The acetyl derivative, N-(7-nitro-3-fluorenyl)acetamide, prepared by the action of acetic anhydride in benzene, melted at 260–260.5° after recrystallization from 60% aqueous acetic acid. Ultraviolet spectrum: max. 230 (ϵ = 22,000), 260–265 (ϵ = 11,200) (inflection point), and 340 mμ (ϵ = 13,600); min. 219 (ϵ = 18,000), and 281 mμ (ϵ = 4,600).

Anal. Calcd. for $C_{15}H_{12}N_2O_3$: C, 67.15; H, 4.51. Found: C, 66.74; H, 4.69.

N-(7-Amino-3-fluorenyl)acetamide. Low pressure (40–50 p.s.i.) hydrogenation in ethanol over platinum oxide of 0.2 g. of the nitro derivative gave 0.12 g. of white crystals, m.p. 212.5–213.5°, after crystallization from 50% ethanol and from benzene. Ultraviolet spectrum: max. 219 (ϵ = 27,800), 251 (ϵ = 24,200), 290 (ϵ = 19,900), and 323 mμ (ϵ = 10,900) (inflection point); min. 236 (ϵ = 16,300), and 269 mμ (ϵ = 12,500).

Anal. Calcd. for $C_{15}H_{14}N_2O$: C, 75.60; H, 5.92. Found: C, 75.42; H, 6.02.

2,6-Fluorenediamine. Catalytic reduction (ethanol, platinum oxide) of 0.35 g. of 7-nitro-3-fluorenamine afforded 0.17 g. of an almost white product, m.p. 153–154.5°, after 2 crystallizations from 50% ethanol and one from benzene. Ultraviolet spectrum: max. 214 (ϵ = 25,800), 247 (ϵ = 11,800), 284 (ϵ = 13,500), and 300 mμ (ϵ = 9,400); min. 237 (ϵ = 9,800), 264 (ϵ = 7,800), and 316 mμ (ϵ = 7,200).

Anal. Calcd. for $C_{13}H_{12}N_2$: C, 79.56; H, 6.17. Found: C, 79.36; H, 6.40.

The *diacetyl derivative, N,N'-2,6-fluorenylenebisacetamide,* prepared with acetic anhydride in benzene, melted at 277–278°, after crystallization from ethanol. Ultraviolet spectrum: max. 217 (ϵ = 29,600), 253 (ϵ = 25,600), 280 (ϵ = 23,000), 289 (ϵ = 23,400), 309.5 (ϵ = 15,500), and 322 mμ (ϵ = 18,400); min. 234 (ϵ = 14,400), 267 (ϵ = 17,600), 283 (ϵ = 22,800), 304.5 (ϵ = 13,600), and 313 mμ (ϵ = 14,600).

Anal. Calcd. for $C_{17}H_{16}N_2O_2$: N, 10.00. Found: N, 9.62.

7-Nitro-3-fluorenol. The outcome of the reaction described below is materially affected by the experimental conditions. The best procedure was found to be as follows: 7-Nitro-3-fluorenamine (1 g.) was dissolved by warming in a mixture of 25 ml. of acetic acid and 10 ml. of water. A thick mush formed upon cooling in an ice bath and adding 25 ml. of concentrated sulfuric acid. A solution of 0.4 g. of sodium nitrite in 10 ml. of water was stirred in, causing most of the precipitate to dissolve. After another 1.25 hr., the diazonium solution was dropped over a period of 15 min. into a refluxing solution of 100 ml. of water, 10 ml. of sulfuric acid, and 20 ml. of acetic acid. The mixture was poured on ice after an additional 15 min. The crude yellow material, 0.95 g., m.p. 247°, was twice recrystallized from 65% ethanol (Norit) to give 0.59 g. of fine yellow needles, m.p. 257°. Ultraviolet spectrum: max. 271 (ϵ = 8,500), and 349 mμ (ϵ = 18,600); min. 251 (ϵ = 6,500), and 281 mμ (ϵ = 5,600).

Anal. Calcd. for $C_{13}H_9NO_3$: C, 68.72; H, 3.99; N, 6.17. Found: C, 68.45; H, 4.12; N, 6.39.

7-Amino-3-fluorenol. Catalytic reduction (platinum oxide) of 0.48 g. of 7-nitro-3-fluorenol in ethanol gave 0.41 g. of amine, m.p. 248° (dec.). Recrystallization of 65 mg. of product from 30 ml. of 50% aqueous ethanol yielded 60 mg. of small white needles, m.p. 255° (dec.). Ultraviolet spectrum: max. 212 (ϵ = 32,700), 243 (ϵ = 7,100) (inflection point), 281 (ϵ = 15,700), 302 (ϵ = 13,200), and 324 mμ (ϵ = 11,800); min. 253 (ϵ = 3,600), 295 (ϵ = 12,200), and 316 mμ (ϵ = 11,000).

Anal. Calcd. for $C_{13}H_{11}NO$: C, 79.16; H, 5.62. Found: C, 78.69; H, 5.60.

N-(6-Hydroxy-2-fluorenyl)acetamide. A hot solution of 0.4 g. of 7-amino-3-fluorenol in 50 ml. of 0.1*N* hydrochloric acid was filtered and cooled. After the addition of 3 g. of sodium acetate and 4 ml. of acetic anhydride the mixture was stirred in an ice bath for 5 hr. The white precipitate, 0.46 g., m.p. 238°, was recrystallized twice from 30% aqueous ethanol giving 0.34 g. of fine needles, m.p. 241°. Further crystallization from aqueous ethanol and xylene raised the melting point to 246°. Ultraviolet spectrum: max. 240 (ϵ = 10,500) (inflection point), 277 (ϵ = 23,100), 284 (ϵ = 23,400), 310 (ϵ = 18,000) (inflection point); and 322 mμ (ϵ = 22,200); min. 252 (ϵ = 5,600), 279 (ϵ = 22,900), and 295 mμ (ϵ = 14,800).

