JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Procyanidin A2 and Its Degradation Products in Raw, Fermented, and Roasted Cocoa

Cédric De Taeye, Gilles Caullet, Victor Jos Eyamo Evina, and Sonia Collin*

Unité de Brasserie et des Industries Alimentaires, Earth and Life Institute, ELIM, Faculté des Bioingénieurs, Université catholique de Louvain, Croix du Sud, 2 bte L07.05.07, B-1348 Louvain-la-Neuve, Belgium

ABSTRACT: Cocoa is known as an important source of flavan-3-ols, but their fate "from the bean to the bar" is not yet clear. Here, procyanidin A2 found in native cocoa beans (9−13 mg/kg) appeared partially epimerized into $A2^{E1}$ through fermentation, whereas a second epimer ($A2^{E2}$) emerged after roasting. At m/z 575, dehydrodiepicatechin A was revealed to be the major HPLC peak before fermentation, whereas F1, a marker of well-conducted fermentations, becomes the most intense after roasting. RP-HPLC-ESI(−)-HRMS/MS analysis performed on a procyanidin A2 model medium after 12 h at 90 °C revealed many more degradation products than those identified in fermented cocoa, including the last epimer of A2, A2 open structure intermediates (m/z 577), and oxidized A-type dimers (m/z 573).

KEYWORDS: *procyanidin A2, epimers, flavan-3-ol, cocoa fermentation, cyanidin*

INTRODUCTION

Proanthocyanidins are phytoallexins produced by plant secondary metabolism in response to various environmental stresses.[1−3] Provided by vegetables and fruits in the human diet, they bring health benefits such as antioxidant,[4,5] anticancer,[6,7] antibacterial,[8] anti-inflammatory,[9] cardioprotective,[10] and immunomodulatory[11] effects.

Constitutive building blocks of proanthocyanidins, usually flavan-3-ols (e.g., (+)-catechin and (−)-epicatechin) can be linked together by a single bond through C4−C8 (e.g., dimers B1−B4) or C6−C8 (e.g., dimers B5−B8) interflavan linkages. An additional C2−O7 bond can be found between monomeric units, giving rise to A-type procyanidins.[12] Procyanidin B5 oxidation may result in two distinct A-type structures (C6−C8 and C2−O−C5 or C2−O−C7), whereas procyanidin B2 is converted only to procyanidin A2 (C4−C8 and C2−O−C7) (Figure 1).

The additional ether bond of procyanidin A2, as compared to procyanidin B2, leads to a very different ESI(−) mass spectrum, with new fragments at m/z 449 (heterocyclic breakdown, −126 Da), 423 (retro-Diels−Alder, −152 Da), and 285 (methylene quinone formation). Contrarily to a study by Osman et al.,[13] A-type procyanidins are colorless because they do not absorb at wavelengths above 300 nm.[14]

Procyanidin A2 occurs naturally in plum,[15,16] hawthorn,[17] peanut,[15,16,18−20] cinnamon,[15,18] avocado,[15] litchi,[21−23] curry powder,[15] cranberry,[15,24−27] and chocolate liquor.[28] Procyanidin A2 has also been found in oligomers.[29] Because thiolysis cannot break A-type linkages, both A2 procyanidin (terminal units) and A2 procyanidin benzylthioether (extension units) have been found in procyanidin extract of litchi.[30]

A2 can derive chemically from procyanidin B2[31] (Figure 1a). The subsequent mechanism involves oxidation of the *o*-diphenol B cycle into its corresponding highly reactive *o*-quinone, which converts to a *p*-methylenequinone. This addition can be mediated by an oxidizing agent (e.g., DPPH[32]) promoting removal of the hydrogen atom on C2 or by an enzyme (polyphenol oxidase or laccase[33]).

In wine, anthocyani(-di)ns could also be precursors of A2.[34] In contrast to flavan-3-ols, the stability of anthocyani(-di)ns and their derived absorption spectra are strongly affected by the pH.[35] The reactivity of anthocyanidins and their ability to link to other phenolic compounds such as epicatechin[34−36] thus depend on the form present at equilibrium (Figure 1b).

Recently,[37,38] a molecule with the same mass as procyanidin A2 was proposed in cocoa as a marker of a well-conducted fermentation. This compound, called F2, exhibited a MS/MS(ESI(−)) fragmentation (major m/z 544) different from that of A2. As it was also found in fermented Criollo beans, it was suggested that it might be derived from A2. Chemically, however, the opposite conversion (F2 to A2) could also occur (Figure 1c).

Like other flavan-3-ols,[39,40] procyanidin A2 can be oxidized. It has been proposed that a second ether bond could be formed, either C5−O−C2′/C5′/C6′ (Figure 1d) or C5−O−C2 (Figure 1e).[40] In the latter case, in contrast to what occurs when A2 is created,[41] cycle B of the terminal unit has to be oxidized first to *p*-methylene quinone before nucleophilic attack by the C5−OH of the upper unit.[42]

The aim of the present work was to investigate the occurrence of procyanidin A2 and analogues in cocoa beans and to assess how they might be synthesized and degraded during cocoa fermentation and roasting. The degradation products of A2 were first evidenced in an aqueous model medium heated for 2 days at 40 °C or for 12 h at 90 °C. Identification and semiquantitation of the derived products were carried out by RP-HPLC-ESI(−)HRMS/MS and

Received: November 23, 2016
Revised: February 6, 2017
Accepted: February 11, 2017
Published: February 16, 2017

