Newbouldiaquinone and Newbouldiamide: A New Naphthoquinone–Anthraquinone Coupled Pigment and a New Ceramide from *Newbouldia laevis*

Kenneth Oben EYONG,[a] Karsten KROHN,*[,b] Hidayat HUSSAIN,[b] Gabriel Ngosong FOLEFOC,[a] Augustin Ephram NKENGFACK,*[,a] Barbara SCHULZ,[c] and Qunxiu HU[c]

[a] *Department of Organic Chemistry, Yaounde University I; P. O. Box 812, Yaounde, Cameroon:* [b] *Department of Chemistry, University of Paderborn; Warburger Straße 100, 33098 Paderborn, Germany: and* [c] *Institute of Microbiology, Technical University of Braunschweig; D-38106 Braunschweig, Germany.*

Received December 24, 2004; accepted February 28, 2005

Newbouldiaquinone (1), a new naphthoquinone–anthraquinone coupled pigment and a new ceramide named newbouldiamide (2), have been isolated from *Newbouldia laevis*, besides the known compounds lapachol (3), canthic acid, oleanolic acid, 2-methyl-9,10-anthracenedione, 2-acetylfuro-1,4-naphthoquinone, 2,3-dimethoxy-1,4-benzoquinone, 2-(4-hydroxyphenyl)ethyl triacontanoate, β-sitosterol and β-sitosterol glucopyranoside. The structure elucidations of the isolated new compounds were performed on the basis of spectroscopic and chemical evidence. Preliminary studies showed that 1 is moderately antibacterial against Gram-positive *Bacillus megaterium* and that 3 has moderate herbicidal and antibacterial activities.

Key words *Newbouldia laevis*; Bignioniaceae; naphthoquinone–anthraquinone; ceramide

The genus *Newbouldia* (Bignioniaceae) comprises only one species distributed throughout the tropical and sub-tropical zones of the world.[1)] *Newbouldia laevis* SEEM. locally called "Oogly C", is a shrub or small tree growing in West and Central Africa (Senegal, Ivory Coast, Gabon and Cameroon, Nigeria *etc.*). Apart from the economic uses of *N. laevis* such as fuel wood and as boundary tree, different parts of this plant have been used in African folk medicine as an astringent in diarrhea, dysentery and in the treatment of various diseases such as worms, malaria, sexual transmitted disease, and in the reduction of dental caries.[1)] Previous studies undertaken on this species have resulted in the isolation of a number of secondary metabolites which belong to the classes of furanonaphthoquinones, pyrazole alkaloids, phenylpropanoid glycosides, and benzofurans.[2)] The medicinal uses and the presence of different classes of compounds in the title plant, prompted the present investigation on root and seed metabolites. We isolated and characterized two new compounds, namely, newbouldiaquinone (**1**), a naphthoquinone–anthraquinone coupled pigment, and newbouldiamide (**2**), a ceramide. The bis-benzoquinones, bis-naphthoquinones and bis-anthraquinones are the most widespread groups of quinone "dimers" found in nature.[3,4)] By contrast, the mixed naphthoquinone–anthraquinones coupled pigments are less common. There are only three recorded examples of similar systems. In the first two examples, a pyranoanthraquinone–naphthoquinone and an furanoanthraquinone–naphthoquinone dimeric system are present.[5,6)] In the third example, anthraquinone and naphthoquinone moieties are linked by a nine-membered lactone ring.[7,8)] Thus, the coupled naphthoquinone–anthraquinone system presented here is unique as a natural product. The ceramides containing non-hydroxyl octadecanoic acid as a fatty acid were reported in the literature.[9)] The presence of the long chain base of twenty-four carbons containing hydroxyl groups at positions 3, 4, 5 make compound **2** unique among ceramides.

Results and Discussion

The MeOH–CH_2Cl_2 (1 : 1) extracts of seeds, stem and roots of *N. laevis* were fractionated by silica gel column chromatography to give 556 fractions, which were further chromatographed on silica gel to give the two new compounds **1** and **2** together with known the constituents lapachol (**3**), oleanolic acid, β-sitosterol, and β-sitosterol glucopyranoside.

