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Dictyopanines A, B and C, New Bicyclic Sesquiterpene Esters from *Dictyopanus* sp. HKI 0181

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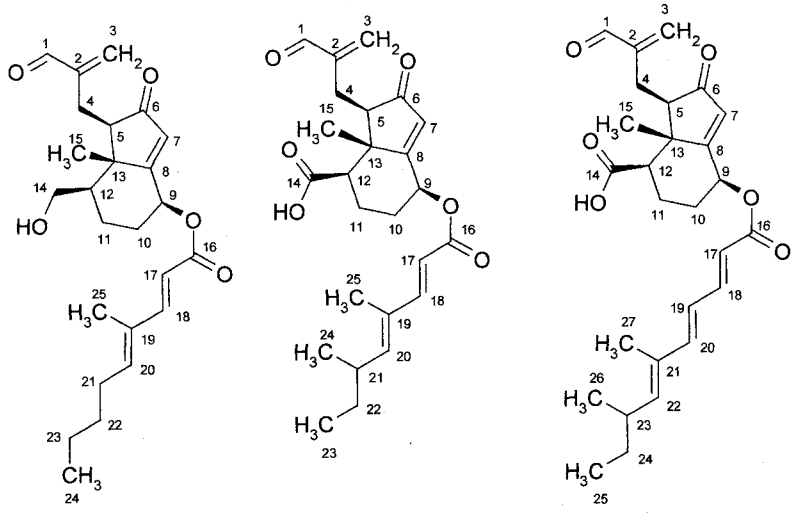
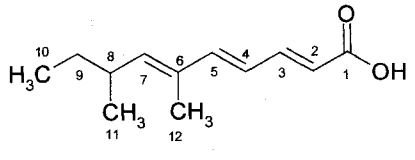
In the course of screening for new antibacterial compounds from tropical fungi[1~3] a strain of *Dictyopanus* sp. HKI 0181 was found to produce three new antibacterial compounds named dictyopanines A (**1**), B (**2**) and C (**3**; Fig. 1). A fourth inactive triene fatty acid metabolite (**4**) was coproduced. This paper describes the strain, production, isolation, structure elucidation and antibacterial activity of compounds **1**~**4**.

The strain HKI 0181 of a species of *Dictyopanus* (ss.SINGER 1986)[1] was isolated in a tropical rain forest region of South America. It forms fruit bodies on wood which are soft fleshy, flexible, with gelantinous trama and yellowish white, poroid, hymenophor spores which are small (4/2.5 μm) and amyloid. The isolated mycelium consists in an early stage of very fine hyaline hyphae with anastomoses (1~1.5 μm diameter) which later develop into deep brown hyphae with 4~6 (~10) μm diameter in part with short cells (*e.g.* 12/6 to 6/6 μm). Rhizomorphs (1~2 mm diameter) are formed with a dark brown bark and central white hyphae which branched later. It has been deposited in the strain collection of the Hans-Knöll-Institute for Natural Products Research, Jena. For submerged cultivation small pieces (1~2 cm^2) of a slant agar culture of the strain HKI 0181 grown on malt agar

Fig. 1. Structures (relative stereochemistry) of dictyopanines A (**1**), B (**2**), C (**3**) and metabolite **4**.



1 **2** **3**



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Dedicated to Professor WOLFGANG STEGLICH at the occasion of his 65th birthday.

Table 1. Physico-chemical properties of compounds **1**, **2**, **3** and **4** from *Dictyopanus* sp.

	1	**2**	**3**	**4**
Appearance	waxy mass	waxy mass	waxy mass	waxy mass
Molecular weight (MS)	414 (M^+: *m/z* 414.2386; calcd.: 414.2366)	428 (M^+-side chain: *m/z* 261.1058; calcd.: 262.1067 for $C_{15}H_{17}O_4$)	454 (M^+: *m/z* 454.2351; calcd.: 454.2347)	194 (M^+: *m/z* 194.1298; calcd.: 194.1291)
Formula	$C_{25}H_{34}O_5$	$C_{26}H_{36}O_5$	$C_{27}H_{34}O_6$	$C_{12}H_{18}O_2$
IR (λ_{max} in KBr (cm^{-1}))	768, 1027, 1092, 1262, 1399, 1460, 1530, 1552, 1588, 1626, 1671, 1678, 2920, 3060, 3400	780, 849, 995, 997, 1054, 1090, 1125, 1179, 1292, 1378, 1409, 1452, 1616, 1685, 1692, 2955, 3425	780, 990, 1088, 1133, 1156, 1180, 1236, 1301, 1337, 1376, 1454, 1610, 1642, 1696, 1700, 2955, 3425	-
$[\alpha]_D^{25}$ (methanol)	+ 31.1 °	+ 35.8 °	+ 25.1 °	-
R_f on TLC ($CHCl_3$/MeOH 95:5 Silica gel)	0.75	0.65	0.70	0.55

