Pogostol *O*-Methyl Ether and Artabotrol: Two Novel Sesquiterpenes from the Stem Bark of *Artabotrys stenopetalus*

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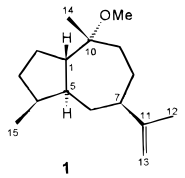
An investigation of the stem bark of *Artabotrys stenopetalus* (Annonaceae) has yielded two novel sesquiterpenes, the guaiane pogostol *O*-methyl ether and an isodaucane that we have named artabotrol, together with the known sesquiterpene β-caryophyllene oxide and the alkaloid artabotrine. The compounds were identified by detailed analysis of their spectral data.

Artabotrys stenopetalus Engl. & Diels (syn. *Artabotrys nitidus* auct.) (Annonaceae) is a climbing shrub that grows in the humid forests of west Africa from Ghana to Zaire.[1] The leaves are eaten for the treatment of enlarged spleen in Zaire, while the twigs form part of a prescription for the promotion of conception, and the sap is considered an aphrodisiac.[2]

This species has not been previously investigated, but other species of *Artabotrys* have yielded bisabolene sesquiterpenes,[3−5] sterols,[6,7] aporphinoid alkaloids,[8−13] and protoberberine alkaloids.[14] We have investigated the stem bark of *A. stenopetalus* and isolated three sesquiterpenes and an alkaloid. Among the sesquiterpenes are two new compounds, and in this paper we wish to report the structure elucidation of these.

A petroleum ether extract of the stem bark of *A. stenopetalus* was initially fractionated by vacuum liquid chromatography (VLC), with individual compounds then being purified by column chromatography and preparative TLC to give β-caryophyllene oxide,[15] a novel guaiane sesquiterpene pogostol *O*-methyl ether (**1**), and a novel isodaucane, which we have named artabotrol (**2**). The non-polar VLC fractions of the EtOAc extract after column chromatography and preparative TLC gave more of compound **1** and the known *N*-methoxy-4,5-dioxoaporphine artabotrine.[12] The structures of the two novel compounds were established by analysis of their spectral data, notably by 2D NMR, while previously reported compounds were identified by comparison of physical and spectral data with those published.



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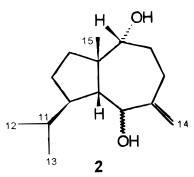

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Compound **1** was obtained as a colorless oil. The HREIMS solved for the empirical formula $C_{16}H_{28}O$ based on the molecular ion peak at *m*/*z* 236.2129. The IR spectrum showed a band at 1643 cm^{-1} for an aliphatic ether, which was confirmed as a methyl ether by the signal at δ 49.0 in the *J*-modulated ^{13}C-NMR spectrum and a fragment ion *m*/*z* 205 ($M^+ - 31$) in the MS. The ^{13}C-NMR spectrum further showed an sp^2 methylene signal at δ 107.8 and a quaternary sp^2 signal at δ 153.0 for an exocyclic methylene, four methine carbons, five sp^3 methylenes, and an oxygen-bearing quaternary sp^3 carbon (δ 78.9). The ^{1}H-NMR spectrum revealed the presence of an isopropenyl group, a tertiary methyl attached to an oxygenated carbon, an *O*-methyl ether, and a secondary methyl. These data required a bicyclic structure for compound **1**.

From analysis of the ^{1}H−^{1}H COSY and TOCSY spectra three spin systems (A, B, and C) were identified. These are shown in Figure 1. On the basis of heteronuclear ^1J, and ^2J and ^3J correlations from HC−COBI[16] and HMBC[17] experiments, respectively, the spin systems (A−C) and a further component (D) were connected to provide the planar structure of compound **1**. Thus, in the HMBC spectrum the vinyl protons (H-12) show what must be a ^3J correlation to the methine carbon at C-7 and to the methyl C-12. The allylic proton (H-7) shows ^2J correlations with C-11 and C-8, and ^3J correlations with C-12, C-13, and C-9. The protons of the secondary methyl (H-15) show a ^2J correlation with the methine carbon at C-4 and ^3J correlation with the methine at C-5 and the methylene at C-3. Another methine proton (H-1) showed ^2J correlation with C-2 and C-10, and ^3J correlation with C-3, C-6, C-9, and C-14. Finally, the *O*-methyl protons showed ^3J correlation with C-10.

