

CXLVI.—*The Constituents of Canadian Hemp.*
Part I. Apocynin.

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THE root of Canadian hemp (*Apocynum cannabinum*) has the useful property of retarding the heart in systole, and is employed as a remedy for dropsy and heart troubles; it is official in the United States. Although Husemann pointed out, in 1876, that the medicinal value of the drug was probably due to the presence of a constituent analogous to digitalin, it does not appear to have been examined closely until 1883, when Schmiedeberg (*Arch. Exp. Path. Pharm.*, **16**, 161) obtained from it two substances, which he named apocynin and apocynein respectively. The first of these had the characteristic physiological properties of *Apocynum*, but as the product appeared to be amorphous and resinous, and was obtained, moreover, only in very small quantity, it was not examined chemically.

The so-called apocynin of American pharmacy is merely the product obtained by precipitating the alcoholic extract with water and subsequently drying, but a definite crystalline substance is now produced commercially in small quantities under the same name, and has also been isolated by Wood (*J. Amer. Med. Assoc.*, 1904, **43**, 1953) from the alcoholic extract. Wood's apocynin was considered by him to be identical with the crystalline commercial product, and melted at 113°; no analysis was made, however, and pharmacological examination seemed to render it doubtful whether it was the active constituent sought. Inquiry having recently arisen as to the cause of certain untoward symptoms following the use of this drug, it was decided to re-investigate the nature of its active constituents on an adequate scale.

Isolation of Apocynin.

Forty kilos. of commercial *Apocynum cannabinum* root,* reduced to a coarse powder, were exhausted with hot 90 per cent. alcohol, for

* The cost of this large quantity of material was defrayed from the grant made by the Royal Society to the Physiological Department of Guy's Hospital, for which the author desires to express his thanks.

which purpose a copper Soxhlet apparatus was constructed capable of taking a charge of about 4 kilos. of the drug. Subsequent extraction of the residues with water yielded nothing further. The greater part of the alcohol having been removed by distillation, the remainder was expelled by heating with water in an open vessel. On cooling the aqueous solution thus obtained, a dark brown, resinous mass was deposited, which, after being twice extracted with boiling water, was reserved for subsequent examination. The united aqueous solutions were then extracted many times with ether, the aqueous residue again being reserved for further examination.

The combined ethereal extracts yielded on removal of the solvent a comparatively large amount of a pale brown, syrupy residue, which speedily deposited a copious, crystalline product. After recrystallisation from ether at the ordinary temperature, and further recrystallisation from dilute alcohol or hot water, this proved to be a well-defined substance identical with the crystalline apocynin of commerce. In all about 80 grams of this compound were obtained, representing a yield of 0·2 per cent. on the original material. Both the resin deposited from the alcoholic extract on treatment with water, and the residue of the aqueous solution after extraction with ether, yielded other equally definite products, although in smaller quantities; these are still under investigation, the results of which it is hoped to communicate later.

Apocynin crystallises in slender, colourless prisms, melts sharply at 115° (corr.), and may be distilled at about 300° without undergoing serious decomposition. It has a faint odour recalling that of vanillin. Although it may be sublimed slowly on a water-bath, apocynin is comparatively slightly volatile with steam; it is readily soluble in hot water, alcohol, ether, or chloroform, sparingly so in cold water, and practically insoluble in light petroleum, by which it may conveniently be precipitated from alcoholic solution. There is no doubt as to its identity with the products already mentioned: the crystalline appearance is similar to that depicted photographically by Wood; the melting point is the same as that of the crystalline apocynin of commerce, and is unaffected by admixture with this substance. Further, the characteristic derivatives (page 1518) are identical:

- I. 0·1947 gave 0·4629 CO₂ and 0·1074 H₂O. C = 64·8; H = 6·1.
 II. 0·1480 „ 0·3531 CO₂ „ 0·0816 H₂O. C = 65·1; H = 6·1.
 0·2051 in 19·65 acetic acid gave a depression of 0·23°. M. W. = 175.
 0·4589 „ 19·65 „ „ „ 0·52°. M. W. = 173.
 C₉H₁₀O₃ requires C = 65·1; H = 6·0 per cent. M. W. = 166.

