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Biotransformation of sinapic acid by the green algae *Stichococcus bacillaris* 155LTAP and *Ankistrodesmus braunii* C202.7a

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Abstract—Sinapic acid was bioconverted by the green alga *Stichococcus bacillaris* into 4-hydroxy-3,5-dimethoxybenzoic acid, 4-hydroxy-3,5-dimethoxybenzaldehyde and 4-hydroxy-3,5-dimethoxybenzylic alcohol. Incubation of sinapic acid in a culture of the alga *Ankistrodesmus braunii* gave 3,6-dihydroxy-2,4-dimethoxy-7*H*-benzocyclohepten-7-one, a new compound formed by bioconversion of thomasidioic acid, the primary oxidative product of sinapic acid.

In a recent study[1] we reported that some strains of green algae are able to degrade phenols, selected from olive-oil mill wastewaters, within 5 days, affording a rate of removal higher than 70%.

During this study it was observed that some algae can biotransform phenols, and good results were obtained especially with sinapic acid (**1**).

Sinapic acid (**1**) is a well-known phenol component of lignocellulose present in phenol-containing wastewaters, such as olive oil mill waste-waters,[2] and it has been isolated from different plants.[3]

Two green microalgae strains were actually capable of biotransforming sinapic acid, namely *Stichococcus bacillaris* 155LTAP and *Ankistrodesmus braunii* C202.7a.

Both strains were cultivated in BBM[4] and the best bioconversion conditions were achieved in cultures with about 4×10^6 cells/mL algal population, sinapic acid (**1**) content of 160 mg/L, for seven days with a photoperiod 16 h light–8 h dark ensured by a fluorescent lamp.

In the case of *S. bacillaris* the biotransformation products, derived by degradative oxidation of the side chain, were identified by comparison of their physical data with authentic standards as 4-hydroxy-3,5-dimethoxybenzoic acid (**2**), 4-hydroxy-3,5-dimethoxybenzaldehyde (**3**) and 4-hydroxy-3,5-dimethoxybenzylic alcohol (**4**). The biotransformation yields for **2**, **3** and **4**, determined by HPLC analysis[5] of the reaction mixture, were 13, 8 and 26%, respectively.

With the alga *A. braunii*, sinapic acid was transformed into one main product **5** (75%) and one minor product 4-hydroxy-3,5-dimethoxybenzaldehyde (**3**) (5%). Biotransformation product **5** was isolated by HPLC chromatography[6] and identified as 3,6-dihydroxy-2,4 dimethoxy-7*H*-benzocyclohepten-7-one on the basis of its physical data.

In the HREI-MS spectrum was present the molecular peak at m/z 248.23 in agreement with the molecular formula $C_{13}H_{12}O_5$. The ^{1}H NMR spectrum shows four aromatic protons, one as a singlet at δ 8.31, two as doublets at δ 7.89 and 7.85, and one as a singlet at δ 7.14, and six protons of two methyls at δ 3.99 and 3.94. In the ^{13}C NMR spectrum can be identified thirteen carbon signals. Beside a carbonyl carbon at δ 176.1 and two methoxyl carbons at δ 61.2 and 61.5, four protonated carbons at δ 129.8, 125.8, 121.0, 104.6 and six non protonated carbons at δ 151.2, 141.9, 140.8, 134.3, 129.3 and 127.2 were detectable.

Keywords: sinapic acid; *Stichococcus bacillaris*; *Ankistrodesmus braunii*; biotransformation; thomasidioic acid; 3,6-dihydroxy-2,4-dimethoxy-7*H*-benzocyclohepten-7-one.

