Tetrahedron Vol. 44, No. 8, pp. 2175 to 2180, 1988
Printed in Great Britain.

0040-4020/88 $3.00 + .00
Pergamon Press plc

SESQUITERPENE ALCOHOLS WITH NOVEL SKELETONS FROM THE FUNGUS *CERATOCYSTIS PICEAE* (ASCOMYCOTINA)

HANS-PETER HANSSEN[1] and WOLF-RAINER ABRAHAM*,[2]

[1]Universitaet Hamburg, Lehrstuhl für Pharmakognosie, Bundesstr. 43, D-2000 Hamburg 13, FRG, [2]GBF-Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-3300 Braunschweig, FRG

(*Received in Germany* 26 *November* 1987)

Summary:
From agar cultures of the ascomycete *Ceratocystis piceae* Ha 4/82 two sesquiterpene alcohols with novel tricyclic skeletons **1** and **2** have been isolated (cerapicol and ceratopicanol). The biogenesis of the newly identified metabolites is briefly discussed.

INTRODUCTION

A number of fungal sesquiterpenes with protoilludane, illudane, marasmane, hirsutane, sterpurane, lactarane and illudalane skeletons have been reported from various Basidiomycetes including species of the orders Aphyllophorales, Agaricales, Russulales, and Nidulariales[1]. A key intermediate in the biosynthesis of these metabolites has been postulated to be 6-protoilludene. *Ceratocystis piceae* (Münch) Bakshi is a sapwood staining ascomycete occurring on coniferous logs and lumber. Recently, we reported that strain *C. piceae* Ha 4/82 forms almost exclusively 6-protoilludene as volatile metabolic product when cultivated on defined synthetic liquid culture media[2]. Using surface cultures on solid media, an increased percentage of oxygenated metabolites could be achieved. We describe here the isolation of two sesquiterpene alcohols with related tricyclic skeleton from agar cultures of the same isolate.

RESULTS AND DISCUSSION

Both compounds were alcohols because they showed infrared absorption in the hydroxyl region at 3530 cm^{-1} and gave molecular ions of the composition $C_{15}H_{26}O$. The main alcohol revealed in the ^{13}C NMR SFORD spectrum a doublet at δ_C = 84.1 ppm and no resonances of sp^2 carbons so it must be a tricyclic secondary alcohol. The ^1H NMR spectrum showed a broad singlet at δ = 3.18 ppm belonging to the α-proton of the alcohol. In the $^1H(^1H)$ homonuclear double resonance or 2D chemical shift correlation ("COSY") this proton (11-H) gave cross peaks to a broad threefold doublet at δ_H = 1.81 (2-H), a double doublet at 1.15 ppm (7α-H), and two methyl groups at δ_H = 1.05 (14-H) and 0.93 (15-H) (Table 1). The results of extensive ^1H NMR decoupling experiments together with the $^1H/^1H$-COSY NMR were only

consistent with the constitution of (1R*,2S*, 6S*,8S*,11R*)-1,4,4,8-tetramethyl-tricyclo [6.2.1.$0^{2,6}$]undecan-11-ol (1) which we named cerapicol. The observed long-range coupling ("W-coupling") of the proton at C-11 with 7ß-and 2ß-H is only possible in an endo-position of the hydroxygroup. The cis annellation of the cyclopentane ring is required for the NOE observed between 2- and 6-H (Table 2).

Table 1: ^{1}H NMR data of cerapicol (1) and ceratopicanol (2)

	1	2	
	$CDCl_3$	$CDCl_3$	C_6D_6
2ß-H	ddd(br) 1.81	ddd 2.36	2.37
3α-H	dd 2.01	dd 1.39	1.49
3ß-H	dd(br) 1.29	ddd 1.34	1.45
5α-H	dd 1.14	dd 1.23	1.37
5ß-H	ddd 1.71	ddd 1.70	1.80
6ß-H	ddddd 2.22	ddddd 2.49	2.52
7α-H	dd(br) 1.46	dd 1.08	1.18
7ß-H	dd 1.15	dd 2.17	2.37
9-H	dddd 1.24	dd 3.72	3.46
9'-H	ddd 1.55	-	-
10-H	ddd 1.40	m 1.91	1.85
10'-H	ddd 1.32	m 1.51	1.55
11-H	s(br) 3.18	m 1.42	1.4
11'-H	-	m 1.56	1.4
12-H	s 0.91	s 0.89	1.03
13-H	s 1.055	s 1.06	1.21
14-H	s 1.05	s 0.90	0.95
15-H	s 0.93	s 1.06	1.12

