# STRUCTURE ELUCIDATION OF NATURAL DITERPENES BY NMR SPECTROSCOPY

PhD Dissertation

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# PUBLICATIONS RELATED TO THE DISSERTATION

## Papers

I. J. Hohmann, **F. Evanics**, A. Vasas, Gy. Dombi, Gy. Jerkovich, I. Máthé, A novel lathyrane diterpenoid from the roots of *Euphorbia lathyris*, *J. Nat. Prod.*, **62**, 176, (1999) 1.641

II. J. Hohmann, **F. Evanics**, L. Berta, T. Bartók, Diterpenoids from *Euphorbia peplus*, *Planta Medica*, **66**, 291, (2000) 1.322

III. J. Hohmann, **F. Evanics**, Gy. Dombi, J. Molnár, P. Szabó, Euphosalicin, a new diterpene polyester with multidrug resistance reversing activity from *Euphorbia salicilfolia*, *Tetrahedron*, **57**, 211, (2001) 2.160

IV. J. Hohmann, **F. Evanics**, Gy. Dombi, P. Szabo, Salicifoline and salicinolide, new diterpene polyesters from Euphorbia salicifolia, *Tetrahedron Letters*, **42**, 6581, (2001) 2.617

V. **F. Evanics**, J. Hohmann, D. Rédei, A. Vasas, L. Berta, Gy. Dombi, Új diterpén poliészterek Magyarországi *Euphorbia* fajokból, *Acta Pharmaceutica Hungarica*, **71** (3), 2001

## Presentations on conferences

I. J. Hohmann, A. Vasas, G. Günther, **F. Evanics**, I. Máthé, Gy. Dombi, Isolation and Structure Elucidation of Macrocyclic Diterpene Polyesters from *Euphorbia esula*, *XXI International Symposium on Macrocyclic Chemistry*, Montecatini Terme, Italy, 1996

II. J. Hohmann, A. Vasas, G. Günther, **F. Evanics**, I. Máthé, Gy. Dombi, Macrocyclic Diterpene Esters of the Jatrophane Type from *Euphorbia esula*, *44nd Annual Congress on Medicinal Plant Research*, Praha, Czech Republic, 1996

III. **F. Evanics**, J. Hohmann, D. Rédei, Gy. Dombi, Structure Elucidation of Diterpene Polyesters from *Euphorbia* Species Using NMR Spectroscopy, *Central European NMR Symposium*, Szeged, Hungary, 1999

IV. J. Hohmann, D. Rédei, **F. Evanics**, J. Molnár, I. Máthé, Biologically Active Diterpene Polyesters from Hungarian *Euphorbia* species, *2000 Years of Natural Products Research - Past, Present and Future*, Amsterdam, The Netherlands, 1999

V. J. Hohmann, D. Rédei, I. Máthé, J. Molnár, I. Mucsi, **F. Evanics**, Gy. Dombi, Diterpene Polyesters with Antiviral Activity from *Euphorbia peplus* and *E. serrulata*, 3rd International Congress on Phytomedicine, Munich, Germany, 2000

VI. J. Hohmann, D. Rédei, I. Máthé, J. Molnár, I. Mucsi, **F. Evanics**, Gy. Dombi, Diterpene Polyesters with Antiviral Activity from *Euphorbia peplus* and *E. serrulata*, *Phytomedicine*, **7**, Supplement II, 85, (2000) (Abstracts from 3rd International Congress on Phytomedicine, Munich, Germany, 2000) 0.281

VII. D. Rédei, J. Hohmann, **F. Evanics**, Gy. Dombi, J. Molnár, K. Wolfard, I. Máthé, New diterpene oligoesters from *Euphorbia salicifolia* and their multidrug resistance reversing activity, *Natural Products Research in the New Millenium, International Congress and 48th Annual Meeting of the Society for Medical Plant Research (GA)*, Zürich, Switzerland, 2000

VIII. A. Vasas, J. Hohmann, I. Máthé, G. Günther, **F. Evanics**, Gy. Dombi, Jatrofánvázas diterpén poliészterek izolálása az *Euphorbia esula*-ból, *Congressus Pharmaceuticus Hungaricus X*, Budapest, Hungary, 1996.

IX. J. Hohmann, A. Vasas, I. Máthé, **F. Evanics**, G. Günther, Új diterpének izolálása magyarországi *Euphorbia* fajokból, *Az MTA NMR Munkabizottságának, az MTA Terpenoidkémiai és Elemorganikus Munkabizottságának és az MKE NMR Szakcsoportjának közös előadói ülése*, Budakalász, Hungary, 1997.

X. J. Hohmann, A. Vasas, G. Günther, **F. Evanics**, G. Blazsó, I. Máthé, A hazai flóra Euphorbia fajainak növénykémiai vizsgálata, diterpének izolálása, szerkezetfelderítése és farmakológiai értékelése, *A Magyar Tudomány Napja Szegeden*, Szeged, Hungary, 1998.

XI. J. Hohmann, G. Günther, **F. Evanics**, D. Rédei, I. Máthé, Magyarországi Euphorbia fajok növénykémiai vizsgálata, *Congressus Pharmaceuticus Hungaricus XI*, Siófok, Hungary, 1999.

XII. J. Hohmann, D. Rédei, **F. Evanics**, I. Máthé, Gy. Dombi, Új jatrofánvázas diterpenoidok izolálása az *Euphorbia serrulata*-ból, *MTA Terpenoidkémiai és Elemorganikus Munkabizottságának Előadóülése*, Budapest, Hungary, 1999.

# 1. INTRODUCTION

## 1.1. General introduction

This PhD dissertation deals with the structure elucidation of natural, mostly biologically active diterpene molecules, isolated from *Euphorbia* (spurge) species found in Hungary.[1]

Species of Euphorbiaceae family occur worldwide and in general they are containing skin-irritant, toxic milky latex. These plants are the source of biologically active compounds with various effects, e.g. irritant, tumor-promoting, cytotoxic, antitumor, antibacterial, HIV-1 transcriptase-inhibiting activities and also with various vascular effects[2-7]. Various diterpene type toxins, mostly based on tigliane, ingenane and daphnane skeleton, the so called "phorboids", were identified and found to be responsible for the tumor-promoting, irritant effects. In recent years, due to their high chemical diversity and therapeutically relevant bioactivity, considerable attention has also been paid to the macrocyclic diterpenes. These investigations on *Euphorbias* have resulted in the discovery of several different classes of diterpenes[8-12],such as casbane, jatrophane, lathyrane, jatropholane, crotofolane, rhamnofolane, tigliane, ingenane and daphnane type diterpenes[13].

The research group lead by Dr Judit Hohmann has reported many new diterpene polyesters, extracted from Hungarian *Euphorbia* species[14,15]. The dissertation covers the structure elucidation of compounds, extracted from three members of this plant family, such as *E. lathyris* L., *E. salicifolia* Host., and *E. peplus* L.

The overall goal of this work was to determine the active compounds of the species growing in Hungary of these plant family, and to find new, pharmacologically active compounds.

## 1.2. Overview of the investigated plants

### 1.2.1. *Euphorbia lathyris* (caper spurge)

*E. lathyris* is widely known throughout the Mediterranean region of Eastern and Middle Europe.[16] Biological studies have demonstrated the proimflammatory effect of the roots, seeds,

aerial parts and milky latex and an antitumor effect of the seeds.[17-19] A series of diterpenes based on the lathyrane skeleton have been isolated from the seeds.[20-24] The roots of caper spurge have been less well investigated, only the isolation of a lathyrane diterpenoid was reported.[19]

## 1.2.2. *Euphorbia peplus*

*E. peplus* is one of the plants in the *Euphorbiaceae* family found to have many biological activities. That's why it has been the target of many pharmacological and phytochemical studies. These investigations were resulted the isolation of irritant ingenane diterpenes.[25-28] Recently, a series of jatrophane diterpenoids and tetracyclic diterpenes based on the pepluane skeleton have also been reported from *E. peplus*.[28-30]

## 1.2.3. *Euphorbia salicifolia*

*E. salicifolia* is a perennial herb distributed throughout Central and South-East Europe.[31] The diterpene constituents of the plant has not been reported yet.

# 2. EXPERIMENTAL

## 2.1. Experimental conditions

NMR spectra were recorded on BRUKER AVANCE DRX 400 and 500 spectrometers. The hydrogen and carbon resonance frequencies were 400.13 MHz, 100.6 MHz and 500.13 MHz and 125.76 MHz respectively. The probeheads were 5 mm inverse Z-gradient devices in both spectrometers. The temperature was usually 300 K except on that cases, when the flexibility of the molecule causes line broadening and the spectrum was hard to interpret. When this occurred, the experiments were carried out on elevated temperature where the intramolecular movements, like conformational changes, become much faster. The sample was solved in perdeuterated solvents, such as chloroform-d, benzene-$d_6$ or pyridine-$d_5$. The solution was filtered and filled in a 5 mm OD NMR tube. The samples were degassed via bubbling with oxygen free, high purity nitrogen gas and ultrasonic stirring.

## 2.2. NMR methods

### 2.2.1. $^1$H spectrum

The $^1$H spectra were acquired as a single-pulse experiment, using Brukers standard zg30 pulse program.

Many important information are recoverable from this simple experiment. The number of hydrogens in the given molecule is determinable via integration of the signals. Very useful knowledge may yielded by the chemical shift and multiplicity analysis about the chemical environment of hydrogens and the geometry of the molecule.

## 2.2.2. The J-modulated spin-echo (JMOD) experiment

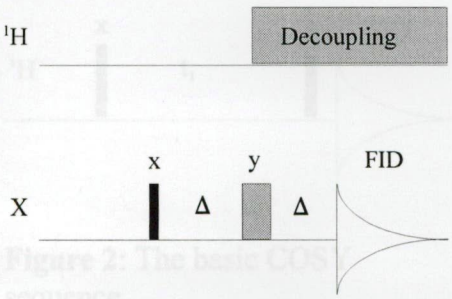



**Figure 1**: Pulse sequence of JMOD experiment

JMOD experiments[32,33] were used to determine the $^{13}C$ chemical shifts. This has the features both of $^{1}H$ coupled and $^{1}H$ decoupled basic $^{13}C$ spectra, while it has the signal enhancements caused by Nuclear Overhauser Effect (NOE, see on page 12) and we can take difference between C, CH, $CH_2$ and $CH_3$ centres on the basis of spectrum editing with spin-echo. This editing makes phases of carbon signals to positive or negative, depending on the odd (1 or 3) or even (0 or 2) number of protons attached to the given carbon atom. The experiment is therefore sometimes called Attached Proton Test (APT)[34]. The pulse sequence of this experiment is shown on **Figure 1**. The X and Y pulses in the sequence are 90° and 180° respectively. The Δ time delays should be optimized for the $^{1}J_{CH}$ coupling. It is obvious that, because of the difference between the coupling constants in a real sample, the Δ delays could not be ideal for all the $^{1}H$-$^{13}C$ couplings. This will cause some intensity loss via insufficient refocusing on signals with extreme couplings. JMOD experiment was preferred against the INEPT[35,36] (Insensitive Nuclei Enhanced by Polarisation Transfer) and DEPT[37] (Distortionless Enhancement by Polarisation Transfer) because these spectra do not show the carbons which are not attached with protons directly. However, it should be noted that the sensitivity of DEPT and INEPT experiments is higher, because of the polarisation transfer.

