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Isolation and Structure Elucidation of Buchholzcine A, a Novel Steroidal Cyanoformamide from the Stem Bark of *Buchholzia coriacae* Engler (*Capparaceae*)

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Abstract: *Isolation and characterization was carried out on the stem bark of Buchholzia coriacea, commonly called wonderful kola. The plant have been used by traditional people to treat hypertension, headache, and various infections. The dried and powdered plant material was extracted with ethanol to get the crude extract which was partitioned between chloroform and n-hexane (1:1). The chloroform fraction was subjected to column chromatography for purification. Thin layer chromatography (TLC) was used to confirm the purity of the isolated compound. The isolated compound was observed as a golden yellow oil, gave a single spot at* R_f *0.62 in n-hexane/chloroform/methanol (25:15:60). The isolated compound was characterized through joint interpretation of its FT-IR,* ^{1}*HNMR,* ^{13}*CNMR spectra, and 2-dimensional techniques including* ^{1}*H-*^{1}*H COSY, HMQC, and HMBC. Buchholzcine A, a steroidal Cyanoformamide was proposed, with IUPAC name: cyano-N- (12E, 14E, 18Z)-19-(hexadecahydro-10,13-dimethyl-1H-cyclopenta[α]phenanthren-17-yl) nonadeca-12,14,16,18-tetraenylformamide,*

Keywords: *Buchholzia coriacea, Buchholzcine A, cyanoformamide, spectroscopy, chromatography*

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1.0 Introduction

Natural products are important sources of compounds with diverse structures and possessing biological activities (Enechi et al., 2022). Isolation, purification, structure elucidation and biological evaluations of natural products play a central role in modern drug discovery efforts, the proper identification of their structures offer useful leads to their further development by chemical synthesis (Hong-Fang Ji, et al., 2009).

Buchholzia coriacea, commonly called ‘Wonderful kola’ belongs to *Capparaceae* family (Erhirie et al., 2015). Called *'Uworo'* in Yoruba, *'Owi'* in Edo, *'Uke'* in Igbo. (Quattrochi-Umberto, 2007). It promotes function of human memory and nervous system. Akunne et al., (2017) reported that the plant is used in traditional medicine to treat various ailments including inflammation and infection. It has been used to treat cardiovascular conditions and hypertension, headache, nasal congestion, sinusitis, bronchitis. (Umeokoli et al., 2016).

Phytochemicals such as Alkaloids, glycosides, saponin, steroids, tannin, flavonoids, terpenes, reducing sugars and phenol have been identified in 'wonderful kola' (Ibrahim and Fagbohun, 2013). (Malomo et al. (2017) reported that the phytochemicals present in the plant may be responsible for its therapeutic effects.

Despite the use of 'wonderful kola' in traditional medicine, there is no scientific validation of its use, moreso, research directed toward isolation of compounds from its stem bark is limited. The gap created hinders the development and standardization of therapeutic agents from the plant. This study was focused on isolating and elucidating the structure of a purified novel cyanoformamide derivative which may be of pharmacological relevance, from the stem bark of *Buchholzia coriacea.* The result of this work is a significant contribution to medicinal chemistry research.

2.0 Materials and Methods

Dried and milled sample (1kg) was macerated in 4 litres of ethanol. (Johnbull et al., 2001). The extract was concentrated by rotary-evaporator which was set to $45^{o}C$ under reduced pressure of 5 kPa, and 40-55 rev / min. The resulting crude extract was dark brown in colour, the mass of the residue was recorded. Crude extract (126 g) was partitioned between *n*-Hexane : Chloroform (1:1). The chloroform fraction (78 g) was purified by gradient elution in a chromatographic column, starting from *n*-hexane (100 cm^3) : chloroform (0 cm^3) to *n*-hexane (0 cm^3) : chloroform (100 cm^3), then chloform (95 cm^3) : methanol (5 cm^3) to chloroform (0 cm^3) : methanol (100 cm^3). The purity and Retention Factor (R*f*) of each fraction was detrmined by Thin-Layer Chromatography.

