Comparison of isolation procedures for essential oils. IV. Leyland cypress

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The introduction of gas chromatography (GC) has opened new avenues for the analysis of complex mixtures in many classes of natural products. The value of this technique for the separation and identification of terpenes from essential oils was recognized a good twenty years ago. Among the earliest applications in this field were those on coniferous oils.[1-6] Since then GC of terpenes has substantially improved,[7] and the interest of workers in several disciplines for the analysis of terpenes and related compounds in *Coniferae* has greatly increased. Thus, terpene composition in conifers has been used successfully in genetic and chemosystematic studies as well as for work on insect attractiveness or repellency and animal damage.[8] The majority of these studies were carried out with oils obtained after steam distillation of the plant material. However, the influence of this method of isolation upon the composition of the coniferous oils is only seldom considered.

Probably the first investigation of this kind in the field of coniferous oils was published by Von Rudloff, who compared different isolation procedures (three steam distillation techniques and single needle injection) for leaf oil of white spruce, *Picea glauca* (Moench) Voss.[9] The author concluded that large scale distillation resulted in the loss of monoterpene hydrocarbons. Sakai and coworkers presumed that many trace components in the needle oil of Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco) were artefacts arising from isolation and processing of the oil, without giving details.[10] Some years later Pauly and coworkers examined the leaf oil from *Pinus pinaster* Ait.[11] It was reported that monoterpene alcohols (mainly α-terpineol and linalool) seemed to be formed during distillation because solvent extracts were devoid of these compounds, which was attributed to the acidity of the distillation water. Von Rudloff and Hunt compared steam distilled oil from *Abies amabilis* (Dougl.) Forbes with the volatile portions of pentane extracts but no changes were observed.[12] In a similar comparison on *Pinus monticola* Dougl. (western white pine), Hunt and Von Rudloff recorded differences in the levels of elemol *vs* cadinol isomers.[13] The authors stated that "it appears that enzymatic or catalytic action during steam distillation increases the amounts of cadinols at the expense of elemol." In contrast, the formation of elemol (and its acetate) as a result of distillation was reported by Von Rudloff in a chemosystematic study on several juniper species.[14] Hörster encountered an artefact in the monoterpene fraction of *Juniperus oxycedrus* L.[15] The compound, tetradecane, was derived from the petroleum ether used to trap the oil during distillation.[16] So strictly speaking this is not an artefact of distillation.

Volatile artefacts may also be introduced by preparation of the plant material. During distillation the oil must be liberated from the plant tissues by diffusion. This process is rather time consuming and therefore frequently facilitated by grinding or cutting the material before distillation. This will speed up the volatilization but may result in the enzymic splitting of nonvolatile precursors (for example, linoleic and linolenic acid) to form volatile substances of which leaf alcohol (*cis*-3-hexenol) and leaf aldehyde (*trans*-2-hexenal) are very common. These and similar substances have been identified in a wide variety of plant material in most divergent concentrations. Thus, conifer oils were reported to contain traces to over 50% of leaf aldehyde, depending on plant species and time of collection.[17]

Recently we investigated the volatile leaf oil of *Abies* x *arnoldiana* Nitz. in the course of a compara-

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tive study on isolation procedures for essential oils.[18] The influence of some distillation parameters on the composition of the oil was described.

In this paper the results of a comparable investigation on the volatile leaf oil of the Leyland cypress, x *Cupressocyparis leylandii* (Dall. & Jacks.) Dall. (*Cupressaceae*) will be considered. This intergeneric hybrid (*Cupressus macrocarpa* Hartw. x *Chamaecyparis nootkatensis* [D.Don] Sudw.) originated spontaneously in 1888.[19] It is a vigorous, rapidly growing tree, frost hardy and very useful for windbreaks.

A report on the monoterpene hydrocarbons present in the oil of the Leyland cypress was given by Scheffer and coworkers as part of a chemotaxonomic investigation on the hybrid.[20] Oil batches obtained after various distillation times showed considerable differences in composition, which were suspected to be due to rearrangements during distillation.

The present paper is intended to provide information on the backgrounds of these changes. At the same time it is intended to identify the higher boiling (oxygenated) compounds present in the volatile leaf oil of the Leyland cypress.

