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CONFIGURATION OF PIPERITONE FROM OIL OF *MENTHA PIPERITA*

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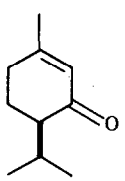
Key Word Index—*Mentha piperita*; Labiatae; peppermint; piperitone; (+)-piperitone from *Mentha piperita*; circular dichroism of piperitone; circular dichroism.

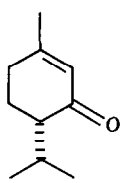
Abstract—Piperitone was isolated from a sample of commercial oil of peppermint (*Mentha piperita*) using gentle procedures to avoid racemization. Comparison of its CD spectrum with the spectra of piperitone standards indicates that the isolated peppermint piperitone is 76–85% the (+)-enantiomer. Piperitone as it occurs in *Mentha piperita* is thus (+)-4*S*-piperitone, or a mixture of enantiomers with the (+)-enantiomer predominating.

INTRODUCTION

The monoterpene piperitone is found in several families of higher plants. It is a major component of the essential oils of some *Cymbopogon* species (synonym *Andropogon*, Gramineae) and some *Eucalyptus* species (Myrtaceae), and a usually minor component of the oil from several species of Labiatae, especially of the genus *Mentha* [1–3]. Piperitone can exist in two steroisomeric forms: (−)-4*R*-piperitone (**1**) and (+)-4*S*-piperitone (**2**). In previous studies, the piperitone enantiomers have been differentiated by optical rotation. Commonly it has been assumed that the physiological piperitone is a pure enantiomer and that partial or complete racemization usually occurs during isolation. Any residual optical activity has then been considered to represent the original enantiomer. Piperitone is very readily racemized during isolation [1–4]. This is especially true of older work in which the conventional bisulfite addition method was used, with resultant base-catalysed enolization–racemization. Even excessive heat can, by itself, cause racemization [5].



1 (−)-4*R*-Piperitone



2 (+)-4*S*-Piperitone

The piperitone from several *Eucalyptus* species has been characterized as (−)-piperitone by a number of investigators [1–3]. Piperitone isolated from *Cymbopogon* has been characterized as the (+)-form, or as the racemic mixture, presumably formed by racemization during isolation and purification [1–3].

Most reported optical rotation values for pipertone from Labiatae are negative: *Mentha pulegium*, α_D −3.10° [4], $[\alpha]_D$ −45.1°, −53° [6]; *Mentha arvensis*, $[\alpha]_D$ −2.2° [7]; a *Mentha longifolia* (L.) Huds. × *M crispa* L. hybrid, negative, no value reported [8]; *Perilla frutescens*, $[\alpha]_D$ −54° [9]. Based on these reports it has been assumed generally that piperitone in the genus *Mentha* is the (−)-form, although there were some obscure reports to the contrary [1–3]. However, recent studies have suggested that (+)-piperitone may be the naturally-occurring form, at least in some species of *Mentha*. Nagasawa and Umemoto [10] reported that piperitone from oil of *Mentha gentilis* had an $[\alpha]_D^{20}$ of +48.2°. Lawrence [11; and personal communication, 1980] purified *ca* 30 μl of piperitone by GC from oil of *Mentha pulegium* and measured an $[\alpha]_D$ of +40°. He suggested that previous reports of (−)-piperitone from *Mentha* were based on inadequately purified samples.

The early work of Kremers [12] is often cited in documentation of the occurrence of piperitone in oil of *Mentha piperita* [2, 3]. However, Kremers' optical rotation data have not been cited previously. Kremers purified piperitone from oil of *Mentha piperita* by the neutral sulfite addition method [13], thus reducing base-catalysed racemization. The isolated piperitone gave an average $[\alpha]_D$ of +9.38°, and he concluded that peppermint oil contains (+)-piperitone [12].

Modern techniques of circular dichroism (CD) allow one to determine sterioisomerism with much smaller samples than are required for optical rotation measurements. Because of the small percentages of piperitone in most *Mentha* oils of the order of 0.1–1% for *Mentha piperita* oils [11, 14]), this is an important advantage. We wish here to report evidence, based on CD measurements of chromatographically-purified material, that the piperitone of *Mentha piperita* is (+)-4*S*-piperitone, or possibly a mixture of (+)- and (−)-forms with (+)-piperitone predominating.

