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Research Article

Syntheses of perdeuterated indoles and derivatives as probes for the biosyntheses of crucifer phytoalexins

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Summary

A simple two-step preparation of [$^2H_4$]indole, a starting material necessary for the synthesis of various crucifer metabolites, starting with readily available ^{1}H NMR solvent [$^2H_5$]nitrobenzene (99% deuterated) was developed. [4,5,6,7-$^2H_4$]Indole 99% deuterated at the specified positions was then used to synthesize [4′,5′, 6′,7′-$^2H_4$]indolyl-3-acetaldoxime, [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-acetaldoxime, [1″,1″,1″,4′,5′,6′,7′-$^2H_7$]1-methoxyindolyl-3-acetaldoxime, [4′,5′,6′,7′-$^2H_4$]1-methoxybrassinin, and [3,3,3,4′,5′,6′,7′-$^2H_7$]1-methoxybrassinin. Copyright © 2005 John Wiley & Sons, Ltd.

Key Words: 1-methoxybrassinin; 1-methoxyindolyl-3-acetaldoxime; brassinin; indolyl-3-acetaldoxime; tetradeuterated indole

Introduction

The interesting array of phytoalexins, i.e. chemical defenses, produced by plants of the family Cruciferae (Brassicaceae) has prompted diverse studies dealing with their syntheses, biosynthesis, biotransformation, and biological activity.[1] In particular, the correlation between phytoalexins and defense pathways operating in plants[2] explains both the interest in and significance of the biosynthetic pathway of crucifer phytoalexins. Knowledge of such a pathway(s) is of great importance in the genetic manipulation of secondary metabolites of crucifers. Crucifer crops are the third largest source of vegetable oils (canola, rapeseed, and mustard oils) and include as well a great variety of vegetables used worldwide as staple food (broccoli, cauliflower, cabbage,

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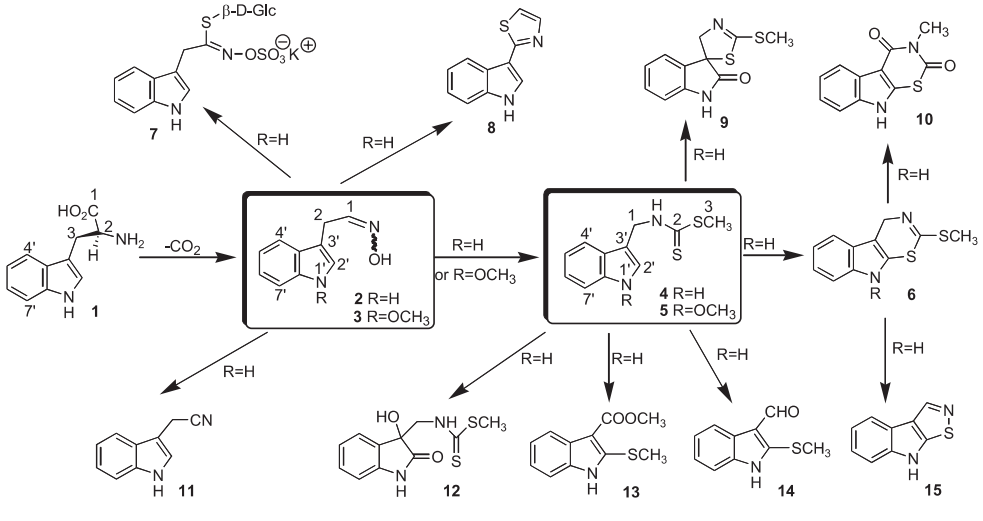
radish, rutabaga, turnip). Importantly, a number of epidemiological studies suggest that cruciferous vegetables protect against cancer by modulating carcinogen metabolism.[3] Related studies attributed this modulation to indole-containing metabolites[4] such as 1-methoxyindole-3-carbinol, which appears to show higher efficiency in the induction of cytochrome P450 hepatic enzymes.[5]

Crucifer phytoalexins,[1] including camalexins,[6] are biosynthetically derived from tryptophan (**1**). Unambiguous biosynthetic studies carried out independently by various groups have demonstrated that (*S*)-tryptophan (**1**) is the precursor of indolyl-3-acetaldoxime (**2**), which in turn is a precursor of the phytoalexins brassinin (**4**), a precursor of several other phytoalexins (**6**, **9**, **10**, **12**−**15**), camalexin (**8**), and indolyl-3-acetonitrile (**11**), and the indole glucosinolate glucobrassicin (**7**). Importantly, the C-2 of ^{13}C-labeled tryptophan (**1**) was incorporated into the dithiocarbamic carbon of brassinin (**4**), suggesting a Lossen-type rearrangement.[7] Despite the importance and number of naturally occurring compounds containing the 1-methoxyindole moiety, the first 1-methoxyindolyl biosynthetic intermediate was established only very recently, using [$^2H_3$]1-methoxyindolyl-3-acetaldoxime (**3**, R = OC^2H_3, Scheme 1).[8] In continuation of those biosynthetic studies, it was crucial to develop synthetic routes to perdeuterated indolyl-3-acetaldoximes **2a**, **3a** and **3b** and brassinins **5a** and **5b**. Here we report the syntheses and characterization of new perdeuterated indoles in which the isotopic composition at the selected positions is about 99%.