J(Hz): 1: 2ß,3α-14; 2ß,3ß-6; 2ß,6ß-8; 3α,3ß-12; 3ß,5ß-0.5; 5α,5ß-13.5; 5α,6ß-3.5; 5ß,6ß-8.5; 6ß,7α-13; 6ß,7ß-6.5; 7α,7ß-13; 9,9'-12; 9,10-12; 9,10'-4; 9',10-4; 9',10'-12; 10,10'-12; unresolved long-range couplings from COSY: 2ß,11ß; 3α,12; 3ß,13; 5α,12; 7α,9α; 7α,15; 7ß,11ß; 9ß,15; 10α,14; 11ß,14; 11ß,15; 12,13.
J(Hz): 2: 2ß,3α-11; 2ß,3ß-8; 2ß,6ß-8; 2ß,14>0; 3α,3ß-12.5; 3α,12>0; 3ß,5ß-1.5; 5α,5ß-13; 5α,6ß-5; 5α,12>0; 5ß,6ß-8; 6ß,7α-6.5; 6ß,7ß-9.5; 7α,7ß-13.7; 9,10-8; 9,10'-8; 12,13>0.

To the best of our knowledge this is the first report of a natural product with such a carbon skeleton very probably derived by rearrangement of a protoilludane cation. Ohfune and co-workers synthetized cerapicol by acidic rearrangement of a compound related to protoilludane[3]. The published data of this compound are in good agreement with ours. Very recently Ayer and McCaskill reported on bullerone from Cyathus bulleri (belonging to the basidiomycetous order Nidulariales) which possesses a 4,5-seco-cerapican skeleton[4].

