from time to time. No harm results if the grains loosen from the agar and crumble as the culture ages. The agar frequently loosens from the bottom of the dishes, whereupon the amoebae will be found in large numbers on the agar film and on the glass bottom of the vessel. It is of interest to note that the amoebae, seldom if ever, are found attached to the under-surface of the dislodged agar films.

Syracuse watch glasses, containing about 8 cc distilled water, may be used instead of finger bowls. These dishes, with the bottom covered with agar, as described for the finger bowls, and with three grains of rice anchored in each, may be seeded with 15 to 25 amoebae from an old culture.

M. B. SHEIB

NEW YORK UNIVERSITY

# SPECIAL ARTICLES

## ON AN ALKALOID FROM ERGOT

DURING the past few months a new development in the chemistry and pharmacology of ergot has occurred through the almost simultaneous reports of the isolation of a new, apparently simpler and clinically more useful alkaloid from the fungus; the ergometrine of Dudley and Moir,[1] the ergotocin of Kharasch and Legault,[2] the alkaloid of Thompson,[3] and very recently the ergobasine of Stoll and Burckhardt.[4] Because of our own investigations of the structure of the ergot alkaloids, we have attempted to satisfy ourselves of the possible relationship of the new alkaloid or alkaloids to the lysergic acid group—ergotinine, ergotamine and ergoclavine—already studied by us.

The ergot powder residue which remained after it had been exhausted in the usual manner of its ether-soluble alkaloids was extracted with hot dilute acid (sulfuric or lactic acid). After neutralization of this extract with sodium carbonate and extraction with chloroform, a substance was obtained in very small yield which was characterized by its very sparing solubility in chloroform. It gave the usual color reactions of the ergot alkaloids. It was recrystallized by solution in methyl alcohol, addition of chloroform and concentration to remove the methyl alcohol. The same substance was obtained from fresh ergot powder by adhering to the exact directions as given by Dudley and Moir. Since the substance melted at 154°, it was assumed at first that we were dealing with the same alkaloid[5] described by Dudley and Moir and Kharasch and Legault. However, since further study of our material has given results which are not in agreement with the analytical results of these workers or with the rotation reported by the former, and since none of the substances of these workers was available to us for direct comparison, the question of identity must be left open for the present.

However, through the kindness of The Sandoz Company we have very recently received a small sample of the ergobasine (as the tartrate) of Stoll and Burckhardt. After isolation of the free base from this salt and crystallization from chloroform, it appeared to be identical with our own material. The former showed $[\alpha]_D^{25} = +32°$, and the latter $[\alpha]_D = +30°$ in methyl alcohol. The analytical results obtained with both samples were also in agreement. When crystallized from chloroform it separates with 1 mol of chloroform. $C_{19}H_{23}O_2N_3 \cdot CHCl_3$. Calculated C 53.98, H 5.44, Cl 23.93. Found C 54.54, H 5.83, Cl 22.83. Thus, these figures are in agreement with the formula presented by Stoll and Burckhardt, who recrystallized their alkaloid from alcohol. Because of the very limited supply of our own material, we have not employed this solvent.

Most important, however, has been the study of the hydrolysis of our alkaloid, which we had practically concluded before the publication of Stoll and Burckhardt. The results obtained appear definitely to substantiate the above formulation. On hydrolysis with 12.5 per cent. KOH in 50 per cent. alcohol, lysergic acid was obtained by our usual procedure and melted at 230°. Calculated: C 71.69, H 6.00. Found: C 71.49, H 5.60. No ammonia was produced during the hydrolysis, and no isobutyrylformic or pyruvic acids could be detected. After removal of most of the lysergic acid the material remaining in the mother liquor was subjected to hydrolysis with strong acid, a procedure heretofore employed by us where amino-acids were to be obtained and which also destroyed the residual lysergic acid. Instead, however, of an amino-acid a base resulted which was isolated as the sulfate. This salt proved to be the sulfate of an amino propanol $(C_3H_9ON)_2H_2SO_4$. Calculated: C 29.00, H 8.12. Found: C 29.25, H 8.05. This was substantiated by the preparation of a di-p-bromobenzoate which melted at 155°, $C_{17}H_{15}O_3NBr_2$. Calcu-

[1] H. W. Dudley and C. Moir, *Brit. Med. Jour.*, 1: 520, 1935.

[2] M. S. Kharasch and R. R. Legault, SCIENCE, 81: 388, 1935; *Jour. Am. Chem. Soc.*, 57: 956, 1140, 1935.

[3] M. R. Thompson, *Jour. Am. Pharm. Assoc.*, 24: 24, 185, 1935; SCIENCE, 81: 636.

[4] A. Stoll and E. Burckhardt, *Compt. rend.*, 200: 1680, 1935.

