Biogenetic Studies in *Mentha* × *piperita*. 2. Stereoselectivity in the Bioconversion of Pulegone into Menthone and Isomenthone

Sabine Fuchs, Thomas Beck, Martin Sandvoss, and Armin Mosandl*

Institut für Lebensmittelchemie, Johann Wolfgang Goethe-Universität Frankfurt, Marie-Curie-Strasse 9, D-60439 Frankfurt (Main), Germany

Mentha × *piperita* shoot tips and first leaf pairs were fed with aqueous solutions of different deuterium-labeled pulegone and various enantiomeric distributions. The essential oil was extracted by solid-phase microextraction and analyzed using enantioselective multidimensional gas chromatography/mass spectrometry. The genuine *p*-menthan-3-ones (−)-menthone and (+)-isomenthone as well as their labeled analogues were analyzed simultaneously. Both enantiomers of labeled pulegone were converted into the corresponding labeled *p*-menthan-3-ones by *Mentha* × *piperita*, indicating an unspecific reduction process. The generation of 4*S*- and 4*R*-configured *p*-menthan-3-ones differed in their stereoselectivities. Labeled (*S*)-pulegone was reduced by *Mentha* × *piperita* more rapidly rather than (*R*)-pulegone. From a comparison of labeled pulegone enantiomers the bioconversion preferrably led to 4*S*-configured diastereomers.

Keywords: *Bioconversion; deuterium-labeled pulegone; Mentha* × *piperita biosynthesis; enantioselective multidimensional gas chromatography/mass spectrometry (enantio-MDGC/MS)*

INTRODUCTION

Peppermint oil is produced by steam distillation of the flowering herb *Mentha piperita* L. It is mainly used for flavoring oral hygiene products, for example, toothpaste, and chewing gum (Bauer et al., 1997). Besides the genetic influence, the oil composition changes during plant development (Kokkini, 1991). Furthermore, effects of light and temperature on the composition of peppermint oil have been observed (Burbott and Loomis, 1967; Clark and Menary, 1980).

In 1958 Reitsema proposed, on the basis of the chemical structures of the monoterpenes, that pulegone may be converted to menthone in peppermint type mints. Several in vitro and in vivo experiments gave evidence for this hypothesis (Battaille and Loomis, 1961; Aviv and Galun, 1978; Murray et al., 1980; Croteau and Venkatachalam, 1986; Park et al., 1993). Genetic aspects on the conversion of pulegone to menthone were also investigated (Lincoln and Murray, 1978; Croteau and Gershenzon, 1994). Due to the biosynthetic conversion of pulegone into menthone and isomenthone, the optical purity of menthone and isomenthone can be used as an indicator of the natural origin of peppermint oil (Kreis et al., 1990; Askari et al., 1992).

This paper deals with in vivo feeding experiments of deuterium-labeled pulegone enantiomers using intact plant material of *Mentha* × *piperita*. The essential oil of each single shoot tip and first leaf pair is analyzed by solid-phase microextraction (SPME) and enantioselective multidimensional gas chromatography/mass spectrometry (enantio-MDGC/MS). In this way genuine and labeled monoterpenes are detectable simultaneously.

MATERIALS AND METHODS

Syntheses of Deuterium-Labeled Precursors and Reference Compounds. The syntheses are described as part 1 of this publication series; abbreviations of compounds investigated are identical with those of the earlier paper (Fuchs et al., 1999).

Plant Material. Cuttings from *Mentha* × *piperita* were kindly provided from the Botanical Garden of Frankfurt University. The plants used for the experiments were 12−15 cm high with six to seven leaf pairs.

Feeding Experiments. The deuterium-labeled pulegone was dissolved in distilled (two times) water containing 0.1 mg/mL Tween 20 to get a solution that was 0.1 mg/mL in the monoterpene. Solutions with the two d_8-pulegone enantiomers **7** and **8** were prepared by mixing both in different ratios. The shoot tip and first leaf pair were cut off and put into the feeding solutions. In any case, a blank experiment was carried out with distilled (two times) water containing 0.1 mg/mL Tween 20. After 24−29 h, the shoot tip and first leaf pair were removed from the solution and the essential oil evaporating from the glandular trichomes was analyzed using SPME.

SPME. The shoot tip and first leaf pair of an actively growing plant were set into a 2 mL vial and equilibrated there for 1 h at room temperature. An SPME fiber, coated with a 100 μm film of poly(dimethylsiloxane), installed in an SPME fiber holder for manual use (both Supelco, Munich, Germany) was used for the headspace sampling (7 min).

