

## Stilbenic Profile of Cocoa Liquors from Different Origins Determined by RP-HPLC-APCI(+)-MS/MS. Detection of a New Resveratrol Hexoside

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*trans*-Resveratrol and *trans*-piceid were recently discovered in chocolate. In the present work, both were quantified by RP-HPLC-APCI(+)-MS/MS in 22 cocoa liquors from 11 different countries. A very large range of concentrations was observed for *trans*-piceid. The most concentrated sample (Arriba 06) reached 0.4 and 2.6 mg/kg of *trans*-resveratrol and *trans*-piceid, respectively, but in other cultivars stilbene levels were five times lower. Neither *cis*-resveratrol nor *cis*-piceid was found in cocoa liquors. An unknown compound eluting 0.5 min before *trans*-piceid and present at concentrations up to 0.8 mg/kg of *trans*-piceid equivalents in cocoa liquors was tentatively identified by HRMS as a *trans*-piceid-like hexoside.

**KEYWORDS:** Resveratrol; piceid; resveratrol galactoside; stilbene; polyphenol; cocoa; chocolate

### INTRODUCTION

Cocoa is increasingly recognized for its health benefits (1–4). It is a source of various polyphenolic compounds, including hydroxybenzoic acids (gallic/syringic/protocatechic/vanillic acids), hydroxycinnamic acids and analogues (caffeic/ferulic/ *p*-coumaric/phloretic acids, clovamide, dideoxyclovamide), flavonols (quercetin), flavones (luteolin, apigenin), flavanones (naringenin), and flavan-3-ols ((+)-catechin, (–)-epicatechin, oligomers, and polymers/procyanidins) (5, 6). Catechin and epicatechin have been found at concentrations of 150–1580 mg/kg in chocolate and 2530–3170 mg/kg in cocoa liquor (4, 7–9). Procyanidins (from monomers to hexamers) have been reported at concentrations from 2200 to 13230 mg/kg in various cocoa liquors (6, 8). Many phenols are found as glycosides in cocoa, mainly glucoside, galactoside, and arabinoside (10, 11).

Besides hydroxyphenolics and flavonoids, two stilbenes were recently identified in a cocoa liquor from the Ivory Coast and in dark chocolate (12). *trans*-Resveratrol is known to exhibit interesting antiinflammatory, anticancer, cardioprotective, and estrogenic activities (13, 14). It has been reported in grapes (0.13–47.6 mg/kg) (15–18), wine (0–18.03 mg/L) (15, 16), grape juice (0.35 mg/kg) (16), cranberry juice (0.24 mg/kg) (16), peanuts (3.7 mg/kg) (19), strawberries and cranberries (3.57 and 19.29 mg/kg, respectively) (20), sorghum (0.5 mg/kg) (21), hop (0.05–2.28 mg/kg) (22–25), and beer (5 μg/L) (26). In cocoa liquor, 0.5 mg/kg of *trans*-resveratrol and up to 1.2 mg/kg of its glucoside *trans*-piceid have been determined (12). More recently, Hurst et al. (27) reported much higher levels in some cocoa-derived

products: from 0.09 to 1.85 mg/kg and from 0.35 to 7.14 mg/kg for *trans*-resveratrol and *trans*-piceid, respectively. Their highest values were obtained for cocoa powder.

The aim of the present work was to determine the range of stilbene concentrations in cocoa liquors from different origins (Asia, South-America, and Africa). To the stilbene extraction method initially developed in our laboratory for hop analysis was added an Oasis cartridge purification step to avoid contamination of the samples by melanoidins (22, 26). Reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with APCI(+) tandem mass spectrometry (MS/MS) was used for quantification.

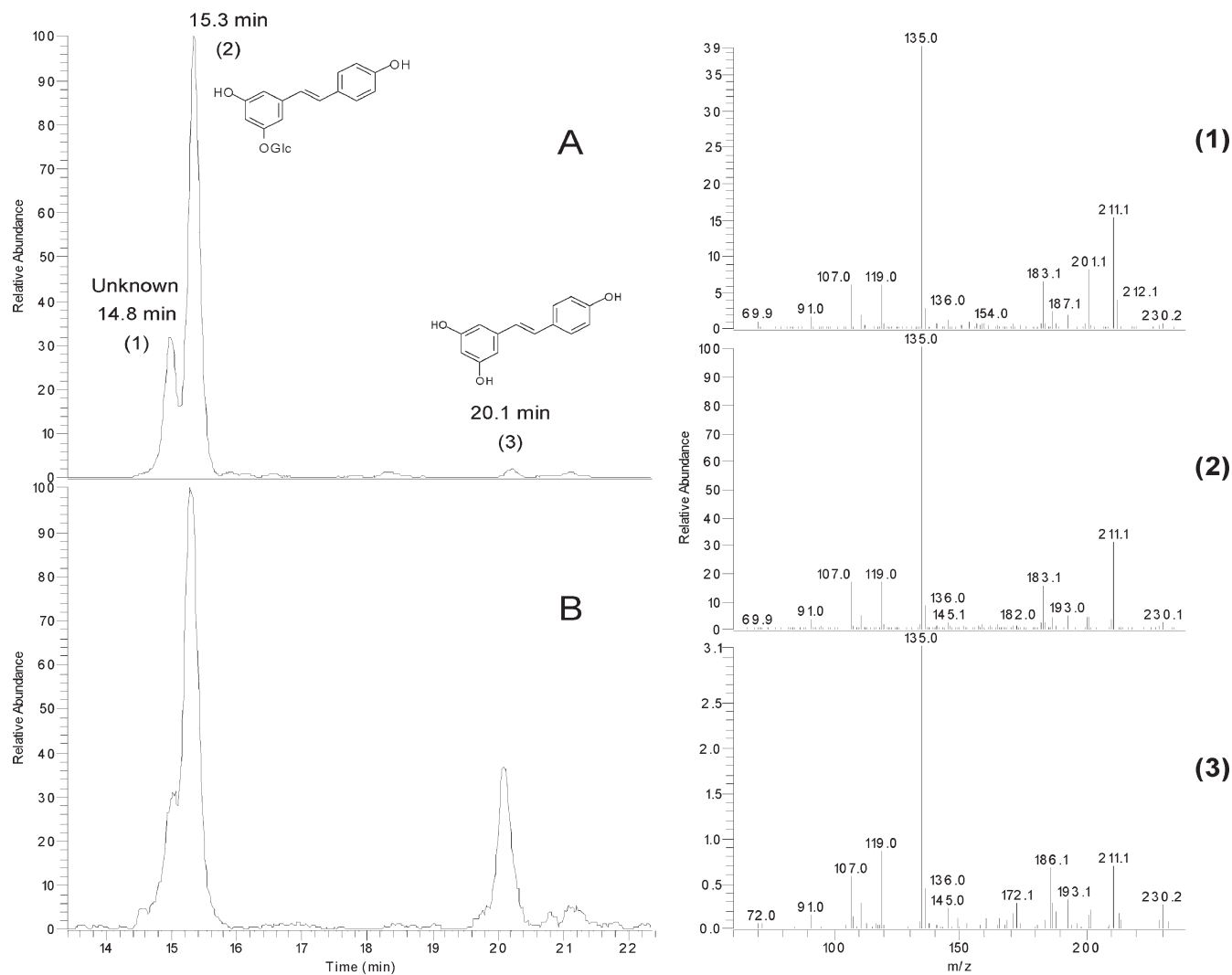
### MATERIALS AND METHODS

**Materials.** Twenty-two cocoa liquors from two successive harvests of cocoa beans (2006 and 2007) were supplied by Belcolade (Puratos Group, Belgium) and investigated just after delivery. All fermented and dried beans imported from the Ivory Coast, New Guinea, Java, Madagascar, Arriba, Tobago, Ghana, Venezuela, Costa Rica, Dominican Republic, or Peru were roasted in the same way (150 °C, 30 min) by the supplier.

