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Research Article

ISOLATION AND CHARACTERIZATION OF INCENSOLE AND DA-23-IV (NEPHTHENOL) FROM ETHYL ACETATE STEM BARK EXTRACT OF PSEUDOCEDRELA KOTSCHYI SCHEINF HARMS (MELIACEAE)

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Abstract

Pseudocedrela kotschyi Schweinf Harms (Meliaceae) is used in ethnomedicine to treat various disease conditions (like ulcers, sores, rheumatism, leprosy, syphilis, fevers and tumors. The dried pulverized bark material was extracted using maceration method with methanol. The methanolic extract obtained was fractionated into water, hexane and ethyl acetate fractions. Repeated Column chromatography and preparative thin-layer chromatography (TLC) of the ethyl acetate fraction yielded two pure compounds, incensole and nephthenol. The structures of the isolated compounds were elucidated using nuclear magnetic resonance spectroscopy (1D and 2D ^{1}HNMR and ^{13}CNMR). This is the first time report of Incensole a cembrane diterpenoid compound in the stem bark of the plant *P. kotschyi.*.

Keywords: *Pseudocedrela kotschyi,* Incensole, Nephthenol, Chromatography.

INTRODUCTION

Pseudocedrela kotschyi Schweinf Harms (Meliaceae) stem bark was observed to have rough texture with brittle and fibrous fracture. The taste is bitter and when dried, pieces assumed curved convex shape. The powder form of the bark is brown to white in colour (Dafam *et al.,* 2019). *P. kotschyi* has numerous uses in traditional medicine, particularly its bark, roots and leaves. Bark decoctions or macerations are applied externally to ulcers, sores, rheumatism, leprosy, syphilis, yaws, itch, caries and gingivitis. Internally they are used to treat fever, stomach-ache, diarrhoea and dysentery, and as a diuretic and aphrodisiac (Burkill, 1997). In Nigeria the stem bark is used in mixtures to treat trypanosomiasis in livestock, and leaves are administered in veterinary medicine against intestinal worms (Burkill, 1997). The Tarok speaking communities use the bark and leaves to treat fevers, pains/inflammation and treatment of tumors in the form of powder and decoction (*Dafam et al.,* 2016). The bark yields a brownish dye that has been used in West Africa for dyeing cloth. The plant is occasionally planted as an ornamental shade tree and roadside tree. In Nigeria the leaves are used as a green manure (Burkill, 1997). Phytochemical screening of the plant revealed the absence of anthraquinones and cardiac glycosides, whereas saponins, tannins, flavonoids, steroids and terpenes were shown to be present (Dafam *et al.,* 2018;). The dental cleansing effect (Akande and Hayashi, 1998), antiepileptic (Anuka *et al.,* 1999), anti-inflammatory activity (Otimenyi *et al.,* 2004), analgesic (Musa *et al.,* 2005), anticonvulsant (Odugbemi, 2006), of the plant have been reported .Okunade *et al.,* (2007) also reported its dental cleansing effects.

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In addition, the plant has other biological effects such as antibacterial (Koné *et al.,* 2004), antimalarial (Asase *et al.,* 2005; Dawet and Yakubu, 2014), antipyretic (Akuodor *et al.,* 2013), heamatinic, antidiabetic activities and protective effect on the renal system (Ojewale *et al.,* 2014; Georgewill and Georgewill, 2009; Bothon *et al.,* 2013.,) and antiproliferative activities of the root (Olakunle *et al.,*2015).

MATERIALS AND METHODS

Experimental

General procedures. Thin-layer Chromatography (TLC) was carried out on Si gel 60 F254 Merck®. Accelerated Gradient Chromatography - AGC (Bæckström, 1993), a form of Medium Pressure Liquid Chromatography (MPLC) was carried out on columns packed with Si gel 60, 0.040- 0.063mm Merck®. The MPLC workstation was from University of Western Cape, South Africa. NMR was carried out on a Brucker 400 MHz spectrometer.

Collection and preparation of Plant Material

Stem bark of *Pseudocedrela kotschyi* was collected from Langtang North local government area of Plateau state, Nigeria in August, 2014. Photographs of the freshly collected plant were taken in its natural habitat.The plant was identified by comparing with voucher specimens deposited at the Herbaria of the Federal College of Forestry Research Institute Jos, and authenticated in the Department of Botany and plant Sciences, Ahmadu Bello University Zaria, Nigeria by the taxonomist, Mr. Mohammed Musa and a voucher specimen number, ABU 960243was given, The stem bark of the plant collected was sliced into pieces to facilitate its drying under the shade.