Enantio-MDGC. The enantiomeric distribution of the d_8-pulegone feeding solution was checked with MDGC by a Siemens SiChromat 2 - 8, equipped with two independent ovens and a live-T-switching device. Conditions were as follows: precolumn, fused silica capillary (30 m × 0.25 mm i.d.) coated with PS 268 (film thickness = 0.38 μm); carrier gas, hydrogen at 150 kPa; split, 30 mL/min; injector temperature, 260 °C; detector, FID, 280 °C; oven temperature, 50 °C (5 min isothermal), raised at 3 °C/min to 200 °C, cut time 27000−28000 min; main column, fused silica capillary (30 m × 0.32 mm i.d.) coated with 30% heptakis(2,3-di-*O*-methyl-6-*O*-*tert*-butyldimethylsilyl)-β-cyclodextrin in SE 52 (film thickness = 0.64 μm); carrier gas, hydrogen at 70 kPa; oven temperature, 80 °C (5 min isothermal) raised at 1 °C/min to 135 °C; detector, FID, 280 °C.

Enantio-MDGC/MS. The same system as previously reported was employed (Fuchs et al., 1999).

RESULTS AND DISCUSSION

Feeding experiments with aqueous solutions of deuterium-labeled monoterpenes have become a very useful

10.1021/jf9901338 CCC: $18.00
Published on Web 07/13/1999

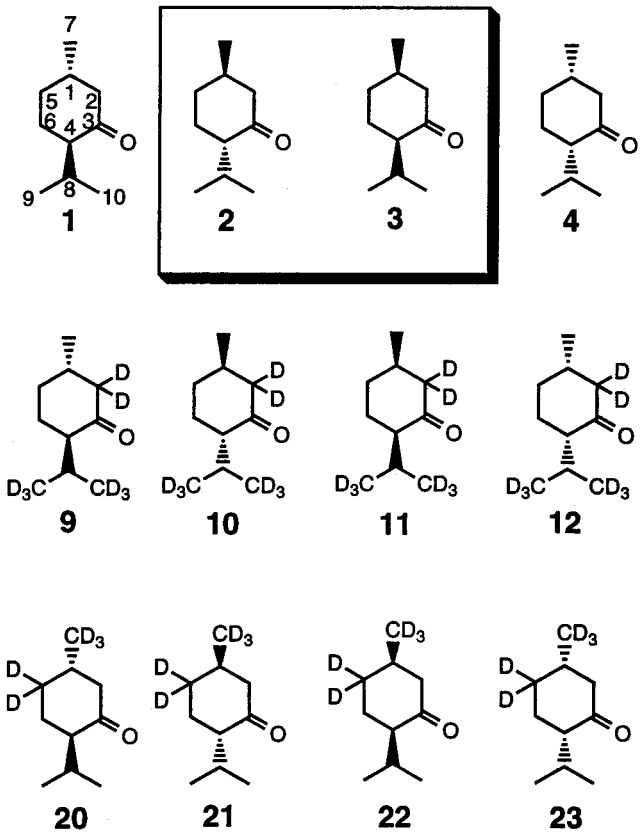


Figure 1. Stereoisomers of (deuterium-labeled) menthone and isomenthone enantiomers. The natural enantiomers of *Mentha* × *piperita* (−)-menthone (**2**) and (+)-isomenthone (**3**) are boxed.

