Malaysian Journal of Science **18**: 63-66 (1999)

Alkaloids from *Litsea elliptibacea* (Lauraccae)

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Abstract. (+)-*N*-methylactinodaphnine, (+)-actinodaphine, (+)-*N*-methyllaurotetanine, (+)-boldine, (+)-norboldine and (+)-reticuline were isolated from the stem-bark of *Litsea elliptibacea*. The structures of the alkaloids were deduced from their spectral data.

Abstrak. (+)-*N*-Metilaktinodafnin, (+)-aktinodafin, (+)-*N*-metillaurotenanin, (+)-boldin, (+)-norboldin dan (+)-retikulin ditapis dari kulit tumbuhan *Litsea elliptibacea*. Struktur alkaloid ditentukan secara spektroskopi.

Introduction

The *Litsea* species has been documented to yield a number of aporphine alkaloids that display hydroxyl substitution in the A or D ring [1-5]. *Litsea elliptica*, growing to a 45 m tall and 2.40 m girth, has short buttresses, grey-brown bark, and a pinkish inner bark that emits a strong spicy odour. It is widely distributed in the lowland forests in Peninsular Malaysia, Borneo and New Guinea.. Ridley (1924) in *Flora of the Malay Peninsula* **3**: 119 reports this species as being highly valued in native medicine. *Litsea turfosa* [6], found in the peat-swamps forest of Sarawak, Malaysia, has been shown to possess anti-fungal and anti-tumour activity. *Litsea glutenosa* var. *glabratia* from West Bengal, India has reported [7] to have spasmolytic activity. The alkaloids isolated from *Litsea* species were reported [8,9] to possess hypotensive activity. A recent phytochemical survey [10,11] of East Malaysian plants included *L. elliptibacea* (Lauraceae) as one of the promising plants for further detailed studies; we now report the alkaloids from the bark of this species, which is highly valued in native medicine.

Experimental

The stem-bark of *L. elliptibacea* was collected from the Danum Valley, Sabah, East Malaysia. The specimen was identified at the herbarium of the Forest Research Centre, Sepilok, Sabah, where a voucher specimen (No. JTP 205) was deposited.

Dried ground bark (500 g) of *L elliptibaceae* was extracted with 95% EtOH at room temperature. The solvent was removed *in vacuo* and the concentrate was acidified with 5% HCl. The mixture was filtered and the filtrate neutralized with 25% NH_4OH to pH 10. The alkaloids were extracted into $CHCl_3$. The crude alkaloids (3.20 g) was separated by silica gel (Merck 9385) column chromatography to afford the **5** (1.35 g). Further separation by Chromatotron (Merck 7749) and preparative TLC (Merek 7730) chromatography gave **1** (6.4 mg), **2** (40.3 mg), **3** (10.8 mg), **4** (131.1 mg) and **6** (14.0 mg).

(+)-*N*-Methylactinodaphnine (**1**). HREIMS, found 325.1326 (calcd. for $C_{19}H_{19}NO_4$, 325.1313), EIMS (70 eV) m/z (rel. int. %): 325 (55, M^+), 324 (100, M^+-H), 305 (20), 282 (40), 251 (25), 222 (24), 205 (40). UV λ_{max} (EtOH) 280 and 307 nm. IR ($CHCl_3$) ν_{max} 3400 - 3500 cm^{-1}. $[\alpha]_D$ +37.4° ($CDCl_3$, c = 0.04). ^{1}HNMR ($CDCl_3$, 270 MHz): δ 7.63 (s, H-11), 6.81 (s, H-8), 6.51 (s, H-3), 6.08 and 5.93 (d, J = 1.5 Hz, -OCH_2O- at C-1/C-2), 3.90 (s, OMe at C-10) 3.15 (m, H-4 & H-5), 3.06 (dd, J = 13.7, 4Hz, H-6a),

3.05 (m, H-7), 2.71 (dd, H-7), 2.60 (m, H- 4), 2.58 (s, NMe) and 2.52 (m, H-5). ^{13}CNMR ($CDCl_3$, 67.8MHz): δ 28.9 (C-4), 33.7 (C-7), 43.8 (NMe), 53.4 (C-5), 56.2 (OMe at C-10), 62.3 (C-6a), 100.6 (-OCH_2O-), 106.7 (C-3), 109.8 (C-11), 114.3 (C-8), 116.8 (C-1a), 122.9 (C-11a), 126.4 (C-3a), 126.7 (C-1b), 128.9 (C-7a), 141.7 (C-1), 145.1 (C-9), 145.4 (C-10), 146.7 (C-2).

