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Rapid identification of some coumarin derivatives in deterpenated citrus peel oil by gas chromatography

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Abstract

Generally on the gas chromatogram of a volatile essential oil, terpenes, oxygenated compounds and sesquiterpenes appear. With temperature programming, it was shown that some non-volatiles are present with the volatiles. They are simple coumarin (2*H*-1-benzopyran-2-one) derivatives such as citropten (5,7-dimethoxycoumarin) and furocoumarins (psoralen, 7*H*-furo[3,2-*g*][1]benzopyran-7-one) such as bergapten (5-methoxypsoralen), some of which are phototoxic. Terpeneless oils are used in perfumes and cosmetics, so it is important to be able to establish rapidly if they contain phototoxic compounds.

1. Introduction

Furocoumarins have been used empirically for centuries in the treatment of depigmentations, especially in Egypt (a papyrus mentions the use against vitiligo of a plant nowadays named *Psoralea caryfolia*).

It has been reported that lemon oil elicits a phototoxic reaction in animal skin [1,2]. Various coumarin and furocoumarin derivatives found in several plants of the Umbelliferae, Rutaceae and Moraceae families are potent photosensitizing agents, and are known to have lethal and mutagenic effects [3,4]. Several coumarin derivatives have been reported to impart a variety of biological manifestations, ranging from antibiotic, anti-inflamatory activity [5], phytotoxic activity, inhibition of seed germination and inhibition of growth of plants and microorganisms [6] to those causing skin dermatitis, liver damage and carcinogenisis.

Some psoralens, such as bergapten, are used in the therapeutic treatment (buvatherapia) of psoriasis and mycosis fungoides [7], but they induce skin dermatitis at high concentrations [8,9].

Lemon oil is an essential oil of considerable commercial importance [10]. Citrus oils are used in the formulation of perfumes and cosmetics and coumarins are used for their flavour quality and are added to sunscreen formulations to enhance tanning induced by ultraviolet radiation [5,11]. Coumarin derivatives are photosensitizing, so the International Fragrance Association [12] guideline limits the lemon oil content in fragrances to 4%.

Coumarin derivatives are separated by extraction and liquid chromatography before the identification [13,14]. HPLC with spectrofluorimetric detection is the most generally used

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method to determine coumarin derivatives [11,15–17] after their separation from the volatiles. In this paper, we report the identification of some coumarin derivatives on the same chomatogram as the volatile compounds of a deterpenated citrus peel oil.

2. Experimental

2.1. Materials

This study was carried out on a lemon essential oil (*Citrus limon* L. var. Eureka) from the Ivory Coast, kindly supplied by Chauvet (Seillans, France). The oil was obtained by cold-pressing of the fruit peel and was "purified" by chilling. The volatile fraction contained 94% (percentage area) of terpenes, 3–4% of oxygenated compounds and 2% of sesquiterpens. The non-volatile fraction was estimated to be 2.5% [18].

2.2. Chromatography and spectroscopy

GC analyses were performed in a Carlo Erba Fractovap 2900 instrument equipped with a flame ionization detector. A fused-silica column (25 m × 0.32 mm I.D.) coated with SE-54 was utilized. The initial temperature was 100°C for 3 min after injection, then increased to 230°C at 3°C/min, with a final hold at 230°C for 20 min. The injector and detector were maintained at 200 and 260°C, respectively. Helium was used as the carrier gas at a flow-rate of 30 ml/min (splitting ratio 1:100) and the auxiliary gases were air (100 ml/min) and hydrogen (1 ml/min).

On the chromatogram, terpene compounds have retention times from 7 to 13 min, oxygenated compounds from 13.5 to 26 min, sesquiterpenes from 26.5 to 40 min and non-volatiles from 40 min to the end of the analyses.

A Finnigan ITD ion trap detector coupled to a gas chromatograph was used to analyse samples, the GC temperature programme was the same as above for GC alone. We compared mass spectra from three databanks: National Institute of Standards and Technology, General Purpose and Toxicology and Terpenes.

Samples were run in ethanol (1:7, v/v) and the retention times and mass spectra were compared with those of pure products: coumarin, 7-methoxycoumarin (herniarin), 5,7-dimethoxycoumarin (limettin, citropten), psoralen, 5-methoxypsoralen (bergapten) and 2,3-dihydroflavone (flavanone).

