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

EXTRACTIVE COMPONENTS FROM INCENSE-CEDAR HEARTWOOD (*Libocedrus decurrens* Torrey). III. OCCURRENCE OF LIBOCEDROL, A NEW PHENOL ETHER, AND ITS *p*-METHOXYTHYMOL ADDITION COMPLEX

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In continuing our investigation of the nature of the extractive components present in the incense-cedar heartwood, *Libocedrus decurrens* Torrey, a considerable quantity of a crystalline chemical product was isolated in 1.38 per cent yield from the acetone extract (1, 2). When a petroleum ether solution of this material was extracted with sodium hydroxide, *p*-methoxythymol, $C_{11}H_{16}O_2$, (2) was isolated from the alkaline solution, while an unknown crystalline compound, $C_{22}H_{30}O_4$, was recovered from the petroleum ether solution. These compounds were recovered in approximately equimolal quantities. When each of these compounds was brought together in petroleum ether, the original compound readily crystallized from solution. This suggested that the crystalline product, as obtained from the extract, was an addition complex. This conclusion was further substantiated, for upon determining the molecular weight of the original product by the Rast camphor method, a value was obtained which was slightly less than one-half the calculated value. This behavior as found later, proved to be due to dissociation of the above material in camphor solution into *p*-methoxythymol and the unknown compound, $C_{22}H_{30}O_4$.

The compound $C_{22}H_{30}O_4$, which we propose to name libocedrol, is optically inactive and contains two methoxyl groups. The third oxygen atom was identified as a hydroxyl group through the preparation of mono-benzoate derivatives. Libocedrol is readily oxidized by potassium permanganate in pyridine; it reacts with bromine in carbon tetrachloride with evolution of hydrogen bromide, and it couples with diazotized amino compounds. With alcoholic ferric chloride it gives a red color, but it does not dissolve even in highly concentrated sodium hydroxide solutions. These reactions indicate that libocedrol is a cryptophenolic compound.

The ultraviolet absorption spectrum of libocedrol exhibited a single maximum at 287 mμ, being very similar to the absorption spectra of *p*-methoxythymol and *p*-methoxycarvacrol (Fig. 1) (2). In the infrared, it exhibited a sharp maximum at 3550 cm.$^{-1}$, corresponding to OH stretching and two benzenoid maxima at 1600 and 1620 cm.$^{-1}$, but no carbonyl band (Fig. 2) (3).

The similarity of the ultraviolet spectra of libocedrol and hydrothymoquinone monomethyl ethers and the nature of the infrared spectrum of the former, together with its molecular formula, strongly suggest that the structure of libocedrol is that of two molecules of either *p*-methoxythymol or *p*-methoxycarvacrol joined with removal of two hydrogens in a way that no strong resonance interaction takes place between the two benzene nuclei.