**Chemicals.** Ethanol (97%) was obtained from Belgaco (Gent, Belgium). Acetonitrile (99.99%) and cyclohexane (99.96%) were supplied by Fisher Scientific (U.K.). Formic acid (99%) was obtained from Aldrich (Germany). Methanol (99.9%) was supplied by Romil (Cambridge, U.K.). Ethyl acetate (97%) came from by Fisher Scientific (U.K.). *trans*-Resveratrol (99%), *trans*-piceid (97%), acetobromo- $\alpha$ -D-galactose (93%), dichloromethane (99.8%), and potassium hydroxide were supplied by Sigma-Aldrich (Bornem, Belgium). Acetic acid (100%) was obtained from Merck (Darmstadt, Germany). Aqueous solutions were made with Milli-Q (Millipore, Bedford, MA) water (resistance = 18 m $\Omega$ ).

**Specific Extraction Procedure for Stilbenes.** This method has been first developed in our laboratory (22, 26) to analyze stilbenes in hop pellets. All extraction steps have been done with protection against day light, in

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**Figure 1.** RP-HPLC-APCI(+)-MS/MS data of (A) Peru 07 and (B) Peru 06 cocoa liquor extracts. MS/MS chromatogram ( $m/z = 229$ ) and experimental mass spectra for the unknown (1), *trans*-piceid (2), and *trans*-resveratrol (3).

78 duplicate. An Oasis cartridge purification step was added to avoid sugar  
79 and melanoidin contamination of the mass spectrometer.

80 **Lipid Removal.** Cocoa liquor (5 g) was reduced to powder and  
81 introduced into a centrifugal vial. By successive 10 min extractions, lipids  
82 were removed with  $3 \times 50$  mL cyclohexane at room temperature under  
83 gentle stirring. At the end of each step, the sample was centrifuged for  
84 10 min at 2500g. At the last step, cocoa powder was dried under vacuum to  
85 get rid of residual solvent.

86 **Stilbene Extraction.** Defatted cocoa liquor was extracted three times  
87 with 40 mL ethanol–water (80:20 v/v), each time for 10 min under gentle  
88 stirring at 60 °C. After each extraction, the sample was centrifuged for  
89 10 min at 2500g and the supernatant collected. The combined supernatants  
90 were concentrated by rotary evaporation (35 °C) under vacuum to obtain  
91 ~20 mL of extract and then filtrated to remove residual particles.

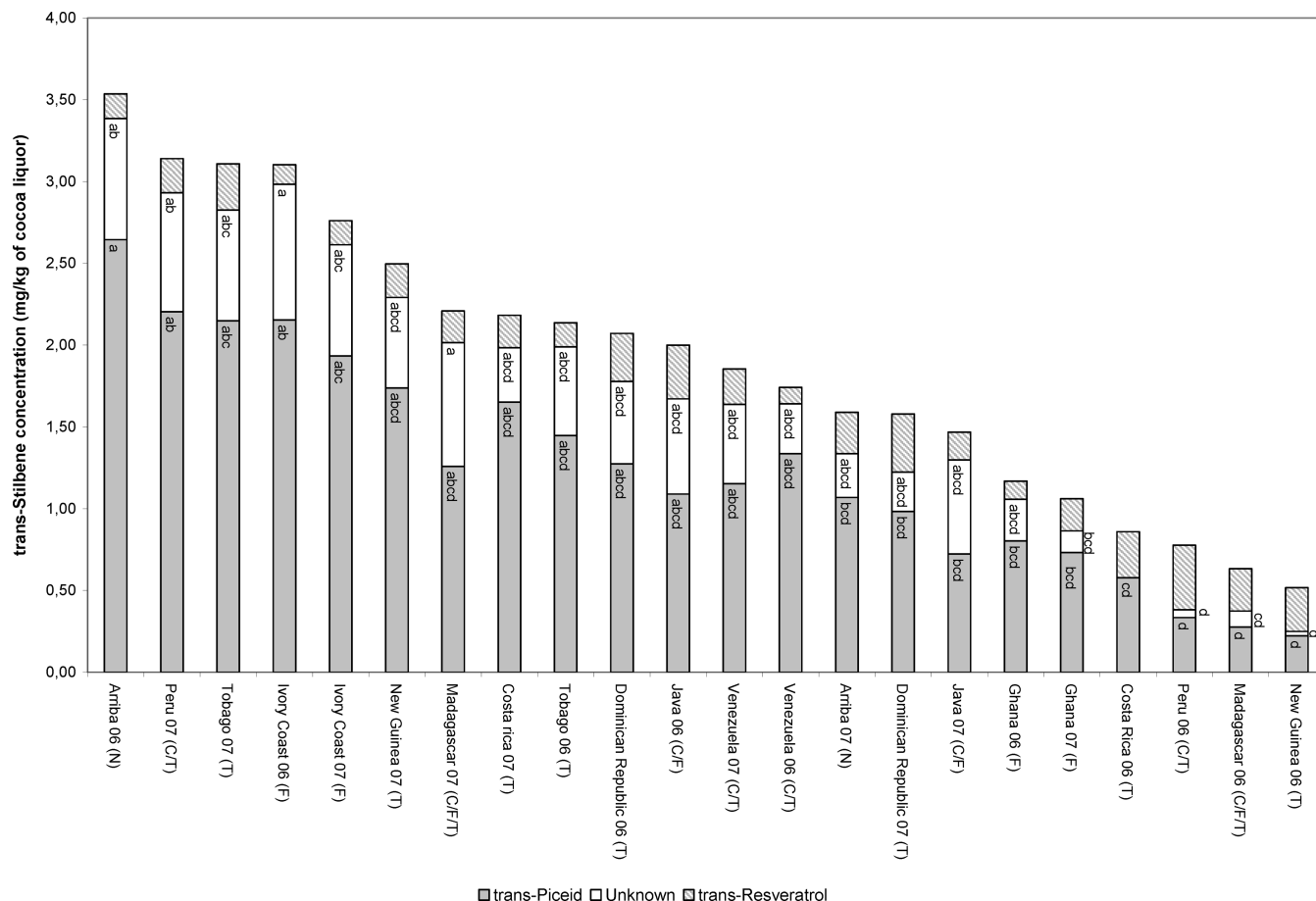
92 **Solid-Phase Extraction Purification Step.** The 500 mg Oasis cartridge  
93 (Waters) was preconditioned with methanol and 1.5 M formic acid.  
94 Approximately 20 mL of stilbene extract was loaded on the cartridge,  
95 and sugar and melanoidin were removed with 15 mL of 1.5 M formic acid  
96 followed by 15 mL of water containing 5% methanol and 2% formic acid.  
97 Stilbenes were then eluted with 24 mL of ethyl acetate. The eluate was  
98 concentrated by rotary evaporation (35 °C) to dryness. The residue was  
99 solubilized in 1 mL of a 50:50 (v/v) mixture of ethanol–water and filtered  
100 before analysis.

