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Potentiometric Study on Acid Properties of Some 4-Hydroxy-6*H*-1,3-oxazin-6-ones. Structure–Biological Activity Relationship

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Abstract — 4-Hydroxy-6*H*-1,3-oxazin-6-ones exhibit properties of weak OH acids. These compounds are readily methylated with diazomethane to give the corresponding 4-methoxy derivatives. According to the potentiometric titration data, the pK_a values of 2-methoxy- and 2-methylsulfanyl-substituted 4-hydroxy-6*H*-1,3-oxazin-6-ones range from 7.45 to 8.42, depending on the substituent in position 5 of the heteroring. 4-Hydroxy-6*H*-1,3-oxazin-6-ones in biological media exist mainly in the neutral form.
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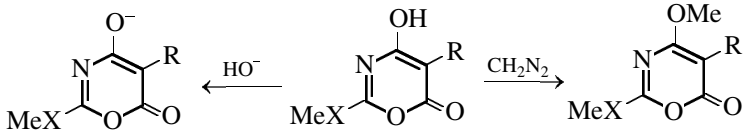
We previously [1] synthesized a series of 2-alkoxy- and 2-alkylsulfanyl-4-hydroxy-6*H*-1,3-oxazin-6-ones and examined their structure, reactions with nucleophiles [2], and biological activity [3]. The presence of a hydroxy group on C^4 in the heteroring endows these compounds with acid properties. Some 4-hydroxy-6*H*-1,3-oxazin-6-ones showed a pronounced sedative activity which, as well as their acute toxicity, was found to strongly depend on the nature of substituents in positions 2 and 5 of the heteroring. It seemed to be important to study their acid properties with a view to reveal how their acidity affects the biological activity and the behavior in biological media.

Oxazines **Ia**–**Ig** are readily soluble in aqueous alkali; their treatment with diazomethane affords the corresponding 4-methoxy derivatives **IIa**–**IIg**. These data may be regarded as indirect evidences of acid properties of these compounds.



Ia–**Ig** **IIa**–**IIg**

I, II, X = S, R = H (**a**), Me (**b**), Ph (**c**), *cyclo*-C_6H_{11} (**d**); X = O, R = Me (**e**), Ph (**f**), *cyclo*-C_6H_{11} (**g**).

The yields, melting points, R_f values, and elemental analyses of 4-methoxyoxazines **IIa**–**IIg** were given in Table 1. Their structure was confirmed by the ^1H and ^{13}C NMR, UV, and IR spectra (Tables 2, 3). The ^1H NMR spectra of **IIa**–**IIg** in DMSO-d_6 (Table 2) contained a singlet at α 3.11–3.20 ppm due to protons of the methoxy group on C^4. The signal at δ 2.46–2.49 ppm in the spectra of compounds **IIa**–**IId** belongs to proton of the methylsulfanyl group on C^2, and the 2-methoxy group in oxazines **IIe**–**IIg** gives rise to a signal at δ 3.93–3.95 ppm. Compound **IIa** also showed in the spectrum signal at δ 5.98 ppm from the 5-H proton. In addition, signals from protons in the alkyl substituents R on C^5.

In the ^{13}C NMR spectra of compounds **IIa**–**IId** (Table 3), we observed a signal at δ_C 12.65–13.15 ppm, which is typical of the MeS group. The signal in the region δ_C 27.81–28.40 ppm belongs to the methoxy group on C^4. The carbon atom in the 2-me-

Table 1. Yields, melting points, R_f values, and elemental analyses of compounds **IIa**–**IIg**

Comp. no.	Yield, %	mp, °C	R_f [a]	Found, %				Formula	Calculated, %			
				C	H	N	S		C	H	N	S
IIa	86	107–109	0.57	48.61	6.49	8.14	18.59	$C_7H_{11}NO_2S$	48.53	6.40	8.09	18.51
IIb	84	86–88	0.59	51.35	7.05	7.54	17.09	$C_8H_{13}NO_2S$	51.31	7.00	7.48	17.12
IIc	86	91–93	0.60	62.71	6.12	5.68	12.78	$C_{13}H_{15}NO_2S$	62.62	6.06	5.62	12.86
IId	90	96–98	0.62	61.20	8.34	5.53	12.49	$C_{13}H_{21}NO_2S$	61.14	8.29	5.48	12.56
IIe	85	100–102	0.54	56.19	7.61	8.11	–	$C_8H_{13}NO_3$	56.13	7.65	8.18	–
IIf	87	97–99	0.57	67.00	6.42	6.05	–	$C_{13}H_{15}NO_3$	66.94	6.48	6.00	–
IIg	89	105–107	0.69	65.31	8.79	5.89	–	$C_{13}H_{21}NO_3$	65.25	8.84	5.85	–

[a] Eluent ethyl acetate.