The samples were obtained by supercritical carbon dioxide fractionation. The description of the experimental procedure will be the subject of another paper.

3. Results and discussion

The chromatogram in Fig. 1 shows a classical representation [19] of the volatile compounds of cold-pressed lemon oil variety Eureka obtained using an SE-54 column with temperature programming from 100 to 190°C. The components are 94% terpenes, 3% oxygenated compounds and 2% sesquiterpenes. Fifty-one constituents accounting for 99.7% of the total volatiles have been identified and quantified in commercial lemon peel oil [20].

Fig. 2 shows the GC analysis of whole peel oil (the same sample as for Fig. 1), with temperature programming from 100 to 230°C. It can be seen that there are two small peaks at 52 and 56 min. The chromatogram in Fig. 3 shows the analysis with temperature programming of a terpeneless fraction. After 40 min many unidentified compounds with volatile can be seen; they represent 0.5–15.0% of the terpeneless oil. Examination of several such chromatograms shows that their content increases with increase in oxygenated compounds in the fractions. This fact is important if we want to separate flavour compounds from non-volatiles.

For preliminary identification, the samples of essential oil were analysed and compared with several coumarins by GC and GC–MS using some pure compounds. Table 1 gives the retention times with temperature programming for the pure products used. The results of the GC–

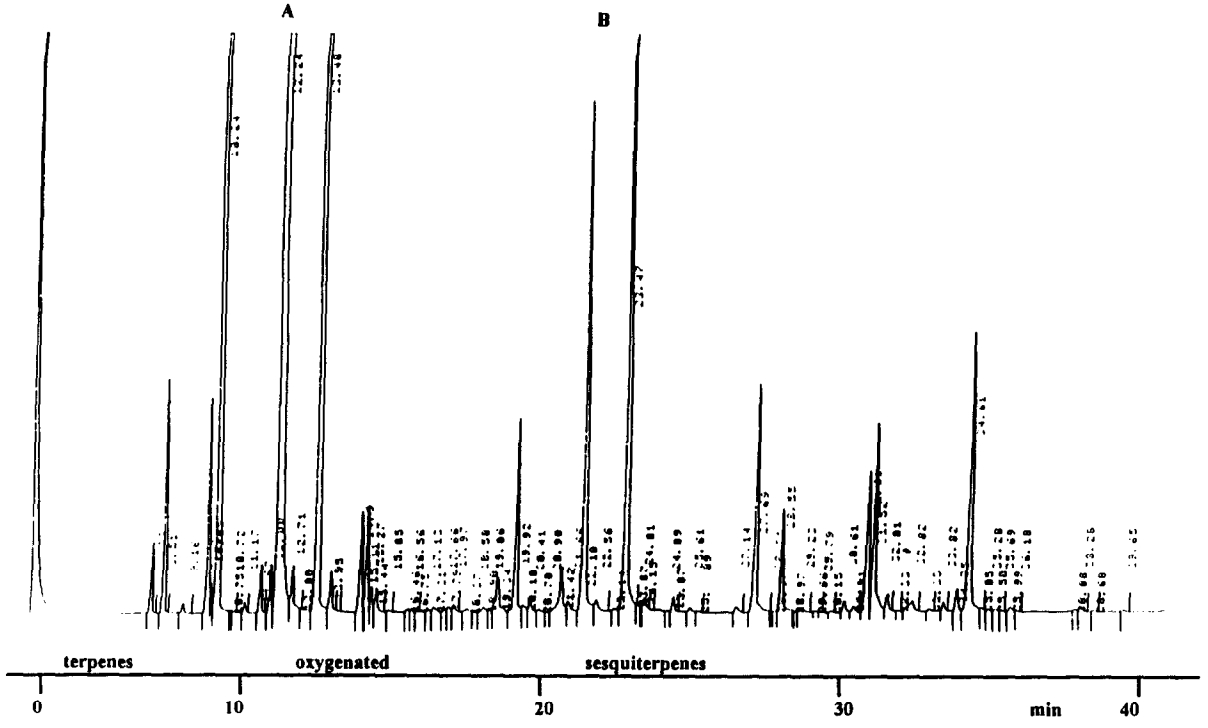

Fig. 1. Classical GC analysis of whole peel oil with a temperature gradient from 100 to 190°C. A = Limonene; B = citral.

