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Cis- and *trans*-isoshinanolone from *Dioncophyllum thollonii*: absolute configuration of two 'known', wide-spread natural products[1]

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Abstract

From the rare West African liana, *Dioncophyllum thollonii* (Dioncophyllaceae), the known acetogenic tetralones, *cis*- and *trans*-isoshinanolone, were isolated. Exemplarily on this material, a new ruthenium-catalyzed oxidative degradation procedure, related to the well-established stereoanalysis of 1,3-dimethyltetrahydroisoquinolines, was elaborated. The method allows to unambiguously attribute the absolute configuration of these natural products, which likewise occur in several other plant species. For the rapid discrimination between the four possible stereoisomeric forms of isoshinanolone (i.e. the *cis*- and *trans*-diastereomers and their enantiomers), an HPLC-analytical procedure on a chiral stationary phase has been developed.

Keywords: *Dioncophyllum thollonii*; Dioncophyllaceae; *cis*-Isoshinanolone; *trans*-Isoshinanolone; Tetralones, naturally occurring; Ruthenium-mediated oxidative degradation; Structural elucidation; Stereochemistry

1. Introduction

Isoshinanolone (**1**) (Tezuka et al., 1973) constitutes an acetogenic (Bringmann, Wohlfarth, Rischer, Rückert, & Schlauer, 1998) natural tetralone of wide-spread occurrence in various plant families, like Iridaceae (Kumar, Meepagala, & Balasubramaniam, 1985), Plumbaginaceae (Gunaherath, Gunatilaka, Sultanbawa, & Balasubramaniam, 1983; Bhattacharyya & de Carvalho, 1986), Nepenthaceae (Likhitwitayawuid, Kaewamatawong, Ruangrungsi, & Krungkrai, 1998), Dioncophyllaceae (Lavault & Bruneton, 1980; Hanson, Crawford, & Thanasingh, 1981), Ancistrocladaceae (Bringmann et al., 1998a; Bringmann & Pokorny, 1995; Bringmann, Schneider, & Aké Assi, 1991; Anh, Ripperger, Porzel, Sung, & Adam, 1997) and Ebenaceae (Tezuka et al., 1973; Zhong, Waterman, & Jeffreys, 1984; Bin Zakaria, Jeffreys, Waterman, & Zhong, 1984; Richomme, Papillon, Cabalion, & Bruneton, 1991). In Ancistrocladaceae and Dioncophyllaceae, isoshinanolone, and its obvious naphthoquinone precursor, plumbagin (**2**), may be formed in major amounts under various conditions of chemical, physical, and biotic stress (Bringmann, François, Aké Assi, & Schlauer, 1998; Bringmann et al., submitted for publication (b)), apparently as an alternative to the normally predomi-

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[1] Part 127 in the series 'Acetogenic isoquinoline alkaloids'. For part 126, see Bringmann et al., submitted for publication (a).

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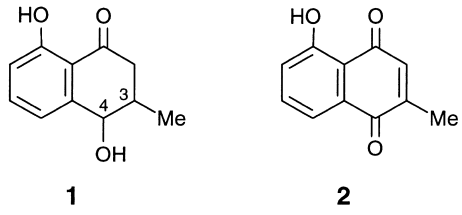


nant formation of acetogenic naphthylisoquinoline alkaloids (Bringmann & Pokorny, 1995).

Despite the importance of isoshinanolone and its possible stereoisomeric forms (see Fig. 1), **1a** (*cis*) and **1b** (*trans*, '*epi*-isoshinanolone' (Zhong et al., 1984)) and their enantiomers, *ent*-**1a** ('*neo*-isoshinanolone' (Kumar et al., 1985)) and *ent*-**1b** (again named '*epi*-isoshinanolone' (Bhattacharyya & de Carvalho, 1986)), astonishingly little work has been done on their unambiguous stereoanalysis. In this paper, we report on a novel, unequivocal procedure for the attribution of the absolute configuration of isoshinanolone (**1**), by ruthenium-mediated oxidative degradation and subsequent stereoanalysis of the resulting 2-methylsuccinic acid. Furthermore, a rapid chromatographic stereoanalysis for the distinction of the four stereoisomeric forms of isoshinanolone, by chromatography on a chiral stationary phase, is presented.

