universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Daphnane diterpenes from the genus *Daphne*“

verfasst von / submitted by
Aaron Pramstrahler

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of
Magister der Pharmazie (Mag.pharm.)

Wien, 2020 / Vienna, 2020

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 449
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Diplomstudium Pharmazie
Betreut von / Supervisor:	Prof. Dr. Olivier Potterat

Table of contents

List of abbreviations

ACN	Acetonitrile
BPC	Base peak chromatogram
br	Broad
COSY	Correlation Spectroscopy
d	doublet
dd	doublet of doublets
DAD	Diode array detector
DCM	Dichloromethane
DMSO	Dimethyl sulfoxyde
ELSD	Evaporate light scattering detector
ESI	Electrospray-ionization
EtOAc	Ethyl acetate
FA	Formic acid
Fr	Fraction
HIV	Human immunodeficiency virus
HMBC	Heteronuclear single quantum coherence
HPLC	High performance liquid chromatography
HSQC	Heteronuclear single quantum coherence
m	Multiplet
MeOH	Methanol
MS	Mass spectrometry
MW	Molecular weight
m/z	Mass to charge ratio
NMR	Nuclear magnetic resonance
NO	Nitric oxide
PDA	Photo diode array
PKC	Protein kinase C
t_R	Retention time
s	Singlet
t	Triplet
TLC	Thin layer chromatography
UV	Ultraviolet

List of tables

List of figures

1 Abstract

Daphnane diterpenes are a group of secondary metabolites characteristic of plants from the Thymelaeaceae and Euphorbiaceae families.

In recent studies carried out at the University of Basel daphnane diterpenes including daphnetoxin and some congeners, isolated from *Daphne gnidium* showed potent antiretroviral activity on multidrug-resistant HIV- strains. In addition, extracts of *Daphne gnidium* and *Daphne giraldii* as well as daphnetoxin showed strong activation of the human vascular system which could be relevant for dermal applications. For further studies, larger amounts of daphnetoxin and the isolation of a structurally diverse collection of daphnane diterpene would be needed. Due to their complex chemical structures, the synthesis of these compounds is hardly feasible.

As a promising source of daphnane diterpenes, *Daphne feddei* has been selected for this Diploma thesis. *D. feddei* is used in the traditional Chinese medicine and can be found in the "Inventory of Existing Cosmetics in China". Up to now, only a small number of publications were dedicated to this plant and only one study focused on daphnane diterpenes.

From the dichloromethane (DCM) extract of the stems of this plant, the main daphnane diterpene has been isolated and its structure established through NMR analysis as 1,2-dihydrodaphnetoxin. Several further daphnane diterpenes have been also detected and tentatively identified by HPLC-ESIMS analysis.

Besides, an exhaustive list of daphnane diterpenes which have been previously isolated from plants of the genus *Daphne,* with their synonyms and their corresponding sources was created through extensive search in several literature databases. While a review on daphnane diterpenes had been recently published, no exhaustive list of the respective sources of these compounds in the genus *Daphne* was available. This list will constitute a valuable source of information for the targeted isolation of various daphnane diterpenes with structurally diverse features.

1.1 Zusammenfassung

Daphnanditerpene sind sekundäre Pflanzenstoffe welche typischerweise in Pflanzen aus den Familien der Thymelaeaceae (Seidelbastgewächse) und Euphorbiaceae (Wolfsmilchgewächse) zu finden sind.

Kürzlich durchgeführte Studien der Universität Basel ergaben, dass Daphnetoxin und andere Daphnanditerpene, welche aus *Daphne gnidium* isoliert wurden, eine starke anti-HIV Wirkung aufweisen. Zudem wurde festgestellt, dass sowohl Extrakte aus *Daphne gnidium*, *Daphne giraldii* als auch Daphnetoxin eine starke Aktivierung des humanvaskulären Systems hervorrufen. Dies könnte eine erfolgsversprechende Anwendungsmöglichkeit für den dermatologischen Bereich bedeuten. Um diese wirksamen Substanzen eingehender zu erforschen werden größere Mengen von Daphnetoxin und weiteren Daphnanditerpenen benötigt, jedoch sind diese aufgrund ihrer strukturellen Komplexität synthetisch kaum zugänglich.

Daphne feddei wurde für diese Diplomarbeit als vielversprechende Quelle zur Isolierung von Daphnanditerpenen ausgewählt. Die Pflanze wird bereits in der traditionellen chinesischen Medizin angewandt und wird im "Verzeichnis der existierenden Kosmetika in China" gelistet. Bisher sind nur wenige Publikationen den Inhaltsstoffen und Wirkungen dieser Pflanze gewidmet und nur eine Studie thematisiert Daphnanditerpene.

Aus dem Dichlormethanextrakt, welches aus den Stämmen dieser Pflanze gewonnen wurde, wurde die Hauptverbindung aus der Klasse der Daphnanditerpene isoliert und mittels NMR-Analyse als 1,2- Dihydrodaphnetoxin identifiziert. Zudem wurden viele weitere Daphnanditerpene mittels HPLC-ESIMS Verfahren detektiert.

Zusätzlich wurde eine umfassende Liste erstellt, welche alle bisher isolierten Daphnanditerpene aus der Gattung *Daphne*, deren Synonyme als auch deren Quelle nennt. Kürzlich war ein Review über Daphnanditerpene veröffentlicht worden, jedoch gab es bisher keine vollständige Aufzählung der Pflanzen aus welchen die einzelnen Substanzen isoliert wurden. Die folgende Auflistung stellt eine nützliche Informationsquelle für die weitere gezielte Isolierung von verschiedensten Daphnanditerpenen dar.

Keywords:

Daphne feddei, Thymelaeaceae, daphnane diterpenes, extraction, HPLC-UV-MS, NMR

2 Aim of the work

The initial aim of this diploma thesis was the isolation of daphnane diterpenes from different plants of the *Daphne* genus and the identification of a sustainable source of daphnetoxin. The isolated compounds should have been tested in collaboration for their activity on the human vascular system. In this context, we started with the investigation of *Daphne feddei*, a plant from the traditional Chinese medicine known to contain daphnane diterpenes.

Unfortunately, due to the rising threat of the emerging SARS-CoV-2 and the resulting shutdown of the laboratory, the experimental work had to be discontinued after 6 weeks. At this stage, one compound had been isolated and several further diterpenes detected.

For the further course of this thesis, the focus has been therefore adapted to the new situation and redirected to a literature search on daphnane diterpenes reported in the whole genus Daphne. No list with the exhaustive sources of these compounds was available. The generated list will therefore serve as a valuable source of information for future experimental studies on daphnane diterpenes

3 Introduction

3.1 Genus *Daphne*

Plants of the genus *Daphne* are part of the Thymelaeaceae family and are native in temperate and tropical areas of the Eurasian continent and northern Africa. Some species have been introduced to North America. Many *Daphne* varieties are also cultivated as ornamental plants. The genus comprises around 90 species, of which roughly the half are endemic in China. It consists of woody plants which grow as shrubs or subshrubs [1], [2], [3], [4].

Various species and parts of the plants and their extracts have been used in the traditional medicine to treat different forms of ache, rheumatism, quadriplegia, tumors, cough and were used as an abortifacient [5], [6], [7].

3.1.1 Toxicity

Plants of the genus *Daphne* are usually considered as poisonous plants. All parts are known to contain toxic components [8]. Ingestion of the berries or other parts of the plant causes strong irritation of the gastrointestinal tract, such as burning throat and stomach, vomiting, bloody diarrhea and spasms and can lead also to nephritis, bradycardia, circulatory arrest and coma. Plants from the genus *Daphne* are known to have a strong skin irritant effect. Yuanhuacine, a daphnane-type diterpene was shown to induce labor in pregnant women and inflammation and necrosis of the decidual membrane which could lead to abortion. The most toxic components are known to be diterpenes of the daphnane-type and coumarins [8], [9], [10].

Only few investigations regarding the toxic principles and effects of compounds found in *Daphne* species are available in literature and most of them are *in vitro* studies and often old.

An *in vivo* study investigated the oral toxicity of daphnane-type diterpenes in rats. The principal outcome of the study was that the toxicity of the compounds in the extract varies due to the different molecular structures and consequently different bioavailability and blood concentrations. The dosages of the diterpene extract administered to the animals in this study ranged from 0.1 to 1 g/kg. All the animals except those who got the lowest concentration of the extract died dose- dependently within 10 hours. The rats on which the lowest dose was administered recovered totally after initial symptoms of intoxication. These results are important clues on the toxicity of the compounds but can not be transferred directly to humans as there were no studies performed [11].

A study by Diogo *et al.* (2009) compared the toxicity of the diterpene daphnetoxin (Figure 1) and the biscoumarin daphnoretin (Figure 1) on different cell lines and mitochondrial liver cells. The authors showed that both compounds were toxic to all cell lines by stopping the cell proliferation while daphnetoxin had slightly a higher toxic effect. Administered concentrations in this study ranged from 150 to 600 µM. A suspected liver toxicity was confirmed as the compounds were administered on mitochondrial cells and

stopped ATP synthesis. Both compounds were previously described as anti-tumor agents but in this study no selectivity on the tested tumor cell lines could be shown [12].

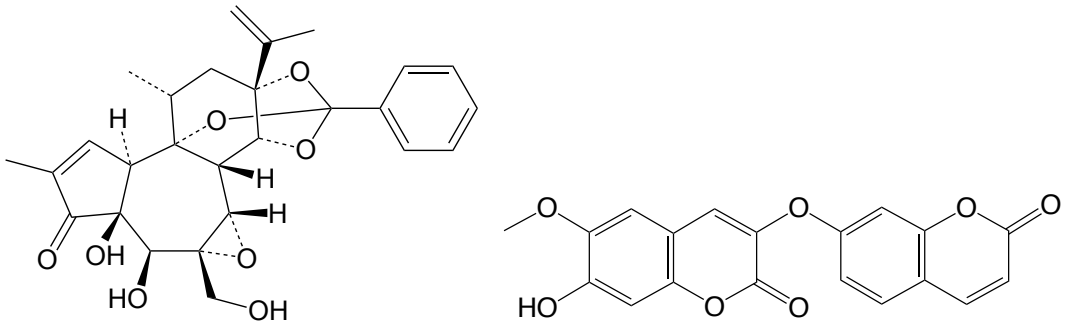


Figure 1: Structures of daphnetoxin (left) and daphnoretin (right)

3.1.2 Chemical constituents

As shown above, coumarins are among the compounds with the strongest bioactivities found in the genus. Daphnin (Figure 2) which showed a potent anti-inflammatory effect was one of the first coumarins ever isolated and named after the genus [13]. Since then over fifty coumarins and their glycosides were reported.

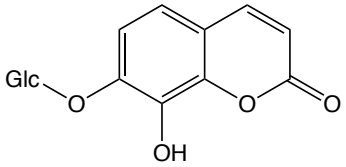


Figure 2: Structure of daphnin

In 1992 the *in vitro* and *in vivo* antimalarial effect of daphnetin was reported [14]. The biscoumarin daphnoretin (Figure 1), a protein kinase C activator, showed strong antiviral activity and also antiproliferative activities in human osteosarcoma cells by inducing apoptosis. These results were obtained with much lower concentrations than in the studies mentioned in the chapter above [15], [16], [17]. The coumarin glycosides daphneretusins A and B showed strong antioxidant activities [18].

Up to now over 100 flavonoids and biflavonoids have been reported from the genus. Beside strong antioxidant properties, various substances have shown inhibition of NO-production, anti-HIV activity, insecticidal activity, K^+ - ATPase inhibition and angiotensin II formation inhibition. A flavonoid which showed high angiotensin II (ATII) inhibition is daphnodorin A (Figure 3) [19],[20], [21].

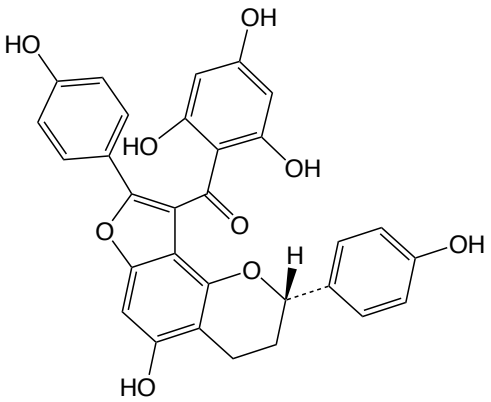


Figure 3: Structure of daphnodorin A

Over thirty lignans have been found in the genus *Daphne* and several of them have shown anti-HIV, cytotoxic and anti-inflammatory effects [19], [22].

Terpenoids are typical secondary metabolites and can be found in different parts of *Daphne* plants. The majority of terpenoids found in the genus are daphnane-type diterpenes. While mono- and sesquiterpenes are common in the flowers of the plants and give them a specific smell [23], diterpenes and triterpenes are found more commonly in other parts of the plants [24]. Daphnauranols [25] (Figure 4) and daphnauranins [26], both sesquiterpenoids isolated from *D. aurantiaca*, have shown insecticidal activity and could be possibly used as natural pesticides.

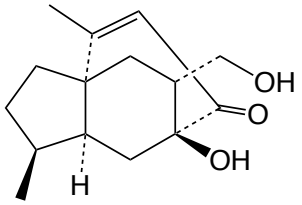


Figure 4: Structure of daphnauranol A

As shown before, daphnane diterpenes are among the substances with the strongest bioactivities found in the genus *Daphne* and are interesting objects for potential application in modern medicine. The following chapter is dedicated to this topic.

3.2 Daphnane diterpenes found in the genus *Daphne*

3.2.1 Phytochemistry

Natural daphnane-type diterpenoids usually possess a 5/7/6-tricyclic ring system with poly-hydroxyl groups located at C-3, C-4, C-5, C-9, C-13, C-14, or C-20 (Figure 5) [27]. Several compounds possess a characteristic orthoester motif connected to C-9, C-13, and C-14. Daphnane diterpenes found in the genus *Daphne* can be divided into five groups according to the substitution pattern of ring A and the oxygen-containing functions at rings B and C [27]. Daphnanes of the ketal-lactone-type differ by an additional ketal-type group located between C-2 and C-4 (Figure 6) [28].

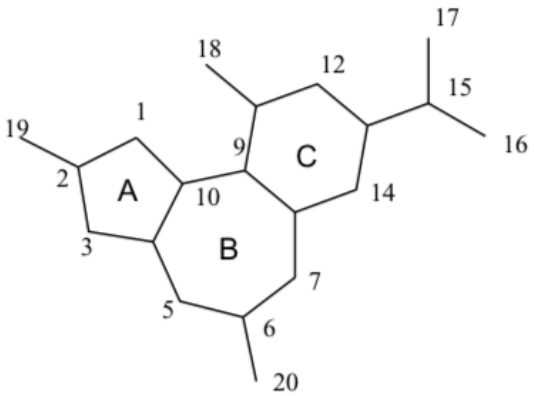


Figure 5: Daphnane-type diterpene skeleton

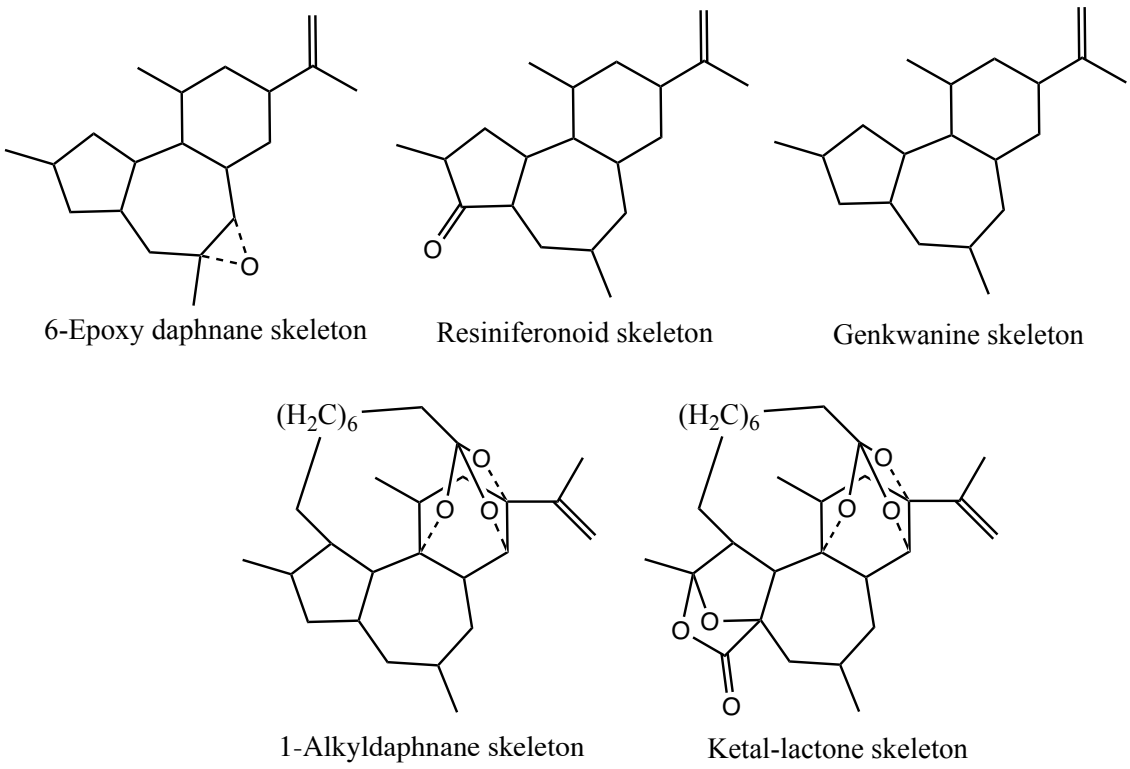


Figure 6: Kinds of daphnane diterpene skeletons

3.2.2 Bioactivity

Daphnane diterpenes are only found in plants of the Thymelaeaceae and Euphorbiaceae families together with structurally related diterpenes of the tigliane and ingenane types. Until now, more than 200 daphnane diterpenes have been isolated and more than 100 are found in the genus *Daphne* [27], [29].

Daphnane diterpenes have been the object of many scientific investigations. Representatives of this substance class have shown potent pharmacological activities including anti-HIV [30], [31], anti-leukemia , antiproliferative on different cancer cell lines [32], [30], cholesterin-lowering [33], neuroprotective [34] and insecticidal [35] effects. Many daphnane diterpenes, including mezerein (Figure 7) and daphnetoxin (Figure 1), two of the first ever isolated compounds of this class [36], [29], have been shown to be potent protein kinase C (PKC) inhibitors. PKC is a family of serine/threonine kinases involved in cellular signaling pathways which play a central role in the control of cell growth and differentiation [37].

A study conducted by Saraiva *et al*. in 2001 [37] showed that the two mentioned substances possess a different selectivity to different PKC isoforms. These different isoforms fulfill several different functions in the cell. This could be the reason for the differing effects mediated by the two substances: Mezerein is an antileukemic agent while daphnetoxin does not share this property but showed antiproliferative activity against other cancerous cell lines [32]. The only difference between the two structures is a lipophilic ester at C12 in mezerein, which is absent in daphnetoxin.

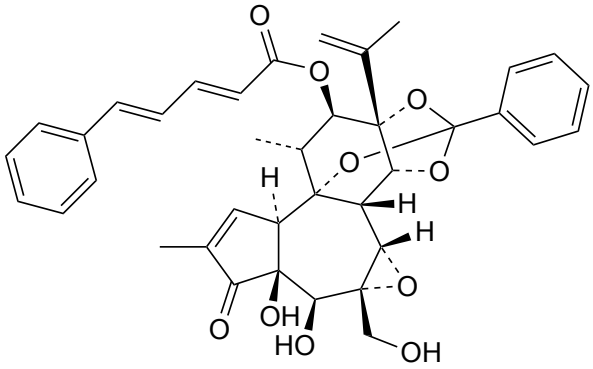


Figure 7: Structure of mezerein

3.3 Daphne feddei

Daphne feddei H. Lév is endemic in the provinces of Yunnan, Sichuan and Guizhou in China and can be found in forests and on shrubby slopes between 1800 and 2600 m above sea level. The plant is an evergreen shrub and grows from 0.6 m to 2 m tall [38].

