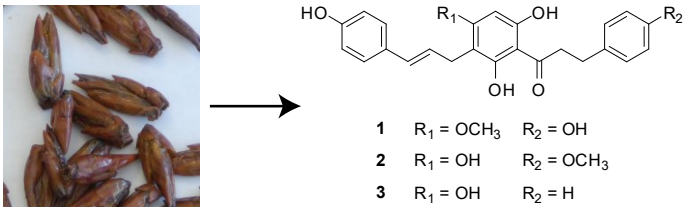
# New antibacterial dihydrochalcone derivatives from buds of *Populus balsamifera*

Serge Lavoie[a], Jean Legault[a], François Simard[a], Éric Chiasson[a] and André Pichette[a,*]

[a] Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, Québec, Canada, G7H 2B1

* Corresponding author. Tel.: +1 418 545 5011; fax: +1 418 545 5012. E-mail address: andre_pichette@uqac.ca (A. Pichette).

## Graphical abstract



**Abstract**

Three new dihydrochalcone derivatives, balsacones A, B and C, along with seven known compounds, were isolated from the buds of *Populus balsamifera*. The structures of the new compounds were elucidated by the analysis of spectroscopic data. Only balsacone A, B and C were significantly active against *Staphylococcus aureus* with MIC values ranging from 3.1 to 6.3 μM.

**Keywords**

Dihydrochalcone, *Populus balsamifera*, Antibacterial activity, *Staphylococcus aureus*, NMR

## 1. Introduction

*Populus balsamifera* L., belonging to the Salicaceae family, is a tree inhabiting almost all part of North America[1]. The American aboriginals prepared ointment from its buds to treat wounds[2] suggesting that this substance might act as a protection against infections. This traditional use, combined to recent results indicating that buds extracts from other *Populus* species possess antimicrobial activity,[3] prompted new investigation on a *P. balsamifera* buds extract to identify antimicrobial compounds. Previous phytochemical studies on *P. balsamifera* buds resulted in the identification of alkanes,[4] fatty acids,[4,5] terpenes,[4,6] phenols,[4,7,8] flavonoids,[4,7-9] chalcones,[4, 7-9] carbohydrates[10] and prostaglandins.[11] Herein are reported the isolation and structural elucidation of three new dihydrochalcone derivatives, characterized as balsacones A–C (**1**–**3**), together with seven known compounds (Figure 1). All isolates were tested for biological activities against *Staphylococcus aureus*, *Escherichia coli* and normal skin fibloblast (WS1).

## 2. Results and discussion

Crushed and air-dried buds of *P. balsamifera* (875 g) were extracted with refluxing aqueous ethanol of increasing polarity (5 % to 30 % $H_2O$). Solvent partition of the combined aqueous EtOH extracts followed by column chromatographic separations and semi-preparative HPLC purifications resulted in the isolation of compounds **1**-**10**.

Compound **1** was isolated as an orange amorphous solid which gave a $[M+K]^+$ quasimolecular ion peak at *m/z* 459.1228 in the HRMS spectrum consistent with the molecular formula $C_{25}H_{24}O_6$.[12] The IR spectrum showed bands at 3354 and 1612 $cm^{-1}$, due to OH and a conjugated carbonyl respectively. The $^{13}C$ and DEPT-135 NMR spectra showed the presence of 21 carbon resonances accounting for one carbonyl, five oxygenated aromatic quaternaries, seven $sp^2$ methines, four aromatic quaternaries, one oxygenated methyl and three aliphatic methylenes (Table 1). Detail analysis of 1D $^1H$, 2D-COSY and HSQC experiments (Figure 2) showed signals for two 1,4-disubstituted aromatic rings at $\delta_H$ 7.05 (2H, d, J = 8.3 Hz), 6.69 (2H, d, J = 8.3 Hz) and 7.11 (2H, d, J = 8.5 Hz), 6.66 (2H, d, J = 8.5 Hz). Two other spin systems could be observed in COSY, an allyl at 6.23 (1H, br d, J = 15.6 Hz), 6.05 (1H, dt, J = 15.6, 6.3 Hz) and 3.36 (2H, m) and two methylenes at 3.31 (2H, m) and 2.87 (2H, t, J = 8.2 Hz). The HMBC correlations of H-2 with $\delta_C$ 156.5 (C-4) and 31.5 (C-7) and of H-7 and H-8 with $\delta_C$ 207.1 (C-9) suggested that the first ring was hydroxylated and branched to a chain consisting of two methylenes and a carbonyl. HMBC signals of H-2" with $\delta_C$ 157.4 (C-4") and 130.3 (C-7") showed that the second ring was also hydroxylated and branched to the allyl group. The six remaining carbons were assigned to a third aromatic ring substituted by three oxygenated functions and the two phenylpropane groups described above. The exact positions of all the substituents were determined from the HMBC correlations of H-9" with $\delta_C$ 165.1 and 164.0 and the methoxy group with $\delta_C$ 165.1. From these data, only two possibilities can be considered for the position of the methoxy group: C-2' or C-4'. Using

