Unsaturated Components in the Essential Oil of Anthemis nobilis L. (Roman Chamomile)

By I. Klimes and D. Lamparsky, Givaudan Research Company Ltd., Dübendorf-Zurich, Switzerland

The identification of new bifunctional esters in the essential oil of *Anthemis nobilis* L., the so-called Roman Chamomile oil, was recently described by two groups of Swiss research chemists.[1,2] A novel structural type of naturally occurring substances derived from 3-hydroxy 2-methylene butyric acid could be ascertained in the case of compounds A to C (figure 1). The same acid is part of a goyazensanolide derivative recently described by Bohlmann et al.[3]

The angelate D derives from a bifunctional isoprene unit whereas the esters E to G are derivatives of C_4-diols exhibiting an angelic and an isobutyric acid moiety. These acids are very typical also for a large number of mono-esters already known to occur in Roman Chamomile oil.[4]

French authors recently presented their analytical findings on the oil using the negative chemical ionization technique in mass spectrometry.[5] They tracked the GLC profile of the complete oil with the mass numbers of the quasi-molecular ions $[M\text{-}H]^-$ in combination with the carboxylate anion $RCOO^-$. Thereby

0272-2666/84/0004-0101$02.00/00—© 1984 Allured Publishing Corp.

Perfumer & Flavorist (ISSN 0272-2666)
An Allured Publication
Volume 9, Number 4

August/September 1984

inter alia the presence of five esters derived from methacrylic or crotonic acid was reported, however, without giving definite structural assignments for the individual components. The same holds for eight esters of the angelate or tiglate type, respectively. We have worked in a more traditional way and wish here to present the results concerning some of these methacrylates, crotonates, angelates and tiglates. All esters mentioned by us have proved by comparison with authentic synthetically prepared samples.

Methacrylates/Crotonates

The acidic fraction of Roman Chamomile oil represents 1.02% by weight of the total oil. Besides a very large portion of free angelic acid (ca. 59%) we found small amounts of methacrylic acid (ca. 2%) thus confirming literature data.[6] It seemed therefore reasonable to assume the presence of its esters in the neutral part since esters play a predominant role in the oil. Indeed, the same alcohols representing the main components as esters of angelic acid and which are part of the above mentioned new esters of 3-hydroxy 2-methylene butyric acid, are also connected with methacrylic acid in the form of the following three methacrylates 1, 2 and 3 (figure 2).

As representative of this group, the 2-methylbutyl methacrylate (2) exhibits in its mass spectrum (figure 3) two prominent peaks at m/z = 69 and 70 (base peak). They are indicative for the acidic part $[C_4H_5O]^+$ (m/z = 69) as well as the olefin C_5H_{10} produced by mass spectrometric elimination of methacrylic acid from the molecule. Two small fragments may give further support to the proposed structure: m/z = 87 is formed by the protonated acid part $[C_4H_7O_2]^+$ of the molecule and m/z = 99 corresponds to the $[M\text{-}C_4H_9]^-$ fragment. No signal is observed for the molecular ion at m/z = 156. To elucidate the molecular weight of such a compound, negative chemical ionization is really more helpful.[7] But all these data originating from MS alone are not yet proving the definite structure. Other structural possibilities are not excluded. Thus, for instance, a corresponding crotonate is expected to have a similar mass display.

For structural assignments we need more information even in such a low molecular aliphatic ester series. The best confirmation of structures proposed especially for newly detected natural constituents is therefore always a comparison with authentic synthetic samples. In the case of compound 2 the synthetic specimen was in all respects identical with the natural product. IR- and ^{1}H-NMR-spectra show all the features (figure 4).

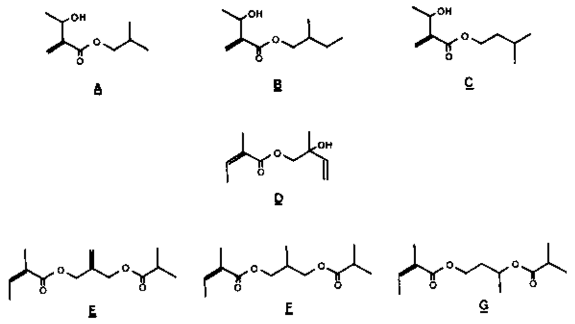

Figure 1. Bifunctional esters of Roman Chamomile oil.

