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METHOXYSTYRENES FROM THE GENUS *ZIERIA**

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Key Word Index—*Zieria smithii*; *Z. chevalieri*; Rutaceae; essential oil; styrene monomers.

Abstract—A new group of aromatic ethers has been isolated from the essential oils of several *Zieria* species. ^{1}H NMR and MS have indicated trimethoxy-, tetramethoxy- and dimethoxymethylenedioxy-styrene structures while chemical evidence has suggested probable methoxylation patterns.

INTRODUCTION

As part of our continuing studies on the phytochemistry of the Australian flora, we are examining the essential oils of the genus *Zieria* (tribe Boronieae, family Rutaceae) which is currently undergoing taxonomic revision [1]. All but one species, *Z. chevalieri* from New Caledonia, are endemic to eastern Australia. Gas chromatography of the essential oils of specimens of *Z. chevalieri*, an undescribed subspecies of *Z. smithii* and an undescribed species of *Zieria* indicated several poorly volatile components which when isolated by liquid chromatography showed strong tendencies to polymerize. This communication reports the isolation of three natural styrene monomers. Spectroscopic examination and chemical modification of the most stable of these has established it as 2,3,4,6-tetramethoxystyrene (**1**). Because of the ease of polymerization of the remaining two monomers, only tentative structures of 2,6-dimethoxy-3,4-methylenedioxystyrene (**2**) and 2,4,6-trimethoxystyrene (**3**) are proposed.

RESULTS AND DISCUSSION

The essential oil of an undescribed subspecies of *Z. smithii* from Mount Warning, N.S.W. contained 59% of a colourless high-boiling liquid (*RR*, 1.94 with respect to safrole) for which combustion analysis and high resolution mass spectrometry indicated a $C_{12}H_{16}O_4$ formula. Four ^{1}H NMR aromatic methoxyl signals at δ 3.81, 3.83, 3.84 and 3.88, one aromatic proton signal at δ 6.29 and an ABX system at δ 5.35, 6.00 and 6.82 indicated a tetramethoxystyrene. That the aromatic proton was flanked by two *ortho*-methoxyl substituents was established in two ways. Two methoxyl groups were shown by double irradiation experiments to be coupled (0.3 Hz) to the aromatic proton consistent with *ortho*-substituted methoxybenzenes [2]. The same methoxyl signals shifted upfield by 50 and 51 Hz when the spectrum was determined using deuterated benzene as solvent. Hence these signals arise from less sterically hindered methoxyl groups than the other two signals which only shifted upfield by 8 and 9 Hz [3]. The biogenetically unlikely 2,3,5,6-tetramethoxystyrene structure (**4**) was positively eliminated by oxidation to the benzoic acid with alkaline potassium permanganate and methylation with methyl iodide. The chemical shift of the aromatic proton of the resultant ester (δ 6.27) was significantly upfield of the proton in the known 2,3,5,6-tetramethoxybenzoate (**5**) (δ 6.60) [4] and hence assigned to methyl 2,3,4,6-tetramethoxybenzoate (**6**). Mayen and Marechal [5] attempted a synthesis of this styrene but were thwarted by polymerization during the oxalic acid dehydration of the 2,3,4,6-tetramethoxyphenylethanol.

The second most abundant styrene constituted 41% of the oil of an undescribed *Zieria* species from Mount Barney, Queensland which had affinities with *Z. arborescens* and *Z. smithii*. GC–MS indicated a component with retention time 2.37 relative to safrole and a $C_{11}H_{12}O_4$ formula. Two ^{1}H NMR aromatic methoxyl signals at δ 3.78 and 3.97, one methylene-dioxy signal at δ 5.88, one aromatic proton signal at δ 6.28 and an ABX system at δ 5.48, 6.26 and 7.20 indicated a dimethoxymethylenedioxystyrene. As with the tetramethoxystyrene, double irradiation and solvent shift experiments ($CDCl_3$ to C_6D_6) were informative. They showed that the one coupled (0.3 Hz) methoxyl was *ortho* to the aromatic proton as its signal moved upfield by 54 Hz in benzene in contrast to the uncoupled signal which moved upfield 21 Hz. Methylenedioxy groups are invariably attached at one end *para* to the alkyl side chain of phenylpropenoids and consequently on biogenetic grounds this styrene can be assigned as either 2,5- or 2,6-dimethoxy-3,4-methylenedioxystyrene, **7** or **2** respectively. In parsley apiole (**8**) which shares similar substitution to **7**, the less hindered methoxyl signal shifts upfield by only 31 Hz when the spectrum was run in C_6D_6 [3]. Thus the greater upfield shift (54 Hz) by the styrene indicates the alternate structure 2,6-dimethoxy-3,4-methylenedioxystyrene (**2**).