101 **Combinatorial synthesis of stilbene galactosides** was achieved according  
102 to the methodology described in the literature for stilbene glucosides (28).  
103 Under red light, 5 mg of *trans*-resveratrol, 9 mg of acetobromo- $\alpha$ -  
104 D-galactose, and 2.45 mg of potassium hydroxide were dissolved in

180  $\mu$ L of ethanol. The reaction medium was stirred for 1 week at room  
105 temperature. Then, 500  $\mu$ L of ethanol was added, and the mixture was  
106 filtered before analysis by RP-HPLC-MS/MS.

107 **RP-HPLC-APCI(+)-MS/MS Analysis of Stilbenes.** Quantifica-  
108 tions were performed on a 150 mm  $\times$  2.1 mm, 2  $\mu$ m C18 Prevail column  
109 (Grace, Deerfield, IL) eluted with a linear gradient from water (containing  
110 1% acetonitrile and 0.1% formic acid) to acetonitrile. Gradient elution  
111 was as follows: from 95% water to 55% in 23 min, 55% to 0% in 7 min,  
112 and isocratic for 10 min at a flow rate of 200  $\mu$ L/min. Ten microliters of the  
113 sample was injected onto the column. A SpectraSystem equipped with an  
114 AS3000 autosampler and a P4000 quaternary pump was used. The system  
115 was controlled with the Xcalibur software version 1.2 (Finnigan Mat).  
116 Mass spectra were acquired using a LCQ mass spectrometer equipped with  
117 an APCI source (Finnigan Mat). The following APCI inlet conditions in  
118 positive mode were applied: vaporization temperature, 470 °C; capillary  
119 voltage, 30 V; capillary temperature, 175 °C; sheath gas, 40 psi; auxiliary  
120 gas, 7 psi; discharge current, 5  $\mu$ A. After the first monitoring on the  $m/z$   
121 229, collision-induced dissociation spectra were recorded at 37% relative  
122 collision energy.  
123

124 **RP-HPLC-APCI(+)-HRMS Analysis of Stilbenes.** RP-HPLC  
125 was performed on an Accela system (Thermo Fisher), as described above.  
126 HRMS analyses were carried out using an LTQ-Orbitrap-XL (Thermo  
127 Fisher) mass spectrometer equipped with an APCI source. The system was  
128 controlled with the Xcalibur software version 2.0.7. The same APCI inlet  
129 conditions were applied as described above. For MS/MS (monitoring on  
130  $m/z$  229), collision-induced dissociation spectra were recorded at 35%  
131 relative collision energy. LC-HRMS applied on the unknown in the



**Figure 2.** Concentration (mg/kg of cocoa liquor) of *trans*-piceid, *trans*-resveratrol, and the unknown (in *trans*-piceid equivalents) in different varieties of cocoa liquor. Key: C, *Criollo*; F, *Forastero*; T, *Trinitario*; N, *Nacional*. Assay in duplicate. For *trans*-piceid or the unknown, all samples that do not share a common letter are significantly different ( $p < 0.05$ ) according to the Tukey test (for *trans*-resveratrol all samples were found in the same group).

132 Tobago 07 extract confirmed an elemental formula of  $C_{20}H_{23}O_8$   
 133 (experimental mass of  $m/z$  391.13932, theoretical mass of  $m/z$  391.13874,  
 134  $\delta = 0.57$  ppm, well in the variation range of the apparatus) with a major  
 135 fragmentation into  $m/z$  229.08630 (theoretical mass of protonated *trans*-  
 136 resveratrol ( $C_{14}H_{13}O_3$ ) = 229.08592,  $\delta = 1.66$  ppm). As expected, the  
 137 MS/MS applied on  $m/z$  229 gave an intense ion at  $m/z$  135.04388.

138 **NP-HPLC-APCI(+)-MS/MS Analysis of Stilbenes.** Separations  
 139 were carried out on a 250 mm  $\times$  2.1 mm, 2  $\mu$ m Alltima Silica column  
 140 (Grace, Deerfield, IL) at a flow rate of 200  $\mu$ L/min with a linear gradient  
 141 from A (dichloromethane) to B (methanol) and a constant 4% level of C  
 142 (acetic acid and water, 1:1 v/v). Gradient elution was 14–50% B, 0–30  
 143 min; 50–56% B, 30–35 min; 56–85% B, 35–40 min; 40–50 min isocratic.  
 144 Ten microliters of the sample was injected into the column. The mass  
 145 spectrometry conditions are the same as for RP analyses.

146 **Statistical Analyses.** The statistical analysis system (SAS Institute,  
 147 Inc., Cary, NC) was used. Significant differences among samples were  
 148 determined by analysis of variance (ANOVA), and the multiple compari-  
 149 sons of means were performed with the Tukey test.

## 150 RESULTS AND DISCUSSION

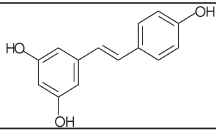
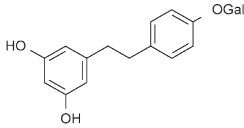
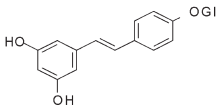
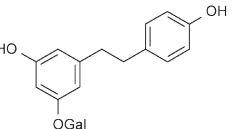
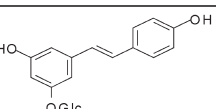
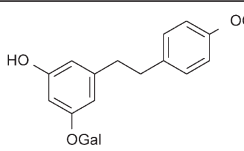
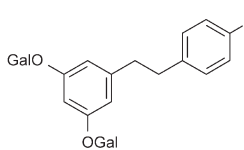
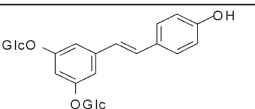
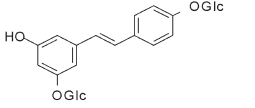
151 **Optimization of Stilbene Extraction.** The method was initially  
 152 developed to analyze resveratrol in hop (22). According to the  
 153 literature (29), preliminary removal of lipids and other hydro-  
 154 phobic constituents is required for recovering high amounts of  
 155 polyphenols. Ether is often chosen for preliminary cleaning (30).  
 156 Unfortunately, because of the S1-type solubility of resveratrol  
 157 (> 3.3% soluble in both water and ether) (22, 29, 31), solvents  
 158 more hydrophobic than diethyl ether were required here for  
 159 precleaning. A three-step solid/liquid precleaning with cyclohexane  
 160 proved sufficient for removing lipids from cocoa liquor. Stilbenes