Newbouldiaquinone (**1**) was obtained as a yellow powder. The UV spectrum of **1** exhibited absorption maxima at 275 and 388 nm, suggesting a naphthoquinone derivative.[2)] This assumption was supported by the IR bands at 1640 and 1630 cm^{-1} for carbonyl absorption. Analysis of the chemical ionization mass spectrum (CI-MS) gave a molecular ion at *m/z* 395.2 $[M+1]^+$, corresponding to the molecular formula $C_{25}H_{14}O_5$, supported by the ^{1}H-, ^{13}C-NMR, and distortionless enhancement by polarization transfer (DEPT) analysis. The mass spectrum also showed peaks at *m/z* 379 $[M-CH_3]^+$, 376 $[M-H_2O]^+$, 351 $[M-CO-CH_3]^+$, 322 $[M-2CO-CH_3]^+$, and 294 $[M-3CO-CH_3-H]^-$, suggesting the presence of hydoxyl, methyl, and carbonyl groups. This fragmentation pattern is typical for hydroxyanthraquinones and/or naphthoquinones.[5,10)]

Preliminary inspection of the ^{1}H-NMR spectrum of newbouldiaquinone (**1**) showed one singlet at δ 2.50 which was in a strongly deshielded environment, characteristic for attachment to an aromatic system.[5)] The ^{1}H-NMR spectrum in $CDCl_3$ (Table 1) also showed two double doublets and two triplets of doublets for four symmetrical AA′BB′ type of aromatic protons at δ 8.22 (dd, *J*=7.5, 1.5 Hz), 8.16 (dd, *J*=7.5, 1.2 Hz), 7.76 (td, *J*=7.5, 1.2 Hz), and 7.67 (td, *J*=7.5, 1.5 Hz), for H-8, H-5, H-6, and H-7, respectively. Two 1H doublets at δ 8.19 and 7.42, with coupling constant of 6.1 Hz each, suggested *ortho* coupled aromatic protons, indicating that compound **1** contains an anthraquinone, possessing an unsubstituted A-ring and a disubstituted C-ring, as shown in partial structure "A" (Chart 1).

* To whom correspondence should be addressed. e-mail: karsten.krohn@upb.de

Table 1. ^{1}H- and ^{13}C-NMR Data of Compound **1** and ^{1}H-NMR Data of **3**

Position	**1**		**3**
	^{1}H (*J*: Hz)[a]	^{13}C[b] (DEPT)	^{1}H (*J*: Hz)[a]
1		137.7 (C)	
2		144.7 (C)	
Me-2	2.50 (s)	21.8 (CH_3)	
3	7.42 (d, 6.1)	135.1 (CH)	
4	8.19 (d, 6.1)	128.9 (CH)	
5	8.16 (dd, 7.5, 1.2)	127.3 (CH)	8.16 (dd, 6.4, 1.3)
5a		127.4 (C)	
6	7.76 (td, 7.5, 1.2)	135.1 (CH)	7.80 (td, 6.4, 1.5)
7	7.67 (td, 7.5, 1.5)	134.1 (CH)	7.73 (td, 6.4, 1.5)
8	8.22 (dd, 7.5, 1.5)	128.9 (CH)	8.11 (dd, 6.4, 1.3)
8a		133.9 (C)	
9		183.4 (C)	
9a		134.6 (C)	
10		181.6 (C)	
10a		129.7 (C)	
1′		183.3 (C)	3.32 (d, 6.6)
2′		150.4 (C)	5.23 (t, 6.7)
3′		124.9 (C)	
4′		183.2 (C)	1.80 (s)
4a′		129.7 (C)	
5′	8.02 (dd, 7.5, 1.4)	127.3 (CH)	1.70 (s)
6′	7.63 (td, 7.5, 1.4)	133.8 (CH)	
7′	7.71 (td, 7.5, 1.3)	133.2 (CH)	
8′	8.12 (dd, 7.5, 1.3)	126.9 (CH)	
–OH	7.25 (br s)		7.30 (br s)

a) ^{1}H-NMR carried out at 500 MHz. *b*) ^{13}C-NMR carried out at 150 MHz.

