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J. Nat. Prod., **1993**, 56 (12), 2166-2169• DOI: 10.1021/np50102a020 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 12, pp. 2166-2169, December 1993

INDOLE ALKALOIDS FROM *KOPSIA TEOI*

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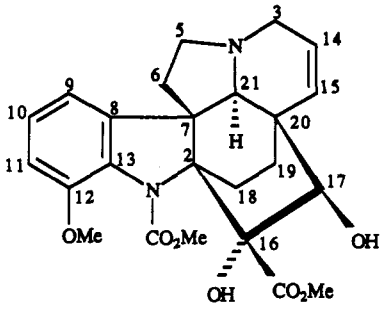
ABSTRACT.—Six monomeric indole alkaloids have been isolated and identified from the stem bark and leaves of *Kopsia teoi* collected in Malaysia. Four of them are known: kopsingine [**1**], isoeburnamine, rhazinilam, and lonicerine [**2**]. The two new alkaloids, 16-*epi*-17-α-hydroxy-$\Delta^{14,15}$-kopsinine [**3**] and 11,12-methylenedioxykopsaporine [**4**], belong to the same aspidofractinine group.

Kopsia (Apocynaceae) is a genus native to China, India, and Southeast Asia. During a systematic investigation of this genus, we have studied a new species, *Kopsia teoi* Allorge Remy (1,2), a small tree endemic to the Johore region (Malaysia). The structural elucidation of two new indole alkaloids, among six alkaloids isolated, is presented.

From the stem bark five indole alkaloids were isolated. Three were identified by their spectral data as kopsingine [**1**] (3), isoeburnamine (4,5), and rhazinilam (6,7). The fourth alkaloid, lonicerine [**2**], had never been isolated from this genus (8). A complete assignment of the ^{1}H- and ^{13}C-nmr spectra of **2** was realized through homo- and heteronuclear COSY spectra. The last alkaloid **3** was obtained as an optically active amorphous compound. The uv spectrum was characteristic of a dihydroindole alkaloid. The eims exhibited a molecular ion peak at *m/z* 352 that analyzed for $C_{21}H_{24}N_2O_3$ (found *m/z* 352.1789, calcd 352.1787). In the ^{1}H-nmr spectrum, four aromatic protons indicated a non-substituted indole ring; two signals at δ 5.87 (ddd, J=9.9, 3.3, and 2.5 Hz) and δ 5.64 (dt, J=9.9 and 2.1 Hz) revealed the presence of two olefinic protons, and an Me singlet at δ 3.77 was indicative of an Me ester. The ^{13}C-nmr spectra were characteristic of the aspidofractinine skeleton; particularly diagnostic were the three quaternary carbons at δ 67.54, 56.07, and 41.00. Comparison of the spectra of **3** with those of venalstonine (9), *N*-carbomethoxy-17β-hydroxy-$\Delta^{14,15}$-kopsinine (10), and 19β-hydroxyvenalstonine (11) led us to propose structure **3** for this alkaloid. The position of the OH at C-17 or C-19 was deduced from an examination of the eims spectrum. The typical retro-Diels-Alder fragmentation (12) (Scheme 1) was observed; the presence of fragments at *m/z* 324 $[M-28]^+$ and 123 was in agreement with a C-17 substituted skeleton as previously observed in the same series (10).

Stereochemistry at C-16 and C-17 was deduced from ^{1}H-nmr spectra. H-16 appeared as a doublet of doublets (J=7.7 and 2.1 Hz) at δ 2.77. The small coupling constant observed between H-16 and H-18 furnished useful information about the stereochemistry of C-16. Such a value is characteristic of long distance coupling of two pseudo-equatorial protons (W coupling) and has already been observed with other alkaloids of this family (10). A similar value between H-17 and H-19 (J=2.0 Hz) indicated the same orientation for H-16 and H-17. From these observations it was concluded that alkaloid **3** was 16-*epi*-17-α-hydroxy-$\Delta^{14,15}$-kopsinine. During this study 17-α-hy-