161 were then extracted from the defatted cocoa liquor with ethanol–  
 162 water (80:20) at 60  $^{\circ}$ C as proposed by Callemien et al. (22). The  
 163 presence of Maillard colored compounds in the ethanol–water  
 164 extract, however, made it necessary to perform an additional  
 165 purification step on an Oasis HLB cartridge (26). Elution with  
 166 ethyl acetate, often used for stilbenoid extraction (16, 31–33),  
 167 enabled us to recover 80% *trans*-resveratrol and 60% *trans*-piceid  
 168 (determined by standard addition in cocoa liquor). These recovery  
 169 values were used for further quantifications.

170 **Stilbenoids in Cocoa Liquors.** The optimized procedure was  
 171 applied to 22 cocoa liquors of different origins (Africa, America,  
 172 and Asia; 11 countries). Indicative data on cocoa varieties (fine  
 173 grades, *Criollo*, *Trinitario*, and *Nacional*; bulk-basic grade, *For-*  
 174 *astero*) were given by the producers. RP-HPLC-APCI(+)-MS/  
 175 MS enabled us to easily separate *trans*-piceid (RT = 15.3 min in  
 176 **Figure 1A**) and *trans*-resveratrol (RT = 20.1 min in **Figure 1A**) in  
 177 all varieties. As expected (28), fragmentation of the monogluco-  
 178 side was characterized by loss of the sugar, leading in all cases to  
 179 an intense aglycon  $M + 1$  ion. Therefore, both *trans*-stilbenes  
 180 were measured just by selecting  $m/z$  229. As in hop, grape juices,  
 181 wine, and cocoa-containing products (23, 27, 34, 35), *trans*-piceid  
 182 emerged as the major form (**Figure 1A**; its lower recovery is  
 183 balanced on the chromatogram by the two times higher sensitivity  
 184 of the mass spectrometer for piceid than for its aglycon). Only  
 185 three cocoa liquors (Peru 06, Madagascar 06, and New Guinea  
 186 06) exhibited a distinct profile with a higher peak for *trans*-  
 187 resveratrol (**Figure 1B**).

188 As depicted in **Figure 2**, concentrations ranged in the 22  
 189 investigated cocoa liquors from 0.1 to 0.5 mg/kg *trans*-resveratrol

**Table 1.** Structures, Retention Times (RT, min) on C18 and Silica Columns, and Relative Retention Times (rRT = 1 for *trans*-Piceid) of Stilbene Glucosides and Galactosides, Potentially Candidates for Being the Unknown (rRT C18 = 0.97; rRT Silica = 1.35) in Cocoa Liquor<sup>a</sup>

structure	name	<i>m/z</i> [M +H] <sup>+</sup>	RT C18 (rRT)	RT Silica (rRT)
<b>Resveratrol</b>				
	<i>trans</i> -Resveratrol or <i>trans</i> -3,4',5-Trihydroxystilbene	229	20.1 (1.31)	4.0 (0.37)
<b>Resveratrol monohexosides</b>				
	<i>trans</i> -Resveratrol-4'-O-β-D-galactopyranoside or <i>trans</i> -3,4',5-Trihydroxystilbene-4'-O-β-D-galactopyranoside	391	13.5 (0.88)	14.9 (1.37)
	<i>trans</i> -Resveratrol-4'-O-β-D-glucopyranoside or <i>trans</i> -3,4',5-Trihydroxystilbene-4'-O-β-D-glucopyranoside	391	13.9 (0.91)	12.5 (1.15)
	<i>trans</i> -Piceid-3-O-β-D-galactopyranoside or <i>trans</i> -3,4',5-Trihydroxystilbene-3-O-β-D-galactopyranoside	391	14.8 (0.97)	11.7 (1.07)
	<i>trans</i> -Piceid-3-O-β-D-glucopyranoside or <i>trans</i> -3,4',5-Trihydroxystilbene-3-O-β-D-glucopyranoside	391	15.3 (1.00)	10.9 (1.00)
<b>Resveratrol dihexosides</b>				
	<i>trans</i> -3,4',5-Trihydroxystilbene-3,4'-O-β-D-digalactopyranoside	553	9.9 (0.65)	24.9 (2.28)
	<i>trans</i> -3,4',5-Trihydroxystilbene-3,5-O-β-D-digalactopyranoside	553	10.6 (0.69)	21.4 (1.96)
	<i>trans</i> -3,4',5-Trihydroxystilbene-3,5-O-β-D-diglucopyranoside	553	11.6 (0.76)	19.1 (1.75)
	<i>trans</i> -3,4',5-Trihydroxystilbene-3,4'-O-β-D-diglucopyranoside	553	10.9 (0.71)	20.6 (1.89)

<sup>a</sup> Chromatograms given in Figure 5. For all compounds, the APCI(+)-MS/MS spectrum obtained after selecting *m/z* = 229 was similar to that of the unknown.

190 (one statistical group according to Tukey test) and from 0.2 to  
191 2.6 mg/kg *trans*-piceid (three statistical groups according to  
192 Tukey test). Fine-cocoa liquors, to which shorter fermentations  
193 are usually applied, are known to contain higher levels of total  
194 procyanidins (6). Stilbenes did not systematically follow this rule.  
195 The fine Arriba/Nacional sample did exhibit the highest level, but  
196 fine samples from Java and Madagascar showed levels at the  
197 bottom of the range.