Grinding was carried out using a grinding machine to powder form and stored in an airtight container to prevent it from microbial attack.This was preserved according to methods described in the African Herbal Pharmacopoeia (AfrHP) (2010) and African Pharmacopoeia (1985).

Extraction and Isolation

The dried, powdered stem bark of *P. kotschyi* was weighed (800 g) and extracted using maceration method with 3.5Litres of methanol for 20hrs at room temperature, with occasional shaking and the extract was filtered. The filterate was concentrated under reduced pressure using rotavaporand the percentage yield determined. The methanol extract (146.30 g) was separately partitioned with water,hexane and ethyl acetate hexane (500 mL) and the aqueous portion (fraction) containing the hexane was concentrated to dryness using rota vapor at a temperature of 65ºC. The aqueous portion was further partitioned with ethyl acetate (400 mL) and distilled water (100 mL) in a separating funnel and shaken. The aqueous layer was collected and concentrated using a rotavapor and later on a water bath. The amount of yield obtained was determined and kept for use when needed. The dry-pack method of column chromatography (CC) was adopted. The column (4 x 60) cm was packed with silica gel. The extract was mixed with an equivalent amount of silica and was initially pre-absorbed with Dichloro methane (DCM) was allowed to dry and added to the previously packed adsorbent in the column. The column was gradiently eluted with different solvent system; 100 % Hexane, (Hexane/EtOAc) in the ratio (9:1; 8:2; 7:3; 3:2; 1:1; 2:3; 3:7; 2:8) and 100 % ethyl acetate. The TLC was carried out for the various fractions collected and was developed in different solvent systems in varying concentrations. The ethyl acetate fraction was profiled using Hexane/EtOAc (3:2) and the developed chromatograms were observed under UV light at 256 and 366 nm wavelengths and spread with vanillin sulphuric acid for the spots separation visibility when heated in an oven gradually up to 105°C. On repeated CC and TLC analysis of the combined fractions eluted with ethyl acetate, pure fraction coded DA-23-I (Incencole) showed chromatograms of single spot signifying a state of purity of the isolated compound as colourless oil characteristic of incensole (21.13 g /40.50 mg). and fraction coded DA-23-IV (Nephthenol) also showed single spot on TLC in different solvent system.

Characterization and Structure Determination of the Isolated Compound

The structures of the isolated compounds were elucidated through spectroscopic methods to include ultraviolet (UV), infra-red spectrometry (IR), hydrogen nuclear magnetic resonance ^{1}(HNMR) and ^{13}CNMR. The most useful data concerning the chemical structures were furnished by 1D and 2D NMR spectroscopy. From 1H NMR, JMOD, 1H-1H COSY, HSQC and HMBC experiments, the constitution of the compound was determined with the aid of the NOESY spectra, the relative configurations were elucidated. In the isolated compounds, the numbers of asymmetric carbons were determined, and they were all characterized stereochemically. NMR studies allowed complete 1H and 13C assignments for the characterization of the compound.

RESULTS AND DISCUSSION

Incencole from Ethyl acetate fraction of the stem bark of *P. kotschyi*

The compound was isolated as thick oil. The NMR data supported a diterpene skeleton of cembrane type. Signals from ^{1}H NMR showed two olefinic protons at 5.04, 5.06 ; hydroxylated proton at 3.27 (d, 9.7 Hz); two high field shift methyls at 1.48, 1.60 (both singlet), each attached to a double bond; methyl signal at 1.04 (s); signal for isopropyl group [two doublet methyl signals at 0.887, 0.889 (J = 7.9 Hz); and a proton at 1.95 (m)] these were supported by 1H-1H Cosy spectra which showed a cross peak between the two methyls and the proton. ^{13}C NMR spectra showed 20 signals of 20 carbons which support the diterpene skeleton. Those signals could be splitted using DEPT-135 into 5 methyls (0.887, 889, 1.04, 1.48, 1.60), 7 methylene (24.8, 30.6, 30.7, 32.4, 33.7, 36.4, 38.6), 4 methines, two of them are olefinic and one hydroxylated (34.8, 75.6, 121.8, 125.1) and 4 quaternary carbons (84.2, 88.6, 134.2, 134.3). Furthermore, the HMBC spectra showed correlations between (δ_H/δ_C): Me's 0.887, 0.889, 5.06/88.6; Me 1.04/84.2; 1.48/121.8, 134.3 and 1.60/125.1, 134.2. The long range C-H correlation deduced from the COLOC spectrum, H-H COSY coupling and NOESY, prove that compound is the macrocyclic diterpenoid: 1,12-epoxy-3,7- cembradien-11-ol, known as incensole.