composed of (g/liter) malt extract 40, yeast extract 4, deionized water (pH 6.0) were inoculated into 500 ml Erlenmeyer flasks containing 100 ml of a culture medium composed of (g/liter): glucose 30, soybean meal 20, yeast extract 1, KH_2PO_4 1, $MgSO_4 \cdot 7H_2O$ 0.5, $ZnSO_4 \cdot 7H_2O$ 0.008 (pH 6.0).

Cultivation occurred as surface culture for 14 days whereby a dense lawn of mycelium developed. Thereafter, 20 liters of the culture broth were extracted twice with 20 liters of ethyl acetate. The extracts were evaporated *in vacuo* to dryness.

The residue (2.2 g) was chromatographed on silica gel 60 (column 100 cm×5 cm, $CHCl_3$/MeOH, 95 : 5). Fractions displaying antibacterial activity and/or staining blueish with 1% vanillin in conc. H_2SO_4 on TLC were collected. Final purification was achieved by preparative TLC (silica gel aluminium sheets Merck, $CHCl_3$ (3 times run) and RP_{18} silica gel sheets Merck, acetonitrile/H_2O, 83 : 17, v/v), yielding 25 mg (**1**), 30 mg (**2**), 11 mg (**3**) and 22 mg (**4**), respectively. All components were isolated as waxy masses. The physico-chemical properties of the new compounds are shown in Table 1. Compounds **1**~**3** displayed absorbances in the IR spectrum attributable to several carbonyl groups. The molecular weights of **1**~**4** as shown in Table 1 were determined from the HREI-MS (AMD Intectra double-focussing sector-field mass spectrometer AMD 402) and ESI-MS spectra (Triple quadrupole mass spectrometer Quattro, VG Biotech, Altrincham, England). Accurate mass measurements of M^+ or related fragments furnished the molecular formulas. In the EIMS of **2** and **3** diagnostic sesquiterpene fragments (see **2** in Table 1: M^+-fatty acid side chain: *m/z* 261.1058, calcd. 261.1067 for $C_{15}H_{17}O_4$) were prominent suggesting that both metabolites contained the same sesquiterpene bicycle. Otherwise, *m/z* 246.5 (60% intensity) was visible in the EI-MS of **1** attesting to the presence of a hydroxymethylene instead of a carboxyl group in this moiety. Suggestions about the molecular weights and composition of molecules **1**~**3** were supported by the results of ESI-MS showing the pertinent $[M-H]^-$, $[M+H]^+$ and $[M+Na]^+$ ions in the negative and positive ion mode, respectively. ESI-CID-MS/MS (Quattro instrument; argon as collision gas) of *m/z* 415 (**1**, $[M+H]^+$) afforded *m/z* 247.3 (sesquiterpene moiety, 30%), *m/z* 151.0 (fatty acid side chain−O, 90%) and *m/z* 122.9 (fatty acid side chain, $-CO_2$, $-CH_3$). Similarly *m/z* 429.3 as the $[M+H]^+$ ion of **2** afforded *m/z* 261.3 (sesquiterpene fragment) and *m/z* 150.9 (side chain−O). Information about the fatty acid constituent of **3** was furnished, too, by CID-MS/MS of *m/z* 455.7 ($[M+H]^+$, 40%) displaying 261.4 (sesquiterpene fragment) and *m/z* 177.1 (side

Table 2. Assignment of ^{1}H and ^{13}C NMR spectra of **1**, **2**, **3** and **4**.

