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Isolation, Characterization, and Determination of 1-*O-trans*-Cinnamoyl-β-D-glucopyranose in the Epidermis and Flesh of Developing Cashew Apple (*Anacardium occidentale* L.) and Four of Its Genotypes

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1-*O-trans*-Cinnamoyl-β-D-glucopyranose was purified from cashew apple (*Anacardium occidentale* L.) juice and unambiguously characterized. Absent at the immature green stage, its concentration bursts at the turning and even more at the mature ripe stage, reaching 6.2 mg/100 g of fresh weight. Whatever the considered cashew apple genotype, this cinnamoyl glucoside ester was preferentially concentrated in the epidermis, which was 4−5 times richer than flesh, reaching 85 mg/100 g of fresh weight for skin of the Brazilian clone EMBRAPA 50. Entire cashew apples contained from 6 to 20 mg of 1-cinnamoylglucose/100 g, a concentration similar to that of red strawberry receptacle. Accumulation of such amounts in this false fruit remains to be explained.

KEYWORDS: *Anacardium occidentale*; Anacardiaceae; cashew apple; immature green; receptacle; epidermis; flesh; 1-*O-trans*-cinnamoyl-β-D-glucopyranose; HPLC-DAD/ESI-MS

INTRODUCTION

The cashew apple, borne by the cashew tree (*Anacardium occidentale* L.), although beyond pineapple and mango in terms of world annual production at 30 millions metric tons (*1*), is often considered to be a byproduct of the high-added-value cashew nut, and part of its production is wasted, left to rot under trees after nut harvest. However, it deserves more attention because it is juicy and nutritious with an ascorbic acid content ca. 5 times that of citrus (*2*, *3*).

Botanically speaking, the cashew apple is a pseudocarp or false fruit supporting the true fruit, an indehiscent dry achene (cashew nut) (**Figure 1**). The development of cashew nut and apple begins after self-pollination of a hermaphrodite flower, after which a fruitlet starts developing to finally give a mature achene ∼30−35 days after anthesis; at that time, the cashew apple starts progressively swelling into a pear-shaped organ and reaches full maturity days 60−70 days after anthesis. The achene is connected to the pedicel via a *fasciculus* of vascular bundles that extends umbrella-like from the pedicel through the cashew apple and then branches out toward the achene (**Figure 2**). The cashew apple is actually a receptacle, which is a nonovarian tissue, that initially bears a sexually functional flower, that is, sepals, petals, and the reproductive organs (*androecium* and *gynoecium*); the apple is indeed the enlarged terminal portion of the pedicel (*4*). It is made of a fibrous parenchyma in which are embedded about a hundred vascular bundles and is covered by a thin and soft epidermis.

Ontogenically speaking, the cashew apple shows strong similarities with the strawberry fruit, as the red edible portion of the strawberry (the minute yellow "seeds" being the achenes) is also a receptacle (*5*, *6*). Recently, a cinnamoyl glucose ester was reported in developing strawberry (*Fragaria* × *ananassa*

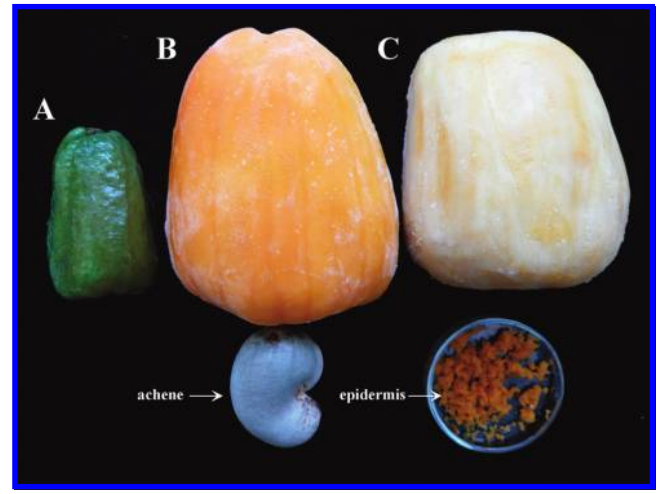

Figure 1. Views of immature green (**A**), mature ripe (**B**), and hand-dissected (**C**) cashew apple (clone CCP 76).

