New Tryptophan Metabolites from Cultures of the Lipophilic Yeast *Malassezia furfur*

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Dedicated to Professor *Rolf Huisgen* on the occasion of his 85th birthday

Eleven new indole alkaloids were isolated from cultures of the human pathogenic yeast *Malassezia furfur* after addition of L-tryptophan as the sole N-source: pityriacitrin B (**2**), the malassezindoles A (**3**) and B (**4**), malassezialactic acid (**6**), the malasseziazoles A (**7**), B (**8**), and C (**9**), pityriazole (**10**), malasseziacitrin (**11**), and malassezione (**12**), along with the known D-indole-3-lactic acid (=(αR)-α-hydroxy-1*H*-indole-3-propanoic acid **5**), and 2-hydroxy-1-(1*H*-indol-3-yl)ethanone (**13**). The structural elucidation of these compounds was performed by spectroscopic methods (MS as well as 1D- and 2D-NMR). The biogenetic relationships (*Scheme*) and biological activities of the new metabolites are discussed.

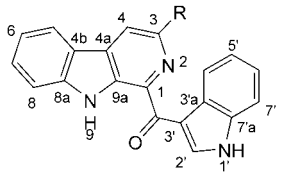
1. Introduction. – Lipophilic yeasts of the genus *Malassezia* belong to the resident flora of the human skin [1]. Of special importance is *M. furfur* that is considered as one of the pathogenic agents of pityriasis versicolor (='Kleienpilzflechte' in German), a *Malassezia*-associated common skin disease, which is characterized by flaky lesions with a hitherto unexplained variation in color and fluorescence [2]. Addition of L-tryptophan to cultures of this yeast induces the production of numerous indole alkaloids including colored and fluorescent compounds [3]. Several of these metabolites may be connected with the pathogenesis of pityriasis versicolor.

Thus, malassezin [4], an aromatic hydrocarbon receptor (AHR) agonist [5], induces apoptosis of melanocytes [6] and may be responsible for the development of depigmentations on the skin of certain pityriasis patients. Pityriacitrin (**1**) [7] has strong UV-protecting properties and may thus prevent lesions of such depigmented areas from UV damage. Bis(indolyl)spiranes like pityriarubin A [8] suppress the 'oxidative burst' of human granulocytes, and their presence *in vivo* could thus explain the lack of inflammation in diseased parts of the skin [9]. The pityriarubins are accompanied by the biosynthetically related pityrianhydride and pityrialactone [8][10]. The latter exhibits a strongly yellowish-green fluorescence which resembles that used for the diagnosis of pityriasis versicolor.

In this work, we describe further indole metabolites from cultures of this remarkable fungus. The compounds were obtained by extraction of the culture medium with AcOEt followed by chromatography on a *Sephadex LH-20* column. The

crude fractions were then subjected to repeated TLC on silica gel followed by careful HPLC of the individual zones.

2. Results and Discussion. – The yellow pityriacitrin B (**2**), showed a characteristically broad UV/VIS spectrum, closely similar to that of the UV-protecting agent pityriacitrin (**1**) [7]. The HR-EI-MS of **2** (*m/z* 355.0955) indicated the molecular formula $C_{21}H_{13}N_3O_3$, and a strong $[M-CO_2]^+$ ion at *m/z* 311 was in accord with the presence of a carboxy group. From the NMR data (*Table 1*), including those derived through HMBC and HSQC experiments, structure **2** could be assigned to pityriacitrin B.



1 R = H
2 R = COOH

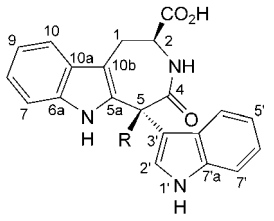
Table 1. *^{1}H- and ^{13}C-NMR Data* (CD_3OD) *for Compound* **2**[a]). At 600 (^{1}H) and 151 MHz (^{13}C)[b]); δ in ppm, *J* in Hz.

	δ(H)	δ(C)[c])		δ(H)	δ(C)[c])
C(1)	–	141.0 (*s*)	*C*O–C(1)	–	190.1 (*s*)
C(3)	–	144.7 (*s*)	CO_2H	–	174.6 (*s*)
H–C(4)	8.99 (br. *s*)	119.6 (*d*)	H–C(2′)	9.60 (br. *s*)	138.0 (*d*)
C(4a)	–	133.3 (*s*)	C(3′)	–	116.6 (*s*)
C(4b)	–	118.4 (*s*)	C(3′a)	–	129.3 (*s*)
H–C(5)	8.29 (*d*, *J* = 6.9)	122.9 (*d*)	H–C(4′)	8.61 (br. '*s*')	123.7 (*d*)
H–C(6)	7.36 (*m*)	121.7 (*d*)	H–C(5′)	7.27 (br. '*s*')	123.4 (*d*)
H–C(7)	7.62 (*m*)	130.2 (*d*)	H–C(6′)	7.27 (br. '*s*')	124.3 (*d*)
H–C(8)	7.74 (*d*, *J* = 7.8)	113.8 (*d*)	H–C(7′)	7.51 (br. '*s*')	113.0 (*d*)
C(8a)	–	143.7 (*s*)	C(7′a)	–	139.0 (*s*)
C(9a)	–	137.8 (*s*)			

[a]) Assignments were made by 2D ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Chemical shifts relative to MeOH (δ(H) 3.35) and CD_3OD (δ(C) 49.3. [c]) By DEPT sequence.

In the ^{1}H- and ^{13}C-NMR spectra of **2**, signals for a 3-monosubstituted and a 2,3-disubstituted 1*H*-indole system could be identified. In (D_6)DMSO, H–C(2′) appeared as *d* at δ(H) 9.72 (*J* = 2.7 Hz) indicating a 3-acyl-1*H*-indole chromophore [7][11]. In accord with structure **2**, H–C(4) (δ(H) 8.99) exhibited HMBC correlations with CO_2H at δ(C) 174.6 as well as with C(5) (δ 122.9) and C(9a) (δ 137.8).

The malessezindoles A (**3**) and B (**4**) represent an interesting new type of indole alkaloid with as yet unknown biological properties. Malassezindole A (**3**), obtained as an optically active colorless powder, had the molecular formula $C_{21}H_{17}N_3O_3$, as determined by HR-ESI-MS (positive-ion mode) of the $[M+1]^+$ ion at 360.1363. From a full assignment of the ^{1}H- and ^{13}C-NMR signals (*Table 2*) by HMBC, HSQC, and ^{1}H,^{1}H-COSY experiments, structure **3** could be established for malassezindole A.



3 R = H
4 R = OH

Table 2. *^{1}H- and ^{13}C-NMR* (CD_3OD) *Data for Compounds* **3** and **4**[a]). At 600 (^{1}H) and 151 MHz (^{13}C)[b]); δ in ppm, J in Hz.

