were diluted with 100 cc. of water before heating and titrating. A number of comparative titrations showed that the use of undiluted urine gives low results.

This modified method was compared with the official volumetric method for phosphoric acid and found to give concordant results. Combustions for the official method were made by the Osborne method.

Of the 46 samples of urine analyzed by the uranium acetate, and the official volumetric methods, the results obtained on 6 samples were identical; one sample showed a maximum difference of $\pm$ 0.015 gram P_2O_5, the mean of all the results being $\pm$ 0.004 gram P_2O_5 in 100 cc. urine.

[CONTRIBUTION FROM THE BIOCHEMIC DIVISION, BUREAU OF ANIMAL INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.]

ESTIMATION OF NICOTINE IN PRESENCE OF PYRIDINE.

BY JAMES A. EMERY.

FOR several years past the writer has made examinations of tobacco powders and extracts submitted by manufacturers throughout the United States to the Bureau of Animal Industry for the endorsement of that Bureau, as to whether certain requirements, recommended by the United States government, were being complied with in regard to the percentage of nicotine present in their products. For the dipping of sheep for scab the dips now approved by the Department of Agriculture are "The Tobacco and Sulphur Dip," and "The Lime and Sulphur Dip."

The regulations, as established for the first named dip, require that it shall be "made with sufficient extract of tobacco or nicotine solution to be a mixture containing not less than five one-hundredths of one per cent of nicotine and 2 per cent. of sulphur."

For the convenience of stockmen, transportation companies, and others, who were compelled by the regulations as cited in the Bureau of Animal Industry, Order No. 108, to use a dip, certain manufacturers in the various States have prepared highly concentrated extracts of tobacco, containing varying percentages of nicotine to be diluted according to the directions accompanying them, so that the resultant liquid will conform with the requirements of the government demand.

These concentrated extracts have been submitted, from time to time, by the manufacturers for analysis as to the percentage of nicotine contained in them, and, until of late, all volatile bases distilling with steam in the ordinary Kissling process were calculated as nicotine. Recently, however, it was found that manufacturers of some of these concentrated preparations were fortifying their product with pyridine, thereby increasing the apparent amount of nicotine, as indicated by the usual process of its estimation. It became necessary, therefore, to devise some method by which, in mixtures of the two bases, the true percentage of nicotine present could be estimated.

As the two bases are so closely allied in their chemical behavior, the problem of estimating one in the presence of the other presented considerable difficulty.

As it is well known that pyridine is unaffected by treatment with chromic or fuming nitric acids, such means being employed to free it from aniline and other impurities of a like character, the attempt was made to destroy the nicotine in a similar manner, after first determining the amount of volatile bases present, and from the results to calculate the percentage thus decomposed. The attempts, however, was unsuccessful, as it was found that a large percentage of nitrogenous bases, which were volatile with steam, resulted from this decomposition, thereby interfering with the result. The action of strong hydrochloric acid and crystals of potassium chlorate was next tried, but similar difficulties were encountered.

As solutions containing nicotine are known to affect polarized light, an attempt was made along this line to effect a method of determination. Solutions of pyridine were found to be optically inactive and as a result a method, which will be described later, for the accurate determination of nicotine was devised. At the same time a fairly accurate determination of the pyridine may be made by subtracting the nicotine figure found, from the total volatile bases, as determined by the Kissling process. The rotary power of nicotine has been used by Propovici as a basis for its quantitative estimation.[1] In his method the nicotine is extracted from the tobacco by Kissling's process, and the ether extract treated with a rather strong solution of phosphomolybdic acid in nitric acid,

[1] *Ztschr. phys. Chem.*, **13**, 445 (1889).

shaken, and the ether poured off from the quickly subsiding precipitate. To this precipitate is then added sufficient water to bring it to a given volume, and the nicotine obtained as a free base in alkaline solution by adding barium hydroxide and shaking frequently for several hours. The solution is then poured off from the yellow precipitate and polarized, and the resulting reading in minutes compared with a table obtained from experiments with known amounts of nicotine in solution.

According to Pribram[1] the value of Sd at 20° for pure nicotine is —161.55°.[2]

The rotation diminishes rapidly, but irregularly on dilution. In a 4 per cent. solution the value of Sd was —77.03°, while below this strength an increase was observed, Sd being —79.32° for a solution containing 0.8826 per cent. nicotine. In the same article it is stated that the rotation is affected by time, not reaching its maximum for forty-eight hours.