The information about the number of carbon atoms, the number of directly attached hydrogens and of course about the chemical environment of the carbons can be explored using this spectrum.

## 2.2.3. Homonuclear Correlation Spectroscopy ($^1$H - $^1$H COSY)

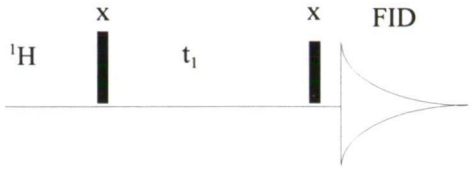



**Figure 2**: The basic COSY sequence

Since Jeener presented the principles of generating two-dimensional spectrum[38] many two- and three-dimensional experiments were developed. The first of these powerful methods was the COSY[39], which is widely in use nowadays. One can see the pulse sequence of the original COSY experiment on **Figure 2**, where $t_1$ is the evolution time. During the last decades, many pulse sequences were published which give us information about *J*-coupled protons[40-42]. A pulsed field gradient selected version of COSY was used, because that is easy to setup, quick and produces the desired information about the *J*-coupled proton pairs. The pulse sequence is described on **Figure 3**. The two gradient pulses are for selecting the desired coherence pathway. The first gradient pulse will dephase all the coherences via inhomogenous phase shifts, than the second one will rephase only the selected coherences. The coherence pathway selection depends on the ratio of gradient pulses. In the case of relatively strong gradient pulses the suppression of undesired coherence pathways is satisfactory. The experiment can be very fast, because of the lack of phase-cycling.

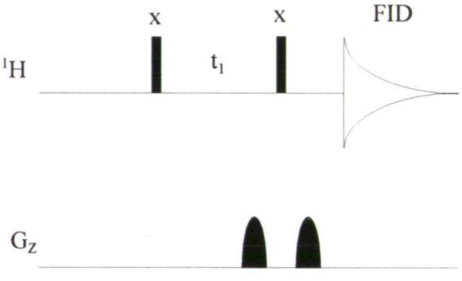



**Figure 3**: Pulse sequence of PFG COSY experiment

Analysing the cross peaks of the COSY spectra, proton pairs can be found, which are *J*-coupled to each other. Using this information, it is possible to determine the members of the spin systems of the molecule step by step. The spin-system is a chain of scalar or spin-spin coupled protons, or other nuclei.

## 2.2.4. Total Correlation Spectroscopy (TOCSY)

When the COSY spectrum is difficult to interpret, the TOCSY[43], which is sometimes described as HOHAHA[44,45] (Homonuclear Hartmann-Hahn spectroscopy), can help in structure determination. (These two experiments differs only in technical details in the sequences, but essentially they are identical.) With the aid of this spectrum type, one can assign the spin-systems of the molecule, which has strongly overlapped signals in $^1H$ and COSY spectra. The pulse sequence is shown on **Figure 4**. After $t_1$ evolution time, we can apply a mixing pulse or a mixing sequence, so called spin-lock or isotropic mixing sequence. The spin-lock in the simplest form may be a single low-power pulse, but in the practice, it is usually one of the many suitable pulse sequences, like MLEV-17[45], MLEV-16, WALTZ-16[46,47] and DIPSI-2.[48,49] The spin-lock will fix the magnetization along the y axis, precessing around it at a $B_1$ dependent frequency. During this mixing time, the coupled spins will precess with the same frequency, so they are strongly coupled that time. Because of this strong coupling, they become labelled with each others frequencies, so we can see crosspeaks between all the spins, which are members of the same spin system.

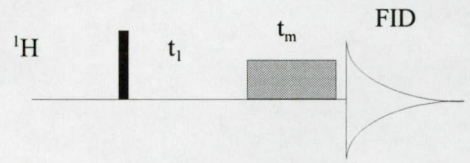



**Figure 4**: Pulse sequence of TOCSY experiment

## 2.2.5. Heteronuclear Multiple-Quantum Correlation (HMQC)

Gradient-selected HMQC[50,51] experiment was used for mapping the connectivities between the directly attached $^1H$ and $^{13}C$ nuclei. This robust and quick method was described many years ago,[52,53] but it was lack widespread use before presenting a scheme[54] that was able to overcome the technical difficulties, related to avoiding the high intensity F1 noise lines. The used method was a magnitude mode experiment, so it had difficulties with small mass samples and highly overlapped proton or carbon signals because of the pure resolution and sensitivity. The sequence scheme is depicted on **Figure 5**. The $G_1$ - $G_3$ gradient pulses are for selecting the

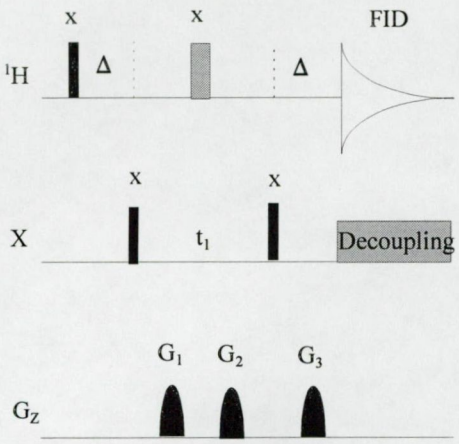



**Figure 5**: Pulse sequence of HMQC experiment

the desired coherences, thus suppressing the noise, arising from non-$^{13}$C bound hydrogens. The Δ values should be optimized for the $^1J_{CH}$ coupling.

## 2.2.6. Heteronuclear Single-Quantum Correlation (HSQC)

To override the HMQC's disadvantages, gradient-selected HSQC experiments were applied. The HSQC spectra give the same single bond $^1$H-$^{13}$C correlations as HMQC, but it has higher sensitivity and resolution, so it is much more effective on dilute samples with overlapped signals. Since the original scheme[55] was published, many papers were dealing with advantages and further development of this experiment.[56,51] The usage and optimising of gradient pulses for selecting HSQC signals (and also for HMQC) were discussed many times, too.[57-61] The **Figure 6** shows the pulse sequence of gradient-selected, phase-sensitive HSQC using the echo-antiecho approach. The echo-antiecho method is used to refocus the magnetization during the long pulse sequence to avoid the intensity loss and suppress the evolution of

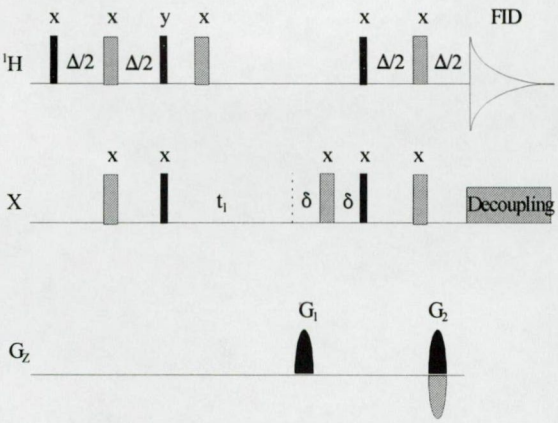



**Figure 6**: Pulse sequence of gradient-selected, phase-sensitive HSQC

homonuclear couplings. The gradient pulses are for suppressing the non-$^{13}C$ bounded hydrogen signals and selecting the desired coherence pathways.

## 2.2.7. Heteronuclear Multiple-Bond Correlation Spectroscopy (HMBC)

To determine the long-range coupling (normally two and three bond) between $^{1}H$ and $^{13}C$ the HMBC[62,63] spectroscopy was devoted. With the aid of this spectrum we can connect the spin-systems separated by quaternary carbons or heteroatoms. The original sequence had some disadvantages, such as phase distortions of crosspeaks, caused by the evolution of proton-proton couplings, during the long (~100 ms) evolution time, and the difficulties in suppression of the parent $^{1}H$-$^{12}C$ signals causing $t_1$-noise. One can mask the phase errors using absolute-value presentation of the spectra, but the effective suppression of $t_1$ noise bands is possible since the introduction of pulsed field gradients.[64,59] You can see the pulse sequence of the gradient-selected HMBC on **Figure 7**

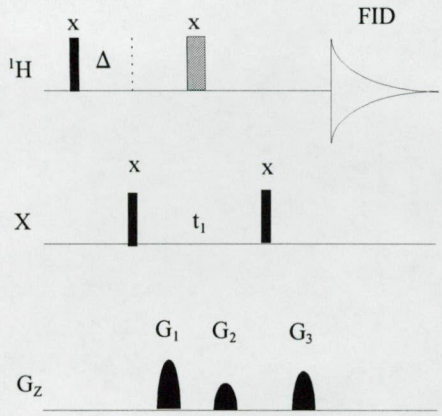



**Figure 7**: Pulse sequence of gradient-selected HMBC experiment

## 2.2.8. The Nuclear Overhauser Spectroscopy (NOESY)

If one has the constitution of the molecule and want to know the configuration and conformation of it, the NOESY[65] (see the pulse sequence on **Figure 8**) is the most powerful method in the NMR spectroscopy. Many times it is more adequate as the widely used X-ray diffraction (XRD), especially when the target is a flexible large molecule in solution. The nuclear Overhauser effect (NOE) is the intensity change

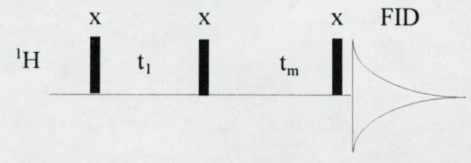



**Figure 8**: Pulse sequence of NOESY experiment

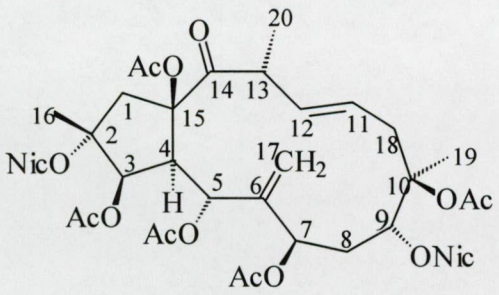
on signals, caused by the transfer of magnetization between the dipolar-coupled spins. This magnetization transfer, called nuclear cross-relaxation, is induced by motional processes[66-70].

