being reached with one of the isomeric racemic forms of 2,4-di-(p-hydroxyphenyl)-3-ethylhexane, which is known as benzestrol.

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[CONTRIBUTION FROM THE EVANS MEMORIAL, MASSACHUSETTS MEMORIAL HOSPITALS, AND THE DEPARTMENT OF MEDICINE, BOSTON UNIVERSITY SCHOOL OF MEDICINE]

Antibacterial Action of an Oxidation Product of Sulfanilamide[1a,b]

BY GEORG BARKAN AND LEONTINE GOLDSMITH

An early theory, now largely abandoned, explained the antibacterial action of the sulfonamide drugs on the basis of the formation *in vivo* of an oxidation product, with enhanced bacteriostatic effect. The history of this theory, and the evidence leading to its present disrepute, may be found in full detail in the monograph by Henry.[2] Barkan[3] observed in 1939 that when molecular oxygen was bubbled through concentrated solutions (0.015 M) of sulfanilamide in the presence of traces of copper sulfate, hydrogen peroxide appeared, demonstrable by the phenolphthalein test of Schales,[4] and a blue color developed, which disappeared with the addition of certain reducing agents (sodium hydrosulfite or ascorbic acid) and which returned on reoxidation by shaking with air.

Ottenberg and Fox[5] had previously obtained a similar colored oxidation product by ultraviolet irradiation of sulfanilamide in the presence of oxygen. In 1940 Barkan[6] reported the formation of an apparently identical blue oxidation product by the similar treatment of arsanilic acid. The identity of the products led to the conclusion that the side chains were removed during the oxidation process. Rosenthal and Bauer[7] had previously postulated the loss of the sulfonamide group from sulfanilamide as a result of similar oxidative changes.

In a short note in 1944, Barkan[8] announced the isolation, purification and analysis of the blue oxidation product, stating that the substance possesses high antibacterial activity. Since Dr. Barkan's death (March 7, 1945), this paper has been prepared to present the data upon which these statements were based.

Experimental

Preparation of the Oxidation Product.—To 200 ml. of 0.03 M (0.5%) aqueous solution of sulfanilamide is added an equal volume of 2% sodium carbonate, then approximately 100 mg. of solid hydrazine sulfate. After this is dissolved, 4 ml. of freshly prepared 0.01 M cupric sulfate will develop a faint blue color. One hundred ml. of benzene (thiophene-free) is added at this stage and oxygen passed through the mixture for ten minutes.

The product, which is blue in aqueous solutions, enters the benzene layer, which is separated, washed with water, and dried over anhydrous sodium sulfate. It is then separated by chromatographic adsorption on aluminum oxide (Alorco activated, chromatographic, mesh minus 80, partially deactivated by exposure to cool, moist air).[9] The adsorbed material is dark blue in color; no color is left in the benzene. After washing away the benzene with petroleum ether, the substance is eluted with ether (peroxide-free), yielding a red solution. The solvent is removed by distillation on a water-bath in an atmosphere of nitrogen. About 4 mg. of blue-black amorphous product is obtained from the above procedure.

Properties of the Oxidation Product.—The product is a blue-black amorphous solid; m. p. about 118° with decomposition; soluble and stable in pure organic solvents; soluble in water up to about 40 mg. per 100 ml., and unstable in that it cannot be recovered completely by extraction; reducible by ascorbic acid or sodium hydrosulfite to a colorless compound, which can be reoxidized by air; in butanol solution, maximal light absorption at 590 millimicrons; $E^{1\%}_{1\,cm.}$ ranges from 820 to 1028, mean

TABLE I

SUMMARY OF ANTIBACTERIAL ACTIONS OF THE OXIDATION PRODUCT

Original inoculum	Minimal bacteriostatic concn. (oxidized form), micrograms/ml.	Minimal bactericidal concn. (oxidized form), micrograms/ml.	Minimal bacteriostatic concn. (reduced form), micrograms/ml.
Strep. hemolyticus			
17 organisms per ml.	0.5	1.8	0.5
65		1.8	
170	1		0.5
8 (serum 1:1)	4	8	
25 (serum 1:1)	4		
35 (serum 1:1)	1.5		1.5
143 (serum 1:1)	3		
Staph. aureus			
275 organisms per ml.	2		
30 (serum 1:1)	8		
150 (serum 1:1)	8		
750 (serum 1:1)	6		
Pneumococcus Type I			
2250 organisms per ml.		1.5	
2250 (serum 1:1)	3		
7500 (serum 1:1)	3		

(1) (a) This paper was compiled from Dr. Barkan's data by Burnham S. Walker, with the assistance of Dr. Goldsmith. (b) This work was aided by a grant from the Johnson & Johnson Research Foundation.

(2) R. J. Henry, "The Mode of Action of Sulfonamides," Josiah Macy, Jr., Foundation Review Series, **2**, No. 1 (1944).

(3) G. Barkan, *Proc. Soc. Exp. Biol. Med.*, **41**, 535 (1939).

