CHEMICAL INVESTIGATION OF A RED SEA SPONGE OF THE GENUS DYSIDEA

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BY

Tesfamariam Yosief

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CONTENTS

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L I S T O F T A B L E S

L I S T O F F I G U R E S

ABSTRACT

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A RED SEA SPONGE
OF THE GENUS DYSIDEA

by

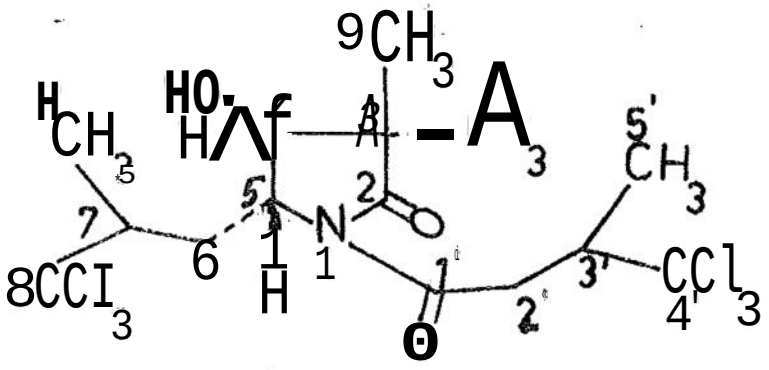
Tesfaraariam Yosief

Advisor: Dr. Tarekegn Gebreyesus

A new hexachlorinated metabolite, for which the name dysidimide is proposed, has been isolated from a Red Sea sponge of the genus Dysidea.

Dysidimide was the major metabolite obtained from the ethanol extract of the dried sponge collected near Massawa, Repeated column chromatography on silica gel with pet.ether: chloroform and recryatallization yielded dysidimide as white needle crystals.

The structure of dysidimide was determined by chemical and spectroscopic studies including IR, MS, and ID and 2D NMR. The relative stereochemistry at C.4 and C.5 was deduced from nOe measurements on the 4.Acetate.



INTRODUCTION

The isolation and characterization of natural products from marine organisms has been a major area of endeavour in the past twenty years. Many structurally and pharmacologically interesting natural products have been isolated and characterized.[1]

The Red Sea being in the Tropics is rich in marine plants and animals both vertebrates and invertebrates. It is peculiar in that its salinity is high being second only to the Dead Sea, It has a pH-range of 7.5 - 8.5, The Red Sea is rich in coral beds that are convienient for sponge growth. The marine sponges are considered to be primitive organisms with relatively simple internal organization. Sponges particularly those without spicules frequently produce large quantities of secondary metabolites that are thought to deter potential predators and to inhibit the growth of fouling organisms.[2,3]

Systematic chemical studies on sponges started in the years after 1968, but not much has been done on the Red Sea marine organisms. It is only in Egypt and Israel that work is being done on the northern area of the sea. Professor Kashman's group in Tel Aviv University has been working on the natural products of soft corals, sponges and tunicates of the northern tip of the Red Sea and has published interesting compounds which might have future economic values.[4-18]

The reasons why people were prompted in the study of natural products of the Red Sea marine organisms are mainly three. First, the under-water observation that soft corals and sponges appear to be remarkably free of predators which led to the hypothesis that chemical substances toxic to fish might be responsible for the protection of the animals. Second, the abundance and variety of soft-corals in the Red Sea and the fascinating spectrum of new molecular structures that appeared from studies of soft corals elsewhere stimulated the study of soft-corals. Thirdly, the relatively large number of uninvestigated sponges in the Red Sea increased the interest in the study of sponge metabolites.[19]

The taxonomic classification of marine organisms is much more difficult than those of terestrial plants. Natural products study of marine organisms has contributed a great deal in the systematization of the taxonomy of these organisms. Chemotaxonomy has been applied to the classification of soft-corals of the northern tip of the *Red* Sea.[1,20]

Sponges in general have been the source of diverse secondary metabolites such as pyrroles, indoles, sesquiterpenes, diterpenes and their derivatives.[21,22] Many sponge metabolites have been found to be biologically active.[1]

In the course of this research work, the chemical

investigation of a Red Sea sponge of the genus Dysidea has been carried out and has yielded several fractions of which the major constituent is a hexachlorinoted compound. This has been characterized based on physical, spectroscopic and chemical data.

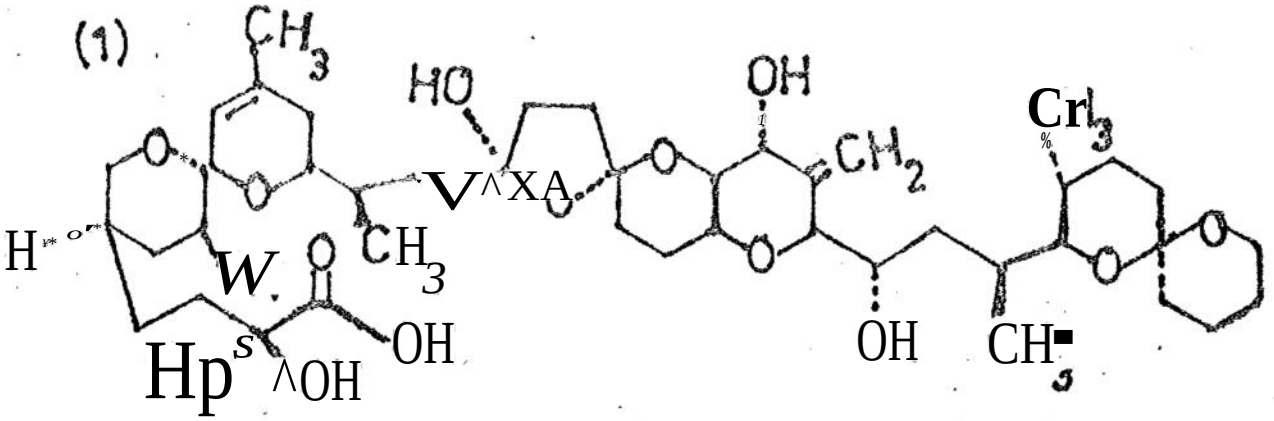
2. MARINE NATURAL PRODUCTS

2,1 Agrochemicals, Pharmaceuticals and Marine Toxins from Marine Organisms

Several marine natural products that have been isolated from algae and marine animals havo found application as agrochemicals) pharmaceuticals and as compounds of ecological significance,[1,2,3] Experimental evidence from studies in animal behaviour and natural products chemistry leads to the conclusion that chemical communication is the paramount mode of communication in most groups of animals in the marine ecosystem. Consequently, chemical communication must be regarded os a very general biological phenomenon.[23]

Okadaic acid (1) a toxic marine metabolite was isolated from the sponge Halichondria okaclai. It has shown antifungal activity and is under field test to be used as a fungicide.

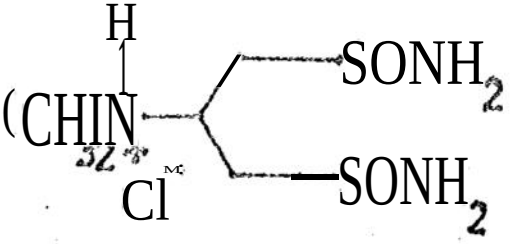
(1)



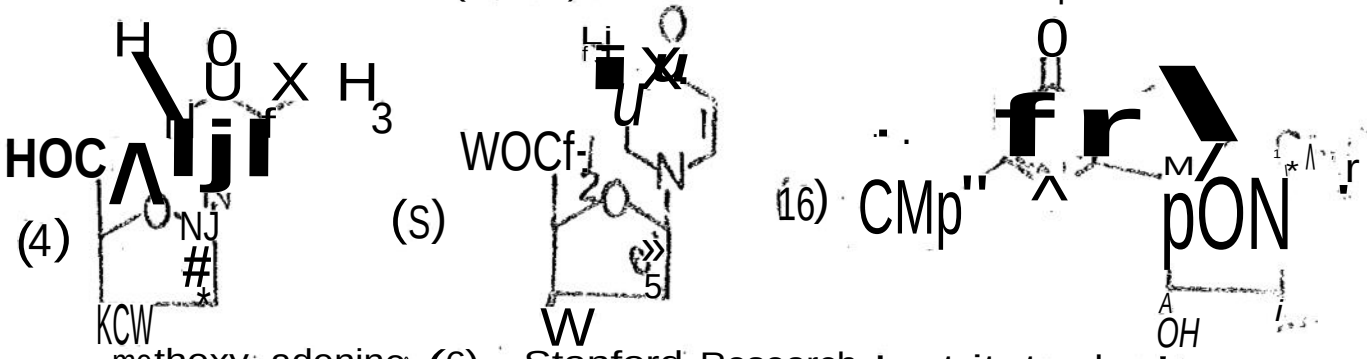
Anglers: In Oepan had long observed that the marine annelid Lumbriconereis: heteropedaV contained a contact poison for flies and ants, Scientific: interest was aroused in 1922,* when a certain person who had handled th@ worms complained about headaches, vomiting and respiratory difficulties. This led, in 1934,* to the iselation of Nereistoxin (2) p the fcexic principle. Its molecular structure was cerrectly deduced in 1060 and its laboratory synthesis followed in 1965V Nereistoxin is sensitive t© heat: and oxygen, thus a stable derivative: cartap hydro- chlorj.de: (3) was prepared. This derivative appeared an the market under the trade name "padan" VyVfl 19G7 and is effective: against larvae ©f the rice: plant: b\er, the rice plant skipper and the: citrus leaf miner.[1*25] There ana a numbear of other agjricultural insecticides whose origin is from marine organisms;

(2)

(3)

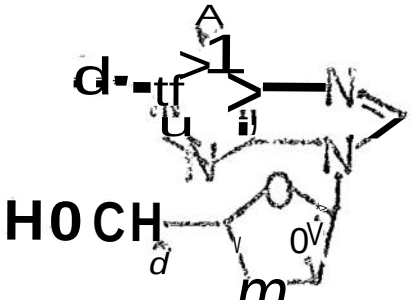


A number of marine natural products are used in the pharmaceutical industry. For instance, chemical investigation of the sponge Cryptotethya crypta yielded three nucleosides[26,27] (4,5,6). Based on the natural product



(4) (5) (6)

methoxy adenine (6), Stanford Research Institute chemists synthesized 9-(5-deoxy-arabinofuranosyl)acicmine (7) which is an antitumor and an antiviral drug [chemical name illegible] under the name vidarabine in 1976.



(7)

Mollusks, [illegible] sponges, soft corals, seaweeds and other marine organisms are known for their toxic secondary metabolites.[21] Even though it is not well known why these organisms produce those toxic metabolites, they seem to have a defensive function. The secondary metabolites may act as antifeedant, repellent or toxic to predators in the marine environment. This gives a clue to the understanding of ecological distribution of organisms.[18,23,25]

2.2 Secondary Metabolites from The Genus Dysidea

The genus Dysidea has been the subject of chemical investigation for the last eighteen years.

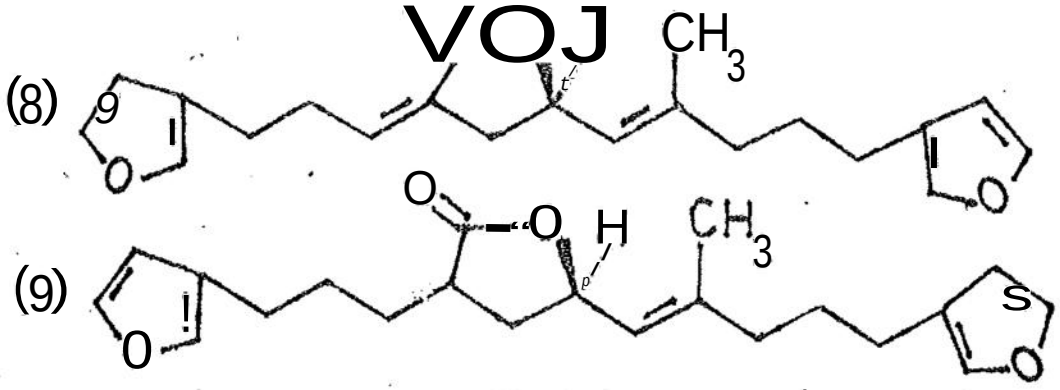
As a result of these chemical investigations many compounds have been isolated, characterized and tested for their biological activities.[28,29] Some of the compounds isolated have shown antiviral, antibacterial, antifungal, antifeedant, insect repellent and plant growth regulation activities.[30,31,32] The studies sofar carried out hove included three unidentified Dysidea sp,,[31,32,33] D, amblia,[34] P. aronaria[35], D, avara,[30136,37] D, etheria[38,39,40] D, fragilis f D, herbacea[44-57] snd D, tupha[58] Extensive studies have been carried out on Q, avara, D, etheria and D, herbacea.

