478. *Submicro-methods for the Analysis of Organic Compounds. Part III.* Determination of* N-*Methyl groups and the Simultaneous Determination of Alkoxyl and* N-*Methyl groups.*

By R. Belcher, M. K. Bhatty, and T. S. West.

N-Methyl groups can be determined with an absolute accuracy of ±0·3% on 50-μg. samples by modification of Herzig and Meyer's method. Methoxyl and *N*-methyl groups can also be determined simultaneously on the same sample with equal accuracy by a slight modification of the procedure.

N-Methyl groups are always determined on the micro-scale by some form of the method originated by Herzig and Meyer.[1] Although the accuracy of the method is inferior to that of most other micro-methods, no alternative approach has yet been advanced. Hence an adaptation of the conventional Herzig–Meyer method was examined on the submicro-scale. Several forms of the usual apparatus were examined and a number of factors which affected results investigated.[2]

One of the major difficulties encountered in the adaptation of Herzig and Meyer's method to the sub-micro-scale was that very high blanks were obtained. This method, as opposed to the alkoxyl method, required the drastic steps of distillation of the hydriodic acid and pyrolysis of the quaternary ammonium salt (at 300—360°), so the method is subject to errors not found in the Zeisel method which it otherwise so closely resembles. High blanks were obtained because (*a*) the hydriodic acid formed an aerosol, (*b*) the vapour-laden gas stream was incompletely washed, and (*c*) the apparatus was not uniformly heated.

Aerosol formation was prevented by passing the hot gas stream from the decomposition flask over hydriodic acid boiling in a flask fitted with a water condenser. Modifications of the apparatus of Pregl and Lieb [3] and of Friedrich [4] were unsuitable but a modification of Steyermark's apparatus [5] was adopted. A second digestion flask, attached to the existing submicro-alkoxyl apparatus,[6] was used for digestion of the sample and for pyrolysing the salt, while the flask with the reflux condenser was used for collecting and boiling the distilled acid. The blanks were thus reduced to 20—30 μl. of 0·01N-sodium thiosulphate. A wide variation in the blank values obtained from successive determination was, however, still obtained.

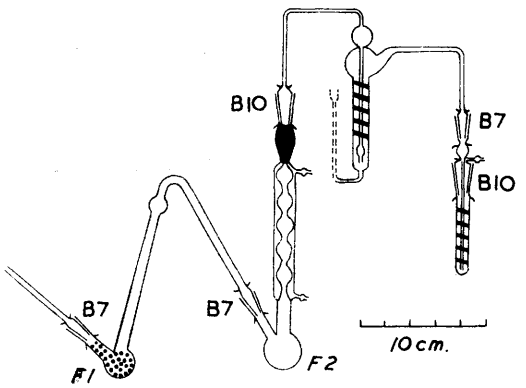
It was suspected that these high values were caused by some hydriodic acid vapour

* Part II, *J.*, 1957, 4480.

[1] Herzig and Meyer, *Monatsh.*, 1894, **15**, 613; 1895, **16**, 599; 1897, **18**, 379.
[2] Bhatty, Ph.D. Thesis, Birmingham University, 1957.
[3] Pregl, "Quantitative Organic Microanalysis," 5th English Edn., J. and A. Churchill Ltd., London, 1951, p. 196.
[4] Friedrich, *Mikrochem.*, 1929, **7**, 195.
[5] Steyermark, "Quantitative Organic Microanalysis," The Blakiston Co., New York, 1951, p. 234.
[6] Belcher, Bhatty, and West, *J.*, 1957, 4480.

passing through the spiral wash-chamber. A second internal scrubbing device was therefore incorporated in the apparatus. The gas stream, before entering the spiral washer, was intimately scrubbed in a silica-wool plug which had been soaked with the wash liquid and inserted in the neck of the condenser; such a washer was used to keep down the dead-space of the apparatus.