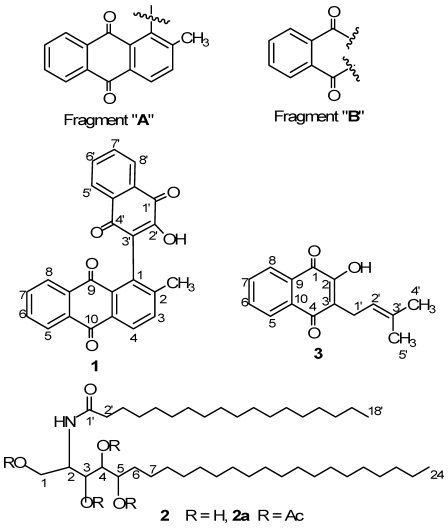

Chart 1. Structures of Fragments A and B, Newbouldiaquinone (**1**), Newbouldiamide (**2**), and Lapachol (**3**)

Another 4H AA′BB′ system at δ 8.12 (dd, J=7.5, 1.3 Hz), 8.02 (dd, J=7.5, 1.4 Hz), 7.71 (td, J=7.5, 1.3 Hz), and 7.63 (td, J=7.5, 1.4 Hz) allowed the assignment of a second partial structure "B" *i.e.* a naphthoquinone, possessing an unsubstituted ring A.

The structures of the two fragments were confirmed by the analysis of the ^{13}C-NMR spectrum (Table 1). The naphthoquinone–anthraquinone coupled skeleton produced four relatively up-field non chelated carbonyl signals for structure "A" and "B" (δ 183.4, 183.3, 183.2, 181.6). In addition, twenty signals are found in the aromatic region (10×CH and 10×C). All signals could be assigned to individual carbon atoms on

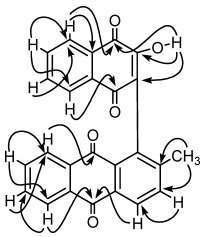

Fig. 1. Important HMBC Data for Compound **1**

the basis of two-dimensional NMR techniques, such as heteroatom multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC). The molecular formula $C_{25}H_{14}O_5$ suggested 19 double bond equivalents. Thus, five rings must form the molecular skeleton of compound **1**, considering the substraction of fourteen double bond equivalents for four carbonyl groups and ten double bonds. The structure was assembled by analysis of the correlation spectra (^{1}H–^{1}H-COSY, HMQC) and HMBC experiments.

On this basis, fragment "B" is a 1,4-disubstituted naphthoquinone structure showing the absence of any quinoidal protons for H-2′ or H-3′, which are usually observed at *ca.* δ 6.87.[6] Consequently, the hydroxyl group is placed at C-2′ on the basis of HMBC correlations (Fig. 1) and the second open position (C-3′) is assigned to be connected with C-1 of the anthraquinone moiety. The ^{13}C-NMR value of C-3′ (δ 124.9) is compatible with the proposed structure.[5] Furthermore, the absence of a proton signal that could be assigned to H-1 in the C-ring of fragment "A" and the position of the methyl group, which is confirmed by the coupling constant (J=6.1 Hz, between H-3 and H-4) as well as by HMBC correlations (Fig. 1), suggests that the site of the coupling in **1** is between C-1 in the anthraquinone and C-2′ in naphthoquinone. For the protons with resonances at δ 8.22 (H-8), 8.19 (H-4), 8.16 (H-5), 8.12 (H-8′), and 8.02 (H-5′), a position next to the carbonyl group was obvious from the strong HMBC correlations to the ^{13}C signals of C-9 (δ 183.4), C-10 (δ 181.6), C-1′ (δ 183.3), and C-4′ (δ 183.2). Further connectivities were established by the HMBC spectrum; the important correlations are shown in Fig. 1. The above data indicated the presence of a coupled naphthoquinone-anthraquinone skeleton at positions C-3′ and C-1. Consequently, the structure was established to be 8-(2-hydroxynaphthoquinon-3-yl)-7-methyl-anthracen-9,10-dione (**1**, Chart 1), named newbouldiaquinone, after the producing organism, *Newbouldia laevis*.

