77. Novel Antifungal Tetracyclic Compounds from *Bauhinia rufescens* LAM.[1])

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As the root bark CH_2Cl_2 extract of *Bauhinia rufescens* showed antifungal activity in a bioassay with the plant pathogenic fungus *Cladosporium cucumerinum*, a phytochemical investigation was undertaken on material collected in Niger. Activity-guided fractionation of this extract, using different preparative chromatographic methods, allowed the isolation of the four new antifungal tetracyclic compounds **1**–**4**. Their structures were established by ^{1}H- and ^{13}C-NMR data, and single-crystal X-ray analyses were used to confirm the structures of **1** and **3**. The isolated compounds seem to be biogenetically related to a stilbene.

Introduction. – *Bauhinia rufescens* LAM. (Leguminosae-Caesalpinioideae) is a small tree of widespread distribution in North and West Africa. The stem bark and root bark are used for the treatment of leprosy and different kinds of venereal diseases, and the roots are reputed to cure fevers [1].

A survey of the previous phytochemical studies on the genus *Bauhinia* showed a certain lack of data. Indeed, only few species of a total of more than 300 have so far been chemically investigated. However, those showed a series of biologically active compounds with different structures such as flavonoids [2–6], phenolic compounds [7], stilbene derivatives [8], protoal' aloids [9], and terpenoids [5]. No reports on the constituents of *B. rufescens* have appeared to our knowledge.

Screening for biologically active substances showed, during preliminary studies, that the CH_2Cl_2 extract of the root bark of *B. rufescens* exhibited antifungal activity against *Cladosporium cucumerinum*, a plant pathogenic fungus. These results led us to the isolation and the characterization of four new tetracyclic antifungal derivatives biogenetically related to a stilbene.

Results. – The root bark of *B. rufescens*, collected in Niger, was extracted with CH_2Cl_2. This extract (10.0 g) which showed significant fungitoxicity was subjected to a bioassay-guided fractionation by flash chromatography on silica gel (see *Exper. Part*). Several of the 12 fractions collected showed strong antifungal activity, namely *Fractions 2, 3, 6,* and *9* (1500, 640, 1690, and 320 mg, resp.). Further purification of *Fr. 2* by reversed-phase low-pressure liquid chromatography on *RP-8* afforded compounds **1** (50 mg). Separations of *Fr. 3* and *9* by low-pressure chromatography on *Diol* allowed the

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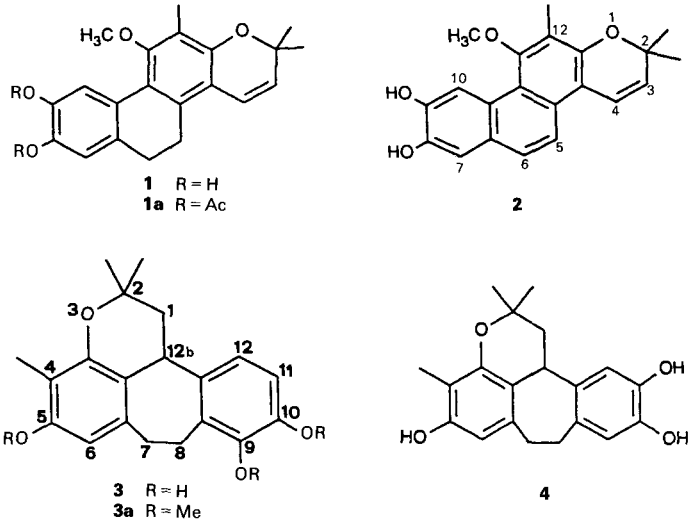


isolation of compounds **2** (15 mg) and **4** (34 mg), and purification of *Fr.* 6 by filtration on a *Sephadex-LH-20* column yielded compound **3** (81 mg).

Compound **1** was obtained as an amorphous powder (M^+ 338). This product was particularly unstable in solution and unsuitable for NMR measurements. However, its IR (chelated OH at 3340 cm^{-1}) and its UV spectrum (λ_{max} 268 nm) indicated the phenolic nature of this product. The presence of 2 OH groups was further confirmed by formation of diacetate **1a** whose structure was supported by its spectral data.

The HR-MS (EI) of **1a** indicated the molecular formula $C_{25}H_{26}O_6$ (M^+ 422.1725). In addition, the ^{1}H- and ^{13}C-NMR data were consistent with a tetracyclic skeleton, the δ(C)'s at 27.9 (2 CH_3) and 75.5 ppm (O-substituted quaternary C) and of 2 sp^2 C-atoms indicating in particular that one of these cycles is a 2,2-dimethyl-2*H*-pyran. High-field ^{13}C-signals for 2 CH_2 groups pointed to a cyclohexadiene moiety, while the two remaining cycles were polysubstituted benzene rings. The ^{1}H-NMR spectrum showed the presence of 1 MeO (3.55 ppm) and an aromatic Me group (2.12 ppm), besides 2 aromatic protons (7.03 and 8.16 ppm).

