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Received for review January 13, 1975. Accepted May 30, 1975. Presented at the Symposium on Chemistry of Essential Oils, 166th National Meeting of the American Chemical Society, Chicago, Ill., Aug 26–31, 1973, Abstract AGFD-57. Some other papers also presented at this Symposium have already appeared in the September–October, 1974, issue of this journal.

Qualitative Analysis of the Odoriferous Fraction of Oakmoss (*Evernia prunastri* (L.) Ach.)

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The composition of the important odoriferous fractions of a commercial oakmoss extract was investigated. The extract had been prepared by steam distillation of an alcoholic extract from Yugoslavian oakmoss (*Evernia prunastri* (L.) Ach.). After removal of the main component ethyl everninate, the residual material was separated by gradient elution over silica gel. Four fractions were analyzed. These gave by combination a product with olfactive properties very similar to those of oakmoss absolute. Analysis was performed by GC–MS combination using glass SCOT columns. Further characterization was accomplished by preparative GC followed by spectral analysis. In some cases confirmation was obtained by synthesis. Carbonyls, phenols, and acids were isolated by chemical methods. A total number of 61 components was identified, 49 of which were not reported previously as constituents of oakmoss extract. A number of aromatic compounds formed by alcoholysis of depsides was found.

Oakmoss (*Evernia prunastri* (L.) Ach.) is a lichen belonging to the family Usneaceae. Solvent extracts of oakmoss are important perfumery materials. The constituents of the lichen *Evernia prunastri* have been described in the literature by many workers. Compilations of the literature concerning oakmoss depsides were published by Culberson (1969, 1970). The depsides found in oakmoss are summarized in Figure 1. Also, usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3-(2*H*,9b*H*)-dibenzofurandione) not belonging to the class of depsides was repeatedly found in this lichen (Culberson, 1969, 1970).

Monoaryl derivatives of depsides reported as constituents of processed oakmoss extracts must be considered to be degradation products of depsides. Reports are given by Hesse (1861), Gattefosse (1911), Walbaum and Rosenthal (1924), Pfau (1924, 1928, 1937), Horel (1930), Stoll and Scherrer (1937), and Zolotowitch et al. (1971). Only Stoll and Scherrer (1937) gave information concerning odoriferous components of oakmoss not related to depsides. Also polysaccharides were isolated and their hydrolysis products were described (Stüde, 1864; Ulander and Tollens, 1906; Pueyo, 1964–1965; Feige, 1967; Boissière, 1968; Mirovic et al., 1969). Recently some lower aliphatic hydroxy acids were identified (Feige, 1967; Rawinskaja, 1971).

EXPERIMENTAL SECTION

Starting Material. A commercial extract from Yugoslavian oakmoss served as starting material for our investigations of olfactive important components of oakmoss. The extract was prepared by extraction of this oakmoss with ethanol denatured with methanol. The absolute obtained was further concentrated by steam distillation.

Concentration of the Odoriferous Components. The major component of the oakmoss extract—ethyl everninate—was removed for the greater part by stirring a mixture of oakmoss extract (168 g) and pentane (850 ml) for 30 min. The insoluble material was filtered off. Then the filtrate was cooled for 30 min at −80° in a CO_2–2-propanol mixture. The precipitate formed was filtered off through a double wall funnel, cooled at −80°, and washed with a small volume of pentane. The filtrate was concentrated by distillation using a Vigreux column. The residue possessed the characteristic odor aspects of oakmoss absolute. The precipitate was almost odorless. Twenty portions were concentrated in this way.

Gradient Elution over Silica Gel. *Preparation of the Column.* Silica gel (Merck, type 60, for column chromatography, 70–230 mesh) was sieved into fractions of 100–120, 80–100, and 60–80 mesh. For each size a 450-g portion was thoroughly mixed with 1 l. of ether and stored overnight. A 50 mm × 1500 mm glass column, closed at the bottom with a fritted disc and a Teflon needle valve (Fisher Scientific Co., Pittsburgh) was filled completely with ether. A slurry of 450 g of silica gel (100–120 mesh) in ether was poured into the column, the needle valve being opened completely after settling of a small layer of silica gel. Then the remaining two portions were poured into the column as a slurry in ether, the last one being sized 60–80 mesh. After settling of the adsorbent the ether in the column was displaced by 4 l. of pentane. The pentane was allowed to drop near the surface of the column. By using this filling procedure air bubbles in the column were avoided.

Elution. The pentane soluble residue of oakmoss extract (50 g) was dissolved in pentane (100 ml), pipetted carefully

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onto the top of the column, and after impregnation the components were eluted successively with: 2.8 l. of pentane, fractions 1 and 2; 2.8 l. of pentane–ether (95:5, v/v), fractions 3 and 4; 2.8 l. of pentane–ether (90:10, v/v), fractions 5 and 6; 2.8 l. of pentane–ether (75:25, v/v), fractions 7 and 8; 2.8 l. of pentane–ether (25:75, v/v), fractions 9 and 10; 2.8 l. of ether, fractions 11 and 12. The flow of the eluting solvent was adjusted to 10 ml/min. The 12 fractions obtained were concentrated to a volume of 100 ml and then evaluated olfactively by a panel of perfumers. Combination of the fractions 7, 8, 9, and 10 gave a product with olfactive properties very similar to those of the absolute of oakmoss. Only these fractions were selected for further analysis.

Analysis of the Fractions 7, 8, 9, and 10. The solvent of each fraction was removed and the residue was separated by preparative gas chromatography using a Varian Aerograph Model 705 as follows. The fractions were injected onto the top of a 1 m × 8 mm i.d. glass column filled with 20% silicone OV-17, coated on Embacel support (60–80 mesh). A nitrogen flow of 100 ml/min and a column temperature of 175° were maintained. Ethyl everninate and all components eluting earlier were collected together in a 1-ml collection flask (Varian) at −80°. After elution of ethyl everninate the carrier gas was reversed and the components left behind in the column were eluted from the column in opposite directions (at 175°) and trapped together at the end of a glass injector liner which was inserted in the injector (septum removed). A 1-ml collection flask held at room temperature was used. The product obtained on recombination of the collected fractions of each sample did not differ olfactively from the starting product injected.

