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THE CONSTITUENTS

OF THE RHIZOME OF

APOCYNUM ANDROSÆMIFOLIUM

BY

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LXXXV.—*The Constituents of the Rhizome of Apocynum androsaemifolium.*

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Under the title of "Apocynum," the Pharmacopœia of the United States recognises "the dried rhizome of *Apocynum cannabinum*, Linné, or of closely allied species of *Apocynum*." In "Gray's *New Manual of Botany*," 1908, 7th ed., p. 662, only three species of *Apocynum* are described, namely, *A. androsaemifolium*, Linné, *A. mèdium*, Greene, which is similar in its characters to the preceding, and *A. cannabinum*, Linné. The last-mentioned species, however, is stated to vary greatly, and several varieties of the plant are regarded by some botanists a definite species, to which distinctive names have been assigned.

An investigation of material, described as the root of *Apocynum cannabinum*, L., was conducted some years ago by Schmiedeberg (*Arch. exp. Path. Pharm.*, 1883, **16**, 161), who obtained two products, designated as apocynin and apocynein, the latter having been regarded a glucoside similar in character to saponin. Both these products were, however, only obtained in an amorphous state, and evidently consisted of mixtures.

Wood (*J. Amer. Med. Assoc.*, 1904, **43**, 1953) obtained from a fluid extract of *Apocynum cannabinum* a crystalline substance, m. p. 112°, which he believed to be identical with the so-called "crystalline apocynin" of commerce, but found the latter to be, physiologically, almost inert. The same investigator was "inclined to believe that the active principle is in the nature of a glucoside," but this opinion was based only on the observation that a solution which had been heated with an acid had thereby become deprived of its physiological activity. The present investigation has shown that the above view is incorrect, but that the active principle, which has now been obtained n a pure state, is decomposed on heating with acids, although it is not a glucoside.

Finnemore (Trans., 1908, **93**, 1513), in a publication entitled "The Constituents of Canadian Hemp, Part I," has recorded the isolation

of a crystalline substance from the root (rhizome) of *Apocynum cannabinum*. This substance, which was described under the name of "apocynin"—having been found to correspond to the crystalline apocynin of commerce—he has shown to be identical with acetovanillone.

The present investigation of the rhizome of *Apocynum androsaemifolium*, Linné, commonly known as "Spreading Dogbane," has resulted in the isolation of its chief active constituent, which is designated *apocynamarin*, $C_{14}H_{18}O_3,H_2O$, m. p. 170—175°. This substance has an intensely bitter taste, and is highly toxic. The rhizome has, furthermore, been shown to contain a considerable proportion of acetovanillone, the glucoside of which, $CH_3\cdot CO\cdot C_6H_3(O\cdot CH_3)\cdot O\cdot C_6H_{11}O_5$, m. p. 218—220°, has also been isolated, and designated *androsin*. A summary of the results of the complete investigation of the rhizome, in the course of which a number of other substances have been isolated, is given in a summary at the end of this paper.

Experimental.

The material employed in this investigation was obtained from the United States, and was supplied as representing the "Apocynum" of the U.S. Pharmacopœia. It conformed in its anatomical characters to the description given of the rhizome of *Apocynum androsaemifolium*, Linné.

A portion of the material (25 grams) was tested for the presence of an alkaloid, but the reactions were so slight as to indicate that it contained not more than traces of such a substance.

Twenty grams of the ground material were successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried at 90—100°, were obtained:

Petroleum (b. p. 35—50°)	extracted	1·19	grams	=	5·95 per cent.
Ether	,,	0·39	,,	=	1·95 ,,
Chloroform	,,	0·09	,,	=	0·45 ,,
Ethyl acetate	,,	0·24	,,	=	1·20 ,,
Alcohol	,,	2·74	,,	=	13·70 ,,
	Total	4·65	grams	=	23·25 per cent.

For the purpose of a complete examination, a quantity (39·92 kilograms) of the ground material 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 10·48 kilograms.

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

A quantity (5 kilograms) of the above-mentioned extract, representing about 19 kilograms of the rhizome, was mixed with water, and steam passed through the mixture for several hours. The distillate, which amounted to about 10 litres, contained some drops of oil floating on the surface. It was thoroughly extracted with ether, and the ethereal liquid shaken with a dilute solution of ammonium carbonate. On acidifying this alkaline liquid, a small quantity of an oily acid separated, which was removed by ether and distilled under the ordinary pressure, when it passed over between 210° and 215° as an almost colourless, viscid liquid, and amounted to about 2·0 grams. It was converted into the ammonium salt, from which five fractions of silver salt were precipitated. The analysis of these salts showed that the acid consisted chiefly of an octoic acid. The ethereal liquid which had been extracted with ammonium carbonate, as above described, was shaken with successive portions of a dilute solution of potassium hydroxide. The alkaline liquids thus obtained were united, acidified, and extracted with ether, when a small amount (1·2 grams) of a crystalline substance, melting at 112—114°, was obtained. This proved to be identical with a substance which was afterwards obtained in larger quantity, and was shown to be acetovanillone.