The NOE intensity (the integrated volume of the crosspeak ($V_{AB}$)) depends on the distance of the interacting nuclei ($r_{AB}$), on the correlation time ($\tau_c$) and the mixing time ($\tau_m$) as given by the equation: $V_{AB} \propto \tau_c \tau_m r_{AB}^{-6}$. To measure the distance on the simplest way, in case of a small molecule with isotropic correlation time, you have to chose a reference nuclei pair (X and Y) with well known interatomic distance ($r_{XY}$). Then one have to determine the volume of the reference ($V_{XY}$), and the interested ($V_{AB}$) NOESY crosspeaks. These data allows to calculate the distance between the interested atom pair with the following equation:[71]

$$r_{AB}^{-6} = r_{XY}^{-6}[V_{AB}/V_{XY}]$$

# 3. RESULTS AND DISCUSSION

During the work presented in this dissertation the structure of 13 natural compounds were determined. All the investigated compounds are diterpene polyesters isolated from Hungarian *Euphorbia* species. Twelve of the described molecules were formerly unknown. In this section a detailed description of the structure elucidation method will be given, applied on the compound **1**, as a representative of new structures.




Ac = Acetyl Nic = Nicotinoyl

**1**

## 3.1. NMR spectra and their interpretation

### 3.1.1. $^1$H spectrum

The $^1$H spectrum of **1**, acquired in $CDCl_3$ at 300 K, shows many broad, unresolved lines (**Figure 9**). This fact suggests that the molecule must contain flexible parts. One has two possibilities to make the spectrum more interpretable: change the solvent or apply higher temperature. In this case both of them were applied, because in $CDCl_3$ solutions you can not elevate the temperature, because of the low boiling point of it. Using perdeuterated benzene ($C_6D_6$) at 340 K temperature, the spectrum became much better defined as shown on **Figure 10**. From the proton spectrum five acetate groups (1.78, 1.78, 1.48, 1.62 and 1.69 ppm) and two nicotinoate residues are easy to identify. Additionally, three methyl signals, one doublet (1.24 ppm) and two singlets (1.57 and 1.59 ppm) are recognizable.

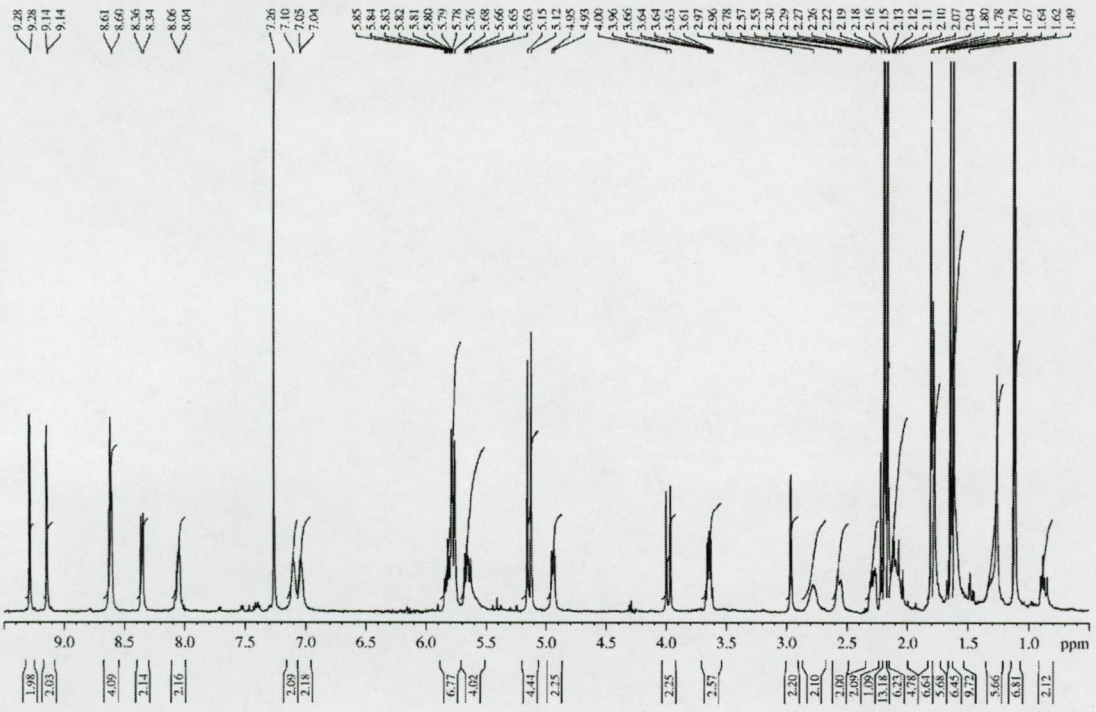


**Figure 9** $^{1}H$ spectrum of **1** at 300 K in $CDCl_3$

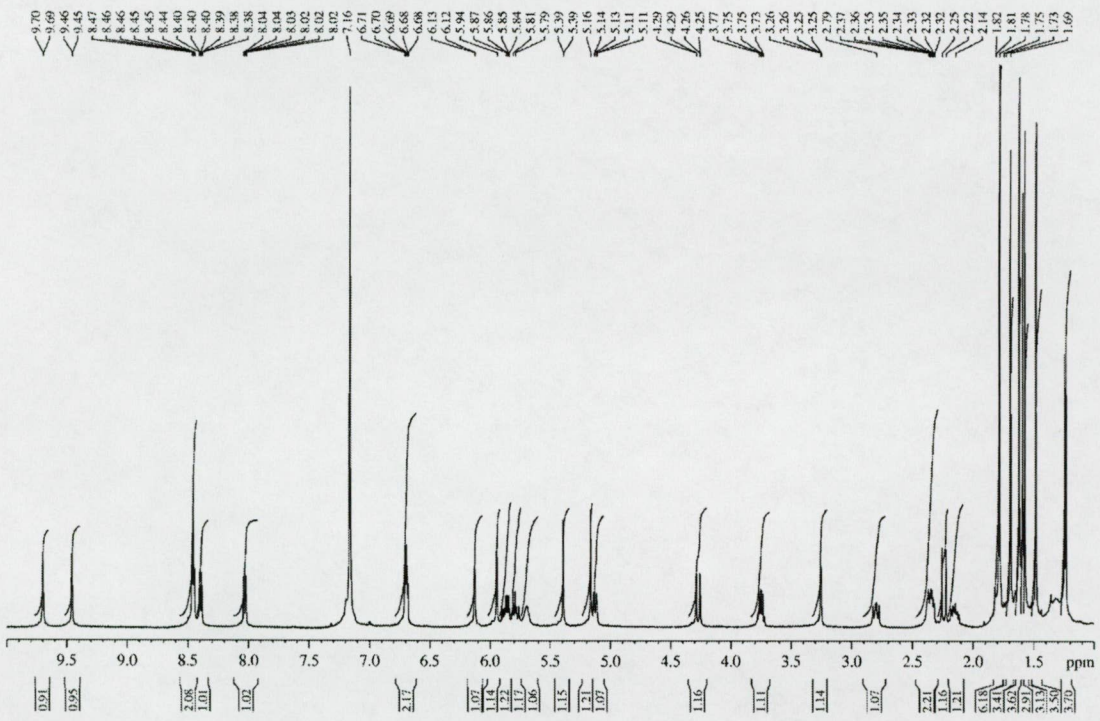


**Figure 10** $^{1}H$ spectrum of **1** at 340 K in $C_6D_6$

## 3.1.2. The JMOD spectrum

Checking the JMOD spectrum on **Figure 11**, the presence of five quaternary carbons (85.9, 88.7, 94.1, 148.6 and 212.9 ppm) , eight methine (45.4, 52.6, 69.5, 70.0, 74.5, 79.0, 127.1 and 137.4 ppm) and four methylene (35.7, 40.7, 49.7 and 112.2 ppm) groups are recognizable. These carbons and the three methyl groups (19.1, 19.1 and 21.5 ppm) build up a 20 carbon containing diterpene core. The carbon and hydrogen chemical shifts are summarized in **Table 1**.

The differences of signal intensities of the JMOD spectrum are originated from two effects. One of them is the heteronuclear overhauser effect. The heteronuclear NOE causes intensity, and on that way, sensitivity enhancements on signals of carbons holding directly attached protons. The enhancement depends on the number of attached protons. On the other hand, loss of intensity can be expected on signals when the pulse repetition time is shorter, then the relaxation time (see the signal of C-14 carbonyl carbon at 212.9 ppm).

