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ORNITHOGALUM THYRSOIDES

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The genus *Ornithogalum* has been stated to contain about one hundred species (Wood and Evans, 'Natal Plants,' 1899, Vol. I., Part 2, page 57), and of these, according to the 'Flora Capensis,' seventy-three are natives of South Africa, eleven species having been found in Natal.

Ornithogalum thyrsoides, Jacq., is a bulbous, liliaceous plant, which is common in Cape Colony, but not yet reported from the Transvaal. It is known in South Africa by the names "Chincher-and-Ching," "Chinkerinchee," and "Viooltje" ('Transvaal Department of Agriculture, Annual Report, 1903-1904,' p. 312). The plant is reputed to be poisonous, and many deaths among horses have been attributed to it when mixed with the forage. A paper on this subject, entitled, "Poisoning of Horses by *Ornithogalum thyrsoides*, or Chinkerinchee," has been contributed by D. Hutcheon, M.R.C.V.S., Acting-Director of Agriculture, to *The Agricultural Journal of the Cape of Good Hope*, 1906, 28, p. 165. In this paper, which is accompanied by a coloured illustration of the flowering plant, a number of cases of poisoning were reported, with the symptoms attending them, and the results of post-mortem examinations of the animals. Although the evidence thus brought forward would appear to have been quite conclusive respecting the destructive action of the plant in question on horses, some doubt has nevertheless been expressed in certain quarters with regard to the correctness of this conclusion.

The subject having been brought to our notice as one that merited further investigation, it was decided to make a com-

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plete examination of the plant with respect to its constituents and their physiological action, and the results obtained are embodied in the present communication.

EXPERIMENTAL.

The material employed in this investigation consisted of the entire flowering plant, including the underground bulbous portion, of the previously mentioned *Ornithogalum thyrsoides*, Jacq., which had been obtained through the kindness of Mr. J. T. Borthwick, chief veterinary surgeon, Department of Agriculture, Cape Town, South Africa. The identity of the material was kindly confirmed for us by Mr. E. M. Holmes, F.L.S., who had compared it with specimens of the plant contained in the Herbarium of the Royal Botanic Gardens, Kew.

Both the bulbs and the entire overground portion of the plant were first separately subjected to a test for the presence of an alkaloid by treatment with Prollius' fluid, but with a negative result.

As a preliminary experiment, 50 Gm. of the dried and ground material were successively extracted in a Soxhlet apparatus with various solvents, when the following amounts of extract, dried in a water-oven, were obtained :—

Petroleum (b.p. 35-50°)	extracted	0·97 Gm.	= 1·94 per cent.
Ether	„	0·58 Gm.	= 1·16 per cent.
Chloroform	„	0·46 Gm.	= 0·92 per cent.
Ethyl Acetate	„	0·50 Gm.	= 1·00 per cent.
Alcohol	„	3·90 Gm.	= 7·80 per cent.
Water	„	7·25 Gm.	= 14·50 per cent.
	Total	13·66 Gm.	= 27·32 per cent.

For the purpose of a complete examination, a quantity (3·46 kilogrammes) of the dried and ground material was thoroughly extracted with hot alcohol, when 495 Gm. of a thick extract were obtained. The greater portion of this extract (475 Gm.) was brought into a large flask, some water added, and steam passed into the mixture until the volatile products were removed. There then remained in the distillation flask a dark-coloured, aqueous liquid (A) and a quantity of a dark-green resin (B). The latter was collected, thoroughly washed with hot water, and the washings added to the aqueous liquid.

Examination of the Steam Distillate.

The distillate obtained as above described was slightly opalescent, and contained on the surface a very small amount of oil, which became partly solid. It was extracted with ether, and the ethereal liquid shaken with a solution of sodium carbonate. The alkaline liquid was then acidified and extracted with ether, when, after the removal of the solvent, a small amount of an oily product was obtained, which solidified to a crystalline mass. This was brought on a porous plate, and subsequently crystallised from glacial acetic acid, after which it melted at 61°-62°, and was found to consist of palmitic acid. The ethereal liquid which had been extracted with sodium carbonate yielded, on evaporation of the solvent, a very small amount of a yellowish essential oil, possessing a persistent odour.