The ethereal liquid which had been extracted with potassium hydroxide, as above described, yielded, after removing the solvent, a small quantity of an essential oil, which distilled between 130° and 250°. It was a pale yellow liquid, possessing a strong persistent odour and the following constants : d 12°/12° = 0·948 ; α_D +0°50′ in a 1-dcm. tube. The amount of this essential oil was 3·0 grams. It gave a strong furfuraldehyde reaction.

Non-volatile Constituents of the Extract.

After the distillation of the extract with steam, as above described, there remained a quantity of a brown resin (*A*) and a dark-coloured aqueous liquid. The latter was separated by filtration while still hot, and the brown resin repeatedly treated with boiling water until nothing further was removed. The aqueous liquid and washings from the brown resin were united and kept 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 Resin (A).

This resin, at the ordinary temperature, was a dark brown, sticky solid, and amounted to 1010 grams. It was first treated with a large volume of cold light petroleum (b. p. 35—50°), the undissolved material being allowed to deposit, and the clear liquid decanted, after which the same operation was repeated. The brown, undissolved residue was then further extracted in a Soxhlet apparatus with light petroleum, and the combined petroleum extracts concentrated as far as possible on the water-bath.

The portion of the resin (*A*) which was insoluble in light petroleum amounted to 368 grams, and formed a dark brown, amorphous powder. This was thoroughly extracted with alcohol in a Soxhlet apparatus, when 140 grams of it were dissolved. The remaining 228 grams consisted of a dark brown powder, which, although originally extracted from the rhizome by alcohol, had evidently suffered some change, and was now insoluble in all the usual solvents. The portion soluble in alcohol was mixed with purified sawdust, the thoroughly-dried mixture being then successively extracted in a Soxhlet apparatus with ether, chloroform, ethyl acetate, and alcohol.

Petroleum Extract of the Resin (A).

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

This extract was a dark-coloured, sticky solid, and amounted to 642 grams. It was dissolved in 4 litres of ether, and the solution allowed to stand for about ten days, when a grey, sparingly soluble substance separated. This was collected, washed with a little ether, and crystallised several times from dilute pyridine, when it formed tufts of colourless, microscopic needles melting at 285—290°:

0·1365 gave 0·3625 CO_2 and 0·1290 H_2O. C = 72·4; H = 10·5.

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

The substance was thus shown to be identical with ipuranol, a dihydric alcohol which has recently been obtained in these laboratories from several plants. The amount of ipuranol obtained in the present instance was 1·5 grams. On boiling the substance with acetic anhydride, it yielded diacetylipuranol, which separated in colourless leaflets melting at 162°.

The ethereal solution, after the removal of the ipuranol as above described, was diluted with ether to about 8 litres, then shaken with successive portions of an aqueous solution of sodium carbonate, and finally washed with water. The alkaline liquids and washings were

united and acidified, when a quantity of solid separated. On attempting to remove this by shaking with ether, an inseparable emulsion was formed. The acid liquid was therefore heated to boiling and cooled, after which the precipitated solid could easily be removed by filtration. The solid material so obtained was distilled under diminished pressure, when most of it passed over between 240° and 260°/25 mm. as a viscid, oily liquid which solidified on cooling. It amounted to 20 grams and consisted of a mixture of fatty acids, which were examined in connexion with a similar product obtained from the neutral portion of the petroleum extract after hydrolysis.

The aqueous liquid from which the above-described fatty acids had been separated yielded, on extraction with ether, about 1 gram of acetovanillone, melting at 112—114°.

Unsaponifiable Constituents of the Petroleum Extract.

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 of a sticky solid was obtained. This was boiled for five hours with an alcoholic solution of potassium hydroxide, the alcohol removed, and water added, when a quantity of amorphous, grey material was precipitated. This was separated by filtration, and well washed with boiling water, after which it was treated with ether, when nearly all of it dissolved. The ethereal solution was concentrated to 1500 c.c., when, after some time, small, colourless needles separated. These, when dry, weighed 200 grams. The material contained in the mother liquor yielded, after solution in dilute alcohol, a further 35 grams of this crystalline material. The final mother liquor contained 215 grams of uncrystallisable products, which will be referred to later.

