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Analysis of Buchu Leaf Oil

Roman Kaiser, Dietmar Lamparsky,* and Peter Schudel

In addition to the previously described stereoisomers of 8-mercapto-*p*-menthan-3-one about 120 further constituents have been identified in Buchu leaf oil of commercial origin. Some new sul-

furated terpenoid ketones as well as *p*-menthan-3-one derivatives oxygenated in the 2 or 4 position found for the first time to occur in nature are discussed in more detail.

Creation of a well-accepted black currant flavor needs the essential oil of Buchu leaves originating from South Africa. Its scarcity during the last few years prompted us to analyze the oil in order to determine its flavorwise important components.

Only three constituents were known in the chemical literature (Gildemeister and Treibs, 1959; Guenther, 1964) until Fluck and coworkers (1961) succeeded in identifying the levorotatory pulegone and the so-called ψ -diosphenol. In 1967 older data were corrected in the sense that not (-)-menthone, but (-)-isomenthone was found to be the major constituent accompanied by (+)-menthone (Klein and Rojahn, 1967).

From our own investigation on Buchu leaf oil of commercial origin, we have been able to verify the occurrence of six main and ten minor constituents cited in the literature. However, we did not succeed in finding any piperitone epoxide also cited to occur in Buchu leaf oil (Klein and Rojahn, 1967), neither in commercial samples of different suppliers nor in samples of specific botanical origin. Being aware of the fact that piperitone epoxide very easily rearranges to diosphenol (Reitsema and Varnis, 1956) which thermally converts to an equilibrium mixture including about 30% ψ -diosphenol (Fluck et al., 1961), both found in Buchu leaf oil, we decided to check our negative findings using synthetic piperitone epoxide as reference material. This was prepared starting from piperitenone via the piperitenone epoxide (Reitsema, 1957) followed by catalytic hydrogenation (Figure 1). The spectra (ir, NMR, MS) thus

obtained differ from those of the isolated components (ψ -diosphenol and diosphenol).

The suspicion that the diosphenols of Buchu leaf oil might be artefacts formed from piperitone epoxide during oil production (Klein and Rojahn, 1967) could be dissipated by the fact that even a diethyl ether extract of dry leaves showed the presence of ψ -diosphenol as well as diosphenol in the same ratio as found in the steam-distilled essential oil (Figure 2).

EXPERIMENTAL SECTION

Procedures. Commercial Buchu leaf oil (660 g) (supplier: White, Tomkins and Courage, Reigate, England) was fractionated by distillation, crystallization, and chromatographic procedures. Heat-sensitive constituents were transformed to more stable derivatives (e.g. by mild acetylation) before being separated or purified by preparative GLC. Trace components detected through GLC-MS coupling experiments were identified by correlation with authentic synthetic specimens.

Apparatus. GLC. Carlo Erba Model GI with FID and Model Fractovap GV were used. (a) Packed glass columns were used for analytical measurements: length 3 m; 3 mm i.d.; 2% Carbowax 20M on Chromosorb G (AW DMCS, 60-80 mesh); temperature programmed from 120 to 240° with a heating rate of 5°/min or isothermal at 160 or 180°; carrier gas flow, 50 ml of He/min. (b) Packed glass columns were used for preparative purposes: length 3 m; 15 mm i.d.; 10 to 5% Carbowax 20M (loading with stationary phase stepwise decreasing with increasing distance from column entrance) on Chromosorb G (AW DMCS, 80-100 mesh); isothermal at 160 or 180°; carrier gas flow, 200 ml of N₂/

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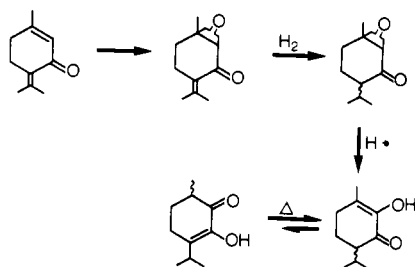


Figure 1. Synthesis of piperitone epoxide and its rearrangement.

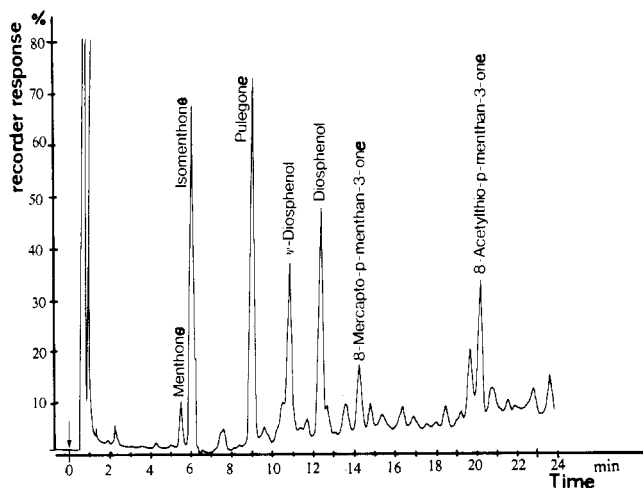


Figure 2. Ether extract of dry Buchu leaves.

min; injection of 5 to 100 μ l samples according to the problem. (c) Glass capillary columns coated with UCON HB 5100 were used: length 50 m; 0.31 mm i.d.; temperature programmed from ambient temperature to 175° with a heating rate of 3°/min; carrier gas flow, 2–3 ml of He/min; splitless injection of 0.8- μ l samples of a 0.1% solution in pentane on cold column (Grob and Grob, 1972).

