THE ISOLATION OF THE TOXIC PRINCIPLE OF *ŒNANTHE CROCATA*

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INTRODUCTION

ŒNANTHE CROCATA Hemlock Water Dropwort, belonging to the family Umbelliferae, is common in wet places all over Western Europe, including the British Isles. Its toxicity has been known for a great many years, Linnaeus having noted it in Sweden in 1746 (Skarman[1]), while Orfila[2] quotes a number of instances of poisoning in the 17th and 18th centuries. Witthaus[3] cites 159 cases, 42 of them (i.e., 26 per cent.) being fatal, while there are numerous references to the subject in more recent medical literature (Thomas[4]: McGarth[5]). Holmes[6] refers to *Œnanthe crocata* as the most poisonous plant in England, while Fenton and Robertson[7] state that it is responsible for more stock poisoning than any other. The frequency with which cases of poisoning occur is probably because it has a pleasant taste, and an attractive smell, rather like celery.

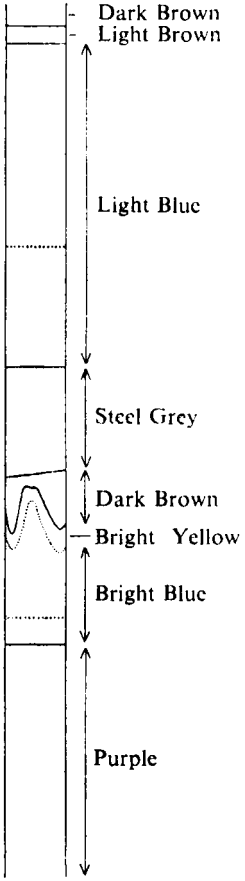

FIG 2. Diagram showing sections of Chromatogram

In spite of its well-established toxicity, very little work has been done on the chemistry of the plant. Cormerais and Pihan-Dufeillay[8] found the active ingredient to be contained in a resinoid material in the root, while Gerding[9] discovered a similar material in *Œ. fistulosa.* By purifying an ethyl alcoholic extract of the root by dissolving in ether, washing with sodium hydroxide and precipitating with light petroleum, Pohl[10] obtained a neutral resinous substance, which he named œnanthotoxin, and to which he assigned the formula $C_{17}H_{22}O_5$. Tutin[11] isolated several non-toxic substances (triacontane, hentriacontane and ipuranol) from an ethyl alcoholic extract of the root, but he also found that the toxicity was associated with the ether-soluble neutral resin. He considered that "Pohl's œnanthotoxin" was not a pure substance. We are not aware of any other chemical investigation of value.

EXPERIMENTAL

Extraction. As a preliminary experiment, ethyl alcoholic extracts of roots, seeds and green

stems were made, and tested for toxicity by intra-peritoneal injection in mice. Only the extract from the root proved lethal, so investigation was confined to this part of the plant. The roots were obtained from the banks of the Thames near Kew, and passed, while still fresh, through an ordinary kitchen mincer. An extract made with 0·9 per cent. sodium chloride solution showed no activity, while there was little to choose in toxicity between extracts made with ethyl alcohol, chloroform or ether. The latter solvent was chosen owing to the ease of removal. Air-dried roots extracted with either ethyl alcohol or ether gave less toxic preparations.

6 kg. of freshly minced roots were gently refluxed in a 20-l. bolthead flask for a total time of 12 hours with 11 l. of ether. The residue was washed with 6 l. of ether, the washings added to the extract, and the whole dried over anhydrous sodium sulphate. The ether was then distilled off, leaving 25 g. of a toxic brown oily residue, with an A.L.D. in mice of approx. 25 mg./kg. This oil was dissolved in 30 ml. of ether and 600 ml. of light petroleum was added. This threw down 17·5 g. of a toxic resinous material. The fraction remaining in the light petroleum was not toxic. The resinous precipitate was dissolved in 300 ml. of ether and shaken with 300 ml. of 40 per cent. sodium hydroxide, then with 300 ml. of 8 per cent. sodium hydroxide solution, and finally washed with saturated sodium sulphate solution (it having been found that water gave troublesome emulsions) until the washings were neutral. The sodium hydroxide extract, neutralised with hydrochloric acid, threw down a dark resinous material that was not toxic. The ethereal solution was dried over anhydrous sodium sulphate and evaporated to dryness, yielding 8 g. of light brown viscous oil, which was highly toxic. This oil deposited a few crystals on standing, but insufficient for investigation.

Chromatography

Chromatography in daylight was unsatisfactory, but on elution on alumina columns under ultra-violet light a series of coloured bands was given by most organic solvents. The best separation was obtained with a mixture of dry benzene and ethyl alcohol (99 : 1). Therefore a solution of the light brown oil in benzene/ethyl alcohol (99 : 1) was placed on an alumina column and developed with the same mixed solvent. The bands seen under ultra-violet light were removed, eluted separately with ethyl alcohol and the extracts tested on mice. The toxicity was found to be associated with a band which gave a steel-grey fluorescence (Figs. 1 and 2).

The 5 g. of pale yellow oily material obtained on evaporating the extract from this band deposited oily crystals on standing. These were recrystallised by dissolving in the minimum of chloroform and cooling to −15°C. 400 mg. of colourless crystals was obtained, which were highly toxic (*v. infra.*). They can be purified, either by recrystallisation from chloroform, methyl alcohol or benzene, dissolving at room temperature

and cooling as above, or by chromatography on an alumina column, eluting with benzene/ethyl alcohol (99:1), when a single steel-grey zone is obtained. These purifications do not alter the melting point or crystalline habit.

