82. *Submicro-methods for the Analysis of Organic Compounds. Part XVI.* The Determination of Nitrogen by a General Procedure.*

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A general procedure has been developed for the determination of nitrogen on the submicro-scale. Decomposition is done at 380° with mercury catalyst regardless of the type of linkage. Iodine or bromine, if present, is expelled by digestion with sulphuric acid before the tube is sealed. Reduction by glucose, when required, is also done in an open tube so that the mercury catalyst can be added after the glucose has been destroyed. Reduction with phosphorus and hydriodic acid reduces substances for which glucose is ineffective. Although the procedure is longer, it is recommended for use when the type of nitrogen linkage is unknown.

The method of Belcher *et al.*[1] for the determination of nitrogen in amines and similar compounds was modified later [2] to enable it to be extended to the analysis of iodine- and bromine-containing compounds and of compounds containing heterocyclic nitrogen or azo-, hydrazo-, or nitro-groups. In general, these modifications required varying the temperature of decomposition, and in some cases mercury(II) was required as catalyst. It would be much more convenient to have a standard temperature for the decomposition and to reduce the number of extra modifications to a minimum, whilst maintaining the applicability of the method to as wide a range of compounds as possible. Further studies were therefore made.

The mercury(II) catalyst accelerates the decomposition in all cases and its use is essential for complete decomposition of some compounds. In the absence of complications such as iodine and bromine and prereduction, all compounds decomposed satisfactorily at 380° in presence of this catalyst; hence it is now added regardless of the nature of the nitrogen linkage. It was thought that if sufficient catalyst were used it would eliminate the need to add more mercury(II) later as indicator at the neutralisation stage. However, the precipitate did not redissolve on neutralisation, and erratic results were obtained; probably some decomposition occurs; so the amount of catalyst was kept as low as possible (about 10 μg.) and mercuric sulphate was later added separately as indicator.

At 350° elemental iodine and bromine are the end-products of the decomposition and can be expelled by heating the opened tube. At 380° iodide is formed, and bromide just above this temperature. In order to maintain the proposed standard digestion temperature at 380°, the iodine and bromine were expelled from the open tube before sealing. The necks of the tube were drawn out to leave an opening 2—3 mm. in diameter; gentle digestion with sulphuric acid expelled all the bromine and iodine. Catalyst was added and, after being sealed, the tube was heated at the standard temperature.

At the standard temperature of 380°, with mercury catalyst, the heterocyclic compounds examined gave satisfactory results. Here too, when iodine or bromine was present, it was first expelled as above.

Previously, reduction at 420° with glucose had been found necessary for azo-, hydrazo-, and nitro-compounds; at lower temperatures decomposition was incomplete and, with catalyst present, the recoveries were even worse. This problem was solved by a two-stage decomposition: the compound was reduced with glucose in the open tube, catalyst was then added, and the tube was sealed. Digestion at 380° then gave satisfactory results.

Unfortunately one compound, cyclohexanone 2,4-dinitrophenylhydrazone, did not give complete recoveries on glucose reduction. The more drastic hydriodic acid–red

* Part XV, *J.*, 1962, 3033.

[1] Belcher, West, and Williams, *J.*, 1957, 4323.
[2] Belcher, Bhasin, and West, *J.*, 1959, 2585.

phosphorus [3] method was then examined. This was effective for all compounds, but it is more tedious than the glucose method; the tubes cannot be drawn out to facilitate their later sealing because of the comparatively large amounts of iodine which have to be expelled, and a long boiling period is required. Another compound, hexamine allyl iodide, gave low recoveries regardless of the treatment except when hydriodic acid and phosphorus were used (exact results were then obtained). Hence, when the nitrogen linkage is unknown it is advisable to use the red phosphorus–hydriodic acid pre-reduction.

Results obtained by glucose reduction and prior expulsion of bromine and iodine are shown in Table 1. Results from hydriodic acid–red phosphorus reduction are shown in Table 2. Compounds which do not require pretreatment are included and indicate that amino-groups are not decomposed by the process.

TABLE 1.

Compounds analysed for nitrogen.

Pre-reduction with glucose–H_2SO_4 at 300°; digestion with $HgSO_4$–H_2SO_4 at 380° for 45 min. Thiosulphate standardised against ammonium sulphate.

Compound	Found (%)	Calc. (%)
8-Hydroxyquinoline	9·7, 9·2	9·65
Acetanilide	10·2, 10·5	10·4
p-Nitroaniline	20·05, 19·9	20·3
8-Hydroxy-7-iodoquinoline-5-sulphonic acid	3·85, 4·2	4·0
trans-2-Dimethylaminocyclohexanol methiodide	4·7, 4·9	4·9
2,4,6-Tribromoaniline	4·5, 4·3	4·2
1-Chloro-2,4-dinitrobenzene	13·7, 13·6	13·8
7,8-Dimethoxy-1,2-dimethylisoquinolinium iodide	3·9, 3·8, 3·8	4·1
o-Bromonitrobenzene	6·9, 6·6, 6·7	6·9
Cyclohexanone 2,4-dinitrophenylhydrazone	18·7, 18·85	20·1
Hexamine allyl iodide	17·6, 17·5	18·2

TABLE 2.

Compounds analysed for nitrogen.

Pre-reduction with red phosphorus and hydriodic acid; digestion with $HgSO_4$–H_2SO_4 at 380° for 45 min.