The entire amount (235 grams) of the above-mentioned crystalline product was recrystallised from dilute alcohol, when nearly 200 grams of the material was obtained in small, colourless needles melting at 153—155°. One hundred grams of this crystallised product were subjected to a prolonged process of fractional crystallisation from ethyl acetate. A large number of fractions was thus obtained, which showed indefinite melting points, ranging from 151—155° to 180—185°. The specific rotatory power of these fractions also varied; thus the fraction melting at 180—185° had $[\alpha]_D$ +31·6°, whilst the more soluble fraction, melting at 151—155°, had $[\alpha]_D$ +54·6°. The percentage composition of the various fractions was uniform within the limits of experimental error; the mean of six analyses being C = 83·9, H = 11·7 per cent. From the results it was evident that the above-described crystalline product consisted of a mixture of substances of very similar

composition, but all attempts to separate them by fractional crystallisation were unsuccessful. The entire mixture was therefore heated with acetic anhydride, after which the acetylated product was submitted to fractional crystallisation from ethyl acetate. Although by this means the separation was far from complete, the isolation of two pure substances was effected.

Isolation of a New Monohydric Alcohol, Androsterol, $C_{30}H_{49}\cdot OH$.

One hundred grams of the above-mentioned acetylated product were repeatedly crystallised from ethyl acetate, when a small fraction (5 grams) was eventually obtained, which melted constantly at 212—214°, and appeared to be a pure substance. This substance was hydrolysed by boiling with an excess of a 10 per cent. alcoholic solution of potassium hydroxide for four hours. The solvent was then removed, water added, and the resulting solid crystallised from a mixture of ethyl acetate and alcohol, from which it separated in small, colourless needles melting at 208—210°:

0·3350, dried at 115°, lost 0·0130 H_2O. $H_2O = 3·9$.

0·1452 of anhydrous substance gave 0·4488 CO_2 and 0·1538 H_2O. $C = 84·3$; $H = 11·7$.

$C_{30}H_{50}O,H_2O$ requires $H_2O = 4·0$ per cent.

$C_{30}H_{50}O$ requires $C = 84·5$; $H = 11·7$ per cent.

This compound is evidently a monohydric alcohol of the formula $C_{30}H_{49}\cdot OH$. As it does not agree in its properties with any substance of this formula which has hitherto been described, it is proposed to designate it *androsterol*, with reference to the specific name of the plant from which it has been obtained.

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

0·2060, in 20 c.c. of chloroform, gave $\alpha_D + 0°37'$ in a 2-dcm. tube, whence $[\alpha]_D + 29·9°$.

If to a solution of androsterol in acetic anhydride a few drops of concentrated sulphuric acid are added, a fine magenta-red colour slowly develops, which is permanent for several hours.

Monoacetylandrosterol, $C_{30}H_{49}O\cdot CO\cdot CH_3$.—On heating androsterol with acetic anhydride, the pure *acetyl* derivative, melting at 212—214°, was regenerated. When crystallised from ethyl acetate, it separates in long, fine needles:

0·1252 gave 0·3760 CO_2 and 0·1250 H_2O. $C = 81·9$; $H = 11·1$.

$C_{32}H_{52}O_2$ requires $C = 82·0$; $H = 11·1$ per cent.

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

0·3025, in 20 c.c. of chloroform, gave $\alpha_D + 1°15'$ in a 2-dcm. tube, whence $[\alpha]_D + 41·3°$.

Monobromoacetylandrosterol, $C_{30}H_{48}BrO·CO·CH_3$.—One gram of acetylandrosterol was dissolved in 50 c.c. of chloroform, and to the cold solution a 5 per cent. solution of bromine in the same solvent was added, drop by drop, until a slight excess of bromine was present. The liquid, which evolved hydrogen bromide, was then at once shaken with a solution of sodium hydrogen sulphite, washed with water, dried, and the solvent removed. A syrup was thus obtained which, after treatment with ethyl acetate, yielded a crystalline product. The latter, after repeatedly crystallising from the same solvent, separated in colourless prisms, melting at 228—230°, and amounted to 0·6 gram:

0·1494 gave 0·3850 CO_2 and 0·1270 H_2O. C = 70·3; H = 9·4.
0·1570 „ 0·4026 CO_2 „ 0·1325 H_2O. C = 69·9; H = 9·3.
0·2614 „ 0·0906 AgBr. Br = 14·7.

$C_{32}H_{51}O_2Br$ requires C = 70·2; H = 9·3; Br = 14·6 per cent.

This substance is evidently a *monobromo*-derivative of acetyl androsterol.

Isolation of a New Monohydric Alcohol, Homoandrosterol, $C_{27}H_{43}·OH$.

Some of the numerous mother liquors obtained during the separation of the above-described acetyl derivative of androsterol (m. p. 212—214°) deposited on standing small amounts of sparingly soluble, prismatic needles, which, after recrystallisation from ethyl acetate, melted constantly at 234—236°. The amount of this substance was 2 grams, and its properties indicated that it was a pure compound. It was hydrolysed by boiling for four hours with an excess of a 10 per cent. alcoholic solution of potassium hydroxide, the solvent removed, water added, and the resulting solid recrystallised from dilute alcohol, from which it separated in small, colourless needles melting at 192°:

0·1201 gave 0·3702 CO_2 and 0·1270 H_2O. C = 84·0; H = 11·7.

