INVESTIGATION OF CHEMICAL CONSTITUENTS OF

ANDRACHNE ASPERA

AND

PORANIA PULVILLUS

BY

MOHAMMAD AJMAL NASIR

THESIS SUBMITTED FOR THE FULFILMENT OF THE DEGREE OF DOCTOR OF PHILOSOPHY

H.E.J. RESEARCH INSTITUTE OF CHEMISTRY

UNIVERSITY OF KARACHI

KARACHI-32, PAKISTAN

1986

L12 - B

IN THE NAME OF ALLAH
THE MOST COMPASSIONATE
THE MOST MERCIFUL

TO,

MY PARENTS

MY GRANDFATHER

AND MY TEACHERS

WHOSE PRAYERS AND EFFORTS

ELEVATED ME TO THIS STAGE

ACKNOWLEDGEMENT

The author wishes to express his deep sense of gratitude and heartfull thanks to the kind and learned supervisor PROFESSOR VIQAR UDDIN AHMAD for his able guidence, valuable suggestions, generous help, patience and keen interest during last five years of association to his group.

My heartfull thanks go to Dr. Lars Bohlin and Mr. Leif Andresson of Department of Pharmacognosy, Biomedical Center, University of Uppsala Sweden for their guidence and interest during the work relating to isolation of steroidal glycosides from starfish performed by the author in their department.

The author wishes to express his deepest sense of gratitude to his late father, whose encouragement, moral support and commitment towards higher education guided him through the stages leading to the highest academic level.

Thanks are also due to his loving mother, brothers and sister in law who took a lot of pain in making all this possible.

Sincere thanks go to PROFESSOR SALIMUZZAMAN SIDDIQUI, F.R.S., H.I., Director, H.E.J. Research Institute of Chemistry, University of Karachi to provide facilities and financial support and whose shear presence inspired him to complete this work.

Prof. Atta-ur-Rahman, T.I., Co-Director, H.E.J. Research Institute of Chemistry and Prof. Zafar H. Zaidi are also highly acknowledged for their valuable suggestions.

Dr. Lennart Kenne of Department of Organic Chemistry, Stockholm University is acknowledged for providing 400 MHz NMR and suger analysis of glycosides.

Thanks are also due to Dr. M.S. Shekhani of H.E.J. Research Institute of Chemistry and Dr. Iltifat Hassan of Glaxo Laboratories, Karachi for the valuable discussions during the course of studies.

The author highly appreciates the cooperation of the technical and non technical staff of this institute.

My colleagues and friends are highly acknolwedged for their help and cooperation during the studies. It was simply a fun to work in such a nice group.

Thanks are due to Miss Maria Iorizzi of Department of Organic Chemistry Napolli University, Italy for valuable discussions.

Mr. Syed Wajihul Hasnain Rizvi is highly acknowledged for composing this thesis, in spite of his sickness.

Last but not least the author wishes to thank Prof. Rune Liminga, Head of International Seminar in Chemistry, University of Uppsala, Sweden for the award of a fellowship for one year from September 1984 - September, 1985.

MOHAMMAD AJMAL NASIR

C O N T E N T S

SUMMARY

The present thesis is divided into two chapters. Chapter 1 relates to chemical investigation of the medicinal plant Andrachne aspera. This study has resulted in the isolation of three novel piperidine alkaloids from this plant which have been named as andrachcine, andrachamine and AA-3. The structures for andrachcine and andrachamine have been proposed on the basis of spectroscopic analysis.

The second chapter deals with isolation of steroidal glycoside named as attenuatoside A-1, halityloside D and E. These glycosides are isolated for the first time from starfish porania pulvillus.

The structures for these isolated saponins have been proposed on the basis of spectroscopic and chemical studies.

INTRODUCTION

Nature has fascinated and challenged mankind from the very beginning.

Man, endowed with the unique capability of intuition has used his instinct and experience to study and unravell the mysteries of nature for his own survival. The use of certain plants to alleviate human sufferings is as old as the civilization.

Since ancient times, man, through his experience has accumulated and compiled considerable knowledge of healing agents from such "Natural Products".

The oldest compilation of medicine in Indo-Pakistan subcontinent are Rigveda (4500-1600 BC) and Ayurveda (255-600 BC). The Ayurveda is still being practised in India and Pakistan. In the advent of Mughals another system of Medicine "Greco Arabic" or Unani Tibb came into practice in this sub continent. This system, which incorporated many medicine from Ayurvedic system, is still being practised in many areas of Pakistan. This system of medicine is providing relief to a vast majority of population in rural areas of Pakistan.

The advantages ascribed to these drugs are their comparatively low toxicity, low cost and the vast experience gained in their therapeutic properties since ancient times.

Pakistan has a varied climate which is suitable for the growth of a rich flora containing enormous varieties of plants or herbs of medicinal importance.

The successful use of these herbs to cure human and animal ailments prompted many natural product chemists to start work on these herbs. This work resulted in many new compounds of medicinal interest with remarkable activities.

In a large number of medicinal plants, their theraputic value was found to be due to the presence of alkaloids which in certain respects rank among the most interesting of naturally occuring substances both from the chemical and physiological stand points.

MEDICINAL IMPORTANCE OF ALKALOIDS

When the "art of healing" was considered as magical or religious; minerals, plants and animal organs were being used by healers to cure various ailemtns. The knowledge was not based on the true scientific facts but on the experience of past generations.

In the period when alchemy evolved into chemistry and the practice of medicine became more scientific, it was found that certain type of compounds are responsible for this magical or spiritual power of these so called miraculons drugs.

Till early ninetcenth century no attempt was made to isolate the principle constituents from these plants. Stork, an Austrian apathocary reintroduced many plant drugs into medical practice of this era which include aconite, colchicum, stromonium, henbane and belladona[1]. Opium which was product of poppy plant and was being used by people of early times as analgasic and also has narcotic properties, was a great challenge for modern researchers and prompted them to isolate active ingradiants of this drug.

In 1803 Derosne was the first person to isolate a semipure constituent narcotine from the opium[1]. In 1805 Serturner isolated morphine and he also repoted the basic character of morphine for the first time[1].

Soon a number of similar substances were recognized and grouped into a new class of compounds known as "vegetable alkalies". These compounds became known as Alkaloids[2]. It was observed that these alkaloids essentially contain nitrogen as one of the elements, and basicity is a common character of organic nitrogenous compounds. Usually simple amines which are also basic compounds are considered as a separate class but as the complexity of structure increases it becomes difficult to draw a sharp line between these two classes i.e. alkaloids and amines[3].

Many attempts are made to provide a system of classification in to which most alkaloids can be placed. One of the most widely accepted classification system was given by Hegnauer[4] which divides the alkaloids into the following three classes

a) True Alkaloid

The compound of this class are toxic, with wide range of physiological activity, basic in nature, normally contain nitrogen in heterocyclic ring, derived from amino acid, have limited taxonomic distibution and are present as salt in the plant.

Some exceptions to these rules are colchicin, (**1**) aristolochic acid (**2**) which are not basic and have no heterocyclic ring and the quaternary alkaloids which are acidic rather than basic in nature.

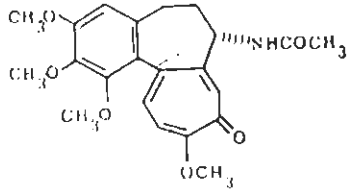
b) **Proto Alkaloids**

These are relatively simple amines having amino acid nitrogen out of the ring, are basic in nature and biosynthesized from amino acid. They are also termed as "Biological amines". Examples are mescaline (**3**), ephidrine (**4**) and N-N-dimethyltryptamine (**5**).

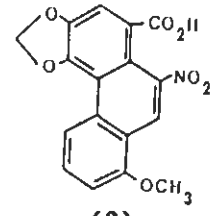
b) **Pseudo Alkaloids**

These are not derived from an amino acid precursors and are usually basic. The examples are steroidal alkaloids e.g. conessine (**6**) and purines e.g. caffeine (**7**).

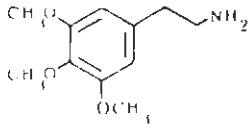
In the period 1817-1820 Pelletier and Caventou of Faculty of Pharmacy in Paris reported many alkaloids which are considered as cornerstones of alkaloidal chemistry[1]. Some examples are strychnine, emetine, brucine, piperine, caffeine, quinine, cinchonine, colchicine.



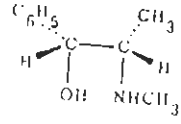
(1)



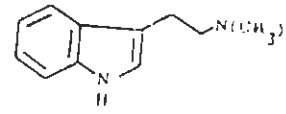
(2)



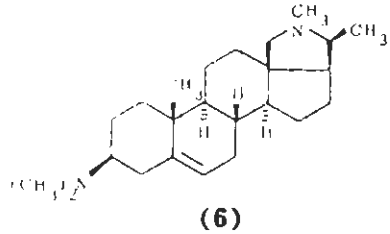
(3)



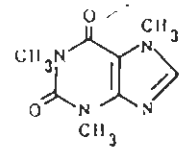
(4)



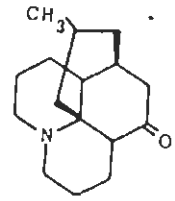
(5)



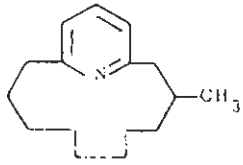
(6)



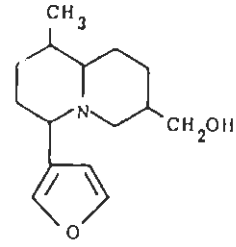
(7)



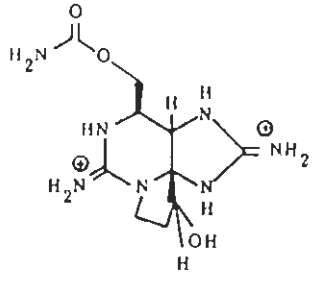
(8)



(9)



(10)



(11)

The first alkaloid which was characterized and synthesized was coniine, its synthesis was completed as early as in 1886[5]. It was isolated from the historical plant Hemlock which was given to Socrates as poisinous drink. The distribution of alkaloids in nature can not be described in any simple and unambigueous way. Usually higher plants are the main source of alkaloids but some alkaloids have also been isolated from lower plants e.g. club mosses (lycopodium) which yield lycopodine (**8**), horse tails (Equisetum spp) and fungi. In the past few years many reports appeard regarding isolation of alkaloids from animals, including insects, marine organisms, microganisms etc. Some examples are muscopyridine (**9**) from musk deer, castoramine (**10**) from canadian beaver and sex pharmone of several insects; saxitoxin (**11**), salamandar compounds and quinazolones excreted as a repellent by millepedes[3].

Some of these alkaloids produced by animals may simply be derived with slight structural modification from plants eaten by animals, but one can not rule out this possibility that these are synthesized in the animal body from simple precursors.

Most of the isolated alkaloids are crystalline solids with a definite melting point or decomposition range. Some alkaloids having amorphrous or liquid state e.g. coniine and nicotine are also reported. Mostly alkaloids are colourless but some complex and highly aromatic compounds are coloured e.g. berberine which is yellow and betanine which is red

TABLE-1

ACTIVITY	ALKALOID	PHARMACAOGICAL ACTION	SOURCE
Analgesic	Morphine	Analgesic, Norcotic	Opium
	Codeine	Analgesic (Non-Adductive effect)	
	Heroin	Powerfull Analgesic (Strongly Adductive)	
Cardiac stimulant	Quinidine	Antiarrythmic of Auricles	Cinchona
	Ajmaline Series Alkaloids	Antiarrythmic of Ventricle	Rauwolfia
Respiratory stimulant	Nicotine	Respiratory Stimulant (Nonclinical use)	Nicotana tobacum
	Cytisine	""	
	Lobeline	Safer Than Nicotine	Lobelia
	Atropine	Respiratory Stimulant, Dilates Puplis	Atropa Baladona
Blood Vessels contrictors	Ergonovine	To Reduce Uterine Hemorrage	Ergot
	Ephedrine	Constriction of Blood Vesses used for ashthama	
Muscle Relaxent	Tubocurarine	Musclerelaxent	Curare
	Toxiferine		
	L.S.D.		Ergot
	Psilocybine	Psychedelic	Spliocybe species

in colour.

The physiological activity of alkaloids is a well known not only in Medicine but also in forensic chemistry.

In medicine alkaloids are employed as narcotics analgasics, anti malarials, local anesthatics, cardiac, uterine and respiratory stimulants. They are also used as materials that rise the blood pressure; causes the pupils to dilate or bring about the relaxiation of the skeletol muscles[2].

Some important medicinal uses of alkaloids according to their physiological activities are listed in Table. 1.

ANDRACHNE ASPERA

The plant Andrachne aspera Spreng belongs to family Euphorbiaceae. Four species of this plant are found in Pakistan i.e. Andrachne cordifolia (Dene) A.routendifolia, A.telephioides L. and Andrachne aspera. Among these four species A.aspera and A.rotundifolia are found in Sind and Baluchistian area of Pakistan. While A.cardifolia and A.telephioides are commonly found in Northern Hilly region of Pakistan where shady places are common. The plant Andrachne aspera Spreng is the only species found in Karachi but it is also found in India. Iran, Arabia and tropical Africa[6,7].

This plant is small perennial undershrub, prostrate or ascending with woodey root, branches 10-30 cm long and covered with short glandular hairs. Leaves are suborbicular or reniform, 6-12 cm. Flowers are small.

This is plant commonly found on stony, sandy and rocky ground[7].

MEDICINAL AND PHARMACOLOGICAL IMPORTANCE OF THE PLANT

In local system of medicine plant is known as Haran Tutia[8] and the roots are usually used in medicinal preparations. The roots are slender, yellowish brown to brown surmounted by knotty crown, which tastes acrid and tobacco like[8].

They are used in local system of medicine for treating eye sore and to improve eye sight[8].

The alkaloidal mixture obtained from alcoholic extract of this plant nas stimulating action on respiration and blood pressure of dog and cat. It showed spasmolytic activity on guineapig ileum, on rat ileum it showed both neurotropic and muscolotropic activity. It also showed spasmolytic activity on tracheal muscles of cat and anti histaminic activity on guineapig ileum[9].

The alkaloidal mixture exhibited antibacterial activity against Staphylococcus aureaus, S.citreus, Salmonella typhi A,B and C, E.scherichia coli, Bacillus magatherium and Bacillus subtilis[9].

During studies on albino rats and mice alkaloids were found nontoxic as none of the animal died upto a dose of 10.0 mg/100 gram body weight on oral administration[9].

The plant is also reported as expectorant in indigenous system of medicine. On pharmacognostic comparison with Indian and Pakistani varieties of senega it was found that Andrachne aspera is the source of the above drugs but is not a suitable substitute for true senega obtained from Polygala senega[10] which grows in North Africa and it's roots are used as expectorant.

INTRODUCTION TO PRESENT WORK

In view of the medicinal and pharmacclogical importance of the plant it was decided to carry out chemical investigation of plant Andrachne aspera.

A survery of the literature revealed that no work on the chemical constituents of this particular species of Andrachne has been reported.

Khalsa et al.[5] did some pharmacological investigation of alkaloids of this plant but they did not report the chemical structural features of the compound.

In 1967 V.V.VIL'YAMS[11] reported isolation of an alkaloid named "andrachnine from another species Andrachne rotundifolia. They reported molecular formula ($C_{11}H_{17}NO_2$) along with some I.R. data in this communication. Another species Andrachne cordifolia is reported to evolve HCN during analysis in an early report a ıd this was stated as a reason for poisonous value of the plant[12].

In 1983 F. Hassan et al. reported isolation of two bisbenzylisoquinoline alkaloids from Andrachne cardifolia[13], but latter in a private communication they informed the present author that the plant was missidentified, it was a species of Cocculus rather than Andrachne. The isolation of isoquinoline alkaloid from Andrachne also appeared surprising from chemotaxonomic point of view.

PRESENT WORK

The present work regarding chemical investigation of Andrachne aspera is the isolation and structure elucidation of alkaloidal constituents of the plant.

ISOLATION AND STRUCTURE ELUCIDATION OF ALKALOIDAL CONSTITUENTS OF ANDRACHNE ASPERA

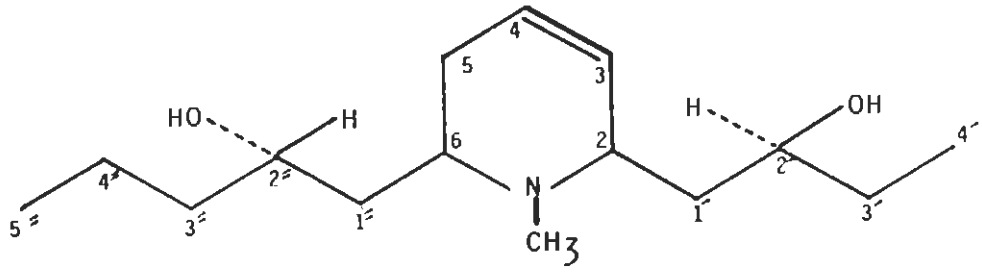
The ethanolic extract of the plant obtained was evaporated under reduced pressure to thick gummy material. The material obtained was defatted by partitioning between water and ethyl acetate. The aqueous portion thus obtained was basified to pH-9 and extracted with chloroform. On evaporation under reduced pressure this $CHCl_3$ extract afforded crude alkaloidal mixture.

The crude alkaloidal mixture of the plant was subjected to various chromatographic steps and it yielded two novel piperidine type alkaloids, andrachcine and andrachamine along with alkaloid AA-3. Due to paucity of amount isolated material only partial structure elucidation of compound AA-3 was possible.

ISOLATION AND STRUCTURE ELUCIDATION OF ANDRACHCINE

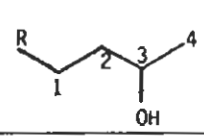
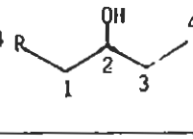
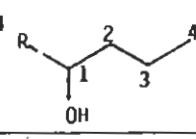
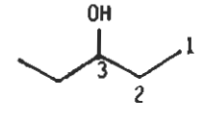
The alkaloidal mixture was submitted to flash chromatography using Si 60 (04-0063 mm) starting from chloroform and amount of MeOH was increased to 20% step wise with the addition of 5% methanol. This gave 5 fractions and fraction 2 was further chromatographed on flash chromatograph using chloroform-methanol 7.5:2.5 as eluate. This yielded 6 fractions and fraction 4 showing one major along with two minor alkaloids was further chromatographed on Lobar column. This chromatographic step yielded andrachcine as gum which did not crystallize. However the purity of the alkaloid was checked on TLC Si60 plates using various solvent system like $CHCl_3$-MeOH 9.5:0.5 9:1, 8:2, 8:2 + 0.1% NH_3. Benzene MeOH 8:2, 9:1 etc. The high resoltuion mass spectrum showed molecular ion peak at m/z 255.22408 corresponding to $C_{15}H_{29}NO_2$ (calcd. 255.21981), indicating the degree of unsaturation was 2. The presence of -OH group was inferred by I.R. spectrum of the compound in $CHCl_3$ which showed a broad peak between 3100-3200 cm^{-1}.

