

# Contribution to the study of the chemical composition of the essential oil from the terminal branches of the cypress tree in the Grasse area

J. Garnero and P. Buil, P. Robertet, Grasse, France; D. Joulain, University of Le Mans, Le Mans, France; and R. Tabacchi, University of Neuchatel, Neuchatel, Switzerland

In a previous study of the chemical composition of the essential oil derived from the Algerian cypress,<sup>1</sup> we compared that oil with oil obtained by steam distillation of the terminal branches of cypress trees from the Grasse area. We then noted some differences that affected the relative proportions of certain constituents. The richness in high constituents, in particular, prompted us to begin the study of this higher fraction. Cypress essential oil possesses a dry-down odor which is both woody and amberlike and is greatly appreciated by perfumers;\* this also makes it worth studying.

We have already shown that fractions with a high boiling point which are very rich in cedrol contain a characteristic odorous constituent: deca-2(E)-4(Z)-dienyl isovalerate (I) and also diterpenic constituents; among the latter sandaracopimaradiene (II), dehydroabietane (III) and manoyl oxide (IV) have been identified (see fig. 1).<sup>1</sup>

Deca-2(E)-4(Z)-dienyl isovalerate contributes to the woody and fruity note of cypress essential

oil, which is reminiscent of the odor of juniper berries. However, we have not been able to single out the constituent or constituents with an amber-like odor responsible for the amber-like feature in cypress essential oil.

---

\*A specialty ("Cephalone" A.C.) manufactured by a Grasse firm before World War II was obtained from the tail fractions of the essential oil yielded by the terminal branches of cypress trees by means of an isolation and purification process (in particular fractional distillation under high vacuum).

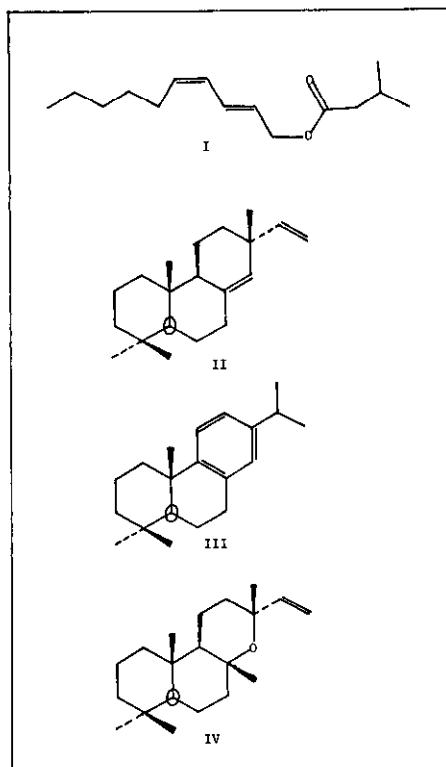


Figure 1

### Physicochemical properties

The essential oil studied was obtained by steam distillation of the terminal branches of *Cupressus sempervirens* Linnaeus (*Cupressus fastigiata* De Candolle = *Cupressus sempervirens* Linnaeus, var. *stricta* Aiten) of the Grasse area. The trees were pruned in autumn 1975, the terminal branches were carefully stripped of large branches, and galbuls were extracted by five to six hours of steam distillation. Distillation water was extracted by hexane before being drained off. Essential oils obtained both by direct distillation and by extraction were blended together, thus representing the total essential oil produced.

The yield in total essential oil was 0.63 p. 100

and thus within the limits usually obtained which range from 0.3 to 0.8 p. 100. The physicochemical properties of this essential oil are given in Table I. These properties are in line with the constants usually accepted for essential oils from the terminal branches of cypress trees.

