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From ‘mad honey’ to hypotensive agents, the grayanoid diterpenes

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ABSTRACT

The toxicity of Rhododendron *species and other members of the Ericaceae which is due to the grayanoid diterpenes, is described. Their structures, biogenesis and biological activity is reviewed.*

Keywords: Rhododendron *species, Ericaceae, grayanotoxins, honey, poisons, hypotensive agents*

1. Introduction

Honey has been a prized food since the dawn of civilisation. Apart from fructose, glucose, water and small amounts of other sugars and traces of proteins, honey can contain natural products that are characteristic of the source of the nectar from which the bees derived the honey. These natural products can impart a distinctive character to a monofloral honey such as manuka honey derived from the New Zealand Tea Tree *(Leptospermum scoparium,* Myrtaceae) or heather honey from *Calluna vulgaris* (Ericaceae). However there are a number of instances in which these additional natural products can have a deleterious effect. The so called ‘mad honey’ is an example in which consumption of the honey leads to temporary intoxication, dizziness, hypotension (reduced blood pressure), vomiting and diarrhoea.

The dramatic consequences of ingesting this honey were known in antiquity. The Greek writer Xenophon in 401 BC, reported these effects on soldiers who, on returning across north-eastern Turkey from victory against a Persian army, had stolen some of this honey and become intoxicated.

In 69 BC, Roman troops under the command of Pompey the Great who were attacking the Heptakometes in Turkey, were poisoned by the toxic honey from honeycombs that had been deliberately placed along their route. The intoxicated Roman troops became an easy prey in this early example of chemical warfare.

Figure 1 Rhododendron luteum*, by Chrumps (own work) CC BY 3.0, via Wikimedia Commons.*

This honey had been produced by bees that had fed on particular *Rhododendron* species, probably *R. ponticum* or *R. luteum* (Figure 1) which grow profusely in the north-eastern Trabzon region of Turkey near the Black Sea. Honey with these intoxicating properties, is still produced as 'deli bal' and at one time it was added to alcoholic drinks to increase their potency. Although medical problems arising from the consumption of this honey are well documented in Turkey, they are by no means restricted to that area. They have been recorded in Japan, Nepal and both North and South America.

Other *Rhododendron* species are known to contain related biologically active constituents. For example, *R. molle* has been used in traditional Chinese medicine for its anodyne and anaesthetic properties. It is also used as an insecticide. Similar compounds were obtained from *R. maximum*, *R. japonicum* and other *Rhododendron* species in the United States. *Rhododendron* species are members of the Ericaceae. A number of other members of this large plant family produce toxic natural products, some of which are found in the nectar obtained by bees whilst others have been extracted from the leaves of plants which are stock poisons. Over the last 50 years considerable progress has been made in identifying the compounds that are responsible for these toxic properties and in understanding the way in which they exert their biological activity. The majority of these compounds belong to the grayanoid group of tetracyclic diterpenoids.

2. The isolation of grayanoids

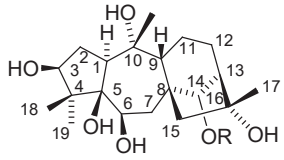
Investigations into the toxicity of leaves from members of the Ericaceae began in the late 19th century. A toxic substance named asebotoxin obtained from *Andromeda (Pieris) japonica,* was described in 1882[1] whilst the name andromedotoxin was given to a compound from *R. ponticum* in the late 1880s[2]. Andromedotoxin, obtained in this instance from *R. maximum,* was thoroughly characterised in 1954[3] and described then as a potent hypotensive agent. Under the alternative name acetylandromedol, it was shown[4] to be present in a number of *Kalmia, Leucothoe, Lyonia, Pernettya, Pieris and Rhododendron*

species. The leaves of the Evergreen Mountain Laurel, *K. latifolia,* which grows in the eastern states of the USA, were known to be stock poisons. A relative, *K. angustifolia* which was a particularly abundant source of these compounds, had the colloquial name 'Lambkill'. A Japanese shrub, *Leucothoe grayana,* sometimes called 'Hanahironoki', the sneeze-causing shrub because of the irritant compounds that it produces, was also an abundant source. It was structural studies on its products, the grayanotoxins, which led to the overall name for this family of diterpenoids[5,6]. Other related compounds have been obtained from *Pieris japonica*[7] and from *Lyonia ovalifolia* (the lyonols)[8]. A stimulus for many of these studies was the powerful hypotensive action of acetylandromedol (grayanotoxin G l) described in 1954[9] and which has subsequently been examined in detail.

