Reactions of carboline

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Abstract. The reactions of carboline under various oxidative conditions are reported. The soft aerobic radiolysis, hydroxylation and rearrangement transformations are studied using isotopic labelling and GC–MS techniques.

Keywords: Soft radiolysis of indolic alkaloid, oxidations of indolic alkaloid

1. Introduction

The oxidative transformation of indole alkaloids, belonging to the tetrahydro-beta-carboline (**1**) family, under various biological, biomimetical or chemical conditions was extensively studied by our group [1–6]. The parent compound **1** easily underwent a series of monohydroxylation with Cytochrom P 450 [6], as well as with Gif [1–4] or FeII/FeIII red-ox systems [5,6]. Although the yields of hydroxylation are generally low, numerous interesting side products, e.g., keto derivatives, diols, etc., are obtained. In all reactions studied an important level of autooxidation of alkaloids as well as several coupling reactions were observed. In this study we report a soft gamma radiolysis of **1** under different aerobic conditions, its Barton's photolysis in presence of N-hydroxypyridone-2-thione (**2**) [7–9], together with its lead tetraacetate oxidation. This last reaction, proceeding via the oxidation of 7a carbon of indole alkaloids to the O-acetate (**3**), lead through a rearrangement of the skeleton to an oxindole lactam **4b** or according to Finch scheme [10] to the indolinone (**4a**). Both reactions should be considered as possible under the biomimetic oxidation of **1** framework.

The application of radiolysis to alkaloids, or to the natural products in general, is a very appealing way to achieve their transformation. However, although unusual, the radiolysis is a highly unspecific process which can enhance several unsuitable and unexpected reactions as well as it can lead to an extremely complex post-reaction mixtures of products. The behavior of natural products under soft radiolysis remains a fascinated and relatively unexplored field.

All reactions presented here were carefully monitored by the GC–MS technique, the identification of tetrahydro-beta-carboline derivatives were confirmed by comparison with available compounds coming from our previous synthesis and by selected deuterium labelling experiments.

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2. Results and discussion

Tetrahydrocarboline (**1a**) irradiated with a visible light in the presence of **2** in aromatic solvent under aerobic conditions (benzene or chlorobenzene) produce, as expected, three disulphides issuing from the disproportionation of intermediate sulphide radicals (**8a**, **8b** and **8c**; total yield of 18.7%) [8,9], as well as two unseparated C-4 and C-6 substituted thiopyridylcarboline (**7d**) (m/z 335) obtained in a 2:1 ratio, with a total yield of 1.8%. The absence of higher yield of these compounds and of three hydroxycarbolines (**7a**, 0.8%) under these conditions could be explained by the small yield of $\text{OH}\cdot$ radical and by the fact that these radicals could abstract hydrogen from the methylenes especially when these are activated by heteroatom and then allow the carboline-S-thiopyridyl coupling reactions.

It is worthy to note that the abstraction of labile hydrogen atom by the $\text{OH}\cdot$ generated by cleavage of **2** is much more efficient when the substrate is at the same time the reaction solvent [8,9]. These conditions cannot be achieved when a high molecular weight substrates are used, as it was in our case (Figs 1 and 2).

When the same reaction, under visible light, is performed under anaerobic conditions (under N_2 atmosphere) in a presence of **2**, the carboline **1a** underwent the same kind of transformation. The

Fig. 1. (i) MeO^- , (ii) H^+ , (iii) chlorobenzene, radiolysis, (iv) chlorobenzene, radiolysis, N-hydroxythiopyridone, (v) N-hydroxy thiopyridone, sunlight or tungsten lamp, dichloromethane or pyridine, (vi) TEMPO, dichloromethane, (vii) cumylperoxide, dichloromethane, (viii) $\text{Co}(\text{AcAc})_3$, toluene, oxygen.

product profile remains the same with a reduction, by half, of hydroxylation products yield. This reduction could be related to two reasons, the autooxidation of indole alkaloid as well as to the influence of atmospheric dioxygen on the oxidation reaction level. Although tempting, the difference in the isolated product yields under aerobic–anaerobic condition shift cannot be unambiguously, because of a very small yield of the 7a products, related to the atmospheric oxygen presence.