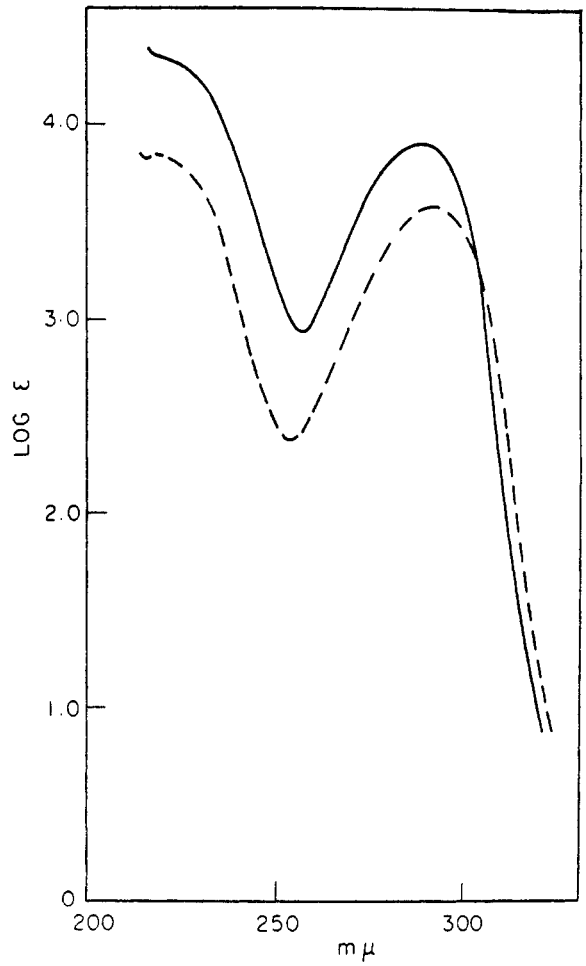


FIG. 1. ULTRAVIOLET ABSORPTION SPECTRA OF LIBOCEDROL (———) AND *p*-METHOXYTHYMOL (- - -)

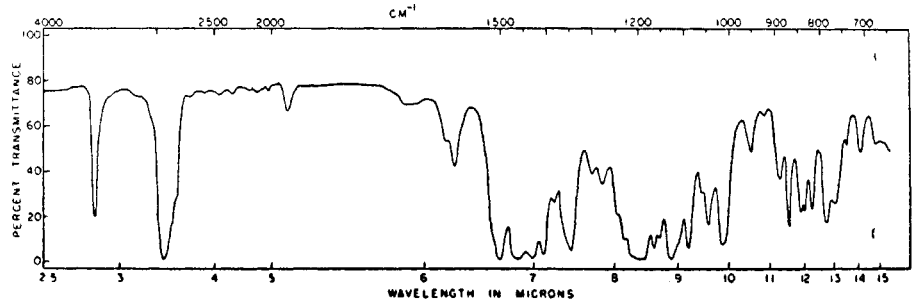


FIG. 2. INFRARED ABSORPTION SPECTRUM OF LIBOCEDROL

Upon oxidation of the libocedrol with ferric sulfate in alcohol solution, a quinone, $C_{21}H_{26}O_4$, libocedroquinone, containing one methoxyl was obtained, an oxidative demethylation having removed one of the methoxyls. In the ultraviolet, this quinone exhibited three maxima at 259, 351–360, and 460 mμ, identified with Braude's A, B, and C quinoid bands and a benzenoid deflection point at 284–285 mμ, resulting from the absorption of the unoxidized portion of the molecule (Fig. 3) (4). In applying Braude's rules for the position of the absorption maxima, it was possible to formulate that the quinoid part of the molecule should contain at least one oxygen, in addition to the two carbonyl oxygens. In the infrared, the quinone exhibited no band corresponding to OH stretching; it possessed, however, two strong bands corresponding to conjugated carbonyl and conjugated double-bond stretching at 1665 and 1620 cm.$^{-1}$, respectively (Fig. 4) (3).