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

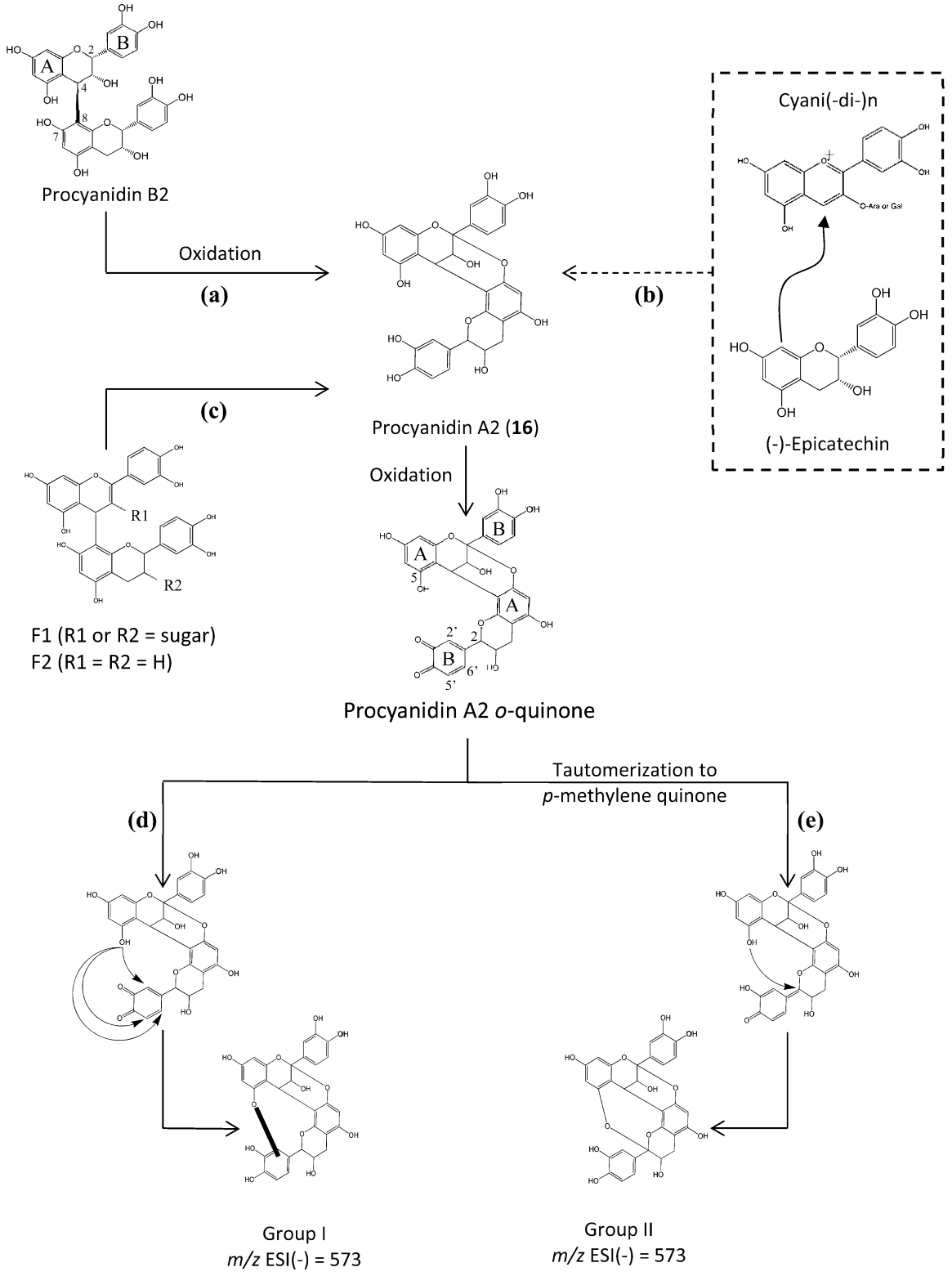


Figure 1. Chemical structures of procyanidin A2, its potential precursors (pathways a, b, and c), and degradation products (pathways d and e).

compared to those found in the previously investigated B2 degradation model medium.[40]

MATERIALS AND METHODS

Chemicals. Acetonitrile (99.9%) and methanol (99.9%) were supplied by VWR (Leuven, Belgium). Formic acid (99%) was obtained from Acros Organic (Geel, Belgium). (−)-Epicatechin (98%) and (+)-catechin (98%) were supplied by Sigma-Aldrich (Bornem, Belgium). (−)-Epicatechin-4β-8-(−)-epicatechin (B2, 90%) and (−)-epicatechin-4β-8,2-*O*-7-(−)-epicatechin (A2, 99%) were supplied by PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). Aqueous solutions were made with Milli-Q water (resistance = 18 mΩ) (Millipore, Bedford, MA, USA).

Cocoa Samples. Raw and fermented cocoa beans from Cuba (Trinitario UF 654 and Criollo C 411, harvest 2015) were provided by the Instituto de Investigaciones para la Industria Alimenticia (Habana,

