PD-AAW-716
52050

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D C 20523

DATE: 9/16/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 6, 562
pd. Jan 1 - June 87

Attachment

2 cys

6. 562

PROGRESS REPORT

A STUDY ON INSECTICIDAL PRINCIPAL OF SEEDS OF ANNONA SPECIES FOUND IN INDONESIA

No. of Project : AID 936.5542.97.1

Periode covered by this report: January 1 to June 1987

Faculty of Pharmacy

Gadjah Mada University

Indonesia

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A Study on Insecticidal Principal of Seeds of Annona species found in Indonesia

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INTRODUCTION

The Annonaceae is a large family of aromatic trees,shrubs or climbers,which grown in tropical and subtropical regions. Economically the family is of appreciable importance as a source of edible fruits.

Several Annona species are found in Indonesia,ia Annona squamosa,Annona reticulata,Annona muricata and Annona cherimolia In this report preliminary study on the insecticidal compounds of Annona squamosa and Annona cherimolia are reported. Annona squamosa is found wildly and cultivated throughout Indonesia up to 1150 m above sea level.The fruits are edible and are in season between August to January.The seeds are oblong and dark brown to black coloured,consist of hard seed coat and white flat endosperm.Annona squamosa seeds contain alkaloids,volatile oil,resinous matter,polyphenols,cyanogen and insecticidal compound.

Annona cherimolia grows on places 500 - 1200 m above sea level on dry places.The fruits are round with short and soft pines. The mesocarpium is yellow,sweet nice smelling and edible with many yellowish brown coloured seeds.the sedds contain alkaloids volatile oils,polyphenols,resin alcohols and insecticidal compounds.

ACTIVITIES DURING PERIODE COVERED IN THIS REPORT.

January 1 to June 30 1987

I.1.Collecting of seeds of Annona squamosa and of A. cherimolia

2.Purchasing equipment and chemicals.

3.Extracting seeds

4.Fractionizing extracts

5.Running IR and UV spectra.

II.1.Preparation for rearing test insects

2.Rearing test insects

3.Testing crude extract upon test insect.

PROCEDURE

The seeds of Annona squamosa were collected from various places Bayat,Wonosari and Yogyakarta.The seeds were from epicarpium and mesocarpium,washed and dried in the sun than ground in a mill.The seeds of Annona cherimolia were collected from Botanical garden of the faculty of Biology.

Extraction of Seeds

100 g of seedswere extracted with 500 ml ether in a Soxhlet extractor for 4 hours.The ether extract was filtered and 5 times of volume of petroleum ether was added and left for 24 hours.A resinous mass was precipitated and filtered. The resinous mass was studied with thin layer chromatography using several kinds of solvent and solvent mixture.A mixture of chloroform and methanol(9:1) was used to fractionize the resinous mass using silicagel H 60 as adsorbent in a column 30 cm diameter.

Spectral data

The isolated compounds proved to be pure by TLC were examined with IR Yasco IRA-II spectrphotometer as film in chloroform and the absorbent in UV with Spectronic 210-Shimadzu in ethanol.The IR spectra of compounds A and B isolated from Annona squamosa and C isolated from Annona cherimolia are as showed in the appendices .

II.Rearing of test insects

Test insects used for preliminary testing of insecticidal compounds were Sitophylus oryzae reared on rice grain in the laboratory.

Whatman no. 1 filter papers (7 cm diameter) and 1 ml graduated pipet were used for testing;plastic jar (5 cm diameter and 5 cm in height) were used to confine the insects to the treated area

Preliminary testing

The crude extract from ether after evaporation of the ether and the precipitated resinous mass were used as master stock solution.Serial dilution using a factor of 1/2 were used.Dilution were carried out using a mixture of petroleum ether and aceton(3:1).

Adult beetles were counted in 16 batches each of 20 insects, into small vials. Filter papers were labelled with pencil before impregnation and supported on glass ring.The 0.5 ml aliqots of diluted stock solution were spread on the filter papers with 1 mlpipettes using a progressively decreasing spi-

ral to ensure even distribution.They were allowed to dry for approximately 1 minute and than transferred to a sheet of plate glass.The batches were than assigned at random to the test filter papers to which they were confined by plastic jars. After exposure the insects were examined and the totals and n numbers responding were recorded.

The criterion of response was defined as inability of insects to stand and to walk.4 controls of solvent without extract were used.

RESULT AND DISCUSSION

I.From the resinous mass of Annona squamosa extract two compounds A and B was isolated ,and from resinous mass of Annona cherimolia compound C has been isolated.

The IR spectra of the three compounds are in the appendix.