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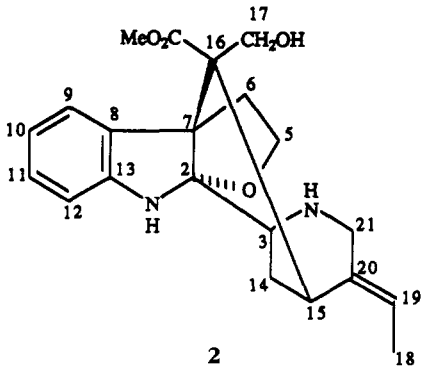

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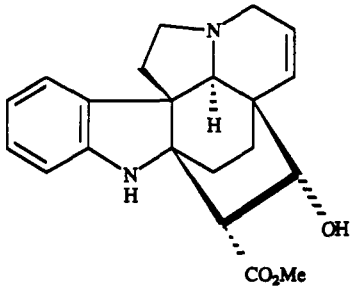

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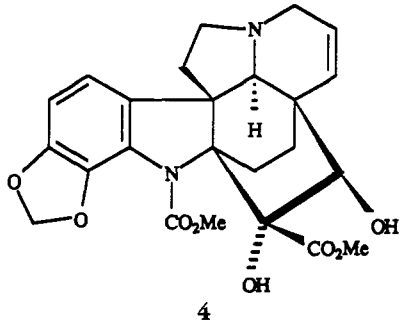
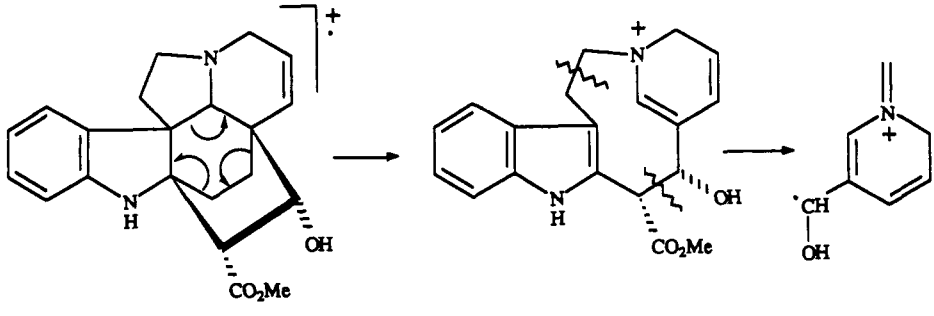


4

droxy-$\Delta^{14,15}$-kopsinine has been isolated from the same species (13). A great similarity in ^1H and ^{13}C nmr exists between these two alkaloids, with the exception of the coupling constant between H-16 and H-18. As this coupling is clearly established for alkaloid **3**, an inversion of C-16 stereochemistry could not be precluded in the first publication.

Among the alkaloids extracted from the leaves of *K. teoi*, kopsingine [**1**] was the major alkaloid. Only one other alkaloid was obtained in sufficient amount to allow a complete structural elucidation. This alkaloid, **4**, was obtained as an optically active amorphous compound; its uv and ir spectra showed similarities with those of kopsingine [**1**]. In the eims the molecular ion was observed at *m/z* 470 and was analyzed for $C_{24}H_{26}N_2O_8$ (found *m/z* 470.1689, calcd 470.1689). In the ^1H- and ^{13}C-nmr spectra, the upfield region showed a complete analogy with those of **1**, with the exception of the MeO signal of kopsingine. A two-proton singlet (δ 5.94) was observed in the spectrum of alkaloid **4** suggesting the presence of a methylenedioxy substituent