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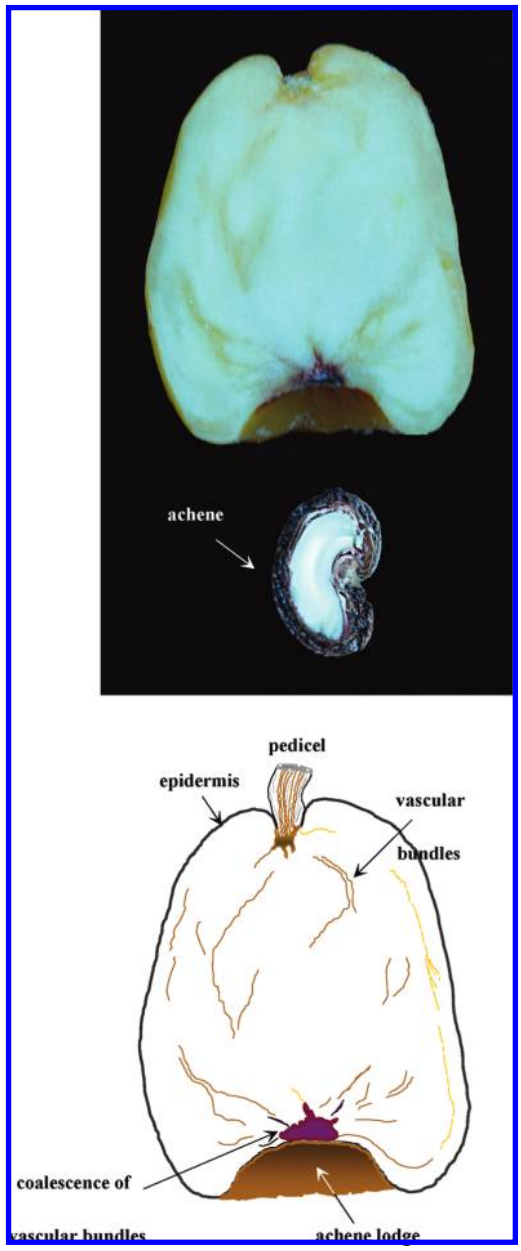

Figure 2. Scheme and view of a longitudinal section of a mature ripe cashew apple.

Duch.) (*6*, *7*); this 1-*O*-cinnamoylglucose is strictly limited to the strawberry receptacle, and its concentration increases with maturation from undetectable in green and white pseudocarps to 0.6 mg/100 g in turning to 1.5−7.5 mg/100 g in red receptacles. Similarly, the spatial and developmental expression pattern of *FaGT2*, a strawberry UDP-glucose:cinnamate glucosyltransferase (*6*), shows a high level in the red strawberry receptacle and a negligible one in other plant organs (leaf, root, achenes), but it is present in flowers (only ∼10% of that of receptacle); it also paralleled the increase of this ester in the developing pseudocarp. These authors showed that expression of *FaGT2* was negatively regulated by auxin from the achenes and positively induced by oxidative stress.

Six cinnamoyl-hexose isomers were also detected by high-temperature gas chromatography in cashew apple (*Anacardium occidentale* L.) without complete characterization and measurement (*8*). Within the framework of the "PAVUC" European Research Project on underutilized tropical fruits, we have described the monomeric phenols (mainly flavonol glycosides) from the cashew apple and incidentally detected an unknown strongly UV-absorbing compound (*9*). We report here the isolation, characterization, and measurement of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose in the epidermis and flesh of developing cashew apple and in four genotypes of mature apples originating from Brazil and Benin (West Africa).