Infrared spectroscopy was recorded on a Perkin Elmer FT Model. IR samples were run in nujol mull. Readings were taken between 4000 cm^{-1} and 625 cm^{-1}. ^1H NMR spectra were recorded on a Bruker AM-500 FT-NMR spectrometer operating at 500 MHz. ^{13}C NMR and DEPT (Distortionless Enhancement by Polarization Transfer) were run under proton decoupled condition at 125 MHz on a Bruker AM-500 spectrometer. Interpretation of spectra, included data from 2-dimensional techniques namely: ^1H-^1H COSY (Correlation Spectroscopy), HMQC (Heteronuclear Multiple Quantum Coherence), and HMBC (Heteronuclear Multiple bond Correlation)

3.0 Results and Discussion

The isolated compound was observed as a golden yellow oil, which gave a single spot at R_f 0.62 in n-hexane/chloroform/methanol (25:15:60).

As shown in table 1 and figure 1, the two bands between 3350 cm^{-1} and 3310 cm^{-1}represent N-H stretching vibrations of a secondary amide, two weak bands between 1710 and 1510 cm^{-1} depict carbonyl (C=O) stretching vibrations of an amide. The higher frequency (1710 cm^{-1}) absorption is called the Amide I band while the lower frequency (1510 cm^{-1}) called Amide II band is largely due to N-H bending and trans to the carbonyl oxygen. The sharp bands of medium intensity between 2240 - 2260 cm^{-1} are typical of nitrile (CN). The very small and sharp peak at 3100 cm^{-1} denotes alkene (C=C), this band is supported by the weak stretching vibration band at 1630 cm^{-1}. The strong bands between 2922 - 2852 cm^{-1} represent aliphatic stretching vibrations specifically of methyl (CH_3) and methylene (CH_2). These absorptions are supported by medium intensity bands between 1350-1470 cm^{-1} accounting for CH_2 & CH_3 deformation, another set of medium intensity bands between 1370-1390 cm^{-1}, specifically for CH_3 deformation and a set of weak bands between 720-725 cm^{-1} for CH_2 rocking. ^{1}H NMR spectrum of NJBC 22 shows the presence of two methyl protons singlets at δ 0.82 (3H-19') and δ 0.88 (3H-18'); four aliphatic methylene protons triplets at δ 2.40 and 1.60 (2H-1), δ 1.62 and δ 2.10 (2H-1'), δ 1.57 and 1.42 (2H-4') and δ 1.30 and 2.04(2H-12').

Table 1: Data from IR Spectrum

S/N	Frequency (cm^1)	Intensity/shape	Functional group	Type of vibration
1.	3350-3310	Medium, one band	N-H	Stretching vibrations
2.	775	Strong	N-H	Wagging
3.	2922	Strong	C-H	Asymmetric stretching for CH_3
4.	2852	Strong	C-H	Symmetric stretching for CH_2
5.	1460	Strong	C-H	Bending vibrations for CH_2
6.	1300	Strong	C-H	Bending vibrations for CH_3
7.	2260	Strong	C≡N	Stretching vibrations
8.	1040	Strong	C≡N	Bending vibrations
9.	1710-1510	Weak	C=O	Stretching vibrations for amide
10.	1630	Weak	C=O	Stretching vibrations of alkene
11.	880-995	Strong	=CH	Out-of-plane bending of alkene

The spectrum also reveals the presence of ten aliphatic methylene protons multiplet at δ 2.40 and 1.30 (2H-2), δ1.29 and 1.32 (2H-3), δ 1.29 and 2.01 (2H-4), δ1.29 and 2.22 (2H-5), δ 1.29 and 2.21 (2H-6), δ 1.29 and 1.31(2H-7), δ 1.29 and 1.30 (2H-8), δ 1.29 and 2.00 (2H-9), δ 1.29 and 2.10 (2H-10) and δ 1.51 and 1.52 (2H-3'). Seven aliphatic methylene protons quartet at δ 1.33 and 1.60 (2H-11), δ 1.72 and 1.47 (2H-2'), δ 1.31 and 1.64 (2H-6'), δ 1.30 and 1.65 (2H-7'), δ 1.52 and 1.17 (2H-11'), δ 1.62 and 1.30 (2H-15') and 1.17 and 1.80 (2H-16') four aliphatic methine quartet at δ 1.41 (1H-8'), δ 1.40 (1H-9'), δ 1.42 (1H-14'), δ 2.19(1H-17'), and one aliphatic methine multiplet at δ 1.18 (1H-5'). Six olefinic methine protons doublet of doublet at δ 5.34 (1H-13), δ 6.51 (1H-14), δ 5.35 (1H-15), δ 5.37 (1H-16), δ 5.60 (1H-17), δ 5.65(1H-18); one olefinic methine protons triplet at δ 5.60 (1H-19); one aliphatic methine quartet at δ 2.19 (1H-17') and one amido NH proton at δ 8.0. The ^{13}C NMR contained signals for a total of forty carbon atoms among which are two methyl/primary carbons, twenty one secondary carbons, eight olefinic tertiary carbons, five aliphatic primary carbons, and two quartenary carbons, one carbonyl carbon and one nitrile carbon. The ^{13}C NMR assingnments made in this study is in agreement with the work of Mayra et al., (2005), who made similar assignments to elucidate the structure of 9α-chloro and bromo-androstane derivatives. Off-diagonal peaks of ^{1}H-^{1}H COSY spectrum showed key couplings which indicate connectivity in the molecule between protons and neighbouring protons. Coupling were observed between 1H olefinic methine proton multiplet centered at δ 6.51ppm H-18 and another 1H olefinic methine at δ 5.60ppm H-19.

