Institut für Pharmazeutische Biologie[1], Universität Heidelberg, Heidelberg and Abtl. Allergologie[2], Dermatologisches Zentrum, Krankenhaus Buxtehude, Germany

1,2,4-Trihydroxy menthane, a contact allergen from oxidized Australian tea tree oil

M. HARKENTHAL[1], B. M. HAUSEN[2] and J. REICHLING[1]

Tea tree oil (TTO), an essential oil derived from leaves of *Melaleuca alternifolia*, is becoming increasingly popular as a naturally occuring agent for a wide range of health care products [1–3]. TTO has been reported to show undesirable side effects such as allergic contact dermatitis and local skin irritation. Oxidized TTO revealed a sensitizing capacity 3 times stronger than that of freshly distilled oil. Therefore it was assumed that mainly oxidized monoterpenes were the sensitizing agents of TTO [4, 5].

Recently, we isolated from oxidized TTO a cristalline compound identified as 1,2,4-trihydroxy menthane (1,2,4-THM) by MS, $^{1}H/^{13}C$ spectroscopy (Fig.). 1,2,4-THM is well known from the oxidized essential oil of *Melaleuca linariifolia* [6]. This compound is formed from terpinen-4-ol by oxidation. By elimination of water the triol passes to *p*-cymen very easily. First 1,2,4-THM is dissolved in TTO, later when the oil is stored for a longer period, it deposits slowly on the bottom of the bottles. To get sufficient material for toxicological and antimicrobial testing we synthesized this compound with terpinen-4-ol as the start molecule. In the present investigation, fifteen patients sensitive to tea tree oil were tested epicutaneously with seven typical constituents and two degradation products of TTO. 1,2,4-Trihydroxy menthane was shown to be an important allergen as well as ascaridol, another degradation product of TTO (Table). Besides 1,2,4-THM and ascaridol, α-phellandrene, α-terpinene, and terpinolene were found to be frequent allergens as well. On the other hand, 1,2,4-THM showed no antimicrobial activity.

In conclusion, TTO kept under practical daily conditions undergoes photooxidation within a short time, leading to the formation of peroxides and subsequently to the generation of degradation products [7]. Compounds like ascaridol and 1,2,4-trihydroxy menthane are formed. These de-

