ISOLATION, IDENTIFICATION, AND CHEMISTRY OF THE ANTIBACTERIAL ALKALOID SOLANOCAPSINE FROM *SOLANUM PSEUDOCAPSICUM* L.

by

Per Mølgaard Boll

A THESIS

Submitted to the School of Advanced Graduate Studies of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

1957

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VITA

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Doctor of Philosophy

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AN ABSTRACT

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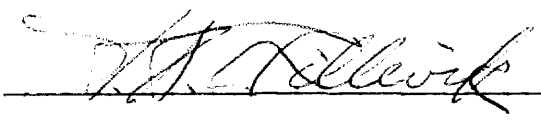
Approved 

Per Mølgaard Boll

It was observed by Lucas and associates (1) that an aqueous extract of the plant Solanum pseudocapsicum L. possessed antibacterial properties. The purpose of this study was to isolate and chemically identify the antibacterial substance(s).

A substance active against Mycobacterium tuberculosis was found associated with the alkaloid fraction of the plant and identified as the steroidal alkaloid solancapsine. The method with modifications for isolation was that used by Schlittler and Uehlinger (2). The antitubercular activity of pure solanocapsine was 3 γ/ml. culture broth. The minimum lethal dose for mice injected peritoneally was 50-100 mg./kg. of body weight for solanocapsine and 200 mg. for its hydrochloride. Subcutaneously, the lethal dose was 300 mg./kg. of body weight for both these compounds.

In addition to solancapsine a colored principle inhibiting growth of Diplococcus pneumoniae was found present. No further work on the purification of this substance was undertaken. Attempts without much success were made to isolate and purify solanocapsine from the colored impurities by column chromatography using alumina, acid alumina, and Dowex 50.

Alkaloid determinations on plants grown over a three year period showed that the alkaloid content varied from 0.83 to 2.24 per cent (based on dry weight) in roots and from 1.53 to 2.15 per cent in leaves plus stems.

Solanocapsine is the only alkaloid from the plant genus Solanum which has not been isolated as a glycoside. Therefore,

the possibility of an extraordinary labile glycosidic bond being acted upon enzymatically or during isolation was carefully examined. Enzyme inactivation by placing the plants in absolute ethanol or in boiling water and using weak acids in the isolation procedure did not yield a glycoside. It is regarded that solanocapsine is present in the plant as a free alkaloid.

It has been reported that the plant in addition to solanocapsine contains an amorphous alkaloid solanocapsidine (3), the alkaloidal glycoside solanine and its aglycone solanidine. Paper chromatography in different solvents revealed only one spot corresponding to the alkaloid solanocapsine. Solanocapsidine, as described by Barger and Fraenkel-Conrat (3) was found to be a crude amorphous alkaloid preparation, which could be crystallized to solanocapsine.

Solanine and solanidine from Solanum tuberosum and a crude alkaloid preparation from Solanum carolinense were isolated. Of these, only solanine showed weak antitubercular activity. Tomatine from Lycopersicon esculentum was likewise found inactive.

Some chemical structural studies on solanocapsine was undertaken. Two derivatives previously thought both to be N,N'-diacetylsolanocapsine was found more identifiable as O,N-diacetylsolanocapsine and O,N,N'-triacetylsolanocapsine. The piperidine ring of the solasodan nucleus was opened by hydrogenolysis.

Schlittler and Uehlinger (2) proposed a solasodan structure for solanocapsine and left the primary amino and hydroxyl groups unassigned. From the present work the amino group was concluded to be in the 3 position and the hydroxyl group provisionally assigned to the 8 position. The tentative structure proposed for solanocapsine is 3α-aminosolasodan-8-ol monohydrate.

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1. Frisbey, A., Roberts, J. M., Jennings, J. C., Gottshall, R. Y., and Lucas, E. H., Quart. Bull. Mich. Agr. Exp. Sta., Mich. State Coll., Vol. 35, 3, 392-404 (1953).

2. Schlittler, E., and Uehlinger, H., Helv. Chim. Acta, 35, 2034-2044 (1952).

3. Barger, G., and Fraenkel-Conrat, H. L., J. Chem. Soc., 1936, 1537-1542.

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I. INTRODUCTION

The discovery and development of antibiotic agents, typified by penicillin (30,31), has eclipsed all previous discoveries and inventions for the safe, effective and rapid treatment of diseases caused by microorganisms. Most antibiotic work since the isolation of penicillin has had as its objective the discovery and isolation of some of the hundreds of naturally occurring antibacterial compounds.

This search has not been restricted to microorganisms. Several surveys of the antibacterial activity shown by extracts from large numbers of higher plants have been published. Lucas and associates (32,33,34,35,40,41,54,55,56) have conducted and reported on an extensive screening program with the purpose of finding seed plants possessing antibacterial activity, especially against Mycobacterium tuberculosis. They found that a considerable number of plants showed such antibacterial activity. Of these, Solanum pseudocapsicum L. appeared quite promising (32). On the basis of these findings this investigation was undertaken with the purpose of isolating and chemically identifying the main antibacterial principle or principles in the plant.

II. HISTORICAL

A. History of Antibacterial Agents from Higher Plants

1. Definition of Antibiotic and Antibiotic Agents

Antibiotic agents are, in the truest sense of the word, chemotherapeutic agents. The term is derived from the word antibiosis which may be defined as the antagonistic association between organisms to the detriment of one of them. The concept is not new, since Pasteur in 1877 (68) suggested that infections might be combated by making use of the antagonism between different organisms.

In this study the term antibacterial agent is applied to a compound (or principle) inhibiting the growth of bacteria, whereas the term antibiotic agent is connected to growth inhibition of both bacteria and other microorganisms.

It should be emphasized at this point that a substance can be classed as an antibiotic or antibacterial agent even though it is without effect in vivo or is too toxic to permit use in the animal body. In vitro activity alone is sufficient to indicate antibiotic activity. However, only if the substance is effective in the presence of body fluids and is sufficiently non-toxic to tissue cells to permit safe administration, hope for its value as a medicinal can be entertained.

2. Early Developments

Recognition of the medicinal value of certain plants occurred very early in the history of mankind. One of the earliest references to the medicinal use of plants is found in the ancient Chinese literature (65). Plant drugs were described in Pên-tsào (the Great Herbal) written by Shên-nung, who is said to have lived about 3000 B.C. In the following centuries additional information was collected in China, and in 1597 A.D. Li Shih-chên published a 52-volume series on Chinese medicinals which included several plants used against septic wounds.

Another early reference to plant drugs from approximately the sixteenth century is the Ebers Papyrus (26), discovered in a tomb at Thebes in 1873. It describes more than seven hundred herbal remedies, including many that are familiar and still used today.

In the Old Testament more stress was laid on the intervention of the deity than on use of drugs in healing the sick. Even the instructions of Isaiah (12) for saving the life of Hezekiah have a somewhat arbitrary character: "And Isaiah said, Take a lump of figs. And they took and laid it on the boil, and he recovered."

Egyptian medicine decayed along with the kingdom of Egypt itself, but in Greece, and later in Rome, a new school of medical thought evolved, and modern medicine is generally considered to date from the time of Hippocrates (460-361 B.C.).

Theophrastus (307 to 256 B.C.) in his Historia Plantarum described the therapeutic uses of plants and Galen (103 to 193 A.D.) advocated in his writings the use of vegetable in place of mineral preparations in medicine. Dioscorides, a Greek surgeon to the army of Nero, dealt with medicinal plants in De universa medicina (3). Celsus, who is believed to have lived during the reign of Tiberius, mentioned in De Medicina the use of a number of plants in providing remedies for purulent wounds.

Hippocrates and Galen together dominated European medicine for some fifteen centuries. In the absence of original thinkers and experimenters their teaching turned into dogma and became distorted and misunderstood. The health of the body was thought by many to depend on a balance of the "four principles," i.e., heat, cold, moisture, and dryness. In the same period or until 1500 A.D. the "doctrine of signatures" was propagated. According to this doctrine, medicinal plants were stamped with some obvious sign of the use for which they were intended. Thus, Solomon's Seal owes its name to the fact that if its root is cut across, the section resembles a seal. Hence, it was supposed that preparations of the plant would be valuable for "sealing" wounds, and it was widely used for this purpose (91). By the sixteenth century the art of herbalism had become firmly established. The herbal remedies of many people and many centuries were recorded in fairly readily accessible form. Paracelsus (1493-1541) in his extensive

writings, began to bridge the gap between medicine and chemistry and thus founded iatrochemistry. John Gerarde, an Englishman, wrote The Herball or Generall Historie of Plantes in 1597. It is a curious book, a mixture of accurate description and contemporary folklore. Some idea of the nature of its content may be gathered from the following quotation (37):

> Dioscorides writeth that the yellowe Wallflower is most used in phisicke, and more than the rest of stocke gilloflowers, whereof this is holden to be a kinde, which hath moved me to prefer it unto the first place. He saith, that the juice mixed with some unctious or oilie thing, and boiled to the form of a lyniment, helpeth the choppes or riftes of the fundament. The herb boiled with white wine, honie, and a little allom, doth cure hot ulcers, and cankers of the mouth.

After the seventeenth century botany became more scientific and the herbal was gradually replaced by the flora and the pharmacopoeia.

3. Modern Developments in Chemotherapy

In 1910 with Paul Ehrlich's synthesis of the organic arsenical Salvarsan used in the treatment of syphilis (27), the modern era of chemotherapeutics was inaugurated. Plasmochin, the synthetic antimalarial, Domagk's Prontosil, and modern sulfa drugs, such as sulfanilamide, sulfathiazole, and sulfadiazine gave hope that one day it would be possible to fight any disease with a synthetic drug. Consequently, the consideration of plants as suppliers of medicinals went more or less into the background and Alexander Fleming's observation

in 1929 (30) that the mold Penicillium notatum could inhibit the growth of Micrococcus pyogenes var. aureus almost passed unnoticed. About ten years later, Florey and Chain at Oxford investigated the same antibiotic agent and succeeded in obtaining crude preparations which had remarkably high activity against many Gram-positive organisms (31).

Consequently, the work of the Oxford group re-established the importance of plants as suppliers of medicinals and after 1940 the isolation of a great many antibiotic agents was announced. Some of these, like streptomycin, aureomycin and terramycin, have showed great promise and are used therapeutically whereas many others have not appeared as promising.

Since one is prone to associate antibiotics with microorganisms, it may be surprising to find that a large number of different species and varieties of higher plants have been examined for antibacterial activity. It may be conceded that the results of the search for antibiotics in higher plants have not as yet met with the dramatic success that has attended the researches on microorganisms.

Insofar as can be determined, no antibiotic isolated from a seed plant has yet shown sufficient promise to warrant its common therapeutic use. Nevertheless, this has not decreased the interest in higher plants as possible sources of antibiotics, since it is noticed that even in the case of microorganisms, only a few of the many antibiotics isolated actually have been useful therapeutically.

4. Plants Possessing Antibacterial Activity

Several surveys over higher plants having antibacterial activity have been published of which the first was by Osborn in 1943 (67). Among the most exhaustive of these investigations are those of Lucas and co-workers at Michigan State University (32,33,34,35,40,41,54,55,56), Sanders, Weatherwax, and McClung in Indiana (74), Hughes and associates in California (4,45), Bishop and MacDonald in Canada (13), and Winter and Willeke in Germany (92). These references do not represent a complete survey of plants having antibacterial activity and are only of preliminary significance; but they indicate that very active screening programs are carried on at several places in the world. One can therefore anticipate a more systematic determination of antibacterial principles in higher plants. That this goal is within reach, can be seen from the first reports of Lucas and co-workers (40,55,32). It was found that activity against M. tuberculosis occurred in 16.8, 21.1, and 43.2 per cent of the tested plants in three consecutive surveys. This demonstrates that through experience it is possible to select plants which probably will exhibit antibacterial activity.

Besides, it may be concluded from the survey results that antibacterial activity occurs most frequently in the flowers and leaves and less frequently in the fruits and seeds. Roots have not been examined to such an extent that similar conclusions can be drawn.

Phenolic and acidic substances. - Since the early days of bacteriology it has been recognized that phenolic substances exhibit antibacterial activity. One of these is thymol, present in Thymus vulgaris. Others are protocatechuic acid and catechol isolated from a pigmented variety of onion, Allium cepa, by Link and co-workers (53,2).

The earlier work on steam-volatile products of certain gymnosperms provoked interest because of the antibacterial properties of many of the compounds. Thujic acid (42), carvacrol (19), and chamic acid (19) are examples of such substances. Pinosylvine and its monomethyl ether isolated from the heartwoods of Pinus sylvestris by Rennerfeldt (73) inhibited the growth of a number of wood-rotting fungi.

Lactones. - Lactones as a group seem to exhibit antibacterial properties. The unsaturated lactone kawain isolated from the roots of Piper methysticum (14) showed such activity, especially against Gonococcus (86). Protoanemonin and anemonin from Anemone pulsatilla (17) showed considerable activity against fungi, moderate activity against several bacteria; but the toxicity of both compounds was fairly high. Parasorbic acid isolated from the ripe berries of the mountain ash, Sorbus aucuparia, is another example of an antibacterial lactone (17). In 1941 Campbell and Link (18) isolated a substance responsible for the hemorrhagic disease of cattle caused by eating sweet clover. The compound responsible for this disease was dicumarol, which was also found to have antibacterial properties (17).

Alkaloids. - Since alkaloids possess interesting pharmacological properties, their possible antibacterial action has been given some attention. Haim (43) tested atropine, hyoscine, aconitine, morphine, and cocaine against a number of bacteria and found that they all showed only negligible activity. This confirms the finding that such well-known drug plants as Atropa and Datura exhibit no detectable antibacterial activity. Lambin and Bernhard (51) tested several alkaloids, such as apomorphine, berberine, conessine, and strychnine against M. tuberculosis and found similarly their activity not appreciable. Bersch and Döpp (9) on the other hand found that cepharatine, berberine, and sanguinarine all were fairly active against M. tuberculosis.

Lambin and Bernhard (loc. cit.) stated that berberine at the concentration of 150 γ/ml. inhibited the growth of M. tuberculosis, whereas Bersch and Döpp (loc. cit.) mentioned a concentration of only 10 to 40 γ/ml. for the same effect. These examples show how difficult it is to compare the results of bacteriological testing. The results obtained are influenced to such a great extent by the methods used for detecting and measuring antibacterial activity. The alkaloid sanguinarine tested by Bersch and Döpp (loc. cit.) inhibited the growth of M. tuberculosis at concentrations 0.5 to 5 γ/ml. It was isolated by Johnson et al. (47) and found active against several species of Escherichia and Aerobacter.

The steroidal alkaloid glycoside, tomatine, reported by Fontaine and Ma (57) showed low activity against Micrococcus pyogenes var. aureus and B. subtilis and no activity against E. coli. On the other hand, it exhibited a high activity toward certain fungi.

B. Chemistry and Pharmacology of Solanum Pseudocapsicum L.

Solanum pseudocapsicum L. (5), commonly called Jerusalem Cherry, Christmas Cherry, or Natal Cherry (66), is a small branching leafy shrub that grows to a height of three to four feet. It has narrow, lanceolate leaves which are dark green and possess a shiny surface. The small and white flowers are solitary or few in lateral clusters. Its bright orange cherry-like fruit makes it an attractive ornamental. The plant is probably a native of the Old World, perhaps the island of Madeira off the northwest coast of Africa. It is widely distributed in tropical and subtropical regions of Africa and has been naturalized in Florida. It is widely grown in greenhouses for ornamental purposes and can be grown in fields anywhere in the United States where tomatoes are cultivated.

Kylin found in 1927 (50) that the berries contain the pigments phyllorhodin and physalin.* Breyer-Brandwijk reported in 1929 (15) that the dried leaves contain 0.25 - 0.53 per cent of an unknown, non-volatile alkaloid, which was toxic and not identical with solaine.

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*Probably physalien

Watt et al. (87,88,89) described the action of the alkaloid product from the leaves of Solanum pseudocapsicum on the circulatory system. The amorphous alkaloidal preparation was called solanocapsine in their second paper. The alkaloid is irritant, produces vomiting when given by the stomach, and gives local irritation when injected subcutaneously. The application of toxic concentrations resulted in wide disorganization of the heart action by producing sinus arrhythmia, auriculoventricular block, and weakening of the muscle. It was possible to administer doses of 0.06 to 0.084 g. to human subjects. Its toxicity is about equal to that of cocaine hydrochloride.

Muenscher (66) mentioned that the plant, in addition to the alkaloid solanocapsine, contained solanine and solanidine. He also stated that the berries should never be eaten as they possess toxic substances that may cause severe poisoning.

Schnell and Thayer (82) tested the berries of S. pseudocapsicum for antibacterial activity and found that an aqueous extract was inactive in vitro against Micrococcus pyogenes and E. coli.

Lucas et al. (32) found that a water extract of the roots of S. pseudocapsicum was promisingly active against both M. tuberculosis and Micrococcus pyogenes, whereas an ethanolic extract of the leaves showed activity only against Micrococcus pyogenes. The alkaloid solanocapsine was first chemically characterized by Barger and Fraenkel-Conrat (6), who thought

that two alkaloids were present. Schlittler and Uehlinger (78) found only one alkaloid, namely solanocapsine.

C. Chemical Studies on Solanocapsine

In 1929 Breyer-Brandwijk prepared from the leaves of Solanum pseudocapsicum an amorphous alkaloid product (15), which had a depressant effect on the heart.

In 1936 Barger and Fraenkel-Conrat (6) isolated from the leaves of the same plant a crystalline alkaloid which they assigned the formula $C_{26}H_{44}N_2O_2$ or $C_{25}H_{42}N_2O_2$ and named this solanocapsine. Furthermore, the two workers obtained an amorphous alkaloid which was found to have the formula $C_{26}H_{42}N_2O_4$ and called this compound solanocapsidine. No alkaloid glycoside was isolated; but the crude alkaloid treated with 2 N hydrochloric acid gave reaction for carbohydrates and for pentoses. The authors conceived that the two bases were secondary products formed during isolation and not present as such in the plant.

Solanocapsine was used for a number of mild degradation reactions, but due to insufficient material for selenium dehydrogenation, this reaction was carried out on the more abundant solanocapsidine. Assuming a similarity of structure in the two alkaloids, they applied the results of selenium dehydrogenation to solanocapsine. Solanocapsine reacted with two equivalents of nitrous acid; a secondary amino group acquired a nitroso group, a primary amino group was converted

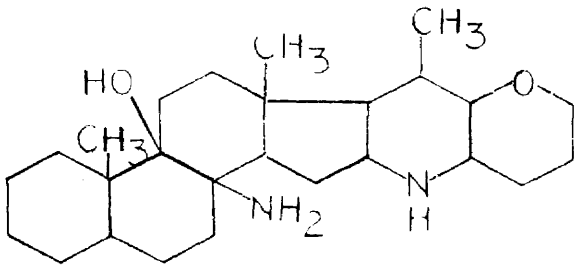
into a hydroxyl group, and probably the hydroxyl originally present was eliminated as water.

According to Barger and Fraenkel-Conrat (ibid.) solanocapsine showed three active hydrogens at room temperature and four at higher temperature, the fourth being the second hydrogen in the primary amino group. Since the secondary amino group accounts for one and the primary amino group for one (or two) of the three (or four) active hydrogens, it was deduced that a hydroxyl group was originally present. On heating with acetic anhydride, solanocapsine yielded a neutral diacetyl derivative. Since these workers (ibid.) found that the hydroxyl group did not acetylate and believed that it was readily eliminated as water with formation of a double bond, they concluded that it was tertiary. Inasmuch as the function of the second oxygen atom could not be determined, it was assumed that it was a member of a heterocyclic ring.

It was also reported by Barger and Fraenkel-Conrat (6) that solanocapsine could be condensed with acetone to yield a compound that showed only one active hydrogen. Acetylation of the acetone complex resulted in a monoacetyl solanocapsine; the acetyl group was assumed attached to the secondary amino group and the acetone part was split off during isolation of this acetyl derivative. By selenium dehydrogenation of solanocapsidine, Diels hydrocarbon (3'-methyl-cyclopentenophenanthrene), 2-methyl-5-ethylpyridine, and 4-methyl-2-ethylpyridine were obtained. Based upon these results the authors

proposed a structure for solanocapsine as presented in drawing number I.

In 1945 Rochelmeyer (74) suggested a structural formula for solanocapsine with 27 carbons, which would bring solanocapsine in line with other alkaloids isolated from the genus Solanum. Without any reported experimental evidence the formula given in drawing number II was suggested.



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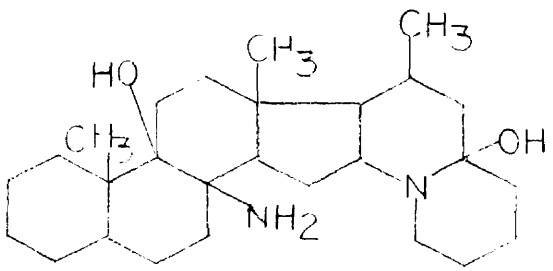


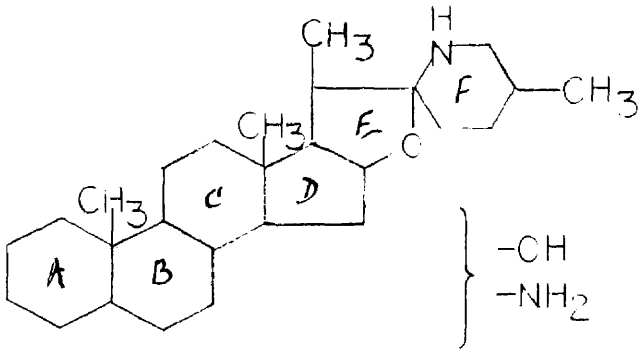
II

Schlittler and Uehlinger (78) published, in 1952, a paper on the structure of solanocapsine. There was no doubt about the identity of their compound with the base isolated by Barger and Fraenkel-Conrat (6); but they decided that the empirical formula $C_{27}H_{46}N_2O_2 \cdot H_2O$ was more in agreement with their elementary analysis of the compound. By this formula solanocapsine was classified as an aglycone with 27 carbons (others being solanidine, tomatidine, solasodine, et cetera). Like Barger and Fraenkel-Conrat (op. cit.) they maintained that the compound had a primary and a secondary amino group and formed a trimethyl derivative. They also concluded that one of the oxygen atoms was either present as a tertiary hydroxyl group

or as a nonreactive secondary hydroxyl group, while the second oxygen was present as an ether.

Schlittler and Uehlinger (6) found that the hydroxyl group could not be acetylated or eliminated as water by alcoholic alkali. By dehydrogenation of solanocapsine with selenium, Diels hydrocarbon was obtained besides 2-ethyl-5-methylpyridine. Short time heating of solanocapsine with selenium gave a basic reacting compound with the formula $C_{27}H_{39}NO$. It contained all the carbon atoms, but had lost the hydroxyl and the primary amino groups and three double bond were introduced into the compound. The ultraviolet spectrum seemed to indicate that the double bonds were conjugated.

The infrared spectrum of trimetyl solanocapsine hydrochloride showed a more intense hydroxyl band than the spectrum of trimetyl solanocapsine, and that is why it was suggested that ring E opened under influence of acid. From these results the authors proposed a structure for solanocapsine as given in drawing number III.



III

III. EXPERIMENTAL

A. Apparatus

Wiley laboratory mill, No. 1. - A small scale rotary knife cutter with interchangeable sieve openings. Sold by Arthur H. Thomas Company, Philadelphia, Pennsylvania.

Flash evaporator. - The rotating evaporator described by Craig and Gregory (22) and built in the shops of the Kedzie Chemical Laboratory. It was operated at 17 to 20 mm. pressure and a temperature below 50°C.

pH-meter. - A Beckman model H 2, glass electrode, line operated. pH meter was used in making pH measurements.

Melting point apparatus. - A mineral oil bath was used with the capillary method. The liquid bath consisted of a beaker with a mechanical stirrer and thermometer supporting the capillary. Melting points were reported without correction.

Chromatography columns. - Two types of columns were used. The first type was a straight glass column (50 x 2.1 cm. i.d.) reduced to a small diameter effluent outlet (0.5 cm. i.d.). a plug of Pyrex glass wool was inserted into the top of the outlet tube and covered with a filter paper disk slightly smaller than the inside diameter of the column. This provided an even base for the adsorbent.

The second type was a glass column (60 x 2.2 cm. i.d.) with the lower end comprised by a ground glass joint ($\frac{T}{S}$ 29/42). The flow rate of the eluent was controlled by a stopcock at the effluent outlet. A plug of Pyrex glass wool and a filter paper disk were inserted as described above.

Paper chromatography. - The ascending method as described by Cramer with Whatman No. 1 filter paper was used (23).

In order to saturate the paper with solvent a piece of bent iron wire was hooked from one side to the other of the paper cylinder. By placing a small magnet on top of the glass plate that covered the cylinder, the iron wire was held by the magnet and the paper cylinder in this way kept above level of the solvent. When ready for development of the spots, the magnet was removed and the bottom of the paper cylinder lowered into the solvent.

Infrared spectrophotometer. - A recording infrared spectrophotometer, model 21, from The Perkin-Elmer Corporation, Norwalk, Connecticut, was used.

Ultraviolet spectrophotometer. - A ratio recording spectrophotometer, model D K2, from Beckman Instruments, Incorporated, South Pasadena, California was used.

Visible spectrophotometer. - A manually operated Bausch and Lomb "Spectronic 20" spectrophotometer was used for measuring optical density in the visible region of the spectrum.

Polarimeter. - The optical rotation was measured in a polarimeter manufactured and sold by O. C. Rudolph and Sons, Caldwell, New Jersey.

Barnstead extractor. - A large capacity extractor working on the principle of complete immersion of solid in the liquid with periodic siphoning off of solvent and replacement by fresh liquid. Fabricated by Barnstead Still and Sterilizer Company, Incorporated, Forest Hills, Boston, Massachusetts and used through courtesy of Dr. H. Sell, Department of Agricultural Chemistry.

