The constituents of the leaves of prunus serotina / by Frederick B. Power and Charles W. Moore.

Power, Frederick B. 1853-1927.
Moore, Charles W.
Wellcome Chemical Research Laboratories.

London : Wellcome Chemical Research Laboratories, [1910?]

No. 108

THE CONSTITUENTS OF THE LEAVES OF PRUNUS SEROTINA

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(From the Transactions of the Chemical Society, Vol. 97, 1910)

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XCI.—*The Constituents of the Leaves of* Prunus Serotina.

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The leaves of *Prunus serotina,* Ehrhart, commonly known as the Wild Black Cherry, have hitherto never been subjected to a complete chemical examination, and apparently the only information concerning them is a statement by Procter (*Proc. Amer. Pharm. Assoc.*, 1858, **6**, 325) that, when macerated with water and distilled, they yield a volatile oil and hydrocyanic acid. As the constituents of the bark of *Prunus serotina* were recently made the subject of a thorough investigation by the present authors (Trans., 1909, **95**, 243), it seemed desirable also to examine the leaves of this tree. It has thus been ascertained that the leaves contain the same cyanogenetic compound as the bark, namely, *l*-mandelonitrile glucoside, although in relatively smaller amount, but, as was anticipated, there are interesting differences with respect to the other constituents. A complete summary of the results of the present investigation is given at the end of this paper.

Experimental.

The material employed in this investigation consisted of the air-dried leaves of *Prunus serotina,* Ehrhart, which had been very kindly supplied to us by Professor J. U. Lloyd, of Cincinnati, Ohio, to whom our thanks are due. The leaves were gathered under the personal supervision of Professor Lloyd during the month of August, at a time when they were fully matured, and when the fruit of the tree was ripe.

In order to determine the amount of hydrogen cyanide yielded by the leaves, a portion (25 grams) of the finely-crushed material was macerated with water in a tightly closed flask for several days 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 material was 0·4 c.c., being thus equivalent to 0·0086 per cent. of HCN.

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

Twenty grams of the crushed leaves were 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	0·74	gram	= 3·70	per cent.
Ether	,,	0·83	,,	= 4·15	,,
Chloroform	,,	0·20	,,	= 1·00	,,
Ethyl acetate	,,	0·34	,,	= 1·70	,,
Alcohol	,,	3·10	,,	= 15·50	,,
Total		5·21	grams	= 26·05	per cent.

For the purpose of a complete examination, a quantity (12·59 kilograms) of the crushed leaves was extracted by continuous percolation with hot alcohol. After the removal of the greater portion of the alcohol, a viscid, dark green extract was obtained, amounting to 6·66 kilograms.

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

A quantity (2 kilograms) of the above-mentioned extract, representing 3·78 kilograms of leaves, was mixed with water, and steam passed through the mixture for several hours. The aqueous distillate, which amounted to about 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 0·2 gram of an aromatic essential oil was obtained. The amount of this oil was not sufficient for its examination.

Non-volatile Constituents of the Extract.

After the distillation of the extract in a current of 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 resin repeatedly treated with boiling water until nothing further was removed. The aqueous liquid and the washings from the resin were united, and may be designated as (B).

Examination of the Green Resin (A).

This resin, at the ordinary temperature, was a dark green, waxy solid, and amounted to 206 grams. It was dissolved in alcohol and mixed with purified sawdust, the thoroughly dried mixture being then successively extracted in a Soxhlet apparatus with petroleum (b. p. 35—50°), ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin.

This extract formed a dark green, waxy solid, and amounted to 99 grams.

Isolation of Pentatriacontane, $C_{35}H_{72}$, *and Hentriacontane,* $C_{31}H_{64}$.

The entire amount of the petroleum extract was dissolved in a large volume of warm ether, and the solution kept for several hours, when a small quantity of an almost colourless substance separated. This was collected and washed with cold ether, after which it was distilled under a pressure of 15 mm. The distillate, which rapidly solidified, was crystallised from ethyl acetate, when 0·3 gram of a substance was obtained in small, colourless, glistening leaflets, melting at 74—75°. (Found, $C=84·9$; $H=14·7$. Calc., $C=85·4$; $H=14·6$ per cent.)