Newbouldiaquinone (**1**) does not show any optical activity. Thus, there should be free rotation around the linking bond. In fact, semi-empirical calculation gave a 15 kcal/mol^{-1} rotation barrier, confirming the possibility of free rotation at room temperature, since a value of 23 kcal/mol^{-1} is required for stable rotamers to exist at room temperature.[11]

Newbouldiamide (**2**) was obtained as a colorless powder, mp 129 °C and assigned molecular formula of $C_{42}H_{85}NO_5$ on the basis of chemical ionization mass spectrometry. The IR spectrum showed an absorption band at 3610 cm^{-1} for a hydroxyl group, a strong absorption band at 1640 cm^{-1}, indicating the presence of a secondary amide group.[12—14] A very strong signal at δ 1.29 in the ^{1}H-NMR spectrum and the lack of up-field methine signals in the ^{13}C-NMR spectrum re-

Table 2. ^{1}H- and ^{13}C-NMR of Compound **2** in py

Position	^{1}H (J: Hz)[a]	^{13}C[b] (DEPT)
1a	4.50 (dd, 6.0, 10.5)	62.9 (CH_2)
1b	4.35 (dd, 6.0, 10.5)	
2	5.11 (m)	53.8 (CH)
3	4.42 (m)	77.6 (CH)
4	4.61 (m)	73.3 (CH)
5	4.27 (m)	73.9 (CH)
6	1.99 (m)	33.0 (CH_2)
7	1.72 (m)	33.0 (CH_2)
8—21	1.29 (br s)	29.9 (CH_2)
22		27.5 (CH_2)
23		23.8 (CH_2)
24	0.86 (t, 6.8)	15.1 (CH_3)
NH	8.57 (d, 9.0)	
1′		176.1 (C)
2′	2.23 (t, 7.2)	36.6 (CH_2)
3′	1.76 (m)	35.0 (CH_2)
4′—15′	1.29 (br s)	29.9 (CH_2)
16′		27.5 (CH_2)
17′		23.8 (CH_2)
18′	0.86 (t, 6.8)	15.1 (CH_3)
–OH	7.60 (d, 5.0)	
–OH	6.68 (d, 6.0)	
–OH	6.25 (d, 5.2)	
–OH	6.22 (d, 6.5)	

a) ^{1}H-NMR carried out at 500 MHz. *b*) ^{13}C-NMR carried out at 150 MHz.

vealed that **1** must be derived from a long-chain fatty acid precursor.[13)]

The ^{1}H-NMR spectrum in C_5D_5N (Table 2) exhibited five exchangeable proton signals due to NH [δ 8.57 (d, J=9.0 Hz)] and four OH [7.60 (d, J=5.0 Hz), 6.68 (d, J=6.0 Hz), 6.25 (d, J=5.2 Hz), and 6.22 (d, J=6.5 Hz)].[12)] Compound **2** also showed the presence of two primary methyl groups (Me-24 and Me-18′), which appeared as a triplet of six protons at δ 0.86 (t, J=6.8 Hz). Furthermore, nonequivalent methylene proton signals were detected at δ 4.50 (dd, J=6.0, 10.5 Hz, H-1a) and 4.35 (dd, J=5.6, 10.5 Hz, H-1b), three methine groups [(δ 4.61 m, H-4), (δ 4.42 m, H-3), (4.27 m, H-5)], and a signal at low field at δ 5.11 (m, H-2) which was identified as a methine proton vicinal to the nitrogen atom of the amide group in the ^{1}H-NMR spectrum.[12)] The usual methylene groups associated with the chain appeared as a broad singlet at δ 1.29. The ^{13}C-NMR spectrum (Table 2) showed characteristic signals typical for an amide carbonyl at δ 176.1, a methine carbon linked to amide nitrogen at δ 53.0, and three other methines at δ 77.6 (CHOH), 73.9 (CHOH), and 73.3 (CHOH).[12)] A downfield signal for a methylene group bearing the hydroxyl function appeared at δ 62.9. On acetylation, compound **2** afforded a tetraacetate (**2a**). The acetylation further confirmed the presence of four hydroxyl groups in compound **2**. The remaining methylene groups of the chain showed their signals in the carbon spectrum at their normal positions.[12—14)] The ^{1}H–^{1}H- and ^{1}H–^{13}C connectivities were supported by the ^{1}H–^{1}H-COSY (Fig. 3) and HMQC spectra. These spectral data and the molecular formula suggest that compound **2** is a ceramide. The position of the hydroxyl groups were ascertained by the mass fragmentation pattern (Fig. 2), ^{1}H–^{1}H-COSY, and HMBC spectra (Fig. 3). Cross peaks in ^{1}H–^{1}H-COSY were observed between an amide proton (δ 8.57) and H-2 methine (δ 5.11), which, in turn, was coupled to three protons at δ 4.50 (H-1a), δ 4.42 (H-3), and δ 4.35 (H-1b). Furthermore, H-3 (δ 4.42) showed correlations with H-2 (δ 5.11) and with H-4 (δ 4.50). The position of the hydroxyl groups in the long chain base was further confirmed from HMBC correlations (Fig. 3).

