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DETERMINATION OF THE ABSOLUTE CONFIGURATION OF NEW PIPERIDIN-4-ONE DERIVATIVE FROM *Pellacalyx saccardianus* BY NOESY SPECTROSCOPY

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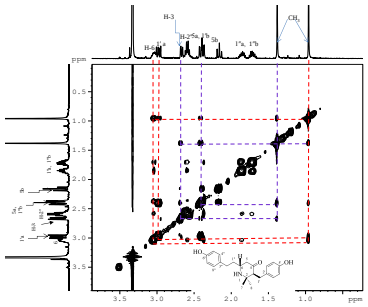
Graphical abstract



Abstract

A new alkaloid, pellacalyxin was isolated from the leaves of *Pellacalyx saccardianus* of Rhizophoraceae family. Pellacalyxin was analyzed using nuclear Overhauser spectroscopy (NOESY) nuclear magnetic resonance (NMR) technique to determine the absolute configuration. The analysis of absolute configuration of pellacalyxin was supported by X-ray crystallography. ^{1}H-^{1}H NOESY NMR spectroscopy exhibited that pellacalyxin possesses two chiral centers (3*S*) and (6*R*).

Keywords: *Pellacalyx saccardianus,* Pellacalyxin, Absolute configuration, X-ray crystallography, NOESY spectroscopy

Abstrak

Alkaloid baharu, pellacalyxin telah diasingkan daripada daun *Pellacalyxsaccardianus* daripada family Rhizophoraceae. Pellacalyxin dianalisis menggunakan teknik spektroskopi resonans magnet nukleus (NMR) Overhouser (NOESY) bagi menentukan konfigurasi mutlak pellacalyxin. Analisis konfigurasi mutlak pellacalyxin disokong oleh kristalografi sinar-X. Spektroskopi NMR ^{1}H-^{1}H NOESY menunjukkan pellacalyxin mempunyai dua pusat kiral (3*S*) dan (6*R*).

Kata kunci: *Pellacalyxsaccardianus,* Pellacalyxin, Konfigurasi mutlak, Kristalografi Sinar-X, Spektroskopi NOESY

1.0 INTRODUCTION

The absolute configuration determination of the chiral compounds is important for the studies of biochemical processing in the living systems and drug developments. Accordingly, in the bioprocess of living systems, the stereochemistry of a substrate controls the binding affinity to its target enzyme where the specific relationship between configurations and activity may be required to reach maximum effect [1,2]. In pharmaceutical studies and drug improvement, the chirality represents critical effect in the interactions between drugs and all receptors, because all receptors in the human body possess chiral centres. Thus the enantiomers exhibit different pharmacologic effects and pharmacokinetics, beside that the pure enantiomer is more influence for a specified pharmacological activity than racemic mixture. It possibly displays different pharmacologic effects as inactive, less active or toxic [3]. Therefore,

determination of absolute configuration (AC) is recently increased and developed, and the available method include X-ray crystallography, chemical synthesis, NMR spectroscopy/chiral derivatization, analytical chemistry, as well as chiroptical approaches which are based on circular dichroism, electronic circular dichroism (ECD) and vibrational circular dichroism (VCD). In spite of these methods have advantages, there are limitations, and the determination of AC is still a challenging task in the structure elucidation especially those with complex structures. Among these, X-ray crystallography maybe remains the most performance and effective method, but is only applicable to crystalline natural products [4].

The present work deals with pellacalyxin**(1)**, a new bioactive diarylalkaloid isolated from the leaves of *Pellacalyx saccardianus.* Pellacalyxin**(1)** was reported to possess significant anti-inflammatory activity against COX-2 enzyme [5,6]. It has two chiral centres located at C-3 and C-6. Therefore, the aim of the present work is to assign the absolute configuration of pellacalyxin**(1)** to support the pharmacokinetics studies. Besides that, we wish to further highlight the significant of nuclear Overhauser spectroscopy, NOESY in the determination of absolute configuration (AC), to support the relative configuration which has been assigned by X-ray crystallography.