The whole plant, which is called "Dian Rui Xiang" (滇瑞香) in its regions of origin [38], has been used in traditional Chinese medicine for treating injuries of bruises and falls [20] and also for treatment of rheumatic arthritis and as an analgesic [39]. As well as all plants from the genus *Daphne*, all parts of *D. feddei* are toxic [8].

Compared to other *Daphne* species, there were not many phytochemical studies conducted on this species. There are also a few Chinese patents mentioning the plant. Most of them are referring to the extract as treatment for several conditions also in combination with extracts from other plants.

3.3.1 Constituents

As described above, coumarins are characteristic secondary metabolites found in the genus *Daphne*. Several substances of this class have been isolated from *D. feddei*. Besides daphnin, daphnetin and other well-known coumarins also new compounds such as bicoumol-7'-O-ß-glucopyranoside (Figure 8) were discovered.

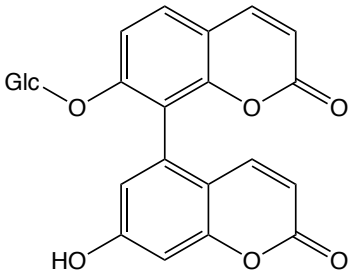


Figure 8: Structure of bicoumol-7'-O-ß-glucopyranoside

Liang *et al*. isolated in 2009 [40] the first substance with a dicoumarinolignoid skeleton from a natural source and named it feddeiticin (Figure 9). Other studies reported a high number of phenylpropanoids and lignans. An example are feddeiketones A-C (Figure 9) which were first found in this plant [41], [42], [43].

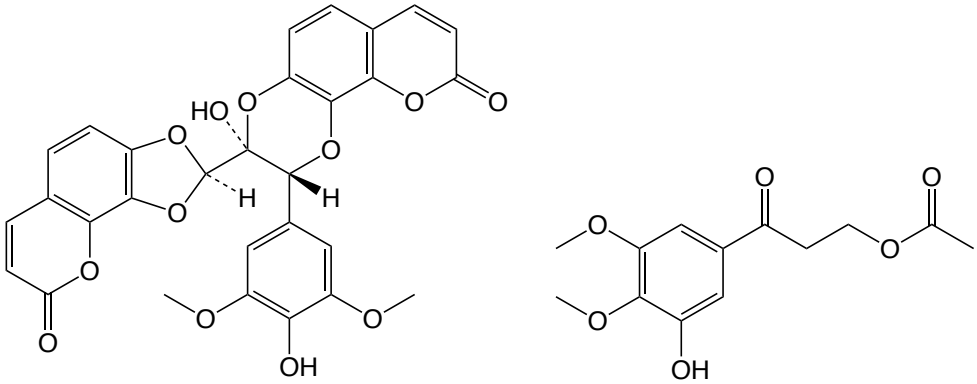


Figure 9: Structures of feddeiticin (left) and feddeiketone A (right)

Among several other flavonoids and biflavonoids, a novel compound, feddeinoid A (Figure 10) was discovered [20], [44], [45]:

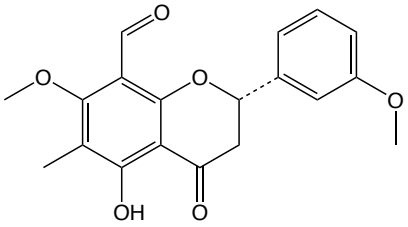
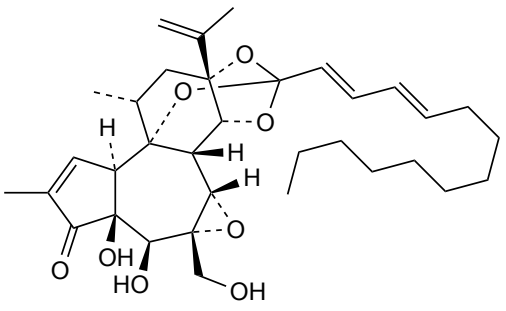
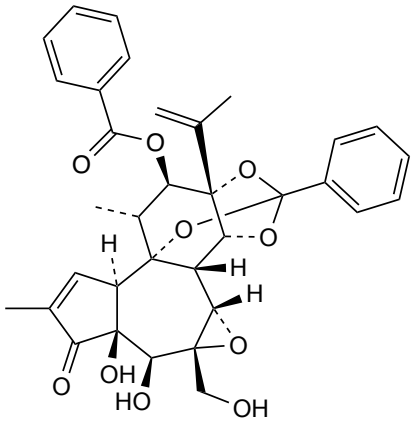


Figure 10: Structure of feddeinoid A

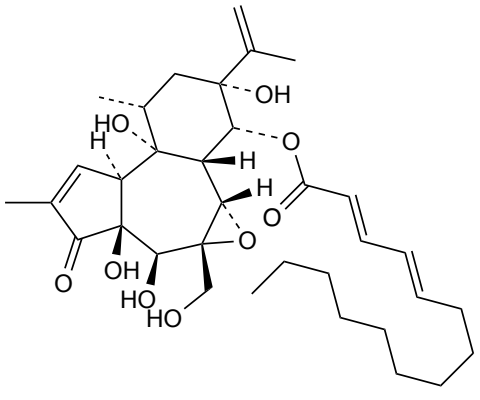
So far four daphnane diterpenes (Figure 11) could be isolated from a methanolic extract of the roots and stem bark of *D. feddei* [39].



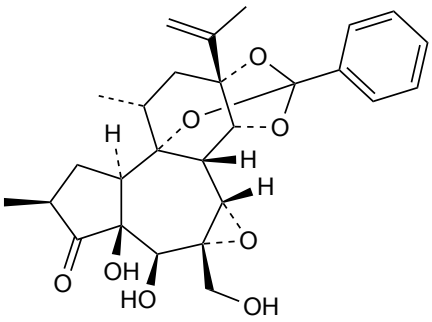
Huratoxin (daphne factor F_1)



Genkwadaphnin (daphne factor F_2)



Prohuratoxin (daphne factor F_3)



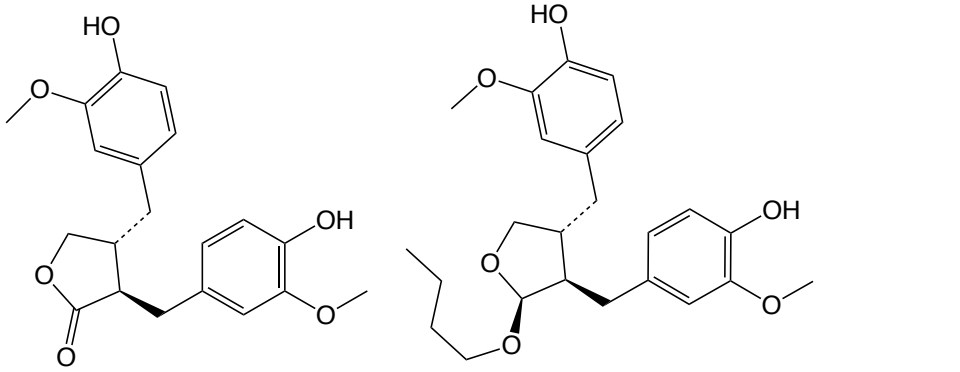
1,2-dihydrodaphnetoxin (daphne factor F_4)

Figure 11: Daphnane diterpenes found in *D. feddei*

3.3.2 Bioactivities

Studies on substances found in *D. feddei* have reported a wide range of bioactivities. Among them, one of the most important findings is the anti-inflammatory activity of several compounds which confirms the traditional usage of the plant for the treatment of falls and bruises [44].

Among the active substances, already known lignans, such as matairesinol, can be found, but also new compounds such as 4,4′-dihydroxy-3,3′-dimethoxy-9-butoxy-9,9′-epoxylignan (Figure 12) [41]. Also several biflavonoids showed anti-inflammatory activity. They include 2"-methoxy-2-epi-daphnodorin C (Figure 13), a new biflavonoid and wikstrol A, an already known substance [44]. The coumarins daphnetin and 7–hydroxycoumarin have also been extracted from the plant and have shown anti-inflammatory activity [46]. A common mechanism is the inhibition of nitric oxide (NO) production in different cell lines [44], [41], [46]. Nitric oxide is an important factor in the regulation of many physiological functions, such as immunologic defense, vasodilation and neurotoxicity. Overabundance of NO has been associated with immunological and inflammatory diseases. As a consequence, inhibition of NO overproduction is an important target for anti-inflammatory agents [44].



Matairesinol 4,4′-dihydroxy-3,3′-dimethoxy-9-butoxy-9,9′-epoxylignan

Figure 12: Structure of NO production - inhibiting lignans

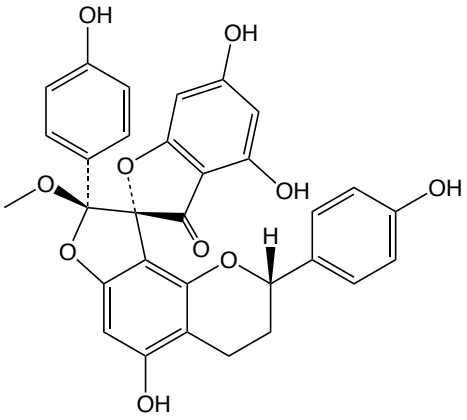


Figure 13: Structure of 2"-methoxy-2-epi-daphnodorin C

Several studies investigated the anti-HIV activity of compounds from different structural classes isolated from *D. feddei* but in the most cases the analyzed substances showed only weak or modest activity [20], [42], [22]. In contrast, the newly discovered lignans feddeiphenols A-C (Figure 14) showed EC_{50} of 3.2-4.1 μg/ml with therapeutic indexes above 30 [43]. The daphnane diterpene prohuratoxin, which was already isolated earlier from this plant [39], was found to be a potent inhibitor of HIV replication [47].

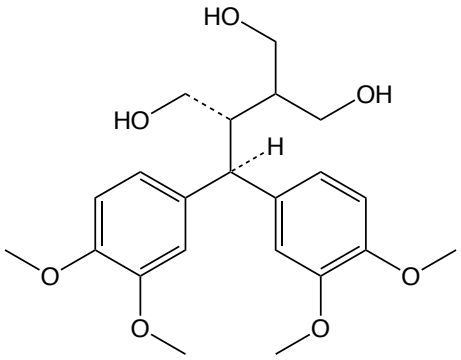


Figure 14: Structure of feddeiphenol A

The skin irritancy of the daphnane diterpenes isolated from *D. feddei* was measured on the mouse ear and the tumor promoting activity was measured on the back skin of mice following standard procedures. Huratoxin showed the highest skin-irritant activity which was on the same level as that of known skin-irritant diterpenes. Prohuratoxin was less irritant than huratoxin, the corresponding orthoester. Genkwadaphnin showed a similar activity as daphnetoxin which was used as a reference substance and has a similar structure.1,2-Dihydrodaphnetoxin was shown to be three times less irritant than daphnetoxin. A tumor promoting assay could only be carried out on 1,2-dihydrodaphnetoxin and showed that the substance was practically inactive at a dose at which other daphnane diterpenes showed strong tumor promoting activity [39].

4 Material and methods

4.1 Equipment

4.1.1 Common instruments and extraction system

Table 1: Common instruments

Instrument	**Manufacturer**	**Model**
Analytical balance	Sartorius	MSA225S
Refrigeration bath	Fryka	KT 18-42
Lyophilisator	Lyo Christ	Gamma 1-16 LSC
Rotary evaporator Water bath Vacuum Pump Condensor	Büchi	Rotavapor R-215
Evaporator for Vials, with N_2	Vögtli	Typ V100
Mill	Retsch	Cutting mill SM 100
Mill	Retsch	Grindomix GM 200

Table 2: Pressurized liquid extraction

Instrument	**Manufacturer**	**Model**
Accelerated solvent extractor	Dionex, Thermo Fisher Scientific	ASE 200

4.1.2 Chromatographic and spectroscopic instrumentation

Table 3: MPLC-Device

Instrument	**Manufacturer**	**Model**
MPLC - Device	Interchim	Puriflash 4100

Table 4: Preparative HPLC-device

Instrument	**Manufacturer**	**Model**
Preparative HPLC column with precolumn	Waters	SunFire Prep C_{18} OBD column (5 µm, 30 x 150 mm i.d.)

		C_{18} Prep guard cartridge (10 x 30 mm i.d.)
Quaternary pump	Agilent Technologies	1200 series, 1290 Infinity II 1260 Prep bin pump
HPLC		1100 series
System controller		1100 series
PDA detector		1100 series
Software		ChemStation software
MS		6120 quadrupole LC/MS

Table 5: Analytical HPLC-DAD-ELSD-MS

Instrument	**Manufacturer**	**Model**
Analytical HPLC column with precolumn	Waters	SunFire C_{18} 3.5 µm, 150 × 3.0 mm SunFire 10 x 3.0 mm
Degasser	Shimadzu	DGU-20A3R
Liquid chromatograph		LC-20AD
Auto sampler		SIL-20ACHT
PDA detector		SPD-M20A
Column oven		CTO-20AC
Communications bus module		CBM-20A
Liquid chromatograph mass spectrometer		LCMS-8030
Software		LabSolutions
ELSD	Alltech	3300

Table 6: NMR

Instrument	**Manufacturer**	**Model**
Probehead	Bruker	1 mm TXI probe
Software		Topspin software
Spectrometer		Bruker Avance III spectrometer operating at 500.13 MHz for ^{1}H and 125.77 MHz for ^{13}C

Table 7: Chromatographic material

Instrument	Manufacturer	Model
Syringe Filter	BGB Analytik AG	Syringe filters, 13 mm, 0.45 µm, PTFE (Polytetra-fluorethylen, hydrophobic)
TLC plates	Merck	Silica gel 60 F_{254}
UV detector	CAMAG	Reprostar 3

4.2 Chemicals and solvents

Table 8: Chemicals

Substance	Manufacturer
Formic acid (FA)	Merck

Table 9: Solvents

Substance	Manufacturer
Ethyl acetate (EtOAc)	Scharlau, redistilled at the Pharmaceutical Biology of the University of Basel
n – Hexane (Hex)	Scharlau, redistilled at the Pharmaceutical Biology of the University of Basel
Methanol (MeOH)	Scharlau, redistilled at the Pharmaceutical Biology of the University of Basel
Dimethyl sulfoxide, deuterated (DMSO d6)	Armar chemicals
Water, HPLC grade	Milli-Q water purification system, Merck
Acetonitrile (ACN), HPLC grade	Macron Fine Chemicals
Dimethyl sulfoxide, HPLC grade (DMSO)	Scharlau
Dichloromethane (DCM)	Scharlau, redistilled at the Pharmaceutical Biology of the University of Basel
Methanol (MeOH), HPLC grade	Macron Fine Chemicals

Table 10: Reagents

Substance	Manufacturer
Vanillin-sulfuric acid reagent for TLC	Merck, compounds mixed at Pharmaceu-tical Biology of the University of Basel

4.3 Plant material

Table 11: Plant material

Plant	Collector/ Identification	Origin	Year of collection
Aerial parts of *Daphne feddei* Lévl.	Prof. Yang Ye (Sanghai Institute of Materia Medica)	China	2019

4.4 Methods

4.4.1 Extraction

As the dried plant material was not homogenous it was sorted manually in three different batches to detect potential differences:

- Leaves
- Small parts (twigs)
- Big parts (stems)

The leaves (23 g) were pulverized in a SM 100 mill and 1 g of the material was extracted by pressurized liquid extraction at 120 bar and 70°C. The sample was extracted twice with 50 ml of dichloromethane (DCM) and subsequently twice with 50 ml methanol (MeOH). The extracts with the same solvent were combined before they were dried using a rotary evaporator under reduced pressure and at a temperature below 40 °C. The small parts and the big parts of the stems were ground in a SM 100 mill separately and pulverized under liquid N_2 in a Grindomix GM 200 mill. ASE extracts from the big parts and the small parts were processed under the same conditions. Instead of methanol ethanol (EtOH) was used for extraction.

For large scale extraction, the big parts (390 g) and the small parts (192 g) were combined and mixed with quartz sand at a ratio of approximately 3:1. The plant material was extracted by percolation by using 9.3 liters of dichloromethane followed by 4.3 liters of methanol. Both extracts were separately evaporated under reduced pressure and below 40°C to provide the dichloromethane (9.55 g) and methanol (86.4 g) extracts, respectively.

4.4.2 HPLC-DAD-ELSD-MS analysis of extracts

The extracts were analyzed by HPLC-DAD-ELSD-MS. The samples were dissolved in DMSO with the help of an ultrasonic bath and filtrated through a membrane filter prior to analysis.

Table 12: Conditions for HPLC-DAD-ELSD-MS analysis

Sample	*Daphne feddei* extracts
Sample concentration	10 mg/ml in DMSO
Injection volume	10 µl
Column with precolumn	SunFireC_{18} 3.5 µm, 3.0 x 150 mm

Mobile phase	[A]: H_2O + 0.1% FA (formic acid) [B]: Acetonitrile (ACN) + 0.1% FA
Gradient	[A:B] 95:5 → 0:100 over 30 min
Flow Rate	0.4 ml/min
Detection	ESI-MS (positive and negative modes), UV and ELSD

4.4.3 Preliminary fractionation of the dichloromethane extract

Chromatographic separation was performed on a Puriflash MPLC system connected to a silica gel glass column. The analysis of the fractions was performed by thin layer chromatography (TLC).

Table 13: Conditions for MPLC - fractionation

Sample	DCM extract: 9.55 g as "dry load"
Column	Glass: l = 35 cm r = 2.5 cm
Solid phase	Silica gel KG60
Mobile phase	[A] Ethyl acetate (EtOAc) [B] Hexane (Hex) [C| Methanol (MeOH)
Gradient	[A:B] 95: 5 → 0:100 over 4 h Afterwards [B:C] 100:0 → 0:100 over 1 h
Flow rate	20 ml/min
Collection	20 ml per vial with a fraction collector
Analysis/detection	Thin layer chromatography (TLC)

4.4.4 Thin layer chromatography of the Puriflash fractions

10 µl of Puriflash fractions were applied on a silica gel TLC plate with a capillary. The DCM extract and pure daphnetoxin were used as reference samples. The bands were detected under UV at 254 nm and 365 nm, followed by spraying with vanillin-sulphuric acid reagent and heating at 110°C for 3 minutes. Fractions with similar TLC patterns were combined to provide 21 fractions (Fractions 1- 21). All fractions were dried on a rotary evaporator.

4.4.5 HPLC-DAD-ELSD-MS analysis of Fractions 1–21

The samples were dissolved in DMSO and filtered before HPLC-DAD-ELSD-MS analysis.

Table 14: Conditions for HPLC-DAD-ELSD-MS analysis of Fractions 1–21

Sample	Fractions 1-21
Solvent	DMSO
Sample concentration	1 mg/ml
Injection volume	10 µl
Column with precolumn	SunFire C_{18} 3.5 µm, 3.0 x 150 mm
Mobile phase	[A]: H_2O + 0.1% FA (formic acid) [B]: Acetonitrile (ACN) + 0.1% FA
Gradient	[A:B] 95:5 → 0:100 over 30 min
Flow Rate	0.4 ml/min
Detection	ESI-MS (positive and negative modes)

4.4.6 Isolation procedure of compounds

Nine peaks contained in Fraction 17 and eight peaks contained in Fraction 18 were separated using a preparative HPLC-MS system which was also equipped with an UV detector. The samples were dissolved in DMSO and filtrated through a 0.45 µm filter before injection. Peaks were collected based mostly on the MS-signal because of the poor UV absorbance of the substances. The eluent between the peaks was collected separately. The acetonitrile was evaporated with a rotary evaporator (<40 °C) under reduced pressure and the fractions were further dried on a lyophilizer.

