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Total synthesis of bioactive frustulosin and frustulosinol

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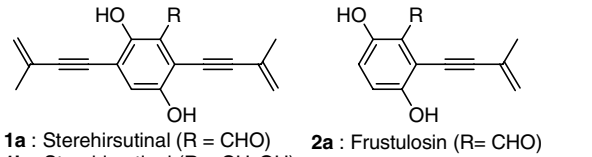
Abstract—*Stereum hirsutum* is a pathogenic fungus involved in 'Esca', a trunk wood disease of grapevine. Among the metabolites isolated from the culture medium of this fungus, frustulosin (**2a**) shows a high phytotoxicity. A new simple and efficient method for the synthesis of frustulosin (**2a**) and frustulosinol (**2b**) was developed. Antibacterial activity and phytotoxicity are reported.

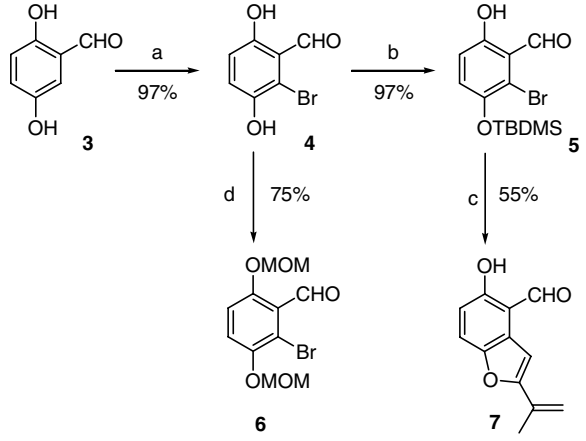
Different fungi infect grapevines and cause severe diseases leading to important economical losses. One of the oldest known is Esca.[1] This disease, progressing worldwide, is associated with a group of fungi that includes *Stereum hirsutum*.[2] To find an alternative control method against Esca, as efficient as the highly toxic sodium arsenite treatment, now forbidden, the search for the pathogenically active secondary metabolites appears to be necessary. *S. hirsutum* seems to play an important role in the wood deterioration process.[2] In a previous work,[3] we described the isolation and the identification of seven secondary metabolites from the culture medium, and the synthesis of two phytotoxic acetylenic compounds, sterehirsutinal (**1a**) and sterehirsutinol (**1b**) (Scheme 1).[4] Frustulosin (**2a**) and frustulosinol (**2b**) were also isolated from the same toxic medium. These compounds were previously described as metabolites of *Stereum frustulosum* and exhibited antimicrobial activity at modest concentrations, particularly against *Staphylococcus aureus*, several Bacilli and *Vibrio cholera*.[5]

In order to understand the role played by compounds **2a** and **2b**, large amount of material is required for more specific biological assays. We thus undertook the synthesis of these two compounds **2a** and **2b**. Two different pathways were already described for the synthesis of frustulosin (**2a**).[6,7] Herein, we report a simpler and more efficient procedure.



Scheme 1. Phytotoxins extracted from *Stereum hirsutum*.

Ronald and co-workers described the preparation of 2-iodo-3,6-dihydroxybenzaldehyde in five steps as intermediate in the frustulosin (**2a**) synthesis.[7] Then, the key step was the coupling reaction between this iodo derivative and appropriate alkyne to lead to frustulosin (**2a**). Thus, we decided to use bromo analogue **4** as key synthon, obtained in one step from commercially available 2,5-dihydroxybenzaldehyde (**3**) (Scheme 2).[8] This



Scheme 2. Reagents and conditions: (a) Br_2, $CHCl_3$, rt, 3 h; (b) TBDMSCl, imidazole, CH_2Cl_2, rt, 15 min; (c) 2-methylbut-1-en-3-yne, CuI, $[PdCl_2(PPh_3)_2]$, Et_3N, DMF, 110 °C, 3 h; (d) MOMCl, iPr$_2$EtN, CH_2Cl_2, rt, 1 h.

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bromination reaction afforded exclusively 2-bromo-3,6-dihydroxybenzaldehyde (**4**) in 97% yield.