Table 2: ^{1}H and ^{13}C Assignments

Carbon No.	^{13}C (ppm)	^{1}H (ppm)	Type	No. of H	Multiplicity
1	31.93	2.40, 1.62	–CH_2–	2H	t
2	29.48	2.40, 1.30	–CH_2–	2H	m
3	25.94	1.29, 1.32	–CH_2–	2H	m
4	29.16	1.29, 2.01	–CH_2–	2H	m
5	39.00	1.18, 2.22	–CH_2–	2H	m
6	29.16	1.29, 2.21	–CH_2–	2H	m
7	32.79	1.30, 1.65	–CH_2–	2H	m
8	29.30	1.29, 1.30	–CH_2–	2H	m
9	29.31	1.29, 2.00	–CH_2–	2H	m
10	31.53	1.29, 2.10	–CH_2–	2H	m
11	34.70	1.33, 1.60	–CH_2–	2H	q
12	135.00	5.60	CH	1H	q
13	129.15	5.34	CH	1H	dd
14	130.20	6.51	CH	1H	dd
15	130.21	5.35	CH	1H	dd
16	129.20	5.37	CH	1H	dd
17	129.15	5.60	CH	1H	dd
18	128.30	6.51	CH	1H	dd
19	138.15	5.60	CH	1H	t
20	173.00	–	C=O	–	–
21	117.00	–	C=N	–	–
1′	31.93	1.62, 2.10	–CH_2–	2H	t
2′	31.53	1.72, 1.47	–CH_2–	2H	q
3′	28.10	1.51, 1.52	–CH_2–	2H	m
4′	34.70	1.57, 1.42	–CH_2–	2H	t
5′	39.00	1.18	–CH_2–	2H	m
6′	24.90	1.31, 1.64	–CH_2–	2H	q
7′	32.79	1.30, 1.65	–CH_2–	2H	q
8′	56.60	1.41	–CH–	1H	q
9′	56.70	1.40	–CH–	1H	q
10′	33.92	–	–C–	–	–
11′	22.71	1.57, 1.17	–CH_2–	2H	q
12′	39.91	1.10, 2.09	–CH_2–	2H	t
13′	33.90	–	–C–	–	–
14′	52.90	1.42	–CH–	1H	q
15′	24.47	1.62, 1.20	–CH_2–	2H	q
16′	39.90	1.17, 2.19	–CH_2–	2H	q
17′	49.50	2.19	–CH–	1H	q
18′	16.00	0.82	–CH_3	3H	s
19′	16.10	0.88	–CH_3	3H	s

****m = multiplet, t = triplet, d = doublet, dd = doublet of doublet, q = quartet, s = singlet.**

Coupling was observed between aliphatic methine protons at δ 2.19ppm H-17’ and the three methyl protons which appears as a singlet at δ 0.82ppm H-18’. Aliphatic methylene protons at δ 1.60ppm H-11 coupled with olefinic methine proton at δ 5.37ppm H-12. Finally, there was coupling between methine proton at σ 2.09ppm H-12’and aliphatic methylene proton at δ 1.57ppm H 11’.

Analysis of HMQC spectrum shows one bond correlation between aliphatic secondary carbons C-1’ at δ 31.93ppm and methylene proton H-1’ at δ 1.62ppm; aliphatic tertiary carbon C-5’ at δ 39.00 and methine proton H-5’ at δ 1.18; aliphatic secondary carbon C-7’ at δ 32.79ppm and two methylene protons at Ha -7’ at δ 1.30, Hb -7’ at δ 1.65; aliphatic secondary carbon C- 16’ at δ 39.90 ppm and the methylene protons H-16’ at δ 2.19ppm and 1.17 ; aliphatic primary carbon C-18’ at δ 16.0ppm and three methyl protons H – 18’ appearing as a singlet at δ 0.82ppm.