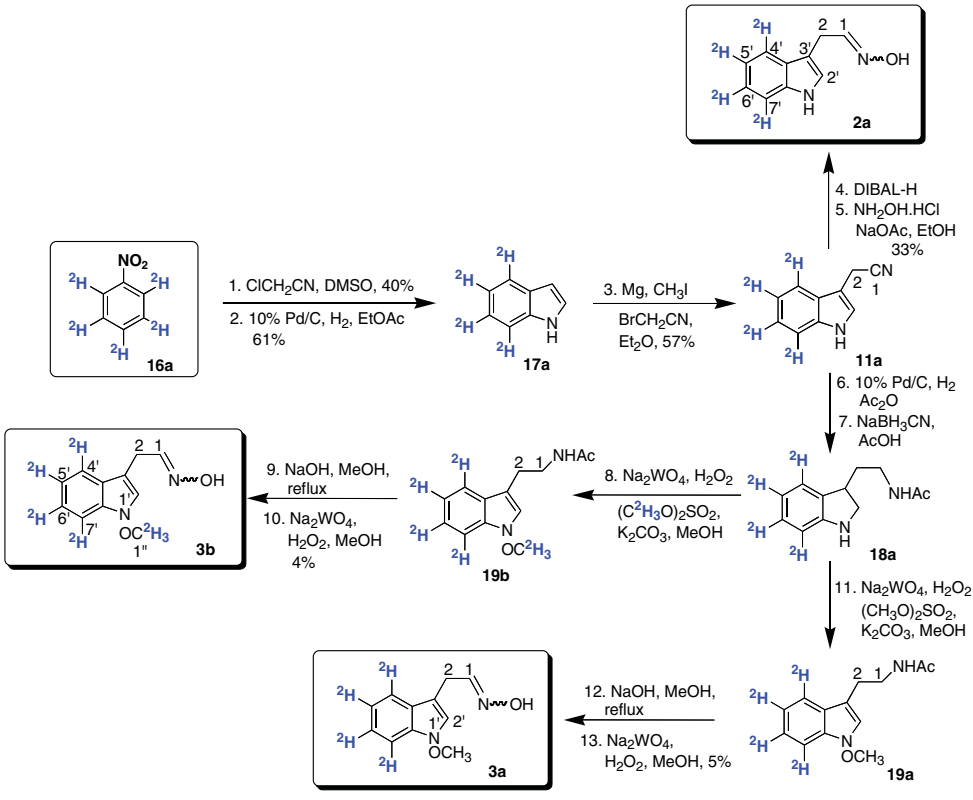


Scheme 1. Biosynthetic pathway of crucifer phytoalexins

Results and discussion

The use of perdeuterated compounds in biosynthetic studies is invaluable as extremely small amounts of deuterated metabolites can be detected unambiguously by mass spectrometry, e.g. ESI-HRMS or APCI-HRMS.[9] The indole derivatives of immediate interest in biosynthetic studies of phytoalexins need to contain at least three deuterium atoms, as this number will allow the unambiguous detection and assignment of labeled phytoalexins using HRMS analysis of m/z $[M+3]^{+/-}$ and corresponding fragment ions m/z $[M-X+3]^{+/-}$. The target compounds were selected containing different deuterium composition: [4′,5′,6′,7′-$^2H_4$]indolyl-3-acetaldoxime (**2a**), [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-acetaldoxime (**3a**), [1″,1″,1″,4′,5′,6′,7′-$^2H_7$]1-methoxyindolyl-3-acetaldoxime (**3b**), [4′,5′,6′,7′-$^2H_4$]1-methoxybrassinin (**5a**), and [3,3,3,4′,5′,6′,7′-$^2H_7$]1-methoxybrassinin (**5b**). The required starting material for these syntheses, [4,5,6,7-$^2H_4$]indole (**17a**), needed to be at least 99% deuterated; however, commercially available perdeuterated indoles either did not offer this percentage of deuterium content at specific sites or were extremely expensive. Subsequently, after consideration of a previous synthesis of 99% tetradeuterated indole,[10] which included the preparation of [$^2H_4$]2-nitrophenylacetonitrile from [$^2H_8$]toluene,[11] a rather difficult and somewhat hazardous preparation, we searched for a shorter route to [$^2H_4$]2-nitrophenylacetonitrile that could potentially provide access to [2,3-$^{13}C_2$]indole as well. The well-known vicarious nucleophilic substitution of stabilized *R*-chlorocarbanions with nitrobenzene[12] appeared to provide a ready entry to various isotopically labeled indoles. Thus, [$^2H_4$]2-nitrophenylacetonitrile was obtained from [$^2H_5$]nitrobenzene and provided access to [4,5,6,7-$^2H_4$]indole (**17a**), from which the readily accessible key intermediate [$^2H_4$]indolyl-3-acetonitrile (**11a**) could be prepared.[13,14] The required deuterated oxime **2a** could be readily obtained from **11a**,[15] but the synthesis of perdeuterated N_b-acetyl-1-methoxytryptamine (**19a**, **19b**) from which the corresponding oximes could be obtained through oxidation, required a higher number of steps. [$^2H_4$]Indole (**17a**) was the starting material also used to prepare deuterated methoxyindole (**21a**) and methoxybrassinins **5a** and **5b** via [$^2H_4$]indole-3-carboxaldehyde oxime (**22a**), using a well-established route for non-labeled materials.[1]

