New Indole Alkaloids from the Roots of *Ochrosia acuminata*

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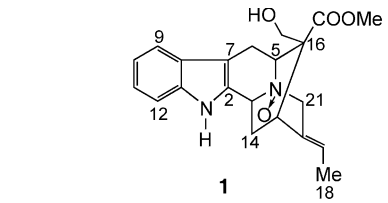
Two new indole alkaloids, polyneuridine-*N*-oxide (**1**) and 17-hydroxy-10-methoxy-yohimbane (**2**), together with seven known alkaloids were isolated from the roots of *Ochrosia acuminata* collected in Savu, Indonesia. 9-Methoxyellipticine (**3**) and ellipticine (**4**) were responsible for the antitumor activities of the extract. The structures of all compounds were elucidated using MS and NMR methods.

Extracts of the roots of *Ochrosia acuminata* Val. (Apocynaceae) have been used by native people in Savu, Indonesia, to treat tumors and ectopic pregnancy.[1] Ochrosia species have been well investigated and contain many indole alkaloids.[2] A previous study on the stem extracts of *O. acuminata* collected from Taiwan afforded two antitumor alkaloids, ellipticine and 9-methoxyellipticine.[3] In this paper we report the isolation and structural identification of two new indole alkaloids (**1** and **2**) and seven known indole alkaloids from the roots of *O. acuminata* collected from Savu, Indonesia. The structures of all alkaloids were solved by MS and NMR methods (^{1}H, ^{13}C, HSQC, HMBC, DQF-COSY, and NOESY) and by comparison of NMR data with those of the known compounds.

Bioassay-guided fractionations of the root extract led to the isolation of 9-methoxyellipticine (**3**)[4] and ellipticine (**4**),[5] as the active components, and a new compound (**1**). A mixture of **3** and **4** had IC_{50} values of 1.4 and 0.75 μg/mL against normal fetal fibroblast and melanoma cancer cell lines, respectively. A conventional acid−base extraction was also employed to isolate other minor alkaloids present in the extract. From this scheme, compounds **3** and **4** were reisolated as the major components, together with a second new compound (**2**). Five other known alkaloids, namely, quebrachidine,[6] voachalotine,[7] isoreserpiline,[8] reserpiline,[8] and strictosamide,[9,10] were also isolated as minor components. To the best of our knowledge, this is the first report of the occurrence of quebrachidine, voachalotine, and strictosamide in the genus *Ochrosia*, and isoreserpiline and reserpiline in *O. acuminata* species.



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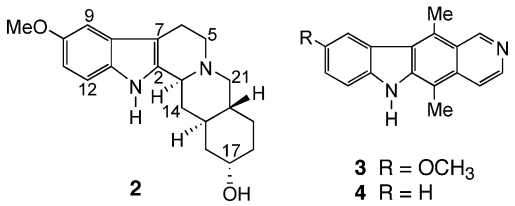

2 **3** R = OCH_3 **4** R = H

Compound **1** was isolated as a light yellow amorphous solid with a molecular formula of $C_{21}H_{24}N_2O_4$ (HRESIMS).

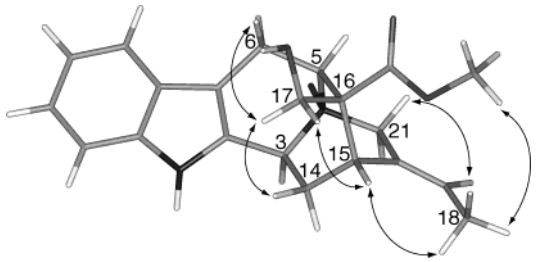

Figure 1. Selected NOE correlations for compound **1**.

