Tetrahedron Vol. 43, No. 6, pp. 1075 to 1082, 1987
Printed in Great Britain.

0040-4020/87 $3.00+.00
Pergamon Journals Ltd.

NOVEL AZEPINE DERIVATIVES FROM THE PUNGENT MUSHROOM CHALCIPORUS PIPERATUS[1]

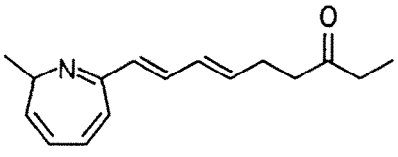
OLOV STERNER[2], BERT STEFFAN AND WOLFGANG STEGLICH*

Institut für Organische Chemie und Biochemie der Universität
Bonn, Gerhard-Domagk-Straße 1, D-5300 Bonn (FRG)

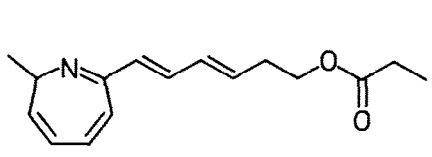
(*Received in Germany* 18 *December* 1986)

Abstract - Two pungent 2H-azepines, chalciporone (1) and norchalciporyl propionate (2), were isolated from ethyl acetate extracts of fruit-bodies of the fungus Chalciporus piperatus, together with the related but nonpungent 3H-azepines isochalciporone (3) and dehydroisochalciporone (4).

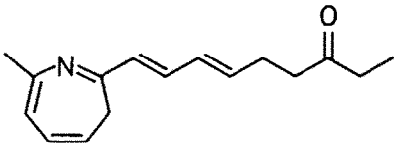
Fruit-bodies of the "Pfefferröhrling" Chalciporus piperatus (Bull. ex Fr.) Bat. (Basidiomycetes) are distinguished from other boletes by their peppery taste as well as by lemon-yellow coloured stem bases. We have investigated the chemical background for both of these characteristics, and in this paper we report on the isolation and structural elucidation of the pungent[3] principles. Pungent tasting extracts were prepared from both frozen and fresh fruit-bodies, and the extracts were subjected to column and preparative thin layer chromatography (tlc). Large amounts of the common fungal steroid ergosterol were excluded from the extracts by Al_2O_3 chromatography, and the major component of the remainings, chalciporone (1), could be isolated by silica gel chromatography. 1 is a very pungent com-