PROPERTIES

Crystalline "œnanthotoxin" prepared by this method forms small colourless irregular crystals, (Fig. 3) m.pt. 80° to 81°C., insoluble in water, light petroleum, alkalis and dilute mineral acids, but freely soluble in ether, ethyl alcohol and chloroform. If dissolved in ethyl alcohol, and the solvent allowed to evaporate, the substance crystallises in flat plates, many of them with a characteristic "bullet-shaped" appearance (Fig. 4). Found C, 71·75; H, 7·29; O, 20·96 per cent. Molt. wt. (cryoscopic in benzene) 292 $C_{18}H_{22}O_4$ requires C, 71·50; H, 7·43; O, 21·16, Mol. wt. 302.

Nitrogen is absent. The substance is extremely unstable, changing comparatively rapidly into a brown insoluble resinous material, decomposing without melting above 200°C., and possessing no pharmacological activity. This change is accelerated by a high temperature and by oxygen. At 4°C. under oxygen-free nitrogen œnanthotoxin is much more stable, only a slight yellow colour developing in a period of weeks. The specific rotation in chloroform is + 14·7°. Œnanthotoxin gives an immediate black colour with concentrated sulphuric acid.

PHARMACOLOGY

Œnanthotoxin, in the form of an emulsion, was injected intraperitoneally into white mice. In the earlier preparations the emulsion had been prepared by dissolving the substance in 0·5 ml. of ethyl alcohol

TABLE I

Results of injecting 0·5 ml. of lecithin-saline suspension of crystalline œnanthotoxin containing the dose shown, intraperitoneally into white mice, *c.*18 g. weight. C—Interval before onset of convulsions. (In minutes from time of injection). D—Time elapsing before death. S—Survived.

Mouse No. ...		1	2	3	4	5	Average
Dose							
·25 mg.	C	3	3	3	2	3	2·8
	D	22	10	27	21	7	17·4
·12 mg.	C	3	4	4	3	4	3·4
	D	23	51	28	51	7	32·0
·06 mg.	C	13	8	4	4	5	6·8
	D	26	45	44	42	59	43·2
·03 mg.	C	11	19	13	20	16	15·8
	D	83	58	57	88	27	62·6
·015 mg.	C	28	20	32	42	23	29·4
	D	S	38	S	S	32	
·007 mg.	C	—	—	—	—	—	
	D	S	S	S	S	S	
0	C	—	—	—	—	—	
	D	S	S	S	S	S	

Controls received 0·5 ml. lecithin saline only.

This gives an A.L.D. of approximately 0·83 mg./kg. Pohl's œnanthotoxin killed one rabbit in 105 minutes at a dose of 24 mg./kg.

and pouring this into 9·5 ml. of 0·9 per cent. sodium chloride. In the case of the crystalline product, the emulsion produced by this method was unstable, owing to the removal of some emulsifying agent by the purification. The emulsion of crystalline œnanthotoxin for injection was made by pouring an ethyl alcoholic solution into a 0·1 per cent. solution of lecithin in 0·9 per cent. sodium chloride solution.

The results of the experiment on mice with the crystalline substance are given in Table I.

Signs

Shortly after injection the animal becomes perceptibly less active, respiration is accelerated but the animal shows no sign of distress. 1 to 20 minutes after injection the animal becomes restless and adopts the characteristic feeding posture with excessive movement of the fore limbs.

Soon tremors of varying intensity are observed which may be confined to only local regions, but more frequently are general. The onset of convulsions may be sudden, but it is usually preceded by a generalised tremor of the whole body. Depending on the dose the convulsive movements vary from those involving tonic contractions and rolling, to wild jumping movements. The convulsive stage varies in its duration: usually several convulsions follow in rapid succession, but occasionally an interval of some minutes may intervene between any two.

The terminal phase is invariably heralded by pedalling movements of the hind limbs and irregular vigorous movements of the fore limbs. Abduction of the digits is marked, and always accompanies the pedalling movements. At this stage hæmorrhages from the buccal cavity may be observed accompanied by trismus of the jaw muscles. Prior to death the animal assumes a characteristic posture, lying on one side with the fore limbs acutely flexed and the hind limbs rigidly extended to the full. Death follows.

These symptoms correspond closely with those recorded in cases of poisoning in man and domestic animals following the ingestion of the roots of *Œnanthe crocata*.

Summary

1. A method is described for the preparation of highly toxic crystals from water dropwort root. Previous workers have only reported oils or resins of far less toxicity.

2. These can be purified by recrystallisation, or by chromatography on an alumina column, when a single steel-grey zone is obtained. These purifications do not alter the melting point or the crystalline habit.

3. The crystalline material, m.pt. 80° to 81° C., is insoluble in water, light petroleum, alkalis, and diluted mineral acids, but readily soluble in chloroform, ethyl alcohol and ether.

4. The crystals are extremely unstable, yielding an insoluble infusible resinous material, with no pharmacological activity.

5. If kept under nitrogen at 4°C., the crystals can be preserved for weeks with little loss of activity.

6. Death after characteristic convulsions follows the intraperitoneal injection of an emulsion of the crystalline material into mice.

7. The A.L.D. is 0·83 mg./kg. of body weight.

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