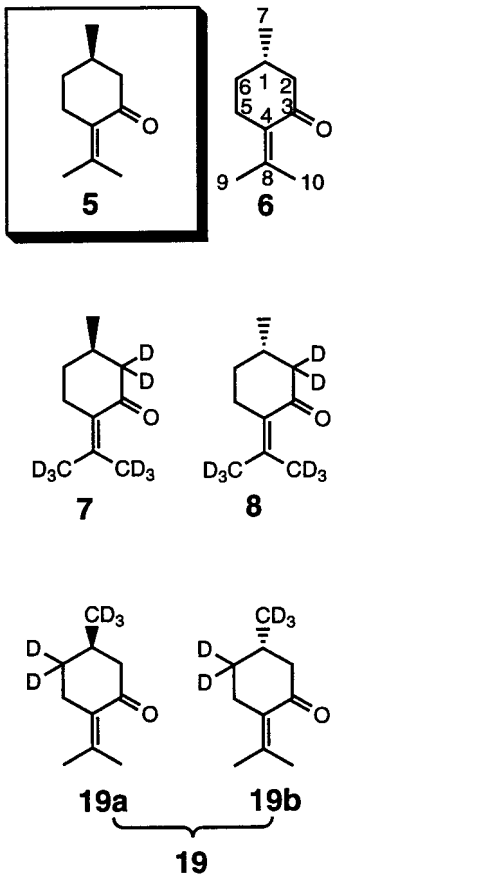


Figure 2. Stereoisomers of (deuterium-labeled) pulegone enantiomers. The natural enantiomer of *Mentha* × *piperita* (*R*)-pulegone (**5**) is boxed.

method for in vivo studies of biogenesis. Using the SPME technique and analysis of the headspace extract by enantio-MDGC/MS, the evaluation of the stereochemical pathways during bioconversion reactions was successfully carried out (Wüst et al., 1996, 1998).

The biogenesis of the monoterepenes menthone and isomenthone in *Mentha* was investigated by incubation of leaf disks, cell-free systems, and cell lines of *Mentha* with pulegone, as reported in earlier literature (Battaile et al., 1961; Aviv and Galun, 1978; Croteau and Venkatachalam, 1986; Park et al., 1993). Both qualitative and quantitative differences were reported. Whereas Croteau and Venkatachalam found menthone the dominant product, Battaile et al. as well as Park et al. reported isomenthone as the preponderant monoterpenoid ketone. Only Aviv and Galun detected no menthone. Exept for Battaile and co-workers, all authors reported on the administeration of (*R*)-pulegone. Battaile et al. used labeled pulegone biosynthesized by *Mentha pulegium* L. Therefore, it is most conceivable that (*R*)-pulegone was administered. On the other hand, after incubation with nongenuine (*S*)-pulegone, no biotransformation was observed (Aviv et al., 1981).This paper reports on the conversion of deuterium-labeled pulegone enantiomers into the corresponding deuterated *p*-menthan-3-ones in *Mentha* × *piperita* using feeding experiments with single shoot tip and first leaf pair and subsequently enantio-MDGC/MS analysis of SPME headspace extracts. A typical precolumn chromatogram of *Mentha* × *piperita* is shown in Figure 3. In Figure 4 are shown main column chromatograms of feeding experiments with d_8-pulegone.

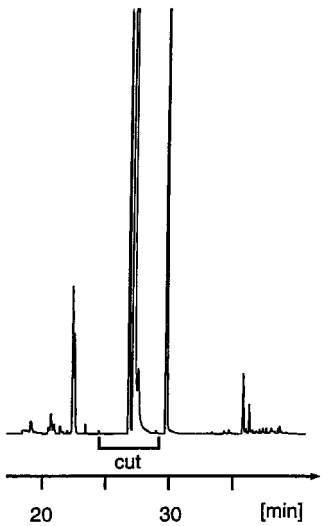


Figure 3. Enantio-MDGC/MS analysis of a headspace extract of *Mentha* × *piperita* (precolumn chromatogram).

After feeding experiments with d_8-(*R*)-pulegone (**7**), the genuine (1*R*)-*p*-menthan-3-ones (−)-menthone (**2**), (+)-isomenthone (**3**), and the analogous deuterium-labeled compounds d_8-(−)-menthone (**10**) and d_8-(+)-isomethone (**11**) were detected. Note that the sign of the optical rotation of the labeled menthone and isomenthone was not determined, because the rotatory contribution of the deuterated carbon to the magnitude and sign of the D-line rotation is assumed to be negligibly small (Verbit, 1970). Mean values indicated that incubation afforded more d_8-(−)-menthone (**10**) than d_8-(+)-

