demonstrated that only phenobarbital was absorbed through the gut, it was also shown that the rate of absorption was independent of the degree of dissociation of the complex in the mucosal fluid. It has been proposed [after Levy and Matsuzawa (4)] that the intestinal membrane has a dissociating effect on the complex, allowing the phenobarbital to be absorbed, but preventing absorption of PEG.

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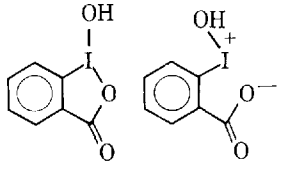
Chemistry and Biochemistry of Polyvalent Iodine Compounds V

Ionization of Heterocyclic Polyvalent Iodine Compounds

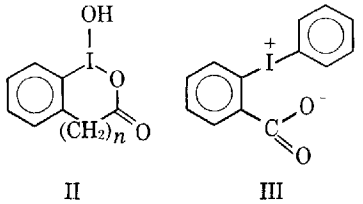
By WALTER WOLF, JAMES C. J. CHEN, and LAUREEN L. J. HSU

The ionization constants of several polyvalent compounds were determined and found to be consistent with the ionization of an hydroxyl group of polyvalent iodine. The pKa of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole is 7.35 ± 0.13, and those of its two immediate higher homologs are 7.54 ± 0.29 and 7.37 ± 0.17, respectively. The behavior of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole was studied in varying concentrations of sulfuric acid. Two protonation steps seem to occur, with apparent pKa values of −0.58 and −5.75, on the H_0 scale. The structural implications of these findings on the heterocyclic nature of the benziodoxole ring are discussed.

RECENT WORK on the structure of certain polyvalent iodine compounds (1, 2) confirms their heterocyclic nature. The structure of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole (I) has been unequivocally determined by X-ray crystallography (3). This study revealed a significant difference between the two I—O bonds; the intra-annular bond between iodine and oxygen is 2.30 Å. long, while the bond between iodine and the hydroxylic oxygen is 2.00 Å. This difference can be ascribed either to the steric strain of a 5-membered ring or to a strong ionic contribution to the iodine-oxygen (ring) bond. An alternative possibility, that a betaine type of ring (iodonium-carboxylate, I*a*) makes a significant contribution to the structure of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole had to be evaluated. The behavior of 2-carboxy-diphenyl iodonium (III), containing such a ring system, had been studied recently (4). The ionization constants of I and its homologs (II, $n = 1$ or 2) were investigated in order to provide further information on the structure of these iodine-containing heterocyclic rings, and to evaluate the possible contribution of betainic structures such as (I*a*). This study was conducted both in dilute aqueous



I Ia



II III

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solutions and in media of very high acidity (H_0 to -10).

EXPERIMENTAL

Products.—1,3 - Dihydro - 1 - hydroxy - 3 - oxo-1,2-benziodoxole was prepared by the method of Meyer and Askenasy (5) as modified by Wolf and Hsu (1). 3,4-Dihydro-1-hydroxy-3-oxo-1*H*-1,2-benziodoxin and 1,3,4,5-tetra-hydro-1-hydroxy-3-oxo-1,2-benziodoxepin were prepared as described previously (1, 2). Baker and Adams reagent grade sulfuric acid (95%) was used.

Methods.—The method used for the determination of the pKa in dilute aqueous solutions was that of Albert and Sarjeant (6), using a Cary 14 recording spectrophotometer, a Beckman DB recording spectrophotometer, and a Beckman-Gilford direct-reading spectrophotometer. The pH measurements were performed on a Photovolt 115 pH meter, which was standardized against Beckman buffers before each measurement. All measurements were conducted at 23 ± 3°.

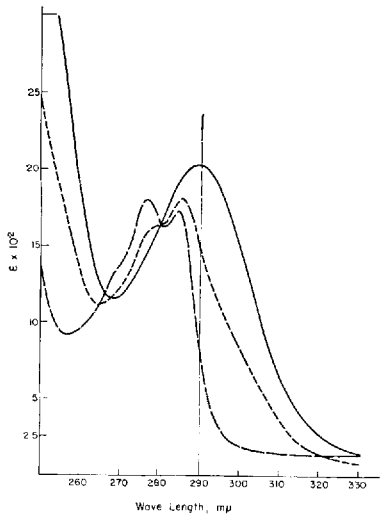


Fig. 1.—Absorption spectra of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole in $^1/_{15}$ *M* phosphate buffer. Key: ——, pH 11.19; - - -, pH 7.5; – – –, pH 1.85.