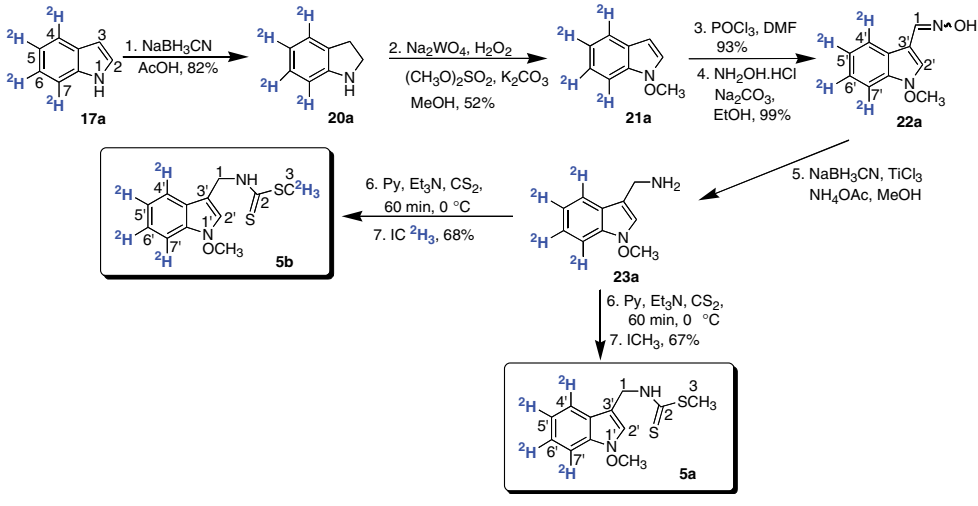
Subsequently, [4,5,6,7-$^2H_4$]indole (**17a**) was obtained in two steps and 24% overall yield, starting with the readily available ^{1}H NMR solvent [2,3,4,5,6-$^2H_5$]nitrobenzene (**16a**, 99%, $^2H_5$) and 2-chloroacetonitrile,[11] as summarized in Scheme 2 and described in the Experimental section. [4′,5′,6′,7′-$^2H_4$]Indolyl-3-acetonitrile (**11a**) was prepared by reaction of indolyl-magnesium iodide (prepared from [$^2H_4$]indole (**17a**), and Mg/methyl iodide) with bromoacetonitrile.[13,14] Reduction of indolyl-3-acetonitrile (**11a**) to indolyl-3-acetaldehyde employing DIBAL-H, followed by hydrolysis[15] and treatment with $HONH_2 \cdot HCl$ and NaOAc yielded the desired



Scheme 2. Synthesis of perdeuterated indolyl-3-acetaldoximes 2a, 3a and 3b

[4′,5′,6′,7′-$^2H_4$]indolyl-3-acetaldoxime (**2a**) in acceptable yields. [$^2H_7$]N_b-acetyl 1-methoxytryptamine (**19b**) was prepared from **18a** after reduction with $NaBH_3CN$, followed by oxidation with Na_2WO_4/H_2O_2,[16] and methylation of the 1-hydroxyindolyl intermediate with $(CD_3O)_2SO_2$. [$^2H_4$]N_b-acetyl 1-methoxytryptamine (**19a**) was prepared similar to the [$^2H_7$] compound **19b** but using $(CH_3O)_2SO_2$,[16] instead of the hexadeuterated methylating reagent. Perdeuterated 1-methoxyindolyl-3-acetaldoximes **3a** and **3b** were then obtained by hydrolysis of the N_b-acetyl group followed by oxidation of the amine to oxime using Na_2WO_4/H_2O_2.[17] This oxidation step proved rather difficult, mostly due to decomposition of the product under the reaction conditions used, however no simple and efficient methods were found to oxidize aliphatic amines to oximes. For example, CH_3ReO_3/H_2O_2 catalyzed oxidation of primary alkylamines possessing the α-C–H bond was found to yield mixtures of oximes, nitroso dimers, and azoxy compounds.[18]

Next, perdeuterated 1-methoxybrassinins **5a** and **5b** were obtained from [4,5,6,7-$^2H_4$]indole as shown in Scheme 3. First, reduction of [$^2H_4$]indole (**17a**) with $NaBH_3CN$ in AcOH followed by oxidation with Na_2WO_4/H_2O_2 and



Scheme 3. Synthesis of perdeuterated 1-methoxybrassinins 5a and 5b

methylation with $(CH_3O)_2SO_2$[16] yielded [$^2H_4$]1-methoxyindole (**21a**) in moderate yield. Standard Vilsmeier-Haack conditions yielded [$^2H_4$]1-methoxyindole-3-carboxaldehyde, which was first treated with $HONH_2 \cdot HCl$ and NaOAc followed by reduction of the resulting oxime with $NaBH_3CN$, $TiCl_3$, and NH_4OAc in MeOH to yield [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-methanamine (**23a**).[19] The synthesis of [$^2H_4$] and [$^2H_7$]1-methoxybrassinins **5a** and **5b**, followed previously set routes,[1] which afforded the desired compounds in reasonable yields.