2.0 EXPERIMENTAL

2.1 General Experimental Procedures

Melting points were determined using Leica Gallen apparatus and were uncorrected. Infrared (IR) spectra were recorded on Perkin Elmer 1650 FTIR spectrophotometer as KBr disc and NaCl cell. ^{1}H-NMR spectra were recorded on Bruker 400 MHz, and ^{13}C-NMR spectra at 100 MHz, and deuterated solvents of $CDCl_3$, $(CD_3)_2CO$ and CD_3OD were used as solvents. Absolute configurations were obtained by X-ray crystallography from the Chemistry Department Laboratory of NUS, Singapore.Mass spectral data were acquired by Bruker Mass Spectrometry Services from National University of Singapore (NUS),. Analytical thin layer chromatography (TLC) was carried out on a silica gel aluminium sheets (Merck Kieselgel 60 F254, 0.20 mm). Spots were visualized with UV light (254 nm and 365 nm) and sprayed with vanillin reagent. Merck silica gel 60 (230-400) mesh was used for vacuum liquid chromatography (VLC), and column chromatography (CC) was carried out by using silica gel 60 (70-230 mesh).

2.2 Plant Materials

Leaves of *Pellacalyx saccardianus* were collected in July 2011 from Kuala Berang, Terengganu. The species was identified by Dr. Shamsul Khamis from Universiti Putra Malaysia, and the voucher specimen SK1941/11 was deposited at Herbarium, Bioscience Institute of Universiti Putra Malaysia.

2.3 Extraction and Purification

Powdered leaves (800 g) were extracted in a soxhlet extractor with methanol (3 L) for 24 hr. The solvent was evaporated to dryness using a rotary evaporator to afford the MeOH extract (PM, 170 g). The extract (PM) was screened for the presence of alkaloid using Mayer's reagent and exhibited very weak precipitate (1+) [7]. The extract (PM) was partitioned between water (400 mL) and $CHCl_3$:methanol (2:1) (3 × 300 mL) using a separatory funnel. Next, the aqueous layer was partitioned with $CHCl_3$:ethanol (2:1). The organic layers were evaporated to dryness to obtain $CHCl_3$:methanol extract (PMC, 30 g) and $CHCl_3$:ethanol extract (PME, 53 g). Finally, the aqueous layer was evaporated and dried to yield the PA extract (80 g).

The PME extract (12.0 g) was fractionated over silica gel using VLC with a solvent gradient of increasing polarity as follows: petroleum ether:diethyl ether (1:0, 1:1, 0:1), diethyl ether:$CHCl_3$ (1:0, 9:1, 3:1, 1:1, 2:3, 0:1), $CHCl_3$:acetone (1:0, 9:1, 3:1, 1:1, 0:1), ethanol, and methanol. The fractions were checked by TLC, and fractions with similar profiles were combined to yield five major fractions (PMEA-PMEE). PMEC (1.4 g) was purified using CC (3 × 50 cm) with silica gel and eluted in the order of increasing polarity as follows: *n*-hexane, *n*-hexane:diethyl ether, diethyl ether:acetone, acetone, and ethanol. Forty fractions were collected and examined by TLC. Similar fractions were combined to obtain six subfractions (PMECa-PMECf). Subfraction PMECb was purified on CC (2 × 30 cm) with silica gel (10 cm in height) using a solvent gradient of increasing polarity, *n*-hexane, *n*-hexane:diethyl ether (6:4, 5:5, 2:8, 0:10), diethyl ether:acetone (1:1), and acetone to yield twenty-seven subfractions PMECb1-PMECb62. Vials of subfractions PMECb (16-17) were combined and washed with acetone to yield white powder that was designated as compound (**1**).

