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Isolation and Characterization of Methoxy, Propoxy Dimethylamine from *Detarium Senegalense* Seed

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Review Article

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Abstract

Detarium senegalense Gmelin. is a leguminous tree widely used in Nigeria for food and medicine. It produces fruits that are nutritious. The seeds have been applied to control blood-glucose levels in diabetic individuals. Report showed that the seeds are used for the treatment of mosquito bites and as an antidote against arrow poison and snake bite. Despite its rich pharmacological potential, the plant has not been scientifically evaluated. Thus the Chemical investigation of the bioactive constituents of the seed *of Detarium senegalense* resulted in the isolation of a new compound, 2^{l}, 2^{l},-dimethyl 1^{1},1^{1} 7, 7- tetrahydroxy- 6 – methoxy –1- propoxy 3,4 –dimethylene 1,2, 5 –triamine. The structure was elucidated using NMR spectroscopy in combination with IR and MS spectral data. Antibacterial studies showed that the isolated compound successfully inhibited *Escherichia coli, Pseudomonas aeruginosa* and *Proteus mirabilis*. This result authenticates the use of the plant in phytomedicine for the treatment of infections and for prevention of diseases.

Keywords: Detarium Senegalense; Amine; Antibacterial; Phytomedicine

Abbreviations: FMC: Federal Medical Centre; MIC: Minimum Inhibitory Concentration

Introduction

Detarium senegalense Gmelin also known as “ofo” in Igbo tribe of Nigeria is a popular plant whose several parts are utilized for a variety of purposes, food, building and for folk medicine. The tree grows up to 38m high with a large very leafy crown. It produces its fruits from November to March. The fruits are 4-6 cm diameter, fibrous, sweet and one seeded [1]. The seed flour is used in food as a stabilizer, thickening and flavouring agent [2]. Recent studies showed that *Detarium senegalense* seed contains a large amount of water soluble, non-starch polysaccharide, zyloglucan. This suggests that it has considerable commercial potential in food, drugs and pharmaceutical industries [3]. The stem bark is macerated for preservation of palm wine in Igbo land, pulp from the bark is eaten as a remedy for tuberculosis. A bark decoction is given to women at childbirth to expel the placenta. It is used for cases of heavy blood loss and for the treatment of anaemia, skin problems, wounds, bronchitis, pneumonia, stomach ache and digestive disorders [4,5]. The liquid from the boiled bark is taken for indigestion. A decoction of the leaves is taken to treat convulsions [2]. The roots are used for treatment against mental conditions and for protection against evil spirits [6].

Herein we report the isolation, characterization and structural elucidation of 2^{l}, 2^{l},-dimethyl 1^{l} ,1^{l} 7, 7-tetrahydroxy- 6 – methoxy –1- propoxy 3,4 –dimethylene 1,2, 5 –triamine.

In addition, we investigated the antibacterial activity of methoxy, propoxy dimethylamine isolated from *D. senegalense* seed.

Material and Methods

Plant Materials

The seeds of *Detarium senegalense* were collected from the forest in Lokpaukwu, Umunneochi Nigeria. Authentication of plant materials was done by Dr. A. Nmeregini of Taxonomy Section, Forestry Department Michael Okpara University of Agriculture Umudike Nigeria. A voucher specimen No. DS/102 has been deposited at the Forestry Department Herbarium of Michael Okpara University of Agriculture Umudike, Nigeria.

Treatment of Plant Material

The seeds were soaked overnight. The loosened testa were then pealed off and dried in air. 3 kg of the seeds were air dried on the laboratory bench at Chemistry Department, Michael Okpara University of Agriculture, Umudike for 10 days. The dry sample was milled and grounded into powder (1.2 kg) using Thomas Wiley machine (model 5 USA). The powdered seed sample (1 kg) was packed into a soxhlet apparatus (2L) and extracted exhaustively with 1000 ml ethanol for 24 hours. The filtrate from the seeds was concentrated with Rotary evaporator at 40°C to a crude oil extract (59.7g). The crude extract was partitioned between $CHCl_3$ and water and a $CHCl_3$ - soluble fraction (18.8g) was obtained. 10.0g of the $CHCl_3$ fraction was then partitioned between petroleum ether (60–80°C) and aqueous methanol The crude extract was partitioned between chloroform and water. A chloroform soluble fraction (20.8 g) was obtained. 15 g of the chloroform fraction were then partitioned between petroleum ether (60-80°C) and aqueous methanol. 4.0 g of the chloroform fraction was subjected to column chromatography over silica gel and eluted gradually with petroleum ether, petroleum ether-chloroform (90:10; 80:20; 70:30) to get a yellow solid 0.52 g, yellow crystal 0.73 g and yellow oil 0.20 g.