Table 15: Conditions for preparative HPLC

Sample(s)	Fraction 17 (174 mg) and Fr 18 (390 mg)
Solvent	DMSO
Sample concentration(s)	64.6 mg/ml (Fr 17) and 78 mg/ml (Fr 18)
Injection volume	30 µl for method optimization 500 µl after optimizing
Column with precolumn	SunFire Prep C_{18} 5 µm 30.0 x 150 mm
Mobile phase	[A]: H_2O + 0.1% FA [B]: ACN + 0.1% FA
Gradient	[A:B] 80:20 → 0:100 over 30 min →10 min 100% [B]
Flow rate	20 ml/min
Detection	MS
Collection	Manual

4.4.7 Analytical investigation of the isolated peaks (17.1-17.9 and 18.1-18.8)

100 µl of each fraction were taken before the fraction was evaporated and dried and analyzed by HPLC-DAD-ELSD-MS. The system is described in Table 5 and the conditions are the same as shown in Table 14.

4.4.8 NMR analysis of Fraction 17.3.

The isolated peak was analyzed by 1D and 2D NMR. For that, 2 mg of compound were dissolved in 10 µl of DMSO-d6. An attempt to dissolve the sample in deuterated chloroform failed. ^{1}H NMR, COSY, HSQC and HMBC spectra were recorded. The spectra were analyzed with ACD/Labs – Spectrus® Software.

4.4.9 Strategy for the literature search of daphnane diterpenes in the genus *Daphne*

The search of the daphnane-type diterpenes found in the genus *Daphne* was performed mostly with SciFinder^{N} ® (Chemical Abstracts Services) and Reaxys® (Elsevier) search engines. The list of daphnane diterpenes found in the review by Moshiashvili *et al*. (2020) [19] was used as a source of additional information. When the original source could not be accessed or was written in Chinese, a footnote was added.

For every compound all the publications listed as "references" on the search engine were looked through to gain an exhaustive insight on the sources of daphnane diterpenes within the genus *Daphne*. The other way around, a search was performed based on every single *Daphne* species listed in "The Plant List" from the Royal Botanic Gardens, Kew and Missouri Botanical Garden [2] to find publications concerning daphnane diterpenes. The chemical structures of the compounds were extracted from the publications and drawn with ChemDraw® (PerkinElmer) software.

5 Results

5.1 Investigation of daphnane diterpenes in *Daphne feddei*

5.1.1 Extraction

As described above, the plant material was sorted in three different batches (leaves, small parts and big parts of the stems). Small amounts of each were extracted through accelerated solvent extraction (ASE) with dichloromethane followed by methanol (leaves) or with dichloromethane and ethanol (stems).

5.1.2 HPLC-DAD-ELSD-MS analysis of extracts and large scale extraction

The chromatograms of the extracts showed that the daphnane diterpenes had a very low UV-absorption and also the signal in the ELSD-trace was in most cases weak. The analysis was therefore mostly based on the MS-data.

The analytical investigation of the different extracts showed that the MeOH extract of the leaves and the EtOH extracts of the stems contained only very small amounts of the searched substances. The DCM leaf extract contained also only very small amounts of daphnane diterpenes. On the basis of these findings it was decided to continue the work with the DCM extracts of the big parts and the small parts. As these two DCM extracts showed a very similar composition, the big parts (390 g) and small parts (192 g) were combined after pulverization for large scale extraction. The pulverized plant material (582 g) was extracted by percolation to afford 9.70 g of dichloromethane extract and 86.4 g of methanol extract.

The masses of all four daphnane diterpenes which were previously isolated from *D. feddei* and described in the paper of Dagang *et al*. (1991) [39] could be found in the DCM extracts of the big parts and the small parts of the stems. As seen in the MS chromatogram (Figure 15), the mass of the main compound 1,2-dihydrodaphnetoxin (*m/z* 484) was found together with the masses of genkwadaphnin and prohuratoxin (both *m/z* 602) and huratoxin (*m/z* 584). Other masses which were found in the extracts can also be tentatively assigned to various daphnane diterpenes: Yuanhuafine can be associated with the *m/z* 540, yuanhuapine with *m/z* 542 and the mass 604 can be attributed to yuanhuatine. The peak at *m/z* 590 could correspond to three daphnane diterpenes, genkwanine M, genkwanine N and yuanhuaoate C.

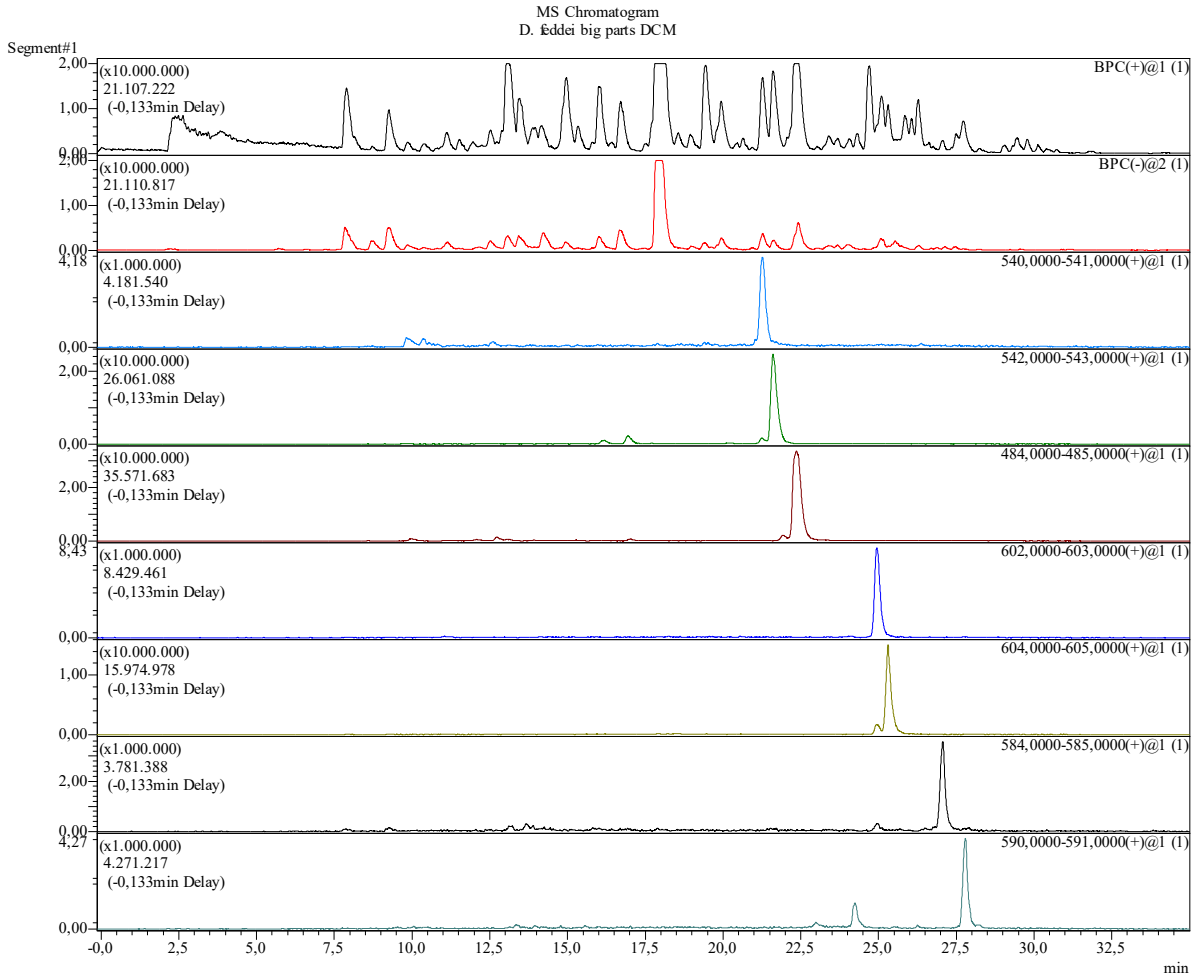


Figure 15: HPLC-MS analysis of the DCM extract of the big parts of the stems. Base peak chromatograms (BPC) in positive and negative ions modes and extracted ion traces

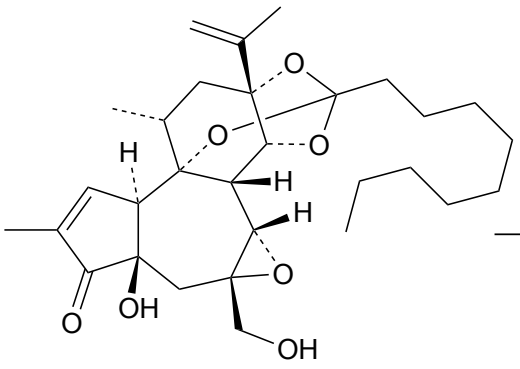
5.1.3 MPLC fractionation of the dichloromethane extract

The dichloromethane extract was separated on a Puriflash MPLC device equipped with a glass column filled with silica gel to afford 300 fractions. During the chromatographic separation, samples of 10 µl from every 5th fraction were taken and analyzed via TLC. Based on the separation on the TLC plate the number of fractions could be reduced from 300 to 21 by pooling the fractions with similar bands.

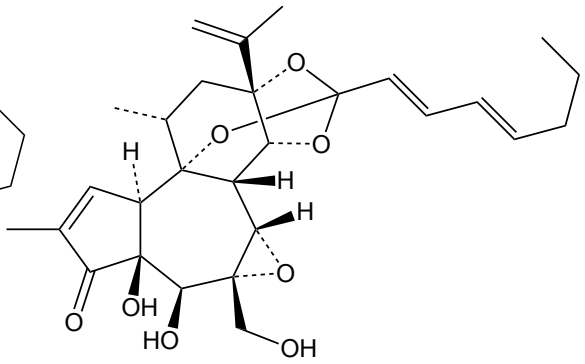
5.1.4 HPLC-DAD-ELSD-MS analysis of Fractions 1 – 21

The HPLC-MS chromatograms of the fractions showed that the Fractions 15 to 20 contained many compounds with masses which could be assigned to daphnane diterpenes. In these enriched fractions all masses which were detected in the extract were found and in addition two additional compounds could be detected. One compound showed a mass to charge ratio of 526 and might be excoecaria factor O1. A substance with *m/z* 516 was detected in the Fractions 15 to 17. To the best of our knowledge up to now no daphnane-type diterpene with this mass has been isolated from any *Daphne* species previously. However, Adolf *et al*. described the extraction of two different daphnane diterpenes with this mass from plants from the genera *Daphnopsis* [48] and *Synaptolepis* [49]: daphnopsis factor R_4 and 24-butylidene-24-deoxy-12-hydroxy-(12β)-crepitol (Figure 16), respectively. Both genera are part of the Thymelaeaceae family, like the genus *Daphne.* Fraction

17 contained the highest amount of the compound with the mass to charge ratio of 484 which was tentatively assigned to 1,2-dihydrodaphnetoxin.



Daphnopsis factor R4



24-butylidene-24-deoxy-12-hydroxy-(12β)-crepitol

Figure 16: Structures of daphnane diterpenes potentially new in *D. feddei*

5.1.5 Analytical investigation of the isolated peaks (17.1-17.9 and 18.1-18.8)

Fraction 17 and Fraction 18 were separated through preparative HPLC and peaks were collected (Isolation scheme, Figure 18)

In Table 16 and Table 17 all the substances which were found in the MPLC fractions and their corresponding retention time are listed. For some molecular weights, different isomers were detected which is revealed by different retention times. An example are compounds with an ion at *m/z* 584 which eluate in Fr 17.6 at 27.5 min and in Fr 18.5 at 27.1 min under the same chromatographic conditions.

Table 16: Compounds found in Fraction 17

Collected peaks	**Base peak 1 (highest intensity), [*m/z*]**	**t_R [min]**	**Base peak 2 (medium intensity), [*m/z*]**	**t_R [min]**	**Base peak 3 (low intensity), [*m/z*]**	**t_R [min]**	**Amount [mg]**
Fr 17.1	256	18.3					1.3
Fr 17.2	299	19.7	516	19.7			3.9
Fr 17.3	484	22.8	484	17.2	276	23.8	12.3
Fr 17.4	278	25.6	604	25.7	602	25.3	1.2
Fr 17.5	278	25.5	278	26.0	604	25.7	1.9
Fr 17.6	584	27.5					1.4
Fr 17.7	586 (noisy)	28.9					1.0
Fr 17.8	664 (noisy)	29.0	586 (noisy)	29.0			1.0
Fr 17.9	noisy						0.1

Table 17: Compounds found in Fraction 18

Collected peaks	Base peak 1 (highest intensity), [*m/z*]	t_R [min]	Base peak 2 (medium intensity), [*m/z*]	t_R [min]	Amount [mg]
Fr 18.1	299	19.2	516	19.2	6.7
Fr 18.2	540	21.2	542	21.5	2.5
Fr 18.3	542	21.5			4.8
Fr 18.4	484	22.2			4.9
Fr 18.5	584	27.2			2.1
Fr 18.6	279	29.6			1.9
Fr 18.7	281	31.9	296	31.4	2.2
Fr 18.8	noisy				0.2

5.1.6 NMR analysis of Fraction 17.3.

Extensive NMR analysis of Fraction 17.3 confirmed that the main compound in this fraction was 1,2-dihydrodaphnetoxin. The NMR data of the compound are shown in Table 18, while the mass spectrum and the ^{1}H NMR spectrum of the fraction can be seen in Figure 24 and Figure 25, respectively, in the appendix. Due to the shutdown of the lab caused by covid-19 no final purification of the compound by semi-preparative HPLC could be carried out. A minor compound contained in the fraction which complicated the structure elucidation was confirmed to be also a daphnane diterpene but its structure could not be elucidated conclusively.

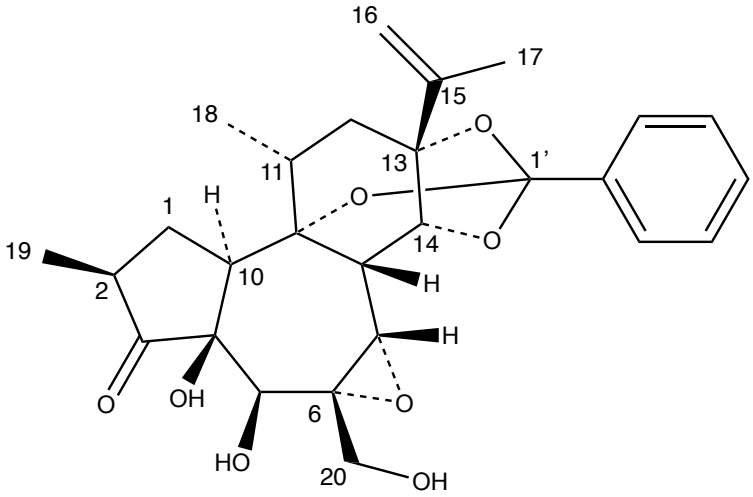


Figure 17: Structure of 1,2-dihydrodaphnetoxin with atom numbering

Table 18: ^{1}H and ^{13}C NMR Spectroscopic data for 1,2-dihydrodaphnetoxin (DMSO-d6; 500.13 Hz for ^{1}H and 125.77 for ^{13}C NMR; δ in ppm

Position	**δ_C[a]**	**δ_H, mult (*J* in Hz)**
1	32.6, CH_2	1.43[b] 2.15[b]
2	41.6, CH	2.44[b]
3	216.3, C	
4	75.5, C	
5	68.5, CH	3.94, br s
6	61.9, C	
7	62.1, CH	3.37, br s
8	35.7, CH	2.88, d (2.4)
9	80.0, C	
10	43.7, CH	3.10, br dd (13.3, 5.7)
11	34.5, CH	2.50, m
12	35.8, CH_2	1.62, d (14.0)
12	35.8, CH_2	2.19[b]
13	84.0, C	
14	81.4, CH	4.65, d (2.4)
15	146.3, C	
16	110.4, CH_2	5.05, m
16	110.4, CH_2	4.88, br s
17	18.8, CH_3	1.80, br s
18	20.6, CH_3	1.23, d (6.7)
19	12.4, CH_3	1.00, d (6.4)
20	63.2, CH_2	3.41 br d (12.2) 3.84, br d (12.2)
1‘	116.5, C	
2‘	136.5, C	
3‘	125.7, CH	7.66, m
4‘	127.7, CH	7.41, m
5‘	129.1, CH	7.41, m
6‘	127.7, CH	7.41, m
7‘	125.7, CH	7.66, m

[a] ^{13}C NMR data extracted from HSQC and HMBC spectra.

[b] Overlapping signals.

5.1.7 Isolation scheme

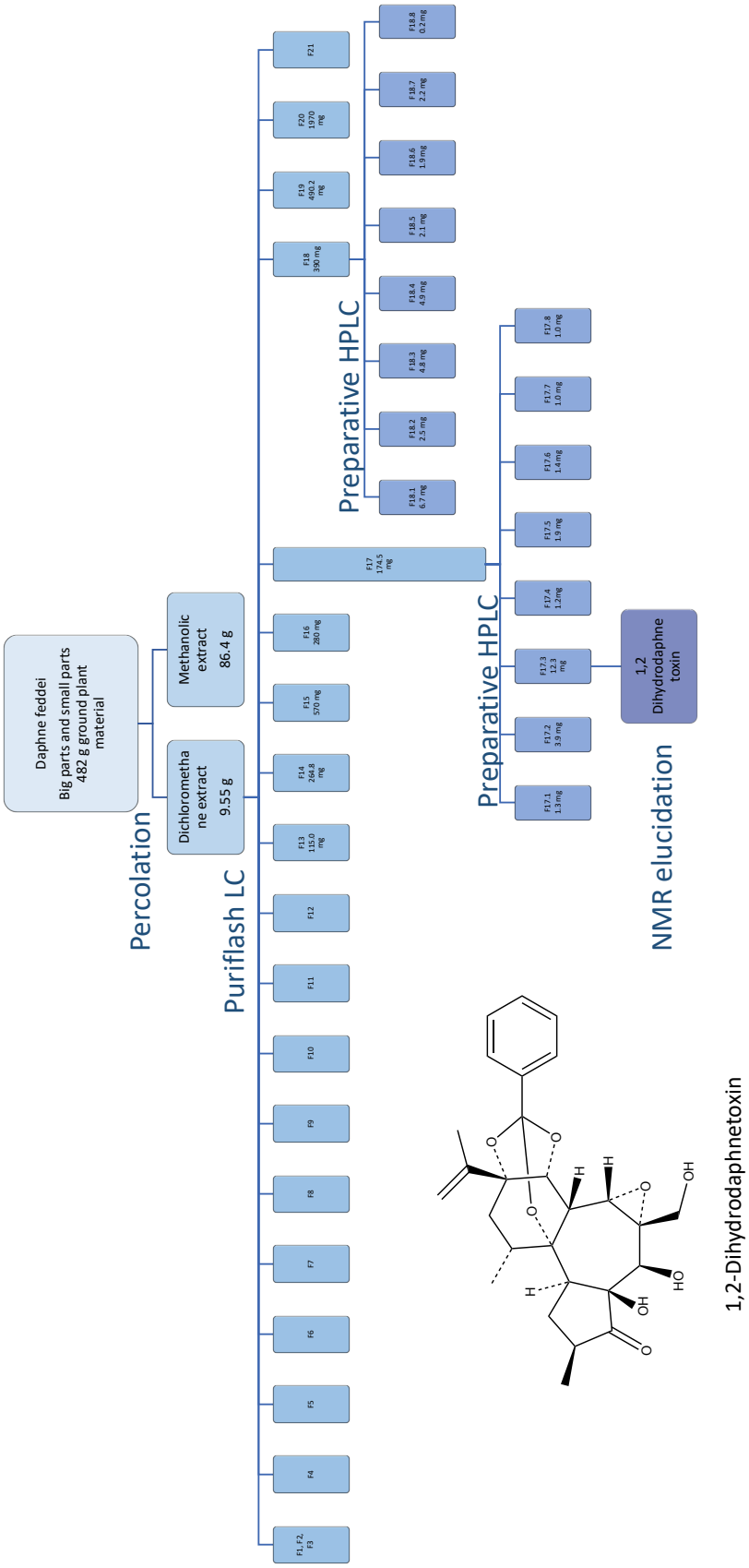


Figure 18: Isolation scheme

5.2 Daphnane diterpenes found in the genus *Daphne*

Daphnane diterpenes which have been found in the genus *Daphne*, their synonyms, sources and bioactivities are shown in the next chapters. The compounds are sorted according to their substructures which can be seen in Figure 6. A table of daphnane diterpenes sorted by plant name can be found in the appendix (Table 24).

