[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF MICHIGAN]

THE PREPARATION OF N^{15}-BUTYLACETAMIDE AND ITS N^{15}-D DERIVATIVE[1]

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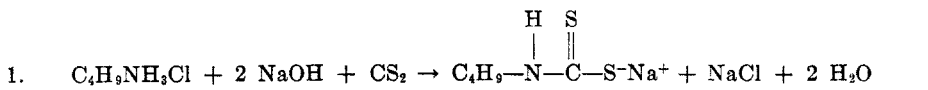
In the elucidation of the structure of proteins and polypeptides by infrared analysis, it is of great value to know the effect of substituting N^{15} for N^{14} on the spectrum of the peptide link (1). This can be most easily done by studying the changes in the spectrum of a simple monosubstituted amide when N^{14} is replaced by N^{15}. The amide selected was N-butylacetamide since much spectroscopic work had already been done on the N^{14} analog, including the effect of deuteration on the NH bond. Moreover, as the spectroscopic effects of substituting D for H in the NH bond of N^{15}-butylacetamide were also of interest, deuterated N^{15}-butyl-acetamide was also desired.

In view of the desirability of preparing N^{15}-butylacetamide with a very high nitrogen-15 content for such investigation, it became necessary to devise a reaction sequence capable of affording the maximum yield on each step in the synthesis. The availability of nitrogen-15 labelled potassium phthalimide with better than 60 atom percent enrichment[2] fixed the starting point of the series, and, accordingly, the well-known Gabriel synthesis of primary aliphatic amines was selected. The reaction between equivalent quantities of potassium phthalimide and butyl bromide in dimethylformamide (2) was essentially quantitative after 4 hr. at 70°, as indicated by the separation of 97.5% of the theoretical quantity of potassium bromide.

Conversion of the N-butylphthalimide to butylamine was next studied, and it was found that the obvious acidic or basic hydrolyses were quite unsatisfactory. Therefore, attention was focused on the reaction of phthalimides with hydrazine (3) to give phthalhydrazide which was removed in the usual way by acidification.

The various obvious means of acetylating the amine (*e.g.*, treatment with acetic anhydride) proved to be unsatisfactory from the standpoint of yield based on nitrogen, and consequently, recourse was had to an unusual series of reactions wherein the amine was converted in good yield to butyl isothiocyanate, and thence to the amide by reaction with acetic acid. The first reaction of this series,



1. $C_4H_9NH_3Cl + 2\ NaOH + CS_2 \rightarrow C_4H_9{-}\underset{\displaystyle H}{\overset{|}{N}}{-}\overset{\displaystyle S}{\overset{\|}{C}}{-}S^-Na^+ + NaCl + 2\ H_2O$

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[2] Eastman Kodak Co., Rochester, New York.

was originally developed by Delepine (4) as a general route to dithiocarbamic acids, and the procedure used was adapted from that used for the preparation of methyl isothiocyanate (5). Two methods for conversion of the dithiocarbamate to the isothiocyanate have been investigated (6): steam-distillation of the lead salt formed by addition of lead nitrate or reaction with ethyl chlorocarbonate and subsequent thermal decomposition of the reaction product. In view of appreciably better yields in the latter procedure (up to 90%) it was adopted:

2. $C_4H_9NHCS_2Na + ClCO_2C_2H_5 \rightarrow C_4H_9NHC(=S)-S-C(=O)-OC_2H_5 + NaCl \xrightarrow{\Delta} C_4H_9N=C=S$

Conversion of alkyl isothiocyanates to amides by reaction with the appropriate acid has been described by von Braun (7), but has not been used as a general reaction. Considerable study of this reaction has been undertaken in these laboratories and is continuing (8). Von Braun's findings have been substantiated and since the yields are satisfactory, the reaction was employed in the present synthesis:

3. $C_4H_9N=C=S + CH_3CO_2H \rightarrow CH_3-C(=O)-N(H)-C_4H_9 + COS$

The entire reaction series has the distinct advantage that the product from each reaction may be used *without isolation and purification* for the succeeding step. Thus, mechanical losses are reduced to the minimum, and under ideal conditions a 93% yield of butyl isothiocyanate is available from potassium phthalimide. Only the final step admits of significant variation in yield, which appears to be a function of reaction time, temperature, and the relative quantity of acetic acid added. Colors and thermal characteristics observed during the reaction do not appear to give any clue as to the actual success of the conversion, and some care must be taken to ensure complete separation of N-butylacetamide from unreacted butyl isothiocyanate.