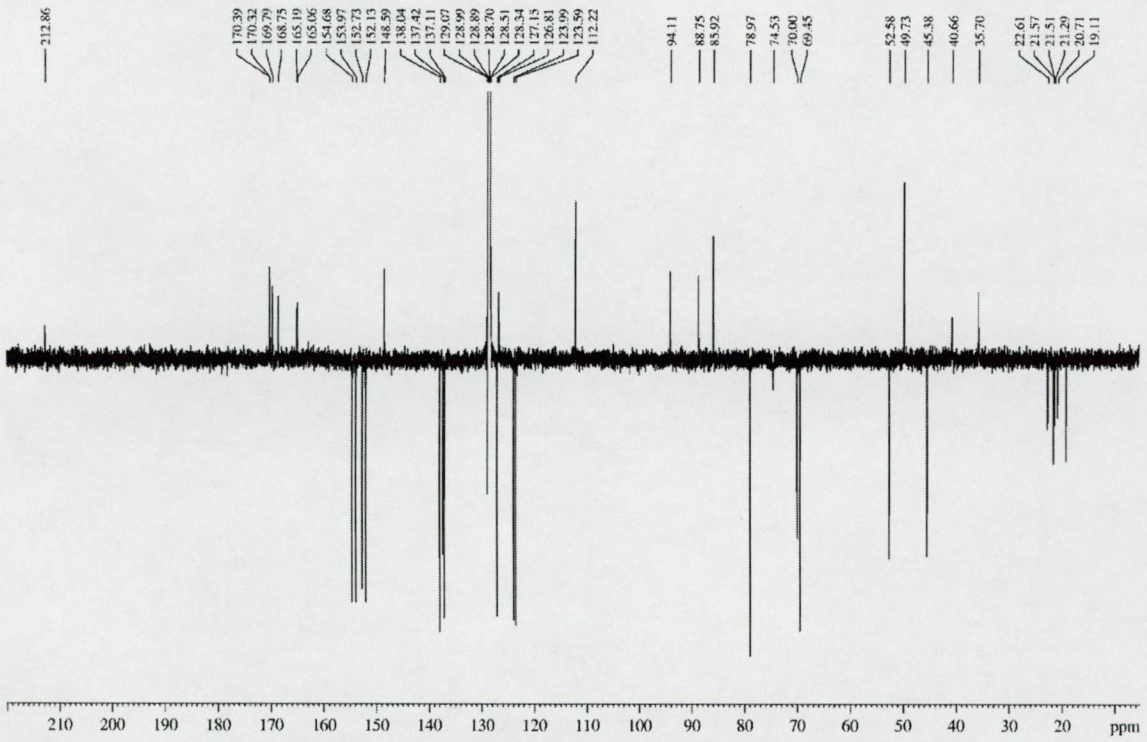



**Figure 11**: JMOD spectrum of **1**, recorded at 340 K in $C_6D_6$

## 3.1.3. Interpretation of the HSQC spectrum

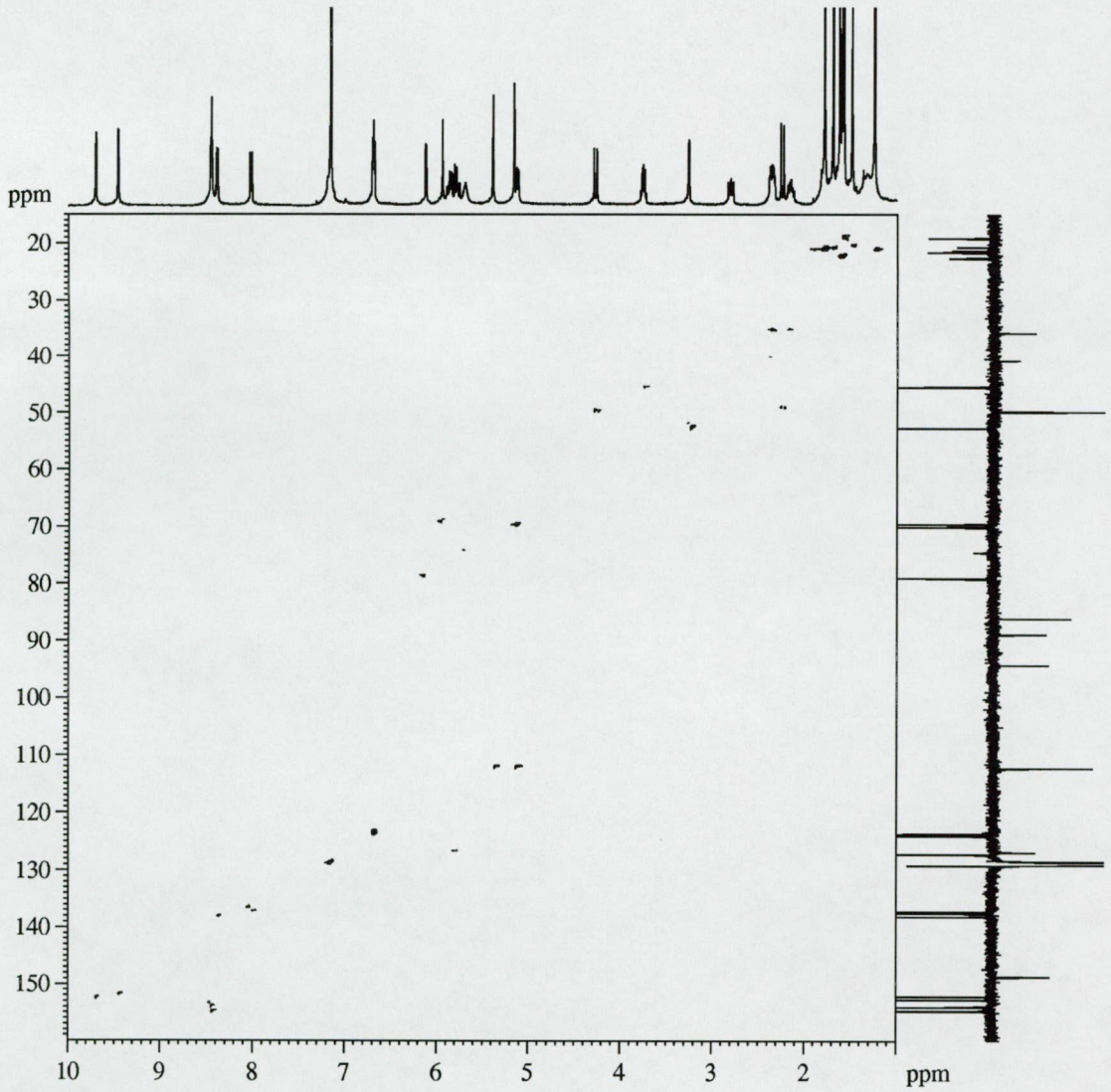



**Figure 12**: HSQC spectrum of **1**, measured in $C_6D_6$, at 340 K

We can associate the carbon signals with the corresponding signals of directly attached protons based on an HSQC experiment. The spectrum is shown on **Figure 12**. The coupled carbon-proton pairs' chemical shifts are listed in **Table 1**.

| No | $^{1}H$ | $^{13}C$ | No | $^{1}H$ | $^{13}C$ |
|---|---|---|---|---|---|
| 1a | 4.27 dd (16.4, 1.3) | 49.7 | 3-OAc | 1.78 s | 21.6 |
| 1b | 2.24 d (16.4) | | | | 168.8 |
| 2 | - | 88.7 | 5-OAc | 1.78 s | 21.6 |
| 3 | 6.12 d (3.1) | 79 | | | 169.8 |
| 4 | 3.25 dd (3.1, 1.3) | 52.6 | 7-OAc | 1.48 s | 20.7 |
| 5 | 5.94 s | 69.5 | | | 170.3 |
| 6 | - | 148.6 | 10-OAc | 1.62 s | 22.6 |
| 7 | 5.12 dd (10.4, 2.5) | 70 | | | 170.4 |
| 8a | 2.35 m | 35.7 | 15-OAc | 1.69 s | 21.3 |
| 8b | 2.14 ddd (16.8, 10.5, 6.8) | | | | 170.4 |
| 9 | 5.69 brs | 74.5 | 2-ONic | | |
| 10 | - | 85.9 | CO | - | 165.2 |
| 11 | 5.87 ddd (15.4, 9.6, 4.8) | 127.1 | 2' | 9.70 d (1.5) | 152.7 |
| 12 | 5.78 dd (15.4, 9.1) | 137.4 | 3' | - | 128.3 |
| 13 | 3.75 dq (9.1, 6.7) | 45.4 | 4' | 8.39 dt (7.9, 1.9) | 138 |
| 14 | - | 212.9 | 5' | 6.70 dd (7.9, 4.8) | 124 |
| 15 | - | 94.1 | 6' | 8.46 dd (4.8, 1.5) | 154 |
| 16 | 1.57 s | 19.1 | 9-ONic | | |
| 17a | 5.39 s | 112.2 | CO | - | 165.1 |
| 17b | 5.16 s | | 2" | 9.45 d (1.5) | 152.1 |
| 18a | 2.80 dd (14.5, 9.6) | 40.7 | 3" | - | 126.8 |
| 18b | 2.35 m | | 4" | 8.03 dt (7.9, 1.9) | 137.1 |
| 19 | 1.59 s | 19.1 | 5" | 6.69 dd (7.9, 4.8) | 123.6 |
| 20 | 1.24 d (6.7) | 21.5 | 6" | 8.45 dd (4.8, 1.5) | 154.7 |

**Table 1**: NMR data on **1** [δ/ppm (*J*/Hz)]

## 3.1.4. COSY correlations

The proton-proton connectivities were studied by means of a COSY measurement. The results of this experiment shows, that there are three chains in the molecule where one can follow the connections on the base of $^{3}J$ and $^{4}J$ proton-proton couplings. These chains may include more than one spin-systems of the molecule, because of the presence of $^{4}J$ $^{1}$H-$^{1}$H couplings. The

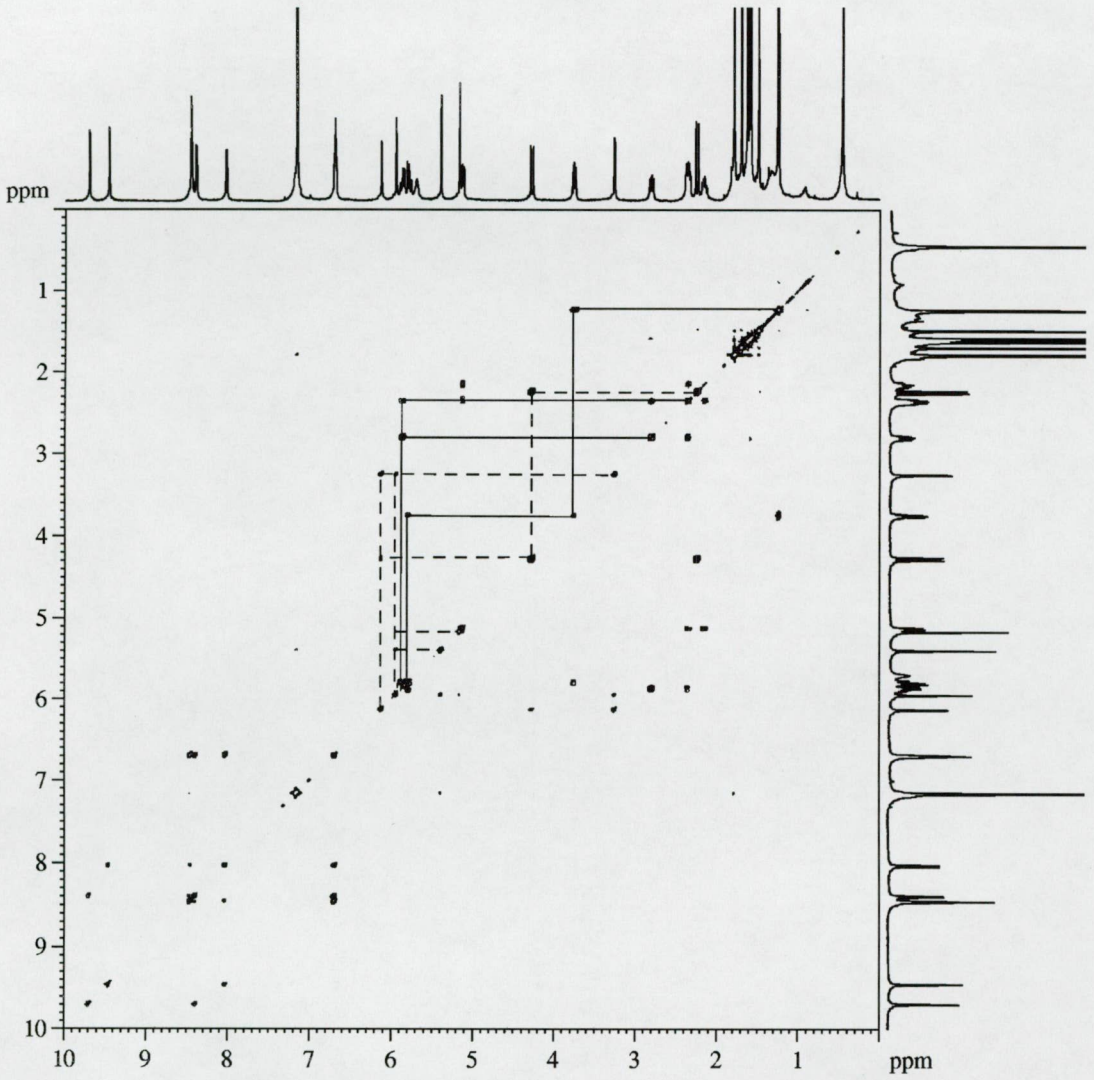



**Figure 13**: COSY spectrum of **1**, acquired at 340 K in $C_6D_6$

sequences are the followings: -$CH_2$-C-CH(OR)-CH-CH(OR)-C(=$CH_2$)- *(A)*, CH(OR)-$CH_2$- *(B)* and $CH_2$-CH=CH-CH($CH_3$)- *(C)*. $^4J$ couplings were detected between the 4.27 and 6.12 ppm , the 5.94 and 5.39 ppm and the 5.94 and 5.16 ppm proton pairs. The coupling constant, $^3J_{(H\text{-}11,H\text{-}12)}$=15.4 Hz, suggests a *trans (E)* configuration on the double bond in chain *(C)*. A gradient selected COSY spectrum is presented on **Figure 13**