TABLE I.—MOLAR ABSORPTIVITIES OF POLYVALENT IODINE COMPOUNDS AND THEIR ANIONS[a]

Compd.		Anion[b]	Un-ionized Molecule[c]
1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole	2900	2044	849
	2845	1915	1739
	2765	1267	1811
3,4-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxin	3200	337	184
	2900	491	521
	2500	1717	1687
1,3,4,5-Tetrahydro-1-hydroxy-3-oxo-1,2-benziodoxepin	3200	326	24
	2900	496	809
	2500	1547	2619

[a] In phosphate buffers, 1/15 *M*. [b] pH 10–12. [c] pH 1–2.

The spectrophotometric method as modified by Davis and Geissman (7) was used to determine the ionization constants in the concentrated sulfuric acid solutions. Stock solutions of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole in 96% H_2SO_4 were prepared and their stability checked periodically. No apparent decomposition could be observed during the course of this work. The stock solutions were diluted, under refrigeration, to the desired final concentrations in 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole and sulfuric acid. The H_0 scale used was that of Paul and Long (8) for the 40–95% sulfuric acid region and that of Bascombe and Bell (9) for the 0–40% sulfuric acid concentration.

RESULTS AND DISCUSSION

The changes in the absorption spectra of a solution of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole in $^1/_{15}$ *M* phosphate buffers at pH 1.85, 7.5, and 11.19 are illustrated in Fig. 1. By using 290 mμ ($\lambda_{max.}$ for the anion, Table I) as the analytical wavelength, the pKa values listed in Table II were obtained.

Similar determinations for 3,4-dihydro-1-hydroxy-3-oxo-1*H*-1,2-benziodoxin and 1,3,4,5-tetrahydro-1-hydroxy-3-oxo-1,2-benziodoxepin are illustrated in Tables III and IV.

The pKa and ionization constants of the 3 heterocyclic polyvalent iodine compounds are listed in Table V.

For comparison, the pKa values, as reported in the literature for some related iodo compounds, are listed in Table VI.

The influence of the iodo group on the ionization of the benzoic and phenylacetic acids is seen to be strongest when the halogen is in the *ortho* position. The decrease in pKa has been ascribed to both inductive and resonance effects (10, 13), which could be enhanced in *o*-iodobenzoic acid by the participation of a betaine type of structure.

The introduction of a methylene group shields the carboxylate function from such effects. Thus, for *o*-iodophenylacetic acid, the ΔpKa is 0.27, compared with a ΔpKa of 1.34 for *o*-iodobenzoic acid. The ΔpKa values are 0.15 and 0.34 for the *m*-analogs, respectively. While no data are available for the corresponding iodophenylpropionic acids, it can be assumed that the introduction of the second methylene group would further shield the carboxylic acid group. [*Cf.* pKa of propionic acid, 4.88 (6).]

In contrast, the authors' results on the ionization constants of the related polyvalent iodine deriva-

TABLE II.—IONIZATION CONSTANTS DETERMINATION FOR 1,3-DIHYDRO-1-HYDROXY-3-OXO-1,2-BENZIODOXOLE AT 23 ± 3°C.

pH	d	$d_I - d$	$\frac{d_I - d}{d - d_M}$	$\log \frac{d_I - d}{d - d_M}$	pKa
6.80	0.355	0.295	3.470	0.5403	7.34
7.00	0.390	0.260	2.160	0.3345	7.33
7.15	0.445	0.205	1.170	0.0682	7.22
7.50	0.480	0.170	0.807	−0.0931	7.40
7.60	0.500	0.150	0.650	−0.1870	7.41

TABLE III.—IONIZATION CONSTANTS DETERMINATION FOR 3,4-DIHYDRO-1-HYDROXY-3-OXO-1*H*-1,2-BENZIODOXIN AT 23 ± 3°C.

pH	d	$d_I - d$	$\frac{d_I - d}{d - d_M}$	$\log \frac{d_I - d}{d - d_M}$	pKa
6.80	0.058	0.052	6.500	0.813	7.61
7.20	0.070	0.040	2.000	0.300	7.50
7.50	0.075	0.035	1.400	0.146	7.65
7.60	0.088	0.022	0.785	−0.105	7.59
7.95	0.100	0.010	0.200	−0.700	7.25