GLC-MS. The GLC-MS coupling system consists of a gas chromatograph (Carlo Erba, Model GI), a mass spectrometer (Varian-MAT, Model CH 5), and a data system (Varian-MAT, Model Spectroscopy 100). The glass capillary column described above was connected to the mass spectrometer via a platinum capillary interface (Neuner-Jehle et al., 1973). Several coupling experiments were done with packed columns using a Watson-Biemann separator.

NMR. The conditions were: Varian A-60 A; 60 MHz; solutions in deuteriochloroform with Me_4Si as internal standard; δ in parts per million.

Infrared. The conditions were: Perkin-Elmer, Models 157 and 257; samples neat as liquid film or solutions in chloroform; frequency in cm^{-1} .

$[\alpha]_D$ values were determined under the following conditions: Perkin-Elmer, polarimeter Model 141; solvents, chloroform or methanol; ambient temperature; cell length 10 cm.

RESULTS AND DISCUSSION

Elimination of the lights and most of the crystalline diosphenol led to the so-called "heavies" of the Buchu leaf oil exhibiting a powerful, very interesting odor complex (Figure 3). Further column chromatography and GLC-MS coupling experiments allowed us to trace the two diastereomeric 8-mercapto-*p*-menthan-3-ones, which showed identical ir, NMR, and mass spectra as well as identical GLC retention times on polar and apolar stationary phases as the two reaction products obtained by alkali-catalyzed addition of hydrogen sulfide to (+)-pulegone (Lamparsky and Schudel, 1971). The fact that the pulegone isolated from

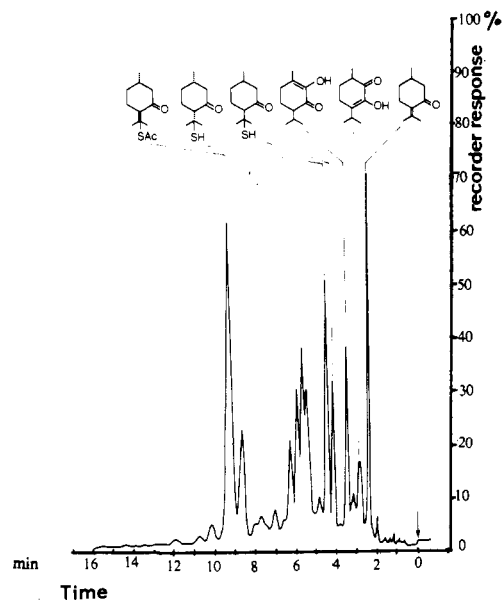


Figure 3. Heavies of Buchu leaf oil.

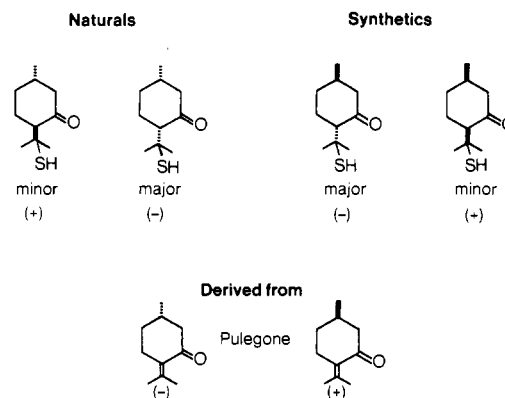


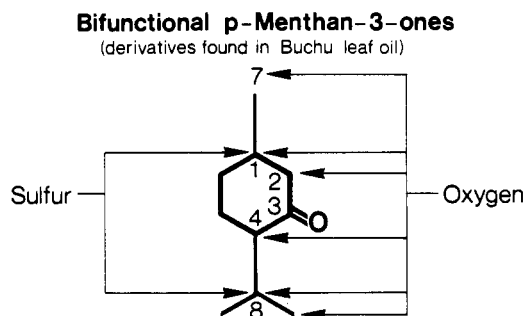
Figure 4. 8-Mercapto-*p*-menthan-3-ones.

Buchu leaf oil in quantities up to about 11% showed specific rotations of $[\alpha]_D^{20} -24.6^\circ$ (CHCl_3 , c 0.7842) and -22.6° (methanol, c 0.7595), which practically excludes the presence of the corresponding enantiomer, allowed us to assign their chirality as depicted in Figure 4. This was completely confirmed by a later publication (Sundt et al., 1971).

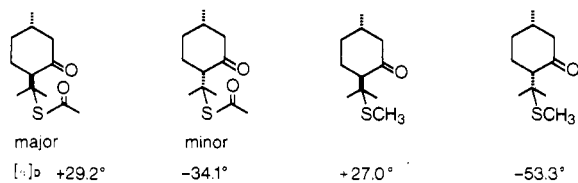
The major isomer found in Buchu leaf oil is a formal derivative of isomenthone. However, the sensorically preferred isomer exhibiting the full fruity character typical for black currants derives from menthone. Incidentally, the latter represents the major isomer one obtains synthetically from pulegone.

Being aware of the fact that often complex mixtures of numerous substances are responsible for typical sensoric effects, we continued with the analysis of Buchu leaf oil even after the isolation of the sensorically important 8-mercapto-*p*-menthan-3-ones. Therefore, 123 substances could be identified. For several other trace components, however, only tentative structures could be assigned (Table I). Characteristic of the investigated oil is the occurrence of bifunctional monoterpene ketones which can be classified as sulfurated and oxygenated derivatives of *p*-menthan-3-one (Figure 5).