This additional spectroscopic evidence, together with the information pre-

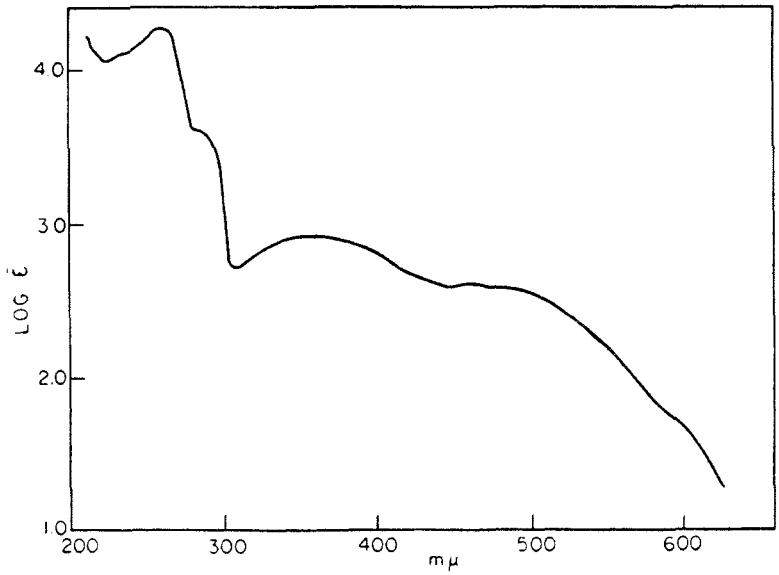


FIG. 3. ULTRAVIOLET ABSORPTION SPECTRUM OF LIBOCEDROQUINONE

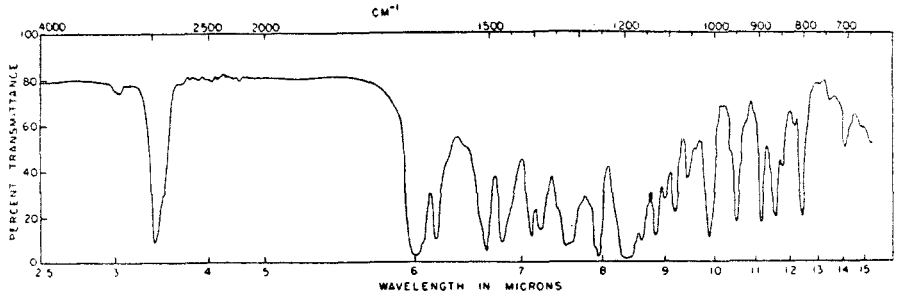
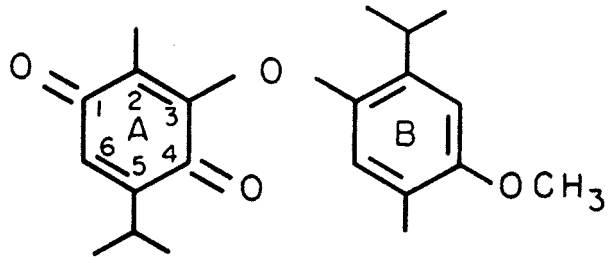


FIG. 4. INFRARED ABSORPTION SPECTRUM OF LIBOCEDROQUINONE

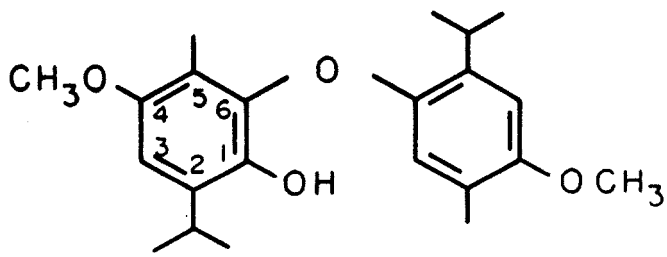
viously obtained, seems to indicate that libocedrol has the structure of a dehydrodihydrothymoquinone monomethyl ether of the phenyl ether type.

Oxidation of libocedroquinone with alkaline hydrogen peroxide resulted in the oxidative destruction of the quinoid nucleus, with *p*-methoxythymol being isolated in 48% yield from the reaction mixture (5). This identifies the nonquinoidal part of the libocedroquinone molecule as being *p*-methoxythymol.

