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Spirocyclization strategy toward indole phytoalexins. The first synthesis of (±)-1-methoxyspirobrassinin, (±)-1-methoxyspirobrassinol, and (±)-1-methoxyspirobrassinol methyl ether

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Abstract—The first syntheses of cruciferous indole phytoalexins (±)-1-methoxyspirobrassinin, (±)-1-methoxyspirobrassinol, (±)-1-methoxyspirobrassinol methyl ether as well as a new syntheses of phytoalexins (±)-spirobrassinin and cyclobrassinin were achieved by dioxane dibromide (DDB)-mediated spirocyclization of brassinin and its 1-substituted derivatives.

Phytoalexins are defined as antimicrobial low molecular weight secondary metabolites, produced by plants after their exposure to physical, biological or chemical stress.[1] About 30 indole phytoalexins were isolated from the economically and dietary important plants of the family *Cruciferae*, cultivated worldwide.[2] Selected examples (except for the synthetic analogs **7**, **8** and **11**) are shown in Fig. 1. With respect to their interesting biological properties, including antitumor activity[3–6] it is important to develop synthetic methods toward cruciferous phytoalexins, since the isolation from plants does not afford sufficient quantities for biological screening.

In the present paper we report the preparation and antiproliferative activity of hitherto synthetically unavailable spiroindoline phytoalexins **3**–**5** and a new approach to the compounds **1** and **10**. (*S*)-(−)-Spirobrassinin **1** was isolated in 1987 from Japanese radish,[7] however, its synthesis by cyclization of dioxibrassinin **2**[8] with $SOCl_2$ or MsCl and absolute stereochemistry were fully described only recently.[9] (−)-1-Methoxyspirobrassinin (**3**) isolated in 1994 from kohlrabi[10] and (−)-1-methoxyspirobrassinol methyl ether **4** as well as optically inactive methoxyspirobrassinol **5**, isolated in 1995 as a minor phytoalexin from Japanese radish[11] have not been synthesized to date and their biological properties are unknown. Compound **5** exists in solution as a mixture of diastereomers **5a** and **5b** in a 4:1 ratio, owing to its unstable hemiaminal structure.[11] The absolute stereochemistries of **3**–**5** remains unknown, formulae **4** and **5** show the diastereomeric structures only, as determined by NOE experiments.[11] Phytoalexins **3**–**5**, **9** and **12** belong to a group of hydroxyindole natural products.[12] Our approach to the target compounds is based on the finding, that spirobrassinin **1** and cyclobrassinin **10** are synthesized in plants from brassinin **6**.[13] Whereas **10** was prepared by cyclization of **6** with pyridinium tribromide[14] or NBS[4] in 34–35% yield and sinalbin B **12** was obtained by treatment of 1-methoxybrassinin **9** with NBS (41%),[15] no transformation of brassinin **6** or its 1-substituted derivatives to spiroindoline phytoalexins has been reported to date.

Retrosynthetic analysis of **1** and **3**–**5** revealed a central role for a spiroindoleninium intermediate **13** (Scheme 1), which can undergo a rearrangement to the cyclobrassinin structure, or nucleophilic addition of water and methanol to a spiroindoline structure.

Keywords: phytoalexins; indoles; dioxane dibromide; spirocyclization; antitumor activity.

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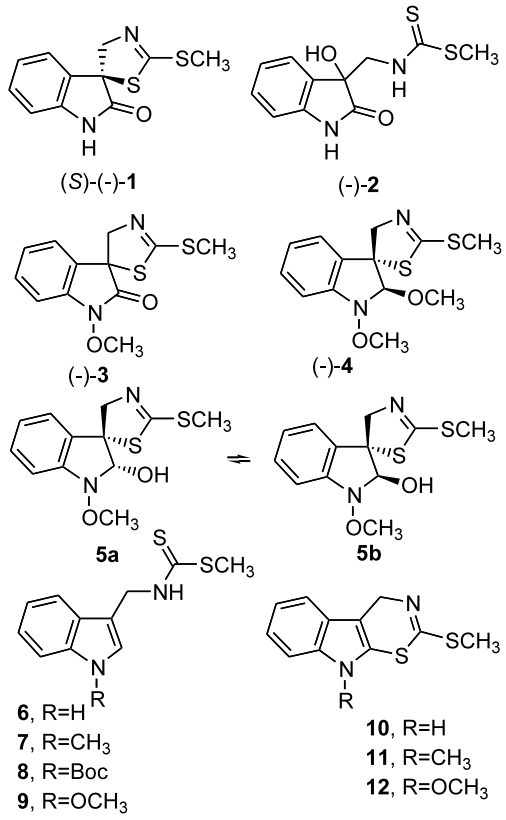
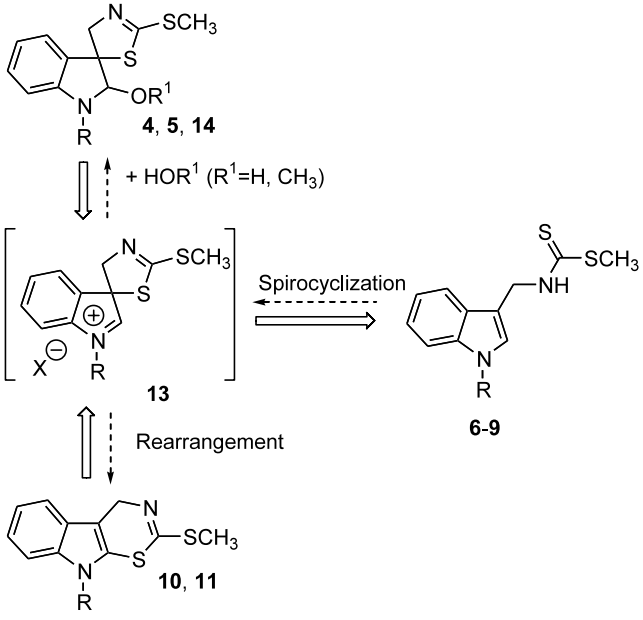


