DIVISION OF NARCOTIC DRUGS
Vienna

RECOMMENDED METHODS FOR TESTING BARBITURATE DERIVATIVES UNDER INTERNATIONAL CONTROL

MANUAL FOR USE BY NATIONAL NARCOTICS LABORATORIES

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INTRODUCTION

Background

Over the past few years there has been a considerable increase in the number of substances newly placed under international control. At the same time, seized quantities of drugs already under control have also shown an alarming and unprecedented increase in certain regions. This new situation, involving an increase both in the frequency and volume of seizures, presents a challenge not only to national law enforcement authorities, but also to the technical and scientific staff of forensic laboratories.

Analysts have to be able to deal with more substances and preparations and to use faster, more accurate and more specific methods of identification and analysis. In addition, the international character of drug trafficking requires the timely exchange of analytical data between laboratories and law enforcement authorities both on the national and the international levels.

The Commission on Narcotic Drugs, at its tenth special session in February 1988, reviewed the technical and scientific assistance programme of the Division of Narcotic Drugs with special emphasis on the development of laboratory methodologies. It noted with satisfaction that the harmonization of laboratory methods and the programme on establishment of recommended methods of testing for national forensic laboratories was pursued vigorously and that such methods had already been developed for heroin, cocaine, cannabis products, opium/crude morphine, amphetamine/methamphetamine, ring-substituted amphetamine derivatives, methaqualone/mecloqualone, LSD and benzodiazepine derivatives.

In emphasizing the importance of the expert group meetings organized by the Division on various scientific and technical aspects of drug control and the high practical value for national law enforcement and laboratory services of the technical manuals as the outcome of the expert meetings, the Commission at its 33rd regular session strongly recommended that such meetings and the publication of laboratory manuals continue on a regular basis. It proposed that a manual for the analysis of barbiturate derivatives be prepared.

Purpose of the manual

In accordance with the recommendation of the Commission on Narcotic Drugs, a group of fifteen experts was convened in June 1989 in Wiesbaden, Federal Republic of Germany, by the Division of Narcotic Drugs in cooperation and with the financial support of the Government of the Federal Republic of Germany through UNFDAC. The present manual published by the United Nations Division of Narcotic Drugs reflects the conclusions of the group of experts and has been designed to provide practical assistance to national authorities by describing recommended methods to be used in

forensic laboratories for the identification and analysis of barbiturate derivatives under international control. The manual may also serve as a guide to national authorities in assessing existing methods used within their own government and university laboratories.

This manual is one in a series of similar publications dealing with the identification and analysis of various groups of drugs under international control; it was preceded by manuals on heroin (ST/NAR/6), cocaine (ST/NAR/7), cannabis (ST/NAR/8), amphetamine/methamphetamine (ST/NAR/9), opium/crude morphine (ST/NAR/11), ring-substituted amphetamine derivatives (ST/NAR/12), methaqualone/mecloqualone (ST/NAR/15), benzodiazepine derivatives (ST/NAR/16) and LSD (ST/NAR/17). A manual on hallucinogenic plant products is in preparation.

These manuals suggest approaches that may help the forensic analyst to select a technique appropriate to the sample currently being examined. The analyst may then choose to follow any of the methods described in the manual, as each method can be expected to produce reliable analytical information with respect to the samples to which they are applied. Each method has been used for a number of years in reputable forensic laboratories and has been published in the scientific literature. In identifying these methods, the expert group was aware that many other useful and acceptable methods produce worthwhile analysis and information for the forensic analyst, and that a number of other acceptable options are recorded in the forensic scientific literature.

Use of the Manual

Few methods are perfect, least of all in forensic drug analysis where the materials under examination are very likely to show significant variation both in their physical form and chemical composition. The choice of methodology and approach to analysis remains within the control of the analyst working within his or her own country. The analyst alone has seen the suspect material and can best judge the correct approach to the problem at hand. Furthermore, the choice of methods may necessarily depend on the availability of reference materials and of instrumentation.

Not all methods listed need to be applied to all samples suspected to contain a barbiturate derivative. Requirements vary, for example, as a result of local trends in samples encountered, facilities available, and the standard of proof acceptable in the prosecution system within which the analyst works. The more complex methods are needed only for certain forensic requirements, such as comparison of samples or for source determination.

In order to establish the identity of any controlled drug, it is suggested that the criteria should be at least two independent analytical parameters. The selection of these parameters in any particular case would take into account the drug involved and the laboratory resources available to the analyst. For example, two uncorrelated TLC systems would count as two parameters. Uncorrelated TLC systems in this context means that either the solvent systems or the coating on the plates are completely different. When possible, three entirely different analytical techniques should be used, for example: colour test, chromatography (TLC, GLC or HPLC) and spectroscopy (IR or UV). The actual choice of parameters is left to the discretion of the chemist.

Attention is also drawn to the vital importance of the availability of textbooks on drugs of abuse and analytical techniques. Furthermore, the analyst must continually keep abreast of current trends in analysis, consistently following current analytical and forensic science literature. For this purpose, attention is drawn to the Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control (ST/NAR/1), and to the Manual of Staff Skill Requirements and Basic Equipment for Narcotics Laboratories (SR/NAR/2), both published by the Division of Narcotic Drugs. The latter publication lists bibliographic references as well as a selection of well-known journals in the field. Analysts should refer to these and to previous manuals in this series for general descriptions of the analytical techniques included in this manual.

It is equally important that the latest information on changes in drugs available in the illicit traffic be quickly disseminated. This may often need to be done prior to publication in specialized periodicals dealing with forensic and other chemical analyses, since these publications are available to the forensic community some two to three years after the changes become known. The value of frequently published national reports on the latest information on such changes in drugs and on work being undertaken and analytical results obtained within individual laboratories cannot be over-emphasized.

The Division of Narcotic Drugs would welcome observations on the contents and usefulness of the present manual. Comments and suggestions may be addressed to:

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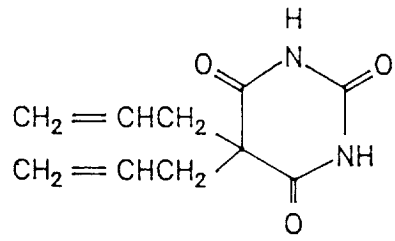
United Nations Office at Vienna
Vienna International Centre
P.O. Box 500
A-1400 Vienna, Austria

I. DESCRIPTION OF THE PURE COMPOUNDS

ALLOBARBITAL
5,5-diallylbarbituric acid
5,5-di-2-propenyl-2,4,6(1H,3H,5H)-pyrimidinetrione
allobarbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Allobarbital — Schedule IV

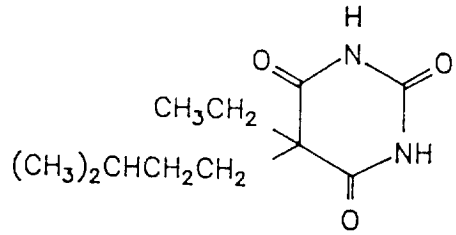


$C_{10}H_{12}N_2O_3$
M.Wt. = 208.2

M.pt. = 171-173°C

AMOBARBITAL
5-ethyl-5-isopentylbarbituric acid
5-ethyl-5-(3-methylbutyl)-2,4,6(1H,3H,5H)-pyrimidinetrione
amylobarbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Amobarbital — Schedule III



$C_{11}H_{18}N_2O_3$
M.Wt. = 226.3

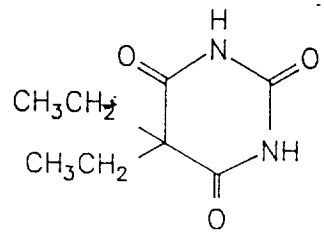
M.pt. = 155-161°C

AMOBARBITAL SODIUM
$C_{11}H_{17}N_2NaO_3$
M.Wt. = 248.3

M.pt. = ca.156°C

BARBITAL
5,5-diethylbarbituric acid
5,5-diethyl-2,4,6(1H,3H,5H)-pyrimidinetrione
barbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Barbital — Schedule IV



$C_8H_{12}N_2O_3$
M.Wt. = 184.2

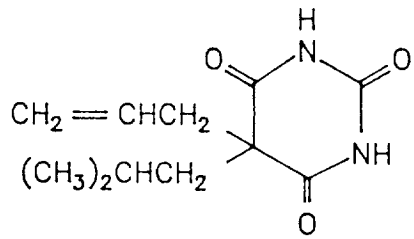
M.pt. = 188-192°C

BARBITAL SODIUM
$C_8H_{11}N_2NaO_3$
M.Wt. = 206.2

M.pt. = ca.190°C

BUTALBITAL
5-allyl-5-isobutylbarbituric acid
5-(2-methylpropyl)-5-(2-propenyl)-2,4,6(1H,3H,5H)-pyrimidinetrione
allylbarbituric acid
allylbarbital

Scheduled under the "Convention on Psychotropic Substances 1971"
Butalbital — Schedule III

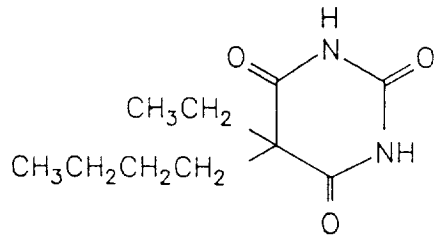


$C_{11}H_{16}N_2O_3$
M.Wt. = 224.3

M.pt. = 138-141°C

BUTOBARBITAL
5-butyl-5-ethylbarbituric acid
5-butyl-5-ethyl-2,4,6(1H,3H,5H)-pyrimidinetrione
butethal
butobarbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Butobarbital Schedule IV

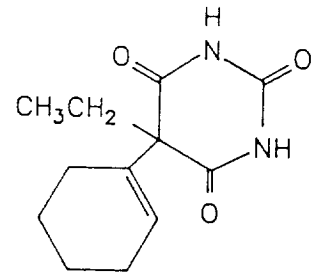


$C_{10}H_{16}N_2O_3$
M.Wt. = 212.2

M.pt. = 122-127°C

CYCLOBARBITAL
5-(1-cyclohexen-1-yl)-5-ethylbarbituric acid
5-(1-cyclohexen-1-yl)-5-ethyl-2,4,6(1H,3H,5H)-pyrimidinetrione
cyclobarbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Cyclobarbital Schedule III



$C_{12}H_{16}N_2O_3$
M.Wt. = 236.3

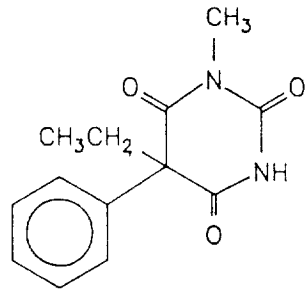
M.pt. = 171-175°C

CYCLOBARBITAL CALCIUM
$C_{24}H_{30}CaN_4O_6$
M.Wt. = 510.6

M.pt. = >300°C

METHYLPHENOBARBITAL
5-ethyl-1-methyl-5-phenylbarbituric acid
5-ethyl-1-methyl-5-phenyl-2,4,6(1H,3H,5H)-pyrimidinetrione
mephobarbital
methylphenobarbitone

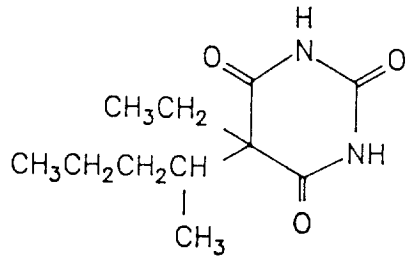
Scheduled under the "Convention on Psychotropic Substances 1971"
Methylphenobarbital Schedule IV



$C_{13}H_{14}N_2O_3$
M.Wt. = 246.3 M.pt. = 176-181°C

PENTOBARBITAL
5-ethyl-5-(1-methylbutyl)barbituric acid
5-ethyl-5-(1-methylbutyl)2,4,6(1H,3H,5H)-pyrimidinetrione
pentobarbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Pentobarbital Schedule III

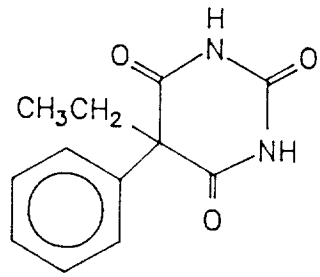


$C_{11}H_{18}N_2O_3$
M.Wt. = 226.3 M.pt. = 127-133°C

PENTOBARBITAL SODIUM
$C_{11}H_{17}N_2NaO_3$
M.Wt. = 248.3 M.pt. = 127°C (decomp.)

PHENOBARBITAL
5-ethyl-5-phenylbarbituric acid
5-ethyl-5-phenyl-2,4,6(1H,3H,5H)-pyrimidinetrione
phenobarbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Phenobarbital Schedule IV



$C_{12}H_{12}N_2O_3$
M.Wt. = 232.2

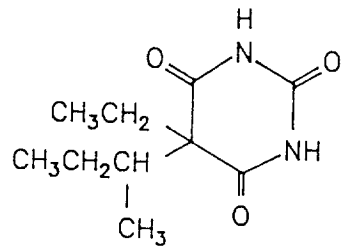
M.pt. = 174-178°C

PHENOBARBITAL SODIUM
$C_{12}H_{11}N_2NaO_3$
M.Wt. = 254.2

M.pt. = ca.175°C

SECBUTABARBITAL
5-sec-butyl-5-ethylbarbituric acid
5-ethyl-5-(1-methylpropyl)-2,4,6(1H,3H,5H)-pyrimidinetrione
secbutobarbitone
butabarbital

Scheduled under the "Convention on Psychotropic Substances 1971"
Secbutabarbital Schedule IV



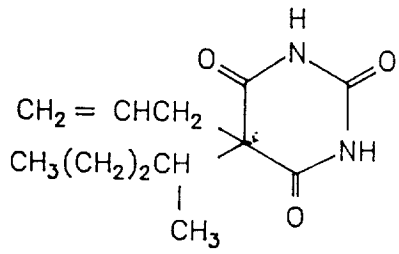
$C_{10}H_{16}N_2O_3$
M.Wt. = 212.2

M.pt. = 165-168°C

SECBUTABARBITAL SODIUM
$C_{10}H_{15}N_2NaO_3$
M.Wt. = 234.2

SECOBARBITAL
5-allyl-5-(1-methylbutyl)barbituric acid
5-(1-methylbutyl)-5-(2-propenyl)-2,4,6(1H,3H,5H)-pyrimidinetrione
quinalbarbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Secobarbital Schedule II

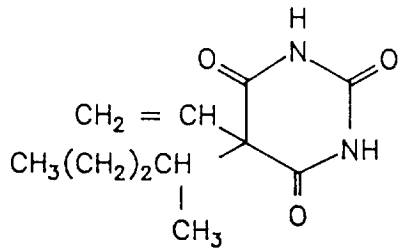


$C_{12}H_{18}N_2O_3$
M.Wt. = 238.3 M.pt. = 100°C

SECOBARBITAL SODIUM
$C_{12}H_{17}N_2NaO_3$
M.Wt. = 260.3

VINYLBITAL
5-(1-methylbutyl)-5-vinylbarbituric acid
5-ethenyl-5-(1-methylbutyl)-2,4,6(1H,3H,5H)-pyrimidinetrione
butylvinal
vinylbitone

Scheduled under the "Convention on Psychotropic Substances 1971"
Vinylbital Schedule IV



$C_{11}H_{16}N_2O_3$
M.Wt. = 224.3 M.pt. = 90-92°C

II. PRODUCTION, PHYSICAL AND CHEMICAL CHARACTERISTICS OF BARBITURATE DERIVATIVES UNDER INTERNATIONAL CONTROL

The barbiturates, therapeutically used as sedatives, hypnotics, anaesthetics and anticonvulsants, are a class of drugs derived from barbituric acid, a synthetic condensation product of malonic acid and urea. They differ mainly in the substitution pattern at the 5 position with some also including an N-methyl at N-1. They are usually classified according to the duration of their clinical effects into "long-", "intermediate-", "short-" and "ultrashort-" acting compounds.

