499. *Submicro-methods for the Analysis of Organic Compounds. Part XI.* Determination of Tertiary Nitrogen by Use of an Ion-exchange Resin.*

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Tertiary nitrogen is determined on the submicro-scale by treating the compound with methyl iodide to form the corresponding quaternary ammonium iodide. The latter is dissolved in water and converted into its hydroxide by passage through a strongly basic anion-exchange resin. The hydroxide is determined by titration with standard acid. The method, which is rapid and accurate to within 0·3%, was applied to the analysis of several alkaloids, but should be generally applicable to bases which react quantitatively with methyl iodide.

The determination of NMe groups by the Herzig–Meyer method is a difficult and tedious operation on the submicro-[1] or micro-scale;[2,3] it requires 6—8 hr. under exacting conditions for a single determination. We have developed a more convenient, very rapid submicro-method[4] of determining the tertiary nitrogen content of certain organic compounds.

With an alkyl iodide many tertiary amines readily form the quaternary ammonium compound: $R_3N + MeI \longrightarrow [R_3NMe]^+I^-$. The rate of the reaction varies with the nature of the amine; certain reactions are exothermic, and others endothermic. The nature of substituents adjacent to the amino-nitrogen is also important. Since the quaternary iodides are formed rapidly in most cases[5] it occurred to us that treatment of the halides with an anion-exchange resin in its free-base form would provide a simple method for the generation of the corresponding quaternary ammonium hydroxide. The hydroxides are usually sufficiently basic to be titrated as strong bases.

The substance under examination was treated with excess of methyl iodide. After the excess had been evaporated, the solution in distilled water was passed down a small ion-exchange column in the free-base form. The eluate was titrated with standard 0·01N-sulphuric acid by using a micrometer syringe burette to a Methyl Red–Methylene Blue end-point. Parallel blank determinations on the reagents and the column itself were done.

Table 1.

	No. of	N (%) †		Diff.		No. of	N (%) †		Diff.
Substance	detns.	Calc.	Found	(%) *	Substance	detns.	Calc.	Found	(%) *
Atropine	7	4·84 (5·19)	4·93 (5·28)	+0·09	Isoquinoline methiodide hydrate	8	4·84 (5·19)	4·75 (5·09)	−0·09
Narcotine	5	3·39 (3·63)	3·42 (3·65)	+0·03	Codeine	4	4·67 (5·01)	4·62 (4·95)	−0·05

* Maximum positive error in individual results was 0·23%; maximum negative error in individual results was 0·28% (*i.e.*, error from theoretical percentage). The sample weights used in these 24 determinations varied from 67·18 to 50·44 μg.

† Figures in parentheses are results calculated in terms of the *N*-Me group.

Results obtained in the analysis of some alkaloids are shown in Table 1. These substances have a methyl group attached to the nitrogen atom and this method therefore provides a simpler and (although indirect) much more rapid method than that previously described for the NMe content of such compounds.[1] The accuracy is satisfactory and only

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* Part X, *J.*, 1959, 3577.

[1] Belcher, Bhatty, and West, *J.*, 1958, 2393.

[2] Herzig and Meyer, *Monatsh.*, 1897, **19**, 379.

[3] Vieböck and Brecher, *Ber.*, 1930, **63**, 3207.

[4] Bhatty, Ph.D. Thesis, Birmingham University, 1957.

[5] Hickinbottom, "Reactions of Organic Compounds," Longmans, Green, London, 1948, pp. 304ff.

very simple apparatus is needed. There are, of course, limitations owing to the failure of certain compounds to react quantitatively with methyl iodide, *e.g.*, *N*-methylacetanilide and morphine. The acid–base end-point is not as sharply defined as the iodine end-point of the corresponding Herzig–Meyer method in such small volumes of solution at these dilutions, but a reproducibility of ±1 µl. of 0·01N-sulphuric acid is obtainable by titrating to a definite shade of the indicator. Protection from fumes such as ammonia, hydrogen chloride, etc., is of course essential.

