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Research Paper

The First Hydroxymanool, a Diterpene and its corresponding Monoacetate from *Xylopia benthamii*

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ABSTRACT

A diterpene (2), (+) 2-hydroxymanool was obtained as the predominant isolate from the CH_2Cl_2 extract of *Xylopia benthamii* using CH_2Cl_2/Hexane as the eluent followed by repeated flash column chromatography of a specific range of fractions using also CH_2Cl_2/Hexane. Its structure was assigned using modern spectroscopic techniques: ^{1}H NMR, ^{13}CNMR, DEPT 135, ^{1}H-^{1}H COSY, HMBC, HMQC. It is the first labdane diterpene of such a nature to be isolated from a *Xylopia* species and for the first time isolated as its pure hydroxymanool form. The pure diterpene (2) was monoacetylated using acetic anhydride in the presence of pyridine at room temperature for 24 hours and was obtained as a brown viscous liquid (9) which was also characterized via the above techniques.

Keywords: Diterpene; *Xylopia benthamii;* 2-hydroxymanool; 2α-acetoxymanool.

INTRODUCTION

Sixty percent of Pharmaceutical drugs currently in use are natural products in nature (Mann, 1986, Mann, et. al, 1994, Asakawa, et. al, 2004). Some diterpenes isolated have the Manool skeleton incorporated in their structure (Bruns, et. al, 1970, Bohlmann et. al, 1978), The latter can exist as the (+) and (-) enantiomeric ent-manool (1), Manool, 13 epimeric, Manool, enantio 13 epimeric (Bruns, et. al, 1970). However, *Ent*-manool was never isolated as its ent-2-hydroxy pure form (3) and (4), α or β, from a *Xylopia species* (Mass, et. al, 2001, Correa, et. al, 1984) but as its corresponding monoacetates (5) and (6) from the aerial parts of another genus of species *Baccharia oxydonta*, DC (Bohlmann, et. al, 1981). Even so, only ^{1}H NMR characterization of the monoacetate was given (Tiansheng, et. al, 1995). Added to this, ^{1}H NMR assignment for H-1α, H-1β, H-3α, H-3β are missing for the 2-acetoxymanool. Based on these shortcomings, we report the isolation, structural elucidation and complete characterization of compound (2) using a combination of ^{1}H NMR, ^{13}CNMR, DEPT 135 NMR, ^{1}H-^{1}H COSY, HMBC and HMQC experiments.

As an ongoing program to isolate, structurally elucidate and investigate biologically active compounds from plants of the Caribbean, this report outline the

isolation and structural elucidation of a diterpene (2) from the stems of *Xylopia bentamii*.

To the very best of knowledge, compound (2), a labdane diterpene has never been isolated from the genus *Xylopia* or the family *annonaceae. Ent-Kaurane and Ent-trachylobutane diterpenes are encountered in the genus Xylopia* (Faulkner, et. al, 1985, Andrade, et. al, 2004*).* Recently, two new diterpenes of the *ent*-trachylobutane type were isolated from the stems of *Xylopia langsdorffiana*, ent- 7a-acetoxytrachyloban-18-oic acid (7) and *ent*-7a-hydroxytrachyloban-18-oic acid (8). The cyctotoxic effect of (11) against V79 fibroblasts and rat hepatocytes was investigated (Tavares, et. al, 2006). Seven new Labdane Diterpenes were isolated from *Xylopia langsdorffiana* and *Juniperus pseudosabina* (Ribeiro, et. al, 2007, Pandita, et. al, 1987). Sesquiterpenes and hydrocarbons were also isolated from the fruits of *Xylopia emarginata* (Moreira, et. al, 2007).

Diterpene (2) was isolated as a white crystalline solid (2.19g) via flash column silica gel chromatography of the CH_2Cl_2 extract (20.11g) using CH_2Cl_2/Hexane as the eluent followed by repeated flash column chromatography of a specific range of fractions; using CH_2Cl_2/Hexane as the eluent.

