New Antifungal 'Quinone Methide' Diterpenes from *Bobgunnia madagascariensis* and Study of Their Interconversion by LC/NMR

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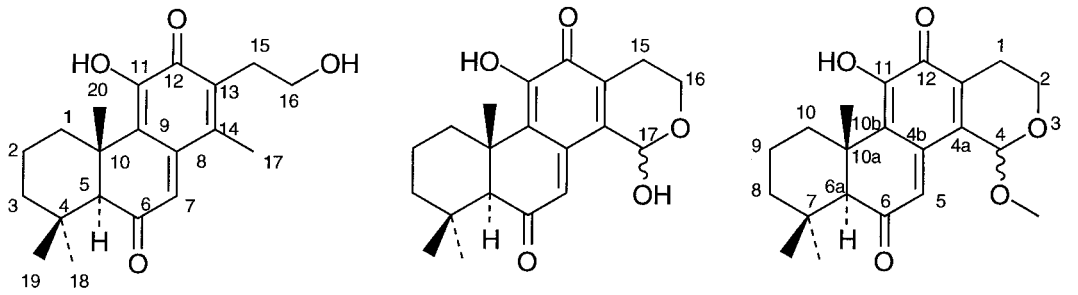
Two new 'quinone methide' diterpenes with a cassane skeleton were isolated from the root bark of *Bobgunnia madagascariensis* (DESV.) J. H. KIRKBR. & WIERSEMA (Leguminosae). The two compounds occurred as a diastereoisomer mixture **1/2**. Their structure was established as (4*R*,6a*S*,10a*S*)- and (4*S*,6a*S*,10a*S*)-1,4,6a,7,8,9,10,10a-octahydro-4,11-dihydroxy-7,7,10a-trimethyl-2*H*-phenanthro[1,2-*c*]pyran-6,12-dione by spectroscopic methods and by comparison with the data obtained for another 'quinone methide' diterpene isolated previously from the same plant and identified by single-crystal X-ray analysis. ^{1}H- and ^{13}C-NMR signals were assigned by extensive in-mixture 2D correlation experiments, and ^{1}H-NMR spectra of the separated diastereoisomers **1** and **2** were recorded by on-flow LC/NMR. The interconversion of **1** and **2** was followed by repeated stop-flow LC/NMR experiments over a two-hour period. Compounds **1** and **2** showed moderate antifungal properties towards human pathogenic fungi, in particular the yeast *Candida albicans*.

Introduction. – *Bobgunnia madagascariensis* (DESV.) J. H. KIRKBR. & WIERSEMA (Leguminosae) is a tree widely distributed in tropical Africa, which was known as *Swartzia madagascariensis* DESV. until April 1997 [1]. Several traditional medicinal uses have been documented [2]. The roots in particular are employed as a cure of leprosy and syphilis. Insecticidal activity against termites has also been reported [3]. *B. magadascariensis* has been the center of several phytochemical investigations, and potent molluscicidal saponins were reported in the fruits [4], a flavonoid has been purified from the seeds [5], and pterocarpans have been isolated from the heartwood [6–8].

As part of our screening aimed at the discovery of new antifungal lead compounds, the lipophilic extract of the root bark of *B. magadascariensis* was investigated, and the main compound **3** of the extract was identified as a new 'quinone methide' diterpene with a cassane skeleton. This compound appeared to be a promising lead for the development of novel antimycotic drugs [9]. In continuation of this study, other antifungal constituents of the lipophilic root-bark extract of *B. magadascariensis* that inhibited the growth of both *Candida albicans* and *Cladosporium cucumerinum* in TLC bioassays were investigated [10][11]. We report here the isolation and the in-mixture structure elucidation of two new antifungal diterpenes **1** and **2** that occur as interconverting diastereoisomers in this plant.

Results and Discussion. – Dried and powdered root bark of *B. magadascariensis* was extracted at room temperature with CH_2Cl_2. An LC/UV/MS analysis of this crude



extract was performed to check for the presence of other 'quinone methide' diterpenes with a cassane skeleton similar to **3** in the extract (*Fig. 1*). Indeed, several spots besides **3** revealed strong inhibition of the growth of both *Candida albicans* and *Cladosporium cucumerinum* in TLC bioassays (*Fig. 1,b*). The LC/UV plot of the extract (330 nm) revealed that three main compounds were present (*Fig. 1,a*). The LC peak corresponding to the previously isolated compound **3** was assigned by co-elution with the pure standard. Compounds **1** and **2** displayed the same type of UV spectra as **3**, with a main absorption band presenting maxima at *ca.* 320 and 330 nm (*Fig. 1,c*). This indicated that these compounds share the same quinonic chromophore as **3**.

