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Antidesmone, a novel type isoquinoline alkaloid from *Antidesma membranaceum* (Euphorbiaceae)[1]

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Abstract: A novel type of tetrahydroisoquinoline alkaloid, antidesmone (**1**), was isolated from *Antidesma membranaceum* Müll. Arg.. The structure of **1** was determined to be (5*S*)-1-hydroxy-4-methoxy-3-methyl-5-octyl-5,6,7,8-tetrahydroisoquinolin-8-one by spectroscopic methods (MS, ^{1}H, ^{13}C 2D NMR, CD) and chemical derivatisation. The absolute (*S*)-configuration was determined by quantumchemical calculation of CD spectra.

Introduction

Antidesma membranaceum Müll. Arg. is a shrub or small tree occurring in equatorial Africa. It belongs to the subfamily of the Phyllanthoideae, which seems to be somewhat different in phytochemistry to the well known monoovulate subfamilies of the Euphorbiaceae [1]. From the genus *Antidesma* hitherto only two alkaloids have been reported [2], both from the cyclopeptide type. In continuation of our studies on the bioactive compounds of tropical and subtropical plants we have recently reported six new benzopyranones as well as feruloyl amides and ferulic acid derivatives to be found in *A. membranaceum* [3]. The present paper describes the structural elucidation of antidesmone (**1**) (Fig. 1), a novel type tetrahydroisoquinoline alkaloid with the nitrogen being located in the aromatic ring.

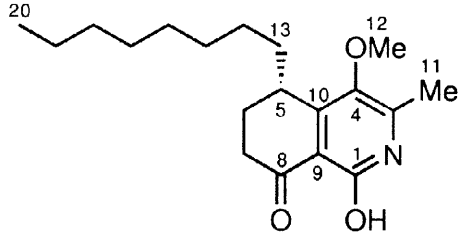

Figure 1: Structure of antidesmone (**1**).

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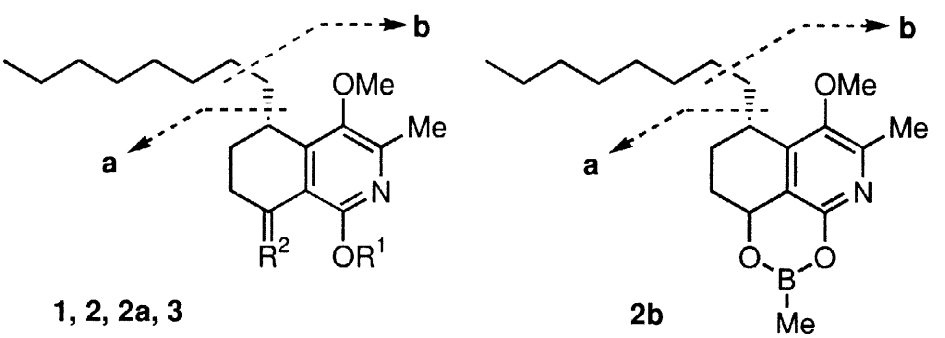
Results and Discussion

Leaves, bark and root material of *A. membranaceum* were air-dried, ground and extracted with 80% MeOH (aq) separately. The extracts were concentrated to the aqueous phase and subsequently extracted with *n*-hexane and ethyl acetate. **1** was first isolated from the *n*-hexane extract of root material by repeated column chromatography performed on silica gel using *n*-hexane-ethyl acetate gradients. Finally preparative HPLC on RP-18 yielded **1** as a pale yellow oil.

In the EIMS spectrum the molecular ion peak at *m/z* 319 indicated the presence of nitrogen in the molecule. The HR-EIMS revealed the molecular composition $C_{19}H_{29}NO_3$. The mass spectral fragmentation is mainly characterised by the formation of ions with *m/z* 318 ($[M-H]^+$), 291 ($[M-CO]^+$), 263 ($[M-CO-C_2H_4]^+$) as well as cleavages in the side chain (**a**, **b**) (Fig. 2). The base peak at *m/z* 207 ($[M-C_8H_{16}]^+$) strongly indicated an octyl side chain. From ^1H, ^{13}C and 2D (COSY, NOESY, HMQC and HMBC) NMR experiments the presence of a cyclohexenone ring became obvious, containing an carbonyl function (δ_{13C} 194.3), two quaternary aromatic carbons (δ 139.0 and 132.2) and three aliphatic carbons (δ 32.2, 30.3 and 24.3). The geminal coupling constant of the two protons attached to C-7 (final counting mode, $J_{H\text{-}7ax/H\text{-}7eq}$ 18.2 Hz) indicates an oxo function at C-8. The alkyl chain was connected to C-5 (δ 30.3) in axial position as shown by the analysis of the coupling constants using homonuclear decoupling of H-5 ($^3J_{H\text{-}5/H\text{-}6ax}$ 4.7 Hz; $^3J_{H\text{-}5/H\text{-}6eq}$ 2.4 Hz). Methyl hydrogens at δ 2.37 showed two HMBC correlations to aromatic carbons at δ 138.9 and 147.5, the latter also having a correlation to a methoxy group. The lack of a further correlation from the methyl group indicated that the



