celium was transferred to two 500-ml. flasks each containing 50 ml. of NL 500 (13) made up with water containing about 3.5% ^{18}O excess. After 7 days of fermentation the alkaloid yield was 65 mg. In both experiments an aliquot of the culture filtrate was distilled to recover water for ^{18}O analysis. From the remainder, chanoclavine-I was isolated as described previously (13).

^{18}O analyses were carried out by pyrolysis of the organic compounds at 650° in a sealed glass tube with break tip (14). For the analysis of water, naphthalene was added as a carbon source. The tubes were opened in a vacuum system connected to the inlet of an Atlas M 86 mass spectrometer. CO_2 was condensed with liquid nitrogen and the noncondensible gases were pumped off. The CO_2 was then introduced into the mass spectrometer and analyzed for its ^{18}O content. As a reference for the natural ^{18}O content, the same chemical compounds containing no ^{18}O excess were analyzed.

RESULTS AND DISCUSSION

The results given in Table I show that the incorporation of oxygen from water into the hydroxyl groups of both chanoclavine-I and elymoclavine is negligible. Since the ^{18}O enrichment of the water was determined after the fermentation, the possibility can be excluded that extensive dilution of the ^{18}O by oxygen of other components of the medium is responsible for this result. The oxygen of the hydroxyl groups of these two alkaloids must therefore originate from molecular oxygen. Attempts to confirm this directly in the case of elymoclavine have been made using *Claviceps* strain SD-58. However, on incubation under an artificial atmosphere containing ^{18}O enriched oxygen gas, the metabolism of the fungus changed drastically, resulting in a consistently low alkaloid production. Nevertheless, the conclusion seems to be valid that the introduction of the hydroxyl groups into these alkaloids does not involve reaction of OH^- with a carbonium ion generated by hydride abstraction from an allyl position. The results suggest that the oxidation reactions involved in ergot alkaloid formation occur by an initial hydroxylation, possibly by a mixed function oxygenase. In the case of the oxidative cyclizations, these hydroxylations may be followed by phosphorylation and phosphate elimination to generate the reactive carbonium ion.

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New Alkaloid from *Lobelia portoricensis* Urban

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A major alkaloid has been isolated from the leaves of *Lobelia portoricensis* Urban. Alkaloids are extracted with ether in alkaline ammoniacal solution, an average of 1.25 percent in the dried leaves. The crystalline alkaloid has a melting point of 115–116°, the hydrochloride, m.p. 187–188°, perchlorate, m.p. 156–158°, and the picrate, m.p. 175–176°. The new alkaloid showed the same sensitivity to reagents as lobeline. Paper chromatography, ascending process, was employed. Stationary phase consisted of formamide, ammonium formate, and formic acid; the mobile phase, equal parts of benzene and chloroform; R_f 75. Infrared absorption spectra showed a R—CO—aromatic organization and the presence of a —NH group; no CH_3 or OH group as in lobeline. The empirical formula is $C_{21}H_{23}NO_{23}$. The formula is shown in structure I. The new alkaloid was found to stimulate the respiratory center.

AMONG THE various members of the *Lobeliaceae* in Puerto Rico, there grows in the wet mountain forests a medium size handsome endemic plant, commonly named tupa and tibey tupa, the *Lobelia portoricensis* Urban, *Tupa portoricensis* Vatke or *Tupa assurgens* A.DC. (1).

The plant is described by Britton (2) and also in *Linnaea* (3) and by Stahl (4). A complete study of the anatomy and histology of the plant is being conducted by the author for publication.

Leaves were collected while the plants were blooming and dried in an air-drier at 45° and powdered in a ball grinder. The material was sifted through a No. 40 sieve.

The powdered material was extracted in a Soxhlet extractor using 200-Gm. samples each time. The material was macerated with a mixture of 4 vol. of strong ammonia T.S., 5 vol. of ethanol, 10 vol. of ether, and mixed well. It was extracted with ether

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until the ether in the Soxhlet became almost colorless and the last extractions gave a negative reaction with the usual alkaloidal reagents.

The liquid extract was evaporated on a water bath and the residue shaken with 50 ml. of ether and 150 ml. of 0.5 *N* sulfuric acid and evaporated again to remove the ether. The acid solution was filtered through a dry filter and the filtrate transferred to a separator. The residue was dissolved again in ether and 0.5 *N* sulfuric acid, shaken, and filtered. This procedure was repeated until a small portion of the last filtrate gave a negative test for alkaloids. Silicotungstic acid reagent was used.

The combined filtered portions in the separator were made slightly alkaline with ammonia T.S. and extracted with 40-ml. portions of ether.