Combination Gas Chromatography–Mass Spectrometry. The fractions containing the volatile constituents were analyzed by the combination GC–MS. The gas chromatograph, a Varian Aerograph Model 1220, was equipped with a 50 m × 0.75 mm i.d. glass SCOT column. This column, coated with a suspension of 3 g of Ucon 50 LB-550 X, 15 mg of Aerosil, Type R-972 (Degussa), and 3 g of ANM rice powder (Neckar Chemie) in 29 g of CCl_4 (dynamic coating method with a constant flow of 2 cm/sec), was programmed from 70 to 170° at a rate of 1°/min (held at upper limit) at a helium flow rate of 3.5 ml/min. The SCOT column was coupled by means of an all glass restriction allowing a helium flow rate of 0.5–1 ml/min to the inlet of a single focusing 90° magnetic sector field mass spectrometer (Varian-MAT, CH-5, Bremen, Germany). The ionization chamber operated at 70 eV electron beam energy.

Preparative Gas Chromatography and Infrared and NMR Spectrometry. Components of the collected volatiles remaining unidentified after GC–MS analysis and the backflushed fractions were separated by preparative GC on a Varian Aerograph Model 705.

The following glass columns were used: (a) 1 m × 2 mm i.d., 10% silicone OV-17 on Embacel, oven temperature programmed from 170 to 250° at a rate of 2°/min; (b) 1 m × 2 mm i.d., 5% silicone OV-225 on Embacel, oven temperature programmed from 150 to 250°, at a rate of 2°/min. The components were trapped at room temperature in glass capillaries of 0.75 mm i.d.

Infrared spectra were measured on a Perkin-Elmer 457 grating spectrometer in CCl_4 and in CS_2 and also in KBr pellets. Trace components were measured in ultramicrocavity cells (Barnes Engineering Co.), thickness 0.5 mm. Wavelength accuracy was 4000–2000 cm^{-1}, ±6 cm^{-1}; 2000–600 cm^{-1}, ±3 cm^{-1}.

NMR spectra were determined on a Varian A 60 A instrument in CCl_4 solution using tetramethylsilane as an internal standard.

Chemical Group Separation. *Isolation of the Carbonyls.* The pentane-soluble residue of oakmoss extract (200 g) was mixed with Girard-P reagent (160 g), a weakly acid ion exchanger (8 g, type no. 4, Merck), and methanol (750 ml).

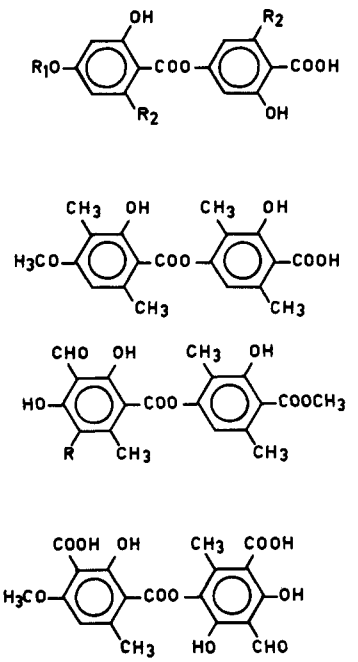

Figure 1. Depsides reported in oakmoss.

This mixture was refluxed for 1 hr, cooled to room temperature, and poured into buffered ice water of pH 7 (4 l.). After extraction of the nonreacted compounds with 8 × 20 vol % of ether, the aqueous solution of Girard hydrazones was acidified to pH 1 with concentrated HCl. After 2 hr the liberated carbonyls were extracted with ether (20 vol %, 5 times, every 2 hr one extraction). The combined ether layers were washed with 10 vol % of water, dried over $MgSO_4$, and concentrated by distillation.

Analysis of the Carbonyls. The carbonyl fraction was analyzed by GC–MS as described earlier. The compounds remaining uncertain or unknown were trapped from a Ucon coated SCOT column at programmed temperature (100–170° at 2°/min). The collected carbonyls were identified by ir analysis.

Isolation of Phenols and Acids. The pentane-soluble residue of oakmoss extract (7 g) was dissolved in ether (500 ml) and extracted with 1 *N* NaOH at 0° (4 times with 50 ml). The ethereal solution was washed with 10 vol % of water. The aqueous layers were combined, brought to pH 10 with 4 *N* HCl, and after addition of NaCl (10 g) extracted with 5 × 20 vol % of ether. The combined ether layers were washed with 10 vol % of water, dried over $MgSO_4$, and concentrated by distillation. This fraction contained the phenolic compounds.

The aqueous layers were combined, acidified to pH 1 with HCl, and extracted with 5 × 20 vol % of ether. The combined ether extracts were dried over $MgSO_4$ and concentrated to a small volume giving the acids.

Analysis of the Phenols and Acids. The phenols and the acids were identified by the combination GC–MS. Unidentified components were trapped from a Ucon coated glass SCOT column at 170° and identified by ir analysis.

Synthesis. Infrared spectra of all compounds described in the following sections are included in the microfilm edition of this volume of the journal (Figures 3–17) (see Supplementary Material paragraph).

3-Methoxy-5-methylphenol (Orcinol Monomethyl Ether). This compound was obtained in 65% yield from orcinol according to the method described by Henrich and Nachtigall (1903).

Methyl 2-Hydroxy-4-methoxy-6-methylbenzoate (Methyl Everninate). Methyl 2,4-dioxo-6-methylcyclohexanecarboxylate (A) was prepared by condensation of meth-

yl crotonate with methyl 3-oxobutyrate in methanol in the presence of sodium methylate analogous to the procedure reported by von Schilling and Vorländer (1899). Reaction of ester A with bromine yielded methyl 3,5-dibromo-2,4-dihydroxy-6-methylbenzoate (methyl dibromoorsellinate), which was reduced with hydrogen in the presence of palladium on charcoal giving methyl 2,4-dihydroxy-6-methylbenzoate, i.e. methyl orsellinate (Sonn, 1928). Methylation of methyl orsellinate with diazomethane afforded methyl everninate (Herzig et al., 1903).

