Andirol A and B, Two Unique 6-Hydroxymethylpterocarpenes from *Andira inermis*[§]

Carola Kraft[a], Kristina Jenett-Siems[a,*], Inga Köhler[a], Karsten Siems[b], Daniel Abbiw[c], Ulrich Bienzle[d] and Eckart Eich[a]

[a] Institut für Pharmazie (Pharmazeutische Biologie), Freie Universität Berlin, Königin-Luise-Str. 2–4, D-14195 Berlin, Germany. Fax: +49 30 83 85 37 29. E-mail: kjsiems@zedat.fu-berlin.de
[b] AnalytiCon Discovery GmbH, D-14473 Potsdam, Germany
[c] Department of Botany, University Legon-Accra, Accra, Ghana
[d] Institut für Tropenmedizin, Medizinische Fakultät Charité der Humboldt-Universität zu Berlin, D-14050 Berlin, Germany

[*] Author for correspondence and reprint requests

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Antiplasmodial Activity, Andirol A–B, Andinermol

From a methanolic extract of the leaves of *Andira inermis* (Fabaceae), andirol A and B, two compounds with a novel type of a rotenoid-related skeleton and andinermol, a new 2-aryl-3-hydroxymethyl-benzofuran could be isolated. Characterisation and structure elucidation of these compounds was achieved on the basis of their spectral data. In addition, the *in vitro* activities of the isolated compounds against both the chloroquine-sensitive strain PoW and the chloroquine-resistant clone Dd2 of *Plasmodium falciparum* have been evaluated.

Introduction

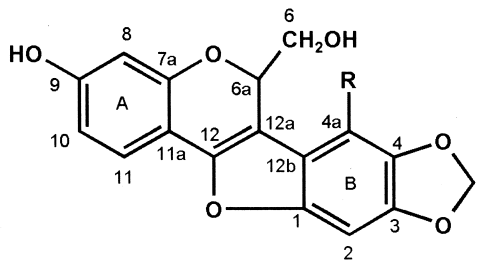
Andira inermis (W. Wright) H. B. K., Fabaceae, a plant remedy native from Mexico to Southern America also occurs often as an ornamental in West Tropical Africa. Recently, we reported the isolation of 2-arylbenzofuran-3-carbaldehydes (andinermals) from the leaves of a Panamanian sample. These, as well as the also isolated isoflavones calycosin and genistein proved to be active against both the chloroquine-sensitve strain PoW and the chloroquine-resistant clone Dd2 of *Plasmodium falciparum* with IC_{50} values between 2.0 μg/ml/7.4 μM to 9.8 μg/ml/34.5 μM (Kraft *et al.*, 2001). In this study we investigated the leaves of this species collected in Ghana and obtained two novel rotenoid-type flavonoids and one new 2-aryl-3-hydroxymethyl-benzofuran. Furthermore, the antiplasmodial effects of the isolated compounds have been assayed.

Results and Discussion

In addition to the already known constituents of *A. inermis* (Kraft *et al.*, 2000, 2001), three novel compounds could be obtained by bioassay-guided fractionation of the $CHCl_3$ fraction. Their structural elucidation was carried out by EIMS, ^{1}H NMR, ^{13}C NMR, HMBC, as well as NOE experiments.

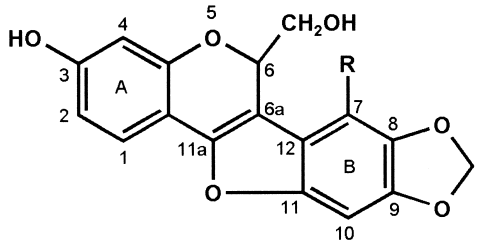
Compound **1** (Fig. 1) gave a molecular ion peak at *m/z* 342 in the EIMS spectrum, corresponding to a molecular formula of $C_{18}H_{14}O_7$. The ^{1}H NMR spectrum (Table I) indicated the presence of a methylendioxy group (6.01 ppm, s, 2 H), one isolated aromatic proton (6.81 ppm, s, 1 H) and one methoxy group (4.16 ppm, s, 3 H). Additionally the ^{1}H NMR displayed characteristic signals of a 1,2,4-trisubstituted aromatic ring and the typical pattern of an ABX-system (4.03 ppm, dd, 1H; 4.46 ppm, dd, 1H; 5.18 ppm, dd, 1H) similar to those described for 12a-hydroxyrotenoids (Abe *et al.*, 1985). In the HMBC (Table I) the protons at C-6 and C-6a showed correlations to a carbon at 117.9 ppm corresponding to a double bond. In addition, H-6a correlated to a carbon at 150.2 ppm. The ^{13}C NMR chemical shifts of the double bond between C-12 and C-12a at δ 150.2 ppm and 117.9 ppm, respectively, were characteristic for an enolether. On the other hand, the ring between C-6 and C-4a which is typical for rotenoids has been opened. Thus, compound **1** represented a re-

