[CONTRIBUTION FROM THE FOREST PRODUCTS LABORATORY, UNIVERSITY OF CALIFORNIA]

EXTRACTIVE COMPONENTS FROM INCENSE-CEDAR HEARTWOOD (*Libocedrus decurrens* Torrey) II. OCCURRENCE AND SYNTHESIS OF *p*-METHOXYTHYMOL AND *p*-METHOXYCARVACROL, TWO NEW PHENOLIC COMPOUNDS

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Received November 2, 1954

In the course of a general investigation of the extractive components present in incense-cedar heartwood, *Libocedrus decurrens* Torrey (family, *Cupressaceae*), three compounds have thus far been identified, namely carvacrol, hydrothymoquinone and thymoquinone (1). These constituents are of functional interest, for among other things, they exhibit varying degrees of toxicity toward wood-destroying fungi, as recently demonstrated by Erdtman and Rennerfelt (2). Thus, the well-known decay resistance of incense-cedar heartwood is partially due to the presence of these three compounds.

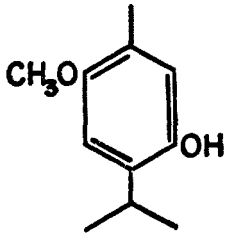
Carvacrol and hydrothymoquinone accounted for approximately 35 per cent of the total steam-volatile phenolic fraction obtained from incense-cedar heartwood (1). While fractionally distilling the phenolic-bearing oil, *in vacuo*, approximately 58 per cent distilled within a narrow boiling range of 119–120° (35 mm.). All attempts to crystallize this fraction failed. This product was insoluble in sodium bicarbonate but soluble in dilute sodium hydroxide. It reacted with bromine in carbon tetrachloride to give hydrogen bromide, was rapidly oxidized with potassium permanganate in pyridine, formed a dark orange color with diazotized sulfanilic acid in alkaline solution, and produced a light orange spot with diazotized benzidine hydrochloride on filter paper. While this fraction exhibited reactions typical of phenols, no color was produced in aqueous or alcoholic ferric chloride.

Preliminary trials indicated that separation of this fraction into pure compounds could best be accomplished through benzoylation. The benzoylated mixture produced a crystalline benzoate in 75 per cent yield, which upon saponification, produced a pure liquid phenolic compound having the molecular formula $C_{11}H_{16}O_2$. The recovered non-crystalline benzoates, upon saponification, yielded a crystalline phenolic compound, which also proved to have the same molecular formula, $C_{11}H_{16}O_2$. The approximate quantity of each of these products isolated from the wood amounted to 1.0 per cent for the liquid phenol and 0.04 per cent for the crystalline product, respectively, based on the dry weight of the wood. The present paper deals with the determination of structure and synthesis of these two new phenolic compounds.

Each of the compounds contained a methoxyl group. When the compounds were oxidized with chromic acid, the primary product formed was thymoquinone. This indicated that these phenols are monomethyl ethers of hydrothymoquinone, namely *p*-methoxythymol (I) and *p*-methoxycarvacrol (II).

A thorough search of the literature indicated that neither *p*-methoxythymol

nor *p*-methoxycarvacrol had been previously isolated from a natural product. Semmler isolated the dimethyl ether of hydrothymoquinone from the oil of *Eupatorium triplinere* Vahl, which upon demethylation with concentrated hydriodic acid and red phosphorus produced hydrothymoquinone, together with what was reported to be a mixture of its monomethyl ethers (3). No attempt, however, was made to separate and identify the isomeric monomethyl ethers. As far as we are aware, no report appears in literature on the characterization or preparation of either *p*-methoxythymol or *p*-methoxycarvacrol.



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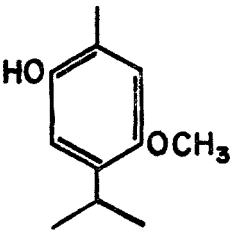
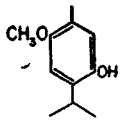


II

The proof that the liquid phenol isolated is *p*-methoxythymol (I) was afforded through synthesis. This was accomplished by preparing the methyl ether of *p*-aminocarvacrol (4) from *p*-aminocarvacrol which upon diazotization and steam distillation in presence of cupric sulfate produced *p*-methoxythymol (I). The properties and derivatives of the synthetic product and the isolated liquid phenol from incense-cedar heartwood were identical. Similarly, when the methyl ether of *p*-aminothymol (4) was diazotized and steam-distilled in the presence of cupric sulfate, *p*-methoxycarvacrol (II) resulted, which proved to have properties identical to that of the crystalline isomeric phenol from the wood.

