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5-(12-HEPTADECENYL)-RESORCINOL, THE MAJOR COMPONENT OF THE ANTIFUNGAL ACTIVITY IN THE PEEL OF MANGO FRUIT

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Key Word Index *Mangifera indica*; Anacardiaceae; mango; *Alternaria alternata*; 5-(12-heptadecenyl)-resorcinol;

Abstract – A mixture of 5-(12-*cis*-heptadecenyl)- and 5-pentadecyl-resorcinol was isolated from the peel of mango fruit; it acts as a preformed agent against *Alternaria alternata*, a fungus responsible for the black spot disease of mango fruits in Israel. The chemical characterization was done by ^{1}H NMR, ^{13}C NMR and MS.

INTRODUCTION

The fungus *Alternaria alternata* (Fr.) Keissler is responsible for the black spot disease of mango (*Mangifera indica* L.) fruits in Israel [1, 2]. The latency in its development is due [3] to a preformed antifungal activity present in the peel of unripe mango fruits.

We now report that this material is a mixture of 5-substituted resorcinols, whose major components are 5-(12-*cis*-heptadecenyl)-resorcinol (**1a**) (65%) and 5-pentadecylresorcinol (**2a**) (15%). Mango belongs to the Anacardiaceae, the main source of mono- [4] and diphenols [5, 6] substituted in the aromatic ring with long aliphatic chains. 5-(10-Pentadecenyl)-resorcinol was isolated from the vesicant exudate of seed pods of *Grevillea pyramidalis* (Proteaceae) [7]. To our knowledge, 5-substituted resorcinols with 17 carbon atoms in the side chain are not described. Higher homologues, 5-nonadecyl- and 5-heneicosylresorcinol were isolated from the unsaponifiable fraction of wheat bran [8].

RESULTS AND DISCUSSION

The biologically active material was purified by repeated chromatography (CC, prep. TLC and reversed phase HPLC) [3]. All stages of purification were monitored by bioassay on TLC plates (assay organisms, *Cladosporium cucumerinum* and *A. alternata*). The partially purified product obtained after the first column chromatography contained sitosterol and esters thereof with palmitic (52.8%), oleic (16.5%), linoleic (10.1%) and linolenic (15.8%) acids, which were identified by GC after methanolysis.

The product obtained after HPLC had λ_{max}^{EtOH} nm (ε): 280 (860), 274 (920) and 233 (1900); after addition of sodium hydroxide (pH ~ 11), λ_{max} 292 (920) and 244 (1400). Such absorptions are characteristic for a resorcinol type moiety [9]. Further characterization was done by ^{1}H (Table 1) and ^{13}C NMR spectra, as well as by EIMS and CIMS. The structural information was completed by analysis of several derivatives: acetates (**1b**, **2b**), trimethylsilyl ethers (**1c**, **2c**), epoxide (**1d**) and the isomeric dimethylamino alcohols **1e** and **1f** obtained from the latter.

The spectra (Table 1) indicated a 3,5-dihydroxyphenyl unit connected to a long, linear aliphatic chain including a disubstituted double bond. These signals can fit only a structure such as **1a** in which $m + n = 14$, without defining the actual values of m and n. The distorted triplet pattern of the primary methyl indicated that it should be at least three bonds away from the double bond. The assignments of the signals due to the benzylic, homobenzylic, allylic and vinylic protons were supported by decoupling experiments. The intensity of the δ 5.34 and 2.01 signals was less than required for two and four protons, respectively, thus pointing to the presence of the saturated companion **2a**.

The ^{13}C NMR data confirm these results. One finds the $ArCH_2CH_2$ unit [156.53 (C-3, 5), 146.10 (C-1), 108.01 (CH, C-2, 6), 100.16 (CH, C-4), 35.84 (CH_2, α), 31.05 (CH_2, β)], as well as proof of the *cis* configuration of the double bond [10] [129.87 (olefinic CH) and 27.22 (allylic CH_2)]. Although the last three carbons of the straight chain appear at the well known positions [10], each of them is split into two or three peaks of unequal intensity, indicating heterogeneity due to double bond positional isomers and/or homologues [14.07, 14.10 (terminal Me); 22.58, 22.66 ($\underline{C}H_2Me$); 31.52, 31.78, 31.91 ($\underline{C}H_2CH_2$ Me)].

The mass spectrum (direct inlet) of the mixture of **1a** and **2a** shows a signal for the molecular ion of **1a** [M]$^+$ 346 (EI) and [MH]$^+$ 347 (CI) and another signal for the molecular ion of **2a** [M]$^+$ 320 (EI) and [MH]$^+$ 321 (CI). The EI mass spectrum is dominated by benzylic fragmentation, common to both compounds, with transfer of one H [11], which leads to the base peak at m/z 124. In addition to the molecular ions and to the above base peak, there are several less abundant signals which are instrumental in determining the position of the double bond in **1a**: (a) m/z 97 and 250 (cleavage of the 9,10-bond with hydrogen transfer in the ion containing the aromatic ring); (b) m/z 303 (cleavage of the 14,15-

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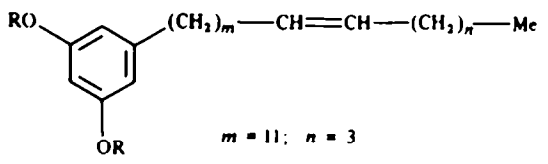
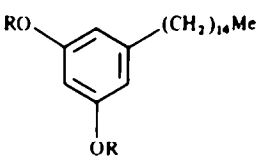
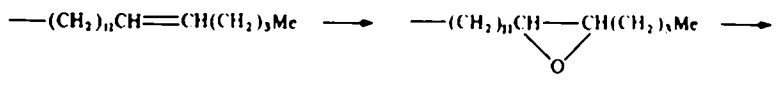