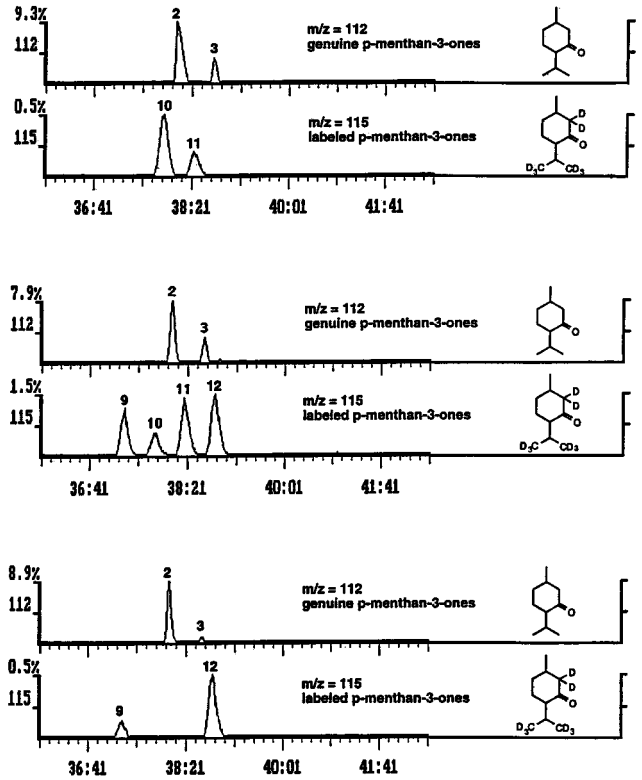


Figure 4. Enantio-MDGC/MS analysis of headspace extracts of *Mentha* × *piperita* fed with d_8-(*R*)-pulegone (**7**) (at the top), with a racemic mixture of d_8-pulegone (**7**, **8**) (middle) and with d_8-(*S*)-pulegone (**8**) (at the bottom) (main column chromatogram).

isomenthone (**11**), although there was a range in the ratio of **10**/**11** from 0.8 to 2.7. Furthermore, it was proved that there was a correlation between the genuine (−)-menthone to (+)-isomenthone ratio (**2**:**3**) and the ratio of the d_8-analogues (**10**/**11**).

The results of feeding experiments with d_8-(*R*)-pulegone **7**, in which the ratio of genuine (−)-menthone/(+)-isomenthone **2**/**3** is high, were in agreement with the findings of Croteau and Venkatachalam. On the other hand, the results of the feeding experiments, in which the ratio **2**/**3** was low, were in agreement with the findings of Battaile et al. and Park et al. The results of all our feeding experiments with d_8-(*R*)-pulegone (**7**) were different from the results of Aviv and Galun, because in all our experiments deuterium-labeled (−)-menthone (**10** and **21**, respectively) was detected. In none of the previous work had the simultaneous enantioselective analysis of genuine *p*-menthan-3-ones and the labeled *p*-menthan-3-ones, caused by administered pulegone precursors, been done. Therefore, no correlation between the genuine *p*-menthan-3-ones and the de novo biosynthesized *p*-menthan-3-ones could be drawn.

After feeding a racemic mixture of d_8-pulegone, the deuterium-labeled *p*-menthan-3-ones **9**−**12** were detected. 1*S*-configured d_8-(+)-menthone (**9**) and d_8-(−)-isomethone (**12**) were detected after feeding with d_8-(*S*)-pulegone (**8**). The data obtained from the feeding experiments with *Mentha* × *piperita*, when d_8-pulegone was administered, are summarized in Table 1.

In Figure 5 the main column chromatogramm of a feeding experiment in which racemic d_5-pulegone (**19**) was administered is given. The deuterium-labeled *p*-menthan-3-ones **20**−**23** were detected. The data obtained

Table 1. Data Obtained from *Mentha* × *piperita* Feeding Experiments with d_8-Pulegone Enantiomers[a]

terpene administered	*p*-menthan-3-ones ratio (%)				labeled *p*-menthan-3-ones ratio (%)			
	1	**2**	**3**	**4**	**9**	**10**	**11**	**12**
7	nd	80	20	nd	nd	67	33	nd
	nd	81	19	nd	nd	55	45	nd
	nd	79	21	nd	nd	73	27	nd
	nd	69	31	nd	nd	54	46	nd
	nd	60	40	nd	nd	45	54	nd
	nd	57	43	nd	nd	45	55	nd
	nd	67	33	nd	nd	49	51	nd
	nd	62	38	nd	nd	50	50	nd
7/**8** (3:1)	nd	84	16	nd	9	34	23	35
	nd	69	31	nd	16	18	38	28
	nd	54	46	nd	12	19	33	36
	nd	75	25	nd	8	23	15	54
	nd	42	58	nd	9	5	17	69
7/**8** (1:1)	nd	80	20	nd	14	29	10	47
	nd	86	14	nd	8	24	9	59
	nd	71	29	nd	21	10	16	53
	nd	87	13	nd	18	26	19	37
	nd	53	47	nd	23	8	36	33
	nd	31	69	nd	27	9	38	26
	nd	70	30	nd	21	8	31	40
	nd	76	24	nd	23	13	30	34
	nd	53	47	nd	26	16	29	30
	nd	73	27	nd	23	19	20	38
	nd	tr	tr	nd	22	6	33	40
	nd	21	79	nd	21	3	29	47
	nd	34	66	nd	27	11	37	25
8/**7** (3:1)	nd	48	52	nd	31	3	10	57
	nd	80	20	nd	29	7	7	57
	nd	88	12	nd	20	6	2	71
	nd	51	49	nd	22	1	9	68
	nd	46	54	nd	21	3	8	68
8	nd	92	8	nd	32	nd	nd	68
	nd	92	8	nd	35	nd	nd	65
	nd	92	8	nd	32	nd	nd	68
	nd	96	4	nd	18	nd	nd	82
	nd	68	32	nd	39	nd	nd	61
	nd	45	55	nd	42	nd	nd	58
	nd	77	23	nd	44	nd	nd	56

