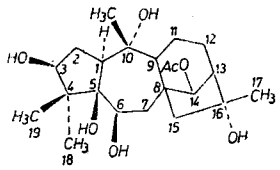
Terpenoids from Some Toxic European Ericaceae Species

P. Pachaly
Pharmazeutisches Institut der Universität Bonn
West Germany

It has been known for a long time that many Ericaceae genera form toxic diterpenes as especially characteristic compounds in addition to phenolic constituents and pentacyclic triterpenes.



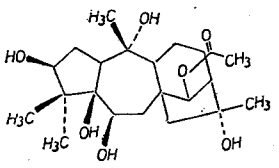
Grayanotoxin I(GI)
=Andromedotoxin
=Rhodotoxin
=Acetylandromedol

One such diterpene is the grayanotoxin I derived from *Rhadodendron* genera and used therapeutically by virtue of its hypotensive properties. It is found not just in the leaves and flowers of these Ericaceae but even appears unchanged in the honey which bees collect during the flowering period of these rhododendrons.

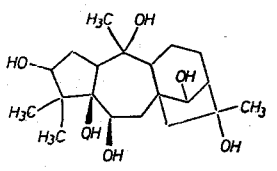
As far back as during Alexander the Great's era such honey led to mass poisoning of Greek soldiers.

Grayanotoxin I itself was first isolated in 1882 by Ejkmann as asebotoxin from *Andromeda japonica*, *Pieris japonica* and subsequently under very varying names also from other Ericaceae. Plugge (1883) called the compound andromedotoxin, Makino (1927) called it rhodotoxin and Mikajima (1935) and Takemoto (1955) named it grayanotoxin. It was not until 1957 that Tallent established the identity of all these compounds and proposed the name acetylandromedol.

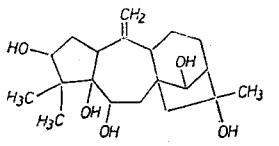
Nonetheless, the name grayanotoxin ultimately prevailed, and the Chemical Abstracts even went over to calling the parent skeleton of such diterpenes "grayanotoxanes". The second figure shows the grayanotoxins I, II and III whose structure had been elucidated by 1966.



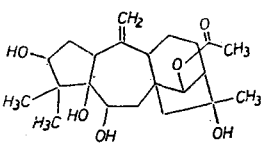
Grayanotoxin I



G II



G III



Δ 10(20)-Acetylandromedenol
= G IV

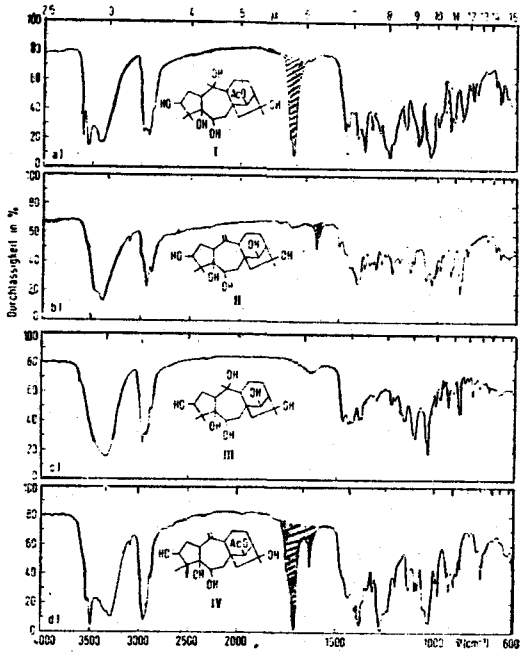
At that time we set ourselves the task of developing a method of assaying the therapeutically used G I - then still called acetylandromedol - which would enable the content to be stated in rhododendron extracts as well. At the time we solved the problem spectrophotometrically by standardizing the known colour reaction with antimony trichloride following thin layer-chromatographic separation of the acetylandromedol from accompanying substances.

At that time we isolated a number of natural substances giving a positive reaction with antimony trichloride that had not been described for *Rhododendron ponticum*. Some of these evidently resembled acetylandromedol= G I very closely.

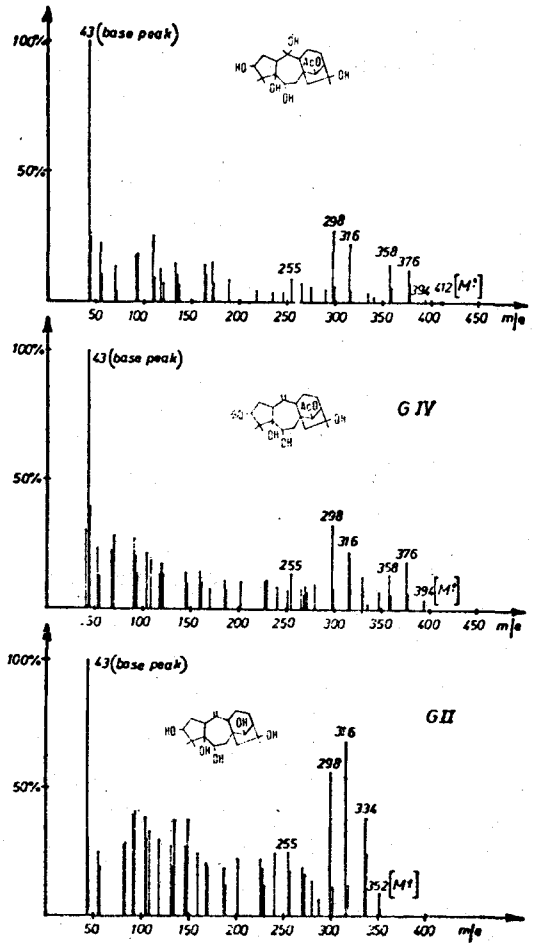
As their quantity increases relative to grayanotoxin I with ageing of the rhododendron extract, there was the suspicion that decomposition products were involved.

After isolation by layer chromatography we were able to obtain a hitherto unknown grayanotoxin in crystalline form as primary substance in addition to the two known grayanotoxins II and III. Spectra and chemical reactions demonstrated that the compound was a 10(20)-acetyl-andromedenol, which we later called grayanotoxin IV.