A further source of variation in blanks was traced to irregular heating of the apparatus. The conventional copper oxide bath was, therefore, replaced by an electrically-heated aluminium block for the pyrolysis flask and another block (flame-heated) for the reflux flask.



When these measures were adopted and a carefully regulated stream of nitrogen was passed through the apparatus, the blank titres in a determination became reproducible at 15 μl. of 0·01N-thiosulphate solution. The bulk of the blank value was obtained generally in the first pyrolysis, whereas the subsequent distillations and decompositions gave negligible values.

Care was taken to ensure that the quaternary salt was decomposed below 300°, but in order to decompose the salt adhering to the outlet tube from the pyrolysis flask, the temperature was raised to 360° after the main treatment. Longer periods were used to ensure complete cleavage of the methyl iodide from the quaternary salt.

Once conditions for low and consistent blanks had been established, determinations were carried out with atropine as a reference standard. Several forms of hydrolysis flask were examined, and that adopted finally is depicted in the Figure. This hydrolysis flask has a condenser tube of the same proportions as the normal micro-apparatus.

In the initial tests recoveries were only about 50% even after three distillations and pyrolyses. Further distillation showed no significant increase in recovery, but when the pyrolysis flask was packed with glass beads (which simultaneously increased the surface area of the liquid and helped to maintain a uniform temperature inside the flask) recoveries were improved. These values are recorded in Tables 1 and 2.

It will be seen from Table 1 that the results obtained for the determination of methylimino-groups are accurate to ±0·3% except in the case of one compound, *viz.*, *N*-methyl-

TABLE 1. *Determination of* N-*methyl group.*

	N-Me (%)				*N*-Me (%)		
Compound	Calc.	Found	Difference (%)	Compound	Calc.	Found	Difference (%)
Atropine ...	5·19	5·14	−0·05	Ephedrine, $\frac{1}{2}H_2O$	8·62	8·39	−0·23
		4·97	−0·22			8·32	−0·30
		4·97	−0·22	*iso*Quinoline methiodide	5·53	5·42	−0·11
		4·89	−0·30			5·34	−0·19
		5·02	−0·17	* *N*-Methylacetanilide	10·07	9·70	−0·37
Morphine ...	5·26	5·38	+0·12			9·52	−0·55
		5·09	−0·17			9·82	−0·25

* Low results on this compound are attributed to its volatile nature. Modification of the apparatus to include a longer air condenser between the flask *F*1 and the rest of the apparatus might yield quantitative recoveries.

acetanilide. Table 2 summarises the results obtained in the combined determination of *O*-methyl and *N*-methyl groups in the same compound. It will be seen that, even in the simultaneous determination of both groups, the accuracy is within ±0·3% in both cases. All these results were obtained on sample weights within the range 49—64 μg.

Low values for the group in *N*-methylacetanilide are attributed to the low melting point and low initial reactivity of this substance towards hydriodic acid, as some of it may be lost by volatilisation when the temperature is raised for distillation and pyrolysis.

TABLE 2. *Determination of* N-*methyl and methoxyl groups.*

	N-Me (%)			OMe (%)		
Compound	Calc.	Found	Difference (%)	Calc.	Found	Difference (%)
Narcotine	3·63	3·81	+0·18	22·52	22·72	+0·20
		3·48	−0·15		22·55	+0·03
		3·83	+0·2		22·76	+0·24
Codeine	5·02	4·96	−0·06	10·37	10·31	−0·06
		4·95	−0·07		10·29	−0·08
Cocaine	4·95	4·72	−0·23	10·23	10·48	+0·25
		4·66	−0·29		10·42	+0·19
		4·77	0·18		10·45	+0·22

Despite the reduction in weight of sample, the total time needed may exceed that required for the micro-determination. Under our conditions it is advisable to adhere to the recommended time schedule. Further modification of the apparatus might enable total time to be reduced.