MS analysis, reported in Table 2, show that there are sesquiterpenes, oxygenated compounds, three coumarins, three psoralens (Fig. 4) and two fatty acid methyl esters. Our interest is mainly in the identification of the coumarin derivatives, because they play an important role

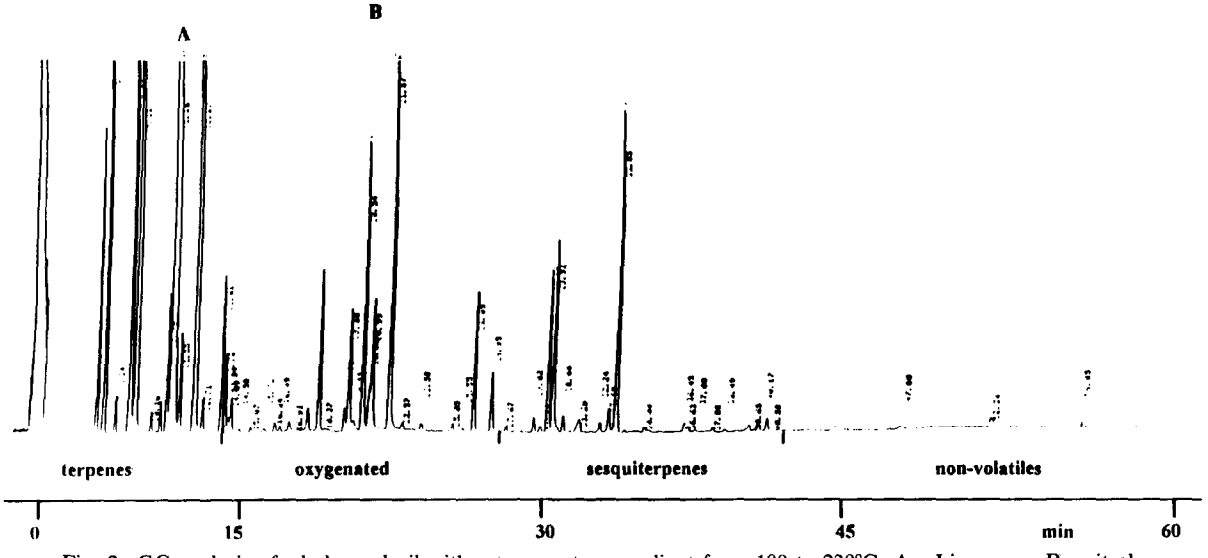

Fig. 2. GC analysis of whole peel oil with a temperature gradient from 100 to 230°C. A = Limonene; B = citral.