1a R = H
1b R = Ac
1c R = $SiMe_3$

2a R = H
2b R = Ac
2c R = $SiMe_3$



1b **1d**

1e **1f**

Table 1. ^{1}H NMR data of 5-substituted resorcinols (δ values)

Proton	Multiplicity	Compound		
		1a + **2a**	**1b** + **2b**	Epoxy-**1b** + **2b**
Me	distorted *t* (6.5)	0.88	0.88	0.88
Benzylic CH_2	*t* (7.5)	2.46	2.59	2.59
Homobenzylic CH_2	*br quintet* (7.5)	1.55	1.59	1.59
Allylic CH_2	*br q* (6)	2.01	2.01	1.49*
Vinylic CH	*t* (5)	5.34	5.34	—
Epoxidic CH	*br t* (4)	—	—	2.91
Other chain CH_2	*m*	1.25–1.34	1.25–1.34	1.25–1.34
Aryl H-4,6	*d* (2.5)	6.24	6.79	6.79
Aryl H-2	*t* (2.5)	6.18	6.73	6.73
OAc	*s*	—	2.28	2.28

*α to epoxide, *br m*.

bond); (c) *m/z* 137 and 166 (cleavage of the 2,3-bond). The fragment *m/z* 166, which contains carbons 3–14, is due to additional allylic cleavage. These fragmentations indicate position 12,13 for the double bond.

The mixture of diacetates **1b** and **2b** was separated and analysed by GC/MS. The faster running fraction (15%; **2b**) gave under CI conditions $[MH]^+$ at *m/z* 405, $[MH - COCH_2]^+$ 363 (base peak) and $[MH - 2COCH_2]^+$ 321. The slower running fraction (65%; **1b**) gave $[MH]^+$ at *m/z* 431, $[MH - COCH_2]^+$ 389 (base peak) and $[MH - 2COCH_2]^+$ 347. The molecular weight of **1a** was further confirmed by the MS of the bis-trimethylsilyl derivative **1c** (EI), $[M]^+$ *m/z* 490 and $[M - C_{16}H_{30}]^+$ (benzylic fragmentation) 268.

Treatment of **1d** with dimethylamine [12] afforded two isomeric vicinal dimethylamino alcohols **1e** and **1f**, whose major fragmentation involves cleavage of the bond between the carbons bearing the hydroxyl and dimethylamino groups, with charge retention on the latter. Indeed, fragmentation of **1e** led to formation of an ion at *m/z* 404, whereas that of **1f** resulted in an ion at *m/z* 114 (EI).

The only doubt concerning the significance of these results is that fragment *m/z* 404 has the same mass as the molecular ion of **2b**. However, this ion was not obtained from **2b** under EI conditions. Under CI conditions, ions of *m/z* 405 may be due to: (a) fragmentation of dimethylamino alcohol **1e**; (b) $[MH]^+$ of **2b**; (c) fragment $[MH - COCH_2]^+$ from **1d**. The relative intensity of the ion current of fragment *m/z* 405 derived from source (a) increased more than fourfold as compared to that derived from source (c). The concentration of **2b** must remain constant, since the two chemical processes, epoxidation

and subsequent opening of the epoxide are not operative with this compound. The above increase in the intensity of the ion current points therefore to source (a) for ion m/z 405, rather than (b) and/or (c).

The double bond in 5-heptadecenylresorcinol is therefore assigned position 12. Naturally occurring 5-(8-pentadecenyl)-resorcinol (cardol) is considered to be biosynthesized by initial coupling of palmitoleyl-coenzyme A with three malonyl-coenzyme A units [13]. A similar process with oleyl-coenzyme A as starter unit would lead to 5-(8-heptadecenyl)-resorcinol, a double bond isomer of **1a**. The different position of the double bond might be due to isomerization, or to a starter unit having a 13-octadecenoyl rather than 9-octadecenoyl structure. In **1a**, the position of the double bond with respect to the end of the alkenyl chain, is the same as in 5-(10-pentadecenyl)-resorcinol [7].

EXPERIMENTAL

NMR spectra were recorded on a Bruker AM-300 Fourier transform instrument operating at 300 (^{1}H) and 75.5 MHz (^{13}C). Chemical shifts correspond to ppm relative to internal TMS for $CDCl_3$ solns. ^{1}H connectivities were proven by double irradiation; ^{13}C signal multiplicities follow from a single frequency off-resonance decoupled (SFORD) spectrum. MS were obtained by direct probe inlet in a Finnigan 4021 quadrupole instrument equipped with a data system. EI conditions were: emission current 0.30 mA; EM 1.7 kV; electron energies, 25–70 eV. CI conditions were: reagent gas, isobutane; ionizing potential, 70 eV. GC/MS was carried out on a SP-2310 glass capillary column (20 m, 0.4 mm i.d.) coupled via a fused silica capillary interface to the Finnigan instrument (CI, 70 eV, 250°). The samples were splitlessly introduced; column temp. was programmed as follows: 100°, 2 min isothermal, then 8°/min up to 270°.

Work-up of plant material and isolation of the product, see ref. [3]. Acetylation (Ac_2O–pyridine, overnight, room temp.), silylation [bis-(trimethylsilyl)-trifluoroacetamide in $HCONMe_2$, 1 hr, 50°) and epoxidation (*m*-chloroperbenzoic acid in $CHCl_3$; overnight, room temp.) were done by standard procedures.

Treatment of **1d** *with dimethylamine.* Excess Me_2NH was added to a soln of **1d** (2 mg) in Et_2O at O° and the obtained soln was kept for 1 hr at this temperature; solvent and excess reagent were then removed with a stream of N_2.

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