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

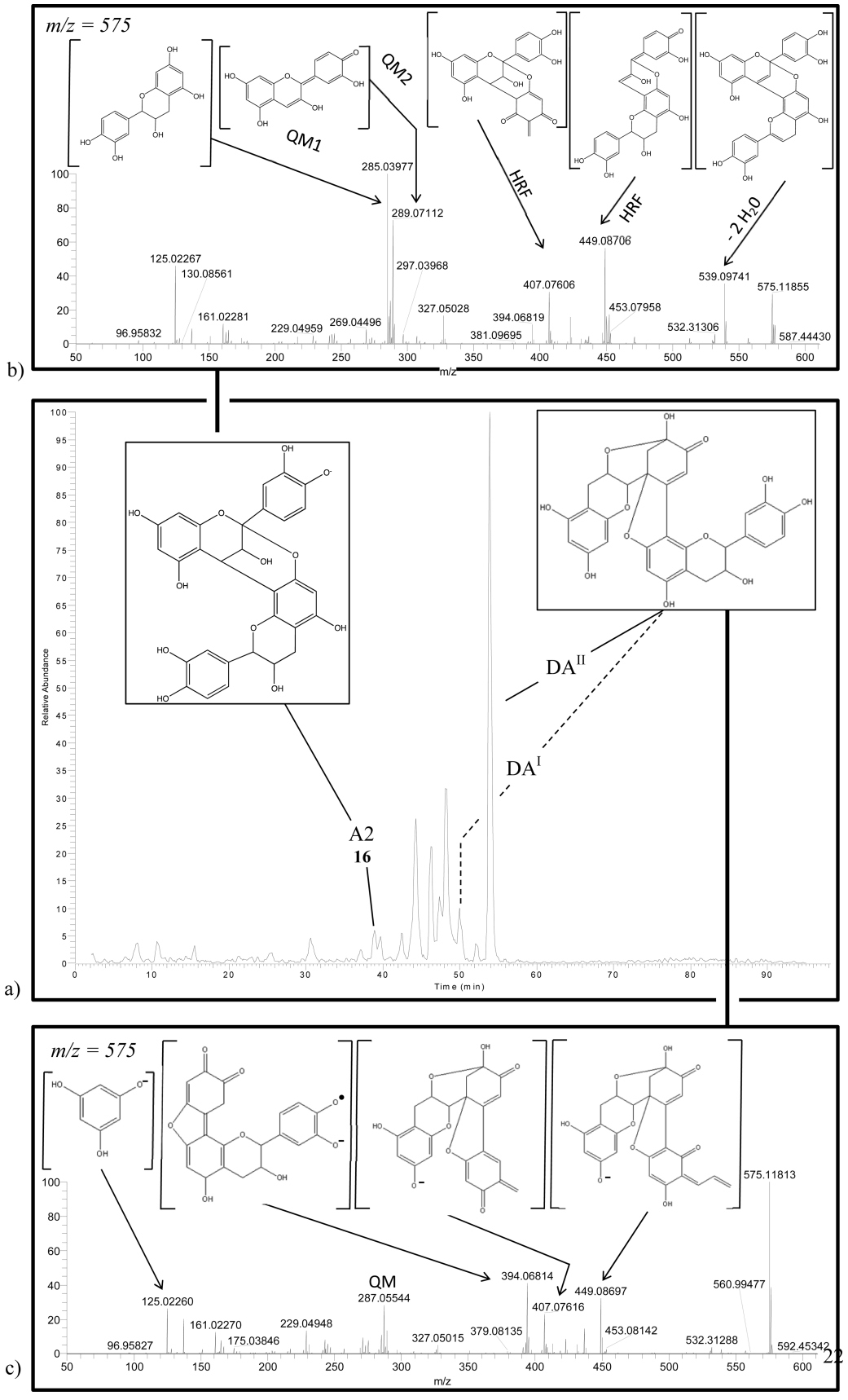


Figure 2. RP-HPLC-MS/MS-ESI(−) chromatogram at *m/z* 575 of (a) raw ICS 40 cocoa bean extract and mass spectra with fragment structures of (b) procyanidin A2 and (c) dehydrodi(epi)catechins A. The dotted lines indicate that these compounds gave an HRMS/MS spectrum very similar to that displayed here.

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

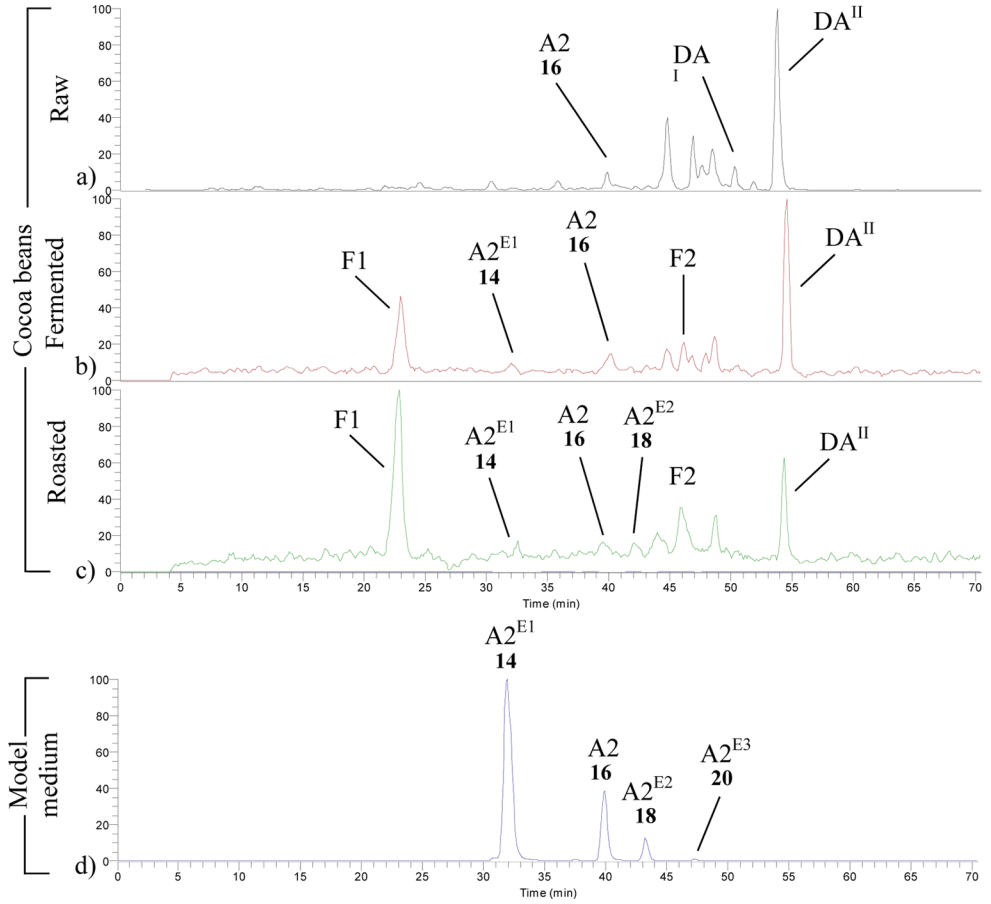


Figure 3. RP-HPLC-MS/MS-ESI(−) chromatogram at m/z 575 of (a) raw, (b) fermented, and (c) roasted (30 min at 150 °C) Criollo C 411 cocoa bean extract; (d) thermal degradation of procyanidin A2 in model medium heated for 48 h at 40 °C (1000 mg/L).

Cuba). Cameroon cocoa samples (Forastero German Cocoa and Trinitario ICS 40, harvest 2015) were collected in the area of Ngoumou and Nkomvoene and fermented for 5 days.[36]

Cocoa Roasting. Beans from Cuba or Cameroon were spread in one layer on a perforated tray and roasted in a ventilated heat chamber (series 4000, EHRET Labor- and Pharmatechnik GmbH & Co. KG, Emmendingen, Germany). Beans were milled at room temperature and homogenized before flavan-3-ol extractions performed in duplicate.

Preparation of Model Media. Aqueous model media were prepared in duplicate by diluting procyanidin A2 to 1000 mg/L in ultrapure water (10000 mg/L stock solution prepared in methanol) before heat treatment of 48 h at 40 °C (temperature close to that encountered in cocoa fermentation) or at 90 °C for 12 h (chosen on the basis of our previous work[39]). The heat treatment of the sealed vials was performed in a water bath (GFL 1003, GFL, Burgwedel, Germany). After filtration (0.22 μm), the samples were kept at −80 °C before injection.

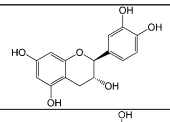
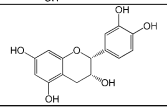
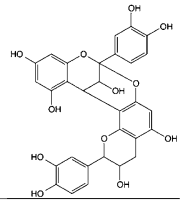
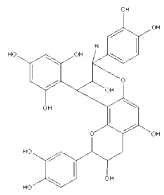
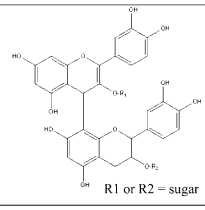
Extraction of Flavan-3-ols from Cocoa Beans. This method was adapted from that developed in our laboratory for the analysis of flavan-3-ols in chocolate.[42] All extraction steps were carried out in duplicate. Cocoa beans (7 g) were defatted with diethyl ether (3 × 50 mL) at room temperature under gentle stirring. After centrifugation, the samples were dried under vacuum. Defatted samples spiked with 500 μL of kaempferol at 10,000 mg/L in methanol (used as internal standard; 714 mg/kg bean weight) were extracted with 3 × 50 mL of acetone/water/acetic acid (70:28:2, v/v/v). The three resulting extracts were kept under nitrogen and then pooled. Each pool was concentrated by rotary evaporation and freeze-dried.