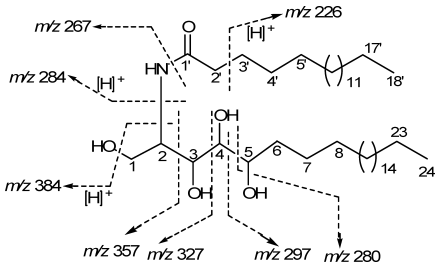

Fig. 2. Mass Fragmentation Pattern of **2**

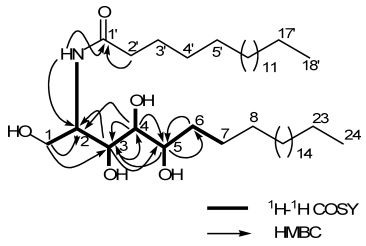

Fig. 3. ^{1}H–^{1}H COSY and HMBC Correlations for **2**

The presence of two proton triplets in the ^{1}H-NMR spectrum at δ 2.02 due to methylene protons connected to the amide carbonyl indicated that *N*-acyl in **2** was a non-hydroxyl fatty acid.[14)] The length of the fatty acid was determined by the characteristic ions (Fig. 2) at *m/z* 267 $[CH_3(CH_2)_{16}CO]^+$, 284 $[CH_3(CH_2)_{16}CONH_2+H]^+$ and 339 $[CH_3(CH_2)_{16}C(OH)=NC(=CH_2)CH_2OH]^+$ in the EI-MS. The length of the long chain base was also determined by the characteristic ions at 326 $[M-CH_3(CH_2)_{18}(CHOH)_3]^+$, 357 $[CH_3(CH_2)_{18}(CHOH)_3]^+$, and 372 $[CH_3(CH_2)_{18}(CHOH)_3-2H]^+$ in the EI-MS.[9,13—17)] The assignments were further confirmed by ^{1}H–^{1}H COSY, HMQC and HMBC correlations. Thus, the long chain base and fatty acid of **2** must be 2-amino-tetracosane-1,3,4,5-tetraol and octadecanoic acid, respectively.

The stereochemistry at C-2, C-3, C-4, and C-5 were not determined because no synthetic 3,4,5-trihydroxy ceramides were reported for comparing NMR and optical rotation values. Some natural 3,4,5-trihydroxy ceramides are reported in literature[18)] but their stereochemical assignments were not given. On the basis of this evidence, the structure of **2** was determined to be 1,3,4,5-tetrahydroxy-2-octadecanoylamino-tetracosane.

Lapachol (**3**) (for ^{1}H-NMR data see Table 1),[19)] canthic acid,[20)] oleanolic acid,[21)] 2-methyl-9,10-anthracenedione,[22)] 2-acetylfuro-1,4-naphthoquinone,[23)] 2,3-dimethoxy-1,4-benzoquinone,[24)] 2-(4-hydroxyphenyl)ethyl triacontanoate,[25)] β-sitosterol[26)] and β-sitosterol glucopyranoside[27)] were identified by comparison with published data.

Three of the isolated metabolites (**1**—**3**) were tested for herbicidal, antibacterial, and antifungal activities (Table 3). Compound **1** showed moderate antibacterial activity against the Gram-positive *Bacillus megaterium*, while compound **3** was moderately herbicidal.

