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Gerronemins A–F, cytotoxic biscatechols from a *Gerronema* species

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Abstract

The gerronemins A–F (**1**–**6**) were isolated as the cytotoxic components of an extract of a *Gerronema* species detected in a screening for new cytotoxic metabolites from basidiomycetes. Their structures were elucidated by spectroscopic techniques, and they are composed of a C_{12}–C_{16} alkane or alkene substituted at both ends by 2,3-dihydroxyphenyl groups. The gerronemins blocked the inducible expression of a hCOX-2 and iNOS promoter driven reporter gene with IC_{50}-values of 1–5 μg/ml. In addition, cytotoxic activities were observed which were due the inhibition of cellular macromolecular syntheses.

Keywords: *Gerronema*; Gerronemins A–F; Biscatechols; Cytotoxic activity

1. Introduction

Species of the genus *Gerronema* Sing. have been described from tropical to subtropical and temperate to arctic zones, and can be considered to be of worldwide occurrence. Each subgenus, *Gerronema* and *Romagnesia*, has characteristic areas of distribution (Singer, 1986). A species frequently encountered in Europe is *Gerronema fibula* (Bull. ex Fr.) Sing. = *Rickenella fibula* (Bull. ex Fr.) Raith, which, from mycelial cultures, has yielded the highly cytotoxic diterpenoid striatal D (Steglich et al., 1993). In the course of a screening for new antibiotic and cytotoxic metabolites from fungal species, the mycelial cultures of a North American *Gerronema* were found to produce a mixture of closely related cytotoxic compounds. This paper describes the production, isolation, biological properties and structure elucidation of six new cytotoxic biscatechols from this fungus.

2. Results and discussion

The gerronemins were isolated as described in the experimental section, and their structures were determined from their spectroscopic data (see Fig. 1 for structures and Tables 1 and 2 for NMR data). In the LC–MS spectrum of gerronemin A (**1**) only a peak for m/z 385 was observed, corresponding to a molecular weight of 386, and this was confirmed by EIMS. High resolution measurements suggested that the elemental composition of gerronemin A (**1**) is $C_{24}H_{34}O_4$, corresponding to a degree of unsaturation of 8. As the NMR data suggested the presence of fewer carbons and protons, it was reasonable to assume that the molecule is symmetric. The presence of an aromatic system was indicated by the UV spectrum and by the NMR data (three aromatic protons and 6 carbons). No other functionalities appear to be present, the data for the remaining part is in agreement with a symmetrical chain of methylene groups, substituted at both ends. The only fragment in the EI mass spectrum has a m/z of 123, which would fit the benzylic fragment obtained from a dihydroxy alkylbenzene, suggesting that gerronemin A (**1**) contains two identical dihydroxyphenyl groups attached to both ends of an alkane. The length of the alkane could be deduced from the integrals in the ^{1}H NMR spectrum and from the molecular weight, and must contain 12 methylene groups. In the HMBC spectrum, a strong correlation can be observed between the benzylic protons and three carbons in the benzene ring, of which one is protonated (according to the HMQC spectrum) and one obviously must be hydroxylated. The dihydroxyphenyl moiety could then be either 3-alkylcatechol, 4-alkylresorcinol

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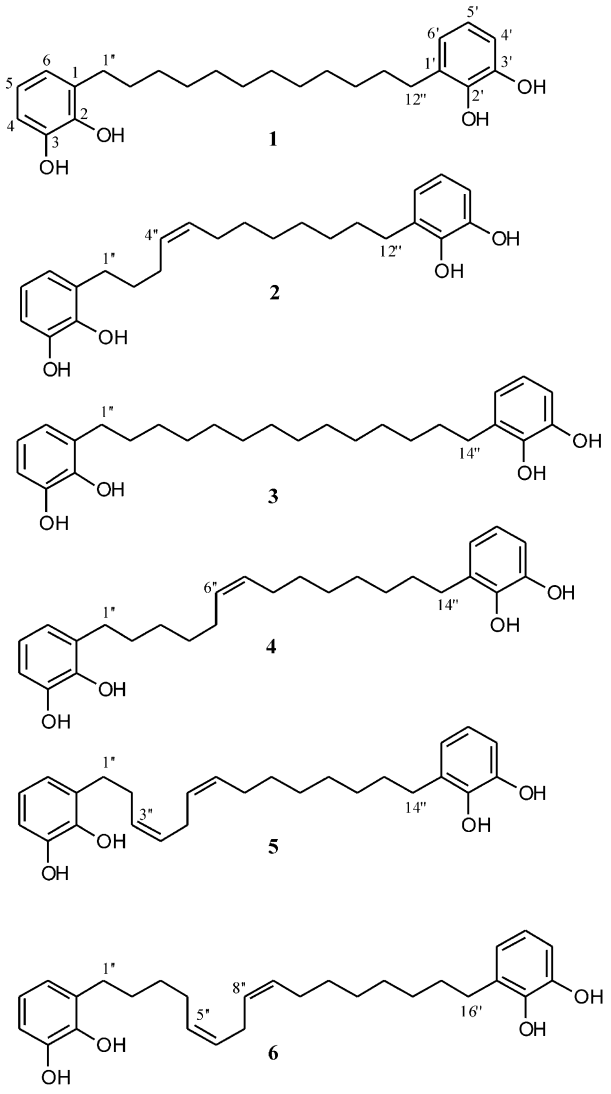