Reportedly, over 2500 barbiturates have been synthesized with more than 50 of these presently marketed for clinical use throughout the world. Twelve of these are subject to international control under the Convention on Psychotropic Substances 1971 as follows:

> secobarbital in Schedule II, amobarbital, butalbital, cyclobarbital and pentobarbital in Schedule III and allobarbital, barbital, butobarbital, methylphenobarbital, phenobarbital, secbutabarbital and vinylbital in Schedule IV.

Abuse of barbiturates is widespread and the international nature of the illegal market means that any forensic laboratory may encounter a range of these compounds. However, virtually all of the barbiturates in the illicit market result from diversion from legitimate sources and there is no evidence of clandestine manufacture.

The twelve scheduled barbiturate derivatives appear mainly as capsules and tablets; however, some are marketed in other pharmaceutical forms such as elixirs, injectable solutions and sterile powders for injection. Pentobarbital sodium is available in some countries as rectal suppositories and barbital sodium is commonly sold in powder form for use as a buffer reagent. Barbiturates often occur as mixtures with other barbiturates (amobarbital/secobarbital), with other drugs (caffeine, aspirin, ephedrine, theophylline, codeine) and with other pharmaceutical excipients. This makes the isolation and identification of a specific barbiturate a considerable analytical challenge.

Analysts should be aware of the particular barbiturates commonly available in their area as well as the characteristics and methodologies for their identification and analysis. Reference should be made to national pharmacopoeias and drug tablet and capsule identification guides for preliminary screening information. The Multilingual Dictionary of Narcotic Drugs and Psychotropic Substances under International Control (ST/NAR/1) published by UNDND includes a listing of many brand names for six of these barbiturate derivatives. A planned future edition of this dictionary will include data on all of the scheduled barbiturates.

In the free acid form, barbiturates are soluble in most organic solvents such as ether, ethyl acetate, chloroform and methanol, but are insoluble in water. Amobarbital, pentobarbital, phenobarbital, secbutabarbital and secobarbital are also available as sodium salts and cyclobarbital as a calcium salt. These salts are generally insoluble in ether, ethyl acetate and chloroform, but are soluble in methanol and water.

III. THE ANALYSIS OF MATERIALS CONTAINING BARBITURATE DERIVATIVES

A. Sampling

The principal reason for a sampling procedure is to produce a correct and meaningful chemical analysis. Because most methods - qualitative and quantitative - used in forensic science laboratories for the examination of drugs require very small aliquots of material, it is vital that these small aliquots be entirely representative of the bulk from which they have been drawn. Sampling should be undertaken to conform to the principles of analytical chemistry, as laid down, for example, in national pharmacopoeias or by such organizations as the Association of Official Analytical Chemists.

There may be situations where, for legal reasons, the normal rules of sampling and homogenization cannot be followed if, for example, the analyst wishes to preserve some part of an exhibit as visual evidence. Alternatively, it may be necessary to perform separate assays on two powder items, rather than combining the powders prior to a single assay being performed on the mixture, because each has been separately exhibited by the seizing officer, and the legal system within which the analyst works requires individual results on every exhibit which is to be taken before the courts.

To save valuable resources and time, forensic analysts should seek, on all possible occasions, to use an approved sampling system to reduce the number of quantitative determinations needed yet guarantee statistically valid results. To facilitate such an approach, the forensic analyst may need to discuss individual situations with both seizing officers and the legal personnel with whom he works.

Barbiturate derivative exhibits are encountered predominantly as capsules and tablets as a result of diversion from the licit market. In some countries, bulk drug powder may be diverted from legitimate use.

1. Powders

(a) Sampling of single package items

The simplest sampling situation is where the submitted item consists of a single package of material. The material should be removed from its container or wrappings, placed in a clean clear plastic bag and the net weight recorded. The material should be thoroughly homogenized prior to the application of the sequence of chemical tests, although presumptive testing may be applied at this stage if it is thought that the sampling or homogenization process will be lengthy and there is still some doubt as to the identity of the material. The simplest way to homogenize a powder is to shake it thoroughly within the clear plastic bag to which it has been transferred. If the powder contains aggregates, these may be broken down by passing through successively finer sieves, or by pounding in a mortar with a pestle, or by using an adapted commercial food-mixer or food-processor.

Alternatively, the technique of coning-and-quartering can be applied, as follows: the sample is mixed by shaking or stirring. Large fragments are reduced if necessary; the material is then poured on a flat surface to form a cone. The "cone" is flattened and the material is then divided at right angles, forming quarters. Opposite quarters are taken for a sample; the remainder of the material is returned to the receptacle from which it was removed. Should further coning-and-quartering be desired to decrease sample size, particle sizes are further reduced, the material mixed thoroughly, poured onto a flat surface, and divided as before.

(b) Sampling of items consisting of more than one package

The analyst should examine the contents of all packages by eye, and possibly screen by using a simple colour test or TLC to determine:

1. If all packages contain suspect material, and/or

2. If one or more packages contain material different to that of the majority of packages. The simplest indicator is the physical appearance of the powder. If one or more packages obviously differ in content, these should be segregated and subjected to separate analysis.

The compositing of multiple container items is as follows:

(a) If there are less than 10 packages - all packages should be sampled.

(b) If there are 10 - 100 packages, randomly select 10 packages.

(c) If there are more than 100 packages - randomly select a number of packages equal to the square root of the total number of packages rounded to the next highest integer.

If the powders are found to be the same then the contents of a number of packages may be combined; the combined bulk material may then be homogenized in, for example, an adapted commercial food-processor. Alternately, the bulk may be subjected to coning-and-quartering.

When different types of material have been identified in the various packages, then each sub-group should be composited in an identical fashion to that previously outlined.

Sampling errors for quantitative methods are reduced if large aliquots of material are subjected to sequential dilution with the dissolving solvent. If the total sample size is large, this approach may be adopted. However, when large amounts of material are used for the first dissolution, it may be necessary to add the solvents by pipette to avoid error due to insoluble materials.

2. Tablets and capsules - Commercial or licit preparations

The preliminary determination of commercial origin is a subjective one. Clear-cut examples of products of commercial origin would be dosage units resembling descriptions as pictorial representations in national compendia of pharmaceutical preparations. Commercial preparations usually undergo quality control by the manufacturer; therefore, little useful information would be gained by screening a large number of units from each package. The amount of ingredient per tablet or capsule determined will be statistically valid for the entire lot.

(a) Single container

1. 1-50 dosage units -- Randomly select 1/2 total number of units to a maximum of 20. Determine average weight, powder to pass through a 20-mesh sieve and mix thoroughly.

2. 51-100 dosage units -- Randomly select 20 units, proceed as above.

3. 101-1,000 dosage units -- Randomly select 30 units, proceed as above.

4. Greater than 1,000 dosage units -- Randomly select a number of units equal to the square root of the total number present, rounded to the next higher integer; proceed as above.

(b) Multiple containers

Segregate containers by lot numbers and treat each group as described in 1 (b) above. Report results separately for each group.

Determine the square root of the total number of packages in each group. Randomly select a number of packages equivalent to the square root, rounded to the next highest integer.

From each of the selected packages, randomly select a number of dosage units equivalent to the square root of the total number of dosage units divided by the square root of the number of packages, rounded to the next higher integer.

Form a composite by grinding, sieving through a 20-mesh sieve and thoroughly mixing. Perform the analysis on the composite.

3. Residues from syringes

Because of the trace amounts of drug usually present on hypodermic syringes seized from individuals, the analyst should not attempt to perform presumptive tests but should proceed directly with conclusive analytical procedures.

Wash the syringe with a minimum amount of methanol and concentrate it to dryness under a stream of nitrogen. Proceed with selected tests.

B. Extraction techniques

Virtually all of the barbiturates encountered in the illicit traffic appear in the form of tablets, capsules and bulk powder diverted from legitimate sources. They are present as the free acid or as the sodium or calcium salt.

1. For qualitative analysis

Both the free acids and the salts are soluble in methanol and this is the solvent of choice for sample preparation for presumptive or qualitative analysis.

METHOD

Triturate a quantity of finely powdered tablet or capsule contents or bulk drug powder with a small amount of methanol sufficient to obtain a solution containing approximately 1 to 20 mg of barbiturate per milliliter. The extract may be used directly or, after filtration, evaporated to dryness under a stream of nitrogen.

2. For quantitative analysis

(a) Capsules and tablets containing barbiturates in the free acid form:

METHOD

Combine the contents of a representative number of capsules or tablets as determined by the sampling procedure above. Transfer an accurately weighed amount of the capsule or tablet contents, equal to the full weight of one or more tablets or capsules to a suitably sized volumetric flask and dilute to volume with ethyl acetate. The extract may be used directly or an aliquot removed, filtered and evaporated to dryness.

(b) Capsules and tablets containing barbiturates in the salt form:

METHOD

Dissolve or suspend an accurately weighed amount of the representative sample of barbiturate as determined by the sampling procedure above, in an appropriate volume of water (10 ml) in a separatory funnel. Add 3N HCl to acidify the solution. Extract with several 10 ml portions of ethyl acetate. Combine these ethyl acetate extracts and filter through glass wool. Bring the filtered extract to a suitable, known volume with ethyl acetate. The extract may be used directly or an aliquot removed and evaporated to dryness.

C. Presumptive tests

1. Tablet and capsule identification guides

As a first test, analysts should refer to national identification guides for presumptive identification of the barbiturate products commonly available in their country.

Some useful guides such as those listed below include pictorial representations of legitimate capsule and tablet forms to assist in identification.

References:

1. "Physicians' Desk Reference", 43rd edition, Medical Economics Company (Oradell, N.J.), 1988.

2. "Compendium of Pharmaceuticals and Specialties" 23rd edition, Canadian Pharmaceutical Association (Ottawa), 1988.

3. "Tablident, EBL Guide", 2 volumes, EBL Publications (Buckinghamshire, U.K.) 1988.

4. "ITAKA 88, Identifiering av Tabletter och Kapslar", Apoteksbolaget AB, (Stockholm) 1988.

5. "The Logo Index for Tablets and Capsules", 1st edition, U.S. Department of Justice, Drug Enforcement Agency (Washington) 1988.

2. Colour tests

It must be stressed that a positive result from a colour test is only a presumptive indication of the possible presence of a barbiturate derivative. Nevertheless, the colour test suggested below is very useful because all compounds in the barbiturate class react in a similar manner and very few other drugs cross-react to give the same colour with the test reagents. No information as to which particular barbiturate is present, however, can be made with this colour test.

Dille-Koppanyi test

Solution K_1: Dissolve 0.1 g cobaltous acetate tetrahydrate in 100 ml absolute methanol, then add 0.2 ml glacial acetic acid.

Solution K_2: Mix 5 ml isopropylamine with 95 ml absolute methanol.

Reference:

1. Rapid Testing Methods of Drugs of Abuse. A manual for use by national narcotics laboratories, ST/NAR/13, United Nations (New York) 1988.

METHOD

Place a small amount of the suspected material in a depression of a spot plate. Add three drops of solution K_1 and three drops of solution K_2. A purple colour indicates the possible presence of barbiturates.

3. Salt determination

For quantitative purposes, it is necessary to know whether the barbiturate is present as a free acid or in a salt form. Test (a) is mainly applicable to bulk powder. As excipients in tablets and capsules may interfere with the observation, test (b) may be more appropriate for those preparations.

a) Solubility:

Place small amounts of the suspect material in each of two test tubes. Add several drops of water to the first test tube and several drops of ethyl acetate to the second. Observe in which solvent the material dissolves. Free acids are soluble in organic solvents such as ethyl acetate, but are insoluble in water. The salt forms of the barbiturates are readily soluble in water, but are insoluble in ethyl acetate. Other organic solvents such as ether and chloroform may be substituted for ethyl acetate.

b) pH determination:

Place a small amount (ca.10-20 mg) of the suspected barbiturate in a test tube and add 1 ml of water. Determine the pH. A pH greater than 8.0 indicates that the barbiturate is present as the sodium or calcium salt.

D. Thin-layer chromatography

PLATES

Activated silica gel G on glass backed plates; the coating (0.25 mm thickness) contains an additive which fluoresces at 254 nm.

DEVELOPING SOLVENTS

SYSTEM A:	Ethyl acetate	85
	Methanol	10
	25% Ammonia	5
SYSTEM B:	Chloroform	80
	Acetone	20

Preparation of solutions to be applied to the TLC plate

Sample: Extract the material using the method outlined in Chapter III B and prepare a solution in methanol containing the equivalent of approximately 5 mg/ml.

Standard solutions: All made at a concentration of 5 mg/ml in methanol.

Apply 1 to 2 ul of the sample and standard solutions to the plate.

VISUALIZATION

The plates must be dried prior to visualization. This can be done at 120°C for 5 minutes in an oven or, more quickly, by using a hot air blower.

Visualization methods

1. UV light at 254 nm both before and after exposure to ammonia vapour.

2. Mercuric chloride-diphenylcarbazone reagent: (NOTE)

Spray reagent

(a) dissolve 0.1 g of diphenylcarbazone in 50 ml of ethanol
(b) dissolve 1 g of mercuric chloride in 50 ml of ethanol.
Prepare the solution daily; mix (a) and (b) just before spraying.