Anal. Calcd. for $C_{15}H_{13}NO_2$: C, 75.29; H, 5.48. Found: C, 75.31; H, 5.55.

BETHESDA 14, MD.

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

Extractive Components from Incense Cedar Heartwood (*Libocedrus decurrens* Torrey). VI. On the Occurrence of 3-Libocedroxythymoquinone

EUGENE ZAVARIN

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A new quinoid pigment has been isolated from the heartwood of incense cedar and its structure deduced on the basis of the presented spectroscopic, degradative, and synthetic experimental evidence. It has been demonstrated that the isolated quinone occurs *in situ* as well as forming through air oxidation of the other incense cedar extractives.

During our investigation of the extractive components of incense cedar heartwood, *Libocedrus decurrens* Torrey,[1] the petroleum ether extract of the sawdust was found to have a reddish color. This could not be caused by the formation of the quinquidrone type compounds between the various phenols present and thymoquinone[2] since it persisted upon removal of the latter either by distillation or by conversion to its semicarbazone followed by extraction with alkali. This indicated the presence of an unknown coloring matter which prompted us to investigate this component.

On extracting incense cedar heartwood sawdust with petroleum ether, a 1.5% yield (dry wood basis) of a mixture of various components was obtained. Chromatography on alumina yielded a red fraction

(1) E. Zavarin and A. B. Anderson, *J. Org. Chem.*, **20**, 788 (1955).

(2) E. Zavarin and A. B. Anderson, *J. Org. Chem.*, **20**, 82 (1955).

which, upon treatment with methanol and cooling, crystallized to yield 1.6% (0.024% dry wood basis) of a dark red coloring matter. The resulting material analyzed for $C_{32}H_{40}O_6$ and contained two methoxy groups. The molecular weight determined by Rast method agreed with the above formula. The C-methyl determination pointed to the presence of at least 6 methyls bonded to carbon.

The ultraviolet and infrared spectra of the compound (Figs. 1 and 2) were similar to those of

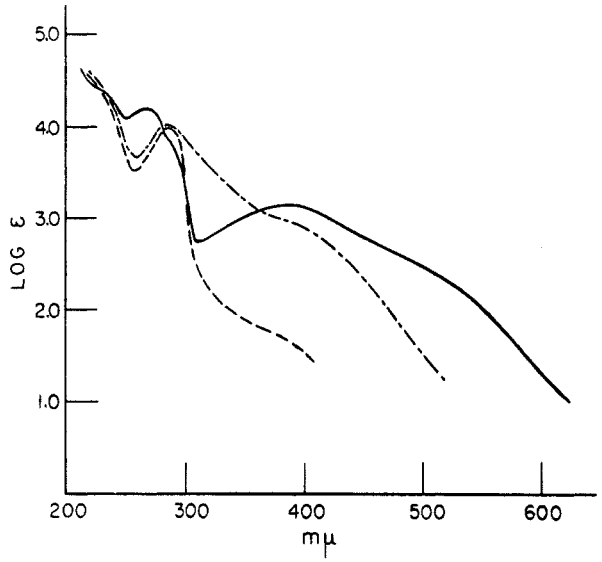


Fig. 1. Ultraviolet absorption spectra, ——— 3-libocedroxythymoquinone, - - - - hydro-3-libocedroxythymoquinone, — - - — epoxy-3-libocedroxythymoquinone

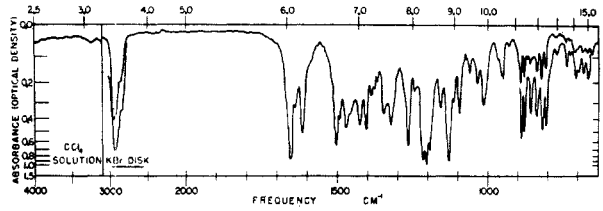


Fig. 2. Infrared spectrum of 3-libocedroxythymoquinone

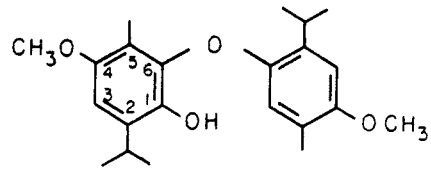
libocedroquinone.[1] The former showed three inflection points at 225, 285, and 450–510 mμ arising from the benzenoid K-band, benzenoid B-band,[3] and the quinoid C-band and two maxima at 267 and 389 mμ arising from the quinoid A and B bands.[4] The increase in intensity of the benzenoid K and B bands seem to indicate the increased proportion of the benzenoid chromophores in the molecule as compared with libocedroquinone. In the infrared, in addition to a weaker band at 1645 cm.$^{-1}$, the compound exhibited a strong carbonyl band at 1660 cm.$^{-1}$, and a strong double bond band at 1620 cm.$^{-1}$ No band corresponding to hydroxyl stretching could be detected.

(3) A. E. Gillam, E. C. Stern, and E. R. H. Jones, *Electronic Absorption Spectroscopy*, Edward Arnold Publishers, Ltd., London 1954, p. 116.

(4) E. A. Braude, *J. Chem. Soc.*, 490 (1945).

The compound is readily reduced to the colorless, amorphous hydroquinone by stannous chloride, sodium hydrosulfite, or catalytically with hydrogen over platinum, or palladium-on-charcoal. The resulting hydroquinone is again easily oxidized with ferric chloride to the original compound. The volume of hydrogen absorbed during hydrogenation corroborates the figure obtained for the molecular weight. The ultraviolet absorption spectrum of the hydroquinone shows a single benzenoid maximum at 286 mμ (Fig. 1). In infrared, a strong hydroxyl band is present but no carbonyl band.

This seems to substantiate the quinoid structure of the red compound and to indicate that the remaining two oxygens must be of an ether nature.

