Total Synthesis of Isotopically Labelled Flavonoids, 2[‡] ^{13}C-Labelled (±)-Catechin From Potassium [^{13}C]Cyanide

Bastien Nay,[a] Valérie Arnaudinaud,[a] Jean-François Peyrat,[a] Alain Nuhrich,[a] Gérard Deffieux,[a] Jean-Michel Mérillon,[a] and Joseph Vercauteren*[a]

Keywords: Flavonoids / Catechin / Isotopic labelling / Carbon 13 / Total synthesis / French paradox

Racemic 4-[^{13}C]catechin **14** has been synthesized in ten steps starting from potassium [^{13}C]cyanide. The intermediate chalcone **9** was formed by acylation of the benzylated phloroglucinol **7** with the caffeic acid synthon **6**, using the trifluoroacetic mixed anhydride. The flavonoid skeleton was then obtained by previously described reactions such as the reduction of chalcone **9** and dihydroxylation of flavene **11**. Such [^{13}C]-labelled polyphenols should prove to be useful tools for human biological investigations.

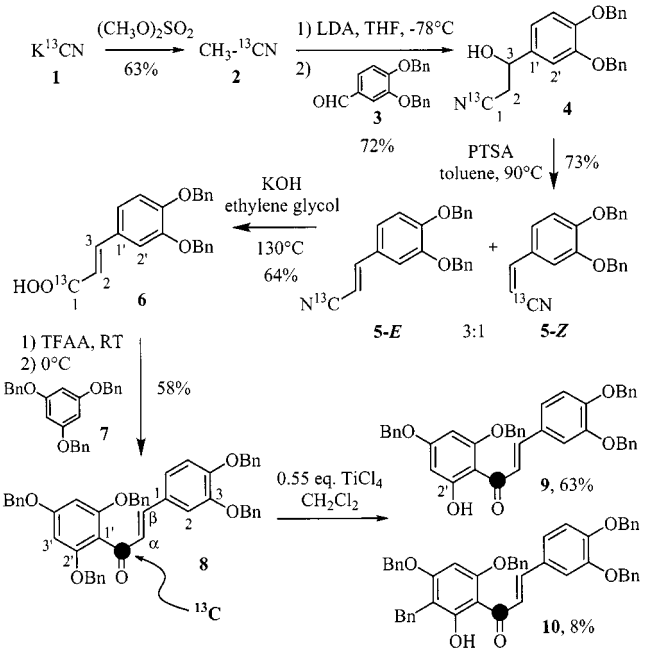
Introduction

Flavonoids have a broad distribution in the plant kingdom, and are thus found in many foodstuffs (fruits, vegetables, drinks). Since the beginning of the 1990's, and especially since the work of Renaud et al.,[1,2] the interest of the medical,[3] scientific or industrial communities in polyphenols has increased enormously. Indeed, they are generally considered to be responsible for most of the benefits that a well-balanced diet can bring to health (diminution of cardiovascular risk and of cancers, increase of the "Mediterranean" people′s life expectancy). Their presence in such a diet is one of the best explanations of the "French paradox",[1,2,4,5] the apparent compatibility between high saturated-fat intakes and a low mortality from cardiovascular diseases. For example, a high level of flavonoids can be found in red wines (about 3 g/l) for which several studies have suggested the beneficial effect of a moderate intake in cardiovascular,[1] cancer[6] and Alzheimer[7] protection. This could be due to the anti-oxidative and radical scavenging properties of these molecules, that could act similarly to vitamin E in fighting against oxidative stress and ageing processes, but also to their anti-thrombotic, anti-atherosclerotic (inhibition of LDL oxidation[8]) and anti-inflammatory activities.[9]

However, there is almost no direct demonstration of their intestinal resorption, and the metabolism of these flavonoids in humans, although some observations have been made in gnotoxenic rats,[10] is poorly known. The preparation of labelled molecules could prove very useful in order to be able to detect them in human biological fluids by noninvasive methods. They could be easily detected by NMR spectroscopy and/or isotopic mass spectrometry. Several studies have started with the synthesis of deuterated and tritiated flavonoids[11,12] and also with the bio-labelling of natural polyphenols by *Vitis vinifera* cell cultures.[13,14] We report herein the first total synthesis of regioselectively labelled flavan-3-ol 4-[^{13}C]catechin **(14)**. A strategy coupling a C_3–C_6 unit (caffeic acid residue) to a C_6-unit (phloroglucinol synthon) was chosen to build the C_6–C_3–C_6 flavonoid skeleton.

Results and Discussion

According to the above strategy, the (*E*)-1-[^{13}C]-*O*-dibenzylcaffeic acid **(6)** (C_3–C_6 unit) was first synthesized starting from labelled acetonitrile (Scheme 1). 1-[^{13}C]Acetonitrile **(2)** was obtained by the method of Walden[15] from potassium [^{13}C]cyanide **(1)** (99% enrichment). Its anion condensed with 3,4-dibenzyloxybenzaldehyde **(3)** to yield **4**;



Scheme 1. Synthesis of the labelled caffeic acid **6** and chalcone intermediates **8** and **9**

[‡] Part 1: M. C. Pierre, C. Chèze, J. Vercauteren, *Tetrahedron Lett.* **1997**, *38*, 5639–5642.

