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Synthesis of Alloxazine Derivatives by Dealkylation of Isoalloxazines at N-10

by

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Summary. Refluxing of isoalloxazines in several solvents and also in urea leads to dealkylation at N–10, allowing a simple synthesis of alloxazines suitably substituted at N–3. Best results were obtained by heating isoalloxazines in the urea–dimethylformamide mixture and this method was successfully applied for the synthesis of 3-undecyllumichrome.

Syntheses of alloxazines substituted at N-3 *via* condensation of *o*-diamines with suitably substituted alloxane [1,2] are handicapped by the unavailability of the latter compounds. As an alternative N-3 alkylation of isoalloxazines and subsequent dealkylation at N-10 can be proposed providing that a suitable method is known (direct alkylation of alloxazines leads to a mixture of derivatives mono- and disubstituted at the N-1 and N-3 positions [3]).

Partial N-10 dealkylation of isoalloxazine derivatives was reported when refluxed in acetic acid containing hydroxylamine sulfate [3, 4], sodium [5] or mercury acetate [4] or in the presence of benzyltrimethylammonium hydroxide [6] and *tert*-butyl hydroxide in dimethylformamide (DMF) [7], during syntheses [8] or alkylation of isoalloxazines [9]. One of these reactions was claimed to be restricted to N-10 methyl [6] and another to C-8 non methyl (H or Cl [7]) isoalloxazine derivatives.

Using 3, 7, 8, 10-tetramethylisoalloxazine as a model compound it has been established that refluxing in acetic acid alone is more efficient in promoting

N-10 dealkylation than when containing the recommended [3–5] additives. Using other solvents of relatively high boiling temperatures, it has been found that N-10 demethylation efficiency similar to that obtained with acetic acid (about 25% in 24 h) can also be achieved with acetic acid anhydride, butyronitrile, DMF, benzonitrile and nitrobenzene (in the last three solvents many transients and/or by-products were formed). In different batches of solvents under standard conditions the reaction was found to proceed with variable efficiency. Attempts to recognize the contaminants or the decomposition products (e.g. aldehydes, amines, peroxides) responsible for these effects, were unsuccessful. No demethylation was observed in pyridine, halogenated benzene derivatives, 1,4-dioxane, 1-butanol or pentanols. In search of proper reactants also inorganic salts (e.g. $Mg(NO_3)_2$) and organic solids (e.g. urea) of proper melting temperatures were checked. In general, it has been found that the dealkylation at N-10 proceeds in the presence of oxygen, at elevated temperatures, in many media of relatively low polarity but not in the presence of strong acids. Under strongly acidic conditions the reaction proceeds very slowly and always *via* transient structures of higher and lower polarity (TLC) which retain the isoalloxazinic structure (characteristic UV spectra).

In preliminary experiments the best results were obtained in urea heated up to 130–150°C (urea slowly decomposes under such conditions), the reaction proceeds smoothly and no major transients or by-products were formed. Heating the isoalloxazines with an excess of urea to which 5–10% (v/v) of DMF was added (to prevent solidification), roughly 50% dealkylation efficiencies were obtained within 30 h of heating in the case of: 10-methyl-, 3,10-dimethyl-, 3,7,8,10-tetramethylisoalloxazine, riboflavin (7,8-dimethyl-10-(1-D-ribityl) isoalloxazine), 3-undecyl- and 3-octadecyl-7,8,10-trimethylisoalloxazine. No dealkylation was found for 10-ethyl-3-carboxymethyl-7,8-dimethyl- and 5-deaza-3,7,8,10-tetramethylisoalloxazine under the same conditions.

The procedure was then carefully optimized for the synthesis of the alloxazine derivatives with long chain substituents (undecyl-, octadecyl-) at N-3 used in studies on the excited state proton transfer in solutions [10], solid state [11] and in reversed micelles. The following procedure is proposed for routine use: 7,8,10-trimethylisoalloxazine is alkylated in position N-3 using undecylbromide (octadecylbromide, methylbromide, etc.) under conditions reported by Hemmerich *et al.* [3]. The recrystallized product (about 30 mg) is placed in a test tube, 3 g of urea and 0.5 cm^3 of DMF is added and heated to 130–140°C for about 30 h. The reaction mixture in the open test tube is occasionally stirred with a glass rod which is also used to take control samples at regular intervals. The reaction is monitored by TLC on silica gel H (Merck) using the solvent mixture: chloroform–2-butanone (14:1, v/v). The spots are located by their fluorescence, yellow for isoalloxazine, blue for alloxazine derivatives (the

latter identified finally by their phototautomeric yellow fluorescence when acetic acid or pyridine is applied on the spot [10]).

In the synthesis of 3-undecylderivative, the following standard R_f values are observed on TLC; 0.78 for 3-undecyl-7,8-dimethylalloxazine, 0.51 for 3-undecyl-7,8,10-trimethylisoalloxazine and 0.63 for an unidentified (orange-yellow fluorescent) product.

When more than 50% of the substrate is demethylated the mixture is taken out and dispersed/dissolved in water, extracted with chloroform, the organic phase washed with water, dried and evaporated to dryness. The mixture is then dissolved in chloroform–2-butanone (14:1, v/v) and separated on a silica gel column (15 cm bed length, 1.5 cm diameter, Kieselgel–Mallincrodt, Serva, 100 mesh). The first blue fluorescent fraction is collected and solvent evaporated. The 3-undecyl-7,8-dimethylalloxazine is recrystallized from ethanol–water mixture (1:1, v/v). The average yields of the two reaction steps are: 85% for alkylation at N-3, 48% for demethylation at N-10.

Analogous results are obtained in the syntheses of 3-octadecyl-7,8-dimethylalloxazine and 3,7,8-trimethylalloxazine [12]. Work is in progress to elucidate the mechanism of N-10 dealkylation of isoalloxazines.

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