$C_{27}H_{44}O$ requires C = 84·4; H = 11·4 per cent.

This compound is evidently a monohydric alcohol of the formula $C_{27}H_{43}·OH$. As it does not agree in its properties with any substance of this formula which has hitherto been described, and differs from androsterol by the elements C_3H_6, it is proposed to designate it *homoandrosterol*.

If to its solution in acetic anhydride a drop of concentrated sulphuric acid is added, a magenta-red colour is at once produced, which is permanent for several hours.

Monoacetylhomoandrosterol, $C_{27}H_{43}O·CO·CH_3$.—On heating homo-

androsterol with acetic anhydride, the pure *acetyl* derivative, melting at 234—236°, was regenerated. It crystallises from ethyl acetate in glistening, prismatic needles:

0·1292 gave 0·3852 CO_2 and 0·1256 H_2O. C = 81·6; H = 10·8.

$C_{29}H_{46}O_2$ requires C = 81·7; H = 10·8 per cent.

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

0·3000, in 20 c.c. of chloroform, gave α_D +2°29′ in a 2-dcm. tube, whence $[\alpha]_D$ +82·8°.

If to a chloroform solution of acetylhomoandrosterol bromine is added, the halogen is very slowly absorbed and hydrogen bromide is evolved. The reaction is accelerated by the addition of a little iodine. From the reaction mixture a small quantity of a *bromine* derivative was obtained, which crystallised in needles melting and decomposing at 212—214°. Owing to the limited amount of material at disposal, this derivative was not further investigated.

After the separation of the two above-described compounds, a large amount of material remained in the mother liquors, from which nothing further could be obtained in a pure state by crystallisation. It has already been mentioned that while acetylandrosterol absorbs bromine very rapidly, acetylhomoandrosterol only does so very slowly. It was, therefore, thought possible that a similar behaviour might be shown by the constituents of this mixture of acetyl derivatives, and that by taking advantage of this property some separation might be effected.

Ten grams of the mixture were therefore dissolved in 150 c.c. of chloroform, and a dilute solution of bromine in the same solvent cautiously added. It was found that 1·7 grams of the halogen were rapidly absorbed, whereas the addition of 1 molecule of bromine would have required 3·6 grams, assuming the constituents of the mixture to have the composition $C_{30}H_{49}OAc$. As soon as the rapid absorption of bromine had ceased, the excess of the halogen was removed and the solvent evaporated. A syrupy mass was thus obtained, which was dissolved in acetic acid, when, on standing, a small quantity (0·5 gram) of monobromoacetylandrosterol separated.

The material remaining in the acetic acid mother liquor was precipitated by water, and dissolved in chloroform. To this solution 5 grams of bromine were added and the mixture kept for twenty-four hours, when copious fumes of hydrogen bromide were evolved. At the end of this time the excess of bromine was removed and the solvent evaporated. A syrupy mass was thus obtained, which was dissolved in a small quantity of hot ethyl acetate, and allowed to stand

for some time, when a substance separated in glistening plates. This was purified by recrystallisation from a mixture of chloroform and ether, when it formed handsome plates, melting at 266—268°, and amounted to 2 grams :

0·1425 gave 0·3681 CO_2 and 0·1210 H_2O. C = 70·4 ; H = 9·4.

0·1382 ,, 0·0475 AgBr. Br = 14·6.

$C_{32}H_{51}O_2Br$ requires C = 70·2 ; H = 9·3 ; Br = 14·6 per cent.

This bromine derivative differs from both of the two monobromoacetyl derivatives described above, and therefore must be derived from a substance other than androsterol or homoandrosterol. In the hope of obtaining the parent substance, attempts were made to replace the bromine by hydrogen, but these were unsuccessful.

It has already been mentioned that from the unsaponifiable material obtained from the petroleum extract of the resin (*A*) only 235 grams of crystalline products could be obtained. The remaining material (215 grams) formed a light-coloured, resinous mass, from which nothing definite could be separated, either by solvents or by fractional distillation under diminished pressure. A quantity (50 grams) of the material was acetylated, when it yielded 25 grams of a mixture of acetyl derivatives similar to that previously described.

Identification of the Fatty Acids.

The alkaline, aqueous solution of potassium salts from which the unsaponifiable material had been removed by filtration, as above described, was allowed to stand for some days, when a small quantity of a sparingly soluble potassium salt separated. This yielded an acid which melted at 72—74°, and appeared to consist chiefly of arachidic acid. The clear alkaline filtrate from which the sparingly soluble potassium salt had been removed yielded nothing on extraction with ether. It was therefore acidified and again extracted with ether, when a quantity (60 grams) of fatty acids was obtained. The latter, when distilled under diminished pressure, passed over between 240° and 260°/25 mm. as a viscid, oily liquid which solidified on cooling.

As these acids distilled within the same range of temperature as those previously obtained, which existed in the rhizome in the free state, equal parts of each portion were mixed for their examination.