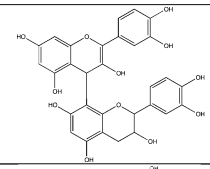
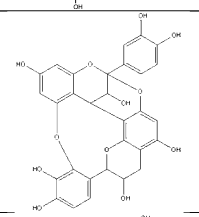
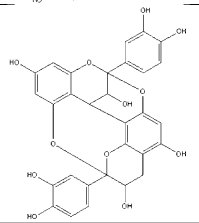
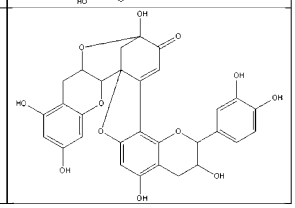
HRMS/MS Identification. High-resolution MS/MS analyses were performed after separation on a C18 Prevail column (150 × 2.1 mm, 2.7 μm) (Grace, Deerfield, IL, USA) using a multilinear gradient produced with A (water containing 1% acetonitrile and 2% formic acid) and B (acetonitrile containing 2% formic acid). Gradient elution was as follows: from 97 to 91% A in 5 min, from 91 to 85% A in 25 min, from 85 to 64% A in 35 min, from 64 to 10% A in 10 min, and isocratic for 20 min at a flow rate of 200 μL/min. Five microliters of sample was injected onto the column kept at 20 °C. The Exactive system (Thermo Fisher Scientific, Austin, TX, USA) was composed of an Accela LC coupled to the Orbitrap mass spectrometer (ionization ESI(−)) and controlled with Xcalibur software version 2.0.7 (ThermoFisher).

RP-HPLC-ESI(−)-MS/MS Semiquantitation. Quantitations were done by connecting the same column with the same elution program to a SpectraSystem equipped (ThermoFisher) with an AS3000 autosampler and a P4000 quaternary pump. The system was controlled with Xcalibur software version 1.2 (ThermoFisher). Compounds were monitored from 200 to 800 nm with a UV6000LP diode array detector. Mass spectra were acquired using an LCQ ion trap mass spectrometer equipped with an ESI source (ThermoFisher). Collision-induced dissociation spectra were recorded at a relative collision energy of 30, 35, or 40%, respectively, for singly charged $[M - H]^{-1}$ ions of m/z 289 (monomers), m/z 577 (B2, **24**–**27**), m/z 575 (A2 and epimers, F1, F2, DA^{I}, and DA^{II}), and m/z 573 (compounds of groups I and II). The ESI inlet conditions were as follows: source voltage, 4.9 kV; capillary voltage, −4 V; capillary temperature, 200 °C; sheath gas, 39 psi. For ESI(−)-MS/MS quantitations in cocoa beans (kaempferol used as internal standard), a relative recovery factor = 1 was applied for all compounds. Procyanidin A2 and its degradation products were quantitated according to the calibration curve of A2 (0, 10, 25, 50, 100 mg/L, R^2 = 0.98901).

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

Table 1. Polyphenols Detected in Cocoa Beans of A2 Model Media Heated at 90 °C for 12 h; RT = Retention Time

Name, formula and peak number [a]	RT [min] (relative retention time)	$[M-H]^{-1}$	MS/MS ions *m/z* (%)	Suspected structure
(-)-Catechin ($C_{15}H_{14}O_6$) **5**	15.0 (0.72)	289	289.1 (100) 245.1 (20) 179.1 (6)	
(-)-Epicatechin ($C_{15}H_{14}O_6$) **6**	21.1 (1.00)	289	289.1 (100) 245.1 (20) 179.1 (6)	
A2 and A2 Epimers * ($C_{30}H_{24}O_{12}$) **16 and 14, 18, and 20**	32.1 (1.52) 39.8 (1.89) 43.7 (2.04) 47.5 (2.25)	575	449.1 (100) 539.2 (47) 423.1 (42) 289.1 (30) 407.2 (15) 557.1 (14)	
Open A2 structures * ($C_{30}H_{26}O_{12}$) **24, 25, 26 and 27**	39.3 (1.86) 42.3 (2.00) 42.9 (2.03) 45.3 (2.24)	577	577.1 (100) 451.1 (37) 541.2 (20) 286.1 (18) 425.1 (17) 298.1 (15) 409.1 (8)	
F1 * (F2-O-sugar) $C_{30}H_{24}O_{12}$ + sugar - 18	23.8	575	544.1 (100)	 R1 or R2 = sugar

Name, formula and peak number [a]	RT [min] (relative retention time)	$[M-H]^{-1}$	MS/MS ions *m/z* (%)	Suspected structure
F2 * $C_{30}H_{24}O_{12}$	46.2	575	530.1 (100)	
Group I * ($C_{30}H_{22}O_{12}$) **15, 17** and **19**	36.3,..., 43.9 (1.72,..., 2.08)	573	125.0 (100) 285.1 (85) 573.1 (29) 163.0 (16)	
Group II * ($C_{30}H_{22}O_{12}$) **28**, **29**, **30** and **31**	47.9,..., 55.8 (2.27,..., 2.64)	573	285.1 (100) 125.0 (92) 163.0 (17)	
DA^{I} and DA^{II} * ($C_{30}H_{24}O_{12}$)	50.6 (2.39) 53.8 (2.55)	575	449.1 (100) 423.0 (75) 539.2 (28) 289.1 (22) 407.1 (20) 557.1 (13)	

[a]From a previous work.[39] An asterisk indicates tentative identification by comparison with the literature[37−41] and/or according to the HRMS/MS fragmentation.

RESULTS AND DISCUSSION

Occurrence of A2 and Other *m/z* 575 Compounds in Raw, Fermented, and Roasted Cocoa Beans. Procyanidin A2 (**16**, RT = 39.8 min) was found in all unfermented red beans (either Trinitario UF 654 and ICS 40 or Forastero German Cocoa; Figure 2a,b) and also in white Criollo beans (C 411; Figure 3a). To our knowledge, this is the first time that procyanidin A2 is reported in raw cocoa beans. Although present at much lower concentration than procyanidin B2 (340−650 mg/kg), A2 reached 9−13 mg/kg in the samples investigated here. Its occurrence in anthocyanin-free beans indicates that, in Criollo at least, A2 does not arise through epicatechin addition on anthocyanin (Figure 1b).

In fermented beans, procyanidin A2 appeared partially degraded to compound **14** (Figure 3b). The very close HRMS/MS spectra of these compounds led us to suspect that **14** might have arisen through epimerization of the terminal unit of A2, leading to (−)-epicatechin-(2β→7,4β→8)-(−)-catechin (not to be confused with procyanidin A1 (−)-epicatechin-(2β→7,4β→8)-(+)-catechin, which cannot derive from A2 through epimerization). As expected,[43] the presence of catechin instead of epicatechin in the extension unit leads to a shorter retention time for **14** (RT = 32.1 min) than for **16** on our apolar HPLC column.