This substance therefore was pentatriacontane.

The ethereal liquid from which the pentatriacontane had been removed, as above described, was successively shaken with aqueous solutions of sodium carbonate and sodium hydroxide, which, however, removed nothing. The ether was accordingly evaporated, and the residue hydrolysed by boiling with an alcoholic solution of potassium hydroxide, after which the alcohol was removed, water added, and the alkaline solution of potassium salts extracted with ether. The ethereal liquid was washed, dried, and then concentrated to a volume of about 150 c.c., when, on cooling, a quantity of a crystalline substance separated. This was collected, washed with a little cold ether, and then recrystallised from a large volume of ethyl acetate, when it was obtained in the form of colourless, glistening leaflets, which melted at 68—69°. The amount of substance so obtained was 5 grams. (Found, $C=85·3$; $H=15·1$. Calc., $C=85·3$; $H=14·7$ per cent.)

The substance was thus identified as hentriacontane.

Isolation of Ceryl Alcohol, $C_{27}H_{56}O$.

The ethereal filtrate from the hentriacontane was evaporated to remove all the solvent, when a quantity of brown, resinous material was obtained, which appeared to consist of a mixture of hydrocarbons and an alcohol of high carbon content. The mixture was accordingly heated for some hours, at 150°, with an equal weight of phthalic anhydride, in order to convert any alcohol present into its acid phthalic ester, and thus effect its separation. The product of this operation was subsequently digested with a mixture of ether and chloroform, the solution filtered, and shaken with aqueous sodium carbonate, when an insoluble compound was deposited, which was evidently the sodium salt of an acid phthalic ester. This was collected, washed with a little ether and water, and then hydrolysed by boiling for some time with an alcoholic solution of potassium hydroxide. On cooling, a quantity of a colourless substance separated, which was collected, washed with a little alcohol, and crystallised from a large volume of alcohol, when it was obtained in colourless, glistening plates, melting at 79°. (Found, C=81·5; H=14·1. Calc., C=81·8; H=14·1 per cent.)

This substance was thus identified as ceryl alcohol.

The ether-chloroform solution from which the ceryl alcohol had been removed, as above described, was evaporated, and the residue heated with alcoholic potassium hydroxide in order to hydrolyse unchanged phthalic anhydride. The alcohol was then evaporated, water added, and the insoluble portion extracted with ether, the ethereal liquid being washed, dried, and the solvent removed. A quantity of a waxy solid was thus obtained, which distilled between 170° and 300°/15 mm., but from which no pure substance could be isolated. It appeared to consist of a mixture of hydrocarbons.

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

The aqueous, alkaline solution of potassium salts, from which the hentriacontane and ceryl alcohol had been removed by extraction with ether, was acidified, and again extracted with ether. The ethereal liquid was washed, dried, and concentrated to a volume of about 500 c.c., when, on keeping for several hours, a small quantity of a dark green powder separated. This was collected, washed with ether, and crystallised from dilute pyridine, when it was obtained in colourless, microscopic needles, melting at 295°. This substance possessed the characteristic properties of ipuranol (Trans., 1909, **95**, 249), with which it evidently was identical. It yielded an acetyl derivative, which separated in glistening leaflets, melting at 163°, and when this was mixed with a little diacetylipuranol the melting point remained unchanged. The amount of substance obtained was too small for analysis.

Examination of the Fatty Acids.

The ethereal filtrate from the ipuranol was concentrated somewhat, and then mixed with a large volume of light petroleum, when a quantity of chlorophyll was precipitated. This was removed by filtration, and the filtrate evaporated, when a quantity (12 grams) of fatty acids was obtained, which, when distilled under diminished pressure, passed over between 225° and 237°/12 mm.