5.2.1 Daphnane diterpenes from the genus *Daphne* sorted by compound

Table 19: 6-epoxy daphnanes

	Compound	MW	Sources	Bioactivity
1	**1,2α-Dihydro-20-palmitoyl-daphnetoxin 1,2-Dihydrodaphnegiraldifin**[1]	722.95	*D. tangutica* [6], [50]	
3	**1,2α-Dihydro-5β-hydroxy-6α,7α-epoxyresiniferonol-14-benzoate**	512.21	*D. tangutica* [51]	
4	**1,2β-Dihydro-5β-hydroxy-6α,7α-epoxyresiniferonol-14-benzoate**	512.21	*D. tangutica* [51]	
5	**12–Hydroxydaphnetoxin**	498.52	*D. giraldii* [52] *D. mezereum* [36]	
6	**12β-Acetoxy-5β-hydroxy-resiniferonol-9,13,14-triyl-ortho[(2E)-dec-2-enoate]**	588.29	*D. genkwa* [53]	
7	**14'-Ethyltetrahydrohuratoxin**	616.83	*D. acutiloba* [30]	- strong anti-HIV activity [30] - cytotoxic against five tumor cell lines [30]
8	**Acutilobin A**	662.27	*D. acutiloba* [30]	- anti-HIV activity [30]
9	**Acutilobin B**	662.28	*D. acutiloba* [30]	- anti-HIV activity [30]
10	**Acutilobin C**	718.29	*D. acutiloba* [30]	- anti-HIV activity [30]
11	**Acutilobin D**	720.80	*D. acutiloba* [30]	- anti-HIV activity [30]
12	**Acutilobin E**	674.69	*D. acutiloba* [30]	- anti-HIV activity [30]
13	**Acutilobin F**	634.76	*D. acutiloba* [30]	- anti-HIV activity [30]
14	**Acutilobin G**	616.70	*D. acutiloba* [30]	- anti-HIV activity [30]
15	**Altadaphnan A**	630.28	*D. altaica* [54]	- antiproliferative activity against human lung carcinoma cell line [54]
16	**Altadaphnan B**	648.28	*D. altaica* [54]	- antiproliferative activity against human lung carcinoma cell line [54]
17	**Altadaphnan C**	646.27	*D. altaica* [54]	- antiproliferative activity against human lung carcinoma cell line [54]
18	**Daphnegiraldifin**	720.93	*D. giraldii* [52]	
19	**Daphnegiraldigin**	500.54	*D. giraldii* [5]	
20	**Daphnegiraldin 12-Lauroyloxydaphnetoxin**	680.83	*D. giraldii* [55]	
23	**Daphnetoxin**	482.52	*D. giraldii* [56] *D. tangutica* [32] *D. gnidium* [31]	- cytotoxic activity against two human nasopharyngeal carcinoma cells [32]

	Compound	MW	Sources	Bioactivity
			D. acutiloba [30] *D. mezereum* [57] *D. altaica* [54]	- selective inhibition of HIV replication [31] - potential cholesterol-lowering activity [33]
24	**Excoecaria factor O_1** **Peddiea factor V1**	526.62	*D. acutiloba* [30]	- cytotoxic against five tumor cell lines [30]
25	**Excoecariatoxin**	528.63	*D. tangutica* [32] *D. gnidium* [31] *D. acutiloba* [30] *D. genkwa* [35] *D. altaica* [54]	- cytotoxic activity against two human nasopharyngeal carcinoma cells [32] - insecticidal activity [35] - selective inhibition of HIV replication [31]
29	**Genkwadane D**	614.72	*D. genkwa* [35]	
30	**Genkwadaphnin** **Daphne factor F_2**	602.63	*D. oleoides* ssp *oleoides* [58] *D. genkwa* [59] *D. feddei* [39] *D. aurantiaca* [60] *D. altaica* [54]	- inhibitory effect on IL1 and TNFα biosynthesis [61] - cytotoxic activity against leukemia and other humancarcinoma cells *in vitro* [59]
31	**Genkwadaphnin-20-palmitate**	840.04	*D. oleoides* ssp *oleoides* [58]	
32	**Genkwanin I^2**	512.19	*D. genkwa* [7]	
36	**Genkwanine M** **Genkwanine VI**	590.66	*D. genkwa* [62]	
37	**Genkwanine N** **Genkwanine VII**	590.66	*D. genkwa* [34]	- nurr1 activation, neuroprotective [34]
38	**Genkwanine N-20-palmitate**	828.07	*D. genkwa* [63] *D. oleoides* ssp. *oleoides* [64]	
39	**Genkwanine O** **Genkwanine II**	504.57	*D. genkwa* [65]	
40	**Gnidicin**	628.66	*D. gnidium* [31] *D. acutiloba* [30] *D. giraldii* [66] *D. tangutica* [51] *D. altaica* [54]	- selective inhibition of HIV replication [30], [31] - cytotoxic against tumor cell lines [30] - antileukemic [67]
41	**Gnidicin-20-palmitate**	866.07	*D. oleoides* ssp *oleoides* [58]	
42	**Gnididin**	648.74	*D. acutiloba* [30]	- anti-HIV activity [30] - cytotoxic against tumor cell lines [30] - antileukemic [67]
43	**Gnidilatidin-20-palmitate**	886.15	*D. odora* [68] *D. genkwa* [63] *D. oleoides* ssp. *oleoides* [58]	
44	**Gnidilatimonoein**	662.81	D. mucronata [69]	- antileukemic activity [70] - inhibition of nucleic acids biosynthesis [69]

	Compound	MW	Sources	Bioactivity
45	**Gnidilatin**	652.77	*D. oleoides* ssp *oleoides* [61]	
46	**Gnidilatin-20-palmitate**	890.18	*D. oleoides* ssp *oleoides* [61]	
47	**Gniditrin** **Daphne factor P_1**	646.72	*D. tangutica* [32] *D. gnidium* [31] *D. acutiloba* [30] *D. giraldii* [66] *D. mezereum* [71] *D. alpina* [71] *D. aurantiaca* [60] *D. papyracea* [72] *D. odora* [73]	- cytotoxic activity against two human nasopharyngeal carcinoma cells and other tumor cell lines [32] - selective inhibition of HIV replication [30], [31] - antileukemic [67] - potential cholesterol-lowering activity [33]
48	**Huratoxin** **Daphne factor F1** **Hippomane factor M1**	584.74	*D. feddei* [39]	
49	**Isovesiculosin**	546.65	*D. tangutica* [51]	
50	**Isoyuanhuacine** **Yuanhuacine B**	648.74	*D. genkwa* [35]	
51	**Isoyuanhuadine**	586.67	*D. genkwa* [35]	
52	**Kirkinine**	672.85	*D. acutiloba* [30]	- anti-HIV activity [30] - cytotoxic against tumor cell lines [30]
53	**Mezerein**	654.70	*D. mezereum* [36]	- cytotoxic [36] - antileukemic [74]
54	**Odoratrin** **Daphne factor P2**	648.74	*D. odora* [68] *D. papyracea* [72]	
55	**Orthobenzoate 2**	486.23	*D. genkwa* [7]	
59	**Prohuratoxin** **Daphne factor F3** **Wikstroelide M**	602.76	*D. acutiloba* [75] *D. feddei* [72] *D. altaica* [54]	- inhibition of HIV replication [47]
60	**Simplexin** **Daphnopsis factor R3** **Pimelea factor P1** **Wikstrotoxin D**	532.67	*D. genkwa* [76] *D. holosericea* [77]	
61	**Tanguticacin**	884.50	*D. tangutica* [78]	
62	**Tanguticadin[1]**	686.79	*D. tangutica* [50]	
63	**Tanguticafin[1]** **Thymeleatoxin A**	628.66	*D. tangutica* [50]	
64	**Tanguticahin[1]** **15,16-Dihydrodaphnetoxin**	484.54	*D. tangutica* [50]	
65	**Tanguticakin** **1,2-Dihydrodaphnetoxin** **Daphne factor F_4**	484.54	*D. oleoides* ssp. *oleoides* [58] *D. giraldii* [66] *D. feddei* [39] *D. altaica* [79] *D. genkwa* [80] *D. tangutica* [50]	- inhibitory effect on IL1 and TNFα biosynthesis [61]
66	**Vesiculosin**	546.65	*D. tangutica* [51] *D. acutiloba* [75] *D. altaica* [54]	

	Compound	MW	Sources	Bioactivity
68	**Wikstroemia factor M1**	636.77	*D. genkwa* [81] *D. acutiloba* [30]	- cytotoxic against tumor cell lines [30] - nurr1 activation [81]
69	**Yuanhuacine** **Gnidilatidin** **Odoracin**	648.74	*D. genkwa* [81], [59] *D. tangutica* [32] *D. odora* [68], [82] *D. oleoides* ssp *oleoides* [58]	- promoting effect on hepatocyte growth factor production in human dermal fibroblasts [68] - nurr1 activation [81] - nematicidal [82] - cytotoxic activity against human leukemia, nasopharyngeal and other carcinoma cells *in vitro* [59], [32] - up-regulation of p21 expression [83] - regulating AMPK/mTOR signaling pathway [84] - selective antagonist of phorbol ester receptor in PKC [85]
70	**Yuanhuadine**	586.67	*D. genkwa* [81], [86] *D. odora* [68]	- promoting effect on hepatocyte growth factor production in human dermal fibroblasts [68]
71	**Yuanhuafine**	540.56	*D. genkwa* [53]	
72	**Yuanhuagine** **Kirkinine D** **Peddiea factor V_2**	584.65	*D. genkwa* [86]	
73	**Yuanhuahine**	600.70	*D.genkwa* [81]	
74	**Yuanhuajine** **Isouanhuajine**	646.72	*D. odora* [68] *D. tangutica* [32] *D. acutiloba* [30] *D. genkwa* [86]	- promoting effect on hepatocyte growth factor production in human dermal fibroblasts [68] - cytotoxic activity against human nasopharyngeal carcinoma cells [32]
75	**Yuanhualine**	614.72	*D. genkwa* [35]	
76	**Yuanhuamine A**	586.67	*D. genkwa* [35]	
77	**Yuanhuamine B**	628.75	*D. genkwa* [35]	
78	**Yuanhuamine C**	642.78	*D. genkwa* [35]	
79	**Yuanhuaoate A**	554.59	*D. genkwa* [87]	
80	**Yuanhuaoate C**	590.66	*D. genkwa* [87]	
81	**Yuanhuaoate E**	666.75	*D. genkwa* [35]	
82	**Yuanhuapine**	542.57	*D. genkwa* [86]	
83	**Yuanhuatine**	604.64	*D. genkwa* [81] *D. aurantiaca* [60]	

[1] Only abstract of original source accessible: [88]
[2] $C_{28}H_{32}O_9$: Possibly genkwanine "1" and genkwanine "i"

Table 20: Genkwanine-type daphnanes

	Compound	MW	Sources	Bioactivity
33	**Genkwanin III**	504.57	*D. genkwa* [89]	
34	**Genkwanin IV**	504.57	*D. genkwa* [89]	
35	**Genkwanin V**	522.58	*D. genkwa* [89]	
84	**Genkwanine A**	504.57	*D.genkwa* [90]	- inhibition of melanogenesis [90]

	Compound	MW	Sources	Bioactivity
85	**Genkwanine B**	654.79	*D. genkwa* [62]	
86	**Genkwanine C**	652.77	*D. genkwa* [62]	
87	**Genkwanine D**	608.68	*D. genkwa* [62]	
88	**Genkwanine E**	652.77	*D. genkwa* [62]	
89	**Genkwanine F**	654.79	*D. genkwa* [90]	- inhibition of melanogenesis [90]
90	**Genkwanine G**	656.80	*D. genkwa* [62]	
91	**Genkwanine H**	608.68	*D. genkwa* [90]	- inhibition of melanogenesis [90]
92	**Genkwanine I**[2]	522.58	*D. genkwa* [80]	
93	**Genkwanine J**	672.80	*D. genkwa* [35]	
94	**Genkwanine K**	626.69	*D. genkwa* [62]	
95	**Genkwanine VIII**	608.25	*D. acutiloba* [30] *D. genkwa* [91]	- anti- HIV activity [30] - cytotoxic against tumor cell lines [91]
96	**Neogenkwanine A**	504.57	*D. genkwa* [92]	- weak cytotoxic activity on three cancer cell lines [92]
97	**Neogenkwanine B** **Genkwanine Q**	504.57	*D. genkwa* [92]	- weak cytotoxic activity on three cancer cell lines [92]
98	**Neogenkwanine C**	654.79	*D. genkwa* [92]	- strong cytotoxic activity on three cancer cell lines [92]
99	**Neogenkwanine D**	654.79	*D. genkwa* [92]	- strong cytotoxic activity on three cancer cell lines [92]
100	**Neogenkwanine E** **Genkwanine T**	654.79	*D. genkwa* [92]	- strong cytotoxic activity on three cancer cell lines [92]
101	**Neogenkwanine F** **Genkwanine U**	654.79	*D. genkwa* [92]	- strong cytotoxic activity on three cancer cell lines [92]
102	**Neogenkwanine G**	608.68	*D. genkwa* [92]	- weak cytotoxic activity on three cancer cell lines [92]
103	**Neogenkwanine H** **Genkwanine R**	504.57	*D. genkwa* [92]	- weak cytotoxic activity on three cancer cell lines [92]

[2] $C_{28}H_{32}O_9$: Possibly Genkwanine "1" and Genkwanine "i"

Table 21: 1-Alkyldaphnanes

	Compound	MW	Sources	Bioactivity
27	**Genkwadane B**	638.79	*D. genkwa* [76]	
28	**Genkwadane C**	656.80	*D. genkwa* [76]	
56	**Pimelea factor P_2** **Daphnopsis factor R1** **Gnilatimacrin** **Linifolin B** **Linimacrin B**	638.79	*D. genkwa* [76]	
67	**Wikstroelide E** **Pimelea factor S_6**	532.67	*D. genkwa* [76]	
106	**Daphneodorin A**	952.38	*D. odora* [93]	- strong anti-HIV activity [93]
107	**Daphneodorin B**	1010.3	*D. odora* [93]	- strong anti-HIV activity [93]
108	**Daphneodorin C**	1010.6	*D. odora* [93]	- weak anti-HIV activity [93]
109	**Gnidimacrin** **Ligustrinin B**	774.89	*D. odora* [93]	- strong anti-HIV activity [93]

Table 22: Resiniferonoid-type daphnanes

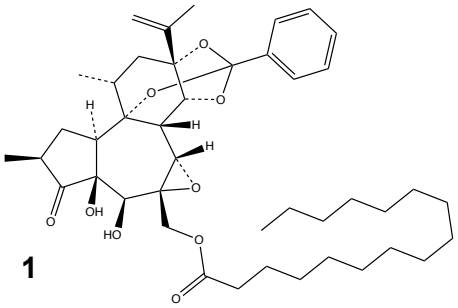
	Compound	MW	Sources	Bioactivity
21	**Daphneresiniferin A**	446.49	*D. genkwa* [90]	
22	**Daphneresiniferin B**	508.56	*D. genkwa* [90]	
26	**Genkwadane A**	620.64	*D. genkwa* [76]	
104	**Genkwanine L**	560.59	*D. genkwa* [53]	
105	**Yuanhuaoate B**	620.64	*D. genkwa* [87]	

Table 23: Ketal- lactone-type daphnanes

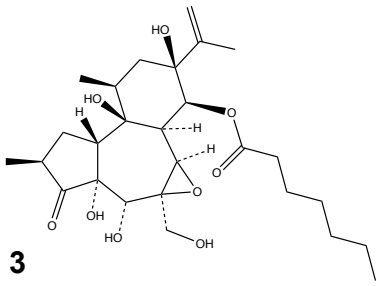
	Compound	MW	Sources	Bioactivity
57	**Pimelotide A**	546.65	*D. genkwa* [76]	
58	**Pimelotide C**	546.65	*D. genkwa* [76]	

