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AGENCY FOR INTERNATIONAL DEVELOPMENT
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PROGRESS REPORT NO. 4

SULFONAMIDE DRUGS AS A MALE ANTIFERTILITY AGENT

A RESEARCH PROJECT

US-ISRAEL CDR PROGRAM

Grant No. 936-5544-G-00-6105-00

Submitted by

Chumpol Pholpramool, Ph.D.

Project Leader

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Mahidol University, Rama VI Road, Bangkok 10400, Thailand

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Project Profile

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Grant No.	:	936-5544-G-00-6105-00
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Project Leader	:	Chumpol Pholpramool, Ph.D.
Organization	:	Faculty of Science, Mahidol University
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Project Consultants	:	None
Authorized Officer	:	Natth Bhamarapravati, M.D., D.Sc. Rector, Mahidol University
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1. Introduction

In the previous report, we have demonstrated that the sulfonamides which possess antifertility activity in the male rat could be a simple sulfonamide such as sulfanilamide (1). Furthermore, the presence of a p-amino or p-nitro group at the benzene ring is required for the activity (2). Thus when the p-amino group of sulfapyridine is deleted (N-2-pyridylbenzene sulfonamide) or is replaced by a methyl group (2-p-toluene benzene sulfonamido pyridine, PTSP) or o-nitro group (2-o-nitrobenzene sulfonamido pyridine, ONSP), these compounds are ineffective for suppression of fertility. On the other hand, 2-(p-nitrobenzene sulfonamido) pyridine (PNSP) and N^4-acetyl sulfanilamide are as potent as sulfasalazine. In order to substantiate these findings, 2-(4-bromobenzene sulfonamido) pyridine, 2-(3-nitrobenzene sulfonamido) pyridine, N^1-N^1 diethyl-N^4-acetyl-sulfanilamide and 2-(p-amino benzenesulfonylamino) pyridine have been synthesized, and the results of biological testing of some of the compounds are described in this study. The complete data from fertility testing of PTSP, ONSP and PNSP are also included in this report.

Since the possible effect of sulfa drugs showing antifertility activity in the male on the gonadal hormones is still unclear, the effect of sulfapyridine on serum testosterone of the male rats has been determined (2). The results indicate that sulfapyridine does not interfere with the testicular production of testosterone. However, whether sulfapyridine would alter the responsiveness of the pituitary gland to gonadotropin releasing hormone (GnRH) and the response of the Leydig cells to the released LH enhanced by GnRH are unknown. The present study attempts to deliniate this question.

2. Objectives

The aim of the work included in this report are :

(a) to synthesize some sulfapyridine analogs which lack the p-amino group in their benzene rings and to synthesize some sulfanilamide analogs, in which the N^1 position is substituted by an alkyl group,

(b) to test whether the compounds synthesized in (a) have any antifertility activity.

(c) to deliniate the possible effect of sulfapyridine on the responsiveness of the pituitary gland and the Leydig cells to GnRH,

(d) to develop suitable methods or tests for determining the function of epididymis on sperm maturation.

3. Materials and Methods

3.1 Fertility testing

Male rats of Fischer strain obtained from the Animal House at the Faculty of Science, Mahidol University aged between 90-120 days were screened for fertility capacity before grouping

and drug treatments. Each male was housed with two virgin female rats of the same strain aged 40-50 days in hanging cages with free access to pellet feeds and water for one week. The females were then separated and kept for 8-10 days at which time pregnancy was examined after sacrifice. The number of fetus and corpus luteum were counted under a dissecting microscope. Per cent fertility of the male was evaluated from the percentage of the eggs successfully fertilized and the embryos implanted, i.e., numbers of fetuses over corpola lutea x 100. Only the males showing high percentage fertility (greater than 80% in at least one of the two females) were selected for further experiments.

3.2 Collection of blood samples

Blood samples were collected from either left or right external jugular vein using aseptic conditions after the rats were anesthetized with diethyl ether. Blood samples were kept overnight in a refrigerator to allow complete coagulation. In the following morning, the blood samples were centrifuged and sera were separated and kept at -20°C for hormone assays.

3.3 Hormone assays

Testosterone was analyzed by RIA using the method of Resko et al. (3). Testosterone antibody (JAR #6) was obtained from Dr. J.R. Resko of Oregon Regional Primate Research Center, U.S.A. The sensitivity of the assay was 10 pg/ml. Intra-and interassay variation coefficients were 3.02% and 15.13%, respectively.

3.4 Experimental procedures

3.4.1 Experiment 1 (Fertility testing of the synthetic compounds)

Thirty nine male rats were divided into 5 groups (7-9 rats each) and were forced-fed with either corn oil, N^1-diethylsulfanilamide (DES) or N^4-acetyl-N^1-diethylsulfanilamide (ADES) at doses of 150 and 450 mg/kg (once daily) for 6 weeks. At the end of weeks 1, 3, 5, 7 and 9 all control and treated animals were subjected to fertility testing as described above except that the females were checked for the presence of spermatozoa in vagina smears done every morning after cohabitation so as to confirm mating with the male. Therefore, fertilities of the males at weeks 1, 3 and 5 during treatment and weeks 1 and 3 after the cessation of treatment were determined.

3.4.2 Expeiment 2 (The responsiveness of the pituitary gland and the testis to LH-RH)

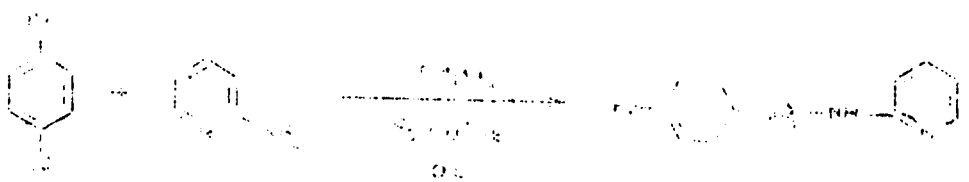
Sixteen male rats with proven fertility were allocated into 2 groups (8 rats each) and were forced-fed with either corn oil or sulfapyridine at a dose of 450 mg/kg (once daily) for 6 weeks. At the end of weeks 1, 3 and 5, all animals were subjected to fertility testing as in Experiment 1. At the end of treatment period (6 weeks), the animals in both groups were anesthetized with diethyl ether and 2-3 ml of blood samples were

collected as described above. Each animal then received a subcutaneous injection of 10 ug LH-RH (Sigma Chemical, U.S.A.) and were allowed to recover. Exactly two hours after LH-RH injection blood samples were collected again from the jugular vein.

In order to reduce large variations of blood gonadotropins and testosterone due to diurnal rhythms, all blood sample collections were made early in the afternoon as in previous experiments.

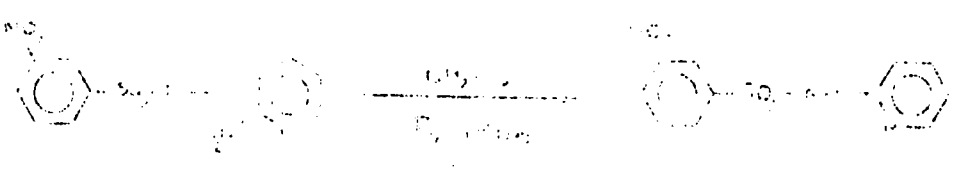
3.5 Synthesis of the proposed compounds

3.5.1 2-(4-Bromobenzene-sulfonamido) pyridine



4-Bromobenzenesulfonyl chloride (5.4 g) was dissolved in dichloromethane (40 ml) and cooled in an ice bath. To the above solution, a mixture of 2-aminopyridine in dichloromethane (20 ml) and pyridine (2 ml) was added dropwise. The mixture was stirred at room temperature for 3 hrs then the orange suspension was filtered and washed several times with cold water. The precipitate was recrystallized from hot methanol and white amorphous solid was obtained (2.06 g, 33% yield). The product was identified by its melting point (203-204° C), IR (Appendix 1a), NMR (Appendix 1b) as well as elemental analysis.
Calculation for $C_{11}H_9BrN_2O_2S$: C = 42.19; H = 2.90; N = 8.94
Analysis : C = 41.92; H = 2.97; N = 9.01

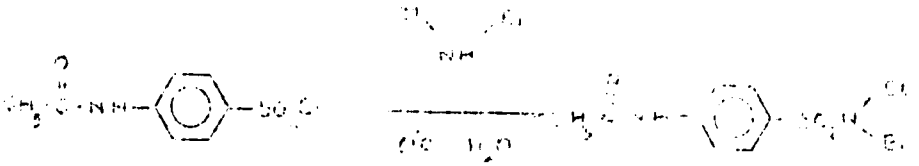
3.5.2 2-(3-Nitro-benzenesulfonamido)-pyridine



To 4.7 g of 3-nitrobenzene sulfonyl chloride in 30 cc. of dichloromethane was added dropwise with vigorously stirring on an ice bath, a mixture of 2 g 2-amino-pyridine in 20 cc. of dichloromethane and 2 cc. pyridine. After 2 hrs, the precipitate was filtered by vacuum filtration and washed several times with cold water then recrystallized from hot methanol, pale yellow amorphous solid was obtained, yield 1.56 g (27.9%). The product was identified by its melting point (228-229° C), IR (Appendix 2a), NMR (Appendix 2b) and elemental analysis.

