rated. After two hours 48 g. of product was collected, m.p. 229–232°. Recrystallization from dioxane raised the melting point to 231–232°.

Anal. Calcd. for $C_7H_4N_2O_4$: C, 46.68; H, 2.24. Found: C, 46.56; H, 2.35.

5-Aminobenzoxazolone.—The 5-nitrobenzoxazolone was hydrogenated using 5% palladium-on-Darco as catalyst. It was crystallized from alcohol–water, m.p. 223°.

Anal. Calcd. for $C_7H_6N_2O_2$: C, 55.99; H, 4.03. Found: C, 55.92; H, 4.03.

5-Carbamidobenzoxazolone was prepared like 6-carbamidobenzoxazolone. It was crystallized from water, m.p. 356°.

Anal. Calcd. for $C_8H_7N_3O_3$: C, 49.74; H 3.65. Found: C, 49.90; H, 3.59.

5,6-Dinitrobenzoxazolone.—A solution of 15 g. of 6-nitrobenzoxazolone in 100 ml. of fuming nitric acid was warmed to 40° on the steam-bath, it was then removed and the temperature climbed to 60°. After standing overnight it was diluted with water to give 17.5 g. Recrystallization from alcohol–water gave 14 g., m.p. 190–195°. A sample was recrystallized for analysis and the melting point was 199–201°.

Anal. Calcd. for $C_7H_3N_3O_6$: C, 37.34; H, 1.34. Found: C, 37.13; H, 1.32.

5,6-Ureidobenzoxazolone.—5,6-Dinitrobenzoxazolone was hydrogenated in alcohol using 5% palladium-on-Darco. The diamine was quite insoluble and considerable difficulty was encountered getting it separated from the catalyst. Some hydrochloride was prepared and this was dissolved in water and phosgene bubbled into it. A pink solid separated. It was purified by dissolving in sodium hydroxide and precipitating with hydrochloric acid.

Anal. Calcd. for $C_8H_5N_3O_3$: C, 50.27; H, 2.64. Found: C, 49.39; H, 2.78.

Acknowledgment.—The authors wish to thank Dr. Lewis H. Sarett for his guidance and encouragement in this investigation.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF TEXAS]

Utilization of α,γ-Dialkoxyacetoacetates in the Synthesis of Certain 2-Thiouracils and Uracils[1]

BY HENRY R. HENZE AND EILHARD N. KAHLENBERG[2]

RECEIVED AUGUST 19, 1957

A series of *sec*-butyl alkoxyacetates was converted into a new series of *sec*-butyl α,γ-dialkoxyacetoacetates, and the latter yielded the corresponding thiouracils by interaction with thiourea. Uracils were formed more readily by desulfurization of the thiouracils than from interaction of the alkoxyacetoacetates with urea.

In light of the early interest in thiouracils[3] and uracils,[4] and more recently, in connection with a study of compounds of possible use as metabolic inhibitors, it was decided to attempt the synthesis of certain pyrimidines containing alkoxy substituents. Production of these compounds was visualized through utilization of well-established procedures involving condensation of substituted acetoacetates with thiourea or urea. During the preliminary investigations in this work, it was demonstrated that *sec*-butyl alkoxyacetates gave better yields of dialkoxyacetoacetates, upon condensation, than did the corresponding methyl, ethyl and propyl alkoxyacetates. Using a method described by Johnson and Caldwell,[5] the *sec*-butyl alkoxyacetoacetates were converted into the corresponding 5-alkoxy-6-alkoxymethyl-2-thiouracils by allowing the esters to react with thiourea in the presence of sodium methoxide. When it was found that the corresponding uracils were better prepared from the thiouracils than from condensation of the keto esters with urea, each thiouracil derivative was in turn desulfurized through use of chloroacetic acid.