A determination of the iodine value of this mixture gave the following result :

0·4807 absorbed 0·3770 iodine. Iodine value = 78·4.

Twenty grams of the 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. The soluble portion of the lead salts yielded 11 grams of liquid acids, whilst the insoluble portion gave 8 grams of solid acids.

The Liquid Acids.—These acids, when distilled under diminished pressure, passed over between 215° and 225°/15 mm.

A determination of the iodine value gave the following result:

0·4250 absorbed 0·6162 iodine. Iodine value = 145.

This indicated that the liquid acids consisted of a mixture of oleic acid and an acid of a higher degree of unsaturation.

In order to obtain more definite information respecting the composition of the above mixture, a quantity of it was oxidised with potassium permanganate according to the method described by Lewkowitsch (*Chemical Technology and Analysis of Oils, Fats, and Waxes*, 1904, Vol. I, 360). This resulted in the formation of approximately equal quantities of dihydroxystearic acid (m. p. 125—127°) and tetrahydroxystearic acid (m. p. 157—160°), and, with consideration of the iodine value, it would thus appear that the liquid acids consisted of a mixture of oleic and linolic acids in about equal proportions.

The Solid Acids.—These acids were fractionally crystallised from glacial acetic acid, but no separation was effected, since all the fractions melted at about 55—62°. The analysis of these acids and of their silver salts indicated that the mixture consisted principally of palmitic and stearic acids, together with a small amount of some acid of higher carbon content.

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

These extracts amounted to 40, 14, 11, and 30 grams respectively. The remainder of the original resin (45 grams) had undergone change and become insoluble. All the extracts were dark brown resins, and with the exception of a small quantity (7 grams) of acetovanillone and a trace of ipuranol, nothing definite could be isolated from them. The ethyl acetate extract appeared to contain some glucosidic material, as on acid hydrolysis it yielded a small quantity of sugar.

Examination of the Resin (B).

This was a dark brown powder and amounted to 110 grams. It was mixed with purified sawdust, and the mixture successively extracted in a Soxhlet apparatus with ether, chloroform, ethyl acetate, and alcohol.

The ether and chloroform extracts together amounted to 7 grams, and consisted of almost pure acetovanillone.

The ethyl acetate and alcohol extracts amounted to 10 and 80 grams respectively, and consisted only of resinous material.

Examination of the Aqueous Liquid (C).

Isolation of Acetovanillone.

The aqueous liquid (*C*), which amounted to about 15 litres, was repeatedly extracted with ether, and the combined ethereal extracts, after drying over anhydrous sodium sulphate, evaporated on the water-bath to a small volume. On keeping, about 45 grams of a substance were deposited in fine prisms. This substance was collected on a filter, washed with a little ether, and recrystallised from ethyl acetate, when it separated in colourless prisms melting at 112—114°.

The ethereal filtrate and washings were united, diluted with ether to about 300 c.c., and the solution shaken with successive portions of an aqueous solution of ammonium carbonate. The alkaline liquids thus obtained yielded, on acidifying, a viscid acid liquid, from which nothing definite could be isolated.

The ethereal liquid was then shaken with successive portions of a solution of sodium carbonate. On acidifying the alkaline extracts thus obtained and extracting with ether, a further quantity (15 grams) of the above-described crystalline substance, melting at 112—114°, was obtained. This, together with the portion previously obtained, was recrystallised from ethyl acetate, when the melting point rose to 115°. The yield of pure material was about 50 grams :

0·1217 gave 0·2900 CO_2 and 0·0670 H_2O. C = 65·0 ; H = 6·1.

$C_9H_{10}O_3$ requires C = 65·1 ; H = 6·0 per cent.

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

0·1612 gave 0·2290 AgI. OMe = 18·7.

$C_8H_7O_2 \cdot OMe$ requires OMe = 18·6 per cent.

This substance is thus seen to be acetovanillone (4-hydroxy-3-methoxyacetophenone), which was first obtained by Tiemann (*Ber.*, 1891, **24**, 2855) from another source, and has quite recently been shown by Finnemore (Trans., 1908, **93**, 1513) to be that constituent of *Apocynum cannabinum*, Linné, which had hitherto been known as "crystalline apocynin."

The identity of the above-described substance with acetovanillone was further confirmed by the preparation of its monoacetyl derivative (m. p. 57°), its methyl ether (m. p. 51°), and its phenylhydrazone (m. p. 126°) (Nietzel, *Ber.*, 1891, **24**, 2863).

The material contained in the mother liquors from which the acetovanillone had been separated, as above described, was distilled, when a

further small amount of acetovanillone was obtained, together with a trace of a substance, melting at about 301°, which corresponded in its properties to dehydrodiacetovanillone (Nietzel, *Ber.*, 1891, **24**, 2688).