[a] GESNIT, Laboratoire de Pharmacognosie, Faculté de Pharmacie, Université Victor Ségalen Bordeaux 2,
146 rue Léo Saignat, F-33076 Bordeaux, France
Fax: (internat.) +33-5/5696-0975
E-mail: Joseph.Vercauteren@gnosie.u-bordeaux2.fr

  1434–193X/00/0407–1279 $ 17.50+.50/0

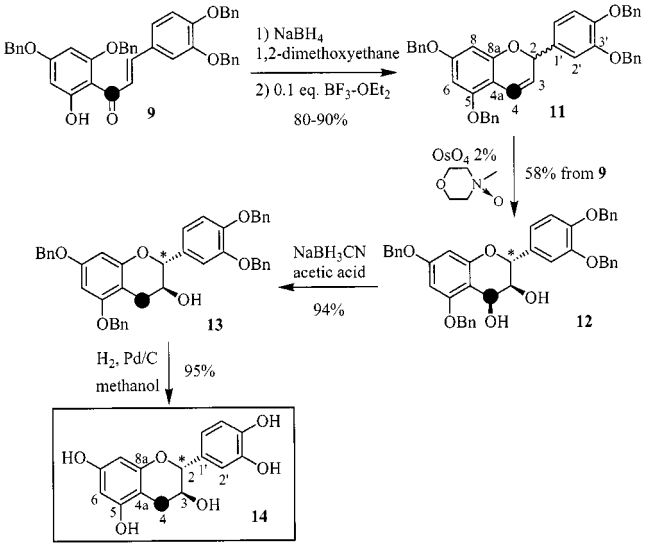
dehydration of **4** gave a mixture of *Z*- and *E*-cinnamonitrile **(5)** in a 1:3 ratio (based on NMR spectroscopy). Compound **5** was hydrolyzed to form **6** in 34% overall yield from **2**. The *Z* isomer could not be recovered, most probably due to the complete isomerization to the *E* form during the hydrolysis step.

The flavonoid skeleton was produced by acylation of the phloroglucinol tribenzyl ether **7** by **6** (Scheme 1), previously activated by trifluoroacetic anhydride (TFAA),[16,17] under mild conditions (0 °C), providing the pentabenzyloxychalcone **8** in 58% yield. Selective deprotection of **8** by $TiCl_4$ gave the 2′-hydroxychalcone **9**, although vicinal *C*-benzylation leading to the by-product **10** could not be avoided (8%).

Flavan-3,4-diol **(12)** was synthesized in 58% yield in three steps from **9** by the Clark-Lewis method (Scheme 2):[18,19] borohydride reduction of **9** and Lewis acid catalyzed cyclization into racemic flavene **11**, which was then directly transformed by an osmium-catalyzed dihydroxylation into **12** with high diastereoselectivity (the all *cis*-isomer was not observed in our hands). As expected,[19,20] **11** was a very labile compound that could not be purified.

Compound **12** is an interesting precursor of several flavonoid classes. $NaBH_3CN$ reduction at C-4,[21] followed by hydrogenolysis of the benzylic protecting groups, yielded the target racemic 4-[^{13}C]catechin **(14)** in quantitative yield (Scheme 2). The structure of **14** was confirmed by analysis of the spectroscopic data and co-migration with the natural (+)-catechin on TLC.

Figures 1 and 2 show the ^{1}H and ^{13}C NMR spectra of **14**. Each one displays the large coupling constants observed for nuclei directly bound to 4-[^{13}C]. One should note for the ^{1}H NMR (Figure 1) that the tiny signals (*dd*) visible at the center of each large doublet correspond to the 1% unlabelled catechin (99% enrichment). As expected, one can observe in the ^{13}C NMR spectrum the high intensity of the C-4 signal (Figure 2) which is about one hundred times higher than the others. This is noteworthy in view of the use of NMR spectroscopy to detect the molecule and to follow its metabolites in vivo.



