Tetrahedron Vol. 42, No. 13, pp. 3587 to 3593, 1986
Printed in Great Britain

0040-4020/86 $3.00 + .00
Pergamon Journals Ltd

ANTIBIOTICS FROM BASIDIOMYCETES. Part 24.[1] ANTIBIOTICS WITH A REARRANGED HIRSUTANE SKELETON FROM PLEUROTELLUS HYPNOPHILUS (AGARICALES)

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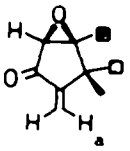
(*Received in Germany* 24 *March* 1986)

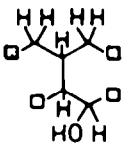
Abstract- The structures of the antibiotically active sesquiterpenoids hypnophilin, pleurotellic acid and pleurotellol have been elucidated by spectral investigations. Pleurotellic acid and pleurotellol contain a rearranged hirsutane skeleton which may be derived biosynthetically from hypnophilin by a Wagner-Meerwein type rearrangement.

Recently we reported on the isolation and biological activity of the sesquiterpenoid antibiotics hypnophilin, pleurotellic acid and pleurotellol from mycelial cultures of Pleurotellus hypnophilus (Berk.) Sacc. (Agaricales).[2,3] In this communication we describe the structural elucidation of these compounds.

Hypnophilin is a colourless, unstable oil which exhibits a UV maximum (MeOH) at 234 nm and IR absorptions ($CHCl_3$) at 1720 and 1620 cm^{-1}, indicating the presence of an α,β-unsaturated carbonyl group. The mass spectrum leads to the molecular formula $C_{15}H_{20}O_3$ which corresponds to the ^1H and ^{13}C NMR data. From the NMR spectra (Table 1) the presence of an α-methylene ketone unit and a trisubstituted epoxide function is easily discerned. Comparison of the corresponding signals with those of complicatic acid (1)[4,5] indicates that in hypnophilin and the other Pleurotellus compounds partial structure I is present which conforms to ring A in 1. In all

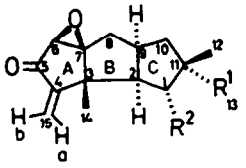


I



II

cases a characteristic long range coupling J_5 = 0.7 Hz between the epoxide proton and H_a of the methylene group can be observed.[6] Complicatic acid is produced by mycelial cultures of Galerina cephalotricha,[2] a species closely related to P. hypnophilus.



1, R^1 = CO_2H, R^2 = H

2, R^1 = CH_3, R^2 = OH

Table 1. ^{1}H NMR data of hypnophilin (2), pleurotellic acid (6) and pleurotellol (7) (2 and 6 at 400 MHz, 7 at 90 MHz; $CDCl_3$; TMS as internal standard)

H-atom	2	6	7†
1	3.87 d	-	
2	2.14 dd	3.36 dm	
6	3.44 s	3.45 s	3.46 s
8	1.93 ABX	2.07 ABX	
	1.84	2.13	
9	2.65 m	2.90 m	
10α	1.87 dd	2.47 m	
10β	1.30 dd	2.90 m	1.74 s,br.
12	1.07 s	2.18 s,br	1.75 s,br
13	0.89 s	-	4.11, 4.31 AB-q
14	1.31 s	1.10 s	1.09 s
15a	5.46 s	5.65 s	5.63 s
15b	6.14 s	6.25 s	6.20 s
OH	1.60 s	-	1.60 s

2: $J_{1,2}$=9 Hz, $J_{2,9}$=12, $J_{8\alpha,8\beta}$=14, $J_{8\alpha,9}$=9 or 10, $J_{8\beta,9}$=9 or 10, $J_{9,10\alpha}$=8, $J_{9,10\beta}$=10.5, $J_{10\alpha,10\beta}$=13, $J_{15a,15b}$=0.4; $^5J_{1,14}$=0.36, $^5J_{6,14}$=0.32, $^5J_{6b15a}$=0.7, $^4J_{10\alpha,12}$=0.2, $^4J_{10\alpha,13}$=0.7; Difference NOE's: δ 3.87 ↔1.31; 3.87 ↔1.07; 2.14 ↔2.65; 2.14 ↔1.87; 2.14 ↔0.89; 2.65 ↔0.89; 1.20 ↔1.07; 1.31 ↔5.46.

