Skin Sensitization to Eugenol and Isoeugenol in Mice: Possible Metabolic Pathways Involving *ortho*-Quinone and Quinone Methide Intermediates

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With the aim of providing further mechanistic insights into the mode of action of eugenol (4-allyl-2-methoxyphenol) and isoeugenol (4-propenyl-2-methoxyphenol), we have synthesized two series of modified compounds which were tested in the mouse local lymph node assay for their skin sensitizing potential. The replacement of the methoxy group by an isopropoxy group led to a complete loss of sensitization for the eugenol derivative **6a**, while no significant effect was observed for the isoeugenol derivative **6b**. In the eugenol series, when methyl groups were present in the 3-, 5-, or 6-position a significant reduction in sensitization potential was observed while in the isoeugenol series only methyl substitution in the 3- and 5-position had a discernable effect. Introduction of three methyl groups on the aromatic ring of eugenol (3,5,6-trimethyl-4-allyl-2-methoxyphenol, **7**) and of a *tert*-butyl substituent at the γ-position of the alkyl chain of isoeugenol (4-[3′,3′,3′-trimethylpropenyl]-2-methoxyphenol, **8**) led to a strong decrease of the sensitizing capacity. Our findings indicate that, at least in the mouse, eugenol could sensitize via a demethylation pathway followed by oxidation to the *o*-quinone which could act directly as a hapten even if we cannot exclude a reaction via its tautomeric *p*-quinone methide. Isoeugenol, on the other hand, could act via a mechanism not involving demethylation and for which the evidence is consistent with a direct oxidation to the *p*-quinone methide.

Introduction

The skin sensitization potential of eugenol **1** and isoeugenol **2** (structures shown in Figure 1) continues to be the subject of mechanistic studies (*1*−3). Although the two compounds appear structurally very similar, they show large differences in their sensitization properties in guinea pig sensitization tests (*1*). In human predictive studies, eugenol seems to be a weaker sensitizer than isoeugenol (*4*), and this is borne out by clinical evidence (*5*). Similarly, eugenol is a very weak sensitizer in the guinea pig, whereas isoeugenol is a strong sensitizer. The clinical evidence also suggests that eugenol and isoeugenol may not cross react since individuals often give positive patch test reactions to one, but not both, of these perfume chemicals. Furthermore, guinea pig cross-challenge studies involving eugenol, isoeugenol, and related compounds have provided some evidence that the two compounds could act by different molecular mechanisms (*1*). Two metabolic pathways are usually considered for skin sensitization to eugenol and isoeugenol. The first one considers the formation of a phenolic radical which can either haptenate proteins by reaction at the unsubstituted *ortho*-position or form a reactive quinone methide intermediate. The second one considers an in vivo demethylation to form 4-alkyl catechols which can then haptenate proteins by the "poison ivy mechanism" involving oxidation to the *o*-quinone, which acts as an electrophilic Michael acceptor for protein nucleophiles. These two mechanisms are shown in Figure 1. Studies in the mouse have recently provided new data on the sensitizing potential of eugenol and isoeugenol. In the local lymph node assay it is found that both eugenol and isoeugenol are potent skin sensitizers, although isoeugenol is still stronger than eugenol (*6*). Furthermore, recent publications by Bolton et al. (*7, 8*) on the isomerization of *o*-quinones to *p*-quinone methides lead us to consider the possibility of modes of action additional to the mechanisms outlined above.

With the aim of providing further mechanistic insights into the modes of action of eugenol, isoeugenol, and related compounds, we now describe the synthesis of two series of compounds and sensitization studies in mice. In these series of compounds the basic structures of eugenol and isoeugenol were modified by introduction of methyl groups in the 3-, 5-, and 6-positions of the aromatic ring and also by replacement of the methoxy group by the isopropoxy group (Figure 2). Futhermore, to confirm our initial findings on skin sensitization to eugenol and isoeugenol, additional compounds **7** and **8** were synthesized and tested.

Materials and Methods

Caution! *Skin contact with eugenol and isoeugenol derivatives must be avoided. As sensitizing substances, these compounds must be handled with care.*

Chemistry. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC200-MHz spectrometer in $CDCl_3$ unless otherwise

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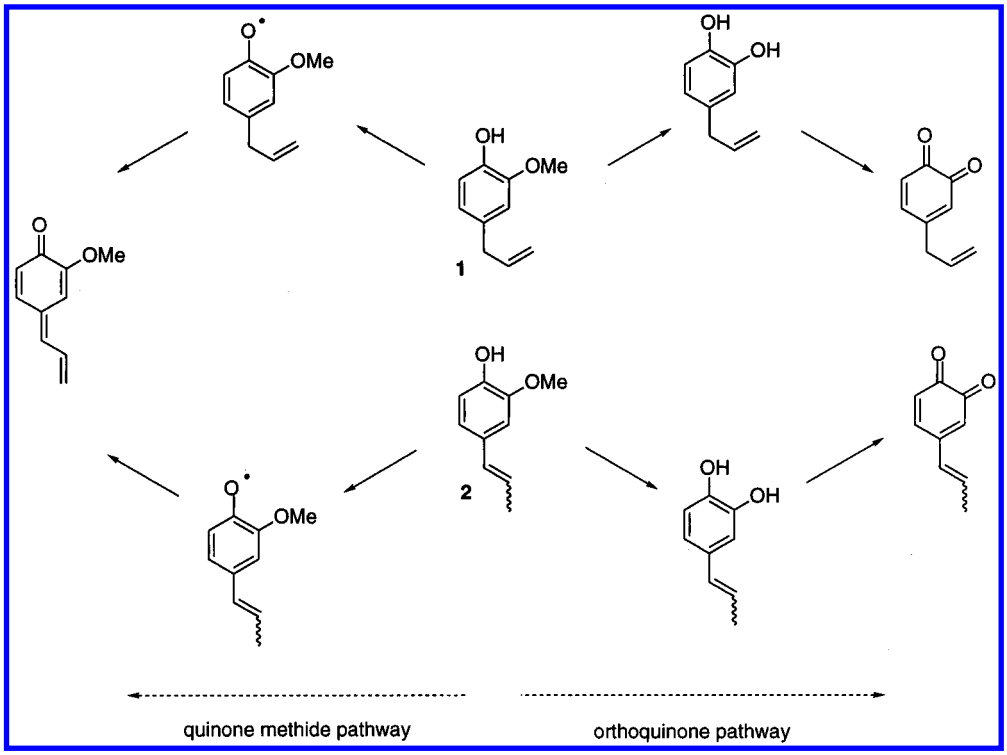


Figure 1. Potential reaction pathways for the skin sensitization to eugenol **1** and isoeugenol **2**.

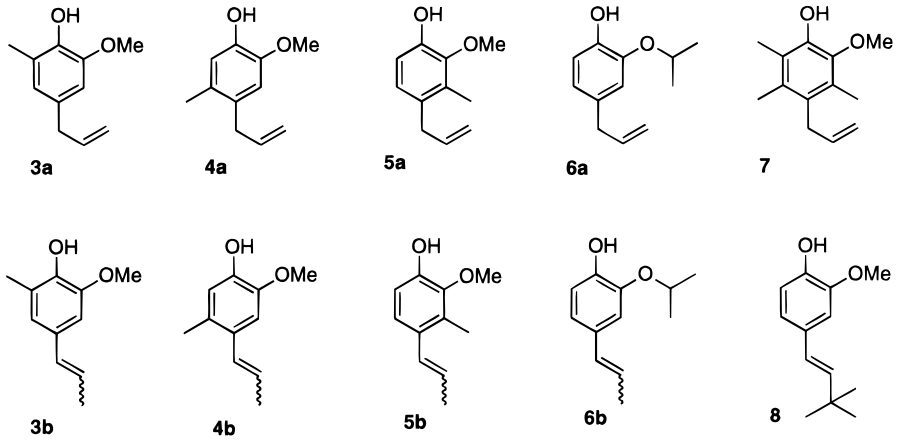


Figure 2. Structures of eugenol and isoeugenol derivatives tested for their sensitizing potential in mice (LLNA).

specified. Chemical shifts are reported in ppm (δ) with respect to TMS, and $CHCl_3$ was used as internal standard ($\delta = 7.27$ ppm). Multiplicities are indicated by s (singlet), d (doublet), t (triplet), and m (multiplet). Infrared spectra were obtained on a Perkin-Elmer FT-IR 1600 spectrometer; peaks are reported in reciprocal centimeters. Melting points were determined on a Buchi Tottoli 510 apparatus and are uncorrected. Dried solvents were freshly distilled before use. Tetrahydrofuran and ethyl ether were distilled from sodium benzophenone. Triethylamine was distilled from powdered calcium hydride. Methylene chloride was dried over P_2O_5 before distillation. All air- or moisture-sensitive reactions were conducted in flame-dried glassware under an atmosphere of dry argon. Chromatographic purifications were conducted on silica gel columns according to the flash chromatography technique.

