

The Synthesis of some Pyrimidine Derivatives Labelled with ^{14}C

V. JEZDIĆ, N. RAZUMENIĆ, M. SKAKUN, S. ALBAHARI, J. ODAVIĆ-JOSIĆ *

Hot Laboratory Department, "Boris Kidrič" Institute of Nuclear Sciences,
Vinča, Yugoslavia.

Received November 20, 1969.

SUMMARY

The procedures for synthesis of the following pyrimidine derivatives have been described (yields are given in brackets) : 2-thiouracil-2- ^{14}C (94 %), uracil-2- ^{14}C (91 %), 2-thiothymine-2- ^{14}C (92 %), thymine-2- ^{14}C (90 %), 5-bromouracil-2- ^{14}C (95 %) and orotic acid-6- ^{14}C (68 %). All these substances are chemically and radiochemically homogeneous and pure. The procedures develop according to the known reaction schemes with some modifications offering better yields. A new apparatus has been constructed for the synthesis of orotic acid-6- ^{14}C enabling continuous work in the dry nitrogen atmosphere. Specific activities are 28-30 mCi/mmole. The activities used were 60 mCi, and for the orotic acid-6- ^{14}C 30-180 mCi.

INTRODUCTION

The isotope-labelled pyrimidines are of great importance for various biological researches. In metabolic processes they are incorporated into nucleic acids enabling an easier process observation.

The present paper describes syntheses of the following pyrimidine derivatives : uracil, 2-thiouracil, thymine, 2-thiothymine and 5-bromouracil labelled with ^{14}C in the position 2, and orotic acid labelled in the position 6.

For syntheses of the above-mentioned compounds we used methods described in the literature. By detailed investigation of certain reactions remarkably better yields were obtained.

The starting radioactive material for the synthesis of pyrimidine derivatives labelled in the position 2 was thiourea- ^{14}C , produced in our laboratory ⁽¹⁾,

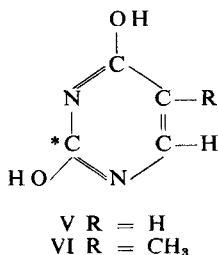
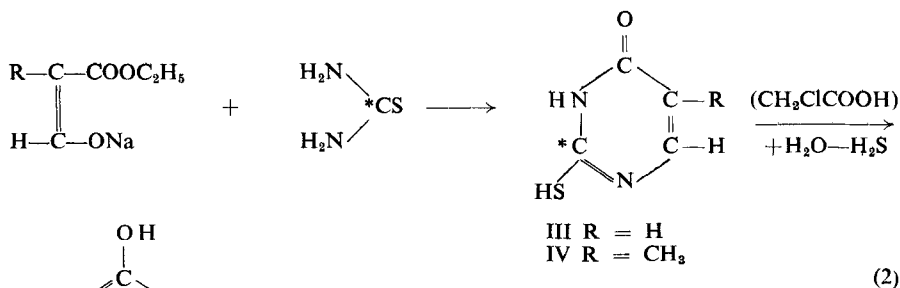
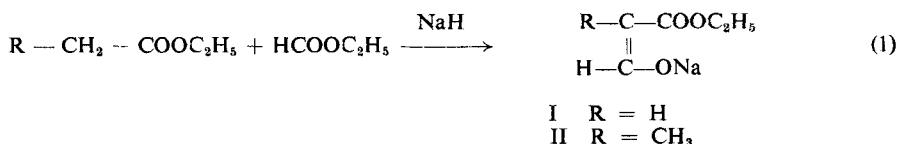
* Present address : Faculty of Technology, Organic Chemistry Department, Novi Sad, Yugoslavia.

and for the orotic acid-6- ^{14}C , sodium acetate-1- ^{14}C synthesized according to the already known procedure ⁽²⁾, and ethyl acetate-1- ^{14}C prepared by the method of Sakami, Evans and Gurin ⁽³⁾. By bromination of uracil-2- ^{14}C 5-bromouracil was prepared.