Terpenes are among the most widely spread groups of natural products. They are mainly of fungal and plant origin, but they have also been isolated from insects and marine organisms. They have been reported todate from algae, coelentrates, molluscs and sponges. Sponges have provided terpenes in large amounts, most of them possessing unique structural features without parallel in terrostrial sources.[59]

The number and variety of marine terpenoids that have been isolate from sponges are large, but

th6rH has been no report on the biosynthetic pathways leading to terpenes. It is not yet known whether biosynthetic mechanism in marine organisms differ from those known in terrestrial organisms or whether microsymbionts participate.[1] Sponges have yielded an astonishing wealth of secondary metabolites many ef which exhibit in vitr© activity against o number of terrestrial organisms.[59]

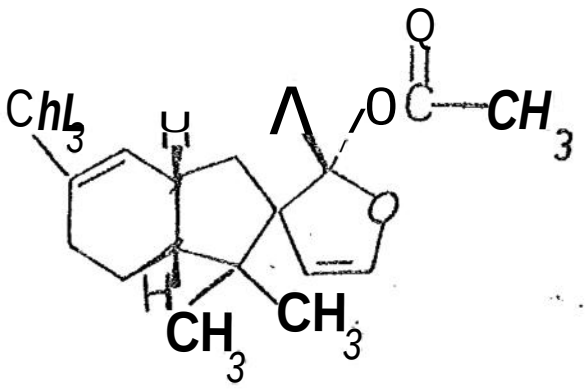
Furan rings occur frequently although hitherto most @»fT the known furanoterpenes were plant piroducta, But sponges have: yielded the linear furan©terpenes containing C~21 atoms which are: the most intriguing compounds from the biogenetic point of view# Spongia nitons for example, has yielded nitenin (8) and dihydronitanin (9) fo



Sesquiterpenes are relatively abundant in sponges in contrast to diterpenes and men©terpenes, Furaneid and non furanoid sesquiterpenes are very common in the genus Dysidaa, Except for D, amblia that: has yielded furane diterpenes, almost all the species of the genus investigated up» to the present contain furano sesquiterpenes in addition to other secondary metabolites. For instance, D, herbacea contains spirdysin (30), a sesquiterpene with

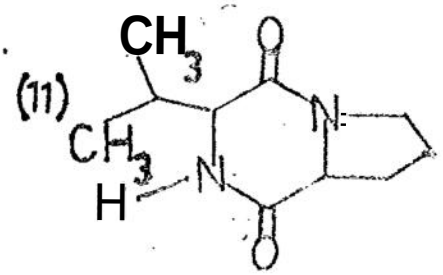
in1k resting structural features„ Many of the sesquitrer- pares isolated frora the genus Dysidea are indicated in Table XV

(10)

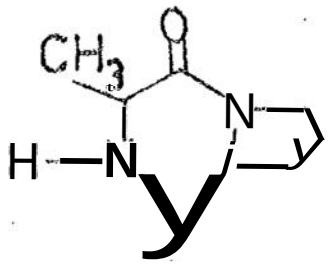


Many &f the nitrogenous metabolites of sponges are thought to be products of amino acid biosynthesis^ This seems to be the case with cyclic peptides such as (11) and (12) isolailod from Tedania ignis.[61]

(11)



02)



DV* horbac&fThas also yielded & chlorinated meiabolitay diketopd/\oerazin (IS), derived from tirichlor© leucin*

03)

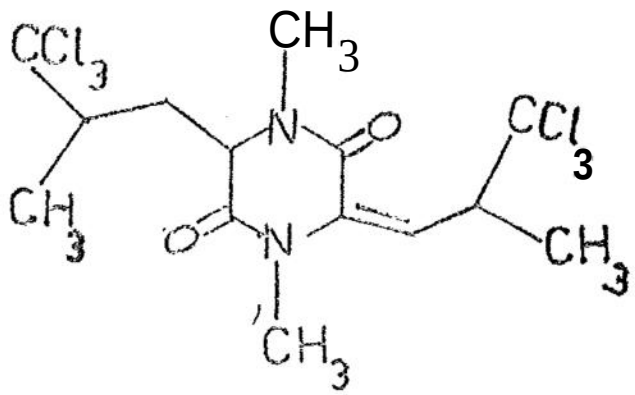


TABLE 1: DISTRIBUTION OF SOME SESEQUITERPENES AND THEIR DERIVATIVES IN THE MANGIFERA

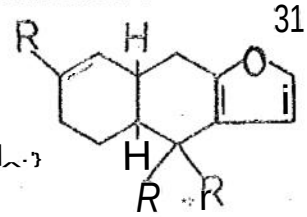
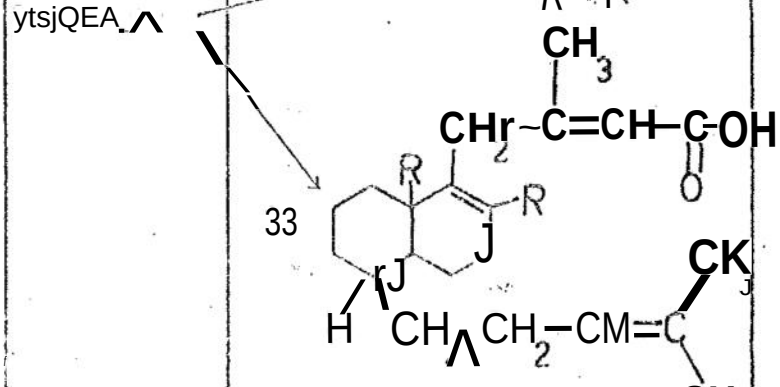
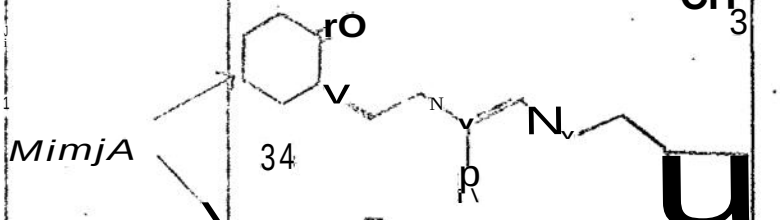
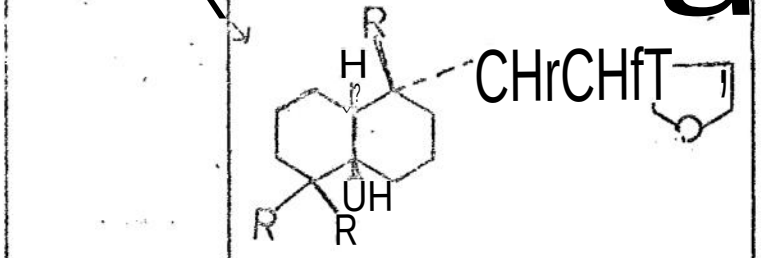
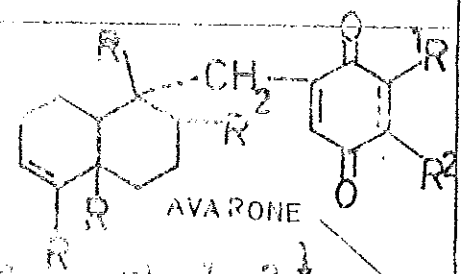
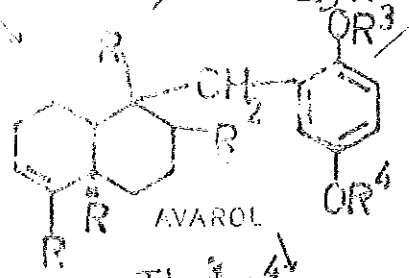
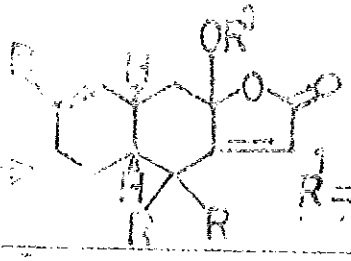
Name of Species	Structure of Metabolite	Biological Activity Reported
Mangifera indica	31  FLORODYSIM  33	PLANT GROWTH REGULATOR
Mangifera indica	 34 	

TABLE I (CONTINUED)

Name of Species	Structure of Metabolite	Biological Activity Reported
D. Ambigua 30	 AVARONE I) $R^1, R^2 = H$ II) $R^1 = H, R^2 = NHEt$ III) $R^1 = NHEt, R^2 = H$	BACTERICIDES VIRUCIDES FUNGICIDES
	 AVAROL I) $R^3, R^4 = H$ II) $R^3, R^4 = Ac$	
D. [illegible] 38	 $R = H, CH_3$	PLANT GROWTH REGULATORS

R = CH_3

TABLE 1 : ...CONTINUED

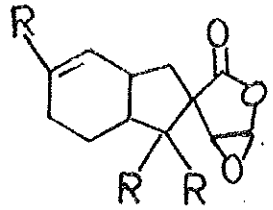
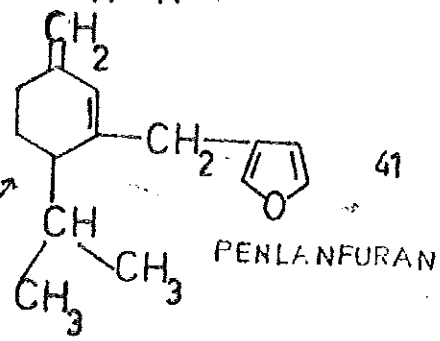
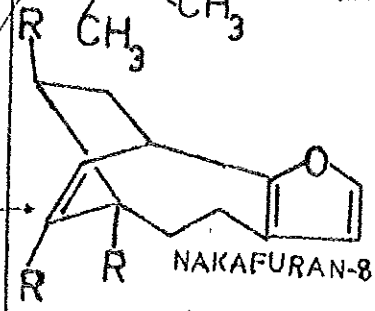
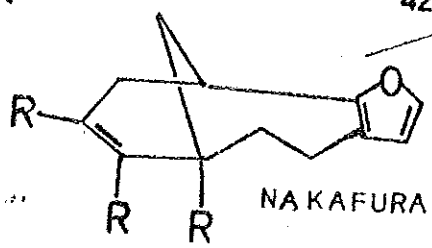
Name of Species	Structure of Metabolite.	Biological Activity Reported
D. ETHERIA	 39	
	 41 PENLANFURAN	
D. FRAGILIS	 NAKAFURAN-8 42	ANTIFEEDANT
	 NAKAFURAN-3	
	$R = CH_3$	

TABLE 1 : ...CONTINUED

Name of Species	Structure of Metabolite	Biological Activity Reported
D. FRAGILIS	43	ANTIFEEDANT
D. HERBACEA	HERBASOLIDE; 46	

$R = CH_3$

TABLE 1 : ...CONTINUED

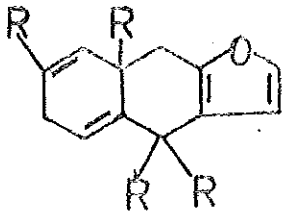
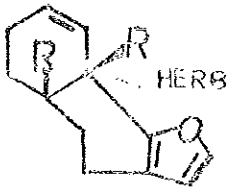
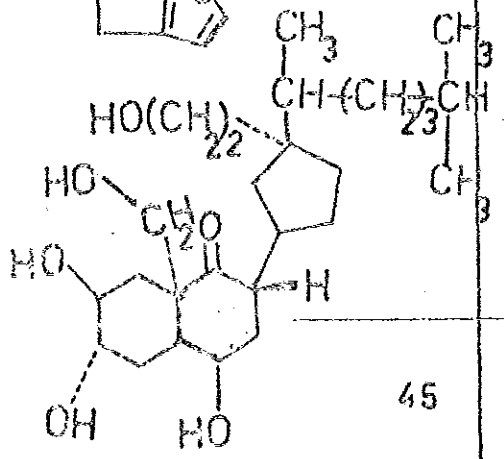
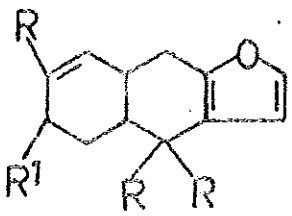
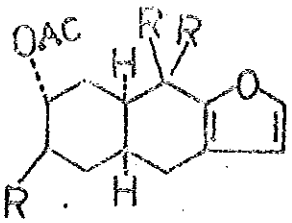
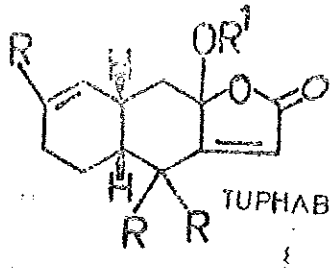
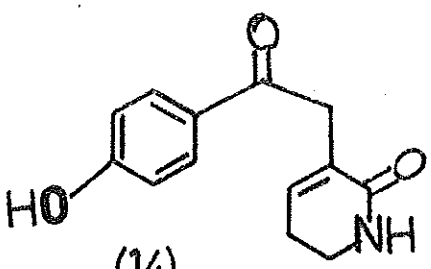
Name of Species	Structure of Metabolite	Biological Activity Reported
D. HERBACEA	  HERBACIN 44  45 $R=CH_3$	TOXIC TO GOLD FISH

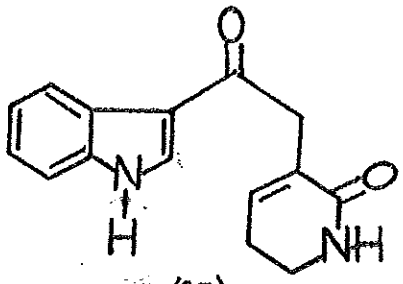
TABLE 1 : ...CONTINUED

Name of Species	Structure of Metabolite	Biological Activity Reported
D. HERBACEA	 R^1 = H or, β, OH	
	 48 49 $R=CH_3$	
	 TUPHABUTENOLIDES $R=CH_3$	

There are many aromatic compounds isolated from marine sponges. Some of these aromatic metabolities are thought to be tyrosin and trypotophan derivatives such as (14) and (15) which have been isolated from Halichondria melanodocia.[62]



(14)



(15)

The genus Dysidea has also yielded aromatic compounds such as polybrominated diphenyl ethers. Some of the polybrominated diphenyl ethers from Dysidea are indicated in Table 2. Moreover, the genus Dysidea has also yielded indoles and polychlorinated metabolites (See Table 2). All polybrominated diphenyl ethers from Dysidea are biologically active as antimicrobal agents.