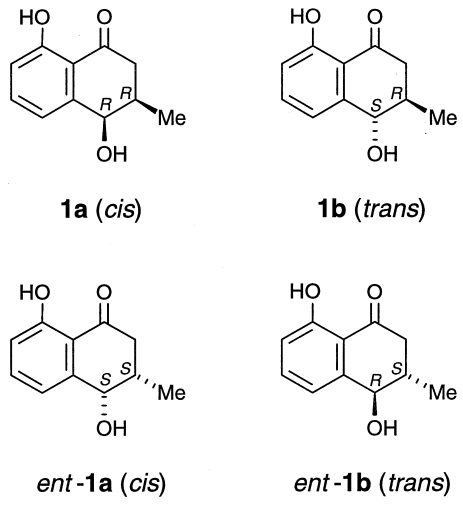


Fig. 1. The four possible stereoisomeric forms of isoshinanolone (**1**).

2. Results and discussion

2.1. Previous methods for the stereoanalysis of isoshinanolones

The determination of the relative configuration at C-3 vs. C-4 poses no particular problem, given the significantly different coupling constants between 3-H and 4-H for the *cis*- and *trans*-isomers (2.5 and 7.5 Hz, respectively (Tezuka et al., 1973)). Most of the isoshinanolone-containing plants seem to produce the *cis*-diastereomer exclusively, exceptions being *Diospyros canaliculata* (Zhong et al., 1984) and *Plumbago scandens* (Bhattacharyya & de Carvalho, 1986). Initial attempts to distinguish the two *cis*-enantiomers (**1a** and *ent*-**1a**) by their optical rotations, led to most ambiguous results, since their α_D-values in the literature range from -7° (Tezuka et al., 1973) to $+200^\circ$ (Lavault & Bruneton, 1980), as shown in more detail in Table 1.

Due to the large variation of the optical rotations, some authors assumed that their samples might constitute enantiomeric mixtures (Kumar et al., 1985; Richomme et al., 1991). The absolute stereostructure of *cis*-isoshinanolone from *Diospyros maritima* was attributed as **1a**, i.e. with 3*R*,4*R*-configuration, by comparison of the CD spectrum of its dibenzoate with that of the dibenzoate of the related natural product shinanolone (**3**) (see Fig. 2) (Tezuka et al., 1973). The absolute configuration of **3**, for its part, had been attributed using the exciton chirality method (Nakanishi, Berova, & Woody, 1994). This conclusion, however (Tezuka et al., 1973), to which most other papers refer (see Table 1) might be critical, given the a priori unknown conformational behavior of **1** compared with that of **3**, due to the methyl group and thus additional stereogenic center in the cyclohexenone ring and the authors themselves (Tezuka et al., 1973) and others (Kumar et al., 1985) have expressed some doubt on the validity of the method in this case. Because of all these uncertainties, the development of an independent analytical tool for the rapid and unambiguous stereoanalysis of the absolute configuration of *cis*- or *trans*-isoshinanolone from any desired plant source, was an urgent demand.

2.2. Degradative stereoanalysis of isoshinanolones

For the rapid attribution of the absolute stereostructures of tetrahydroisoquinolines or -β-carbolines **A**, we have established (Bringmann, Geuder, Rübenacker, & Zagst, 1991) and further improved (Bringmann, God,

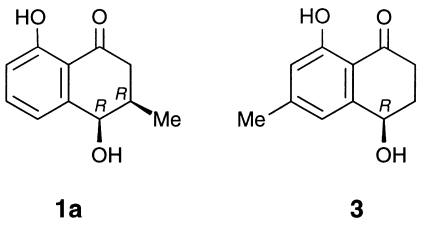


Fig. 2. *cis*-Isoshinanolone (**1a**) and the structurally 'related' tetralone shinanolone (**3**), as configuratively attributed by CD spectroscopy.

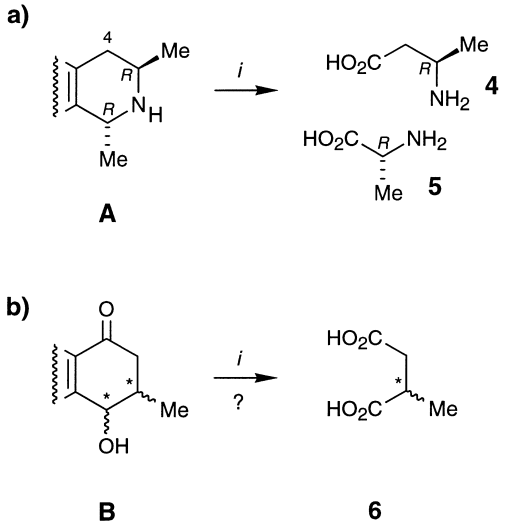
Table 1
Largely divergent α_D-values of isoshinanolone (**1**) from various plant sources, as reported in the literature