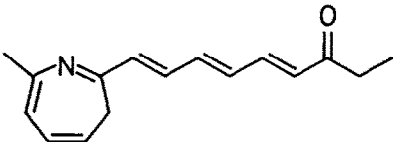
1



2



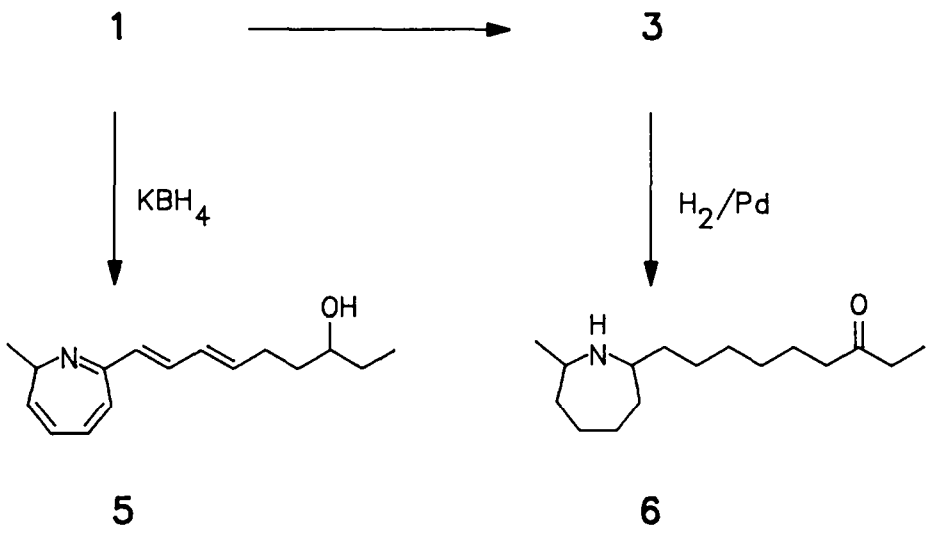
3



4

pound: a few µg applied on the tongue is sufficient to cause an intense burning sensation. In addition, small amounts of norchalciporyl propionate (2), which is approximately as pungent as 1, various amounts of the nonpungent isochalciporone (3), and small amounts of the nonpungent dehydroisochalciporone (4) were isolated.

Chalciporone (1) spontaneously isomerizes to isochalciporone (3), for example during prolonged NMR experiments in $CDCl_3$ solution at room temperature. Further derivatives were prepared by KBH_4 reduction of 1 which yielded the alcohol 5, and by hydrogenation of isochalciporone (3) to form the perhydro derivative 6 (see Scheme 1).



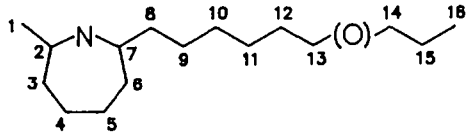
Scheme 1

Spectral data (including the high resolution mass spectrum) and elemental analysis established the atomic composition of chalciporone (1) as $C_{16}H_{21}NO$. The ^{13}C NMR and IR spectra indicated the presence of a saturated keto functionality, and the fragmentation pattern of both 1 and its dihydro derivative 5 suggested that the carbonyl group is part of a 3-oxopentyl chain. Double resonance ^1H NMR experiments showed that this moiety in turn is linked to a -CH=CH-CH=CH- chain, resulting in a 7-oxonona-1,3-dienyl residue. The presence of such a nine-carbon chain was also supported by the fact that the MS base peak of the perhydro derivative 6 was $M^+ - C_9H_{17}O$ ($M^+ - (CH_2)_6COCH_2CH_3$). The odd number of olefinic carbon signals found in the proton noise decoupled ^{13}C NMR spectrum of 1 suggested that the nitrogen is part of an imine functionality, and two characteristic carbon signals[4] (a singlet at δ =161 and a doublet at 55 ppm) indicated that the imino group is fully substituted. ^1H-^1H and ^1H-^{13}C correlation experiments showed that the saturated carbon atom bound to the nitrogen is further connected to a methyl group and a -CH=CH-CH=CH- unit. In the ^1H NMR of the isomer isochalciporone (3), this methyl group (C-1) was shifted downfield and has become a singlet, indicating that the carbon (C-2) binding the methyl group has become olefinic. The strong optical activity of chalciporone (1) is totally lost

Table 1. ^{1}H NMR data of chalciporone **1**, oxychalciporone **2**, isochalciporone **3** and dehydroisochalciporone **4**, δ in ppm and J in Hz. The spectra were recorded at 400 MHz in $CDCl_3$ with TMS as internal standard.