Three different procedures were explored for producing the N-D amide: the first involved the same reaction by which the N^{15} amide was prepared, using O-D acetic acid (9); the second made use of ethanol-d, a new preparation for which was developed for this purpose; and the third, which proved most satisfactory, involved simple exchange with an excess of deuterium oxide. The first mentioned process was abandoned when it became apparent that an obscure side reaction was interfering and affording hydrogen for the nitrogen, inasmuch as no better than 40% deuteration was ever effected by this process, even under the most stringently controlled conditions (8).

The second process, exchange by means of ethanol-d, was successful where only minute amounts of deuterated amide were required. Suitable quantities of ethanol-d were added to the amide, the solution was placed in a Dry-Ice-chloroform bath, and the ethanol was removed under a good vacuum. This was repeated until the spectrum showed complete exchange. The ethanol-d was prepared without recourse to a vacuum-lattice by using aluminum ethoxide (instead of the usual

magnesium ethoxide) which was *distilled* into *n*-decane stirred magnetically to afford a fine slurry. Deuterium oxide was added to the stirred slurry, and the ethanol-d was distilled directly. A yield of 93% was obtained, and the product showed no 3μ band.

Deuteration of larger quantities of the amide was best effected by addition of a substantial excess of deuterium oxide followed by removal of the water at atmospheric pressure and a bath temperature of 150–160°, the distilling column being heated to 110°.

EXPERIMENTAL

N-Butylacetamide. To a mixture of 10 g. (0.054 mole) of potassium phthalimide and 25 ml. of dimethylformamide contained in a 100-ml. flask equipped with a stirrer and condenser was added 7.4 g. (5.7 ml., 0.054 mole) of *n*-butyl bromide. The resulting mixture was heated at 70° with stirring for 4 hr. during which time potassium bromide separated and the mixture became light tan in color. At the end of the heating period, the mixture was cooled to 0° and the potassium bromide was filtered off (6.25 g., 97.5%). Following the removal of dimethylformamide at the water pump, 12.52 g. of residue remained in the flask. To this were added 20 ml. of absolute methanol and 2.7 g. (0.054 mole) of hydrazine hydrate, and the resulting mixture was heated on the steam-bath for 1 hr., during which time a white solid separated. Water (20 ml.) was added and the methanol was removed at the water pump. Then 20 ml. of conc'd hydrochloric acid was added and the mixture was heated for 1 hr. on the steam-bath. After cooling, 8.6 g. (98.3%) of phthalhydrazide was removed by filtration. Water was removed from the filtered solution of butylamine hydrochloride under an air stream on a hot plate, whereupon there was obtained 7.9 g. of residue.

The crude butylamine hydrochloride thus obtained was dissolved in water in a flask equipped with an efficient stirrer and condenser, and to the solution were added 4.1 g. (0.054 mole) of reagent carbon disulfide and enough 40% aqueous sodium hydroxide (dropwise) to bring the solution to *p*H 10–11. Next, 2.16 g. (0.054 mole) of sodium hydroxide in 3 ml. of water was added dropwise over 5–10 min. The resulting mixture was heated gently on the steam-bath until an oil had separated, after which the mixture was cooled and 5.9 g. (5.4 ml., 0.054 mole) of ethyl chlorocarbonate was added over a period of 5–10 min. Next, the mixture was warmed on the steam-bath until no further evolution of carbon oxysulfide could be observed. In this manner there was obtained 6.0 g. (97%) of crude butyl isothiocyanate. In a separate run a sample of the crude product was fractionated at 70–71°/35 mm. (n_D^{25} 1.4986) in order to obtain a sample for spectral analysis.

