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The Irpexans, a New Group of Biologically Active Metabolites Produced by the Basidiomycete *Irpex* sp. 93028†

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Five novel antibiotics described as irpexans (**1**, **2**, **3a**, **3b**, **4**) were isolated from fermentations of an *Irpex* species in the course of a screening for new inhibitors of AP-1 and NF-κB mediated signal transduction pathways in COS-7 cells using secreted alkaline phosphatase (SEAP) as a reporter gene. The expression of an AP-1 and NF-κB driven SEAP reporter gene was inhibited in a dose dependent manner with 14-acetoxy-15-hydroxyirpexan (**3b**) being the most potent compound, followed by 14,15-irpexanoxide (**2**), 14,15-dihydroxyirpexan (**3a**) and 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**). Irpexan (**1**) exhibited no activity. The irpexans (**1**, **2**, **3a**, **3b**, **4**) are characterized by weak cytotoxic but neither antibacterial nor antifungal activities. All five compounds are terpenoids with a mannose moiety. The structures were elucidated by spectroscopic methods.

Mammalian signal transduction pathways contain a number of components which are attractive targets[1] for pharmacological interventions *e.g.* in inflammatory processes or cancer[2]. In order to search for new inhibitors of the AP-1 and NF-κB mediated signal transduction COS-7 cells were transiently transfected with reporter gene constructs containing the reporter gene secreted alkaline phosphatase (SEAP) under the control of three copies of the TPA responsive element or five copies of the NF-κB binding site, respectively. A screening of 280 strains of basidiomycetes, ascomycetes and fungi imperfecti resulted in the isolation of five new compounds from fermentations of an *Irpex* sp. Here we describe the taxonomy of the producing strain, the fermentation, isolation, some biological activities and the structure elucidation of the metabolites which we named irpexan (**1**), 14,15-irpexanoxide (**2**), 14,15-dihydroxyirpexan (**3a**), 14-acetoxy-15-hydroxyirpexan (**3b**) and 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**).

Materials and Methods

General

UV spectra were obtained with a Perkin Elmer λ16, and IR spectra with a Bruker IFS 48. The optical rotation was measured with a Perkin-Elmer 1541 polarimeter with a cell path of 10 cm. FAB-MS spectra (direct inlet, positive ions) were recorded with a Jeol JMS-SX102 spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX 500 spectrometer with an inverse 5 mm probe equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were performed with gradient enhancements using sine shaped gradient pulses, and for the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^1J_{CH}=145$ Hz and $^2J_{CH}=10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001).

Producing Organism

Fruiting bodies of the basidiomycete *Irpex* sp. (Fr.)[3],

† Dedicated to Sir EDWARD P. ABRAHAM.

strain 93028, were collected in North America. The specimen showed all characteristics of the genus, the species, however could not be unequivocally determined. The fruiting bodies have no stem, are resupinate and more or less laterally fused, with the edges curling up 0.5~1 cm from the substratum. Caps are 2~5 cm across, 0.5~2 mm thick, have cream to pale buff color and are densely hairy. The hymenophor is irpicoid, to the edge irregular poroid. Spines or teeth are up to 0.3 (0.5) cm long, white to cream. Spores are ellipsoid to cylindrical (5~5.5 μm×2~3 μm), hyalin and smooth. The dimitic hyphal system consists of simple septate generative hyphae (2~3 μm in diameter) without buckles but heavily encrusted cystidia (20~27 μm×3~5 μm)[4,5]. Voucher specimen and mycelial cultures are deposited in the culture collection of the LB Biotechnologie, Universität Kaiserslautern.