6-Methylguaiacol (9). A mixture of *o*-vanillin (5 g, 33 mmol), monohydrated hydrazine (16 mL, 330 mmol, 10 equiv), and potassium carbonate (20 g, 145 mmol, 4.4 equiv) in triethyleneglycol (100 mL) was heated under reflux for 1.5 h and then at 200 °C for an additional 3 h. After it was cooled, the mixture was diluted in ether (100 mL) and washed with water (3 × 100 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude phenol which was purified by column chromatography over silica (AcOEt 20%, hexane) to give 4.09 g of **9** (29.7 mmol, 90% yield) as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.27 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 5.70 (s, 1H, OH), 6.70−6.80 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.5, 56.0, 108.3, 119.2, 123.3, 124.0, 143.9, 146.3. IR ($CHCl_3$): ν 3540 (OH). Anal. Calcd for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.43; H, 7.53.

3-Methylguaiacol (11). To a solution of 3-methylcatechol (5.0 g, 40.3 mmol) in THF (200 mL) were added, at 0 °C, NaH (2.9 g, 121 mmol, 3 equiv) and *tert*-butyldimethylsilyl chloride (6.1 g, 40.5 mmol, 1 equiv). The reaction was stirred at room temperature for 4 h, MeI (5 mL, 80.3 mmol, 2 equiv) was then added, and the mixture was stirred for an additional 4 h before hydrolysis with 10% HCl (50 mL) under reflux for 4 h. The reaction was extracted with ether (2 × 200 mL), the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure, and the crude product was purified by column chromatography over silica (AcOEt 10%, hexane) to give 3.9 g (28.2 mmol, 70% yield) of **11** as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.31 (s, 3H, CH_3), 3.80 (s, 3H, OCH_3), 5.63 (s, 1H, OH), 6.60−7.00 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.8, 60.5, 113.4, 122.5, 124.6, 131.0, 145.6, 149.0. IR ($CHCl_3$): ν 3532 (OH). Anal. Calcd for $C_8H_{10}O_2$: C, 69.54; H, 7.30. Found: C, 69.17; H, 7.05.

6-Methylallylguaiacol (13). To a solution of 6-methylguaiacol **7** (2.5 g, 18 mmol) in acetone (50 mL) were added potassium carbonate (10.0 g, 72 mmol, 4 equiv) and allyl bromide (3.2 mL, 36 mmol, 2 equiv). The reaction mixture was stirred for 12 h, diluted with hexane (50 mL), filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography over silica (AcOEt 20%, hexane) to give 2.74 g (15.4 mmol, 85% yield) of **13** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.48 (ddd, dt-like, 2H, $J_{H\beta-H\alpha} = 6$ Hz, $J_{H\alpha-H\gamma cis} = 2$ Hz, $J_{H\alpha-H\gamma trans} = 1$ Hz, Hα), 5.22 (ddt, 1H, $J_{H\beta-H\gamma trans} = 10$ Hz, $J_{H\gamma trans-H\gamma cis} = 2$ Hz, Hγ*trans*), 5.37 (ddt, 1H, $J_{H\beta-H\gamma cis} = 17$ Hz, Hγ*cis*), 6.12 (ddt, 1H, Hβ), 6.70−7.00 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 16.3, 55.8, 73.5, 110.1, 117.2, 122.9, 123.7, 132.3, 134.7, 146.3, 152.8. Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.73; H, 8.22.

5-Methylallylguaiacol (14). Same procedure as for **13** starting from 5-methylguaiacol **10** (5.0 g, 36 mmol) to give 5.74 g (32.2 mmol, 89% yield) of **14** as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.86 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 4.60 (ddd, dt-like, 2H, $J_{H\beta-H\alpha} = 5$ Hz, $J_{H\alpha-H\gamma cis} = 1$ Hz, $J_{H\alpha-H\gamma trans} = 1$ Hz, Hα), 5.28 (ddt, 1H, $J_{H\beta-H\gamma trans} = 10$ Hz, $J_{H\gamma trans-H\gamma cis} = 1$ Hz, Hγ*trans*), 5.41 (ddt, 1H, $J_{H\beta-H\gamma cis} = 17$ Hz, Hγcis), 6.10 (ddt, 1H, Hβ), 6.65−6.80 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.0, 56.1, 69.9, 111.9, 114.9, 117.8, 121.4, 130.4, 131.6, 147.5, 147.9. Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.04; H, 8.24.

3-Methylallylguaiacol (15). Same procedure as for **13** starting from **11** (2.5 g, 18 mmol) to give 3.16 g (17.7 mmol, 98% yield) of **15** as colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 3.84 (s, 3H, OCH_3), 4.58 (ddd, dt-like, 2H, $J_{H\beta-H\alpha} = 5$ Hz, $J_{H\alpha-H\gamma cis} = 2$ Hz, $J_{H\alpha-H\gamma trans} = 1$ Hz, Hα), 5.28 (ddt, 1H, $J_{H\beta-H\gamma trans} = 10$ Hz, $J_{H\gamma trans-H\gamma cis} = 2$ Hz, Hγ*trans*), 5.43 (ddt, 1H, $J_{H\beta-H\gamma cis} = 17$ Hz, Hγcis), 6.09 (ddt, 1H, Hβ), 6.70−7.00 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.9, 53.5, 69.5, 112.0, 117.2, 123.2, 123.6, 132.1, 133.6, 147.9, 151.8. Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.03; H, 8.19.

2-Isopropoxyallylphenol (16). Same procedure as for the synthesis of **13** starting from 2-isopropoxyphenol **12** (4 mL, 27 mmol) to give 5.2 g (27 mmol, quantitative yield) of **16** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (d, 6H, $J = 6$ Hz, $\underline{Me}_2$CH-O), 4.51 (m, 1H, $Me_2C\underline{H}$-O), 4.59 (ddd, dt-like, 2H, $J_{H\alpha-H\beta} = 5$ Hz, $J_{H\alpha-H\gamma cis} = 2$ Hz, $J_{H\alpha-H\gamma trans} = 2$ Hz, Hα), 5.26 (m, 1H, Hγ*trans*), 5.51 (m, 1H, Hγcis), 6.08 (ddt, 1H, $J_{H\beta-H\gamma cis} = 17$ Hz, $J_{H\beta-H\gamma trans} = 10$ Hz), 6.80−7.00 (m, 4H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.3, 70.1, 72.1, 115.3, 117.1, 118.0, 121.5, 121.8, 133.9, 148.2, 150.1. Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.96; H, 8.39. Found: C, 74.88; H, 8.63.