The crude butyl isothiocyanate was added to 5 ml. of glacial acetic acid, and the resulting solution was heated in an oil-bath maintained at 160°. After about 9 hr. the color of the solution became dark brown and the temperature reached 155°; heating was continued for three more hours. It then was fractionated at 144.5–146.0°/33 mm. to give 3.75 g. (67% based on crude isothiocyanate) of N-butylacetamide. Redistillation at 69–70°/2.5 mm. afforded 3.1 g., n_D^{25} 1.4380.

N^{15}-Butylacetamide. The above procedure was duplicated exactly and with nearly identical results[3] using Eastman Kodak potassium phthalimide-N^{15} (62.5 atom-% N^{15}). A sample of crude labelled butyl isothiocyanate was removed in order to obtain its spectrum. Careful fractionation gave 2.18 g. of colorless oil boiling at 141°/30 mm. (corr.), n_D^{25} 1.4404.

Anal. Calc'd for $C_6H_{13}NO$: C, 62.24; H, 11.31; N, 12.63.

Found:[4] C, 62.21; H, 10.98; N, 12.56.

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[3] In the course of the distillation of the final product, the reflux head of the distillation column cracked and admitted some 25 ml. of water to the distillation flask. This was removed and separation from a small amount of water-insoluble oil was effected by filtration through Norit contained in a wet filter paper which was carefully washed with additional water.

[4] Microanalysis by Goji Kodama, University of Michigan.

The analytical sample was analyzed for nitrogen by the Dumas method, and the nitrogen was analyzed mass-spectrometrically (8, 9)[4] along with a similarly prepared nitrogen sample from the original labelled potassium phthalimide: atom-per cent N^{15} 62.7% for each sample (EKC value 62.5%).

Ethanol-d. An 11-g. sample of aluminum foil which had been freshly washed with acetone and dried at 140° for several hours was added to 350 ml. of commercial absolute ethanol contained in a 500-ml. flask equipped with a reflux condenser which carried a calcium sulfate drying tube. The reaction was initiated by addition of a crystal of iodine followed by a few small crystals of mercuric chloride. After about 0.5 hr. the reaction had subsided, so the mixture was heated to the reflux temperature to complete the transformation. At this point the flask was completely filled with fluffy impure aluminum ethoxide. The excess alcohol was removed at water pump pressure, and the aluminum ethoxide was distilled through a Claisen head equipped with a short air condenser. The main fraction boiled at 185°/0.4 mm. (59.5 g., 20%). This product was redistilled directly into a 200-ml. 2-necked round-bottomed flask containing 75 ml. of dry *n*-decane. During the distillation the decane was stirred by means of a magnetic stirrer in order to obtain a fine slurry of aluminum ethoxide. The flask containing the slurry then was attached to a reflux condenser protected from the atmosphere by a series of drying towers, and 12 ml. (0.6 mole) of 99–100% deuterium oxide was introduced with a syringe. The resulting mixture was stirred magnetically for 3 hr. and was refluxed for 1 hr. Then the condenser was arranged for distillation and the ethanol-d was carefully distilled. In this manner, there was obtained 32 ml. (93%) of ethanol-d whose infrared spectrum showed no absorption due to O—H at 3μ.

Exchange reactions. A drop of N-butylacetamide was placed in a small tube contained in a cylindrical flask, and to it was added several drops of ethanol-d. The flask then was immersed in a Dry-Ice-chloroform bath and connected to an efficient pump, which was used to remove the ethanol. The exchange process was repeated as often as required to effect disappearance of the N—H absorption, as well as of absorption due to ethanol.

A 1.0-ml. sample of N^{15}-butylacetamide was mixed with 4.0 ml. of 99–100% deuterium oxide to give a somewhat cloudy solution. Mixing was done in a micro-distillation flask which then was connected to a total reflux distillation column and placed in an oil bath heated at 150–160°. The water was removed by distillation (column temperature 110°) and the residue in the distillation flask was heated at 150° for an additional 2 hr. Upon cooling, the remaining oil was placed in a vacuum desiccator over phosphorus pentoxide for 12 hr. The sample obtained in this manner showed virtually no absorption due to N—H.

SUMMARY

N^{15}-Butylacetamide has been prepared and the amide hydrogen replaced by deuterium. A convenient preparation of ethanol-d is described.

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