D. herbacea is extensively studied mainly due to its wide tropical distribution. It is rich in secondary metabolities and has yielded more than 25 novel natural products. Since some tropical sponges, in particular D. herbacea, have large quantities of photosynthetic micro-organisms and are the source of several different classes of metabolites, it has been suggested that some of the metabolites isolated from sponges may be produced by the epiphytes. Various sample of D. herbacea from different locations have yielded polybrominated diphenyl

TABLE 2 : Polyhalogenated metabolites from D. herbacea

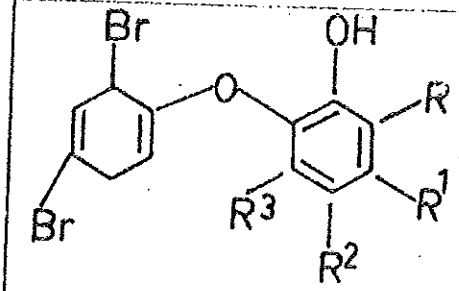
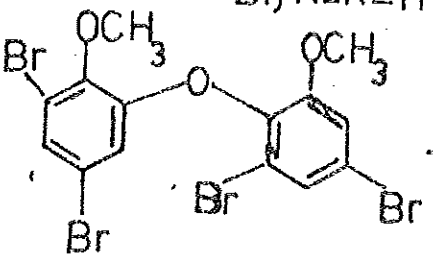
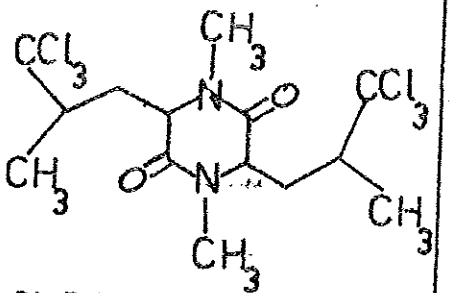
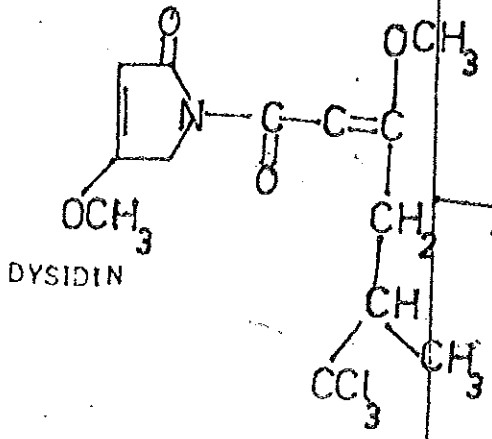
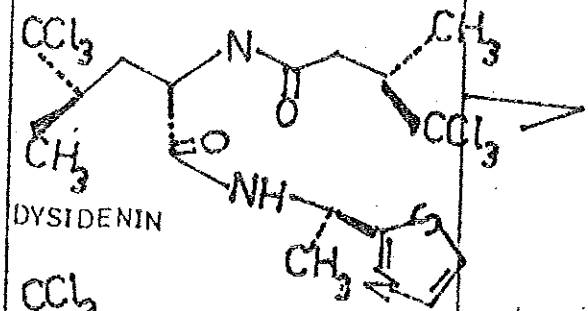
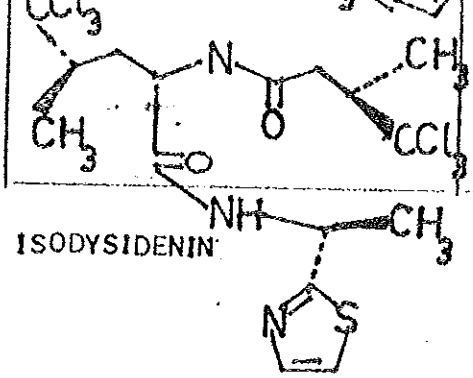
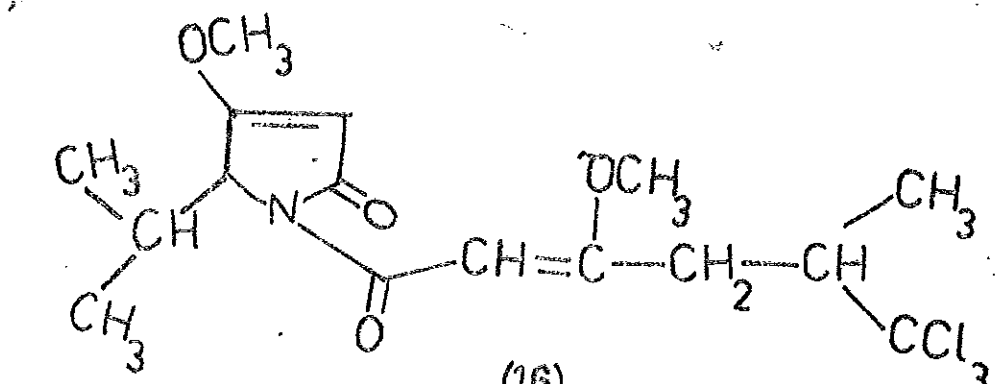
Structure of Metabolite	Biological Activity
	ANTIBACTERIAL
I) $R=R^2=Br$, $R^3=H$ II) $R=R^2=H$, $R^1=R^3=Br$ III) $R=R^2=Br$, $R^1=R^3=H$	
	ANTIBACTERIAL
 DIKETOPIPERAZIN	

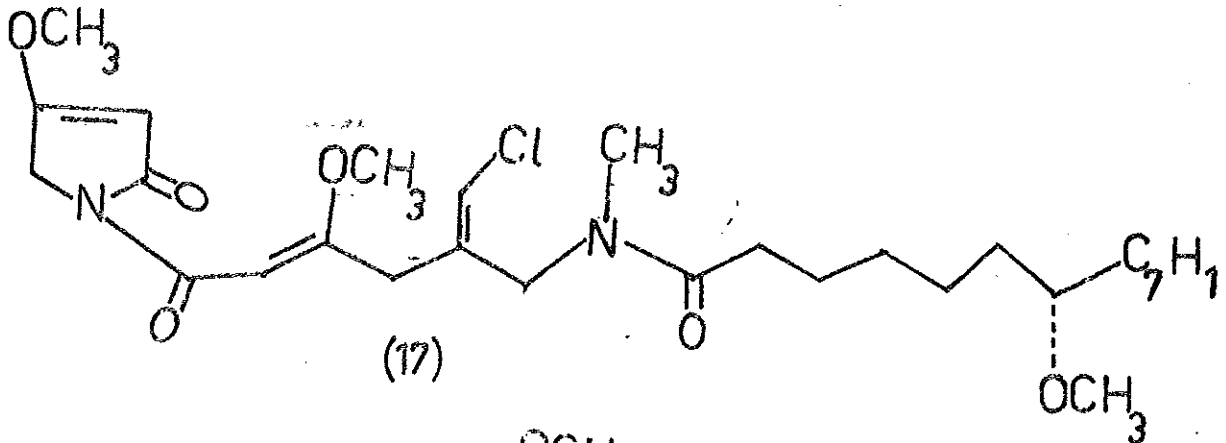
TABLE 2 : Continued

Structure of Metabolite	Biological Activity
 DYSIDIN	SLIGHLY TOXIC TO PREDATORS
 DYSIDENIN	TOXIC TO PREDATORS
 ISODYSIDENIN	

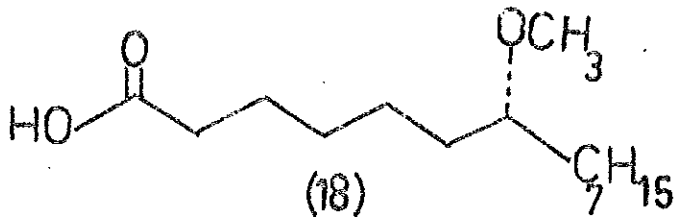
ethers and polychlorinated metabolites derived from amino acids. The chlorinated metabolites from D. herbacea show remarkable resemblance to metabolities of blue green algae. This is particularly true of dysidin (16) which is a molecule that hears a striking resemblance to the tetramic portion of Malyngamide (A) (17) from Lyngbye majuscula.[64] Malyngamide (A) is an imide of 7-methoxy-tetra dec-4-enoic acid (18). Both (17) and (18) have been found together in L. majuscula.[65]



(16)



(17)



(18)

3. MATERIALS AND METHODS

3.1 Apparatus

IR spectrum (KBr) was recorded on a PYE-UNICAM Pu 9512 spectrophotometers. UV spectrum was recorded on a Philipa PU 8600 spectrophotometer in methanol solution. Optical rotation was determined on a Perkin Elmer - 24 polarimeter. Melting point was determined on a Thomas Hoover capillary melting apparatus and is uncorrected. Mass Spectra were taken with Dupont 21-491B instrument. ^{13}C-NMR spectra were measured on a Jeol FX 90Q at (22.5 MH_z) and Bruker WH-360 at (90.0 MH_z) in $CDCl_3$. ^1H NMR spectra were recorded on a Bruker WH-360 MH_z and Bruker WH-400 MH_z instruments in acetone d_6 and $CDCl_3$ respectively. Column chromatography was performed on silica gel 60. All solvents used were either freshly distilled or analytical reagent grade.

3.2 Collection, Extraction and Fractionation of A Dysidea sp.

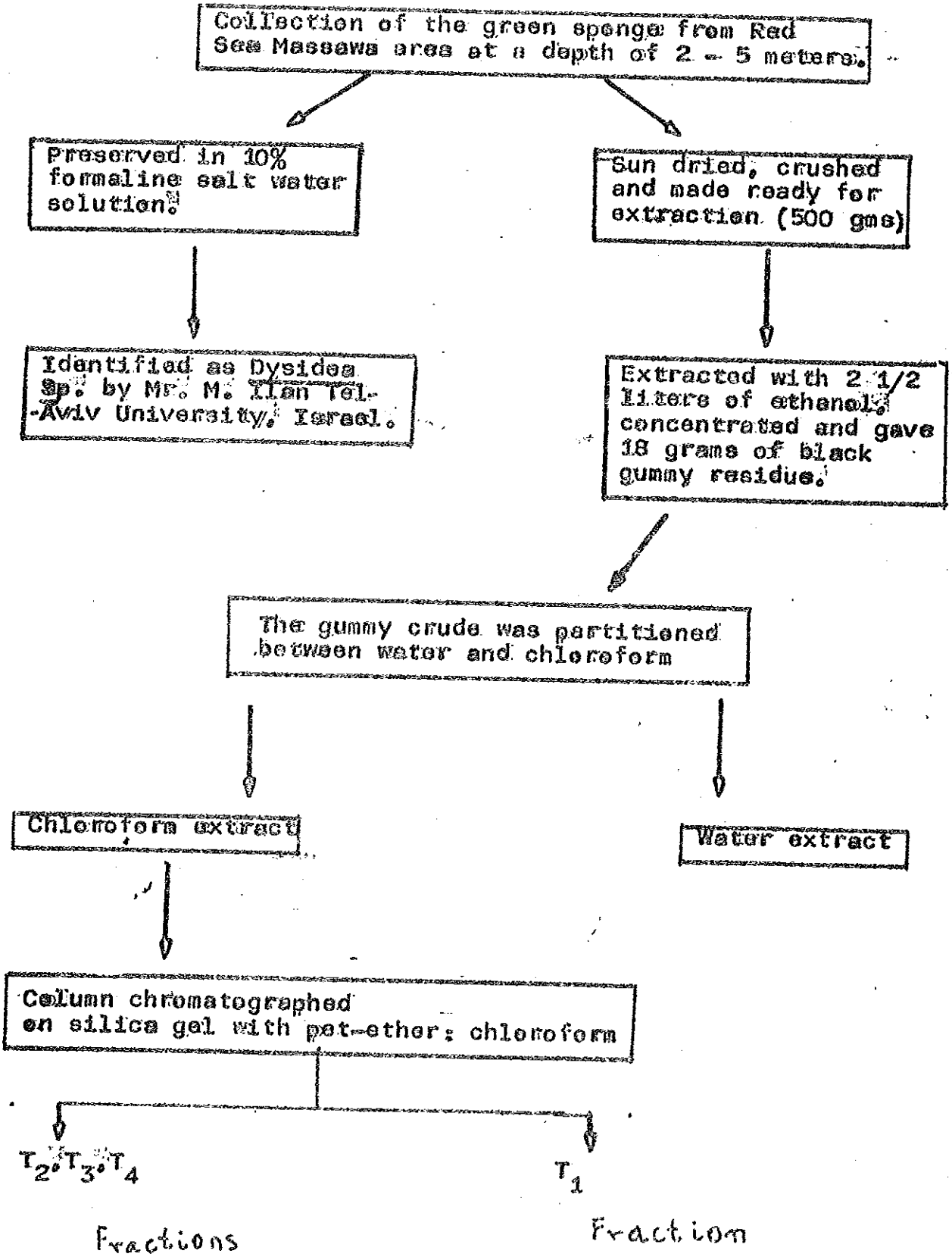
The grassy shaped, green sponge was collected from the Red Sea around the marine Biology Station of Asmara University (AU) in Massawa at a depth of 2 - 5 meters with the help of a specimen preserved in the Department of Biology, AU. A specimen was preserved in 10% formalin salt water solution and identified as Dysidea sp. by Mr. M. Ilan in Tel Aviv

University, Israel. The sponge was dried on open air and crushed with mortar and pestle. 500 grams of the dried and crushed sponge was extracted with 2.5 liters of ethanol (cold extraction). The extraction and fractionation is shown in Flow-chart 1 on page 21. This gave 10 grams of a black gummy residue. The gummy crude was partitioned between water and chloroform (See Flow-chart 1).[66] The chloroform extract was concentrated, column chromatographed on silica gel and eluted with varying concentration of pet-ether: chloroform. 100 ml volumes were collected and fractions 21 - 28 yielded T_1. The pet-ether: chloroform 1:1 fractions yielded T_1 which is a white crystalline solid. It was purified using repeated column chromatography on silica gel with pet-ether: chloroform 1:1 as the solvent system. T_1 recrystallized from pet-ether as white needles m.p 123 - 124^{o}C and giving a yield of 300 mg.