NOTE

Mercuric chloride - diphenylcarbazone is the most sensitive spray reagent amongst the many tested for the detection of barbiturates. However, the use of mercury salts cannot be recommended because of environmental concerns. Detection by visualization method 1 is sufficient, but should the use of this reagent still be required, the spraying procedure must be performed with special care to guard against harmful mercury vapours.

METHOD

First observe the plate under short wavelength UV light (254 nm). Expose the plate to concentrated ammonia vapours and observe again under UV light at the same wavelength. If necessary, spray with mercuric chloride-diphenylcarbazone reagent. Barbiturates give blue-violet spots on a pink background. Detection limit is about 1-5 ug.

RESULTS

R_f x 100 values:

COMPOUND	Developing System A	Developing System B
Allobarbital	31	50
Amobarbital	40	52
Barbital	33	41
Butalbital	44	54
Butobarbital	39	50
Cyclobarbital	35	50
Methylphenobarbital	41	70
Pentobarbital	44	55
Phenobarbital	29	47
Secbutabarbital	44	50
Secobarbital	42	55
Vinylbital	40	38

References:

1. "Thin-layer Chromatographic R_f Values of Toxicologically Relevant Substances on Standardized Systems" DFG/TIAFT, VCH Verlagsgesellschaft, (Weinheim) 1987.

2. "Clarke's Isolation and Identification of Drugs." 2nd edition, The Pharmaceutical Press (London) 1986.

E. Gas liquid chromatography

1. Packed column technique

(a) Without derivatization

The use of the packed column technique for the analysis of underivatized barbiturates is not recommended.

(b) With derivatization

Operating conditions:

Detector:	FID
Column:	6 ft (or 2 m), 2 to 4 mm ID glass
Packing:	3% SE-30 on 80-100 mesh Chromosorb G HP
Carrier gas:	Nitrogen at 45-50 ml/min
Column temperature:	190-200°C
Injector/detector temperature:	220°C
Internal standard:	n-alkanes
Derivatizing agent:	trimethyl anilinium hydroxide 0.2M in methanol (Meth Elute)

METHOD

Preparation of internal standard solution

Dissolve an appropriate n-alkane in ethyl acetate to give a concentration of 1 mg/ml.

Preparation of the standard solution

To an accurately weighed amount of the barbiturate (free acid) standard, add internal standard solution to give a barbiturate concentration of 1 mg/ml.

Preparation of sample solutions

Add internal standard solution to an accurately weighed amount of the sample extract obtained under III B above. The concentration of the barbiturate and internal standard should be approximately equal to that of the standard solution (1 mg/ml).

Inject 1 ul of the standard solution together with 1 ul of the derivatizing reagent solution onto the column using the on-column derivatizing technique. Then proceed with injecting the sample and derivatizing reagent solutions, also together, and calculate the barbiturate's content (%) in the sample using the general formula:

$$C_x\,\% = \frac{C_{r.std.}}{C_{sam.}} \times \frac{A_x/A_{int.std.\ in\ sam.\ chrom}}{A_{r.std.}/A_{int.std.\ in\ std.\ chrom}} \times 100$$

where:

$C_x\,\%$ = content of component x in the sample (w/w %)

$C_{r.std.}$ = concentration of substance x in the standard reference solution (w/v %)

$C_{sam.}$ = concentration of the sample (w/v%)

A_x = peak area for substance x during the sample chromatography

$A_{r.std.}$ = peak area for standard obtained during the standard chromatography

$A_{int.std.\ in\ sam.\ chrom}$ = peak area of the internal standard obtained during the sample chromatography

2. Capillary column technique

(a) Without derivatization

Operating conditions:

Detector:	FID
Column:	Fused silica, chemically bonded and cross-linked methylsilicone or methylphenylsilicone, such as OV-1, SE-30, SE-54 or equivalent
Film thickness:	0.52 um
Length:	25m, 0.35mm ID

Carrier gas:	Nitrogen at 1 ml/min
Split ratio:	20:1
Column temperature:	isothermal at 200°C or programmed from 200°-260°C at 4°C/min
Injector/detector temperature:	275°C
Internal standard:	n-alkanes

METHOD

Prepare internal standard, drug standard and sample solution at a concentration of 1 mg/ml as described above. Inject successively 1 ul of the sample and standard solutions into the gas chromatograph.

RESULTS

Compound	Retention Indices		
	packed SE-30 with derivatization	SE-30 capillary	SE-54 capillary
allobarbital	1491	1575	1629
amobarbital	1600	1695	1751
barbital	1415	1465	1519
butalbital	1553	1642	1698
butobarbital	1557	1642	1695
cyclobarbital	1850	1946	2026
methylphenobarbital	1832	1875	1950
pentobarbital	1632	1719	1778
phenobarbital	1831	1934	2012
secbutabarbital	1564	1635	1692
secobarbital	1670	1770	1827
vinylbital	1629	1712	1774

References:

1. J. Chromatography 204 (1981) 275-284.
2. J. Chromatography 192 (1980) 363-374.

F. High performance liquid chromatography

Reverse phase

METHOD 1

Column:	250 mm x 4.6 mm ID
Packing material:	Octadecyl-silica HPLC grade, 5 um (Spherisorb 5 ODS-2 or equivalent)
Mobile Phase:	Acetonitrile 30 Water 70
Flow rate:	0.9 ml/min.
Detection:	UV at 220 nm

Sample and standard solutions:

Dissolve an accurately weighed amount of the standard in methanol at a concentration of 1 mg/ml. Similary, dissovle an accurately weighed amount of the powdered sample, obtained under one of the extraction procedures outlined in Chapter III B above in methanol to give an approximate barbiturate concentration of 1 mg/ml.

Injection volume:	1-5 ul by syringe or loop injector
Quantitation:	By peak area, external standard method

METHOD 2

Column:	150 mm x 4.6 mm ID
Packing material:	Octadecyl-silica HPLC grade, 5 um (ODS-Hypersil or equivalent)
Mobile phase A:	0.1M sodium dihydrogen phosphate buffer 60 Methanol 40 pH 3.5 adjusted with phosphoric acid
Mobile phase B:	0.1M sodium dihydrogen phosphate buffer 60 Methanol 40 pH 8.5 adjusted with sodium hydroxide solution
Flow rate:	2.0 ml/min.
Detection:	UV at 216 nm

Sample and standard solutions:	1 mg/ml in methanol, prepared as described above.
Injection volume:	1-5 ul by syringe or loop injector.
Quantitation:	By peak area, external standard method.

RESULTS

The capacity ratios (k' values) are as follows:

COMPOUND	METHOD 1	METHOD 2	
		Mobile phase A	Mobile phase B
allobarbital	1.35	2.46	1.33
amobarbital	4.86	10.91	7.05
barbital	0.60	1.11	0.63
butalbital	2.90	6.17	3.48
butobarbital	2.56	5.43	3.42
cyclobarbital	2.56	5.25	2.61
methylphenobarbital	5.72	7.27	3.84
pentobarbital	4.63	10.96	8.07
phenobarbital	1.94	3.09	1.23
secbutabarbital	2.24	4.89	3.32
secobarbital	6.81	16.28	11.47
vinylbital	4.86	10.40	7.05

References:

1. J. Chromatography 427 (1988) 172-180 (modified).
2. J. Chromatography 204 (1981) 275-284.

G. Spectroscopic techniques

In some countries, confirmation of identity by spectroscopic means is required.

1. Ultraviolet spectroscopy

Ultraviolet spectroscopy (UV) has been used for barbiturate analysis because of its simplicity and availability of reliable and relatively inexpensive instrumentation. However, because of its lack of specificity and the short wavelength for most barbiturates' absorbance maxima, UV is not recommended for identification purposes. If it is used for quantitative analysis, UV should be employed in conjunction with chromatographic techniques to ensure that the absorbance of the barbiturate is the only one being measured.

METHOD

Borax buffer solution (0.05M): dissolve 19.07 g of borax in sufficient water to produce 1000 ml. Prepare solutions containing 1 to 2.5 mg of barbiturate per 100 ml of borax buffer. Measurements are made versus the appropriate solvent blank. The amount of barbiturate present in the sample is calculated against the absorbance of a standard treated in the same way.

Absorbance maxima for the barbiturates are listed in the following reference publication.

Reference

1. "Clarke's Isolation and Identification of Drugs." 2nd edition, The Pharmaceutical Press (London) 1986.

2. Mass spectroscopy

Because of the similarity of many barbiturates, mass spectroscopy is not very discriminative. Unless the barbiturates are derivatized, very weak molecular ions are obtained.

References:

1. Anal. Chem. 45 (3) 1973, pp. 574-576.
2. Microgram 6 (12), 1973, pp. 188-193.
3. Beitr. Gerichtl. Med. 37 1979, pp. 337-345.

3. Nuclear magnetic resonance spectroscopy (NMR)

NMR enables the analyst to unequivocably distinguish between particular barbiturates even in the presence of diluents and other adulterants. Because of the cost and technical expertise required, NMR is not recommended for routine sample analysis.

References:

1. T. Mills and J.C. Roberson. "Instrumental Data for Drug Analysis", 2nd edition, volumes. 1-4, Elsevier (New York) 1987.

2. United Nations Scientific and Technical Notes, UNDND, SCITEC/7, November 1989.

4. Infrared spectroscopy

Theoretically, each substance has a unique infrared spectrum and this method would permit the unequivocal identification of any barbiturate.

For powders, considered from prior chromatographic analysis to be reasonably pure, the infrared spectrum may be run directly in a KBr disk for comparison with the spectra of the barbiturate free acids or salts included in this manual. However, with licit pharmaceutical tablet and capsule samples, separation of individual barbiturates from excipients and their isolation in pure form is essential. For tablets, capsules and powders suspected to be mixtures, the extraction procedures outlined in Chapter III B above may be used to isolate the barbiturate as the free acid.

An additional difficulty in comparing infrared spectra arises from the existence of more than one crystalline form for some of the barbiturates. This phenomenon, known as polymorphism, gives rise to differences in the infrared spectra obtained from one crystalline form to the other.

To overcome this difficulty, analysts should subject the standard barbiturate to the same manipulations as the sample. This should convert the standard to the same crystalline form as the sample and give good comparative infrared spectra.

METHOD

For a description of the standard methods (halide disk, microhalide, nujol mull and thin-film techniques) see previous manuals in this series.

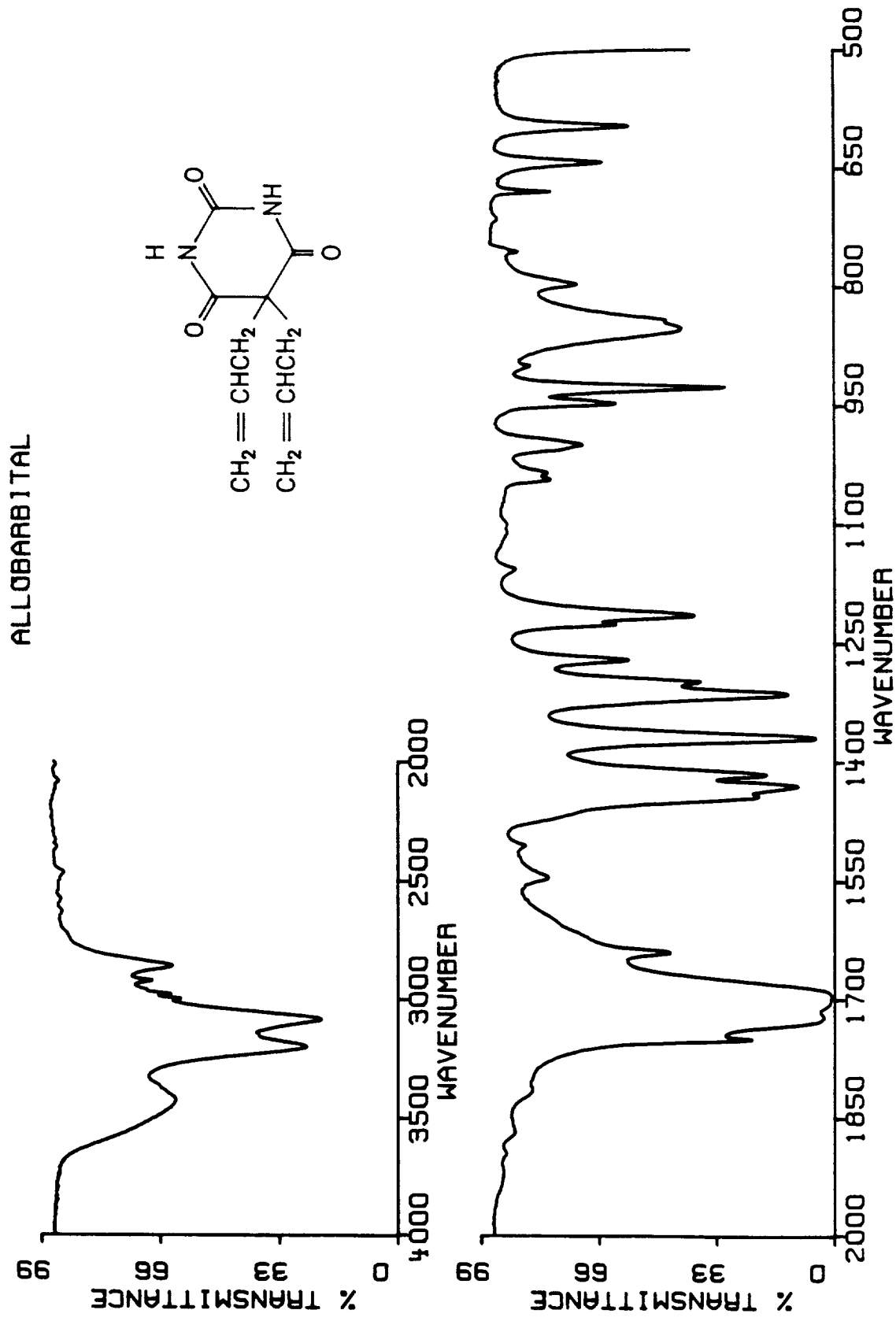
RESULTS

The following barbiturate reference standard spectra were recorded at 2 cm^{-1} resolution on a Fourier Transform instrument using samples prepared by the halide disk method. Other spectral collections are found in the following references:

References:

1. "Clarke's Isolation and Identification of Drugs." 2nd edition, The Pharmaceutical Press (London) 1986.

