and isoeugenyl isovalerate were prepared by means of the thionyl chloride method [2]. These esters showed, after purification, the same MS and GLC R_t values as those of the compounds in fraction 5. The MS of synthesised eugenyl valerate and isoeugenyl valerate did not show great differences from those of the isovalerates but the fragment at *m/e* 191 could not be observed in these spectra. The GLC R_t values were also different. The NMR-spectra of the isovalerates showed a doublet at δ 2.4 (J = 1.5 Hz) for $Me_2CH.\underline{CH_2}—COOR$, whereas the valerates showed a triplet at δ 2.5 (J = 7 Hz) for $Me—CH_2—CH_2.\underline{CH_2}.COOR$. The IR-spectra of the isovalerates showed a double band at 1185–1200 cm^{-1}; the valerates one band at 1200 cm^{-1}.

The distinction between the eugenyl- and isoeugenyl esters could be easily made with the aid of NMR- and IR-spectral data (see Experimental). The presence of eugenol has been reported in the essential oil of *Valeriana officinalis* L. var. *angustifolia* Mig [3]. Eugenyl isovalerate and isoeugenyl isovalerate recently have been reported in *Felicia* species [4].

EXPERIMENTAL

Isolation of the essential oil. 7300 g of *Valerian* root (air dried) was submitted to steam distillation according to ref. [5]. Yield 48.3 g (0.66%). 43.0 g of the essential oil was submitted to chromatography on a Si gel column (100 × 4 cm) using petrol (bp < 40°) containing an increasing percentage of Et_2O (0 → 100%). Twenty fractions of about 250 ml were collected. Fraction 5 was investigated by GLC using a 2 m × 2 mm column of Carbowax 20 M on chromosorb W HP 100–200 mesh; temp. 80–200° (4°/min); flow 25 ml N_2/min; FID. R_t bornylacetate (17% normalization method) 950 sec, valeranone (32%) 1810 sec, eugenyl isovalerate (3%) 2200 sec and isoeugenyl isovalerate (5%) 2580 sec. R_t eugenyl valerate 2290 sec and isoeugenyl valerate 2850 sec.

GC-MS. GC-MS was performed on a Finnigan 3300 quadrupole computerized system under the above gaschromatographic conditions. Electron energy 70 eV; scan speed 2 sec/scan; ion source 200°.

Spectral data. Eugenyl isovalerate. MS 248 (M^+), 191, 166, 165, 164 (100), 163, 149, 137, 133, 132, 131, 121, 104, 103, 91, 85, 77, 65, 57, 55, 51, 43, 41, 39. NMR, 60 MHz, CCl_4: δ 1,1 (6H, *d*, J = 6 Hz); ~1,9 (1H, *m*) 2,4 (2H, *d*, J = 1,5); 3,3 (2H, *d*, J = 7 Hz); 3,7 (3H, *s*); 5,1 (2H, *d*, J = 14 Hz); ~6,0 (1H, *m*); 6,7 (3H, arom.). IR ν_{max}^{NaCl} cm^{-1}: 910, 990, 1185–1200 (double band), 1640. Isoeugenyl isovalerate. MS: 248 (M^+), 191, 166, 165, 164 (100), 163, 149, 137, 133, 132, 131, 121, 104, 103, 91, 85, 77, 65, 57, 55, 51, 43, 41, 39. NMR: δ 1,1 (6H, *d*, J = 6 Hz); 1,8 (3H, *d*, J = 5 Hz); 1,9 (1H, *m*); 2,4 (2H, *d*, J = 15 Hz); 3,7 (3H, *s*); 5,5–6,4 (2H, *m*); 6,7 (3H, arom). IR ν_{max}^{NaCl} cm^{-1}: 960, 1185–1200 (double band). Eugenyl valerate. MS: 248 (M^+), 166, 165, 164 (100), 163, 149, 137, 133, 132, 131, 121, 104, 103, 91, 85, 77, 65, 57, 55, 51, 43, 41, 39. NMR: δ = 0,9 (3H, *t*, J = 6 Hz); 1,6 (4H, *m*); 2,5 (2H, *t*, J = 7 Hz); 3,3 (2H, *d*, J = 6 Hz); 3,7 (3H, *s*); 5,1 (2H, *d*, J = 14 Hz); ~6,0 (1H, *m*); 6,7 (3H, arom). IR ν_{max}^{NaCl} cm^{-1}: 910, 990, 1200, 1640. Isoeugenyl valerate. MS: 248 (M^+), 166, 165, 164 (100), 163, 149, 137, 133, 132, 131, 121, 104, 103, 91, 85, 77, 65, 57, 55, 51, 43, 41, 39. NMR: δ = 0,9 (3H, *t*, J = 6 Hz); 1,6 (4H, *m*); 1,8 (3H, *d*, J = 5 Hz); 2,5 (2H, *t*, J = 7 Hz); 3,7 (3H, *s*); 5,5–6,4 (2H, *m*); 6,8 (3H arom). IR ν_{max}^{NaCl} cm^{-1}: 960, 1200.