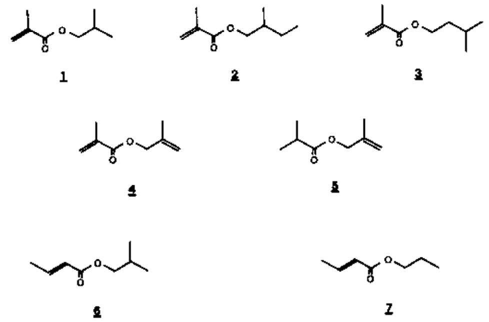

Figure 2. Methacrylates and other unsatured acid esters.

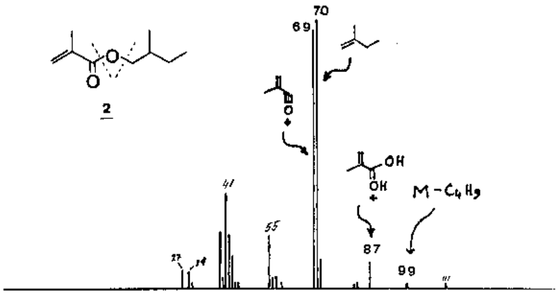

Figure 3. Mass spectrum of 2-methylbutyl methacrylate (2).

Regarding the above mentioned publication of Georges and Fellous[5] we thus confirmed their indication of a methacrylate or crotonate appearing at 16.3 min. (figure 5). According to our findings this compound with the quasi-molecular ion $[M\text{-}H]^-$ at m/z = 155 is identical with 2-methylbutyl methacrylate (2).

Interestingly enough, Georges and Fellous also indicated a trace component eluting before the 2-methylbutyl methacrylate. This constituent appears with $[M\text{-}H]^-$ at m/z = 139. It has therefore the molecular weight of 140 which corresponds to the empirical formula $C_8H_{12}O_2$. We detected the same compound when we analyzed a distillation fraction of Roman Chamomile oil by GLC-MS. It locates under peak number 49 of the GLC of the total oil whereas the just mentioned 2-methylbutyl methacrylate appears later as peak number 59 (figure 6).

Once again, the mass spectrum of this new compound does not reveal too much of its structural secrets, but the base peak at m/z = 69 indicates clearly enough the presence of the methacrylic or crotonic acid moiety. Some reflections in other connections led us to the synthesis of methallyl methacrylate (4) whose spectra are shown in figure 7.