Phenolic Derivatives of Apocynin.

Apocynin gives the usual phenolic reactions. It is soluble in cold alkalis, from which it is precipitated unchanged by carbon dioxide or dilute acids. It is particularly characterised by the formation of a deep bluish-violet coloration with ferric chloride in aqueous solution, which disappears on boiling with excess of the reagent, owing to the formation of an insoluble product.

The *potassium* derivative was prepared by the addition of the calculated amount of alcoholic potassium hydroxide to an alcoholic solution of apocynin (1.66 gram) at the ordinary temperature, and in the course of twenty-four hours was deposited as a crystalline mass. After recrystallisation from a mixture of moist ethyl and amyl alcohols, it formed well-defined, colourless needles. It is an unstable compound, however, and, after being kept for some weeks, was no longer soluble in alcohol :

0.4080 gave 0.1540 K_2SO_4 . $K = 16.9$.

$C_9H_9O_3K, 1\frac{1}{2}H_2O$ requires $K = 16.9$ per cent.

The *acetyl* derivative was obtained by heating apocynin (2 grams) with acetic anhydride (10 grams) and dry sodium acetate (2 grams) in a reflux apparatus for four hours. After shaking the clear product with water, a crystalline substance slowly separated, which, after recrystallisation from dilute alcohol, formed colourless needles and melted at 57° (corr.) :

0.1401 gave 0.3125 CO_2 and 0.0742 H_2O . $C = 60.8$; $H = 5.8$.

$C_{11}H_{12}O_4$ requires $C = 60.8$; $H = 5.8$ per cent.

The *benzoyl* derivative was prepared by shaking apocynin (1 gram) with benzoyl chloride and aqueous sodium hydroxide. The solid product, when washed with water and recrystallised from dilute alcohol, was obtained in silky, needle-shaped crystals melting at 106° (corr.). This derivative is especially well adapted for the identification of apocynin, as the crystals are particularly beautiful and characteristic :

0.1695 gave 0.4405 CO_2 and 0.0829 H_2O . $C = 70.9$; $H = 5.4$.

$C_{16}H_{14}O_4$ requires $C = 71.1$; $H = 5.2$ per cent.

An identical product was made from crystalline commercial apocynin.

The *methyl ether* was prepared by adding sodium (0.6 gram) to a solution of apocynin (4 grams) and methyl iodide (4 grams) in methyl alcohol. After boiling in a reflux apparatus on a water-bath for two hours, the alcohol was distilled off and the residue poured into water. On extracting the whole with ether and removing the solvent from the dried solution, an oil was obtained which soon crystallised. After recrystallisation from dilute alcohol, it formed colourless, nearly rectangular prisms, and melted at 51° (corr.) :

0.1568 gave 0.3825 CO_2 and 0.0955 H_2O . C = 66.5; H = 6.7.

$\text{C}_{10}\text{H}_{12}\text{O}_3$ requires C = 66.6; H = 6.6 per cent.

This compound is insoluble in alkalis, and does not give the phenolic reactions described above.

Presence of a Methoxyl Group in Apocynin.

Three determinations by the Zeisel-Perkin method gave results indicating the presence of a single methoxyl group; these, however, although concordant, were somewhat low (17.0, 17.0, 17.2 per cent.). On repeating the estimation with the addition of acetic anhydride, as recommended by Hewitt and Moore (*Trans.*, 1902, **81**, 318), the following result was obtained:

0.3471 gave 0.4929 AgI. OMe = 18.7.

$\text{C}_8\text{H}_7\text{O}_2\cdot\text{OMe}$ requires OMe = 18.7 per cent.

On extracting the residue with ether, after the estimation a small quantity of a crystalline substance was obtained, giving a green colour with ferric chloride, which changed to purple on the addition of sodium carbonate, the characteristic reaction of catechol derivatives. Apocynin is itself characterised by forming a bright red colour when boiled with 50 per cent. sulphuric acid, accompanied by the liberation of a volatile product closely resembling guaiacol in odour.