Percolator type extractor. - A Soxhlet type of extractor which instead of a siphoning device was furnished with a porous disk allowing the extract to pass through. Used through courtesy of Dr. H. Sell, Department of Agricultural Chemistry.

Hydrogen chloride generator. - Hydrogen chloride was generated by dropping concentrated sulfuric acid from a separatory funnel (connected to a suction flask) onto solid sodium chloride. The gas was dried by passing it through a concentrated sulfuric acid washer.

B. Reagents, Materials and Analytical Methods

1. Reagents and Materials

Chemicals. - All chemicals used for analytical purposes were reagent grade or C.P. quality.

Diethyl ether. - This was obtained from Merck and Company, washed with a solution of ferrous sulfate, dried over calcium chloride and magnesium sulfate. Finally it was distilled over sodium and stored over sodium wire.

Alumina. - Alcoa alumina, grade F-20, 80 mesh, "activity I." When dispersed in a small amount of water, this alumina reacted to produce a pH of around 12.

Acid alumina. - Aluminum oxide (Woelm); activity grade I for chromatographic analysis. Obtained from Alupharm Chemicals, 54 C Street, Elmont, Long Island, New York.

Dowex 1. - Anion exchange resin of 50 to 100 mesh size and 12 per cent cross linkage. Obtained from Dow Chemical Company, Midland, Michigan.

Dowex 50. - Cation exchange resin of 100 to 200 mesh size and 4 per cent cross linkage. Obtained from Dow Chemical Company, Midland, Michigan.

0.02 N HCl. - Prepared by diluting 3 ml. of concentrated hydrochloric acid to 1 liter. Standardized against borax, $Na_2B_4O_7,10\ H_2O$.

0.02 N NaOH. - Twenty-five milliliters of N sodium hydroxide was diluted to 1 liter with freshly boiled water. N sodium hydroxide was prepared from 50 per cent sodium hydroxide. Standardized against 0.02 N hydrochloric acid.

Tomatine. - Obtained through courtesy of Dr. P. S. Schaffer, Eastern Utilization Research Branch, U.S.D.A. and may be prepared by the procedure of Fontaine et al. (31a).

Molisch's reagent. - A 5 per cent ethanolic solution of α-naphtol.

Mayer's reagent. - 1.36 g. of mercuric chloride was dissolved in 60 ml. of water and 5 g. of potassium iodide in

10 ml. of water. The two solutions were mixed and diluted to 100 ml. with water (24). It precipitates the hydrochlorides of most alkaloids in very dilute solution. The reagent should be added to solutions rendered distinctly acid with dilute hydrochloric acid or sulfuric acid. The alkaloid solution should not contain acetic acid and more than a small amount of ethanol or else the alkaloid precipitate is soluble. A few drops of the reagent should be added as the precipitates of some alkaloids are soluble in excess of the reagent.

Aniline hydrogen phthalate reagent. - For reducing sugars 1.66 g. phthalic acid and 0.93 g. of aniline was dissolved in 100 ml. of water saturated with 1-butanol. The paper was sprayed with this mixture and warmed to 105°C. for 10 minutes. Aldopentoses produce reddish-brown spots and other sugars olive-brown spots (23).

Modified Dragendorff reagent. - A mixture of 2.6 g. of basic bismuth carbonate, 7.0 g. of sodium iodide, and 25 ml. of glacial acetic acid were boiled for a few minutes. After standing overnight the supernatant was decanted from the crystal deposit of sodium acetate. Twenty milliliters of this decantate was added to 80 ml. of glacial acetic acid for use as stock reagent. For spot development 20 ml. of stock reagent was mixed with 50 ml. of glacial acetic acid and 120 ml. of ethyl acetate. While shaking 10 ml. of water was added to the mixture.

Paper can be dipped in this solution for development and it does not spread the developed colors (85).

Buffers. - The buffers were prepared according to the tables by Gortner (39).

Anhydrous acetone. - The acetone was distilled over anhydrous calcium sulfate.

Aluminum phenoxide. - The reagent used for an Oppenauer oxidation was prepared followed the procedure of Fuchs and Reichstein (36).

Source of crude material. - In order to obtain sufficient material of uniform quality of S. pseudocapsicum about 500 specimens were grown in the field. These were harvested just before the first frost and stored under refrigeration. Tests were conducted in order to determine the stability of the antibacterial substance. It was found that the activity was retained regardless whether the material was refrigerated or dried. For this reason all plant material was dried in a forced draft oven at a maximal temperature at 60°C. Green parts and roots were separated before drying and later stored separately. All plant material was ground in a Wiley mill to 20 mesh.

2. Analytical Procedures

Determination of acetyl groups. - The number of acetyl groups was determined by the procedure of Kuhn and Roth as described by Milton and Waters (60). N ethanolic potassium

hydroxide was used for hydrolysis except in the case of the triacetyl solanocapsine, where N 1-butanolic potassium hydroxide was used. The latter reagent has been regarded to be more efficient in the hydrolysis of N-acetyl compounds. The compound was dissolved in pyridine before addition of hydrolytic reagent.

The method used by Alicino (1) was employed for determination of O-acetyl groups.

Rast's molecular weight determination. - Molecular weight was determined according to the procedure of Belcher and Godbert (7). A beaker of mineral oil outfitted with mechanical stirrer served as the heating bath. A thermometer calibrated from 90° to 200°C. with 0.2° subdivisions was used for temperature measurements. Twice sublimed camphor was used as solvent.

Determination of methyl groups attached to carbon. - The procedure and reagents described by Milton and Waters (61) were adopted.

Neutralization equivalent. - A weighed sample of solanocapsine was dissolved in ethanol containing a known excess of 0.0102 N hydrochloric acid, and then back-titrated with 0.0120 N potassium hydroxide. From the data obtained, the neutralization equivalent was calculated. The pH change during the titration was followed with a Beckman pH-meter.

Semi-micro Kjeldahl analysis. - Procedure and reagents according to Clark (21) were followed. Digestion and

distillation equipment for analysis was that as modified and used in this laboratory. A digestion time of at least 6 hours was necessary to insure complete digestion.

Van Slyke amino nitrogen determination. - The method is based on measurement of nitrogen gas liberated in the reaction:

$$RNH_2 + HNO_2 \longrightarrow ROH + N_2 + H_2O$$

During the reaction, excess nitrous acid decomposes spontaneously resulting in the formation of nitric oxide:

$$3\ HNO_2 \longrightarrow HNO_3 + 2\ NO + H_2O.$$

Procedure and reagents were followed according to Morrow and Sandstrom (63).

Elementary analysis. - The analyses were performed by either Huffman Microanalytical Laboratories, P. O. Box 125, Wheatridge, Colorado or Geiler Laboratories, 473 Blanchard Terrace, Hackensak, New Jersey.

Microhydrogenation (e.g. determination of "double bonds").- A Warburg apparatus fitted with hollow-stoppered vessels is adaptable for following hydrogenation reactions and with reduced platinum oxide as catalyst was used for hydrogenation. Procedure as described by Milton and Waters (62) was followed.

Periodate oxidations.- Procedure and reagents as given by Dyer (25) were used. The periodate oxidations were performed at three different hydrogen ion concentrations (pH 2, 5, and 8). Aliquots were titrated after 3, 6, 12, 24, 48, 120, and 600 minutes as described in the procedure.

Test for acetone. - The iodoform test was an adaptation from the procedure described by Cheronis and Entrikin (20). Reagents used were those mentioned by the two authors; but the procedure was scaled down such that only a tenth of both compound and reagents were used.

Bioassay. - The purification procedures were followed by biological assay* against Mycobacterium tuberculosis and Diplococcus pneumoniae. Isolation work could have been hampered by S. pseudocapsicum's specific activity against M. tuberculosis because of its long incubation period. For this reason an extended bacterial spectrum was studied in order to find fast-growing organisms which are at least to some extent inhibited by the substance in question. It was found that the antitubercular substances of S. pseudocapsicum could be indicated with a sufficient degree of certainty by testing against Diplococcus pneumoniae. The assays were designed and directed by Dr. R. Y. Gottshall at the Division of Laboratories, Michigan Department of Health. A discussion of the methods of assay will be found in the Appendix I. The activities are expressed as the least number of micrograms (γ) per milliliter of culture broth which inhibits growth. In the introductory experiments the activity is also expressed in a relative way: As the highest dilution which inhibits bacterial growth.

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*The author would like to thank Mrs. Shirley Geis for conducting the bioassays on samples from this project.

C. Experimental Procedures

ISOLATION WORK

1. Preliminary Experiments

Lucas and co-workers (32) found that an aqueous extract of the macerated roots of Solanum pseudocapsicum contained the antibacterial principle. Therefore, the first experiments were mostly concerned with fractionation of a water extract into different active fractions. By extraction with various water-immiscible solvents or addition of different solvents to the water extract, collection and testing of the precipitates for antibacterial activity was carried out. During these introductory experiments the active principle was found considerably more soluble in ethanol than in water. Furthermore, the once water-extracted roots re-extracted with ethanol released still more of the antibacterial principle. Thus a study was undertaken to investigate the efficiency of various solvents.

a. Extraction agents. - Fifteen-gram portions of the dried root powder were extracted with 150 ml. of the appropriate solvent for 150 minutes in a Soxhlet apparatus. The obtained extracts were filtered, concentrated under diminished pressure in a flash evaporator to 15 ml. to give a proportion of root weight to solvent volume of 1:1. The once-extracted root powder was re-extracted, now with 150 ml. of a different solvent. The extracts were filtered and concentrated as

before to 15 ml. All concentrates were tested for antibacterial activity. The antibacterial activity of the extracts made with different solvents can be seen from the data given in Table I.

TABLE I

RESULTS OF VARIOUS EXTRACTION SOLVENTS WITH ROOTS OF S. PSEUDOCAPSICUM

15 g. Root Sample No.	Extraction Order	Extraction Solvent*	Inhibit Growth of: D.pneumoniae in a Dilution of	Inhibit Growth of: M.tuberculosis in a Dilution of
1.	1. extraction	absol.ethanol	1:1024	1:640
	2. extraction	water	inactive	inactive
2.	1. extraction	80% ethanol	>1:128	>1:1280
	2. extraction	water	inactive	inactive
3.	1. extraction	water	1:64	1:80
	2. extraction	80% ethanol	1:64	1:80

* All solutions have a relative concentration of 1 g. of roots to 1 ml. of solvent.

b. Possibility of an antibacterial alkaloid. - Addition of 6 N ammonium hydroxide or 6 N sodium hydroxide changed the straw yellow color of the ethanolic root extract to a deep yellow and a fluffy precipitate appeared. Addition of 6 N hydrochloric acid to the same extract did not change its appearance.

Since all Solanum species investigated so far have contained alkaloids, it was thought that the precipitate appearing

after addition of ammonium hydroxide could be an alkaloid. A drop of Mayer's reagent, a common alkaloid reagent, when added to 1 ml. of the root extract prepared with 80 per cent ethanol (Table I) gave a white precipitate. This indicated the possibility of an alkaloid. After some time the color of the solution changed to a blue. This may be due to a reaction between iodine (from the reagent) and starch present in the solution.

One milliliter of the water extract prepared by extracting the roots previously extracted with 80 per cent ethanol (refer with Table I) gave only a very slight cloudiness with Mayer's reagent. This indicates that the alkaloids for the most part were extracted the first time by ethanol.

c. Fractionation using ion-exchange. - The ion-exchange fractionation was carried out in a batch process and may be schematically represented as follows:

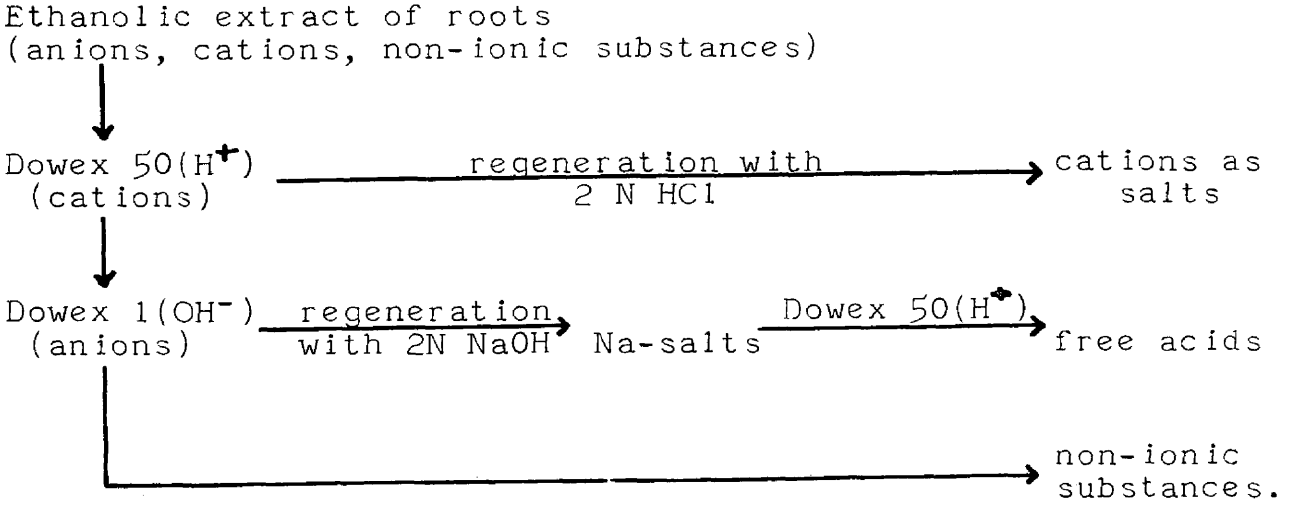

Figure 1. Scheme of ion-exchange fractionation of root extract.

Dowex 50 was in the free sulfonic acid form (RSO_2OH). Twenty grams was prepared by washing the resin twice, each time with 100 ml. of 2 N hydrochloric acid, and then washing with water until the washings did not give precipitate with 0.05 N silver nitrate. Dowex 1 was in the quaternary ammonium base form. Twenty grams of the resin was washed twice, each time with 100 ml. of N sodium hydroxide, and finally with water until neutral to litmus paper.

The following describes stepwise the procedure of separation:

Step 1. - Thirty grams of root meal was extracted with 300 ml. of 80 per cent ethanol in a Soxhlet apparatus for three hours. The extract was concentrated in a flash evaporator to 150 ml. and gave a solution of pH 5.4.

Step 2. - The ethanolic extract was mixed with the Dowex 50 resin and the pH change followed on a Beckman pH-meter. After one minute the pH was 1.9 and both after three and five minutes 1.85. The resin was now washed with 50 ml. of 80 per cent ethanol.

Step 3. - The effluent plus wash liquids were added to the Dowex 1 resin. The pH was after one minute 4.0, and after three and five minutes 6.5 and 9.5, respectively. The resin was washed with 50 ml. of 80 per cent ethanol.

Step 4. - The effluent plus washings which contain the non-ionic substances were evaporated at 50°C. to dryness in a flash evaporator. The residue was dissolved in 30 ml. of

80 per cent ethanol and tested for antibacterial activity.

Step 5. - The Dowex 50 resin was regenerated twice, each time with 100 ml. of 2 N hydrochloric acid and the greenish colored effluent was evaporated to near dryness. After neutralization, ethanol was added to produce a final volume of 30 ml. of 80 per cent ethanol. This solution presumed to contain the organic cationic materials was tested for antibacterial activity.

Step 6. - The Dowex 1 resin was regenerated twice, each time with 100 ml. of N sodium hydroxide. The sodium salts of organic acids present in the effluents were converted to free acids by adding the effluents to a Dowex 50 resin on the free acid form. The effluents, of a greenish color before addition to the Dowex 50 resin, were colorless after having passed over the resin. The effluent was evaporated to dryness; the residue was dissolved in 30 ml. of 80 per cent ethanol and then tested for antibacterial activity.

The activities of the fractions are given in Table II.

TABLE II

ANTIBACTERIAL ACTIVITY OF FRACTIONS OBTAINED BY ION-EXCHANGE

Fraction*	Inhibit Growth of: *D. pneumoniae* in a Dilution of	*M. tuberculosis* in a Dilution of:
Inorganic cations nitrogenous compounds	1:8	1:5
Free acids	inactive	inactive
Non ionic substances	inactive	inactive

* All fractions had a relative concentration of 1 g. of roots to 1 ml. of solvent.

d. Extraction of basic principles. - As reported above it was found that the activity was associated with the cationic substances, which would include nitrogenous compounds. In the following experiment isolation of the basic principles by ether extraction was attempted.

Thirty grams of root powder was extracted in a Soxhlet apparatus with 300 ml. of 80 per cent ethanol for 2 hours. The ethanol extract was concentrated in a flash evaporator to a volume of 50 ml. and made distinctly ammoniacal. Then it was extracted five times with ethyl ether, the first time with 250 ml. and the next four times with 100-ml. portions. The ether-extracted water phase was tested for antibacterial activity.

The combined ether extracts were dried over anhydrous sodium sulfate and evaporated to remove solvent. The residue was redissolved in 300 ml. of ethyl ether and a one-sixth aliquot (or 50 ml.) was evaporated to dryness. This residue was dissolved in 5 ml. of ethanol and tested for antibacterial activity. A stream of dry hydrogen chloride gas from a generator was passed through the remaining five-sixth of the ether extract. A white precipitate occurred which was filtered off and washed with ether and dried. The weight of the precipitate was 275 mg. To test for antibacterial activity the precipitate was dissolved in 25 ml. of ethanol.

The antibacterial activity of the various fractions of the ethanolic extract can be seen in Table III.

TABLE III

ANTIBACTERIAL ACTIVITY OF FRACTIONS OBTAINED FROM A ROOT ETHANOLIC EXTRACT

Fraction a)	Inhibit Growth of: *D. pneumoniae* in a Dilution of:	Inhibit Growth of: *M. tuberculosis* in a Dilution of:
Ether extracted water phase	1:8	1:5
Basic substances in ether	1:64	1:160
Crude alkaloid hydrochloride	1:256	1:640
Ether, alkaloid free	inactive	inactive

a) All fractions have a relative concentration of 1 g. of roots to 1 ml. of solvent.

From Table III it can be seen that the activity followed the alkaloid fraction.

2. Isolation and Purification of Solanocapsine

a. Antibacterial activity in extracts from roots and leaves. - It was originally found by Lucas and associates (32) that only the roots of *S. pseudocapsicum* showed activity against *M. tuberculosis*. In the preceding paragraph it was reported that the antibacterial principle was closely related to the alkaloids in the plant. Since the alkaloid solanocapsine, which is present in the plant, only had been isolated from the leaves, it was decided to test whether the leaves also possessed antibacterial activity.

Twenty-five grams dried and ground leaves and the same weight of root meal were extracted for four hours, each in a

Soxhlet apparatus. Each 25-g. sample was extracted with 250 ml. of 80 per cent ethanol. Both extracts were concentrated in a flash evaporator to 25 ml. and, after dry weight determinations, tested for antibacterial activity.

TABLE IV

ANTIBACTERIAL ACTIVITY IN EXTRACTS FROM ROOTS AND LEAVES

Plant Part	mg.Dry Wt./ml.	γ/ml.* Inhibit Growth of: D. pneumoniae	M. tuberculosis
Leaves	108	840	340
Roots	56	440	44

* See Appendix for details and definitions of units.

The activities shown in Table IV do not indicate that one part of the plant is better than the other as a source of the antibacterial principle. Different amounts of ballast material dissolved during extraction could have changed the activities reported. But it is confirmed that the leaves too contain the antibacterial principle.

b. Isolation of crude alkaloid from roots. - As a result of previous considerations, the isolation of the alkaloid from roots of S. pseudocapsicum was attempted. Besides determining whether this alkaloid had any antibacterial activity, it remained to be ascertained whether solanocapsine was the alkaloid present in the roots. The method used for isolation was, except for minor changes, that given by Schlittler and

Uehlinger (78) for the isolation of solanocapsine from the leaves. The method used is outlined in Figure 2.

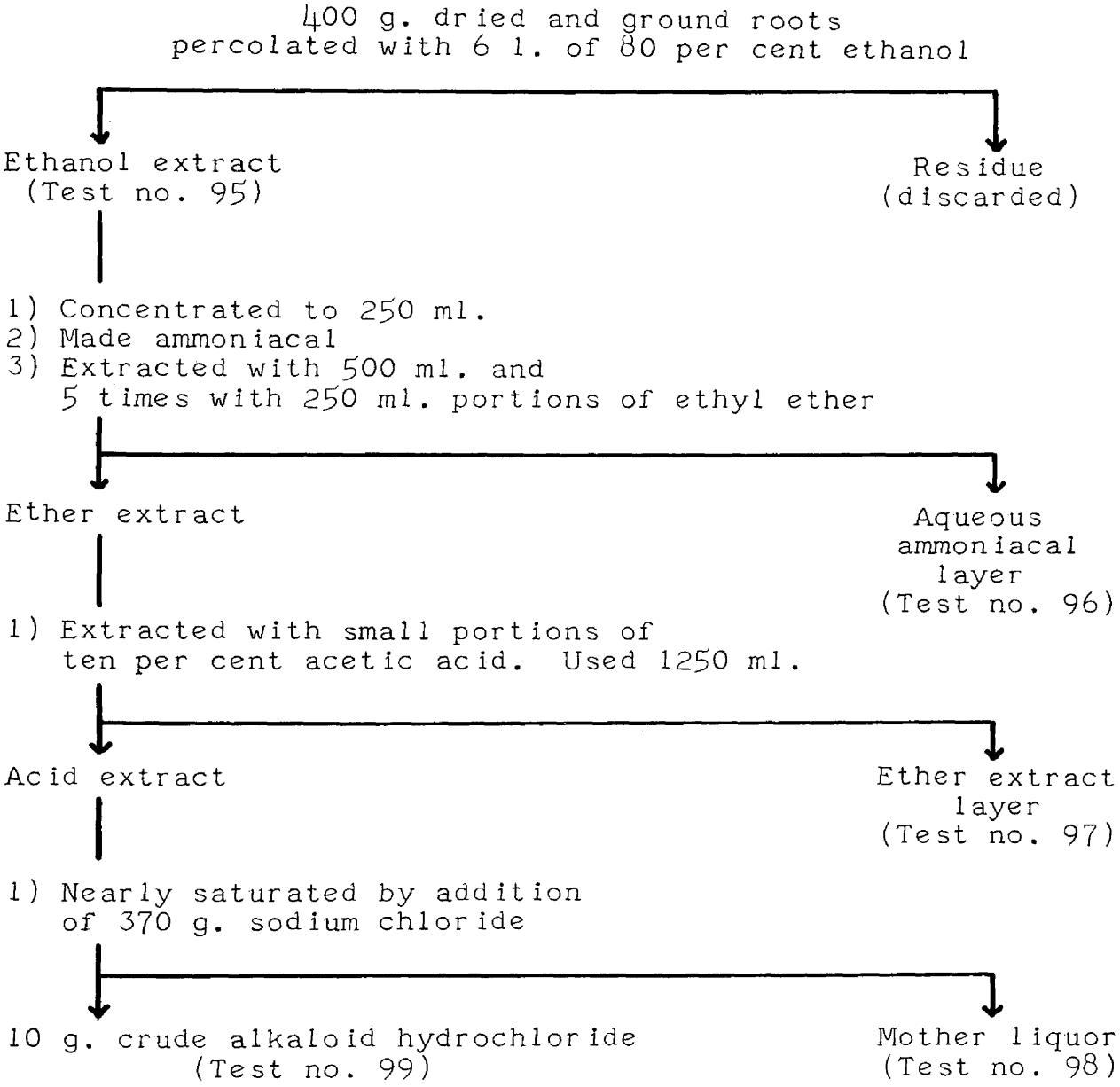

Figure 2. Scheme of isolation of alkaloid from roots.

Dried and ground roots (400 g.) were macerated with 6 l. of 80 per cent ethanol for 48 hours in a conical glass percolator equipped with glass stop cock and a plug of cotton to prevent the root powder from plugging the stop cock. After 48 hours, percolation was performed at a speed of 10 drops per minute and a golden brown extract (5.6 l.) was obtained.

The extract was concentrated in a flash evaporator to 250 ml. The concentrate was made distinctly ammoniacal and extracted once with 500 ml. of ethyl ether and 5 times with 250 ml. portions of the same solvent. The remaining ammoniacal phase was tested for antibacterial activity.

The combined ether extracts were placed in a 2-liter separatory funnel and extracted with small amounts of 10 per cent acetic acid until 1250 ml. of 10 per cent acetic acid was used. An aliquot of the acid extracted ether phase was evaporated to dryness, dissolved in absolute ethanol and tested for antibacterial activity.

Sodium chloride was added to the acetic acid solution in an amount (370 g.) sufficient to precipitate the alkaloid as the hydrochloride. After standing overnight in the refrigerator the crystalline alkaloid hydrochloride was collected on a filter and dried at room temperature. The yield of crude alkaloid hydrochloride was 10.0 g. and found contaminated with a large amount of sodium chloride. Both the crude alkaloid hydrochloride and the acidic mother liquor were tested for antibacterial activity.

Two grams of crude alkaloid hydrochloride was dissolved by heating in 20 ml. of water. The water solution made distinctly ammoniacal was extracted three times with 100-ml. portions of ethyl ether. The combined ether extracts were dried over anhydrous sodium sulfate and evaporated to dryness to give 260 mg. of crude alkaloid base. The antibacterial activity of both the base and the ether extracted water phase was determined. The antibacterial activity of the various fractions can be seen in Table V.

TABLE V

ANTIBACTERIAL ACTIVITY DURING ROOT ALKALOID ISOLATION

Test no.	Fraction	mg. Dry Wt./ml.	γ/ml. Inhibit Growth of: D.pneumoniae	M.tuberculosis On Agar	M.tuberculosis On Blood Agar
95	Ethanol extract	138	30	54	54
96	Ammoniacal layer	188	>100	>100	>100
97	Ether extract layer	4.6	72	inactive	inactive
98	Mother liquor	55.5	>100	>100	>100
99	Crude alkaloid HCl	2.0	16	25	25
100	Alkaloid base	1.0	16	12	12
101	Aqueous phase from extr. of alkaloid base	85	>100	inactive	inactive

c. Isolation of solanocapsine from leaves. - Several batches of solanocapsine were isolated during this investigation.