The yellow crystal (0.73 g) isolated from the seed was recrystallized from hexane, it afforded compound 1 yellow crystal solid (0.15 g). Thin layer chromatography (chloroform: methanol 7:3) iodine vapour shows the presence of one band Rf (0.81). IR Vmax 3122.01cm^{-1} (OH), 1650cm^{-1} (N-H), 2840cm^{-1} (C-H), 1260cm^{-1} (C-O) and 1211cm^{-1} (C-N). HEREIMS m/z 297 [m^{+}] calculated for $C_{10}H_{21}O_6N_3$ and m/z 149(M^{+}) base peak calculated for $C_5H_{11}O_3N_2$ (m/z 147).

Bioassay Procedures

The in vitro antibacterial activity of compound (1) was carried out for 24 hours culture of five selected bacteria. The bacteria organisms used were *Escheria* coli, *Staphylococcus aureus, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumonia*. All the test organisms were clinical isolates of human pathogens obtained from the Federal Medical Centre (FMC) Umuahia, Nigeria.

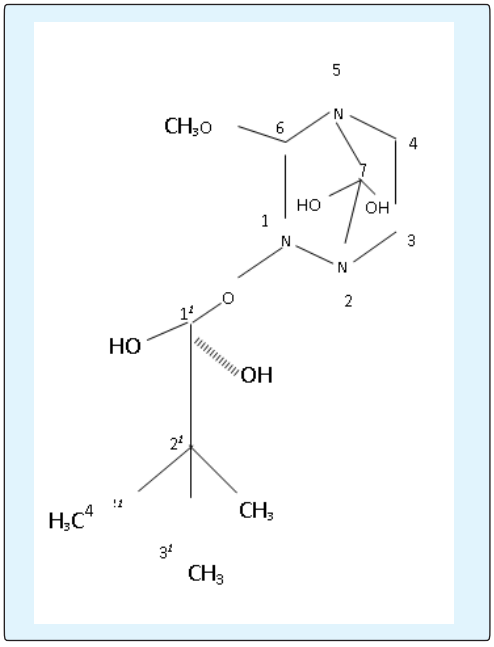
Cultures were brought to laboratory conditions by resuscitating the organism in buffered peptone broth and thereafter agar medium and incubated at 37°C for 24 hours. The antibacterial activity was performed by filter paper disc diffusion technique. The medium (7g nutrient agar in 250 ml distilled water, auto claved at 115°C for 15 mins) was cooled to 50°C. 20ml of the medium was poured into a sterile petri dish and allowed to solidify. It was allowed to stay for 8 hours and observed for contamination. The sterility of the medium was tested. 1.0 g of compound (1) was dissolved in 1ml of absolute ethanol and made up to 10ml with distilled water to give a concentration of l00mg/ml (10% dilution), a colony of each test organism was sub- cultured on nutrient broth and incubated at 37°C for 8 hours. This was then used to flood the agar plates. Sterilized filler paper disc soaked in compound (1) was placed on the plates with test organisms. The plates were incubated at 37°C for 24 hours. After incubation, plates were observed for zones of inhibition (in mm diameter). The minimum inhibitory concentration was determined by comparing the different concentrations of compound (1) having different zones of inhibition and selecting the lowest concentration. The sensitivity susceptibility of the test bacteria to the standard drug was tested using inoculated agar plate and ciprofloxacin. The zones of inhibition were measured and compared with those of compound (1).

Results and Discussion

Compound (1) has R_f value of 0.394 with infrared absorption 3122.01cm^{-1} (OH) hydroxyl, 1650cm^{-1} (N-H), 2840cm^{-1} (C-H), 1260cm^{-1} (C-O) and 1211cm^{-1} (C-N) (Figure 3). HEREIMS m/z 297[M^{+}] calculated for $C_{10}H_{21}O_6N_3$ and m/z 149(M^{+}) base peak calculated for $C_5H_{11}O_3N_2$ (m/z 147). ^{1}HNMR ($CDCl_3$) has chemical shift

absorption peaks at 0.98 (3Hs), 1.40 (9Hs), 1.60 (bs) OH and 2.40 (4Ht).

