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The Alkaloids of *Ophiorrhiza communis* and *O. tomentosa*

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ABSTRAK

Dua alkaloid, harman dan asid striktosidinik telah diasingkan daripada daun dan kulit *Ophiorrhiza communis* Linn. Asid striktosidinik juga telah diasingkan dari *Ophiorrhiza tomentosa* Jack. Sebatian tersebut telah dikenalpasti melalui analisis spektroskopi.

ABSTRACT

Two alkaloids, harman and strictosidinic acid, were isolated from the leaves and bark of *Ophiorrhiza communis* Linn. Strictosidinic acid was also isolated from *Ophiorrhiza tomentosa* Jack. These structural assignments were based on their physical and spectroscopic data.

Keywords: ***Ophiorrhiza communis, O. tomentosa*, strictosidinic acid, harman**

INTRODUCTION

Ophiorrhiza communis (Rubiaceae) and *O. tomentosa* are small herbs, 0.1 to 0.3 metre tall, commonly found in Peninsular Malaysia except in the far south. The former is known locally as pokok peparu (lung plant) or pengerak nasi. Being a soft and easily pulped plant, it is used as a poultice and also for treating coughs (Burkill 1936). Previous chemical studies on this genus included *O. japonica, O. pumila, O. kuroiwai, O. filistipula, O. major* and one unidentified *Ophiorrhiza* species (Aimi *et al.* 1985a, 1985b, 1986, 1989, 1990; Arbain *et al.*

1993). This paper reports the results from work on the isolation and identification of harman from *O. communis* and strictosidinic acid from both *O. communis* and *O. tomentosa.*

MATERIALS AND METHODS

Plant materials

Ophiorrhiza communis was collected from the National Park, Ulu Tembeling, Malaysia and from Bungus Beach, Padang, West Sumatera, Indonesia. *Ophiorrhiza tomentosa* was collected from the Cameron Highlands in Malaysia. Voucher specimens collected in Malaysia were deposited at the herbarium of Universiti Pertanian Malaysia and that collected in Sumatera was deposited at the herbarium of Universitas Andalas.

General

Melting points were determined on a Kofler hot stage apparatus and were uncorrected. UV spectra were recorded on Shidmazu UV-VIS 160, and IR spectra on Perkin Elmer 1600 FTIR spectrometers. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker CPX 300 at 300 and 75 MHz, respectively. Column and centrifugal chromatography utilized Merck 7729 and 7749 silica gel, respectively. Preparative TLC was carried out using Merck 60 F_{254} Art 5717 and analytical TLC on Merck DC-Plastikfollen 60 F_{254}.

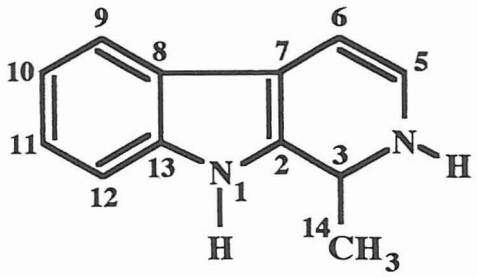
Extraction of Plant Materials

A general extraction method for alkaloids was employed for each plant sample. The fresh leaves and stems of *O. communis* (357.7 g) were macerated with methanol for 48 h. The methanol was removed by filtration and fresh methanol was added to the plant material. This process was repeated until the residue gave a negative test for alkaloids (Meyers reagent). The methanol extracts were combined and evaporated under reduced pressure to give a dark green viscous mass. The extract was then triturated using light petroleum. The residue was taken up in chloroform and extracted repeatedly with 1M H_2SO_4 and the extracts were combined, basified with Na_2CO_3 and extracted successively with chloroform at pH 8 and butanol at pH 12. The crude alkaloids were present in both the chloroform and butanol fractions.

Isolation of Harman (I)

The crude alkaloid (40 mg) from the chloroform extract was subjected to preparative TLC using $CHCl_3$/MeOH (95:5) as the solvent. The major band was recovered using a mini column eluted with methanol. The alkaloid harman (8 mg) was recrystallized from light petroleum/dichloromethane as yellowish crystals, m.p. 234-237°C (lit. m.p. 237-238°C, Southon and Buckingham 1989). UV λ_{max} nm (log ε) EtOH: 348 (0.78), 335 (0.78), 289 (0.87); IR υ cm^{-1} (KBr disk): 3422, 3130, 1625, 1565, 1506, 1453, 1324, 741; ^{1}H-NMR δ (300 MHz, $CDCl_3$): 8.33 (d, 1H, $J_{5,6}$= 5.4 Hz, H-5), 8.10 (d, 1H, $J_{9',10'}$= 7.9 Hz, H-9), 7.83