compound	1		2		3		4	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	193.19	9.54 s	193.06	9.45 s	193.10	9.54 s	171.91	-
2	148.06	-	147.75	-	147.71	-	118.29	5.85 d, 11.0
3	136.19	6.25 d, 0.7, 6.4, d, 0.7	136.31	6.24 d, 0.5; 6.4, d, 0.5	132.46	6.25 d, 1; 6.45 d, 1.1	147.91	7.41 d,d, 11.0, 14.3
4	43.23	3.85 d, 11.0	43.17	3.72 dd, 11.2, 6.0	43.21	3.85 dd, 11.3, 6.1	123.48	6.25 d,d, 14.3, 15.0
5	42.95	2.15 dd, 8.1, 3.3	43.13	2.25 dd	43.18	2.15 m	147.51	6.52 d, 15.0
6	197.21	-	196.90	-	196.81	-	132.49	-
7	128.77	6.05 s	129.40	6.08 s	129.55	6.1 s	145.91	5.5 d, 9.4
8	161.18	-	159.08	-	158.91	-	35.05	2.41 m
9	73.78	5.5 s,dd, 3.2, 0.9	72.51	5.6 dd, 3.1, 1.0	72.46	5.5 dd, 3.2, 1.8	30.12	1.2, 1.4 m
10	30.47	2.15 m	29.75	1.89 m	30.12	2.2 m	11.91	0.91 t, 7.2
11	20.10	1.85 m	20.05	1.85 m	20.05	1.85 m	20.39	0.98 d, 7.2
12	50.21	1.55 dd,br	53.34	2.49 dd, 6.1, 2.2	52.22	2.45 dd, 8.8, 2.8	12.48	1.80 d, 0.5
13	38.58	-	38.34	-	38.32	-	-	-
14	63.0	3.48 d, 6.8	177.63	-	177.01	-	-	-
15	18.95	1.45 s	19.63	1.55s	19.59	1.5 s	-	-
16	166.18	-	172.26	-	165.70	-	-	-
17	115.09	5.72 d, 16.0	114.40	5.75 d, 15.0	118.72	5.8 d, 15.0	-	-
18	150.77	7.3 d, 16.0	152.44	7.43 d, 15.0	146.53	7.42 dd, 15.o, 11.0	-	-
19	131.48	-	131.47	-	123.44	6.25 dd, 11.0, 15.0	-	-
20	149.11	5.6 t, 7.2, 7.3	150.88	7.38 s	147.15	6.56 d, 15.0	-	-
21	20.10	1.45 m	34.97	2.49 m	132.46	-	-	-
22	29.36	1.7 m	29.92	1.35 m, 1.48 m	145.63	5.5 d, 7.3	-	-
23	29.96	1.7 m	11.58	0.85 t, 7.1	35.01	2.45 m	-	-
24	11.91	0.8 t, 7.5	20.03	1.0 d, 7.1	30.1	1.45 m	-	-
25	12.41	1.75 s	12.84	1.81 d	11.91	0.85 t, 7.1	-	-
26	-	-	-	-	20.29	1.0 d, 7.3	-	-
27	-	-	-	-	12.48	1.75 s		

In $CDCl_3$; chemical shift (δ) in ppm; TMS as internal standard; coupling constants in Hz. Abbreviations: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad.

chain+O, 80%) as the product of carbon-heteroatom cleavage between the sesquiterpene and fatty acid moiety.

