ICETEXANE DITERPENOIDS ISOLATED FROM THE ROOTS OF *PREMNA ODORATA* BLANCO

Pa Pa Aung[1], Hnin Yu Win[2], Myint Myint Sein[3]

Abstract

In the present work, one Myanmar Medicinal Plant, *Premna odorata* Blanco was selected for chemical investigation. Phytochemical screening of the selected medicinal plant was carried out by using the standard methods. Two icetexane diterpenoids were isolated from the ethyl acetate extracts of the root of *Premna odorata* Blanco by chromatographic techniques. The structure elucidation of pure compounds was based on 1D and 2D-NMR spectral analysis and mass spectrometry. Moreover, the antimicrobial activities of various solvent extracts of the root of *Premna odorata* and the two isolated compounds were tested against six different microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilis*, *Candida albicans* and *E.coli*.

Keywords: icetexane, diterpenoids, antimicrobial

INTRODUCTION

Since the time immemorial, plants are a good source of information for novel drug compounds. The plant derived medicines contributed a lot of human health and well-being. According to the World Health Organization (WHO) in 2012, more than 90% of the world's population relies on traditional medicine for their primary healthcare needs, particularly in rural areas.

All over the world, traditional medicines are composed of herbs, animal products, minerals and marine products; completely of natural origin. Actually, nature has provided a complete remedy to cure all ailments of mankind. Plants can synthesize many miraculous compounds with medicinal properties beyond any human imagination and creation.

Several herbs are traditionally used in the treatment of a variety of ailments particularly in the rural areas of Myanmar where herbal medicine is mainly the source of health care system. Many of these herbs have not been assessed for safety or toxicity to tissue or organs of the mammalian recipients. Many commonly used herbs cause acute toxicity effects and in the long term may be toxic. Therefore, scientific study on phytochemical constituents of Myanmar medicinal plants and their biological activity is very important for the utilization of traditional medicine.

According to literature survey, Myanmar medicinal plant, *Premna odorata*, which is locally known as Pyae-sone has anti-inflammatory, anticancer and antibiotic properties. Moreover, scientific study of the constituents of *Premna odorata* has not been reported. Therefore, this drew our attention to select *Premna odorata* for chemical analysis.

Materials and Methods

General Experimental Procedures

UV lamp, Lambda - 40 Perkin- Elmer Co. England, FT-IR spectra were measured on spectrophotometer (Shimadzu, Japan), ^{1}H NMR spectra were measured on varian Unity 300 (300.542 MHz), Bruker AMX 300 (300.542 MHz), Varian Inova 500 (499.8 MHz). Coupling constant (J) in Hz. Abbreviations: s = singlet, d = doublet, t = triplet, m = multiplet, br = broad. ^{13}C NMR spectra: Varian Unity 300 (75.5 MHz), Varian Inova 500 (125.7 MHz). Chemical

[1] Assistant Lecturer, Dr., Department of Chemistry, University of Mandalay
[2] Associate Professor, Dr. rer. nat., Department of Chemistry, University of Mandalay
[3] Professor & Head (Rtd.), Dr., Department of Chemistry, University of Mandalay

shift were measured relatively to tetramethylsilane as internal standard. EI-Mass spectrometer (JEOL, Japan) Mass spectra: ESI-MS with Quattro Triple Quadruple mass spectrometer Finnigan MAT-Incos 50, ESIMSL CQ (Finnigan). Thin Layer chromatography (TLC): DC-folien polygram SIL G/U254 (Ma-cherey-Nagel & Co.). Glass plates for chemical screening: Merck silica gel 60 F 254, (10x20 cm). Analytical preparative thin layer chromatography was performed by using precoated silica gel (Merk. Co.Inc, Kiesel gel 60 F256). Silica gel (70-230 mesh, ASTM) was used for column chromatography.

Sample Collection

The roots of *Premna odorata* Blanco were collected from Mandalay, Myanmar. The plant materials were cut into small pieces and allowed to air dry at room temperature for two weeks.

Extraction and Isolation of Pure Compounds

The air dried sample (800 g) was percolated in 3.5 L of ethanol for two months and the ethanol extracts were filtered and evaporated the solvent under reduced pressure. Then, the residue was extracted with ethyl acetate and the solvent was removed under reduced pressure to obtain 6.46 g of crude extracts. The obtained crude extracts were subjected to silica gel column by using stepwise gradient of n-hexane and ethyl acetate.

After recrystallization of fraction V, pure compound (PPA-1) (15.5 mg) was isolated as white amorphous. It showed UV absorbing band at 256 nm. Pure compound (PPA-2) (10.0 mg) was isolated from fraction VII after purification on Sephadex LH-20 using methanol. It was isolated as pale yellow solid and it showed UV absorbing band at 256 nm.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening of *Premna odorata*

According to phytochemical screening, the roots of *Premna odorata* gave positive tests for alkaloids, glycosides, terpenoids, steroids, saponins, polyphenols, phenolics, tannins, reducing sugars and lipophilics.