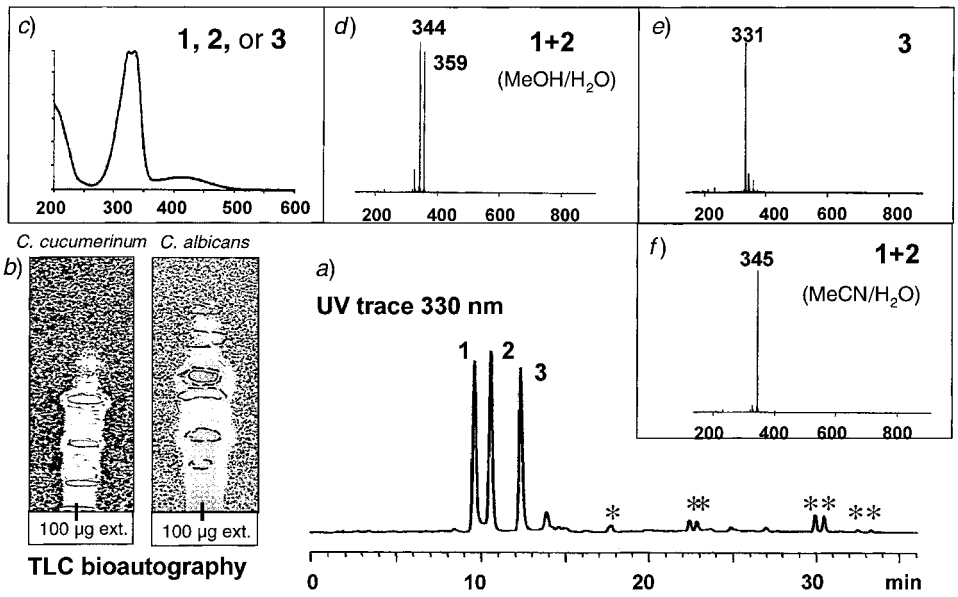


Fig. 1. a) *HPLC/UV Trace of the crude CH_2Cl_2 extract of the root bark of* B. madagascariensis *obtained with the MeOH/H_2O gradient* (see *Exper. Part*); b) *TLC bioautography performed on 100 µg of extract against both* Cladosporium cucumerinum *and* Candida albicans; c) *typical UV spectrum recorded for* **1**, **2**, or **3** *as well for all the minor peaks marked with* *; d) *and* e) *LC/TSP-MS obtained with the MeOH/H_2O gradient*; f) *LC/TSP-MS obtained with the MeCN/H_2O gradient.*

Interestingly, almost all the minor constituents of this extract also exhibited the same type of UV spectra (see peaks marked with an * in *Fig. 1,a*). The LC/thermospray (TSP)-MS analysis revealed a protonated molecule $[M+H]^+$ at *m/z* 331 for **3** (M_r 330), while **1** and **2** presented both ions at *m/z* 344 and 359 when the extract was analyzed with a MeOH/H_2O gradient (*Fig. 1,e* and *d*, resp.). Curiously, **1** and **2** displayed only one ion at *m/z* 345 when the separation was carried out with MeCN/H_2O (*Fig. 1,f*). These different measurements suggested that **1** and **2** were isomers and indicated a M_r of 344 for them. The ion observed at *m/z* 359 was probably due to in-source methylation of these constituents when MeOH was used as an organic modifier for HPLC.

These first on-line data revealed that **1** and **2** were closely related to the new antimycotic agent **3**, and their targeted isolation was undertaken.

The CH_2Cl_2 extract of *B. madagascariensis* was fractionated by column chromatography (silica gel), which yielded 8 fractions. *Fraction D* containing compounds **1** and **2** was further separated by medium-pressure liquid chromatography (MPLC) on a *Diol* support followed by semi-prep. HPLC on a *C-18* column. However, the isomers **1** and **2** could not be isolated at the prep. scale. The semi-prep. HPLC separation yielded two well-resolved LC peaks that were collected separately. The HPLC/UV analysis of the fractions, performed directly after purification under the same conditions as for the separation of the extract, established that an enrichment was obtained, but both compounds were still present in the fractions. The same LC/UV analysis performed 5 h after the isolation indicated, however, an equimolar ratio of **1** and **2**. Thus, an interconversion between these two isomers occurred in solution.

