Phytochemistry, Vol. 21, No. 2, pp. 367–369, 1982.
Printed in Great Britain.

0031-9422/82/020367-03$03.00/0

CHALMICRIN, A MANNITOL ETHER OF METHYLATED MONOCYCLOFARNESOL, FROM *CHALARA MICROSPORA*

TOMAS FEX

Organic Chemistry 2, Chemical Center, Lund Institute of Technology, P.O.B 740, S-220 07 Lund 7, Sweden

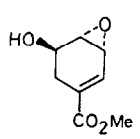
(*Revised received* 1 *June* 1981)

Key Word Index—*Chalara microspora*; fungus; metabolites; chalmicrin; 10-methyl-*trans*-monocyclofarnesol; sesquiterpene; D-mannitol.

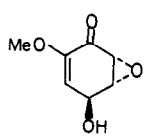
Abstract—The structure of chalmicrin, isolated from *Chalara microspora*, has been determined. It consists of 10-methyl-*trans*-monocyclofarnesol linked to D-mannitol as an allyl ether.

In an investigation of fungal metabolites produced by *Chalara microspora* (Corda) Hughes,* chalmicrin (**1**) was isolated. The methyl ester of (+)-3,4-anhydroshikimic acid (**2**) [1], chaloxone (**3**) [2] and three new antibiotic cytochalasins [3] were previously isolated from the same fungus.

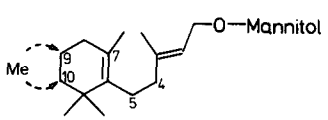
Chalmicrin crystallized as a white powder from an Et_2O solution of the crude extract of the fungus. Recrystallization from Me_2CO gave mp 101–102° and $[\alpha]_D^{25} - 20.6°$ (EtOH; *c*0.66). The compound showed only residual absorption in UV below 230 nm (ϵ = 2300 at 220 nm). The IR spectrum (KBr) revealed the presence of OH (3440, 3320 cm^{-1}), C=C (1675 cm^{-1}) and the absence of C=O functions. Elemental analysis suggested the molecular formula $C_{22}H_{40}O_6$.



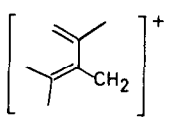
2



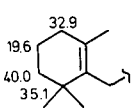
3



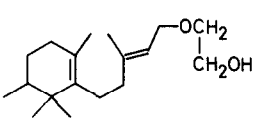
6



7

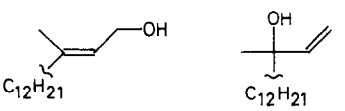


8



9

*The identification was performed at Centralbureau voor Schimmelkultures, Baarn, Netherlands, and the fungus is incorporated in their collection under this name.



4 5

Fig. 1.

Hydrolysis of chalmicrin produced two nonpolar compounds (**4** and **5**) and a sugar moiety. The sugar proved to be identical in all respects with D-mannitol. **4** and **5** both had a MW of 236, indicating the molecular formula $C_{16}H_{28}O$. According to ^1H NMR and IR, **4** and **5** contain the isomeric allyl alcohol units shown in Fig. 1. The ^1H NMR spectrum of **4** is nearly superimposable on that of chalmicrin, apart from the signals due to mannitol. The mannitol unit in chalmicrin is obviously bound as an allyl ether.

^1H NMR (including decoupling experiments) and ^{13}C NMR of chalmicrin (Table 1) revealed the presence of the molecular subunits shown in Fig. 2. Three CH_2 groups bear allylic hydrogens, and the CH in Me–CH is not allylic. According to spectral data (see below), these fragments could be assembled to form structure **6**. The six-membered ring is supported by the mass spectrum of **4**, where a peak at *m/z* 109 indicates the formation of the ion **7** via fragmentations involving a retro-Diels–Alder fragmentation of the cyclohexene ring. The hydrocarbon moiety of chalmicrin is identical with monocyclo-

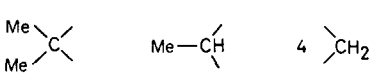


Fig. 2.