Table I

$d_{20}^{20}$	= 0.8803
$\alpha_D$	= + 18°65
$n_D^{20}$	= 1.4768
I.A	= 2.2
I.E	= 16.5
I.E <sub>Classical acetylation</sub>	= 33.5
I.E <sub>Cold formylation</sub>	= 75.5
I.CO <sub>Cold (2 hours)</sub>	= 6.8
I.CO <sub>Hot (3 hours)</sub>	= 25.1

### Isolation and identification of constituents

In carrying out this research, we used the isolation techniques: fractional distillation, liquid chromatography on alumina and silica gel columns liquid chromatography on silica gel columns impregnated with silver nitrate, analytical and preparatory gas chromatography. Most of these techniques have already been described in our previous work.

Tables II and III give a general summary of the way the different groups of isolated constituents were separated from each other.

Sixteen new constituents were isolated and identified in addition to the thirty already known. They were:

**Monoterpenes** (fig. 2).  $\alpha,\rho$ -Dimethylstyrene (V) (peak 35 of the general chromatogram) identified by direct comparison with an authentic sample ( $t_R$ , IR, NMR)<sup>3,4</sup>;  $\rho$ -cymen-8-ol or 8-hydroxy- $\rho$ -cymene (VI) (peak 104) identified by direct comparison with an authentic sample ( $t_R$ , IR, NMR)<sup>4,5</sup>; karahanaenone or 2,2,5-trimethylcyclohept-4-en-1-one (VIIA) (peak 49) and karahanaenol or 2,2,5-trimethylcyclo-hept-4-en-

1-ol (VIIb) (peak 54). The ketone was isolated by analytical and preparatory gas chromatography and identified by direct comparison with an authentic hemi-synthetic sample prepared from linalol, according to the method developed by Demole and Enggist.<sup>6,7</sup> This alcohol is a new natural monoterpene. Similarly isolated by preparative gas chromatography, it is identical to the hemi-synthetic product obtained by reduction  $\text{LiAlH}_4$  (=aluminium lithium hydride) (IR, NMR, MS).

3,6,6-Trimethylcyclohexa-2,4-dienone (VIII) (peak 78), a ketone identified by its IR and NMR spectra, was recently found among the constituents of black pepper essential oil and in the excrement of a species of coleoptera that attacks conifers, *Hylotrupes bajulus* Linnaeus.<sup>8,9,27</sup>

It is worth noting that the simultaneous presence in an essential oil of 3 monoterpenoids with the skeleton of 1,1,4-trimethylcycloheptane is unprecedented so far as we know.

Carvacrol methyl ether (IX) (peak 64), identified by direct comparison with an authentic sample ( $t_r$ , IR and NMR), was identified by Naves as the major constituent in the essential oil derived from the wood of *Cupressus sempervirens* Linnaeus.<sup>10</sup>

Note that para-cymenol-8,  $\alpha$ ,  $\rho$ -cimethylstyrene and karahanaenone could constitute artifacts due to steam distillation. It has been pointed out elsewhere that these substances could be caused by various isomerizations of terpinolene epoxide.<sup>11</sup>

**Sesquiterpenes** (fig. 2). In addition to the sesquiterpenes already recognized in the essential

oil of Algerian cypress:  $\alpha$ -cedrene,  $\beta$ -cedrene, thuyopsene, and  $\delta$ -cadinene, we have isolated and identified  $\alpha$ -cubene (X) (peak 59) (IR<sup>12</sup> and NMR),  $\epsilon$ -cadinene (XI) (peak 87) (IR<sup>12</sup> and NMR), and  $\alpha$ -cadinol (XII) (peak 157) (IR,<sup>12</sup> NMR and MS). The latter sesquiterpenic alcohol, which is quite widespread in the plant world, has been found in particular in the essential oils of the terminal branches of the lentisc and of Provence verbena.<sup>14,15</sup>

Analysis of the IR and NMR spectra of an unknown secondary sesquiterpenic alcohol (cupressol),  $\text{C}_{15}\text{H}_{24}\text{O}$ , reveals the presence of 2  $>\text{C}=\text{CH}_2$  exocyclic groups and of a third disubstituted double bond (E) conjugated with one of the exocyclic methylene which favors germacrane or humulane skeleton (see fig. 3). Moreover high resolution mass spectrometry (HRMS) indicates the presence of a secondary alcohol possessing a gem-dimethyl that certainly belongs to an isopropyl (according to the NMR). These data when assembled could correspond with the structure in figure 4 which is bio-