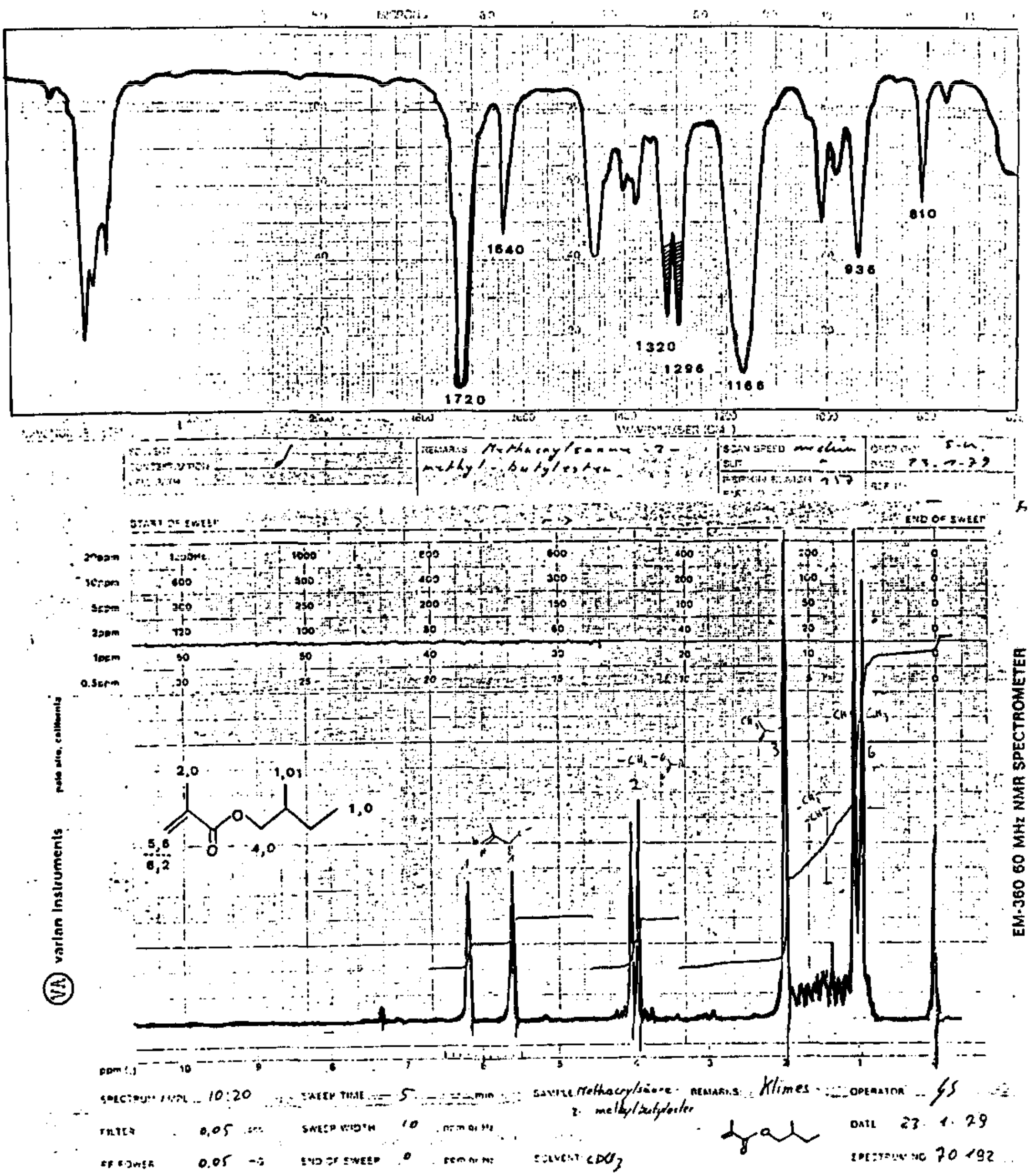

Figure 4. IR- and ^{1}H-NMR-spectra of compound 2.

The IR data fit very well into the picture we already gained with our first methacrylate sample: 1720 and 1162 cm^{-1} for the ester group, 1636, 942 and 820 together with 1320 and 1298 cm^{-1} for the unsaturated acidic part and, in addition, 1656 and 906 cm^{-1} for the terminal methylene group in the alcoholic part of compound 4.

The NMR-spectrum shows even at 60 MHz all features we need for full information and proof of the molecular structure.

The gas chromatographic behaviour on three different columns is identical for the synthetic compound and its natural counterpart. The mass spectra do not differ the one from the other. Thus, the occurence of methallyl methacrylate (4) in Roman Chamomile oil is adequately proved. In this context, we furthermore confirm the presence of methallyl isobutyrate (5) (constituent 36 in the GLC curve) which has recently been mentioned by Thomas.[8]

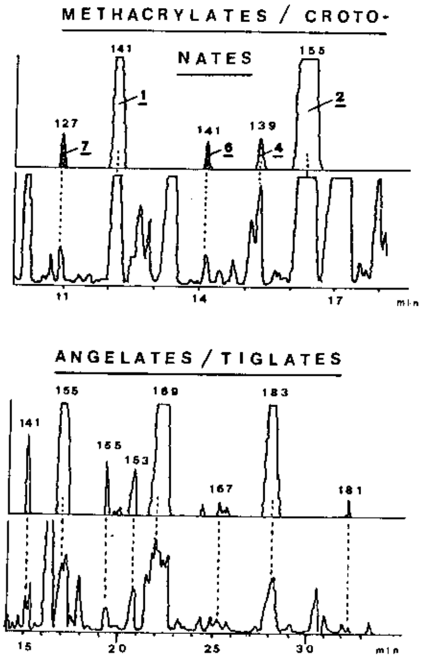

Figure 5. Chromatographic profiles according to negative ion mass spectrometry (*Georges and Fellous* 1982).

Supposing that the elution sequence of this class of compounds on Carbowax 20 M which was used by Georges and Fellous does not differ too much from the one we applied in our studies (UCON HB 5100), one can deduce some further conclusions from a comparison of various data now available: With respect to the retention behaviour, crotonates are eluted later than the corresponding methacrylates. On our column, for instance, isobutyl methacrylate (1) is eluted as peak number 35 whereas isobutyl crotonate (6) comes as peak number 58. Comparing the respective quantitative data (ca. 1.7% and ca. 0.1%, respectively) with the indications given in the publication of Georges and Fellous (see figure 5), one can assume that the broad peak appearing at 12.2 min with $[M\text{-}H]^-$ at m/z = 141 is due to isobutyl methacrylate (1) and the small, third peak at 14.1 min with the same quasi-molecular ion $[M\text{-}H]^-$ to isobutyl crotonate (6).