II.Resultsof preliminary testing of insecticidal activities are in table as follow:

Contact poison test on Sitophylus oryzae

No.	Test solution %	Material	Number of insects	Mortality
1.	100	Extr. A squa mosa	20	20
2.	50	,,	20	20
3.	25	,,	20	20
4.	12.5	,,	20	18
5.	100	Extr. A cheri-molia	20	20
6.	50	,,	20	20
7.	25	,,	20	

8.	100	Filtrate of A.Sq. after precipit.	20	20
9.	50	,,	20	20
10.	25	,,	20	11
11.	12.5	,,	20	5
12.	12.5	Resinous mass of A. Squam.	20	3

Discussion:

The insecticidal activities of ether extract of Annona squamosa seeds and the precipitated mass from ether extract by adding petroleum ether had been investigated before(T.D. Mukeryee and Govind,1957).In those investigation it had been proved that the resinous precipitate contains most of the insecticidal principal.

In this preliminary study the precipitated resinous mass was studied.By fractionation in a column with chloroform and methanol(9:1) 2 compounds was isolated from A. Squamosa seeds ie compounds A and B,and from A. cherimolia seeds compound C.The IR spectra of compounds B and C showed very similar characteristics,so were the UV absorptions.

However preliminary testing showed that the precipitated resinous mass does not contain most of the insecticidal principal as suggested by previous investigator(table ,testing no. 12).A further fractionation and testing using several methods of the seeds extract will be carried out.

References:

N.B.Naidu et al,Jour. Indian Chem.Soc.,Ind. & News ed., vol.

16 ,No. 4,1953.

T.D. Mukerjea & Ram Govind,J. Sci. Industr. Res.,vol. 17C, January 1958.

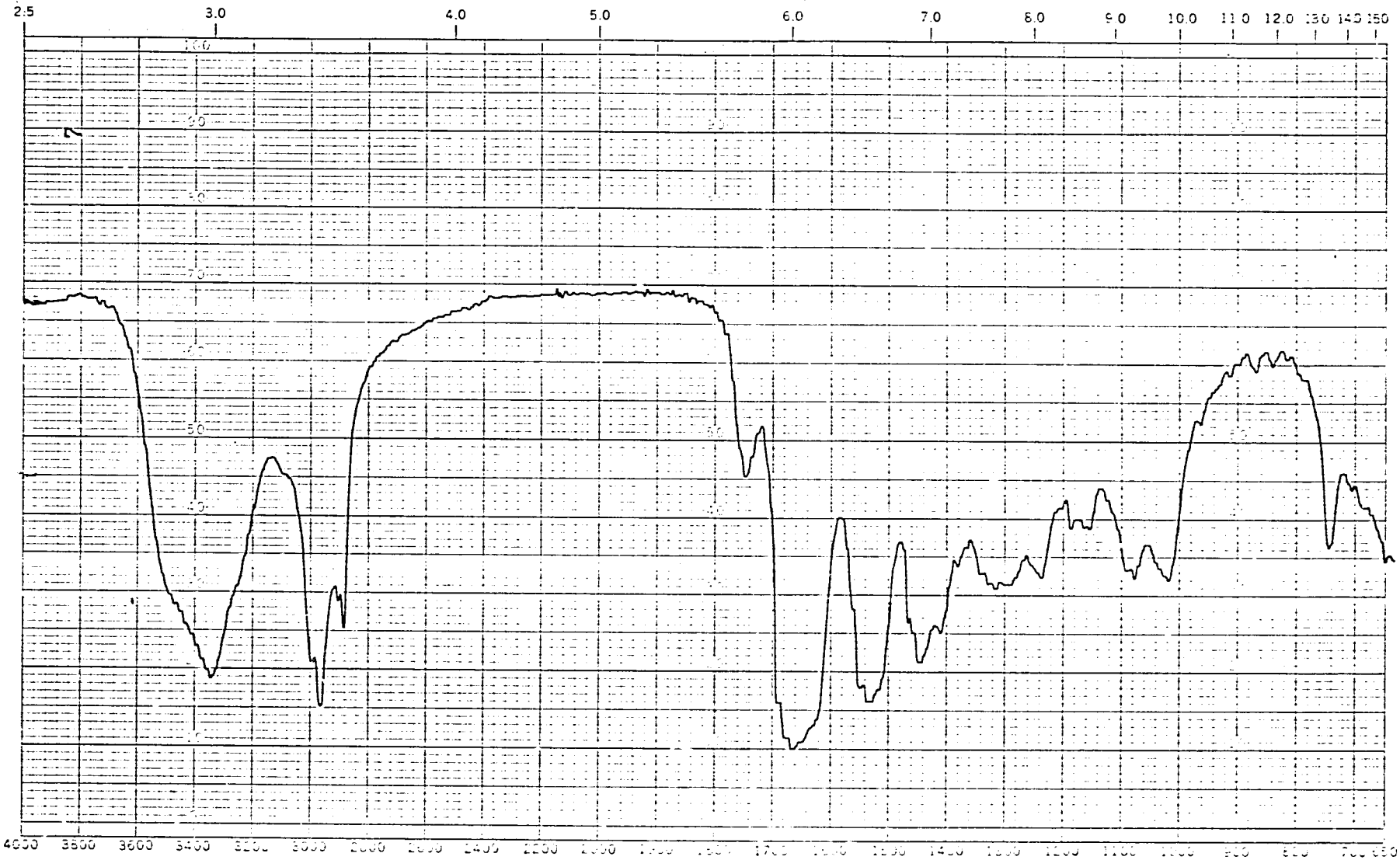
M.Leboeuf et al,Phytochemistry,vol. 21 ,No. 12,2783-2813,1982.