MATERIALS AND METHODS

Plant Materials. Two batches of mature cashew apples (clones CCP 76 and EMBRAPA 50) (20 kg each) were harvested in November 2006 at EMBRAPA Paraipaba Experimental Station (Ceará State, Brazil), immediately frozen, and air-freighted to our laboratory. Two batches of mature cashew apples with cashew nuts still attached (varieties Parakou Rouge and Parakou Jaune) (20 kg each) were collected in February 2006 from orchards in the Parakou District (Bénin, West Africa) and treated as above; nuts were removed before analysis. All cashew apples were at the mature stage (60−65 days after anthesis). Two batches of green immature and turning apples (clone CCP 76; 30 and 53 days after anthesis) were also used. Ten frozen cashew apples of each batch were randomly chosen; they were, being still frozen, weighed, and rapidly, carefully, and entirely peeled by gently grating the epidermis with a razor blade (**Figure 1**); skin pieces (∼ 0.2−0.5 mm maximum thickness) were immediately dipped in liquid nitrogen to prevent oxidation and pulverized to a very fine powder with a pestle in a mortar; epidermis pieces appeared translucent upon dissection, meaning that one or two epidermal cell layers only were collected. Similarly, skinned cashew apples were cleared from the corky lignified zone of achene attachment ($\phi = 1$ cm, $h = 1$ cm; see **Figure 2**, achene lodge), and then frozen flesh was rapidly cut into bits (1 × 1 cm) and ground in liquid nitrogen as above. Epidermis and flesh liquid nitrogen powders were stored at −80 °C under nitrogen before analyses.

Standard and Enzymes. *trans*-Cinnamic acid, sweet almond β-D-glucosidase, and *Escherichia coli* β-D-galactosidase were from Fluka (Buchs, Switzerland). All other reagents and solvents were of analytical grade.

Preparation of Cashew Apple Juice. Cashew apples from the Brazilian clone EMBRAPA 50 (10 kg) were hand cut in cubes (5 × 5 cm), and then juice was extracted with a hydraulic press. Juice (7.8 L) was then filtered on muslin and frozen at −20 °C.

Extraction of Phenolics. For preparative purpose, acetone was added to cashew apple juice (7.8 L) to a 60% (v/v) final concentration, and the medium was stirred for 1 h at ambient temperature. After centrifugation for 15 min at 15000 rpm (20 °C), acetone was removed from the supernatant by vacuum evaporation (30 °C), and the water phase was further concentrated to ∼100 mL. Extract was then mixed with hexane and, after phase separation, the water phase was passed at 20 mL h^{-1} on a column packed with Amberlite XAD-2 (500 g) previously washed with methanol and then distilled water; after extensive washing of the column with 10 L of water, adsorbed materials were recovered by desorption with ethanol (2 L). The ethanolic extract was brought to dryness under vacuum (crude extract).

For analytical purposes, ca. 200−500 mg of fresh epidermis or flesh powders was extracted for 1 h with 25 mL of an acetone/water mixture (60:40, v/v), after which the medium was filtered, brought to dryness under vacuum (40 °C), redisolved in 1 mL of methanol/water (50:50, v/v), and then injected onto the HPLC column. Triplicate extractions were made for each sample, each extract being injected twice.

Purification of 1-*trans*-Cinnamoyl-β-D-glucose and *trans*-Cinnamic Acid. 1-*trans*-Cinnamoyl-β-D-glucose was purified by semi-preparative HPLC as follows: the crude extract was redisolved in distilled water (50 mL) and injected onto an Agilent 1100 separation system (Agilent Technologies, Waldbronn, Germany) including a quaternary pump coupled to a diode array detector and controlled by Chemstation A.10.02 software. Separations were achieved using a (250 × 10 mm i.d.) Lichrospher 100 RP-18 e column (Merck, Darmstadt, Germany) with a guard column, operated at 30 °C. Mobile phase consisted of water/formic acid (98:2, v/v) (eluant A) and water/acetonitrile/formic acid (18:80:2, v/v/v) (eluant B). Flow rate was 2 mL min^{-1}. The elution program was as follows: 50−75% B (0−4 min); 75−84% B (4−8 min); 84−50% B (8−40 min). Because a trace of an

anthocyanin eluted at the same retention time, 1-*trans*-cinnamoyl-β-D-glucose was rechromatographed under the same conditions but without formic acid added. Injected volumes were 200 μL.

trans-Cinnamic acid was extracted with 50 mL of pentane/ether (1:1, v/v) from the crude extract redissolved in distilled water (50 mL). The organic phase was recovered, washed twice with water saturated with NaCl (50 mL), and dried under vacuum.