Ronald and co-workers achieved the substitution of the iodide by the alkenynyl chain thanks to copper(I) isopropenylacetylide in DMF. A low conversion was observed and a large amount of starting material was recovered. A mixture of frustulosin (**2a**) (less than 10% yield) and benzofuran derivative **7** was obtained. Using the same procedure, we attempted this transformation with the same poor results. We investigated the cross-coupling reaction between 2-methylbut-1-en-3-yne and the aryl bromide **4** by the Sonogashira reaction[9] in the presence of cuprous iodide, bis(triphenylphosphine)-palladium dichloride and triethylamine in DMF. Unfortunately, the results were similar to the cuprate pathway and we obtained mainly the benzofuran derivative **7**.

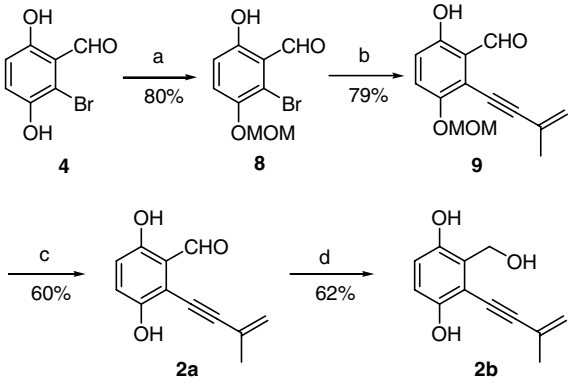
In order to avoid the formation of **7**, the hydroxyl group in *meta* position of the aldehyde had to be protected. TBDMS protecting group was chosen and introduced according to the Hu procedure.[8] This method allowed a regioselective silylation of the most reactive hydroxyl group of **4**, the hydroxyl group in *ortho* position of bromide. Indeed, the other hydroxyl group is less acidic because of an intramolecular H-bond with aldehyde function that stabilizes the hydroxyl group. Thus, compound **5** was obtained in 97% yield (Scheme 2).

At this step, the 2-methylbut-1-en-3-ynyle chain had to be introduced into compound **5**. No reaction occurred when the Sonogashira cross-coupling was realized at 70 °C. But beyond this temperature, the silyl ether was cleaved and the benzofuran derivative **7** formed as the major product (Scheme 2). Tentatively, the reaction using the cuprate[6] was also tested but unsuccessfully.

The MOM group was used to protect hydroxyl groups as in the syntheses of siccayne,[10] eutypine,[11] and stere-hirsutinal,[4] that include a Sonogashira reaction for the acetylenic side chain introduction. Thus, we had obtained the protected compound **6** in 75% yield (Scheme 2). No reaction occurred in Sonogashira conditions between **6** and acetylenic derivative.

The regioselective protection of the most nucleophilic hydroxy group of **4** was achieved with 1.05 equiv of MOMCl affording **8** in 80% yield (Scheme 3). The Sonogashira reaction between bromide **8** and 2-methylbut-1-en-3-yne led to the protected frustulosin **9** in 79% yield.[12] In this case, the presence of MOM group in *meta* position of the bromine atom is deactivating for the Sonogashira reaction. Finally, frustulosin (**2a**) was obtained after MOM cleavage using a solution of 10% TFA in CH_2Cl_2 (v/v) in 60% yield.[13] Biologically interesting frustulosinol (**2b**) was obtained in 62% yield by chemioselective reduction of frustulosin (**2a**) with $NaBH_4$ in aqueous medium.[14]

Preliminary phytotoxic tests were carried out on grapevine leaf discs. Solutions of frustulosin (**2a**) and frustulosinol (**2b**) in 98:2 H_2O/ethanol were prepared in



Scheme 3. Reagents and conditions: (a) MOMCl, *i*Pr_2EtN, CH_2Cl_2, rt, 1 h; (b) 2-methylbut-1-en-3-yne, CuI, [$PdCl_2(PPh_3)_2$], Et_3N, DMF, 60 °C, 3 h; (c) 10:90 TFA/CH_2Cl_2 (v/v), rt, 5 h; (d) $NaBH_4$, 1% (w/v) aq NaOH soln, rt, 2 h.

different concentrations. The same test was realized simultaneously with eutypine as reference, another fungal toxin of grapevine, previously isolated in our laboratory that shows a high phytotoxic activity.[15]

Necroses already appeared at different levels on leaves before 18 h at 25 °C the concentrations of 500, 250, 100 and 50 mg/L for frustulosin (**2a**). At the concentration of 25 mg/L, necroses were observed only between 18 and 24 h. The appearance of necroses caused by eutypine was less important and no phytotoxicity was observed at the concentration of 25 mg/L. Frustulosinol (**2b**) was less toxic, necroses were only observed at 250 and 500 mg/L.