All the synthesis described above afforded perdeuterated products 99% labeled at the indicated sites.

Experimental

General

All chemicals were purchased from Sigma-Aldrich Canada Ltd., Oakville, Ont., except for deuterated solvents that were purchased from Cambridge Isotopes Laboratories Inc., Andover, MA. All solvents were high-performance liquid chromatograph (HPLC) grade and used as such, except $CHCl_3$ and CH_2Cl_2 that were redistilled. Preparative TLC: (Merck, Kieselgel 60 F_{254}) $20 \times 20\,cm \times 0.25\,mm$; analytical TLC (Merck, Kieselgel 60 F_{254}, aluminum sheets) $5 \times 2.5\,cm \times 0.2\,mm$; compounds were visualized by exposure to UV and by dipping the plates in a 5% aqueous (w/v) phosphomolybdic acid solution containing a trace of ceric sulfate and 4% (v/v) H_2SO_4, followed by heating at 200°C. Flash column chromatography (FCC): silica gel Merck, grade 60, mesh size 230–400, 60 Å. HPLC analysis was carried out with an

HPLC equipped with quaternary pump, automatic injector and diode array detector (wavelength range 190–600 nm), degasser and a Hypersil ODS column (5 μm particle size silica, 4.6 i.d. × 200 mm) equipped with an in-line filter. HPLC mobile phase H_2O–CH_3CN: 75%/25%–0%/100%, for 45 min, linear gradient and flow rate 1.0 ml/min. NMR spectra were recorded on Bruker 500 MHz Avance spectrometers; for ^1H (500 MHz), values were referenced to $CHCl_3$ (7.27 ppm) or CHD_2CN (1.94 ppm), for ^{13}C (125.8 MHz) referenced to $CHCl_3$ (77.2 ppm) or CD_3CN (118.7 ppm). Mass spectra (MS) were obtained on a VG 70 SE mass spectrometer (high resolution (HR), electron impact (EI)), employing a solids probe.

*[4′,5′,6′,7′-$^2H_4$]Indolyl-3-acetaldoxime (**2a**)*

A solution of DIBAL-H (850 μl, 1.28 mmol) in toluene was added drop wise to a solution of [4′,5′,6′,7′-$^2H_4$]indolyl-3-acetonitrile (100 mg, 0.63 mmol) in dry toluene (8.0 ml) cooled to −78°C under atmosphere of argon. The reaction mixture was allowed to stir at −78°C for 10 min, was diluted with ice-cold HCl (10.0 ml, 2 M) and immediately extracted in EtOAc (20.0 ml × 3).[15] The combined organic extract was washed with H_2O (10.0 ml × 2), was dried over Na_2SO_4, and was concentrated under reduced pressure to yield crude [4′,5′,6′,7′-$^2H_4$]indolyl-3-acetaldehyde, which was used for the next step without purification. A solution of $HONH_2 \cdot HCl$ (111 mg, 1.60 mmol) and CH_3COONa (131 mg, 1.60 mmol) in water (1.0 ml) was added to a cooled solution (1°C) of crude [4′,5′,6′,7′-$^2H_4$]indolyl-3-acetaldehyde in EtOH (8.5 ml). The reaction mixture was allowed to stir at 1°C for 10 min, and then at r.t. for 15 min. The reaction mixture was concentrated, the residue was dissolved in H_2O (15.0 ml), extracted with EtOAc (15.0 ml × 4), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure. Separation by FCC (CH_2Cl_2–MeOH, 97:3, v/v) yielded [4′,5′,6′,7′-$^2H_4$]indolyl-3-acetaldoxime (**2a**, 36.8 mg, 33% yield from indolyl-3-acetonitrile (**11a**)).

^1H NMR (500 MHz CD_3CN): (two isomers, 1.1:0.9) major isomer: δ 3.60 (d, $J = 6$ Hz, H_2-2), 7.12 (d, $J = 2$ Hz, H-2′), 7.49 (t, $J = 6$ Hz, H-1), 8.37 (s, OH), 9.17 (br s, NH); minor isomer: δ 3.78 (d, $J = 5$ Hz, H_2-2), 6.83 (t, $J = 5$ Hz, H-1), 7.15 (d, $J = 1$ Hz, H-2′), 8.85 (s, OH), 9.17 (br s, NH). HREIMS m/z measured 178.1044 (178.1044 calculated for $C_{10}H_6D_4N_2O$); EIMS m/z (relative abundance) 178 $[M]^+$ (76), 134 (100).