Fermentation

Fermentations were carried out in 20 liters of HA medium composed of: Yeast extract 0.4 %, glucose 0.4 %, malt extract 1%, pH 5.5. For solid media 1.5 % agar was added. Fermentations were carried out in 20 liters of YMG medium in a Biolafitte C6 fermenter at 24°C with an aeration rate of 3 liters/minute and agitation (120 rpm). 250 ml of a well grown culture were used as inoculum. During fermentation 100 ml samples were withdrawn, the culture broth separated by filtration and the mycelia extracted with methanol - acetone 1 : 1. After evaporation of the organic solvents the remaining aqueous residue was extracted with EtOAc, dried with Na_2SO_4, concentrated *in vacuo* (40°C) and the residue dissolved in 1 ml of MeOH. The content of 14-acetoxy-15-hydroxyirpexan (**3b**) was determined by analytical HPLC of 10 or 20 μl samples (Merck Li Chrospher 100 RP 18, 5 μm; column 125×4 mm; flow: 1.5 ml/minute; gradients: H_2O - acetonitile 0~70% in 5 minutes, 70~100% in 10 minutes; Rt 14-acetoxy-15-hydroxyirpexan (**3b**)=8.21 minutes).

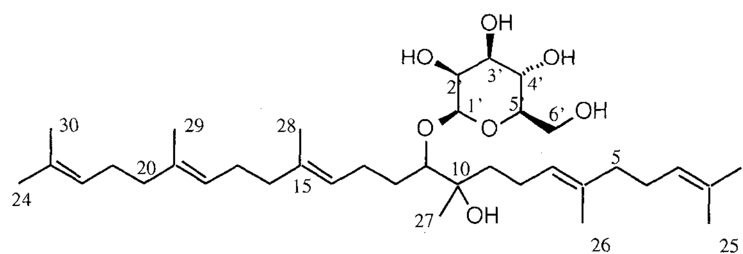
Isolation of the Compounds

After 4 days of fermentation the mycelia were separated from the culture broth and extracted twice with a total amount of 4 liters of methanol - acetone 1 : 1. The organic solvents were evaporated and the remaining aqueous residue extracted with ethyl acetate, dried with Na_2SO_4 and concentrated *in vacuo* (40°C). The crude extract (1.83 g) was applied onto a column (30×2.5 cm) containing silica gel (Merck 60, 0.063~0.2 mm). An enriched product (346 mg) was obtained after elution with 100% ethyl acetate. Preparative HPLC (Merck Li Chrospher WP 300 RP 18, 12 μm; column 250×25 mm; flow 5 ml/minute; gradients: H_2O - acetonitrile 0~75% in 30 minutes, 75~100% in 50 minutes) yielded 100 mg of 14-acetoxy-15-hydroxyirpexan (**3b**) (Rt=57.86 minutes), 3.6 mg of 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**) (Rt=42.59 minutes), 2.8 mg of 14,15-dihydroxyirpexan (**3a**) (Rt=49.56 minutes), 1.1 mg of 14,15-irpexanoxide (**2**) (Rt=66.43 minutes) and 5.7 mg of irpexan (**1**) (Rt=91.34 minutes) (Fig. 1), all five as oils.

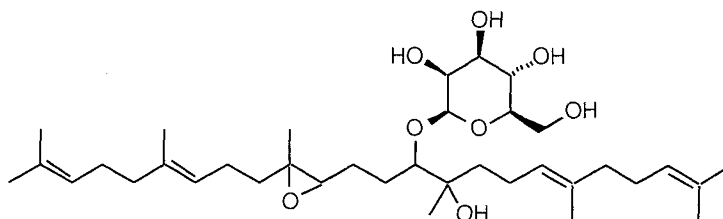
The retention times (Rt) of the compounds **1**, **2**, **3a**, **3b**, **4** were determined by analytical HPLC (Merck Li Chrospher 100 RP 18, 5 μm; column 125×4 mm; flow: 1.5 ml/minute; gradients: H_2O - acetonitile 0% for 0.5 minutes, 0~100% in 19.5 minutes). The retention times are 8.21 minutes for 14-acetoxy-15-hydroxyirpexan (**3b**), 5.44 minutes for 14-acetoxy-15,23-dihydroxyirpexan (**4**), 5.84 minutes for 14,15-dihydroxyirpexan (**3a**), 6.57 minutes for 14,15-irpexanoxide (**2**) and 7.4 minutes for irpexan (**1**) respectively.