Nine grams of the mixed acids were converted into their lead salts, and the latter digested with ether, when the greater 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 the lead salts yielded 6 grams of liquid acids, whilst the insoluble portion gave 2·5 grams of solid acids.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over at about 230°/12 mm. An analysis and a determination of the iodine value gave the following results:

0·1232 gave 0·3490 CO_2 and 0·1255 H_2O. C = 77·2; H = 11·3.
0·2190 absorbed 0·4337 iodine. Iodine value = 198·0.
$C_{18}H_{32}O_2$ requires C = 77·1; H = 11·4 per cent. Iodine value = 181·4.
$C_{18}H_{30}O_2$ „ C = 77·7; H = 10·8 „ Iodine value = 274·0.

In order to obtain more definite information respecting the nature of the above mixture, a quantity of it was oxidised according to the method described by Lewkowitsch (*Chemical Technology and Analysis of Oils, Fats, and Waxes*, 1904, vol. I, p. 360). This resulted in the formation of tetrahydroxystearic acid (m. p. 157—160°) and a very small quantity of a hexahydroxystearic acid, melting at 170—172°. It may thus be concluded that the liquid acids consisted chiefly of a mixture of linolic and *iso*linolenic acids, the former in predominating amount.

The Solid Acids.—These acids melted at 53—55°, and on analysis gave the following result:

0·1564 gave 0·4305 CO_2 and 0·1750 H_2O. C = 75·1; H = 12·4.
$C_{16}H_{32}O_2$ requires C = 75·0; H = 12·5 per cent.
$C_{18}H_{36}O_2$ „ C = 76·1; H = 12·7 „

From this result it would appear that the solid acids consisted of a mixture of palmitic and stearic acids, the former predominating.

Ethereal Extract of the Resin.

This extract, after the complete removal of the solvent, amounted to 70 grams. As originally obtained, it consisted of a dark green, ethereal liquid, containing a quantity of an amorphous, green, sparingly soluble powder. The latter was collected on a filter, and washed well with ether, when it amounted to about 35 grams. On concentrating the ethereal filtrate to about 500 c.c., a further quantity (7 grams) of a similar amorphous powder was obtained.

Isolation of a New Crystalline Substance, Prunol, $C_{31}H_{48}O(OH)_2,H_2O$.

The above-mentioned portions of solid material were separately examined, but proved to be identical. Its purification was found to be best accomplished by the following method: 30 grams of the material were mixed with 300 c.c. of cold alcohol, and subsequently about 300 c.c. of dilute sodium carbonate solution added. The liquid was filtered to remove a considerable quantity of a dark green resin, and the pale green filtrate boiled for a short time with animal charcoal, filtered while still hot, and concentrated on a water-bath until nearly all the alcohol had been expelled, when a substance separated in long, colourless needles. This was collected, washed with a little cold water, and recrystallised from a solution of sodium carbonate in dilute alcohol, in the manner described above. The product thus obtained, consisting of a *sodium* derivative, was readily soluble in strong or dilute alcohol, but almost insoluble in water. When boiled with water, it appeared to undergo partial dissociation. On adding hydrochloric acid to a solution of the sodium derivative in dilute alcohol, the parent substance is liberated. The latter separates from dilute alcohol in colourless, hair-like crystals, melting at 275—277°:

0·3262, on heating at 125°, lost 0·0126 H_2O. $H_2O = 3·8$.

0·1502 * gave 0·4335 CO_2 and 0·1474 H_2O. $C = 78·7$; $H = 10·9$.

$C_{31}H_{50}O_3,H_2O$ requires $H_2O = 3·7$ per cent.

$C_{31}H_{50}O_3$ requires $C = 79·1$; $H = 10·6$ per cent.

The sodium derivative gave on analysis the following results:

0·4474, on heating at 125°, lost 0·0299 H_2O. $H_2O = 6·7$.

0·3136 * gave, on ignition, 0·0330 Na_2CO_3. $Na = 4·6$.