In the solutions with which the accompanying experiments were made by the writer, the percentage of nicotine did not exceed 1.5 per cent., and with a sugar scale reading to tenths, the degree of rotation of solutions containing varying small percentages of nicotine has been directly proportional to the amount of nicotine contained. The rotating power of a solution of nicotine (about 0.5 per cent.) was taken, but no change was noted after several days. The method which has been employed for estimating nicotine in the presence of pyridine, and which depends, as previously stated, upon the rotary power of nicotine, is as follows:

Weigh out, in a small beaker, about 5 grams of the tobacco extract, or 20 grams of dry, finely powdered tobacco. To this add 10 cc. of a solution containing 6 grams of sodium hydroxide in 60 cc. of 90 per cent. alcohol and 40 cc. of water, followed, in the case of the tobacco extract, by a sufficient quantity of dry chemically pure calcium carbonate to form a moist but not lumpy mass. The whole is well mixed and transferred to a Soxhlet extractor and extracted with ether for five hours. The ether is evaporated at a low temperature on the steam-bath, care being taken that the last portion is not heated sufficiently to volatilize the nicotine or pyridine.

[1] *Ber. d. chem. Ges.*, **20**, 1840.

[2] Attention should be called to the error in Allen's "Commercial Organic Analysis," Vol. III, Part II, 2nd edition, page 180, in which the value Sd of —161.55° is given for a 20 per cent. aqueous solution of nicotine, and a value Sd of —79.32° for a solution of nicotine of 0.8826 specific gravity.

To the residual liquid in the extraction flask add 50 cc. of an approximately N/10 sodium hydroxide solution and transfer to a round-bottomed flask of about 300 cc. capacity, by means of 150 cc. of water. Distil in a current of steam, passing the vapor through a well-cooled condenser, the condensing tube being allowed to dip in a small amount of water in the receiving flask. A three-bend outflow tube is used and a few pieces of pumice stone or broken glass, and a small piece of paraffin added to prevent bumping and frothing.

The rate of distillation and passage of steam through the liquid in the flask should be so regulated that when 400 cc. to 450 cc. of distillate are collected the amount of liquid left in the distillation flask shall not exceed 10 cc. to 20 cc., when all nicotine will have passed over. The distillate is now made up to a volume of 500 cc. with distilled water, and aliquot portions taken for titration against standard acid, using methyl orange as an indicator. This is the regular Kissling method, and gives the total volatile bases extracted by ether. The following method was then used for estimating the nicotine present: A solution of chemically pure nicotine of approximately 1 per cent. strength is made, and an aliquot portion carefully titrated against acid of known strength (N/5 hydrochloric acid), using methyl orange as an indicator, and the percentage of nicotine it contains definitely established. A separate portion of the same solution is then placed in a 40 cm. tube, and its rotation in terms of the sugar scale determined. By dividing the percentage of nicotine found above by the reading on the sugar scale, the value of 1° of the scale in terms of per cent. of nicotine is established, and the figure so obtained can be used as a factor in all determinations of nicotine made, providing the same polariscope is used. A portion of the distillate from the treatment of the powder or extract by the Kissling process, as given above, is placed in the 40 cm. tube and its degree of polarization noted. From this reading the percentage of nicotine in the solution can be calculated, using the value of 1° of the sugar scale as previously determined. By multiplying this percentage by 5 the amount of nicotine in the whole distillate is obtained, and can be calculated back to the original amount of powder or extract used. Now, if the total amount of nicotine found be expressed in terms of N/5 acid, and the number of cubic centimeters

thus indicated be subtracted from the amount of N/5 acid required to neutralize the entire distillate (as calculated from the titration of an aliquot portion), a fairly accurate determination of pyridine (if present) can be made.

In case a rapid method for the estimation of the nicotine alone is desired, the tedious process of extracting by ether, etc., is obviated by proceeding as follows: A weighed amount of the sample is rendered alkaline with 50 cc. of approximately N/10 soda and transferred to a round-bottomed flask of about 300 cc. capacity with 150 cc. of distilled water. The whole is then subjected to distillation in a current of steam in the usual manner, and the distillate made to a volume of 500 cc. A portion of the distillate is then placed in a 40 cm. tube and a polariscopic reading taken. The result, calculated on the value of 1° on the sugar scale, as previously established for chemically pure nicotine, will give the amount present in the total distillate, from which the percentage of nicotine in the sample taken can be obtained. In tobacco extracts, especially, this modification yielded very satisfactory results, but in tobacco powders, although the figures compared favorably, some difficulty was experienced, as it was almost impossible to reduce the volume of liquid in the distillation flask sufficiently without causing considerable bumping and splashing of the wet powdered substance contained in it; the result, as compared with a determination made by treating another portion of the same sample of tobacco powder by the Kissling process, and then determining the nicotine in the distillate by use of the polariscope, was slightly lower.

Experiment 1.—(*a*) A solution containing 1.2334 grams of chemically pure nicotine in 100 cc. of water was prepared. A portion of this solution in a 40 cm. tube gave a reading of —11° on the sugar scale, which represents 1.233 per cent. of nicotine, or 0.112 per cent. for each degree of rotation.