Appendices:

IR spectra of compound A

IR spectra of Compound B

IR spectra of compound C



DATE	SCAN SPEED	SLIT	SAMPLING CONC.	SAMPLE	REMARKS
OPERATOR	GAIN CONTROL	EXPANSION	CELL LENGTH SOLVENT	F 25 A	

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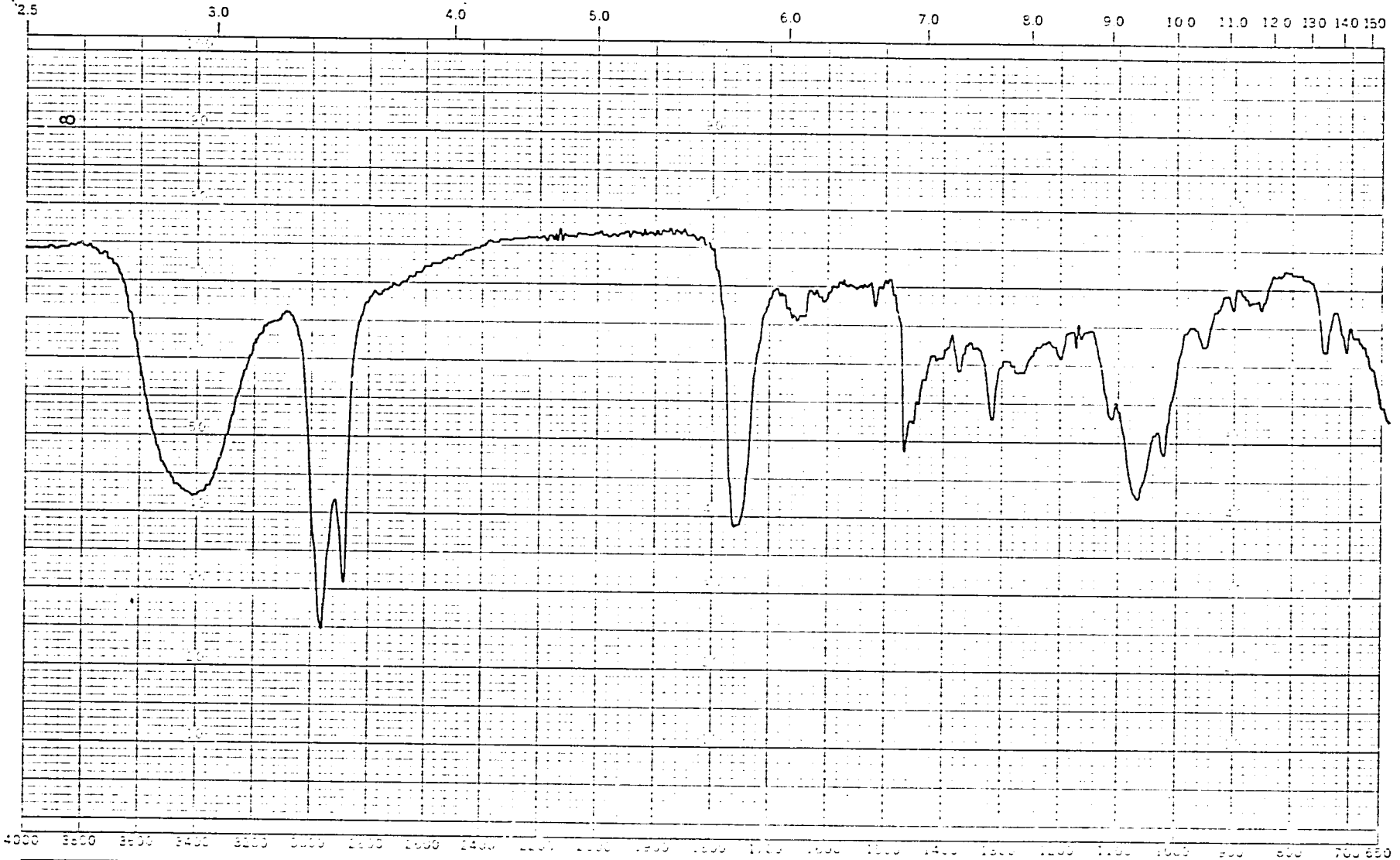