Just the comparison of the IR-spectrum with those of the known grayanotoxins I, II and III indicated that G IV evidently contains an acetyl ester group like G I and a C=C double bond like G II.

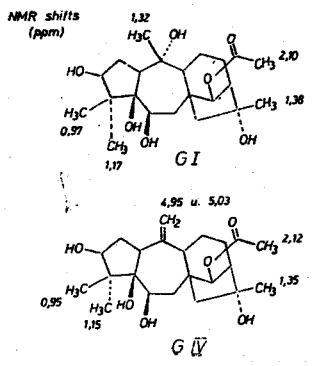


The mass spectra confirmed this finding. Although the fragmentation in these compounds is not very informative, comparing the mass specrta does reveal some characteristic fragments: the stepwise loss of water is noteworthy and, from G I and G IV, the cleaving of acetic acid



(M-60).

On account of the low volatility of G I its mass spectrum reveals an only very small molecule peak at m/e 412, and the first large fragment is M-H_2O at m/e 394; in this sense



the mass spectra of G I and G IV are essentially identical.

The NMR-spectra show that the C-20 methyl group of G I at 1.32 ppm is absent in G IV; instead vinyl proton signals at 4.95 and 5.03 ppm appear additionally.

The other signals of the two compounds are the same including that of the acetyl-methyl-protons at 2.10 ppm.

It required the determination of the position of the acetyl group to establish the final structure of grayannotoxin IV.

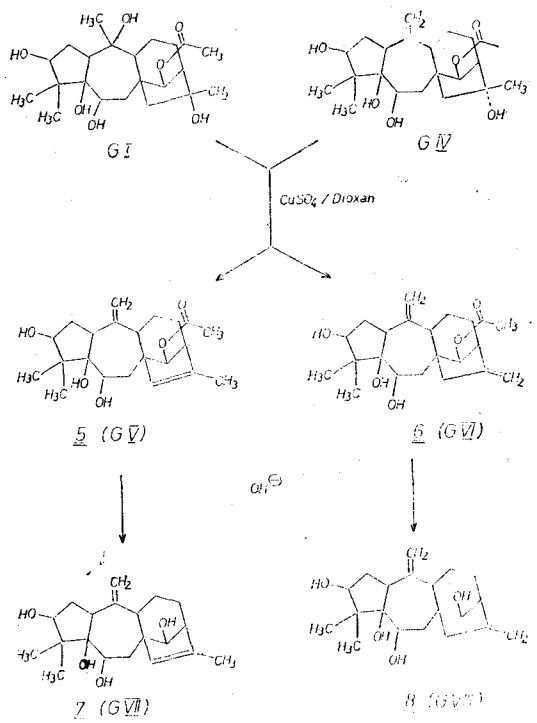
The following observation led us on the right track: the fresher the leaf drug is and the sooner the extracts of *R. ponticum* are investigated, the greater is the G I content and the less is the amont of the three other grayanotoxins.

In the event that grayanotoxin I is the parent substance of the other grayanotoxins, dehydration of G I-if it occurs on C-10/C-20-should yield G IV.

On attempting to dehydrate G I with copper sulphate in acetone we did, in fact, obtain a transformation product identical in all its properties with G IV in addition to three other products. Simultaneous G IV was revealed to be substantially identical with the △-10/20-acetyl- andromedenol obtained by Tallent in 1964 during such a dehydration of G I, i.e. a compound in which the acetoxy group remains unchanged on C-14 like in G I. Alkaline saponification of G IV expectedly gave grayanotoxin II, and thus the structure of our novel grayanotoxin IV was securely established.

Evidently without a knowledge of our results, Matsumoto *et al.* in Japan only a few months later described the same substance also as grayanotoxin IV, but these authors has isolated this substance from *Leucothoe grayana.* (Ericaceae).

We were also able to prepare the second grayanotoxinlike diterpene that we had obtained crystalline from our rhodendron extract by dehydrating G I, but also G IV, with anhydrous copper sulphate in dioxane.



This substance, too, had not previously been found in nature but been obtained during dehydration of G I.

According to Kakisawa it had the structure 5, while according to Tallent this reaction should afford a 1 : 4 mixture of 5 and 6. NMR spectrum measurements even on several samples of our substance, shows 3, 5 vinylprotons instead of the 3 or 4 protons expected from structures 5 or 6. This fact thus speaks in favour of a 1 : 1 mixture of 5 and 6 being present. Alkaline hydrolysis produced a mixture of compounds 7 and 8 which could now be separated without difficulty by chromatography. Ultimately we found these two substances also in older rhododendron extracts in the same fraction containing

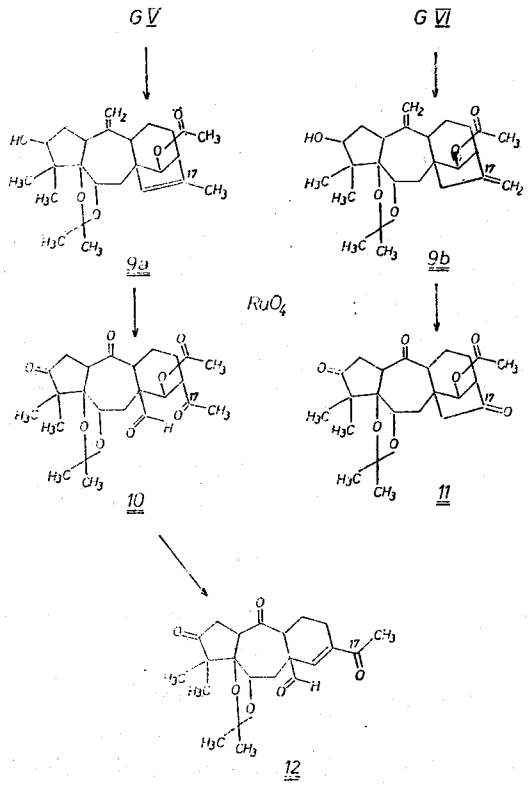
the G IV. We called these new grayanotoxins G V, G VI, G VII and G VIII.

