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Short Communication

Synthesis and X-Ray Structure of 2-(3-Methyl-2-butenyl)-3,4,5-trimethoxyphenol: a Potent Anti-Invasive Agent Against Solid Tumours

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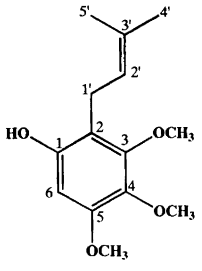
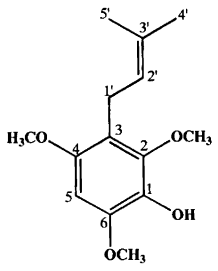
The isolation of the title compound **1** (Fig. 1) from the dichloromethane extract of the leaves and stems of *Piper clarkii* has recently been reported by us.[1] The structure was elucidated on the basis of UV, IR, ^{1}H NMR, ^{13}C NMR and mass spectral data, the relative positions of the hydroxyl group and three methoxyl groups were established on the basis of NOE experiments.[1] Recently activity testing revealed that the compound **1** possesses strong anti-invasive activity against human breast carcinoma cells.[2] The activity shown by the compound prompted us to make the compound in larger amounts for evaluation of its detailed biological activity. The X-ray diffraction studies carried out on the natural sample confirmed its structure as 2-(3-methyl-2-butenyl)-3,4,5-trimethoxyphenol.



1



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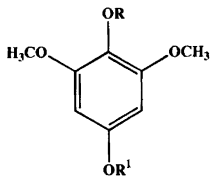

3 R=R^1=H
4 R=H, R^1=CH_3
5 R=CH_3, R^1=H

Fig. 1. Structures of investigated compounds.

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Results and discussion

The synthesis of 2-(3-methyl-2-butenyl)-3,4,5-trimethoxyphenol (**1**) was completed in five steps starting from 1,2,3-trimethoxybenzene, which was subjected to nitric acid oxidation to give 2,6-dimethoxybenzoquinone,[3] followed by reduction with sodium dithionite to give 2,6-dimethoxyhydroquinone (**3**). Partial methylation of **3** with one equivalent of dimethyl sulfate gave a mixture of two compounds, which was separated by column chromatography over silica gel to yield 2,4,6-trimethoxyphenol (**4**)[4] as a brown viscous oil and 3,4,5-trimethoxyphenol (**5**) as a light brown crystalline solid. Compound **5** is also known as antiarol, and is reported to occur in the needles of *Antiaris toxicaria*;[5] the melting point of our synthetic sample of **5** was quite comparable with the natural sample. The detailed and complete spectral data for **4** and **5** have not been reported thus far. We have characterised both **4** and **5** fully from their spectral data (cf. Experimental section). Compound **5** on prenylation with 1 equiv. of 2-methylbut-3-en-2-ol in dry dioxane in the presence of boron trifluoride etherate yielded, after purification by preparative TLC, a white coloured solid, identical in all

respects with the natural sample of **1**.[1] The mixed melting point of synthetic **1** and the natural sample of **1** remained undiminished and the two samples recorded superimposable IR spectra.

The X-ray crystal structure of the molecule [(Fig. 2), of the natural sample] reveals that the prenyl group is flanked by a hydroxy and a methoxy group, and the three methoxy groups are on adjacent carbon atoms of the benzene ring.

Compound **4** was also subjected to prenylation to get, after purification by preparative TLC 3-(3-methyl-2-butenyl)-2,4,6-trimethoxyphenol (**2**), the positional isomer of **1** as a light brown viscous oil. It showed a molecular ion peak at *m/z* 252 and showed a broad absorption band at 3410 cm^{-1} in its IR spectrum, showing the presence of an -OH group. In the ^{1}H-NMR spectrum two singlets (each integrating for three protons) at δ 1.67 and 1.77 for the *gem* dimethyl group, a triplet at δ 5.16 for the vinylic proton and a doublet at δ 3.27 for the benzylic protons showed the presence of a nuclear prenyl group. A singlet for one aromatic proton was obtained at δ 6.32; three singlets at δ 3.77, 3.83 and 3.87 (each integrating for three protons) could be assigned to three methoxy groups. The mass spectral fragmentation pattern and ^{13}C-NMR spectral data were also in agreement with the proposed structure.

Experimental

All the melting points, determined in a sulfuric acid bath by the capillary method, are uncorrected. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AC-250 spectrometer at 250 and 62.9 MHz, respectively. The ^{1}H-NMR spectra were also recorded on Hitachi R-600 FTNMR spectrometer at 60 MHz. The mass spectra were recorded on a Varian MAT 311A mass spectrometer at 70 eV.

X-Ray crystallography. All measurements were made using a Siemens P3R3 four-circle diffractometer equipped with an Oxford Cryosystems cryostream cooler (version 2.4). Graphite monochromated Mo K_α radiation (λ = 0.710 73 Å) was used to collect the intensity data in the ω-2θ mode. Unit cell parameters and orientation matrices were obtained by least-squares refinement of the setting angles of 22 high-angle reflections.