The first peak in the same figure of the French publication must be, according to our findings, the propyl crotonate (7), which is present to the extent of 0.02% in the oil analyzed.

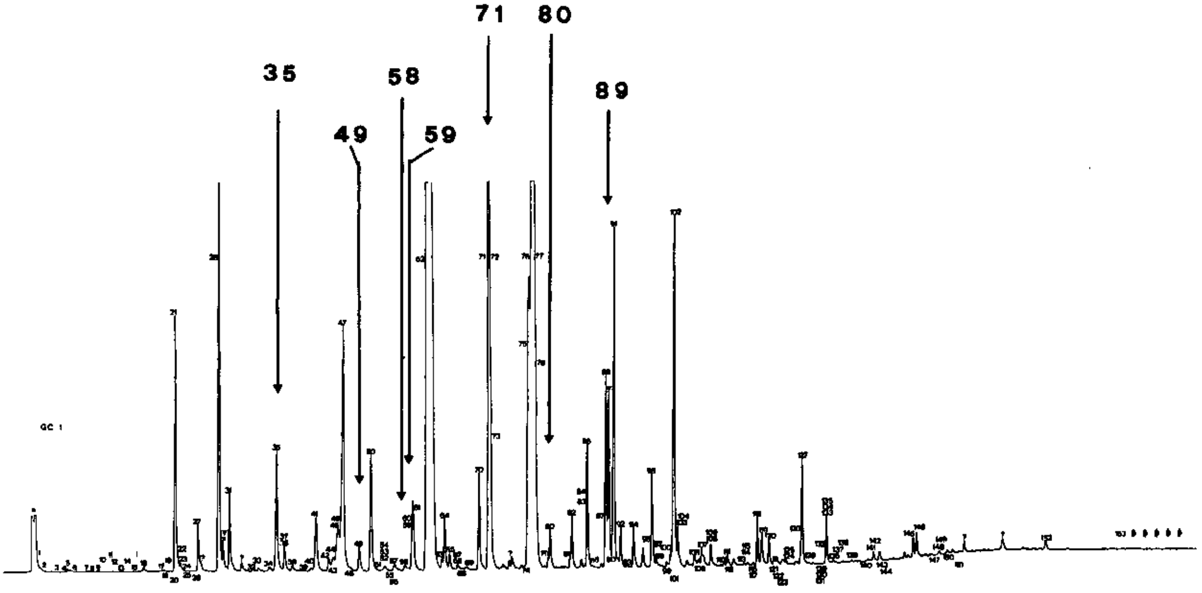

Figure 6. GLC of Roman Chamomile oil (glass capillary column Ø 0.35 mm, 50 m length, UCON HB 5100 as stationary phase, temperature program: 10′ room temp., 10′ 50°C, then 50-180°C with ΔT = 3°/min, carrier gas helium, 1.5 atm).

