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# Chemotaxonomy of the tribe Antidesmeae (Euphorbiaceae): antidesmone and related compounds

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**Abstract**

Selected species of the tribe Antidesmeae (Euphorbiaceae, subfamily Phyllanthoideae) have been screened for antidesmone occurrence and its content by quantitative HPLC (UV) and qualitative LC–MS/MS analysis. The LC–MS analysis allowing the additional detection of 17,18-bis-nor-antidesmone, 18-nor-antidesmone, 8-dihydroantidesmone and 8-deoxoantidesmone was carried out in the selected reaction monitoring (SRM) mode. Leaf material from herbarium specimens of 13 *Antidesma* spp., *Hyeronima alchorneoides* and *Thecacoris stenopetala* (all subtribe Antidesminae), as well as *Maesobotrya barteri*, *Aporosa octandra* (both Scepinae) and *Uapaca robynsii* (Uapacinae) were analysed. Additionally, freshly collected samples of different plant parts of two *Antidesma* spp. were investigated to ensure the significance of the results on herbarium specimens and to compare the antidesmone content in bark, root and leaves. Antidesmone could be unambiguously identified in 12 of 13 *Antidesma* spp., as well as in the two other investigated genera of subtribe Antidesminae, in levels of up to 65 mg/kg plant dry weight. Antidesmone was not found in specimens from other subtribes. Antidesmone-derived compounds occur in much lower concentrations than antidesmone.

*Keywords:* Antidesmone; Alkaloids; *Antidesma*; Euphorbiaceae; Chemotaxonomy

## 1. Introduction

*Antidesma* is a relatively homogeneous genus of dioecious shrubs and trees in the Old World tropics (Pax and Hoffmann, 1922). Within the subfamily Phyllanthoideae of the Euphorbiaceae it belongs to the tribe Antidesmeae, subtribe Antidesminae (Webster, 1994; Radcliffe-Smith, 2001). Pax and Hoffmann (1922) listed 142 species, Webster (1994) estimated ca. 200 species, and Radcliffe-Smith (2001) 170 species. However, other investigations indicate that the actual number might be noticeably lower due to the high number of synonyms (Hoffmann, 1999a,b, 2002). The subtribe Antidesminae includes furthermore the genera *Hyeronima* (20 spp. the Americas), *Thecacoris* (20 spp., Africa and Madagascar), *Leptonema* (2 spp., Madagascar), *Phyllanoa*, and *Celianella* (both monotypic, South America).

Some interesting compounds have been reported from *Antidesma* species: from *A. montanum* (formerly also known as *A. acuminatum*, *A. pentandrum* and *A. menasu*) antidesmanol (3-keto-16α-hydroxy-friedelan), *n*-tritiacontane, friedelin, canophyllal, canophyllol (Rizvi et al., 1980a), 16α-hydroxy-3-ketoisomultiflorene and 3β-hydroxy-16-ketoisomultiflorene (Rizvi et al., 1980b), the tannins antidesmin A, geraniin and carpusin (Namba et al., 1991; Yoshida et al., 1992), lupeolactone (Kikuchi et al., 1983) and two cyclopeptide alkaloids (Arbain and Taylor, 1993) and from *A. tetrandrum* also two cyclopeptide alkaloids (Taylor, 1994). Further reports show the negative results in general tests for alkaloids, bitterness, essential oil, cyanogenic compounds, saponins and triterpenoids for *A. ghaesembilla* (Arthur, 1954), only weak activity of the ethanolic extract in the brine shrimp assay for *A. tomentosum* (Rahamani et al., 1992), the presence of sitosterol for *A. diandrum* (synonym of *A. acidum*) (Misra et al., 1973) and the occurence of phenolic acids for *A. montanum* (Hemalatha and Radhakrishnaiah, 1994). The presence of cucurbitacins (Hegnauer, 1989) and diterpenoid esters (Seigler, 1994) in *Antidesma* spp. is doubtful, as the initial reports rely only on a single result (Beutler et al., 1989; J. Beutler, personal communication) or non-specific bioassays. In other instances the taxo-

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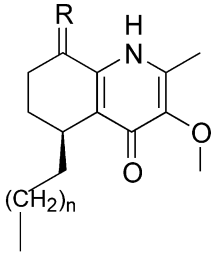
nomic identification of the plant material is equivocal (Tessier and Paris, 1978).