6: $J_{2,9}$=10 Hz, $J_{8\alpha,8\beta}$=14, $J_{8\alpha,9} \approx J_{8\beta,9}$=8.5; $^5J_{2,12}$=2, $^5J_{2,10} \approx$1.5, $^5J_{6,15a}$=0.7.

† Other signals not resolved.

Extensive ^{1}H NMR decoupling experiments lead to a second partial structure II, which leaves only a unit $C(CH_3)_2$ to be incorporated into the formula. In this manner structure 2 containing a hirsutane skeleton is derived for hypnophilin. The coupling constant $J_{2,9}$ = 12 Hz indicates a cis-junction between rings B and C.[7] The absolute configuration depicted in formula 2 follows from the similarity of the CD spectrum (Figure 1) with that of complicatic acid (1), whose absolute configuration is known.[8] The α-configuration of the hydroxyl group was established

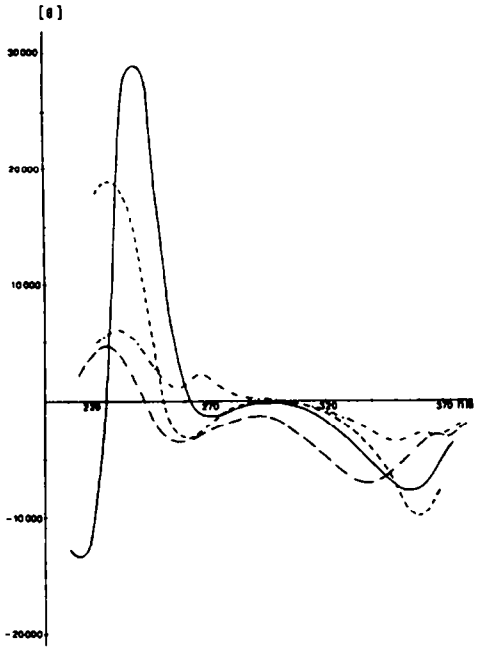
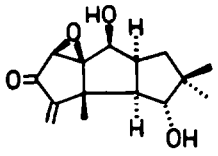


Fig. 1. Circular dichroism spectra of complicatic acid (-·-·-·), hypnophilin (— — —), pleurotellic acid (———) and pleurotellol (------) in acetonitrile.

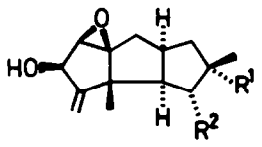
by a NOE experiment in which irradiation at the frequency of 14-CH_3 (δ = 1.31) leads to a 20% signal enhancement of 1-H (δ = 3.87). 1-H exhibits a vicinal coupling $J_{1,2}$ = 9 Hz in agreement with the value known from 3 and related synthetic compounds.[9]

Reduction of 2 with sodium borohydride gives rise to the dihydro derivative 4 which shows close correspondence in its ring A ^1H NMR signals to hirsutic acid C (5).[10]

Pleurotellic acid, $C_{15}H_{16}O_4$, is a colourless solid, mp 148°C, which is soluble in aqueous sodium hydrogen carbonate. The presence of a carboxyl function is indicated by IR absorptions (KBr) at 3600-2400 and 1690 cm^{-1}. In the ^1H NMR spectrum (400 MHz, Table 1) singlets



3

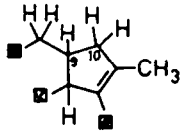


4, R^1 = CH_3, R^2 = OH

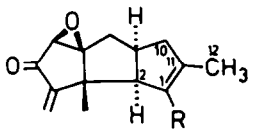
5, R^1 = CO_2H, R^2 = H

for two methyl groups (δ = 1.10 and 2.18), an epoxide-H (δ = 3.45) and two exo-methylene protons (δ = 5.65 and 6.25) are easily recognized. With the exception of the AB-part of an ABX-system (δ = 2.07 and 2.13, J_{AB} = 14 Hz, J_{AX}=J_{BX}= 8.5 Hz) the remaining signals at δ = 2.47 (1H), 2.90 (2H) and 3.36 (1H) appear as complicated multiplets. Decoupling experiments indicate long range couplings between the methyl group at δ = 2.18 and these complicated multiplets. Surprisingly, irradiation at the multiplet δ = 2.90 leads to changes in all the other multiplets. By combining the results of this and other decoupling experiments one arrives at partial formula III. The difficulties in interpreting the line shapes of the three multiplets are then explained by the fact that 9-H and one of the protons at C-10 are isochronous which causes virtual coupling.