Plant source	relative configuration	absolute configuration[a]	$[\alpha]_D$ (solvent)
Iridaceae			
Aristea ecklonii (Kumar et al. 1985)	*cis*	3*S*,4*S*[b] (partly racemized?) (Tezuka et al., 1973; Gunaherath et al., 1983)	+24° ($CHCl_3$)
Plumbaginaceae			
Plumbago zeylanica (Gunaherath et al., 1983)	*cis*	3*R*,4*R*[c] (Tezuka et al., 1973)	+24.17° [d]
Plumbago scandens (Bhattacharyya & de Carvalho, 1986)	*cis*/*trans*[e]	3*S*,4*S*/3*S*,4*R*[f] (4:1) (Tezuka et al., 1973; Gunaherath et al., 1983)	+19.7° ($CHCl_3$)
Nepenthaceae			
Nepenthes thorelii (Likhitwitayawuid et al., 1998)	*cis*	3*S*,4*S*[f,g] (Tezuka et al., 1973; Bhattacharyya & de Carvalho, 1986)	+20.2° ($CHCl_3$; *c* 0.2)
Dioncophyllaceae			
Dioncophyllum thollonii (Lavault & Bruneton, 1980)	*cis*	'attribution not possible' (Tezuka et al., 1973)	+200° ($CHCl_3$; *c* 1)
Habropetalum dawei (Hanson et al., 1981)	*cis*	3*S*,4*S*[b] (Tezuka et al., 1973)	+33° ($CHCl_3$; *c* 0.97)
Ancistrocladaceae			
Ancistrocladus barteri (Bringmann & Pokorny, 1995; Bringmann et al., 1991)	*cis*	3*R*,4*R*[b] (Tezuka et al., 1973)	+20° ($CHCl_3$)
Ancistrocladus cochinchinensis (Anh et al., 1997)	*cis*	3*S*,4*S*[f,g] (Tezuka et al., 1973; Hanson et al., 1981)	[d], positive [d]
Ebenaceae			
Diospyros maritima (Tezuka et al., 1973)	*cis*	3*R*,4*R*[b,c]	−7° ($CHCl_3$; *c* 0.078)
Diospyros canaliculata (Zhong et al., 1984)	*cis*/*trans*[e]	3*R*,4*R*/3*R*,4*S* (Tezuka et al., 1973) (1:1)	+16° [d]
Diospyros samoensis (Richomme et al., 1991)	*cis*	racemic?	0° ($CHCl_3$; *c* 1)
Diospyros siamang (Bin Zakaria et al., 1984)	*cis*	3*R*,4*R*[g]	[d]
Diospyros wallichii (Bin Zakaria et al., 1984)	*cis*	3*R*,4*R*[g]	[d]

[a] Attributed by comparison with data from the refs. given.
[b] Tentative or uncertain attribution (e.g. due to contradictory or inconsistent α_D-data).
[c] Attributed by CD investigations on the dibenzoate.
[d] Corresponding data or solvent not given.
[e] Diastereomers not separated.
[f] Configuration not verbally expressed, but graphically shown.
[g] No argument given.

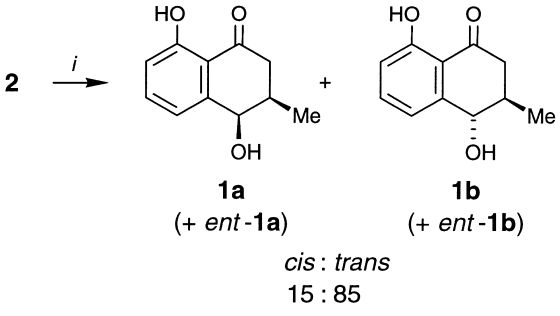
& Schäffer, 1996) a ruthenium-mediated oxidative degradation procedure[2], which neatly cuts out the stereocenters in the form of known and easy-to-analyze aminoacids (see Scheme 1(a)). This useful analytical device works even on a submilligram scale and is routinely applied to any new naphthylisoquinoline alkaloid isolated (Bringmann & Pokorny, 1995; Bringmann et al., 1998b). Besides the 'regular' degradation products from **A**, 3-aminobutyric acid (**4**) and alanine (**5**), an additional loss of a C_1-unit of **4** may occur (Bringmann et al., 1996), in particular if the α-atom next to the originating carboxylic function is already functionalized; thus dioncophyllinol D (i.e. a 4-hydroxytetrahydroisoquinoline) gives alanine (**5**) as the only degradation product, no 2-hydroxy-3-aminobutyric acid (Bringmann et al., 1998c). This led us to anticipate that isoshinanolone might be degraded, under identical conditions, to 2-methylsuccinic acid (**6**) as a stereochemically well known and thus easily analyzable degradation product (see Scheme 1(b)).