	3		**4**	
	δ(H)	δ(C)[c])	δ(H)	δ(C)[c])
CH_2(1)	2.95 ('*dd*', J = 16.1, 12.2)	31.7 (*t*)	2.91 (*dd*, J = 16.0, 13.0)	31.4 (*t*)
	3.58 ('*d*', J = 16.1)		3.50 (*d*, J = 16.0)	
H–C(2)	4.55 ('*d*', J = 12.2)	55.2 (*d*)	4.28 (*d*, J = 13.0)	56.0 (*d*)
C(4)	–	175.0 (*s*)	–	175.3 (*s*)
R–C(5)	5.34 (*s*)	48.0 (*d*)	–	73.5 (*s*)
C(5a)	–	130.1 (*s*)	–	133.7 (*s*)
C(6a)	–	137.5 (*s*)	–	136.7 (*s*)
H–C(7)	7.36 (*d*, J = 7.7)	112.1 (*d*)	7.47 (*d*, J = 7.7)	112.5 (*d*)
H–C(8)	7.16 (*m*)	123.1 (*d*)	7.19 (*m*)	123.1 (*d*)
H–C(9)	7.08 (*m*)	120.6 (*d*)[d])	7.09 (*m*)	120.3 (*d*)
H–C(10)	7.52 (*d*, J = 7.7)	119.3(*d*)	7.51 (*d*, J = 7.7)	119.4 (*d*)
C(10a)		130.3 (*s*)		130.1 (*s*)
C(10b)		110.5 (*s*)		108.4 (*s*)
H–C(2′)	6.69 (*s*)	125.2 (*d*)	6.77 (*s*)	126.8 (*d*)
C(3′)	–	114.0 (*s*)	–	119.0 (*s*)
C(3′a)	–	127.6 (*s*)	–	126.0 (*s*)
H–C(4′)	7.73 (*d*, J = 8.0)	120.3 (*d*)[d])	7.56 (*d*, J = 7.8)	120.7 (*d*)[d])
H–C(5′)	7.03 (*m*)	120.4 (*d*)[d])	7.01 (*m*)	120.8 (*d*)[d])
H–C(6′)	7.14 (*m*)	123.3 (*d*)	7.13 (*m*)	123.2 (*d*)
H–C(7′)	7.37 (*d*, J = 8.0)	112.8 (*d*)	7.36 (*d*, J = 7.8)	112.8 (*d*)
C(7′a)	–	138.9 (*s*)	–	138.9 (*s*)
CO_2H	–	177.2 (*s*)	–	176.8 (*s*)

[a]) Assignments were made by 2D ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Chemical shifts relative to MeOH (δ(H) 3.35) and CD_3OD (δ(C) 49.3). [c]) By DEPT sequence. [d]) Assignments interchangeable.

The NMR spectra of **3** as well as the data derived from HMBC and HSQC experiments revealed the presence of two 1*H*-indole systems, one doubly substituted at C(2) and C(3), the other 3-monosubstituted. In addition to the 1*H*-indole signals, the ^{1}H-NMR spectra (CD_3OD) showed signals for an aliphatic *ABX* system ($\delta(H_A)$ 2.95, $\delta(H_B)$ 3.58, $\delta(H_X)$ 4.55; J_{AB} = 16.1, J_{AX} = 12.2, $J_{BX} \approx 0$ Hz) and a *s* at δ(H) 5.34, connected in the ^{1}H,^{1}H-COSY spectrum by a ^4J(H,H) coupling with the 1*H*-indole proton at C(2′) (δ(H) 6.69). In the ^{13}C-NMR spectrum (CD_3OD), 16 1*H*-indole signals were visible as well as signals for one CH_2 (δ 31.7), two CH (δ 55.2, 48.0), one CO_2H (δ 177.2), and one CONH group (δ 175.0). The *s* for H–C(5) appeared at δ(H) 5.34 and exhibited correlations to the neighboring amide C-atom C(4) as well as to C(5a), C(10b), C(2′), and C(3′) of the attached 1*H*-pyrrole rings.

Feeding of [1′-^{13}C]-DL-tryptophan to cultures of *M. furfur* [8] resulted in the formation of racemic **3** with 67.1% incorporation of label into the CO_2H group. According to this finding, the malassezindole system can be produced either from L- or DL-tryptophan yielding the (2*S*)- or (2*RS*)-compounds, respectively. The relative

configuration of malassezindole A was determined by a NOESY experiment at 240 K, in which a strong correlation between H–C(2) and H–C(2′) at the opposite 1*H*-indole substituent was observed. This established the configuration given in formula **3**, in which H–C(2) and the 1*H*-indol-3-yl group occupy positions on the same side of the lactam ring. Molecular-modeling calculations [12] led to a low-energy conformation (*Fig. 1*) in which H_α–C(1) and H_α–C(2) adopt a dihedral angle near 90°, whereas H_β–C(1) and H_α–C(2) are in a pseudo-diaxial relationship, in accord with the observed coupling constants for the *ABX* system.

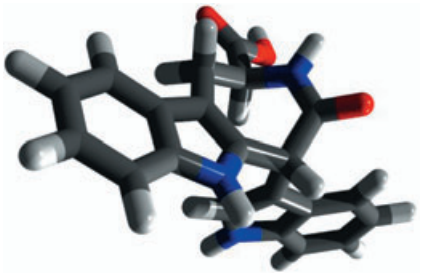


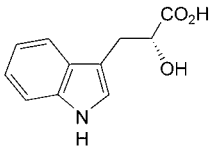
Fig. 1. *Molecular model of the most-stable conformation for compound* **3** *according to force-field calculations* [12]

The molecular formula of malassezindole B (**4**), $C_{21}H_{17}N_3O_4$, was determined by an accurate mass measurement of the $[M + HCO_2H - H]^-$ ion appearing at *m/z* 420.1203 in the ESI-MS (negative-ion mode). The compound exhibited ^{1}H- and ^{13}C-NMR properties similar to those exhibited by congener **3**, including the magnitude of the coupling constants of the *ABX* system. Compared to **3**, malassezindole B lacked the characteristic *s* at δ(H) 5.34 in the ^{1}H-NMR spectrum, and, instead of the corresponding ^{13}C-NMR signal at δ(C) 48.0, a quaternary C-signal at δ(C) 73.5 was visible. The additional O-atom must, therefore, be attached to C(5), which established structure **4** for malassezindole B. The correspondence of the CD curves for both malessezindoles pointed to the same configuration. Unfortunately, the minute amount of **4** available precluded the acquisition of a NOESY spectrum.

One of the major metabolites of *M. furfur* was identified as the known D-indole-3-lactic acid (=(αR)-α-hydroxy-1*H*-indole-3-propanoic acid; **5**) [13]. It is accompanied by the structurally related malassezialactic acid (**6**), the structure of which followed from the NMR data given in *Table 3* as well as from HMBC, HSQC, and ^{1}H,^{1}H-COSY experiments. In keeping with the spectral properties of other 2,3′-methylenebis[1*H*-indole] derivatives, the ^{13}C-NMR signal of the bridging CH_2 group occurred at unusual high field (δ(C) 23.7) [4]. The fact that D-indole-3-lactic acid (**5**) and malassezialactic acid (**6**) both showed a negative optical rotation suggested that each possesses the (*R*)-configuration at C(α).

Interestingly, **6** resembles 2-(1*H*-indol-3-ylmethyl)tryptophan (IMT), which has been identified as a toxic impurity associated with the eosinophilia myalgia syndrome in commercial L-tryptophan [14]. IMT is formed by reaction of 1*H*-indole-3-methanol with tryptophan [15], which suggests a similar origin of **6** from 1*H*-indole-3-methanol and D-indole-3-lactic acid (**5**). The 1*H*-indole-3-carboxaldehyde, a possible precursor of 1*H*-indole-3-methanol, has been isolated before from cultures of *M. furfur* [3].

Another group of *Malassezia* metabolites are the malasseziazoles A–C (**7**–**9**). Malasseziazole A (**7**), obtained as a yellow solid, was determined to have the molecular



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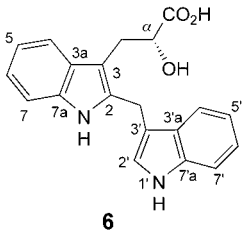

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Table 3. *^{1}H- and ^{13}C-NMR* (CD_3OD) *Data for Compound* **6**[a]. At 600 (^{1}H) and 151 MHz (^{13}C)[b]); δ in ppm, J in Hz.