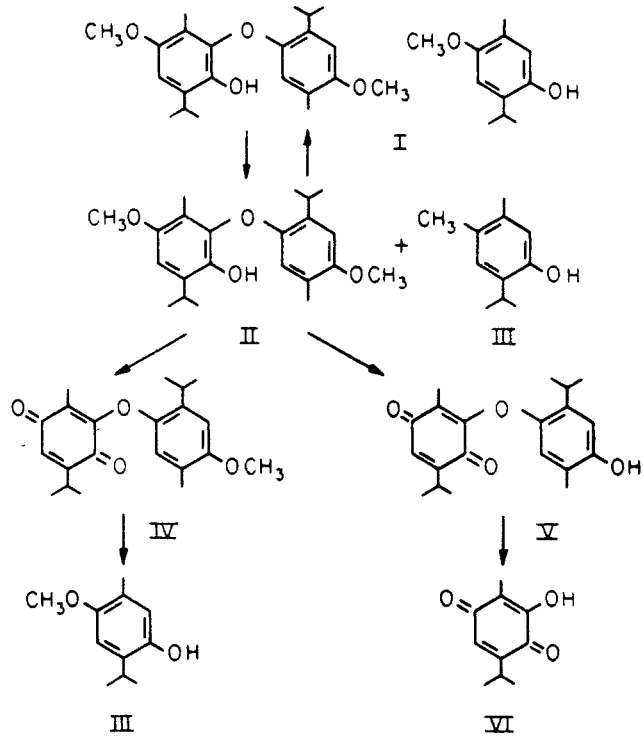
Demethylation of libocedrol by refluxing in a mixture of 48 per cent hydrobromic acid and acetic acid, followed by oxidation of the resulting mixture with cupric acetate produced a quinoid compound, $C_{20}H_{24}O_4$, norlibocedroquinone. Pyrolysis of the latter produced 3-oxythymoquinone, $C_{10}H_{12}O_3$, in 39 per cent yield, identified by a mixture melting point with synthetically prepared 3-oxythymoquinone (6) and its aniline derivative (7). The same oxy-quinone was obtained, in lower yield, however, when norlibocedroquinone was oxidized with chromic acid in acetic acid solution. The identification of 3-oxythymoquinone determines the nature of the quinoid portion of the libocedroquinone and establishes the structure of the latter compound as being:



Since 3-oxythymoquinone could be derived from either *p*-methoxythymol or *p*-methoxycarvacrol, it was next necessary to ascertain which one of these isomeric phenols represents part A of libocedrol. It is well known that dehydrogenation of phenols by oxidases frequently causes the formation of coupled products such as phenol ethers (8). Since enzymatic reactions of this type take place in the *o*- or *p*-position to the hydroxyl, it would strongly suggest that part A of libocedrol must correspond to *p*-methoxythymol rather than *p*-methoxycarvacrol, since the latter would not conform to the structure for libocedroquinone. Thus libocedrol appears to be a dehydrodi-*p*-methoxythymol or 6-*p*-methoxythymyl-*p*-methoxythymol.



The various reactions and structure of compounds discussed may be summarized as follows:



I Libocedrol · p-methoxythymol complex
II Libocedrol
III p-Methoxythymol
IV Libocedroquinone
V Norlibocedroquinone
VI 3-Oxythymoquinone

The determination of the structure of libocedrol and its addition complex 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, namely, *p*-methoxythymol, *p*-methoxycarvacrol, carvacrol, hydrothymoquinone, and thymoquinone (1, 2).

EXPERIMENTAL[1]

Extraction of wood. The acetone extraction of a composite sample of sound incense-cedar heartwood sawdust (8,640 g. dry basis) to yield 1547 g. of crude extract (17.9% yield dry basis) was previously reported (1).

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

Isolation of libocedrol-p-methoxythymol addition complex. The dark red-brown acetone-soluble extract (1547 g.) was thoroughly triturated with 700 ml. of chloroform. The resulting insoluble material was removed by filtration and washed with chloroform. The brown precipitate was again extracted with 1000 ml. of chloroform, filtered, and washed with the solvent leaving 506 g. of brown powdery, largely phlobaphenic material (5.8% dry wood basis). The chloroform filtrates were combined and the solvent was removed by distillation from a water-bath. The residual, tarry mixture was next triturated with 700 ml. of petroleum ether and the petroleum ether was decanted. This extraction procedure was repeated seven times using 250-ml. portions of petroleum ether. The petroleum ether extracts were combined and most of the solvent was removed on a water-bath. Upon permitting the concentrated petroleum ether extract to stand at room temperature, crystals gradually deposited. After one week, the crystals were removed by filtration and the filtrate upon dilution to 200 ml. with petroleum ether was allowed to stand at −5° for one week after being inoculated with the crystalline material. The additional crystals which formed were filtered and washed with petroleum ether. A total of 120 g. of fine white crystals was recovered (1.38% yield on dry weight of wood basis). Recrystallization from isoöctane gave 92.4 g. of colorless needles, m.p. 91.5–92.0°, the melting point of which remained constant after repeated recrystallizations.

