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Lichen extracts as raw materials in perfumery. Part 1: oakmoss

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ABSTRACT: A comprehensive review is presented on extracts of a lichen, oakmoss (*Evernia prunastri*), that are used in the fragrance industry. Analytical aspects are discussed in detail, from both qualitative and quantitative standpoints, mainly in relation to the industrial processing of the lichen. It is shown that more than 170 constituents have been identified so far in oakmoss extracts, including 47 depsides or depside-derived compounds and 25 triterpenes or steroids. A survey of industrially relevant synthetic products with an oakmoss odour is included. Toxicology issues related to the use of oakmoss extracts in cosmetics and fragrance formulations are critically reviewed.

Keywords: oakmoss; *Evernia prunastri*; fragrances; depsides; triterpenes; steroids; contact allergy

Introduction

Due to many misunderstandings and inaccurate statements published over the years and even during a recent period,[1,2] it is timely to publish a comprehensive review on lichen extracts that are used in the fragrance industry. In order to ensure clarity, this first part of the review will deal with oakmoss only, while a second article dealing with treemoss will appear in the next issue of this journal.

The fragrance industry uses two species of lichen, *Evernia prunastri* var. *prunastri* (L.) Ach. (Parmeliaceae), commonly called 'oakmoss', collected on oak trees, and *Pseudevernia furfuracea* (L.) Zopf., which grows especially on pine and cedar trees and is usually called 'treemoss'. About 700 tons of oakmoss are currently processed every year in France. The Chemical Abstracts Service (CAS) Registry has assigned the three following numbers to oakmoss extracts:

- 68917-10-2 and 90028-68-5, both defined as 'extractives and their physically modified derivatives of *Evernia prunastri*'.
- 9000-50-4, defined as 'extractives and their physically modified derivatives of *Evernia prunastri*, which may contain resin acids and their esters'.

The assignment of the latter is noteworthy, since it concerns extracts of oakmoss obviously combined with other lichen species that grow on pine trees (see below), which are contaminated by diterpenoid resin acid coming from the host plant (pine trees).

The same three numbers are cited in the Standard on Oakmoss Extracts of the International Fragrance Association (IFRA). However, an additional CAS RN: 94944-94-2, has been assigned to '*Evernia prunastri* extracts, ethanolized', which would likely apply better to many existing absolute oils offered on the market.

Two species of lichens are exploited in China under the name 'Chinese oakmoss', *Evernia mesomorpha* Nyl. and *Cetrariastrum nepalensis*, but without one knowing the exact importance of this activity.[3,4] Until recently, the fragrance industry indiscriminately indicated under the name of 'oakmoss' the lichen collected on oak trees or the lichen collected on other trees. Indeed, it should be noted that oakmoss extracts, in general more expensive than treemoss extracts, can be mixed with the latter, but without this being necessarily motivated by economic considerations, but rather to satisfy needs relating to odour, colour or solubility.

Among other applications in fragrance compounding, oakmoss absolute oil is one of the basic components of the famous 'Chypre Accord', which was used for the first time with great success in the creation of *Chypre*, a milestone fragrance by Coty in 1917. Since then, with many variations,[5,6] this type of accord has been constantly used in numerous successful fragrances. For this reason, the importance of lichen extracts, and of oakmoss in particular, as raw materials for the creation or perfumes, does not need to be demonstrated.

The Industrial Processing of Oakmoss

Since the introduction of volatile solvents to manufacture extracts of fragrant plants, ethanol was used first (at the beginning of the nineteenth century), then benzene and petroleum ether in 1860–1870.[7] Other systems have been used (Table 1). Benzene was banned more than 25 years ago and has totally disappeared from manufacturing plants. In the case of lichens, benzene produced extracts of a remarkable quality, and one must admit today that all attempts to substitute other solvents, or binary or tertiary azeotropic mixtures of solvents, of comparable volatility and selectivity, have so far been only moderately successful. To date, the current solvents are either pure hexane or mixtures of hexane with more polar solvents, in general acetates and more rarely alcohols such as isopropanol.

The crude solvent extracts of lichens are called resinoids, which generally are further treated with ethanol to obtain 'soluble

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Table 1. Typical solvents for extracting oakmoss

Solvent	Method	Yield (%)	Reference
Hexane	Extraction	2	9
Hexane	Extraction	1.5–3	8
Dichloromethane	Extraction	5	9
Benzene	Hydrolysis/ extraction	7	9
Benzene	Extraction	5.8–6.6	8
Acetone	Hydrolysis/ extraction	10	9

resinoids', usually called 'absolutes' and offered on the market. It happens that these resinoids or absolutes are submitted to physical treatments intended to attenuate or to remove the original colour. These treatments may involve discoloration with charcoal or high-vacuum distillation, in general molecular distillation. In the latter case, it is often advisable to use suitable auxiliary solvents for the distillate and/or the residue.

Given the multiple possibilities in treating the lichens—type of solvent, temperature, durations and number of contacts—it is not surprising that the characteristics of the obtained resinoids vary, whether in chemical composition or colour.[8] As a result, the olfactory properties of extracts can also vary a lot. Another degree of variability is caused by the fact that the lichen is desiccated beforehand, then humidified again before being subjected to solvent extraction. This treatment by water can be drastic, sometimes involving steam. Actually, each manufacturer has developed its own products and proprietary processes.

Treatment of lichen is carried out after desiccation, since the fragrance has long been thought to develop during storage. Initially, it was also thought advantageous to restore its partial permeability by soaking the lichen in tepid water for several hours before extraction.[7] Whatever the real importance of this, it is now clear that hydrolysis of odourless depsides, such as evernin **9**, is beneficial, as it can generate by cleavage a cascade of monoaromatic compounds, such as everninic acid **29**, which can in turn undergo a decarboxylation to orcinol monomethylether **19** and methyl β-orcinolcarboxylate **24**, which are mostly responsible for the typical odour of moss extracts (Figure 1).[9,10] When a reactive solvent like ethanol is used for extracting the lichen, direct trans-esterification of evernin to ethyl everninate 31 can occur, either partially during the extraction step or later during the processing of the resinoid to the absolute.