There was no significant absorption in U.V. spectrum other than the end absorption at 220 nm indicating the absence of conjugated double bond. The fragmentation pattern in mass spectrum of the compound indicated the presence of straight alkyl chain with hydroxyl group.



ANDRACHCINE

TABLE II

C.NO.	1	2	3	4	OBSERVED
					
1	δ 26.3	δ 32	δ 71.8	δ 9.8	δ 9.6
2	δ 44.8	δ 81	δ 44.8	δ 29.7	δ 30.00
3	δ 71.2	δ 32.9	δ 16.6	δ 73.8	δ 73.6
4	δ 24.1	δ 7.8	δ 13.7		δ 40.73

The fragments at m/z 240, 226 indicated the loss of CH_3 and C_2H_5 groups from molecular ion. The fragment at m/z 182 on peak matching was found to have an exact m/z value of 182.1545 suggestive of loss of a butanol ($C_4H_{10}O$) group from molecular ion.

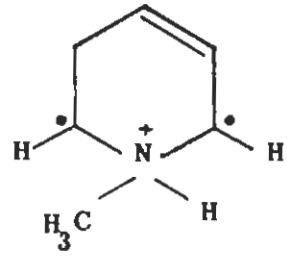
3 positional isomers of butanol i.e. butan-1-ol,2-ol, 3-ol were possible for fragment $C_4H_{10}O$, attached as side chain to the rest of the molecule.

For all these isomers ^{13}C chemical shifts were calculated using standard methods[14] (see table II).

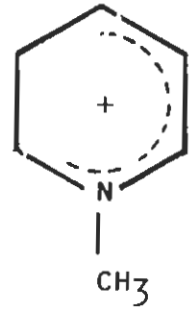
The table shows that the observed value of andrachcine are closest to those reported for 3-pentanol[15].

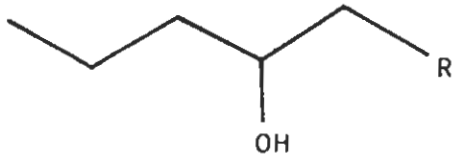
On further analysis of mass spectrum it was concluded that there is a CH_3-CH_2-CHOH-CH_2 group attached to the molecules as the presence of fragments at m/z 240, 226, 196 and 182 are due to sequential loss of CH_3, C_2H_5, C_3H_7O and $C_4H_{10}O$. This was also a supporting fact to place hydroxyl group at 3rd carbon atom of this alkyl side chain.

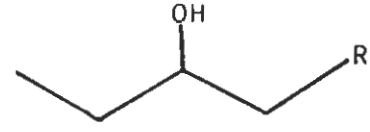
However fragments at m/z 212 and 168 were indicated the presence of another alkyl chain of 5 carbon atoms having one hydroxyl group at 4th carbon atom and fifth carbon atom is attached to the rest of the molecule. The fragment at m/z 96, on peak matching, found to have the exact value 96.082098 which was in close agreement for $C_6H_{10}N$



(Fig. Ia)







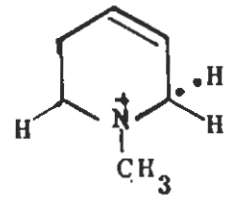
R = ANY SUBSTITUENT

TENTATIVE FRAGMENTS OF ANDRACHCINE

Fig.1

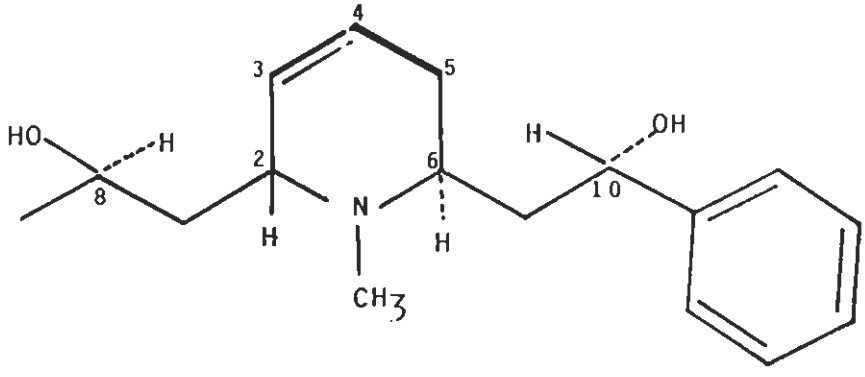
(calc. 96.081320). The most possible structure for this molecular formula was a protonated species having structure as shown in Fig. (Ia).

As our further studies showed this ion had the following formula.



On the basis of above evidences and observations following tentative fragments of the molecule (Fig. I) were deduced.

In ^{1}H-NMR ($CDCl_3$, 300 MHz) there was a sharp triplet was centered at δ 0.98 with integration of 3H (J = 7.4 Hz) and a destorted triplet centered at δ 0.91 (3H J = 6.7 Hz). These two signals indicate the presence of two methyl groups adjacent to methylenes. A singlet at δ 2.52 (3H) was indicative of presence of N-CH_3. This confirmed the position of methyl group in species shown in Fig. I, on nitrogen rather than on the carbon atoms of the ring system. The ^{13}C values for this species provided further support to the proposed structure. The observed values are δ 34.68, 63.12, 126.03, 124.62, 24.92, and 51.02 ppm for N-CH_3, C_2, C_3, C_4, C_5, and C_6 respectively. The downfield shift of C_2 and C_6 indicate the points of substitutions[10].



SEDENINE (FIG. II)

Two signals at δ5.63 (1H) and δ 5.88 doublet of doublets (1H) indicated the presence of double bond in the system which was further confirmed by the ^{13}C signals at δ 124.67 and 126.03 ppm. The only possibility to accomodate a double bond was in the piperidine ring. These signals were assigned to a double bond in piperidine ring betwen C_3 and C_4. Another broad signal at δ 3.48 (2H) was assigned to two protons at H-2 and H-6 with the assumption that both alkyl chains are attached on C-2 and C-6 of Δ 3 piperidine ring. Which is already proved by ^{13}C spectrum of the compound. This was further supported by the reported 60 MHz spectrum of sedenine (fig II)[17] in which both protons at 2 and 6 positions of piperidine ring, which are also sites for the attachment of side chains resonate at δ3.16 ppm as a broad signal.

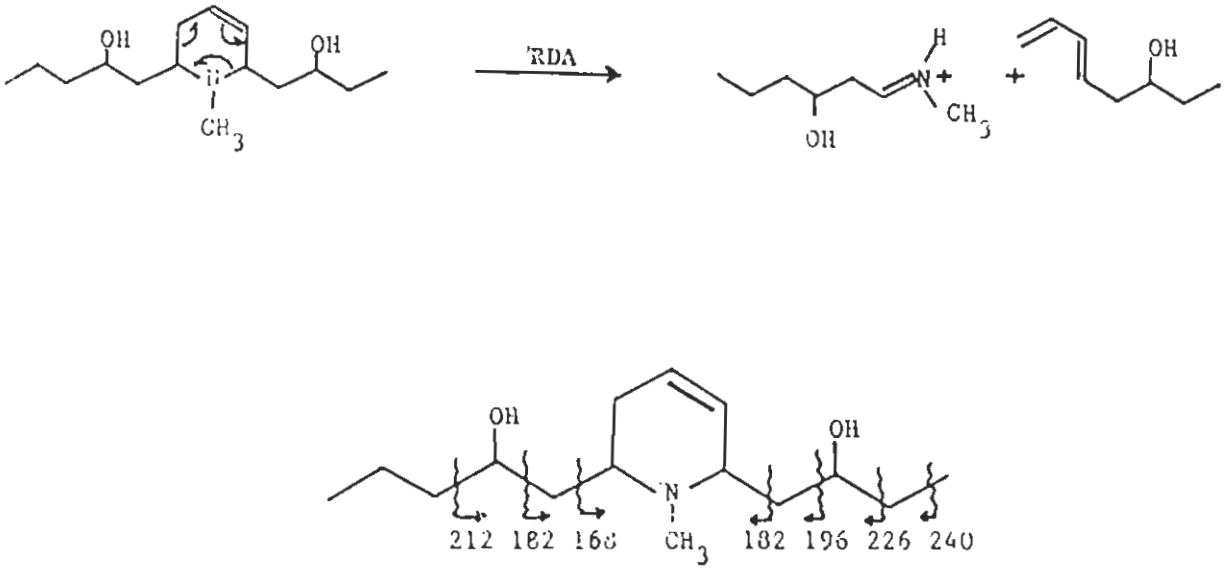
On irradiation at δ3.48 (H-2 and H-6) the signal due to H-3 collapsed into a clear doublet showing $J_{3,4}$ = 10.3 Hz whereas signal due to H_4 was ddd J = 10.3, 4.7, 2.2 Hz. On irradiation at δ 5.88 (H-4) signal at δ5.63 due to H-3 collapsed into a doublet with a very small coupling constant (J = 1 Hz). This irradiation experiment led to the conclusion that there is a Δ 3-piperidine ring having alkyl substituents attached to C-2 and C-6 as no coupling is evident. This was further confirmed by COSY-45 experiment.

A broad signal at δ3.77 (2H) was assigned to two protons geminal to -OH groups.

TABLE-III

^{13}C (75 MHz $CDCl_3$) ASSIGNMENTS OF ANDRACHCINE

C.NO	CHEMICAL SHIFT	C.NO.	CHEMICAL SHIFT	C.NO.	CHEMICAL SHIFT
$(N\text{-}CH_3)$	δ 34.68	$C_{1'}$	δ 40.74	$C_{1''}$	δ 38.2
C_2	δ 63.12	$C_{2'}$	δ 73.30	$C_{2''}$	δ 69.9
C_3	δ 126.03	$C_{3'}$	δ 30.83	$C_{3''}$	δ 38.9
C_4	δ 124.67	$C_{4'}$	δ 9.6	$C_{4''}$	δ 18.7
C_5	δ 24.92			$C_{5''}$	δ 14.13
C_6	δ 51.02				



Scheme I

The fragment at m/z 130 which on peak matching was found to have the exact m/z 130.12388 lead to formula $C_7H_{16}NO$ (calc. 130.123182). If it is assumed that alkyl chain C_4H_9O is attached at C_2 of Δ-3 piperidine ring, then this peak at m/z 130 will appear as a result of retro Diels - Alder fragmentation. If the $C_5H_{11}O$ alkyl chain is attached to C-2 it would have led to the formation of fragment at m/z 115 which was however not observed; this confirms that alkyl chain C_4H_9O is attached to C2 and $C_5H_{11}O$ chain at C6 of the piperidine ring. The other important fragments observed in mass spectra are shown in Scheme (I).

The configuration at C_2 and C_6 was assigned by comparing reported NMR of sedenine[1]. H-6 showed a broad signal with $W_{\frac{1}{2}}$ = 15 Hz due to trans diaxial coupling between H-6 and the axial H-5. In the case of andrachcine signal due to H_2 and H_6 was also broad with $W_{\frac{1}{2}}$ = 12 Hz. On the basis of this observation and considering biogenesis of compound trans configuration of alkyl chains at C-2 and C-6 was concluded. Trans configuration of sedenine is already confirmed by X-ray crystallography[18].

In ^{13}C spectrum of andrachcine (75 MHz) it was possible to assign all signals and chemical shift values were consistent to the proposed structure (Table-III). The absolute configuration of -OH group at alkyl chains was confirmed by application of Horeau's method[19]. This method is based on the reaction of racemic mixture of ± phenyl-butyric anhydride to secondary hydroxyl group. After completion of

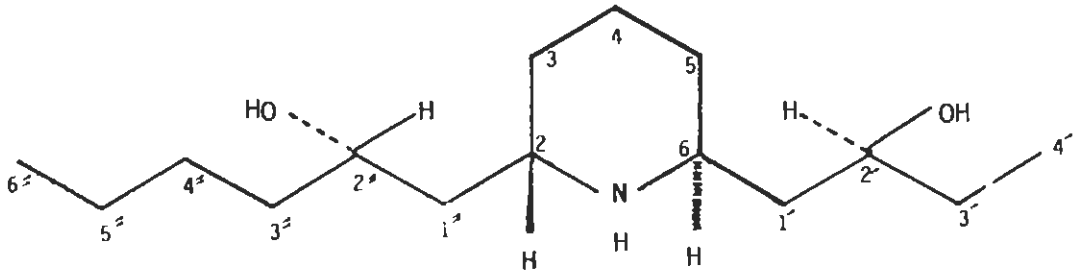
the reaction the mixture is quenched with water and the unreacted phenylbutanoic acid is isolated and the optical rotation of unreacted acid is determined. It is observed that + anhydride will react with + hydroxyl group and vice versa. The sign of isolated acid will be opposite to the configuration of hydroxyl group.

The isolation of + Phenylbutric acid and this assumption that both hydroxyl groups are reacting with equal rate due to similar environment it was concluded that both hydroxyl groups has "S" configuration at $C_{2'}$ and $C_{2''}$.

ISOLATION AND STRUCTURE ELUCIDATION OF ARNDRACHAMINE

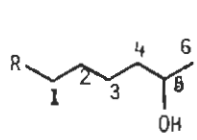
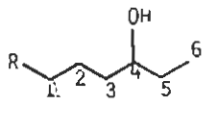
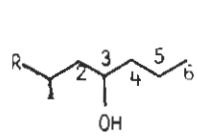
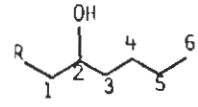
The crude alkaloidal mixture was subjected to flash chromatography using Si60 (04-0063mm) as stationary phase and $CHCl_3$ MeOH 8:2 as mobile phase, yielding 5 fractions. Fraction 2 was further chromatographed on flash chematograph using 8.5 : 1.5 $CHCl_3$, MeOH as eluate and this step afforded 5 fractions. Fraction 2 was further chromatographed by low pressure chromatography on Lobar Si 60 column using $CHCl_3$ MeOH 8.5:1.5. Fraction 3 of this step was subjected to HPLC on Si column using Z module (Waters Associates) with mobile phase 9.5:0.5 $CHCl_3$ MeOH with flow rate of 2ml/min. Andrachamine was obtained as fine needle like crystals , hygroscopic in nature, weighing 14 mg.

The molecular ion of the compound appeared at m/z 257. Peak matching of M^+ afforded molecular formula $C_{15}H_{31}NO_2$ showing 257.23461 (calc. 257.235466). Hydrogen deficiency index was 1. The I.R. ($CHCl_3$) spectrum exhibited a broad peak at 3100-3200 cm^{-1} signifying the presence of -OH groups. U.V. spectrum only showed end absorption at 220 nm. This indicates that there is no conjugated double bond system in the molecule. The presence of double bond was also ruled out, on the basis of ^{13}C and ^{1}H-nmr spectra of the compound.



ANDRACHAMINE

TABLE-IV

C.NO.	1 	2 	3 	4 	OBSERVED IN ANDRA-CHAMINE
6	δ 24.1	δ 8.00	δ 14.0	δ 14.0	δ13.9
5	74.6	23.1	26.8	22.2	23.0
4	33.2	81.1	32.9	26.8	29.4
3	23.5	42.2	81.5	42.3	39.6
2	22.8	16.9	33.2	72.0	71.0
1	23.1	23.1	16.9	33.0	31.0

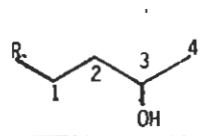
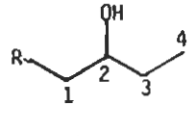
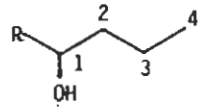
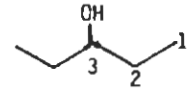
The fragmentation pattern in mass spectrum was very simillar to andrachcine and it was assumed that there is a piperidine ring in the compound. The strong peak at 82.065485 corresponds to the formula C_5H_8NO (calc. 82.965671) is apparently due to the ions arising as a result of cleavage of the two side chains from the molecular ion. Tnis conclusion was further confirmed by singals in ^{13}C NMR spectrum at δ59.05, 40.06, 18.7 showing that the ring is substituted at C_2 and C_6 position[16].

In the mass spectrum, peaks are present at m/z 242, 227, 214, 200, 170 and 156 indicating the loss of CH_3, C_2H_5, C_3H_7, C_3H_7, $C_5H_{11}O$ and $C_6H_{13}O$ respectively from the molecular ion. This was confirmed through high resolution mass spectrometery of these peaks. The loss of $C_6H_{13}O$ indicates the presence of a side chain with 6 carbon atoms contaning one OH group.

For the placement of the hydroxyl group on this six carbon atom alkyl chain all possible position were considered and their chemical shifts were calculated using standard calculation method[14]. Chemical shifts thus obtained are listed in Table IV.

On comparing observed chemical shifts with calculated values the most possible structure was 4 in Table having -OH group at C-2. Observed chemical shifts are 31.0, 71.0, 39.6, 29.4, 23.0, and 13.9 for C-1',

TABLE V

C.NO.	1	2	3	4	OBSERVED
					
1	δ 26.3	δ32	δ 71.8	δ 9.8	δ 9.6
2	44.8	81	44.8	29.7	30.00
3	71.2	32.9	16.6	73.8	73.6
4	24.1	7.8	13.7		40.73

C-2', C-3', C-4', C-5' respectively. In mass spectrum of the compound oxygen was found in fragments appeared at m/z 87.08078 and 101.096535 corresponding to $C_5H_{11}O$ and $C_6H_{13}O$ respectively. This signifies that hydroxyl bearing carbon appear at fifth carbon atom rather than 6th or any other carbon atom.

Another fragment at m/z 184.169520 suggested the presence of species $C_{11}H_{22}N_1O_1$ which could be due to the loss of C_4H_9O fragment from molecular ion. This leads to the assumption of the presence of another alkyl chain having 4 carbon atoms with hydroxyl group at one of these carbon atoms. It was possible to put hydroxyl group at 3 possible positions and chemical shifts of all these possibilities were calculated[14] (see table V).

On comparison of the observed chemical shifts of andrachamine structure II of Table and reported ^{13}C values of 3 propanol[15] it was possible to place -OH on C2 of the alkyl chain.