Table 1. Summary of NMR Data on DA-23-I, of Ethyl acetate fraction from the stem bark of *P. kotschyi*

Position	δc (ppm)	DEPT	δH(ppm)/HSQC	H-H COSY	HMBC
1	88.53	C			H-2;H-14;H-16/17;H-13
2	33.62	CH_2	2.00	H-3	H-14;H-15
3	121.74	CH	5.08	H-2	H-18;H-5;H-14;H-15
4	134.16	C			H-2;H-6;H-18H-5
5	34.79	CH_2	1.88	H-6	H-18;H-3;H-6
6	24.8	CH_2	1.48	H-5;H-7	H-7;H-5;H-9
7	125.10	CH	5.05	H-6	H-5;H-19;H-9
8	134.23	C			H-10;H-5;H-6;H-11
9	32.31	CH_2	1.85	H-10	H-7;H-19;H-11
10	36.32	CH_2	1.89	H-9;H-11	OH;H-11;
11	84.12	CH		H-10	H-20;H-9;H-10;OH
12	77.31	C			H-20;OH;H11;H-14;H-10
13	30.68	CH_2	I.34	H-14	H-20;H-11
14	30.59	CH_2	1.33	H-13	H-15;H-2;H-13
15	38.58	CH	2.04	H-16/17	H-14;H-2;H-3;H-16/17
*16	17.96	CH_3	1.27	H-15	H-2;H-14
*17	18.04	CH_3	1.27	H-15	H-2;H-14
18	20.63	CH_3	1.29	H-3	H-3;H-5;H-6
19	18.14	CH_3	1.30	H-7	H-7;H-9;
20	16.10	CH_3	1.32	H-11	H-11;H-13;

N.B.: * assignments are interchangeable.

Table 2. NMR data for Nephthenol, of ethyl acetate fraction from the stem bark of *p. kotschyi*

Position	δc (ppm)	DEPT	δH(ppm)/ HSQC	H-H COSY	HMBC
1	48.47	CH	1.54	H-2;H14	
2	28.45	CH_2	1.58	H-1;H-3	H-14;H-16/17;H-4
3	125.96	CH	5.07	H-2	H-1;H-18
4	133.35	C			H-18;H-5;H-3;H 6;H 2
5	38.81	CH_2	2.12	H-6	H-3;H-7
6	24.67	CH_2	2.08	H-5;H-7	H-3;H-18;H-19
7	125.78	CH	4.98	H-6	H-9;H-19;H-5;H-6
8	133.03	C			H-10;H-19;H-9
9	39.39	CH_2	2.09	H-10	H-20;H-7
10	24.02	CH_2	2.11	H-9;H-11	H-7;H-13;H-20
11	125.00	CH	4.97	H-10	H-9;H-14;H-20;H-13
12	134.02	C			H-14;H-1;H-10;H-20
13	37.42	CH_2	1.65	H-14	H-11;H-1
14	28.26	CH_2	1.54	H-13;H-1	H-2
15	73.96	C			H-16/17;H-14;H-2;OH
16	27.67	CH_3	1.29		OH;H-17;H-1
17	27.51	CH_3	1.29		OH;H-16;H-1
18	29.71	CH3	1.61		H-3;H-5
19	15.32	CH3	1.61		H-7;H-9
20	15.57	CH3	1.61		H-11;H-13

