Hydrochloride: Colorless needles (from MeOH-EtOAc), m.p. 212~214° (decomp.). Yield, 80%. *Anal.* Calcd. for $C_{16}H_{23}ON \cdot HCl$: C, 68.12; H, 8.53; N, 4.96. Found: C, 68.01; H, 8.65; N, 4.72.

Summary

A new synthetic route for N,N-dimethyl-1-*m*-hydroxyphenylcyclohexaneëthylamine (IX) from ethyl cyclohexylidenecyanoacetate is described. 1-(*m*-Methoxyphenylcyclohexyl)trimethylamine(XII) was prepared by the Curtius degradation of 1-*m*-methoxyphenylcyclohexaneacetic acid (V) and subsequent dimethylation.

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172. Goro Chihara*[1] and Keiichi Tanikawa*[2]: Analysis of Compounds containing Heavy Nitrogen (^{15}N) by Infrared Absorption Spectra.

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The use of an isotopic element has become one of the most important measures in studying biochemical and organic reactions. Analysis of radioisotope compounds can be carried out accurately and in a simple manner by the use of a Geiger-Müller, gas-flow, or a scintillation counter. However, analysis of compounds containing stable isotopes like ^{15}N, with the exception of deuterium, had to depend on the mass spectrograph and, consequently, studies using stable isotopes were far behind those using radioactive isotopes.

Infrared spectrophotometer is far more common in laboratories than mass spectrometer and it would be very convenient if stable isotopes could be measured by infrared spectrophotometer. Under such consideration, infrared absorption spectra were measured with ammonium sulfate, *dl*-alanine, phenylalanine, and ephedrine contatining ^{15}N. If strict assingment of absorptions has been determined, infrared spectrophotometer would theoretically be more useful than the mass spectrometer. In compounds examined, those with 6~12% of total nitrogen atoms in ^{15}N did not show any marked isotopic shift. Measurement of compounds with higher percentage of ^{15}N was therefore desired.

A modified apparatus for the Kjeldahl method was therefore devised by combination with the gas cell for infrared spectral measurement so as to convert the sample quantitatively into ammonia and to make the measurement simple and accurate. The apparatus is shown graphically in Fig. 1.

The spectrum obtained by measurement by this means is an overlapping of the absorptions of $^{15}NH_3$ and $^{14}NH_3$, and this makes it possible to prove qualitatively the presence of ^{15}N. Quantitative consideration is also possible.

Establishment of such an apparatus and method will make it possible to carry out analyses of compounds containing isotopic nitrogen in any laboratories equipped with infrared spectrophotometer and open a new field in the study using isotopic elements. However, this method requires a larger quantity of sample than with mass spectrometer and somewhat inferior in accuracy, so that it is not suitable where a slight

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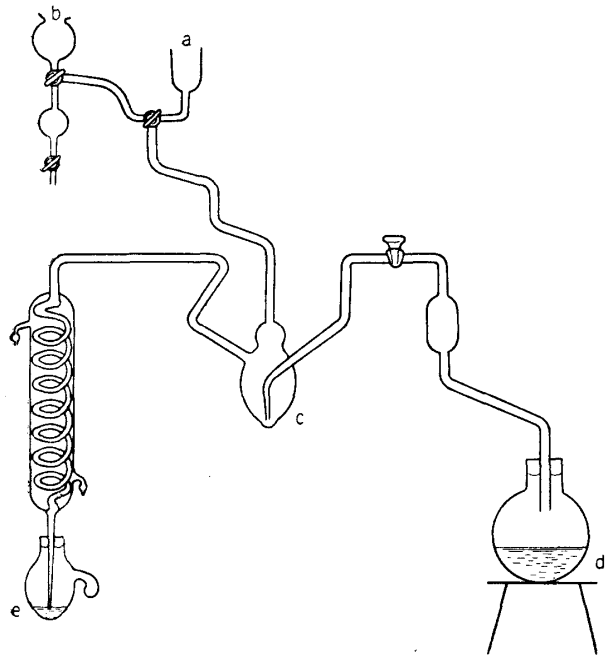
change in the content of heavy nitrogen has to be measured with a minute quantity of a sample, such as in biochemical work but would be an important measure in the field of organic reaction kinetics or in various kinds of preliminary testing.