Proton-Carbon long range coupling (HMBC) gave the following correlations: A two bond coupling was observed between aliphatic tertiary carbon C-17’ at δ 49.50 and 2H aliphatic methylene proton at δ 1.20; a three-bond coupling between aliphatic methylene carbon C- 16’ at δ 39.90 and the 2H aliphatic methylene protons H-12’ at δ 2.09 and 1.10; a three bond coupling between 2H aliphatic secondary carbon C-4 at δ 29.16 and 2H aliphatic methylene protons H-2 at δ 2.40; aliphatic secondary carbon C-11’ at δ 22.71 coupled with one of the 2H aliphatic methylene protons H-12’ δ 1.10 through a two bond coupling. Interpretation of data from the various spectra gave the following structure:

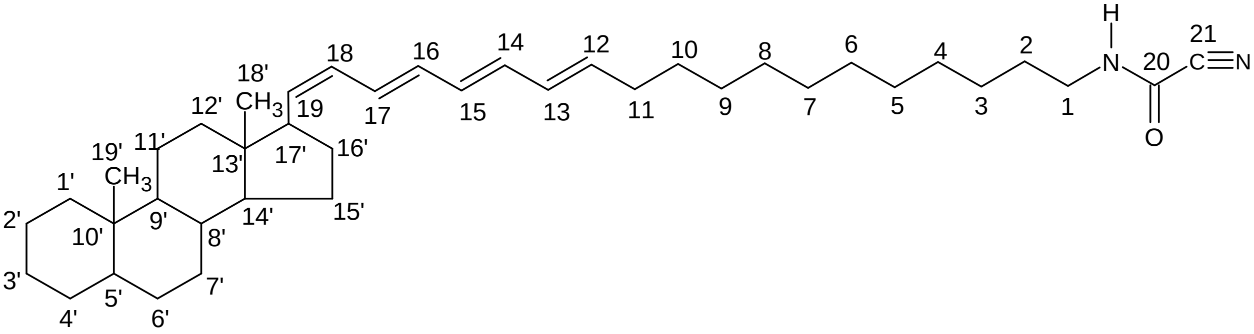

Fig.1 1 Buchholzcine A,
IUPAC Name: Cyano-N-((12E, 14E,16E, 18Z)-19-(hexadecahydro-10,13-dimethyl-1H cyclopenta[α]-12,14,16,18- tetraenyl)formamide
Molecular formula: $C_{40}H_{62}N_2O$ Molecular wt. 586

4.0 Conclusion

This study reports the successful isolation of a novel steroidal cyanoformamide, a golden-yellow oil, soluble in methanol and chloroform. Data from comprehensive analyses of the provided spectra led the researchers to propose the structure of the isolated compound as having the trivial name Buchholzcine A, and IUPAC name cyano-N-((12E, 14E,16E, 18Z)-19-(hexadecahydro-10,13-dimethyl-1H cyclopenta[α]-12,14,16,18-tetraenyl)formamide. This is the first time isolation of this compound will be reported from any plant source. This discovery contributes to the growing insights into the chemical properties of *B. coriacea*, and its potential in natural products research.

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This work was sole collaboration among all the authors

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Authors Contributions

All authors were involved in the work. CPN designed the works and others were in involved in al the stages

