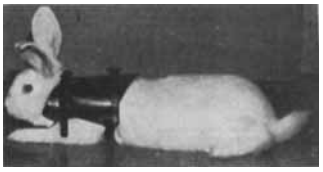

Fig. 2—Control animal in Newmann harness.

All of the animals treated with a pyridinethione salt, either in DMSO or water, showed skin irritation at the site of application which cleared when the treatment was terminated.

DISCUSSION

Dimethylsulfoxide apparently aided in the penetration of both NaPT and ZnPT in comparison with water. The sodium salt readily penetrated the skin when applied in an aqueous solution, but when applied in DMSO, the onset of toxic signs was earlier and paralysis and death resulted earlier in the treatment regimen. The animals all lost weight which is attributed to their inability to eat because of the hind quarter paralysis.

In the ZnPT treated group, DMSO apparently facilitated percutaneous absorption with the production of toxic signs and, in most cases, death. ZnPT in water did not produce paralysis or death except in two cases where the animals were observed to lick applied material from the sides of their cages. A precaution that must be taken in percutaneous studies is to avoid the transfer of applied material from the backs of animals to the sides of the cages. If this occurs, the study becomes an oral ingestion study and not a percutaneous study. In all other cases the material remained on the backs of the animals and was not observed on the sides of the cages. Adequate-size cages can prevent the transfer in most cases.

DMSO apparently did not aid the penetration of CdPT. The present study indicates an apparent lack of penetration regardless of the vehicle. As indicated in Table III, two animals developed toxic signs and lost weight, but recovered completely when treatment ended. All animals were comparable with the water control grossly, except for the animal treated with CdPT in an aqueous vehicle. This animal had lost considerable weight, but returned to normal weight within 1 week after treatment ended. Whether or not the skin was abraded apparently had no effect on penetration. Further study of CdPT in DMSO and aqueous vehicles is planned to gain evidence for the lack of penetration of this salt.

The concurrent use by the public of DMSO and other drugs, cosmetics, and soap products can be a potential hazard, as this study and others indicate. Substances that do not penetrate the skin and have been shown to be safe may become hazardous if applied concurrently with DMSO. This may be the strongest indication for restrictions on the use of DMSO regardless of the safety of DMSO itself.

As a continuation of this study and in an attempt to explain the difference in penetration between the two insoluble salts, ZnPT and CdPT, when applied in DMSO, an analytical method for the analysis of pyridinethione in tissues is in progress. A possible explanation for the difference in penetration is that Cd^{2+} is known to have a higher affinity for sulfur in the lower oxidation states, *i.e.*, S^{2-}, while Zn^{2+} does not show so great an affinity. This might cause a stronger complex with hydroxypyridinethione (PT) as compared with DMSO for Cd^{2+}, and the reverse of this for Zn^{2+}, thereby freeing the pyridinethione for penetration.

REFERENCES

(1) Snyder, F. H., Buehler, E. V., and Winek, C. L., *Toxicol. Appl. Pharmacol.*, **7**, 425(1965).
(2) Winek, C. L., and Buehler, E. V., *ibid.*, **9**, 269(1966).
(3) Jacob, S. W., Bischell, M., and Herschler, R. J. *Current Therap. Res.* **6**, 193(1964).
(4) Jacob, S. W., *Headache*, **5**, 78(1965).
(5) Kligman, A. M., *J. Am. Med. Assoc.*, **193**, 140(1965).
(6) Newmann, E. A., *Lab. Animal Care*, **13**, 207(1963).

Biosynthesis of Ergot Alkaloids. Origin of the Oxygens of Chanoclavine-I and Elymoclavine

By H. G. FLOSS, H. GUENTHER*, D. GROEGER†, and D. ERGE†

The oxygen of the hydroxyl groups of chanoclavine-I and elymoclavine has been shown not to be derived from water. This indicates that these hydroxyl groups do not originate from the reaction of an allylic carbonium ion with water, but may be introduced by direct hydroxylation.

