SPECIALIA

Reduction of aflatoxin B_1 with zinc borohydride: An efficient preparation of aflatoxicol[1]

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Summary. A simple and mild reduction of aflatoxin B_1, involving treatment of aflatoxin B_1 with ethereal zinc borohydride to give 57–65% yield of diastereomeric aflatoxicols, is described.

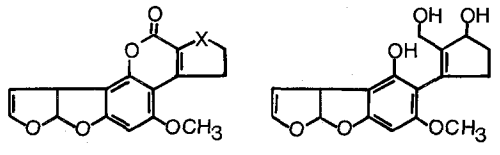
Current investigations on aflatoxin B_1 (afla B_1, **I**) have shown that the in vivo metabolism of this toxin plays a significant role for the toxicity of this potent hepatocarcinogen[2]. The only identified afla B_1 metabolite known to be produced by a soluble enzyme system is aflatoxicol (**II**) or aflatoxin R_0, in which the carbonyl group in the cyclopentenone ring of afla B_1 is reduced to a hydroxyl group[3,4]. It has been shown to be produced by the protozoan Tetrahymena pyriformis[4], the steroid-hydroxylating fungus Dactylium dendroides[3] and animal liver preparations[5]. Because of its importance in afla B_1 metabolism, synthetic aflatoxicol is needed for additional biochemical and toxicological studies. In our ongoing radioimmunological research, aflatoxicol was needed for the preparation of a protein-mycotoxin conjugate. However, both the microbial and enzymatic conversion of afla B_1 to aflatoxicol are not practical for synthetic purposes[6]. Viewing the recent success of employing zinc borohydride in the total synthesis of extremely base-sensitive prostaglandins[7], we set out to test the applicability of this mild reducing agent to the preparation of aflatoxicol.

Materials and methods. Afla B_1 was prepared according to the method of Chu[8]. ^{3}H-Afla B_1 was purchased from Moravek Biochemicals. The following materials and reagents were used: Adsorbosil 5 (Applied Science Laboratories), Silica Gel 60 F-254 thin layer chromatography (TLC) plates (Brinkmann Instruments Co.), sodium borohydride (Fisher Scientific Co.), zinc chloride (Ventron Corp.).

All organic solvents were analytical reagent grade. UV-spectra were determined in a Beckman DU spectrophotometer modified with Gilford system with 1 cm light path. Mass spectra were taken at 70 eV with a Finnegan Model 1015 mass spectrometer equipped with a direct-insertion probe and a Finnegan 6000 MS data system. Radioactivity data were obtained by counting in a Beckman Model 335 liquid scintillation spectrometer.

In a typical preparation, 6.34 mg of afla B_1 was dissolved in 3 ml of anhydrous chloroform and cooled to 0 °C. The flask was septumed and flushed with nitrogen. 10 molar equivalent of freshly prepared zinc borohydride[9] was added through a syringe. The whole content was stirred magnetically for 30 min at 0 °C. The reduction was completed after another 30 min at room temperature. Excess reagent was destroyed by dropwise addition of 0.05 N hydrochloric acid at ice-bath temperature until hydrogen gas evolution ceased. Chloroform (30 ml) was then added and the organic layer washed with water, dried over anhydrous magnesium sulfate, and filtered through a short path of Adsorbosil 5. The filtrate was evaporated to dryness to give 65% yield of chromatographically pure aflatoxicol (R_f 0.33 in ethyl acetate:chloroform/3:1).

The same procedure was used in the reduction of 100 μCi of ^{3}H-afla B_1. The diastereomers of aflatoxicols were separated by TLC on 0.25 mm plate with multiple developments. Each blue fluorescent spot was scraped from the TLC plate and mixed with 10 ml of Bray's solution[10] and subjected to counting for 10 min.



I X = >C=O

II X = >CHOH

III

Structures of aflatoxin B_1 (**I**), aflatoxicol (**II**) and trihydroxyl aflatoxin B_1 (**III**).