Fig. 1. Structures of gerronemins A–F.

or 2-alkylhydroquinone, and due to overlap of the aromatic proton signals it was not possible to determine which it is. However, the inspection of reference spectra of 3-alkylcatechols, 4-alkylresorcinols and 2-alkylhydroquinones (Pouchert and Behnke, 1993) clearly shows that the gerronemins contain 2,3-dihydroxyphenyl groups. The ^{13}C NMR shifts of 3-methylcatechol are very similar to those observed for the aromatic part of the gerronemins, and the signals for the aromatic protons are also overlapping. In contrast, the carbon signals of 4-alkylresorcinols and 2-alkylhydroquinones are significantly different and their proton signals are well separated (Pouchert and Behnke, 1993).

According to the MS data of gerronemin B (**2**), which indicated the elemental composition $C_{24}H_{32}O_4$, it contains an additional unsaturation compared to **1**. This is in the form of a C–C double bond in the C_{12} chain, its position could be determined by COSY correlations and the *Z* configuration was indicated by the coupling constant between 4″-H and 5″-H (11 Hz). The spectroscopic data for gerronemin C (**3**) are very similar to those of gerronemin A (**1**), the only difference is that it contains two additional methylene groups. Gerronemin C is consequently the 1,14-disubstituted tetradecane (**3**). Similar to the situation in gerronemin B (**2**), gerronemin D (**4**) contains an additional unsaturation. However, the C-C double bond is situated farther away from the aromatic system, and the signals for the two olefinic protons in the isolated double bond overlap. The EIMS fragmentation does not reveal the position of the double bond. A 4″-5″ double bond as in gerronemin B (**2**) is easily detected (due to the large shift difference between for example 2″-H and 11″-H in **2**), and a 5″–6″ double bond would be expected to show some significant shift differences as well (as was observed for gerronemin F (**6**), vide infra). On the other hand, a 7″–8″ double bond would make the compound completely symmetric with only 7 signals for saturated carbons. This is not the case, some of the signals for the saturated carbons are doubled, although the shift difference is small, and the signal for the isolated double bond protons is not a symmetric triplet. The conclusion is therefore that the double bond in **4** is between C-6″ and C-7″. The *Z* configuration could not be established by coupling constants, but is indicated by the ^{13}C NMR shift of the two allylic carbons (27.0 ppm) (Gonzalez et al., 1993). Oxidative cleavage of the double bond to produce the two aldehydes would have confirmed the structure for **4**, but too small amounts were available for the preparation of derivatives. The positions of the two double bonds in gerronemin E (**5**) could easily be determined by COSY correlations, and their *Z* configurations was established by the NOESY correlations observed between 5″-H_2 and 2″-H_2 as well as 8″-H_2. Also the structure of gerronemin F (**6**), which is based on a disubstituted hexadecane with two isolated double bonds in the chain, could be determined by following the proton spin system in the COSY experiment, and again it was possible to assign a *Z* configuration to both double bonds based on the correlations observed between 7″-H_2 and 4″-H_2 as well as 10″-H_2 in the NOESY experiment.