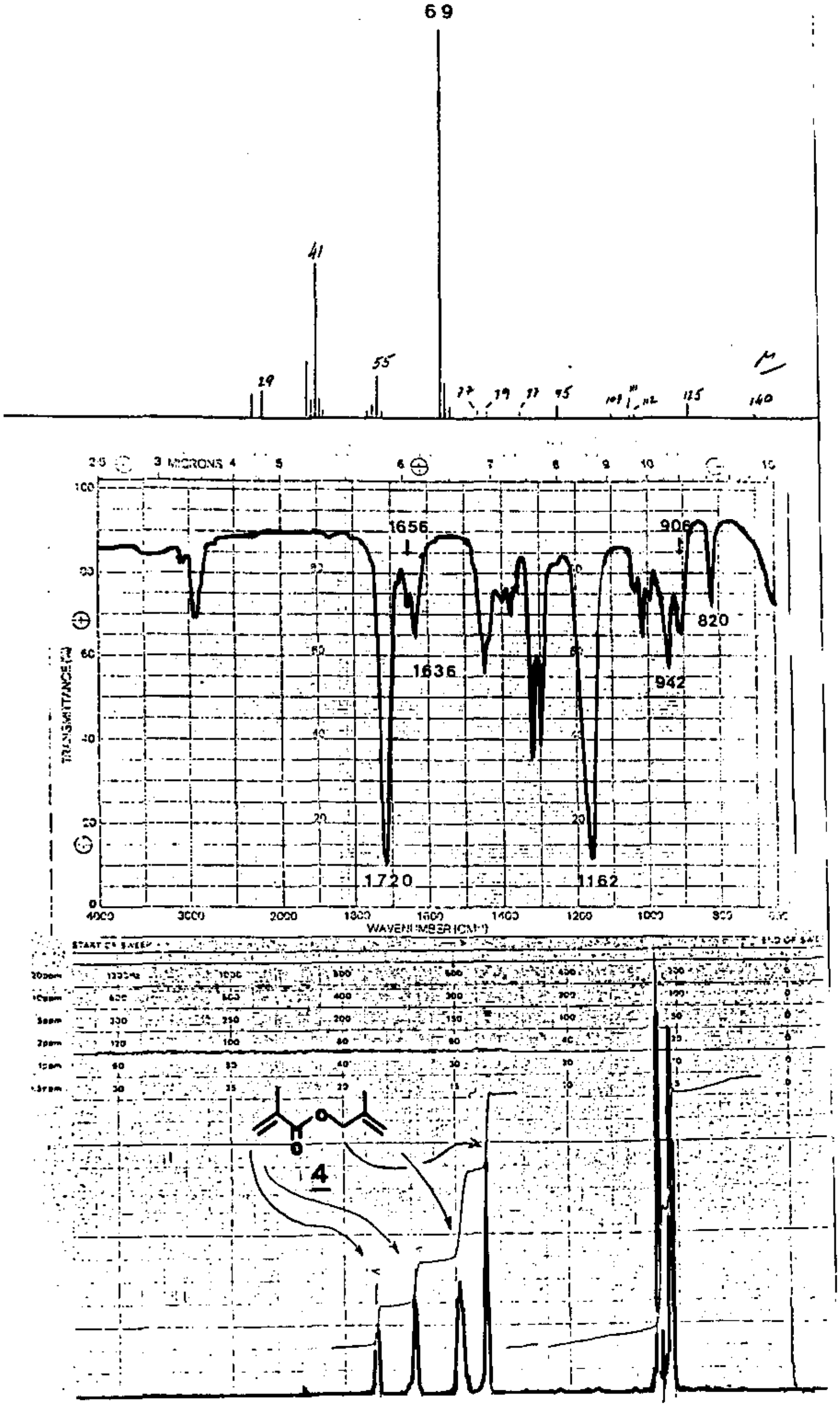

Figure 7. Spectra of methallyl methacrylate (4).

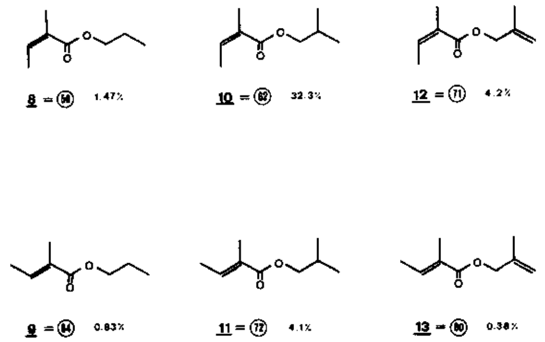

Figure 8. Some pairs of angelates/tiglates in Roman Chamomile oil. The approximate percentages in the oil as well as the position in the GC (figure 6) is given under the structural formula.

Tiglates/Angelates

Let us now turn to the presence of tiglic acid esters besides the esters of angelic acid, known to be the main components of Roman Chamomile oil. According to Thomas and Willhalm,[9] one can clearly differentiate between these two isomeric forms on the basis of mass spectrometry.

Thus, the (E)-isomer presents not only a much more abundant mass fragment at m/z = 101 (corresponding to the protonated acid moiety), but also the ratio of the fragments at m/z = 83 and m/z = 82 is always in favor of the tiglate. The authors furthermore showed that synthetically prepared esters like methallyl and phenylethyl angelates/tiglates do not exhibit signals at m/z = 100 or 101, respectively.

We could not find any reference to the natural occurence of these esters in Roman Chamomile oil itself, but Georges and Fellous[5] localized an ester of this type which may possibly be the methallyl angelate (12) (cp. their figure 9, retention time 20.9 minutes, quasi-molecular ion [M-H] at m/z = 153).

Because the differentiation of angelates and tiglates is strongly facilitated by isolation of the natural product and comparison of all spectroscopic data with its synthetic counterpart, we proceeded in this manner in all cases where it was possible. We now confirm the presence of methallyl angelate (12) and methallyl tiglate (13) in the oil together with two other pairs of angelates/tiglates (8/9, 10/11) as presented in figure 8.