[a] Accuracy ± 1%; nd, not detectable; tr, traces.

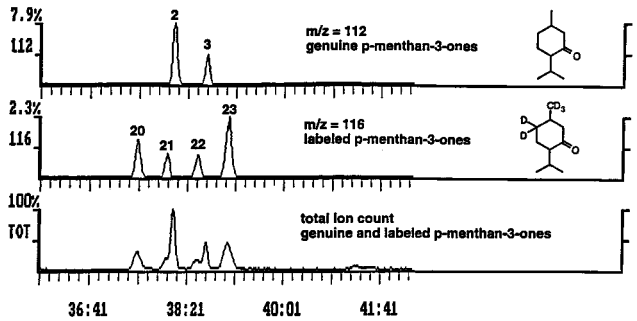


Figure 5. Enantio-MDGC/MS analysis of a headspace extract of *Mentha* × *piperita* fed with racemic d_5-pulegone (**19**) (main column chromatogram).

from the feeding experiments with *Mentha* × *piperita*, when d_5-pulegone was administered, are summarized in Table 2.

In *Mentha* × *piperita* exclusively 1*R*-configured (−)-menthone (**2**) and (+)-isomenthone (**3**) were identified; 1*S*-configured (+)-menthone (**1**) and (−)-isomenthone (**4**) remained undetectable. This is in agreement with previous investigations (Askari et al., 1992). In all experiments the genuine diastereomers (−)-menthone

Table 2. Data Obtained from *Mentha* × *piperita* Feeding Experiments with d_5-Pulegone Enantiomers[a]

terpene administered	*p*-menthan-3-ones ratio (%)				labeled *p*-menthan-3-ones ratio (%)			
	1	**2**	**3**	**4**	**20**	**21**	**22**	**23**
19a/**19b** (3:1)	nd	60	40	nd	14	15	45	25
	nd	64	36	nd	16	16	28	40
	nd	34	66	nd	15	9	30	45
	nd	22	78	nd	9	2	42	47
	nd	32	67	nd	13	9	34	44
19	nd	31	67	nd	27	7	23	43
	nd	70	30	nd	25	14	17	45
	nd	64	36	nd	22	13	15	49
	nd	75	25	nd	20	13	10	57
	nd	68	32	nd	27	11	21	41
19b/**19a** (9:1)	nd	77	23	nd	24	1	1	74
	nd	75	25	nd	30	2	1	67
	nd	63	37	nd	21	1	1	76
	nd	71	29	nd	26	2	2	71
	nd	57	43	nd	23	1	1	75

[a] Accuracy ± 1%; nd, not detectable.

(**2**) and (+)-isomenthone (**3**) occurred in the range of 3:1, but there was much variety; the minimum ratio was 0.3:1, the maximum ratio 26:1. Thus far, it was not surprising that the ratios of the deuterated *p*-menthan-3-one analogues were varying considerably.

It has been found that two *p*-menthenone-$\Delta^{4,8}$-reductases exist, one responsible for the reduction of (*R*)-pulegone (**5**) to (−)-menthone (**2**) and the other enzyme responsible for the formation of (+)-isomenthone (**3**) from (*R*)-pulegone (**5**). Different combination of the alleles, which induce the exprimation of these reductases, were thought to be responsible for the variability of the (−)-menthone/(+)-isomenthone (**2**/**3**) ratio in *Mentha* essential oils (Croteau and Gershenzon, 1994). In our investigations we used cuttings exclusively from one parent plant, so there could be no genetic differences. A possibility for the observed variability of the (−)-menthone/(+)-isomenthone ratio **2**:**3** may be due to the analysis of single cuttings, the young leaf age, or their different stages of plant development.

