RESEARCH ON ISOALLOXAZINE DERIVATIVES

III. Synthesis and Some Reactions of 9-(β-Amino-ethyl)Isoalloxazines*

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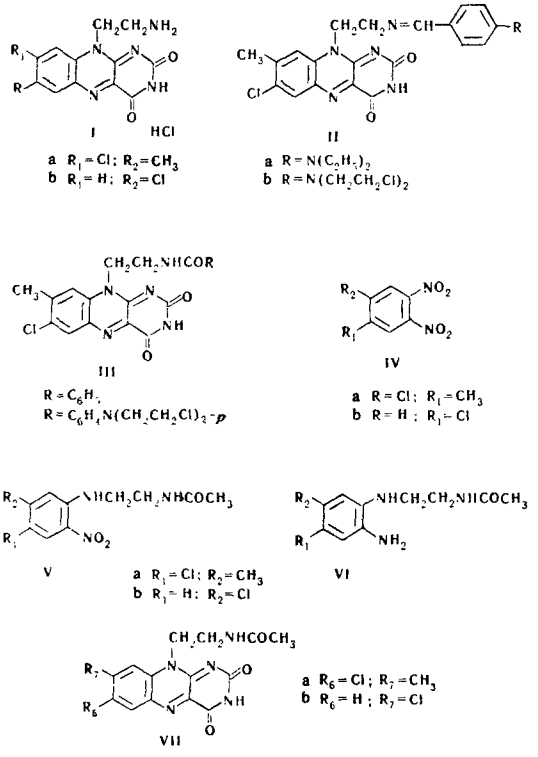
A convenient route to 9-(β-aminoethyl)isoalloxazines is offered, based on monoacetylethylenediamine. 6-Chloro-7-methyl-9-(β-aminoethyl)-isoalloxazine is used to synthesize some acyl derivatives and azomethines, among them some with the bis(β-chloroethyl)amino group, for biological testing.

Alloxazines with a β-hydroxyethyl group at position 9 in the isoalloxazine ring, as well as some of their esters, are known to be riboflavin antagonists, sometimes with antitumor activity [1, 2]. Then there is information in the literature about the varied biological activity of 9-(aminoalkyl) derivatives of isoalloxazine [3, 4, 5]. In that connection amino analogs of 9-(β-hydroxyethyl)isoalloxazine, viz. 9-(β-aminoethyl) derivatives of isoalloxazine of general formula **I** have been synthesized.



6-Chloro-7-methyl-9-(β-aminoethyl)isoalloxazine (**Ia**) was used as a starting material for preparing azomethines of general formula **II** and acyl derivatives of general formula **III**, among them some with a cytotoxic bis(β-chloroethyl)amino group (**IIb**, **IIIb**).

The kinds of substituents at positions 6 and 7 in the isoalloxazine ring were based on known connections in the isoalloxazine series between structure and biological activity.

The starting material for synthesizing 9-(β-aminoethyl)isoalloxazines of general formula **I**, not substituted at the amino group, was monoacetylethylenediamine, obtained in high yield by acetylating 98% ethylenediamine with ethyl acetate [6]. Condensation of monoacetylethylenediamine with the appropriate o-dinitro derivative **IV** [7] in amyl alcohol gives o-nitro-N-(β-acetylaminoethyl)anilines **V**, from which the corresponding 9-(β-acetylaminoethyl)isoalloxazines of general formula **VII** are obtained, by reducing the nitro groups and then condensing the resultant o-diamines **VI** with alloxan. Deacylation of compounds **VII** with 20% hydrochloric acid gives 25% yields of amines **Ia** and **Ib**, as hydrochlorides, the figure being based on the corresponding o-dinitro derivative **IV**. This method of synthesis is superior to those described in the literature [8, 9], for 9-(aminoalkyl)isoalloxazans, because of the high yields at the individual stages, the comparatively small number of stages, the simplicity of each one of them, and the high purities of the products.

As was to be expected, the amino group of 6-chloro-7-methyl-9-(β-aminoethyl)isoalloxazine (**Ia**) reacts readily with aromatic aldehydes, among them p-bis(β-chloroethyl)aminobenzaldehyde, giving the azomethines **II**. Acid hydrolysis of the latter led to isolation of the starting **Ia** as hydrochloride.

Acetic anhydride acylation of **Ia** gives a product identified by paper chromatography as 6-chloro-7-methyl-9(β-acetylaminoethyl)isoalloxazine (**VIIa**), which we previously prepared as an intermediate in the synthesis of **Ia**. Correspondingly benzoyl chloride benzoylation of **Ia** in the presence of triethylamine gives 6-chloro-7-methyl-9-(β-benzoylaminoethyl)isoalloxazine (**IIIa**). When the acylating agent was p-bis(β-chloroethyl)aminobenzoyl chloride, the product was 6-chloro-7-methyl-9-[p-bis(β-chloroethyl)aminobenzoylaminoethyl)]isoalloxazine (**IIIb**).