The acetylated derivative **3c** was prepared by treating 14-acetoxy-15-hydroxyirpexan (**3b**) (20 mg) with acetic anhydride (0.5 ml) in pyridine (1 ml) at room temperature over night. **3c** was obtained as a colourless oil, $[\alpha]_D^{22}$ −8.4° (*c* 1.4 in $CHCl_3$). ^1H (500 MHz) NMR data in $CDCl_3$ (δ; multiplicity; *J*): 5.58, d, $J_{1'\text{-}2'}$=3.1, 2′-H; 5.19, dd, $J_{3'\text{-}4'}$=$J_{4'\text{-}5'}$=10, 4′-H; 5.12, m, 18-H; 5.10, m, 3-H and 22-H; 5.07, m, 7-H; 5.00, dd, $J_{2'\text{-}3'}$=3.1, $J_{3'\text{-}4'}$=10.1, 3′-H; 4.83, dd, $J_{13a\text{-}14}$=1.0, $J_{13b\text{-}14}$=9.6, 14-H; 4.74, s, 1′-H; 4.21, dd, $J_{5'\text{-}6'a}$=6.5, $J_{6'a\text{-}6'b}$=12.2, 6′-Ha; 4.17, dd, $J_{5'\text{-}6b}$=3.0, $J_{6'a\text{-}6'b}$=12.2, 6′-Hb; 3.71, ddd, $J_{4'\text{-}5'}$=10.0, $J_{5'\text{-}6'a}$=6.5, $J_{5'\text{-}6'b}$=3.0, 5′-H; 3.56, dd, $J_{11\text{-}12a}$=$J_{11\text{-}12b}$=6.4, 11-H; 3.36, s, 10-OH; 2.14, s, 2′-OAc; 2.10 and 1.99, m, 4-H_2 and 21-H_2; 2.09, s, 6′-OAc; 2.06, m, 8-H_2 and 17-H_2; 2.05, s, 14-OAc; 1.99, s, 4′-OAc; 1.96, s, 3′-OAc; 1.97, m, 5-H_2 and 20-H_2; 1.77, dt, $J_{13a\text{-}13b}$=12.9, $J_{13a\text{-}14}$=6.4, 13-Ha; 1.66, s, 1-H_3 and 24-H_3; 1.61, s, 26-H_3; 1.60, m, 13-Hb; 1.59, s, 25-H_3, 29-H_3 and 30-H_3; 1.53, m, 9-Ha; 1.52, m, 16-Ha; 1.42, m, 16-Hb; 1.41, m, 12-H_2; 1.27, m, 9-Hb; 1.18, s, 28-H_3; 1.03, s, 27-H_3. ^{13}C (125 MHz) NMR data in $CDCl_3$ (□; multiplicity): 170.9 14-OCO; 170.6 6′-OCO; 170.3 2′-OCO; 170.0 3′-OCO; 169.6 4′-OCO; 135.6 C-6; 134.9 C-19; 131.4 C-23; 131.3 C-2; 124.8 C-22; 124.3 and 124.3 C-3 and C-7; 124.0 C-18; 100.5 C-1′; 91.7 C-11; 76.9 C-14; 73.8 C-15; 73.6 C-10; 72.4 C-5′; 71.0 C-3′; 69.0 C-2′; 66.0 C-4′; 62.5 C-6′; 39.7 C-5 and C-20; 38.6 C-16; 35.6 C-9; 27.3 C-12; 26.7 and 26.7 C-4 and C-21; 25.6 C-1 and C-24; 25.6 C-13; 23.4 C-27; 22.5 C-28; 21.7 C-17; 21.6 C-8; 21.0 14-Ac; 20.7 2′-Ac; 20.6, 20.6 and 20.6 3′-Ac, 4′-Ac and 6′-Ac; 17.6 and 17.6 C-26 and C-29; 16.0 and 15.9 C-25 and C-30. HRFAB-MS: 873.4988 ($M+Na^+$), calculated for $C_{46}H_{74}O_{14}Na$ 873.4976.