To confirm that **14** is an A2 epimer, commercial procyanidin A2 was thermally degraded for 48 h at 40 °C (temperature close to that of a cocoa fermentation medium) in an aqueous model medium. As depicted in Figure 3d, four peaks were found: **16** (A2), **14** (named $A2^{E1}$), **18** (named $A2^{E2}$), and **20** (named $A2^{E3}$), all sharing the same HRMS/MS profile. As expected from the relative stabilities of catechin and epicatechin,[40] the major peak was **14**. This confirms the above-mentioned hypothesis regarding its structure and explains its occurrence in fermented cocoa beans. On the other hand, compounds **18** and **20** were assessed as (−)-catechin-(2β→7,4β→8)-(−)-epicatechin for $A2^{E2}$ and (−)-catechin-(2β→7,4β→8)-(−)-catechin for $A2^{E3}$. Much less stable, they did not occur in raw or fermented cocoa beans (Figure 3a,b). Traces of **18** were found, however, after roasting, because of the higher temperature applied (Figure 3c).

In raw, fermented, and roasted cocoa beans, two other compounds (DA^{I} and DA^{II}) with the same mass-to-charge ratio (*m/z* 575) and the same mass spectrum (different from that of A2; Figure 2b,c) were found between RT = 50.6 and 53.8 min. The absence of a signal at *m/z* 539 indicated that the loss of two molecules of water was not favored as in the case of A2 (C3−OH group on the upper and terminal heterocycles),[39] The very small signal at *m/z* 423 indicates that retro-Diels−Alder (RDA) fragmentation was prevented on the heterocycles. Production of fragment 449 through heterocyclic ring fission

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

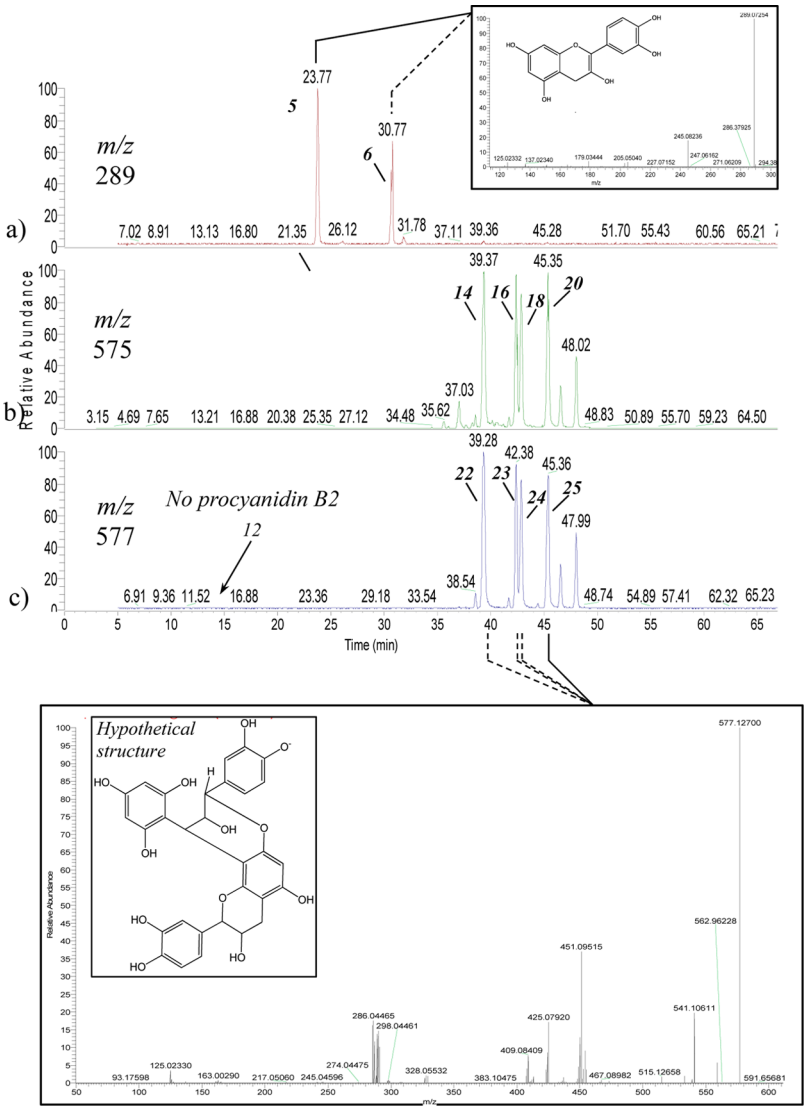


Figure 4. RP-HPLC-MS/MS-ESI(−) chromatograms at (a) *m*/*z* 289, (b) *m*/*z* 575, and (c) *m*/*z* 577 of procyanidin A2 model medium heated for 12 h at 90 °C. The dotted lines indicate that these compounds gave an HRMS/MS spectrum very similar to that displayed here. (Bottom) Mass spectrum of the open A2 structure with hypothetical structure.

(HRF) in A2 remained possible, however. Dehydrodi(epi)-catechins A (here called DA^{I} and DA^{II}), oxidation products of the "chemical dimers" previously found in (−)-epicatechin degradation model medium,[40] were logically suspected. HRMS confirmed their molecular composition ($C_{30}O_{12}H_{23}$ experimental *m*/*z* 575.11813 and 575.11798; theoretical *m*/*z* 575.1195; δ 2.38 and 2.64).

Also worth stressing is the presence of two more *m*/*z* 575 compounds, one (F1, RT = 26.9 min) eluting close to (−)-epicatechin and the other (F2, RT = 48.3 min), between $A2^{E2}$ (**18**) and $A2^{E3}$ (**20**) (Figure 3b). Both have recently been evidenced by Eyamo et al.[37] as good markers of well-conducted cocoa fermentations. F1 and F2 were absent, as is logical, from all our raw samples (Figure 3a), but were detected after fermentation (Figure 3b) or roasting (Figure 3c). As proposed by the authors just mentioned, F2 and its glucoside F1 (Table 1) could arise in vivo through A2 degradation (instead of deriving from anthocyanin). This would explain its occurrence in Criollo beans. Because of the relatively stronger degradation of A2 and its epimers (−27%) and of F2 (34%) and dehydrodi(epi)catechins A (−58%) through roasting, F1, suspected of being a glycoside, emerged for the first time as the highest peak in the HPLC chromatogram (Figure 3c).

Degradation of A2 in Aqueous Model Medium at Higher Temperature. In a previous study, the products arising through flavan-3-ol degradation were the same in aqueous and lipidic medium, although their concentrations were very different.[40] Therefore, A2 degradation was investigated here only in an aqueous model medium (12 h at 90 °C) requiring no long extraction procedures, which are sources of artifacts.