The relative stereochemistry was established from a NOESY experiment and by comparison with similar bicyclic guaianes.[18−20] In this spectrum, the two bridgehead protons did not show NOE correlation, indicating a *transoid* relationship for H-1 and H-5, while H-1 did show an NOESY correlation with H-14 and H-15. H-5 did not show correlation with the methyl or vinylic protons of the isopropenyl unit. Other NOESY correlations observed were those between H-14 and H-1, H-8b and the vinylic protons (H-13), while the *O*-methyl protons showed correlation with H-8a. Thus, compound **1** was identified as the novel pogostol *O*-methyl ether. The spectral data were similar to those of 11-hydroxypogostol isolated from the aerial parts of *Leuceria floribunda*,[18] except for the presence of a 10-*O*-methyl group and the loss of 11-OH to provide an isopropenyl unit. The parent skeleton pogostol was isolated from the oil of *Pogostemon cablin*.[21]

Compound **2**, to which we have given the trivial name artabotrol, was obtained as an apparently optically inactive colorless oil that showed the presence of hydroxyl (3415 cm^{-1}) and exomethylene (2956 and 1641

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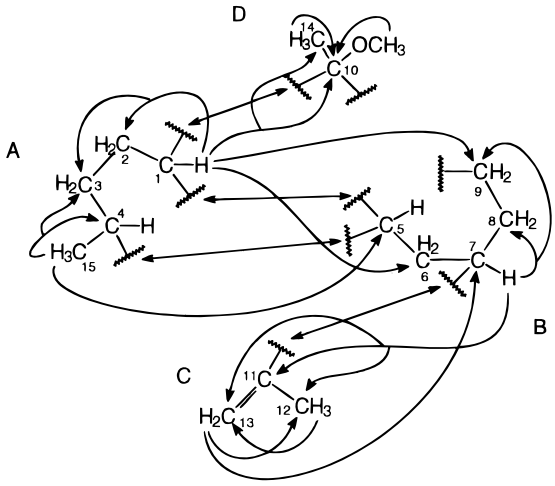

Figure 1. Long-range heteronuclear coupling interactions (single arrows, H→C) in pogostol *O*-methyl ether (**1**) identified from an HMBC experiment. Double-headed arrows (↔) indicate direct bonds between fragments identified from this procedure.

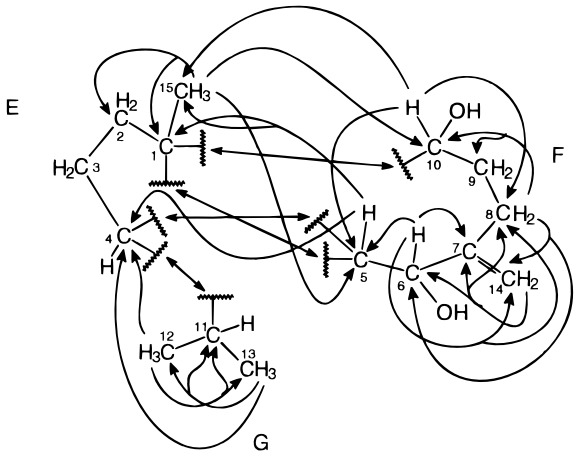

Figure 2. Long-range heteronuclear coupling interactions (single arrows, H→C) in artabotrol (**2**) ether identified from an HMBC experiment. Double-headed arrows (↔) indicate direct bonds between fragments identified from this procedure.

cm^{-1}) functional groups in the IR spectrum. The HREIMS gave a molecular ion peak at *m/z* 238.1890, which solved for $C_{15}H_{26}O_2$, and showed fragment ions at *m/z* 220 and *m/z* 202 for the loss of the elements of H_2O twice. The ^{13}C-NMR spectrum revealed signals for two oxygenated methines; an sp^2 exocyclic methylene; two quaternary carbons, one of which was olefinic; three methines; five methylenes; and three methyls. These data suggested a bicyclic sesquiterpene alcohol.

The ^{1}H-NMR spectrum showed signals due to a tertiary methyl group, an isopropyl group, an exomethylene group, and two oxygenated methines. Analysis of the ^{1}H−^{1}H COSY spectrum provided the three spin systems (D, E, and F) shown in Figure 2. From the HC−COBI and HMBC spectra the planar structure for compound **2** was deduced. From the former all the methines and the methyls were assigned unambiguously. From the HMBC spectrum, the protons of the tertiary methyl (H-15) show ^2J correlation to the signal at C-1, and ^3J correlations to C-2, C-5, and C-10. The H-5 proton also shows ^2J correlations to C-1 and C-4 and a ^3J correlation to C-15. The methyl protons of the isopropyl unit show ^2J and ^3J correlations to C-11 and C-4, respectively. These and other significant correlations are shown in Figure 2.