[2] For other ruthenium-mediated oxidative degradation reactions see Carlsen, Katsuki, Martin, and Sharpless (1981) and Gill, Gimenez, Jhingran, and Palfreyman (1990).

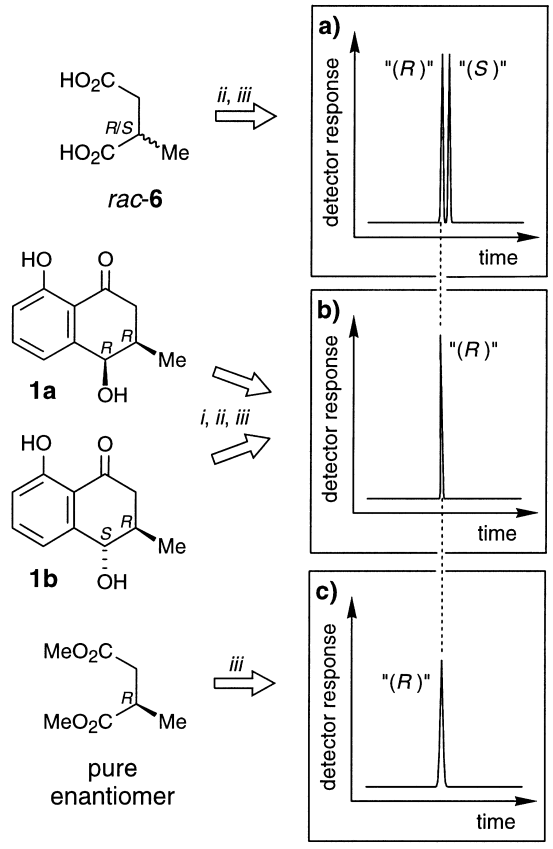


Scheme 1. Oxidative degradation of 1,3-disubstituted tetrahydroisoquinolines and expected 'analogous' degradation of isoshinanolones to give 2-methylsuccinic acid (**6**). Reagents: (i) $RuCl_3$, $NaIO_4$.

Recently, plant material of the very rare tropical liana *Dioncophyllum thollonii* (Dioncophyllaceae) was collected by one of us (A.M.L.) in Gabon. The closely related Dioncophyllaceae species *Triphyophyllum peltatum* is a rich source of a broad series of naphthylisoquinoline alkaloids (Bringmann & Pokorny, 1995; Bringmann et al., 1998b). By contrast, first LC-NMR investigations on *D. thollonii* revealed only very low alkaloid concentrations, but high quantities of naphthoquinones (Bringmann, Rückert, Messer, Schupp, & Louis, in press). Furthermore, in contrast to previous reports (Lavault & Bruneton, 1980), the occurrence of both *cis*- and *trans*-isoshinanolone, with an 8.2:1 *cis–trans* ratio of the two diastereomers in the *n*-hexane extract, was found. Exemplarily on this stereomeric mixture from *D. thollonii*, we have, for the first time, resolved and separately characterized the two diastereomeric forms of isoshianolone, allowing a full characterization, now even for the *trans*-isomer. Its very low optical rotation value, which is not distinctly different



Scheme 2. Partial synthesis of isoshinanolone (**1**) from plumbagin. Reagents and conditions: (i) $LiAlH_4$, Et_2O, $-78° \rightarrow 25°$.



Scheme 3. GC/MSD chromatograms of racemic (a) and *R*-configured methyl 2-methylsuccinate (c), as well as the result of the oxidative degradation of both *cis*- and *trans*-isoshinanolones (**1a** and **1b**) from *D. thollonii* (b). Reaction conditions: (i) $RuCl_3 \cdot H_2O$, $NaIO_4$; (ii) MeOH, $SOCl_2$; (iii) chromatography on a chiral β-DEX 325 column.

from zero, is *not* due to the presence of a racemate (see below). This underlines the necessity of an unambiguous and α_D-independent stereoanalysis.

