RESEARCHES ON ALLO- AND ISOALLOXAZINES

XVII. Hydroxyethylation of 7-Aminoallo- and Isoalloxazines*

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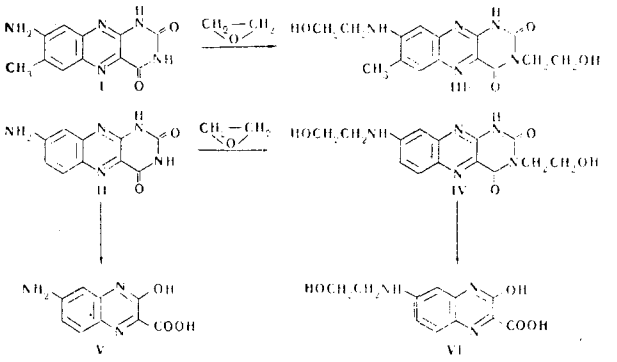
The reaction of 7-aminoallo- and isoalloxazines with ethylene oxide is investigated, and its optimum conditions are found. Addition of ethylene oxide to 7-aminoisoalloxazine involves only the cyclic amino group at position 3, while addition to 7-aminoalloxazine involves the cyclic imino group at position 3 and the amino group at position 7.

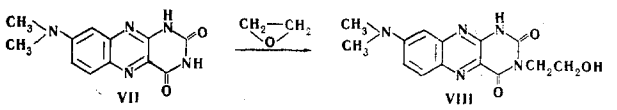
As a continuation of work done in this laboratory on the synthesis of various amino derivatives of allo- and isoalloxazines [2], and in studying their reactivities, the present work made a study of the reaction of 7-aminoallo- and 7-aminoisoalloxazines with ethylene oxide. The reaction of slightly basic amines, among which may be numbered aminoallo- and aminoisoalloazines, with ethylene oxide usually takes place under drastic conditions, that is, under pressure and at 160° [3]. Hydroxyethylation of 7-aminoallo- and 7-aminoisoalloxazoles under such conditions in a heterogeneous phase (due to low solubility) gave a mixture, separable with difficulty, of starting amine and its hydroxyethylation products. Fractional crystallization from ethanol led to the isolation of a small yield of a product of di-hydroxyethylation of 7-aminoalloxazine.

The amines investigated did not hydroxyethylate in pyridine or acetic acid solution, while with 1.5% alkali solution there was great difficulty in separating the reaction products, due to ethylene oxide polymerization. The optimum conditions for effecting hydroxyethylation have now been found, viz. passing ethylene oxide into heated aqueous solutions of 7-aminoallo- and 7-aminoisoalloxazines. Hydroxyethylation of 6-methyl-7-aminoalloxazine (I) and 7-aminoalloxazine (II) gave 3-(β-hydroxyethyl)-6-methyl-7-(β-hydroxyethyl)aminoalloxazine (III) and 3-(β-hydroxyethyl)-7-(β-hydroxyethyl)aminoalloxazine (IV) respectively. Unlike 7-aminoalloxazine [4], compounds III and IV do not diazotize, indicating the absence of a primary amine group.

The alkaline degradation reaction of alloxazines [5] was used to confirm the structures of compounds III and IV. In the case of compound IV it gave 2-hydroxy-7-(β-hydroxyethyl)aminoquinoxaline-3-carboxylic acid (VI), while the starting 7-aminoalloxazine (II) gave 2-hydroxy-7-aminoquinoxaline-3-carboxylic acid (V).

That hydroxyethylation of 7-aminoalloxazines does not proceed simultaneously at positions 1 and 3, is confirmed by the preparation of 3-(β-hydroxyethyl)-7-dimethylaminoalloxazine (VIII) by hydroxyethylation of 7-dimethylaminoalloxazine (VII).





The occurrence of hydroxyethylation at the ring imino group at position 3 (and not at position 1) is obviously due to the position 3 imino group between two carbonyls being more acidic and more mobile than the position 1 imino group, and capable of splitting off a proton to give a strongly nucleophilic center, which reacts with the electrophilic carbon atom of the ethylene oxide.

The presence of a dialkylamino group at position 7 in the alloxazine molecule causes, in comparison with an unsubstituted amino group, a bathochromic shift (by 35 mμ) of the long wave band of the absorption spectrum. The absorption spectra of the hydroxyethyl derivatives of 7-aminoalloxazine and 7-dimethylaminoalloxazine resemble the spectra of the starting amines. This confirms the fact that compounds III and IV do not have the structure of a 7-di(β-hydroxyethyl)aminoalloxazine (Fig. 1).