^{13}C NMR and HSQC data showed the presence of two methyls, four methylenes, eight methines, and seven quaternary carbon atoms. The ^{1}H and ^{13}C chemical shifts of **1** were comparable with the published values for polyneuridine ($C_{21}H_{24}N_2O_3$),[7] except for the shifts at positions 3, 5, and 21, which were more downfield for **1**. The downfield shifts around N-4 and an extra oxygen confirmed that **1** is an oxide of polyneuridine and is hence named polyneuridine-*N*-oxide. The relative stereochemistry about C-16 and the double bond at C19−C20 were determined from a NOESY experiment. Selected NOE correlations for **1** are shown in Figure 1. NOESY cross-peaks were observed between H-15/Me-18, H-19/H-21α, and H-19/H-21*β*, indicating the *E* configuration about the C-19−C-20 double bond. NOEs were also observed between H-6*β*/H-17α, H-14*β*/H-17α, H14*β*/H-17*β*, H-15/H-17α, H-15/H-17*β*, and Me-18/OMe, suggesting an *R* stereochemistry at C-16.

Compound **2** was isolated as a white amorphous solid with a molecular formula of $C_{20}H_{26}N_2O_2$ (HRESIMS). ^{13}C NMR and HSQC data showed the presence of one methyl, seven methylenes, seven methines, and five quaternary carbon atoms. The presence of a substituted indole moiety and a carbomethoxy group could be established from characteristic ^{1}H and ^{13}C NMR signals. A quaternary carbon at δ 155.1 showing HMBC cross-peaks from H-9, H-11, H-12, and OMe was assigned to C-10. The methylene signals of H-5α/*β* (δ2.67, 3.13) and H-6α/*β* (δ 2.97, 2.70) are scalar coupled and were placed between C-7 and N-4 on the basis of the HMBC data. HSQC and DQF-COSY data established the presence of two six-membered rings, {N-4, C-3, C-14, C-15, C-20, C-21} and {C-15, C-16, C-17, C-18, C-19, C-20}, giving a yohimbinoid ring system. The ^{1}H and ^{13}C shifts at position 17 (δ 4.10 and 67.2, respectively) are consistent with hydroxyl substitution at this position.

The relative stereochemistry of **2** was determined from a NOESY spectrum and by comparison of the ^{13}C NMR spectra with literature data. Comparison of ^{13}C NMR data with the series of yohimbinoids described by Wenkert et al.[11] showed that **2** has the same stereochemistry as yohimbane at the asymmetric centers C-3, C-15, and C-20.

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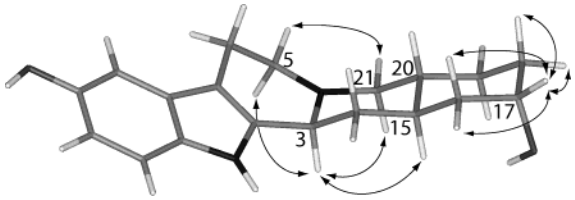

Figure 2. Selected NOE correlations for compound **2**.

Both rings D and E have chair conformations on the basis of the NOE correlations and the values of coupling constants. Selected NOE correlations for **2** and the 3D model are shown in Figure 2. The coupling constants of H-14β (J = 12.9, 12.5, 11.5 Hz) are consistent with trans-diaxial relationships to both H-3 and H-15. A large coupling constant between H-21α and H-20 (J = 11.0 Hz) is consistent with the β position for H-20. Compound **2** ($[\alpha]^{25}_D$ +3.3°, c 0.14, MeOH) has the same optical rotation sign as a related compound, 10-methoxy-yohimbine, a methyl ester of **2**, at C-16 ($[\alpha]^{25}_D$ +56.6°, c 1.2, EtOH),[12] which indicated that they probably have the same stereochemistry. In 10-methoxy-yohimbine, the absolute stereochemistry at C-3 is S;[12] therefore the stereochemistry in **2** at both C-15 and C-20 would be R relative to C-3. An S configuration at C-17 was then deduced since H-17 (m, J = 2.8 Hz) showed NOEs to H16α/β and H-18α/β and is consistent with the equatorial position of H-17. The new compound **2** is a derivative of yohimbane, and hence named 17-hydroxy-10-methoxy-yohimbane.