	1	2	3	4
H	δ mult. J	δ mult. J	δ mult. J	δ mult. J
1	1.62; d; 6.5	1.60; d; 6.6	2.12; s;	2.23; s
2	2.92; dq; 6.5, 5.2	2.94; dq; 6.6, 5.4	- - -	- - -
3	5.67; dd; 5.2, 9.2	5.64; dd; 5.4, 9.3	5.93; d; 6.4	6.06; d; 6.7
4	6.23; dd; 9.2, 5.2	6.22; m;	6.18; dd; 6.4, 8.8	6.30; dd; 6.7, 8.8
5	6.83; dd; 5.2, 11.4	6.82; dd; 5.1, 11.5	5.04; dt; 8.8, 6.8	5.15; dt; 8.8, 6.8
6	7.09; d; 11.4	7.08; d; 11.5	a	a
8	6.27; d; 16.0	6.33; d; 15.9	6.07; d; 15.4	6.40; d; 15.5
9	6.63; dd; 16.0, 10.5	6.63; dd; 15.9, 10.5	6.78; dd; 15.4, 10.8	6.96; dd; 15.5, 10.8
10	6.16; dd; 10.5, 15.2	6.22; m;	6.09; dd; 10.8, 15.2	6.68; dd; 10.8, 15.0
11	5.87; dt; 15.2, 7	5.81; dt; 15.1, 7	5.87; dt; 15.2, 6.9	6.52; dd; 15.0, 11.0
12	2.40; m;	2.44; dq; 7, 6.6	2.35; m;	7.21; dd; 11.0, 15.5
13	2.53; t; 7.0	4.11; t; 6.6	2.53; t; 6.1	6.24; d; 15.5
15	2.40; m;	2.29; q; 7.6	2.35; m;	2.61; q; 7.3
16	1.07; t; 7.2	1.10; t; 7.6	0.98; t; 7.3	1.12; t; 7.3

a/ See text and Figure 1.



upon isomerization to **3**, and this is in accordance with the loss of the asymmetry at C-2. The structural elucidation of **3** was greatly facilitated by its relative stability which permitted the recording of coupled ^{13}C NMR spectra with selective irradiation of pertinent proton frequencies. In addition, a large NOE was observed on 3-H when the C-1 protons were irradiated. The ^{1}H NMR spectrum of norchalciporyl propionate (**2**) showed almost exactly the same signals as that of **1** (see Table 1), the only difference being a downfield shift of the 13-H_2 triplet. The MS indicated the presence of an extra oxygen, and the fragmentation pattern suggested that the keto functionality has been replaced by an ester. This was nicely confirmed by the IR spectrum (neat) in which the carbonyl signal was shifted from 1705 to 1735 cm^{-1}. The ^{1}H NMR spectrum of dehydroisochalciporone (**4**), on the other hand, was very similar to that of isochalciporone (**3**), the major differences being ascribed to the replacement of the $-CH_2-CH_2-$ unit of C-12 and C-13 by a -CH=CH- unit. All other spectroscopic data (IR, UV, MS) were also in accordance with the suggested structure.

In the ^{1}H NMR spectra of isochalciporone (**3**) and dehydroisochalciporone (**4**), the signals for C-6 protons are not observable, although the integral suggested the presence of extremely broad signals at about δ=2.5 ppm. Furthermore, in the ^{1}H-^{13}C correlation spectrum of **3**, no signal for C-6 appeared. These observations

Table 2. ^{13}C NMR data of chalciporone **1** and isochalciporone **3** (100.2 MHz, in $CDCl_3$, with TMS as internal standard).

	1			3		
Carbon	δ (ppm)	mult.	J (Hz)	δ (ppm)	mult.	J (Hz)
1	21.4	qd	126, 4	24.3	qd	127, 4
2	54.8	ddq	134, 4, 4	149.6	dqd	7, 7, 2
3	135.8	ddt	160, 6, 6	113.6	ddqd	155, 9, 4, 1
4	126.4	ddd	159, 11, 5	127.4	dt	156, 6
5	136.7	dd	155, 11	114.2	ddt	163, 6, 6
6	129.2	ddd	156, 8, 4	32.6	tddd	133, 9, 6, 4
7	161.4	m		143.3	dt	5, 5
8	130.5	dd	155, 3	130.4	dd	156, 4
9	136.0	ddd	150, 7, 3	137.1	ddd	151, 8, 3
10	130.3	dm	151	130.2	dm	149
11	136.3	dm	151	137.7	dm	151
12	26.5	tm	129	26.7	tm	128
13	41.0	tm	125	41.0	tm	125
14	209.6	m		209.7	m	
15	35.5	tq	125, 4	35.6	tq	124, 4
16	7.4	qt	127, 4	7.4	qt	127, 4