Diacetate **1a** crystallized easily from $MeCN/CHCl_3$ as colorless needles. An X-ray analysis of a single crystal of **1a** (see *Fig. 1* and *Exper. Part*) confirmed its structure to be the

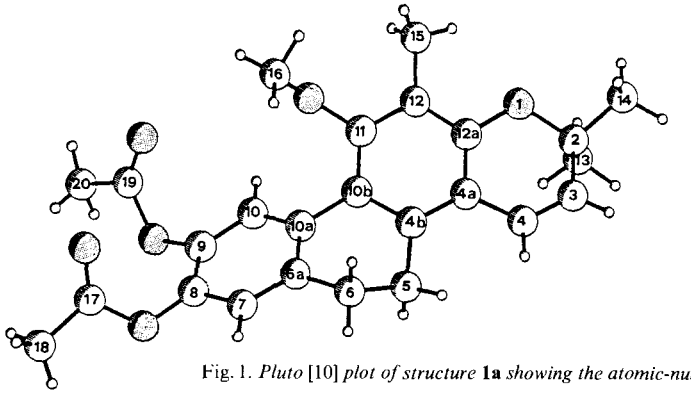


Fig. 1. *Pluto* [10] *plot of structure* **1a** *showing the atomic-numbering scheme*

diacetate of the parent 5,6-dihydro-11-methoxy-2,2,12-trimethyl-2*H*-naphtho[1,2-*f*][1]-benzopyran-8,9-diol (**1**), a new natural product. The best planes through the two benzene rings of **1a** were twisted by 151.2° with respect to each other, and the pyran ring was best described as having a twist conformation with C(3) and C(12a) lying on one two-fold axis and the second two-fold axis bisecting bonds O(1)–C(2) and C(4)–C(4a). The C–O bond lengths in the pyran moiety (1.372 and 1.459 Å) indicated some degree of π-electron delocalization over the C–O bonds (*cf.*: C=O bond length, 1.215 Å; C–O bond length in aliphatic system, 1.430 Å). The π-electron delocalization into the pyran ring and the conjugation of the two aromatic rings explained the presence of an absorption band at 307 nm in the UV spectrum of **1**. The cyclohexadiene ring (C(4b), C(5), C(6), C(6a), C(10a), C(10b)) was also best described as having a twist conformation with C(4b) and C(6a) lying on the first two-fold axis and the second two-fold axis bisecting bonds C(5)–C(6) and C(10a)–C(10b).

The structure of the closely related compound **2** was deduced mainly by comparison of its ^{1}H- and ^{13}C-NMR spectra with those of **1** and **1a** and postulated to be 11-methoxy-2,2,12-trimethyl-2*H*-naphtho[1,2-*f*][1]benzopyran-8,9-diol, another new natural product. This was confirmed by a series of NOE difference experiments [11].

The EI-MS of **2** showed a $M^{+\cdot}$ at 336 (*cf.* **1**, $M^{+\cdot}$ at 338), and no ^{13}C-NMR signals for CH_2 groups were observed. In the ^{1}H-NMR spectrum, signals for 1 MeO (3.74 ppm, *s*), 1 aromatic Me (2.37 ppm, *s*), and 1 Me_2C group (1.47 and 2.09 ppm, 2*s*) were observed, while the aromatic region was characterized by 2*d* (J = 10 Hz, each) for the 2 pyran protons (H–C(3) at 5.80 and H–C(4) at 7.15 ppm), 2*d* (J = 9 Hz each) for the 2 protons of the inner aromatic ring (H–C(6) at 7.56 and H–C(5) at 7.80 ppm), and 2*s* (1 H each, at 7.27 (H–C(7)) and 9.00 ppm (H–C(10)). The large downfield shift of the H–C(10) *s* was explained by the spatial proximity of the O-atom of MeO–C(11).

Presaturation of the H–C(4) resonance (7.15 ppm) of **2** produced enhancement of the H–C(5) (7.80 ppm) and H–C(3) signals (5.80 ppm), irradiation of the H–C(3) signal enhanced 1*s* (1.47 ppm) of the Me_2C unit and the H–C(4) *d*, and upon presaturation of the H–C(5) *d*, enhancement of the H–C(6) (7.56 ppm) and H–C(4) resonances were observed. Finally, irradiation of the MeO *s* (3.75 ppm) enhanced the signals of Me–C(12) (2.37 ppm) and H–C(10) (9.00 ppm).

After purification, compound **3** was obtained as a microcrystalline product with a molecular weight of 326 (see MS in *Exper. Part*). Its IR and UV spectra pointed to a phenolic product. Permethylation of **3** using CH_3I gave the trimethyl ether **3a**. The ^{1}H- and ^{13}C-NMR spectra of **3** and **3a** allowed the identification of **3** as 1,7,8,12b-tetrahydro-2,2,4-trimethyl-2*H*-benzo[6,7]cyclohepta[1,2,3-*de*][1]benzopyran-5,9,10-triol, a new natural product related to racemosol, a compound previously isolated from the heartwood of the Indian species *Bauhinia racemosa* LAMK. [12].