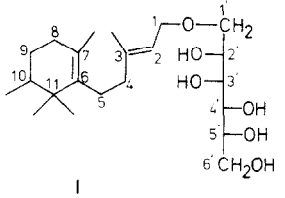
Table 1. ^{13}C NMR (CD_3OD) and ^1H NMR (CD_3OD) of chalmicrin **1**

	δ (TMS)	δ (TMS)
C(1)H_2	65.2[a]	4.10 (*d*, *J* = 7 Hz)
C(2)H	121.9	5.42 (*t*, *J* = 7 Hz)
C(3)	142.3	
C(4)H_2	41.4	2.12*
C(5)H_2	28.3[b]	2.12*
C(6)	138.4	
C(7)	128.4	
C(8)H_2	32.6	1.9–2.05 (*m*)
C(9)H_2	29.0[b]	1.35–1.6 (*m*)
C(10)H	40.8	1.45 (*m*)
C(11)	39.4	
3-Me	16.7[c]	1.74 (*s*)
7-Me	20.2	1.63 (*s*)
10-Me	22.3	0.90 (*d*, *J* = 6 Hz)
11-Me(ax)	17.1[c]	0.86 (*s*)
11-Me(eq)	27.7	1.04 (*s*)
C(1')H_2	78.0	
C(2')H	78.0[d]	
C(3')H	77.7[d]	
C(4')H	76.5[d]	3.5–3.9 (*m*)
C(5')H	76.0[d]	
C(6')H_2	68.9[a]	

a, b, c, d may be interchanged.
*Appear very much as a singlet (AA'BB' spectrum).

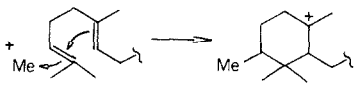
farnesol [4] except for an extra methyl group in the 9- or 10-position.

^1H NMR data for the 7-Me and the 4- and 5-CH_2 groups of chalmicrin are very similar to those of *trans*-monocyclofarnesol [5]. An alternative structure with the *gem*-dimethyl group in the 8-position seems very unlikely from a biogenetic point of view. The noncyclic double bond was assigned a *trans* configuration after comparison of the ^{13}C NMR data of chalmicrin with those of geraniol (*trans*) and nerol (*cis*) [6]. The position of the extra methyl group was also determined with the aid of ^{13}C NMR, taking advantage of the fact that alkyl substituents give rise to large α- and β-effects. Thus, adding a methyl group to the 9- or 10-position of the known structural element **8** [7] and calculating new chemical shifts [8, 9] clearly indicated that the extra methyl group must be in the 10-position, as drawn in Fig. 3. The absolute configuration of this 10-methyl-*trans*-monocyclofarnesol remains unknown. One may speculate on the origin of the extra methyl group. Cyclizations



I

Fig. 3.



Scheme 1.

in for example, the diterpene class are frequently initiated by protons. Methylations are often thought of as involving a Me^+ equivalent, and this might in an analogous manner initiate a cyclization as depicted in Scheme 1.

To determine which alcohol group in mannitol is involved in bonding, chalmicrin was oxidized with $NaIO_4$ and the products reduced with $NaBH_4$. According to mass spectroscopy the final product had a MW of 280, which corresponds to structure **9**. Mannitol is thus bonded via one of the identical CH_2OH groups. In addition, the large chemical shift difference in ^{13}C NMR between the two CH_2OH groups in mannitol indicated that one of them was alkylated, the alkyl group giving rise to a β-effect.

EXPERIMENTAL

Cultivation. The fungus was grown as a stationary culture on 30 l. of a medium containing 2% malt extract (Difco), 0.5% yeast extract (Difco), 0.2% asparagin, 3% glucose and 0.15% agar (Difco). The temp. was 16–19°. After 4 weeks, the medium and the mycelium were extracted with EtOAc. Drying and evaporation yielded 13.6 g of crude extract. Most of the very polar and nonpolar parts were removed by extraction (polar: CCl_4–H_2O, MeOH (1:1); nonpolar: H_2O, MeOH (2:8)–hexane). From an Et_2O soln of the fraction of intermediate polarity, 0.8 g of chalmicrin crystallized.