It was next of interest to investigate the cleavage of the ether linkages of both the thiouracil and uracil derivatives, since the ease of cleavage and/or replacement of their alkoxyl groups would have a direct bearing on their possible use as intermediates in the synthesis of pyrimidines of more complex structure. It was recalled that, in a search for a synthesis of orotic acid,[5] the cleavage of both ethoxyl groups of 5-ethoxy-6-ethoxymethyluracil had been accomplished under pressure by means of concentrated hydrochloric acid at 120–140°; such treatment resulted in formation of 5-hydroxy-6-hydroxymethyluracil. Since hydrogen iodide is the common reagent for scission of ether linkages, although frequently extensive reduction of the product is noted, the behavior of certain thiouracil and uracil derivatives toward hot concentrated hydriodic acid was investigated. It was found that 5-isobutoxy-6-isobutoxymethyl-2-thiouracil could be reductively cleaved in a stepwise fashion. The initial stage involved cleavage of the alkoxyl group at the 6-position with reduction to form 5-isobutoxy-6-methyl-2-thiouracil. The next step comprised splitting, unaccompanied by reduction at the 5-position, resulting in formation of 5-hydroxy-6-methyl-2-thiouracil. The latter resulted, also, from similar treatment of 5-isopropoxy-6-isopropoxymethyl-2-thiouracil.

Similarly, reductive cleavage was found to occur with the corresponding uracils. Thus, using boiling, concentrated hydriodic acid, 5-isobutoxy-6-isobutoxymethyluracil was converted into 5-isobutoxy-6-methyluracil and 5-hydroxy-6-methyluracil. The latter compound had previously been prepared through oxidation of 6-methyluracil by Behrund and Grünewald.[6] They reported that uracils containing an hydroxyl substituent at the 5-

(1) From the Ph.D. dissertation of E. N. Kahlenberg at the University of Texas, June, 1954.

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(3) W. B. Baker, *J. Am. Pharm. Assoc., Pract. Pharm. Ed.*, **6**, 359 (1945).

(4) T. G. Klumpp and J. B. Rice, *Record Chem. Progr., Kresge-Hooker Sci. Lib.*, **7**, 15 (1946).

(5) T. B. Johnson and W. T. Caldwell, THIS JOURNAL, **51**, 873 (1929).

(6) R. Behrund and R. Grünewald, *Ann.*, **323**, 186 (1902).

TABLE I

sec-BUTYL ALKOXYACETATES $ROCH_2COOC_4H_9$-*sec*

R	B.p., °C.	Mm.	Yield,[a] %	$n^{25}D$	d^{25}_4	*MR*D Calcd.	*MR*D Found	Sapn. equiv. Calcd.	Sapn. equiv. Found
Methyl[c]	166–168	751	60	1.4072[b]	0.9519[b]	37.81	37.83	146.2	140.6
Ethyl	178–179	743	89	1.4070	.9281	42.44	42.50	160.2	160.4
Propyl	195–196	742	86	1.4103	.9171	47.06	47.11	174.2	174.4
Isopropyl	85–86	16	80	1.4089	.9141	47.06	47.12	174.2	174.0
Butyl	212–213	744	89	1.4144	.9118	51.68	51.65	188.3	188.1
Isobutyl	204–205	743	90	1.4121	.9029	51.68	51.89	188.3	188.5
sec-Butyl	203–204	743	60	1.4150	.9146	51.68	51.55	188.3	188.2

[a] Yield based on alkoxyacetic acid. [b] These values were determined at 20°. [c] M. H. Palomaa, E. J. Salmi, J. I. Jansson and T. Salo [*Ber.*, 68B, 303 (1935)] reported b.p. 62–64° (22–23 mm.), $n^{20}D$ 1.40711, d^{20}_4 0.9522.

TABLE II

sec-BUTYL α,γ-DIALKOXYACETOACETATES $ROCH_2COCHRCH(OR)COOC_4H_9$-*sec*

R	B.p., °C.	Mm.	Yield, %	$n^{25}D$	d^{25}_4	*MR*D Calcd.	*MR*D Found	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found
Methyl	146–148	15–16	61	1.4341	1.080	53.32	53.58	55.03	54.75	8.29	8.01
Ethyl	153–156	16–17	72	1.4310	1.012	62.57	63.01	58.52	58.32	9.01	8.90
Propyl	133–135	1.5–2.5	74	1.4334	1.003	71.80	71.06	61.29	61.02	9.56	9.31
Isopropyl	128–130	1.5–2.5	70	1.4252	0.9903	71.80	70.88	61.29	60.98	9.56	9.69
Butyl	165–167	2.5–3.5	76	1.4354	.9771	81.05	80.84	63.55	63.45	10.00	10.09
Isobutyl	145–147	1.5–2.5	78	1.4325	.9604	81.05	81.77	63.55	63.31	10.00	10.18
sec-Butyl	143–145	1.5–2.5	56	1.4340	.9748	81.05	80.80	63.55	63.60	10.00	10.26

position imparted a deep blue color to a dilute, aqueous solution of ferric chloride. In the present study, it has been found that a similar color test is given by 5-hydroxy-2-thiouracils.