Ethyl 2-Hydroxy-4-methoxy-6-methylbenzoate (Ethyl Everninate). Ethyl orsellinate was prepared analogous to the synthesis of the methyl ester. Partial methylation with diazomethane (Fischer and Hoesch, 1912) produced ethyl everninate.

3-Methoxy-2,5-dimethylphenol (β-Orcinol Monomethyl Ether). β-Orcinol monomethyl ether was prepared starting from methyl 2,4-dihydroxy-3,6-dimethylbenzoate (methyl β-orcinolcarboxylate), which was partially methylated with diazomethane (Herzig et al., 1903) giving in 80% yield methyl 2-hydroxy-4-methoxy-3,6-dimethylbenzoate (methyl ester of rhizoninic acid). Saponification with potassium hydroxide in methanol afforded the corresponding acid. Decarboxylation of this acid yielded β-orcinol monomethyl ether (Pfau, 1928).

Methyl 2,4-Dihydroxy-3,6-dimethylbenzoate (Methyl β-Orcinolcarboxylate). Methyl 2,4-dioxo-3,6-dimethylcyclohexanecarboxylate was synthesized by condensation of methyl crotonate with methyl 3-oxovalerate in methanol in the presence of sodium methylate (Sonn, 1929). Aromatization of this compound with bromine yielded methyl 3-bromo-4,6-dihydroxy-2,5-dimethylbenzoate, which was debrominated with hydrogen in the presence of palladium on charcoal to give β-orcinolcarboxylic acid methyl ester (Sonn, 1929).

3-Methoxy-5-propylphenol (Divarine Monomethyl Ether). Divaricatinic acid (0.3 g, 0.00143 mol), prepared from the corresponding ethyl ester by saponification, was dissolved in pyridine (30 ml) and after addition of a small amount of copper powder refluxed for 1.5 hr. After cooling the mixture was poured into 2 *N* HCl and subsequently extracted with ether. The organic layer was dried ($MgSO_4$) and after evaporation of the solvent at reduced pressure the oily residue was chromatographed over silica gel. Elution with ether–pentane (6:1, v/v) afforded 0.17 g (72%) of divarine monomethyl ether.

Methyl 2-Hydroxy-4-methoxy-6-propylbenzoate (Divaricatinic Acid Methyl Ester). Methyl 2-hexenoate was condensed with methyl 3-oxobutyrate giving methyl 2,4-dioxo-6-propylcyclohexanecarboxylate, which was converted to methyl 3,5-dibromo-2,4-dihydroxy-6-propylbenzoate on treatment with bromine. Subsequent debromination yielded methyl 2,4-dihydroxy-6-propylbenzoate (Sonn, 1931). Methylation with diazomethane gave the title compound.

Ethyl 2-Hydroxy-4-methoxy-6-propylbenzoate (Divaricatinic Acid Ethyl Ester). The ethyl ester of divaricatinic acid was prepared analogous to the synthesis of the corresponding methyl ester starting from ethyl 2-hexenoate and ethyl 3-oxobutyrate.

Methyl 3-Formyl-2,4-dihydroxy-6-methylbenzoate (Hematommic Acid Methyl Ester). This compound was obtained from methyl orsellinate by a modified Gattermann reaction according to Whally (1949).

Ethyl 3-Formyl-2,4-dihydroxy-6-methylbenzoate (Hematommic Acid Ethyl Ester). The ethyl ester of hematommic acid was synthesized analogous to the corresponding methyl ester.

3-Chloro-2,6-dihydroxy-4-methylbenzaldehyde (Chloroatranol). Methyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (methyl hematommate) was chlorinated with chlorine in a carbon tetrachloride solution giving methyl 3-chloro-5-formyl-4,6-dihydroxy-2-methylbenzoate (methyl chlorohematommate). Saponification of this ester with concentrated H_2SO_4 afforded the corresponding acid (Pfau, 1933). A solution of this acid (4.6 g, 0.02 mol) in pyridine (25 ml) was refluxed for 2 hr in the presence of a catalytic amount of copper powder. After cooling the reaction mixture was poured into water and extracted with ether. The organic layer was washed and dried ($MgSO_4$) and the solvent removed at reduced pressure. The crude residue was recrystallized several times from water affording 2.8 g (75%) of chloroatranol (mp 140–141°).

2-Chloro-3-methoxy-5-methylphenol (Monochloroorcinol Monomethyl Ether). Diethyl 2-methyl-4,6-dioxo-1,3-cyclohexanedicarboxylate (A) was prepared in 25% yield by condensation of diethyl malonate with ethyl 2-ethylidene-3-oxobutyrate in ethanol in the presence of sodium ethylate (Knoevenagel, 1894). Ester A (13.5 g, 0.05 mol) was dissolved in acetic acid (50 ml) and a solution of bromine (9.6 g) in acetic acid (25 ml) was added dropwise over a period of 15 min. After the addition was complete the mixture was stirred for 2 hr. The reaction mixture was poured into water; the precipitate was filtered off and washed successively with an aqueous solution of sodium bisulfite and with water. The precipitate was dried and recrystallized from methanol giving diethyl 5-bromo-4,6-dihydroxy-2-methyl-1,3-benzenedicarboxylate (B) in 90% yield (mp 141–143°). A solution of ester B (6.7 g, 0.02 mol) and sodium hydroxide (4 g, 0.1 mol) in water (50 ml) was hydrogenated for 2 hr in the presence of palladium on charcoal. After the uptake of hydrogen was complete the catalyst was filtered off and the filtrate acidified with hydrochloric acid. The precipitate formed was filtered off, washed with water, and dried ($MgSO_4$) giving 4.8 g (90%) of diethyl 4,6-dihydroxy-2-methyl-1,3-benzenedicarboxylate (mp 91–92°). To a solution of this ester (4 g, 0.015 mol) in chloroform (25 ml) was added a solution of chlorine (1.5 g, 0.021 mol) in chloroform (25 ml) at 20°. When the addition was complete the mixture was stirred for 2 hr at room temperature. After evaporation of the solvent the crude residue was crystallized from pentane to give 4 g (90%) of diethyl 5-chloro-4,6-dihydroxy-2-methyl-1,3-benzenedicarboxylate (mp 131–132°).