In the course of our investigations on bioactive compounds from tropical and subtropical plants, we reported the isolation and structure elucidation of six new benzopyranones and ferulic acid derivatives from *A. membranaceum* (Buske et al., 1997), as well as antidesmone (**3**) (Fig. 1), a novel quinoline type alkaloid from *A. membranaceum* and *A. venosum* (Buske et al., 1999; Bringmann et al., 2000a) and its (17*R*:*S*)17-β-D-glucopyranosyloxy- and (17*R*:*S*) 8-deoxo-17-β-D-glucopyranosyloxy-derivatives from *A. membranaceum* (Buske et al., 2001). The alkaloid hyeronimone found in *Hyeronima alchorneoides* (Tinto et al., 1991) can be regarded as the 8-dihydroderivative (**4**) of antidesmone (Fig. 1). From *Hyeronima oblonga*, two alkaloids, hyeronine A and B were reported (Alves and Zani, 1999). The former compound shows striking spectroscopic similarities to antidesmone (**3**) and has been proved to be identical with **3** by HPTLC- and HPLC-analysis in various solvent systems (Carlos L. Zani, personal communication), whereas the latter can be regarded as 17,18-bis-nor-antidesmone (**1**) (Fig. 1). It is noteworthy that antidesmone (**3**) has strong fungitoxic activity against *Cladosporium cucumerinum* (Buske et al., 1999) and hyeronine A shows activity in the brine shrimp assay (Alves and Zani, 1999). The biosynthesis of antidesmone in *A. membranaceum* proceeds via formation of a linear $C_{16}$-polyketide chain and direct incorporation of glycine (Bringmann et al., 2000b).

The structure of **3** and its easy identification by HPLC with UV and fluorescence detection as well as LC–ESI–MS in the positive ion mode makes it a valuable candidate for chemotaxonomic investigations to clarify the somewhat difficult taxonomy of the Euphorbiaceae–Phyllanthoideae.

For quantitative analysis of antidesmone (**3**), an HPLC assay was developed and applied to leaf material from herbarium specimens of 13 *Antidesma* spp. and one of each *Thecacoris*, *Hyeronima* (both Antidesminae), *Uapaca* (Uapacinae), *Maesobotrya* and *Aporosa* (both Scepinae). These measurements were completed by the analysis of recently collected plant material of two *Antidesma* spp., which also gave information on the distribution of **3** within the plant. Since levels of **3** were lower than the detection limit in some samples, a more sensitive LC–ESI–MS/MS method, selected reaction monitoring (SRM), was additionally employed. Using this method 17,18-bis-nor-antidesmone (**1**), 18-nor-antidesmone (**2**), 8-dihydroantidesmone (**4**) and 8-deoxoantidesmone (**5**) could also be identified (Fig. 1).



1 : R = O, n = 4
2 : R = O, n = 5
3 : R = O, n = 6
4 : R =α-H, ß-OH, n = 6
5 : R = H, H, n = 6

Fig. 1. Structures of 17,18-bis-nor-antidesmone (**1**), 18-nor-antidesmone (**2**), antidesmone (**3**), 8-dihydroantidesmone (**4**) and 8-deoxoantidesmone (**5**).

## 2. Results and discussion

### 2.1. Identification of compounds

The isolation and structure elucidation of antidesmone (**3**, Fig. 1) has been described (Buske et al., 1999; Bringmann et al., 2000a). The $[M+H]^+$ ion of antidesmone (**3**) displays a significant collision induced dissociation (CID) spectrum being specific for the bicyclic system (Buske et al., 2001). In agreement with **3**, the $[M+H]^+$-ions of **1** and **2** (*m*/*z* 292 and 306 respectively) show very similar CID spectra indicating the same bicyclic system, but a shortened side chain at C-5. Therefore, the mass spectral fragmentation allows an unambiguous identification of these compounds. A sample of hyeronine B, obtained from C.L. Zani, was identical with **1** by both the retention time and CID spectrum.

Reduction of **3** with $LiAlH_4$ or $NaBH_4$ yields 8-dihydroantidesmone (**4**). Both diastereomers were observed by HPLC and upon trimethylsilylation and analysis by GC–MS in a 15:1 ratio. In the $^1H$ NMR spectrum of the major diastereomer, the exact coupling constants of the protons at C-6 and C-7 could not be determined. However, 5-H shows, as in **3**, a broad singlett signal ($\Delta_{1/2}$ = 18 Hz), indicating the axial position of the side chain. 8-H has in contrast vicinal coupling constants of 10.0 and 6.3 Hz, revealing the equatorial position of 8-OH. The resulting configuration is the same as proposed for hyeronimone (Tinto et al., 1991) and the identity of **4** and hyeronimone is verified by excellent agreement of $^1H$ and $^{13}C$ NMR data.