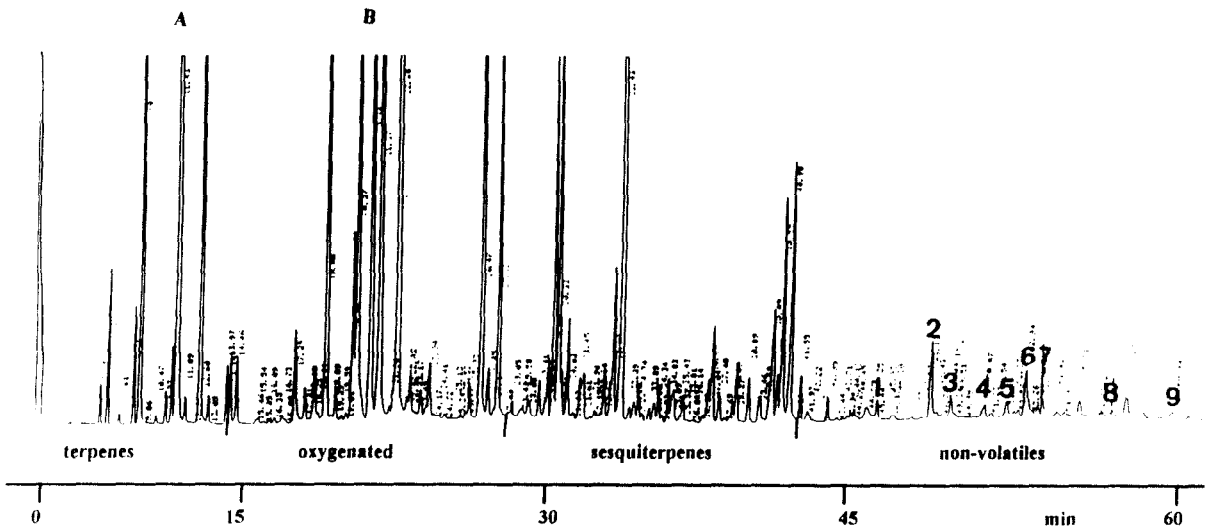

Fig. 3. GC analysis of terpeneless citrus oil with a temperature gradient from 100 to 230°C. See Table 2 for products identified.

Table 1
Retention times of pure compounds

Compound	t_R (min)[a]	M_r
Limonene (A)	11	136
Citral (B)	19–21	154
Coumarin	30	146
Herniarin	46	176
Citropten	51	206
Bergapten	55	216
Flavanone	61	224

[a] Retention time ±1 min.

in the fragrance and flavour quality of the treated oil.

From Fig. 3 and Tables 1 and 2, it can be seen that the retention time increases with increasing molecular mass for coumarin derivatives and with increasing number of methoxyl groups [21]. With our technique we can identify only the compounds that have molecular masses up to about 300; polymethoxyflavones [22], carotenoids and other pigments should be analysed with more appropriate conditions (*e.g.*, SE-30 column and higher temperatures) [23,24]. An

Table 2
Compounds identified on the same chromatogram of volatiles

No.	Compound	M_r	Retention time (min)
1	7-Methoxycoumarin (herniarin)	176	46
2	Bicyclic sesquiterpen	208	48
3	5-Methoxy-8-(3′-methylbut-2′-enyloxy)psoralen (phellopterin)	300	49
4	Methylpentadecanoate	256	50
5	6,7-Dimethoxycoumarin (scoparone)	206	51
6	5,7-Dimethoxycoumarin (citropten)	206	52
7	Methylhexadecanoate	286	55
8	5-Methoxypsoralen (bergapten)	216	56
9	5-(2′,3′-Epoxy-3′-methylbutyloxy)psoralen (oxypeucedanin)	286	59

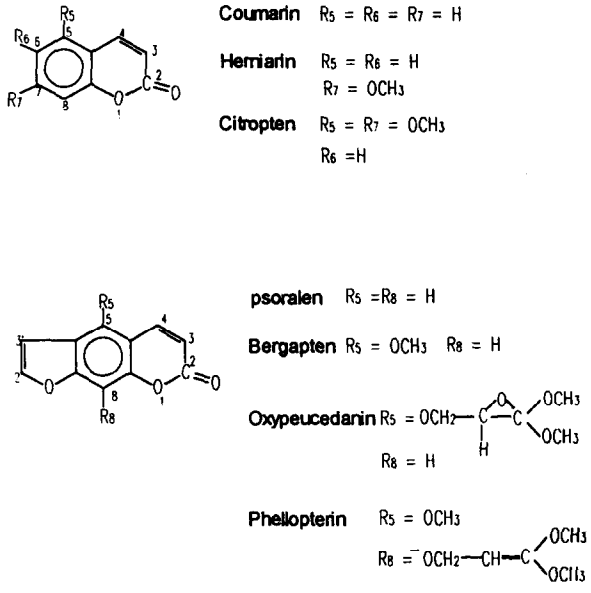

Fig. 4. Formulae of coumarins and psoralens identified by GC analysis.

increase in oxygenated sesquiterpenes in the terpeneless fractions occurs. However, we did not analyse them because terpenoids generally have a complex skeleton, making them difficult to identify, and in addition they have several isomers. Some thermal degradation during the GC analysis was observed [25]. Some compounds such as furanone or pyranone and lactone derivatives resulting from the oxidation of linalool and linalool ester have been found only in old essential oils [26].

