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Simple Synthesis of Deuterated Pterosines

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Abstract: Ptaquiloside, a potent carcinogen present in bracken fern, a plant consumed by farm animals, may be detected in traces by converting it into the bromopterosine. A simple synthesis of bromopterosine d_2, to be used as standard in GC/MS or LC/MS analyses, is described.

Keywords: Deuterated compounds, pterosines, ptaquiloside, sesquiterpene glucoside

Ptaquiloside (Pta, **I**) is a sesquiterpene glucoside, isolated from a variety of bracken fern (*Pteridium aquilinum*, var. *latiusculum*), whose structure was fully elucidated in 1987.[1] The same substance and several analogs have been identified in other *pteridacae*.[2] Bracken fern is a plant widely distributed all over the world, and it is currently consumed by farm animals. Because Pta is a potent carcinogen, the consumption of fern causes a number of well-known syndromes.[3] In large ruminants, chronic enzootic haematuria, the clinical expression of multiple neoplasia of the urinary bladder, occurs. Pta has been shown also in laboratory experiments to be carcinogenic,[4] and it seems to act synergistically with bovine papilloma virus type 2 (BPV-2), a well-known virus associated with neoplastic pathology of the bovine urinary bladder.[5] These adverse properties of Pta have been related to its electrophilic nature and its capability to react with (alkylate)[6] DNA.

The growing public awareness of the risk implied in continuous exposure to carcinogenic substances present in foods has prompted some researchers to

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evaluate the possibility that Pta may represent a pollutant of foods derived from cattle.[7] Methods of detection of this substance at ppb level are changelling: Pta (I), a glucoside, is unstable in both acid and basic conditions. In basic conditions, Pta undergoes a β-elimination, affording the dienone(II), which is converted by acids into pterosin B (Ptb, III)[8] (Fig. 1).

These difficulties have been overcome by converting Pta into the stable Ptb during sample preparation. Detection and quantitative determinations of this product (an aromatic ketone) are currently achieved using high pressure liquid chromatography (HPLC)[9] with ultra violet (UV) detection. To our knowledge, no methods that directly detect the Pta have been published.

The wide interest in this substance, considered a potential dangerous pollutant of some foods (i.e., milk, cheese, meat), prompted us to study the whole analytical problem with the aim to use MS in detecting traces of ptaquiloside. We found[10] that Ptb can be revealed by gas chromatography/mass spectrometry (GC/MS), and ptaquiloside may be converted in a bromopterosine during sample preparation. Bromopterosine may be detected by GC/MS as whitness of the presence of ptaquiloside: this procedure appears to be more reliable than those based on the identification of Ptb, a possible decay product of Pta in body fluids. Pterosines are naturally occurring indanones, isolated from plants and certain fungi.[11]

Quantitative analyses by GC/MS require standards, and to this purpose the best substances are deuterated analogs of analytes. Then we decided to prepare deuterated Ptb and bromopterosine: a straightward synthesis of these substances is reported here.

Several synthetic routes have been proposed since 1974 to prepare pterosine derivatives.[12] The benzene moieties with appropriate substituents are the starting material to add the (condensed) five-terms ring, and inter alia, the use of a methacrylic ester in a one-step reaction—PPA as condensing agent—has been reported.[13] This route seemed to be suitable for a synthesis