A solution of this ester (3.0 g, 0.01 mol) and sodium hydroxide (4.0 g, 0.1 mol) in water (50 ml) was refluxed for 8 hr. After acidification the reaction mixture was refluxed for 4 hr and extracted with chloroform (4 × 50 ml). The organic layer was dried ($MgSO_4$) and evaporated. The crude residue was crystallized from carbon tetrachloride to give 1.2 g (77%) of 2-chloro-5-methyl-1,3-benzenediol (monochloroorcinol) (mp 136–138°).

To a solution of this compound (0.8 g, 0.005 mol) and sodium hydroxide (0.2 g, 0.005 mol) in water (25 ml) was added dimethyl sulfate (0.7 g, 0.0055 mol). After stirring for 2.5 hr at 50–60° the reaction mixture was acidified with hydrochloric acid and extracted with chloroform (2 × 20 ml). The combined organic layers were washed, dried ($MgSO_4$), and evaporated. Pure 2-chloro-3-methoxy-5-methylphenol (monochloroorcinol monomethyl ether) was obtained by preparative GC of the crude residue after evaporation.

Ethyl 3-Chloro-6-hydroxy-4-methoxy-2-methylbenzoate (Monochloroeverninic Acid Ethyl Ester). To a solution of ethyl everninate (1.0 g, 0.0047 mol) in chloroform (20 ml) was added a solution of chlorine (0.5 g, 0.007 mol) in chloroform (50 ml) over a period of 30 min. The mixture was stirred for an additional 30 min. Evaporation of the solvent afforded a residue which was recrystallized several times from carbon tetrachloride to give 0.58 g (50%) of ethyl monochloroeverninate.

1-Isopentyl-1H-pyrrole-2-carboxaldehyde. 1*H*-Pyrrole (40.2 g, 0.6 mol) was added dropwise over a period of 10 min to a solution of sodium amide (23.4 g, 0.6 mol) in liquid

ammonia (500 ml). After the addition was complete 1-bromo-3-methylbutane (90.6 g, 0.6 mol) was added in 30 min. After addition of ethanol (10 ml) the ammonia was removed under reduced pressure and the residue was steam distilled. The distillate was extracted with *n*-hexane and dried ($MgSO_4$) and after evaporation of the solvent the crude material was purified by distillation affording 30.3 g (37%) of 1-isopentyl-1*H*-pyrrole (Treibs and Dietl, 1958). To a solution of dimethylformamide (11.0 g, 0.15 mol) in dichloromethane (150 ml) was added phosphoryl chloride (18.4 g, 0.12 mol). After the addition was complete 1-isopentyl-1*H*-pyrrole (13.7 g, 0.1 mol) was added at 20–30° over a period of 15 min. Stirring was continued for an additional 2 hr. The reaction mixture was poured into a solution of sodium acetate (82 g, 1 mol) in water (200 ml). After steam distillation, the distillate was extracted with dichloromethane (2 × 75 ml). The combined organic layers were washed and dried ($MgSO_4$). After evaporation of the solvent at reduced pressure the oily residue was distilled to afford 7.3 g (44%) of 1-isopentyl-1*H*-pyrrole-2-carboxaldehyde (bp 96° (6 mm)).

1-Ethyl-1H-pyrrole-2-carboxaldehyde. This aldehyde was synthesized analogous to the isopentyl homolog. The ir spectrum is shown in Figure 16 of the supplementary material.

RESULTS AND DISCUSSION

The oakmoss extract investigated consisted mainly of ethyl everninate (ir and NMR analysis) which had been formed by alcoholysis of evernic acid (see Figure 1) during the preparation of the extract. By removal of most of this compound by crystallization from pentane the extract was made more suitable for analysis. A seven-fold concentrated product was obtained. The concentrate possessed all the characteristic odor aspects of oakmoss absolute. Because of the complex composition of this concentrate narrowing of the analysis was attempted. A gradient elution over silica gel using pentane and ether as solvents was carried out. It appeared that only 4 of the 12 fractions collected contributed to the characteristic oakmoss odor. The fractions of low polarity containing among others hydrocarbons and esters and those containing the very polar compounds (high fraction numbers) were of minor olfactive importance. Therefore, these fractions were not investigated.

Carbonyls, phenols, and acids were isolated separately from the pentane-soluble part of oakmoss extract. These isolates had interesting odor aspects.

A total number of 61 components was identified. These are summarized in Table I.

Many of the volatile components were identified by the combination GC–MS. A Ucon coated SCOT column provided the separations necessary in obtaining good mass spectra of most of the constituents of the complicated fractions. Checks on the identity as derived from mass spectra were made by comparing the GC retention times and the mass spectra with those of reference compounds. Components of which the structure was uncertain or unknown were isolated by preparative GC and analyzed by ir and NMR spectrometry. Various tentative structures were confirmed by synthesis. This applies especially for compounds to be regarded as degradation products of depsides. The structures of the synthesized products were elucidated by MS, ir, and NMR techniques.

A total of 32 carbonyl compounds was identified. With the exception of 2-undecanone these carbonyls have never been reported in oakmoss extracts. Most of these carbonyls are common constituents of natural products.

Interesting is the occurrence of three irone isomers. Their identity was proven by comparison of mass and ir spectra of the isolated compounds with those of authentic samples. The ir spectra were also in agreement with reported spectra of isomers (Seidel and Ruzicka, 1952; Rautenstrauch and Ohloff, 1971). Irones have been found almost exclusively in orris root oil (Gildemeister and Hoffmann, 1963).

Valerophenone (1-phenyl-1-pentanone) was identified by ir analysis. The spectrum of the isolated compound was identical with a published spectrum (Pouchert, 1970) and with that of an authentic sample. As far as we could trace valerophenone has hitherto only been detected in the oil from stalks and leaves of celery (Wilson, 1970).

In the carbonyl fraction two pyrrole-2-carboxaldehydes were found. The compound with the shorter retention time was identified as 1-ethyl-1*H*-pyrrole-2-carboxaldehyde on the basis of agreement of mass and ir spectra with those of a synthesized product (ir spectrum in the microfilm edition; Figure 17). This compound was repeatedly described in the literature as a constituent of natural products. Published mass and ir spectra were in agreement (Stoll et al., 1967; Bricout et al., 1967; Tatum et al., 1967; van der Wal et al., 1968).