The assigned ^{13}C value are 40.3, 29.4, 9.9 ppm for C1', C2', C3', and C4' respectively. 73.6.

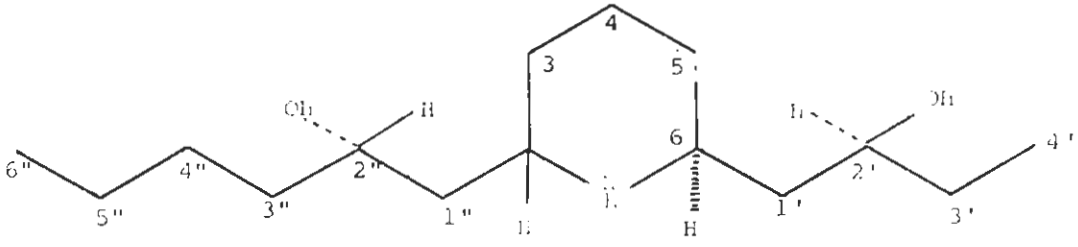
^1H-NMR ($CDCl_3$ 300 MHz) showed a signal centered at δ 0.91 (2 x CH_3) due to two overlapped triplets, indicating the presence of the two methyl groups adjacent to the methylene groups.

The presence of two hydroxyl groups was further confirmed by the appearance of a broad singlet at δ 3.85 with the integration of 2 protons geminal to two different secondary hydroxyl groups. Another broad signal with the integration of 2 protons appeared at δ3.02 and was assigned to C_2 and C_6 protons of 2,6 disubstituted piperidine ring. On the basis of above observation it was concluded that andrachamine contains 2, 6-disubstituted piperidine ring system. This was also confirmed by ^{13}C signals appearing at δ 59.05, 40.06 and 18.7 corresponding to $C_{2',6}$, $C_{3',5}$ and C_4 respectively. By irradiating signals at δ3.2037 and 3.850 no effect was observed on both of these signals. This indicates that protons at 2 and 6 of piperidine ring are not coupled with protons geminal to -OH groups. This observation support the conclusion based on ^{13}C values to place -OH groups at C-2′ and C-2″ in the alkyl chains. This was further confirmed by COSY-45 experiment which did not show any coupling between C_1', C_1'' and C_6 C_2 protons. The mass spectra of the compound also support this fact.

The ^{13}C ($CDCl_3$ 75 MHz) broad band showed 12 signals at δ 9.9, 13.9, 18.7, 23.0, 29.4, 31.0, 39.6, 40.0, 40.3, 59.05, 71.8 and 73.53 ppm.

Table showing ^{13}C chemical shifts in edited DEPT. δ

CH	59.0, 71.8, 73.5
CH_2	18.7, 23.08, 29.3, 31.0, 39.6, 40.04
CH_3	9.9, 13.9.



C_2 59.05, C_3 40.06, C_4 18.7, C_5 40.6, C_6 59.05, $C_{1''}$ 31.0, $C_{2'}$ 71.0, $C_{3'}$ 39.0, $C_{4''}$ 29.4, $C_{5''}$ 23.0, $C_{6''}$ 13.9, $C_{1'}$ 40.3, $C_{2'}$ 73.6, $C_{3''}$ 29.4, $C_{4'}$ 9.9

Figure-3

In the edited DEPT ^{13}C spectrum observed sets of carbon atoms are shown in Table. Table shows the ^{13}C-chemical shifts of andrachamine as separated into CH_3 CH_2, CH signals on that basis of edited DEPT spectrum. The compounds contains no quarternary carbon atoms because there were no extra peaks in the broad band ^{13}C-nmr spectrum not found in the DEPT spectrum.

On the basis of above observations and data it was possible to assign the ^{13}C values to the proposed structure (Fig. III).

Trans configuration of substitutents on C_2 and C_6 was conformed by the chemical shift of C_4 i.e. at 18.7. The reported value of C_4 in trans 2,6 di substituted piperidine is 19.5[20] whereas in cis 2,6 disubstitueted piperdine is 25.1 ppm which however was not observed.

Application of Horeau's method led to the isolation of + phenylbutric acid. It was assumed that both hydroxyl groups at $C_{2'}$ and $C_{2''}$ are reacting with ± phenylbutanoic anhydride with equal optical yield. It was concluded that both these chiral centers have S configruation.

ISOLATION AND PARTIAL CHARACTERIZATION OF AA-3

After repeated chromatographic steps (see scheme III) alkaloid AA-3 was obtained in very low amount (4.0 mg), showing a tendency towards crystallization. The high resolution M.S. of this compound showed molecular ion at 267.218903 corresponding to $C_{16}H_{29}NO_2$ (calc. 267.219817).

Hydrogen defficiency index of the compound indicated the 3 double bond equivalents. I.R. ($CHCl_3$) showed a broad peak at 3100 - 3600 cm^{-1}, signifying the presence of -OH groups.

U.V. of the compound showed end absorption at 220 nm.

Mass (E.I.) showed pattern very similar to the piperidine type of the alkaloids. Other important fragments were at m/z 224, 198, 180, 168, 139, 128, 124, 96, 112 corresponding to $C_{13}H_{22}NO_2$, $C_{11}H_{20}NO_2$, $C_{11}H_{18}NO$, $C_9H_{14}NO_2$, $C_9H_{15}O$, $C_7H_{14}NO$, $C_8H_{14}N$, $C_6H_{10}N$, $C_6H_{10}NO$ respectively. The composition of these ions was determined by peak matching of these fragments.

^{1}H-NMR of AA3 (300 MHz in $CDCl_3$) showed two overlapping triplets centerd at δ 0.85 and 0.88 integrating for 9 protons. This signal show the presence of three methyl groups adjacent to methylene.

The region between 1.00 PPM to 2.2 PPM was complex accomodating many signals. A ddd resonated at δ 2.46 integrating for one proton. One broad signal was centered at δ 3.32 partially overlaped by a doublet centered at δ 3.39 with integration of 1H. One septet showed its presence at δ 3.65 with integration of 1H.

Another doublet with integration of 1H resonated at δ 4.17.

One doublet of doublets integrating for 1H was present at δ 5.33 and one doublet of triplets resonated at δ 5.51 with integration of 1H.

By homodecoupling and COSY 45° experiment it was observed that the doublet of triplets at δ 5.51 on irradiation left an unresolved signal and signal at δ 5.33 which was dd collapsed into a triplet. Signal at δ 1.95 also show some changes but are not very clear. This was confirmed by COSY 45° experiment which was clearly showing coupling between 5.5, 5.33 and 1.95 ppm signals. Irradiation at δ 5.33 effected two signals at δ 5.51 and 2.46. The signal at δ 5.51 which was doublet of triplets collapsed into a doublet and the multiplet at δ 2.46 collapsed into a doublet. This indicate that signals at δ 5.33, 5.51 and 2.46 are coupled to each other. This conclusion was further confirmed by COSY

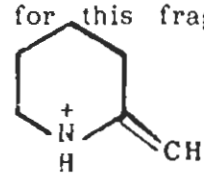
45° experiment. On irradiation at δ 4.71 signal at δ 3.9 collapsed into a singlet and this was also evident in the COSY 45° that these two sets of protons are coupled only to each other.

On irradiation at δ 3.65 we observed slight changes in the region 1.5-2.0 ppm. In the irradiation experiment this region was very complex it was concluded from COSY 45 that sigal at δ 3.65 is coupled to signals at δ 1.5 and 1.9 PPM.

The signal at δ 3.22 was partially overlaped by doublet centered at δ3.39 and it did not gave any information on irradiation. But in COSY 45° it was quite clear that this signal is coupled to the signals at δ1.5 which is a complex signal.

Signals at δ5.33 and δ5.51 with the multiplicity of dd and dt respectively were indicating the presence of double bond having a carbon atom with one H at one end and CH_2 group at the other end.

Mass fragment appearing at m/z 96.08192 was in very close range for $C_6H_{10}N$ (calc. 96.081320). As we do not have any signal for $N-CH_3$ so the most plausiable structure for this fragment was a charged species as shown in fig.



This was further confirmed by ^{13}C-NMR of the compound showed the carbon atoms at δ 58.62 and 60.90 which could be assigned to C_2 and

C_6 of the 2,6 disubstitueted piperidine[16]. The signals at δ 41.66 and 42.69 may be assinged to C_3 and C_5 atom of this ring and 18.49 was assignable to C_4 of this ring system and this final value also suggest the trans configuration of substituents at C_2 and C_6[20].

The status of these carbon atoms was confirmed by EDITED DEPT Experiment which proved that C_2 and C_6 are CH, C_3 C_5 and C_4 are CH_2 in nature.

Two methyl carbon atoms were resonating at δ 13.64 and 14.068 while another methyl carbon was at δ 9.70 but it was very uncertain to assign it due to very low intensity of peak.

Two carbon atoms at δ67.84 and δ 77.20 could be the carbon with hydroxyl group and it was clear in DEPT that -OH are secondary in nature.

One signal resonating at δ 84.79 showed the presence of CH_2 group which is next to oxygen atom and one other electron withdrawing group as the chemical shift is too low for a carbon atom next to oxygen atom.

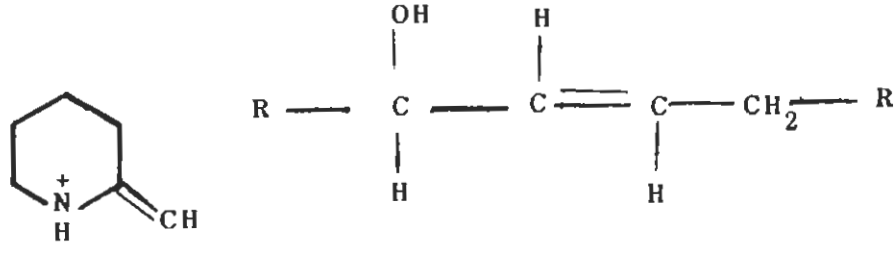
In ^{13}C Broad band spectrum two carbon atoms were resonating at δ132.84 and 131.56 signifying the presence of double bond which was also consistent to the ^{1}H-NMR. In GASPE ^{13}C spectrum of the same

compound these two carbon atoms were not visible and two new signals at δ 29.689 and δ28.556 showed their presence.

In order to confirm this ^{13}C spectrum using EDITED DEPT program was scanned and again carbon atoms appearing previously in broad band spectrum at δ132.84 and 131.56 did not show any signal. This point was one of the main problem in this compound as every time ^{1}H-NMR and ^{13}C broad band showed the presence of double bond but GASPE and edited DEPT both did not show these carbon atoms.

Mass spectrum of this compound showed pattern simillar to the piperidine type of alkaloids with straight chain substituents.

Important fragment appeared at m/z 267 M^{+}, 252, 238, 224, 210, 198, 168, 154, 128, 112, 98, 81, 67 and 56. On the basis of above data we can conclude following fragments in this molecule.



E X P E R I M E N T A L

GENERAL EXPERIMENTAL NOTE

The uv spectra were recorded on Shimadzu UV-240 Ultraviolet spectrophotometer. The infrared spectra were scanned on JASCO-IRA-I and JASCO A-302 infrared spectrophotometers.

^{1}H- and ^{13}C spectra were recorded on Bruker WP-100 SY and Bruker AM-300 nuclear magnetic resonance spectrometer. Mass spectra were measured at Varian MAT 112 and Varian 312 mass spectrometer connected to MAT 188 data system with PDP 11/34 DEC computer system.

The thin layer chromatography was performed on silica gel precoated TLC plates (E. Merck and Riedel De Haen). Low pressure chromatography was performed on LiChroprep Si60 (40-65 μm) column (E.Merck) using Duramat pump for solvent delivery.

Flash chromatography was performed on Eyela Flash chromatograph EF-10. HPLC was performed on silica column Z-Module (Water Associates) using CONSTAMETRIC III Pump (LDC - MILTON ROY), REFRECTOMONITOR III RI detector (LDC - MILTON ROY).

The solvents mostly used were obtained from E. Merck (Unless otherwise specified).

ISOLATION AND STRUCTURE ELUCIDATION OF ALKALOIDS OF ANDRACHNE ASPERA

PLANT MATERIAL

The plant Andrachne aspera was collected form the hills near University campus. Specimen was identified by the Taxonomist of the Department of Botany, University of Karachi. A voucher specimen was deposited in the Herberium of Department of Botany, University of Karachi.

ISOLATION OF CRUDE ALKALOIDAL FRACTION

The fresh plant material (40 kg) was initially separated into aerial parts and roots and were crushed separately with ultra turax homogeniser in 40 litres of 90% ethanol.

The homogenized material was filtered and filtrates were evaporated under reduced pressure to a thick gummy material separately.

These ethanolic extracts were partitioned between water and ethylacetate separately. The aqeuous layer was extracted 3 times with ethylacetate and basified with NH_3 upto pH 9.0. This basified aqueous portion was

extracted with $CHCl_3$. The crude alkaloidal mixture of aerial parts and roots was obtained.

By comparing on TLC using $CHCl_3$ MeOH, 8:2 and 9:1, Benzene MeOH 8:2 as mobile phase on Si60 plates it was found that both fractions are same and hence were mixed. None of the spot on TLC showed presence of U.V active compound under U.V. lamp at 254 nm.

The total yield of crude alkaloid was 5 grams.

ISOLATION OF ANDRACHCINE

Crude alkaloidal mixture was subjected to flash chromatography using $CHCl_3$ with step wise increment of 5% MeOH upto 20% MeOH. This gave 5 fractions.

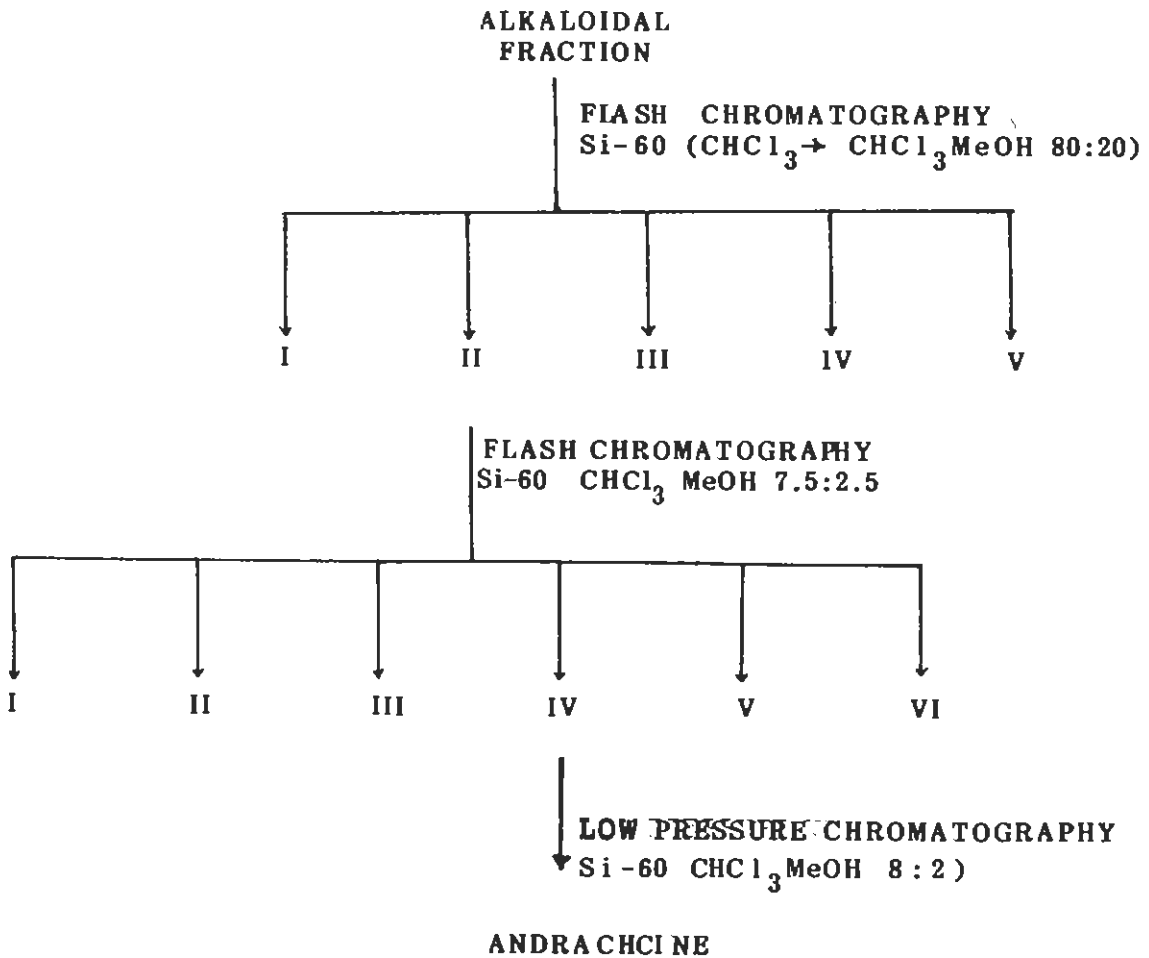
Fraction 2 was rechromatographed on silica gel column with flash chromatograph, using $CHCl_3$ MeOH 7.5 : 2.5 as eluate. This afforded 6 fractions and fraction 4 showed one major spot along with two minor spots on TLC. It was further purified by low pressure liquid chromatography using Lobar column Lichroprep Si 60 (40-65 µm) (E.Merck) using $CHCl_3$ MeOH 8:2 as mobile phase. This step gave andrachcine in pure form as a gum $[\alpha]_D^{25}$ - 87° (methanol C= 1.2). The purity of alkaloid was checked by TLC Si60 using $CHCl_3$ MeOH 8:2 and 9:1 as mobile phase.

I.R. ($CHCl_3$) showed a broad peak at 3100-3200 cm^{-1} (-OH).

U.V. showed only end absorption at 220 nm.

^{1}H-NMR $CDCl_3$ (400 MHz) δ 0.98 (t, 3H, J = 7.4 Hz) δ 0.91 (3H, J = 6.7 Hz) δ 2.52 (s, 3H, N-CH_3) δ 3.77 (b.s 2x1H H2′ and H2″) δ 3.48 (b.s. 2 x 1H, H-2 and H-6) δ 5.63 (1H, H-3) δ 5.88 (1H, H-4).

Isolation Scheme of andrachcine



^{13}C-NMR $CDCl_3$ (75 MHz) N-CH_3 (34.68) C_2 (63.12) C_3 (126.03) C_4 (124.67) C_5 (24.92) C_6 (51.02) C_1' (40.74) C_2' (73.30) C_3' (30.83) C_4' (9.6) C_1'' (38.2) C_2'' (69.9) C_3'' (38.9, C_4'' (18.7) C_5'' (19.13).