To determine the structure of the isomers **1** and **2**, on-flow and stop-flow LC/NMR experiments [12] with a MeCN/D_2O gradient were performed on the enriched fraction containing both **1** and **2**, the NMR solvent signals being suppressed by the WET solvent suppression sequence [13]. The on-flow LC/NMR analysis demonstrated that almost no difference between the signals of **1** and **2** was detected. Indeed as shown on the 2D plot (*Fig. 2*), all the signals in the high-field region of the NMR dimension (1.0–4.5 ppm) were common for both compounds showing their important similarity. However, the signals detected in the 5.5–7.0 ppm region differed significantly between these two compounds; two *s* appeared at 5.9 and 6.5 ppm for **1**, while they were shifted to 5.8 and 6.7 ppm in the case of **2**. The full LC/^{1}H-NMR spectra recorded in the stop-flow mode (*Fig. 3*) for **1** and **2** confirmed these observations and also indicated that these compounds were closely related to the previously identified 'quinone methide' diterpene **3**.

A stop-flow LC/NMR analysis performed on compound **2** demonstrated also very clearly the interconversion of both constituents in solution. The LC separation was stopped exactly when peak **2** was eluting in the LC/NMR cell. The first stop-flow LC/NMR spectrum of this peak obtained after 15 min showed that **2** was indeed the major constituent in this peak, but already small signals of **1** were observable (*Fig. 2*). A comparison of the integration of the signals of **1** and **2** indicated that **2** was present to more than 93% in the LC/NMR flow cell after 15 min. This stop-flow experiment was repeated over a two-hours period, and spectra were recorded every 15 minutes. As displayed on the expansion of the stop-flow spectra in *Fig. 2*, the signals of compound **1** were increasing while those of **2** were decreasing with time. The amount of **1** reached one third of that of **2** after 105 min thus establishing that indeed **1** and **2** were

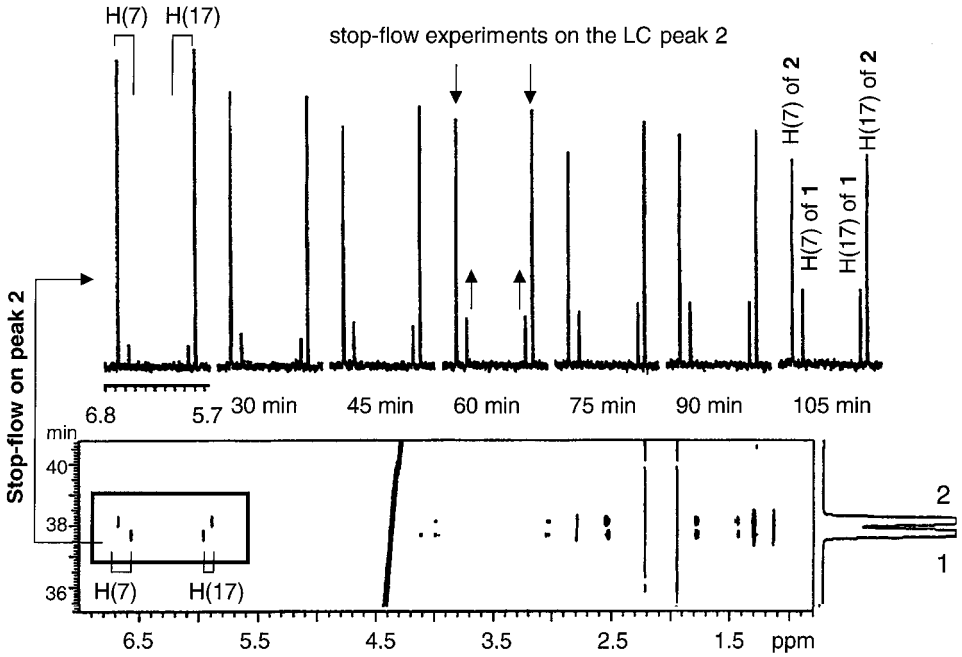


Fig. 2. *On-flow LC/NMR analysis of the* **1/2** *mixture and expansion* (5.7–6.8 ppm) *of the stop-flow LC/NMR spectra recorded for peak 2* (upper part). Stop-flow spectra (256 transients) recorded every 15 min are displayed. H(7) = H–C(7) and H(17) = H–C(17)[1]).

interconverting in solution. A 1:1 ratio was finally obtained after storage of these compounds in the LC/NMR flow cell overnight. The sum of the integrals of H–C(7) and H–C(17)[1]) of **1** and **2** remained constant during the whole experiment, demonstrating also that no other compound was formed.