DESCRIPTION OF THE PROCEDURES

The uracil-2- ^{14}C and 2-thiouracil-2- ^{14}C were synthesized by a method representing the modification of the procedure of Plentl and Schoenheimer ⁽⁴⁾. By modifying reaction conditions considerably higher yields were obtained — 94 % of thiouracil and 91 % of uracil based on the thiourea- ^{14}C . Differing from previous authors ^(4, 5) we found out that the best yield in the synthesis of 2-thiouracil-2- ^{14}C (III, R = H) could be reached by condensation of thiourea- ^{14}C with the sodium salt of ethyl formylacetate (I) in absolute ethanol without the presence of sodium ethoxide as catalyst in the mole ratio 1 : 2.5. The uracil-2- ^{14}C (V, R = H) was obtained by desulphurization of 2-thiouracil-2- ^{14}C , by Wheeler's procedure ⁽⁶⁾ in the presence of the aqueous solution of monochloroacetic acid. After investigating the yield obtained from this reaction at various mole ratios of 2-thiouracil-2- ^{14}C and of monochloroacetic acid, we observed that the best yield could be obtained when this ratio was 1 : 2.5. The sodium salt of ethyl formylacetate was obtained according to the method of Grossman and Visser ⁽⁵⁾ in a 50 % yield.

The chemical reactions for the uracil and thiouracil syntheses are :



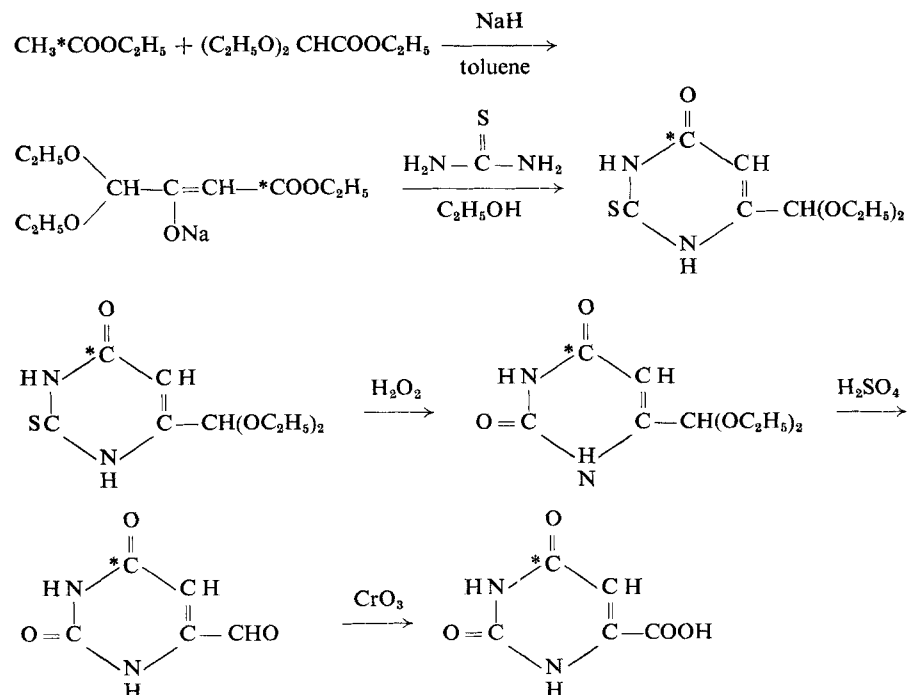
The thymine-2- ^{14}C (VI, $\text{R} = \text{CH}_3$) and 2-thiothymine-2- ^{14}C (IV, $\text{R} = \text{CH}_3$) were prepared by the same chemical reactions as it was the case with the uracil-2- ^{14}C and 2-thiouracil-2- ^{14}C , except that the sodium salt of ethyl formylpropionate (II, $\text{R} = \text{CH}_3$) was used for condensation with thiourea- ^{14}C with the same molecular proportions as I.

In reaction (1) sodium salt of ethyl formylpropionate was obtained also with the yield of 50 %; 2-thiothymine-2- ^{14}C was obtained by the reaction (2) with the yield of 92 %, while the yield of thymine-2- ^{14}C was 90 % (based on thiourea- ^{14}C).