Scheme 2. Synthesis of labelled catechin **14** from chalcone **9**

Conclusion

Compound **14** has been synthesized in 10 steps from $K^{13}CN$ via $CH_3^{13}CN$ (surprisingly enough, only $^{13}CH_3CN$ is commercially available, although very expensive) in 4% overall yield, based on a (C_6–C_3 + C_6)-type strategy. This strategy has seldom been used to build this skeleton,[20,22,23] the traditional synthesis being (C_6–C_2 + C_1–C_6),[24] and, to the best of our knowledge, it is the first time that "TFAA chemistry" has been used to build a chalcone since Bourne's work back in the fifties,[16] although as can be seen from this work it is the only successful method with this hydroxylation pattern. Improvement of this total syn-

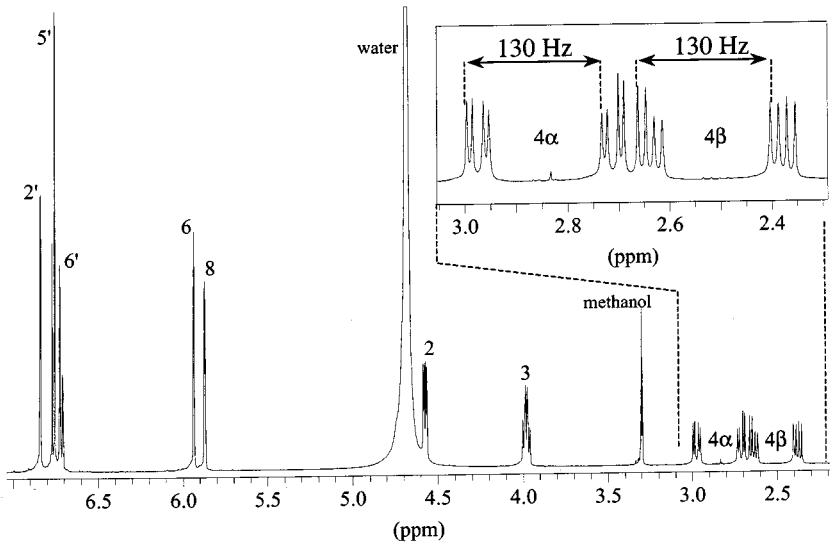


Figure 1. ^{1}H NMR spectrum of ^{13}C-labelled catechin **14** (500 MHz, CD_3OD); the box shows the large doublet of 4-H

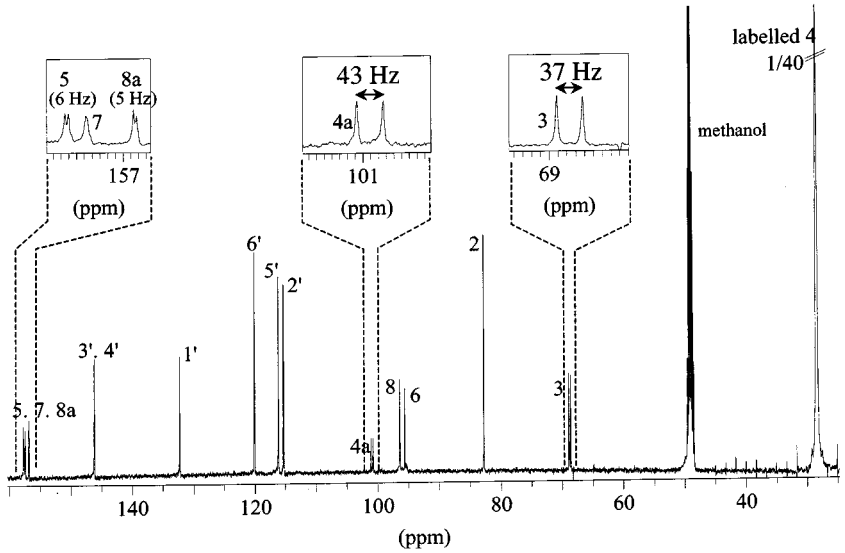


Figure 2. ^{13}C NMR spectrum of ^{13}C-labelled catechin **14** (125 MHz, inverse gated mode, CD_3OD); the boxes show the doublet of C-3 and C-4a

thesis would depend on the preparation of optically active natural catechin. It is versatile enough to be easily extended to other carbon isotopes: ^{14}C (in vitro studies) and ^{11}C (for positron camera detection allowing the direct follow-up of the molecules in the organism). Intermediate **12** has also been used to prepare labelled tanins (forthcoming publication). Investigations of the bioavailability directly from supplemented wine in volunteers, and the elucidation of the molecular mechanisms of some of the fascinating biological properties, is currently in progress.

Experimental Section

General Remarks: $K^{13}CN$ was made available through Euriso-top (Gif-sur-Yvette, France) with a 99% ^{13}C enrichment. All reactions were performed under nitrogen. Benzylations of 3,4-dihydroxybenzaldehyde and phloroglucinol were made in DMF for **3** and in DMSO for **7** with benzyl bromide in the presence of K_2CO_3. Reactions were monitored on TLC (silica gel 60 F_{254}, Merck). Products were purified by circular centrifuged chromatography on Merck silica gel 60 PF_{254} (ref. = 1.07749) with CH_2Cl_2/hexane 1:1 as eluents for **8**, 4:6 for **9**, 9:1 for **12** and **13**, and by flash chromatography on SDS silica gel 60 (35–70 μm) (eluents: hexane/ethyl acetate 7:3 for **4**, CH_2Cl_2/hexane 9:1 for **5**, CH_2Cl_2/methanol 99:1 for **6**). All spectral data for known compounds were identical to those published in the literature. Only modifications due to ^{13}C-labelling or new data are described herein. – UV/Vis: Hitachi U2000. – IR: BOMEM MB 100. – High resolution NMR: Bruker AMX-500 spectrometer (500.13 MHz and 125.73 MHz for ^1H and ^{13}C, respectively).