EXPERIMENTAL

Reagents.—Most of the reagents were as already described;[5] the following were also required: (1) ammonium iodide, reagent grade; (2) gold chloride, 2% solution; (3) glass beads (2—3 mm. diam.) used for packing the decomposition flask, *F*1; (4) silica wool.

Apparatus.—An electrically heated aluminium block carried the decomposition flask *F*1 which was heated to 360°, while a gas-heated aluminium block supported the flask *F*2 of the alkoxyl apparatus which was heated at 150°. The apparatus is identical with that used for the determination of alkoxyl groups except that a pyrolysis flask is attached to the inlet tube.

Procedure.—A weighed [7] sample (about 50 μg.) was transferred to a platinum boat which was inserted through the B7 side arm into the flask *F*1; 20 mg. of ammonium iodide, 0·05 g. of phenol, 2 drops of gold chloride solution, and 0·5 ml. of iodine-free hydriodic acid were also added in that order. The flask was then packed with clean, dry glass beads (as indicated in the Figure) and the gas-inlet tube connected to it, silicone grease being used to seal the joint. The wash-chamber was charged with 8 ml. of a solution of sodium antimonyl tartrate, and the absorber with 1 ml. of a solution of sodium acetate in glacial acetic acid and about 0·05 ml. of bromine. The neck of the condenser was plugged with a 1·5 cm.-long wad of silica wool which had been soaked moderately with the sodium antimonyl tartrate liquid, excess being avoided.

The apparatus was assembled as indicated in the Figure. Silicone grease was used for lightly sealing all the joints except that of the absorber.

The spaces between the aluminium blocks and the flasks *F*1 and *F*2 were filled with powdered copper oxide, and thermometers placed in the oxide in contact with the flasks. A regulated stream of nitrogen (1 bubble per sec. as seen in the scrubber) was connected with the inlet tube of flask *F*1.

The following scheme was observed for heating the two metal blocks.

Time (hr.)	½	½	1	1½	2	3
Temp. for *F*1	100°	160°	260°	300°	360°	360°
Temp. for *F*2	50	50	50	150	150	150

At the end of 3 hr., heating was discontinued and the scrubber and the absorber were disconnected.

The two flasks *F*1 and *F*2 were raised together from the heating blocks and allowed to cool to room temperature. The flow of nitrogen was then stopped. The flasks were suitably tilted so that the hydriodic acid in flask *F*2 was re-introduced into flask *F*1. Meanwhile the blocks had cooled to 160° and 50° respectively. Another absorber was attached to the scrubber and the apparatus was reassembled. The nitrogen supply was connected as before and the flasks

[7] Astbury, Belcher, and West, *Mikrochim. Acta*, 1956, 598.

were lowered into the heating blocks. The second and subsequent distillations and pyrolyses were each completed in 2½ hr., starting from the 30-min. step of the heating scheme above. Three distillations and pyrolyses for a sample and two for a blank determination sufficed to give a net titration value corresponding to the *N*-methyl content of the sample.

In the presence of methoxyl groups flasks *F*1 and *F*2 were gradually heated to 160° and 50° in 30 min. as before. These temperatures were then maintained for a further 1 hr., during which the gas stream was passed through the apparatus at the rate of one bubble per second. Methyl iodide formed from the methoxyl group was thus removed quantitatively into the absorber. Blanks were similarly determined to make allowance for the 1½ hours' digestion time. The methoxyl estimation was carried out as described previously.[4] A fresh absorber was added and the determination for *N*-methyl groups was then completed as described already, by starting from the 30-min. step in the heating scheme.

Titration of the solution containing iodine was performed in the same way as described for the alkoxyl determination.[4]

1 μl. of 0·01N-$Na_2S_2O_3$ ≡ 0·025 μg. of CH_3

CHEMISTRY DEPARTMENT, THE UNIVERSITY,
EDGBASTON, BIRMINGHAM.

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