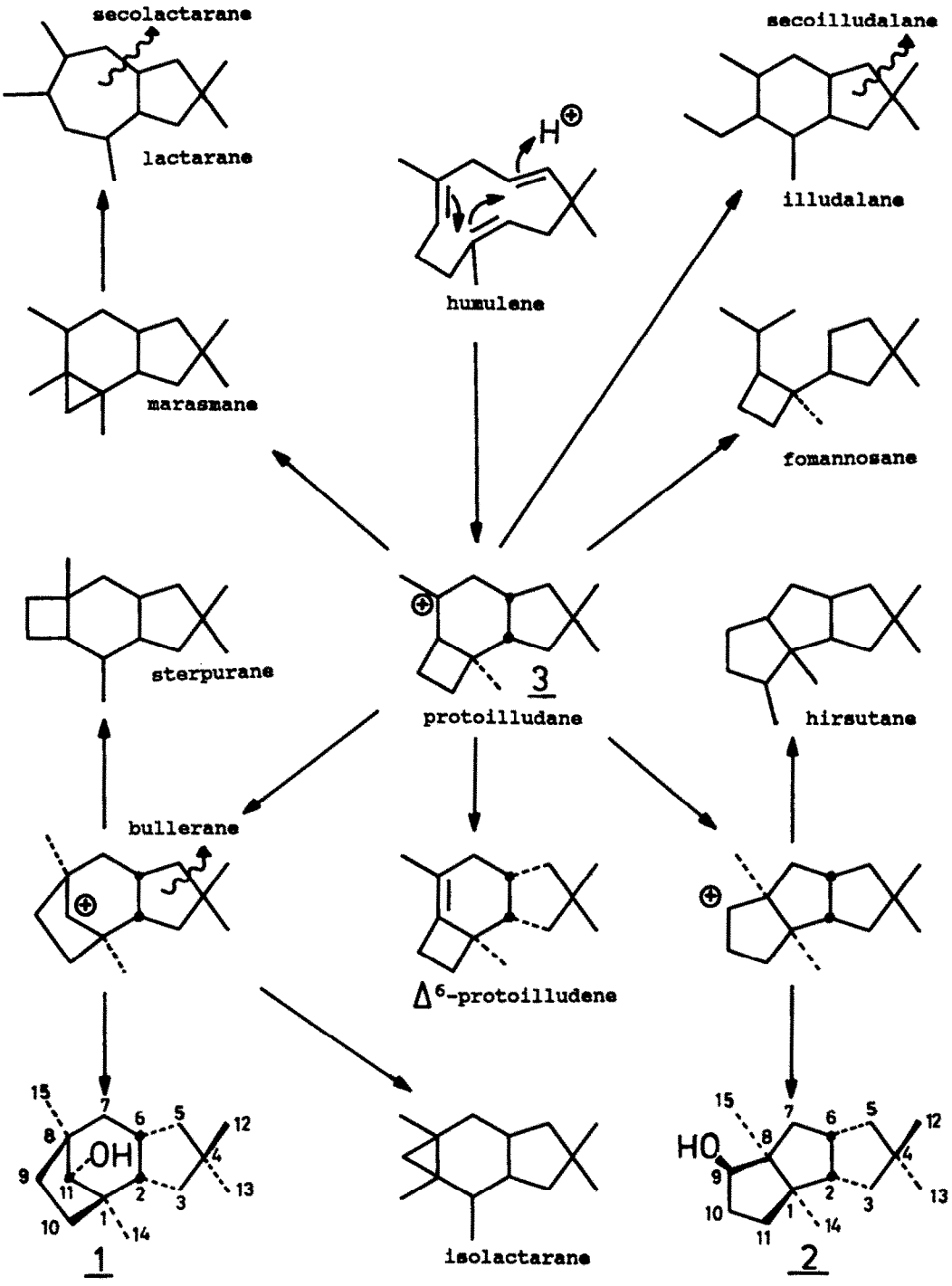

Figure 1: Cerapicol (1) and ceratopicanol (2) and their position in the "protoilludane-tree" of sesquiterpenoids

The second alcohol was isolated only in minor amounts. The ^1H NMR spectrum gave no hints for a double bond in the molecule so again a tricyclic skeleton was assumed. A double doublet at $\delta_H = 3.72$ in the ^1H NMR spectrum pointed to the α-proton of a secondary alcohol. The coupling pattern of this compound was in part similar to that one of 1. A fivefold doublet at 2.49 ppm coupled with resonances at 2.36 (ddd, 2ß-H), 1.08 (dd, 7α-H), 2.17 (dd, 7ß-H), 1.23 (dd, 5α-H), and 1.70 (ddd, 5ß-H). A ^4J-coupling between 3ß- and 5ß-H, between 3α- and 12-H, and between 5α- and 12-H is found in the COSY spectrum. Such couplings are only possible if 3β- and 5β-H are in a pseudo-equatorial and 3α-, 5α-, and 12-H in

Table 2: Results of NOE experiments on 1 and 2

Compound	Resonance irradiated	Resonance enhanced
1	2ß	6ß > 3ß
	11	14, 15 > 10', 9'
2	2ß	3α, 12
	9α	10α, 15
	7ß	7α > 6ß
	5ß	5α > 6ß > 7ß > 12

a pseudo-axial position. Decoupling experiments and $^1H/^1H$ NMR COSY led to the constitution (1R*, 2S*,6S*,8S*,11R*)-1,4,4,8-tetramethyl-tricyclo[6.3.0.$0^{2,6}$] undecan-9-ol (2), for which we choose the name ceratopicanol. A nuclear Overhauser enhancement at 15-H was observed on irradiation at the resonance of 9-H which requires an anti-orientation of the hydroxyl- and the 15-methyl group. The configuration of the ring system is cis-anti-cis because of the appearance of a NOE between 7ß- and 5ß-H and the lack of a NOE between 7ß- and 15-H. The observed W-coupling between 2-H and 14-H requiring the intervening dihedral angles to be both close to 180° is another confirmation for this configuration. The carbon skeleton of ceratopicanol is also unprecedented.

Biosynthetically both skeleton can be derived by the rearrangement of the protoilludyl cation 3. Comer and co-workers postulated the ceratopicanyl cation to be an intermediate in the biosynthesis of the hirsutane sesquiterpenoids[5]. Feline and Mellows[6] came to the same conclusion in their discussion of the ^{13}C labelling pattern in hirsutic acid C.

Until recently sesquiterpenoids with protoilludane skeleton or hereof by rearrangement or carbon-carbon cleavage derived compounds were restricted to basidomycetous fungi[7]. Ceratocystis piceae Ha 4/82 was the first and so far only sole ascomycete producing this kind of tricyclic terpenoids and it became the first known source of these often postulated intermediates in sesquiterpene biosynthesis.

EXPERIMENTAL

The ^{1}H NMR spectra were recorded at 400 MHz on a Bruker WM 400 spectrometer and the ^{13}C NMR spectra at 75.5 MHz on a Bruker AM 300 instrument[8]. If not stated otherwise, $CDCl_3$ was used as solvent, and TMS was an internal standard. MS analyses were carried out on a Varian MAT 111 (GNOM) mass spectrometer (80 eV); HR-MS (7500) analyses were performed on a Varian MAT 311 instrument (70 eV)[9]. Infrared spectra were measured in chloroform on a IR Spectral-Photometer 297, Perkin Elmer. Optical rotation were obtained on a Perkin-Elmer Polarimeter 241 in chloroform.

GC analyses were performed using a glass capillary WG-11 column (22 m x 0.3 mm i. d.), FID and a computing integrator. Operating conditions: linear temperature programme 80-200°, 2°/min; injection vol. 1.0 μl. Quantities of the sesquiterpenes were calculated using 6-methyl-5-hepten-2-one as an internal standard and FID specific substance factors.