The crystallographic program system was SHELXTL PLUS[6] and SHELXL-93;[7] the refinement program uses atomic scattering factors taken from Ref. 8. The structure was solved by direct methods and refined using full-matrix least-squares on F^2. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were inserted using a riding model and given isotropic thermal parameters equal to 1.2 (or 1.5 for methyl groups) times the equivalent isotropic displacement parameter of the atom to which it is attached. The weighting scheme was of the form $w^{-1}=[\sigma^2(F_o)^2+(0.0669P)^2+0.4184P]$ where $P=[\text{max. } (F_o^2,0)+2F_c^2]/3$. The R factors are defined as follows: $R_1=\sum\|F_o|-|F_c\|/\sum|F_o|$ and $wR_2=[\sum[w(F_o^2-F_c^2)^2]/\sum[w(F_o^2)^2]]^{1/2}$.

A summary of the crystal data and refinement details is given in Table 1; atomic coordinates and selected bond lengths and angles are given in Tables 2 and 3. Additional material, available from the Cambridge Crystallographic Data Centre, includes a full list of bond lengths and angles, thermal parameters and hydrogen coordinates.

2,4,6-Trimethoxyphenol (4) and 3,4,5-trimethoxyphenol (5). 2,6-Dimethoxyhydroquinone *(3)* (680 mg) was dissolved in dry acetone; K_2CO_3 (ca. 700 mg) and dimethyl

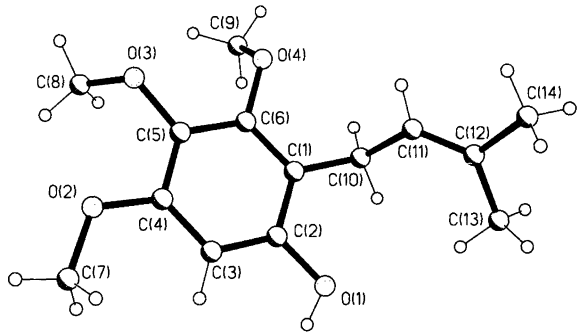

Fig. 2. X-Ray structure of a molecule of the natural sample.

Table 1. Crystal data and structure refinement for natural sample of **1**.

Identification code	sc1del
Empirical formula	$C_{14}H_{20}O_4$
Formula weight	252.30
Temperature/K	220(2)
Wavelength/Å	0.71073
Crystal system	Monoclinic
Space group	$P2_1/C$
Unit cell dimensions/Å,°	$a=11.124(8)$ $b=10.729(7)$ $c=11.914(8)$ $\beta=101.99(5)$
Volume/Å	1391(2)
Z	4
Density (calculated)/Mg m^{-3}	1.205
Absorption coefficient/mm^{-1}	0.087
$F(000)$	544
Crystal size/mm^3	0.78 × 0.28 × 0.22
θ range for data collection/°	1.87–25.05
Index ranges	$0\leqslant h\leqslant 13$, $-1\leqslant k\leqslant 12$, $-14\leqslant l\leqslant 13$
Reflections collected	2888
Independent reflections	2453 [R(int) = 0.038]
Absorption correction	None
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2453/1/173
Goodness-of-fit on F^2	1.022
Final R indices [$I>2\sigma(I)$]	$R_1=0.045$, $wR_2=0.118$
R indices (all data)	$R_1=0.060$, $wR_2=0.131$
Extinction coefficinet	0.073(5)
Largest diff. peak and hole/e $Å^{-3}$	0.19 and −0.16

sulfate (0.5 ml) were added and the contents were refluxed for 3 h. The reaction mixture was filtered, and acetone was removed from the filterate, water was added to the residue and the contents were extracted with ethyl acetate; the organic layer was dried on anhydrous Na_2SO_4, concentrated *in vacuo* and chromatographed over silica gel using petroleum ether – EtOAc as eluent. The fractions eluted with ethyl acetate–petrol (1:19) yielded 2,4,6-trimethoxyphenol (**4**)[4] as a brown viscous oil (100 mg). UV (MeOH)λ_{max}:256 and 282 nm; IR (KBr)ν_{max}:3410, 2900, 2850, 1600, 1500, 1470, 1350, 1200, 1120, 1100, 1030, 960 and 800 cm^{-1}; ^1H NMR ($CDCl_3$): δ 3.77 (3H, s, OCH_3), 3.87 (6H, s, $2 \times OCH_3$), 5.12 (1H, s, -OH) and 6.18 (2H, s, Ar-H); ^{13}C NMR ($CDCl_3$): δ 55.65 (C-4 OCH_3), 56.15 (C-2 OCH_3 and C-6 OCH_3), 91.61 (C-3 and C-5), 128.87 (C-1), 147.19 (C-2 and C-6) and 152.96 (C-4); MS(EI, eV), *m/z*(%): 184$[M]^+$ (100), 169 (87), 141 (65), 126 (32), 109 (30), 95 (30), 69 (92) and 53 (80). The fractions eluted with ethyl acetate–petrol (1:9) gave 3,4,5-trimethoxyphenol (5) as a light brown crystalline solid (120 mg), m.p. 146–48 °C (lit.[5] 148 °C). ^1H NMR ($CDCl_3$): δ 3.75 (9H, s, $3 \times OCH_3$), 5.15 (1H,s,-OH) and 6.10 (2H, s, Ar-H); ^{13}C NMR ($CDCl_3$): δ 55.98 (C-3 OCH_3 and C-5 OCH_3), 61.03 (C-4 OCH_3), 93.04 (C-2 and C-6), 131.85 (C-4), 152.44 (C-1) and 153.76 (C-3 and C-5); MS(EI, eV), *m/z*(%): 184$[M]^+$ (100), 169 (98), 141 (24), 126 (9), 111 (15) and 69 (22).