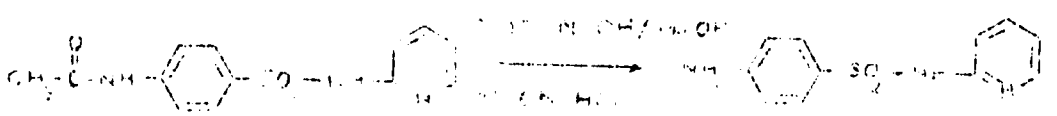
Calculation for $C_{11}H_9N_3O_4S$: C = 47.31; H = 3.25; N = 15.05
Analysis : C = 47.70; H = 3.33; N = 14.96

3.5.3 N'N'-Diethyl-N⁴-acetylsulfanilamide



To 5 cc. of diethylamine in 100 cc. three-necked flask, equipped with a condenser and a mechanical stirrer, cooled to 0°C, was added 2 g of N-acetylbenzenesulfonyl chloride in small portions with vigorous stirring. After the addition, about 20 cc. of water was added to the reaction mixture and stirring was continued until a homogeneous suspension was obtained. The suspended product was filtered, friturated and washed several times with cold water and recrystallized from methanol-water, white amorphous solid was obtained,yield 1.0 g (82.6%). The produced was identified by its melting point (60-62°C), IR (Appendix 3a), NMR (Appendix 3b), mass spectrum (Appendix 3c) and elemental analysis.
Calculation for $C_{12}H_{18}O_3N_2S$: C = 53.33; H = 6.71; N = 10.36
Analysis : C = 53.21; H = 6.84; N = 10.19.

3.5.4 2-(p-aminobenzenesulfonylamino) pyridine



2-N⁴-Acetylsulfanilylaminopyridine (1 g) was dissolved in 12% sodium hydroxide in methanol (10 ml) with stirring at ambient temperature for 1 hr. The mixture was carefully neutralized with 6 N hydrochloric acid. The precipitate was then filtered and recrystallized from hot methanol to give pale yellow amorphous solid (0.4 g, 53% yield), The product was identified by its melting point (189-191°C), IR (Appendix 4a), NMR (Appendix 4b) and mass spectrum (Appendix 4c).

4. Results and Discussions

4.1 Fertility testing of the synthetic compounds

Table 1 shows the complete data on the effect of 3 sulfonamides on fertility including the recovery period (after drug withdrawal) which were lacking in the previous report (2). It would appear that per cent fertility by weeks 1 and 3 after

drug withdrawal was not different from the control. However, comparison within a group between before treatment and after drug withdrawal indicates that per cent fertility is still significantly suppressed ($P<0.05$) by week-1 after withdrawing 2-(p-nitrobenzenesulfamido) pyridine (PNSP) at a dose of 450 mg/kg, whereas the fertility is recorved as soon as by one week after withdrawing PNSP at a lower dose.

Table 2 shows the effect of DES and ADES on fertility of the male rats. It should be noted that per cent fertility of all groups before treatment were relatively high in this series of experiment compared to other series, c.f. (1) and (2). In addition, per cent fertility was slightly decreased ($P<0.05$) by week 3 after feeding with corn oil in the control group. Furthermore, the number of males that inseminated the cohabited females was also decreased. The reason for the reduction in fertility of the control group during this period is not known However, it was noted that the body weights of these animals slightly decreased during 2-3 weeks after forced feeding with corn oil.

It is apparent from Table 2 that both DES and ADES markedly suppressed fertility of the male rats. ADES is more potent than DES. Thus, by week-5 after treatment with DES at doses of 150 and 450 mg/kg fertility was decreased to 37.8% and 22.8%, respectively. On the other hand, a substantial reduction in fertility occurred as soon as one week and reached a nadir by five weeks after treatment with ADES at both 150 mg/kg and 450 mg/kg doses. The potency of ADES is comparable to those of sulfapyridine (see Table 3) and sulfanilamide. However, it is worth noting that all animals receiving ADES at a high dose were suffered from urinary tract infections and one of them died during treatment (by week-5), whereas two of seven rats treated with a lower dose were illed. By contrast, all animals were healthy in the group treated with DES at low dose, but one of eight rats fed with high dose DES was illed and dead during treatment (by week-3). The causes of illness and death are unknown at present, but it is thought that this series of compound may be toxic to the urinary system since it caused an early hematuria followed by oligourea and dripping of urine. Pathological lesions in these animals are being investigated.

4.2 <u>The responsiveness of the pituitary gland and the testis to LHRH</u>

By the time of this report, hormone assays for LH, FSH prolactin and testosterone are still incomplete. Only the latter had been determined and the data are shown in Table 4. By 2 h after LHRH stimulation, serum testosterone of the control rats was elevated from 2.7+0.6 (n = 8) to 17.7+1.9 (n = 7) ng/ml, a 6.5 folds increase from the basal level. On the other hand, a 10.4 folds increase of serum testosterone was found in the sulfapyridine treated rats. Thus, serum testosterone was enhanced from the basal value of 2.2+0.6 (n=7) to 22.9+0.9 (n=7) ng/ml. It would appear that LHRH caused a significantly ($P<0.05$)

greater stimulation of the Leydig's cells in the sulfa-treated animals compared to the controls. Since the serum concentration of LH released in response to LHRH is not known, whether the responsiveness of Leydig's cells to endogenous LH is altered by sulfapyridine can not be speculated at present. The finding that levels of basal serum testosterone in both control and treatment groups are not different confirms our previous data (2). However, these values as well as the LHRH-induced serum testosterone levels in the control group in our study are relatively lower than those previously reported by Hodgson and de Krestser (7). These authors found approximately 9 folds increase in serum testosterone by 2 h after subcutaneous injection of 1.0 ug LHRH in normal rats.

4.3 Development of methods

We have tried to develop methods for testing the epididymal function of the sulfa-treated and control animals. This has proved to be difficult since there are no standard method available at present. However, several approaches have been explored and the preliminary results of which are summarized below.

4.3.1 Sperm morphology

Testicular spermatozoa and immature spermatozoa in the caput epididymidis still retain their cytoplasmic droplets which were attached to the neck region. These droplets move distally and are lost when spermatozoa enter the cauda epididymidis (3). Thus, we expected that cytoplasmic droplets would be present in the caput epididymal sperm but not in the cauda epididymal sperm of rats. However, our preliminary experiment showed 60-70% of the cauda epididymal sperm from both sulfa treated and untreated rats contained cytoplasmic droplets. This test was therefore not useful.

4.3.2 Sperm motility

There are several methods for the assessment of sperm motility (4). However, each method is suitable for only a limited conditions. Direct visual observation by using a haematocytometer or a similar chamber like a Makler cell seems to be more versatile. Indeed, we have tested the Makler cell with the rat sperm but sperm motility declined rapidly especially after washing the sperm with rat fertilizine medium. It is best to determine sperm motility in the sample freshly removed from the rat epididymis and without washing. Another method for measurement of sperm motility is being tested by using a glass slide and a cover slip with a fixed depth of 25 um of the solution containing sperm. This clearance for sperm movement is selected such that the sperm still has freedom of movement yet its path is always in focus. Motility will be evaluated by both subjective and objective methods. The former is based on grading system and the latter is measured interms of swimming speed after multiple exposure photography. At present, we do not have

sufficient information to evaluate the effect of sulfapyridine treatment on sperm motility. To test the in vitro effect of sulfapyridine is somewhat difficult due to poor water solutility of the drug itself.