Table 3. Biological Activity of the Pure Compounds[a)]

Compound	Herbicidal	Antifungal	Antibacterial
	Chl	Ust	Bm
1	0	n.t[b)]	0.5
2	0	0	0
3	0.5	0.7	0.6

a) 5 mg/ml of compounds **1**—**3** were tested for inhibitions of *Chlorella fusca* SHIH KRAUSS (Chl), *Ustilago violacea* (PERS.) ROUSSEL (Ust) and *Bacillus megaterium* DE BARY (Bm). *b*) n.t=not tested.

Experimental

General ^{1}H, 2D ^{1}H–^{1}H COSY, ^{13}C, 2D HMQC and HMBC spectra were recorded with a Bruker Avance 500 MHz spectrometer. Chemical shifts are referenced to internal TMS (δ=0) and coupling constants J are reported in Hz. Optical spectra were recorded with a NICOLET 510P FT-IR spectrometer, a UV-2101PC spectrometer, and Perkin-Elmer 241 polarimeter.

Plant Material The plant *Newbouldia laevis* SEEM. (Bignioniaceae) was collected at Mamfe, South West province of the Republic of Cameroon, in December 2002, and identified by Mr. Ndive Elias (Plant taxonomist), Botanical Garden, Limbe Cameroon. A voucher specimen (No. 1754/SRFK) has been deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and Isolation Dried and powdered seeds (1 kg), root bark (2.5 kg), and stem bark (3 kg) of *N. laevis* were separately extracted with a mixture of MeOH/CH_2Cl_2 (1 : 1) at room temperature for 24 h. The suspensions were filtered and each filtrate was concentrated under vacuum to give 80 g, 80 g and 30 g of crude residue, respectively. The crude extract from the seeds (80 g) was subjected to column chromatography (silica gel, hexane, hexane–EtOAc and EtOAc, in order of increasing polarity) yielding 205 fractions ($F_{1—205}$). Fractions $F_{35—40}$, were eluted with a mixture of hexane–EtOAc (9 : 1) yielding β-sitosterol (200 mg), and fractions $F_{107—112}$, which were eluted with hexane–EtOAc (8.5 : 1.5) and subjected to a second CC, afforded oleanolic acid (100 mg). Column fractions $F_{151—162}$ [hexane–EtOAc (5.5 : 4.5)] and $F_{170—172}$ [hexane–EtOAc (3 : 7)] were similarly subjected to second CC yielded compound **2** (100 mg) and β-sitosterol glucopyranoside (500 mg). Similarly, the crude extract of the root bark (80 g) was also chromatographed on a silica gel column and eluted with a gradient of hexane–EtOAc and yielding 200 fractions ($F_{1—200}$). Fractions F_{20}—F_{30} were eluted with hexane–EtOAc (9.5 : 0.5) afforded 2-acetylfuro-1,4-naphthoquinone (6.5 mg) and 2-methylanthraquinone (8.1 mg). Factions $F_{31—35}$, eluted with a mixture of hexane–EtOAc (9 : 1), gave compound **3** (200 mg), while fractions $F_{96—106}$ (hexane–EtOAc 8.5 : 1.5) gave compound **1** (80 mg). Fractions $F_{136—138}$ on CC using hexane–EtOAc (8 : 2), gave canthic acid (10 mg). Finally, the crude extract of stem bark (30 g) was subjected to CC using hexane–EtOAc yielding 151 fractions ($F_{1—151}$). Fractions $F_{17—20}$ eluted with hexane afforded 2,3-dimethoxy-1,4-benzoquinone (8 mg) and fractions $F_{48—50}$ gave 2-(4-hydroxyphenyl)ethyl triacontanoate on subjecting to CC using hexane–EtOAc (9.5 : 0.5).

Newbouldiaquinone (**1**): Yellow powder. mp 206 °C. IR ν_{max} ($CHCl_3$ (1 ml)+5 drops of MeOH): 3610, 1640, 1630 cm^{-1}. UV ($CHCl_3$) λ_{max} nm: 250, 275, 388. ^{1}H- and ^{13}C-NMR, see Table 1. CI-MS (CH_4): *m/z* 394.2 $[M+1]^+$. EI-MS *m/z* (rel. int.): 394 $[M]^+$ (20), 379 $[M-CH_3]^+$ (9), 376 $[M-H_2O]^+$ (11), 351 $[M-CO-CH_3]^+$ (25), 322 $[M-2CO-CH_3]^+$+ (12), 294 $[M-3CO-CH_3-H]^+$ (9), 282 $[M-4CO]^+$ (8), 197 (15), 151 (7), 126 (8), 76 (6), 43 (4).

