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THE SYNTHESIS OF [^{3}H]-INDOLE-3-CARBINOL, A NATURAL ANTI-CARCINOGEN FROM CRUCIFEROUS VEGETABLES

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SUMMARY

Indole-3-carbinol is a natural anti-carcinogen found as a glucosinolate in cruciferous vegetables such as cabbage, cauliflower and broccoli. A complete understanding of the mechanisms of anti-carcinogenesis by this dietary inhibitor requires improved insight into the disposition and metabolic fate of indole-3-carbinol *in vivo*. Such metabolic studies have been hampered by the lack of a commercial source of radiolabelled compound. This provided the main impetus for the work reported here, the synthesis of 5-[^{3}H]-indole-3-carbinol from 5-bromoindole.

KEY WORDS: Indole-3-carbinol, anti-carcinogen, cruciferous vegetables, tritium labeling.

INTRODUCTION

Cruciferous vegetables such as cabbage, cauliflower and broccoli contain a variety of naturally-occurring compounds which modulate the carcinogenic process (1-6). One such compound which has received increasing interest is indole-3-carbinol (I3C). Found as a glucosinolate (7) in cruciferous vegetables, I3C was shown a decade ago to inhibit tumorigenesis in rodents exposed to polycyclic aromatic hydrocarbons (8). More recently, I3C was found to inhibit aflatoxin B1 (AFB1)-induced hepatocarcinogenesis in rainbow trout (9) and rat (Selivonchick et al. in preparation). Further studies into the mechanism of I3C anti-carcinogenesis revealed significant alterations in the *in vivo* uptake, distribution and metabolism of AFB1 in trout, such that carcinogen-induced DNA damage in the target organ was

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attenuated (10). This inhibitory effect on in vivo DNA binding subsequently was studied in trout over a range of both AFB1 and I3C doses, providing evidence for an inhibitory response which was linearly related to I3C at low doses of inhibitor (11). These observations may be indicative of a protective effect of I3C at levels which are encountered in human diets.

While considerable effort has been devoted to clarifying the effects of I3C on carcinogen disposition in vivo, only recently has attention been focused on the in vivo disposition of I3C itself. It has been suggested that the mechanism by which dietary indoles inhibit DNA binding and tumorigenesis may be related to their potency as inducers of cytochrome P-448 monooxygenases (4), perhaps through the involvement of condensation products of I3C formed in the acid conditions of the stomach (12). After treatment in vitro with 0.05 M hydrochloric acid, I3C forms a series of linear and cyclic dimers, trimers and tetramers (12), some of which resemble 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in shape and molecular dimensions. Like TCDD, several of these condensation products bind to the Ah receptor protein and induce hepatic monooxygenase activity (12).

To date, these induction studies have been reported only for the rat, and have been limited to studies of I3C acid-condensation products generated in vitro. Since I3C is not available commercially in radiolabelled form, only limited information is available on the metabolic fate of I3C in vivo (13). This report describes the synthesis of 5-[^{3}H]-I3C, for use in studies on the in vivo disposition of this dietary anti-carcinogen.

EXPERIMENTAL

MATERIALS AND METHODS

Radioactivity determinations were conducted using a Beckman LS7500 scintillation counter. Scintillation fluids (OCS, ACS) were from Amersham

(Arlington Heights, IL). Thin layer chromatography (TLC) was performed on commercially prepared Silica Gel 60 aluminium sheets (0.2 mm; E. Merck, Darmstadt), and plates were analyzed using a Berthold Varian Aerograph 4-pi TLC scanner. High-pressure liquid chromatography (HPLC) was performed with a Shimadzu SPD-6AV UV-VIS spectrophotometer detector and two LC-6A pumps linked to an SCL-6A system controller and a Beckman 171 Radioisotope detector/110B solvent delivery module. Samples of labelled or unlabelled I3C in 10% acetonitrile/0.33mM potassium acetate buffer, pH 5 ("buffer") were subjected to HPLC on a Nova-Pak C_{18} reverse-phase column (Waters Assoc., 3.9 mm X 15 cm). HPLC was performed under the following conditions: 10% acetonitrile in buffer to 55% acetonitrile in buffer using a linear gradient over 25 min, maintained at 55% acetonitrile in buffer for 10 min before returning to 10% acetonitrile in buffer over a 5 min linear gradient. The flow rate was maintained at 0.8 ml/min throughout the program. Unlabelled I3C was purchased from two sources (Sigma Chemical Company, St. Louis, MO and Aldrich Chemical Company, Milwaukee, WI). All other chemicals and reagents were of purest grade available and from sources described previously (11).

SYNTHESIS

a) Synthesis of 5-bromoindole-3-carbinol

The synthetic pathway for preparing 5-[^{3}H]-I3C from 5-bromoindole is presented in Figure 1. 5-Bromoindole-3-carboxaldehyde (2) was synthesized from 5-bromoindole (1) according to the methods of Noland & Reich (14) and James & Snyder (15). 5-Bromoindole-3-carbinol (3) was synthesised from 5-bromoindole-3-carboxaldehyde by the method of Silverstein et al. (16) with the following modifications. 5-Bromoindole-3-carboxaldehyde (6.0 g, 0.027 mol) was added to 90 ml of methanol and the mixture was stirred for 5 min. With rapid stirring, powdered anhydrous sodium borohydride (1.5 g) was added directly to the mixture in small increments. The solution was