[5] The results of a preliminary study of our material which was perhaps erroneously considered by us to be ergometrine are soon appearing in the *Journal of Biological Chemistry*.

lated: C 46.26, H 3.43. Found: C 46.27, H 3.66. $[\alpha]_D^{28} = +48°$ (C = 0.125 in pyridine). The natural thought at once occurred that this base might have a biogenetic relationship to alanine. Hydroxyisopropylamine (2-aminopropanol-1) was prepared by reduction of l-alanine ester with sodium in butyl alcohol. The resulting base gave a di-p-bromobenzoate (C 45.97, H 3.75), which melted at 155° and showed no depression with the above derivative obtained from the alkaloid. However, $[\alpha]_D^{28} = -41°$ (C = 0.415 in pyridine). Therefore, the alkaloid amine is the natural form corresponding to d-alanine.

The alkaloid obtained by us (the ergobasine of Stoll and Burckhardt) is therefore the hydroxyisopropylamide of lysergic acid. In the formation of this substance, as in the case of the other ergot alkaloids studied by us, amino-acids or their derivatives are involved. It will be of interest to see what relationship to this alkaloid ergotocin will be shown to possess. Kharasch and Legault have given for it a formula $C_{21}H_{27}O_3N_3$, which thus differs from the former by $C_2H_4O$ (or $C_2H_5OH$, alcohol?). The analyses of ergometrine reported by Dudley and Moir were made with the alkaloid recrystallized from benzene and could possibly have contained solvent.

WALTER A. JACOBS
LYMAN C. CRAIG

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH,
NEW YORK

## A SEROLOGICAL ESTIMATE OF THE ABSOLUTE CONCENTRATION OF TOBACCO MOSAIC VIRUS

ROBBINS[1] has assumed the molecular weight of tobacco mosaic virus to be in the neighborhood of 100,000, judging by the filtration studies of Duggar and Karrer, Waugh and Vinson, and MacClement and Smith. On the basis of infectivity tests on *Nicotiana glutinosa,* Caldwell[2] has estimated the concentration of infective particles to be of the order of $3 \times 10^7$ per cubic centimeter. If these two assumptions are correct, according to Robbins's calculations, the concentration of tobacco mosaic virus is such that 1 milligram is contained in 200,000 liters of expressed juice.

The most highly antigenic substance known, according to the serological literature, is the soluble specific carbohydrate of Pneumococcus, which gives a positive precipitin test with immune serum at about 1:2,500,000 (= .0004 milligrams per cubic centimeter). The most highly antigenic protein known is egg albumin, which gives a positive precipitin test at about 1:250,000 (= .004 milligrams per cubic centimeter). The writer has submitted evidence indicating that expressed saps of mosaic-diseased tobacco plants give precipitin titers of about 1:250,[3] and that it is the tobacco mosaic virus itself which is the antigen involved in this reaction.[4]

If tobacco mosaic virus sap is no more antigenic than the most highly antigenic substance known, the Pneumococcus carbohydrate, and if the minimal precipitating concentration of Pneumococcus carbohydrate is .0004 mg/cc, then it follows, *ipso facto,* that 1 cubic centimeter of tobacco mosaic expressed sap contains at least 0.1 milligram of virus (.0004 milligram × 250).

One might question the advisability of comparing the serological behavior of tobacco mosaic virus, which is entirely specific, with that of the Pneumococcus carbohydrate, which exhibits a striking lack of source-specificity. The serological behavior of egg albumin is more typically comparable with that of tobacco mosaic virus. Comparable precipitin tests of egg albumin and tobacco mosaic virus have been performed by the writer, and the results gave a precipitin titer of 1:250,000 for egg albumin. Comparing the precipitin titer of tobacco mosaic virus with that of egg albumin, one finds that if tobacco mosaic virus is no more antigenic than egg albumin, 1 cubic centimeter of virus sap would contain at least 1 milligram of virus antigen. From the serological results with tobacco mosaic virus and on the basis of the premises stated above, it is hence estimated that the concentration of virus in expressed virus sap is no less than 0.1–1.0 milligram per cubic centimeter.

Furthermore, it is known that crude tobacco mosaic sap when diluted to 1:1,000,000 gives approximately one necrotic lesion per leaf on *Nicotiana glutinosa,* and that about 0.1 cubic centimeter of sap dilution is used in making the inoculation. From these facts, and assuming that Robbins's hypothesis of a molecular weight of 100,000 is correct for tobacco mosaic virus, it would follow that a cubic centimeter of virus sap contains $6.06 \times 10^{14}$ to $6.06 \times 10^{15}$ molecules of virus, and that a single minimal infective dose on *N. glutinosa* corresponds to 60–600 million molecules of virus antigen. The enormous ratio of 60–600 million virus molecules to a single infection, if our premises are correct, may be due to (1) a great loss of opportunity to infect in the cases of the myriads of virus particles which fail to fall in a position suitable for infection, *i.e.,* on the naked protoplast exposed in the breaking of a leaf hair, and (2) a possible aggregation of particles such that many antigenic molecules may coalesce, thus greatly reducing the actual number of discrete infective units.

KENNETH STARR CHESTER

THE ROCKEFELLER INSTITUTE
FOR MEDICAL RESEARCH
PRINCETON, N. J.

[1] William J. Robbins, SCIENCE, 80: 275, 1934.
[2] John Caldwell, *Ann. Appl. Biol.,* 20: 100, 1933.
[3] Kenneth S. Chester, *Phytopath.* (in press).
[4] *Ibid.*