The second pyrrole derivative was identified as 1-isopentyl-1*H*-pyrrole-2-carboxaldehyde on the basis of its ir spectrum. The spectrum of a synthesized product was conclusive (ir spectrum in the microfilm edition; Figure 16). This product is known as a constituent of dried mushroom (Thomas, 1973), Burley tobacco (Demole and Berthet, 1972), and coffee (Stoll et al., 1967).

The structures of three ketones could not be elucidated unquestionably. Their tentative structures were derived from interpretation of the mass and ir spectra.

4,5- or 4,6-Dimethyl-3-octanone. A saturated ketone was concluded from a band (ir spectrum) at 1714 cm^{-1} (CCl_4). There is also a band at 1101 cm^{-1} due to an ethyl ketone. A band of low intensity at 1411 cm^{-1} indicated the presence of one $CH_2C{=}O$ group. A methyl absorption band occurred at 1378 cm^{-1}. Fingerprint agreement with the spectrum of 2-methyl-3-pentanone suggested the presence of an α-methyl substituent with regard to the carbonyl group. Bands due to acetyl, isopropyl, and four adjacent methylene groups were absent. From these elements and from interpretation of the mass spectrum two possible structures could be designed, namely 4,5- or 4,6-dimethyl-3-octanone: MS *m/e* (%) 156 (M^+, <0.5), 127 (1), 109 (1.5), 99 (7), 86 (23), 57 (100), 55 (6), 43 (1.5), 41 (2), 29 (3); ir (CCl_4) 2961, 2928, 2872, 2851, 1714, 1459, 1411, 1378, 1348 (br) cm^{-1}; ir (CS_2) 1160, 1155, 1101, 1019, 973, 795, 769, 763, 754 cm^{-1}.

(*E*)-5,6-Dimethyl-3-hepten-2-one. The ir spectrum of the trapped component indicated a carbonyl (ketone) group conjugated with a trans HC=CH bond (bands in CCl_4 at 1623, 1677, and 1697 cm^{-1}), an acetyl group (bands at 1158, 1180, and 1358 cm^{-1}), trans HC=CH bond (977 cm^{-1}), a methyl group (1374 cm^{-1}), and an isopropyl group (1366, sh, and 1386 cm^{-1}). Additional ir absorptions (CCl_4) were found at 2960, 2928, 2871, 1250, 1100, 1067, 1042, 1017, and 930 cm^{-1}. Mass fragments were found at *m/e* (%) 140 (M^+, 3), 125 (7), 98 (33), 97 (25), 83 (38), 70 (12), 55 (32), 43 (100), 41 (23), 27 (12). On the basis of these data it was concluded that the compound must be (*E*)-5,6-dimethyl-3-hepten-2-one.

5-Methyl-2-cyclohexen-1-one. The ir spectrum of the third ketone indicated the presence of a conjugated ketone group (band at 1684 cm^{-1} in CCl_4 and CS_2). A strong absorption band at 797 cm^{-1} (CS_2) originates from a cis HC=CH bond. Further support is given by a band at 3032 cm^{-1}. These bands were also present in a reference spectrum of 4,4-dimethyl-2-cyclohexen-1-one. A band at 1410 cm^{-1} indicated an $H_2CC{=}O$ group. A cyclic structure was concluded from the complex pattern in the fingerprint region. Because of strong resemblance with the ir spectrum of cryptone (4-isopropyl-2-cyclohexen-1-one) and from the mass spectral data 5-methyl-2-cyclohexen-1-one was the structure proposed: ir (CCl_4) 3032, 2964, 2930, 2872, 2860,

Table I. Compounds Identified in Oakmoss Extract[a]

Compound	Evidence	t_R, min, phase, Ucon	Reported earlier in oakmoss
n-Octanal	MS, t_R	39.6	–
n-Nonanal	MS, t_R	53.4	–
n-Decanal	MS, t_R	67.4	–
n-Undecanal	MS, t_R	81	–
Benzaldehyde	MS, t_R	45.8	–
2,5-Dimethylbenzaldehyde	MS, t_R	74.2	–
1-Ethyl-1*H*-pyrrole-2-carboxaldehyde	ir, MS, t_R, synth.	54	–
1-Isopentyl-1*H*-pyrrole-2-carboxaldehyde	ir, t_R, synth.	85	–
2-Heptanone	MS, t_R	26	–
2-Octanone	MS, t_R	38.4	–
2-Nonanone	MS, t_R	51.8	–
2-Decanone	MS, t_R	65.6	–
2-Undecanone	MS, t_R	79.4	+
2-Dodecanone	MS, t_R	92.6	–
2-Tridecanone	MS, t_R	105.4	–
2-Tetradecanone	MS, t_R	122	–
2-Pentadecanone	MS, t_R	140	–
6-Methyl-2-heptanone	MS, t_R	33.4	–
4,5- or 4,6-dimethyl-3-octanone	ir, MS	44.6	–
(*E*)-5,6-Dimethyl-3-hepten-2-one	ir, MS	50	–
5-Methyl-2-cyclohexen-1-one	ir, MS	40.6	–
6-Methyl-5-hepten-2-one	MS, t_R	39.9	
8(9)-*p*-Menthen-2-one (dihydrocarvone)	MS, t_R	39.8	–
Carvone	MS, t_R	71.2	–
α-Ionone (4-(2,6,6-trimethyl-2-cyclohexen-1-yl)-3-buten-2-one)	MS, t_R	102	–
β-Ionone (4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-3-buten-2-one)	MS, t_R	108.2	–
(*Z*)-2,6-(*E*)-2_1,2_2-α-Irone (4-(2,5,6,6-tetramethyl-2-cyclohexen-1-yl)-3-buten-2-one)	ir, MS, t_R	111.4	–
(*E*)-2,6-(*E*)-2_1,2_2-α-Irone	ir, MS, t_R	107	–
(*Z*)-2,6-(*E*)-2_1,2_2-γ-Irone (4-(2,2,3-Trimethyl-6-methylenecyclohexyl)-3-buten-2-one	ir, MS, t_R	114.2	–
			–
Acetophenone (1-phenylethanone)	ir, MS, t_R	60.8	–
4′-Methylacetophenone	MS, t_R, ir	76.4	–
1-Phenyl-1-pentanone (valerophenone)	ir, t_R	96.6	–
1,8-Cineol	MS, t_R	39	+
Bornyl acetate	MS, t_R	77.8	–
Ethyl phenylacetate	MS, t_R	82.4	–
Ethyl-2-furancarboxylate	MS, t_R	58.4	–
Borneol	MS, t_R	79.2	+
3,7,11,15-Tetramethyl-1-hexadecen-3-ol (isophytol)	ir, t_R	310	–
Phenol	MS, t_R	86	+
Hexanoic acid	MS, t_R	83.2	–
Heptanoic acid	MS, t_R	95.5	–
Octanoic acid	MS, t_R	107.6	–
Nonanoic acid	MS, t_R	124.2	+
(*Z*,*Z*)-9,11-Octadecadienoic acid (linoleic acid)	ir, t_R (OV-225 column)		–
3-Methoxy-5-methylphenol (orcinol monomethyl ether)	ir, MS, t_R, synth.	152.8	+
Methyl-2-hydroxy-4-methoxy-6-methylbenzoate (methyl everninate)	ir, MS, t_R, synth.	147.8	+
Ethyl 2-hydroxy-4-methoxy-6-methylbenzoate (ethyl everninate)	ir, MS, t_R, synth.	181	+
3-Methoxy-2,5-dimethylphenol (β-orcinol monomethyl ether)	ir, MS, t_R, synth.	147.4	–
Methyl 2,4-dihydroxy-3,6-dimethylbenzoate (methyl β-orcinolcarboxylate)	ir, NMR, t_R, synth.	556	+
3-Methoxy-5-propylphenol (divarine monomethyl ether)	ir, MS, t_R, synth.	230	+
Methyl 2-hydroxy-4-methoxy-6-propylbenzoate (methyl divaricatinate)	ir, MS, t_R, synth.	202	–