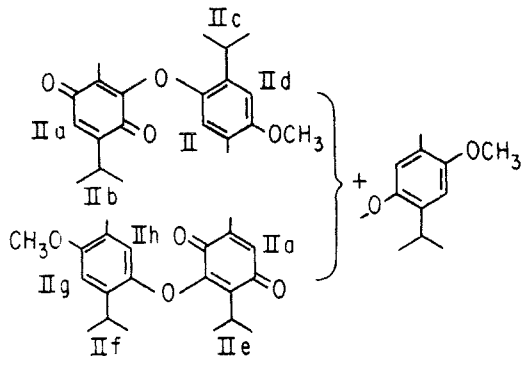


I

Further insight into the structure of the isolated quinone was obtained by the study of the reaction of libocedrol (I) with alkaline ferricyanide. The reaction gave a greenish-yellow solution which, upon evaporation, yielded a yellow amorphous solid. After shaking its ethyl ether solution with 10% hydrochloric acid, the organic phase assumed an intense red coloration and gave, in addition to a 19% yield of the starting material, 11% of *p*-methoxythymol, 4% of libocedroquinone, and 46% yield of a red quinone which was found to be identical with the quinone from the heartwood of incense cedar by mixed melting point and infrared techniques.

This synthesis, together with information obtained previously, seems to demonstrate that the red compound is composed of three units each derived from *p*-methoxythymol. One unit is present in the form of quinone, the other two units are benzenoid. The attachment seems to take place through ether linkages, since the carbon to carbon bonds would necessitate the presence of the free hydroxyls which is excluded on the basis of infrared evidence. This attachment must also involve the aromatic nuclei or, less likely, the tertiary positions on the isopropyl groups[5]; the isopropyl methyls are too far away and the attachment to the aromatic methyls is excluded on the basis of the C-methyl determination. The quinoid nucleus must carry at least one oxygen in addition to the carbonyl oxygens, since according to Braude's rules[4] for the positions of the absorption maxima in ultraviolet, the bathochromic shift of the quinoid B band is far too great to be caused by the alkyl substituents alone. All this leaves nine theoretically possible formulations for the red quinone.

(5) C. D. Cook, N. G. Nash, and H. R. Flanagan, *J. Am. Chem. Soc.*, **77**, 1783 (1955).



The application of the nuclear magnetic resonance methods[6] narrowed the number of possible structures still further. Several preliminary runs on model compounds including *p*-methoxythymol, libocedrol, benzoquinone, thymoquinone, 3-bromothymoquinone, and libocedroquinone indicated that absorption arising from the quinoid hydrogens falls in approximately the region of the absorption of benzenoid hydrogens. Contrary to the benzenoid state, the quinoid state promotes strong band splitting resulting from the spin-spin coupling of the quinoid hydrogens with the hydrogens in the α-position of the chain. Thus, in the case of thymoquinone, there resulted a split of quinoid maxima into 1:1 doublet (hydrogen in the 6 position), and into 1:3:3:1 quadruplet (hydrogen in the 3 position). Other quinones fell into the same line. This permits the differentiation between benzenoid and quinoid hydrogens and, in favorable cases, between the quinoid hydrogens.

The nuclear magnetic resonance spectrum of the unknown quinone together with the spectra of some of the model compounds are reproduced in Fig. 3. The spectrum bears a great similarity to the spectrum of libocedroquinone and shows two isopropyl doublets, one seems to result from the methyls of the two isopropyl groups on the benzenoid nuclei and the other from the methyls of the isopropyl group on the quinoid nucleus. This is followed by two peaks resulting from three aromatic methyls, a multiplet corresponding to the tertiary hydrogens of the isopropyl groups split by the corresponding hydrogens from methyl groups, and two peaks resulting from the hydrogens on two methoxy methyls. In the aromatic region, we find three maxima resulting from the hydrogens on the benzenoid nuclei and one quinoid doublet. The identification of the quinoid maximum eliminates formulation IIa which involves a completely substituted quinoid nucleus. The appearance of this maximum as a doublet instead as a 1:3:3:1 quadruplet seems to favor the quinone's being substituted in the 3 rather than in the 6 position; the latter needs further substantiation however, inasmuch as the resolution is not entirely satisfactory. The presence of the three benzenoid hydrogen peaks with 1:1:1 intensities seems to exclude the structures IIb, IIc, IIe, and IIf which possess four benzenoid hydrogens.

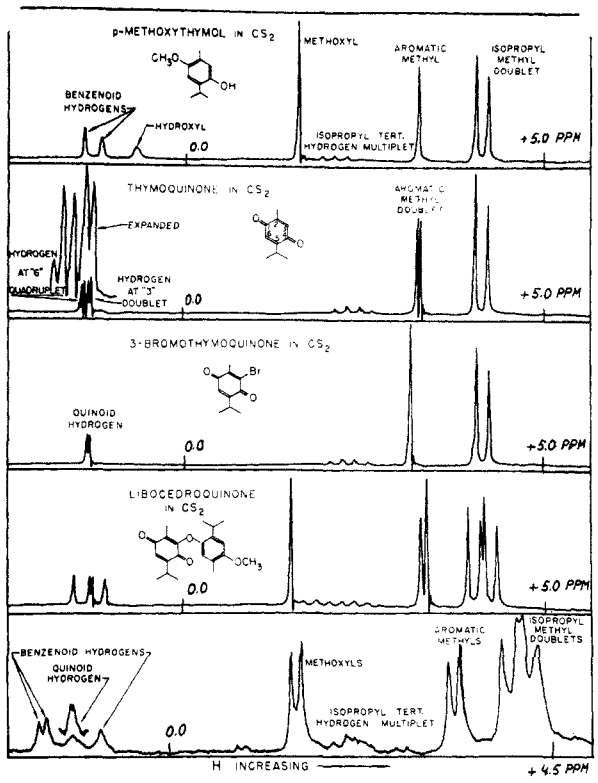
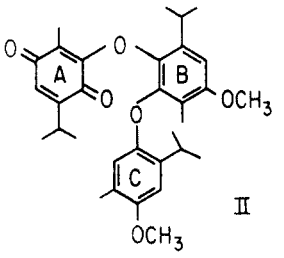


Fig. 3. Nuclear magnetic resonance spectra of various model compounds and 3-libocedroxythymoquinone. In the latter case the quinoid peak was resolved into doublet (upper curve) by using the heated sample in the experiment.