ERGOT ALKALOIDS are formed from tryptophan and mevalonic acid (1, 2). A hypothetical biogenetic scheme which accounts for the experimental results so far available has been proposed recently (Scheme I) (3). The formation of the tetracyclic ergoline skeleton, as well as many of the known alkaloid interconversions, involves a number of oxidative cyclizations and hydroxylations. As pointed out by Agurell *et al.* (4), most of these reactions can be accounted for by oxidations at positions allylic to the exocyclic double bond. Very little experimental evidence about the mechanism of these oxidations is, however, available. Ramstad, Taylor, and their co-workers (5) have shown that the conversion of agroclavine to setoclavines and of elymoclavine to penniclavines can be brought about by horseradish peroxidase in the presence of hydrogen peroxide. This oxidation was

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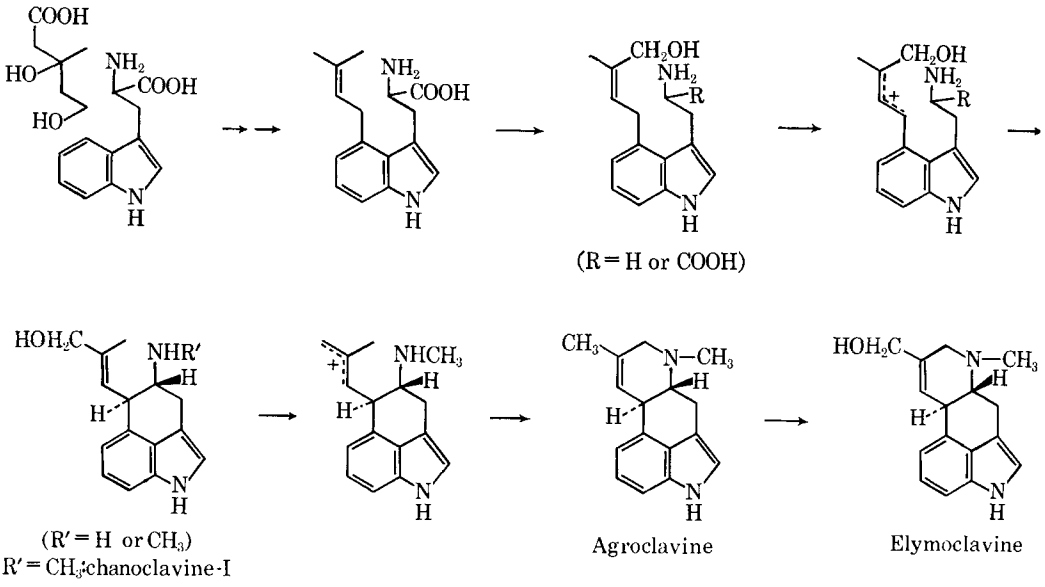

Hypothetical Scheme of Ergot Alkaloid Formation

Scheme I

shown to involve an initial attack at position 10 and rearrangement of the product to give the 8-hydroxylated compound (5). It is also known that these conversions cannot only be carried out by the ergot fungus but also by a whole variety of other organisms (6–10). However, agroclavine was not converted to elymoclavine by either the peroxidase system or by the organisms that do not produce ergot alkaloids. These findings suggest that the mechanism of 8-hydroxylation differs from that of the other oxidations involved in ergot alkaloid formation.

It seems to be a reasonable assumption that the oxidative cyclizations involve formation of allylic carbonium ions as the reactive species. Evidence for this, for instance, is the fact that the hydroxymethyl group of chanoclavine-I reacts with the amino nitrogen to form ring D (11, 3). Several mechanisms can, however, be discussed for the formation of these carbonium ions. The above-mentioned conversion of chanoclavine-I to agroclavine would suggest that initially a hydroxyl group is introduced at an allylic position, followed by phosphorylation and phosphate elimination. In this case the oxygen of the hydroxyl groups would originate from molecular oxygen. It has, however, not been assessed with certainty that chanoclavine-I is a natural intermediate in ergot alkaloid formation. It may only be in equilibrium with an intermediate, the corresponding allylic carbonium ion. In this case, the latter could be formed by hydride abstraction from the allylic position. Reaction of the carbonium ion with the nitrogen would lead to agroclavine, whereas its reaction with water would lead to chanoclavine-I. The oxygen of the hydroxyl group would then originate from the oxygen of the water. The same considerations hold for the formation of elymoclavine from agroclavine, except that cyclization is not possible here.

