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3′,4′-DIHYDRONORSTEPHASUBINE, A NEW BISBENZYLISOQUINOLINE FROM THE BARK OF *ALSEODAPHNE CORNERI*

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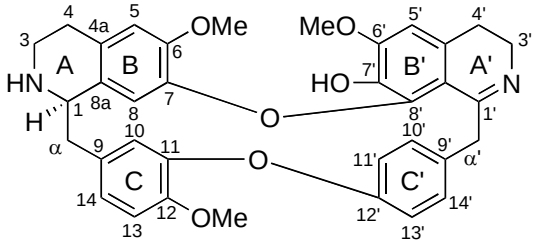
Abstract – A new bisbenzylisoquinoline, 3′,4′-dihydronorstephasubine (**1**) together with two known alkaloids, norstephasubine (**2**) and gyrolidine (**3**) were isolated from the stem bark of *Alseodaphne corneri* (Lauraceae). The ^{13}C-NMR data for norstephasubine (**2**) and gyrolidine (**3**) were also reported. Structural elucidation of **1** was performed by spectral methods such as 1D- and 2D- NMR, IR, UV, and HRMS. 3′,4′-Dihydronorstephasubine (**1**) and gyrolidine (**3**) showed moderate vasorelaxant effect on rat aorta.

Alseodaphne corneri belongs to the family of Lauraceae. It is a tree of moderate size growing in Singapore, Malaysia, Jawa, Sumatra, and Borneo.[1] Five *Alseodaphne* species; *A. perakensis*, *A. andersonii, A. hainensis, A. semicarpifolia*, and *A. archboldiana* have been chemically studied and reported to contain phenantrenes, aporphines, morphinandienones, lactones, and furanones.[2-8] There has been no report on the phytochemical study and medicinal value of *Alseodaphne corneri*, although the fruits of this species were known to be poisonous.[9-11]

Recently we have isolated three new bisbenzylisoquinoline alkaloids, α′-oxoperakensimines A – C, showing vasorelaxant activity from the bark of *Alseodaphne perakensis*.[12] Our continued effort on identifying additional new alkaloids with biological activities from *A. corneri* led to the isolation of a new bisbenzyisoquinoline, 3′,4′-dihydronorstephasubine (**1**) and two known alkaloids, norstephasubine (**2**) and

gyrolidine (**3**). This paper reports the isolation and structural elucidation of 3′,4′-dihydronorstephasubine (**1**), which showed a moderate vasorelaxant activity on isolated rat aorta. This finding indicated that *Alseodaphne corneri* might also become useful as a source of these pharmacologically interesting molecules.

Chromatographic purification of the base fraction from the bark of *A. corneri* afforded a new alkaloid (+)-3′,4′-dihydronorstephasubine (**1**) together with two known alkaloids, norstephasubine (**2**) and gyrolidine (**3**). [13-15]



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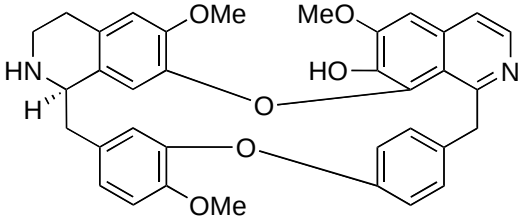

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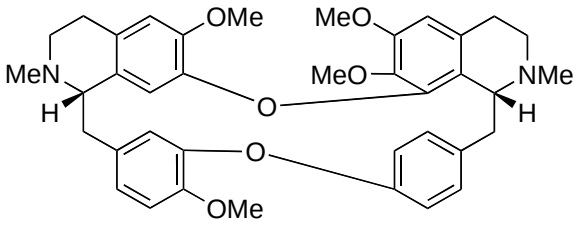

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(+)-3′, 4′-Dihydronorstephasubine (**1**) was isolated as a brown amorphous solid, with $[\alpha]_D^{23}$ +22 (*c* 0.5, MeOH). The HRESIMS spectrum of **1** showed a pseudomolecular ion peak at *m/z* 579.2535 $(M+H)^+$ corresponding to the molecular formula of $C_{35}H_{34}N_2O_6$ (calcd 579.2495). An absorption band at 1604 cm^{-1} in the IR spectrum is typical of an imine stretching band.[14]