6-Methyleugenol (3a). 6-Methylallylguaiacol **13** (2.0 g, 11.2 mmol) was stirred under argon at 180 °C for 4 h. The reaction mixture was purified by column chromatography over silica (AcOEt 20%, hexane) to give 1.52 g (8.5 mmol, 76% yield) of **3a** as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.25 (s, 3H, CH_3), 3.30 (bd, 2H, $J_{H\alpha-H\beta} = 7$ Hz, Hα), 3.89 (s, 3H, OCH_3), 5.07 (m, 1H, Hγ*trans*), 5.11 (m, 1H, Hγ*cis*), 5.59 (s, 1H, OH), 5.97 (ddt, 1H, $J_{H\beta-H\gamma cis} = 17$ Hz, $J_{H\beta-H\gamma trans} = 10$ Hz, Hβ), 6.70−6.90 (m, 2H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.5, 40.1, 56.0, 108.7, 115.4, 123.2, 123.7, 131.0, 138.1, 142.1, 146.3. IR ($CHCl_3$): ν 3544 (OH). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.93; H, 8.24.

5-Methyleugenol (4a). To a solution of 5-methylallylguaiacol **12** (2.0 g, 11.2 mmol) in CH_2Cl_2 (20 mL) was added a solution of diethylaluminum chloride (6.2 mL, 11.2 mmol, 1.8 M, 1 equiv). The reaction mixture was stirred for 15 min, hydrolyzed with 5% HCl (10 mL), and extracted with CH_2Cl_2 (3 × 20 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum to give a crude product which was purified by column chromatography over silica (AcOEt 10%, hexane) to give 840 mg (4.7 mmol, 42% yield) of **4a** as a yellow oil. ^{1}H NMR (200 MHz, C_6D_6) δ 2.01 (s, 3H, CH_3), 3.15 (ddd, dt-like, 2H, $J_{H\beta-H\alpha} = 6$ Hz, $J_{H\alpha-H\gamma cis} = 2$ Hz, $J_{H\alpha-H\gamma trans} = 2$ Hz, Hα), 3.20 (s, 3H, OCH_3), 4.95 (ddt, 1H, $J_{H\beta-H\gamma cis} = 17$ Hz, $J_{H\gamma trans-H\gamma cis} = 2$ Hz, Hγ*cis*), 5.02 (ddt, 1H, $J_{H\beta-H\gamma trans} = 10$ Hz, Hγ*trans*), 5.40 (s, 1H, OH), 5.87 (ddt, 1H, Hβ), 6.46 (s, 1H, H_3), 6.91 (s, 1H, H_6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.7, 37.4, 56.2, 112.1, 115.4, 116.4, 129.1, 129.4, 137.1, 143.8, 144.7. IR ($CHCl_3$): ν 3536 (OH). Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.03; H, 8.24.

3-Methyleugenol (5a). To a solution of pentabromophenol (11.0 g, 22.5 mmol, 4 equiv) in CH_2Cl_2 (500 mL) was added, dropwise, a solution of trimethylaluminum (5.6 mL, 11.2 mmol, 2 equiv, 2 M). After 1 h at room temperature, the solution was cooled down to −78 °C and a solution of **15** (1 g, 5.6 mmol) in CH_2Cl_2 (100 mL) was added. The reaction mixture was stirred at room temperature for 2 weeks, hydrolyzed with 5% HCl (20 mL), and extracted with CH_2Cl_2 (50 mL). Organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum, and the residue was purified by column chromatography over silica (AcOEt 10%, hexane) to give 560 mg (3.1 mmol, 56% yield) of **5a** as a yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.22 (s, 3H, CH_3), 3.29 (ddd, dt-like, 2H, $J_{H\beta-H\alpha} = 6$ Hz, $J_{H\alpha-H\gamma cis} = 2$ Hz, $J_{H\alpha-H\gamma trans} = 2$ Hz, Hα), 3.77 (s, 3H, OCH_3), 4.97 (ddt, 1H, $J_{H\beta-H\gamma cis} = 16$ Hz, $J_{H\gamma trans-H\gamma cis} = 2$ Hz, Hγ*cis*), 5.04 (ddt, 1H, $J_{H\beta-H\gamma trans} = 10$ Hz, Hγ*trans*), 5.53 (s, 1H, OH), 5.93 (ddt, 1H, Hβ), 6.70−6.90 (m, 2H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 11.7, 37.2, 60.5, 112.8, 115.2, 125.3, 129.4, 130.7, 136.8, 145.5, 147.1. IR ($CHCl_3$): ν 3536 (OH). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.99; H, 8.17.

2-Isopropoxy-4-allylphenol (6a). Same procedure as for the synthesis of **3a** starting from **16** (2.3 g, 10.4 mmol) to give 600 mg (3.1 mmol, 30% yield) of **6a** as a colorless oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.36 (d, 6H, $J = 6$ Hz, $\underline{Me}_2$CH-O), 3.31 (bd, 2H, $J_{H\alpha-H\beta} = 7$ Hz, Hα), 4.58 (m, 1H, $Me_2\underline{CH}$-O), 5.05 (m, 1H, Hγ*trans*), 5.06 (m, 1H, Hγ*cis*), 5.59 (s, 1H, $\underline{OH}$), 5.96 (ddt, 1H, $J_{H\beta-H\gamma cis} = 17$ Hz, $J_{H\beta-H\gamma trans} = 9$ Hz, Hβ), 6.60−7.00 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 22.3, 40.0, 71.7, 114.1, 114.6, 115.5, 121.4, 131.8, 138.1, 144.7, 145.1. IR ($CHCl_3$): ν 3536 (OH). Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.96; H, 8.39. Found: C, 74.73; H, 8.68.

6-Methylisoeugenol (3b). To a solution of 6-methyleugenol **3a** (2.5 g, 14 mmoles) in Me_2SO (DMSO)[1] (50 mL) was added potassium *tert*-butanolate (3.2 g, 28.5 mmol, 2 equiv). The reaction mixture was heated at 110 °C for 2 h, diluted with water (50 mL), and extracted with ether (3 × 50 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The resulting crude oil was purified by column chromatography over silica (AcOEt 20%, hexane) to give 2.27 g (12.7 mmol, 91% yield) of **3b** as a mixture of *cis*/*trans* isomers (1/9). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.86 (dd, 3H, $J_{H\beta-H\gamma} = 6$ Hz, $J_{H\alpha-H\gamma} = 1$ Hz, allyl-CH_3), 2.24 (s, 3H, CH_3), 3.89 (s, 3H, OCH_3), 5.64 (s, 1H, OH), 6.11 (dd, 1H, $J_{H\alpha-H\beta} = 15$ Hz, Hβ), 6.31 (dd, 1H, Hα), 6.65−6.80 (m, 2H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.5, 18.4, 56.0, 105.7, 121.3, 123.0, 123.7, 129.6, 131.1, 143.1, 146.4. IR ($CHCl_3$): ν 3540 (OH). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.05; H, 8.26.

5-Methylisoeugenol (4b). Same procedure as for **3b** starting from **4a** (2.5 g, 14 mmol) to give 2.24 g (12.6 mmol, 90% yield) of **4b** as a *cis*/*trans* mixture (15/85). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.89 (dd, 3H, $J_{H\beta-H\gamma} = 6$ Hz, $J_{H\alpha-H\gamma} = 2$ Hz, allyl-CH_3), 2.24 (s, 3H, CH_3), 3.88 (s, 3H, OCH_3), 5.48 (s, 1H, OH), 5.97 (dd, 1H, $J_{H\alpha-H\beta} = 16$ Hz, Hβ), 6.52 (dd, 1H, Hα), 6.70 (s, 1H, H_6), 7.91 (s, 1H, H_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.6, 19.0, 56.1, 108.1, 116.3, 124.7, 128.1, 128.8, 128.9, 144.6, 144.9. IR ($CHCl_3$): ν 3540 (OH). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.31; H, 7.90.