The result of these spectroscopic examinations IR, ^{1}HNMR, ^{13}CNMR and mass spectroscopy carried out on the isolate afforded compound (16). Compound (16) was identified as 2^{1},2^{1} – dimethyl 1^{1},1^{1},7,7 –tetrahydroxy – 6 – methoxy - 1 – propoxy 3,4 dimethylene 1,2,5 – triamine with the molecular formula $C_{10}H_{21}O_6N_3$ based on HEREIMS m/z 279 with base peak at m/z 149(M^+) calculated for $C_5H_{11}O_3N_2$ (m/z 147).



(I) 2^{1} , 2^{1} - dimethyl 1^{1},1^{1} 7, 7- tetrahydroxy 6 – methoxy –1- propoxy 3,4 – dimethylene 1,2, 5 – triamine

The Infrared (IR) spectrum indicated the presence of hydroxyl and tertiary amine bands (3000cm^{-1} and 1211cm^{-1}) respectively. The relative molecular mass of 279 with base peak at m/z 147 ($C_5H_{11}O_3N_2$) confirmed compound (1) as $2^{1,}2^{1}$-dimethyl 1^{1},1^{1},7,7 tetrahydroxy – 6- methoxy-1-propoxy 3,4-dimethylene- 1,2,5 – triamine. The ^{1}HNMR spectrum showed four signal protons signals appearing at d 0.98 (3Hs), 1.40 (9Hs), 1.60 (OH) and 2.40 (4Ht). The three methyl protons attached to tertiary carbon ($C_2{}^{1}$) appeared as a singlet absorption band at 1.40 ppm. The –CH_2 –CH_2- attached to C_3 and C_4 appeared at lower d 2.40 ppm. The ^{13}CNMR spectrum indicated that two carbons were bonded to the hydroxyl group C-7 (δ C 33.98) and C-1^{1} (δ 32.02). The tertiary butyl carbon C-2^{1} appeared at δ C 29.16. Also ^{13}CNMR showed that three carbons were bonded to a tertiary amine C-3 (δ C22.78), C-4 (δ C- 22.79) and C-6 (δ C 14.20). The results of these spectral data suggested compound (1) as 2^{1}, 2^{1} –dimethyl, 1^{1}, $1,^{1}$ 7,7 – tetrahydroxy –6- methoxy -1- propoxy 3,4 – dimethylene 1, 2,5 –triamine.

This paper reported the isolation and characterization of an amine from the seed of *D. senegalense*. Amines are largely used in pharmaceutical industry. Some of them are used as analgesics, anesthetic, decongestant, antidepressants etc. Several simple amine compounds exhibit antiplasmodial and antibacterial activities. Not only are the molecules potent central nervous system stimulants but also increase cardiovascular activity and raise body temperature and cause loss of appetite. Amines are used to create amino acids, the building blocks of proteins in living beings thus they play an important role in the survival of life, many vitamins are also built from amino acids. An amine, Serotonin is an important amine that functions as one of the primary neurotransmitters for the brain. It controls the feelings of hunger and is critical for the speed with which the brain operates in general. It also affects the state of happiness and helps in regulating the sleeping and waking-up cycle of the brain [7].

The antibacterial activity of the compound isolated from the bark showed potent inhibition on some micro-organisms. The seed extracts inhibited *P. mirabilis, P. aeruginosa and E.coli* but did not show any activity against *K. pneumonia*, and *S. aureus*. The inability of the extracts of the seed to inhibit the growth of *K. pneumonia* and *S. aureus* indicates that the extracts of the seed cannot be used in the treatment of diseases caused by these organisms. The minimum inhibitory concentration (MIC) of the compound was 12.5 – 25 mg/ml (Table 3). *P. mirabilis* and *E. coli* are the common cause of urinary track infection and traveler's diarrhoea, This finding showed that the seed of *D. senegalense* can be used in the treatment of diarrhea and urogenital infections in herbal medicine [8-11]. And thus authenticates the use of the plant in phytomedicine for the treatment of infections and prevention of diseases.

IR Absorption(CM^{-1})	Bond	Compound Type
3122.01	OH	Alcohol
2917.00	C-H	Alkyl
1650.00	N – H	Amine
2840.00	C – H	Alkyl
1260	C – O	Ether
1211	C - N	Amide

Table 1: Infra-Red Analysis of Compound (1) from *Detarium Senegalense* Seed.

