ACTA CHEMICA SCANDINAVICA 7 (1953) 114–118

The Degradation of Pyrimidines for Tracer Work

ULF LAGERKVIST

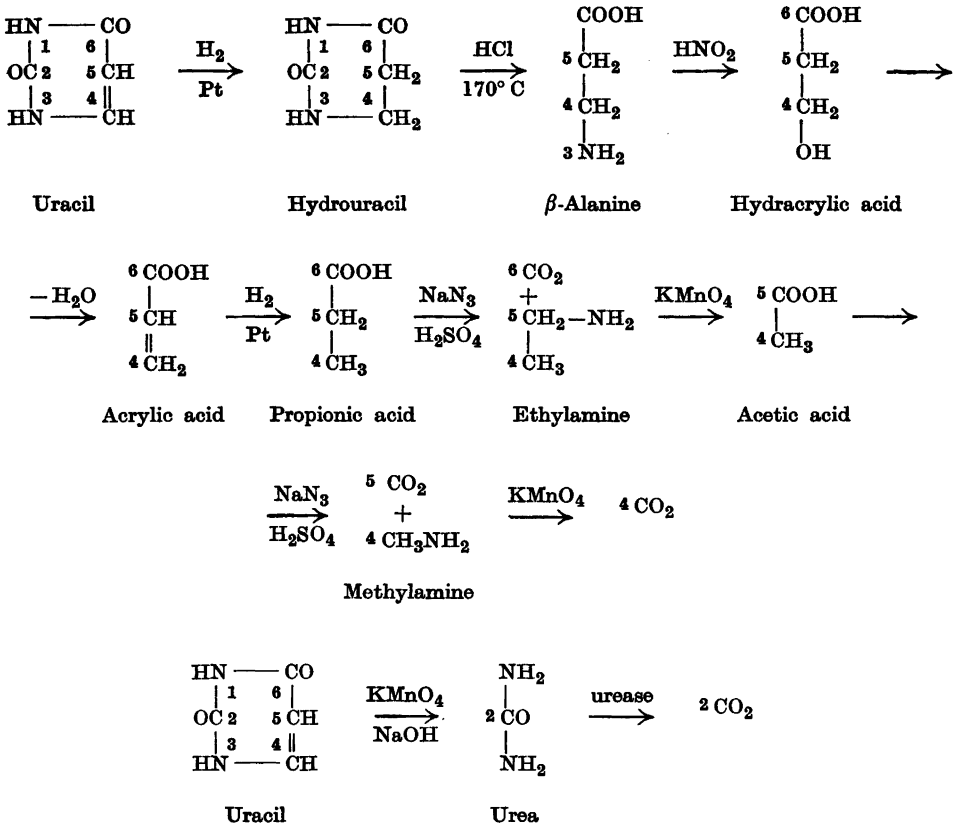
Biochemical Department, Karolinska Institutet, Stockholm, Sweden

During the past few years much work has been done on the metabolism of the pyrimidines. In order to secure full understanding of these problems it is necessary to be able to determine the distribution of isotope within the pyrimidine ring. In a preliminary report [1] a degradation of uracil was outlined in which oxalic acid and urea were obtained after oxidation with potassium permanganate. Following the work of Offe [2] oxalic acid was thought to be representative of carbon atoms 5 and 6. A similar degradation was published at the same time by Heinrich *et al.*[3] However, by degrading uracil labeled in position 5 with C^{13} and in position 6 with C^{14} it could be shown that oxalic acid was also formed to a certain extent from carbon atoms 4 and 5. A new degradation method for uracil has therefore been worked out.

The main steps in this degradation are as follows: After hydrogenation of uracil to hydrouracil according to Brown *et al.*[4] and subsequent hydrolysis with hydrochloric acid, nitrogen atom 3 and carbon atoms 4, 5 and 6 where obtained as β-alanine [5]. Deamination of this compound led to hydracrylic acid and dehydration to acrylic acid [6], which upon subsequent hydrogenation yielded propionic acid. This substance was degraded with two Schmidt decarboxylations according to Phares [7].

Carbon atom 2 was obtained in essentially the same way as described before [1]. After oxidation with potassium permanganate and hydrolysis with alkali carbon dioxide was liberated from the resulting urea with urease.

This degradation procedure makes it possible to determine the isotope concentration in every carbon atom directly. It has been tested using labeled uracil and was found to give results in good agreement with the theoretical values.