Anal. Calc'd for $C_{33}H_{46}O_6$: C, 73.57; H, 8.61; OCH_3, 17.28.

Found, C, 73.77; H, 8.94; OCH_3, 17.04.

Mol. wt. (camphor) Calc'd: for undissociated compound, 538; for dissociated compound, 269.

Found: 256 ± 10%.

Cleavage of addition complex into libocedrol and p-methoxythymol. The addition complex, m.p. 91.5–92.0° (34.2 g.) was taken up in 250 ml. of petroleum ether (b.p. 30–70°) and extracted with 450 ml. of 10% sodium hydroxide solution in several portions. The petroleum ether solution was dried over sodium sulfate, filtered, and the volume of the filtrate reduced to 100 ml. on a water-bath. The solution was stored at −5° and the resulting crystalline precipitate filtered. The filtrate was reduced in volume, stored at −5°, and an additional crop of crystals was obtained The total yield of libocedrol was 19.74 g. (87%) m.p. 86.5–88°. Recrystallization from petroleum ether did not raise the melting point of the material.

Anal. Calc'd for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44; OCH_3, 17.32.

Found: C, 73.81; H, 8.47; OCH_3, 16.74.

The above alkaline solution was acidified with an excess of concentrated hydrochloric acid and extracted with a total of 150 ml. of petroleum ether. The combined petroleum ether extract was dried over sodium sulfate, filtered, the solvent removed by evaporation, and the residual oil distilled with the main portion going over between 149.5 and 153.5° at 19 mm., n_D^{21} 1.5278. An oil was recovered amounting to 10.25 g. (90% yield), which was identified as *p*-methoxythymol (2).

Properties and characterization of libocedrol. Libocedrol, m.p. 87–88°, (372 mg.), and *p*-methoxythymol n_D^{20} 1.5272 (184 mg.) were added to 15 ml. of petroleum ether and the whole was heated until solution was complete. The volume of the resulting mixture was reduced to 4 ml. on a water-bath and the solution was cooled on ice. Upon seeding, the addend crystallized and was filtered and dried to give 515 mg. (93% yield) of crystalline product, m.p. 91–92.5°, undepressed on admixture with the natural libocedrol addition complex. Attempts to prepare analogous addition compounds failed when using other phenolic compounds isolated from incense-cedar heartwood, *i.e.*, carvacrol, hydrothymoquinone, and *p*-methoxycarvacrol.

The monobenzoate derivative of libocedrol was prepared in the usual manner. Upon recrystallization from alcohol, colorless needles were formed, m.p. 137.5–138.5°.

Anal. Calc'd for $C_{29}H_{34}O_5$: C, 75.30; H, 7.41.

Found: C, 75.27; H, 7.26.

Similarly the *p*-nitrobenzoate derivative of libocedrol was prepared in the usual manner. This derivative was recrystallized from alcohol acetone (1:1) to give fine, light yellow needles, m.p. 173.8–174.6°.

Anal. Calc'd for $C_{29}H_{33}NO_7$: C, 68.35; H, 6.92, N, 2.75.
Found: C, 68.69: H, 6.64; N, 2.72.

