chemical yield of 23.2%*. The melting point was 128-130°C (according to literature data mp 129-131°C). Specific activity was 17.7 mCi/g. Total activity was 14.7 mCi. Radiochemical yield was 22.8%. RCP was determined by counting a chromatogram of the product on Silufol in the system butanol–acetic acid–water (4:1:5). The radiochemical content of the main product was not less than 99.5% (R_f 0.5). The product contained a radioactive contaminant producing a spot (R_f = 0.2) on the chromatogram containing less than 0.5% activity.

The chemical content of (III) in the preparation determined by comparing its UV absorption at 230 and 276 nm with the absorption of a standard sample (prepared in the Farmakon works) was 100%.

*On working out the synthesis starting from inactive sodium cyanide the yield of (III) reached 40% with careful working.

LITERATURE CITED

1. V. A. Dobrina, D. V. Ioffe, S. G. Kuznetsov, et al., Khim. Farm. Zh., No. 6, 14 (1976).
2. J. D. Roberts and C. M. Regan, J. Amer. Chem. Soc., 75, 2069 (1953).
3. A. G. Chigarev and D. V. Ioffe, Zh. Org. Khim., No. 3, 85 (1967).
4. A. V. El'tsov, A. G. Chigarev, and N. T. Starykh, Zh. Obshch. Khim., 34, 3344 (1964).
5. Swiss Patent No. 575944 (1976); British Patent No. 1,364,993 (1974).
6. A. G. Chigarev, Inventor's Certificate No. 215,215 (USSR); Otkrytiya No. 13 (1968).
7. F. J. Dominiani, R. P. Piochi, and R. L. Goung, J. Med. Chem., 14, 1018 (1971).
8. A. V. El'tsov, S. S. Krylov, N. T. Starykh, et al., Inventor's Certificate No. 166,348 (USSR); Otkrytiya No. 22 (1964).
9. A. G. Chigarev, Inventor's Certificate No. 234,410 (USSR); Otkrytiya No. 4 (1969).

THIOLATION OF HYDROXY DERIVATIVES OF PURINE AND PYRIMIDINE IN A SULFONE MEDIUM

I. L. Ozola and U. Ya. Mikstais

UDC 615.272:[547.857.4+547.854.83].012.1

Thio derivatives of purine and pyrimidine possess a multiplicity of biological activities chiefly connected wiht their antimetabolite action. The compound 6-mercaptopurine (I) is securely established in oncological practice for the treatment of various neoplasms.

Thio derivatives of purine and pyrimidine are usually obtained from the corresponding hydroxy derivatives by thiolation with phosphorus pentasulfide. Thus, 6-mercaptopurine (I) is obtained from hypoxanthine (II) in yields of 32 to 85% depending on the solvent and conditions of carrying out the reaction [1-3]. The compounds 2,6-dithiopurine (III) and 2,6,8-trithiopurine (IV) have not been obtained from the corresponding hydroxy derivatives of purine, viz. xanthine (V) and uric acid (VI). On reacting these hydroxy compounds with phosphorus pentasulfide products were obtained with only partial replacement of hydroxy by mercapto groups [4]. Compounds (III) and (IV) have been obtained by a multistage synthesis involving formation of the purine ring [4-6]. The compound 2,6-dithiouracil (VII) may be obtained from uracil (VIII) by direct thiolation with phosphorus pentasulfide in yields of up to 54% [7].

The thiolation of hydroxy derivatives of purine (II, V, VI) and pyrimidine (VIII) with phosphorus pentasulfide in a sulfone medium has been investigated by us. Sulfones were selected by us as reaction media for thiolation because of their high chemical and thermal stability [8]. Thiolation was carried out in tetramethylenesulfone and dimethylsulfone at 160-185°C with an excess of phosphorus pentasulfide. No significant decomposition of sulfone occurred under the reaction conditions and a high degree of replacement of hydroxy groups by

All-Union Scientific-Research Institute for Applied Biochemistry, Olaine.

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 12, No. 9, pp. 85-87, September, 1978. Original article submitted February 28, 1978.

0091-150X/78/1209-1190$07.50

mercapto groups was achieved. Up to 94% in the case of hypoxanthine and up to 97.2% in the case of uracil were found [9]. The synthesis of 2,6-dithiopurine and 2,6,8-trithiopurine was achieved by thiolation in sulfones by direct replacement of the hydroxy groups of xanthine in 65% yield and of uric acid in 90% yield [10]. Thus the developed methods of thiolation of purine and pyrimidine hydroxy groups make it possible to obtain thio derivatives in high yield and may be recommended for preparative use.