Many syntheses of the labelled 5-bromouracil have been recorded ⁽⁷⁻¹³⁾. Bromination could be done by bromine water ^(7, 8), bromine in carbon tetrachloride ^(10, 11) and dioxanedibromide in dioxane ^(11, 12). The yields are from 40 to 96 %. The best yield has been obtained by means of dioxanedibromide ⁽¹²⁾, but 5-bromouracil obtained was of a considerably lower melting point (294-296° C) in comparison with that stated in the literature (312° C) ⁽⁸⁾.

We prepared 5-bromouracil-2- ^{14}C by bromination of the aqueous suspension of uracil-2- ^{14}C by means of bromine water with stirring and efficient ice-cooling till the appearance of a permanent light yellow coloring. In this way 95 % yield was obtained.

For the orotic acid-6- ^{14}C synthesis a slightly modified reaction scheme of Johnson and Schroeder ⁽²¹⁾ has been adopted.



In our procedure notable modifications were made in respect to the previous work (15-21). An assembly enabling continuous work in nitrogen atmosphere was constructed for the condensation of ethyl acetate-1- ^{14}C with ethyl diethoxyacetate and cyclization of sodium ethyl 4,4-diethoxy-acetoacetate-1- ^{14}C with thiourea to 2-thio-4-diethoxymethyl uracil-6- ^{14}C . The next part of the synthesis included the oxidation of the thio group into the keto group by hydrogen peroxide, conversion of the obtained 4-diethoxymethyluracil-6- ^{14}C by sulphuric acid to 4-uracil-carboxaldehyde-6- ^{14}C and in the oxidation of aldehyde into the acid by action of CrO_3 . All these reactions were performed in a centrifuge tube and the reaction products were not isolated except the final one.

The orotic acid-6- ^{14}C yield calculated relatively to the starting sodium acetate-1- ^{14}C was 68 % for larger quantities (5-6 mmole) and 59 % for a smaller scale (1-2 mmole).

The chemical purity of the final products was checked by the ultraviolet absorption spectrum and by ascending chromatography on paper; also the melting points were verified for the inactive substances : for uracil 333-335° C, for thymine 318-320° C, for thiouracil 338-340° C, for 5-bromouracil 307-309° C, for orotic acid 343-345° C (uncorrected).

The radiochemical purity was checked by the radioscanning of the paper chromatograms.

The labelled compounds synthesized by the described methods showed a satisfactory chemical and radiochemical purity.

The specific activities were 28-30 mCi/mmole.

EXPERIMENTAL

For syntheses A, B, C and D freshly dehydrated solvents (ether, benzene and ethanol) and reagents (ethyl formate and ethyl propionate) were used.

A. THIOURACIL-2- ^{14}C .

(a) *Sodium salt of ethyl formylacetate.*

To a suspension of 0.53 g of dry sodium hydride (22 mmole) in 10 ml of ether, 2.2 g (30 mmole) of ethyl formate was added dropwise with stirring and then 1.76 g (20 mmole) of ethyl acetate. After long stirring the reaction mixture was allowed to stand overnight. The sodium salt was separated by centrifugation. The yield was 1.38 g.

(b) *2-Thiouracil-2- ^{14}C .*

A mixture of 0.15 g (2 mmole) of thiourea- ^{14}C , 0.69 g (5 mmole) of the sodium salt of ethyl formylacetate and 10 ml of ethanol was refluxed three hours while stirring. The ethanol was then evaporated, the precipitate dissolved

in water and after acidification with acetic acid the thiouracil was precipitated. By filtration and drying 2-thiouracil-2-¹⁴C was obtained with a quantitative yield to be used immediately for the uracil synthesis.

If pure thiouracil is wanted, the precipitate after the evaporation of ethanol should be dissolved in water, treated with activated charcoal and again precipitated by adding acetic acid. The pure product was obtained by recrystallization from water.

