by this relationship. Extensive binding of nicotinic acid to the thiolated gelatin film occurred in this study. When binding simultaneously occurs with diffusion, Eq. 1 may still be approximately valid as long as the binding occurs rapidly in relation to experimental times as in the case of nicotinic acid. With the resultant loss of diffusing solute, $C_a + C_b < C_0$, and the y intercept of the diffusion plot is negative. (See Fig. 5.) The diffusion constant for methscopolamine bromide was greater than would be predicted from its molecular weight.

REFERENCES

(1) Fraenkel-Conrat, H., and Mecham, D. K., *J. Biol. Chem.*, **177**, 477(1949).
(2) Danielsson, C. E., *Svensk Kem. Tidskr.*, **60**, 142(1948).
(3) Pouradier, J., *et al.*, *Bull. Soc. Chim.*, **19**, 928(1952).
(4) Benesch, R., and Benesch, R. E., *Proc. Natl. Acad. Sci. U.S.*, **44**, 848(1958).
(5) Gregor, H. P., and Kantner, E., *J. Phys. Chem.*, **61**, 1169(1957).
(6) Wiegand, R. G., *Anal. Chem.*, **31**, 1745(1959).
(7) Jost, W., "Diffusion in Solids, Liquids, Gases," 3rd ed., Academic Press Inc., New York, N. Y., 1960, p. 44.
(8) Thovert, J., *Compt. Rend. Acad. Sci.*, **135**, 579(1902).
(9) Ballard, B. E., and Nelson, E., *J. Pharmacol. Exptl. Therap.*, **135**, 120(1962).

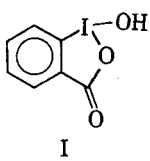
Chemistry and Biochemistry of Polyvalent Iodine Compounds III. Acute Toxicity of 1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole

By WALTER WOLF, ALLEN WEINER, and ROBERT WEISBERG

The acute toxicity of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole was re-evaluated. An LD_{50} of 175 mg./Kg. was determined for mice upon intraperitoneal injection of the title compound in a buffered suspension at pH 7.48. The symptoms observed upon injection of this compound in toxic doses were incoordination, lethargy, and respiratory depression. Upon autopsy, no observable lesions could be noted.

CHINARD REPORTED (1) in 1942 that intraperitoneal injections of *o*-iodosobenzoic acid to mice resulted in symptoms of shock with death within 15 minutes. His form of administration was to dissolve the *o*-iodosobenzoic acid in an equivalent amount of potassium hydroxide in a total volume of 1.5 ml. On examination of the dead animals, he found approximately 5 ml. of a gelatinous clear exudate with scattered petechial hemorrhages on the mesenteries and intestines. A dose of 15 mg. administered subcutaneously failed to produce an acute toxic effect. He observed a gelatinous exudate, but it appeared to be reabsorbed within a short period with no observed after effects.

Recent work (2, 3) has substantiated the suggestion that *o*-iodosobenzoic acid is actually a heterocyclic compound of structure I, 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole, whose pKa at 25° is 7.35. A check of the pH of the solutions, prepared as indicated by Chinard, gave values of pH 11 and above, thus allowing for the possibility that the type of toxic effects observed by this author were due to the high alkalinity of the injected solutions rather than to their content in polyvalent iodine derivatives.



I

In view of the continued interest in compounds of polyvalent iodine, it was felt desirable to redetermine the true toxicity of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole.

EXPERIMENTAL

Products.—1,3 - Dihydro - 1 - hydroxy - 3 - oxo-1,2-benziodoxole was prepared by the method of Meyer and Askenasy (4), as modified by Wolf and Hsu (2, 5). The product used had an iodometric purity of at least 95%. All other products used were of commercial origin and purified when necessary.

Preparation of Suspension.—Compound I is dried well and ground in a mortar to an extremely fine powder. This finely divided powder is triturated with a few milliliters of a Sorensen phosphate buffer, pH 7.48, $^1/_{15}$ *M*, containing 0.3% methylcellulose. Once a homogenous mass is achieved, taking care that no clumps are left, the mixture is brought up to the final volume desired with the same 0.3% methylcellulose-buffer solution. A drop of *n*-octyl alcohol should be added at this moment to prevent foaming and clumping upon shaking. Several glass beads may be added to aid agitating in the suspension. Suspensions prepared in this manner were still perfectly homogeneous after shaking, 3 months after originally prepared, and the starting material had suffered no chemical changes.[1]

Received August 26, 1964, from the School of Pharmacy, University of Southern California, Los Angeles.
Accepted for publication October 6, 1964.
Presented to the Scientific Section, A.PH. A., New York City meeting, August 1964.
This investigation was supported in part by an Interdepartmental Cancer Research Committee grant from the American Cancer Society, a grant-in-aid from the Sigma Xi-RESA Societies, and grant GM-10721 from the Institute of General Medical Sciences, U. S. Public Health Service, Bethesda, Md.
The authors acknowledge discussions with Professors J. Bester, D. Berman, and O. Miller. The technical assistance of Miss S. Benson and Mr. L. Gonick is gratefully acknowledged.

[1] The authors have observed, however, that nonmethylated cellulose (paper) readily reduces the benziodoxole ring, even in the dry state. (Wolf, W., and Liberman, P., unpublished results.)