Experimental

Preparation of *sec*-Butyl Alkoxyacetates.—Chloroacetic acid (1.5 moles) was heated for 2 hr. with an appropriate sodium alkoxide (3 moles) in the corresponding anhydrous alcohol. The alcohol was largely removed by distillation, and addition of concentrated hydrochloric acid (1.7 moles) liberated the alkoxy acid (yields of distilled products 80–88%). The alkoxy acids were treated with thionyl chloride, and the crude acid chlorides were mixed with excess *sec*-butyl alcohol; after 5 hr. refluxing, the mixtures were neutralized, extracted and fractionated to give the butyl esters (yields of 60–90%). Analytical samples were prepared by fractionation through a 25-cm. column; saponification equivalents were determined by the procedure of Cheronis and Entriken.[7] Certain data for these esters appear in Table I.

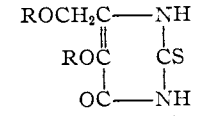
Preparation of *sec*-Butyl α,γ-Dialkoxyacetoacetates.—The *sec*-butyl alkoxyacetates were self-condensed in the presence of alcohol-free sodium methoxide suspended in anhydrous toluene. After the reaction mixture had refluxed for 12 hr., the toluene was distilled to leave a volume of about 125–150 ml. The cooled material was treated with acetic acid, then with 10% sodium carbonate solution until neutral. The ether extract was dried and fractionated under diminished pressure. Data for these esters are collected in Table II.

Preparation of 5-Alkoxy-6-alkoxymethyl-2-thiouracils.—The procedure was patterned after that used by Johnson and Caldwell[5] for the preparation of 5-ethoxy-6-ethoxymethyl-2-thiourea. To a solution of sodium methoxide (prepared from 100 ml. of methanol and 5.6 g. (0.244 g.-atom) of sodium) at room temperature was added 9.2 g. (0.122 mole) of thiourea followed by *sec*-butyl α,γ-dialkoxyacetoacetate (0.10 mole) dissolved in three volumes of methanol. The mixture was refluxed for 6 hr., then the alcohol was removed *in vacuo*, the viscous residue dissolved in the minimum amount of water and acidified (litmus) with glacial acetic acid; in each case a voluminous, buff-colored solid immediately separated. Next day, the thiouracil derivative was filtered off and washed with water before being recrystallized from dilute alcohol as white crystals (yields 62–85%). In general, three recrystallizations were necessary to obtain a sample sharp melting point and suitable for analysis. The thiouracils are listed in Table III.

(7) N. D. Cheronis and J. B. Entriken, "Semimicro Qualitative Organic Analysis," T. Y. Crowell Co., New York, N. Y., 1947, p. 469.

TABLE III

5-ALKOXY-6-ALKOXYMETHYL-2-THIOURACILS



R	M.p., °C.	Yield, %	Nitrogen, % Calcd.	Nitrogen, % Found
Methyl	187.0–188.5	68	13.85	13.79
Ethyl	174.2–176.0[a]	62	12.17	12.26
Propyl	136.5–137.0	82	10.85	11.00
Isopropyl	177.0–177.5	67	10.85	11.06
Butyl	110 –111	82	9.78	9.82
Isobutyl[b]	158.5–160.0	85	9.78	9.92
sec-Butyl[c]	142.0–143.5	79	9.78	9.88

[a] T. B. Johnson and W. T. Caldwell [THIS JOURNAL, 51, 876 (1929)] reported m.p. 178°. [b] Calcd. for $C_{13}H_{22}N_2SO_3$: mol. wt., 286.4. Found: mol. wt., 291. [c] Calcd. for $C_{13}H_{22}N_2SO_3$: S, 11.19. Found: S, 11.04.