(4) O. Schales, *Ber.*, **71**, 447 (1938).

(5) R. Ottenberg and C. L. Fox, Jr., *Proc. Soc. Exp. Biol. and Med.*, **38**, 479 (1938).

(6) G. Barkan, *Science*, **92**, 107 (1940).

(7) S. M. Rosenthal and H. Bauer, *ibid.*, **91**, 509 (1940).

(8) G. Barkan, *Federation Proc.*, **3**, 65 (1944).

(9) H. Brockman and H. Schadder, *Ber.*, **74**, 74 (1941).

931; molecular weight (by melting point method) ranges from 473 to 526, mean 493; analysis (mean of three): C, 73.5%; H, 5.2%; N, 13.5%; S absent; proposed empirical formula $C_{30}H_{25}N_5O_2$ (mol. wt. 487.54).

Antibacterial Action (These studies were carried out by Miss Alice McDonald and Miss Marjorie Jewell in the laboratories of the Evans Memorial).—Bactericidal or bacteriostatic effects were determined by adding the compound (dissolved in physiological salt solution) to cultures of several organisms in veal infusion broth, with or without horse serum. Plate counts were made at intervals of eight, twenty-four and forty-eight hours.

The results are shown in tabular form. The effect is characterized as bactericidal if no organisms survive after eight hours, as bacteriostatic if no significant increase in the number of organisms occurs for forty-eight hours. Control tubes were carried through all experiments.

Discussion

The results of the antibacterial tests show no consistent relationship between bacteriostatic concentration and the size of the inoculum. Two conclusions are apparent: (1) The reduced form has approximately equal activity with the oxidized form, and (2) the activity is diminished in the presence of serum.

Attention should be called to the remarkable antibacterial potency of the compound. Comparable results with the sulfonamides would require concentrations higher by a factor of about 25 (against streptococci in the presence of serum). The action of gramicidin is more comparable; gramicidin is bactericidal against pneumococci in concentrations of 1 microgram per ml., and against streptococci in concentrations of 5 micrograms per ml., under approximately similar conditions.[10]

It should also be emphasized that Dr. Barkan left no significant data in regard to the toxicity of the product. A few preliminary tests with mice indicated that it is not prohibitively toxic.

There are no data in regard to antibacterial action *in vivo*.

Summary

A water-soluble oxidation product of sulfanilamide is blue in its oxidized form and colorless when reduced. The oxidation and reduction are reversible. In both forms, the compound is bacteriostatic against streptococci, staphylococci, and pneumococci.

(10) D. H. Heilman and W. E. Herrell, *Proc. Staff Meetings Mayo Clinic*, **17**, 321 (1942).

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[CONTRIBUTION FROM RESEARCH AND DEVELOPMENT DEPARTMENT, COMMERCIAL SOLVENTS CORPORATION]

Reaction of Some Cyclic Acetals with Acid Anhydrides[1]

BY MURRAY SENKUS

Haskins, Hann and Hudson[2] observed that when an acetylating mixture (II) composed of 35 volumes of acetic anhydride, 15 volumes of acetic acid and 1 volume of sulfuric acid is allowed to react at 20–25° with some cyclic benzaldehyde acetals (I) of polyhydric alcohols, the benzylidene radical is eliminated from the substituted 2-phenyl-1,3-dioxanes (I) and substituted diacetates (III) are formed

>C<(C—O)(C—O)>CH—C_6H_5 + Acetylating Mixture ⟶
I II

>C<(C—O—$COCH_3$)(C—O—$COCH_3$)
III

Ness, Hann and Hudson[3] extended this reaction to some cyclic formals prepared from polyhydric alcohols and found that the methylene radical is not eliminated from each of the substituted cyclic formals (IV) during the acetolysis but leads to the formation of an acetoxymethoxy group

>C<(C—O)(C—O)>CH_2 + II ⟶
IV

>C<(C—O—$COCH_3$)(C—O—CH_2—O—$COCH_3$)
V

They were also able to show that in each of the products from the cyclic formals which contain a primary carbon atom, the acetoxy group is attached to this atom and the acetoxymethoxy group is attached to the secondary carbon atom.

We now wish to report the acetolysis of some cyclic acetals derived from dihydric alcohols. Each of the formals that was investigated gave as a main product a compound similar in structure to the compounds prepared by Hudson, *et al.* 1,3-Dioxolane and 1,3-dioxane gave small amounts of other products whose structures are discussed herein. The direct conversion of 4,5-dimethyl-1,3-dioxolane to formaldehyde and 2,3-butanediol diacetate was also studied with some success and is reported.

The cyclic acetals other than formals failed to react with 1% sulfuric acid acetic anhydride at 100° in two hours. Reactions did take place on

(1) Prepared for the 1945 Meeting-in-Print of the Division of Organic Chemistry, A. C. S.

(2) Haskins, Hann and Hudson, THIS JOURNAL, **64**, 134 (1942).

(3) Ness, Hann and Hudson, *ibid.*, **65**, 2215 (1943).