EXPERIMENTAL

Degradation procedure. Uracil (0.8—1 millimole) was dissolved with warming in 10 ml of a freshly prepared 0.5 % solution of gum arabic. To this was added 0.2 ml of a 10 % solution of chloroplatinic acid, and the reaction vessel was freed of air and filled with hydrogen to a pressure of 2.5 atmospheres. The sample was hydrogenated with shaking at 75° C for 24 hours. By that time the light absorbtion had completely disappeared, indicating that the hydrogenation was complete.

Hydrolysis of hydrouracil. After the hydrogenation the solution was evaporated to dryness *in vacuo*. The residue was dissolved in 5 ml concentrated hydrochloric acid and transferred to a bombtube. The tube was sealed and heated at 170° C for 24 hours. The tube was opened and the contents were centrifuged and the precipitate washed twice with a few ml of water. The supernatant plus washings was evaporated to dryness *in vacuo*.

Chromatography of the hydrolysate. The residue from the evaporation was taken up in 3 ml + 1 ml + 1 ml of 2 *N* HCl and applied to the top of a Dowex 50 column (200 mm × 10 mm). The column was eluted with 2 *N* HCl and the position of the peak determined with ninhydrin according to Moore and Stein [8]. Under these conditions β-alanine and

ammonia were obtained together in a single peak that appeared after an elution volume of about 20 ml. An identical chromatogram was obtained with a sample of authentical β-alanine. The β-alanine from the hydrolysate was further identified by its chromatographic behaviour on starch using 2 : 1 propanol — 0.5 *N* HCl as eluant. Finally, a sample of the β-alanine from the hydrolysate was mixed with known β-alanine and subjected to chromatography on starch in the same way. A single apparently homogenous peak was obtained. The yield of β-alanine after chromatography on both Dowex 50 and starch was about 60 % calculated from the nitrogen value after Kjeldahl combustion. The yield after chromatography on Dowex 50 only, calculated in the same way after removal of the ammonia by steam distillation, was about 80 % indicating that the β-alanine at this stage of purification was contaminated with a nitrogen-containing substance other than ammonia. This contamination, however, did not affect the accuracy of the degradation, as can be seen in the experiment with labeled uracil.

The transformation of β-alanine to acrylic acid. The chromatographic fractions containing β-alanine were pooled and repeatedly evaporated to dryness *in vacuo* to remove HCl as completely as possible. The residue was dissolved in 5 ml of water, neutralized and 2 g of sodium nitrite were added. The flask was equipped with a dropping funnel and an outlet tube connected with a mercury valve. The air was replaced with carbon dioxide and 25 ml of 2 *N* H_2SO_4 introduced through the funnel. After 3 hours 800 mg urea were added to destroy the remaining sodium nitrite. The test for nitrite with potassium iodide-starch was negative after about one hour. The solution was transferred to a 500 ml double necked, round-bottom flask and diluted to 75 ml. An equal volume of concentrated H_2SO_4 was added with cooling in ice water. The flask was connected to a condenser and equipped with a dropping funnel. Distillation over a free flame was maintained until 500 ml of distillate had been collected. During the distillation water was dropped into the solution to keep the volume constant. The operation took at least two hours.

Hydrogenation of acrylic acid to propionic acid. Two and one-half grams of gum arabic were dissolved in the distillate and 1 ml of a 10 % solution of chloroplatinic acid added. A slow stream of hydrogen was passed through the solution for 3 hours during which time it was refluxed on a boiling water bath. After neutralization the volume was reduced to about 50 ml *in vacuo*. After acidification with an excess of 5 *N* H_2SO_4, a little silver sulfate was added and the propionic acid distilled over with steam. The distillate was neutralized and evaporated to dryness. It was acidified with a slight excess of 10 *M* H_2SO_4 and extracted with 5 ml + 2 ml + 2 ml chloroform. The extract was applied to the top of a Celite column (200 mm × 20 mm), and the column was eluted first with 100 ml of chloroform saturated with 0.5 *N* H_2SO_4 and then with chloroform — 1 % butanol saturated with 0.5 *N* H_2SO_4 as described by Mosbach *et al.*[9] The yield of propionic acid was 30 % calculated on the basis of uracil.

The degradation of propionic acid. Phares' method[7] with two Schmidt decarboxylations was used. Starting with 0.9 millimole uracil at least 0.1 millimole $BaCO_3$ for isotope analysis was obtained in the final step.