As depicted in Figure 4, no procyanidin B2 (**12**) was found in the aqueous A2 medium heated for 12 h at 90 °C. This suggests that the additional C2−O−C7 ether bond is more stable than expected. On the other hand, traces of the monomer (−)-epicatechin (**6**) and of its epimer (−)-catechin (**5**) were detected at *m*/*z* 289 (experimental *m*/*z* 289.07303; theoretical *m*/*z* 289.07121; δ 3.29) (Figure 4a,c). As previously evidenced,[40] the (−)-catechin/(−)-epicatechin ratio remained in favor of the (−)-catechin monomer (ratio = 2.3 after epimerization).

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

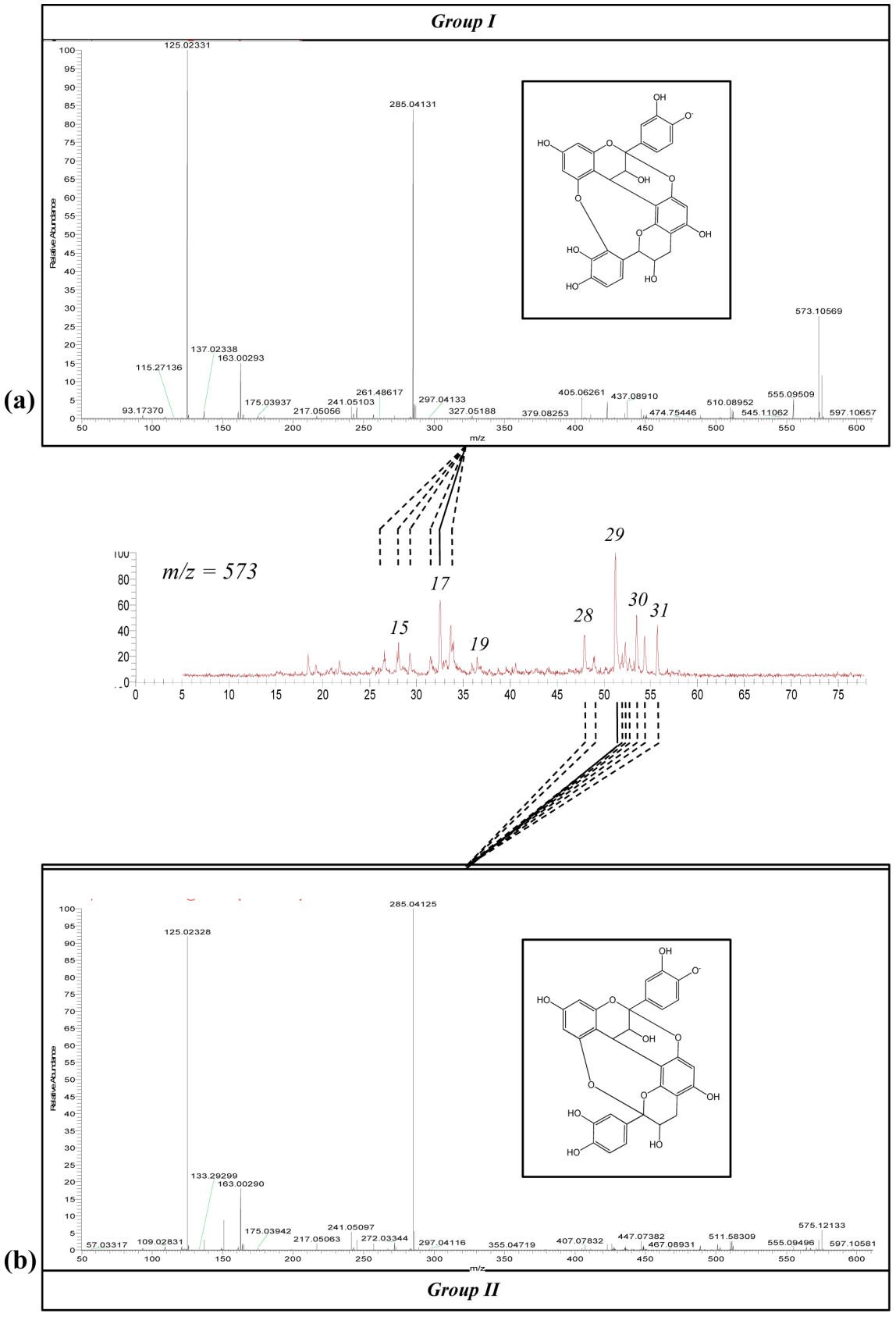


Figure 5. RP-HPLC-MS/MS-ESI(−)chromatogram at *m/z* 573 of procyanidin A2 model medium heated for 12 h at 90 °C: mass spectra of compounds eluting in (a) group I or (b) group II.

As shown at 40 °C (Figure 3d), procyanidin A2 (**16**) epimerized also at 90 °C, yielding four peaks, all detected in ESI-(−) at *m/z* 575 (compounds **14**, **16**, **18**, and **20**, Figure 4b). All of these compounds shared a very close HRMS/MS-

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

ESI(−) spectrum. Even compound **20**, suspected of arising through epimerization on both heterocycles, was produced in significant amount (more abundantly than **18**). The catechin moiety being more stable on the terminal unit, the major epimer was not A2 (22% **16** versus 30, 19, and 29% **14**, **18**, and **20**, respectively). A2 and its three epimers totaled 619 mg/kg A2 equiv, accounting for 62% of the initial concentration of A2.

With retention times very close to those of the A2 epimers discussed above (*m*/*z* 575; **14**, **16**, **18**, **20**), four compounds (**22**, **23**, **24**, **25**) were found in ESI(−) at *m*/*z* 577 (Figure 4c). The HRMS/MS ESI(−) was the same for all, with an *m*/*z* range of 577.12700−577.13318, indicative of the molecular formula $C_{30}H_{25}O_{12}$ (theoretical *m*/*z* 577.13460, δ range 1.32−2.46). Although exhibiting the same molecular weight as B2, they eluted much later, suggesting strong modifications of the heterocycle. The open intermediates required for epimerization of the A2 upper unit were therefore suspected. This hypothesis was supported by the HR mass spectra, showing a shift of 2 mass units for most A2 fragments. The new compounds at *m*/*z* 577 totaled 57 mg/kg A2 equiv. None of these compounds was detected after cocoa roasting at 150 °C, even in the MRM mode (*m*/*z* 577 → 451).

Although very small, various peaks with *m*/*z* 573 were observed (Figure 5; $C_{30}H_{22}O_{12}$, RT = 25−37 and 47−47 min). A first group (**I**, RT = 25−37 min; Figure 5a), more hydrophilic and previously evidenced in B2 degradation media, was characterized by a relatively stable pseudomolecular ion in ESI(−) (573.10569; 27%). In the second group, not found in the previous B2 model media, the pseudomolecular ion was much less abundant (3%) (II, RT = 47−56 min; Figure 5b). For both groups, the A2 fragment at *m*/*z* 449.08919 ($C_{24}H_{17}O_9$) was absent, confirming a more rigid structure probably involving three interunit linkages.