[§] Part 7 in the series "Herbal remedies traditionally used against malaria". For part 6 see Onegi *et al.*



1 R = OCH_3
2 R = H

numbering system based on the rotenoids



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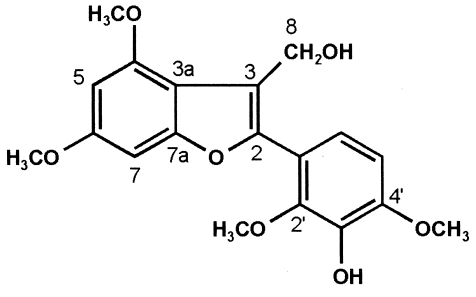

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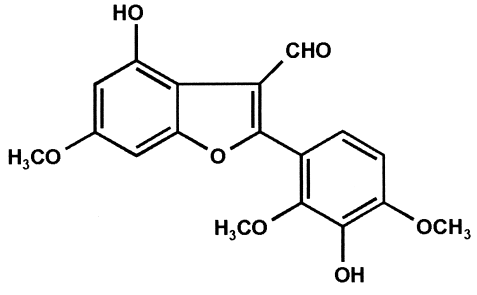

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Fig. 1. Compounds isolated from the leaves of *Andira inermis*.

arranged rotenoid derivative (the numbering system of the rotenoids has been kept, Fig. 1). The positions of the methylendioxy group and of the hydroxy group could be deduced from the HMBC, whereas the position of the methoxy group at C-4a could be determined by NOE experiments: irradiation of the singlet at 4.16 ppm led to the enhancement of the H-6a signal (5.18 ppm). Compound **2** showed similar signals in the ^{1}H NMR, its molecular formula was determined as $C_{17}H_{12}O_6$. In contrast to **1** no methoxy group could be observed. The singlets at 7.08 and 7.14 ppm represented the *para*-positioned protons at C-2 and C-4a, respectively. The shifts and coupling patterns of the other aromatic signals and the methylendioxy group were nearly identical to those of **1**. Thus, **2** is the 4a-demethoxy derivative of **1**; for these compounds we propose the names andirol A (**1**) and B (**2**).

The molecular formula of **3** was calculated as $C_{19}H_{20}O_7$ by HRMS. The ^{1}H NMR displayed characteristic signals of a pair of *meta*-coupled protons at 6.38 ppm (d, J = 1.9 Hz, H-5) and 6.69 ppm (d, J = 1.9 Hz, H-7), as well as of two *ortho*-coupled protons at 6.75 ppm (d, J = 8.5 Hz, H-5′) and 6.98 ppm (d, J = 8.5 Hz, H-6′) and four methoxy groups. Furthermore, one hydroxymethyl moiety could be characterized in the ^{1}H-NMR by a doublet at 4.71 ppm (J = 7.0 Hz, 2H) and by a downfield signal at 56.5 ppm in the ^{13}C NMR. The spectral data were comparable to those of 2-(3′, 4′-dimethoxyphenyl)-3-hydroxymethyl-7-methoxybenzofuran which could be prepared by reduction of the corresponding 3-formyl derivative by Stom-

Table I. ^{1}H NMR data [400 MHz, CD_3COCD_3, δ_H (ppm), *J* (Hz)], ^{13}C-NMR data [100.6 MHz, CD_3COCD_3, δ_C (ppm)] and HMBC correlations of andirol A (**1**) and ^{1}H NMR data [400 MHz, CD_3COCD_3, δ_H (ppm), *J* (Hz)] of andirol B (**2**).

