Isolation and Identification of Lysergic Acid Amide and Isolysergic Acid Amide as the Principal Ergoline Alkaloids in *Argyreia nervosa*, a Tropical Wood Rose

By MICHAEL D. MILLER[1] (Bureau of Narcotics and Dangerous Drugs, Department of Justice, Dallas Regional Laboratory, Dallas, Tex. 75202)

Lysergic acid amide and isolysergic acid amide have been extracted and isolated from the seed of Hawaiian baby wood rose. The ergoline alkaloids were identified by TLC, melting point determination, and UV and IR spectrophotometry.

The introduction of Federal and State legislation regulating the possession and sale of hallucinogenic drugs has given impetus to the utilization of natural products with mind-altering properties by drug abusers. *Argyreia nervosa*, more commonly referred to as Hawaiian baby wood rose, is a closely related genus to *Ipomoea* (morning glory). An excellent, concise botanical description of *Argyreia nervosa* is given by McJunkins *et al.* (1). Our laboratory has received No. 2 gelatin capsules containing a light brown powder alleged to be mescaline. Preliminary microscopic examination of the powder revealed fibrous plant segments indicative of a natural origin. The powder also gave a positive test with Van Urk reagent for indole-related compounds. The brown powder in all such samples was identified as baby wood rose. The appearance on the psychedelic drug scene of capsules containing the ground-up seed of baby wood rose suggested that, in addition to its commercial value in the field of floral decorations, baby wood rose may also have an illicit value when sold as a psychotomimetic agent.

Morning glory seeds, usually "Heavenly Blue" or "Pearly Gates," have been subject to abuse for a number of years by those seeking hallucinatory experiences due to their lysergic acid and clavine alkaloid content (2). The psychic phenomena produced by the alkaloids in morning glory seed is similar to that of LSD (3). Dissociative reactions and schizophrenic breakdowns are major adverse psychotic effects that may be incurred through seed ingestion (4). Lysergic acid amide (LAA) and isolysergic acid amide (iso-LAA) were tentatively identified as the principal alkaloidal constituents of "Heavenly Blue," "Pearly Gates," and "Ololiuqui" by Taber *et al.* (5), who used thin layer (TLC) and paper chromatographic systems. Later work by Marderosian and Youngken (6) and Genest (7) corroborated the earlier chromatographic study of the alkaloids in certain varieties of morning glory. Hylin and Watson (8) were also able to tentatively identify LAA and a clavine alkaloid (penniclavine) as the principal alkaloids in baby wood rose by TLC and paper chromatography.

The determination of LAA and total alkaloids in baby wood rose, given in Table 1, was made by Hylin and Watson (8), using a colorimetric method with Van Urk's reagent after elution of the alkaloidal bands from the plate. Genest (7) determined the alkaloid content of the morning glory (Table 1) by direct densitometry on TLC plates. It is evident from Table 1 that the total alkaloidal content of baby wood rose is almost ten-fold that of the morning glory varieties.

Table 1. LAA and total alkaloidal content of baby wood rose and selected varieties of morning glory

Variety	LAA, %	Total Alkaloids, % by Wt
Hawaiian baby wood rose	0.04	0.30
Ololiuqui	0.02	0.04
Heavenly Blue	0.01	0.02
Pearly Gates	0.02	0.03
Wedding Bells	0.01	0.03

Hylin and Watson (8) have tentatively identified LAA as one of the ergoline alkaloidal constituents of baby wood rose by comparison of its mobility on both TLC and paper chromatographic systems with that of an authentic standard. We undertook to positively identify the alkaloidal constituents by several different techniques. Such evidence was provided by Hofmann (9) for the presence of LAA and iso-LAA in the Mexican magic drug "Ololiuqui." We have isolated LAA and iso-LAA from the seed of baby wood rose and used IR spectrophotometry for identification.

---

[1] Former address: Food and Drug Administration, 1521 W. Pico Blvd., Los Angeles, Calif. 90015.

Received August 22, 1969.