Antimicrobial Activities

According to the antimicrobial tests, the n-hexane extracts showed low activities on *Bacillus subtilis, Bacillus pumilus* and *E. coli*. The ethyl acetate extracts showed medium activities on *Staphylococcus aureus, Bacillus pumilus* and *E. coli*, the low activities on *Bacillus subtilis, Pseudomonas aeruginosa* and *Candida albicans* respectively. The ethanol extracts gave rise to low activities on all selected organisms. The compound (PPA-1) showed medium activity against *Bacillus pumilus* and weak activities against *Bacillus subtilis, Staphylococcus aureus, Candida albicans* and *E. coli*. The compound (PPA-2) showed medium activities against *Bacillus pumilus, Candida albicans* and weak activity against *E. coli*.

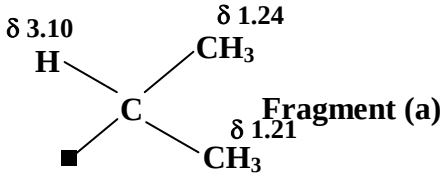
Structure Elucidation of Pure Compound (PPA-1)

According to ^{1}H NMR spectrum, compound (PPA-1) contained 26 protons. In the sp^2 region of ^{1}H NMR spectrum, one singlet at δ 6.42 ppm and one doublet at δ 6.03 ppm (J = 1.44 Hz) with the integration of one proton in each signal were detected. Moreover, two broad singlets at δ 5.11 and δ 4.96 were assigned to two hydroxyl groups and there was one doublet at δ 5.09 ppm (J = 1.98 Hz). In the aliphatic region of ^{1}H NMR spectrum, two methyl doublets at δ 1.24 and 1.21 ppm were detected. Moreover, the septet proton at δ 3.10 ppm was observed. Furthermore, ^{1}H NMR spectrum showed two doublet signals at δ 2.87 ppm (J = 16.4 Hz) and δ 2.65 ppm (J = 16.4 Hz) with the integration of one proton in each signal. In addition, ^{1}H NMR spectrum exhibited a doublet at δ 2.13 ppm (J = 10.7 Hz) with the integration of one

proton. Moreover, there were signals between δ 1.80 and δ 1.33 responsible for five protons and two methyl singlets at δ 1.12 and δ 1.01 ppm. Therefore, ^{1}H NMR spectrum displayed 26 proton signals.

The ^{13}C NMR and DEPT spectra revealed a total of 20 carbon signals. Among them, in the sp^2 region, 8 carbon signals (two methine and six quaternary) were visible. In the aliphatic region, two oxygenated sp^3 carbons (one quaternary and one methine) at δ 83.5 ppm and δ 79.9 ppm were assigned according to the chemical shifts. Furthermore, ^{13}C NMR spectrum displayed 10 sp^3 carbon signals which were comprised of four methyls, four methylenes, one methine and one quaternary.

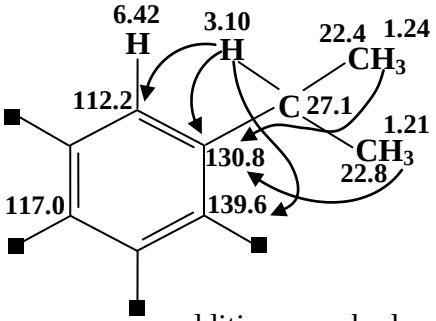
In the aliphatic region of ^{1}H NMR spectrum, two methyl doublets at δ 1.24 ppm (J = 6.84 Hz) and δ 1.21 ppm (J = 6.90 Hz) coupled with a proton responsible for septet at δ 3.10 ppm (J = 6.90 Hz). These signals indicated the presence of an isopropyl moiety (fragment a).



Fragment (a)

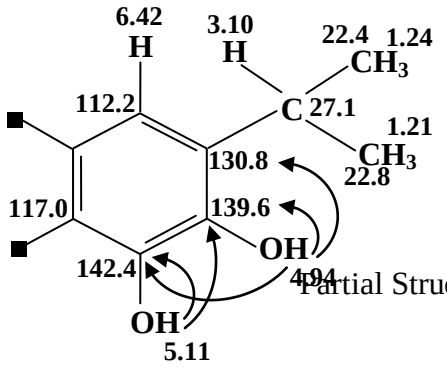
In the upfield aromatic region of ^{1}H NMR spectrum, a singlet at δ 6.42 ppm ascribed to one aromatic methine proton. In HMBC spectrum, it showed strong correlations to two quaternary carbons at δ 117.0 and δ 139.6 ppm.