MATERIALS AND METHODS

***Instrumentation*:** Melting points were measured on a Geahaka model PF 1500 version apparatus and are uncorrected. ^1H and ^{13}C NMR, ^{13}C- DEPT 135 NMR, COSY, HMQC and HMBC spectra were recorded on a Bruker DRX-500 spectrophotometer using $CDCl_3$ as the solvent. Chemical shifts are quoted in δ ppm with reference to TMS internal standard and coupling constants (*J)* expressed in Hertz (Hz). HREIMS and EIMS were recorded on a KRATOS/AEI MS-50 spectrometer. Silica gel 60A (70-230 mesh, Merck) was used for flash column chromatography (CC). All solvents were redistilled prior to use. Analytical TLC analyses were done on precoated Kiesegel 60 F_{254} (Merck) plates and were 0.25mm thick. TLC plates were viewed under a UV lamp, (Spectroline Longlife Filter) and developed chromatograms were visualized via spraying with iodine. IR spectrum was recorded on a Perkin Elmer FT_IR spectrometer connected to a Hewlett-Packard Colour Pro. Plotter. $(\alpha)_D$ was recorded in $CHCl_3$ on an ADP220 Polarimeter ADS220 Saccharimeter.

***Plant Collection*:** *Xylopia benthamii* (Family-*Annonaceae*) was obtained from the Coastal Plain (Berbice-Corentyne region) of Guyana by T. McDowell with D. Gopaul on the 14th of April, 1990. It was identified by a Taxonomist at the University of Guyana and a Voucher specimen (# 2260) is deposited at the Biodiversity centre of the University of Guyana.

***Plant Extraction*:** The stems of the plant were dried, ground and exhaustively extracted in distilled methanol for 48 hours. Solvents were removed in *vacuo* to yield a crude dark viscous brown liquid of weight 47g. This crude extract was subjected to sequential extraction using solvents of increasing polarity: C_6H_{14}, CH_2Cl_2, EtOAc and CH_3OH. For each solvent type, three extractions were done. Each solvent type extract was dried over sodium sulphate. Na_2SO_4, filtered and solvents removed in *vacuo* to yield viscous oils and paste. The CH_2Cl_2 extract was a crude black viscous liquid of weight 20.11g. The physical nature of the C_6H_{14}, EtOAc and CH_3OH extract was oil, light brown solid and brown solid respectively.

***Isolation of Natural Products*:** The CH_2Cl_2 extract (20.11g) was loaded on a silica gel column and was eluted with CH_2Cl_2/Hexane (9:3, v/v) via Flash column

chromatography (CC). It was further gradient eluted with CH_2Cl_2/EtOAc (20: 1 to 2:18, v.v). Several bands were observed and fractions were collected and combined based on similar R_f values via TLC analyses on precoated Kieselgel 60 F_{254} and chromatogram developed using iodine and viewed using a UV/Vis lamp. All together a total of 260 fractions were collected of which fractions 240-254 (3.4g) were combined and re-chromatographed using CH_2Cl_2/Hexane (10: 3, v/v). Fractions 15-25 were collected from the second chromatographic separation and combined based on similar R_f values. Solvents were removed in *vacuo to* yield a white solid of 2.19 g which was found to be spectroscopically pure as seen from NMR profiles. Further purification via re-crystallization was not required.