RESULTS

The absorption spectra of the (+)- and (−)-piperitone standards were measured in 95% ethanol (Fig. 1) for comparison with previous results [15–18]. For the (−)-piperitone standard the shape of the spectrum agrees extremely well and the intensity of the spectrum fairly well with the literature (Table 1). Presumably, the weak absorption band at 318 nm is the carbonyl $n\pi^*$, while the intense absorption band at 233 nm is the first $\pi\pi^*$. This

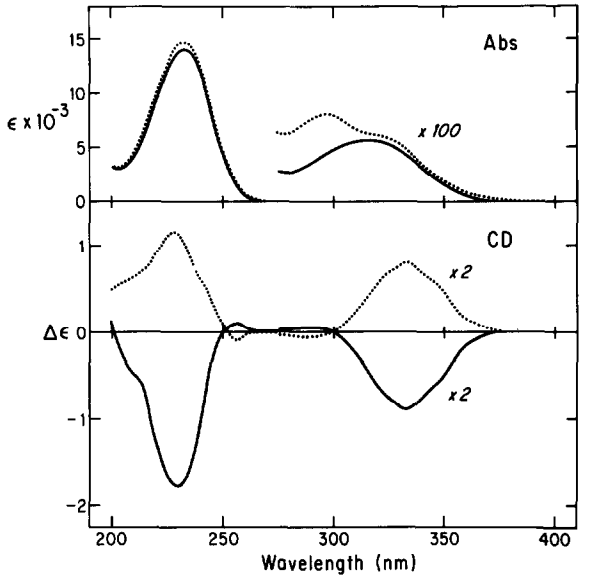


Fig. 1. Absorption and CD spectra of the (−)-piperitone (———) and (+)-piperitone (. . . .) standards in 95% ethanol. $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$.

Table 1. Comparison of absorption data for piperitone in 95% ethanol

	First band (nm)		Second band (nm)	
Reference	λ_{max}	ε_{max}	λ_{max}	$\varepsilon_{max}(\times 10^{-4})$
[15]	319	48.8	—	—
[16]	319	53.7	235	1.58
[17]	323	54.9	235	1.78
[18]	324	39.8	—	—
This work	318	55.9	233	1.39

sample will be considered spectroscopically and chemically pure (but not necessarily a pure optical isomer) and used as the basis for comparison with other samples. The (+)-standard contains some spectroscopic impurities. The CD spectra of the standards (Fig. 1) reveal a band corresponding to the $n\pi^*$ with an extremum at 333 nm, and a $\pi\pi^*$ band with an extremum at 229 nm. The very small CD band at 256 nm could be due to a third transition, the 0–0 vibronic band of the $\pi\pi^*$, a hot band, or an impurity.

Absorption and CD spectra were measured for both of the standards and our peppermint piperitone in diethyl ether, and are compared in Fig. 2. The absorption spectrum of the (+)-piperitone standard shows the same spectroscopic impurities seen in 95% ethanol. Since our peppermint piperitone was isolated in ether solution, its concentration must be determined spectroscopically. The shape of the absorption spectrum indicates that this preparation also contains impurities. We normalize the absorption spectrum of the peppermint piperitone to the (−)-piperitone standard at the long wavelength end (340–400 nm) where the effect of the impurities is minimal

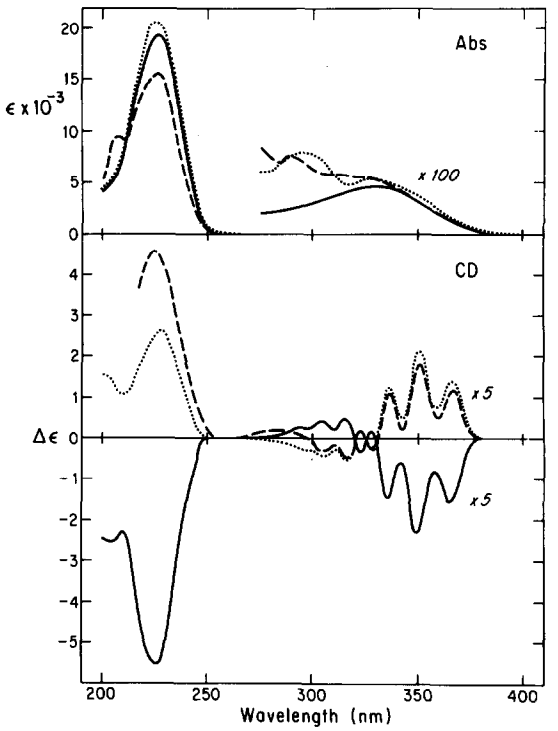


Fig. 2. Absorption and CD spectra of (−)-piperitone (———), (+)-piperitone (. . . .) and peppermint piperitone (– – – –) in ether. The spectra for peppermint piperitone are normalized using $\varepsilon_{340}-\varepsilon_{400}$. $\Delta\varepsilon = \varepsilon_l - \varepsilon_r$.