Experimental

Material. Terminal branches were picked at random from a windbreak consisting of approximately nine-year-old Leyland cypresses, growing in Merenwijk, Leiden (The Netherlands). As fluctuations in terpene composition appear to be less in winter and late fall,[21,22] the material was collected in December 1978-January 1979 and kept in a cold storage chamber (−15°C) until use (within three weeks).

Isolation of the oil was performed by distillation from 20 g samples of plant material using the modified Clevenger-type apparatus described in the European Pharmacopoeia.[23] Distillation liquid was deionized water (500 ml). In experiments on pH influence, water was replaced by the same amount of McIlvaine's citrate-phosphate buffer solutions of various pH between 2.2 and 8. Distillations were conducted for 6 h at a rate of 2.5 ml/min. In a few cases oil samples (1 μl) were taken every hour over a period of 12 h to see if the oil composition varied with the duration of distillation. Samples were diluted with 1 ml redistilled pentane.

To obtain sequential oil fractions a slightly modified Likens and Nickerson apparatus was utilized,[24] the solvent vessel charged with 10 ml redistilled pentane and the sample flask with 20 g leaf material in 500 ml deionized water. Distillation-extraction lasted for 12 h, during which the solvent vessel was replaced every 30 min.

For comparison, 10 g leaf samples were ground with solid carbon dioxide (to prevent evaporation) and extracted with 300 ml pentane/diethyl ether (1:1) during 6 h. Subsequently the volume was brought to 5 ml under reduced pressure in a rotary evaporator at 0°C.

Column Chromatography. To facilitate isolation of compounds 0.25 ml of the oil was chromatographed on 20 g Silica gel 60 (70-230 mesh; Merck), deactivated by addition of 5% water,[25] using a glass tube (18 mm I.D.) cooled to 10°C. The column was eluted with 150 ml pentane followed by 150 ml diethyl ether yielding hydrocarbons and oxygenated compounds respectively. The volume of both fractions was reduced to 5 ml as above.

Gas Chromatography. The composition of the oil was determined by analytical GC over Carbowax 20M (10%) on Chromosorb W AW (60-80 mesh). The column was an 8 m x 1.5 mm I.D. copper coil housed in a Packard GC Model 409.[a] Separation conditions were: oven 90°C (monoterpene hydrocarbons) or 150°C (higher boiling compounds); injector and detector (FID) 200°C. N_2 at 20 ml/min (90°C) or 12 ml/min (150°C) was used as carrier gas. Sample size 1-2 μl. Quantification was performed with an Infotronics CRS-208 integrator.[b] To resolve some areas of overlap, separation was also achieved by GC over 10% SF 96 as stationary phase. Apparatus and conditions were essentially the same as with Carbowax 20M, with exception of the oven temperature, which was held at 80°C for monoterpene hydrocarbons and at 130°C for higher boiling compounds. N_2 flow rates were 27 and 20 ml/min respectively.

Preparative GC made use of a Packard GC Model 419 provided with a Packard Model 798 Preparative Attachment. The aluminum column (6 m x 7 mm I.D.) was packed with 5% Carbowax 20M on Chromosorb W AW (60-80 mesh). Operating conditions were: over 100-120°C; injector and detector (FID) 200°C. N_2 flow rate 90-110 ml/min. Sample size 50-100 μl. Separated compounds were collected by passing the major portion (split ratio 1:30) of the eluent through one of the sample traps cooled to −50°C with an ethanol-solid carbon dioxide mixture.

Identification of Compounds. In general, identification was based on mass spectra. Compounds isolated by preparative GC were submitted to mass spectrometry at 70 eV in an AEI MS 902.[c] Temperature of ion source was 130°C, inlet system 150°C above ambient. Alternatively, in some cases IR spectroscopy was employed, using a Hitachi Model EPI-G2 Grating Infrared Spectrophotometer.[d] Spectra were run from pure liquids as thin films (0.025 mm) between AgCl plates. Identity was verified by comparing GC retention times of isolated compounds with those of authentic samples on columns with stationary phases of different polarity, *viz.* Carbowax 20M, SF 96, β,β-oxydipropionitrile and Poly-*m*-phenyl ether (6 ring).