The alkaloids present in the combined ether extracts were purified by the addition of 20-ml. portions of 0.5 *N* sulfuric acid, the separation of the aqueous layer, and extraction with ether in slightly alkaline solution.

The combined ether extracts were evaporated *in vacuo* at a temperature of 40° until the volume was markedly reduced. The temperature was changed to about 20° and the evaporation continued. Abundant crystallization was observed. The last liquid residue, about one-tenth the original volume, was also collected. The crystals in the flask were carefully washed with a small portion of ether and the washings added to the liquid residue collected. The crystals were dried *in vacuo* over phosphoric acid. The melting point of the dried crystalline alkaloidal base was 115–116°. The determination was repeated after the material was recrystallized.

Preparation of Various Alkaloidal Salts—Crystalline hydrochloride, picrate, and perchlorate were prepared and carefully purified, m.p. 187–188°, 175–176°, and 156–158°, respectively.

Quantitative Alkaloidal Determination—The quantitative determination of the alkaloids was performed using the principles of Lynch and Evers (5), who recommended a procedure involving their careful isolation and titration with 0.02 *N* sulfuric acid. The official method of the "Pharmacopoeia of Japan" (6) for lobelia using a Soxhlet extractor was followed. It was found that the leaves contain an average of 1.25% alkaloids.

Sensitivity of the New Alkaloid to Several Common Alkaloid Precipitating Reagents—Martello and Farnsworth (7) published a detailed study on the sensitivity of several common alkaloid precipitating reagents. Their observations included alkaloids of broad spectrum of the various groups.

Seven different alkaloidal reagents for the pyridine–piperidine group of alkaloids were selected for testing the new alkaloid. Five drops of the alkaloidal reagent was added to 1 ml. of a 1 mg. in 100 ml. aqueous solution of the alkaloid dissolved in tartaric acid solution. The amount and nature of the precipitate was observed. Mercuric chloride 1% solution gave a negative result. The rest of the reagents gave a positive reaction in the following order: phosphomolybdic acid reagent, only slightly positive reaction; picric acid, silicotungstic acid, Dragendorff, Mayer, and Wagner reagent.

The same reagents were respectively added to a 1-ml. solution of lobeline sulfate of the same concentration. The same results were obtained.

Chromatography—Several studies in chromatography with the lobelias and their alkaloids are reported in the literature. Steinegger and Ochsner determined the alkaloids in *Lobelia salicifolia* (8) and in *L. inflata* (9) using chromatographic procedures. They also described a method for the determination of lobeline in small quantities in alkaloidal extracts followed by spectrometric work (10). Schmidt and Steinegger (11) reported the separation of the alkaloids of *L. syphilitica* with the aid of paper and column chromatography. In 1959 Steinegger and Ochsner (12) reported new alkaloids in *L. salicifolia* also by means of paper chromatography.

Paper chromatography, ascending process, was employed for the separation of the alkaloids in the extract of *L. portoricensis*. Scheibler and Schull paper No. 204-B was used.

The stationary phase consisted of a solution of 9 vol. formamide saturated with ammonium formate, the excess salt precipitated with 20 vol. of acetone, and the mixture filtered and 1 vol. of formic acid added to the filtrate. The mobile phase best adapted for the resolution of the alkaloids consisted of a mixture of equal parts of benzene and chloroform.

Microburets with ether–formamide solution of the total alkaloidal extract of the leaves of *L. portoricensis*, crystals of the main alkaloid, and residue of the extract after the main alkaloid was separated were used.

A run of about 6 hr. in the solution at a room temperature of 24° sufficed. Then it was placed in the drying oven at a temperature of about 85° for about 1 hr.; it was then examined with ultraviolet light. Alkaloidal spots were clearly seen. Dragendorff reagent with Muniere's modification (13) was used for the development of the chromatogram. Orange-colored precipitates were obtained.

In the first column where the total alkaloidal extract was placed, good resolution of the alkaloids was obtained. A distinct spot of the main alkaloid was observed in the upper part away from the rest of the alkaloids. Six other small size spots were observed.

In the second column where the main alkaloid was placed, a large spot was observed just at the same level as the main spot in the upper part of the first column. Both spots consisted of the same alkaloid. Repeated procedures revealed an R_f of 75.

In the third column, where the rest of the alkaloidal extracts were placed, successive spots were observed at the same level as those of the total alkaloidal extract. No spot was observed in the upper part of the paper in this case, a confirmation that the main alkaloid was already separated and observed in the second column.

Chromatographic studies were conducted under the same conditions with the total alkaloidal extract of *L. inflata*, lobeline alkaloid, lobelanine, lobelanidine, lobelidine, and the total alkaloids of *L. portoricensis*.