Appendix

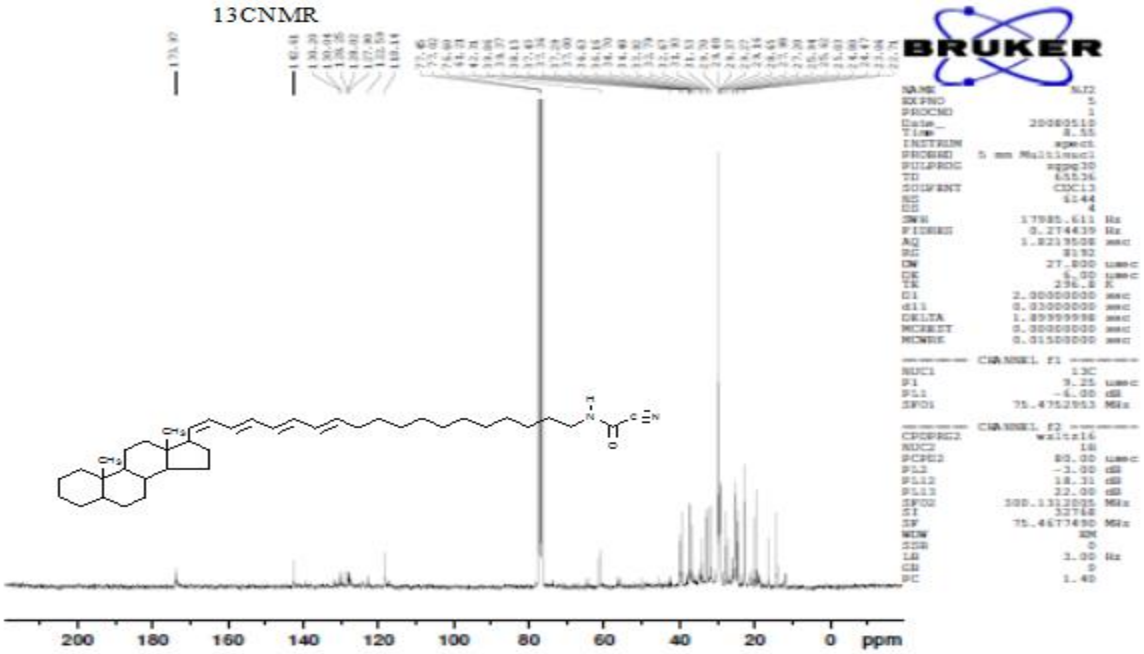

Fig. 2 ^{13}C NMR of Buchholzcine A

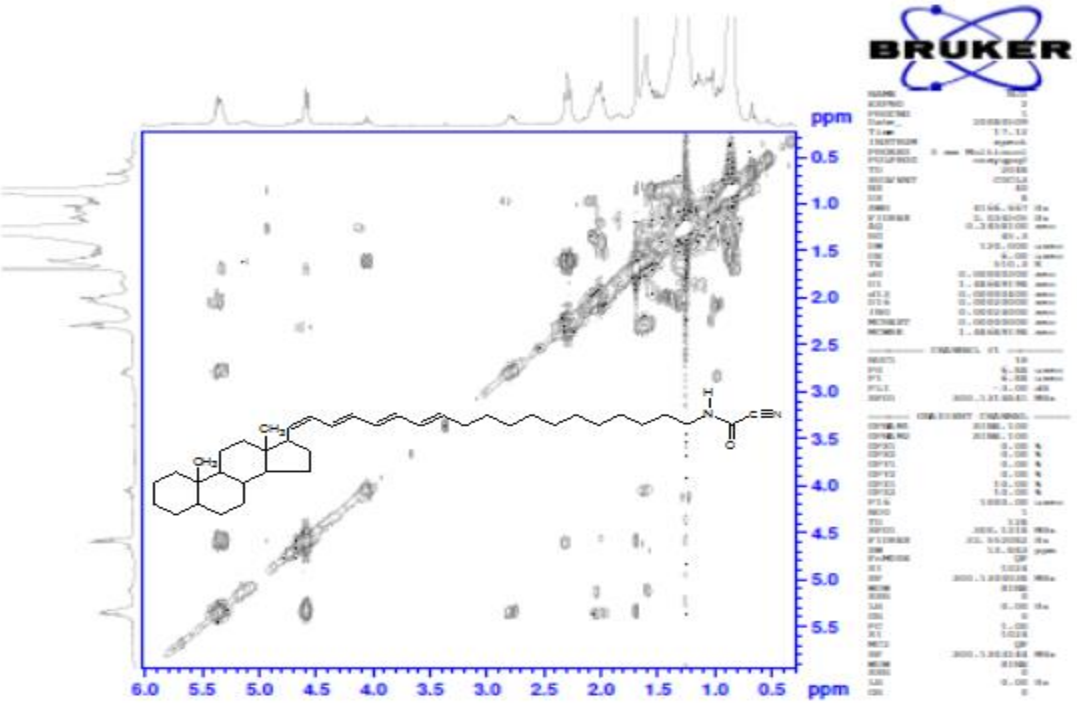