Such large variations between samples have been previously  
198 observed in grapes and hop (18, 23–25). As stilbenes are  
199 phytoalexins, growing area, climate conditions, interactions with  
200 pathogens, and many other factors can modulate their concen-  
201 trations. Counet et al. (12) found values in the same range, with  
202 0.5 mg/kg *trans*-resveratrol and 1 mg/kg *trans*-piceid in their  
203 cocoa liquor from the Ivory Coast. On the other hand, this range  
204 is lower than the values reported by Hurst et al. (27) for baking  
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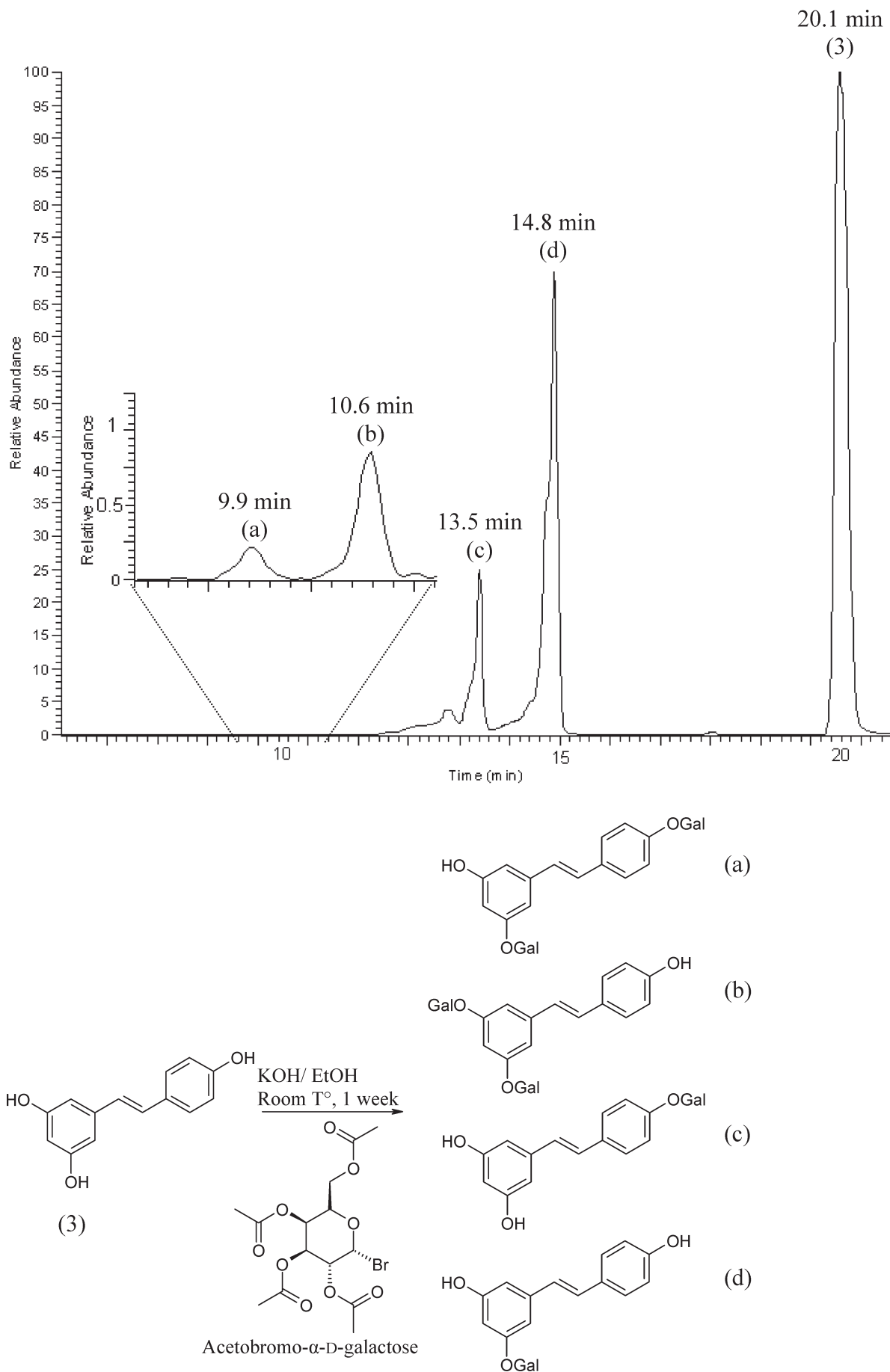
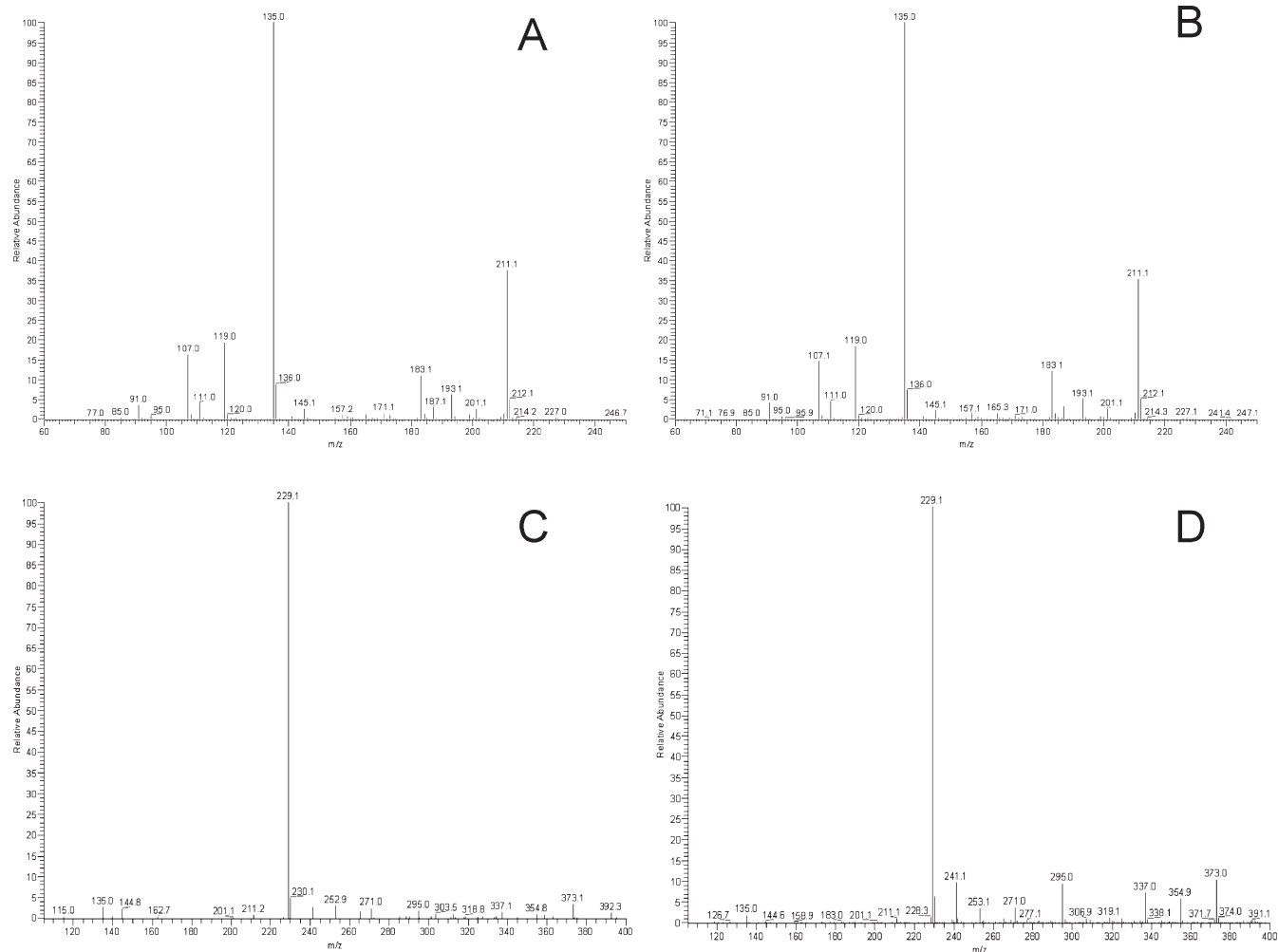
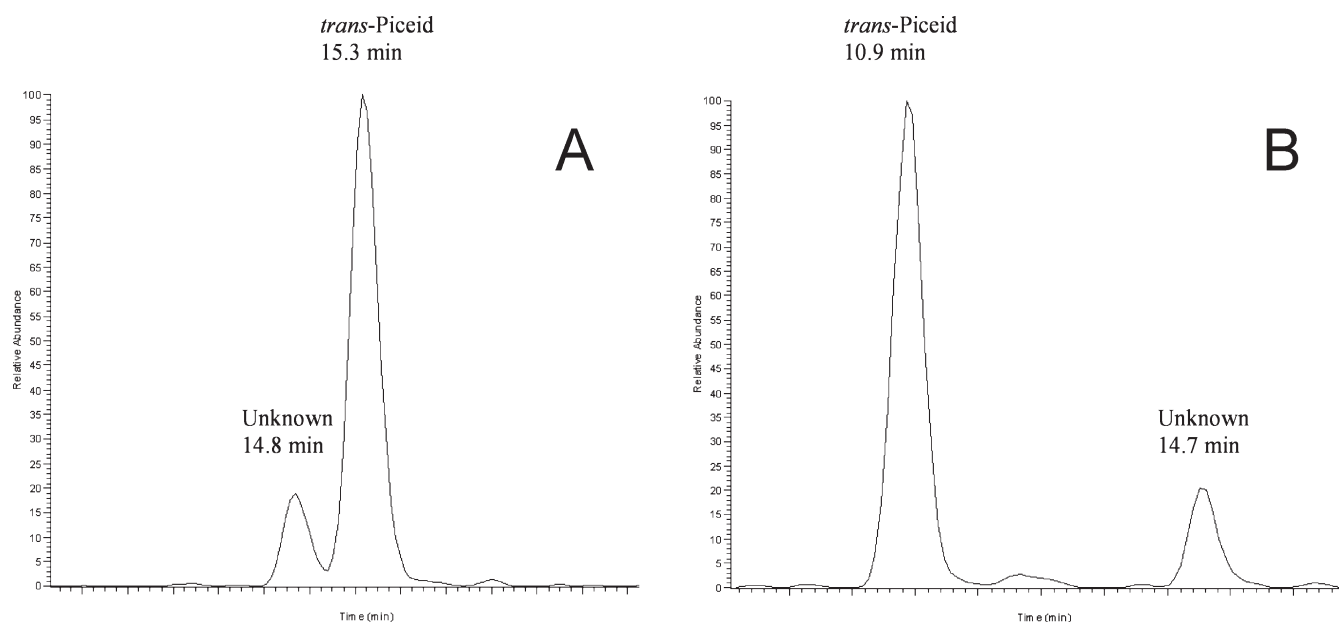


