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ISOLATION AND SYNTHESIS OF *p*-HYDROXYPHENYLETHYLAMINE AN ACTIVE PRINCIPLE OF ERGOT SOLUBLE IN WATER

BY

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CXXVII.—*Isolation and Synthesis of* p-*Hydroxyphenylethylamine, an Active Principle of Ergot soluble in Water.*

By George Barger.

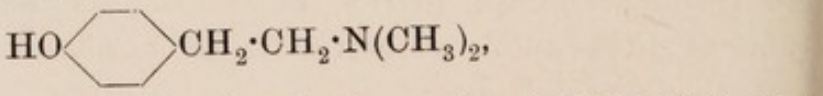
In a paper on the alkaloids of ergot, F. H. Carr and the author (Trans., 1907, **91**, 337) described the amorphous alkaloid ergotoxine, to which many of the characteristic physiological effects of ergot are due. This alkaloid is, however, only present in small quantities in most specimens of the pharmacopœial preparations of ergot, and, in discussing the latter, H. H. Dale and the author (*Biochem. J.*, 1907, **2**, 286) postulated the presence of a second active principle soluble in water. Attempts to isolate this hypothetical principle were for a long time unsuccessful. Vahlen (*Arch. exper. Path. Pharm.*, 1906, **55**, 131) seems also to have been aware of the existence of such a principle, but his so-called "clavin" has been shown to be an inert mixture of amino-acids, in which leucine predominates (Barger and Dale, *Biochem. J.*, 1907, **2**, 288).

The physiological properties of *p*-hydroxyphenylethylamine, recently isolated from putrid meat by Barger and Walpole (*J. Physiol.*, 1909, **38**, 343), suggested that this base might be the above-mentioned active principle of aqueous ergot-extracts. It has indeed been possible to prove that *p*-hydroxyphenylethylamine occurs in such extracts, and that the presence of this base accounts in a satisfactory manner for such of the activity as is not due to small quantities of ergotoxine (see Barger and Dale, *Proc. physiol. Soc.*, May 15th, 1909; the physiological experiments have been performed by Dr. H. H. Dale). The method of isolation, described below, was based on the process employed in the case of putrid meat, but required further elaboration on account of the large amount of resinous constituents of ergot which are soluble in water.

p-Hydroxyphenylethylamine has figured several times in chemical literature. It was first prepared in small quantities by Schmitt and Nasse (*Annalen*, 1865, **133**, 214), who obtained it by heating tyrosine. Subsequently it was isolated by Emerson (*Beitr. chem. Physiol. Path.*, 1902, **1**, 501) from autolysed pancreas, and by Langstein (*ibid.*, 1902, **1**, 507) in prolonged peptic digestion of egg-albumin. Gautier (*Bull. Soc. chim.*, 1906, [iii], **35**, 1195) isolated the base, together with a lower and a higher homologue, from the mother liquors obtained in the putrefaction of cod-livers. He suggested for these bases the general name "tyrosamine," since they may be considered as being derived from tyrosine and its

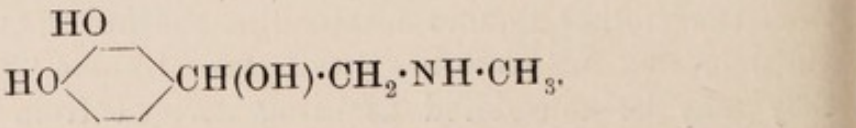
homologues by loss of carbon dioxide. That *p*-hydroxyphenylethylamine is indeed one of the products of the action of bacteria on tyrosine was shown by Barger and Walpole (*loc. cit.*), and their recognition of its physiological action enabled Rosenheim (*J. Physiol.* 1909, **38**, 337) to trace the substance in placental extracts, the action of which on blood pressure and uterus was first observed by Dixon and Taylor (*Brit. Med. Journ.*, 1907, ii, 1150). Rosenheim also showed that such extracts are only active if the placenta has undergone a certain amount of putrefaction. Similarly, Van Slyke and Hart (*Amer. Chem. J.*, 1903, **30**, 8) found *p*-hydroxyphenylethylamine in Cheddar cheese, but not in cheese prepared under sterile conditions. Quite recently the base was also isolated from Emmenthaler cheese by Winterstein and Künz (*Zeitsch. physiol. Chem.*, 1909, **59**, 138).