1-[^{13}C]Acetonitrile (2): Synthesized from $K^{13}CN$ (6.5 g) by the method of Walden[15] (CAUTION !). Only one distillation. Water was eliminated with molecular sieves 4Å (beads). About 5% methanol remained. 63% of **2** was recovered. – IR (thin film) $\tilde{\nu}$: = 2203 cm^{-1} ($^{13}C{\equiv}N$). – ^1H NMR ($CDCl_3$): δ = 1.99 (d, *J* = 9.9 Hz, 2-H). – ^{13}C NMR ($CDCl_3$): δ = 1.7 (d, *J* = 58 Hz, C-2), 116.3 (s, labelled C-1). – MS (GC-coupled EI, 70 eV); *m/z* (%): 42 [M^+·] (100).

1-[^{13}C]-β-Hydroxynitrile (4): Compound **2** (330 μL) was added at –78 °C to a solution of LDA (4.7 mL of a 2M solution in THF) in 10 mL THF. Then **3** (2 g diluted in 5 mL THF) was added dropwise. After 3 h, the reaction was quenched with aqueous NH_4Cl and extracted with dichloromethane. Purification yielded 1.59 g of **4** (72%) as a pale yellow oil. UV/Vis (MeOH): λ_{max} = 227 nm, 277. – IR (thin film): $\tilde{\nu}$ = 3444 cm^{-1} (OH), 2197 ($^{13}C{\equiv}N$). – ^1H NMR ($CDCl_3$): δ = 2.67 (ddd, *J* = 16.0, 9.4, 6.5 Hz, 2-Ha), 2.71 (ddd, *J* = 12.8, 9.4, 5.7, 2-Hb), 4.93 (m, 3-H), 5.16, 5.18 (s, 2 benzylic OCH_2), 6.90 (dd, *J* = 8.3, 2.0 Hz, 6′-H), 6.94 (d, *J* = 8.3 Hz, 5′-H), 7.02 (d, *J* = 2.0 Hz, 2′-H), 7.30–7.48 (m, 10 benzylic H). – ^{13}C NMR ($CDCl_3$): δ = 27.8 (d, *J* = 58 Hz, C-2), 69.5 (C-3), 71.5, 71.6 (3′- and 4′-OCH_2), 112.6 (C-2′), 115.2 (C-5′), 117.3 (labelled C-1), 118.8 (C-6′), 127.3, 127.4, 127.9, 128.3 (benzylic ArH), 134.3 (C-1′), 137.0, 137.1 (2 benzylic C-*ipso*), 149.3, 149.5 (C-3′, C-4′). – MS (HR-EI); *m/z*: calcd. for $^{13}C^{12}C_{22}H_{21}NO_3$ 360.1555; found 360.1557.

(*Z*/*E*)-1-[^{13}C]Cinnamonitrile (5-*E*, 5-*Z*): Compound **4** (1.5 g) in toluene (40 mL) was heated for 1 h in the presence of PTSA (390 mg) and 10 g of MS 4Å (beads). After washing with saturated aq. $NaHCO_3$, water and brine, purification gave 1.04 g of **5** (73% yield) as a mixture of the two isomers **5-*Z*** and **5-*E*** in a 1:3 ratio based on NMR analysis. Pale yellow oil. – UV/Vis (MeOH): λ_{max} = 219 nm, 235, 292, 318. – IR (thin film): $\tilde{\nu}$ = 2160 cm^{-1} ($^{13}C{\equiv}N$). – ^1H NMR ($CDCl_3$): δ = 5.18, 5.21 (2 s, 2 benzylic OCH_2, *E*), 5.23 (s, 2 benzylic OCH_2, *Z*), 5.28 (dd, *J* = 11.8, 1.1 Hz, 2-H *Z*), 5.63 (dd, *J* = 16.5, 2.2 Hz, 2-H *E*), 6.93 (d, *J* = 8.4 Hz, 5′-H *E*), 6.95 (d, *J* = 8.6 Hz, 5′-H *Z*), 6.98 (d, *J* = 11.8 Hz, 3-H *Z*), 6.99 (dd, *J* = 8.4, 2.0 Hz, 6′-H *E*), 7.02 (d, *J* = 2.0 Hz, 2′-H *E*), 7.25 (d, *J* = 16.5 Hz, 3-H *E*), 7.26 (d, *J* = 8.6 Hz, 6′-H *Z*), 7.32–7.51 (m, benzylic H *E* and *Z*), 7.63 (d, *J* = 2.1 Hz, 2′-H *Z*). – ^{13}C NMR ($CDCl_3$): δ = 71.0 (4′-OCH_2 *Z* and *E*), 71.2 (3′-OCH_2 *Z*), 71.6 (3′-OCH_2 *E*), 92.2 (d, *J* = 80 Hz, C-2 *Z*), 93.9 (d, *J* = 82 Hz, C-2 *E*), 113.2 (C-2′ *E*), 114.0 (C-5′ *Z*), 114.3 (C-2′ *Z* and C-5′ *E*), 117.9 (labeled C-1 *Z*), 118.5 (labeled C-1 *E*), 122.3 (C-6′ *E*), 124.2 (C-6′ *Z*), 127.1–128.6 (benzylic ArH, *Z* and *E*), 129.0 (C-1′ *Z*), 129.8 (C-1′ *E*), 136.5, 136.7 (2 benzylic C-*ipso*, *E*), 136.6, 136.8 (2 benzylic C-*ipso*, *Z*), 148.1 (C-3 *Z*), 148.8 (C-3′ *Z*), 149.2 (C-3′ *E*), 150.0 (C-3 *E*), 151.4 (C-4′ *Z*), 151.9 (C-4′ *E*). – MS (HR-EI); *m/z*: calcd. for $^{13}C^{12}C_{22}H_{19}NO_2$ 342.1449; found 342.1453.

