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# 4-β-AMINOETHYLGLYOXALINE (β-IMINAZOLYLETHYLAMINE) AND THE OTHER ACTIVE PRINCIPLES OF ERGOT



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# CCLXV.—4-β-*Aminoethylglyoxaline* (β-*Iminazolylethylamine*) *and the other Active Principles of Ergot.**

By George Barger and Henry Hallett Dale.

For many years ergot has been notorious among drugs on account of the ignorance and division of opinion concerning the nature of its active principles. The problem had, indeed, approached solution in 1875, with Tanret's discovery of ergotinine and Buchheim's suggestion that ergot owes its activity to decomposition products of proteins produced by putrefaction. This discovery and suggestion were largely obscured by the work of subsequent investigators, which resulted rather in the physiological characterisation of impure products (sphacelinic acid, sphacelotoxin, etc.) than in chemical isolation of active principles.

Of late years, however, a considerable measure of agreement has been reached. The alkaloid ergotoxine (Barger and Carr) was also found by Kraft, who named it hydroergotinine, and the formulæ assigned to ergotinine and ergotoxine (Trans., 1907, **91**, 337) have been confirmed by Tanret and by Kraft respectively. There is also a consensus of opinion regarding the effect of ergotoxine on the blood-vessels and uterus, and its activity in producing gangrene.

Thus ergot, in common with many drugs, contains a complex physiologically active alkaloid. In addition, however, there are present in ergot a number of simpler bases, derived from amino-acids by the elimination of carbon dioxide. Such bases are generally formed in putrefaction. Ergot, as a fungus, is more closely related to bacteria than to the higher plants, from which all other important vegetable drugs are derived. Thus the peculiar nature of these active principles of ergot is due to its peculiar systematic position in the vegetable kingdom.

Putrefaction bases were first isolated from ergot by Rieländer (*Sitzungsber. Ges. Naturw. Marburg*, August 5th, 1908), who found putrescine and cadaverine, which only have a feeble physiological action. The first markedly active base of this class, *p*-hydroxyphenylethylamine, was isolated from ergot by ourselves (Barger and Dale, *Proc. Physiol. Soc.*, May 15th, 1909, in *J. Physiol.*, 1909, **38**, lxxvii; Barger, Trans., 1909, **95**, 1123). It is formed from tyrosine during putrefaction, but appears to be present also in fresh ergot. It is the chief pressor constituent of most aqueous ergot extracts, but does not produce contraction of the isolated uterus of the non-pregnant cat. In addition, we showed that *iso*-

* A preliminary note on this subject was read at the meeting of May 26th, 1910.

amylamine (from leucine) is probably present in ergot, but in such proportion that it makes no significant contribution to the physiological action.

After the isolation of *p*-hydroxyphenylethylamine, there still remained unaccounted for the powerful action of certain aqueous ergot extracts in producing contraction of the isolated uterus, even of the non-pregnant cat, as observed by Kehrer. Since it was found quite impossible to remove the active substance from aqueous solution by means of organic solvents, a precipitation method had to be employed, and the ergot extract was subjected to the process worked out by Kutscher for the isolation of bases from meat extract. In this way we obtained a minute quantity of a crystalline picrate which gave Pauly's reaction with *p*-diazobenzenesulphonic acid, and exhibited in an intense degree the physiological action in question. It was not histidine picrate, for histidine was found to be inert; we therefore supposed it to be the picrate of 4-β-aminoethylglyoxaline (β-iminazolylethylamine), the base which would result from histidine by the loss of carbon dioxide:

$$\begin{matrix}CH{=}N\\ |\\ NH{\cdot}CH\end{matrix}\!\!>C{\cdot}CH_2{\cdot}CH(NH_2){\cdot}CO_2H = \begin{matrix}CH{=}N\\ |\\ NH{\cdot}CH\end{matrix}\!\!>C{\cdot}CH_2{\cdot}CH_2{\cdot}NH_2 + CO_2$$

and we confirmed this supposition by chemical and physiological comparison with a specimen of 4-β-aminoethylglyoxaline, very kindly sent us at our request by Dr. D. Ackermann, who a short time before had obtained this base by the putrefaction of histidine (*Zeitsch. physiol. Chem.*, 1910, **65**, 504). Simultaneously with ourselves, Kutscher (*Zentr. Physiol.*, 1910, **24**, 163) obtained a very active base from ergot, which he considered to be closely related to 4-β-aminoethylglyoxaline, although not identical with it, on account of a supposed difference in the physiological action of the two bases. It has, however, recently been shown that the differences in physiological action observed by Kutscher were presumably due to differences in the animals employed. One and the same base, whether from ergot or from histidine, can be made to produce the different effects described by Kutscher. On the other hand, the base from ergot and that from histidine, when tested successively on the same animal, gave identical effects. We have also analysed the picrate of the base from ergot, and have compared it and the picrolonate with the corresponding salts of 4-β-aminoethylglyoxaline (from histidine). As a result, we maintain our original conclusion (Proc., 1910, **26**, 128) that, contrary to Kutscher's view, the base in question is identical with 4-β-aminoethylglyoxaline. It is therefore the second active principle of ergot belonging to the class of putrefaction bases derived from amino-acids. Its physiological activity is very great. A marked contraction of the isolated uterus

is produced by adding to the bath sufficient of the base to give a concentration of one part in 25 million parts of Ringer's solution; the effect of one part in 250 millions is often quite definite.