Compounds were also tested on grapevine callus (kindly supplied by Roustan, INRA, Toulouse, France). Callus cultures of *Vitis vinifera* cv. Gamay were grown on a synthetic medium, 12 h daylight for 28 days at 28 °C. Calli were weighed before and after incubation with different concentrations of **2a**, **2b** and eutypine and percent growth was calculated. Frustulosin (**2a**) and frustulosinol (**2b**) inhibit 100% callus growth at 20 mg/L and 50 mg/L, respectively.

In addition, antibacterial tests were realized with frustulosin (**2a**). Activities were measured on TLC by bioautography on thin-layer plates. Test solutions (10 μL) were applied as small spots on TLC plates (Silicagel G) to give a concentration series of 0.1–30 μg/application zone. The organic solvent was evaporated by steam air and plates were eluted by the adequate solvent to obtain different R_fs. The solvent was evaporated once again and the plates homogeneously sprayed with 10 mL of nutrient agar infected with 1 ml of the nutrient broth containing bacteria (10^8–10^9 CFU/ml). Plates were incubated for 2 days at 30 and 37 °C in the dark. The appearance of a blank after spraying the plate with a solution of thiazolyl blue tetrazolium bromide, indicated antibacterial activity. Minimum inhibitory concentrations (MIC) against *Bacillus subtilis* and *Escherichia coli* were determined from the lowest tested compound concentration causing recognizable bacterial growth inhibition. Chloramphenicol was used as posi-

tive control and acetone as negative. Antibacterial activity for frustulosin (**2a**) was observed at 3 and 10 μg, respectively, against *B. subtilis* and *E. coli*.

In conclusion, this new synthetic route supplies rapidly a large amount of frustulosin (**2a**) with a total yield of 37% in only four steps. Frustulosinol (**2b**) is also obtained in good yield. This procedure can be applied to the synthesis of other phytotoxins belonging to the same family, that is, sterehirsutinal, isolated from *S. hirsutum*. Biological activities of synthesized compounds have been checked. Phytotoxicity has been exhibited on grapevine and the antibacterial activity has been shown against *B. subtilis* and *E. coli*. The efficiency of the method allows the large scale synthesis of such compounds in order to investigate more advanced biological tests.