1D-selective NOESY experiment, a clear correlation between the methoxy group and H-5' was observed meaning that the methoxy group was branched at C-4'. Examination of the mass spectrum (APCI) revealed a fragment at *m/z* 301 (Figure 2) thus confirming the proposed structure. Based on the above spectral evidence, **1** was characterized as 4,2',6'-trihydroxy-3'-(4"-hydroxycinnamyl)-4'-methoxydihydrochalcone and named balsacone A.

Compound **2** was isolated as an orange amorphous solid which gave a $[M+K]^+$ quasimolecular ion peak at *m/z* 459.1209 in the HRMS spectrum consistent with the molecular formula $C_{25}H_{24}O_6$.[13] The IR spectrum showed bands at 3335 and 1611 $cm^{-1}$, due to OH and conjugated carbonyl respectively. The $^{13}C$ and DEPT-135 NMR spectra afforded signals accounting for 21 carbons (one carbonyl, five oxygenated armomatic quaternaries, seven $sp^2$ methines, four aromatic quaternaries, one oxygenated methyl and three aliphatic methylenes). The $^1H$ and $^{13}C$ NMR spectra were similar to those of compound **1** suggesting that **2** was an isomer. Indeed, after detail analysis of NMR spectra, the only difference was accounted for the position of the methoxy group, which was determined as C-4 by the HMBC correlation at $\delta_H$ 3.73 (3H, s, $OCH_3$) and $\delta_C$ 159.4 (C-4). Compound **2** was thus characterized as 2',4',6'-trihydroxy-3'-(4"-hydroxycinnamyl)-4-methoxydihydrochalcone and named balsacone B.

Compound **3** was isolated as an orange amorphous solid which gave a $[M+H]^+$ quasimolecular ion peak at *m/z* 391.1500 in the HRMS spectrum consistent with the molecular formula $C_{24}H_{22}O_5$.[14] The IR spectrum showed bands at 3332 and 1611 $cm^{-1}$, due to OH and conjugated carbonyl respectively. The $^{13}C$ and DEPT-135 NMR spectra afforded signals accounting for 20 carbons (one carbonyl, four oxygenated aromatic quaternaries, eight $sp^2$ methines, four aromatic quaternaries and three aliphatic methylenes). Detail analysis of 1D $^1H$, 2D-COSY and HSQC experiments showed signals for one mono-substituted aromatic at $\delta_H$ 7.23 (2H, m), 7.23 (2H, m) and 7.14 (1H, m) and one 1,4-disubstituted aromatic ring at $\delta_H$ 7.12 (2H, m) and 6.66 (2H, d, J = 8.5 Hz). The remaining signals were almost identical to those of compound **1** and **2**. Compound **3** was thus characterized as 2',4',6'-trihydroxy-3'-(4"-hydroxycinnamyl)-dihydrochalcone and named balsacone C.

All other isolates were known substances identified as trans-cinnamic acid (**4**),[15] trans-4-hydroxycinnamic acid (**5**),[16] 4,2',6'-trihydroxy-4'-methoxydihydrochalcone (**6**),[17] 2',4',6'-trihydroxy-4-methoxydihydrochalcone (**7**),[17] 2',4',6'-trihydroxydihydrochalcone (**8**),[18] 2',6'-dihydroxy-4,4'-dimethoxydihydrochalcone (**9**)[19] and 2',6'-dihydroxy-4'-methoxydihydrochalcone (**10**).[20]

Conserva and coworkers proposed a biosynthetic pathway for similar compounds isolated from *Iryanthera laevis* in 1990.[21] From their results, the biosynthesis of the balsacones A, B and C could be explained by cinnamylation reactions of compounds **6**, **7** and **8** respectively. Indeed, Conserva *et al.* supposed the existence of compound **2** without being able to isolate it.