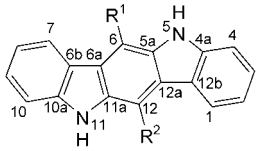
	δ(H)	δ(C)[c]		δ(H)	δ(C)[c]
C(2)	–	137.9 (*s*)	H–C(α)	4.40 (*dd*, *J* = 7.7, 5.2)	73.1 (*d*)
C(3)	–	107.4 (*s*)	CO_2H	–	178.2 (*s*)
C(3a)	–	130.5 (*s*)	H–C(2′)	7.01 (*s*)	124.3 (*d*)
H–C(4)	7.55 (*d*, *J* = 7.3)	119.3 (*d*)	C(3′)	–	114.1 (*s*)
H–C(5)	6.96 (*m*)	119.8 (*d*)	C(3′a)	–	129.0 (*s*)
H–C(6)	6.96 (*m*)	121.7 (*d*)	C(4′)	7.46 (*d*, *J* = 8.3)	119.8 (*d*)[d])
H–C(7)	7.19 (*d*, *J* = 7.3)	111.8 (*d*)	C(5′)	6.96 (*m*)	119.9 (*d*)[d])
C(7a)	–	137.5 (*s*)	C(6′)	7.06 (*m*)	122.6 (*d*)
CH_2–C(2)	4.27 (*AB* (‘*d*’), *J* = 16.0)	23.7 (*t*)	C(7′)	7.32 (*d*, *J* = 8.3)	112.4 (*d*)
	4.28 (*AB* (‘*d*’), *J* = 16.0)				
CH_2(β)	3.16 (*dd*, ‘*J*’ = 14.5, 7.7)	31.4 (*t*)	C(7′a)	–	138.6 (*s*)
	3.34 (*dd*, *J* = 14.5, 5.2)				

[a]) Assignments were made by 2D ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Chemical shifts relative to MeOH (δ(H) 3.35) and CD_3OD (δ(C) 49.3). [c]) By DEPT sequence. [d]) Assignments interchangeable.

formula $C_{20}H_{12}N_2O_3$ by virtue of an exact mass measurement of the $[2M-H]^-$ ion appearing at *m/z* 665.1623 in the ESI-MS (negative-ion mode). In the EI-MS, the M^+ ion was absent, and only an intense fragment ion arising from a consecutive loss of CO_2 and CO was observed at *m/z* 256. The NMR spectra (*Table 4*) showed signals for two 2,3-disubstituted 1*H*-indole moieties interconnected by a $C_4H_2O_3$ unit containing a $-C(=O)CO_2H$ group. By means of the HMBC correlations, malasseziazole A could be identified as 5,11-dihydro-α-oxoindolo[3,2-*b*]carbazole-6-acetic acid (**7**).

Of special value were the correlations between H–C(12) and C(5a), C(6), C(6a), and C(12b) of **7**. The NMR data of **7** were in excellent agreement with those of 5,11-dihydroindolo[3,2-*b*]carbazole-6-carboxaldehyde, a potent synthetic AHR receptor agonist described by *Bergman* and co-workers [16].

Malasseziazole B (**8**), obtained as a red solid, had the molecular formula $C_{21}H_{12}N_2O_4$, as determined by HR-ESI-MS (*m/z* 355.0707, $[M-H]^-$). The NMR data (*Table 4*) suggested a derivative of indolo[3,2-*b*]carbazole, and, by analysis of the HMBC correlations, structure **8** could be established for malasseziazole B. The aldehyde proton at δ(H) 11.55 exhibited correlations with C(11a), C(12), and C(12a), which allowed the full assignment of the ^{13}C-NMR signals.



7 R^1 = COCOOH, R^2 = H
8 R^1 = COCOOH, R^2 = CHO
9 R^1 = COOH, R^2 = CHO

Table 4. *^{1}H- and ^{13}C-NMR* (CD_3OD) *Data for Compounds* **7**–**9**[a]). At 600 (^{1}H) and 151 MHz (^{13}C)[b]); δ in ppm, J in Hz.

	7		**8**		**9**	
	δ(H)	δ(C)[c])	δ(H)	δ(C)[c])	δ(H)	δ(C)[c])
H–C(1)	8.19 (*d*, *J* = 8.3)	121.2 (*d*)	8.44 (*d*, *J* = 7.9)	125.2 (*d*)	8.69 (*d*, *J* = 7.8)	125.0 (*d*)
H–C(2)	7.24 (*m*)	120.3 (*d*)	7.23 (*m*)	120.8 (*d*)	7.23 (*m*)	120.7 (*d*)
H–C(3)	7.44 (*m*)	127.5 (*d*)	7.49 (*m*)	128.2 (*d*)	7.45 (*m*)	127.5 (*d*)
H–C(4)	7.57 (*d*, *J* = 8.3)	112.2 (*d*)	7.68 (*d*, *J* = 7.5)	112.7 (*d*)	7.64 (*d*, *J* = 8.2)	112.3 (*d*)
C(4a)	–	142.8 (*s*)	–	143.7 (*s*)	–	143.4 (*s*)
C(5a)	–	138.3 (*s*)	–	135.6 (*s*)	–	134.2 (*s*)
C(6)	–	112.8 (*s*)	–	112.6 (*s*)	–	126.6 (*s*)
C(6a)	–	122.5 (*s*)	–	125.1 (*s*)	–	124.5 (*s*)
C(6b)	–	123.9 (*s*)	–	122.5 (*s*)	–	122.9 (*s*)
H–C(7)	8.77 (*d*, *J* = 8.3)	126.6 (*d*)	8.48 (*d*, *J* = 7.9)	125.6 (*d*)	8.43 (*d*, *J* = 7.8)	125.3 (*d*)
H–C(8)	7.14 (*m*)	119.3 (*d*)	7.30 (*m*)	121.1 (*d*)	7.26 (*m*)	120.5 (*d*)
H–C(9)	7.44 (*m*)	127.6 (*d*)	7.54 (*m*)	128.5 (*d*)	7.50 (*m*)	127.9 (*d*)
H–C(10)	7.50 (*d*, *J* = 8.3)	111.6 (*d*)	7.67 (*d*, *J* = 7.5)	113.1 (*d*)	7.66 (*d*, *J* = 8.2)	113.0 (*d*)
C(10a)	–	143.6 (*s*)	–	143.7 (*s*)	–	143.5 (*s*)
C(11a)	–	137.4 (*s*)	–	137.1 (*s*)	–	138.4 (*s*)
H–C(12)	8.40 (*s*)	108.4 (*d*)	–	116.0 (*s*)	–	113.5 (*s*)
C(12a)	–	125.4 (*s*)	–	122.8 (*s*)	–	121.2 (*s*)
C(12b)	–	123.9 (*s*)	–	122.4 (*s*)	–	123.1 (*s*)
CO	–	197.9 (*s*)	–	198.6 (*s*)	–	–
CO_2H	–	175.8 (*s*)	–	172.6 (*s*)	–	174.7 (*s*)
CHO	–	–	11.55 (*s*)	192.3 (*d*)[d])	11.44 (*s*)	191.8 (*d*)[e])

[a]) Assignments were made by 2D ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Chemical shifts relative to MeOH (δ(H) 3.35) and CD_3OD (δ(C) 49.3). [c]) By DEPT sequence. [d]) ^2J(H,C) = 23.8 Hz. [e]) ^2J(H,C) = 23.3 Hz.

The orange-red malasseziazole C (**9**), $C_{20}H_{12}N_2O_3$, is closely related to **8**, and from the ^{1}H- and ^{13}C-NMR spectra (*Table 4*), HSQC, HMBC experiments, and by comparison of the spectral data with those of **8**, structure **9** could be assigned to the new metabolite.

The close structural resemblance of the malasseziazoles A–C (**7**–**9**) to synthetic indolo[3,2-*b*]carbazoles [16], which are potent agonists of the aromatic hydrocarbon receptor, suggests a similar biological activity for these natural products. Experiments to test this possibility are under way (induction of cytochrome P450 isoenzymes, EROD activity).

The colorless pityriazole (**10**) represents a different type of carbazole alkaloid. An accurate mass measurement on the ion appearing at *m/z* 341.0933 ($[M-1]^-$) in the ESI-MS (negative-ion mode) established the molecular formula $C_{21}H_{14}N_2O_3$. From

the ^{1}H-NMR data (*Table 5*) as well as various HMBC and HSQC correlations, structure **10** could be assigned to pityriazole.