The degradation of depsides through hydrolysis and decarboxylation has been textbook knowledge for 60 years.[11] However, even recently, some authors persist in claiming, without any evidence, that this degradation proceeds mainly through the decarbalcoxylation of intermediate esters produced by transesterification with exogenous alcohols (viz. ethanol).[1]

Qualitative Composition of Oakmoss Extracts

Given the above considerations, the qualitative composition of extracts can be extremely variable, depending on the method of preparation that has been used. Industrial extracts are expected to contain less depsides, in both number and concentration. On the other hand, they may also contain minor components that originate from contamination during the collection and transportation of large quantities of biomass, which may themselves be slightly contaminated by other plant species during the industrial processing, as we shall discuss hereunder. Conversely, academic studies would involve careful extractions of the selected and cleaned lichen, under mild conditions and without any prior

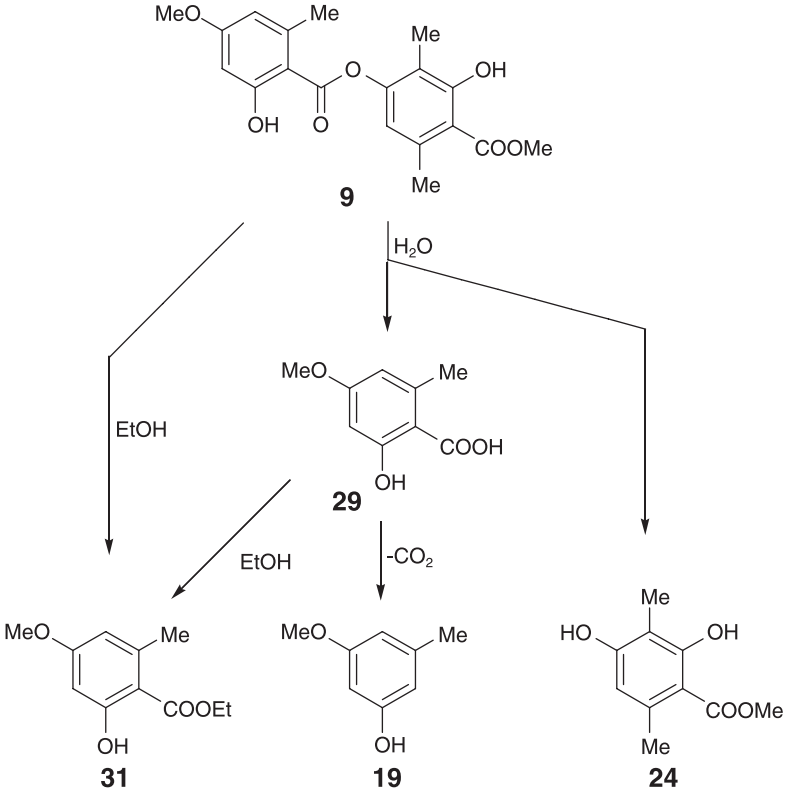
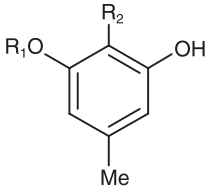


Figure 1. Degradation of evernin

Table 2. Depsides and a dibenzofuran identified in oakmoss

Compound	Name	CAS RN	MW	Reference
1	Evernic acid	537-09-7	332.3	13
2	Barbatic acid	17636-16-7	360.3	12
3	Atranorin	479-20-9	374.3	12
4	Chloroatranorin	479-16-3	408.8	14
5	Thamnolic acid	484-55-9	420.3	12
6	Divaricatic acid	491-62-3	388.4	15
7	Lecanorin	72947-55-8	274.3	16
8	Lecanoric acid	480-56-8	318.3	17
9	Evernin	61631-65-0	360.3	18
10	Prunastric acid	NA	406.3	17
11	Methyl 3′-methyllecanorate	70342-22-3	346.3	17
12	3′-Methylevernic acid	70342-22-2	346.3	17
13	2′-*O*-Methylevernic acid	NA	346.3	16,17
14	2′′,4-di-*O*-Methylgyrophoric acid	NA	496.4	16,17
15	(+)-Usnic acid	7562-61-0	344.3	19

Table 3. Mono-aromatic compounds identified in oakmoss extracts



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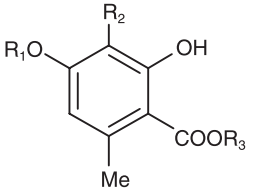

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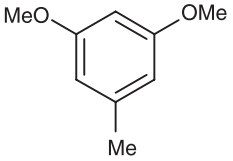


3,5-dimethoxytoluene

Compound	Substitution	Name	CAS RN	MW	References
16	$R_1 = R_2 = H$	Orcinol	504-15-4	124.1	20,21
17	$R_1 = H, R_2 = Me$	β-Orcinol	488-87-9	138.1	20,21
18	$R_1 = H, R_2 = CHO$	Atranol	526-37-4	152.1	12,20,21
19	$R_1 = Me, R_2 = H$	Orcinol monomethylether	3209-13-0	138.1	20,21
20	$R_1 = R_2 = Me$	β-Orcinol monomethylether	56526-87-5	152.2	21
21	$R_1 = R_2 = R_3 = H$	Orsellinic acid	480-64-8	168.1	23
22	$R_1 = R_2 = H, R_3 = Et$	Ethyl orsellinate	2524-37-0	196.1	24
23	$R_1 = R_2 = H, R_3 = Me$	Methyl orsellinate	3187-58-4	182.1	12–14
24	$R_1 = H, R_2 = R_3 = Me$	Methyl β-orcinolcarboxylate	4707-47-5	196.2	12
25	$R_1 = H, R_2 = Me, R_3 = Et$	Ethyl β-orcinolcarboxylate	31581-32-5	210.2	21
26	$R_1 = H, R_2 = CHO, R_3 = H$	Haematommic acid	479-25-4	196.1	15
27	$R_1 = H, R_2 = CHO, R_3 = Me$	Methyl haematommate	34874-90-3	210.1	15
28	$R_1 = H, R_2 = CHO, R_3 = Et$	Ethyl haematommate	39503-14-5	224.2	15,22
29	$R_1 = Me, R_2 = R_3 = H$	Everninic acid	570-10-5	182.2	12–14
30	$R_1 = Me, R_2 = H, R_3 = Me$	Sparassol	520-43-4	196.2	23
31	$R_1 = Me, R_2 = H, R_3 = Et$	Ethyl everninate	6110-36-7	210.2	21,22
32	$R_1 = R_2 = Me, R_3 = H$	Rhizonic acid	479-26-5	196.2	12–14
33	$R_1 = R_2 = R_3 = Me$	Methyl rhizonate	19104-04-2	210.2	12–14
		3,5-Dimethoxytoluene	4179-19-5	152.2	21

treatment with water, and are therefore expected to characterize more depsides and produce bias-free results. The depsides identified in oakmoss are listed in Table 2, and their corresponding structures are shown in Figure 2.