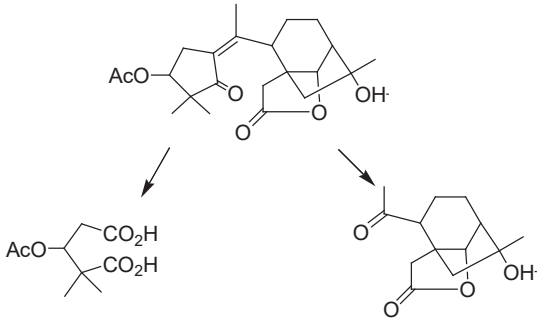
3. The structures of the grayanoids

Studies leading to the elucidation of the structure and stereochemistry of the grayanotoxins were reported between 1957 and 1962[4–6,10,11]. They reveal the interesting transition between the role of degradative, particularly oxidative, chemistry and the application of spectroscopic methods, especially NMR and IR. The nature of the 5 : 7 : 6 : 5 grayanane ring system of the grayanotoxins meant that classical dehydrogenation reactions to form readily identifiable aromatic hydrocarbons which had provided valuable structural information with other diterpenoids containing the 6 : 6 : 6 ring system, were less helpful in this case (for a detailed review of the structural evidence for the grayanotoxins see ref.[12]; the occurrence of the various grayanoids has been reviewed in ref.[47]). The key to the chemical degradation lay in the presence of the *cis*-5,6-diol. Oxidative cleavage of the diol led to the opening of the central ring[5,13,14]. Acetylation and dehydration of the tertiary alcohols generated alkenes which allowed the cleavage of the molecule by ozonolysis, into two fragments (Scheme 1) which were then identified by further degradation. The rudimentary 40 MHz ^{1}H NMR spectra which were available at the time, nevertheless gave valuable information on the chemical environment of various protons, particularly alkenes, secondary alcohols and methyl groups. Gross structures (Scheme 1) for grayanotoxins G I and G III, and in a separate study, grayanotoxin G II, were proposed in 1961. There was some ambiguity over the stereochemistry of the ring junctions[11,15–19], but this was eventually resolved by the formation of a series of transannular ethers involving reactions between the 5-hydroxyl group and C-3, C-9, C-10 and C-15 as well as between C-6 and C-14. The structures were finally established by an X-ray crystal structure in 1970[20].

Subsequently over 100 compounds related in various ways to the grayanotoxins, hence the generic name grayanoids, have been isolated from members of the Ericaceae. The degradative chemistry which had been carried out in the earlier structural studies had involved breaking the molecule down into smaller identifiable fragments and then bringing this information together



Grayanotoxin I R = Ac
Grayanotoxin II [10 = CH_2] R = H
Grayanotoxin III R = H



Scheme 1

to establish the overall structure. The successful experiments often required 0.1 – 0.5 g of material and hence an abundant source of the original natural product. Modern spectroscopic methods have been particularly useful in this polyfunctional, polycyclic bridged series of compounds. They have been employed not only to identify particular functional groups but also, using two-dimensional NMR spectra, to establish the connectivity between groups of carbon and hydrogen atoms and to reveal through-space interactions. These experiments can be carried out with 1 – 10 mg of material. Consequently coupled with modern separation techniques, this has meant that many more grayanoids from the same plant source, can be identified.

Common structural features of these compounds include the geminal dimethyl group at C-4 and either a 3β-hydroxyl group, a 3-ketone or 2β,3β-epoxide, the vicinal 5β,6β-diol and either a tertiary alcohol or an alkene at C-10, an alcohol at C-14 and a tertiary alcohol at C-16. Less common features are hydroxyl groups at C-7α, C-11 and C-12. In some cases, the hydroxyl groups particularly at C-14, are masked as their acetate or propionate esters or at C-3 as their glycosides. Although the majority of the grayananes have a *trans* A/B ring junction (C-1αH) a few have been isolated in which the A/B ring junction is *cis* (C-1βH). Many of these structures have also been confirmed by X-ray crystallography. In recent years, a number of compounds have been isolated in which the C – C bonds of the underlying 5 : 7 : 6 : 5 tetracyclic grayanane ring system have been broken leading to 1,5-[21,22], 3,4-[23], and 9,10-*seco*-grayananes[24] and 1,10 : 2,3-*diseco*-grayananes[25]. These include the grayanols.

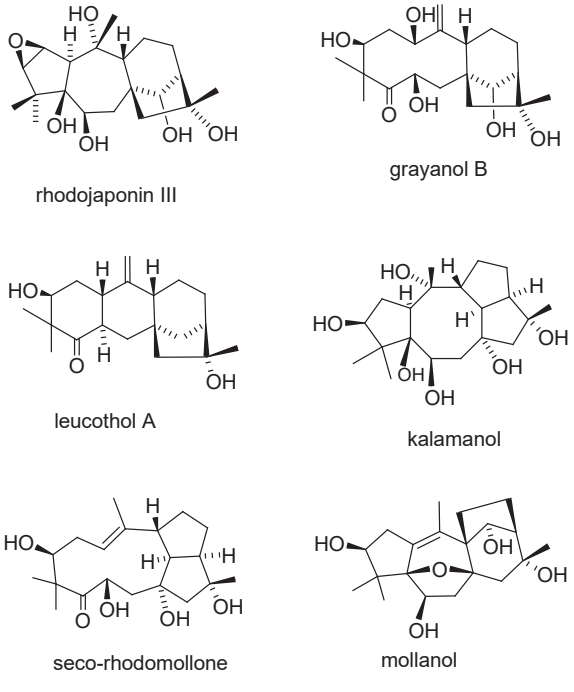
A number of rearrangement products including the kalmanes[26], 1,5-*seco*-kalmanes[27], micranthanes[28], leucothanes[29] and mollanols[30] have been detected. *Rhododendron molle, Leucothoe grayana, Pieris japonica* and *Craiobiodendron yunnanense* have been particularly fruitful sources of these compounds. Some examples of these structures are shown in Scheme 2.

