Table 1. Table showing the presence of different amino acids at 35 and 50 °C in the mycelium of *Mucor miehei*

Amino acids	4th day		8th day		12th day	
	35 °C	50 °C	35 °C	50 °C	35 °C	50 °C
1 Asparagine	+	−	+	−	+	−
2 Aspartic acid	+	−	+	−	+	−
3 α-Alanine	+	+	+	+	+	+
4 γ-Amino-n-butyric acid	+	−	+	−	+	−
5 Glutamine	+	+	+	+	+	+
6 Glutamic acid	+	+	+	+	+	+
7 Glycine	+	+	+	+	+	+
8 Histidine	+	+	+	+	+	+
9 Leucine	+	−	+	−	+	−
10 Lysine	+	+	+	+	+	+
11 Phenylalanine	+	−	+	−	+	−
12 Serine	+	+	+	+	+	+
13 Valine	+	+	+	+	+	+

+, Present; −, absent.

Table 2. Effect of aspartic acid and phenylalanine on zygospore formation at 50 °C

Amino acid	Dextrose	Nitrogen concentration (g/l)		
		1.5	1	0.5
Aspartic acid	10 g	−	++	−
	5 g	+	+++	+
	4 g	+	−	−
	2 g	−	−	−
Phenylalanine	10 g	+	++	+
	5 g	+	+++	+++
	4 g	+	−	−
	2 g	−	−	−

−, No zygospore; + few zygospores; ++, many zygospores; +++, abundant zygospores.

Excluding asparagine, which had already been tested, being a constituent of the initial medium, the remaining 4 amino acids were supplied to the fungus grown at 50 °C by incorporating them singly in the basal medium. The basal medium used was the same as for the previous experiments except that instead of 40 g of dextrose, 5 g was used and asparagine was replaced by 1 of the remaining 4 amino acids singly in quantities so as to supply the same amount of nitrogen as in asparagine. It was observed (table 2) that out of the 4 amino acids tested aspartic acid and phenylalanine were able to induce abundant zygospore formation at 50 °C in *M. miehei*. Further studies on the role of amino acids in zygospore formation at high temperatures in thermophilic fungi are in progress.

1 Present address: Botany Department, Kumaun University, Naini Tal 263001 (India).
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Preformed azulene pigments of *Lactarius indigo* (Schw.) Fries (Russulaceae, Basidiomycetes)[1]

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Summary. The structure of a new naturally-occurring azulene, 1-stearoyloxymethylene-4-methyl-7-isopropenylazulene, obtained from an acetone extract of the blue mushroom *Lactarius indigo,* has been determined by chemical and spectral methods.

Azulenes have been obtained from a wide variety of plant sources[4], but in almost all cases, these pigments are not present in the living plant but are formed during isolation from suitable precursors[5]. We wish to report the occurrence of evidently pre-existing, exceedingly sensitive azulenes in the mushroom *Lactarius indigo,* and the determination of the structure of the main blue azulene from this species.

Fresh sporophores of fungi from this large genus contain a true latex[6] which, in a number of species, is highly colored. The only such species studied chemically so far, the orange *L. deliciosus* (Fr.) S.F. Gray, has yielded 2 azulenes, the blue lactarazulene (**1**)[7] and the reddish-violet lactaroviolin (**2**)[8]. However, neither of these occurs as such[9]; the orange color of the fungus is due, in European specimens, to the extremely sensitive dihydroazulenes (**3**) and (**4**)[10], while fungi from California have yielded lactarofulvene (**5**)[11]. The isolation of these pigments from a *Lactarius* species suggested the intriguing possibility that the color of the deep blue latex of young specimens[12] of *L. indigo* might be due to pre-existing azulenes. We have now shown that this is indeed true.

Preliminary chromatographic studies on 1 young specimen of *L. indigo* found in Potomac, Maryland, several years ago seemed to show the presence of 1 blue pigment, extractable with acetone and soluble in hexane. The absorption spectrum of the crude pigment suggested an azulene related to **1**

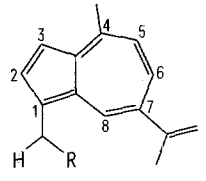
or its isopropyl analog, guaiazulene (**6**). The unknown pigment differed from these stable azulenes by its extraordinary sensitivity, being almost instantaneously converted to an intractable green substance upon addition of methanol to its solution in acetone, on gentle warming, or on attempted chromatography on any but the least polar adsorbents (e.g. paper, calcium phosphate). No further characterization was possible with the extremely limited amount of material available[13].

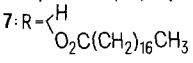
Filtered acetone extracts from a collection of fresh, young mushrooms from Florida[14] (58 g) were concentrated in vacuo, and the crude pigments were transferred to hexane. Column chromatography (silica gel 60, activity III, hexane/acetone 6:1)[15] provided 4 colored zones: blue, violet, blue-green, and blue (in order of elution). The pigments from the first 2 zones were purified further by repeated chromatography.