In addition to the standard techniques that have been used over the years for the identification of lichen substances, modern techniques are based on spectroscopic data, ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR), mass spectrometry (MS), etc.,[17,18,40] as in the typical case of evernin **9**,[18] and structures are preferably confirmed by total synthesis, like in the cases of **1**, **8**, **9**, **11**, **12**, **13** and **14**.[17,39,41] Further information on the crystal structures of **9** and **24** have been provided by X-ray diffraction analysis,[42,43] which demonstrate that the carbonyl O-atom of the ester group and the *ortho*-hydroxyl group form a strong intramolecular H-bond (Figure 2).

Mono-aromatic degradation products of depsides are listed in Tables 3–5, and structures are shown in Figure 3. An in-depth analysis of the non-volatile part of the neutral fraction by tandem

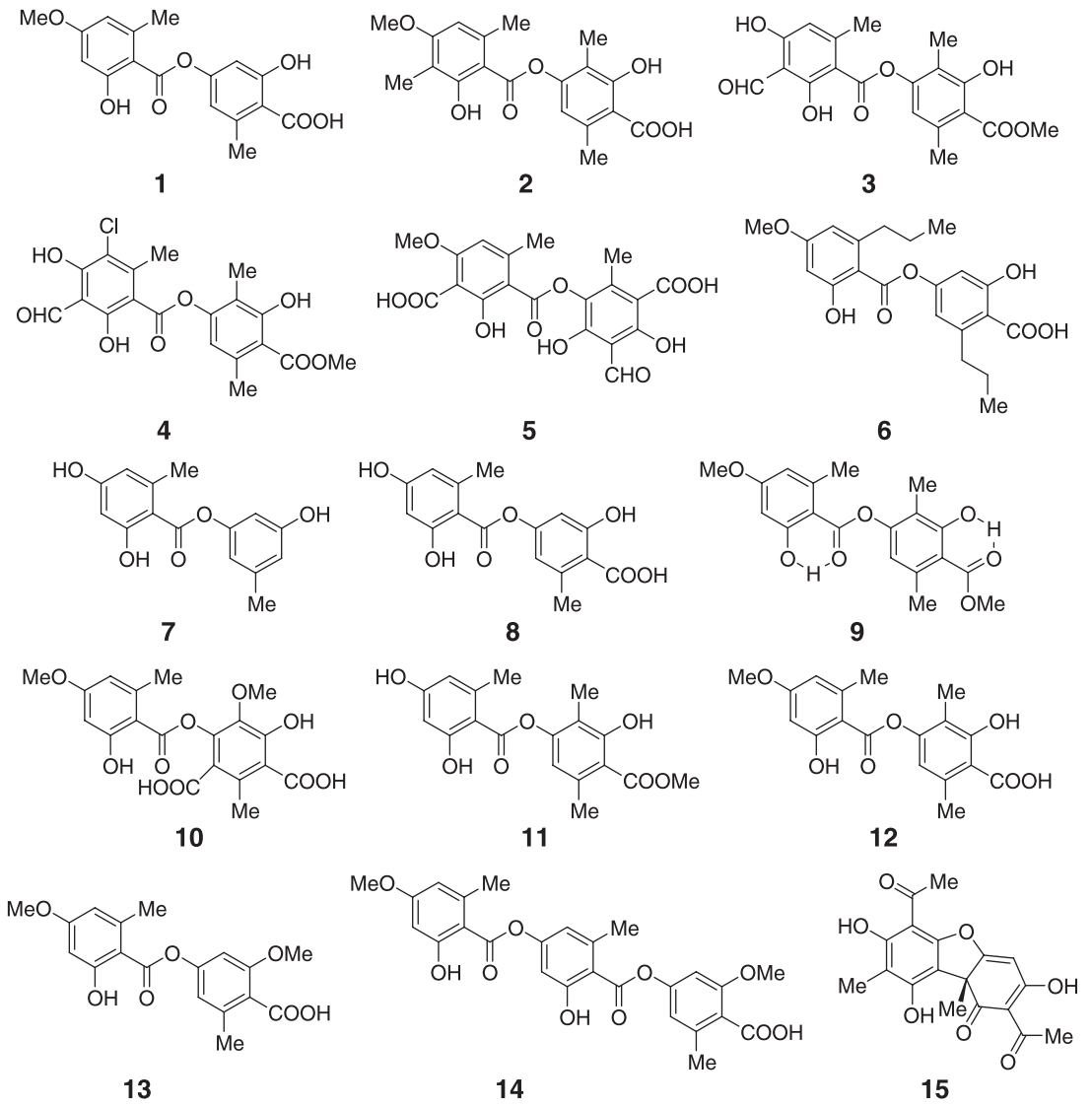


Figure 2. Depsides and a dibenzofuran identified in *Evernia prunastri*

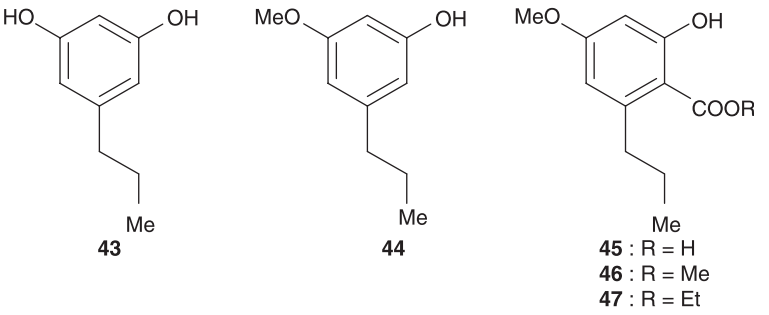
Table 4. Chlorinated mono-aromatic compounds identified in oakmoss