Sulfur Derivatives of *p*-Menthan-3-one. Structurally closely related to the 8-mercapto-*p*-menthan-3-ones are the two diastereomeric pairs 8-acetylthio-*p*-menthan-3-one and 8-methylthio-*p*-menthan-3-one, for the first time found in nature and not yet described in the literature (Figure 6). All four compounds exhibit clearly distinguish-

Figure 5. Bifunctional *p*-menthan-3-ones.

Natural isolates



Synthetics (starting from (+)-pulegone)

Figure 6. Derivatives of 8-mercapto-*p*-menthan-3-one.Table I. Composition of Buchu Leaf Oil^a

| | Mono- terpenes | Sesqui- terpenes | Ali- phatic | Aro- matic |
|------------------|-------------------|---------------------|----------------|---------------|
| Hydrocarbons | 15 | 5 | | 2 |
| Oxides | 7 | | | |
| Alcohols | 24 (+3) | 1 (+1) | 3 (+2) | 1 |
| Esters, lactones | 12 | | 1 | |
| Aldehydes | 2 | | | 2 (H) |
| Ketones | 10 | | 3 (A) | |
| Bifunct. ketones | 30 (+5) | | | |
| Phenols, ethers | | | | 5 |
| Total | 100 (+8) | 6 (+1) | 7 (+2) | 10 |

^a A, alicyclic; H, heterocyclic. The numbers in parentheses refer to the components only tentatively identified.

able GLC retention times whereby the *trans*-configured menthone derivatives were eluted first. Their structures, derived from the ir, NMR, and mass spectra, were completely confirmed by comparison with the corresponding synthetic products. Since the diastereomers synthesized from (+)-pulegone by addition of thioacetic acid or methyl mercaptan, respectively, exhibit opposite specific rotations compared to the corresponding natural isomers, one has to conclude that the latter are derived from (-)-pulegone. In order to confirm this, (+)-*trans*-8-acetylthio-*p*-menthan-3-one was synthesized from (-)-pulegone and purified by preparative GLC. It showed identical spectra as well as an optical rotation of $[\alpha]_D^{20} +29.6^\circ$ (CHCl₃, *c* 1.02) compared to $[\alpha]_D^{20} +29.2^\circ$ (CHCl₃, *c* 1.03) of the natural material from Buchu leaf oil.

The ir spectra of the sulfur derivatives of menthone and isomenthone exhibit typical differences as listed in Table II (ν in cm⁻¹), where w = weak and st = strong. The NMR spectra reveal the same features as observed in the case of the free mercapto derivatives. Assuming that the bulky thioisopropyl grouping prefers the equatorial position, the relative stereochemistry within each pair of diastereomers could be assigned using the chemical shift of the equatorial

Other sulfurated ketones

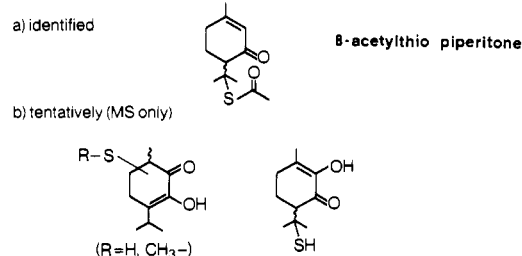


Figure 7. Further sulfurated ketones in Buchu leaf oil.

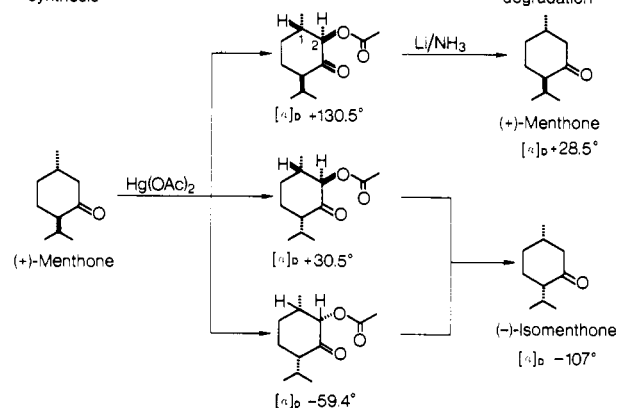
Oxygenated Ketones
synthesisFigure 8. 2-Acetoxy-*p*-menthan-3-ones.

Table II

| S-derivatives of | 1382- 1390 | 1362- 1372 | 1200- 1204 | 1074- 1078 |
|------------------|---------------|---------------|---------------|---------------|
| Menthone | w | st | st | |
| Isomenthone | st | w | | st |

(δ 1.02 ppm, badly resolved doublet) or axial (δ 0.96 ppm, sharp doublet) secondary methyl group on the six-membered ring (cf. Fetizon et al., 1966).

Furthermore, four other new sulfur-bearing terpenoids were detected (Figure 7) of which only one could be identified as 8-acetylthiopiperitone by comparison of its GLC behavior and its mass spectrum with an authentic synthetic specimen, additionally characterized by ir, uv, and NMR spectra. The chirality of the natural product still remains undetermined.

The three other constituents occur in extremely small quantities. They could be detected with GLC-MS coupling experiments only. Their mass spectra are to the best of our knowledge compatible with the tentatively assigned diosphenol and ψ -diosphenol structures given in Figure 7.