The procedure with minor modifications was that followed by Schlittler and Uehlinger (78).

Dried and ground leaves (500 g.) were macerated with 9 l. of 80 per cent ethanol in a cylindrical glass jar for 48 hours. The leaf material was filtered on a Büchner funnel and again extracted for 48 hours with 9 l. of 80 per cent ethanol. The combined, deep green filtrates were concentrated in a flash evaporator to 1250 ml. During concentration large amounts of chlorophyll separated on the side of the flask as a viscous coating. To precipitate chlorophyll 200 ml. glacial acetic acid was added to the concentrate. After standing overnight in the refrigerator the chlorophyll was removed by filtration.

The concentrate was then divided into fractions of 500 ml. and each made distinctly ammoniacal. In a 2-liter separatory funnel each fraction was extracted once with 1 l. of ethyl ether and five times with 500-ml. portions. The ether phase separated readily from the aqueous ammonia layer during the first three extractions. However, during the last two extractions it was very difficult to separate the two phases due to emulsification. Some of the lipids present in the extract were possibly saponified by the ammonium hydroxide.

The combined ether extracts were extracted with small amounts of 10 per cent acetic acid. To the combined acetic acid extracts was added sodium chloride (330 g.) until cloudiness persisted and the solution was then placed in the refrigerator. After 10 hours the precipitate was collected on

a filter and dried at room temperature. The mother liquor gave only an insignificant cloudiness with Mayer's reagent. The yield of crude solanocapsine hydrochloride was 8.0 g., which corresponds to a 1.6 per cent yield based on the dry weight of the leaves.

Several additional isolations using the procedure described above gave yields of 1.26 per cent and 1.54 per cent of crude alkaloid hydrochloride based on dry weight of leaves.

Barnstead extractor. - To simplify the cumbersome procedure a large scale isolation was attempted by making use of a Barnstead extractor. A 2 kg. batch of root meal was allowed to absorb 2 l. of water and then extracted in the apparatus with 12 l. of ethanol for seven hours. The main portion of the alcohol was removed by distillation and the remaining extract was further concentrated to 2 l. in a flash evaporator. The concentrate was divided into 500-ml. fractions and worked up as described above. The yield of crude alkaloid hydrochloride was only 2.2 g. corresponding to a 0.11 per cent yield based on dry weight of roots.

d. The completeness of alkaloid extraction. - Schlittler and Uehlinger (78) noticed that the concentrated and aqueous ammoniacal layer obtained from an extract of leaves after ether extraction gave a highly positive alkaloid test with Mayer's test. When this particular step was checked only a very slight cloudiness with Mayer's reagent was observed. Nevertheless, it was decided to re-extract the ether extracted

ammoniacal concentrate with a chloroform-ethanol mixture to determine if more alkaloid could be removed.

For this purpose a liquid-liquid extractor as described by Morton (64) was used and 1250 ml. of ammoniacal concentrate was extracted with 1400 ml. of chloroform-ethanol (2:1). Usually it is a very convenient type of extractor for extraction of a liquid with a heavier liquid; but in this case chloroform and ethanol formed an azeotropic mixture. Since ethanol was more hydrophilic than organophilic the volume of the extractant increased so much that it was necessary to stop the extraction.

Instead the mother liquor was extracted with chloroform in a separatory funnel. Two 750-ml. portions of the mother liquor were extracted five times with 250-ml. portions of chloroform and the combined chloroform extracts were saturated with dry hydrogen chloride gas. The precipitated alkaloid hydrochloride was filtered and dried to give a yield of 1.1 g. The ammoniacal concentrate used in this experiment was originally obtained from 500 g. of root meal, hence the yield in per cent was 0.22 based on dry weight.

e. Purification of solanocapsine. - In an attempt to determine whether the antibacterial activity of pure solanocapsine was greater than the activity of the crude alkaloid fraction, part of the preceding crude material was purified.

One gram of the crude, greenish preparation of leaf alkaloid hydrochloride was dissolved in 70 ml. of 80 per

cent ethanol, heated to 60-70°C. and decolorized with Norit. The filtered solution was tested for antibacterial activity to determine if the active carbon had adsorbed any antibacterial material.

The ethanolic solution was evaporated to dryness, suspended in 3 N ammonium hydroxide and extracted in a separatory funnel seven times with 100-ml. portions of ethyl ether. The remaining ammonia layer was saved and tested for antibacterial activity, and the combined ether fractions were dried with anhydrous sodium sulfate and evaporated to dryness. The yield of colorless alkaloid base obtained was 450 mg. Recrystallization from ethanol-water(1:1) gave colorless, crystalline solanocapsine of melting point 212-214°C. A second recrystallization gave a compound melting at 215-216°C. The results of testing the materials for antibacterial activity can be seen in Table VI.

Table VI shows that the antitubercular activity of the alkaloid increased four-fold during the purification, while its activity against D. pneumoniae decreased one and one-half times. This may suggest that another antibacterial compound besides solanocapsine was present in the plant.

Often during recrystallization of different batches of solanocapsine an amorphous, resinous material was encountered, which was difficult to crystallize. But by repeated solution and cautious precipitation from ethanol-water(1:1) solanocapsine could be obtained in crystalline form.

TABLE VI

ANTIBACTERIAL ACTIVITY DURING SOLANOCAPSINE PURIFICATION

Fraction	mg. Dry Wt./ml.	γ/ml. Inhibit Growth of: D.pneumoniae	M.tuberculosis On Agar	M.tuberculosis On Blood Agar
Crude alkaloid hydrochloride	1	20	13	13
Decolorized alkaloid hydrochloride	14	14	44	22
Ether extracted ammonia layer	20	78	>100	inactive
Alkaloid base (solanocapsine)	2	8	25	25
Solanocapsine, recrystallized four times	0.45	30	3	3

f. Column chromatography. - It was found a very difficult task to purify crude solanocapsine. The colored impurities were difficult to remove, even after treatment with activated carbon. Only through repeated crystallization of solanocapsine from ethanol-water(1:1) was it possible to obtain a pure compound; but the yield was extremely small. Therefore, it was decided to try separation of the alkaloid fraction by adsorption on alumina, acid alumina, or Dowex 50. An extract of the roots was used for alumina, whereas a crude solanocapsine preparation from the roots was used for trials on acid alumina and Dowex 50.

Column chromatography on alumina. - Preparation of extract: A 175 g. portion of root powder was extracted with 1500 ml. of 80 per cent ethanol in a percolator type of extractor (described in Apparatus Section). The extraction was continued for seven hours to secure complete removal of alkaloids. However, some doubt existed about the effectiveness of the extraction. The solvent flowed so rapidly through the root powder that it was impossible to maintain a head of liquid over the root sample. The extract obtained had a volume of 1270 ml. and an antibacterial activity of 330 γ/ml. against M. tuberculosis. When the extract was concentrated to 125 ml. and filtered, the antibacterial activity became 125 γ/ml. against same organism.

Column preparation: The 50 x 2.1 cm. column was "dry-packed" with 200 g. of Alcoa alumina, grade F-20, 80 mesh, in the following fashion. The alumina was poured into the column in small portions and after each addition, the column was tapped gently with a rubber mallet. After addition of all the alumina a filter paper disk was placed on top of the adsorbent.

Introduction of extract: About 400 ml. of absolute ethanol was applied into the column and the first effluent was discarded. When only a 2 mm. height of solvent remained above the alumina, the root extract was added to the column. After the root extract had passed so far down into the alumina that only a 2 mm. liquid height remained above the alumina,

the column was eluted with absolute ethanol. Fractions of 20 ml. were collected in tared 50-ml. beakers. Elution with absolute ethanol was continued until no residue showed after evaporation of the solvent.

Table VII and Figure 2 shows that the amount of basic compounds (=alkaloids) followed the antibacterial activity. Apparently not much separation of impurities from alkaloids was accomplished. It should be reported here that fractions 1 to 11 were all yellow-colored with the highest intensity in fraction 4.

Extrusion: The upper one-fourth of the alumina was of a deep yellow color, while the rest was colorless. The upper colored part gave no fluorescence in ultraviolet light, whereas the lower part did. The alumina was extruded and divided into 3 parts. Part 1 consisted of the material in the yellow zone and parts 2 and 3 were obtained by dividing the lower colorless part into 2 equal segments. Each section was extracted in a Soxhlet apparatus for five hours with 300 ml. of 80 per cent ethanol. The extract, evaporated to dryness in a flash evaporator, was dissolved in 20 ml. of 80 per cent ethanol and tested for antibacterial activity. None of the 3 fractions showed activity against D. pneumoniae; but the fraction with its origin in the colored upper one-fourth of the column contained 1 mg. of dry material, which showed an antibacterial activity of 3 γ/ml.

TABLE VII

DATA FOR ELUTION CHROMATOGRAPHY OF EXTRACT FROM ROOTS OF *S. PSEUDOCAPSICUM* ON ALUMINA

20 ml. Fraction Eluted	ml. of N HCl Necessary for Neutralization	Total Weight in mg.	Relative Activity Against:		γ/ml. Inhibit Growth of:	
			D.pneumoniae	M.tuberculosis	D.pneumoniae	M.tuberculosis
1	0.05	98	1:32	1:80	188	75
2	0.10	272	1:32	1:160	425	85
3	0.20	1508	1:128	1:320	587	235
4	0.35	2396	1:128	1:640	935	172
5	0.60	1792	1:512	1:2560	113	35
6	0.50	1296	1:512	1:1280	127	51
7	0.50	812	1:256	1:640	159	63
8	0.20	160	1:256	1:320	63	50
9	0	60	1:64	1:160	94	37
10	0	32	inactive	1:80	inactive	40
11	0	16	inactive	inactive	inactive	inactive

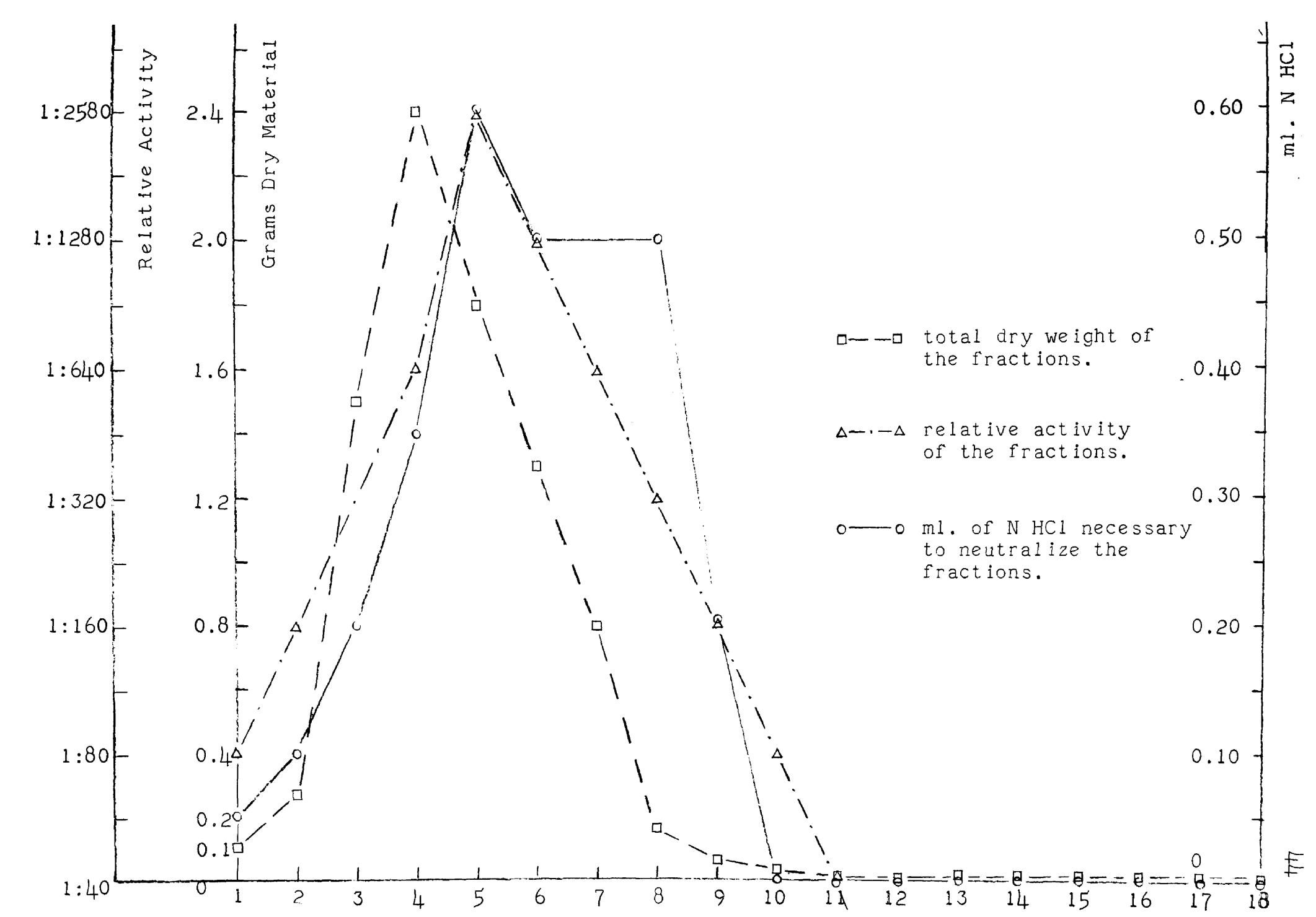

Figure 3. Data for Elution Chromatography of Extract from Roots of S. pseudocapsicum on Alumina.

Ion-exchange chromatography. - Column preparation: The Dowex 50 resin was prepared by washing the resin several times, alternately with 2 N hydrochloric acid and 2 N sodium hydroxide. After each addition of either acid or base the resin was washed with water until neutral. After a final washing with 2 N hydrochloric acid, the resin was washed until neutral first with water and then with 60 per cent ethanol. The resin, now filled into the column in a slurry, had the dimensions 39 x 2.1 cm. corresponding to a volume of 135 ml. During the chromatographic process the length of the resin in the column decreased to 34 cm. When the resin had settled, a filter paper disk was placed on top of the resin and 60 per cent ethanol was allowed to percolate through the column to aid the final packing.

Introduction of crude alkaloid solution: A solution of 2.2 g. of crude solanocapsine from roots in 100 ml. of 60 per cent ethanol and 5 ml. of glacial acetic acid was introduced into the column and allowed to flow down through the resin until only a 2 mm. height remained above the resin. Then a small volume of 60 per cent ethanol was applied to the column and again allowed to pass down until only a 2 mm. height of liquid remained above the resin. Now the developing solvent of 5 per cent ammonium hydroxide in 60 per cent ethanol was added, allowed to flow through, and fractions of 100 ml. eluate were collected. When 1300 ml. were collected, the developing solvent was changed to 10 per cent ammonium hydroxide in 60 per cent ethanol.

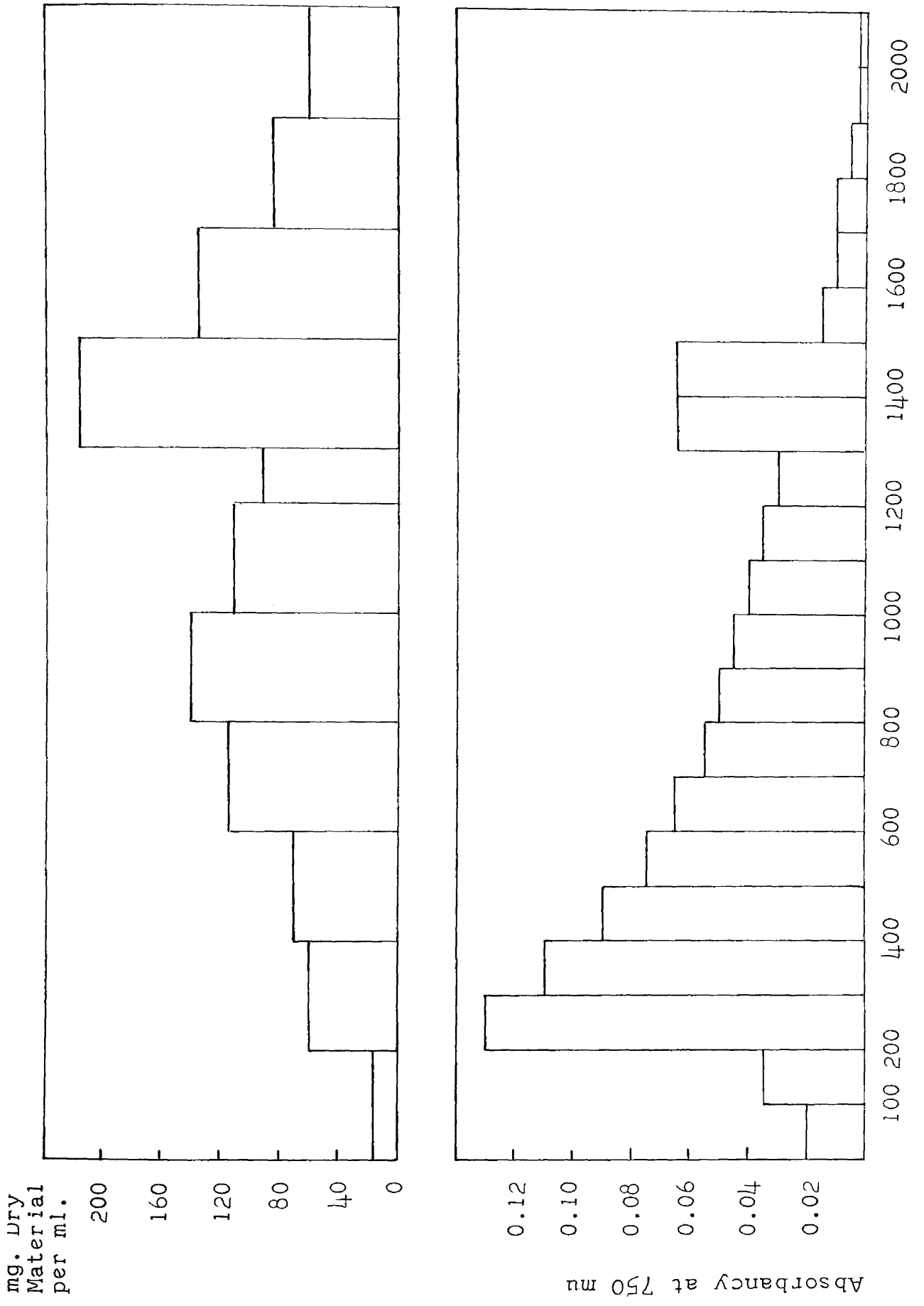

Figure 4. Color Intensities and Amounts of Dry Material in Fractions Obtained by Elution Chromatography on Dowex 50.

In addition to dry weight determinations, the optical density of each fraction was determined at 750 mμ in a Bausch and Lomb spectrophotometer. The spectrophotometric data were intended to give an idea about the distribution of colored impurities. The wavelength of 750 mμ was selected, since spectra of several fractions in the region 400-950 mμ showed maximum absorption at 750 mμ. Figure 4 records color intensities and amount of dry material in the different eluate fractions.

Paper chromatography was applied to all eluted fractions to determine the distribution of solanocapsine and to decide whether only one alkaloid was present in what is called "crude solanocapsine." The composition of both solvent and color reagent is described in the section on paper chromatography.

Result: The overall result of this experiment was negative. No separation of alkaloid from colored impurities was accomplished. The R_f-value of the alkaloid from all fractions was 0.57, the value found characteristic for solanocapsine. The "washing" contained a compound with $R_f = 0.48$; but if this compound was eluted from the paper and re-chromatographed, the R_f-value was 0.57.

Column chromatography on acid alumina. - The acid alumina used was Woelm's anionotropic aluminum oxide, activity grade 1, and specified for chromatographic analysis. One hundred grams of the alumina was "dry-packed" as described before. Water saturated 1-butanol was applied to the column before

TABLE VIII

DATA FOR ELUTION CHROMATOGRAPHY OF CRUDE SOLANOCAPSINE FROM ROOTS ON ACID ALUMINA

Fraction Eluted	Amount of Dry Material in mg.	Color of Fraction	γ/ml. Inhibits Growth of: D.pneumoniae	M.tuberculosis
1	1	colorless	inactive	inactive
2	1	colorless	inactive	inactive
3	2	colorless	inactive	inactive
4	8	weak yellow	25	inactive
5	106	weak yellow	16	13
6	327	yellow	16	13
7	354	yellow	16	13
8	244	weak yellow	16	13
9	81	weak yellow	8	6
10	38	weak yellow	60	47
11	10	colorless	8	6
12	3	colorless	5	4
13	4	colorless	6	5
14	2	colorless	3	2.5
15	3	colorless	5	4
16	2	colorless	3	2.5
17	1	colorless	1.5	1.25
18	1	colorless	3	2.5

application of the alkaloid. A solution of 1.2 g. of crude solanocapsine from roots in 20 ml. of water saturated 1-butanol was introduced into the column. When only a 2 mm. height of liquid remained above the alumina the adsorbent was eluted with water saturated 1-butanol. Eluate fractions of 10 ml. were collected, the solvent was evaporated and the amount of dry material was determined. All fractions were tested for antibacterial activity. Table VIII gives the data for the experiment.

Result: Some separation of colored impurities from alkaloid was accomplished. Fractions 11-18 consisted of pure solanocapsine indicated by fairly constant antibacterial activity*, but the yield was extremely small.

g. Alkaloid content in the plant. - Since it was not known to what extent solanocapsine occurs in S. pseudocapsicum, alkaloid determinations were carried out. The procedure used is an adaptation of the method given for Belladonna Leaf in Pharmacopeia of The United States:

Extraction: Ten grams of dried leaf or root powder (40 mesh) was placed in an extraction thimble, and the thimble was inserted in a Soxhlet extractor. The plant material was

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* The activity of the fractions can easily vary, since the dilution technique has experimental limitations. Besides, the amount of dry material is only determined to the nearest mg. When the amount of dry material in a fraction is in the range of 1 mg. a slight deviation in the weight determination can easily change the activity considerably.

moistened with a mixture of 8 ml. concentrated ammonium hydroxide, 10 ml. of absolute ethanol, and 20 ml. of ethyl ether, mixed thoroughly, and macerated for six hours. The thimble was then covered with a tuft of purified cotton, and the plant material was extracted for six hours with 150 ml. of ethyl ether.

Purification: The alkaloidal solution obtained by this maceration was contaminated with other extractives which would interfere with the volumetric determination of the alkaloid. To effect the purification, the alkaloids were removed from the solvent by extracting with an acid, then the aqueous solution was made ammoniacal and extracted with an immiscible solvent such as ethyl ether:

The ether extract was transferred to a separatory funnel; the Soxhlet extractor was rinsed twice with about 10 ml. of ethyl ether each time, and these rinsings were added to the separatory funnel. The alkaloid was removed completely from the ether layer by extracting with 7 successive 10-ml. portions of N acetic acid, filtering each portion drawn off. The combined acidic solutions were rendered distinctly ammoniacal, and the free alkaloid was immediately extracted with 5 successive 50-ml. portions of ethyl ether. To determine the completion of extraction, 1 ml. of the last extract was evaporated and redissolved in 0.5 ml. of approximately 0.5 N hydrochloric acid. This solution should not show more than a slight turbidity upon the addition of a drop of Mayer's reagent.

Determination: The solution of purified alkaloid in ethyl ether was dried by shaking with anhydrous sodium sulfate and filtered. The filter was washed with 10 ml. of ethyl ether, and the washing was added to the ether solution. This solution was then evaporated to dryness on a steam bath and heated there for 15 minutes. The resulting residue was softened by addition of about 1 ml. of absolute ethanol and dissolved in a measured and sufficient volume of 0.02 N hydrochloric acid to give an acidic solution. The mixture was gently heated to insure complete solution of the alkaloid. The excess acid was titrated with 0.02 N sodium hydroxide in the presence of methyl red as indicator.

Alkaloid determinations on plant material from the past three years can be found in Table IX.

TABLE IX

ALKALOID CONTENT IN S. PSEUDOCAPSICUM ON DRY WEIGHT BASIS

Year of Harvest	Nitrogen Level in Soil	Alkaloid Percentage in: Leaves and Stems	Roots
1953	normal	1.53	0.83
1954	normal	2.14	1.53
1955	low	2.21	1.86
1955	normal	2.15	2.24

These determinations showed that the plant contains a fairly uniform amount of alkaloid. Seasonal and other

environmental factors may cause fluctuations within limits; but so far they have not been extensive.

h. Derivatives of root and leaf alkaloid. - To confirm that the isolated antibacterial alkaloid isolated from both roots and leaves was identical with the alkaloid solanocapsine isolated by both Barger and Fraenkel-Conrat (6) and Schlittler and Uehlinger (78) physical constants were determined and a number of derivatives prepared. Details of procedure are described in the second part of this investigation. Only melting points and physical constants are reported in Table X. From this table the identity of both the root and leaf alkaloid with solanocapsine can be seen.

TABLE X

CHEMICAL CONSTANTS OF ROOT AND LEAF ALKALOIDS

Determination	Alkaloids from Leaves			Alkaloids from Roots
	Found	Schlittler et al.	Barger et al.	Found
Base, m.p.	215-6°	216-7°	222°	215-6°
Dihydrochloride, m.p.	>300°	>300°	280°	>300°
Picrate, m.p.	194-6°	200-1°	194°	198-9°
Oxalate, m.p.	>300°	288-9°		287-9°
Isopropylidene derivative, m.p.	232-3°		233°	
Nitroso derivative, m.p.	194-6°	200°	194°	
Trimethyl derivative, m.p.	205-6°	209°		208°
Nitrogen, per cent	6.20	6.29		
Optical rotation, $[\alpha]_D^{25}$	+25°	+24°	+25.5°	+23.5°
Rast's molecular weight determination	456,481		460	
Neutralization equivalent	482			

3. The Antibacterial Spectrum of Solanocapsine

Solanocapsine isolated from the leaves of S. pseudocapsicum gave an antibacterial spectrum as shown in Table XI.