(d, 1H, $J_{6',5'}$= 5.4 Hz, H-6), 7.55 (m, 1H, H-12), 7.54 (m, 1H, H-11), 7.30 (m, 1H, H-10), 2.85 (s, 3H, H-14); ^{13}C-NMR (75.4 MHz, $CDCl_3$; DEPT-experiment): 141.6 (C-3), 140.3 (C-2), 138.0 (C-5), 134.8 (C-13), 128.5 (C-11), 128.4 (C-8), 121.9 (C-7), 121.6 (C-9), 120.2 (C-10), 112.9 (C-6), 111.6 (C-12), 20.0 (C-14); Ms m/z (%): 183 (M^+ + 1, 13), 182 (M^+, 100), 181 (29), 154 (26), 127 (11).

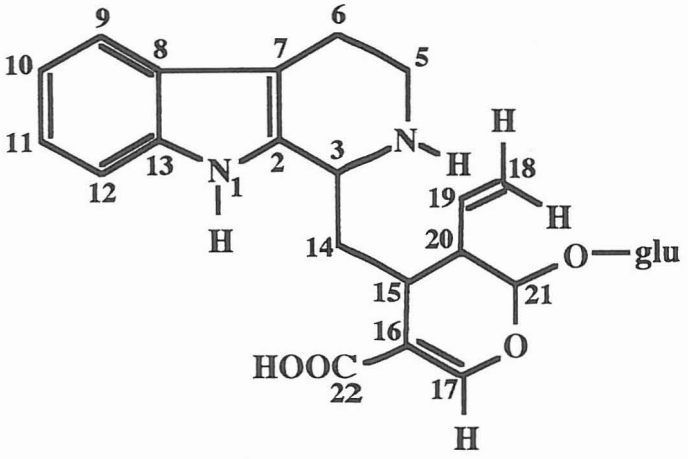


(I) Harman

Isolation of Strictosidinic Acid (II)

The crude alkaloids from butanol fraction (0.6 g from *O. communis* and 1.5 g from *O. tomentosa*) were further fractionated by centrifugal chromatography with increasing concentrations of methanol in ethyl acetate as the eluant. The major component was obtained after evaporation of the solvent as a brown gum. This was further purified by preparative TLC. Recrystallization from methanol gave yellowish crystals (0.12 g from *O. communis* and 0.3 g from *O. tomentosa*), $[\alpha]_D$ = -184°, m.p. 238-241°C, (c = 0.85 in EtOH) (lit. $[\alpha]_D$ = -223°, c = 0.19 in H_2O, Arbain *et al.* 1993). Isolates from both plants co-chromatographed on TLC. The UV, IR, ^{1}H- and ^{13}C-NMR spectra were consistent with literature (Arbain *et al.* 1993). UV λ_{max} nm (log ε) MeOH: 272 (0.96), 223 (2.41); IR υ_{max} cm^{-1} (KBr disk): 3404, 3600-2700, 1640, 1076, 740; ^{1}H-NMR δ (300 MHz, MeOH-d_4): 7.55 (s, 1H, H-17), 7.43 (d, 1H, $J_{9,10}$= 7.8 Hz, H-9), 7.31 (d, 1H, $J_{12,11}$= 8.1 Hz, H-12), 7.12 (ddd, 1H, $J_{11,12}$= 7.1 Hz, $J_{11,10}$= 7.1 Hz, $J_{11,9}$= 1.1 Hz, H-11), 7.02 (ddd, 1H, $J_{10,9}$= 7.1 Hz, $J_{10,11}$= 7.1 Hz, $J_{10,12}$= 1.1 Hz, H-10), 5.85 (ddd, 1H, $J_{19,18\text{-}E}$ = 17.5 Hz, $J_{19,18\text{-}Z}$= 10.6 Hz, $J_{19,20}$= 7.5 Hz , H-19), 5.82 (d, 1H, $J_{21,20}$= 9.6 Hz, H-21), 5.30 (br-ddd, 1H, $J_{18\text{-}E,19}$= 17.4 Hz, J= 1.38 Hz, $J_{18\text{-}20}$= 1.2 Hz, H-18-E), 5.18 (br-ddd, 1H, $J_{18\text{-}Z,19}$= 10.7 Hz, $J_{18,18}$= 1.40 Hz, $J_{18\text{-}18}$= 1.2 Hz, H-18-Z), 4.81 (d, 1H, $J_{1',2'}$= 7.9 Hz, H-1'), 4.37 (br-d, 1H, $J_{3,14}$= 11.9 Hz, H-3), 4.00 (dd, 1H, $J_{6',6'}$= 11.8 Hz, $J_{6',5'}$= 2.08 Hz, H-6'), 3.67 (dd, 1H, $J_{6',5'}$= 6.8 Hz, $J_{6',6'}$= 11.8 Hz, H-6'), 3.42 (dd, 1H, $J_{3',2'}$= 9.07 Hz, $J_{3',4'}$= 9.07 Hz, H-3'), 3.39 (ddd, 1H, $J_{5',6'}$= 2.0 Hz, $J_{5',6'}$= 6.9 Hz, $J_{5',4'}$= 9.7 Hz, H-5'), 3.21 (dd, 1H, $J_{4',3'}$= 8.9 Hz, $J_{4',5'}$= 9.7 Hz, H-4'), 3.17 (dd, 1H, $J_{2'}$= 9.09 Hz, $J_{2',1'}$= 7.9 Hz, H-2'), 2.97 (m, 1H, H-15), 2.68 (br-ddd, 1H, H-20), 2.34 (ddd, 1H, $J_{14',3}$= 3.21 Hz, $J_{14',15}$= 14 Hz, $J_{14',14}$= 14.0 Hz, H-14α). 2.10 (ddd, 1H, $J_{14,15}$= 4.4 Hz, $J_{14',3}$= 12.4 Hz, $J_{14',14}$= 14 Hz, H-14β); ^{13}C-NMR (75.4 MHz, MeOH-d_4, DEPT-experiment): 176.03 (C-22), 153.24 (C-17), 138.16 (C-13),