(*E*)-1-[^{13}C]-*O*-Dibenzylcaffeic Acid (6): Compound **5** (1 g, *E*/*Z* mixture) in 30 mL ethylene glycol was heated at 130 °C, in the presence of KOH (3 g). After 5 h, the medium was acidified and extracted with ethyl acetate, giving 676 mg of **6** after purification (64% yield) as white to beige crystals, m.p. 195 °C. – UV/Vis (MeOH): λ_{max} = 235 nm, 290, 313. – IR (KBr): $\tilde{\nu}$ = 3200–2500 cm^{-1} (broad, OH), 1649 (C=O). – ^{1}H NMR ([D$_6$]DMSO): δ = 5.18 (s, 4′-OCH$_2$), 5.19 (s, 3′-OCH$_2$), 6.41 (dd, *J* = 15.9, 2.7 Hz, 2-H), 7.07 (d, *J* = 8.4 Hz, 5′-H), 7.19 (dd, *J* = 8.4, 1.9 Hz, 6′-H), 7.30–7.48 (m, 10 benzylic H and 2′-H), 7.50 (dd, *J* = 15.9, 6.8 Hz, 3-H). – ^{13}C NMR ([D$_6$]DMSO): δ = 69.9, 70.0 (3′ and 4′-OCH$_2$), 113.0 (C-2′), 114.0 (C-5′), 117.0 (d, *J* = 73 Hz, C-2), 122.7 (C-6′), 127.3, 127.5, 127.7, 128.3 (benzylic ArH and C-1′), 137.0, 137.2 (2 benzylic C-*ipso*), 143.8 (C-3), 148.3 (C-3′), 150.1 (C-4′), 167.8 (labeled C-1). – MS (HR-EI); *m*/*z*: calcd. for $^{13}C^{12}C_{22}H_{20}O_4$ 361.1395; found 361.1387.

[^{13}CO]-3,4,2′,4′,6′-Pentabenzyloxychalcone (8): TFAA (400 µL) was added to a suspension of **6** (500 mg) in 5 mL of 1,2-dichloroethane. After 5 min stirring at room temp., the solution was cooled at 0 °C before adding **7** (825 mg in 5 mL of 1,2-dichloroethane). After 1 h, the deep purple solution was quenched with aq. NaHCO$_3$ (stirring until the solution became pale yellow), and then extracted with dichloromethane. The crude extract was washed with diethyl ether to precipitate **8**, and the remaining solution was purified by chromatography. A total of 586 mg of **8** was obtained in all (58% yield). White to beige crystals, m.p. 151–153 °C. – UV/Vis (MeOH): λ_{max} = 228 nm, 336, 346. – IR (KBr): $\tilde{\nu}$ = 1596 cm^{-1} (C=O). – ^{1}H NMR (CDCl$_3$): δ = 5.02 (s, 4′-OCH$_2$), 5.05 (s, 2′-OCH$_2$, 6′-OCH$_2$), 5.14 (s, 4-OCH$_2$), 5.21 (s, 3-OCH$_2$), 6.29 (s, 3′-H, 5′-H), 6.86 (dd, *J* = 16.0, 2.2 Hz, α−H), 6.92 (d, *J* = 8.3 Hz, 5-H), 7.03 (dd, *J* = 8.3, 1.9 Hz, 6-H), 7.14 (d, *J* = 1.9 Hz, 2-H), 7.28 (dd, *J* = 16.1, 5.8 Hz, β−H), 7.24–7.48 (m, 25 benzylic H). – ^{13}C NMR (CDCl$_3$): δ = 70.3 (4′-OCH$_2$), 70.5 (2′-OCH$_2$, 6′-OCH$_2$), 71.0 (3-OCH$_2$), 71.4 (4-OCH$_2$), 93.8 (C-3′, C-5′), 113.4 (d, *J* = 58 Hz, C-1′), 114.1 (C-2), 114.4 (C-5), 123.2 (C-6), 126.9, 127.2. 127.3, 127.5, 127.7, 127.9, 128.4, 128.5 (benzylic ArH and C-1), 128.4 (d, *J* = 61 Hz, C-α), 136.4 (4′-benzylic C-*ipso*), 136.6 (2′ and 6′-benzylic C-*ipso*), 136.8 (4-benzylic C-*ipso*), 136.9 (3-benzylic C-*ipso*), 144.3 (C-β), 149.0 (C-3), 151.0 (C-4), 157.8 (C-2′, C-6′), 161.1 (C-4′), 193.7 (labeled C=O). – MS (HR-FAB+, nitrobenzyl alcohol), *m*/*z*: calcd. for $^{13}C^{12}C_{49}H_{43}O_6$ (M + H) 740.3093; found 740.3080.