The results from the feeding experiments in which d_8-pulegone (**7**, **8**) or d_5-pulegone (**19a**, **19b**) were fed are comparable. This makes evident that the diastereomeric ratios of the deuterium-labeled *p*-menthan-3-ones remain unaffected by the position of the deuterium marker in the molecule. Furthermore, no effect due to the small differences in the deuterium labeling rate of the d_8-pulegone enantiomers was observed.

If enantiopure deuterium-labeled pulegone (*R*)-d_8-pulegone (**7**) or (*S*)-d_8-pulegone (**8**) (*R* > 98%, *S* > 98%, proved by enantio-MDGC) was fed alternatively, only the two *p*-menthan-3-one diastereomers, corresponding to the symmetry of C-1 of the precursor, were generated; these were 1*R*-configured d_8-(−)-menthone (**10**) and d_8-(+)-isomenthone (**11**) by feeding (*R*)-d_8-pulegone (**7**) and 1*S*-configured d_8-(+)-menthone (**9**) and d_8-(−)-isomenthone (**12**) by feeding (*S*)-d_8-pulegone (**8**). Using mixtures of d_8- and d_5-pulegone enantiomers **7** (**19a**) and **8** (**19b**), all four conceivable deutero *p*-menthan-3-one stereoisomers were detected. In this case, the deutero pulegone enantiomers **7** (**19a**) and **8** (**19b**) were in competition, so it is possible to check whether there was enantiodiscrimination.

Only in feeding experiments with enantiopure d_8-pulegone could the diastereomeric ratio d_8-(4*S*)-*p*-menthan-3-one (**10**)/d_8-(4*R*)-*p*-menthan-3-one (**11**) be compared with the genuine ratio (4*S*)-*p*-menthan-3-one (**2**)/(4*R*)-*p*-menthan-3-one (**3**), because the conversion is not affected by enantiodiscrimination. The diastereomeric ratios (4*S*)-d_8-(−)-menthone/(4*R*)-d_8-(+)-isomenthone **10**/**11** [feeding (*R*)-d_8-pulegone (**7**)] and (4*S*)-d_8-(−)-isomenthone/(4*R*)-d_8-(+)-menthone **12**/**9** [feeding (*S*)-d_8-pulegone (**8**)] corresponded with the genuine ratio (4*S*)-(−)-menthone/(4*R*)-(+)-isomenthone **2**:**3** but were mostly a little bit lower than the genuine ratios.

Exclusively in feeding experiments with both deutero pulegone enantiomers the bioconversion can be checked for an enantiodiscrimination with regard to the absolute configuration in the C-4 position leading to the (4*R*)- and (4*S*)-*p*-menthan-3-ones. Therefore, the diastereomeric ratio of the deuterium-labeled analogue of genuine (1*R*,4*S*)-(−)-menthone (**2**) to the deuterium-labeled analogue of the nongenuine (1*S*,4*S*)-(−)-isomenthone (**4**) as well as the ratio of the deuterium-labeled analogues of genuine (1*R*,4*R*)-(+)-isomenthone (**3**) to the deuterium-labeled analogues of nongenuine (1*S*,4*R*)-(+)-menthone (**1**) was determined. The diastereomeric ratios (1*R*,4*S*)-d_8(d_5)-(−)-menthone to (1*S*,4*S*)-d_8(d_5)-(−)-isomenthone **10**/**12** (**21**/**23**) and (1*R*,4*R*)-d_8(d_5)-(+)-isomenthone/(1*S*,4*R*)-d_8(d_5)-(+)-menthone **11**/**9** (**22**/**20**) were compared with the enantiomeric ratios of d_8(d_5)-(1*R*)-pulegone/d_8(d_5)-(1*S*)-pulegone **7**/**8** (**19a**/**19b**) of the feeding solutions. It was found that the ratio (1*R*,4*R*)-d_8(d_5)-(+)-isomenthone/(1*S*,4*R*)-d_8(d_5)-(+)-menthone **11**/**9** (**22**/**20**) was roughly the same as the ratio d_8(d_5)-(1*R*)-pulegone/d_8-(d_5)-(1*S*)-pulegone **7**/**8** (**19a**/**19b**), but the ratio (1*R*,4*S*)-d_8(d_5)-(−)-menthone/(1*S*,4*S*)-d_8(d_5)-(−)-isomenthone **10**/**12** (**21**/**23**) is almost smaller than the ratio d_8(d_5)-(1*R*)-pulegone/d_8(d_5)-(1*S*)-pulegone **7**/**8** (**19a**/**19b**). This indicated that there was no enantioselectivity in the reduction generating the (4*R*)-*p*-menthan-3-ones, but a discrimination against d_8(d_5)-(*R*)-pulegone **7** (**19a**), the deuterium analogues of the genuine (*R*)-pulegone, favoring the generation of the (4*S*)-*p*-menthan-3-ones.