Ketonic Derivatives of Apocynin.

Although apocynin, like most phenols, readily reduces ammoniacal silver nitrate, it is without action on alkaline copper solutions, and does not restore the colour to leuco-magenta. The third oxygen atom is therefore not aldehydic; its ketonic character, however, is established by the formation of an oxime, a hydrazone, and a semicarbazone.

The *oxime* was prepared by dissolving apocynin (1 gram) in 90 per cent. alcohol (2 c.c.), diluted with water (10 c.c.), and then adding hydroxylamine hydrochloride (1 gram) and crystallised sodium carbonate (2 grams). After heating the whole on a water-bath for forty-five minutes, the product, from which an oil had separated, was poured into water and extracted with ether. The ethereal extract was washed and dried and the solvent removed, when the oxime was obtained as an oil, which only crystallised after keeping for some days in a vacuum desiccator. It formed groups of radiating prisms, and melted about 80—90°. As the yield of this product was very poor and the amount of material limited, it was not thought necessary, in view of the very well-defined character of the phenylhydrazone and the semicarbazone, to prepare a further quantity for analysis.

The *phenylhydrazone* was obtained by heating apocynin (1.66 gram)

in aqueous alcoholic solution (10 c.c. of alcohol and 20 c.c. of water) with phenylhydrazine hydrochloride (1.44 grams in 10 c.c. of water) and crystallised sodium acetate (1 gram) on a water-bath for an hour, when a brown oil had separated, together with a few slender crystals. On dissolving this product in alcohol and reprecipitating with water, it soon solidified. After recrystallisation from alcohol, the hydrazone formed flat, yellow needles, becoming reddish-brown after some time, and melting at 126° (corr.):

0.1515 gave 14.7 c.c. N₂ at 26° and 764 mm. N = 11.2.

C₁₅H₁₆O₂N₂ requires N = 10.9 per cent.

The *semicarbazone* was prepared by adding a solution of semicarbazide hydrochloride (2 grams) and crystalline sodium acetate (2 grams) in water (5 c.c.) to a solution of apocynin (2 grams) in 50 per cent. alcohol (20 c.c.). After three hours, crystals deposited, which, after recrystallisation from dilute alcohol, formed colourless needle-shaped prisms, and melted at 166° (corr.):

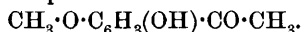
0.2180 gave 35.0 c.c. N₂ at 28° and 764 mm. N = 18.4.

C₁₀H₁₃O₃N₃ requires N = 18.8 per cent.

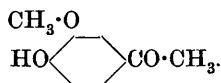
An identical product was obtained from crystalline commercial apocynin.

Constitution of Apocynin. Identity with Acetovanillone.

The above results not only account for the whole of the oxygen in the apocynin molecule, but also practically establish its constitution, except as regards the orientation of the groups. A phenolic ketone of the formula C₉H₁₀O₃, containing a methoxyl group, cannot be other than a derivative of acetophenone of the constitution



The fact that the diphenol obtained in the course of the methoxyl determination shows the colour reaction characteristic of catechol, and that the substance itself when heated with sulphuric acid gives the odour of guaiacol, indicates with considerable probability that it is a derivative of the latter. The resemblance of the odour of the substance, albeit faint, to that of vanillin (4-hydroxy-3-methoxybenzaldehyde) also suggests very strongly that it is the methyl ketone corresponding with this compound, and having therefore the constitution represented by the formula :



This conclusion was entirely confirmed. Apocynin is identical with the acetovanillone which was isolated by Tiemann (*Ber.*, 1892, 24, 2855) in small quantity from a resinous by-product of the oxidation of

isoeugenol, and subsequently obtained by Goldschmiedt and Hemmelmayr (*Monatsh.*, 1904, **35**, 338) by the hydrolysis of scoparin. The substances and their derivatives so completely correspond in composition and properties that further work in this direction was superfluous.

