(decomp.) (CH_2Cl_2–hexane), $[\alpha]_D^{25}$ −36.4° (*c* 7.7; $CHCl_3$); voachalotine (**8**) (roots, bark): mp 210–212° (C_6H_6), $[\alpha]_D^{25}$ 7.7° (*c* 1.0; $CHCl_3$); vobasine (**9**) (bark): $[\alpha]_D^{25}$ −141.3° (*c* 0.86; $CHCl_3$); 12-methoxy-N_b-methylvoachalotine iodide (**10**) (roots, bark): mp 256 (decomp.) (MeOH); $[\alpha]_D^{25}$ −65.6° (*c* 6.7; $CHCl_3$).

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3-DEHYDROMITRAGYNINE: AN ALKALOID FROM *MITRAGYNA SPECIOSA*

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Key Word Index—*Mitragyna speciosa*; Rubiaceae; leaves; indole alkaloids; 3-dehydromitragynine.

Abstract—An investigation of the fresh leaves of *Mitragyna speciosa* has resulted in the isolation of a new alkaloid in addition to the indole alkaloids previously reported. The new alkaloid is the 3-dehydro derivative of mitragynine and its structure was elucidated by spectral means and chemical transformations. (−)-Epicatechin was also isolated from the leaves.

INTRODUCTION

Much phytochemical work on the alkaloids of *Mitragyna* species has been carried out over the last 20 years. Interest in this genus arose primarily from the fact that the leaves of *M. speciosa* have been used as a drug of abuse in Thailand and were used as a substitute for opium. The alkaloidal content of different morphological, geographical and chronological samples of *M. speciosa* has been extensively studied [1–3]. The major alkaloid present in many samples of leaves is mitragynine together with its isomers. This compound has undergone a reasonable degree of testing of its pharmacological actions but there is little evidence to suggest that it is responsible for the sensations for which this plant is used as a drug of abuse.

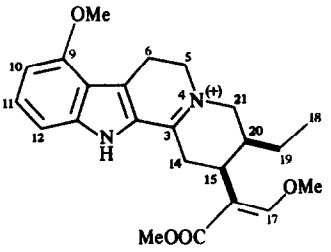
Most of the work previously carried out has been done using dried plant material. This paper describes the analysis of fresh leaves and the isolation from them of (−)-epicatechin, a procyanidin, and a new type of indole alkaloid consisting of a 3-dehydro heteroyohimbine.

RESULTS AND DISCUSSION

(−)-Epicatechin was identified by comparison with published spectral data for the substance isolated and its acetate derivative. This is the first report of this type of compound from *Mitragyna* although it is well-known as the basis of the condensed tannins present in the related genus *Uncaria*.

Mitragynine, paynantheine, speciogynine, speciociliatine and mitraciliatine were all identified by comparison with their TLC behaviour and ^{1}H NMR spectra with authentic samples.

3-Dehydromitragynine (**1**) had an $[M]^+$ of *m/z* 397, one less than mitragynine. The ^{1}H NMR spectrum showed that three methoxyl groups were present and also the presence of an ethyl group. This shows that the alkaloid must have an open E ring as in corynantheidine and be 9-methoxy substituted. The UV spectrum showed that the conjugated system in the molecule was greater than that in the mitragynine-like molecules, the bathochromic shift seen in a neutral or acidic environment indicating the possible presence of a quaternary N. This would also explain the polar nature of the molecule evidenced by its low R_f values on TLC. The downfield shift of the methine and methylene protons at C-5, C-14, C-15 and C-21 (δ3.5–4.0) compared with signals given in mitragynine (δ2.4–3.4) [4] indicate the presence of an unsaturated bond in their vicinity. The lack of any signal below 4.0 for



1

H-3 means that if present this proton has a β configuration [5]. It is impossible to say if such a 3-H signal exists because it would be mixed with the signals for the other protons seen between δ3.5 and 4.0. If an unsaturated bond exists it must either be between C-3 and C-14 or C-3 and N-4. The latter is preferred because no doublet at *ca* δ5.50 is observed such as is seen in similar alkaloids with a C-3–C-14 double bond [6]. The well-resolved triplet for the C-18 methyl means that the ethyl group at C-20 is in the β configuration such as is seen in mitragynine [7].

Compound **1** is therefore the most plausible structure for the alkaloid isolated and it was named 3-dehydromitragynine. This structure was confirmed by the formation of mitragynine by $NaBH_4$ reduction of **1** [8] and by the synthesis of **1** by lead tetraacetate oxidation of mitragynine [9]. This is the first alkaloid of this type to be isolated from natural sources although a similar compound prepared synthetically from yohimbine forms a key intermediate in the Woodward synthesis of reserpine [8]. Presumably **1** is unstable and does not withstand the drying process which is why it had not previously been isolated.

EXPERIMENTAL

Fresh leaves of *M. speciosa* were collected from trees growing on the campus of the Universiti Kebangsaan Malaysia. The material was authenticated at source and a specimen voucher is deposited in the herbarium of the Botany Dept., Universiti Kebangsaan Malaysia. Fresh leaves (500 g) were macerated in cold MeOH for 3 days. The MeOH extract was filtered off and concd under red. pres. to yield 33.5 g of residue. H_2O (350 ml) was added to this and filtered. The filtrate was red in colour, had a pH of 3.5 and gave a positive Dragendorff's test for alkaloids. The ppt was washed with 100 ml H_2O and the washings added to the filtrate. The filtrate was washed with 2 × 50 ml petrol (bp 60–80°) and then extracted with 3 × 50 ml $CHCl_3$. The combined $CHCl_3$ extracts were washed with H_2O, dried (Na_2SO_4) and concd under red. pres. to yield 540 mg of residue from which the alkaloids were obtained as described below.