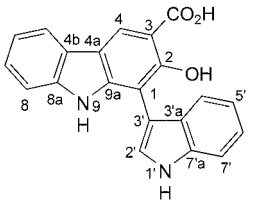


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Table 5. *^{1}H- and ^{13}C-NMR* ((D$_6$)DMSO) *Data for Compound* **10**[a]. At 600 (^{1}H) and 151 MHz (^{13}C)[b]); δ in ppm, *J* in Hz.

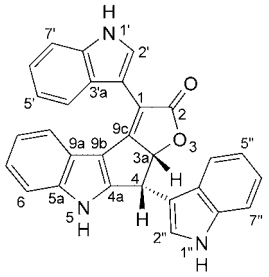
	δ(H)	δ(C)[c])		δ(H)	δ(C)[c])
C(1)	–	104.9[d])	CO_2H	–	172.1
C(2)	–	160.3	H–C(2′)	7.47 (*s*)	124.7
C(3)	–	104.9[d])	C(3′)	–	109.3
H–C(4)	8.40 (*s*)	122.8	C(3′a)	–	127.1
C(4a)	–	112.7	H–C(4′)	7.30 (*d*, *J* = 7.8)	121.0
C(4b)	–	124.3	H–C(5′)	6.94 (*m*)	118.4
H–C(5)	7.89 (*d*, *J* = 7.4)	118.4	H–C(6′)	7.09 (*m*)	121.0
H–C(6)	7.02 (*m*)	118.4	H–C(7′)	7.45 (*d*, *J* = 8.3)	111.3
H–C(7)	7.13 (*m*)	122.9	C(7′a)	–	136.1
H–C(8)	7.30 (*d*, *J* = 7.8)	110.8	OH–C(2)	17.10 (br.)	
C(8a)	–	140.3	H–N(9)	10.16 (*s*)	
C(9a)	–	142.5	H–N(1′)	11.21 (*s*)	

[a]) Assignments were made by 2D ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Chemical shifts relative to DMSO (δ(H) 2.49) and (D$_6$)DMSO (δ(C) 39.7). [c]) Deduced from HMBC and HMQC experiments. [d]) Deduced from HMBC spectrum in CD_3OD; assignments interchangeable.

Once again, a 3-monosubstituted and a 2,3-disubstituted 1*H*-indole system were clearly discernible in **10**. Of special diagnostic importance were strong HMBC correlations between H–C(4) (δ 8.40) and C(2) (δ 160.3), C(4b) (δ 124.3), C(9a) (δ 142.5), and *C*O$_2$H (δ 172.1). It should be mentioned that due to the limited amount of **10** available, the ^{13}C-NMR data given in *Table 5* could only be obtained from the HMBC experiments.

The most complex of the hitherto known *Malassezia* metabolites is malasseziacitrin (**11**). The compound, obtained as a brown-red powder, showed an M^+ at *m/z* 441.1456 in the HR-EI-MS, which corresponded to the molecular formula $C_{29}H_{19}N_3O_2$. This conclusion was supported by high-resolution FAB-MS (positive-ion mode) and MALDI-MS experiments. In the ^{1}H- and ^{13}C-NMR spectra, three 1*H*-indole systems could be discerned, two of them 3-mono-, the third one 2,3-disubstituted. From HMBC correlations (*Table 6*) the 1*H*-indole moieties could be extended to partial structures **A**–**C** (*Fig. 2*), which on connection with the still unconsidered $-CO_2-$ group **D** gave structure **11** for malasseziacitrin.

Structure **11** was confirmed by the HMBC correlations (*Fig. 3*). In the NOESY plot of **11**, the protons at C(3a) and C(4) exhibited a strong correlation indicating a *cis*-relationship (*Fig. 4*). The NOEs observed between the different 1*H*-indole moieties were in excellent agreement with the minimum-energy conformation



11

Table 6. *^{1}H- and ^{13}C-NMR Data* (D_6(DMSO), *Including HMBC Correlations, for Compound* **11**[a]). At 600 (^{1}H) and 151 MHz (^{13}C)[b]); δ in ppm, J in Hz.

	δ(H)	δ(C)[c])	HMBC
C(1)	–	108.5 (*s*)	H–C(3a), H–C(2′)[g])
C(2)	–	175.0 (*s*)	H–C(3a)[f])
H–C(3a)	6.09 (*d*, *J* = 6.7)	87.3 (*d*)	H–C(4)
H–C(4)	5.33 (*d*, *J* = 6.7)	41.5 (*d*)[f])	H–C(3a), H–C(2″)[g])
C(4a)	–	156.1 (*s*)	H–C(4), H–N(5)
C(5a)	–	141.3 (*s*)	H–C(7), H–C(9)
H–C(6)	7.40 (*d*, *J* = 7.8)	113.1 (*d*)	H–C(8)
H–C(7)	7.16 (*m*)	122.8 (*d*)	H–C(9)
H–C(8)	7.01 (*m*)	121.1 (*d*)	H–C(6)
H–C(9)	7.33 (*d*, *J* = 7.8)	120.7 (*d*)[e])	H–C(7)
C(9a)	–	121.3 (*s*)[d])	H–C(6), H–C(8), H–N(5)
C(9b)	–	112.6 (*s*)	H–C(4), H–C(9), H–N(5)
C(9c)	–	161.0 (*s*)	H–C(3a), H–C(4)
H–C(2′)	7.59 (*d*, *J* = 2.5)	125.3 (*d*)	H–N(1′)
C(3′)	–	106.8 (*s*)	H–C(2′), H–C(4′)
C(3′a)	–	126.8 (*s*)	H–C(2′), H–C(5′), H–C(7′), H–N(1′)
H–C(4′)	7.66 (*d*, *J* = 7.8)	120.8 (*d*)[e])	H–C(6′)
H–C(5′)	7.01 (*m*)	119.0 (*d*)	H–C(7′)
H–C(6′)	7.17 (*m*)	121.7 (*d*)	H–C(4′)
H–C(7′)	7.48 (*d*, *J* = 7.8)	111.8 (*d*)	H–C(5′)
C(7′a)	–	136.5 (*s*)	H–C(2′), H–C(4′), H–C(6′), H–N(1′)
H–C(2″)	6.77 (*d*, *J* = 2.4)	123.6 (*d*)	H–C(4), H–N(1″)
C(3″)	–	109.7 (*s*)	H–C(4), H–C(3a), H–C(2″), H–C(4″), H–N(1″)
C(3″a)	–	126.5 (*s*)	H–C(4), H–C(2″), H–C(5″), H–C(7″)
H–C(4″)	7.26 (*d*, *J* = 8.0)	118.8 (*d*)	H–C(6″)
H–C(5″)	6.84 (*m*)	118.9 (*d*)	H–C(7″)
H–C(6″)	7.05 (*m*)	121.3 (*d*)[d])	H–C(4″)
H–C(7″)	7.34 (*d*, *J* = 8.0)	111.7 (*d*)	H–C(5″)
C(7″a)	–	136.3 (*s*)	H–C(2″), H–C(6″), H–C(4″), H–N(1″)
H–N(5)	12.18		
H–N(1′)	11.42		
H–N(1″)	10.97 (*s*)		

[a]) Assignments were made by 2D ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Chemical shifts relative to DMSO (δ(H) 2.49) and (D_6)DMSO (δ(C) 39.7). [c]) By DEPT sequence. [d]) Assignments interchangeable. [e]) Assignments interchangeable. [f]) Value from spectrum in CD_3OD (in (D_6)DMSO obscured by solvent signal). [g]) Correlation only visible in CD_3OD.