Table 2. IR, UV, and ^{1}H NMR spectra of compounds **IIa**–**IIg**

Comp. no.	IR spectrum (KBr), ν, cm^{-1}		UV spectrum (ethanol) λ, nm (log ε)	^{1}H NMR spectrum (DMSO-d_6), δ, ppm		
	C^6=O	C^2=N		CH_3X	C^4H_3O	R
IIa	1760	1630	206 (3.82), 280 (4.10)	2.48 s (3H, CH_3)	3.11 s (3H, CH_3)	5.98 s (1H)
IIb	1770	1630	209 (3.80), 283 (4.11)	2.49 s (3H, CH_3)	3.13 s (3H, CH_3)	1.79 s (3H, CH_3)
IIc	1770	1620	208 (3.83), 235 (4.23), 285 (4.09)	2.46 s (3H, CH_3)	3.20 s (3H, CH_3)	7.26–7.44 m (5H, C_6H_5)
IId	1765	1640	207 (3.89), 283 (4.15)	2.49 s (3H, CH_3)	3.12 s (3H, CH_3)	1.17–1.88 m (11H, *cyclo*-C_6H_{11})
IIe	1775	1630	208 (3.81), 251 (4.20)	3.95 s (3H, CH_3)	3.14 s (3H, CH_3)	1.68 s (3H, CH_3)
IIf	1780	1630	209 (3.79), 253 (4.26)	3.93 s (3H, CH_3)	3.19 s (3H, CH_3)	7.27–7.48 m (5H, C_6H_5)
IIg	1760	1620	211 (3.80), 250 (4.23)	3.92 s (3H, CH_3)	3.14 s (3H, CH_3)	1.19–1.68 m (11H, *cyclo*-C_6H_{11})

Table 3. ^{13}C NMR spectra of compounds **IIa**–**IIg** in DMSO-d_6, δ_C, ppm

Comp. no.	CH_3X	C^4H_3O	C^2	C^4	C^5	C^6	R
IIa	13.15	27.81	147.80	159.64	96.77	168.23	–
IIb	12.65	28.24	147.92	159.31	105.22	160.38	10.29 (CH_3)
IIc	12.93	28.40	147.69	159.44	111.06	161.93	128.20–130.69 (C_6H_5)
IId	12.94	28.35	147.25	160.07	113.40	161.36	25.26–29.37 (*cyclo*-C_6H_{11})
IIe	56.52	28.31	146.43	160.17	84.41	163.31	6.82 (CH_3)
IIf	56.36	28.29	145.34	161.08	87.15	162.15	127.34–130.41 (C_6H_5)
IIg	56.30	28.21	145.09	161.15	90.85	163.11	25.37–29.41 (*cyclo*-C_6H_{11})

thoxy group of compounds **IIe**–**IIg** appears as δ_C 56.50–56.59 ppm; the C^5 atom is characterized by δ_C 84.12–111.06 ppm. The C^2, C^4, and C^6 signals are at δ_C 146.43– 147.91, 159.31–160.17, and 161.93–168.23 ppm, respectively. In addition, signals from carbon atoms in alkyl substituents R on C^5 (**IIb**–**IIg**) are present.

Compounds **IIa**–**IIg** characteristically showed in the IR spectra (Table 2) absorption bands in the regions 1780–1760(C^6=O) and 1650–1640 cm^{-1} (C=N,

C=C). The UV spectra of solutions of **IIa**–**IId** in ethanol (Table 2) contain absorption maxima at λ 206–209 and 280–285 nm, and the corresponding maxima of compounds **IIe**–**IIg** are located at λ 208–211 and 250–253 nm.