TABLE IV.—IONIZATION CONSTANTS DETERMINATION FOR 1,3,4,5-TETRAHYDRO-1-HYDROXY-3-OXO-1,2-BENZIODOXEPIN AT 23 ± 3°C.

pH	d	$d - d_I$	$\frac{d - d_I}{d_M - d}$	$\log \frac{d - d_I}{d_M - d}$	pKa
6.80	0.680	0.225	3.200	0.5050	7.31
7.20	0.560	0.215	1.950	0.2900	7.49
7.50	0.600	0.145	0.850	−0.0700	7.42
7.70	0.530	0.075	0.314	−0.5031	7.20

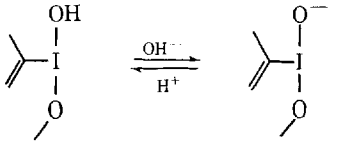
TABLE V.—pKa AND IONIZATION CONSTANTS OF THREE HETEROCYCLIC POLYVALENT IODINE COMPOUNDS

	pKa	Ionization Constant
1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole	7.35 ± 0.13	$(2.2 \pm 0.5) \times 10^{-7}$
3,4-Dihydro-1-hydroxy-3-oxo-1*H*-1,2-benziodoxin	7.54 ± 0.29	$(3.4 \pm 1.6) \times 10^{-7}$
1,3,4,5-Tetrahydro-1-hydroxy-3-oxo-1,2-benziodoxepin	7.37 ± 0.17	$(2.3 \pm 0.8) \times 10^{-7}$

TABLE VI.—pKa VALUES OF SOME *o*-IODOPHENYL CARBOXYLIC ACIDS

Compd.	H	*o*-Iodo	*m*-Iodo	*p*-Iodo	Ref.
Benzoic acid	4.20	2.86	3.86	3.93	(10, 11)
Phenylacetic acid	4.31	4.04	4.16	...	(10, 12)
β-Phenyl propionic acid	4.66	...	...	...	(10)

tives fail to detect any such variations in their pKa values, and this suggests that the ionizable function is not located on a carboxylic acid. Instead, the authors suggest that a common ionizable function is present in the three heterocyclic polyvalent iodine systems, namely, the iodine-bonded hydroxyl group.



This function is independent, except for steric factors, of the number of methylene groups between the carbonyl group and the benzene ring.

Further arguments supporting this assignment can be made if we consider the pKa values of the proven betaine structures reported by Beringer (4), who studied the acidities of carboxydiphenyliodonium compounds in acetonitrile–water solutions at 24°. Under their conditions the apparent pKa values are 6.92 for benzoic acid, and 6.24, 6.45, and 6.64, respectively, for the *o*-, *m*-, and *p*-iodobenzoic acids, and 3.5 ± 1, 5.55, and 4.6 for the *o*-, *m*-, and *p*-phenyliodoniumbenzoic acids (2,3 and 4-carboxydiphenyliodonium). The iodonium substituted benzoic acids appear to be stronger acids than the corresponding iodo-benzoic acids under the conditions studied. Thus, while in all the above examples, the *o*-iodo or the *o*-iodonium derivatives were stronger acids than unsubstituted benzoic acid, the contrary is the case for 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole, which is a weaker acid than either benzoic or *o*-io-

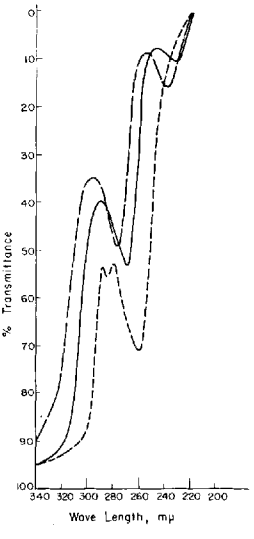


Fig. 2.—Absorption spectra of 1, 3-dihydro - 1 - hydroxy- 3-oxo - 1,2 - benziodoxole in sulfuric acid solution. Key: - - -, in 9.6% H_2SO_4; ——, in 48% H_2SO_4; – – –, in 86.3% H_2SO_4.