4.3.3 Sperm surface bindings

Changes in sperm surface, which include increases in net negative charge and changes in surface proteins revealed by binding with lectins or specific antigens, have been associated with sperm maturation during their transit through the epididymis (3). The binding of specific antigens to sperm can be assessed by using fluorescence microscopy and selected monoclonal antibodies specific for these antigens. Preliminary experiments of this approach have been done but the results are at best unclear and nonquantitative.

4.3.4 Measurement of sperm surface SH groups

Sperm maturation has been shown to involve the oxidation of SH to SS bonds in the proteins of head and tail (3). A method for quantitative measurement of the SH groups has been worked out by Dr. Ruth Schalgi's group. The preliminary experiments have served to standardize the method. No conclusions are available at present.

4.3.5 Epididymal secretion of carnitine

It is known that in the presence of androgen, carnitine is concentrated by the epididymis and other accessory glands (5, 6). Attempts have been made to determine accumulation of ^{14}C-L-carnitine in the epididymis and various accessory sex organs of the male rats treated with sulfapyridine and control animals. Preliminary experiments in non-treated rats showed high level of radioactivity in the epididymis as well as in the seminal vesicles and coagulating glands. Sufficient data on the sulfa-treated animals have not yet been obtained to make any conclusion.

4.4 Synthesis of the proposed compounds

2-(4-Bromobenzenesulfonamido) pyridine was synthesize according to the developed procedure previously described by the reaction of 4-Bromobenzenesulfonyl chloride and 2 aminopyridine using pyridine as a base in the condensation reaction. The product was purified by recrystallization and the yield of 33% was obtained. Similarly, 2-(3-Nitrobenzenesulfonamido) pyridine was obtained by the reaction of 3-nitrobenzenesulfonyl chloride and 2-aminopyridine in 28% yield. The product was also purified by recrystallization.

To direct from the old trend of using 2-aminopyridine as the base moiety in the traditional sulfonamide drugs, we decided to synthesize and study the antifertility effect of simple sulfonamide drugs lacking the aminopyridine moiety. Our

first target in this series was directed to the synthesis of N^1N^1-diethyl-N^4-acetylsulfanilamide which was synthesized directly by the reaction of N-acetylbenzenesulfonyl chloride and diethylamine. Excess of diethylamine was used as a reactant and at the same time as a base in this reaction. The product was obtained in good yield (82%) after purification by recrystallization.

All the compounds we have successfully synthesized have been fully characterized by various spectroscopic methods (IR, NMR and MS).

5. Conclusion and Remarks

In this report we have synthesized 4 more compounds. Two of which contains either bromo or nitro substitution in the benzene ring, and one compound has a diethyl group at the N^1 position. Two synthetic compounds (DES and ADES) have been tested for possible antifertility activity. These compounds markedly suppressed fertility of the male rats and had potency comparable to that of sulfapyridine or sulfanilamide. However, ADES appears to have a toxic effect on the urinary system of the animals. The responsiveness of Leyldig's cells to endogenous LH released after LHRH stimulation has also been investigated. A definite conclusion can not be made at present in view of the lack of data concerning levels of serum LH.

In addition, we have done some preliminary experiments to develop methods for studying the function of the epididymis. Although conclusions are not presently available, it would appear that we may succeed in developing some of these methods for practical uses later on.

6. Workplan for the next reporting period

Three to four sulfonamides having dimethyl and/or diethyl substitution at the N^4 position as well as 3- or 4-(benzenesulfonamido) pyridine will be synthesized. Fertility testing of the compounds synthesized in this and somes in the next reporting period including N^4-acetyl sulfapyridine will be performed. Effects of sulfapyridine on spermatogenesis, sperm motility epididymal sperm reserve, metabolism and morphology of the epididymal spermatozoa will be studied. In addition, further development of the methods for studying epididymal functions will be investigated.

7. References

1. Pholpramool, C. (1987). Sulfonamide drugs as a male antifertility agent. Prog. Report. No. 2 US-Israel CDR program.

2. Pholpramool, C. (1988). Sulfonamide drugs as a male antifertility agent. Prog. Report No. 3 US-Israel CDR program.

3. Bedford, J.M. (1975). Maturation, transport and fate of spermatozoa in the epididymis. In : Handbook of Physiology, Sec. 7, Vol. 5, D.W. Hamilton & R.O. Greep, eds., Am. Physiol. Soc., Washington, D.C., pp. 307-317.

4. Mann, T. and Lutwak-Mann, C. (1981). Male Reproductive Function and Semen. Springer-Verlag, Heidelberg, pp. 71-74.

5. Marquis, N.R. and Fritz, IB (1965). Effects of testosterone on the distribution of carnitine, acetylcarnitine, and carnitine acetyl transferase in tissues of the reproductive system in the male rat. J. Biol. Chem. 240, 2197-2200.

6. Cooper, T.G. (1986). The Epididymis, Sperm Maturation and Fertilisation, Springer-Verlag, Heidelburg, pp. 180-191.

7. Hodgson, Y.M. and de Kretser (1982). Serum testosterone response to single injection of hCG, ovine-LH and LHRH in male rats. Internat. J. Androl. 5, 81-91.

Table 1. The effect of some synthetic sulfonamides on fertility of the male rats.

Treatment group	Dose (mg/kg)	Before treatment	Percentage Fertility				
			During treatment wk-1	wk-3	wk-5	After drug withdrawal wk-1	wk-3
Control	-	93.7±2.2 (8)	83.6±4.7 (7)	91.8±4.2 (7)	82.3±7.6 (8)	83.8±5.6 (8)	81.3±5.5 (8)
PNSP	150	90.9±4.5 (7)	82.7±7.9 (7)	54.3±11.9*** (7)	40.5±4.7***++ (6)	61.1±10.9 (7)	72.3±12.8 (7)
	450	91.5±3.8 (8)	82.3±6.3 (4)	36.2±15.5** (6)	56.2±10.7+ (7)	57.2±12.5+ (7)	82.0±8.5 (7)
ONSP	150	98.7±3.0 (7)	82.3±5.0 (7)	75.5±13.9 (7)	97.3±1.6 (5)	92.1±4.1 (7)	71.6±8.9+ (7)
	450	92.8±3.2 (8)	93.7±2.5 (7)	70.6±12.4 (5)	82.4±5.9 (8)	81.4±10.9 (8)	83.0±6.7 (8)
PTSP	150	95.5±2.9 (7)	87.1±7.3 (5)	90.0±6.8 (5)	92.6±7.4 (6)	84.9±6.9 (6)	68.4±14.6 (7)
	450	94.0±3.0 (7)	93.7±3.3 (6)	95.0±3.0 (7)	89.4±6.9 (7)	77.7±4.9+ (7)	71.2±13.4 (7)

Values are mean ± SEM (n)
*P<0.05; **P<0.01; ***P<0.001 when compared to the corresponding period in the control group (t-test).
+P<0.05; ++P<0.001 when compared to the control period (before treatment) within a group (t-test).

Table 2. The effect of some synthetic sulfonamides on fertility of the male rats.

Treatment group	Dose (mg/kg)	Before treatment	Percentage Fertility				
			During treatment			After drug withdrawal	
			wk-1	wk-3	wk-5	wk-1	wk-3
Control	-	95.1+2.3 (9)	89.5+4.4 (8)	63.5+18.0* (5)	95.3+2.2 (7)	-	-
DES	150	97.6+1.6 (7)	60.5+19.6 (6)	59.1+20.0 (6)	37.8+18.1**,++ (4)	-	-
	450	95.5+2.7 (8)	60.0+24.5 (5)	91.9+4.8 (3)	22.8+8.1***,+++ (5)	-	-
ADES	150	95.6+2.2 (7)	44.2+20.5*,+ (5)	47.8+17.0*+ (6)	30.9+9.2***,+++ (5)	-	-
	450	98.5+1.0 (8)	73.8+9.7*+ (5)	11.3+5.8*,++ (7)	21.0+11.6***,+++ (3)	-	-

Values are mean + SEM (n)

*P<0.05; **P<0.01; ***P<0.001 when compared to the corresponding period in the control group (t-test).

+P<0.05; ++P<0.001; +++P<0.001 when compared to the control period (before treatment) within a group (t-test).

Table 3. Fertility of the male rats to be tested for their responsiveness to LHRH.