Examination of the Aqueous Liquid (A).

The aqueous liquid which, as previously mentioned, had been separated from the dark green resin, was thoroughly extracted with ether, but this removed only traces of resinous matter. The liquid was then treated with a slight excess of a solution of basic lead acetate, which produced an abundant, deep yellow precipitate. This precipitate was collected on a filter, washed with water, and then suspended in water and decomposed by hydrogen sulphide. On filtering the mixture a light yellow liquid was obtained, which was concentrated under diminished pressure, but nothing definite could be isolated from it.

The filtrate from the above-mentioned basic lead acetate precipitate was treated with hydrogen sulphide for the removal of the excess of lead, the mixture filtered, and the filtrate concentrated under diminished pressure to the consistency of a syrup. On allowing the syrupy liquid to stand, it deposited a small amount of crystalline material, which consisted of potassium and sodium chlorides, with a trace of nitrate. After the removal of this material, the liquid was found to contain sugar, since it reduced Fehling's solution and yielded *d*-phenylglucosazone, melting at 212-213°. By the subsequent treatment of the aqueous liquid with alcohol and ethyl acetate a further amount (25 Gm.) of inorganic matter was separated, which consisted chiefly of magnesium and sodium acetates, and represented by far the larger proportion of the constituents of the liquid.

Examination of the Resin (B).

The dark green resinous material, which had been separated from the aqueous liquid as above described, amounted to 141 Gm. This material, with the exception of a small portion reserved for physiological tests, was dissolved in alcohol, mixed with purified sawdust, and the thoroughly dried mixture extracted successively in a Soxhlet apparatus with I. light petroleum (boiling-point, 35-50°); II. ether; III. chloroform; IV. ethyl acetate; and V. alcohol.

I. Petroleum Extract.

This extract, after the complete removal of the solvent, formed a soft, green solid, and amounted to 47 Gm. It was dissolved in a large volume of ether, when a small quantity of a sparingly soluble substance separated. The latter was found to consist chiefly of pentatriacontane, with a trace of a fatty acid. The respective hydrocarbon was subsequently isolated in larger amount. The ethereal liquid from which the solid substance had separated was afterwards shaken with a solution of ammonium carbonate, but this removed nothing. It was then shaken with several successive portions of aqueous sodium carbonate, the resulting alkaline liquids being acidified and extracted with ether. On removing the ether a quantity (20 Gm.) of a solid acid was obtained, and this was distilled under 15 Mm. pressure, when it passed over between 240° and 280°. It was crystallised from ethyl acetate, when it melted at 60°-61°, and gave the following result on analysis:—

0·1140 gave 0·3134 CO_2 and 0·1302 H_2O. C = 74·9; H = 12·7.
$C_{16}H_{32}O_2$ requires C = 75·0; H = 12·5 per cent.

A portion of the acid was converted into a silver salt, and the latter analysed.

0·2840 of salt gave on ignition 0·0834 Ag. Ag = 29·4.
$C_{16}H_{31}O_2Ag$ requires Ag = 29·7 per cent.

The above-described substance was thus identified as palmitic acid.

The ethereal liquid, which had been treated with ammonium and sodium carbonates, as above described, was shaken with a 10 per cent. solution of sodium hydroxide, but this only removed a further small amount of palmitic acid. It was subsequently washed, dried, and the ether evaporated, when a residue was obtained which amounted to 25 Gm. This product was hydrolysed by boiling for six hours in a reflux apparatus with an alcoholic solution of 7·5 Gm. of potassium hydroxide, after which the alcohol was removed, water added, and the alkaline liquid repeatedly extracted with ether. The ethereal liquid was dried and the solvent evaporated, when a viscid liquid was obtained, which became solid on cooling. This was dissolved in about 600 C.c. of hot alcohol, and the solution allowed to cool, when a quantity of solid separated. The latter was collected on a filter, washed with alcohol and dried, when it amounted to 2·7 Gm. It was distilled under 15 Mm. pressure, when it passed over as a nearly colourless oil, which solidified on cooling. On crystallisation from ethyl acetate it separated in small, lustrous leaflets, melting at 74°-75°.