*[4′,5′,6′,7′-$^2H_4$]1-Methoxyindolyl-3-acetaldoxime (**3a**)*

Ten percent Pd/C (80 mg) was added to a solution of [4′,5′,6′,7′-$^2H_4$]indolyl-3-acetonitrile (80 mg, 0.50 mmol) in Ac_2O (4.0 ml), and the mixture was stirred at r.t. under H_2 atmosphere (balloon pressure). After 14 h the catalyst was filtered

off, the filtrate was diluted with toluene and concentrated under reduced pressure, the residue was dissolved in CH_2Cl_2 (15.0 ml), was washed with a 10% solution of $NaHCO_3$ (8.0 ml), H_2O (8.0 ml × 2), and dried over Na_2SO_4. After removal of the solvent under reduced pressure, crude [4′,5′,6′,7′-$^2H_4$]N_b-acetyltryptamine was obtained (87.9 mg, 85%) in sufficient purity to use directly in the next step. To a solution of [4′,5′,6′,7′-$^2H_4$]N_b-acetyltryptamine (100 mg, 0.495 mmol) in glacial acetic acid (2.0 ml) at r.t., $NaBH_3CN$ (47 mg, 0.74 mmol) was added in portions through a canula. The reaction mixture was allowed to stir for 3 h at room temperature, was diluted with H_2O (5.0 ml), was basified with NaOH, and was extracted with Et_2O (15.0 ml × 3). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure to yield crude [4′,5′,6′,7′-$^2H_4$]N_b-acetyl-2,3-dihydrotryptamine (117 mg, 99%), which was used in the next step without purification. A solution of $Na_2WO_4 \cdot 2H_2O$ (17.2 mg, 0.0943 mmol) in H_2O (199 µl) was added to a solution of [4′,5′,6′,7′-$^2H_4$]N_b-acetyl-2,3-dihydrotryptamine (112.8 mg, 0.542 mmol) in MeOH (2.2 ml) cooled to −20°C under stirring, followed by drop wise addition of a solution of H_2O_2 (520 µl, 5.42 mmol) in MeOH (540 µl). After being stirred at r.t. for 10 min, K_2CO_3 (601 mg, 4.44 mmol) and $(CH_3O)_2SO_2$ (78 µl, 0.87 mmol) were added under vigorous stirring at r.t.[16] After 60 min, the reaction mixture was diluted with H_2O (12.0 ml), was extracted in Et_2O (20.0 ml × 3), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure to yield crude [4′,5′,6′,7′-$^2H_4$]N_b-acetyl-1-methoxytryptamine (**19a**, 79.4 mg, 62%). Crude [4′,5′,6′,7′-$^2H_4$]N_b-acetyl-1-methoxytryptamine in (79.4 mg) was dissolved in 15% methanolic solution of NaOH (20.0 ml) and the mixture was allowed to reflux for 24 h. After removing the solvent under reduced pressure, the residue was dissolved in water, was extracted with CH_2Cl_2–MeOH (95:5, v/v), was dried over Na_2SO_4 and was concentrated under reduced pressure to yield crude [4′,5′,6′,7′-$^2H_4$]1-methoxytryptamine (41 mg, 63%). An aqueous solution of $Na_2WO_4 \cdot 2H_2O$ (1.4 mg, 0.0042 mmol) in H_2O (60 µl) was added to the solution of [4′,5′,6′,7′-$^2H_4$]1-methoxytryptamine (41 mg, 0.21 mmol) in MeOH (400 µl), the mixture was cooled to −15°C and H_2O_2 (48 µl, 0.5 mmol) was added under stirring.[17] After being stirred for 60 min at r.t., the mixture was diluted with H_2O (5.0 ml), was basified with NaOH, was extracted with CH_2Cl_2 (20.0 ml × 3), the combined organic extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford a crude residue (24.3 mg). The crude residue was separated by preparative TLC (CH_2Cl_2–MeOH, 95:5, v/v) to yield [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-acetaldoxime (**3a**, 4.8 mg, 5% over five steps).

^{1}H NMR (500 MHz $CDCl_3$): (two isomers, 1.1:0.9) major isomer δ 3.64 (d, J = 6 Hz, H_2-2), 4.08 (s, OC$\underline{H}_3$), 7.15 (s, H-2′), 7.60 (t, J = 6 Hz, H-1); minor isomer: δ 3.83 (d, J = 5 Hz, H_2-2), 4.09 (s, OC$\underline{H}_3$), 6.93 (t, J = 5 Hz,

H-1), 7.17 (s, H-2′). HREIMS *m/z* (relative abundance) measured 208.1142 (208.1149 calculated for $C_{11}H_8D_4N_2O_2$); EIMS *m/z* (relative abundance) 208 $[M]^+$ (100), 190 (25), 164 (45), 159 (54), 132 (70).