DATE	SCAN SPEED	SLIT	SAMPLING	SAMPLE	REMARKS
8/8/87	4	normal	CONC.	F 10.	
OPERATOR	GAIN CONTROL	EXPANSION	CELL LENGTH	B	
R.R.	5	1	SOLVENT		

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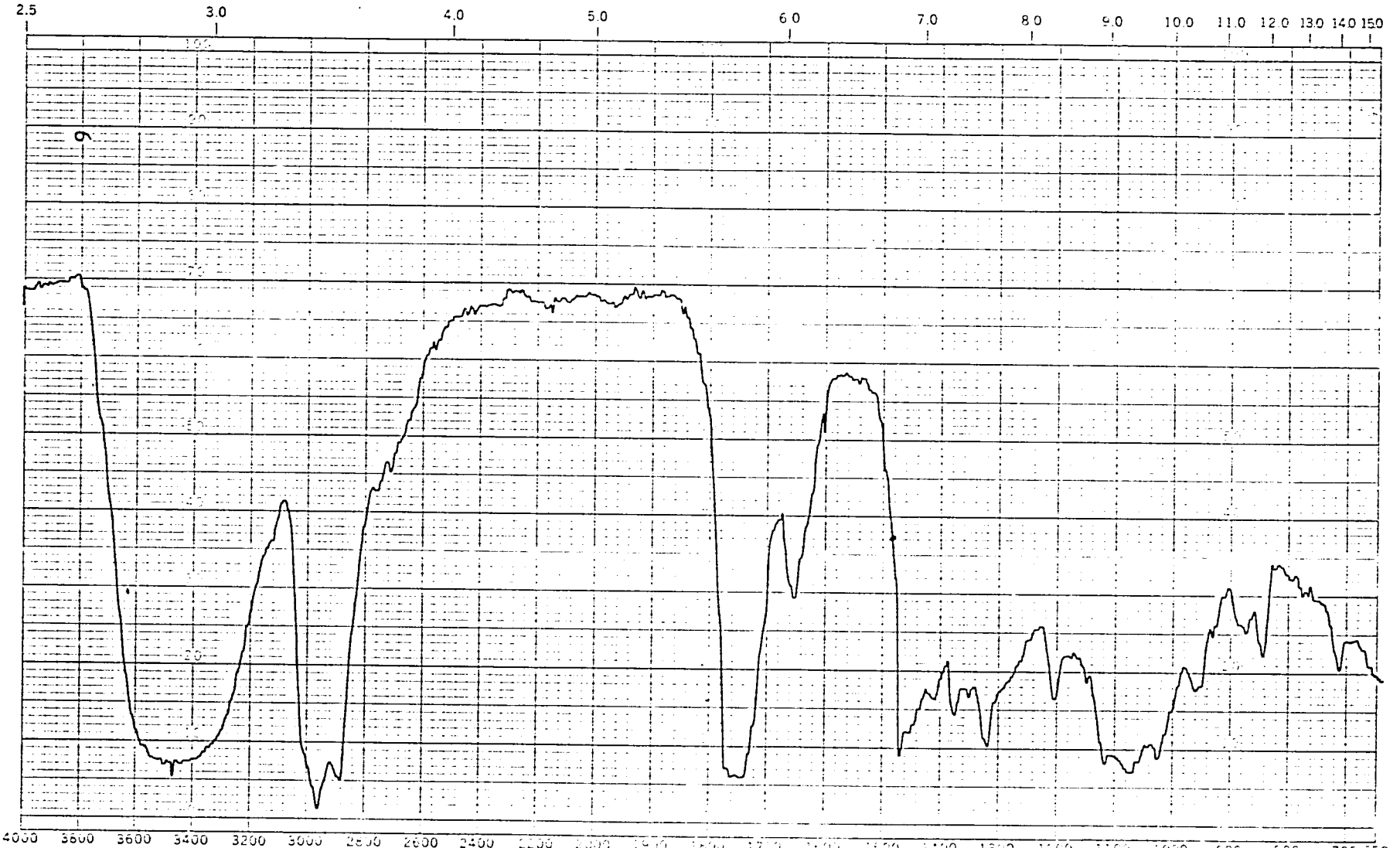

DATE	SCAN SPEED	SLIT	SAMPLING	SAMPLE	REMARKS
9/5/19	4	1	NaCl	B C	
OPERATOR	GAIN CONTROL	EXPANSION	CONC.		
PR	5	1	CELL LENGTH		
			SOLVENT Ether		

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