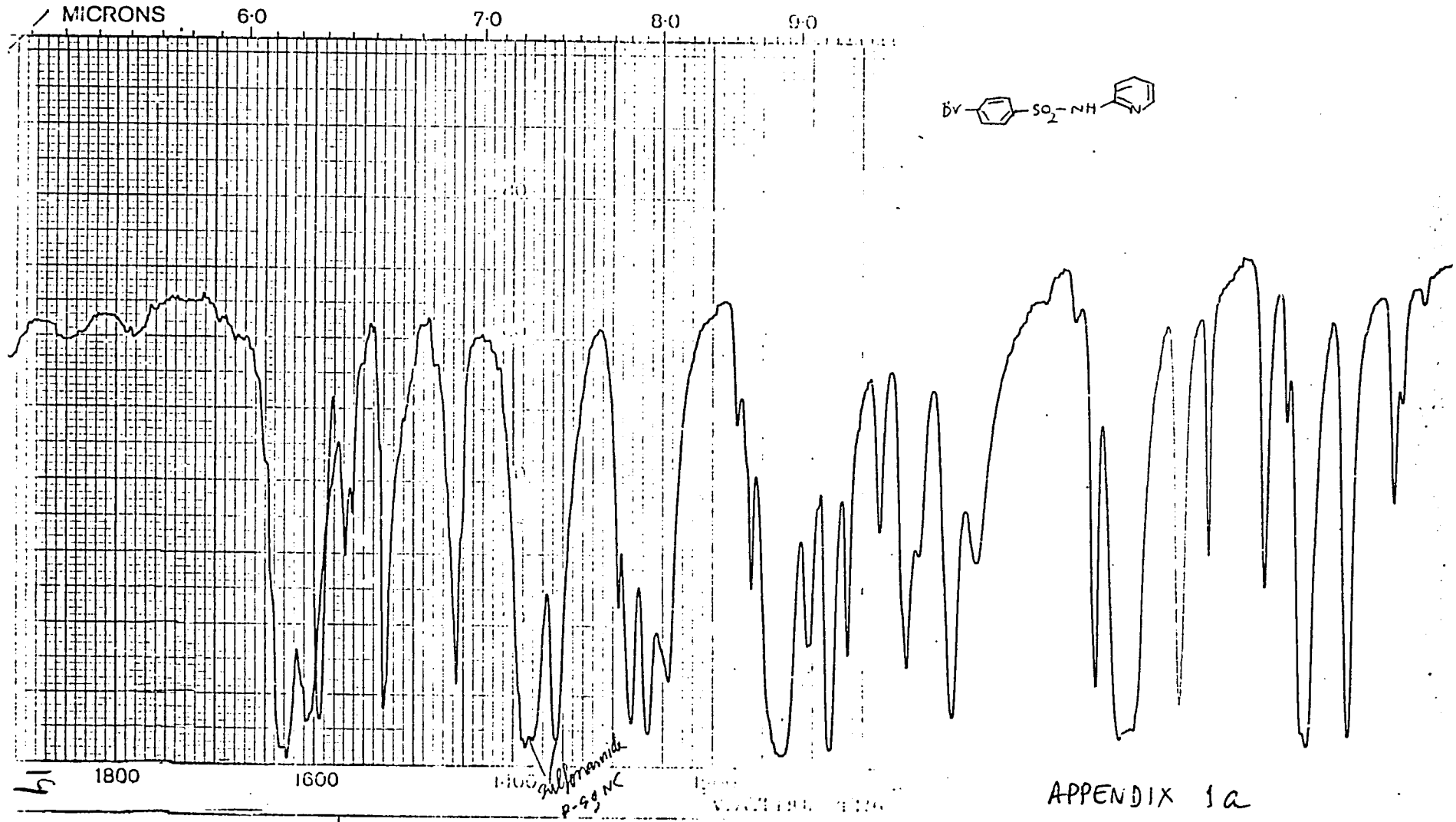
Treatment group	Dose (mg/kg)	Percentage Fertility			
		Before treatment	During treatment wk-1	wk-3	wk-5
Control	-	96.5±1.4 (8)	91.9±1.9 (8)	95.8±2.4 (4)	78.5±9.6 (5)
Sulfapyridine	450	90.7±2.3 (8)	91.2±2.7 (5)	32.4±7.8* (4)	14.3±11.9** (5)

Values are mean ± SEM (n)
*P<0.01; **P<0.001 when compared to the corresponding period of the control group.

Table 4. The effect of LH-RH on serum testosterone of sulfapyridine treated male rats.

Treatment group	Serum testosterone (ng/ml)		fold increase
	Before	2-hr after LH-RH	
Control	2.7±0.6 (8)	17.7±1.9 (7)	6.5
Sulfapyridine (450 ng/kg for 6 weeks)	2.2±0.6 (7)	22.9±0.9* (7)	10.4

Values are mean ± SEM (n)
*$P<0.05$ when compared to the control group.