3.3 Test for Halogens, Nitrogen and Sulphur

100 mg of T_1 was treated with 130 mg of a piece of freshly cut sodium metal in a small test tube. The bottom of the test tube was heated gradually to a dull red and allowed to cool for some time. It was heated again and while still hot dropped into a small porcelain dish containing 15 ml of distilled water with care. The tube was broken up with a stirring rod. The solution was diluted to 30 ml,

Table 3. Flow chart for collection, extraction and fractionation of the sponge sample



heated to boiling and filtered. The filtrate was tested for halogens sulphur and nitrogen. For halongens, 5 ml of the original stock solution was acidified with 3 ml of 2N nitric acid and treated with 4 ml of 0.5 N silver nitrate solution. A white milky precipitate appeared confirming Beilstein's test result. For sulphur, 5 ml of stock solution was acidified with 5 ml of 2N acetic acid solution and treated with a few drops of 1N lead acetate solution showing a negative test for sulphur. For nitrogen, 5 ml of the stock solution was treated with a few drops of saturated ferrous amonium sulphate solution and a few drops of 30% potassium fluoride solution, boiled for one minute and acidified with 30% sulphuric acid solution until the ferric hydroxide completely dissolved. A prussian blue green solution appeared.[67]

3.4 Acetylation of T_1

85 mg of T_1 was acetylated using 30 ml of acetic anhydride and two drops of pyridine with continuous stirring for 24 hrs at room temperature. The progress of acetylation was followed using TLC. 40 ml of water was added and the mixture extracted with 50 ml chloroform twice. The combined chloroform extract was washed twice with 40 mls of water and dried with sodium sulphate. Finally, 5 ml benzene was added to the dried chloroform extract and the solvent evaporated at a reduced pressure. An oily

residue remained as the acetate which was purified by recrystallizing from n-hexane and m.p 118°C.

Hydrolysis of T_1

100 mg of T_1 was hydrolyzed with 150 mg of sodium methoxide in 50 ml of dried methanol solution, at room temperature for 24 hrs with continuous stirring. 60 ml of 2N sodium hydroxide solution was added to the reaction mixture and extracted with three 40 ml fractions of chloroform successively. The combined chloroform extract was washed with 50 ml of 2N sodium hydroxide solution. It was further washed with 40 ml of water and dried using sodium sulphate. The solvent was evaporated and a white powder appeared that recrystallized from pet-ether-chloroform 1:1 mixture and m.p 155°C.

The solvent system used to follow up the purity of the derivative, the hydrolysis product and the compound itself is Toluene: Ethylacetate: Acetic acid in the ratio of 41:8:1. The R_f values are 0.81, 0.70, 0.55 for the acetate, the compound itself, and the hydrolysis product respectively.

4. RESULTS AND DISCUSSION

4.1 Physical Properties and Chemical Tests on T_1

T_1 is a white crystalline sold m.p 123 - 124°C, $(\ )_o^{21}$ = -14.9°C (C = 1, $CHCl_3$) and soluble in polar solvents such as methanol and chloroform. T_1 recrystallizes from pet-ether as needle crystals.

T_1 gave a positive Beilstein's test in methanol solution. Sodium fusion test gave a positive test for halogen. The milky precipitate was soluble in dilute ammonia solution suggesting the precipitate is silver chloride. The test for sulphur was negative. T_1 also gave a positive test for nitrogen. Hence, these tests indicate the presence of chlorine and nitrogen in T_1.

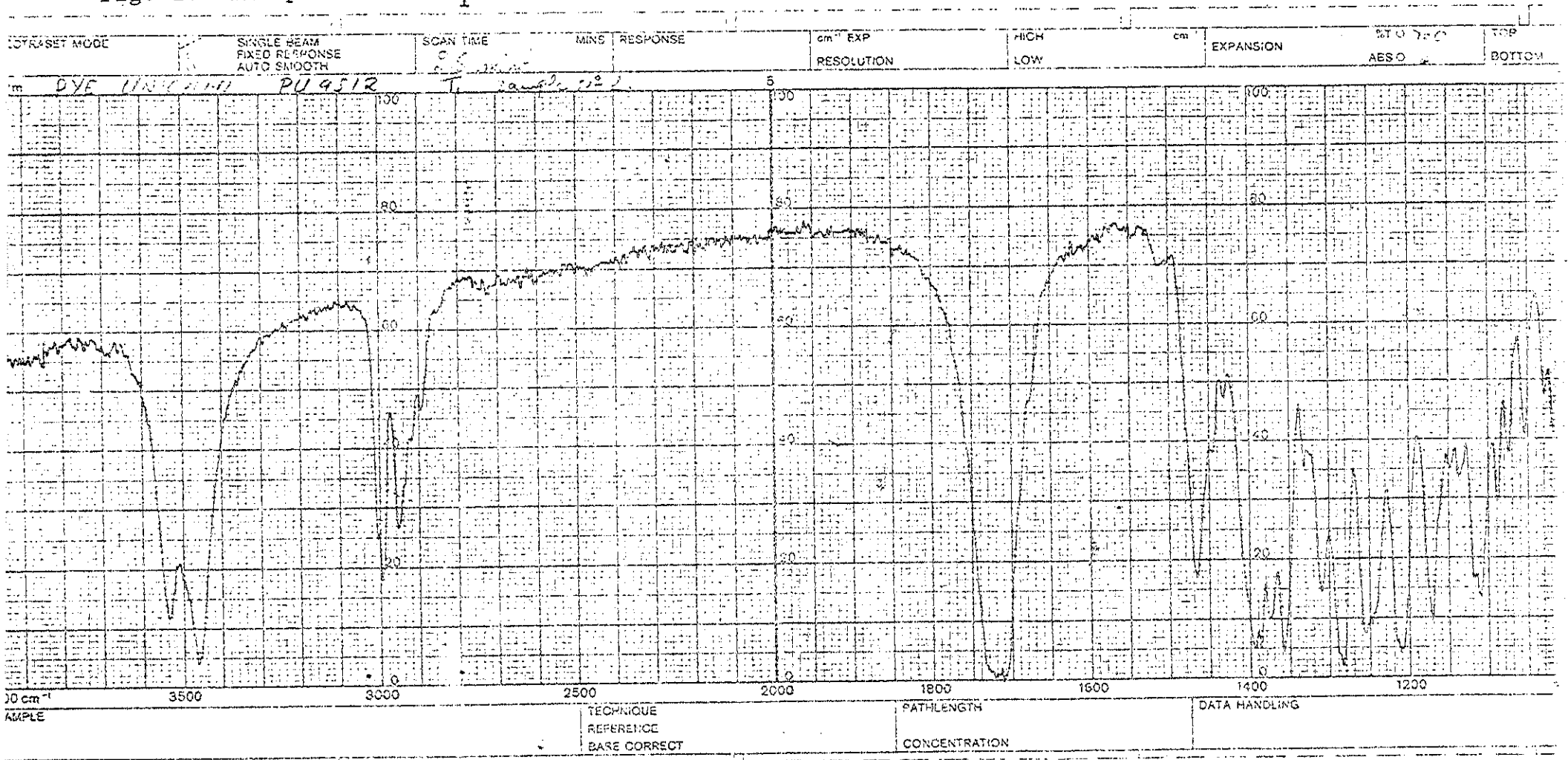
4.2 Structural Elucidation of T_1

The IR spectrum of T_1 (Fig. 1) indicates the presence of a hydroxyl (3540, 3460 cm^{-1}), carbonyl (broad, 1720 - 1730) and a gem dimethyl (doublet 1380 cm^{-1}) group. The broadness of the carbonyl absorption band might indicate the presence of more than one carbonyl groups.[68]

The UV spectrum of T_1 showes an end absorption at 195 nm.

The CIMS of T_1 exhibited a $MH^{\oplus}$ ion peak at M/Z

Fig. 1. IR-spectrum of T_1



475.9 (See Fig. 2 and Table 4) corresponding to the molecular formula $C_{15}H_{21}Cl_6NO_3$. The EIMS doesn't show a molecular ion peak (See Fig. 3). The highest mass observed in the EIMS is at M/Z 440.5 which is due to loss of Cl from the molecular ion.

The ion clusters observed in both CIMS and EIMS of T_1 offer important structural features in the molecule. The ion cluster at M/Z 475.9 corresponds to six chlorine atoms and fits the calculated values 51.2:100:81.2:35.2: 8.5:1.1:0.06 (See Fig. 2). The ion cluster appearing at 440.5 corresponds to five chlorine atoms and the ion clusters at M/Z 358 and 316 correspond to three chlorine atoms each. The ion peak at M/Z 358 is due to loss of a CCl_3 group. Thus, one might be able to conclude that there are two CCl_3 groups in the molecule.[54] Moreover, loss of one and two molecules of HCl is evident from the ion peaks at M/Z 439.5 and 404.

Several important features of the structure of T_1 were obtained from the ^{13}C-NMR spectrum (See Fig. 4). The ^{13}C-NMR spectrum reveales 15 carbon signals out of which five are due to quaternary, four due to tertiary, two due to secondary and four due to primary carbons. The quaternary carbon signals at 179.22 and 172.44 ppm are assignable to carbonyl carbons. The presence of two CCl_3 groups can be deduced from the quaternary carbon signals at 105.97 and 105.05 ppm.[54] This assignment is also

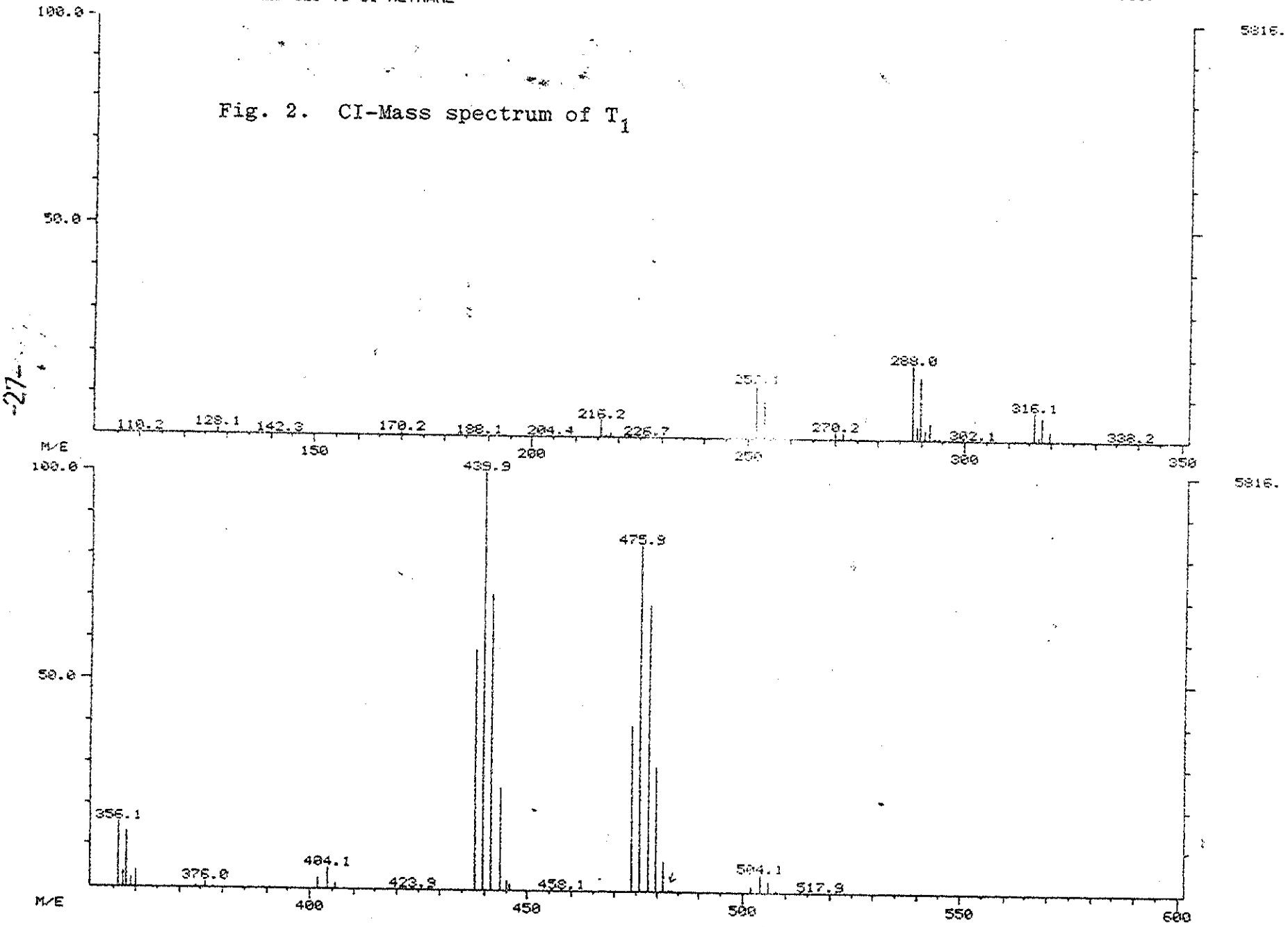


Fig. 2. CI-Mass spectrum of T_1

MASS LIST
02/28/88 10:51:00 + 1:43
SAMPLE: GEB-T1 CI METHANE

DATA: SHMULIKC # 103
CALI: CALI1 # 12

BASE M/E: 440
RIC: 37056.

110 1.00 0.00 MINIMA
520 # 100 MAXIMA

MIN INTEN: 58.