Compound (2) **$C_{20}H_{34}O_2$**, White solid; mp 134.5-136°C, R_f 0.059($CHCl_3$), $(\alpha)_D$ -45.31(c 0.06, $CHCl_3$); HREIMS: m/z [M^+] = 306.47; Calcd for $C_{20}H_{34}O_2$ = 306.47; IR bands (KBr): 3371.4, 2964.6, 1640.5, 1458, 1381.7, 1162.4, 1038.5, 917.1, 697.6 cm^{-1} ^{1}HNMR ($CDCl_3$, 400MHz) δ: 5.92 (dd, J = 10.8Hz; 1H), 5.26 (d, J = 17.6 Hz; 1H), 5.08 (d, J= 10.8 Hz; 1H), 4.85 (brs; 1H), 4.54 (brs; 1H), 3.89 (m; 1H), 2.39 (d; J = 12.8 Hz; 1H), 2.13(d, J = 10.8Hz; 1H), 1.96 (m; 1H), 1.73-1.80 (m) 1.57-1.84 (m), 1.35-1.43 (m), 1.29 (s; 3H), 1.16 (t; J = 12Hz; 1H), 1.11(d; J = 9.3 Hz; 1H), 0.94 (d, J = 6.63Hz; 1H), 1.0 (s; 3H), 0.96 (s; 3H), 0.82 (s; 3H), **^{13}C NMR ($CDCl_3$, 400MHz**) δ: 15.58(C-20),17.97(C-11),22.73(C-19), 24.07(C-6), 28.18 (C-16), 33.77(C-18), 35.27 (C-4), 38.28 (C-7), 41.45 (C-12), 48.43 (C-1), 51.15 (C-3), 55.12(C-5), 57.2 (C-9), 35.27 (C-4), 38.28 (C-7), 41.45 (C-12), 48.43(C-1), 51.15 (C-3), 55.12 (C-5), 57.2 (C-9), 65.92 (C-2), 73.83 (C-13), 111.97 (C-15), 145.29 (C-14),107.50 (C-17),148.02 (C-8). **COSY:**H-1/H_2-1, H-2, H-3; H-2/H_2-1, H_2-3, H-18; H-3/H-2, H_2-3; H-5/H-6, H_2-7; H-6/H-5, H-6, H-7; H-7/H-5, H-6, H_2-7, H-9, H-9/H-5, H_2-6, H_2-7, H-11, H-12; H-11/H_2-6, H-7, H-9, H_2-11, H-12; H-12/H-6, H_2-7, H_2-9, H-11, H-12, H_2-17; H-14/H_2-15; H-15/ H-14; H-17/H_2-11, H_2-12, H_2-17; **HMBC CORRELATIONS:** H-1/C-1, C-2, C-3, C-6, C-9, C-10, C-19, C-20; H-3/C-1, C-2, C-6, C-11, C-16; H-5/C-1, C-6, C-9, C-10, C-16; H-7/C-5, C-6, C-8, C-17, C-20; H-9/C-7, C-8, C-10, C-13, C-17, C-20; H-11/C-6, C-7, C-8, C-9, C-10, C-13, C-17, C-20; H-12/C-5, C-7, C-8, C-9, C-10, C-11, C-13; H-13/ C-8, C-10; H-14/C-6, C-13; H-15/C-13, C-16; H-15/C-13, C-16; H-16/C-5, C-7, C-13, C-15, C-18, C-20; H-17/C-5, C-18; H-18/C-2, C-3, C-5, C-20; H-19/C-3, C-5, C-20; H-20/C-1, C-10, C-11.

Monoacetate of 2-hydroxymanool (9), **2α-acetoxymanool**: Diterpene (0.022g, 7.2 x 10^{-6} mol) was treated with acetic anhydride in the presence of pyridine. The reaction mixture was left stirring for twenty four hours under nitrogen after which it was quenched with ice and worked up. Solvent was removed in *vacuo* to yield a light brown viscous oil (0.03g, 95%), **$C_{20}H_{36}O_2$**. ^{1}H NMR ($CDCl_3$, 400MHz) δ: 5.91 (dd; J =17.2 Hz), 5.08 (dd; J = 12Hz), 5.24 (dd; J =17.2 Hz), 5.08 (dd; J = 12 Hz), 5.026 (m), 4.85 (brs), 4.51 (brs), 2.38 (dd, J = 12.8Hz), 2.078 (d, J = 9.6Hz), 2.04 (s, CH_3), 1.245(t, J =), 1.121 (d = 2.8Hz), 1.052 (d; J = 12 Hz), 0.94 (s, CH_3), 0.89 (s, CH_3), 0.76 (s, CH_3); ^{13}CNMR ($CDCl_3$, 400MHz) δ: 170.669, 147.522, 144.948, 111.781, 107.430, 73.616, 69.34, 56.95, 54.98, 46.82, 44.15, 41.147, 41.13, 38.011, 34.95, 33.58, 28.31, 23.89, 22.45, 21.55, 17.83, 15.17. ^{13}CNMR (DEPT 135 ($CDCl_3$, 400MHz) δ: 144.949, 69.34, 56.95, 54.99, CH_3 (33.58, 28.31, 22.45, 21.55, 15.17; CH_2(111.78, 107.43, 46.82, 44.15, 41.12, 38.01, 22.45, 17.82); **COSY:** H-1/H_2-1, H-2, H_2-3, H-5, H-20; H-2/H_2-1, H_2-3, H-5; H-3/H_2-2, H_2-3, H_2-1, H-5; H-5/H_2-1, H_2-3, H_2-6, H_2-7, H-9, H-11, H-20; H-6/H-5, H_2-6, H_2-7, H-9; H-7/H-5, H-6,H_2-7,H-9, H-11, H-12; H-9/H-5, H_2-6, H_2-7, H-11, H_2-12; H-11/H-5, H_2-7, H_2-9,H_2-12; H-12/H-6, H-7, H-9, H_2-11, H_2-12, H-17; H-14/H_2-15; H-15/H-14; H-17/H_2-7, H-9, H_2-17; H-