Mass M^+ 255, other important fragments appeared at m/z 240 (M^+-CH_3) 226 (M^+-C_2H_5) 212 (M^+-C_3H_7) 196 (M^+-C_3H_2O) 182 (M^+-C_4H_90) 168 (M^+-$C_5H_{11}O$) 130 (M^+-$C_7H_{16}NO$ R.D.A. Fragment) 110 (M^+-$C_8H_{18}O_2$) 96 (M^+-$C_9H_{20}O_2$) 58 (M^+-$C_{12}H_{22}NO$).

APPLICATION OF HOREAU'S METHOD

2 mg andrachcine was dissolved in about 0.2 ml of dry pyridine 2 molar equivalents of ± 2 phenylbutanoic anhydride were added. The reaction mixture was allowed to stand for 1 hour at room temp, 3 ml of water was added to reaction mixture and was allowed to stand for 3 hours at room temperature. It was basified with 0.1N NaOH solution and extracted 3 times with $CHCl_3$ to remove esters formed.

The aqueous phase was then acidified with 1.0 NHCl and extracted with benzene. Benzene extract was dried over anhydrous sodium sulphate, filtered and the volume adjusted to 1 ml reading at D line afforded + 1.399°.

ISOLATION OF ANDRACHAMINE

The crude alkaloidal mixture was subjected to flash chromatography on Si60 column using $CHCl_3$ MeOH 8:2 as mobile phase. This gave 5 fraction, the fraction II was further chromatographed on flash chromatograph using 8.5:1.5 $CHCl_3$ MeOH as eluate. This afforded 3 fractions. The fraction 2 of this step was further chromatographed by low pressure liquid chromatography on Labor Lichroprep Si60 column (E.Merck) using 8.5:1.5 $CHCl_3$ MeOH as eluate. This yielded 4 fraction, fraction 3 showed two alkaloidal spots with very close R_f value. These were finally separated by HPLC using straight phase column with 9.5:0.5 $CHCl_3$ MeOH as mobile phase with flow rate 2 ml/min.

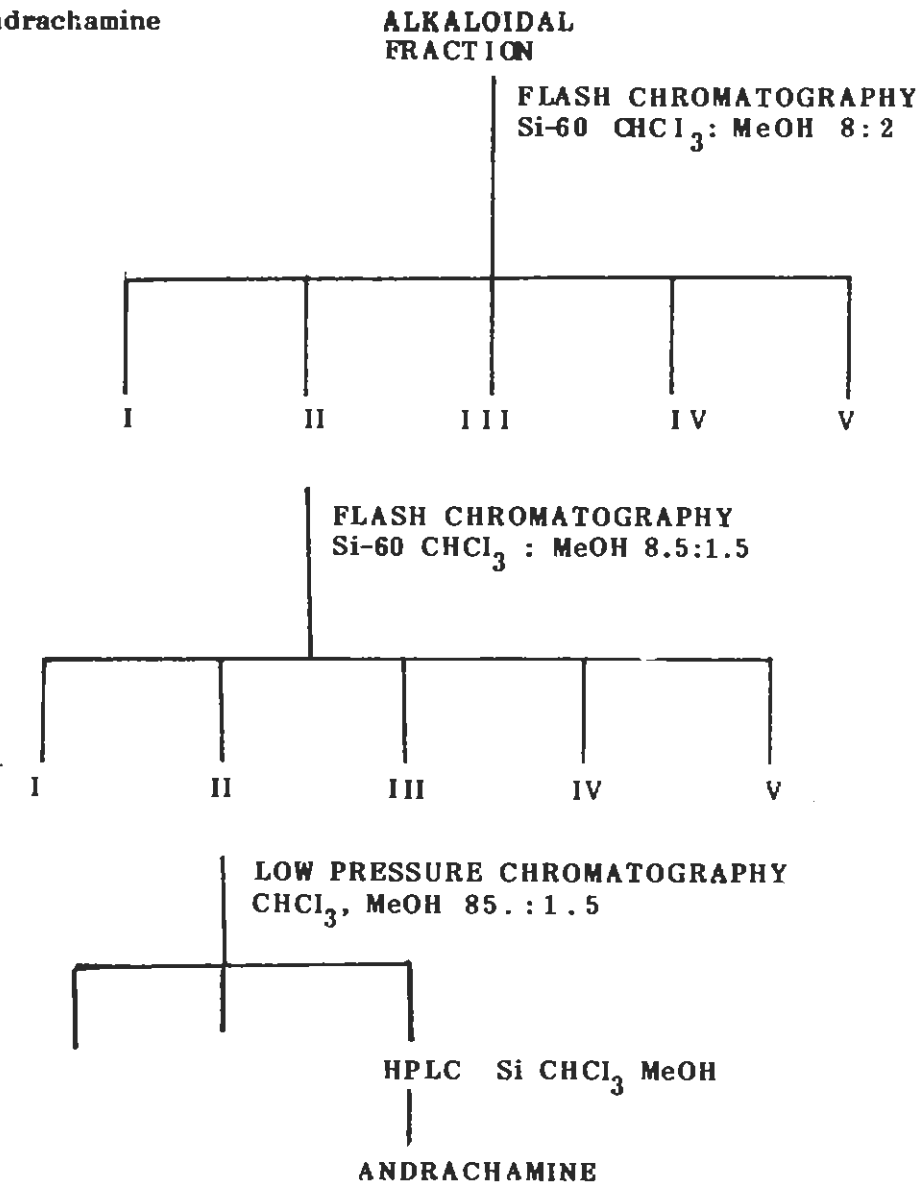
This gave us Andrachamine as needles in pure form which were hygroscopic in nature.

I.R. ($CHCl_3$) showed a broad peak at 3100-3200 cm^{-1} (-OH).

U.V. showed only end absorption at 220 nm.

^{1}H-NMR $CDCl_3$ (300 MHz) δ 0.91 (t, 2 x CH_3 J=7Hz) δ 3.20 (b.s. 2 x 1H H-2, and H-6) δ3.85 (b.s. 2x1H H-2' and H-2'').

Isolation Scheme of andrachamine



^{13}C-NMR $CDCl_3$ (75 MHz) C_2 59.059, C_3 40.06, $C_4$18.7, $C_5$40.06 $C_6$59.059, C_1'' 3.10, C_2'' 71.0, C_3'' 39.6 C_4'' 29.4, C_5''23.0, C_6'' 13.9, C_1' 40.3 C_2'73.6, C_3' 29.4, C_4' 9.9

Mass M^+ = 257 m/z other important fragments were at m/z 242 (M^+-CH_3) 227 (M^+-C_2H_5), 214 (M^+-C_3H_7), 200 (M^+-C_4H_9) 198 (M^+-C_3H_7O) 184 (M^+-C_4H_9O) 170 (M^+-$C_5H_{11}O$) 156 (M^+-$C_6H_{13}O$).

APPLICATION OF HOREAU'S METHOD

4 mg andrachamine was dissolved in 0.2 ml of dry pyridine in a small reaction vial and 2 molar equivalents of ± pher ylbutanoic anhydride were added and reaction mixture was left at room temperature for about 1 hour. 2.5 ml of water was added and was allowed to stard at room temperature for 3 hours.

This mixture was basified with 0.1N NaOH and was extracted with $CHCl_3$ to remove of esters formed.

The aqueous portion thus obtained was acidified with 1N HCl and was extracted with benzene three times. The benzene extract was dried over anhydrous sodium sulphate and filtered. Volume of this was made to 2 ml and reading at D line afforded + 0.899° signifying that both -OH groups are "S" in configuration

REFERENCES

1. G.A. Cordell, **"Introduction to Alkaloids" a biogenetic approch**, page **1** (John Willey and Sons. New York).

2. Encyclopeadia **Britanica** Vol. **1** 595 (15th Edition Pub. Encyclopaedia Britanica Inc.)

3. Trevor Robinson, In "The Biochemistry of Alkaloids. page **2** (Springer Verlag, New York Inc.)

4. G.A. Cordell, **"Introduction to Alkaloids' a biogenetic approach** page **5-6**, (John Willey and Sons, New York).

5. G.A.Cordell, **"Introduction to Alkaloids" a biogenetic approach** page **2** (John Willey and Sons.)

6. R.R. Stewart, **Flora of West Pakistan** (Ed. E. Nasir and S.I. Ali) page **444** (1972) (Fakhri Printing Press, Karachi, Pakistan).

7. S.M. Jafri, **"The Flora of Karachi"** page **195**. (The Book Corporation Karachi).

8. G.Gopalan, **Medicinal Plants of India** Vol. **I** page 64 (Indian Council of Medical Research, New Dehli (1976).

9. H.R. Derasari and J.H. Khalsa. **Indian J. Pharm. 28**(9), 237 (1966).

10. N.A. Qazilbash, **Pharm. J.**, 163-108 (1949).

11. V.V.Vil'yams, L.P. Nadovich, B.K. Rostotskii and I.A. Gubanov, **Khim. Prir. Soedin 2**(4), 257 (1966).

12. R.E. Churchward and E.H.Gurney, **Queensland Ag. J., 50**, 180, (1938).

13. M. Israr Khan, M.Ikaram and S. Fazal Hussain, **Planta Med., 47**, 191 (1983).

14. Pretsch, Clepc, Seibl, Simon, **"Tabellen Zur Strukturaufklarung Organischer Verbindungen mit Speketroskopischen Methoden.** page **C-10** (Springer Verlag Berlin).

15. Robert M.S. Silverstein and G. Clayton Bassler "Spetrometric identification of Organic compounds. 5th ediction page 268 (1984).

16. Maurice Shamma and David M. Hindenlang **"Carbon-13 NMR shift assignment of Amines and Alkaloids** (Plenum Publishing Corporation, New York).

17. B.Frank **Ber**, **93**, 2361 (1960).

18. C.Hootele, B. Cloau, F. Halim, J.P. Decleroq, G. Germaian and M. Van Meerssche. **Tet.Lett.**, **21**, 5063 (1980).

19. A. Horeau. (Stereochemistry Fundamentals and methods (Ed. Henri. B. Kagan) Vol. **3**, 51 (Georg Thieme Publishing Stuttgard 1977).

20. E.L. Eliel, D. Kandasamy, Chen-yu-yen, and K.D. Hargrane. **J.Am.Chem.Soc., 102:11** 3698 (1980).

C H A P T E R - 2

INTRODUCTION

The oceans of the world, which cover 75% of the planet earth are believed to be the place from where life emerged. About 90% of the living species on this earth are found in the oceans.

The environment and ecosystem in oceans is totally different as compared to the terrestrial environment.

The presence of secondary metabolites in the living organisms depend on the conditions available to the animal to live. This different environment available to the marine animals provided a chance to produce an enormous number of new secondary metabolities having unusuall functionalities and novel physiological activities.

It was also observed that the animals found in the oceans react and behave in totally different manner. In order to overcome certain difficulties in feeding and movement they have also developed certain mechanisms to facilitate their living.

This unusual behaviour of marine animals has been of great interest for mankind from the begnning. With the development of natural science biologists and chemists have start showing interest to understand the mechanism of and to find agents responsible for this special behaviour.

Chemistry which is interdisciplinary subject in its nature played a very vital role to explore and understand this kind of phenomenon.

Starfishes (Echinodermata) show one of the typical behaviour. It was observed that some marine invertebrates e.g. some sea anemones, sea urchins, some starfishes and particularly mollusks show"escape response" and "avoidence reaction" in the presence or on contact with other star fishes[1,2,3,4,5].

On the other hand starfishes in dried form and their extracts are also reported to be used to kill fly maggots in Japan. It was suggested that this extract act as surfactent agents in preventing ecdysis of maggots[6]. Toxicity of star fishes is a very common observation over many years. Cooked and dried star fish fed to domestic animals caused vomiting, retarded growth and even death in some cases. Hashimoto also mentioned the attempt to make meal from starfishes by some group but it was found to cause nausea[7,8,9]

In 1960 Hashimato reported that the toxicity is due to the compounds similar to plant saponins[10]. This was based on the observation that water containing dead star fish also start foaming and on extraction of star fishes by the same procedure being used to extract plant saponins they found a mixture of saponin like compounds which was toxic and also haemolytic. In 1962 Mackie et al. reported that in some species of star fishes asterosaponins are the compounds to evoke escape response in marine invertabrates[11].

Keeping haemolytic and toxic properties of the saponins in mind one can say that the major function of saponins could be chemical defense against predators, infactious microorganisms, parasites etc. Even the star fish larvae are reported to have the ability to repell predatory planktivorus fishes[14].

On the other hand potential prey species have developed senses to detect the approach of predatory star fishes by sensing these saponin like water soluble compounds.

This help these prey species to protect and to give them a better chance to survive and escape[13]. Later some groups found that fairly

dilute solution of saponins are toxic to many marine invertebrates[7,10,12,13]. It was also observed that amount of saponins in different body parts of starfish varies during the annual metabolic cycle[13] and saponins not only play their part as defensive weapon but they also act as regulatory factor in the body of the animal.[13] The reports regarding regulatory function of saponins are however contradictory . Some authors suggest saponins as inhibitors during spawning and others suggest that these chemicals promote the reproductive process[13,16]. The toxicity of these compounds attracted attentions of pharmacologists. They were interested to see their effect on different testing systems and they found that extracts of startfishes and pure saponins showed pharmacological activities like antineoplastic,[17] toxicity towards human K.B. carcinoma cells in vitro[17], and inhibition of influenza virus multiplication[19].

The saponins of the starfish Asterias forbesi are reported to show anti-inflammatory activity[20]. Nigrelli et al. reported the considerable cytotoxicity of some asterosaponins towards human KB oral carcinoma cells in vitro. On the other hand Owlen et al. found no anti-tumour activity (P-388 leukemia) in a soponin fraction from Asterias forbesi[21].

Acanthaster planci, the multiarmed starfish species found almost at all coasts of the Indian and western Pacific Oceans is also reported with

high degree of toxicity. It is reported that even on touching this star fish a great pain is felt which may last for hours and occasional'y area around the wound will swell, lose sensation and appear to be paralysed. Some times these symptoms may be accompanied by nauesa and vomiting[25]. Later Acanthaster planci soponins exhibited weak anti-fungal activity[18] against Candida albicans and Saccharomyces cerevisiac.

Many starfish extracts are reported to show haomolytic activity with fish and mammalian erythrocytes. Lymphocytes were found more stable in the same solution[13]. Mackic et al. proposed that cytolysis occur due to removal of cholesterol from cell membrane. This ruins the osmoregulatory function of the cell membrane. The presence of 7 sterols rather than 5 in other animals and presence of cholesteryl sulfate in star fish could be the reason towards the immunity of star fish towards asterosaponins. This was concluded from the experiment in which addition of cholesterol to the haemolytic mixture reduced the rate of haemolysis and this fact that 5Δ cholest 7 en-3 -ol does not form a complex with digitonin [16].

Thus the toxicity and pharmacological activity of star fishes prompted many groups to work in this field to explore the compounds responsibile for these kind of activities.

TABLE-I

RECENT EXAMPLES OF ASTEROSAPONINS

Species, saponin name	mp °C	$[\alpha]_D$ (H_2O) (°C)	Carbohydrate components †	References
Acanthaster planci				
AP-I	228-235	-	-	19,38
AP-II	215-219	-	Quin-Fuc (2:1)	37,38
Thornasteroside A	203-204	-7.0(25)	Quin-Fuc-Xyl-Gal (2:1:1:1)	39
Thornasteroside A	200(dec)	-4.0(22)	Quin-Fuc-Xyl-Gal (2:1:1:1)	40
Mixture	180-185	-	Quin.Fuc.Xyl.Gal.Glu.Arab	41
Aphelasterias japonica				
-	-	-	Fuc.Gal	42
Archaster angulatus				
-	-	-	Quin.Fuc.Glu	42
Asterias amurensis				
Asterosaponin A	185-190(dec)	+0.03(20)	Quin-Fuc(2:2)	43
Asterosaponin B	189-191(dec)	-2.66(20)	Quin-Fuc-Xyl-Gal(2:1:1:1)	44
Glycoside B_2	-	-	Quin-Xyl-Gal(3:1:1)	45
Mixture	-	-	Quin.Fuc.Xyl.Gal	43
Mixture	-	-	Quin.Fuc.Xyl.Gal	46

Table-I (Contd.)

Asterias forbesi				
AF-I	Amorph.	-	-	19,38
AF-II	205-210	-	-	38
AF-III	219-223	-	-	19,38
Asterias rubens				
-	-	-	Quin.Fuc	11
Asterina pectinifera				
ASP-I	181-185	-	-	19,38
ASP-II	229-232	-	-	19,36
Mixture	-	-	Xyl.Glu.Rham	10
Asteropsis corinifera				
-	-	-	Glu	42
-	-	-	Quin.Fuc.Xyl	42
Coscinasterias calamaria				
-	-	-	Fuc.Xyl.Glu	42
-	-	-	Fuc.Gal	42
Echinaster sepositus				
Sepositoside A	-	-68.5	Gal-Glu-Glucur (1:1:1)	47
47b	-	-56.5	Gal-Glu-Glucur (1:1:1)	48
17c + 47d (2:1)	-	-71	Gal-Glu-Glucur (1:1:1)	48

Table-1 (Contd.)

Evasterias retifera				
-	-	-	Quin.Fuc.Glu	42
Evasterias troschelii				
-	-	-	Quin-Fuc-Xyl(9:9:2?)	49
Lethasterias fusca				
-	-	-	Fuc.Xyl.Gal	42
Linckia guildingi				
Asterosaponin 1	222-225	-22.5(20)	Quin.Fuc.Xyl	50
Linckia laetigata				
-	-	-	Quin.Fuc.Xyl	42
-	-	-	Quin.Fuc.Xyl.Glu	42
Luidia maculata				
-	194-196(dec)	-	Quin.Fuc.Xyl.Gal	40
-	198-201(dec)	+25(19)	Quin.Fuc.Glu	40
Lysastrosoma anthosticta				
-	-	-	Quin.Fuc.Xyl.Gal	42
-	-	-	Quin.Fuc	42
-	-	-	Quin.Fuc.Xyl.Gal	42

Table-I (Contd.)