The aqueous liquid from which the acetovanillone had been removed by extraction with ether was 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. The liquid was evaporated to dryness under diminished pressure, when about 200 grams of a brown, amorphous product were obtained, from which, however, nothing definite could be isolated.

Isolation of a Toxic, Bitter Principle, Apocynamarin, $C_{14}H_{18}O_3,H_2O$.

The filtrate from the above-mentioned 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 4·5 litres. The syrupy liquid thus obtained was kept for about four weeks, during which time a crystalline substance slowly separated. This substance was collected, washed with cold water, and dried in the air, when it amounted to 12 grams. It was purified by crystallisation from dilute alcohol, when it formed handsome, colourless prisms melting and decomposing at about 170—175°.

The air-dried substance was analysed :

0·1324 gave 0·3175 CO_2 and 0·0965 H_2O. C = 65·4 ; H = 8·1.

1·2010 of the air-dried substance, on drying in a vacuum, lost 0·0205 H_2O. H_2O = 1·7 per cent.

The substance after drying in a vacuum was analysed :

0·1390 gave 0·3390 CO_2 and 0·1020 H_2O. C = 66·5 ; H = 8·1.

After recrystallisation and drying in a vacuum, it was again analysed :

0·1372 gave 0·3352 CO_2 and 0·1005 H_2O. C = 66·6 ; H = 8·1.

0·4020 of the substance, dried in a vacuum, when heated at 110—115°, very slowly lost 0·0286 H_2O. H_2O = 7·1.

$C_{14}H_{18}O_3,H_2O$ requires C = 66·7 ; H = 8·0 ; H_2O = 7·1 per cent.

A determination of the molecular weight of the substance, dried in a vacuum, was kindly conducted by Mr. A. J. Ewins, B.Sc, according to Barger's microscopic method (Trans., 1904, **85**, 286), and gave the following result :

0·1100 in 2·41 pyridine was between 0·18 and 0·19 mol. benzil in pyridine, hence M.W. 254—240.

$C_{14}H_{20}O_4$ requires M.W. = 252.

It is evident that this substance possesses the formula $C_{14}H_{18}O_3$, and that it crystallises with one molecule of water, the small additional amount of water contained in the air-dried substance being doubtless occluded moisture. As it does not agree in its properties with any substance of the above formula which has hitherto been described, it is proposed to designate it *Apocynamarin*, with reference to the generic name of the plant from which it has been obtained, and the fact that it possesses an extremely bitter taste.

If to a solution of apocynamarin in acetic anhydride a few drops of concentrated sulphuric acid are added, a red colour is produced, which rapidly changes, first to blue and then to green, the green solution showing a fine reddish-bronze fluorescence.

On dehydrating apocynamarin by heating it at 115°, the water is only very slowly eliminated, the substance at the same time undergoing some further change, becoming brown and amorphous. The water of crystallisation may, however, be removed by prolonged boiling with chloroform, when the substance slowly dissolves. On evaporating the solvent a syrup, consisting of anhydrous apocynamarin, is obtained, and on treating this with water it at once crystallises, regenerating the original substance.

Apocynamarin is a neutral substance, but is changed by prolonged boiling with acids or alkalis. It contains no methoxyl group, and attempts to prepare an acetyl derivative resulted only in the formation of a syrup. Apocynamarin combines with phenylhydrazine, but the resulting hydrazone was amorphous and could not be obtained pure. It contained 5·6 per cent. of nitrogen, whereas theory requires 8·5 per cent. It appears, therefore, that apocynamarin contains a carbonyl group, and may be an aldehyde, since it readily reduces ammoniacal silver nitrate and alkaline potassium permanganate solutions.

Isolation of a New Glucoside, Androsin, $CH_3{\cdot}CO{\cdot}C_6H_3(O{\cdot}CH_3){\cdot}O{\cdot}C_6H_{11}O_5,2H_2O$ (*Acetovanillone Glucoside*).

The aqueous liquid from which the apocynamarin had been removed by filtration, as above described, was concentrated to the volume of 2 litres and kept for some weeks, but nothing further separated from it. The concentrated liquid, which formed a viscid syrup, contained a considerable quantity of sugar, as it readily reduced Fehling's solution, and yielded *d*-phenylglucosazone, melting at 208—210°.

One-eighth of the total liquid was diluted with water to 1 litre, and

then shaken with successive portions of chloroform in order to remove any possible traces of acetovanillone. About 50 grams of concentrated sulphuric acid, diluted with an equal weight of water, were subsequently added, and the solution boiled for three hours. The contents of the flask, which consisted of a dark brown aqueous liquid and a quantity of resinous material, were allowed to cool, and then filtered. The clear, aqueous filtrate yielded on extraction with chloroform 1·5 grams of acetovanillone. It thus appears probable that the glucoside of acetovanillone was present in the original aqueous liquid and in considerable quantity, as the amount of acetovanillone liberated by hydrolysis corresponded to about 24 grams of its glucoside in the total aqueous liquid.