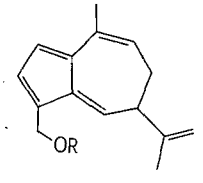
The violet band yielded **2** (< 0.5 mg), identified by mass spectrometry and by its electronic and ^{1}H-NMR spectra.

The major blue pigment (97 mg) from the least polar zone crystallized from hexane as dark blue prisms, m.p. 48.5–49 °C. The similarity of its electronic spectrum to those of **1** and **6** showed that the compound was indeed an azulene. An IR-band at 1729 cm^{-1} indicated an ester linkage. Chemical ionization mass spectrometry gave a parent ion at m/e 478 which, together with the preceeding information, suggested the molecular formula $C_{35}H_{50}O_2$ [Found: C, 83.02; H, 10.22; Calc. C, 82.79; H, 10.53]. The ^{1}H-NMR spectrum showed AB and ABX signals similar to those of the aromatic protons of **6** (table), proving that the azulene from *L. indigo* possesses a guaiane skeleton. In addition, 1 of the methyl groups has been replaced by $-CH_2-O_2C(CH_2)_{16}CH_3$; NMR signals from this moiety [δ 0.87 (complex t, 3 H), 1.25 (br s, 28 H), 1.61 (t, 2 H), and 2.30 (t, 2 H)][16] are in agreement with this interpretation. These data indicate that the blue pigment should be the stearate ester of an hydroxymethyl analog of **1**. The base peak in the mass spectrum, m/e 195 ($C_{15}H_{15}^+$), supports such a structure.

NMR data show that the stearoyloxy group modifies the methyl at C-1 of **1**. It could be excluded from the isopropenyl side chain because long-range allylic coupling [J = 1 Hz] was observed. The resonances of methyl groups from a series of substituted azulenes have been examined[17], and it has been shown that the methyl at C-1 resonates near δ 2.65, while the signal for the one at C-4 occurs near δ 2.85.



1: R = H_2
2: R = =O
6: R = H_2; isopropyl replaces isopropenyl
7: R = 



3: R = H
4: R = $CH_3(CH_2)_{16}CO-$

Ring proton NMR resonances for azulenes **2**, **6**, and **7**

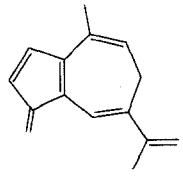
Compound	C-2	C-3	C-5	C-6	C-8
2	8.23	7.34	7.52	7.89	9.96
6	7.61	7.20	6.98	7.42	8.18
7	7.84	7.29	7.15	7.71	8.62

Coupling constants for compounds **2**, **6**, and **7**: $J_{2,3} = 4.0 \pm 0.2$ Hz; $J_{5,6} = 10.8 \pm 0.3$ Hz; $J_{6,8} = 2.0 \pm 0.1$ Hz.

A peak at δ 2.88 (s, 3 H) was recorded for the *Lactarius* pigment, which has therefore been assigned structure **7**.

The presence of a stearic acid residue was conclusively proved by hydrolysis of the pigment (5 mg) with 5% alcoholic KOH at room temperature or, alternatively, by filtration of its hexane solution through a short column of silica gel (activity I), whereby pure stearic acid, m.p. 68–69.5 °C (Beilstein[18] 70.1 °C) was obtained. This was converted (CH_2N_2) to the methyl ester, which was identified as methyl stearate by m.p. 35–36 °C (Beilstein[19] 38.5–39.5 °C), gas chromatography, and mass spectrometry. The parallelism in the pigmentation of *L. indigo* and *L. deliciosus* is striking, the main pigment (**4**) of the latter species being a dihydro derivative of **7**.

The 2 more polar minor pigments decomposed even on brief storage at − 5 °C and have not yet been investigated further. Remarkable instability was also demonstrated by the addition of a few drops of methanol to acetone solutions of the pigments, whereupon they were almost instantaneously transformed into green material. In contrast, the analogous transformation of **7** required more than 30 min. On the other hand, addition of a trace of HCl gas to a $CDCl_3$ or hexane solution of **7** produced an instant color change to green. The resulting solution retained the NMR signals of stearic acid, while those from the azulene had disappeared. The green product thus seems to be polymeric; it may be related to the green material obtained by Hafner and Bernhard[20] from acid-treatment of 1-hydroxymethylazulene, by way of very unstable intermediates of type **8**.



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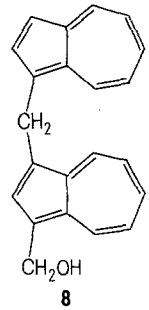

8

The natural blue color of *L. indigo* and the isolation of a substantial amount of blue pigment by employing mild extraction and purification conditions supports the occurrence of this azulene in the living fungus. We hope to study the remaining blue pigments if additional material becomes available.