Antibacterial activity of compounds **1-10** were evaluated against *E. coli* (gram-) and *S. aureus* (gram+). The results presented in Table 2 show that all compounds were inactive against *E. coli*. In contrast, dihydrochalcones **1-3** were found significantly active against *S. aureus* with MIC values of 6.3 µM, 6.3 µM and 3.1 µM, respectively. All other compounds (**4-10**) were inactive with MIC higher than 100 µM. These results suggest that the presence of the 4-hydroxycinnamyl group at position 3' of ring A of balsacones (**1-3**) is important to obtain significant biological activities. Indeed, compounds **6-10,** which are missing the 4-hydroxycinnamyl group, are inactive. Hufford and Oguntimein reported also that the presence of an alkyl group in position 3' of dihydrochalcone ring A is essential for antibacterial activity.[22] In addition, Awouafack and coworkers suggested that a prenyl group at the same position is an antibacterial inducer.[23] Surprisingly, their compounds were active against *E. coli*, but not against *S. Aureus* suggesting that the structure of alkyl groups could modulate the selectivity toward gram+ or gram- bacteria. The cytotoxicity of compounds **1-10** was also evaluated on human skin fibroblasts, WS1. The results presented in Table 2 show that compounds **1, 3, 8-10** were moderately cytotoxic with $IC_{50}$ values ranging from 20 to 35 µM. Interestingly, compound **2** possesses antibacterial properties without cytotoxicity on WS1 cells.

**Acknowledgments**

This study was supported by a FQRNT fonds forestier 02 grant . The authors also wish to thank Marcel Pichette for buds collection, Guillaume Desbiens for extraction and Catherine Dussault for biological evaluation.

**References and notes**

1. Marie-Victorin, F.E.C. La flore Laurentienne, 3rd ed.; Les presses de l'Université de Montréal: Montréal, Québec, 1995; pp 162.
2. Van Wyk, B.-E.; Wink, M. *Medicinal plants of the world*; Timber Press, 2004.
3. Vardar-Ünlü, G.; Silici, S.; Ünlü, M. *World J. Microbiol. Biotechnol.* **2008**, 24(7), 1011-1017.
4. Isidorov, V.; Vinogorova, V. T. *Z. Naturforsch C: biosci.* **2003**, *58*, 355-360.
5. Polyakov, V. V.; Orlov, V. K.; Shukenova, R. Z.; Mullaeva, N. I. *Chem. Nat. Compd.* **1985**, *21*, 795.
6. Mattes, B. R.; Clausen, T. P.; Reichardt, P. B. *Phytochemistry* **1987**, *26*, 1361-1366.
7. Sentsov, M. F.; Braslovskii, V. B.; Kurkin, V. A.; Zapesochnaya, G. G.; Bakulin, V. T.; Pravdivtseva, O. E. *Rastitel'nye Resursy* **1997**, *33*, 51-56.
8. Greenaway, W.; May, J.; Whatley, F. R. *J. Chromatogr.* **1989**, *472*, 393-400.
9. Kurkin, V. A.; Zapesochnaya, G. G.; Braslavskii, V. B. *Chem. Nat. Compd.* **1990**, *26*, 224-225.
10. Isaeva, E. V.; Ryazanova, T. V. *Khimiya Rastitel'nogo Syr'ya* **2006**, 33-36.