0·1334 gave 0·4156 CO_2 and 0·1782 H_2O. C = 84·9 ; H = 14·8.
$C_{35}H_{72}$ requires C = 85·4 ; H = 14·6 per cent.

The above substance was thus identified as pentatriacontane.

The alcoholic mother-liquors from the pentatriacontane were concentrated to about 250 C.c., and allowed to stand, when a further quantity of this hydrocarbon separated. This was removed, and the filtrate evaporated to a small bulk, when, after standing for some time, a solid separated in the form of plates. The latter, when collected and dried, amounted to 0·5 Gm. This substance was crystallised twice from a mixture of ethyl acetate and dilute alcohol, when it formed colourless laminæ, melting at 133°-134°, and gave the colour reactions yielded by the phytosterols.

0·1042, on heating at 110°, lost 0·0052 H_2O. H_2O = 4·9.
0·0590 of anhydrous substance gave 0·1820 CO_2 and 0·0662 H_2O.
C = 84·1 ; H = 12·4
$C_{27}H_{46}O, H_2O$ requires H_2O = 4·5 per cent.
$C_{27}H_{46}O$ requires C = 83·9 ; H = 11·9 per cent.

This substance was thus identified as a phytosterol. Its specific rotatory power was determined with the following result:—

0·0990 of anhydrous substance in 20 C.c. of chloroform gave a_D − 0° 20′ in a 2-Dcm. tube, whence $[a]_D$ −33·6°.

The alkaline, aqueous liquid, from which the above-mentioned substances had been removed by means of ether, was acidified and distilled with steam. The distillate was a slightly opalescent, acid liquid, and contained a few oily drops floating on the surface. It was, therefore, extracted with ether, which removed a small amount of an oily acid, from which a silver salt was prepared. The latter was analysed with the following result:—

0·2958 of salt gave on ignition 0·1568 Ag. Ag = 53·0.
$C_4H_7O_2$ Ag requires Ag = 55·4 per cent.
$C_5H_9O_2$ Ag requires Ag = 51·7 per cent.

The above-mentioned oily acid was, therefore, evidently a mixture. After extraction with ether the distillate was found to contain small amounts of formic and butyric acids.

The liquid remaining in the distillation flask contained a green, fatty solid, floating on the surface. It was extracted with ether, when, on evaporation of the solvent, 4 Gm. of a product were obtained, which was purified by distillation under 15 Mm pressure. When subsequently dissolved in ethyl acetate, it was obtained in colourless laminæ, melting at 59-60°.

0·1770 gave 0·4856 CO_2 and 0·2042 H_2O. C=74·8; H=12·8,
$C_{16}H_{32}O_2$ requires C=75·0; H=12·5 per cent.

The above product was thus identified as palmitic acid.

II. ETHER EXTRACT.

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

This extract, after the complete removal of the solvent, formed a dark green solid, and amounted to 24·2 Gm. Its ethereal solution, on standing, deposited a quantity (4·7 Gm.) of a dark green substance, which was collected on a filter. This substance was subsequently brought into a Soxhlet apparatus, and extracted with absolute alcohol, when a quantity of green, resinous material was quickly removed. On continuing the extraction with a fresh portion of alcohol, a light coloured, sparingly soluble solid was slowly taken out. The latter was purified by crystallisation from dilute pyridine, from which it separated in small rosettes of microscopic needles, melting at 285-290°.

0·1028 gave 0·2726 CO_2 and 0·0968 H_2O. C=72·3; H=10·5
$C_{23}H_{40}O_4$ requires C=72·6; H=10·5 per cent.

This substance yielded a colour reaction similar to that produced by the phytosterols, and was identified as ipuranol, $C_{23}H_{38}O_2(OH)_2$ (*Amer. Journ. Pharm.*, 1908, **80**, 264, 576; *Journ. Chem. Soc.*, 1908, **93**, 907; 1909, **95**, 249; 1910, **97**, 7). When boiled with acetic anhydride it yielded diacetylipuranol, $C_{23}H_{38}O_4(CO{\cdot}CH_3)_2$, which separated in leaflets melting at 162°.