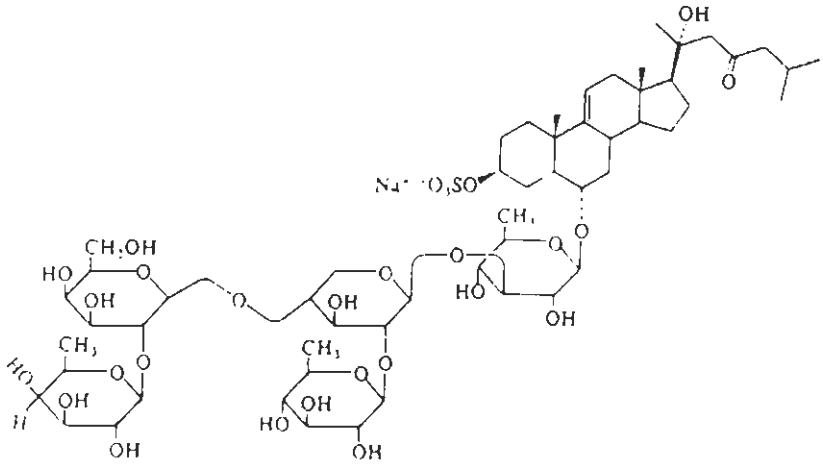
Marthasterias glacialis				
Glycoside M_1	-	-	Quin.Fuc.Xyl	11
Glycoside M_2	-	-	Quin-Fuc-Glu(2:1:1)	51
Ophidiaster hemprichi				
-	-	-	Quin.Fuc.Xyl	42
Patiriella calcar				
Calcarsaponin	199-200(dec)	-	Quin.Fuc.Gal(2:1:1)	52
Pycnopodia heliantnoides				
Mixture	-	-	Xyl.Glu.Rham.Unident	53

+Abbreviations: Quin.quinovose; Fuc.fucose; Xyl.xylose; Gal.galactose; Glu.glucose; Arab.arabinose; Rham.rhamnose; Glucur. glucuronic acid.

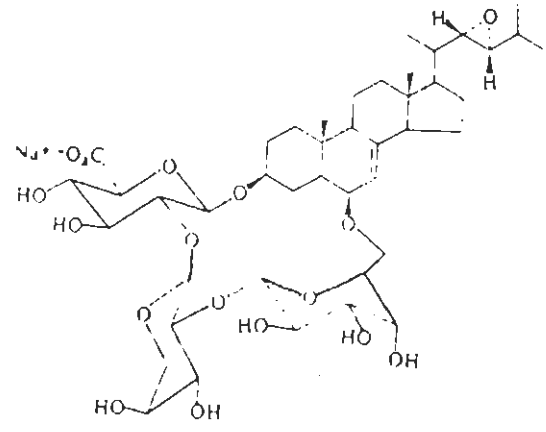
Hashimoto et al. in 1960 isolated for the first time crude soponin mixture from dried star fish using same isolation procedure being used to isolate plant saponins. This isolated mixture was found to be toxic and hemolytic in nature[26].

Uptil now a large number of saponins and glycosides are isolated from various species of starfishes. Some recent examples are listed in Table (1). Saponins having steroidal or triterpenic aglycone attached to sugar moiety, which are water scluble compounds, found commonly in terrestrial plants but in animal kingdom are found only in Echinodermates. The living Echinoderms contain five classes Crinoidea (see lilies) Echinoidea (sea cucumber) Echinaidea (sea Urchins, sand dollars and heart urchins) Ophiuroidea (Brittle star) and Asteroidea (star fishes). It is established that only Asteroidea and Holothuraidea have appricable amount of saponins.

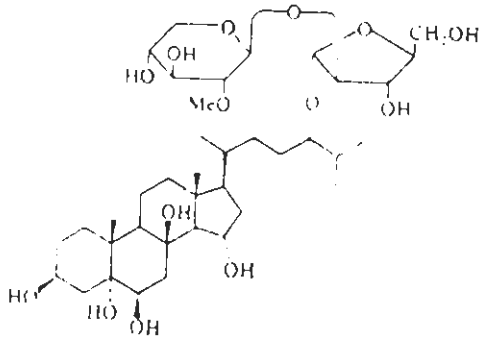
Steriodal Glycosides on the basis of their chemical structure can be divided into three classes a) Steroidal glycoside sulphates (Astero soponins) (I) b) Steroidal cyclic glycosides (II) c) Glycosides of polyhydroxy steroids (III)



I



II



III

PRESENT WORK

Keeping in view the pharmacological activity and toxicity of starfish saponins and this fact that no saponin has been isolated from starfish Porania pulvillus present work was carried out. Chemical literature indicated the detection of Gallium (0.07 ppm) by spectrophotometeric method in porania pulvillus[23].

In 1977 Mackie et al. reported two steroids marthasteron (4) and dihydro-marthasterone (5) from starfish[16] porania pulvillus.

These were the first steroids from asterosoponins[24] for which structure was completely determined.

PORANIA PULVILLUS

Porania pulvillus a brightly coloured starfish, belonging to the phylum Echinodermata, subphylum Asterozoa, class Stelleraidea, sub class Asteroidea order Spinulosida and Family Poranidae.

It is usually 15 cm in diameter and is acushion like deep bodied starfish with prominent gills. It feeds on small organic particles which are

caught in strings of mucus and transferred to the mounth by cilary action. It is usually found on rocky bottom at the depth of 20-25 meters[27].

The material for present study was collected from Koster Fiord at Northern west coast of Sweden. The organisms were collected by SCUBA diving from 15-25 m depth and were immediately frozen in dry ice. The starfish was identified by Dr. Lars Afzelius of Tjarno Marine Biology station and finally were stored at -23°C.

ISOLATION AND CHARACTERIZATION OF STEROIDAL GLYCOSIDES OF PORANIA PULVILLUS

1.3 kg Porania pulivillus was extracted with water. The aqueous extract was desalted by passing through Amberlite XAD-2 column and the adsorbed organic material was eluted with methanol. The methanolic eluate was chromatographed in different steps using various chromatographic techniques as shown in Scheme I. Details of isolation procedure are discussed in the Experimental. This yielded three glycosides provisionally named as glycoside 1,2 and 3.

GLYCOSIDE 1

This compound was isolated from glycosidic fraction of LH60 and was further chromatographed on D.C.C.C. (See Scheme II) and was finally purified by HPLC using μBondapack C-18 column. The amount obtained was 3.2 mg which did not crystallize $[\alpha]_D^{20}$ = - 21.2° (C = 1 MeOH). The FAB mass spectrum of this compound exhibited M^+ at 753 $[M+Na]^+$ corressponding to molecular formula $C_{38}H_{66}O_{13}$. Other important fragments appeared at m/z 170 [147 + Na]$^+$ due to species having formula $C_6H_{11}O_4^+$, 186 [163+ Na] due to formation of ion $C_6H_{11}O_5^+$. This signifies the presence of hexose or O-CH_3 pentose. Methoxypentose was a favourable choice due to the presence of -OCH_3 signal in ^1H NMR at δ 3.62. The ion at m/z 139 [116 + Na] was due to the presence of $C_5H_8O_3^+$ and 155 [132 + Na] due to $C_5H_8O_4^+$. this signified the loss of pentose from the molecular ion. The fragment at m/z 302 [295 + Na] was due to $C_{11}H_{19}O$ and m/z 318 was due to [295 + Na] corresponding to $C_{11}H_{19}O_9$. This indicated the presence of pentose and -OCH_3 pentose linked glycosidically to each other and they are in turn linked glycosidically to rest of the molecule with formula $C_{27}H_{47}O_4$. A small portion of compound

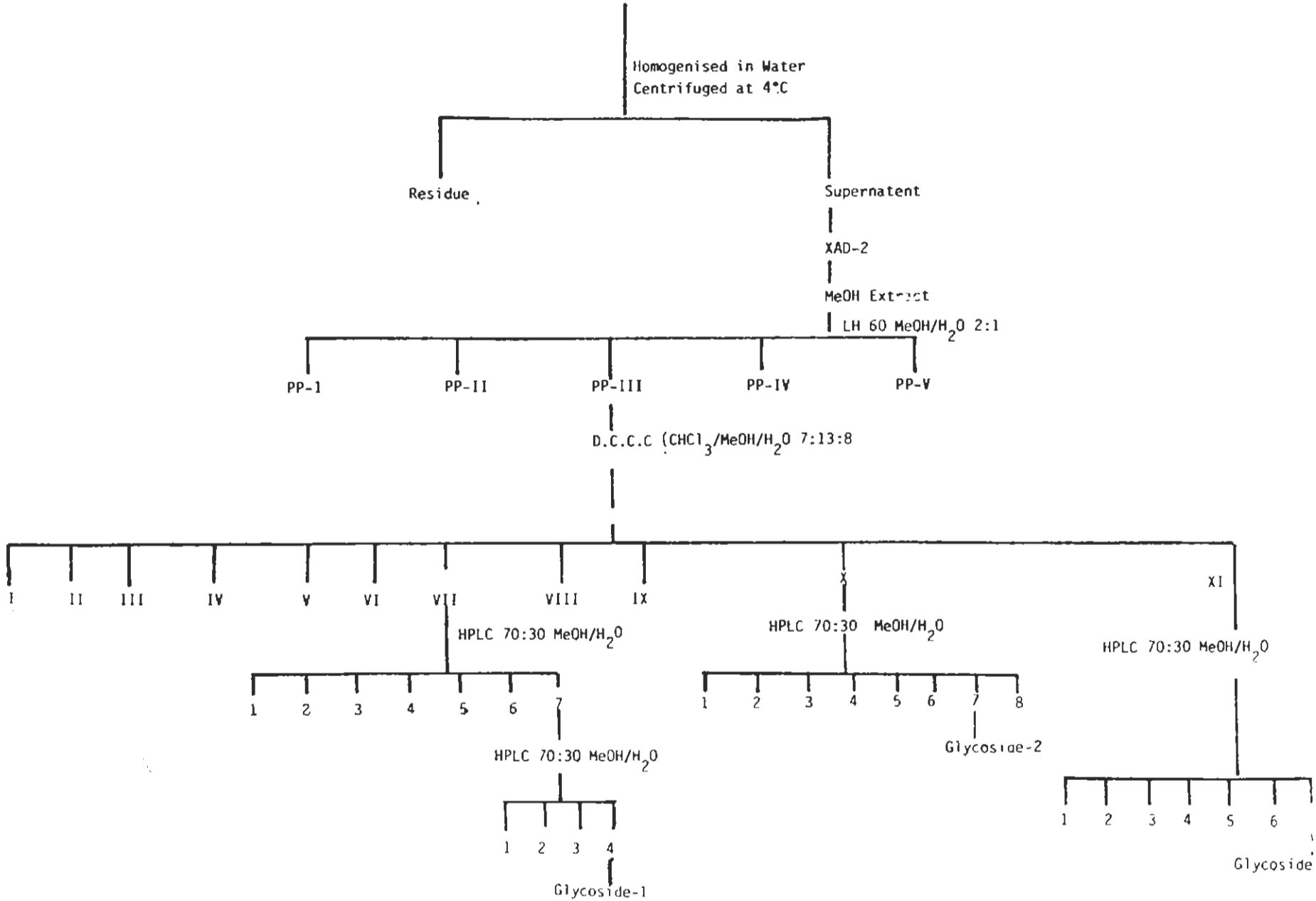

Homogenised in Water
Centrifuged at 4°C
Residue
Supernatent
XAD-2
MeOH Extract
LH 60 MeOH/H2O 2:1
PP-1
PP-II
PP-III
PP-IV
PP-V
D.C.C.C (CHCl3/MeOH/H2O 7:13:8
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7
HPLC 70:30 MeOH/H2O
1 2 3 4
Glycoside-1
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7 8
Glycoside-2
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6
Glycoside

TABLE-II

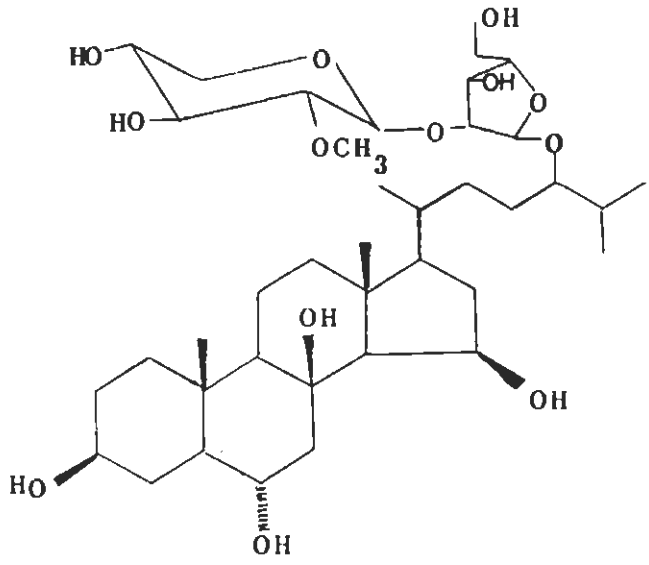
^{13}C CHEMICAL SHIFT OF SUGARS IN NODOSOSIDE AND GLYCOSIDE 1

SUGAR	C. NO.	NODOSOSIDE	GLYCOSIDE 1
ARABINOSE	1	δ 107.6	δ 107.9
	2	93.1	92.66
	3	77.6	77.90
	4	85.0	84.02
	5	62.4	62.77
2-OMe xylose	1	105.2	105.25
	2	84.1	84.88
	3	77.8	77.48
	4	71.0	71.26
	5	67.1	66.96
	OMe	60.7	61.06

TABLE-III

^1H NMR ASSIGNMENTS OF SUGARS IN NODOSOSIDE AND G-LYCOSIDE 1

SUGARS	^1H NO.	NODOSOSIDE	GLYCOSIDE 1
ARABINOSE MOIETY	H-1	δ 5.146 (s)	δ5.12 (s)
	H-2	4.153 (d, J=3.80 Hz)	4.10(d, J = 3.70Hz)
	H-3	4.129 (dd, J = 7.20,3.80Hz)	4.02(dd J = 7.1, 3.70Hz)
	H-4	4.034 (ddd, J=7.20,4.60,3.52Hz)	3.97(ddd, J = 7.1,4.5, 3.4Hz)
	H-5	3.83(dd, J = 12.5, 4.60Hz)	3.80(dd, J = 12.4, 4.5Hz)
	H-5	3.76 (dd, J = 12.50, 3.52Hz)	3.67 (dd, J = 12.4, 3.4Hz)
2-O-Methyl xylose Moiety	H-1	4.53(d, J = 7.7Hz)	4.45 (d, J = 7.5Hz)
	H-2	3.036(dd, J = 7.75, 9.02Hz)	2.90 (dd, J = 7.5, 9.1Hz)
	H-3	3.472 (dd, J = 9.02, 9.02Hz)	3.43 (dd, J = 9.1, 9.1Hz)
	H-4	3.631 (ddd, J = 10.3, 9.02, 5.65Hz)	3.75(dd, J = 10.1, 9.1, 5.5Hz)
	H-5ax	3.261 (dd, J = 11.6, 10.3Hz)	3.17 (dd, J = 11.4, 10.1Hz)
	H-5eq	3.90 (dd, J = 11.65, 5.65Hz)	3.70 (dd, J = 11.4, 5.5Hz)
	OCH	3.60 (s)	3.62 (s)
		(500 MHz CD_3OD)	(400 MHz CO_3OD)



GLYCOSIDE-1

(ATTENUATOSIDE-A1)

was subjected to acid hydrolysis and then reduced with $NaBD_4$ and finally was acetylated. This acetylated mixture was submitted to GC/MS and it was revealed that one sugar is 2-O-methyl pentose showing fragment at m/z 261 (M^+-CHD-OAc) and other is pentose showing fragment due to M^+ - CHODAc at 288. For further confirmation of sugars these derivatives were co-chromatographed on G.C with standard samples and it was found that one sugar is 2-O-methyl xylose and other is arabinose.

In order to confirm, the ^{13}C signals due to these two sugars were compared with spectrum of nodososide[28] a glycoside from starfish protoreaster nodosus. It was found that the literature values are in very close agreement to the observed chemical shift (see table-II).

The proton NMR signals of glycoside 1 and nodonoside were also in close agreement. In case of glycoside 1 coupling constants and chemical shifts of both these sugars were consistant to the asssigned values for both sugars in nodososide (see table-III).

On the basis of detailed ^{13}C and ^{1}H analysis it has already been proved that the arabinose and 2-O-methyl xylose are in furanose and pyranose forms respectively. This has also been established that 2-O-

TABLE-IV

SINGLE FREQUENCY OFF RESONANCE DECOUPLED ^{13}C SPECTRUM OF GLYCOSIDE 1

C NO	CHEMICAL SHIFT δ	MULTI-PLICITY	C NO	CHEMICAL SHIFT δ	MULTI-PLICITY
1	39.49	t	21	19.07	q
2	31.61	t	22	33.00	t
3	72.24	d	23	28.77	t
4	32.40	t	24	84.64	d
5	53.95	d	25	31.51	d
6	67.70	d	26	18.27	q
7	49.4	t	27	18.27	q
8	77.48	s	1	107.91	d
9	57.56	d	2	92.66	d
10	38.02	s	3	77.90	d
11	19.75	t	4	84.02	d
12	43.47	t	5	62.72	t
13	44.44	s	1	105.25	d
14	62.72	d	2	84.88	d
15	71.25	d	3	77.48	d
16	42.53	t	4	71.26	d
17	58.14	d	5	66.96	t
18	16.52	q	OCH_3	61.06	q
19	14.06	q	OCH_3		
20	36.44	d			

methylxylose is linked to arabinose by 1→2 linkage and arabinose is glycosidically linked to aglycone moiety[28].

On comparing ^{1}H-NMR (400 MHz) it was concluded that aglycone is also simillar to attenuatoside A-1[28], a member of cholestane structure. Its off resonance single frequency decoupled spectrum showed the presence of 5 hydroxyl groups, 4 of which are secondary and one is tertiary (see table-IV).

Three doublets centered at δ 0.94, 0.96 and 0.98 (J = 7 Hz) showed the presence of δ 27,26, and 21 secondary methyl group of steroidal aglycone. Two singlets resonating at δ1.02 and 1.32 showed the presence of 18 and 19 angular methyl groups tertiary in nature.

The multiplet at δ3.45 showed pattern usually observed for A/B trans 3-β hydroxy steroids. The position and stereochemistry of 6 hydroxyl group was also established by the signal appearing at δ 3.7, which showed multiplicity ddd J = 12.0 and 4.0 Hz due to the axial proton geminal to 6-α-hydroxy group[29]. Apperance of a doublet of doublets at δ 2 42 (J = 12, 4.0 Hz) due to βH-7 was also a supporting evidence for α-hydroxyl group at C_6. This is possible if βH-7 is in such an angle that there is an interaction with 6α-hydroxy group.