136.24 (C-2), 130.5 (C-19) 127.52 (C-8), 123.33 (C-11), 120.53 (C-10), 119.05 (C-9), 118.98 (C-18), 113.65 (C-16), 112.24 (C-12), 107.29 (C-7), 100.34 (C-1'), 96.58 (C-21), 78.71 (C-5'), 77.97 (C-3'), 74.73 (C-2'), 71.79 (C-4'), 63.09 (C-6'), 52.26 (C-3), 45.68 (C-20), 42.99 (C-5), 35.11 (C-14), 34.03 (C-15), 19.59 (C-6).



(II) Srictosidinic acid

RESULTS AND DISCUSSION

Extraction of the fresh leaves of *O. communis* and *O. tomentosa* followed by purification of each extract afforded harman (from *O. communis* only) and strictosidinic acid. Harman, isolated from the chloroform extract, m.p. 234-237° C, gave a mass spectrum with a molecular ion peak at m/z 182 corresponding to a molecular formula of $C_{12}H_{10}N_2$. The UV spectrum displayed strong absorption bands at λ_{max} 348, 335 and 289 nm and the IR spectrum indicated the presence of pyridoindole skeleton (1625, 1565, 1506, 1453 cm^{-1}). The ^{1}H-NMR of the compound was assigned by spin decoupling of the aromatic protons. A singlet at δ 2.85 which integrated for three protons was assigned to a methyl group attached to the C-14 carbon. The C-^{13}NMR spectrum indicated that the compound contained six unsubstituted aromatic, one primary and three substituted aromatic carbons.

Strictosidinic acid has absorption maxima at 272 nm and 223 nm, characteristic of an indole skeleton. The IR spectrum showed strong absorptions at 3404 cm^{-1} and 1640 cm^{-1}, which are consistent for N-H and C=O stretching absorptions, respectively. A broad absorption band around 3600-2700 cm^{-1} suggested the presence of a hydrogen-bonded OH group. The H-NMR spectrum showed the presence of four aromatic protons, and these are assigned to protons of the indole skeleton. Multiplets at δ 5.30 and δ 5.18 were assigned to olefinic protons. At higher field signals for the protons of a sugar unit were also present. The ^{13}C-NMR of strictosidinic acid has six quartenary, five methine and sixteen methylene carbons. A low field signal at 176.03 p.p.m. is

assigned for carbonyl carbon while those at 110-150 p.p.m. are assigned to the aromatic and olefinic carbons. Signals for the sugar and other saturated carbons appeared at a higher field.

Strictosidinic acid was earlier reported to occur in *Rhayza orientalis* (De Silva 1971) and was recently isolated from *O. filistipula* (Arbain *et al.* 1993). Harman or 1-methyl-β-carboline was previously isolated from *Arariba rubra* and also from *Passiflora incarta* (Southon and Buckingham 1989).

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