2. T. Mills and J.C. Roberson. "Instrumental Data for Drug Analysis." volumes 1-4, 2nd edition, Elsevier (New York) 1987.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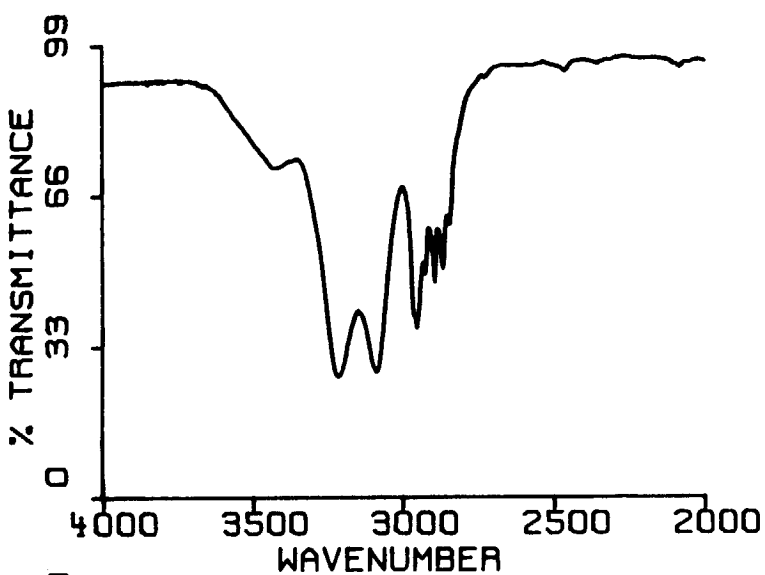

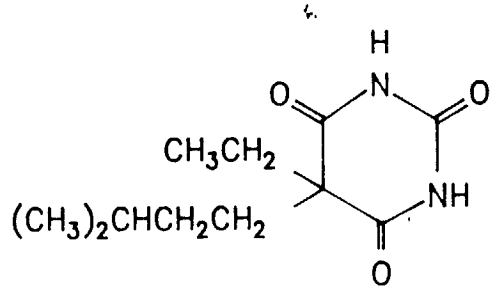

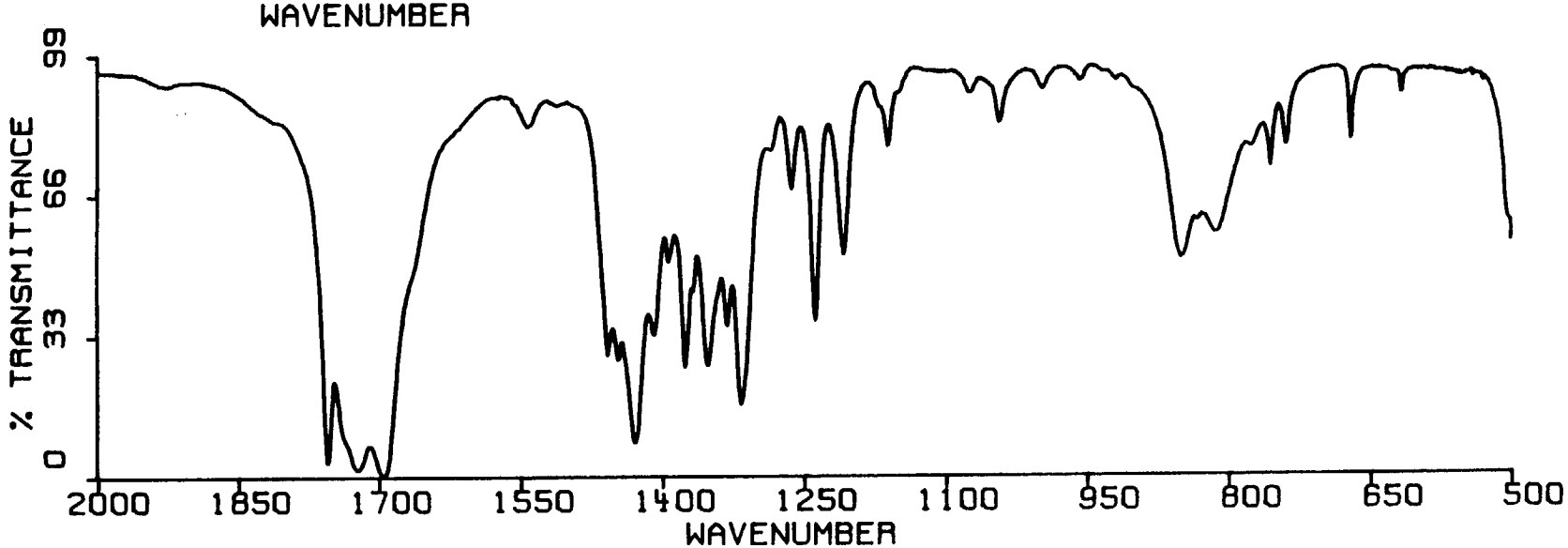

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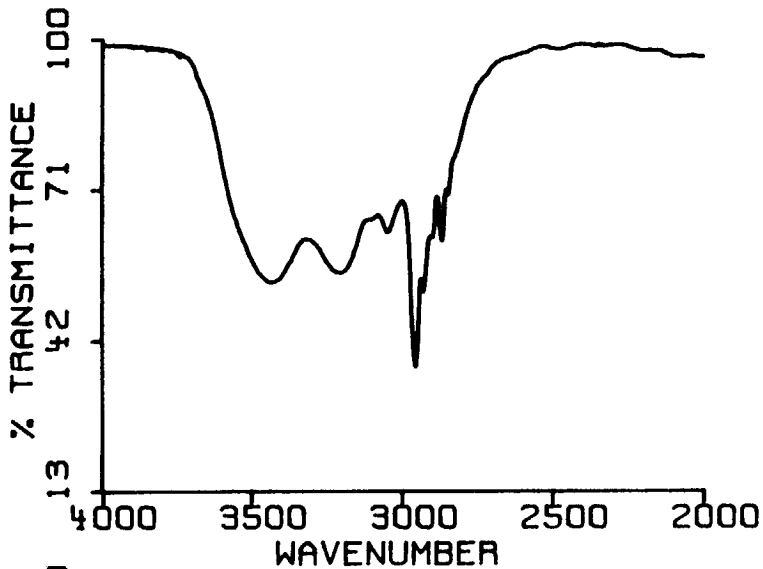