(Fig. 2). However, with this normalization the 226 nm absorption band is unrealistically low for our pepperimint piperitone. Normalizing at 226 nm would lead to a 24% increase in intensity for the spectra of peppermint piperitone as compared to those given in Fig. 2. These two normalizations give the two limits of enantiomeric purity discussed below.

The CD spectra shown in Fig. 2 demonstrate that the isolated peppermint piperitone is predominantly (+)-piperitone, in agreement with Kremers [12]. Table 2 gives the intensity of the 350 nm vibronic band for the (−)- and (+)-piperitone standards as measured, and for our peppermint piperitone as normalized in concentration. In

Table 2. Physical measurements of piperitone samples

Sample	$\Delta\varepsilon_{350}$ (in diethyl ether)	$[\alpha]_D^{20}$ (neat)
(−)-Piperitone standard (as measured)	−0.46	−48.4°
(+)-Piperitone standard (as measured)	+0.43	+41.9°
Peppermint piperitone (normalized using $\varepsilon_{340}-\varepsilon_{400}$ as in Fig. 2)	+0.36	—
Peppermint piperitone (normalized using ε_{226})	+0.45	—
Pure optical isomer of (−)-piperitone [19]	—	−67.8°

Table 2 the measured optical rotation data reflect the similarity seen in the CD data of the two piperitone standards. The best $[\alpha]_D^{20}$ values for the pure piperitone enantiomers are $\pm 67.8°$ [1–3, 19]. This is based on the maximum value of $-67.8°$ reported by Huggett [19] for (−)-piperitone from *Eucalyptus dives* purified to constant rotation by crystallization (three times) at $-50°$. Our value of $-48.4°$ for the (−)-piperitone standard is close to the $-49.8°$ Huggett [19] obtained with his original (−)-piperitone sample before crystallization, a procedure to which our standards were not subjected. Using $\pm 67.8°$ as the true $[\alpha]_D^{20}$, our (−)-piperitone standard is 87% optically pure, and the (+)-piperitone standard is 81% optically pure. These estimates of the optical purity of our standards, and the CD spectra of our samples in diethyl ether, give an optical purity for our *Mentha piperita* piperitone of 76–85% (+)-piperitone and 24–15% (−)-piperitone. Calculations based on the two piperitone standards give essentially identical values, agreeing with each other to within 1%. Normalization at 340–400 nm gives 76% (+)-piperitone, and normalization at 226 nm gives the 85% value.

DISCUSSION

As discussed in the Introduction, piperitone racemizes with extreme ease. The procedures used here were designed to be as mild as possible, and to avoid use of heat as far as possible. The only stage at which heat was used was in the initial steam distillation of the commercial oil. This is done on the farm, usually in galvanized iron stills. Ethyl ether was chosen as the solvent for spectroscopy of the piperitone samples because it is sufficiently polar to elute piperitone readily from Si gel, and sufficiently volatile to permit easy concentration of samples without application of heat and without volatilization of piperitone; and it possesses the necessary UV-transparency. In addition, ether resolves vibronic structure in the $n\pi^*$ band, thereby facilitating detection of contaminants.

Mentha piperita is a sterile hybrid propagated exclusively by vegetative means. Virtually all of the oil produced in the U.S.A. is from the Black Mitcham cv or from new varieties produced from it by radiation-induced somatic mutation. The new varieties have all been tested extensively for the ability to produce an oil as nearly as possible identical to that of Black Mitcham. The peppermint oil used in this study was almost certainly derived from the Black Mitcham cv or the Todd's Mitcham cv. We feel confident, however, that it is representative of all varieties of *Mentha piperita*.