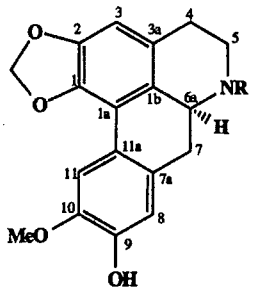
(+)-Actinodaphnine (**2**). $C_{18}H_{17}NO_4$, EIMS (70 eV) m/z (rel. int. %): 311 (58, M^+), 310 (100, M^+-H), 296 (7), 282 (10), 279 (9), 265 (6). UV λ_{max} (EtOH) 282 and 310 nm. $[\alpha]_D$ +41.4° (CDC_{13}, c = 0.24). ^{1}HNMR ($CDCl_3$, 270 MHz): δ 7.63 (s, H-11), 6.76 (s, H-8), 6.51 (s, H-3), 6.06 and 5.91 (d, J = 1.6 Hz, -OCH_2O- at C-1/C-2), 3.88 (s, OMe at C-10), 3.87 (m, H-6a), 3.35 (m, H-5), 2.97 (m, H-4 & H-5), 2.80 (m, H-7) and 2.65 (m, H-4). ^{13}C NMR ($CDCl_3$, 67.8 MHz): δ 29.4 (C-4), 36.4 (C-7), 43.3 (C-5), 54.1 (C-6a), 56.1 (OMe at C-10), 100.6 (-OCH_2O-), 107.1 (C-3), 110.1 (C-11), 114.2 (C-8), 116.5 (C-1a), 123.1 (C-11a), 126.8 (C-3a), 127.5 (C-1b), 129.0 (C-7a), 141.7 (C- 1), 145.0 (C-9), 145.3 (C- 10), 146.6 (C-2).

(+)-*N*-Methyllaurotetanine (**3**). $C_{20}H23NO_4$, EIMS (70 eV) m/z (rel. int. %): 341 (87, M^+), 340 (100, M_+-H), 329 (16), 327 (49), 267 (20), 278 (1 1). UV λ_{max} (EtOH) 220, 273sh, 280, 303 and 312sh nm. $[\alpha]_D$ +89.5° ($CDCl_3$, c = 0.06). ^{1}HNMR ($CDCl_3$, 270 MHz): δ 8.06 (s, H-1 1), 6.82 (s, H-8), 6.58 (s, H-3), 3.88 (s, OMe at C-10), 3.90 (s, OMe at C-2), 3.64 (s, OMe at C-1), 3.10 (m, H-4, H-S & H-7), 2.99 (dd, J = 15.9, 3.5Hz, H-6a), 2.67 (dd, H-7), 2.60 (m, H-5), 2.56 (s, NMe) and 2.50 (m, H-4). ^{13}CNMR ($CDCl_3$, 67.8 MHz): δ 29.1 (C-4), 34.1 (C-7), 43.8 (NMe), 53.3 (C-5), 55.7 (OMe at C-2), 56.0 (OMe at C-10), 60.1 (OMe at C-1), 62.5 (C-6a), 11 1.2 (C-3), 110.2 (C-11), 113.9 (C-8), 123.9 (C-11a), 127.0 (C-1a), 127.2 (C-1b), 128.7 (C-3a), 130.0 (C-7a), 144.1 (C-1), 144.8 (C-10), 145.2 (C-9),151.9 (C-2).