In order to distinguish between these possibilities the authors have conducted experiments in ^{18}O enriched water and determined the incorporation of ^{18}O into chanoclavine-I and elymoclavine.

METHODS

Claviceps strain SD-58 was grown for 3 days in the medium given previously (12). The mycelium from three 100-ml. shake cultures was then transferred under sterile conditions into a flask containing 110 ml. of the same medium made up with water containing about 1.3% excess ^{18}O. After 14 days of fermentation the culture was harvested and found to contain 40 mg. of alkaloid. The culture filtrate was lyophilized to recover the ^{18}O water for analysis. The residue was dissolved in normal water, and elymoclavine was isolated and purified from this solution (12).

Two fermentations were carried out to produce chanoclavine-I using *Claviceps paspalum* strain Li 342/SE 156. The fungus was grown for 8 days in medium NL 563 (13). In the first experiment the mycelium was then transferred to a 500-ml. flask containing 100 ml. of the following medium (NL 619), made up with water containing about 0.6% excess ^{18}O: ammonium succinate, 3 Gm.; sorbitol, 5 Gm.; propylene glycol, 2 ml.; KH_2PO_4, 0.1 Gm.; $MgSO_4$, 0.03 Gm.; water to 100 ml.; pH adjusted to 5.4 with HCl. After 5 days of fermentation in this medium the alkaloid yield was 44.6 mg. In the second experiment the my-

TABLE I—INCORPORATION OF ^{18}O FROM $H_2{}^{18}O$ INTO ELYMOCLAVINE AND CHANOCLAVINE-I BY *Claviceps*

Claviceps Strain Used	Alkaloid Isolated	Atom % ^{18}O Excess in Water of Medium	Atom % ^{18}O Excess in Isolated Alkaloid	Atoms of Oxygen Incorporated
SD 58	Elymoclavine	1.111	0.006	0.005
Li 342/SE 156	Chanoclavine-I	0.496	0	0
Li 342/SE 156	Chanoclavine-I	3.397	0.021	0.006

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.

REFERENCES

(1) Weygand, F., and Floss, H. G., *Angew. Chem. Intern. Ed.*, **2**, 243(1963).
(2) Agurell, S., *Acta Pharm. Suecica*, **3**, 71(1966).
(3) Floss, H. G., Hornemann, U., Schilling, N., Groeger, D., and Erge, D., *Chem. Commun.*, **1967**, 105.
(4) Agurell, S., Ramstad, E., and Wolinsky, J., *Svensk Farm. Tidskr.*, **66**, 741(1962).
(5) Ramstad, E., personal communication.
(6) Brack, A., Brunner, R., and Kobel, H., *Helv. Chim. Acta*, **45**, 276(1962).
(7) Yamano, S., Kozu, Y., Yamada, S., and Abe, M., *Ann. Repl. Takeda Res. Lab.*, **21**, 88(1962).
(8) Groeger, D., *Planta Med.*, **4**, 444(1963).
(9) Beliveau, J., and Ramstad, E., *Lloydia*, **29**, 234(1966).
(10) Taylor, E. H., Goldner, K. J., Pong, S. F., and Shough, H. R., *ibid.*, **29**, 239(1966).
(11) Fehr, T., Acklin, W., and Arigoni, D., *Chem. Commun.* **1966**, 801.
(12) Floss, H. G., and Groeger, D., *Z. Naturforsch.*, **18b**, 519(1963).
(13) Groeger, D., Erge, D., and Floss, H. G., *ibid.*, **21b**, 827(1966).
(14) Guenther, H., Simon, H., and Floss, H. G., unpublished data.

New Alkaloid from *Lobelia portoricensis* Urban

By ESTEBAN NÚÑEZ MELÉNDEZ*, LUIS CARRERAS, and JOSÉ RUÍZ GIJÓN

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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