TABLE XI

ACTIVITY OF CRUDE SOLANOCAPSINE BASE AGAINST VARIOUS MICROORGANISMS

Organisms and Strain No. or Source	Concentration Required for Inhibition of Growth (γ/ml.)
Aerobacter aerogenes 618	500
Bacillus subtilis 231	78
Corynebacterium diphteriae PW8	125
Diplococcus pneumoniae Type III	20
Escherichia coli 621	500
Histoplasma capsulatum Duke Univ., yeast phase	15.5
Histoplasma capsulatum Duke Univ., mold phase	125
Klebsiella pneumoniae NIH III	500
Micrococcus pyogenes var. aureus 209P	312
Mycobacterium phlei	125
Mycobacterium smegmatis	125
Mycobacterium tuberculosis D.T.	31
Mycobacterium tuberculosis H37	31
Mycobacterium tuberculosis H37Rv	31
Proteus sp. OXK 1414	500
Pseudomonas aeruginosa R-P-CC	312
Salmonella typhimurium 9	500
Sarcinia lutea PCI 1001	156
Shigella paradysenteriae 3009	312
Streptococcus hemolyticus 286	39
Xanthomonas phaseoli	78

Since the alkaloid was not one of the most highly purified preparations the inhibitory action against M. tuberculosis was less than that of other preparations. However, the table serves for comparative purposes, provided that a possible enhancing effect of impurities is given due consideration.

4. Toxicity Studies on Solanocapsine

The toxicity determinations were directed by Dr. R. Y. Gottshall and carried out at the Division of Laboratories, Michigan Department of Health*.

A 1 per cent solution of both solanocapsine and solanocapsine hydrochloride was made by dissolving 100 mg. of the compound in 2 ml. of sterile propylene glycol and slowly adding 8 ml. of sterile 0.9 per cent sodium chloride solution.

The compounds were injected subcutaneously and into the peritoneal cavity of white mice, Webster strain, in amounts of 1, 2, 4, and 6 mg. The control mice were injected with 0.1 ml. of propylene glycol.

By the intraperitoneal route only 50-100 mg./kg. of body weight of solanocapsine was tolerated, whereas with the free base it was necessary to inject 200 mg./kg. before the animal succumbed. The lethal dose by subcutaneous injection was 300 mg./kg. for either solanocapsine or solanocapsine hydrochloride.

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*The author would like to thank Mrs. Shirley Geis for conducting the toxicity determinations.

After injection of as little as 0.25 mg. of solanocapsine the mouse became very nervous. From 9-15 minutes after inoculation short jerky convulsions started with the animal demonstrating complete collapse between seizures. After 15 minutes the posterior limbs were almost immobile. Following 30-60 minutes the mouse usually collapsed; breathing was rapid and shallow. The animal usually remained collapsed from one to three days and then expired, if lethal dose had been administered. Otherwise, the animal recovered and appeared normal and active.

In many cases the mice developed sores, which often turned into a crusty eczema over the entire body or else it localized to the back. A scabby sore usually appeared at the site of injection.

5. Attempts to Isolate an Alkaloidal Glycoside

All alkaloids isolated so far from the genus Solanum have been glycosides with the exception of solanocapsine. If one were to attach any significance to the genetic relationships among similar compounds isolated from closely related species, one would conclude that solanocapsine probably was an artifact and that the alkaloid was present in the plant as a glycoside. Two plausible reasons for finding an alkaloid and not a glycoside may be proposed: Either a very active glycosidase catalyses the hydrolysis of the compound at the time of harvesting the plant or the glycoside is so labile that the

weak acid used in the isolation procedure will hydrolyze the compound.

a. Possibility of a glycoside. - The following observations supported the theory of solanocapsine occurring as a glycoside in the plant: 1) The isolated alkaloid was very difficult to purify to such an extent that the physical constants were identical with those found for solanocapsine. 2) Different batches of crude root solanocapsine usually gave a weak, but positive Molisch test (43a), a test characteristic for furfural and furfural-yielding substances. Batches of crude leaf solanocapsine only in one case have given a positive test. Pure solanocapsine gave a negative Molisch test. In all cases the test was compared with a blank test. Incidentally, the blank test with distilled water was found to show a faint purple ring at the interface. 3) The mother liquor from recrystallization of the root alkaloid gave, after concentration, a positive Molisch test. 4) The same mother liquor gave upon paper chromatography and detection of sugar spots with aniline hydrogen phthalate, a reddish spot with an R_f-value comparable with the R_f of arabinose.

b. Paper chromatography. - Under the assumption that the crude root solanocapsine was partly present as a glycoside, both acid and enzymatic hydrolysis was attempted.

Acid hydrolysis: A solution of 4.8 mg. of crude alkaloid in 0.5 ml. of 0.1 M sulfuric acid was refluxed on steambath. After 0, 5, 10, 15, 20, 40, and 130 minutes 5-µl. aliquots

were removed with a micropipet and deposited on a sheet of Whatman no. 1 filter paper. The chromatogram was developed with 1-butanol-ethanol-water(80:20:20) and a single reddish spot of R_f-value 0.25-0.26 was detected with aniline hydrogen phthalate reagent (23). Only the aliquot hydrolyzed for 130 minutes gave the reddish spot expected for a pentose.

In another experiment 10 mg. of the crude alkaloid was dissolved in 1 ml. of N hydrochloric acid in a small test tube which then was sealed and heated at 100°C. for five hours in an oven. Aliquots were spotted on paper alone and together with different pentoses. Again a sugar was detected and it was most identifiable with arabinose.

Enzymatic hydrolysis: Other hydrolysis experiments were conducted with Parke, Davis and Company Taka-Diastase in neutral buffered solutions; but chromatography on paper for sugar was without any success. Neither did methanolysis of the compound produce any such result.

Crude root solanocapsine, crude leaf solanocapsine, and crystalline solanocapsine were each chromatographed. As solvent, the upper phase of ethyl acetate-glacial acetic acid-water(3:1:3) to which was added 15 per cent of a 85 per cent ethanol (49), was used. All four materials produced spots of the same R_f-value when detected with a modified Dragendorff reagent (85). Table XII records the R_f-values for the compounds developed in the above mentioned and other solvents.

TABLE XII

R_f-VALUES FOR SOLANOCAPSINE, CRUDE LEAF ALKALOID, AND CRUDE ROOT ALKALOID

Solvent	Crystalline Solano-capsine	Amorphous Crude Leaf Alkaloid	Amorphous Crude Root Alkaloid
1-butanol-ethanol-water (80:20:20) 1)	no spot	0.25	no spot
Upper phase of ethyl acetate-acetic acid-water (3:1:3) to which is added 15% of 85% ethanol 2)	0.57	0.56	0.57
1-butanol-ethanol-phosphate buffer (pH8.2) (80:20:20) 2)	0.74	0.75	0.74
Ethanol- N HCl -water (50:2:48) 2)	0.73	0.74	0.73

1) Spots developed with aniline hydrogen phthalate reagent.
2) Spots developed with modified Dragendorff reagent.

These findings indicated that the alkaloid isolated from S. pseudocapsicum was the free alkamine solanocapsine and not a glycoside. Nevertheless, the results did not disclose the existence of solanocapsine in the plant as a glycoside.

It had been stated in the literature that solanine and solanidine were present in S. pseudocapsicum (66). Therefore, alcoholic extracts from the roots and leaves and samples of solanine, solanidine, and tomatine were paper chromatographed side by side in the aforementioned solvent for developing alkaloids. The alkaloids were detected with the modified

Dragendorff reagent. The root and leaf ethanolic extracts gave only one spot with each an R_f of 0.22. Then, when eluted and re-run these spots had a R_f of 0.57. Solanidine followed the liquid front, while solanine and tomatine had R_f-values of 0.08 and 0.07, respectively. Thus it was found that the one and only alkaloid spot given by the root and leaf extracts was identified in R_f-value as that given by a pure sample of solanocapsine.

c. Isolation attempts. - As mentioned previously, the possibility of a glycosidase catalyzing the hydrolysis of a glycoside could not be excluded. Therefore, inactivation of such an enzyme was attempted as fast as possible after harvesting the plants. The method used for isolating the basic solanocapsine utilized ethyl ether as a solvent, but since ether probably is a poor solvent for more hydrophilic glycosides, a standard method for isolating this class of compounds was employed (84).

Two methods of enzyme inactivation were utilized: 1) Submersion of the harvested plants in absolute ethanol and 2) immediate freezing in dry ice and storage in deep freeze until the day of isolation when they were boiled with water.

1) The roots and tops of 15 fresh plants were immediately separated and each part covered with absolute ethanol and stored until time for isolation. A total of 1400 g. of dehydrated tops was macerated in a Waring blender with

14 l. of 2 per cent acetic acid and allowed to stand for 48 hours with occasional stirring. The supernatant was removed by filtration and the plant pulp was similarly re-extracted with 5.1 of 2 per cent acetic acid for 24 hours and filtered.

The combined filtrates were made distinctly alkaline and heated to about 50°C. to flocculate a precipitate. After standing overnight the supernatant was decanted and the precipitate collected on a filter. After drying at room temperature the precipitate weighed 0.76 g. The dark brown precipitate, which contained large amounts of inorganic salts, was refluxed five times with 100-ml. portions of methanol, each time for one hour. During concentration of the green colored methanol extracts in a flash evaporator a compound crystallized in fine needle clusters, but adsorbed rapidly green impurities from the mother liquor. The crystalline precipitate was removed and dried at 45°C. to give 20 mg. of product. It was recrystallized from 60 per cent methanol with not much success in decolorizing.

The roots (350g.) were treated in the same way as just described for the tops. For amounts of extraction agent and yield of isolated compound see Table XIII.

2) For the second part of the experiment 13 plants were harvested, immediately placed in dry ice and preserved in deep freeze. At the time of glycoside extraction the plants were placed in boiling water for 10 minutes and then isolation procedure continued in the same way as above.

The various data concerned with these experiments are summarized in Table XIII.

TABLE XIII

DATA CONCERNING ATTEMPTED ISOLATION OF ALKALOID GLYCOSIDE

Number of Plants	Part of Plants	Stored in	Weight (Dehydrated)	1st Time Macerated with 5% Acet.Acid	2nd Time Macerated with 5% Acet.Acid	Yield of Crude "glycoside"	Yield of Crude "glycoside"
15	stems and leaves	6 l. abs. ethanol	1400 g.	14.0 l.	5.0 l.	0.76 g.	20 mg.
	roots	3 l. abs. ethanol	350 g.	3.5 l.	1.0 l.	1.28 g.	61 mg.
13	whole plants	deep freeze	2200 g.	14.0 l.	5.0 l.	12.5 g.	128 mg.

The three compounds isolated all melted in the board range of 175-195°C. which suggested impurities. Chromatography in the solvents mentioned in Table XIV showed that the three compounds all gave same alkaloid R_f-value as solanocapsine. If the compounds were chromatographed in 1-butanol-ethanol-water(80:20:20) and the spots detected with aniline hydrogen phthalate, only the compound isolated from the plant tops and the compound isolated from the frozen plants showed spots of D-glucose.

The result of these experiments indicate that it is not possible to isolate an alkaloid glycoside from S. pseudocapsicum by the conventional method used for isolating glycosides. Besides it suggests very strongly that solanocapsine probably does

not exist in the plant as a glycoside, but as a genuine alkaloid, since inactivation of a possible glycosidase did not yield a glycoside.

TABLE XIV

R_f-VALUES OF COMPOUNDS ISOLATED BY A STANDARD PROCEDURE FOR ALKALOIDAL GLYCOSIDES

Solvent	Solanocapsine	R_f-Value of: Compound Isolated from Tops	Compound Isolated from Roots	Compound Isolated from Whole Plant
1-butanol-ethanol-water (80:20:20) 1)	no spot	0.20	no spot	0.19
Upper phase of ethyl acetate-acetic acid-water (3:1:3) to which is added 15% of 85% ethanol 2)	0.57	0.57	0.56	0.57
1-butanol-ethanol-phosphate buffer (pH 8.2) (80:20:20) 2)	0.74	0.74	0.75	0.74
Ethanol-N HCl-water (50:2:48) 2)	0.73	0.74	0.74	0.73

1) Spots developed with aniline hydrogen phthalate reagent.
2) Spots developed with modified Dragendorff reagent.

6. Principle with Antibacterial Activity Against D. pneumoniae

In several instances during this study it was observed that the antibacterial activity against D. pneumoniae seemed to be associated with the colored impurities. Such examples were found in some of the fractions of an ethanolic extract

from roots chromatographed on alumina (page 41). Also in the purification of crude solanocapsine, the activity of solanocapsine against D. pneumoniae decreased as it was freed of colored impurities.

An attempt was made to isolate this principle by ether extraction of an ethanolic root extract made acidic to prevent the alkaloid fraction going over into the ether phase.

Twenty grams of roots were refluxed for four hours with 250 ml. of 80 per cent ethanol in a Soxhlet extractor. The obtained extract was concentrated to 50 ml., 10 ml. of acetic acid was added, and the solution was extracted twice with 200-ml. portions of ethyl ether. The combined ether fractions were evaporated to dryness in a flash evaporator to give a yield of 210 mg. of material. This residue was partly dissolved in 50 ml. of 80 per cent ethanol (solution I), and the remaining residue was dissolved in 50 ml. of absolute ethanol (solution II). The two preparations were then tested for antibacterial activity with results shown in Table XV.

Table XV indicates that another antibacterial principle besides solanocapsine is present, since a fraction was obtained which only showed activity against D. pneumoniae. The fairly high antibacterial activity of solutions I and II suggested that the other principle was in fact organophilic. This was tested by an attempt to isolate this principle by a direct ether extraction of the root powder.

TABLE XV

ANTIBACTERIAL ACTIVITY OF FRACTIONS DURING ISOLATION OF PRINCIPLE ACTIVE AGAINST D. PNEUMONIAE

Fraction	γ/ml. Inhibits Growth of: D. pneumoniae	M. tuberculosis
ethanolic root extract	720	255
solution I	11	inactive
solution II	22	inactive
water phase (mother liquor)	19	8

Four hundred grams of dry root powder was extracted with 1.6 l. of ethyl ether. An apparatus as described by Morton (64) for continuous extraction of a liquid with a heavier liquid was used. A plug of glass wool was inserted into the outlet tube of the column and it was then filled with the root powder, which could be continuously extracted with ether. By this process 550 ml. of a strongly yellow colored solution was obtained which, when tested for antibacterial activity, did not show any inhibiting effect against D. pneumoniae.

If the root powder was re-extracted with 80 per cent ethanol, a solution was obtained, which had high activity against both D. pneumoniae and M. tuberculosis. These activity results corresponded very closely to the antibacterial activities encountered during isolation of solanocapsine from the roots.

From this experiment it can be seen that although ethyl ether can extract the principle active against D. pneumoniae

from an ethanolic extract, it can not be extracted from the plant material directly. The significance of this observation has not been further examined.

7. Antitubercular Activity of Other Solanum Alkaloids

Since solanocapsine exhibited antitubercular activity it was decided to test alkaloids or their glycosides of other plants belonging to Solanaceae.

a. Tomatine. - Tomatine is an alkaloidal glycoside isolated from the leaves of the tomato plant Lycopersicon esculentum Mill. Ma and Fontaine (57) found it to have only very low activity against most bacteria, but higher activity against certain fungi. In this experiment it was tested against M. tuberculosis and found to be completely inactive.

b. Solanine and solanidine. - Solanine is an alkaloid glycoside which together with its aglycone solanidine is present in the potato plant, Solanum tuberosum L. The highest concentrations of solanine are found in potato sprouts (52). The concentration is especially high if the potato tubers have been allowed to sprout in daylight.

Isolation. - The isolation method for solanine was a modification of the conventional method for alkaloid glycosides from Solanaceae (93). Green potato sprouts (970 g.) was macerated with 2 l. of 2 per cent acetic acid in a Waring blender and allowed to stand for 48 hours. The suspension was then centrifuged in an International centrifuge (size 2) and the

supernatant was filtered with the aid of a little Hyflo super-cel. The remaining plant residue was re-extracted, this time with 750 ml. of 2 per cent acetic acid. After 24 hours the suspension was centrifuged and filtered as before, and the filtrate was combined with the first filtrate.

Concentrated ammonium hydroxide was added to the light brown filtrates (3.5 l.) until the ammonia odor remained and the turbidity of the solution was permanent. By heating the solution to 50-70°C. for a short time the crude alkaloid flocculated. After 24 hours the precipitate was collected on a filter, washed with 50 ml. of 5 per cent ammonium hydroxide, and dried over phosphorus pentoxide at 65°C. in an Abderhalden drying pistol. The yield of crude solanine and solanidine was 3.6 g. The product was greatly contaminated with inorganic salts.

Purification. - The separation of solanine from solanidine is based upon the fact that solanine is insoluble in ethyl ether, whereas the basic aglycone solanidine is soluble. Therefore, the 3.6 g. mixture of solanine and solanidine was extracted three times with 200-ml. portions of ethyl ether which were combined and dehydrated over anhydrous sodium sulfate. After evaporation to dryness the yield was 30 mg. of crude, slightly brownish colored solanidine. Upon recrystallization from acetone the compound melted at 204-207°C. (Lit. value 217°C. [93]). No further attempt was made to

purify solanidine. The compound was tested for antitubercular activity and found to be inactive.

The remaining residue of crude solanine was refluxed five times, each time for one hour, with 250 ml. of 80 per cent ethanol. The undissolved residue (1.3 g.) was tested for antitubercular activity and the combined ethanol fractions were concentrated to 250 ml. in a flash evaporator. During concentration the solanine crystallized. The yield of crude solanine was 280 mg. of melting point 255-260°C. After recrystallization from 80 per cent ethanol the compound melted at 278-280°C. (Lit. value 285-287°C. [93]). Without further purification the solanine was tested for antitubercular activity.

The results were that solanine showed weak antibacterial activity against D. pneumoniae (125 γ/ml.) and M. tuberculosis (25 γ/ml.). Solanine showed no antibacterial activity in the presence of blood. The insoluble residue obtained above did not show any inhibitory effect.

c. Crude alkaloid preparation from S. carolinense. - Solanum carolinense has been recorded as containing solanine (90). This information is in early literature and requires confirmation since the identification was superficial and therefore a possibility of other alkaloidal glycosides can not be excluded.

A 1000 g. sample of dried and ground (20 mesh) leaves and stems was macerated for 48 hours in a large glass

cylindrical jar with 30 l. of water and 600 ml. of glacial acetic acid. The plant residue was removed by filtration, the filtrate was made ammoniacal until the odor persisted and the turbidity of the solution was permanent. Heating the solution for a short time at 50-70°C. flocculated the crude alkaloid. After 24 hours the precipitate was collected on a filter, washed with 200 ml. of 5 per cent ammonium hydroxide, and dried at room temperature to give a yield of 11 g. of crude alkaloid base. Both the mother liquor and the crude alkaloid were tested for antitubercular activity. The mother liquor which contained 32 mg. of dry matter inhibited growth of M. tuberculosis in a dilution of 1:5, whereas the crude alkaloid product was completely inactive. In view of this result no further attempt to purify the alkaloid was made.

The results obtained from the testing of the above mentioned alkaloids are summarized in Table XVI.

TABLE XVI

ANTIBACTERIAL ACTIVITY OF DIFFERENT *SOLANUM* ALKALOIDS

Alkaloid	γ/ml. Inhibits the Growth of: *D. pneumoniae*	*M. tuberculosis*	
		On Agar	On Blood Agar
Tomatine	inactive	inactive	inactive
Solanine	125	25	inactive
Solanidine	inactive	inactive	inactive
Alkaloid preparation from *S. carolinense*	inactive	inactive	inactive

CHEMICAL STUDIES ON SOLANOCAPSINE

8. Physical Constants

a. Solanocapsine. - Five hundred milligrams of solanocapsine dihydrochloride was dissolved in 10 ml. of ethanol-water (1:1). By addition of 6 N ammonium hydroxide solanocapsine precipitated. The yield of crude solanocapsine was 370 mg. After recrystallization from ethanol-water (1:1) it crystallized as platelets which melted with decomposition at 215-216°C. (Lit. value 216-217°C. [78], 222°C. [6]).

For analysis the compound was dried 48 hours at 17 mm. pressure over phosphorus pentoxide and paraffin at room temperature.

Anal. Calc'd for $C_{27}H_{46}N_2O_2 \cdot H_2O$: N, 6.24 %

Found: N, 6.20, 6.29 % (Kjeldahl)

Determination of water crystallization: The compound was dried in an Abderhalden drying pistol for 72 hours at 2 mm. pressure over phosphorus pentoxide. The pistol was heated by refluxing 1-butanol (b.p. 117-118°C.). The compound (642.3 mg.) lost 25.6 mg. by drying, which corresponds to 3.98 per cent (calculated for 1 mole of water 4.01 per cent). On exposure to air the anhydrous substance regained its original weight.

Optical rotation: A solution of 0.206 mg. in 5 ml. of methanol showed a rotation of + 2.061° in a 20 cm. tube. Calculated specific rotation $[\alpha]_D^{25} = + 25.01°$.

Rast's molecular weight determination: 11.3 mg. in 194.2 mg. camphor depressed the melting point 5.1°C., corresponding to a molecular weight of 456. Seventeen and four-tenths milligrams in 19.3 mg. camphor depressed the melting point 7.6°C., corresponding to a molecular weight of 481.

Neutralization equivalent: Solanocapsine (63.5 mg.) dissolved in 10 ml. ethanol and 40 ml. 0.0102 N hydrochloric acid consumed 12.05 ml. 0.0120 N sodium hydroxide corresponding to a molecular weight of 482.

Determination of methyl groups attached to carbon: Solanocapsine (8.6 mg.) used after oxidation and titration 5.74 ml. 0.0103 N sodium hydroxide. Solanocapsine (7.1 mg.) used after oxidation and distillation 4.80 ml. 0.013 N sodium hydroxide (oxidation time was six hours).

Calculated for 3 methyl croups: 10.05 %

Found: 10.28, 10.42 %

Oppenauer oxidation following the procedure later described did not oxidize the hydroxyl group. Solanocapsine was recovered and identified through melting point and infrared spectrum.

One milligram in methanol showed no absorption in the ultraviolet part of the spectrum.

No unsaturation was detected with potassium permanganate (80) or bromine (81).

