TABLE I

No.	*n*-Heptane, g.	D_2O, g.	Time, hr.	Catalyst	Temp., °C.	Phase[b]	K[a]
				Liquid phase experiments			
1	7.5	9.2	21	2 g. Raney nickel[i]	Reflux	L	0
2	10.5	16.4	24	Trace of sulfuric acid	Reflux	L	0
3	30.3	13.0	18	5 g. U. O. P. nickel[c] finely powdered	Reflux	L	0
4	30.0	12.0	24	2 g. platinized asbestos[c]	Reflux	L	0
5	24.0	9.0	24	0.8 g. platinum(IV)[c] oxide	25	L	0
6	30.0	9.0	55	3 g. platinum oxide[c]	80–100	L	0
7	55.0	12.5	36	1.5 g. platinum oxide[c]	150	L	0
				Vapor phase experiments			
			No. of passes,[d]				
8	39.6	14.3	6	Pelleted U. O. P. nickel[e]	165–180	V	1.1×10^{-1}
9	36.8	13.6	4	Pelleted U. O. P. nickel[e]	160–180	V	1.3×10^{-1}
10	24.0	16.2	5	Pelleted U. O. P. nickel[e]	120–135	V	8.0×10^{-3}
		H_2O, g.					d^{20}_4[f]
11	24.5	9.1	4	Pelleted U. O. P. nickel[e]	160–180	V	-8×10^{-4}
12	24.6[g]	10.1	4	Pelleted U. O. P. nickel[e]	170–180	V	-5×10^{-4}
13	100.5	36.5	72[h]	Pelleted U. O. P. nickel[e]	150–155	V[h]	1.4×10^{-3}[j]

[a] This constant is a measure of extent of reaction, see Discussion. [b] L, liquid; V, vapor. [c] Catalyst reduced in hydrogen atmosphere at room temperature before introduction of heavy water. [d] Number of times the entire charge of water and hydrocarbon passed through the catalyst bed. [e] Catalyst reduced by heating at 200–250° for eight hours in a stream of hydrogen. [f] Difference in density between the hydrocarbon product and the *n*-heptane. [g] *n*-Octane. [h] See experimental for description of apparatus used. [i] Viscosity increased $(7 \pm 1) \times 10^{-3}$ cs. [k] Pressure rose gradually to 1.5 atmospheres several times during the reaction period. The gas which was being produced was intermittently vented. [l] Raney nickel prepared by the procedure described in Adkins, "Reactions of Hydrogen," Wisconsin University Press, Madison, Wisc., 1937, p. 20. It was washed three times with acetone and twice with heptane before use.

ment 11 and the original *n*-heptane were identical. If the hydrocarbon were cracking to give principally lower molecular weight straight chain fragments, the density would be a much more sensitive criterion than the infrared spectra. Experiment 13, which was of much greater duration, produced not only very material changes in physical properties of the product hydrocarbon but also considerable quantities of gaseous material. It appears that in addition to the cracking some considerable carbon skeletal rearrangements may have taken place to produce a product with an increased viscosity and density.

Evidently the preparation of a completely deuterated straight-chain hydrocarbon by successive equilibrations of hydrocarbon and heavy water is a poor procedure if a product of unchanged carbon skeleton is desired.

Experimental

Materials.—*n*-Heptane was obtained from the Westvaco Chlorine Products Co., d^{20}_4 0.68397, $n^{20}D$ 1.3870, f.p. −90.66°; NBS values[10] 0.68368, 1.38764, −90.595°, respectively. The hydrocarbon was passed through a short column of silica gel before use.

n-Octane was prepared by the modified[11] Wolff–Kishner reduction of 2-octanone, d^{20}_4 0.7026; NBS[10] 0.7026. The octane was passed through silica gel before use.

Deuterium oxide was supplied by the Stuart Oxygen Co. and had a minimum deuterium content of 99.5%.