In order to save material, initial degradation experiments were done on partial-synthetic isoshinanolone, as prepared by $LiAlH_4$-reduction of plumbagin (**2**) (see Scheme 2), by slightly modifying a literature procedure (Tezuka et al., 1973). In this reaction, a 15:85 *cis–trans* ratio was obtained, both diastereomers being formed in a racemic form, due to the use of the achiral hydride transfer reagent.

The racemic *trans*-isoshinanolone (**1b**/*ent*-**1b**) was submitted to our standard degradation conditions ($RuCl_3/NaIO_4$) previously optimized for tetrahydropyridine systems (Bringmann et al., 1996). Subsequent GC–MSD analysis after esterification with MeOH/$SOCl_2$ gave the dimethyl ester of 2-methylsuccinic acid (**6**) as the only detectable fragment of **1** with the mass numbers (*m/z* (rel. int.) 129 (27), 128 (18), 101 (14), 100 (11), 87 (10), 69 (11), 59 (100)), fully identical with an authentic racemic sample (Aldrich).

With the successful identification of 2-methylsuccinic acid (**6**) as a degradation product, the next step was to

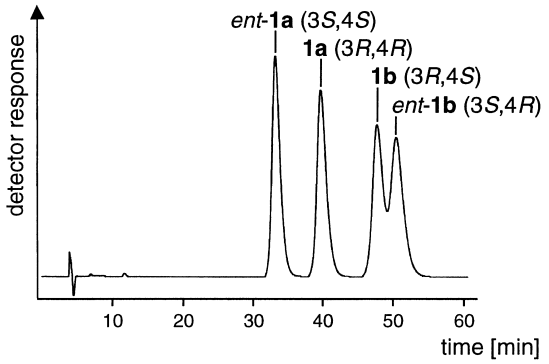


Fig. 3. HPLC chromatogram of all four possible stereoisomeric forms of isoshinanolone (**1**), on a Chiralcel OD-H phase (eluent: *n*-hexane–*i*-PrOH; 98:2).

establish an enantiomer analysis, which was achieved by capillary GC of the diester on a chiral β-DEX 325 stationary phase (see Scheme 3). For an attribution of the two peaks thus obtained for racemic 2-methylsuccinic acid (see Scheme 3(a)) to its two enantiomers, an authentic sample of dimethyl (*R*)-2-methylsuccinate (Aldrich) was submitted to the same analytical conditions (see Scheme 3(c)), clearly indicating the chromatographically 'earlier' peak at $R_t = 13.0$ min belonging to the *R*-enantiomer, while the *S*-enantiomer of methyl 2-methylsuccinate elutes at $R_t = 13.1$ min (exact elution time difference: 7 s).

With this stereoanalysis established, degradation of both *cis*- and *trans*-isoshinanolone from *D. thollonii* and GC–MSD analysis after esterification gave one single peak (see Scheme 3(b)), corresponding to the dimethyl ester of the *R*-enantiomer of **6**. Besides the important information that even the optically inactive *trans*-isoshinanolone is not racemic, but enantiomerically pure, this clearly indicates both *cis*- and *trans*-isoshinanolones of *D. thollonii* to be *R*-configured at C-3. From this and the known relative configuration at C-3 vs. C-4 (through NMR, see above), the two natural products must have the full stereostructures **1a** and **1b**, i.e. with 3*R*,4*R* and 3*R*,4*S*-configuration, respectively.

2.3. Chromatographic stereoanalysis of isoshinanolones

Based on this degradative method for the unequivocal stereo-attribution of isoshinanolones, and with pure **1a** and **1b**, and the partial-synthetic racemic *cis*/*trans* mixture available, a generally applicable HPLC analytical device was developed for the rapid identification of all four stereoisomeric forms of isoshinanolone, by chromatography on a Chiralcel OD-H column as the stationary phase (see Fig. 3). The attribution of the signals to the individual tetralone isomers was achieved by coelution experiments of the isoshinanolones of known configuration as well as the corresponding racemates. Not only *cis*-isoshinanolone (**1a**), but also its optically nearly inactive *trans*-isomer (**1b**) from *D. thollonii* were thus found to be enantiomerically pure, in the plant.