Table 2. TLC of ergoline alkaloids in *Argyreia nervosa*

Control Standards			*Argyreia nervosa*		
Std	R_f	DMBA	R_f	DMBA	Tentative Identification
LSD	0.90	blue-violet	0.92	blue	unknown
			0.85	green	unknown
			0.72	blue-violet	unknown
Iso-LAA	0.61	blue-violet	0.61	blue-violet	iso-LAA
			0.45	blue-violet	unknown
LAA	0.34	blue-violet	0.34	blue-violet	LAA
			0.20	green	unknown
			0.06	blue-green	penniclavine
			0.00	blue	unknown

Experimental

Extraction of Alkaloids

Specimens of *Argyreia nervosa* were obtained at a local florist shop which specialized in flowers from Hawaii. The seeds were removed from the pods and were ground with a tablet grinder. The resulting coarse mixture was defatted by refluxing 15 min with petroleum ether. (Defatting reduces the occurrence of emulsions in later extractions.) The seed composite was then powdered in a Wiley mill to pass a 20-mesh sieve and the seed powder was then refluxed an additional 15 min with petroleum ether. (Loss of ergoline alkaloids during petroleum ether refluxing is minimal.) Powdered seed (10 g) was transferred to a 250 ml Erlenmeyer flask and wetted with 50 ml 10% NH_4OH. Two hundred ml ether was added and the flask was put on a mechanical shaker 15 min; then the ether extract was decanted into an 800 ml beaker. The ether extraction was repeated for a total of three extractions. The combined ether extracts were evaporated to ca 100 ml under nitrogen and at ca 35°C. The ether was then extracted with three 25 ml portions of 2% H_2SO_4. The combined acid extracts were made alkaline with solid sodium carbonate and extracted with three 25 ml portions of chloroform. The combined chloroform extracts were passed through anhydrous Na_2SO_4 and allowed to evaporate under nitrogen in dim light at room temperature. The residue was dissolved in 500 μl chloroform for preparatory TLC.

Thin Layer Chromatography

Genest (7) found that chloroform-ethanol (96 + 4) gave a good overall separation of the ergoline alkaloids in morning glory seeds on aluminum oxide plates. This system also gave an excellent separation of the ergoline alkaloids in baby wood rose. Precoated aluminum oxide plates (types T and F, Brinkmann Instruments, Inc.) were prewashed with acetone-water (1 + 1) and then reactivated 30 min at 100°C. Ten μl of the chloroform extract was spotted on a type-T aluminum oxide plate and developed for 1 hr in an unlined glass tank. After the plate was air-dried, the spots were examined under both long and short wave UV light. Momentary observations of the chromatogram under short wave UV is necessary to view some of the clavine alkaloid spots. This was followed by spraying with the DMBA spray[2] to cause the separated ergoline alkaloids to appear as blue-violet spots. After 3 min the plate was sprayed with 1% aqueous sodium nitrite solution to intensify and stabilize the color of the spots (*see* Table 2).

The size and color intensity of the blue-violet spots at R_f values 0.61 (iso-LAA) and 0.34 (LAA), when compared with the other spots, identify these alkaloids as the major ergoline alkaloidal constituents of baby wood rose. The blue-green spot at R_f 0.06 is probably an unresolved pair of clavine alkaloids, since the ergoline alkaloids found thus far in morning glory seeds have appreciably higher R_f values in this particular chromatographic system. Penniclavine and chanoclavine would be likely candidates due to their limited mobility on aluminum oxide with a chloroform-ethanol solvent system (7). Penniclavine gives a green color with the DMBA reagent and was identified as being present in baby wood rose by TLC and paper chromatography (8). Tryptophan and tryptamine also give a green color but they react more slowly to Van Urk reagent than ergoline alkaloids. The alkaloid at R_f

[2] Prepared as follows: Sixty-five ml H_2SO_4 was cautiously added to 35 ml water and the solution was allowed to cool. Then 125 mg *p*-dimethylaminobenzaldehyde (DMBA) was dissolved in the solution and 1–2 drops of ferric chloride T.S. were added.

0.92 has a distinctive blue-green fluorescence under UV light and an R_f value very close to that of LSD. This alkaloid could be agroclavine, because LSD and agoclavine have very similar mobilities in this system (7). A sufficient quantity of the alkaloid was isolated to record the IR spectrum as the free base. The spectrum is somewhat suggestive of a clavine structure but more work will have to be done to establish the identity of the alkaloid.

The remainder of the extract was applied as a line, ca ¾″ above the bottom edge of a type-F aluminum oxide plate for preparatory TLC. The plate was developed in the chloroform-ethanol solvent system for 90 min; after this the fluorescent bands corresponding to the lysergamides were outlined under long wave UV light and removed from the plate with the aid of a sealing tube with constricted ends and fitted with a medium porosity disk. The alkaloids were eluted from the adsorbent with two 5 ml portions of chloroform-methanol (2 + 1) and evaporated to dryness under nitrogen at ca 35°C. The residues were dissolved in 500 μl chloroform in 1 dram vials wrapped with aluminum foil to protect the isolated alkaloids from excessive light. Thirty μl of the above solutions were used to prepare micro-KBr disks for preliminary IR identification.

