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Determination of the enantiomeric purity of the phytoalexins spirobrassinins by ^{1}H NMR using chiral solvation

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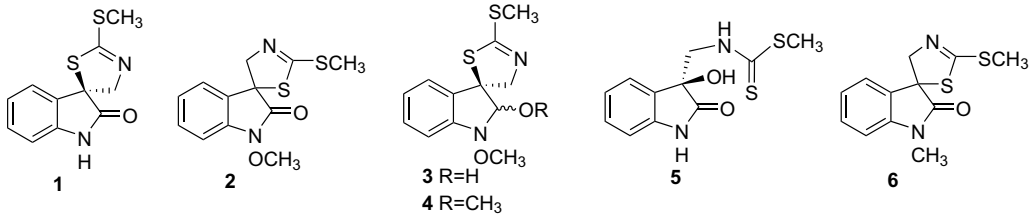
Abstract—A simple and inexpensive method for enantiomeric discrimination of the phytoalexins spirobrassinin (**1**), 1-methoxyspirobrassinin (**2**) and synthetic analog 1-methylspirobrassinin (**6**) using the chiral solvating agent 2,2,2-trifluoro-1-(9-anthryl)ethanol in C_6D_6 is described. Using this method the enantiomeric composition of each sample can be determined accurately by ^{1}H NMR and the compounds can be recovered readily by chromatography.

Phytoalexins are important chemical defenses produced de novo by plants under stress conditions.[1] Spirobrassinin (**1**) and 1-methoxyspirobrassinin (**2**) are unique phytoalexins produced by a large number of cruciferous plants, including turnip (*Brassica rapa* L.), canola (*B. napus*), broccoli (*B. oleracea*), brown mustard (*B. juncea*), and radish (*Raphanus sativus*).[2] Spirobrassinins **1** and **2** are among the over 30 known cruciferous phytoalexins, of which only 5 (**1**–**5**) are chiral.[3] Recently, resolution of racemic synthetic spirobrassinin and determination of its absolute configuration established that naturally occurring (−)-spirobrassinin (**1**) had the *S* configuration,[4] and that naturally occurring **1** isolated from turnip was not enantiomerically pure (95% ee). Thus, subsequent to our isolation of spirobrassinin (**1**) and 1-methoxyspirobrassinin (**2**) from cultivated (rutabaga, *B. napus* L. ssp. *rapifera*),[5] and wild (*Erucastrum gallicum*)[6] plants, it was of interest to determine the enantiopurity of these compounds. Because chiral HPLC[7] did not give baseline resolution of racemic spirobrassinin (**1**), and the specific optical rotation values of small amounts of sample were not sufficiently accurate to determine the enantiomeric purity, NMR methods were sought. Chiral solvating agents (CSA) are a simple and inexpensive choice to determine enantiomeric purity using NMR spectroscopy. CSA have been used for more than three decades to analyze mixtures of enantiomers and measure the enantiomeric composition of samples of chiral compounds of unknown enantiomeric purity using ^{1}H NMR.[8] Here we report a simple and inexpensive method for the determination of enantiomeric purity of the phytoalexins spirobrassinin (**1**), 1-methoxyspirobrassinin (**2**, absolute configuration unknown) and synthetic analog 1-methylspirobrassinin (**6**), using (*R*)-2,2,2-trifluoro-1-(9-anthryl)ethanol (TFAE) in C_6D_6. The enantiomeric composition of samples can be easily determined by ^{1}H NMR, after which the compounds can be recovered by chromatography.



Keywords: Spirobrassinin; 1-Methoxyspirobrassinin; Enantiomeric purity; Chiral solvation.
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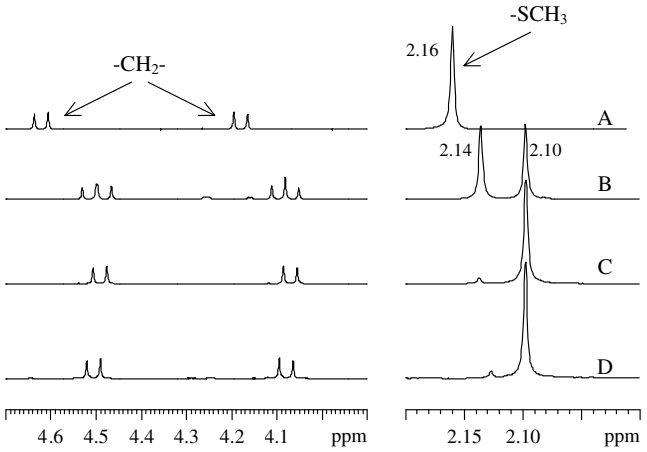

Figure 1. ^{1}H NMR spectra of spirobrassinin (**1**): A—racemic mixture (1.8 mg) in C_6D_6 (500 μL);[14] B—racemic mixture containing 6 equiv of (*R*)-TFAE in C_6D_6 and D_2O (ca. 20 μL); C—synthetic *S* enantiomer containing 6 equiv of TFAE in C_6D_6 and D_2O (ca. 20 μL); D—naturally occurring from cauliflower containing 6 equiv of TFAE in C_6D_6 and D_2O (ca. 20 μL).