[^{13}CO]-3,4,4′,6′-Tetrabenzyloxy-2′-hydroxychalcone (9): A 1 M solution of TiCl$_4$ in CH$_2$Cl$_2$ (280 µL) was added to a solution of **8** (373 mg) in 5 mL CH$_2$Cl$_2$. The reaction was performed at –15 °C and slowly warmed to room temperature. It was then quenched with saturated aq. NaHCO$_3$ (1 h stirring), and extracted with CH$_2$Cl$_2$. Purification gave 203 mg of **9** (63% yield) and 32 mg of by-products **10** (8%).

9:[19] IR (KBr): $\tilde{\nu}$ = 1623 cm^{-1} (C=O). – ^{1}H NMR (CDCl$_3$): δ = 7.68 (dd, *J* = 15.5, 6.2 Hz, β-H), 7.78 (dd, *J* = 15.5, 5.1 Hz, α-H). – ^{13}C NMR (CDCl$_3$): δ = 70.3 (4′-OCH$_2$), 71.1, 71.3 (3-, 4- and 6′-OCH$_2$), 92.7 (C-5′), 95.2 (C-3′), 106.6 (d, *J* = 58 Hz, C-1′), 114.7 (C-5), 115.4 (C-2), 122.4 (C-6), 125.9 (d, *J* = 55 Hz, C-α), 127.2, 127.4, 127.7, 127.9, 128.0, 128.3, 128.4, 128.5, 128.6, 128.7, 128.9 (benzylic ArH), 129.0 (d, *J* = 6.1 Hz, C-1), 135.7, 136.0, 137.0 (4-benzylic C-*ipso*), 142.7 (C-β), 148.9 (C-3), 150.8 (C-4), 161.7 (C-6′), 165.2 (C-4′), 168.6 (C-2′), 192.6 (labeled CO). – MS (HR-FAB+, nitrobenzyl alcohol) *m*/*z*: calcd. for $^{13}C^{12}C_{42}H_{37}O_6$ (M + H) 650.2624; found 650.2651.

10: Yellow crystals. UV/Vis (MeOH): λ_{max} = 206 nm, 372. – IR (thin film): $\tilde{\nu}$ = 1605 cm^{-1} (C=O). – ^{1}H NMR (CDCl$_3$): δ = 4.05 (s, 3′-CH$_2$), 4.97 (s, 3-OCH$_2$), 5.05 (s, 6′-OCH$_2$), 5.15 (s, 4′-OCH$_2$), 5.21 (s, 4-OCH$_2$), 6.15 (s, 5′-H), 6.69 (d, *J* = 8.3 Hz, 6-H), 6.75 (d, *J* = 8.3 Hz, 5-H), 6.95 (s, 2-H), 7.14–7.48 (m, 25 benzylic H), 7.66 (dd, *J* = 15.5, 6.1 Hz, β-H), 7.74 (dd, *J* = 15.5, 5.0 Hz, α-H). – ^{13}C NMR (CDCl$_3$): δ = 28.2 (3′-CH$_2$), 70.2 (4′-OCH$_2$), 71.1 (4-OCH$_2$), 71.2 (3-OCH$_2$), 71.3 (6′-OCH$_2$), 89.1 (C-5′), 106.7 (d, *J* = 58 Hz, C-1′), 110.6 (C-3′), 114.6 (C-5), 115.2 (C-2), 122.4 (C-6), 126.2 (d, *J* = 57 Hz, C-α), 125.4, 127.1, 127.2, 127.3, 128.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8 (benzylic ArH), 129.0 (d, *J* = 4 Hz, C-1), 135.7, 136.3, 136.9 (3-, 4-, 4′- and 6′-benzylic C-*ipso*), 141.6 (3′-benzylic C-*ipso*), 142.4 (C-β), 148.8 (C-3), 150.7 (C-4), 160.5 (C-6′), 162.4 (C-4′), 165.2 (C-2′), 192.9 (labeled CO). – MS (HR-FAB+, nitrobenzyl alcohol) *m*/*z*: calcd. for $^{13}C^{12}C_{49}H_{43}O_6$ (M + H) 740.3093; found 740.3110.