Moreover, in HMBC spectrum, one methine proton at δ 3.10 ppm from fragment (a) showed correlations to two sp^2 quaternary carbons at δ 130.8 and δ 139.6 ppm and one sp^2 methine carbon at δ 112.2 ppm. Therefore, isopropyl group could be attached to sp^2 quaternary carbon at δ 130.8 ppm. Two methyl doublets at δ 1.24 and 1.21 ppm showed β coupling with sp^2 quaternary carbon at δ 130.8 ppm. These two methyl doublets showed HMBC cross signals between each other and coupled additionally to methine carbon at δ 27.1 ppm. According to these spectroscopic data, partial structure (I) could be confirmed.



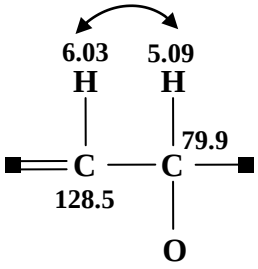
Partial structure (I)

In addition, one hydroxyl group at δ 4.94 ppm gave correlations with the three sp^2 quaternary carbons at δ 130.8, 139.6 and 142.4 ppm. Another hydroxyl group at δ 5.11 ppm exhibited correlations with two sp^2 quaternary carbons at δ 139.6 and 142.4 ppm. Therefore, two hydroxyl groups at δ 4.94 ppm and δ 5.11 ppm could be attached to two sp^2 quaternary carbons at δ 139.6 and 142.4 ppm respectively. According to these data, partial structure (II) could be drawn.



Partial Structure (II)

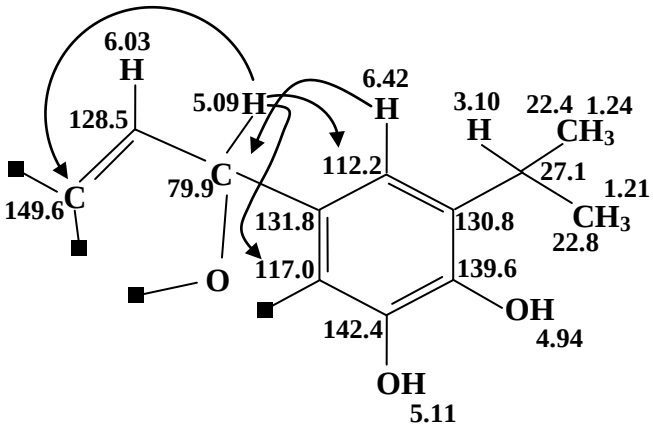
Moreover, in DQF-COSY spectrum, one sp^2 methine proton at δ 6.03 ppm which is attached to carbon at δ 128.5 ppm correlated with one sp^3 oxygenated methine proton at δ 5.09 ppm. Therefore, fragment (b) could be assigned.



COSY (↔) correlations in fragment (b)

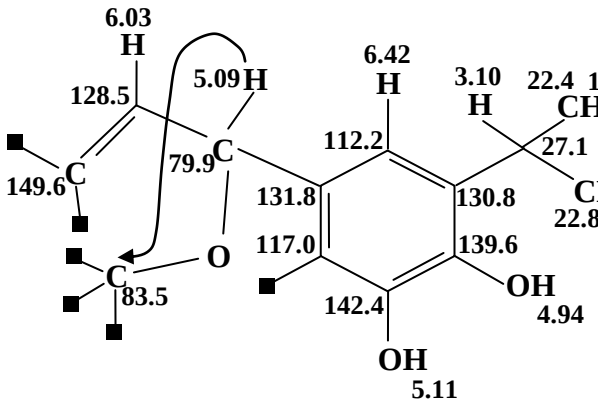
Furthermore, in HMBC spectrum, one doublet methine proton at δ 5.09 ppm from fragment (b) showed β-couplings with one sp^2 methine carbon at δ 112.2 ppm and two sp^2 quaternary carbons at δ 117.0 and 149.6 ppm.

Moreover, one aromatic methine proton at δ 6.42 ppm showed β-correlation to one oxygenated methine carbon at δ 79.9 ppm. According to these spectroscopic data, fragment (b) could be attached to partial structure II as shown in partial structure (III).



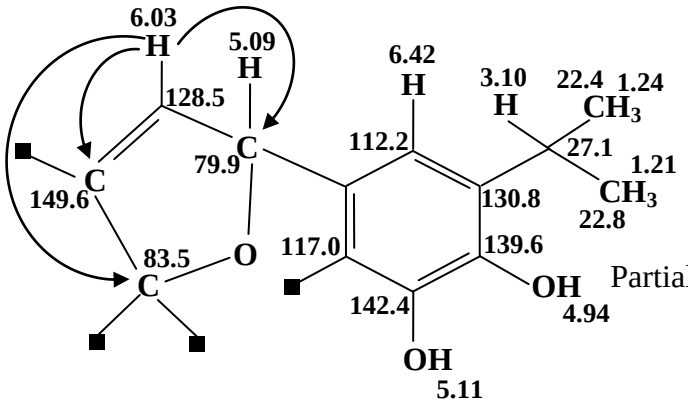
Partial Structure (III)

In addition, one methine doublet at δ 5.09 ppm showed β-correlation with one oxygenated methine carbon at δ 83.5 ppm. The partial structure (IV) could be elucidated.