11. Levin, E. D.; Isaeva, E. V.; Cherepanova, V. E. *Phytochemistry* **1990**, *29*, 2325-2326.
12. Balsacone A (**1**): orange amorphous solid; UV (MeOH) $\lambda_{max}$ 194, 208 (sh), 268, 288 nm; IR (film) $\nu_{max}$ 3354, 2925, 1612, 1513, 1446, 1420, 1220, 1138, 1106, 968, 830 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR, see Table 1; APCIMS (pos.) *m*/*z* 421 $[M-H]^+$ (21), 301 (100); HRMS *m*/*z* 459.12276 (calcd for $C_{25}H_{24}O_6K$, 459.12099).
13. Balsacone B (**2**): orange amorphous solid; UV (MeOH) $\lambda_{max}$ 194, 208 (sh), 268, 288 nm; IR (film) $\nu_{max}$ 3335, 2930, 1611, 1512, 1438, 1244, 1176, 1137, 828 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR, see Table 1; APCIMS (pos.) *m*/*z* 421 $[M-H]^+$ (43), 301 (100); HRMS *m*/*z* 459.12085 (calcd for $C_{25}H_{24}O_6K$, 459.12099).
14. Balsacone C (**3**): orange amorphous solid; UV (MeOH) $\lambda_{max}$ 192, 206 (sh), 266, 290 nm; IR (film) $\nu_{max}$ 3332, 2919, 1611, 1512, 1442, 1250, 1217, 1139, 1080, 833 $cm^{-1}$; $^{1}H$ NMR and $^{13}C$ NMR, see Table 1; APCIMS (pos.) *m*/*z* 391 $[M-H]^+$ (36), 271 (100). HRMS *m*/*z* 391.15000 (calcd for $C_{24}H_{23}O_5$, 391.15455).
15. Kalinowska, M.; Swislocka, R.; Lewandowski, W. *J. Mol. Struct.* **2007**, *834-836*, 572-580.
16. Massow, F.; Smith, M. A. R. *J. Chem. Soc., Perkin Trans. 2* **1976**, 977-980.
17. Mizuno, M.; Kojima, H.; Tanaka, T.; Iinuma, M.; Kimura, R.; Zhi-Da, M.; Murata, H. *Phytochemistry* **1987**, *26*, 2071-2074.
18. Mustafa, K.; Kjaergaard, H. G.; Perry, N. B.; Weavers, R. T. *Tetrahedron* **2003**, *59*, 6113-6120.
19. Nilsson, M. *Acta Chem. Scand*. **1961**, *15*, 154-158.
20. Tanaka, H.; Ichino, K.; Ito, K. *Phytochemistry* **1984**, *23*, 1198-1199.
21. Conserva, L. M.; Yoshida, M.; Gottlieb, O. R.; Martinez V., J. C.; Gottlieb, H. E. *Phytochemistry* **1990**, *29*, 3911-3918.
22. Hufford, C.D.; Oguntimein, B.O. *J. Nat. Prod.* **1982**, 45(3), 337-342.
23. Awouafack, M.D.; Kusari, S.; Lamshöft, M.; Ngamga, D.; Tane, P.; Spiteller, M. *Planta med.* **2010**, 76(6), 640-643.

**Supplementary material**

Supplementary data (Experimental details on the isolation of **1**-**3** and their spectral data) associated with this article can be found, in the online version, at doi:

**Table 1**

$^{1}H$ (400 MHz) and $^{13}C$ (100 MHz) NMR data for balsacones A (**1**), B(**2**) and C (**3**) in $CDCl_3$