## 3.1.5. The long-range correlations

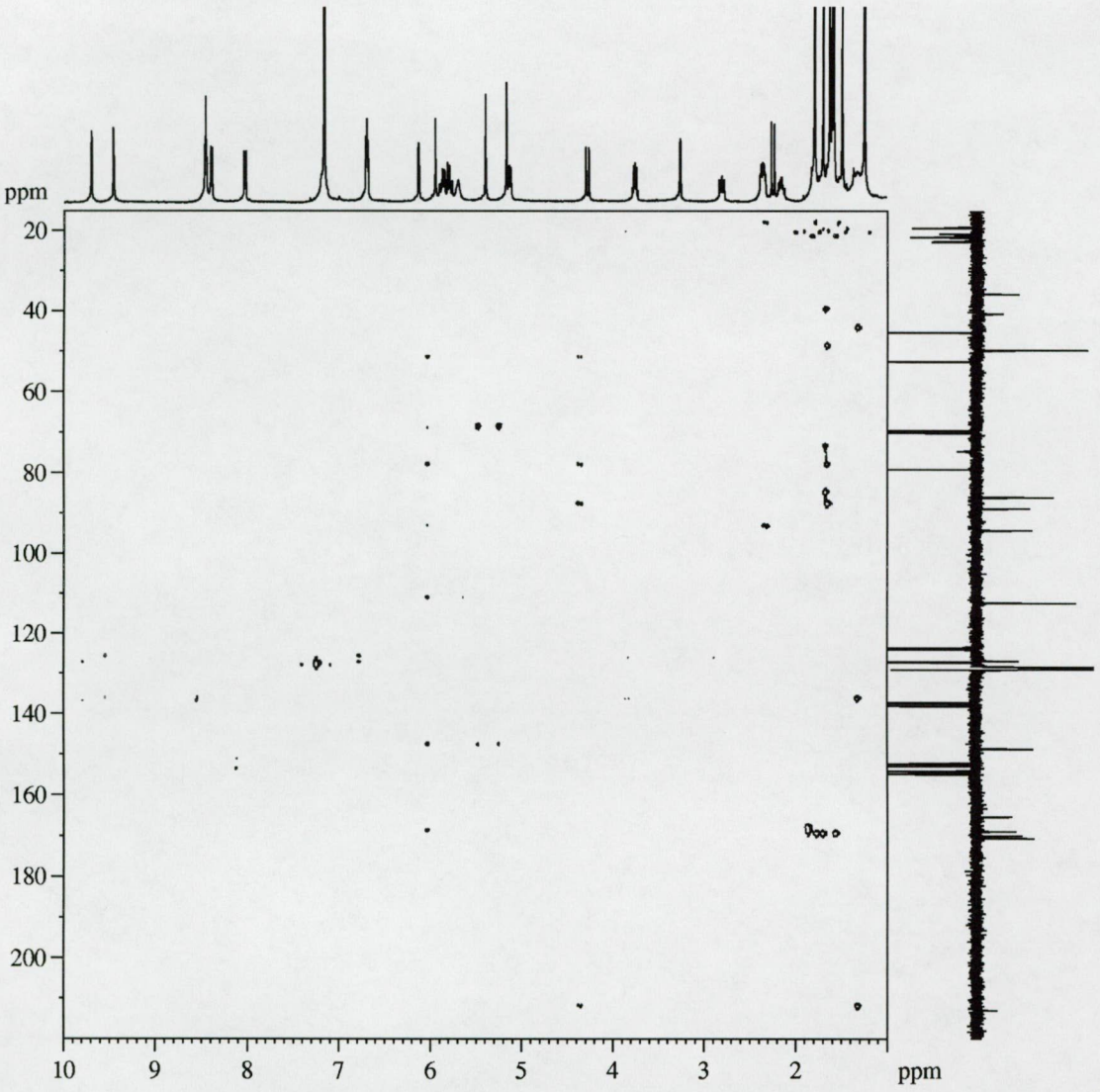



**Figure 14**: HMBC spectrum of **1** measured in $C_6D_6$ at 340 K using 60 ms evolution time

| Carbon No | HMBC Correlations to H |
|---|---|
| 1 | 3, 16 |
| 2 | 1a, 3, 16 |
| 3 | 1a, 4, 5, 16, 3-OAc |
| 4 | 1a, 5 |
| 5 | 4, 17a, 17b |
| 6 | 5, 17a, 17b |
| 7 | 5, 7-OAc |
| 8 | - |
| 9 | 19 |
| 10 | 19 |
| 11 | 12, 13 |
| 12 | 11, 13, 20 |
| 13 | 11, 12, 20 |
| 14 | 1a, 1b, 4, 13, 20 |
| 15 | 1a, 1b, 3, 5, 15-OAc |
| 16 | 1b |
| 17 | 5 |
| 18 | 19 |
| 19 | - |
| 20 | 12, 13 |

**Table 2**: HMBC correlations of **1**

The assembling of the structure was carried out by analysis of long-range $^{1}H$-$^{13}C$ couplings. The corresponding HMBC spectrum, acquired with 60 ms evolution time, is shown on **Figure 14**. The cross-peaks in the $\delta_C$=88.7 ppm (C-2) and $\delta_C$=94.1 ppm (C-15) rows show (the detailed long-range connectivities are listed in **Table 2**), that the substructure *A* involves the C-1 - C-6(C-17) part of the molecule and contains a five-membered ring, which is common in Euphorbiacea diterpenes[13]. The HMBC correlations of $\delta_C$=212.9 ppm carbon (C-14) locates a keto group at C-14 and indicated that substructure *C* embodies the C-18 - C-11 - C-13 - C-20 section of the molecule. Further analysis of the HMBC cross-peaks (see **Table 2**) between C-18 - H-19, C-10 - H-19, C-9 - H-19 and C-7 - H-5 guides to the conclusion that the parent diterpene involves a new carbon skeleton. The new skeletal system is similar to the jatrophane carbon core, but one of the geminal methyl groups is incorporated in the carbocycle, resulting in a 13-membered cycle. (This similarity is the reason, why the jatrophane atom numbering was used in case of this molecule.) The presence of this

macrocycle explains the flexibility of the molecule. The positions of the ester groups were determined via long-range correlations. On the bases of $^{3}J_{C\text{-}H}$ couplings, the presence of acetyl groups at C-3, C-5 and C-7 was evident (see **Table 2**). The localization of acyl groups connected to quaternary carbon atoms is more difficult, because of the lack of $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ correlations. To override this problem, an HMBC experiment with 100 ms evolution time was used. This evolution time (optimal for $J$=5 Hz) allows to develop the cross-peaks belonging to $^{4}J_{C\text{-}H}$. Part of

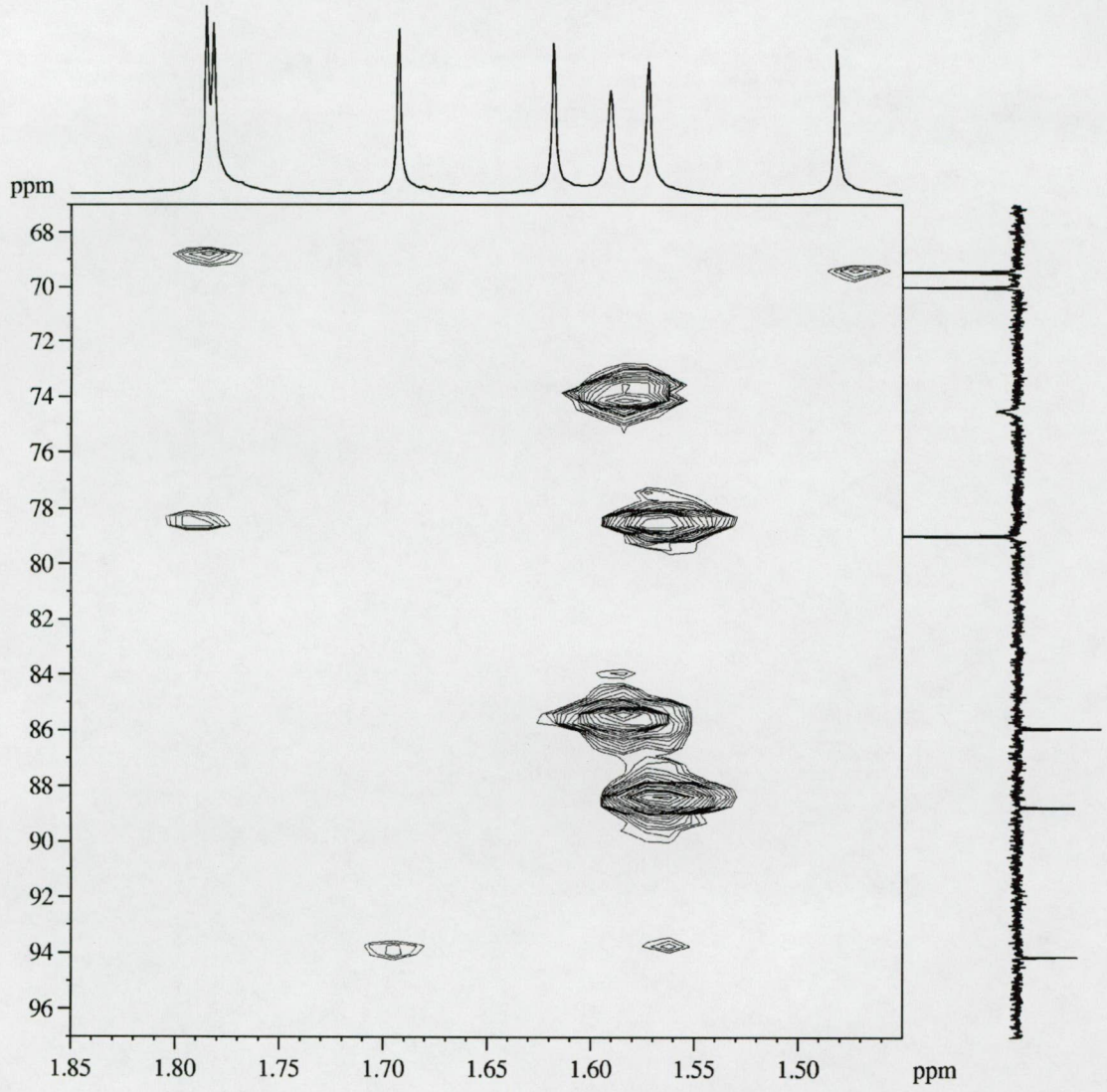



**Figure 15**: HMBC spectrum of **1** in $C_6D_6$ at 340 K, using 100 ms evolution time

the spectrum is shown on **Figure 15**. Such couplings observed between C-10 (85.9 ppm), C-15

(94.1 ppm) and the corresponding acetyl methyl protons (1.62 and 1.69 ppm). One of the nicotinoyl groups was located with the aid of the $^4J_{C\text{-}H}$ of its carbonyl carbon and H-16. The other nicotinoyl group was of necessity situated on C-9. The fact, that this group has no 4-bond correlations as the other is explained by the flexibility of the molecule.