1, 2, 2a, 3 **2b**

	R^1=	R^2=	**a** *m/z* (rel.int.)	(**a** +H) *m/z* (rel.int)	**b** *m/z* (rel.int.)
1	H	O	206 (26)	207 (100)	220 (23)
2	H	H, OH	208 (28)	209 (69)	222 (16)
2a*	TMSi	H, OTMSi	352 (13)	353 (23)	366 (10)
2b*			-	233 (100)	246 (21)
3	CH_3	O	220 (30)	221 (100)	234 (22)

Figure 2: Structure and main key fragments of the EI mass spectra of **1**, **2**, **2a**, **2b** and **3**, * obtained by GC-MS.

connected aromatic carbon must be neighboured by the nitrogen. HMBC correlations relevant for the structural elucidation are shown in Tab.2. No HMBC correlations could be found to the fifth aromatic carbon (δ 172.8), but from chemical shift and the molecular formula one can deduce the presence of a hydroxyl substituent.

For a further proof of structure, **1** was reduced with $LiAlH_4$ (4 h, r.t., abs. ether) at C-8 giving the dihydro compound **2**. Upon trimethylsilylation to **2a** both diastereomers could be separated by GC-MS in a 15:1 ratio. Since NMR experiments on **2** did not give any further information about the position of the aromatic hydroxy group, **2** was methylboronated to afford **2b**. The detection of **2b** by GC-MS proved the 1-position of the aromatic hydroxy group, since the formation of methylboronates is limited to 1,2 and 1,3-diols [4]. Therefore, the structure of antidesmone was elucidated as 1-hydroxy-4-methoxy-3-methyl-5-octyl-5,6,7,8-tetrahydroisoquinolin-8-one (**1**).

Table 1: ^{1}H and ^{13}C NMR data of **1**, **2** and **3** (in $CDCl_3$ unless otherwise noted).

	1		**2**		**3**
Pos.	δ_C[1]	δ_H[1,2]	δ_C[3]	δ_H[1,2]	δ_H[1,4]
1	172.8 s		162.7		
3	138.9 s		142.7		
4	147.5 s		145.6		
5_{eq}	30.3 d (133)	3.266 br s ($\Delta_{1/2}$= 18)	31.8	2.904 br s	3.334 m
6_{ax}	24.3 t (129)	2.084 dddd (14.7/14.0/4.7/4.4)	22.8	1.620 m	2.057 tdd (13.6/6.0/4.8)
6_{eq}		2.200 dddd (14.0/5.3/2.4/2.4)		1.94	2.124 ddt (13.6/6.4/2.3)
7_{ax}	32.2 t (128)	2.750 ddd (18.2/14.7/5.3)	26.0	1.90	2.819 ddd (18.2/13.6/6.4)
7_{eq}		2.578 ddd (18.2/4.4/2.4))		2.069 m	2.632 ddd (18.2/6.0/2.3)
8	194.6 s		66.0	4.743 dd (9.6/7.0)	
9	132.2 s		128.0		
10	139.0 s		149.5		
11	14.5 q (130)	2.366 s	14.1	2.347 s	2.464 s
12	59.4 q (145)	3.935 s	62.0	3.739 s	3.801 s
13	30.5 t (127)	1.765 m; 1.41	32.5	1.76; 1.38	n.d.
14	29.4 t (125)	1.46	28.0	1.46	n.d.
15 - 17	29.6, 29.5, 29.2	1.4 - 1.2	29.6, 29.5, 29.4	1.4 - 1.2	n.d.
18	31.8 t (127)	1.25	32.0	1.25	n.d.
19	22.6 t (126)	1.26	22.7	1.27	n.d.
20	14.0 q (126)	0.874 t (7.0)	14.1	0.856 tr (7.0)	0.892 t (7.1)