(*b*) A solution was prepared containing 2.021 grams of chemically pure nicotine in 100 cc. of distilled water. Ten cc. of this solution was made to a volume of 50 cc. A portion of this last solution, containing 0.404 per cent. nicotine in a 40 cm. tube, gave a reading of —3.6° on the sugar scale, which is equivalent to 0.112 per cent. for 1°.

Experiment 2.—(*a*) 2.5275 grams of a sample of tobacco ex-

tract were next taken and carried through the regular Kissling process, the final distillate being made to a volume of 500 cc. A portion of this distillate in the 40 cm. tube gave a reading of —4° on the sugar scale. Now, as determined in Experiments 1 and 2, each degree of sugar scale is equivalent to 0.112 per cent. of nicotine; therefore, —4° would mean that the distillate contained 0.448 per cent., or 2.24 grams of nicotine, and this, calculated back to the weight of the sample taken, gave a percentage of 88.6 per cent.

By the titration of a portion of the distillate against N/5 hydrochloric acid, as in an ordinary Kissling determination, a percentage of 89.73 of nicotine was obtained.

(*b*) 3.125 grams of the same sample of tobacco extract were carried through the regular process and the distillate made to 500 cc. A portion of this distillate gave a reading of —4.95°, or 0.554 per cent. of nicotine, equivalent to 2.772 grams in the total distillate, which, calculated back to weight of sample taken, gave 88.7 per cent. of nicotine. By titration of a portion of distillate as in the Kissling process, 89.4 per cent. nicotine was shown.

Experiment 3.—(*a*) To 1.470 grams of same tobacco extract as that used in Experiment 2 (*a* and *b*) a solution containing 1.066 grams of pyridine was added. The mixture was then carried through the usual Kissling process and the distillate made to 500 cc. A portion of this distillate gave a reading of —2.32°, which, upon calculation, gives 0.26 per cent. of nicotine, or 1.30 grams in distillate, equivalent to 88.4 per cent. in weight of sample taken. A portion of this distillate was titrated against standard acid, the required amount in cubic centimeters of acid was noted, and the number of cubic centimeters equivalent to 1.30 grams of nicotine subtracted. The figure thus obtained, calculated to pyridine, gave 0.968 gram, or about 91 per cent. of the original amount added. (In this experiment the flask containing the ether extract was evidently heated sufficiently to volatilize a small amount of the pyridine. In Experiment 3 (*b*), which follows, the ether was driven off with extra precaution as to the loss of pyridine.)

(*b*) 1.3025 grams of extract were taken and a solution of pyridine containing 0.3989 gram added. This mixture was then treated as in (*a*) and the distillate gave a reading of —2.05°, or 88.3 per cent. nicotine. The pyridine in the distillate was also

calculated as in (*a*) and gave 0.3969 gram, or 99.2 per cent. of pyridine recovered.

Experiment 4.—(*a*) Twenty grams of tobacco powder were treated according to the Kissling method and the distillate made to 500 cc., as usual. A portion of this distillate gave a reading of —1.4°, or an equivalent of 0.1568 per cent., or 0.784 gram of nicotine in total distillate, which is equivalent to 3.92 per cent. of the powder.

Titration of another portion, as in an ordinary Kissling method, gave 4.25 per cent.

(*b*) Twenty grams of the same powder gave a reading corresponding exactly to the reading in (*a*), the percentage of nicotine, therefore, being identical with (*a*).

Titration of another portion of this distillate, as in an ordinary Kissling method, showed 4.30 per cent.

Experiment 5.—1.7148 grams of an extract of tobacco containing 1.5175 grams of nicotine were added to 2.5365 grams of a solution containing 0.5036 gram of pyridine. This mixture, which contained 35.7 per cent. nicotine and 11.8 per cent. pyridine, was well shaken and 2.524 grams taken for analysis. It was treated in the usual way and the distillate made to 500 cc. A portion in the 40 cm. tube gave —1.6°, corresponding to 35.5 per cent. nicotine contained in the mixture. The pyridine, as determined by titration of a portion of distillate, etc., was found to be 10.8 per cent.

Experiment 6.—5.017 grams of a tobacco extract, treated as in the Kissling process and the distillate made to a volume of 500 cc., gave a reading of —3.5° on the sugar scale, corresponding to 39.07 per cent. nicotine contained in the sample.

By titration of another portion of the distillate, as in an ordinary Kissling method, 40.3 per cent. was obtained.

Experiment 7.—5.076 grams of the same sample of tobacco extract as that used in Experiment 6 were transferred to a 300 cc. round-bottomed flask by means of 150 cc. of distilled water. Fifty cc. of approximately N/10 sodium hydroxide were added and the whole subjected to distillation with steam in the usual manner. The distillate was made to 500 cc.

A portion in the 40 cm. tube gave a reading of —3.5°, or a percentage in the sample of 38.61 per cent. nicotine.

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