The ^1H (400 MHz) and ^{13}C-NMR (100 MHz) spectral assignments performed by extensive 2D NMR experiments (COSY, NOESY, HMQC, and HMBC) were summarized in Table 1. In the ^1H-NMR spectrum, signals for ten aromatic protons, three methoxy singlets, two -CH_2-CH_2-N- groups, and a set of isolated none equivalent methylene protons were observed, suggesting a bisbenzylisoquinoline type of skeleton.[13,14] Among the ten aromatic proton signals, three singlets at δ_H 6.43, 6.52, and 6.10 were attributed to H-5, H-5′, and H-8, respectively. In addition, an upfield signal of H-10 (δ_H 4.95, broad singlet) was observed, which is the characteristic peak of head to head and tail to tail bisbenzylisoquinoline alkaloid (two ether linkages between 7-8′, 11-12′ of type VI).[15] H-10 signal appeared as a broad singlet because it was *meta*-coupled with H-14, which was placed vicinal to H-13 (δ_H

6.72, d), indicating that ring C was trisubstituted. Other prominent peaks of an AX spin system were observed at δ_H 3.96 and 4.50 (J = 13.7 Hz), supporting the presence of two geminal protons of the methylene adjacent to the imine function (H_2-α′). Three broad doublet signals of H-10′ (δ_H 7.27), H-13′ (δ_H 6.74), and H-14′ (δ_H 7.41), and one doublet of doublets of H-11′ (δ_H 6.40), indicating that ring C′ is a *para* disubstituted (AA′BB′) ring system.[17]

The ^{13}C NMR spectrum showed 35 carbon resonances, which were in agreement with the molecular formula. The signals at δ_C 54.5 and δ_C 165.1 could be assigned as the chiral C-1 and iminium C-1′ carbons, respectively.

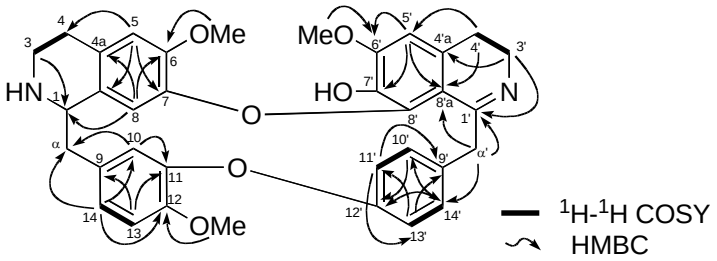

Figure 1. Selected 2D NMR Correlations for 3′, 4′-dihydronorstephasubine (**1**).

Selected 2D NMR correlations for 3′,4′-dihydronorstephasubine (**1**) were shown in Figure 1. The position of $\Delta^{1'\text{-}N'}$ double bond on the right side of the dimer was confirmed by the HMBC correlations of H-3′ to C-1′ (δ_C 165.1) and H-α′ to C-1′ and C-8a′ (δ_C 116.3).

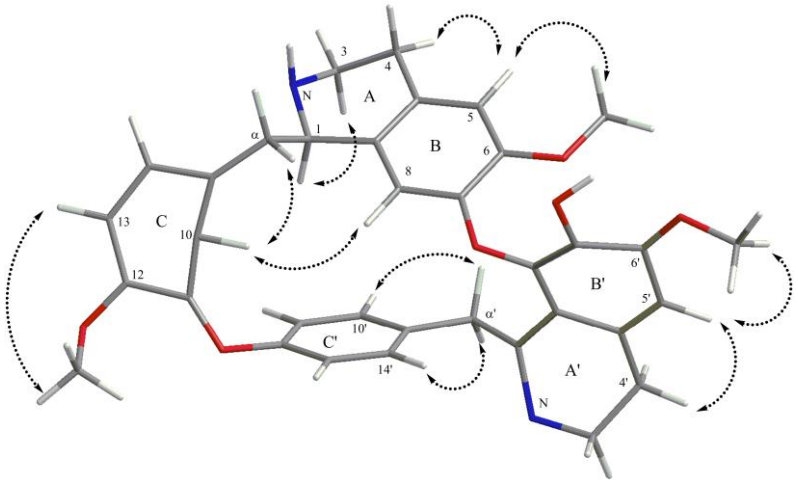

Figure 2. Selected NOESY correlation of a minimum energy conformer of 3′, 4′-dihyronorstephasubine (**1**).

The position of the three methoxy groups, was assigned by detecting the cross-peaks in the NOESY spectrum between H-5 (δ_H 6.43)/6-OCH_3 (δ_H 3.91), H-5’ (δ_H 6.52)/6’-OCH_3 (δ_H 3.86), and H-13 (δ_H

6.72)/12-OCH_3 (δ_H 3.86) respectively. Furthermore, the positive sign of the specific rotation indicated the absolute configuration at C-1 was *R* as in the known alkaloids, (+)-norstephasubine (**2**), (+)-3′, 4′-dihydrostephasubine, (+)-stephasubine, (+)-norstepharanthine and pangkorimine.[13-15] In addition, the cross-peaks in the NOESY spectrum were observed between H-1/H-3α and H-10/ H-α and H-8, supporting the configuration at C-1 of **1** as depicted in Figure 2.