By this procedure, any isoshinanolone (mixture) can now be analyzed rapidly and unambiguously for its relative and absolute configuration(s) by HPLC analysis on that chiral phase.

3. Experimental

3.1. General

Mps: uncorr. NMR spectra were recorded in $CDCl_3$ with the solvent as int. standard ($CDCl_3$, δ 7.26 and δ 77.01, resp.). EIMS: 70 eV. CC: silica gel (60–200 mesh, Merck).

3.2. Materials and reference compounds

The catalyst $RuCl_3 \cdot H_2O$ was purchased from Heraeus Feinchemikalien und Forschungsbedarf GmbH, Germany. 0.1 M Na–Pi buffer was prepd. by dissolving 1.38 g (10 mmol) $NaH_2PO_4 \cdot H_2O$ in 100 ml water and adjusting pH to 6 with 0.1 M NaOH. Racemic reference material of 2-methylsuccinic acid and the enantiopure dimethyl (*R*)-2-methylsuccinate were obtained from Aldrich. Plumbagin was purchased from Sigma.

3.3. Plant material

Plant material of *D. thollonii* was collected by one of us (A.M.L) in Rabi Kounga (Gabon) in October 1996. A voucher specimen has been deposited at Herb. Bringmann, University of Würzburg.

3.4. Extraction and isolation

The air-dried and powdered roots (300 g) were extracted with *n*-hexane. From this extract, **1a** and **1b** were isolated by repeated CC (*t*-BuOMe–*n*-hexane 5:1, EtOAc–*n*-hexane 3:2 and Et_2O–*n*-hexane 1:1).

3.5. cis-Isoshinanolone (*1a*, 3R,4R)

Oil, yield 250 mg. $[\alpha]_D^{25}$ +22.2° ($CHCl_3$, *c* 1.0). UV λ_{max}^{EtOH} nm: 215, 262, 333. CD: $\Delta\varepsilon_{226}$ −49, $\Delta\varepsilon_{265}$ +12 (EtOH; *c* 0.01). IR (CCl_4) ν_{max} cm^{-1}: 3617 (O–H), 2966 (C=C), 1645 (C=O), 1543, 1456, 1341, 1238, 1210, 1164 (C–O), 973. ^1H NMR (250 MHz, $CDCl_3$): δ 1.19 (3H, d, $J = 6.72$ Hz, CH_3), 2.44 (1H, m_c, H-3), 2.56 (1H, ddd, $J = 17.7$, 4.3, 0.9 Hz, H_{eq}-2), 2.87 (1H, dd, $J = 17.7$, 11.0 Hz, H_{ax}-2), 4.75 (1H, d, $J = 2.45$ Hz, H-4), 6.92 (1H, d, $J = 7.3$ Hz, H-5), 6.94 (1H, dd, $J = 8.5$,

1.2 Hz, H-7), 7.48 (1H, dd, $J=8.3$, 7.3 Hz, H-6), 12.42 (1H, s, OH-8). ^{13}C NMR (150.9 MHz, $CDCl_3$): δ 16.1 (CH_3), 34.4 (C-3), 40.7 (C-2), 71.2 (C-4), 114.9 (C-8a), 118.2 (C-7), 118.6 (C-5), 136.9 (C-6), 145.0 (C-4a), 162.7 (C-8), 204.7 (C-1). The ^{13}C assignments were achieved by HMQC and HMBC experiments. EIMS *m/z* (rel. int.): 192 $[M]^+$ (49), 177 $[M-CH_3]^+$ (13), 150 (33), 121 (100). HRMS: $C_{11}H_{12}O_3$. Found 192.078, calc. 192.079.

3.6. *trans*-Isoshinanolone (*1b*, 3*R*,4*S*)

