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The first isolation and crystal structure of a boron difluoro complex (isoflavone yellow). Biologically active intermediates produced during isoflavone synthesis

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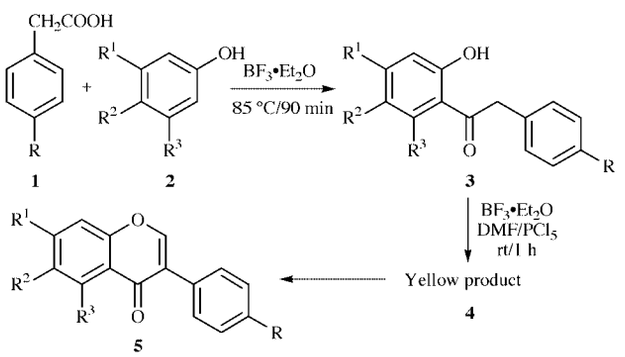
The yellow intermediate **4f** produced during the reaction of the deoxybenzoin **3f** with *N*,*N*′-dimethyl(chloromethylene)ammonium chloride in the presence of $BF_3{\cdot}Et_2O$ prior to the formation of the corresponding isoflavone was isolated for the first time and characterized by NMR, MS and single-crystal X-ray diffraction techniques. These yellow intermediates showed anticancer, nematicidal and mosquitocidal activities.

Introduction

Isoflavones (3-phenyl-4*H*-1-benzopyran-4-ones) which exist in plants, fruits, seeds and flowers of species belonging to the *Leguminosae* family are known to possess many biological activities. These compounds are extensively studied for their estrogenic,[1a] anticancer,[1b] antibacterial,[1c] antifungal[1d] and antimicrobial[1e] activities. We have shown that[1f–l] the isoflavone formononetin (Mycoform®) is very effective in stimulating the growth of vesicular arbuscular mycorrhizal (AM) fungi and enhances the growth of many plant species that are host to AM fungi. Over the years many synthetic methods, such as the oxidative rearrangement of chalcones,[2a–c] the ring closure of benzyl phenyl ketones,[2d–i] the rearrangement of flavonone to isoflavone by hydroxy(tosyloxy)iodobenzene[2j] and other methods[2k–m] have been developed for the synthesis of isoflavones. However, these methods are unsuccessful for their scale-up due to economic reasons. We have recently reported[3] a 'one pot' and a two-step process for the synthesis of isoflavones. In order to optimize the reaction conditions, we have studied the mechanism for the formation of isoflavones by monitoring the intermediates formed under different conditions using various reagents. The deoxybenzoin route used for the synthesis of isoflavones involved the addition of a single carbon at the benzylic position followed by cyclization. In our reported method, the cyclization step, which was carried out in the same vessel, involved the treatment of a deoxybenzoin **3** with *N*,*N*′-dimethyl(chloromethylene)ammonium chloride[4] in the presence of $BF_3{\cdot}Et_2O$. This reaction, carried out under very mild conditions, yielded a mixture of the desired isoflavone **5** and an unstable yellow compound **4**. Treatment of this yellow product with dilute acid converted it into the corresponding isoflavone **5**, making it a simple process for the production of isoflavones in high yields and purity.

Results and discussion

The overall procedure consisted of the reaction of a phenylacetic acid **1** and a phenol **2** in the presence of a Lewis acid ($BF_3{\cdot}Et_2O$) to form the corresponding deoxybenzoin **3**, and its treatment with *N*,*N*′-dimethyl(chloromethylene)ammonium chloride to form the isoflavone **5** (Scheme 1). Attempts to purify the yellow product generated during the synthesis of several isoflavones like formononetin **5d**, daidzin **5c**, genistin **5j** and biochanin-A **5k** using column chromatography were unsuccessful. In the case of formononetin and daidzin, the purification



5a R = R^2 = R^3 = H, R^1 = OH
5b R = R^2 = R^3 = H, R^1 = OCH_3
5c R = R^1 = OH, R^2 = R^3 = H
5d R = OCH_3, R^1 = OH, R^2 = R^3 = H
5e R = OH, R^1 = OCH_3, R^2 = R^3 = H
5f R = R^1 = OCH_3, R^2 = R^3 = H
5g R = R^2 = OH, R^1 = R^3 = H
5h R = R^3 = OH, R^1 = R^2 = H
5i R = OCH_3, R^1 = R^2 = H, R^3 = OH
5j R = R^1 = R^3 = OH, R^2 = H
5k R = OCH_3, R^1 = R^3 = OH, R^2 = H

Scheme 1

was extremely difficult due to overlapping R_f-values for the isoflavones and their respective yellow products. However, genistin **5j** and biochanin-A **5k** contained very little of the yellow product and converted rapidly on the column to their corresponding isoflavones. The reaction involving the formation of 4′,7-dimethoxyisoflavone **5f** yielded a yellow, oily mixture and further purification yielded a product in pure form.