The signal due to H-7 α is masked with upfield signals ^{13}C chemical shift for C_6 also indicate the presence of -OH group at 6th position of aglycone. It appeard at 67.70 rather than 29.2[30].

The third hydroxyl group which is tertiary in nature was placed at C_8 of steroidal moiety which is a common feature of starfish saponins and poly hydroxysteroids[30]. This was further confirmed by measuring spectrum in pyridine d-5. It was observed that signals due to both angular methyles shifted 0.3 ppm. This shift is due to 1,3 diaxial interaction of these methyl groups with tertiary hydroxyl group at C-8 which is suffering hydrogen bonding with pyridine solvent and is called pyridine induced solvent shift[31].

A typical hydroxy methyne signal at δ4.5 was observed when signal at δ2.90 was irradiated and signal due to H-1 of xyloside at δ 4.45 collapsed into a singlet. The shape and chemical shift of this signal suggested the presence of 15 β hydroxy group[29]. The downfield shift of 18 methyl signal and ^{13}C chemical shift also support the presence of 15β hydroxy group in the compound.

The position of 5th secondary hydroxyl group was determined by comparing spectrum with nodososide[28] and attenuatoside A-1[32] with glycoside 1. It was found that 5th hydroxyl group is at C-24 and that is

TABLE-V

^{1}H NMR OF ATTENUATOSIDE A-1 AND GLYCOSIDE 1

ATTENUATOSIDE-AI δ	GLYCOSIDE 1 δ
0.78 (27-CH_3, d J = 6.9)	0.94 (27-CH_3, d, J = 7Hz)
0.912 (26-CH_3, d J = 6.7Hz)	0.96 (26-CH_3,d, J = 7Hz)
0.910 (21-CH_3 d, J = 6.9Hz)	0.98 (21-CH_3 d, J = 6.8Hz)
1.266 (18-CH_3, s)	1.02 (18-CH_3, s)
0.986 (18-CH_3, s)	1.32 (19-CH_3, s)
3.50 (H-3 ddd J = 11.0, 11.0, 4.5)	3.45 (H-3, m)
	3.7 (H-6 ddd J = 12.6, 4.0Hz)
	2.42 (H-7 dd J = 12.0, 4.0)
4.412 (ddd H-15 J=5.1, 5.1, 1.7)	4.5 (b, s, H-15)
5.706 (H-1, d, J=1.1Hz)	5.12 (1-H, s)
4.058 (H-2 dd, J = 4, 11.0Hz)	4.10 (H-2, dd, J = 3.70, 11.0Hz)
3.978 (H-3 dd J = 7.8, 4.0Hz)	4.02 (H-3, dd, J = 7.1, 3.70 Hz)
3.932 (H-4, ddd, J = 7.8, 5.1, 2.9Hz)	3.97 (H-4, ddd, J = 7.1, 4.5, 3.4Hz)
3.750 (H-5, dd, J = 12.5, 2.9)	3.80 (H-5, dd, J = 12.4, 4.5)
3.634 (H-5, dd, J = 12.5, 5.1Hz)	3.67 (H-5, dd, J = 12.4, 3.4 Hz)
4.408 (H-1 d J = 7.8 Hz)	4.45 (H-1, d, J = 7.5Hz)
2.846 (H-2, dd, J = 9.1, 7.8Hz)	2.90 (H-2, dd, J = 7.5, 9.1)
3.35 (H-3),	3.43 (H-3 dd J = 9.1, 9.1Hz)
3.460 (H-4, ddd, J = 10.2, 9.1, 5.6Hz)	3.75 (H-4, ddd, J = 10.1, 9.1, 5.5Hz)
3.130 (H-5ax, dd, J = 11.3, 10.2 Hz)	3.17 (H-5 ax, dd, J = 11.4, 10.1Hz)
3.780 (H-5eq, dd, J = 11.3, 5.6Hz)	3.70 (H-5eq, dd, J = 11.4, 5.5Hz)
3.529 (OCH_3,s)	3.62 (OCH_3, s)

TABLE-V

^{13}C-CHEMICAL SHIFTS OF ATTENUATOSIDE-A-I AND GLYCOSIDE I

C.NO.	ATTENUATO-SIDE-A-1 δ	GLYCOSIDE -1 δ	C.NO.	ATTENUATO-SIDE-I δ	GLYCOSIDE 1 δ
1	39.2	39.49	21	18.9	19.7
2	32.2	31.61	22	32.1	33.00
2	71.4	72.24	23	28.1	28.77
4	33.2	32.40	24	83.3	84.64
5	54.0	53.95	25	30.8	35.51
6	66.5	67.70	26	18.1	18.27
7	50.0	49.4	27	18.2	18.27
8	76.7	77.48	1	107.5	107.91
9	56.8	57.56	2	92.8	92.66
10	37.4	38.02	3	77.6	77.90
11	19.3	19.75	4	85.0	84.02
12	42.6	43.47	5	62.7	62.72
13	43.7	44.44	1	105.1	10.525
14	61.8	62.72	2	84.3	84.88
15	70.2	71.25	3	77.8	77.48
16	42.2	42.53	4	71.1	71.26
17	57.2	58.14	5	67.1	66.9
18	16.6	16.52	OCH_3	60.6	61.06
19	14.3	14.06	OCH_3		
20	35.6	36.44			

also the site of glycosidation. The stereochemistry at C-24 is proposed 24-S on comparing the shifts of signals due to side chain carbons of nodososide[28] which has been determined and corelated to the ^{13}C spectra of the nodososide which are in very close agreement to the glycoside 1.

On comparing ^{1}H and ^{13}C data of glycoside 1 and attenuatoside A-1 it was concluded that glycoside 1 is the attenuatoside A-1 (see table-V) and is first time is isolated from this species of starfish (Porania pulvillus).

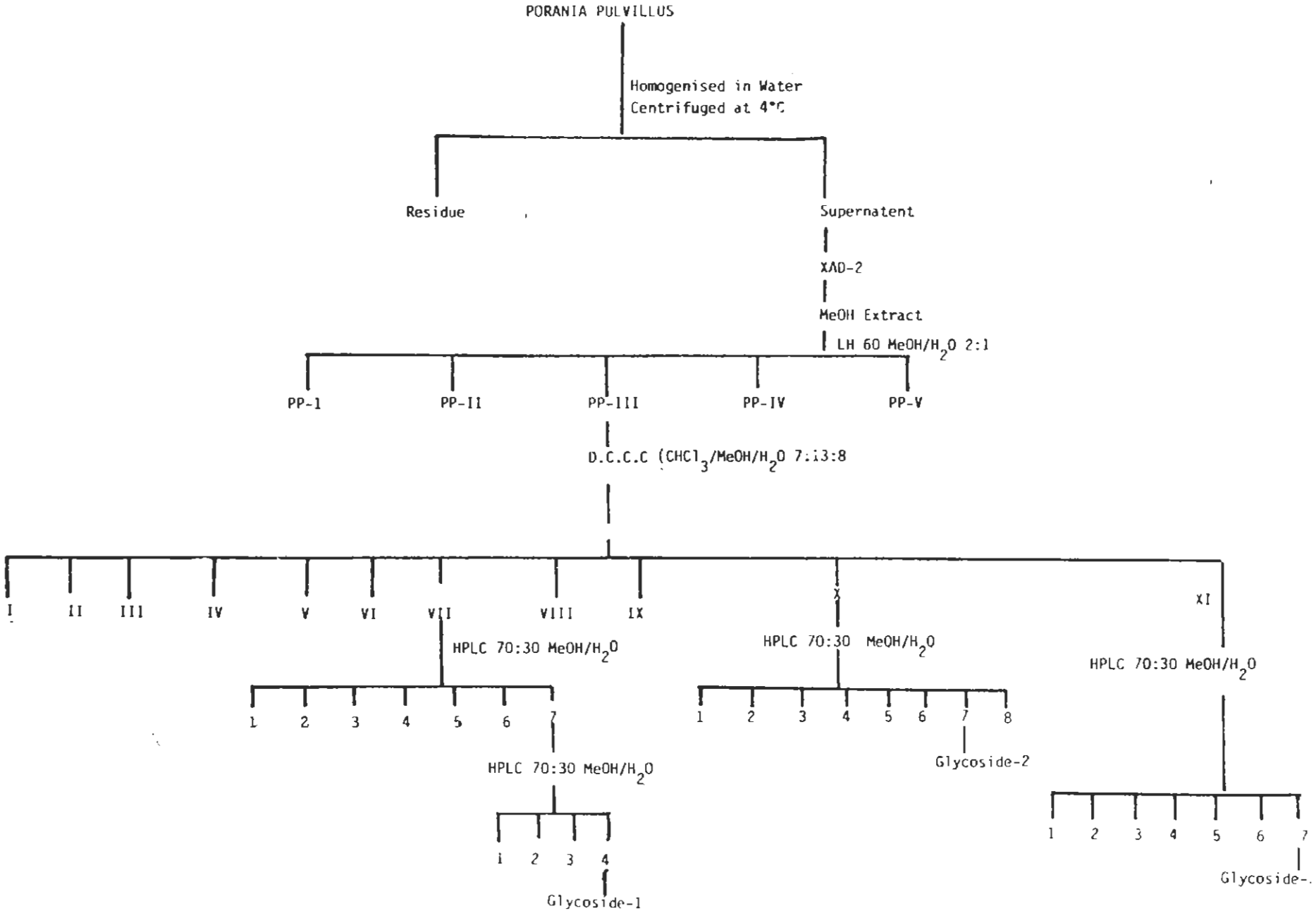
GLYCOSIDE 2

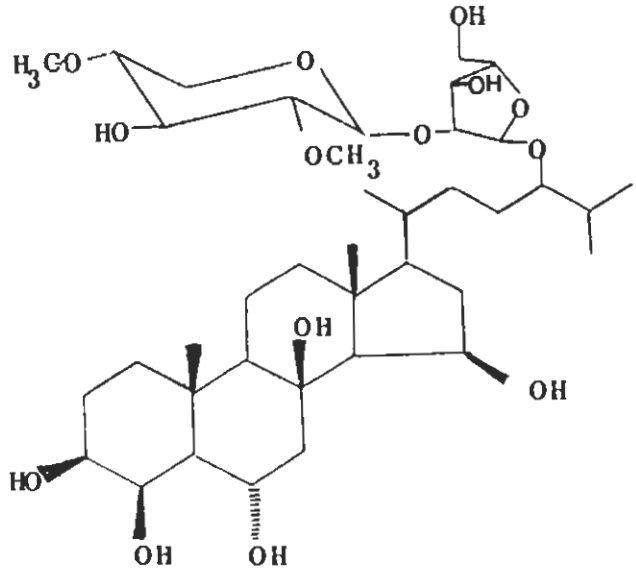
This compound was isolated from glycosidic fraction of LH 60 which was further chromatographed on DCCC (see scheme II) and was finally purified by HPLC using μ Bondapack C-18 column.

Total amount isolated was 7.1 mg which did not crystallize $[\alpha]_D^{20}$ = -13.5°.

The FAB mass spectrum showed molecular ion at m/e 783 $(M+Na)^+$ corresponding to $C_{39}H_{68}O_{14}$. Other important fragments appeard at m/e 184 [161 + Na] due to fragment $C_7H_{13}O_4$, 200 [177 + Na]$^+$ due to species $C_7H_{13}O_5$. These two fragments signified the presence of sugar with 7 carbon atoms linked glycosidically to another moiety. A pentose with 2 methoxy group was a favourable choice on the basis of two-OCH_3 singlets at δ3.51 and 3.62.

Another fragment at 139 [116 + Na] corresponding to $C_5H_8O_3^+$ and 155 [132 + Na] due to $C_5H_8O_4$. This indicated the presence of glycosidically linked pentose.


PORANIA PULVILLUS
Homogenised in Water
Centrifuged at 4°C
Residue
Supernatent
XAD-2
MeOH Extract
LH 60 MeOH/H2O 2:1
PP-1
PP-II
PP-III
PP-IV
PP-V
D.C.C.C (CHCl3/MeOH/H2O 7:13:8
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7
HPLC 70:30 MeOH/H2O
1 2 3 4
Glycoside-1
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7 8
Glycoside-2
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7
Glycoside-



GLYCOSIDE-2
(HALITYLOSIDE "D")

The presence of fragment at m/z 316 [293 + Na] corresponding to $C_{12}H_{21}O_8$ and 332 [309 + Na] $C_{12}H_{21}O_9$, gave evidence of one di-O-methyl pentose and one pentose linked glycosidically to each other and this unit is in turn linked glycosidically to the rest of the molecule having molecular formula $C_{27}H_{47}O_5$.

A small amount of the compound was subjected to acid hydrolysis reduced with $NaBD_4$ and finally was acetylated. G.C/M.S. of this acetylated mixture showed two peaks showing fragments at m/z 234 due to M^+-CHDOAc and 288 due to M^+-OHDOAc. The fragment at 234 signify the presence of 2, 4-di-O-methylpentose triacetate, which appeared due to 2,4-di-O-methyl pentose.

On the other hand fragment at m/z 288 is due to loss of CHD-OAc from pentos penta-acetate. This signifies the presence of pentose.

In order to confirm the sugars, standard sugars were derivatized and were co-chromatographed on G.C. and on the basis of retention time it was concluded that one sugar is 2,4-di-O-methylxylose and other is arabinose.

TABLE-VI

^1H (400 MHz CD_3OD) CHEMICAL SHIFTS OF SUGARS IN HALITYLOSIDE-D AND GLYCOSIDE-2

SUGAR	^1H NO	HALITYLOSIDE-D δ	GLYCOSIDE-2 δ
ARABINOSE MOIETY	H-1	5.11 (bs)	5.12 (bs)
	H-2	4.08 (dd)	4.10 (dd)
	H-3	4.02 (m)	4.02 (m)
	H-4	3.98 (m)	3.97 (m)
	H-5	3.79 (dd J = 12.5, 3.0Hz)	3.80 (J = 12.3, 2.9Hz)
	H-5	3.65 (dd 12.5, 4.8Hz)	3.67 dd (J = 12.3, 8.8Hz)
2,4-di-O-Methyl xyloside moiety	H-1	4.44 (d J = 7.6)	4.45 (d J = 7.8Hz)
	H-2	2.90 (ddd J = 9.0, 7.6Hz)	2.92 (dd J = 8.8, 7.8Hz)
	H-3	3.42 (t J = 90Hz)	3.43 (t J = 8.9Hz)
	H-4	3.20 (m)	3.22 (m)
	H-5ax	3.14 (t J = 10.6Hz)	3.17 (t J = 9.8 Hz)
	H-5eq	4.02 (dd, J = 10.6, 4.0)	3.70(dd J = 9.8, 4.2 Hz)
	OCH_3	3.50 (s)	3.51 (s)
	OCH_3	3.61 (s)	3.62 (s)

TABLE-VI

^{13}C CHEMICAL SHIFTS OF SUGARS IN HALITYLOSIDE-D AND GLYCOSIDE-2

SUGAR	C NO	HALITYLOSIDE-D δ	GLYCOSIDE-2 δ
ARABINOSE MOIETY	1	108.0	107.78
	2	92.4	92.64
	3	77.9	77.76
	4	84.2	83.83
	5	62.8	62.51
2,4-di-O-Methyl xyloside Moiety	1	105.1	105.14
	2	84.9	84.89
	3	76.9	76.50
	4	81.0	80.81
	5	64.4	64.36
	OMe	61.0	61.13
	OMe	59.0	59.04

The difference in sugar portion between attenuatoside A-1 and glycoside 2 is that there is one more methyl groups at 4-OH of 2-OMe xylose. ^{13}C and ^{1}H of this compound was also indicating the presence of arabinose and 2-4-di-O-Me xylose. Assigned ^{13}C and ^{1}H values for both these sugars are compared with the halityloside D a glycoside recently isolated from starfish Halityla regularis,[32] (see table-VI).

As observed ^{13}C and ^{1}H values are in very good agreement to the reported values of both above sugars it was co cluded that arabinose is α-L-arabino furanosyl and 2-4-di-O-me xylose is$^{\beta}$?,4-di-O-Me xylopyranosyl in nature[32]. The significant shift of C-2 of arabinofuranosyl 92.64 indicate the point of glycosidation of at C-2 and this also signifies the presence of terminal 2,4-di-O-methyl xylopyranosyl moiety attached to the C-2 of arabino-furanosyl.

After excluding signals due to sugars portion both in ^{13}C and ^{1}H-NMR, remaining signals showed the presence of hydroxysteroid. On measuring off resonance single frequency decoupled spectrum it was inferred that there are 6 hydroxyl groups, 5 are secondary in nature while one is quarternary in nature. The methyl groups are 3 secondary in nature and two are tertiary in nature. On the basis of above observations saturated steroid with 6 hydroxyl group was a candidate for above

TABLE-VII

SINGLE FREQUENCY OFF RESONANCE DECOUPLED ^{13}C SPECTRUM OF GLYCOSIDE-2

C NO	CHEMICAL SHIFT δ	MULTI-PLICITY	C NO	CHEMICAL SHIFT δ	MULTI-PLICITY
1	39.67	t	21	19.20	q
2	26.16	t	22	32.91	t
3	73.66	d	23	28.58	t
4	69.06	d	24	84.44	d
5	57.24	d	25	31.49	d
6	67.75	d	26	18.19	q
7	49.76	t	27	18.30	q
8	77.41	s	1	107.78	d
9	58.02	d	2	92.64	d
10	38.13	s	3	77.76	d
11	19.02	t	4	83.83	d
12	43.34	t	5	62.51	t
13	44.38	s	1	105.14	d
14	62.72	d	2	84.49	d
15	71.12	d	3	76.50	d
16	42.54	t	4	80.81	d
17	58.41	d	5	64.36	t
18	16.49	q	OCH_3	61.13	q
19	16.98	q	OCH_3	59.04	q
20	36.47	q			

data (see table-VII).

In ^{1}H-NMR (400 MHz CD_3OD) two singlets at δ 1.21 and 1.31 showed presence of two angular methyl groups at C_{19} and C_{18} of the chalestane respectively. Three doublets centered at δ 0.94, δ0.96, and 0.98 with coupling constant of 6.9 Hz were showing the presence of 27, 26, 21 methyl groups respectively.