$C_{31}H_{49}O_3Na,2H_2O$ requires $H_2O = 6·8$ per cent.

$C_{31}H_{49}O_3Na$ requires $Na = 4·7$ per cent.

These results render it evident that the above-described substance possesses the formula $C_{31}H_{50}O_3$. The only other known substance of this formula is that obtained by Power and Tutin from olive leaves, which was designated oleanol (Trans., 1908, **93**, 896). Although these two substances are very similar in their chemical properties, and are evidently closely related, they are not identical. The substance obtained from the leaves of *Prunus serotina* being therefore a new compound, it is proposed to designate it *prunol*, with reference to the generic name of the plant.

Prunol, $C_{31}H_{48}O(OH)_2,H_2O$, is sparingly soluble in ether, chloroform, ethyl acetate, and cold alcohol, but dissolves readily in hot

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* Anhydrous substance.

alcohol. If to its solution in acetic anhydride a few drops of concentrated sulphuric acid are added, a fine pink colour is produced, which slowly passes through violet to blue, finally becoming green.

Two of the oxygen atoms contained in prunol are, as shown below, present in the form of hydroxyl groups, one of which possesses phenolic properties, whilst the other is evidently alcoholic, as only a mono-sodium derivative could be obtained. The state of combination of the third oxygen atom is not known, but it was ascertained by means of Perkin's modification of the Zeisel method that no methoxyl or ethoxyl group was present.

Diacetylprunol, $C_{31}H_{48}O_3(CO\cdot CH_3)_2$.—This was obtained by heating prunol with acetic anhydride. The resulting liquid, when concentrated and kept for some time, slowly deposited the *diacetyl* derivative in handsome, glistening needles, which melted at 181°:

0·1442 gave 0·3995 CO_2 and 0·1255 H_2O. C = 75·6; H = 9·7.
$C_{35}H_{54}O_5$ requires C = 75·8; H = 9·7 per cent.

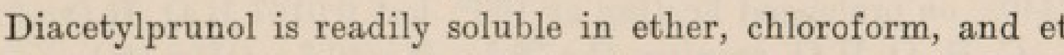
A determination of its specific rotatory power gave the following result:

0·1814, made up to 20 c.c. with chloroform, gave α_D + 1°8′ in a 2-dcm. tube, whence $[\alpha]_D$ + 62·4°.

Diacetylprunol is readily soluble in ether, chloroform, and ethyl acetate, and also in hot alcohol, but is only moderately soluble in cold alcohol. As already stated, it melts at 181°, but if the temperature is raised to about 220°, acetic anhydride is evolved and the mass solidifies, after which it only melts above 300°. The substance so obtained is insoluble in boiling alcohol, but dissolves readily in benzene. If to its solution in boiling benzene hot alcohol is added, the substance is obtained in small, glistening, colourless plates, which melt at about 315°:

0·1546 gave 0·4434 CO_2 and 0·1404 H_2O. C = 78·2; H = 10·1.
$C_{66}H_{102}O_7$ requires C = 78·7; H = 10·1 per cent.

This result indicates that on heating diacetylprunol two molecules of the compound lose one molecule of acetic anhydride, the residual complexes becoming united by an oxygen atom, as follows:



$$2(CH_3\cdot CO\cdot O)_2\cdot C_{31}H_{48}O=(CH_3\cdot CO)_2O+\begin{matrix}CH_3\cdot CO_2\cdot C_{31}H_{48}O\\ CH_3\cdot CO_2\cdot C_{31}H_{48}O\end{matrix}\!>O.$$

A somewhat similar change takes place on heating diacetyloleanol (*loc. cit.*, p. 898), but in this case both the acetyl groups are eliminated from one molecule of the compound.