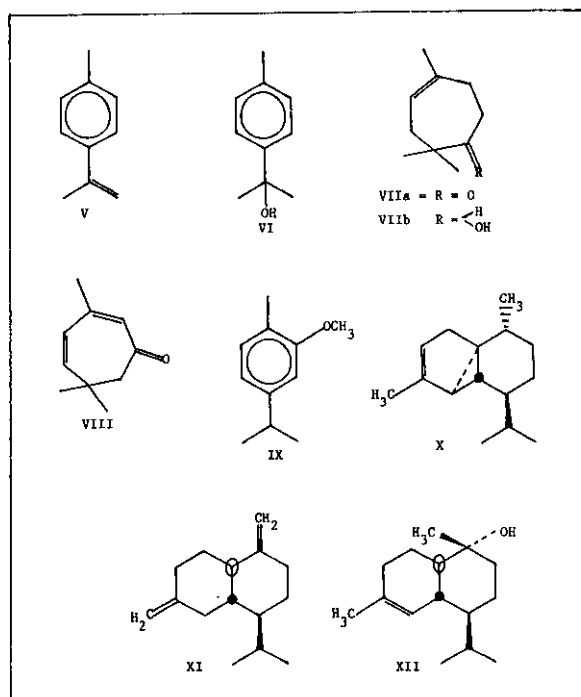


Figure 2. Monoterpene and sesquiterpene constituents of the essential oil of the Grasse cypress tree.

genetically possible. Study of this product is being continued with a view to establishing its structure conclusively.

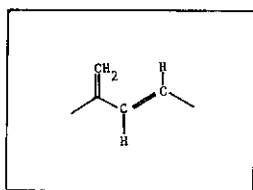


Figure 3

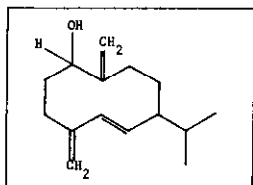


Figure 4

**Diterpenes** (fig. 5). The diterpenic constituents most of which are not eluted in gas chromatography form quite an unusual combination of products belonging to the labdane, pimarane, abietane, and totarol groups.

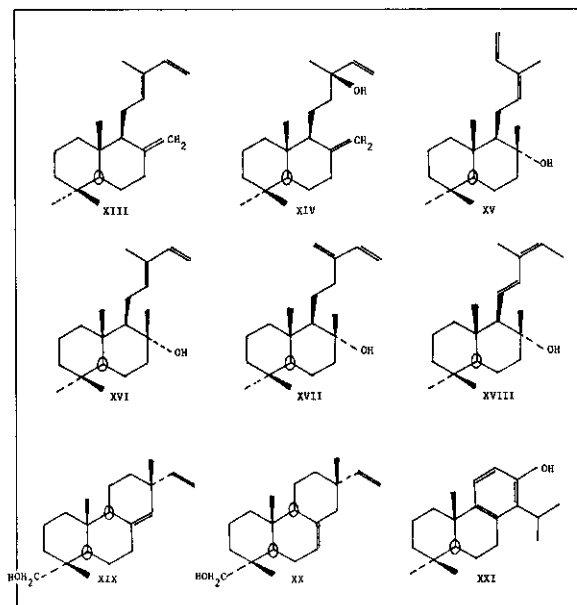


Figure 5. Diterpenic constituents of the essential oil of *C. sempervirens* Linnaeus of Grasse.

### Labdane Group

— Biformene (XIII) is identified by comparing its IR and NMR spectra with those mentioned in scientific literature.<sup>16</sup> In particular the S-trans stereochemistry of the dienic system is deduced from the study of the chemical displacement of the ethylenic protons in NMR.<sup>17a,17b</sup> The biformene was extracted from the wood of *Dacrydium biforme* and can be obtained by acetic dehydration of manool, of sclareol and of abienol.<sup>16b,17c</sup> It consists of blends of cis and trans biformenes a and b in conjunction with sclarene c (see fig. 6).