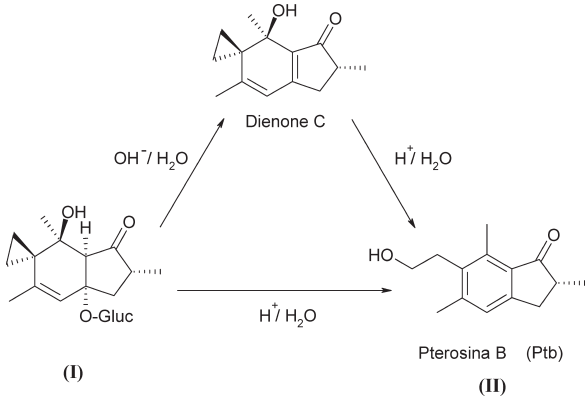
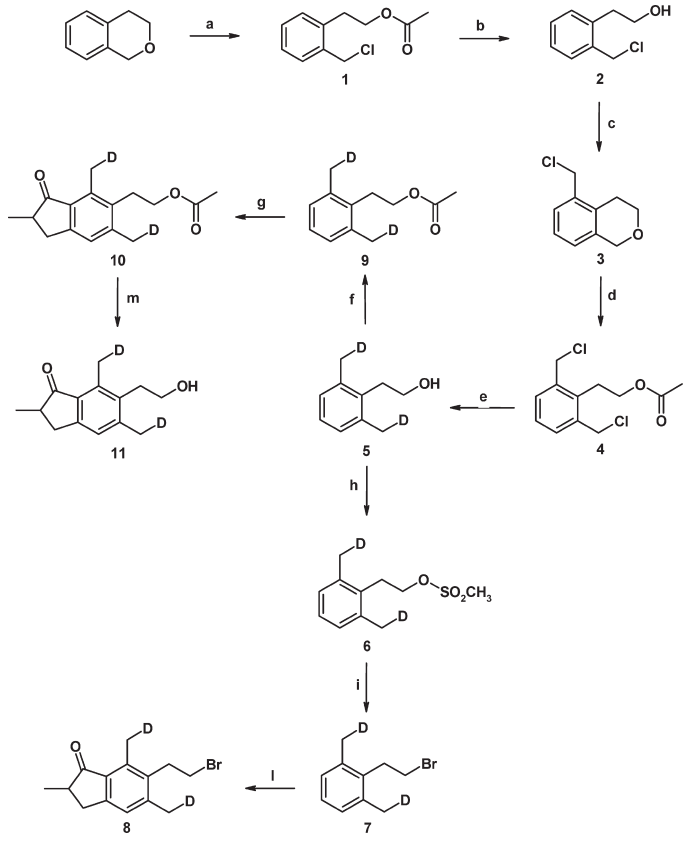


Figure 1. Instability of Pta (I) in acid and basic conditions.

of deuterate analogs: its drawback was the very low yield reported for the last step (15%). We succedeed in modifying this reaction by using the anhydride of methacrilic acid (commercially available) instead of the ester and by carring out the reaction at room temperature for 5 days. Yields are fairly good ($>80\%$) in these conditions.

The synthetic route of deuterated substrates is reported in Scheme 1. All the substances isolated gave satisfactory NMR spectra, and pure bromopterosine-d_2 is now currently used in our laboratories for analytical purposes. In the



Scheme 1. (a) Acetyl chloride, $ZnCl_2$, CH_2Cl_2, 1 h, room temperature, yield 100%; (b) HCl 37%, CH_3OH, 1 night, room temperature, yield 100%; (c) paraformaldheide, HCl 37%, 2 h, T = 60 °C, yield 87%; (d) acetyl chloride, $ZnCl_2$, CH_2Cl_2, 4 h, reflux, yield 100%; (e) lithium alluminium deuteride, THF, 1 h, room temperature, yield 100%; (f) acetyl chloride, CH_2Cl_2, pyridine, 1 night, room temperature, yield 75%; (g) PPA, metacrilic anidride, 5 days, room temperature, yield 86%, (h) MsCl, Et_3NH, DMAP, CH_2Cl_2, room temperature, yield 86.2%; (i) lithium bromide, acetone, 1 night, reflux, yield 90%; (l) PPA, metacrilic anidride, 5 days, room temperature, yield 82%; (m) HCl 37%, CH_3OH, 1 night, room temperature, yield 100%.

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experimental section, the reactions not yet reported in literature are described (Scheme 1).

EXPERIMENTAL

Chemistry

Melting points were determined with a Buchi 530 capillary apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Spectrum-One spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AC 400 spectrometer, using tetramethylsilane (Me_4Si) as an internal standard. All compounds were routinely checked by thin-layer chromatography (TLC) and ^{1}H NMR. TLC was performed by using aluminium-baked silica-gel plates (Fluka F_{254}) and aluminium-baked aluminium oxide plates (Fluka F_{254}). Concentration of solutions after reactions and extractions involved the use of a rotatory evaporator operating at a reduced pressure of approximately 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Mass spectra were recorded with gascromatography HP 5890 supplied with mass spectrometer (MS) HP 5971A at 70 eV.