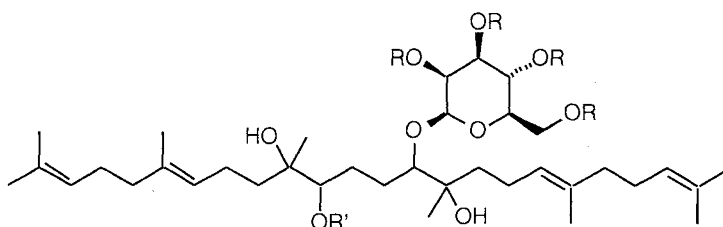
Fig. 1. Structures of irpexans.



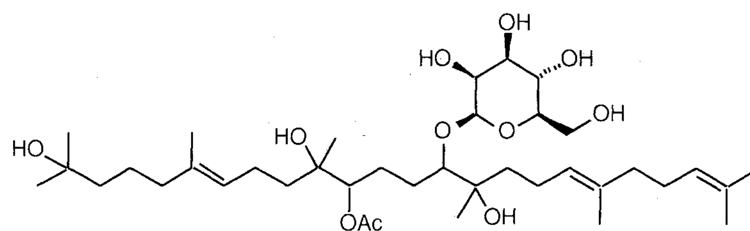
Irpexan (**1**)



14,15-Irpexanoxide (**2**)



14,15-Dihydroxyirpexan (**3a,** R=R'=H)
14-Acetoxy-15-dihydroxyirpexan (**3b**, R=H, R'=Ac)
3c, R=R'=Ac



14-Acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**)

Biological Assays

Assay for cytotoxic activity: The assay was performed as described previously with slight modifications[6]; L1210 and HL-60 cells were grown in RPMI 1640 medium (GIBCO, BRL), BHK-21, COS-7 and HeLa S3 cells in D-MEM (GIBCO, BRL), supplemented with 10% fetal calf serum (FCS) (GIBCO, BRL), 65 μg/ml of penicillin G and 100 μg/ml of streptomycin sulfate. The assays contained 1×10^5 cells/ml medium.

Reporter gene assays: The plasmids pGE2-AP1 and pGE2-NF1[7] contained the reporter gene secreted alkaline phosphatase (SEAP)[8] under the contol of an enhancerless

Table 1. Physico-chemical properties of irpexan (**1**), 14,15-irpexanoxide (**2**), 14,15-dihydroxyirpexan (**3a**), 14-acetoxy-15-hydroxyirpexan (**3b**) and 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**).

	1	**2**	**3a**	**3b**	**4**
Appearance	Colourless oil	Colourless oil	Colourless oil	Colourless oil	Colourless oil
$[\alpha]_D^{22}$	± 0° (c 0.5 in $CHCl_3$)	- 22° (c 0.3 in $CHCl_3$)	+ 2° (c 0.9 in $CHCl_3$)	- 2° (c 0.9 in $CHCl_3$)	- 5° (c 0.4 in $CHCl_3$)
Molecular formula	$C_{36}H_{62}O_7$	$C_{36}H_{62}O_8$	$C_{36}H_{64}O_9$	$C_{38}H_{66}O_{10}$	$C_{38}H_{68}O_{11}$
HRFAB-MS (*m/z*) Observed	629.4387 M + Na^+	645.4349 M + Na^+	663.4439 M + Na^+	705.4555 M + Na^+	723.4666 M + Na^+
Calculated	629.4393 for $C_{36}H_{62}O_7Na$	645.4342 for $C_{36}H_{62}O_8Na$	663.4448 for $C_{36}H_{64}O_9Na$	705.4553 for $C_{38}H_{66}O_{10}Na$	723.4659 for $C_{38}H_{68}O_{11}Na$
UV (CH_3CN) λ_{max} nm (ε)	No maxima observed above 220 nm				
IR (KBr) cm^{-1}	3415, 2925, 1630, 1450, 1375, 1165, 1070 and 1030	3420, 2925, 1630, 1450, 1380, 1170, 1070 and 1025	3425, 2925, 1635, 1450, 1375, 1170, 1070 and 1025	3420, 2925, 1740, 1630, 1450, 1375, 1245, 1070 and 1020	3420, 2935, 1735, 1640, 1450, 1375, 1240, 1075 and 1025