The ^{13}C-NMR spectrum and DEPT subspectra of **3** accounted for 20 C-atoms. Their multiplicities, together with the molecular weight of 326, established the molecular formula $C_{20}H_{22}O_4$. The ^{1}H-NMR spectrum indicated the presence of 2 aliphatic Me *s* (1.24 and 1.45 ppm) and 1 aromatic Me group (1.85 ppm). Signals for 3 protons were observed in the aromatic region (1 H as *s* (6.03 ppm) and 2 H *ortho* to each other as an *AB* system (6.56 ppm, J = 8.5)). Of the 7 additional protons, 4 aliphatic nonequivalent CH_2 protons appeared as a complex *m* between 2.70 and 3.40 and a unique CH proton at 4.40 ppm ('*dd*', J = 12.6, 6.3). This latter showed coupling with 2 other protons (*ABX* system) at 2.24 ('*dd*', J = 12.6, 12.6) and 1.90 ppm ('*dd*', J = 12.6, 6.3) and supported, together with the 2 aliphatic Me *s* the presence of a 2,2-dimethylchroman moiety. This partial structure was confirmed by the loss of 56 amu in the EI-MS of **3**, due to a *retro-Diels-Alder* fragmentation. The remaining fragment included another aromatic ring and a C_2 fragment bearing the 4 aliphatic nonequivalent protons. Extensive NMR experiments, including COSY and NOE, gave further evidences for the structure of **3**.

In order to establish the relative configuration of **3**, the crystalline material was subjected to single-crystal X-ray analysis, which confirmed the overall connectivity de-

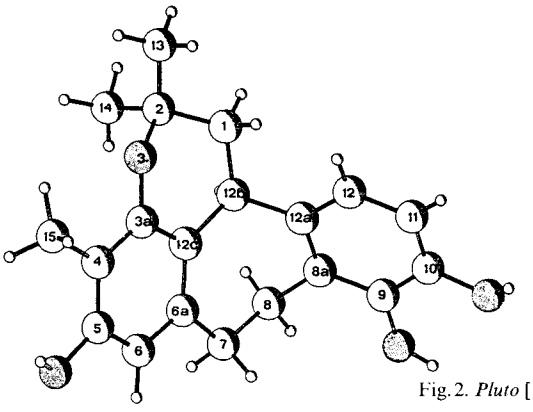


Fig. 2. *Pluto* [10] *view of molecule* **3**

duced by NMR analysis and revealed the relative configuration at C(12b) (see *Fig. 2* and *Exper. Part*). In the crystal structure of **3**, a strong intramolecular H-bond was present, together with three intermolecular H-bonds involving symmetry-related molecules. The torsion angles associated with the atoms of the cycloheptane ring clearly indicated that the best plane through atoms C(7), C(6a), C(12c), C(12b) was inclined by 58.9° to the best plane through atoms C(12b), C(12a), C(8a), C(8). Under these conditions, the two aromatic rings were no more coplanar and the best planes through them were inclined to

Table. *Comparison of* ^{13}*C-NMR Data* (50.1 MHz) *of Compounds* **3**, **3a**, *and* **4**[a])

	3[b])	**3**[c])	**3a**[d])	**4**[c])
C(1)	37.4 (*t*)	38.9	38.3	38.7
C(2)	72.7 (*s*)	73.4	73.1	73.4
C(3a)	153.3 (*s*)	154.3	156.1	154.1
C(4)	108.4 (*s*)	110.0	111.6	109.7
C(5)	151.8 (*s*)	153.4	152.1	152.9
C(6)	108.9 (*d*)	110.0	105.0	109.7
C(6a)	135.9 (*s*)	137.4	136.8	137.0
C(7)	33.6 (*t*)	34.8	35.1	35.8
C(8)	21.8 (*t*)	22.9	22.7	31.1
C(8a)	133.8 (*s*)	135.7	136.1	135.0 (*s*)
C(9)	143.4 (*s*)	144.3	151.1	115.4 (*d*)
C(10)	141.3 (*s*)	142.2	142.1	143.1 (*s*)
C(11)	111.6 (*d*)	112.3	109.1	143.5 (*s*)
C(12)	114.8 (*d*)	115.2	120.2	113.1 (*d*)
C(12a)	129.3 (*s*)	130.2	135.6	134.1
C(12b)	31.1 (*d*)	32.5	32.1	32.1
C(12c)	113.4 (*s*)	113.7	114.7	114.3
C(13)	29.9	n.d.	30.3	31.0
C(14)	22.8	23.4	23.1	23.0
C(15)	8.4	8.7	8.2	8.7
3 CH_3O			55.7, 55.3, 61.1	

[a]) For convenience, the atomic-numbering scheme of *Fig. 2* is used.
[b]) (D_6)DMSO.
[c]) (D_6)acetone.
[d]) $CDCl_3$.

one another by 63.5°, which indicated a *cis*-configuration of the two aromatic rings relative to the plane generated by C(12b), C(7), and C(8). In addition, the pyran ring could best be described as having a sofa conformation with atom C(2) displaced by 0.67 Å from the best plane through the remaining five atoms (planar to within 0.02 Å). The best plane through all six atoms of the pyran ring was inclined by 6.3° to the adjacent benzene ring. Finally, the C–O bond lengths in the pyran moiety (1.383 and 1.450 Å) indicated some degrees of π-electron delocalization over the C–O bonds.