Compound **5** exhibits a protonated molecular ion at *m*/*z* 306. The daughter ion spectrum that matches that of (17*R*:*S*) 8-deoxo-17-β-D-glucopyranosyloxy-antidesmone (Buske et al., 2001). Compound **5** could not be isolated, but was found enriched in subfractions of leaf and root extracts of *A. membranaceum.*

The fragmentation patterns (Fig. 2) and elemental composition of antidesmone (**3**), 8-dihydroantidesmone (**4**) and 8-deoxoantidesmone (**5**) were determined by

ESI–HR–MS/MS measurements. The mass spectral fragmentation of the $[M+H]^+$ ion of **3** and **5** is mainly characterized by successive losses of CO and $C_2H_4$ from the key ions at *m*/*z* 220 and 206, respectively (Fig. 2). In case of compound **4** the base peak ion at *m*/*z* 163 ($C_9H_9NO_2$) comprises the nitrogen-containing ring.

### 2.2. HPLC assay

For the quantification of **3**, an HPLC assay with UV-detection was employed. In order to ensure maximum chromatographic separation isocratic conditions were chosen. The pH of the aqueous solvent component had to be adjusted to 7.5 as a decrease of signal intensity was observed under acidic conditions. A linear response was obtained for injections of pure external standard of **3**, as well as for extracts of defined amounts of bark from *A. venosum*. The detection limit was determined to be 0.25 µg ml$^{-1}$ (ca. 1.25 ppm in final assay setup). An additionally recorded fluorescence signal was used for verification only as it showed less reproducibility and precision as well as higher detection limits.

### 2.3. LC–MS assay

LC–ESI–MS in the positive ion mode with selected reaction monitoring (SRM) provides a very specific and sensitive detection of antidesmone (**3**) and the related alkaloids **1**, **2**, **4**, and **5**. This assay was performed on samples prepurified by SPE rather than on raw extracts to ensure highest sensitivity. For each compound, the reaction from the $[M+H]^+$ ion to the base peak ion in the CID spectrum was monitored. A detection of all five alkaloids within one run is possible by changing the monitoring parameters during the HPLC run.