HPLC-DAD Analysis. HPLC analyses were performed using an Agilent 1100 separation system (Agilent Technologies) including a quaternary pump coupled to a diode array detector and controlled by Chemstation A.10.02 software. Separations were achieved using a (250 × 4.6 mm i.d.) Modulocart QS-Lichrospher 5 μm ODS2 column (Interchim, Montluçon, France) with a guard column, operated at 30 °C. Mobile phase consisted of water/formic acid (98:2, v/v) (eluant A) and water/acetonitrile/formic acid (18:80:2, v/v/v) (eluant B). Flow rate was 0.5 mL min^{-1}. The elution program was as follows: 5−10% B (0−4 min); 10−16% B (4−8 min); 16−25% B (8−45 min); 25−35% B (45−55 min); 35−80% B (55−72 min); 80−100% B (72−75 min); 100−5% B (75−80 min). Duplicate samples were injected at a level of 10 μL. The column effluent was monitored from 230 to 600 nm. Quantification was achieved by injection of solutions of known concentrations of 1-*O*-*trans*-cinnamoyl-β-D-glucose; it was expressed as milligrams per 100 g of fresh weight, isomers being added to the 1-*O*-ester (see further).

HPLC-DAD/ESI-MS Analysis. Separations were performed on a (250 × 4.6 mm i.d.) Modulocart QS-Lichrospher 5 μm ODS2 column (Interchim) with a guard column, operated at 30 °C. Mobile phase consisted of water/formic acid (99.9:0.1, v/v) (eluant A) and water/acetonitrile/formic acid (18.0:81.9:0.1, v/v/v) (eluant B). Flow rate was 0.5 mL min^{-1}. The elution program was the same as above. The column eluate was then split, and 0.25 mL min^{-1} was directed to an LCQ ion trap spectrometer fitted with an electrospray interface (Thermo Finnigan, San Jose, CA). Experiments were conducted in both negative and positive modes. Scan range was 100−2000 amu and scan rate 1 scan/s. The desolvation temperatures were 250 and 300 °C in the positive and negative ion modes, respectively. High spray voltage was set at 4000 V (positive) and 3500 V (negative) ion modes. Nitrogen was used as the dry gas at a flow of 5 for the auxiliary gas and a flow of 55 for the sheath gas. Identifications were achieved on the basis of the ion molecular masses and UV−visible spectra.

Enzymatic, Acid, and Alkaline Hydrolyses. To a solution (50 μL) of the isolated compound in 50 mM acetate buffer (pH 5.2) was added 5 μL of sweet almond β-glucosidase (150 μg; 0.9 unit) in acetate buffer; the mixture was incubated at 35 °C for 5 h. A solution (50 μL) of the isolated compound in 75 mM phosphate buffer (pH 7.4) was amended with 5 μL of *E. coli* β-D-galactosidase (50 μg; 7 units) in phosphate buffer and incubated overnight at ambient temperature. Blanks were run the same way with buffers instead of enzymes.

A solution (50 μL) of the isolated compound in methanol was brought to dryness, and 50 μL of 4 M HCl was added; the medium was kept at 45 °C for 1 h and brought to dryness, and then 50 μL of water/methanol (50:50) was added.

A solution (50 μL) of the isolated compound in methanol was brought to dryness, and 50 μL of 2 N NaOH containing 10 mM EDTA and 1% ascorbic acid were added (*10*); the medium was kept at 45 °C for 30 min, then 10 μL of fuming HCl was added. In all assays, the injected volume was 10 μL.

Reaction products were analyzed by HPLC.

NMR Spectroscopy. ^{1}H and ^{13}C NMR spectra were recorded at 30 °C on a Varian iNOVA 500 spectrometer; solvent was methanol-d_4. Chemical shifts are given in δ values (ppm), based on those of the CD_3OD signals (δ_H 3.30 and δ_C 29.8).

RESULTS AND DISCUSSION

Isolation and Characterization of 1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose. A typical HPLC-UV−vis chromatogram of a crude extract from cashew apple juice is shown in **Figure 3**. A huge unknown UV-absorbing peak (**X**) was observed at 50.7 min (λ_{max} 283 nm, no shoulder), eluting between flavonol glycosides, namely, quercetin 3-*O*-galactoside and a quercetin-3-*O*-pentoside (*9*). Its retention time suggested a compound of medium polarity. Another compound (**Y**) of lower polarity eluted at 64.9 min (λ_{max} 275 nm) and was suspected to be related to the first one. Whatever the conditions used, this last compound gave no response in HPLC/ESI-MS (negative and positive modes); we incidentally found that this compound was soluble in the pentane/ether (1:1) azeotrope, whereas compound **X** was not. Compound **Y** was shown to be *trans*-cinnamic acid by GC-MS of its methyl ester derivative.