Oxygenated Derivatives of *p*-Menthane-3-one. Twenty bifunctional *p*-menthan-3-ones isolated from Buchu leaf oil are dealt with in the next section.

Oxygen Functions in Position 2. Three 2-acetoxy-*p*-menthan-3-ones occurring to the extent of about 0.3% of the total weight of the oil were isolated in the ratio of 6:3:1. In order to determine their exact structures, (+)-menthone was oxidized with mercuric acetate (Jefferies et al., 1954). The reaction mixture, which had a minty, herbaceous odor, contained among others three 2-acetoxy-*p*-menthan-3-ones identical with the natural isomers with respect to GLC retention times, ir, NMR, and mass spectra (Figure 8).

The synthetic (+)-*trans*-2-acetoxymenthone had a specific rotation of $[\alpha]_D +126.3^\circ$ (CHCl₃, *c* 1.90) compared to

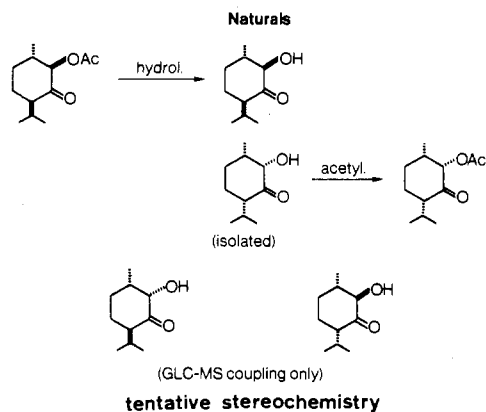


Figure 9. 2-Hydroxy-*p*-menthan-3-ones from Buchu leaf oil.

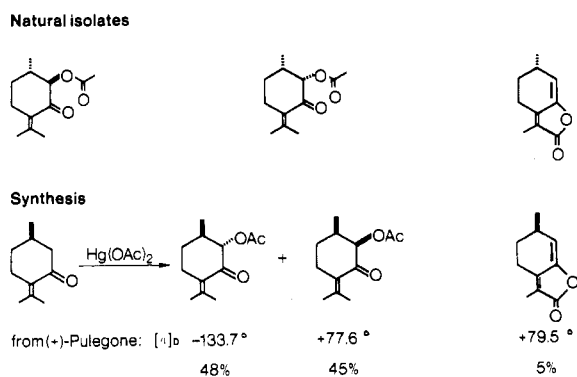


Figure 10. Acetoxylation of pulegone.

$[\alpha]_D +130.5^\circ$ (CHCl_3 , c 0.597) of the natural one. Based on the fact that the coupling constant of the two hydrogen atoms at C_1 and C_2 ($J_{H_1-H_2} = 10.5$ Hz) in the NMR spectrum clearly indicated a *trans* diaxial relationship, we have to assign the *trans* configuration between the methyl group at C_1 and the acetoxy group at C_2 . Reductive elimination of the acetoxy group with lithium in ammonia led to (+)-menthone ($[\alpha]_D +28.5^\circ$ (CHCl_3 , c 0.843)). Since pure (+)-menthone from Buchu leaf oil showed the same optical rotation ($[\alpha]_D +31.8^\circ$ (CHCl_3 , c 0.667)), the relative and absolute stereochemistry of the naturally occurring (+)-*trans*-2-acetoxymenthone is defined as depicted in Figure 8.

Analogous deductions were made for the two remaining compounds, (+)-*trans*- and (-)-*cis*-2-acetoxymenthones, which were correlated to (-)-isomenthone ($[\alpha]_D -107^\circ$ (CHCl_3 , c 0.371)), pure (-)-isomenthone from Buchu leaf oil ($[\alpha]_D -114.4^\circ$ (CHCl_3 , c 1.256)). Besides the 2-acetoxy-*p*-menthan-3-ones we were able to isolate two diastereomeric 2-hydroxy-*p*-menthan-3-ones. The first one represents (+)-*trans*-2-hydroxymenthone (Figure 9). It shows identical properties (GLC, ir, NMR, MS) as the saponification product of (+)-*trans*-2-acetoxymenthone. The specific rotation is very low for both the natural and the synthetic sample ($[\alpha]_D$ 0 to $+5.1^\circ$ (CHCl_3 , c 5440)). In order to ascertain the stereochemistry, the *trans*-2-hydroxymenthone obtained was reacylated with acetic anhydride in pyridine. Thereby, the specific rotation of $[\alpha]_D +129.4^\circ$ (CHCl_3 , c 0.736) was again obtained, thus indicating that the 2-hydroxy compound in question does not undergo racemization and must exhibit the same stereochemistry as the parent *trans*-2-acetoxymenthone before and after its chemical transformation.