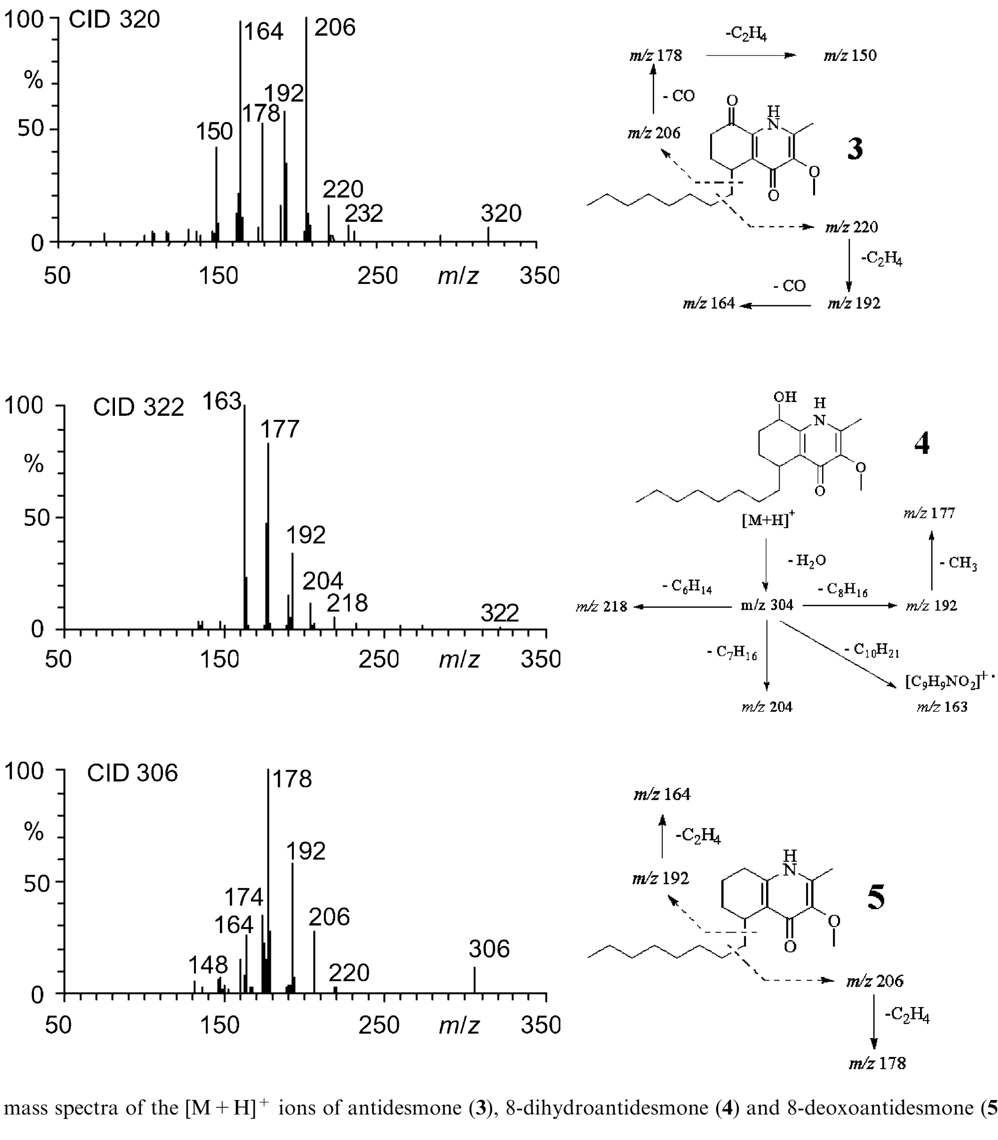


Fig. 2. 40 eV-CID mass spectra of the $[M+H]^+$ ions of antidesmone (**3**), 8-dihydroantidesmone (**4**) and 8-deoxoantidesmone (**5**) obtained by LC–ESI–MS.

### 2.4. Detection of antidesmone and related alkaloids in herbarium specimens

Levels of secondary metabolites depend on a number of biotic and abiotic factors, i.e., the age of the organism or tissue, season or possible pathogenic infection. Furthermore, compounds can be degraded by time and sometimes herbarium specimens are soaked in alcohol upon collection to assist drying. This treatment must be regarded as an extraction process and results in lower amounts of extractable matter.

Antidesmone (**3**) could be quantified in 8 samples of 13 *Antidesma* spp. as well as in one sample of *Hyeronima alchorneoides* (Table 1). The highest concentration found was about 65 ppm in a sample of *A. montanum* from 1935, indicating the stability of antidesmone over long periods. There are no obvious correlations between antidesmone concentration and extractable matter, age

Table 1
Antidesmone content and distribution of compounds **1**–**5** in herbarium specimens

| Species[a] | Collected: locality/date/ collector/specimen number | Antidesmone content (mg/kg) (*n*) | LC–MS/MS (SRM) analysis, compounds[b] | | | | | Soluble matter (%)[c] |
|---|---|---|---|---|---|---|---|---|
| | | | **1** | **2** | **3** | **4** | **5** | |
| *Antidesma neurocarpum* Miq. | Sarawak/21–10–1986/ Awa and Lee/ S 47545 | 19.9±2.3 (3) | – | – | x | – | – | 6.2 |
| *A. tomentosum* Blume | Sabah/25-04-?? (illegible, prob. 1960)/Meijer/SAN 21270 | 18.7±3.9 (3) | – | – | x[d] | – | – | 3.8 |
| *A. ghaesembilla* Gaertn. | China/26-04-1928/Tsang/16796 | 6.8±0.8 (3) | – | 0.004 | x[d] | – | – | 11.4 |
| *A. velutinosum* Blume | Malay Peninsula/04-01-1935/ Corner/SF 30210 | 30.4±4.0 (2) | 0.002 | 0.09[d] | x[d] | x[d] | x | 12.5 |
| *A. montanum* Blume | Malay Peninsula/19-08-1935/ Corner/SF 29836 | 64.9±25.5 (3) | – | 0.009 | x | – | x | 18.0 |
| *A. parvifolium* Thwaites and F. Muell. | Australia/30-04-1991/Forster/ PIF 8245 | – | – | – | x | – | x | 22.3 |
| *A. excavatum* Miq. | Solomon Islands/23-04-1964/ Whitmore/BSIP 3941 | 18.3 (1) | – | – | –[e] | – | – | 10.5 |
| *A. venosum* Tul. | Tanzania/09-10-1939/Vaughan 2921 | 6.1±1.0 (3) | – | 0.004 | x | | – | 8.1 |
| *A. membranaceum* Müll. Arg | Mozambique/13-12-1984/ Groendijk and Dungo/1602 | 2.0 (1) | – | 0.04 | x | – | – | 4.9 |
| *A. madagascariense* Lam. | Mauritius/24-04-1976/Coode 5050 | – | – | – | – | – | – | 9.3 |
| *A. laciniatum* Müll. Arg. | Cameroon/15-06-1987/Nemba & Thomas/545 | – | – | – | x | – | – | 4.5 |
| *A. acidum* Retz. | Thailand/28-06-1974/Larsen/ 33908 | – | – | – | x | – | – | 22.0 |
| *A. japonicum* Siebold and Zucc. | Thailand/10-06-1930/Kiah/ SFN 24284 | – | – | – | x | – | – | 18.1 |
| *Thecacoris stenopetala* Müll. Arg. | Liberia/03-12-1947/Baldwin/ 10501 | – | – | – | x | – | – | 16.4 |
| *Hyeronima alchorneoides* Fr. Allem. | Peru/03-01-1963/Iltis and Ugent/ 1113 | 4.4±1.3 (3) | 0.09 | 0.006 | x | – | – | 13.0 |
| *Maesobotrya barteri* (Baill.) Hutch. | Ivory Coast/16-10-1961/ de Wilde/3150 | – | – | – | – | – | – | 22.3 |
| *Uapaca robynsii* De Wild. | Malawi/03-05-1960/Adlard/354 | – | – | – | – | – | – | 8.5 |
| *Aporosa octandra* (Buch.-Ham. ex D. Don) Vickery var. *chinensis* (Champ. ex Benth.) Schot | Hong Kong/24–04–1969/Hu/ 7086 | – | – | – | – | – | – | 16.1 |