With the object of isolating the glucoside, 1 litre of the above-described aqueous liquid was mixed with 3 litres of alcohol, and allowed to stand for twelve hours at 0°. The clear alcoholic liquid was then decanted from the syrup which had separated, and the latter again extracted with alcohol in a similar manner. The combined alcoholic extracts were evaporated to about 2 litres, when a further quantity of syrup separated, from which the supernatant liquid was decanted. The syrup was repeatedly extracted with small quantities of absolute alcohol, and the combined alcoholic liquids were evaporated. From the resulting alcoholic extract, after a prolonged process of extraction, a quantity of a syrup was eventually obtained which was entirely soluble in ethyl acetate containing a little alcohol. This syrup was dissolved in water, and the solution repeatedly extracted with chloroform, after which it was extracted twice with cold amyl alcohol. The aqueous liquid was concentrated as far as possible under diminished pressure, the residue dried, and then extracted many times with small quantities of dry, boiling ethyl acetate. The product removed by the latter solvent was dissolved in a small quantity of alcohol, when the solution, after some days, deposited a crystalline substance. This was collected and washed with alcohol, after which it was crystallised, first from 70 per cent. alcohol and finally from water. By this means about 1 gram of a substance was obtained in long, colourless needles, melting at 218—220°:

0·2039, heated at 115°, lost 0·2100 H_2O. $H_2O = 10·4$.
$C_{15}H_{20}O_8,2H_2O$ requires $H_2O = 9·9$ per cent.

0·0980 of anhydrous substance gave 0·1969 CO_2 and 0·0560 H_2O.
$C = 54·8$; $H = 6·3$.
$C_{15}H_{20}O_8$ requires $C = 54·9$; $H = 6·1$ per cent.

Half a gram of the substance was boiled for two hours with 5 per cent. aqueous sulphuric acid, when, on extracting the solution with chloroform, acetovanillone was obtained. The aqueous liquid, after

being deprived of sulphuric acid and concentrated, yielded, on treatment with phenylhydrazine, *d*-phenylglucosazone, melting at 208—210°.

It is evident that the above-described substance is the glucoside of acetovanillone, and, being a new compound, it is proposed to designate it *androsin*.

Androsin is readily solub in hot water or hot dilute alcohol, but sparingly soluble in cold water or in absolute alcohol. It is a β-glucoside, since it is hydrolysed by emulsin.

When androsin is heated with acetic anhydride, it yields an *acetyl* derivative, which crystallises from absolute alcohol in colourless needles melting at 154°.

Physiological Tests.

The physiological action of a number of the products obtained in the course of this investigation was kindly determined by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories.

The only active products obtained from resin (*A*) were the ethyl acetate and alcohol extracts, and these, when administered *per os* to dogs in doses of 0·5 gram, caused death, preceded by vomiting. The ethyl acetate and alcohol extracts of the resin (*B*) had only an emetic action. Acetovanillone, when injected intravenously into a cat, produced a small and very evanescent rise of blood pressure, whilst its glucoside, androsin, had no definite effect. One decigram of apocynamarin, when administered *per os* to a dog, had a powerful emetic action and no after effects. When, however, 10 milligrams of this substance were injected intravenously into a cat, a very large increase of blood pressure occurred, soon terminated by heart failure. Apocynamarin, when injected into the circulatory system, has also a pronounced diuretic action. The aqueous liquid from which the apocynamarin had been obtained produced some effects similar to those caused by the latter compound.

The conclusion, therefore, seems warranted that the emetic, diuretic, and cardiac tonic actions for which apocynum has been employed in therapeutics are all attributable to apocynamarin. It is probable, however, that the toxic action of the products obtained from resin (*A*) is due to another active principle. The physiological action of apocynamarin is being further investigated by Dr. H. H. Dale.

Summary.

The results of this investigation may be summarised as follows:

The material employed was the air-dried rhizome of *Apocynum androsaemifolium*, Linné. An alcoholic extract of the rhizome, when