Using GC–MS, some compounds were identified with volatile compounds as shown in Table 2. For preliminary identification, the samples of essential oil were analysed and compared with some pure compounds by GC and GC–MS. We used also a comprehensive databank that contains 40 000 reference spectra for identification, and we compared the mass spectra with those in the literature.

About fifteen coumarin derivatives have been found in different citrus oils [3]. A study of the volatile compositions of several citrus species showed that the qualitative and quantitative composition of the product depends on the climatic and geographical origin, the harvesting season and the isolation procedure [27–29]. We may draw the same conclusions for the Eureka lemon, but data are scarce.

In the literature, these compounds are considered as non-volatiles, and are normally analysed separately from volatiles, as it is assumed that they are not present in the volatile fractions, *e.g.*, after a vacuum distillation. However, Shu *et al.* [16] reported that the content of bergapten was 11–88 ppm in a lemon cold-pressed oil from the Ivory Coast, and Naganuma et al. [30] gave 69–87 ppm for the natural oil of the same origin and 5–17 ppm for oil from Sicily.

It is generally accepted that psoralens are more important than coumarins in the phototoxicity of lemon oil. Of the psoralens, oxypeucedanin [30] is said to be 2–100 times more important than bergapten in citrus oils. The content of bergapten in citrus oils is difficult to specify with any certainty because its content depends on the origin of the oil and the way in which it is identified. The highest concentration of bergapten is found in bergamot and lime oils (0.3–0.4% and 0.21%, respectively) [31], and it must be purified when it is employed in perfumes and in the fragrance and flavour industry.

We can identify six coumarin derivatives, herniarin, scoparone, citropten, bergapten, phellopterin and oxypeucedanin, of those mentioned in the literature [17]; for the other compounds we do not have enough data because of their very low concentration. We can say that some of them are coumarin derivatives because their mass spectra show a fragmentation pattern characteristic of the psoralen nucleus [32–34].

We identified by GC–MS as phellopterin a compound for which other workers [35] gave the same mass spectrum but were not sure of its structure (*m/z* 217 and 232). We found herniarin (7-methoxycoumarin) and a dimethoxycoumarin, which we tentatively identify as 6,7-dimethoxycoumarin because of its retention time (50–51 min), differing by about 1 min from that of citropten (51–52 min) (*cf.*, Furuya and Kojima [18]), whereas 4,7-dimethoxycoumarin appears 3 min later. The major coumarin in this oil is citropten, which is not phototoxic for human skin [7]. It precipitates as a white powder in terpeneless samples, suggesting the presence of

other coumarins. After treatment with hexane and ethanol it gives white, filamentous crystals.

In addition to the six coumarins that we report, Rouzet *et al.* [17] identified in the non-volatile fraction of lemon oil 5-geranoxypsoralen (bergamottin), 5-geranoxy-7-methoxycoumarin (Same), 5-isopentenoxy-7-methoxycoumarin, 8-geranoxypsoralen, 5-methoxy-8-(2′,3′-epoxy-3′-methylbutyloxy)psoralen (byakangelicol), 5-(2′,3′-dihydroxy-3′-methylbutyloxy)psoralen (oxypeucedaninehydrate) and 5-methoxy-8-(2′,3′-dihydro-3′-methylbutyloxy)psoralen (byakangelicin). He estimated their concentration to be 0.91% [36]. Only traces of other compounds have been found by TLC, so it makes them difficult to identify.

Glandian [36] analysed extracts of non-chilled Eureka lemon oil to determine the coumarin and psoralen compounds. There are differences between our results and his, which may be explained by the different extraction methods used (solvent extraction and steam distillation by Glandian and supercritical fractionation in this work). The chilling step is used to precipitate some non-volatile compounds [10].

4. Conclusions

We have demonstrated the possibility of the qualitative and rapid analysis of lemon peel oil by a single capillary GC run to obtain data rapidly on components, principally coumarins, important for the fragrance and phototoxicity quality of the sample. The major important coumarin (citropten) could be used as a marker of the other non-volatile components of the oil. The presence of coumarins in chilled oils shows that the process is not sufficient to remove all coumarin compounds from cold-pressed lemon oil.

5. Acknowledgements

6. References

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