The antifungal compound **4** isolated from the root bark of *B. rufescens* showed several spectroscopical analogies with compound **3**, including a similar EI-MS fragmentation (326 (M^+), 311 ($[M - 15]^+$), 270 ($[M - 56]^+$)) and similar ^{1}H- and ^{13}C-NMR spectra (see the *Table*) which finally allowed to deduce the structure of **4** to be 1,7,8,12b-tetrahydro-2,2,4-trimethyl-2*H*-benzo[6,7]cyclohepta[1,2,3-*de*][1]benzoypran-5,10,11-triol, a new natural product.

In the ^{1}H-NMR spectrum of **4**, apart from the typical signals for the *ABX* system of 2 H–C(1) and H–C(12b) (see *Exper. Part*) and the poorly defined complex *m* at 2.5–3.8 ppm of the 2 CH_2 groups, 3's' (1 H each) appeared in the aromatic region at 6.08, 6.69, and 6.84 ppm. None of them showed visible coupling; however, long-range 2D-delayed-COSY revealed that the two latter signals arose from *para*-related protons. The off-resonance and DEPT ^{13}C-NMR data of **4** were almost the same as that measured for **3** (see *Table*), except for the D-ring C-atoms and C(8) (different substitution pattern of D-ring). Extensive 2D-NMR experiments, including long-range 2D-COSY and NOE difference measurements, finally confirmed the structure of **4**.

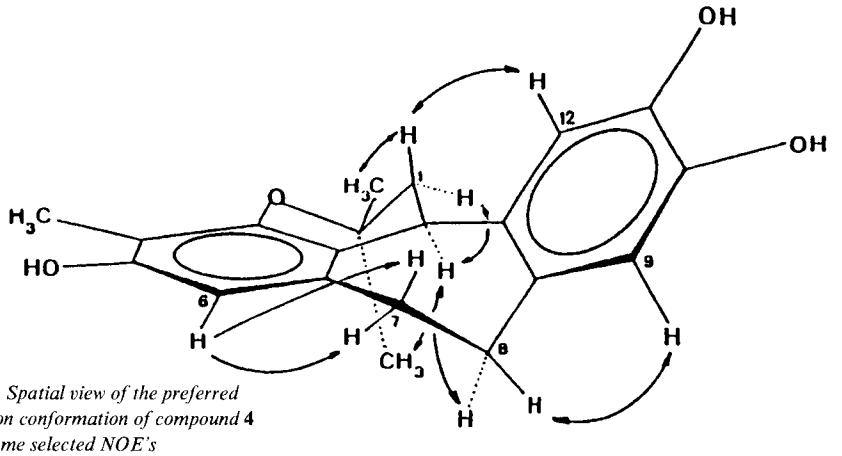
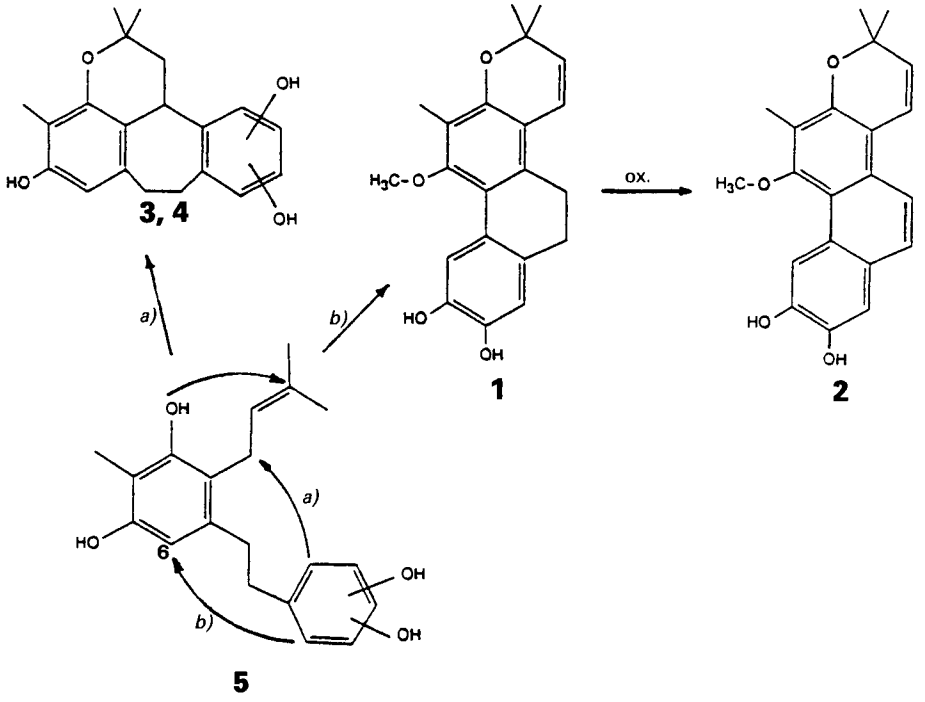