Preliminary Infrared Identification of LAA and iso-LAA

A Perkin-Elmer infrared grating spectrophotometer (Model 257), with a refracting beam condenser, reference beam attenuator, and micro-KBr die, was used for the preliminary IR analysis.

The 30 μl aliquot, containing the alkaloids, was applied in 5 μl portions to ca 3–5 mg of well pulverized KBr. The KBr-alkaloid mixture was dried 1 min in an air-draft oven at 35°C before preparation of the micro-KBr disk. The IR spectra recorded were compared to the spectra of authentic LAA and iso-LAA reported by Hofmann (9). Our spectra matched the absorption patterns of LAA and iso-LAA in Hofmann's paper very closely. Sufficient quantities of LAA and iso-LAA for melting point determination, macro-IR, and UV spectrophotometry were subsequently obtained by repetitive preparatory TLC.

Identification of LAA

The chloroform-methanol eluates from repetitive preparatory TLC isolations of LAA were combined and evaporated to dryness under nitrogen at ca 35°C. The residue was taken up with 10 ml alcohol in a 4 dram vial. The vial was set aside in the dark at room temperature to allow the formation of LAA crystals. The finely splintered, solvent-free crystals that resulted had a melting point range of 230–244°C with noticeable decomposition. This corresponds favorably with the range of 232–240°C reported for LAA by Hofmann (9). The UV spectra of the crystals in methanol and 0.1*N* HCl (Fig. 1) are characteristic of the lysergic acid ring system and identical with that of LSD taken in the same solvents. This is to be expected since LAA and LSD differ only in the substitution of ethyl groups for the two hydrogen atoms in the amide group. The macro-IR spectrum of the crystals (Fig. 2) was identical in absorption pattern with that reported by Hofmann (9) for LAA.