distilled with steam, yielded a small amount of an essential oil, together with some acetovanillone. The essential oil, deprived of acidic substances, distilled between 130° and 250°, and possessed a strong, persistent odour. The non-volatile constituents of the rhizome, as obtained after treating the alcoholic extract with steam, consisted of a brown resin (*A*) insoluble in either hot or cold water; a brown resin (*B*) 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 brown resin (*A*), amounting to about 5·3 per cent. of the weight of the rhizome, yielded small quantities of ipuranol, $C_{23}H_{38}O_2(OH)_2$ (m. p. 285—290°), and acetovanillone, palmitic, stearic, oleic, and linolic acids, and a large quantity of unsaponifiable material. From the latter, two new alcohols, *androsterol*, $C_{30}H_{49}\cdot OH$ (m. p. 208—210°), and *homoandrosterol*, $C_{27}H_{43}\cdot OH$ (m. p. 192°), were obtained, whilst the presence of a third *alcohol*, apparently isomeric with androsterol, was proved by the isolation of its *bromoacetyl* derivative (m. p. 265—268°). Androsterol yields an *acetyl* derivative (m. p. 212—214°) and a *monobromoacetyl* derivative (m. p. 228—230°). *Acetylhomoandrosterol* melts at 236°. The brown resin (*B*), amounting to 0·58 per cent. of the weight of the rhizome, yielded a further small quantity of acetovanillone. The portion of the alcohol extract of the rhizome which was soluble in cold water, and from which the above-described resins had been removed, contained large amounts of sugar and tannin. It yielded a quantity of acetovanillone (m. p. 115°), which was also present in the form of its glucoside, *androsin*, $CH_3\cdot CO\cdot C_6H_3(O\cdot CH_3)\cdot O\cdot C_6H_{11}O_5,2H_2O$ (m. p. 218—220°), and a new substance, *apocynamarin*, $C_{14}H_{18}O_3,H_2O$ (m. p. 170—175°), which possesses an intensely bitter taste, is highly toxic, and represents the chief active constituent of the rhizome.

Addendum.

Since the above paper was written, a preliminary note by Horace Finnemore (Proc., 1909, **25**, 77) has appeared, in which he records the isolation of an active principle from the root (rhizome) of *Apocynum cannabinum*. This substance he proposes to designate "cynotoxin," and assigns to it the formula $C_{20}H_{28}O_6$. The properties attributed by Finnemore to "cynotoxin" are practically the same as those of apocynamarin, the composition of which agrees with the empirical formula $C_{14}H_{18}O_3,H_2O$. In this connexion it is significant that apocynamarin, when air-dried, yields results on analysis which are in accordance with the formula assigned by Finnemore to "cynotoxin." It has been shown, however, that air-dried apocynamarin contains, not only water of crystallisation, but also some occluded water, the latter

being lost on drying in a vacuum. It therefore seems probable that the active principle of *Apocynum cannabinum* is identical with apocynamarin.

With consideration of the formula assigned by Finnemore (*loc. cit.*) to the active principle of *Apocynum cannabinum*, and his conclusion that "it is a dilactone, either of Kiliani's digitic acid, $C_{20}H_{32}O_8$ (*Ber.*, 1891, **24**, 339), or of a closely related isomeride," the present author has redetermined the molecular weight of apocynamarin.

The substance, after drying at 115° until constant in weight, gave the following result :

0·3420 in 25·00 nitrobenzene gave Δ^t −0·192°. M.W. = 492.

As, however, apocynamarin when dried at 115° suffers some decomposition, the molecular weight was also determined by the cryoscopic method in acetic acid solution, first with the hydrated substance, and then with anhydrous material, as obtained after removing the water of crystallisation by means of chloroform, the last traces of the latter having been expelled by anhydrous acetic acid :

0·5305 of hydrated substance in 24·65 acetic acid gave Δ^t −0·307° M.W. = 268.

0·4990 of anhydrous substance in 21·50 acetic acid gave Δ^t −0·187° M.W. = 473.

$C_{14}H_{18}O_3,H_2O$ requires M.W. = 252.
$C_{28}H_{36}O_6$ „ M.W. = 468.

Apocynamarin, when dehydrated by boiling with chloroform, does not appear to suffer any change other than the loss of water of crystallisation, as the material thus obtained is instantly converted into the crystalline hydrated substance when brought in contact with water. It would therefore appear probable that the values given by the anhydrous substance indicate the correct molecular weight of the compound, and that the lower figure yielded by the hydrated compound is due to the water of crystallisation.

Dr. G. Barger has kindly conducted two determinations of the molecular weight with the hydrated substance by his microscopic method, which gave the following results, and therefore support the above view.

(1) 0·0933 in 0·887 acetic acid at 80° was between 0·225 and 0·250 mol.

2) 0·1072 in 1·914 alcohol was between 0·111 and 0·147 mol.

(1) Mean M.W. = 442. (2) Mean M.W. = 441.

The correct formula for apocynamarin would thus appear to be $C_{28}H_{36}O_6,2H_2O$, that is, double the formula assigned to it in the preceding part of this paper. The lower value obtained for the

substance in pyridine solution, which indicated its formula to be $C_{14}H_{18}O_3,H_2O$, cannot as yet be explained.

The result obtained by the analysis of the phenylhydrazone of apocynamarin (p. 746) is also in agreement with the double formula, since this compound was found to contain N = 5·6, and $C_{34}H_{42}O_5N_2$ requires N = 5·0 per cent.

In view of the above facts it is, therefore, possible that apocynamarin is the dilactone of Kiliani's oxydigitogenic acid, $C_{28}H_{40}O_8$ (*loc. cit.*), or of an isomeride.

In conclusion, the author wishes to express his thanks to Dr. F. B. Power for suggesting this research, and for advice and assistance given throughout the course of the work.

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