Proton[a]	Carbon[a]	Compound **1**			Compound **2** δ_H
		δ_H	δ_C	HMBC Correlations[e]	
–	C-1	–	148.4[b]		–
H-2	C-2	6.81, 1 H, s	136.7		7.08, 1 H, s
–	C-3	–	133.5		–
–	C-4	–	148.9[b]		–
H-4a	C-4a	–	88.8	1, 3, 4, 12a, 12b	7.14, 1 H, s
H-6	C-6	4.03, 1 H, dd (1.9 and 12.4)	75.1	6, 12a	4.12, 1 H, dd (2.2 and 12.2)
H-6′		4.46, 1 H, dd (3.8 and 12.4)			4.41, 1 H, dd (4.4 and 12.2)
H-6a	C-6a	5.18, 1 H, dd (1.9 and 3.8)	65.9	12, 12a	5.06, 1 H, m
–	C-7a	–	159.5[c]		–
H-8	C-8	6.60, 1 H, d (2.3)	108.2	7a, 9, 10, 11a	6.60, 1 H, d (2.3)
–	C-9	–	159.8[c]		
H-10	C-10	6.71, 1H, dd (2.3 and 8.6)	111.8	11a	6.72, 1H, dd (2.3 and 8.8)
H-11	C-11	7.76, 1 H, d (8.6)	127.8	7a, 9, 12	7.76, 1 H, d (8.8)
–	C-11a	–	114.2		–
–	C-12	–	150.2		–
–	C-12a	–	117.9[d]		–
–	C-12b	–	116.5[d]		–
4a-OC**H**$_3$	O**C**H$_3$	4.16, 3 H, s	60.6	2	–
-O-C**H**$_2$-O-	-O-**C**H$_2$-O-	6.01, 2 H, s	102.2	3, 4	6.03, 2 H, s

[a] Numbering system based on the rotenoids; [b,c,d] Interchangeable; [e] CD_3OD as solvent.

berg *et al.* (1997). In addition they were similar to andinermal A (**4**), a 2-aryl-3-carbaldehyde, previously isolated from *A. inermis* (Kraft *et al.*, 2001). Thus, **3** represented the reduced form of **4** with an additional methyl group. The position of the three methoxy groups at 3.86, 3.95, and 3.98 ppm (6-OCH_3, 4′-OCH_3, 4-OCH_3, respectively) were established by NOE experiments whereas the ^{13}C NMR chemical shift of the fourth methoxy group at 61.13 ppm pointed to the C-2′ position in ring B as in **4**. This could be confirmed by NOE irradiation of the doublet at 4.71 ppm which led to the enhancement of the H-6′ signal (6.98 ppm). **3** represents a novel compound, which we named andinermol.

In the *in vitro* antiplasmodial assay against *Plasmodium falciparum* the methanolic extract exhibited no activity. After successive extraction we found an enhanced activity in the $CHCl_3$ fraction (IC_{50}: 10.6 μg/ml [Dd2 clone of *P. falciparum*]). Although isolated from the most active fractions, andirol A (**1**) showed no remarkable antiplasmodial activity (IC_{50} values: 15.0 μg/ml/43.9 μM [PoW strain of *P. falciparum*]; 42.5 μg/ml/124.3 μM [Dd2]). Andinermol (**3**) exhibited slightly lower IC_{50} values (8.6 μg/ml/23.9 μM [PoW]; 13.7 μg/ml/38.1 μM [Dd2]), but it was less active than the related carbaldehyde andinermal A (**4**; IC_{50} values: 2.1 μg/ml/6.1 μM [PoW]; 3.9 μg/ml/11.3 μM [Dd2]; Kraft *et al.*, 2001). Due to the lack of sufficient amounts of andirol B (**2**), it could not be tested.

In conclusion, during our investigation on the phytochemical spectrum of *A. inermis*, we could isolate from the leaves two compounds (**1**, **2**) with a unique heterocyclic structure. In contrast to the 2-arylbenzofuran-3-carbaldehydes they exhibited no antiplasmodial activity. Their carbon skeleton seems to be related to that of the rotenoids. Rotenoids are a class of isoflavonoids characterized by the presence of an extra carbon atom in an additional heterocyclic ring. This system originates in nature by oxidative cyclization of a 2′-methoxyisoflavone (Harborne, 1988). In the case of **1** and

2, the cyclization must be due to the enolic hydroxyl group in position 12 and a hydroxy group in position 1. Up to now, no similar compounds are described in literature.