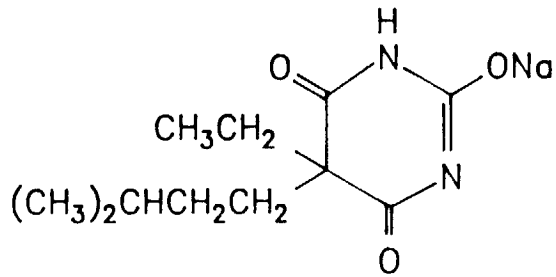

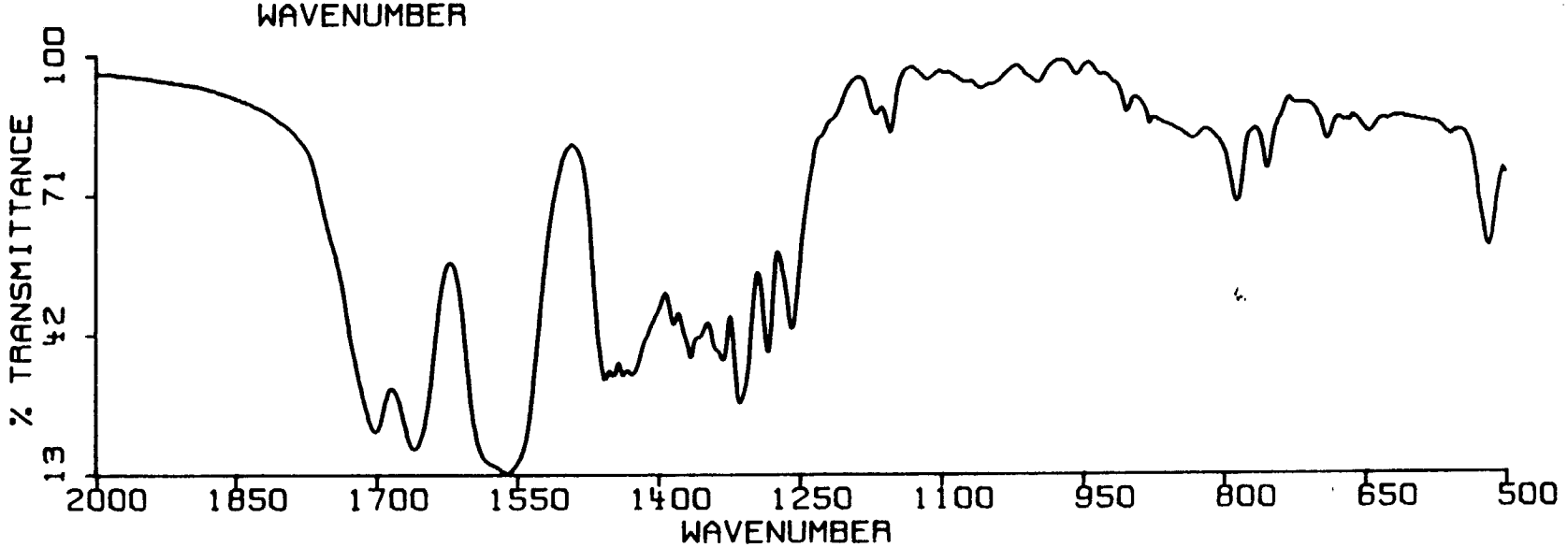

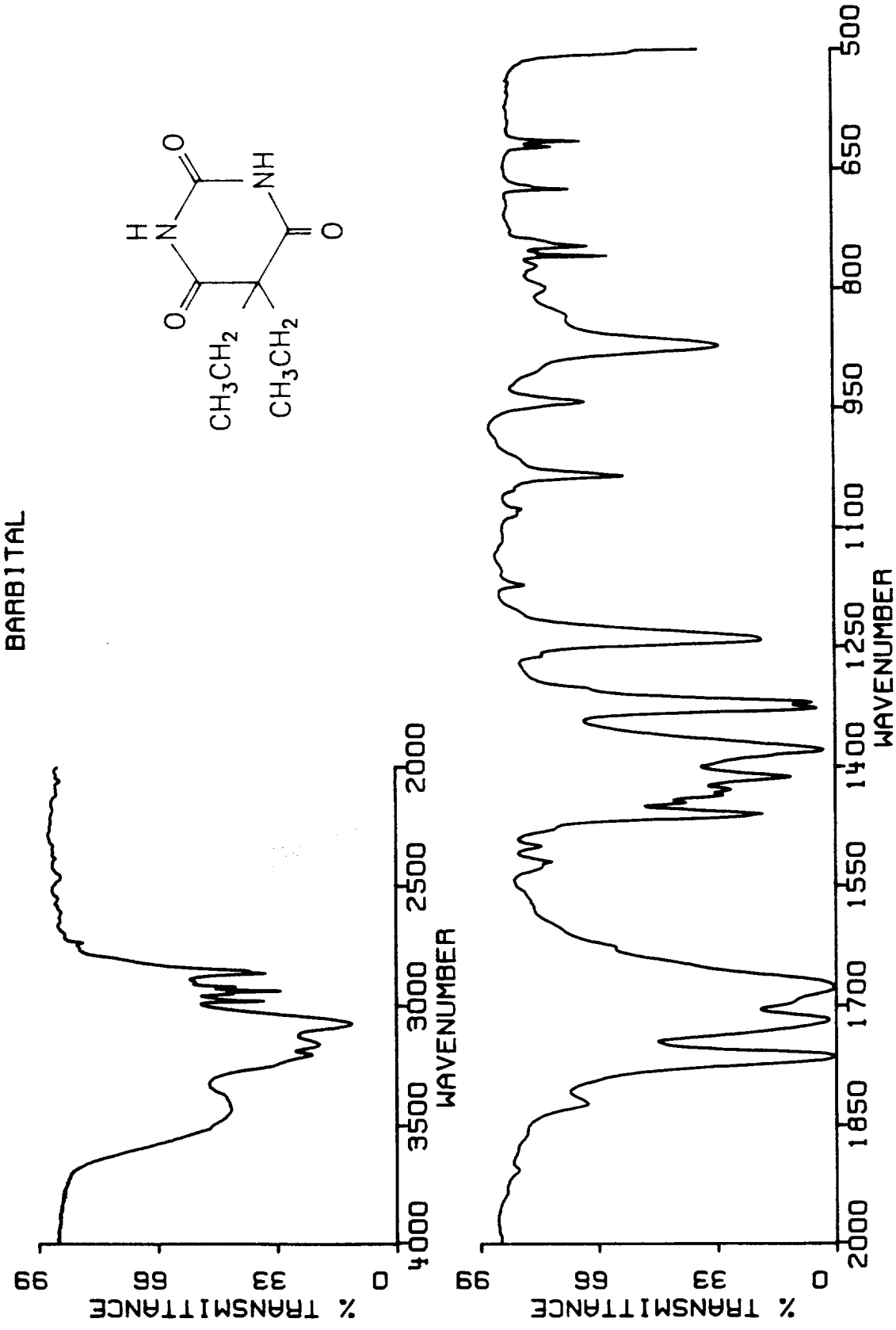
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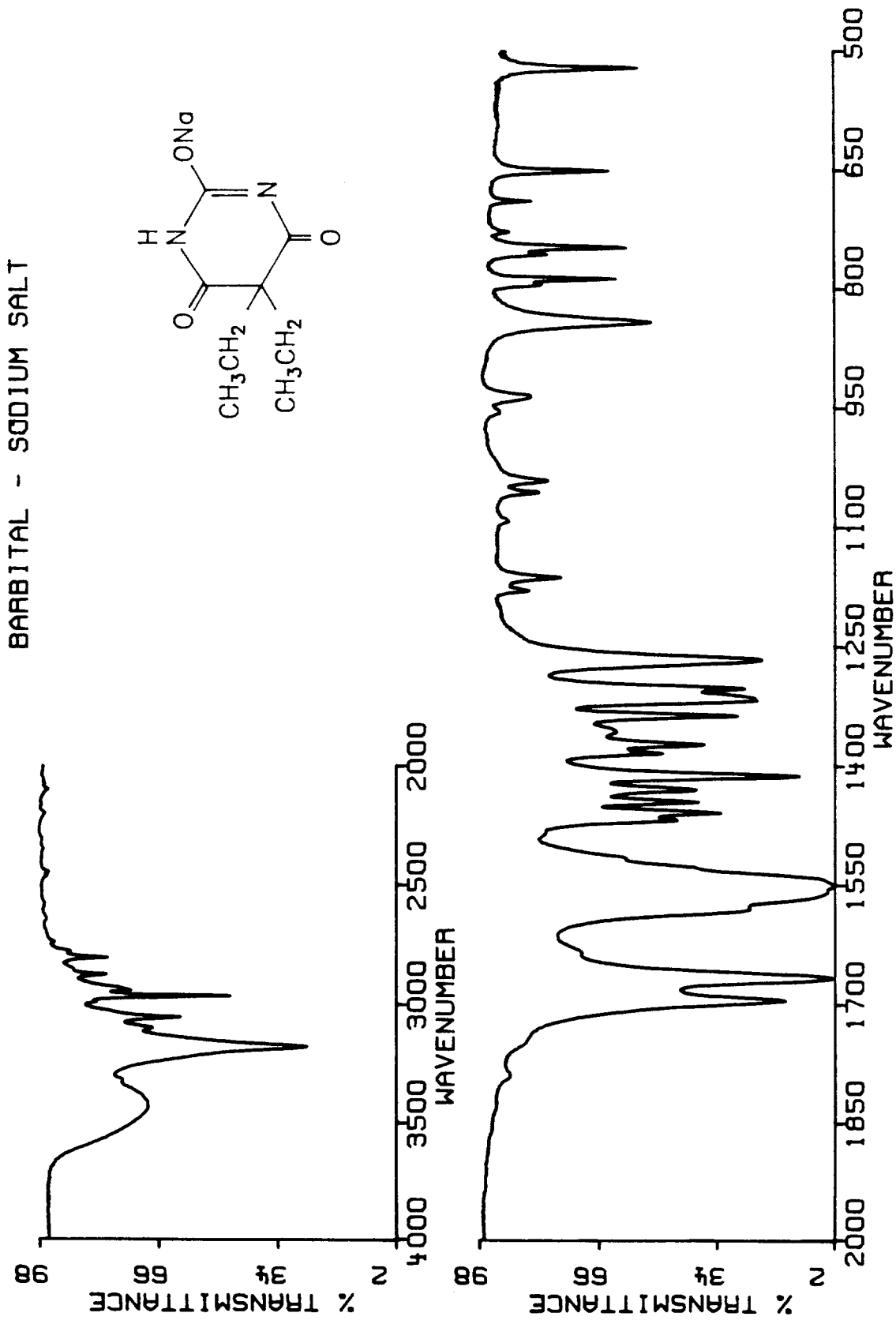
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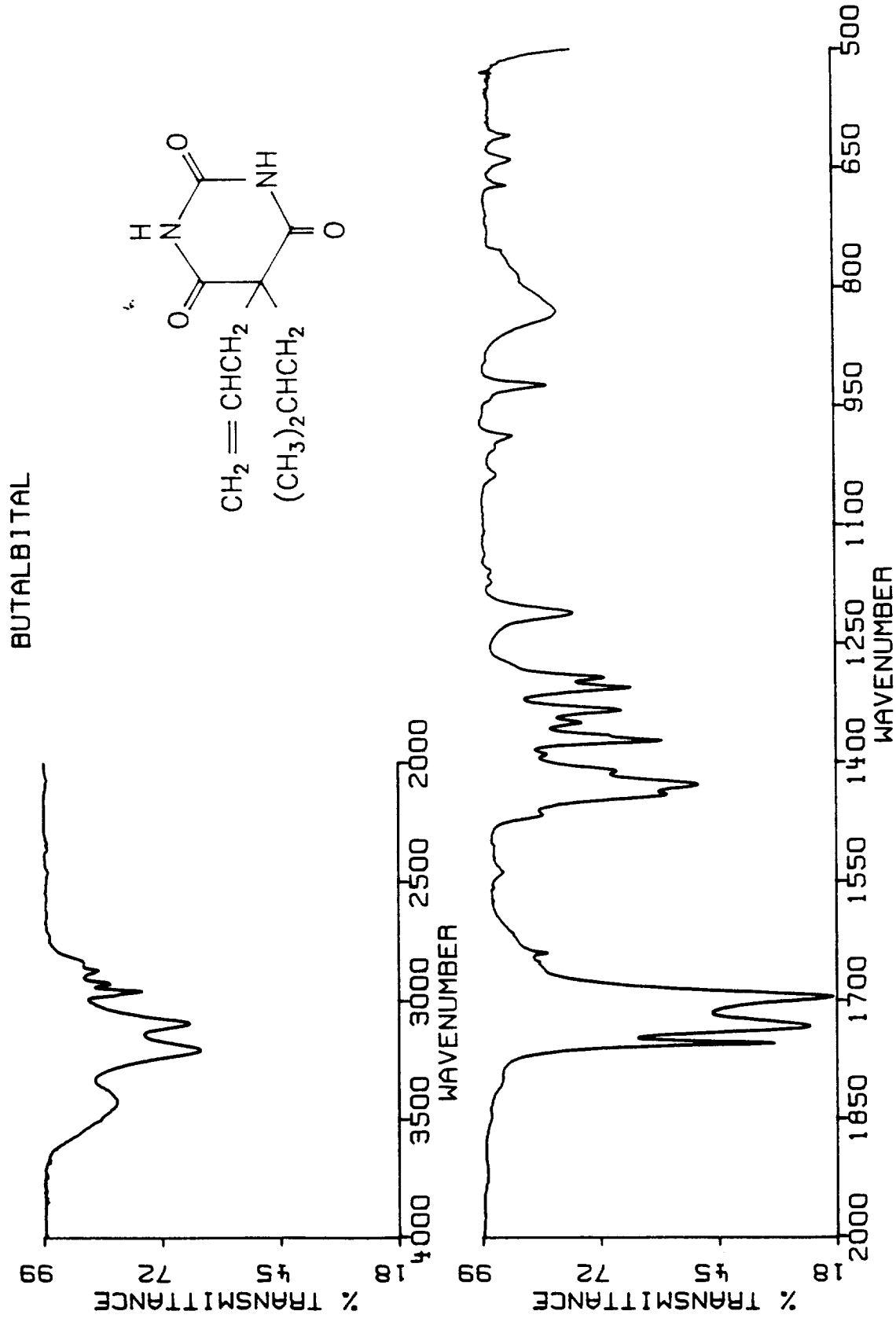
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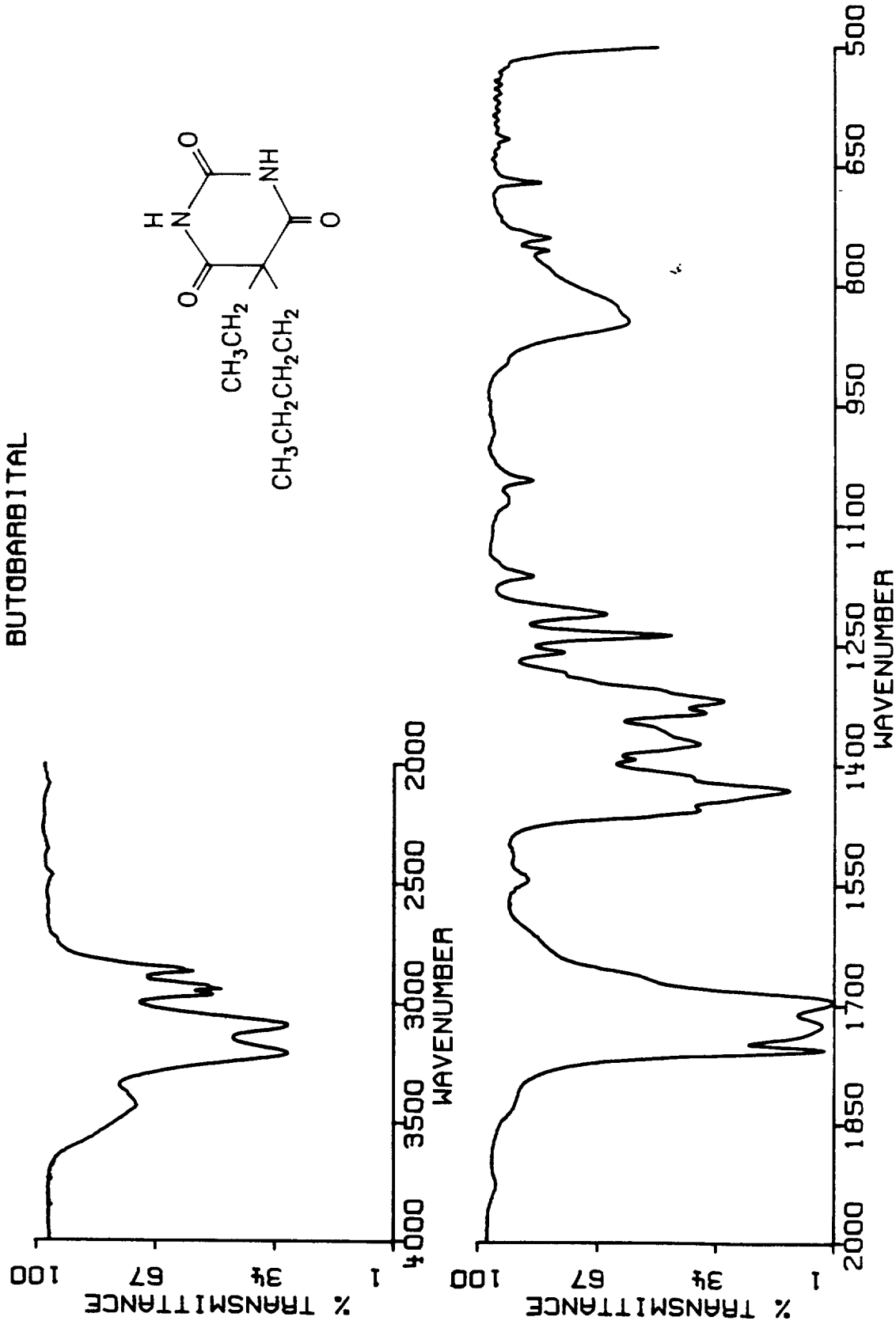