Oil, yield 30 mg. $[\alpha]_D^{25}$ 2.9° ($CHCl_3$, *c* 1.0). UV λ_{max}^{EtOH} nm: 215, 262, 333. CD: $\Delta\varepsilon_{225}$ −11, $\Delta\varepsilon_{263}$ −4 (EtOH; *c* 0.01). IR (KBr) ν_{max} cm^{-1}: 3246 (O–H), 2892 (C=C), 1636 (C=O), 1456, 1341, 1249, 1217, 1069 (C–O), 1043, 810, 756. ^1H NMR (200 MHz, $CDCl_3$): δ 1.19 (3H, d, $J=6.5$ Hz, CH_3), 2.25 (1H, m_c, H-3), 2.43 (1H, dd, $J=17.0$, 10.1 Hz, H_{ax}-2), 2.91 (1H, dd, $J=16.9$, 3.8 Hz, H_{eq}-2), 4.50 (1H, d, $J=8.0$ Hz, H-4), 6.90 (1H, ddd, $J=8.4$, 1.1, 0.6 Hz, H-5), 7.10 (1H, dd, $J=7.6$, 1.0 Hz, H-7), 7.49 (1H, t, $J=8.0$ Hz, H-6), 11.74 (1H, s, OH-8). ^{13}C NMR (50 MHz, $CDCl_3$): δ 17.8 (CH_3), 37.4 (C-3), 43.4 (C-2), 73.7 (C-4), 115.3 (C-8a), 117.2 (C-5), 117.3 (C-7), 137.0 (C-6), 145.9 (C-4a), 162.5 (C-8), 203.6 (C-1). The ^{13}C assignments were achieved by HMQC and HMBC experiments. EIMS *m/z* (rel. int.): 192 $[M]^+$ (87), 177 $[M-CH_3]^+$ (16), 150 (40), 121 (100). HRMS: $C_{11}H_{12}O_3$. Found 192.078, calc. 192.079.

3.7. Partial synthesis of the isoshinanolone stereoisomers

38.6 mg (210 μmol) plumbagin (**2**) was reduced with $LiAlH_4$ (47.8 mg, 1.3 mmol) in Et_2O at −19°. The soln. was stirred for 4 h while warming up to room temp., then 0.1 N HCl was added until pH 5 was reached. The mixt. was extracted with Et_2O and the org. extract was washed with H_2O, dried ($MgSO_4$) and the solvent was evpd in vacuo. The residue was passed through a silica gel column using CH_2Cl_2–MeOH (50:1). Further purification by prep. RP-HPLC with Waters cartridge system RCM 8 × 10 (4 μm, 200 mm × 7.8 mm, Nova-Pak C18) eluting with MeOH–H_2O (32.5:67.5) and 1‰ TFA at a flow rate of 1.5 ml min^{-1} yielded racemic *cis*-isomer **1a**/*ent*-**1a** (1.61 mg, 8.4 μmol, 4%) as a yellow semi-solid and *trans*-isomer **1b**/*ent*-**1b** (6.86 mg, 35.7 μmol, 17%) as needles (from CH_2Cl_2–*n*-hexane), mp 114–115°C. Spectroscopic data (IR, NMR, MS) identical with those of natural **1a** and **1b**.

3.8. Oxidative degradation procedure

The reactions were performed in analogy to Bringmann et al. (1996). To a stirred two-phase mixt. of 150 μl MeCN, 50 μl CCl_4 and 300 μl 9.1 M Na–Pi buffer (pH 6) in a 2.5 ml Wheaton screw-cap vial 3.3 mg (15.3 μmol) of isoshinanolone and the catalyst $RuCl_3 \cdot H_2O$ (0.1 mg, 0.4 μmol) were added at room temp. Over a period of 60 min, 40 mg (183 μmol) of $NaIO_4$ were added in small portions, followed by stirring for another 1.5 h. The resulting 2-methylsuccinic acid (**6**) was extracted with 700 μl H_2O and washed two times with 300 μl $CHCl_3$ and subsequently lyophilized. The residue was extracted with 1.5 ml of dry MeOH, followed by sepn of the insol. inorganic salts by centrifugation. The resulting soln of 2-methylsuccinic acid (**6**) in MeOH was used for derivatization.

3.9. Derivatization procedure

For esterification 100 μl (1.4 mmol) of freshly dist. $SOCl_2$ were added at 0° to a stirred MeOH soln of 2-methylsuccinic acid (**6**). The mixt. was then allowed to stand at room temp. for 12 h and another portion of 100 μl (1.4 mmol) of $SOCl_2$ was added in the same manner. After 6 h standing at room temp. and evapn of the solvent, the residue was dissolved in CH_2Cl_2 and analyzed by GC–MS.