	δC		δH		
Position	**Chemical Shift(δ)**	**Carbon**	**Chemical Shift(δ)**	**Multiplicity**	**Proton**
1					
2					
3	22.78	CH_2CH_2	2.40	4Ht	CH_2CH_2
4	22.79	CH_2CH_2	2.40	4Ht	CH_2CH_2
5					
6	14.20	OCH_3 – N	0.98	3Hs	OCH_3
7	33.98	OH-C-OH	1.60	Bs	OH
1^1	32.02	OH-C-OH	1.80	Bs	OH
2^1	29.16	$(CH_3)_3C$ -	1.40	9Hs	$(CH_3)_3C$
3^1	29.33	CH_3			
4^{11}	29.46	CH_3			
5^{11}	29.53	CH_3			

Table 2: ^{1}h and ^{13}c Nmr Data Of 2^1,2^1 Dimethyl 1^1, 1^1,7,7 – Tetrahydroxy 6- Methoxy – 1 – Propoxy 3, 4 –Dimethylene 1,2,5 – Triamine.

Organism	**Ciprofloxacin**	***D.senegalense Seed***
		$CHCl_3$ extract
Proteus mirabillis	19.0 ± 0.05	6.00 ± 0.01
Klebsiella pneumonia	16.0 ± 0.02	-
Staphylococus aureus	11.0 ± 0.10	-
Pseudomonas aeruginosa	26.0 ± 0.01	4.00 ± 0.10
Echerichia coli	11.0 ± 0.20	2.00 ± 0.02

Table 3: Diameter of zones of inhibition (mm) of Compound (1) isolated from *Detarium senegalese* seed and ciprofloxacin.

Pathogens	**Concentration of seed isolate (100 mg/ml)**					**MIC mg/ml**
	100	**50**	**25**	**12.5**	**6.25**	
	Zone of inhibition (mm)					
Escherichia coli	6.00	3.00	2.00	-	-	25
Pseudomonas aeruginosa	9.00	6.00	2.00	1.00	-	12.5
Staphylococcus aureus	-	-	-	-	-	-
Kebsiella pneumonia	-	-	-	-	-	-
Proteus mirabilis	6.00	7.00	3.00	2.00	-	12.5

Data are means of triplicate determinations - No zone of inhibition.

Table 4: Minimum inhibitory concentration of Compound (1) isolated from seed of *Detarium senegalese* on the pathogens mg/ml.

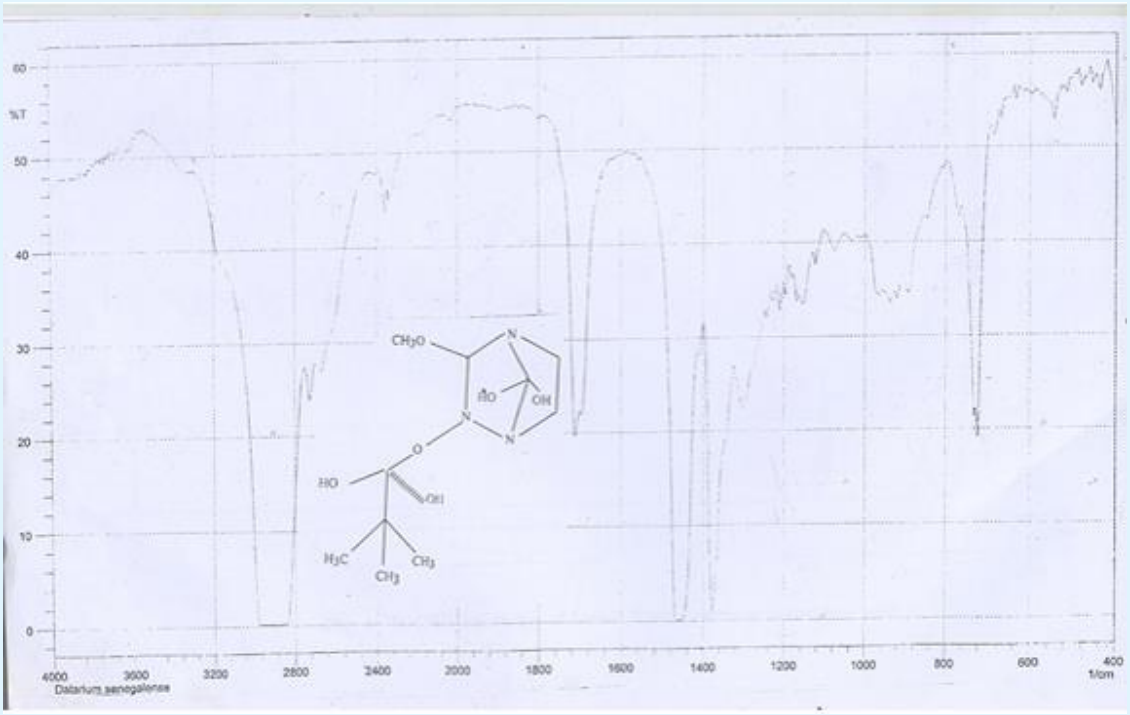


Figure 1: Infrared Spectrum of Compound (1).

