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SHORT REPORTS

6-PROTOILLUDENE, THE MAJOR VOLATILE METABOLITE FROM *CERATOCYSTIS PICEAE* LIQUID CULTURES

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Key Word Index—*Ceratocystis piceae*; Ascomycetes; 6-protoilludene; sesquiterpenes; fungal terpenoids; volatiles; culture conditions.

Abstract—A newly isolated strain of the ascomycete *Ceratocystis piceae* was grown on defined synthetic liquid culture media. Volatiles were obtained by steam distillation or pentane extraction, and analysed by GC and GC/MS. 6-Protoilludene was found to be the major volatile constituent. Structure elucidation resulted from NMR data using 2D-$^{13}C/^{1}H$-shift correlation. The influence of different nitrogen sources on 6-protoilludene accumulation was investigated.

INTRODUCTION

So far, illudanes and related sesquiterpenoids have been considered to be exclusive metabolic products of Basidiomycetes [1, 2]. It has been assumed that the sesquiterpene hydrocarbon 6-protoilludene (**1**) might be a biogenetically significant precursor of illudanes, marasmanes, and lactaranes, and that 6-protoilludene itself is formed from farnesyl pyrophosphate via humulene or humulenium ion [3, 4]. In the present communication, we describe the isolation of 6-protoilludene from the ascomycete *Ceratocystis piceae* (Münch) Bakshi, a sapwood staining species occurring on coniferous logs and lumber [5]. Furthermore, the influence of different inorganic and organic nitrogen sources on 6-protoilludene accumulation in defined synthetic liquid cultures of the fungus was studied.

RESULTS AND DISCUSSION

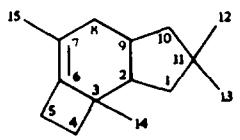
The sapwood staining fungus *C. piceae* Ha 4/82 was cultivated on liquid glucose–mineral salt media differing only in the nitrogen source (Table 1). Under these culture conditions, only a weak submerse mycelium was formed. The steam distillates and pentane extracts, respectively, obtained from cultures of different ages, consisted almost exclusively of a predominant compound which was characterized as a sesquiterpene hydrocarbon by its GC/MS data, and some 10–20 trace components. The fragmentation pattern of the major constituent was in good agreement with data given for 6-protoilludene, a metabolite isolated for the first time from mycelia of the basidiomycete *Fomitopsis insularis* [6]. This compound has been postulated to be a biogenetically significant intermediate in the formation of various sesquiterpenoids hitherto found only in Basidiomycetes [1, 2]. Structure elucidation was achieved using homonuclear double resonance ^{1}H NMR. The ^{13}C NMR spectrum of the hydrocarbon was in good agreement with the data published by Nozoe *et al.* [6] (Table 2) and was assigned by 2D-$^{13}C/^{1}H$-NMR shift correlation (Table 3).

Using different nitrates and amino acids (Table 1) the highest yields of 6-protoilludene (60.6 and 34.2 mg/l, respectively) were obtained, when phenylalanine and isoleucine were offered as sole N-sources. Similar observations had been made with *Lentinus lepideus* FPRL 7B, a brown-rot fungus producing besides cinnamic acid derivatives mainly cadinane-type sesquiterpenes [7, 8].

With surface cultures grown on solid media, an increased percentage of oxygenated metabolites could be achieved. The structure elucidation of these compounds is under way.

EXPERIMENTAL

Cultivation. Ceratocystis piceae Ha 4/82 was isolated from pine logs in Friedrichsruh, West Germany and cultivated in defined synthetic liquid media with glucose (2%), a nitrogen source (concn for the different assays, see Table 1), thiamine and



1

Table 1. Effect of nitrogen source on 6-protoilludene accumulation in liquid cultures of *Ceratocystis piceae* Ha 4/82

N-source	%	N/l. culture medium (mM)	Maximum of protoilludene accumulation (mg/l)
Ammonium nitrate	0.05	12.50	1.0
Calcium nitrate	0.10	8.47	0.2
Asparagine	0.10	13.33	15.7
Glutamine	0.10	13.89	4.9
Alanine	0.10	11.23	17.9
Leucine	0.15	11.40	3.6
Isoleucine	0.15	11.40	34.2
Phenylalanine	0.15	9.09	60.6

Table 2. ^{1}H NMR data of 6-protoilludene (300 MHz, $CDCl_3$, TMS internal standard)

H	δ, multiplicity	J (Hz)
1α	1.31 *dd*	1α, 1β = 12.5
1β	1.36 *ddd*	1α, 2β = 10
2β	2.16 *ddd*	1β, 2β = 8
4	1.80 *m*	1β, 10β = 2
4′	1.73 *m*	2β, 9β = 11
5	2.73 *ddddq*	4, 5 = 8
5′	2.54 *ddddq*	4, 5′ = 3.5
8α	1.65 *dddd*	4′, 5 = 8
8β	1.83 *dd*	4′, 5′ = 7
9β	2.35 *dddd*	5, 5′ = 14.5
10α	0.95 *m*	5, 8α = 2
10β	1.53 *ddd*	5′, 8α = 2
12	1.06 *s*	5, 15 = 2
13	1.05 *s*	5′, 15 = 2
14	0.93 *s*	8α, 8β = 14
15	1.57 *dd*	8α, 9β = 11
		8β, 9β = 7
		9β, 10α = 11
		9β, 10β = 8
		10α, 10β = 12.5

Table 3. ^{13}C NMR data of 6-protoilludene (75.5 MHz, $CDCl_3$, TMS internal standard) 2D-^{13}C/^{1}H-shift correlation

Carbon	δ, multiplicity	Correlation with proton at
1	41.1 *t*	1.31 and 1.36
2	47.2 *d*	2.16
3	45.8 *s*	—
4	36.9 *t*	1.73 and 1.81
5	25.6 *t*	2.73 and 2.54
6	141.8 *s*	—
7	123.1 *s*	—
8	34.2 *t*	1.65 and 1.83
9	40.6 *d*	2.35
10	48.6 *t*	0.95 and 1.53
11	39.3 *s*	—
12	20.5 *q*	1.06
13	29.9 *q*	1.05
14	27.5 *q*	0.93
15	17.4 *q*	1.57

mineral salts [9] in 1 l. Fernbach flasks containing 250 ml of culture medium without shaking. Volatiles were determined four to eight times during a culture period of 3–4 months.

Identification of 6-protoilludene. Volatile metabolites were obtained by circulation steam distillation [10] or pentane extraction. The hydrocarbon was separated by TLC using *n*-hexane. NMR spectra were recorded on Bruker AM 300 in $CDCl_3$ with TMS as int. standard.

MS were recorded on a GC/MS system at 80 eV, *m/z* (rel. int.): 204 $[M]^+$ (20), 189 $[M-Me]^+$ (15), 176 $[M-C_2H_4]^+$ (14), 175 $[M-C_2H_5]^+$ (72), 119 $[M-C_5H_{11}]^+$ (100), 105 $[M-99]^+$ (69). GC analyses were performed using a glass capillary WG-11 column (22 m × 0.3 mm i.d.), FID and a computing integrator. Operating conditions: linear temp. programme 80–200°, 2°/min; injector, 180°; detector, 180°; carrier gas, N_2 at 1 ml/min; injection vol. 1.0 μl. Quantities of 6-protoilludene were calculated using 6-methyl-5-hepten-2-one as an internal standard and FID specific substance factors.

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