(*n*) Number of independently processed samples.

[a] All specimen have been kindly provided by the Royal Botanic Gardens, Kew.

[b] Concentrations for compounds **1** and **2** are given relative to **3**.

[c] Proportion of dry weight soluble in 80% aqueous MeOH.

[d] CID spectrum was obtained.

[e] Extraordinary high noise levels at the reaction trace 320= >206 prohibited secure identification of the antidesmone peak.

Table 2
Antidesmone content and distribution of compounds **1**–**5** in recently collected samples

| Species | Collected: locality/date/collector/specimen number | Plant part | Antidesmone content (mg/kg) (*n*) | LC–MS/MS (SRM) analysis, compounds[a] | | | | | Soluble matter (%)[b] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **1** | **2** | **3** | **4** | **5** | |
| *Antidesma venosum* Tul. | Dar es Salaam, Tanzania/ 26-8-1997/L.B. Mwasumbwi/111100[d] | Leaves | 41.0±3.0 (4) | 0.0002 | 0.01 | x[c] | – | – | 21.1 |
| | | Root | 14.9±1.3 (5) | 0.001 | 0.01 | x[c] | – | – | 8.4 |
| | | Bark | 18.3±0.90 (5) | – | 0.02 | x | – | – | 6.4 |
| *A. ghaesembilla* Gaertn. | Tam Dao, Vietnam/ March 1997/Nguyen Van Tap/647[e] | Leaves | 3.37±0.65 (3) | – | – | x | – | – | 11.2 |
| | | Bark | 2.44±0.12 (2) | – | 0.01 | x[c] | – | – | 9.5 |
| *A. ghaesembilla* Gaertn. | Vinh Phu, Vietnam/15-8-1998/Ngo Van Trai/647A[e] | Aerial parts[f] | | – | 0.02 | x | – | – | n. d. |

(*n*) Number of independently processed samples; n.d. = not determined.

[a] Concentrations for compounds **1** and **2** are given relative to **3**.

[b] Proportion of dry weight soluble in 80% aqueous MeOH.

[c] Daughter ion spectrum was obtained.

[d] A voucher specimen is deposited in The Herbarium, Department. of Botany, University of Dar es Salaam, Dar es Salaam, Tanzania.

[e] Voucher specimens are deposited in the Herbarium of the Institute of Materia Medica, Hanoi, Vietnam.

[f] From this specimen only a ready-made extract (repetitive extraction with 80% aqueous MeOH) was available.

or geographic origin. Comparison of herbarium and recently collected specimens gives a heterogeneous picture: Whereas samples from *A. ghaesembilla* show good agreement in antidesmone levels and extractable matter, the herbarium specimen of *A. venosum* is lower in extractable matter and antidesmone compared to the recently collected sample (Tables 1 and 2).

Antidesmone could be unambiguously identified by LC–ESI–SRM measurements in 12 of 13 samples from *Antidesma* herbarium specimens, as well as in *Hyeronima alchorneoides* and *Thecacoris stenopetala* (Table 1). The alkaloids **1**, **2**, **4** and **5** were observed only in low concentrations compared to **3** and only in samples with high antidesmone content. This indicates that they might also be present in samples with lower antidesmone concentration, but do not reach the detection limit. 18-Nor-antidesmone (**2**) has been found in five *Antidesma* spp. in concentrations of 0.004 to 0.09 relative to antidesmone. 17,18-Bis-nor-antidesmone (**1**) was detected only in *A. velutinosum* at levels of 0.002 relative to antidesmone and 0.02 relative to **2**. Although **2** occurs in *Hyeronima alchorneoides* in levels comparable to that in *Antidesma* spp. (0.006 relative to **3**), **1** was found in remarkably higher concentration (0.09 relative to **3**). This result is supported by the isolation of **1** and **3** from *Hyeronima oblonga* in equal amounts as described by Alves and Zani (1999).