Figure 3. Synthesis scheme and RP-HPLC-APCI(+)-MS/MS chromatograms of synthesized galactosides (a, b, c, d) issued from *trans*-resveratrol (3).



**Figure 4.** APCI(+)-MS/MS spectra ( $m/z$  229 for **A** and **B**,  $m/z$  391 for **C** and **D**) of *trans*-piceid (**A**, **C**) and *trans*-piceid-like galactoside (**B**, **D**).

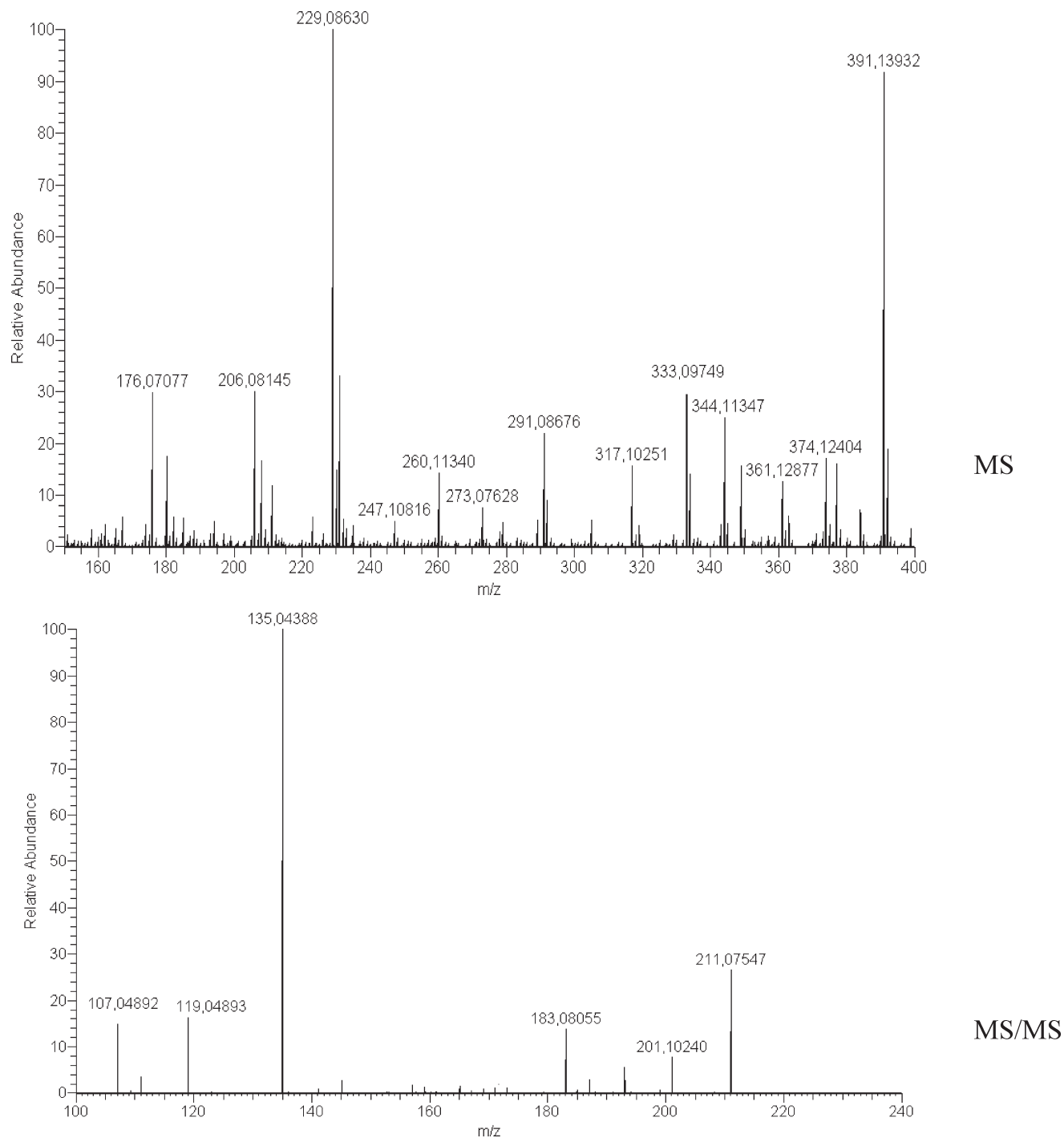


**Figure 5.** Elution of *trans*-piceid and the unknown in the Tobago 07 cocoa liquor extract by RP- (**A**) and NP- (**B**) HPLC-APCI(+)-MS/MS ( $m/z$  229).