To establish the structure of **1** and **2**, 1D and 2D NMR correlation experiments were performed in-mixture taking into account the attribution of individual signals of **1** and **2** based on the LC/^{1}H-NMR measurements.

The EI-MS of the mixture **1/2** exhibited a molecular ion at *m/z* 344, confirming the information obtained on-line by LC/TSP-MS in MeCN/H_2O. The study of the ^{13}C-NMR and DEPT spectra together with the M_r of 344 indicated a molecular formula of $C_{20}H_{24}O_5$ for these two isomers. In comparison with **3** [9], the differences suggested an additional CH and one Me fewer in **1/2**. As a mass difference of 14 Da was observed between **3** and **1/2**, an additional O-atom was also present in these latter compounds. The ^{13}C-NMR spectra of **1/2** exhibited 40 signals corresponding, in fact, to 20 doubled resonances for each pair of signals from **1** and **2**. A comparison of the ^{13}C- and ^{1}H-NMR spectra of **1/2** with those of **3** [9] demonstrated that all the signals corresponding to the tricyclic skeleton of **3** were found for **1/2**, and only the signals of C(8), C(13), and C(14) differed slightly[1]). However, significant differences were observed for the atoms of the hydroxyethyl (C(15)–C(16)) side chain at C(13) and for Me(17) at C(14). In the case of **3**, CH_2(15) and CH_2(16) were observed at δ 31.0 and 61.3 [9], while they were shifted to 22.9/22.7 and δ 56.2/56.3, respectively, for **1/2**. The signal of Me(17) of **3** was not observed for **1/2**; it was replaced by a CH at δ 87.5/87.8 for **1/2**. In the ^{1}H-NMR of **1/2**, the two CH_2 groups were shifted compared to **3**, and two distinct signals were observed for CH_2(16) of **1/2** (4.15 (*m*, H_a–C(16)); 3.87

[1]) The cassane numbering (see *Formulae* **1**–**3**) is used in the *General Part* and for the spectroscopic data; systematic names are given in the *Exper. Part* (see *Formula* **4**/**5**).

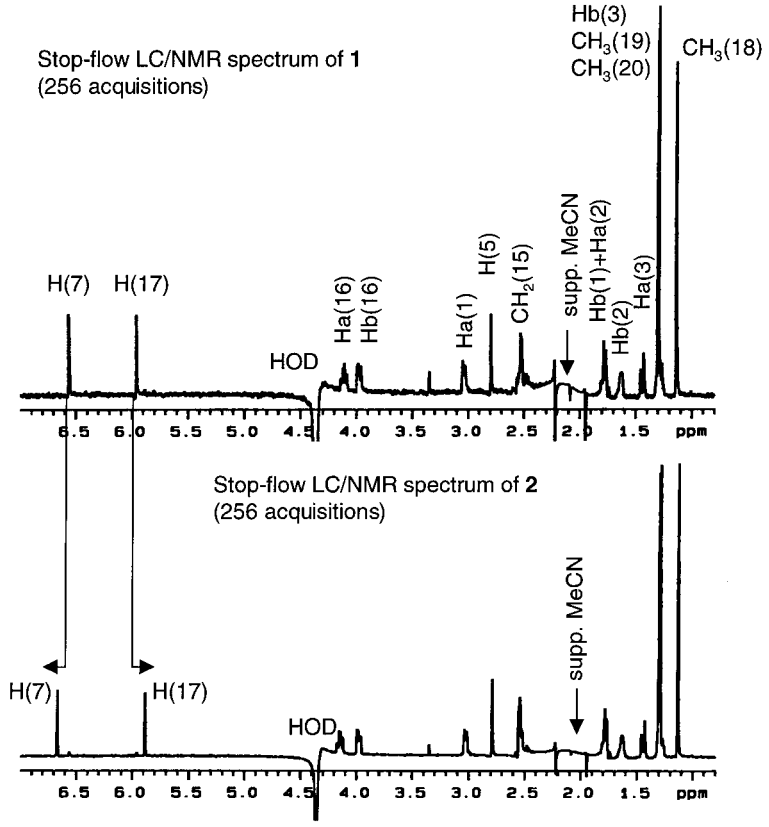


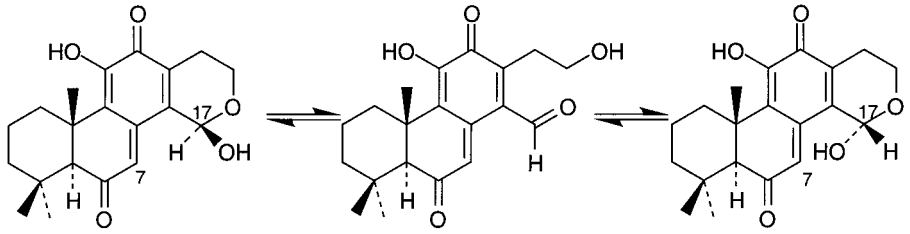
Fig. 3. *Stop-flow LC/NMR spectra recorded for* **1** *and* **2**. Hb(1) = H_b–C(1), H(7) = H–C(7) *etc.*[1]).