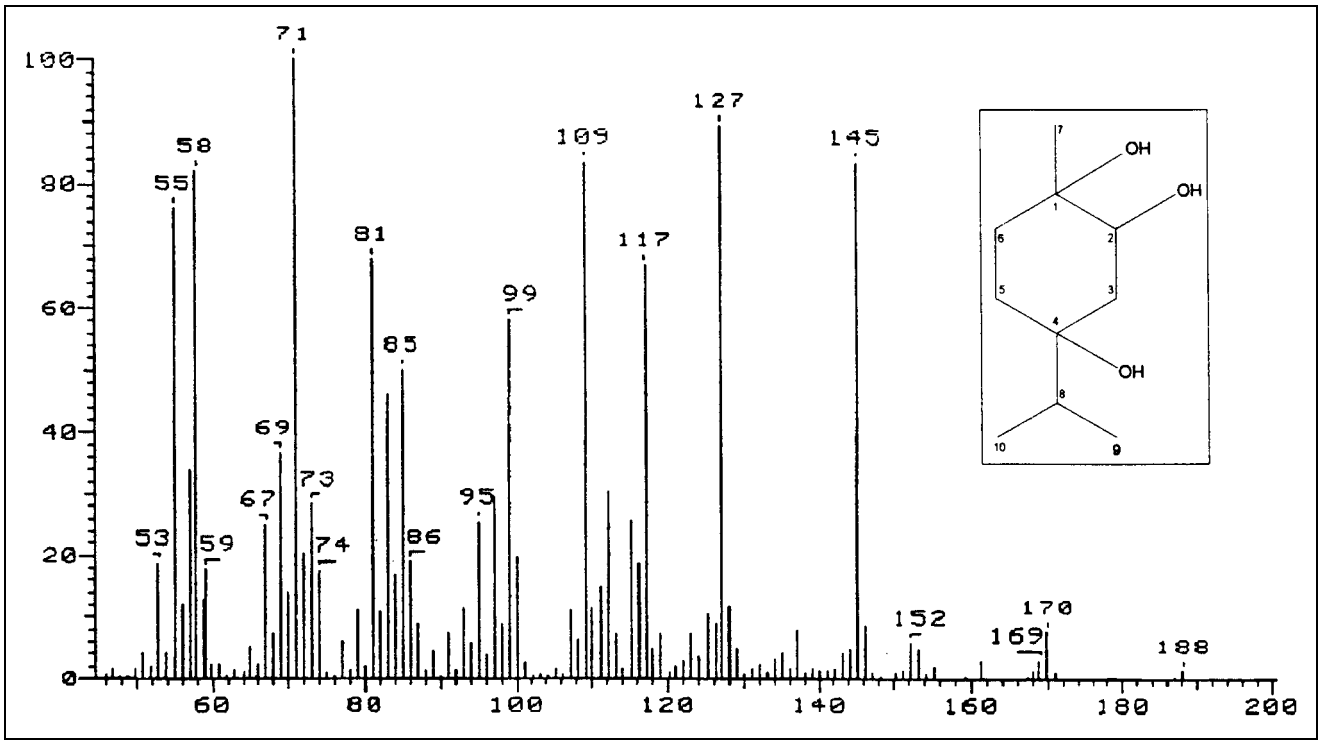

Fig.: Mass spectrum of 1,2,4-trihydroxy menthane

Table: Results of patch tests with constituents of tea tree oil (72-h-readings)

Compounds	Concentration	Patients															Σ
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
α-Terpinene	5%	++	++	++	+	++	0	+	+	++	0	0	0	0	+++	+++	10
α-Phellandrene	5%	0	+	+	0	++	0	0	0	0	0	0	0	++	+++	+	6
Terpinolene	10%	++	++	+++	+	++	++	++	+	++	+	+	+	++	+++	+++	15
Myrcene	5%	+	0	0	0	0	0	0	0	0	0	0	0	0	++	0	2
D/L-Carvone	5%	0	0	0	0	0	0	0	0	0	0	++	0	0	0	0	1
Aromadendrene	5%	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	1
Viridiflorene	5%	+++	0	0	0	0	0	0	0	0	0	0	0	0	0	+	2
Ascaridol	5%	++	++	++	++	++	0	++	+	0	0	++	0	0	+++	+++	10
1,2,4-THM	5%	++	++	0	+	++	+	0	+	++	++	0	0	++	+++	+++	11

(+ weak, ++ moderate, +++ strong reaction)

gradation compounds are moderate to strong sensitizers. They must be considered responsible – together with the other mentioned monoterpenes – for the induction of contact allergy developing in individuals treating themselves with TTO.

Due to our results the German Contact Dermatitis Group (DKD) has decided to perform routine patch tests with oxidized TTO and different constituents such as 1,2,4-trihydroxy menthane in patients of 11 different clinics in Germany and Austria who have used tea tree oil as a cosmetic or remedy.

Experimental

1. Terpene standards

Aromadendrene, α-phellandrene, and viridiflorene were purchased from Fluka, Buchs, Switzerland, α-terpinene, terpinolene, myrcene, and d/l-carvone derived from Roth, Karlsruhe, Germany.

2. Synthesis of 1,2,4-THM

The synthesis of 1,2,4-trihydroxy menthane was performed according to Thappa et al. [8]. The compound was synthesized from terpinen-4-ol with H_2O_2 and HOAc. According to the spectroscopical data the natural occuring compound (in the oil) appears to be identical with the synthetic compound.