5.2.2 Structures of daphnane diterpenes found in the genus *Daphne*

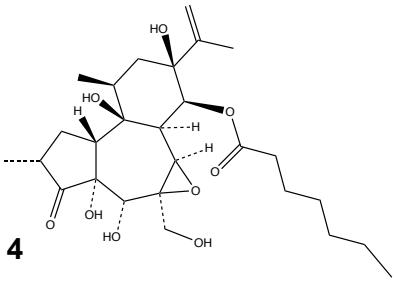
5.2.2.1 6-Epoxy daphnanes



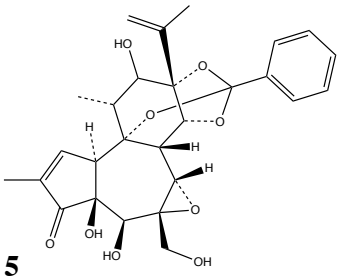
1



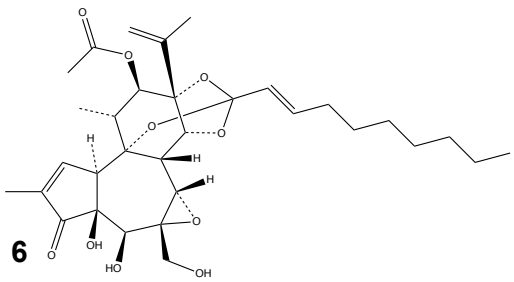
3



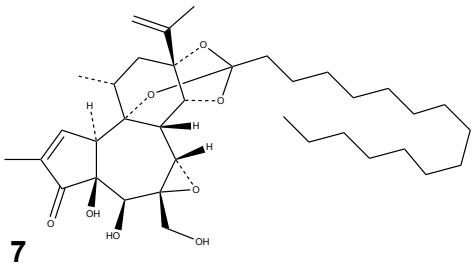
4



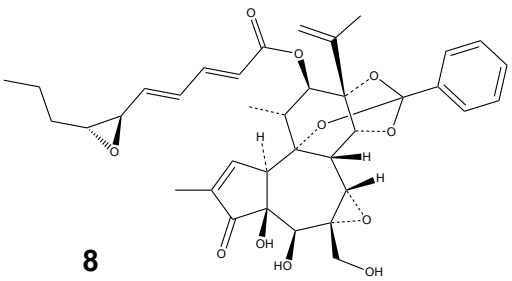
5



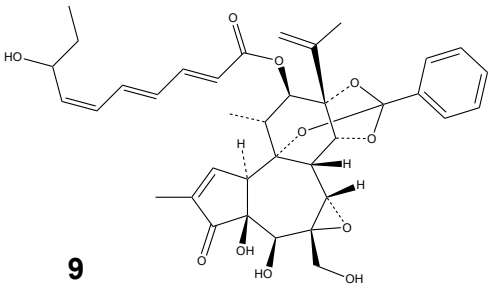
6



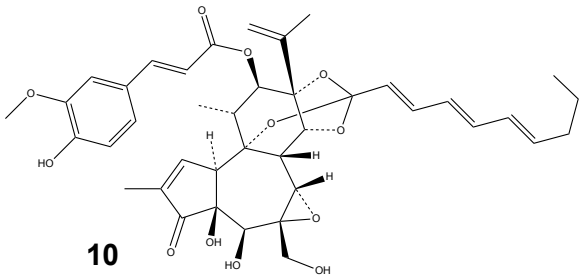
7



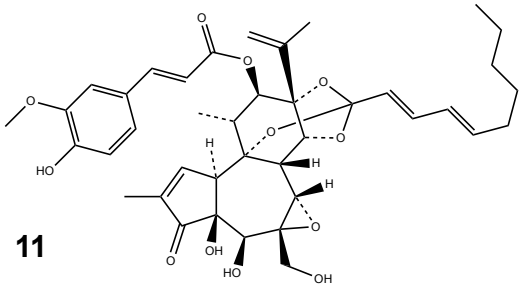
8



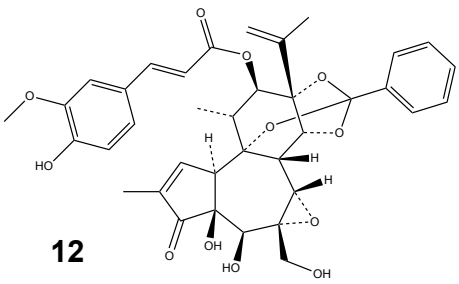
9



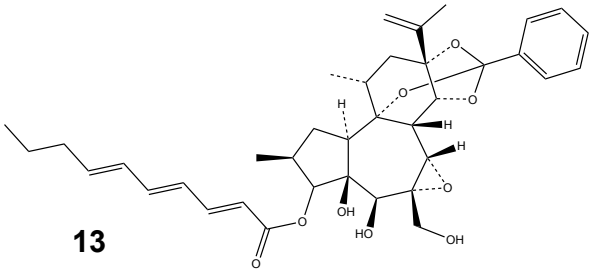
10



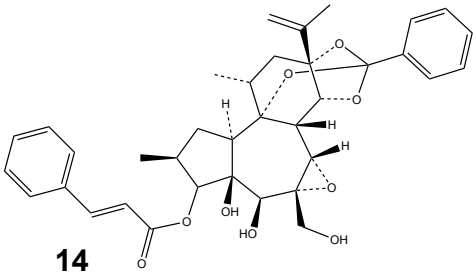
11



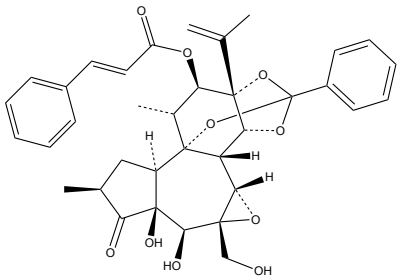
12



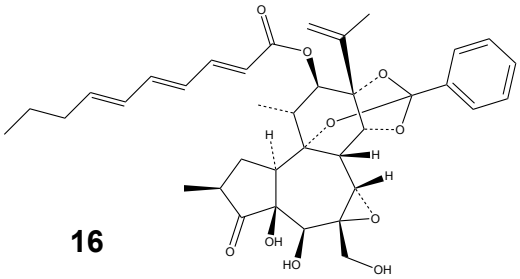
13



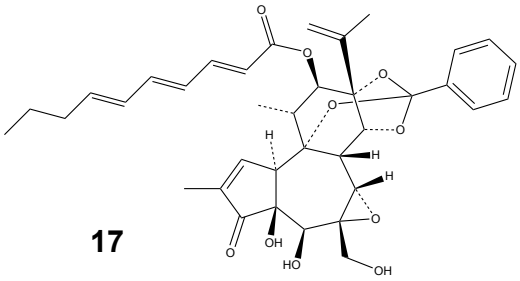
14



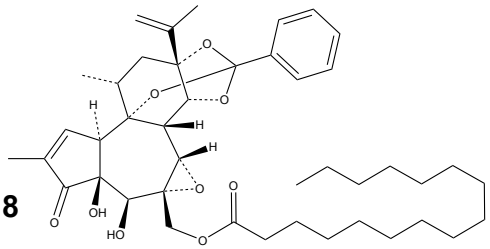
15



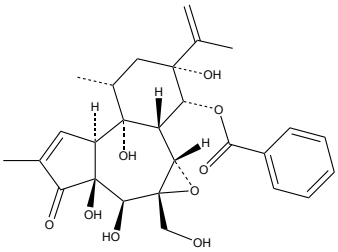
16



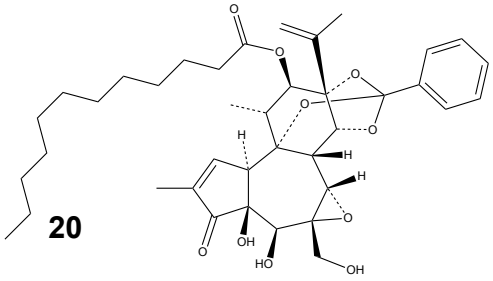
17



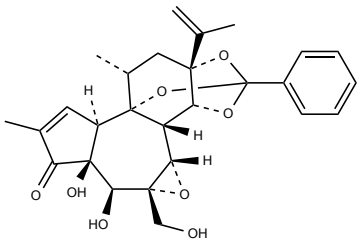
18



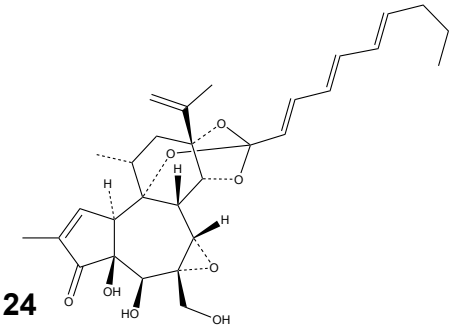
19



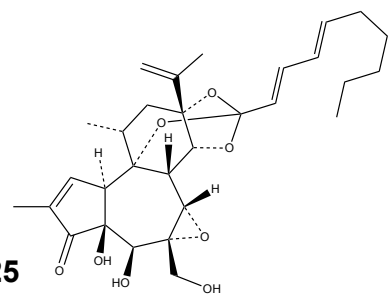
20



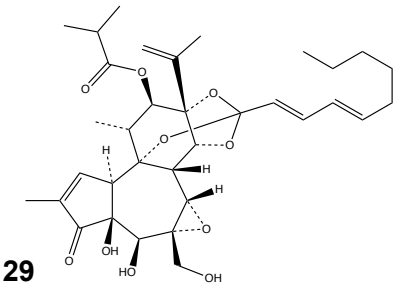
23



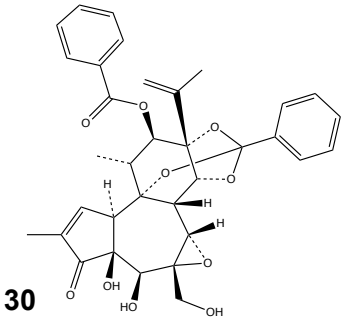
24



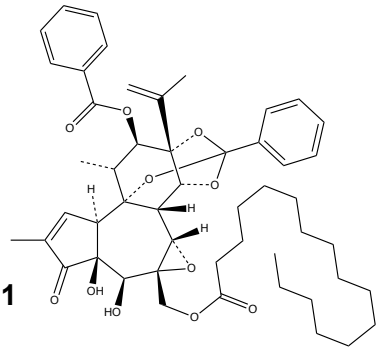
25



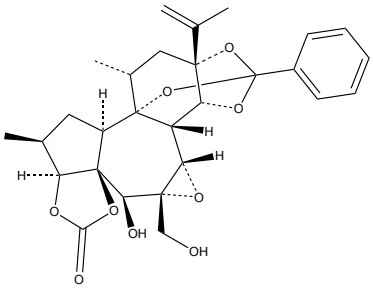
29



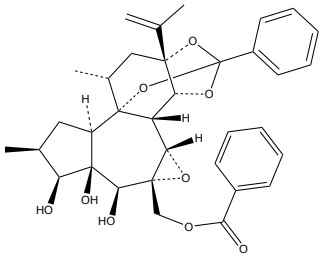
30



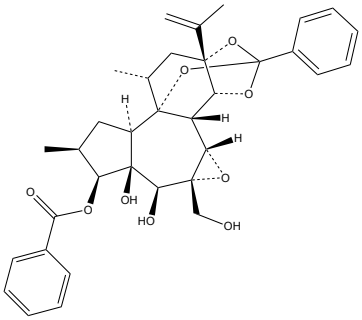
31



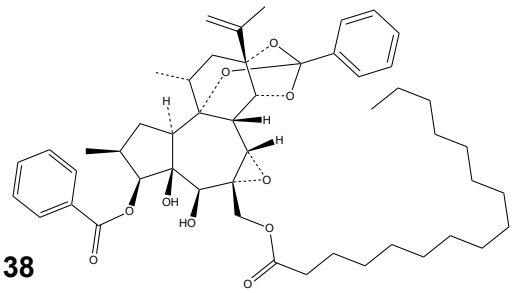
32



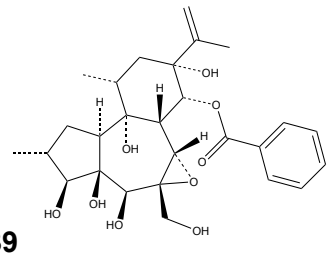
36



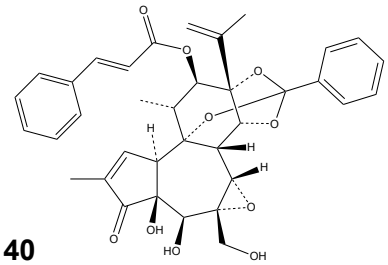
37



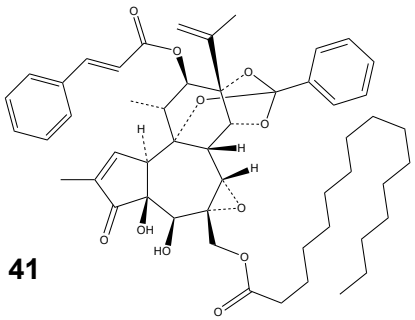
38



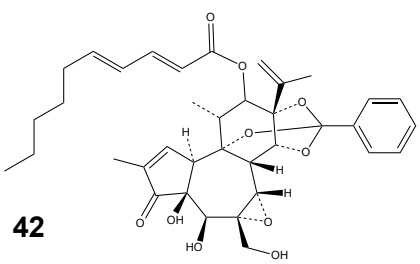
39



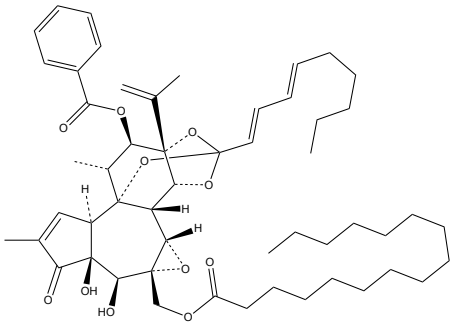
40



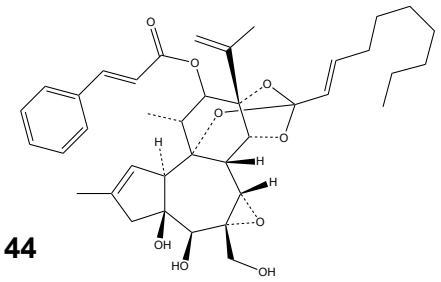
41



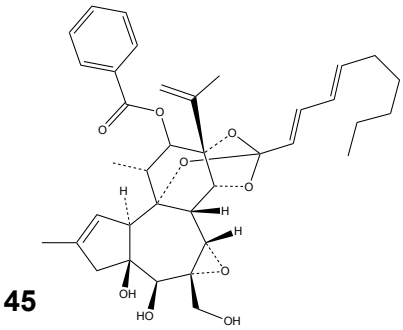
42



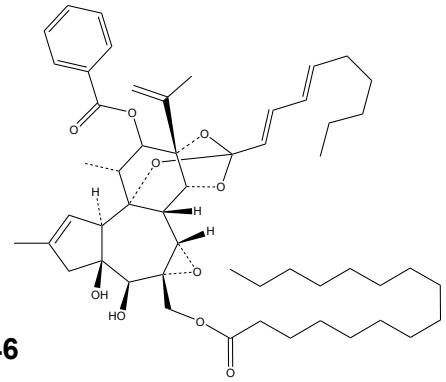
43



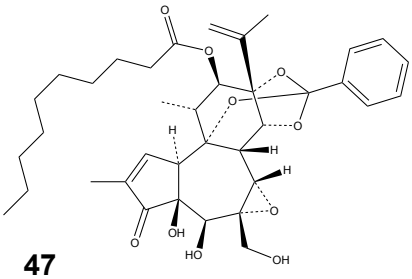
44



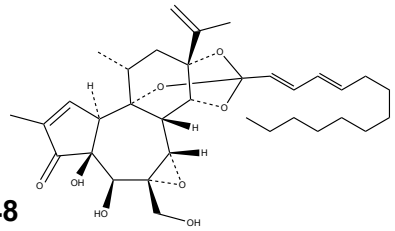
45



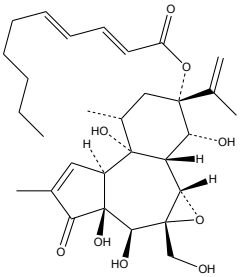
46



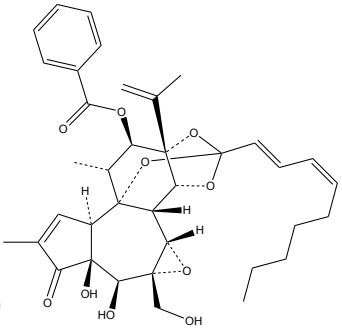
47



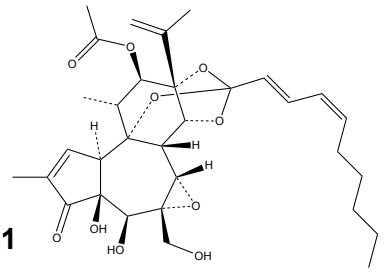
48



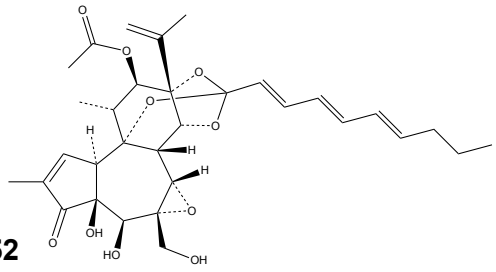
49



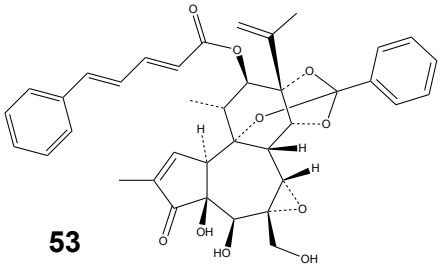
50



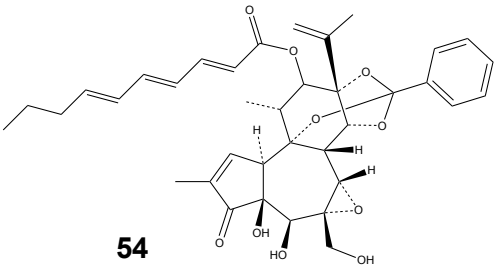
51



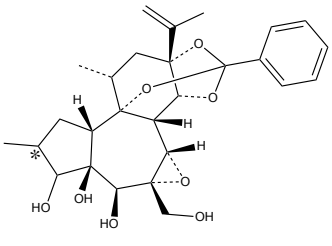