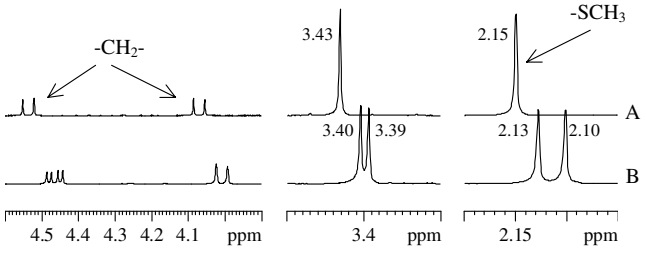

Figure 2. ^{1}H NMR spectra of 1-methoxyspirobrassinin (**2**): A—racemic mixture (1.5 mg) in C_6D_6 (500 μL);[15] B—racemic mixture containing 6 equiv of (*R*)-TFAE in C_6D_6 and D_2O (ca. 20 μL).

(±)-Spirobrassinin (**1**) was synthesized and resolved as previously published,[4] whereas 1-methoxyspirobrassinin (**2**) and 1-methylspirobrassinin (**6**) were synthesized in racemic form[9] only. Initially, the ^{1}H NMR spectra of (±)-spirobrassinin (**1**) was obtained in $CDCl_3$ containing increasing amounts of TFAE. Enantiodifferentiation with peak baseline resolution was observed for the signals corresponding to protons of the (S)CH_3 group when the concentration of TFAE was four times that of **1**. Close inspection of the ^{1}H NMR spectra showed several additional resonances related to spirobrassinin, suggesting modifications in its structure. Eventually we discovered that spirobrassinin (**1**) decomposed slowly (<5% in 24 h) on standing in $CDCl_3$ to yield a mixture of undetermined compounds. Next, additional deuterated solvents in which spirobrassinin was stable were tested. Although spirobrassinin (**1**) appeared stable in both CD_3OD and CD_3CN, these solvents did not allow sufficient chiral discrimination of both spirobrassinin enantiomers. Finally, chiral discrimination of the (S)CH_3 groups of spirobrassinins **1**, **2**, and **6** was achieved in C_6D_6 containing 6 equiv of (*R*)-TFAE and D_2O (to exchange OH of TFAE, whose chemical shift at δ_H 2.20 is close to that of SCH_3–δ_H 2.16–2.10; the presence of D_2O did not affect the chemical shifts of SCH_3 group), as shown in Figures 1–3. As expected, naturally occurring samples of spirobrassinin (**1**) isolated from rutabaga ($[\alpha]_D^{20}$ −53; *c* 0.30 g/100 mL in $CHCl_3$),[5] and cauliflower[10] ($[\alpha]_D^{20}$ −109; *c* 0.35 g/100 mL in CD_2Cl_2) were determined to have the *S* configuration (Fig. 1D) upon comparison with an authentic sample of (*S*)-spirobrassinin (**1**) synthesized[11] and resolved as previously reported (Fig. 1C).[12] The enantiomeric excess of resolved synthetic and naturally occurring spirobrassinin (**1**) samples could be accurately measured by integration of the areas of the ^{1}H NMR peaks corresponding to the (S)CH_3 group of each enantiomer (δ_H 2.14 for *R* and 2.10 for *S*). The signals corresponding to the CH_2 group were only partially differentiated. The enantiomers of 1-methoxyspirobrassinin (**2**) and 1-methylspirobrassinin (**6**)[13] could also be discriminated, and the percentage of each enantiomer could be measured accurately by integration of areas of the ^{1}H NMR peaks corresponding to the (S)CH_3 group of each one (**2**, δ_H 2.13 and 2.10; **6**, δ_H 2.16 and 2.13), as shown in Figures 2 and 3, respectively. However, since the absolute configuration of naturally occurring 1-methoxyspirobrassinin (**6**) is unknown, the peak corresponding to each enantiomer could not assigned. In the case of **2** and **6**, the peaks corresponding to the additional methyl groups were also partially resolved (Figs. 2 and 3, respectively).