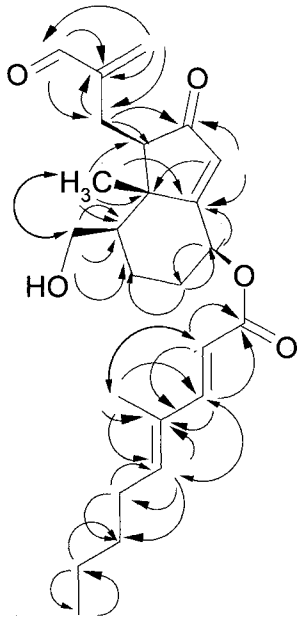
The structures of **1**, **2**, **3** and **4** (Fig. 1, relative stereochemistry) were settled conclusively on the basis of ^{1}H and ^{13}C one- and two-dimensional NMR experiments (COSY, DEPT, HSQC, HMBC, NOESY) as shown in Table 2. Characteristic features of the ^{1}H NMR spectra of **1**~**4** were a series of olefinic singlet, doublet and multiplet protons. Analysis of the H-17/H-18 coupling pattern in **1**, **2** and **3** ($^{3}J_{H\text{-}17,\,H\text{-}18}$=16 Hz and 15 Hz, respectively) attested to an (*E*)-configuration at this position. The observable NOESY correlation of H-18 and H-20 in **1** and **2** was consistent with the same configuration of the second double bond as shown in Fig. 1. Due to $^{3}J_{H\text{-}19/H\text{-}20}$=15.0 Hz and visible NOESY correlations between H-20 and H-22 the relative stereochemistry of the olefinic side chain in compound **3** appears to be the same as in **1** and **2**. In the same manner the olefinic $^{3}J_{H\text{-}2,\,H\text{-}3}$ and $^{3}J_{H\text{-}4,\,H\text{-}5}$ coupling constants (11.0 Hz and 15.0 Hz, respectively) and the strong NOESY correlations between H-5 and H-7 supplied evidence for the structure of **4**. In addition to the double bond protons of the side chains the ^{13}C and DEPT spectra of **1**, **2** and **3** displayed an olefinic methylene group. The presence of an aldehyde proton in **1**, **2** and **3** was suggested by the resonances at δ 9.45 ppm and δ 9.54 ppm, respectively.

The $^{3}J_{H,H}$ couplings of the methyl groups in **1**~**4** supported the assignments of their bonding at the molecular positions shown in Fig. 1. The ^{13}C NMR spectra of **1**~**3** displayed two aldehyde and ketone carbonyl signals around 193 and 197 ppm which owed their upfield shift to the neighbouring conjugated double bonds. In addition, compound **1** displayed one but **2** and **3** showed two carboxyl signals (Table 2). In the ^{13}C NMR spectrum of **4** a carboxyl signal at 171.91 ppm was visible, too. Moreover, the ^{13}C NMR and DEPT spectra confirmed the number of double bonds, methylene, methine and quarternary carbons as shown in Fig. 1.

The connectivity of protons and carbons both in the sesquiterpene and the fatty acid structures was conclusively

settled by the HSQC, COSY and HMBC experiments (Table 2). C, H long-range heteronuclear couplings (HMBC), in particular, confirmed the bicyclic terpene structures of **1**~**3** and the positions of the methyl and oxymethylene groups, respectively, at the bicycle and the olefinic chain (Fig. 2). The above results suggested that **1**, **2** and **3** are new structures of terpenoid natural products. Although the bicyclic ring system is frequently found amongst the fungal metabolites[2] the special type of substitution renders it as a new type of sesquiterpene. **1**~**3** contain mono- and dimethylated olefinic fatty acids similar to those found earlier in other basidiomycete products[4]. However, in **1** the methylated fatty acid contains a nonadiene backbone as an unusual feature. The structure of coproduced **4** was similar to those of the fatty acid substituents in **1**~**3**.

Fig. 2. Instructive C, H long-range and NOE's correlations in the HMBC (one-sided arrow) and NOESY spectrum (two-sided arrow) of **1**.



Dictyopanines A (**1**), B (**2**) and C (**3**) displayed moderate antimicrobial activities against a narrow spectrum of filamentous fungi and Gram-positive bacteria (Table 3). The comparably good activity against resistant *Staphylococcus aureus* 134/94 strains appears as an interesting fact.

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Table 3. Antibiotic activities of 50 μl of a methanolic solution (1 mg/ml) of **1**, **2** and **3** during the agar well diffusion assay[4].

	diameter of inhibition zone (mm)		
	1	**2**	**3**
1 *Staphylococcus aureus* SG 511	19	15	15
2 *Staphylococcus aureus* 134/94 (MRSA)	19	15	15
3 *Enterococcus facalis* 1528 (VR)	19	15	15
4 *Bacillus subtilis* ATCC 6633	21	16	16
5 *Candida albicans* BMSY 212	0	0	0
6 *Sporobolomyces salmonicolor* SBUG 549	0	0	0
7 *Penicillium notatum* JP 36	13 p	12 p	12 p

1 - 4 Standard I nutrient agar (SERVA)

5 - 6 Yeast morphology agar (DIFCO)

7 Malt agar

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