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# Reduction of Aflatoxin $B_1$ to Aflatoxicol

Aflatoxin $B_1$ was reduced to aflatoxicol (aflatoxin $R_0$) with lithium tris(*tert*-butoxy)aluminum hydride in 80% yield. The diastereomers of aflatoxicol were resolved and their ultraviolet extinction coefficients were redetermined.

Aflatoxicol (aflatoxin $R_0$) has been formed by the biological reduction of aflatoxin $B_1$. Microorganisms (Detroy and Hesseltine, 1970; Cole et al., 1972), soluble duck liver enzymes (Patterson and Roberts, 1972), and rainbow trout liver enzymes (Schoenhard, et al., 1976) have been used for the reduction. Aflatoxicol is reported to be less active than the parent aflatoxin $B_1$ in the duckling bioassay (Detroy and Hesseltine, 1970) and in a microsomal mediated-bacterial assay. Recently, we reported that the acute toxicity of aflatoxicol to rainbow trout was similar to that of aflatoxin $B_1$, but its diastereomer was less toxic (Schoenhard et al., 1974). Recent studies in our laboratories show that aflatoxicol is a carcinogen to trout similar in activity to aflatoxin $B_1$ (Schoenhard, 1974). Development of a high-yield chemical reduction of aflatoxin $B_1$ to aflatoxicol and subsequent requests by several laboratories for this method prompt this communication.

There are at least four metal hydrides which appear to be potentially satisfactory for the specific reduction of a carbonyl conjugated with a olefinic double bond. Such hydrides must be inert toward lactones and double bonds conjugated with lactones. A further restriction on the hydride is that it must retain these desired properties while present in a large excess with microgram quantities of aflatoxin. Lithium tris(*tert*-butoxy)aluminum hydride was found to be a suitable reducing agent.

## EXPERIMENTAL PROCEDURES

Thin-layer chromatographic plates were coated with MN-Kieselgel G-HR and developed with benzene–acetone–ethyl acetate (100:12:24). Under these conditions the two diastereomers of aflatoxicol separate and have $R_f$ 0.53 (natural isomer) and 0.48, while $B_1$ migrates to $R_f$ 0.24. Preparative separation of the aflatoxicol diastereomers was effected on a column packed with aluminum oxide G, type E, EM Brinkman No. 1090 (pH 7.5) with 3% added water. The sample was applied in a minimum of chloroform, and washed with 2 vol of hexane. The aflatoxicols were eluted with benzene–acetone–ethyl acetate (100:12:24), at a flow rate of 24 ml/h on a 20-mm column.

Metal hydrides are highly reactive, moisture-sensitive reagents. All glassware was oven dried and cooled to slightly above room temperature before use. Tetrahydrofuran was refluxed over and distilled from calcium hydride. Since the reaction between dilute solutions of aflatoxin $B_1$ and lithium tris(*tert*-butoxy)aluminum hydride in equimolar amounts is slow, the hydride was taken in excess. When using the hydride in excess, we found that the use of glass-stoppered flasks was a suitable substitute for a nitrogen atmosphere. The following conditions were optimized by over 40 trials.

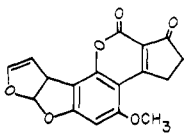
Aflatoxin $B_1$ (purchased from Calbiochem) was dried by adding benzene and evaporating under vacuum to a small volume. This procedure was carried out three times. One hundred milligrams of lithium tris(*tert*-butoxy)aluminum hydride dissolved in 10 ml of tetrahydrofuran (THF) at 0 °C was added to 10 mg of aflatoxin $B_1$ in 10 ml of THF. The reaction was held in an ice–water bath for exactly 60 min. The time–temperature relationship is extremely important. Longer times at lower temperatures give unsatisfactory results. Apparently, the desired reduction has a higher free energy of activation than the side reactions and shows a larger temperature effect.