EXPERIMENTAL

Chromatography was carried out on Silufol UV-254 plates in system A: butyl alcohol—acetic acid—water (12:3:5), system B: isopropyl alcohol—ammonia—water (7:1:2), and system C: ispropyl alcohol—water—ammonia (85:15:1.2). Visualization was by UV.

UV spectra were taken on a Unicam 800 spectrometer. The data obtained corresponded to literature data [3, 7, 11, 12].

6-Mercaptopurine (I). A. A mixture of hypoxanthine (1.36 g, 0.01 mole), phosphorus pentasulfide (6 g), and tetramethylenesulfone (10 ml) was heated for 5 h at 180-185 °C. The reaction mixture was poured into water, left until the following day, and then heated on a water bath for 3 h. Active carbon was added to the hot solution which was then filtered. On cooling, yellowish crystals separated from the solution and were recrystallized from hot water. The yield of (I) was 1.6 g (94%) of mp 314-315 °C (with decomposition). Rf 0.68 (system A). Found, %: C 34.97; H 3.42; N 32.11; S 18.84. Calculated, %: C 35.32; H 3.58; N 32.08; S 18.82.

B. Compound (I) (1.4 g, 82%) was obtained by an analogous method from hypoxanthine (1.36 g; 0.01 mole), phosphorus pentasulfide (6 g), and dimethylsulfone (15 g).

2,6-Dithiopurine (III). A mixture of xanthine (1.52 g, 0.01 mole), phosphorus pentasulfide (10 g), and tetramethylenesulfone (15 ml) was heated for 5 h at 160-165°C. The reaction mixture was poured into water, left until the following day, heated on a water bath for 3 h, and ammonia added to pH 7.0-8.0 The hot solution was treated with activated carbon and filtered. The filtrate was acidified with hydrochloric acid to pH 5.0. On cooling yellow crystals separated and were recrystallized from hot water. The yield of (III) was 1.2 g (65%). R_f 0.66 (system B). Found, %: C 32.80; H 2.30; N 30.10; S 34.91. Calculated, %: C 32.69; H 2.27; N 30.40; S 34.68.

2,6,8-Trithiopurine (IV). A mixture of uric acid (9.2 g, 0.05 mole), phosphorus pentasulfide (20 g), and tetramethylenesulfone (30 ml) was heated for 5 h at 160-165°C. Isolation of the product was analogous to (III). The yield of (IV) was 9.83 g (90%). R_f 0.94 (system C). Found, %: C 27.95; H 1.85; N 24.89; S 42.7. Calculated, %: C 27.80; H 1.85; N 25.90; S 44.4.

2,6-Dithiouracil (VII). A mixture of uracil (5.4 g, 0.05 mole), phosphorus pentasulfide (15 g), and tetramethylenesulfone (15 ml) was heated for 4 h at 160-165°C. Isolation of the product was analogous to that of (III). The yield of (VII) was 7.0 g (97.2%). R_f 0.84 (system C). Found, %: C 33.25; H 2.80; N 18.78; S 44.7. Calculated, %: C 33.30; H 2.78; N 19.44; S 44.4.

B. By a similar method using uracil (5.4 g, 0.05 mole), phosphorus pentasulfide (15 g), and dimethylsulfone (25 g) compound (VII) was obtained in a yield of 6.73 g (93.5%).

LITERATURE CITED

1. Polish Patent No. 67,025 (1973).
2. US Patent No. 2,691,654 (1954): Chem. Abs., 50, 1933h (1956).
3. Japanese Patent No. 30,832 (1970); Chem. Abs., 74, 42388g (1971).
4. G. B. Elion, S. Mueller, and G. H. Hitchings, J. Amer. Chem. Soc., 81, 3042 (1959).
5. Japanese Patent No. 10789 (1969): Chem. Abs., 71, 124506e (1969).
6. A. E. Beman, J. F. Gerster, and R. K. Robins, J. Org. Chem., 27, 986 (1962).
7. G. B. Elion and G. H. Hitchings, J. Amer. Chem. Soc., 69, 2138 (1947).
8. E. M. Arnet and C. F. Douty, J. Am. Chem. Soc., 86, 409 (1964).
9. I. L. Ozola and U. Ya. Mikstais, Byull. Izobret., No. 43 (1976), Inventor's Certificate No. 536178.
10. I. L. Ozola and U. Ya. Mikstais, Byull. Izobret, No. 32 (1977), Inventor's Certificate No. 570612.