Upon examination of the chromatogram of these alkaloidal materials, it was observed that none of the alkaloidal spots were located in a position near or at the same level as the main alkaloid of *L. portoricensis*. Their R_f's were less than 50.

Preliminary Chemical Tests—Chemical tests were

performed with the new alkaloid and with lobeline to test for the formation of acetophenone in alkaline solution. Nitroprussate 5% solution and glacial acetic acid were employed. A positive reaction was obtained; it suggested a lobeline type of alkaloid.

The presence of a carbonyl nucleus was confirmed by the formation of an oxime, which was prepared with various mg. amounts of the crystalline alkaloid. The crystalline compound obtained has a melting point of 237°.

Spectrometric Studies—Infrared absorption spectra analysis was conducted with the pure alkaloid using also pure lobeline for comparison (Fig. 1.). A Perkin-Elmer infrared instrument, model 125, was employed as well as a Perkin-Elmer nuclear magnetic resonance, 60 Mc. apparatus.

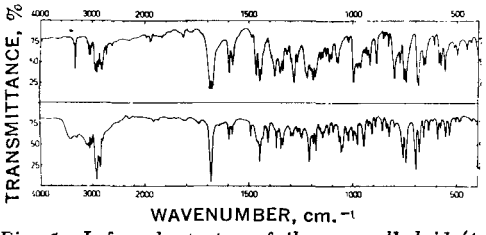
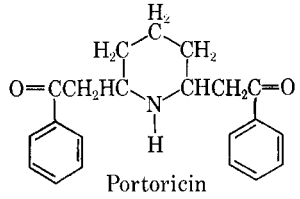


Fig. 1—Infrared spectra of the new alkaloid (top) and lobeline (bottom).

The spectra showed the presence of benzene rings at 1601, 1587, 1495, and 1452 cm.$^{-1}$ confirmed by the presence of small bands at 3085 and 3005 cm.$^{-1}$.

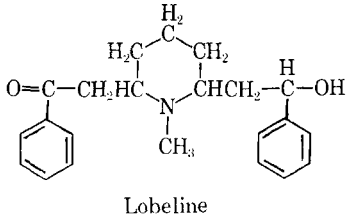
The band at 1690 cm.$^{-1}$ showed valence vibration of —CO groups, in R—CO—Ar organization. The band at 3260 cm.$^{-1}$ is due to the presence of a —NH group. No CH_3 group as in lobeline was indicated nor any —OH group. The suggested empirical formula for the new alkaloid of *L. portoricensis* is $C_{21}H_{23}NO_2$.

The formula suggested is shown in structure I and for lobeline in II.



Portoricin

I



Lobeline

II

Pharmacologic Studies—Though lobelia was introduced into medicine as an emetic by the Rev. Mannasse Gutler in 1775, the first important pharmacologic work was done by Von Ott in 1875. The separation of the alkaloids was done by Heinrich Wieland in 1921 and the studies of their pharmacologic properties by Herman Wieland and Mayer in 1922 (13). Alpha lobeline, lobelidine, and the base B were found. It was determined that α-lobeline has a marked effect on the respiration and at the same time is free from the emetic action which characterizes the administration of the crude lobelia preparations.

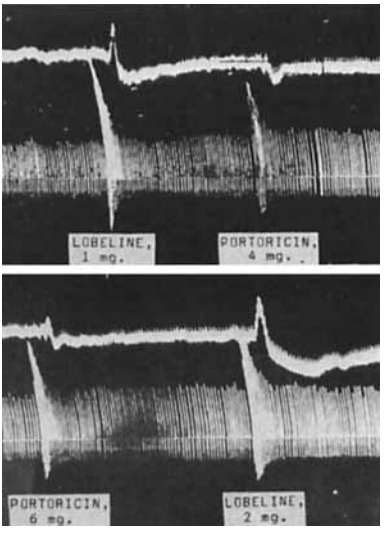


Fig. 2—Action of the new alkaloid on the respiration and blood pressure in dogs.

