Journal of Chromatography A, 1217 (2010) 6422–6427

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Evaluation of tea tree oil quality and ascaridole: A deep study by means of chiral and multi heart-cuts multidimensional gas chromatography system coupled to mass spectrometry detection

Danilo Sciarrone[a], Carla Ragonese[a], Caterina Carnovale[a], Anna Piperno[a], Paola Dugo[a], Giovanni Dugo[a], Luigi Mondello[a,b,*]

[a] Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, viale Annunziata, 98168 Messina, Italy
[b] Università Campus Bio-medico di Roma, Via Alvaro del Portillo, 21, 00128 Roma, Italy

ARTICLE INFO

Article history:
Received 14 June 2010
Received in revised form 4 August 2010
Accepted 6 August 2010
Available online 13 August 2010

Keywords:
Ascaridole
Deans switch
Chiral chromatography
ISO/FDIS 4730:2004
MDGC/MS
Multidimensional chromatography
Preparative GC
Tea tree oil

ABSTRACT

The natural-like assessment of essential oils is a demanding task due to the growing trend toward adulterations. Usually chiral chromatography was used for this purpose due to the capability of assessing stereospecificity which is directly related to the enzymatic pathways of each plant species. On the other hand, the quality of an essential oil involves also the evaluation of its oxidative state, mainly connected with the age and storage conditions. In fact, some modifications in the chemical profile of the oil can occur if not properly preserved. Alterations of the components due to oxidative reactions lead to the formation of peroxides, endoperoxides and epoxides, such as ascaridole and 1,2,4-trihydroxymenthane, usually present in very low amount, formed by the oxidation of terpinen-4-ol and α-terpinene, respectively. Therefore, in the present research, the quality of Australian Tea Tree oil (*Melaleuca alternifolia* (Maiden & Betche) Cheel, Myrtaceae) was investigated by means of a multi heart-cut multidimensional gas chromatographic system coupled to a mass spectrometer detector and by conventional enantio-GC. The MDGC system allowed the complete separation of the compounds of interest transferred from the first column to a second dimension based on a different separation mechanism. The MS detector at the end of the second column provided the identification of the peaks with high similarity values because of their high purities after the multidimensional separation. Method validation was carried out, in order to use this procedure for routine application, monitoring the repeatability of 1D retention times and 2D peak areas, LoD and LoQ. Finally, enantiomeric ratios for chiral compounds were established to support quality data obtained.

1. Introduction

Melaleuca is a genus of plants in the family Myrtaceae. The plants are generally found in open forest, woodland or shrub land, particularly along watercourses and the edges of swamps. Tea tree essential oil (TTO) is a complex mixture of compounds obtained by steam distillation from the leaves and twigs of *Melaleuca alternifolia* (Maiden & Betche) Cheel, Myrtaceae with a fresh camphoraceous fragrance. Sometimes, however, other essential oils from *Leptospermum* spp. and other *Melaleuca* spp. may be summarised under this name like, for instance, cajuput oil obtained from *Melaleuca leucadendra* (L.) L. and niaouli oil obtained from *Melaleuca viridiflora* Sol. ex Gaertn., which are typical of the northeast coast of New South Wales, Australia. The main components of TTO are terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols. The oil has beneficial medical (including antiseptic and antifungal action), as well as cosmetic properties. Monoterpenes and sesquiterpenes have been evaluated for their various bioactivities, including antimicrobial [1] and antioxidant activities [2]. Terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, *p*-cymene, α-terpineol, α-pinene, terpinolene, limonene and sabinene account for 80–90% of the oil. Terpinen-4-ol has been found to suppresses inflammatory mediator production by activated human monocytes [3]. TTO also exhibited strong cytotoxicity towards human lung cancer cell line (A549), human breast cancer cell line (MCF-7) and human prostate cancer cell line (PC-3) [4]. From about 100 terpenes found in TTO more than 60 individual substances have been identified by means of gas chromatography coupled to mass spectrometry detection. The natural content of the individual terpenes may vary consider-

* Corresponding author at: Dipartimento Farmaco-chimico, Facoltà di Farmacia, Università di Messina, viale Annunziata, 98168 Messina, Italy.
Tel.: +39 090 6766536; fax: +39 090 358220.
E-mail address: lmondello@unime.it (L. Mondello).

0021-9673/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2010.08.019

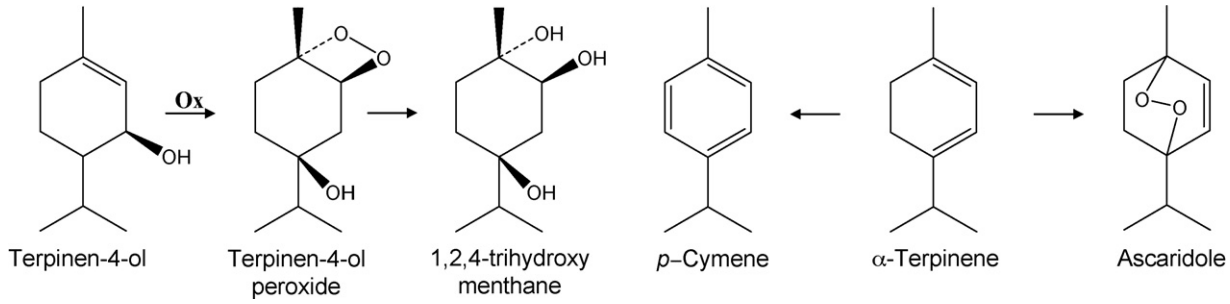

Fig. 1. Terpinen-4-ol (a) and α-terpinene (b) oxidation process pathways.