The relative stereochemistry of compound **2** was established by a NOESY experiment in which correlation was observed between the tertiary methyl (H-15) and H-5, suggesting a *cisoid* relationship at the ring junction. H-15 further showed NOE correlation with the oxymethine proton at δ 3.70 (H-10) and the methyl groups of the isopropyl unit (H-12 and H-13). The relative stereochemistry of the oxymethine at δ 4.22 (H-6) could not be resolved.

The occurrence of the guaiane skeleton in sesquiterpenes of the Annonaceae has been reported only recently from *Uvaria narum*,[22] *Piptostigma fugas*,[23] and *Neostenanthera hamata*[23] (T. C. Fleischer, unpublished data), while the isolation of artabotrol represents the first report of the rare isodaucane class of sesquiterpenes[24] in the family.

Experimental Section

General Experimental Procedures. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. UV spectra were recorded in absolute EtOH or MeOH using a Perkin-Elmer 552 UV-vis spectrophotometer. IR data were recorded in $CHCl_3$ on a Mattson Genesis Series FT-IR. Specific rotations were determined at the sodium D line on a ADP 220 polarimeter (Bellingham Stanley Ltd.). NMR spectra were recorded on a Bruker AMX-400 instrument (for solvents see text). MS were recorded on an AEI-MS 902 double-focusing instrument using a direct probe insert at 70 eV.

Plant Material. The plant material used for this study (stem bark of *Artabotrys stenopetalus* Engl. & Diels) was collected from the Bia-Tano Forest Reserve in the Brong-Ahafo Region of Ghana, and identified by comparison with a herbarium specimen at the Forestry Department in Kumasi, where a voucher sample is stored.

Extraction and Isolation of Compounds. The stem bark of *A. stenopetalus* (1 kg) was extracted in a Soxhlet with petroleum ether (bp 60−80 °C), then EtOAc, and finally MeOH. Each extract was dried *in vacuo* at 40 °C under reduced pressure to give 11.80 g, 10.20 g, and 27.60 g of material, respectively. The MeOH extract was reconstituted in aqueous MeOH and extracted with EtOAc, the soluble part being added to the original EtOAc extract.

VLC fractionation of the petroleum ether extract (6 g) over Si gel, eluting with petroleum ether, petroleum ether−EtOAc mixtures, EtOAc, and EtOAc−MeOH mixtures yielded six fractions: A (100% petroleum ether), B (up to 5% EtOAc), C (up to 10% EtOAc), D (up to 15% EtOAc), E (up to 30% EtOAc), and F (up to 10% MeOH in EtOAc). Fraction B was column chromatographed over Si gel, eluting with increasing concentrations of EtOAc in petroleum ether (up to 10% EtOAc); fractions 16−23 afforded β-caryophyllene oxide (6 mg). The same procedure was applied to fraction C, with fractions 13−19 yielding more β-caryophyllene oxide (6 mg), and fractions 53−65 giving **1** (11 mg). Column chromatography of fraction E over Si gel, eluting with 10−20% EtOAc in petroleum ether afforded a series of fractions (11−30) that on preparative TLC (solvent: petroleum ether−EtOAc, 8:2) gave **2** (12 mg).

The early fractions from VLC of the EtOAc extract (5 g) were bulked and column chromatographed over Sephadex LH-20, eluting with $CHCl_3$ to give two fractions (A and B). Preparative TLC of fraction A (petroleum ether−EtOAc 8:2) gave more β-caryophyllene oxide (6 mg). Fraction B was repeatedly column chromatographed, initially over alumina, then over Si gel, eluting with $CHCl_3$, and was then finally purified by preparative TLC ($CHCl_3$−MeOH 9.8:0.2, multiple development) to give artabotrine (4 mg).