Newbouldiamide (**2**): Colorless powder (100 mg): mp 129 °C. $[\alpha]_D^{20}$ +12.0° (c=0.001). IR ν_{max} ($CHCl_3$+MeOH): 3610, 2930, 2860, 1640, 1297 cm^{-1}. ^{1}H- and ^{13}C-NMR, see Table 2. CI-MS (CH_4): *m/z* 684.4 $[M+1]^+$. EI-MS *m/z* (rel. int.): 683.4 $[M]^+$ (10), EI-MS data and important HMBC correlations are illustrated in Figs. 2 and 3.

Acetylation A solution of dry pyridine (0.5 ml) and Ac_2O (1.0 ml) were added to compound **2** (10 mg), and left overnight. After usual workup, the tetraacetate **2a** was isolated (7 mg). mp 65 °C. $[\alpha]_D^{20}$ +13.4° (c=0.001). IR ν_{max} ($CHCl_3$): 2990, 1725, 1630, 1280 cm^{-1}. ^{1}H-NMR (C_5D_5N): δ: 0.87 (t, J=6.7 Hz, Me-18′, Me-24), 1.29 (for CH_2 chain), 1.74 (m, H-7), 1.78 (m, H-3′), 2.02 (m, H-6), 2.24 (t, J=6.7, H-2′), 2.05, 2.03, 2.02, 1.99 (12H, all s, 4×OAc), 4.43 (dd, J=5.6, 10.1 Hz, H-1b), 4.67 (dd, J=5.6, 10.1 Hz, H-1a), 5.34 (m, H-5), 5.50 (m, H-2), 5.58 (m, H-3), 5.70 (m, H-4), 9.50 (d, J=9.6 Hz, NH). ^{13}C-NMR (C_5D_5N): δ: 15.2 (q, C-24, C-18′), 21.5, 21.6, 21.7 (s, $CO\underline{C}H_3$), 23.8 (t, C-23, C-17′), 26.9 (t, C-16′), 27.0 (t, C-22), 30.6 (t, C, 8-21, C, 4′-15′), 33.0 (t, C-6, 7), 33.6 (t, C-3′), 33.8 (t, C-2′), 49.1 (d, C-2), 64.2 (t, C-1), 73.5 (d, C-4), 74.1 (d, C-5), 75.8 (d, C-3), 171.2, 171.4, 171.6, 171.8 (s, $\underline{C}OCH_3$), 172.0 (s, C-1′). EI-MS *m/z* (rel. int.): 788 $[M-HOAc-3H]^+$ (10), 731.2 $[M-2HOAc]^+$ (8), 672.2 $[M-3HOAc+H]^+$ (5), 611 $[M-4HOAc]^+$ (4), 534 (70), 450 (20), 310 (10), 43 $[CH_3COO]^+$ (100).

Bioactivity Tests Agar Diffusion Test: The compounds **1**—**3** were dissolved in methanol : acetone (1 : 1) at a concentration of 5 mg/ml. 50 μl of the solution were pipetted onto a sterile filter disc, which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the respective test organism.[27)] The test organisms were *Bacillus megaterium* (NB medium), *Microbotryum violaceum* (MPY) and *Chlorella fusca* (MPY); the radius of zone of inhibition was measured in cm.

Grass Test: Nine microliters of substance at 5 mg/ml (as above) were placed in each well of an ELISA plate (96 wells), each containing 30 seeds of *Agrostis stolonifer* and 250 μl of Gamberg B-5 Basal Salt Mixture (Sigma 65768) at pH 6.0. 2,4-Dichlorphenoxyacetic acid (2,4-D; 50 mg/ml) served as a control. Final concentration of the substance was 0.18 mg/ml, that 2,4-D was 0.018 mg/ml. Growth inhibition was evaluated as +, +/0 or 0. Growth of the negative control was +, that with 2,4-D was 0.

