# Coniferyl Aldehyde as a Constituent of Oils Containing Eugenol

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**The presence of coniferyl aldehyde in eugenol, clove oil, myrica oil, pimenta oil, and sassafras oil has been demonstrated. The characteristic cherry red developed by coniferyl aldehyde with phloroglucinol and hydrochloric acid can be used for a colorimetric assay of coniferyl aldehyde and contributes a significant portion of the color developed by eugenol-containing oils with these reagents.**

A CHARACTERISTIC cherry red is produced when a phloroglucinol-hydrochloric acid reagent is allowed to react with clove oil (1). Eugenol has been considered responsible for this test (2, 3), and since many allyl compounds give a positive test, it has been suggested as a functional group test for this moiety (2).

Adler (4), however, has explained the positive phloroglucinol reaction of lignin and its prototype compounds upon the formation of coniferyl aldehyde or related compounds. He was able to isolate the 2,4-dinitrophenylhydrazone of methylconiferyl aldehyde from methyleugenol and to demonstrate that a purified sample of eugenol does not give a positive phloroglucinol test.

Although coniferyl aldehyde has not been listed as a constituent of *Eugenia caryophyllata* (5–7), it appears that it may be present in this plant and other eugenol-containing species as well as oils obtained from such plants. This investigation was undertaken to detect the presence of coniferyl aldehyde in eugenol-containing oils and to isolate the compound from eugenol U.S.P.

## EXPERIMENTAL

**Apparatus and Materials.**—Spectrophotometric determinations were made with Beckman model DB, Zeiss PMQ II, and Perkin-Elmer model 137 spectrophotometers. All melting points were made on a Köfler hot stage and are corrected. Samples of eugenol, clove oil, myrica oil, pimenta oil, and sassafras oil met the present or former requirements for official purity. All chemicals were of reagent grade and were used without further purification.

**Chromatographic Procedures.**—One-tenth of a milliliter of alcoholic solutions containing 2% clove oil, myrica oil, pimenta oil, or eugenol; 0.01% coniferyl aldehyde, or 0.2% vanillin were chromatographed as suggested by Higuchi (9) on Whatman No. 1 filter paper, using the aqueous phase of a mixture of water:petroleum ether (90–100°):benzene:methanol (50:50:50:1) or butanol saturated with 3% aqueous ammonia.

Presence of vanillin and coniferyl aldehyde was detected by spraying with a 0.5% solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid or with a 1% solution of phloroglucinol in a mixture of 10 ml. of hydrochloric acid and 90 ml. of alcohol.

Since sassafras oil has a low concentration of eugenol, a 0.5-ml. alcoholic solution containing 50% of this oil was chromatographed as a band on a 23 × 57-cm. sheet of Whatman No. 1 paper using the butanol-ammonia system. The zone corresponding in $R_f$ to coniferyl aldehyde was extracted with alcohol and the concentrated extract rechromatographed as a single spot with the butanol-ammonia system. Coniferyl aldehyde was then detected with the phloroglucinol spray.

**Isolation of Coniferyl Aldehyde.**—The 2-Gm. brown residue remaining after distillation up to 250° of the major portion of 200 ml. of eugenol was dissolved in 5 ml. of ethanol. One-milliliter portions were chromatographed as bands on 23 × 57-cm. sheets of Whatman No. 1 filter paper using the aqueous system described above in a descending technique.

The coniferyl aldehyde zone was located with the aid of the phloroglucinol spray on a portion of this paper. This zone was extracted with alcohol, the solution concentrated to 1 ml. under reduced pressure, and rechromatographed in the same manner. The coniferyl aldehyde zone was then extracted with boiling benzene and the extract concentrated to 1 ml. Ten milligrams of yellow needles were isolated after cooling the solution overnight.

**Colorimetric Determination of Coniferyl Aldehyde.**—To a 2-ml. solution of from 1 to 6 mcg. of coniferyl aldehyde in acetone was added 1 ml. of a 0.2% solution of phloroglucinol in a mixture of 5 ml. of hydrochloric acid and 95 ml. of acetone. The cherry red which develops must be measured at 540 mμ within 10 to 15 minutes after the start of the reaction.

**Rate of Formation of Coniferyl Aldehyde.**—A mixture of 200 ml. of eugenol and 20 ml. of a 1% alcoholic solution of 2,4-dinitrophenylhydrazine was distilled under nitrogen. The fraction collected at 248° was tested at a concentration of 20 mg. in 2 ml. of acetone as outlined under the *Colorimetric Determination of Coniferyl Aldehyde.* Determinations were made immediately following distillation and at intervals up to 48 hours after the distillation.