11. A. Viout and P. Rumpf, Chem. Abs., 54, 18532g (1960).
12. C. W. Noel and R. K. Robins, J. Amer. Chem. Soc., 81, 5997 (1959).

PREPARATION OF AMINO ACID MIXTURES FROM AUTOLYZATES OF BAKERS' YEAST. II. IMPROVEMENT OF THE CONTENT OF PURIFIED AUTOLYZATES BY HYDROLYTIC TREATMENT

A. P. Kiselev, T. A. Loginova, and A. D. Neklyudov

UDC 615.31:547.466].012:663.14

The autolyzate of bakers' yeast after ion-exchange purification usually contains from 15 to 30% peptides of low and medium molecular weight [1]. Furthermore, as was shown in the previous communication, there is also a certain quantity of cyclic peptide fragments (of molecular weight up to 1500) in the final product and contaminants of a glycopeptide nature. Attempts have been made in the present work to convert the autolyzate into an amino acid mixture with minimal concentration of low-molecular-weight peptides. At the same time an increase in the content of essential amino acids in the product was achieved due to hydrolysis of peptidic material. The developed methods for hydrolytic treatment of the autolyzate do not require the use of poorly accessible reagents and complex technological equipment. Practically no loss of amino acids or increase in color of the autolyzate were observed in the process of hydrolysis.

EXPERIMENTAL

For the laboratory investigations an autolyzate of bakers' yeast was taken which had been obtained in the experimental unit of the Institute of Heteroorganic Compounds of the Academy of Sciences of the USSR (ANSSR)* and had been subjected to ion-exchange purification according to the optimized scheme [2]. Crystalline toluene-p-sulfonic acid (Merck, Federal Republic of Germany) was added to 4 N concentration and also a small quantity (0.1-0.15%) of antioxidant, viz. tryptamine hydrochloride (Reanal, Hungary), to a solution containing 54 g/liter free amino acids and 16 g/liter peptides (according to data of amino acid analysis and titration in nonaqueous medium) for chemical hydrolysis. Hydrolysis was carried out for 1-24 h at 95-120°C in evacuated glass ampuls or in an open glass reactor (sulfonation flask of 250 ml capacity; Jena, German Democratic Republic) with a stirrer and bubbler for passing argon. At the end of hydrolysis the excess of toluene-p-sulfonic acid was removed by absorption on columns of AV-17 resin in the OH^- form or with the bifunctional ion-exchange resin AG-11 A8 (Bio-Rad, USA). The amount of resin required was determined by calculating a 10% excess of the total exchange volume for the content of toluene-p-sulfonic acid in the solution. Purification of the hydrolyzate on a column of cation exchange resin Ku-2 × 8 in H^+ form was also used (desorption of amino acids with 2% ammonia solution).

Complex preparations of the bacterial protease pronase (Merck, Federal Republic of Germany) and protosubtilin of specific activity 190 units/g [2] chemically immobilized on silica gel were taken for enzymic hydrolysis. Preliminary activation of the silica gel was achieved by treatment with a solution of γ-aminopropyltriethoxysilane in acetone. Covalent bonding of enzymes with carrier was accomplished by the formation of azomethine derivatives with glutaric dialdehyde [3] or by way of further treatment of the activated carrier with o-phenylenediamine and subsequent stages of diazotization and azo coupling with the enzyme [4]. The amount of enzyme fixed onto the carrier was determined spectrophotometrically at λ = 280 nm and by Lowry's method.

Enzymatic treatment of the purified autolyzate (solution with a content of 71 g/liter amino acids and 17 g/liter peptides) was carried out for 1.5-3 h at 40-50°C, pH 7.5-8.5 and an enzyme/substrate ratio of 1:100 (ratio of weights of immobilized enzyme and autolyzate

*The authors are grateful to V. M. Belikova, S. V. Gordienko, and V. K. Latova for kindly providing the samples.

All-Union Scientific-Research Technological Institute for Blood Substitutes and Hormonal Preparations, Moscow. Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 12, No. 9, pp. 87-91, September, 1978. Original article submitted March 23, 1978.

 0091-150X/78/1209-1192$07.50