The aerobic radiolysis of 1a under Co bomb gamma rays (150 min) conditions in chlorobenzene was then performed with and without presence of 2. The radiolysis of 1 in chlorobenzene alone mainly lead to formation of three major compounds chlorodiphenyl, dichlorodiphenyl and diphenyl, 6a–6c, respectively, as well as to smaller quantities of the dibenzene dimer (6d) and to a small quantity of hydroxycarboline (7a), chlorophenylcarboline (7c), dihydroxycarboline (7a) and phenylcarboline (7c), respectively, with following yields: 2.7, 1.9, 3.1 and 2.3%, expressed as a percentage of transformation of the substrate 1a. The position of substitution of these radicals on the carboline ring was not rigorously established, however the usually activated position of alpha to N₅ and benzylic carbon C₇ are the preferred target of these reactions.

When this radiolysis is performed in the presence of 2 three groups of products were obtained. The two most abundant groups corresponded to chlorobenzene radiolysis products (ca. 45% of the mixture of 6a–6d compounds mentioned above; yields of 2.3, 27.0, 2.1 and 13.7%); and to three thiopyridone originated dimers (8a–8c) in the yields range of 40% of transformation of compound 2 and the ratio of 3 : 2 : 2 of compounds 8a : 8b : 8c. The last group of products containing the carboline moiety showed in order of decreasing abundance: S-2-pyridyloxycarboline (3.1%), chlorophenylcarboline (2.6%),

Fig. 2.

S-2-pyridylcarboline (1.9%), hydroxycarbolines (1.7%) and traces of dihydroxy (1.1%), chloro (0.9%) and phenyl (0.7%) carbolines. Comparing these last two oxidations the addition of **2** has increased the relative yield of hydroxycarbolines (**7a**) by a factor of two and in general has activated the carboline coupling reaction. On the other hand the addition of thiols to methylenes seems to be catalysed by the dioxygen, because under anaerobic conditions the autooxidation of indole alkaloids under radiolysis was slightly lowered [12–15].

Although the structure of isomeric hydroxy carbolines **7a** and S-pyridylcarbolines **7d** was not precisely established, it seems, from the isotopic labelling experiments and from the fragmentation of these compounds under mass spectrometry electronic impact conditions, that the S-pyridyl radicals are placed on the alicyclic part of the carboline **1a** alpha to nitrogen N₅ position, and that the major hydroxylation takes place on the C₆ of the **1** as well. At this moment there is no evidence for the addition of the ortho thiopyridyl radical to the pyrrolic double bond of the indole cycle.

The radiolysis of **2** alone in chlorobenzene leads to obtaining seven identified products, with a total yield of 7%, which were in order of decreasing abundance dithiopyridine dimer (1.9%) followed by S-phenyl 2-thiopyridine (1.8%), chlorodiphenyl (1.3%), chlorohydroxydiphenyl (0.6%), bis-diphenylether (0.45 %), dichlorodiphenyl (0.4%) and diphenyl (0.1%) as well as six unidentified minor reaction products.

The radiolysis described here has certainly improved the yield of the oxidation products of this alkaloid, probably due to the hydroxylation via the OH· radical. However, the necessary use of high boiling point solvent (chlorobenzene) induced several secondary reactions of this fragile molecule. Radiolysis remains a very uncontrolled and chaotic reaction and further optimisation of reaction conditions is difficult because of a still limited access to the experimental reactors, a little say in choosing the experimental conditions and the generally speaking high cost of these experiments. Despite these factors and because of the important biological role of indolic compounds, the study of the behavior of this moiety under radiolysis is both interesting and necessary.

Most popular chemical oxidants are readily oxidizing the indole ring. In order to complete this study, the search for rearrangement reaction products from **1** under different oxidative conditions was performed. These products could be present in many biomimetic oxidation reactions, however, they have never been detected yet in post-oxidative mixtures. Among the others, the fragility of the indolic aromatic ring under oxidation justified this rearrangement. The lead tetraacetate oxidation of carboline **1a**, according to the Finch procedure [10,11,16,17] developed for more substituted indole alkaloids is leading to expected beta-acetoxyindoleine (**3a**). This compound when treated in mild acidic or basic (e.g., sodium methoxide) conditions should rearrange to oxindole lactam (**4b**) or to the indolinone (**4a**) skeleton systems. The rearrangement of **3a** under basic conditions led to obtaining the oxidised product at m/z 242 which was identified as indolinone **4a** (m/z 130, 96, etc.).