Combining partial structure III with I and a carboxyl group leads to formula



III



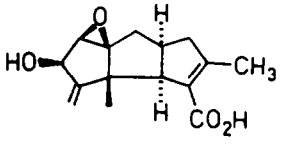
6, R= CO_2H

7, R= CH_2OH

8, R= CHO

6 for pleurotellic acid. The position of the carboxyl group at C-1 instead at C-11 is preferred because of the homoallyl coupling (^5J = 2 Hz) between 2-H and the 12-methyl group. The W-coupling of the latter with each of the two protons at C-10 is less than 1 Hz. Structure **6** is further supported by the ^{13}C NMR data. In the ^1H coupled spectrum the signals of the carboxyl carbon (C-13) and the 12-methyl group appear as singlets which indicates dihedral angles of around 80° between the corresponding C-H and C-C-bonds[11].

On reduction with $NaBH_4$ pleurotellic acid is converted into the dihydro derivative **9**.



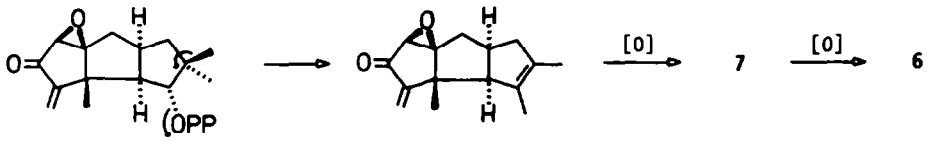
9

Pleurotellol, $C_{15}H_{18}O_3$, is a colourless oil which exhibits IR bands at 3500 (OH), 1720 and 1620 cm^{-1} (α,ß-unsaturated CO). The ^1H NMR spectrum shows great similarity with that of **6**. Besides signals typical for partial structure I, two methyl singlets at δ = 1.09 and 1.75 are visible. The latter can be assigned to a methyl group situated at a double bond because of its chemical shift and its broadening by long range couplings. The broad AB quartet centred at δ = 4.20 is sharpened by addition of D_2O and can therefore be assigned to a CH_2OH group. This leads to formula **7** for pleurotellol, in good agreement with the spectroscopic data.

To confirm this formula pleurotellol was converted into pleurotellic acid (**6**). Oxidation of **7** with MnO_2 in ether yielded aldehyde **8**, which was further oxidized to carboxylic acid **6** with AgO/NaCN by Corey's method.[12] The R_f values and MS spectrum of this product were identical with those of authentical pleurotellic acid. The CD spectra of pleurotellic acid (**6**) and pleurotellol (**7**) (Figure 1) show characteristic differences in comparison to the 'normal' hirsutane derivatives **1** and **2**, which can be ascribed to the presence of the second chromophore.

Pleurotellic acid (**6**) and pleurotellol (**7**) constitute a new group of sesquiterpenoids which contain a carbon skeleton which may arise biogenetically from hypno-

philin (2) via a Wagner-Meerwein rearrangement:



EXPERIMENTAL

^{1}H NMR spectra were obtained on Bruker WH 90 and WM 400 spectrometers in deuteriochloroform solutions with tetramethylsilane as internal standard. IR spectra were recorded on a Pye Unicam SP 1100 spectrophotometer. UV spectra were taken with a Varian Cary 17 spectrometer, and the CD spectra with a Jouan-Roussel III spectropolarimeter. High resolution mass spectra were obtained on an AEI MS 50 instrument. Melting points were determined using a Reichert hot-stage microscope and are uncorrected. The R_F values were obtained on silica gel plates (Merck) with carbon tetrachloride/ethyl acetate = 1:1. The antibiotics **2**, **6** and **7** were isolated from mycelial cultures of Pleurotellus hypnophilus as described before.[2] CD spectrum of complicatic acid (1) in CH_3CN: $[\theta]_{232}$ = 6105, $[\theta]_{267}$ = 2245, $[\theta]_{301.5}$ = 0, $[\theta]_{352}$ = -3430, $[\theta]_{369}$ = -2840.