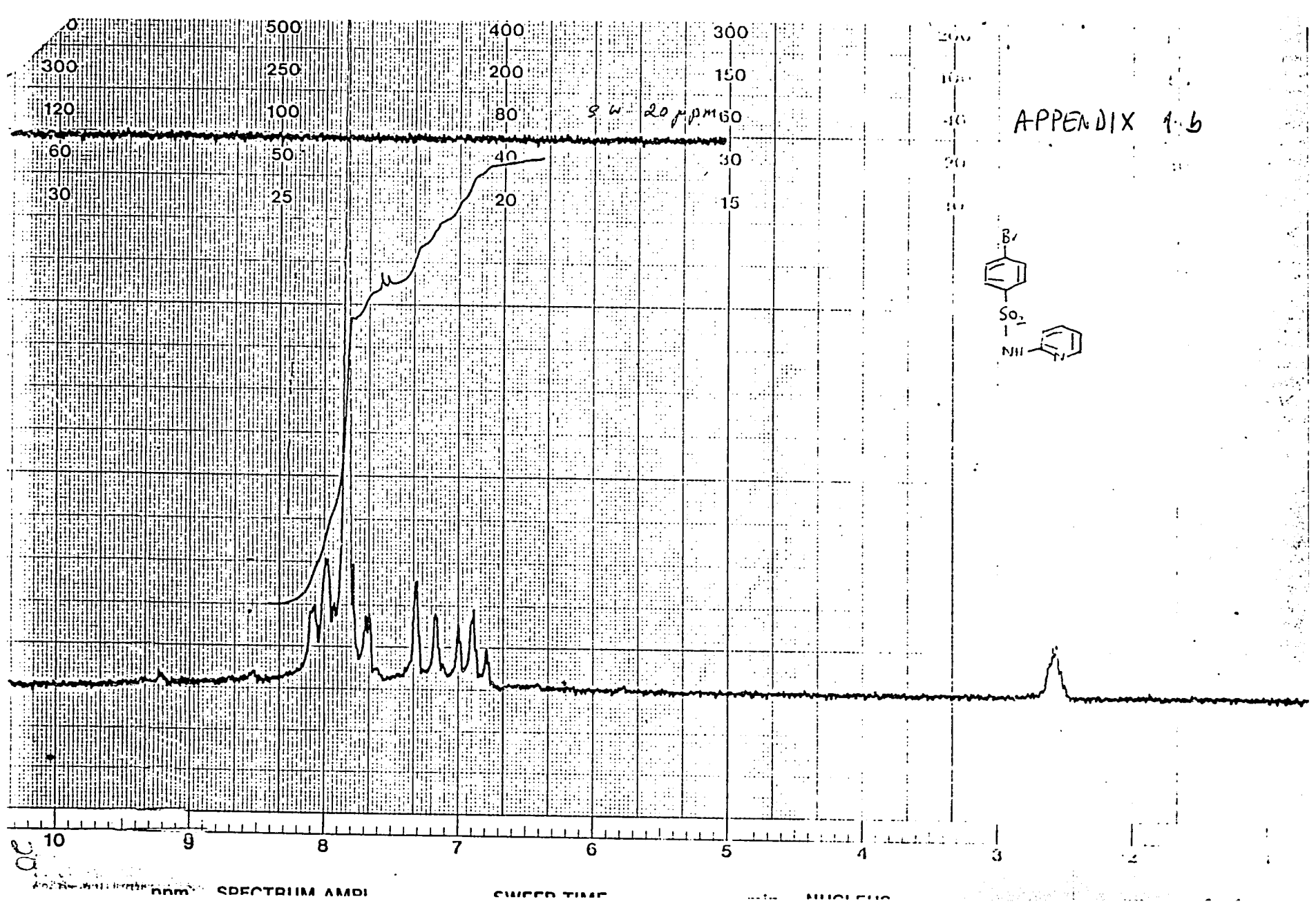


APPENDIX 1a

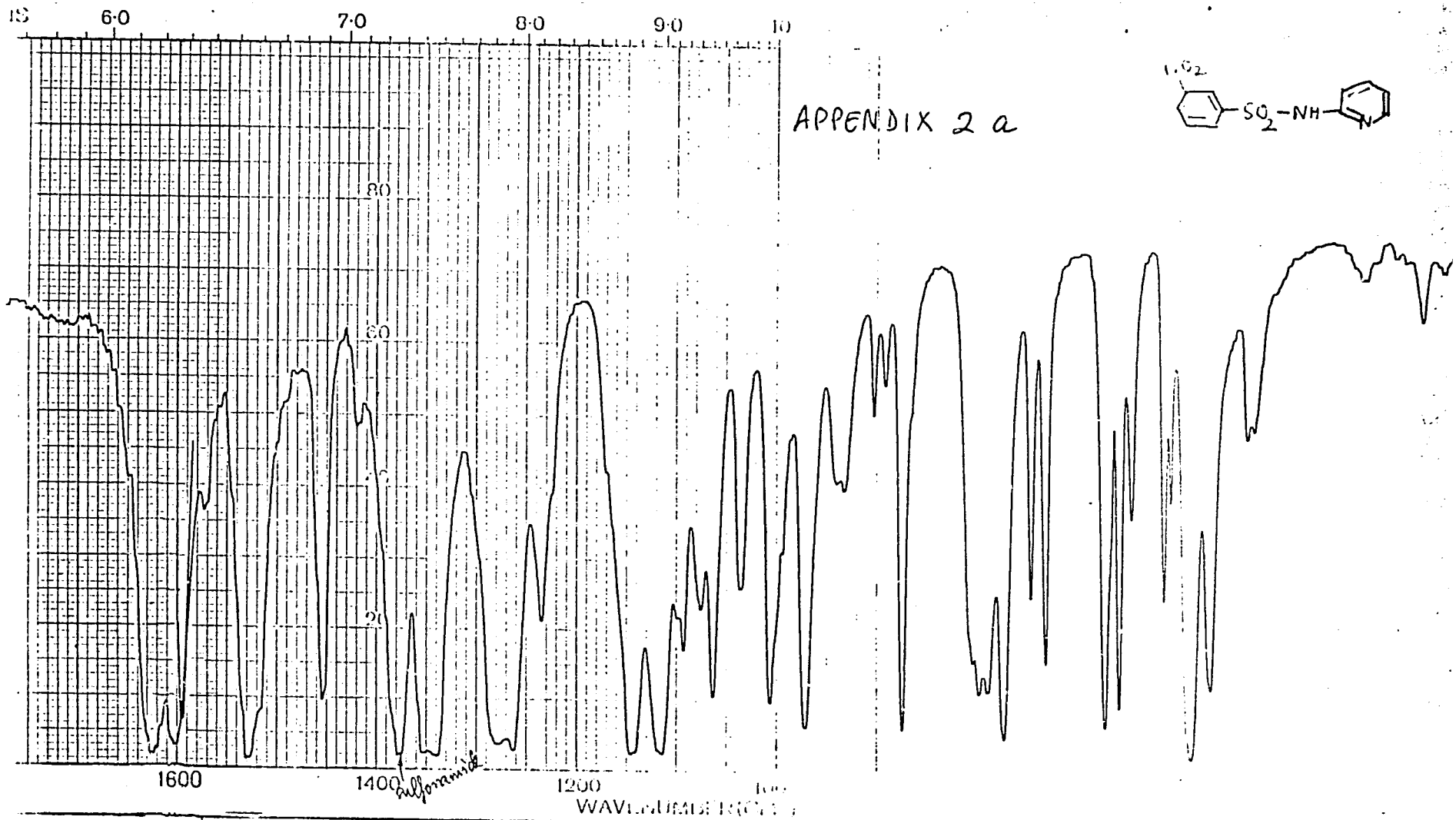
SLIT PROGRAM
SCAN TIME
MULTIPLIER
TIME CONSTANT
ORDINATE

$-SO_2-NH-$

204°C


APPENDIX 1.b
SW 20 ppm
Br
SO2
NH
N
10
9
8
7
6
5
4
3
2
ppm SPECTRUM AMPL
SWEEP TIME
min NUCLEUS