Whereas several isoflavones (e. g. genistein, formononetin, and calycosin) and the 2-arylbenzofuran-3-carbaldehyde andinermal A (**4**) as well as andirol A (**1**) could be detected in both the Panamanian plant material and the leaves harvested in Ghana, andirol B (**2**) and andinermol (**3**), a 2-aryl-3-hydroxymethyl-benzofuran could only be found in the African plant. 2-Aryl-benzofurans are normally derived from flavonoids or pterocarpanes. They seem to occur rarely in nature. From literature simple benzofurans like 2-(2,4-dihydroxyphenyl)-5,6-methylendioxy-benzofuran from *Artemisia indica* Willd., Asteraceae, (Chanphen *et al.*, 1998) or ebenfuran I from *Onobrychis ebenoides* Bioss & Spuner, Fabaceae (Halabalaki *et al.*, 2000) are known, as well as 3-methyl-benzofurans like parvifuran from *Dalbergia parviflora* Prain, Fabaceae, (Muangnoicharoen and Frahm, 1981), benzofuran-3-carbaldehydes like ebenfuran II and III (Halabalaki *et al.*, 2000) or andinermal A-C (Kraft *et al.*, 2001), and benzofuran-3-carboxylic acids like norwedelic acid from *Wedelia calendulaceae* Less., Asteraceae (Govindachari and Premila, 1985). In contrast, **3** represents the first natural 2-aryl-*3-hydroxymethyl*-benzofuran. Up to now, such structural feature was only described in semisynthetic reduction products from the related carbaldehydes (Stomberg *et al.*, 1997) or as synthetic educts in chemical syntheses (Fukui *et al.*, 1969).

Experimental

General

For fractionation silica gel 60 (63–200 μm) was used. Preparative HPLC was performed on a Knauer instrument equipped with Eurochrom 2000 on a Nucleosil P 300 C-18 (10 μm) column and medium pressure liquid chromatography (MPLC) on a LiChroprep® RP-18 (40–63 μm) column. EIMS were recorded on a Finnigan MAT CH7A (70 eV); FAB MS and HR-EIMS on a Finnigan MAT 711 (80 eV). ^{1}H NMR, ^{13}C NMR, NOE, and HMBC spectra were obtained on a Bruker AVANCE DPX 400 MHz or a Bruker DRX 500 MHz spectrometer (TMS as int. standard; CD_3COCD_3 or $CDCl_3$ as solvent).

Plant material

The leaves of *A. inermis* were collected in the Upper East Region of Ghana during March 2000. Voucher specimens (GC 47682) were identified by Dr. D. Abbiw and deposited at the Department of Botany, University Legon-Accra, Ghana.

Extraction and isolation

Ground leaves (400 g) were extracted four times with 1.5 l MeOH-H_2O (8:2, v/v) for 24 h at room temperature. MeOH was evaporated and the remaining aqueous phase (ca. 500 ml) was successively extracted with petrol ether, $CHCl_3$, and EtOAc (four times with 300 ml each) to yield four fractions, which were tested against *P. falciparum*. The $CHCl_3$ fraction proved to be most active.

The oily residue (3.4 g) was subjected to column chromatography on silica gel 60 (50 g) and eluted with $CHCl_3$, $CHCl_3$-MeOH mixtures, and MeOH to yield seven fractions. Due to the results of the bioassay, fraction 3 (190 mg, eluted with $CHCl_3$-MeOH 9:1 [v/v]; IC_{50}: 4.2 μg/ml [PoW]) and fraction 4 (420 mg, eluted with $CHCl_3$-MeOH 8:2 [v/v]; IC_{50}: 8.0 μg/ml [PoW]) were further separated. From fraction 3 we gained three main peaks by preparative HPLC (RP-18) with a gradient of MeOH-H_2O 3:7 (v/v) to MeOH-H_2O 7:3 (v/v) in 40 min at a flow rate of 5.5 ml/min, which were purified by preparative TLC. Peak 1 and 2 were purified on TLC (cyclohexane-EtOAc 3:7 [v/v]) and yielded **3** (R_f: 0.35; 1 mg) and **1** (R_f: 0.37; 6.3 mg), respectively. From peak 3 compound **4** could be identified by comparison of spectral data (Kraft *et al.*, 2001). The purification of fraction 4 was performed by MPLC with MeOH-H_2O mixtures. Again the fourth fraction (350 mg, eluted with MeOH-H_2O 7:3 [v/v]; IC_{50}: 4.7 μg/ml [PoW]) was the most active one. After a second column chromatography on silica gel (10 g) with $CHCl_3$, $CHCl_3$-MeOH mixtures and MeOH we gained again **1** (2 mg; eluted with $CHCl_3$-MeOH 99:1 [v/v]). By preparative TLC ($CHCl_3$-MeOH 95:5 [v/v]; developed twice) of the fraction eluted with $CHCl_3$-MeOH 99:1 and 95:5 [v/v], **2** could be isolated (R_f: 0.46; < 1 mg).