4-[^{13}C]-3′,4′,6,8-Tetrabenzyloxy-3-flavene (11): Synthesized by the Clark–Lewis method[18] modified by Kawamoto et al.[19] 190 mg of **9** gave 188 mg of crude product **11** (no purification, 80–90% yield based on NMR). – ^{1}H NMR[19] (CDCl$_3$): δ = 6.88 (ddd, *J* = 166, 9.9, 1.7 Hz, 4-H). – ^{13}C NMR (CDCl$_3$): δ = 70.1, 70.3, 70.7, 71.3 (benzylic 5, 7, 3′ and 4′-OCH$_2$), 77.0, (C-2), 93.9 (C-6), 95.2 (C-8), 105.0 (d, *J* = 53 Hz, C-4a), 114.3 (C-2′), 114.9 (C-5′), 118.9 (labeled C-4), 119.7 (broad s, C-3), 120.5 (C-6′), 127.2, 127.3, 127.4, 127.5, 127.7, 127.9, 128.0, 128.4, 128.5 (benzylic ArH), 134.2 (C-1′), 136.7, 136.9, 137.2, 137.3 (4 benzylic C-*ipso*), 149.1, 149.2 (C-3′, C-4′), 154.9 (C-8a), 155.3 (C-5), 160.3 (C-7). – MS (EI); *m*/*z* (%): 633 [M$^+$] (40), 542 (30), 91 (100).

4-[^{13}C]-3′,4′,6,8-Tetrabenzyloxyflavan-3,4-diol (12): Synthesized from 188 mg of **11** by the Clark-Lewis method[18] modified by Kawamoto et al.[19] Yield: 112 mg of the 2,3-*trans*-3,4-*cis* diol **12** (58% from **9**). – ^{1}H NMR[19] (CDCl$_3$): δ = 5.09 (dd, *J* = 152.0, 3.9 Hz, 4-H). – ^{13}C NMR (CDCl$_3$): δ = 61.5 (labeled C-4), 70.2 (d, *J* = 34 Hz, C-3), 70.1, 70.3, 71.3, 71.4 (4 benzylic OCH$_2$), 76.6 (C-2), 94.1 (C-6), 94.6 (C-8), 105.2 (d, *J* = 48 Hz, C-4a), 114.6 (C-2′), 115.1 (C-5′), 121.2 (C-6′), 127.2, 127.3, 127.4, 127.5, 127.7, 128.0, 128.1, 128.4, 128.6, 128.7 (benzylic ArH), 130.9 (C-1′), 136.4, 136.6, 137.2, 137.3 (4 benzylic C-*ipso*), 149.2, 149.5 (C-3′, C-4′), 156.0 (C-8a), 158.7 (C-5), 160.8 (C-7). – MS (HR-FAB+, nitrobenzyl alcohol) *m*/*z*: calcd. for $^{13}C^{12}C_{42}H_{38}O_7Li$ (M + Li) 674.2811; found 674.2868.

(±)-4-[^{13}C]Catechin-5,7,3′,4′-tetrabenzyl Ether (13): Synthesized from diol **12** (105 mg) by the method of Brown and Fuller.[21] Yield: 96 mg of **13** (94%). – ^{1}H NMR[25] (CDCl$_3$): δ = 2.68 (ddd, *J* = 130.5, 16.5, 8.8 Hz, axial 4β-H), 3.13 (ddd, *J* = 133.2, 16.5, 5.6 Hz, equatorial 4α-H). – ^{13}C NMR (CDCl$_3$): δ = 27.6 (labeled C-4), 68.1 (d, *J* = 36 Hz, C-3), 69.9, 70.1 (5- and 7-OCH$_2$), 71.3, 71.4 (3′- and 4′-OCH$_2$), 81.5 (C-2), 93.9 (C-6), 94.5 (C-8), 102.3 (d, *J* = 44 Hz, C-4a), 114.1 (C-2′), 115.1 (C-5′), 120.5 (C-6′), 127.1, 127.2, 127.5, 127.8, 127.9, 128.4, 128.5, 128.6 (benzylic ArH), 131.1 (C-1′), 136.9, 137.0, 137.2 (4 benzylic C-*ipso*), 149.2, 149.4 (C-3′, C-4′), 155.3 (C-8a), 157.8 (C-5), 158.8 (C-7). – MS (EI, 70 eV); *m*/*z* (%): 651 [M$^+$·] (1), 320 (10), 91 (100).