Nickel on kieselguhr was purchased from the Universal Oil Products Co. No exchange was observed over this catalyst unless it was first reduced in a stream of hydrogen at elevated temperatures. In the present work, the reduction was performed at 200 to 250° for eight hours.

Platinized asbestos obtained from Fisher Scientific Co. had 10% platinum by weight.

Liquid Phase Experiments.—Experiments 1 to 5, Table I were performed in a 100-ml. three-neck flask equipped with a mercury-seal stirrer, gas inlet tube and condenser. The hydrocarbon and catalyst were charged to the flask and the catalyst reduced in a hydrogen atmosphere. The hydrogen was replaced by nitrogen and the deuterium oxide charged to the flask. At the completion of the run the water was frozen, the hydrocarbon layer separated and distilled from powdered calcium hydride in an all-glass distilling system. No hydrogen exchange takes place between the hydride and hydrocarbon under these conditions. Densities were determined with an accuracy of ±0.0001.

Experiment 6 was accomplished in a Burgess–Parr low pressure hydrogenation apparatus. The catalyst was reduced *in situ* before the introduction of the heavy water. At the end of the reaction period the products were isolated as above.

Experiment 7 was performed in an Aminco high pressure hydrogenation apparatus.

Vapor Phase Experiments.—Runs 8 to 12 Table I were made by simply distilling the water and hydrocarbon together through a horizontal catalyst chamber, which was 22-mm. o.d. Pyrex, packed with catalyst pellets for a length of six inches and surrounded by a 750-watt heater. The temperatures recorded in the table are those of the outside wall.

Experiment 13 was accomplished in an apparatus which provided continuous and repeated passage of the hydrocarbon and water vapors through a heated catalyst chamber.

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(10) Selected Values of Properties of Hydrocarbons, Circular of the National Bureau of Standards, C 461, 1947.

(11) C. H. Herr, F. C. Whitmore and R. W. Schiessler, THIS JOURNAL, **67**, 2061 (1945).

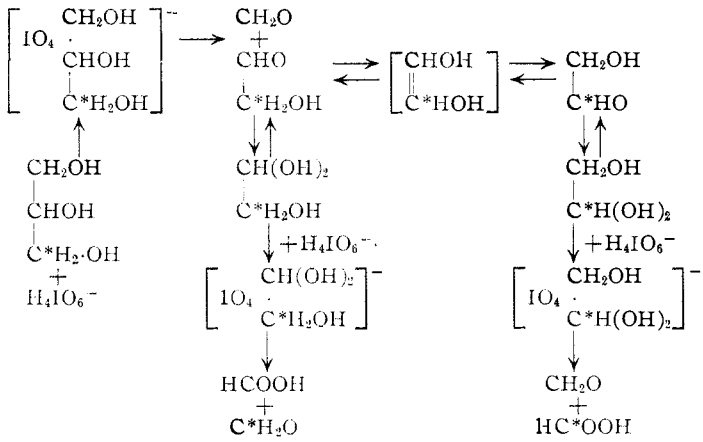
On the Oxidation of Glycerol-1-C^{14} by 1,2-Glycol-cleaving Reagents[1]

BY ALBERT P. DOERSCHUK

The reaction that occurs between periodic acid in buffered aqueous solution and glycerol to yield quantitatively formaldehyde and formic acid is well known. A comparison of the glycerol reaction with reactions of other 1,2-glycols and peri-

(1) This work was supported in part by a grant from the Nutrition Foundation, Inc.

odic acid suggests that the formaldehyde has as its exclusive origin the glycerol primary alcohol carbons, with the formic acid arising exclusively from the glycerol secondary alcohol carbon.[2] Further oxidation beyond the glycol-cleaving stage could convert some primary alcohol carbons into formic acid, although the quantitative nature of the reaction predicts that this type of oxidation would be slight. A reaction path can, however, be postulated, employing a coördination complex disproportionation mechanism analogous to that written for the periodate oxidation of ethylene glycol,[3] that could transform some of the glycerol primary alcohol carbons to formic acid without altering the quantitative relationship between reactants and products.