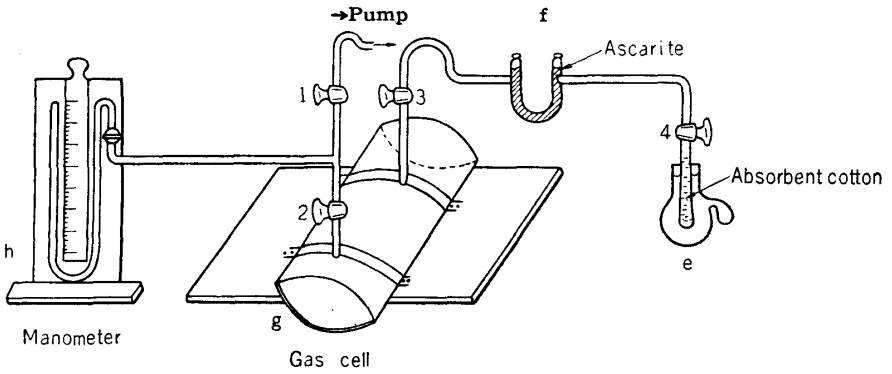
Infrared absorption spectra of compounds containing stable isotopes have not been measured to date with the exception of deuterium compounds. Report on compounds containing heavy nitrogen is found in the physicochemical discussion on the structure of $^{15}NO_2$ through infrared spectrum[1] but application of infrared spectral measurement for such purposes is non-existent.*3

Method

The present method is a combination of the usual Kjeldahl method and gas analysis by infrared spectrum, with various modifications for simplification of the procedure, to allow quantitative determination, and removal of acid gas and moisture.



A



B

Fig. 1.

Apparatus for Measuring Infrared Spectrum of Compounds containing Heavy Nitrogen by generation of $^{15}NH_3$ from such Compounds

*3 Infrared spectrum of glycine(^{15}N) has recently beed examined by Tsuboi and Shimanouchi (Paper presented at the 6th Symposium on Infrared and Raman Spectra, Tokyo, September, 1959).

1) E. T. Arakawa, A. H. Nielsen : J. Mol. Spectroscopy, **2**, 413(1958).

Apparatus—The apparatus to be used is illustrated in Fig. 1. A is the decomposition flask in which the sample compound is decomposed, alkali is added to the decomposed sample, and ammonia thereby generated is distilled, to be absorbed in H_2SO_4 and form pure $(NH_4)_2SO_4$. In A, a is an inlet for a sample, b the inlet for alkali solution, c is the distillation flask, d the steam generator, and e the receiver containing conc. H_2SO_4. This is a part of the usual Kjeldahl apparatus except that a modified egg-shaped flask is used for the flask (e).

The apparatus A in Fig. 1 is used only when strict accuracy is necessary or when the sample available is extremely minute in quantity. This apparatus may be omitted in mere qualitative estimation of ^{15}N.

B in Fig. 1 is an apparatus in which alkali is added to the $(NH_4)_2SO_4$ obtained as above to generate NH_3 and to supply this NH_3 into the gas cell for infrared spectral measurement.

The modified egg-shaped flask (e) is provided with cotton wool in its opening to serve as a filter. The U-tube, f, is filled with Ascarite which is thought to be the best available material for removing acid gas and water. The gas cell, g, 20 cm. long, is provided witn three cocks, 1, 2, 3, as shown in the diagram, at one end and a modified manometer, h, is placed at the other end. This manometer is provided so as to allow constant measurement of internal pressure of the gas cell.

This apparatus was designed to be used in conjunction with the Hilger Model H-800 infrared spectrophotometer. This apparatus as a unit should be designed to suit each of the various kinds of spectrometers.

Procedure—The sample is placed in the decomposition flask by the usual procedure, calculated amount of conc. H_2SO_4 is added, and heated in a decomposition chamber with $CuSO_4$ and K_2SO_4 as catalysts, to be converted to $(NH_4)_2SO_4$.