(*m*, H_b–C(16))). CH_2(15) and CH_2(16) showed a clear coupling in a gDQCOSY experiment. A careful study of the gHMBC, gDQCOSY, and NOE correlations of **1**/**2** finally permitted the attribution of a hemiacetal function (–CH_2–CH_2–O–CHOH–) between C(13) and C(14), which explains well the presence of the CH(17) resonance at δ 87.5/87.7. The position of the –CH_2CH_2–O–C*HOH– moiety between C(13) and C(14) was established by NOE and gHMBC correlations. Irradiation of both H–C(7) signals of **1**/**2** showed a clear NOE effect at the corresponding H–C(17) signals, indicating that H–C(17) was attached to the quaternary C(14). Furthermore, HMBC correlations between H–C(17) and C(14), as well as between H–C(15) and C(13), confirmed the structure assignment.

The absolute (5*S*,10*S*) configuration of **1**/**2** was assigned by analogy to **3**. In the case of **3**, this configuration was assigned by X-ray analysis of a brominated derivative [9]. To the best of our knowledge, **1** and **2** are new natural products.

The presence of the hemiacetal group in **1**/**2** explains well the observed equilibrium between the two diastereoisomers. Indeed, a new stereogenic center is present at C(17), and the instability of **1** and **2** in solution is due to the epimerization at this position of chirality. The diastereoisomers **1** and **2** could thus be interconverted by ring opening, which yields a hydroxy aldehyde, followed by recyclization (*Scheme*). The nature of the ions observed in the LC/TSP-MS spectra of **1** and **2** can also be explained by the presence of the hemiacetal function. As mentioned before, the molecular ions of

Scheme. *Interconversion of the Diastereoisomers* **1** *and* **2** *in Solution*



1 and **2** were shifted by 14 Da when the analysis was performed in presence of MeOH (*Fig. 1*). In this case, the alcohol was reacting in the thermospray source with the hemiacetal to form the more stable acetal diastereoisomers **4/5**, a well-known type of reaction. The mixture of artefacts **4/5** was formed even when the mixture **1/2** was left in the presence of MeOH at room temperature for 5 min. A longer exposure of **1/2** to MeOH led to their complete decomposition. The artefacts **4/5** were isolated from the last fraction of the column-chromatography separation (silica gel) of the extract after washing with MeOH. The structures of **4/5** were established by ^{1}H- and ^{13}C-NMR spectroscopy (δ(C) 55.3 for MeO, all the other signals corresponding to those of **1/2**). This indicates that **1** and **2** are very unstable in the presence of MeOH and form rapidly the artefacts **4/5**.

With regards to biological activity, 0.5 µg of **1/2** 1:1 was sufficient to inhibit the growth of both *Candida albicans* and *Cladosporium cucumerinum* on TLC plates. This prompted us to compare the antifungal activity of **1/2** with that of commercial antifungal agents towards *Candida albicans* in a dilution assay. The diastereoisomers **1/2** were tested in a modification of the NCCLS microbroth assay in the presence of the tetrazolium salt XTT, to quantitate viable fungi cells [14][15]. The 50% inhibition concentration IC_{50} of **1/2** (7.0 µg/ml) was significantly higher than that of the other 'quinone methide' **3** previously isolated from the same plant (IC_{50} 0.2 µg/ml) [9]. The antifungal activity of the artefacts **4/5** was not evaluated.

Contrary to the 'quinone methide' diterpene **3**, the new hemiacetals **1/2**, the two other major constituents of the CH_2Cl_2 extract of the root bark of *B. madagascariensis*, displayed only a moderate antifungal activity against *C. albicans* and thus were not retained for further testings. These results suggest that the presence of a hemiacetal function at position C(17) significantly lowers the activity of this type of product. These natural products of a new type have to be investigated more deeply. The characterization of the minor constituents of the extract is currently underway.

Financial support was provided by the *Swiss National Science Foundation*. We are grateful to *Phytera, Inc.*, Worcester, MA, USA, for the evaluation of the antifungal properties and for financial support. The compounds described in this publication are the subject of a patent, US 5.929.124 ('antimicrobial Diterpenes'), issued on July 27, 1999.