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EIN NEUES THYMOL-DERIVAT AUS *CALLILEPIS LAUREOLA*

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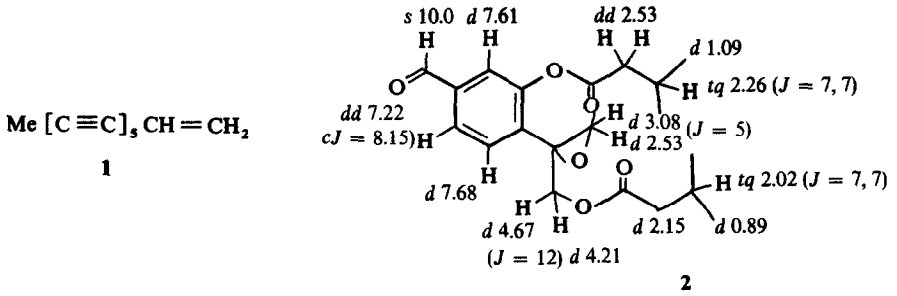
Key Word Index—*Callilepis laureola*; Compositae; new thymol derivative.

Die Wurzeln der in Südafrika heimischen *Callilepis laureola* DC. sind sehr toxisch, sie werden jedoch auch in der Volksmedizin als Wurmmittel sowie gegen verschiedene andere Krankheiten benutzt. Die Inhaltsstoffe der Wurzelknollen sind jedoch noch nicht untersucht worden. Neben dem weitverbreiteten Pentainen **1** [1] isoliert man praktisch als einzigen wesentlichen Inhaltsstoff einen Aldehyd der Summerformel $C_{20}H_{26}O_6$. Das ^{1}H-NMR-Spektrum bei 270 MHz läßt sofort erkennen, daß es sich um ein Epoxid handelt, das neben einer Aldehyd-Gruppe zwei Esterreste enthält, bei denen es sich um Isovaleriansäureesterreste handeln muß. Alle Daten sind nur vereinbar mit der Konstitution **2**. Während Thymolepoxide bei Compositen sehr verbreitet sind [2], haben wir bisher erst einen Aldehyd isoliert [2]. Weiterhin handelt es sich stets um Phenolisobutyrate. Insofern ist **2** doch ein neuartiger Typ. Die pharmakologische Prüfung diese Substanz ist noch nicht abgeschlossen.

Die Untersuchung weiterer Arten muß zeigen, ob Verbindungen vom Typ **2** für die Gattung charakteristisch sind.

EXPERIMENTELLES

IR: Beckman IR 9 in CCl_4; ^{1}H-NMR. Bruker WH 270, δ-



$$Me\,[C\equiv C]_5\,CH=CH_2$$

1

2

Werte in ppm, $CDCl_3$, TMS als innerer Standard. Die lufttrockenen Wurzeln (560 g, im Februar 1977 in Natal gesammelt, Herbar Nr. 77/56) wurden zerkleinert und mit Ether–Petrol (1:2) bei RT extrahiert. Den Extrakt trennte man zunächst grob durch SC (Si gel, Akt.-St. II) und anschließend durch DC (Ether–Petrol 1:3). Man erhielt 0.2 mg **1** und 1.8 g **2**.

7-Oxo-10-isovaleryloxy-8,9-dihydro-8,9-epoxy-thymolisovalerat (**2**). Farbloses Öl. IR: PhOCOR 1765; ROCOR 1740; CHO 2710, 1703; Aromat 1610, 1565 cm^{-1}. MS: M^+ *m/e* 362 (<0.1%) (mit Cl (Isobutan also Stoßgas: 363); $-Me_2CHCH_2CO_2H$ 260.105 (6) (ber. für $C_{15}H_{16}O_4$ 260.105); 260 $-Me_2CH-CH=O$ 176 (22); $C_4H_9CO^+$ 85 (100); 85 $-CO$ 57 (96).

$$[\alpha]_{24}^{\lambda} = \frac{589 \quad 578 \quad 546 \quad 436\,nm}{-37.4 \quad -39.2 \quad -45.4 \quad -87.7^\circ} (c = 7.55,\ CHCl_3)$$

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MECHANISM OF ACTION OF LIGNINS AND LIGNIN MODEL COMPOUNDS WITH HORSERADISH PEROXIDASE*

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Key Word Index—lignins; lignin model compounds; horseradish peroxidase.

Abstract— Horseradish peroxidase displayed a ping-pong kinetic reaction mechanism with lignin model compounds and lignins. Oxidation of the α carbon on acetosyringone or acetovanillone failed above pH 6.5, while conversion of α-methylsyringyl (or guaiacyl) alcohol to acetosyringone (or vanillone) occurred optimally at pH 7.8. Small MW fragments were not formed from lignins at pH 6.4 and 7.8. These observations provide evidence for the growing concept that freely soluble peroxidase is not a lignolytic enzyme.

INTRODUCTION

Lignins are heteropolymeric molecules composed mainly of singly or doubly methoxylated phenylpropyl phenolic units [1]. They are degraded by microorganisms [2–4]. The enzymological approaches are virtually unknown [5], though polyphenoloxidases such as peroxidase and laccase have long been suspected [3]. The reaction between laccase and maple milled wood lignin involves simultaneous depolymerization processes [6], and although long-lived phenoxy radicals are produced during the oxidative reaction of monomeric and dimeric syringyl derivatives with horseradish peroxidase [7], subsequent coupling reactions between mesomeric aryloxy radicals that are produced in these reactions, as well as in laccase-catalyzed reactions, virtually negate any lignolytic effect these enzymes may have [8].

In lignin model studies it has been shown that α-methylsyringyl alcohol is converted by horseradish peroxidase to acetosyringone, which is oxidized further to a mixture of syringyl hydroquinone and 3-methoxy-5-acetyl-*o*-hydroquinone [9]. Free radical intermediates are involved in these reactions, as well as in reactions between horseradish peroxidase and hardwood lignins

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