Spectroscopic data

3-Hydroxy-7-methoxy-8,9-methylendioxy-6-hydroxymethylpterocarpene (**1**, andirol A): Yellow needles; $[\alpha]_D^{20}$: + 5.33° ($CHCl_3$, c = 0.075); EIMS *m/z* (rel. Int.): 342 $[M]^+$ (92), 324 $[M\text{-}H_2O]^+$ (100), 313, (53), 309, (40); (+)-FABMS: *m/z*: 342 $[M]^+$, 325 $[M\text{-}H_2O\text{+}H]^+$; (–)-FABMS: *m/z*: 341 $[M-H]^-$; HRMS *m/z*: 342.07368 ($C_{18}H_{14}O_7$, calculated for 342.07396), 324.06315 ($C_{18}H_{12}O_6$, calculated for 324.06339); ^{1}H NMR and ^{13}C-NMR: see Table I.

3-Hydroxy-8,9-methylendioxy-6-hydroxymethylpterocarpene (**2**, andirol B): Yellow needles; EIMS *m/z* (rel. Int.): 312 $[M]^+$ (31), 294 $[M\text{-}H_2O]^+$ (43), 167 (20) ,149 (49), 28 (100); HRMS *m/z*: 312.06352 ($C_{17}H_{12}O_6$, calculated for 312.063390), 294.05271 ($C_{17}H_{10}O_5$, calculated for 294.05283); ^{1}H NMR: see Table I.

2-[2′,4′-Dimethoxy-3′-hydroxyphenyl]-4,6-dimethoxy-3-hydroxymethyl-benzofuran (**3**, andinermol): Yellow needles; EIMS *m/z* (rel. Int.): 360 $[M]^+$ (100), 342 $[M\text{-}H_2O]^+$ (10); HRMS *m/z*: 360.12078 ($C_{19}H_{20}O_7$, calculated for 360.12091); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.98 (1H, d, *J* = 8.5 Hz, H-6′), 6.75 (1H, d, *J* = 8.5 Hz, H-5′), 6.69 (1H, d, *J* = 1.9 Hz, H-7), 6.38 (1H, d, *J* = 1.9 Hz, H-5), 5.95 (1H, brs, 3′-OH), 4.71 (2H, d, *J* = 7.0 Hz, $H_{a,b}$-8), 3.98 (3H, s, 4-OCH_3), 3.95 (3H, s, 4′-OCH_3), 3.86 (3H, s, 6-OCH_3), 3.71 (3H, s, 2′-OCH_3), 3.24 (1H, t, *J* = 7.0 Hz, 8-OH); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 159.8, 159.2, 156.5 (C-4, C-6, C-7a), 153.2 (C-4′), 148.5 (C-2′), 138.7 (C-3′), 121.7 (C-6′), 116.6 (C-3), 108.3, 106.8 (C-3a, C-5′), 94.3 (C-5), 88.7 (C-7), 61.1 (2′-OCH_3), 56.5 (C-8), 56.3, 55.8, 55.8 (4-OCH_3, 6-OCH_3, 4′-OCH_3), n. d. (C-2, C-1′).

In vitro antiplasmodial activity

The bioassay was performed as previously described (Kraft *et al.*, 2000). *P. falciparum* strain PoW (IC_{50} for chloroquine = 0.008 μg/ml/0.015 μM) and the clone Dd2 (IC_{50} = 0.092 μg/ml/0.18 μM) were maintained in continuous culture in human red blood cells (A^+) as previously described (Trager and Jensen, 1976). Substances were dissolved in DMSO (20 mg/ml) and diluted in medium to final concentrations between 50 and 0.78 μg/ml. The antiplasmodial assay, according to Desjardins *et al.* (1979) was analysed by means of the microculture radioisotope technique after 42 h.

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