Fig. 3. *Spatial view of the preferred solution conformation of compound* **4** *and some selected NOE's*

The detailed ^{1}H-NMR data of **4** (see *Exper. Part*) showed that the preferred solution conformation of the tetracyclic skeleton of **4** did not correspond to the solid conformation of its isomer **3** as established by X-ray analysis: compound **4** in solution adopted a twisted conformation of the cycloheptadiene moiety, indicating that the two aromatic rings had a *trans*-configuration relative to the plane generated by C(12b), C(7), and C(8) (see *Fig. 3*).

Discussion. – In spite of the originality of the structure of the four isolated compounds, they seem to be biogenetically related to a common precursor, the stilbene derivative **5** shown in the *Scheme*. Indeed, intramolecular cyclization of **5** to the chroman

Scheme. *Postulated Biosynthetic Pathway for Compounds* **1–4**



(or chromene) ring and oxidative cyclization at C(6) or C(1″) would lead to products **1**, **3**, and **4**. Compound **2** could be an oxidation product of **1**. Although during this investigation, it was not possible to isolate or even detect any traces of the postulated precursor **5**, support for this biosynthetic scheme is provided by the presence in some other species of the genus *Bauhinia* of stilbene derivatives such as *trans*-resveratrol or pacharine [8].

The coloring of the amorphous compounds **1** and **2** and of the crystalline **3** and **4** (see *Exper. Part*) might arise from the presence of trace amounts of the corresponding *ortho*-quinone derivatives, possibly formed by oxidation during isolation. The intermediacy of such *ortho*-quinone derivatives could also be responsable for the fact that compounds **3** and **4** were optically inactive. Another explanation for that would be the involvement of a nonenzymatic step on cyclization of **5** at C(1″) (see *a*) in the *Scheme*).

Compounds **1–4** were tested for their fungitoxicity against *Cladosporium cucumerinum* using a TLC bioassay [13]. In this assay, the four products had similar activity, and amounts of 2 μg deposited on the TLC plate were sufficient to prevent growth of the fungus. Under the same conditions, 0.5 μg of the commercially available miconazole were sufficient to exhibit antifungal action on the TLC plate. In addition, at concentrations of 10 μg/ml, compounds **1** and **2** showed slight antifungal activity against the pathogenic microorganisms *Candida albicans* and *Saccharomyces cerevisiae* in a standard dilution assay [14]. On the other hands, these two products were inactive against *Aspergillus fumigatus* and *Trichophyton mentagrophytes var. asteroides*. Compounds **3** and **4** were inactive in these assays.

Experimental Part

General. TLC: silica gel *60* F_{254} precoated Al sheets (*Merck*), $CHCl_3$/MeOH 95:5; *RP-8*-precoated glass plates (HPTLC; *Merck*) MeOH/H_2O 8:2; *Diol*-precoated glass plates (HPTLC; *Merck*), AcOEt/petroleum ether 1:1; detection at 254 and 366 nm and with *Godin* reagent [15]. Column chromatography (CC): silica gel (63–200 μm; *Merck*) and *Sephadex LH-20* (*Pharmacia*). Low-pressure liquid chromatography (LPLC): *Lobar Lichroprep RP-8* (40–63 μm, 27 × 2.5 cm i.d.; *Merck*) or *Lobar Lichroprep Diol* (40–63 μm, 27 × 2.5 cm i.d.; *Merck*) equipped with a *Duramatic 80* pump (*Chemie und Filter*); flow rate 1 ml/min. Purity of compounds was checked by HPLC with a *Spectra-Physics-8700* pump and a photodiode array detector *HP-1040A* coupled with a *HP-85* personal computer and a *HP-7470A* plotter (*Hewlett-Packard*); column, *μ-Bondapak C-18* (10 μm, 300 × 3.9 mm i.d.; *Waters*). M.p.: *Mettler FP 80/82* hot stage apparatus; uncorrected. $[\alpha]_D$: *Perkin-Elmer-241* polarimeter. UV spectra: *Perkin-Elmer-Lambda-3* spectrophotometer. IR spectra: *Perkin-Elmer-681* apparatus. ^{1}H- and ^{13}C-NMR: *Varian VXR 200* equipped with switchable 5-mm probe; 200 and 50.1 MHz, resp.; solns. in $CDCl_3$, (D_6) acetone, or (D_6)DMSO; chemical shifts in δ (ppm) rel. to TMS as internal standard; multiplicities determined by DEPT experiments; NOE difference measurements were carried out with a standard pulse sequence, an irradiation of 5 s was used, with no additional relaxation delay after data acquisition.