MASS	% RA	% RIC
128.14	1.15	0.18
216.16	4.25	0.67
252.12	12.86	2.02
253.98	9.23	1.45
270.18	1.62	0.25
272.08	1.50	0.23
288.02	17.95	2.82
289.14	3.16	0.50
290.08	15.11	2.37
291.02	2.32	0.36
292.08	4.01	0.63
316.08	7.05	1.11
318.10	5.71	0.90
319.90	2.27	0.36
356.06	15.13	2.37
357.22	3.51	0.55
358.12	13.07	2.05
359.16	2.15	0.34
360.16	3.83	0.60
376.00	1.38	0.22
402.00	2.44	0.38
404.10	4.69	0.74
406.10	1.38	0.22
437.90	57.57	9.03
439.86	100.00	15.70
441.82	70.56	11.08
443.76	24.42	3.83
445.24	2.17	0.34
446.08	1.48	0.23
473.94	39.89	6.26
475.92	82.67	12.97
477.92	68.50	10.75
479.88	29.54	4.64
481.74	6.91	1.08
502.04	1.27	0.20
504.08	4.04	0.63
506.02	2.56	0.40

Fig. 3. EI-Mass spectrum of T_1

m/z = 439,9935 Δm = 3 m.u.

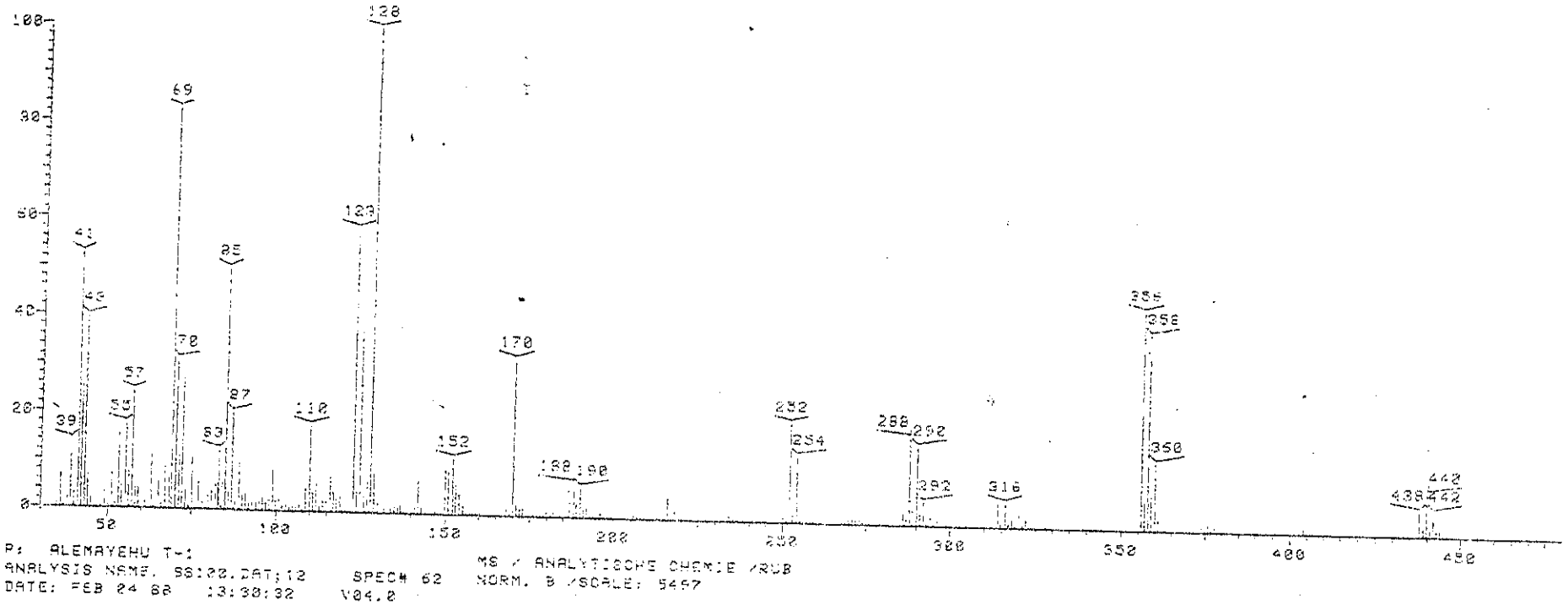
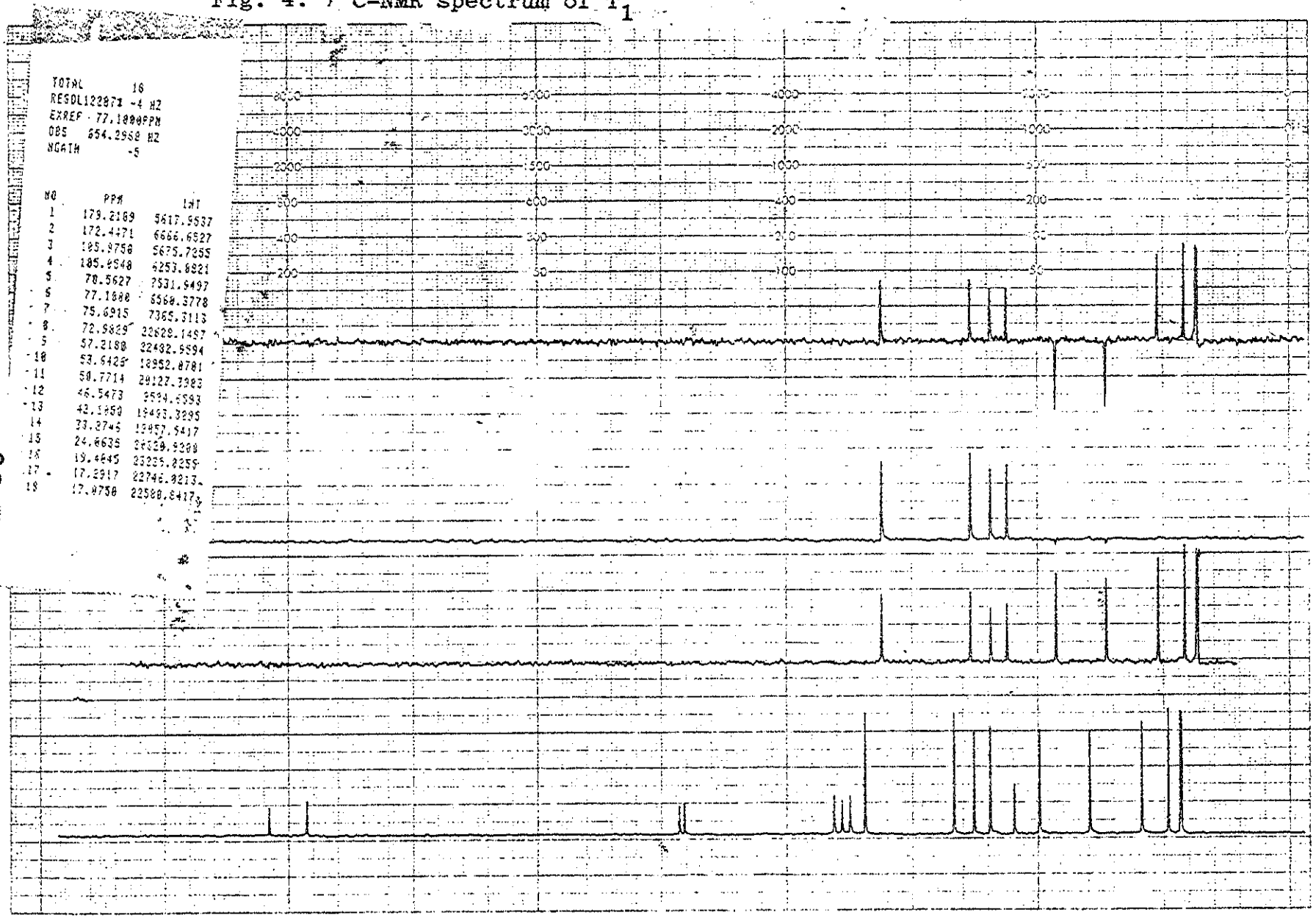
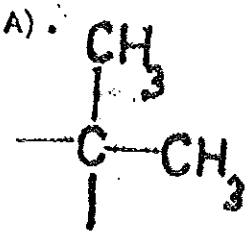


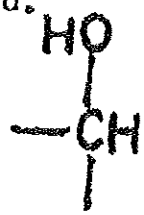
Fig. 4. ^{13}C-NMR spectrum of T_1



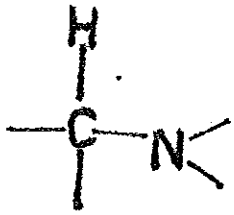
substantiated by EIMS and CIMS ion clusters (see Figs 2 and 3). Out of the four primary signals two appear at 17.29 and 17.07 ppm. The small difference in chemical shift between these methyls (0.2 ppm) suggests that these groups are attached to the quaternary carbon appearing at 46.53 ppm. Based on the above argument, it is possible to write the partial structure (A).

(A) 

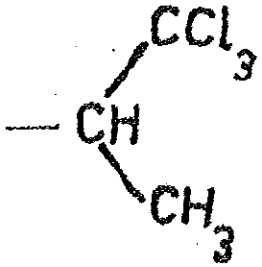
From the tertiary carbon signal at 72.98 ppm, the following partial structure (B) can be deduced.[69]

(B) 

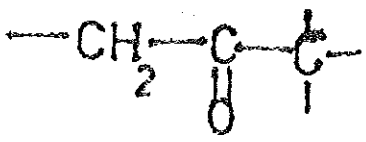
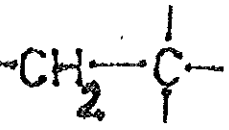
The carbon signal at 57.21 ppm can be assigned to a carbon atom bearing a nitrogen as in the following partial structure (C).[69]

(C) 

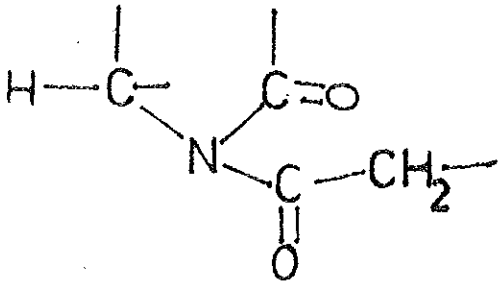
The remaining two tertiary carbon signals at 53.64 and 50.77 ppm may be assigned to two trichloroisopropyl groups as in the partial structure (D).

(D) 

The remaining two primary carbon signals at 19.40 and 24.06 ppm are assignable to the trichloroisopropyl methyl carbons. The two carbon signals at 42.10 and 33.27 ppm are due to two methylene carbons. The difference in their chemical shift (~9 ppm) may suggest that one of the $-CH_2-$ groups is attached to a carbonyl carbon whereas the other is not suggesting following partial structures (E and F).

(E)  (F) 

The carbonyl resonances at 179.22 and 172.44 ppm may be assignable to an ester, a carboxylic acid and an amide or imide carbonyl carbons.[69] Since the molecule contains three oxygen atoms one can exclude the presence of an ester or carboxylic acid groups. If the molecule were to contain two amide groups, the presence of two nitrogen atoms would be required. Since T_1 contains only one nitrogen atom, it may be rationalized that the carbonyl resonances observed are due to an imide functionality in the molecule. Hence, assuming the presence of an imide and based on the discussion above, one can write the following partial structure (G).

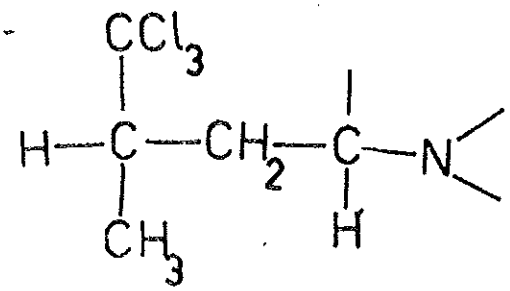
(G) 

The double bond equivalent of the molecule shows the presence of one ring and two double bonds.