Experimental Part

General. TLC: Silica gel *60 F_{254}* Al sheets (*Merck*); petroleum ether/AcOEt 1:1; detection at 254 and 366 nm and with *Godin*'s reagent [16]. CC = column chromatography. Medium-pressure liquid chromatography (MPLC): home-packed *LiChroprep Diol* (25–40 µm; 460 × 25 mm i.d.; *Merck*). UV: *Perkin-Elmer Lambda-20* UV/VIS spectrometer, in EtOH; λ_{max} [nm] (ε). NMR: *Varian Inova-500* spectrometer equipped with a

^{1}H/multinuclear inverse probe head at 499.870 MHz; ^{13}C at 125.704 MHz, multiplicities from DEPT sequences; attributions[1]) by use of gradient double quantum filtered correlation spectroscopy (gDQCOSY), gradient heteronuclear single quantum coherence (gHSQC), and gradient heteronuclear multiple bond correlation (gHMBC) experiments. EI- and D/CI-MS: *Finnigan-MAT-TSQ-700* triple-stage quadrupole instrument; EI: *m*/*z* (rel. intensity in %), ionization energy 70 eV; D/CI: NH_3, positive-ion mode.

Bioassays. Bioautography against *Cladosporium cucumerinum* and *Candida albicans* was carried out as previously reported [10][11]. *MIC* (= minimum inhibitory concentration) and IC_{50} values were determined in a modification of the NCCLS microbroth assay in the presence of the tetrazolium salt XTT [14][15]. Fungi were grown on *Sabouraud* dextrose agar plates and incubated overnight. On day 2, the 1:1 mixture **1/2** was diluted (8 concentrations) in DMSO. Then, the diluted solns. (2 µl each) were added to test wells of a 96 well sterile culture plate, followed by addition of 98 µl of suspension of fungi into RPMI-MOPS [14] to a concentration of $5 \cdot 10^2$ cells/ml. After incubation, *MIC* values were determined, followed by addition of XTT [15]. Plates were read at OD_{450}, and percent inhibition and IC_{50} values were then calculated.

Plant Material. *Bobgunnia madagascariensis* (DESV.) J. H. KIRKBR. & WIERSEMA was collected in 1996 near Murewa (Zimbabwe). A voucher specimen has been deposited at the National Herbarium and Botanical Garden of Zimbabwe, Causeway, Harare.

Fungal Strains. Human pathogenic fungal strains were commercially obtained from the *ATCC* and from *Chrisopee Technologies.*

Extraction and Separation. Dried powdered root bark (1090 g) of *B. madagascariensis* was extracted at r.t. by CH_2Cl_2 (65 g). A portion of that extract (64.5 g) was fractionated by CC (silica gel, petroleum ether/AcOEt 2:1): *Fraction D* (18.7 g). Final purification of a portion of this material (6.0 g) by MPLC (*Diol*, petroleum ether/AcOEt 7:1) provided **1/2** (2.59 g). A portion of **1/2** (0.5 g) was separated by semi-prep. HPLC (*C-18* column, MeCN/H_2O (containing 0.05% of CF_3COOH), isocratic). An HPLC analysis of the fraction collected just after this semi-prep. separation demonstrated that a significant enrichment of one of the isomer was obtained in each fraction of interest, but the same analysis performed 5 h after collection established the interconvertion of **1** and **2** in solution, yielding an 1:1 mixture from both fractions.

The artefacts **4/5** were found in the last fraction of the CC (silica gel, after washing with MeOH): *Fraction H* (0.9 g). A final purification of this fraction was performed by CC (silica gel, petroleum ether/AcOEt 2:1), followed by gel filtration (*Sephadex LH-20*, CH_2Cl_2/^{i}PrOH 1:1): **4/5** (86 mg).

LC/UV/MS Analysis of the Extract. LC/UV/MS setup: *Finnigan-MAT TSQ-700* mass spectrometer equipped with a *Thermospray 2* interface; solvent delivery by a *Waters 600-MS pump*; UV recording by a *Hewlett-Packard HP-1050* photodiode array detector, addition of 0.5M NH_4OAc post-column (0.2 ml/min) with a *Waters 590-MS* pump to help ionization; MS recording in positive-ion thermospray mode, source 200°, vaporizer 95°. The separation was performed with 300 µg of extract on a *Waters NovaPak-RP-18* column (4 µm; 150 × 3.9 mm i.d.) with a linear gradient MeOH/H_2O (0.05% CF_3COOH) 70:30 → 100:0 within 30 min. Another separation was performed with 120 µg of extract on a *Waters NovaPak-RP-18* column (4 µm; 250 × 4.6 mm i.d.) with a linear gradient MeCN/H_2O (0.05% CF_3COOH) 25:75 → 55:45 within 45 min.