Hypnophilin (2)

Colourless oil, R_F 0.43; $[\alpha]_D^{20}$ -82.9° (c 0.9, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 234 (3.65); CD (CH_3CN): $[\theta]_{226}$ = 4590, $[\theta]_{242.5}$ = 0, $[\theta]_{257}$ = -3530, $[\theta]_{337.5}$ = -7060, $[\theta]_{371.5}$ = -3135; IR ($CHCl_3$): 3600-3500 (s, br.), 2950 (s), 2850 (s), 1720 (s), 1620 (s), 1460 (s), 1450 (s), 1400 (s), 1370 (m), 1320 (w), 1280 (w), 1250 (m), 1220 (m), 1160 (w), 1100 (m), 1080-1040 (m, br), 1020 cm^{-1} (m); ^{1}H NMR see Table 1; ^{13}C-NMR ($^{12}CDCl_3$): δ 81.3 (d, C-1), 56.2 (d, C-2), 45.6 (s, C-3), 153.8 (s, C-4), 197.7 (s, C-5), 61.2 (d, C-6), 76.1 (s, C-7), 30.8 (t, C-8), 34.6 (d, C-9), 46.2 (t, C-10), 44.2 (s, C-11), 17.7, 19.7, 26.4 (each q, 3 CH_3), 121.7 (t, C-15); MS: m/z 248.1404 (M^+, 10.2%, calc. for $C_{15}H_{20}O_3$ 248.1412), 233 (6, $C_{14}H_{17}O_3$), 231 (2, $C_{15}H_{19}O_2$), 230 (10, $C_{15}H_{18}O_2$), 220 (8, $C_{14}H_{20}O_2$), 219 (13, $C_{14}H_{19}O_2$), 217 (6, $C_{14}H_{17}O_2$), 215 (8, $C_{14}H_{15}O_2$), 207 (12, $C_{13}H_{19}O_2$), 201 (11, $C_{14}H_{17}O$), 177 (60), 158 (45), 147 (20), 146 (20), 135 (30), 133 (28), 121 (28), 111 (38), 109 (46), 107 (45), 105 (75).

Sodium borohydride reduction of 2

To an ice-cold solution of 2 (10 mg) in dioxane/H_2O (4:1, 4 ml) $NaBH_4$ (30 mg) was slowly added and the mixture was stirred for 2 h. The excess of $NaBH_4$ was destroyed by addition of aqueous oxalic acid and the dioxane was removed i. vac. After addition of some water the residue was repeatedly extracted with EtOAc. Evaporation of the dried extracts (Na_2SO_4) yielded a colourless oil (10 mg), R_F 0.23; ^{1}H NMR ($CDCl_3$ + D_2O, 90 MHz): δ 0.89, 1.04, 1.31 (each s, 3 CH_3), 3.46 (d, J =2 Hz, 6-H), 3.90 (m, 1-H), 4.62 (m, 5-H), 5.15 (dd, J = 1.9, 0.5 Hz, 15-H), 5.33 (d, J = 1.9 Hz, 15-H), other signals unresolved (1.00-2.60, m, 6H).

Pleurotellic acid (6)

Colourless crystals, mp 148°C, R_F 0.58 (benzene/acetone 10:4); $[\alpha]_D^{20}$ -70.8° (c 0.07, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 226 nm (3.70); CD (CH_3CN): $[\theta]_{215}$ = -13265; $[\theta]_{226}$ = 0, $[\theta]_{271}$ = -265,

