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# Fatty acid derivatives and dammarane triterpenes from the glandular trichome exudates of *Ibicella lutea* and *Proboscidea louisiana*

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ABSTRACT

*Ibicella lutea* and *Proboscidea louisiana*, both of the Martyniaceae family, are known for rich glandular trichomes on their leaves and stems. Chemical investigations of the glandular trichome exudates on leaves of the two plants furnished three types of secondary metabolites, glycosylated fatty acids, glycerides (2-*O*-(3,6-diacetyloxyfattyacyl)glycerols and 2-*O*-(3-acetyloxyfattyacyl)glycerols) and dammarane triterpenes. The glycosylated fatty acids from *I. lutea* were determined to be 6(*S*)-(6-*O*-acetyl-*β*-D-glucopyranosyloxy)-octadecanoic acid (**1A**), -eicosanoic acid (**1B**) and -docosanoic acid (**1C**), as well as their respective deacetyl congeners (**2A**, **2B** and **2C**), whereas *P. louisiana* furnished 8(*S*)-(6-*O*-acetyl-*β*-D-glucopyranosyloxy)-eicosanoic acid (**3A**) and -docosanoic acid (**3B**) and their respective deacetyl congeners (**4A** and **4B**), together with **2B**. Both plants contained 12 identical 2-*O*-[(3*R*,6*S*)-3,6-diacetyloxyfattyacyl]glycerols (**5A**-**L**), in which the fatty acyl moieties contained between 17 and 21 carbon atoms. The corresponding monoacetyloxy compounds, 2-*O*-[(3*R*)-3-acetyloxyfattyacyl]glycerols (**6A**–**L**) were detected in both plants. Among these glycerides, ten compounds (**5A**, **5C**, **5F**, **5H**, **5K**, **6A**, **6C**, **6F**, **6H** and **6K**) had iso-fattyacyl structures and four (**5E**, **5J**, **6E** and **6J**) had anteiso-fattyacyl structures. A previously unknown dammarane triterpene, betulatriterpene C 3-acetate (**7**), was isolated together with three known dammarane triterpenes, 24-*epi*-polacandrin 1,3-diacetate (**8**), betulatriterpene C (**9**) and 24-*epi*-polacandrin 3-acetate (**10**) from *I. lutea*, whereas 12 dammarane triterpenes, named prosbosciderols A–L (**12**–**23**), and the known compound betulafolienetriol (**11**) were isolated from *P. louisiana*. The structures of these compounds were elucidated by spectroscopic analysis including 2D-NMR techniques and chemical transformations. The 6-*O*-acetylglucosyloxy-fatty acids **1A**–**C** (42%) and the dammarane triterpenes **7**–**10** (31%) were the two most abundant constituents in the glandular trichome exudate of *I. lutea*, whereas the dammarane triterpenes **11**–**23** (47%) and the glucosyloxy-fatty acids (**4A**, **4B** and **2B**) (38%) were the most abundant constituents in the glandular trichome exudate of *P. louisiana*.



## 1. Introduction

Glandular trichomes are highly specialized epidermal cells common to many plant groups and are involved in the synthesis, storage, and secretion of a variety of secondary metabolites. Glandular trichomes can be divided into two main types, capitate and peltate, in accordance with their mode of secretion. Certain types of peltate glandular trichomes release substances, mainly essential oils such as those containing mono- and sesqui-terpenes when, for example, they are ruptured by touching by predators. On the other hand, capitate glandular trichomes freely exude durable sticky materials (Werker, 2000). The secondary metabolites produced by glandular trichomes may have diverse biological activities, such as acting to protect the aerial parts of plants against herbivores and pathogens (Duke, 1994).

*Ibicella lutea* (Lindl.) Van Eselt. of the Martyniaceae family is native to Brazil, but has since spread to neighboring countries. Its infusion has been used in popular medicine as an antimicrobial for the treatment of eye and skin infections in Uruguay (Alonso et al., 1995). Previous phytochemical investigations of this plant's aerial parts led to the isolation of a glucosyloxy-fatty acid assigned as 11-(6-*O*-acetyl-*β*-D-glucopyranosyloxy)stearic acid (Cerderias et al., 2000), the dammarane triterpenes, 24-*epi*-polacandrin 1,3-diacetate, 24-*epi*-polacandrin 3-acetate and (20*S*,24*R*)-epoxydammarane-3*β*,12*β*,25-triol, and a flavone, apigenine (Simirgiotis et al., 2003).

*Proboscidea louisiana* (Mill.) Thell. of the Martyniaceae family is a native of the southwestern United States and northern Mexico (Martin and Hutchins, 1980). Previous phytochemical investigations of this plant led to the isolation of glycosides of phenylpropanoids, an abscisic acid analogue (roseoside), a quinol and various iridoids (Sasaki et al., 1978).