Plant Material. B. rufescens was collected in September 1989 near Niamey (Niger). A voucher specimen of the plant material is retained at the University of Niamey (Niger).

Extraction and Isolation. The powdered root bark of *B. rufescens* (200 g) was extracted at r.t. with CH_2Cl_2. A 10.0-g portion of this extract (12.0 g) was submitted to flash chromatography on a silica-gel column (63–200 μm, 60 × 5.0 cm i.d.) with a $CHCl_3$/MeOH gradient (99:1→95:5→50:50), and 12 fractions were collected. Purification of *Fr. 2* (500 mg) by LPLC on *RP-8* (MeOH/H_2O 7:3) afforded **1** (50 mg). LPLC of *Diol* of *Fr. 3* (200 mg) and *Fr. 9* (100 mg) using toluene/AcOEt 9:1 and 2:1, resp., yielded **2** (15 mg) and **4** (34 mg), resp. Finally, purification of *Fr. 6* (500 mg) by gel filtration on *Sephadex LH-20* (MeOH) afforded **3** (81 mg). Microcrystals of **3** were obtained by further repeated crystallization from MeCN/hexane.

*5,6-Dihydro-11-methoxy-2,2,12-trimethyl-2*H*-naphtho[1,2-f][1]benzopyran-8,9-diol* (**1**). Grey-red amorphous powder. M.p. 75–80°. TLC (SiO_2, $CHCl_3$/MeOH 95:5): R_f 0.42, violet carmine with *Godin* reagent. UV (MeCN): 307 (4500), 268 (8500), 228 (7700), 203 (6700). IR (KBr): 3340, 3000–2900, 1610, 1420, 1140. ^{1}H-NMR (200 MHz, (D_6)DMSO): 1.43 (*s*, Me_2C); 2.16 (*s*, Me–C(12)); 3.51 (*s*, MeO–C(11)); 5.61 (*d*, J = 10, H–C(3)); 6.56 (*d*, J = 10, H–C(4)). EI-MS: 338 ($M^{+\cdot}$), 323 ($[M-15]^+$), 308 ($[M-30]^{+\cdot}$). DCI-MS (NH_3, positive-ion mode): 356 ($[M+NH_4]^+$), 339 ($[M+H]^+$).

Acetylation of **1**. A soln. of **1** (40 mg) in Ac_2O/pyridine 1:1 was stirred at r.t. for 24 h. The mixture was then poured into ice/H_2O and the precipitate purified by CC (silica gel, CH_3Cl/MeOH 99:1): diacetate **1a** (30 mg), which crystallized easily from MeCN/$CHCl_3$. Colorless flat needles. M.p. 125–129°. TLC (SiO_2, petroleum ether/AcOEt 1:1): R_f 0.5, red with *Godin* reagent. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.44 (*s*, Me_2C); 2.12 (*s*, Me–C(12)); 2.28, 2.29 (2*s*, 2 Ac); 2.70 (br. *s*, CH_2(5), CH_2(6)); 3.55 (*s*, MeO–C(11)); 5.62 (*d*, J = 10, H–C(3)); 6.55 (*d*, J = 10, H–C(4)); 7.03 (*s*, H–C(7)); 8.16 (*s*, H–C(10)). ^{13}C-NMR (50.1 MHz, $CDCl_3$): 168.5 (2 CH_3COO); 157.1 (C(12a)); 151.4 (C(11)); 140.3 (C(9)); 139.6 (C(8)); 136.0, 133.0, 131.7 (C(4b), C(10a), C(6a)); 129.5 (C(3)); 122.3 (C(4)); 121.4 (C(7)); 119.0 (C(10)); 118.7, 117.9, 114.5 (C(10b), C(4a), C(12)); 75.5 (C(2)); 60.1 (CH_3O); 28.9 (C(6)); 27.9 ($CH_{3\alpha}$–C(2), CH_3COO); 24.3 (C(5)); 20.1 ($CH_{3\beta}$–C(2)); 8.5 (CH_3–C(12)). HR-MS (EI): 422.1725 ($M^{+\cdot}$), 407.1512 ($[M-CH_3]^{+\cdot}$), 380.1625 ($[M-C_2H_2O]^{+\cdot}$), 365.1371, 323.1247.