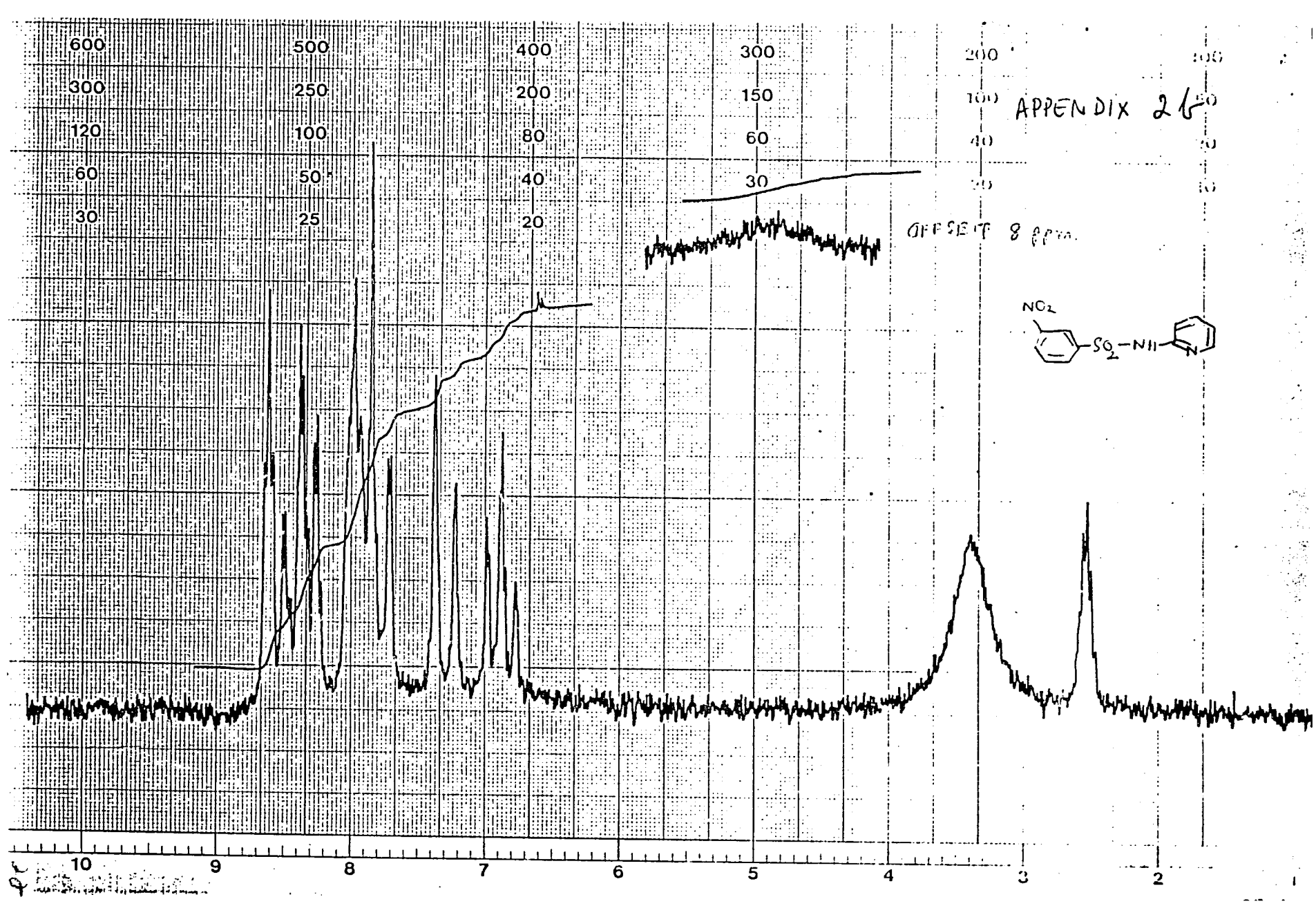
APPENDIX 2 a

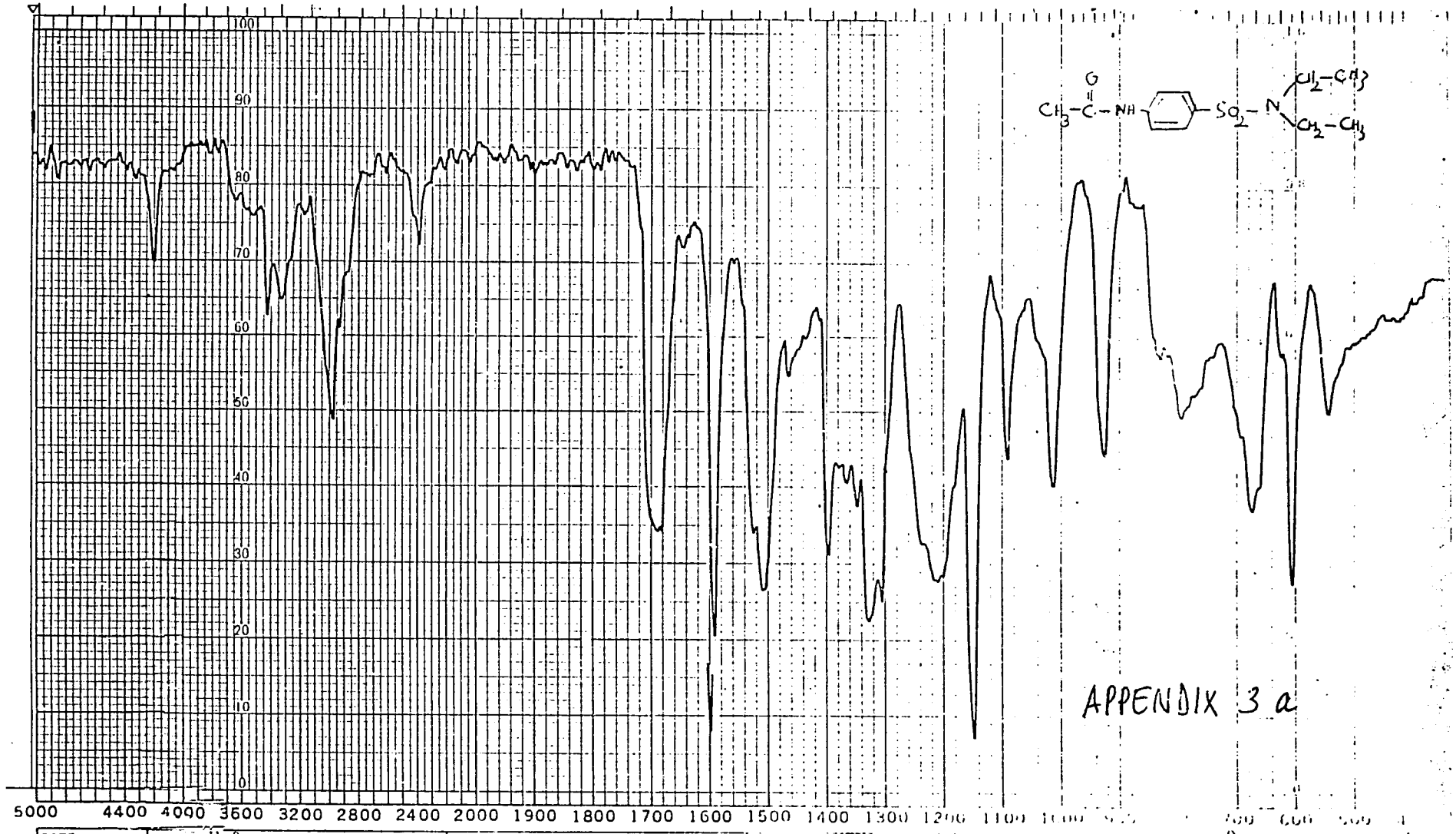


2

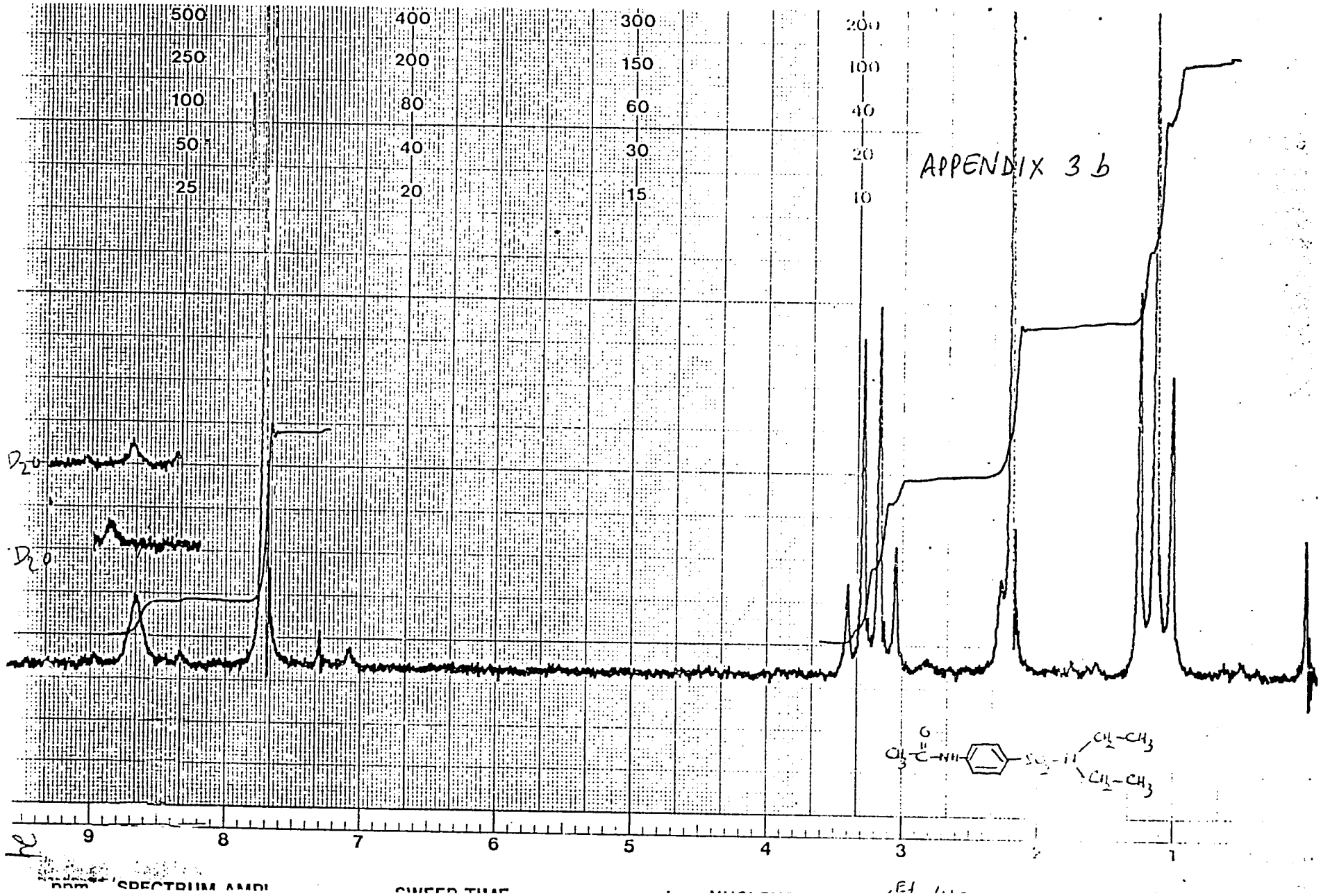
SLIT PROGRAM
SCAN TIME
MULTIPLIER
TIME CONSTANT

ORDINATE EXP


APPENDIX 26
OFFSET 8 ppm.
NO2
SO2-NH
600
300
120
60
30
500
250
100
50
25
400
200
80
40
20
300
150
60
30
200
100
40
20
10
9
8
7
6
5
4
3
2
1

