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BENZOPYRANONES AND FERULIC ACID DERIVATIVES FROM *ANTIDESMA MEMBRANACEUM*

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Key Word Index—*Antidesma membranaceum*; Euphorbiaceae; bark; roots; benzopyranones; 8,8-bis-(dihydroconiferyl)-diferuloylate; feruloyl amides.

Abstract—From *Antidesma membranaceum*, besides three feruloyl amides and (−)-syringaresinol, new phenolic compounds have been isolated. Their structures were established as two series of 2-alkylated 5,7-dihydroxychromones and 2,5,7-trihydroxychromanones, and the dimeric compound, 8,8-bis-(dihydroconiferyl)-diferuloylate, respectively, from their spectroscopic data. © 1997 Elsevier Science Ltd

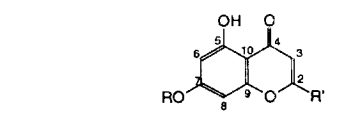
INTRODUCTION

Antidesma membranaceum is a shrub or small tree occurring in the rain forests of tropical Africa, which has not been investigated phytochemically until now. It belongs to the subfamily Phyllanthoideae, which is somewhat distinct in appearance and phytochemistry from the mono-ovulate subfamilies of the Euphorbiaceae. In the genus *Antidesma*, the occurrence of triterpenoids [1–4], a dimeric ellagitannin [5] and of cyclopeptide alkaloids [6] has been published.

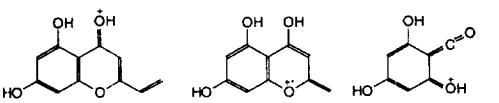
In continuation of our studies on the bioactive constituents of tropical and subtropical plants [7], the present paper deals with the isolation and structural determination of 2-alkyl-5,7-dihydroxychromones and 2-alkyl-2,5,7-trihydroxychromanones, two related groups of benzopyranones, and 8,8-bis-(dihydroconiferyl)-diferuloylate obtained, besides *N-trans*-feruloyl tyramine, *N-trans*-feruloyl octopamine, *N-cis*-feruloyl octopamine and (−)-syringaresinol, from *A. membranaceum*.

RESULTS AND DISCUSSION

Leaves, stem bark and roots were collected, dried and processed separately using a standardized extraction scheme. From the *n*-hexane extract of root material a mixture of the benzopyranones **1a–c** could be isolated by silica gel chromatography. Their elemental compositions were determined by high resolution mass spectrometry. The mass spectral behaviour of compounds **1a–1c** is mainly characterized by



1a	R=H	R'=$C_{19}H_{39}$	5,7-Dihydroxy-2-nonadecyl-chromone
1b	R=H	R'=$C_{20}H_{41}$	5,7-Dihydroxy-2-eicosyl-chromone
1c	R=H	R'=$C_{21}H_{43}$	5,7-Dihydroxy-2-heneicosyl-chromone
1d	R=Ac	R'=$C_{19}H_{39}$	
1e	R=Ac	R'=$C_{20}H_{41}$	
1f	R=Ac	R'=$C_{21}H_{43}$	



a: *m/z* 205 **b**: *m/z* 192 **c**: *m/z* 153

Fig. 1. Structure and main key fragments in the EI mass spectra of 2-alkyl-5,6-dihydroxy-chromones (**1a–c**).

the formation of the key ions **a**, **b** and **c** (Fig. 1). While ion **b** originates from a McLafferty rearrangement, ion **c** is formed by a retro-Diels–Alder-reaction. Cleavage of the C-2′/C-3′ bond leads to the energetically favoured ion **a**. Besides the signals of the alkyl side-chain, the ^{1}H NMR revealed two aromatic protons in a *meta*-position, two hydroxyl protons (one of these strongly hydrogen-bonded) and one other downfield-shifted proton. From these data and ^{13}C NMR measurements (Table 1), a 5,7-dihydroxychromone skeleton with alkyl side chains of 18, 19 and 20 carbon atoms at C-2 is indicated for **1a–c**. Compound **1b** is known as a hydration product of the 5,7-dihydroxy-2-nonadeca-4,7,10,13,16-pentenyl-chromone found in the brown algae *Zonaria tournefortii* [8] and its ^{1}H NMR and ^{13}C NMR values fit well with our data. The relative composition of the

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Table 1. ^{1}H and ^{13}C NMR data of 2-alkyl-5,6-dihydroxy-chromones (**1a–c**)

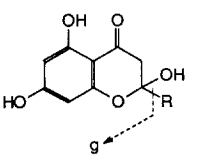
Position:	2	3	4	5	6	7	8	9	10	1′
^{13}C NMR†	170.8	107.7	182.6	158.3	99.3	162.3	94.1	162.2	105.1	34.2
^{1}H NMR‡	—	6.03	—	12.7	6.34	6.52	6.28	—	—	2.57
		s		OH	*d*, J = 2Hz	OH	*d*, J = 2Hz			*t*, J = 7.3Hz

† 125.7 MHz, $CDCl_3$.
‡ 500 MHz, $CDCl_3$.

benzochromanones **1a–c** was deduced from the total ion chromatogram obtained by GC-mass spectrometry of their monoacetates **1d–f**. Accordingly, compound **1a** with 83% represents the main component (**1b**: 5%, **1c**: 12%).