The identification of these isomeric methoxyphenols is of interest from the taxonomic and biochemical standpoints, because of their close structural relationship with the other compounds thus far isolated from incense-cedar heartwood. These include carvacrol (III), hydrothymoquinone (IV), and thymoquinone (V).



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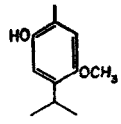

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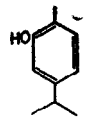

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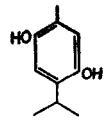

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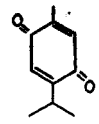

V

The bioassay of the relative toxicity of these new phenolic compounds against various wood-destroying fungi is under investigation. Also, these compounds are being tested in certain virus chemotherapy studies.

EXPERIMENTAL[1]

Fractionation of phenolic oil. The details of the procedure used in obtaining the steam-volatile phenolic fraction from incense-cedar heartwood and its fractionation, *in vacuo*, have been described (1). The intermediate fractions 3 (22.53 g.) and 4 (7.0 g.), b.p. 119–120° (3.5 mm.), n_D^{23} 1.5264–1.5274, representing 58% of the total phenols present, were combined. This material exhibited the reactions characteristic for phenols, except that no color was produced with ferric chloride in alcohol or water. All attempts to crystallize the oil failed.

Benzoylation and separation of phenols as benzoates. The phenolic fraction, b.p. 119–120° (3.5 mm.) (15.0 g.), was dissolved in 50 ml. of dry pyridine, 20 g. of benzoyl chloride was added, and the mixture was heated on a steam-bath for one hour. Upon cooling, 50 ml. of saturated solution of sodium bicarbonate and 100 ml. of water were added, and the resulting mixture was extracted with 100 ml. of ethyl ether. The organic phase was washed in sequence with 100 ml. of a 50% saturated solution of sodium bicarbonate, 50 ml. of water, and 20% hydrochloric acid to remove the pyridine present. The ether solution was dried over sodium sulfate, filtered, and ether removed by distillation from a water-bath. Methanol (100 ml.) was added to the residue and then stored at −4° for three days. The resulting crystalline precipitate was filtered and washed with 10 ml. of methanol. The air-dried crystals, 16.7 g., had a melting point of 86.5–87.5°. An additional quantity (1.025 g.) of crystalline product was obtained by concentrating the mother liquid to 45 ml. and allowing it to stand overnight at −4°. Total yield of phenolic benzoate was 17.7 g. or 75% of the phenolic fraction (44% of total phenolic oil and 1.0% based on weight of dry wood). The methanol mother liquor, containing benzoates, was subsequently used for the isolation of the isomeric crystalline phenolic compound.

Hydrolysis of phenolic benzoates and recovery of phenols. The crystalline benzoate, m.p 85.5–86.5°, (8.0 g.) was added to a mixture containing 20 ml. of ethanol, 10 ml. of water, and 1.5 g. of sodium hydroxide. This solution was refluxed for one hour, cooled, diluted to 150 ml. with water, and extracted four times with 150-ml. portions of chloroform. The chloroform solution was dried over sodium sulfate, filtered, and the solvent removed by distillation. The residue was distilled, *in vacuo*, to give 4.30 g. (85%) of phenolic product, b.p. 155–156° (25 mm.), n_D^{20} 1.5272; $d_4^{24.5}$ 1.0325.

Anal. Calc'd for $C_{10}H_{13}O{\cdot}OCH_3$: C, 73.30; H, 8.95; OCH_3, 17.22.

Found: C, 73.21; H, 8.66; OCH_3, 16.64.

The methanol was distilled from the above methyl alcohol filtrates containing the non-crystalline benzoates. A solution of 1.5 g. of sodium hydroxide in 80 ml. of alcohol-water (1:1) was added to the residue and the resulting solution was refluxed for 2 hours on a steam-bath. The mixture was cooled, diluted with 100 ml. of water, acidified with dilute hydrochloric acid, and an excess of a saturated solution of sodium bicarbonate was added. This mixture was extracted three times with 35-ml. portions of chloroform and the combined chloroform solution was washed with 50 ml. of a saturated solution of sodium bicarbonate. The chloroform solution was dried over sodium sulfate, filtered and solvent removed by distillation on a steam-bath at 16–20 mm. to insure complete removal of solvents present. Petroleum ether (25 ml.) was added to the solvent-free residue and the resulting solution was allowed to stand several days at −4°. The separated crystals were filtered, washed with a little cold petroleum ether, and air-dried. A yield of 0.98 g. of crystals was obtained with m.p. 61–64°, which is equivalent to 6.5 per cent of the distilled phenolic fraction, b.p. 119–120° (3.5 mm.). This represents 1.75 per cent of the total phenolic oil or 0.04 per cent, based on the dry weight of the wood. The filtrate was concentrated, but no additional crystals formed. The product recovered was recrystallized from *n*-hexane to constant m.p. 66–67°.