## RESULTS AND DISCUSSION

It was not only possible to indicate the presence of coniferyl aldehyde by paper chromatography but also to isolate this aldehyde from eugenol U.S.P. The residue after distillation of the major portion of a sample of eugenol was chromatographed on paper using the aqueous phase of the system suggested by Higuchi (9) for the separation of coniferyl aldehyde in his lignin studies. While the presence of two carbonyl compounds was indicated through the use of a 2,4-dinitrophenylhydrazine spray, only one of the compounds gave a cherry red test with a phloroglucinol-hydrochloric acid spray. The red produced by this latter spray was used to locate the coniferyl aldehyde zone. Extractions of this zone yield crystals melting at 78° which did not show a depression when mixed with an authentic sample of coniferyl aldehyde.[1] A 2,4-dinitrophenylhydrazone was also prepared from the isolated material and melted at 246–248°. A mixture of this hydra-

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TABLE I.—CHROMATOGRAPHIC CHARACTERISTICS OF CONIFERYL ALDEHYDE AND VANILLIN

| Compd. | $R_f$ Values[a] System I[b] | System II[c] | Phloroglucinol Color Reaction |
|---|---|---|---|
| Coniferyl aldehyde | | | |
| Reference | 0.61 | 0.58 | Cherry red |
| Isolated | 0.60 | 0.58 | Cherry red |
| Vanillin | | | |
| Reference | 0.68 | 0.46 | Pale yellow |
| Isolated | 0.67 | 0.46 | Pale yellow |

[a] These values are for Whatman No. 1 paper at 21° and are averages with a standard deviation of less than .015. [b] The aqueous phase of a mixture of water: petroleum ether (90–100°): benzene: methanol (50:50:50:1). [c] Butanol saturated with 3% aqueous ammonia.

zone and one prepared from the authentic sample showed no depression in melting point. The infrared spectra of both the aldehyde and its hydrazone were identical with the reference materials.

The chromatographic characteristics of the isolated and reference coniferyl aldehyde are summarized in Table I. The second aldehyde present in the sample of eugenol is consistent in its chromatographic characteristics with those of vanillin as also summarized in Table I. For both coniferyl aldehyde and vanillin, a mixture of the isolated and reference aldehyde chromatographed as a single spot with characteristic $R_f$ values. It is evident that coniferyl aldehyde can be separated from any vanillin present.

In addition to the isolation of coniferyl aldehyde from eugenol, the presence of this aldehyde in eugenol-containing oils was demonstrated by paper chromatography. When clove, myrica, and pimenta oils were chromatographed on paper using the butanol-ammonia system, a characteristic red developed with the phloroglucinol-hydrochloric acid spray and had the same $R_f$ value as the reference coniferyl aldehyde.

The presence of coniferyl aldehyde can even be demonstrated for sassafras oil which contains eugenol as a minor constituent in the order of 0.5% (8). Thus, sassafras oil yields a chromatogram with the characteristic color and $R_f$ value for coniferyl aldehyde but requires a preliminary concentration of the coniferyl aldehyde.

Coniferyl aldehyde can be determined colorimetrically with phloroglucinol under a variety of conditions. In this investigation, maximum stability was obtained with acetone as a solvent and by limiting the amount of hydrochloric acid used. For a concentration of 6 mcg. in 3 ml. of the reaction mixture, a maximum absorbance of 0.485 was obtained after 10 minutes and was stable for another 5 minutes. The color developed during this period was proportional to concentration up to 6 mcg. in 3 ml. of the reaction mixture. However, it should be noted that although coniferyl aldehyde contributes significantly to the color developed by eugenol-containing oils upon treatment with phloroglucinol and hydrochloric acid, it is not advisable to use this reaction for the direct determination of coniferyl aldehyde in these oils. Other constituents produce color under the same conditions. For example, vanillin produces a pale yellow, while safrol yields an intense red.

A positive cherry red phloroglucinol test on freshly pressed oil and clove buds demonstrated that the formation of coniferyl aldehyde also occurs at this point and is not an artifact due to processing of the oil or to the method of isolation used in this investigation. It becomes evident that coniferyl aldehyde must be widely present in eugenol preparations when the rapid rate of formation of the compound is noted. Eugenol was distilled under nitrogen and a colorless oil obtained which turns pale yellow after a 1-hour exposure to the atmosphere. A 20-mg. sample of the colorless distilled eugenol failed to give a positive test with phloroglucinol when examined immediately. However, when the distilled eugenol was exposed to atmospheric oxygen, a concentration of 20 mg. in 3 ml. of reaction mixture gave an absorbance of 0.010 after 5 minutes and at the same concentration reached a maximum absorbance of 0.325 after 22 hours of exposure to the atmosphere.

## SUMMARY

Coniferyl aldehyde has been isolated from eugenol and shown to be present in eugenol-containing volatile oils.

Coniferyl aldehyde forms rapidly from eugenol and is essentially responsible for the characteristic cherry red color developed by eugenol-containing oils with phloroglucinol and hydrochloric acid.

A colorimetric procedure has been developed for the estimation of coniferyl aldehyde.

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