The compounds **2a–c** isolated as a mixture from the same column chromatography show $[M]^+$ at m/z 462, 476 and 490 as obtained by LC-mass spectrometry using negative ion electrospray detection. The EI mass spectral fragmentation pattern is similar to that found for **1a–c**. Upon water expulsion from the $[M]^+$, key fragments of the types **a–c** are formed (Fig. 1). The most prominent ion **g** is formed by α-cleavage of the alkyl side chain moiety C-2 (Fig. 2), suggesting an additional hydroxyl group. In the ^{1}H NMR, instead of the 3-H singlet from compounds **1a–c**, two doublets with geminal coupling constants of 17 Hz appear. Therefore, the structures were identified as 2-nonadecyl-, 2-eicosyl- and 2-heneicosyl-2,5,7-trihydroxychromanone. These three compounds have not been reported in the literature before, but spectral data are in good coincidence with the known 2-isopropyl-2,5,7-trihydroxychromanone isolated from *Helichrysum callicomum* [9].

From the ethyl acetate extract of roots, three feruloyl amides, (−)-syringaresinol and the new 8,8-bis(dihydroconiferyl)-diferuloylate (**3**) were isolated. Compound **3** displays in the EI mass spectrum a $[M]^+$ at m/z 714 and two key ions **h** and **i**, indicating the expulsion of one and two $C_{10}H_{10}O_4$ ferulic acid moieties, respectively (Fig. 3). Fragments **j** and **l** are characteristic of the feruloyl moiety. Expulsion of $C_8H_9O_2$ from ion **i** leads to ion **k**. ^{1}H NMR data showed that compound **3** represents a dimer with two 1,3,4-substituted phenolic systems, whereas HMQC- and HMBC-measurements revealed the structures of dihydroconiferyl and feruloyl moieties. Since H-8 shows in the ^{1}H NMR spectrum a multiplet at δ 2.21 with



2a R = $C_{19}H_{39}$ 2-Nonadecyl-2,5,7-trihydroxy-chromanone
2b R = $C_{20}H_{41}$ 2-Eicosyl-2,5,7-trihydroxy-chromanone
2c R = $C_{21}H_{43}$ 2-Heneicosyl-2,5,7-trihydroxy-chromanone

Fig. 2. Structure of chromanones (**2a–c**).

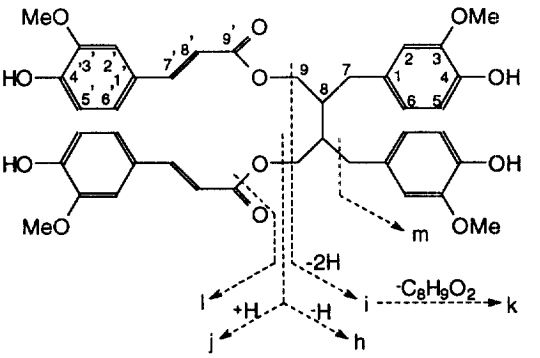
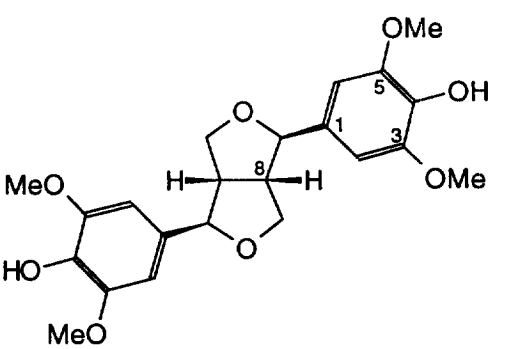


Fig. 3. Structure and main key fragments in the EI mass spectra of 8,8-bis(dihydroconiferyl-feruloylate) (**3**).