Compound **X** was purified to homogeneity from juice by semipreparative HPLC, yielding nonquantitatively 310 mg from 7.8 L of starting juice.

Sweet almond β-D-glucosidase liberated stoichiometrically (peak area basis) cinnamic acid from compound **X**, indicating that this compound was a cinnamoyl β-D-glucoside; however, because this enzyme shows a broad specificity being able to also degrade β-D-galactosides (*11*), we checked that a β-D-galactosidase had no action on this compound. Acid and alkaline hydrolyses also liberated quantitatively cinnamic acid from **X**, indicating that this compound contained both a glycoside and an ester.

In HPLC-DAD/ESI-MS analysis (negative mode), **X** showed a deprotonated molecular ion at *m*/*z* 355 corresponding, as already observed by Lunkenbein and co-workers (*6*), to the formiate adduct $[M + HCOO]^-$ and, thus, a molecular weight of 310; fragments obtained in MS^2 were *m*/*z* 207 and 147, the latter corresponding to $[\text{cinnamic acid} - H]^-$. It gave in positive mode *m*/*z* 333 corresponding to $[M + Na]^+$ and, surprisingly enough, no molecular ion at 311.

The compound was further submitted to NMR analysis (**Figure 4** and **Table 1**). The existence of glucopyranosyl moiety in the molecule was confirmed by resonances of six carbons (*12*): one anomeric at δ 95.51 ppm, one ($-CH_2OH$) at 62.81 ppm, and four [−CH(OH)−] in the range 0f 71.43−79.12 ppm. The $^{1}H-^{1}H$ coupling constant ($J = 7.2$ Hz) measured at the C-1′ level revealed a β monomeric configuration of the glucopyranosyl moiety (*7*, *12*). Cinnamic acid was characterized by nine carbon atoms: six aromatic (C4−C9) between 129.75 and 135.79 ppm, two alkenic (C2 and C3) at 117.75 and 148.95 ppm, respectively, and one carboxylic (C1) at 168.15 ppm. Five aromatic protons (H5−H9) resonated between 7.39 and 7.63 ppm. The *trans* configuration of cinnamic acid was corroborated by proton resonances at δ 6.61 (H-2) and 7.82 (H-3) and a typical $^{1}H-^{1}H$ coupling constant ($J = 16$ Hz) (*7*, *13*, *14*).

Thus, we conclude unambiguously that compound **X** was 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose, the same molecule previously characterized in red strawberry receptacle (*6*, *7*). Finally, 1-O-glucosylation of cinnamic acid induces a bathochromic shift analogue to the one observed by later authors.

On the basis of total area of peaks detected in HPLC at λ 280 nm, the purity of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose was estimated on an anhydrous basis at 98.2%; measurement of cinnamic acid after hydrolysis with β-glucosidase provided a 99.4% purity index. Recovery of cinnamoylglucose in its following quantification analyses was measured by adding known quantities of our purified compound to fresh epidermis or flesh powders; it was found to be 99.5 ± 1.9 ($n = 5$).

In addition to 1-*O*-cinnamoylglucose, three isomers were detected at 48.3, 51.8, and 53.7 min (*m*/*z* 355; **Figure 3**), and their common λ_{max} values were at 280 nm. The presence of these compounds derived from 1-*O*-cinnamoylglucose was also noted in strawberry extracts (*6*).