The NMR spectra of G VII expectedly shows signals of three methyl groups (C-18, C-19, acetyl), while the NMR of G VIII has signals of 2 methyl groups and 4 vinyl protons.

Finally, we also succeeded in unambiguously separating the mixture of G V and G VI by thin-layer chromatography on silver nitrate impregnated silica gel. There was thus clear evidence that a mixture of two isomeric grayanotoxins is present in the second diterpene substance.

In order to prove the structure chemically the mixture of G V and G VI was initially converted into the corresponding ketals (9a and 9b) with acetone and then oxidised with ruthenium tetroxide.

Using this type of oxidation for locating isolated double bonds had already been used earlier for splitting double bonds in steroids and for oxidising secondary alcohols.



The mixture of the ketals 9a/9b was to form the compound **10** and **11** with ruthenium tetroxide. In addition to small quantities of impurities we found two main products in approximately equel proportions (3 mg of each) which we separated by layer chromatography. High-resolution mass spectrometry gave the empirical formula of the one main product as $C_{23}H_{30}O_7$ corresponding to the structure **11**.

The IR spectrum shows a strong carbonyl band at 1745cm^{-1} for the two five-membered ring carbonyl groups, absorption at 1729cm^{-1} for the ester carbonyl group and a further carbonyl band at 1691cm^{-1} assignable to the 7-membered α, α' branched ring carbonyl. The CD and NMR spectra are also in accord with structure 11. The second main product revealed an empirical formula of $C_{24}H_{32}O_8$ in its mass spectrum corresponding to the expected structure 10. On prolonged standing compound 10 is transformed into a 13-en-16-one (compound **12**) by loss of a molecule acetic acid. This ready cleavage of an acetoxy group beta to the C-17 carbonyl had also been observed earlier by Kakisawa.

Overall, our findings show that **10** and **12** could only have been formed from G V(5) or its ketal **9a**, while **11** could only have been formed from G VI (6) or the ketal **9b**.

They prove, though, that our natural product does indeed consist of the grayanotoxin mixture G V and G VI with the structural formulae shown here. They also prove the structures of the diterpenes G VII and G VIII obtainable from these by hydrolysis but later also found in the rhododendron extract.

It remained to resolve whether these novel grayanotoxins should be regarded as genuine

Table I. TLC detection of Grayanotoxines in *Rhododendron* sp.
(Comparison of spot extension and colour)

Spec.	*Rhododendron ponticum*				*Rhod. catawbiense*	*Rhod. metternichii*	*Rhod. flavum*
Origin:	Germany		Turkey	Caucasus	Germany (?)	Japan	Turkey
	fresh leaves	dried extract	dried leaves	dried leaves	dried leaves	dried leaves	dried leaves
G I	###	+	##	+	###	###	##
G II	—	+	—	—	—	—	—
G III	?	+	—	—	+	—	—
G IV	+	##	—	—	##	—	—
G V/VI	—	##	—	—	+	—	—
G VII	—	##	—	—	—	—	—
G VIII	—	+	—	—	—	—	—

constituents of *R. ponticum* or as artefacts that are only formed on prolonged storage.

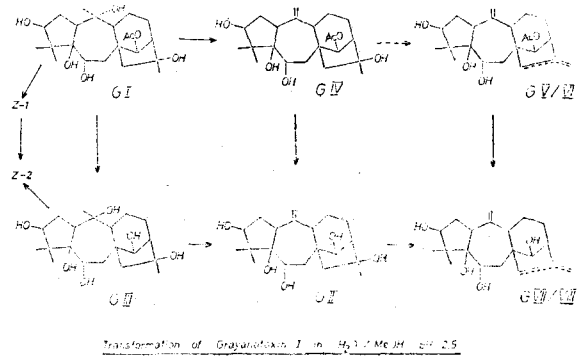
The result of these thin-layer chromatographic studies of several species of *Rhododendron* is shown in the next figure:

It is revealed clearly by this table that in fresh leaves of *Rhododendron ponticum* only G I and, after concentrating, also grayanotoxin IV can be identified. In contrast, all eight known grayanotoxins G I to G VIII are found in the dry extract. Under the similar conditions, in the dried leaves of other *Rhododendron* species investigated by us here, too, substantially only G I can be identified.

There are 2 phases during the normal procedure used to obtain the grayanotoxins which could provoke decomposition of G I: (1) the addition of calcium carbonate to the aqueous suspension of the drug in order to neutralise acid constituents, (2) severalhour perfusion of the aqueous extract with chloroform leading to formation of traces of hydrochloric acid by decomposition of the chloroform.

For this reason, we tested the effect of weak alkali and acid on the individual grayanotoxins by thin-layer chromatography. It was indeed found that after only a few hours at both pH 8 and pH 2.5 in aqueous methanol solution appreciable decomposition gives rise to the other grayanotoxins by splitting off of water and ester hydrolysis.

The result of these investigations at, for example, pH 2.5 is shown in the next figure:



Transformation of Grayanotoxin I in H_2O / MeOH pH 2.5

Thus G I gives rise primarily to 3 transformation products G IV, G III and the not further elucidated ZK_{15}; the next observed transformation product is G II and the also not further investigated ZK_{16}. G II then changes into the mixture of G VII and G VIII, which is also formed from G V and G VI under these conditions.

While conversion of G IV into G V and G VI is very probable, we were unable to detect it side by side with the formation of G II. These results show that grayanotoxins G V to

G VIII are formed in dependence on the nature of the workingup and storage but occur naturally in *R. ponticum* and other *Rhododendron* species in only undetectable amountsif at all.

Intravenous administration of both G I and G IV (in larger doses) produces a lowering of blood pressure and bradycardia in the cat. In rats G I and G III have an approximately equal hypotensive effect, whild here G IV or the mixture of G V and G VI show only small blood pressure effects.

Our new grayanotoxins G V, G VI, G VII and G VIII were also isolated by Takemoto in Japan from *Leucothoe grayana* a few months later, *i.e.* again almost at the same time, and their structure was elucidated.