Table I (*Continued*)

Compound	Evidence	t_R, min, phase, Ucon	Reported earlier in oakmoss
Ethyl 2-hydroxy-4-methoxy-6-propylbenzoate (ethyl divaricatinate)	ir, NMR, MS, t_R, synth.	242	–
Methyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (methyl hematommate)	ir, MS, t_R, synth.	180	–
Ethyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (ethyl hematommate)	ir, NMR, t_R, synth.	221	+
3-Chloro-2,6-dihydroxy-4-methylbenzaldehyde (chloroatranol)	ir, MS, t_R, synth.	227	+
2-Chloro-3-methoxy-5-methylphenol (monochloro-orcinol monomethyl ether)	ir, NMR, MS, t_R, synth.	116	–
1-Chloro-2,4-dimethoxy-6-methylbenzene (monochloroorcinol dimethyl ether)	ir, MS	120	–
Ethyl 3-chloro-4,6-dihydroxy-2-methylbenzoate (ethyl monochloroorsellinate)	ir	398	–
Ethyl 3-chloro-6-hydroxy-4-methoxy-2-methylbenzoate (ethyl monochloroeverninate)	ir, NMR, t_R, synth.	320	–
Ethyl 3-chloro-4,6-dihydroxy-2,5-dimethylbenzoate (ethyl monochloro-β-orcinol carboxylate)	ir, MS	452	–
2,4-Dichloro-3-methoxy-5-methylphenol or 2,6-dichloro-3-methoxy-5-methylphenol (dichloro-orcinol monomethyl ether)	ir, MS	166	–

[a] Abbreviations used are: MS, mass spectrum; ir, infrared spectrum; NMR, nuclear magnetic resonance; t_R, retention time in minutes; synth., synthesis. The retention times were determined on a glass SCOT column (50 m × 0.75 mm i.d.) coated with 3 g of Ucon 50LB-550 X on rice powder (see Experimental Section). The column temperature was programmed from 70 to 170° at a rate of 1°/min and held at the upper limit.

2830, 1684, 1426, 1410 (br), 1385, 1377, 1355, 1317, 1230, 1212, 1179, 1128, 1108, 1095, 1079, 1026, 949, 885, and 797 (CS_2) cm^{-1}; mass spectrum *m/e* (%) 110 (M^+, 22), 95 (4.5), 82 (4.5), 68 (100), 55 (13), 53 (12), 43 (28), 41 (24), 39 (36), 27 (25). There were no reports on the occurrence of these three ketones in natural products.

Major components of fraction 9 were isophytol and linoleic acid. Isophytol was identified by ir analysis, the spectrum being in agreement with a published one (Demole and Lederer, 1958). To our knowledge isophytol has only been reported as a constituent of jasmine oil (Demole, 1956).

Linoleic acid was trapped from a silicon OV-225 column and the structure was determined by ir analysis. The spectrum was in accordance with that reported by Sammul et al. (1964).

The monoaryl derivatives which must be considered as degradation products of depsides were found in all fractions analyzed. Seventeen aromatic compounds were identified of which ten were not reported previously. Thirteen of these were synthesized (see Experimental Section). The ir spectra of these products are included in the microfilm edition (Figures 3–15). Structures of the aromatic compounds found in oakmoss extract are depicted in Figure 2. Analytical data are mentioned in Table I. The compounds that could not be related to known depsides are depicted at the right column of Figure 2.

The structures of four components were based on mass and infrared interpretation.

1-Chloro-2,4-dimethoxy-6-methylbenzene. A component of fraction 7 showed a molecular ion at *m/e* 186. The presence of chlorine was evident. Mass peaks were found at m/e (%) 188 (32), 186 (M^+, 100), 143 (39), and 121 (30). The ir spectrum showed a band at 2838 cm^{-1} due to a methoxy group. The presence of an aromatic-type structure was recognized from absorptions at 3001, 3066 (=C—H), and 1590–1580 cm^{-1} (vs) due to C=C vibrations. Medium bands in the region 850–800 cm^{-1} indicated the presence of only isolated aromatic hydrogens. As a consequence the aromatic ring carries at least three substituents. Considering the molecular weight the presence of a fourth substituent is very probable. Support for a 1,2,3,5-tetrasubstituted benzene was obtained from the strong resemblance of the ir spectrum with that of 1-chloro-2,6-dimethoxy-4-methylbenzene, being available as a reference compound. The ir spectrum further shows the absence of hydroxyl and carbonyl. The arrangement of the substituents was based on an assumed relation to other oakmoss constituents. Taken together, this information indicated the compound to be 1-chloro-2,4-dimethoxy-6-methylbenzene: ir (CCl_4) 3066, 3001, 2838, 1590, 1438, 1413, 1377, 1341, 1331, 1202, 1161, 1099, and 1044 and (CS_2) 839 and 825 cm^{-1}.