Similarly, the second 2-hydroxy-*p*-menthan-3-one isolated could be correlated to the *cis*-2-acetoxymenthone. The other two diastereomeric 2-hydroxy-*p*-menthan-3-ones traced by GLC-MS coupling experiments exhibited

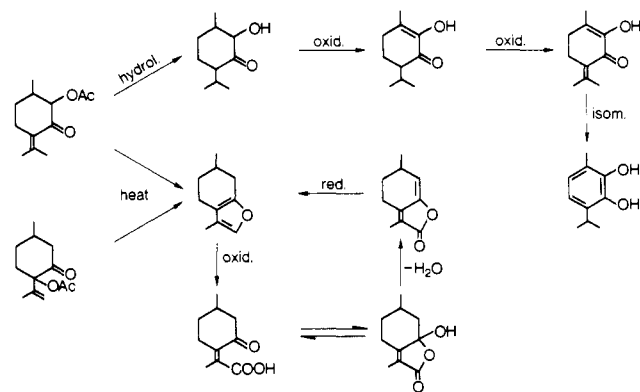


Figure 11. Relationships in the acetoxypulegone series without stereochemical assignment.

very similar mass spectra to the ones just described. Since their fragmentation patterns differ remarkably from the 4-hydroxy-*p*-menthan-3-ones, we believe them to be the *cis*-2-hydroxymenthone and the *trans*-2-hydroxyisomenthone. Assuming that they are biogenetically related to (-)-pulegone, (+)-menthone, and (-)-isomenthone, all known as main constituents of Buchu leaf oil, we might also assign them the *S* configuration at C_1 .

2-Acetoxypulegones have not yet been reported to occur in nature. We now could find them in Buchu leaf oil. For the identification of their structures, (+)-pulegone was oxidized with mercuric acetate (Zalkow, 1963). In addition to the 1:1 mixture of the *cis*- and *trans*-2-acetoxypulegones, a 5% impurity was formed which proved to be (ir, NMR, and MS) the dehydration product of the so-called Woodward-Eastman pseudo acid (Woodward and Eastman, 1950). Interestingly enough, this enol lactone could also be traced in Buchu leaf oil by GLC-MS experiments (Figure 10).

The ir, NMR, and mass spectra of the natural 2-acetoxypulegones were identical with the synthetic products. Unfortunately, we have not been able to measure the optical rotation of the natural compounds, for experimental reasons. Assuming again, however, that the 2-acetoxypulegones from Buchu leaf oil are formally related to (-)-pulegone, their (1*S*) configuration seems to be the most probable one.

In regard to odor characteristics, the mixture of 2-acetoxypulegones imparts a particular minty, hay-like note to the odor complex of Buchu leaf oil. Treatment of the synthetic enol lactone mentioned above with LiAlH_4 led to menthofuran, which represents another trace component of the oil (positively identified by GLC-MS). Previous investigations (Zalkow, 1963; Zalkow and Ellis, 1964) showed that 2-acetoxypulegone as well as 4-acetoxypulegone are transformed to various products listed in Figure 11 under pyrolytic, hydrolytic, oxidative, and/or reductive reaction conditions. Therefore, one is tempted to assume that oxidation of position 2 of the *p*-menthan-3-one skeleton represents in one way or another a key step leading to numerous trace components of Buchu leaf oil.

Oxygen Functions in Position 4. Two further trace components were identified as diastereomeric 4-hydroxy-*p*-menthan-3-ones. Their structures were correlated with authentic compounds synthesized from (+)-(*1R*)-pulegone via the (*1R*)-pulegone oxides (Djerassi et al., 1965; Katsuhara, 1967; Reusch and Mattison, 1968) as depicted in Figure 12. A different synthesis was described by Suga et al. (1967). The natural 4-hydroxyisomenthone obtained shows identical ir, NMR, and mass spectra, but opposite chiral properties ($[\alpha]_D -116.2^\circ$ (CHCl_3 , c 1.706) as the synthetic compound ($[\alpha]_D +114.2^\circ$ (CHCl_3 , c 2.409)). This clearly indicates the (1*S*) chirality of the natural isomer (Figure 13), which again is in agreement with the findings mentioned above.

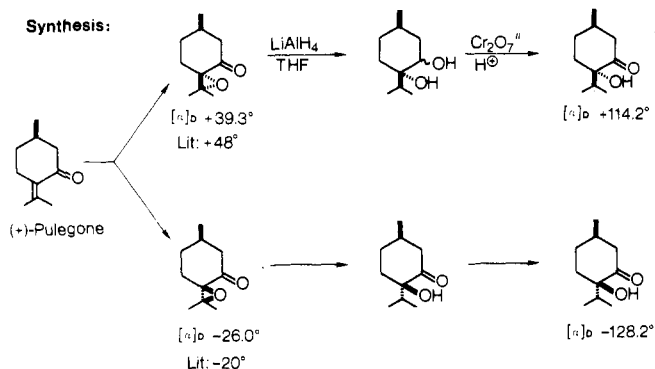


Figure 12. Synthesis of 4-hydroxy-*p*-menthan-3-ones from (+)-(1*R*)-pulegone.

Natural isolates

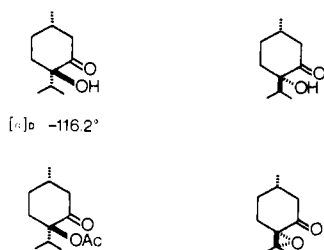


Figure 13. Natural isolates from Buchu leaf oil oxygenated at C₄.

The second natural diastereomer exhibited identical GLC retention times and mass fragmentation pattern as the corresponding synthetic 4-hydroxymenthone. Under the assumption that it is also related to (-)-pulegone, we can assign it the (1*S*,4*S*) configuration.

Two other trace components, namely the acetate of 4-hydroxyisomenthone first synthesized by Zalkow and Ellis (1964) and the *trans*-pulegone oxide, were detected by GLC-MS experiments and confirmed by comparison with the synthetic specimens. Again, we tend to assign them the (1*S*) chirality as shown in Figure 13.