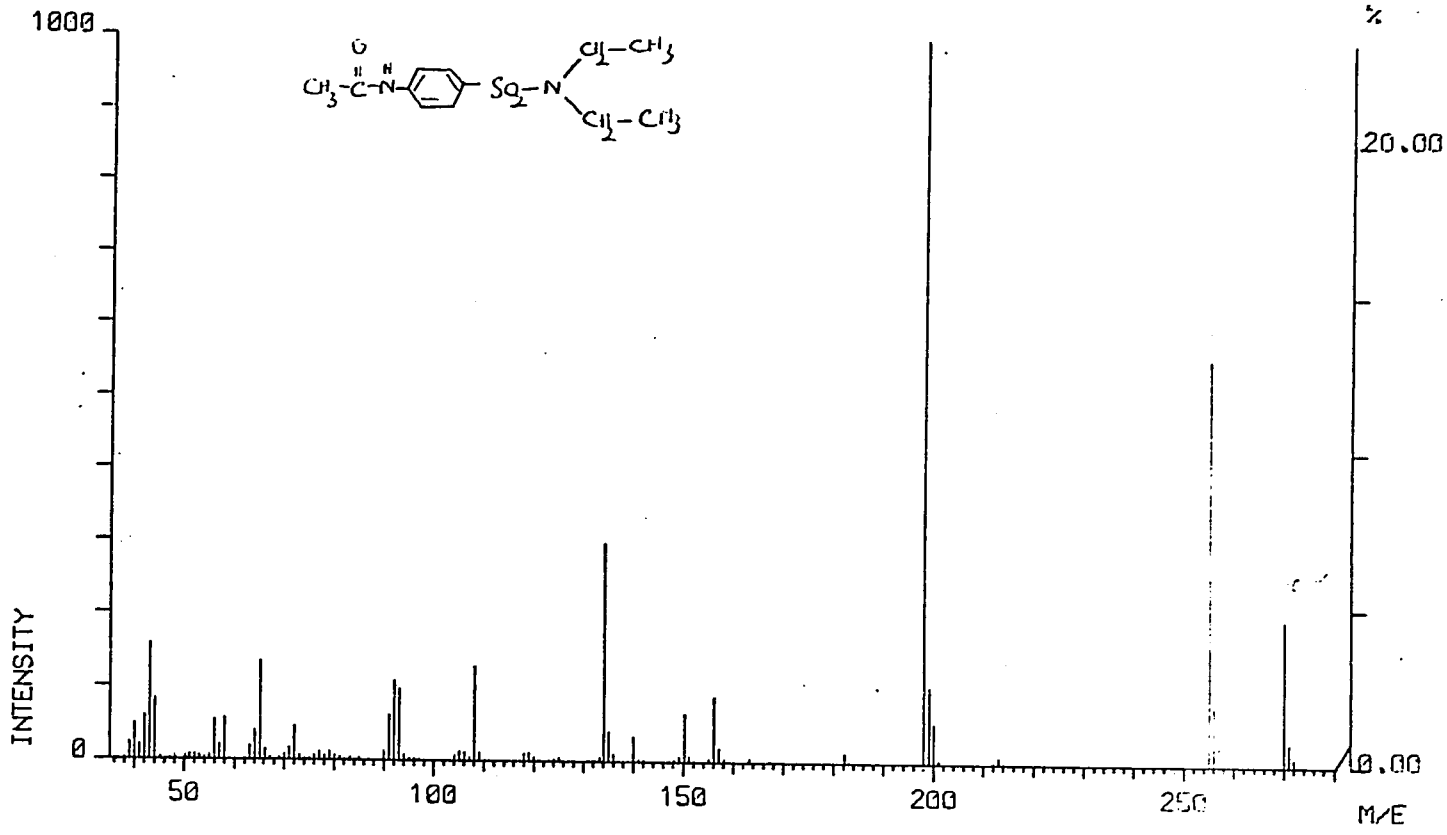


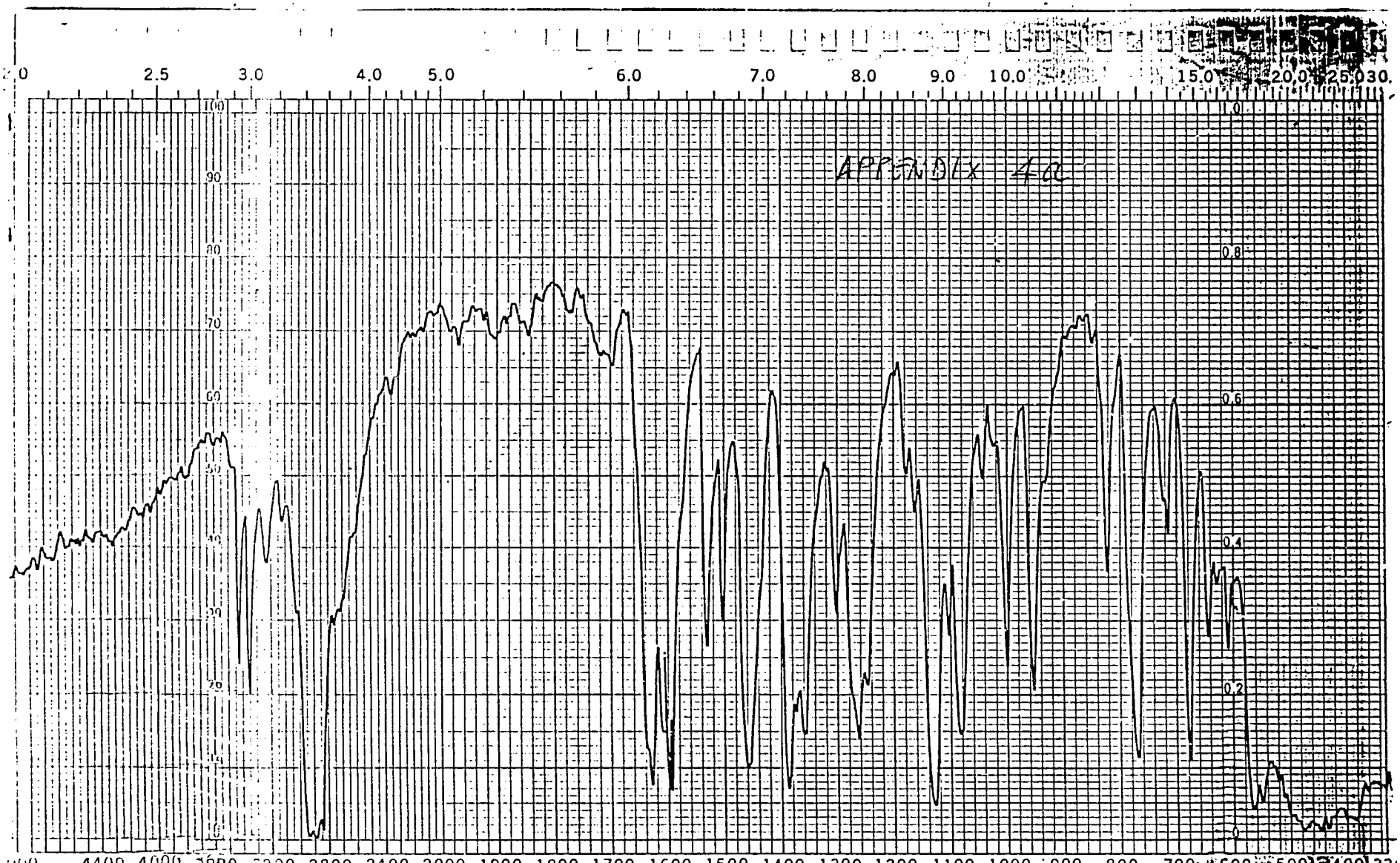
DATE	SPECTRAL-CONDITION		EXPANSION		SAMPLE
17/5/31	1	MAN. SLIT-WIDTH	T%	½ 1 2 5 10 MAN	SAMPLING-METHOD
OPERATOR	2	GAIN	ABS		CELL-LENGTH
ถ้วยแก้ว	3	SCAN SPEED	ν	½ 1 2 5 10 20 50 100	CONCENTRATION
					SOLVENT