IR, UV and GC/MS analysis of *Z. chevalieri* oil revealed evidence of 31% of a third styrene with a retention time 2.05 relative to safrole and a $C_{11}H_{14}O_3$ formula. MS comparison showed similar fragmentation to other styrenes. UV comparison showed identical absorption to authentic 3,4,5-trimethoxystyrene. All isolation procedures resulted in polymerization. On biogenetic grounds, structures unsubstituted *para* to the

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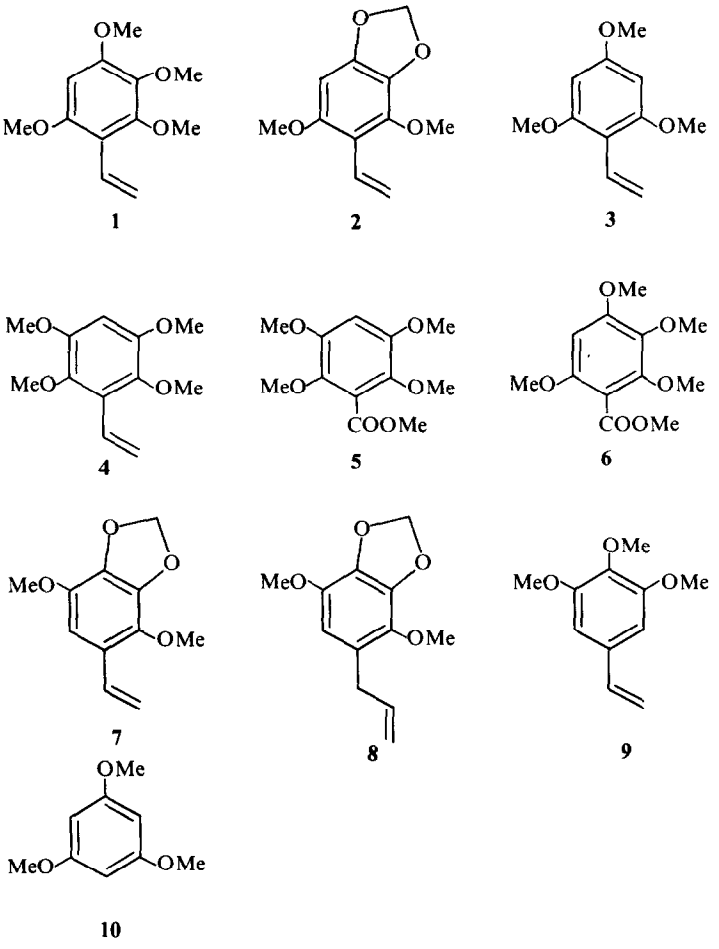

alkyl group are unlikely and comparison with authentic 3,4,5-trimethoxystyrene (**9**) eliminated the elemicin analogue. Co-occurrence in *Z. chevalieri* with 1,3,5-trimethoxybenzene (**10**) and styrenes **1** and **2** suggest the tentative structure of 2,4,6-trimethoxystyrene (**3**).

This is, to our knowledge, the first report of polymethoxylated styrene monomers in essential oils. Their isolation in significant quantities presents opportunity for much speculation concerning their taxonomic significance, their biogenetic origins and their potential as precursors of a new group of bipolymers.

EXPERIMENTAL

IR spectra were determined on liquid films or nujol mulls. UV absorption maxima were measured in MeOH. ^{1}H NMR spectra were run at 100 Hz in $CDCl_3$ or C_6D_6 with TMS as an internal standard. GLC retention times were measured relative to safrole as internal standard on a 30 m by 0.5 mm i.d. glass SCOT column coated with FFAP as stationary phase and programmed after 3 min at 80° to 170°, at 6° per min. MS were determined at 70 eV for both high resolution and GC/MS analyses.

Plant material. An as yet undescribed subspecies of *Z. smithii* was collected from Mt. Warning near Murwillumbah in north-eastern N.S.W. (N.S.W. Herbarium: J. Armstrong 49, I. Southwell 80.10). An as yet undescribed species of *Zieria* with affinities to *Z. smithii* and *Z. arborescens* was collected from Mt. Barney near Beaudesert in south-eastern Queensland (N.S.W. Herbarium: J. Armstrong 1141, 1142; I Southwell 80.17). *Z. chevalieri* was collected from a plant cultivated from material originally collected from Mt. Kaala in New Caledonia (N.S.W. Herbarium: J. Armstrong 1212). Leaf and terminal branchlets of these species were steam distilled in an all glass apparatus [6].