the same relative intensity as H-7′, the two dihydroconiferyl moieties must be connected *via* C-8. This leads to the structure of 8,8-bis-(dihydroconiferyl)-diferuloylate, which is a new natural product. *N-trans*-feruloyl tyramine was readily identified by its characteristic EI mass spectrum [10]. In the ^{1}H NMR spectrum, the signals assigned to the vinylic protons at δ 6.50 (J = 15.6 Hz) and at δ 7.45 (J = 15.6 Hz) revealed the *trans*-configuration of the double bond. *N-trans*-feruloyl octopamine and *N-cis*-feruloyl octopamine were isolated as a mixture, and determined using EI- and LC-mass spectrometry (positive ion electrospray) and ^{1}H NMR data in comparison with published data [10]. In the LC chromatograms (25% MeCN, 0.2% HOAc), two peaks at R_t 3.05 and 3.52 min with identical mass spectra appeared. In the ^{1}H NMR spectrum of the mixture, the intensities of the *trans*- and *cis*-olefinic proton signals gave a *cis-trans* ratio of 17:83.

(−)-Syringaresinol (**4**) could be unambiguously



(-)-Syringaresinol (**4**)

identified by direct comparison of ^1H and ^{13}C NMR data [11] and $[\alpha]_D$ value [12]. Syringaresinol is a widespread lignan compound occurring mostly in its (+)-form.

EXPERIMENTAL

General. NMR: Unity 500 and Gemini 300 (Varian) with TMS as int. standard. EIMS: 70 eV. GC-MS: EI (70 eV), source temp. 200°, column DB-5MS (J and W, 15 m × 0.32 mm, 0.25 μm film thickness), inj. temp. 260°, interface temp. 300°, carrier gas He, flow rate 1 ml min^{-1}, splitless injection; column temp. programme: 170° for 1 min, then raised to 270° at a rate of 25° min^{-1} and then elevated to 290° at a rate of 2° min^{-1}. RR_t values were calculated with respect to 5α-cholestane (R_t = 5.95 min). Acetylation was done in pyridine and acetic anhydride for 12 hr at room temp. LC-MS: column LiChrospher RP-18 (5 μm, 2 × 100 mm), flow: 0.2 ml min^{-1}. Silica gel Merck 0.063–0.2 mm for CC and 0.04–0.063 mm for flash CC were used.

Plant material. Antidesma membranaceum Müll. Arg. 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 bark (1.541 g) and roots (314 g) were extracted separately with 80% MeOH (bark: 30 l and root: 9 l), concd *in vacuo* and successively extracted with *n*-hexane (2.3 l, 1.7 l) and EtOAc (2.5 l, 1.5 l). The dried extracts (bark: *n*-hexane extract 2.51 g; EtOAc extract 10.63 g, root: 1.35 and 1.34 g, respectively). Chromatography (*n*-hexane with increasing amounts of EtOAc) of 1 g of the *n*-hexane-extract of the roots yielded, with 30% EtOAc, a complex fr. (79 mg) that was further purified by CC (*n*-hexane–EtOAc from 49:1 to 4:1); 5 mg of benzochromanones **1a–c** and 12 mg of **2a–c** were obtained.

Compounds **1a–c**. EI-MS *m/z* (rel. int.): 472.3555 [M **1c**]$^+$ (20) (calcd for $C_{30}H_{48}O_4$: 472.3552), 458.3408 [M **1b**]$^+$ (8) (calcd for $C_{29}H_{46}O_4$: 458.3396), 444.3229 [M **1a**]$^+$ (48) (calcd for $C_{28}H_{44}O_4$: 444.3239), 205 (**a**, 100), 192 (**b**, 31), 153 (**c**, 12). NMR: Table 1. A part of the mixt. (0.5 mg) was acetylated and examined by GC-MS (rel. int.): **1d**: RR_t 2.76, 486 [M]$^+$ (48), 247 (92), 234 (25), 205 (**a**, 100), 192 (**b**, 35), **1e**: RR_t 3.11, 500 [M]$^+$ (23), 247 (74), 234 (25), 205 (**a**, 100), 192 (**b**, 36), **1f**: RR_t 3.54, 514 [M]$^+$ (39), 247 (82), 234 (25), 205 (**a**, 100), 192 (**b**, 36).

Compounds **2a–c**. EI-MS *m/z* (rel. int.): 490.3682 [M **2c**]$^+$ (5) (calcd for $C_{30}H_{50}O_5$: 490.3658), 472 [M **2c**-H_2O]$^+$ (12), 462.3315 [M **2a**]$^+$ (9) (calcd for $C_{28}H_{46}O_5$: 462.3345), 444 [M **2a**-H_2O]$^+$ (20), 205 (**a**, 30), 195 (**d**, 100), 192 (**b**, 11), 153 (**c**, 31). LC-MS (95% MeCN, 0.2% HOAc, negative ion ESI MS): **2a**: R_t 12.53 min, 462 [M]$^-$ (66), 461 [M-H]$^-$ (100), **2b**: R_t 16.1 min, 476 [M]$^-$ (84), 475 [M-H]$^-$ (100), **2c**: R_t 20.93 min, 490 [M]$^-$ (100), 489 [M-H]$^-$ (88). ^1H NMR ($CDCl_3$ + CD_3OD, 300 MHz): δ 0.880 (3H, *t*, *J* = 6.7 Hz, —CH_3), 2.89 (1H, *d*, *J* = 17 Hz, H-3a), 2.73 (1H, *d*, *J* = 17 Hz, H-3b), 5.902 (1H, *d*, *J* = 2 Hz, H-8), 5.948 (1H, *d*, *J* = 2 Hz, H-6). $[\alpha]_D^{21.2} = -12.9°$ ($CHCl_3$, *c* 0.064).