Figure 1.



Scheme 1.

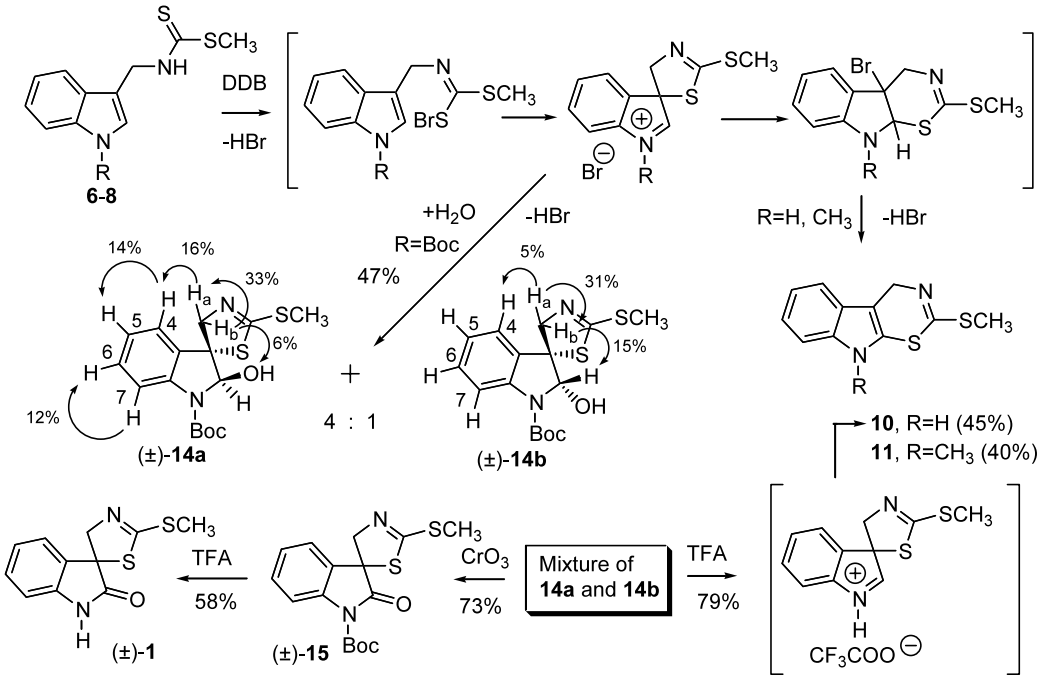
The preferred pathway should be dependent on the reactivity of iminium intermediate **13**, towards nucleophiles. A low reactivity is expected to produce cyclobrassinin, and on the other hand its high reactivity should result in nucleophilic trapping resulting in the formation of spiroindolines.