SV promoter, the vector pGE2-NF1 contains 5 copies of the NF-κB binding site. Transfections of COS-7 cells were performed by electroporating $1\sim2\times10^6$ cells suspended in 1 ml phosphate buffered saline (PBS) containing 30 μg of the reporter plasmids at 500 V/cm and τ=20~25 ms using a gene pulser apparatus (BioRad). After electroporation the cells were diluted to $4\times10^4\sim8\times10^4$ cells/ml in Opti-MEM medium (GIBCO, BRL) supplemented with 10% fetal calf serum (FCS), 65 μg/ml penicillin G and 100 μg/ml streptomycin sulfate in 24 well tissue culture plates and allowed to recover for 16 hours. For inducing SEAP expression, TPA (50 ng/ml) and various concentrations of **1**, **2**, **3a**, **3b**, **4** were added in Opti-MEM containing 0.5% FCS and antibiotics (see above). 48 hours after transfection the activity of the SEAP in the culture medium was measured by using the Phospha-Light chemiluminescent reporter gene assay (TROPIX, MA) according to the manufacturer's instructions with a liquid scintillation counter.

Results and Discussion

The fermentation of *Irpex* sp. 93028 was performed in HA medium as described in materials and methods. The production of 14-acetoxy-15-hydroxyirpexan (**3b**) in the mycelia started after 15 hours and peaked after nearly 75 hours, as detected by HPLC. The fermentation was determined after 90 hours when maltose and glucose were used up and the concentration of 14-acetoxy-15-hydroxyirpexan (**3b**) started to decrease. The culture broth did not contain antibiotic activity and was discarded. The active compounds were isolated by activity guided fractionation as described in materials and methods. The content of the compounds **1**, **2**, **3a** and **4** were not monitored during fermentation because they were produced in too small amounts as described in the experimental section.

Structure Determination of the Irpexans

The structure determination of the irpexans was based on data obtained by NMR spectroscopy (see Table 2 for ^1H NMR data, Table 3 for ^{13}C NMR data and Figure 2 for HMBC correlations observed with compound **3c**) and mass spectrometry (see Table 1). The five natural products all gave NMR spectra with relatively poor resolution, and

Table 2. ^{1}H (500 MHz) NMR data (δ; multiplicity; J) for irpexan (**1**), 14,15-irpexanoxide (**2**), 14,15-dihydroxyirpexan (**3a**), 14-acetoxy-15-hydroxyirpexan (**3b**) and 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**), in $CDCl_3$ with the $CHCl_3$ signal (7.26 ppm) as reference.

H	**1**	**2**	**3a**	**3b**	**4**
1	1.67; s	1.68; s	1.67; s	1.66; s	1.68; s
3	5.09; m	5.08; m	5.09; m	5.08; m	5.10; m
4	2.05; m	2.06; m	2.04; m	2.04; m	2.04; m
5	1.98; m	1.97; m	1.96; m	1.96; m	1.96; m
7	5.09; m	5.08; m	5.09; m	5.08; m	5.10; m
8	2.05; m	2.06; m	2.04; m	2.04; m	2.12/2.00; m
9	1.56/1.32; m	1.60/1.30; m	1.59/1.31; m	1.60/1.28; m	1.62/1.29; m
11	3.54; m	3.65; m	3.29; m	3.56; m	3.59; m
12	1.53; m	1.68; m	1.50; m	1.45; m	1.54/1.42; m
13	2.05; m	1.76/1.44; m	1.84; m	1.79/1.60;m	1.78/1.73; m
14	5.09; m	2.68; dd; 3.6, 7.6	3.46; m	4.85; dd; 1, 9.9	4.86; m
16	1.98; m	1.43; m	1.41; m	1.43; m	1.45; m
17	2.05; m	2.06; m	2.04; m	2.04; m	2.04; m
18	5.09; m	5.08; m	5.09; m	5.08; m	5.10; m
20	1.98; m	1.97; m	1.96; m	1.96; m	1.92; m
21	2.05; m	2.06; m	2.04; m	2.04; m	1.45; m
22	5.09; m	5.08; m	5.09; m	5.08; m	1.41; m
24	1.67; s	1.68; s	1.67; s	1.66; s	1.20; s
25	1.59; s	1.61; s	1.60; s	1.59; s	1.60; s
26	1.59; s	1.61; s	1.60; s	1.59; s	1.60; s
27	1.12; s	1.17; s	1.16; s	1.11; s	1.12; s
28	1.59; s	1.26; s	1.21; s	1.15; s	1.16; s
29	1.59; s	1.61; s	1.60; s	1.59; s	1.60; s
30	1.59; s	1.61; s	1.60; s	1.59; s	1.20; s
1'	4.52; s	4.56; s	4.55; s	4.59; s	4.60; s
2'	4.04; s	4.03; s	4.17; s	4.10; s	4.09; s
3'	3.50; m	3.57;m	3.61; m	3.58; m	3.57; m
4'	3.86; m	3.90; m	3.89; m	3.85; m	3.85; m
5'	3.20; m	3.22; m	3.20; m	3.23; m	3.25; m
6'	3.86; m	3.94/3.79; m	3.89/3.80	3.85; m	3.85; m
Ac	-	-	-	2.07; s	2.09; s