APPENDIX 3 b
500
250
100
50
25
400
200
80
40
20
300
150
60
30
15
200
100
40
20
10
D_2O
D_2O
9
8
7
6
5
4
3
1

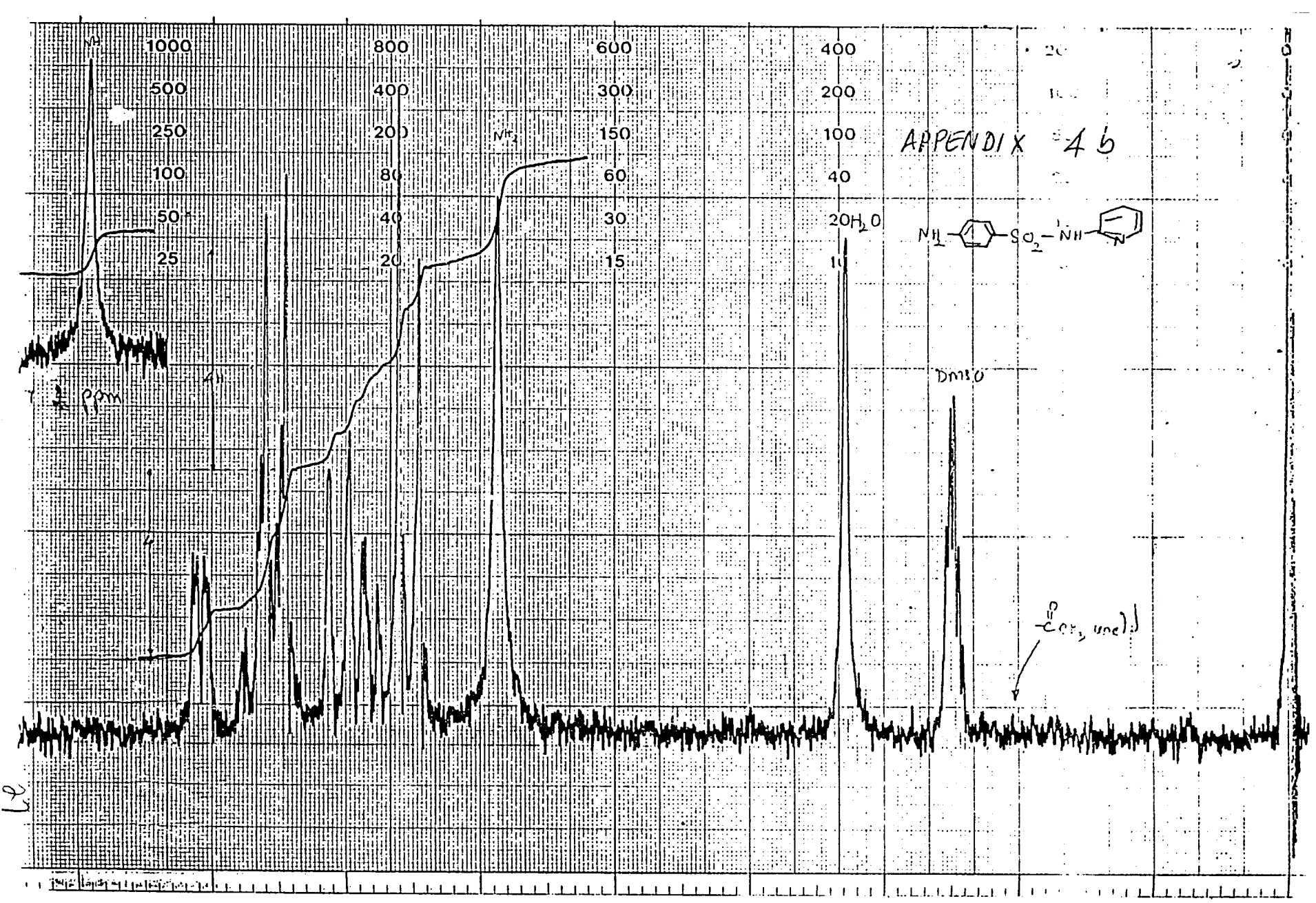
SAMPLE:EP-15-111 27 JUNE 88
NOTE :458/1 EI,70V,300UA.CHAMB.TEMP.100
BASE PEAK : M/E 198.0 INT. 568.6

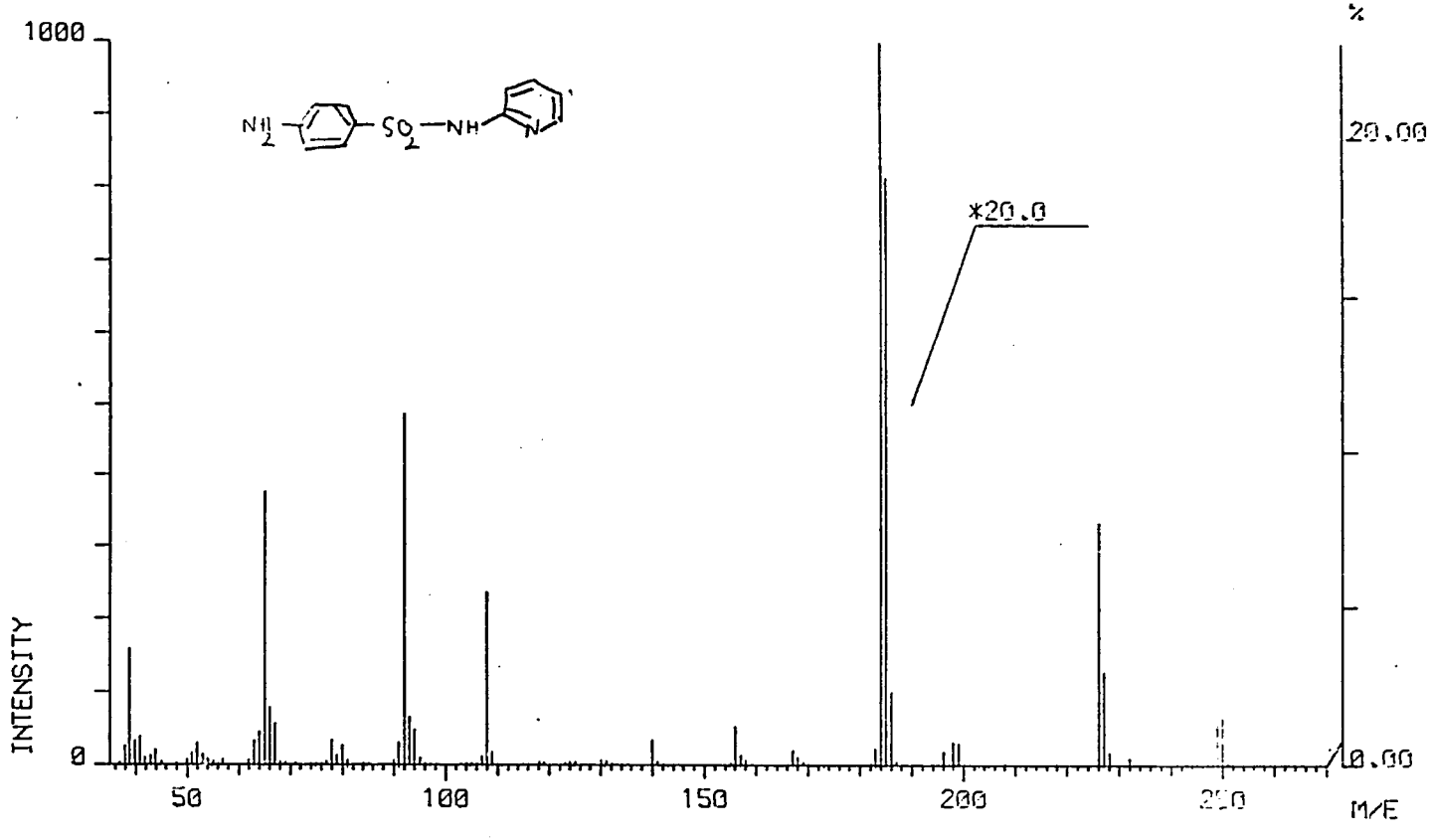
APPENDIX 3 C.





DATE	SPECTRAL-CONDITION	EXPANSION	SAMPLE
19 mar 81	1 MAN. SLIT-WIDTH	T% ½ 1 2 5 10 MAN	CP-13
OPERATOR	2 GAIN	ABS	SAMPLING-METHOD mull
			CELL-LENGTH


APPENDIX 4b
1000
500
250
100
50
25
800
400
200
80
40
20
600
300
150
60
30
15
400
200
100
40
20
10
H_2O
DMSO
ppm

SAMPLE:CP-13. M.P.189. 8 MAR 88.
NOTE :21/1.EI,70V,300UA.CHAMB.TEMP.180.
BASE PEAK : M/E 184.0 INT. 566.9

APPENDIX 4C