To verify this prediction, brassinin **6**[14] and 1-methylbrassinin **7**[16] with low reactivity of the iminium intermediate versus 1-Boc-brassinin **8**[16] with expected high reactivity of the corresponding acyliminium ion were selected as a model compounds. After several unsuccessful experiments with NBS, which seems to react by a different mechanism, involving bromination at the indole 3-position, we have found that dioxane dibromide (DDB) in dioxane in the presence of water (0.5%) is a suitable reagent for generation and rearrangement or trapping of iminium intermediate **13**. The reaction probably starts at the thiocarbamoyl functionality with the formation of a sulfenyl bromide in which the electrophilic sulfur attacks the 3-position of indole to provide an iminium intermediate (Scheme 2). As anticipated, the iminium ions with R = H and CH_3 afforded cyclobrassinin **10** (45%) and 1-methylcyclobrassinin **11** (40%) identical with previously described compounds,[14,16] whereas the acyliminium ion generated from the 1-Boc derivative **8** afforded a diastereomeric mixture of 1-Boc spirobrassinols **14a** and **14b** in a ratio 4:1 in 47% yield. Individual diastereomers were separated by flash chromatography and their structure determined on the basis of NOE-difference NMR experiments.[17] However, under anhydrous conditions, 1-Boc-brassinin **8** reacted with the formation of cyclobrassinin **10** (45%), probably because the HBr liberated during the reaction deprotected the 9-Boc-cyclobrassinin, formed by the rearrangement of Boc-substituted intermediate **13**. Compound **8** was consumed within 5 min and if, within the next 5 min, 0.5% of water was added, the spirobrassinols **14a** and **14b** were formed. However, when stirring was continued under anhydrous conditions, slow rearrangement and deprotection took place. We have found that the clean reaction with water as a nucleophile proceeds with up to 5% of water contained in the reaction medium. Therefore all further experiments were performed in dioxane/water or dioxane/methanol 95:5 as the solvent.

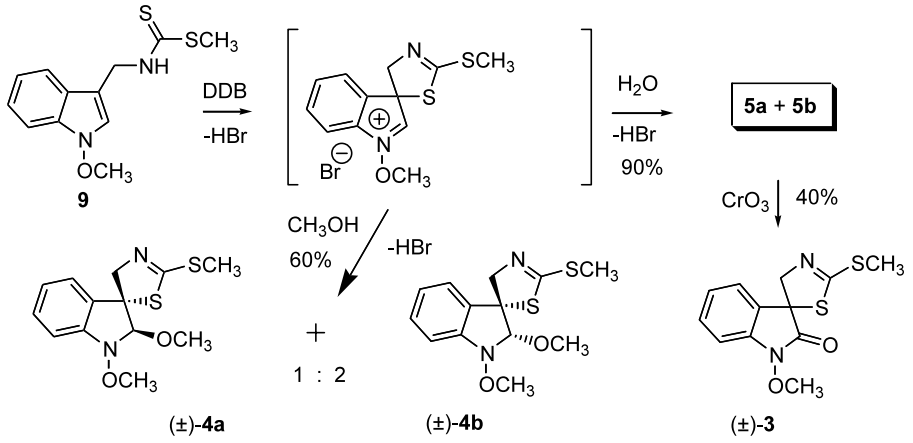
A mixture of **14a** and **14b** was oxidized with CrO_3 to 1-Boc-spirobrassinin (±)-**15**, which after deprotection with TFA afforded spirobrassinin (±)-**1**. Treatment of a mixture of **14a** and **14b** with TFA proceeded with deprotection and water elimination toward an iminium cation with a trifluoroacetate counterion, which smoothly rearranged to cyclobrassinin **10** (Scheme 2).

Next, with the experimental conditions in hand for the preparation of 1-Boc-spirobrassinol **14**, we attempted the cyclization of 1-methoxybrassinin **9**.[15,18,19] It appeared that the methoxyiminium intermediate formed is a highly reactive species, probably because of the activating effect of the electronegative oxygen atom. Consequently, the water present in the reaction mixture smoothly added to the carbon atom of the iminium intermediate to produce methoxyspirobrassinol **5** in 90% yield (Scheme 3).

Oxidation of **5** with CrO_3 afforded racemic 1-methoxyspirobrassinin (±)-**3** in 40% yield. If methanol was added as a nucleophile, instead of water, the methoxyiminium intermediate reacted with the formation of a mixture of the natural **4a** and unnatural **4b**



Scheme 2.



Scheme 3.

diastereomers of methoxyspirobrassinol methyl ether in a ratio of 1:2 which separated by flash chromatography (ether/hexane, 1:2). Spectroscopic data of the synthesized phytoalexins **1**, **3**–**5** and **10** are identical with those of the natural products.[7,10,11,14]