52



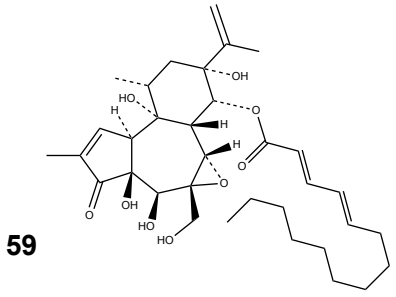
53



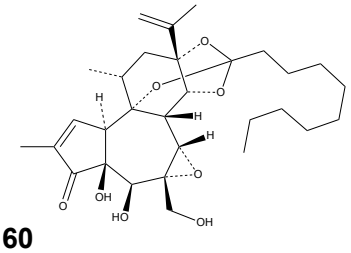
54



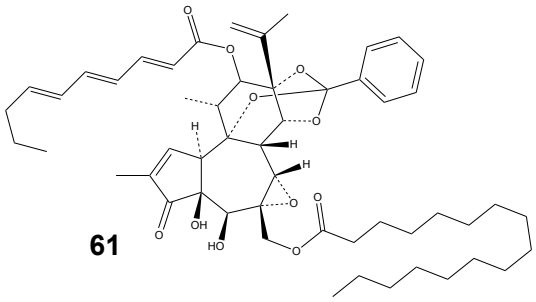
55



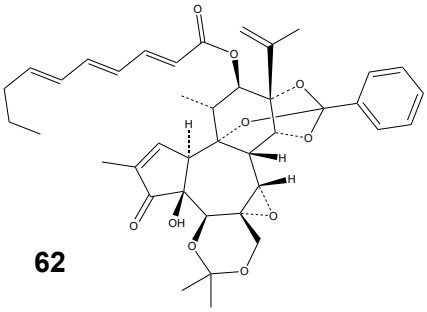
59



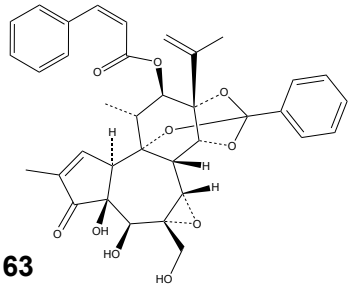
60



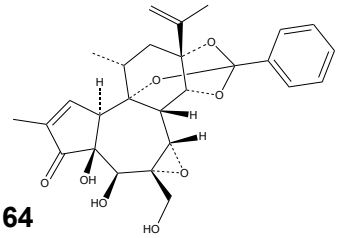
61



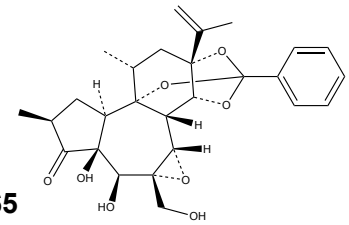
62



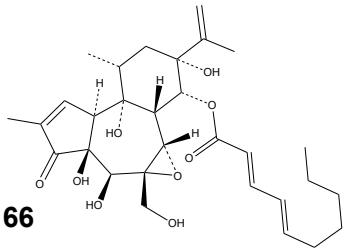
63



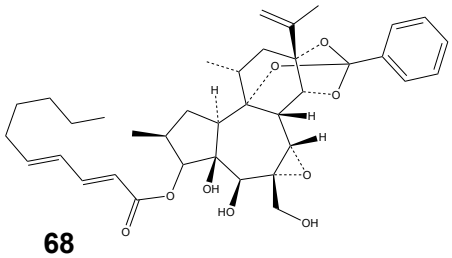
64



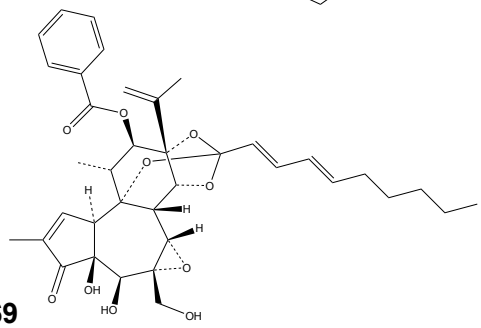
65



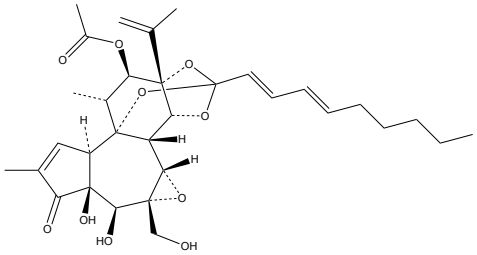
66



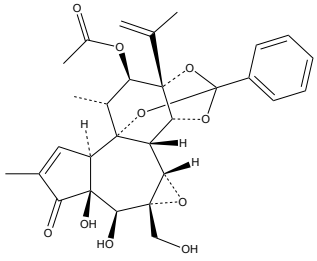
68



69



70



71

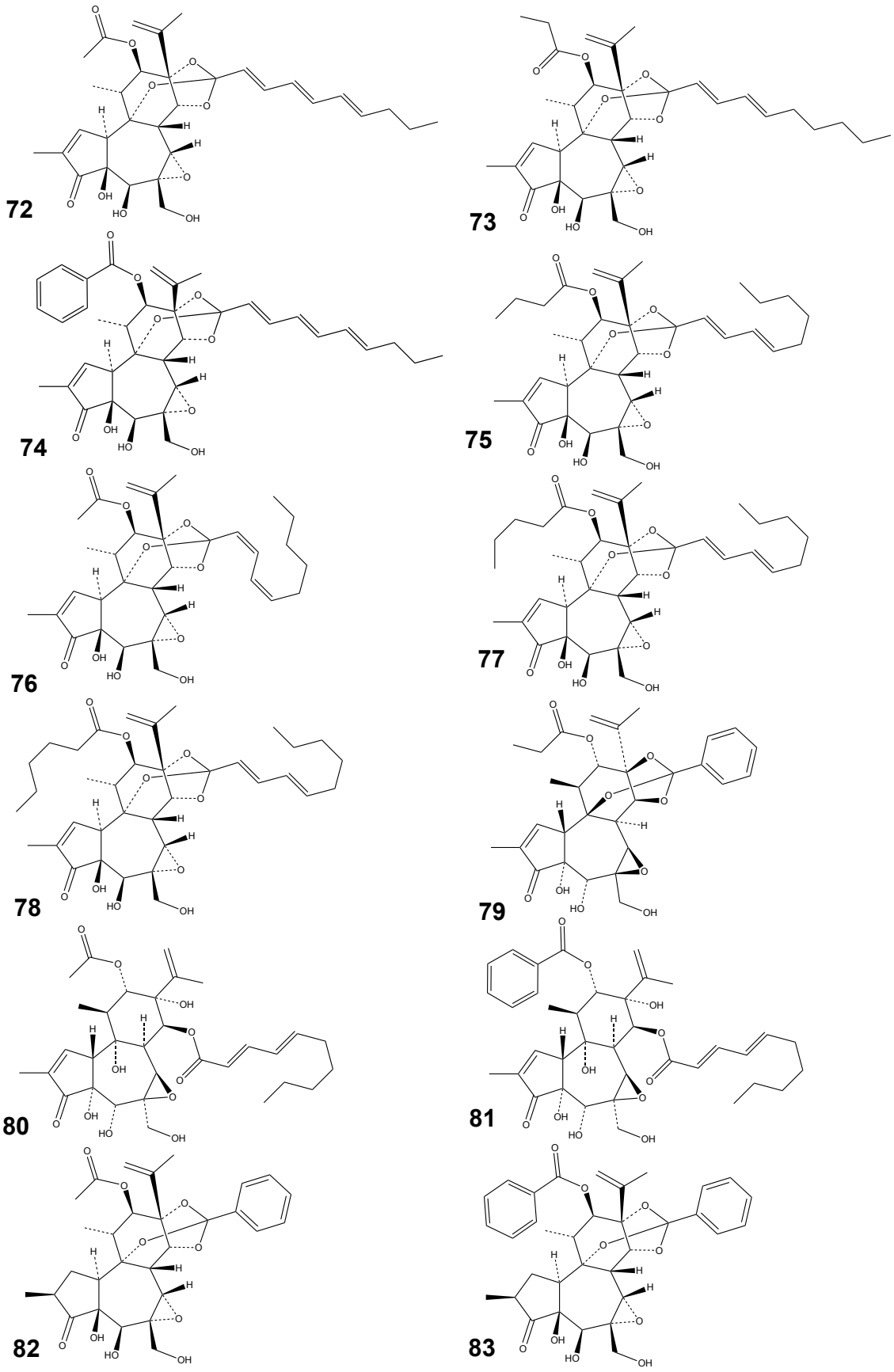
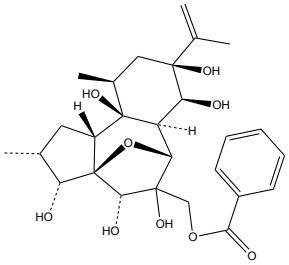
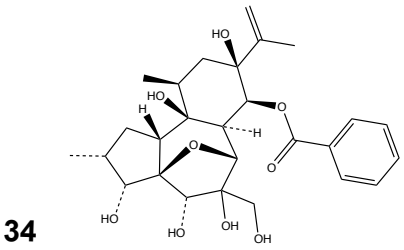


Figure 19: Structures of 6-epoxy daphnanes

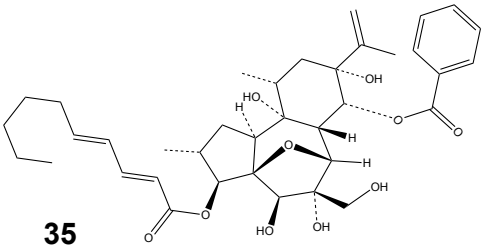
5.2.2.2 Genkwanine–type daphnanes



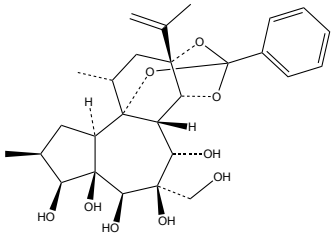
33



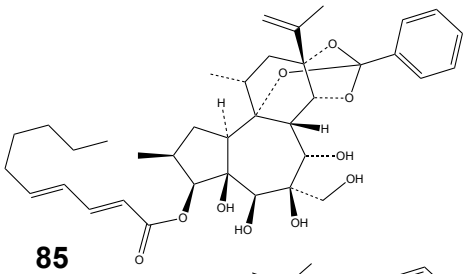
34



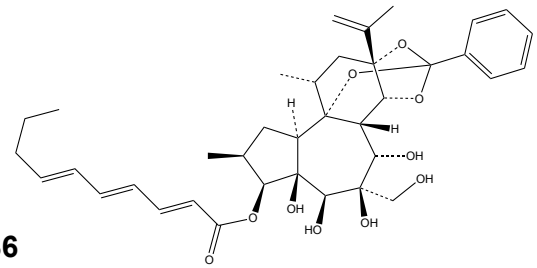
35



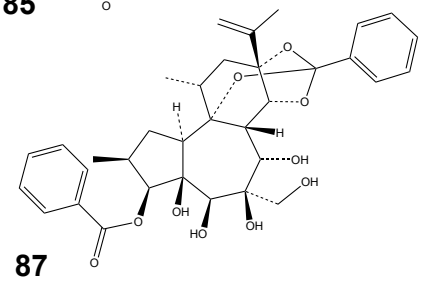
84



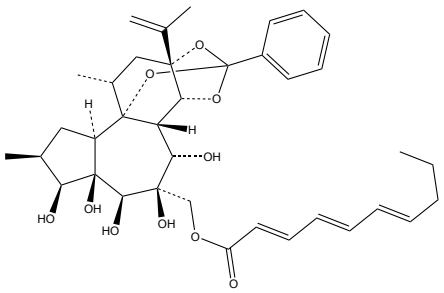
85



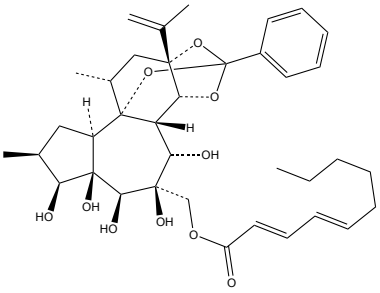
86



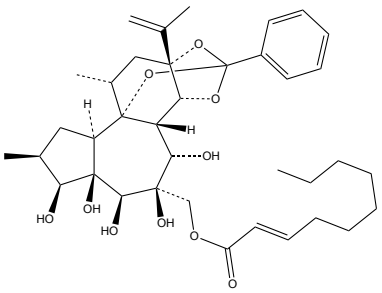
87



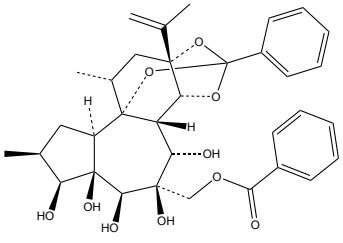
88



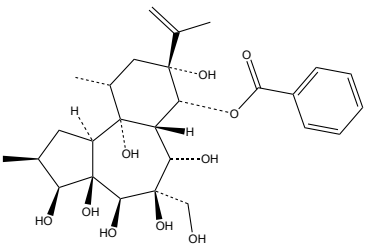
89



90



91



92

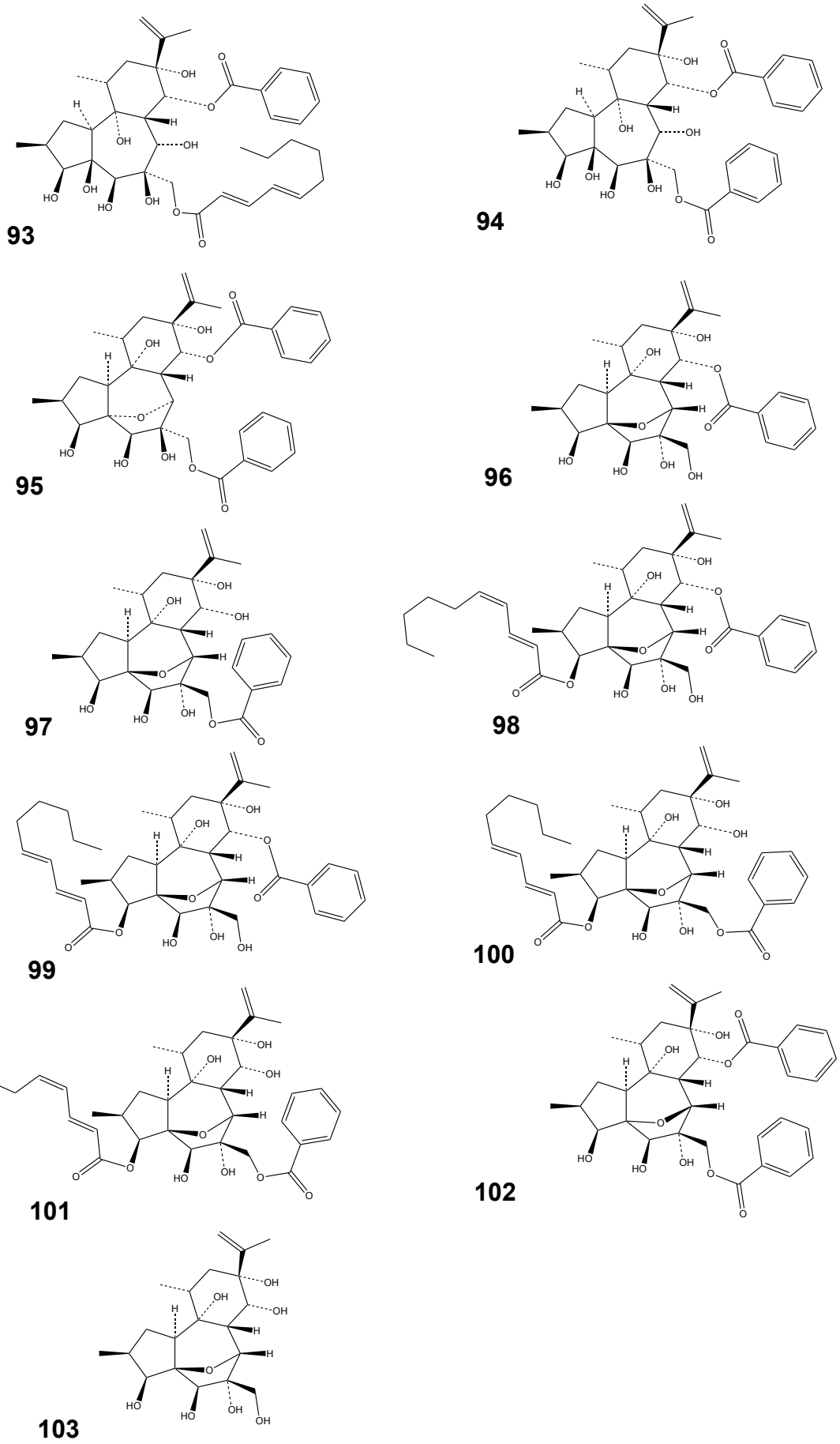


Figure 20: Structures of genkwanine-type daphnanes

5.2.2.3 1-Alkyldaphnanes

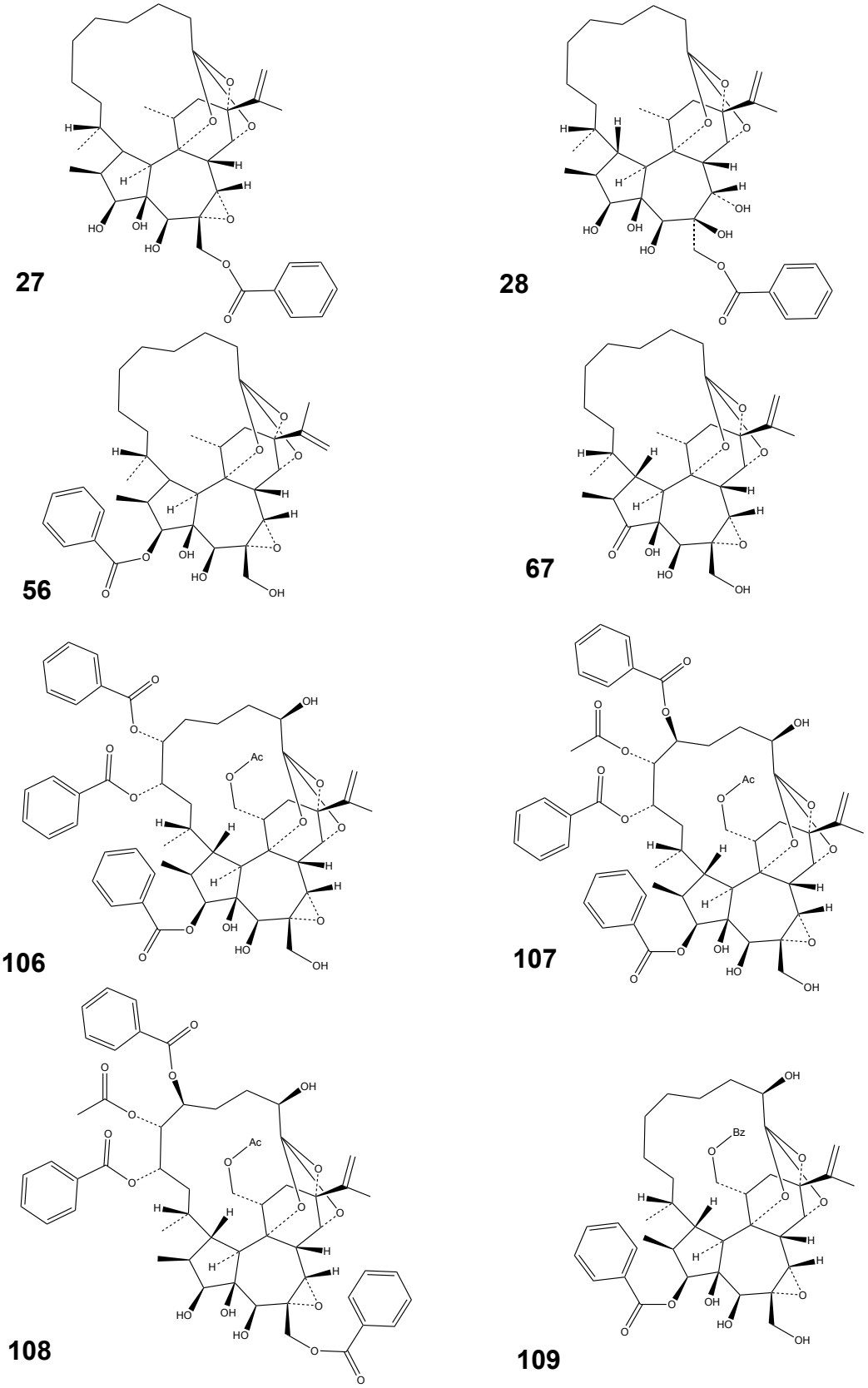


Figure 21: Structures of 1-alkyldaphnanes

5.2.2.4 Resiniferonoid-type daphnanes

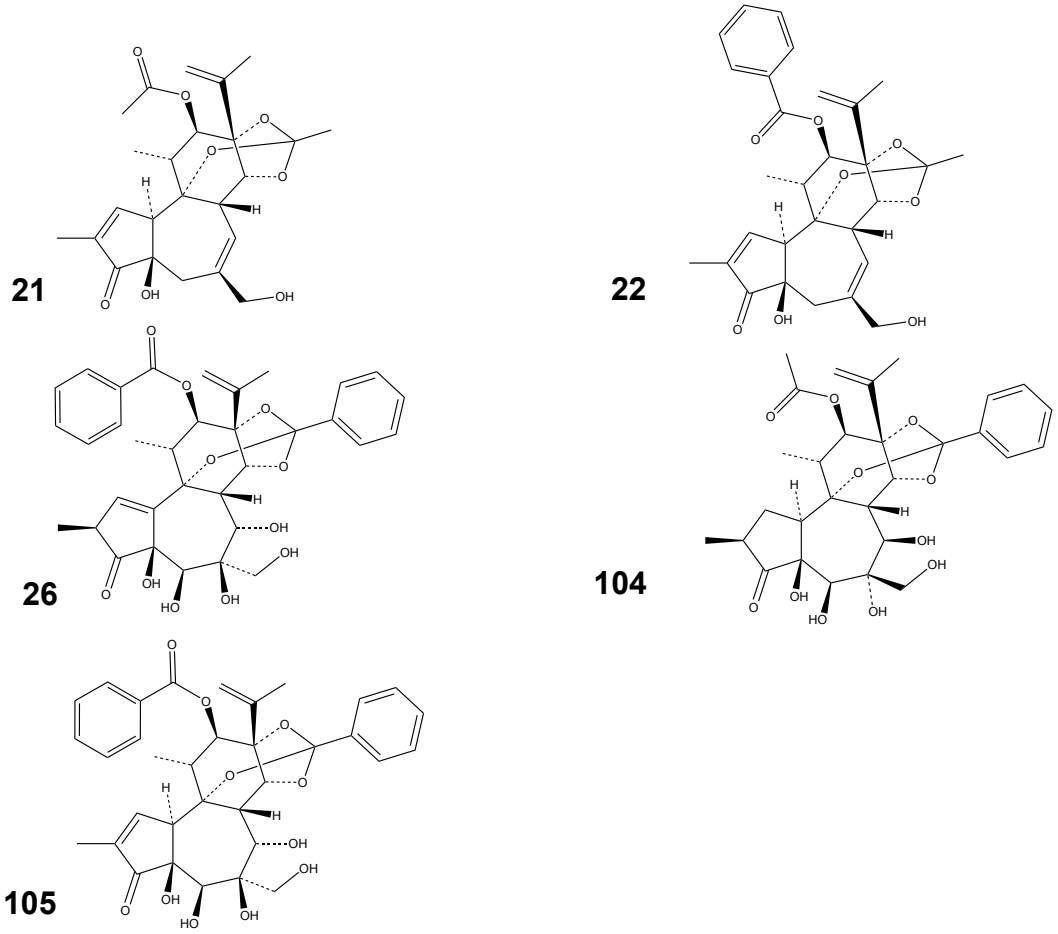


Figure 22: Structures of resiniferonoid-type daphnanes

5.2.2.5 Ketal-lactone-type daphnanes

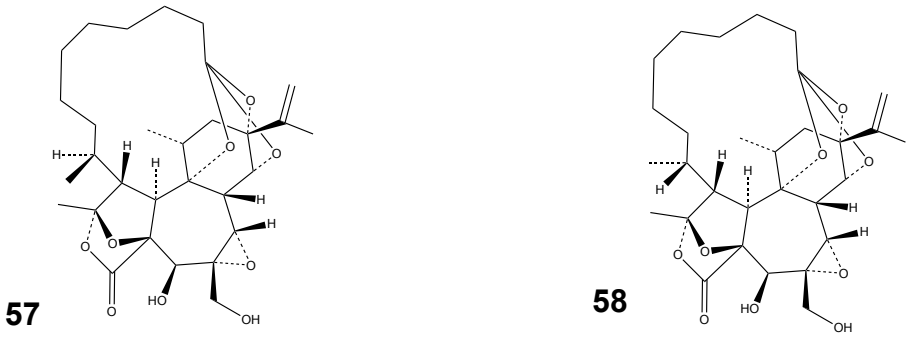


Figure 23: Structures of ketal-lactone-type daphnanes

6 Discussion

6.1 Isolation of daphnane diterpenes from *Daphne feddei*

The analysis of extracts of the stems and twigs of *D. feddei* showed the presence of several diterpenes while the leaves contained only low concentrations of the substances. Because of the weak UV absorption of the compounds, the analyses had to be based therefore mostly on MS-data.