The degradation of uracil to urea. Uracil (0.1—0.15 millimole) was dissolved in 3 ml water with warming. The solution was cooled to roomtemperature, and a 5 % solution of $KMnO_4$ was added dropwise until the permanganate colour persisted slightly. A drop of 0.1 *N* H_2SO_4 was added now and then to facilitate the precipitation of MnO_2. The excess $KMnO_4$ was destroyed with hydrogen peroxide and the resulting precipitate centrifuged down and washed twice with a few ml of water. One ml of 5 *N* NaOH was added to the combined supernatant and washings, and the sample was hydrolyzed for 15 minutes on

the boiling water bath. The solution was neutralized with 5 *N* H_2SO_4 with phenol red as indicator and 0.5 ml H_2SO_4 added in excess. After a rapid aeration for 5 minutes the solution was neutralized with carbonate-free 5 *N* NaOH until it became faintly red. A small amount of urease, in solution, was added, and the mixture allowed to stand for 30 minutes at 42° C. It was then acidified with 5 *N* H_2SO_4 and aerated for half an hour with warming to 100° C. The CO_2 was precipitated as $BaCO_3$. Yield 75 % calculated on the basis of uracil.

The degradation of labeled uracil. Uracil labeled in position 5 with C^{13} and in position 6 with C^{14} was synthesized from aspartic acid labeled in the β-carbon atom with C^{13} and in the β-carboxyl group with C^{14} *. The aspartic acid was deaminated to malic acid with an excess of potassium nitrite in glacial acetic acid. The resulting solution was acidified with concentrated H_2SO_4, the precipitate of potassium sulfate filtered off and the filtrate extracted with ether. The acetic acid was removed by distillation *in vacuo* and the remaining syrup used directly for the synthesis of uracil according to Davidson *et al.*[10]. Yield 25 %, calculated on the basis of aspartic acid.

Labeled uracil (0.9 millimole) was degraded as described above. The result is given in Table 1.

Table 1. Comparison of the theoretical values for C^{13} and C^{14} in labeled uracil with actual values found by degradation. The theoretical values were calculated from the C^{13}- and C^{14}-values of the total uracil molecule. They agreed quite satisfactorily with the values for the aspartic acid used in the synthesis of the uracil. The C^{14}-values are reported as counts/min. at infinite thickness. All samples were counted as $BaCO_3$.

Carbon atoms	Theoretical values		Values obtained from degradation	
	C^{14} counts/min.	C^{13} % excess	C^{14} counts/min.	C^{13} % excess
C_4	—	—	0	0.00
C_5	—	0.17	0	0.16
C_6	600	—	604	0.00

The degradation outlined in a preliminary report [1], in which carbon atoms 5 and 6 were supposed to be obtained as oxalic acid, was also tested by degrading uracil labeled as described above. The $BaCO_3$ obtained from oxalic acid was analysed for C^{13} and C^{14}. The value for C^{13} was 0.083 % excess, compared to the theoretical value 0.085 %. The value for C^{14}, however, was 210 counts/min. compared to the theoretical value 300 counts/min. Since the uracil was labeled in position 5 with C^{13} and in position 6 with C^{14}, it appears that oxalic acid was formed not only from carbon atoms 5 and 6 but also to an appreciable extent (30 %) from carbon atoms 4 and 5. This degradation is, therefore, quite unsatisfactory for use in tracer studies.

* I am greatly indebted to Dr. Gösta Ehrensvärd for his generous gift of the labeled aspartic acid.

SUMMARY

A degradation of uracil for tracer work has been described. Carbon atoms 4, 5 and 6 were split off as β-alanine, which after conversion to propionic acid, was degraded by two Schmidt decarboxylations according to Phares. Carbon atom 2 was obtained as urea.

Using this method every carbon atom in the ring can be analyzed directly for its isotope content. The degradation has been tested by degrading labeled uracil; the values obtained were found to agree quite satisfactorily with the theoretical ones.

REFERENCES

1. Lagerkvist, U. *Acta Chem. Scand.* **4** (1950) 1151.
2. Offe, G. *Ann.* **353** (1907) 267.
3. Heinrich, M. R., and Wilson, D. W. *J. Biol. Chem.* **186** (1950) 447.
4. Brown, E. B., and Johnson, T. B. *J. Am. Chem. Soc.* **45** (1923) 2702.
5. Weidel, H., and Roithner, E. *Monatsh. Chemie* **17** (1896) 183.
6. Broude, L. *Z. Physiol. Chem.* **173** (1928) 1.
7. Phares, E. F. *Arch. Biochem.* **33** (1951) 173.
8. Moore, S., and Stein, W. H. *J. Biol. Chem.* **176** (1948) 367.
9. Mosbach, E. H., Phares, E. F., and Carson, S. F. *Arch. Biochem.* **33** (1951) 179.
10. Davidson, D., and Baudisch, O. *J. Am. Chem. Soc.* **48** (1926) 2379.

Received June 16, 1952.