Table II

Cypress terminal branches (91 kg)  
 Total essential oil of Grasse Cypress (575 g)

Fraction (545 g)  
 (packed column 1.2 m under low pressure)

Volatile fractions (486.5 g)

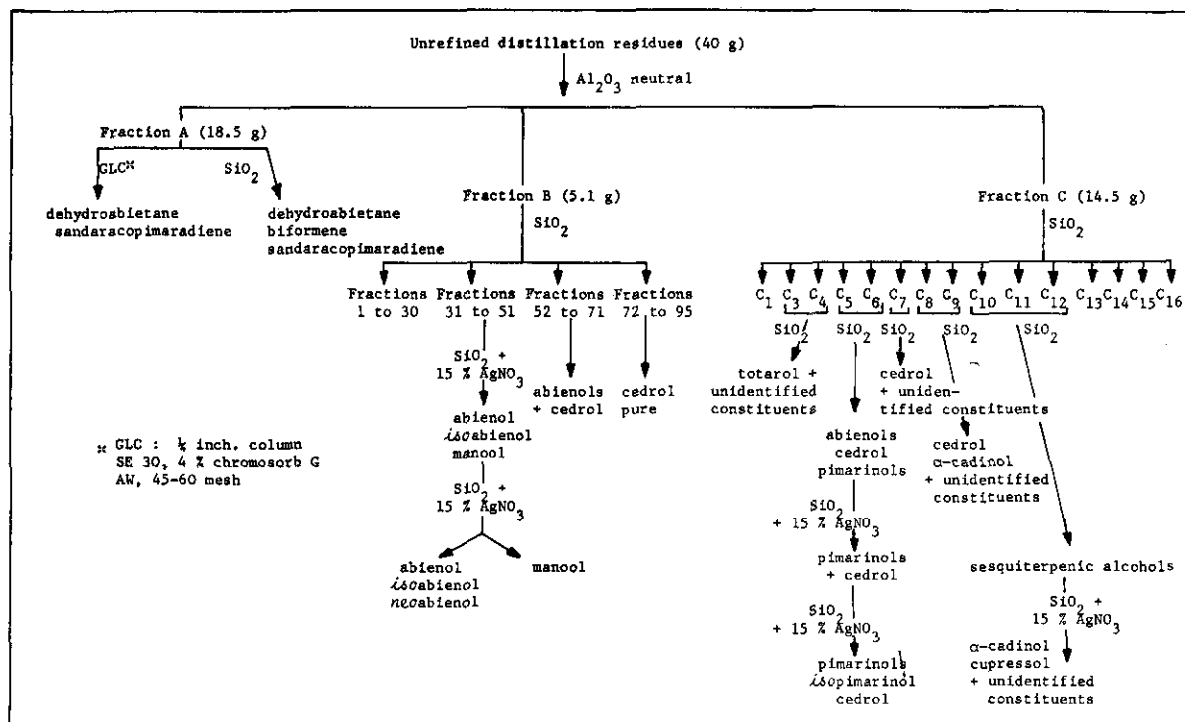
Residue (58.5 g)

Separation into fraction (packed column after spinning band adiabatic column Nester-Faust/Perkin-Elmer, under lower pressure)