When procyanidin A2 undergoes oxidation, a reactive intermediate (i.e., *o*-quinone) can be formed on the lower unit. Its reaction with the 5-OH of the upper unit, acting as a nucleophilic site, leads to two kinds of oxidized dimers (Figure 1d,e). Group I involves the attack on the B-ring (position 2′, 5′, or 6′), whereas group II is probably issued from the attack on C2. In the latter case, the *o*-quinone generated first has to tautomerize to *p*-methylene quinone, leading to a reactive C2. Epimerization probably also occurred on these molecules, explaining the large number of peaks observed. Deeper investigation is needed to assign an accurate structure to each retention time.

The raw, fermented, and roasted cocoa samples were analyzed at *m*/*z* 573 → 285 (MRM) for the presence of compounds of group I or II. Unfortunately, like the open intermediates, both group I and group II compounds were absent at all stages of the process.

AUTHOR INFORMATION

Corresponding Author

*(S.C.) E-mail: sonia.collin@uclouvain.be. Fax: +32 10 47 21 78. Phone: +32 10 47 29 13.

ORCID

Sonia Collin: 0000-0001-6574-8237

Funding

We thank the WBI (Wallonie-Bruxelles International) for financial support. V.J.E. is grateful to the "Agence Universitaire de la Francophonie" (AUF) for a research fellowship.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank our academic fellows at the Instituto de Investigaciones para la Industria Alimenticia in La Habana, Cuba (Pr. María Cristina Jorge Cabrera and Ir. Lumey Llera) for supplying samples.

ABBREVIATIONS USED

Da, dalton unit; DA, dehydrodi(epi)catechin A; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ESI, electrospray ionization; HPLC, high-performance liquid chromatography; HR, high resolution; MS/MS, tandem mass spectrometry; *m*/*z*, mass on charge ratio; RP, reverse phase

REFERENCES

(1) Ceballos, L.; Andary, C.; Delescluse, M.; Gibernau, M.; McKey, D.; Hossaert-McKey, M. Effects of sublethal attack by a suckling insect, *Hyalymenus tarsatus*, on *Sesbania drummondii* seeds: impact on some seed traits related to fitness. *Ecoscience* **2002**, *9*, 28−36.

(2) Ward, D.; Young, T. P. Effects of large mammalian herbivores and ant symbionts on condensed tannins of *Acacia drepanolobium* in Kenya. *J. Chem. Ecol.* **2002**, *28*, 921−937.

(3) Stoutjesdijk, P. A.; Sale, P. W.; Larkin, P. J. Possible involvement of condensed tannins in aluminium tolerance of *Lotus pedunculatus*. *Aust. J. Plant Physiol.* **2001**, *28*, 1063−1074.

(4) Serafini, M.; Bugianesi, R.; Maiani, G.; Valtuena, S.; De Santis, S.; Crozier, A. Plasma antioxidants from chocolate. *Nature* **2002**, *424*, 1013.

(5) Bagchi, D.; Bagchi, M.; Stohs, S. J.; Das, D. K.; Ray, S. D.; Kuszynski, C. A.; Joshi, S. S.; Pruess, H. G. Free radicals and grape seed proanthocyanidn extract: importance in human health and disease prevention. *Toxicology* **2000**, *148*, 187−197.

(6) Ahmad, N.; Gupta, S.; Mukhtar, H. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kB in cancer cells versus normal cells. *Arch. Biochem. Biophys.* **2000**, *376*, 338−346.

(7) Kandil, F. E.; Smith, M. A. L.; Rogers, R. B.; Pepin, M. F.; Song, L. L.; Pezzuto, J. M.; Seigler, D. S. Composition of a chemopreventive proanthocyanidin-rich fraction from cranberry fruits responsible for the inhibition of 12-*O*-tetradecanoyl phorbol-13 acetate (TPA)-induced ornithine decarboxylase (ODC) activity. *J. Agric. Food Chem.* **2002**, *50*, 1063−1069.

(8) Foo, L. Y.; Lu, Y.; Howell, A. B.; Vorsa, N. The structure of cranberry proanthocyanidins which inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* in vitro. *Phytochemistry* **2000**, *54*, 173−181.

(9) Subarnas, A.; Wagner, H. Analgesic and anti-flammatory activity of the proanthocyanidin shellegueain A from *Polypodium feei* METT. *Phytomedicine* **2000**, *7*, 401−405.

(10) Pataki, T.; Bak, I.; Kovacs, P.; Bagchi, D.; Das, D. K.; Toskai, A. Grape seed proanthocyanidins improved cardiac recovery during reperfusion after ischemia in isolated rat hearts. *Am. J. Clin. Nutr.* **2002**, *75*, 894−899.

(11) Lin, L. C.; Kuo, Y. C.; Chou, C. J. Immunomodulatory proanthocyanidins from *Ecdysanthera utilis*. *J. Nat. Prod.* **2002**, *65*, 505−508.

(12) Poupard, P.; Sanoner, P.; Baron, A.; Renard, C.; Guyot, S. Characterization of procyanidin B2 oxidation products in an apple juice model solution and confirmation of their presence in apple juice by high-performance liquid chromatography coupled to electrospray ion trap mass spectrometry. *J. Mass Spectrom.* **2011**, *46*, 1186−1197.

(13) Osman, A. M.; Wong, K. K. Y.; Fernyhough, A. The laccase/ABTS system oxidizes (+)-catechin to oligomeric products. *Enzyme Microb. Technol.* **2007**, *40*, 1272−1279.