indicated that the azepine ring is capable of ring inversion. Consequently, when the ^1H NMR spectrum of **3** was recorded at -63 and 55 °C in $CDCl_3$, the signals for the C-6 protons appeared as two double doublets at δ =1.35 and 3.96 ppm, and as a broad singlet at δ =2.62 ppm (see Figure 1). The coalescence temperature was found to be 2 ±1 °C, and ΔG = 49.4 kJ/mol. Upon irradiating 5-H (δ =5.04 ppm) at -63 °C, the signals for C-6 protons appeared as two doublets with J=11 Hz (see Figure 1). Almost identical observations have previously been reported for similar 3H-azepines[5].

For azepines, the order of stability is known to be 3H>4H>2H, and 2H-azepines are well known to be unstable compounds that *via* consecutive 1,5-H shifts readily rearrange to more stable isomers[6]. The fact that only isochalciporone (**3**) is formed from chalciporone (**1**) (the isomerization was followed by tlc and ^1H NMR), indicates that the most stable isomer is obtained by a single 1,5-H shift[7]. The ready isomerization of **1** raises the question of whether **3** is a true natural product or if it is formed during the extraction and isolation procedures. Actually, when young and seemingly unaffected specimens of *C. piperatus* were collected with great care, extracted rapidly, and investigated immediately by tlc, no trace of isochalciporone (**3**) was seen. However, when specimens were collected the normal way and not handled with extreme care, the fruit-bodies contain significant amounts of **3**. Dehydroisochalciporone (**4**) was always found to be present in small amounts in crude extracts. This is probably also the case for norchalciporyl propionate (**2**), although the large initial amount of ergosterol makes the tlc analysis uncertain. It is interesting to note that the pungency of chalciporone (**1**) is completely lost upon isomerization to isochalciporone (**3**). In **1** the pungency is obviously linked to the 2H-azepine part of