When the quinone was reduced to hydroquinone and heated under reflux with hydrogen bromide in acetic acid, a 22% yield of 3-hydroxythymoquinone was obtained upon oxidizing the resulting mixture with ferric chloride at room temperature. This excludes structures IIa, IIb, IIe, IIf, and IIg which should not yield this compound.

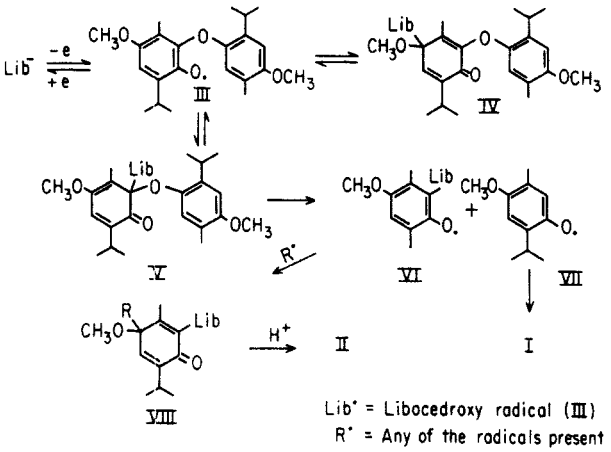
Of the remaining three structures, structure IIh appears to be rather improbable in view of the nuclear magnetic resonance indications of a free quinoid hydrogen in 6 position and also because in the hydrogen bromide cleavage, it would be expected to yield either 6-hydroxythymoquinone or a mixture of 6- and 3-hydroxythymoquinone, whereas pure 3-hydroxythymoquinone was obtained. As far as structure IId is concerned, the point of attachment of the third *p*-methoxythymol derived unit seems to indicate that the rather improbable meta coupling had taken place; with structure II, the corresponding attachment involves the same ortho linkage found in libocedrol. Thus, it would seem that the structure of the isolated pigment should correspond to formulation II.

(6) W. A. Anderson, *Phys. Rev.*, **102**, 151 (1956); J. T. Arnold, *Phys. Rev.*, **102**, 136 (1956); L. H. Meyer, A. Saika, and H. S. Gutowsky, *J. Am. Chem. Soc.*, **75**, 4567 (1953).



II

The nature of the synthetic sequence leading from libocedrol to 3-libocedroxythymoquinone can be rationalized in connection with the work on oxidation of the hindered phenols with alkaline ferricyanide done by Mueller, Cook, Waters, and others. The evidence obtained indicates that an equilibrium sets in between the ferricyanide and phenoxide ions on one side and ferrocyanide ion and phenoxy radical (III) on the other[7] with the latter again existing in equilibrium with its cyclohexadienone dimers[8] (IV, V) (depicted for libocedrol).



The yellowish, crude, amorphous oxidation product of libocedrol exhibited in infrared a strong carbonyl band at 1665 cm.$^{-1}$ while the intensity of the hydroxyl band decreased nearly fivefold; in ultraviolet in additon to the expected strong benzenoid B-band at 289 mμ, it exhibited two characteristic inflection points at 231 and 328 mμ. This is quite in agreement with the data given by Mueller for the oxidation dimers of some related compounds.[8] In the visible portion of the spectrum the material exhibited three peaks at 377, 397, and 650 mμ with the extinction coefficients increasing with dilution, temperature, or polarity of the solvent. This suggests the reversible dissociation of the material into colored components.

The electron paramagnetic resonance studies indicated the presence of unpaired electrons in the solid; the intensity of the signal markedly increased upon dissolving the material in chloroform. This seems to suggest the free radical nature of the dissociating compounds and substantiates further the depicted scheme.

Among the several possible cyclohexadienones existing in equilibrium with the libocedroxy radicals, structure V may dissociate into *p*-methoxythymoxy (VII) and 6-libocedroxy-*p*-methoxythymoxy radicals (VI) also. Once formed, the *p*-methoxythymoxy radicals will irreversibly dimerize to give libocedrol.[9] The 6-libocedroxy-*p*-methoxythymoxy radicals, on the other hand, should associate with the other radicals largely to give the corresponding 2,5-cyclohexadienones (VIII),[10] which, being ketals, should easily hydrolyze upon acidification to 3-libocedroxythymoquinone.[11] The preferential formation of libocedroquinone in ferric sulfate oxidation of libocedrol[1] can be explained by the acidic conditions forcing the formed, 2,5-cyclohexadienone to hydrolyze before *p*-methoxythymoxy substituent had time to exchange.

Oxidation of the isolated quinone with alkaline hydrogen peroxide resulted in formation of the corresponding quinone mono-epoxide which was stable to treatment with strong acids. It represents a convenient derivative for characterization of the compound.

Treatment of the quinone with semicarbazide hydrochloride did not result in formation of the semicarbazone. Libocedroquinone gave a very good yield of the adduct under these conditions. Originally this was held to support structure IIa. Examination of the molecular models revealed, however, that in the case of structure II, ring C with its side chains can also interfere sterically with the carbonyl of ring A.

Attempts to prepare the *p*-nitrobenzoate ester of hydro-3-libocedroxythymoquinone resulted in isolation of yellow materials that could not be induced to crystallize. Extension of the heating time converted the material into mixtures consisting largely of the original quinone.