C. piceae Ha 4/82 was isolated from pine logs in Friedrichsruh, West Germany, and cultivated on a glucose (2%)-asparagine (0.15%)-malt extract (0.25%)-mineral salt medium[10] solidified with 1.5% agar for 21 days. Volatiles were obtained by circulation steam distillation in pentane. The crude extract was further separated on a Si-60 column with a n-hexane/ethyl acetate gradient (changing from pure hexane to 19 : 1). Beside 14.5 mg/l of 6-protoilludene as the main component two oxygenated metabolites could be isolated in amounts of 9.4 mg/l cerapicol (1) and 1.6 mg/l ceratopicanol (2).

Cerapicol (1):
Colorless liquid, R_f 0.72 (dichloromethane, Si-60).

$\alpha^{20°}$ =	589nm	578nm	546nm	436nm	365nm	
	+24.7°	+26.0°	+29.3°	+48.8°	+73.9°	(c=1.00)

HR-MS: M^+ 222.1977 (222.1982 calculated for $C_{15}H_{26}O$); GLC/MS (m/z): 222 (M^+, 14% rel. intensity), 207 ($M^+-CH_3^\cdot$, 26), 204 (M^+-H_2O, 18), 191 (52), 189 (40), 175 (32), 161 (26), 109 (40), 95 (100).

Ceratopicanol (2):
Colorless gum, R_f 0.27 (dichloromethane, Si-60).

$\alpha^{20°}$ =	589nm	578nm	546nm	436nm	365nm	
	+6.4°	+6.6°	+7.4°	+10.8°	+14.8°	(c=0.50)

HR-MS: M^+ 222.1966 (222.1982 calculated for $C_{15}H_{26}O$); GLC/MS (m/z): 222 (M^+, 1%), 207 (1), 204 (3), 163 (66), 121 (10), 107 (21), 95 (17), 89 (24), 73 (45), 59 (17), 58 (16), 45 (100).

REFERENCES AND NOTES

1 for review see: Turner, W. B.; Aldridge, D. C.; Fungal Metabolites II, Academic Press, London (1983); recent work: Bradshaw, A. P. W.; Hanson, J. R.; Hitchcock, P. B.; Phytochemistry 21, 942 (1982); Favre-Bonvin, J.; Gluchoff-Fiasson, K.; Bernillon, J.; Tetrahedron Lett. 23, 1907 (1982); Donelly, D. M. X.; Sanada, S.; O'Reilly, J.; Polonsky, J.; Prange, T.; Pascard, C.; J. C. S. Chem. Commun., 1982, 135; Midland, S. L.; Izac, R. R.; Wing, R. M.; Zaki, A. I.; Munnecke, D. E.; Sims, J. J.; Tetrahedron Lett. 23, 2515 (1982); Donelly, D. M. X.; Coveney, D. J.; Polonsky, J.; Tetrahedron Lett. 26 5343 (1985); Giannetti, B. M.; Steffan, B.; Steglich, W.; Kupka, J.; Anke, T.; Tetrahedron 42, 3587 (1986); Arnone, A.; Cardillo, R.; Nasini, G.; Intern. Symp. on Org. Chem. of Medicinal Nat. Prod., Shanghai, B-033 (1985); Arnone, A.; Cardillo, R.; Nasini, G.; Phytochemistry 25, 471 (1986); Arnone, A.; Nasini, G.; Assante, G.; Roeijmans, H. J.; van Eijk, G. W.; Phytochemistry 26, 1869 (1987).
2 Hanssen, H.-P.; Sprecher, E.; Abraham, W.-R.; Phytochemistry 25, 1979 (1986).
3 Ohfune, Y.; Shirahama, H.; Matsumoto, T.; Tetrahedron Lett. 32, 2795 (1976).
4 Ayer, W.A.; McCaskill, R.H.; Can. J. Chem. 65, 15 (1987).
5 Comer, F.W.; McCapra, F.; Qureshi, I.H.; Scott, A.I; Tetrahedron 23, 4761 (1967).
6 Feline, T.C.; Mellows, G.; J. Chem. Soc., Chem. Commun. 1974, 63-64.
7 Ayer, W.A.; Browne, L.M.; Tetrahedron 37, 2199 (1981).
8 Particulars of NOE and 2D-$^1H/^1H$- and $^{13}C/^1H$- chemical shift correlation experi-

ments are described in: Abraham, W.-R.; Ernst, L.; Witte, L.; Hanssen, H.-P. and Sprecher, E.; Tetrahedron 42, 4475 (1986).
[9] Conditions are described in: Hanssen, H.-P.; Sinnwell, V.; and Abraham, W.-R.; Z. Naturforsch. 41c, 825 (1986).
[10] Sprecher, E.; Planta 53, 565 (1959).