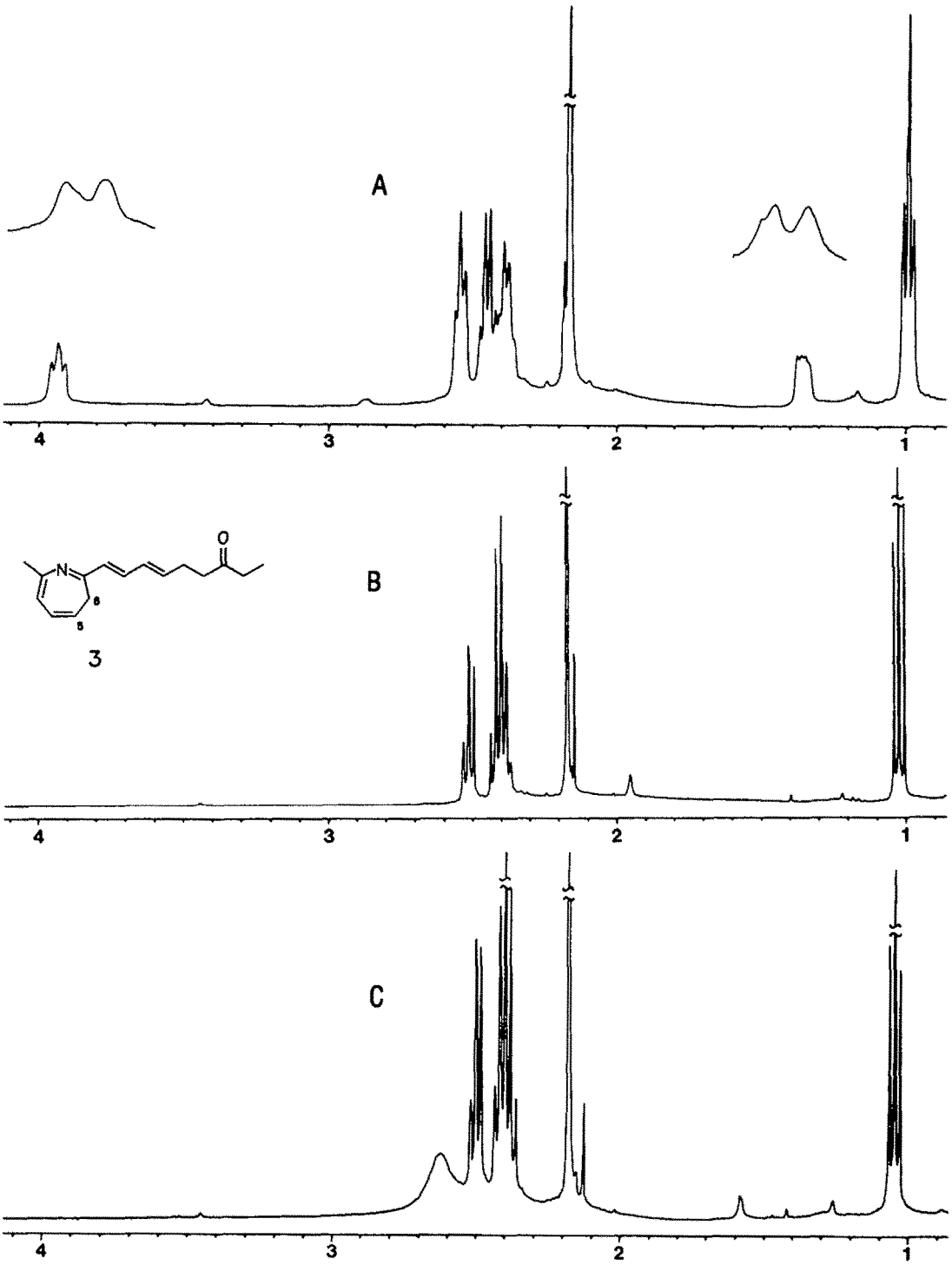


Figure 1. The pertinent parts of the ^{1}H NMR spectra (400 MHz) of isochalciporone (3) recorded at A -63, B 2, and C 55 °C. The signals for the C-6 protons appear as two double doublets at δ=1.35 and 3.96 ppm at -63 °C, are not observable at 2 °C, and appear as a broad singlet at 55 °C. Irradiation of the 5-H signal (δ=5.04 ppm) resulted in the collapse of the signals for the C-6 protons into two doublets, shown separately in spectrum A.

the molecule. Reduction of the keto function with KBH_4 resulted in a separable mixture of two diastereomers of alcohol 5, and both isomers were approximately as pungent as 1. Whether 2H-azepines are pungent in general, or if a certain substitution pattern (as in compounds 1, 2 and 5) is required, is not known.

Pungent compounds have been isolated from a variety of organisms, which may use them to discourage parasites and predators[8]. In the fruit-bodies of peppery Russulaceae species (genera Lactarius and Russula), the enzymatic formation of pungent unsaturated dialdehyde sesquiterpenes, as a response to physical damage, appears to constitute a refined chemical defense system[9]. No enzymatic conversions of the azepines were observed in C. piperatus, nevertheless, the presence of pungent compounds may offer a protection for the fruit-bodies. Similar to the pungent sesquiterpenes of the Russulaceae species, chalciporone (1) shows high antimicrobial activity[10], and fruit-bodies of C. piperatus that have been attacked and damaged by parasites are rarely observed (contrary to most mushrooms). Interestingly however, in spite of their pungency to man, the fruit-bodies of C. piperatus were not found to be distasteful to the opossum (Didelphis virginiana)[11] which is a natural fungivore.

The azepines isolated from C.piperatus represent a new type of natural products. A yellow dihydroazepine derivative, muscaflavin, has been isolated from fruit-bodies of Amanita muscaria and from several Hygrocybe species[12]. However, muscaflavin has been shown to be biogenetically derived from dihydroxyphenylalanine, and this is clearly not the case for the azepines discussed above. Previous investigations of Chalciporus piperatus have yielded several pulvinic acid derivatives, e.g. the red-violet variegatorubin[13].