The ultraviolet absorption spectrum of libocedrol in alcohol showed a maximum at 287 mμ, (log ε 3.91) (Fig. 1). The spectrum is very similar to the spectra of *p*-methoxythymol and *p*-methoxycarvacrol (2).

The infrared spectrum of libocedrol in carbon tetrachloride-carbon bisulfide exhibited a strong maximum at 3550 cm.$^{-1}$ in the 1600–4000 cm.$^{-1}$ region, corresponding to OH stretching and at 1620 and 1600 cm.$^{-1}$ two maxima corresponding to phenyl vibrations (3) (Fig. 2).

Libocedroquinone. Libocedrol, m.p. 83–86° (536 mg.) was dissolved in 25 ml. of 95% ethanol and a solution of ferric ammonium sulfate (6.0 g.) in 10 ml. of warm water was added. The whole was heated on a steam-bath for 45 minutes with occasional stirring. Upon cooling, the resulting liquid was diluted to 150 ml. with water and extracted with a total of 70 ml. of petroleum ether. The petroleum ether solution was dried over sodium sulfate, filtered, and the solvent removed by distillation from a steam-bath. Methanol (10 ml.) was added to the residue and the material was allowed to crystallize at −5°, to give 418 mg. of black-brown crystals, m.p. 95–96°. A second crop was obtained by reducing the filtrate to 3 ml. and cooling at −5°. The total yield was 441 mg. (86%). The crystals were purified by sublimation at 150–160° (6–8 mm.), and then recrystallized from methanol-water, giving diamond shaped dark red-black prisms, m.p. 95.3–95.5°.

Anal. Calc'd for $C_{21}H_{26}O_4$: C, 73.66; H. 7.65; OCH_3, 9.06.
Found: C, 73.62; H, 7.58; OCH_3, 8.69.

Anal. for C•CH_3: Calc'd for 4 C•CH_3: 17.6; for 5C•CH_3, 22.0.
Found: 14.4 (82% of 4 C•CH_3; 66% of 5 C•CH_3) (9).

The ultraviolet absorption spectrum of libocedroquinone in *n*-heptane showed three maxima at 259, 351–360, and 460 mμ, identified with Braude's A, B, C quinoid bands and a benzenoid band at 284–285 mμ, (4) (Fig. 3).

The intrared spectrum of libocedroquinone presented a very strong conjugated carbonyl maximum at 1665 cm.$^{-1}$ and at 1620 cm.$^{-1}$ a strong conjugated double-bond maximum, agreeing very well with those found in benzoquinone. No band corresponding to OH stretching was present (3) (Fig. 4).

Monoxime of libocedroquinone. Libocedroquinone m.p. 95–96° (504 mg.) was dissolved in a mixture of 5 ml. of ethanol and 2 ml. of water, to which 400 mg. of hydroxylamine hydrochloride followed by 3 drops of conc'd hydrochloric acid were added and the whole refluxed for 75 minutes on a steam-bath. Upon cooling the resulting mixture to −5°, orange-yellow crystals appeared, which upon filtering and drying weighed 518 mg., m.p. 151° d. (98.5%). The monoxime was recrystallized from methanol-water giving long yellow plates, m.p. 152–153° (d).

Anal. Calc'd for $C_{21}H_{27}NO_4$: C, 70.56; H, 7.61; N, 3.92.
Found: C, 70.48; H, 7.65; N, 4.00.