Fig. 3: 1H-1H COSY of Buchholzcine A

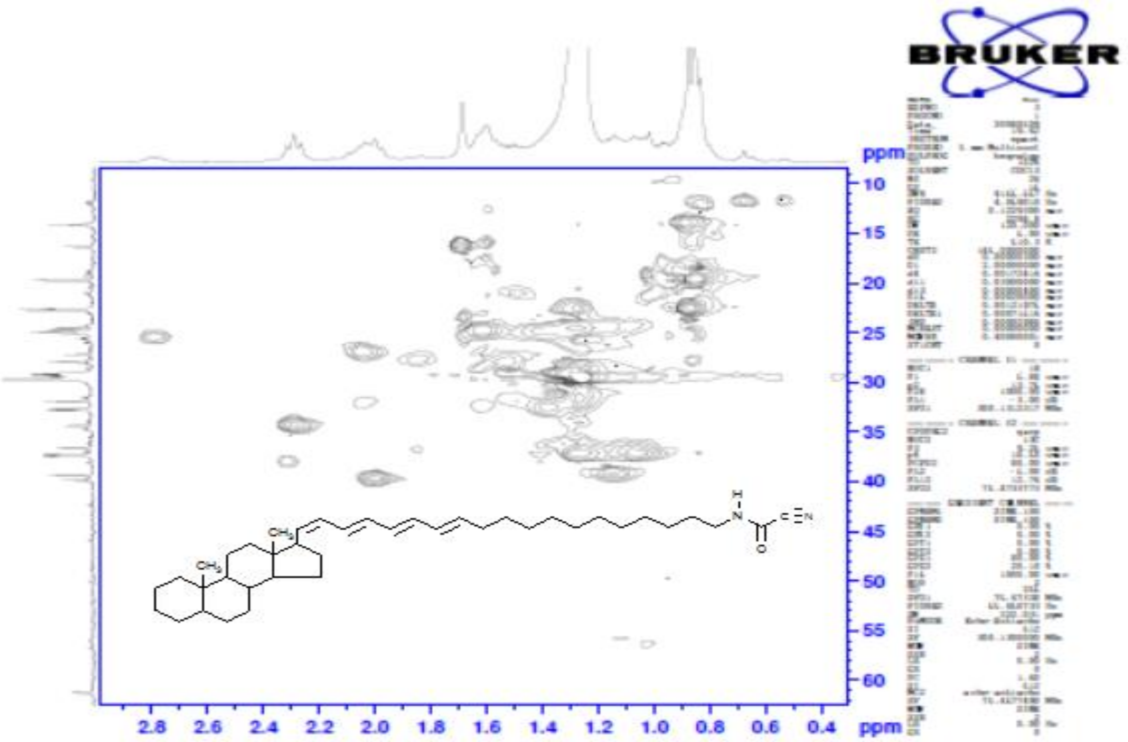

Fig. 4: HMQC spectrum I of Buchholzcine A

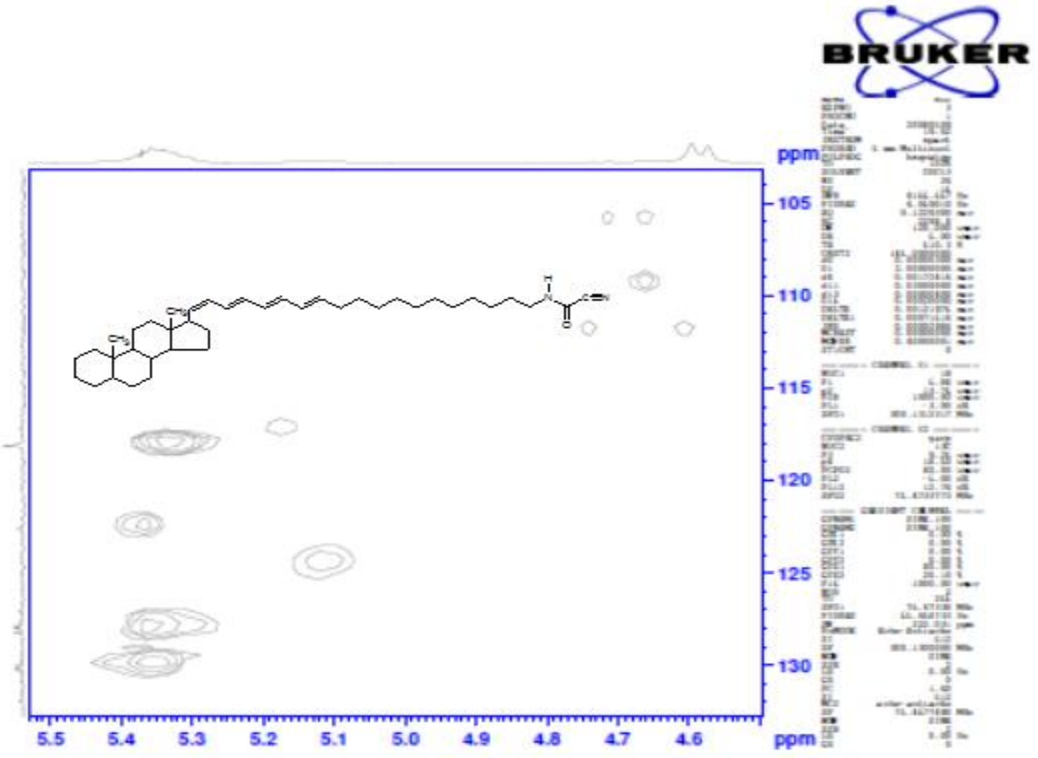