Compound	Name	CAS RN	MW	References
34	Ethyl 5-chloroorsellinate	57074-23-4	230.6	15
35	Ethyl chloroatrarate	57074-25-6	244.7	15
36	Ethyl chlorohaematommate	57857-81-5	258.7	22
37	Ethyl 5-chloroeverninate	NA	244.7	15,24
38	Chloroatranol	57074-21-2	186.6	14,15,21
39	2-Chloro-3-methoxy-5-methylphenol	57074-22-3	172.6	15
40	4-Chloro-3-methoxy-5-methylphenol	78135-47-4	172.6	15
41	2,4-Dichloro-3-methoxy-5-methylphenol	NA	207.0	15
42	2,6-Dichloro-3-methoxy-5-methylphenol	96682-22-3	207.0	15

mass spectrometry (MS/MS) allowed the identification of several triterpenes and steroids listed in Table 6, some of them being new natural products (Figure 4).[26,28] Although they also occur in fungi, triterpenes are typically metabolites of higher plants. The presence of these compounds in lichens can be explained as a result of symbiosis (algae–fungi) or of migration from the host tree. In the case of *E. prunastri*, only friedelin, ursolic acid, taraxerol, lanosterol and hopane derivatives have also been identified in the leaves of some *Quercus* (oak tree) species.[25]

The volatile part of oakmoss extracts represent a minor proportion. With the exception of the aforementioned mono-aromatic compounds, which are derived from depsides, the other remaining

Table 5. Divarinol derivatives identified in oakmoss extracts



Compound	Name	CAS RN	MW	References
43	Divarinol	500-49-2	151.2	21
44	Divarinol monomethyl ether	55136-70-4	166.2	21
45	Divaricatinic acid	6245-56-3	210.2	15
46	Methyl divaricatinate	57074-20-1	224.2	15
47	Ethyl divaricatinate	53530-26-0	238.2	3,15

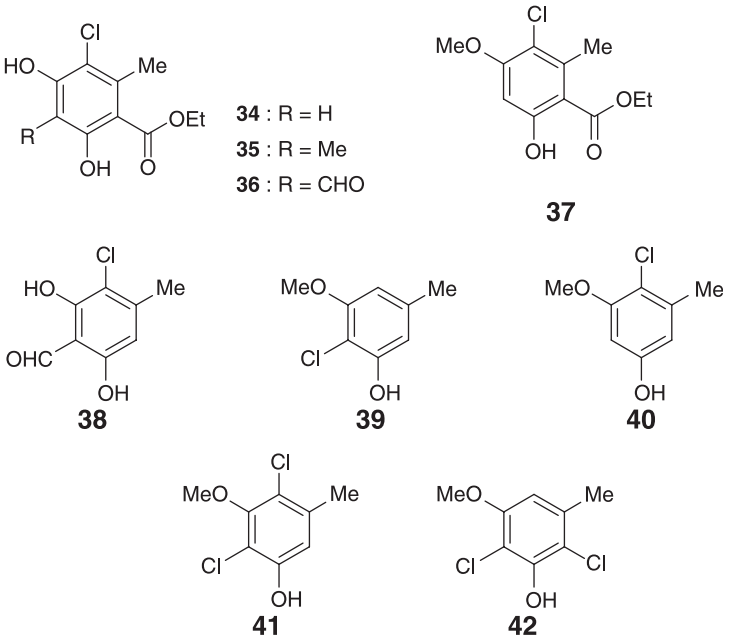


Figure 3. Structures of chlorinated mono-aromatic compounds identified in oakmoss

components of this fraction can be considered as trace elements. However, they do contribute to the characteristic odour of this natural product. After appropriate separation of this fraction, gas chromatography (GC)–MS with 50 m long wall coated open tubular (WCOT) or support coated open tubular (SCOT) columns was first used for the identification of the different components.[30] Those that have been reported to date are either terpenoid in nature (Table 7) or not terpenoid, either aliphatic or aromatic (Table 8).

Apart from environmental contaminants such as *N*,*N*-dimethyl-*p*-toluenesulphonamide (Table 8), a few items call for attention. The α- and β-thujones (Table 7) have been observed independently at a 40-year interval by two groups,[20,21] but their real presence in the lichen is still questionable. Indeed, when the pure lichen is extracted under strict conditions with diethyl ether, and the neutral fraction of the crude extract is examined by GC–MS in selective ion monitoring (SIM) mode, thujones are not detected. Spiking experiments allow the claim to be made that, if thujones are present, their concentration in the lichen does not exceed 50 p.p.b.[48] This suggests that, when collecting the lichen on the trees, some contamination with other plant species occurs. This has actually been observed in the case of a contamination of oakmoss with a liverwort (*Frullania* spp.), which contains high levels of a strong skin sensitizer (frullanolide).

The irone isomers reported in a commercial oakmoss extract suggest a contamination during the processing of the lichen

Table 6. Triterpenes and steroids identified in *Evernia prunastri*

Compound	Name	CAS RN	MW	References
48	3β-Hydroxy-olean-12-ene (=β-amyrin)	559-70-6	426.7	26
49	3β-Hydroxy-urs-12-ene (=α-amyrin)	638-95-9	426.7	26
50	Ursolic acid	77-52-1	456.7	25
51	3β-Hydroxy-olean-12-en-11-one (=β-amyrenonol)	38242-02-3	440.6	28
52	3β-Hydroxy-30-*nor*-hopan-22-one	NA	428.7	28
53	3β-Hydroxy-30-*nor*-hop-5-en-22-one	NA	426.7	27
54	30-*nor*-Lupan-3,22-dione	NA	426.7	27
55	3β-Hydroxy-30-*nor*-lupan-22-one	NA	428.7	27
56	3β-Hydroxy-6α-acetoxy-30-*nor*-hop-5-en-22-one	NA	486.7	28
57	Taraxerol	127-22-0	426.7	25
58	Isomultiflorenone	22611-26-3	424.7	26
59	Friedelin	559-74-0	426.7	25
60	29-*nor*-21α*H*-Hopan-3,22-dione	60686-85-3	426.7	25
61	Moretenone	1812-63-1	424.7	25
62	Lanosterol	79-63-0	426.7	25,26
63	Lupeol	545-47-1	426.7	25
64	Lup-20(29)-en-3-one	1617-70-5	424.7	26
65	β-Amyrenone	638-97-1	424.7	26
66	Betulin	473-98-3	440.7	26
67	3β-Hydroxyhopan-29-oic acid	NA	458.7	26
68	3β-Hydroxy-6-acetoxyhopan-29-oic acid	NA	516.7	26
69	24-Methylenecycloartan-3-one	1449-08-7	438.7	26
70	Isomultiflorenol acetate	22611-24-1	468.7	26
71	Ergosterol-5α,8α-peroxide	2061-64-5	428.6	26
72	3β-Hydroxy-5,8-*epi*dioxyergosta-6,9(11),24(28)-triene	78342-37-7	426.6	26
73	(22*S*)-6-*O*-Acetyl-21β*H*-hopane-3β,6β,22,29-tetrol	752234-69-8	518.8	29

by orris resinoid (Table 7).[15] Irones are formed by oxidative degradation of specific triterpenes (irridals) biosynthesized in Iridaceae, which are not found in this lichen family.