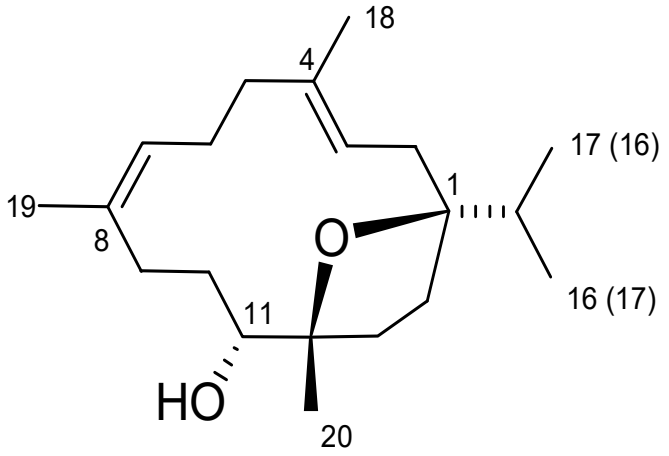

Fig. 1. The structure of Incensole from the ethyl acetate fraction of the stem bark of *P. kotschyi*

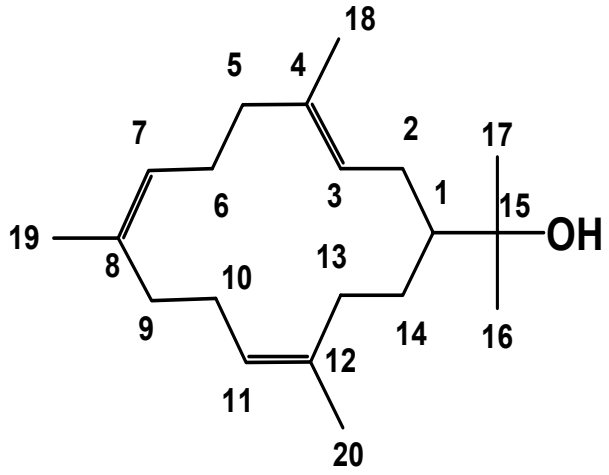

Fig. 2. The depicted structure of Nephthenol from ethyl acetate fraction of the stem bark of *P. kotschyi*

The isolated compound is soluble in dichloro methane (CH2Cl2) and Ethyl acetate (EtOAc). The 1H NMR data for this compound revealed that it is in agreement with that obtained by previous researchers (Corsano and Nicoletti, 1967), who isolated and characterized the same compound, incensole, from the neutral fraction of frankincense, produced by *Boswellia carteri*. Its chemical synthesis (Kato, 1976) and 13C NMR spectral data (Gacs-Baitz, *et al.,* 1978) have also been reported. It is to be noted that this is the first time Incensole is reported in *P. kotschyi* Schweinf Harms (Meliaceae). The second isolated compound showed similar NMR data with incensole, which indicated a cembrane diterpene derivative. ^{1}H NMR showed three olefinic protons signals at 5.07, 4.98, and 4.97(br t, J=6.1 Hz), 5.11 (br t, 6.0 Hz); 5 methyl singlet signals 1.19 (X2), 1.55, 1.56 (X2). ^{13}C NMR spectra showed 20 signals of 20 carbons which support the diterpene skeleton. This was splitted with DEPT-135 which showed five methyls, (27.67, 27.51, 29.71, 15.32 and 15.57), seven methylenes (28.45, 38.84, 24.67, 39.43, 24.02, 37.42, 28.26), four methines (48.47, 125.96, 125.78, and 125.00).and four quaternary carbons (133.35, 133.03, 134.02 and 73.93). The HMBC showed correlations between (δ_H/δ_C): 1.58/28.45, 2.12/38.84, 2.08/24.02, 4.98/125.78, 2.09/39.43 1.65/37.42, 1.54/28.26 and 2.11/23.99. The data indicated cembrane diterpene with three double bonds and oxygenated isopropyl group.