(+)-Boldine (**4**). $C_{19}H_{21}NO_4$, EIMS (70 eV) m/z (rel. int. %): 327 (80, M^+), 326 (100, M^+-H), 313 (8), 312 (42), 311 (9), 310 (5), 297 (5), 296 (21), 284 (18), 269 (13). UV λ_{max} (E5OH) 218, 274sh, 281, 303 and 313sh nm. $[\alpha]_D$ +11.1° ($CDCl_3$, c = 1.31). ^{1}HNMR ($CDCl_3$, 270 MHz): δ 7.90 (s, H-1 1), 6.75 (s, H-8), 6.53 (s, H-3), 3.83 (s, OMe at C-10), 3.56 (s, OMe at C-1), 3.02 (m, H-4, H-5 & H- 7), 2.92 (dd, J = 13.5, 3.8Hz, 6a), 2.60 (m, H-4 & H-7), 2.56 (s, NMe) and 2.52 (m, H-5). ^{13}CNMR ($CDCl_3$, 67.8MHz): δ 28.5 (C-4), 33.7 (C-7), 43.6 (NMe), 53.2 (C-5), 55.9 (OMe at C-10), 60.1 (OMe at C- 1), 62.4 (C-6a), 110.3 (C-11), 113.4 (C-3), 114.4 (C-8), 123.4 (C-11a), 126.1 (C-1b), 126.2 (C-1a), 129.5 (C-3a), 129.8 (C-7a), 142.3 (C-1), 145.1 (C-10), 145.7 (C-9),148.3 (C-2).

(+)-Norboldine (**5**). $C_{18}H_{19}NO_4$, EIMS (70 eV) m/z (rel. int. %): 313 (90, M^+), 312 (100, M^+-H), 298, 284, 282, 269, 253. UV λ_{max} (EtOH) 280 and 307 nm. $[\alpha]_D$ +64.2° ($CDCl_3$, c = 0.50). ^{1}HNMR ($CDCl_3$, 270M Hz): δ 7.92 (s, H-11), 6.77 (s, H-8), 6.63 (s, H-3), 3.84 (s, OMe at C-10), 3.78 (dd, J = 12.7, 5.4Hz, 6a), 3.60 (s, OMe at C-1), 3.36 (m, H-5), 2.96 (m, H-4 & H-5), 2.72 (m, H-7) and 2.61 (m; H- 4). ^{13}CNMR ($CDCl_3$, 67.8 MHz): δ 28.7 (C-4), 36.3 (C-7), 42.9 (C-5), 53.6 (C-6a), 55.9 (OMe at C-10), 60.1 (OMe at C-1), 110.5 (C-11), 114.0 (C-3), 114.5 (C-8), 123.4 (C-11a), 125.8 (C-1b), 127.3 (C-1a), 129.7 (C-7a), 129.7 (C-3a), 142.3 (C- 1), 145.3 (C-10), 145.8 (C-9), 148.5 (C-2).

(+)-Reticuline (**6**). $C_{19}H_{19}NO_4$, EIMS (70 eV) m/z (rel. int. %): 329 (10, M^+), 328, 327, 326, 324, 310, 311, 310. $[\alpha]_D$ +49.3° ($CDCl_3$, c = 0.04). ^{1}HNMR ($CDCl_3$, 270 MHz): 8 6.75 (d, J = 2.0 Hz, H-2'), 6.72 (d, J = 8.3 Hz, H-2'), 6.57 (dd, J = 8.3 & 2.0 Hz, H-6'), 6.53 (s, H-5), 6.37 (s, H-8), 3.67 (t, J = 6.3Hz, H-1), 3.83 (s, OMe at C-6) 3.84 (s, OMe at C-4'), 3.16m & 2.78m (H-3α & H-3β), 3.02 (dd, J = 14.2, 6.3, H-laα), 2.78 (m, H-laβ), 2.57m & 2.78m (H-4α & H-4β) and 2.44 (s, NMe). ^{13}CNMR (CDC13, 67.8 MHz): δ 24.7 (C-4), 40.7 (C- la), 42.1 (NMe), 46.4 (C-3), 55.7 (OMe at C-4'), 55.7 (OMe at C-6), 64.3 (C-1) , 110.5 (C-5'), 110.6 (C-5), 113.9 (C-8), 115.8 (C-2'), 120.7 (C-6), 124.8 (C-4a), 129.9 (C-1'), 132.9 (C-8a), 143.4 (C-3'), 145.2 (C-4'), 145.2 (C-7), and 145.4 (C-6).