*2-(3-Methyl-2-butenyl)-3,4,5-trimethoxyphenol (**1**).* To a stirred solution of 3,4,5-trimethoxyphenol (5) (92 mg) in dry dioxane (10 ml) was added boron trifluoride–etherate (0.025 ml) and 2-methylbut-3-en-2-ol (0.052 ml) in dioxane (2 ml). After 1 h the reaction mixture was diluted with moist ether (15 ml) and washed with water (3×10 ml). The ethereal solution after concentration was subjected to preparative TLC in the solvent system ethyl acetate-benzene (3:17) to give 2-(3-methyl-2-butenyl)-3,4,5-trimethoxyphenol (30 mg) as a white coloured solid, m.p. 88–90 °C. UV (MeOH) λ_{max}: 232 and 282 nm; IR(KBr)ν_{max}:3308, 3004, 2964, 2931, 1610, 1510, 1466, 1418, 1243, 1128, 1078 and 1044 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.75(3H, s, H-4′), 1.81 (3H, s, H-5′), 3.34 (2H d, *J* 7, H-1′), 3.80 (6H, s, $2 \times OCH_3$), 3.84(3H, s, OCH_3), 5.13(1H, s, -OH), 5.21(1H, t, *J* 7, H-2′) and 6.24 (1H, s, H-6); ^{13}C NMR ($CDCl_3$): δ17.83(C-5′), 22.79(C-4′), 25.75(C-1′), 55.93(C-4 OCH_3), 61.01 and 61.21 (C-3 OCH_3 and C-5 OCH_3), 96.53(C-6), 112.30(C-2), 122.31(C-2′), 134.71(C-3′ and C-4), 150.99(C-1) and 152.10 and 152.18(C-3 and C-5); MS(EI, eV), *m/z*(%): 252(100)(M^+), 237(17), 197(59), 196(25), 181(24), 153(9), 91(2), 77(3) and 69(6).

*3-(3-Methyl-2-butenyl)-2,4,6-trimethoxyphenol (**2**).* To a stirred solution of 2,4,6-trimethoxyphenol (**4**) (92 mg) in dry dioxane (10 ml) were added boron trifluoride–etherate (0.025 ml) and 2-methylbut-3-en-2-ol (0.052 ml) in dioxane (2 ml). The solution was stirred at room temperature for 5 h, diluted with moist ether (15 ml) and washed with water (3×10 ml). The ethereal layer after concentration was subjected to preparative TLC in the solvent system ethyl acetate–benzene (1:9) to furnish a light brown viscous oil. UV(MeOH)λ_{max}: 258 and 285 nm; IR(KBr)ν_{max}: 3410, 2900, 2810, 1625, 1490, 1450, 1320, 1250, 1200, 1100, 1020 and 900 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.67(3H, s, H-4′), 1.77(3H, s, H-5′), 3.27(2H, d, *J* 7.5, H-1′), 3.77(3H, s, OCH_3), 3.83(3H, s, OCH_3), 3.87(3H, s, OCH_3), 5.16(1H, t, *J* 7.5, H-2′) and 6.32(1H, s, H-5); ^{13}C NMR ($CDCl_3$): δ 17.67(C-5′), 22.53(C-4′), 25.68(C-1′), 56.28 and 56.40 (C-2 OCH_3 and C-6 OCH_3), 60.71 (C-4 OCH_3), 92.87(C-5), 116.52(C-3), 123.37(C-2′), 130.85(C-1), 132.83(C-3′), 145.25 and 145.70 (C-2 and C-6) and 150.46(C-4); MS(EI, eV), *m/z*(%): 252 (85) (M^+), 237(30), 221(8), 197(10), 196(16), 183(22), 167(10), 153(12), 91(30), 77(38), 69(100), 53 (58) and 41 (90).

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