The mass spectra of 12 and 13 were already discussed by Thomas and Willhalm[9] and showed the mentioned differences of the ratio at m/z = 83 and m/z = 82 whereas the mass fragments at m/z = 100 and m/z = 101 could also under our experimental conditions not be observed.

A most important hint came from NMR, because the chemical shift for the olefinic proton in the acidic moiety differs considerably (δ = 6.04 ppm for 12 versus 6.96 ppm for 13). Also the shape of some infrared absorption bands is very characteristic (figure 9).

In addition to the three pairs of esters mentioned above, others of both the angelic and tiglic acid type were identified. Thus, 2-butyl tiglate (14), n-butyl tiglate (15) and n-amyl tiglate (16) were found in small percentages between 0.2% and 0.8% in the oil (figure 10).

Furthermore the in-depth analysis has revealed the presence of additional angelates (17 to 20) which have been detected for the first time in Roman Chamomile oil.

Another angelate exhibiting an unsaturated al-

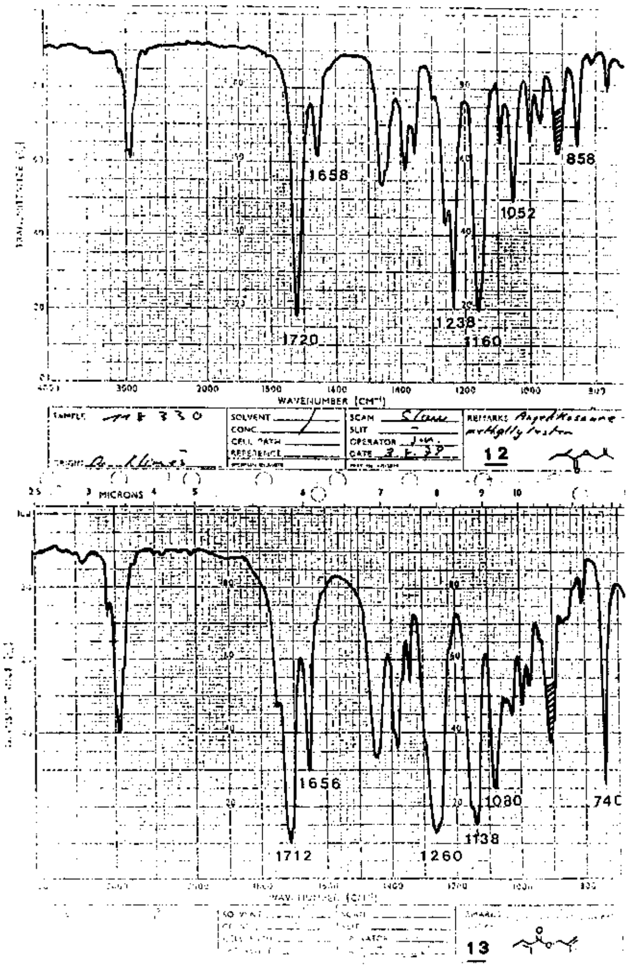

Figure 9. IR-spectra of compounds 12 and 13.

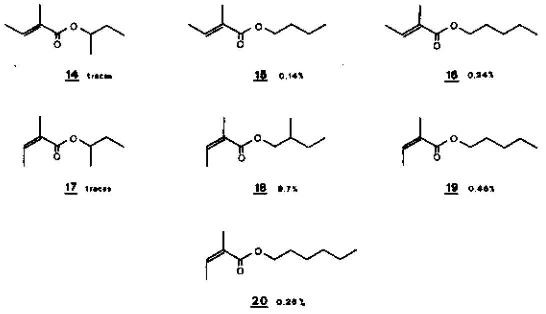

Figure 10. Some further tiglates and angelates.