The spectroscopic data of **2** and **3** were reported in comprehensive reviews but they were lacking of ^{13}C NMR data.[13-16] In view of that, complete assignments were established through various NMR measurements; DEPT, HSQC, and HMBC spectra. The ^{13}C-NMR spectra of norstephasubine (**2**) and gyrolidine (**3**) indicated the presence of 35 and 38 carbons, respectively, as shown in Table 1. The ^{13}C chemical shifts of **1** is similar to the known (+)-norstephasubine (**2**) except the presence of the olefinic carbon signals (-C3′=C4′-) observed at δ_C 140.3 and 119.0 in **2**.

Vasodilators are useful for treatment of cerebral vasopasm and hypertension, and for improvement of peripheral circulation.[18] When phenylephrine (PE) 3×10^{-7} M was applied to thoracic aortic rings with endothelium after achieving a maximal response, we added 3′,4′-dihydronorstephasubine (**1**), norstephasubine (**2**), and gyrolidine (**3**). 3′,4′-Dihydronorstephasubine (**1**) and gyrolidine (**3**) showed a moderate and slow vasorelaxant activity on isolated rat aorta (65% relaxation at 3×10^{-5}M), whereas norstephasubine (**2**) did not show a significant vasorelaxant activity (20% relaxation at 3×10^{-5}M). Vasodilation seems to be influenced by the aromaticity of ring A′ and potency of **1** and **3** was higher than that of α′-oxoperakensimines A – C from *A. perakensis*.[12] The mode of actions of these bisbenzylisoquinoline alkaloids on vasorelaxant activities are under investigation.

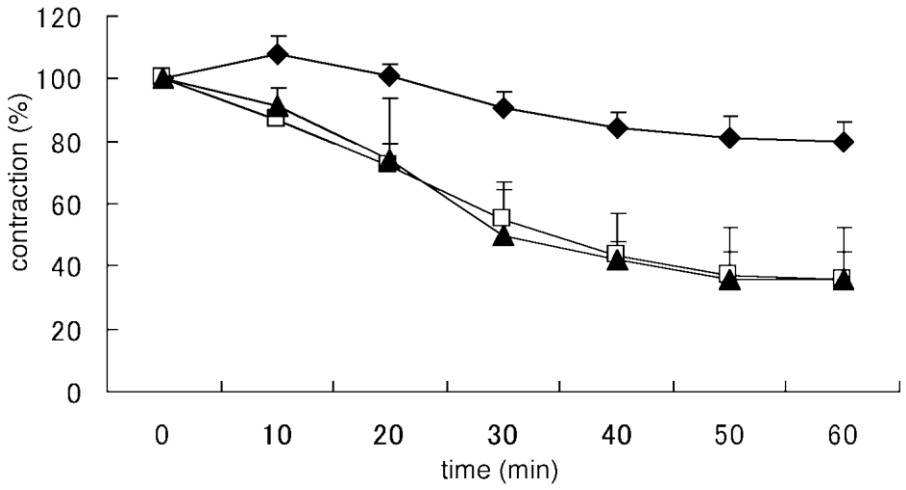

Figure 3. Relaxation responses induced by 3', 4'-dihydronorstephasubine (**1**), norstephasubine (**2**), and gyrolidine (**3**) in aortic rings precontracted with 3×10^{-7} M phenylephrine (PE); Symbols: －▲－, **1** at 3×10^{-5}M; －◆－, **2** at 3×10^{-5}M; －□－, **3** at 3×10^{-5}M. Values are means ±SE (*n*=3).

Table 1: ^{1}H and ^{13}C NMR spectral data of 3′, 4′-dihydronorstephasubine (**1**), norstephasubine (**2**), and gyrolidine (**3**) in $CDCl_3$.