In all the above investigations, *p*-hydroxyphenylethylamine was only obtained in small quantities (generally as the dibenzoyl derivative). Even the method of preparation by the destructive distillation of tyrosine yields only small quantities. After the powerful and interesting physiological action of the substance became known, and after the substance had been recognised as the chief active principle of aqueous extracts of ergot, it became desirable to have a more convenient method of preparation, and this was found in the reduction of *p*-hydroxyphenylacetonitrile. The latter substance had already been prepared by Pschorr, Wolfes, and Buckow (*Ber.*, 1900, **33**, 171), and readily yielded the desired amine on reduction with sodium and alcohol. It thus became possible to study the properties of the substance a little more closely, and particularly to investigate its behaviour towards methylating agents. Since the alkaloid hordenine, obtained by Léger (*Compt. rend.*, 1906, **142**, 108) from malt germs, is regarded as having the structure



its synthesis, by methylation of *p*-hydroxyphenylethylamine, was attempted. By the action of methyl iodide on this base, a quaternary iodide is readily obtainable, which proved to be identical with hordenine methiodide. It is hoped that an account of these experiments, which are as yet incomplete, may be given later.

Attention may also be drawn to the somewhat close chemical and physiological relationship between the active principle of ergot under discussion and the active principle of the adrenal gland,



Experimental.

Isolation of p-*Hydroxyphenylethylamine from Ergot.*

Preliminary experiments had shown that the active principle in question could not be removed to any appreciable extent from aqueous extracts by extraction with ether, chloroform, or ethyl acetate. Nor could the substance be obtained by precipitation; it was, indeed, carried down to a large extent whenever a bulky precipitate was formed, as, for instance, by basic lead acetate, but it could not be recovered from these precipitates. In amyl alcohol, a solvent was, however, found which extracted the active principle from an aqueous extract, rendered alkaline by sodium carbonate, but not from one containing a sufficient quantity of sodium hydroxide or hydrochloric acid. The active substance therefore behaved like a phenolic amine. In the case of putrid meat, the purification, suggested by these properties, was sufficient to allow of the crystalline dibenzoyl derivative being obtained at once, but in the case of ergot this was not so by any means. A certain portion of the large quantity of inert matter, which still accompanied the active principle after the use of amyl alcohol, was removed by precipitation in alcoholic solution by mercuric chloride. Its final isolation, however, only became possible by utilising the (very slight) solubility of the substance in ether, which solubility had originally been overlooked. Eventually the following process was employed:

The aqueous extract from 1·5 kilos. of ergot was concentrated to 375 c.c. in a vacuum on the water-bath; it was then rendered alkaline with sodium carbonate and extracted ten times with 150 c.c. of amyl alcohol (a very tedious process on account of the formation of emulsions). The amyl alcohol extract was evaporated to 200 c.c., and extracted ten times with 30 c.c. of 1 per cent. sodium hydroxide. The sodium hydroxide was neutralised with hydrochloric acid and evaporated to dryness, and the residue was extracted with absolute alcohol, which left sodium chloride behind. The alcoholic filtrate, measuring 250 c.c., was precipitated with a saturated alcoholic solution of mercuric chloride until no further immediate precipitate occurred (about 10 c.c. were required). After filtration by the aid of the pump, the filtrate was concentrated; the remainder of the alcohol was removed by distillation in a current of steam, so that the solution was never evaporated to dryness. A precipitate, insoluble in water, was formed, and was collected; the excess of mercury was then removed by hydrogen sulphide, and the solution was concentrated to 30 c.c.

This solution, which was still strongly pigmented, was very active

physiologically, 0·5 c.c. producing a large rise of blood-pressure. At each step in the purification, it was shown by physiological experiment that the loss of activity was only of such an order as the manipulations necessarily entailed. In the same way it was shown that the active substance could be removed from solution as an insoluble benzoyl derivative, and recovered by hydrolysis, but it was impossible to crystallise the benzoyl derivative or to separate it from the complex mixture of other benzoyl derivatives. The final purification was made possible by the observation that the active principle is very slightly soluble in ether.

To the above-mentioned concentrated solution sufficient sodium hydroxide was added to make it semi-normal; the solution was then extracted ten times with half its volume of ether. This ethereal extract possessed no physiological activity. After neutralising the aqueous solution and rendering it slightly alkaline with sodium carbonate, it was again extracted ten times with half its volume of ether. On evaporation the ether left behind 0·1 gram of a dark brown, syrupy residue, which gave an intense Millon reaction, and was very active physiologically. The aqueous solution was again extracted ten times with ether, and the ethereal extract was found to possess about one-quarter of the activity of the first extract, showing that presumably nearly all, but not all, the active substance had been removed; in accordance with this the aqueous solution was now found to possess a very slight activity, much less than that of the second extract. The conclusion therefore seems justified that the whole of the active substance is (very slightly) soluble in ether.

The first ethereal extract containing the bulk of the active substance was dissolved in 3 c.c. of 10 per cent. sodium hydroxide, and benzoylated by the Schotten-Baumann method. The dark brown benzoyl derivative was boiled in alcoholic solution with animal charcoal, and separated as an amorphous, pigmented solid. This was redissolved in hot alcohol, and then separated overnight in large, almost white, sphæro-crystals, which were collected and washed with alcohol. These crystals melted at 167°. On mixing with an equal weight of synthetic dibenzoyl-*p*-hydroxyphenylethylamine (m. p. 170°), the melting point was 168·5°.