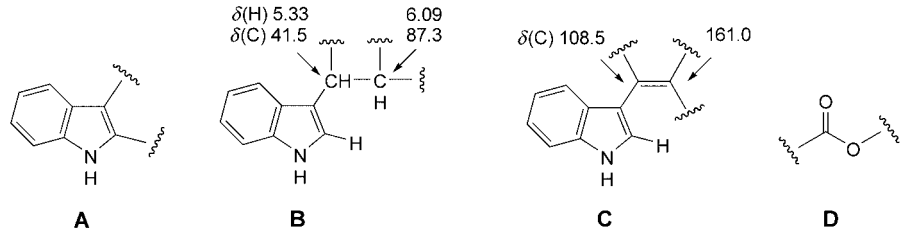


Fig. 2. *Partial structures* **A**–**D** *of malasseziacitrin* (**11**)

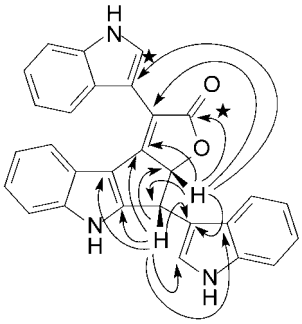


Fig. 3. *Selected HMBC correlations* ((D$_6$)DMSO) *for compound* **11**. * = Only visible in CD_3OD.

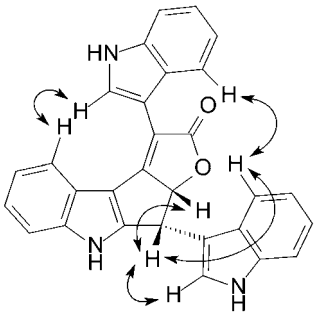


Fig. 4. *Selected NOE correlations for compound* **11**

of **11** (*Fig. 5*), obtained by force-field calculations [12]. Malasseziacitrin (**11**) was optically inactive. The possibility that the compound possesses a small optical rotation that could not be determined with the minute amount of material at hand cannot be excluded.

The colorless malassezione (**12**) showed, in the HR-EI-MS, an M^+ at *m/z* 288.1282, which corresponds to the molecular formula $C_{19}H_{16}N_2O$. The ^{1}H- and ^{13}C-NMR spectra of **12** only contained signals for a CH_2 group and a 1*H*-indol-3-yl moiety, suggesting a symmetrical structure. Considering the molecular formula, malassezione could be identified as the hitherto unknown 1,3-di(1*H*-indol-3-yl)propan-2-one (**12**).

Due to keto-enol tautomerism, the ^{1}H- and ^{13}C-NMR signals for the CH_2 groups of **12** were broadened, and the ^{13}C-NMR signal for the C=O group could not be observed. The ketone character of **12** could be demonstrated by formation of an orange 2,4-dinitrophenylhydrazone.

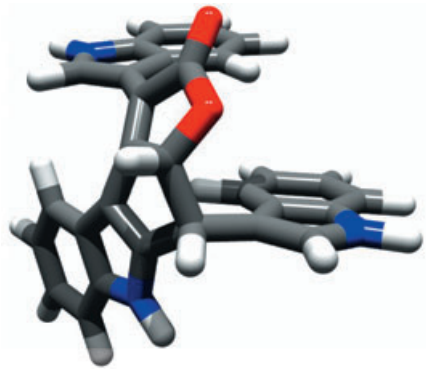
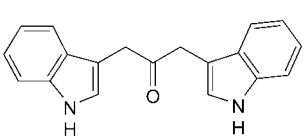
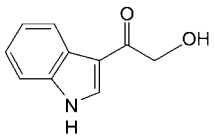


Fig. 5. *Molecular model of the most-stable conformation for compound* **11** *according to force-field calculations* [12]



12



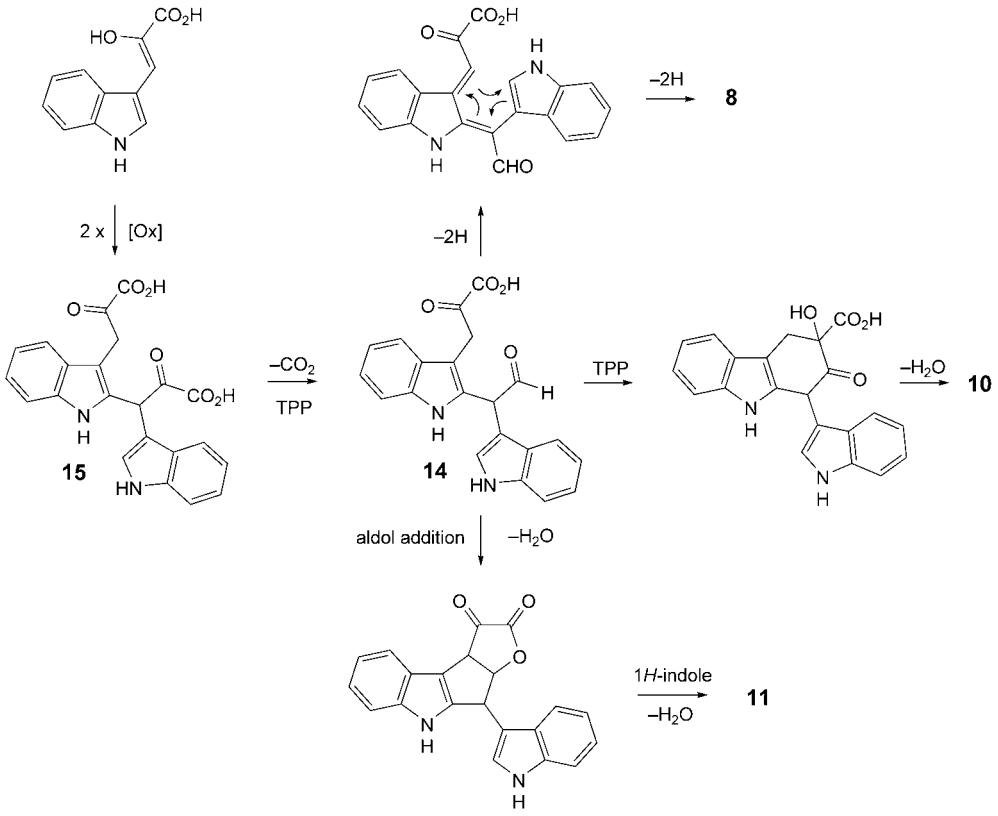
13

Malassezione (**12**) was discovered by an effect-guided separation of the crude *Malassezia* extract in search for inhibitors of tyrosinase, an enzyme involved in the formation of melanin in human melanocytes. Compound **12** inhibits the conversion of L-DOPA into a brown pigment in melanocytes of human epidermis preparations, a reaction used as model in the screening for such compounds. It may, therefore, contribute to the long-lasting depigmentations observed in pityriasis versicolor, in addition to the apoptosis-inducing malassezin. Compound **12** is accompanied by a second tyrosinase inhibitor, ketomalassezine (= 2-(1*H*-indole-3-carbonyl)-1*H*-indole-3-carboxaldehyde) [17], known as a synthetic intermediate [18], which will be described in a separate publication.

Finally, the known 2-hydroxy-1-(1*H*-indol-3-yl)ethanone (**13**) was isolated from cultures of *M. furfur* and identified by direct comparison with a synthetic sample [19].

3. Conclusion. – *Malassezia* converts tryptophan into an astonishing variety of indole alkaloids, which is suggestive of the operation of a 'combinatorial-biosynthesis' regime by this pathogen. Several of the metabolites contain a structural motif in which two 1*H*-indole units are connected by a C-atom bridging the 2- and 3′-positions. It is tempting to suggest that malasseziazole B (**8**), pityriazole (**10**), and malasseziacitrin (**11**) are all derived from a common precursor **14** by way of the transformations depicted in the *Scheme*. Dehydrogenation of **14** followed by 6π-electrocyclization and aromatization could lead to malasseziazole B (**8**), the most complex of the indolo[3,2-b]carbazoles. Intramolecular aldol addition followed by lactone formation could yield a keto lactone, which on condensation with 1*H*-indole might afford malasseziacitrin (**11**). Finally, pityriazole (**10**) could be formed from **14** by a thiamine pyrophosphate (TPP) catalyzed C–C bond formation, followed by dehydration of the resulting hydroxy acid.