Anal. Calc'd for $C_{10}H_{13}O{\cdot}OCH_3$: C, 73.30; H, 8.95; OCH_3, 17.22.

Found: C, 73.24; H, 8.83; OCH_3, 17.01.

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[1] All melting points are corrected, microanalyses by Microchemical Laboratory, University of California, Berkeley.

Oxidation of methoxyphenols to thymoquinone. The crystalline phenol (206 mg.) m.p. 66–67° was dissolved in 3 ml. of acetic acid and 1.3 g. of chromic oxide in 3 ml. of water was added. The reaction mixture was cooled in ice, allowed to stand 3 minutes, and poured into 100 ml. of water. Upon seeding the separated oil with a crystal of thymoquinone, it solidified to a yellow crystalline material. The crystals were filtered, washed with water, and air-dried to give 147 mg. (78.5%) of thymoquinone, m.p. 44.5–45.0°, which showed no depression in melting point when mixed with authentic thymoquinone [reported m.p. 45.5° (5)].

The liquid phenol (520 mg.) n_D^{20} 1.5272, when similarly oxidized, gave 390 mg. (82.3%) of thymoquinone.

The oxidation product was further identified as thymoquinone by reducing it with stannous chloride to hydrothymoquinone, m.p. 143–144°, undepressed on admixture with authentic sample [reported m.p. 141.5° (6)].

Various derivatives of the methoxyphenols. The aryloxyacetic and *p*-nitrobenzoate derivatives of the purified liquid phenol n_D^{20} 1.5272 were prepared in the usual manner. The melting point and analyses of each of these derivatives together with the above prepared benzoate derivative follow:

1. *Aryloxyacetic acid*, fine colorless needles from water, m.p. 132.8–133.6°.

Anal. Calc'd for $C_{13}H_{18}O_4$: C, 65.52; H, 7.61; N.E., 238.3.
Found: C, 65.81; H, 7.56; N.E., 245.

2. *p-Nitrobenzoate*, long yellow needles from alcohol, m.p. 127.6–128.6°.

Anal. Calc'd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.82; N, 4.25.
Found: C, 65.74; H, 5.85; N, 4.33.

3. *Benzoate*, white needles from methyl alcohol, m.p. 85.7–86.7°.

Anal. Calc'd for $C_{18}H_{20}O_3$: C, 76.03; H, 7.09.
Found: C, 75.88; H, 7.19.

The aryloxyacetic acid, *p*-nitrobenzoate, and *p*-bromobenzenesulfonate derivatives of the purified crystalline phenol, m.p. 66–67°, were likewise prepared in the usual manner. The melting points and analyses of each of these derivatives follow:

1. *Aryloxyacetic acid*, fine colorless needles from water, m.p. 122.2–122.6°.

Anal. Calc'd for $C_{13}H_{18}O_4$: C, 65.52; H, 7.61; N.E., 238.
Found: C, 65.86; H, 7.76; N.E., 236.

2. *p-Nitrobenzoate*, long yellow needles from alcohol, m.p. 125.3–125.7°.

Anal. Calc'd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.85; N, 4.25.
Found: C, 65.76; H, 5.62; N, 4.34.

3. *p*-Bromobenzenesulfonate, colorless needles from alcohol, m.p. 101.6–102.4°.

Anal. Calc'd for $C_{17}H_{19}BrO_4S$: C, 51.13; H, 4.80; Br, 20.01.
Found: C, 51.10; H, 4.95; Br, 20.21.

Synthesis of p-methoxythymol. The methyl ether of N-acetyl *p*-aminocarvacrol (3 g., 0.013 mole), m.p. 140.5–141.5°, [reported m.p. 140° (4)] was added to a mixture of 60 ml. of water and 6 ml. of conc'd sulfuric acid and then refluxed for 6½ hours. Upon cooling, a white precipitate of the methyl ether of *p*-aminocarvacrol hydrosulfate appeared. The mixture was diluted with 50 ml. of water, cooled to 5° and diazotized in the usual manner, by portionwise addition of 10% sodium nitrite solution to the point where the mixture, after allowing to stand for 5 minutes, gave a distinct color with starch-iodine paper. The reaction mixture was allowed to stand for an additional 5 minutes and the excess nitrous acid was destroyed by the addition of some sulfanilic acid.