Takemoto called his compounds (in the same sequence) G IX, G X, G VII and G VIII; thus this time identical naming retained only for G VII and G VIII.

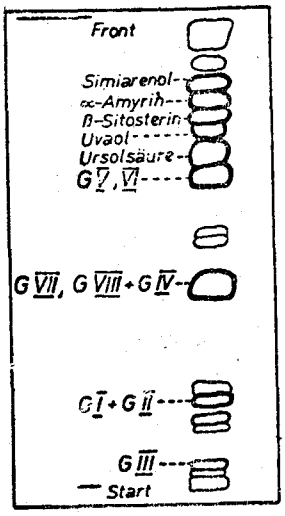
We belive, though, that subsequent formation must be considered as a possibility for Takemoto's substances as well.

Meanwhile, overall more than 30 different grayanotoxins have been isolated from the Ericaceae genera *Pieris*, *Andromeda*, *Leucothoe* and *Rhododendron* under the names pieristoxins, asebotoxins, rhodojaponins and grayanotoxins.

Further investigation of the antimony trichloride positive rhododendron extract yielded no additional new grayanotoxins but, instead, led us to obtain some triterpenes and steroids.

The next two figures show a thin-layer chromatogrphic separation of the extract in various eluents following spraying with antimony trichloride.

Clear evidence of the variously coloured zones is seen; unless they are grayanotoxins the zones turn grey after a few days. The coloured grayanotoxin-antimony trichloride complexes are stable for much longer periods.



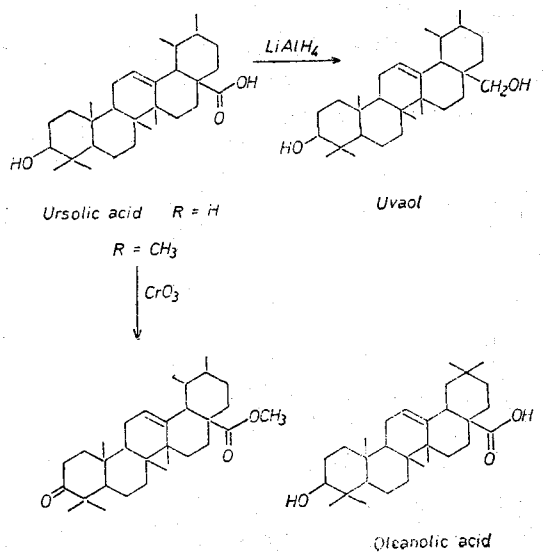
Dünnschichtchromatogramm eines alten Trockenextraktes von *Rhododendron ponticum* Fließmittel: $CHCl_3$/MeOH 9:1 (zweimal entwickelt Detektionsmittl: $SbCl_3$

In the second colour slide the polar substances of this substance class are shown in a different eluent.

The first two substances which we isolated here were the red-violet and blue zones.

Without authentic reference substances we confirmed the structure by chemical reactions and spectra evaluation. Accordingly, the compounds are the triterpenes ursolic acid and uvaol.

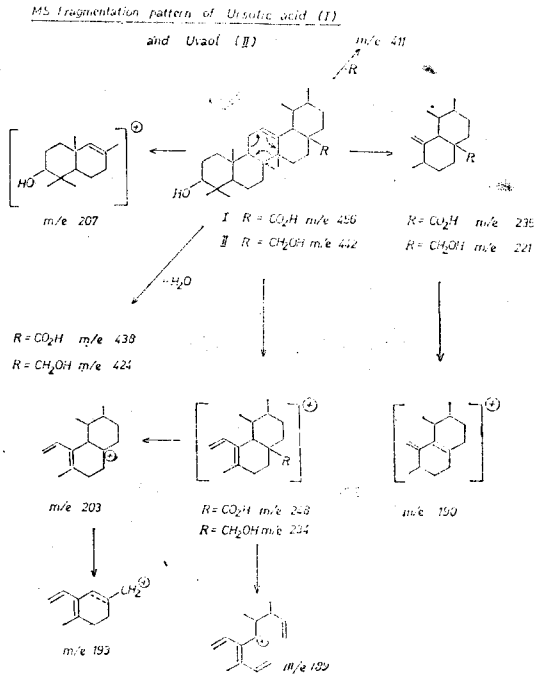
Expectedly, the ursolic acid could be converted



into uvaol by lithium tetrahydroaluminate reduction. With chromium(VI) oxide methyl ursolate gives methyl ursonate. All these derivatives have physical constants matching the literature data.

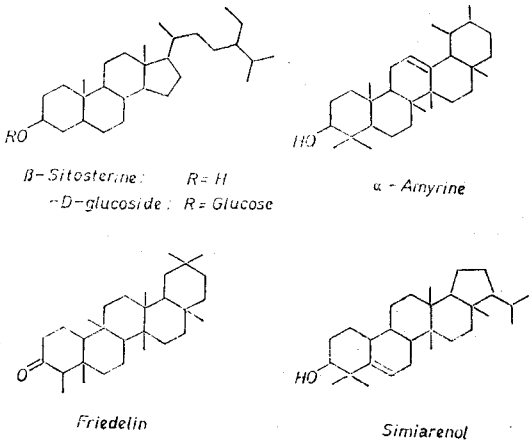
The CD spectrum methyl ursonate shows the expected positive Cotton effect at 293 nm ($\varepsilon = +0.367$) and a weakly negative Cotton effect at 322 nm ($\varepsilon = -0.077$).

A smaller amount of the isomeric oleanolic acid accompanies the ursolic acid, but we confirmed it only by thin-layer chromatography.