In the plate diffusion assay the gerronemins exhibit low antibacterial (all) and weak antifungal (only gerronemin A) acivities starting from 50 μg/paper disk. Their cytotoxic activities against cells growing in suspension are pronounced, with IC_{50} values between 1 and 5 μg per ml for all compounds, while monolayer cultures are less sensitive (Table 3). Table 4 shows the effect of the gerronemins A–E on the incorporation of radioactive thymidine, uridine and leucine into TCA precipitable material (DNA, RNA, and proteins) of HL-60 cells. In this assay the syntheses of all three macromolecules (with a small preference for protein synthesis) were inhibited at concentrations of 5 μg/ml. To our knowledge, the gerronemins are the first biscathechols of this

Table 1
^{1}H (500 MHz) NMR spectral data (δ; multiplicity; J) for gerronemin A (**1**), B (**2**), C (**3**), D (**4**), E (**5**) and F (**6**). The spectra were recorded in $CDC1_3$: CD_3OD 19:1, and the solvent signal for $CHC1_3$ (7.25 ppm) was used as reference. The coupling constants J are given in Hz

H	1	2	3	4	5	6
4/4′	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*
5/5′	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*
6/6′	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*	6.58; *m*
1″	2.52; *t*; 8.0	2.54; *t*; 8.0	2.52; *t*; 8.0	2.52; *t*; 8.0	2.59; *t*; 8.0	2.54; *t*; 8.0
2″	1.52; *quin*; 7.5	1.58; *quin*; 7.5	1.52; *quin*; 7.5	1.52; *quin*; 7.5	2.31; *dt*, 7, 8	1.55; *quin*; 7.5
3″	1.26; *m*	2.01; *dt*; 7, 7	1.26; *m*	1.26; *m*	5.39; *m*	1.35; *quin*; 7.5
4″	1.22; *m*	5.31; *td*; 7, 11	1.22; *m*	1.22; *m*	5.28; *m*	2.02; *dt*; 7, 8
5″	1.20; *m*	5.29; *td*; 7, 11	1.20; *m*	1.93; *m*	2.68; *dt*; 7, 7	5.29; *m*
6″	1.18; *m*	1.92; *dt*; 7, 7	1.18; *m*	5.26; *m*	5.21; *m*	5.25; *m*
7″	1.18; *m*	1.26; *m*	1.18; *m*	5.26; *m*	5.27; *m*	2.69; *dt*; 7, 7
8″	1.20; *m*	1.20; *m*	1.18; *m*	1.93; *m*	1.95; *dt*; 7, 7	5.25; *m*
9″	1.22; *m*	1.22; *m*	1.18; *m*	1.22; *m*	1.25; *m*	5.28; *m*
10″	1.26; *m*	1.26; *m*	1.20; *m*	1.20; *m*	1.22; *m*	1.96; *dt*; 7, 7
11″	1.52; *quin*; 7.5	1.52; *quin*; 7.5	1.22; *m*	1.22; *m*	1.22; *m*	1.26; *m*
12″	2.52; *t*; 8.0	2.52; *t*; 8.0	1.26; *m*	1.26; *m*	1.26; *m*	1.22; *m*
13″	–	–	1.52; *quin*; 7.5	1.52; *m*	1.52; *m*	1.22; *m*
14″	–	–	2.52; *t*; 8.0	2.52; *t*; 8.0	2.52; *t*; 8.0	1.27; *m*
15″	–	–	–	–	–	1.52; *m*
16″	–	–	–	–	–	2.52; *t*; 8.0

Table 2
^{13}C (125 MHz) NMR spectral data (δ multiplicity) for gerronemin A (**1**), B (**2**), C (**3**), D (**4**), E (**5**) and F (**6**). The spectra were recorded in $CDCl_3$:CD_3OD 19:1, and the solvent signal for $CDCl_3$ (77.0 ppm) was used as reference. The multiplicities of the carbon signals were determined indirectly from HMQC experiments