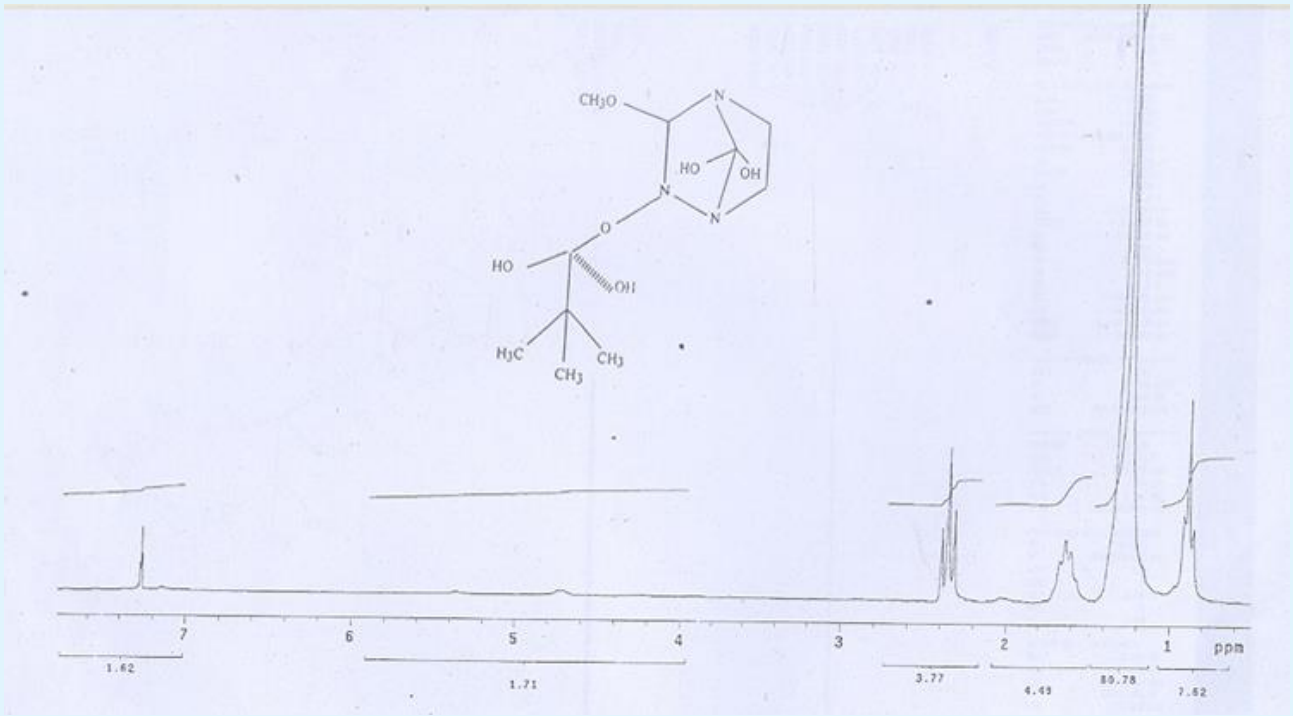


Figure 2: HNMR Spectrum of Compound (1).