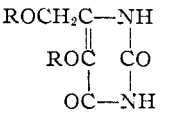
Preparation of 5-Alkoxy-6-alkoxymethyluracils.—Again, the procedure of Johnson and Caldwell[5] was utilized, in that a mixture of 100 ml. of water, 25.4 g. (0.27 mole) of chloroacetic acid and 0.04 mole of a thiouracil derivative was refluxed for 6 hr.; compounds of lower molecular weight dissolved completely during this treatment, but the higher members formed dark-brown insoluble layers or droplets. Chilling overnight produced crystals, which were recrystallized from aqueous methyl alcohol (yields 50–87%). The uracils are very stable to fusion and showed no change in melting point behavior after fusion and resolidification. Data for these compounds appear in Table IV.

Interaction of 5-Isobutoxy-6-isobutoxymethyl-2-thiouracil with Concentrated Hydriodic Acid. (A).—A mixture of 8.4 g. of this thiouracil and 65 g. of concentrated hydriodic acid (58%) was heated; during the first 15 min. of refluxing, the thiouracil either went into solution or melted, for no solid could be seen. After 40 min., a thick crust of solid appeared at the surface of the solution and iodine vapors were obvious. After being chilled overnight, the mixture was filtered and the solid product was recrystallized from dilute alcohol as colorless crystals, m.p. 261–262°, 3 g. (48% yield). From the mother liquor was recovered 2.5 g. of starting material. The product, **5-isobutoxy-6-methyl-2-thiouracil**, gave no color to a neutral, aqueous ferric chloride solution.

Anal. Calcd. for $C_9H_{14}N_2SO_2$: C, 50.42; H, 6.58; N, 13.08. Found: C, 50.34; H, 6.43; N, 12.88.

TABLE IV

5-ALKOXY-6-ALKOXYMETHYLURACILS



R	M.p., °C.	Yield, %	Nitrogen, % Calcd.	Found
Methyl	191.5–193.5	50	15.05	14.97
Ethyl	168.5–170.0[a]	76	13.08	13.06
Propyl	122.5–124.0	79	11.56	11.35
Isopropyl	205–206	82	11.56	11.49
Butyl	106.0–107.5	81	10.36	10.44
Isobutyl[b]	130–131	87	10.36	10.28
sec-Butyl	163.5–164.0	84	10.36	10.28

[a] T. B. Johnson and W. T. Caldwell [THIS JOURNAL, 51, 873 (1929)] reported m.p. 168°. [b] Calcd. for $C_{13}H_{22}N_2O_4$: mol. wt., 270.4. Found: mol. wt., 275.

(B).—A mixture of 5 g. of this thiouracil was refluxed for 4 hr. with 75 g. of concentrated hydriodic acid; the crust of brown solid, which appeared during the initial half-hour of refluxing, subsequently disappeared. From the chilled reaction mixture was obtained 1.8 g. (59% yield) of **5-hydroxy-6-methyl-2-thiouracil** as white needles melting above 310°. The product was added to a dilute, neutral, aqueous solution of ferric chloride and produced immediately an intense, blue coloration.

Anal. Calcd. for $C_5H_6N_2SO_2$: N, 17.71. Found: N, 17.64.

The same product (1.3 g., 55% yield) was obtained after 4 g. of 5-isopropoxy-6-isopropoxymethyl-2-thiouracil had been heated for 4 hr. with 65 g. of hydriodic acid.

Anal. Calcd. for $C_5H_6N_2SO_2$: C, 37.95; H, 3.80; N, 17.71. Found: C, 37.77; H, 3.86; N, 17.76.

Interaction of 5-Isobutoxy-6-isobutoxymethyluracil with Concentrated Hydriodic Acid. (A).—Three grams of this uracil derivative was heated for 2.5 hr. with 35 g. of concentrated hydriodic acid. After being chilled, the reaction mixture was filtered, and the brown, fluffy solid was recrystallized as white needles from diluted methyl alcohol; m.p. 238–240°, 1.2 g. (55% yield) of **5-isobutoxy-6-methyluracil**; the latter did not impart color to a ferric chloride solution.

Anal. Calcd. for $C_9H_{14}N_2O_3$: N, 14.14. Found: N, 14.04.