Not all of the isoalloxazine derivatives prepared have sharp melting points; they begin to decompose at 280–320°.

The table gives some properties of the compounds synthesized.

EXPERIMENTAL

2-Nitro-4-chloro-5-methy-N-(β-acetylaminoethyl)aniline (Va). 9 g (0.041 mole) IVa and 21 g (0.21 mole) monoacetylethylenedi-

*For Part II see [10].

Properties of Intermediates and Isoalloxazine Derivatives Synthesized

Compound	Name	Mp, °C	R^*_{f1}	R^{**}_{f2}	Formula	Found, %				Calculated, %				Yield, %
						C	H	N	Cl	C	H	N	Cl	
Va	2-Nitro-4-chloro-5-methyl-N-(β-acetylaminoethyl)-aniline	136	—	—	$C_{11}H_{14}ClN_3O_3$	49.04	5.22	—	13.04	48.73	5.16	—	13.10	83
Vb	2-Nitro-5-chloro-N-(β-acetylaminoethyl)aniline	153	—	—	$C_{10}H_{12}ClN_3O_3$	47.11	4.73	16.57	—	46.76	4.67	16.31	—	83
VIa	2-Amino-4-chloro-5-methyl-N-(β-acetylaminoethyl)aniline	110	—	—	$C_{11}H_{16}ClN_3O$	—	—	—	14.51	—	—	—	14.70	74
VIb	2-Amino-5-chloro-N-(β-acetylaminoethyl)aniline	120	—	—	$C_{10}H_{14}ClN_3O$	—	—	—	15.43	—	—	—	15.60	70
VIIa	6-Chloro-7-methyl-9-(β-acetylaminoethyl)isoalloxazine***	—	0.61	0.67	$C_{15}H_{14}ClN_5O_3$	51.94	4.14	21.15	—	51.80	4.04	20.82	—	70
VIIb	7-Chloro-9-(β-acetylaminoethyl)-isoalloxazine	—	—	—	$C_{14}H_{12}ClN_5O_3$	50.59	3.69	20.73	—	50.50	3.61	21.00	—	65
Ia	6-Chloro-7-methyl-9-(β-aminoethyl)isoalloxazine hydrochloride	—	—	—	$C_{13}H_{12}ClN_5O_2 \cdot HCl \cdot H_2O$	43.53	4.50	19.47	—	43.41	4.17	19.45	—	84
Ib	7-Chloro-9-(β-aminoethyl)-isoalloxazine hydrochloride	—	—	—	$C_{12}H_{10}ClN_5O_2 \cdot HCl \cdot H_2O$	—	—	20.14	—	—	—	20.23	—	70
IIa	6-Chloro-7-methyl-9-[p-(diethylamino)benzylideneaminoethyl] isoalloxazine	—	—	0.81	$C_{24}H_{25}ClN_6O_2$	61.94	5.59	18.18	—	62.00	5.38	18.08	—	60
IIb	6-Chloro-7-methyl-9-[p-bis(β-chloroethyl)aminobenzylideneaminoethyl]-isoalloxazine	—	—	0.75	$C_{24}H_{23}Cl_3N_6O_2$	54.21	4.26	15.54	—	54.00	4.32	15.74	—	60
IIIa	6-Chloro-7-methyl-9-(β-benzoylaminoethyl)isoalloxazine	—	0.34	0.83	$C_{20}H_{16}ClN_5O_3$	58.60	4.16	16.92	—	58.70	3.91	17.00	—	87
IIIb	6-Chloro-7-methyl-9-[p-bis(β-chloroethyl)aminobenzoylaminoethyl] isoalloxazine	—	0.18	—	$C_{24}H_{23}Cl_3N_6O_3$	52.04	4.35	15.49	18.97	52.41	4.19	15.39	19.44	81

*Found with the system BuOH saturated water-AcOH (10:1).

**Found with the system water–pyridine–BuOH (2:1:1).

***Obtained as an intermediate.

amine were dissolved together in 60 ml amyl alcohol, the mixture kept at 100° for 4 hr., then cooled. The orange precipitate which formed was filtered off and washed with water, giving yellow plates from EtOH or aqueous dioxane.

2-Nitro-5-chloro-N-(β-acetylaminoethyl)aniline (Vb) was prepared similarly to **Va**, starting from 3,4-dinitrochlorobenzene.