## 3. Conclusions

Antidesmone (**3**) was unambiguously identified in 12 of 13 species of *Antidesma* herbarium specimen as well as in *Hyeronima alchorneoides* and *Thecacoris stenopetala*, the other investigated species of subtribe *Antidesminae*. However, antidesmone was not found in samples from outside this subtribe.

The relatively low levels of **3** ($<65$ mg/kg dry weight) suggest that it is a minor secondary metabolite. Antidesmone derivatives occur in even lower concentrations. The large amount of 8-dihydroantidesmone (**4**) isolated from roots of *Hyeronima alchorneoides* (Tinto et al., 1991) could not be verified using leaf material from the same species.

## 4. Experimental

### 4.1. General methods

NMR: $^1$H and 2D Varian Unity 500, 499.87 MHz, $^{13}$C Varian Unity 500, 125.7 MHz, with TMS ($^1$H) and $CDCl_3$ ($^{13}$C), respectively; 70 eV EIMS and HR–EIMS (resolution ca. 7.500): AMD 402 (AMD Intectra); CD and UV: Jasco J-710.

### 4.2. Gas chromatography–mass spectrometry

Fisons MD 800: EI (70 eV), source temp. 200 °C, column DB-5MS (J&W, 15 m×0.32 mm, 0.25 μm film thickness), inj. temp. 250 °C, interface temp. 300 °C, carrier gas He, flow rate 1.2 ml min$^{-1}$, splitless injection; column temp. program: 60 °C for 1 min, then raised to 110 °C at a rate of 25 °C min$^{-1}$, 110 °C held for for 3 min and then elevated to 200 °C at a rate of 10 °C min$^{-1}$; trimethylsilylating agent: MSTFA (*N*-methyl-*N*-trimethylsilytrifluoroacetamide). The $RR_t$ values were calculated with respect to 5α-cholestane ($R_t$ 5.95 min).

### 4.3. *High pressure liquid chromatography*

Merck/Hitachi D-7000 system, consisting of L-7100 low pressure gradient pump, L-7250 autosampler, L-7420 UV-detector, L-7480 fluorescence-detector and D-7000 interface; data processing: Merck-Hitachi D-7000 HSM software; column: Merck LiChroCart LiChrospher 100 RP-18 (5 μm, 250×4 mm) with guard column (4×4 mm, same material), flow rate: 1 ml min$^{-1}$, solvent: 80% MeOH, 20% 10 mM $K_2HPO_4/KH_2PO_4$-buffer (pH 7.5), after each run the column was eluted with MeOH for 10 min; detection: UV at 248 nm UV, fluorescence at 480 nm absorption and 525 nm emission.

### 4.4. *Liquid chromatography/electrospray tandem mass spectrometry*

Finnigan TSQ 7000, capillary temperature: 220 °C, ESI positive ion mode: spray voltage 4.5 kV, collision energy 40 eV, CID pressure 1.8 mT, collision gas: Ar; LC-Tech Ultra Plus pumps, Linear UVIS 200 detector, Sepserv Ultrasep ES RP-18 1×100 mm, 4 μm column, flow rate: 70 μl min$^{-1}$, gradient program: solvent A: water with 0.2% acetic acid, solvent B: MeCN with with 0.2% acetic acid, gradient starting at 50% B, then a linear increase to 90% B over 15 min, left at 90% B for 5 min; SRM program: **1**: *m/z* 292.4 = > 206.1: 0–6 min, **2**: *m/z* 306.4 = > 206.1: 6–9 min, **3:** *m/z* 320.4 = > 206.1: 9–12 min, **4:** *m/z* 322.4 = > 163.0: 12–15 min, **5:** *m/z* 306.4 = > 192.1: 15–25 min.

### 4.5. *Liquid chromatography/high resolution electrospray mass spectrometry*

API QSTAR Pulsar (Applied Biosystems) with Ion-Spray source, positive ion mode, spray voltage 5.5 kV, collision energy −40 eV, collision gas: $N_2$; LCPackings LC-system consisting of Famos autosampler, Ultimate Micro HPLC pump and UV detector and F&S $C_{18}$ PepMap 3 μm 150×0.3 mm column, flow rate 4 μl min$^{-1}$, gradient program: solvent A: water with 0.05% formic acid, solvent B: MeCN with 0.05% formic acid, starting at 50% B, then a linear increase to 98% B over 15 min, left at 98% B for 10 min (**3**: $t_R$ = 6.27 min; **4**: $t_R$ = 7.63 min; **5**: $t_R$ = 8.43 min).