Irrespective of the amount of hydride used, the time or temperature, there always remained a spot at $R_f$ 0.25 which may be easily mistaken for aflatoxin $B_1$. The identity of this spot has not yet been determined. The reaction mixture is quickly quenched by adding 30 ml of water containing 5 drops of 6 N HCl which should bring the pH near 7. If emulsification occurs during chloroform extraction, the pH is above 7. Yields of aflatoxicol ran as high as 80% (both diastereomers). The mass spectrum showed a strong parent at $m/e$ 314 (100%); $m^+ - 18$ ($H_2O$) = 296 (53%); $m^+ - 46$ ($H_2O + CO$) = 268 (33%); $m^+ - 47$ ($H_2O + CHO$) = 267 (18%). Copies of the mass spectrum are available from the authors upon request. The NMR

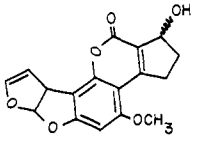
spectrum matched that reported by Detroy and Hesseltine (1970): ($CDCl_3$) δ 2.38–2.6 (m, 3 H), 3.31 (m, 2 H), 3.88 (s, 3 H), 4.80 (m, 1 H), 5.36 (m, 1 H), 5.50 (t, $J$ = 4 Hz, 1 H), 6.40 (s, 1 H), 6.48 (t, $J$ = 5 Hz, 1 H), and 6.80 (d, $J$ = 7 Hz, 1 H). The infrared spectrum has been previously reported (Schoenhard et al., 1976).

## DISCUSSION

Cole et al. (1972) report divergent extinction coefficients in the ultraviolet for the two diastereomers of aflatoxicol. The two diastereomers have identical traces in the UV, and differ only by the configuration of a saturated carbon bearing an unhindered hydroxyl. It seems unlikely that they should possess different probabilities (extinction coefficients) for identical electronic transitions. Both epimers gave maxima at 330, 260, and 253 nm, with absorbance ratios of 1.373 for $A_{330}$:$A_{260}$ and 0.881 for $A_{253}$:$A_{260}$. For the front running (natural) epimer, we measured the following extinction coefficients, $\epsilon_{max}$ 9600, 6950, and 6400, and for the slower migrating epimer, $\epsilon_{max}$ 8220, 5950, and 5460.




AFLATOXIN $B_1$




AFLATOXICOL
(AFLATOXIN $R_0$)

Secondary allylic hydroxyl functions such as the hydroxyl in aflatoxicol are readily acid labile. In 50% dioxane–phosphate buffer at 22 °C, aflatoxicol racemizes with a half-life of 18.6 h at pH 2.1. Increasing the pH suggests that the racemization is 1.67 order in acid, rather than first order as expected (Streitwieser, 1956).

Garner et al. (1972) used sodium borohydride in 2-propanol to chemically reduce aflatoxin $B_1$ to aflatoxicol, but only reported a yield of 10%. Sodium borohydride in ethanol gave us very little, if any, aflatoxicol, which in itself is not surprising since this hydride is known to reduce double bonds when conjugated with an ester grouping. It appeared that this problem could be overcome by basic hydrolysis of the lactone ring in aflatoxin $B_1$ to its sodium salt, followed by sodium borohydride reduction of the ketone. Neutralization then recloses the lactone ring. This method also failed to produce aflatoxicol, yielding nonfluorescent products.

Ashoor and Chu (1975) used sodium borohydride in 85% chloroform. The double bond resisted reduction, but both the lactone and keto groups were reduced, yielding a trihydroxy product.

Sodium tris(methoxy)borohydride performs similarly to lithium tris(*tert*-butoxy)aluminum hydride but gives slightly inferior results.

## ACKNOWLEDGMENT

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## CORRECTION

### PEROXIDASE ACTIVITY IN GOLDEN DELICIOUS APPLES AS A POSSIBLE PARAMETER OF RIPENING AND SENESCENCE

In this article by Natalio Gorin and Frouwke T. Heidema [*J. Agric. Food Chem.* **24**(1), 200 (1976)], on p 200, the last line of column 2 should read "the phosphate buffer (pH 7) (1.8 ml), 67 mmol/l. phosphate buffer (pH 7) (1.0 ml), an aqueous solution".