B. URACIL-2-¹⁴C.

A mixture of 256 mg (2 mmole) of crude 2-thiouracil-2-¹⁴C, 7 ml of water and 472.5 mg (5 mmole) of monochloroacetic acid was refluxed for one hour during which time the entire thiouracil was dissolved. The solution was then evaporated in vacuum, the residue treated with hot ethanol and after that filtered off. By recrystallization from water the pure product was obtained.

The purity of uracil and thiouracil was checked by ascending chromatography on Whatman No. 1 paper in the following sets of solvents: *n*-butanol saturated with water and *n*-butanol/0,6 N NH₄OH(6/1) (v/v).

C. 2-THIOTHYMIN-2-¹⁴C.

(a) *Sodium salt of ethyl formylpropionate.*

To a well stirred suspension of 0.53 g (22 mmole) of sodium hydride in 20 ml of benzene 1.48 g (22 mmole) of ethyl formate was added dropwise at first, then 2.0 g (20 mmole) of ethyl propionate. The reaction mixture was stirred for another two hours and then permitted to stand overnight. The precipitate was separated by centrifugation, washed with ether and dried in vacuum. We obtained 1.52 g of the sodium salt of ethyl formylpropionate.

(b) *2-Thiothymine-2-¹⁴C.*

A mixture of 0.15 g (2 mmole) of thiourea-¹⁴C, 0.76 g (5 mmole) sodium salt of ethyl formylpropionate and 10 ml of ethanol was refluxed for three hours with good stirring. After the reaction was completed the solvent was evaporated, the dry precipitate was dissolved in 6 ml of water and the 2-thiothymine-2-¹⁴C was precipitated by adding dilute acetic acid (1:1). The precipitate was collected by filtration and then dried. Such crude thiothymine could be used immediately for the thymine synthesis.

The pure product can be obtained when the product after the ethanol evaporation is dissolved in water, treated with activated charcoal, precipitated with acetic acid and recrystallized from water.

D. THYMINE-2- ^{14}C .

A mixture of 284 mg (2 mmole) of crude 2-thiothymine-2- ^{14}C , 7 ml of water and 5 mmole of monochloroacetic acid was refluxed for one hour, during which time a clear solution resulted. By evaporation in vacuum a crystalline precipitate was obtained. This was treated with warm ethanol, then filtered off, washed with ethanol and recrystallized from water.

The purity of thymine and thiothymine was examined by the ascending chromatography on Whatman No. 1 paper in the set of solvents already mentioned for the uracil.

E. 5-BROMOURACIL-2- ^{14}C .

A suspension of 250 mg (2.2 mmole) of uracil-2- ^{14}C in 15 ml of water was stirred with constant cooling in the ice-bath and freshly prepared bromine water was added dropwise till the appearance of a constant yellow colour. The solution obtained was evaporated in vacuum (temp. of the bath 40°C), and dissolved in about ten milliliters of ethanol, evaporated to dryness and dried. By recrystallization from water with activated charcoal the pure product was obtained. The ascending chromatography was performed on Whatman 1 paper in the following sets of solvents: *n*-butanol saturated with water; *n*-butanol/acetic acid/water (5 : 2 : 1).

F. OROTIC ACID-6- ^{14}C .

Two procedures for the synthesis are given :

- I) for quantities of 5-6 mmole, where the synthesis was performed in the apparatus shown in Figure 1.
- II) for quantities of 1-2 mmole, apparatus Figure 2.

Materials.—Anhydrous solvents were used as well as freshly distilled diethyl sulphate.

PROCEDURE I.**(a) Ethyl acetate-1- ^{14}C .**

In the flask C (Fig. 1) 0.3 g (12.5 mmole) of sodium hydride and 10 ml of toluene were placed, and in the flask A 0.5 g (6.1 mmole) of dry sodium acetate-1- ^{14}C and 3 ml of diethyl sulphate were used. The tube D was attached to the apparatus for supply of nitrogen and through the whole apparatus dry nitrogen was swept for 10 minutes. The tube D was removed and replaced with a stopper. After the circulation of water started through the condenser of the flask A, the mixture in the flask was refluxed (temperature of the bath 160°C) for half an hour. After that water from the condenser was drained off, and the flask B chilled with liquid nitrogen. The bath temperature under the

flask A was increased to 190° C and ethyl acetate-1-¹⁴C was distilled in the flask B. The cooling with the liquid nitrogen was stopped and the flask A disconnected from the apparatus (Fig. 1).