In connection with isolation of 3-libocedroxythymoquinone from incense cedar heartwood, the question might be posed as to whether the compound is present in the wood, *in situ*, or whether it was formed during the process of isolation. To resolve this point, fresh green incense cedar heartwood was cut cross grain into three slabs. These slabs were immediately extracted with petroleum ether in nitrogen atmosphere and protected from direct light. The extract was stored under nitrogen at −5°. Chromatography of the extract gave a

(7) C. G. Haynes, A. N. Turner, and W. A. Waters, *J. Chem. Soc.*, 2829 (1956).

(8) E. Mueller, K. Ley, and G. Schlechte, *Ber.*, **90**, 2660 (1957); K. Ley, E. Mueller, and G. Schlechte, *Ber.*, **90**, 1530 (1957) and preceding papers.

(9) E. Zavarin and A. B. Anderson, *J. Org. Chem.*, **22**, 1122 (1957).

(10) The 2,5-cyclohexadienones should exist preferentially over 2,4-cyclohexadienones in the equilibrium mixture because of the known enhanced stability of the *p*-quinoid system and in this case, also, on sterical grounds.

(11) *p*-Methoxythymoxy radical could also participate in formation of various cyclohexadienones. The 2,5-cyclohexadienones would be expected to be particularly stable to redissociation. This would be one way of explaining the occurrence of *p*-methoxythymol in the reaction products.

fraction in which the above quinone could be identified. The amount of the quinone present was, however, considerably below that occurring in air-seasoned incense cedar heartwood used originally, indicating the possibility that the quinone might still form, in part, through air oxidation of the extractives during storage. Impregnation of the acetone-extracted Port Orford cedar sawdust with a mixture of libocedrol, *p*-methoxythymol, and thymoquinone, exposure of the sawdust for about three months to the action of air, extraction, and analysis of the extract resulted in identification of 3-libocedroxythymoquinone in the material obtained.

The importance in the determination of the structure of the 3-libocedroxythymoquinone is in its relationship to the other closely related materials isolated from incense cedar heartwood.[1,2,12] It extends the chain of compounds connected through the oxidative coupling mechanism. It is possible that, biosynthetically, the quinone is formed also by addition of libocedroxy radical to thymoquinone since quinones in general are known to undergo similar reactions.[13,14]

EXPERIMENTAL[15]

Isolation of 3-libocedroxythymoquinone. Sound air-dried heartwood from the butt log of incense cedar was ground in a Wiley mill to pass a 2-mm. screen. A total of 2900 g. of sawdust was extracted in Soxhlet extractors with petroleum ether to the point where no more coloring matter appeared. The petroleum ether extracts were combined and the solvent removed by evaporation on a steam bath to give 43 g. of a red viscous extract (1.65% yield, dry wood basis).

A 14.85-g. portion of the extract was dissolved in 50 ml. of *n*-hexane and chromatographed using 350 g. of alumina (Merck's acid-washed alumina, "suitable for chromatographic analysis"). The column was successively washed with 500 ml. of *n*-hexane, and 500 ml. of benzene. A portion of the benzene used produced a red-brown fraction that, upon removal of the solvent, weighed 7.7 g. This material was rechromatographed using 177 g. of the Woelm alumina. The column was successively washed with 250 ml. of *n*-hexane, 100 ml. of 10% benzene in hexane, 300 ml. of benzene, and four 100-ml. portions of 2, 5, 10, and 50% ether in benzene. The last 100 ml. of benzene and all of the subsequent fractions were reddish in color. They were combined and evaporated to dryness to give 2.6 g. of red viscous matter. The latter was dissolved in 20 ml. of methanol, and cooled to −5° for 10 days; the precipitate which formed was filtered, and washed with methanol to give 242 mg. of dark red crystalline material, m.p. 141–145° (1.63% yield from extract, 0.027% from wood). Further cooling produced no additional precipitate.

Purification of the material was achieved by repeated sublimation at 0.5–0.1 mm. pressure at 140–180° and crystallization from methanol, to give dark red crystals m.p. 154.5–155°.

Anal. Calcd. for $C_{32}H_{40}O_6$: C, 73.82; H, 7.74; OCH_3, 11.92; C—CH_3, 17.3; mol. wt., 521. Found: C, 73.84; H, 7.75; OCH_3, 12.05; C—CH_3, 14.0 (81%); mol. wt., 550 (camphor).

Libocedroquinone gave 82% and norlibocedroquinone 78% yield in the C—CH_3 analysis.[1]

The ultraviolet absorption spectrum of 3-libocedroxythymoquinone showed two maxima at 267 mμ (log ε 4.22) and 389 mμ (log ε 3.17) identified with Braude's A and B quinoid bands and three inflection points at 225 mμ (log ε 4.42), 285 mμ (log ε 3.88), and 450–510 mμ (log ε 2.8–2.3) identified with benzenoid K and B bands and quinoid C band[4] (Fig. 1).

In the infrared, using the potassium bromide pellet technique, the compound exhibited a strong carbonyl maximum at 1660 cm.$^{-1}$, a strong conjugated double bond maximum at 1620 cm.$^{-1}$, and a weaker band at 1645 cm.$^{-1}$ In carbon tetrachloride solution no hydroxyl band could be detected (Fig. 2).

The nuclear magnetic resonance spectra (Fig. 3) were obtained at 40 mc. frequency, using carbon disulfide as the solvent in case of *p*-methoxythymol, libocedrol, thymoquinone, 3-bromothymoquinone, and libocedroquinone and deuterated chloroform in case of 3-libocedroxythymoquinone. Due to unfavorable solubility characteristics, this compound produced a satisfactory spectrum only when the sample was heated during the experiment.

Hydrogenation of 3-libocedroxythymoquinone. The experiment was conducted in a micro apparatus at room temperature and atmospheric pressure using acetic acid as a solvent and platinum as a catalyst. The reaction was essentially over in 15 min., when the mixture became colorless and the absorption figure was equal to 523 mg. sample per mmole of hydrogen (calcd. 521 mg. sample per mmole of hydrogen). This fast uptake was followed by somewhat slower absorption which after 85 min. from the beginning of the reaction, lessened the above figure to 480 mg. of sample per mmole of hydrogen and was probably due to some side reactions.