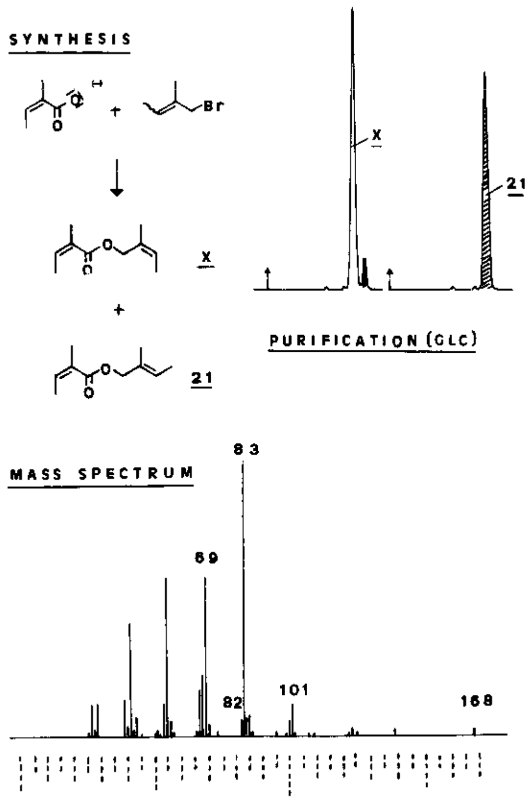

Figure 11. A novel unsaturated angelate (21) of Roman Chamomile oil.

cohol moiety constitutes a novel ester and is shown to be the (E)-2-methyl-2-buten-1-yl angelate (21), (figure 11). It corresponds to compound 89 in figure 6.

The new ester was synthesized from the potassium salt of angelic acid and 2-methyl-2-butenyl bromide in the presence of crown ether 18:6. We obtained a mixture of two angelates which could

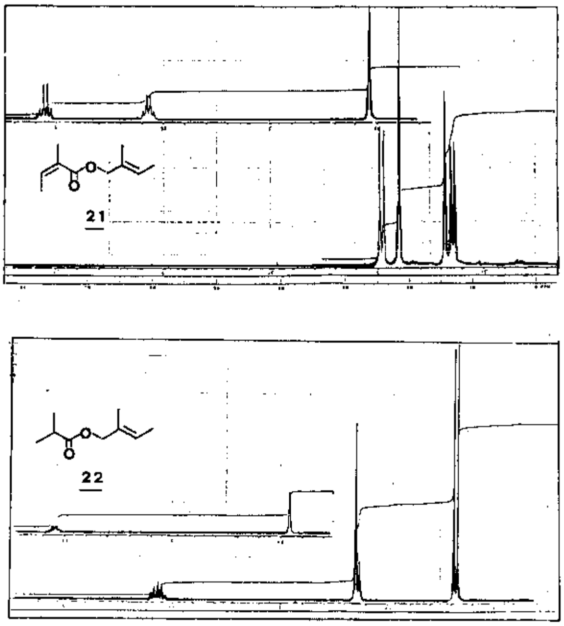

Figure 12. 400 MHz-¹HNMR-spectra of compounds 21 and 22.

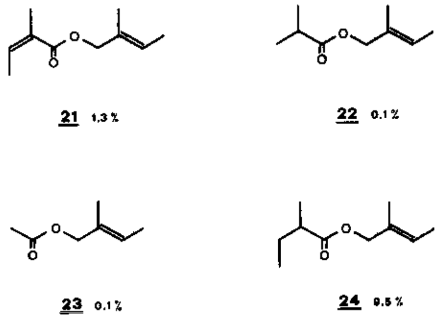

Figure 13. Esters of (E)-2-methyl-2-buten-1-ol in Roman Chamomile oil.

be separated and isolated by preparative GLC. This separation has enabled us to compare the 400 MHz-¹HMNR spectra with respect to the (Z)- or (E)-configuration of the double bond in the alcoholic part of the molecule. From this we deduce that it is the (E)-configurated isomer (tiglyl angelate) which is present in the natural substrate.

The spectral data lead to the following observations: The mass spectrum exhibits a molecular ion peak at m/z = 168. The base peak appears at m/z = 83. The abundance of the mass fragment at m/z = 101 does not immediately presume the (Z)-isomer, but the infrared spectrum seems to indicate its presence by the characteristic shape of the absorption bands (sharp at 1230, broad at 1156, sharp at 1042 and 850 cm^{-1}, see also reference 10). No doubt however remains about the structure when the 400 MHz-¹HNMR data are analyzed (figure 12).

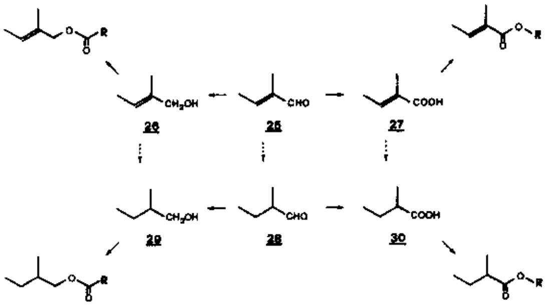

Figure 14. Compounds with (E)-2-methyl-2-butene skeleton and their saturated analoga identified in Roman Chamomile oil.