Acknowledgements

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12. Sonogashira reaction procedure: 2-Hydroxy-5-(methoxymethyloxy)-6-(3-methylbut-3-en-1-ynyl)benzaldehyde **9**. A solution of **8** (4.73 g, 18.1 mmol, 1.0 equiv), CuI (1.72 g, 9.06 mmol, 0.5 equiv), $[PdCl_2(PPh_3)_2]$ (3.18 g, 4.53 mmol, 25 mol %), Et_3N (10.1 mL, 72.5 mmol, 4.0 equiv) and 2-methylbut-1-en-3-yne (6.9 mL, 72.5 mmol, 4.0 equiv) in 72 mL of DMF was heated at 60 °C under N_2 atmosphere for 3 h. The mixture was then diluted with Et_2O, washed twice with 5% aq HCl soln and satd aq NaCl soln, dried over $MgSO_4$, and concentrated. Purification by flash chromatography on silica gel (eluant: *n*-hexane/acetone 97:3) provided 3.52 g (79%) of **9** as an orange oil: IR (film) 2924, 1652, 1466, 1272, 1154, 1016; ^{1}H NMR δ ($CDCl_3$, 400 MHz): 11.40 (s, 1H, C_6-OH), 10.42 (s, 1H, CHO), 7.33 (d, $J = 9.1$ Hz, 1H, C_4-H), 6.89 (d, $J = 9.1$ Hz, 1H, C_5-H), 5.48 (qt_{app}, $^2J = {}^4J = 1.0$ Hz, 1H, CH_2=), 5.40 (qt_{app}, $^2J = {}^4J = 1.6$ Hz, 1H, CH_2=), 5.19 (s, 2H, OCH_2O), 3.56 (s, 3H, OCH_3), 2.03 (dd, $^4J_{cisoïde} = 1.1$ Hz, $^4J_{transoïde} = 1.4$ Hz, 3H, CH_3); ^{13}C NMR δ ($CDCl_3$, 100 MHz): 197.2, 158.2, 151.6, 127.1, 126.7, 123.8, 119.5, 119.0, 117.6, 102.3, 96.8, 80.1, 56.8, 23.5; MS (ESI^-) $m/z = 245.1$ $[M-H]^-$.
13. Deprotection of compound **9**: 2,5-Dihydroxy-6-(3-methylbut-3-en-1-ynyl)benzaldehyde (frustulosin) **2a**: To a solution of **9** (1.85 g, 7.51 mmol, 1.0 equiv) in 67.5 mL of CH_2Cl_2 was added 7.5 mL of TFA at 0 °C. The reaction mixture was stirred for 6 h and then TFA was removed in vacuo. Purification by flash chromatography on silica gel (eluant: *n*-hexane/EtOAc 95:5) provided 915 mg (60%) of frustulosin **2a** as a yellow solid: IR (KBr disc) 3281, 2923, 1641, 1464, 1280, 1149; ^{1}H NMR δ ($CDCl_3$, 400 MHz): 11.25 (s, 1H, C_6–OH), 10.33 (d, $^5J = 0.5$ Hz, 1H, CHO), 7.19 (d, $J = 9.1$ Hz, 1H, C_4–H), 6.92 (dd, $^3J = 9.1$ Hz, $^5J = 0.5$ Hz, 1H, C_5–H), 5.58 (s, 1H, C_3–OH), 5.54 (qt_{app}, $^2J = {}^4J = 1.0$ Hz, 1H, CH_2=), 5.40 (qt_{app}, $^2J = {}^4J = 1.6$ Hz, 1H, CH_2=), 2.06 (dd, $^4J_{cisoïde} = 1.1$ Hz, $^4J_{transoïde} = 1.5$ Hz, 3H, CH_3); δ ($CDCl_3$, 100 MHz): 196.3, 157.1, 150.6, 125.9, 124.9, 124.8, 120.0, 118.3, 110.7, 104.4, 78.3, 23.6; MS (ESI^-) $m/z = 201.5$ $[M-H]^-$.
14. Reduction of frustulosin (**2a**): 2-Hydroxymethyl-3-(3-methylbut-3-en-1-ynyl)benzene-1,4-diol (frustulosinol) **2b**: To **2a** (85 mg, 0.42 mmol, 1.0 equiv) in 1% aq NaOH soln (10 mL) was added dropwise 10% aq NaOH soln (1 mL) of $NaBH_4$ (21 mg, 0.55 mmol, 1.3 equiv). After 2 h, the reaction mixture was diluted with EtOAc and acidified with 5% aq HCl soln. The organic layer was then washed with aq $NaHCO_3$ soln, dried over $MgSO_4$ and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (eluant: *n*-hexane/EtOAc 80:20) to give frustulosinol **2** (86 mg, 62%) as a yellow solid: IR (KBr disc) 3371, 2937, 1473, 1237, 1169; ^{1}H NMR δ ($CDCl_3$, 400 MHz): 7.61 (s, 1H, OH), 6.78 (s, 2H, C_5–H, C_6–H), 5.52 (s, 1H, OH), 5.44 (qt_{app}, $^2J = {}^4J = 0.8$ Hz, 1H, CH_2=), 5.38 (qt_{app}, $^2J = {}^4J = 1.5$ Hz, 1H, CH_2=), 2.94 (s, 1H, OH), 2.02 (dd, $^4J_{cisoïde} = 0.8$ Hz, $^4J_{transoïde} = 1.4$ Hz, 3H, CH_3); ^{13}C NMR δ ($CDCl_3$, 100 MHz): 150.2, 149.5, 125.9, 124.8, 123.2, 118.6, 114.8, 107.8, 101.7, 79.9, 62.6, 23.4; MS (ESI^-) $m/z = 203.6$ $[M-H]^-$.
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