In the two-step synthesis of isoflavones, a minimum of 3 mole equivalents of $BF_3{\cdot}Et_2O$ was required for the completion of the reaction. The yellow product was absent when the reaction was carried out in the absence of $BF_3{\cdot}Et_2O$ in DMF. Similarly, the yellow product was not formed when the reaction was conducted with $BF_3{\cdot}Et_2O$ and DMF in the absence of reagent *N*,*N*′-dimethyl(chloromethylene)ammonium chloride. Also, extending the reaction time to two hours did not yield the yellow product. Similar results were obtained for the 'one-step' process as well. Our results indicate that *N*,*N*′-dimethyl(chloromethylene)ammonium chloride is required for the reaction and that the yellow product is the key intermediate in the formation of isoflavone products.

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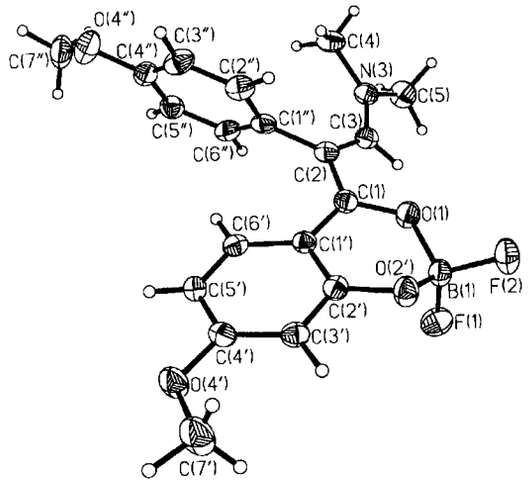


Fig. 1 The structure of 1-(2′-difluoroboryloxy-4′-methoxyphenyl)-3-dimethylamino-2-(4″-methoxyphenyl)-prop-2-enone **4f**. Relevent bond distances (Å) and bond angles (°): F(1)–B(1) 1.387(3), F(2)–B(1) 1.383(2), B(1)–O(2′) 1.452(3), B(1)–O(1) 1.501(3), O(1)–C(1) 1.327(2), C(1)–C(2) 1.411(3), C(1)–C(1′) 1.458(3), C(2)–C(3) 1.406(3), C(2)–C(1″) 1.496(3), C(3)–N(3) 1.319(2), C(2′)–O(2′) 1.350(2); F(2)–B(1)–F(1) 110.35(17), F(2)–B(1)–O(2′) 109.74(17), F(1)–B(1)–O(2′) 111.48(17), F(2)–B(1)–O(1) 106.48(16), F(1)–B(1)–O(1) 108.64(16), O(2′)–B(1)–O(1) 110.02(16), C(1)–O(1)–B(1) 123.02(15), O(1)–C(1)–C(2) 115.93(17), O(1)–C(1)–C(1′) 116.91(16), C(2)–C(1)–C(1′) 127.17(17), C(3)–C(2)–C(1) 115.43(18), C(3)–C(2)–C(1″) 121.59(17), C(1)–C(2)–C(1′) 122.66(17), N(3)–C(3)–C(2) 129.4(2), N(3)–C(3)–H(3) 113.2(12), C(3)–N(3)–C(4) 124.99(18), C(2′)–O(2′)–B(1) 116.45(15).

The ^{1}H NMR spectrum of the yellow product **4f**, isoflavone yellow, showed peaks similar to those of its corresponding isoflavone. Peaks at δ 3.74 and 3.83 were assigned to two methoxy groups, a doublet of a doublet at δ 5.98 for H-6, a doublet at δ 6.43 for H-8, a doublet at δ 6.60 for H-5, and two doublets at δ 6.89 and 7.08 for B-ring protons H-2′, -3′, -5′ and -6′. In addition, two broad signals at δ 2.54 and 3.31 integrating for 3 protons each, and a singlet at δ 8.32 integrating for one proton, observed in its ^{1}H NMR spectrum confirmed the presence of an *N*,*N*′-dimethyleneamine moiety in the molecule. A comparison of ^{1}H NMR chemical shifts with that of the isoflavone **5f** showed appreciable upfield shifts for H-5, H-6 and H-7 of the A-ring protons as well as the absence of the ubiquitous singlet around δ 7.8 for the vinylic proton on C-2. The ^{13}C NMR spectrum of the yellow product showed peaks at δ_C 36.25, 44.52 and 159.96 and confirmed the presence of a DMF moiety as part of the molecule (DMF signals are at δ_C 31.1, 36.2 and 162.4, respectively). Another interesting feature in its ^{13}C NMR spectrum was the upfield shift of the carbonyl peak. The carbonyl peak in **4f** appeared at δ_C 161.76, as compared with δ_C 174.65 for its corresponding isoflavone.