Chromatography of the EtOAc-extract of roots (*n*-hexane with increasing amounts of EtOAc) gave, with EtOAc, a fr. (78 mg) which was further purified by flash CC ($CHCl_3$–MeOH, 100:0 to 19:1) to afford successively 3.4 mg **4** [11, 12], 1.7 mg 8,8-bis-(dihydroconiferyl)-diferulate (**3**), 7.7 mg *N-trans*-feruloyl tyramine [10] and 5 mg of *N-trans*-feruloyl-octopamine [10] and *N-cis*-feruloyl-octopamine.

Compound **3**. EI-MS *m/z* (rel. int.): 714.2774 [M]$^+$ (10) (calcd for $C_{40}H_{42}O_{12}$ 714.2676), 520.2093 (**h**, 9;

Table 2. ^1H and ^{13}C NMR data of 8,8-bis-(dihydroconiferyl)-diferuloylate (**3**)

Position:	1	2	3	4	5	6	7	8	9	3-OCH_3
^1H NMR*	—	6.53 1H, *d*, *J* = 2 Hz	—	—	6.78 1H, *d*, *J* = 8 Hz	6.61 1H, *dd*, *J* = 2 Hz, *J* = 8 Hz	2.71 2H, *m*	2.21 1H, *m*	4.15 2H, ABq	3.77 3H, *s*
^{13}C NMR†	131.8	111.2	146.2	144	114.1	121.7	35.2	40.0	64.4	55.7
Position:	1′	2′	3′	4′	5′	6′	7′	8′	9′	3′-OCH_3
^1H NMR*	—	7.01 1H, *d*, *J* = 2 Hz	—	—	6.89 1H, *d*, *J* = 2 Hz	7.06 1H, *dd*, *J* = 8 Hz, *J* = 2 Hz	7.59 1H, *d*, *J* = 16 Hz	5.85 1H, *d*, *J* = 16 Hz	—	3.91 3H, *s*
^{13}C NMR†	126.5	109.4	146.5	148.2	119.6	123.0	145.1	116.1	167.0	55.9

* 500 MHz, $CDCl_3$.

† Derived from HMBC and HMQC, 500 MHz, $CDCl_3$.

calcd for $C_{30}H_{32}O_8$ 520.2097), 344 (8), 326.1527 (**i**, 54; calcd for $C_{20}H_{22}O_4$ 326.1518), 194 (**j**, 17), 198 (**k**, 52), 177 (**l**, 100), 145 (20), 137 (**m**, 88). ^{1}H and ^{13}C NMR: Table 2.

N-trans-*feruloyl tyramine*. ^{13}C NMR ($CDCl_3$, 125.7 MHz: δ 35.7 (C-7), 41.9 (C-8), 56.2 (C-10′), 111.3 (C-2′*), 116.0 (C-5′*), 116.1 (C-3, C-5), 120.1 (C-8′†), 122.5 (C-6′†), 128.3 (C-1′‡), 130.5 (C-2, C-6), 131.2 (C-1‡), 140.3 (C-7′), 148.6 (C-4′§), 149.1 (C-3′§), 156.7 (C-4), 166.46 (C-9′).

N-*feruloyl octopamine*. ^{1}H NMR (acetone-d_6, 500 MHz): δ 5.884 (0.17 H, *d*, J = 12.8 Hz, *cis*-H-7′), 6.584 (0.17 H, *d*, J = 12.8 Hz, *cis*-H-8′), 6.594 (0.83 H, *d*, J = 15.6 Hz, *trans*-H-8′), 7.476 (0.83 H, *d*, J = 15.6 Hz, *trans*-H-7′).

Compound **4**. $[\alpha]_D^{24.4} - 10.3°$ (*c* 0.1, $CHCl_3$). EI-MS (rel. int.): *m/z*: 418 $[M]^+$ (81), 193 (31), 181 (100), 167 (78), 161 (34).

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*, †, ‡, § Assignments may be interchanged.

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