22/H-1, H-2, H-3, H-19; **HMBC:** H-1/C-1, C-2, C-3, C-6, C-9, C-10, C-19, C-20; H-3/C-1, C-2, C-6, C-11, C-16; H-5/C-1, C-6, C-9, C-10, C-16; H-6/C-2, C-5, C-9, C-10, C-11, C-13, C-18, C-20; H-7/C-5, C-6, C-8, C-17, C-20; H-9/C-7, C-8, C-10, C-13,C-17,C-20; H-11/C-6, C-7, C-8, C-9, C-10, C-13, C-17, C-20; H-12/C-5, C-7, C-8, C-9, C-10, C-11, C-13; H-13/C-8, C-10; H-14/C-6, C-13; H-15/C-13, C-16; H-16/C-5, C-7, C-13, C-15, C-18,C-20; H-17/C-5, C-18; H-18/C-2, C-3, C-5, C-11, C-19; H-19/C-3, C-5, C-20; H-20/C-5, C-9, C-16

RESULTS AND DISCUSSION

The structure of the white solid (2) was elucidated using a combination of ^{1} H NMR, ^{13}C NMR, DEPT 135, ^{1}H-^{1}H COSY, HMQC and HMBC spectroscopy. The combined spectral data are shown in Table 1.0 and 2.0. The ^{1}H NMR spectrum recorded in $CDCl_3$ displayed a doublet of doublet at 5.92 ppm, arising from alkene proton H-14 (J=10.8Hz; J=6.4Hz). This results from *trans* and *cis* coupling with terminal alkene protons H-15. Terminal alkene protons H-15 resonate as two doublets at 5.26 ppm (J = 17.6 Hz) and 5.09 ppm (J = 10.8 Hz) as a result of trans and *cis* coupling with alkene H-14 protons. Terminal alkene protons, H-17 adjacent to a quaternary carbon C-8 resonate as a broad singlet at 4.87 and 4.54 ppm. As anticipated, protons on adjacent carbon of the fused cyclohexane ring exhibit axial-axial and axial-equatorial coupling whereas protons on the same carbon exhibit germinal coupling (axial-equatorial interaction). For example, H-1 axial protons interact with H-2 axial to give a doublet which is further split into a doublet due to Haxial- H equatorial interaction. Thus, two doublets of doublets are seen for H-1 protons at 2.133 and 0.9 ppm in the ^{1}H NMR profile. H-7 proton is seen as a doublet and a broad singlet at respectively. For each axial-equatorial protons, the equatorial protons resonate downfield since its position in the deshielding zone. For example, H-1 equatorial (J = 2.13Hz) resonate at 2.13 ppm whereas the axial proton resonate upfield at 0.9 ppm. The spectrum exhibit complexity in the region 1.3-1.8 ppm with three multiplets resonating in the region 1.70-1.80 ppm, 1.57-1.70 ppm and 1.34-1.43 ppm. In this multiplet are found overlapping signals. For example, in the first multiplet, one proton for H-6, H-3 and H-12 resonate whereas in the second, one proton for H-11 and H-9 resonate. In the third multiplet, one proton for H-6, H-11 and H-12 resonate. The other proton for H-3 is seen as a triplet at 1.17(J = 12Hz). Four (4) different methyl signals arising from H-16, H-18, H-19 and H-20 are seen as singlets at 0.75, 0.89, 0.99 and 1.27 ppm respectively.