Solanocapsine (10 mg.) dissolved in 10 ml. of ethanol and mixed with 10 ml. of a 1 per cent digitonin solution in

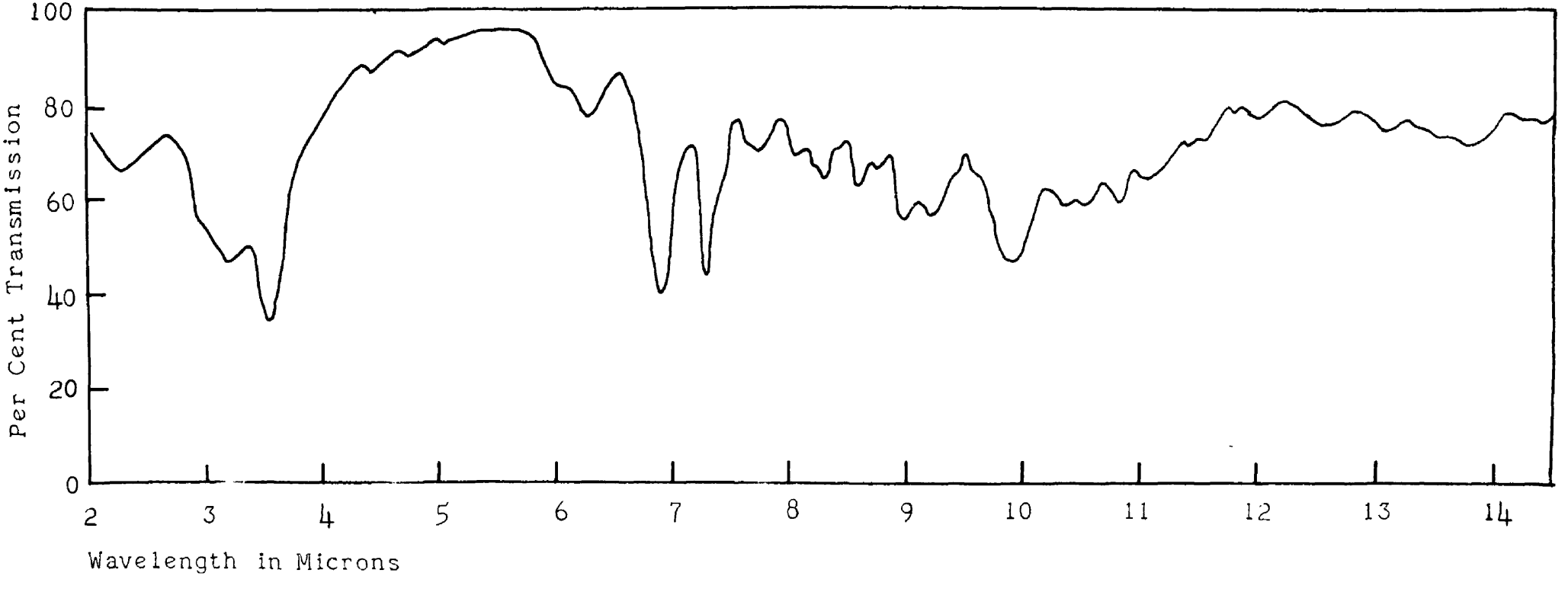

Figure 5. Infrared Spectrum of Solanocapsine (Nujol Mull)

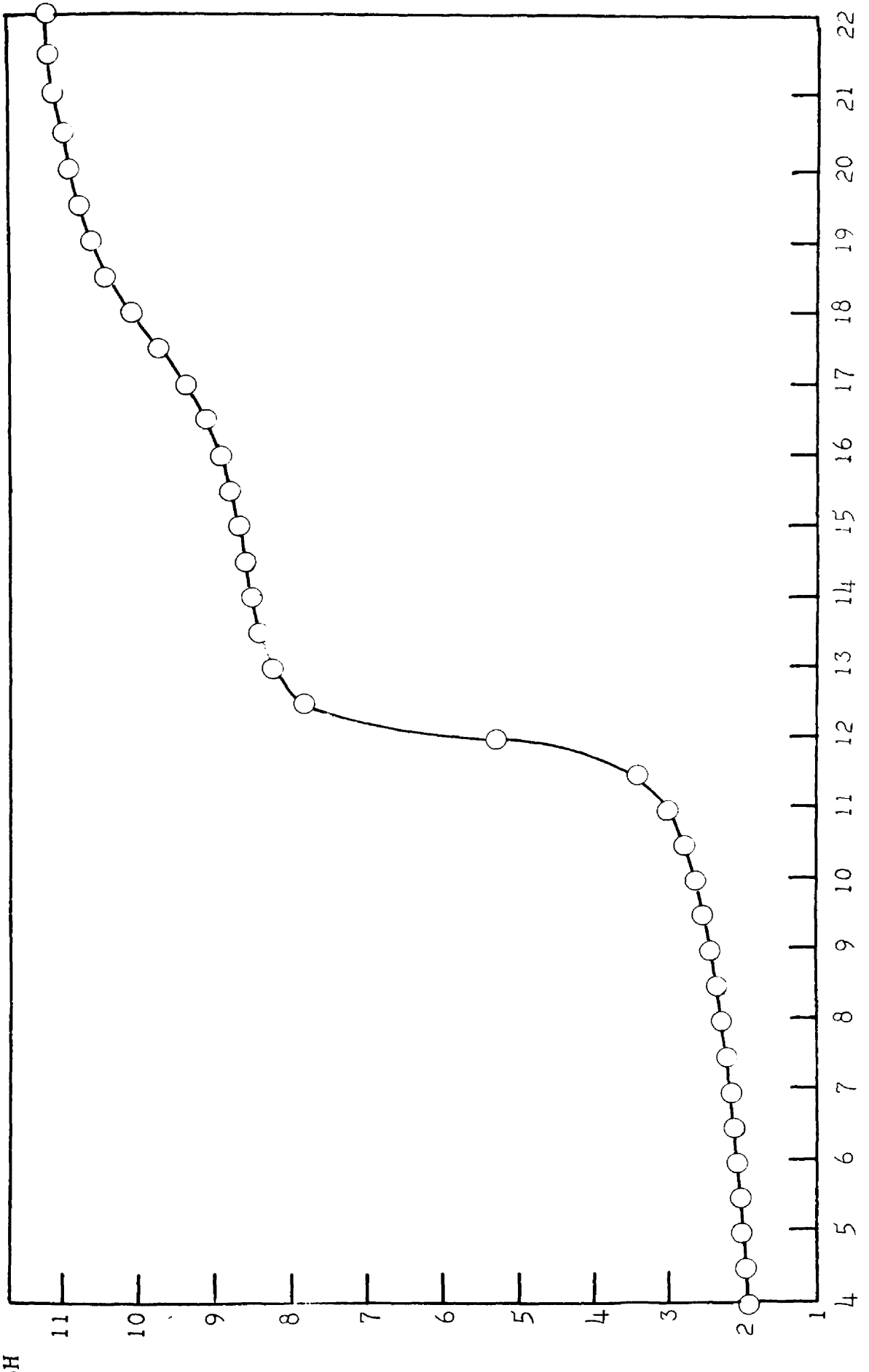

Figure 6. Titration Curve of Solanocapsine.

(63.5 mg. of solanocapsine dissolved in 10 ml. ethanol and 40 ml. 0.01020 N HCl)

90 per cent ethanol remained clear, even after 10 hours of standing.

No consumption of periodic acid was observed, even after 10 hours. The oxidation was attempted at 3 different hydrogen ion concentrations:

pH 2: Solanocapsine (20 mg.) dissolved in 10 ml. ethanol and 10 ml. 0.0216 molar periodic acid.

pH 5: Solanocapsine (20 mg.) dissolved in 10 ml. ethanol, 2 ml. pH 8 buffer, and 10 ml. 0.0216 molar periodic acid.

pH 8: Solanocapsine (14 mg.) dissolved in 10 ml. ethanol, 6 ml. pH 9 buffer and 7 ml. 0.0216 molar periodic acid.

b. Solanocapsine dihydrochloride. - Two hundred milligrams of crude solanocapsine hydrochloride was recrystallized twice from water. The yield of pure solanocapsine dihydrochloride was 105 mg. and did not melt below 300°C. (Lit. value >300°C. [78], 324° [6]).

Before analysis the compound was dried for 48 hours at 17 mm. pressure over phosphorus pentoxide and paraffin at room temperature.

Anal. Calc'd for $C_{27}H_{46}N_2O_2 \cdot 2HCl \cdot H_2O$: C, 62.17; H, 9.66; N, 5.37 %
Found: C, 62.27; H, 9.40; N, 5.59 %

Determination of water crystallization: A sample of the compound was dried in an Abderhalden drying pistol for 72 hours at 2mm. pressure over phosphorous pentoxide. The pistol was heated by refluxing 1-butanol (b.p. 117-118°C.). The compound (576.8 mg.) lost 19.4 mg. by drying, corresponding to 3.36 per

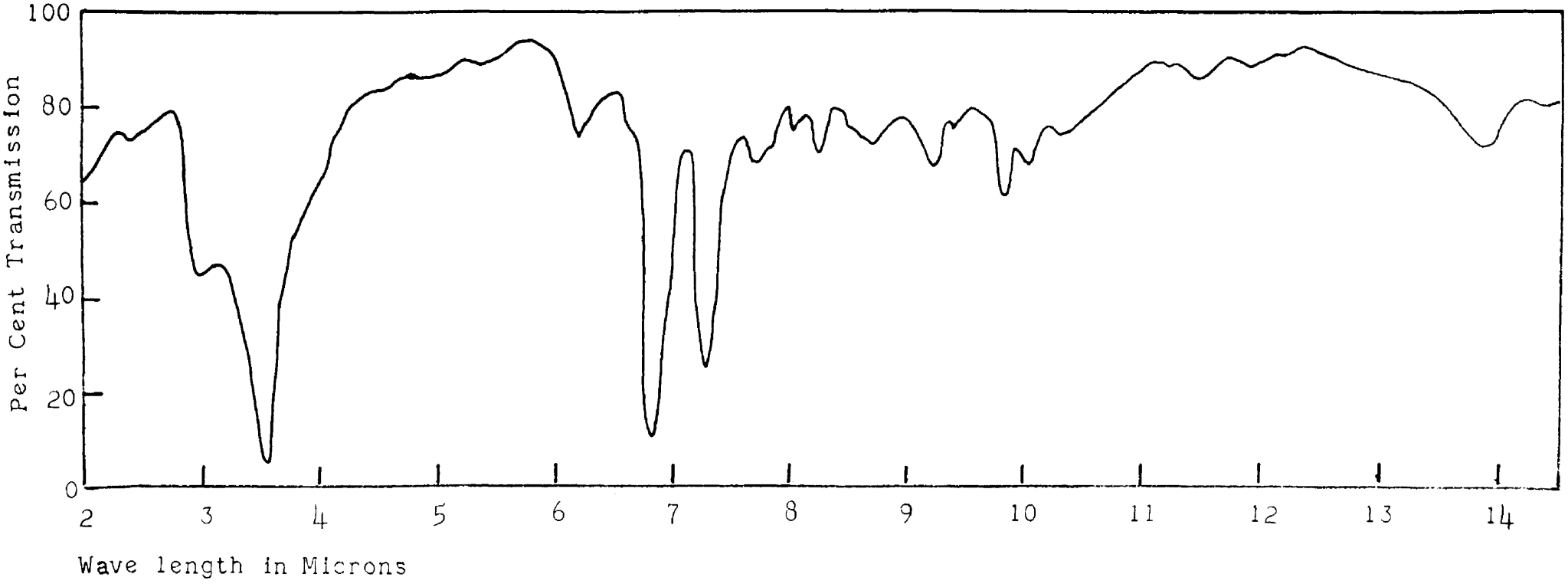

Figure 7. Infrared Spectrum of Solanocapsine Dihydrochloride (Nujol Mull)

cent (calculated for 1 mole of water 3.36 per cent). On exposure to air the anhydrous substance regained its original weight.

As with solanocapsine no absorption in the ultraviolet part of the spectrum was observed.

9. Reaction with Nitrous Acid

a. Nitrososolanocapsine. - This derivative was prepared following the procedure of Barger and Fraenkel-Conrat (6). When a solution of 110 mg. of solanocapsine in 6 N acetic acid was treated with 1 equivalent of sodium nitrite (16.9 mg.), only a slight precipitate was formed. But addition of a second equivalent of sodium nitrite produced a copious precipitate. The yield of compound was 80 mg. Recrystallized from absolute ethanol it melted at 194-196°C. (Lit. value 194°C. [6], 200°C. [78]).

The ultraviolet spectrum of 0.25 mg. nitrososolanocapsine/ml. methanol showed a maximum at 355 mμ (log E 2.42).

Unsaturation could be detected with bromine in glacial acetic acid (81).

Microhydrogenation: One and one-half milligram of the compound should theoretically absorb 75 μl. of hydrogen (1 double bond). It was found that 71 μl. was absorbed.

Van Slyke amino nitrogen determination: Nitrososolanocapsine (21.3 mg.) in 7 ml. of 6 N acetic acid gave evolution of 1.300 mg. nitrogen, corresponding to 0.99 mole of nitrogen.

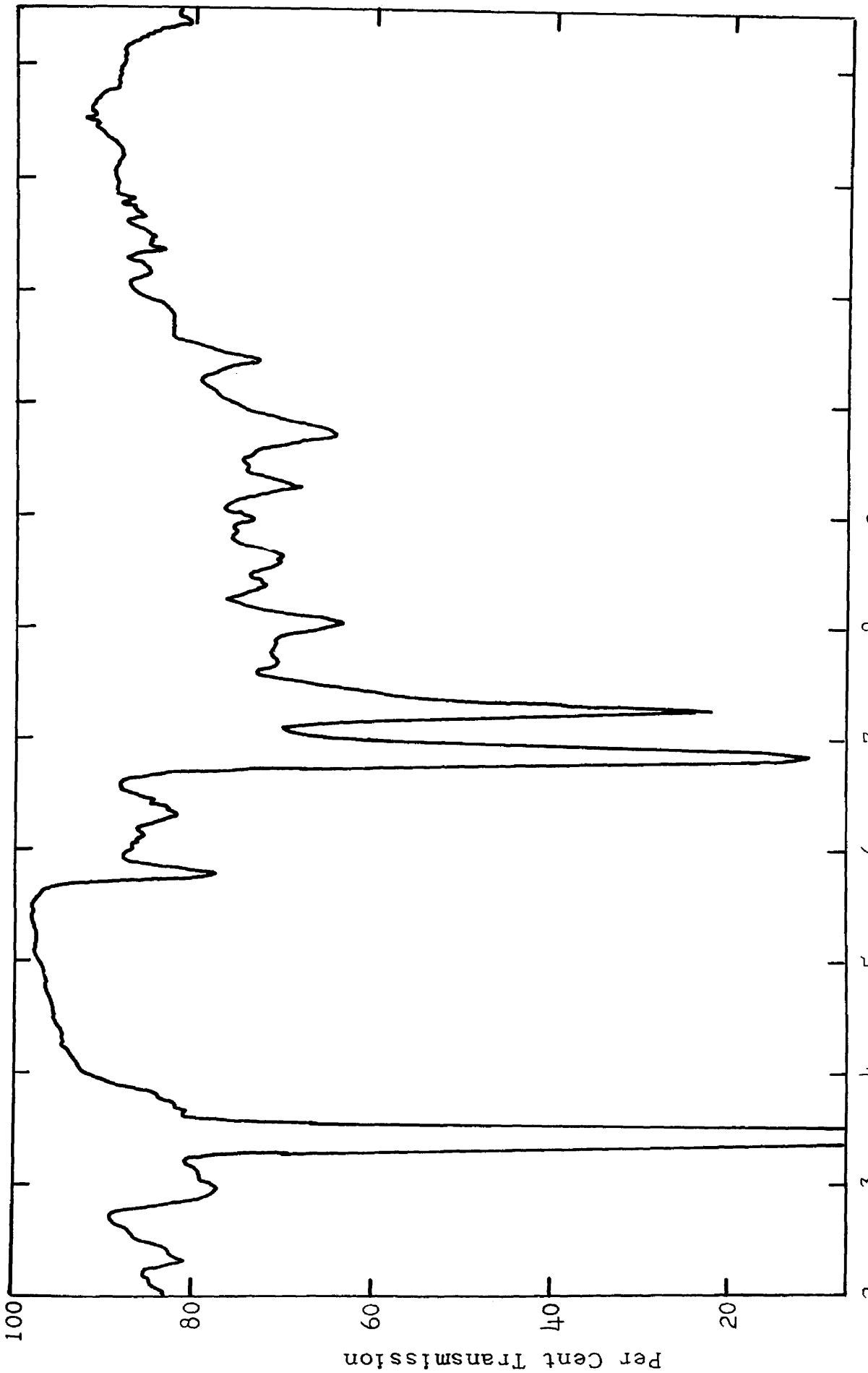

Figure 8. Infrared Spectrum of Nitrososolonocapsine (Nujol Mull)

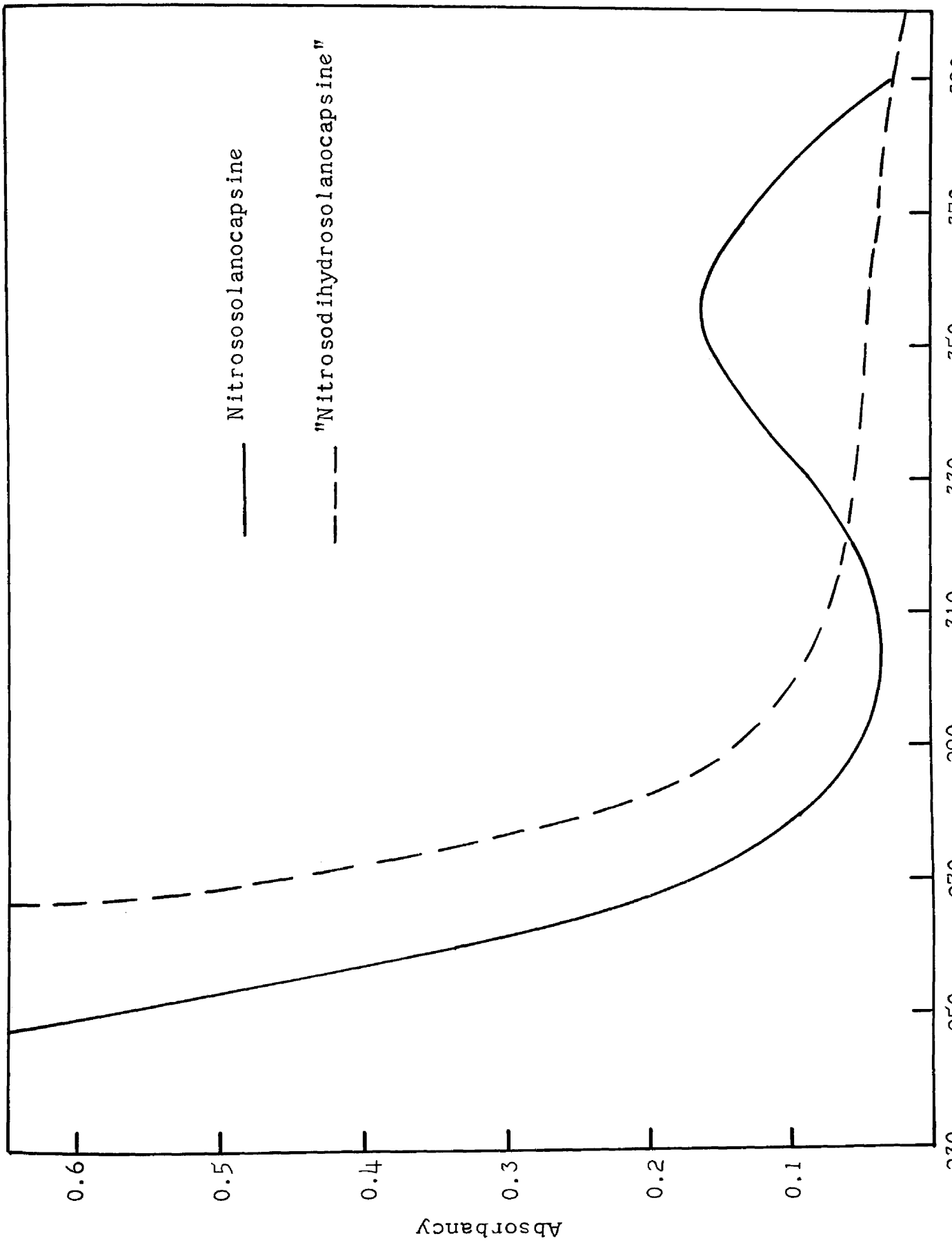

Figure 9. Ultraviolet Spectra of Nitrososolanocapsine and "Nitrosodihydrosolanocapsine."

b. Digitonide of nitrososolanocapsine. - To a solution of 70 mg. nitrososolanocapsine in 20 ml. ethanol was mixed 20 ml. of a 1 per cent digitonin solution in 80 per cent ethanol. After standing overnight the copious precipitate was filtered, washed with water and dried to give 45 mg. of digitonide. The derivative was washed with 5 ml. of ethanol and dried for 48 hours at 17 mm. pressure over phosphorous pentoxide.

Anal. Calc'd for $C_{83}H_{134}N_2O_{32}$: N, 168 %

Found: N, 182, 1.90 % (Kjeldahl)

c. Dihydrosolanocapsine. - A mixture of 200 mg. solanocapsine and 200 mg. of platinum oxide in 10 ml. of glacial acetic acid and 5 ml. of ethanol was shaken under 3 atmospheres of pressure for 12 hours in an Adams hydrogenation apparatus.

After filtration of the catalyst, the solvent was removed by distillation under diminished pressure in a flash evaporator. The residue was dissolved in ethanol-water (1:1) and 6 N ammonium hydroxide was added for precipitation to give a yield of 170 mg. of the compound. It was recrystallized from ethanol-water (1:1), melted at 210-212°C. and a mixed melting point with solanocapsine did not show any depression.

Optical rotation: A solution of 0.112 mg. of the compound in 5 ml. of methanol showed a rotation of 1.792°. Calculated specific rotation $[\alpha]_D^{25} = +40°$.

Van Slyke amino nitrogen determination: Dihydrosolanocapsine (6.7 mg.) in 7 ml. of 6 N acetic acid gave evolution

of 0.791 mg. of nitrogen, corresponding to 1.97 mole of nitrogen.

Active hydrogen determination: 0.42 per cent active hydrogen was found at room temperature and 0.84 per cent at 100°C. Recalculated to numbers of active hydrogens the result was 3.64 active hydrogens.

d. "Nitrosodihydrosolanocapsine." - Dihydrosolanocapsine (100 mg.) was treated with nitrous acid in the same manner as described under nitrososolanocapsine and yielded 82 mg. After recrystallization from absolute ethanol the compound metled at 165-168°C.

For analysis the derivative was dried 48 hours at 17 mm. pressure over phosphorus pentoxide and paraffin at room temperature.

Anal. Calc'd for $C_{27}H_{44}O_3$: C, 77.84; H, 10.64 %
Found: C, 68.67; H, 9.38 %
Calc'd for $C_{25}H_{40}O_6$: C, 68.77; H, 9.24 %

A solution of 0.20 mg. compound/ml. methanol showed only endabsorption in the ultraviolet part of the spectrum.

Sodium fusion following the microprocedure of Schneider (79a) did not detect any nitrogen in the compound.

Unsaturation could be detected with bromine in glacial acetic acid (81).

Microhydrogenation: One and seven-tenths of the compound should theoretically absorb 93 µl of hydrogen (1 double bond). It was found that 109 µl was absorbed.

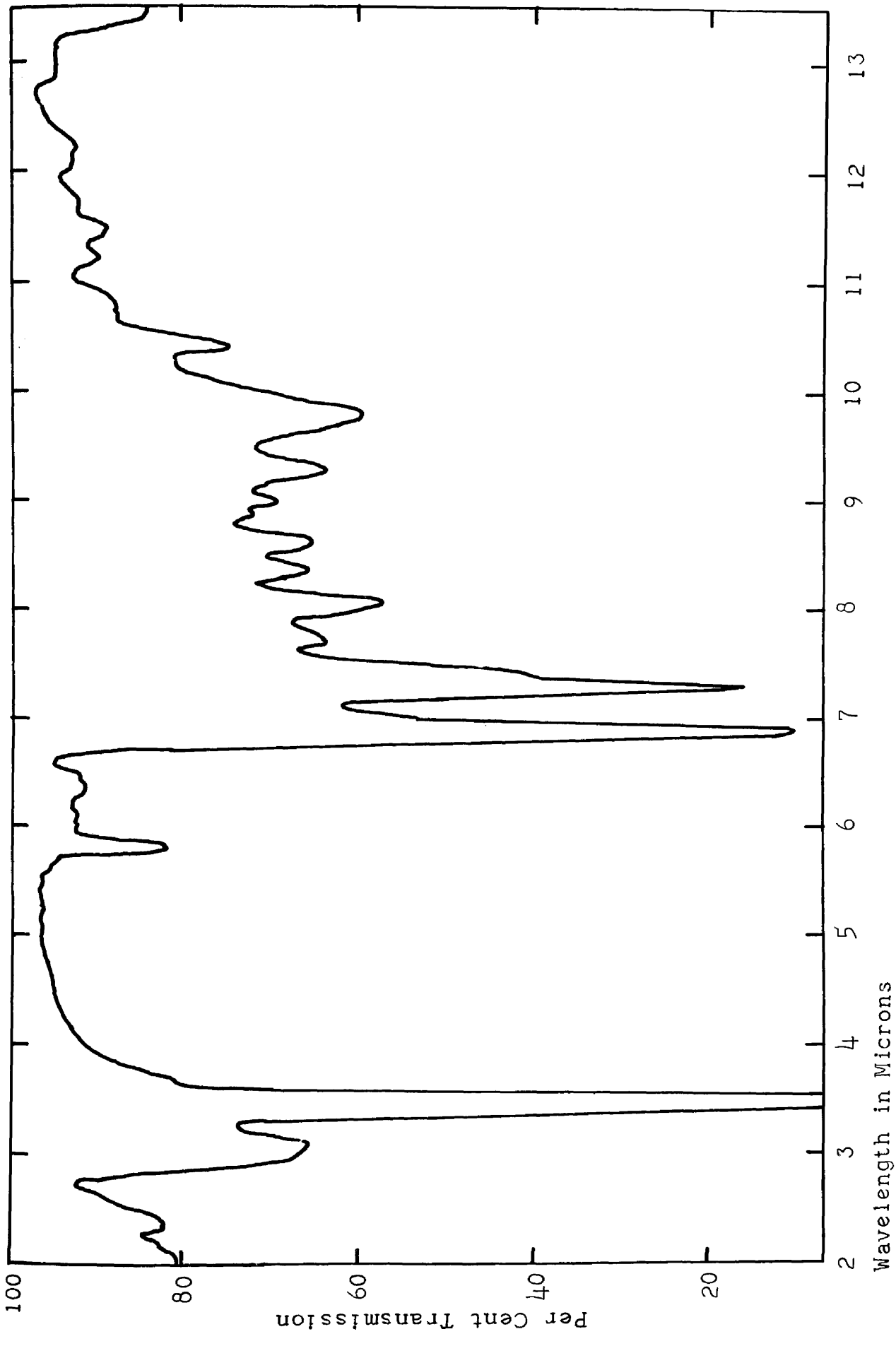

Figure 10. Infrared Spectrum of "Nitrosodihydrosolanocapsine" (Nujol Mull).

A solution of 10 mg. of the compound in 10 ml. of ethanol gave with 10 ml. of a 1 per cent digitonin solution in 90 per cent ethanol a copious precipitate.

10. Acetylation Studies

a. Diacetylsolanocapsine. - The compound was prepared using the procedure of Barger and Fraenkel-Conrat (6). Solanocapsine (150 mg.) was dissolved in 4 ml. of pyridine and allowed to stand with 5 ml. of acetic anhydride for 48 hours. The yellow solution was evaporated to dryness under diminished pressure at 50°C. The brown residue, after dissolving in 20 ml. of chloroform, was extracted four times with 10-ml. portions of 10 per cent sodium bicarbonate solution and washed neutral with water. After drying over anhydrous sodium sulfate and evaporation of the chloroform solution 135 mg. of gelatinous precipitate was produced. This acetylated alkaloid was dissolved in 5 ml. of benzene and chromatographed on 8.7 g. of alumina. Eluate fractions of 20 ml. were collected. The composition of the fractions was as follows in Table XVII.

Fractions 10-13 were combined and reprecipitated three times from methanol-ethyl ether (1:5). It was not possible to obtain a crystalline product. The amorphous, gelatinous precipiate after drying melted at 198-200°C. (Lit. value 193-196°C. [78]).

For analysis the derivative was dried at 17 mm. pressure over phosphorus pentoxide and paraffin at room temperature.

TABLE XVII

FRACTIONATION OF DIACETYL SOLANOCAPSINE BY COLUMN CHROMATOGRAPHY ON ALUMINA

Fraction	Eluant	Residue	
1-3	benzene	9 mg.	brown oil, soluble in ethyl ether
4-5	benzene + 5% chloroform	7 mg.	colorless oil, soluble in ether
6-7	benzene + 10% chloroform	9 mg.	colorless oil, soluble in ether
8-9	benzene + 25% chloroform	5 mg.	colorless oil, soluble in ether
10-13	chloroform + 1% methanol	79 mg.	white foam, insoluble in ether
14-16	chloroform + 2% methanol	6 mg.	greenish oil, insoluble in ether

Anal. Calc'd for $C_{31}H_{50}N_2O_4 \cdot H_2O$: C, 70.77; H, 9.62 %

Found: C, 69.90; H, 9.84 %

Determination of equivalents of acetyl groups:

1.3 equivalents of O,N-acetyl*) (6.5 mg. consumed after hydrolysis for 1.5 hour and distillation 1.56 ml. of 0.0103 N sodium hydroxide).