The coupling constants J are given in Hz.

signal overlap between most of the signals made coupling constants difficult to determine. The best NMR data were actually obtained with the acetylated derivative **3c**, obtained from 14-acetoxy-15-hydroxyirpexan (**3b**) which could be isolated in reasonable amounts from the extracts. High resolution measurements of the molecular ion obtained with FAB ionisation of **3c** suggested that the composition of the compound is $C_{46}H_{74}O_{14}$, and this composition is in agreement with the 1D NMR data. Due to the similarity of the two ends of the triterpene chain only 44 of the 46 carbon signals could be observed in the ^{13}C NMR spectrum. The ^{1}H NMR spectrum reveals the presence of 5 acetyl groups, as the signals for all acetyl CH_3 groups were well separated. As mentioned above, many of the ^{1}H signals overlap, but it was still possible to determine the chemical shifts for all protons from the data in the COSY spectrum, and thereby the short- and long-range ^{1}H-^{13}C correlations observed in HMQC and HMBC spectra (see Figure 2) facilitated the structure determination. The presence of a monosaccharide unit was suggested by the correlations shown in Figure 2, and 4 of the 5 acetyl groups was found to be attached to this sugar. The ^{1}H-^{1}H coupling constants of the sugar ring protons, both $J_{3'-4'}$ and $J_{4'-5'}$ are approximately 10 Hz while $J_{1'-2'}$ was less than 1 Hz and $J_{2'-3'}$ 3.2 Hz, suggested that 3′-H. 4′-H and 5′-H are axial, while at least 2′-H is equatorial. NOESY correlations

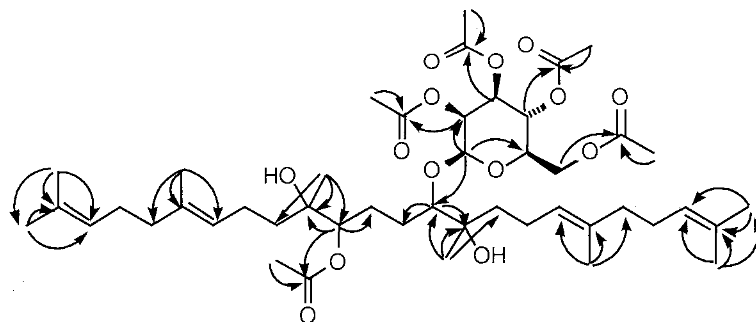
Table 3. ^{13}C (125 MHz) NMR data (δ; multiplicity) for irpexan (**1**), 14,15-irpexanoxide (**2**), 14,15-dihydroxyirpexan (**3a**), 14-acetoxy-15-hydroxyirpexan (**3b**) and 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**), in $CDCl_3$ with the $CDCl_3$ signal (77.0 ppm) as reference.