Oxidation of libocedroquinone to p-methoxythymol. Libocedroquinone, m.p. 95–96° (1.005 g.) was dissolved in 15 ml. of acetone, 2 ml. of 30% hydrogen peroxide was added, and the whole was heated to 50° (5). A solution of 200 mg. of sodium carbonate in 2 ml. of water was added to the mixture and the resulting solution was refluxed for 5 minutes, until the original red-brown solution became orange-yellow. Then a solution of 0.5 g. of sodium hydroxide in 2 ml. of water was added and the whole was refluxed for an additional 5 minutes until the color of the solution became less intense and light red. The mixture then was acidified with 10% sulfuric acid, diluted to about 100 ml. with water, and then extracted with a total of 50 ml. of petroleum ether. The petroleum ether was removed by distillation from a steam-bath and the residue was heated with 20 ml. of 10% sulfuric acid for 10 minutes. The resulting mixture was diluted to 100 ml. with water and extracted with a total of 50 ml. of petroleum ether. The petroleum ether solution then was extracted with 25 ml. of 10% sodium bicarbonate solution followed by extraction with 55 ml. of 10% sodium hydroxide solution. The sodium hydroxide-soluble extract was acidified with an excess of 10% sulfuric acid and extracted with a total of 35 ml. of chloroform. The chloroform solution was dried over sodium sulfate, filtered, and the solvent removed by distillation from a steam-bath. The oily residue

distilled at 140° (19 mm.) and weighed 255 mg., n_D^{21} 1.5268 (48% yield). The *p*-nitrobenzoate derivative was prepared in the usual manner, giving light yellow needles, m.p. 127.5–128.5, undepressed on admixture with the corresponding derivative of *p*-methoxythymol (2). Similarly, the monobenzoate and the aryloxyacetic acid derivatives were prepared melting at 84.5–85.3° and 132.7–133.7 respectively. The melting points remained undepressed on admixture with authentic derivatives (2).

Oxidation of libocedrol to norlibocedroquinone. Libocedrol, m.p. 87–88° (1.00 g.) was dissolved in 20 ml. of acetic acid and 10 ml. of 48% hydrobromic acid containing a few crystals of stannous chloride to discharge a slight bromine coloration. The resulting mixture was refluxed for 1½ hours, cooled, and diluted with 50 ml. of 50% ethanol. A solution of 1.5 g. of cupric acetate monohydrate (2.68 oxidation equivalents) was added to the mixture. The resulting brown mixture was diluted with 200 ml. of water and then extracted with a total of 90 ml. of chloroform. The chloroform solution was dried over sodium sulfate, filtered, and the solvent was removed on a steam-bath. The residue was crystallized from a mixture of 30 ml. of methanol and 15 ml. of water to give 600 mg. of brick-red needles, m.p. 134–137° (66% yield). Recrystallization from isoöctane, followed by crystallization from methanol-water raised the m.p. to 137.5–138°.

Anal. Calc'd for $C_{20}H_{24}O_4$: C, 73.14; H, 7.37.
Found: C, 73.12; H, 7.30.

Anal. for C•CH_3: Calc'd for 4 C•CH_3: 18.3; for 5 C•CH_3, 22.8.
Found: 14.3 (78% of 4 C•CH_3; 63% of 5 C•CH_3).

Norlibocedroquinone exists in two forms, as brick-red needles and as grey-brown needles. While heating the red modification in a melting-point apparatus, it transforms into the grey modification between 105 and 120°. The red modification seems to form from cold solution, while its grey modification seems to be favored by crystallization from hot solvents. The grey modification was frequently obtained also in the cold when the solution was inoculated with a crystal of that modification. There exist several examples of dimorphism of phenylated quinones (10).

Monoxime of norlibocedroquinone. Norlibocedroquinone m.p. 135–136° (508 mg.) was refluxed with a mixture of 0.4 g. of hydroxylamine hydrochloride, 5 ml. of ethanol, 2 ml. of water, and 3 drops of conc'd hydrochloric acid for 75 minutes. The yellow-red solution was allowed to stand at room temperature overnight, then cooled to −5° and the separated crystals were filtered to give 447.5 mg. of long red-orange needles, m.p. 178.5–179.5° d. An additional crop of crystals, 75 mg., was obtained from the mother liquor. The total yield was 522.5 mg. (98%). Recrystallization from methanol gave red needles m.p. 179.5–180° d.