In conclusion, we found a very simple and inexpensive method to determine the enantiopurity of naturally occurring spirobrassinins **1** and **2**. Considering that crucifers do not appear to produce enantiomerically pure phytoalexins,[4] this method will be very useful to establish the enantiomeric purity of these naturally occurring

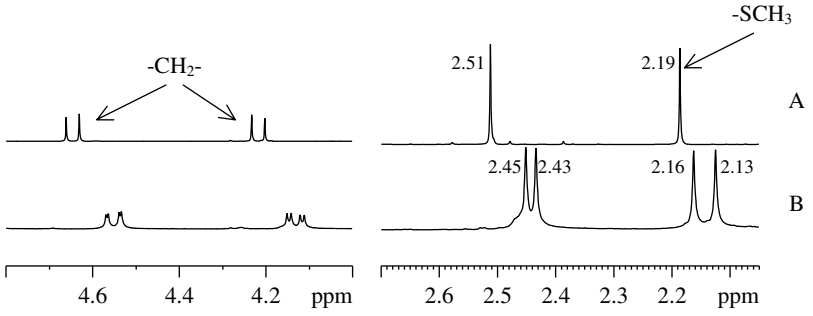

Figure 3. ^{1}H NMR spectra of 1-methylspirobrassinin (**6**): A—racemic mixture (1.4 mg) in C_6D_6 (500 μL);[13] B—racemic mixture containing 6 equiv of (*R*)-TFAE in C_6D_6 and D_2O (ca. 20 μL).

compounds. Furthermore, because spirobrassinins appear to display antiproliferative activity against various cancer cell lines and that such activity is associated with the modulation of activity of factors that regulate cell cycle,[16] the enantiomeric purity of these compounds may be of significance. Consequently, this method should be valuable to establish the enantiopurity of these phytoalexins and related compounds in future structure–activity studies.[17]

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13. 1-Methylspirobrassinin (**6**) was prepared by methylation of (±)-spirobrassinin (**1**) (MeI/NaH, 1.5 equiv in dry THF). ^{1}H NMR (500 MHz, C_6D_6) δ 7.07 (dd, J = 7.5, 1 Hz, 1H), 6.89 (ddd, J = 7.5, 7.5, 1 Hz, 1H), 6.71 (ddd, J = 7.5, 7.5, 1 Hz, 1H), 6.08 (d, J = 7.5 Hz, 1H), 4.65 (d, J = 15.1 Hz, 1H), 4.22 (d, J = 15.1 Hz, 1H), 2.51 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (125 MHz, CD_3CN): δ 176.0 (s), 163.2 (s), 143.5 (s), 130.6 (s), 130.2 (d), 124.1 (d), 123.6 (d), 109.2 (d), 75.0 (t), 64.5 (s), 25.7 (q), 15.3 (q). HRMS-EI *m*/*z*: measured 264.0389 (M^+, calcd 264.0391 for $C_{12}H_{12}N_2OS_2$). MS-EI *m*/*z* (relative intensity): 264 (M^+, 67), 217 (82), 191 (100), 130 (41), 87 (41), 71 (32). FTIR ν_{max}: 2928, 1712, 1611, 1583, 1491, 1470, 1370, 1345, 1091, 991, 939 cm^{-1}.
14. Spirobrassinin (**1**): ^{1}H NMR (500 MHz, C_6D_6) δ 6.99 (d, J = 7.5 Hz, 1H), 6.93 (br s, 1H, D_2O exchangeable), 6.81 (dd, J = 7.5, 7.5 Hz, 1H), 6.65 (dd, J = 7.5, 7.5 Hz, 1H), 6.11 (d, J = 7.5 Hz, 1H), 4.62 (d, J = 15 Hz, 1H), 4.18 (d, J = 15 Hz, 1H), 2.16 (s, 3H).
15. 1-Methoxyspirobrassinin (**2**): ^{1}H NMR (500 MHz, C_6D_6) δ 6.97 (d, J = 7.5 Hz, 1H), 6.89 (dd, J = 7.5, 7.5 Hz, 1H), 6.68 (dd, J = 7.5, 7.5 Hz, 1H), 6.54 (d, J = 7.5 Hz, 1H), 4.54 (d, J = 15.5 Hz, 1H), 4.07 (d, J = 15.5 Hz, 1H), 3.43 (s, 3H), 2.15 (s, 3H).
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