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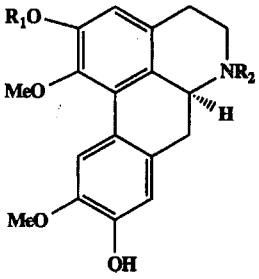

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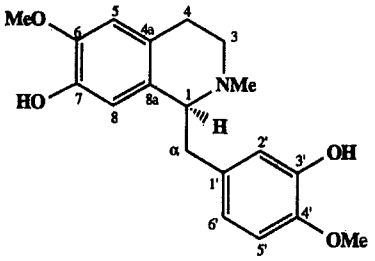

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Assignment of structure

Compound **1** was established from the HREIMS spectrum, which showed an exact molecular mass of 325.1326 that corresponds to $C_{19}H_{19}NO_4$ (calcd. 325.1313). The UV absorption λ_{max} at 280 and 307 nm indicated a 1,2,9,10-tetra-substituted aporphine skeleton; the presence of phenolic function was shown by a bathchromic shift in alkaline solution and confirmed by the IR absorption band at 3400-3500 cm-1. The ^{1}HNMR spectrum showed three one-proton singlets at 7.63, 6.81 and 6.51 ppm in the aromatic region and two methylene protons of a methylenedioxy group at 8 6.08 and 5.93 ppm (J = 1.5 Hz), which were characteristic for a 1,2,9,10-tetraoxygenated aporphinel. Among the aromatic protons, those for H-3 and H-11 are readily assignable, the former being observed as a singlet at 6.51 ppm and the latter, which is comparatively deshielded, at 7.63 ppm. The remaining singlet observed at 6.81 ppm was assigned to H-8. The substitution pattern in ring D was confirmed by the chemical shift of H-8 and H- 11, which is typical of 9-hydroxyl-10-methoxyl- substitution. The nonequivalence of the methylenedioxy protons owing to the torsion of the biphenyl system that give an AB quartet pattern can only be attributed to substitution at C(1)/C(2) whereas substitution at C-9/C-10 will give a singlet. The chemical shift of H-11 at 7.63 ppm indicated the presence of a C(1)/C(2) methylenedioxy group whereas if the C-1 is substituted with hydroxyl or methoxyl it will be in the 8.00 - 8.20 ppm range. Other signals included a C-10-methoxyl singlet at 3.90 ppm and an NMe singlet at 2.58 ppm. The aliphatic protons appeared in the region between 3.2 and 2.5 ppm. The 13CNMR data, not reported previously, also support the substitution pattern in ring D with C-8 and C-11 observed at 114.5 and 109.8 ppm. The optical rotation +37.4° of **1** indicated an α [12] configurations at C-6a.

Compound **2** showed similar UV absorption and mass fragmentation pattern as those of 1. In the EIMS spectrum, a molecular ion at m/z 311 corresponded to $C_{18}H_{17}NO_4$ and the $[M-291]^+$ ion at m/z 282 indicated a secondary aniine functionality. This was corroborated by the absence of the NMe resonances in the ^{1}HNMR spectrum. The ^{1}H and ^{13}CNMR data (carbon data not previously reported [1]) and its optical rotation value were in agreement with the assignment of (+)-actinodaphnine.

The EIMS of **3** showed a molecular ion at m/z 341 which corresponds to $C_{20}H_{23}NO_4$. The MS fragmentation pattern of **3** was similar to that of **1**. In its The presence of three methoxyl singlets at 3.88 (C-10), 3.90 (C-2) and 3.64 ppm (the most deshielded methoxyl group at C-1), one NMe signal at 2.56 ppm and three aromatic singlets at 6 6.58 (H-3), 6.82 (H-8) and 8.06 ppm (H-1 1) were in agreement with the assignment of (+)-*N*-methyllaurotetaninel. The ^{13}CNMR data of 3, not reported previously, was in agreement with the assignment (+)-*N*-methyllaurotetanine.