Compound	Found (%)	Calc. (%)
Acetanilide	10·3, 9·9	10·4
m-Dinitrobenzene	16·5, 16·2	16·7
Cyclohexanone 2,4-dinitrophenylhydrazone	19·9, 20·1, 20·25	20·1
8-Hydroxy-7-iodoquinoline-5-sulphonic acid	4·2, 3·95	4·0
4-Nitrobenzyl bromide	6·55, 6·4	6·5
2,4,6-Tribromoaniline	3·9, 4·4	4·2
o-Bromonitrobenzene	7·1, 7·0	6·9
Hexamine allyl iodide	18·0, 17·9	18·2
8-Hydroxyquinoline	9·35, 9·95, 9·9	9·65
trans-2-Dimethylaminocyclohexanol methiodide	4·7, 4·6	4·9
7-Chlorobenzocycloheptenone 2,4-dinitrophenylhydrazone *	15·3, 14·8	15·6
Perfluorobenzaldehyde azine ($C_{14}H_2F_{10}N_2$)	7·0, 6·9	7·2
3,3′-Bistrifluoromethylazobenzene	8·5, 8·7, 9·0	8·8
3,3′-Bistrifluoromethylazoxybenzene	7·95, 8·3	8·4

* Ring Index nomenclature.

EXPERIMENTAL

Reagents and Apparatus.—These were the same as those described previously [1,2] except that 0·01N-sodium thiosulphate, 0·02N-sodium hypochlorite, and 2% sodium hydrogen carbonate solution were used. Reagent-grade red phosphorus and "M.A.R." hydriodic acid (*d* 1·7) were used for the prereduction.

Agla syringe burettes containing 0·02N-sodium hypochlorite should be protected from daylight (black paper round the barrel of the burette proved suitable).

[3] Friedrich, *Z. physiol. Chem.*, 1933, **216**, 68.

The thiosulphate solution was standardised against weighed submicro-portions of solid ammonium sulphate taken through the entire procedure.

Pre-reduction with Hydriodic Acid and Red Phosphorus (for the analysis of all compounds when the form of nitrogen linkage is unknown).—The sample (40—80 μg) was transferred to the digestion tube, and red phosphorus (100 μg.) and hydriodic acid (0·05 ml.; *d* 1·7) were added. The tubes were held vertical whilst resting on a hot-plate which was raised from 150° to 190° during 30 min. and then held at 190° for 30 min. The tubes were allowed to cool and water (0·05 ml.) and concentrated sulphuric acid (20 μl.) were added. The tubes were then heated in the cavities of the heating block (the cavities were of similar depth to the length of the tube) at 150°, rising to 200° during 30 min. Most of the hydrogen iodide and iodine was eliminated in this time. The tubes were then heated in the hot-plate (300°) for 1 hr. to complete the elimination of iodine (and bromine if present in the sample). The tubes were then cooled, mercuric sulphate (about 10 μg.) was added, and the tubes were sealed and heated at 380° in the heating block for 45 min. The tubes were cooled and opened; [1] both portions were heated in an oven at 100° for 10 min. to expel sulphur dioxide and then cooled in a metal block. After the cap of the tube had been rinsed, two drops of 2% mercuric sulphate was added (0·1 ml.). 2N-Sodium hydroxide was added carefully from a micrometer syringe burette, with stirring, against a black back-ground. This addition was stopped as soon as the cloudy precipitate failed to redissolve. Final neutralisation was by addition of six drops of 2% sodium hydrogen carbonate solution(an excess). Two drops of a 30% solution of potassium bromide were added and the solution was stirred vigorously for 0·5 min. to dissolve the precipitate. A faint cloudiness sometimes persisted but had no ill effects.

0·02N-Sodium hypochlorite (200 μl.) was added and the vessel set aside in the dark for 5 min. It was then replaced on the magnetic stirrer, and one drop of 30% potassium iodide and four drops (0·25 ml.) of 4N-sulphuric acid were added. The liberated iodine was titrated with 0·01N-sodium thiosulphate. Near the end-point, Thyodene was added as indicator. Blank determinations were done under the same conditions.

Amino- and Heterocyclic Compounds.—(*a*) *Bromine and iodine absent.* The sample (30—60 μg.) was transferred to the digestion tube, sulphuric acid (10 μl.) and mercuric sulphate (10 μg.) were added, the tube was sealed and digested at the standard temperature (380°), and the determination completed as above.

(*b*) *Iodine or bromine present.* The sample and sulphuric acid (20 μl.) were added to digestion tubes and the necks of the tubes were drawn out to leave an opening 2—3 mm. in diameter. The tubes were held vertically whilst resting on a hot-plate (300°) for 1 hr. The tubes were covered with an inverted Petri dish and the temperature at the necks rose to about 100—110°. Bromine and iodine were eliminated in this time. A small crystal of mercuric sulphate (about 10 μg.) was then added and the tubes were sealed off and treated as above.

Azo-, Hydrazo-, and Nitro-compounds.—The sample and glucose (0·7—1·0 mg.) were transferred to each tube and well tapped down. Concentrated sulphuric acid (20 μl.) was added. The necks of the digestion tubes were drawn out to leave a 2—3 mm. opening as above. Pre-digestion at 300°, sealing, and digestion at 380° were then carried out as described in the preceding paragraph.

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