Results and discussion

As we demonstrated in a preceding paper, the sequence of volatilization of terpenes during distillation

[a]Packard-Becker B. V., Delft, The Netherlands
[b]Infotronics Corporation Ltd., Shannon, Ireland
[c]AEI Scientific Apparatus Ltd., Manchester, England
[d]Hitachi Ltd., Tokyo, Japan

from uncomminuted plant material is governed by the rate of diffusion of the different compounds through the cell membranes, which in turn is determined by their degree of solubility in the distillation water.[26] Therefore, this type of diffusion with water as the carrier was called hydrodiffusion by Von Rechenberg.[27] This means that the higher-boiling oxygenated compounds, being more polar—hence more soluble—will distill before the hydrocarbons. In consequence, the ratio of both fractions may change drastically as distillation proceeds. For example, the portion of hydrocarbons in caraway oil increased from 12.5% after 1 h to over 42% after 12 h.[26]

When Leyland cypress oil, sampled after different distillation times (every hour; European Pharmacopoeia apparatus) was analyzed, the ratio of hydrocarbons *vs* oxygenated compounds appeared to be almost constant throughout the entire distillation period of 12 h. However, the amount of sabinene in the oil steadily declined from 33% after 1 h to 18% after 12 h.

A subsequent experiment was performed with the Likens and Nickerson apparatus, whereby the distilling oil was collected in separated fractions every 30 min. Although GC analysis revealed that the hydrocarbon portion of the distillate slightly increased, it became also evident that all fractions contained a considerable amount of the oxygenated monoterpene terpinen-4-ol. Apparently the hydrodiffusion described above plays no prominent role during the distillation of the oil from Leyland cypress. However, striking differences were noticed by comparing the composition of distilled oil with the volatile fraction present in the solvent extract.

The results are presented in figures 1 and 2. In figure 1, gas chromatograms are given for the oil of Leyland cypress after 6 h of distillation (European Pharmacopoeia apparatus) and for the volatile fraction of the solvent extract. As can be seen, both *trans*- and *cis*-sabinene hydrate (peaks 16 and 19 respectively)

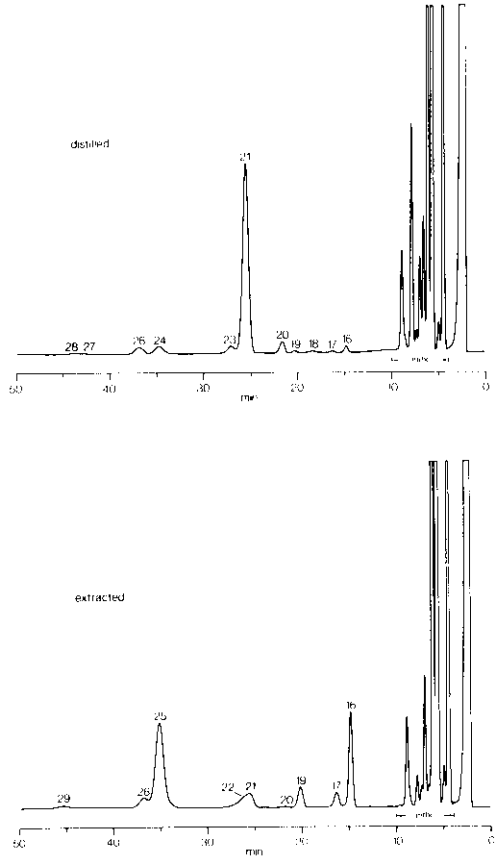

Figure 1. Gas chromatograms of Leyland cypress volatiles obtained by distillation (6 h) and by solvent extraction. Column: Carbowax 20M at 150°C. Peak numbering according to Table I. mthc = monoterpene hydrocarbons (see figure 2).