The leaves, stems, flowers and immature fruits of *I. lutea* and *P. louisiana* are covered with dense capitate glandular trichomes that

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are ca. 1.5 mm length, and that exude unpleasant smelling oily materials. The sticky materials on the trichome head are visible to the naked eye. Small insects such as aphids are often entrapped on the leaf surface. Unlike carnivorous plants, it has been reported that *I. lutea* does not produce digestive enzymes, and therefore should be considered quasi-carnivorous rather than truly carnivorous (Schnell, 2002). We have been interested in phytochemical studies aimed at further understanding the biological roles of the secondary metabolites contained in micro-organs of plants. In previous papers, we reported the characterization of oxygenated fatty acylglycerols, such as 1-*O*-acetyl-2-*O*-[(3*R*,6*S*)-3-acetyloxy-6-hydroxyeicosanoyl]-*sn*-glycerol, 2-*O*-[(3*R*,8*R*)-3,8-diacetyloxyeicosanoyl]glycerol and 2-*O*-[(3*R*,9*R*)-3,9-diacetyloxyeicosanoyl]glycerol from the glandular trichome exudate on the leaves of *Paulownia tomentosa* (Asai et al., 2009) and geranylated flavanones from the oily secretions on the surface of the plant's immature fruit surface (Asai et al., 2008). As a continuation of our work, we have investigated the glandular trichome exudates on the leaves of *I. lutea* and *P. louisiana*. In this article, we describe the identification of three classes of secondary metabolites (i.e., glycosylated fatty acids, glycerides (2-*O*-(3,6-diacetyloxyfattyacyl)- and 2-*O*-(3-acetyloxyfattyacyl)-glycerols) and dammarane triterpenes) in glandular trichome exudates of the two plants.

## 2. Results and discussion

The glandular trichome exudate obtained from the leaves of *I. lutea* was separated by silica gel chromatography to give Fr. 1*i* (6-*O*-acetylglucosyloxy-fatty acid fraction, **1A**–**C**), Fr. 2*i* (glucosyloxy-fatty acid fraction, **2A**–**C**), Fr. 3*i* (2-*O*-(3,6-diacetyloxyfattyacyl)glycerol fraction, **5A**–**L**), Fr. 4*i* (2-*O*-(3-acetyloxyfattyacyl)glycerol fraction **6A**–**L**) and four dammarane triterpenes **7**–**10**. The exudate from the leaves of *P. louisiana* was collected and separated in a similar manner to give four fractions, Fr. 1*p* (6-*O*-acetylglucosyloxy-fatty acid fraction, **3A** and **3B**), Fr. 2*p* (glucosyloxy-fatty acid fraction, **4A** and **4B**), Fr. 3*p* (**5A**–**L**) and Fr. 4*p* (**6A**–**L**), as well as compound **2B** and the dammarane triterpenes **11**–**23**.

### 2.1. Glycosylated fatty acids

Fr. 1*i* from *I. lutea* showed pseudo-molecular ion peaks at *m*/*z* 531.3508, together with smaller peaks at 559.3892 and 503.3268 $[M-H]^-$, in the negative HRFABMS, which corresponded to the molecular formulae $C_{28}H_{52}O_9$, $C_{30}H_{56}O_9$, and $C_{26}H_{48}O_9$, respectively. The $^1$H NMR spectrum showed signals from seven protons including an anomeric proton at $\delta$ 4.35 (*d*, *J* = 7.8 Hz) due to a hexose moiety, longer-chain methylene protons, as well as one methyl triplet characteristic of a fatty acyl moiety, one oxymethine proton at $\delta$ 3.62 and one acetyl methyl singlet at $\delta$ 2.10 (Table 1). H–H COSY correlations and the coupling constants of the sugar hydrogens established the hexose moiety to be a $\beta$-linked glucopyranosyl group acetylated at C-6. The $^{13}$C NMR spectrum of Fr. 1*i* (Table 1) confirmed the presence of a 6-*O*-acetylglucopyranosyl moiety and provided further proof that the fatty acyl group was linear and mono-oxygenated along the methylene chain (i.e., signals at $\delta$ 176.9 and 80.3). The nature of the linkage between the sugar and fatty acyl moieties was delineated by a HMBC correlation from the anomeric proton to the oxymethine carbon at $\delta$ 80.3. At this stage of investigation, based on their molecular formulae, these analyses suggested a mixture of $C_{18}$, $C_{20}$ and $C_{22}$ oxygenated fatty acids, although the exact position of the oxygenation along the methylene chain remained to be elucidated.