Pogostol O-methyl ether (**1**): colorless oil, $[\alpha]_D$ −33.3° (*c* 0.066, $CHCl_3$), IR ν_{max} ($CHCl_3$) 1643 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) 2.21 (ddd J = 5.6, 10.2 H-1), 1.65 (m H-2a), 1.53 (dt J = 11.0, 6.6 H-2b), 1.71 (m H-3a), 1.21 (m H-3b), 2.02 (m H-4), 1.94 (ddd J = 2.6, 3.4, 6.0 H-5), 1.40 (dd J = 3.4, 13.6 H-6a), 1.18 (dd J = 3.2, 12.2 H-6b), 2.39 (ddd J = 10.2, 5.6 H-7), 1.82 (m H-8a), 1.41 (m H-8b), 1.81 (m H-9a), 1.62 (m H-9b), 1.72 (s H-12), 4.67 (dd J = 1.5 2.2 H13a), 4.60 (dd J = 1.4, 2.1 H-13b), 1.12 (s H-14), 0.91 (d J = 6.8 Hz, H-15), 3.24 (s H-16); ^{13}C NMR (100.56 MHz, $CDCl_3$) δ 52.7 (C-1), 26.1 (C-2), 30.4 (C-3)*, 39.0 (C-4), 45.9 (C-5), 27.7 (C-6), 45.3 (C-7), 28.4 (C-8), 30.5 (C-9)*, 78.9 (C-10), 153.0 (C-11), 20.2 (C-12), 107.8 (C-13), 25.1 (C-14), 16.5 (C-15), 49.0 (C-16); HREIMS *m/z* (rel int) 236 $[M]^+$ (9), 221 (12) 219 (33), 205 (16), 203 (58)191 (49), 154 (6), 145 (43),-121 (34), 107 (53), 91 (46) 95 (62), 81 (82), found 236.2129; calcd 236.2140 for $C_{16}H_{28}O$; signals with an asterisk are interchangeable.

Artabotrol (2): colorless oil, $[\alpha]_D$ 0° (*c* 0.06, $CHCl_3$); IR ν_{max} ($CHCl_3$) 3415 (OH), 2956, 1641 (>C═H_2) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.50 (m H-2a), 1.25 (m H-2b), 1.59 (m H-3a), 1.32 (m H-3b), 1.67 (m H-4), 1.82 (dd J = 4.1, 9.4 Hz H-5), 4.22 (d J = 4.1 Hz H-6), 2.76 (dt J = 5.3, 13.4 Hz H-8a), 2.12 (dt J = 10.3, 3.8 Hz H-8b), 1.88 (m H-9a), 1.88 (m H-9b), 3.70 (dd J = 1.7, 4.3 Hz H-10), 1.67 (m H-11), 0.94 (d J = 6.7 Hz H-12), 0.87 (d J = 6.7 Hz H-13), 4.89 (ddt J = 0.7, 1.8, 3.4 Hz H-14a), 4.70 (d J = 2 Hz H-14b), 1.19 (s H-15); ^{13}C NMR (100.56 MHz, $CDCl_3$) 49.8 (C-1), 24.3 (C-2), 38.2 (C-3), 50.4 (C-4), 55.7 (C-5), 78.9 (C-6), 149.4 (C-7), 27.1 (C-8), 29.9 (C-9), 77.7 (C-10), 29.7 (C-11), 22.8 (C-12), 17.5 (C-13), 113.2 (C-14), 30.6 (C-15); HREIMS *m/z* (rel int) 238 $[M]^+$ (4), 220 (11), 202 (8), 191 (49), 163 (18), 145 (43), 139 (17), 121 (23), 107 (27), 95 (31), 91 (46), 85 (100), 83 (72); found 238.1890; calcd 238.1933 for $C_{15}H_{26}O_2$.

***β*-Caryophyllene oxide:** colorless oil $[\alpha]_D$ −70.8 (*c* 1.2, $CHCl_3$) (lit value[15] −54.7°); HREIMS found 220.1875, calcd 220.1828 for $C_{15}H_{24}O$, ^1H and ^{13}C NMR, MS, in agreement with published data.[15]

Artabotrine: Yellow-orange amorphous solid; UV λ_{max} (MeOH) 236, 279, 301, 310, 423, 445 nm; IR ν_{max} ($CHCl_3$) 1693, 1662 cm^{-1}, in agreement with literature data,[25] ^1H and ^{13}C NMR in agreement with literature data;[26] EIMS *m/z* $[M]^+$ 321 (85), 305 (5), 291 (48), 278 (100), 263 (52), 177 (26); HREIMS found 321.0625; calcd 321.0637 for $C_{18}H_{11}NO_5$.

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