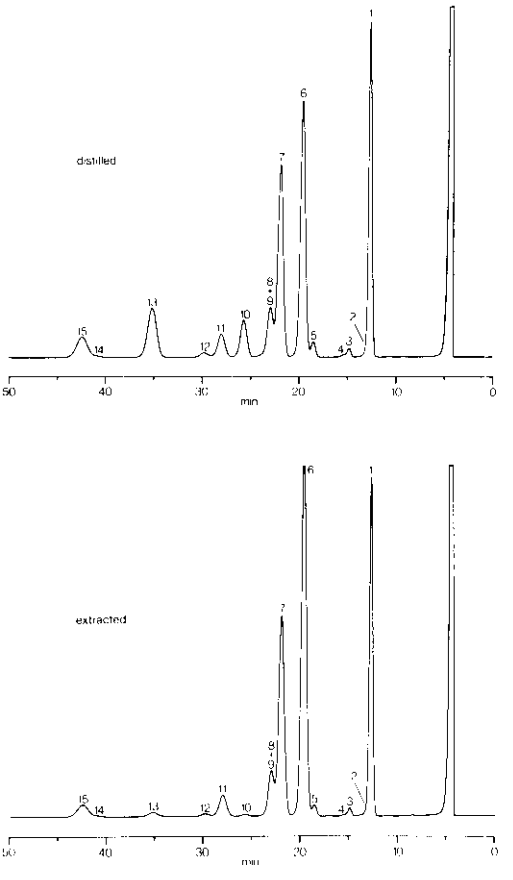

Figure 2. Gas chromatograms of monoterpene hydrocarbons present in distilled oil (6 h) and in solvent extract of Leyland cypress. Column: Carbowax 20M at 90°C. Peak numbering according to Table I.

were greatly reduced in distilled oil. Instead, a substantial quantity of terpinen-4-ol (peak 21) was present, which was of only secondary importance in solvent extracted oil. Furthermore in the distillate small amounts (< 1%) of linalool (peak 18), *cis*- and *trans*-p-2-menthen-1-ol (peaks 20 and 23 respectively), α-terpineol (peak 24), *cis*-piperitol (peak 27), and citronellol (peak 28) were found, whereas in the extract only traces (if any) of these compound could be detected. On the other hand, the extract contained more citronellal (peak 17) than the distillate. Moreover, some sesquiterpene hydrocarbons (peaks 22, 25, and 29) were encountered, which were absent from the distilled oil. Further differences came to light when distillate and extract were gas chromatographed at lower temperature, as reported in figure 2 (see also Table I). The solvent extract contained, in addition to α-pinene (peak 1) and δ_3-carene (peak 7), sabinene (peak 6) as a major component. In distilled oil the relative amount of the latter compound was much smaller, but a significant increase in the content of α-terpinene (peak 10) and γ-terpinene (peak 13) was observed. From these findings the conclusion may be drawn that distillation produces several transformations among the monoterpenes.

The pH of plant material in water usually tends to be slightly acidic (pH 5-6), although it was also reported that aqueous distillation medium rapidly presented a much lower acidity (pH 3).[11] Rearrangements of monoterpenes under acidic conditions have been described in the literature.[28-32] More particularly, the tendency of sabinene to be converted into terpinen-4-ol by the action of dilute sulfuric acid was reported as early as 1907 by Wallach.[33,34] Several decades later the formation of terpinen-4-ol from sabinene was reinvestigated by Tolstikov and coworkers.[35] More recently, kinetics and products of the acid catalyzed hydration of sabinene were studied by Norin and Smedman[36] and by Cooper and coworkers[37] yielding terpinen-4-ol, α-terpinene, γ-terpinene, and terpinolene. The first mentioned authors isolated, in addition, small quantities of *cis*- and *trans*-sabinene hydrate. Furthermore, the acid catalyzed reaction of sabinene hydrate leads to terpinen-4-ol, as was originally observed by Wallach.[38] Granger and coworkers performed the same conversion under various conditions,[39] while details about the mechanism were published by Taskinen.[40] In addition, these authors reported α- and γ-terpinene as products.

In view of these reports it was supposed that the differences between solvent extracted and distilled oil of Leyland cypress were due to the acidity of the distillation water. So the influence of pH during isolation of the volatile components present in Leyland

cypress was examined by distilling the oil from plant material soaked in buffer solutions covering the pH range 2.2-8. The results are given in Table II. As is evident, the concentration of sabinene in the oil differs widely over the investigated pH range. At low pH values there is only a small quantity of sabinene, *ca.* 6%. When pH is raised, however, this percentage increases enormously to more than 37% at pH 8. Simultaneously a decrease of some other compounds is observed: the amounts of both α- and γ-terpinene as well as terpinen-4-ol are greatly reduced, while the content of terpinolene also lessens notably. In addition to these changes, *trans*-sabinene hydrate diminishes as the acidity of the water is enhanced; the *cis*-isomer shows the same behaviour. As will be seen, the structurally related α-thujene also declines when the distillation water is rendered acidic.