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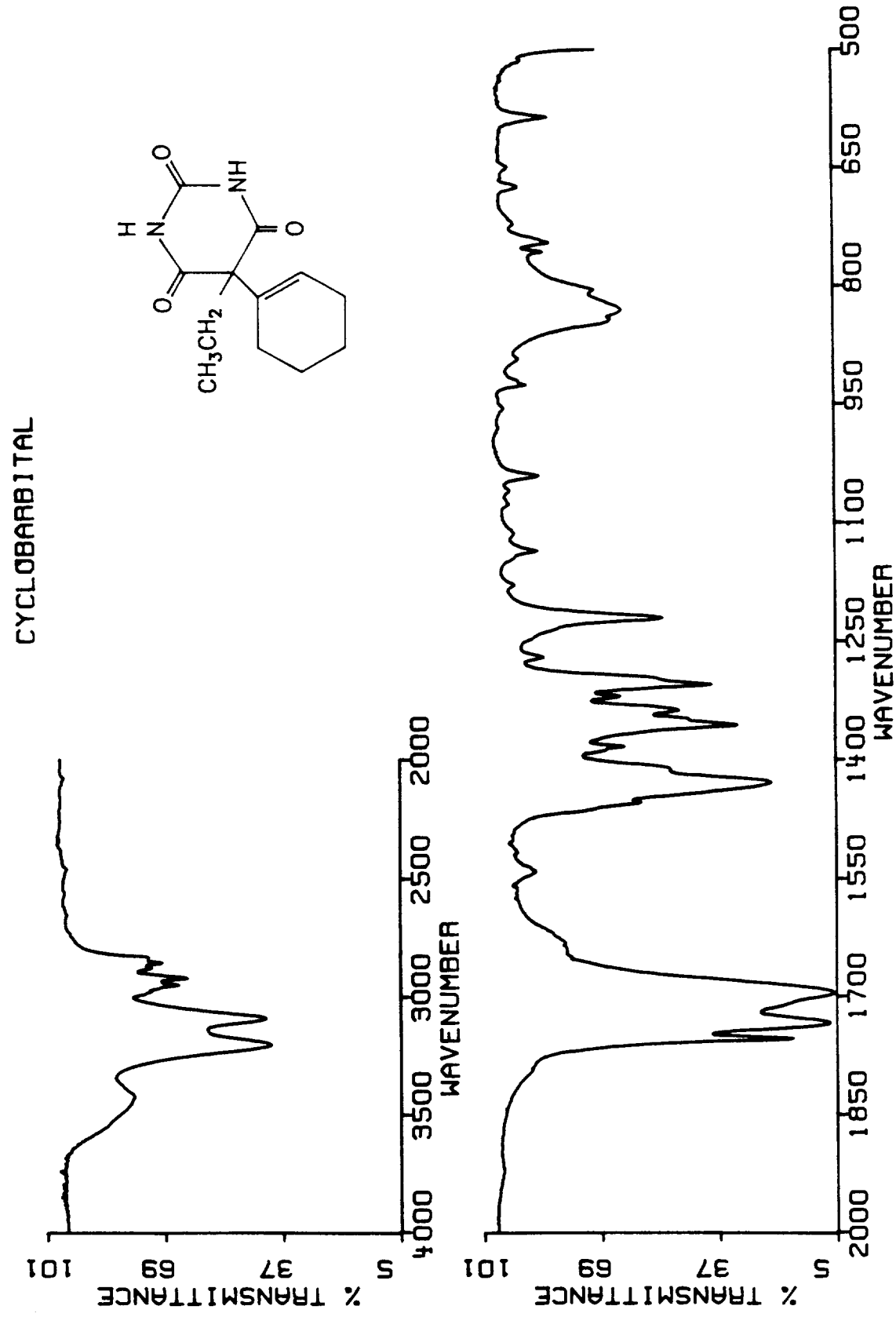
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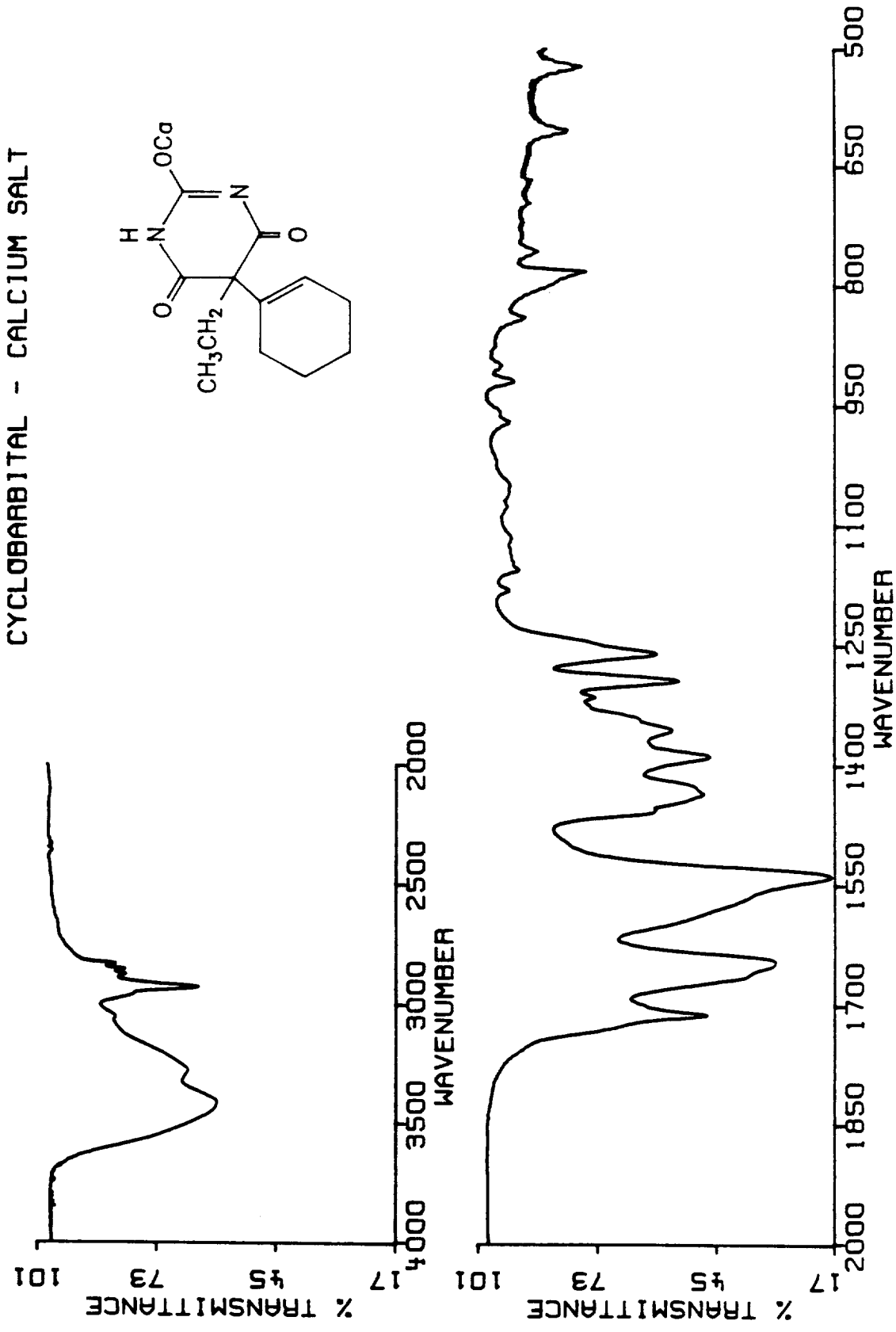
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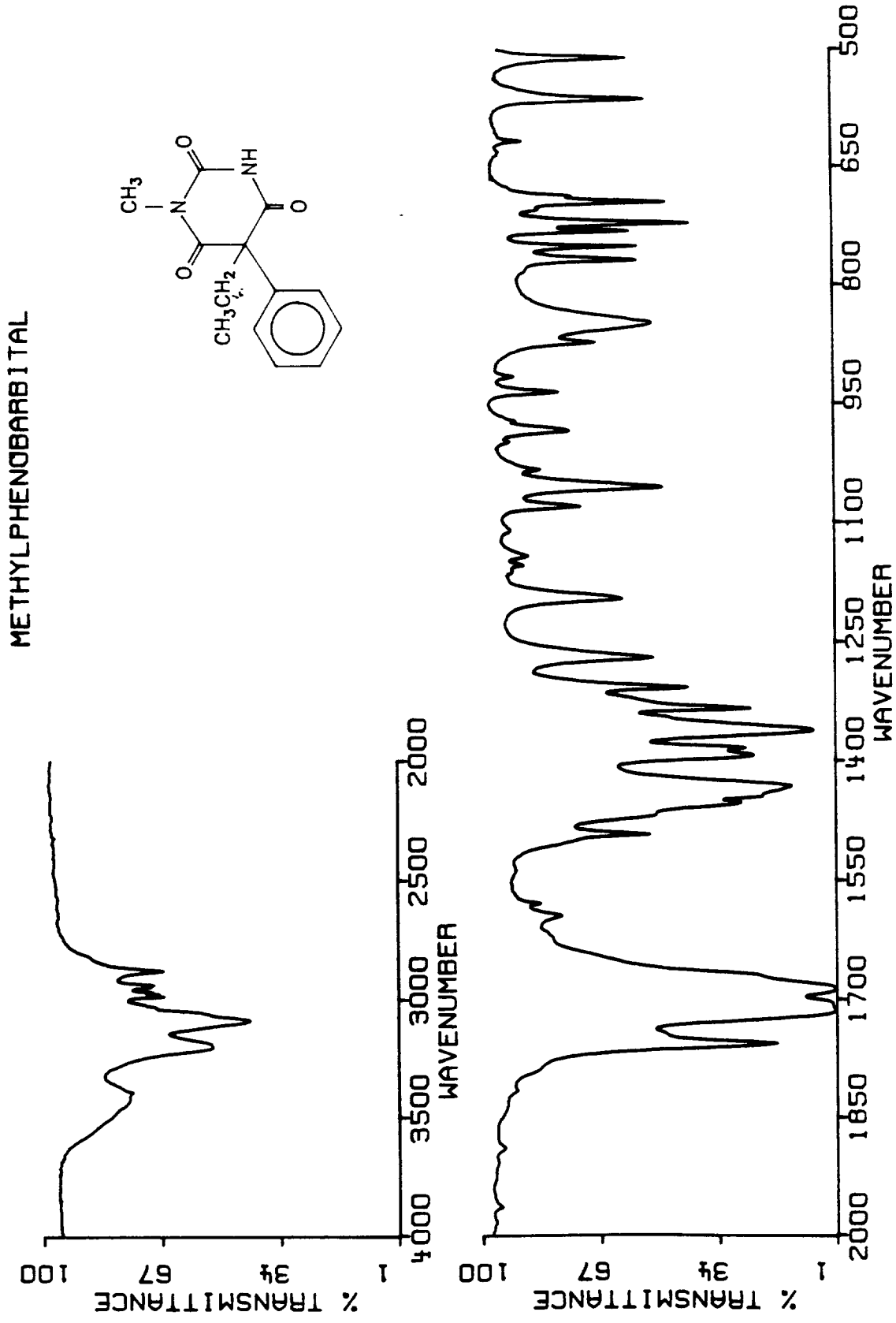
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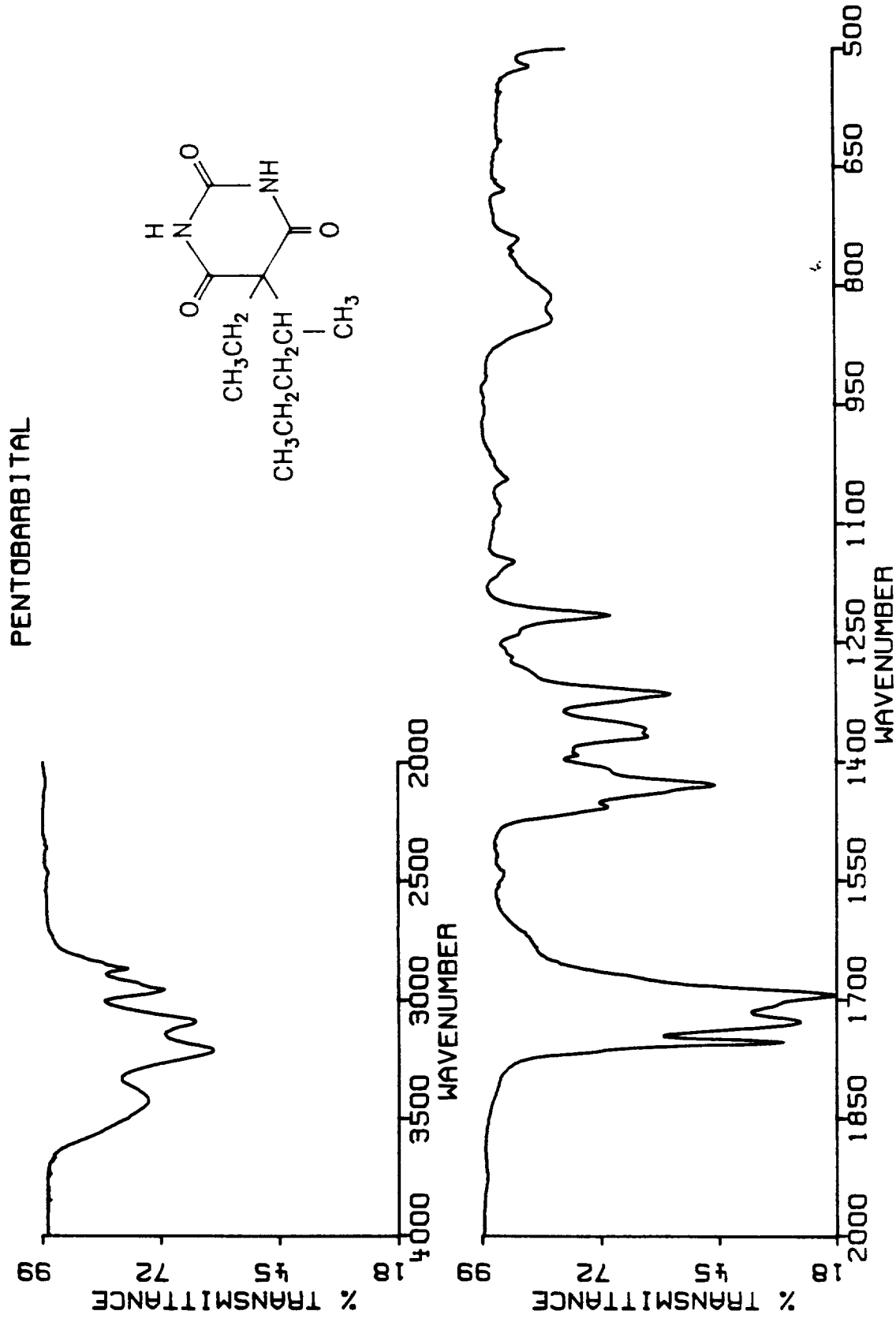