EXPERIMENTAL

Fruit-bodies of Chalciporus piperatus were collected near Bonn in the autumns of 1984 and 1986. Those collected in 1984 were kept at -20 °C for 15 months before they were extracted, while those obtained in 1986 were extracted immediately. The fruit-bodies were ground in a turbomixer with ethyl acetate (2 l/kg), whereafter the ethyl acetate phases were dried with Na_2SO_4 and evaporated to dryness. The crude extract was subjected to chromatography on an Al_2O_3 column eluted with diethyl ether:petroleum ether 1:1, the azepines 1-4 were eluted as a mixture while ergosterol remained on the column. The azepine mixture was then separated by repeated column and preparative thin layer chromatography on SiO_2. Column chromatography was performed on "Merck Aluminiumoxid 90, standardized according to Brockmann, activity II-III" (0.063-0.200 mm, water content 4%), and "Merck Lobar pre packed" silica gel columns eluted with ethyl acetate:petroleum ether mixtures. Preparative tlc was performed on "Merck DC Fertigplatten, Kieselgel 60" (layer thickness 0.25 mm) with diethyl ether, or ethyl acetate:petroleum ether mixtures containing 5% methanol. Analytical tlc was performed on "Merck DC Alufolien, Kieselgel 60" with ethyl ether. ^1H and ^{13}C NMR spectra were obtained on a Bruker WM 400 spectrometer in $CDCl_3$ solutions with tetramethylsilane as the internal standard. The coupling constants J are given in Hz. IR spectra were recorded on a Perkin Elmer 1420 instrument. UV spectra were taken with a Varian Cary 17 spectrometer. High resolution mass spectra were obtained on an AEI MS 50 instrument.

2-Methyl-7-(7-oxonona-1,3-dienyl)-2H-azepine (chalciporone, 1)

1 was isolated as a slightly yellow oil (approx. 50 mg/kg fresh mushroom). R_F 0.35.

$[\alpha]_D^{22}$ -452 ° (c 1.3 in diethyl ether). UV (ethanol) λ_{max} (ε): 253 (21.000), 260 (infl.), 270 (infl.) and 315 nm (infl.). IR (neat): 2970, 1705, 1570, 1530, 1370, 1240, 1110, 990 and 745 cm^{-1}. ^1H NMR see Table 1. ^{13}C NMR see Table 2. MS, m/z: 243.1623 (M^+, 5%, calculated for $C_{16}H_{21}NO$ 243.1623), 186 (13%) $M^+ - C_3H_5O$, 172 (15%) $M^+ - C_4H_7O$, 158 (100%) $M^+ - C_5H_9O$, 144 (10%), 79 (24%). Elemental analysis: Calculated for $C_{16}H_{21}NO$: C 78.97, H 8.70; Found C 78.73, H 8.53%.

2-Methyl-7-(6-propionoxyhexa-1,3-dienyl)-2H-azepine (norchalciporyl propionate, 2)

2 was isolated as a slightly yellow oil (approx. 2 mg/kg fresh mushroom). R_F 0.39. $[\alpha]_D^{22}$ -290 ° (c 0.2 in diethyl ether). UV (ethanol) λ_{max} (ε): 246 (15.900), 260 (infl.) and 270 nm (infl.). IR (neat): 2960, 2930, 1735, 1460, 1370, 1180, 990 and 745 cm^{-1}. ^1H NMR see Table 1. MS, m/z: 259.1516 (M^+, 6%, calculated for $C_{16}H_{21}NO_2$ 259.1572), 244 (2%) $M^+ - CH_3$, 202 (2%) $M^+ - C_3H_5O$, 186 (24%) $M^+ - C_3H_5O_2$, 172 (19%) $M^+ - C_4H_7O_2$, 158 (54%) $M^+ - C_5H_9O_2$, 43 (100%).