We have observed that hydroxyacetaldehyde, postulated as an intermediate, yields quantitatively one mole of formaldehyde under the conditions of the glycerol oxidation. Enediol configurations have been suggested before as intermediates in reactions of 2-hydroxyketo functions. Several enediol structures adjacent to electron-attracting groups are stable (reductones).[4] The existence of two hydrogens on the alcohol carbon of hydroxyacetaldehyde should increase the statistical probability of enolization and transient enediol formation. If the enediol-mediated equilibrium were completely attained, one-fourth of the primary alcohol carbons of glycerol would appear finally as formic acid without any alteration of the 1:2:1 molar ratio of glycerol, formaldehyde and formic acid. An oxidation-reduction exchange between the formaldehyde and the formic acid produced by the reaction is another process the operation of which results in glycerol primary alcohol carbons appearing as formic acid with no alteration in the observed 1:2:1 molar ratio between glycerol, formaldeleyde, and formic acid.

The extent to which these mechanisms are operating is also of biochemical interest from the standpoint of using periodate degradation in carbon tracer studies of the origin of the glycerol residue in physiologically important materials structurally related to glycerol. Oxidation of glycerol-1-C^{14} provides a sensitive method for determining the degree to which 1,2-glycol cleavage with periodate in aqueous buffer converts glycerol primary alcohol carbons to formic acid.

The experiment was repeated using lead tetraacetate in aqueous acetic acid, a 1,2-glycol-cleaving reagent that quantitatively oxidizes formic acid to carbon dioxide.[5]

Experimental

Eighteen milligrams of glycerol-1-C^{14}, possessing a specific activity of 3.12×10^5 counts/minute/millimole[6] and dissolved in 2 ml. of aqueous solution, was oxidized with periodic acid buffered with sodium bicarbonate according to the procedure of Reeves.[7] The formaldehyde was steam distilled out of the reaction mixture, previously made alkaline to phenol red, and oxidized to carbon dioxide with potassium permanganate. The distillation residue was evaporated to a solid *in vacuo* under nitrogen at 50° and dissolved in 10 ml. of water; the process was repeated three times and the final solution, after being made acid to congo red, was oxidized with mercuric oxide. Formaldehyde is not oxidized under these conditions, while formic acid is converted to carbon dioxide.[8]

Twenty-nine and six-tenths mg. of hydroxyacetaldehyde, prepared from tartaric acid[9] by way of dihydroxymaleic acid,[10] was oxidized using the quantities described by Reeves[7] for eighteen milligrams of glucose; the formaldehyde-dimedon derivative was precipitated using 10 ml. of molar sodium acetate solution, 5 ml. of 5% dimedon in 95% ethanol and 20 ml. of water. Formaldehyde: found, 14.6 mg.; calcd., 14.8 mg.

Eighteen milligrams of glycerol-1-C^{14} in 2 ml. of water and 5 ml. of glacial acetic acid was treated with 290 mg. of lead tetraacetate for thirty minutes, during which time the temperature was raised from 35 to 45°.[5] The carbon dioxide was collected quantitatively in saturated barium hydroxide solution using a stream of carbon dioxide-free nitrogen. Hydroxyacetaldehyde and ethylene glycol were oxidized in similar fashion.

Repeating the experiments with glycerol-1-C^{14} that had been benzoylated, purified by recrystallization as the tribenzoate from ligroin and hydrolyzed back to glycerol, gave the same results. All radioactivities were determined as barium carbonate mounted on filter paper.

RADIOACTIVITIES OF REACTANTS AND PRODUCTS

Glycerol	312,000 c./min./mmole
Formaldehyde from periodate oxidation	312,000 c./min./2 mmoles
Formic acid from periodate oxidation	789 c./min./mmole
Formic acid from lead tetraacetate oxidation	13,000 c./min./mmole

The values represent an incorporation of radioactivity in the formic acid of 0.25% for periodate oxidation and 4.2% for lead tetraacetate oxidation. In all cases of lead tetraacetate oxidation, carbon dioxide yields were obtained enough in excess of those predicted on the basis of one mole from glycerol, one mole from hydroxyacetaldehyde and none from ethylene glycol to explain the 4.2% radioactivity incorporation by further oxidation of the formaldehyde resulting from the 1,2-glycol-cleaving reaction.