GLC-MS coupling experiments revealed another bifunctional monoterpene ketone with a molecular ion peak at *m/e* 168 whose fragmentation pattern reminded us of those of the 4-hydroxy-*p*-menthan-3-ones. The idea that we probably are dealing with an isomer of 4-hydroxyisopulegone had to be substantiated by syntheses. For this reason, (+)-pulegone was treated with lead tetraacetate followed by alkaline saponification (Zalkow and Ellis, 1964). The two synthetic diastereomeric 4-hydroxyisopulegones were separated and purified by preparative GLC (Figure 14).

The natural compound exhibited an identical GLC retention time as well as an identical mass spectrum as the synthetic (1*R*,4*S*) isomer. Assuming again that the natural isomer belongs to the (1*S*) series, we assigned the (1*S*,4*R*) configuration to the 4-(iso)hydroxyisopulegone. 4-Hydroxyisopulegones were also described as reaction products of the photooxidation of (+)-pulegone (Schulte-Elte et al., 1971).

Contrary to the above-mentioned 4-hydroxylated compounds which all are known in the chemical literature, the 4-hydroxydiosphenol is new and occurs together with the 1-hydroxy- ψ -diosphenol (Cusmano and Cattini, 1924) as a trace component in Buchu leaf oil. The structure was determined by comparison of its ir, NMR, and mass spectra with those of diosphenol and ψ -diosphenol. Thereby, characteristic differences of the NMR spectra were extremely helpful (Figure 15).

Other chemically known diosphenol derivatives such as the methyl ethers of diosphenol and ψ -diosphenol as well as

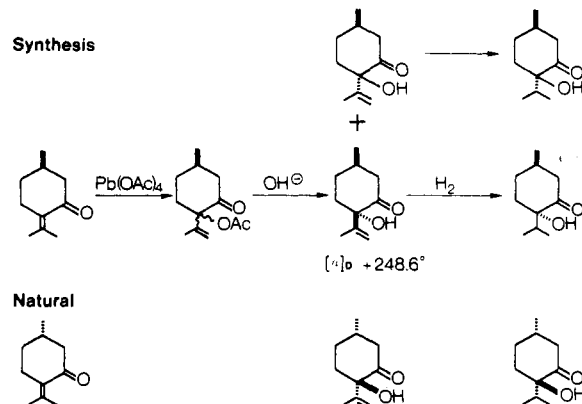


Figure 14. Relationships between synthetic and natural 4-hydroxyisopulegones.

Diosphenol derivatives

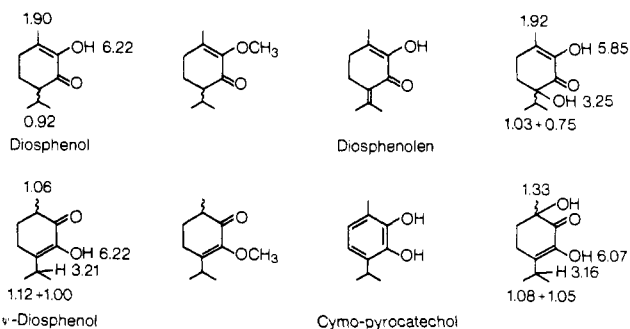


Figure 15. Diosphenol derivatives found in Buchu leaf oil.

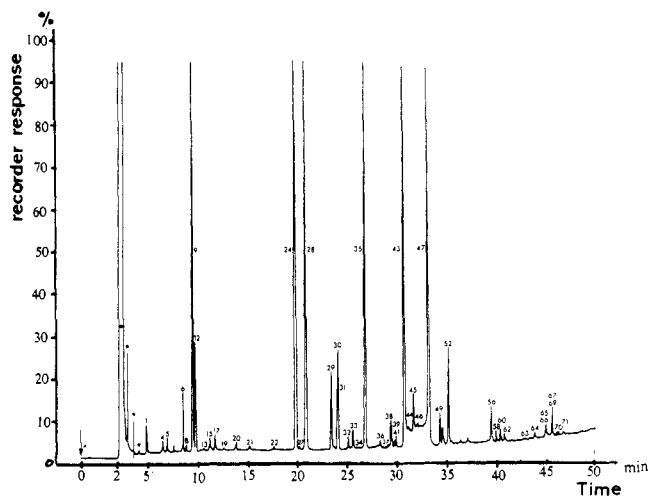


Figure 16. Capillary GLC of Buchu leaf oil.

the dehydrogenation products, namely diosphenolene (Naves, 1966) and cymopyrocatechol (Treibs and Albrecht, 1959), are trace components in Buchu leaf oil and were identified by comparison of GLC retention times and mass spectra with the synthetic specimens prepared according to the literature.

Composition of Buchu Leaf Oil. Table III lists all the components identified in Buchu leaf oil according to chemical considerations and within each group according to increasing GLC retention times. The peak numbers refer to the capillary GLC curve shown in Figure 16.