33 fractions

Number of fraction	Boiling point	Pressure (Torr)	Weight of distillate	Identification of constituents
1 to 15	41-46°	12	206.5	$\alpha$ -pinene
16 to 18	46-49°	12	60.0	$\alpha$ -pinene, camphene, sabinene, $\beta$ -pinene, 3-carene, myrcene $\alpha$ -pinene, camphene, sabinene, $\beta$ -pinene, 3-carene, myrcene, $\alpha$ -terpinene, limonene, cineole, $\gamma$ -terpinene, $p$ -cymene, terpinolene
19 to 20	49-57.5°	12	50.0	
21 & 22	24.5-26.5°	0.7	31.0	
23	26.5°	0.7	19.0	3-carene mainly
24 & 25	26.5-45°	0.5	11.4	3-carene almost pure
26	45-55°	0.5	4.5	Terpinolene mainly and $\alpha$ , $p$ -dimethylstyrene
27	55-62°	0.5	7.9	
28	60-76°	0.7	6.2	
29	76-77°	0.7	7.1	
30	77-82°	0.7	8.0	linalol, borneol, terpinen-1-ol-4, $\alpha$ -terpineol, karahanaenol, $p$ -cymenol, karahanaenone, bornyl acetate, terpinen-1-ol-4 acetate, $\alpha$ -terpenyle acetate, $\alpha$ -cedrene, $\beta$ -cedrene, thuyopsene, $\delta$ -cadinene, $\epsilon$ -cadinene, carvacrol methyl ether
31	82-88°	0.7	10.5	
32	88-96°	0.7	25.1	- idem + 3,6,6-trimethylcyclohexa-2,4-dienone and decadienyl isovalerate
33	< 128°	0.5	24.5	
				hydrocarbons and alcohols sesquiterpenics (especially cedrol), $\alpha$ -cadinol, dehydroabietane, sandaracopimaradiene

Table III



—Manool (XIV) is identified by comparing its IR and NMR spectra with those of an authentic sample derived from the essential oil of *Dacrydium biforme* Pilg.<sup>18,19</sup> Manool is an important constituent of the gum from the wood of *Cupressus sempervirens* Linnaeus.<sup>20</sup>

—Cis (VI), trans (XVI), iso (XVII) and neo (XVIII) abienols are identified by their IR, NMR and MS and by comparing them with data from scientific literature.<sup>21</sup> Abienol can be isolated from Canada balsam derived from *Abies balsamea* Linnaeus. In the case of balsam of *Abies sibirica* Ledebeva, the abienol is found together with isoabienol.<sup>22</sup>

$\Delta_{13}$ -(Z)-Neoabienol is an artifact formed during successive chromatographies of the abienols on  $\text{SiO}_2$  impregnated with  $\text{AgNO}_3$ . The difficulties encountered in separating the isomers from the abienol as well as the formation of artifacts have already been emphasized.<sup>21a</sup>

—Manoyl oxide (IV) has already been described.<sup>1</sup>

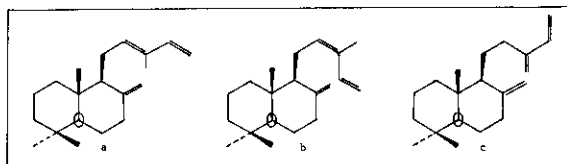


Figure 6

### Pimarane Group

—Sandaracopimaradiene (II) has already been described.<sup>1</sup> —Sandaracopimara-8(14),15-dien-18-ol or pimarinol (XIX) and isopimara-7(8),15-dien-18-ol or isopimarinol (XX) are identified by their IR, NMR and MS and by comparison with data obtained from scientific literature.<sup>23,24</sup> Isopimarinol is the main product.

### Abietane Group

—Dehydroabietane (III) or abietatriene has already been described.<sup>1</sup>

### Totarol Group

—Totarol (XXI) is identified by its IR and NMR spectrum. This diterpenic alcohol has also been found in the gum from the wood of *Cupressus sempervirens* Linnaeus.<sup>25</sup>

At the beginning of this paper we assumed the presence in cypress essential oil of a product that was partly or wholly responsible for the characteristic amber-like note of the superior fraction. It is a fact that peak no. 152 of the general chromatogram has the same retention time as one of the "amber oxides" (3a,6,6,9a-tetramethyl-dodecahydro-2(2,1b)-naphthofurane) (XXII) (see fig. 7) when enriched with an authentic sample. However, by means of a very careful check analysis by thin layer chromatography (TLC) using at least six distinct solvent systems we have shown that a fraction highly enriched with a substance corresponding to

peak no. 152 was completely devoid of amber oxide. Similarly we have had to rule out the possible presence of cedranoxide, which also possesses a characteristic amber-like note (XXIII) (see fig. 7).