It should be mentioned that in 70% sulfuric acid solution compound IV readily undergoes dealkylation to the starting 7-aminoalloxazine (II), which is diazotized by sodium nitrite, and then undergoes diazo coupling with β-naphthol to give 7-(β'-naphthyl-1'-azo) alloxazine (IX). This was proved by paper chromatography of the reaction products using the appropriate markers (Fig. 2).

Hydroxyethylation of 7-amino(desmethyl)lumiflavin (X) having two active centers, a ring imino group and a primary amino group at positions 3 and 7 respectively, gave 3-(β-hydroxyethyl)-7-amino(desmethyl)-lumiflavin (XI).

*For Part XVI see [1].

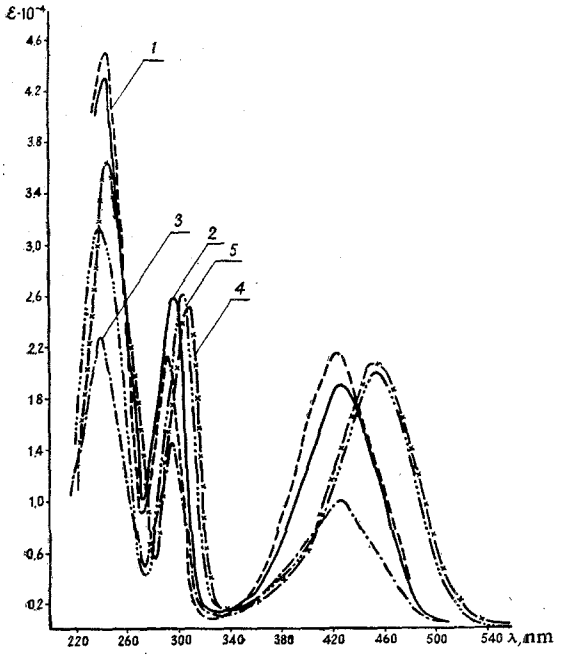


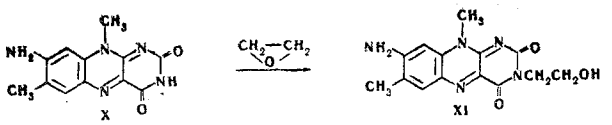
Fig. 1. Absorption spectra (in ethanol): 1) 3-(β-hydroxyethyl)-6-methyl-7-(β-hydroxyethyl) aminoalloxazine (III); 2) 3-(β-hydroxyethyl)-7-(β-hydroxyethyl) aminoalloxazine (IV); 3) 7-aminoalloxazine (II); 4) 3-(β-hydroxyethyl)-7-dimethylaminoalloxazine (VIII); 5) 7-dimethylaminoalloxazine (VII).

Properties of the Compounds Prepared

Compound	Name	Fluorescence	R_f* of system			Absorption spectrum in EtOH						Formula	Found %			Calculated, %		
			1	2	3	λ_{max1}, nm	$\varepsilon_1 \times 10^{-4}$	λ_{max2}, nm	$\varepsilon_2 \times 10^{-4}$	λ_{max3}, nm	$\varepsilon_3 \times 10^{-4}$		C	H	N	C	H	N
III	3-(β-Hydroxyethyl)-6-methyl-7-(β-hydroxyethyl)aminoalloxazine	Yellowish green	0.60	—	0.60	244	4.50	292	2.15	424	2.12	$C_{15}H_{17}N_5O_4$	54.20 54.58	5.35 5.28	21.34 20.88	54.38	5.24	21.14
IV	3-(β-Hydroxyethyl)-7-(β-hydroxyethyl)aminoalloxazine	Yellowish green	0.51	—	0.60	244	4,31	298	2.60	424	1.90	$C_{14}H_{15}N_5O_4$	52.50 52.67	4.85 4.78	21.98 22.46	52.99	4.77	22.07
V	2-Hydroxy-7-aminoquinoxaline-3-carboxylic acid	Blue	0.44	0.31	—	—	—	—	—	—	—	$C_9H_7N_3O_3$	52.10 52.02	3.57 3.88	20.41 20.25	52.68	3.44	20.48
VI	2-Hydroxy-7-(β-hydroxyethyl)aminoquinoxaline-3-carboxylic acid	Blue	0.51	0.50	—	—	—	—	—	—	—	$C_{11}H_{11}N_3O_4$	—	—	16.82 16.43	—	—	16.90
VIII	3-(β-Hydroxyethyl)-7-dimethylaminoalloxazine	Bright yellow	0.73	0.93	—	246	3.65	308	2.53	452	2.08	$C_{14}H_{15}N_5O_3$	55.53	5.61	22.58	55.80	5.02	23.25
XI	3-(β-Hydroxyethyl)-7-amino(desmethyl)lumiflavin	Yellowish green	0.36	0.64	—	252	5.09	—	—	482	5.11	$C_{14}H_{15}N_5O_3$	56.22 56.41	5.25 5.46	23.15 22.97	55.80	5.02	23.25
XII	7-(β-Hydroxyethyl)amino(desmethyl)lumiflavin**	Yellow	0.26	0.49	—	254	5.36	314	0.786	484	5.72	—	—	—	—	—	—	—