$[\theta]_{356}$ = -7625; IR (KBr): 3600-2400 (s, br), 1730 (s), 1690 (s), 1640 (s), 1450-1410 (m, br), 1290 (m), 1270 (w), 1250 (w), 1230 (m), 1200 (w), 1135 (w), 1100 (w), 1085 (w), 950 (w), 890 (w), 845 (w), 770 (w), 750 (w), 730 (w), 710 (w), 680 cm^{-1} (w); ^1H NMR see Table 1; ^{13}C NMR ($^{12}CDCl_3$): δ 126.5 (s, C-1), 58.5 (d, C-2), 48.2 (s, C-3), 151.4 (s, C-4), 198.1 (s, C-5), 61.0 (d, C-6), 75.6 (s, C-7), 31.4 (t, C-8), 35.1 (d, C-9), 48.7 (t, C-10), 160.7 (s, C-11), 16.9 (q, C-12), 170.8 (s, C-13), 16.8 (q, C-14), 124.1 (t, C-15); MS: m/z 260.1048 (M^+, 3%, calc. for $C_{15}H_{16}O_4$ 260.1048), 242 (20, $C_{15}H_{14}O_3$), 232 (8, $C_{14}H_{16}O_3$), 224 (4, $C_{15}H_{12}O_2$), 217 (28, $C_{13}H_{13}O_3$), 214 (16, $C_{14}H_{14}O_2$), 187 (21, $C_{13}H_{15}O$), 157 (7), 143 (12), 137 (17), 136 (62, $C_8H_8O_2$), 129 (11), 128 (12), 124 (38, $C_7H_8O_2$), 108 (67), 107 (89, C_7H_7O), 96 (12, C_6H_8O), 91 (27).

Sodium borohydride reduction of **6**

From **6** (5 mg) in the same manner as decribed for **2**; colourless crystals (3 mg), mp 145°C, R_F 0.14; ^1H NMR ($CDCl_3$, 90 MHz): δ 0.98 (s, 14-CH_3), 2.14 (s, br, 12-CH_3), 3.50 (d, J = 2 Hz, 6-H), 4.44 (m, 5-H), 5.15 (dd, J = 1.9, 0.5 Hz, 15-H), 5.44 (d, 1.9 Hz, 15-H); other signals not resolved (1.00-2.60, m, 6H).

Pleurotellol (**7**)

Colourless oil, R_F 0.34; $[\alpha]_D^{20}$ -86.5° (c 0.015, $CHCl_3$); UV (MeOH): λ_{max} (log ε) = 226 nm (sh, 3.70); CD (CH_3CN): $[\theta]_{228}$ = 18910, $[\theta]_{251}$ = 0, $[\theta]_{261}$ = -3200, $[\theta]_{360}$ = -9835; IR ($CDCl_3$): 3600--3500 (s, br), 2950 (s), 2900 (s), 1720 (6), 1640 (s), 1460 (s), 1450 (s), 1410 (s), 1380 (m), 1315 (w), 1250 (m), 1230 (m), 1170 (m), 1130 (m), 1080 (w), 1070 (w), 1055 (m), 1000 cm^{-1} (w); ^1H NMR see Table 1; ^{13}C NMR ($^{12}CDCl_3$): δ 13.9, 15.9, 30.7, 35.8, 47.9, 48.2, 58.5, 58.8, 60.9 (C-6), 76.5 (C-7), 121.6 (C-15), 133.1 (C-11), 139.7 (C-1), 151.9 (C-4); CO signal not visible due to the small amount of material; MS: m/z 246.1253 (M^+, 5%, calc. for $C_{15}H_{18}O_3$ 246.1256), 228 (100, $C_{15}H_{16}O_2$), 218 (10), 217 (9), 216 (26, $C_{14}H_{16}O_2$), 215 (27, $C_{14}H_{15}O_2$), 213 (14, $C_{14}H_{13}O_2$), 203 (51, $C_{13}H_{15}O_2$), 200 (31, $C_{14}H_{16}O$), 199 (18), 189 (20), 187 (63, $C_{13}H_{15}O$), 185 (31, $C_{13}H_{13}O$), 171 (20), 157 (7), 136 (9), 110 (11), 108 (13), 95 (14), 93 (28), 91 (23), 81 (16), 79 (20).