m/z 324 *m/z* 123

SCHEME 1

which was confirmed by the typical methylene signal in the ^{13}C nmr at δ 100.79. From these data, structure **4** was proposed. Observation of two aromatic doublets at δ 6.54 and 6.62 allowed location of the methylenedioxy on C-11 and C-12 in accordance with previously described alkaloids (5). Stereochemistry of the OH group on C-17 was further confirmed by observation of a long range coupling between H-17 and H-21 along a (W) path and absence of coupling between H-17 and H-19, as previously described. We concluded that the OH group on C-17 is in a β pseudoequatorial position. The same pattern for kopsingine [**1**] (see Experimental) indicated that these two alkaloids possess the same relative configuration. During this study, the same alkaloid, named 11,12-methylenedioxykopsaporine, had been isolated from *Kopsia singapuriensis* (14) and was analyzed by X-ray analysis. This analysis confirmed the stereochemistry on C-17 and gave the configuration of C-16 as indicated.

The structural study of alkaloids from *K. teoi* indicates a similarity of these species with *K. singapurensis*, suggesting a correlation between morphological evolution (nose appendage and trichasial cyme) (1) and phytochemistry.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotation was determined on a Perkin-Elmer model 141 polarimeter. Spectra were recorded with the following instruments: uv, Perkin-Elmer Lambda 5; ir, Nicolet 205 FT-IR spectrometer; eims, AEI MS50; ^1H nmr (300 MHz) and ^{13}C nmr (75.3 MHz) on a Bruker AC 300. Chemical shifts are given in ppm relative to TMS (δ=0), and coupling constants (*J*) are given in Hertz.

PLANT MATERIAL.—The aerial parts of *K. teoi* were collected in November 1990, near Keluang in Malaysia. The plant was identified by L. Allorge and L.E. Teo. A voucher speciment (KL 3976) was deposited at the Kepong herbarium (Kepong, Malaysia).

EXTRACTION AND ISOLATION.—The dried powdered stem bark (1.0 kg) was made alkaline with 0.5 liter of a saturated Na_2CO_3 aqueous solution and stirred with EtOAc (10 liters). Alkaloids were extracted from the EtOAc solution with 2% aqueous H_2SO_4 until a Mayer's reagent test was negative for the organic phase. The aqueous phase, made alkaline with NH_4OH, was extracted with $CHCl_3$. The $CHCl_3$ layers were washed with H_2O, dried (Na_2SO_4), and evaporated in vacuo to give 4.0 g of crude alkaloid mixture. Kopsingine [**1**] (0.5 g) crystallized from MeOH. Other alkaloids obtained by Si gel chromatography and preparative tlc of the mother liquor were isoeburnamine (15 mg), rhazinilam (7 mg), lonicerine [**2**] (23 mg), and 16-*epi*-17-α-hydroxy-$\Delta^{14,15}$-kopsinine [**3**] (47 mg). The same procedure conducted on leaves (0.7 kg) furnished 12.0 g of crude alkaloids, from which kopsingine [**1**] (7.7 g) was obtained pure by crystallization. Chromatography of the mother liquor eluted 11,12-methylenedioxykopsaporine [**4**] in small amounts (5 mg).