X-Ray Analysis of **1a**. Suitable crystals were grown from $CHCl_3$/MeCN 1:5. Crystal data: $C_{25}H_{26}O_6$, M_r 422.2, space group $P2_1/c$, a = 13.3141(3), b = 11.731(1), c = 14.320(1) Å, α = 90°, β = 107.96(1)°, γ = 90°, V = 2127.6 Å^3, $F(000)$ = 8.96, Z = 4, D_c = 1.319 g·cm^{-3}, MoK_α, λ = 0.71073 Å, μ = 0.9 cm^{-1}. One crystal (0.49 × 0.42 × 0.38 mm) was used for data collection. Intensity data with index limit h −15 to 15, k 0 to 13, l 0 to 17, and θ_{max} = 25° were measured on a *Stoe Siemens AED2* four-circle diffractometer (graphite-monochromated MoK_α radiation). There was no significative intensity variation for 3 standard reflections measured every h. A total of 3723 unique reflections were measured of which only 2714 could be considered observed ($I > 2.5\,\sigma(I)$). Cell parameters from ± ω of 25 reflections in the range 30° < 2θ < 38°. No absorption corrections were applied. The structure was solved by direct methods using the program SHELXS-86 [16]. The program NRCVAX [17] was used for all further calculations. The H-atoms were located in differences maps and optimized in an isotropic manner. Weighted anisotropic refinement for 2714 reflections converged at R = 0.044, R_w = 0.069; $w^{-1} = \sigma^2(F_o) + 0.00208(F_o)^2$. Average parameter shift was 0.234. Heights in final difference map ρ_{max} = 0.22; ρ_{min} = −0.32 eÅ^{-3}. Supplementary material, see below (X-ray analysis of **3**).

*11-Methoxy-2,2,12-trimethyl-2*H*-naphtho[1,2-f][1]benzoypran-8,9-diol* (**2**) Grey-red amorphous powder. M.p. 86–91°. TLC (SiO_2, $CHCl_3$/MeOH 95:5): R_f 0.13, violet carmine with *Godin* reagent. UV (MeCN): 380 (780), 360 (700), 300 (3500), 267 (8000), 235 (6200), 202 (4500). ^{1}H-NMR (200 MHz, (D_6)acetone): 1.44 (*s*, Me–C(2)); 2.07 (*s*, Me–C(2)); 2.37 (*s*; Me–C(12)); 3.75 (*s*, MeO–C(11)); 5.80 (*d*, J = 10.0, H–C(3)); 7.15 (*d*, J = 10.0, H–C(4)); 7.27 (*s*, H–C(7)); 7.56 (*d*, J = 9.0, H–C(6)); 7.80 (*d*, J = 10.0, H–C(5)); 9.00 (*s*, J = 9.0, H–C(10)). ^{13}C-NMR (50.1 MHz, $CDCl_3$): 157.4 (C(12a)); 150.9 (C(11)); 144.5, 143.6 (C(8), C(9)); 129.5 (C(3)); 128.0, 127.9 (C(4b), C(6a)); 127.0 (C(4)); 125.2 (C(10a)); 120.6 (C(10b)); 119.8, 119.6 (C(7), C(10)); 118.1 (C(4a)); 112.7 (C(12)); 112.6 (C(7)); 112.5 (C(10)); 76.3 (C(2)); 60.2 (CH_3O–C(11)); 28.4 ($CH_{3\alpha}$–C(2)); 28.2 ($CH_{3\beta}$–C(2)); 9.0 (CH_3–C(12)). EI-MS: 336 ($M^{+\cdot}$), 321 ($[M-15]^+$), 306 ($[M-30]^{+\cdot}$), 277 ($[M-59]^+$), 265 ($[M-71]^+$).

*1,7,8,12b-Tetrahydro-2,2,4-trimethyl-2*H*-benzo[6,7]cyclohepta[1,2,3-de][1]benzopyran-5,9,10-triol* (**3**). Red prisms from MeCN/hexane. M.p. 208–212°. TLC (SiO_2, $CHCl_3$/MeOH 95:5): R_f 0.16, red with *Godin* reagent. UV (MeCN): 277 (1600), 203 (14500). IR (KBr): 3400, 2980, 2920, 1540, 1480, 1270. ^{1}H-NMR (200 MHz, (D_6)DMSO): 1.24, 1.45, (2*s*, Me_2C); 1.85 (*s*, MeC(4)); 1.90 ('*dd*', J = 6.3, 12.6, H_α–C(1)); 2.24 ('*dd*', J = 12.6, 12.6, H_β–C(1)); 2.70–3.40 (*m* CH_2(7), CH_2(8)); 4.40 ('*dd*', J = 12.6, 6.3, H–C(12b)); 6.03 (*s*, H–C(6)); 6.53 (*A* of *AB*, J = 8.5, H–C(11)); 6.69 (*B* of *AB*, J = 8.5, H–C(12)). ^{13}C-NMR (50.1 MHz, (D_6)DMSO): *Table*. EI-MS: 326 ($M^{+\cdot}$), 309 ($[M-15]^+$), 270 ($[M-56]^{+\cdot}$). DCI-MS (NH_3, positive-ion mode): 344 ($[M+NH_4]^+$), 327 ($[M+H]^+$), 270 ($[M+H-57]^+$).