2-Amino-4-chloro-5-methyl-N-(β-acetylaminoethyl)aniline (VIa). a) 10 g (0.038 mole) **Va** was dissolved in 600 ml hot 50% EtOH, then hydrosulfite added until decolorization was complete (20 g). The colorless solution was filtered, and vacuum concentrated until turbidity appeared. On cooling, a copious precipitate of yellow needles formed.

b) 5 g **Va** was dissolved in 75 ml EtOH, the solution heated to boiling and 3 g hydrazine hydrate added, followed by Raney Ni until decolorization was complete. The products were filtered, the filtrate vacuum concentrated to half volume and poured into water, whereupon a white flocculent precipitate formed.

2-Amino-5-chloro-N-(β-acetylaminoethyl)aniline (VIb) was prepared similarly to **VIa**.

6-Chloro-7-methyl-9-(β-acetylaminoethyl)isoalloxazine (VIIa). A solution of 3 g (0.012 mole) **VIa** in 15 ml glacial AcOH was added to a hot solution of 8.2 g (0.12 mole) boric acid and 7.5 g (0.053 mole) alloxan in 250 ml glacial AcOH. The mixture was refluxed for 1 1/2 hr, vacuum evaporated to half volume, and the residue diluted with two volumes of water. The yellow precipitate was filtered off and washed with water. Minute bright yellow crystals, ex AcOH, for the preparation of **VIIa** by acetylating **Ia**, see below.

7-Chloro-9-(β-acetylaminoethyl)isoalloxazine (VIIb) was prepared similarly to **VIIa**.

6-Chloro-7-methyl-9-(β-aminoethyl)isoalloxazine hydrochloride (Ia). 4.5 g **VIIa** was refluxed with 150 ml 20% HCl for 12 hr. The products were vacuum evaporated to dryness, and the residue recrystallized from 50 ml water. Bright yellow crystals.

7-Chloro-9-(β-aminoethyl)isoalloxazine (Ib) was prepared similarly to **Ia**.

6-Chloro-7-methyl-9-[p-(diethylamino)benzylideneiminoethyl]-isoalloxazine (IIa). 1 g (0.0028 mole) **Ia** was mixed with 50 ml dimethylformamide, 0.4 ml (0.0032 mole) Et_3N, and 0.5 g (0.0036 mole) p-diethylaminobenzaldehyde. The mixture was stirred for 2 1/2 hr at 75°, vacuum evaporated to half volume, and then poured into water. The resultant dark brown precipitate was filtered off and washed with boiling EtOH. For purification, a solution of 1 g reaction product in 70 ml dioxane was run through an alumina column, concentrated under vacuum, and the residue poured into water. The resultant pale brown precipitate was filtered off, washed with EtOH, and vacuum dried.

6-Chloro-7-methyl-9-[p-bis(chloroethyl)aminobenzylideneimino-ethyl]isoalloxazine (IIb) was prepared similarly to azomethine **IIa**. Heating time was 6 hr. The product was purified by precipitating a few times from dimethylformamide with water-EtOH. Amorphous pale brown powder.

6-Chloro-7-methyl-9-(β-acetylaminoethyl)isoalloxazine (VIIa). 0.74 g (0.002 mole) **Ia** was dissolved in 50 ml water which had been made alkaline (pH 8). 0.3 g (0.0025 mole) Ac_2O was added to the solution, and the mixture kept at 40° for 30 min. The yellow precipitate was filtered off, washed with water, and crystallized from AcOH, yield 85%.

6-Chloro-7-methyl-9-(β-benzoylaminoethyl)isoalloxazine (IIIa). 0.25 ml (0.0028 mole) Et_3N was added to a mixture of 0.5 g (0.0014 mole) **Ia** and 30 ml dimethylformamide. 0.24 g (0.0017 mole) benzoyl chloride was gradually added, then the mixture heated for 1 1/2 hr on a steam bath, the products vacuum concentrated to half volume, and then poured into water. The orange precipitate was filtered off, washed with EtOH, and recrystallized from 70% AcOH.

6-Chloro-7-methyl-9-[p-bis(β-chloroethyl)aminobenzoylamino-ethyl isoalloxazine (IIIb). 1.9 g (0.073 mole) p-bis(β-chloroethyl)aminobenzoyl chloride and 1.25 ml (0.01 mole) Et_3N were added to a solution of 1.5 g (0.0049 mole) **Ia** in 70 ml dimethylformamide, and the whole stirred for 2 hr at 75°-80°. The products were vacuum evaporated, and the residue poured into EtOH. The precipitate was filtered off and washed with hot EtOH, orange crystals ex 70% AcOH.

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