These findings are consistent with the data given for the acid catalyzed reactions of sabinene and the sabinene hydrates.[33-40] Therefore, it was concluded that the acidity of the water accounts for the production of α-terpinene, γ-terpinene, terpinolene, and terpinen-4-ol during the distillation of Leyland cypress oil. The rearrangement of α-thujene is essentially the same as that of sabinene, but was reported to proceed at a much lower rate.[36,37] Hence sabinene must be considered as the main source of these compounds, but the sabinene hydrates also contribute to the conversions. The observation of Norin and Smedman that the latter compounds were also products of hydration of sabinene[36] must be attributed to the low temperature (20°C) at which sabinene was treated, as was already suggested by Cooper and coworkers.[37] This was confirmed by our experiment: under the conditions of distillation *trans*- and *cis*-sabinene hydrate disappeared, although the possibility cannot be excluded that they are formed intermediately. It may also be concluded from Table II that the statement of Granger and coworkers that the transformation of sabinene hydrate only proceeds under strongly acidic conditions is not of general validity.[39]

Previously we described the isomerization of sabinene during column chromatography on Silica gel, producing mainly α-terpinene, γ-terpinene, and terpinolene.[25] It is noteworthy that a similar transformation of sabinene proceeds during distillation.

From the data presented in Table II another correlation may also be derived. As will be seen, the drop in the content of α-pinene is associated with the decreasing pH of the distillation water. α-Terpineol

TABLE I. Compounds detected in volatile oil of Leyland cypress obtained by distillation (6 h) and by solvent extraction

No.	Compound	percentage: Distilled oil	Solvent extract
1	α-Pinene	16.1	20.4
2	α-Thujene	1.6	1.5
3	α-Fenchene	0.7	0.9
4	Camphene	t	t
5	β-Pinene	0.3	0.8
6	Sabinene	29.3	34.7
7	Δ_3-Carene	21.4	19.4
8	Myrcene	2.6	1.9
9	α-Phellandrene	0.3	0.2
10	α-Terpinene	0.9	0.5
11	Limonene	2.6	2.5
12	β-Phellandrene	0.8	0.7
13	γ-Terpinene	4.3	2.7
14	p-Cymene	0.1	0.1
15	Terpinolene	4.3	2.3
16	*trans*-Sabinene hydrate	1.3	0.4
17	Citronellal	0.1	0.8
18	Linalool	t	n.d.
19	*cis*-Sabinene hydrate	0.1	1.0
20	*cis*-p-2-Menthen-1-ol	0.5	t
21	Terpinen-4-ol	5.6	5.8
22	δ-Elemene	n.d.	t
23	*trans*-p-2-Menthen-1-ol	0.5	n.d.
24	α-Terpineol	1.7	n.d.
25	Unknown (M$^+$ 204)	n.d.	n.d.
26	α-Terpinyl acetate	0.9	1.7
27	*cis*-Piperitol	t	n.d.
28	Citronellol	t	n.d.
29	δ-Cadinene	n.d.	0.3

t = trace (< 0.1%) ; n.d. = not detected

shows the inverse pattern, being most abundant at low pH. The conversion of α-pinene into α-terpineol with aqueous mineral acids has been described in the literature and finds technical application.[41,42] It is likely that this reaction also occurs under the (acidic) conditions during distillation, for α-terpineol was not detected in the solvent extract.

In addition, some minor compounds, not listed in Table II, fluctuated as the acidity of the distillation water was varied. These changes were, however, not undirectionally, for example, *cis*- and *trans*-p-2-menthen-1-ol gave maximum readings (*ca.* 0.5%) at pH 5, whereas *cis*-piperitol was subjected to a similar, though inverse process. Presumably, these compounds are converted by allylic rearrangement, which is known to proceed quite easily in the p-2-menthen-1-ol ⇆ piperitol equilibrium.[43] As only traces of one of these compounds were found in the extract, this reaction may well be of a secondary nature, but a possible progenitor has thus far not been ascertained. Furthermore, in all distilled oils irrespective of pH, the amount of citronellal was relatively low (0.1-0.2%). In a study on the effect of pH on recovery of components from a model system using a modified Likens and Nickerson apparatus, Schultz and coworkers noticed instability of citronellal in the lower part of the pH range, but no further explanation was given.[44] The strong tendency of citronellal towards cyclization on treatment with acids to give *iso*-pulegol is well-known,[45] but we could not detect the latter compound.

From these findings it will be seen that the pH exhibits the most drastic influence on sabinene and its hydrates. Recently we demonstrated the same feature in pepper oil (*Piper nigrum* L.) and in savin oil (*Juniperus sabina* L.).[46] It is our opinion that the conversion of these compounds during distillation is quite common, but only seldom recognized.