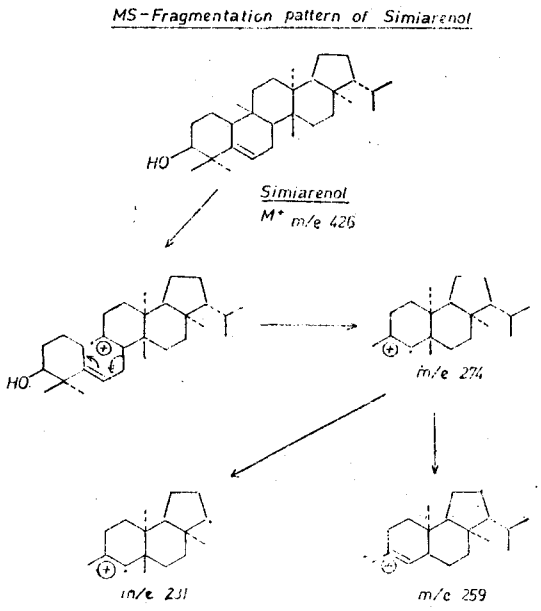


The mass spectra of both triterpenes show the typical fragments for such pentacyclic triterpenes. Fragments 248 and 207 for ursolic acid and 234 for uvaol are formed by the diene decomposition characteristic of $\Delta^{11,12}$-unsaturated ursanes.

Further substances isolated by us were ß-sitosteriol, ß-sitosterol-D-glucoside and the three further triterpenes α-amyrine, simiarenol and friedelin.



While α-amyrine furnishes the fragments corresponding to ursolic acid and uvaol in the mass spectrum, the mass spectrum of simiarenol must proceed differently because of the $\Delta^{5(6)}$ double bond, which is relatively seldom encountered in Ericaceae tritepenes.



Here the anticipated diene decomposition gives the fragments m/e 277, 231 and 259. The comparison with authentic substances confirmed these substance assignments in each case.

After we had now gathered some analytical experience with such Ericaceae terpenoids, and investigation of *Vaccinium uliginosum* L. appered to us to be of interest also in chemotaxonomic respect. *Vaccinium uliginosum* is distributed over high-altitude moors in Europe for example in the Black Forest and the "Hohe Venn" in Belgium.

This dwarf shrub carries blue berries rich in Vitamin C which are sometimes confused with the tasty berries of *Vaccinium myrtillus.* However, these berries of *Vaccinium uliginosum* are suspected of being poisonous and it was thus an interesting step to investigate this plant for toxic diterpenes of the grayanotoxin type.

The investigation of the leaves, however, gave no indication of grayanotoxins and we once more found only β-sitosterol, β-sitost erol-D-glucoside, α-amyrine, friedelin and ursolic acid accompanied by oleanolic acid. All substances could now be identified by means of reference substances. On the basis of these terpenoids *V. uliginosum* readily takes its place as a member of the hitherto investigated group of *Vaccinium* species; the other *Vaccinium* species studied also gave no indication of the presence of grayanotoxins.

The alleged poisonous nature of *V. uliginosum* berries thus appears to have a different cause.

The third Ericacea genus which we investigated is *Andromeda polifolia*, the Marsh-Andromeda, which is called Rosmarinheide in Germany. This small dwarf shrub is also ubiquitously found on European high altitude moors and within the Ericaceae, like Rhododendron, belongs to the Arbutoideae sub-family.

Chemotaxonomically the Arbutoideae genera are characterised by the frequently occurence of grayanotoxin-type toxic diterpenes.

While several grayanotoxins could be isolated from *Andromeda japonica* (=*Pieris japonica*) this had hitherto not been acccmplished with the European *Andromeda polifolia.* Solely a publication by P.C. Plugge dating from 1883 was available in which the author postulated andromedotoxin (GI) as the toxic ingredient in European *A. polifolia* on the basis of pharmacological findings and some colour reactions. Plugge caused frogs and rabbits to be killed with an aqueous alcoholic extract while showing the same manifestations of poisoning as with GI or *Pteris japonica* extract.

An investigation of *A. polifolia* attracted us and we hoped to track down further hitherto unknown representatives of the meanwhile discovered numerous variations of the grayanotoxin skeleton.

Strangely enough, however, the thin-layer chromatographic investigation of the methanol extract of stalks and leaves gave no indications of GI or grayanotoxins reacting similarly with antimony trichloride.

To separate the constituents the concentrated agueous methanol extract was extracted successively with chloroform and ether. The grayanotoxins were to be concentrated in the chloroform phases, while the ether phases contain several flavonoids such as quercetin and hyperoside among other substances.

By contrast, development of a blue colour on adding acid to the aqueous phase, which is also observed in at least two substances after spraying with antimony trichloride, points to the presence of monoterpenoid compounds, namely iridoids.

Although iridoids occur among Ericales, they had hitherto been described among Ericaceae themselves only in *Arbutus* genera and in recent years in *Vaccinium bracteatum.*

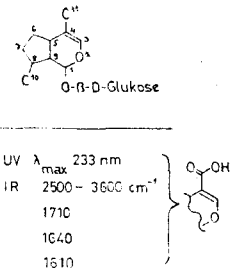
Since suchch emically very labile iridoid glycosides could produce the toxic effect observed by Plugge, we initially studied this aqueous

phase more closely and ultimately isolated four different iridoids (1, 2, 3, 4) after column chromatography using polyamide.

From 600g fresh plant material we obtained three substances in amounts of 120～170mg, while not enough of the fourth iridoid (compound 2) has so far been obtained for further processing.

Thin layer chromatography on silica gel revealed compound 1 to be considerably more polar than compounds 2, 3 and 4, which with their higher R_F value cannot be separated under very widely different conditions on either silica gel or Sephadex.

Separation requires polyamide and water; the compounds are eluted from the column in the order of their names 1-4.



The basic skeleton typical of the iridoids is a cyclopentane-pyran ring system that is generally linked to glucose as a β-glycoside. Carbon atoms 10 and 11 are present as free or esterified carboxyl, hydromethyl, or simply as methyl, or may be entirely absent.