Acknowledgements We thank Brigitta Elsässer for conducting the calculation of rotation barrier of compound **1**, using the Spartan program, Unix Version 1.07.

References

1) Burkill H. M., "The Useful Plants of West Tropical Africa," Vol. I, Royal Botanic Gardens, Kew. London, 1985.
2) Gorman R., Kaloga M., Li X.-C., Ferreira D., Bergenthal D., Kolodziej H., *Phytochemistry*, **64**, 583—587 (2003).
3) Thomson R. H., "Naturally Occurring Quinones III. Recent Advances," Chapman and Hall, London, 1987.
4) Thomson R. H., "Naturally Occurring Quinones IV. Recent Advances," Chapman and Hall, London, 1996.
5) Aguinaldo A. M., Ocampo O. P. M., Bowden B. F., Gray A. I., Waterman P. G., *Phytochemistry*, **33**, 933—935 (1993).
6) Hassanean H. A., Ibraheim Z. Z., Takeya K., Itorawa H., *Pharmazie*, **55**, 317—319 (2000).
7) Gill M., Gimenez A., *Tetrahedron Lett.*, **1990**, 3505—3508 (1990).
8) Gill M., Gimenez A., *J. Chem. Soc. Perkin Trans. 1*, **1995**, 645—651 (1995).
9) Raith K., Neubert R. H. H., *Rapid Commun. Mass Spectrom.*, **12**, 935—938 (1998).
10) Singh J., *Phytochemistry*, **25**, 1985—1987 (1986).
11) Eliel E. L., Wilen S. H., Mander L. N., "Stereochemistry of Organic Compounds," John Wiley & Sons, Inc., New York, 1994, p. 1143.
12) Natori T., Morita M., Akimoto K., Yasuhiko K., *Tetrahedron*, **50**, 2771—2784 (1994).
13) Ahmad V. U., Hussain J., Hussain H., Farooq U., Akber E., Nawaz S. A., Choudhary M. I., *Z. Naturforsch.*, **59b**, 329—333 (2004).
14) Mukhtar N., Iqbal K., Anis I., Malik A., *Phytochemistry*, **61**, 1005—1008 (2002).
15) Yaoita Y., Kohata R., Kakuda R., Machida K., Kikuchi M., *Chem. Pharm. Bull.*, **50**, 681—684 (2002).
16) Inagaki M., Isobe R., Kawano Y., Miyamoto T., Komori T., Higuchi R., *Eur. J. Org. Chem.*, **1998**, 129—131 (1998).
17) Chebaane K., Guyot M., *Tetrahedron Lett.*, **27**, 1495—1496 (1986).
18) Garg H. S., Sharma M., Bhakuni D. S., Pramanik B. N., *Tetrahedron Lett.*, **36**, 1641—1644 (1992).
19) Khan R. M., Mlungwana S. M., *Phytochemistry*, **50**, 439—442 (1999).
20) Chatterjee T. K., Basak A., Borua A. K., Mukherjee K., Roy L. N., *Trans. Bose Res. Inst.*, **42**, 85—87 (1979).
21) Ikuta A., Hokawa H., *Phytochemistry*, **27**, 2813—2815 (1988).
22) Charkraborty D. P., Islam A., Roy S., *Phytochemistry*, **17**, 2043 (1978).
23) Rao M. M., Kingston D. G. I., *J. Nat. Prod.*, **45**, 600—604 (1982).
24) Attygale A. B., Xu S.-C., Meinwald J., *J. Nat. Prod.*, **56**, 1700—1706 (1993).
25) Mat A. R., Houghton P. J., *Planta Medica*, **65**, 455—456 (1999).
26) Rubinstein I., Goad L. J., Clague A. D. H., *Phytochemistry*, **15**, 195—200 (1976).
27) Seo S., Tomita Y., Tori K., Yoshimura Y., *J. Am. Chem. Soc.*, **100**, 3331—3339 (1978).
28) Schulz B., Sucker J., Aust H.-J., Krohn K., Ludewig K., Jone P. G., Doering D., *Mycol. Res.*, **1995**, 1007—1015 (1995).