The sensoric properties of Buchu leaf oils originating either from different producers or from plant materials of different botanical origin differ strongly. We have compared: (a) a very powerful so-called "english distilled" oil;

Table III. Constituents Identified in Buchu Leaf Oil^a

| Peak no. | Compound | Means of ident. | Peak no. | Compound | Means of ident. |
|----------------------------------|--|-----------------|----------|--|-----------------|
| Monoterpene Hydrocarbons | | | | | |
| 1 | α -Pinene* | ir, MS, RT | | β -Phellandrene | MS, RT |
| | Thujene | MS, RT | 13 | <i>p</i> -Mentha-1,3,6-triene | ir, MS, NMR |
| | Camphene* | MS, RT | | <i>cis</i> - β -Ocimene | MS, RT |
| 4 | β -Pinene* | ir, MS, RT | 15 | γ -Terpinene | ir, MS, RT |
| 5 | Sabinene | ir, MS, NMR | | <i>p</i> -Cymene | MS, RT |
| 6 | Myrcene* | ir, MS, RT | 17 | <i>trans</i> - β -Ocimene | ir, MS, RT |
| | α -Phellandrene | MS, RT | | Terpinolene | MS, RT |
| 8 | α -Terpinene* | ir, MS, RT | 22 | α , <i>p</i> -Dimethylstyrene | ir, MS, NMR |
| 9 | (+)-Limonene* | ir, MS, RT | | | |
| Monoterpene Oxides | | | | | |
| | 2,6,6-Trimethyl-6-vinyl-tetrahydropyran | MS, RT | | <i>cis</i> -Linalool oxide | MS, RT |
| | 1,4-Cineol | MS, RT | 20 | <i>trans</i> -Linalool oxide | MS, RT |
| 12 | 1,8-Cineol | ir, MS, RT | | Pinol | ir, MS, NMR |
| | | | | Menthofuran | MS, RT |
| Monoterpene Alcohols | | | | | |
| 31 | (+)-Linalool | ir, MS, RT, NMR | | Citronellol | ir, MS, RT |
| | Menthol | ir, MS, RT | | <i>p</i> -Cymen-8-ol | ir, MS, RT |
| | Neomenthol | MS, RT | | Nerol | MS, RT |
| | Neoisomenthol | MS, RT | | <i>trans</i> -Carveol | MS, RT |
| 33 | Terpinen-4-ol* | ir, MS, RT, NMR | | <i>cis</i> -Carveol | MS, RT |
| | β -Terpineol | MS, RT | | Myrtanol | ir, MS, NMR |
| 36 | δ -Terpineol | ir, MS, NMR | 54 | <i>p</i> -Menth-1-en-9-ol | ir, MS, NMR |
| | Borneol | MS, RT | | <i>p</i> -Menth-8-en-7-ol | ir, MS, NMR |
| 39 | α -Terpineol | ir, MS, RT | | α -Phellandrol | ir, MS, NMR |
| | Myrtenol | ir, MS, RT | | β -Phellandrol | ir, MS, NMR |
| | Isopulegol | ir, MS, RT | | <i>p</i> -Menth-4(8)-en-7-ol | ir, MS, NMR |
| | Neoisopulegol | ir, MS, RT | | <i>p</i> -Mentha-1,8-dien-10-ol | MS, RT |
| Monoterpene Esters and Lactones | | | | | |
| | Menthyl acetate | MS, RT | | Neryl acetate | MS, RT |
| | Neoisomenthyl acetate | MS, RT | | Myrtanyl acetate | MS, RT |
| 41 | Myrtenyl acetate | MS, RT | | <i>trans</i> -Carveyl acetate | MS, RT |
| | <i>x</i> -Isopulegyl acetate | MS | | <i>cis</i> -Carveyl acetate | MS, RT |
| | <i>y</i> -Isopulegyl acetate | MS | | <i>p</i> -Menth-1-en-9-yl acetate | MS, RT |
| | Citronellyl acetate | MS, RT | | Enol lactone (Woodward) | ir, MS, NMR |
| Monoterpene Aldehydes | | | | | |
| | <i>p</i> -Menth-1-en-9-al | MS, RT | | <i>p</i> -Mentha-1,8-dien-10-al | MS, RT |
| Monoterpene Ketones | | | | | |
| 24 | (+)-Menthone | ir, MS, NMR | 35 | (-)-Pulegone | ir, MS, RT, NMR |
| 28 | (-)-Isomenthone | ir, MS, NMR | | Piperitone | ir, MS, NMR |
| 29 | (+)-Isopulegone | ir, MS, NMR | | Piperitenone | MS, RT |
| 30 | (-)-Isopulegone | ir, MS, NMR | | Carvone | MS, RT |
| 32 | Dihydrocarvone | ir, MS, NMR | | Nerylacetone | MS, RT |
| Monoterpene Bifunctional Ketones | | | | | |
| 34 | (-)-4-Hydroxyisomenthone | ir, MS, NMR | | (-)- <i>cis</i> -2-Acetoxyisomenthone | ir, MS, NMR |
| 37 | (+)-4-Hydroxymenthone | MS, RT | | <i>trans</i> -2-Acetoxy-pulegone | ir, MS, NMR |
| 38 | <i>trans</i> -2-Hydroxymenthone | MS, RT | | <i>cis</i> -2-Acetoxy-pulegone | ir, MS, NMR |
| 43 | ψ -Diosphenol* | ir, MS, NMR | 69 | (+)- <i>trans</i> -8-Acetylthio- <i>p</i> -menthan-3-one | ir, MS, NMR |
| 44 | (+)-8-Hydroxymenthone | ir, MS, NMR | | (-)- <i>cis</i> -8-Acetylthio- <i>p</i> -menthan-3-one | ir, MS, NMR |
| 45 | <i>cis</i> -2-Hydroxyisomenthone | MS, RT | 70 | 8-Acetylthiopiperitone | MS, RT |
| 47 | Diosphenol* | ir, MS, NMR | | Pulegone epoxide A | MS, RT |
| 49 | (+)- <i>trans</i> -8-Mercapto- <i>p</i> -menthan-3-one | ir, MS, NMR | | | |
| 52 | (-)- <i>cis</i> -8-Mercapto- <i>p</i> -menthan-3-one | ir, MS, NMR | | 4-Acetoxyisomenthone | MS, RT |
| | | | | 8-Acetoxymenthone | MS, RT |