7-Methyl-2-(7-oxonona-1,3-dienyl)-3H-azepine (isochalciporone, 3)

3 was isolated as a yellow oil. Spontaneous isomerization of 1 in $CDCl_3$ solution at room temperature yielded 3 as the only identifiable product. R_F 0.65. UV (ethanol) λ_{max} (ε): 241 (28.000), 246 (infl.), 292 (14.700) and 328 nm (infl.). IR (neat): 2970, 1705, 1565, 1310, 1200, 1110, 990, 755 and 720 cm^{-1}. ^1H NMR see Table 1. ^{13}C NMR see Table 2. MS, m/z: 243.1628 (M^+, 5%, calculated for $C_{16}H_{21}NO$ 243.1623), 186 (5%) $M^+ - C_3H_5O$, 172 (9%) $M^+ - C_4H_7O$, 158 (100%) $M^+ - C_5H_9O$, 79 (17%). Elemental analysis: Calculated for $C_{16}H_{21}NO$: C 78.97, H 8.70; Found C 79.06, H 8.65%.

7-Methyl-2-(7-oxonona-1,3,5-trienyl)-3H-azepine (dehydrochalciporone, 4)

4 was isolated as an intensely yellow oil (approx. 2 mg/kg fresh mushroom). R_F 0.67. UV (ethanol) λ_{max} (ε): 226 (21.800), 285 (infl.) and 338 nm (23.000). IR (neat): 2970, 1650, 1600, 1400, 1170, 1000, 750 cm^{-1}. ^1H NMR see Table 1. MS, m/z: 241.1460 (M^+, 82%, calculated for $C_{16}H_{19}NO$ 241.1467), 226 (20%) $M^+ - CH_3$, 212 (16%) $M^+ - C_2H_5$, 184 (100%) $M^+ - C_3H_5O$, 158 (34%) $M^+ - C_5H_7O$, 106 (16%) $M^+ - C_9H_{11}O$, 79 (54%).

7-Methyl-2-(7-hydroxynona-1,3-dienyl)-3H-azepine (5)

5 was obtained by KBH_4 reduction of 1 in EtOH and separation of the diastereomeric mixture by preparative tlc chromatography. The data are given for the diastereomer that is less polar in the analytical tlc system. R_F 0.20. $[\alpha]_D^{22}$ -351 ° (c 0.9 in diethyl ether). UV (ethanol) λ_{max} (ε): 253 nm (21.000). IR (neat): 3350, 2960,2930, 1570, 1375 and 990 cm^{-1}. ^1H NMR: 7.10, d, 6-H, J_{5-6}=11.5; 6.83, dd, 5-H, J_{4-5}=5.1, J_{5-6}=11.5; 6.64, dd, 9-H, dd, J_{8-9}=15.9, J_{9-10}=10.5; 6.26, d, 8-H, J_{8-9}=16.2; 6.21, dd, 4-H, J_{3-4}=9.2, J_{4-5}=5.2; 6.16, dd, 10-H, J_{9-10}=10.3, J_{10-11}=15.4; 5.89, dt, 11-H, J_{10-11}=15.2, J_{11-12}=7; 5.65, dd, 3-H, J_{2-3}=5.0, J_{3-4}=9.3; 3.53, m, 14-H; 2.95, m, 2-H; 2.24, m, 12-H_2, 1.61, d, 1-H_3, J_{1-2}=6.6; 1.60-1.36, m, 13-H_2 and 15-H_2; 0.93, t, 16-H_3, J_{15-16}=7.2. MS, m/z: 245.1777 (M^+, 23%, calculated for $C_{16}H_{23}NO$ 245.1780), 230 (5%), 216 (7%), 186 (20%), 172 (15%) and 158 (100%).