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Results and discussion. The synthetic aflatoxicol was found to have the same R_f-value as that of an authentic aflatoxicol standard provided to us by Professor Dennis P. H. Hsieh of University of California, Davis. The UV-spectrum was identical to the standard. The mass spectrum contained the following major ions which are consistent with the structure of alfatoxicol: m/e 314 (M^+, base peak), 313, 297, 296, 268 and 267. The fragmentation patterns for both synthetic and natural aflatoxicols were identical.

Chemical reduction of afla B_1 by sodium borohydride has been reported to give either low yield of aflatoxicol[6] or quantitative conversion to the trihydroxy derivative (**III**)[11]. These results must be related to the sensitivity of afla B_1 toward the hydroxide or ethoxide ions present in alcoholic borohydride. In the preparation of ^{3}H-aflatoxicol, essentially equal radioactivity was found for each diastereomer. Thus, a 50:50 mixture was obtained from this reduction, indicating no steric preference for the hydride attack. Therefore, our present procedure represents an efficient preparation for obtaining quantities of synthetic aflatoxicol under essentially neutral conditions. We are presently investigating the synthesis of a protein-mycotoxin conjugate starting with labeled aflatoxicol and succinic anhydride.

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Fe(II)-induced decomposition of epidioxides. A chemical model for prostaglandin E, prostacyclin and thromboxane biosynthesis

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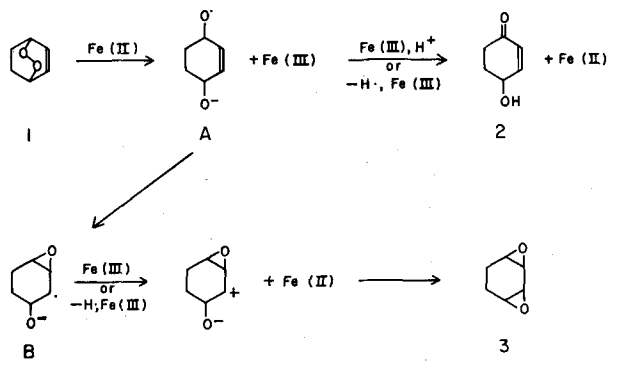
Summary. A chemical model for biosynthesis of PGE, PGX and the thromboxanes from the prostaglandin endoperoxides is presented which is based on known reactions of other endoperoxides with the Fe(**II**)-Fe(**III**) redox system in vitro.

The peroxy radical cyclization mechanism for the cyclooxygenase-mediated[2–4] conversion of arachidonic acid to the prostaglandin endoperoxides (PPG_2 and PGH_2)[5] has been supported by di-tert-butyl peroxyoxalate-initiated reactions of lipid hydroperoxides[6] and by a recent model study[7]. We now suggest that Fe(II)-induced isomerizations of epidioxides[8] provide not only a model for the transformation of PGG_2 and PGH_2 to the PGE's in vivo as already pointed out briefly by us earlier[8b], but also a model for the biosynthesis of the thromboxanes[4,9,10] and prostacyclin (PGX)[11,12]. This is in line with recent suggestions[13,14] that the in vivo fragmentation of PG-endoperoxides is probably a catalyzed process.

We have provided evidence[8] that the previously little-studied isomerization of epidioxides of type **1** to ketols **2** or diepoxides **3** by $FeSO_4$ in H_2O-THF actually involves a redox process in which the first step is reduction of **1** by Fe(II) to anion radical A (Scheme I). The latter may be oxidized by Fe(III), generated in the first step, to ketol **2** or, if a double bond is present can isomerize to B which is oxidized to **3**[15]. In appropriately constituted anion radicals A, intramolecular 1,5-hydrogen transfer from remote carbon to oxygen may intervene prior to oxidation by Fe(III)[8a,c].

The oxidations of A and B by Fe(III) can be viewed[8b] as equivalent to loss, by fragmentation, of H which is subject to oxidation by Fe(III). Such fragmentations, with loss of an isopropyl radical which migrates intra- or intermolecularly (the latter if a radical trap is introduced) or is subsequently oxidized to propylene, have been observed on treatment of ascaridole[17] or dihydroascaridole[18] with $FeSO_4$.



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