The olefinic proton centered at δ = 6.05 ppm can be assigned to the angelic part of the molecule, whereas the second one centered at δ = 5.57 ppm and exhibiting the same fine structure probably due to an allylic coupling belongs to the 2-methyl-2-buten-1-ol part of the ester. The singlet at δ = 4.53 for the $-O-CH_2$ group as well as the signals for the four methyl groups on C = C double bonds (doublets at δ = 1.98 and 1.64 ppm, singlets with fine structure at δ = 1.90 and 1.68 ppm) finally confirm the presence of all structural elements we are expecting for 21.

Further support for the occurrence of the (E)- rather than the (Z)-2-methyl-2-buten-1-yl esters in Roman Chamomile oil is given by the fact that we also identified (E)-2-methyl-2-buten-1-yl isobutyrate (22), the NMR spectrum of which is shown in figure 13. In addtion, tiglyl acetate (23) and tiglyl 2-methylbutyrate (24) were also found in the oil.

Last but not least we would like to mention the occurrence of traces of tiglic aldehyde (25), tiglic alcohol (26) and tiglic acid (27) in Roman Chamomile oil. They are accompanied by the corresponding saturated compounds 28-30 (figure 14).

The occurrence of all degrees of oxidation of the isoprene unit together with the fully hydrogenated counterparts may be indicative of the biogenetic possibilities in the plant of *Anthemis nobilis* L. Hydroxylation, oxidation or reductive steps will often be followed by esterification. Very active enzyme systems thus furnish the various esters which constitute the majority of the constituents in Roman Chamomile oil. With respect to the effects of this oil in perfumery, the predominance of angelic acid esters, i,e., the (Z)-configurated 2-methyl-2-butenyl system, seems to be the most interesting feature.

Summary

Esters play a predominant role in Roman Chamomile oil. The main components are derivatives of (Z)-2-methyl-2-butenoic acid (angelic acid). These angelates are accompanied by a large number of other esters. The present communication now definitely confirms the natural occurrence of the methacrylates 1-4, the crotonates 6 and 7, the tiglates 9, 11, 13-16 and further angelates 12, 17-21. Final proof for all mentioned compounds was established by comparison with authentic synthetically prepared specimens.

Acknowledgment

We thank our colleagues Dr. E. Billeter and Dr. J. Schmid for valuable discussions and assistance in instrumental analysis.

This paper is based on a lecture given at the 13th International Workshop on Essential Oils, September 1982, Würzburg/Germany.

References

Address correspondence to D. Lamparsky, Givaudan Research Company Ltd., Uberlandstrasse 138, CH-8600 Dubendorf, Zurich, Switzerland.

1. I. Klimes, D. Lamparsky and E. Scholz, Helv. Chim. Acta **64,** 2338 (1981)
2. a.) A. F. Thomas, M. Schonwey and J.-Cl. Egger, Helv. Chim. Acta **64,** 1488 (1981)
2. b.) A. F. Thomas and J.-Cl. Egger, Helv. Chim. Acta **64,** 2393 (1981)
2. c.) A. F. Thomas, Helv. Chim. Acta **64,** 2397 (1981)
3. F. Bohlmann, P. Singh, C. Zdero, A. Ruhe, R. M. King and H. Robinson, Phytochemistry **21,** 1669 (1982)
4. G. M. Nano, T. Sacco and C. Frattini, Ess. Der. Agrum. **43,** 107 (1973); **46,** 171 (1976)
5. G. Georges and R. Fellous, Parfum., Cosmet., Arômes **43,** 37 (1982)
6. E. Gildemeister and W. Treibs, Die Ätherischen Oele, Vol. 7, Akademie-Verlag, Berlin, 1961, p. 637
7. R. Bos in Vorkommen und Analytik ätherischer Oele, K.-H. Kubeczka, ed., Georg Thieme Verlag Stuttgart, 1982, p. 25
8. A. F. Thomas, unpublished work cited in reference 2c
9. A. F. Thomas and B. Willhalm, Org. Mass Spectrometry **11,** 831 (1976)
10. Y. Chrétien-Bessière, L. Peyron, L. Benezet and J. Garnero, Bull. Soc. Chim. France 1970, 381