1.6 equivalents of O,N-acetyl*) (5.4 mg. consumed after hydrolysis for five hours and distillation 1.63 ml. of 0.0103 N sodium hydroxide).

*) The calculations are based on a diacetylsolanocapsine.

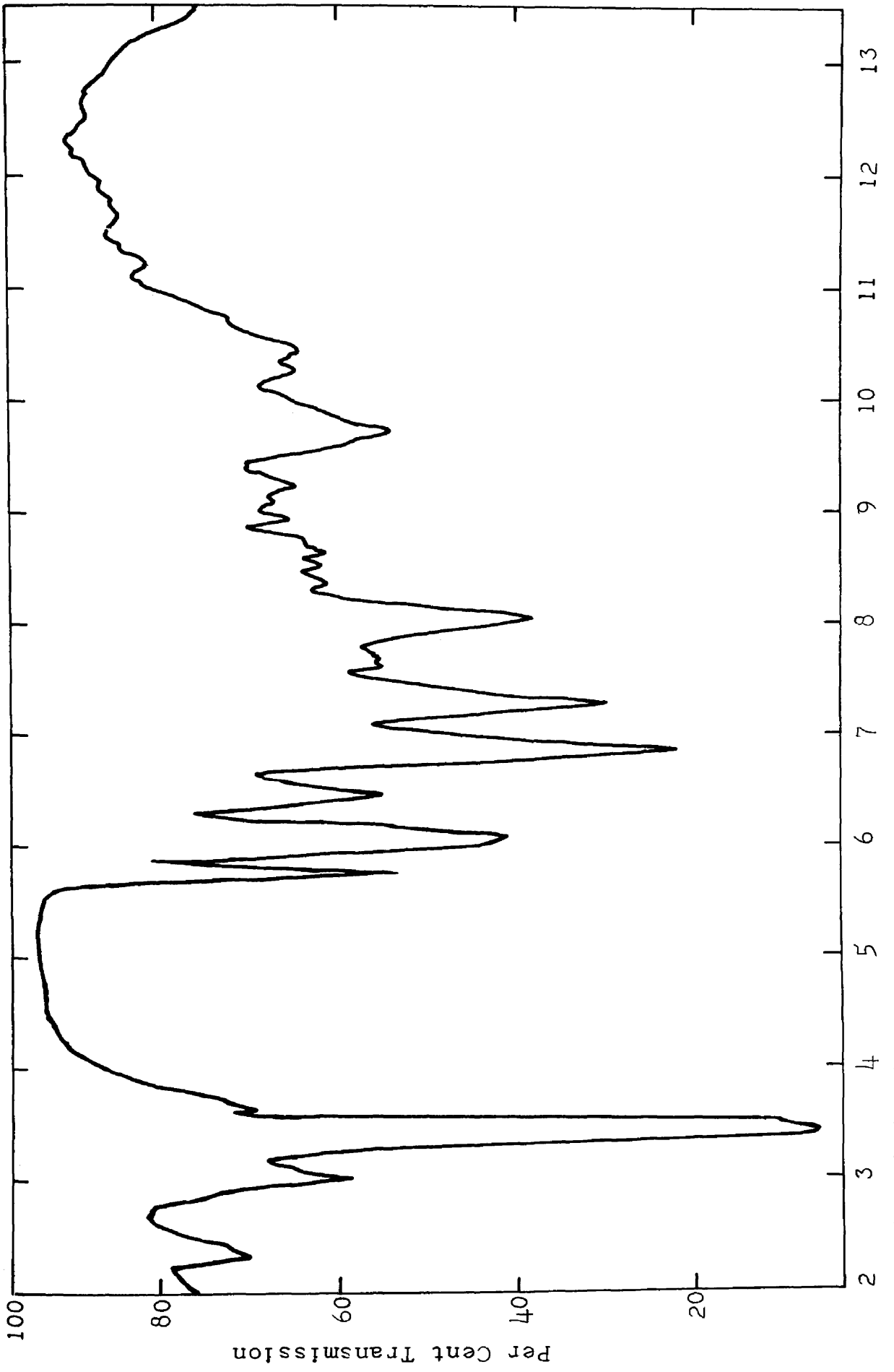

Figure 11. Infrared Spectrum of O,N - Diacetylsolanocapsine (Nujol Mull).

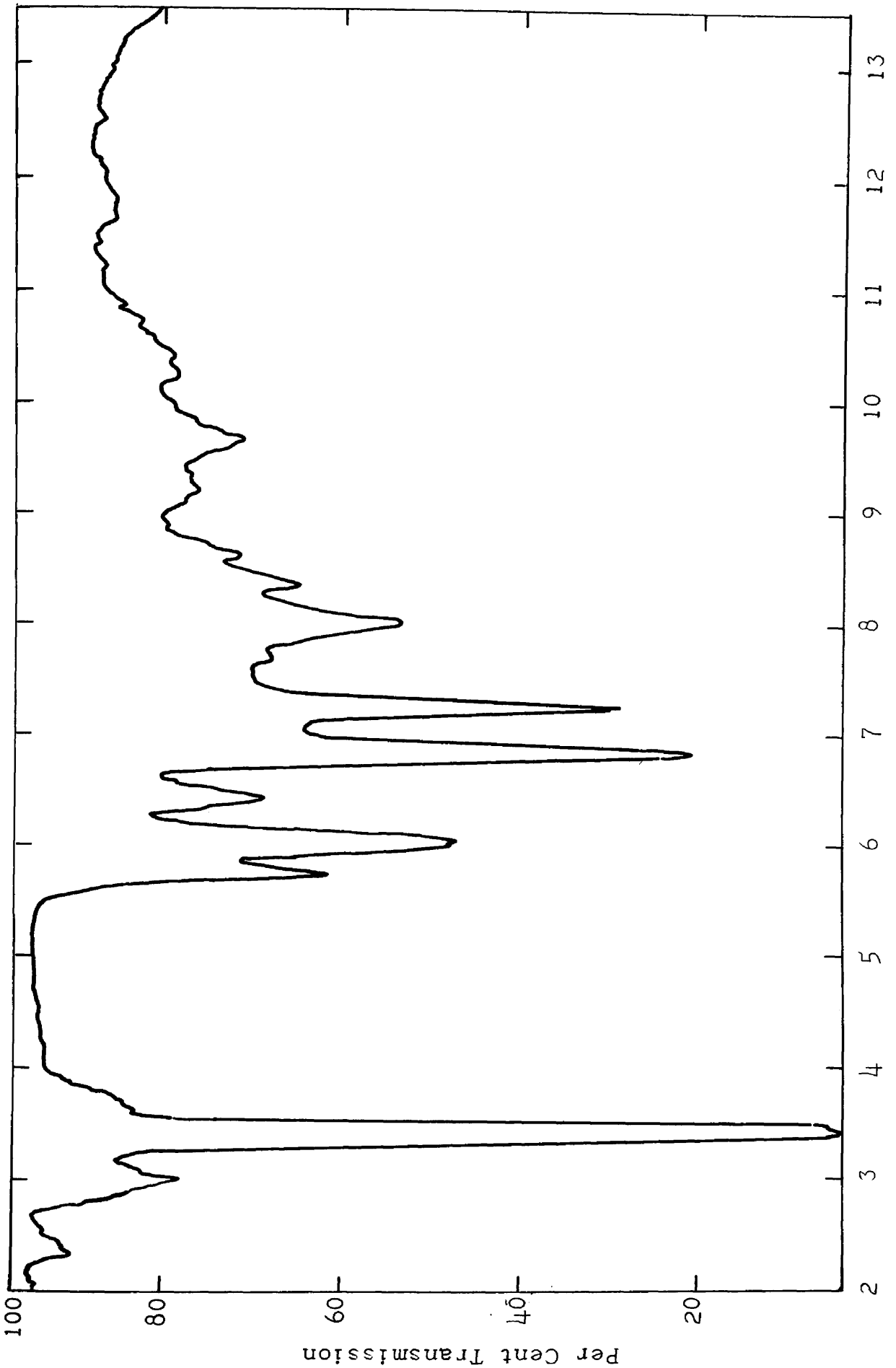

Figure 12. Infrared Spectrum of O,N,N' - TriacetylIsolanocapsine (Nujol Mull).

0.9 equivalents of O-acetyl*) (5.7 mg. consumed after hydrolysis and titration 0.91 ml. of 0.0103 N sodium hydroxide).

Diacetylsolanocapsine was soluble in 6 N acetic acid.

A solution of 0.20 mg. of the compound/ml. of methanol showed no absorption in the ultraviolet part of the spectrum.

b. Triacetylsolanocapsine. - Barger and Fraenkel-Conrat's procedure (6) was followed. A mixture of 500 mg. of solanocapsine and 10 ml. of acetic anhydride was refluxed in an oil bath for five hours. The solvent was removed under diminished pressure and the brown, oily residue dissolved in ethanol. Water was added to slight turbidity and the compound allowed to crystallize. Thus a yield of 400 mg. of product melting at 140-150°C. was obtained. The compound was amorphous and even repeated solution in hot benzene-ligroin did not change it to a crystalline compound. The melting point of the purified compound was 150-155°C. (Lit. value 150-160°C. [6]).

A further attempt was made to convert the amorphous material into a crystalline compound by column chromatography on alumina. A solution of 83 mg. of acetylated solanocapsine in 5 ml. of benzene was chromatographed on 9 g. of alumina

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*) The calculations are based on a diacetylsolanocapsine.

and eluate fractions of 20 ml. were collected. The composition of the fractions was as follows in Table XVIII.

TABLE XVIII

FRACTIONATION OF TRIACETYL SOLANOCAPSINE BY COLUMN CHROMATOGRAPHY ON ALUMINA

Fraction	Eluant	Residue
1-3	benzene	5 mg. brownish oil, soluble in ethyl ether
4-5	benzene + 5% chloroform	4 mg. colorless oil, soluble in ether
6-7	benzene + 10% chloroform	6 mg. colorless oil, soluble in ether
8-9	benzene + 25% chloroform	4 mg. colorless oil, soluble in ether
10-13	chloroform + 1% methanol	56 mg. white foam, insoluble in ether
14-16	chloroform + 2% methanol	5 mg. greenish oil, insoluble in ether

Fractions 10-13 were combined and after evaporation of solvent gave an amorphous product still melting at 150-155°C.

For analysis the expected triacetyl derivative was dried at 17 mm. pressure over phosphorus pentoxide and paraffin at room temperature.

Anal. Calc'd for $C_{33}H_{52}N_2O_5$: C, 71.17; H, 942 %

Found: C, 71.61; H, 949 %

Determination of equivalents of acetyl groups:

2.8 equivalents of O,N-acetyl*) (4.3 mg. consumed after hydrolysis for eight hours and distillation 2.11 ml. of 0.0103 N sodium hydroxide).

Triacetylsolanocapsine was found insoluble in 6 N acetic acid.

A solution of 0.20 mg. of the compound/ml. methanol showed only endabsorption in the ultraviolet part of the spectrum.

11. Miscellaneous Derivatives

a. Trimethylsolanocapsine. - The derivative was prepared following the procedure of Schlittler and Uehlinger (78). Solanocapsine (150 mg.) was refluxed for four hours on a water bath with 0.7 ml. of 40 per cent formaldehyde and 0.8 ml. of 100 per cent formic acid. Evolution of gas soon began in the yellow colored oil. The reaction product was diluted with 5 ml. of water and 2 ml. of N hydrochloric acid. The acidic solution was first extracted four times with 10 ml. of ethyl ether, then made distinct ammoniacal with dilute ammonium hydroxide and exhaustively extracted with ether. The latter ether extracts were combined, dried over anhydrous sodium sulfate and evaporated to dryness. The residue was crystalline and weighed 140 mg. Recrystallized twice from absolute methanol, the compound melted at 208°C. (Lit. value 209°C. [78]).

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*) The calculation is based on a triacetylsolanocapsine.

For analysis the expected trimethylsolanocapsine was dried over phosphorus pentoxide and paraffin at 17 mm. pressure.

Anal. Calc'd for $C_{30}H_{52}N_2O_2$: N, 5.93 %

N, 586, 572 % (Kjeldahl)

b. Solanocapsine picrate. - The picrate was prepared as described by Schlittler and Uehlinger (78). Solanocapsine (100 mg.) was dissolved in 3 ml. of ethanol. A small excess of saturated ethanolic picric acid (1.25 ml.) was added. The solution was heated to the boiling point and 5 ml. of boiling water was added. By slowly cooling, fine needles crystallized. After standing 12 hours in the refrigerator, 166 mg. of crude picrate was obtained. The derivative, recrystallized from ethanol-water (1:1) and dried, gave a melting point of 198-199°C. (Lit. value 200-201°C. [78]).

c. Solanocapsine oxalate. - Again the procedure of Schlittler and Uehlinger (78) was followed. A solution of 40 mg. solanocapsine in 3 ml. of absolute ethanol was reacted dropwise with 10 per cent oxalic acid in absolute ethanol. When the calculated equimolar amount of exalic acid was added, a copious precipitate appeared. The derivative was thoroughly washed with absolute ethanol and gave after drying 42 mg. of the derivative. Its melting point was 287-289°C. (Lit. value 228-289°C. [78]).

d. Isopropylidenesolanocapsine. - The compound was made following the procedure of Barger and Fraenkel-Conrat (6).

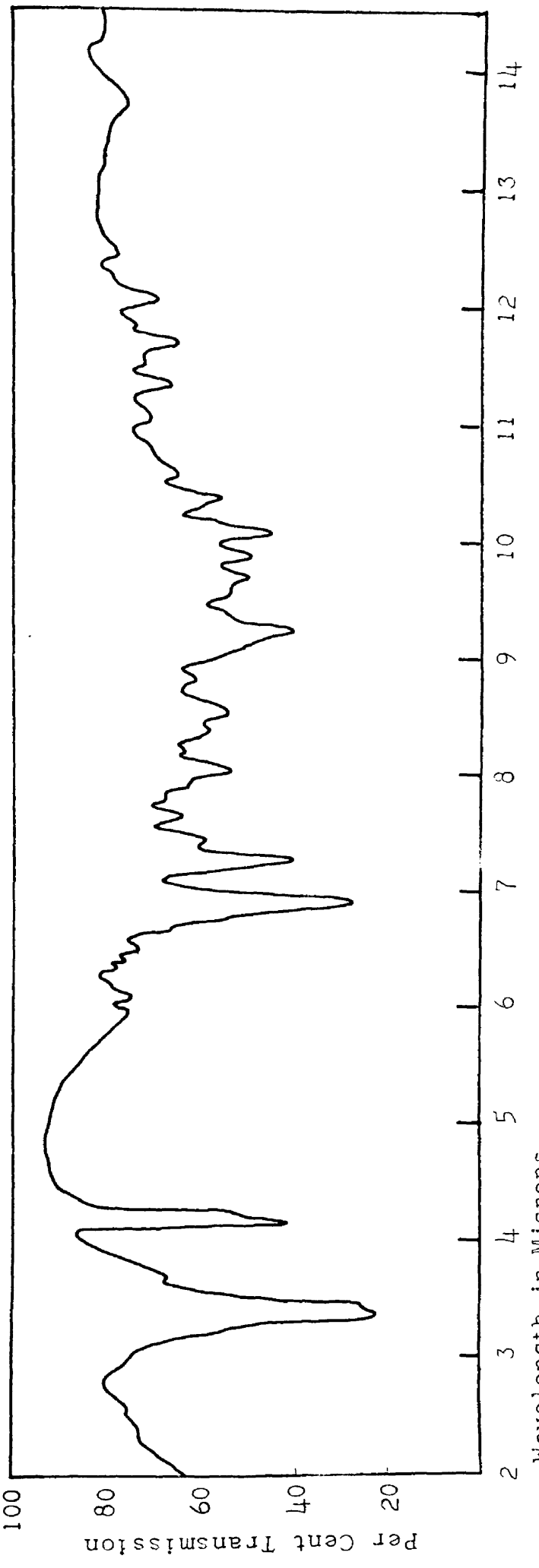


Figure 13. Infrared Spectrum of Trimethylsolanocapsine (Nujol Mull).

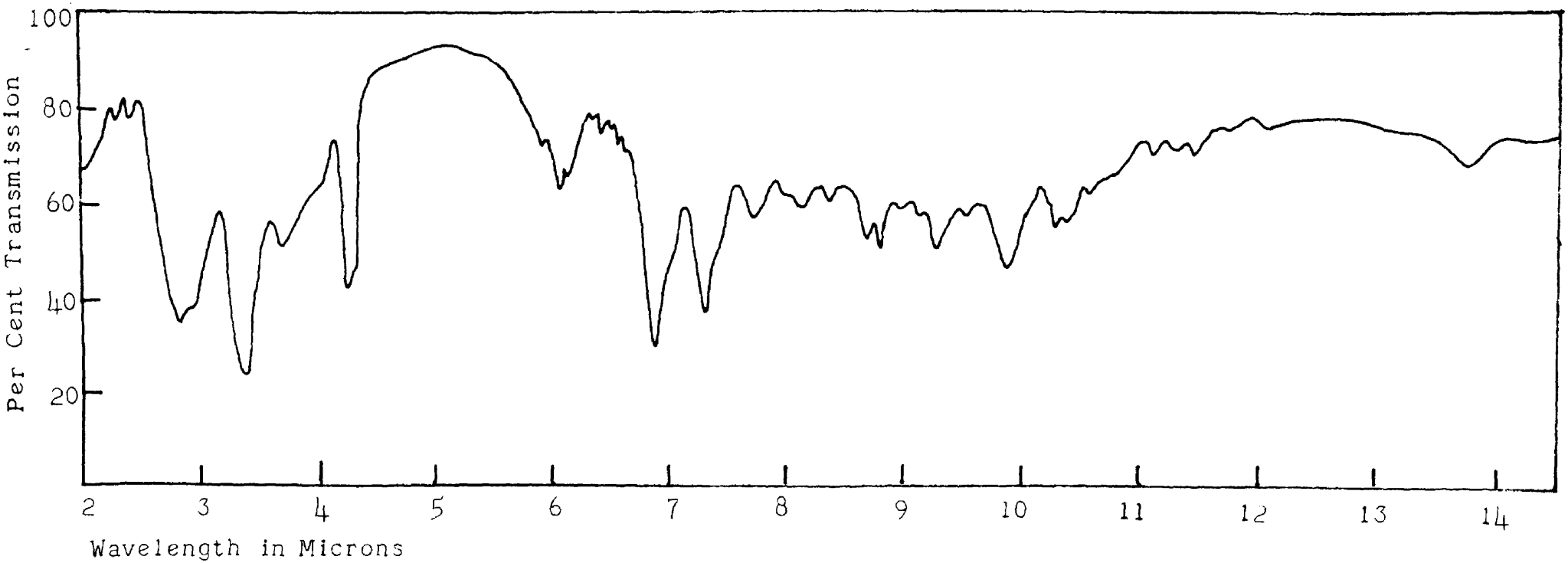


Figure 14. Infrared Spectrum of Trimethylsolanocapsine Hydrochloride (Nujal Mull).

Solanocapsine (70mg.) and 10 ml. of acetone was heated on a water bath for a few minutes until crystals began to appear. After standing for eight hours in the refrigerator, the crystals were separated by centrifugation and yielded 67 mg. of product. Recrystallized from ethyl acetate-acetone, the compound melted at 232-233°C. (Lit. value 233°C. [6]).

The stability of the complex was examined by shaking the compound with water or with hydrochloric acid and then testing for acetone:

1) Ten milligrams of the compound was stirred with 0.1 ml. of water in a centrifuge tube for a few minutes. The suspension was centrifuged, and the supernatant drawn off with a micropipet. The supernatant gave a negative iodoform test.

2) Ten milligrams of the compound was stirred with 0.1 ml. of 2 N hydrochloric acid for a few minutes. Three-tenths of a milliliter of 10 per cent sodium hydroxide solution was added and the content was centrifuged. The supernatant was tested for acetone. The iodoform test was positive.

e. Attempted tosylation of solanocapsine. - A solution of 100 mg. solanocapsine in 2.5 ml. of pyridine was treated with 100 mg. of p-toluene sulfonyl chloride. After standing 12 hours, 5 ml. of water was added and the resulting precipitate was washed with much water. The product was recrystallized from ethanol-water (1:1). Only solanocapsine was received as evidenced by mixed melting point and infrared spectrum of the compound.

f. Attempted dehydration of solanocapsine. -

By acid catalysis. - A solution of 50 mg. solanocapsine in 15 ml. concentrated hydrochloric acid and 35 ml. ethanol was refluxed for 10 hours. After 50 ml. of water was added, the solution was made distinctly ammoniacal and then extracted four times with 100-ml. portions of ethyl ether. The combined ether fractions were dried over anhydrous sodium sulfate and evaporated to dryness. The residue was recrystallized from ethanol-water (1:1). Only solanocapsine was recovered as evidenced by mixed melting point and infrared spectrum of the compound.

By base catalysis. - Dehydration in basic solution by the procedure of Barger and Fraenkel-Conrat (6) was as follows: A solution of 60 mg. of solanocapsine dihydrochloride in 5 ml. of 10 per cent methanolic potassium hydroxide was heated at 100°C. for four hours. Upon addition of water, extraction with ethyl ether, drying and removal of solvent, an amorphous residue was obtained. The yield of product was 36 mg. Recrystallized from ethanol-water (1:1) the product was found by melting point and infrared spectrum to be solanocapsine.

The recovered solanocapsine gave a condensation product with acetone, which is contrary to the compound thus obtained by Barger and Fraenkel-Conrat (6).

12. Oxidation Studies

a. Oppenauer oxidation of nitrososolanocapsine. - The oxidation was performed following the procedure of Prelog and

Szpilfogel (71). Nitrososolanocapsine (100 mg.) was refluxed on steam bath with 8 ml. of anhydrous acetone, 20 ml. of absolute benzene, and 1 g. of aluminum phenoxide for 24 hours. The condenser was equipped with drying tube containing anhydrous calcium sulfate.

The reaction mixture was reacted with water and 6 N sodium hydroxide and extracted three times with 30-ml. portions of ethyl ether. The yield of yellow residue was 60 mg. Recrystallized from ethanol-water (4:1) the solid transformed to colorless crystals melting at 258-260°.

For analysis the oxidized nitrososolanocapsine was dried over phosphorus pentoxide and paraffin at 17 mm. pressure. The drying pistol was heated by refluxing methanol.

Anal. Calc'd for $C_{27}H_{40}O_3N_2$: C, 73.60; H, 9.15; N, 6.36 %

Found: C, 72.89; H, 9.16; N, 6.03 %

A solution of 0.20 mg. compound/ml. methanol showed only endabsorption in the ultraviolet part of the spectrum.

The compound did not give any precipitate with digitonin contrary to nitrososolanocapsine.

b. Chromic acid oxidation of triacetyl solanocapsine. - The oxidation was performed after the procedure used for oxidation of tomatidine by Sato et al. (76). To a solution of 600 mg. of triacetyl solanocapsidine in 20 ml. of glacial acetic acid was added dropwise with stirring 500 mg. of chromic acid in 10 ml. of 80 per cent acetic acid. The reaction mixture was kept at ca. 10°C. by cooling in ice-water. After

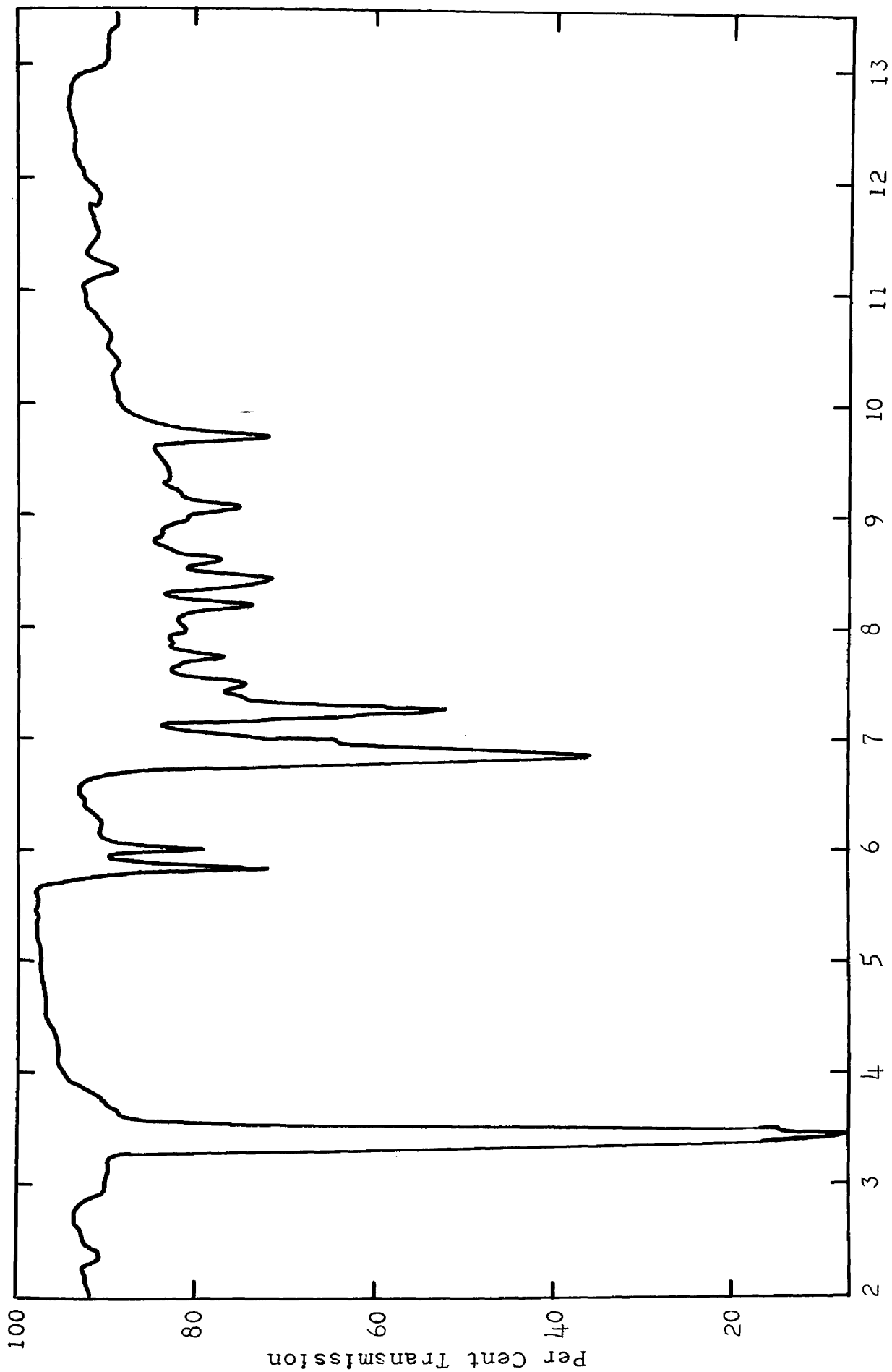


Figure 15. Infrared Spectrum of Derivative Obtained after Oppenauer Oxidation of Nitrososolanocapsine (Nujol Mull).

standing at room temperature for 1.5 hours, the dark-brown solution was poured into ice-water and extracted with ether. The ethereal solution was washed with water, dilute sodium carbonate solution and water again, dried over anhydrous sodium sulfate and evaporated. The yield of semi-crystalline product was 135 mg. Recrystallized from dilute methanol the melting point was 134-135°C.