Evolution of 1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose during Maturation. Cashew apples from the Brazilian CCP 76

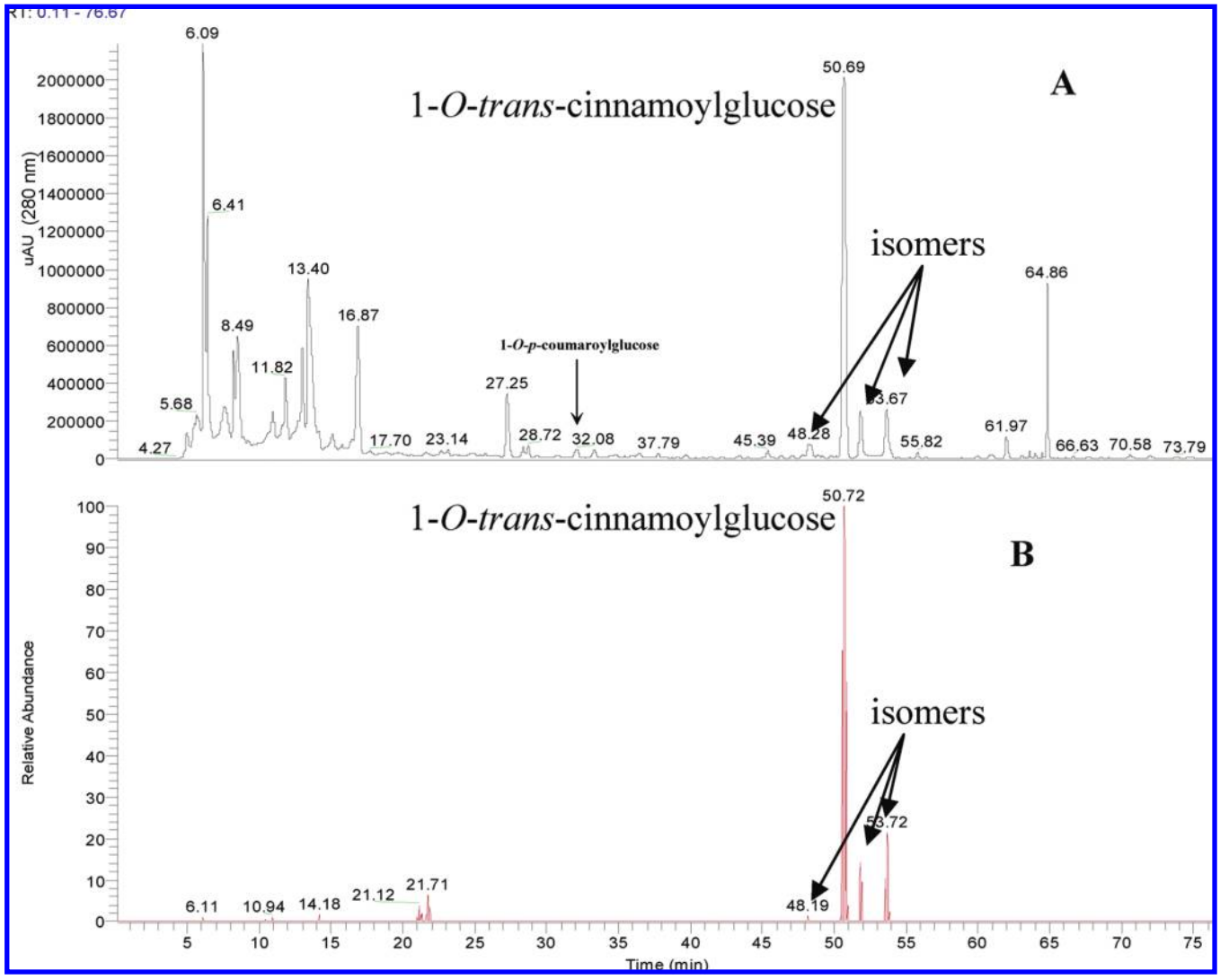

Figure 3. HPLC-DAD/ESI-MS chromatogram of a juice extract showing the four cinnamoylglucose isomers: (**A**) UV trace; (**B**) ion selective monitoring at *m*/*z* 355.

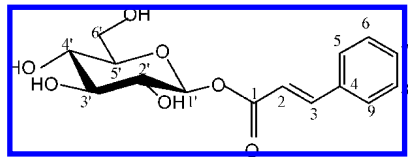

Figure 4. Structure of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose.