Monoacetylprunol, $C_{31}H_{48}O_2(OH)\cdot CO\cdot CH_3$.—If diacetylprunol is dissolved in hot 70 per cent. alcohol, and the solution boiled for about two hours, one acetyl group is removed, and, on cooling the solution, the *monoacetyl* derivative separates in small, colourless needles, which melt at 290°:

0·1202 gave 0·3394 CO_2 and 0·1120 H_2O. C=77·0; H=10·4.
$C_{33}H_{52}O_4$ requires C=77·3; H=10·2 per cent.

This monoacetyl derivative is neutral towards sodium hydroxide, and it may therefore be concluded that the hydroxyl group regenerated by the above-described change is not the one which possesses a phenolic character.

Monomethylprunol, $C_{31}H_{48}O(OH)\cdot O\cdot CH_3$.—Two grams of prunol were dissolved in absolute alcohol, and an excess of both sodium ethoxide and methyl iodide added. The mixture was then boiled for about an hour, after which the greater portion of the solvent was removed. On the subsequent addition of water, the *monomethyl* derivative slowly separated in long, hair-like crystals. It was recrystallised from dilute alcohol, when it melted indefinitely between 110° and 115°. If dried at 100°, and then crystallised from absolute alcohol, it melted at 164—165°. After drying in a desiccator, it gave on analysis the following results:

0·1440 gave 0·4044 CO_2 and 0·1380 H_2O. C=76·6; H=10·6.
0·2580 gave, by Perkin's modification of Zeisel's method, 0·1190 AgI. OMe=6·1.
0·4405, on prolonged heating at 100°, lost 0·0167 H_2O. H_2O=3·8.
$C_{32}H_{52}O_3,H_2O$ requires C=76·5; H=10·7; OMe=6·1; H_2O=3·6 per cent.

Acetylmethylprunol, $C_{31}H_{48}O_2(O\cdot CH_3)\cdot CO\cdot CH_3$. — Monomethylprunol was dissolved in acetic anhydride, and the solution boiled for an hour. The liquid was then somewhat concentrated, and allowed to cool, when *acetylmethylprunol* separated in glistening leaflets, which melted at 235°:

0·1512 gave 0·4290 CO_2 and 0·1390 H_2O. C=77·4; H=10·2.
$C_{34}H_{54}O_4$ requires C=77·6; H=10·3 per cent.

The original ethereal liquid from which the crude prunol had been separated was thoroughly examined, but, apart from a further small quantity of prunol, nothing definite could be isolated from it.

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

These extracts amounted to 7, 5, and 15 grams respectively, and consisted entirely of resinous material.

Examination of the Aqueous Liquid (B).

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

Isolation of Benzoic Acid.

The aqueous liquid, which, on being kept for some time, had deposited a quantity of a brown, amorphous powder, was thoroughly extracted with ether, the combined ethereal liquids being washed, dried, and the solvent removed. A quantity of an amorphous product was thus obtained, which was subjected to prolonged distillation with steam. The distillate had an acid reaction, and, on extraction with ether, yielded about 1 gram of a crystalline substance, melting at 120°. (Found, $C=68{\cdot}7$; $H=5{\cdot}0$. Calc., $C=68{\cdot}8$; $H=4{\cdot}9$ per cent.) This substance thus proved to be benzoic acid.

Isolation of Quercetin.

After completely removing the benzoic acid from the ether extract by distillation with steam, as above described, the solid material remaining in the distillation flask was repeatedly extracted with boiling water. On cooling the aqueous liquid, a small quantity (0·7 gram) of a light yellow, amorphous powder was precipitated. This was collected, washed with a little ether, and crystallised from dilute alcohol, when it separated in small, yellow needles, melting at 310—312°.

This substance showed all the properties of quercetin; it was soluble in alkalis, gave crystalline salts with sulphuric and hydrochloric acids, and responded to the colour test with ferric chloride. Its identity with quercetin was confirmed by an analysis (Found, $C=59{\cdot}8$; $H=3{\cdot}5$. Calc., $C=59{\cdot}6$; $H=3{\cdot}3$ per cent.), and the preparation of its penta-acetyl derivative, which formed small, colourless needles, melting at 194°, and when mixed with a little penta-acetylquercetin the melting point remained unchanged.

