PHARMACOLOGICAL AND CHEMICAL OBSERVATIONS ON SOME TOXIC NECTARS

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Received April 20, 1959

Acetylandromedol (andromedotoxin) has been isolated from the nectar of *Rhododendron thomsonii*. The pharmacological properties of toxic nectars obtained from several *Rhododendron* sp. and hybrids have been investigated and these indicate that the poisonous principle is acetylandromedol.

BEES which consume nectars containing toxic substances may produce poisonous honey. Reports of poisoning of bees by nectars from plants of the *Ericaceae* are not numerous and reports of systematic chemical and pharmacological investigations of the nectars themselves have not appeared in the literature. Honey found near Trebizonde in northern Asia Minor poisoned Xenophon's troops[1] and Pliny[2] describes the poisonous honey found at Heraclea in the Pontus in Asia Minor as having a strong odour causing sneezing and if eaten, thirst, perspiration and pain. Strabo,[3] a native of the Pontus, mentions the intoxication of three of Pompey's armies by honey eaten as they were crossing the mountains of Themiscyra in Asia Minor and their consequent destruction by a local tribe. Thresh[4], Krause[5], Pulewka[6] and Ungan[7] have given more recent descriptions of the history and properties of poisonous honey.

Nectars taken from several families are believed to produce poisonous honey but most of it comes from the *Ericaceae*. *Rhododendron*, *Azalea*, and *Andromeda* sp. are especially important. Plants from these species contain the complex, non-nitrogenous compound andromedotoxin (Plugge[8–10]) isolated from Trebizonde honey[4,6]; from *Andromeda japonica* by Eykman[11,12] who called it asebotoxin and from *Rhododendron* sp.[8–10,13,14]. It was also obtained by Horning and his co-workers[15,16] from *R. maximum* and *Kalmia angustifolia* var. *caroliniana* and identified as the acetyl derivative of the glycol, andromedol and hence called acetylandromedol. Rhodotoxin[17–20] and grayanotoxin I[20,21] are identical with andromedotoxin[16,20]. There are reports of a number of plants being poisonous to bees and in some instances *Rhododendron* sp. have been suspect[22,24]. A serious outbreak of poisoning of bees by *Rhododendron* sp. on the island of Colonsay led us to investigate the nature of the toxic substance, to attempt to identify it and the rhododendrons which produced it.

METHODS

Nectars were collected from rhododendron flowers (Tables I and II) with a Pasteur pipette and stored in glass stoppered bottles at 4° after removal of pollen grains by centrifugation.

Pharmacology

Bees. For toxicity tests with bees, measured volumes of centrifuged nectars were put into glass vials inverted over holes in the tops of cages. These contained 15 to 25 bees and were kept in a dry incubator at 28°. Control solutions contained 30 per cent w/v of sucrose or 10 and 100 μg./ml. of andromedotoxin in 30 per cent w/v of sucrose. The observation period was 48 hours.

Mice. Groups of from six to eight female mice weighing 30 to 40 g. were given the nectars by intraperitoneal injection. A 1 mg./ml. solution of andromedotoxin was used as control. A few nectars were tested by injecting them intraperitoneally into frogs.

Cat respiration and blood pressure. Cats of either sex weighing between 1·5 and 3·5 kg. were anaesthetised with intraperitoneal sodium pentobarbitone or chloralose. Blood pressure was recorded from the common carotid artery and injections made into the cannulated external jugular vein. Respiration was recorded directly from the movements of the epigastrium by means of a thread attached to a lever, or from the movements of a tambour connected to a cannula inserted into the trachea.

Other experiments. Doses of up to 1 ml. of nectar were injected into the cannula supporting the isolated cat heart perfused by Langendorff's method[26]. The effects were compared with those after injection of 0·1 to 0·5 mg. of andromedotoxin. In a few experiments the effects were investigated of intravenous injection of up to 100 μg./kg. of andromedotoxin and 0·5 ml. of nectar upon the twitch height of the cat gastrocnemius muscle stimulated indirectly *via* the sciatic nerve. The direct action of nectars on isolated strips of guinea pig ileum suspended in Tyrode's solution at 36° and on acetylcholine-induced contractions of the frog rectus muscle suspended in frog Ringer's solution at room temperature were also investigated.

Results

Toxic nectars (Tables I and II) produced the following effects. Bees became dull, sluggish or inert, flew only in short spurts, falling from time to time to the floor of the cage. They then lay on their sides or backs, the abdomen turned upwards as if the bee was supported by its wings. There was ataxia and disorientation, bees climbing downwards not upwards as is usual. When taken out of the cage the bees spun round characteristically and vibrated their wings very rapidly but could not fly. There was increasing weakness and prostration and death followed, the tongue often being extended.

Mice became quiet, the flanks drawn in and there was a tendency to drag the hind limbs and abdomen along the ground. Sharp spasmodic contractions of the diaphragm and gasping movements of the mouth followed. There was salivation, dyspnoea and weakness but when disturbed the animals were still able to move fairly rapidly. Convulsions ensued and after one of these episodes respiration did not return. The heart continued to beat for about one minute after respiration failed.