The availability of sufficient material from the Japanese *L. grayana,* has meant that a number of aspects of the chemistry of the grayanotoxins have been explored. These include some interesting oxidative and rearrangement reactions which inter-relate grayanotoxin G II with compounds possessing a grayanol, leucothol and ent-kaurene carbon skeleton[31–36].

The juxtaposition of the hydroxyl groups and their participation in transannular and other neighbouring group reactions has implications for the biological activity of these compounds and suggests that the interactions between more than one centre may facilitate hydrogen bonding to receptors involved in the expression of their biological activity.

4. The biogenesis of the grayanoids

The grayananes are tetracyclic diterpenoids, *i.e*. they belong to the large family of diterpenoids which are biosynthesised from four isoprene units, the basic building block of all terpenes. The tetracyclic diterpenoids include some well-known natural products such as the gibberellin plant hormones, steviol, the



Scheme 2

aglycone of the sweetener stevioside and cafestol from coffee. Ent-kaurene has been shown to be the parent hydrocarbon of the gibberellins[37] and steviol[38] and it is presumed to fulfil this role for many other tetracyclic diterpenoids.

Despite the variety of modifications to the underlying grayanane carbon skeleton that have been isolated, it is possible to rationalise these skeleta in terms of plausible biogenetic relationships although the specific substrates for the individual steps are not known. We can consider these skeletal modifications in terms of rearrangement and ring cleavage reactions.

A plausible mechanism for the biosynthesis of the 5:7:6:5 ring system from ent-kaurene could involve the oxidative removal of the C-1α hydrogen atom to initiate the rearrangement of the C-5:C-10 bond to form the C-5:C-1 bond. A similar oxidative removal of a C-14 hydrogen could initiate the rearrangement of the C-8:C-9 bond to C-14β that leads to the kalmane skeleton whilst oxidative removal of the C-9β hydrogen could initiate the rearrangement of the C-8:C-14 bond to C-9 which leads to the mollanols. In these cases, the remaining carbocations are discharged either by hydration or ether formation.

An oxidative process has been proposed for the formation of the grayanols[22]. Alternatively the ring-opening may occur by fragmentation of the 5β,10α-diol to generate the 10-membered ring and the C-5 carbonyl group that is characteristic of the grayanols. Cyclisation of the grayanols may lead to the leucothols and the A/B *cis*grayananes. The 3,4-*seco*-grayananes may arise by fragmentation of a C-3 hydroperoxide.

5. Biological activity of the grayanotoxins

Many of the grayanotoxins have been identified as stock poisons and they may have a role in protecting plants against predation. A number of the parent plants are evergreens, some grayanotoxins have been shown to act as insect antifeedants[39,40] and the parent plants such as *R. molle* have been used in the control of insect pests.

Whilst the intoxicating effects of the accidental ingestion of grayanotoxins in humans are rarely fatal, in ruminant animals, their ingestion may have more serious fatal consequences. In 1954, it was reported[9] that grayanotoxin G I (tested under the old name of andromedotoxin) produced a 20–40% lowering of blood pressure in test animals at a concentration of 5–10 μg kg^{-1}. This hypotensive effect has attracted considerable interest. It has been reported[41] that rhodojaponin III (from *R. molle)* has been used since 1972 in medical practice in China at doses of 1–2 mg for this purpose. Structure : activity studies[42] have implicated the role of the 3β-hydroxyl group, the 5β,6β-diol, the C-10β methyl group and a C-14β acyl substituent.

The mode of action has been thoroughly investigated. The grayanotoxin is reported (for a review see ref.[43]) to bind specifically to the sodium ion channels in the cell membrane. This binding modifies the configuration of the proteins that make up these channels to such an extent that it prevents sodium channel

inactivation. The continued activation of these cells has effects on the central nervous system and on the heart, producing bradycardia and respiratory depression resulting in hypotension and the loss of consciousness. The hypotensive cardiovascular activity of these compounds and their bioavailability has made them suitable templates for further structure : activity studies.

The novel 5 : 7 : 6 : 5 bridged grayanane ring system and their biological activity, has made these compounds targets for partial and total synthesis and these have been reported[44,45]. Their detection is also important[46] because of the more widespread availability of speciality food stuffs such as monofloral honeys and the potential harm that may arise from their unwitting consumption. There is also a greater interest in adding edible flowers to salads and there is the possibility that mistakes may arise. However it is worth noting that not all plants from the Ericaceae contain toxic levels of the grayanotoxins. Thus berries such as the cranberry, bilberry and whortleberry are produced by *Vaccinium* species and the fruits of the Strawberry Tree*, Arbutus unedo,* are edible whilst the honey produced by bees that have fed on heather, *Calluna vulgaris*, is a popular monofloral honey.

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