HPLC-MS analysis of the stem extract and fractions thereof showed masses of at least 9 substances which could be tentatively assigned to various daphnane diterpenes. Among them all masses of the daphnane diterpenes previously described in *D. feddei* [39] have been detected. As there are only 4 daphnane diterpenes described up to now in *D. feddei*, new substances which have never been reported in this plant are expected. A substance with *m/z* of 516 is possibly a daphnane diterpene which has never been described before, to the best of our knowledge, in any plant of the *Daphne* genus. It was tentatively assigned to daphnopsis factor R_4 or 24-butylidene-24-deoxy-12-hydroxy-(12β)-crepitol, two substances which have been found in plants of the genera *Daphnopsis* [48] and *Synaptolepis* [49]. Both genera are part of the Thymelaeaceae family.

Due to the emerging Sars-CoV-2 the experimental work had to be stopped before it was possible to purify the substances detected in preparative HPLC fractions 17 and 18 through semipreparative HPLC and analyze them via NMR. Only one enriched fraction (17.3) could be analyzed via NMR, although limited purity complicated the structure elucidation. Nonetheless it was possible to confirm the structure of 1,2-dihydrodaphnetoxin as the main daphnane diterpene in the plant.

These results confirm the potential of this plant for future research on daphnane diterpenes. Especially the main diterpene, 1,2-dihydrodaphnetoxin, is a very promising substance due to its almost absent tumor promoting activity [39] in conjunction with its relative high occurrence in the plant.

6.2 Daphnane diterpenes found in the genus *Daphne*

After a comprehensive literature search, a table including all daphnane diterpenes found in the genus *Daphne* with their respective sources was built. To the best of our knowledge it is the first time that a complete list of all daphnane diterpenes in the genus *Daphne* with their exhaustive natural sources was established.

This list will serve as a base for future research on this very promising class of substances. Due to their complex chemical structures, the synthesis of these compounds is hardly feasible. Therefore, it is essential to precisely know the sources of these substances for targeted isolation.

References

[1] Plants of the world online, Kew Science. *Daphne* Tourn. ex L. Plants of the World Online 2020; Available from: http://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:30024583-2, [accessed 24 July 2020]

[2] Royal Botanic Gardens, Kew and Missouri Botanical Garden. *Daphne*. The plant list 2020; Available from: http://www.theplantlist.org/browse/A/Thymelaeaceae/Daphne/, [accessed 16 June 2020]

[3] Flora of China. *Daphne*. Flora of China 2020; Available from: http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=109294, [accessed 5 June 2020]

[4] Halda, Josef Jakob. Some taxonomic problems in the genus *Daphne* L. Act Mus Richnoviensis 1999; 6: 195–233

[5] Su J, Wu Z. A new daphnane-type diterpenoid from *Daphne giraldii*. Chem Nat Compd 2014; 50: 285–287

[6] Pan L, Zhang XF, Wu HF, *et al*. A new daphnane diterpene from *Daphne tangutica*. Chin Chem Lett 2006; 17: 3

[7] Li LZ, Gao PY, Peng Y, *et al*. A novel daphnane-type diterpene from the flower bud of *Daphne genkwa*. Chem Nat Compd 2010; 46: 380–382

[8] Wink M. Mode of action and toxicology of plant toxins and poisonous plants. Mitt Julius Kühn-Inst 2009; 421: 93–112

[9] Lewis RA. Lewis' dictionary of toxicology. Boca Raton, Fla.: Lewis Publishers, 1996, 382-383

[10] Yang BY. Mechanism of the action of Yuanhuacine to induce labor during mid pregnancy. Chin Med J (Taipei, Taiwan) 1981; 61: 613–616

[11] Chen Y-Y, Guo J-M, Qian Y-F, *et al*. Toxicity of daphnane-type diterpenoids from *genkwa* flos and their pharmacokinetic profile in rat. Phytomedicine 2013; 21: 82–89

[12] Diogo CV, Félix L, Vilela S, *et al*. Mitochondrial toxicity of the phyotochemicals daphnetoxin and daphnoretin – Relevance for possible anti-cancer application. Toxicol *In Vitro* 2009; 23: 772–779

[13] Leone, P. Constitution and synthesis of daphnin. Gazz Chim Ital 1925; 55: 8

[14] Yang Y-Z, Ranz A, Pan H-Z, *et al*. Daphnetin: A novel antimalarial agent with *in vitro* and *in vivo activity*. Am J Trop Med Hyg 1992; 46: 15–20

[15] Ko FN, Chang YL, Kuo YH, *et al*. Daphnoretin, a new protein kinase C activator isolated from *Wikstroemia indica* C.A. Mey. Biochem J 1993; 295: 321–327

[16] Ho W-S, Xue J-Y, Sun SSM, *et al*. Antiviral activity of daphnoretin isolated from *Wikstroemia indica*. Phytother Res 2010; 24: 657–661

[17] Gu S, He J. Daphnoretin induces cell cycle arrest and apoptosis in human osteosarcoma (HOS) cells. Molecules 2012; 17: 598–612

[18] Mansoor F, Anis I, Ali S, *et al*. New dimeric and trimeric coumarin glucosides from *Daphne retusa* Hemsl. Fitoterapia 2013; 88: 19–24

[19] Moshiashvili G, Tabatadze N, Mshvildadze V. The genus *Daphne*: A review of its traditional uses, phytochemistry and pharmacology. Fitoterapia 2020; 143: 104540

[20] Hu L, Zhang Q, Ye Y, *et al*. A new flavonoid from *Daphne feddei* and its anti-HIV-1 Activity. Asian J Chem 2012; 24: 5391–5392

[21] Takai S, Sakaguchi M, Jin D, *et al*. Effects of daphnodorin A, daphnodorin B and daphnodorin C on human chymase-dependent angiotensin II formation. Life Sci 1999; 64: 1889–1896

[22] Wang L, Chen Z-Y, Jiang C-Q, *et al*. Lignans from *Daphne feddei* Levl. and their cytotoxicity. Asian J Chem 2010; 22: 5

[23] Watanabe, Ichiro, Yanai, Tetsuya, Awano, Kenichi, *et al*. Volatile components of zinchoge flower (*Daphne odora* Thunb.). Agric Biol Chem 1983; 47: 483–490

[24] Sovrlić MM, Manojlović NT. Plants from the genus *Daphne*: A review of its traditional uses, phytochemistry, biological and pharmacological activity. Serb J Exp Clin Res 2017; 18: 69–80

[25] Huang S-Z, Li X-N, Ma Q-Y, *et al*. Daphnauranols A–C, new antifeedant sesquiterpenoids with a 5/6/7 ring system from *Daphne aurantiaca*. Tetrahedron Lett 2014; 55: 3693–3696

[26] Huang S-Z, Ma Q-Y, Kong F-D, *et al*. Daphnauranins A and B, two new antifeedants Isolated from *Daphne aurantiaca* roots. Fitoterapia 2017; 122: 11–15

[27] Jin Y-X, Shi L-L, Zhang D-P, *et al*. A review on daphnane-type diterpenoids and their bioactive studies. Molecules 2019; 24: 1842

[28] Hayes PY, Chow S, Somerville MJ, *et al*. Pimelotides A and B, diterpenoid ketallactone orthoesters with an unprecedented skeleton from *Pimelea elongata*. J Nat Prod 2009; 72: 2081–2083

[29] He W, Cik M, Appendino G, *et al*. Daphnane-type diterpene orthoesters and their biological activities. Mini-Rev Med Chem 2002; 2: 185–200

[30] Huang SZ, Zhang XJ, Li XY, *et al*. Daphnane-type diterpene esters with cytotoxic and anti-HIV-1 activities from *Daphne acutiloba* Rehd. Phytochemistry 2012; 75: 99–107

[31] Vidal V, Potterat O, Louvel S, *et al*. Library-based discovery and characterization of daphnane diterpenes as potent and selective HIV inhibitors in *Daphne gnidium*. J Nat Prod 2012; 75: 414–419

[32] Yin Z-Y, Cheng Y-F, Wei J-K, *et al*. Chemical constituents from *Daphne tangutica* and their cytotoxicity against nasopharyngeal carcinoma cells. Fitoterapia 2018; 130: 105–111

[33] Zhang Y, Zhang H, Hua S, *et al*. Identification of two herbal compounds with potential cholesterol-lowering activity. Biochem Pharmacol 2007; 74: 940–947

[34] Han B-S, Kim K-S, Kim YJ, *et al*. Daphnane diterpenes from *Daphne genkwa* activate nurr1 and have a neuroprotective effect in an animal model of parkinson's disease. J Nat Prod 2016; 79: 1604–1609

[35] Zhao H-D, Lu Y, Yan M, *et al*. Rapid recognition and targeted isolation of anti-HIV daphnane diterpenes from *Daphne genkwa* guided by UPLC-MS. J Nat Prod 2020; 83: 134–141

[36] Ronlán A, Wickberg B. The structure of mezerein, a major toxic principle of *daphne mezereum* L. Tetrahedron Lett 1970; 11: 4261–4264

[37] Saraiva L, Fresco P, Pinto E, *et al*. Differential activation by daphnetoxin and mezerein of PKC-isotypes α, βI, δ and ζ. Planta Med 2001; 67: 787–790

[38] Flora of China. Daphne feddei. Flora of China 2020; Available from: http://www.efloras.org/florataxon.aspx?flora_id=2&taxon_id=242316855, [accessed 28 April 2020]

[39] Dagang W, Sorg B, Adolf W, *et al*. Oligo- and macrocyclic diterpenes in Thymelaeaceae and Euphorbiaceae occurring and utilized in Yunnan (Southwest China) 1. Daphnane type diterpene esters from *Daphne feddei*. Phytother Res 1991; 5: 163–168

[40] Liang S, Shen Y-H, Tian J-M, *et al*. Three new dicoumarins from *Daphne feddei*. Helv Chim Acta 2009; 92: 133–138

[41] Liang S, Shen Y-H, Tian J-M, *et al*. Phenylpropanoids from *Daphne feddei* and their inhibitory activities against NO production. J Nat Prod 2008; 71: 1902–1905

[42] Lu Y, Li X, Mu H, *et al*. Bioactive phenylpropanoids from *Daphne feddei*. J Braz Chem Soc 2012; 23: 656–660

[43] Hu Q-F, Mu H-X, Huang H-T, *et al*. Secolignans, neolignans and phenylpropanoids from *Daphne feddei* and their biological activities. Chem Pharm Bull 2011; 59: 1421–1424

[44] Liang S, Tang J, Shen Y-H, *et al*. Biflavonoids from *Daphne feddei* and their inhibitory activities against nitric oxide production. Chem Pharm Bull 2008; 56: 1729–1731

[45] Liang S, Xiong Z, Tian J, *et al*. Flavones from *Daphne feddei*. Chem Nat Compd 2011; 47: 816–817

[46] Liang S, Feng Y, Tian J-M, *et al*. Coumarins from *Daphne feddei* and their potential anti-inflammatory activities. J Asian Nat Prod Res 2011; 13: 1074–1080

[47] Zhang X. Wikstroelide M potently inhibits HIV replication by targeting reverse transcriptase and integrase nuclear translocation. Chin J Nat Med 2014; 12: 186–193

[48] Adolf W, Hecker E. On the active principles of the Thymelaeaceae-skin irritant and cocarcinogenic diterpenoid factors from *Daphnopsis racemosa*. Planta Med 1982; 45: 177–182

[49] Adolf W, Seip EH, Hecker E, *et al*. Irritant principles of the mezereon family (Thymelaeaceae), V. New skin irritants and tumor promoters of the daphnane and 1a-alkyldaphnane type from *Synaptolepis kirkii* and *Synaptolepis retusa*. J Nat Prod 1988; 51: 662–674

[50] Xu W-C, Shen J-G, Jiang J-Q. Phytochemical and biological studies of the plants from the genus *Daphne*. Chem Biodivers 2011; 8: 1215–1233

[51] Pan L, Zhang XF, Deng Y, *et al*. Chemical constituents investigation of *Daphne tangutica*. Fitoterapia 2010; 81: 38–41

[52] Wang C. Studies on the bioactive diterpenes from Daphne giraldii. Acta Chim Sin (Chin Ed) 1987; 45: 993–996

[53] Akhtar K, Khan SB, Ali I. Structural determination of diterpenes from *Daphne genkwa* by NMR spectroscopy. Magn Reson Chem 2006; 44: 1063–1066

[54] Nugroho AE, Chin-Piow W, Hirasawa Y, *et al*. Daphnane diterpenoids from *Daphne altaica*. Nat Prod Commun 2016; 11: 1934578X1601100

[55] Zhiheng H, Yiming Y, Manli D, *et al*. Studies on the mass spectra of diterpene orthoesters isolated from Thymelaeaceae species. Acta Chim Sin (Engl Ed) 1985; 43: 529

[56] Liao S-G, Zhang B-L, Wu Y, *et al*. New phenolic components from *Daphne giraldii*. Helv Chim Acta 2005; 88: 2873–2878

[57] Stout GH, Balkenhol WJ, Poling Mitchell, *et al*. Isolation and structure of daphnetoxin, the poisonous principle of *Daphne* species. J Am Chem Soc 1970; 92: 1070–1071

[58] Taninaka H, Takaishi Y, Honda G, *et al*. Terpenoids and aromatic compounds from *Daphne oleoides* ssp. *oleoides*. Phytochemistry 1999; 52: 1525–1529

[59] Hall IH, Liou Y-F, Oswald CB, *et al*. The effects of genkwadaphnin and gnidilatidin on the growth of P-388, L-1210 leukemia and KB carcinoma cells *in vitro*. Eur J Cancer Clin Oncol 1986; 22: 45–52

[60] Liang S, Shen Y-H, Feng Y, *et al*. Terpenoids from *Daphne aurantiaca* and their potential anti-inflammatory activity. J Nat Prod 2010; 73: 532–535

[61] Yeşilada E, Taninaka H, Takaishi Y, *et al*. *In vitro* inhibitory effects of *Daphne oleoides* ssp. *oleoides* on inflammatory cytokines and activity-guided isolation of active constituents. Cytokine 2001; 13: 359–364

[62] Zhan Z-J, Fan C-Q, Ding J, *et al*. Novel diterpenoids with potent inhibitory activity against endothelium cell HMEC and cytotoxic activities from a well-known TCM plant *Daphne genkwa*. Bioorg Med Chem 2005; 13: 645–655

[63] Jiang H-L, Wang R, Li J-Y, *et al*. A new highly oxygenated daphnane diterpene esters from the flower buds of *Daphne genkwa*. Nat Prod Res 2015; 29: 1878–1883

[64] Riaz M, Saleem A, Siddique S, *et al*. Phytochemistry of *Daphne oleoides*. Nat Prod Res 2016; 30: 880–897

[65] Li L-Z, Peng Y, Wang L-H, *et al*. Daphnane-type diterpenoids from the flower buds of *Daphne genkwa*. Helv Chim Acta 2010; 93: 1172–1179

[66] Zhou G-X, Yang Y-C, Shi J-G. Study of chemical constituents in stem rind of *Daphne giraldii*. China J Chin Mater Med 2006; 31: 555–557

[67] Kupchan SM, Sweeny JG, Baxter RL, *et al*. Tumor inhibitors. 102. Gnididin, gniditrin, gnidicin, novel potent antileukemic diterpenoid esters from *Gnidia lamprantha*. J Am Chem Soc 1975; 97: 672–673

[68] Shigemori H, Nakasone R, Kurisu M, *et al*. Promoting effects on hepatocyte growth factor production of daphnane diterpenoids from *Daphne odora*. Heterocycles 2013; 87: 1087

[69] Yazdanparast R, Sadeghi H. Nucleic acid synthesis in cancerous cells under the effect of gnidilatimonoein from *Daphne mucronata*. Life Sci 2004; 74: 1869–1876

[70] Hedayati M, Yazdanparast R, Yeganeh MZ, *et al*. A new diterpene extracted from *Daphne mucronata*. Effects on human K562 and CCRF-CEM cell lines. J Cancer Ther 2011; 02: 71–75

[71] Görick C, Melzig MF. Gniditrin is the main diterpenoid constituent in the bark of *Daphne mezereum*. Pharmazie 2013; 68: 640–642

[72] Dagang W, Sorg B, Adolf W, *et al*. Oligo- and macrocyclic diterpenes in Thymelaeaceae and Euphorbiaceae occurring and utilized in Yunnan 5. Daphnane type diterpene 9,13,14-orthoesters from roots of *Daphne papyracea*. Phytother Res 1993; 7: 197–199

[73] Ohigashi H, Hirota M, Ohtsuka T, *et al*. Resiniferonol-related diterpene esters from *Daphne odora* Thunb. and their ornithine decarboxylase-inducing activity in mouse skin. Agric Biol Chem 1982; 46: 2605–2608

[74] Kupchan, SM, Baxter, R. Mezerein: antileukemic principle isolated from *Daphne mezereum*. Science 1975; 187: 652–653

[75] Huang S-Z, Ma Q-Y, Liu Y-Q, *et al*. Chemical constituents from *Daphne acutiloba*. China J Chin Mater Med 2013; 38: 64–69

[76] Li F, Sun Q, Hong L, *et al*. Daphnane-type diterpenes with inhibitory activities against human cancer cell lines from *Daphne genkwa*. Bioorg Med Chem Lett 2013; 23: 2500–2504

[77] Ma Q-Y, Chen Y-C, Huang S-Z, *et al*. Chemical constituents from the stems of *Daphne holosericea* (Diels) Hamaya. Chem Biodiversity 2016; 13: 1469–1474

[78] Chengrui W, Qinglain W, Bainian L, *et al*. Studies on the bioactive diterpenes isolated from *Daphne tangutica* I. Isolation and structure of an antifertility active diterpene, tanguticacine. Acta Chim Sin (Engl Ed) 1987; 45: 982

[79] Kizaibek M, Pferschy-Wenzig E, Kretschmer N, *et al*. LC-MS-based phytochemical characterization of an antiproliferative *Daphne altaica* stem bark extract. Planta Med 2015; 81: s-0035-1565491

[80] Minh NV, Han B-S, Choi H-Y, *et al*. Genkwalathins A and B, new lathyrane-type diterpenes from *Daphne genkwa*. Nat Prod Res 2018; 32: 1782–1790

[81] Han B-S, Minh NV, Choi H-Y, *et al*. Daphnane and phorbol diterpenes, anti-neuroinflammatory compounds with nurrl activation from the roots and stems of *Daphne genkwa*. Biol Pharm Bull 2017; 40: 2205–2211

[82] Kogiso S, Wada K, Munakata K. Odoracin, a nematicidal constituent from *Daphne odora*. Agric Biol Chem 1976; 40: 2119–2120

[83] Zhang R, Wang Y, Li J, *et al*. The chinese herb isolate yuanhuacine (YHL-14) induces G2/M arrest in human cancer cells by up-regulating p21 protein expression through an p53 protein-independent cascade. J Biol Chem 2014; 289: 6394–6403