LC/NMR Analysis of **1/2**. LC/NMR setup: *Varian Inova-500* spectrometer equipped with a ^{1}H[^{13}C] pulse-field gradient LC/NMR flow-probe (60 µl, 3 mm i.d.), a *Varian 9012* ternary HPLC pump, a *Waters 490MS* UV detector, and a *Valco* stop-flow valve. For the stop-flow experiments, the UV detector was used to trigger the stop-flow valve and trap precisely the LC/peak of interest in the LC/NMR flow cell [12]. Solvent suppression was achieved by applying the WET sequence [13]. For the on-flow experiments, 24 transients per increments were recorded, in the stop-flow mode, 256 transients were accumulated for each spectrum. Reference for the LC signal was set at δ 2.10 for MeCN. The separation was performed with 300 µg of *Fraction D* on a *Waters NovaPak-RP-18* column (4 µm; 250 × 4.6 mm i.d.) with a linear gradient MeCN/D_2O 25:75 → 55:45 within 45 min.

*Mixture of (4*R*,6a*S*,10a*S*)- and (4*S*,6a*S*,10a*S*)-1,4,6a,7,8,9,10,10a-Octahydro-4,11-dihydroxy-7,7,10a-trimethyl-2*H*-phenanthro[1,2-*c*]pyran-6,12-dione* (**1** and **2**, resp.). Yellow crystalline powder. TLC (SiO_2, petroleum ether/AcOEt 1:1): R_f 0.44; dark green with *Godin*'s reagent [16]. UV (EtOH): 410 (2700), 331 (19000), 322 (18000). EI-MS: 344 (100, $M^{+\bullet}$, $C_{20}H_{24}O_5^{+\bullet}$), 329 (25), 299 (22), 285 (28), 283 (36), 262 (26), 255 (21), many fragments below 250. D/CI-MS: 363 ($[M+NH_4]^+$), 345 ($[M+H]^+$), 330 ($[M+H-15]^+$).

Data of Isomer **1**[2]): ^{1}H-NMR ((D$_6$)acetone): 6.58 (*s*, H–C(7)); 5.98 (*s*, H–C(17)); 4.15 (*m*, H_a–C(16)); 3.87 (*m*, H_b–C(16)); 3.07 (*m*, H_a–C(1)); 2.67 (*s*, H–C(5)); 2.47 (*m*, CH_2(15)); 1.75 (*m*, H_b–C(1), H_a–C(2)); 1.57 (*m*, H_b–C(2)); 1.38 (*m*, H_a–C(3)); 1.28 (*m*, H_b–C(3)); 1.27 (*s*, Me(19), Me(20)); 1.11 (*s*, Me(18)). ^{13}C-NMR ((D$_6$)acetone): 201.0 (C(6)); 181.9 (C(12)); 145.5 (C(11)); 143.7 (C(14)); 138.2 (C(8)); 133.3 (C(13));

131.3 (C(7)); 127.5 (C(9)); 87.5 (C(17)); 62.4 (C(5)); 56.2 (C(16)); 43.3 (C(4)); 43.0 (C(3)); 37.8 (C(1)); 33.4 (C(10)); 33.4 (C(18)); 22.9 (C(15)); 22.2 (C(19)); 21.8 (C(20)); 19.2 (C(2)).

Data of Isomer **2**[2]): ^{1}H-NMR ((D$_6$)acetone): 6.73 (*s*, H–C(7)); 5.90 (*s*, H–C(17)); 4.17 (*m*, H$_a$–C(16)); 3.87 (*m*, H$_b$–C(16)); 3.07 (*m*, H$_a$–C(1)); 2.70 (*s*, H–C(5)); 2.47 (*m*, CH$_2$(15)); 1.75 (*m*, H$_b$–C(1), H$_a$–C(2)); 1.57 (*m*, H$_b$–C(2)); 1.38 (*m*, H$_a$–C(3)); 1.28 (*m*, H$_b$–C(3)); 1.27 (*s*, Me(19)); 1.26 (*s*, Me(20)); 1.10 (*s*, Me(18)). ^{13}C-NMR ((D$_6$)acetone): 201.1 (C(6)); 181.9 (C(12)); 145.5 (C(11)); 143.5 (C(14)); 138.8 (C(8)); 133.1 (C(13)); 131.5 (C(7)); 127.5 (C(9)); 87.8 (C(17)); 62.6 (C(5)); 56.3 (C(16)); 43.3 (C(4)); 43.0 (C(3)); 37.7 (C(1)); 33.4 (C(10)); 33.4 (C(18)); 22.7 (C(15)); 22.2 (C(19)); 21.7 (C(20)); 19.2 (C(2)).