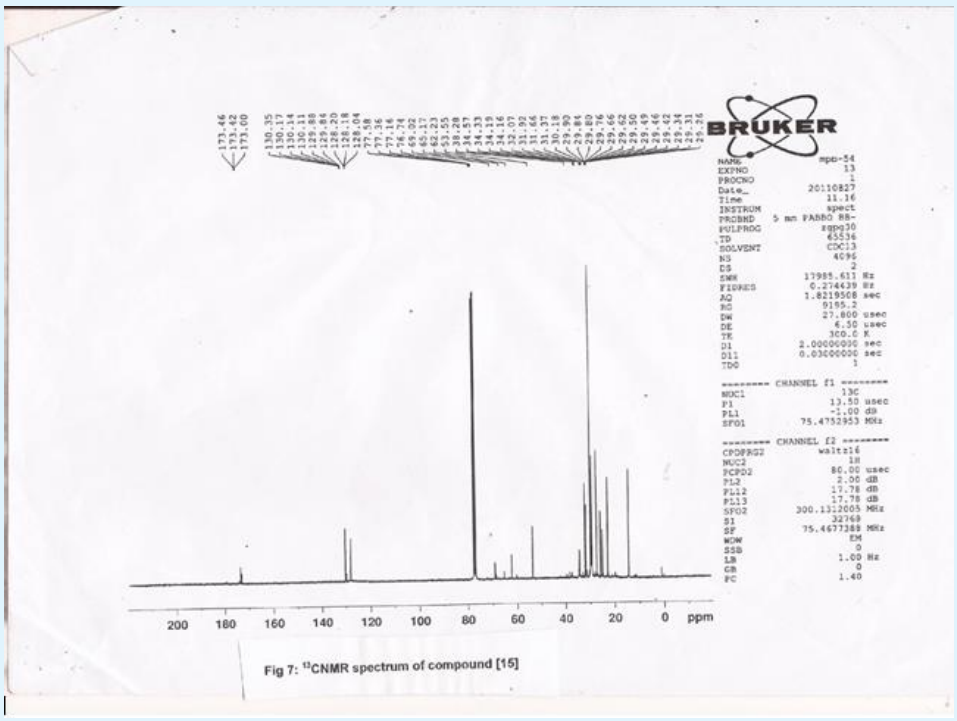


Figure 3: ^{13}CNMR Spectrum of Compound (1).

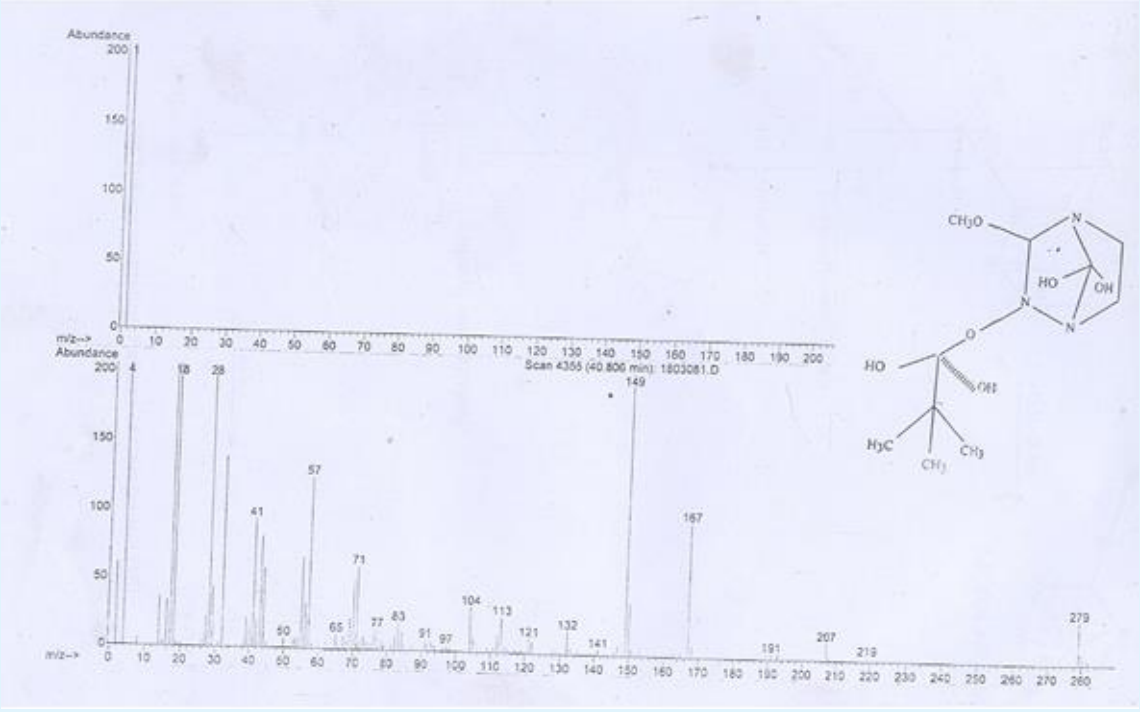


Figure 4: Mass Spectrum of Compound (1).

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