$$r + db = 3$$

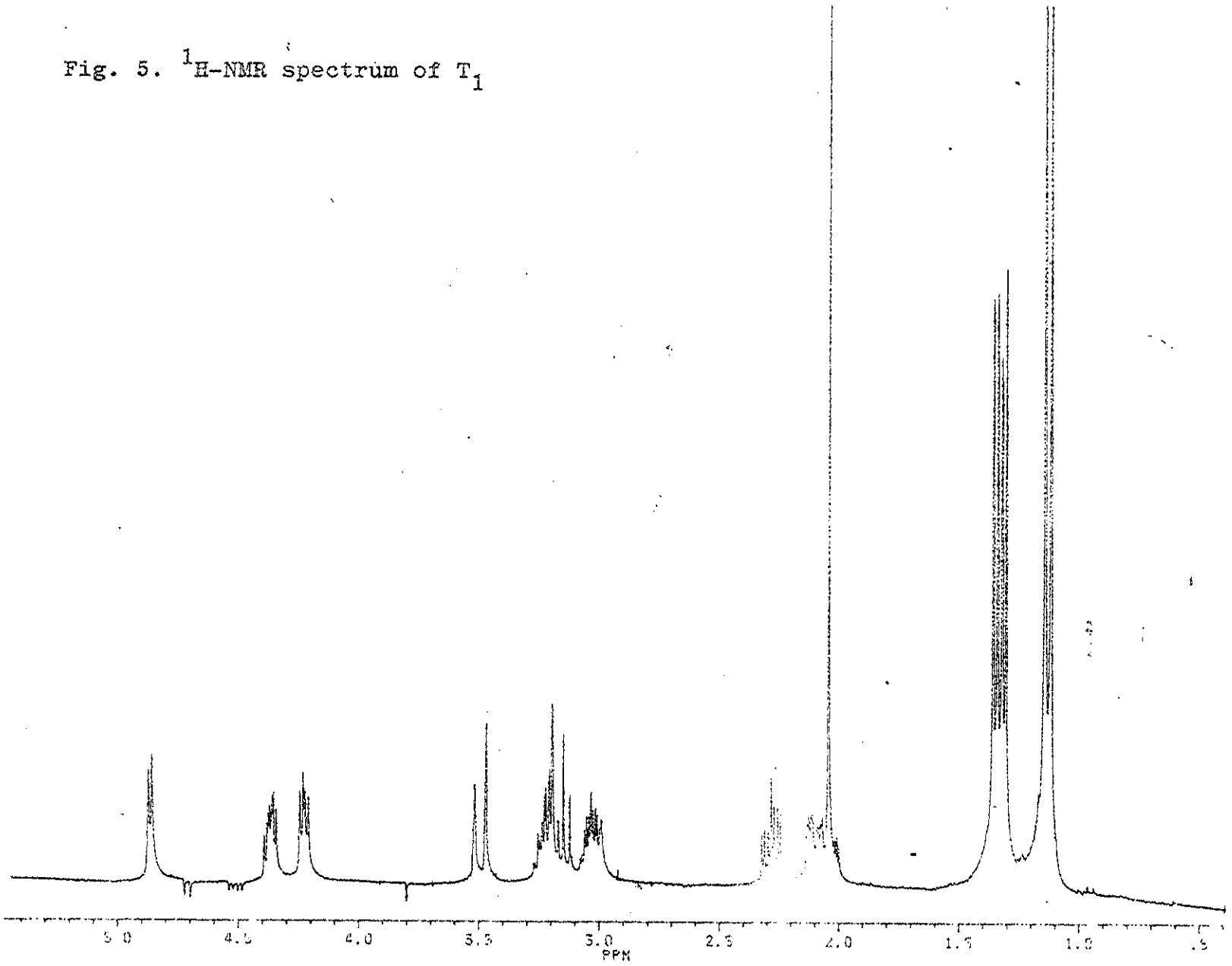
A great deal of information about the structure of T_1 was obtained from its ^1H NMR spectrum (See Fig. 5). The ^1H NMR spectrum reveals the presence of two methyl singlets at 1.14 and 1.16 ppm supporting the partial structure (A). The two methyl doublets at 1.37 and 1.33 ppm (J = 6.4 and 6.3 Hz respectively) are assignable to the two trichloroisopropyl methyl groups. The one-proton ddq centred at 3.07 ppm (J = 9.7, 6.4 and 2.6 Hz) is assignable to one of the trichloroisopropyl methine protons. The one-proton ddd centred at 2.30 ppm and the one-proton ddd appearing at 2.10 ppm (J = 14.1, 9.7, 4.2 and 14.1, 5.9, 6.2 Hz respectively) are attributed to the methylene protons not bearing an α carbonyl group (partial structure F). These methylene protons are coupled with the one proton ddd signal appearing at 4.40 ppm (J = 7.6, 5.9, 4.2 Hz). The above arguments lead to the partial structure (H).

(H)

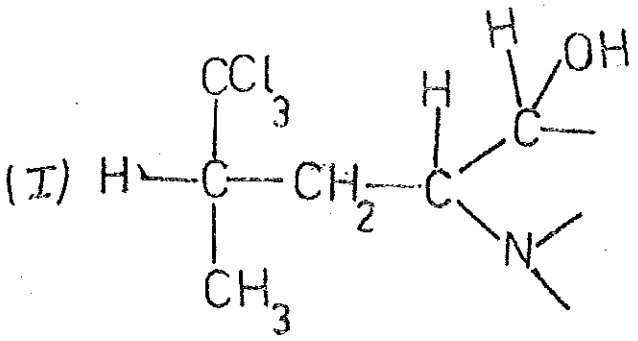


GEB T1 IN ACETONE-D6 FOR NETCOSY EXP.

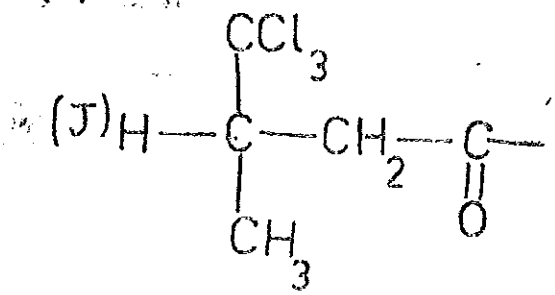
Fig. 5. ^{1}H-NMR spectrum of T_1



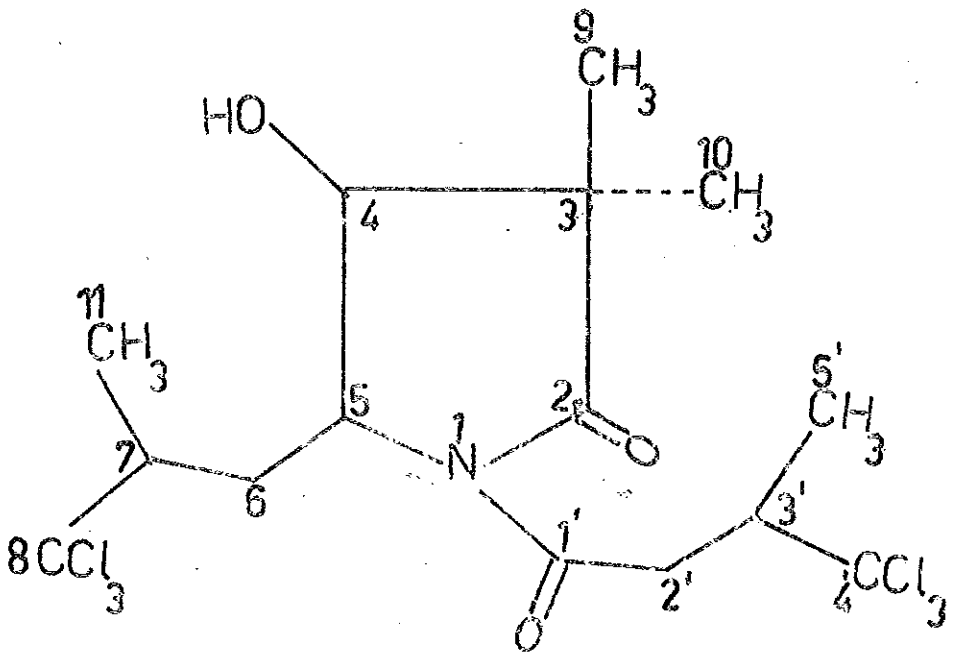
The one-proton double doublet centred at 4.24 ppm (J = 5.2, 7.6 Hz) is coupled with the one-proton doublet at 4.90 ppm (J = 5.2) and with the one-proton ddd appearing at 4.40 ppm. It is thus possible to extend partial structure (H) to (I).



The one-proton ddq at 3.23 ppm (J = 9.5, 6.3, 1.7 Hz) is coupled with the remaining trichloroisopropyl methyl protons at 1.37 ppm (J = 6.3 Hz). It is also further coupled with the two protons appearing at 3.20 and 3.52 ppm(J = 16.9, 9.5 and 16.9, 1.7 Hz respectively) which are methylene protons bearing an α-carbonyl group as in partial structure (E). These protons are coupled with each other and the large coupling constant (J = 16.9 Hz) shows that they are geminal protons suggesting the partial structure (J).

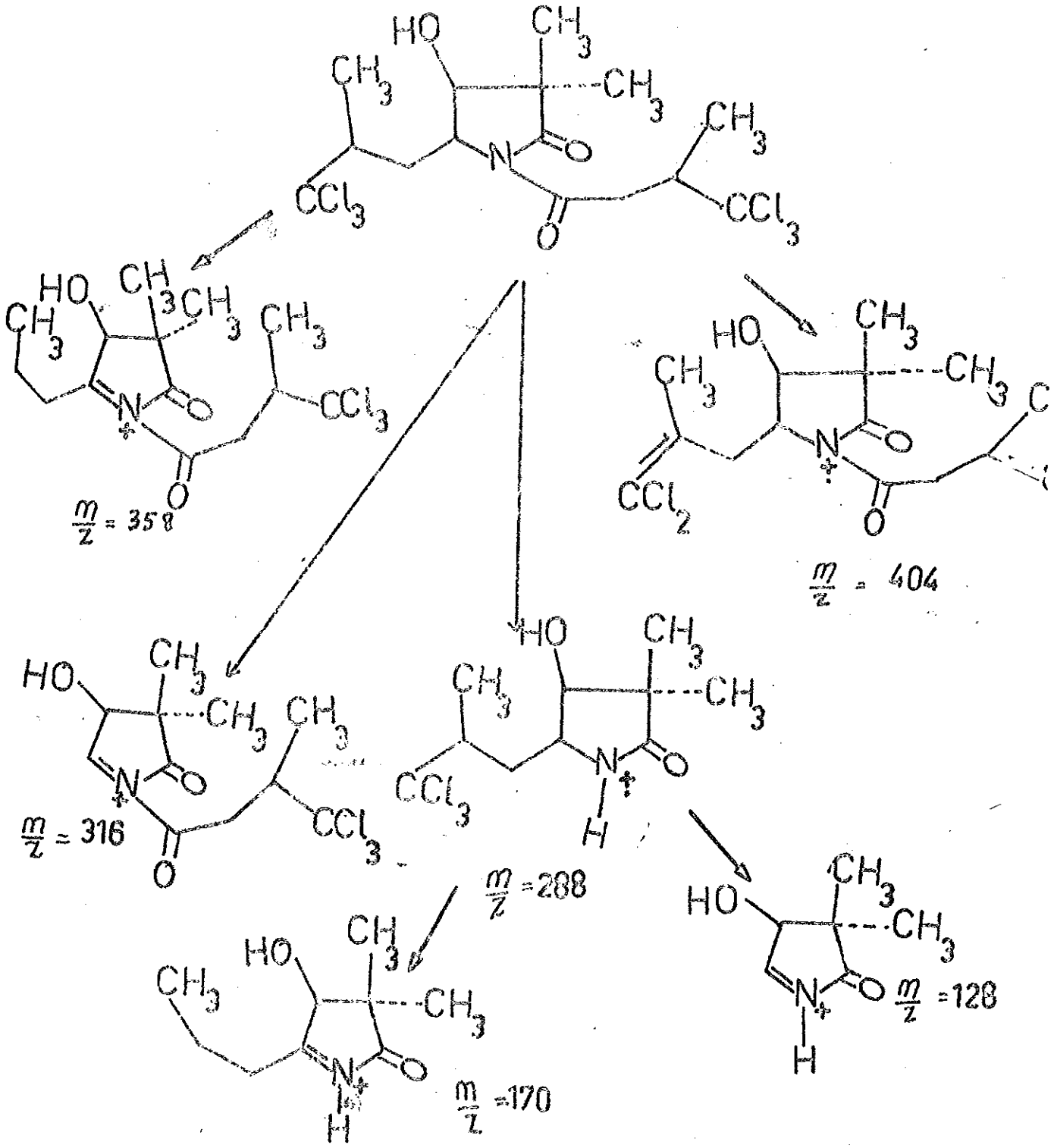


Combining all the partial structures (A - J) deduced from IR, MS, ^{1}H and ^{13}C-NMR; one can suggest the following structure for T_1 (19).



(19)

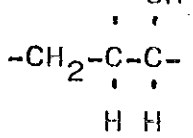
The EIMS also supports the structure assigned to T_1. The base peak is at M/e 128. The following Scheme shows the EIMS fragmentation patterns.



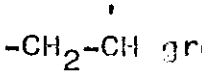
Further support for the structure of T_1 has been obtained from COSY ^1H-^1H and COSY ^1H-^{13}C experiments. Table 5 gives 1D and 2D NMR data. The COSY ^1H-^1H spectrum (See Fig. 6) showes correlation between C-4 and C-5 protons. The C-4 proton also correlates with the OH proton. Furthermore, the correlation of C-5 and C-6 protons is also observed. The C-7 proton correlates with C-6 methylene and C-11 methyl protons. Likewise, C-3' methineproton correlates with C-2' methylene and C-5' methyl protons.

The COSY ^1H-^{13}C spectrum of T_1 shows couplings of ^{13}C-carbons with ^1H protons including the long range interactions (See Fig. 7) C-2' carbon shows long range interaction with H-9 and H-10 protons. C-1' carbon correlates with C-2' methylene protons. C-4' carbon shows long range couplings with one of the two C-2' methylene and C-5 methyl protons. Likewise C-8 carbon is coupled with one of the two C-6 methylene protons and C-11 methyl protons. Moreover, C-3 carbon correlates with H-4, H-9 and H-10 protons.