Anal. Calc'd for $C_{20}H_{25}NO_4$: C, 69.94; H, 7.34; N, 4.08.
Found: C, 69.96; H, 7.43; N, 4.02.

Pyrolysis of norlibocedroquinone to 3-hydroxythymoquinone. Norlibocedroquinone, m.p. 135–136° (248 mg.) was heated for 30 minutes in a Pyrex test tube at 203–204°. A yellow sublimate formed on the cooler parts of the tube. The sublimate was dissolved in 30 ml. of chloroform and extracted with 50 ml. of 10% sodium carbonate. The dark-violet aqueous solution was separated, acidified, and extracted with a total of 40 ml. of chloroform. The chloroform solution was dried over sodium sulfate, filtered, and the solvent was removed by heating on a steam-bath. The residue was sublimed at 100° (20 mm.) to give 52.5 mg. (39%) of yellow-orange needles, m.p. 166–168° (sealed capillary), undepressed on admixture with an authentic sample of 3-hydroxythymoquinone prepared according to the method of Carstanjen (6).

Anal. Calc'd for $C_{10}H_{12}O_3$; C, 66.63; H, 6.72.
Found: C, 66.82; H, 6.81.

The 3-hydroxythymoquinone was further identified by the preparation of its aniline derivative according to Schulz (7), m.p. 159–159.5°, undepressed on admixture with an authentic sample.

Oxidation of norlibocedroquinone to 3-hydroxythymoquinone. Norlibocedroquinone, m.p. 137–138° (202 mg.) was dissolved in 4 ml. of acetic acid and to this was added dropwise, with swirling, a solution of 41 mg. of chromic oxide (2 oxidation equivalents) in an ice-

cooled mixture of 4 ml. of acetic acid and 1 ml. of water. The whole was stirred for 3–4 minutes at 0°, diluted with 25 ml. of water, and then extracted with a total of 40 ml. of chloroform. The chloroform solution was extracted with 160 ml. of 5% solution of sodium carbonate to the point where the extracts did not assume a violet color. The combined aqueous extracts were acidified with conc'd hydrochloric acid and the resulting yellow solution was extracted with a total of 30 ml. of ethyl ether. The ether extract was dried over sodium sulfate, filtered, and the solvent was removed from a water-bath. The yellow liquid residue solidified upon scratching, to give 29 mg. of a yellow product, m.p. 150–162°. Sublimation at 100° (20 mm.) gave 17 mg. (15%) of 3-oxythymoquinone, m.p. 166.2–167.7°, undepressed on admixture with authentic sample.

Acknowledgment. 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

A new cryptophenolic compound, libocedrol, $C_{22}H_{30}O_4$, has been isolated from incense-cedar heartwood in the form of an addition product with one mole of *p*-methoxythymol in 1.38 per cent yield (dry wood basis). Libocedrol contains one phenolic hydroxyl group, two methoxy groups, and one ether oxygen. It was oxidized by ferric sulfate to libocedroquinone, which in turn, was readily oxidized by hydrogen peroxide to *p*-methoxythymol. This identified the non-quinoidal part of the above quinone molecule. Demethylation of libocedrol, followed by oxidation with cupric acetate produced norlibocedroquinone, which upon pyrolysis gave 3-oxythymoquinone. This established the nature of the quinoidal portion of libocedroquinone. Spectroscopic evidence has been presented to support the above conclusions. Assuming that libocedrol is formed by enzymatic coupling of two molecules of hydrothymoquinone monomethyl ether and since such coupling occurs normally in *o*- or *p*-positions to the hydroxyl, it would appear that libocedrol is *p*-methoxythymyl-*p*-methoxythymol.

This new compound, together with its addition complex, is of interest from the taxonomic and biochemical standpoint, since they are closely related structurally to *p*-methoxythymol, *p*-methoxycarvacrol, hydrothymoquinone, carvacrol, and thymoquinone previously identified among the extractive components present in incense-cedar heartwood.

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