The specific rotatory power of ipuranol and of its acetyl derivative not having previously been ascertained, and the amount of material at present available having been sufficient for this purpose, these determinations have now been made, with the following results:—

0·2150 of ipuranol, dissolved in 20 C.c. of pure pyridine, gave α_D —0° 24′ in a 1 Dcm. tube, whence $[\alpha]_D$ —37·2°.

0·0982 of diacetylipuranol, dissolved in 20 C c. of ethyl acetate, gave α_D —0° 15′ in a 2 Dcm. tube, whence $[\alpha]_D$ —25·4°.

The green, resinous material which was separated from the ipuranol by means of alcohol, as mentioned above, was only very sparingly soluble in ether. After treatment with animal charcoal it was obtained as a light-brown resin, from which, however, nothing but a further small quantity of ipuranol could be isolated.

The original ethereal solution, from which the ipuranol and sparingly soluble resin had been separated, was shaken with a solution of ammonium carbonate, but this removed nothing. It was then shaken with a solution of sodium carbonate, which extracted only a small quantity of resinous material. It was, finally shaken with a 10 per cent. solution of sodium hydroxide, which removed practically all of the material from the ether. The alkaline liquid was acidified and extracted with ether, when it yielded a further small quantity of ipuranol, together with about 17 Gm. of resinous material. As nothing definite could be separated from the latter it was heated in a reflux apparatus with dilute alcoholic sulphuric acid, but after this treatment there were obtained, besides resinous products, only a small amount of a neutral volatile oil and a little formic acid. As no sugar was formed it is evident that the ether extract of the resin contained nothing glucosidic.

The original ethereal solution, after extraction with alkalies as above described, was found to contain only a small amount of substance which had escaped extraction by the preceding treatment with petroleum.

III. Chloroform Extract.

This extract, after the complete removal of the solvent, was a brown resinous mass, and amounted to 12 Gm. As nothing could be directly separated from it, it was dissolved in chloroform and treated with alkalies. Sodium carbonate removed only a very small amount of resinous substance, but subsequent treatment with a solution of sodium hydroxide extracted practically all the material from the chloroform. The alkaline liquid thus obtained was acidified and shaken with chloroform, the chloroform solution being dried with anhydrous sodium sulphate and the solvent removed, when a quantity (10·5 Gm.) of resin was obtained. This was heated with dilute, alcoholic sulphuric acid in a reflux apparatus for five hours, after which the alcohol was evaporated, water added, and the mixture distilled with steam, but the distillate contained only traces of formic acid. The distillation flask contained a dark yellow liquid, on the surface of which was a soft, resinous mass. The latter was extracted with ether, and the ethereal liquid shaken with a solution of sodium carbonate. The alkaline liquid thus obtained was acidified and extracted with ether, the ethereal liquid being dried and the solvent removed, when a small amount of a syrupy product was obtained which deposited crystals on standing. This substance was purified by recrystallisation from ethyl acetate with the use of animal charcoal, when it melted at 176°-180°, but the amount was too small for further examination. The ethereal liquid, after being shaken with a solution of sodium carbonate as above described was found to contain only resinous material.

The acid, aqueous liquid, from which the above-mentioned, soft, resinous mass had been removed by means of ether, was treated with baryta for the removal of the sulphuric acid, and concentrated. A small amount of syrup was thus obtained, which reduced Fehling's solution, but no osazone could be prepared from it.

IV. and V. Ethyl Acetate and Alcohol Extracts.

These extracts, after the complete removal of the solvents, formed dark, resinous masses, and amounted to 11·5 and 9·4 Gm. respectively. They were treated with dilute alcoholic sulphuric acid, as described in connection with the preceding ether extract, but nothing definite could be obtained from them.

Physiological Tests.

For the purpose of ascertaining whether the plant under examination possesses the poisonous properties ascribed to it, some tests were kindly conducted for us by Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories.

As a preliminary experiment, 5 Gm. of the ground entire plant, mixed with food, were given to each of two guinea-pigs, and, although all was not eaten, the result in both cases was fatal.

Further tests were then made on guinea-pigs with various products obtained in the course of the chemical examination of the plant, with the endeavour, if possible, to locate the active principle. The administration was in all cases by the mouth.

One Gm. of the original alcoholic extract, representing 7 Gm. of the entire dried plant, quickly produced a fatal result. All the symptoms were in agreement with those which have been recorded as typical of the poisoning produced in horses by some South African fodder.