TABLE I

TOXICITY OF NECTARS FROM CERTAIN *Rhododendron* SPECIES TO MICE AND BEES

Series	Species	Bees		Mice
		No. per group	48 hr. mortality per cent	Approx. LD50 ml./kg.
Arboreum	*R arboreum*	21	62	>20+
,,	*R. arboreum* var. *album*	46	87	>20+
,,	*R. arboreum* var. *hermisinum*	45	16	—
,,	*R. niveum*	27	70	>20++
Barbatum	*R. barbatum*	32	40	—
Falconeri	*R. fictolacteum*	45	0	2·5
Fulvum	*R. fulvum*	23	26	>30++
Grande	*R. sinogrande*	41	44	10
	R. macabeanum	35	14	—
Neriiflorem	*R. sperabile*	32	0	>20++
	R. neriiflorem	107	0	>30++
	R. sperabiloides	32	0	>20++
	R. scyphocalyx	29	0	>20++
	R. euchaites	25	0	>20++
	R. haematodes	25	0	>20++
Taliensi	*R. pratti*	47	85	>20+
Thomsonii	*R. thomsonii*	168	100	10
Andromedotoxin	100 µg./ml.	49	84	1 mg./kg.
,,	10 µg./ml.	51	24	

+ no deaths but toxic symptoms
++ no deaths or toxic symptoms
} at highest dose used.

TABLE II

TOXICITY OF NECTARS FROM CERTAIN *Rhododendron* HYBRIDS TO MICE AND BEES

Name	Parents	Bees		Mice
		No. per group	48 hr. mortality per cent	Approx. LD50 ml./kg.
R. Dicharb.	*R. arboreum* × *R. dicroanthum*	37	0	>20*
R. Red Admiral	*R. arboreum* × *R. thomsonii*	53	59	—
R. Fiery Cross	*R. barbatum* × *R. griffithianum*	25	100	7
R. Abbot	*R. thomsonii* × *R. delavayi*	36	31	720*
R. Barclayi	*R. thomsonii* × R. Glory of Penjerrick (R. Glory of Penjerrick = *R. arboreum* × *R. griffithianum*)	88	97	7
R. Barclayi var. Helen Fox	ditto	84	100	—
R. Red Star	*R. thomsonii* × R. Ascot Brilliant (R. Ascot Brilliant = *R. thomsonii* × —)	34	100	7
R. Redwing	R. Barclayi (see above) × R. shilsonii (R. shilsonii = *R. thomsonii* × *R. barbatum*)	29	0	>20*
R. J. G. Millais	*R. thomsonii* × —	27	100	7
—	R. Barclayi × *R. meddianum*	27	100	5
R. May Day	*R. griersonianum* × *R. haematodes*	20	0	—
R. Ascot Brilliant	*R. thomsonii* × —	19	37	—
—	*R. thomsonii* × —	26	100	5
—	*R. thomsonii* × —	19	100	5
Andromedotoxin	100 µg./ml.	49	84	1 mg./kg.
,,	10 µg./ml.	51	24	

* No deaths, but toxic symptons at highest dose used.

In frogs, respiration became gasping, the animals became weak, prostration followed and there were occasional convulsive movements.

Pulewka[6] used mice and frogs to identify poisonous honey. After subcutaneous injection of extracts the animals showed characteristic respiratory disturbances with convulsive movements of the diaphagm, contraction of the bronchial and glottal muscles and movements which simulated vomiting.

When injected into cats, toxic nectars (0·1 to 0·5 ml./kg.) caused depression of respiration associated with contractions of the diaphragm, bradycardia and either a sharp, short-lived fall in blood pressure often followed by a smaller rise, or a sustained hypertensive effect. Similar effects were obtained when 10 to 40 μg./kg. of andromedotoxin were injected. Bradycardia and hypotension were abolished by 1 mg./kg. of atropine sulphate. Cutting the vagi abolished the respiratory and hypotensive effects. The pressor response was abolished by 2 mg./kg. of phentolamine.

Moran and his colleagues[25] similarly showed intravenous injections of low doses of andromedotoxin (2 to 3 μg./kg.) into dogs to cause bradycardia, hypotension and respiratory depression. At higher dose levels (40 to 80 μg./kg.) there was hypertension due to release of adrenaline from the adrenal medullae. Bradycardia was prevented by atropine which also reduced the hypotensive effect. Bradycardia, hypotension and respiratory depression were abolished by vagotomy. Hypertension was prevented by adrenalectomy or phentolamine. Hardikar[13] obtained similar results.

Toxic nectars and andromedotoxin slowed the isolated cat heart and increased the amplitude of the beat. No effect was observed upon twitch height in the gastrocnemius muscle. The nectars (0·01 to 0·03 ml./ml.) caused direct contractions of the isolated guinea pig ileum (Fig. 1) which were inhibited by atropine sulphate. There was no inhibition of acetylcholine-induced contractions of the frog rectus by up to 0·3 ml./ml. of nectar.