3-Methylisoeugenol (5b). Same procedure as for the preparation of **3b** starting from **5a** (2.5 g, 14 mmol) to give 880 mg (4.9 mmol, 35% yield) as a mixture of *cis*/*trans* isomers (3/17). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.88 (dd, 3H, $J_{H\beta-H\gamma} = 6$ Hz, $J_{H\alpha-H\gamma} = 2$ Hz, allyl-CH_3), 2.26 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 5.61 (s, 1H, OH), 5.98 (dd, 1H, $J_{H\alpha-H\beta} = 16$ Hz, Hβ), 6.49 (dd, 1H, Hα), 6.77 (d, 1H, $J_{H5-H6} = 8$ Hz, H_6), 7.09 (d, 1H,

[1] Abbreviations: AAO, acetone−olive oil; DMSO, dimethyl sulfoxide; LLNA, local lymph node assay; PBS, phosphate-buffered saline; TBDMS, *tert*-butyldimethylsilyl.

H_5). ^{13}C NMR (50 MHz, $CDCl_3$): δ 15.3, 18.7, 60.8, 113.0, 122.2, 122.6, 128.1, 128.5, 130.8, 145.3, 147.8. IR ($CHCl_3$): ν 3532 (OH). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.83; H, 7.98.

2-Isopropoxy-4-propenylphenol (6b). Same procedure as for the synthesis of **3b** starting from **6a** (2.5 g, 13 mmol) to give 2.23 g (11.6 mmol, 89% yield) of **6b** as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$): δ 1.37 (d, 6H, J = 6 Hz, $\underline{Me_2}CH$-O) 1.86 (dd, 3H, $J_{H\beta-H\gamma}$ = 6 Hz, $J_{H\alpha-H\gamma}$ = 2 Hz, allyl-$\underline{CH_3}$), 4.60 (m, 1H, $Me_2C\underline{H}$-O), 5.67 (s, 1H, OH), 6.05 (dd, 1H, $J_{H\alpha-H\beta}$ = 16 Hz, Hβ), 6.49 (dd, 1H, Hα), 6.75−7.00 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.4, 22.2, 71.8, 111.0, 114.6, 119.4, 123.2, 130.6, 130.9, 144.7, 145.9. IR ($CHCl_3$): ν 3536 (OH). Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.96; H, 8.39. Found: C, 74.83; H, 8.61.

6-Acetylisopseudocumenol (18). Acetyl isopseudocumenol **17** (13.0 g, 73 mmol) and aluminum chloride (10.0 g, 75 mmol, 1 equiv) were heated at 100 °C for 90 min. The reaction mixture was hydrolyzed with crushed ice (50 g) and extracted with CH_2Cl_2 (3 × 50 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude residue. Purification by column chromatography over silica (AcOEt 5%, hexane) gave 11.0 g (62 mmol, 85% yield) of **18** as a yellow solid, mp = 32−34 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 2.20 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.60 (s, 3H, $\underline{CH_3}$-CO), 7.36 (s, 1H, H_4), 12.55 (s, 1H, OH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 11.3, 16.7, 20.2, 26.5, 116.7, 125.2, 126.5, 128.1, 145.0, 158.9, 204.1. IR ($CHCl_3$): ν 3400−3050 (OH), 1628 (C=O). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.12; H, 7.92. Found: C, 73.88; H, 7.64.

6-Acetylallylisopseudocumenol (19). To a solution of 6-acetylisopseudocumenol **18** (10.0 g, 56 mmol) in acetone (200 mL) were added potassium carbonate (31 g, 224 mmol, 4 equiv) and allylbromide (10 mL, 116 mmol, 2.1 equiv). The reaction mixture was heated under reflux for 2 days, diluted with hexane (200 mL), filtered, and concentrated under reduced pressure. The crude oil was taken up in hexane (50 mL), filtered, concentrated, and purified by chromatography over silica (AcOEt 5%, hexane) to give 11.8 g (54 mmol, 96% yield) of **19** as a yellow oil. ^1H NMR (200 MHz, $CDCl_3$): δ 2.20 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 2.61 (s, 3H, CH_3-CO), 4.25 (bd, 2H, $J_{H\alpha-H\beta}$ = 6 Hz, Hα), 5.26 (dd, 1H, $J_{H\gamma trans-H\beta}$ = 10 Hz, $J_{H\gamma cis-H\gamma trans}$ = 1 Hz, Hγ*trans*), 5.41 (dd, 1H, $J_{H\gamma cis-H\beta}$ = 17 Hz, Hγ*cis*), 6.07 (ddt, 1H, Hα), 7.35 (s, 1H, H_4). ^{13}C NMR (50 MHz, $CHCl_3$): δ 12.8, 16.4, 20.1, 30.5, 75.7, 117.6, 128.0, 130.6, 130.7, 132.2, 133.5, 141.5, 154.3, 200.7. IR ($CHCl_4$) ν 1672 (C=O). Anal. Calcd for $C_{14}H_{18}O_2$: C, 77.02; H, 8.32. Found: C, 76.70; H, 8.36.

6-Hydroxyethylallylipsopseudocumenol (20). To a solution of 6-acetyl allylisopseudocumenol **19** (10.0 g, 46 mmol) in ether (200 mL) was added, by portions and at 0 °C, $LiAlH_4$ (1.7 g, 45 mmol, 1 equiv). The reaction mixture was stirred at room temperature for 30 min, cooled down to 0 °C, hydrolyzed with water (20 mL), washed with 5% HCl (50 mL), and extracted with ether (5 × 50 mL). Combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude product. Purification by column chromatography on silica (AcOEt, 30% hexane) gave 9.5 g (43 mmol, 94% yield) of **20** as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$): δ 1.51 (d, 3H, $\underline{CH_3}$-CHOH), 2.18 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.28 (s, 3H, $\underline{CH_3}$), 2.65−2.80 (bs, 1H, OH), 4.30 (bd, 2H, $J_{H\alpha-H\beta}$ = 5 Hz, Hα), 5.17 (quint, 1H, C$\underline{H}$OH), 5.30 (dd, 1H, $J_{H\gamma trans-H\beta}$ = 10 Hz, $J_{H\gamma cis-H\gamma trans}$ = 1 Hz, Hγ*trans*), 5.47 (dd, 1H, $J_{H\gamma cis-H\beta}$ = 17 Hz, Hγ*cis*), 6.13 (ddt, 1H, Hβ), 7.12 (s, 1H, H_4). ^{13}C NMR (50 MHz, $CHCl_3$): δ 13.1, 15.9, 20.5, 24.0, 65.0, 74.7, 117.3, 124.8, 129.5, 132.5, 133.9, 135.2, 135.9, 152.5. Anal. Calcd for $C_{14}H_{20}O_2$: C, 76.31 H, 9.16. Found: C, 76.52; H, 9.19.

6-Hydroxyallylipsopseudocumenol (21). To a solution of 6-hydroxyethyl allylipsopseudocumenol **20** (8.8 g, 40 mmol) in THF (100 mL) were added a solution of H_2O_2 (50 mL, 35% aqueous) and *p*-toluenesulfonic acid (760 mg, 4 mmol, 0.1 equiv). The reaction mixture was stirred at room temperature for 3 days and extracted with hexane (3 × 100 mL), and combined organic layers dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by chromatography on silica (AcOEt 10%, hexane) to give 5.84 g (30 mmol, 76% yield) of **21** as a colorless oil. ^1H NMR (200 MHz, $CDCl_3$): δ 2.12 (s, 3H, CH_3), 2.23 (s, 6H, 2 CH_3), 4.36 (bd, 2H, $J_{H\alpha-H\beta}$ = 6 Hz, Hα), 5.25−5.40 (m, 1H, Hγ*trans*), 5.40−5.60 (m, 1H, Hγ*cis*), 5.62 (s, 1H, OH), 6.14 (ddt, 1H, $J_{H\gamma cis-H\beta}$ = 17 Hz, $J_{H\gamma trans-H\beta}$ = 10 Hz, Hβ), 6.68 (s, 1H, H_4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.2, 15.2, 20.4, 74.5, 114.3, 118.3, 127.3, 129.4, 132.8, 133.8, 142.5, 146.3. IR ($CHCl_3$): ν 3536 (OH). Anal. Calcd for $C_{12}H_{16}O_2$: C, 74.96 H, 8.39. Found: C, 74.62; H, 8.46.