### 4.6. *Extraction of plant material*

Ca. 200 μg plant material were crushed and weighed in a glass tube and then extracted three times with MeOH/H2O (4:1, v/v, 5 ml each, 15 min in an ultrasonic bath, then 23 h shaking at room temperature). Each portion of solvent was pipetted off and finally the residue was washed with 1 ml solvent. The combined extracts were taken to dryness in a vacuum centrifuge, the residue then redissolved in 1 ml $MeOH/H_2O$ (4:1, v/v), centrifuged and ca. 0.8 ml transferred into a vial. The extraction efficiency was 100%, because after two extraction steps no further extraction of antidesmone could be detected. For determination of soluble matter, 0.5 ml of the extract was pipetted after HPLC analysis into a weighed glass tube and, after solvent evaporation, re-weighed.

For LC–MS analysis, this material was then dissolved in 1 ml MeOH/H2O (1:1, v/v), placed onto a preconditioned (5 ml MeOH, then 5 ml $MeOH/H_2O$, 1:1, v/v) RP-18 SPE cartridge, washed with 3 ml $MeOH/H_2O$ (1:1, v/v) and eluted with 1 ml $MeOH/H_2O$ (19:1, v/v). This solution was taken to dryness again and redissolved in a suitable amount MeOH (ca. 20–200 μl). The recovery of antidesmone was determined to be >95%.

### 4.7. *HPLC assay*

The assay was carried out using the external standard method. Antidesmone (33.87 mg) was dissolved in a 100 ml volumetric flask in MeOH. For the first standard (33.87 μg ml$^{-1}$), 1 ml of this solution was diluted with 9 ml $MeOH/H_2O$ (4:1, v/v). Further standard solutions (6.774, 1.655 and 0.271 μg ml$^{-1}$) were prepared by appropriate dissolution with $MeOH/H_2O$ (4:1, v/v). Triple injections of each standard led to a linear calibration function (intercept: $2.11\times10^{-2}$, slope: $1.76\times10^{-5}$, $R^2$ = 0.9999). The limit of detection (0.25 μg ml$^{-1}$) was determined by extrapolation of the S/N ratios to S/N = 3. For establishing the reproducibility, six samples of homogeneous bark material were extracted and analysed.

*17,18-Bis-nor-antidesmone (**1**)*: The isolation procedure and spectroctroscopic data are given in Alves and Zani (1999). LC–MS (TSQ): $t_R$ = 5.30 min, CID–MS of *m/z* 292 ($[M+H]^+$), *m/z* (rel. int.): 292 (18), 220 (38), 206 (98), 192 (100), 178 (54), 164 (92), 150 (32).

*18-Nor-antidesmone (**2**)*: LC–MS (TSQ): $t_R$ = 7.75 min, CID–MS of *m/z* 306 ($[M+H]^+$), *m/z* (rel. int.): 306 (8), 220 (30), 206 (100), 192 (54), 178 (36), 164 (70), 150 (22).

*Antidesmone (**3**):* Spectroscopic data are given in Buske et al. (1999) and Bringmann et al. (2000a). LC–MS (TSQ): $t_R$ = 11.33 min, CID-MS of *m/z* 320 ($[M+H]^+$), *m/z* (rel. int.): 320 (26), 220 (24), 206 (100), 192 (70), 178 (30), 164 (72), 150 (26); ESI–CID–TOF–MS (QStar) *m/z*: 320.2224 (100, $[M+H]^+$, calc. for $C_{19}H_{30}NO_3$: 320.2220), 220.0959 (23, calc. for $C_{12}H_{14}NO_3$ 220.0968), 206.0814 (51, calc. for $C_{11}H_{12}NO_3$ 206.0812), 193.0720 (44, calc. for $C_{10}H_{11}NO_3$ 193.0733), 192.0654 (26, calc. for $C_{10}H_{10}NO_3$ 192.0655), 178.0849 (12, calc. for $C_{10}H_{12}NO_2$ 178.0863), 164.0707 (18, calc. for $C_9H_{10}NO_2$ 164.0706), 150.0533 (8, calc. for $C_8H_8NO_2$ 150.0550).