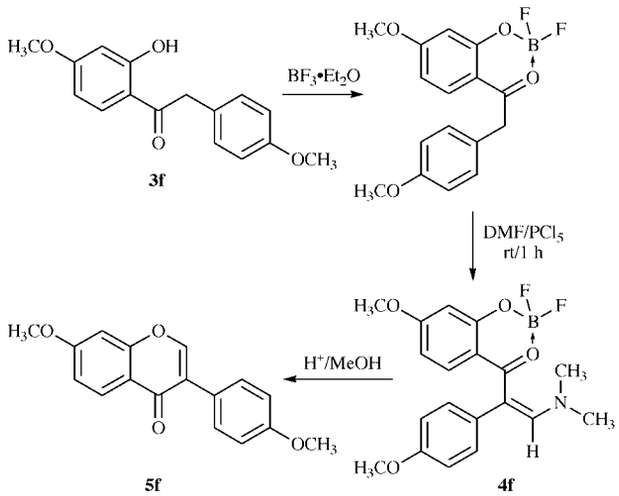
The ^{1}H NMR spectrum for the yellow product **4d** isolated from the reaction involving the formation of formononetin **5d** also showed two broad singlets integrating for three protons each at δ 2.55 and 3.33, respectively, and a singlet integrating for one proton at δ 8.33. Again, this confirmed the presence of an *N*,*N*′-dimethyleneamine moiety.

Fast-atom bombardment mass spectroscopic (FAB MS) analysis of **4f** gave a molecular ion at *m*/*z* 375 and indicated the presence of additional moieties in the product that were not revealed by its NMR spectra. The fragment ions at *m*/*z* 356, 331, and 199 indicated the loss of a fluorine atom, $N(CH_3)_2$, and $C_{11}H_{14}NO$ (176), respectively. A similar result was obtained for **4d** which gave a molecular ion at *m*/*z* 361.

After several attempts to produce single crystals of the yellow product **4f** in different solvent systems, a DMSO–acetone mixture yielded suitable crystals for X-ray diffraction study. The structure of **4f**, as confirmed by the X-ray diffraction data, is depicted in Fig. 1.

The structural data for 1-(2′-difluoroboryloxy-4′-methoxyphenyl)-3-(dimethylamino-2-(4″-methoxyphenyl)-prop-2-enone, **4f**, indicated that the bond lengths for F–B and B–O bonds are similar to those reported for other boron compounds, and the bond angles for F–B–F, F–B–O, O–B–O were in agreement with the published data.[5] Similarly the bond lengths for C–N, C–C and N–C were in agreement with the reported data for *N*,*N*′-dimethyleneamine, $(H_3C)_2N$–CH=C, bonds.[6]

The presence of BF_3·Et_2O in the reaction mixture prior to the addition of *N*,*N*′-dimethyl(chloromethylene)ammonium chloride leads to the existence of a difluoroboron deoxybenzoin complex followed by the formation of the isoflavone yellow. In the case of 4′,7-dimethoxyisoflavone **5f**, the mechanism for the formation of the isoflavone yellow can be explained by the initial formation of a difluoroboryl complex of 2-hydroxy-4,4′-dimethoxydeoxybenzoin followed by attack of the electrophilic reagent *N*,*N*′-dimethyl(chloromethylene)ammonium chloride at the benzylic position to yield **4f** (Scheme 2). Under acidic



Scheme 2

conditions, the difluoroboryl complex converts to the isoflavone **5f** *via* cyclization.

A comparison of the spectral data (NMR, MS and UV–visible) for isoflavone yellow and the X-ray diffraction data for the yellow product **4f** confirmed that a difluoroboryl complex similar to **4f** is a common yellow intermediate formed during the formation of isoflavones. Therefore, it is possible that the formation of difluoroboryl complex (isoflavone yellow) is crucial for the conversion of deoxybenzoins to isoflavones under mild conditions in high yield and purity.