More than 20 linear alkylated benzenes (LABs) have been identified.[19] They are well-known environmental pollutants.

Quantitative Composition of Oakmoss Extracts

Determining the composition of industrial lichen extracts, either resinoids or absolutes, is a very difficult task for two reasons: one is the intense variability within one type of extract, and the other, much more severe, is the lack of analytical reference compounds that are necessary to perform quantitative measurements by standardization. These reference materials, such as **3**, **4** or **9**, can be prepared by isolation from the natural extract, involving tedious separation steps: solvent partition, precipitation, liquid chromatography, crystallization, etc. Very few are commercially available. In general, mono-aromatic compounds such as **18**, **24** and **35** are accessible by total synthesis. Some typical quantitative data obtained with four types of extracts A, B, C and D, are presented in Table 9.

It appears that high-pressure liquid chromatography (HPLC)–UV may not be satisfactory for analysing certain mixtures, due to co-elutions and lack of selectivity.[35,36] GC–MS with or without silylation gives good results with mono-aromatic compounds. Obviously, HPLC–MS/MS is the method of choice for the quantitative determination of these compounds, whether it be for measuring large concentrations in industrial extracts[37] or for trace determinations in cosmetic products.[38]

Direct analysis of the volatile fraction of an oakmoss extract (an absolute) by GC, for example, is not straightforward if one bothers about the non-volatile fraction, which remains stuck in the chromatographic system, and, consequently, the necessity to use standardization.

This drawback is relatively attenuated in using silylation. Several reports have been published on the 'semi-quantitative' direct analysis of oakmoss absolutes by GC without standardization, and their interest is therefore limited.[31,44] This drawback is circumvented by using the atomatic liner exchange (ALEX®) technique, which allows determination in the low p.p.m. range of some minor components (e.g. **37**: 0.05%) or the residual concentrations of atranol and chloroatranol in oakmoss absolutes which have been processed to selectively eliminate these components (see below).[45]

Other Analytical Aspects

Whereas labelling with ^{14}C has been used with success to establish the biosynthesis of orsellinic acid **21**, the quantitative distribution of stable isotopes of carbon, hydrogen and oxygen in lichen substances had not been investigated until very recently. Hopefully, collection of data on the abundances of stable isotopes would provide further information on some biosynthetic pathways. One would expect also that it would allow us to determine the origin of certain oakmoss constituents, viz, natural vs. synthetic, in natural extracts or fragrance compounds. Global concentrations of ^{13}C, ^{2}H or ^{18}O can be measured by stable isotope ratio analysis (SIRA)–MS,[46,47] with or without prior

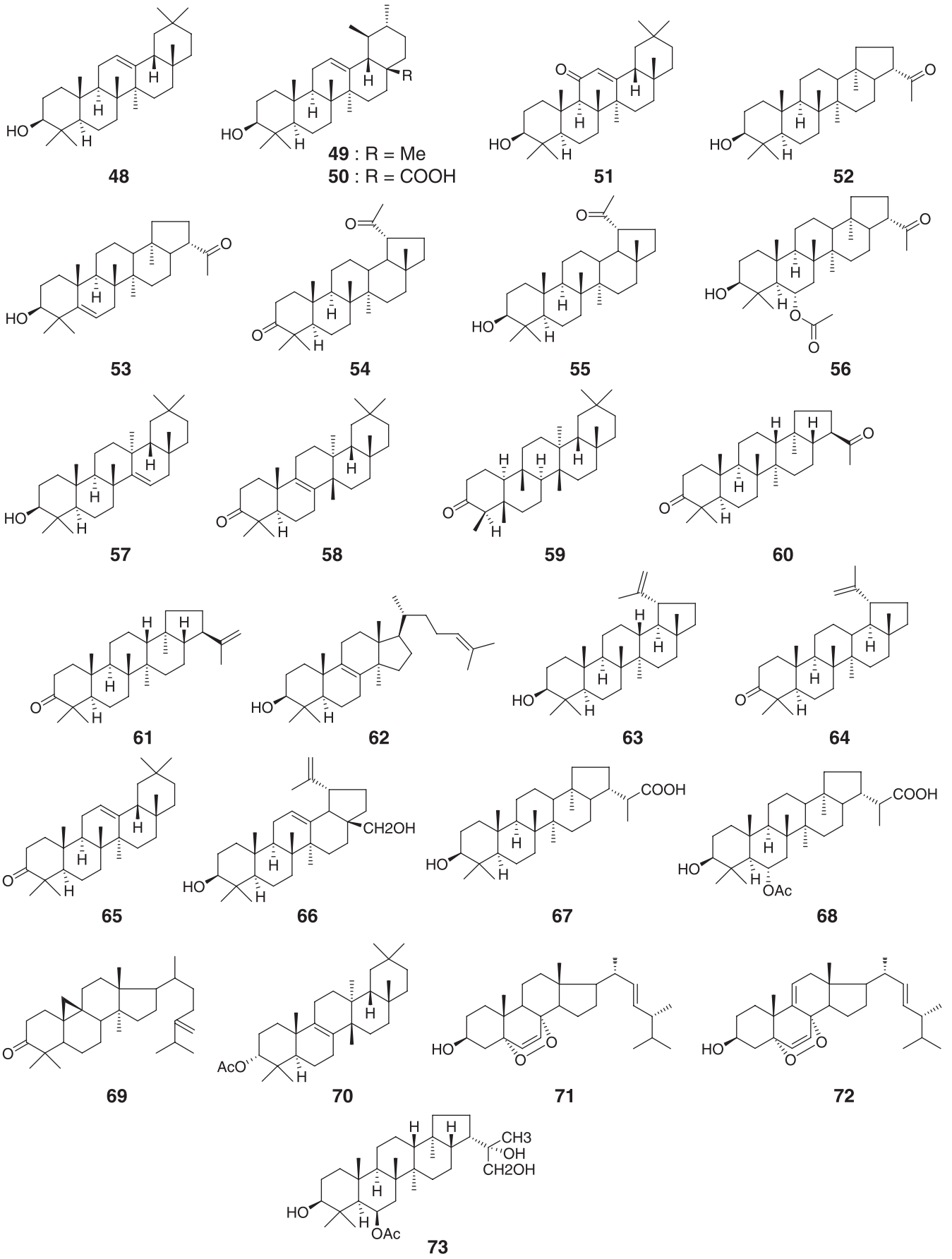


Figure 4. Structures of triterpenes and steroids identified in *Evernia prunastri*