Position	^{1}H (δ_H, $CDCl_3$, Hz)	^{13}C (δ_C, $CDCl_3$)		
	1	**1**	**2**	**3**
1	3.90 m	54.5	54.6	63.9
N-Me				43.7
3	2.12 m 2.77 m	41.2	41.5	50.8
4	2.14 m 2.28 m	29.4	29.7	28.4
4a	-	130.2	129.8	130.7
5	6.43 s	112.7	112.4	110.9
6	-	148.0	147.5	148.3
6-OMe	3.91 s	55.9	56.1	54.9
7	-	145.5	144.6	143.8
8	6.10 s	112.5	110.9	116.7
8a	-	127.0	127.9	127.3
α	2.77-2.82 m	38.6	38.6	37.5
9	-	127.9	127.9	130.7
10	4.95 br s	116.9	116.6	116.4
11	-	150.5	150.4	149.0
12	-	147.0	146.9	146.6
12-OMe	3.86 s	56.2	55.9	55.9
13	6.72 d (8.7 Hz)	110.8	110.8	110.7
14	6.68 d (8.7 Hz)	122.6	122.6	123.5
1′	-	165.1	157.0	61.5
N′-Me				42.1
3′	3.54-3.62 m 3.82-3.84 m	46.7	140.3	45.3
4′	2.62-2.65 m	27.0	119.0	25.4
4′a	-	131.5	133.4	127.2
5′	6.52 s	105.9	101.6	105.7
6′	-	150.9	151.3	151.6
6′-OMe	3.86 s	56.2	56.3	56.0
7′	-	136.1	135.7	137.0
7′-OMe				60.5
8′	-	142.0	145.2	147.5
8′a	-	116.3	137.0	138.9
α′	3.96 d (13.7 Hz) 4.50 d (13.7 Hz)	44.7	45.2	39.5
9′	-	135.3	137.6	127.7
10′	7.27 d (8.2 Hz)	132.1	129.2	131.4
11′	6.40 dd (2.0, 8.2 Hz)	121.9	122.6	121.1
12′	-	152.6	152.3	152.2
13′	6.74 dd (8.6, 2.0 Hz)	122.4	121.9	122.3
14′	7.41 d (8.6 Hz)	128.8	131.2	127.8

EXPERIMENTAL

General Experimental Procedures. Spectra were recorded on the following instruments: UV, Shimadzu UV-250 uv-visible spectrophotometer; IR, Perkin Elmer 1600; optical rotations at 25^{o} were taken on Jasco DIP-1000 Digital polarimeter. UV and optical rotation were recorded in MeOH. LC-EIMS, Waters Micromass ZQ; NMR, Bruker AV 400 MHz and JEOL ECA 400 MHz. All solvents, except those used for bulk extraction are AR grade. Silica gel 60 F_{254} for thin layer chromatography (TLC) was used for column chromatography. Glass and aluminium supported silica gel 60 F_{254} plates were used for TLC. TLC spots were visualized under UV light (254 and 365 nm) followed by spraying with Dragendorff's reagent for alkaloid detection.

Plant Material. The bark of *Alseodaphne corneri* was collected at Piah Reserve Forest, Sungai Siput, Perak by the phytochemical group of the Department of Chemistry, Faculty of Science, University of Malaya. The voucher specimen (KL5501) of this plant has been deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation. Dried, grounded bark of the plant (1.0 kg) was first defatted with hexane for twice for 3-days period. The hexane extract were first taken up to dryness. The plant material was dried up and then soaked with 25% NH_4OH for 2 hours. They were then macerated with CH_2Cl_2 twice for 3-days periods. The supernatant obtained was concentrated using rotary evaporator under reduced pressure to a volume of 500 ml and were examined for their alkaloid content (using TLC and confirmed by spraying with Dragendorff's reagent). The extract was finally concentrated to give crude alkaloids (8.0g). The crude alkaloid (3.0 g) was subjected to column chromatography over silica gel using dichloromethane and methanol solvent (100:0, 99:1, 98:2, 95:5, and 90:10) and finally with 100% methanol was used as eluent to obtain six fractions. Further purification of fraction two by a Preparative Thin Layer Chromatography (PTLC) yielded alkaloid (**1**) (15 mg, 95:5: saturated with NH_4OH) and alkaloid (**2**) (25mg, 95:5: saturated with NH_4OH). Alkaloid (**3**) (20 mg, 98:2: saturated with NH_4OH) was obtained from a Preparative Thin Layer Chromatography (PTLC) of fraction one.

3′, 4′-Dihydronorstephasubine (1): a brown amorphous solid, $[\alpha]_D^{23}$ +22 (*c* 0.5, MeOH). UV (MeOH) 203, 286 nm. IR ($CHCl_3$) λ_{max}: 3383, 2934, and 1604 cm^{-1}; HRESIMS *m/z* 579.2535 $[M+H]^+$; calcd for $C_{35}H_{34}N_2O_6$, 579.2495. ^{1}H and ^{13}C-NMR see Table 1.

Vasodilation Assay.[18] A male Wistar rat weighting 260 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 1.2 mM NaH_2PO_4, 1.2 mM $MgSO_4$, and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2, 5% CO_2) bath of 5

mL KHS solution at 37 °C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic ring was contracted by treatment with 3×10^{-7} M PE. The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (ACh), and aortic ring in which 80% relaxation occurred, were regarded as tissues with endothelium. When the PE-induced contraction reached a plateau, each sample (**1**-**3**, 3×10^{-5}) was added.

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

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