6-Methoxyallylisopseudocumenol (22). To a solution of 6-hydroxyallylipsopseudocumenol **21** (5.0 g, 26 mmol) in acetone (200 mL) were added potassium carbonate (14.3 g, 104 mmol, 4 equiv) and methyl iodide. The reaction mixture was stirred for 12 h, diluted with hexane (100 mL), and filtered, and the solid residue was washed with hexane (10 mL). Combined organic layers were concentrated under reduced pressure, and the crude residue was purified by column chromatography over silica (hexane) to give 5.15 g (25 mmol, 96% yield) of **22** as a colorless liquid. ^1H NMR (200 MHz, $CDCl_3$): δ 2.17 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.87 (s, 3H, OCH_3), 4.47 (bd, 2H, $J_{H\alpha-H\beta}$ = 6 Hz, Hα), 5.20−5.35 (m, 1H, Hγ*trans*), 5.35−5.50 (m, 1H, Hγ*cis*), 6.19 (ddt, 1H, $J_{H\gamma cis-H\beta}$ = 17 Hz, $J_{H\gamma trans-H\beta}$ = 10 Hz, Hβ), 6.65 (s, 1H, H_4). ^{13}C NMR (50 MHz, $CDCl_3$): δ 13.0, 15.4, 20.7, 55.9, 73.9, 111.8, 117.0, 127.8, 130.7, 131.6, 134.8, 144.4, 150.2. Anal. Calcd for $C_{13}H_{18}O_2$: C, 75.68; H, 8.80. Found: C, 75.42; H, 8.81.

3,5,6-Trimethyleugenol (7). To a solution of 6-methoxyallylisopseudocumenol **22** (3.0 g, 14.6 mmol) in dry toluene (50 mL) was added, dropwise, a solution of Et_2AlCl in toluene (8.1 mL; 14.6 mmol; 1.8 M). The reaction was heated at 100 °C for 1 h, cooled down to 0 °C, and hydrolyzed with water (20 mL) and then 10% HCl (50 mL). The mixture was extracted with ether (3 × 50 mL), and combined organic layers were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give a crude product. Purification by column chromatography on silica (AcOEt 10%, hexane) gave 2.34 g (11.4 mmol, 78% yield) of **7** as a yellow solid, mp = 55−57 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 2.12 (s, 3H, CH_3), 2.16 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 3.46 (ddd, dt-like, 2H, $J_{H\alpha-H\beta}$ = 5 Hz, $J_{H\alpha-H\gamma cis}$ = 2 Hz, $J_{H\alpha-H\gamma trans}$ = 2 Hz, Hα), 3.74 (s, 3H, OCH_3), 4.92 (ddt, 1H, $J_{H\gamma cis-H\beta}$ = 10 Hz, $J_{H\gamma cis-H\gamma trans}$ = 4 Hz, Hγ*cis*), 5.04 (ddt, 1H, $J_{H\gamma trans-H\beta}$ = 17 Hz, Hγ*trans*), 5.58 (s, 1H, OH), 5.97 (ddt, 1H, Hβ). ^{13}C NMR (50 MHz, $CDCl_3$): δ 12.5, 15.7, 16.1, 31.3, 61.8, 115.3, 121.8, 127.1, 127.7, 132.2, 137.1, 143.5, 147.1. IR ($CHCl_3$): ν 3536 (OH). Anal. Calcd for $C_{13}H_{18}O_2$: C, 75.68; H, 8.80. Found: C, 76.00; H, 8.90.

9,9,9-Trimethylisoeugenol (8). 4-Bromoguaiacol **23** (4 g, 20 mmol), palladium acetate (440 mg, 2 mmol, 0.1 equiv), tri-*o*-tolylphosphine (600 mg, 2 mmol, 0.1 equiv), neohexene (26 mL, 0.2 mol, 10 equiv), and Et_3N (11 mL, 80 mmol, 4 equiv) were placed in a sealed tube which was heated at 110 °C for 3 days. The reaction mixture was cooled down and filtered on Celite, and the organic layer was washed with 5% HCl, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography over silica (AcOEt 30%, hexane) to give 3.45 g (16.7 mmol, 85% yield) of **8** as a white solid, mp = 63−64 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 1.12 (s, 9H, t-Bu), 3.92 (s, 3H, OCH_3), 5.56 (s, 1H, OH), 6.09 (d, 1H, $J_{H\alpha-H\beta}$ = 16 Hz, Hβ), 6.24 (d, 1H, Hα), 6.80−6.90 (m, 3H, ArH). ^{13}C NMR (50 MHz, $CDCl_3$): δ 29.7, 33.2, 55.9, 108.0, 114.4, 119.5, 124.4, 130.7, 139.7, 144.8, 146.6. IR ($CHCl_3$): ν 3544 (OH). Anal. Calcd for $C_{13}H_{18}O_2$: C, 75.68; H, 8.80. Found: C, 75.29; H, 8.77.

Mouse Local Lymph Node Assay (LLNA). This assay was carried out as described (*9*) but with the exception that wherever possible chemicals were tested at equimolar concentrations. Briefly, groups of mice (n = 4) received 25 μL of the test chemical dissolved in vehicle (acetone-olive oil 4:1) in the concentrations indicated in Tables 1−3, on the dorsum of both ears daily for 3 consecutive days. Control animals were treated in the same way with the vehicle alone. All mice were injected intravenously 5 days after the first treatment, with 250 μL of phosphate-

Table 1. Cell Proliferation Induced by Eugenol 1 and Derivatives 3a−6a in the Local Lymph Node Assay[a]

chemical	concentration (w/w %)	[3H]thymidine incorporation (dpm/node)	stimulation index
eugenol **1**	control	380	−
	10 (0.61 M)	899	2.4
	25 (1.52 M)	2091	5.5
	50 (3.05 M)	6100	**16.1**
6-methyleugenol **3a**	control	655	−
	11 (0.62 M)	1263	1.9
	27 (1.52 M)	3182	**4.9**
	54 (3.03 M)	5436	**8.3**
5-methyleugenol **4a**	control	713	−
	11 (0.62 M)	1927	2.7
	27 (1.52 M)	3477	**4.9**
	54 (3.03 M)	3035	**4.3**
3-methyleugenol **5a**	control	713	−
	11 (0.62 M)	1037	1.5
	27 (1.52 M)	1621	2.3
	54 (3.03 M)	4578	**6.4**
compound **6a**	control	655	−
	12 (0.62 M)	1160	1.8
	29 (1.51 M)	1185	1.8
	59 (3.07 M)	1442	2.2

[a] Groups of mice (n = 4) received 25 μL of the test chemical dissolved in vehicle (AOO) in the concentrations indicated, on the dorsum of both ears daily for 3 consecutive days. Control animals were treated in the same way with the vehicle alone. All mice were injected intravenously 5 days after the first treatment with 250 μL of PBS containing 20 μCi of [3H]thymidine. 5 h later, draining auricular lymph nodes were excised and pooled for each group and a single-cell suspension of lymph node cells was prepared. The thymidine incorporation was measured by β-scintillation counting. The increase in thymidine incorporation relative to vehicle-treated controls was derived for each experimental group and recorded as a stimulation index.

buffered saline (PBS) containing 20 μCi of [3H]thymidine. 5 h later, draining auricular lymph nodes were excised and pooled for each group, and a single-cell suspension of lymph node cells was prepared. The thymidine incorporation was measured by β-scintillation counting. The increase in thymidine incorporation relative to vehicle-treated controls was derived for each experimental group and recorded as a stimulation index.

Results

Chemistry. The synthesis of eugenol derivatives **3a**−**6a** was based on the thermal or catalyzed Claisen transposition of appropriately functionalized allylphenol compounds **13**−**16**. The further transformation of eugenol into isoeugenol derivatives **3b**−**6b** was achieved by potassium *tert*-butanolate treatment in DMSO.