The *Z. smithii* subspecies yielded 0.7% of a pale yellow oil, n_D^{20} 1.532, $[\alpha]_D$ + 1.8° (neat oil), d_4^{20} 1.058. This oil (1.0 g) was applied to an activated alumina (100 g) column in petrol (40–60°) and eluted consecutively with 500 ml of each of the following solvent systems: (a) petrol, (b) 1% Et_2O/petrol, (c) 2% Et_2O/petrol, (d) 5% Et_2O/petrol, (e) 10% Et_2O/petrol, (f) 20% Et_2O/petrol, (g) 50% Et_2O/petrol and (h) Et_2O. Fraction (f) contained 2,3,4,6-tetramethoxystyrene (**1**) (0.27 g, 96% pure by GLC). Found: C 64.6; H 7.3. $C_{12}H_{16}O_4$ requires C 64.3; H 7.2%, M^+ 224.1047. $C_{12}H_{16}O_4$ requires 224.1048. IR ν_{max} cm^{-1}: 1624, 1602, 1582, 1500, 1476, 1415, 1390, 1348, 1237, 1195, 1130, 1112, 1026, 972, 920, 862, 805. UV λ_{max} nm (ε): 225 (21 500), 262 (13 300), 295 (3900). ^{1}H NMR: δ ($CDCl_3$) 3.81 (3 H, *s*, C2 or C3 OMe), 3.83 (3 H, *d*, $J_{C5-H,OMe}$ = 0.3 Hz, C4 or C6 OMe), 3.84 (3 H, *s*, C2 or C3 OMe), 3.88 (3 H, *d*, $J_{C5-H,OMe}$ = 0.3 Hz, C4 or C6 OMe), 5.35 (1 H, *dd*, J_{gem} = 3 Hz, J_{cis} = 12 Hz, (E)-C_βH), 6.00 (1 H, *dd*, J_{gem} = 3 Hz, J_{trans} = 18 Hz, (Z)-C_βH), 6.29 (1 H, *br. s* $Wh/2$ = 1.5 Hz, C5-H), 6.82 (1 H, *dd*, J_{cis} = 12 Hz, J_{trans} = 18 Hz, C_αH); δ (C_6D_6) 3.33 (3 H, *d*, $J_{C5-H,OMe}$ = 0.3 Hz, C4 or C6 OMe), 3.37 (3 H, *d*, $J_{C5-H,OMe}$ = 0.3 Hz, C4 or C6 OMe), 3.73 (3 H, *s*, C2 or C3 OMe), 3.75 (3 H, *s*, C2 or C3 OMe), 5.52 (1 H, *dd*, J_{gem} = 3 Hz, J_{cis} = 12 Hz, (E)-C_βH), 6.01 (1 H, *br. s*, C5-H), 6.38 (1 H, *dd*, J_{gem} = 3 Hz, J_{trans} = 18 Hz, (Z)-C_βH), 7.24 (1 H, *dd*, J_{cis} = 12 Hz, J_{trans} = 18 Hz, C_αH). MS, 70 eV, *m/z* (rel. int.): 224 (100), 209 (90), 166 (23), 225 (13), 95 (13).