Signal resonating at δ 4.30 as a broad singlet indicated the presence of hydroxyl group at C-4 which is typical signal due to alpha proton at C-4[29]. This was further confirmed by the downfield shift of H6 signal appeard at δ4.20 as ddd (12.3, 12.3, 4 Hz) rather than at 3.75. The ddd signal at δ 3.75 is typical for the βH at 6 position with hydroxyl group at C-6 with α orientation[29]. The low field shift of this proton is due to through space interaction of 6-α-H with 4 β-hydroxyl group. One tertiary hydroxyl group was suspected at C_8 on the basis of ^{13}C-NMR showing resonance at δ 77.41 and also on the biogenetic point of view. The hydroxyl group at C-8 with orientation is very common is stereoids and glycosides of many starfish[34]. The presence of this hydroxy group was further confirmed by measuring ^{1}H-NMR in pyridine d-5. The shift of 0.3 ppm on both the angular methyle groups suggested the presence of hydroxyl group at C-8 and

this shift is attributed to the pyridine inducced solvent shift[31]. Signal at δ 4.47 showed typical shape of signal observed for alpha proton at C-15, ^{13}C chemical shift of C-15 and 71.12 also suggest the presence of hydroxyl group at C-15. This confirms the 15-β-OH-group at C-15. The low field shift of C-18 angular methyl group at δ 1.3 is also supporting fact to put hydroxyl group at C-15 with β-orientation. The low field shift of this methyl group is due to space interaction with 8 and 15 -OH group.

The signal with pattern of ddd resonating at δ2.40 with coupling constant J = 15, 12.5 and 5 Hz is due to 16βH which appeard at a low field due to interaction with 15 β hydroxyl group, H-16α is masked with upfield signals.

In ^{13}C-NMR the chemical shift of C-24 was significantly downfield. In the off resonance spectrum it resonated at δ 72.24 with multiplicity of a doublet. This indicated the position of the remaining hydroxyl group, which was also site of glycosidation. On the basis of above data and conclusion, the assigned structure for glycoside 2 was (24S)-24-O-[2,4, di-O-methyl-β-D-xylopyranosyl]-(1→2)α-L-arabinofuranosyl]-5α-cholastane 3β, 4β, 6α, 8, 15β 24 hexaol.

On comparison of data with Halityloside D it was found that both of these compounds are same (see table-VI). However this compound was isolated first time from starfish Porania pulvillus.

Later in a private communication IORIZZI informed the present author about the isolation of same compound from starfish Halityla regularis.

GLYCOSIDE 3

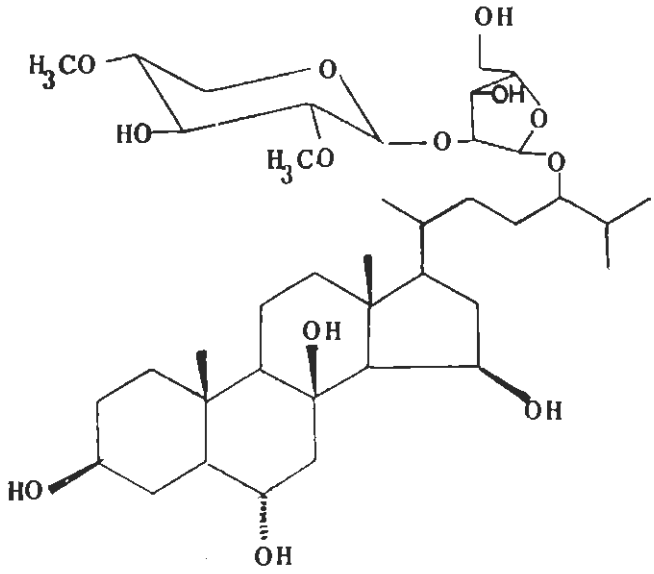
This compound was isolated from glycosidic fraction of LH-60 and was further chromatographed on D.C.C.C. Finally it was purified by HPLC on μ Bondapack column. The amount of this compound was 16.5 mg showing $[\alpha]_D^{20}$ = - 21.0° (C = 0.4 MeOH).

The FAB mass spectrum of the compound showed molecular ion peak at m/z 767 $[M + Na]^+$ corresponding to the molecular formula $C_{39}H_{68}O_{14}$.

Other important fragments appeared at m/z 184 [161 + Na] and 200 (177 + Na] corresponding to $C_7H_{13}O_4$ and $C_7H_{13}O_5$ respectively. These fragments suggested the presence of di-O-methyl pentose glycosidically linked to other moiety, di-methoxy pentose was considered on the basis of two $-OCH_3$ signals in ^{1}H-NMR resonating at δ 3.51 and 3.62 respectively.

The presence of fragment at m/z 139 [116 + Na] and 155 [132 + Na] showed the presence of fragments $C_5H_8O_3$ and $C_5H_8O_4$ respectively. This observation led to the conclusion that one pentose is present linked glycosidically to another moiety.


PORANIA PULVILLUS
Homogenised in Water
Centrifuged at 4°C
Residue
Supernatent
XAD-2
MeOH Extract
LH 60 MeOH/H2O 2:1
PP-I
PP-II
PP-III
PP-IV
PP-V
D.C.C.C (CHCl3/MeOH/H2O 7:13:8
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7
HPLC 70:30 MeOH/H2O
1 2 3 4
Glycoside-1
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7 8
Glycoside-2
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7
Glycoside-3



GLYCOSIDE-3
(HALITYLOSIDE "E")

The presence of fragment of 316 [293 + Na] and 332 [309 + Na] corresponding to $C_{12}H_{21}O_8$ and $C_{12}H_{21}O_9$ showed that both pentose and di-O-methyl pentose are glycosidically linked to each other and are again linked to the rest of molecule having molecular formula $C_{27}H_{47}O_4$.

Acid hydrolysis followed by reduction with $NaBD_4$ and acetylation of the compound afforded two sugars. On GC/MS of these sugars it was revealed that one sugar is pentose showing fragment at m/z 218 due to M^+-CHDOAc and other is 2-4-di-O-methyl pentose showing fragment at m/z 233 due to loss of M^+-CHDOAc. On comparing with standard sugar derivatives by GC the retention times indicated the presence of arabinose and 2,4-di-O-methyl xylose. On comparison of ^{13}C and ^1H-NMR spectra with nodososide it was revealed that there is one more methyl group at 4-OH of the xyloside.

On comparison of ^{13}C spectrum of glycoside **3** and nodososide the values were identical and difference in chemical shift of C_4 was within the expected range

^1H-NMR of both these sugars in nodososide and glycoside 3 was also consistent to each other. This also supports the facts that both of these sugars are arabinose and 2,4-di-O-methylxylose and also they

TABLE-IX

^1H CHEMICAL SHIFTS OF SUGARS IN NODOSOSIDE AND GLYCOSIDE 3

SUGARS	^1H NO	NODOSODIE δ	GLYCOSIDE-3 δ
	H-1	5.14 (b.s)	5.11 (b.s)
	H-2	4.153 (d, J = 3.8 Hz)	4.10 (d, J = 4.0 Hz)
	H-3	4.129 (dd, J = 7.20, 3.80Hz)	4.03 (ddd, J = 7.3, 4.0 Hz)
	H-4	4.034 (ddd, J = 7.20, 4.6, 3.5Hz)	3.96 (m)
	H-5	3.83 (dd, J = 12.5, 4.60Hz)	3.81 (dd, J = 12.3, 2.9Hz)
	H-5	3.76 (dd, J = 12.50, 3.52Hz)	3.67 (dd, J = 12.3, 4.8Hz)
	H-1	4.53 (d, J = 7.7Hz)	4.44 (d, J = 7.8 Hz)
	H-2	3.036 (dd, J = 7.75, 9.02Hz)	2.92 (dd, J = 8.8, 7.8Hz)
	H-3	3.472 (dd, J = 9.02, 9.02)	3.43 (t, J = 8.87)
	H-4	3.631 (ddd, J = 10.3, 5.02, 5.65Hz)	3.22 (m)
	H-5ax	3.261 (dd, J = 11.6, 10.3Hz)	3.17 (t = 9.8Hz)
	H-5eq	3.90 (dd J = 11.6, 5.65Hz)	3.70 (dd, J = 9.8, 4.2Hz)
	OCH_3	3.60 (s)	3.53 (s)
	OCH_3		3.62 (s)

TABLE-IX

^{13}C-CHEMICAL SHIFTS OF SUGAR CARBONS IN NODOSOSIDE AND GLYCOSIDE-3

SUGARS	C.NO.	NODOSOSIDE δ	GLYCOSIDE-3 δ
ARABINOSE MOIETY	1	107.6	107.78
	2	93.1	92.62
	3	77.76	77.76
	4	85.0	83.83
	5	62.4	62.52
2,4-di-O-Methyl xylapyranoxyl	1	105.2	105.13
	2	84.1	84.84
	3	77.8	76.49
	4	71.0	80.80*
	5	67.1	64.36
	OCH_3	60.7	61.12
	OCH_3		59.04

*Low field shift due to the presence of -OCH_3 group at 4 position.

TABLE-X

SINGLE FREQUENCY OFF RESONANCE DECOUPLED ^{13}C SPECTRUM OF GLYCOSIDE-3

C.NO.	CHEMICAL SHIFT δ	MULTI-PLICITY	C.NO.	CHEMICAL SHIFT δ	MULTI-PLICITY
1	39.41	t	21	19.04	q
2	31.49	t	22	32.91	t
3	76.16	d	23	28.60	t
4	32.34	t	24	84.45	d
5	53.83	d	25	31.45	d
6	67.64	d	26	18.22	q
7	49.35	t	27	18.31	q
8	77.46	s	1	107.78	d
9	57.43	d	2	92.62	d
10	37.97	s	3	77.76	d
11	19.72	t	4	83.83	d
12	43.39	t	5	62.52	t
13	44.37	s	1	105.13	d
14	62.58	d	2	84.84	d
15	71.11	d	3	76.49	d
16	42.54	t	4	80.80	d
17	58.03	d	5	64.36	t
18	16.53	q	OCH_3	61.12	q
19	14.08	q	OCH_3	59.04	q
20	36.47	q			

are in furanose and pyranose forms respectively. The configuration of both these sugars is well documented and are commonly found in starfish glycoside. On the basis of reported data of ^{1}H and ^{13}C NMR it was concluded that sugars are α-L-arabinofuranoside and β D-2,4-di-O-methyl xylopyranoside. (see table-IX).

The D and L configuration of both these sugars is determined on the basis of biogenetic grounds as most of these sugars encountered in starfish saponins have above said configuration[35]. The glycosidation shift for the C-2 of arabinose advocates the presence of terminal 2,4-di-O-mexylopyranoside. On substracting $C_{13}H_{22}O_8$ of both sugars the remaining molecule was $C_{27}H_{46}O_5$. Single frequency off resonance ^{13}C spectrum of the compound showed presence of 5 hydroxyl groups, 4 secondary and one tertiary in nature. On the basis of ^{1}H, ^{13}C NMR and above conclusion a pentahydroxy saturated cholastane steroid was the closest candidate for the basic skeleton to the above formula (see table X).

^{1}H-NMR (CD_3OD, 400 MHz) showed two methyl singlets at δ 1.03 and 1.31 due to presence of 19 and 18 angular methyl groups respectively.

Three methyl doublets appeard at δ 0.94 (J = 6.7 Hz) 0.96 (J = 6.7 Hz) and 0.98 (J = 6.8 Hz) due to the presence of 27, 26 and 21 methyl groups.

A multiplet at δ 3.56 showed the pattern normally observed in A/B trans 3 beta hydroxylsteroid system having proton at C-3 with α-orientation.

The signal at δ 3.75 which was partially overlapped by other signals showed the pattern usually observed for 6 -hydroxysteroid[29] having proton. The coupling constant was 11.00, 11.00 and 4.6 Hz. The third hydroxyl group was placed at C-8 as a tertiary hydroxy group. The presence of tertiary hydroxy group at C-8 is very common in steroidal glycosides of starfishes[34]. The chemical shift and multiplicity of signal in ^{13}C also indicate the presence of hydroxyl group at C-8 (see table-X).

This in turn was proved by measuring ^{1}H of the same compound in pyridine d-5. A shift of 0.3 ppm was observed for both of the angular methyl groups at C-18 and C-19. This pyridine induced solvent shift is due to complex formation of pyridine with 8 hydroxyl group[31].

The multiplet at δ 4.45 which was masked by the signal of H-1 of xyloside became clear after irradiation at δ 2.92 and appeared as multiplet. The shape and chemical shift of the signal due to 15α-H signified the presence of hydroxyl group at C-15 -OH group as this proton was

having through space interaction with -OH[29]. On comparison of ^{13}C data with nodososide the position of 5th hydroxyl group was confirmed which appeared at δ84.45 and correspond to C-24 and this is also the site for glycosidation. The S configuration at C-24 is determined by comparing the ^{13}C values for 26 and 27 methyls to the reported values of nodososide. Recently it is determined that in 24 S isomer the signal due to 26 and 27 carbons are very close to each other i.e 0.1 to 0.2 ppm. On the other hand in 24R isomer the difference between two signals is about 1 ppm[36].

^{13}C chemical shift for C-26 and C-27 was found to be δ 18.22 and 18.31. Hence it was concluded that configuration at 24 is "S".

On the basis of above conclusion and spectral data the structure of glycoside **3** was found to be (24S)-24-O[2,4-di-O-methyl-β-D-xylopyranosyl-(1→2)-α-L-arabinofuranosyl]5 -cholestane-3β,6α8, 15β24-pentaol.

This compound was isolated first time from any starfish at the time of isolation. Latter in a private communication Iorizzi informed present author the isolation of the same compound from starfish Halityle regularis.

Now this and glycoside 2 are the compounds isolated first time from starfish Poronia pulvillus.

EXPERIMENTAL

GENERAL EXPERIMENTAL NOTE

^{1}H and ^{13}C spectra were recorded on JEOL 90MHz and 400MHz Nuclear magnetic resonance spectrometer.

Mass spectra were recorded on Varian MAT 312 equipped with FAB source.

Thin layer chromatography was performed on silica gel precoated plates (E. Merck). D.C.C.C. was performed on D.C.C. apparatus by TOKYO RIKAKIKAI Co., using 400 tubes.

HPLC was performed on μ Bondapack C-18 semi preparative column using LDC constametric pump and waters 401 Differential R.I. detector.

Aqueous extract was desalted on Amberlite XAD-2 resin supplied by Rohm and Hauus.

The solvents mostly used were obtained by E. Merck and were distilled before use.

Organism was freezed immediately after collection in dry ice and was stored at - 23°C.

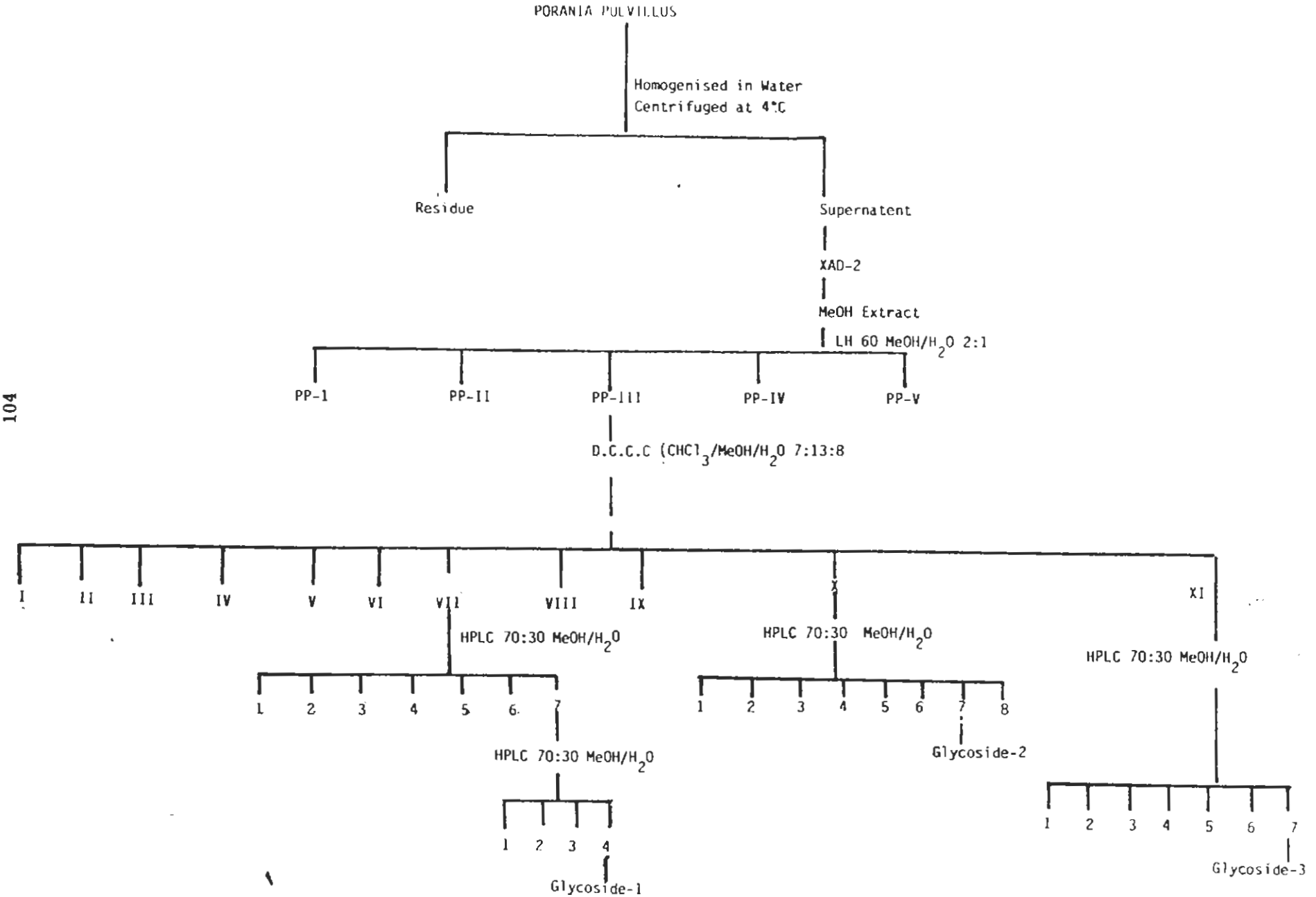
EXPERIMENTAL

Starfish Porania pulvillus was collected from the swedish west coast in May 1985 at Tjarno Marine Biology Laboratory. The material was collected by SCUBA diving between 15-25 meter depth. and was frozen immediately and stored at -23°.

1.3 kg of the starfish was homogenized in 5 litre of distilled water by an ultraturax and was centrifuged at +4°C. The supernatent liquid obtained was desalted by passing through a column of Amberlite XAD-2 (1 kg). The charged column was washed with 3 litre of distilled water in order to remove maximum salts.