To purify the compound further, 70 mg. of the compound was treated with Girard's reagent (T) to separate the ketonic material from impurities or non-ketonic material. The procedure described by Reichstein (72) was followed. Yield of "non-ketonic" material was 41 mg. and of "ketonic" material 10 mg. The melting point of the "ketonic" material was after recrystallization from methanol 158-160°C. The ultraviolet spectrum of the derivative (0.20 mg./ml. methanol) showed an absorption peak at 285 mμ.

For elementary analysis the derivative was dried at room temperature over phosphorus pentoxide and paraffin at 17 mm. pressure.

Anal. Found: C, 42.82; H, 8.80; N, 13.06 %

It has not been possible from these analyses to construct a reasonable empirical formula for the oxidation product.

The derivative gave a yellow precipitate with 2,4-dinitrophenylhydrazine (79).

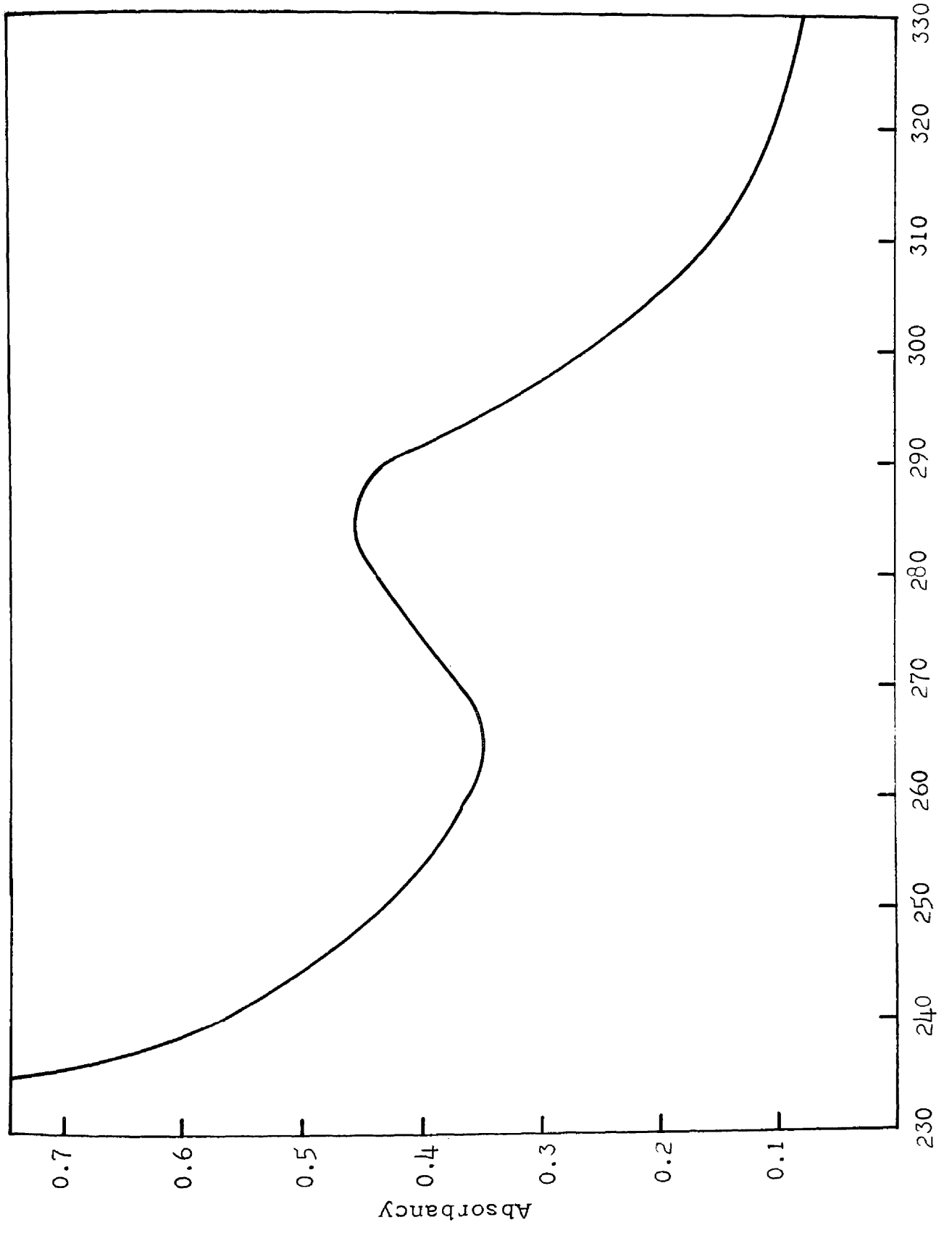


Figure 16. Ultraviolet Spectrum of Oxidation Product of Triacetylsolanocapsine.

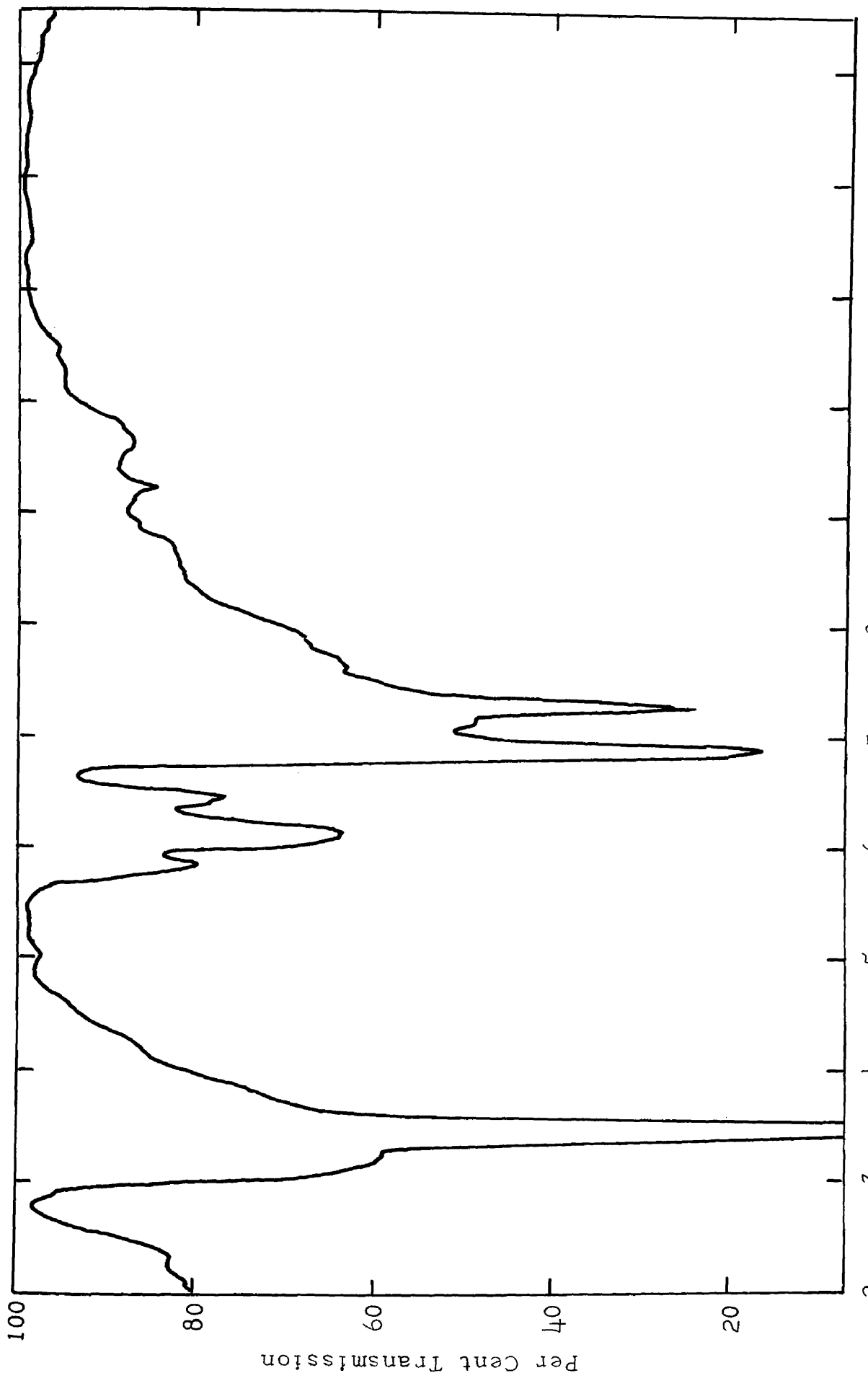


Figure 17. Infrared Spectrum of Oxidation Product of Triacetylsolanocapsine (Nujol Mull).

13. Color Reactions

Liebermann-Burchard color test as described by Fieser (28) was negative with solanocapsine, but positive with nitrosolanocapsine and "nitrosodihydrosolanocapsine."

Rosenheim color test (83) gives an immediate and intense red color with compounds containing a conjugate double bond system or a double bond in the α,β -position to a hydroxyl group. Nitrososolanocapsine and "nitrosodihydrosolanocapsine" gave no color reaction.

Selenium dioxide test (28). The mild oxidation agent selenium dioxide in acetic acid can introduce an acetoxy group adjacent to a double bond. The ease of oxidation depends on the position of the double bond in relation to the hydroxy group in the steriod. The selenium dioxide is reduced to selenium, detectable first as a yellow colloidal solution and then as a red precipitate. Both nitrososolanocapsine and "nitrosodihydrosolanocapsine" gave a red precipitate at 100°C., but not at 20°C. or 40°C.

IV. DISCUSSION AND CONCLUSIONS

Isolation. - The purpose of this research was to isolate and chemically identify the antibacterial substance(s) in Solanum pseudocapsicum L.

Lucas and associates (32) reported that the antitubercular activity was associated with the roots and that no such activity was present in the tops. This was not found to be the case, since the antitubercular activity of the roots and of the tops was approximately the same. The principal antitubercular substance was found to be the steroidal alkaloid solanocapsine.

Besides, a substance inhibiting the growth of Diplococcus pneumoniae was present. It could be removed from an ethanolic extract of the plant with ethyl ether, but no activity was found if the plant was directly extracted with ethyl ether. The substance seemed to be connected with the green colored fraction of the plant extract. It had a certain affinity for solanocapsine and was very difficult to separate from the alkaloid. No attempt was made to isolate and chemically identify this substance.

Solanocapsine is the only alkaloid from the genus Solanum which has not been isolated as a glycoside. In this study it was assumed until contrary evidence that solanocapsine was present in the plant as a glycoside. Either a glycosidase

catalyzed the hydrolysis of an existing glycoside at the time of harvesting or the acid used in the isolation procedure was able to hydrolyze the precursor compound to solanocapsine and a carbohydrate moiety. The attempt to inactivate enzyme before isolation by either placing the plants in boiling water or in absolute ethanol did not yield a glycoside, but always solanocapsine. Fairly large amounts of D-glucose were present in the partially purified alkaloid preparations. It was first thought that a glycoside was present in these and that it was hydrolyzed in the fairly acidic solvent used for chromatography. A basic reacting solvent was tried for developing the alkaloid, but it did not change the result; only solanocapsine could be detected.

One must therefore conclude that solanocapsine is not an artifact, but present in the plant in the same form as isolated. That it is a reasonable conclusion will be seen from the discussion of the chemical structural studies, where it was found that solanocapsine has a primary amino group in position 3 on the steroid nucleus. All other steroidal alkaloid glycosides have a hydroxyl group in this position and the carbohydrate moiety has been found to be attached to this hydroxyl through an acetal linkage (84). The possibility for an N-glycoside seems remote.

Barger and Fraenkel-Conrat (6) reported that besides solanocapsine they found an amorphous compound called solanocapsidine. During the isolation and purification of solanocapsine

an amorphous, resinous material was often obtained; but it was always possible by repeated precipitation from aqueous ethanol to change this material to crystalline solanocapsine. Schlittler and Uehlinger (78) observed the same result. This together with the fact that only one compound was obtained by paper chromatography, leads to the conclusion that solanocapsidine is non-existent and what Barger and Fraenkel-Conrat (op. cit.) called solanocapsidine is impure, amorphous solanocapsine.

It has been stated that the plant S. pseudocapsicum contains solanine and solanidine (66). These two compounds were isolated from potato sprouts but did not, by paper chromatography, give the same R_f-value as solanocapsine. Therefore such a statement should be revised.

Now that the compound "solanocapsidine" apparently is non-existent, it might be suggested that the name for solanocapsine be changed to solanocapsidine. Such a change would bring the name in line with the nomenclature for other steroidal alkaloids. For this class of alkaloids it is customary to let the name of the aglycone end in -idine, whereas the name of the corresponding glycoside has the suffix -ine. (16,49,52,57,84).

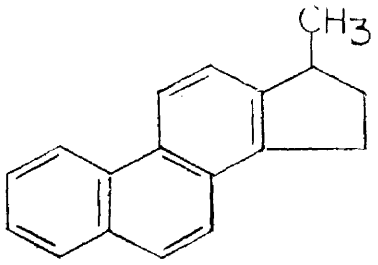
Concerning the possibility of solanocapsine as an antitubercular drug, it can be said that the toxicity is not extremely large, but the side effects of the compound when injected into mice are serious. Before one could even test

the compound clinically, it would be necessary to modify it in such a way that the undesirable side effects disappeared.

Chemical studies. - By means of carbon, hydrogen, and nitrogen analyses of solanocapsine and its derivatives, it can be concluded that solanocapsine, in agreement with Schlittler and Uehlinger (78) has the empirical formula $C_{27}H_{46}N_2O_2 \cdot H_2O$. Rast's molecular weight determination and the neutralization equivalent for solanocapsine gave values of 456, 481, and 482. These results agree more with a 27-carbon atom compound than with a 25-or 26-carbon atom compound as suggested by Barger and Fraenkel-Conrat (6). Therefore, solanocapsine can be classified together with the other 27-carbon atom alkaloids isolated from the genus Solanum. A double bond could not be detected by neither bromine in glacial acetic acid nor permanganate reagent. No O-methyl or N-methyl groups were present, but analyses for methyl groups attached to carbon gave a value of three. No precipitate was formed with digitonin in ethanol.

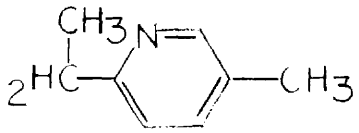
Ring structure. - Barger and Fraenkel-Conrat (6) isolated after dehydrogenation of "solanocapsidine" with selenium, Diels hydrocarbon (3'-methylcyclopenteno-phenanthrene) (I). After 12 hours heating of solanocapsine with selenium at 320° C. in an evacuated tube, Schlittler and Wehlinger (78) isolated the same compound. Since this compound was identical with the authentical compound with respect to melting point, mixed melting point and ultraviolet spectrum, no doubt remains

about solanocapsine being a steroidal alkaloid. Through acid extraction of the material obtained from selenium dehydrogena-

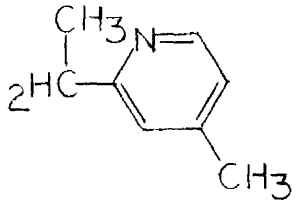


(I)

(II)

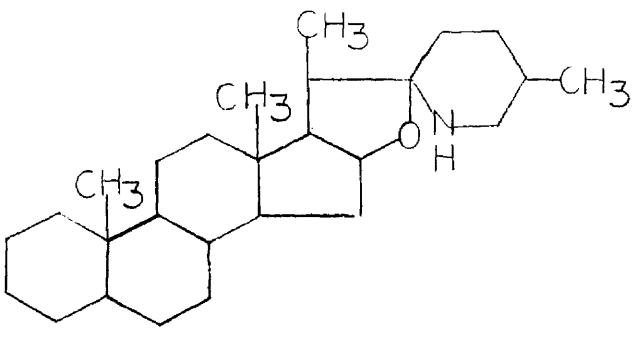


(III)

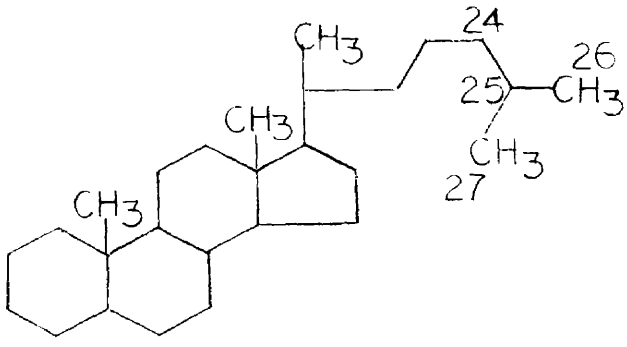


tion of solanocapsine, the latter authors isolated as the only compound 2-ethyl-5-methylpyridine (II). Barger and Fraenkel-Conrat (6) isolated after selenium dehydrogenation both 2-ethyl-5-methylpyridine and its isomer 4-methyl-2-ethylpyridine (III).

If one can attach any importance to biogenetic relationships, one will assume that solanocapsine has a solasodan



(IV)



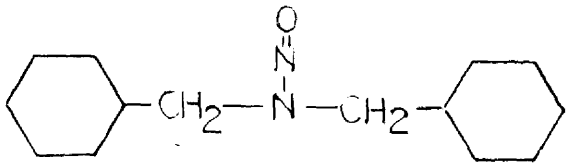
(V)

structure (IV). Since it is generally thought that steroids with 27 carbon atoms are precursors for the steroidal alkaloids

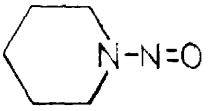
(16), it does not seem likely from biogenetic relationships that Barger and Fraenkel-Conrat's 4-methyl-2-ethylpyridine (III) was correctly identified. If this compound really was isolated, an unexpected precursor for solanocapsine should have had a methyl group at carbon 24 instead of at the usual carbon 25 position (V).

Functional groups. - Both Barger and Fraenkel-Conrat (6) and Schlittler and Uehlinger (78) found that solanocapsine possesses a primary amino, a secondary amino group, and a hydroxyl group. The evidence for having two amino groups came from methylation of the nitrogen atoms with formic acid and formaldehyde, which gave a trimethyl derivative $C_{30}H_{52}N_2O_2$. This observation was confirmed by the present investigation.

Further evidence for the existence of the two amino groups was given by Schlittler and Uehlinger (78) by reacting solanocapsine with nitrous acid. In this study the same was observed and it was further determined that by reacting solanocapsine with 2 equivalents of nitrous acid, a neutral, unsaturated nitroso derivative, $C_{27}H_{42}N_2O_3$, resulted. A Van Slyke analysis indicated that 1 mole of nitrogen was liberated per mole of solanocapsine, from which can be concluded that a primary amino group was present. The ultraviolet spectrum of the obtained nitroso derivative (Figure 9) showed a peak of low intensity at 355 mμ (log E 2.42) and suggested the presence of a nitrosamino group. Di(cyclohexylmethyl)-N-nitrosamine (VI) shows two absorption peaks at 240 mμ (log E 3.9) and



(VI)



(VII)

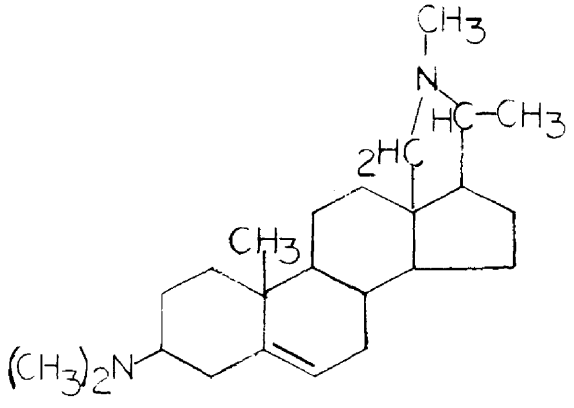
355 mμ (log E 4.25), while 1-nitrosopiperidine (VII) has peaks at 255 mμ (log E 4.25) and 350 mμ (log E 2.0) (38). Only the peak at the longer wavelength was present in nitrososolanocapsine.

It was assumed by Barger and Fraenkel-Conrat (6) that during the formation of nitrososolanocapsine the hydroxyl group, originally present in the molecule, was eliminated as water, whereas Schlittler and Uehlinger (78) thought it more likely that the action of nitrous acid on the primary amino group would result in the formation of a double bond and leave the hydroxyl group intact. The latter seems like a more plausible explanation, since deamination of primary amino groups often results in the formation of both unsaturated compounds and the corresponding hydroxy compounds. From experiences in this study it was not possible to dehydrate solanocapsine, which is contradictory to Barger and Fraenkel-Conrat's findings and consequently supports Schlittler and Uehlinger's conclusions.

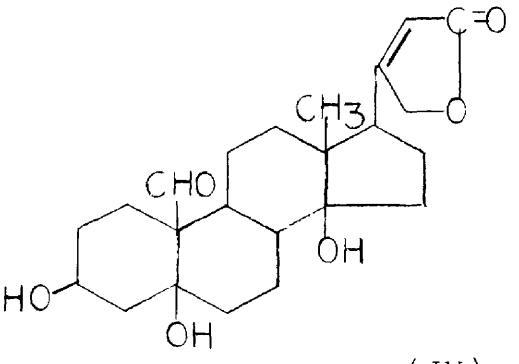
Nevertheless, there may be validity in the contentions of Barger and Fraenkel-Conrat (op. cit.) due to the following: Solanocapsine does not give any precipitate with digitonin and

this is why the hydroxyl group should not be 3β-hydroxy. It can not be a 3α-hydroxy group either, since it would have been possible to dehydrate such a group. Nitrososolanocapsine gives a precipitate with digitonin, and it must be assumed, therefore, that the primary amino group was in the 3 position.

Digitonin is a glycoside, which is generally regarded to form sparingly soluble complexes with 3β-hydroxy, but not with 3α-hydroxy steroids. The latter type produces complexes that are much more soluble. Usually, only 3β-hydroxy steroids



(VIII)

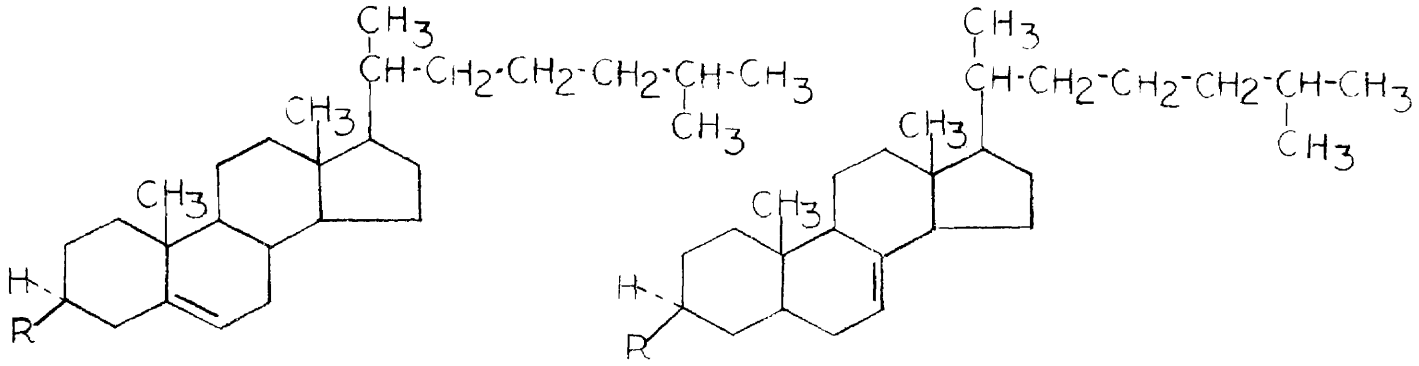


(IX)

(with 3-OH and 10-CH_3 cis to each other) such as the steroidal alkaloids tomatidine, solanidine, and solasodine are precipitated as digitonides. Exceptions among naturally occurring compounds are conessine (VIII), which has a 3β-dimethylamino group and is precipitated with digitonin (10) and strophantidin (IX), which has an aldehyde group in the 10 position besides a 3β-hydroxy group and is precipitated with digitonin (70).