As already noted, the aqueous liquid (B), on being kept for some time, deposited a quantity of a brown, amorphous powder. This was not removed before the above-described extraction with ether, and by this treatment it became dissolved in the aqueous liquid. On subsequently removing the ether contained in the aqueous liquid by means of a current of air, the brown powder was again precipitated. It was collected, and amounted to 35 grams, but, although thoroughly examined, nothing definite could be isolated from it.

Isolation of a New Glucoside, Serotrin, $C_{21}H_{20}O_{12},3H_2O$.

The aqueous liquid, from which the benzoic acid and quercetin had been removed by extraction with ether, and from which the above-described brown powder had been separated by filtration, was thoroughly extracted with successive portions of amyl alcohol. These extracts were united, washed with water, and concentrated under diminished pressure to a syrupy consistency. The dark-coloured residue thus obtained was dissolved in a small quantity of hot ethyl alcohol, and the solution kept for some time, when a substance was deposited in light yellow needles. This was collected, washed with a little alcohol, and recrystallised, first from alcohol, and then from dilute pyridine, when it separated in golden-yellow leaflets, melting at 245°. The amount of substance so obtained was 4 grams:

0·3270 of air-dried substance, when heated at 125°, lost 0·0347 H_2O. $H_2O = 10·6$.

0·1505 * gave 0·2994 CO_2 and 0·0620 H_2O. $C = 54·3$; $H = 4·5$.

$C_{21}H_{20}O_{12}$ requires $C = 54·3$; $H = 4·3$ per cent.

$C_{21}H_{20}O_{12},3H_2O$ requires $H_2O = 10·4$ per cent.

The anhydrous substance, on exposure to the air, readily takes up its three molecules of water of crystallisation:

0·5340 of anhydrous substance absorbed 0·0640 H_2O. $H_2O = 12·0$.

$C_{21}H_{20}O_{12}$, to form $C_{21}H_{20}O_{12},3H_2O$, requires $H_2O = 11·6$ per cent.

The glucosidic character of the substance was ascertained by boiling it for a few minutes with 5 per cent. aqueous sulphuric acid. The products thus obtained were quercetin, $C_{15}H_{10}O_7$, and a sugar, the latter yielding *d*-phenylglucosazone, melting at 205—208°:

0·7410 of anhydrous substance gave, on hydrolysis, 0·4785 of quercetin. $C_{15}H_{10}O_7 = 64·6$.

$C_{21}H_{20}O_{12}$ requires $C_{15}H_{10}O_7 = 65·1$ per cent.

The quercetin thus obtained was identified by its analysis (Found, $C = 59·6$; $H = 3·7$. Calc., $C = 59·6$; $H = 3·3$ per cent.), and also by that of its acetyl derivative (Found, $C = 58·6$; $H = 4·1$. Calc., $C = 58·6$; $H = 3·9$ per cent.).

From these results it is evident that the above-described substance is a glucoside of quercetin. It was thought that it might be identical with a glucoside recently isolated by A. G. Perkin (Trans., 1909, **95**, 2181) from cotton flowers, and designated quercimeritrin, since the latter possesses the formula $C_{21}H_{20}O_{12},3H_2O$, melts at 247—249°, and yields quercetin on hydrolysis. Through the courtesy of Professor A. G. Perkin, who had kindly supplied us with a specimen of quercimeritrin, it was possible to compare the two substances, and they were found not to be identical. The above-described glucoside is more readily soluble in water, and also more readily hydrolysed, than quercimeritrin. Furthermore, on

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* Dried at 125°.

mixing the two substances, the melting point of the mixture was found to be considerably lower than that of either of the constituents. Inasmuch as the glucoside obtained from the leaves of *Prunus serotina* does not agree in its properties with any of the previously-described glucosides of quercetin, it must be regarded as a new compound. It is therefore proposed to designate it *serotrin*, with reference to the specific name of the plant.