*Mixture of (4*R*,6a*S*,10a*S*)- and (4*S*,6a*S*,10*S*)-1,4,6a,7,8,9,10,10a-Octahydro-11-hydroxy-4-methoxy-7,7,10a-trimethyl-2*H*-phenanthro[1,2-c]pyran-6,12-dione* (**4** and **5**, resp.). Yellow crystalline powder. UV (EtOH): 413 (3630), 331 (25703), 322 (25118). EI-MS (70 eV): 359 (100, $M^{+\cdot}$, $C_{21}H_{26}O_5$), 344 (23), 343 (75), 328 (11), 327 (31), 326 (24), 315 (22), 311 (17), many fragments below 300. D/CI-MS: 376 ($[M+NH_4]^+$), 359 ($[M+H]^+$), 344 ($[M+H-15]^+$), 329 ($[M+H-30]^+$).

Data of Isomer **4**[2]): ^{1}H-NMR ($CDCl_3$): 7.40 (*s*, OH–C(11)); 6.31 (*s*, H–C(7)); 5.36 (*s*, H–C(17)); 4.02 (*m*, H$_a$–C(16)); 3.94 (*m*, H$_b$–C(16)); 3.52 (*s*, MeO–C(17)); 2.98 (*m*, H$_a$–C(1)); 2.64 (*s*, H–C(5)); 2.57 (*m*, CH$_2$(15)); 1.70 (*m*, H$_b$–C(1), H$_a$–C(2)); 1.59 (*m*, H$_b$–C(2)); 1.41 (*m*, H$_a$–C(3)); 1.27 (*s*, Me(19), Me(20)); 1.20 (*m*, H$_b$–C(3)); 1.12 (*s*, Me(18)). ^{13}C-NMR ($CDCl_3$): 201.0 (C(6)); 181.0 (C(12)); 144.2 (C(11)); 141.2 (C(14)); 137.0 (C(8)); 132.7 (C(13)); 130.9 (C(7)); 127.3 (C(9)); 93.4 (C(17)); 62.2 (C(5)); 56.0 (C(16)); 55.3 (C(21)); 42.9 (C(4)); 42.3 (C(3)); 37.0 (C(1)); 33.1 (C(18)); 32.8 (C(10)); 22.1 (C(15)); 21.9[3]) (C(19)); 21.8[3]) (C(20)); 18.6 (C(2)).

Data of Isomer **5**[2]): ^{1}H-NMR ($CDCl_3$): 7.39 (*s*, OH–C(11)); 6.41 (*s*, H–C(7)); 5.32 (*s*, H–C(17)); 4.02 (*m*, H$_a$–C(16)); 3.94 (*m*, H$_b$–C(16)); 3.53 (*s*, Me(21)); 2.98 (*m*, H$_a$–C(1)); 2.60 (*s*, H–C(5)); 2.57 (*m*, CH$_2$(15)); 1.70 (*m*, H$_b$–C(1), H$_a$–C(2)); 1.59 (*m*, H$_b$–C(2)); 1.41 (*m*, H$_a$–C(3)); 1.27 (*s*, Me(19), Me(20)); 1.20 (*m*, H$_b$–C(3)); 1.12 (*s*, Me(18)). ^{13}C-NMR ($CDCl_3$): 200.9 (C(6)); 181.1 (C(12)); 144.2 (C(11)); 141.1 (C(14)); 137.7 (C(8)); 132.6 (C(13)); 131.0 (C(7)); 127.3 (C(9)); 93.7 (C(17)); 62.5 (C(5)); 56.1 (C(16)); 55.3 (C(21)); 42.8 (C(4)); 42.4 (C(3)); 37.0 (C(1)); 33.2 (C(18)); 32.7 (C(10)); 21.9 (C(15)); 21.8[3]) (C(19)); 21.7[3]) (C(20)); 18.6 (C(2)).

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[2]) It was not possible to distinguish which ^{1}H-NMR signals were attributable to the isomers having the (17*R*)- or the (17*S*) configuration. The reported resonances were assigned arbitrarily to isomers **1** and **2**, but these assignments may be interchanged. The same comment is also valid for the artefacts **4** and **5**.

[3]) Assignments interchangeable.