The sample is placed in the flask c through the opening a of A, excess of 50% KOH solution is added through the opening b, and the mixture is steam distilled by sending steam from d. The distillate ammonia is collected in the receiver e containing conc. H_2SO_4. By this procedure, compounds with heavy nitrogen can be converted completely quantitatively into $(NH_4)_2SO_4$.

Besides the modified egg-shaped flask serving as the receiver e, 50% KOH solution is placed in another chamber, so as not to mix with the sample, and attached to B (Fig. 1).

The cocks 1, 2, and 3 are then opened, cock 4 is closed, and gas cell is evacuated with a vacuum pump. The cock 4 is then opened, the receiver e is left in reduced pressure, cocks 1 and 2 are closed, and cocks 3 and 4 are opened. The two kinds of liquid in e are mixed, by which a vigorous reaction sets in, generating NH_3. The reaction apparently subsides but when cook 4 is closed and the flask e is heated, a fair amount of NH_3 evolves further. Finally, the flask is heated over an open flame for a few minutes with the cock 4 open and NH_3 is almost completely transferred to the gas call. The cock 3 is then closed and infrared spectral measurement is carried out.

Measurement of infrared spectrum is made at a speed about 10 times slower than the usual. With the Hilger H-800 spectrometer used in the present series of experiments, the range of 1200~700 cm^{-1} is covered in about 25 minutes.

Results and Discussion

By the procedure described above, NH_3 stretching vibration at around 3500 cm^{-1} and its fine structure were measured with lithium fluoride prism. The degenerate NH_3 bending vibration and its fine structure in the region of 1450~1800 cm^{-1} were measured with sodium chloride prism. The spectra of $^{15}NH_3$ and $^{14}NH_3$ were obtained as overlappings when a compound containing ^{15}N was used as the sample. There was distinct difference between the spectrum of ordinary ammonia and that of ammonia containing 12.88% of ^{15}N. However, this difference is not necessarily suitable as a key band for carrying out qualitative and quantitative analyses of compounds containing ^{15}N.

Measurement of symmetric bending vibration of ammonia at around 730~1170 cm^{-1} and its fine structure with sodium chloride prism provides a key band most suitable for the analyses of such compounds containing ^{15}N. Intensity of absorption in this region is much stronger than that in the afore-mentioned regions and the difference in wave number of absorptions between $^{14}NH_3$ and $^{15}NH_3$ is distinct.

Comparison of the absorption of ordinary ammonia and that of ammonia containing ^{15}N in the region of 800~1100 cm^{-1} is given in Fig. 2. As will be clear from this chart, there is a new absorption in the spectrum of ammonia containing 12.88% of ^{15}N, generated from 60 mg. of ammonium(^{15}N) sulfate (curve A), which is not present in the

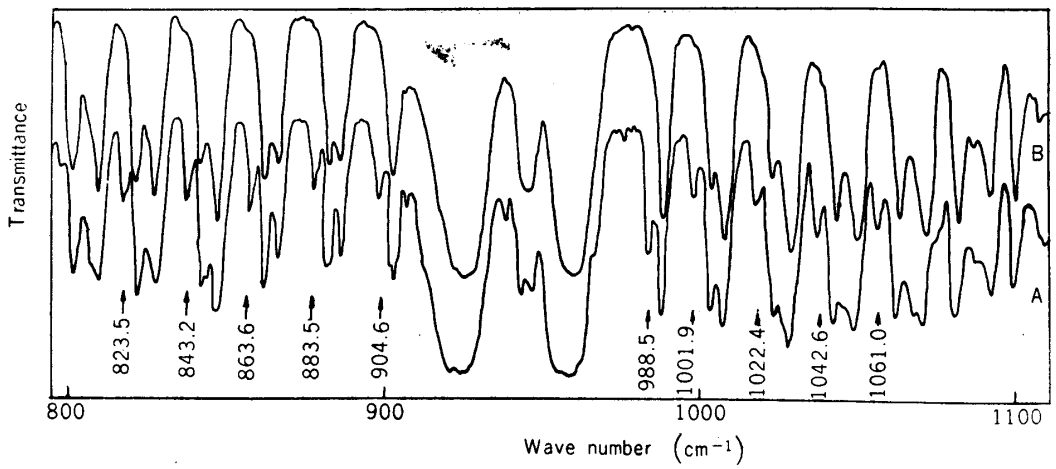


Fig. 2. Comparison of Absorptions of Ammonia

A: Ammonia containing 12.88% of ^{15}N
B: Ordinary ammonia
Arrow indicates the key band for ^{15}N