Compound **(1):** (10 mg, 0.083%), white crystals with m.p. 109-111°C, R_f = 0.52 in diethyl ether. IR ν_{max} (KBr) cm^{-1}: 3430 (OH), 3258.0 (N-H), 2925 (C-H), 1685 (C=O), 1612, 1514 and 1458 (aromatic ring); ^{1}H-NMR ($CDCl_3$/CD_3OD): δ 7.02 (4H, d, *J* = 8.2 Hz, H-3', H-4", H-7', H-8"), 6.71 (4H, d, *J* = 8.2 Hz, H-5", H-7"), 6.66 (2H, d, *J* = 8.2 Hz, H-4', H-6'), 3.05 (1H, m, H-6), 2.97 (H, dd, *J* = 10.0, 14.0 H-1'a), 2.39 (1H, m, H-1'b), 2.65 (1H, d, *J* = 10.0 H-3), 2.59 (2H, dt, *J* = 2.4, 6.4 Hz, H-2"), 2.42 (1H, m, H-5a overlapped with H-1'b), 2.16 (1H, t, *J* = 12.0 Hz, H-5b), 1.86 (1H, m, H-1"a), 1.72 (1H, m, H-1"b), 1.38 (s, CH_3), 0.95 (s, CH_3); ^{13}C-NMR (100 MHz, ($CDCl_3$)/CD_3OD): δ 210.7 (C-4), 155.7 (C-6"), 155.5 (C-5'), 132.5 (C-3"), 132.4 (C-2'), 129.9 (C-3', C-7'), 129.9 (C-3', C-7'), 129.5 (C-4", C-8"), 115.6 (C-5", C-7"), 114.9 (C-4', C-6'), 63.8 (C-3). 58.4 (C-2), 52.1 (C-6) 48.4 (C-5), 38.4 (C-1"), 30.8 (C-2"), 28.7 (C-1'), 28.6 (CH_3), 19.81 (CH_3); EIMS: *m/z* 354 (7%), [M$^+$] ($C_{22}H_{26}O_4$), 353 [M$^+$-H], 334 (25%) [M-H_2O-2H], 333 (100%), 107 (67%); HR-EIMS: *m/z* 353.1972 [M]$^+$ (calcd. for $C_{22}H_{27}O_3N$, 353.1969).

3.0 RESULTS AND DISCUSSION

Compound (**1**) was obtained from the PMECb subfraction of the EtOH:$CHCl_3$ extract as white crystals (10 mg, 0.083%) with m.p. 109-111°C and R_f value of 0.52 in diethyl ether. Compound (**1**) has been identifiedas a novel metabolite, 3-(4'-hydroxybenzyl)-6-(6''-hydroxyphenethyl)-2,2-dimethyl-piperidin-4-one **(1)** [5,6].

The structure of pellacalyxin**(1)**showed the presence of two chiral centres at C-3 and C-6. Thus, according to the 2^n rule it might have four stereoisomers [8]. Therefore, the configuration of compound (**1**) was achieved by single X-ray crystallography to obtain ORTEP view as shown in **Figure 1**. Based on that, the chiral centres at C-3 and C-6 were relatively determined to be 3*S* and 6*R*, respectively. This configuration was further confirmed by ^{1}H-^{1}H NEOSY NMR spectroscopy. In this application, the NOE occurs interaction correlation through space [9], not through chemical bonds. The ^{1}H-^{1}H NOESY spectrum displayed cross-peak correlation for long-range interactions between proton H-6 at δ 3.01 and methyl protons H-7 at δ 0.95 as shown in **Figure 2**, revealing the presence of similar co-facial orientation for both H-6 and H-7 in **Figure 1** (H-9 and H-14 in the ORTEP view). Similarly, H-3 (δ 2.65) showed spatial correlation with methyl protons H-8 (δ 1.38) and H-1'b (δ 2.39), indicating the same alignment face of those protons as shown in **Figure 1** (H-12, H-15 and H-16 in the ORTEP view). Thus, the configurations of H-3 and H-6 were absolutely confirmed to be 3*S* and 6*R*, respectively. The protons of two methyl groups at, δ 0.95 (H-7) and H-8 (δ 1.38) exhibited spatial interaction supporting the location of both methyl groups at C-2. Additional long-range correlations resulted from protons interaction in the space is shown in ^{1}H-^{1}H NOESY spectrum (**Figure 2)**.

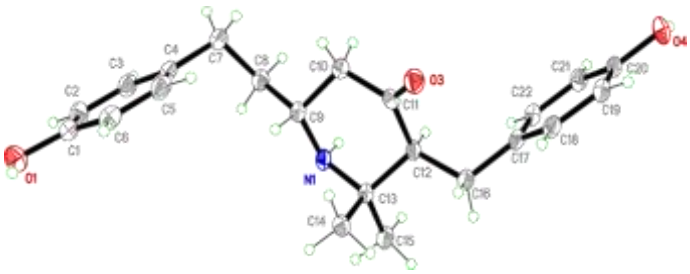
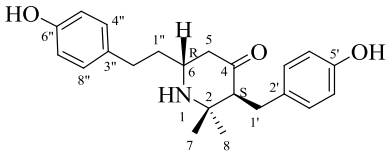


Figure 1 The ORTEP view of pellacalyxin**(1)** obtained by X-ray crystallography.