The ^{13}C NMR spectrum indicates the presence of twenty different carbon signals consistent with the diterpene (C_{20}) structure. This in combination with DEPT 135 NMR confirmed the presence of four methyls (15.35, 17.89, 22.65 and 23.94 ppm), four methine (-CH), eight methylenes (CH_2) (15.15, 18.11, 24.02, 28.18, 38.06, 48.22ppm) of which two are from the terminal olefinic carbons, resonating at 111.827 and 107.364 ppm.

The ^{1}H-^{1}H COSY spectrum shows the relationship or coupling of one proton with another. It is noticeable that H-14 (J = 6.4 Hz; J = 10.8 Hz) are coupled to both H-15 protons (J = 17.56 Hz; J = 10.95 Hz). H-17 protons exhibit germinal coupling. Interstingly, H-17 protons also show some coupling with H-11 and H-12 protons, suggesting a nearby side chain at C-9. The ^{1}H-^{1}H COSY spectrum also indicates that H axial and H equatorial protons of the cyclohexane ring scaffold couple with one another and also with adjacent axial and equatorial protons. For example, H-1 axial protons which resonate as a broad doublet at 2.13 ppm shows cross peak coupling

with H-1 equatorial double doublet at 0.9 ppm. Furthermore, both protons show cross peak coupling with H-2 protons at 3.90 ppm. H-7 cross peak which arise from the axial proton at 2.39 ppm show coupling with H-7 equatorial proton at 1.99 ppm. Also, further cross peak coupling is seen with H-6 protons in the multiplet envelope 1.69-1.80 ppm and the other H-6 proton of the other multiplet 1.36-1.43 ppm. The four methyl groups resonate as a singlet and show no cross peak coupling with the ring protons.

HMQC (Heteronuclear Multiple Quantum Correlation) indicates which protons are attached to a specific carbon i.e. ^{1}H-^{13}C correlations of protonated carbon: For example, alkene H-14 proton shows connectivity to C-14 carbon at 145.28 ppm. Both terminal alkene protons, H-15 and H-17 show connectivity at 111.97 ppm (C-15) and 107.5 ppm (C-17) respectively. Both axial and equatorial H-7 and H-3 protons show connectivity to C-7 and C-3 at 38.28 ppm and 51.15 ppm respectively. Likewise, one of the H-1 proton at 0.9 ppm and the other at 2.13 ppm shows connectivity to the same carbon at 1.16 ppm. The three protons of the four CH_3s, H-16, H-18, H-19 and H-20 show connectivity to carbon C-16, C-18, C-19 and C-20 at 28.18, 33.91, 22.87 and 15.58 ppm respectively. An HMBC long range correlation was used for the assignment of non-protonated carbon. For example, a proton on C-1 shows long range HMBC correlation with that of C-3, C-6, C-9, C-10 and C-20. Protons on C-15 show HMBC correlation with protons on C-13 and C-16. H-14 shows strong HMBC to C-13 (73.83ppm) and a weak HMBC to C-6 at 24.16. H-1 shows correlation to carbons: C-2, C-3, C-9, C-10, C-19, C-20, suggesting that these carbons are nearby. HMBC data were particularly useful in determining the location of the CH_3 groups. For example, C-20 protons show HMBC correlation to C-10, C-11 and C-1 whereas, C-19 carbons cross peaks point to C-3, C-5 and C-20, suggesting that these carbons are nearby as shown in Figure. 4.0. No HMBC correlation was obtained for proton on C-4, C-10 indicating that they are quaternised. Figure-2 shows structure of the molecule and an assignment of the specific carbon and proton chemical shifts based on ^{1} H NMR, ^{13} NMR, ^{1} H-^{1}H COSY, HMQC and HMBC experiments.

The IR spectrum shows a broad O-H stretch at 3371.4 cm^{-1}. Alkene C=C and C-H stretch occurs at 1640.5 cm^{-1} and 3084.3 cm^{-1} respectively. Alkene C-H bend occurs at 996.6 cm^{-1}. Methyl asymmetric stretch occurs at 2964.6 cm^{-1} in comparison to methylene asymmetric stretch at 2843.7 cm^{-1}. Methylene rock is seen at 739.9 cm^{-1}.