2-(2-Chloromethylphenyl)-ethanol acetate (1)[13]

This compound was prepared as reported in Reference 13.

2-(2-Chloromethyl-phenyl)-ethanol (2)

To a solution of compound **1** (23.5 mmol, 5.0 g) in methanol (35 ml), HCl 37% (1.5 ml) was added. The solution was stirred at room temperature for 1 night. Water was added, and the resulted suspension was extracted with chloroform (3 × 50 mL). The organic extracts were collected, washed with brine (3 × 100 mL), and dried. Evaporation of the solvent gave 4.58 g of compound **2** (yield 100%). IR: ν 3325 cm^{-1} (OH); ^{1}H NMR ($CDCl_3$): δ3.08 (t, 2H, CH_2), 3.97 (t, 2H, CH_2), 4.7 (s, 2H, CH_2), 7.28–7.43 (m, 4H, benzene H). ^{1}H NMR ($CDCl_3$) δ3.00 (t, 2H, CH_2), 4.09 (t, 2H, CH_2), 4.64 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 7.04 (m, 1H, benzene H), 7.23–7.28 (m, 2H, benzene H).

5-chloromethyl Isochromane (3)[13]

This compound was prepared as reported in Reference 13.

2-(2,6-Bis-chloromethylphenyl) Ethanol Acetate (4)[13]

This compound was prepared as reported in Reference 13.

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2(2,6-Diethyl-phenyl)-ethanol-d_2 (5)

A solution of compound **4** (11.5 mmol, 3.0 g) in anhydrous tetrahydrofuran (8.0 ml) was slowly added to a suspension of lithium aluminum deuteride (34.5 mmol, 1.45 g) in anhydrous tetrahydrofuran (11.0 ml). The reaction was stirred at room temperature for 1 h and diluted with water. The suspension was filtered, and the residue was washed with THF. The organic solvent was evaporated, and then the acqueous layer was extracted with chloroform. The organic extracts were collected, washed with brine, and dried. The evaporation of the solvent gave 1.75 g of compound **5** (yield 100%). IR: ν 3328 cm^{-1} (OH); ^1H NMR ($CDCl_3$): δ 2.41 (m, 4H, CH_2), 3.03 (t, 2H, CH_2), 3.82 (t, 2H, CH_2), 7.09 (m, 3H, benzene H).

Methanesulfonic Acid 2-(2,6-Diethyl-phenyl)-ethyl Ester-d_2 (6)

A solution of methanesulfonyl chloride (5.99 mmol, 0.46 ml) in dichloromethane (31.5 ml) was slowly added to a solution of compound **5** (0.9 g, 5.99 mmol), triethylamine (12.0 mmol, 1.6 ml), and a catalytic amount of DMAP in dichloromethane (31.5 ml). The reaction was stirred at room temperature for 1 night. Water was added, and the suspension was extracted with chloroform. The organic extracts were collected, washed with brine, and dried. The evaporation of the solvent gave the crude product (1.10 g), which was chromatographed on a silica-gel column (ethyl acetate/n-hexane, 1:2, as eluent) to obtain 0.72 g of compound **6** (yield 86.2%). IR: See inter alia ν 1351 cm^{-1} (SO_2); ^1H NMR ($CDCl_3$): δ 2.41 (m, 4H, CH_2), 2.93 (s, 3H, CH_3), 3.21 (t, 2H, CH_2), 4.35 (t, 2H, CH_2), 7.08–7.14 (m, 3H, benzene H).