ably depending on the *M. alternifolia* population used, the climate, the leaf maceration, the age of the leaves and the duration of distillation. Enantio-GC is commonly adopted for the genuineness assessment of essential oils since the enantiomeric ratios of chiral compounds are peculiar of each plant chemotype and family [5]. In this concern, this technique can easily demonstrate adulteration of genuine oils as for example for the detection of spiking with cheaper distilled oil. The composition of tea tree oil changes along with aging particularly in presence of atmospheric oxygen but also depending on the storage conditions, i.e. if the oil is exposed to light and high temperatures. The oil is usually used diluted, as adverse reactions can occur with the use of pure tea tree oil, due to the presence of sensitizers compounds. In fact, alterations of some components due to oxidative reactions lead to the formation of peroxides, endoperoxides and epoxides: moreover, *p*-cymene concentration can rise to levels approaching its upper limit while α-terpinene and terpinolene decrease. Two pathways are involved in this reactions (Fig. 1), one based on the oxydation of the π bond of terpinen-4-ol leading to its peroxide form and then to 1,2,4-trihydroxy menthane (a), the other involving oxidation of the α-terpinene, γ-terpinene and terpinolene to their benzene analogue, *p*-cymene, or to the endoperoxide ascaridole (b). The two pathways are well known in terpene chemistry [6]. As a consequence, a range for a list of compounds has been proposed to evaluate the quality of the oil (Table 2).

Recently TTO has been determined by means of HPTLC in cosmeceutical formulations [7] even if GC methods are often preferred for the determination of the essential oils. The gas chromatographic approach offers a significant improvement in sensitivity, thus GC and GC/MS methods for tea tree oil have been reported [8,9]. Owing to the widespread use of GC in routine essential oils analysis, it is necessary that good GC methods are developed and that these are thoroughly validated. The complexity of the matrices sometimes is a limitation for monodimensional analytical approaches because of the limited peak capacity of a single column driving to coelutions. This hinders peak identification and quantification even using mass spectrometry detection because of the non-specific fragmentation of this class of compounds. Based on the SCCP opinion [10], listed compounds have to be investigated for the quality assessment of tea tree oil, while some further investigation is required for oxidation products that could lead to allergic problems for which a range of concentration in the oil have not been evaluated. The latter could be important for the quality assessment of the oil as raw material for cosmetic purposes in the industrial field. In the last decades multidimensional techniques have been recognized as the best approach due to their higher capability for the separation of complex matrices. Comprehensive two-dimensional chromatography has been extensively applied for food analysis [11–14]. Comprehensive chromatography has been exploited for enantioselective analysis of TTO [15,16] but the high cost per analysis and the lack of skilful operators have limited the use of this powerful technique for routine purpose. Heart-cutting MDGC technique based on Deans switch device [17,18], which involves the transfer of one or more unresolved fraction from a first to a second dimension, has already proven its utility in essential oils field for separations that require very high efficiencies [19,20]. This technique could be considered the most suitable approach for this purpose due to the user-friendly instrumentation nowadays available and the lower costs per analysis, in comparison with comprehensive techniques employing cryogenic focusing gas and interfaces. MDGC finds particular application in essential oil analysis, i.e. for chiral separation, due to the complex nature of the materials, and the need for highly efficient separation for specific analysis goals. Heart-cut GC has been used after stir bar sorptive extraction for the enantioselective analysis of TTO by Mosandl and co-workers in 2002 [21]. The same multidimensional instrumentation used in this study coupled to mass spectrometry has been recently employed in the essential oils field for enantiomeric ratio assessment by our group [22,23], as well as in the identification and quantification of allergens in flavour and fragrance matrices [24]. The aim of this study was to develop a rapid and accurate multidimensional method for the determination of the compounds reported in ISO/FDIS 4730:2004 for the quality assessment of pure tea tree oil. Moreover, the presence of the two possible allergenic agents ascaridole and 1,2,4-trihydroxymenthane was investigated. While the standard of 1,2,4-trihydroxymenthane was available, ascaridole, due to his peroxide nature could not be purchased from suppliers since its shipment is strictly prohibited to prevent terrorism actions.

2. Experimental

2.1. Materials

Pure standard of α-pinene, sabinene, α-terpinene, p-cymene, limonene, 1,8-cineole, γ-terpinene, terpinolene, terpinen-4-ol, α-terpineol, aromadendrene, ledene, caryophyllene, farnesol and globulol were kindly provided by Sigma–Aldrich (Supelco, Milan, Italy) while 1,2,4-trihydroxymenthane was kindly provided by University of Tasmania (ACROSS center). For ascaridole, due to the unavailability of the standard, a synthesis with a final yield of 37% was carried out followed by a purification step by means of preparative GC. Calibration curves for each chemical class, for 1,2,4-trihydroxymenthane and ascaridole, with regression factors always higher then 0.9992, were built using nonane as internal standard (IS) at a fixed concentration of 3% (w/v). γ-Terpinene was used for monoterpenes, α-terpineol for oxygenated monoterpenes, caryophyllene for sesquiterpenes and farnesol for oxygenated sesquiterpenes. All the solutions were prepared in ethanol (GC grade) and injected at different concentrations (from 0.1 to 50%, w/v) considering their original purity. The identification of single components in tea tree oil has been achieved by means both of mass spectra comparison with a LRI filter of ±5 units and by means of injection of pure standards.