TABLE I.—SOLUBILITY OF 1,3-DIHYDRO-1-HYDROXY-3-OXO-1,2-BENZIODOXOLE IN WATER AT DIFFERENT pH VALUES

pH	9.2	7.6	7.3	7.0	6.0
Solubility of compd. I					
mg./ml.	14.2	1.34	0.84	0.53	0.46
mole/L. × 10^3	53.8	5.07	3.17	2.01	1.75

TABLE II.—ACUTE TOXIC EFFECTS OF 1,3-DIHYDRO-1-HYDROXY-3-OXO-1,2-BENZIODOXOLE AS A FUNCTION OF DOSE ADMINISTERED INTRAPERITONEALLY TO 25 ± 5 Gm. SWISS WHITE MICE

Group	Dose Range, mg./Kg.	Animals, No.	% Mortality
A	57–100	10	10
B	101–120	7	14
C	121–140	13	30
D	141–160	18	16
E	161–180	11	64
F	181–200	8	87
G	201–250	11	100
H	251–300	12	100
I	301–400	15	100
J	400–700	12	100

Animal Experiments.—The mice used were white Swiss mice of 25 ± 5 Gm. weight. No selection based on sex was made. Controls of the same batch, using only 1 ml. of the 0.3% methylcellulose-buffer solution were used for each run, with no observable effects on the animals. No control animals died during the period of this work. The injections were performed intraperitoneally, using a 0.5-in. 24-gauge needle. All volumes injected were 1 ml. or less.

Solubility Determination.—Phosphate buffers were saturated with compound I at 25°, and the pH of the final solution was measured. The concentration of compound I was determined spectrophotometrically at 288 mμ. (ϵ = 2044, pH 11.0.)

RESULTS AND DISCUSSION

The solubility of compound I in aqueous media at pH values of a physiological interest is given in Table I.

The concentrations achieved while maintaining the pH of the solution at a value appropriate for intraperitoneal injection were thus too low to allow a study of the acute toxic effects. To achieve the high concentrations desired for this study (in the 1 to 0.1 mole/L. range), several suitable dosage forms were prepared and assayed.

Several concentrations of methylcellulose, with or without glycols, (propylene, polyethylene), were tried but resulted in low stability of the suspension. Clogging occurred frequently, and the pH of the suspension was 4.0 when no buffer was added. Other agents tested for a suitable suspension without success were polysorbate-80,[2] sorbitan monooleate,[3] glycerin, and combinations thereof. A suitable suspension was obtained finally by using a 0.3% solution of methylcellulose in phosphate buffer, 1/15 *M*, pH 7.48. The good pulverization and subsequent wetting of the powder are essential; otherwise, clumping will occur readily. Suspensions prepared by this method have retained their stability for months, and chromatographic analysis of the product failed to detect any reduction products of compound I.

The toxicological assay results are summarized in Table II. The LD_{50} was determined graphically by the method of Trevan (6), giving a value of 175 mg./Kg. Subsequent work in this laboratory on related biological aspects of polyvalent iodine biochemistry repeatedly confirmed the above figure.

The main symptom observed in animals receiving an intraperitoneal injection of compound I (but not in those receiving only the suspension media) was a loss of coordination, especially in the hind limbs, which produced an erratic jumping movement. The animals gradually became very unresponsive and moved around the cage very slowly until they became practically motionless. They did not respond, even to external stimuli. The respiratory rate appears to be decreased. No impairment of motility of the front legs was noted, other than general loss of reflexes. Upon dissection of the dead animals, no observable lesions were noted, nor was there evidence of a white exudate, as described by Chinard.

The LD_{50} for compound I determined in the present work agrees well with that for iodosobenzene (7) (150 to 200 mg./Kg. in mice) and is 4.6 times higher than that of *o*-iodobenzoic acid (8), its main metabolite (9).

The present result suggests a reappraisal of the interest of polyvalent iodine compounds in biological systems. Thus, *o*-iodosobenzoic acid (I) had been used as a specific sulfhydryl reagent (10), a potent bactericidal (1, 11), and a "radiomimetic" (12, 13). The toxicity values obtained allow for a possible utilization of compound I or related compounds in one of the above areas.

REFERENCES

(1) Chinard, F. P., *Proc. Soc. Exptl. Biol. Med.*, **51**, 317 (1942).
(2) Wolf, W., and Hsu, L. L. J., Abstracts of papers presented to the American Chemical Society, Denver, Colo., meeting, 1964, 54C.
(3) Shefter, E. and Wolf, W., *Nature*, **203**, 512(1964).
(4) Meyer, V., and Askenasy, P., *Ber.*, **26**, 1357(1893).
(5) Hsu, L. L. J., M.S. thesis, University of Southern California, Los Angeles, 1954.
(6) Trevan, J. W., *Proc. Roy. Soc. London, Ser. B* **101**, 483(1927).
(7) Luzzatto, R., and Satta, G., *Chem. Z.*, **I**, 753(1910).
(8) Knoefel, P. K., Huang, K. C., and King, N. B., *J. Pharmacol. Exptl. Therap.*, **117**, 307(1956).
(9) Wolf, W., and Olson, R., unpublished data.
(10) Hillerman, L., Chinard, F. P., and Dietz, V. R., *J. Biol., Chem.*, **147**, 443(1943).
(11) Akiya, S., *Japan J. Exptl. Med.*, **26**, 91(1956).
(12) Hicks, S. P., *J. Cellular Compt. Physiol., Suppl. 1*, **43**, 151(1954).
(13) Hagström, B. E., *Biol. Bull.*, **124**, 55(1963).

[2] Marketed as Tween 80 by the Atlas Powder Co., Wilmington, Del.

[3] Marketed as Span 80 by the Atlas Powder Co., Wilmington, Del.