Both 1,2-glycol-cleaving reagents tested with glycerol-1-C^{14} yielded small quantities of radioactivity in the formic acid produced; the magnitudes of the radioactivity incorporations

(2) E. L. Jackson, "Periodic Acid Oxidation" in "Organic Reactions," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1944, p. 341.

(3) F. R. Duke, THIS JOURNAL, **69**, 3054 (1947).

(4) W. W. Pigman, "Chemistry of the Carbohydrates," Academic Press, New York, N. Y., 1948.

(5) J. M. Grosheintz, THIS JOURNAL, **61**, 3379 (1939).

(6) A. P. Doerschuk, *ibid.*, **73**, 821 (1951).

(7) R. E. Reeves, *ibid.*, **63**, 1476 (1941).

(8) Y. J. Topper and A. B. Hastings, *J. Biol. Chem.*, **179**, 1255 (1949).

(9) H. O. L. Fischer and L. Feldman, *Ber.*, **62B**, 854 (1929).

(10) H. O. L. Fischer and C. Taube, *ibid.*, **60B**, 1704 (1927).

are such that both can be explained by further oxidations of the formaldehyde resulting from the 1,2-glycol-cleaving reaction and no rearrangement of an intermediate or oxidation–reduction exchange between reaction products need be postulated. The periodate system is the more suitable of the two reagents studied for carrying out degradations in carbon tracer studies of the origin of the glycerol residue in physiologically important materials structurally related to glycerol.

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RECEIVED MAY 14, 1951

β-(2-Thienyl)-serine

BY MATTHEW E. DULLAGHAN AND F. F. NORD

The preparation of β-phenylserine by the condensation of benzaldehyde and glycine has been reported by Erlenmeyer.[1,2] In the course of studies on the chemistry of heterocyclics,[3] initiated in this Laboratory,[4] it became necessary to have available α-amino-β-hydroxy-β-(2-thienyl)-propionic acid, *i.e.*, β-(2-thienyl)-serine.

Experimental

A mixture of 2-thenaldehyde (0.5 mole),[3] glycine (0.25 mole) and 100 ml. of absolute ethanol was, therefore cooled to 3° in an ice-bath. A cold solution of potassium hydroxide (0.5 mole) in 150 ml. of absolute ethanol was added with stirring at such a rate that the temperature of the mixture remained below 10°. After all the alkali had been added, and a white precipitate started to form, the mixture was allowed to remain below 10° overnight to enable maximum precipitation. Upon filtration of precipitate, it was washed with absolute ethanol, then dissolved in water (75 ml.) and the solution acidified with 15 ml. of glacial acetic acid. Ethanol (75 ml.) was added and the mixture again allowed to stand in an ice-bath at 5° for two hours. The resulting solid upon filtration was recrystallized from 50% water–ethanol. The yield of white needles amounted to 19 g. (41%). The substance started to soften and turned brown at 185–186°, melting at 194–195° (uncor.) under decomposition.

Anal. Calcd. for $C_7H_9NO_3S \cdot H_2O$: C, 40.97; H, 5.36; N, 6.87. Found: C, 41.13; H, 5.29; N, 6.80.

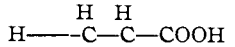
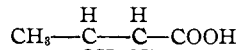
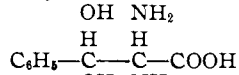
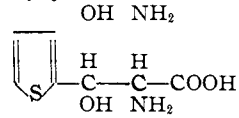
It is presumed that the β-(2-thienyl)-serine so obtained belongs to the DL-threose series. This belief is based on the fact that this was shown to be the case with phenylserine.