In conclusion, the root extract of *O. acuminata* collected in Savu, Indonesia, showed significant variation in indole alkaloids composition. Two new compounds, **1** and **2**, were isolated, and the study also showed the more widespread occurrence of compounds quebrachidine, voachalotine, and strictosamide in the Apocynaceae family. The antitumor activity of the extract is associated with ellipticine and 9-methoxyellipticine.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. HRESIMS was measured on a Finnigan MAT 900 XL double focusing magnetic sector mass spectrometer. ESIMS was measured on a Micromass ES-TOF LCT quadrupole electrospray time-of-flight mass spectrometer. ^{1}H, ^{13}C, HSQC, HMBC, NOESY, and DQF-COSY spectra were recorded on a Bruker AMX 400 MHz or a Bruker DRX 500 NMR MHz spectrometer. ^{1}H spectra were referenced relative to MeOH-d_4 (δ = 3.30 ppm), and ^{13}C spectra were referenced relative to MeOH-d_4 (δ = 49.0 ppm). Flash chromatography was carried out using Scharlau silica gel 60 (0.04−0.06 mm). RP-HPLC was carried out using a Phenomenex Synergi Hydro-RP C_{18} column (250 × 10 mm, 4 μm).

Plant Material. Roots from *O. acuminata* were collected in Savu Island in April 2001 and air-dried. The plant was identified by Dr. Irawati at the Bogoriense Herbarium in Bogor, Indonesia. Voucher specimens (AS016) are kept at the IMB, University of Queensland, Brisbane, Australia.

Extraction and Isolation of Compounds. Dried roots (400 g) were extracted with MeOH/CH_2Cl_2 (1:1) (6 × 3 L) at room temperature and evaporated in vacuo (<40 °C) to afford 8 g of crude extract. For bioassay-guided fractionation the crude extract (1 g) was partitioned by NP flash chromatography using CH_2Cl_2, EtOAc, and MeOH in order of increasing polarity. The fraction eluting in EtOAc/MeOH (3:1) (40 mg) was active in the antitumor assay and was further purified using semipreparative RP HPLC (H_2O/MeOH, 0% to 100% MeOH in 100 min, flow rate 2 mL/min, UV detection at λ 250 nm) to afford alkaloids **1** (1.5 mg), **3** (2.5 mg), and **4** (3.0 mg), in order of decreasing polarity. For acid−base extraction the crude extract (7 g) was partitioned between 3% HCl and Et_2O, and the aqueous layer was basified to pH 10 with NaOH and extracted with $CHCl_3$ to give a crude alkaloidal fraction (1.8 g). The crude alkaloidal fraction was partitioned by NP flash chromatography using mixtures of $CHCl_3$ and MeOH in order of increasing polarity. The fraction eluting in 10% MeOH (200 mg) was further purified using semipreparative RP HPLC (H_2O/CH_3CN, 30% to 100% CH_3CN in 80 min, flow rate 3 mL/min, UV detection at λ 250 nm) to afford quebrachidine (10.5 mg), voachalotine (6.3 mg), isoreserpiline (7.2 mg), and reserpiline (12.0 mg) in order of decreasing polarity. The fraction eluting in 30% MeOH (80 mg) was similarly purified to afford alkaloid **2** (5.1 mg) and strictosamide (1.8 mg). The fraction eluting in 20% MeOH (300 mg) contained **3** and **4** as the major components (evidence from ^{1}H NMR data) and was not purified further.