This point may be underlined by the following references. The effect of solutions of various pH at room temperature and at 100°C on the volatile constituents of cardamom seeds (*Elettaria cardamomum*) was examined by Brennand and Heinz.[47] Among the identified components were sabinene, *trans*-sabinene hydrate and terpinen-4-ol. A correlation between these compounds was not mentioned, although it follows from the tables presented. Schultz and coworkers found that oil of vinegar weed (*Trichostema lanceolatum* Benth.) contained as much as 55% terpinen-4-ol.[48] Second, third, and fourth most abun-

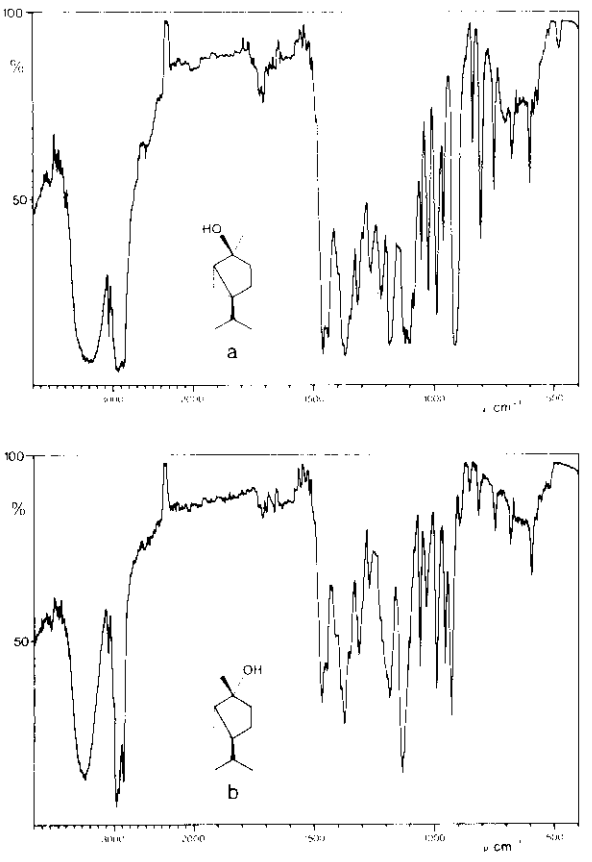

Figure 3. Infrared spectra of **trans**-sabinene hydrate (a) and of **cis**-sabinene hydrate (b).

dant constituents were γ-terpinene (15%), α-terpinene (7.7%), and terpinolene (3.2%) respectively. Concentration of sabinene was found to be 0.13%. Since the authors reported that "the pH of the solution in the still pot was about 4.8," a certain degree of isomerization might be expected. Taskinen compared distilled oil of sweet marjoram (*Majorana hortensis* Moench) with the pentane extract of an alcoholic distillate of the herb (obtained by percolation of the material with ethanol-water (55:45) followed by distillation under reduced pressure).[49] Oil obtained by the first method contained less sabinene and sabinene hydrates, but terpinen-4-ol was increased. In a following paper the author mentioned this rearrangement, stating further that "it is possible in steam distilled oils in general that for compounds structurally related only one is present in the plant and the others are generated during the isolation procedure."[40] An interesting remark on this matter can be found in a discussion on criteria for quality assessment of cold pressed citrus oils, where Di Giacomo draws attention to the fact that the presence of terpinen-4-ol may indicate the addition of distillates.[50,51] In this connection it is also of interest to note that Briggs and Sutherland put forward that sabinene is found in all the oils in which terpinen-4-ol occurs.[52] In view of our findings it is probably more realistic to formulate the reverse.

With regard to the lability of sabinene and its hydrates, comparable changes of these compounds are likely to occur during processing of food products and, in fact, during heat sterilization of black pepper, Maarse and Nijsen observed a decrease of sabinene and both sabinene hydrates, whereas the concentration of α- and γ-terpinene, terpinolene and terpinen-4-ol showed an increase.[53] However, as the experiments were performed with aqueous solutions it seems reasonable to suggest that these changes were brought about by the acidity of the water rather than by high temperature conditions.

It is also plausible that the rearrangements described above, as well as similar conversions, will be catalyzed by organic acids present in the plant material. To prevent such changes during distillation the addition of $CaCO_3$ and $NaHCO_3$ has been recommended.[54-56] On the basis of the data given in Table II it can be concluded that this might be in favour of the genuine composition of an oil.