Table 7. Terpenoids identified in the volatile fraction of oakmoss extracts

Monoterpenes	References	Sesquiterpenes	References
Fenchone	19	β-Elemene	16,30,31
Linalool	16,19	α-Copaene	16,30
Myrtenal	19	β-Gurjunene	16,30
Myrtenol	16	α-Cedrene	16
Terpinolene	16	β-Caryophyllene	16,30
cis-Carveol	16	Longifolene	16,30,31
p-Cymen-8-ol	16	β-Humulene (probably α-)	30
α-Thujone	20,21	α-Muurolene	30
β-Thujone	20,21	β-Selinene	30
Citronellol	20	Thujopsene	30
Geraniol	20	α-Guaiene	16
Camphor	20	(*E*)-β-Farnesene	16
Borneol	20		
1,8-Cineole	20,21	*Diterpenes*	
α-Pinene	20	Rimuene	19
β-Pinene	21,30	Isophytol	15
Camphene	16		
Myrcene	16	*Miscellaneous terpenoids*	
Limonene	16	α-Ionone	15
p-Cymene	16	β-Ionone	15
α-*p*-Dimethylstyrene	16	*cis*-γ-Irone	15
γ-Terpinene	16	*trans*-α-Irone	15
Fenchyl alcohol	16	*cis*-α-Irone	15
1-Terpinen-4-ol	16		
1-Terpinen-8-ol	16		
Thymol	16		
trans-Pinocarveol	16		
1-*p*-Menthen-4-ol	30		
6-Methyl-5-hepten-2-one	15		
Carvone	15		
Dihydrocarvone	15		
Bornyl acetate	15		

separation by GC, whereas site-specific abundances of ^{2}H and ^{13}C can be determined by site-specific natural isotope fractionation (SNIF)–NMR[46,47] and natural abundance ^{13}C–electronic reference to access *in vivo* concentration (ERETIC)–NMR,[48] respectively (Table 10).

From these experiments, it was observed that both the 8-Me in **24** and the methanol involved in the esterase-mediated esterification of natural β-orcinolcarboxylic acid are similarly deuterium-depleted. Incorporation of the C_1 fragment to form the 8-Me in **24**, then the CHO group in **18** or **27**, is thought to be achieved by formate prior to completion of the aromatic ring. Without ruling out a possible isotopic effect, these results are consistent with the involvement of these two one-carbon units, viz. formic acid and methanol, at a later stage during the biosynthesis of **24**. Since the biosynthesis of orsellinic acid **21** involves one acetyl-coenzyme-A C_2 unit and three malonyl-coenzyme-A C_3 units,[49] it appears that the 9-Me of **24** originates from one initial acetic acid unit. As a result, significant deuterium abundance discrepancies are observed for the 8-Me and 9-Me groups in **17** and **24**. Interestingly, in **24**, one can observe an important ^{13}C enrichment (δ, –6.2‰) for 8-Me, whereas the 9-Me is slightly depleted (δ, –39‰) from the mean content of the molecule (ca. –30‰).[48]

Usnic Acid

Usnic acid is one of the most common lichen substances. Whereas (+)-usnic acid **15** occurs in high amounts in *E. prunastri*,[50] it is not detected in *Pseudevernia furfuracea* (treemoss) by thin layer chromatography (TLC). While it is possible to isolate >50 g pure **15** from 350 g of a resinoid,[19] the absolutes are practically free of **15**. It is therefore easier to isolate usnic acid from the 'waxes', which are insoluble in ethanol during the preparation of the absolutes,[51] as they may contain up to 70% of it (HPLC). Isolated usnic acid can find applications as an antioxidant in cosmetic preparations[52] and other applications of this interesting natural products have been reviewed recently.[53]

Synthetic Compounds

Although methyl β-orcinolcarboxylate **24** was initially described as odourless,[54] it became rapidly obvious that it is the major contributor to the odour of oakmoss extracts. Strangely enough, one had to wait until the middle of the 1960s to see a number of processes appear in the patent literature for the production of **24** and other structurally related artificial resorcylates with possible applications in fragrance formulations.

Table 8. Miscellaneous compounds

Compound	References	Compound	References
Octanal	15	Phenol	15,20
Nonanal	15	Acetophenone	15
Decanal	15	4-Methylacetophenone	15
Undecanal	15	1-Phenyl-1-pentanone	15
Benzaldehyde	15	Vanillin	15
3,5-Dimethylbenzaldehyde	15	Ethyl phenylacetate	15
2-Heptanone	15	Ethyl furoate	15
6-Methyl-2-heptanone	15	Methyl salicylate	16
2-Octanone	15	Hexanoic acid	15
2-Nonanone	15	Heptanoic acid	15
2-Decanone	15	Octanoic acid	15
2-Undecanone	15	Nonanoic acid	15
2-Dodecanone	15	Hexadecanoic acid	20
2-Tridecanone	15	Octadecanoic acid	20
2-Tetradecanone	15	Linoleic acid	15,33
2-Pentadecanone	15	Linolenic acid	24
4,5/4,6-Dimethyl-3-octanone	15	Ethyl linoleate	24
5,6-Dimethyl-3-hepten-2-one	15	Ethyl linolenate	24
5-Methyl-2-cyclohexen-1-one	15	Methyl tetradecanoate	16
4,6-Dimethyl-4-decen-3-one	32	Methyl oleate	16
(8*Z*)-1,8-Heptadecadiene	32	Methyl hexadecanoate	16
(8*Z*,11*Z*)-1,8,11-Heptadecatriene	32	Methyl octadecanoate	16
1,2-di-*p*-Tolylethane	25		

Nitrogen-containing compounds	References
1-Ethyl-1*H*-pyrrole-2-carboxaldehyde	15
1-Isopentyl-1*H*-pyrrole-2-carboxaldehyde	15
N,*N*-Dimethyl-*p*-toluenesulphonamide	19,23