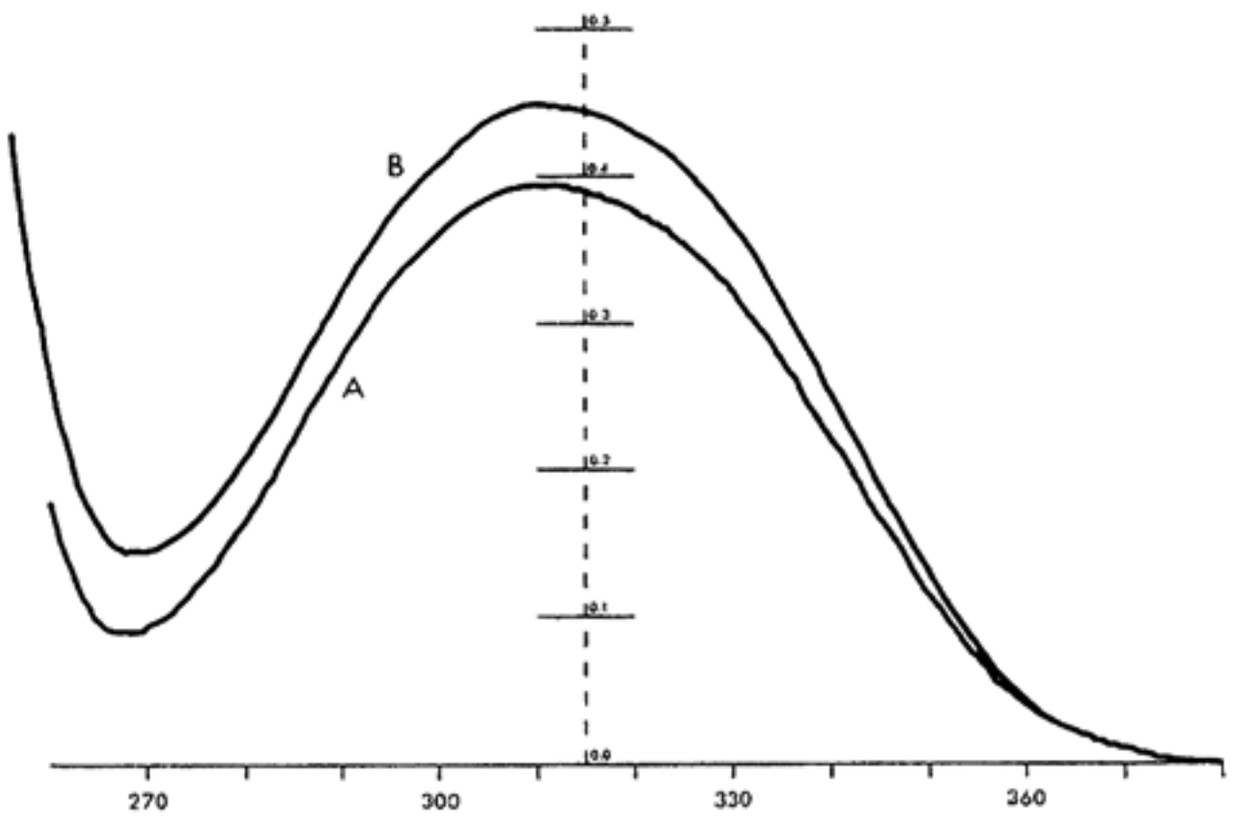

FIG. 1—UV spectra of LAA in 0.1*N* HCl: A, LAA standard (20 μg/ml); B, LAA isolated from baby wood rose.

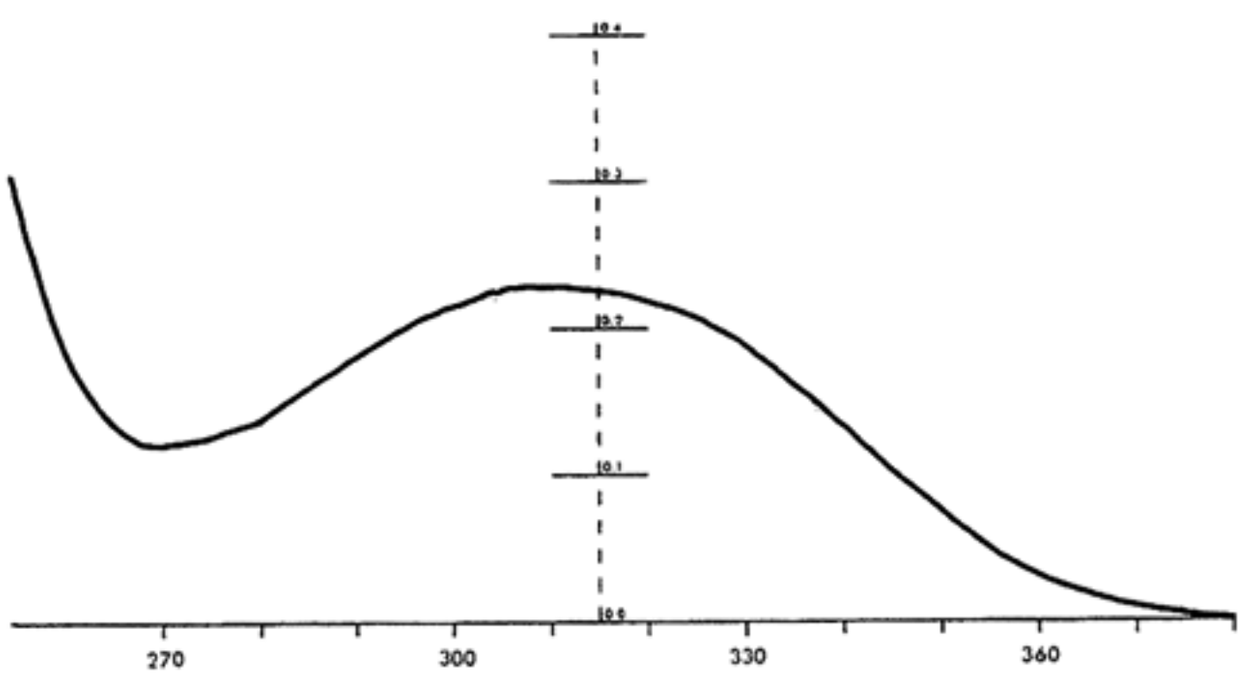

FIG. 2—UV spectrum of iso-LAA in 0.1*N* HCl.