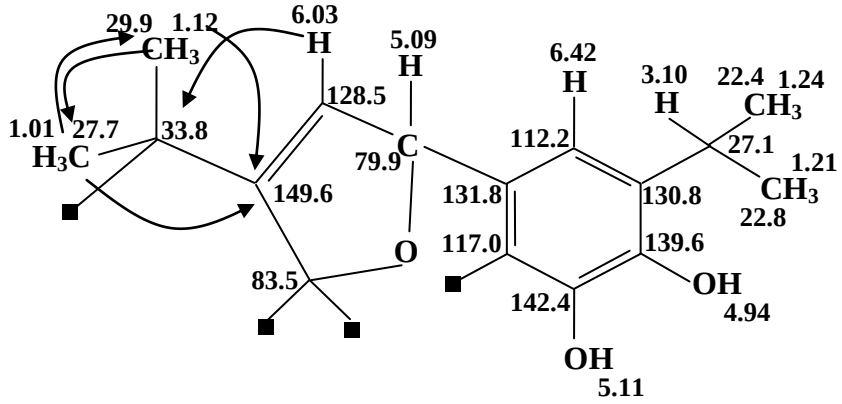
Partial Structure (IV)

Moreover, in HMBC spectrum, one methine doublet at δ 6.03 ppm which is attached to carbon at δ 128.5 ppm showed α and β– correlations with two oxygenated carbons at δ 79.9 and δ 83.5 ppm and α correlation to one quaternary carbon at δ 149.6 ppm. Therefore, the ring could be closed as shown in partial structure (V).



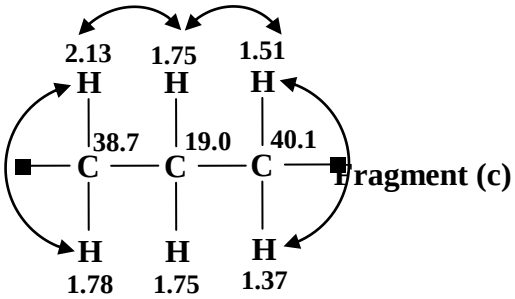
Partial Structure (V)

Furthermore, in HMBC spectrum, the methine doublet at δ 6.03 ppm from partial structure (V) showed β-correlation with one quaternary carbon at δ 33.8 ppm. Two methyl singlets at δ 1.12 and 1.01 ppm showed HMBC cross peaks between each other and additionally to one sp^2 quaternary carbon at δ 149.6 ppm, one sp^3 quaternary carbon at δ 33.8 ppm. According to these data, partial structure (VI) could be assigned.



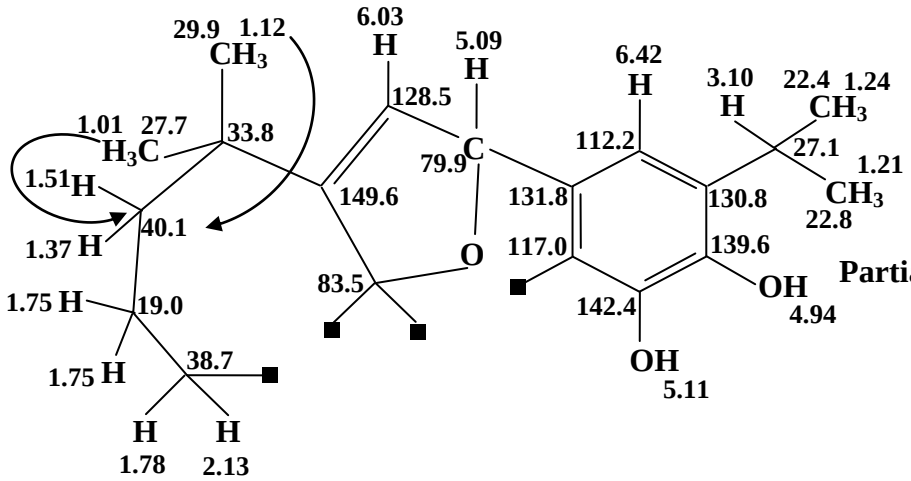
Partial Structure (VI)

In DQF-COSY spectrum, methylene protons at δ 1.75 ppm showed correlations with two methylene protons at δ 2.13 and 1.78 ppm and another methylene protons at δ 1.51 and 1.37 ppm. Therefore, fragments (c) could be drawn.