In order to verify the possible co-occurrence of other minor oxygenated fatty acids, and more importantly, to determine the oxygenation position along the methylene chain, the following experiments were carried out. Acidic hydrolysis of Fr. 1*i* gave an hydroxy-fatty acid fraction which was converted to the corresponding methyl esters (**1a**–**c**) by treatment with ethereal diazomethane. This methylated product was trimethylsilylated and subjected to GLC and GC–MS analyses (EI mode). The chromatogram displayed three peaks in a 5:85:10 ratio, which eluted in that order. The major peak (retention time 16.7 min) showed intense fragment ion peaks at *m*/*z* 299 $[CH(OTMS)(CH_2)_{13}CH_3]^+$ due to C-5/C-6 cleavage, and at *m*/*z* 217 $[CH_3OOC(CH_2)_4CH(OTMS)]^+$ due to C-6/C-7 cleavage (Table 2); thus, the location of the oxygenation was determined to be at C-6. The molecular weight of the TMS ether, 414, was deduced from the $[M-Me]^+$ ion (*m*/*z* 399) and the fragment ions described above, which were in agreement with the FABMS data from the original molecule. The major peak was thus unequivocally assigned as methyl 6-(trimethylsilyloxy)eicosanoate. The two minor peaks, which eluted at 13.7 and 21.7 min, were similarly characterized as methyl 6-(trimethylsilyloxy)octadecanoate and methyl 6-(trimethylsilyloxy)docosanoate, respectively, from interpretation of the MS data (the fragment ions due to C-5/C-6 cleavage were observed at *m*/*z* 271 $[CH(OTMS)(CH_2)_{11}CH_3]^+$ and 327 $[CH(OTMS)(CH_2)_{15}CH_3]^+$, respectively) (Table 2).

The absolute configuration at the C-6 position of these acids was elucidated by the application of the 2-naphthyl-2-methoxyacetic acid (2NMA) ester method (Kusumi et al., 1994) into the above hydroxy-fatty acid methyl ester mixture. The methyl ester mixture (**1a**–**c**) was converted into its (*R*)-2NMA and (*S*)-2NMA esters (80% yield) by treatment with (*R*)-2NMA and (*S*)-2NMA in the same manner as described in our previous paper (Asai et al., 2009). The positive $\Delta\delta_{R-S}$ values for the $H_2$-2 (+0.37 ppm) and $COOCH_3$ (+0.08 ppm) signals of the (*R*)- and (*S*)-2NMA derivatives established a 6*S* configuration. Fr. 1*i* was therefore determined to be a 5:85:10 mixture of 6(*S*)-(6-*O*-acetyl-$\beta$-D-glucopyranosyloxy)-octadecanoic acid (**1A**), -eicosanoic acid (**1B**) and -docosanoic acid (**1C**), respectively. Finally, Fr. 1*i* was separated by reversed-phase HPLC to give the major constituent **1B**, whose physicochemical properties are described in the Section 4.

Fr. 2*i* showed pseudo-molecular ion peaks at *m*/*z* 517.3749, 489.3455 and 461.3120 in the negative HRFABMS, which correspond to the formulae $C_{28}H_{54}O_8$, $C_{26}H_{50}O_8$ and $C_{24}H_{46}O_8$, respectively. The $^1$H NMR spectrum of Fr. 2*i* resembled that of Fr. 1*i*, except for the lack of an acetyl methyl signal and for an upfield shift of the glucose C-6 methylene proton signals ($\delta$ 3.84 and 3.67) compared to those of Fr. 1*i*. It was therefore suggested that the compounds in Fr. 2*i* were the deacetylated derivatives of Fr. 1*i*. The $^{13}$C NMR spectroscopic data of Fr. 2*i* (Table 1) corroborated the compounds' structures. The chain length and the glucosylated position of the fatty acyl moiety were investigated in the same manner as described for Fr. 1*i*. GC–MS analysis of the TMS methyl ester fraction derived from Fr. 2*i* gave the same results as those found in Fr. 1*i*. The C-6 configuration of the oxygenated fatty acid moiety of Fr. 2*i* was determined to be *S* in the same manner as described for Fr. 1*i*. Hence, Fr. 2*i* was determined to be a 5:85:10 mixture of 6(*S*)-($\beta$-D-glucopyranosyloxy)-octadecanoic acid (**2A**), -eicosanoic acid (**2B**) and -docosanoic acid (**2C**), respectively. Finally Fr. 2*i* was separated by reversed-phase HPLC to give the major constituent **2B**, whose physicochemical properties are described in the Section 4.

Fr. 1*p* from *P. louisiana* gave pseudo-molecular ion peaks in the HRFABMS spectrum at *m*/*z* 559.3842 and 531.3557 $[M-H]^-$, which corresponded to the molecular formulae $C_{30}H_{56}O_9$ and $C_{28}H_{52}O_9$, respectively. The $^1$H and $^{13}$C NMR spectra of Fr. *1p* (Table 1) closely resembled those of Fr. 1*i*, which suggested that Fr. 1*p* could be a mixture of 6-*O*-acetylglucopyranosyloxyl substituted eicosanoic and docosanoic acids.