Identification of iso-LAA

The chloroform-methanol eluates from repetitive preparatory TLC isolations of iso-LAA were combined and evaporated to dryness under nitrogen at ca 35°C. The residue was dissolved in 10 ml methanol and allowed to stand at room temperature in the dark. Long massive crystals were

easily crystallized out and determined to have a melting point range of 131–134°C with decomposition, which correlates well with the literature value of 132–134°C (10). The UV spectra of the crystals in methanol and 0.1*N* HCl (Fig. 3) are again characteristic of the tetracyclic lysergic acid ring system and identical with that of LSD. The macro-IR spectrum of the crystals (Fig. 4) exhibits enhanced resolution of the micro-IR spectrum and is identical in absorption pattern with that reported by Hofmann (9) for iso-LAA.

Discussion

Lysergic acid amide and isolysergic acid amide have been isolated and identified as the principal ergoline alkaloids in Hawaiian baby wood rose. The comparative fluorescent intensities of the lysergamides and their relative color intensities when sprayed with the DMBA reagent would indicate that the iso-form is the predominant isomer. Unfortunately, the use of methanol in the isolation procedure precludes any general statement as to which isomer is more prevalent in the seed. The elution of the lysergamides from the aluminum oxide absorbent with chloroform-methanol and subsequent evaporation to dryness may have caused some rearrangement of LAA to iso-LAA. LAA dissolved in methanol recrystallizes as its stereoisomer iso-LAA (11). Further investigations into the identity of the various clavine alkaloids believed present in Hawaiian baby wood rose are planned.

Acknowledgments

The author wishes to thank Kurt Rupke, Food and Drug Administration, Los Angeles, for his assistance in translating Hofmann's paper from German; Donald Dechert, Food and Drug Administration, Los Angeles, for providing literature references; Dr. Albert Sperling, Special Testing and Research Laboratory, Washington, D.C., for providing authentic lysergic acid amide; Mary Mandujan, Food and Drug Administration, Los Angeles, for editorial review of this paper; and William Krueger, Flowers of Hawaii, for providing authentic Hawaiian baby wood rose.

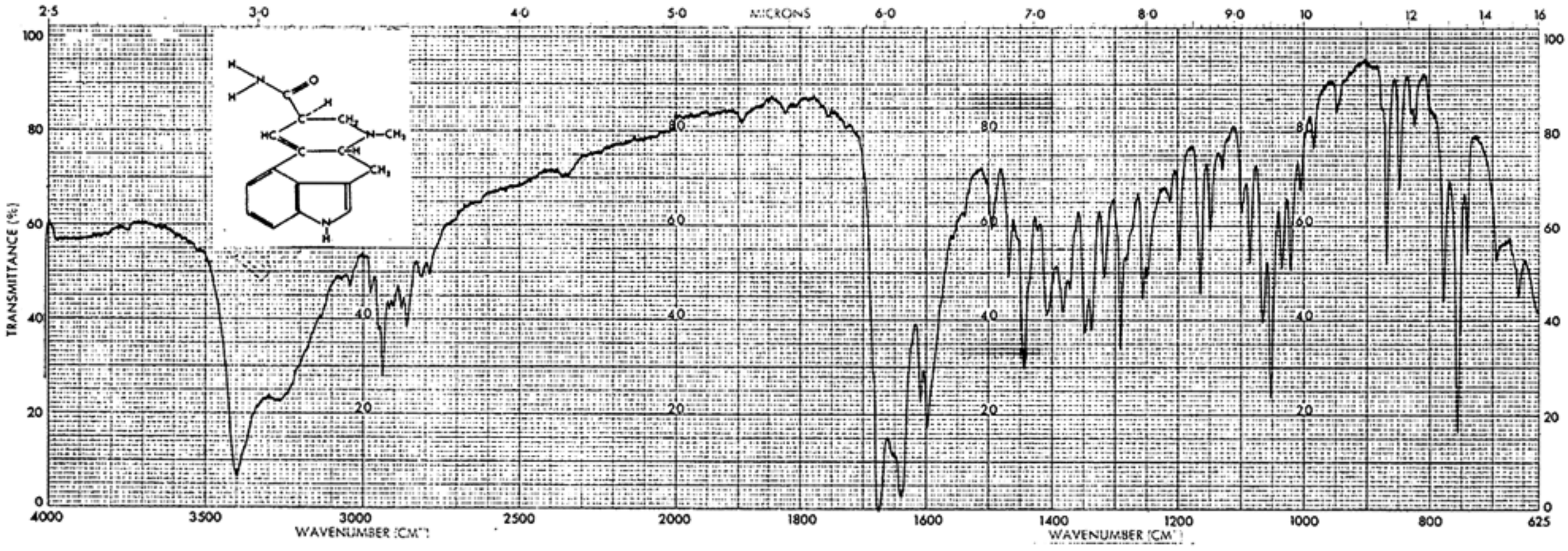

FIG. 3—IR spectrum of LAA isolated from baby wood rose.