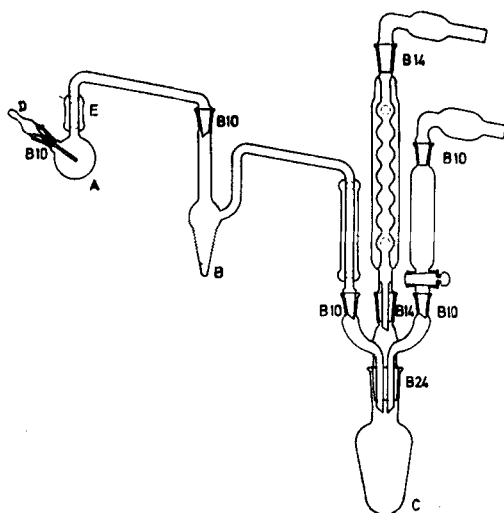


FIG. 1

(b) *Ethyl 4,4-diethoxysodiumacetoacetate-1-¹⁴C.*

A solution of 2 g (11,35 mmole) of ethyl diethoxyacetate in 4 ml of toluene was placed in the dropping funnel. The bath under the flask C was heated to 95° C and half of ethyl diethoxyacetate solution was added dropwise while stirring by means of magnetic stirrer. The flask B was heated slowly to 120° C during the adding of ethyl diethoxyacetate so that the ethyl acetate-1-¹⁴C was carried from B to C. In order to transfer quantitatively ethyl acetate, heating of the flask B was stopped; 1 ml of toluene was added to it, the flask heated again and the entire toluene transferred to C. After that dropwise adding of ethyl diethoxyacetate was finished while stirring and the funnel was washed with 0.5-1 ml of toluene. The reaction mixture in the flask C was heated to 95-100° C during two hours with stirring by magnetic stirrer during which time the whole sodium hydride was dissolved.

After the reaction was complete, toluene was evaporated in vacuum and crude ethyl 4,4-diethoxysodiumacetoacetate-1-¹⁴C was obtained to be used immediately for further work.

(c) *2-Thio-4-diethoxymethyluracil-6- ^{14}C* .

To the crude ethyl 4,4-diethoxysodiumacetoacetate-1- ^{14}C 0.7 g (9.2 mmole) of thiourea and 10 ml of ethanol were added, then refluxed for three hours while stirring (temperature of the bath 95-100° C). Then ethanol was evaporated in vacuum, precipitate dissolved in water, evaporated to a smaller volume (about 8 ml) and acidified with 6N HCl to pH 4. Crystals that are precipitated on standing in refrigerator were filtered, washed with cold water and dried. The yield was 1.08 g (77.5 % based on sodium acetate-1- ^{14}C).

(d) *4-Diethoxymethyluracil-6- ^{14}C* .

In 7 ml of water 1.08 g of 2-thio-4-diethoxymethyluracil was suspended, 3 ml of hydrogen peroxide 30 % was added, cooled with ice and added dropwise slowly while stirring 3 ml of 5N NaOH. In this way the substance was completely dissolved. Cooling was continued for another 10 minutes and stirred at room temperature for two hours. The reaction mixture was heated then on water bath until the excess of hydrogen peroxide was decomposed.

(e) *4-Uracilcarboxaldehyde-6- ^{14}C* .

The solution of 4-diethoxymethyluracil was cooled with ice, stirred and added 1.5 ml of conc. sulphuric acid. White crystals were precipitated. The reaction mixture was heated and allowed to boil for a short time in order to perform hydrolysis and expel sulphur dioxide, then it was cooled in the ice-bath. In this 4-uracilcarboxaldehyde-6- ^{14}C was crystallized.

(f) *Orotic acid-6- ^{14}C* .