Decoupling experiments on T_1 also indicated



grouping. When C-4 proton was irradiated, C-5 proton appeared as a clean triplet suggesting a



group. Furthermore, when C-5 proton was irradiated

Table 5 (360 MHz) ^{13}C and ^{1}H NMR, COSY ^{13}C-^{1}H and COSY ^{1}H-^{1}H correlations of T_1 (Acetone)

C No	δ_C & m	δ_H & m	J(H)	Long Range CH-correl ^{2}JCH	^{3}JCH	COSY-^{1}H-^{1}H correlation
2	179.84s				H-4,9,10	
3	46.55s			H-9,10	H-5,OH	
4	72.5od	4.24dd	5.2,7.6		H-6,9,10	H-5,6
4OH		4.90d	5.2			
5	57.64d	4.40ddd	4.2,5.9,7.6	H-4,6,6'	OH	H-4,OH
6	33.70t	2.30ddd	4.2,9.7,14.1		H-11	H-7,4
H'6	33.70t	2.10ddd	2.6,5.9,14.1		H-11	H-7,4
7	54.27d	3.07ddq	2.6,9.7,6.4	H-6,6',11	H-5	H-6,11
8	106.97s				H-6,11	
9	19.50q	1.14s			H-10,4	
10	23.87q	1.16s			H-9,(4)	
11	16.65q	1.37d	6.4		H-6,6'	H-7
1'	172.39s			H-2','2'		
2'	42.00t	3.52brd	16.9,1.7		H-5'	
'2'	42.00t	3.20dd	16.9,9.5			
'3	51.34d	3.23ddq	1.7,9.5,6.3	H-2',2',5'		H-2'
4'	105.86s			H-2',2',5'		
5'	17.00q	1.33d	6.3		H-2'	

δ_C = chemical shift of carbon

δ_H = " " " protons

J = coupling constant in Hz

m = multiplicity

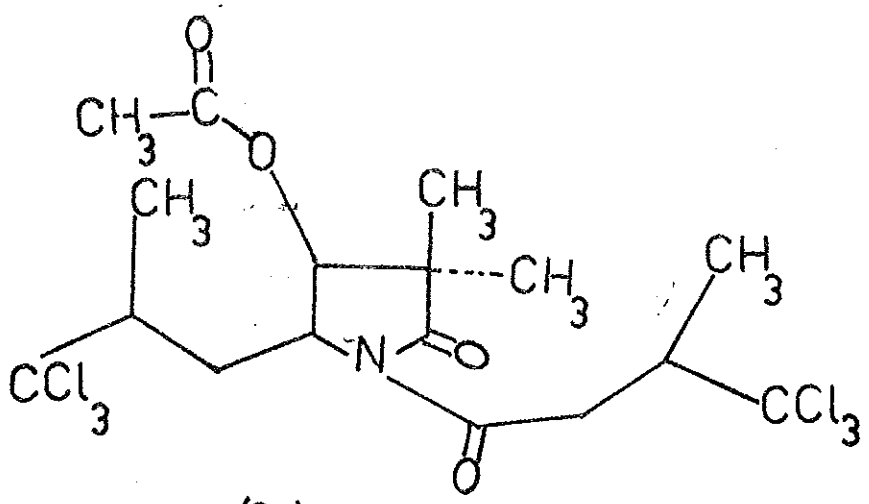
^{2}JCH = coupling accross two bonds

^{3}JCH = " " three bonds

C-4 proton appeared as a clean doublet indicating $\overset{OH}{\underset{H}{C}} - C -$ group. Hence, $-CH_2-\overset{HO}{\underset{H}{CH}}-CH$ group was evident from the decoupling spectrum of T_1

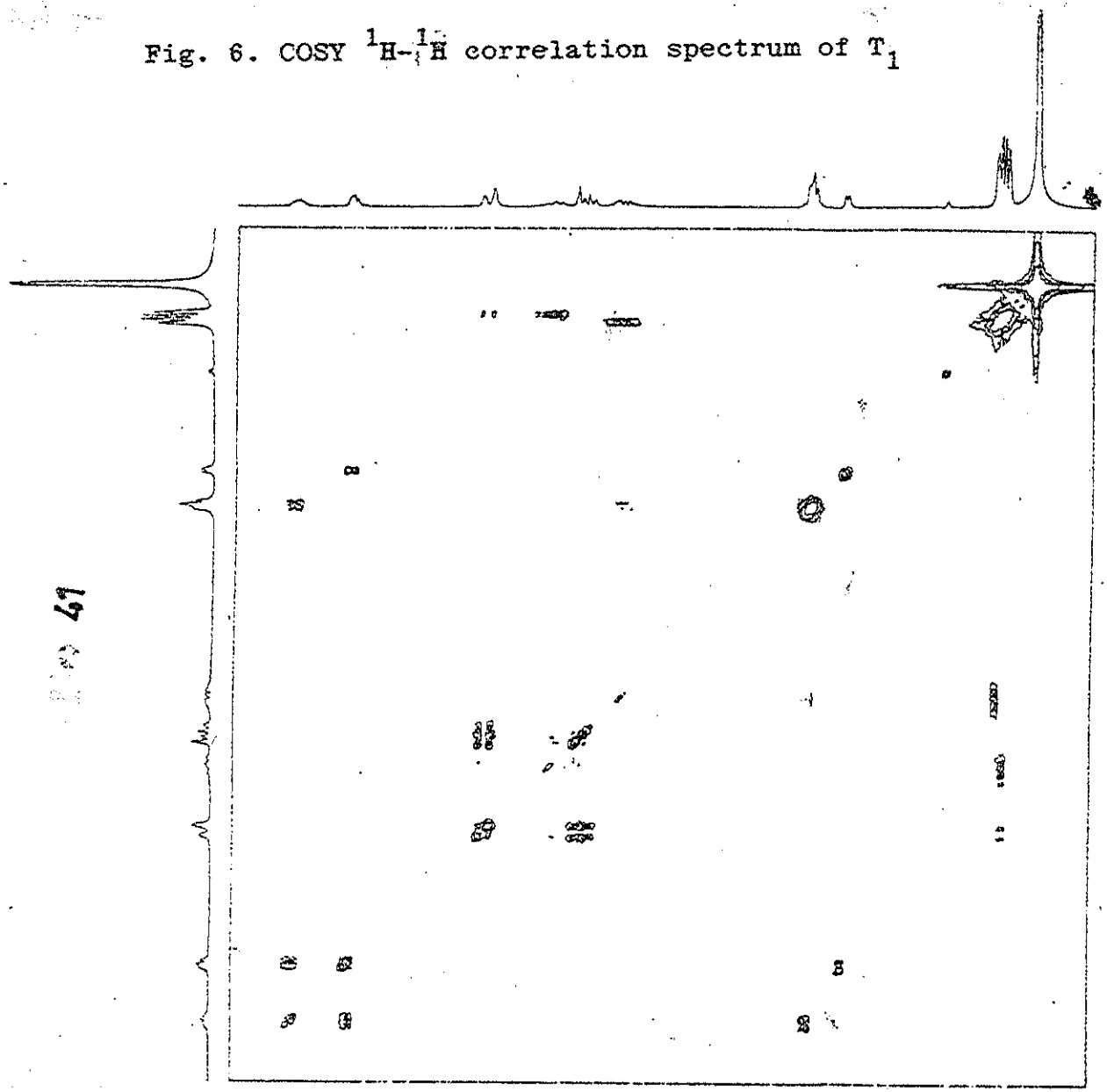
The structure assigned to T_1 based on spectroscopic arguments has also been supported by converting T_1 to its acetate derivative and by degradation to the corresponding pyrrolidinone by hydrolysis.

Treatment of T_1 with acetic anhydride and pyridine gave a white crystalline solid whose IR absorption band showes the disapperance of the OH group. The ^1H NMR of the acetate reveals an acetylation shift of 1.04 ppm downfield for the C-4 proton from 4.15 to 5.19 ppm (See Fig.8). The ^{13}C-NMR spectrum of the acetate (20) displays signals due to 17 carbon atoms (See Fig. 9). Table 6 showes ^1H and ^{13}C-NMR data of T_1 acetate.



(20)

Fig. 6. COSY ^{1}H-^{1}H correlation spectrum of T_1



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TEMPCOSY.SMX
F1 PROJ:
 INTERNAL
F2 PROJ:
 INTERNAL
AU PROG:
 COSY.AU
DATE 31-1-88

SI2   1024
SI1    512
SW2  1470.588
SW1   735.294
NDO      1


WDW2     S
WDW1     S
SSB2     5
SSB1     5
MC2      M
PLIM ROW:
F1     3.675P
F2      .007P
AND COLUMN:
F1     3.675P
F2      .007P

D1     .7000000
P1       12.60
D2     .0300000
DD     .0002000
P2         6.30
RD    0.0
PW         0.0
DE       427.00
NS           16
DS            2
NE          129
IN     .0006800
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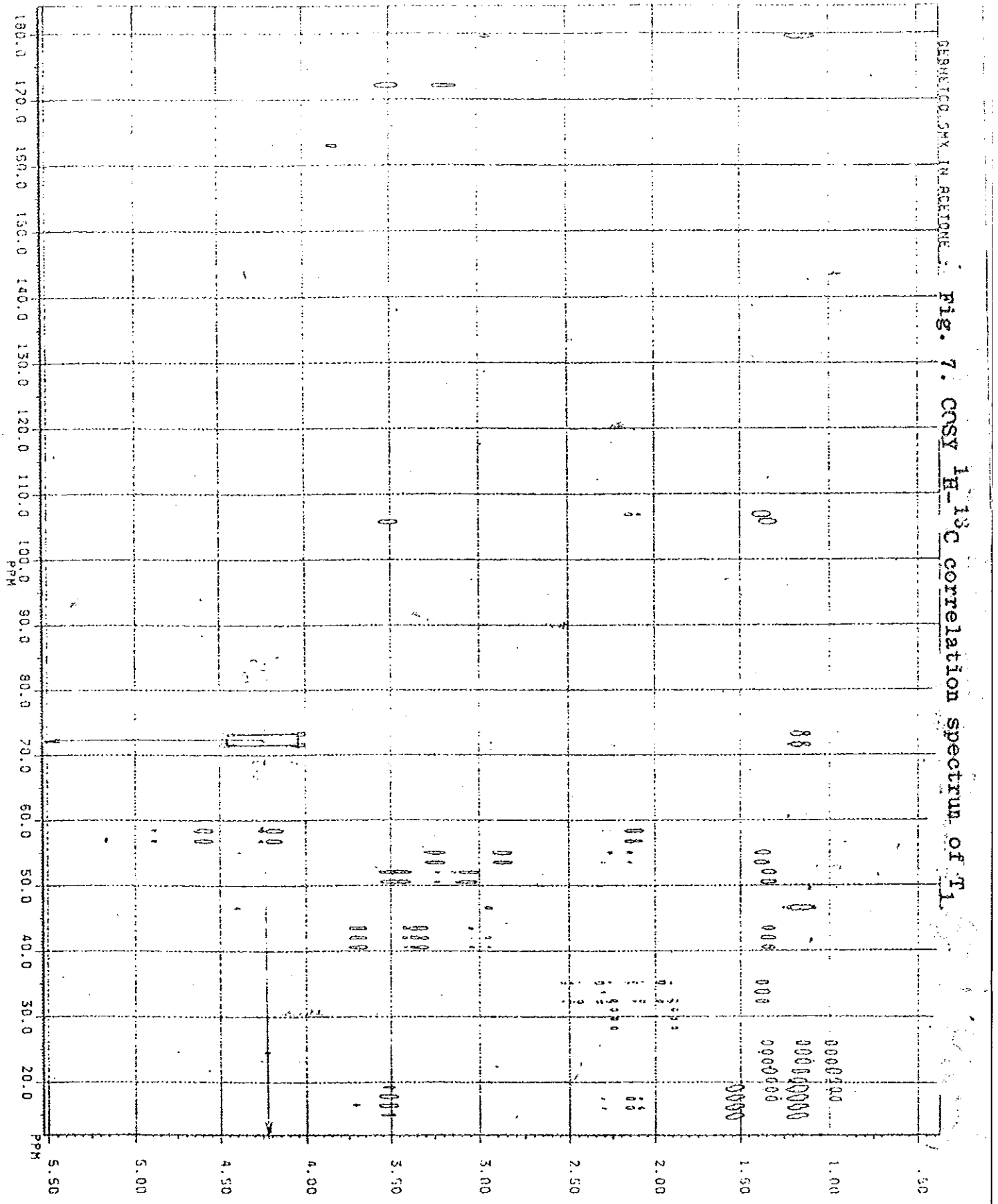