A third active principle of this class was quite recently found in ergot by Engeland and Kutscher (*Zentr. Physiol.*, 1910, **24**, 479). This is agmatine, $NH_2 \cdot C(:NH) \cdot NH \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot NH_2$, discovered in herring roe by Kossel (*Zeitsch. physiol. Chem.*, 1910, **66**, 257). Its relation to arginine is analogous to that of 4-$\beta$-aminoethylglyoxaline to histidine, and it is said to have a similar action on the uterus.

## Experimental.

Among the ergot preparations examined by Kehrer, the *ergotinum dialysatum* of Wernich is one of the most potent. We soon found, in making this extract on a small scale, that the activity of the substance which passed through the dialysing membrane finally exceeded that of the original extract, suggesting that more of the active principle was being formed by an enzyme or by bacteria. The active principle is, however, also present in perfectly fresh ergot, for the physiological effect was produced by a sample of ergot grown by ourselves and tested within half an hour of plucking. The effect was also produced to a smaller extent by commercial extracts of meat and of yeast, and this observation led to the adoption of Kutscher's method for the isolation of bases from meat extract. To 500 c.c. of commercial dialysed ergot extract, 500 c.c. of a 20 per cent. tannin solution were added, which quantity just ensured complete precipitation; next day the clear, supernatant liquid was decanted, and freed from tannin by the addition of barium hydroxide; after filtration, the excess of barium hydroxide was removed by dilute sulphuric acid, and the excess of sulphuric acid, together with the last traces of tannin, were precipitated by adding a suspension of freshly prepared lead hydroxide. After filtration, the liquid was concentrated to 300 c.c., and acidified with phosphoric acid. After adding excess of silver nitrate (400 c.c. of a 20 per cent. solution) and filtering, we found that the whole of the active substance was in the filtrate. To the latter, 150 c.c. more silver nitrate were added, when a drop of the solution at once produced a brown precipitate of silver oxide on mixing with barium hydroxide. The whole of the solution was then precipitated with barium hydroxide, until a sample, on filtration, gave only a slight opalescence with ammoniacal silver nitrate. This precipitate (silver II of Kutscher's method) in one preliminary experiment contained nearly the whole of the active substance, but afterwards it was found convenient to add at once to the filtrate from the first silver precipitate enough barium hydroxide for complete precipitation,

thus collecting together silver precipitates II and III of Kutscher.

After washing, the silver precipitate was carefully suspended in very dilute sulphuric acid, and decomposed by hydrogen sulphide. The filtrate from the silver sulphide was freed from hydrogen sulphide, neutralised, and evaporated to dryness. The residue was extracted several times with hot ethyl alcohol, in which the active principle was found to be sparingly soluble; a large quantity of inert matter was left behind. The residue remaining on evaporating the alcoholic solution was dissolved in a little water, and a hot saturated aqueous solution of picric acid was added. After keeping for some days, a brown, imperfectly crystalline picrate was collected, washed, and recrystallised from water. This picrate was converted into a solution of the hydrochloride, which was very active physiologically, and gave an intense red coloration with sodium *p*-diazobenzenesulphonate (Pauly's reaction), suggesting a relationship to histidine. We had previously detected some activity in the crude histidine mother liquors obtained by hydrolysis of hæmoglobin with hydrochloric acid; histidine itself was found to be inactive, but it became so to a slight extent on heating to 300°. We were thus led to suppose that the picrate we had isolated was that of 4-β-aminoethylglyoxaline. After two crystallisations from water, the picrate from ergot formed dark yellow, rhombic plates, melting and decomposing at 234—235°. A specimen of the picrate sent us by Dr. D. Ackermann, when heated simultaneously in a tube attached to the same thermometer, also melted at 234—235°, and when recrystallised by evaporation of the solution in a desiccator, yielded rhombic plates exactly similar to those of the ergot base. Windaus and Vogt (*Ber.*, 1907, **40**, 3695) give the melting point as 239° on rapid heating, and the same crystalline form for a synthetic specimen of 4-β-aminoethylglyoxaline dipicrate. For analysis, the picrate was dried at 100° until constant:

0·0590 gave 0·0776 $CO_2$ and 0·0192 $H_2O$. C = 35·8; H = 3·6.

$C_5H_9N_3(C_6H_3O_7N_3)_2$ requires C = 35·8; H = 2·6 per cent.

We also prepared the very sparingly soluble picrolonate of the ergot base; it decomposed at 261° (Windaus and Vogt give 266° for 4-β-aminoethylglyoxaline dipicrolonate).

As the physiological action of the ergot base is also the same as that of 4-β-aminoethylglyoxaline, there is no room for doubt that the two bases are identical.

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