[84] Kang JI, Hong J-Y, Lee H-J, *et al*. Anti-tumor activity of yuanhuacine by regulating AMPK/mTOR signaling pathway and actin cytoskeleton organization in non-small cell lung cancer cells. PLoS ONE 2015; 10: 1-17

[85] Zhang DC, Cao CX, Zhang CJ, *et al*. Yuanhuacin A is a selective antagonist of phorbol ester receptor in protein kinase C. Sci China, Ser B: Chem, Life Sci, Earth Sci 1993; 36: 803–808

[86] Zhang S, Li X, Zhang F, *et al*. Preparation of yuanhuacine and relative *Daphne* diterpene esters from *Daphne genkwa* and structure–activity relationship of potent inhibitory activity against DNA topoisomerase I. Bioorg Med Chem 2006; 14: 3888–3895

[87] Zeng Y-M, Zuo W-J, Sun M-G, *et al*. Three new *Daphne* diterpenes from the buds of *Daphne genkwa* Sieb. et Zucc. processed by rice vinegar. Helv Chim Acta 2009; 92: 1273–1281

[88] Gu Z, Wang, C, *et al*. Study on the antifertility activity of daphnane diterpenoid orthoester isolated from four species of Thymelaeaceae in China. Reprod Contracept 1989; 9: 48-52

[89] Shaojiang S, Ying P, Lingzhi L, *et al*. New daphnane diterpene compounds in *Daphne genkwa* as well as preparation method and application of same. Available from: https://worldwide.espacenet.com/publicationDetails/biblio?FT=D&date=20090916&DB=EPODOC&locale=en_EP&CC=CN&NR=101531644A&KC=A&ND=4, [accessed 24 July 2020]

[90] Bang, K-K, Yun C-Y, *et al*. Melanogenesis inhibitory daphnane diterpenoids from the flower buds of *Daphne genkwa*. Bioorg Med Chem Lett; 2013; 23: 3334-3337

[91] Li S, Chou G, Hseu Y, *et al*. Isolation of anticancer constituents from flos genkwa (*Daphne genkwa* Sieb.et Zucc.) through bioassay-guided procedures. Chem Cent J 2013; 7: 159

[92] Li L-Z, Song S-J, Gao P-Y, *et al*. Neogenkwanines A–H: Daphnane-type diterpenes containing 4,7 or 4,6-ether groups from the flower bud of *Daphne genkwa*. RSC Adv 2014; 5: 4143–4152

[93] Otsuki K, Li W, Asada Y, *et al*. Daphneodorins A–C, anti-HIV gnidimacrin related macrocyclic daphnane orthoesters from *Daphne odora*. Org Lett 2020; 22: 11–15

Acknowledgment

I would like to express my gratitude to Prof. Dr. Olivier Potterat for giving me the possibility to carry out my diploma thesis in Basel and for the excellent collaboration and supervision. Further I want to thank Ao. Univ. Prof. Mag. pharm. Dr. Gottfried Reznicek for helping me to organize my semester abroad. My thanks go also to the whole team of the laboratory of the Pharmaceutical Biology at the University of Basel for the great work atmosphere and for answering all my questions and helping me with any kind of problems.

Furthermore, I want to thank my parents for their continuous support in any situation, for giving me the opportunity to study and especially for being my firm basis wherever I can return.

I want to thank my girlfriend Mäggi for being always with me and especially for brighting up every day also in this time where we both wrote our theses and gave us mutual support and made the best out of it.

Appendix

ESI-Mass spectrum of Fraction 17.3

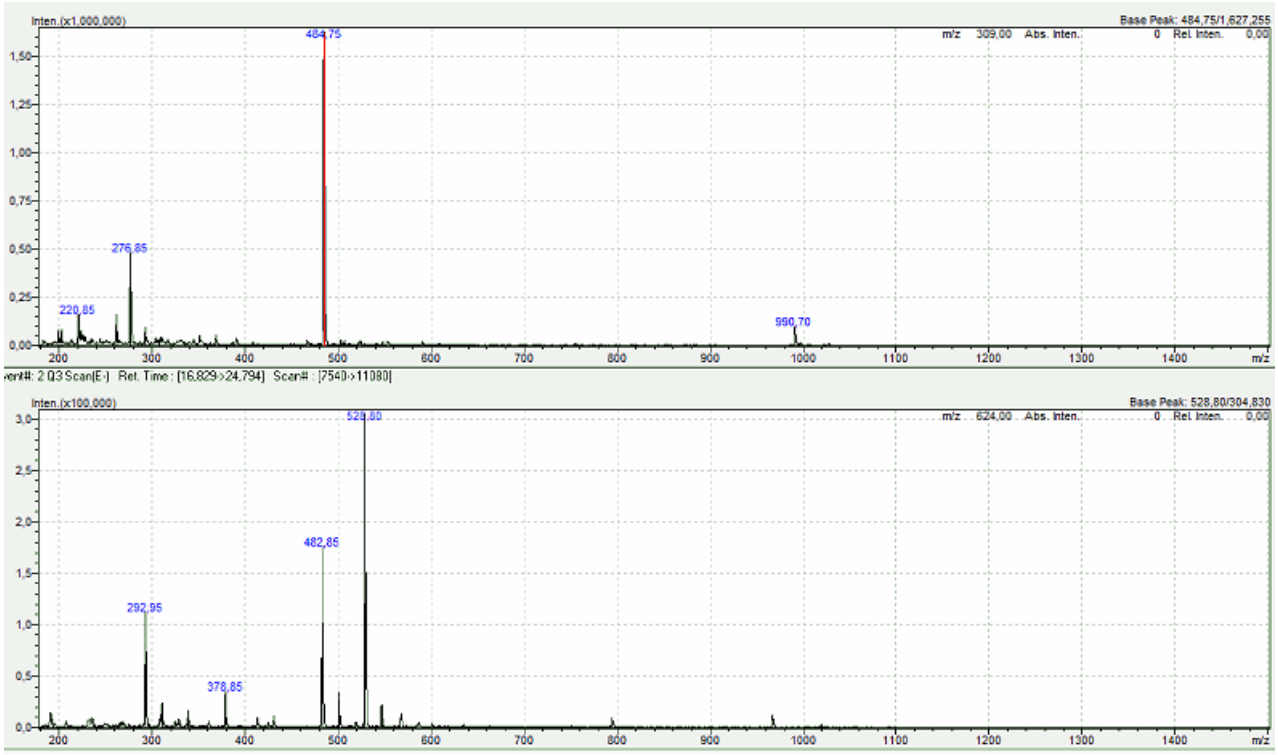


Figure 24: ESI Mass spectrum of Fraction 17.3

^{1}H NMR spectrum of Fraction 17.3

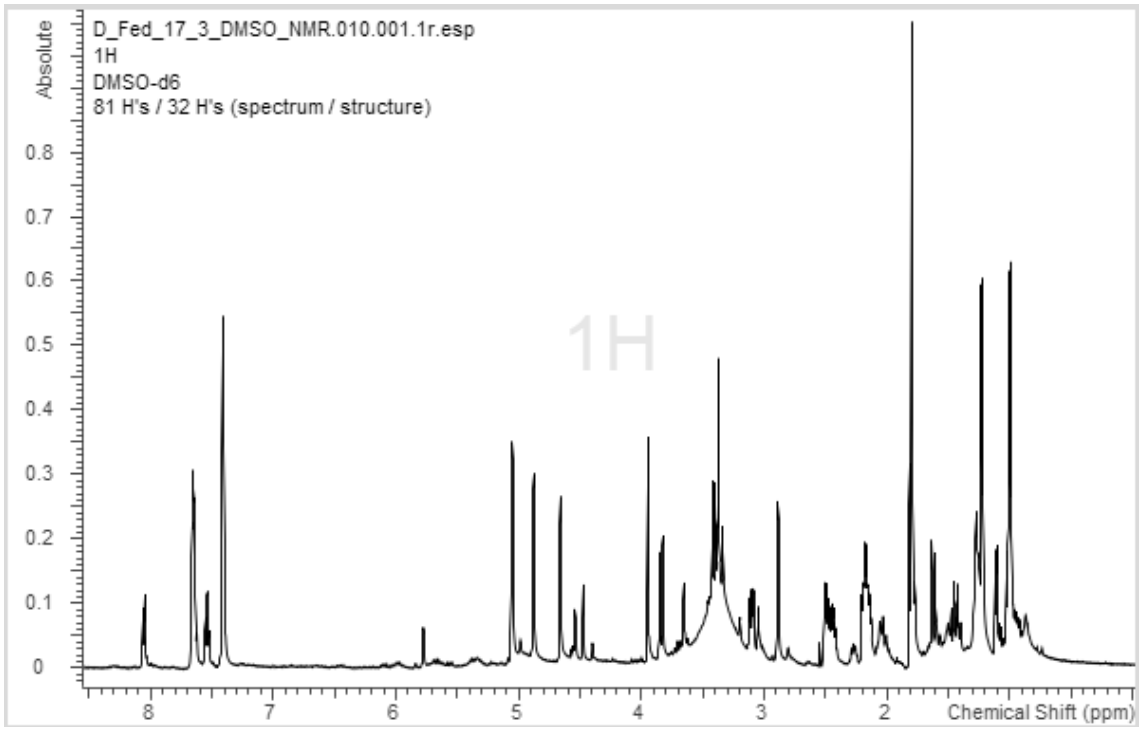


Figure 25: ^{1}H NMR Spectrum of Fraction 17.3 in DMSO-d6

Table of daphnane diterpenes sorted by source

Table 24: Daphnane diterpenes sorted by plant name

Source	No	Compound	MW	
Daphne acutiloba	**7**	14'-Ethyltetrahydrohuratoxin	616.83	[30]
	8	Acutilobin A	662.27	[30]
	9	Acutilobin B	662.28	[30]
	10	Acutilobin C	718.29	[30]
	11	Acutilobin D	720.80	[30]
	12	Acutilobin E	674.69	[30]
	13	Acutilobin F	634.76	[30]
	14	Acutilobin G	616.70	[30]
	23	Daphnetoxin	482.52	[30]
	24	Excoecaria factor O_1 Peddiea factor V1	526.62	[30]
	25	Excoecariatoxin	528.63	[30]
	40	Gnidicin	628.66	[30]
	42	Gnididin	648.74	[30]
	47	Gniditrin Daphne factor P_1	646.72	[30]
	52	Kirkinine	672.85	[30]
	59	Prohuratoxin Daphne factor F3 Wikstroelide M	602.76	[75]
	66	Vesiculosin	546.65	[75]
	68	Wikstroemia factor M1	636.77	[30]
	74	Yuanhuajine Isouanhuajine	646.72	[30]
	95	Genkwanine VIII	608.25	[30]
Daphne alpina	**47**	Gniditrin Daphne factor P_1	646.72	[71]
Daphne altaica	**15**	Altadaphnan A	630.28	[54]
	16	Altadaphnan B	648.28	[54]
	17	Altadaphnan C	646.27	[54]
	23	Daphnetoxin	482.52	[54]
	25	Excoecariatoxin	528.63	[54]
	30	Genkwadaphnin Daphne factor F_2	602.63	[54]
	40	Gnidicin	628.66	[54]
	59	Prohuratoxin Daphne factor F3 Wikstroelide M	602.76	[54]
	65	Tanguticakin 1,2-Dihydrodaphnetoxin Daphne factor F_4	484.54	[54]
	66	Vesiculosin	546.65	[54]
Daphne aurantiaca	**30**	Genkwadaphnin Daphne factor F_2	602.63	[60]
	47	Gniditrin Daphne factor P_1	646.72	[60]

Source	No	Compound	MW	
	83	Yuanhuatine	604.64	[60]
Daphne feddei	**30**	Genkwadaphnin Daphne factor F_2	602.63	[39]
	48	Huratoxin Daphne factor F1 Hippomane factor M1	584.74	[39]
	59	Prohuratoxin Daphne factor F3 Wikstroelide M	602.76	[72]
Daphne genkwa	**6**	12β-Acetoxy-5β-hydroxyresinif-eronol-9,13,14-triyl-ortho[(2E)-dec-2-enoate]	588.29	[53]
	21	Daphneresiniferin A	446.49	[90]
	22	Daphneresiniferin B	508.56	[90]
	25	Excoecariatoxin	528.63	[35]
	26	Genkwadane A	620.64	[76]
	27	Genkwadane B	638.79	[76]
	28	Genkwadane C	656.80	[76]
	29	Genkwadane D	614.72	[35]
	30	Genkwadaphnin Daphne factor F_2	602.63	[59]
	31	Genkwadaphnin-20-palmitate	840.04	[58]
	32	Genkwanin I^2	512.19	[7]
	33	Genkwanin III	504.57	[89]
	34	Genkwanin IV	504.57	[89]
	35	Genkwanin V	522.58	[89]
	36	Genkwanine M Genkwanine VI	590.66	[62]
	37	Genkwanine N Genkwanine VII	590.66	[34]
	38	Genkwanine N-20-palmitate	828.07	[63]
	39	Genkwanine O Genkwanine II	504.57	[63]
	43	Gnidilatidin-20-palmitate	886.15	[63]
	50	Isoyuanhuacine Yuanhuacine B	648.74	[35]
	51	Isoyuanhuadine	586.67	[35]
	55	Orthobenzoate 2	486.23	[7]
	56	Pimelea factor P_2 Daphnopsis factor R1 Gnilatimacrin Linifolin B Linimacrin B	638.79	[76]
	57	Pimelotide A	546.65	[76]
	58	Pimelotide C	546.65	[76]
	60	Simplexin Daphnopsis factor R3 Pimelea factor P1 Wikstrotoxin D	532.67	[76]

Source	No	Compound	MW	
	65	Tanguticakin 1,2-Dihydrodaphnetoxin Daphne factor F_4	484.54	[80]
	67	Wikstroelide E Pimelea factor S_6	532.67	[76]
	68	Wikstroemia factor M1	636.77	[81]
	69	Yuanhuacine Gnidilatidin Odoracin	648.74	[81], [59]
	70	Yuanhuadine	586.67	[81], [86]
	71	Yuanhuafine	540.56	[53]
	72	Yuanhuagine Kirkinine D Peddiea factor V_2	584.65	[86]
	73	Yuanhuahine	600.70	[81]
	74	Yuanhuajine Isouanhuajine	646.72	[86]
	75	Yuanhualine	614.72	[35]
	76	Yuanhuamine A	586.67	[35]
	77	Yuanhuamine B	628.75	[35]
	78	Yuanhuamine C	642.78	[35]
	79	Yuanhuaoate A	554.59	[87]
	80	Yuanhuaoate C	590.66	[87]
	81	Yuanhuaoate E	666.75	[35]
	82	Yuanhuapine	542.57	[86]
	83	Yuanhuatine	604.64	[81]
	84	Genkwanine A	504.57	[90]
	85	Genkwanine B	654.79	[62]
	86	Genkwanine C	652.77	[62]
	87	Genkwanine D	608.68	[62]
	88	Genkwanine E	652.77	[62]
	89	Genkwanine F	654.79	[90]
	90	Genkwanine G	656.80	[62]
	91	Genkwanine H	608.68	[90]
	92	Genkwanine I^2	522.58	[80]
	93	Genkwanine J	672.80	[35]
	94	Genkwanine K	626.69	[62]
	95	Genkwanine VIII	608.25	[91]
	96	Neogenkwanine A	504.57	[92]
	97	Neogenkwanine B Genkwanine Q	504.57	[92]
	98	Neogenkwanine C	654.79	[92]
	99	Neogenkwanine D	654.79	[92]
	100	Neogenkwanine E Genkwanine T	654.79	[92]
	101	Neogenkwanine F Genkwanine U	654.79	[92]
	102	Neogenkwanine G	608.68	[92]

Source	No	Compound	MW	
	103	Neogenkwanine H Genkwanine R	504.57	[92]
	104	Genkwanine L	560.59	[53]
	105	Yuanhuaoate B	620.64	[87]
Daphne giraldii	**18**	Daphnegiraldifin	720.93	[52]
	19	Daphnegiraldigin	500.54	[5]
	20	Daphnegiraldin 12-Lauroyloxydaphnetoxin	680.83	[55]
	23	Daphnetoxin	482.52	[56]
	40	Gnidicin	628.66	[66]
	47	Gniditrin Daphne factor P_1	646.72	[66]
	65	Tanguticakin 1,2-Dihydrodaphnetoxin Daphne factor F_4	484.54	[66]
Daphne gnidium		Daphnetoxin	482.52	[31]
	25	Excoecariatoxin	528.63	[31]
	40	Gnidicin	628.66	[31]
	47	Gniditrin Daphne factor P_1	646.72	[31]
Daphne holosericea	**60**	Simplexin Daphnopsis factor R3 Pimelea factor P1 Wikstrotoxin D	532.67	[77]
Daphne mezereum	**5**	12–Hydroxydaphnetoxin	498.52	[36]
	23	Daphnetoxin	482.52	[57]
	47	Gniditrin Daphne factor P_1	646.72	[71]
	53	Mezerein	654.70	[36]
Daphne mucronata	**44**	Gnidilatimonoein	662.81	[70]
Daphne odora	**43**	Gnidilatidin -20-palmitate	886.15	[68]
	47	Gniditrin Daphne factor P_1	646.72	[73]
	54	Odoratrin Daphne factor P2	648.74	[68]
	69	Yuanhuacine Gnidilatidin Odoracin	648.74	[68]
	70	Yuanhuadine	586.67	[68]
	74	Yuanhuajine Isouanhuajine	646.72	[68]
	106	Daphneodorin A	952.38	[93]
	107	Daphneodorin B	1010.3	[93]
	108	Daphneodorin C	1010.6	[93]
	109	Gnidimacrin Ligustrinin B	774.89	[93]
Daphne oleoides ssp. ***oleoides***	**30**	Genkwadaphnin Daphne factor F_2	602.63	[58]
	31	Genkwadaphnin-20-palmitate	840.04	[58]

Source	No	Compound	MW	
	38	Genkwanine N-20-palmitate	828.07	[64]
	41	Gnidicin-20-palmitate	866.07	[58]
	43	Gnidilatidin-20-palmitate	886.15	[58]
	45	Gnidilatin	652.77	[61]
	46	Gnidilatin-20-palmitate	890.18	[61]
	65	Tanguticakin 1,2-Dihydrodaphnetoxin Daphne factor F_4	484.54	[58]
	69	Yuanhuacine Gnidilatidin Odoracin	648.74	[58]
Daphne papyracea	**47**	Gniditrin Daphne factor P_1	646.72	[72]
	54	Odoratrin Daphne factor P2	648.74	[72]
Daphne tangutica	**1**	1,2-Dihydrodaphnegiraldifin[1] 1,2α-dihydro-20-palmitoyl-daphnetoxin	722.93	[50], [6]
	3	1,2α-Dihydro-5β-hydroxy-6α,7α-epoxyresiniferonol-14-benzoate	512,21	[51]
	4	1,2β-Dihydro-5β-hydroxy-6α,7α-epoxyresiniferonol-14-benzoate	512.21	[51]
	23	Daphnetoxin	482.52	[32]
	25	Excoecariatoxin	528.63	[32]
	40	Gnidicin	628.66	[51]
	47	Gniditrin Daphne factor P_1	646.72	[32]
	49	Isovesiculosin	546.65	[51]
	61	Tanguticacin	884.50	[78]
	62	Tanguticadin[1]	686.79	[50]
	63	Tanguticafin[1] Thymeleatoxin A	628.66	[50]
	64	Tanguticahin[1] 15,16-dihydrodaphnetoxin	484.54	[50]
	65	Tanguticakin[1] 1,2-Dihydrodaphnetoxin Daphne factor F_4	484.54	[50]
	66	Vesiculosin	546.65	[51]
	69	Yuanhuacine Gnidilatidin Odoracin	648.74	[32]
	74	Yuanhuajine Isouanhuajine	646.72	[32]

[1] Only abstract of original source accessible: [88]
[2] $C_{28}H_{32}O_9$: Possibly genkwanine "1" and genkwanine "i"