Bioassays performed on **4c**, **4d** and **4f** showed nematicidal,[7a] mosquitocidal[7b] and topoisomerase I- and II-inhibitory[7c] activities at 250 ppm concentration. Further work is in progress to study the anticancer activity of these yellow intermediates.

Experimental

The melting points were determined on a Bristoline micro melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Varian VXR 300 MHz spectrometer. The fast-atom bombardment mass spectroscopic (FAB MS) analysis was carried out on a JEOL JMS-HX110 using a NBA matrix. The UV-Visible analysis was performed on a Shimadzu UV-260 spectrophotometer. The X-ray diffraction studies were conducted on a Siemens SMART CCD Diffractometer. The starting materials, solvents and reagents used were purchased from Aldrich Chemical Company and were used without further purification.

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1-(2′-Difluoroboryloxy-4′-methoxyphenyl)-3-dimethylamino-2-(4″-methoxyphenyl)prop-2-enone 4f

A mixture of 1-(2-hydroxy-4-methoxyphenyl)-2-(4-methoxyphenyl)ethanone **3f** (0.816 g, 3 mmol) and $BF_3{\cdot}Et_2O$ (1.2 mL, 9 mmol) was cooled to 10 °C and DMF (4.6 mL) was added dropwise. In another flask, DMF (8 mL) was cooled to 10 °C and PCl_5 (0.939 g, 4.5 mmol) was added in small portions. The mixture was then kept at 55 °C for 20 min. The pale pink solution containing *N*,*N*′-dimethyl(chloromethylene)-ammonium chloride was then added to the above reaction mixture slowly. During the addition the temperature of the reaction mixture was maintained below 27 °C. The mixture was then stirred at room temperature for 5 min. The orange-yellow, viscous solution was then poured into aq. NaOAc (12.5%; 200 mL) and the product was extracted with EtOAc (stored over K_2CO_3). The orange layer was washed with brine and dried over Na_2SO_4. The solvent was removed using a rotary evaporator and the crude product was vacuum dried to yield 1.05 g of crude title product (90%). This product was purified by column chromatography on silica using EtOAc–light petroleum (distillation range 68–70 °C) (30%) to yield 1.01 g (87%) of **4f** as a bright yellow amorphous powder, mp 162–164 °C; λ_{max} 417.4 (ε_{max} dm^3 mol^{-1} cm^{-1} 37 050), 291.4 (8694) and 256.8 nm (9526); ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1H), 7.08 (d, *J* 8.9 Hz, 2H), 6.89 (d, *J* 8.9 Hz, 2H), 6.60 (d, *J* 9.7 Hz), 6.43 (d, *J* 2.7 Hz, 1H), 5.98 (dd, *J* 9.2 and 2.8 Hz, 1H), 3.83 (s, 3H), 3.74 (s, 3H), 3.31 (s, 3H), 2.54 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 161.76, 159.96, 155.00, 154.78, 128.60, 128.18, 123.18, 110.20, 103.70, 97.77, 50.94, 50.78, 44.52, 36.25 [Found: *m*/*z* 375.1444 (M$^+$). $C_{19}H_{20}BF_2NO_4BF_2$ requires *M*, 375.1457]; *m*/*z* 375 (M$^+$), 356, 331, 199, 154 (100%) and 136.

Crystallographic data for 1-(2′-difluoroboryloxy-4′-methoxyphenyl)-3-dimethylamino-2-(4″-methoxyphenyl)-prop-2-enone †

Single crystals of 1-(2′-difluoroboryloxy-4′-methoxyphenyl)-3-dimethylamino-2-(4″-methoxyphenyl)prop-2-enone **4f**, were recrystallized from DMSO–acetone, mounted in 'Paratone-N' and transferred to the cold gas stream of the diffractometer. The structure was solved using direct methods and refined by full matrix least-squares on F^2.

$C_{19}H_{20}BF_2NO_4$, 375.17, orthorhombic, space group *Pbca*, a = 14.056(3), b = 13.921(3), c = 18.250(4) Å, V = 3571.3(12) A^3, T = 173 K, Z = 8, D_c = 1.396 Mg m^{-3}, $F(000)$ = 1568, absorption coefficient = 0.110 mm^{-1}, crystal size = 0.08 × 0.10 × 0.36 mm, 30962 reflections measured, 3147 unique (R_{int} = 0.0658) which were used in all calculations. The final $wR(F^2)$ was 0.095 (all data).

† CCDC reference number 207/386. See http://www.rsc.org/suppdata/p1/a9/a908915b for crystallographic files in .cif format.

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