3-Chloro-4,6-dihydroxy-2-methylbenzoic Acid Ethyl Ester. From fraction 9 a component was isolated by preparative GC which is supposed to be 3-chloro-4,6-dihydroxy-2-methylbenzoic acid ethyl ester. The structure was elucidated by ir analysis. Isolated aromatic hydrogen was concluded from a medium absorption band at 849 cm^{-1}. Furthermore, the absence of bands at 3005 and 3080 and a medium band at 1591 cm^{-1} indicated the presence of five substituents attached to the benzene ring. The presence of a hydroxyl adjacent to an ethyl ester group was concluded from a broad band at 3500–2500 cm^{-1} caused by an intramolecular bonded OH with an ethyl ester group, further from the position of the carbonyl at 1659 cm^{-1} and from the intensity ratio of bands in the region 3000–2900 cm^{-1}, which indicated the presence of an ethyl ester. There is also a band at 3525 cm^{-1} due to a halogen (most likely chlorine) adjacent to a hydroxyl at a benzene ring. This band is also found in the spectra of simple ortho-substituted chlorophenols and ascribed to intramolecular interaction between the chlorine and the hydroxyl group (Tichy, 1965). The presence of a methyl substituent is likely on the basis of strong resemblance with the ir spectrum of 3-chloro-6-hydroxy-4-methoxy-2-methylbenzoic acid ethyl ester, which is also an oakmoss component. Intensities of bands in the 3000–2850 cm^{-1} region exclude the presence of ethyl or

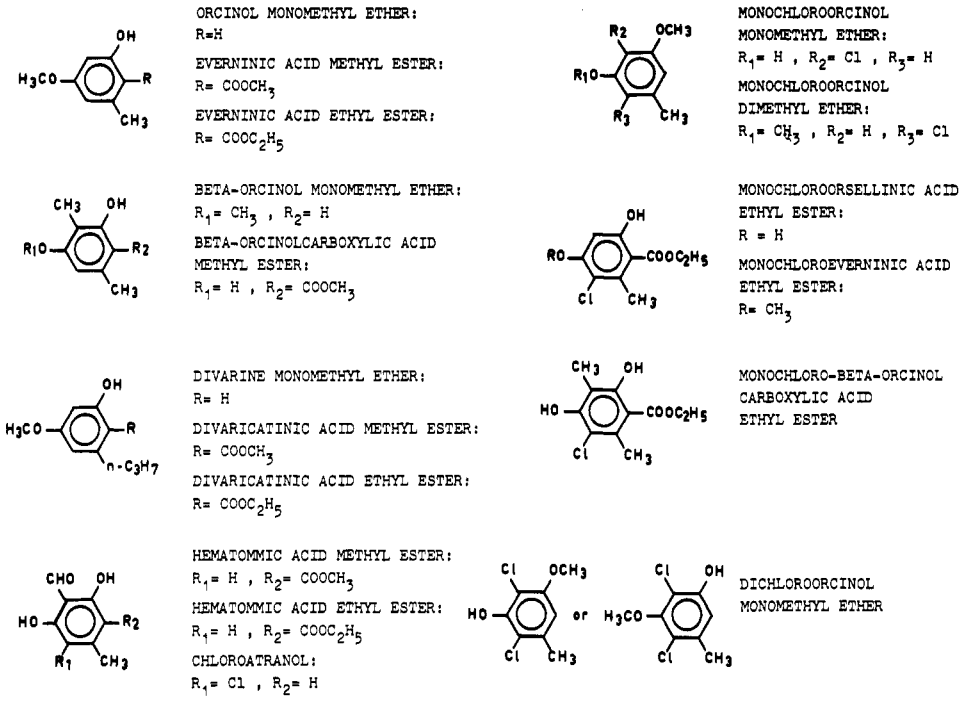

Figure 2. Chemical structures of aromatic compounds identified in oakmoss extract and discussed in the text. The compounds depicted at the left side of the figure can be related to known depsides.

higher alkyl substituents. Absorptions due to a methoxy group were not found. Based on these spectral data this compound was concluded to have the proposed structure: ir (CCl_4) 3525, 3500–2500 (vb), 3060, 2983, 2957, 2928, 2873, 2855, 1659, 1600, 1591, 1461, 1428, 1394, 1378, 1365, 1308, 1250 (sh), 1232, 1162, 1118 (sh), 1107, 1081, 1028, 1015 (sh), 995 (sh), 865 (b), 849, 623, 611, and 605 (sh) and (CS_2) 798, 753 (b), and 699 cm^{-1}.

3-Chloro-4,6-dihydroxy-2,5-dimethylbenzoic Acid Ethyl Ester. A constituent of fraction 8 isolated by preparative GC was supposed to be 3-chloro-4,6-dihydroxy-2,5-dimethylbenzoic acid ethyl ester on the basis of mass and infrared spectra. The molecular ion in the mass spectrum was found at *m/e* 244. The ir spectrum showed absorptions at 3520 cm^{-1} indicating a hydroxyl adjacent to halogen (being a chlorine from mass spectrum), a very broad band at 3500–2500 cm^{-1} due to chelated OH, and a carbonyl band shifted to 1655 cm^{-1}. The absorption pattern at 3000–2900 cm^{-1} indicated an ethyl ester. The presence of an ethyl substituent or a higher alkyl is very unlikely (intensity of bands in the region 3000–2850 cm^{-1}). A methoxy group is absent. As the molecular weight is 244 the benzene ring must carry two methyl substituents. The absence of aromatic hydrogens is not in contradiction with the ir spectrum. The arrangement of the substituents was established as being related to the other compounds identified: ir (CCl_4) 3517, 3500–2500 (vb), 3000 (sh), 2976, 2958, 2929, 2866, 2854, 1655, 1599, 1461, 1455 (sh), 1443, 1402, 1375, 1356, 1335, 1308, 1295, 1286, 1266, 1216, 1184, 1129, 1108, 1050, 1016 and 970 and (CS_2) 815, 802, 762, 703, and 672 cm^{-1}; mass spectrum *m/e* (%) 246 (5), 244 (M^+, 17), 200 (33), 198 (100), 174 (75), 172 (23), 164 (8.5), 155 (11.5), 107 (12.5).