The undescribed species of *Zieria* yielded 0.3% of a pale yellow oil, n_D^{20} 1.517, $[\alpha]_D$ + 15.6° (neat oil), d_4^{20} 0.996. This oil (1.4 g) was applied to an activated alumina column in petrol (b.p. 60–80°) and eluted consecutively with 500 ml of (a) petrol, (b) 1% Et_2O/petrol, (c) 2% Et_2O/petrol and (d) 1000 ml of 5% Et_2O/petrol. The second 250 ml fraction of (d) contained dimethoxymethylenedioxystyrene (**2**) (0.05 g, 95% pure by GLC). IR ν_{max} cm^{-1}: 1650, 1630, 1520, 1500, 1470, 1445, 1410, 1230, 1200, 1132, 1117, 1075, 1040, 935, 910, 805. UV λ_{max} nm (ε): 227 (23 700), 267 (14 200), 313 (6900). ^{1}H NMR: δ ($CDCl_3$) 3.78 (3 H, *br. s*, *W h*/2 = 0.8 Hz, C6-OMe), 3.97 (3 H, *s*, *W h*/2 = 0.6 Hz, C2-OMe), 5.32 (1 H, *dd*, J_{gem} = 3 Hz, J_{cis} = 12 Hz, (E)-C_βH), 5.88 (2 H, *s*, OCH_2O), 5.93 (1 H, *dd*, J_{gem} = 3 Hz, J_{trans} = 18 Hz, (*Z*)-C_βH), 6.28 (1 H, *br. s*, *W h*/2 0.8 Hz, C5-H), 6.83 (1 H, *dd*, J_{cis} = 12 Hz, J_{trans} = 18 Hz, C_αH); δ (C_6D_6) 3.24 (3 H, *d*, *J* = 0.3 Hz, C6-OMe), 3.76 (3 H, *s*, C2-OMe), 5.27 (2 H, *s*, OCH_2O), 5.48 (1 H, *dd*, J_{gem} = 3 Hz, J_{cis} = 12 Hz, (E)-C_βH), 6.09 (1 H, *br. s*, *W h*/2 1 Hz, C5-H), 6.26 (1 H, *dd*, J_{gem} = 3 Hz, J_{trans} = 18 Hz, (Z)-C_βH), 7.20 (1 H, *dd*, J_{cis} = 12 Hz, J_{trans} = 18 Hz, C_αH). GC/MS, 70 eV, *m/z* (rel. int.): 208 (100), 163 (72), 193 (50), 135 (29), 107 (28), 77 (18), 53 (18), 165 (16), 64 (14), 109 (14). This fraction also contained an ether-insoluble white solid polydimethoxymethylenedioxystyrene. IR ν_{max} cm^{-1}: 1645, 1625, 1190, 1125, 1070, 1040, 935. ^{1}H NMR δ (C_6D_6) 2.3 (2*n*H, *br.* nCH_2), 3.3 (4 *n*H, *br. n* C6-OMe, *n* Ar-CH), 3.85 (3 *n*H, *br. n* C2-OMe), 5.4 (2 *n*H, *br.*, *n* OCH_2O), 6.0 (*n* H, *br.*, *n* Ar-H).

Z. chevalieri yielded 0.5% of a colourless oil with major component (31%) suspected 2,4,6-trimethoxystyrene. IR (whole oil) ν_{max} cm^{-1}: 1625, 1610, 1590, 1500, 1198, 1186, 1149, 1122, 1060, 1035, 812, 785. UV (whole oil) λ_{max} nm (ε): 213 (6800), 226 (10 700), 270 (6300). GC/MS, 70 eV, *m/z* (rel. int.): 194 (88), 179 (100), 151 (26), 121 (59), 91 (22), 77 (23), 69 (23), 51 (19). Attempted separation of the major component on alumina yielded an ether insoluble polytrimethoxystyrene. ^{1}H NMR δ (C_6D_6): 2.3 (*br. m*, CH_2's) 3.4 (*br. m*, OMe's), 5.9 (*br. m*, ArH's). The other significant oil components were found to be myrcene (23%), naphthalene (17%), 1,3,5-trimethoxybenzene (9%) and 2,3,4,6-tetramethoxystyrene (3%).

Methyl 2,3,4,6-tetramethoxybenzoate (**6**). 2,3,4,6-Tetramethoxystyrene (**1**) (0.5 g) was added to a solution of $KMnO_4$ (1.5 g) and KOH (0.2 g) in cold water and stirred for 1 hour at room temp. [7]. $Na_2S_2O_5$ and dil. HCl were added to dissolve the MnO_2 sludge and the aq. layer was extracted with Et_2O. The acid product was extracted from the organic phase with $NaHCO_3$ soln, re-acidified and extracted into Et_2O to reveal a brown gummy product which was methylated with $MeI/K_2CO_3/Me_2CO$ at room temp. for 16 hr. Pure methyl 2,3,4,6-tetramethoxybenzoate (**6**) was then eluted from an alumina column in 5% Et_2O/petrol to yield a colourless liquid. IR ν_{max} cm^{-1}: 1760, 1620, 1520, 1365, 1240, 1200, 1140, 1116, 1050, 1005, 970, 920, 858, 813. ^{1}H NMR δ ($CDCl_3$) 3.79, 3.88, 3.90, (3 × 3 H, *s*, C1-COOMe, C2-OMe, C3-OMe) 3.84, 3.93 (2 × 3 H, *br. s*, C4-OMe, C6-OMe), 6.27 (1 H, *s*, C5-H).

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