Fig. 5 HMQC spectrum II of Buchholzcine A

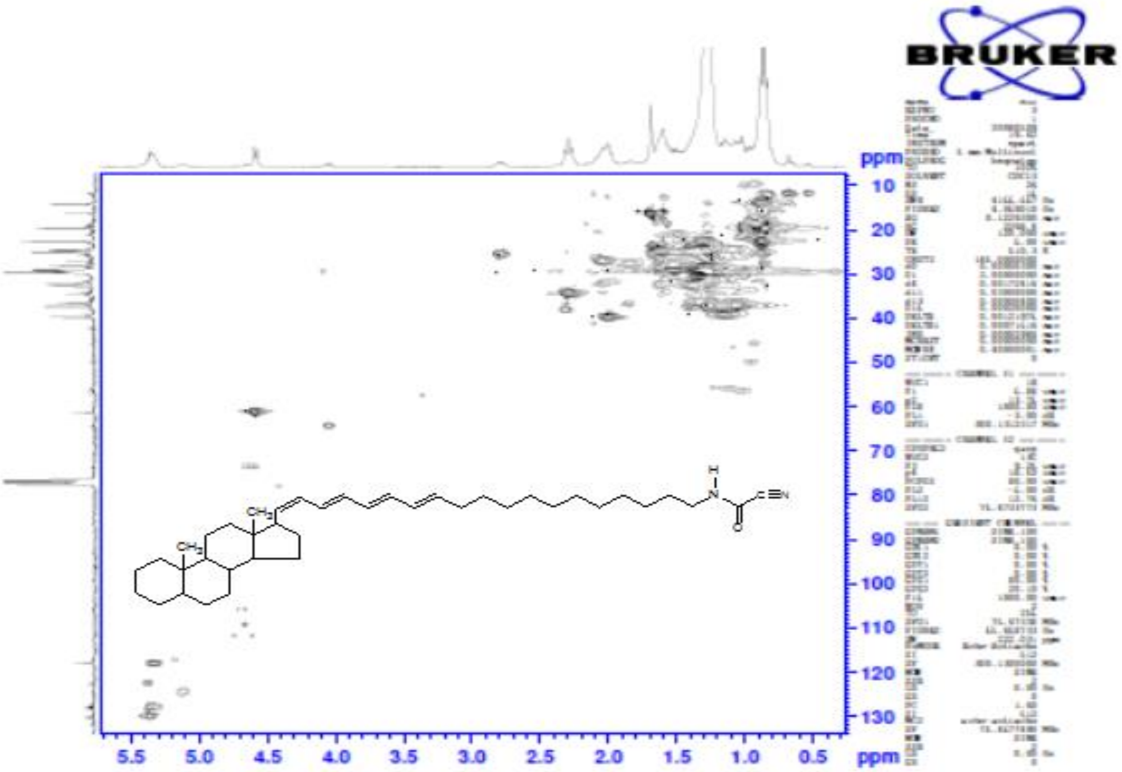

Fig. 6 HMBC spectrum III of Buchholzcine A