[1] In parenthesis: coupling constants in Hz; [2] values without multiplicity are chemical shifts of HMQC correlation peaks; [3] $CDCl_3$ + CD_3OD; [4] -OMe at δ 3.765

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

	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-13	C-14
$H\text{-}5_{eq}$									X	
$H\text{-}6_{ax}$			X		X	X		X		
$H\text{-}6_{eq}$			X					X		
$H\text{-}7_{ax}$			X	X		X				
$H\text{-}7_{eq}$			X	X		X	X			
Me-11	X	X								
OMe-12	X									
H-13A			X							
H-13B			X							X

For the elucidation of the absolute configuration of antidesmone (**1**), the comparison of calculated and experimental CD spectra proved to be a particularly valuable tool as shown earlier in our group in the case of most different classes of compounds [5]. In order to avoid complications in the CD calculations by the possible existence of tautomeric mixtures, the quantumchemical investigations were not performed on **1** itself, but on its methylether **3**, which was formed upon reaction with diazomethane (r.t., 4h) and purified by HPLC.

In a first step a conformational analysis of one of the two possible enantiomers of **3** was performed, arbitrarily for *S*-**3**. For each of the conformers obtained, a theoretical CD spectrum was calculated and subsequently added up by the means of Boltzmann statistic (see Computational). In order to take into account a systematic shift of the calculated CD spectrum compared with the experimental one, a 'UV correction' was carried out as recently introduced by our group [5]. The good correspondence of the CD spectrum calculated for the *S*-configured enantiomer establishes this absolute configuration for the methylated product **3**. Thus, the natural product antidesmone (**1**) must be *S*-configured, too. By contrast, as expected, the spectrum calculated for the *R*-enantiomer is near-opposite to the experimental one.

Antidesmone showed strong fungitoxic activity down to amounts of 1.25 nmol in a bioassay based on *Cladosporium cucumerinum* [6].

Antidesmone (**1**) represents an unprecedented novel type of alkaloids. In contrast to other tetrahydroisoquinoline alkaloids, the nitrogen is located in the aromatic ring and the substitution pattern, in particular the unusual *n*-octyl residue on the isocyclic ring, is unique, too. These structural peculiarities suggest a likewise novel biosynthetic pathway to isoquinoline alkaloids apparently not from aromatic amino acids.

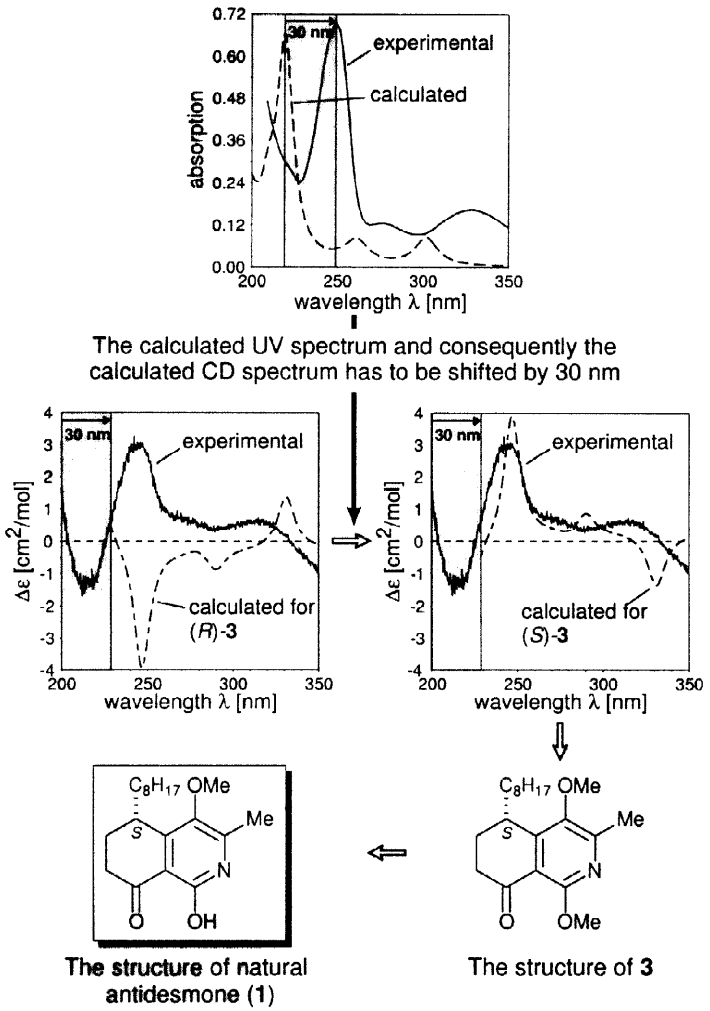


Figure 3: Calculated UV and CD spectra of **3** and comparison with the experimental ones.