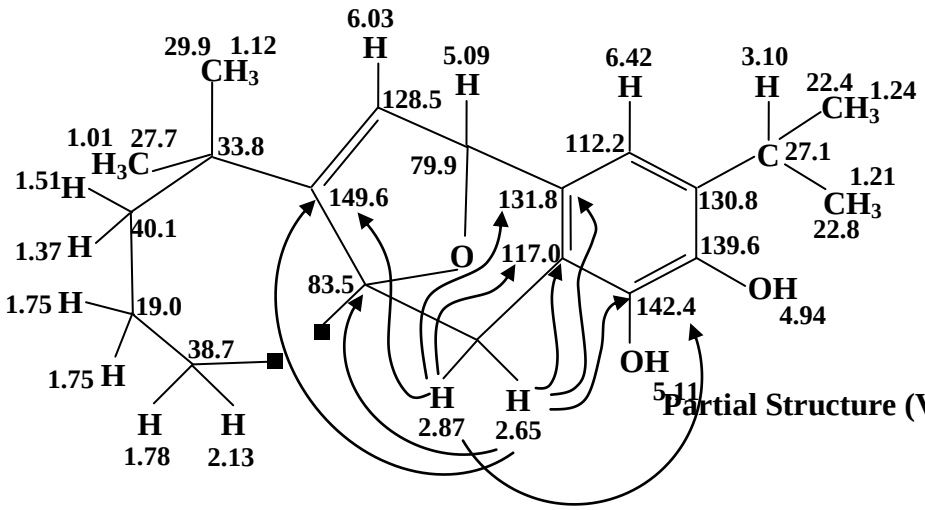
Fragment (c)

In HMBC spectrum, two methyl singlets at δ 1.12 and 1.01 ppm showed β-coupling with one sp^3 methylene carbon at δ 40.1 ppm from fragment (c). According to these spectroscopic data, methylene group at δ 40.1 ppm from fragment (c) could be connected to quaternary carbon at δ 33.8 ppm and partial structure (VII) could be assigned.



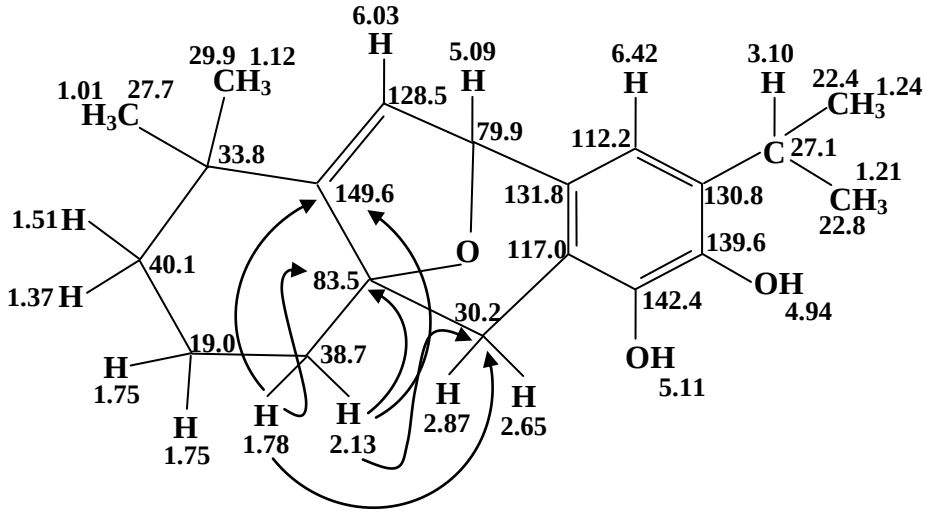
Partial Structure (VII)

Furthermore, in HMBC spectrum, two methylene protons at δ 1.37 and 1.51 ppm which are attached to carbon at δ 40.1 ppm showed correlation with sp^2 quaternary carbon at δ 149.6 ppm and partial structure (VII) could be confirmed. In addition, two diastereotopic methylene protons at δ 2.65 and 2.87 ppm showed α-correlations to sp^2 quaternary carbons at δ 117.0 ppm, β-correlations to δ 131.8, 142.4, 149.6 ppm and one of the methylene protons at δ 2.65 ppm showed α-correlation to one sp^3 oxygenated quaternary carbon at δ 83.5 ppm.