Table 9. Concentrations (%, w/w) of depsides and mono-aromatic compounds in oakmoss extracts

Compound	Resinoid-A[22] HPLC[a]	Abs.-A[22] HPLC[a]	Resinoid-B[24] HPLC[a]	Abs.-B[24] HPLC[a]	Abs.-C[24] GC–MS[b]	Abs.-C[24] HPLC[a]	Abs.-D[34] GC[c]
1	0.1–0.7	≤0.1					
3	0.3	<0.01	0.77	≤0.005			0.30
4	0.7	0.1	0.37	≤0.005			
9			0.61	0.83			
18					2.71	2.41	2.83
24	10.2	21.4			11.8	10.65	
28	1.0	2.4			0.81	2.49	3.53
31	0.2	0.1					
36	<0.01	1.0			0.30	0.61	1.44
38					1.10	0.99	1.40

[a]UV detection.
[b]After silylation.
[c]GC–FID.

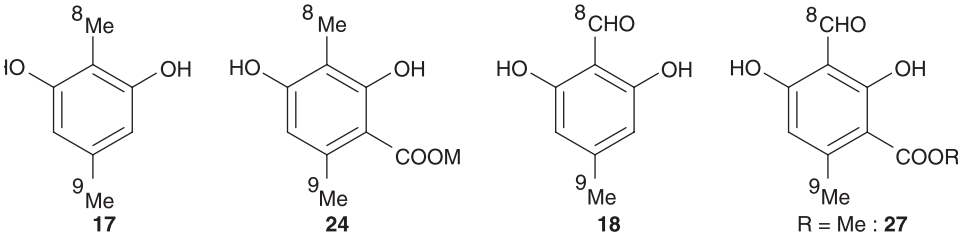
Synthetic routes to various lichen substances, either mono- or poly-aromatic, have been reviewed by Huneck.[41] However, most of them are of academic interest only, and would not apply to the industrial production of **24** and analogous compounds.

The synthesis of β-resorcylic esters is largely based on the aromatization of dihydroresorcylates (viz. **74**), which can be obtained by two methods (Figure 5), the 'acetylacetate route', first described by Sonn,[55] and the 'malonate route'.[56] Whatever the route, this remarkable simultaneous condensation/cyclization can be performed in good to excellent yields. The aromatization of dihydroresorcylates **74** was first achieved by dehydrogenation with palladium,[55] but this has no interest at an industrial level.

Rather than strict dehydrogenation processes, globally oxidative processes were preferred. Halogenation and dehydrohalogenation involve chlorine[57] and bromine under various conditions.[58–60] Other related processes deal with haloamides,[61] hypochlorite,[62]

Table 10. Stable isotope content of selected oakmoss constituents[46,47]

Compound	Source	$\delta^{13}C \pm 0.2‰$ (PDB)	D/H ± 0.2 (p.p.m.)
17	*E. prunastri*	–31.3	128.5
24	*E. prunastri*	–30.1	123.1
18	*E. prunastri*	–28.4	139.0
27	*E. prunastri*	–29.1	132.3
17	Synthetic	–32.0	142.6
24	Synthetic	–29.7	148.0
18	Synthetic	–33.6	140.4
28	Synthetic	–31.3	136.3



Compound		CHO	$-COOCH_3$	8-CH_3	9-CH_3
17	*E. prunastri*			111.2 ± 1.7	96.4 ± 1.0
24	*E. prunastri*		98.0 ± 1.6	112.8 ± 0.9	92.8 ± 1.2
18	*E. prunastri*	112.5 ± 7.4		106.6 ± 1.0	
17	Synthetic			125.6 ± 0.9	145.3 ± 1.1
24	Synthetic		143.7 ± 4.3	126.5 ± 0.6	142.4 ± 2.2
18	Synthetic	102.0 ± 6.6		127.9 ± 0.5	

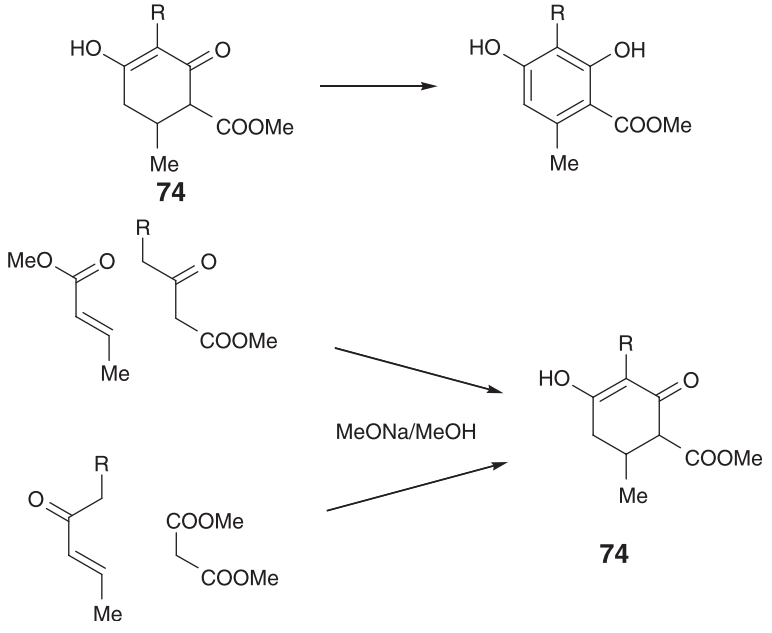


Figure 5. Synthetic routes to hihydroresorcylates and resorcylates

cupric chloride[63] and sulphuryl chloride.[64] However, the use of halogens under certain conditions and media can generate colour problems, which may render the final purification steps tedious. In principle, such risks would not exist with other oxidative processes that use, for example, H_2SO_4/Ac_2O.[65,66]

More recently, a few methods for the direct formation of resorcylates from acyclic precursors have been proposed. They involve α-acetoxy-α-alkenones and malonate[67] or substituted diketenes and acetoacetate.[68] Neither these methods nor those involving a dihydropyranone,[69] nor the direct substitution of

an existing aromatic ring by a Mannich-like aminomethylation[70] or by carboxylation[71] appear to be of practical importance.

Commercial names for methyl β-orcinolcarboxylate **24** are: Veramoss®, Evernyl®, Everniate®, LRG201 (Roure Bertrand Dupont) and Synthetic Oakmoss.