*8-Dihydroantidesmone (**4**)*: Synthesis is described in Buske et al. (1999). Corrected spectroscopic data: UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 200 (4.11), 220 (4.06), 273 (3.78); CD $\lambda^{MeOH}$ nm ($\Delta\varepsilon$): 204 (−2.89) 228 (2.67), 263 (4.77), 285 (−1.91); EI–MS (rel. int.): *m/z* 321.2308 ($M^+$ calcd. for

$C_{19}H_{31}NO_3$: 321.2303, 19), 320 ($[M-H]^+$, 18), 293 ($[M-CO]^+$, 27), 292 (22), 265 ($[M-CO-C_2H_4]^+$, 28), 222 (13) 209 (67), 208 (26), 191 (36), 190 (100), 181 (56), 175 (42), 166 (72), 153 (60), 150 (25), 130 (35); $^1$H–NMR, $CDCl_3$, $\delta$ ($J$ [Hz]): 4.74 (8-H, *dd*, 9.6/7.0), 3.74 (3-OMe, *s*), 2.90 (5-H, br *s*), 2.35 (2-Me, *s*), 2.07 (7-$H_{eq.}$, *m*), *1.94* (6-$H_{eq.}$), *1.90* (7-$H_{ax.}$), *1.76* (11-$H_A$), 1.620 (6-$H_{ax.}$, *m*), *1.46* (12-H), *1.4–1.2* (13-, 14-, 15-H), *1.38* (11-$H_B$), *1.27* (17-H), *1.25* (16-H), 0.856 (18-H, *t*, 7.0); values printed in *italics* are chemical shifts of HMQC correlation peaks; $^{13}$C NMR, $CDCl_3$, $\delta$: 162.7 (C-4), 149.5 (C-10), 145.6 (C-3), 142.7 (C-2), 128.0 (C-9), 66.0 (C-8), 62.0 (3-OMe), 32.5 (C-11), 32.0 (C-16), 31.8 (C-5), 29.6, 29.5, 29.4 (C-13, -14, -15), 28.0 (C-12), 26.0 (C-7), 22.8 (C-6), 22.7 (C-17), 14.1 (C-18, 2-Me); LC–MS (TSQ): $t_R = 12.98$ min, CID–MS of $m/z$ 322 ($[M+H]^+$), $m/z$ (rel. int.): 322 (4), 218 (6), 204 (12), 192 (32), 178 (50), 177 (84), 163 (100); ESI–CID–TOF–MS (QStar) $m/z$: 322.2362 (13, $[M+H]^+$, calc. for $C_{19}H_{32}NO_3$: 322.2377), 304.2282 (77, calc. for $C_{19}H_{30}NO_2$ 304.2271), 218.1186 (5, calc. for $C_{13}H_{16}NO_2$ 218.1176), 204.1024 (8, calc. for $C_{12}H_{14}NO_2$ 204.1019), 192.1026 (100, calc. for $C_{11}H_{14}NO_2$ 192.1042), 178.0856 (96, calc. for $C_{10}H_{12}NO_2$ 178.0868), 177.0778 (50, calc. for $C_{10}H_{11}NO_2$ 177.0784), 163.0635 (58, calc. for $C_9H_9NO_2$ 163.0628); GC–MS (of a trimethylsilylated sample): $RR_{t1}$ 0.667 (93.6% of TIC), $RR_{t2}$ 0.695 (6.3% of TIC, similar spectrum), $m/z$ (rel. int.): 465 ($M^+$, 100), 450 ($[M-Me]^+$, 88), 435 ($[M-CH_2O]^+$, 45), 376 (28), 353 (23), 352 (13) 311 (21), 306 (30), 263 (63), 262 (58), 246 (22), 232 (55), 130 (26).

*8-Deoxoantidesmone (**5**)*: LC–MS (TSQ): $t_R = 18.43$ min, CID-MS of $m/z$ 306 ($[M+H]^+$), $m/z$ (rel. int.): 306 (20), 206 (24), 192 (66), 178 (100), 177 (84), 174 (24), 168 (22), 160 (10); ESI–CID–TOF–MS (QStar) $m/z$: 306.2432 (100, $[M+H]^+$, calc. for $C_{19}H_{32}NO_2$: 306.2428), 206.1167 (22, calc. for $C_{12}H_{16}NO_2$ 206.1176), 192.1009 (32, calc. for $C_{11}H_{14}NO_2$ 192.1019), 179.0931 (59, calc. for $C_{10}H_{13}NO_2$ 179.0941), 178.0854 (52, calc. for $C_{10}H_{12}NO_2$ 178.0863), 175.0975 (21, calc. for $C_{11}H_{13}NO$ 175.0992), 164.0712 (10, calc. for $C_9H_{10}NO_2$ 164.0711).

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