C	1	2	3	4	5	6
1/1′	129.4; *s*	129.5; *s*	129.4; *s*	129.7; *s*	129.3; *s*	129.2; *s*
2/2′	142.5; *s*	142.5; *s*	142.5; *s*	142.5; *s*	142.6; *s*	142.5; *s*
3/3′	143.7; *s*	143.7; *s*	143.7; *s*	143.7; *s*	143.7; *s*	143.7; *s*
4/4′	112.4; *d*	112.5; *d*	112.4; *d*	112.4; *d*	112.4/112.7; *d*	112.4/112.5; *d*
5/5′	119.4; *d*	119.4; *d*	119.3; *d*	119.3; *d*	119.4; *d*	119.4; *d*
6/6′	121.2; *d*	121.2; *d*	121.2; *d*	121.2; *d*	121.2/121.3; *d*	121.2; *d*
1″	29.7; *t*	29.6; *t*	29.7; *t*	29.7; *t*	29.9; *t*	29.7; *t*
2″	29.7; *t*	29.7; *t*	29.7; *t*	29.5; *t*	27.3; *t*	29.7; *t*
3″	*29.5; t*	27.1; *t*	29.6; *t*	29.1; *t*	129.3; *d*	29.6; *t*
4″	29.5; *t*	130.3; *d*	29.5; *t*	29.6; *t*	128.5; *d*	27.1; *t*
5″	29.4; *t*	130.3; *d*	29.5; *t*	27.0; *t*	25.5; *t*	130.1; *d*
6″	29.4; *t*	27.0; *t*	29.5; *t*	129.8; *d*	127.8; *d*	127.9; *d*
7″	29.4; *t*	29.6; *t*	29.4; *t*	129.8; *d*	130.1; *d*	25.5; *t*
8″	29.4; *t*	29.3; *t*	29.4; *t*	27.0; *t*	27.1; *t*	127.8; *d*
9″	29.5; *t*	29.1; *t*	29.5; *t*	29.6; *t*	29.4; *t*	130.0; *d*
10″	29.5; *t*	29.4; *t*	*29.5; t*	29.1; *t*	29.1; *t*	27.0; *t*
11″	29.7; *t*	29.6; *t*	29.5; *t*	29.4; *t*	29.3; *t*	29.7; *t*
12″	29.7; *t*	29.6; *t*	29.6; *t*	29.3; *t*	29.5; *t*	29.4; *t*
13″	–	–	29.7; *t*	29.5; *t*	29.6; *t*	29.1; *t*
14″	–	–	29.7; *t*	29.7; *t*	29.7; *t*	29.3; *t*
15″	–	–	–	–	–	29.6; *t*
16″	–	–	–	–	–	29.7; *t*

type to be reported. Similar compounds, bis-5-alkylresorcinols with 3,5-dihydroxyphenyl group at each end of a tetradecane or tetradecene chain, have been described from the plant *Panopsis rubescens* (Deng et al., 1999) as inhibitors of DNA polymerase β. As this enzyme involved in the repair of DNA damage, Deng et al. (1999) suggested a potential application of these compounds in combination with DNA damaging antitumor drugs. In addition, several bis-(dihydroxyalkylbenzenes) were capable of mediating Cu^{2+}-dependent DNA cleavage (Starck et al., 2000). Related polyphenols like resveratrol, epigallocatechin-3-gallate and resorcinols have been described to inhibit the inducible expression of the proinflammatory enzymes COX-2 and

Table 3
Cytotoxic activities of the gerronemins A, B, D, E and F after 48 h of incubation

Cell line	IC_{50} (μg/ml) Gerronemin				
	A	B	D	E	F
HL-60	2.5	2.5	4	2.5	4–5
U937	1–2	3–4	1–2	2	1.5
L1210	2.5	2.5	2.5	2.5	2.5
COS-7	15	15	15	15	15
HeLa S3	>40	>40	>40	>40	>40

Table 4
Effect of gerronemins on the incorporation of [2-^{14}C]-thymidine, [2-^{14}C]-uridine, and [1-^{14}C]-leucine into TCA-precipitable material (DNA, RNA, proteins) of HL-60 cells[a]

Gerronemin	(μg/ml)	Incorporation (% of controls)		
		(^{14}C)-thymidine	(^{14}C)-uridine	(^{14}C)-leucine
A	5	74	100	36
	12.5	71	80	18
B	5	65	80	49
	12.5	44	76	22
D	5	62	86	61
	12.5	63	86	41
E	5	72	79	61
	12.5	52	74	24
F	5	71	68	68
	12.5	71	64	62

[a] Controls (100%): thymidine 4750 cpm; uridide 26105 cpm; leucine 21903 cpm.