The rearrangement of **3a** under acidic conditions led to obtaining two products, one being de-protected, beta hydroxy carboline **3b**, the second one underwent the rearrangement to the oxindole displaying the lactam bond (m/z 241 M–H, 149, 97, etc.). When the crude mixture of the lead tetraacetate oxidation of **1a** is treated with NaBD₄ according to the Gribble procedure [18–20] the intriguing isomeric compound is obtained. This compound is displaying characteristic M–D⁺ ion at m/z 225 (100%). During the carboline **1** synthesis, when reaction mixture is treated with NaBD₄ instead of NaBH₄, the carboline is monodeuterated in position 4 (m/z 227 M⁺), displaying the 100% ion at m/z 226. The same reaction carried out in deuterated methanol (CH₃OD) is producing a triple deuterated product (on the indolic N, C₃ and C₄) which is displaying a 100% ion at m/z 228. One of these deuterium atoms (N–D) is easily reexchanged with an addition of the methanol.

In order to investigate the intermediates of the lead tetraacetate oxidation of 1a the reduction of the intermediate 3a, with either sodium borohydride or lithium aluminium hydride was performed. When this compound is reacted with lithium aluminium deuteride the monodeuterated 12b-d₁ carboline 1a formation is observed. This result means that the lead tetraacetate oxidation of 1a could proceed via the immonium 5a intermediate as well. In a similar manner the presence of the intermediate 5b was confirmed (M⁺ ion at 224, didehydrogenated carboline, followed by an M-H⁺ ion at 223) during the carboline synthesis. The addition of a CH₃O-ion to the imine N₅-C_{12a} intermediate was not observed. The results obtained from deuteration experiments on both intermediates (5 and 6) enabled to better characterise the enamine N₅-C₄=C₃, azadiene and imine N₅-C_{12a} systems present in the C/D rings junction proximity of this tetracyclic alkaloid according to the intermediates discussed by Barton [7, 17] and postulated by Gribble [18–20]. It is finally interesting to notice that both rearranged carboline derivatives, both bearing the spiro C ring, were not detected in various biomimetic (e.g., Gif IV, Gif-Orsay, Fenton, picolinate FeII) as well as in the cytochrome P450 and the radiolytic oxidations of 1a reported in this paper [5,6].

In order to evaluate the radical formation capacity of several methylenes of the carboline the alkaloid was reacted with TEMPO. This reagent, as the natural source of the radicals, can perform the hydrogen abstraction as well as when used in excess, which was the case, it can form the 2,2,6,6-tetramethyl-1-piperidinyloxy substituted products and eventually under aerobic conditions the oxidised compounds as well. The use of TEMPO is sometimes performed in order to evaluate the capacity of specific substrate molecule to give the radicals via the hydrogen abstraction and to mark the most active methylenes [21]. When the carboline 1a is treated with TEMPO in methylene chloride solution and worked up with water the formation of approximately 0.01% of hydroxycarbolines 7a trace quantities were observed.

One more attempt was performed to form the hydroxy carbolines and oxidise chemically this alkaloid. The CoIII-acetylacetonate oxidation of 1 in toluene and constant oxygen flow for 12 h is leading to several products. In this reaction, for instance [22], the allylic hydroxylation and allylic ketonisation of the cyclohexene system together with four vinylic compounds formation was observed: the epoxidation and the rearrangement of this epoxide to the ketone which in the case of carboline (1) should lead to either aminoketone or spiro lactame rearranged products. The last expected product in this reaction is the simple hydroxylation on either of two vinylic carbons of the alpha, beta indolic double bond usually absent in the indolic oxidation, except in the lead tetraacetate oxidation previously described in this paper. The clear identification of hydroxycarbolines (7a), representing 17% of transformed starting material together with benzyl alcohol originated from the allylic hydroxylation of the solvent (toluene) as well as a small quantity of the ketocarboline (7f) was confirmed. However, the cobalt III acetylacetonate catalysed oxidation of the model cyclohexene has led respectively to a major fraction corresponding to the allylic ketonisation (90%) followed by cyclohexanone, hydroxycyclohexene and 1,2-epoxycyclohexene (total yield of these three compounds 7%) and some cyclohexanol and cyclopentyl carboxylic acid (3%). In this respect, our results, when combined to an overwhelming quantity of benzyl alcohol somewhat overlooked by the Mexican report, are giving a more complete picture of this reaction [22,23] analysed with more rigorous control by the GC-MS technique (Fig. 3).

The last attempted experiment was the cumene hydroperoxide oxidation of 1a in a methylene chloride solution, leading to traces of hydroxylation of the alkaloid, probably due to the autooxidation and the absence of both cumenoxyl-alkaloid coupling products or the addition of cumenoxyl radical to the indolic double bond expected.