Starting Materials. The 6-methylguaiacol **9** was prepared, in one step and with 90% yield, from *o*-vanillin using a Wolf−Kischner reduction of the aldehydic function (*10*). The 3-methylcatechol was transformed into 3-methylguaiacol **11** through a one-pot selective protection of the phenol function at position 1 with *tert*-butyldimethylsilyl chloride (NaH, THF) (*11*) followed by the methylation of the second phenolic function with MeI and potassium carbonate and the subsequent hydrolysis of the silyl protective group (*11*) under acidic conditions (10% HCl, reflux). The 3-methylguaiacol **11** was thus obtained with an overall yield of 70%. The 5-methylguaiacol **10** and the 2-isopropylcatechol **12** were commercially available.

Synthesis of Eugenol and Isoeugenol Derivatives. Phenols **9**−**12** were allylated with 2 equiv of allyl bromide in refluxing acetone with an excess of K_2CO_3 as base with yields ranging from 85% to 98% (Scheme 1). Claisen transpositions of allyl derivatives were performed under

Table 2. Cell Proliferation Induced by Isoeugenol 2 and Derivatives 3b−6b in the Local Lymph Node Assay[a]

chemical	concentration (w/w %)	[3H]thymidine incorporation (dpm/node)	stimulation index
isoeugenol **2**	control	416	−
	2.5 (0.15 M)	3545	**8.5**
	5 (0.30 M)	4990	**12.1**
	10 (0.61 M)	6859	**16.5**
6-methylisoeugenol **3b**	control	659	−
	2.5 (0.14 M)	3879	**5.9**
	5.5 (0.31 M)	7341	**11.1**
	11 (0.62 M)	10329	**15.7**
5-methylisoeugenol **4b**	control	655	−
	2.5 (0.14 M)	3514	**5.4**
	5.5 (0.31 M)	3379	**5.2**
	11 (0.62 M)	4559	**7.0**
3-methylisoeugenol **5b**	control	659	−
	2.5 (0.14 M)	1435	2.2
	5.5 (0.31 M)	2822	**4.3**
	11 (0.62 M)	3963	**6.0**
compound **6b**	control	659/449*	−
	0.6 (31 mM)	1333*	**3.0***
	1.2 (62 mM)	2574*	**5.7***
	3 (0.16 M)	7320/4823*	**11.1/10.7***
	6 (0.31 M)	7657	**11.6**
	12 (0.62 M)	7869	**11.9**

[a] Groups of mice (n = 4) received 25 μL of the test chemical dissolved in vehicle (AOO) in the concentrations indicated, on the dorsum of both ears daily for 3 consecutive days. Control animals were treated in the same way with the vehicle alone. All mice were injected intravenously 5 days after the first treatment, with 250 μL of PBS containing 20 μCi of [3H]thymidine. 5 h later, draining auricular lymph nodes were excised and pooled for each group and a single-cell suspension of lymph node cells was prepared. The thymidine incorporation was measured by β-scintillation counting. The increase in thymidine incorporation relative to vehicle-treated controls was derived for each experimental group and recorded as a stimulation index. Two sets of experiments were carried out with compound **6b**, and data marked with asterisks correspond to the same set.

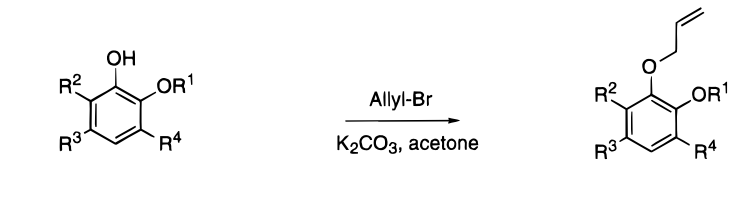
Table 3. Cell Proliferation Induced by Eugenol Derivative 7 and Isoeugenol Derivative 8 in the Local Lymph Node Assay[a]

chemical	concentration (w/w %)	[3H]thymidine incorporation (dpm/node)	stimulation index
eugenol derivative **7**	control	612	−
	12.6 (0.61 M)	1018	1.9
	31.4 (1.52 M)	1313	2.1
	62.8 (3.05 M)	865	1.4
isoeugenol derivative **8**	control	612	−
	6.3 (0.30 M)	1984	**3.2**
	12.6 (0.61 M)	2850	**4.7**
	31.4 (1.52 M)	4903	**8.0**

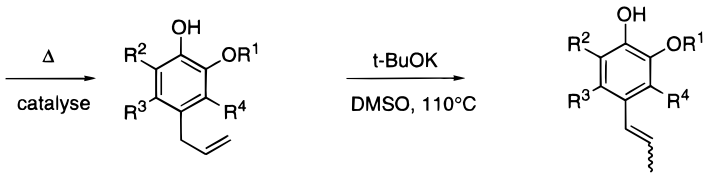
[a] Groups of mice (n = 4) received 25 μL of the test chemical dissolved in vehicle (AOO) in the concentrations indicated, on the dorsum of both ears daily for 3 consecutive days. Control animals were treated in the same way with the vehicle alone. All mice were injected intravenously 5 days after the first treatment, with 250 μL of PBS containing 20 μCi of [3H]thymidine. 5 h later, draining auricular lymph nodes were excised and pooled for each group and a single-cell suspension of lymph node cells was prepared. The thymidine incorporation was measured by β-scintillation counting. The increase in thymidine incorporation relative to vehicle-treated controls was derived for each experimental group and recorded as a stimulation index.

thermal condition when both *o*-positions were functionalized and under aluminum catalyze when one *o*-position was free. It is known in the literature that aluminum derivatives are able to catalyze Claisen transposition (*12, 13*) by chelating the *O*-allyl oxygen and that voluminous groups will orientate the allyl function and promote the transposition at the substituted *ortho*-position, leading then to the *para*-transposition product (*12*). Thus compound **13** gave the eugenol derivative **3a** with 76% yield

Scheme 1. Synthetic Pathway to Eugenol 3a−6a and Isoeugenol 3b−6b Derivatives

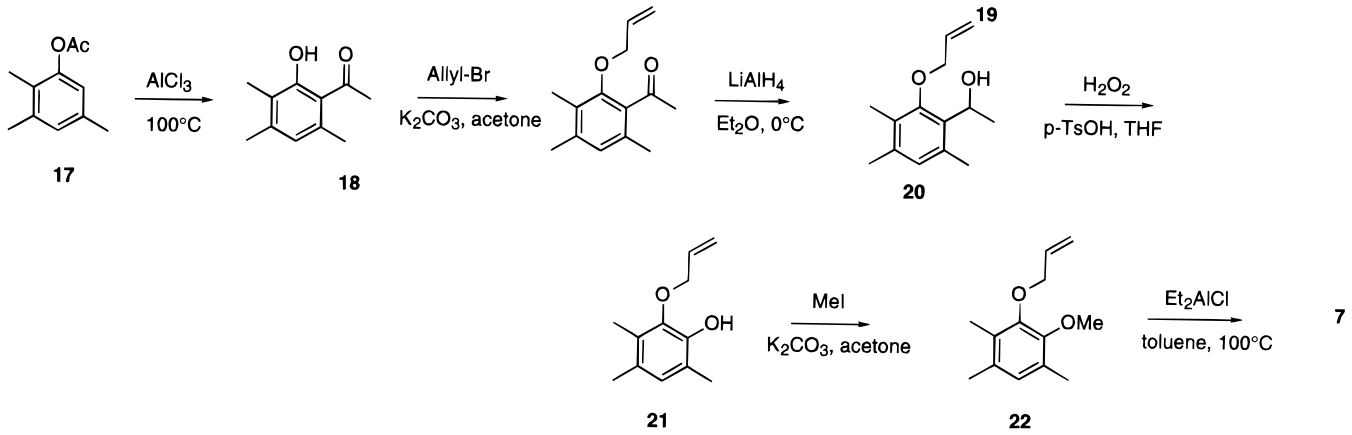


R^1 = Me	R^2 = Me, R^3 = R^4 = H	**9**	R^1 = Me	R^2 = Me, R^3 = R^4 = H	**13**
	R^3 = Me, R^2 = R^4 = H	**10**		R^3 = Me, R^2 = R^4 = H	**14**
	R^4 = Me, R^2 = R^3 = H	**11**		R^4 = Me, R^2 = R^3 = H	**15**
R^1 = i-Pr	R^2 = R^3 = R^4 = H	**12**	R^1 = i-Pr	R^2 = R^3 = R^4 = H	**16**