iNOS in various cells (Mutoh et al., 2000; Surh et al., 2001). The anti-inflammatory properties of these compounds have been mainly attributed to an interference of several signal transduction pathways leading to the expression of immediate early genes involved in immune, acute phase and inflammatory responses. To evaluate the effect of the gerronemins on the expression of COX-2 and iNOS, transient transfection of Jurkat cells were performed using either a human COX-2 or a human iNOS promoter luciferase reporter plasmid. Treatment of Jurkat cells with TPA/ionomycin increased COX-2 dependent promoter activity 15–17 fold and iNOS dependent promoter activity 75–80 fold. As shown in Fig. 2A and B, the gerronemins A, B, D and F inhibited the COX-2 and iNOS promoter activity in a concentration dependent manner with IC_{50} values ranging from 1 μg/ml (gerronemin F) to 5 μg/ml (gerronemin B). Further investigation on the mode of action of the gerronemins are now under way to characterize the cellular targets of these compounds.

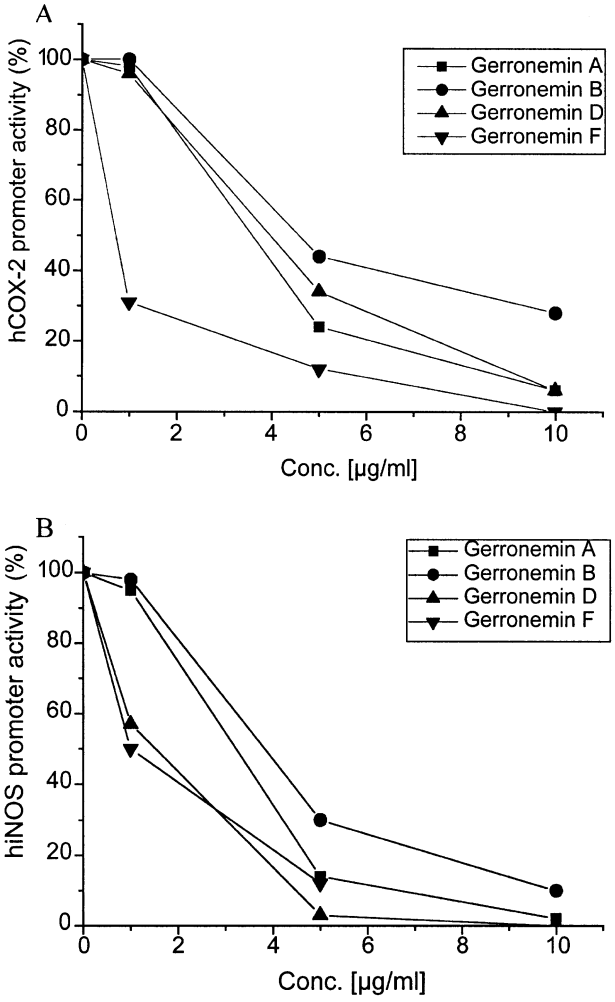

Fig. 2. Effect of the gerronemins A, B, D and F on the TPA/ionomycin induced hCOX-2 and iNOS promoter activity. (A) Jurkat cells were transfected with a hCOX-2 promoter dependent reporter plasmid and stimulated with 10 ng/ml TPA and 2.5 μM ionomycin for 24 h with or without test compounds. (B) Jurkat cells were transfected with a hiNOS promoter dependent reporter plasmid and stimulated with 20 ng/ml TPA and 2.5 μM ionomycin for 24 h with or without test compounds. Control (100%): stimulation only. The expression of the luciferase reporter gene was determined as described in the experimental section.

3. Experimental

3.1. General experimental procedures

For analytical HPLC a Hewlett Packard 1090 series II instrument and for preparative HPLC a Jasco model PU-980 instrument was used. The UV and the IR spectra were recorded with a Perkin Elmer λ 16 and a Bruker IFS 48 spectrometer. The content of gerronemins in samples taken during fermentation and in fractions during purification was determined by analytical HPLC (Merck LiChrosphere RP-18; 5 μm, column 125×4 mm; H_2O—acetonitrile gradient (% acetonitrile); flow 1.5 ml/min; 0–20 min, 0–70%, 20–30 min, 70–100%; retention

times: Gerronemin A 20,2 min; B 21 min, C 21,4 min, D 22 min, E 22,6 min E 23,4 min). APCI mass spectra were acquired on a Hewlett-Packard MSD 1100 spectrometer (negative mode, V_{cap}: 2200 V, nebulizer: 50 psig, drying gas: 5 l/min, drying gas temp.: 350 °C, corona: 6 μA, vaporizer: 400 °C, fragmentator: 70 V). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$: CD_3OD 19:1, and the solvent signals for $CHCl_3/CDCl_3$ (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). EI mass spectra were recorded with a Jeol SX102 spectrometer.