Study of acid properties and substituent effect on the acidity may be helpful for optimization of the conditions of synthesis and isolation of substances, determination of the state of tautomeric equilibrium, and correlation with their biological activity [4]. We have determined the acid ionization constants of 2-methoxy- and 2-methylsulfanyl-4-hydroxy-6*H*-1,3-oxazin-6-ones **Ia**–**Ig** by potentiometric titration [5, 6]. Spectrophotometric method [7] turned out to be inapplicable to the compounds under study, for the electronic absorption spectra of their neutral and anionic forms were similar.

In order to make sure that the potentiometric method is applicable in our case, we performed potentiometric titration of structurally related barbituric acid under analogous conditions. The pK_a value thus determined coincided with the reported value (pK_a 4.04 [8]). The ionization constants were calculated, and the titration curves were plotted, using a program developed by V.F. Apraksin [9].

The results are given in Table 4; they show that the acidity of 4-hydroxyoxazines **Ia**–**Ig** strongly depends on substituents in the 2 and 5 positions of the heteroring. α-Methylsulfanyl-substituted compounds **Ia**–**Id** are stronger acids than their 2-methoxy analogs **Ie**–**Ig**. This is consistent with a stronger electron-donor effect of the methoxy group compared to methylsulfanyl as a result of less effective S–C orbital overlap; correspondingly, the anions derived from 2-methoxy derivatives **Ie**–**Ig** are stabilized to a lesser extent. Introduction in the 5 position of methyl and cyclohexyl groups which exhibit a positive inductive effect reduces the acidity; likewise, the reason is weaker stabilization of the corresponding anions. 5-Phenyl-substituted compound **Ic** is a weaker acid than oxazin **Ia** having no substituent on C^5, presumably, due to weak donor effect of the phenyl substituent.

Our previous biological activity studies [3, 10] showed that oxazines **Ia**–**Ig** exhibit a sedative effect. Judging by their pK_a values (Table 4), oxazines **Ia**–**Ig** should exist in the acid form at pH values corresponding to biological media [11]; obviously, this should facilitate their transport through cell membranes.

EXPERIMENTAL

The electronic spectra were recorded from solutions in ethanol on an SF-2000 spectrophotometer. The IR spectra were measured in KBr on an FSM-1201 Fourier spectrometer. The ^1H and ^{13}C NMR spectra were obtained from solutions in DMSO-d_6 on a Bruker AM-500 instrument. The progress of reactions was monitored by TLC on Sorbfil plates using ethyl acetate as eluent (spots were visualized under UV light). The melting points were determined in a capillary.

Table 4. Ionization constants of oxazines **Ia**–**Ig**

Compound	pK_a [K_a]
Id	8.35 [$(4.8 \pm 0.8) \times 10^{-9}$]
Ib	7.65 [$(2.3 \pm 0.2) \times 10^{-8}$]
Ic	7.55 [$(2.81 \pm 0.06) \times 10^{-8}$]
Ia	7.45 [$(3.5 \pm 0.1) \times 10^{-8}$]
Ig	8.42 [$(3.8 \pm 0.4) \times 10^{-9}$]
Ie	8.00 [$(10.10 \pm 0.07) \times 10^{-9}$]
If	7.47 [$(3.37 \pm 0.06) \times 10^{-8}$]
Barbituric acid	4.05, 4.04 [8]

The ionization constants were determined by potentiometric titration of aqueous–alcoholic solutions [5% of ethanol; $c = (1–5) \times 10^{-3}$ M] of oxazines **Ia**–**Ig** with a 0.05 M carbonate-free solution of potassium hydroxide using a 0.03 M solution of KNO_3 as supporting electrolyte. The pH values were measured on a pH-121 potentiometer with an accuracy of ±0.05 pH unit at a constant ionic strength; an ESL-43-07 glass electrode and an EVL-1M3 silver chloride electrode (reference) were used; the temperature was maintained at 25°C.

5-Substituted 4-methoxy-2-methylsulfanyl- and 2,4-dimethoxy-6*H*-1,3-oxazin-6-ones IIa–IIg *(general procedure).* Oxazine **Ia**–**Ig**, 2 g, was dispersed in 5 ml of diethyl ether, and a solution of diazomethane in diethyl ether was slowly added at 5–10°C until nitrogen no longer evolved. The solvent was distilled off, and the precipitate was filtered off and dried.

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