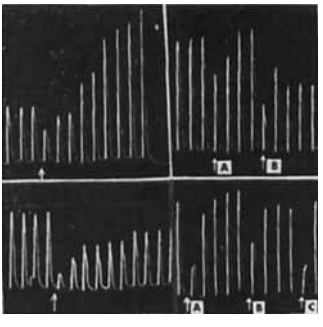


Fig. 3—Action on parasympathetic nervous system of the guinea pig intestine. Contractions with acetylcholine, 0.4 ml. solution, 2:1,000 each, 4 min. Top left: 0.8 mg. portoricin. Top r ght: A, *0.4 mg. lobeline;* B, *0.2 mg. portoricin. Bottom left: 0.8 mg. lobeline. Bottom right:* A, *0.002 mg. atropine sulfate;* B, *0.200 mg. portoricin:* C, *0.001 mg. atropine sulfate.*

Action of the New Alkaloid on the Respiration and Blood Pressure in the Dog—Dogs were anesthetized with pentobarbital using 50 mg./Kg. intravenously and the animal positioned for respiration and blood pressure records. Twenty milligrams of the alkaloid was dissolved in 20 ml. of distilled water with the aid of an equivalent quantity of tartaric acid. Different doses were injected intravenously. A solution of lobeline of the same concentration was prepared and injected periodically to compare the action in the anesthetized dog.

It was clearly shown that the alkaloid stimulates the respiratory center, though the effect is not so marked as with the same concentration of lobeline.

However, the effect in lowering the blood pressure was very slightly demonstrated. (Fig. 2.)

Action in the Cat—Cats were anesthetized with chloralose, 150 mg./Kg. Respiration and arterial pressure were recorded when the alkaloidal solution was injected through the femoral vein. Intravenous injection of the alkaloid stimulates the respiration, though not so marked as when administered to dogs. The action is stronger in the case of lobeline.

Action on the Isolated Intestine of the Guinea Pig—It was demonstrated that the alkaloid blocks the action of acetylcholine in a way similar to atropine, though in a lesser degree, approximately 1/200. Lobeline exerts less activity than the alkaloid (Fig. 3).

Action on the Isolated Intestine of the Rabbit—Trendelenburg technique was employed. When 30 mcg. of the alkaloid was used, inhibition of intestinal motility was observed. With an increased dosage of 100 mcg., the effects were stronger and persisted even after several washings.

Action on the Bronchi of the Isolated Lungs of the Guinea Pig—Konsett technique on the isolated lungs of the guinea pig was used, except that for the registration of the bronchial tonus a water manometer was employed instead of the Piston-Rekorder. Part of the experiments were done on the opened thorax and the rest on the intact organ. Results did not vary, especially when the animal received adequate anesthesia. The experiment was repeated many times.

Results showed that neither the alkaloid nor lobeline inhibited the bronchiospasms produced by the intravenous injection of 12.5 and 25 mcg. of histamine. These effects were neutralized by some synthetic antihistaminics in low concentration.

LD_{50} Determination—White mice in sets of six were used, each of uniform size and weighing 20 to 25 Gm. The determination was carefully performed. The LD_{50} was found to be 95 mg.

SUMMARY

A major alkaloid has been isolated from the leaves of *Lobelia portoricensis* Urban, an endemic woody plant of the wet mountain forests of Puerto Rico.

The alkaloids are extracted with ether in an alkaline ammoniacal medium, an average of 1.25% in the dried leaves. The main alkaloid is separated in monoclinic prisms upon the partial vacuum evaporation of the ether extract at low temperature. The crystalline alkaloid has a melting point of 115–116°. The crystalline hydrochloride has a m.p. of 187–188°; the crystalline alkaloidal perchlorate, m.p. of 156–158°, and the crystalline alkaloidal picrate, m.p. of 175–176°.

The new alkaloid exhibits the same sensitivity as lobeline to the alkaloidal reagents applied to the alkaloids with a pyridine or piperidine structure. This alkaloid was used to compare results and showed the same degree of sensitivity as the new alkaloid in the following order: Wagner, Mayer, Dragendorff, silicotungstic acid, picric acid, and phosphomolybdic acid reagents.

The new alkaloid is separated with paper chromatography, ascending process, using a liquid phase which consisted of formamide, ammonium formate, and formic acid. The mobile phase consisted of a mixture of equal parts of benzene and chloroform. The alkaloid separates in the uppermost portion with an R_f of 75 and quite far from the next six visible much smaller spots.

Infrared absorption spectra showed the presence of benzene rings, a carbonyl structure in a R—CO— aromatic organization, and the presence of a —NH group. No CH_3 or OH group as in lobeline is indicated.

The name portoricin is suggested for the new alkaloid. The suggested empirical formula is $C_{21}H_{23}NO_2$ and the developed formula is shown in structure I.

It stimulates the respiratory center in various animals, particularly in the dog, though the effect is not so marked as with lobeline in the same concentration. However, it has only a very slight effect in lowering the blood pressure.

It inhibits the action of acetylcholine on the isolated intestine of the guinea pig and the rabbit.

Neither the new alkaloid nor lobeline inhibited the bronchiospasms produced by the intravenous injections of small amounts of histamine.

The LD_{50} was determined with white mice and was found to be 95 mg.

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