*[1″,1″,1″,4′,5′,7′-$^2H_7$]1-Methoxyindolyl-3-acetaldoxime (***3b***)*

A solution of $Na_2WO_4 \cdot 2H_2O$ (32.3 mg, 0.098 mmol) in H_2O (206 µl) was added to a solution of [4′,5′,6′,7′-$^2H_4$]N_b-acetyl-2,3-dihydrotryptamine (117 mg, 0.563 mmol) in MeOH (2.3 ml) under stirring.[16] The mixture was cooled to −20°C and a solution of H_2O_2 (541 µl, 5.63 mmol) in MeOH (562 µl) was added drop wise. After being stirred for 10 min at r.t., K_2CO_3 (623 mg, 4.50 mmol) and $(C^2H_3O)_2SO_2$ (85 µl, 0.90 mmol) were added to the reaction mixture under vigorous stirring. After being stirred for 60 min at r.t., the reaction mixture was diluted with H_2O (12.0 ml), was extracted with Et_2O (20.0 ml × 3), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure to yield crude [1″,1″,1″,4′,5′,6′,7′-$^2H_7$]N_b-acetyl-1-methoxytryptamine (84.8 mg, 63%). Crude [1″,1″,1″,4′,5′,6′,7′-$^2H_7$]N_b-acetyl-1-methoxytryptamine (84.8 mg) was treated as reported above for [4′,5′,6′,7′-$^2H_4$]N_b-acetyl-1-methoxytryptamine (**19a**) to yield [1″,1″,1″,4′,5′,6′,7′-$^2H_7$]1-methoxyindolyl-3-acetaldoxime (**3b**, 4.2 mg, 4% over five steps).

^{1}H NMR (500 MHz $CDCl_3$): (two isomers, 1.1:0.9) major isomer δ 3.64 (d, $J = 6$ Hz, H_2-2), 7.15 (s, H-2′), 7.60 (t, $J = 6$ Hz, H-1); minor isomer: δ 3.83 (d, $J = 5$ Hz, H_2-2), 6.93 (t, $J = 5$ Hz, H-1), 7.17 (s, H-2′). HREIMS *m/z* (relative abundance) measured 211.1335 (211.1338 calculated for $C_{11}H_5D_7N_2O_2$); EIMS *m/z* (relative abundance) 211 $[M]^+$ (100), 193 (29), 167 (48), 159 (51), 132 (61).

*[4′,5′,6′,7′-$^2H_4$]1-Methoxybrassinin (***5a***)*

$NaBH_3CN$ (195 mg, 1.68 mmol) was added to a solution of [4,5,6,7-$^2H_4$]indole (**17a**, 135 mg, 1.12 mmol) in glacial acetic acid (2.0 ml) under an argon atmosphere. The reaction mixture was stirred at r.t. for 60 min, was diluted with H_2O (4.0 ml), was basified with NaOH, was extracted with Et_2O (30.0 ml × 3), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure. The crude material was separated by FCC (CH_2Cl_2) to yield [4,5,6,7-$^2H_4$]indoline (**20a**, 113.2 mg, 82%). A solution of $Na_2WO_4 \cdot 2H_2O$ (53 mg, 0.16 mmol) in H_2O (0.35 ml) was added to the stirred solution of [4,5,6,7-$^2H_4$]indoline (113.2 mg, 0.920 mmol) in MeOH (3.0 ml) and the suspension was then cooled to −20°C. A solution of H_2O_2 (766 µl, 7.96 mmol) in MeOH (1.0 ml) was added drop wise to the cooled suspension, the reaction mixture was stirred at r.t. for 10 min, after which solid K_2CO_3 (1.02 g, 7.36 mmol) and $(CH_3O)_2SO_2$ (133 µl, 1.47 mmol) were added