Haworth et al. (44) have synthesized several aminocholestene derivatives and found that Δ^5-cholestene-3β-amine (X)

and Δ^5-cholestene-3β-isopropylideneamine (XI), but not the 3α-epimers, readily yielded precipitates with digitonin. Bertho *et al.* (11) reported that Δ^7-cholestene-3β-dimethylamine (XII) and Δ^7-cholestene-3β-piperidine (XIII) gave no precipitate with digitonin.



(X) R = $-NH_2$

(XI) R = $(CH_3)_2C{=}N-$

(XII) R = $(CH_3)_2N-$

(XIII) R = $C_5H_{10}N-$

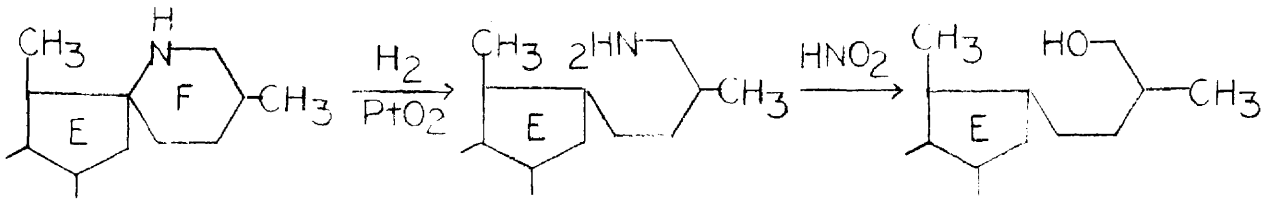
Since solanocapsine gave no precipitate with digitonin, it can now be assumed by analogy with other 3-amino compounds that the amino group probably has a 3α-configuration. An inversion of configuration must have happened during the deamination. Ingold (46) explains the deamination reaction as involving an intermediate formation of a diazonium ion which decomposes by an S_N1 process and affords an alcohol of large inverted configuration, e.g.:

$$\text{L-RNH}_2 + \text{HNO}_2 \longrightarrow \text{RN}_2^+ \longrightarrow \text{R}^+ \longrightarrow \text{D-ROH} + \text{D,L-ROH}$$

Mills (59) has described deamination of aminodecalins with nitrous acid and found it to be a true stereospecific reaction. Equatorial amino groups afforded alcohols of same

configuration, while axial amino groups reacted by inversion of configuration. That is in agreement with the concept that the 3β-hydroxy group in steroids with the same stereochemical configuration as cholesterol is equatorial and therefore, thermodynamically more stable.

Ring opening. - Hydrogenation of solanocapsine at room temperature and about 3 atmospheres pressures with reduced platinum oxide catalyst gave a derivative with melting point 210-212°C. This derivative showed no depression of melting point mixed with solanocapsine, but the specific rotation had changed from +20° to +40°. Schlittler and Uehlinger (78) observed the same results by reduction of solanocapsine with lithium aluminum hydride. A Van Slyke amino nitrogen analysis on the dihydro compound showed that 2 moles of nitrogen were given off per mole of compound and that the resulting product did not give the ultraviolet absorption characteristic for a nitrosamine (Figure 9). It is therefore concluded that ring F was opened as illustrated in the following:



The hydroxy steroid obtained after reaction of dihydro-solanocapsine with nitrous acid absorbed a little more than 1 mole of hydrogen and gave a precipitate with digitonin. This hydroxy steroid derivative is not completely identified,

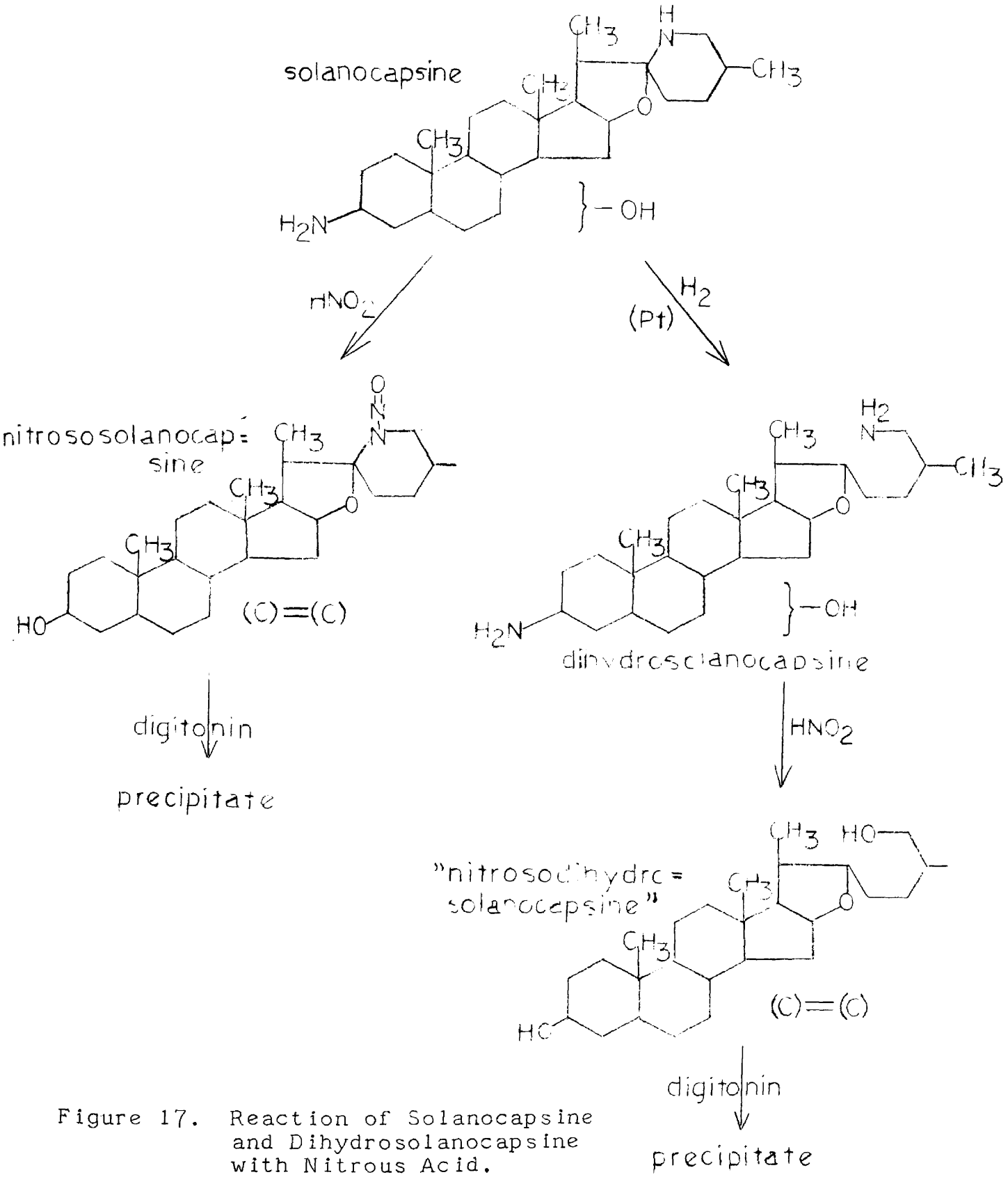
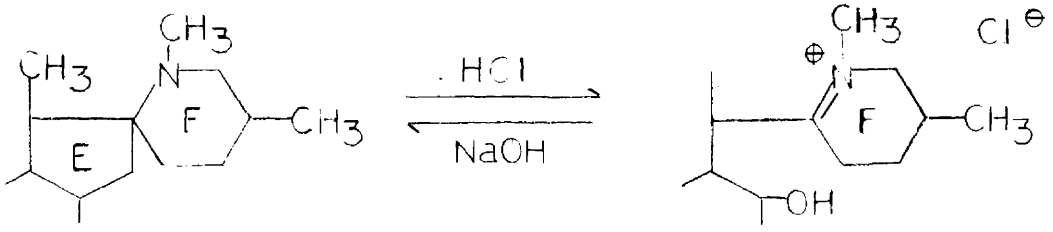


Figure 17. Reaction of Solanocapsine and Dihydrosolanocapsine with Nitrous Acid.

since the elementary analysis is more in agreement with a 25-carbon atom compound than a 27-carbon atom compound.

The reactions of nitrous acid with solanocapsine and dihydrosolanocapsine as described above are summarized in Figure 17.

Schlittler and Uehlinger (78) observed that the infrared spectrum of trimethylsolanocapsine hydrochloride showed a more intense hydroxyl band than the spectrum of its corresponding free base. Besides, the spectrum of the hydrochloride showed a band at 6.1 μ characteristic of a C=N band. To explain this the authors suggested that in the production of the hydrochloride of the trimethyl derivative ring E of the free base (XIV) opened under influence of acid (XV):



(XIV) (XV)

Furthermore, they proposed that ring E of solanocapsine under influence of acid opened in the same manner, but found no similar evidence of this in the infrared spectrum.

These observations as can be seen from the infrared spectra (Figures 13 and 14) have been confirmed.

Acetyl derivatives. - Schlittler and Uehlinger (78) prepared an amorphous acetyl derivative of solanocapsine, considered by them to be a N,N'-diacetylsolanocapsine. Based

upon calculation for 2 acetyl groups they found that acetyl analysis gave 14.97 per cent of the total weight of the compound whereas theoretically it should be 16.73 per cent. In this work the amorphous diacetyl derivative was found to contain 14.22 per cent acetyl and suggests that the compound is rather an O,N-diacetyl derivative than a N,N'-diacetyl derivative.

The reasons for such a conclusion are the following: 1) The derivative was soluble in dilute acetic acid indicating that a basic group probably is present. 2) The infrared spectrum of the derivative showed the characteristic absorption bands for an O-acetyl group (5.78 μ, 8.00 μ, and 9.75 μ) and also the bands characteristic for a secondary amide or N-acetyl (CH_3CO-NHR) (6.06 μ and 6.45 μ). The spectrum of the diacetyl solanocapsine is given in Figure 11.

Normally the absorption instensity of an ester is approximately the same as that of a secondary amide (8). This was found to be the case for the spectrum of the diacetyl derivative and therefore it is concluded that a primary amine is acetylated. Furthermore, for a secondary amide the intensity of the 6.06 μ band is usually the same as that of the 6.45 μ band; but this was not found to be the case for diacetylsolanocapsine. The 6.06 μ band showed a little more intense absorption than the 6.45 μ band, and since a tertiary amide (CH_3CO-NRR') only has one characteristic absorption band occurring at 6.06 μ, it may be concluded that the secondary

amino group present in solanocapsine was partly acetylated.

Several attempts to prepare a diacetate never gave the exact elementary analysis expected for a diacetylsolanocapsine, just as the dermination of acetyl groups always gave a value of less than two acetyl groups. Therefore, the conclusion is that the amorphous diacetate prepared after the procedure of Schlittler and Uehlinger (78) is not a pure compound. But the acetate is not, as suggested by the two authors, a N,N'-diacetate, but rather an O,N-diacetate.

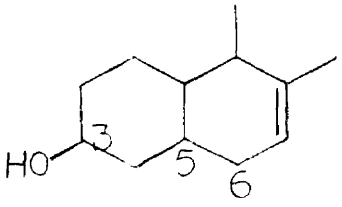
Barger and Fraenkel-Conrat (6) acetylated solanocapsine by refluxing with acetic anhydride for five hours and obtained an amorphous acetate with melting point 150-160°C. On the basis of the elementary analysis it was considered to be a diacetate. But it seems that the authors based their calculation on the wrong empirical formula. When recalculated on basis of a solanocapsine with 27-carbon atoms, the analysis is more in agreement with a triacetate. It was considered to be a N,N-diacetate since it was insoluble in dilute acids. The acetate prepared in this investigation after their procedure gave an elementary analysis which is in agreement with a triacetate and acetyl group analysis also indicated 3 acetyl groups. The infrared spectrum showed the bands characteristic for an O-acetyl group and a secondary amide (CH_3CO-NHR). Furthermore, the intensity of the 6.06 μ band was considerably higher than the 6.45 μ band. Since a tertiary amide (CH_3CO-NRR') only shows one characteristic absorption band at 6.06 μ,

it is concluded that the third acetyl group was attached to the secondary amino group. The acetyl derivative is therefore considered to be O,N,N'-triacetylsolanocapsine.

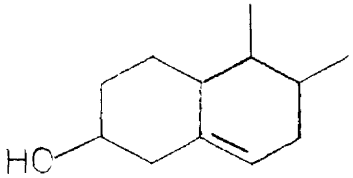
The hydroxyl group was by both Barger and Fraenkel-Conrat (6) and Schlittler and Uehlinger (78) considered to be a tertiary or an unreactive hydroxyl group. This conclusion was based on the acetyl derivatives. This investigation has shown as previously discussed that the hydroxyl group can be acetylated, but not tosylated.

Location of hydroxyl group. - Nitrososolanocapsine gives like cholesterol a positive Lieberman-Burchard test. The requirements necessary to give a positive reaction are not known, except that a double bond seems necessary together with a hydroxyl group.

Lathosterol (XVI) is oxidized by selenium dioxide at 20°C., whereas cholesterol (XVII) requires 60°C. (28) for oxidation.



(XVI)

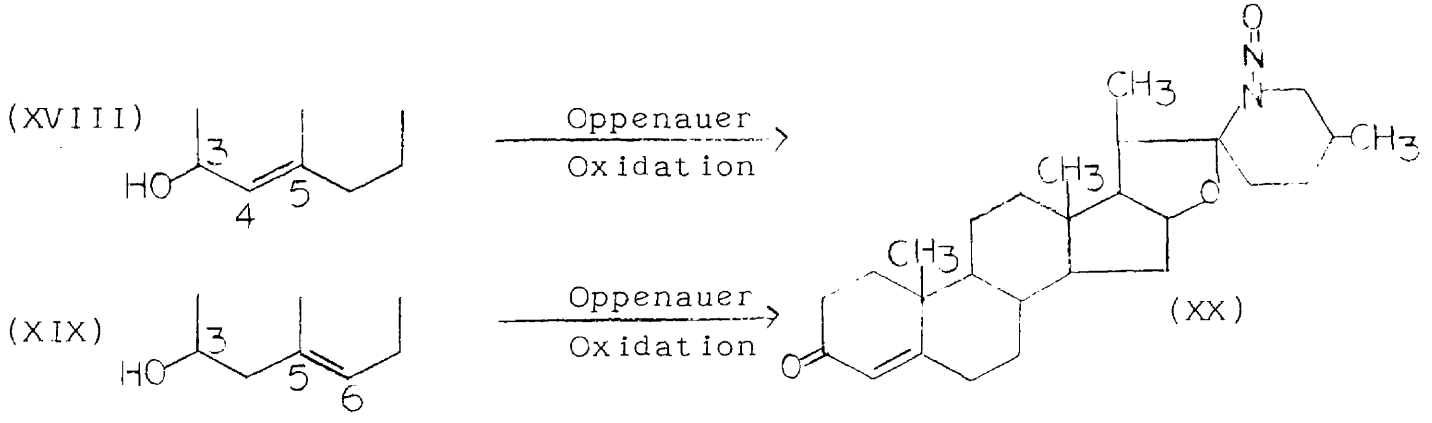


(XVII)

Nitrososolanocapsine which was found to contain a hydroxyl group (presumably at the 3 position) and a double bond is first oxidized by selenium dioxide at 100°C. Therefore, it is concluded that the double bond does not occupy a position as found in either of the two previously mentioned sterols. The

Rosenheim test is characteristic for either a conjugated double bond system or a double bond in the α,β-position to the hydroxyl group. Since nitrososolanocapsine is negative to this test, it suggests that the double bond is not between carbons 4 and 5.

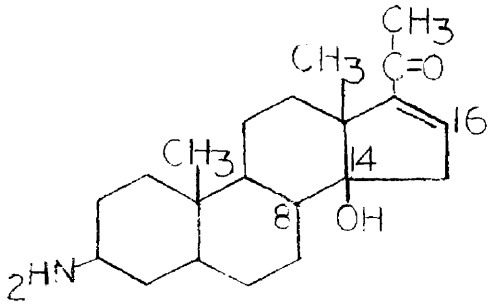
An Oppenauer oxidation of nitrososolanocapsine should oxidize the hydroxyl group in the 3 position to a keto group. If the double bond present in the compound is between either carbons 4 and 5 (XVIII) or carbons 5 and 6 (XIX) an α,β-unsaturated ketone (XX) should be obtained. Such a compound



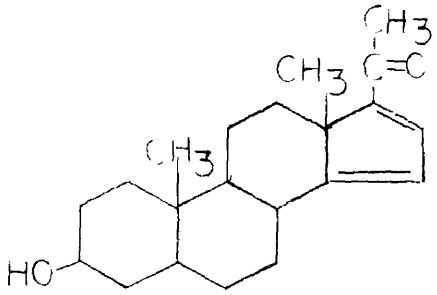
should show ultraviolet absorption about 240 mμ. This was not found to be the case, hence it is concluded that the double bond could not occupy any of the mentioned positions.

It has been observed that most compounds with a solasodan nucleus can be oxidized to a pregnenolone derivative. This has not as yet been accomplished in the case of solanocapsine, since the chromic acid oxidation product of triacetylsolanocapsine was not completely identified. Nevertheless, it contained a carbonyl group as indicated by a precipitate

with 2,4-dinitrophenylhydrazine, and the ultraviolet spectrum showed absorption at 285 mμ. This might be indicative of two double bonds in conjugation with a carbonyl group. If that is the case, one could imagine that before dehydration the



(XXI)

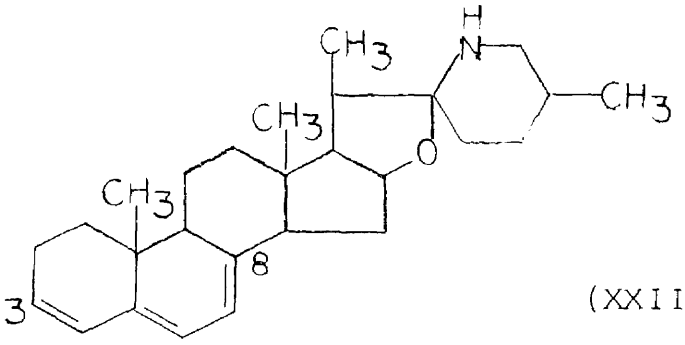


(XXII)

hydroxyl group was attached to carbon 14 in a hypothetical aminopregnenolone (XXI). However, $\triangle^{14,16}$-allopregnene-3-ol-20-one (XXII) shows absorption at both 239 and 306 mμ (28). This possibility is therefore excluded. As a new working hypothesis it is then suggested a hydroxyl group is attached to carbon 8. Following this hypothesis it is conceivable that a possible oxidation product could be $\triangle^{8,16}$-allogregnene-20-one-3-amine and that this derivative would show absorption about 280-290 mμ.

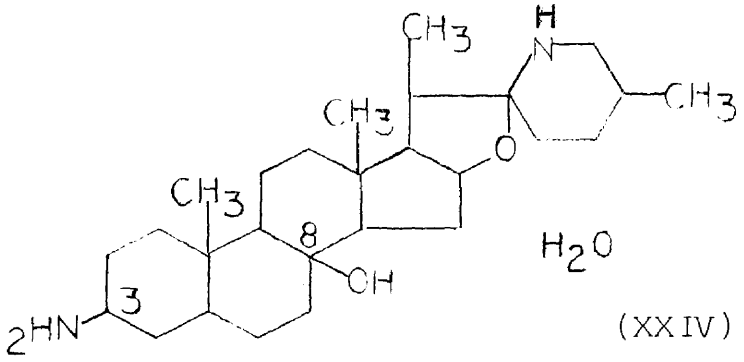
Placing the hydroxyl group in the 8 position will be in line with these observations by Schlittler and Uehlinger: If solanocapsine was heated with selenium for 75 minutes a small yield of a basic compound $C_{27}H_{39}NO$ was obtained. This compound had lost a primary amino group and a hydroxyl group. Not two, but three double bond seemed to have been introduced. An ultraviolet spectrum indicated conjugated double bonds

(λ_{max}=287 mu). Since it is proposed in this work that the primary amino group is in the 3 position, one can write formula XXIII for the dehydrogenation product obtained by Schlittler and Uehlinger.



(XXIII)

As a final conclusion of this study the following formula (XXIV) is proposed for solanocapsine, although it is realized that not very much evidence for a hydroxyl group in the 8 position is submitted. The systematic name for solanocapsine will then be 3α-aminosolasodan-8-ol monohydrate.



(XXIV)

Concerning future work it would be very desirable to succeed in oxidizing solanocapsine to a pregnenolone derivative. If such a type of compound can be prepared, another potentially abundant raw material for the synthesis of cortisone and other steroid hormones would be assured.

V. SUMMARY

1. The antitubercular principle in the plant Solanum pseudocapscum L. is found to be the alkaloid solanocapsine, which in a concentration of 3 γ/ml. inhibits growth of Mycobacterium tuberculosis. The minimum lethal dose injected into the peritoneal cavity of mice is for solanocapsine 50-100 mg./kg. of body weight and for its hydrochloride 200 mg. whereas by subcutaneous injection the minimum lethal doses are 200 mg./kg. of body weight for both compounds. The alkaloid was tested for antibacterial activity against 21 different bacterial organisms.

2. In addition to solanocapsine a colored principle inhibiting growth of Diploccus pneumoniae is present.

3. Attempts to isolate the alkaloid as a glycoside have not been successful. Contrary to findings about other alkaloids from the genus Solanum, it is regarded that solanocapsine occurs in the plant as a free alkaloid and not as a glycoside.

4. Modifications and variations of procedure for extracting and purifying the alkaloid are described. Purification by column chromatography on alumina, acid alumina, or Dowex 50 was not successful.

5. It has been stated that S. pseudocapsicum in addition to solanocapsine contains solanine (66), solanidine (66), and solanocapsidine (6). No indication of the presence of these

additional compounds has been found. Paper chromatography in different solvents revealed only one alkaloid, solanocapsine. Solanocapsidine, as reported by Barger and Fraenkel-Conrat (6), is in reality impure, amorphous solanocapsine, which can be changed to the crystalline form.

6. The solanocapsine content varied from 1.53 to 2.15 per cent in (dry weight basis) leaves plus stems and from 0.83 to 2.24 per cent in roots of plants grown during a three year period.

7. The alkaloidal glycoside solanine and its aglycone from Solanum tuberosum together with a crude alkaloid fraction from Solanum carolinense were isolated. Of these only solanine showed weak antitubercular activity. Besides, the alkaloidal glycoside tomatine from Lycopersicon esculentum was tested and found to be inactive against M. tuberculosis.

8. It has been verified that solanocapsine has the empirical formula $C_{27}H_{46}N_2O_2 \cdot H_2O$ as proposed by Schlittler and Uehlinger (78).

9. Assuming a solasodan structure, solanocapsine has been found to have a primary amino group in the 3 position. The configuration is probably 3 α.

10. Schlittler and Uehlinger's amorphous N,N'-diacetylsolanocapsine (78) is found to be more identifiable as O,N-diacetylsolanocapsine.

11. Evidence for Barger and Fraenkel-Conrat's N,N'-diacetylsolanocapsine (6) being O,N,N'-triacetylsolanocapsine is presented.

12. Opening of the piperidine ring in the solasodan structure has been accomplished by hydrogenolysis.

13. The hydroxyl group in solanocapsine may be provisionally assigned to carbon 8.

14. The tentative systematic chemical name proposed for solanocapsine is 3α-aminosolasodan-8-ol monohydrate.

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APPENDICES

APPENDIX I

ANTIBACTERIAL ACTIVITY

Bioassay

The samples from Solanum pseudocapsicum L. were tested for activity against Mycobacterium tuberculosis, strain H-37, and Diplococcus pneumoniae, Type III, N.I.H., using the serial dilution method:

All cold-water and any ethanol extracts found to be contaminated were sterilized by passing them through a Seitz filter. Hot-water extracts were sterilized by autoclaving.

The extracts were tested for antibacterial properties by making serial dilutions in liquefied glycerol beef extract agar, pH 7.0. The agar was allowed to harden in a slanting position, and after solidification the tubes were inoculated with an 18-to-40-day-old culture of M. tuberculosis, strain H-37, grown on Petragnani's medium. To prevent evaporation during the long incubation period screw-capped tubes were used for making the cultures. After six weeks of incubation at 37°C. the cultures were examined. If they were inhibited, the highest dilution in which no growth occurred was recorded. The active extracts were ten retested on glycerol beef extract agar to which 10% whole sheep blood had been added. With the alcoholic extracts allowance had to be made for the inhibitory

effect of the alcohol. This solvent was found to inhibit the tubercle bacillus at a dilution of 1:40 and therefore only extracts in which there was no growth at a dilution higher than this were considered active. The antibacterial activity of the extracts against Diplococcus pneumoniae, Type III, N.I.H., was determined by making serial dilutions in nutrient broth (F.D.A. formula). The broth was inoculated with 1.0 ml. of an 18-to-24-hour broth culture of the organism per 100 ml. of medium just before making the dilutions. Results were read after incubation for 24 and 48 hours at 37°C.

It was found that ethanol inhibited Diplococcus pneumoniae at a 1:8 dilution. Consequently, only extracts showing an inhibition greater than this dilution were recorded as being antibacterial.

APPENDIX II

EXPRESSION AND CALCULATION OF ANTIBACTERIAL ACTIVITY

The specific antibacterial activity of each sample was expressed as the smallest number of micrograms (γ) per milliliter of culture broth which inhibited bacterial growth.

Example: A 1 per cent (w/v) sample solution was sent for bioassay and was found to inhibit bacterial growth at a dilution of 1:256. The concentration at this solution is 1/256 of the original sample (which in this case was 0.01 g./ml.). A formula for calculating the activity is given below:

$$\text{Specific antibacterial activity (in } \gamma/\text{ml.}) = \frac{C_s \times 10^4}{D}$$

C_s = concentration of original sample in per cent (w/v).

D = maximum observed dilution inhibiting bacterial growth.

For the above example then:

$$\gamma/\text{ml.} = \frac{C_s \times 10^4}{D} = \frac{1 \times 10^4}{256} = \underline{\underline{39}}$$