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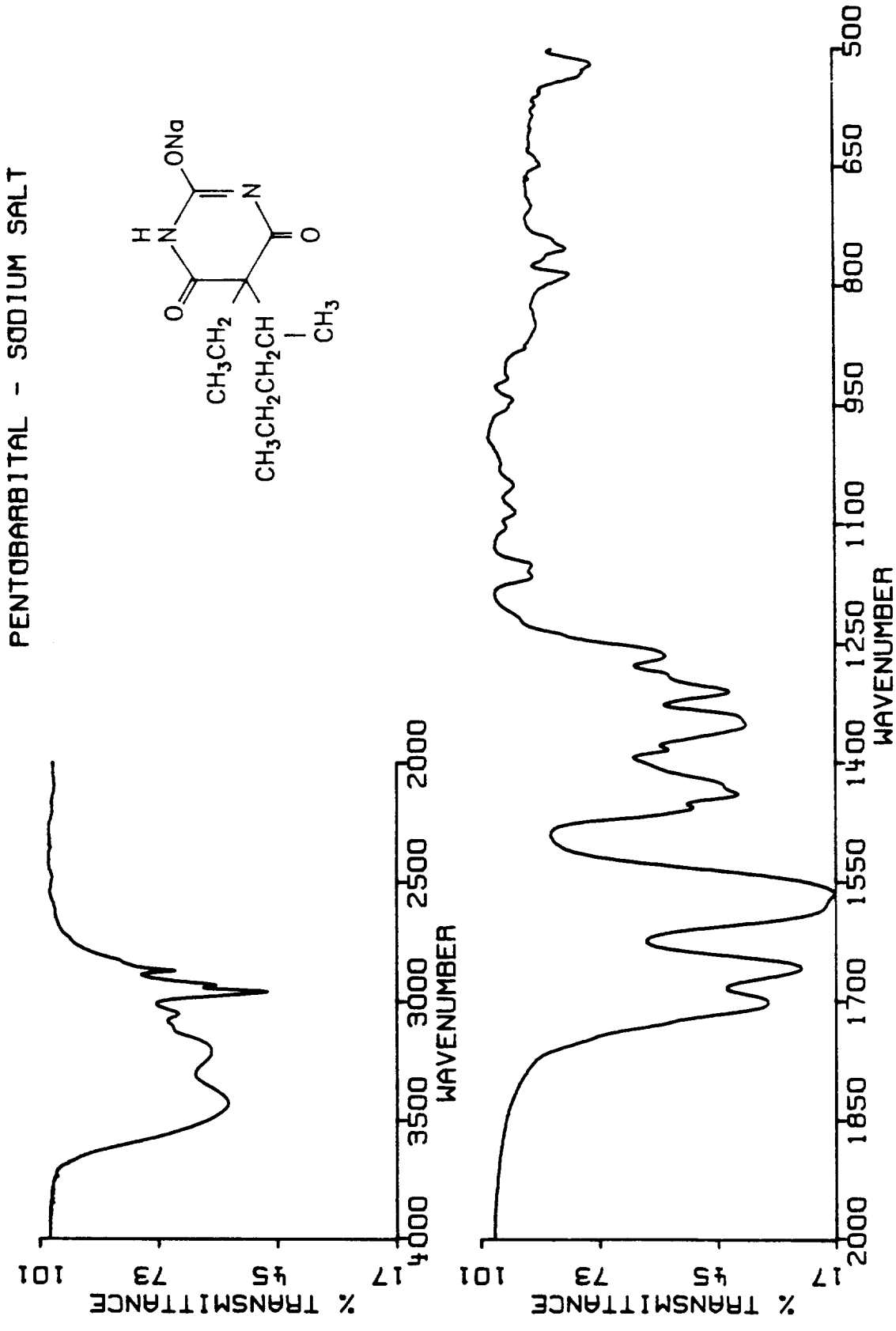
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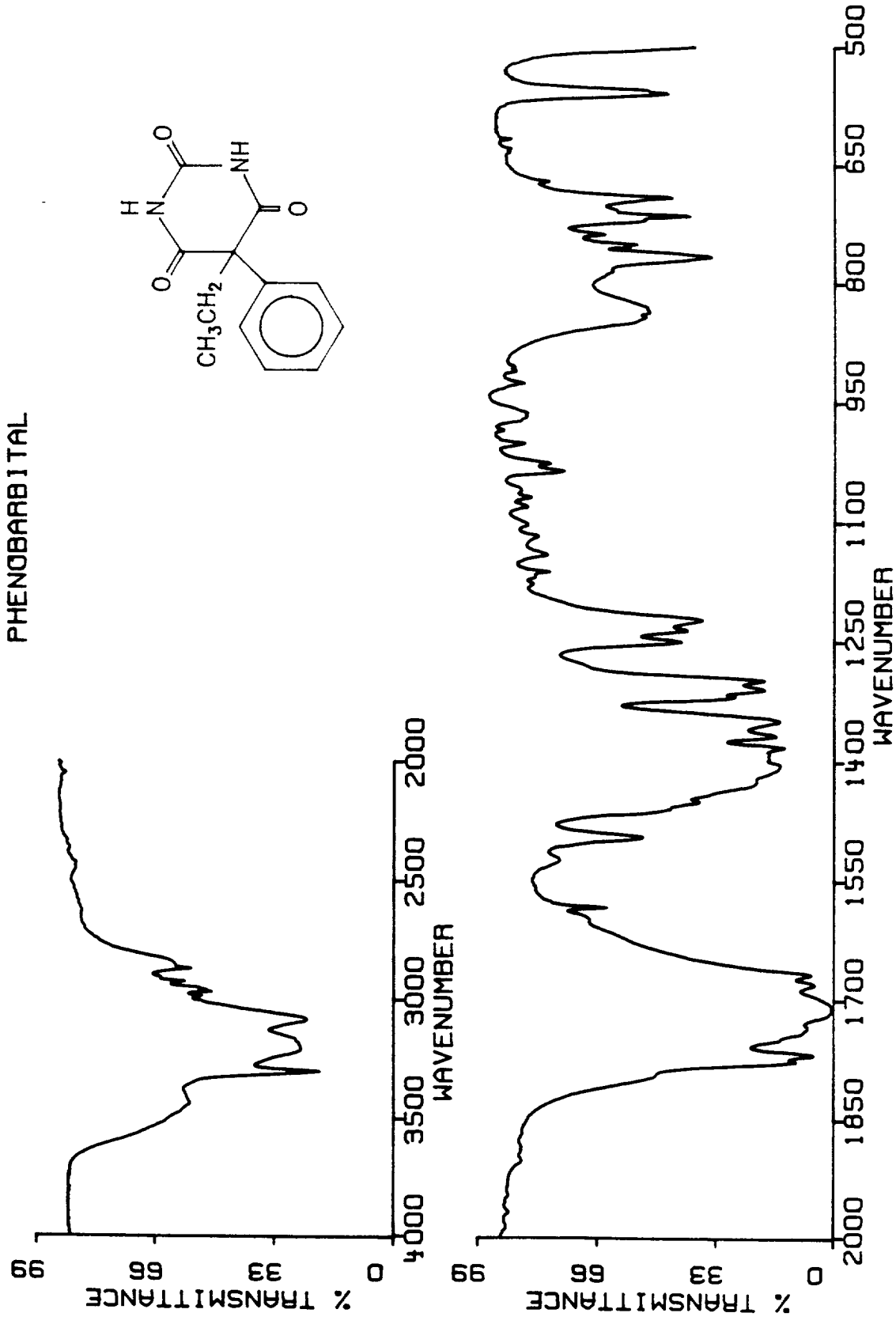
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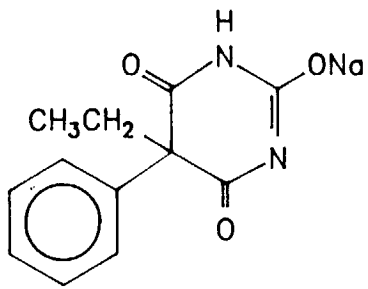
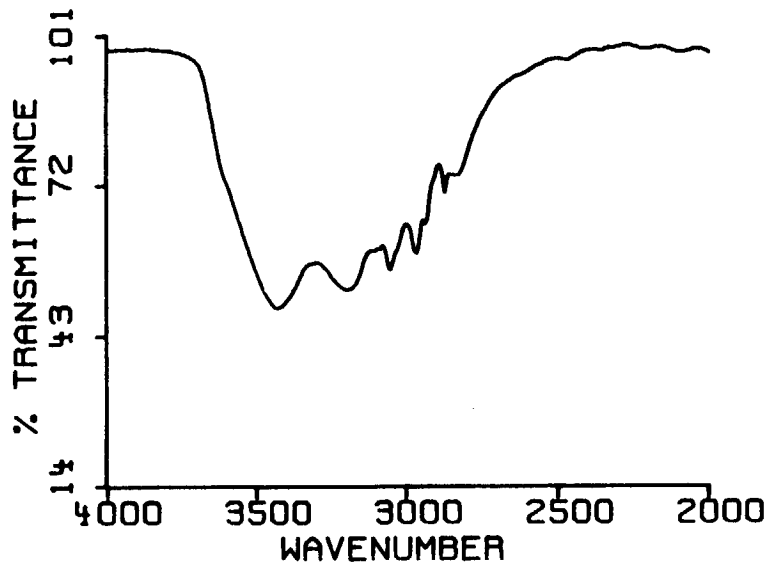
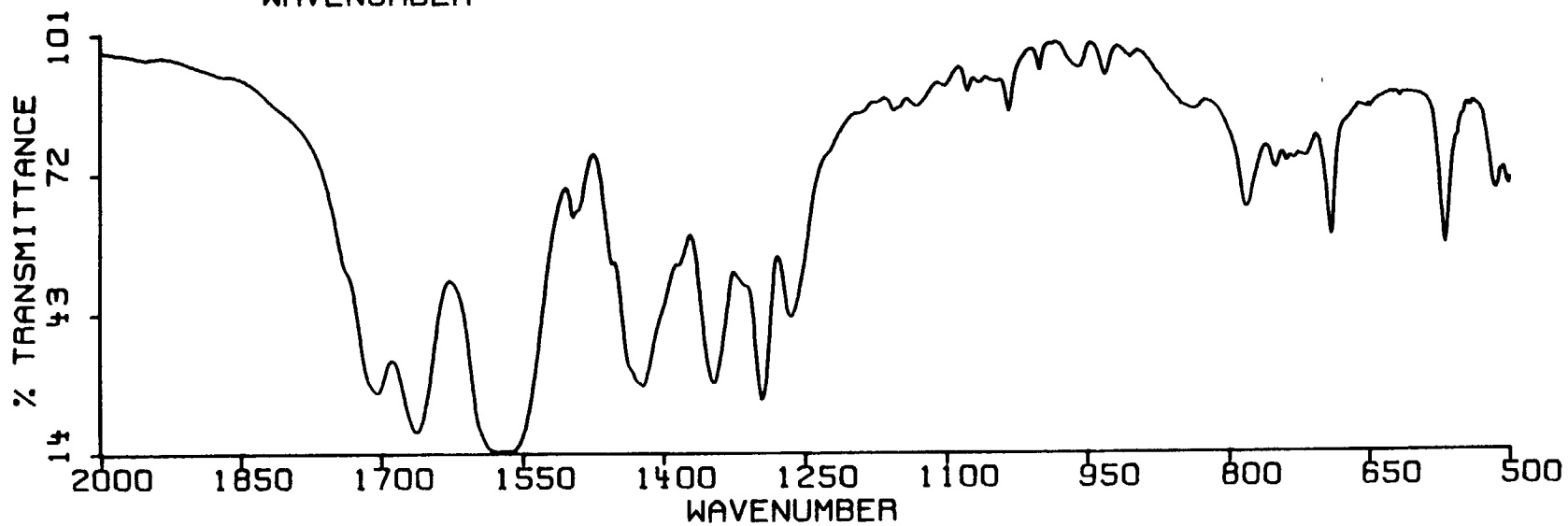
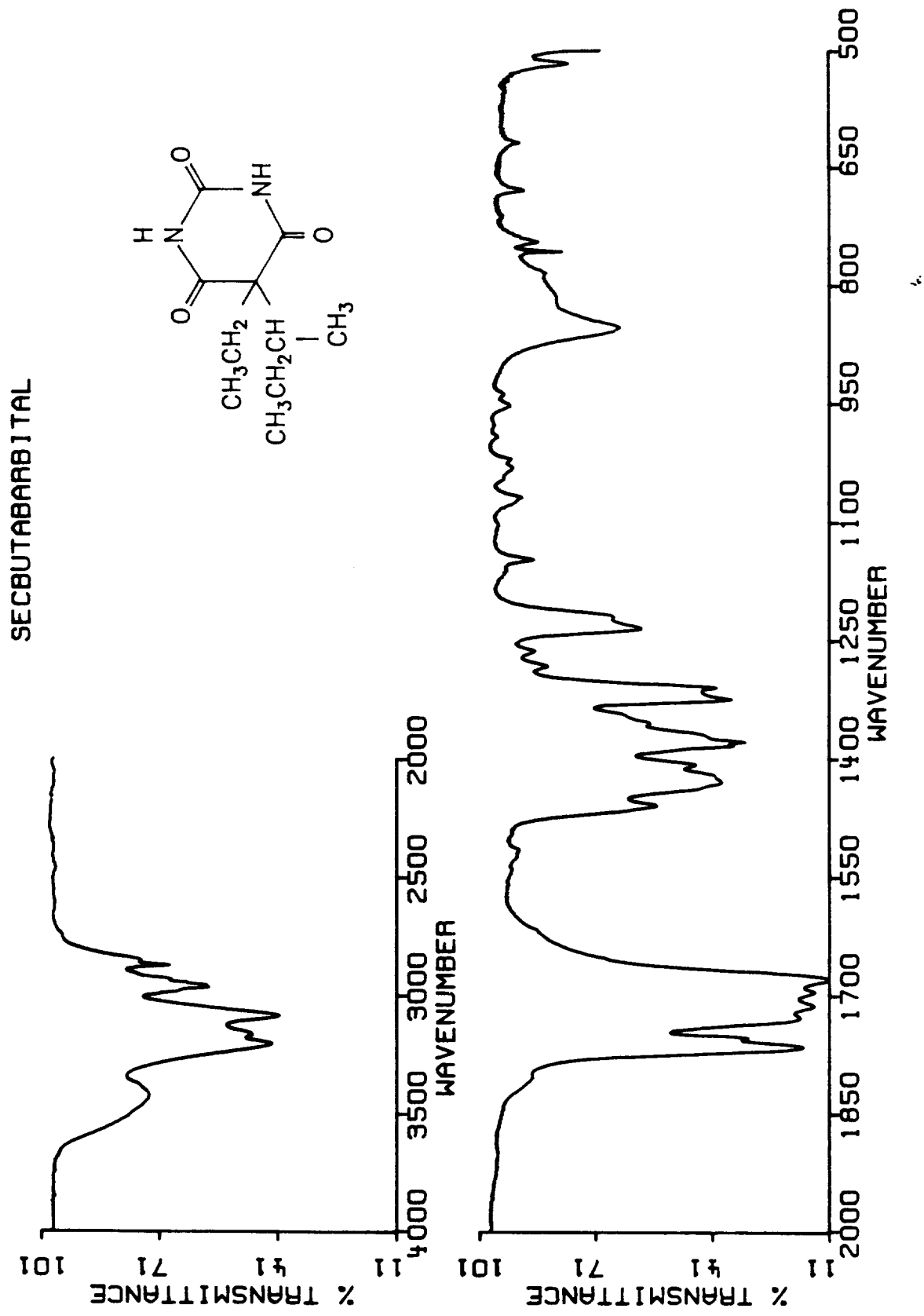
PHENOBARBITAL - SODIUM SALT