under vigorous stirring.[16] After being stirred for 10 min, the reaction mixture was diluted with H_2O (20.0 ml), was extracted with Et_2O (30.0 ml × 3), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure. The crude material was separated by FCC (hexane–CH_2Cl_2, 70:30, v/v) to yield [4,5,6,7-$^2H_4$]1-methoxyindole (**21a**, 73 mg, 52%). Freshly distilled $POCl_3$ (69 µl, 0.75 mmol) was added to a solution of [4,5,6,7-$^2H_4$]1-methoxyindole (102 mg, 0.68 mmol) in DMF (0.6 ml) and the reaction mixture was stirred at r.t. After 40 min, a solution of aqueous NH_3 (4.0 ml, 28%) was added to the reaction mixture, the reaction mixture was extracted with Et_2O (10.0 ml × 3), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure to yield [4′,5′,6′,7′-$^2H_4$]1-methoxyindole-3-carboxaldehyde (113 mg, 93%). A solution of $HONH_2 \cdot HCl$ (80.6 mg, 1.16 mmol) and Na_2CO_3 (61.5 mg, 0.580 mmol) in H_2O (810 µl) was added to a solution of [4′,5′,6′,7′-$^2H_4$]1-methoxyindole-3-carboxaldehyde (103 mg, 0.580 mmol) in EtOH (2.7 ml). The resulting reaction mixture was refluxed at 85°C for 60 min, was diluted with H_2O (3.0 ml) and was extracted with Et_2O (20.0 ml × 3). The combined organic extract was washed with brine (10.0 ml × 2), was dried over Na_2SO_4 and was concentrated under reduced pressure to yield [4′,5′,6′,7′-$^2H_4$]1-methoxyindole-3-carboxaldehyde oxime (**22a**, 122.1 mg, 99%). $NaBH_3CN$ (163 mg, 2.58 mmol) and NH_4OAc (219 mg, 2.84 mmol) were added to a solution of [4′,5′,6′,7′-$^2H_4$]1-methoxyindole-3-carboxaldehyde oxime (50 mg, 0.26 mmol) in MeOH (640 µl) cooled to 0°C and the resulting mixture was treated with $TiCl_3$ in 2 M HCl (795 µl, 2.04 mmol) neutralized with NaOH.[19] After being stirred for 15 min at 0°C, the reaction mixture was diluted with H_2O (2.2 ml), was basified with NaOH and was extracted with CH_2Cl_2 (15.0 ml × 3). The combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure to yield crude [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-methanamine (**23a**, 40.3 mg) which was used in the next step without purification. Et_3N (34 µl, 0.25 mmol) and CS_2 (13 µl, 0.22 mmol) were added to a solution of [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-methanamine (40.3 mg, 0.224 mmol) in pyridine (105 µl) cooled to 0°C. After being stirred for 60 min at 0°C, CH_3I (14 µl, 0.22 mmol) was added to the reaction mixture which was kept at 5°C for 90 min. The reaction mixture was diluted with H_2O (2.0 ml), was extracted with Et_2O (10.0 ml × 2), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure. The reaction mixture was separated by FCC (hexane–CH_2Cl_2) to yield [4′,5′,6′,7′-$^2H_4$]1-methoxybrassinin (**5a**, 46.5 mg, 67% from [4′,5′,6′,7′-$^2H_4$]1-methoxyindole-3-carboxaldehyde oxime (**22a**)).

^1H NMR (500 MHz $CDCl_3$): δ 2.67 (s, SCH_3), 4.12 (s, OCH_3), 5.04 (d, $J = 4.5$ Hz, H_2-1), 7.04 (br s, NH), 7.35 (s, H-2′). HREIMS m/z (relative abundance) measured 270.0794 (270.0795 calculated for $C_{12}H_{10}D_4N_2OS_2$);

EIMS *m/z* (relative abundance), 270 $[M]^+$ (6), 239 (64), 222 (15), 191 (8), 164 (100), 149 (15), 133 (43).

*[3,3,3,4′,5′,6′,7′-$^2H_7$]1-Methoxybrassinin (***5b***)*

$NaBH_3CN$ (179 mg, 2.84 mmol) and NH_4OAc (241 mg, 3.13 mmol) were added to a solution of [4′,5′,6′,7′-$^2H_4$]1-methoxyindole-3-carboxaldehyde oxime (**22a**, 55 mg, 0.284 mmol) in MeOH (700 µl) cooled to 0°C. The resulting reaction mixture was treated with a solution of $TiCl_3$ in 2 M HCl (876 µl, 2.25 mmol) neutralized with NaOH.[19] After being stirred for 15 min at 0°C, the reaction mixture was diluted with H_2O (2.4 ml), was basified with NaOH and was extracted with CH_2Cl_2 (15.0 ml × 3). The combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure to yield [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-methanamine (**23a**, 44 mg) which was used in the next step without purification. Et_3N (37 µl, 0.27 mmol) and CS_2 (15 µl, 0.24 mmol) were added to a solution of [4′,5′,6′,7′-$^2H_4$]1-methoxyindolyl-3-methanamine (44 mg, 0.24 mmol) in pyridine (118 µl) cooled to 0°C. After being stirred for 60 min at 0°C, CD_3I (16 µl, 0.244 mmol) was added and the reaction mixture was kept at 5°C for 90 min. The reaction mixture was diluted in H_2O (2.0 ml), was extracted with Et_2O (10.0 ml × 2), the combined organic extract was dried over Na_2SO_4 and was concentrated under reduced pressure. The reaction mixture was separated by FCC (hexane–CH_2Cl_2) to yield [3,3,3,4′,5′,6′,7′-$^2H_7$]1-methoxybrassinin (**5b**, 52.7 mg, 68% yield from [4′,5′,6′,7′-$^2H_4$]1-methoxyindole-3-carboxaldehyde oxime (**22a**)).

^1H NMR (500 MHz $CDCl_3$): δ 4.11 (s, (OCH_3), 5.02 (br s, H_2-1), 7.04 (br s, NH), 7.32 (br s, H-2′). HREIMS *m/z* (relative abundance) measured 273.0984 (273.0987 calculated for $C_{12}H_7D_7N_2OS_2$); EIMS *m/z* (relative abundance) 273 $[M]^+$ (7), 242 (100), 191 (12), 164 (85), 149 (19), 133 (40).