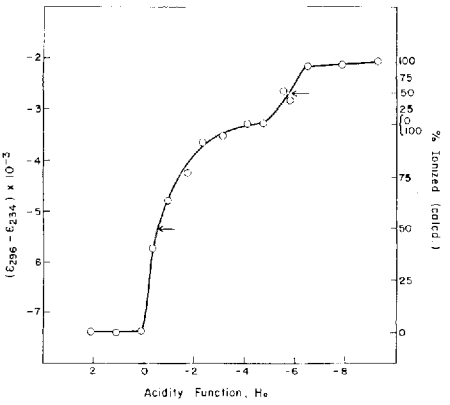


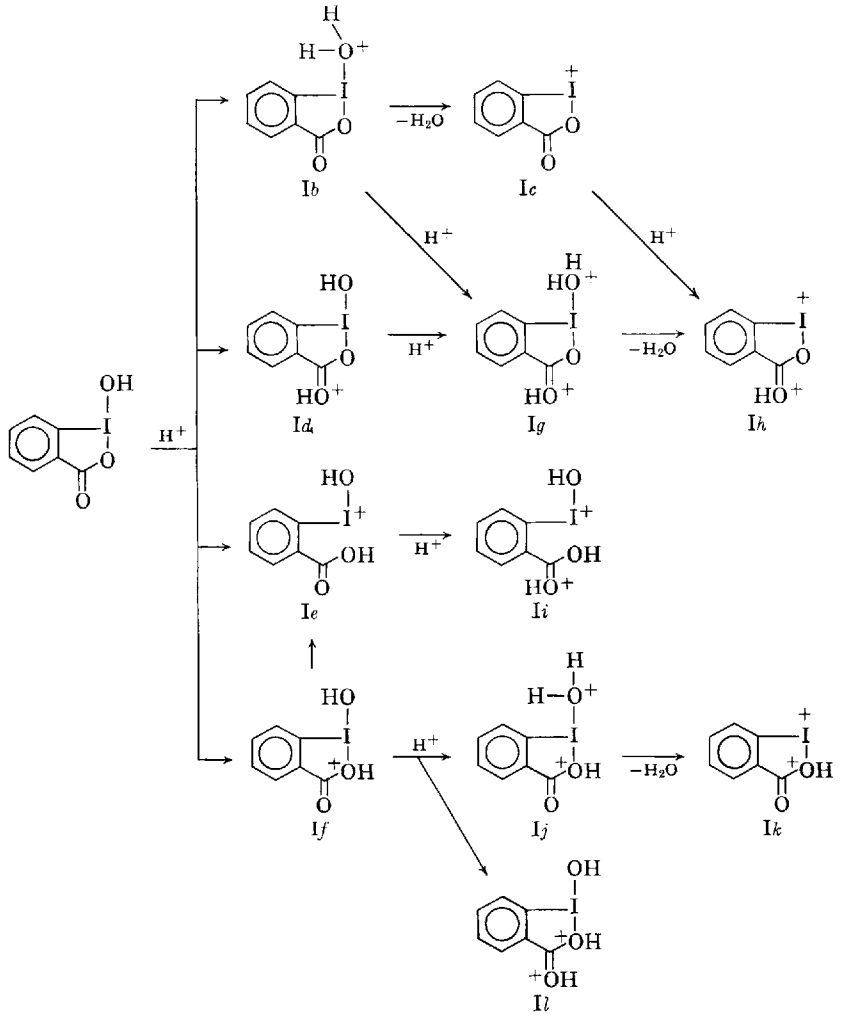
Fig. 3.—1,3-Dihydro-1-hydroxy-3-oxo-1,2-benziodoxole: acidity function *vs.* (ϵ 296–ϵ 234); points from spectral data, pKa's calculated from curve, −0.58 and −5.75.

dobenzoic acid. We can, therefore, safely assume that structures such as I*a* make only an extremely small, if any, contribution to 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole.

Carboxylic acids exhibit a second protonation in strongly acidic media (14), and the pKa's determined in the acidity function (H_0) region are −7.38, −7.78, −7.64, and −7.50 for benzoic acid, and its *o*-, *m*-, and *p*-iodo analogs, respectively. A single protonation step is observed, and the suggestion has been made that protonation occurs on the carboxylic oxygen atom rather than on the hydroxyl group (15).