Reported melting points for **24** are quite diverse, and so are the purities: 130.8–136.6°;[57] 139–139.6°;[67] 139.5–140.5°;[61] 142°;[54] 142–143° from CH_3OH/H_2O;[64] 142.5–144° from $CHCl_3$;[65] 142–144° from H_2O;[69] 143–144°;[63] 146° from toluene.[62]

Toxicological Issues

Allergic contact dermatitis (ACD) of lichens has been observed in 1948 with loggers,[72] and the skin sensitization testing with oakmoss extracts has been reported for the first time only ca. 30 years later.[73] Several isolated depsides which are common in many lichen species have been cited as sensitizing components, such as atranorin, evernic acid, perlatolic acid, divaricatic acid, fumarprotocetraric acid and usnic acid.[74] In oakmoss extracts (absolute oils from *E. prunastri*), the constituents that are known to induce ACD are atranorin 3 and its degradation product, atranol **18**, through haematommic acid **26** and chloroatranorin **4** and its degradation product chloroatranol **38** (through chlorohaematommic acid).[34,37,75,76] The alleged identification of 'a second atranol isomer' requires confirmation.[37]

As the free haematommic acids are very labile, or because they are mainly present as ethyl esters, which are formed during the processing of the resinoids with ethanol, attention was rather focused on ethyl haematommate **28** and ethyl chlorohaematommate **36**.[22,34] Actually, very little is known about the sensitizing potential of the acids, either isolated or present at low levels, in oakmoss extracts. Would they decarboxylate *in vivo* and generate atranols?

All these compounds have in common the 2,6-dihydroxybenzaldehyde substructure (**18**, **26**, **28**, **36**, **38**), and two of them have an additional 'phenyl ester' function (**3**, **4**). Cosmetic manufacturers and oakmoss extracts manufacturers have been concerned by this health issue for more than 25 years, when they started working on methods to reduce the sensitization potential of oakmoss (and treemoss) extracts. Determining the components to be reduced or eliminated in industrial extracts was the first task. Using for the first time bioassay-guided gel permeation chromatography (GPC) separations,[77] it was soon confirmed that atranorin **3** and chloroatranorin **4**, together with haematommates **28** and **36**, are sensitizers. In particular, using a modified guinea pig maximization test (GPMT), positive reactions were elicited when the animals were treated ('challenged') with ethyl chlorohaematommate **36** at 0.01% concentration.[22]

Several techniques for the selective removal of these compounds from industrial oakmoss extracts have been patented. They involve various physical methods, such as chromatographic techniques, including GPC,[77] or chemical methods, such as catalytic hydrogenation, alkaline treatment,[77] reduction with hydride,[77] or reaction with amino acids.[34,79] All extracts with reduced levels of targeted sensitizers gave good results when tested on animals (by GPMT),[22,34,77] or humans [by human repeated insult (occlusive) patch test (HRIPT)].[34,79] It is noteworthy that atranol and chloroatranol have been cited among the targeted compounds,[34] and when they were not cited, they would have been eliminated as well in using the patented chemical methods.[77–79] In any case, when a standard oakmoss absolute is treated to remove the aldehyde-containing compounds (such as **3**, **4**, **18**, **28**, **36** and **38**), so that the residual concentrations of both atranol **18** and chloroatranol **38** are <100 p.p.m., no induction of sensitization is observed when 100 tested patients are treated at 5% concentration.[34] With regard to this, published allegations claiming that exposure to these compounds had been 'hidden and uncontrolled' are therefore unfounded and misleading.[76] More recently, these observations were confirmed when two HRIPTs made with oakmoss extracts containing <100 p.p.m. each of **18** and **38** induced no induction on 100 patients tested at 3% concentration.[80] As of today, 'pure' atranols **18** and **38** give EC3 values [i.e. the concentration of chemical required in the local lymph node assay (LLNA) to evince a three-fold stimulation of proliferation in lymph nodes draining the site of application compared to the vehicle-treated controls] of 0.6% and 0.4%, respectively, classifying them as moderate allergens (Class 2) in the LLNA test.[81] An uncertainty still exists about the real contribution of the atranols in the sensitization potential of oakmoss extracts, either intact or after treatment, as the LLNA applies poorly to such complex mixtures. Moreover, patch-testing on sensitized humans was performed only with *synthetic* atranol and chloroatranol, the purity of which was not known accurately,[75] despite repeated statements in the literature of a purity >99%.[1,38] Secondly, the removal of atranols leading to 'hyposensitizing' oakmoss extracts may not be as strictly selective as one would expect, thus leading possibly to biased conclusions regarding the true contribution of these components.[82] Undoubtedly, given the complexity of this problem and the present tendency to consider elicitation rather than induction, further bias-free experiments on both analytical and toxicological standpoints are desirable to establish the contributions of sensitizers in oakmoss extracts, either depsides or mono-aromatic compounds or both. This seems all the more necessary as the regulation of chloroatranol and atranol in oakmoss absolute-containing products appears to be more important than that of oakmoss absolute itself.[83] It has recently been pointed out that oakmoss alone is not suitable for diagnosing treemoss allergy,[84] a topic that will be discussed in the second part of this review. Last, using a sample of uncertified origin, it was found that oakmoss exerted prominent phototoxic effects in an *in vitro* assay.[85]

Allegations regarding the increase of the number of patients who are sensitized to oakmoss should be examined with great care, since it does not correspond to an increase of usage of moss extracts in fragrances during the last 20–30 years. As a matter of fact, the industrial processing of oakmoss in France has dramatically decreased during the last 25–30 years by a factor of at least three.[86] This apparent contradiction is a concern that must be remedied.

Conclusion

The fragrance industry has been using oakmoss extracts for almost a century. With the exception of jasmine absolute oil, no other complex extract has been subjected to such detailed analytical investigations. In the present review, we show that more than 170 components have been reported in oakmoss extracts, including 47 depsides or depside-derived compounds and 25 triterpenes or steroids. Any claim stating that this natural product is poorly known is therefore ungrounded. The same applies to treemoss extracts, which will be reviewed in the next issue of this journal.

This does not mean that everything is known, and there is still a lot to learn about this fascinating natural raw material. Modern techniques that were not available 20–30 years ago, such as liquid

chromatography (LC)–NMR, comprehensive liquid chromatography (LC × LC)–MS and LC–MS/MS, offer interesting perspectives to motivated and skilled analytical chemists.

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