The reaction mixture was cooled well in the ice-bath and while stirring a solution containing 1.4 g of CrO_3 in 2 ml of water was added dropwise; then 1.5 ml of concentrated sulphuric acid was added. The mixture was stored at room temperature for three hours then heated to 85° C and kept at the temperature for ten minutes. Orotic acid precipitate appeared. After cooling it was permitted to stand overnight in refrigerator. The precipitate was filtered then and suspended in 8 ml of hot water, then added carefully a saturated solution of potassium hydroxide till the product was dissolved completely. Activated charcoal was added, boiled for 15 minutes and filtered off. Hot filtrate was acidified with hydrochloric acid to pH 1. The precipitated product was separated by filtration, washed with water and acetone, then dried in the mechanical pump vacuum. The yield 0.65 g.

PROCEDURE II.

(a) *Ethyl acetate-1-¹⁴C*.

In the flask A (Fig. 2) 88 mg (1.08 mmole) of dry sodium acetate-1-¹⁴C was placed. After sweeping the apparatus for a few minutes with dry nitrogen, 0.53 ml of diethyl sulphate was added. The flask C was then fitted, with previously added 53 mg (2.17 mmole) of sodium hydride and 1.77 ml of toluene. Also the wash bottle D was attached. The apparatus was washed by a stream of nitrogen for 10 min, and mounted the immersion pocket F with the reflux condenser E cooled with water (the condenser and the immersion pocket are connected with polyethylene tubing). The flask A was heated at 160° C for 30 minutes and after that esterification was complete. In order to transfer quantitatively ethyl acetate-1-¹⁴C, the condenser E was separated from the immersion pocket F and the latter filled with hot glycerin. In order to maintain the atmosphere of nitrogen for further work, Bunsen's valve was attached to the wash bottle D enabling nitrogen supply.

Now begins the transfer of ethyl acetate-1-¹⁴C. The flask B is cooled with liquid nitrogen and the flask A heated from 160° C to 190° C and then ethyl acetate-1-¹⁴C redistilled in B. The ethyl acetate is then transferred to the flask C in a similar way : C is cooled with the liquid nitrogen and B is gradually heated to 140° C. Pure ethyl acetate is redistilled, and A and B are washed with 0.5 ml of toluene as in the Procedure I (b).

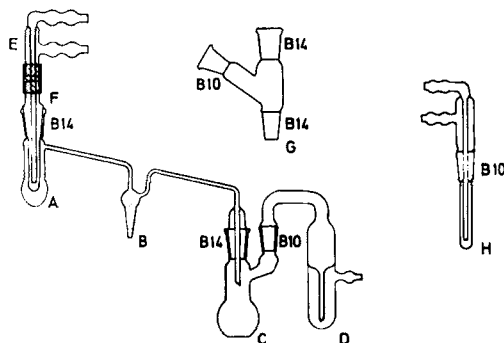


FIG. 2.

(b) *Ethyl 4,4-diethoxysodiumacetoacetate-1-¹⁴C*.

On final distillation the flask C was cooled further on, A and B detached, the adapter G was fitted to the flask C, nitrogen current conducted on the joint B 14, and 0.25 ml (2 mmole) of ethyl diethoxyacetate added with another 0.7 ml of toluene. The adapter G is replaced by the "cold finger" condenser H. Reaction started immediately the same being visible by evolving of hydrogen

bubbles. The cooling bath is now replaced by oil bath of 95-100° C and heated for two hours while stirring. In this the whole sodium hydride was dissolved and a yellow-redish liquid was obtained.

Toluene was then removed in vacuum and an oily yellow mass was gained. Crude ethyl 4,4-diethoxyacetoacetate was used without purification for further work.

(c) *2-Thio-4-diethoxymethyluracil-6- ^{14}C* .

The method was the same as the Procedure I. Quantities of reagents were : solution of 124 mg (1.62 mmole) of thiourea in 1.77 ml of ethanol. Yield 171 mg (69 % based on sodium acetate-1- ^{14}C).

