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New neryl esters from *Helichrysum italicum* (Roth) G. Don (Asteraceae) essential oil

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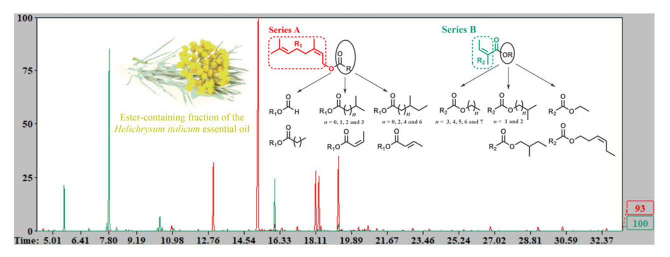
ABSTRACT

Helichrysum italicum (immortelle) is a dwarf aromatic shrub native to the Mediterranean region. The typical subspecies (*italicum*) produces an essential oil rich in neryl acetate and characteristic β-diketones, italidiones, highly valued in the perfume industry. As esters are an important group of aroma-active volatiles, herein the composition of the ester fraction of this immortelle chemotype essential oil was studied in detail. Chromatographic separation of Corsican immortelle essential oil enabled the discovery of numerous potentially olfactory-interesting esters of nerol and/or angelic acid, undetectable by direct GC-MS analyses of the unfractioned oil. Four esters of nerol and medium-chain branched fatty acids represent new natural products, while several other esters have a rather restricted occurrence in the Plant Kingdom.



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1. Introduction

Helichrysum italicum (Roth) G. Don (Asteraceae) is a small Mediterranean aromatic shrub with golden yellow flowers that do not wither, and their lively color and signature curry-like scent prevail even when the flowers are collected and dried. This is why it is also called immortelle or curry plant (Sarkić and Stappen 2018; Andreani et al. 2019; Oliva et al. 2020). This taxon shows remarkable adaptability in terms of growing conditions, thriving both on the coast and in the mountains, that translates into a heterogeneous morphology and phytochemical polymorphism. There are several

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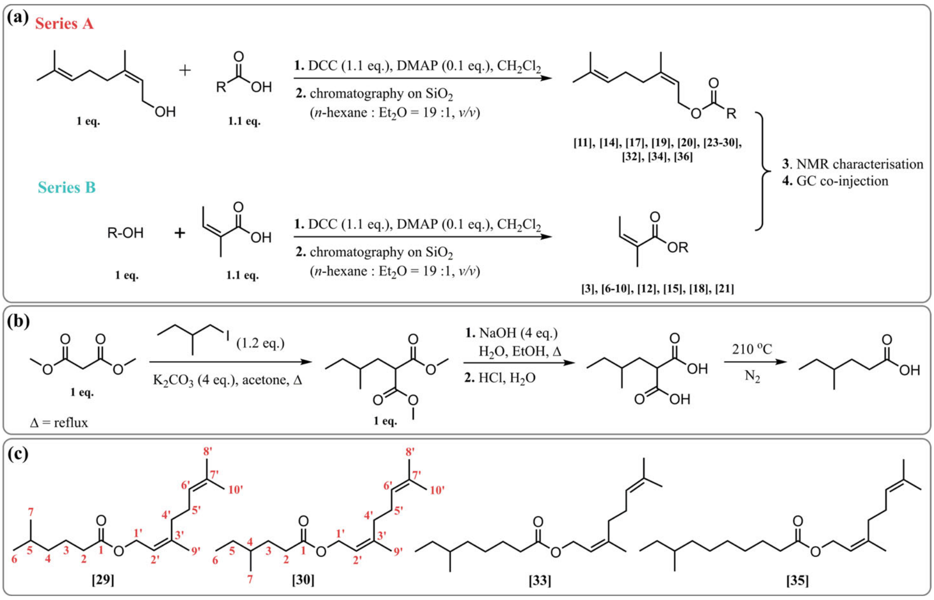