The crude resin, in amounts of 0·25 Gm., representing about

6 Gm. of the entire dried plant, proved fatal, with the usual gastro-intestinal effects.

The aqueous liquid, which had been separated from the resin and purified by means of basic lead acetate, was given in amounts corresponding to about 14 Gm. of the entire dried plant. It produced temporary illness, but the effects soon passed off, and the animal recovered.

The product obtained by the decomposition of the basic lead acetate precipitate proved fatal in amounts corresponding to about 14 Gm. of the entire dried plant.

The various products obtained from the crude resin by its successive extraction with light petroleum, ether, chloroform, ethyl acetate, and alcohol, were then tested with the following results :—

All these extracts had a lethal effect in doses of 0·25 Gm., with the exception of the portion extracted by light petroleum, which was quite inactive. Doses of 0·1 Gm. of the ether, chloroform, ethyl acetate, and alcohol extracts of the resin were then separately administered to guinea-pigs. The animal which received the ether extract died about nineteen hours after ingestion. The animals to which the chloroform and ethyl acetate extracts had been administered showed signs of pronounced diarrhœa, and were found dead on the second morning after ingestion. The animal fed with the alcohol extract was dead on the third morning after ingestion. A post-mortem examination showed in all cases the condition of gastro-enteritis characteristic of that produced by the drug. The various extracts were therefore qualitatively similar in their action, but the ether extract was most toxic, and appeared to be capable of producing death before any considerable gastro-enteritis had developed.

SUMMARY.

The results of the proceeding investigation of *Ornithogalum thyrsoides*, Jacq., may be summarised as follows :—

It was found by a preliminary test that the plant contained no alkaloid.

An alcoholic extract of the plant, when distilled with steam, yielded a very small amount of an essential oil, with traces of palmitic acid.

The portion of the extract which was soluble in water contained a considerable quantity of inorganic salts, together with amorphous products, and a sugar which yielded *d*-phenylglucosazone, melting at 212-213°.

The portion of the extract which was insoluble in water consisted of a dark green resin, and amounted to about 4 per cent. of the weight of the entire dried plant. This resin, when extracted successively with light petroleum (boiling-point 35-50°), ether, chloroform, ethyl acetate, and alcohol, was resolved into a number of products, each of which was separately examined.

The petroleum extract yielded a considerable amount of palmitic acid, which was present both in a free and combined state, together with pentatriacontane, $C_{35}H_{72}$, a phytosterol $C_{27}H_{46}O$ (melting-point 133-134°; $[\alpha]_D - 33{\cdot}6°$), and a very small amount of volatile fatty acids.

The ether extract consisted chiefly of resinous material, but from it there was isolated a small amount of the dihydric alcohol, ipuranol, $C_{23}H_{38}O_2(OH)_2$ (*Amer. Journ. Pharm.*, 1908, **80**, 264, 576 ; *Journ. Chem. Soc.*, 1908, **93**, 907 ; 1909, **95**, 249 ; 1910, **97**, 7). The specific rotatory power of ipuranol is $[\alpha]_D - 37{\cdot}2°$, whilst its acetyl derivative has $[\alpha]_D - 25{\cdot}4°$.

The chloroform, ethyl acetate, and alcohol extracts were all dark-coloured, amorphous products. The chloroform extract, after acid hydrolysis, yielded a very small amount of a crystalline substance melting at 176-180°, but otherwise nothing definite could be obtained from any of these products.

The reputed poisonous properties of the plant have been fully confirmed, inasmuch as the administration of 5 grammes of the ground air-dried material to guinea-pigs was attended with fatal results. The toxic principle appears to be chiefly contained in the resin, and especially in that portion of the latter which is soluble in ether. As, however, all the extracts obtained by the successive treatment of the resin with various solvents were physiologically active, with the exception of the portion removed by light petroleum, there are apparently several poisonous substances present. The attempts to obtain a definite active principle from these various products were, however, unsuccessful.

In conclusion, we desire to express our best thanks to Dr. H. H. Dale, Director of the Wellcome Physiological Research Laboratories, for having kindly conducted for us the physiological experiments involved in this investigation.

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