Scheme. *Possible Biosynthetic Relationship between Malasseziazole B* (**8**), *Pityriazole* (**10**), *and Malasseziacitrin* (**11**)



Metabolite **14** could in turn be obtained by oxidative coupling of two 1*H*-indol-3-ylpyruvate molecules at C(2) and C(3′), followed by TTP-catalyzed decarboxylation of the resulting dimer **15** to formyl derivative **14**. Similarly, coupling of the two keto acid molecules at their 3′-positions would yield the symmetric precursor of the pityriarubin group of *Malassezia* metabolites [8].

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Experimental Part

General. M.p.: *Büchi SMP 535* and *B-540;* uncorrected. TLC: silica gel 60 F_{254} precoated plates (*Merck*); solvent system: toluene/HCO_2Et/HCO_2H 10:5:3. UV Spectra: *Perkin-Elmer Lambda-16* spectrophotometer; λ_{max} (log ε) in nm. CD Spectra: *Instruments S. A. Jobin Yvon CD-6-Dichrograph*; λ_{max} ($\Delta\varepsilon$). FT-IR Spectra: *Perkin-Elmer FT-1000* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^1H-, ^{13}C-, and 2D-NMR Spectra: *Bruker AMX-600* spectrometer; δ in ppm rel. to solvent, J in Hz. MS and HR-MS: *Finnigan MAT-90* and *MAT-95-Q* mass spectrometers; *m/z* (rel. %).

Cultivation of Malassezia furfur. Cultivation of *Malassezia furfur* (CBS 1878) was carried out according to [3] on an agar medium consisting (for 1 l of medium) of 30 ml of *Tween® 80 ultra* (*Sigma*, St. Louis, USA), 0.5 g of cycloheximide (*Sigma-Aldrich*, Steinheim, Germany), and 20 g of agar ('reinst'; *Merck*, Darmstadt, Germany) under addition of 3.06 g (15 mmol) of L-tryptophan in 215 *Petri* dishes of 10 cm diameter for 14 days at 30 °.

Isolation. The medium was cut up and then extracted for 12 h with AcOEt. The extract was washed with H_2O, dried ($MgSO_4$), and evaporated. Pre-purification was achieved by column chromatography (*Sephadex LH-20*, MeOH). The individual fractions, recognized by their brown color or green-yellow fluorescence under UV light (366 nm), were then separated by TLC (silica gel *60*, toluene/HCO_2Et/HCO_2H 10:5:3), after evaporation and redissolution in a small volume of EtOH. The R_f values of the zones with the same colors or yellow-green fluorescence corresponded to those of the crude extract. Each of the colored zones, still consisting of mixtures, was then extracted with AcOEt and each extract further separated by prep. HPLC I *LiChrospher RP-8* (8 μm, 25 cm × 30 mm; *Merck*, Darmstadt); linear gradient within 180 min from 100 % H_2O to 100 % MeCN; flow 5 ml min^{-1}; 180 fractions (5 ml each); UV detection at 220 nm). The t_R values of the components separated in this manner are summarized in *Table 7*. Fractions were combined according to retention time and color or fluorescence at 366 nm, lyophilized, and repurified by HPLC II (same column, conditions as for HPLC I, with optimized H_2O/MeCN gradients as given in *Table 7*). The purity of the resulting compounds was examined by anal. HPLC (*RP-18*, *Shandon ODS Hypersil* (3 μm, 25 cm × 4 mm; *Life Science International Ltd.*, Cheshire, England); autosampler (100-μl samples); linear gradient within 100 min from 100% H_2O to 100% MeCN; flow 1 ml min^{-1}; UV detection at 220 nm): (t_R [min]): 55 (**2**), 27 (**3**), 23 (**4**), 17 (**5**), 40 (**6**), 42 (**7**), 35 (**8**), 41 (**9**), 49 (**10**), 58 (**11**), 25 (**12**), 12 (**13**).

Table 7. *Important Parameters and Conditions for the HPLC Separation of the Metabolites*

R_f of TLC zone	HPLC I	HPLC II		Compound (amount)
	t_R [min]	gradient MeCN/H_2O [%] → MeCN/H_2O [%][a])	t_R [min]	
0.44	141–160	50 → 85	26–29	**2** (1.7 mg)
0.38	60–100	0 → 30	92–117	**3** (3.8 mg)
0.14	10–40	0 → 20	20–33	**4** (2.7 mg)
0.28	40–60	20 → 50	59–63	**5** (10.0 mg)
0.28	50–86	20 → 50	143–156	**6** (2.0 mg)
0.27	64–75	10 → 15	85–125	**7** (1.0 mg)
0.38	79–90	30 → 50	29–33	**8** (1.0 mg)
0.54	121–132	10 → 30	8–98	**9** (1.7 mg)
0.54	133–147	45 → 55	39–48	**10** (1.0 mg)
0.38	124–145	50 → 70	60–78	**11** (1.5 mg)
0.41	68–74	15 → 40	18–27	**12** (1.5 mg)
0.14	26–35	0 → 20	33–36	**13** (2.1 mg)

[a]) Gradient within 180 min.

Pityriacitrin B (= *1-(1*H-*Indol-3-ylcarbonyl)-9*H-*pyrido[3,4-*b*]indole-3-carboxylic Acid*; **2**): Yellow solid. M.p. >300° (dec.). UV (MeOH): 214 (4.49), 266 (4.18), 280 (4.17), 289 (4.17), 391 (3.74). IR (KBr): 3414*m*, 3059*m*, 2925*m*, 2854*m*, 1707*m*, 1603*s*, 1513*m*, 1494*m*, 1458*m*, 1430*m*, 1384*m*, 1323*w*, 1306*m*, 1277*m*, 1239*m*, 1165*m*, 1150*w*, 1129*m*, 1099*m*, 1063*w*, 1025*m*, 1011*m*, 953*m*, 906*w*, 842*w*, 808*m*, 781*m*, 746*m*, 724*m*, 638*m*, 621*w*, 598*w*, 428*w*. ^{1}H-NMR (600 MHz, CD_3OD): *Table 1*. ^{1}H-NMR (600 MHz, (D_6)DMSO): 7.29 (*m*, H–C(5′), H–C(6′)); 7.35 (*dd*, *J* = 7.4, 7.4, H–C(6)); 7.55 (*d*, *J* = 7.8, H–C(7′)); 7.63 (*dd*, *J* = 7.4, 7.8, H–C(7)); 7.89 (*d*, *J* = 7.8, H–C(8)); 8.45 (*d*, *J* = 7.4, H–C(5)); 8.59 (*d*, *J* = 7.0, H–C(4′)); 9.13 (*s*, H–C(4)); 9.72 (*d*, *J* = 2.7, H–C(2′)); 12.26 (H–N(1′)); 12.38 (2 *s*, H–N(9)); 13.10 (br. *s*, OH). ^{13}C-NMR (151 MHz, CD_3OD): *Table 1*. EI-MS: 355 (75, M^+), 338 (51) 311 (35), 294 (63), 144 (100), 116 (55), 89 (42), 44 (62). HR-EI-MS: 355.0955 (M^+, $C_{21}H_{13}N_3O_3^+$; calc. 355.0957).