| | **1** | | **2** | | **3** | |
|---|---|---|---|---|---|---|
| no. | $\delta_C$[a] | $\delta_H$[a,b] | $\delta_C$[a] | $\delta_H$[a,b] | $\delta_C$[a] | $\delta_H$[a,b] |
| 1 | 134.0 (s) | | 135.2 (s) | | 143.3 (s) | |
| 2,6 | 130.4 (d) | 7.05 (2H, d, 8.4) | 130.4 (d) | 7.13 (2H, d, 8.4) | 129.5 (d) | 7.23 (2H, m) |
| 3,5 | 116.2 (d) | 6.69 (2H, d, 8.4) | 114.8 (d) | 6.80 (2H, d, 8.4) | 129.4 (d) | 7.23 (2H, m) |
| 4 | 156.5 (s) | | 159.4 (s) | | 126.9 (d) | 7.14 (1H, m) |
| 7 | 31.5 (t) | 2.86 (2H, m) | 31.6 (t) | 2.88 (2H, m) | 32.4 (t) | 2.95 (2H, t, 8.3) |
| 8 | 47.7 (t) | 3.31 (2H, m) | 47.4 (t) | 3.29 (2H, m) | 47.1 (t) | 3.33 (2H, m) |
| 9 | 207.1 (s) | | 206.4 (s) | | 206.2 (s) | |
| 1' | 105.8 (s) | | 105.3 (s) | | 105.3 (s) | |
| 2' | 164.0 (s) | | 165.1 (s) | | 165.2 (s) | |
| 3' | 107.7 (s) | | 106.7 (s) | | 106.7 (s) | |
| 4' | 165.1 (s) | | 163.9 (s) | | 163.9 (s) | |
| 5' | 91.2 (d) | 6.04 (1H, s) | 95.0 (d) | 5.94 (1H, br s) | 95.0 (d) | 5.94 (1H, br s) |
| 6' | 162.4 (s) | | 161.8 (s) | | 161.8 (s) | |
| 1" | 131.2 (s) | | 131.3 (s) | | 131.4 (s) | |
| 2",6" | 128.1 (d) | 7.11 (2H, d, 8.5) | 128.1 (d) | 7.12 (2H, d, 8.4) | 128.1 (d) | 7.12 (2H, m) |
| 3",5" | 116.2 (d) | 6.66 (2H, d, 8.5) | 116.2 (d) | 6.66 (2H, d, 8.4) | 116.2 (d) | 6.66 (2H, d, 8.5) |
| 4" | 157.4 (s) | | 157.4 (s) | | 157.4 (s) | |
| 7" | 130.3 (d) | 6.22 (1H, br d, 15.8) | 130.2 (d) | 6.26 (1H, br d, 15.7) | 130.2 (d) | 6.25 (1H, br d, 15.7) |
| 8" | 126.6 (d) | 6.05 (1H, dt, 15.8, 6.3) | 126.8 (d) | 6.10 (1H, dt, 15.7, 6.4) | 126.8 (d) | 6.11 (1H, dt, 15.7, 6.3) |
| 9" | 26.4 (t) | 3.35 (2H, m) | 26.5 (t) | 3.36 (2H, m) | 26.5 (t) | 3.36 (2H, m) |
| $OCH_3$ | 56.1 (q) | 3.83 (3H, s) | 55.7 (q) | 3.73 (3H, s) | | |

[a]s: singlet, d: doublet, t: triplet, m, multiplet. [b]Data in parentheses refer to coupling constant (Hz)

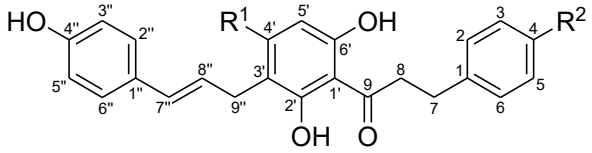
**Table 2**

Antibacterial and cytotoxic activities of compounds **1-10**

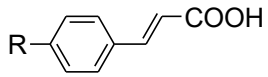
| Compounds | MIC (μM)[a] | | $IC_{50}$ (μM) |
|---|---|---|---|
| | *E. coli* | *S. aureus* | WS1 |
| **1** | > 200 | 6.3 | 25 ± 2 |
| **2** | > 200 | 6.3 | > 200 |
| **3** | > 200 | 3.1 | 23.6 ± 0.8 |
| **4** | > 200 | > 200 | > 200 |
| **5** | > 200 | > 200 | > 200 |
| **6** | > 200 | 100 | 130 ± 7 |
| **7** | NT[b] | NT[b] | > 200 |
| **8** | > 200 | 100 | 35.0 ± 0.3 |
| **9** | > 200 | > 200 | 23 ± 1 |
| **10** | > 200 | > 200 | 20 ± 1 |
| Gentamicin[c] | 0.04 | 0.02 | NT[b] |

[a]Minimum inhibitory concentration. [b]Not tested.[c]Positive control.

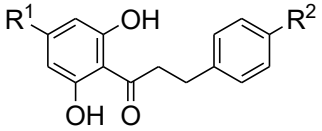
**Figure 1.** Structure of compounds **1-10**.




**1** $R^1 = OCH_3$ $R^2 = OH$
**2** $R^1 = OH$ $R^2 = OCH_3$
**3** $R^1 = OH$ $R^2 = H$




**4** R = H
**5** R = OH




**6** $R^1 = OCH_3$ $R^2 = OH$
**7** $R^1 = OH$ $R^2 = OCH_3$
**8** $R^1 = OH$ $R^2 = H$
**9** $R^1 = OCH_3$ $R^2 = OCH_3$
**10** $R^1 = OCH_3$ $R^2 = H$

**Figure 2**. COSY, HMBC, NOESY and MS key data for identification of compound **1**.