Fig. 3. Model reaction.

3. Experimental

All products and reagents were purchased from Coger Company; most of them originated from Aldrich Chemicals or affiliated suppliers.

3.1. GC–MS identification of reaction mixtures

The gas chromatography–mass spectrometric analysis of reaction mixtures for most of the reaction involving the alkaloids and the model oxidation reactions were carried out on a Nermag R-10-10 mass spectrometer (electron impact, positive ions, 70 eV) coupled to a Delsi DC 700 gas chromatograph using CP SIL 50m ID 0.3u column and the appropriate temperature programming.

3.2. Photolysis of 1a in presence of 2

The reaction was carried out as 10% chlorobenzene solution of the alkaloid using the UV Hanovia 1L photochemical reactor lamp, 100 W for 10 h or sunlight for two months. The ratio of 1a to 2 products was ca. 3:1. The whole reaction mixture was analysed by GC–MS. The overall reaction yield referred to the starting alkaloid conversion was 17 and 9%, respectively.

The same reaction carried out in benzene or in methylene chloride led to the same product profile.

3.3. Anaerobic photolysis of 1a in the presence of 2

The photolysis of 1a under N_2 atmosphere was carried out as in the previous case using the UV reactor for 10 h. The reaction mixture was analysed by the GC–MS (11% of conversion of the alkaloid).

3.4. Radiolysis

The 10% solution of carboline 1a in chlorobenzene was radiolysed under aerobic conditions with Co 60 bomb (Cigal, CEA) for 150 min using 1.8 kGy h^{-1} . The mixture was cooled down and analysed by GC–MS.

- (A) Radiolysis of pure chlorobenzene witness sample yielded the mixture of four compounds: diphenyl (2.3%), chlorodiphenyl (27.1%), dichlorodiphenyl (2.1%) and dibenzene dimer (13.7%). All yields are referred to conversion of starting material.

- (B) Radiolysis of ca 10% solution of 1a in chlorobenzene resulted in ca 23% conversion of the starting alkaloid.
- (C) Radiolysis of ca 10% solution of carboline 1a in chlorobenzene in a presence of 3% of the N-hydroxythiopyridone (2) yielded in 40% transformation of the alkaloid and in ca 90% transformation of 2.

The radiolysis reaction product ratio strongly depends on the radiation dose and time, substrate concentration, evaporation of solvent during radiolysis, aerobic or anaerobic conditions, etc., and usually displays a considerable variation from one experiment to the other.

3.5. Oxidation of 1a with lead tetraacetate

This oxidation was modeled under the Finch [9,12] experimental procedures. Yield of crude 3a product: 21.2%.

3.6. Rearrangement of 3a

The reaction, carried out by refluxing product 3a in methylene chloride for 90 min according to the Finch procedures in methanolic sodium methoxide (12) yielded 4a (35%) or in diluted acetic acid yielded compound 4b (29%), respectively. Both yields are referred to the GC data and the structures were compared to the previously synthesised model alkaloids.

3.7. Deuterated tetrahydro-beta-carbolines

These products were synthesised using the deuterated reagents or solvents according to the experimental procedures of Gribble [18,19] or ourselves [20] for the carboline synthesis; the 4-d₁ (1b), 3,4-d₂ (1e) and 3,4,N₁₂-d₃ (1d) isomers were obtained.

3.8. Reaction of 1a with TEMPO or with cumene hydroperoxide

The equimolar methylene chloride solution of 1a and TEMPO (2,2,6,6-tetramethyl-1-piperdinyloxy radical) or cumene hydroperoxide were stirred at room temperature for 24 h. The excess of reagent was filtered and the solution mixture was washed with water and analysed by the GC-MS. The reaction yields, expressed in starting material conversion for TEMPO was 12 and 23% for cumene hydroperoxide, respectively.

3.9. Oxidation of 1a or cyclohexene with Co(AcAc)₃

The 10% solution of 1a or cyclohexene in toluene was refluxed in presence of catalytic quantities of the cobalt III acetylacetonate for 12 h under an oxygen flow. The solution was concentrated in vacuo and examined by the GC-MS. The benzyl alcohol yield, as originating from the the solvent (toluene) oxidation and being the major product of this reaction, is omitted from the reaction yield calculation. All oxidation product yields are calculated from the GC results and reported in the text to the substrate conversion.

Cyclohexene oxidation: total conversion 47%; carboline 1a oxidation: total conversion 18% (products 7a and 7f).

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