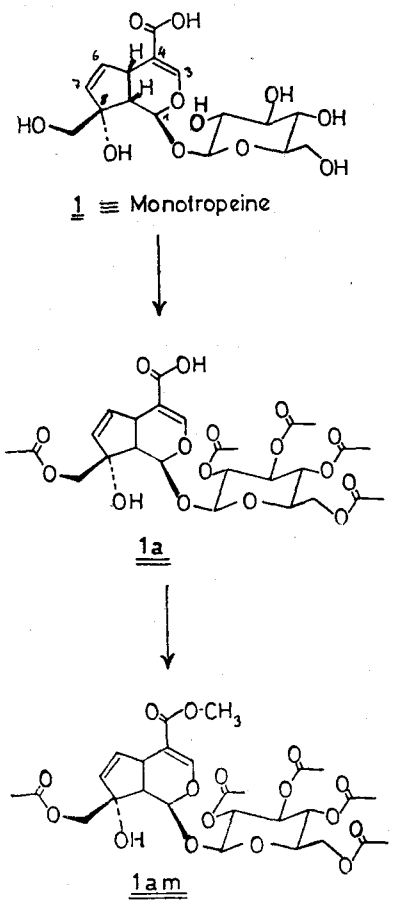
The likewise characteristic enol ether grouping is the cause of the UV absorption of these compounds. In our compound 1, obtained as a white amorphous powder, the UV maximum at 233nm, the IR carbonyl band at 1710cm^{-1} and the shape of the OH bands between 2,500 and 3,600cm^{-1} point to a free α,β-unsaturated carboxyl group as C-atom 11.

Ester and lactone groups were definitely ruled out by the IR spectrum.

The red colour with 2,6-dichlorophenolindophenol sodium and the shift of the carbonyl band by salt formation with caustic soda to 1590 and 1,400cm^{-1} are further evidence for the free carboxyl group.

Acetic anhydride/pyridine gave a crystalline acetylation product **1a** from **1**. Its elementary analysis, mass spectrum and ^{1}H-NMR spectrum revealed **1a**, to be the iridoid pentaacetate with a non-acetylated tertiary OH group, the unchanged carboxyl and a second isolated double bond.

A second noteworthy feature is that no methyl group signal occurs in the NMR spectra. All this spoke in favour of a monotropeine



type pentaacetate.

Monotropeine had already been isolated earlier from Pyrola, Monotropa and other plant genera, and its absolute structure had been elucidated by Inouye.

Compound **1a** deviates from Inouye's data for monotropeine pentaacetate only by its lower melting point (154° instead of 173°) and the higher specific rotation (−103.6 instead of −82.5°).

However, methylation of **1a** with diazomethane gave a methyl ester **1am** which agreed in all properties with the corresponding monotropeine derivative; this represents indirect proof that compound 1 as well must be identical with monotropeine.

By contrast, the iridoids 3 and 4 were found to be different from all hitherto known iridoids. We therefore proposed the names Andromedoside **I** and **II** for these new compounds.

Andromedoside I (3) shows typical UV absorptions characteristic of aromatics at 312, 300 and 292nm speaking in favour of a cinnamic ester; they are like those found in other iridoids, for example durantoside or scutellaroside. This postulate is supported by the IR spectrum, which shows a split carbonyl absorption at 1,710 and 1,690cm^{-1} and typical aromatic absorptions in the 1,500 to 1,680cm^{-1} region.

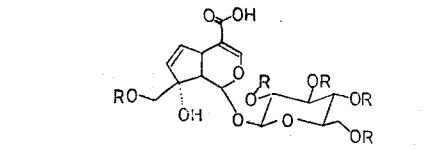
Acetylation gives a pentaacetate **3a**, whose ^{1}H-NMR spectrum has the signal for 4 acetyl groups between 1.96 and 2.05ppm and that of a further acetylated phenolic OH group at 2.28ppm.

A strong band at 860cm^{-1} in the IR spectrum of pentaacetate **3a** points to a *p*-hydroxycinnamic ester partial structure.

Like in **1a**, the OH band at 3,400cm^{-1} speaks for a reluctantly acetylated tertiary OH group, while the wide absorption between 2,800 and 3,400cm^{-1} supports a free carboxyl group.

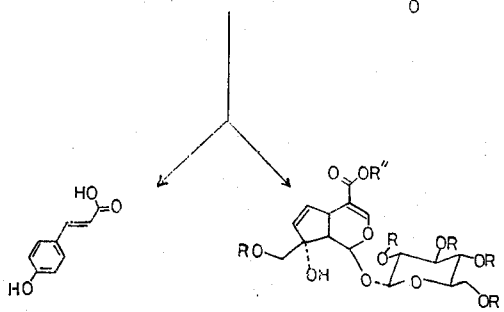
All these findings and the elementary analysis of the pentaacetate **3a** mean that **3** is a *p*-hydroxycinnamic ester of an iridoid which at the least resembles monotropeine.

The typical signals of a *p*-hydroxycinnamic ester in the ^{13}C-NMR spectrum such as recently published by Weinges, *et al.* for scutellaroside II agree with this idea.



3 , 3a : 4 x R = H bzw H_3C-C(=O)-

1 x R = R'O-C$_6$H$_3$(OH)-CH=CH-C(=O)-



3h R, R'' = H

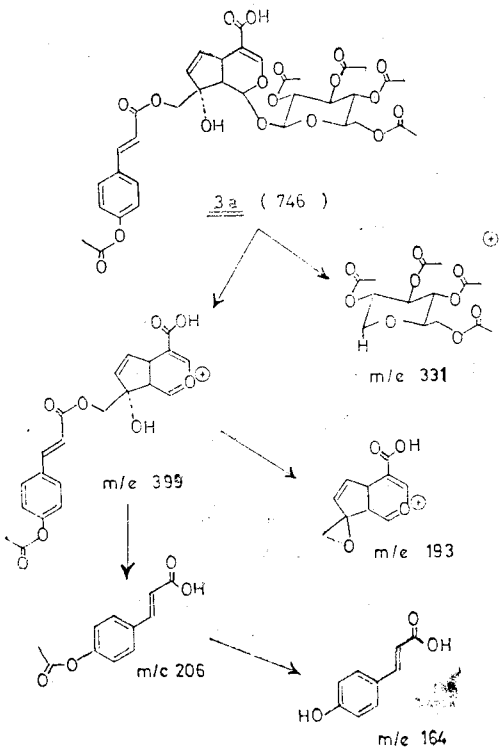
3ha R = H_3C-C(=O)- ≡ 1a
R'' = H

3ham R = H_3C-C(=O)- ≡ 1am
R'' = CH_3

Expectedly, careful hydrolysis of **3** with 0.05N caustic soda (24 hours at 20°) ultimately led to the isolation of trans-*p*-hydroxycinnamic acid, which was identical with authentic reference material. The iridoid cleavage product **3h** obtained was purified by chromatography, acetylated to **3ha** and methylated with diazomethane to **3ham**.