## 3.1.6. Interpretation of the NOESY spectrum

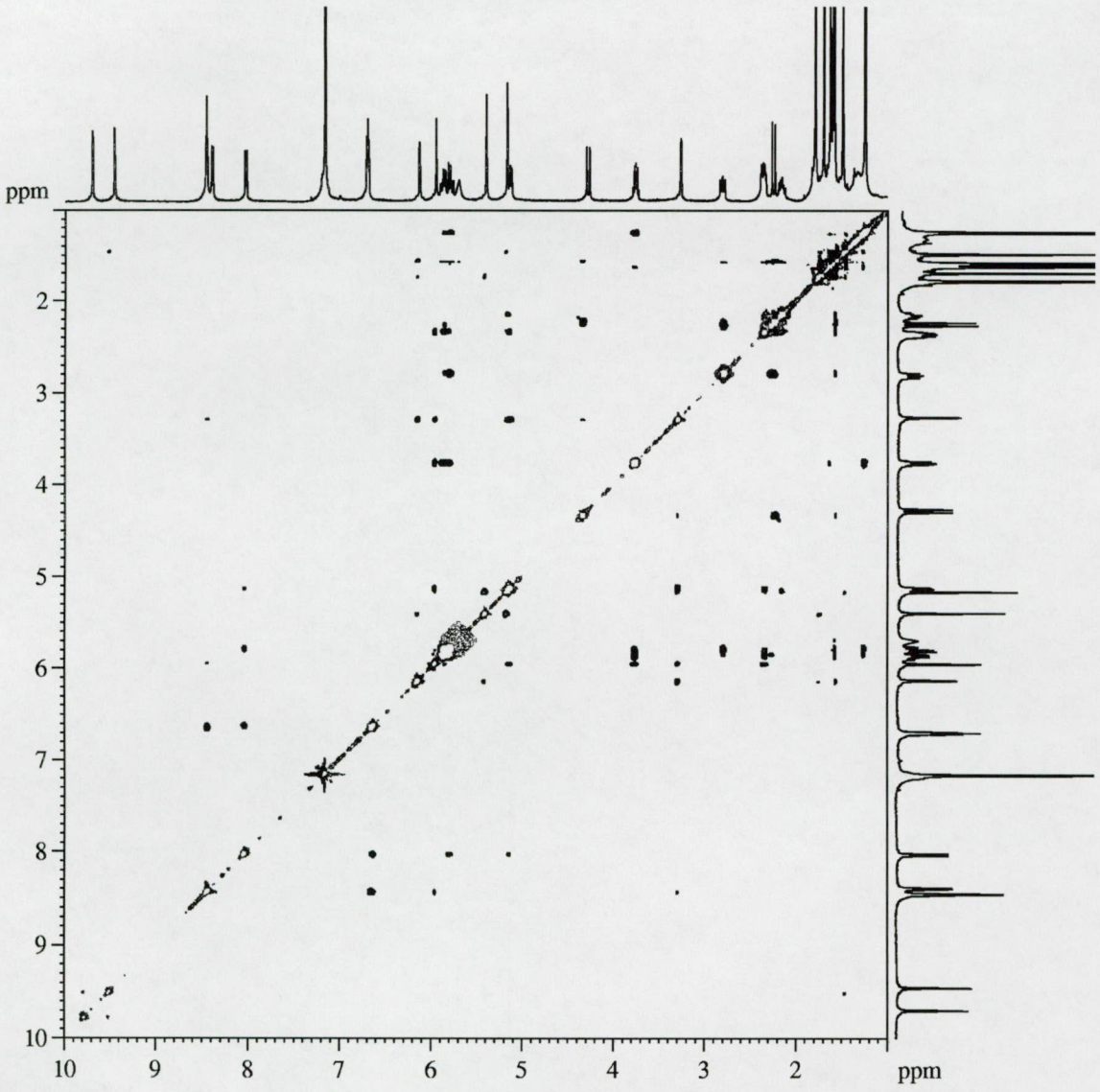



**Figure 16**: NOESY spectrum of **1** in $C_6D_6$ at 340 K, using 340 ms mixing time

The stereochemistry of the molecule were deduced from the NOESY spectrum (see **Figure 16**). The H-4 proton at the ring junction was assumed to be α and it was used as reference point.

| H No | NOESY correlation to H |
|---|---|
| 1a | 1b, 16 |
| 1b | 1a, 16 |
| 3 | 4, 16 |
| 4 | 3, 7, 6', 6" |
| 5 | 13, 15-OAc |
| 7 | 4, 8a |
| 8a | 7, 11, 19 |
| 8b | 19 |
| 9 | 19 |
| 11 | 8a, 13, 18a, 19, 20 |
| 12 | 13, 18a, 18b, 19, 20, 4" |
| 13 | 5, 11, 12, 20, 15-OAc |
| 16 | 1a, 1b, 3 |
| 17a | 3-OAc, 5-OAc |
| 17b | 7-OAc, 10-OAc |
| 18a | 12, 19 |
| 18b | 19 |
| 19 | 8a, 8b, 9, 11, 12, 18a, 18b |
| 20 | 11, 12, 13 |

**Table 3**: NOE correlations in **1**

The NOE interaction (for the detailed list of NOE interactions see **Table 3**) between H-4 - H-5 and H-4 - H-7, together with the small coupling constants of the corresponding protons, suggested that the 6,17-exomethylene group is parallel to the plane of macrocycle, and H-4 and H-5 are almost orthogonal, as was described for jatrophanes.[72,73] This conformation involves the β orientation of the H-5 hydrogen, which points inwards the macrocycle. The NOE effects between the H-5 and H-13 protons and between H-13 and 15-OAc demonstrates the β position of H-13 and 15-Oac. The NOE correlation between H-4 - H-3 and H-4 - H-7 exposes β oriented acyl groups on C-3 and C-7. The interaction from H-4 to nicotinoyl protons H-6' and H-6" offers the α orientation of the two nicotinoyl groups. This position is confirmed by the nuclear Overhauser effect between the nicotinoyl hydrogens H-2' and H-2". Important NOE interaction is observable between the H-17a and the 3-OAc and 5-OAc groups and between H-17b and 7-OAc and 10-OAc, which allows to conclude the β orientation of 10-Oac. We did not detect characteristic correlations for the C-9 area, especially for the nicotinoyl group located on C-9. The

stereochemical assignments of this area are proven by the well established biogenetics of this compound family. The fact, that the other compounds extracted from the same plant have the same configuration on that positions,[74] improves the reliability of this assignment.

## 3.2 Summary of structure elucidation of compound 1

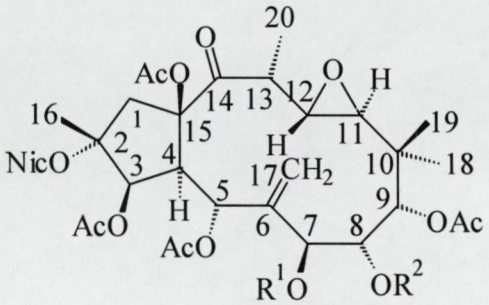
As was described above, different types of NMR experiments were used to determine the structure of molecules. With the help of $^{1}H$, $^{13}C$ JMOD and HSQC spectra, the number of quaternary carbons, CH, $CH_2$ and $CH_3$ groups were measured. The number and types of hetero atoms and also the molecular mass and formula were determined by mass spectroscopy. With these methods, five quaternary carbons, eight methine, four methylene, three methyl signals, five acetate groups and two nicotinoate residues were assigned. The connectivities between these building blocks were followed by COSY, which provided three substructures (-$CH_2$-C-CH(OR)-CH-CH(OR)-C(=$CH_2$)- *(A)*, CH(OR)-$CH_2$- *(B)* and $CH_2$-CH=CH-CH($CH_3$)- *(C)*). HMBC experiments were applied to collect information about long-range $^{1}H$-$^{13}C$ correlations, which allow to assemble the core of the molecule from those substructures, and situate the acyl groups on the diterpene core. The long range carbon-proton correlations of C-2 and C-15 carbons suggest, that substructure *A* includes a five membered ring. The correlations of C-14 keto-carbon locates the substructure *C* as the C-18 - C-20 section of the compound. The analysis of HMBC cross-peaks of the C-10 C-18 area suggest the incorporation of the C-18 carbon in the core ring system of the molecule. $^{1}J_{C\text{-}H}$ correlations, observable in a 100 ms evolution time HMBC, specifically for C-10, C-15 carbons reveal the position of the acetyl groups. Another characteristic signal shows correlation between a nicotinyl carbonyl carbon and H-16, helping to locate that acyl group. Characteristic NOE correlations were used for the stereochemical assignments. These investments lead to the structure **1**, named euphosalicin, which is the first representative of a new, unusual type of natural diterpenes, having a novel bicyclic core, including one five- and one 13-membered ring. This new carbon skeleton is formally derived from the jatrophane framework by incorporation of one of the geminal methyl groups in the ring system.

## 3.3. Compounds from Euphorbiaceae species

During my PhD work five different species of Euphorbiaceae family were investigated. The compounds, extracted from those plants and assigned are described in this section. Detailed NMR data are included in the attached papers, the proton and carbon chemical shifts of skeletons of newly identified compounds are summarized in **Table 4** and **Table 5**. The procedure of structure determination and signal assignement was the same as described on the previous pages.