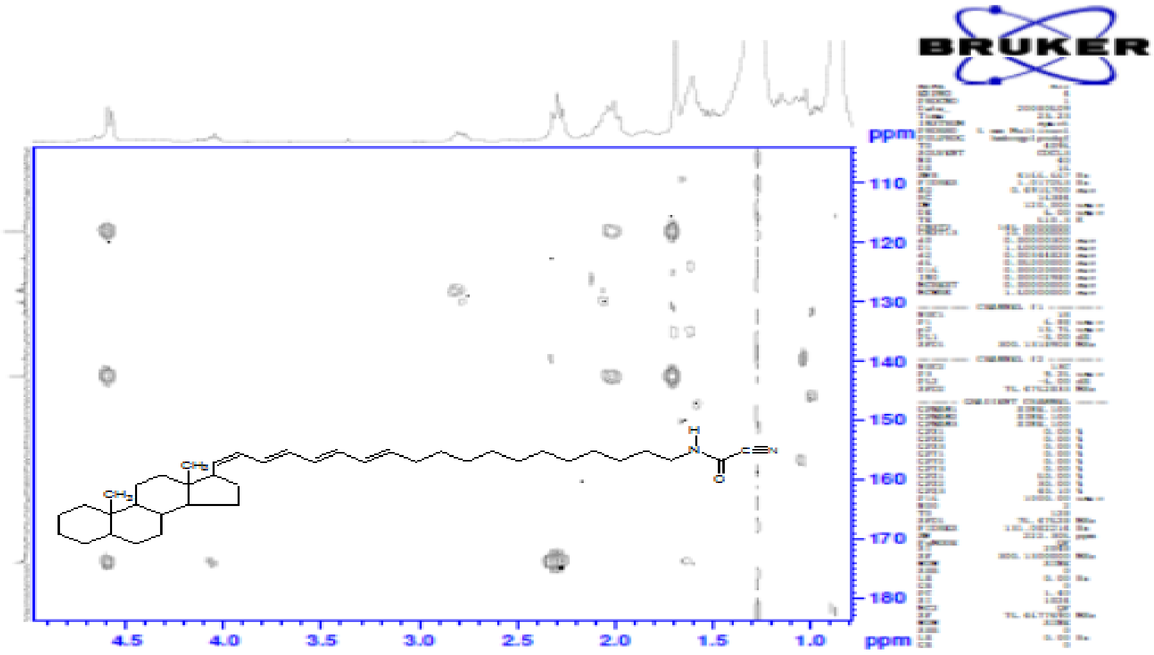

Fig. 7 HMBC spectrum IV of Buchholzcine A

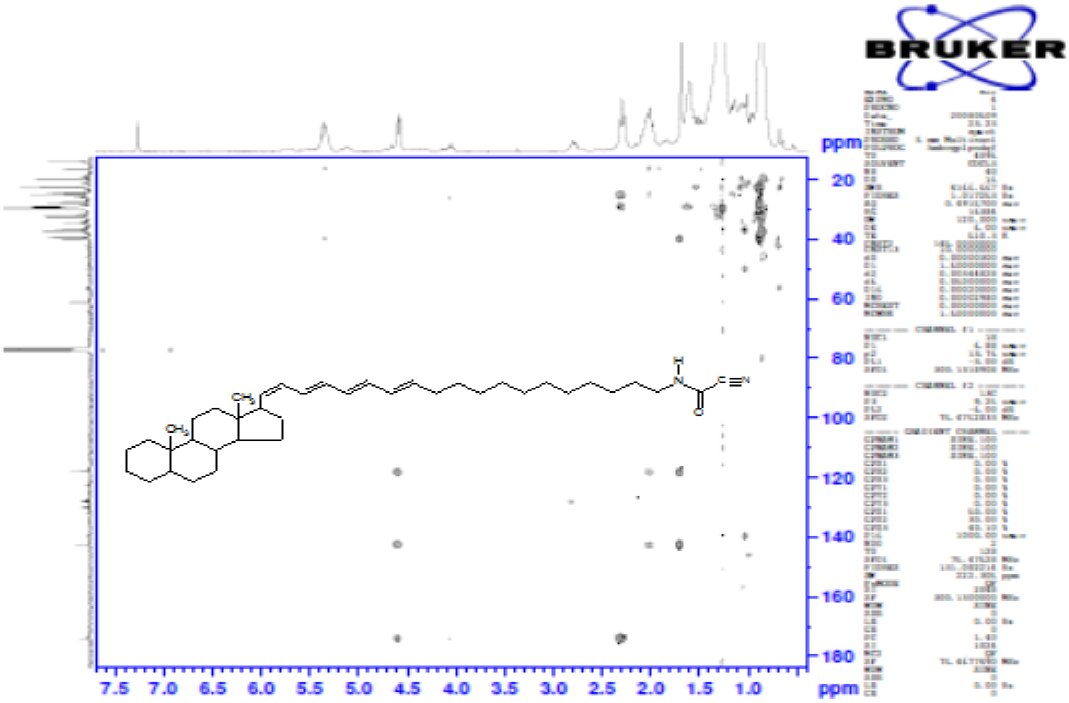

Fig. 8 HMBC V spectrum of Buchholzcine A