EXPERIMENTAL

Apparatus.—(1) *Ion-exchange column.* For the preparation of the 1-cm. wide Pyrex column, a wad of glass wool, about 5 mm. thick, was inserted at the bottom of the stem and was pressed to the walls. The column was filled with water up to the wider end and small amounts of resin were added from the tip of a spatula. The particles settled under gravity. A length of about 3·5 cm. of resin bed was obtained in the column. The filling was secured by a second glass-wool plug, about 2 mm. thick.

Before the column was used it was regenerated with 100 ml. of 4% sodium hydroxide solution which passed through the resin bed at the rate of 1 drop per 5 sec. The column was afterwards washed exhaustively till the alkalinity in 12 ml. of the eluate was not more than 5 µl. of 0·01N-sulphuric acid. It required a total volume of about 1½ l. of the wash water to give a blank of the above order.

The column, thus prepared, lasted more than a fortnight under constant use for the conversion of submicro-samples of the quaternary salts into the hydroxide form. The efficiency of such a column was established by passing through the resin known volumes of 0·01N-sodium chloride solution. Recoveries of sodium hydroxide in the eluate were almost theoretical on the submicro-scale. It was, however, necessary to use about 10 ml. of water in order to remove completely the alkali from the column. Blanks were determined identically at the same time. The recoveries given in Table 2 were obtained.

TABLE 2. *Titration of* NaOH *after generation from* NaCl.

Vol. of 0·01N-NaCl soln. added (µl.)	Vol. of 0·01N-NaOH recovered (µl.)
99·0	99, 97, 98, 98, 98, 98
198·0	198, 199, 198, 199, 198

(2) *Conical flasks.* (*a*) 20 ml. Pyrex-glass flasks for the collection and the titration of the eluate. (*b*) 5 ml. Pyrex-glass flasks for carrying out the reaction of a substance with methyl iodide.

(3) *Titration equipment.* The titration equipment and arrangement have been described in the previous publications in this series. A graduated 2 ml. glass syringe was used for adding known volumes of wash-water.

Reagents.—(1) *Ion-exchange resin.* Analytical reagent grade Amberlite IRA-400(OH^-) resin was used for making the column. The resin is supplied in 20—50 mesh, but for procuring a longer life of the same column, 60—80 mesh resin is better.

(2) *Methyl iodide.* Reagent grade.

(3) 0·01N-*Sulphuric acid.* The acid was standardised on the micro-scale.

(4) *Indicator solutions.* Alcoholic solutions (0·01%) of Methyl Red and Methylene Blue were added separately to the eluate before titration. The use of too much indicator obscured the end-point.

(5) 4% *Sodium hydroxide solution.* The column was treated with about 100 ml. of this solution for the regeneration of the resin.

Procedure.—About 50 µg. of substance were transferred to a 5 ml. conical flask. 10 Drops of methyl iodide were added from a pipette, and the contents of the flask were mixed completely by gentle shaking. The flask was covered with filter paper and placed in water at about 40° in order to evaporate excess of methyl iodide.

In the meantime, the flow-rate on the ion-exchange column was adjusted to about 1 drop per 5 sec. The residue in the flask was dissolved in 1 ml. of water and transferred to the column which had nearly drained to the top of the resin. The flask and the column were then washed in turn with three lots of 1 ml. and four lots of 2 ml. of water. A teat-pipette was used for transference of the wash-solution from the flask. The effluent was collected in a 20 ml. flask, 4 drops of Methyl Red and 3 drops of Methylene Blue indicator solutions were added, and the whole was titrated with 0·01N-sulphuric acid. The blanks were determined before the sample titrations and were considered acceptable when they agreed within 1 µl. of the standard sulphuric acid titre. When the blanks were more than 7—8 µl. of acid, the column was washed further until the values were reduced; the determinations were then carried out. The last blank solution was kept after titration for comparison.

Once set, the rate of flow of the liquid from the column was not altered, because, otherwise, the blanks were also disturbed. Titrations were made without undue delay.

Factor: 1 µl. of 0·01N-H_2SO_4 ≡ 0·1401 µg. of N.

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