R^1 = Me	R^2 = Me, R^3 = R^4 = H	**3a**	R^1 = Me	R^2 = Me, R^3 = R^4 = H	**3b**
	R^3 = Me, R^2 = R^4 = H	**4a**		R^3 = Me, R^2 = R^4 = H	**4b**
	R^4 = Me, R^2 = R^3 = H	**5a**		R^4 = Me, R^2 = R^3 = H	**5b**
R^1 = i-Pr	R^2 = R^3 = R^4 = H	**6a**	R^1 = i-Pr	R^2 = R^3 = R^4 = H	**6b**

Scheme 2. Synthetic Pathway to the Trimethyl Eugenol Derivative 7



after treatment at 180 °C for 4 h, but a similar treatment applied to compounds **14**−**16** gave mainly the formation of the expected *ortho*-substituted products. The use of aluminum derivatives allowed us to decrease both reaction time and temperature and to increase the *para*/*ortho* ratio to a reasonable level. Thus the Claisen transposition of **14** and **15** catalyzed respectively with Et_2AlCl and methylaluminum bis(pentabromophenol) (MAP) gave derivatives **4a** and **5a** with 42% and 56% yield, respectively. The lowest yield (30%) was obtained for the transposition of the isopropyl derivative **16** into **6a**.

Isoeugenol derivatives **3b**−**6b** were prepared in high yields from **3a**−**6a** by treatment with 2 equiv of potassium *tert*-butanolate in DMSO at 110 °C for 2 h, and *trans* isomers were predominantly formed.

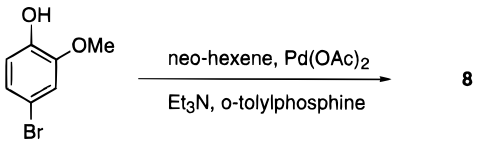
The 3,5,6-trimethyl eugenol **7** was synthesized from 6-acetyl-isopseudocumenol **17** (Scheme 2). A Fries rearrangement *(14)* of **17** with $AlCl_3$ gave the phenol derivative **18** (85% yield) which was allylated under classical conditions. The oxidation of **19** with H_2O_2, under either basic or acidic conditions, gave poor results so we decided to reduce the carbonyl function with $LiAlH_4$ in ether. Oxidation of the alcohol **20** with H_2O_2 under acidic conditions (*15*) (*p*-TsOH) gave with 76% yield the phenol **21** which was subsequently methylated under classical conditions. The Claisen transposition of **22** under thermal conditions gave mainly decomposition products, while treatment at room temperature with Et_2AlCl gave the *ortho*-transposition intermediate with 91% yield. We thus decided to combine thermal and catalytic conditions. As a result, the treatment of **22** in refluxing toluene with 1 equiv of Et_2AlCl gave the expected eugenol derivative **7** with 78% yield.

The 9,9,9-trimethylisoeugenol **8** was prepared in one step (Scheme 3) from neohexene and 4-bromoguaiacol **23** using a classical Heck-type reaction with palladium acetate (*16*). Only the *trans* isomer was formed (85% yield).

LLNA. The mouse local lymph node assay results on the eugenol and isoeugenol derivatives are shown in Tables 1−3. The following points should be noted.

Eugenol Derivatives. In contrast to the situation in guinea pigs, a significant degree of sensitization to eugenol was induced in the mouse, i.e., a dose dependent

Scheme 3. Synthesis of the Isoeugenol Derivative 8



22

increase in proliferation and at least one test concentration inducing a 3-fold or greater increase (SI value ≥ 3) in isotope incorporation compared with vehicle-treated controls (Table 1). However, it should be noted that in the LLNA testing, higher concentrations were used for eugenol (10−50%) and its derivatives than for isoeugenol (2.5−10%) and its derivatives; taking this into account, it can be inferred that isoeugenol is actually a stronger sensitizer than eugenol in the mouse, but the difference is less than in the guinea pig.

The replacement of the methoxy group in eugenol by the isopropoxy group (compound **6a**) led to a complete loss of sensitization potential, and when a methyl group was present in the 3-position (compound **5a**) a significant degree of sensitization was induced only at the highest concentration of 54%. Introduction of methyl substituents in the 5- or 6-position (compounds **4a** and **3a**, respectively) also led to similar decreases in the sensitizing potential, while introduction of three methyl groups on the aromatic ring (compound **7**) led to a complete loss of sensitization potential.

Isoeugenol Derivatives. All isoeugenol derivatives fulfill the criteria for a chemical to be classified as a sensitizer in the LLNA. The sensitization potential of isoeugenol in the mouse was not substantially affected when the methoxy group was replaced by the isopropoxy group (compound **6b**). Methyl substitution in the 6-position of isoeugenol (**3b**) had no discernible effect on the sensitization potential, whereas methyl substitution in the 3- and 5-positions of isoeugenol (**5b** and **4b**, respectively) led to a reduction in sensitization potential. Introduction of a *tert*-butyl substituent at the γ-position of the alkyl chain (compound **8**) resulted in a strong decrease of the sensitizing capacity.

Discussion

The skin sensitization reaction to a chemical is a multistep process which involves T-cells of the immune system. During the induction phase, the chemical penetrates into the epidermis, beneath the stratum corneum, and there it binds to protein, thus modifying the protein's structure. The modified protein is then processed by epidermal antigen presenting cells and is presented, in a form which can be recognized as antigenic, to uncommitted T-cells. Those T-cells whose receptors match the modified protein are stimulated to multiply, producing expanded clones of circulating T-cells capable of recognizing the modified protein.

It follows from the above simple description of the biological mechanism that for a chemical to be a sensitizer it must have the ability to bind, either directly or after appropriate biochemical transformation, to protein so that a non-self-antigen can be produced.

Since it is the chemical which is the driving force, it is reasonable to consider that it is possible to relate chemical structure with the propensity to behave as a skin sensitizer. One of the earliest structure−activity studies in skin sensitization was reported by Landsteiner and Jacobs in the 1930s (*17*). Although the biological mechanism of sensitization was not at that time understood in any detail, they had already come to the view that sensitization to chemicals involved covalent bonding to protein. Their results on aromatic halides and pseudohalides showed a complete correspondence between ability to react with aniline, chosen as a model for protein and ability to sensitize. Further evidence for a relationship between reactivity and ability to sensitize came from a study of the guinea pig sensitization potential of α-substituted *p*-nitrotoluenes, $XCH_2C_6H_4NO_2$, reported in 1984. The compounds with X = H or OH did not react with *n*-butylamine, used as a model for protein nucleophiles, and were non-sensitizing, whereas the compounds with X = F, Cl, Br, I, and *p*-toluenesulfonyl all reacted as electrophiles with butylamine, the X group being displaced in an S_N2 reaction, and were all sensitizers (*18*). More recently, developments on quantitative structure−activity relationships have shown that the ability of a chemical to induce a skin sensitization reaction was a function of three parameters: the dose, the partition coefficient, and the rate constant toward a model nucleophile such as *n*-butylamine (*19*−*21*).

With the development of the local lymph node assay (*9*) it is now possible to apply conventional QSAR approaches in skin sensitization studies. The main reasons for this are that the LLNA uses a single dose per test group and then the extend of sensitization induced by this dose is quantified via measurement of the cellular proliferation induced in the lymph nodes draining the site of chemical application. The data are presented as a ratio, T/C, which compares the proliferation for each test group to that from a vehicle-treated control group and is taken as a quantifier of the degree of sensitization induced.

Within each series—eugenol and isoeugenol derivatives—compounds have been tested at the same molar concentration and have little differences in their partition coefficients. The difference in skin sensitization is therefore strongly related to their differences in chemical reactivity toward epidermal proteins.