The melting points of the various products are given in the following table. The first and second columns give those obtained by the author in the course of the present investigation; the third and fourth give those obtained by Neitzel and by Goldschmiedt from acetovanillone prepared from *isoeugenol* and scoparin respectively; in the fifth are added the melting points of apocynin and its derivatives as prepared synthetically by the present author (see following paper).

	Apocynin, crystalline commercial.	Apocynin, natural. (Finne- more).	Acetovanillone.		Apocynin, synthetic (Finne- more).
			from <i>isoeugenol</i> . (Neitzel).	from scoparin. (Gold- schmiedt).	
Ketone	115°	115°	115°	115°	115°
Acetyl derivative ...	—	57	58	57—58	—
Benzoyl „	106	106	106	—	106
Methyl ether.....	—	51	48—49	—	—
Oxime	—	80—90	95	—	—
Phenylhydrazone.....	—	126	126	125	126
Semicarbazone.....	166	166	—	—	166

Otto's and Neitzel's Syntheses of Acetovanillone.

The constitution of acetovanillone was determined by Tiemann from the above and other experimental data of Neitzel, the orientation of the groups being conclusively established by the oxidation of the acetyl compound to vanillic acid and of the methyl ether to veratric acid. The identity with 4-hydroxy-3-methoxyacetophenone was further confirmed by two syntheses of acetovanillone. Neitzel (*loc. cit.*) describes its production, although only in minute quantity, by distilling calcium vanillate with calcium acetate. Otto (*Ber.*, 1891, **24**, 2869), also working in Tiemann's laboratory, describes its synthesis, in amount sufficient for analysis, by the condensing action of aluminium chloride and zinc chloride on a solution of guaiacol in acetic acid.

As it was desirable to obtain a synthetic specimen of apocynin, in order to compare its physiological properties with those of the natural product, the synthetic experiments of Neitzel and of Otto on acetovanillone were repeated. The results, however, were unsatisfactory, and it was found impracticable to form sufficient material for detailed examination.

Distillation of calcium vanillate with calcium acetate according to Neitzel's directions yielded mainly acetone, the only crystalline product

obtained being a few milligrams of a substance melting about 98° , which gave the characteristic colour reactions of catechol (m. p. 104°). A repetition of the dry distillation under the more favourable conditions afforded by conducting the operation under reduced pressure in an atmosphere of carbon dioxide and with the aid of a bath of fusible metal, gave a minute amount of a crystalline product which may have been acetovanillone, but it is impossible to say definitely. Neitzel himself, indeed, does not even give the melting point of his product, but merely states that "Das so hergestellte Präparat zeigte alle Eigenschaften des Acetovanillons."

Otto's method proved less unsatisfactory, but the yield was too small for any practical purpose. From 60 grams of guaiacol, following the directions of this worker, 0.6 gram of crude, dark-coloured acetovanillone and 0.4 gram of fairly pure substance was obtained. This was still somewhat coloured, however, and melted indefinitely at $96-109^{\circ}$, but it gave the colour reactions of apocynin and yielded the characteristic benzoyl derivative, melting at 106° . In the light of Gattermann's later observation (*Ber.*, 1892, 25, 3232) that guaiacol is readily converted into catechol by the action of aluminium chloride, it is now difficult to see how Otto's method could afford any notable yield of the condensation product he sought.

The physiological properties of apocynin and the other substances which the author has isolated from *Apocynum cannabinum*, and of the compounds which have been produced incidentally, are in course of determination by Mr. P. P. Laidlaw, M.B., in the Physiological Laboratory of this Hospital, and communication of the results is deferred until the work is completed.

The author desires to express his thanks to Dr. Wade for the interest he has taken in this investigation, and the advice he has given throughout its progress. Acknowledgment is also due to Dr. F. B. Power for his courtesy in suspending the investigation of *Apocynum*, which had been commenced in his laboratory, on hearing that the author was already engaged on the same problem.

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