Finally, it was remarkable that normally the most abundant *p*-menthan-3-one was d_8(d_5)-(−)-isomenthone **12** (**23**), the deuterium-labeled analogues of the nongenuine 1*S*,4*S*-configured (−)-isomenthone (**4**).

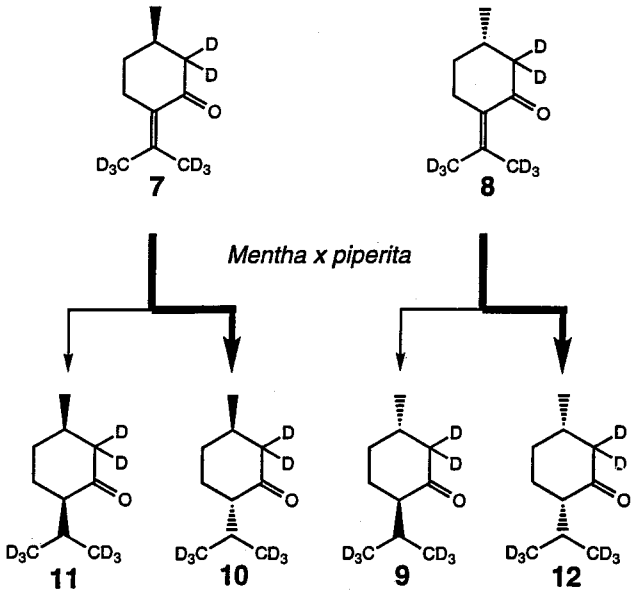
All of the results obtained from the feeding experiments unambiguously prove that the bioconversion of pulegone to the corresponding *p*-menthan-3-ones is not very specific, because both deutero pulegone enantiomers **7** and **8** (**19a** and **19b**) will be converted. Furthermore, in the bioconversion generating the deuterium-labeled (4*S*)-*p*-menthan-3-ones, the deuterium-labeled analogues of the nongenuine (*S*)-pulegone were preferred (Scheme 1).

The 1*S*-configured *cis*-*p*-menthan-3-ones **12** (**23**) were detected as the most abundant stereoisomers after administration of all the mixtures used. Consequently, 1*S*-configured pulegones **8** (**19b**) were preferably converted in *Mentha* × *piperita*.

CONCLUSIONS

Mentha × *piperita* was able to convert both enantiomers of the deuterium-labeled precursor pulegone into menthone and isomenthone, as conclusively demonstrated by feeding experiments. In view of earlier literature the obtained results on the bioconversion of pulegone to menthone and isomenthone in *Mentha* × *piperita* were rather unexpected. It was surprising that the deuterium-labeled analogue of (*S*)-pulegone, which does not occur in *Mentha* species, was preferably

Scheme 1. Proposed Pathway for the Conversion of Pulegone Enantiomers to Menthone and Isomenthone



converted and not the deuterium-labeled analogue of the genuine (*R*)-pulegone when *Mentha* × *piperita* was administered with both enantiomers of pulegone. Furthermore, in the generation of the deutero (4*S*)-*p*-menthan-3-ones a discrimination against the deuterium-labeled (*R*)-pulegone was observed, whereas there was no stereoselectivity in the formation of the deutero (4*R*)-*p*-menthan-3-ones.

ACKNOWLEDGMENT

We thank W. Girnus, Botanical Garden, for cultivating the plant material.

LITERATURE CITED

Askari, C.; Kreis, P.; Mosandl, A.; Schmarr, H.-G. Quality Evaluation of Mentha Oils, Using Enantioselective CGC-Analysis of Monoterpenoid Constituents. *Arch. Pharm. (Weinheim)* **1992**, *325*, 35−39.

Aviv, D.; Galun, E. Biotransformation of Monoterpenes by Mentha Cell Lines: Conversion of Pulegone to Isomenthone. *Planta Med.* **1978**, *33*, 70−77.