Figure 1. Neryl (series A) and angeloyl esters (series B) standards synthesis by the Steglich method (a), the synthetic route to 4-methylhexanoic acid (b), and the structures of the newly found neryl esters (c). The designations of ester synthetics samples correspond to the designations given in Table S1.

chemotypes of *H. italicum* characterized by distinct essential-oil composition, and besides the natural variability, the change in the oil composition may occur due to geographical origin, plant growth stage and various environmental factors (soil type, altitude, sun exposure, etc.; Appendino et al. 2015; Ornano et al. 2015; Tundis et al. 2005; Venditti et al. 2016). The typical subspecies (*italicum*) from Corsica produces an essential oil rich in the acetate ($\geq$30%) and propionate ($\geq$5%) of nerol, and italidiones characteristic β-diketones of this species ($\geq$10%; Ćavar Zeljković et al. 2015; Andreani et al. 2019). This Corsican oil is the most appreciated immortelle chemotype by perfumers because of its spicy saffron character, well complemented with curry, nutty, and celery facets (Hellivan 2009). A recent GC-O study of the Corsican oil revealed the identity of several key odorants (i.e. 4,6-dimethyloctane-3,5-dione, 1,8-cineole, and nerol). However, it was found that many other, still unidentified constituents present in low amounts also participate in the global odor of this essential oil (Andreani et al. 2019).

Esters of short- and medium-chain fatty acids and acyclic monoterpene alcohols are extremely important both as fragrances and flavor substances. For example, neryl acetate has a very sweet, refreshing, fruity-floral scent and it is used in perfumery for blossom compositions (e.g. orange blossom or jasmine; Bauer et al. 2008). Also, it has been established that with the increase of the number of C-atoms in the acid part of the ester the fruity note starts to dominate over the flower one, so neryl hexanoate has a refreshing fruity-herbaceous odor (Arctander 1969). Although angelates do not represent standard items on a perfumer's shelf, mostly due to the cost of their production, some of them have quite complex fragrances – e.g. butyl angelate has a warmherbaceous, winey odor with a distinct caramel undertone, while hexyl angelate has an additional tea-like note instead of the caramel undertone (Arctander 1969).

As esters are an important group of aroma-active volatiles, in this work, we aimed to study in detail the composition of the rather neglected ester fraction of the mentioned immortelle chemotype essential oil.

2. Results and discussion

The chromatographic separation on SiO_2 of a Corsican oil sample, using *n*-hexane/Et_2O mixtures with a very shallow gradient at low Et_2O content, yielded fractions that were dominated either by the acetate or propionate of nerol. In this work, we focused our attention on the first eluting ester-containing fraction (**VIII**) as it was proved to contain numerous minor neryl and angeloyl esters initially undetectable in the direct GC-MS analyses of the unfractioned oil (Figure S1; Table S1).

Although, esters of the most common isomeric acyclic monoterpene alcohols, i.e. nerol, geraniol, linalool, and lavandulol, exhibit very similar mass spectra characterized by the base ion at *m/z* 93 and a very intense ion at *m/z* 69 (Paolini et al. 2005; Figure S1), we anticipated that these esters from fraction **VIII** were those of nerol due to the well-known preference of *H. italicum* to biosynthesize neryl esters (NEs). Good matching of RI values for some of the detected NEs with the RI values available in the literature (Radulović et al. 2017) confirmed this. The fraction total ion chromatogram contained three groups of NEs showing regularities in their GC-retention behavior (a constant RI difference of *ca.* 100 units; Table S1). The most plentiful group was the one containing NEs of short- and medium-chain *n*-fatty acids (*n*-FAs; C_1-C_4, C_6, C_8, C_{10}, and C_{12}). The identity of these NEs was unambiguously corroborated by GC co-injection runs of synthetic standards prepared from nerol and the corresponding FAs by the general Steglich procedure with fraction **VIII** (Figure 1(a)). Interestingly, neryl decanoate [**34**] has been only previously reported as a major volatile compound present in the extracts of pheromone glands of the click beetle *Betarmon bisbimaculatus* (König et al. 2016), and as a minor volatile of the stingless bee *Geotrigona mombuca* mandibular gland extract (Stangler et al. 2009). Moreover, neryl dodecanoate [**36**] has been thus far only found as a minor constituent of the Bulgarian rose concrete (Watanabe et al. 1977).