A last remark on the sabinene hydrates concerns their identification. Based on GC retention characteristics peaks 16 and 19 were tentatively identified as *trans*- and *cis*-sabinene hydrate respectively. By preparative GC the compounds were isolated as white felted crystals. Melting points (60 and 37°C resp.) as well as infrared spectra (fig. 3) were found to agree with data reported by Daly and coworkers[57] and by Lossner.[58] In contrast, the mass spectra, which were much the same, did not correspond with the one published for sabinene hydrate by Stenhagen and coworkers[59] and by Yukawa and Itô.[60] However, as spectral (IR, MS) and GC retention data of peaks 16 and 19 were the same as those of authentic samples of *trans*- and *cis*-sabinene hydrate (respectively) we suggest that the mass spectrum of sabinene hydrate shown in figure 4 should substitute the one given in the literature. The spectrum represents that of *trans*-sabinene hydrate; that of the *cis*-isomer is almost identical, the only practical difference being the intensities at m/e 136 and 139, which are the same for the latter compound.

A final paragraph must be devoted to the sesquiterpene hydrocarbons present in the solvent extract. It is to be noted that these substances were not detected in oil distilled from uncomminuted plant material. Distillation for 6 h yielded an average of 1.0% of oil. When this material was ground after distillation and subsequently submitted once again to distillation (6 h) an additional oil yield of 0.7% was obtained, consisting for *ca.* 40% of sesquiterpene hydrocarbons. Evidently quantitative recovery could not be achieved

TABLE II. Percentages of some compounds of cypress oil involved in rearrangements caused by pH of distillation water

	pH						
	2,7	3	4	5	6	7	8
α-Pinene	13,4	14,7	15,3	15,9	16,1	16,4	16,4
α-Thujene	0,6	0,8	1,0	1,4	1,6	1,7	1,7
Sabinene	8,0	13,8	21,4	26,9	32,3	35,5	37,0
α-Terpinene	10,0	7,6	5,0	3,6	2,8	1,8	1,5
γ-Terpinene	17,3	13,7	7,9	5,9	4,6	3,1	2,4
Terpinolene	5,8	4,9	4,1	3,8	3,3	3,0	2,9
trans-Sabinene hydrate	0,2	0,2	0,3	1,0	2,0	3,7	4,2
cis-Sabinene hydrate	t	t	0,2	0,3	0,5	0,9	1,1
Terpinen-4-ol	29,3	29,7	27,0	12,6	8,4	6,3	5,2
α-Terpineol	2,6	2,1	1,4	1,2	0,9	0,8	0,8

t = trace (< 0.1%)

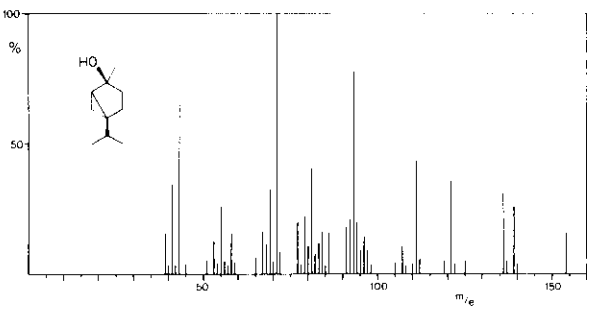

Figure 4. Mass spectrum of **trans**-sabinene hydrate.

within 6 h. To see if extended distillation sufficed to exhaust the intact plant tissue, some distillations were conducted over a 24 h period. Oil yield was then 1.6%. Surprisingly, this oil contained only traces of sesquiterpenes. However, upon grinding followed by 6 h of distillation another 0.3% of oil was recovered consisting almost exclusively of sesquiterpene hydrocarbons (*ca.* 95%). β-Elemene and δ-cadinene were found, but the identity of the other member of this class of compounds represented by peak 25 could not be ascertained. To our knowledge, the infrared and mass spectra given in figure 5 do not match with spectra in the literature. At this point it must be emphasized that these compounds were also found in solvent extracts from ground material. Moreover, they were present in the same proportion when freshly gathered material was pulverized under liquid N_2 and immediately covered under pentane/diethyl ether. Thus, these sesquiterpene hydrocarbons must be considered as genuine components of Leyland cypress. Apparently they are not easily liberated by distillation from the plant tissue in which they occur. A possible explanation could be found in the permeability of the cell membranes: swollen during distillation diffusion is limited to the monoterpenes, while the larger sesquiterpenes are held back. If, on the other hand, intact plant material was soaked overnight in