X-Ray Analysis of **3**. Suitable crystals were grown from $CHCl_3$/MeCN 1:5. Crystal data: $C_{20}H_{22}O_4$, M_r = 326.4, space group $P\bar{1}$, a = 5.240(1), b = 10.650(1), c = 14.774(1) Å, α = 100.51(1)°, β = 94.10(1)°, γ = 95.47(1)°, V = 803.6 Å^3, $F(000)$ = 348, Z = 2, D_c = 1.349 g·cm^{-3}, MoK_α, λ = 0.71073 Å, μ = 0.9 cm^{-1}. One crystal (0.53 × 0.30 × 0.11 mm) was used for data collection. Intensity data with index limit h −6 to 6, k −12 to 12, l −17 to 17, and θ_{max} = 25° were measured on a *Stoe Siemens AED2* four-circle diffractometer (graphite-monochromated MoK_α radiation) using the ω/θ scan mode. There was no significative intensity variation for 4 standard reflections measured every h. A total of 5641 unique reflections were measured of which only 1868 could be considered observed ($I > 2.5\sigma(I)$). Cell parameters from ± ω of 22 reflections in the range 25° < 2θ < 35°. No absorption corrections were applied. The structure was solved by direct methods using the program SHELXS-86 [16]. The program NRCVAX [17] was used for all further calculations. The H-atoms were located in differences maps and optimized in an isotropic manner. Weighted anisotropic refinement for 1868 reflections converged at R = 0.042, R_w = 0.058; $w^{-1} = \sigma^2(F_o) + 0.00109(F_o)^2$. Average parameter shift was 0.012. Heights in final difference map ρ_{max} = 0.15; ρ_{min} = −0.23 eÅ^{-3}. Supplementary material is available from *H.St.-E.* and has been deposited with the *Cambridge Crystallographic Data Centre*.

Methylation of **3**. To a soln. of **3** (5 mg) and K_2CO_3 (excess) in acetone, CH_3I (1 ml) was added in small portions. The mixture was kept at r.t. for 20 h, then poured in H_2O, and extracted with $CHCl_3$. Further purification by LPLC (silica gel, $CHCl_3$) yielded 4 mg of tri-*O*-methyl derivative **3a**. Yellow oil. TLC (SiO_2, $CHCl_3$/MeOH 99:1): R_f 0.74, red with *Godin* reagent. ^{1}H-NMR (200 MHz, $CDCl_3$): 1.34, 1.55 (2*s*, Me_2C); 1.98 (*s*, Me–C(4)); 1.95 ('*dd*', J = 6, 12.5, H_α–C(1)); 2.40 ('*dd*', J = 12.5, 12.5, H_β–C(1)); 2.95–5.50 (*m*, CH(7), CH(8)); 3.69, 3.83, 3.85 (3*s*, 3 MeO); 4.55 ('*dd*', J = 12.5, 6, H–C((12b)); 6.10 (*s*, H–C(6)); 6.58 (*d*, J = 10, H–C(11)); 7.02 (*d*, J = 10, H–C(12)). ^{13}C-NMR (50.1 MHz, $CDCl_3$): *Table*. EI-MS: 368 ($M^{+\cdot}$), 312 ($[M-56]^{+\cdot}$), 294, 283.

*1,7,8,12b-Tetrahydro-2,2,4-trimethyl-2*H*-benzo[6,7]cyclohepta[1,2,3-de][1]benzopyran-5,10,11-triol* (**4**). Orange microcrystalline powder. M.p. 238–242°. TLC (SiO_2, $CHCl_3$/MeOH 95:5): R_f 0.13, red with *Godin* reagent. UV (MeCN): 351 (200), 283 (1850), 202 (15200). IR (KBr): 3400, 3000–2900, 2920, 1530, 1480, 1270. ^{1}H-NMR (200 MHz, (D_6)acetone): 1.30, 1.50 (2*s*, Me_2C); 1.95 (*s*, Me–C(4)); 1.95 (*dd*, J = 12.6, 6.0, H_α–C(1)); 2.25 (*dd*, J = 12.6, 6.0, H_β–C(1)); 2.57 (*ddd*, J = 4.0, 4.0, 13.5, H_β–C(8)); 2.87 (*ddd*, J = 14.0, 4.0, 13.5, H_β–C(7)); 3.05 (*ddd*, J = 14.0, 4.0, 4.0, H_α–C(7)); 3.40 (*ddd*, J = 4.0, 13.5, 13.5, H_α–C(8)); 4.48 (*dd*, J = 6.0, 12.5, H–C(12b)); 6.08 (*s*, H–C(6)); 6.69 (*s*, H–C(9)); 6.84 (*s*, H–C(12)). ^{13}C-NMR (50.1 MHz, (D_6)acetone): *Table*. EI-MS: 326 ($M^{+\cdot}$), 309 ($[M-15]^+$), 270 ($[M-56]^{+\cdot}$). DCI-MS (NH_3, positive-ion mode): 344 ($[M+NH_4]^+$), 327 ($[M+H]^+$), 270 ($[M+H-57]^+$).

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