Experimental procedure for 1-methoxyspirobrassinin: *(±)-Methoxyspirobrassinol* ***5***: To a stirred solution of 1-methoxybrassinin **9** (63 mg, 0.24 mmol) in a mixture of dioxane/water (95:5, 5 mL) was added a freshly prepared solution of DDB (2 mL, 0.26 mmol; the stock solution was obtained by dissolving of 0.04 mL of bromine in 6 mL of dioxane). The reaction mixture was stirred for 10 min at room temperature, then triethylamine (0.26 mmol) was added, the mixture poured into water (50 mL), the product extracted with diethyl ether (2×10 mL), the extract washed with brine (20 mL), then dried over Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to flash chromatography on 5 g of silica gel (cyclohexane/ethyl acetate, 5:1), affording 60 mg (90%) of **5** as a semi-solid colourless material with all the spectral data fully identical with the described natural product.[11] *(±)-1-Methoxyspirobrassinin* ***3***: To a stirred solution of **5** (91 mg, 0.32 mmol) in 98% acetic acid (9 mL) was added CrO_3 (160 mg, 1.6 mmol) and the mixture stirred for 1 h at room temperature. After pouring into water (40 mL), the product was extracted with diethyl ether (2×30 mL), the extract washed with 10% NaOH (2×15 mL) and brine (15 mL), dried over Na_2SO_4 and the residue obtained after evaporation of the solvent subjected to flash chromatography on 30 g of silica gel (diethyl ether/light petroleum,1:2) to afford 36 mg (40%) of **3** as colourless needles, mp 129–131°C (ethyl acetate/hexane). The spectral data was fully identical with those of the natural product.[10]

The antiproliferative activity of the synthesized compounds was examined by MTT (thiazolyl blue) test,[20] using the selected human cancer cell lines (MDA-MB-231, MCF-7, U-87 MG and CACO-2). The highest activity was found with the natural diastereomer of methoxyspirobrassinol methyl ether **4a** which at concentration of 0.2 μmol L^{-1} inhibited the growth of CACO-2 (colon adenocarcinoma) cell line to 38% of solvent control after 72 h incubation. This finding is consistent with the recommendation of consuming brassica vegetables as a protection against cancer.[21]

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17. **Data for 14a**: White solid, mp 72–74°C ($CHCl_3$/light petroleum); R_f (benzene/acetone, 19:1) 0.21; [Found: C, 54.4; H, 5.5; N, 8.2. $C_{16}H_{20}N_2O_3S_2$ requires C, 54.52; H, 5,72; N, 7.95]; ν_{max} ($CHCl_3$) 3607, 2993, 2940, 2880, 1687, 1570, 1480, 1370, 1163 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 7.59 (1H, d, *J* 8.1 Hz, H-7), 7.42 (1H, dd, *J* 7.6, 0.7 Hz, H-4), 7.28 (1H, ddd, *J* 7.9, 7.9, 1.4 Hz, H-6), 7.04 (1H, ddd, *J* 7.4, 7.4, 1.2 Hz, H-5), 6.95 (1H, d, *J* 6.3 Hz, OH–D_2O exchangeable), 5.63 (1H, d, *J* 6.4 Hz, CH), 4.83 (1H, d, *J* 15.6 Hz, H_b), 4.38 (1H, d, *J* 15.9 Hz, H_a), 2.54 (3H, s, SCH_3), 1.53 (9H, s, $C(CH_3)_3$); δ_C (100 MHz, DMSO-d_6) 161.6 (C), 150.9 (C), 140.6 (C), 130.0 (C), 129.2 (CH), 123.6 (CH), 122.8 (CH), 114.2 (CH), 90.8 (CH), 81.0 (C), 70.6 (C), 65.9 (CH_2), 27.6 (CH_3), 14.3 (SCH_3); *m*/*z* (EIMS) 352 (15, M^+), 296 (72), 252 (20), 234 (17), 161 (32), 145 (71), 117 (23), 87 (20), 57 (100). **Data for 14b**: Colourless plates, mp 126–128 °C (CH_2Cl_2/light petroleum); R_f (benzene/acetone, 19:1) 0.29; [Found: C, 54.3; H, 5.9; N, 8.1. $C_{16}H_{20}N_2O_3S_2$ requires C, 54.52; H, 5,72; N, 7.95]; ν_{max} ($CHCl_3$) 3587, 3013, 2986, 2940, 1683, 1560, 1480, 1366, 1163 cm^{-1}; δ_H (400 MHz, DMSO-d_6) 7.61 (1H, d, *J* 7.8 Hz, H-7), 7.29–7.24 (2H, m, H-4, H-6), 7.05–7.00 (2H, m, H-5, OH-D_2O exchangeable), 5.49 (1H, d, *J* 6.0 Hz, CH), 4.36 (1H, d, *J* 15.4 Hz, H_b), 3.82 (1H, d, *J* 15.1 Hz, H_a), 2.49 (3H, s, SCH_3), 1.54 (9H, s, $C(CH_3)_3$); δ_C (100 MHz, DMSO-d_6) 164.1 (C), 151.0 (C), 139.2 (C), 132.1 (C), 128.8 (CH), 132.2 (CH), 122.6 (CH), 114.1 (CH), 87.6 (CH), 80.9 (C), 74.4 (CH_2), 73.7 (C), 27.6 (CH_3), 14.2 (SCH_3); EIMS identical with **14a**.
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