The remaining aq. layer was freeze-dried to give 29 g of a viscous residue. A portion (20 g) of this was taken, dissolved in 100 ml H_2O and extracted with 4 × 30 ml EtOAc. The EtOAc layers were combined, dried (Na_2SO_4) and concd under red. pres. to give 1.6 g residue. TLC analysis showed the presence of one major and several minor UV-quenching, non-alkaloidal spots. The residue (400 mg) was fractionated by CC (silica gel Merck 70–230 mesh) eluted with $CHCl_3$ (400 ml) and 200 ml fractions containing increasing proportions of MeOH. The major compound present as determined by TLC was isolated from the $CHCl_3$ eluates (76 mg). It was identified as (−)-epicatechin by comparison of its spectral data and those of its acetate with the lit. Other compounds present were not identified although they gave similar colours on TLC to epicatechin and are likely to be related in structure.

TLC examination of the combined $CHCl_3$ extracts showed the presence of the 9-methoxy indole alkaloids previously reported from *M. speciosa* but also the presence of a yellow alkaloidal spot of low R_f value. This compound was isolated by centrifugal TLC. The alkaloid-containing extract (200 mg) was dissolved in $CHCl_3$ and fractionated using a Chromatotron (silica gel Merck kieselgel 60 PF_{254} gipshaltig 2 mm thick) eluting with 200 ml $CHCl_3$, 100 ml $CHCl_3$–MeOH (9:1) and 100 ml $CHCl_3$–MeOH (4:1), successively. Fractions (2.5 ml) were collected and like fractions combined to give the following alkaloids in pure form: mitragynine 43 mg, paynantheine 25 mg, speciogynine 22 mg, speciociliatine 15 mg, mitraciliatine 16 mg and 18 mg of a yellow-coloured alkaloid 3-dehydromitragynine **1**. The latter could not be crystallized but its purity was established by homogeneity in several TLC systems.

TLC. The following solvent mixtures were used on silica gel layers (1) $CHCl_3$–MeOH (9:1) (2) $CHCl_3$–MeOH (19:1) (3) EtOAc–*iso*-PrOH–NH_4OH (16:3:1) (4) $CHCl_3$–Me_2CO (5:4). The following spray reagents were used for detection. Dragendorff's reagent (for alkaloids); 0.2 M Fe(III)Cl_3 in 35% $HClO_4$ then heating at 105° for 10 min (for heteroyohimbine alkaloids); 0.5% anisaldehyde in HOAc–H_2SO_4–MeOH (2:1:17) and heating at 105° for 10 min (for terpenes and procyanidins); 1% vanillin in 50% aq. H_3PO_4 and heating at 105° for 10 min (for alkaloids and terpenes).

3-Dehydromitragynine (**1**). On silica gel it gave a pale green–brown colour on spraying with Fe(III)Cl_3/$HClO_4$ reagent and heating at 105° for 10 min. R_f values (1) 0.25 (2) 0.05 (3) 0.78 (4) 0.02: UV λ_{max}^{MeOH} nm (log ϵ): 205 (4.2), 247 (4.0), 317sh (3.75), 342 (3.85), 395sh (3.70); + 1 drop 0.1 M NaOH 205 (4.03), 230sh (4.1), 280 (3.85), 302 (3.82), 318sh (3.79); IR ν_{max}^{KBr} cm^{-1}: 1704 (C=O), 1633 (C=C), 1582, 1555, 1523, 1387, 1254, 1108; EIMS (probe) 35 eV *m/z* (rel. int.): 397 $[M]^+$ (34), 396 (100), 381 (97), 367 (42), 349 (45), 335 (13), 281 (12), 251 (7), 239 (8); ^1H NMR (250 MHz, $CDCl_3$): δ7.49 (1H, *s*, H-17), 7.37 (1H, *d*, J = 8.5 Hz, H-10), 7.23 (1H, *dd*, J = 8.5, 7.6 Hz, H-11), 6.38 (1H, *d*, J = 7.6 Hz, H-12), 3.89 (3H, *s*, 9-OMe), 3.75 (3H, *s*, 17-OMe), 3.61 (3H, *s*, COOMe), 3.5–4.0 (6H, *m*, H-14, H-15, CH_2-5, CH_2-21), 3.44 (2H, *t*, J = 7.9 Hz, 6-CH_2), 3.28 (1H, *t*, J = 12.0 Hz, H-20), 1.31 (2H, *m*, 19-CH_2), 0.97 (3H, *t*, 18-Me).

Conversion of **1** *to mitragynine.* Compound **1** (10 mg) was dissolved in dry MeOH and 2 ml of 0.1 M $NaBH_4$ in MeOH added. The reaction mixture was left for 16 hr and then taken to dryness. The residue was dissolved in $CHCl_3$, washed with H_2O and again taken to dryness. TLC revealed the presence of a spot corresponding in R_f value to mitragynine. Prep. TLC (system 4) resulted in the isolation of 5 mg of an alkaloid with identical spectral characteristics to an authentic sample of mitragynine.

Conversion of mitragynine to **1**. Authentic mitragynine (30 mg) was dissolved in 7.5 ml HOAc containing 30 mg lead tetraacetate and refluxed at 100° for 20 min. H_2O (10 ml) was added to the reaction mixture which was then made alkaline and extracted with 2 × 15 ml $CHCl_3$. The combined $CHCl_3$ layers were washed, dried (Na_2SO_4) and subjected to prep. TLC (system 1) to give 12 mg of an alkaloid identical in spectral and chromatographic characteristics with **1**.

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