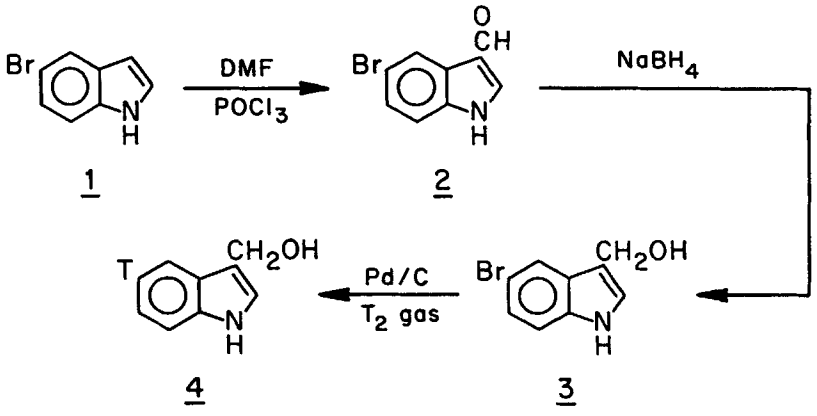

Figure 1. Route of synthesis of 5-[^{3}H]-indole-3-carbinol (4).

stirred for a further 60 min prior to dilution with 100 ml water. After 5 min an additional 750 ml water was added, resulting in a white flocculent mixture. The solid was collected by filtration and dried under vacuum at room temperature. Yield: 4.5 g (75%), small white needles, mp 122.5°C; λ max(MeOH) 287.8 ($\log_\epsilon$ 4.38), 281.0(4.37), 297.1 inflection(4.25); TLC (silica 60)- ethyl acetate/chloroform (1:1) Rf of product 0.40 vs I3C, 0.45. Mass spectrum; m/z(% intensity): 227(41), 225(44), 210(62), 208(61), 145(22), 144(31), 129(52), 128(79), 117(100), 116(33) 102(28), 101(33), 90(35), 89(63).

b) Synthesis of 5-[^{3}H]-I3C from 5-bromoindole-3-carbinol

5-Bromoindole-3-carbinol was converted to 5-[^{3}H]-I3C as follows: potassium hydroxide (20 mg) was dissolved in 1.5 ml methanol, and 8 mg palladium on activated carbon (10% Pd/C, dried at 60°C) was added to the solution. To the latter was added 5-bromoindole-3-carbinol (30 mg, 10.6 mmol). After air evacuation, tritium (10 Ci)/hydrogen gas (Amersham) was introduced at 12-15 psi and the mixture was stirred for 60 min at room temperature. Pd/C was removed by filtration and the reaction mixture diluted with an equal volume of distilled water prior to extraction (twice) with 1.7 parts diethyl ether peroxide-free. The ether fractions

were combined and taken to dryness at room temperature under a stream of nitrogen gas in order to recover the product, 5-[^{3}H]-I3C.

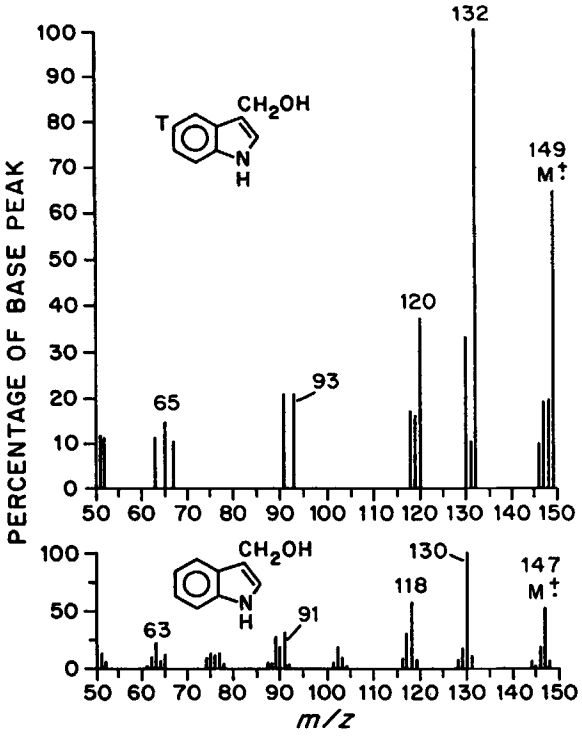

Figure 2. Mass spectra of (top) 5-[^{3}H]-I3C (4) and (below) unlabelled indole-3-carbinol.

ANALYSES OF 5-[^{3}H]-I3C

Yield: 17.0-18.5 mg (85-90%). Specific activity: 21.14 Ci/mmole (21.14 X [2.07 X 10^{19} atoms per Ci/6.02 X 10^{20} molecules per mM] X 100 = ~73% of the molecules labelled for I3C labelled exclusively in the 5-position). The 5-[^{3}H]-I3C product is a white flaky solid, stable in 95% ethanol when stored under nitrogen at -20°C protected from light, which gradually turns orange if left at room temperature or if exposed to air. Radiochemical/chemical purity: The 5-[^{3}H]-I3C was shown to be >99% radiochemically pure, giving a single radioactive peak following HPLC

(retention time 5.15 min), and a single spot following TLC (Berthold TLC scanner). Rf value and UV spectra for 5-[^{3}H]-I3C corresponded with unlabelled I3C purchased from Aldrich and Sigma. Mass spectrum of 5-[^{3}H]-I3C; m/z(% intensity): 149(65), 148(20), 147(19), 132(100), 130(34), 120(38), 118(17), 93(21), 91(21). For comparative purposes, the mass spectrum of 5-[^{3}H]-I3C is presented in Figure 2 together with that of unlabelled I3C purchased from Aldrich.

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