206 chocolate products (i.e., from 3.81 to 4.20 mg/kg for *trans*-piceid).  
 207 The use of a different preextraction procedure (acidic hydrolysis  
 208 which could release bound fractions) and of UV detection  
 209 (less selective than MS) may partially explain these differences.

Moreover, as polyphenols are known to be degraded through  
 fermentation (6, 36), we can suspect that samples issued from less  
 fermented cocoa beans (often used in the United States) were  
 investigated.

210  
 211  
 212  
 213



**Figure 6.** HRMS and MS/MS data of the unknown in the Tobago 07 cocoa liquor extract.

214 **Identification of the Unknown Compound Eluting 0.5 min before**  
 215 ***trans*-Piceid in Cocoa Liquors.** Worth stressing is the presence of  
 216 another peak (peak 1 in **Figure 1A**), closely related to piceid, in the  
 217 cocoa liquor MS/MS chromatograms ( $m/z$  229). The identical  
 218 MS/MS spectra for both peaks (after selecting either  $m/z$  229 or  
 219  $m/z$  391) suggest a glycoside structure. In *trans*-piceid equivalents,  
 220 from 0 to 0.8 mg/kg of the unknown compound were quantified  
 221 in the 22 investigated cocoa liquors (**Figure 2**).

222 A library of stilbene glucosides previously obtained by combi-  
 223 natorial chemistry (28) enabled us to rule out *trans*-resveratrol-  
 224 oside and two *trans*-stilbene diglucosides (3,4',5-trihydroxystilbene-  
 225 3,4'-*O*- $\beta$ -D-digluco-pyranoside and 3,4',5-trihydroxystilbene-3,5-  
 226 *O*- $\beta$ -D-digluco-pyranoside) (**Table 1**), all exhibiting similar MS/  
 227 MS ( $m/z$  229) spectra but eluting at retention times different from

228 that of the unknown compound. Methoxylated and tetrasubsti-  
 229 tuted analogues (28) also proved not to be potential candidates,  
 230 because of their very distinctive mass spectra.

231 As galactoside phenols are known in cocoa, resveratrol galacto-  
 232 sides were synthesized in the present work. Two monogalactosides  
 233 and two digalactosides were characterized (RP-HPLC elution data  
 234 given in **Figure 3** and **Table 1**). As previously emphasized for *trans*-  
 235 piceid, the first fragmentation of the monogalactoside was the loss  
 236 of the sugar, leading to an MS/MS spectrum ( $m/z$  229) very similar  
 237 to that of resveratrol and piceid (**Figure 4A,B**). The MS/MS spectra  
 238 recorded after selecting the total molecular weight ( $m/z$  391) also  
 239 proved similar for glucosides and galactosides (**Figure 4C,D**).

240 The *trans*-resveratrol-like galactoside revealed slightly more  
 241 polar than the unknown on the reversed phase. Both digalactosides

were also ruled out because of their much higher polarity. On the other hand, the galactoside analogue of *trans*-piceid (RT = 14.8 min) (**Table 1**) revealed to coelute with our unknown in RP-HPLC. However, complementary analysis performed on a polar phase led us to discard the piceid-like galactoside as well (less polar than the unknown: RT on Silica = 11.7 min against 14.7 min for the unknown) (**Figure 5** and **Table 1**).

LC-HRMS applied on the Tobago 07 extract confirmed an elemental formula of C<sub>20</sub>H<sub>23</sub>O<sub>8</sub> for the unknown compound (**Figure 6**, experimental mass of *m/z* 391.13932), corresponding to a resveratrol hexoside structure. According to the HPLC retention times, a sugar slightly more polar than galactose is required. Mannose and fructose could be potential candidates. Unfortunately, the corresponding acetobromo reagents are not commercially available, requiring therefore another methodology to synthesize piceid-like mannoside and fructoside.

In conclusion, cocoa liquor is a significant source of stilbene hexosides, especially *trans*-piceid. Compared to the aglycons, stilbene glycosides are known for their higher resistance through the intestinal tract and for their better bioavailability (5). Therefore, both stilbene hexosides here investigated could be key elements for the nutritional properties of cocoa. More investigations are still needed to identify the sugar linked to resveratrol in the unknown compound.

#### LITERATURE CITED

- (1) Kattenberg, H. Nutritional functions of cocoa and chocolate. *Manuf. Confect.* **2000**, 33–37.
- (2) Buijsse, B.; Feskens, E. J. M.; Kok, F. J.; Kromhout, D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly study. *Arch. Intern. Med.* **2006**, *166*, 411–417.
- (3) Vinson, J. A.; Proch, J.; Bose, P.; Muchler, S.; Taffera, P.; Shuta, D.; Samman, N.; Agbor, G. A. Chocolate is a powerful *ex vivo* and *in vivo* antioxidant, an antiatherosclerotic agent in an animal model, and a significant contributor to antioxidants in the European and American diets. *J. Agric. Food Chem.* **2006**, *54*, 8071–8076.
- (4) Lamuela-Raventos, R. M.; Romero-Perez, A. I.; Andres-Lacueva, C.; Tornero, A. Review: health effects of cocoa flavonoids. *Food Sci. Technol. Int.* **2005**, *11*, 159–176.
- (5) Borchers, A.; Keen, C.; Hannum, S.; Gershwin, M. Cocoa and chocolate: composition, bioavailability and health implications. *J. Med. Food* **2000**, *3*, 77–105.
- (6) Counet, C.; Ouwerx, C.; Rosoux, D.; Collin, S. Relationship between procyanidin and flavor contents of cocoa liquors from different origins. *J. Agric. Food Chem.* **2004**, *52*, 6243–6249.
- (7) Cooper, K. A.; Campos-Gimenez, E.; Jimenez Alvarez, D.; Nagy, K.; Donovan, J. L.; Williamson, G. Rapid reversed phase ultra-performance liquid chromatography analysis of the major cocoa polyphenols and inter-relationships of their concentrations in chocolate. *J. Agric. Food Chem.* **2007**, *55*, 2841–2847.
- (8) Gu, L.; House, S. E.; Wu, X.; Ou, B.; Prior, R. L. Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. *J. Agric. Food Chem.* **2006**, *54*, 4057–4061.
- (9) Manach, C.; Scalbert, A.; Morand, C.; Remesy, C.; Jimenez, L. Polyphenols: food sources and bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747.
- (10) Sanchez-Rabaneda, F.; Jauregui, O.; Casals, I.; Andres-Lacueva, C.; Izquierdo-Pulido, M. Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *J. Mass Spectrom.* **2003**, *38*, 35–42.
- (11) Rice-Evans, C.; Miller, N.; Paganda, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci.* **1997**, *2*, 152–159.
- (12) Counet, C.; Callemien, D.; Collin, S. Chocolate and cocoa: new sources of *trans*-resveratrol and *trans*-piceid. *Food Chem.* **2006**, *98*, 649–657.
- (13) King, R. E.; Bomser, J. A.; Min, D. B. Bioactivity of resveratrol. *Compr. Rev. Food Sci. Food Saf.* **2006**, *5*, 65–70.