Conversion of pleurotellol (**7**) into pleurotellic acid (**6**)

A) To a solution of **7** (5 mg) in ether (20 ml) MnO_2 (30 mg) was added and the mixture was stirred overnight at 20°C. The solid was filtered off after addition of some celite and the residue was washed several times with ether. Evaporation of the combined solutions yielded **8** (4 mg) as a colourless oil which solidified after keeping at 5°C; R_F 0.43; $[\alpha]_D^{20}$ -27.0° (c 0.1, $CHCl_3$); IR (KBr): 2960 (s), 2800 (s), 1735 (s), 1675 (s), 1640 (m), 1465 (m), 1380 (m), 1260 (m), 1200 (w), 1060 (m), 1040 cm^{-1} (m); ^1H NMR ($CDCl_3$ + D_2O, 90 MHz): δ 1.01 (s, 14-CH_3), 2.21 (s, br, 12-CH_3), 3.44 (s, 6-H), 5.88 ('t', J = 0.5 Hz, 15_a-H), 6.33 (d, J = 0.5 Hz, 15-H_b), 10.07 (s, 13-CHO); further signals not resolved (0.80-2.70, m, 6H).

B) To a solution of aldehyde **8** (3 mg) in absolute methanol (10 ml), silver(II)oxide (50 mg) and sodium cyanide (20 mg) were added and the mixture was stirred overnight at 20°C. After adjustment of pH 5 by addition of dilute acetic acid the solvent was evaporated i. vac. and the aqueous solution extracted several times with ethyl acetate (30 ml portions). Evaporation yielded the crude acid which exhibited the same R_F (0.58) and MS data as authentic pleurotellic acid (**6**).

Acknowledgements

We thank the Deutsche Forschungsgemeinschaft for the support of this work, Dr. G. Eckhardt, Bonn, for the mass spectra and Mr. C. Schmidt for NMR measurements.

REFERENCES AND NOTES

1. For Part 23, see B. M. Giannetti, B. Steffan and W. Steglich, Tetrahedron, preceding paper.
2. J. Kupka, T. Anke, B.-M. Giannetti and W. Steglich, Arch. Mikrobiol. **130**, 223 (1981).
3. This work has been presented in part at the 12th IUPAC Symposium on the Chemistry of Natural Products, Puerto de la Cruz, Tenerife 1980; W. Steglich, Pure Appl. Chem. **53**, 1233 (1981).
4. G. Mellows, P.G. Mantle, T.C. Feline and D.J. Williams, Phytochemistry **12**, 2717 (1973).
5. ^{13}C NMR spectrum of 1: M.G. Venegas and R.D. Little, Tetrahedron Lett. **1979**, 309.
6. T. Anke, J. Kupka, U. Mocek, B. Steffan and W. Steglich, Angew. Chem. **97**, 714 (1985); Angew. Chem. Int. Ed. Engl. **24**, 714 (1985).
7. E.g., S. Takahashi, N. Naganawa, H. Iinuma, T. Takita, K. Maeda and H. Umezawa, Tetrahedron Lett. **1971**, 1955.
8. Complicatic acid has been reduced to hirsutic acid,[4] whose absolute configuration has been established by an X-ray structural analysis: F. W. Comer and J. Trotter, J. Chem. Soc. B **1966**, 11.
9. S. Danishefsky, R. Zamboni, M. Kahn, S.J. Etheredge, J. Am. Chem. Soc. **103**, 3460 (1981). Further examples for synthetic compounds with a 1α-OH group: K. Iseki, M. Yamasaki, M. Shibasaki and S. Ikegami, Tetrahedron **37**, 4411 (1981); B.M. Trost and D.P. Curran, J. Am. Chem. Soc. **103**, 7380 (1981); G. Mehta, A.V. Reddy, A.N. Murthy and D.S. Reddy, J. Chem. Soc. Chem. Commun. **1982**, 540; T. Ito, N. Tomiyoshi, K. Nakamura, S. Azuma, M. Izawa, F. Moruyama, M. Yanagiya, H. Shirahama and T. Matsumoto, Tetrahedron Lett. **1982**, 1721; Tetrahedron **40**, 241 (1984).
10. F.W. Comer, F. McCapra, J.H. Qureshi and A.I. Scott, Tetrahedron **23**, 4761 (1967).
11. See e.g. H.-O. Kalinowski, S. Berger and S. Braun, ^{13}C-NMR-Spektroskopie, pp. 475, G. Thieme, Stuttgart, New York 1984 and references cited therein.
12. E.J. Corey, N.W. Gilman and B.E. Ganem, J. Am. Chem. Soc. **90**, 5616 (1968).