(d) *4-Diethoxymethyluracil-6- ^{14}C* .

The synthesis was the same as in Procedure I. Quantity of reagent was : water 1 ml, hydrogen peroxide 30 % 0.44 ml. It was used 0.44 ml of 5N NaOH.

(e) *4-Uracilcarboxaldehyde-6- ^{14}C* .

Precipitation was performed as in the Procedure I, with 0.22 ml of sulphuric acid.

(f) *Orotic acid-6- ^{14}C* .

Procedure was identical to the Procedure I and quantity of reagents was : solution 0.2 g of CrO_3 in 0.3 ml of water; 0.22 ml of concentrated sulphuric acid. Yield 100 mg.

Ascending chromatography was performed on Whatman No. 1 paper in the following sets of solvents : isopropanol/conc. HCl/water (170 : 41 : 39); aqueous solution of NH_4Cl (3 %, pH 5.3)/sec. butanol/acetic acid (2 : 1 : 1); *n*-butanol/propionic acid/water (10 : 5 : 7).

REFERENCES

1. JEZDIĆ, V. and RAJNVAJN, J. — *Bull. Inst. Nucl. Sci. "Boris Kidrich"*, **12** : 127 (1961).
2. MURRAY, A. and WILLIAMS, D. L. — *Organic Syntheses with Isotopes*, Interscience Publishers, New York, 1958, vol. I, p. 34; CALVIN, M., HEIDELBERGER, C., REID, J. C., TOLBERT, B. M. and JANKWICH, P. E. — *Isotopic Carbon*, J. Wiley and Sons, New York, 1949, p. 177.
3. SAKAMI, W., EVANS, W. E. and GURIN, S. — *J. Am. Chem. Soc.*, **69** : 1111 (1947).
4. PLENTL, A. and SCHOENHEIMER, R. — *J. Biol. Chem.*, **153** : 211 (1944).
5. GROSSMAN, L. and VISSER, W. D. — *J. Biol. Chem.*, **209** : 447 (1954).

6. WHEELER, L. H. and LIDDLE, M. L. — *Am. Chem. J.*, **40** : 547 (1908).
7. LEVENE, A. P. — *J. Biol. Chem.*, **63** : 653 (1925).
8. HILBERT, G. E. and JANSEN, E. F. — *J. Am. Chem. Soc.*, **56** : 134 (1934).
9. WEYGAND, F., WACKER, A. and GRISEBACH, H. — *Z. Naturforsch.*, **6b** : 177 (1951).
10. WEYGAND, F., WACKER, A., TREBST, A. and SVOBODA, O. P. — *Z. Naturforsch.*, **9b** : 764 (1954).
11. FILIP, J. and MORAVEK, J. — *Chem. and Ind.*, **10** : 260 (1960).
12. MORAVEK, J. and FILIP, J. — *Coll. Czech. Chem. Comm.*, **25** : 2697 (1960).
13. WANG, Shih-Yi. — *J. Org. Chem.*, **24** : 11 (1959).
14. HEIDELBERGER, C. and HURLBERT, B. R. — *J. Am. Chem. Soc.*, **72** : 4704 (1950).
15. PICHAT, L., AUDINOT, M. and CARBONNIER, P. — *Bull. Soc. Chim. France*, 1798 (1959).
16. KÖRTE, F., PAULUS, W. and STÖRIK, K. — *Liebigs Ann. Chem.*, **619** : 63 (1958).
17. WEED, I. L. and WILSON, W. D. — *J. Biol. Chem.*, **189** : 435 (1951).
18. TAMAYO, L. M., GAMBOA, M. J. and ALVAREZ, F. E. — *Anales real. soc. espan. fis. y quim.*, **48B** : 889 (1952).
19. DREHMAN, U. and BORN, H. J. — *J. Prakt. Chem.*, **5** : 200 (1957).
20. LANGLEY, W. B. — *J. Am. Chem. Soc.*, **78** : 2136 (1956).
21. JOHNSON, I. B. and SCHROEDER, E. I. — *J. Am. Chem. Soc.*, **53** : 1989 (1931).