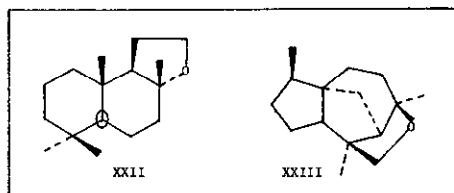


Figure 7

We are continuing to study substance no. 152, whose structure remains for the time being unknown.

### GLC analysis using a capillary column

GLC analysis using a 100 m capillary column impregnated with Carbowax 20M shows that the essential oil from the Grasse cypress tree contains at least 180 constituents, of which it has been possible to identify about 40, representing 96% of the essential oil (Table IV). Manool, trans-abienol, iso and neoabienols as well as pimarinol and its isomer 7(8),15 and totarol are not eluted on the chromatogram (see fig. 9).

Table IV

Peak n°	Relative %	Identification
1	40.90	$\alpha$ -pinene
2	0.50	camphene
3	0.20	tricyclene
4	1.17	sabinene
5	0.80	$\beta$ -pinene
6	15.20	3-carene
7	2.70	myrcene
9	0.09	$\alpha$ -terpinene
10	2.55	limonene
11	0.30	cineole
16	0.30	cis- $\beta$ -ocimene
18	0.10	trans- $\beta$ -ocimene
19	0.30	$\gamma$ -terpinene
20	0.20	p-cymene
21	2.45	terpinolene
35	0.09	$\alpha$ , $\beta$ -dimethylstyrene
49	0.70	karahansenone
50	0.81	linalol
54	0.35	karahansenol
59	0.90	bornyl acetate + $\alpha$ -cubebene
60	1.90	$\alpha$ -cedrene + terpin-1-en-4-ol
64	0.40	carvacrol methyl ether
66	1.20	terpin-1-en-4-ol acetate
67	0.40	$\beta$ -cedrene
72	0.10	thuyopsene
78	1.40	$\alpha$ -terpineol
80	1.00	borneol
82	4.30	$\alpha$ -terphenyl acetate
86	2.60	$\delta$ -cadinene
94	1.00	$\epsilon$ -cadinene
104	0.70	p-cymenol
128	0.35	2(E)-4(Z)-decadienylisovalerate
143	7.00	cedrol
152	0.26	( ? )
157	0.70	$\alpha$ -cadinol
170	1.36	sandaracopimaradiene
173	0.40	manoyl oxide
175	0.10	biformene
180	0.50	dehydroabietane