### 3.3.1. Compounds from *Euphorbia salicifolia*

Five new compounds assigned from *E. salicifolia*[74,75]. The structure determination and NMR data of euphosalicin (**1**) were described in details in previous chapters. After careful analysis of $^{1}H$, $^{13}C$ and the correlation spectra of **2** and **3**, it was obvious that they are based on the same parent ring system as a formerly identified compound, esulatin A[76] and differed only in esterification. In case of molecule **2** the absence of the signal of an acetate group and the appearance of further isobutanoate signals indicated the change of an acetate to isobutanoate. The positions of the substituents were concluded by the analysis of HMBC spectra. The correlation between the H-8 and the 176.0 ppm carbon signal (*i*-butanoyl CO) lead to the position of the additional isobutanoate group. Careful comparison of NOESY spectra enabled us to assume the

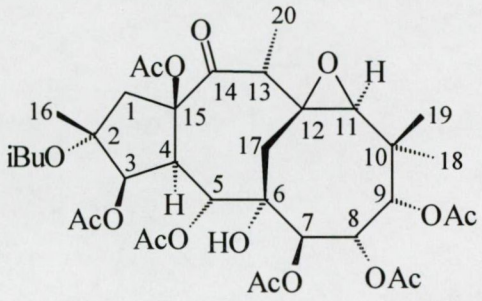



| | $R^1$ | $R^2$ |
|---|---|---|
| **2** | iBu | iBu |
| **3** | Ac | iBu |

Ac = Acetyl
iBu = Isobutanoyl
Nic = Nicotinoyl

stereochemistry of **2** as of esulatin A, whos configuration was established by X-ray chrystallography. Mass spectrometry and NMR experiments have shown only a minor difference between esulatin A and **3**. Analysis of NMR results revealed the different positioning of the isobutanoyl group.

The $^{1}H$ and $^{13}C$ JMOD spectra of **4** (named salicifoline) let us easily identify seven ester residues, such as six acetate and one isobutanoate groups. The heteronuclear correlation spectra shwed the existence of a 20 membered diterpene core. The $^{1}H$ - $^{1}H$ COSY connectivities indicated only short correlated proton sequences such as -CH(OR)-CH-CH(OR)-, CH(OR)-CH(OR)-CH(OR)-, and $CH_3$-CH-. Further analysis of correlation spectra lead to a modified, tricyclic jatrophane structure, where a methylene bridge is formed by coupling of C-17 to C-12. Three acetyl groups were placed at C-3, C-5 and C-9, based on the $^{3}J_{C\text{-}H}$ couplings of oxymethine



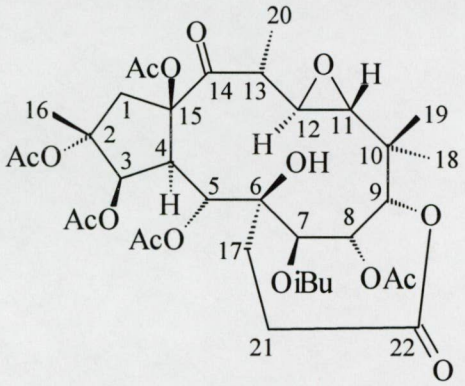

Ac = Acetyl iBu = Isobutanoyl

**4**

protons and carbonyl carbons. The presence of a fourth acetyl group at C-15 was evident because of the $^{4}J_{C\text{-}H}$ coupling observed in the HMBC spectrum, recorded at 120 ms evolution time. The NOESY correlations between the isobutanoyl methyl protons and H-3 and H-6 indicated the position of the isobutanoyloxy group at C-2. The remaining two acetyls were placed of necessity at C-7 and C-8. Considering the chemical shifts, the molecular formula and degree of unsaturation, an epoxy and a hydroxy group were placed at C-11-C-12 and C-6, respectively. The stereochemistry of salicifoline (**4**) was established by the analysis of NOESY signals. Correlations between H-4 and H-3, H-3 and H-1α, H-1β and H-16, and H-16 and 15-OAc showed the β orientation of the C-3 and C-15 acetyl groups, and the C-2 methyl. The correlations

between 15-OAc and H-13 and H-13and H-17β concluded the β position of H-13 and the C-17 bridge. NOE crosspeaks for the H-17β - H-5, H-17α - H-9, H-9 - H-8, H-9 - H-19 proton pairs indicated the β orientation of them. Similarly, the crosspeaks for H-18 - H-7, H-18 - H-11, H-20 - H-11 pairs suggested the α position of those hydrogens and methyls.

The analysis of $^{1}H$ and JMOD spectra of **5** (salicifolinide) showed the presence of five acetate and one isobutanoate groups. Besides the signals of esther moieties, the spectra showed the existence of six quaternary carbons, nine methines, three methylenes, three tertiary methyl groups and one secondary methyl group. Five structural fragments were identified by proton-proton correlations, extracted from the COSY spectrum. These fragments were $-CH_2-$, -CH(OR)-CH-CH(OR)-, -CH(OR)-CH(OR)-CH(OR)-, -CH -CH $-CH(CH_3)-$ and $-CH_2$ $-CH_2-$. Careful examination of the heterocorrelation spectra revealed a 22-membered jatrophane type bishomoditerpene core, bearing a two-carbon segment connected to C-17. The $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$




Ac = Acetyl iBu = Isobutanoyl

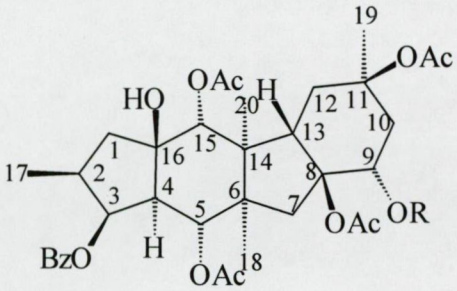
**5**

couplings between C-21 and H-17b, C-22 and H-21, C-22 and H-9 indicated the existence of a lactone ring between C-17 and C-9. The acyl residues were located using the corresponding three- and four-bond HMBC correlations.An epoxy group was placed at C-11-C-12, supported by molecular formula and the chemical shifts of the corresponding atoms. A hydroxyl group was located at C-6, based on the HMBC correlation of its hydrogen to the C-6 carbon. Similar structures, based on 17-ethyljatrophane skeleton were isolated from *Euphorbia terracina*, involving the C-5 or C-3 hydroxyl groups in the lactone ring[10,77]. Investigation of NOESY

spectrum lead to the stereochemical assignment of hydrogens and acyl groups. Characteristic correlations between H-4 and H-3, H-4 and H-1α, H-4 and H-21, H-4 and H-17, H-17 and H-7 supported the α orientation of those protons, and placed the lactone ring below the plane of the macrocycle. The similar correlations between the H-1β and H-16 methyl protons, 6-OH and H-5, H-5 and H-8, H-8 and H-18, H-8 and H-11, H-8 and H-9, H-11 and H-13, H-13 and 15-OAc reveal β oriented protons at C-5, C-8, C-9, C-11 and C-13, and the β position of the methyl group at C-2 and the acetyl group at C-15. The NOE effect between H-19 and H-12 established a *trans* epoxy group at C-11-C-12.

## 3.3.2. Compounds from *Euphorbia peplus*

Two new (**6**, **8**) and four known (**9-12**) compound were identified from the extract of *E. peplus*[78]. One of the new structures (**6**) is a tetracyclic pepluane derivative, closely related to a formerly published compound (**7**)[29]. $^{1}H$ and $^{13}C$ spectra of **6** revealed the existence of a benzoyl and four acetyl groups. The careful comparison of HMQC and $^{1}H$ spectra concluded in the presence of two hydroxy groups (3.07 and 1.91 ppm $^{1}H$ chemical shifts). The skeleton of the molecule consists three tertiary methyls, one isolated methylene, one methine and the following sequences: $-CH_2-$

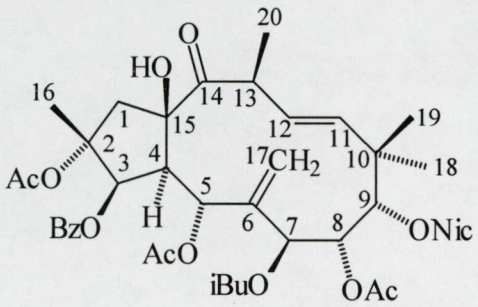



| | R | |
|---|---|---|
| **6** | H | Ac = Acetyl |
| **7** | Ac | Bz = Benzoyl |

$CH(CH_3)-CH(OR)-CH-CH(OR)-$, $-CH-CH_2-$ and $-CH-CH_2-$. Analysis of $^{2}J_{C\text{-}H}$ and $^{3}J_{C\text{-}H}$ couplings a pepluane type diterpene core was concluded. The location of the substituents were determined by the observation of $^{1}H$-$^{13}C$ long range correlations and by comparison of NMR data to those

of the known compound (**7**). The stereochemistry of the molecule was checked by NOESY spectroscopy. Diagnostic NOE interactions were detected 16-OH to H-5, H-13, H-15, H-1β and H-1β to H-17 indicating their β position. Further characteristic signals were found between H-4, H-3 and H-18 and also between H-18 and H-20. These signals prove the α position of these hydrogens. Furthermore, the interactions between the ortho and meta benzoyl protons and 8-OAc and 11OAc and between H-9 and H-10β suggest the same stereochemistry on C-8, C-9 and C-11 as previously found in **7**.

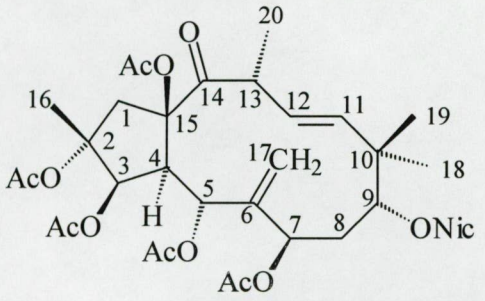
The other formerly unknown structure (**8**) is a jatrophane type diterpene polyester. Six ester groups were recognized from the $^1$H spectrum, such as one nicotinate, one benzoate, one isobutanoate and three acetates. The substructures, such as -$CH_2$-, CH-(OR)-CH-CH(OR)-, -C(=$CH_2$)-CH(OR)-CH(OR)-CH(OR)-, -CH=CH-CH($CH_3$), three tertiary methyl groups,




Ac = Acetyl iBu = Isobutanoyl
Bz = Benzoyl Nic = Nicotinoyl

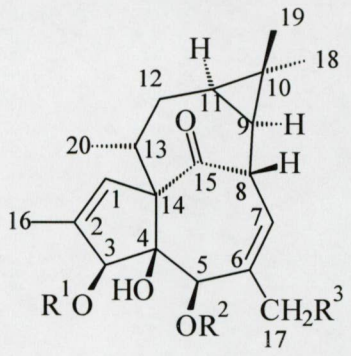
**8**

provided by the COSY and HMQC spectra, were joined together with the help of HMBC spectrum. The stereochemical analysis of the molecule was carried out by NOESY spectroscopy. The corresponding cross peaks between H-4 and H-3, H-7 and H-13, H-13 and H-1α determinated the α position of these hydrogenes. Such as the β orientation of the 15-OH, H-5, H-8, H-9, H-16, H-18, H-19 were concluded from their NOE correlations. The *trans* geometry of the olefin bond was suggested by the *3J*=15.8 Hz coupling constant between H-11 and H-12.