3.2. Gerronema species strain 86053

Fruiting bodies of *Gerronema* sp. 86053 were collected in the Smokey Mountains, USA. The orange red carpophore showed all characteristics of the genus, the species, however could not be unequivocally assigned. Mycelial cultures were derived from spore prints. Voucher specimen and cultures of the strain 86053 are deposited in the collection of the LB Biotechnology, University Kaiserslautern. For maintenance the fungus was grown on YMG medium composed of (g/l): glucose, 10, malt extract 10, yeast extract 4 and agar 20.

3.3. Fermentation and isolation of gerronemins A–F (**1**–**6**)

Fermentations were carried out in 20 l of YMG medium in a Biolafitte C6 fermenter at 22 °C with aeration (3 l/min) and agitation (120 rpm). The pH was adjusted to 5.5. A well grown culture in the same medium (250 ml) was used as inoculum. After 560 h of fermentation the mycelia were separated from the culture fluid by filtration and lyophilized. Extraction of the lyophilisate (210 g) with 3 l of methanol yielded 13 g of crude extract. The crude extract was applied to a column (8×45 cm) with silica gel (Merck 60) and eluted with cyclohexane ethyl acetate 25:75. The gerronemins were purified from the enriched extraxt (178 mg) by preparative HPLC (Nucleosil C18, 250×25 mm; flow: 5 ml/min; H_2O/acetonitrile gradient (% acetonitrile), 0–10 min, 0%; 10–40 min, 0–70%; 40–55 min, 70%; 55–70 min, 70–75%; 70–85 min, 75%; 85–100 min, 75–80%; 100–115 min, 80%; 115–130 min, 80–100%). Yields (retention times): Gerronemin B 7 mg (69 min); E 6 mg (73.7 min), A 32 mg (77.7 min), D 8 mg (82.5 min), F 7 mg (88 min), C 2 mg (100.5 min).

Gerronemin A (**1**) (1,2-dihydroxy-3-[12-(2,3-dihydroxyphenyl)dodecyl]benzene) was obtained as a colourless oil. UV (MeOH), λ_{max} (ε) in MeOH: 277 nm (4300). IR (KBr): 3375, 2925, 2850, 1625, 1595, 1480, 1345, 1285, 1185, 965 and 735 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), m/z (rel. int.): 386.2467 (100%, M^+, $C_{24}H_{34}O_4$ requires 386.2457), 123 (63%).

Gerronemin B (**2**) (1,2-dihydroxy-3-[12-(2,3-dihydroxyphenyl)-(*Z*)-dodec-4-enyl]benzene was obtained as a colourless oil. UV (MeOH), λ_{max} (ε) in MeOH: 277 nm (4700). IR (KBr): 3430, 2925, 2855, 1625, 1595, 1475, 1280, 1190, 830, 780 and 735 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), m/z (rel. int.): 384.2298 (53%, M^+, $C_{24}H_{32}O_4$ requires 384.2300), 262 (5%), 177 (11%), 163 (100%), 149 (12%), 136 (13%), 123 (86%).

Gerronemin C (**3**) (1,2-dihydroxy-3-[14-(2,3-dihydroxyphenyl)tetradecyl]benzene) was obtained as a colourless oil. UV (MeOH), λ_{max} (ε) in MeOH: 277 nm (3000). IR (KBr): 3425, 2925, 2850, 1625, 1475, 1280, 1060 and 735 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), m/z (rel. int.): 414.2763 (100%, M^+, $C_{26}H_{38}O_4$ requires 414.2770), 123 (83%).

Gerronemin D (**4**) (1,2-dihydroxy-3-[14-(2,3-dihydroxyphenyl)-(*Z*)-tetradec-6-enyl]benzene) was obtained as a colourless oil. UV (MeOH), λ_{max} (ε) in MeOH: 277 nm (3300). IR (KBr): 3425, 2925, 2855, 1625, 1595, 1475, 1280, 1195, 780 and 735 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), m/z (rel. int.): 412.2619 (23%, M^+, $C_{26}H_{36}O_4$ requires 412.2613), 177 (10%), 163 (100%), 149 (17%), 136 (13%), 123 (73%).