### Equipment

GLC analysis: Hewlett-Packard 5750

Girdel 75 FD2 equipped with capillary column

Intersmat IGL 15

Integrator Intersmat minigrator

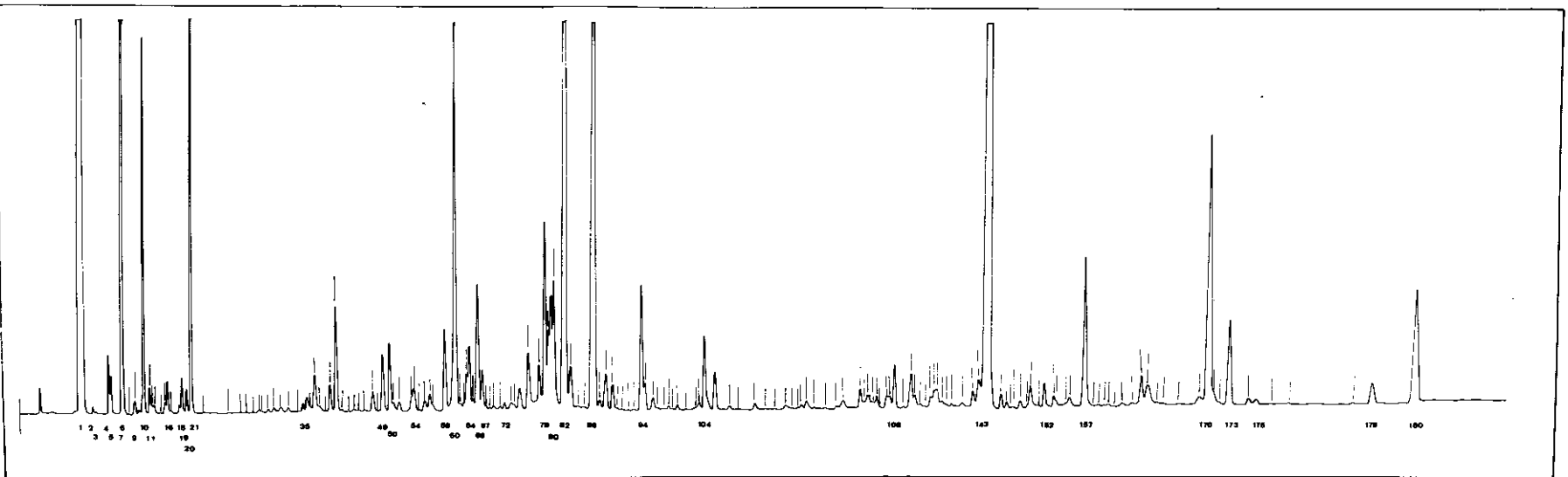


Figure 8

GLC preparatory apparatus: Varian 90 P  
 Liquid chromatography: silica gel Merck 60  
 -230-400 mesh- silica gel impregnated with  
 15% of  $\text{AgNO}_3$  = silver nitrate  
 IR: Perkin-Elmer apparatus 157 G and 257  
 NMR of  $^1\text{H}$ : Varian apparatus A-60  
 Mass spectrometry: Varian apparatus Mat 311—  
 resolution 1800, energy 70 eV  
 Fractional distillation: adiabatic column with a  
 spinning band Nester-Faust/Perkin-Elmer  
 with 30 theoretical plates

### Acknowledgments

We thank Messrs Jean and Paul Maubert for permission to read this paper. We also express our thanks to Professor Rouessac of the laboratory for the total synthesis of natural products at Le Mans University; and to Professor Jacot-Guillarmod of the Institute of Chemistry at the University of Neuchatel for the keen personal interest they have shown in this research.

In addition, we are grateful to Mr. Allemand of the Center for Botany and Plant Pathology at Antibes, who helped us with the identification of plant matter, and to Mr. Guenot of the Scientific Research Training division of the University of Rennes who carried out the high resolution mass spectrometry measurements.

This paper was presented at the 7th International Congress of Essential Oils in Kyoto, Japan, October 1977.