Melting point, UV spectrum and IR spectrum of these two products agree fully with those of the corresponding derivatives **1a** and **1am** of the monotropeine. Thus, Andromedoside I is

proven to be a *p*-hydroxycinnamic ester of monotropeine. The position of the ester link of the *p*-hydroxycinnamic acid in Andromedoside I (3) was identified with the aid of the mass spectrum of pentaacetate **3a**.



The appearance of the fragment m/e 331 points to a tetraacetylhexose group, while the fragments 399, 193, 206 and 164 are explained readily if the *p*-hydroxycinnamic acid is esterified with the aglycone.

Since elementary analysis and IR spectrum show that a free tertiary OH group is present unchanged in **3a**, it is the C-10 hydroxymethyl group of the aglycone that must be esterified. Andromedoside I is thus a C-10 monotropeine *p*-hydroxycinnamic ester.

Andromoside II (4) shows the same UV absorption and the same mass spectrum fragmentation pattern as Andromedoside I. The IR spectra of the two substances show only minimal differences. Thus Andromedoside II must be a very similar compound to Andromedoside I. Differences are revealed only in their chromatographic behaviour on polyamide-Andromedoside I is eluted fasterin their melting points, in the specific rotation and in the IR and NMR spectra of the acetylation products.

From Andromedoside II, too, hydrolysis produces *p*-hydrocinnamic acid, but 0.1N caustic soda is needed and not just 0.05 N alkali.

Andromedoside II also affords a pentaacetate with an unchanged IR OH-band that we likewise assign to an unesterified OH group.

As the fragments of tetraacetylglucose (*e.g.* m/e 331) once again appear in the mass spectrum, the *p*-hydroxycinnamic acid in Andromedoside II must be bound to the C-10 in the same way. It follows that Andromedoside II is an isomer of Andromedoside I.

In principle there are 5 different possible isomeric iridoid skeletons: (1) interchange of the 5-membered ring substituents with the C6-C7 double bond, (2) a sugar other than glucose might be involved, (3) epimerisation on C-1′, i.e. α-glycoside linking of the sugar, (4) a change of configuration on the C-1 of the iridoid portion, (5) epimerisation on C-8 of the iridoid portion. A 6th possibility is the epimerisation on hydroxycinnamic acid portion.

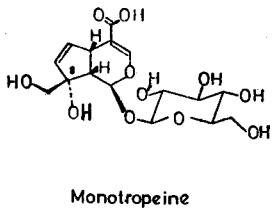
The first possibility appears to be the least likely because of the relatively small differences between the molecular spectra of Andromedosides I and II.

From the ^{1}H and ^{13}C NMR spectra of the pentaacetates 3a and 4a no significant differences in the signals for H-1 and C-1 and all ^{13}C signals of the sugar moiety are revealed. The conclusion is that in Andromedoside II glucose is once more linked to the iridoid portion as a β, β-glycoside.

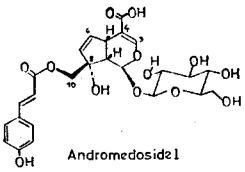
Thus, really only the two last alterna-

tives-namely epimerisation on C-8 and epimerisation of hydroxycinnamic acid portion-are left.

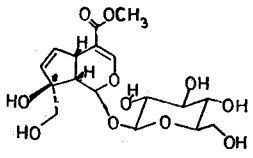
In the first case, Andromedoside II could be a C-8-epiandromedoside I.



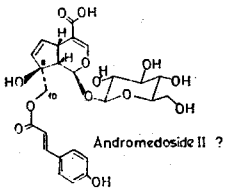
Monotropeine



Andromedoside I



Gardenoside



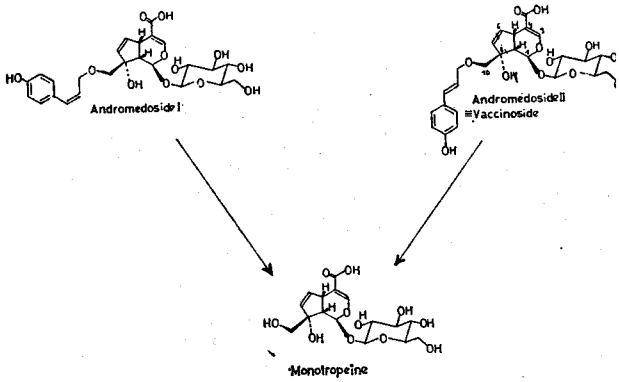
Andromedoside II ?

This assignment was supported by the more difficult hydrolysis of the *p*-hydroxycinnamic ester grouping and by the altered ^{1}H NMR signal of the C-10 methylene group: while these two protons furnish a widened singlet together with the C-6′ methylene protons at 4.18 ppm in Andromedoside I pentaacetate **3a**, a multiplet between 4.11 and 4.31 ppm appears in the pentaacetate of Andromedoside II.

The epimerisation on C-8 should lead to a firmer locating of the now alpha C-10 methylene group in Andromedoside II within the angle of the cis-linked iridoid ring skeleton, so that the two C-10 protons are magnetically nonequivalent. Final proof was to be furnished by the iridoid portion resulting during alkali hydrolysis of Andromedoside II. After acetylation and esterification with diazomethane it ought to be identical with gardenoside pentaacetate, a C-8-epi-monotropeine methyl ester from *Gardenia jasminoides*.

In fact, however, this alkali hydrolysis of Andromedoside II followed by acetylation and methylation of the iridoid portion gave the same pentaacetylmonotropeine methyl ester that we had obtained by an analogous sequence of reactions from Andromedoside I.