Serotrin, $C_{21}H_{20}O_{12},3H_2O$, is sparingly soluble in cold, and fairly readily soluble in boiling water or alcohol. It dissolves in alkalis with a dark yellow colour, and in aqueous solution it gives with lead acetate an orange-red precipitate.

Octa-acetylserotrin, $C_{21}H_{12}O_{12}(CO\cdot CH_3)_8$.—Two grams of serotrin were boiled for two hours with 25 grams of acetic anhydride. The solution so obtained was poured into water, and, after some time, the whole was extracted with ether. The ethereal liquid was washed with dilute aqueous sodium hydroxide, and then with water, after which it was dried and the solvent removed. The residue so obtained was crystallised, first from a mixture of alcohol and acetic acid, and finally from ether, when *octa-acetylserotrin* separated in colourless needles, melting at 150°:

0·1434 gave 0·2910 CO_2 and 0·0630 H_2O. C = 55·3; H = 4·8.

$C_{21}H_{12}O_{12}(CO\cdot CH_3)_8$ requires C = 55·5; H = 4·5 per cent.

Octa-acetylserotrin is sparingly soluble in ether or cold alcohol, but readily soluble in acetic acid. A determination of its specific rotatory power gave the following result:

0·2005, made up to 20 c.c. with chloroform, gave α_D $-1°32'$ in a 2-dcm. tube, whence $[\alpha]_D$ $-76·4°$.

The glucoside serotrin appears to be accompanied in the leaves of *Prunus serotina* by a closely related substance containing a methoxyl group, but the amount of this constituent was too small to permit of its isolation.

Identification of l-*Mandelonitrile Glucoside.*

The original alcoholic filtrate from the crude serotrin was mixed with water and subjected to a vigorous distillation in a current of steam until all the amyl and ethyl alcohols had been removed. The aqueous liquid so obtained was cooled, filtered, and treated with a solution of basic lead acetate. This produced a voluminous yellow precipitate, which was removed by filtration and carefully examined, but nothing definite could be isolated from it.

The filtrate from the basic lead acetate precipitate, after the addition of a little sodium acetate, was treated with hydrogen sulphide for the removal of the excess of lead, and the filtered liquid concentrated under diminished pressure to dryness. A small quantity of the residue thus obtained was dissolved in water and treated with emulsin, when hydrogen cyanide and benzaldehyde were produced. The residue was accordingly extracted with absolute alcohol, and the alcoholic solution concentrated to the volume of 200 c.c. On cooling, a small quantity of resinous material separated, which was removed, and to the clear liquid an equal volume of dry ethyl acetate was added, when a further precipitation occurred. The clear liquid was separated by decantation, concentrated to the volume of 50 c.c., and dissolved in 200 c.c. of ethyl acetate. On the addition of an equal volume of dry ether, a further quantity of resinous material was deposited. After some hours the clear liquid was evaporated to dryness, and the residue extracted with small quantities of dry ethyl acetate, when, after the removal of the solvent, an uncrystallisable syrup was obtained. This was subsequently boiled for a few minutes with twenty times its weight of acetic anhydride, in the presence of a little camphorsulphonic acid, with the view of converting the glucoside into a more readily crystallisable acetyl derivative. The mixture so obtained was poured into water, and after some hours 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 inoculating it with a trace of tetra-acetyl-*l*-mandelonitrile glucoside, the acetyl derivative at once separated in long needles. It was crystallised from alcohol, when it melted at 136°, and when mixed with a little pure tetra-acetyl-*l*-mandelonitrile glucoside the melting point remained unchanged. The amount of this acetyl derivative obtained from 2 kilograms of the alcoholic extract, representing 3·78 kilograms of the leaves, was only about 0·25 gram (Found, $C=57{\cdot}0$; $H=5{\cdot}6$. Calc., $C=57{\cdot}0$; $H=5{\cdot}6$ per cent.).