Reduction of 3-libocedroxythymoquinone with stannous chloride. A 164-mg. sample of 3-libocedroxythymoquinone (m.p. 153–154°) was dissolved in 5 ml. of ethanol. To the resulting solution 0.5 g. of stannous chloride dihydrate was added and the mixture was heated on a steam bath until colorless. This required 15 to 20 min. To this material 25 ml. of petroleum ether was added followed by 10 ml. of 10% hydrochloric acid. The organic phase was separated and the aqueous phase washed with 10 ml. of petroleum ether. The organic extracts were combined, dried over anhydrous sodium sulfate, filtered, evaporated to dryness, and the residue kept in a desiccator evacuated to 0.1 mm. pressure for several days to remove the rest of the solvent. The material represented a fluffy, amorphous solid which weighed 150 mg. (91% yield). It did not possess a sharp melting point but slowly sintered to a thick liquid between 40 and 50°. It slowly oxidized in air assuming a red color.

Anal. Calcd. for $C_{32}H_{42}O_6$: C, 73.53; H, 8.10. Found: C, 73.05; H, 8.00.

In ultraviolet it exhibited a maximum at 286 mμ, identified with the benzenoid B band and an inflection point at 220 mμ identified with benzenoid K band. In infrared, using potassium bromide technique, it did not show the carbonyl stretching band; in carbon tetrachloride solution, it exhibited a strong band corresponding to the OH vibration at 3520 cm.$^{-1}$, and a somewhat weaker one at 3600 cm.$^{-1}$

Oxidation of the hydro-3-libocedroxythymoquinone with ferric chloride. A 37-mg. portion of 3-libocedroxythymoquinone (m.p. 154–155°) was dissolved in 2 ml. of acetic acid to which 100 mg. of stannous chloride dihydrate was added,

(12) E. Zavarin and A. B. Anderson, *J. Org. Chem.*, **20**, 443 (1955).

(13) F. J. L. Aparicio and W. A. Waters, *J. Chem. Soc.*, 4666 (1952).

(14) L. F. Fieser and A. E. Oxford, *J. Am. Chem. Soc.*, **64**, 2060 (1942).

(15) All melting points are corrected; microanalysis by Microchemical Laboratory, University of California, Berkeley. Ultraviolet and infrared spectra were run on Beckman DK II and Perkin Elmer Model 21 recording spectrophotometers, respectively, and NMR and EPR spectra on Varian Associates spectrometers.

and the resulting mixture was heated on a steam bath until the red color disappeared. To this solution 1.0 g. of ferric chloride was added and the material heated over steam for 1 min. The resulting liquid was diluted with 30 ml. of water and extracted with 20 ml. of chloroform. The organic extract was dried over anhydrous sodium sulfate, filtered, evaporated to dryness, and the residue crystallized from 2 ml. of methanol to give 28 mg. (76%) of red crystals, m.p. 154–155° that did not depress the melting point of the original 3-libocedroxythymoquinone.

p-Nitrobenzoylation of 3-libocedroxythymoquinone. Hydro-3-libocedroxythymoquinone (101 mg.) was dissolved in 3 ml. of pyridine and 0.3 g. of *p*-nitrobenzoylchloride was added. This mixture was heated on a steam bath for 5 min. whereupon it acquired a yellow coloration, and was allowed to stand overnight at room temperature. To the resulting material 25 ml. of 10% sodium carbonate solution and 25 ml. of ethyl ether was added; the aqueous phase was separated and the ether washed with 10 ml. of 10% sodium carbonate followed by 50 ml. of 10% hydrochloric acid in two portions. The ether extract was dried over sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved in 10 ml. of iso-octane, filtered from 5 mg. of some insoluble impurities, and cooled. At this point the ester separated in the form of very fine droplets with unsharp melting point. Attempts to induce crystallization failed. Removal of iso-octane by evaporation on a steam bath and later under vacuum gave an amorphous yellow residue weighing 139 mg.

Repeating the reaction using 52 mg. of hydro-3-libocedroxythymoquinone but with the heating period increased to 90 min., crystallization of the reaction product from methanol-water resulted in the separation of 13 mg. of impure 3-libocedroxythymoquinone, m.p. 145–150° (25% yield).

Attempted preparation of 3-libocedroxythymoquinone semicarbazone. A 103-mg. portion of 3-libocedroxythymoquinone, m.p. 153.5–154.5°, was refluxed on a steam bath in 10 ml. of methyl alcohol with 0.5 g. of semicarbazide hydrochloride and 2 drops of concentrated hydrochloric acid for 5 min. The resulting mixture was then cooled and filtered, and the solid was washed with 10 ml. of hot methanol. To the filtrate was added 20 ml. of water and the separated precipitate filtered and recrystallized from 10 ml. of methanol to give 60 mg. (58%) of 3-libocedroxythymoquinone, m.p. 152.5–153.5° undepressed on admixture with authentic material.

Reaction of 3-libocedroxythymoquinone with alkaline hydrogen peroxide. A 50.6-mg. portion of 3-libocedroxythymoquinone, m.p. 154–155° was dissolved in 5 ml. of acetone to which 0.5 g. of potassium carbonate was added. To this mixture 1 ml. of 30% superoxol was added and then refluxed for 30 min. on a steam bath during which time the color of the solution changed from reddish to yellow. Most of the acetone was removed from the solution by evaporation and the residue diluted to 10 ml. with water. The resulting material was extracted once with 15 ml. of petroleum ether and once with 15 ml. of ethyl ether, and the combined organic extracts dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The residue was dissolved in 7 ml. of methanol, filtered from a small amount of white impurity and the epoxide crystallized by addition of water and cooling to −5°. The reaction product represented a yellow powder, m.p. 124–126°, and weighed 33 mg. (67%).