The EIMS of **4** showed a molecular ion at m/z 327 which corresponds to $C_{19}H_{21}NO_4$. The fragmentation pattern was consistent with those of **3**. The ^{1}HNMR spectrum is similar to that of **3**, except that two methoxyl groups were observed. The two methoxyl singlets at 3.56 and 3.83 ppm were assigned to substitutions at C-1 and C-10. The hydroxyl substitution at C-2 was consistent with the ^{1}H and ^{13}CNMR assigmnent of (+)-boldinel [12,13].

Compound **5** was the major alkaloid isolated which amount to 86.6% of the alkaloids but it was unstable as it darkened rapidly on contact with air. The EIMS spectrum showed a molecular ion at m/z 313, which corresponded to $C_{18}H_{19}NO_4$; the cleavage M+ to $[M-29]^+$ at m/z 284 that indicated a secondary amine functionality. The fragmentation pattern was similar to those of **3** and **4**, which indicated a similar skeletal type.

Benzyitetrahydroisoquinoline **6** was identified as (+)-reticuline based on its spectral data. The ^{13}CNMR assignments reported earlier [13] were reassigned based on 2DNMR data, which were similar to those reported [14]. Reticuline is probably the precursor of the 1,2,9,10- tetrasubstituted aporphines.

The isolation of five aporphine alkaloids having the 9-hydroxyl-10-methoxyl substitution together with **6** provided additional information as chemotaxononiical marker for *L elliptibacea.*

Acknowledgments

We thank the Malaysian govennent for IRPA grants and University of Malaya for financial support.

References

1 Guinaudeau, H., Leboeuf, M. and Cave, A. (1975). *Llyodia* **38**: 275.

2 Guinaudeau, H., Leboeuf, M. and Cave, A. (1979). *J. Nat. Prod.* **42**: 325.

3 Guinaudeau, H., Leboeuf, M. and Cave, A. (1983). *J. Nat. Prod.* **46**: 761.

4 Guinaudeau, H., Leboeuf, M. and Cave, A. (1988). *J. Nat. Prod.* **51**: 389.

5 Guinaudeau, H., Lebocuf, M. and Cave, A. (1994). *J. Nat. Prod.* **57**: 1033.

6 Holloway, D.M. and Scheinmann, F. (1973). *Phytochemistry* **12**: 1503.

7 Tewari, 5., Bhakuni, D.S. and Dhar, M.M. (1972). *Phytochemistry* **11**: 1149.

8 Bhakmi, D.S. and Gupta, S. (1983). *Planta Med.* **48**: 52.

9 Chen, W. Y., Ko, F.N., Wu, Y.C., Lu, S.T. and Teng, C.M. (1994) *J. Pharma. & Pharmaco.* **46**: 380.

10 Ooh, S.H., Lee, K.H., Ee, G.C.L., Ong, H.C., Geh, S.L., and Sylvester, R. (1995). *J. of Herbs, Spices & Medicinal Plants* **3**: 55.

11 Ooh, S.H., Lee, K.H., Chuah, C.H., Ong, H.C., Leopold, M. and Pereira, J.T. (1996) *J. of Herbs, Spices & Medicinal Plants.* In press.

12 Shamma, M. and Moniot, J.L. (1978). *Isoquinoline Alkaloids Research* 1972-1977. Plenum Press, New York.

13 Shamma, M and Hindenlang, D.M. (1979). *Carbon-13 NMR Shift Assignment of Amines and Alkaloids.* Plenum Press, New York.

14 Janssen, R.H.A.M., Wijkens, P., Kruk, C., Biessels, H.W.A., Menichini, F. and Thuens, H.G. (1990). *Phytochemistry* **29**: 3331.