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723

(14) Remy-Tanneau, S.; Le Guenervé, C.; Meudec, E.; Cheynier, V. Characterization of a colorless anthocyanin-flavan-3-ol dimer containing both carbon-carbon and ether interflavanoid linkages by NMR and mass spectrometry. *J. Agric. Food Chem.* **2003**, *51*, 3592−3597.
(15) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Zhang, Z.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R. L. Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. *J. Mass Spectrom.* **2003**, *38*, 1272−1280.
(16) Hellstrom, J. K.; Torronen, A. R.; Mattila, P. H. Proanthocyanidins in common food products of plant origin. *J. Agric. Food Chem.* **2009**, *57*, 7899−7906.
(17) Shahat, A. A.; Hammouda, F.; Ismail, S. I.; Azzam, S. A.; DeBruyne, T.; Lasure, A.; Van-Poel, B.; Pieters, L.; Vlietinck, A. J. Anticomplementary activity of *Crataegus sinaica*. *Planta Med.* **1996**, *62*, 10−13.
(18) Gu, L.; Kelm, M. A.; Hammerstone, J. F.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S.; Prior, R. L. Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *J. Nutr.* **2004**, *134*, 613−617.
(19) Monagas, M.; Garrido, I.; Lebron-Aguilar, R.; Gomez-Cordoves, M. C.; Rybarczyk, A.; Amarowicz, R.; Bartolome, B. Comparative flavan-3-ol profile and antioxidant capacity of roasted peanut, hazelnut, and almond skins. *J. Agric. Food Chem.* **2009**, *57*, 10590−10599.
(20) Lou, H. X.; Yamazaki, Y.; Sasaki, T.; Uchida, M.; Tanaka, H.; Oka, S. A-type proanthocyanidins from peanut skins. *Phytochemistry* **1999**, *51*, 297−308.
(21) Liu, L.; Xie, B.; Cao, S.; Yang, X.; Xu, X.; Guo, S. A-type procyanidins from *Litchi chinensis* pericarp with antioxidant activity. *Food Chem.* **2007**, *105*, 1446−1451.
(22) Sun, J.; Jiang, Y.; Wei, X.; Zhao, M.; Shi, J.; You, Y.; Yi, C. Identification of procyanidin A2 as polyphenol oxidase substrate in pericarp tissue of litchi fruit. *J. Food Biochem.* **2007**, *31*, 300−313.
(23) Le Roux, E.; Doco, T.; Sarni-Manchado, P.; Lozano, Y.; Cheynier, V. A-type proanthocyanidins from pericarp of *Litchi chinensis*. *Phytochemistry* **1998**, *48*, 1251−1258.
(24) Hellstrom, J. K.; Torronen, A. R.; Mattila, P. H. Proanthocyanidins in common food products of plant origin. *J. Agric. Food Chem.* **2009**, *57*, 7899−7906.
(25) Koerner, J. L.; Hsu, V. L.; Lee, J.; Kennedy, J. A. Determination of proanthocyanidin A2 content in phenolic polymer isolates by reversed-phase high-performance liquid chromatography. *J. Chromatogr. A* **2009**, *1216*, 1403−1409.
(26) Kylli, P.; Nohynek, L.; Puupponen-Pimia, R.; Westerlund-Wikstrom, B.; Leppanen, T.; Welling, J.; Moilanen, E.; Heinonen, M. Lingonberry (*Vaccinium vitis-idaea*) and European cranberry (*Vaccinium microcarpon*) proanthocyanidins: isolation, identification, and bioactivies. *J. Agric. Food Chem.* **2011**, *59*, 3373−3384.
(27) Maatta-Riihinen, K. R.; Kahkonen, M. P.; Torronen, A. R.; Heinonen, I. M. Catechins and procyanidins in berries of *Vaccinium* species and their antioxidant activity. *J. Agric. Food Chem.* **2005**, *53*, 8485−8491.
(28) Hatano, T.; Miyatake, H.; Natsume, M.; Osakabe, N.; Takizawa, T.; Ito, H.; Yoshida, T. Proanthocyanidin glycosides and related polyphenols from cacao liquor and their antioxidant effects. *Phytochemistry* **2002**, *59*, 749−758.
(29) Lv, Q.; Luo, X.; Zhao, X.; Liu, Y.; Hu, G.; Sun, C.; Li, X.; Chen, K. Identification of proanthocyanidins from litchi (*Litchi chinensis* Sonn.) pulp by LC-ESI-Q-TOF-MS and their antioxidant activity. *PLoS One* **2015**, *10*, 1−17.
(30) Le Roux, E.; Doco, T.; Sarni-Machado, P.; Lozano, Y.; Cheynier, V. A-type proanthocyanidins from pericarp of *Litchi chinensis*. *Phytochemistry* **1998**, *48*, 1251−1258.
(31) Kondo, K.; Kurihawa, M.; Fukuhara, K.; Tanaka, T.; Suzuki, T.; Miyata, N.; Toyoda, M. Conversion of procyanidin B-type (catechin dimer) to A-type: evidence for abstraction of C-2 hydrogen in catechin during radical oxidation. *Tetrahedron Lett.* **2000**, *41*, 485−488.
(32) Tanaka, T.; Kondou, K.; Kouno, I. Oxidation and epimerization of epigallocatechin in banana fruits. *Phytochemistry* **2000**, *53*, 311−316.
(33) Guidouche, S.; Es-Safi, N.-E.; Ducrot, P.-H. Mechanistic study on the enzymatic oxidation of flavonols. *Tetrahedron Lett.* **2008**, *49*, 619−623.
(34) Giusti, M. M.; Wrolstad, R. E. Anthocyanins. Characterization and measurement with UV-visible spectroscopy. In *Current Protocols in Food Analytical Chemistry*; Wrolstad, R. E., Ed.; Wiley: New York, 2001.
(35) Sadilova, E.; Carle, R.; Stintzing, F. C. Thermal degradation of anthocyanins and its impact on color and *in vitro* antioxidant capacity. *Mol. Nutr. Food Res.* **2007**, *51*, 1461−1471.
(36) Dueñas, M.; Fulcrand, H.; Cheynier, V. Formation of anthocyanin-flavanol adducts in model solutions. *Anal. Chim. Acta* **2006**, *563*, 15−25.
(37) Eyamo, E. V. J.; De Taeye, C.; Niemenak, N.; Youmbi, E.; Collin, S. Influence of acetic and lactic acids on cocoa flavan-3-ol degradation through fermentation-like incubations. *LWT−Food Sci. Technol.* **2016**, *68*, 514−522.
(38) De Taeye, C.; Eyamo Evina, V. J.; Caullet, G.; Niemenak, N.; Collin, S. Fate of anthocyanins through cocoa fermentation. Emergence of new polyphenolic dimers. *J. Agric. Food Chem.* **2016**, *64*, 8876−8885.
(39) Poupard, P. *Oxydation des procyanidines en solution modèle jus de pomme: caractérisation structurale des produits et études des propriétés tannantes*. Thesis, Université de Rennes 1, France, 2008.
(40) De Taeye, C.; Cibaka, M. L.; Jerkovic, V.; Collin, S. Degradation of (−)-epicatechin and procyanidin B2 in aqueous and lipidic model systems. First evidence of "chemical" flavan-3-ol oligomers in processed cocoa. *J. Agric. Food Chem.* **2014**, *62*, 9002−9016.
(41) Sanoner, P. *Les polyphénols de la pomme à cidre: diversité variétale et oxydation*. Thesis, Université de Caen, France, 2001.
(42) 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.
(43) Collin, S.; Callemien, D.; Nizet, S. Extraction et analyse des polyphénols. In *Polyphénols et procédés − Transformation des polyphénols au travers des procédés appliqués à l'agro-alimentaire*; Collin, S., Crouzet, J., Eds.; Tec & Doc Lavoisier: Paris, France, 2011.

DOI: 10.1021/acs.jafc.6b05262
J. Agric. Food Chem. 2017, 65, 1715−1723