clone were harvested at three stages of development: immature green, 40 days; turning, 53 days; and mature ripe, 65 days after anthesis; they were analyzed for their levels of 1-*O*-cinnamoylglucose (**Table 2**). Not even traces of cinnamoylglucose were found in the epidermis and flesh of immature green apples; at the turning stage, this ester was present in both compartments and finally reached, at full maturity, 23.1 and 5.1 mg/100 g of fresh weight in skin and flesh, respectively. The higher richness of skin compared to flesh in mature apples was expected because similar observations were made for their monomeric phenols, mainly flavonol glycosides (*9*). No comparison could be done with strawberry epidermis due to the difficulty of peeling such a pseudocarp. Given a mass distribution of epidermis 3.7% and flesh 96.3%, a content of 6.2 mg/100 g in mature ripe apples was calculated, a value close to the 7.7 mg/100 g reported for mature red strawberries at 28 days after anthesis (*7*). This concentration was quoted as considerable by these authors, who were probably comparing it to the minute amounts of flavor alkylcinnamates present in cashew apple, cinnamoylglucose being supposed to be their precursor. In the end, this is, to our knowledge, the second time that 1-*O*-cinnamoyl-

Table 1. ^{1}H and ^{13}C NMR Spectral Data (in Methanol-d_4) of 1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose Isolated from Cashew Apple

	^{1}H	^{13}C
β-D-glucosyl		
1′	5.58 (d, J = 7.2 Hz)	95.51
2′	3.31−3.85 (m)	74.62
3′	3.31−3.85 (m)	78.09
4′	3.31−3.85 (m)	71.43
5′	3.31−3.85 (m)	79.12
6′	3.31−3.85 (m)	62.81
cinnamoyl		
1		168.15
2	6.61 (d, J = 16 Hz)	117.75
3	7.82 (d, J = 16 Hz)	148.95
4		135.79
5	7.39−7.63 (m)	130.79
6	7.39−7.63 (m)	129.75
7	7.39−7.63 (m)	131.83
8	7.39−7.63 (m)	129.75
9	7.39−7.63 (m)	130.79

Table 2. Concentrations of 1-*O*-Cinnamoylglucose in Epidermis and Flesh of Developing and Four Genotypes of Cashew Apple

clone or variety	wt (g)	epidermis[a] (%)	flesh[a] (%)	epidermis (mg/100 g)	flesh (mg/100 g)	apple (mg/100 g)
CCP 76 (green)	11.3 ± 1.5	8.5	91.5	nd[b]	nd	
CCP 76 (turning)	90.1 ± 3.0	4.3	95.7	7.8 ± 0.4	1.6 ± 0.1	1.6
CCP 76 (mature ripe)	160.6 ± 2.3	3.7	96.3	23.1 ± 0.7	5.1 ± 0.2	6.2
EMBRAPA 50	102.2 ± 1.2	4.2	95.8	84.8 ± 2.1	17.3 ± 1.3	20.1
Parakou Rouge	81.2 ± 7.2	4.4	95.6	62.4 ± 1.1	13.0 ± 0.9	15.2
Parakou Jaune	62.3 ± 4.9	4.9	95.1	63.7 ± 1.9	11.8 ± 0.2	13.9

[a] Skin and flesh mass percents of the whole apple. [b] Not detected.

glucose has been found in such an amount in an edible false fruit. A high concentration was also found in leaves of *Spiraea thunbergii* (*12*).

Contents of 1-*O*-*trans*-Cinnamoyl-β-D-glucopyranose in Four Genotypes of Mature Ripe Cashew Apples. Mature ripe cashew apples (clone EMBRAPA 50, varieties Parakou Rouge and Parakou Jaune) were analyzed for their levels in 1-*O*-cinnamoylglucose (**Table 2**) in epidermis and flesh in comparison with the mature CCP 76 clone. It must be noted that the mass percentage of epidermis is in inverse ratio to apple weight. As noted above, epidermis was ∼4−5 times richer than flesh, reaching for EMBRAPA 50 0.09% of the fresh weight. Clone CCP 76 was the poorest of all studied genotypes, EMBRAPA 50 being the richest in this compound. Both West African apples from Bénin appear to be similar with contents ∼2 times that of red strawberry (*7*).

1-*O*-*p*-Coumaroylglucose was also detected in hardly measurable amounts in all genotypes, but CCP 76, at 32.1 min elution time (typical spectrum with λ_{max} 315 nm bathochromically shifted from *p*-coumaric acid at λ_{max} 310 nm; *m*/*z* $[M - H]^-$ 325) (**Figure 3**). Again, the epidermis was richer than the flesh: EMBRAPA 50, epidermis 1.2 mg/100 g, flesh 0.1 mg/100 g; Parakou Rouge, epidermis 0.5, flesh 0.1; Parakou Jaune, epidermis 1.1, flesh 0.1.