Kopsingine [**1**].—^1H nmr ($CDCl_3$) 1.14 (H-19, ddd, *J*=13.0, 10.2, and 1.5 Hz), 1.42 (H-18, ddd, *J*=13.0, 10.8, and 7.6 Hz), 1.72 (H-19, ddd, *J*=12.3, 10.2, and 7.6 Hz), 1.75 (H-6, dd, *J*=13.1 and 3.9 Hz), 2.12 (H-18, ddd, *J*=12.3, 10.8, and 1.5 Hz), 2.68 (H-5, ddd, *J*=11.7, 8.4, and 3.9 Hz), 2.77 (H-21, d, *J*=2.0 Hz), 2.94 (H-5, dd, *J*=8.4 and 6.0 Hz), 3.20–3.30 (H-3 and H-6, m), 3.48 (H-3, ddd, *J*=15.8, 4.3, and 1.8 Hz), 3.80 (H-17, d, *J*=2.0 Hz), 3.81, 3.82, and 3.87 (3×OMe, 3s), 5.68 (H-15, dt, *J*=9.7 and 1.8 Hz), 5.73 (OH, s), 5.98 (H-14, ddd, *J*=9.7, 4.3, and 1.5 Hz), 6.78 (H-11, d, *J*=7.4 Hz), 6.84 (H-9, d, *J*=8.3 Hz), 7.04 (H-10, dd, *J*=8.3 and 7.4 Hz); ^{13}C nmr ($CDCl_3$) 25.80 (C-19), 27.23 (C-18), 39.21 (C-20), 40.66 (C-6), 48.70 (C-3), 49.96 (C-5), 51.95 (OMe), 53.09 (OMe), 56.11 (OMe), 56.53 (C-7), 68.27 (C-21), 76.36 (C-2), 80.04 (C-16), 81.56 (C-17), 111.94 (C-11), 113.35 (C-9), 125.12 (C-10), 128.49 (C-8), 128.75 (C-14), 131.36 (C-15), 144.29 (C-13), 149.44 (C-12), 155.67 (N-CO_2Me), 171.95 (16-CO_2Me).

Lonicerine [**2**].—^1H nmr ($CDCl_3$) 1.70 (Me 18, d, *J*=6.5 Hz), 2.22 (H-14, dt, *J*=14.0 and 2.0 Hz), 2.60–2.80 (2×H-6, m), 2.95–3.05 (H-14 and H-15, m), 3.04 (CO_2Me, s), 3.44 (H-5, m), 3.70 (H-21, dd, *J*=13.6, and 1.5 Hz), 3.80-3.95 (H-21 and 2×H-17, m), 4.30 (H-5, ddd, *J*=8.2, 7.2, and 1.0 Hz), 5.76 (qd, *J*=6.5 and 1.0 Hz), 6.60 (H-12, d, *J*=7.6 Hz), 6.72 (H-10, t, *J*=7.6 Hz), 7.07 (H-11, t, *J*=7.6 Hz), 7.12 (H-9, d, *J*=7.6 Hz); ^{13}C nmr ($CDCl_3$) 13.59 (Me-18), 25.12 (C-14), 31.85 (C-15), 37.90 (C-6), 46.20 (C-21), 51.90 (C-3 and OMe), 55.90 and 57.31 (C-16 and C-7), 65.29 (C-17), 66.72 (C-5), 99.94 (C-2), 107.66 (C-12), 118.94 (C-10), 124.45 (C-9), 127.34 (C-8), 128.26 (C-19), 128.72 (C-11), 132.92 (C-20), 148.09 (C-13), 175.65 (COOMe).

16-epi-*17*-α-*Hydroxy*-$\Delta^{14,15}$-*kopsinine* [**3**].—Amorphous powder: $[\alpha]^{20}D$ −23° (*c*=1.48,