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SUPPLEMENTARY DATA

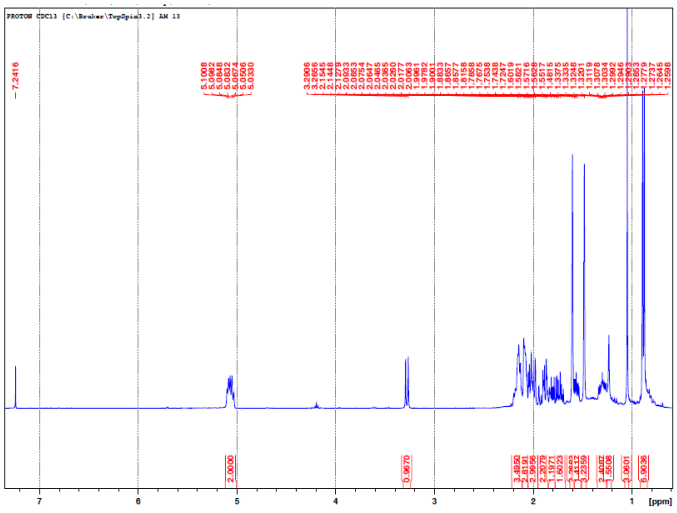

Fig. 1:[1]H-NMR spectra of Icensole from the ethyl acetate fraction of the stem bark of *P. kotschyi.*

DA_23 _1_C1

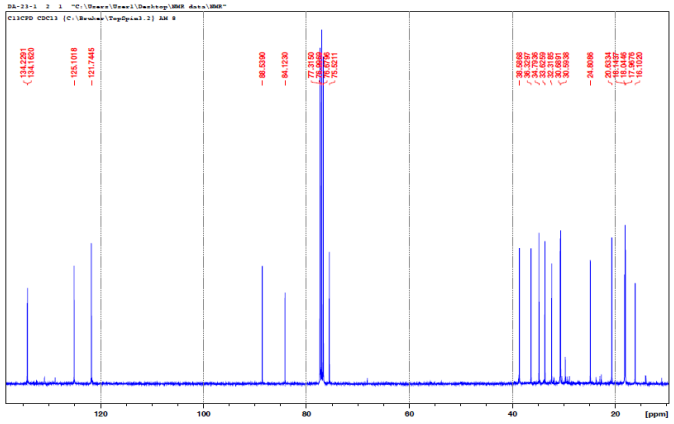

Fig. 2. [13]C-NMR spectra of Icencole from the ethyl acetate fraction of the stem bark of *P. kotschyi.*

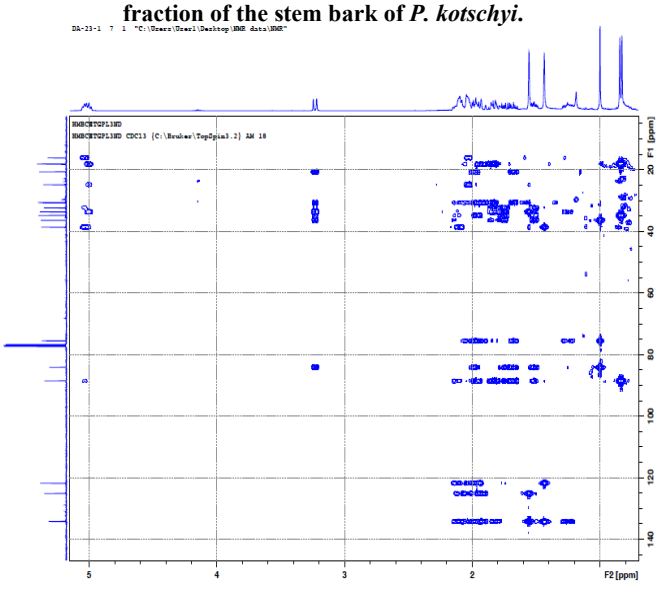

Fig. 3. The HMBC1 spectra of Icencole from the ethyl acetate fraction of the stem bark of *P. kotschyi.*

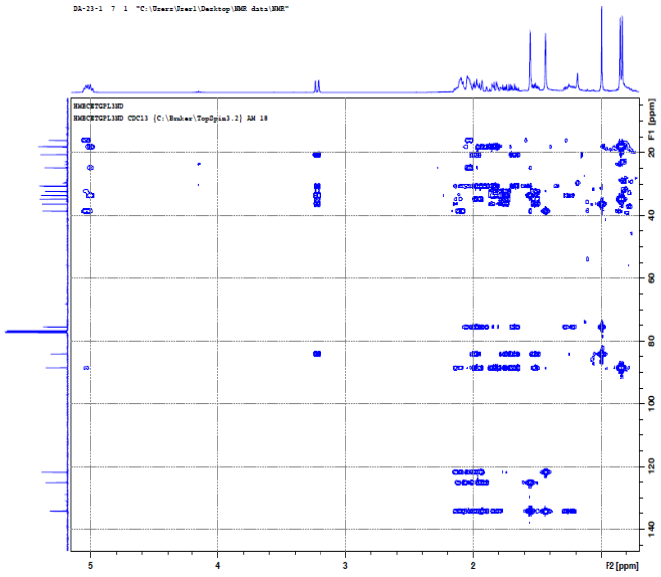

Fig. 4. The HSQC1 spectra of Icencole from the ethyl acetate fraction of the stem bark of *P. kotschyi*.