Lunkenbein and co-workers (*6*), studying cinnamate metabolism in ripening strawberry, isolated a cDNA encoding for a UDP-glucose:cinnamate glucosyltransferase (*FaGT2*), an enzyme catalyzing the transfer of a cinnamoyl group to the C1 of glucose with production of 1-*O*-cinnamoyl-β-D-glucopyranose. This compound had been formerly detected in developing strawberry (*7*). Both groups of authors showed that production of this metabolite is developmentally regulated because no production occurs before 20 days after anthesis (from green to white stage) and then it bursts up to 1.5−7.6 mg/100 g of fresh weight until 28 days after anthesis. The expression pattern of *FaGT2* was strikingly superimposable to that of cinnamoyglucose presence. The absence of 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose in immature green cashew apples and its ultrafast emergence up to the mature stage suggest a similar mechanism. The last authors demonstrated that this absence in the first stages of development was due to a negative regulation of the expression pattern of *FaGT2* by auxin transport from the achenes; in other words, when the achene grows and approaches maturity (30−35 days after anthesis for cashew nut), this repression would be progressively leveled out by the end of auxin synthesis and transport from the achenes to the pseudocarp. These authors examined the spatial regulation pattern of this enzyme in various parts of the strawberry plant, and, as noted above, it is poorly expressed in the flower and petiole and barely in leaves and roots; unfortunately, due to the above-mentioned unavailability of strawberry epidermis, they did not look for expression of *FaGT2* in this compartment. Because the relative concentration of cinnamoylglucose is remarkably stable at 4−5 times higher in skin than in flesh of all cashew apple genotypes and keeping in mind the similarities between strawberry and cashew apple, it is likely that a higher expression level of UDP-glucose:cinnamate glucosyltransferase would be observed in the epidermis of these two receptacles in comparison with their inner flesh compartments; another alternative is that the 1-cinnamoylglucose could be transported from another compartment and accumulate in the epidermis. The reason for this remains obscure. However, the stronger richness of epidermis in this compound was not unexpected because phenolics and related molecules are known to play in fruit epidermis a defense mechanism against pathogens (*15*).

A distinct difference with strawberry has to be noted because, contrary to red mature strawberry, cashew apples did not accumulate massive amounts of hydroxycinnamoylglucose esters, in particular *p*-coumaroyl-D-glucose (16.8 mg/100 g in ref *6*); in our case, only traces were measured. This raises the question of substrate specificity of a putative cashew cinnamate glucosyltransferase different from that of strawberry, which accepts *p*-coumaric acid as well as cinnamic acid (*6*), or of unavailability of a sufficient quantity of *p*-coumarate. Hydroxycinnamoylglucoses play a role in sugar acylation of anthocyanidin glycosides (*16*); thus, the absence of hydroxycinnamoylated anthocyanins in cashew apple epidermis (*9*) could be related to limited availability of these acyl donors.

The role of cinnamoylglucose, a high-energy compound, is not yet fully understood; it was suggested to be, for instance, the precursor, although of minor quantitative importance, of flavor ethyl and methyl cinnamates in ripe strawberry (*7*); however, levels of these aroma compounds in cashew apple and strawberry as well differ considerably by factors of 10^3-10^4 less from that of cinnamoylglucose (*17, 18*). A novel strawberry alcohol acyl-CoA transferase (*SAAT*) able to transfer the acetyl group from acetyl-CoA to aliphatic alcohols was identified in strawberry (*19*), but unfortunately the authors did not test this enzyme activity with cinnamoyl-CoA as acyl donor. However, 1-*trans*-cinnamoyl-β-D-glucose must serve in aroma compound biosynthesis because a 1-*O*-*trans*-cinnamoyl-β-D-glucopyranose: alcohol cinnamoyltranferase has been isolated from cape gooseberry (*20*). In the end, it is not yet understood why cashew apple accumulates such amounts of this cinnamoylglucose ester; one has to mention that sinapoylglucose accumulation has been described in an *Arabidopsis* homozygous mutant (*21*).

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