7-Methyl-2-(7-oxononyl)-perhydroazepine (6)

6 was obtained as a colourless oil as the only product by hydrogenation of 3 with Pd as catalyst. UV (ethanol): no maxima above 210 nm. IR (neat): 2930, 1710, 1450, 1370 and 1130 cm^{-1}. ^1H NMR: 2.74 and 2.56, m, 2-H and 7-H; 2.37, m, 13-H_2 and 15-H_2; 1.80-1.10, m, 3-H_2, 4-H_2, 5-H_2, 6-H_2, 8-H_2, 9-H_2, 10-H_2, 11-H_2 and 12-H_2; 1.06, d, 1-H_3, J_{1-2}=6.5; 1.02, t, 16-H_3, J_{15-16}=7.5. ^{13}C NMR: 211.7 C-14; 59.0 and 54.8 C-4 and C-7; 42.4, 38.9, 38.0, 36.8, 35.8, 29.5, 29.2, 26.5, 25.4, 25.3, 24.1 and 23.9 C-1, C-3, C-4, C-5, C-6, C-8, C-9, C-10, C-11, C-12, C-13 and C-15; 7.8 C-16. MS, m/z: 253.2392 (M^+, 2%, calculated for $C_{16}H_{31}NO$ 253.2405), 224 (6%) $M^+ - C_2H_5$, 182 (11%) $M^+ - C_4H_7O$, 126 (12%) $M^+ - C_8H_{15}O$, 125 (46%), 124 (11%), 112 (100%) $M^+ - C_9H_{17}O$, 110 (14%), 57 (11%), 55 (12%).

Acknowledgement - This work was financially supported by the Deutsche Forschungsgemeinschaft. An Alexander von Humboldt-Fellowship for O.S. is gratefully acknowledged. High resolution mass spectra were recorded by Dr. G. Eckhardt, Bonn.

REFERENCES AND NOTES

1. Dedicated to Professor Börje Wickberg, Lund, on the occasion of his 60th birthday.
2. Permanent address: Organic Chemistry 2, Lund Institute of Technology, P.O.B. 124, 221 00 Lund (Sweden).
3. Compounds were classified as pungent or nonpungent by O.S., by licking a small spatula that had been dipped in a solution of respective compound in diethyl ether (approx. 1 mg/ml).
4. H.O. Kalinowski, S. Berger and S. Braun, ^{13}C-NMR-Spektroskopie, Georg Thieme Verlag, Stuttgart, p. 220 (1984).
5. T.J. van Bergen and R.M. Kellogg, J. Org. Chem., **36**, 978 (1971).
6. R.K. Smalley in Comprehensive Heterocyclic Chemistry (Eds. A.R. Katritzky and C.W. Rees), vol. **7**, 491 Pergamon Press, Oxford (1984).
7. The corresponding isomerizations probably also occur with norchalciporyl propionate (**2**) and the alcohol **5**, although the products formed were not isolated and characterized.
8. For examples, see: G. Cimino, S. De Rosa, S. De Stefano, G. Sodano and G. Villani, Science, **219**, 1237 (1983) and R. Baker, P. Briner and D. Evans, J.Chem.Soc., Chem. Commun., 410 (1978).
9. O. Sterner, R. Bergman, J. Kihlberg and B. Wickberg, J. Nat. Prod., **48**, 279 (1985).
10. Dr. H. Anke, University of Kaiserslautern, unpublished results. We thank Dr. Anke for performing these experiments.
11. S.M. Camazine, J.F. Resch, T. Eisner and J. Meinwald, J. Chem. Ecol., **9**, 1439 (1983).
12. H. Barth, G. Burger, H. Döpp, M. Kobayashi and H. Musso, Liebigs Ann. Chem., 2164 (1981), and R. von Ardenne, H. Döpp, H. Musso and W. Steglich, Z. Naturforsch., **29c**, 637 (1974).
13. W. Steglich, W. Furtner and A. Prox, Z. Naturforsch., Teil B, **25**, 557 (1970).