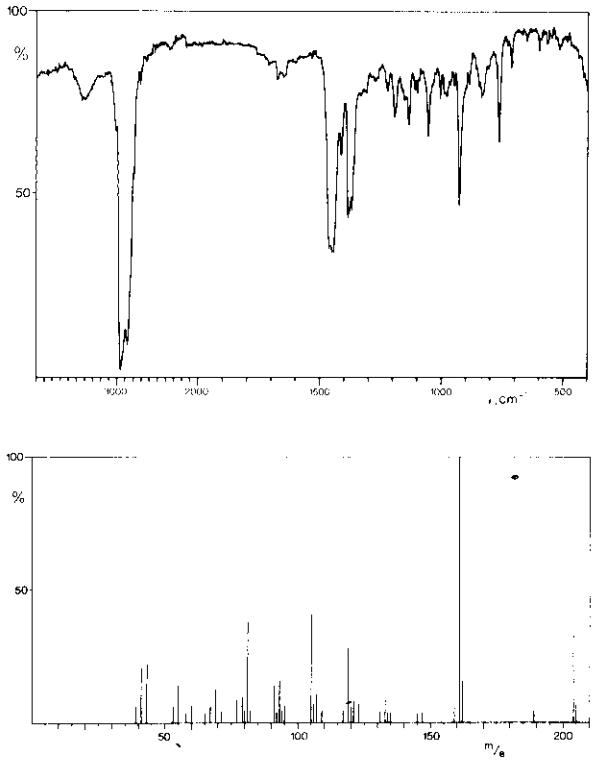

Figure 5. Infrared and mass spectra of unidentified sesquiterpene hydrocarbon (peak 25).

diethyl ether or chloroform, an extract was produced containing considerable amounts of sesquiterpene hydrocarbons. These compounds were not detected after similar treatment using pentane. Obviously, the sesquiterpenes are fairly accessible to polar solvents from the outside, which could be taken to mean that these substances are linked with structures of the cellular walls or even enclosed in specialized cell components. Presumably this requires different biosynthetic sites or at least separate accumulation for mono- and sesquiterpenes.

It is of interest to note that recent findings by Bernard-Dagan and coworkers demonstrated that biosynthesis of monoterpene hydrocarbons in needles of *Pinus pinaster* (maritime pine) is devoted to the epithelial cells of the resin ducts, whereas the site of synthesis for sesquiterpenes is located in secretory structures throughout the whole needle and not related merely to the ducts.[61] Earlier work by Croteau and coworkers revealed that compartmentalization of biosynthetic sites for mono- and sesquiterpenes also holds in the case of peppermint (*Mentha piperita* L.).[62,63] An account of the current state of this matter was recently published by Heinrich.[64]

With respect to the puzzling feature of the recovery of the sesquiterpenes it is noteworthy that Von Rudloff reported some years ago that extended distillation (18-24 h) of *Juniperus horizontalis* Moench (creeping juniper) produced an oil consisting mainly of cadinene-muurolene isomers and their alcohols and acetates.[14] It was suggested that different biosynthetic sites might be concerned. A few years earlier, the same author (together with Pauly)[65] had noted that distillation of leaf oil from *Pinus contorta* Dougl. (lodgepole pine) was not complete even after 24 h. Unfortunately, no specifications as to particular compounds were given, but the idea was advanced that leaf anatomy was involved, since the oil yield could be improved by cutting the leaves. However, as definite evidence to solve the problem of the incomplete (sesquiterpene) distillation is still lacking, further examination of this phenomenon could be rewarding.

Acknowledgement

Thanks are due to Dr. F.-J. Hammerschmidt, Dragoco, Holzminden, G.F.R., for reference substances and for valuable suggestions on the identification of the sabinene hydrates. Appreciation also goes to Dr. Jyrki Taskinen, Orion Pharmaceutical Co., Helsinki, Finland, for the provision of some mass spectra. The technical assistance of Miss Anja Looman is especially acknowledged.

This article is based on a paper presented by Arthur Koedam at the "10. Internationale Abeitstagung Vorkommen und Analytik ätherischer Öle," Würzburg, G.F.R., in May 1979.

Part III of this study appeared in Journal of Agricultural and Food Chemistry **28,** 862, 1980.

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