Further purification was achieved by repeated crystallizations from iso-octane and methanol-water whereupon the melting point was raised to 127–128°.

Anal. Calcd. for $C_{32}H_{40}O_7$: C, 71.62; H, 7.51. Found: C, 71.82; H, 7.71.

In the ultraviolet the compound exhibited an absorption maximum at 285–286 mμ, log ε 4.04 and an inflection point at 370–400 mμ, log ε 3.03–289. In the infrared, in carbon tetrachloride solution, and in the 4000–3000 cm.$^{-1}$ region it did not show an OH stretching band. Pressed into a KBr pellet it exhibited a conjugated double bond band at 1615 cm.$^{-1}$, and two carbonyl maxima at 1675 and 1700 cm.$^{-1}$

Reaction of the 3-libocedroxythymoquinone with hydrobromic acid. A 91.5-mg. portion of 3-libocedroxythymoquinone, m.p. 153–154°, was dissolved in 7 ml. of glacial acetic acid. This solution was heated on a steam bath while stannous chloride dihydrate was added in very small portions to the point of discoloration. To this mixture, 4 ml. of 40–42% hydrobromic acid containing a few small crystals of stannous chloride was added, and the solution was refluxed for 2 hr. Upon cooling, ferric chloride was added to the solution to the point at which the mixture assumed a yellow color. The resulting liquid was diluted with 25 ml. of water and extracted with 25 ml. of ethyl ether in two portions. The organic extract was shaken with 50 ml. of 10% ammonia in three portions, whereupon the aqueous solution assumed an intense violet color. Upon acidification with hydrochloric acid, the aqueous phase was extracted with 10 ml. of ethyl ether in two portions, the extract dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The yellow residue was purified by sublimation at 3 mm. and 100° to give 15 mg. of yellow crystals, m.p. 167.5–168° (sealed capillary), undepressed on admixture with an authentic sample of 3-hydroxythymoquinone. This represents a 24% yield assuming complete cleavage of all ether linkages in the molecule. The infrared absorption spectrum of the yellow material, obtained by using the potassium bromide technique, was found to be identical with that of 3-hydroxythymoquinone. Aside from the strong chelated OH absorption at 3240 cm.$^{-1}$, the compound exhibited a conjugated double bond maximum at 1612 cm.$^{-1}$, and two bands at 1640 and 1665 cm.$^{-1}$ corresponding to the chelated and nonchelated quinoid carbonyl stretching. The split of the quinoid carbonyl absorption band into two bands due to the neighboring hydroxyl has also been noted in the case of various hydroxylated anthraquinones.[16]

Reaction of libocedrol with alkaline ferricyanide. A 3.0-g. portion of libocedrol, m.p. 86.5–88°, was dissolved in 30 ml. of ethyl ether and stirred for 1 hr. with a solution of 6 g. of potassium ferricyanide and 1.6 g. of sodium hydroxide in 60 ml. of water, whereupon the ether phase became green in color. The organic phase was washed with 200 ml. of water in four portions and shaken with 50 ml. of 10% hydrochloric acid, the liquid becoming dark red. The ether solution was separated, dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. The residue was dissolved in 25 ml. of methanol and allowed to crystallize overnight at −5°. The combined aqueous extracts and washings were acidified with hydrochloric acid and extracted with ether. Upon drying, filtering, and evaporation of the solvent, the extract left only a negligible residue.

The precipitated material was filtered, washed with methanol, and dried to give 994 mg. of dark red crystals, m.p. 142–147° (45.5% yield). Recrystallization of the red crystals from methanol raised the melting point to 153–154°. The material was found to be identical with the naturally occurring 3-libocedroxythymoquinone by mixed melting point technique, and by comparison of the infrared spectra using the compounds themselves and their epoxy derivatives.

The mother liquors from the crystallization of 3-libocedroxythymoquinone were combined and evaporated to dryness. The residue was dissolved in 20 ml. of pyridine and 2.0 g. of *p*-nitrobenzoyl chloride was added. The resulting liquid was heated on a steam bath for 20 min., diluted with 40 ml. of 5% sodium carbonate solution, and extracted with 60 ml. of ethyl ether. The organic phase was washed with 60 ml. of water, followed by 80 ml. of 10% hydrochloric acid, dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. The residue was crystallized from 20 ml. of methanol, the precipitate was filtered and recrystallized from methanol-acetone to give the first crop of 806

(16) M. St. C. Flett, *J. Chem. Soc.*, 1441 (1948).

mg., m.p. 171–171.5°. The filtrate was evaporated to dryness and crystallized from ethanol to give 297 mg. of a material melting at 116–120°. Recrystallization of the first crop from ethanol-acetone raised the melting point of the material to 173–174°, undepressed on admixture with the original libocedrol *p*-nitrobenzoate. The amount isolated accounts for 19% of the libocedrol used.

The second crop was recrystallized from *n*-hexane to give a material melting at 126–127.5°. The melting point was undepressed on admixture with an authentic *p*-methoxythymol *p*-nitrobenzoate. The amount of the material isolated accounts for 11% of the libocedrol used.

One half of the residue of the *p*-nitrobenzoate crystallization was distilled at 1 mm. pressure and 200°. The distillate was collected and crystallized from methanol to give a mixture of dark red crystals and some reddish material. The crystals were separated by virtue of their solubility in 10 ml. of iso-octane. Evaporation of the iso-octane left a residue that was recrystallized several times from methanol to give 58 mg. of a material melting at 93–94.5°. Admixture with the authentic libocedroquinone did not depress the melting point. The amount isolated accounts for 4% of the original libocedrol.