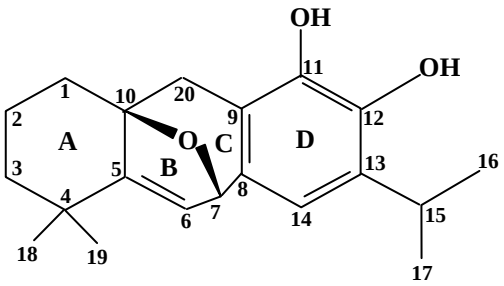


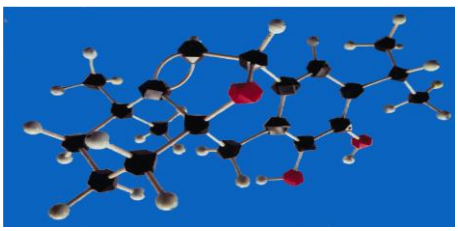
Partial Structure (VIII)

Moreover, two methylene protons at δ 2.13 and 1.78 ppm showed α-correlation to sp^3 oxygenated quaternary carbon at δ 83.5 ppm, β-correlation to sp^3 methylene carbon at δ 30.2 ppm and one sp^2 quaternary carbon at δ 149.6 ppm. Therefore, the complete structure of compound (PPA-1) could be elucidated with eight double bond equivalence. The isolated compound (PPA-1) was assigned as an icetexane diterpenoid.



According to model studies and theoretical background, the ring A in the structure of PPA-1, adopts a distorted chair conformation. Moreover, the five and six-membered heterocyclic rings B and C exhibit envelope and sofa conformation respectively and are having the oxygen atom as a common flap. The essentially planar aromatic ring D is almost coplanar. The relative stereochemistry of C-7 and C-10 could be assigned as S and S respectively. In some literature, the numbering system of icetexane diterpenoids was also given as shown below. Therefore, the isolated compound (PPA-1) was named (4aS,10S)-1,2,3,4,5,10-hexahydro-1,1-dimethyl-8-(1-methylethyl)-4a,10-epoxy-4aH-dibenzo[a,d]cycloheptene-6,diol.





Structure Elucidation of Compound (PPA-2)

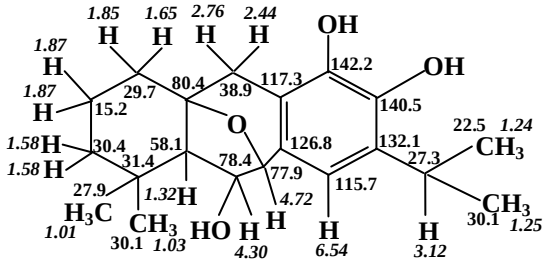
Another icetexane diterpenoid (PPA-2) showed IR bands for hydroxy phenolic groups at 3380 cm^{-1} and aromatic double bonds at 1631 and 1589 cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra of (PPA-2) were similar to those of (PPA-1). The ^{1}H NMR spectrum of (PPA-2) showed signals for two secondary methyl groups at δ 1.25 ppm (d, J = 6.9 Hz) and δ 1.24 ppm (d, J = 6.9 Hz) coupled with a proton responsible for a heptet or septet at δ 3.12 ppm (J = 6.9 Hz). These signals indicated the presence of an aromatic isopropyl group. A singlet at δ 6.54 ppm was ascribed to one aromatic proton and it indicated the presence of pentasubstituted aromatic ring, similar to that present in (PPA-1). Moreover, the ^{13}C NMR spectrum showed signals for four methylene groups at δ 29.7 ppm (C-1), 15.2 ppm (C-2), 30.4 ppm (C-3) and 38.9 ppm (C-20), four methyl groups at δ 22.5 ppm (C-16), 30.1 ppm (C-17), 30.1 ppm (C-19) and 27.9 ppm (C-18), one methine at δ 27.2 ppm (C-15) and one quaternary carbon at δ 31.4 ppm (C-4). These sp^3 carbon signals were similar to those of (PPA-1).

In the sp^2 region of ^{13}C NMR spectrum, there were six carbon signals in compound (PPA-2). However, (PPA-1) possesses two additional sp^2 carbon signals. Therefore, they revealed that the compound (PPA-2) was devoid of the 5, 6-double bond. Furthermore, in the ^{13}C NMR spectrum of compound (PPA-2), three signals due to oxygenated sp^3 carbon atoms were observed. Two methine signals at δ 78.4 and 77.9 ppm were ascribed to C-6 and C-7 respectively and one quaternary carbon at δ 80.4 ppm was assigned to C-10. However, in compound (PPA-1), only two oxygenated sp^3 carbon atoms were detected. In addition, the ^1H NMR spectrum of (PPA-2) also displayed one more additional carbinol proton than in (PPA-1). So, it can be concluded that the double bond between C-5 and C-6 in (PPA-1) was substituted with hydroxyl group or dehydration at C-5 and C-6 in (PPA-2) lead to the formation of double bond in (PPA-1). Moreover, one sp^3 methine carbon at δ 58.1 ppm (C-5) was also observed in ^{13}C NMR spectrum of compound (PPA-2) which was absent in compound (PPA-1).