Compared to the mentioned *n*-homologs, two other groups of NEs eluted slightly faster from the GC column implying that these were their branched-chain isomers. Neryl 2-methylpropanoate [**19**], neryl 2-methylbutanoate [**23**], and neryl 3-methylbutanoate [**24**], common constituents of essential oils, were readily identified (Radulović et al. 2017). Five additional branched NEs, eluting at RI 1693, 1789, 1799, 1996, and 2195, were detected, as well, but their identity was not inferred from MS and RI comparison. Based on the established biosynthetic pathways, by which higher plants produce branched medium-chain FAs (Kroumova et al. 1994), it was assumed that these were also NEs of methyl branched, *iso*- or *anteiso*- (C_6, C_7, C_9, and C_{11}) FAs. Thus far, branched NEs, with *iso*-C_4-C_8 FAs, have been exclusively found in the Australian geranium essential oil (Doimo et al. 1999). In this paper, it was reported that neryl 4-methylpentanoate had a RI value of 1692 on a BPX-5 column, which fits one of the RI values of our detected branched NEs (1693). However, there appears to exist an excessive disparity between the reported RI value for neryl 5-methylhexanoate (1825) by Doimo and coworkers (1999), and the RI values of the herein detected NEs with branched C_7 FAs (1789 and 1799).

To rectify the situation, synthetic samples of esters of nerol with both *iso*- and *anteiso*-C_6 and C_7 FAs were prepared and spectrally characterized (NMR, IR, MS; Figures S2–S21; Table S2). Commercial standards of 3-methylpentanoic, 4-methylpentanoic, and 5-methylhexanoic acids were in our stock, while we needed to prepare a synthetic sample of 4-methylhexanoic acid using the malonic ester synthesis (Figure 1(b)). After GC-coinjection experiments of the obtained NEs standards and fraction **VIII**, it was unequivocally confirmed that the ester eluting at RI 1693 was neryl 4-methylpentanoate [**27**], the ester eluting at RI 1789 – neryl 5-methylhexanoate [**29**], while the ester eluting at RI 1799 – neryl 4-methylhexanoate [**30**]. It seems that the alleged neryl 5-methylhexanoate [**29**], reported by Doimo et al. (1999), was most probably misidentified for neryl heptanoate for which we also prepared a synthetic standard and found the RI value of 1827. In addition, other inconsistencies between our RI data and the RI data reported by Doimo et al. (1999) for several other NEs were noted. It appears that they have most probably misidentified neryl heptanoate (RI = 1882) for neryl 6-methylheptanoate, neryl 6-methylheptanoate for neryl octanoate (RI = 1919; we found the RI of 1926 units for synthetic neryl octanoate) and neryl heptanoate (RI = 1762) for an ester of some other isomeric monoterpenol. As the identification of the mentioned NEs in this study was solely based on MS-library matching and comparison with literature RI values, and a dozen of geranyl esters with both normal- and branched-chains were detected as well, it would be wise to re-examine the chemical composition of the essential oil of this Australian *Pelargonium* hybrid and to unambiguously confirm the identification of these esters by coinjections.