Based on the comparison of the two methods, X-ray Crystallography and ^{1}H-^{1}H NOESY NMR spectroscopy, the absolute configuration (AC) of compound (**1**) was assigned as (3*S*)(6*R*)-3-(4'-hydroxybenzyl)-6-(6''-hydroxyphenethyl)-2,2-dimethylpiperidin-4-one **(1)**, which was isolated for the first time from the leaves of *Pellacalyx saccardianus*.



(1)

4.0 CONCLUSION

In conclusion, pellacalyxin**(1)** has two chiral centres, 3*S* and 6*R*. This finding confirmed the significant contribution of NOESY spectroscopy to the determination of absolute configuration. NOESY method is applicable to crystalline, amorphous and liquid samples of natural products.

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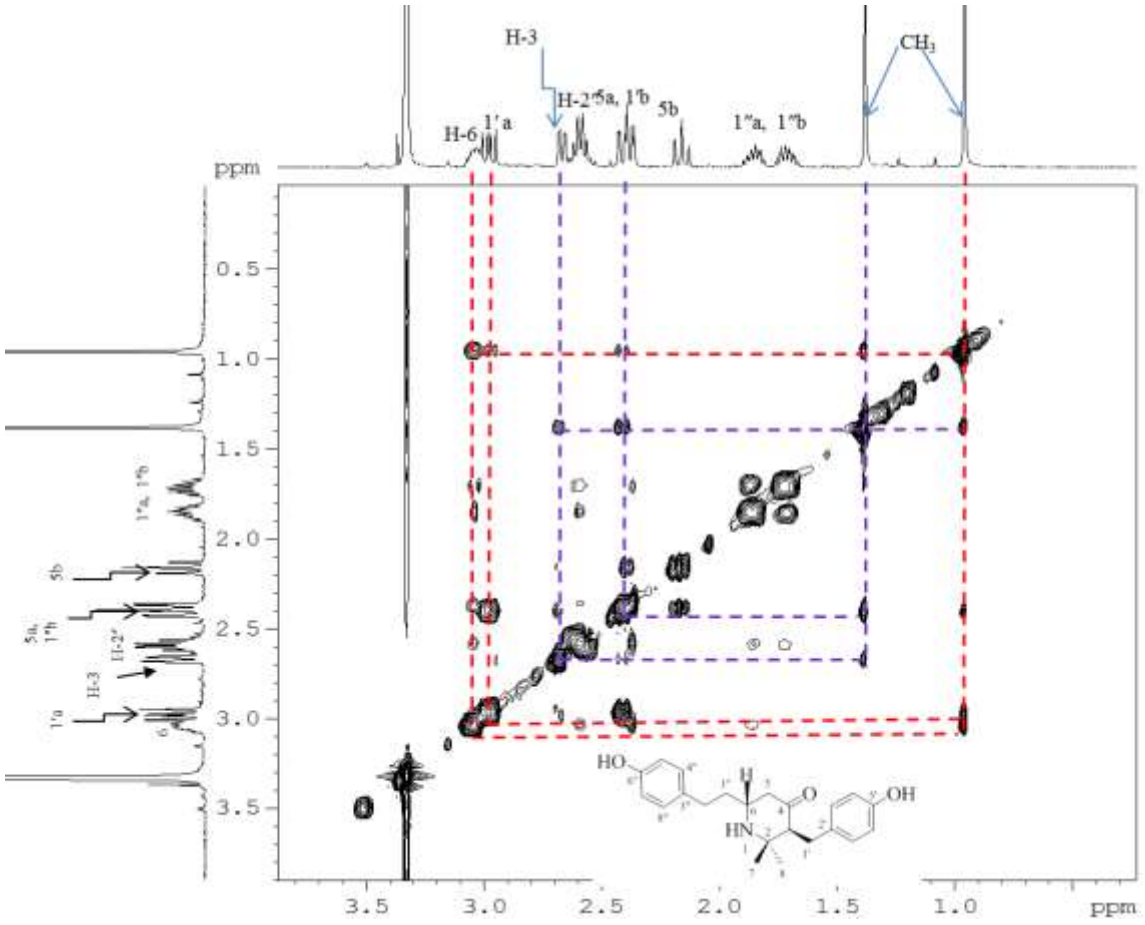

Figure 2 NOESY spectrum of compound **(1)**

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