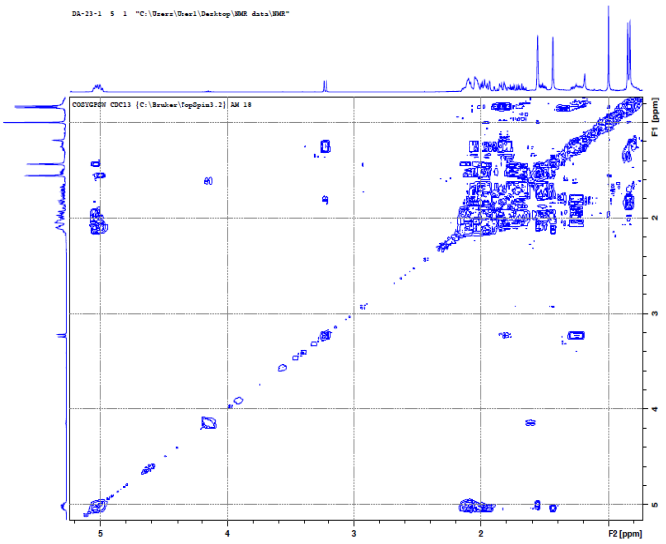

Fig 5. The COSY1 spectra of Icencole from the ethyl acetate fraction of the stem bark of *P. kotschyi*.

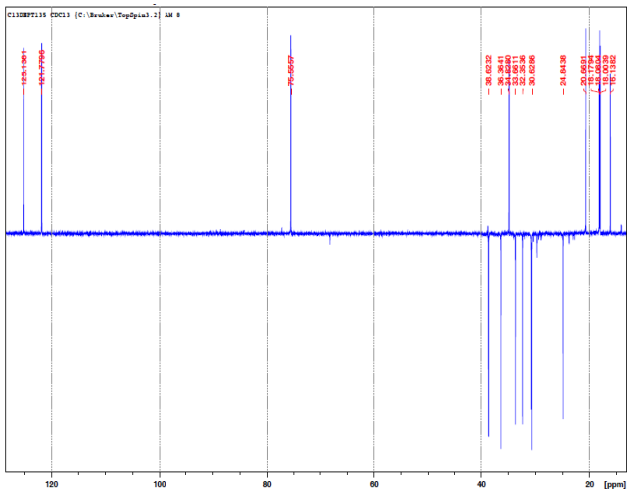

Fig 6. The DEPT1 of Icencole from the ethyl acetate fraction of the stem bark of *P. kotschyi*.

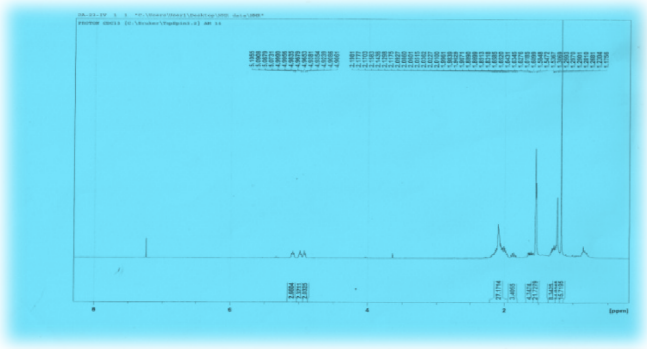

Fig. 7. The ^{1}HNMR spectra of Nephthenolfrom the ethyl acetate fraction of the stem bark of *P. kotschyi*.