C	1	2	3a	3b	4
1	25.3; q	25.7; q	25.5; q	25.4; q	25.7; q
2	131.2; s	131.3; s	131.3; s	131.3; s	131.2; s
3	124.3; d	124.2; d	124.3; d	124.4; d	124.4; d
4	26.8; t	26.8; t	26.8; t	26.8; t	26.8; t
5	39.7; t	39.8; t	39.6; t	39.7; t	39.8; t
6	136.1; s	135.7; s	135.6; s	135.6; s	136.6; s
7	124.7; d	124.6; d	124.5; d	124.6; d	124.7; d
8	21.8; t	21.8; t	21.7; t	21.8; t	21.8; t
9	35.3; t	35.5; t	35.9; t	35.3; t	35.5; t
10	74.1; s	73.9 s	73.8; s	73.8; s	74.0; s
11	88.5; d	87.2; d	87.9; d	88.0; d	88.1; d
12	31.6; t	28.5; t	27.3; t	27.3; t	27.1; t
13	25.6; t	26.0; t	25.9; t	25.7; t	25.3; t
14	124.0; d	63.4; d	76.5; d	77.5; d	77.4; d
15	134.0; s	60.6; s	74.2; s	74.0; s	73.9; s
16	39.7; t	38.7; t	38.7; t	38.8; t	38.8; t
17	26.6; t	23.7; t	21.8; t	21.8; t	21.9; t
18	123.3; d	123.4; d	123.9; d	123.9; d	124.1; d
19	135.1; s	135.0; s	135.0; s	135.0; s	135.0; s
20	39.7; t	39.7; t	39.8; t	39.8; t	39.8; t
21	26.8; t	26.7; t	26.8; t	26.7; t	22.5; t
22	124.4; d	124.4; d	124.2; d	124.3; d	43.3; t
23	131.2; s	131.4; s	131.2; s	131.2; s	71.3; s
24	25.3; q	25.7; q	25.4; q	25.4; q	29.2; q
25	17.6; q	17.7; q	17.6; q	17.7; q	17.7; q
26	16.0; q	16.0; q	16.1; q	16.0; q	16.0; q
27	23.6; q	23.9; q	23.7; q	23.7; q	23.7; q
28	16.0; q	16.7; q	22.5; q	22.5; q	22.6; q
29	16.0; q	16.0; q	16.1; q	16.0; q	16.0; q
30	17.6; q	17.7; q	17.7; q	17.7; q	29.2; q
1'	100.5; d	100.1; d	101.0; d	100.9; d	101.0; d
2'	71.2; d	71.1; d	71.2; d	71.3; d	71.1; d
3'	73.8; d	73.9; d	74.0; d	74.0; d	74.0; d
4'	66.5; d	66.2; d	66.3; d	66.2; d	66.5; d
5'	76.3; d	76.2; d	76.2; d	76.3; d	76.4; d
6'	60.8; t	60.7; t	61.5; t	60.9; t	61.6; t
14-Ac	-	-	-	171.0; s	171.0; s
-	-	-	-	21.0; q	21.0; q

between 1′-H and 3′-H as well as 5′-H show that 1′-H in fact is axial as well, and that the sugar unit is β-mannose. The β configuration of the anomeric carbon was further supported by the heteronuclear coupling constant between 1′-H and C-1′, which is 158 Hz. In Figure 1 and Figure 2 it is shown as β-D-mannose, but the absolute configuration was not determined.

The HMBC correlations between 1′-H and C-11 as well as between 11-H and C-1′ demonstrated the point of attachment of the monosaccharide to the triterpene, and HMBC correlations from the two central methyl groups (from 27-H_3 to C-9, C-19 and C-11, and from 28-H_3 to C-14, C-15 and C-16) as well as COSY correlations in the spin system C-11/C-12/C-13/C-14 gave the structure of the segment C-9 to C-16. The presence of a 2-methylpropene unit substituted in position 1 at both ends of the triterpene was demonstrated by the typical shifts observed and by the HMBC correlations from both 1-H_3 and 25-H_3 to C-2 and C-3, as well as the correlations from 1-H_3 to C-25 and from 25-H_3 to C-1 (the corresponding correlations were observed

Fig. 2. Pertinent HMBC correlations observed with compound **3c** in $CDCl_3$.



for 24-H_3 and 30-H_3). The two remaining methyl groups, C-26 and C-29, are part of two additional trisubstituted double bonds as demonstrated by the HMBC correlations from 26-H_3 to C-5, C-6 and C-7, and from 29-H_3 to C-18, C-19 and C-20. Finally, COSY correlations between the methylene protons 4-H_2 and 5-H_2, between 8-H_2 and 9-H_2, between 16-H_2 and 17-H_2, and between 20-H_2 and 21-H_2, connect the different substructures to compound **3c**. The sterochemistry of the oxygenated carbons could not be determined.