Experimental

General Methods. **NMR**: Unity 500 and Gemini 300 (VARIAN) with TMS as internal standard; 70 eV EIMS and HR-EIMS (resolution ca. 7.500): AMD 402 (AMD Intectra); HPLC: Merck-Hitachi L-7100 Pump, L-7450A DAD, CD and UV: Jasco J-710.

Gas Chromatography-Mass Spectrometry. MD-800 (Fisons Instruments): EI (70 eV), source temp. 200°, column DB-5MS (J&W, 15 m x 0.25 mm, 0.25 mm film thickness), inj. temp. 250°, interface temp. 300°, carrier gas He, flow rate 1 ml min^{-1}, splitless injection; column temp. program: 170° for 1 min, then raised to 270° at a rate of 25 grd min^{-1} and then elevated to 290° at a rate of 2 grd min^{-1}. The RR_t values were calculated with respect to 5α-cholestane (R_t=5.95 min).

HPLC. Separation was done on a 125 x 8 mm, LiChrospher RP-18, 5 μm column (Bischoff) with 80% MeOH (isocratic) at a flow of 3ml min^{-1} and detection at 248 nm.

Plant Material. A. membranaceum was collected on a Frontier Expedition of the Society for Environmental Exploration, London, in August 1994 in Margrotto Hill, East Usambaras, Murenga District, Tanga Region,

Tanzania. It was identified by Mr. Leonard Mwasumbi, Herbarium, Department of Botany, University of Dar es Salaam, Tanzania. The voucher specimen number is Mwasumbi NO 17130.

Extraction and Purification. Dried plant material of leaves (1391 g), bark (1.541 g) and root (314 g) was extracted separately with 80 % MeOH (leaves: 24 l, bark: 30 l and root: 9 l), concd *in vacuo* to aq. phase, successively extracted with *n*-hexane (2.5 l, 2.3 l, 1.7 l) and EtOAc (2.5 l, 2.5 l, 1.5 l) and the organic phases concd *in vacuo* to yield the dry extracts (leaves: *n*-hexane extract: 1.55 g, EtOAc extract: 15.91 g, bark: 2.51 g; 10.63 g, root: 1.35 g and 1.34 respectively).

Chromatography (*n*-hexane with increasing amounts of EtOAc on 100g silica gel Merck 0.063-0.2mm) of 1g of the *n*-hexane-extract of the roots gave with 50% EtOAc a fraction (22.7 mg) which was further purified by flash chromatography ($CHCl_3$ on 20g silica gel Merck 0.04-0.063 mm) and preparative HPLC to yield 1.8 mg **1**. Isolation of **1** from the other extracts was done in a similar procedure.

Antidesmone (**1**) ((5*S*)-1-hydroxy-4-methoxy-3-methyl-5-octyl-5,6,7,8-tetrahydroisoquinolin-8-one): pale yellow oil, $[\alpha]_D^{26.5}$ = 24° (c=0.2, $CHCl_3$), UV λ_{max}^{MeOH} nm (log ε): 247 (4.21), 275 (3.44), 327 (3.52), CD λ^{MeOH} nm (Δε): 212 (-4.38) 246 (5.63), 279 (1.87), 315 (2.36), 354 (-3.19), IR $\nu_{max}^{CCl_4}$ [cm^{-1}]: 3380, 2926, 2854, 1735, 1618, 1581, 1530, 1457, 1378, 1353, 1327, EI-MS (rel. int.): *m/z* 319.2143 (M^+ calc. for $C_{19}H_{29}NO_3$: 319.2147, 4), 318 ($[M-H]^+$, 5), 292 (12), 291.2211 ($[M-CO]^+$, calc. for $C_{18}H_{29}NO_2$ 291.2198, 45), 263.1890 ($[M-CO-C_2H_4]^+$, calc. for $C_{16}H_{25}NO_2$ 263.1885, 18), 234 ($[M-C_6H_{13}]^+$, 26), 220.0988 (**b**, calc. for $C_{12}H_{14}NO_3$ 220.0974, 23), 207.0880 (**a**+H, calc. for $C_{11}H_{13}NO_3$ 207.0895, 100), 206 (**a**, 26), 189 (**b**-H-CH_2O, 10), 178 (**a**-CO, 9), 164 (6).