2-(2-Bromo-ethyl)-1,3-diethyl-benzene-d_2 (7)

Lithium bromide (2.19 mmol, 0.19 g) was added to a solution of compound **6** (2.19 mmol, 0.5 g) in acetone (8.0 ml). The reaction was stirred at room temperature for 27 h. Lihium bromide was added (2.19 mmol, 019 g) and then refluxed for 3 h. After evaporation of the solvent, the crude product was diluted with water and extracted with chloroform. The organic layers were washed with brine and dried. The evaporation of the solvent gave a crude yellow oil, which was chromatographed on a silica-gel column (ethyl ether/n-hexane, 1:10, as eluent) to obtain 0.72 g of compound **7** (yield 90%), mp 65 °C (n-hexane). ^1H NMR ($CDCl_3$): δ 2.40 (m, 4H, CH_2), 3.28 (m, 2H, CH_2), 3.46–3.48 (m, 2H, CH_2), 7.08–7.15 (m, 3H, benzene H).

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6-(2-Bromo-ethyl)-5,7-diethyl-2-methyl-indan-1-one-d_2 (8)[13]

MS data m/z (abundance) 282–284 (25), 203 (100), 189 (35), 161 (7).

Acetic Acid 2-(2,6-Diethyl-phenyl)-ethyl Ester-d_2 (9)

Acetyl chloride (6.70 mmol, 0.47 ml) was added to a solution of compound **5** (0.85 g, 5.58 mmol) and pirydine (6.70 mmol, 0.54 ml) in anhydrous dichloromethane (44 ml). The reaction was stirred at room temperature for 1 night, diluted with water, and extracted with chloroform. The organic extracts were washed with brine and dried. The evaporation of the solvent gave 1.12 g of crude product, which was chromatographed on a silica-gel column (ethyl acetate/n-hexane, 1:4, as eluent) to obtain 0.81 g of compound **9** (yield 75%). IR: ν 1736 cm^{-1} (CO); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, 3H, $COCH_3$), 2.41 (m, 4H, CH_2D), 3.04–3.08 (m, 2H, CH_2), 4.20–4.24 (m, 2H, CH_2), 7.07–7.10 (m, 3H, benzene H).

Acetic Acid 2-(4,6-Diethyl-2-methyl-3-oxo-indan-5-yl)-ethyl Ester-d_2 (10)

Methacrilic anhydride (3.83 mmol, 0.59 g, 0.61 ml) was added to a suspension of compound **9** (2.57 mmol, 0.5 g) in PPA (15.7 g). The reaction was stirred for 5 days, diluted with water and ice, and extracted with chloroform. The organic layers were collected, washed with brine, and dried. The evaporation of the solvent gave the crude product (580 mg), which was chromatographed on a silica-gel column (ethyl ether/n-hexane, 1:7, as eluent) to obtain compound **10** (yield 86%). ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, CH_3), 2.09 (s, 3H, $COCH_3$), 2.48 (m, 2H, CH_2), 3.62–2.73 (m, 4H, CH_2D), 3.08–3.12 (t, 2H, CH_2), 3.27–3.33 (q, 1H, CHCO), 4.18–4.22 (t, 2H, CH_2), 7.15 (s, 1H, benzene H).

5,7-Diethyl-6-(2-hydroxy-ethyl)-2-methyl-indan-1-one-d_2 (11)

HCl 37% (0.1 ml) was added to a solution of compound **10** (0.28 mmol, 0.06 g) in methanol (2 ml). The solution was stirred at room temperature for 1 night. Water was added, and the resulting suspension was extracted with chloroform (3 × 50 mL). The organic extracts were collected, washed with brine (3 × 100 mL), and dried. The evaporation of the solvent gave 0.06 g of compound **11** (yield 100%); IR: ν 3325 cm^{-1} (OH); ^{1}H NMR ($CDCl_3$): δ 1.33 (d, 3H, CH_3), 2.48 (m, 2H, CH_2), 2.61–2.72 (m, 4H, CH_2D), 3.07 (t, 2H, CH_2), 3.30 (q, 1H, CH), 3.81 (t, 2H, CH_2), 7.15 (s, 1H, benzene H); mass data m/z (abundance) 220 (50); 203 (71); 189 (100); 161 (8); 130 (17).

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