The monoacetate (9) was prepared from the parent diterpene (2) by stirring in 1.5 equivalents of acetic anhydride. Acetylation of the OH at position C-2 was more favourable than acetylation at position C-13 because of the less steric nature of C-2 in comparison to C-13. Solvent removal, followed by workup yielded the monoacetate in quantitative yield. The ^{1}H NMR of the monoacetate exhibit a similar spectroscopic splitting pattern as that of the parent diterpene. However, the Chemical s shifts differ due to the presence of the electron withdrawing effect of the acetate group at position C-2. This effect is transmitted through the σ-framework of the molecule. Significantly, H-2 proton which resonate as a multiplet in the parent diterpene, exhibit a downfield shift of ΔS = 1.13 ppm (3.90 ppm-5.03 ppm). Other protons deshielded are H-6 and H-9. Protons such as H-14, H-15, H-17, H-7, H-16 etc. are deshielded. The complexity of the three multiplet envelope occurring at is also reduced. For example, in the second multiplet (), the peaks are unresolved. However, for the monoacetate, the peaks are discernible with a doublet for H-9 and a triplet for H-11. Also, H-1, H-3 and H-5 triplet, doublet and triplet are shifted downfield. An

additional CH_3 proton resonance,, due to the CH_3 group of the acetyl moiety is seen as a sharp singlet at 2.04 ppm.

The ^{13}C NMR spectrum indicates twenty two carbons consistent with the structure. Dept 135 differentiated the hybridization of carbons. It indicates the presence of five tertiary methyls at 15.17, 21.58, 22.45, 28.31 and 33.58 ppm, four methine at 54.99, 56.95, 69.34 and 144.95 ppm with the oxygenated one at 69.34 ppm, eight methylenes at (17.84, 23.89, 38.012, 41.12, 46.80, 107.43 and 111.78 ppm and the four quaternary carbons at 147.52, 73.62, 41.15 and 34.95 ppm with the oxygenated one at 73.62 ppm. The acetyl carbonyl is seen at 170.67 ppm.

The ^{1}H-^{1}H COSY spectrum of the monoacetate shows a similar interproton relationship as that described for the parent diterpene. Additionally, the acetyl CH_3 protons show a ^{1}H-^{1}H COSY interaction with H-1, H-2, H-3 and H-19.

HMQC establish ^{1}H-^{13}C connectivity of protonated carbons. Compared to the diterpene, with the exception of C-5, C-16, all carbons exhibit an upfield shift of signal relative to that of the parent diterpene. For example, the parent diterpene exhibit HMQC values for H-1, H-2, H-7, H-11, H-17 and H-22 at 44.15, 65.34, 38.01, 17.33 and 107.43 ppm respectively. For the corresponding monoacetate, these values occur at 48.43, 65.92, 38.28, 17.97 and 107.50 ppm respectively.

The monoacetate also exhibit a similar ^{2j}HC-C and ^{3J}HC-C-C HMBC correlations as that of the parent diterpene. However, there are some additional HMBC correlations. For example, for both compounds, H-protons show HMBC correlation at C-2, C-3, C-5 and C-10. However, for the parent diterpene, an additional HMBC is seen at C-9 and C-20. For the acetyl moiety, the protons of the acetyl group exhibit an HMBC at only C-2, suggesting that it is connected to the latter. For the acetyl moiety, the protons of the acetyl group exhibit an HMBC at only C-2, suggesting that it is connected to the latter.

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Table- 1: NMR data for compound (2) ($CDCl_3$)[a].