3. Spectroscopy of 1,2,4-THM

The ^{13}C NMR spectra were recorded at 50 MHz; solvent: MeOD; ^{13}C NMR data, chemical shifts in δ-values (ppm): C-1, 72,07; C-2/4, 75,72/75,77; C-3, 34,86; C-5/6, 30,31/30,41; C-7, 27,12; C-9/10, 17,16/17,26. The ^{1}H NMR spectra were recorded at 300 MHz; solvent: MeOD; ^{1}H NMR data, chemical shifts in δ-values (ppm): H-7 (CH_3), 1,23; H-9/10 (CH_3), 0,9; H-8, 1,57; H-2, 3,50; H-3/3, 1,62/1,96; H-5/5, 0,74/1,88; H-6/6, 0,74/1,88. The MS spectra were recorded on a Finnigan MAT 4500 mass spectrometer. EI ionizing voltage 70 eV. MS data see Fig.

4. Patients

Short case histories.

Pat. 1: Pilot, with a known contact allergy to fragrances, treated his seborrhoea with TTO. Pat. 2: Computer specialist, used a shampoo to wash his hair to which his wife had added TTO. Pat. 3: Waitress, treated her foot eczema with a TTO soap. Pat. 4: Woman, treated her foot eczema with TTO. Pat. 5: Woman, tried to treat her gingivitis with TTO capsules. Pat. 6: Employee, used a TTO-containing tooth paste which subsequently caused cheilitis and gingivitis. Pat. 7: Groom, used various essential oils, not knowing that TTO was among them. Pat. 8: Priest, used a hand-made TTO ointment to treat itching and dry skin. Pat. 9: Man, used TTO against "sun pimples" on the face and breast. Pat. 10: Woman, rubbed TTO into the skin against pain of the neck, treated skin lesions of the feet too. Pat. 11: Female secretary, used TTO for skin lesions and as a mouth wash against tooth pain. Pat. 12: Woman, treating her psoriasis with TTO. Pat. 13: Female cashier, treated a sunburn with TTO. Pat. 14: Florist, suffering from chronic eczema, used TTO to treat his lesions. Pat. 15: Woman, used TTO against herpes simplex, mosquito bites and various skin lesions.

5. Patch test

Patch tests were performed on the back of the TTO-sensitive patients with the nine compounds listed in the Table, using Finn chambers on Scanpor (Hermal, Reinbek, Germany). The patches were left on for 24 h. Readings were performed according to the recommendation of the ICDRG and scored after 72 h.

References

1 Reichling, J.; Harkenthal, M.; Geiss, H.-K.; Saller, R.: Österreich. Apoth. Ztg. **51**, 652 (1997)
2 Saller, R.; Berger, T.; Reichling, J.; Harkenthal, M.: Phytomedicine **5**, 489 (1998)
3 Harkenthal, M.; Reichling, J.; Geiss, H.-K.; Saller, R.: Pharmazie **54**, 460 (1999)
4 Hausen, B. M.; Vieluf, I.K.: Allergiepflanzen – Pflanzenallergene. Handbuch und Atlas der allergieinduzierenden Wild- und Kulturpflanzen, 2. Aufl., p. 183. Ecomed Verlagsgesellschaft, Landsberg, München (1998)
5 Hausen, B. M.; Reichling, J.; Harkenthal, M.: Am. J. Cont. Derm. **10**, 68 (1999)
6 Jones, T. G. H., Oakes, H. C.: Univ. Queensl. Pap., Dept. Chem. **1**, 1 (1940)
7 Harkenthal, M.; Reichling, J.; Geiss, H.-K., Saller, R.: Pharm. Ztg. **143**, 4092 (1998)
8 Thappa, R. K., Dhar, K. L., Atal, C. K.: Phytochemistry **15**, 1568 (1976)

Received August 27, 1999
Accepted October 5, 1999

Prof. Dr. Jürgen Reichling
Institut für Pharmazeutische Biologie
Universität Heidelberg
Im Neuenheimer Feld 364
D-69120 Heidelberg
Juergen.Reichling@t-online.de