Gerronemin E (**5**) (1,2-dihydroxy-3-[14-(2,3-dihydroxyphenyl)-(*Z,Z*)-tetradeca-3,6-dienyl]benzene) was obtained as a colourless oil. UV (MeOH), λ_{max} (ε) in MeOH: 277 nm (5100). IR (KBr): 3430, 2925, 2855, 1620, 1595, 1475, 1280, 1195, 835 and 735 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), m/z (rel. int.): 410.2455 (34%, M^+, $C_{26}H_{34}O_4$ requires 410.2457), 217 (4%), 203 (8%), 189 (13%), 176 (12%), 163 (27%), 149 (11%), 136 (8%), 123 (100%).

Gerronemin F (**6**) (1,2-dihydroxy-3-[16-(2,3-dihydroxyphenyl)-(*Z,Z*)-hexadeca-5,8-dienyl]benzene) was obtained as a colourless oil. UV (MeOH), λ_{max} (ε) in MeOH: 277 nm (4100). IR (KBr): 3420, 2925, 2855, 1625, 1595, 1475, 1280, 1195, 1060 and 730 cm^{-1}. See Tables 1 and 2 for NMR data. EIMS (70 eV), m/z (rel. int.): 438.2777 (100%, M^+, $C_{28}H_{38}O_4$ requires 438.2770), 315 (5%), 302 (5%), 217 (36%), 203 (25%), 189 (41%), 176 (39%), 163 (94%), 149 (28%), 136 (34%), 124 (60%), 123 (42%).

3.4. Biological assays

The antimicrobial activity was assayed as described previously by Anke et al. (1989). The cytotoxic activity was assayed with HL-60 cells (promyelocytic leukemia,

human, ATCC CCL 240), U937 cells (histiocytic leukemia, human, ATCC CRL 1593) and L1210 cells (lymphocytic leukemia, mouse, ATCC CCL 219) grown in RPMI 1640 medium, Gibco. COS-7 cells (kidney cells, monkey ATCC CRL 1651) and HeLa S3 cells (cervix carcinoma, human, ATCC CCL 2.2) were grown in D-MEM medium (GIBCO), all supplemented with 10% fetal calf serum. All media were supplemented with 10% fetal calf serum and contained 65 μg/ml benzylpenicillin and 100 μg/ml streptomycin sulfate. The cells (10^5/ml) were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 h incubation with or without gerronemins the cells were examined microscopically. The effect on the growth of monolayer cell lines was measured with Giemsa stain and the viability of suspension cell lines was measured by the XTT test as described previously (Erkel et al., 1996). The incorporation of appropriate precursors into DNA, RNA and proteins in whole COS-7 cells was measured as described previously (Becker et al., 1994). HL-60 cells were harvested by centrifugation (1000 × *g*) and resuspended in phosphate-buffered saline containing 0.1% glucose to a cell density of 2×10^6 cells/ml. After incubation for 15 min (100 rpm, 37 °C) with or without gerronemins, 1 ml of the cell suspensions were incubated at 37 °C with gentle shaking with 0.1 μCi of the radioactive precursors [2-^{14}C]thymidine, [2-^{14}C]uridine, and [1-^{14}C]leucine, all 50–60 mCi/mmol). After 30 min the cells were suspended in 5% cold trichloroacetic acid (TCA), the precipitate was collected on cellulose nitrate filters, washed with 5% cold TCA and the radioactivity measured in a liquid scintillation counter. The 1123-nucleotide human iNOS promoter fragment was amplified by PCR from genomic DNA extracted from THP-cells cells as described recently (Fukuda et al., 2000). The PCR product was cloned into the XhoI-KpnI site of pGL3-Basic (Promega) to generate the iNOS promoter driven luciferase reporter plasmid. The human COX-2 promoter fragment (1442 bp), −1387 to +55 relative to the transcription start site, was amplified by PCR from genomic DNA from HeLa S3 cells using primers derived from published sequences (GenBank D28235, U20548). The PCR product was cloned into the XhoI-HindIII site of pGL3-Basic (Promega) to generate the hCOX-2 promoter driven luciferase reporter plasmid. The transfection of Jurkat cells (ATCC TIB 152) was performed and as described recently (Erkel et al., 2000). The activity of the luciferase in whole cell extracts was determined 24 h after transfection using the luciferase assay system (Promega) according to the manufacturer's instructions with a luminometer.

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