In the HMBC spectrum of compound (PPA-2), one oxygenated methine proton at δ 4.72 ppm (C-7) showed correlations to sp^3 methine carbon at δ 58.1 ppm (C-5), one sp^3 quaternary carbon at δ 80.4 ppm (C-10), one sp^2 methine carbon at δ 115.7 ppm (C-14) and one sp^2 quaternary carbon at δ 117.3 ppm (C-9).

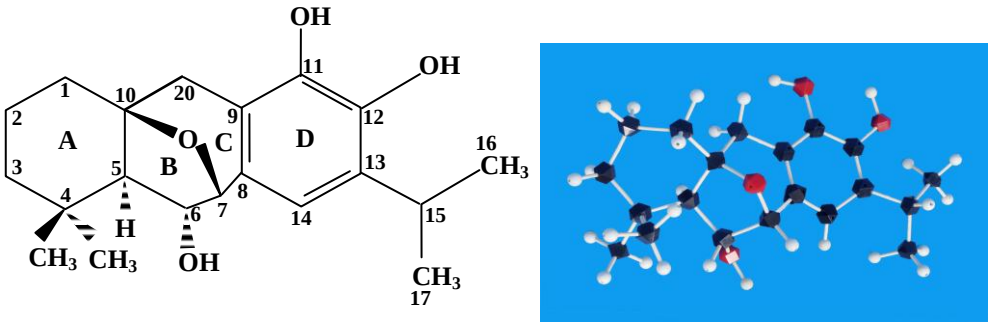
The EI mass spectrum of compound (PPA-2) showed the molecular ion peak at *m/z* 332 with the molecular formula $C_{20}H_{28}O_4$. The EI mass spectrum of compound (PPA-1) showed molecular ion peak at *m/z* 314 and molecular formula was $C_{20}H_{26}O_3$. The difference in molecular mass was 18.

Therefore, compound (PPA-2) possesses one more hydroxyl group (OH) and one proton (H). By comparison of the spectroscopic data of (PPA-1) and (PPA-2) and other HMBC correlations, the structure of compound (PPA-2) could be elucidated. The compound (PPA-2) also possess icetexane skeleton.



The isolated compounds (PPA-1 and PPA-2) belong to the second subclass of icetexanes named barbatusol family (Simmons, et. al., 2009)

According to the splitting pattern of ^1H NMR, theoretical background and model studies, the most stable conformation for six-membered ring (A) in the structure of (PPA-2) could be assigned as chair conformation. In NOESY spectrum, the two methine protons at δ 4.72, 4.30 ppm and methyl singlet at δ 1.01 showed spatial correlations. Therefore, they must be on the same side of the ring. The coupling constant of carbinol proton H-6 group (δ 4.30 ppm, J = 5.94) indicated an α-orientation of the hydroxyl group which is located at C-6 position. This proton was coupled with H-7, the germinal proton of the etheral ring, which was responsible for a doublet at δ 4.72. The trans fusion depicted in compound PPA-2 was established with the aid of coupling constant (J = 6.72 Hz) found for H-5. Moreover, the methyl group at δ 1.01 ppm which exists in axial position showed spatial correlation with one of the methylene protons at δ 2.76 ppm in NOESY spectrum. Therefore, the ring B and C are enveloped and sofa conformation respectively. Thus, the relative stereochemistry of four chiral centers of compound (PPA-2) could be assigned as C_5-R, C_6-R, C_7-R and C_{10}-S respectively.



It was named (4aS, 10R, 11R, 11aR)- 1,2,3,4,5,10,11,11a-octahydro-1,1-dimethyl-8-(1-methylethyl)-,4a,10-epoxy-4aH-dibenzo[a,d]cycloheptene-6, 7,11-triol.

Icetexane diterpenoids are a family of diterpenoid natural products which have been isolated from a variety of terrestrial plant sources. The compounds in this family exhibit an array of interesting biological activities (Esquivel, et. al., 1995 & Fraga, et. al., 2005).

The icetexane natural products that have been discovered to date vary widely in the degree of oxygenation and oxidation in each ring, leading to a diverse array of structures and biological activities. Although a formal classification scheme for the icetexanes does not currently exist, they can be logically divided into various subclasses based on the presence or absence of oxygenation at the C3, C11, C14 and C19 positions (Simmons, et. al., 2009).