spectrum of ordinary ammonia (curve B). Such specific absorption of $^{15}NH_3$ was identified in the region of degenerate bending vibration and symmetric bending vibration of NH_3. These absorptions are due to the shift of absorptions to a smaller frequency region because the molecular weight of isotopic nitrogen is greater than the ordinary nitrogen atom.

Of these absorptions, 10 bands (823.5, 843.2, 863.6, 883.5, 904.6, 988.5, 1001.9, 1022.4, 1042.6, and 1061.0 cm^{-1}) are of strong intensity and sharp, and these can be selected as the suitable key band for qualitative and quantitative analyses of compounds containing ^{15}N. These absorptions are indicated with arrows in the curve (A) in Fig. 2. The absorptions at 863.6 and 1042.6 cm^{-1} are especially useful as the key band.

If the infrared spectrum of a sample, treated as described above, exhibits such absorptions, the sample may be considered to contain ^{15}N. On the basis of such a fact, the present method is probably the most suitable one for simple qualitative analysis of compounds containing ^{15}N.

This procedure was applied to various substances. For example, estimation of 35.409 mg. of *dl*-alanine containing 6.92% of ^{15}N among nitrogen atoms constituting the molecule and 26.350 mg. of phenylalanine containing 10.33% of ^{15}N by the present procedure indicated strong absorptions sufficient for their qualitative analyses.

The infrared spectral curves indicated in Fig. 2 were obtained from 60 mg. of ammonium sulfate containing 12.88% of ^{15}N, but in actual case, it is not necessary to use such a large quantity of the sample. Examinations were also made on whether the qualitative estimation is possible with micro-quantity of the sample.

Ammonium sulfate containing 12.88% of ^{15}N out of total nitrogen was diluted with ordinary ammonium sulfate to prepare samples with decreasing percentage of ^{15}N. Determination of 100 mg. of such samples by the foregoing procedure showed that the afore-mentioned key band can be identified sufficiently even when the percentage of ^{15}N in total nitrogen has been reduced to 0.5%. This signifies that the measurement is sufficiently possible with 0.1 mg. of pure ^{15}N. In future, therefore, the quantity of sample should be calculated from this standard in applying the present procedure.

In the present series of experiments, a gas cell of 20 cm. in length and 5 cm. in diameter was used but the use of a recently developed cell of around 10 m. will probably further reduce the quantity of a sample to one-twentieth of the above.

The use of these key bands may make it possible to carry out quantitative determination of the absolute quantity of ^{15}N by the ordinary procedure. Actually, however, it would suffice to know the ratio of ^{15}N to ^{14}N and, in such a case, the percentage of ^{15}N can be estimated by the usual method from relative ratio of the optical density of absorption of $^{15}NH_3$ at 863.6 cm^{-1} and that of $^{14}NH_3$ at 872.5 cm^{-1}.

The procedure described in this paper requires larger quantity of the sample than in mass spectrometer and quantitative precision is far inferior, but the simplicity of sample preparation and measurement are much better than those of mass spectrometer. Consequently, the present procedure is considered to be effective as a means of daily routine method in the work using ^{15}N.

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Summary

A new analytical procedure using infrared absorption spectra was established for determination of compounds containing heavy nitrogen(^{15}N). This procedure is a combination of the Kjeldahl method and gas determination by infrared spectrum. Compounds containing heavy nitrogen is converted to ammonia, and qualitative and quantitative analyses are carried out after determination of key bands of $^{15}NH_3$ from the difference between $^{14}NH_3$ and $^{15}NH_3$ in the symmetric deformation vibration of NH_3 in the region of 800~1200 cm^{-1}. Precision of the present procedure is somewhat lower than that by mass spectrometer but the procedure is far more simple and will be effective for various experiments using heavy nitrogen(^{15}N).

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