(B).—A mixture of 4.1 g. of the diisobutoxyuracil was refluxed for 4.5 hr. with 60 g. of concentrated hydriodic acid. Filtration of the chilled mixture left a dark-brown solid. The latter was first boiled with diluted alcohol, then was dissolved in concentrated, ammonium hydroxide solution; reprecipitation, by means of concentrated hydrochloric acid, gave 1.1 g. (51% yield) of white, granular crystals of **5-hydroxy-6-methyluracil**. The product,[8] when heated began to decompose at 220°, but did not melt below 310°. The material produced an intense blue coloration in a dilute, aqueous solution of ferric chloride.

Anal. Calcd. for $C_5H_6N_2O_3$: N, 19.71. Found: N, 19.53.

(8) This compound had previously been prepared, by oxidation of 6-methyluracil with potassium permanganate in dilute acetic acid solution, by R. Behrund and R. Grünewald (*Ann.*, **323**, 186 (1902)), who reported that it "decomposed from 220° on."

AUSTIN, TEXAS

[CONTRIBUTION FROM THE MARINE BIOLOGICAL LABORATORY]

The Mechanism of Disulfide Interchange in Acid Solution; Role of Sulfenium Ions

BY RUTH E. BENESCH AND REINHOLD BENESCH[1]

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Evidence is presented to show that disulfide interchange in strongly acid solution proceeds through sulfenium ions, which arise from the acid hydrolysis of the disulfide bond. A number of substances which can be regarded as precursors of sulfenium ions were found to catalyze this interchange.

Introduction

Disulfide interchange (RSSR + R'SSR' = 2 RSSR') recently has attracted the attention of a number of investigators, notably Huggins and his collaborators,[2] Calvin,[3] Kauzmann, *et al.*,[4] and Sanger.[5,6] From the results obtained by these workers it has become clear that disulfide interchange in neutral and alkaline solution probably occurs through an anionic attack of the mercaptide ion, RS^-, on a disulfide bond. Thus, addition of a thiol to a mixture of disulfides, at *p*H values which allow significant concentrations of RS^-, results in disulfide interchange. The unexpected finding of Ryle and Sanger[6] that the addition of thiols to disulfide mixtures in *acid solution* has exactly the opposite effect, *i.e.*, suppression of spontaneous interchange, suggested, of course, that in this medium the mechanism was quite different. It is the purpose of this paper to explain this difference and to propose a mechanism for disulfide interchange in strongly acid solution.

(1) Established Investigator of the American Heart Association.

(2) C. Huggins, D. F. Tapley and E. V. Jensen, *Nature*, **167**, 592 (1951).

(3) M. Calvin in "Glutathione," Academic Press, Inc., New York, N. Y., 1954, p. 9.

(4) W. Kauzmann and R. G. Douglas, Jr., *Arch. Biochem. and Biophys.*, **65**, 106 (1956).

(5) F. Sanger, *Nature*, **171**, 1025 (1953).

(6) A. P. Ryle and F. Sanger, *Biochem. J.*, **60**, 535 (1955).

Methods and Materials

The system used to study disulfide interchange was that employed by Ryle, *et al.*,[6] *i.e.*, the reaction between cystine and bis-(2,4-dinitrophenyl)-cystine (bis-DNPcystine) to form mono-DNPcystine. Since bis-DNPcystine is soluble in ether, but mono-DNPcystine is not, the extent of interchange can be followed very conveniently by measuring the absorption at 355 mμ in the aqueous phase after extraction with ether. The reaction mixtures were prepared from stock solutions of 2×10^{-4} *M* bis-DNPcystine in 12 *N* HCl and 4×10^{-4} *M* cystine in 9 *N* HCl in such a way that the bis-DNPcystine was mixed with the catalyst to be tested (in aqueous solution except where otherwise indicated) and the interchange then started by adding cystine. The final concentrations of reactants were: bis-DNPcystine, 10^{-4} *M*; cystine, 10^{-3} *M*; and HCl, in the majority of experiments, 9.5 *N*. All reactions were carried out at 35°. Under these conditions the spontaneous interchange between the two disulfides was sufficiently slow to permit the observation of catalytic effects.

L-Cystine was a commercial sample standardized by optical rotation. The sample of 2,6-dichlorophenolindophenol used (Harleco) had a molar extinction coefficient of $1.88 \times$