It follows that the two iridoids can differ only in the *p*-hydroxycinnamic ester portion. Since there is no doubt that C-8 ester is concerned in both cases, *cis*-and *trans*-*p*-hydroxycinnamic esters must be involved.



Andromedoside I

Andromedoside II ≡Vaccinoside

Monotropeine

Although we had isolated crystalline *trans*-*p*-hydroxycinnamic acid by hydrolysis in both cases, we were able to interpret a coupling constant of 12.7 Hz for cinnamic ester olefin protons in the Andromedoside I ^{1}H-NMR spectra also or rather as a *cis*-coupling; in Andromedoside II we observed the typical *trans*-coupling of 16 Hz.

Hydrolysis of Andromedoside I was accompanied by partial isomerisation gives both *cis*- and *trans*-*p*-hydroxycinnaic acids, which are undoubted detected in the ^{1}H NMR spectra, while from Andromedoside II only the *trans*-acid arises.

The *trans*-acid obtained from Andromedoside I by recrystallization had initially confused us.

During carrying out of this work we came across a paper by M. Yasue who had isolated vaccinoside from *Vaccinium bracteatum* and determined it as the C-8 *trans*-*p*-hydroxycinna-

mic ester of monotropeine. Thus, Andromedoside II must be identical with Vaccinoside. A direct comparison of the two natural substances actually proved the identity of both substances.

Whether these iridoids are really the cause of the toxicity must be answered by a pharmacological study, however, once we have accumulated sufficient material. The occurrence of these iridoids seems to us to be noteworthy also in chemotaxonomic respects.

Both Andromedoside and Vaccinoside can also be detected directly in fresh leaves; thus the two monotropeine esters are shown to be natural substances. Our further investigations will be devoted to the remaining triterpenoid constituents that have already been identified as being present by thin-layer chromatography. In particular, we want to find out whether the postulated grayanotoxins cannot also be discovered.

In concluding my lecture I should like to introduce to you the Bonn team that has played a decisive role in this work and whom I owe particular thanks for their unflagging effort and patience. They are the following Ladies and Gentlemen from the Pharmaceutical Institute in Bonn-Poppelsdorf: Dr. B.Z. Ahn, Dr. Sun Gan Chung, Dr. S. auf dem Keller, Dr. S. von Kürten, Dr. H. Nees, Prof. Dr. F. Zymalkowski.

I wish to acknowledge my thanks to Prof. Dr. M. Yasue (Niigata) and his team for her kindly gift of Vaccinoside and some copies of IR and NMR spectra, Prof. Fr. O. Sticher for Monotropein, Prof. Dr. G. Snatzke (Bochum) for the recording and discussion of the CD spectra and to Prof. Dr. G. Tauberger and Mr. M. Moussawi of the Pharmacological Institute in Bonn for carrying out the pharmacological tests.

References

1. Xenophon: Anabasis IV, 8, 20-21
2. J.F. Ejkman: *Rec. Trav. Chim.*, Pays-Bas **1**, 224 (1882)
3. P.C. Plugge: *Arch. Pharm.*, (Weinheim) **221**, 813 (1883); dto **223**, 905 (1885).
4. M.Makino: *Okayama-Igakui Zasshi*, **39**, 2099 (1927); *C.A.* **23**, 1691, 3027 (1929)
5. M.Miyajima and S.Takei: *C.A.*, **29**, 1133 (1935)
6. T.Takemoto: *J.Pharm. Soc. Japan*, **75**, 1442 (1955)
7. W.H. Tallent: *J.Am. Chem. Soc.*, **79**, 4548 (1957)
8. H.Kakisawa: T.Kozima, M. Yanai and K. Nakanishi: *Tetrahedron*, **21**, 3091 (1965); W.H. Tallent: *J. Org. Chemistry*, **27**, 2968 (1962); H. Meguri: *J. Pharm. Soc. Japan*, 79, 1060, 1057 (1959)
9. F. Zymalkowski, P. Pachaly and S. auf dem Keller; *Planta Medica*, **17**, 8 (1969)
10. S. auf dem Keller, P. Pachly and F.Zymalkowski: *Arch. Pharm.* (Weinheim), **303**, 249 (1970)
11. W.H. Tallent: *J.Org. Chemistry*, **29**, 2756 (1964)
12. T. Okuno, N. Hamanaka, H. Mijakoshi and T. Matsumoto: *Tetrahedron*, **26**, 4765 (1970)
13. M. Yanai, H. Mishima, T. Kozima, H. Kakisawa and K. Nakanishi: *Chem. Pharm. Bull.* **17**, 2036 (1969)
14. S.v. Kürten, P. Pachaly, F. Zymalkowski and G. Snatzke: *Liebigs Annalen*, **741**, 142 (1970)
15. H. Kakisawa: *J. Chem. Soc. Japan*, **82**, 1096, 1216 (1961)
16. S.v. Kürten, S.auf dem Keller, P. Pachaly, F. Zymalkowski, G. Tauberger and M.

Moussawi: *Arch. Pharm.* (Weinheim), **304**, 753 (1971)
17. H. Hishino. T. Ohta, S. Koriyama, Y. Hikino and T. Takemoto: *Chem. Rharm. Bull.*, **19** (6), 1289 (1971)
18. S.auf dem Keller, P.Pachaly and F. Zymalkowski: *Arch. Pharm.* (Weinheim), **303**, 243 (1970)
19. S.auf dem Keller, S.v. Kürten, P. Pachaly and F. Zymalkowski: *Pharmazie*, **25**, 621 (1970)
20. H. Nees, P. Pachaly and F. Zymalkowski: *Planta Medica*, **24**, 320 (1973)
21. S. G. Chung, B. Z. Ahn and P. Pachaly: Planta Medica (in press) dto. Arch. Pharm., (Weinheim) (in press)
22. H. Inouye, T. Arai, and M. Yasue: *Chem. Pharm. Bull.* (Japan), **19**, 1979 (1971); *Yakugaku Zasshi*, **93**, 164 (1973)