Malassezindole A (= *(2*S,*5*R*)-1,2,3,4,5,6-Hexahydro-5-(1*H-*indol-3-yl)-4-oxoazepino[4,5-*b*]indole-2-carboxylic Acid*; **3**): Colorless powder. M.p. >250°. $[\alpha]_D^{25} = -87$ (*c* = 0.045, MeOH). UV (MeOH): 222 (4.30),

280 (3.68). CD (MeOH): 206 (−4.59), 225 (−3.41), 231 (0), 239 (+2.71), 254 (0), 286 (−2.55). IR (KBr): 3429v*s* (NH), 2926*w*, 1621*s* (CONH), 1459*m*, 1398*m*, 1098*w*, 745*m* (1,2-disubst. benzene), 433*w*. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (151 MHz, CD_3OD): see *Table 2*. ESI-MS (pos.): 719 (61, $[2M+H]^+$), 360 (100, $[M+H]^+$). HR-ESI-MS: 360.1363 ($[M+H]^+$, $C_{21}H_{18}N_3O_3^+$; calc. 360.1348).

Malassezindole B (= *(2*S,5S*)-1,2,3,4,5,6-Hexahydro-5-hydroxy-5-(1*H*-indol-3-yl)-4-oxoazepino[4,5-*b*]indole-2-carboxylic Acid*; **4**): Colorless powder. M.p. 213–215° (dec.). $[\alpha]_D^{25} = -15$ ($c = 0.12$, MeOH). UV (MeOH): 193 (4.23), 218 (4.10), 261 (3.56), 287 (3.48). CD (MeOH): 204 (−3.65), 220 (0), 235 (+1.18), 266 (0), 292 (−0.57), 314 (0). IR (KBr): 3430*s*, 2926*m*, 2854*w*, 1618*s*, 1459*w*, 1385*m*, 1207*m*, 1132*m*, 933*w*, 803*w*, 745*m*, 724*w*, 704*w*, 580*w*, 460*w*. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (151 MHz, CD_3OD): *Table 2*. ESI-MS (neg.): 750 (100, $[2M]^-$), 420 ($[M+HCO_2H-H]^-$), 374 (2, $[M-H]^-$). HR-ESI-MS: 420.1203 ($[M+HCO_2H-H]^-$, $C_{22}H_{18}N_3O_6^-$; calc. 420.1195).

*(*αR*)-α-Hydroxy-1*H*-indole-3-propanoic Acid* (**5**). M.p. 100–103° ([13]: 98–99°). $[\alpha]_D^{25} = -6$ ($c = 0.28$, H_2O). UV (MeOH): 221 (4.56), 281 (3.80), 290 (3.74). ^{1}H-NMR (CD_3OD, 600 MHz): 3.06 (br. *s*, 1 H, CH_2), 3.27 (*d*, $J = 13.3$, 1 H, CH_2); 4.37 (br. *s*, C*H*OH); 6.98, 7.05 (2 *dd*, $J = 8.2, 8.2$, 2 CH); 7.11 (*s*, CH); 7.30, 7.60 (2 *d*, $J = 8.2$, 2 CH). ^{13}C-NMR (151 MHz, CD_3OD): 32.1 (CH_2); 73.2 (CH); 112.0 (quat. C); 112.4, 119.8, 119.9, 122.5, 124.9 (CH); 129.4, 138.2 (quat. C); 179.1 (CO_2H). EI-MS: 205 (10, M^+), 187 (2), 159 (4), 144 (5), 131 (18), 130 (100), 103 (7), 89 (2), 77 (8). HR-EI-MS: 205.0731 ((M^+, $C_{11}H_{11}NO_3^+$; calc. 205.0738).

Malassezialactic Acid (= *α-Hydroxy-2-(1*H*-indol-3-ylmethyl)-1*H*-indole-3-propanoic Acid*; **6**): Colorless powder. M.p. 135–137°. UV (MeOH): 209 (4.14), 225 (4.12), 276 (3.75). $[\alpha]_D^{25} = -8$ ($c = 0.17$, MeOH). IR (KBr): 3430*s* (br.; OH, CO_2H), 2915*w*, 2849*w*, 1716*w* (CO_2H), 1630*s*, 1524*w*, 1484*w*, 1459*m*, 1422*m*, 1381*w*, 1336*m*, 1233*w*, 1200*w*, 1089*m*, 1038*w*, 1008*w*, 745*m* (2,3-disubst. 1*H*-indole). ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (151 MHz, CD_3OD): *Table 4*. ESI-MS (neg.): 677 (1, $[2M-2H+Na]^-$), 655 (30, $[2M-H]^-$), 327 (100, $[M-H]^-$). HR-ESI-MS: 655.1623 ($[2M-H]^-$, $C_{40}H_{23}N_4O_6^-$; calc. 655.1618).

Malasseziazole A (= *5,11-Dihydro-α-oxoindolo[3,2-*b*]carbazole-6-acetic Acid*; **7**): Yellow solid. M.p. >250° (dec.). UV (MeCN): 211 (0.88), 225 (0.97), 255 (1.00), 305 (0.31), 375 (0.38), 447 (0.19). ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (151 MHz, CD_3OD): *Table 4*. ESI-MS (neg.): 677 (1, $[2M-2H+Na]^-$), 655 (30, $[2M-H]^-$), 327 (100, $[M-H]^-$). HR-ESI-MS (neg.): 655.1623 ($[2M-H]^-$, $C_{40}H_{23}N_4O_6^-$; calc. 655.1618).

Malasseziazole B (= *12-Formyl-5,11-dihydro-α-oxoindolo[3,2-*b*]carbazole-6-acetic Acid*; **8**): Red solid. M.p. >300° (dec.). UV (MeOH): 209 (4.39), 230 (sh, 4.15), 242 (sh, 4.05), 275 (sh, 3.70), 306 (3.55), 409 (3.33), 482 (3.16). IR (KBr): 3434*m*, 3384*m*, 2925 *m*, 2854*m*, 1662*m*, 1634*s*, 1614*m*, 1515*m*, 1462*m*, 1384*m*, 1343*m*, 1321*m*, 1298*m*, 1225*m*, 1207*m*, 1169*m*, 1127*m*, 1102 *m*, 1026*m*, 1001*m*, 945*w*, 920*w*, 829*w*, 803*m*, 764*m*, 743*m*, 723*m*, 703*m*, 685*m*, 641*m*, 623*m*, 552*m*, 492*w*. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (151 MHz, CD_3OD): *Table* 4. ESI-MS (neg.): 355 (100, $[M-H]^-$). HR-ESI-MS: 355.0707 ($[M-H]^-$, $C_{21}H_{11}N_2O_4^-$; calc. 355.0719)

Malasseziazol C (= *12-Formyl-5,11-dihydroindolo[3,2-*b*]carbazole-6-carboxylic Acid*; **9**): Orange solid. M.p. >300° (dec.). UV (MeOH): 214 (3.76), 226 (sh, 3.71), 247 (3.66), 268 (3.51), 308 (3.23), 391 (3.19), 462 (2.95). IR (KBr): 3430*m*, 2926*m*, 2855*w*, 1682*s*, 1617*m*, 1515*w*, 1460*m*, 1439*w*, 1385*m*, 1322*m*, 1296*w*, 1259*w*, 1211*m*, 1183*m*, 1134*m*, 1024*w*, 964*w*, 895*w*, 841*w*, 804*m*, 747*w*, 725*m*, 700*w*, 518*w*. ^{1}H-NMR (600 MHz, CD_3OD) and ^{13}C-NMR (151 MHz, CD_3OD): *Table 4*. ^{1}H-NMR ((D_6)DMSO): 7.13 (*m*, 2 CH); 7.37, 7.42 (2 *dd*, $J = 8.0$, 7.5, 2 CH); 7.71 (*d*, $J = 8.0$, 2 CH); 8.50, 8.91 (2 *d*, $J = 8.0$, 2 CH), 11.32 (*s*, CHO), 11.35, 11.66 (2 *s*, 2 NH). ESI-MS (neg.): 327 (28, $[M-H]^-$). HR-FAB-MS: 327.0737 ($[M-H]^-$, $C_{20}H_{11}N_2O_3^-$; calc. 327.0770).