The column was then eluted with MeOH and this methanolic extract was evaporated under reduced pressure at + 25°. This afforded 2.5 grams of methanolic extract.

1.6 grams of the methanolic extract was loaded on a sephadex LH-60 column (2.5 x 90 cm) and eluted with methanol:water 2:1 with flow rate of 0.2 ml/min. and fractions of 5 ml were collected. Usually this step devides the methanolic extract of the starfishes into tryptophane


PORANIA PULVILLUS
Homogenised in Water
Centrifuged at 4°C
Residue
Supernatent
XAD-2
MeOH Extract
LH 60 MeOH/H2O 2:1
PP-1
PP-II
PP-III
PP-IV
PP-V
D.C.C.C (CHCl3/MeOH/H2O 7:13:8
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7
HPLC 70:30 MeOH/H2O
1 2 3 4
Glycoside-1
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7 8
Glycoside-2
HPLC 70:30 MeOH/H2O
1 2 3 4 5 6 7
Glycoside-3

fraction containing mostly tryptophane and other derivatives, saponin fraction, glycosidic fraction and finally lipids and other fats. On TLC analysis of these fraction on Si60 plates using $CHCl_3$:MeOH:H_2O 80:18:2 it was found that fraction 1 62, 63 73, 74 102, 103 130, 130 last fraction can be divided into 5 fraction. Fraction-III (525 mg) was the glycosidic fraction and hence was submitted to D.C.C.C.

The solvent system used in D.C.C.C. was $CHCl_3$ MeOH H_2O 7:13:8. The aqueous phase was used as mobile phase with asending mode with flow rate of 0.15 ml/min. Fractions of about 6 ml were collected.

These fractions were checked on tlc using $CHCl_3$ MeOH H_2O 80:18:2 and Butanol Acetic acid water 12:3:5. These fraction were divided into eleven main fractions. Fraction VII, X and XI showed the presence of glycosides.

These fractions were further chromatographed on μ Bondapack C-18 semipreparative column, using 70:30 MeOH: H_2O as eluate.

RI detector was used to detect glycosides and flow rate was kept at 5 ml/min. This chromatographic step afforded 3 glycoside named glycoside 1,2, and 3 (see scheme-II).

SPECTRAL DATA

(1) ATTENUATOSIDE A-1

1

Amorphous

$[\alpha]_D^{20}$ = - 21.2° (C = 1.0 MeOH)

MASS FAB (glycerol + NaCl) M^+ Na 753 m/z M^+ = 730 m/z other peaks were at m/z 170 [147 + Na $C_6H_{11}O^+_4$], m/z 186 [163 + Na, $C_6H_{11}O^+_5$] m/z 302 [279+Na, $C_{11}H_{19}O^+_8$], m/z 318 [295 + Na, $C_{11}H_{19}O^+_9$]

^{1}H-NMR : - (400 MHz CD_3OD) δ

δ 0.94 (27 - CH_3 d, J = 7Hz), 0.96 (26-CH_3 d, J = 7Hz) 0.98 (21-CH_3 d, J = 6.8 Hz), 1.02 (18-CH_3,s), 1.32 (19-CH_3,s), 3.45 (m, 3∝H) 3.7 (ddd, J = 12.0, 4.0 Hz, 6-H), 2.42 (H-7 , dd, J = 12.0, 4.0 Hz), 4.5 (b.s. H-15)

ARABINOFURANOSE MOIETY

δ 5.12 (H-1 s), 4.10 (H-2, dd, J = 3.70, 11.0Hz) 4.02 (H-3 dd, J = 7.1, 3.70 Hz) 3.97 (H-4 ddd, J = 7.1, 4.5, 3.4 Hz), 3.80 (H-5 dd, J = 12.4, 4.5Hz) 3.67 (H-5 dd, J = 12.4, 3.4 Hz).

2,O-METHYXYLOPYRANOSYL MOIETY

δ 4.45 (H-1 d, J = 7.5 Hz), 2.90 (H-2 dd, J = 7.5, 9.1), 3.43 (H-3 dd, J = 9.1, 9.1 Hz) 3.75 (H-4 ddd, J= 10.1, 9.1, 5.5 Hz), 3.17 ($H\text{-}5_{ax}$ H dd, J = 11.4, 10.1 Hz), 3.70 ($H\text{-}5_{eq}$ dd, J = 11.4, 5.5 Hz). 3.62 ($2\text{-}OCH_3$, s).

^{13}C (100 MHz CD_3OD)

δ C^1 39.49, C^2 31.61, C^3 72.24 C^4 32.40, C^5 53.95, C^6 67.70, C^7 49.4, C^8 77.48 C^9 57.56 C^{10} 38.02, C^{11} 19.75, C^{12} 43.47, C^{13} 44.44 C^1 462.72, C^{15} 71.26, C^{16} 42.53, C^{17} 58.14, C^{18} 16.52, C^{19} 14.06, C^{20} 36.44, C^{21} 19.07, C^{22} 33.00, C^{23} 28.77, C^{24} 84.64 C^{25} 31.51, C^{26} 18.27, C^{27} 18.27. C^1 107.91, $C^2$9 2.66, C^3 77.90, C^4 84.02, C^5 62.72, C^1 105.25, C^2 84.88, C^3 77.48, C^4 71.26, C^5 66.96, $OCH_3$61.06.

HALITYLOSIDE - "D"

Amorphous

$[\alpha]_D^{20}$ = - 13.5° (C = 0.6 MeOH)

MASS FAB (Glycerol + NaCl) $[M^+ + Na]$ = m/z 783 M^+ = m/z 760 corresponding to $C_{39}H_{68}O^+_{24}$. Other important peaks appeard at m/z 184 [161 + Na, $C_7H_{13}O^+_4$] m/z 200 [177 + Na, $C_7H_{13}O^+_5$], m/z 316 [293 + Na $C_{12}H_{21}O^+_8$] m/z 332 [309 + Na, $C_{12}H_{21}O^+_9$].

^{1}H-NMR (400 MHz CD_3OD) δ 0.94 (27-CH_3, d, J = 6.9 Hz) 0.96 (26-CH_3, d, J = 6.9 Hz) 0.98 (21-CH_3 d, J = 6.8 Hz), 1.21 (19-CH_3, s) 1.31 (18-CH_3 s) 4.30 (b.s, H-4-α) 4.20 (H-6 β ddd, J = 12.3, 12.3, 4.0 Hz) 4.47 (H-15-α) 2.40 (H-16, ddd, J = 15, 12.5 and 5Hz).

ARABINOFURANOSE MOIETY

δ 5.12 (H-1, b.s) 4.10 (H-2, ddd) 4.02 (H-3 m,) 3.97 (H-4, m 3.80 (H-5, dd, J = 12.3, 2.9 Hz). 3.67 (H-5 dd J = 12.3, 4.8).

2-4, DI-O-METHYLXYLOPYRANOSYL MOIETY

δ 4.45 (H-1, d) 2.92 (H-2 dd, J = 8.8, 7.8 Hz) 3.43 (H-3 t, J = 8.9 Hz) 3.2 2 (H-4 ddd) 3.17 (H-5 t, J = 9.8 Hz) 3.70 ($H\text{-}5_{eq}$ dd, J = 9.8, 4.2 Hz) 3.51 ($-OCH_3$, s) 3.62 ($-OCH_3$, s).

^{13}C(100 MHz CD_3OD) C^1 39.67, C^2 26.16, C^3 73.66 C^4 69.06, C^5 57.24, C^6 64.75, C^7 49.76, C^8 77.41 C^9 58.02, C^{10} 38.13, C^{11} 19.02, C^{12} 43.34, C^{13} 44.38, C^{14} 62.72, C^{15} 71.12, C^{16} 42.54, C^{17} 58.41, C^{18} 16.49, C^{19} 16.98, C^{20} 36.47, C^{21} 19.20, C^{22} 32.91, C^{23} 28.58, C^{24} 84.44, C^{25} 31.49, C^{26} 18.19, C^{27} 18.30 C^1 107.78, C^2 92.64, C^3 77.76, C^4 83.83 C^5 62.51, C^1 105.11, C^2 84.89, C^3 76.50, C^4 80.81, C^5 64.36, OCH_3 61.13, OCH_3 59.04.

HALITYLOSIDE-"E"

Amorphous

$[\alpha]_D^{20}$ = 21.0° (C = 0.4 MeOH)

MASS FAB (Glycerol + NaCl)

$[M^+ Na]$ = m/z 767 M^+ = 744 corresponding to $C_{39}H_{68}O_{13}$. Other important fragments appeard at m/z 184 [161 + Na, $C_7H_{13}O^+_4$], m/z 200 [177 + Na, $C_7H_{13}O^+_5$], m/z 316 [293 + Na, $C_{12}H_{21}O_8$], m/z 332 [309+Na, $C_{12}H_{21}O_9$].

^{1}H-NMR (400 MHz CD_3OD)

δ 0.94 (27-CH_3, d, J = 6.7 Hz) 0.96 (26-CH_3, d, J = 6.7 Hz) 0.98 (21-CH_3 d, J = 6.8 Hz) 1.03 (19-CH_3 s) 1.31 (18-CH_3, s) 3.56 (H-3,α b.m.) 3.75 (H-6β ddd, J = 11.00, 4.6 Hz). 4.45 (H-15, m).

ARABINOFURANOSE MOIETY

δ 5.11 (H-1, b.s.), 4.10 (H-2, dd) 4.03 (H-3, m) 3.96 (H-4, m), 3.81 (H-5 dd, J = 12.3, 2.9) 3.67 (H-5 dd, J = 12.3, 4.8 Hz).

2-4, DI-O-METHYLXYLOPYRANOSYL MOIETY

δ 4.44 (H-1 d J = 7.8 Hz) 2.92 (H-2 dd, J = 8.8, 7.8 Hz), 3.43 (H-3, t, J = 8.8) 3.22 (H-4, m) 3.17 ($H\text{-}5_{ax}$ t, J = 9.8 Hz) 3.70 ($H\text{-}5_{eq}$ dd, J = 9.8, 4.2 Hz) 3.53 ($-OCH_3$, s) 3.62 ($-OCH_3$, s).

^{13}C (100 MHz CD_3OD)

δ C^1 39.41, C^2 31.49, C^3 76.16, C^4 32.34, C^5 53.83, C^6 67.64, C^7 49.35, C^8 77.46, C^9 57.43, C^{10} 37.97, C^{11} 19.72, C^{12} 43.39 C^{13} 44.37, C^{14} 62.58, C^{15} 71.11, C^{16} 42.54, C^{17} 58.03 C^{18} 16.53, C^{19} 14.08, C^{20} 36.47, C^{21} 19. 04, C^{22} 32.91 C^{23} 28.60, C^{24} 84.45, C^{25} 31.45, C^{26} 18.22, C^{27} 18.31 C^1 107.78, C^2 92.62, C^3 77.76, C^4 83.83, C^5 62.52, C^1 105.13, C^2 84.84, C^3 76.49, C^4 80.80, C^5 64.36, OCH_3 61.12 OCH_3 59.04.

REFERENCES

1. T.H. Bullock. **Behavior**, 5, 130 (1953).

2. A.J. Kohn, **Amm.Zool. 1**, 291 (1961).

3. H.Feder, A.M. Christensen, **Physiology of Echinodermata** (ed. R.A. Boolootian) pp 87-127 Willey Interscience New York (1966).

4. H.M. Feder, **Sarsia 29**, 371 (1967)

5. H.M. Feder, **Sarsia, 29**, 371 (1967)

5. H.M. Feder, **Sci.Amm., 227**, 92 (1972).

6. Y-Hashimoto "**Marine Toxins and other Bioactive Marine Metabolites**" pp 369, **Jap. Sci.Soc.Press Tokyo** (1979).

7. B.W. Halstead, **Poiscnous and Venomus Marine Animals of the World**, 1 -537 U.S. Government Printing office Washington, D.C. (1965).

8. C.D. Alender and E.E. Russell, **Physiology of Echinodermata** (ed. R.A. Boolootian pp. 529 (Willy Interscience New York) (1966).

9. H.M. Baslow, **Marine Pharmacology**, p. 171 (Williams and Wilkins Baltimore).

10. Y.Hashimoto and Y. Yasumoto, **Bull. Japan. Soc.Scient.Fish, 26**, 1132, (1960).

11. A.M. Mackie, R. Lasker and P.T. Grant **Comp. Biochem.Physiol, 26**, 415 (1968).

12. Y. Hashimoto, **Marine Toxins and other Bioactive Metabolites**, p-268, **Jap.Sci.Soc.Press. Japan** (1979).

13. D.J. Burnell and J.W. Apsimon, **Marine Natural Products. Chemical and Biological Prespectives"** Vol. V (ed. P.J. Scheuer) Academic Press, New York (1983).

14. A.M. Mackie, R. Lasker and P.T. Grant, **Comp. Biochem.Physiol, 26B**, 415 (1968).

15. J.S.Lucas, R.J. Hart, M.E. Howden and R. Salthe, **J. Exp. Mar.Biol. Ecol., 40**, 155 (1979).

16. A.M. Mackie, H.T. Singh and J.M.Owen, **Comp.Biochem.Physiol. B-56**, 9, (1979).

17. G.R. Pettit, J.F. Day, J.L. Hartwell and H.B. Wood, **Nature, 227**, 962 (1970).

18. G.D. Ruggieri and R.F. Nigerelli in **Bioactive compounds from the sea** p-183 (ed. H. Humm and C.Lane) Marcel Dekker, New York (1974).

19. Y. Shimizu, **Experientia, 27**, 1188 (1971).

20. L.A. Goldsmith and G.P. Carlson, **Food drugs from the sea.** Proceedings (Ed. H.H. Webber and G.D. Ruggieri) p-**354**. Marine Tech.Soc. Washington, D.C.

21. R.J. Owellen, R.G. Owellen, A.M. Gorog and D. Klein **Toxicon, 11**, 319 (1973).

22. A.M. Mackie, T.H. Singh and T.C. Fletcher, **Mar.Biolo., 29**, 307 (1975).

23. F. Culkin and J.P. Riley, **J.Marine Biol.Assoc.U.K., 37**, 607 (1958).

24. A.B. Turner, D.S.H. Smith and A.M. Mackie, **Nature, 233**, 209 (1971).

25. Bernhard Grizmek, **Grizmekes Animal Life Encyclopedia** pp-838 (Van Nostrand Reinhald Company).

26. Y. Hashimoto and Y.Yasumoto, **Nippon. Snisan Gakkaishi, 26**, 1132, (1960).

27. Encyclopeadia of marine sciences, Hamyln books Ltd.,

28. R. Ricco, L. Minale, C. Pizza, F. Zollo, and J. Pusset, **Tet. Lett., 23**(28), 2899 (1982).

29. J.E. Bridgeman, P.C. Cherry, A.S. Clegg, J.M.Evans, Sir. E.R. Jones, A. Kasal, V.Kumar, **J.Chem.Soc.(C)** 250 (1970).

30. Hanne Eggert, Craigl. Van Antiwerp, Norman, S. Bhacca and Carl Djerassi, **J.Org.Chem. 41**(1)-72 (1976).

31. P.V. Demarco, E. Farkas, D. Doodrell, B.L. Mylari, and E. Wenkert, **J.Am.Chem.Soc., 90:20**, 5480 (1968).

32. M.Iorizzi, L. Minale, R. Ricco M. Debray, J.L. Menou, **J.Nat. Prod., 49**, (1), 67 (1968).

34. E. Minale, C. Pizza, F. Zollo, R. Ricco, **J.Nat.Prod., 46**(5) 736 (1983).

35. R.Ricco, L. Minale, C. Pizza, F. Zollo and J. Pusset, **Tet. Lett., 23**(28), 2899 (1982).

36. Private Communication with M. Iorizzi.

37. Y. Shimizu, **J.Am.Chem.Soc., 94**, 4051 (1972).

38. Y. Shimizu, **In "Food Drugs from the Sea Proceedings**, (Ed. L.R. Worthen) pp-292-297, Marine Technology Soc. Washington D.C. (1973.

39. I. Kitagawa, M. Kobayashi, T. Sugawara, **Chem.Pharm.Bull., 26**, 1852 (1978).

40. T.Komori, K. Sakamoto, J. Matuso, Y. San Echika, T. NMohara, Y. Ito, T. Kawasaki and R.H. Schulten, **Presented at I.U.P.A.C. Int. Symp.Chem. Natural Products"** 11th Golden Sands Bulgaria Symp. Pap. **Vol.4** pp. 120 (1978).

41. M.E.H. Howden, S.J. Lucas, M. McDuff and R. Salathe In **Crown of Thorns Starfish Seminar Proceedings Brisbane** pp. 67 (Australian Government Publication Service Canberra).

42. G.B. Elyakov, E.V. Levina, V.S. Levin and I.I. Kapustina, **Comp. Biochem. Physiol** B-55 57- (1976).

43. T. Yasumoto and Y. Hashimoto, **Agric.Biol.Chem.**, 29, 804 (1965).

44. T. Yasumoto and Y. Hashimoto, **Arg.Biol.Chem.**, 31, 368 (1967).

45. S. Ikegamia, K. Okano, and H. Muragaki, **Tet.Lett.**, p-1769 (1979).

46. E.V.Levina and I.I. Kapustina, In **Vesoyuznyi Simpozium po Bioorganischekoi Khimii** (Tezisy Dokladon) p-23 (Akademiya Nauk SSR, Dal' noveostochnyi Nauchnyi Tsentr Vladivostok (1975).

47. F. De Simone, A. Dini, E.Finamore, L. Minale, C. Pizza, R.Ricco and F. Zollo. **J.Chem.Soc.Perkin. Tr. I.**, 1855 (1981).

48. R.Ricco, E. DeSimone, A. Dini, L. Minale, C. Pizza, F. Senatore and F. Zollo, **Tet. Lett.**, 22, 1557 (1981).

49. M. J.Patterson, J. Bland and E.W. Lindgren, **J.Exp.Mar.Biol. Ecol.**, 33, 51 (1978).

50. I.I. Kapustina, E.V. Levina, and V.A. Stonik, **Khim.Prir. Soedin**, 14, 411 (1978).

51. A.M. Mackie and A.B. Turner **Biochemie J.** 117, 543 (1970)

52. J.A. Croft and H.E.M. Howden, **Comp.Biochem.Physio. B-48**, 535 (1974).

53. G.J.Rio, G.D. Ruggieri, M.F. Stempien Jr. and R.F. Nigrelli, **Am.Zool. 3**, 554 (1963).