Cupric sulfate pentahydrate (40 g.) was mixed with 40 ml. of water and heated to boiling under reflux. The above prepared solution of the diazotized amine was then slowly added over a period of 15 minutes to the cupric sulfate, maintaining a gentle boiling of the mixture. After all the solution had been added, the resulting mixture was refluxed for an additional 5 minutes, and the reaction product was steam-distilled until no oil came over. The residue remaining in the flask was a dark brown tar. The total distillate (400 ml.) was extracted 3 times with 60 ml. of *n*-hexane. The *n*-hexane solution was extracted twice with 60 ml. of 5% sodium hydroxide and washed with 30 ml. of water. The organic phase contained 0.48 g. of neutral material which was discarded. The combined aqueous extracts were acidified

with an excess of 18% hydrochloric acid and then extracted with 3 portions of 60 ml. of ethyl ether. The ether solution was washed with water, dried over sodium sulfate, and filtered. The ether was removed by distillation, and the phenolic oil was distilled, *in vacuo*, to give 1.19 g. (48.7%) of *p*-methoxythymol, b.p. 155–156° (25 mm.), n_D^{20} 1.5263.

The benzoate, *p*-nitrobenzoate, and aryloxyacetic acid derivatives of the synthetic product were prepared with m.p.'s 85.7–86.7°, 127.6–128.6°, and 132.8–133.6°, respectively. The mixture melting points with the corresponding derivatives prepared from the purified liquid methoxyphenol, b.p. 155–156° (25 mm.), n_D^{20} 1.5272, isolated from incense cedar heartwood remained unchanged.

Ultraviolet absorption spectrum of the naturally occurring *p*-methoxythymol in 95% ethyl alcohol was measured on a Beckman D. U. spectrophotometer. It exhibited a maximum at 291 mμ (log ε 3.578) and a minimum at 253 mμ (log ε 2.316). The synthesized *p*-methoxythymol had an identical spectrum.

Synthesis of p-methoxycarvacrol. The methyl ether of N-acetyl-*p*-aminothymol (3.0 g., 0.013 mole) m.p. 139–141.5° [reported m.p. 139° (4)] was diazotized and treated in the same manner as described above for the synthesis of *p*-methoxythymol. The crude semicrystalline residue of *p*-methoxycarvacrol, recovered from the ether extract of the steam-distillate after treatment with cupric sulfate pentahydrate, was recrystallized from 15 ml. of *n*-hexane at 0°, and the separated crystals were filtered. The recovered product (960 mg.) (42.5%) had a melting point of 67–67.5°, and the m.p. was not depressed when mixed with the crystalline phenol isolated from incense-cedar heartwood.

The aryloxyacetic acid and *p*-nitrobenzoate derivatives of the synthesized *p*-methoxycarvacrol were prepared, m.p.'s 122.5° and 125.5°, respectively. The mixture melting points with the corresponding derivatives prepared from the purified crystalline monomethoxy phenol, $C_{11}H_{16}O_2$, m.p. 66–67°, isolated from incense-cedar heartwood remained unchanged.

Ultraviolet absorption spectrum of the naturally occurring *p*-methoxycarvacrol in 95% ethyl alcohol showed a maximum at 291 mμ (log ε 3,590) and a minimum at 254 mμ (log ε 2.370). The synthesized *p*-methoxycarvacrol exhibited an identical absorption. The spectrum is almost identical with that of its isomer, *p*-methoxythymol.

Acknowledgement. The authors are indebted to the California Cedar Products Company for sponsoring a research grant supporting this investigation and to Mr. Charles P. Berolzheimer for his interest and cooperation.

SUMMARY

Two new isomeric phenolic compounds, $C_{11}H_{16}O_2$, have been isolated from incense-cedar heartwood. These compounds proved to be *p*-methoxythymol, 1.00 per cent dry wood basis, and *p*-methoxycarvacrol, 0.04 per cent dry wood basis, and their structures were established through synthesis. In addition to the interest from the taxonomic standpoint, preliminary bioassays against various wood destroying fungi indicated that each of these new isomeric phenols has fungicidal properties and thus they appear to be responsible, in part, for the decay resistance of incense-cedar heartwood.

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