- (14) Fremont, L. Biological effects of resveratrol. *Life Sci.* **2000**, *66*, 663–673.
- (15) Burns, J.; Yokota, T.; Ashihara, H.; Lean, M. E. J.; Crozier, A. Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.* **2002**, *50*, 3337–3340.
- (16) Wang, Y.; Catana, F.; Yang, Y. N.; Roderick, R.; van Breemen, R. B. An LC-MS method for analyzing total resveratrol in grape juice, cranberry juice, and in wine. *J. Agric. Food Chem.* **2002**, *50*, 431–435.
- (17) Landraut, N.; Larronde, F.; Delaunay, J.-C.; Castagnino, C.; Vercauteren, J.; Mérillon, J.-M.; Gasc, F.; Cros, G.; Teissède, P.-L. Levels of stilbene oligomers and astilbin in french varietal wines and in grapes during noble rot development. *J. Agric. Food Chem.* **2002**, *50*, 2046–2052.
- (18) Romero-Perez, A. I.; Lamuela-Raventos, R. M.; Andres-Lacueva, C.; de la Torre-Boronat, M. Method for the quantitative extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J. Agric. Food Chem.* **2001**, *49*, 210–215.
- (19) Tokusoglu, O.; Kemal Unal, M.; Yemis, F. Determination of the phytoalexin resveratrol (3,5,4'-trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatography diode array (HPLC-DAD) and gas chromatography-mass spectrometry (GC-MS). *J. Agric. Food Chem.* **2005**, *53*, 5003–5009.
- (20) Ehala, S.; Vahe, M.; Kaljurand, M. Characterization of phenolic profiles of Northern European berries by capillary electrophoresis and determination of their antioxidant activity. *J. Agric. Food Chem.* **2005**, *53*, 6484–6490.
- (21) Jerkovic, V.; Collin, S. Resveratrol analogues in malt, hop and adjuncts. 2007, Proceedings of the European Brewery Convention, Fachverlag Hans Carl, Nürnberg, Germany, pp 1190–1198.
- (22) Callemien, D.; Jerkovic, V.; Rozenberg, R.; Collin, S. Hop as an interesting source of resveratrol for brewers: optimization of the extraction and quantitative study by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 424–429.
- (23) Jerkovic, V.; Callemien, D.; Collin, S. Determination of stilbenes in hop pellets from different cultivars. *J. Agric. Food Chem.* **2005**, *53*, 4202–4206.
- (24) Jerkovic, V.; Collin, S. Occurrence of resveratrol and piceid in American and European hop cones. *J. Agric. Food Chem.* **2007**, *55*, 8754–8758.
- (25) Jerkovic, V.; Collin, S. Fate of resveratrol and piceid through different hop processings and storage times. *J. Agric. Food Chem.* **2008**, *56*, 584–590.
- (26) Jerkovic, V.; Nguyen, F.; Timmermans, A.; Collin, S. Comparison of procedures for resveratrol analysis in beer: assessment of stilbenoids stability through wort fermentation and beer aging. *J. Inst. Brew.* **2008**, *114*, 143–149.
- (27) Hurst, W. J.; Glinski, J. A.; Miller, K. B.; Apgar, J.; Davey, M. H.; Stuart, D. A. Survey of *trans*-resveratrol and *trans*-piceid content of cocoa-containing and chocolate products. *J. Agric. Food Chem.* **2008**, *56*, 8374–8378.
- (28) Jerkovic, V.; Nguyen, F.; Nizet, S.; Collin, S. Combinatorial synthesis, reversed-phase and normal-phase high-performance liquid chromatography elution data and liquid chromatography/positive atmospheric pressure chemical ionization tandem mass spectra of methoxylated and glycosylated resveratrol analogues. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2456–2466.
- (29) Callemien, D.; Collin, S. Structure, organoleptic properties, quantification methods and stability of phenolic compounds in beer. A review. *Food Rev. Int.* **2010**, *26*, 1–84.
- (30) Counet, C.; Collin, S. Effect of the number of flavanol units on the antioxidant activity of procyanidin fractions isolated from chocolate. *J. Agric. Food Chem.* **2003**, *51*, 6816–6822.
- (31) Shriner, R. L.; Hermann, C. K. F.; Morrill, T. C.; Curtin, D. Y.; Fuson, R. C. *The systematic identification of organic compounds*, 8th ed.; John Wiley & Sons: Hoboken, NJ, 1997.
- (32) Urpi-Sarda, M.; Jauregui, O.; Lamuela-Raventos, R. M.; Jaeger, W.; Miksits, M.; Covas, M. I.; Andres-Lacueva, C. Uptake of diet resveratrol into the human low-density lipoprotein. Identification and quantification of resveratrol metabolites by liquid chromatography

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- 379 coupled with tandem mass spectrometry. *Anal. Chem.* **2005**, *77*,  
380 3149–3155.
- 381 (33) Lamuela-Raventos, R. M.; Waterhouse, A. L. Occurrence of resver-  
382 atrol in selected California wines by a new HPLC method. *J. Agric.*  
383 *Food Chem.* **1993**, *41*, 521–523.
- 384 (34) Romero-Perez, A. I.; Lamuela-Raventos, R. M.; Waterhouse, A. L.;  
385 de la Torre-Boronat, M. C. Levels of *cis*- and *trans*-resveratrol and  
386 their glucosides in white and rose *Vitis vinifera* wines from Spain.  
387 *J. Agric. Food Chem.* **1996**, *44*, 2124–2128.
- 388 (35) Romero-Perez, A. I.; Ibern-Gómez, M.; Lamuela-Raventos, R.;  
389 Carmen de la Torre-Boronat, M. Piceid, the major resveratrol  
derivative in grape juices. *J. Agric. Food Chem.* **1999**, *47*, 1533–  
1536.
- (36) Camu, N.; De Winter, T.; Addo, S. K.; Takrama, J. S.; Bernaert, H;  
De Vuyst, L. Fermentation of cocoa beans: influence of microbial  
activities and polyphenol concentrations on the flavour of chocolate.  
*J. Agric. Food Chem.* **2008**, *88*, 2288–2297.
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