In another similar experiment using 0.704 g. of libocedrol in 50 ml. of *n*-hexane, 20 ml. of 2.5% sodium hydroxide solution, and 20 ml. of 2% potassium ferricyanide solution, the reaction was conducted in nitrogen atmosphere using deaerated water. After 30 min. of stirring, the organic phase became yellowish green and about as intensive in color as when the reaction was conducted in presence of air. Allowing air into the system did not produce any color change. The yield of the 3-libocedroxythymoquinone was 47%.

In another similar experiment, 550 mg. of libocedrol, m.p. 86.5–88°, was dissolved in 50 ml. of ethyl ether and stirred for 1 hr. with a solution of 2.5 g. of potassium ferricyanide in 50 ml. of 5% sodium hydroxide solution. The organic phase was washed thoroughly with water, dried over sodium carbonate, filtered, and evaporated to dryness on a steam bath. The last traces of solvent were removed in 0.1 vacuum at room temperature. The resulting material was slightly yellow, amorphous, fluffy solid. It weighed 539 mg. (98%) and melted, not very sharply, at 45–50°.

The substance was soluble in most of the organic solvents. It dissolved in petroleum ether, and similar hydrocarbon solvents with a yellow-green color, and in more polar solvents such as chloroform or acetone, with a green color. Heating intensified the green color and cooling decreased its intensity.

The infrared absorption spectrum obtained by potassium bromide technique was found to be similar to that of 3-libocedroxythymoquinone, with a carbonyl band at 1665 $cm.^{-1}$, a band at 1640 $cm.^{-1}$, and a conjugated double bond band at 1615 $cm.^{-1}$; the intensity of all three bands was, however, appreciably less than with the quinone. In carbon tetrachloride solution a hydroxyl stretching band was present at the same position as with libocedrol (3550 $cm.^{-1}$) but with intensity reduced nearly five times. In the ultraviolet region between 220 and about 330 mμ, in methylcyclohexane solution, the material seemed to obey Beer's law. It showed a benzenoid B-band with λ_{max} 289, log ϵ = 4.07 and λ_{max} 265 mμ, log ϵ = 3.82 and two inflection points at 231 and 328 mμ with log ϵ 4.57 and 3.15, respectively, assuming the molecular weight of the dimer of the libocedroxy radical for the calculation. Above about 330 mμ Beer's law was not obeyed. The nature of the spectrum was found to be dependent on the concentration and the solvent used. In methylcyclohexane solution, the material had the absorption maxima at 377, 397, and 650 mμ and in chloroform solution at 387, 402, and 650 mμ. The absorbance decreased less than proportional to the dilution and with about the same concentration of the solute was several times larger in chloroform. Thus, with methylcyclohexane $E^{1\%}_{1\,cm.}$ was 4.8 and 4.2 at a concentration of 0.51 g./l. and 3.6 and 3.1 at a concentration of 2.2 g./l. for the first two bands mentioned. With chloroform the respective values were 10.5 and 12.2 at a concentration of 0.50 g./l. and 7.8 and 8.7 at a concentration of 2.1 g./l. The third, 650 mμ band ($E^{1\%}_{1\,cm.}$ 0.66, cond. 2.1 g./l., chloroform as the solvent) behaved similarly in all respects. The absorbance tended to decrease with time particularly when chloroform was used as the solvent; with the more concentrated solution in the above experiment, the E-values decreased to 8.4 and 9.3 in 10 min.

In the electron paramagnetic resonance measurements a strong signal was obtained using 8.4 mg. of the solid material; dilution with a few drops of chloroform intensified the signal roughly 10 times (g about 2.0, line width around 8 gauss).

Determination of the presence of 3-libocedroxythymoquinone in fresh incense cedar heartwood. From fresh moist incense cedar heartwood, three cross grain slats with combined weight of 307 g. (dry weight) were prepared (25 × 6 × 1.8 cm.). All surfaces were freshly cut and cleaned by rubbing with a cloth moistened in acetone. The slats were extracted under nitrogen atmosphere with petroleum ether, b.p. 30–60° for 15 hr. From the extract most of the solvent was removed by evaporation on a steam bath and the remainder under vacuum at room temperature. The residue weighed 10.6 g. (2.9%) and was stored under nitrogen atmosphere at −5°.

A 9.2-g. portion of the above residue was treated with 50 ml. of cold *n*-hexane and filtered from the separated libocedrol/*p*-methoxythymol complex (2.4 g.), m.p. 91–92°. The filtrate was chromatographed using 50 g. of Woelm alumina. The red fraction which came with *n*-hexane was evaporated to dryness, dissolved in 20 ml. of *n*-hexane, and allowed to stand for 3 days at −5°, then was filtered from 1.65 g. of separated libocedrol/*p*-methoxythymol complex, m.p. 88–91°, and rechromatographed three times using 50-g. portions of Woelm alumina. In the latter case, a 200-ml. portion of 40% ethyl ether in *n*-hexane brought a red fraction that was evaporated to dryness. Spectroscopic examination of the residue revealed the presence of about 10 mg. of 3-libocedroxythymoquinone (0.004% of the dry wood weight). Crystallization from methanol yielded 1.0 mg. of the above compound, m.p. 152–153° undepressed on admixture with authentic sample.

Formation of 3-libocedroxythymoquinone by air oxidation. One hundred grams of acetone-extracted Port Orford cedar sawdust was mixed with a solution of 2.0 g. of libocedrol/*p*-methoxythymol addition complex and 0.1 g. of thymoquinone in 100 ml. of acetone and the solvent removed by evaporation. The prepared sawdust was spread on the paper and allowed to stand for 80 days with an air current gently blowing over it. Extraction of the sawdust with acetone for 8 hr., evaporation of the solvent, chromatography of the residue, and crystallization of the appropriate fraction from methanol gave 11 mg. of 3-libocedroxythymoquinone m.p. 145–148°. Further crystallization from the same solvent raised the melting point to 151–152° which remained undepressed upon admixture with an authentic sample of the quinone.

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