1 This paper is dedicated to Prof. André Dreiding, Zürich, on the occasion of his 60th birthday.
2 Present address: National Heart, Lung, and Blood Institute, Bethesda, Maryland 20205, USA.
3 To whom inquiries should be addressed.
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14 Collected in July, 1977, by H.E. Barnhart at Gainsville, Florida. We wish to thank Col. Barnhart for his help in collecting and shipping the mushrooms.
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The jump of *Orchestia cavimana* Heller, 1865 (Crustacea, Amphipoda, Talitridae)

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Summary. The saltatory locomotion of the Talitrid (Crustacea) *Orchestia cavimana* Heller, 1865, was studied by high frequency cinematography (1000 fps). The jump lasts about 350–400 msec and covers a distance of 18 cm due to an average acceleration of 300 m/sec^2. About 4–6 somersaults are performed in the course of each jump.

The conspicuous locomotory jumps of the beachhopper, *Orchestia cavimana* (Talitridae, Amphipoda), first mentioned in 1879[2], were studied by high frequency cinematography at 1000 frames/sec (figure 1).

In getting ready for the jump, the 7th pereopods stretch fully and the thorax bulges upwards. The abdomen is lifted off the ground so that the urosome, so far flexed under the body, can be stretched downwards at an angle of about 45°. At the end of stretching, the 7th pereopods, and also the tips of uropods 1–3, touch the substratum.

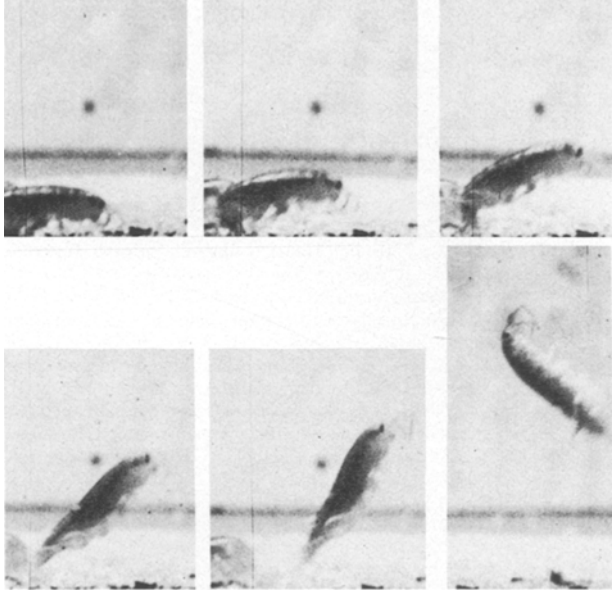

Fig. 1. *Orchestia cavimana,* take-off. Frames 0, 2, 4, 6, 8, and 18 of a film taken at 1000 frames/sec. Frame 18 shows the beginning of the somersault movement.

Thus, in the launching phase (figure 2), the bent metasome is increasingly stretched and the uropods are pressed against the ground, serving as a catapult. Immediately before take-off, the 6th and 7th pereopods are clear off the ground, and only the 5th pereopods give lateral support to the body. A jerking and stretching movement of the metasome pushes the animal into a ballistic trajectory along which 4–6 somersaults tail over head are performed. Depending on the animal's posture at take-off, additional rotations around its longitudinal axis occur ('Schraubensalto').

The jump lasts between 350 and 400 msec varying with height and distance. The average angle of take-off is 43°, and the average distance is 18 cm. Initial acceleration is brought about by 2 thrusts (figure 3b): a 1st one by

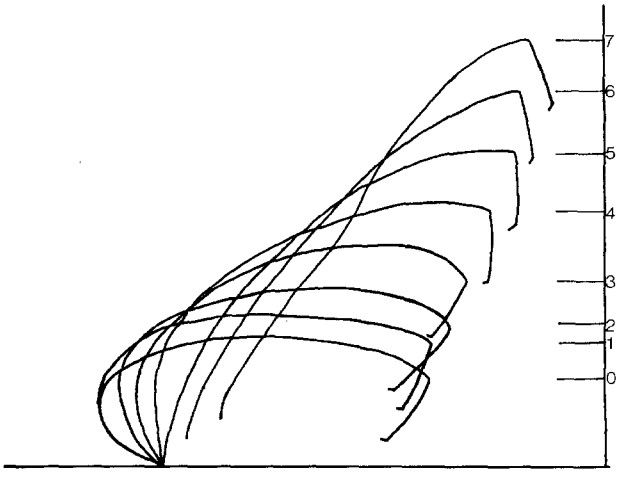

Fig. 2. Profiles of *O. cavimana* from the same film from which figure 1 is taken. Frame numbers are shown on the right.