Chalmicrin **1**. (Found: C, 66.0; H, 10.2. $C_{22}H_{40}O_6$ requires: C, 66.3; H, 10.1%.) Other physical data are given in the text.

Hydrolysis. Chalmicrin (46 mg) was hydrolysed in a mixture of 0.1 M HCl (1 ml) and refluxing cyclohexane (3 ml) for 30 hr. The organic layer was separated, and the H_2O phase extracted twice with Et_2O. The H_2O phase was evaporated to yield a compound (21 mg) identical in all respects with D-mannitol. (The optical rotation was measured in a molybdate soln [10].) The organic solvents were also removed to yield a mixture of **4** and **5**, which were separated by chromatography on Si gel using CH_2Cl_2 as eluant.

4 (5 mg). MS (probe) 70 eV *m/z* (% rel. int.): 236 (M^+, 3) 151 (100), 150 (31), 109 (67), 95 (78), 81 (33). ^1H NMR (60 MHz, $CDCl_3$): δ 5.4 (1H, *t*, *J* = 7 Hz), 4.15 (2H, *d*, *J* = 7 Hz), 2.1–1.3 (5H, *m*), 2.1 (4H), 1.75 (3H, *s*), 1.6 (3H, *s*), 1.05 (3H, *s*), 0.9 (3H, *d*, *J* = 6 Hz), 0.85 (3H, *s*). IR (neat): 3340 (OH), 1670 cm^{-1} (C=C). **5** (15 mg) MS (probe) 70 eV *m/z* (% rel. int.): 236 (M^+, 12) 203 (38), 151 (34), 150 (31), 137 (61), 135 (73), 121 (43), 119 (36), 109 (100), 107 (55), 95 (91), 93 (57), 81 (61). ^1H NMR (60 MHz, $CDCl_3$): δ 6.3–5.0 (3H, ABC spectrum), 2.3–1.3 (9H, *m*), 1.6 (3H, *s*), 1.35 (3H, *s*), 1.0 (3H, *s*), 0.9 (3H, *d*, *J* = 6 Hz), 0.85 (3H, *s*). IR (neat): 3400 (OH), 1670 (C=C), 1000, 925 cm^{-1} (–CH=CH_2).

$NaIO_4$ *oxidation and reduction.* Chalmicrin (1 mg) was oxidized with excess $NaIO_4$ in MeOH–H_2O. The products were isolated and reduced with KBH_4 in MeOH. The final product was isolated and was pure according to TLC (Si gel, Et_2O). MS (probe) 70 eV: 280 (M^+), corresponding to structure **9**.

Acknowledgements—I wish to thank Professor B. Wickberg for his encouragement of this work. I am also indebted to Professor M. Johansson and Dr. A. Hyppel, Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, for providing the fungus, and for pointing out its antibiotic properties. Financial support from the Swedish Natural Science Research Council is gratefully acknowledged.

REFERENCES

1. Fex, T., Trofast, J. and Wickberg, B. (1981) *Acta Chem. Scand. Ser. B* **35**, 91.
2. Fex, T. and Wickberg, B. (1981) *Acta Chem. Scand. Ser. B* **35**, 97.
3. Fex, T. (1981) *Tetrahedron Letters* 2703.
4. Kato, T., Kanno, S. and Kitahara, Y. (1970) *Tetrahedron* **26**, 4287.
5. Negishi, E., King, A. O. and Klima, W. L. (1980) *J. Org. Chem.* **45**, 2526.
6. de Haan, J. W. and van de Ven, L. J. M. (1973) *Org. Magn. Reson.* **5**, 147.
7. Englert, G. (1975) *Helv. Chim. Acta* **58**, 2367.
8. Pehk, T. and Lippmaa, E. (1971) *Org. Magn. Reson.* **3**, 679.
9. Senda, Y., Suda, H., Ishiyama, J. and Imaizomi, S. (1976) *Tetrahedron Letters* 1983.
10. Sagel, J. and Bouwmeester, R. J. F. (1974) *Pharm. Weekbl.* **109**, 241.