3.10. Capillary GC

GC–EI-MSD analyses were performed with a transfer-line temp. maintained at 280° resulting in a source temp. of 170° and an ionizing energy of 70 eV. RIC was measured in the mass range of *m/z* 40 to 250. A β-DEX 325 (Supelco), 30 m × 0.25 mm (i.d.) × 0.25 μm column, with an on-column injector maintained at 210° was used for chromatographic enantiomer separation of dimethyl 2-methylsuccinate. Helium was used as the carrier gas with a column head pressure of 90 kPa. Column temp. was prog. 80° 5 min, 80–100° 5° min^{-1} and finally held at 100° for 10 min.

3.11. HPLC separation of the four stereoisomeric forms of *1*

The analytical separation was carried out on a Chiralcel OD-H column (10 μm, 250 mm × 4.6 mm, Daicel Chemical Industries Ltd) at room temp., elution with *n*-hexane–*i*-PrOH (98:2), at a flow rate of 1.0 ml min^{-1} and detection at 254 nm.

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References

Anh, N. H., Ripperger, H., Porzel, A., Sung, T. V., & Adam, G. (1997). *Phytochemistry*, *44*, 549.

Bhattacharyya, J., & de Carvalho, V. R. (1986). *Phytochemistry*, *25*, 764.

Bin Zakaria, M., Jeffreys, J. A. D., Waterman, P. G., & Zhong, S.-M. (1984). *Phytochemistry*, *23*, 1481.

Bringmann, G., Wohlfarth, M., Rischer, H., Rückert, M., & Schlauer, J. (1998a). *Tetrahedron Lett*., *39*, 8445.

Bringmann, G., François, G., Aké Assi, L., & Schlauer, J. (1998b). *Chimia*, *52*, 18.

Bringmann, G., Geuder, T., Rübenacker, M., & Zagst, R. (1991). *Phytochemistry*, *30*, 2067.

Bringmann, G., God, R., & Schäffer, M. (1996). *Phytochemistry*, *43*, 1393.

Bringmann, G., Messer, K., Wohlfarth, M., Kraus, J., Dumbuya, K., & Rückert, M. *Anal. Chem*., submitted for publication (a).

Bringmann, G., & Pokorny, F. (1995). In G. Coroell, *The alkaloids*, *Vol. 46* (p. 127). New York: Academic Press.

Bringmann, G., Rückert, M., Messer, K., Schupp, O., & Louis, A. M. (1999). *J. Chromatogr. A*, in press.

Bringmann, G., Schlauer, J. Rückert, M., Wiesen, B., Ehrenfeld, K., Proksch, P., & Czygan F. C. *Bot. Acta (Section Ecology)*, submitted for publication (b).

Bringmann, G., Schneider, C., & Aké Assi, L. (1991). *Planta Med*., *57*(Suppl. 2), 10.

Bringmann, G., Wenzel, M., Rückert, M., Wolf, K., Busemann, S., Schäffer, M., & Aké Assi, L. (1998c). *Heterocycles*, *47*, 985.

Carlsen, H. J., Katsuki, T., Martin, V. S., & Sharpless, K. B. (1981). *J. Org. Chem*., *46*, 3936.

Gill, M., Gimenez, A., Jhingran, A. G., & Palfreyman, A. R. (1990). *Tetrahedron Lett*., *31*, 1203.

Gunaherath, G. M. K. B., Gunatilaka, A. A. L., Sultanbawa, M. U. S., & Balasubramaniam, S. (1983). *Phytochemistry*, *22*, 1245.

Hanson, S. W., Crawford, M., & Thanasingh, D. P. J. (1981). *Phytochemistry*, *20*, 1162.

Kumar, V., Meepagala, K. M., & Balasubramaniam, S. (1985). *Phytochemistry*, *24*, 1118.

Lavault, M., & Bruneton, J. (1980). *Planta Med.* (Suppl.), 17.

Likhitwitayawuid, K., Kaewamatawong, R., Ruangrungsi, N., & Krungkrai, J. (1998). *Planta Med*., *64*, 237.

Nakanishi, K., Berova, N., & Woody, R. W. (1994). *Circular dichroism: principles and applications*. Weinheim, New York: VCH.

Richomme, P., Papillon, B., Cabalion, P., & Bruneton, J. (1991). *Pharm. Acta Helv*., *66*, 88.

Tezuka, M., Takahashi, C., Kuroyanagi, M., Satake, M., Yoshihira, K., & Natori, S. (1973). *Phytochemistry*, *12*, 175.

Zhong, S.-M., Waterman, P. G., & Jeffreys, J. A. D. (1984). *Phytochemistry*, *23*, 1067.