### References

1. R. Tabacchi, J. Garnero, and P. Buil, a. Paper, Vith International Congress of Essential Oils (San Francisco, 8-12 September 1974); b. Riv. Ital. E.P.P.O.S., **57** 4, 221 (1975)
2. A. Chiris, Les Parfums de France, **10**, 366 (1932)
3. a. J. Garnero, L. Benezet, L. Peyron, and Y. Chretien-Bessiere, Bull. Soc. Chim. France, 4679 (1967); b. J. Garnero and Y. Chretien-Bessiere, La France et ses Parfums, **11** 6, 332 (1968)
4. F. Porsch, H. Farnow, and K. Winkler, Dragoco report, **12** 2, 27 (1965)
5. B. M. Mitzner, V. J. Mancini, S. Lemberg, and E. T. Theimer, Applied Spectroscopy, **22** 1, 34 (1968)
6. E. Demole and P. Enggist, Helv. Chim. Acta, **54**, 456 (1971)
7. Y. Naya and M. Kotake, Tetrahedron Lett., 1645 (1968); Nippon Kagaku Zasshi, **89**, 1113 (1968)
8. J. de Brauwere and M. Verzele, Bull. Soc. Chim. Belges, **84**, 167 (1975)
9. D. A. Evans and M. D. Higgs, Tetrahedron Lett., 585 (1975)
10. Y. R. Naves, Parfümerie und Kosmetik, **48**, 461 (1962)
11. E. Klein and W. Rojahn, Dragoco report, **18** 8, 159 (1971)
12. J. A. Wenninger, R. L. Yates, and M. Dolinsky, Journal of the A.O.A.C., **50** 6, 1313 (1967)
13. B. M. Lawrence, J. W. Hogg, and S. J. Terhune, Amer. Perf., **86**, 34 (1971)
14. P. Buil, J. Garnero, and G. Guichard, Riv. Ital. E.P.P.O.S., **56** 5, 249 (1974)
15. P. Buil, J. Garnero, and G. Guichard, Riv. Ital. E.P.P.O.S., **57** 8, 455 (1975)
16. a. R. M. Carman and N. Dennis, Austral. J. Chem., **20**, 157 (1967); b. A. Ahond and B. Gastambide, Bull. Soc. Chim. France, 4533 (1967)
17. a. R. M. Carman, Austral. J. Chem., **19**, 1535 (1966) and references there cited; b. P. W. Atkinson and W. D. Crow, Tetrahedron, **26**, 1935 (1970) and references there cited; c. R. M. Carman and P. K. Grant, J. Chem. Soc., 2187 (1961)
18. J. R. Hosking and C. W. Brandt, Bull. dtsh. Chem. Ges., **68**, 1311 (1935); **69**, 180 (1936)
19. The sample of manool used for comparison was provided by Messrs Bronson and Jacobs of New Zealand
20. a. C. Enzell and H. Erdtman, Acta Chem. Scand., **11** 5, 902 (1957); b. G. Ohloff, Helv. Chim. Acta, **41**, 845 (1958); c. J. W. Rowe and J. M. Scroggins, J. Org. Chem., **29**, 1554 (1964); d. L. Mangoni and R. Caputo, Gazz. Chim. Ital., **97**, 908 (1967)
21. a. P. F. Vlad, K. S. Khariton, N. M. Koltza, and O. D. Bordakh, Khim. Prirod. Soedin Akad. Nauk SSR, **30** (1974); b. K. Hannus and G. Pensar, Paperija Puu (7), 1 (1973)
22. M. A. Chirkova, A. E. Gordunova, A. I. Lisina, and V. A. Pentegova, Khim. Prirod. Soedin Akad. Nauk SSSR, **99** (1966)
23. P. K. Grant, C. Huntrakul, and J. M. Robertson, Austral. J. Chem., **22**, 1265 (1969); I. Yanagisawa, O. Tanaka, and S. Shibata, Tetrahedron Lett., 1683 (1969)
24. a. E. Wenkert, A. Afonso, P. Beak, R. W. J. Cartney, P. W. Jeffs, and J. D. McChesney, J. Org. Chem., **30**, 713 (1965); b. A. Gaudemer, J. Polonsky, E. Wenkert, Bull. Soc. Chim. France, 407 (1964); c. J. Polonsky, Z. Baskevitch, N. Cagnoli-Ballavita, and P. Ceccherelli, Bull. Soc. Chim. France, 1912 (1970)
25. L. Mangoni and R. Caputo, Gazz. Chim. Ital., **97**, 908 (1967); W. F. Short, H. Wang, J. Chem. Soc., 2979 (1951); R. C. Cambie, L. N. Mander, Tetrahedron, **18**, 469 (1962)
26. a. J. A. Barltrop and N. A. J. Rogers, J. Chem. Soc., 2566 (1958); b. R. C. Cambie, R. J. Madden, J. C. Pernell, Austral. J. Chem., **24**, 217 (1971)
27. W. D. P. Burns, M. S. Carson, W. Cocker, and P. V. R. Shannon, J. Chem. Soc., 3073 (1968)
28. T. Hirata, J. Sci. Hiroshima Univ., **35** 3, 239 (1971)