Aviv, D.; Krochmal, E.; Dantes, A.; Galun, E. Biotransformation of Monoterpenes by Mentha Cell Lines: Conversion of Menthone to Neomenthol. *Planta Med.* **1981**, *42*, 236−243.

Battaile, J.; Loomis, W. D. Biosynthesis of Terpenes. II. The Site and Sequence of Terpene Formation in Peppermint. *Biochim. Biophys. Acta* **1961**, *51*, 545−552.

Bauer, K.; Garbe, D.; Surburg, H. *Common Fragance and Flavor Materials*; Wiley-VCH: New York, 1997.

Burbott, A. J.; Loomis, W. D. Effects of Light and Temperature on the Monoterpenes of Peppermint. *Plant Physiol.* **1967**, *42*, 20−28.

Clark, R. J.; Menary, R. C. Environmental Effects on Peppermint (*Mentha piperita* L.). I. Effect of Daylength, Photon Flux Density, Night Temperature and Day Temperature on the Yield and Composition of Peppermint Oil. *Aust. J. Plant Physiol.* **1980**, *7*, 685−92.

Croteau, R.; Gershenzon, J. Genetic Control of Monoterpene Biosynthesis in Mints (*Mentha*: Lamiaceae). *Recent Adv. Phytochem.* **1994**, *28*, 193−229.

Croteau, R.; Venkatachalam, K. V. Metabolism of Monoterpenes: Demonstration that (+)-*cis*-Isopulegone, Not Piperitone, Is the Key Intermediate in the Conversion of (−)-Isopiperitenone to (+)-Pulegone in Peppermint (*Mentha piperita*). *Arch. Biochem. Biophys.* **1986**, *249* (2), 306−315.

Fuchs, S.; Beck, T.; Burkhardt, S.; Sandvoss, M.; Mosandl, A. Biogenetic Studies in *Mentha* × *piperita*. 1. Deuterium-Labeled Monoterpene Ketones: Synthesis and Stereoselective Analysis. *J. Agric. Food Chem.* **1999**, *47*, 3053−3057.

Kokkini, S. Chemical races within the genus *Mentha*. In *Essential Oils and Waxes*; Linskens, H. F., Jackson, J. F., Eds.; Springer-Verlag: New York, 1991.

Kreis, P.; Mosandl, A.; Schmarr, H.-G. Enantioselektive Analyse des Menthylacetats zur Qualitätsbeurteilung von Mentha-Ölen. *Dtsch. Apoth. Ztg.* **1990**, *130* (47), 2579−2581.

Lincoln, D. E.; Murray, M. J. Monogenic Basis for Reduction of (+)-Pulegone to (−)-Menthone in *Mentha* oil Biogenesis. *Phytochemistry* **1978**, *17*, 1727−1730.

Murray, M. J.; Lincoln, D. E.; Hefendehl, F. W. Chemogenetic Evidence supporting Multiple Allele Control of the Biosynthesis of (−)-Menthone and (+)-Isomenthone Stereoisomers in *Mentha* species. *Phytochemistry* **1980**, *19*, 2103−2110.

Park, S.-H.; Chae, Y.-A.; Lee, H. J.; Kim, S.-U. Menthol biosynthesis pathway in *Mentha piperita* suspension cells. *J. Korean Agric. Chem. Soc.* **1993**, *36* (5), 358−363.

Reitsema, R. H. A Biogenetic Arrangement of Mint Species. *J. Am. Pharm. Assoc.* **1958**, *47*, 267−269.

Verbit, L. Optically Active Deuterium Compounds. In *Progress in Physical Organic Chemistry*; Streitwieser, A., Jr., Taft, R. W., Eds.; Interscience Publishers: New York, 1970; Vol. 7.

Wüst, M.; Beck, T.; Dietrich, A.; Mosandl, A. On the Biogenesis of Rose Oxide in *Pelargonium graveolens* L'Héritier and *Pelargonium radens* H. E. Moore. *Enantiomere* **1996**, *1*, 167−176.

Wüst, M.; Rexroth, A.; Beck, T.; Mosandl, A. Mechanistic Aspects of the Biogenesis of Rose Oxide in *Pelargonium graveolens* L'Héritier. *Chirality* **1998**, *10*, 229−237.

Received for review February 9, 1999. Revised manuscript received April 30, 1999. Accepted May 9, 1999. Financial support from the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged.

JF9901338