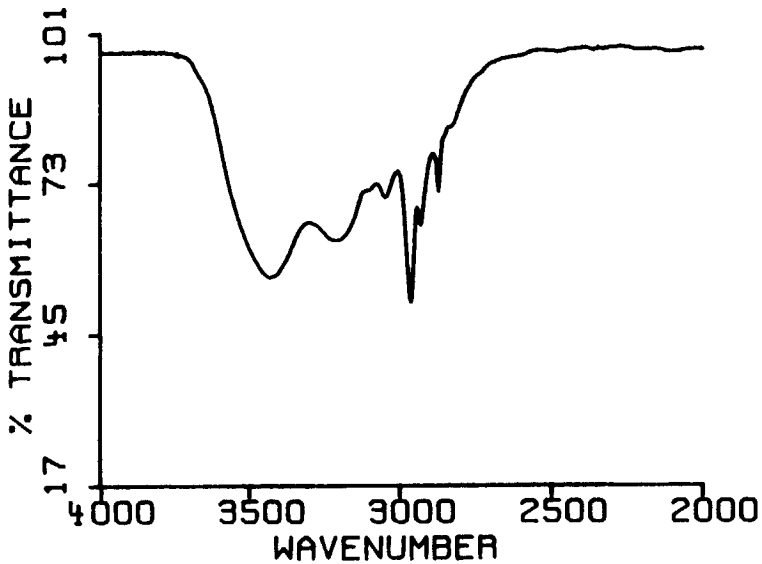


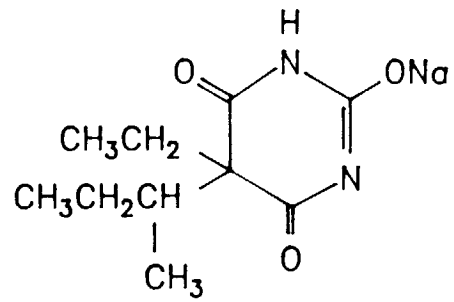


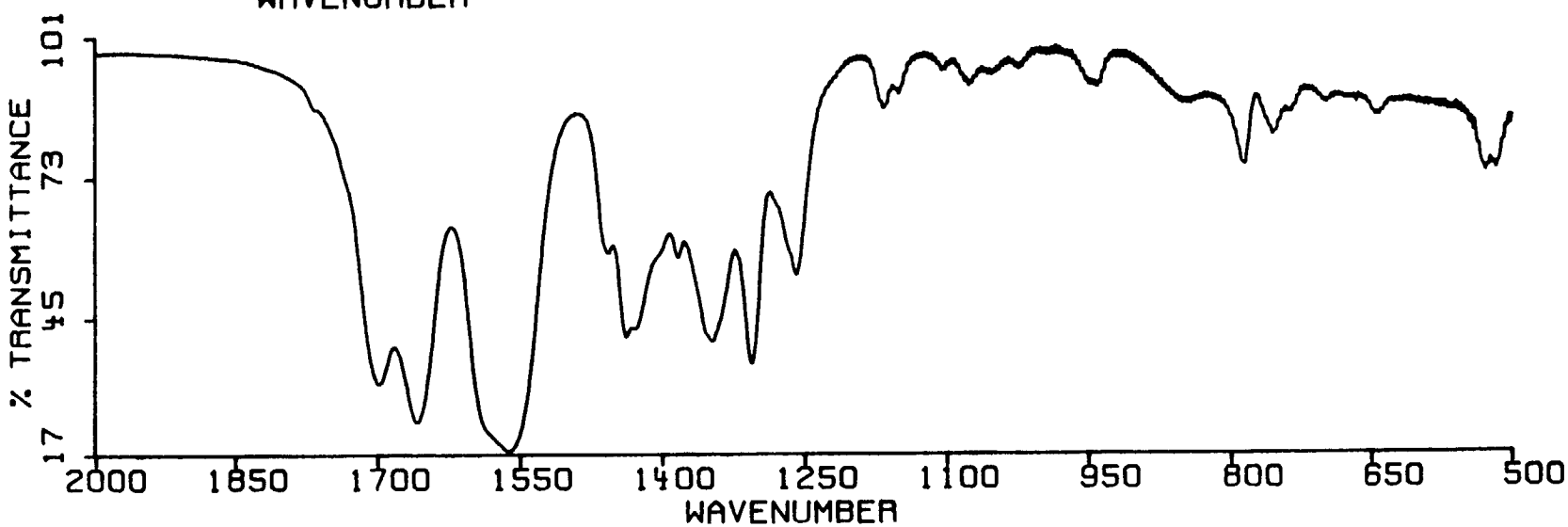


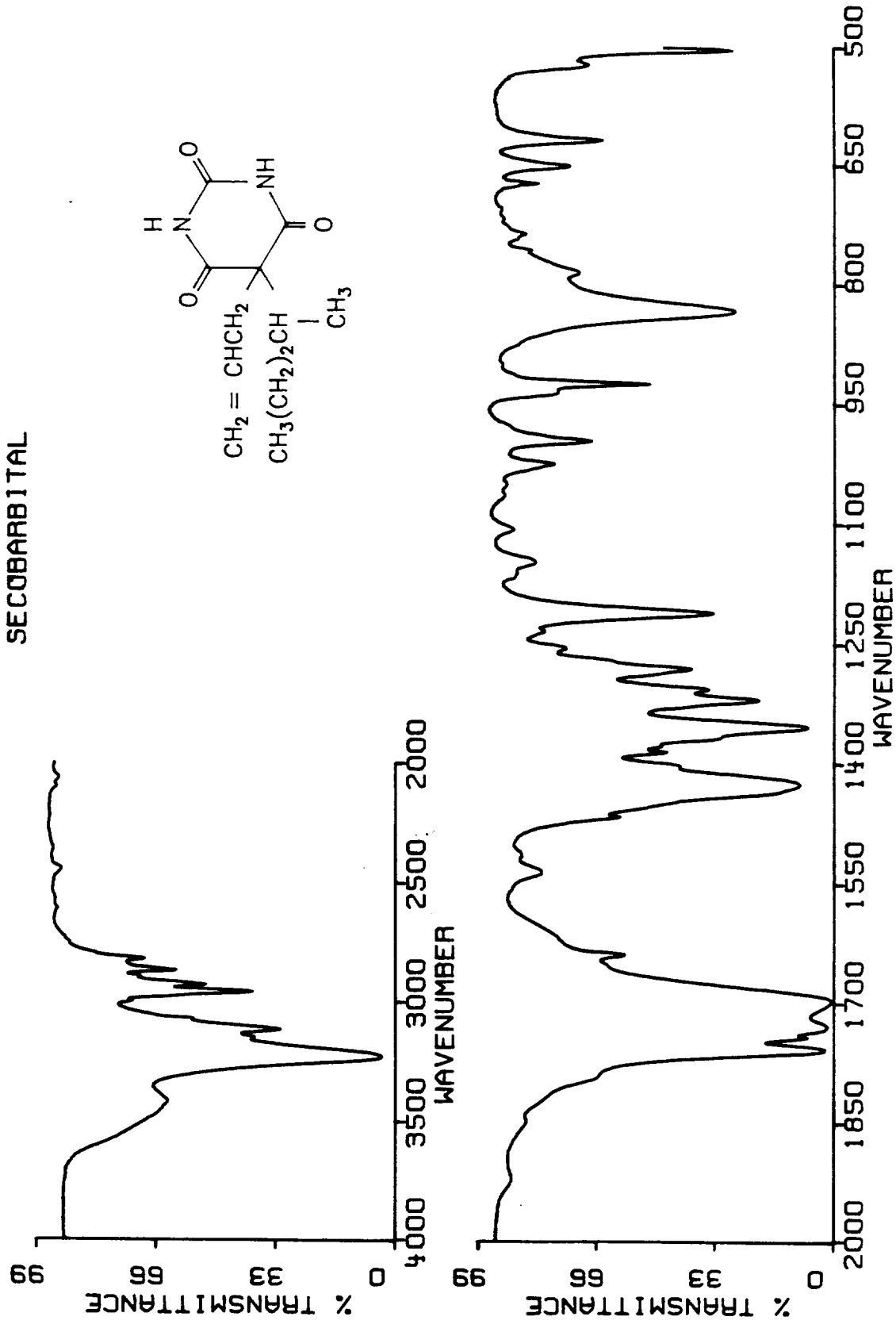

SECBUTABARBITAL
% TRANSMITTANCE
WAVENUMBER
4000
3500
3000
2500
2000
1850
1700
1550
1400
1250
1100
950
800
650
500
NH
H
N
O
CH_3CH_2
CH_3CH_2CH
CH_3

SECBUTABARBITAL - SODIUM SALT

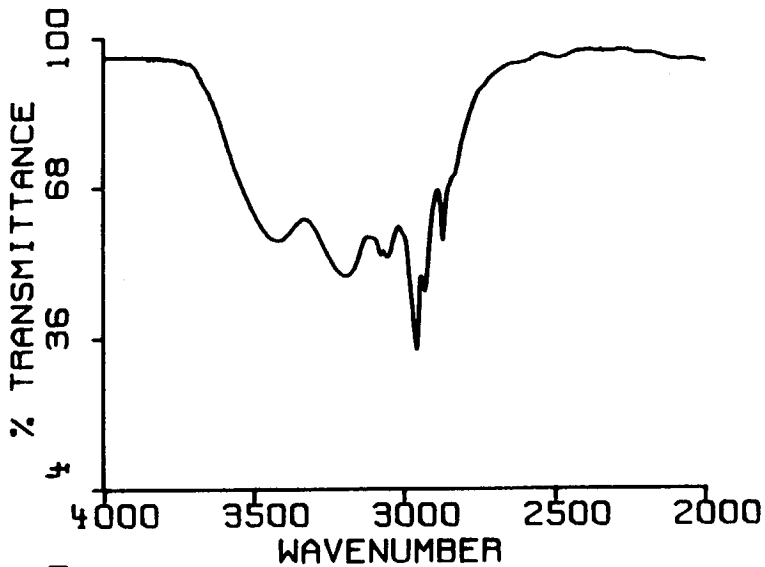


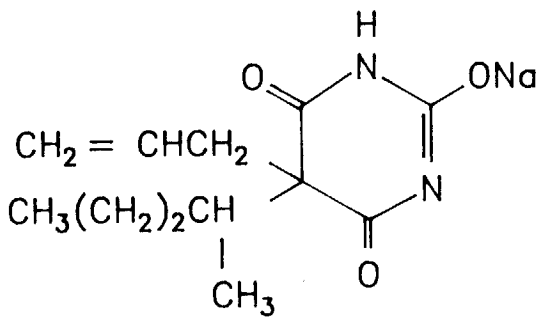


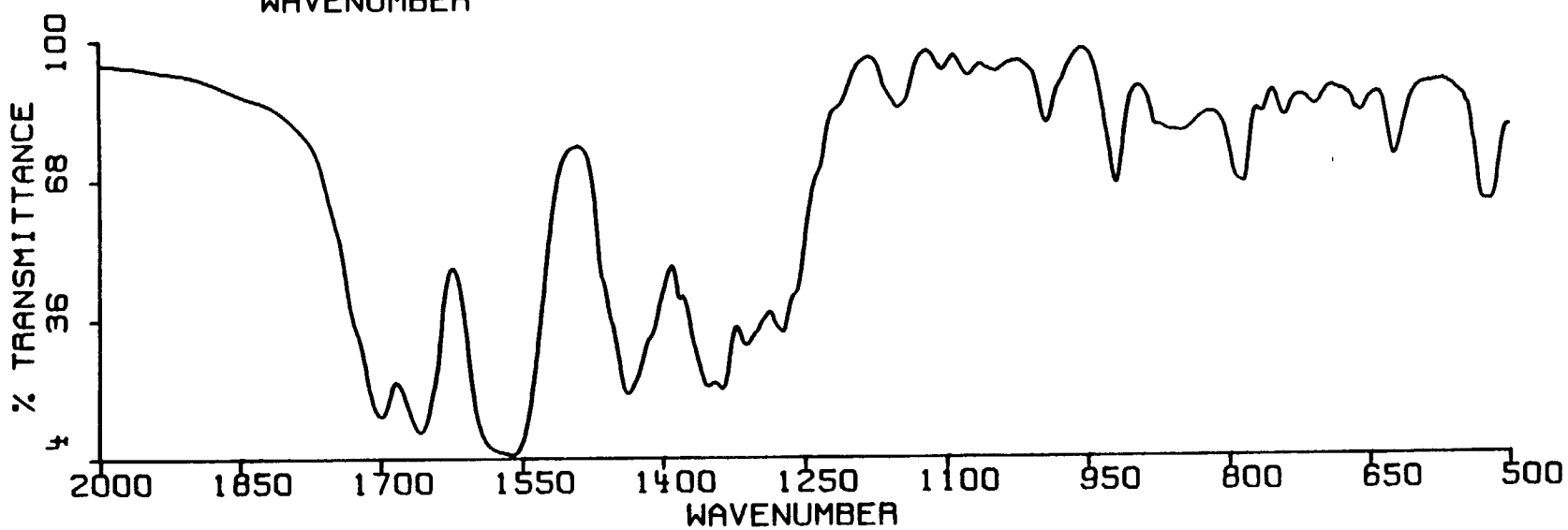


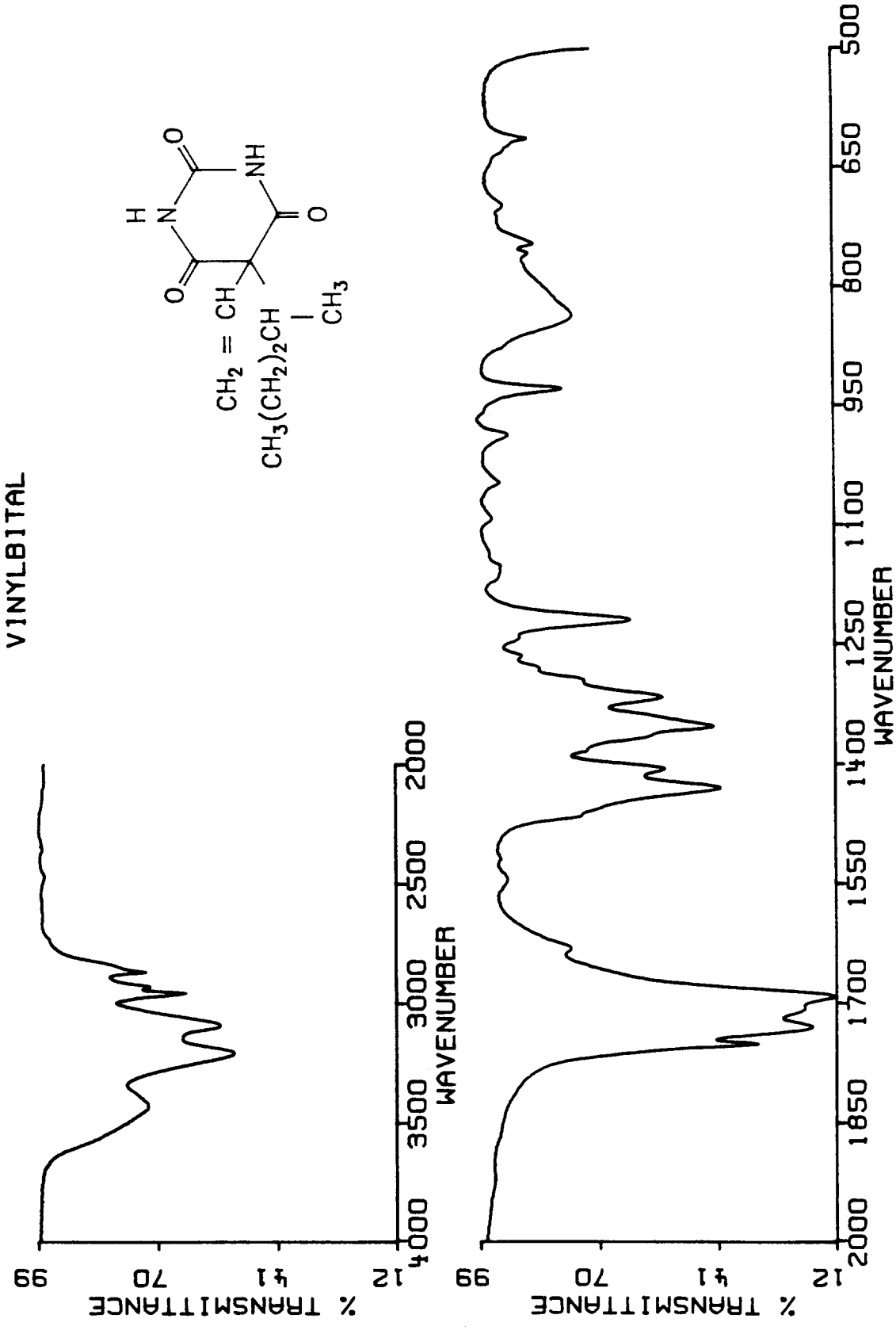

SECOBARBITAL
$CH_2 = CHCH_2$
$CH_3(CH_2)_2CH$
CH_3
% TRANSMITTANCE
99
66
33
0
4000
3500
3000
2500
2000
WAVENUMBER
% TRANSMITTANCE
99
66
33
0
2000
1850
1700
1550
1400
1250
1100
950
800
650
500
WAVENUMBER

SECOBARBITAL - SODIUM SALT








VINYLBITAL
% TRANSMITTANCE
99
70
41
12
4000
3500
3000
2500
2000
WAVENUMBER
2000
1850
1700
1550
1400
1250
1100
950
800
650
500
WAVENUMBER

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