Ac = Acetyl Nic = Nicotinoyl

**9**

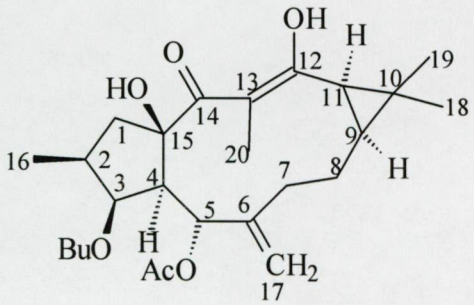


| | $R^1$ | $R^2$ | $R^3$ |
|---|---|---|---|
| **10** | angeloyl | H | OH |
| **11** | angeloyl | H | H |
| **12** | H | angeloyl | H |

The known compounds are ingenane (**10-12**) and jatrophane (**9**) type structures. These molecules (**9-12**) were characterized by compering their NMR data to formerly published data[25, 26, 28, 79]

## 3.3.3. Compound from *Euphorbia lathyris*

A new lathyrane diterpene was isolated from the roots of *E. lathyris*[80]. The detailed NMR spectroscopic analysis revealed that the structure of that compound (**13**) is that of a diester of an unknown, polyfunctional diterpene parent alcohol. The $^1$H NMR spectrum of **13** showed the



Ac = Acetyl Bu = Butyl

**13**

presence of one acetate and one butanoate groups. The characteristic signals ($\delta_H$ 1.10dd, 1.50dd, 1.06s, 1.05s and $\delta_C$ 35.9, 28.3, 25.2, 28.3, 15.7 ppm) suggested a *gem*-dimethyl-substituted cyclopropane ring, present in many types of Euphorbiaceae diterpenes[13]. Based on COSY correlations, two sequences of correlated protons were recognizable: $-CH_2-CH(CH_2)-CHR-CH-CHR-$ and $-CH_2-CH_2-CH-CH-$. Besides these fragments, six quaternary carbon were observable. The two broad singlets of $^1H$ spectrum were assigned as hydroxyl groups because of their missing HMQC correlations. The relative stereochemistry was followed by NOESY. The corresponding interactions showed the *trans*-junction of cyclopentane ring and the *cis*-fused cyclopropane. Other characteristic NOE correlations suggested the orientation of substituents. The depicted enolic form of the molecule could be stable because of hydrogen bonding, which can exist between the 14-keto group and 12-OH and 15-OH groups. A similar enolic structure was published by Torrance in the presumed biogenetic route of jatrophatrione[81]. The relative stereochemistry of this compound (**13**), based on NOESY experiments, supports suggestion of Manners that lathyrane and related jatrophane type diterpenes should be stereochemically similar regarding to the C-3, C-4, C-5 and C-15 positions[82].

| | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|
| | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ |
| 1a | 3.75 | 46.9 | 3.73 | 46.3 | 4.03 | 50.1 | 3.27 | 47.8 |
| 1b | 2.04 | | 2.06 | | 2.19 | | 2.29 | |
| 2 | | 87.5 | | 86.7 | | 86.9 | | 88.9 |
| 3 | 5.58 | 78.8 | 5.58 | 78.2 | 5.55 | 82.4 | 5.76 | 80.7 |
| 4 | 2.96 | 50.6 | 2.99 | 49.5 | 3.03 | 50.8 | 3.08 | 51.1 |
| 5 | 6.09 | 68.4 | 6.04 | 67.8 | 5.89 | 75.2 | 5.5 | 71.6 |
| 6 | | 142.5 | | 140.6 | | 76.3 | | 78.4 |
| 7 | 5.42 | 69.4 | 5.36 | 68.4 | 5.52 | 72.2 | 4.97 | 78 |
| 8 | 5.51 | 69.4 | 5.5 | 69.2 | 6 | 71.3 | 5.66 | 70 |
| 9 | 4.94 | 77.4 | 4.93 | 77.7 | 5.45 | 72.2 | 4.78 | 79.9 |
| 10 | | 40.2 | | 39.3 | | 38.2 | | 38.6 |
| 11 | 3 | 59.2 | 3.02 | 58.2 | 3.1 | 65.7 | 3.84 | 62.3 |
| 12 | 3.33 | 57.8 | 3.32 | 57 | | 63.5 | 2.85 | 58.9 |
| 13 | 3.67 | 37.8 | 3.66 | 37 | 3.26 | 49 | 2.68 | 44.1 |
| 14 | | 210.9 | | 210.4 | | 213.2 | | 216.7 |
| 15 | | 93.3 | | 93 | | 92.2 | | 94.6 |
| 16 | 1.52 | 18.8 | 1.52 | 18.1 | 1.42 | 19.5 | 1.49 | 19.5 |
| 17a | 5.07 | 116 | 5.12 | 111.7 | 2.72 | 48.6 | 2.4 | 39.8 |
| 17b | 5.01 | | 5.03 | | 2.62 | | 2.32 | |
| 18 | 0.98 | 24.3 | 0.99 | 23.3 | 1.35 | 25 | 0.99 | 22.7 |
| 19 | 0.69 | 18.3 | 0.71 | 17.5 | 1.1 | 25.7 | 0.73 | 19.3 |
| 20 | 1.18 | 15.9 | 1.18 | 15 | 1.1 | 12.3 | 1.27 | 18.2 |
| 21a | | | | | | | 2.47 | 31.2 |
| 21b | | | | | | | 2.3 | |
| 22 | | | | | | | | 172.1 |

**Table 4** Chemical shifts of the identified compounds (I)

| | 6 | | 8 | | 13 | |
|---|---|---|---|---|---|---|
| | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ | $^{1}H$ | $^{13}C$ |
| 1a | 2.14 | 45.1 | 2.96 | 51.5 | 3.47 | 50.3 |
| 1b | 1.52 | | 2.15 | | 2.04 | |
| 2 | 2.52 | 36.5 | | 89 | 2.4 | 37.9 |
| 3 | 5.77 | 76.8 | 5.95 | 78 | 5.83 | 79.9 |
| 4 | 2.38 | 49 | 3.6 | 48 | 3.01 | 53.4 |
| 5 | 5.84 | 70.4 | 5.39 | 67.9 | 6.56 | 67.5 |
| 6 | | 49.3 | | 143.9 | | 149.8 |
| 7a | 1.92 | 40.3 | 5.77 | 67.4 | 2.54 | 35.3 |
| 7b | 2.4 | | | | 2.13 | |
| 8 | | 89.6 | 5.14 | 70.7 | 1.94 | 21.6 |
| 9 | 4.66 | 65.6 | 5.19 | 81.6 | 1.1 | 35.9 |
| 10a | 2.44 | 41.2 | | 41.2 | | 25.2 |
| 10b | 1.7 | | | | | |
| 11 | | 81 | 6.15 | 137.3 | 1.5 | 28.3 |
| 12a | 1.8 | 31.8 | 5.61 | 130.4 | | 152.4 |
| 12b | 2.25 | | | | | |
| 13 | 4.1 | 46.9 | 4.02 | 44.1 | | 134.2 |
| 14 | | 51.8 | | 212 | | 202.2 |
| 15 | 5.02 | 74.4 | | 87.6 | | 88.1 |
| 16 | | 84.6 | 1.59 | 20.5 | 1.03 | 14.1 |
| 17a | 1.05 | 16.7 | 5.05 | 113.2 | 5.1 | 114.7 |
| 17b | | | 4.89 | | 5.07 | |
| 18 | 1.09 | 16.8 | 1.02 | 26.3 | 1.06 | 28.3 |
| 19 | 0.89 | 29 | 1.36 | 23.3 | 1.05 | 15.7 |
| 20 | | 16.6 | 1.35 | 19.9 | 1.91 | 12.2 |

**Table 5** Chemical shifts of the identified compounds (II)

# 4. SUMMARY

The diterpene contents of tree species of the Euphorbiaceae family, such as *E. lathyris* L., *E. salicifolia* Host., and *E. peplus*, all growing in Hungary, were investigated, because of the well known high biological activity of this type of compounds. Methanol extraction, followed by solvent-solvent partitioning and different chromatographic methods was used to extract and purify the target components. The structures of the isolated molecules were determined by homo- and heteronuclear 1D ($^1$H and $^{13}$C JMOD) and 2D ($^1$H-$^1$H COSY, $^1$H-$^1$H TOCSY, $^1$H-$^{13}$C HMQC, $^1$H-$^{13}$C HSQC, $^1$H-$^{13}$C HMBC and $^1$H-$^1$H NOESY) NMR spectroscopy.

With the help of $^1$H, $^{13}$C JMOD and HSQC spectra, the number of quaternary carbons, CH, $CH_2$ and $CH_3$ groups were measured. The connectivities between these building blocks were followed by COSY, which allowed to map the spinsystems of the molecules. HMBC experiments were applied to assemble the molecule from substructures, and situate the acyl groups on the diterpene core. Characteristic NOE correlations were used for the stereochemical assignments. In addition to the number and types of hetero atoms, the molecular formula was determined by mass spectroscopy.

Five formerly unknown structures were characterized from *E. salicifolia* (**1-5**). One of them, named euphosalicin (**1**) is a novel structure, the first representative of a new class of bicyclic diterpenes. The skeleton of this molecule is formally derived from the jatrophane framework by incorporation of a geminal methyl group in the ring system. Two of them (**2** and **3**) are closely related jatrophane type diterpenes. The fourth structure, salicifoline (**4**) showed a known, but rare structural feature, forming a tricyclic skeleton via C-17 bridging between C-6 and C-12 carbons. Salicinolide (**6**) has a unique lactone ring, involving a C-6 - C-17 - C-9 bridge, what was formerly unknown in natural diterpene molecules.

One pepluane (**6**) and one jatrophane (**8**) type compound were isolated and identified from *E. peplus* as formerly unknown structures, together with four molecules (**9-12**) described earlier. The ingenol 3-angelate (**10**), a highly irritant toxin was reported first time from this plant by our group.

One new diterpene (**13**) has been characterized from the roots of *E. lathyris*. Detailed NMR spectroscopic analysis revealed that the structure of this compound is that of a diester of a formerly unknown diterpene alcohol.

All together, eight new diterpene polyesters with some formerly known compounds were identified from Hungarian Euphorbiaceae species. One of the new structures (**1**) was based on a unique diterpene core, which is hitherto unknown in the pool of natural and synthetic compounds. Some of the characterized compounds have shown significant pharmacological effects, such as multidrug resistance reversing activity, cytotoxicity and antiviral effect against herpes simplex virus type 2.

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# 8. COPIES OF PAPERS