol, $C_{27}H_{46}O$ (m. p. 135—136° ; $[\alpha]_D$ − 34·0°) ; palmitic, stearic, oleic, and linolic acids, with apparently a very little *iso*linolenic acid ; a small amount of ipuranol, $C_{28}H_{38}O_2(OH)_2$, melting at 285—290° ; and, after acid hydrolysis, oleic acid, dextrose, and β-methylæsculetin, $C_{10}H_8O_4$ (m. p. 204°). The brown resin, likewise amounting to about 1 per cent. of the weight of the bark, yielded, after acid hydrolysis, a trace of a phytosterol, small amounts of oleic acid, β-methylæsculetin, and dextrose, together with insoluble, red, resinous material, which, on fusion with potassium hydroxide, gave formic, acetic, butyric, and protocatechuic acids. The portion of the alcoholic extract of the bark which was soluble in cold water, and from which the above-described resins had been removed, contained *l*-mandelonitrile glucoside, together with a quantity of sugar and tannin. It yielded, furthermore, benzoic, trimethylgallic, and *p*-coumaric acids, traces of a substance melting at 240—241°, and, after heating with dilute sulphuric acid, *l*-mandelic acid and β-methylæsculetin were obtained. The two latter compounds were evidently formed

by the hydrolysis of *l* mandelonitrile glucoside and methylæsculin respectively.

In conclusion, the authors desire to express their thanks to Dr. S. J. M. Auld for having kindly provided them with a small specimen of *l*-mandelonitrile glucoside, obtained by the partial hydrolysis of amygdalin.

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