The amount of the crystalline benzoyl derivative thus obtained was too small to admit of analysis. 4·5 Milligrams were hydrolysed by boiling with 20 per cent. hydrochloric acid for twelve hours; after removal of the hydrochloric and benzoic acids, the solution gave an intense coloration with Millon's reagent, and was found to have a powerful physiological effect, almost as large as that of pure synthetic *p*-hydroxyphenylethylamine

The slight difference in effect was doubtless due to a small portion of the benzoyl derivative not having been fully hydrolysed, or to decomposition during the prolonged boiling with acid.

After the active principle had thus been identified, 0·12 gram of synthetic *p*-hydroxyphenylethylamine was added to the inactive ergot solution, and the extraction with ether was repeated exactly as before. In the first ten extractions, 0·08 gram was now removed, and in the second ten extractions 0·02 gram, thus showing a close agreement in the partition between ether and water to that observed before by physiological means.

It is, indeed, possible, by using a sufficient quantity of ether, to show that a crude aqueous ergot extract contains a phenolic base giving the Millon reaction, but as the amount present is wholly insignificant compared with that of the inert substances accompanying it, this method would scarcely be suitable for the identification of the substance. It will be clear from the above that it is very difficult to form an estimate of the amount of *p*-hydroxyphenylethylamine in ergot by chemical means, but on physiological grounds this amount would appear to be of the order of 0·1—0·01 per cent.

Synthesis of p-*Hydroxyphenylethylamine from* p-*Hydroxyphenylacetonitrile.*

The preparation of *p*-hydroxyphenylacetonitrile from phenylacetonitrile proceeded in close agreement with the description given by Pschorr, Wolfes, and Buckow (*loc. cit.*), except that the yield on diazotisation fell somewhat short of that given by these authors, and amounted at most to 60 per cent. The intermediate products were used in their crude condition.

For the reduction, 5 grams of *p*-hydroxyphenylacetonitrile are dissolved in a small quantity of absolute ethyl alcohol, and 7—10 grams of sodium (2—3 times the theoretical quantity) are added in small pieces. The solution is kept boiling and as concentrated as possible; when sodium ethoxide separates out, more alcohol is added to dissolve it. When all the sodium has dissolved, the solution is neutralised with hydrochloric acid and evaporated to dryness. The residue, consisting mostly of sodium chloride, is extracted with absolute alcohol, and from this alcoholic solution *p*-hydroxyphenylethylamine hydrochloride can be precipitated with ether. It is, however, preferable to purify the free base. For this purpose, sodium carbonate is added to the alcoholic solution of the hydrochloride, and the solution is evaporated to dryness on the water-bath under diminished pressure; the residue may be further dried by evaporation with absolute alcohol, and is then extracted

with boiling xylene until no more of the base crystallises from the filtered extracts on cooling. It is not easy to obtain the base perfectly white by recrystallisation, but this may be readily done by distillation, the boiling point being 161—163°/2 mm. and 175—181°/8 mm. For this a suitably shaped receiver is necessary, on account of the high melting point of the substance.

When crystallised from alcohol, *p*-hydroxyphenylethylamine forms hexagonal leaflets, m. p. 161°. It is soluble in about 10 parts of boiling ethyl alcohol, somewhat less in boiling water, very much less in boiling xylene, and hardly soluble in cold xylene. Xylene is a convenient solvent for recrystallisation, as it does not dissolve the resinous impurities. According to Gautier, the base is soluble in 95 parts of water at 15°. According to Schmitt and Nasse, *p*-hydroxyphenylethylamine is easily decomposed, but this the author is unable to confirm. The base gives Millon's and Mörner's reactions for tyrosine. When benzoylated in 10 per cent. sodium hydroxide with excess of benzoyl chloride, the dibenzoyl derivative, m. p. 170°, previously prepared by Emerson, is obtained. With one molecular equivalent of benzoyl chloride a certain amount of the N-*monobenzoyl* derivative, $OH \cdot C_6H_4 \cdot CH_2 \cdot CH_2 \cdot NH \cdot CO \cdot C_6H_5$, is formed, which crystallises from alcohol in hexagonal plates, melting at 162°, and is somewhat more soluble than the dibenzoyl derivative:

0·1300 gave 6·5 c.c. N_2 (moist) at 15° and 750 mm. N = 5·8.

$C_{15}H_{14}O_2N$ requires N = 5·8 per cent.

When picric acid is added to a hot aqueous solution of *p*-hydroxyphenylethylamine, a *picrate* melting at 200° slowly crystallises in short prisms.

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