Spectral Data of Isolated Compounds

PPA-1: white amorphous, 15.5 mg, R_f = 0.26 (n-hexane : EtOAc; 8:2), UV absorbing band 256 nm -FT-IR (KBr) 3390 cm^{-1} (O-H stretching), 3028 cm^{-1} (sp^2 C-H stretching), 2947, 2872 cm^{-1} (sp^3 asymmetric and symmetric C-H stretching), 1640 cm^{-1} (C=C ring skeleton stretching), 1520,1450 cm^{-1} (C-H in plane bending vibration), 1369 cm^{-1} (O-H in plane bending vibration), 1294,1205,1165 cm^{-1} (C-C-O stretching vibration), 1099,1004 cm^{-1} (C-O-C stretching vibration), 862 cm^{-1} (C-H out of plane bending vibration). -^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.42 (s, 1H, H-14), 6.03 (d, ^3J = 1.44 Hz, 1H, H-6), 5.11 (s, 1H, H-11), 5.09 (d, ^3J = 1.98 Hz, 1H, H-7), 4.94 (s, 1H, H-12), 3.08 (sept, ^3J = 6.90 Hz, 1H, H-15), 2.87 (d, 3J = 16.4 Hz, 1H, H-20), 2.65 (d, ^3J = 16.5 Hz, 1H, H-20), 2.13 (d, 3J = 10.7 Hz, 1H, H-1), 1.78 (m, 1H, H-1), 1.75 (m, 2Hs, H-2), 1.51 (m, 1H, H-3), 1.37 (m, 1H, H-3), 1.24 (d, ^3J = 6.84 Hz, 3Hs, H-17), 1.21 (d, ^3J = 6.90 Hz, 3Hs, H-16), 1.12 (s, 3Hs, H-19), 1.01 (s, 3Hs, H-18). -^{13}C NMR ($CDCl_3$, 150 MHz), δ 149.6 (C_q-5), 142.4 (C_q-11), 139.6 (C_q-12), 131.8 (C_q-8), 130.8 (C_q-13), 128.5 (CH-6), 117.0 (C_q-9), 112.2 (CH-14), 83.5 (C_q-10), 79.9 (CH-7), 40.1 (CH_2-3), 38.7 (CH_2-1), 30.2 (CH_2-20), 29.9 (CH_3-19), 27.7 (CH_3-18), 27.1 (CH-15), 22.8 (CH_3-16), 22.4 (CH_3-17), 19.0 (CH_2-2). - EI-MS *m/z* 314 (25%).

PPA-2: pale yellow solid, 10.0 mg, R_f = 0.17 (n-hexane : EtOAc; 8 : 2), UV absorbing band at 256 nm - FT-IR (KBr) 3380 cm^{-1} (O-H stretching), 2925, 2872 cm^{-1} (sp^3 asymmetric and symmetric C-H stretching), 1631, 1589 cm^{-1} (C=C ring skeleton stretching), 1481 cm^{-1} (C-H in plane bending vibration), 1316 cm^{-1} (O-H in plane bending vibration), 1241, 1165 cm^{-1} (C-C-O stretching vibration), 1035 cm^{-1} (C-O-C stretching vibration), 843 cm^{-1} (C-H out of plane bending vibration).-^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.54 (s, 1H, H-14), 4.72 (d, ^3J = 6.18 Hz, 1H, H-7), 4.30 (d, ^3J = 5.94 Hz, 1H, H-6), 3.12 (sept, 1H, H-13), 2.76 (d, ^3J = 15.97 Hz, 1H, H-20), 2.44 (d, ^3J = 15.9 Hz, 1H, H-20), 1.87 (m, 2Hs, H-2), 1.85 (m, 1H, H-1), 1.65 (m, 1H, H-1), 1.58 (m, 2Hs, H-3), 1.32 (d, ^3J = 6.72 Hz, 1H, H-5), 1.25 (d, ^3J = 3.4, 3Hs, H-17), 1.24 (d, ^3J = 3.4, 3Hs, H-16), 1.03 (s, 3Hs, H-19), 1.01 (s, 3Hs, H-18).-^{13}C NMR ($CDCl_3$, 150 MHz), δ 142.2 (C_q-11), 140.5 (C_q-12), 132.1 (C_q-13), 126.8 (C_q-8), 117.3 (C_q-9), 115.7 (CH-14), 80.4 (C_q-10), 78.4 (CH-6), 77.9 (CH-7), 58.1 (CH-5), 38.9 (CH_2-20), 31.4 (C_q-4), 30.4

(CH_2-3), 30.1 (2CH_3-17,19), 29.7 (CH_2-2), 27.9 (CH_3-18), 27.3 (CH-15), 22.5 (CH_3-16), 15.2 (CH_2-1).-EI-MS *m/z* 332 (100%).

CONCLUSION

The phytochemical studies of roots of *Premna odorata* Blanco led to the isolation of two icetexane diterpenoids (PPA-1 and PPA-2). The complete structure elucidation of isolated compounds was performed based on 1D- and 2D-NMR spectral analysis and mass spectrometry. Moreover, the conformational analysis and relative stereochemistry of isolated compounds were investigated. In biological screening, compound (PPA-1) showed medium activity against *Bacillus pumilus* and weak activities against *Bacillus subtilis, Staphylococcus aureus, Candida albicans* and *E. coli*. The compound (PPA-2) showed medium activities against *Bacillus pumilus, Candida albicans* and weak activity against *E. coli.*

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