Fig. 7. COSY ^{1}H-^{13}C correlation spectrum of T_1

HETCOSY-

$^{1}J + {}^{n}J_{C\text{-}H}$ corr

Fig. 8. ^{1}H-NMR spectrum of T_1-acetate

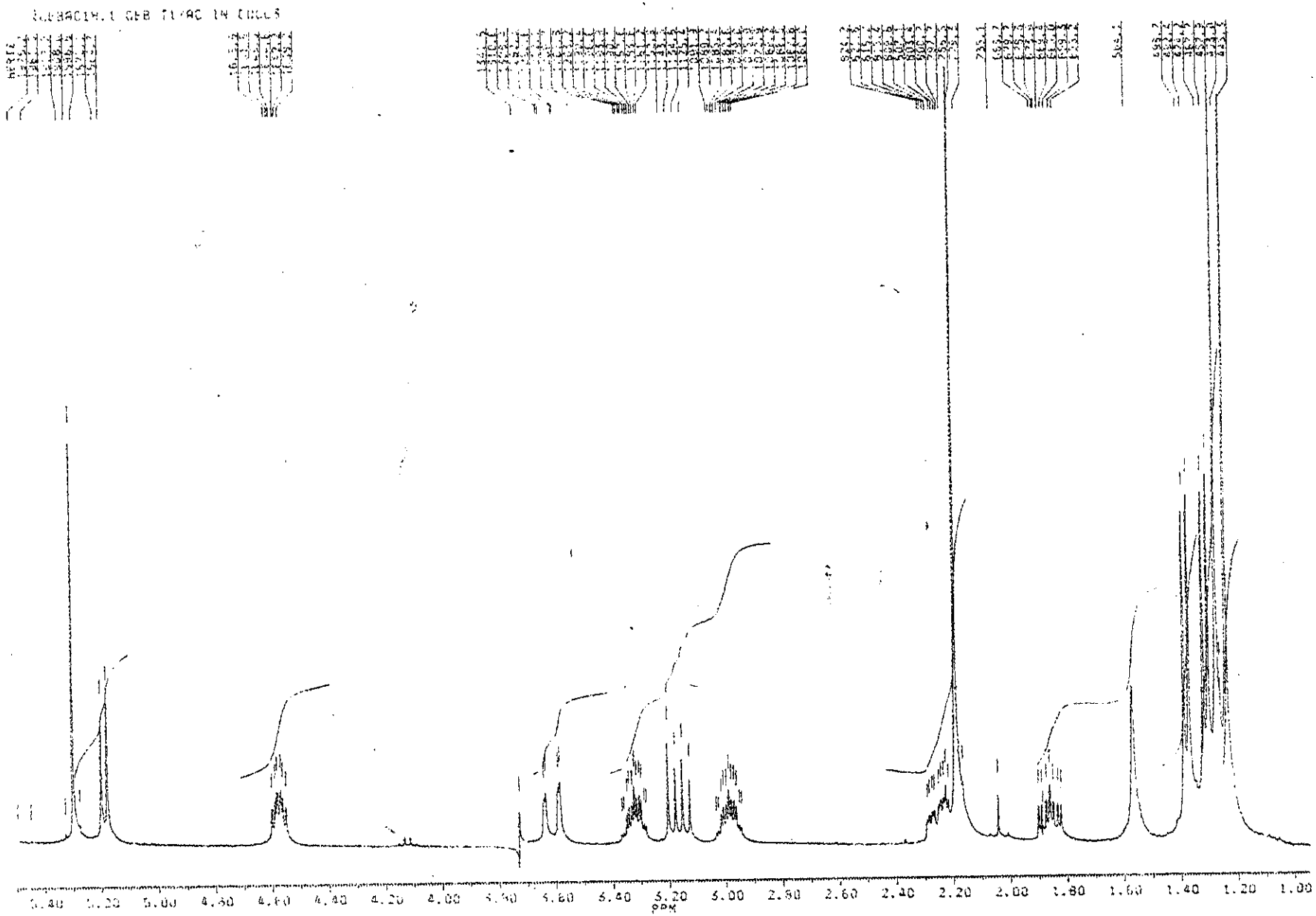


Fig. 9. ^{13}C-NMR spectrum of T_1 acetate

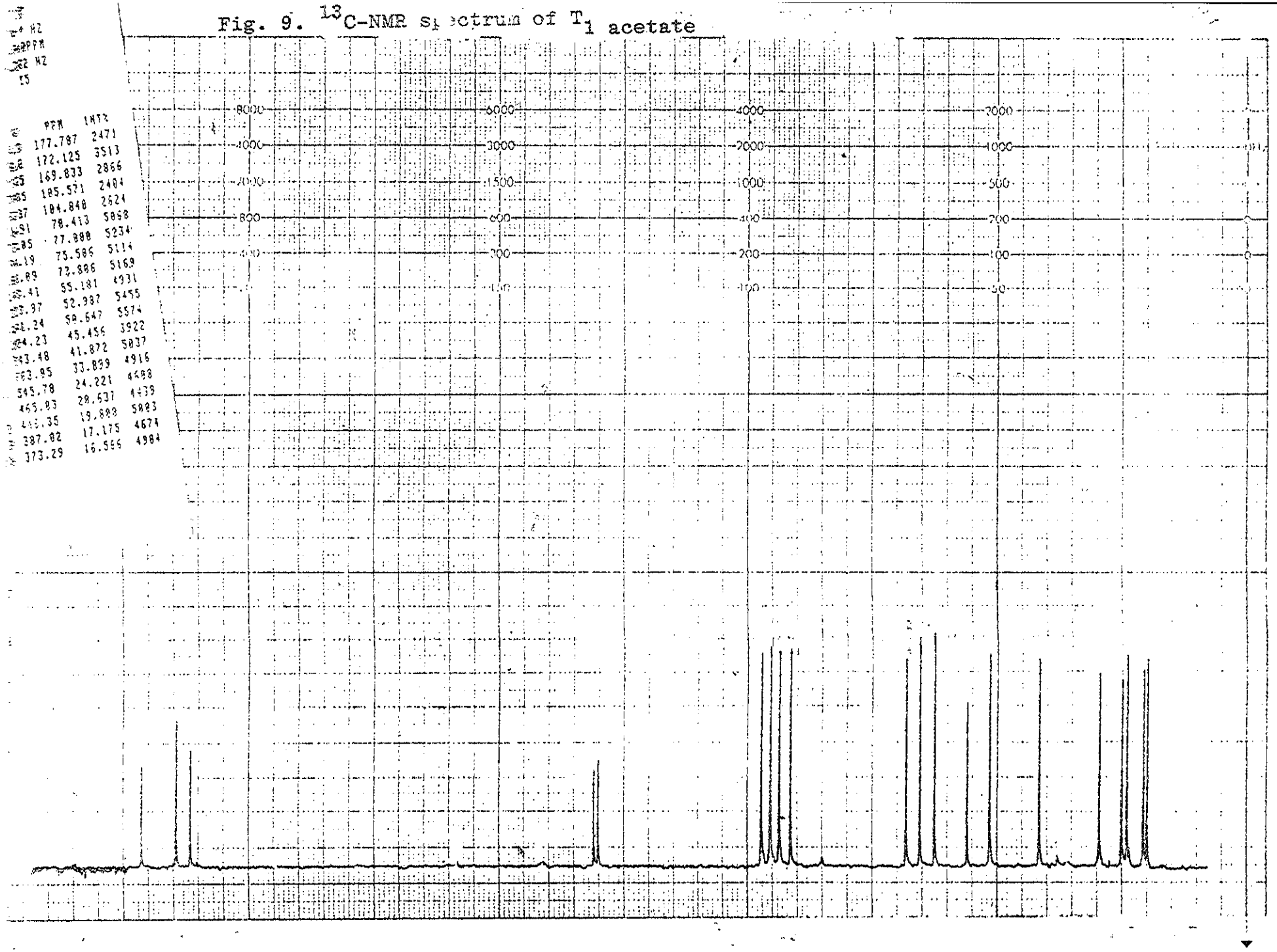


Table 6. ^{1}H and ^{13}C-NMR of T_1 acetate ^{1}H NMR, 360 MHz in $CDCl_3$ and ^{13}C-NMR, 22.5 MHz in $CDCl_3$

C No	δ_C	δ_H & m	J(Hz)
2	177.70		
3	45.45		
4	73.80		
4oAC	169.8 and	5.19 d	7.4
	24.22	4oAC=2.20 s	
5	55.18	4.57 ddd	7.4,7.0,2.3
6	33.89	2.25 ddd	14.5, 6.1, 2.3
		1.86 ddd	14.5, 9.8
7	52.98	2.99 ddq	9.8,6.4,2.6
8	104.84		
9	19.80	1.28 s (Me)	
10	20.63	1.24 s (Me)	
11	16.56	1.32 d	6.4
1"	172.12		
2"	41.87	3.61 dd	18.1,2.3
		3.17 dd	18.1,9.4
3'	50.64	3.32 ddq	9.4,6.4,2.3
4'	105.57		
5'	17.17	1.39 d	6.4

δ_C = chemical shift of carbon.

δ_H = " " " protons.

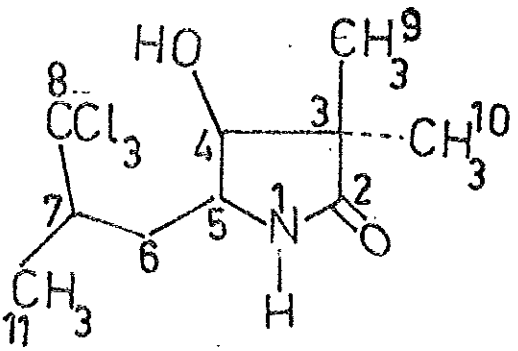
J = coupling constant in Hz

m = multiplicity.

Hydrolysis of T_1 has been carried using sodium methoxide in methanol solution at room temperature and gave the corresponding pyrrolidinone.

The ^{13}C-NMR of the pyrrolidinone shows 10 carbon signals out of which three are quaternary carbons at 179.69, 44.20 and 106.30 ppm assignable to $-\overset{\overset{O}{\|}}{C}-$, $-\overset{CH_3}{\overset{|}{C}}-CH_3$ and CCl_3 groups respectively. The carbon signals at 76.29, 52.78 and 51.54 ppm correspond to $-\overset{OH}{\overset{|}{C}}-H$, $-\overset{CCl}{\overset{|}{C}}-$ and $-\overset{|}{\underset{N}{C}}-H$ groups respectively. Moreover, the three methyl signals appear at 22.49, 17.60 and 15.16 ppm and the one methylene signal appears at 33.36 ppm. The pyrrolidinone has the structure (21). Table 7 shows ^{1}H and ^{13}C-NMR data of the pyrrolidinone.

(21)



The ^{1}H NMR spectrum of the pyrrolidinone shows a one proton signal at δ6.80 ppm attributable to the NH proton. The double doublet at 3.94 ppm and the doublet at 4.30 ppm are due to C-4 and OH protons respectively. The one-proton multiplet centred at 3.37 ppm corresponds to the CH-5 proton. The signals appearing at 2.22 and 1.60 ppm

Table 7. ^{13}C and ^{1}H-NMR of the hydrolysis product of T_1 (360 MHz, $CDCl_3$)

C No	δ_C & m	δ_H & m	J(Hz)
1		6.8 br s	
2	179.69 s		
3	44.20 s		
4	76.29 d	3.94 br d	5.1 Hz
		4.30 br s (OH)	
5	51.54 d	3.77 ddd	10.2,5.1,3.6
6	33.36t	2.22 ddd	13.4,10.2,2.4
		1.60 ddd	13.4,10.4,3.6
7	52.78 d	1.93 ddq	10.4,6.4,2.4
8	106.43 s		
9	22.49 q	0.97 s	
10	17.60q	0.96 s	
11	15.16 q	1.26 d	6.4

δ_C = chemical shift of carbon atoms.

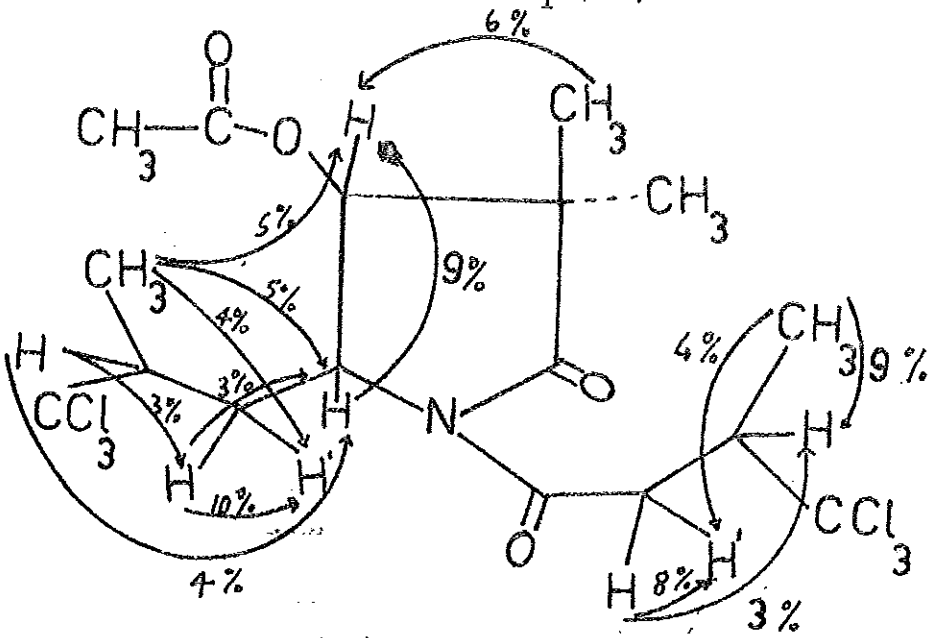
δ_H = " " " protons.

J = coupling constant of protons.

m = multiplicity.

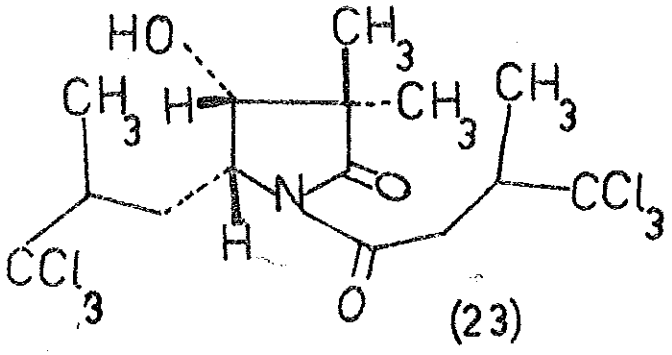
are assignable to the C-6 methylene protons. The one-proton multiplet centred at 1.93 ppm is due to the C-7 methine proton. There are three methyl proton signals appearing at 1.26, 0.97 and 0.96 ppm corresponding to C-11, C-9 and C-10 on C-10 and C-9 protons respectively.

T_1 has four assymetric centres at C-4, C-5, C-7 and C-3' carbons. One can say that T_1 is fully characterized when the absolute stereochemistry at these assymetric centers is fully determined. NOe measurements have been carried on the acetate of T_1 (22).



(22)

The 9% enhancement of the C-4 proton signal due to irradiation of the C-5 proton suggests that cis relationship exists between the C-5 and C-4 protons. Had there been a trans-relationship, a 9% enhancement would have not been observed. Based on the above argument the relative stereochemistry at C-4 and C-5 is indicated below (23).



(23)

X-ray crystallography would give the absolute stereochemistry of T_1.

The name dysidimide has been proposed for T_1. It is the major secondary metabolite of the Dysidea sp.. There are also some more fractions that are under investigation.

5. CONCLUSION

Dysidimide is the major secondary metabolite of a sponge Dysidea sp.. Its structure was determined from physical, chemical and spectroscopic data. Dysidimide has four assymetric centers C-4, C-5, C-7 and C-3', The relative stereochemistry at C-4 and C-5 was determined from nOe measurements on dysidimide-4-acetate. Dysidimide has been submitted for X-ray crystallography. Further investigation on the minor secondary metabolities will be carried out.

Some of the polychlorinated secondary metabolites of the genus Dysidea have shown toxic activities against predators such as fish. Toxicity tests will be carried on dysidimide to see if it also possesses a similar property.

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