*[4′,5′,6′,7′-$^2H_4$]Indolyl-3-acetonitrile (***11a***)*

CH_3I (400 µl, 2.80 mmol) was added to Mg (turnings, 80 mg, 3.3 mmol) in dry Et_2O (5.0 ml) with stirring, under an argon atmosphere. After the Mg was consumed, excess CH_3I was distilled off and fresh dry Et_2O (4.0 ml) was added. A solution of [4,5,6,7-$^2H_4$]indole (100 mg, 0.830 mmol) in dry Et_2O (700 µl) was added drop wise and the mixture was allowed to stir for 15 min at r.t., followed by drop wise addition of bromoacetonitrile (160 µl, 2.70 mmol).[13,14] After being stirred for 20 min, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in water H_2O (40.0 ml). The resulting mixture was neutralized with HCl (2.0 ml, 1 M) and was extracted with CH_2Cl_2 (30.0 ml × 3). The combined organic extract was dried over Na_2SO_4 and was concentrated to dryness under reduced pressure.

Separation by FCC (hexane–CH_2Cl_2, 1:1–1:4) yielded [4′,5′,6′,7′-$^2H_4$]indolyl-3-acetonitrile (**11a**, 75.7 mg, 57% yield).

^{1}H NMR (500 MHz $CDCl_3$): δ 3.86 (s, H_2-2), 7.24 (t, $J = 1$ Hz, H-2′), 8.23 (br s, NH). HREIMS *m/z* (relative abundance) measured 160.0940 (160.0939 calculated for $C_{10}H_4D_4N_2$); EIMS *m/z* (relative abundance) 160 $[M]^+$ (62), 159 (100), 134 (32).

*[4,5,6,7-$^2H_4$]Indole (**17a**)*

A solution of [2,3,4,5,6,-$^2H_5$]nitrobenzene (**16a**, 4.20 ml, 39.4 mmol) and chloroacetonitrile (250 µl, 3.94 mmol) in DMSO (3.9 ml) was added drop wise over 30 min to a thoroughly stirred suspension of NaOH (1.60 g, 39.4 mmol) in DMSO (3.9 ml) at 20°C.[12] After 60 min, the reaction mixture was poured into ice-cold HCl (25.0 ml, 1 M), was diluted with H_2O (10.0 ml), and was extracted with $CHCl_3$ (50.0 ml × 3). The combined organic extract was washed with H_2O (70.0 ml), was dried over Na_2SO_4 and was concentrated under reduced pressure. Separation by FCC (hexane–CH_2Cl_2, 100:0–25:75) yielded a mixture of [3,4,5,6,-$^2H_4$]2-nitrophenylacetonitrile and [2,3,5,6,-$^2H_4$]4-nitrophenylacetonitrile (in a 10:1 ratio, 40% yield). Next, 10% Pd on C (220 mg) was added to the solution of [$^2H_4$]2- and [$^2H_4$]4-nitrophenylacetonitriles (200 mg) in EtOAc (20.0 ml) and the resulting reaction mixture was stirred under H_2 atmosphere (balloon pressure) at r.t. After 40 h, the catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The residue was taken in CH_2Cl_2 (15.0 ml), was washed with HCl (10.0 ml × 2, 1 M), was dried over Na_2SO_4 and was concentrated under reduced pressure to yield [4,5,6,7-$^2H_4$]indole (**17a**, 97.7 mg, 61% yield).

^{1}H NMR (500 MHz $CDCl_3$): δ 6.61 (br s, H-3), 7.23 (br s, H-2), 8.12 (br s, NH). HREIMS *m/z* (relative abundance) measured 121.0827 (121.0830 calculated for $C_8H_3D_4N$); EIMS *m/z* (relative abundance) 121 $[M]^+$ (100), 94 (22), 71 (11), 69 (12), 57 (17).

Conclusion

To the best of our knowledge, only one synthesis of perdeuterated indole (**17a**) and indole containing phytoalexins with 99% incorporation at multiple sites has been reported to date;[10] however, this new route to indole starting with [$^2H_5$]nitrobenzene (**16a**) is simpler, less hazardous and more efficient, providing an accessible method for the synthesis of perdeuterated brassinins (**5a**, **5b**). Furthermore, a simple route to perdeuterated indolyl-3-acetonitrile (**11a**), a key intermediate for the preparation of perdeuterated indolyl-3-acetaldoxime **2a** and 1-methoxyindolyl-3-acetaldoximes **3a** and **3b** is described for the first time. The availability of 99% labeled indole derivatives **2a**, **3a**, **3b**,

5a, **5b** and **11a** will facilitate probing the various biosynthetic relationships among crucifer phytoalexins using LC-HRMS techniques. As well, considering that indolyl-3-acetaldoxime (**2**) is an intermediate in other important metabolic pathways of Brassicaceae, such as those of plant hormones (auxins) and indole glucosinolates,[20] availability of synthetic methodology to make these perdeuterated compounds will have additional applications.

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