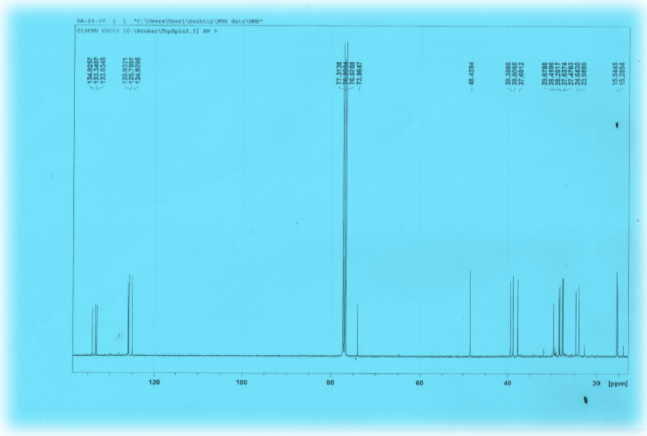

Fig. 8. ^{13}CNMR spectra of Nephthenol from the ethyl acetate fraction of the stem bark of *P. kotschyi*.

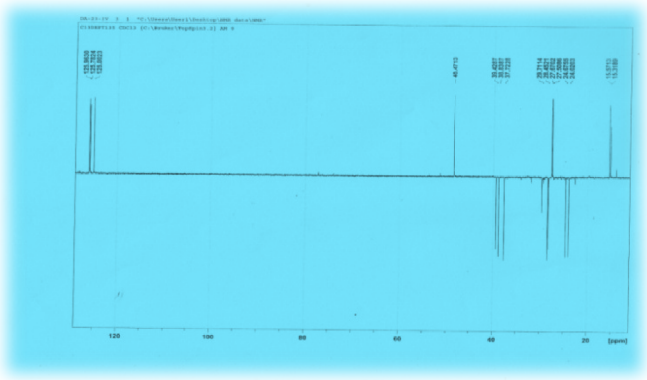

Fig. 9. The DEPT spectra of nephthenol from the ethyl acetate fraction of the stem bark of *P. kotschyi*.

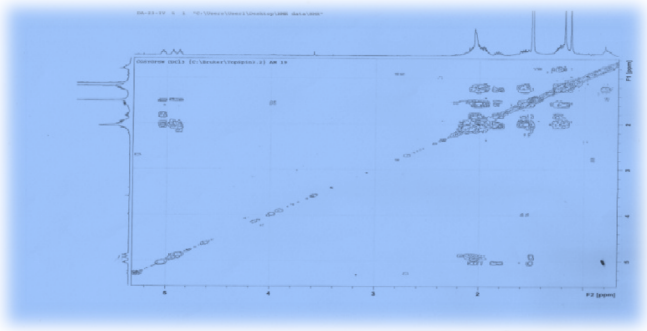

Fig. 10. The COSYGPSW spectra of Nephthenolfrom the ethyl acetate fraction of the stem bark of *P. kotschyi*.

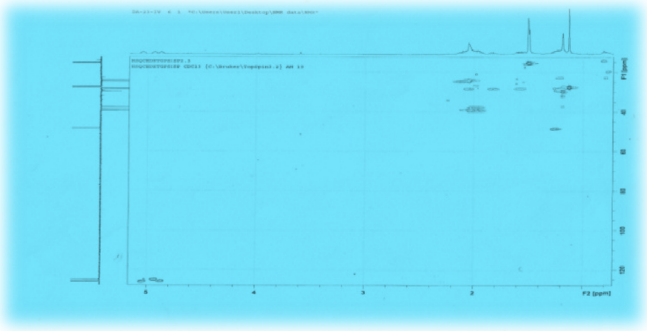

Fig.11. The HSQC spectra of Nephthenol from the ethyl acetate fraction of the stem bark of *P. kotschyi*.

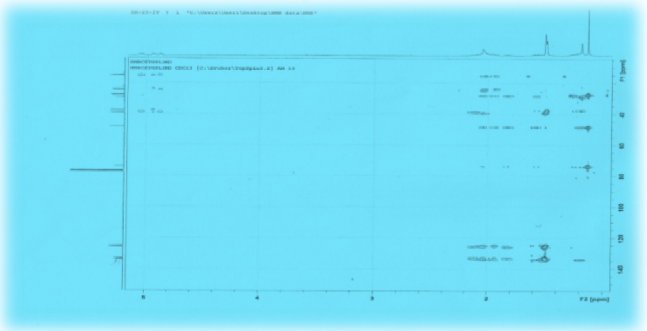

Fig.12. The HMBC spectra of nephthenol from the ethyl acetate fraction of the stem bark of *P. kotschyi*.

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