*System 1: n-BuOH—water—AcOH 4:5:1; system 2: pyridine—iso-BuOH—water-AcOH 33:33:33:1; system 3: n-BuOH—pyridine—water 6:4:3.

**Compound XII was prepared according to [6].



Compound XI does not form a sodium salt, unlike isoalloxazines with a free ring imino group at position 3. It is not identical with 7-(β-hydroxyethyl)amino (desmethyl)lumiflavin (XII), unequivocally prepared by condensing 7-chloro(desmethyl)lumiflavin with monoethanolamine [6] (table).

That compound XI contains a free amino group is confirmed by the fact that it undergoes diazotization, then diazo coupling with β-naphthol to give 3-(β-hydroxyethyl)-6-methyl-7-(β'-naphthyl-1'-azo)-9-methylisoalloxazine (XIII). Moreover, the azo dye molecule is deprived of the fluorescent properties inherent in the isoalloxazine. The occurrence of hydroxyethylation of 7-amino(desmethyl)lumiflavin at the position 3 ring nitrogen atom of the isoalloxazine molecule, is in agreement with known facts regarding the alkylation of 7-amino(desmethyl)lumiflavin by methyl iodide in alkaline solution, to 3-methyl-7-amino(desmethyl)-lumiflavin [7].

The table gives fluorescence colors, R_f values, absorption spectra, and elementary analyses of the compounds prepared.

EXPERIMENTAL

3-(β-Hydroxyethyl)-6-methyl-7-(β-hydroxyethyl)aminoalloxazine (III). A suspension of 2.0 g Na salt of 6-methyl-7-aminoalloxazine was prepared from 2.2 g 6-methyl-7-aminoalloxazine (I), using 40 ml 0.1 N NaOH in 600 ml water. It was heated to 100° and, with stirring, gaseous ethylene oxide passed into the solution for 6 hr. The progress of the reaction was checked by chromatographing (until the starting amine disappeared). The solution was vacuum concentrated to 200 ml, and after 72 hr, 0.85 g (25.1%) III was separated off. It was purified by two recrystallizations from water, washed with MeOH (10 ml), yellow needles, which did not melt up to 300°.

3-(β-Hydroxyethyl)-7-(β-hydroxyethyl)aminoalloxazine (IV). a) Ethylene oxide was passed into suspension of 1.0 g Na salt of 7-aminoalloxazine (II) prepared from 1.1 g 7-aminoalloxazine and 15 ml 0.1 N NaOH as when synthesizing compound III, to give 0.45 g (43%) alloxazine IV, purified similarly to III. Yellow plates, which did not melt up to 300°.

b) 2.29 g (0.01 mole) II and 1 ml (0.02 mole) ethylene oxide were heated together for 7 hr in a sealed tube at 150°. Chromatography showed the products to contain 7-aminoalloxazine and two new compounds. Fractional crystallization from EtOH, followed by recrystallization gave 0.03 g IV, as yellow crystals, which did not melt up to 300°.