Interestingly, NEs of *anteiso*-C_5 and *anteiso*-C_6 FAs eluted from the herein used DB-5MS column faster than the corresponding *iso*-isomers, while the opposite elution order was observed for the esters with *iso*- and *anteiso*-C_7 FAs (Figure S22). Thus, a careful analyst is warned of this elution-order change in the branched C_5-C_8 acid range, and it appears necessary to confirm identifications by coinjections, while a relatively constant difference in retention index values (ΔRIs) of 36 and 28 units, between *iso*- (C_9–C_{20}) and *anteiso*-isomers (C_{11}–C_{19}) of medium and long-chain FA esters, respectively, and the related *n*-chain isomer is well established (Ilic-Tomic et al. 2015). Herein, similar ΔRI values were found for NEs with *iso*- and *anteiso*-C_7 FAs (37 and 27, respectively). As the remaining two branched NEs, eluting at RI 1996 and 2195, had ΔRI values of 28 units, we are quite certain that these two NEs also belonged to the *anteiso*-series, i.e. that they were esters of nerol and 6-methyloctanoic acid [**33**], and nerol and 8-methyldecanoic acid [**35**], respectively (Figure 1(c)). To the best of our knowledge, these three *anteiso*-NEs ([**30**], [**33**], and [**35**]) represent new natural products.

Several angeloyl esters (AEs) were detected in fraction **VIII** as well. The tentative identification of the compounds in this ester series was based on the presence of a characteristic base ion at *m/z* 100 (free acid) and intense ions at *m/z* 55 and 83 that are derived from α-cleavage next to the C = O bond in their mass spectra (Thomas and Willhalm 1976; Figure S1). As mass spectrometry with electron-impact ionization sometimes fails to provide distinguishing features of angelates and their geometric isomers, tiglates, and that there are scarce RI data in the literature, we had to unequivocally confirm these tentative identifications by co-injections of AE synthetic samples with fraction **VIII**. Again, synthetic samples were prepared by the standard Steglich esterification DCC/DMAP method (Figure 1(a)). The most abundant esters were AEs with lower weight branched-chain saturated alcohols (i.e. *iso*-C_4 [**3**], *iso*-C_5 [**6**], and *anteiso*-C_5 [**7**]). Octyl and neryl angelate ([**18**] and [**25**]) were also present in relatively higher percentage, while AEs with C_4-C_7 *n*-chain saturated alcohols ([**6**], [**9**], and [**12**]), (*Z*)-3-hexen-1-ol [**10**] and 2-phenyl-1-ethanol [**21**] were found in trace amounts. We find it worth mentioning that octyl and heptyl angelates, [**18**] and [**12**], have been previously found only in *Anthemis nobilis* L. essential oil (Akio and Takashi 1989), while phenethyl angelate [**21**] has been previously reported only as a minor constituent of *Pinus halepensis* Miller essential oil (Fekih et al. 2014), and as a volatile of Malay rose apple (Pino et al. 2004).

In addition to the already mentioned neryl and angeloyl esters, we have identified four esters of low weight branched alcohols with low weight branched FAs ([**1**], [**2**], [**4**], and [**5**]), three esters of benzoic acid ([**13**], [**16**], and [**22**]), one tiglate [**26**], as well as one ester of geraniol [**31**] in fraction **VIII** (Table S1). To verify the presence of the newly detected constituents in a non-commercial oil sample, we analogously analyzed a sample of *H. italicum* oil hydrodistilled in our laboratory. A targeted screening of the composition for compounds [**1**]–[**36**], combined with dry-flash chromatography, revealed the presence of all esters.

3. Conclusions

In conclusion, in total 36 esters were identified in the studied ester-containing chromatographic fraction of a *H. italicum* subsp. *italicum* essential oil sample from Corsica. Among them, 29 represented minor constituents that were not detected during the GC-MS runs of the unfractionated oil. The identity of the majority of the detected esters was unequivocally confirmed by GC co-injection experiments with synthetic standards and the chromatographic fraction. The most numerous were neryl esters (18 in total), followed by angeloyl esters (10 in total). Among them, neryl 5-methylhexanoate [**29**], neryl 4-methylhexanoate [**30**], neryl 6-methyloctanoate [**33**], and neryl 8-methyldecanoate [**35**] (Figure 1(c)) were found for the first time in the samples of natural origin, while several other esters (i.e. neryl 4-methylpentanoate, decanoate and dodecanoate, as well as phenethyl, heptyl, and octyl angelates) have a rather restricted occurrence in the Plant Kingdom.

Disclosure statement

The authors declare that there are no conflicts of interest.

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