$CHCl_3$); uv λ max nm (log ε) (EtOH) 207 (4.07), 241 (3.64), 287 (3.26); ms *m/z* (%) 352 (100), 324 (6), 322 (7), 293 (6), 251 (12), 211 (25), 202 (28), 182 (31), 150 (23), 138 (37), 137 (47), 135 (55), 123 (37), 107 (23); hr ms 352.1789 (calcd 352.1787 for $C_{21}H_{24}N_2O_3$); ir neat ν max cm^{-1} 3350, 1730, 1455; ^1H nmr ($CDCl_3$) 1.11 (H_b-19, ddt, J=12.7, 11.0, and 2.1 Hz), 1.30 (H_b-18, ddt, J=12.8, 10.9, and 2.1 Hz), 1.31 (H-6, m), 1.87 (H_a-18, ddd, J=12.8, 11.0, 7.5 Hz), 2.10 (H_a-19), ddd, 12.7, 10.9, and 7.4 Hz), 2.45 (H-6), ddd, J=12.2, 9.8, and 6.6 Hz), 2.73 (H-21, s)), 2.80–2.70 (H-5, m), 2.77 (H-16, dd, J=7.7 and 2.1 Hz), 2.96 (H-5, ddd, J=8.2, 6.6, and 1.5 Hz), 3.43 (2×H-3, m), 3.77 (OMe, s), 5.00 (H-17, dd, J=7.7 and 2.0 Hz), 5.64 (H-15, dt, J=9.9 and 2.1 Hz), 5.87 (H-14, ddd, J=9.9, 3.3, and 2.5 Hz), 6.70–7.00 (H-9, H-10, H-11, and H-12, m); ^{13}C nmr ($CDCl_3$) 23.68 (C-19), 34.65 (C-18), 37.13 (C-6), 41.00 (C-20), 49.78 (C-3 and C-5), 52.11 (OMe), 54.04 (C-16), 56.07 (C-7), 66.11 (C-21), 67.54 (C-2), 73.53 (C-17), 111.26 (C-12), 119.51 (C-10), 121.36 (C-9), 127.11 (C-11), 129.44 (C-14), 130.70 (C-15), 139.23 (C-8), 149.30 (C-13), 173.32 (COOMe).

11,12-Methylenedioxykopsaporine [**4**].—Amorphous powder: $[\alpha]^{20}D$ +21° (*c*=0.8, $CHCl_3$); uv λ max nm (log ε) (EtOH) 212 (4.42), 253 (4.01), 287 (3.27); ms *m/z* (%) 470 (100), 456 (33), 442 (7), 426 (30), 411 (11), 410 (11), 409 (11), 382 (27), 351 (31), 271 (53), 103 (55), 91 (76); hrms 470.1689 (calcd 470.1689 for $C_{24}H_{26}N_2O_8$); ir neat ν max cm^{-1} 3400, 1742, 1675, 1476, 1270, 1250; ^1H nmr ($CDCl_3$) 1.23 (H_a-19, ddd, J=12.8, 10.5, and 1.5 Hz), 1.50 (H_b-18, ddd, J=12.8, 10.5 and 7.6 Hz), 1.72 (H-6, dd, J=13.0 and 4.0 Hz), 1.77 (H_b-19, ddd, J=12.4, 10.5 and 7.6 Hz), 2.10 (H_a-18, ddd, 12.4, 10.5, and 1.5 Hz), 2.65 (H-5, ddd, J=11.6, 8.5 and 4.0 Hz), 2.77 (H-21, d, J=1.9 Hz), 2.92 (H-5, dd, J=8.5 and 6.1 Hz), 3.13 (H-6, ddd, J=13.0, 11.6, and 6.1 Hz), 3.20 (H-3, ddd, J=15.7, 1.8, and 1.5 Hz), 3.50 (H-3, ddd, J=15.7, 4.2, and 1.8 Hz), 3.81 (H-17, d, J=1.9 Hz), 3.81 and 3.83 (2×OMe, 2×s), 5.70 (H-15, dt, J=9.8 and 1.8 Hz), 5.94 (OCH_2O, m), 6.02 (H-14, ddd, J=9.8, 4.2 and 1.5 Hz), 6.54 (H-9*, d, J=7.8 Hz), 6.62 (H-10*, d, J=7.8 Hz); ^{13}C nmr ($CDCl_3$), 26.05 (C-19), 26.89 (C-18), 39.25 (C-20), 40.69 (C-6), 48.45 (C-3), 50.02 (C-5), 52.03 and 53.29 (2×OMe), 55.76 (C-7), 68.59 (C-21), 76.01 (C-2), 79.91 (C-16), 81.72 (C-17), 100.79 (OCH_2O), 103.34 (C-10), 113.21 (C-9), 126.00 (C-8), 128.90 (C-14), 131.48 (C-15), 137.27 (C-13), 148.79 (C-12*), 149.21 (C-11*), 154.59 (N-CO_2Me), 171.85 (16-CO_2Me).

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Received 23 February 1993