CXXIII. RESEARCHES ON THE FERMENTATION OF DRIED TOBACCO.

I. THE METHODS FOR SEPARATING NICOTINE AND AMMONIA.

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OUR object in this research was to separate the nicotine from the other volatile nitrogen-containing bases contained in extracts or expressed juices from tobacco leaves, and to estimate them. In the earlier processes insufficient consideration has been given to this separation.

Kissling's method [1920] as usually carried out has the disadvantage that when extracting the leaves with ether, ammonia among other things also passes into the solvent; comparative tests made by us have shown this to be the case in a manner which places the result beyond the possibility of doubt; further, the distillate obtained by Kissling's method always gives the familiar Nessler's ammonia reaction, whilst pure nicotine gives with Nessler's reagent only a white precipitate, which on the addition of the slightest amount of ammonia becomes coloured.

Toth [Kissling, 1920, p. 98] seeks to avoid the presence of ammonia by allowing the latter to be adsorbed by gypsum, but according to Rasmussen's [Kissling, 1920, p. 99] and Schröder's [1911] investigations the Toth process gives inexact results. König extracts tobacco leaves with toluene and estimates the nicotine polarimetrically. Toth [1911], however, holds this method to be unsuitable because dark-coloured solutions are unpolarisable.

Bertrand and Javilliers [1909] precipitate the nicotine as silicotungstate, decompose this compound in a current of steam and ascertain its amount by titration. This method was unsuitable for our purposes because it did not permit of the estimation of the ammonia; moreover silicotungstic acid is an expensive reagent and difficult to obtain.

Harrison and Self [1912] estimate the ammonia and nicotine obtained by steam-distillation of tobacco after the addition of alkali, absorbing the distillate in titrated acid and then titrating back; they then precipitate the nicotine with iodine and, after destroying the colour of the iodine by means of thio-sulphate, distil off the ammonia. The difference between the two titration values gives the nicotine content.

We used the following method. The nicotine, in the mixture of ammonia and nicotine obtained from tobacco leaves by direct steam-distillation, is, after making the liquid weakly acid with sulphuric acid, precipitated by means of a solution of mercuric iodide in potassium iodide (3 g. per 100 cc. water). Under these conditions, ammonia gives no precipitate. The precipitate is allowed to remain overnight and is washed by decantation; it has a wax-like crystalline character. The filtrate obtained is quite clear and free from nicotine. The precipitate can be estimated by the Kjeldahl method, whilst the ammonia can be distilled over from the filtrate in the usual way, an excess of potassium iodide being added before making alkaline to prevent the formation of a Nessler precipitate and consequent loss of ammonia.

For this series of researches it is sufficient to know the total titration value of the volatile nicotine and ammonia, and then, after precipitating the nicotine, to estimate the ammonia content of the filtrate. The difference between the two titration values gives the nicotine content.

The following figures show that this method gives satisfactory results.

We examined a control solution made up as follows:

20 cc. nicotine (pur. prep. Merck) + 20 cc. $N/10$ NH_3 + 60 cc. $N/10$ H_2SO_4 .

The nicotine solution was of about 2 % concentration, 10 cc. corresponding to 13.2 cc. $N/10$ H_2SO_4 .

(Here and in the following the titrations were made against sodium alizarinsulphonate as indicator.)

20 cc. of this mixture were treated in the way described above.

After distillation the filtrate gave a titre corresponding to 4.4 cc. $N/10$ H_2SO_4 . The nicotine precipitate gave after a Kjeldahl determination a titre equivalent to 5 cc. $N/10$ H_2SO_4 .

It was found that when one commenced with the same quantity of nicotine, without however adding ammonia, a titration value corresponding to 0.3 cc. $N/10$ H_2SO_4 was always obtained. (Each determination was repeated several times.)

After correcting the experimental value the following data emerge (cc. $N/10$ H_2SO_4):

Value	Nicotine	Ammonia
Calculated	5.3	4.0
Found experimentally	5.0	4.4
Corrected	5.3	4.1

It appears that the nicotine preparation (Merck) contains, though in very small amount, some nitrogen-containing impurities. Thus, for example, pyridine in weakly acid solution gives just the same reaction as nicotine towards KI-mercuric iodide but in contrast to it is readily soluble in excess of the precipitating agent and is therefore driven over and estimated with the ammonia.

The method reduces itself to two distillation processes:

(1) Driving over the nicotine together with the ammonia by steam-distillation.

(2) Distilling over the ammonia out of the filtrate from the nicotine mercury compound (as in the usual NH_3 -distillation method).

In the application of this method to tobacco extracts there is of course the danger that the value in the first distillation may be too high, because besides nicotine and ammonia, other more or less volatile bases may pass over at the same time.

If these are also precipitated by the nicotine-precipitating agent, too high a nicotine value will be obtained, since the ammonia values (in the second distillation) will be correct.

If, however, no precipitation occurs, then conversely accurate nicotine values but too high ammonia figures are obtained (*e.g.* with pyridine).

Usually the latter will be the case, so that therefore the value, which when working with tobacco is calculated as NH_3 , will generally be more correctly expressed as the value obtained for volatile non-nicotine bases.

Finally, it is to be observed that it is part of our object to find purely physical methods of separation, of practical feasibility, which will next be dealt with.

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