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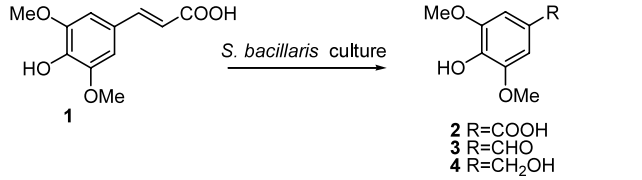
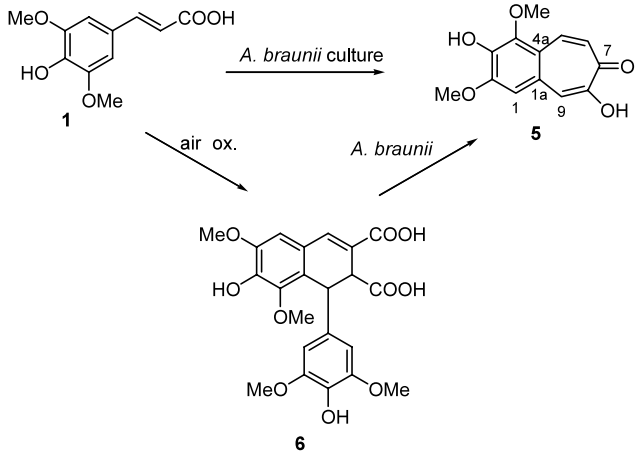
The correlations between protons and carbons were established on the basis of HMQC and HMBC experiments. The H-1 proton, linked to the carbon at δ 104.6, is correlated to the C-1a and C-2 carbons at δ 134.3 and 151.2 across two bonds, and to the C-4a, C-3 and C-9 carbons at δ 127.2, 140.8 and 129.9, respectively, across three bonds. The H-9 proton at δ 8.31, linked to the carbon at δ 129.9, shows heterocorrelations across two bonds with the C-1a and C-8 carbons at δ 134.3 and 129.3, and across three bonds with the C-4a, C-2 and C-7 at δ 176.1 carbons. The C-7 carbon is also correlated to the H-6 proton at δ 7.85, linked to the carbon at δ 121.0. This proton has correlations with the C-4a and C-7 carbons. Finally the H-5 proton at δ 7.89, linked to the carbon at δ 125.8, gives correlations with the C-4a, C-6 and C-4 carbons.





In order to understand the pathway that leads to the benzotropolone **5** with *A. braunii*, we followed the biotransformation at shorter times. The algal cultures after 4, 8, 24, 72 h (3 days) and 168 h (7 days) were directly injected in the HPLC system, after centrifugation to eliminate the algal suspension. We observed that in 4 h appeared a peak (compound A) in the HPLC chromatogram, this peak grew, reached a maximum after 1 day and disappeared after 7 days, while the benzotropolone peak appeared after 3 days and reached a maximum after 7 days.

Compound A was isolated and identified as the already known thomasidioic acid (**6**).[7] It has been shown that thomasidioic acid is an air oxidation product of sinapic acid in alkaline aqueous solutions. The conversion of sinapic acid into thomasidioic acid is depending from the solution pH[8] and the medium of the *A. braunii* culture (pH 6.5) was compatible with the observed transformation.[9]

Thomasidioic acid (**6**) kept 7 days in BBM was stable without formation of 6-hydroxy,5,7-dimethoxy-2-naphthoic acid and 2,6-dimethoxy-*p*-benzoquinone, reported as secondary oxidation products.[10]

Thomasidioic acid (**6**) was subsequently used for biotransformation, and it was in turn incubated in the culture of *A. braunii* for 5 days. The HPLC analysis results and the spectroscopic data revealed that **6** was quantitatively biotransformed into benzotropolone **5**. As far as we know, this is a novel type of biosynthesis of a benzotropolone.

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5. Reverse phase C-18 HPLC [H_2O–MeOH–CH_3CN, (6:3:1)].
6. Reverse phase C-18 HPLC [H_2O–MeOH–CH_3CN (7:2:1)].
7. EIMS: m/z 446; ^{1}H NMR: δ 7.47 (1H, s, H-4), 6.84 (1H, s, H-5), 6.34 (2H, s, H-2′ e H-6′), 4.85 (1H, brs, H-1), 3.75 (1H, brs, H-2), 3.69 (3H, s, 8-OMe), 3.57 (6H, s, 3′-OMe e 5′-OMe), 3.53 (3H, s, 6-OMe); ^{13}C NMR: δ 178.9 (2-COOH), 173.8 (3-COOH), 147.5 (C-3′), 147.5 (C-5′), 147.5 (C-6), 145.2 (C-8), 140.2 (C-7), 135.6 (C-4), 135.4 (C-1′), 132.6 (C-4′), 127.9 (C-3), 124.8 (C-9), 123.6 (C-10), 109.0 (C-5), 105.0 (C-2′), 105.0 (C-5′), 61.0 (8-OMe), 56.2 (3′-OMe), 56.2 (5′-OMe), 56.2 (6-OMe), 49.8 (C-2), 40.3 (C-1).
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