Discussion

While the amino acid is named as an analog of serine, it could also be considered as one of threonine. Since β-(2-thienyl)-alanine is a known antagonist for β-phenylalanine, the series of analogous amino acids listed in Chart I indicate interesting possibilities in further studies of this type.

Even though serine is not an essential amino acid in that it can be synthesized by the animal from glycine, it is possible that the now available β-(2-thienyl)-serine could act as a competitor for serine in protein synthesis, leading to a serine deficiency. Too, penicillamine is thought to act as a competitor for the decarboxylase converting serine to amino ethanol causing a choline deficiency.[5] β-(2-Thienyl)-serine because of its similarity to both penicillamine and serine could act in a similar manner.

CHART I

Serine	
Threonine	
Phenylserine	
β-(2-Thienyl)-serine	

(1) Erlenmeyer, *Ber.*, **25**, 3445 (1892).

(2) Erlenmeyer and Frűstück, *Ann.*, **284**, 36 (1895).

(3) King and Nord, *J. Org. Chem.*, **13**, 635 (1948); Dullaghan and Nord, Abstracts of the 119th Meeting of the Am. Chem. Soc., 34M (1951).

(4) This work was carried out under the aegis of the Office of Naval Research.

(5) Wilson and du Vigneaud, *J. Biol. Chem.*, **184**, 63 (1950).

COMMUNICATION NO. 233 FROM THE DEPARTMENT OF ORGANIC CHEMISTRY AND ENZYMOLOGY
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RECEIVED JUNE 13, 1951

The Synthesis of Compounds for the Chemotherapy of Tuberculosis. II. Hydroxamic Acid Derivatives

BY THOMAS S. GARDNER, E. WENIS AND F. A. SMITH

In the course of the preparation of pyridine derivatives[1] for testing as anti-tubercular agents, nicotinohydroxamic acid was prepared. This was found to be inactive. Shortly afterwards, Urbánski[2] reported tuberculostatic activity of salicylhydroxamic acid in mice and, on these grounds, we prepared further members of the pyridine hydroxamic acid series, namely, picolinohydroxamic acid, isonicotinohydroxamic acid, 3-pyridineacetohydroxamic acid and 5-cyano-6-hydroxy-2-methylisonicotinohydroxamic acid. As a representative of another heterocyclic system, 5-methyl-3-isoxazolecarbohydroxamic acid was made. For control purposes salicylhydroxamic acid was prepared according to Jeanrenaud,[3] and on the ground of the tuberculostatic activity of *p*-aminosalicylic acid, *p*-aminosalicylhydroxamic acid was also made. None of these compounds, including the salicylhydroxamic acid, displayed any anti-tubercular activity in a mouse prophylactic test in which nicotinamide, *p*-aminosalicylic acid, thiosemicarbazones[1] and streptomycin showed activity.

Acknowledgment.—We are grateful to Drs. R. J. Schnitzer and E. Grunberg of our Chemotherapy Laboratories for testing the compounds and to Dr. A. Steyermark and his associates for the microchemical analyses.

Experimental

The method described in the first preparation was employed in all cases (Table I) except that the hydrochloride was not prepared when the hydroxamic acid crystallized.

4-Amino-2-hydroxybenzohydroxamic Acid Hydrochloride.—A solution of sodium methylate was prepared by treating 12 g. of sodium with 300 ml. of anhydrous methanol. To this was added 35 g. of hydroxylamine hydrochloride, and after 30 minutes of stirring, 36 g. (0.215 mole) of methyl *p*-aminosalicylate was added. The reaction mixture was stirred at 25° for 16 hours and filtered. The filtrate was evaporated to dryness and the residue extracted with boil-

(1) T. S. Gardner, F. A. Smith, E. Wenis and J. Lee, *J. Org. Chem.*, **6**, 1121 (1951).

(2) T. Urbánski, *Nature*, **166**, 267 (1950).

(3) A. Jeanrenaud, *Ber.*, **22**, 1270 (1889).