2-Hydroxy-7-aminoquinoxaoline-3-carboxylic acid (V). 0.5 g II in 30 ml 2 N NaOH was heated for 24 hr at 150° in a sealed tube. When the tube was opened, there was a marked smell of ammonia. After filtering, the solution was acidified with dilute HCl. At pH 7-8, silicic acid, extracted from the glass by the alkali, precipitated and was separated off. In acid solution 0.12 g (27%) acid V came down, and was purified by recrystallizing from absolute EtOH, brownish-yellow precipitate, subliming at 292°.

2-Hydroxy-7-(β-hydroxyethyl)aminoquinoxaline-3-carboxylic acid (VI). 0.5 g IV was heated with 30 ml 2 N NaOH for 24 hr at 150°, and the products worked up as for compound V, to give 0.1 g (25.5%) VI, recrystallized from EtOH, yellow crystals, mp 151° (decomp).

3-(β-Hydroxyethyl)-7-dimethylaminoalloxazine (VIII). 1.0 g Na salt of 7-dimethylaminoalloxazine, obtained by reacting VII with 0.1 N NaOH, was prepared in 200 ml water, 20 ml 0.1 N NaOH added, the solution stirred and held at 100° for 6 hr, while ethylene oxide was passed in. The products were vacuum evaporated to 40 ml, when 0.52 ml (48.1%) VIII separated, purified by recrystallizing twice from water, then from MeOH, orange yellow plates which did not melt at 300°.

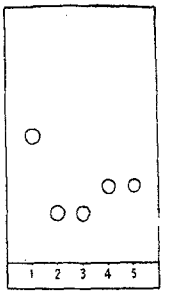


Fig. 2. Paper chromatography (in system 1: n-BuOH—water—AcOH, 4:5:1): 1) 3(β-hydroxyethyl)-7-(β-hydroyethyl)-aminoalloxazine (IV), R_f 0.51 (yellowish-green fluorescence); 2) compound IV in 70% H_2SO_4, R_f 0.20 (yellowish-green fluorescence); 3) 7-aminoalloxazine (II), R_f 0.20 (yellowish-green fluorescence); 4) compound IV in 70% H_2SO_4, after diazotizing and coupling with β-naphthol (red nonfluorescing spot); 5) 7-(β'-naphthyl-1'-azo)alloxazine (IX), R_f 0.30 (red nonfluorescing spot).

7-(β'-Naphthyl-1-azo)alloxazine (IX). 0.1 g IV was dissolved in 3 ml 70% H_2SO_4, and paper chromatography gave a spot fluorescing in UV light with R_f 0.56 (system 2, cf. Fig. 2), and 0.42 (system 3), identical with the marker 7-aminoalloxazine, having R_f 0.56 (system 2) and R_f 0.42 (system 3). After cooling to 0°, it was diazotized with 1 ml 10% $NaNO_2$ solution. After half an hour the products were diluted with water (5 ml), then filtered, and a solution of 0.12 g β-naphtol in 30 ml 15% NaOH was added to the filtrate; it turned an intense raspberry red. After leaving for half an hour, the solution was neutralized to pH 4-5, with AcOH. The black precipitate of azo dye was filtered off (0.09 g), R_f 0.30 (system 1) and 0.69 (system 2), red nonfluorescent spot. It was chromatographically identical with 7-(β'-naphthyl-1-azo)alloxazine, prepared from 7-aminoalloxazine as described in [4]. R_f 0.3 (system 1) and 0.69 (system 2), red nonfluorescent spot.

3-(β-Hydroxyethyl)-7-amino(desmethyl)lumiflavin (XI). 0.5 g X was suspended in 500 ml water, 50 ml 0.1 N NaOH added, and ethylene oxide passed into the solution at 100° for 8 hr. The resultant red solution was cooled, acidified to pH 6, and after 24 hr, 0.4 g (67.8%) XI separated. Red needles ex water or 50% MeOH, which did not melt at 300°.

3-(β-Hydroxyethyl)-6-methyl-7-(β'-naphthyl-1'-azo)-9-methylisoalloxazine (XIII). A suspension of 0.08 g XI in 8 ml 6 N HCl was cooled to 0° and diazotized with 1 ml 5% aqueous $NaNO_2$. After stirring for an hour at 18-20°, a solution of 0.05 g β-naphthol in 15 ml NaOH was added. Half an hour later, the solution was neutralized to pH4-5 with AcOH. 0.09 g azo dye XIII was filtered off, dark cherry, which did not melt at 300°. R_f 0.36 (system 1) and 0.70 (system 2), red nonfluorescent spot.

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