Table III (Continued)

| Peak no. | Compound | Means of ident. | | Peak no. | Compound | Means of ident. | |
|----------|--|--------------------------|-----|----------|--|-----------------|-----|
| 55 | (+)- <i>trans</i> -8-Methylthio- <i>p</i> -menthan-3-one | ir, MS, | NMR | 4 | (Iso)acetoxyisopulegone | MS, RT | |
| 56 | Diosphenolene | ir, MS, | NMR | 4 | (Iso)hydroxyisopulegone | MS, RT | |
| 57 | (-)- <i>cis</i> -8-Methylthio- <i>p</i> -menthan-3-one | ir, MS, | NMR | | Diosphenol methyl ether | MS, RT | |
| 58 | (+)- <i>trans</i> -2-Acetoxy-isomenthone | ir, MS, | NMR | | ψ -Diosphenol methyl ether | MS, RT | |
| 60 | (+)- <i>trans</i> -2-Acetoxy-menthone | ir, MS, | NMR | | Diosphenolene methyl ether | MS, RT | |
| | | | | | 4-Hydroxydiosphenol | ir, MS, | NMR |
| | | | | | 1-Hydroxy- ψ -diosphenol | ir, MS, | NMR |
| | | Sesquiterpenes | | | | | |
| | β -Elemene | ir, MS, RT | | | γ -Muuroleone | ir, MS, RT | |
| | Aromadendrene | ir, MS, RT | | | Calamenene | MS, RT | |
| | α -Muuroleone | ir, MS, RT | | | Spathulenol | ir, MS, RT | |
| | | Aliphatic Compounds | | | | | |
| | <i>cis</i> -4-Decenol | ir, MS, | NMR | | <i>cis,cis</i> -3,6-Dodecadienol | ir, MS, | NMR |
| | <i>trans</i> -4-Decenol | MS, RT | | | <i>cis,cis</i> -3,6-Dodecadienyl acetate | ir, MS, | NMR |
| | | Cycloaliphatic Compounds | | | | | |
| | 3-Methylcyclohexanone | ir, MS, RT | | | | | |
| | β -Damascenone | MS, RT | | | | | |
| | β -Ionone | MS, RT | | | | | |
| | | Aromatic Compounds | | | | | |
| | Phenethyl alcohol | MS, RT | | 66 | Eugenol | ir, MS, | NMR |
| 62 | Methyl eugenol | ir, MS, | NMR | | Guaiacol | MS, RT | |
| 65 | Vinyl guaiacol | ir, MS, | NMR | | Cymopyrocatechol | ir, MS, RT | |
| | | Heterocyclic Compounds | | | | | |
| 21 | Furfural | MS, RT | | | | | |
| 27 | 5-Methylfurfural | MS, RT | | | | | |

^a Known compounds are marked with an asterisk. Means of identification: ir, infrared spectrum; MS, mass spectrum; RT, GLC behavior; NMR, nuclear magnetic resonance spectrum.

Table IV. Comparison of Buchu Leaf Oils of Different Origins (Percentage of Main Constituents)

| Constituents | Variety | | Producer | |
|---------------------------------------|--------------------|---------------------|----------|---------|
| | <i>B. betulina</i> | <i>B. crenulata</i> | A (old) | B (new) |
| Limonene, etc. | 17 | 9 | 14 | 9,5 |
| Menthone | 17 | 6 | 34 | 7 |
| Isomenthone | 43 | 22 | 25 | 20 |
| Isopulegone | 4 | 10 | 2 | 3 |
| Pulegone | 3 | 50 | 7 | 9 |
| ψ -Diosphenol | 8 | 1 | 8 | 22 |
| Diosphenol | 9 | 1 | 9 | 24 |
| 8-Mercapto- <i>p</i> -menthan-3-one | ++ | + | + | ++ |
| 8-Acetylthio- <i>p</i> -menthan-3-one | + | ++ | + | + |

(b) an oil distilled in South Africa, originating from the leaves of the species *Barosma betulina*; (c) an oil distilled in South Africa, originating from leaves of the species *Barosma crenulata*. The latter seems to be of no great sensoric interest. This may be due to the high (-)-pulegone content as well as its low share of diosphenols. Table IV compiles numerically the rough estimations of the major constituents in four oils of various origins.

In addition, we have also observed that minor constituents of high flavor impact quantitatively differ within the two botanical species analyzed. Thus, the more important, flavorwise, 8-mercapto-*p*-menthan-3-one seems to be present in larger amounts in the oil of *Barosma betulina*, whereas the content of the less important 8-acetylthio-*p*-menthan-3-one is higher in the oil of *Barosma crenulata*.

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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*,9*bH*)-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 \times 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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