The transesterification of the tetraacetate **3c** in ethanol containing sodium ethoxide yielded 14,15-dihydroxy-irpexan (**3a**), identical in all detail with the compound isolated from the fungus. The position of the acetoxy group in 14-acetoxy-15-hydroxyirpexan (**3b**) could be established by the HMBC correlations between 14-H and the carbonyl carbon, and by the differences in the ^{1}H NMR shifts of the monosaccharide moiety compared to the acetate **3c**. The HRMS data of irpexan (**1**) suggest that it's composition is $C_{36}H_{62}O_7$, thus having an additional unsaturation compared to 14,15-dihydroxyirpexan (**3a**). The NMR spectra of **1** are similar to those of **3a**, but besides the monosaccharide and C-10 and C-11 no other oxygenated carbons are present. Instead, the increased integral of the signal for the olefinic protons and the methyl groups attached to a double bond show that irpexan (**1**) has a double bond between C-14 and C-15, and the carbon shift for C-28 shows that this double bond has an *E* configuration (as the shifts of C-26 and C-29 in all compounds demonstrate that both the C-6/C-7 and the C-18/C-19 double bonds in general are *E*). 14,15-Irpexanoxide (**2**) is oxidised compared to irpexan (**1**), with the chemical composition $C_{36}H_{62}O_8$. The chemical shifts of 14-H/C-14 and 15-H/C-15 indicate that one double bond of irpexan (**1**) has been epoxidised, and COSY/HMBC correlations form 14-H/C-14 via the $-CH_2-CH_2-$ linker to 11-H/C-11 demonstrate the position of the epoxide ring. Finally, the chemical composition of 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**) ($C_{38}H_{68}O_{11}$) and the differences in the NMR spectra suggested that had been formed by adding water to one of the double bonds of 14-acetoxy-15-hydroxyirpexan (**3b**). The position of the additional hydroxyl group at either C-2 or C-23 was indicated by the changes of the ^{1}H shifts of 24-H_3 and 30-H_3, together with the HMBC correlations from these two methyl groups to a quaternary hydroxylated carbon and a CH_2 carbon, and detailed analysis of COSY as well as HMBC correlations established that the structure of **4** is as suggested in Figure 1.

Biological Properties

14-Acetoxy-15-dihydroxyirpexan (**3b**) inhibited the phorbol ester TPA induced expression of the AP-1 dependent reporter gene (pGE2-AP1) and NF-κB dependent reporter gene with IC_{50} values of 6~7 μg/ml followed by 14,15-irpexanoxide (**2**), 14,15-dihydroxyirpexan (**3a**), and 14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**) whereas irpexan (**1**) exhibited no activity (Table 4). All isolated compounds showed no antibacterial and antifungal activities. Cytotoxic effects were observed starting at 20 μg/ml.

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Table 4. IC_{50} values for reporter gene assays (pGE2-AP1, pGE2-NF1) with COS-7 cells and cytotoxic activities on COS-7 cells after 48 hours incubation with irpexans.

	Reporter gene assays pGE2-AP1, pGE2-NF1; COS-7	Cytotoxic activity COS-7
Compound	IC_{50} values [μg/ml]	
Irpexan (**1**)	> 50	> 100
14,15-Irpexanoxide (**2**)	10 - 15	30 - 35
14,15-Dihydroxyirpexan (**3a**)	15 - 20	30 - 40
14-acetoxy-15-dihydroxyirpexan (**3b**)	6 - 7	20
14-acetoxy-22,23-dihydro-15,23-dihydroxyirpexan (**4**)	30 - 40	70 - 80

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