Position	δ_C	δ_H ($J_{HH, HZ}$)	^1H-^1H COSY	HMBC
1	8.43	2.13, d(J=10.8 Hz);0.9, dd; J=)	H_2-1, H-2, H-3	C-1, C-2, C-3, C-6, C-9, C-10, C-19, C-20
2	65.92	3.90 , m	H_2-1,H_2-3,H-18	-
3	51.15	1.17, t(J = 12 Hz); d in m(1.8-1.69)	H-2, H_2-3	C-1, C-2, C-6, C-11, C-16
4	35.27	-	-	-
5	5.12	1.11, d (J = 12.4 Hz)	H-6, H_2-7	C-1,C-6,C-9,C-10, C-16
6	24.07	1.72, m 1.297 m	H-5,H-6, H_2-7	C-2,C-5,C-9,C-10,C-11, C-13,C-18, C-20
7	38.28	2.39,brd(J=12.8 Hz);1.99, br t	H-5, H-6, H_2-7, H-9,H-11,H-12	C-5, C-6, C-8, C-17, C-20
8	148.02	-	-	-
9	57.2	1.64, m	H-5, H_2-6, H_2-7, H-11, H_2-12	C-7,C-8,C-10, C,13 C-17, C-20
10	41.45	-	-	-
11	17.97	1.62, m	H_2-6, H-7, H-9 H_2-11, H-12	C-6,C-7,C-8,C-9, C-10, C-13, C-17, C-20
12	41.45	1.39, m	H-6, H-7, H-9,H_2-11, H_2-12, H-17	C-5,C-7,C-8, C-9, C-10, C-11,C-13
13	73.83	-	-	C-8, C-10,
14	145.29	5.92, dd(J = 6.4 Hz; 10.8 Hz)	H_2-15	C-6, C-13
15	111.97	5.26, d(J = 17.6 Hz) 5.09, d(J = 10.8 Hz)	H-14	C-13, C-16
16	28.18	1.29 , s	-	C-5,C-7,C 13,C-15, C-18,C-20
17	107.50	4.87, br s 4.54, br s	H_2-11,H_2-12, H_2-17	C-5,C-18
18	33.77	1.06, s	-	C-2, C-3, C-5, C-11, C-16
19	22.73	0.96 , s	-	C-3, C-5, C-20
20	15.58	0.75 , s	-	C-1, C-10, C-11

- [a] 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR.
- All Chemical shifts (relative to TMS) are given in δ (ppm) and coupling constants in Hz.

Table-2: NMR data for compound (9) ($CDCl_3$) [a].

Position	δ_C	δ_H ($J_{HH, HZ}$)	^1H-^1H COSY	HMBC
1	44.15	2.08, d(J = 6.6 Hz);1.08, t; J = 4Hz)	H_2-1, H-2, H_2-3, H-5, H-20	C-2, C-3, C-5, C-10
2	65.34	5.03 , m	H_2-1,H_2-3, H-5	C-21
3	46.82	1.17, t(J = 4.8 Hz); d in m(1.8-1.69)	H_2-2, H_2-3, H_2-1, H-5	C-1, C-2, C-5, C-19
4	35.27	-	-	-
5	54.98	1.05, d (J = 12.4 Hz)	H_2-1,H_2-3, H_2-6,H_2-7, H-9, H-11, H-20	C-1,C-6,C-9,C-10, C-16
6	23.89	1.74-1.78, m 1.27-1.39 m	H-5,H_2-6, H_2-7, H-9	C-5, C-7, C-9, C-20
7	38.01	2.32,brd(J=12.8 Hz);1.97, br t, J=4.8Hz	H-5, H_2-6, H_2-7, H-9, H-11	C-5, C-6, C-7, C-9
8		-	-	-
9	56.95	1.52-1.66, m	H-5, H_2-6, H_2-7, H-11, H_2-12,H-17, H-20	C-12, C-13
10	41.45	-	-	-
11	17.33	1.52-1.66, m 1.27-1.37 m	H-5, H_2-7, H_2-9, H_2-12	C-9, C-12, C-13,
12	41.12	1.74-1.78, m	H_2-9,H_2-11, H_2-12, H-17	C-9, C-13
13	73.62	-	-	C-8, C-10,
14	144.95	5.91, dd(J = 6.4 Hz; 10.8 Hz)	H_2-15	C-6, C-12, C-13, C-15, C-16
15	111.78	5.24, d (J = 17.2 Hz) 5.08, d (J = 12 Hz)	H-14	C-13, C-14
16	28.34	1.26 , s	-	C-5,C-7,C 13, C-15, C-18,C-20
17	107.43	4.85, br s 4.51, br s	H_2-7, H_2-9, H_2-17	C-7,C-9
18	33.58	0.94, s	-	C-2, C-3, C-5, C-11, C-19
19	22.45	0.89 , s	-	C-3, C-4, C-5,
20	15.17	0.76 , s	-	C-5, C-9, C-16
21	170.67	-	-	-
22	21.55	2.04, s	H-1, H-2, H-3, H-19	C-2

- [a] 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR.
- All Chemical shifts (relative to TMS) are given in δ (ppm) and coupling constants in Hz.