Dichloro-3-methoxy-5-methylphenol. Fraction 9 contained a component with a molecular weight 206 (mass spectrum) bearing two chlorines. Also, the presence of a methoxy group, a hydroxyl group, and methyl-substituted benzene was indicated by the mass spectrum. The ir spectrum showed an absorption band being characteristic for an isolated aromatic hydrogen (847 cm^{-1}) and further the presence of a hydroxyl adjacent to a halogen (3538 cm^{-1}) was shown. A methyl band was found at 1380 cm^{-1} and a methoxy band as a shoulder at 2838 cm^{-1}. A carbonyl band was absent. From these data three structures were designed, i.e.: 2,6-dichloro-3-methoxy-5-methylphenol, 2,4-dichloro-3-methoxy-5-methylphenol, and 2,4-dichloro-5-methoxy-3-methylphenol. The latter compound was excluded because the ir spectrum of the synthesized product did not agree with that of the oakmoss compound investigated: mass spectrum *m/e* (%) 210 (11), 208 (66), 206 (M^+, 100), 195 (1.5), 193 (10), 191 (15), 173 (4), 171 (12), 167 (5.5), 165 (32), 163 (52), 128 (14); ir (CCl_4) 3538, 1433, 1380, 1352, 1290, 1223, 1177 and in CS_2: 1098, 1080, 1006, 935, 847, 751, 598 cm^{-1}.

Resuming the results of our investigation of oakmoss extract it can be concluded that the high content of evernic acid in the lichen *Evernia prunastri* is responsible for the presence of the major components in the steam volatile part of the alcoholic extract. These components are orcinol monomethyl ether, methyl everninate, and ethyl everninate. Chloroatranol is formed from chloroatranorin by alcoholysis followed by decarboxylation during the extraction procedure. The methyl ester of β-orcinol carboxylic acid may be formed from atranorin as can be the esters of hematommic acid, which were identified in fraction 7. The propyl-substituted derivatives must be originated from divaricatic acid. The presence of a number of monoaryl derivatives with chlorine substituents which could not be related to known depsides found in *Evernia prunastri* makes it reasonable to assume that this lichen contains also evernic acids with one or more chlorine substituents.

ACKNOWLEDGMENT

The authors wish to thank Leendert M. van der Linde for NMR analysis, Rob Boudestein for his able assistance in carrying out the separations, and Hans M. van Dort for synthesis of the pyrrole-2-carboxaldehydes.

Supplementary Material Available. Infrared spectra of all synthesized products (Figures 3–17) will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary

material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JAFC-75-950.

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Received for review January 24, 1975. Accepted June 2, 1975. Presented at the 6th International Congress of Essential Oils, San Francisco, Calif., Sept 8–12, 1974.

Studies on the Mechanism of Formation of 4-Hydroxy-5-methyl-3(2*H*)-furanone, a Component of Beef Flavor, from Amadori Products

Kevin B. Hicks and Milton S. Feather*

1-Deoxy-1-dibenzylamino-D-fructuronic acid (**2**) was found to readily dehydrate at 100° and pH 7 to 4-hydroxy-5-methyl-3(2*H*)-furanone (**1**). When **1**, labeled with ^{14}C at C-1 of the hexuronic acid, was used as a starting material, **1** was produced exclusively labeled on the methyl group. On performing the reaction in deuterium oxide solution, **1** was produced having essentially all the methyl protons exchanged with deuterium. In 2 *N* sulfuric acid **2**, 1-benzylamino-1-deoxy-D-fructuronic acid (**3**), and 1-benzylamino-1-deoxy-D-xylulose (**4**) all gave rise to 2-furaldehyde. With the exception of **4**, all gave rise to reductic acid (2,3-dihydroxy-2-cyclopenten-1-one) as well. At pH 7, all three produced **1** as the major dehydration product. At pH 2.5, **1** was observed as a major product from **2**, while it was produced in yields of 6% or less from **3** and **4**.

There is abundant indirect evidence (Hodge et al., 1963) which indicates that a number of flavor and aroma constituents found in food preparation are produced during the cooking process by the condensation of carbohydrates with basic amino groups to form Amadori compounds (1-amino-1-deoxy-2-ketoses) and their subsequent dehydration products. Thus, both maltol (3-hydroxy-2-methylpyran-4-one) and isomaltol (3-hydroxy-2-acetylfuran) which contribute to the flavor of, and are found in baked breads, can be produced synthetically from Amadori compounds (Hodge and Nelson, 1961). 4-Hydroxy-5-methyl-3(2*H*)-furanone (Figure 1), a constituent of beef broth, has also been reported produced from Amadori compounds. This furanone has been prepared by heating D-xylose (Severin and Seilmeier, 1968), D-ribose (Peer et al., 1968a) and D-ribose 5-phosphate (Peer et al., 1968b) with amine salts. The compound has a caramel-like or burnt aroma and its isolation from beef broth (Tonsbeek et al., 1968) indicates that it is a constituent of cooked beef flavor. In an earlier communication from this laboratory, we reported that this furanone is also produced during the decomposition of 1-deoxy-1-dibenzylamino-D-fructuronic acid (Hicks et al., 1974) which was synthesized by the condensation of D-glucuronic acid with dibenzylamine, thus showing that hexuronic acids can also serve as a source of this material during the cooking process.

The present study was aimed, in part, at elucidating aspects of the mechanism for formation of the furanone from an Amadori compound derived from D-glucuronic acid. These studies involved isotopic tracer experiments and deuterium incorporation measurements.

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