Reduction of 1: 4 mg **1**, dissolved in 2 x 2 ml abs. ether were given to a solution of 7.5 mg $LiAlH_4$ in 4 ml abs. ether, stirred 4 h at r.t., then EtOAc was added and the solution filtered. After purification by HPLC 2.5 mg **2** were obtained.

1,8-Dihydroxy-4-methoxy-3-methyl-5-octyl-5,6,7,8-tetrahydroisoquinoline (**2**): amorphous, UV λ_{max}^{MeOH} nm (log ε): 200 (4.11), 220 (4.06), 273 (3.78), CD λ^{MeOH} nm (Δε): 204 (-2.89) 228 (2.67), 263 (4.77), 285 (-1.91), EI-MS (rel. int.): *m/z* 321.2308 (M^+ calc. 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 (**b**, 13) 209 (**a**+H, 67), 208 (**a**, 26), 191 (**a**+H-H_2O, 36), 190 (**a**-H_2O, 100), 181 (**a**+H-CO, 56), 175 (42), 166 (72), 153 (60), 150 (25), 130 (**a**-2CO, 35).

Trimethylsilylation of 2. **2** (ca. 100 µg) was reacted with a mixture of *N,O*-bis (trimethylsilyl)acetamide/trimethylchlorosilane (4:1 v/v) for 30min at r.t.. GC-MS: 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 (**a**+H, 23), 352 (**a**, 13) 311 (21), 306 (30), 263 (63), 262 (58), 246 (22), 232 (55), 130 (26).

Methylboronation of 2. Upon reaction of ca. 100 µg **2** with methylboronic acid in pyridine at 70° for 30 min **2b** was formed. GC-MS (inj. temp. 300°, column temp. program: 170° for 1min, then raised to 270° at a rate of 30 grd min^{-1}): RR_t 1.076, *m/z* (rel.int.): 345 (M^+, 45), 302 (13), 246 (**b**, 21), 233 (**a**+H, 100), 130 (7).

Methylation of 1.. 2 mg of **1** were dissolved in 2 ml of a solution of diazomethane in ether with a trace of water added and left for 4 h at r.t.. Then the solvent was evaporated at atmospheric pressure and the substance purified by HPLC to yield 1.1mg of **3**.

(5S)-1,4-Dimethoxy-3-methyl-5-octyl-5,6,7,8-tetrahydroisoquinolin-8-one (**3**): UV λ_{max}^{MeOH} nm (log ε): 246.6 (3.149), 272.4 (2.55), 326,8 (2.53), CD λ^{MeOH} nm (Δε): 242.2 (0.53), 314.6 (0.09), 356.0 (-0.17), EI-MS *m/z* (rel.int.): 333 (M^+, 7), 318 (8), 305 (32), 290 (4), 277 (10), 248 (26), 234 (**b**, 22), 221 (**a**+H, 100), 220 (**a**, 30), 207 (22), 203 (22), 193 (23), 188 (24), 178 (35), 149 (38), 145 (27), 129 (10).

Computational

The conformational analysis was performed on SGI IRIS 4D INDIGO (R4000) and SNI LinuX *i*P6 workstations using two methods: the random search algorithm as implemented in the molecular modeling program SYBYL 6.4 [7] and the semiempirical AM1 [8] parametrization as implemented in the program package VAMP 5.5 [9].

For the calculation of the rotational strenghts R_{0a} of an electronic transition from the groundstate ψ_0 to an excited state ψ_a, the following equation according to the dipol velocity formalism [10] was used:

$$R_{0a} = \Im\left\{\frac{e\hbar}{im(E_a - E_0)} p_{0a} \cdot m_{a0}\right\}$$

The rotational strength R_{0a} thus calculated gives origin-independent results even for approximated wavefunctions. The wavefunctions of the excited states ψ_a were obtained by a CNDO/S-CI [11, 12] calculation, in which the CI expansion takes into account the ground state ψ_0 and all n and π orbitals. These calculations were carried out on LinuX workstations using the BDZDO/MCDSPD [11] program package.

$$\sigma_{0a}(\lambda) = -\frac{1}{\Delta m\sqrt{\pi}} e^{-\left(\frac{\lambda-\lambda_a}{\Delta m}\right)^2}$$

For a better comparison of the theoretical CD spectrum with the experimental one, a Gaussian band shape function of the type with an empirical halfband width Δm was generated over the calculated rotational strength values.

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