Compound 1: light yellow amorphous powder; $[\alpha]^{25}_D$ −19.5° (c 0.10, MeOH); ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.50 (1H, td, J = 7.9, 1.0 Hz, H-9), 7.33 (1H, td, J = 7.9, 1.0 Hz, H-12), 7.10 (1H, dt, J = 7.9, 1.0 Hz, H-11), 7.03 (1H, dt, J = 7.9, 1.0 Hz, H-10), 5.39 (1H, tq, J = 7.0, 2.2 Hz, H-19), 4.70 (1H, br d, J = 6.2 Hz, H-5), 4.48 (1H, ddd, J = 11.0, 2.5, <1.0 Hz, H-3), 4.30 (1H, td, J = 16.4, 2.2 Hz, H-21β), 4.07 (1H, td, J = 16.4, 2.2 Hz, H-21α), 3.73 (3H, s, OCH_3), 3.68 (1H, d, J = 10.7 Hz, H-17α), 3.64 (1H, d, J = 10.7 Hz, H-17β), 3.56 (1H, dd, J = 16.6, 6.2 Hz, H-6α), 3.45 (1H, d, J = 16.6 Hz, H-6β), 3.20 (1H, ddd, J = 3.5, 2.3, <1.0 Hz, H-15), 2.40 (1H, ddd, J = 13.7, 11.0, 2.3 Hz, H-14β), 2.06 (1H, ddd, J = 13.7, 3.5, 2.5 Hz, H-14α), 1.66 (3H, td, J = 7.0, 2.2 Hz, H_3-18); ^{13}C NMR (MeOH-d_4, 100 MHz) δ 174.9 (COO), 138.3 (C-13), 133.8 (C-2), 131.6 (C-20), 127.0 (C-8), 122.8 (C-11), 120.4 (C-10), 119.7 (C-19), 119.4 (C-9), 112.3 (C-12), 104.1 (C-7), 71.3 (C-21), 69.9 (C-5), 65.6 (C-3), 63.9 (C-17), 57.5 (C-16), 53.1 (OMe), 31.7 (C-15), 31.5 (C-14), 20.1 (C-6), 12.8 (Me-18); HRESIMS m/z $[M + H]^+$ 369.1818 (calcd for $C_{21}H_{25}N_2O_4$, 369.1814).

Compound 2: white amorphous powder; $[\alpha]^{25}_D$ +3.3° (c 0.14, MeOH); ^{1}H NMR (MeOH-d_4, 500 MHz) δ 7.16 (1H, dd, J = 8.7, 0.5 Hz, H-12), 6.88 (1H, br d, J = 2.5 Hz, H-9), 6.69 (1H, dd, J = 8.7, 2.5 Hz, H-11), 4.10 (1H, m, J = 2.8 Hz, H-17), 3.79 (3H, s, OMe), 3.41 (1H, br d, J = 11.5 Hz, H-3), 3.13 (1H, ddd, J = 11.2, 4.0 Hz, H-5β), 2.97 (1H, dddd, J = 16.7, 10.4, 4.0, 2.5 Hz, H-6α), 2.91 (1H, dd, J = 11.5, 2.8 Hz, H-21β), 2.70 (1H, ddd, J = 16.7, 4.7 Hz, partial overlap with H-5α, H-6β), 2.67 (1H, ddd, J = 11.2, 10.4, 4.7 Hz, partial overlap with H-6β, H-5α), 2.23 (1H, br t, J = 11.0 Hz, H-21α), 2.18 (1H, td, J = 12.9, 3.2 Hz, H-14α), 1.84 (2H, br d, J = 13.4 Hz, H-16α and H-18β), 1.73 (1H, m, H-15), 1.58 (1H, m, H-18α), 1.47 (1H, m, overlap with H-20, H-19β), 1.46 (1H, m, overlap with H-19β, H-20), 1.40 (1H, m, H-19α), 1.33 (1H, dt, J = 13.4, 2.5 Hz, H-16β), 1.27 (1H, ddd, J = 12.9, 12.5, 11.5 Hz, H-14β); ^{13}C NMR (MeOH-d_4, 125 MHz) δ 155.1 (C-10), 136.5 (C-2), 133.2 (C-13), 128.6 (C-8), 112.6 (C-12), 111.8 (C-11), 107.5 (C-7), 100.9 (C-9), 67.2 (C-17), 62.6 (C-21), 62.3 (C-3), 56.3 (OMe), 54.4 (C-5), 42.3 (C-20), 39.9 (C-16), 36.9 (C-14), 36.1 (C-15), 33.1 (C-18), 25.1 (C-19), 22.3 (C-6); HRESIMS m/z $[M + H]^+$ 327.2068 (calcd for $C_{20}H_{27}N_2O_2$, 327.2072).

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Supporting Information Available: Figure S1: ^{1}H and ^{13}C NMR spectra for compound **1**. Figure S2: ^{1}H and ^{13}C NMR spectra for compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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