Chemical Observations

Isolation and identification of acetylandromedol (andromedotoxin). 240 ml. of nectar of *R. thomsonii* was divided into four 60 ml. portions and each portion simultaneously extracted twenty times in succession with 50 ml. portions of chloroform on a power-driven shaker, allowing five minutes each time for equilibration. Previous experiments had shown that a rise in temperature caused by the use of a continuous chloroform extractor caused extensive decomposition of the chloroform-soluble material. The combined chloroform extracts were concentrated to about 10 ml. at 30° under reduced pressure. The concentrate was treated three times in succession with 20 ml. portions of dry benzene and taken to dryness at room temperature under reduced pressure to ensure removal of all traces of water. A solid residue (42 mg.) was obtained. Fractional crystallisation from chloroform using the Craig tube method[27,28] gave as the least soluble fraction colourless needles

(29 mg. representing 10·8 mg./100 ml. of nectar). The remaining material, which was soluble in light petroleum, was not investigated further. The crystalline solid showed appreciable variations in melting point depending upon the rate of heating but the values always fell within the limits 260 to 272°.

The material showed no absorption in ultra-violet light and was laevo-rotatory. $[\alpha]_D = -8°$ ($c = 1{\cdot}83$ in ethanol); the figure reported for acetylandromedol[15] was $[\alpha]_D = -8{\cdot}8°$ in ethanol. On treatment of the

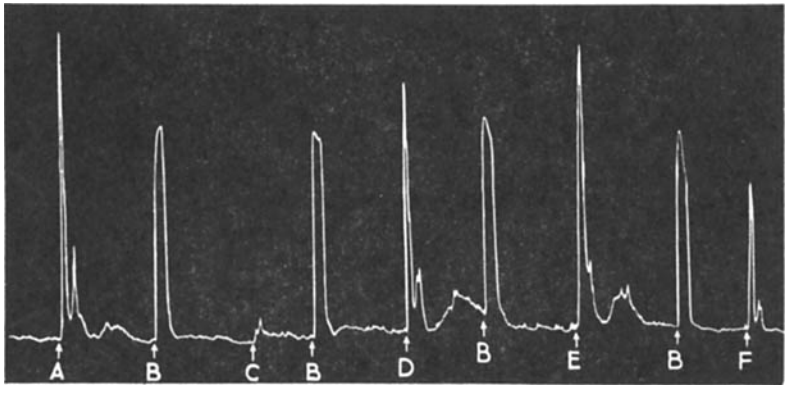

FIG. 1. The effects of rhododendron nectars on the isolated guinea pig ileum 8 ml. bath containing Tyrode's solution at 36°.
At B. 0·3 μg. of acetylcholine chloride.
At E. 30 μg. of acetylandromedol.
At A. 0·3 ml. of nectar from R. Barclayi × *R. meddianum.*
At C. 0·3 ml. " " *R. arboreum.*
At D. 0·3 ml. " " R. Red Star.
At F. 0·3 ml. " " *R. thomsonii.*

solid with mineral acids a deep red colouration was produced and the compound gave a positive reaction with periodic acid. These properties are all in agreement with those reported for acetylandromedol[15]. A direct comparison of the infra-red spectra in KCl discs of our specimen with that of a specimen of authentic acetylandromedol kindly supplied by Dr. E. C. Horning and with the specimen used in the pharmacological tests, confirmed the identity of the three specimens. There was no depression of the melting point on admixture of the compounds.

DISCUSSION

This investigation has shown that some rhododendron nectars are toxic to bees, mice and cats. *R. thomsonii* and some of its hybrids are especially poisonous. *R. arboreum* var. *album* and *R. pratti* are also toxic. The isolation and identification of acetylandromedol (andromedotoxin) from the nectar of *R. thomsonii* and the similar effects seen when toxic nectars and acetylandromedol are tested pharmacologically, indicate that this is the poisonous substance. It is not, however, possible to predict that a particular rhododendron will secrete a toxic nectar: for example,

the hybrid R. Redwing which is derived from four species, three of which are toxic, has been found to secrete a non-toxic nectar (Table II). The significance of these results to bee-keepers will be discussed elsewhere.

Acknowledgements. Permission to collect nectars from their garden on the Isle of Colonsay was kindly given by the Dowager Lady Strathcona and Mount Royal. We thank Mr. and Mrs. J. W. H. Younger of Eckford. near Dunoon, for samples from their garden, Mr. H. N. Davidian of the Royal Botanic Gardens of Edinburgh who identified the rhododendrons, Dr. Glenn Ullyot of Smith, Kline and French Laboratories, Philadelphia, U.S.A., for the sample of andromedotoxin used in the pharmacological studies, Miss Irene Moon and Mr. Charles Dzewu for technical assistance.

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After Miss Carey presented the paper there was a DISCUSSION.