Skin Sensitization to Eugenol and Its Derivatives. In contrast to the situation in guinea pigs, a significant degree of sensitization to eugenol was induced in the mouse, i.e., a dose dependent increase in proliferation and at least one test concentration inducing a 3-fold or greater increase (SI value ≥ 3) in isotope incorporation compared to vehicle-treated controls. The replacement of the methoxy group in eugenol by the isopropoxy group (compound **6a**) led to a complete loss of sensitization, indicating that this derivative is no longer metabolized into a reactive intermediate. This would suggest that a demethylation step occurs as part of the eugenol sensitization pathway (Figure 3). Previously it was assumed that demethylation is followed by oxidation to the *o*-quinone, which reacts as a Michael acceptor with protein to form antigen. Several studies on the reactivity of *o*-quinones (*22*, *23*) have demonstrated that attacks of nucleophiles were following the hard and soft acids and bases (HSAB) theory (*24*), i.e., 1,4 Michael addition for oxo and amino groups and 1,6 Michael addition for thio groups. This has been further confirmed by Bolton et al. on the *o*-quinone derived from hydroxychavicol with a major addition of glutathione in the 6-position (*7*). Previous studies on the sensitizing potential of 3-*n*-alkylcatechols, which are believed to be active through

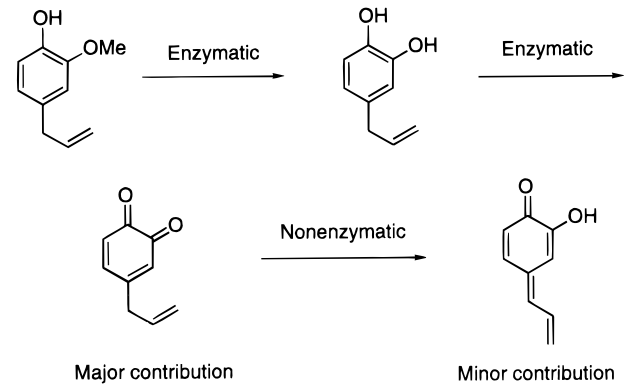


Figure 3. Proposed metabolic pathway for the sensitizing properties of eugenol **1**.

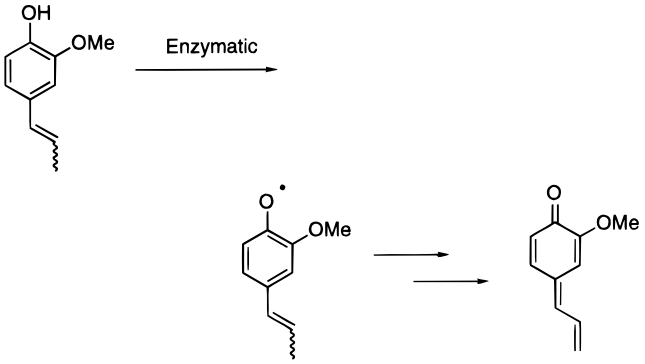


Figure 4. Proposed metabolic pathway for the sensitizing properties of isoeugenol **2**.

the formation of an *o*-quinone intermediate, have highlighted the fact that 1,4 addition positions, and thus amino nucleophiles were largely responsible for the allergenic activity (*25*−*27*). If the same theory applied to eugenol derivatives, introduction of a methyl group at the 5-position (compound **4a**) should have a pronounced effect on the sensitizing potential, while methylation at the 6-position should be less important. This is indeed what was observed with a decreased SI of 8.3 at 3.05 M for compound **3a** and a SI of only 4.3 for compound **4a** at the same concentration (the SI at 3.05 M for eugenol **1** is 16.1).

For the present we do not consider the data to be conclusive, but two additional pieces of evidence tend to favor the involvement of *o*-quinone. When a methyl group was present in the 3-position (compound **5a**) a significant degree of sensitization was induced only at the highest concentration of 54% (we could attribute this to a steric effect hindering attack on the methoxy group in demethylation) while the presence of three methyl substituents on the aromatic ring (compound **7**) completely abolished the sensitizing potential even at the highest concentration (3.05 M).

Skin Sensitization to Isoeugenol and Its Derivatives. In contrast to the situation with eugenol derivatives, the sensitization potential of isoeugenol in the mouse was not substantially affected when the methoxy group was replaced by the isopropoxy group (compound **6b**). This suggests that a demethylation step is probably not necessary for the formation of reactive metabolites and the findings of Bolton et al. (*7*, *8*) prompt us to consider direct oxidation of isoeugenol to a methoxy substituted *p*-quinone methide as the isoeugenol sensitization pathway (Figure 4).

The effects of methyl substitution on the sensitization potential of isoeugenol in the LLNA should provide a good test of the oxidation−quinone methide proposal. On the basis of the findings of Bolton et al. we can assume that if this proposal is correct, conjugation of the quinone methide to protein to produce antigen occurs by nucleophilic attack at the α-carbon atom. This reaction should be susceptible to steric retardation by methyl groups in the 3- or 5-positions but not in the 6-position. In complete agreement with this reasoning, 6-methylisoeugenol **3b** is very similar in sensitization potential to isoeugenol while 3-methylisoeugenol **5b** and 5-methylisoeugenol **4b** (Table 2) are significantly weaker sensitizers.

It is also important to note that the *tert*-butyl derivative **8** which cannot be oxidized directly to a quinone methide has a very low sensitizing potential comparable to the one of eugenol **1**. It could be assumed that when the direct oxidation into a *p*-quinone methide is not available, the more classical demethylation−*o*-quinone pathway is still available.

***p*-Quinone Methide vs *o*-Quinone Pathway.** In two recent papers (*7*, *8*) Bolton and co-workers have demonstrated a bioactivation pathway for the hepatocarcinogen safrole (1-allyl-3,4-methylenedioxybenzene) involving initial *O*-dealkylation of the methylene dioxy ring to form the catechol hydroxychavicol, oxidation of hydroxychavicol to the *o*-quinone, and tautomerization of the *o*-quinone to the *p*-quinone methide. As discussed by Bolton et al. *p*-quinone methides are much more electrophilic than their *o*-quinone tautomers and could account in the toxicity of such compounds. Bolton et al. studied the reactions with the nucleophile glutathione on oxidation intermediates generated from hydroxychavicol using either mushroom tyrosinase, silver oxide, or rat liver microsomes (cytochrome P450). The *o*-quinone generated by oxidation with tyrosinase in the presence of glutathione led to the formation of adducts on the aromatic ring, mainly in the C-6 position while the quinone methide produced by reaction with silver oxide was found to give, under kinetic control, the product resulting from attack of the nucleophile at the α-position, while under thermodynamic control, the product of γ-attack was obtained. Treatment of safrole or hydroxychavicol with rat liver microsomes in the presence of tritiated glutathione gave mainly adducts on the aromatic ring resulting from a reaction with the *o*-quinone intermediate.

When this paper was at an advanced stage of preparation, we received information in a private communication from Bolton of unpublished work in which isoeugenol oxidation was studied. Consistent with our hypothesis, Bolton and Leslie found that isoeugenol is more readily oxidized than eugenol. However, in experiments in which glutathione was added to trap the expected quinone methide, only minimal amounts of the expected quinone methide adducts were detected. One should keep in mind that the balance of metabolic activities in the skin does not necessarily reflect that found in the liver and that the biological mechanism of skin sensitization which involves the covalent modification of epidermal proteins mainly through amino groups is far from the detoxification process involving glutathione.

Conclusion

To summarize, our findings indicate that, in the mouse, eugenol seems to sensitize via a demethylation pathway

followed by oxidation to the *o*-quinone which seems to acts directly as a hapten even if we cannot exclude a reaction via its tautomeric quinone methide. Isoeugenol on the other hand could act via a mechanism not involving demethylation, for which the evidence reported here is consistent with a direct oxidation to the *p*-quinone methide.

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