Although the amount of the cyanogenetic compound present in the leaves was too small to permit of its direct isolation, the characters of the above-described acetyl derivative afford conclusive evidence of its identity as *l*-mandelonitrile glucoside.

The *l*-mandelonitrile glucoside is accompanied in the leaves by an enzyme, which may be obtained by adding to a cold aqueous infusion of them a large volume of alcohol. The precipitate thus produced formed, when dry, a dark brown powder, and amounted to about 0·9 per cent. of the weight of leaves employed. It contains a large proportion of inorganic material, but slowly hydrolyses β-glucosides.

The aqueous liquid, after having been extracted with amyl alcohol as above described, was subjected to a vigorous distillation in a current of steam, in order to remove any dissolved amyl alcohol. The liquid was then allowed to cool, and treated with a solution of basic lead acetate. This produced a voluminous, orange-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 evidently contained a quantity of tannin, but nothing definite 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. It contained a considerable quantity of a sugar, since it readily yielded *d*-phenylglucosazone, melting at 210°.

Isolation of l-*Mandelic Acid.*

To the above-mentioned aqueous liquid 150 grams of sulphuric acid were added, and the mixture boiled for some hours. A quantity of insoluble, red, resinous material was thus formed, which was removed by filtration and examined, but nothing definite could be obtained from it. The aqueous filtrate was thoroughly extracted with ether, the united ethereal liquids being washed with water, dried, and the solvent removed. A quantity (2·5 grams) of a crystalline residue was thus obtained, which, after recrystallisation from benzene, separated in handsome, flat needles, melting at 132° (Found, $C = 63·3$; $H = 5·2$. Calc., $C = 63·2$; $H = 5·2$ per cent.).

A determination of the specific rotatory power of this substance gave the following result:

0·3112, made up to 20 c.c. with water, gave $\alpha_D -4°54'$ in a 2-dcm. tube, whence $[\alpha]_D -157·4°$.

The substance was thus identified as *l*-mandelic acid, and its isolation from the aqueous liquid, after hydrolysis, would indicate that only a small part of the *l*-mandelonitrile glucoside present had been removed by extraction with amyl alcohol, as above described.

Summary.

The results of this investigation may be summarised as follows:

The material employed, consisting of the air-dried leaves of *Prunus serotina*, Ehrhart, yielded, on maceration with water, an amount of hydrogen cyanide equivalent to 0·0086 per cent. of its weight. The leaves contain a relatively small amount of *l*-mandelonitrile glucoside, $C_{14}H_{17}O_6N$, which was identified by means of its tetra-acetyl derivative (m. p. 136°), together with an enzyme which hydrolyses β-glucosides.

An alcoholic extract of the leaves, when distilled in a current of steam, yielded a very small amount of an essential oil, but no hydrogen cyanide. The non-volatile constituents of the leaves, as obtained after treating the alcoholic extract with steam, consisted of a green resin, which was insoluble in either hot or cold water, and material which remained dissolved in the aqueous liquid. The resin, which amounted to about 5·5 per cent. of the weight of the leaves, yielded hentriacontane, pentatriacontane, ceryl alcohol, palmitic, stearic, linolic, and *iso*linolenic acids, a small amount of ipuranol, $C_{23}H_{38}O_2(OH)_2$, and a new, crystalline substance, $C_{31}H_{48}O(OH)_2$, melting at 275—277°, which has been designated *prunol*.

The portion of the alcoholic extract of the leaves which was soluble in water, and from which the above-described resin had been removed, contained benzoic acid, quercetin, a new glucoside of quercetin, $C_{21}H_{20}O_{12},3H_2O$ (m. p. 245°), which has been designated *serotrin*, and *l*-mandelonitrile glucoside, together with a quantity of sugar and tannin. The aqueous liquid, after heating with dilute sulphuric acid, yielded, furthermore, *l*-mandelic acid, which had evidently been formed by the hydrolysis of the *l*-mandelonitrile glucoside.

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