The behavior of the benziodoxole ring in strongly acidic media is of interest, since this system provides a number of potential protonation sites. Particularly, it might be possible to observe if ring opening occurs or if a protonation of the carbonyl function, similar to that of other benzoic acids, is detectable.

The change in absorption of a solution of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole in various concentrations of sulfuric acid is illustrated in Fig. 2. A plot of $\Delta\epsilon$ (ϵ 296 − ϵ 234) *versus* H_0 is given in Fig. 3, and it shows a complex curve, which appears to have two inflection points at H_0 = −0.58 and at H_0 = −5.75. These results indicate



Scheme I

a two-step protonation. As a check of the method, the pKa of *o*-iodobenzoic acid was determined concurrently, yielding the value of −7.4 which is in good agreement with previously published data (12).

A first protonation of compound I could lead to structures I*b*, I*d*, I*c*, or I*f*; loss of water from I*b* will yield I*c*. (Scheme I.)

Of these structures for a single protonation, I*b* and I*f* are considered least likely. Protonation of a conjugated carbonyl function, such as that of benzophenone, occurs at pKa = −6.41 (16), and protonation of a lactone occurs at the carbonyl rather than the ether oxygen; an electronic shift would convert "structure" I*f* into I*e*. Examination of Fig. 2 reveals that protonation of compound I gives rise to a new peak at 256 mμ, while the doublet at 276–284 coalesces to a single peak at 288 mμ. By comparison, *o*-iodobenzoic acid shows a peak at 285 mμ which, in strong acid, shifts to 328 mμ. Thus, the 288 mμ peak of mono-protonated 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole could be indicative of the presence of a carboxylic acid function (I*e*). However, not enough is known about the spectral characteristics of iodonium compounds to rule out the possible contribution of structure I*d*.

The possibility of a sulfonation reaction appears unlikely, as dilution of the above concentrated sulfuric acid solutions resulted in an absorption spectra identical to that obtained by mild acidification of an aqueous solution.

The second protonation (H_0 = −5.75) may be associated with that of a carbonyl group, leading to structures such as I*g*, I*h*, I*i*, I*j*, or I*l*; loss of water could lead to I*k*, or I*h*. The shift of the absorption band from 288 mμ to 296 mμ is quite small. Structures I*j* and I*l* would contain a protonated lactone oxygen, and are therefore considered far less likely than structure I*i*, which represents a classically protonated carboxylic acid and is resonance stabilized. Although the pKa observed is significantly less than those recorded for other *o*-substituted benzoic acids (−6.78 to −7.78), the effect of an *o*-iodonium function must be taken into consideration. Thus, no conclusion can be made at present on the possible significance of these data on deciding between structures I*i* and I*h* for the doubly protonated species, or between I*e* and I*d* for the singly protonated molecule.

The ionization properties of 1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole and homologs thus support the heterocyclic nature of these compounds.

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Pharmacokinetic Model for Nalidixic Acid in Man II

Parameters for Absorption, Metabolism, and Elimination

By G. A. PORTMANN, E. W. McCHESNEY, H. STANDER, and W. E. MOORE

The absorption, metabolism, and excretion of nalidixic acid in man is illustrated by a model, and appropriate equations are derived. A total of 7 rate constants are calculated: 4 metabolic constants, 2 excretory constants, and 1 availability constant. All 5 components of the urine compartment and 2 components of the plasma compartment are measured. Comparisons between experimental and calculated values are good.

THE CONSTRUCTION of pharmacokinetic models, their utility in dosage form design, and the mathematical description of the fate of a drug in the body has been adequately treated by Wagner (1, 2), Nelson (3), Levy (4), and others (5).

A complex model illustrating the pharmacokinetic parameters relating to the absorption, metabolism, and elimination of nalidixic acid[1] in man has been presented previously (6). A practical model was developed which enabled calculation of the biologically active and inactive forms as separate groups. Another article by the authors describes a pharmacokinetic

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Previous paper: Portmann, G. A., McChesney, E. W., Stander, H., and Moore, W. E., *J. Pharm. Sci.*, **55**, 59 (1966).

[1] Nalidixic acid is 1-ethyl-1,4-dihydro-7-methyl-4-oxo-1,8-naphthyridine-3-carboxylic acid. Marketed as NegGram by Winthrop Laboratories, New York, N. Y.