The results described here could be due to partial racemization of native (+)-piperitone, or to the physiological presence of both enantiomers, with (+)-piperitone predominating. Traditional thinking would favor the former view. However, Huggett [19] suggested that the native piperitone of *Eucalyptus dives* is always a mixture, with (−)-piperitone predominating. Studies with cell-free enzymes from *Mentha piperita* cv Black Mitcham [A. J. Burbott and W. D. Loomis, unpublished results] indicate that (−)-piperitone can be reduced to isomenthone, while (+)-piperitone is inert in these enzyme systems. It is possible that *Mentha piperita* produces both enantiomers of piperitone (presumably by means of separate, stereospecific enzymes) and accumulates (+)-piperitone while reducing (−)-piperitone, in part, to (+)-isomenthone.

EXPERIMENTAL

Piperitone standards. Samples of (−)-piperitone and (+)-piperitone were obtained from Dr. E. Klein of Dragoco, Holzminden, West Germany, for use as standards. The (−)-piperitone was purified from *Eucalyptus dives* oil. The (+)-piperitone was synthesized by oxidation of phellandrene hydrochloride [from (+)-α-phellandrene] with $Na_2Cr_2O_7$ [E. Klein, personal communication]. These materials have been authenticated by Dr. Klein using a number of techniques.

Peppermint piperitone. Peppermint piperitone was isolated from a sample of commercial oil of *Mentha piperita* L. obtained from Mr. R. G. Carrington of I. P. Callison and Sons, Chehalis, Washington. This oil was produced in the Madras area of central Oregon and was from an 'early' harvest (early bloom stage), the usual harvest for that area.

Initial fractionation was by CC in 10 batches of 0.5 ml each on 1.0 × 20 cm columns packed with 8 g Mallinckrodt AR silicic acid (100 mesh) plus 2 g Johns Manville Celite (140 mesh). The columns were packed and loaded dry, as this gave the most effective binding of oxygenated terpenes. Each column was developed successively with 40 ml AR hexane, 250 ml CH_2Cl_2–hexane (25:75), 100 ml CH_2Cl_2–hexane (40:60) and finally with 100 ml CH_2Cl_2. GC analysis of column fractions showed that piperitone was concd in the final CH_2Cl_2 fractions. These fractions contained no hydrocarbons, but did contain traces of piperitenone and unidentified (oxygenated) components. The pooled piperitone fractions were concd under a stream of N_2. Further purification by TLC was carried out on Merck Si Gel G, 0.5 mm thick × 20 cm wide × 30 cm long. Development was with hexane–EtOAc (88:12). This solvent system gives an especially good separation of the oxygenated monoterpenes and, thus, a final purification of piperitone [20]. The piperitone bands were scraped from the plates and packed into 0.5 × 8 cm columns, and eluted with Et_2O. The Et_2O eluate was concd under a stream of N_2. The chromatographically purified sample appeared on assay by GC and GC/MS to be pure piperitone. Nevertheless, it could have contained non-volatile contaminants, such as terpene oxidation products.

GC. and GC/MS. Routine monitoring of chromatographic fractions was carried out by FID/GC as described previously [21]. The column employed was 6.1 m × 3.175 mm stainless steel with 1% phenyl diethanolamine succinate (PDEAS) and 1.5% sucrose acetate isobutyrate (SAIB) coated on 100–120 mesh Chromosorb G and was programmed from 125° to 165° at 1°/min with a N_2 flow rate of 25 ml/min.

GC/MS of piperitone was performed on a Finnigan MAT Model 4023 GC/MS computer system with electron ionization (70 eV), and ion source temp. of 275°. This was coupled to a 2 mm × 1.8 m Pyrex column packed with 7% OV-101 on 100–120 mesh HP Chromosorb W and operated at 150°.

Optical methods. Optical rotation measurements were carried out on a Rudolph Model 80 spectrapolarimeter. Calibration was checked with a soln of (+)-maltose, $[\alpha]_D^{20}$ $+130.4 \pm 1.3°$ (ml/g·dm). Temp. was maintained at 20°, and the rotation was observed at 589 nm (Na D line) using a path length of 1.0 or 2.0 cm. The two piperitone standards were run neat.

CD spectra were measured on a Jasco J-41 CD spectrometer calibrated with (+)-10-camphorsulfonic acid, $\Delta\varepsilon = 2.42$ (cm l/mol) at 290.5 nm [22]. Samples of piperitone standards were prepared volumetrically to produce solns of known concns. A sample concn of 13.16 mM was used for all CD analyses, with a pathlength of 50 μm for the 230 nm band and 1.00 cm for longer wavelength bands.

Absorption spectra for the same samples were measured on a Cary 14 spectrometer.

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