Pityriazole (= *2-Hydroxy-1-(1*H*-indol-3-yl)-9*H*-carbazole-3-carboxylic Acid*; **10**): Colorless solid. M.p. >250°. UV (MeOH): 221 (3.77), 279 (3.52), 336 (2.95, sh). IR (KBr): 3435*s* (br.), 2926*w*, 2855*w*, 2074*w*, 1631*s*, 1487*w*, 1456*m*, 1410*m*, 1354*w*, 1268*w*, 1237*m*, 1104*w*, 745*s*, 585*w*, 452*w*. ^{1}H-NMR (600 MHz, (D_6)DMSO) and ^{13}C-NMR (151 MHz, (D_6)DMSO): *Table 5*. ESI-MS (neg.): 341 (100, $[M-H]^-$). HR-ESI-MS (neg. mode): 341.0933 ($[M-H]^-$, $C_{21}H_{14}N_2O_3^-$; calc. 341.0926).

Malasseziacitrin (= *4,5-Dihydro-1,4-di(1*H*-indol-3-yl)furo[3′,2′:3,4]cyclopent[1,2-*b*]indol-2-(3*αH*)-one*; **11**): M.p. >200° (dec.). UV (MeOH): 222 (4.72), 269 (4.23), 285 (4.21), 357 (3.88). IR (KBr): 3414*s*, 2925*w*, 1726*m*, 1660*s*, 1618*m*, 1541*w*, 1460*s*, 1384*w*, 1334*w*, 1243*w*, 1138*w*, 1011*w*, 940*w*, 745*s*, 428*w*. ^{1}H-NMR (600 MHz, (D_6)DMSO) and ^{13}C-NMR (151 MHz, (D_6)DMSO): *Table 6*. EI-MS: 443 (7), 442 (32), 441 (100, M^+), 440 (14), 439 (30), 398 (14), 397 (23), 396 (23), 385 (28), 384 (71), 383 (19), 382 (42), 381 (10), 324 (14), 201 (28), 191 (16), 157 (24), 130 (62). HR-EI-MS: 441.1456 (M^+, $C_{29}H_{19}N_3O_2^+$; calc. 441.1477).

Malassezione (= *1,3-Di-(1*H*-indol-3-yl)propan-2-one*; **12**): Colorless solid. M.p. (dec.) >150°. UV (MeOH): 207 (3.04), 216 (sh, 3.01), 266 (2.57), 280 (2.56), 289 (sh, 2.54). IR (KBr): 3421*s*, 2925*m*, 2854*m*, 1583*s*, 1458*m*, 1420*m*, 1399*m*, 1385*m*, 1354*w*, 1340*w*, 1276*w*, 1226*m*, 1206*w*, 1180*w*, 1124*w*, 1024*w*, 1010*w*, 800*w*, 786*w*, 743*m*, 426*w*. ^{1}H-NMR (600 MHz, CD_3OD): 3.67 (br. *s*, 2 CH_2); 7.00 (*dd*, $J = 7.7, 7.5$, 2 CH); 7.09 (*dd*, $J = 8.1$,

7.5, 2 CH); 7.16 (*s*, 2 CH); 7.33 (*d*, *J* = 8.1, 2 CH); 7.63 (*d*, *J* = 7.7, 2 CH). ^{1}H-NMR (600 MHz, (D_6)DMSO): 3.36 (br., 2 CH_2, partially obscured by H_2O); 6.89 (*dd*, *J* = 7.8, 7.4, 2 CH); 6.99 (*dd*, *J* = 8.1, 7.4, 2 CH); 7.14 (*s*, 2 CH); 7.27 (*d*, *J* = 8.1, 2 CH): 7.51 (*d*, *J* = 7.8, 2 CH); 10.66 (*s*, 2 NH). ^{13}C-NMR (151 MHz, CD_3OD): 35.9 (br., 2 CH_2); 112.2 (2 CH); 112.3 (2 quart. C); 119.7, 120.1, 122.3, 124.4 (4 × 2 CH), 129.5, 138.3 (2 quart. C); CO signal not detectable. EI-MS: 288 (4, M^+), 175 (18), 144 (6), 130 (100), 117 (39), 89 (14), 77 (19), 63 (10). HR-EI-MS: 288.1282 (M^+, $C_{19}H_{16}N_2O^+$; calc. 288.1263).

REFERENCES

[1] G. Midgley, *Med. Mycol.* **2000**, *38 (Suppl. 1)*, 9.
[2] A. K. Gupta, R. Bluhm, R. Summerbell, *J. Eur. Acad. Dermatol. Venereol*. **2002**, *16*, 19.
[3] P. Mayser, G. Wille, A. Imkampe, W. Thoma, N. Arnold, T. Monsees, *Mycoses* **1998**, *41*, 265.
[4] G. Wille, P. Mayser, W. Thoma, T. Monsees, A. Baumgart, H.-J. Schmitz, D. Schrenk, K. Polborn, W. Steglich, *Bioorg. Med. Chem*. **2001**, *9*, 955.
[5] A. Baumgart, P. Mayser, H.-J. Krämer, W. Thoma, T. Monsees, G. Wille, K. Polborn, W. Steglich, D. Schrenk, H.-J. Schmitz, *Organohalogen Compd*. **2003**, *65*, 138.
[6] H.-J. Krämer, M. Podobinska, A. Bartsch, A. Battmann, W. Thoma, A. Bernd, W. Kummer, B. Irlinger, W. Steglich, P. Mayser, *ChemBioChem* **2005**, *6*, 860.
[7] P. Mayser, U. Schäfer, H.-J. Krämer, B. Irlinger, W. Steglich, *Arch. Dermatol. Res.* **2002**, *294*, 131.
[8] B. Irlinger, H.-J. Krämer, P. Mayser, W. Steglich, *Angew. Chem.* **2004**, *116*, 1119; *Angew. Chem., Int. Ed.* **2004**, *43*, 1098.
[9] H.-J. Krämer, D. Kessler, W. Steglich, P. Mayser, *Arch. Derm. Res.* **2003**, *294*, 492.
[10] P. Mayser, H. Stapelkamp, H.-J. Krämer, M. Podobinska, W. Wallbott, B. Irlinger, W. Steglich, *Antonie van Leeuwenhoek* **2003**, *84*, 185.
[11] M. Salmoun, C. Devijver, D. Daloze, J.-C. Braekman, R. W. M. van Soest, *J. Nat. Prod.* **2002**, *65*, 1173.
[12] F. Mohamadi, N. G. J. Richards, W. C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson, W. C. Still, *J. Comput. Chem*. **1990**, *11*, 440.
[13] T. Sasaki, I. Otsuka, *Biochem. Z.* **1921**, *121*, 167.
[14] R. H. Hill Jr., S. P. Caudill, R. M. Philen, S. L. Bailey, W. D. Flanders, W. J. Driskell, M. L. Kamb, L. L. Needham, E. J. Sampson, *Arch. Environ. Contam. Toxicol*. **1993**, *25*, 134.
[15] B. Müller, C. Pacholski, T. Simat, H. Steinhart, *Lebensmittelchem.* **1999**, *53*, 55.
[16] J. Tholander, J. Bergman, *Tetrahedron* **1999**, *55*, 6243; J. Bergman, T. Janosik, N. Wahlström, *Adv. Heterocycl. Chem*. **2001**, *80*, 1.
[17] H.-J. Krämer, K. Dahms, M. Podobinska, A. Bartsch, W. Steglich, P. Mayser, *Mycoses* **2004**, *47*, 363.
[18] J. Bergman, N. Wahlström, L. N. Yudina, J. Tholander, G. Lidgren, *Tetrahedron* **2002**, *58*, 1443.
[19] S. Tsujii, K. L. Rinehart, S. P. Gunasekera, Y. Kashman, S. S. Cross, M. S. Lui, S. A. Pomponi, M. C. Diaz, *J. Org. Chem.* **1988**, *53*, 5446.

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