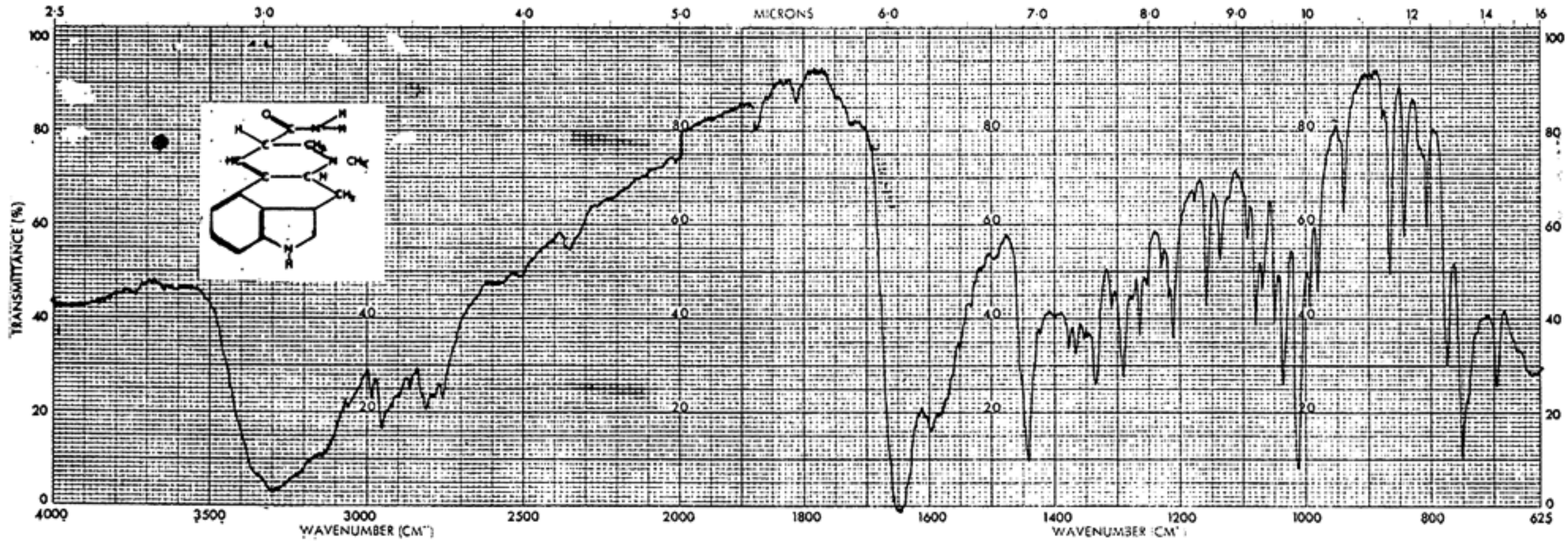

FIG. 4—IR spectrum of iso-LAA isolated from baby wood rose.

References

(1) McJunkins, S. P., Thornton, J. I., and Dillon, D. J., *Forensic Sci. Soc.* **8**(2 & 3), 121–124 (1968).

(2) Communication, *Microgram*, Vol. 1, No. 10, Bureau of Narcotics and Dangerous Drugs, U.S. Department of Justice (Sept. 1968).

(3) Ingram, A. L., Jr., *J. Amer. Med. Ass.* **190**, 1133–1134 (1964).

(4) Cohen, S., *Amer. J. Psychiat.* **102**, 1024–1025 (1964).

(5) Taber, W. A., Vining, L. C., and Heacock, R. A., *Phytochemistry* **2**, 65–70 (1963).

(6) Marderosian, A. D., and Youngken, H. W., *Lloydia* **29**(1), 35–41 (1966).

(7) Genest, K., *J. Chromatog.* **19**, 531–539 (1965).

(8) Hylin, J. W., and Watson, D. P., *Science* **148**, 499–500 (1965).

(9) Hofmann, A., *Planta Medica* **9**, 354–367 (1961).

(10) Stoll, A., and Hofmann, A., *Helv. Chim. Acta* **38**, 421–433 (1955).

(11) Stoll, A., and Hofmann, A., *The Alkaloids*, Vol. 8, R. H. F. Manske (Ed.), Academic Press, New York, 1965, p. 746.