(±)-4-[^{13}C]Catechin (14): A suspension of **13** (95 mg) in methanol (2 mL) was hydrogenolysed at room temp. in the presence of 10 mg Pd/C, under a hydrogen atmosphere. After 5 h, methanol was evaporated and the residue was taken up by ethyl acetate before filtration through celite and evaporation. Purification by preparative HPLC [6 µm RP-18 Silica column (250 × 20 mm), linear gradient elution from 100% H$_2$O to 100% MeOH over 60 min at 7 mL/min] yielded 40 mg of pure catechin **14** (95% yield). – UV/Vis (MeOH): λ_{max} = 279 nm, 341. – IR (KBr): $\tilde{\nu}$ = 3326 cm^{-1} (OH). – ^{1}H NMR (CD$_3$OD): δ = 2.51 (ddd, *J* = 130, 16.2, 8.0 Hz, 4β-H), 2.84 (ddd,

J = 130, 16.2, 5.4 Hz, 4α-H), 3.98 (m, 3-H), 4.57 (dd, J = 7.4, 3.3 Hz, 2-H), 5.87 (d, J = 2.2 Hz, 8-H), 5.94 (d, J = 2.0 Hz, 6-H), 6.72 (dd, J = 8.1, 2.0 Hz, 6′-H), 6.76 (d, J = 8.1 Hz, 5′-H), 6.84 (d, J = 2.0 Hz, 2′-H). – ^{13}C NMR (CD_3OD): δ = 28.4 (labeled C-4), 68.8 (d, J = 37 Hz, C-3), 82.7 (C-2), 95.6 (C-6), 96.4 (C-8), 100.9 (d, J = 43 Hz, C-4a), 115.3 (C-2′), 116.2 (C-5′), 120.1 (C-6′), 132.2 (C-1′), 146.2 (C-3′, C-4′), 156.8 (d, J = 5 Hz, C-8a), 157.5 (C-7), 157.7 (d, J = 6 Hz, C-5). – MS (HR-EI); *m/z*: calcd. for $^{13}C^{12}C_{14}H_{14}O_6$ 291.0824; found 291.0827.

Acknowledgments

We thank MENRT and the ONIVins for financial supports. B. N. and V. A. gratefully acknowledge receipt of a scholarship from MENRT and the ONIVins, respectively. We thank Dr. Chantal Castagnino, Eric Bezançon, Nathalie Chéron and Marie-France Pommier for technical assistance, and Philippe Sigaut for HR-MS analysis.

[1] S. Renaud, M. De Lorgeril, *Lancet* **1992**, *339*, 1523–1526.
[2] S. Renaud, J. Ruf, *Circulation* **1994**, *90*, 3118–3119.
[3] E. Haslam, *J. Nat. Prod.* **1996**, *59*, 205–215.
[4] A. S. St. Leger, A. L. Cochrane, F. Moore, *Lancet* **1979**, 1017–1020.
[5] D. Fitzpatrick, S. Hirschfield, R. Coffey, *Amer. J. Physiol.* **1993**, *265*, H774–H778.
[6] S. F. Asad, S. Singh, A. Ahmad, S. M. Hadi, *Med. Sci. Res.* **1998**, *26*, 723–728.
[7] J. M. Orgogozo, J. F. Dartigues, S. Lafont, L. Letenneur, D. Commenges, R. Salamon, S. Renaud, M. B. Breteler, *Rev. Neurol.* **1997**, *153*, 185–192.
[8] E. Frankel, J. Kanner, J. German, E. Parks, J. Kinsella, *Lancet* **1993**, *341*, 454–457.
[9] C. R. Pace-Asciak, S. Hahn, E. P. Diamandis, G. Soleas, D. M. Goldberg, *Clin. Chim. Acta* **1995**, *235*, 207–219.
[10] C. Brézillon, S. Rabot, C. Philippe, J. Durao, C. Chèze, J. Vercauteren, in *Polyphenols Communications 98, XIXth International Conference on Polyphenols,* Lille (France), 1–4 sept. 1998, F. Charbonnier, J.-M. Delacotte, C. Rolando, Eds., Lille, **1998**, vol. 1, p 11–12.
[11] M. C. Pierre, C. Chèze, J. Vercauteren, *Tetrahedron Lett.* **1997**, *38*, 5639–5642.
[12] S. Deprez, S. Buffnoir, A. Scalbert, C. Rolando, *Analusis* **1997**, *25*, 43–46.
[13] J. Laparra, J. Michaud, M. F. Lesca, P. Blanquet, J. Masquelier, *Bull. Soc. Pharm. Bordeaux* **1977**, *116*, 17–20.
[14] S. Krisa, P. Waffo Téguo, A. Decendit, G. Deffieux, J. Vercauteren, J.-M. Mérillon, *Phytochemistry* **1999**, *51*, 651–656.
[15] P. Walden, *Chem. Ber.* **1907**, 3214–3217.
[16] E. J. Bourne, M. Stacey, J. C. Tatlow, J. M. Tedder, *J. Chem. Soc.* **1951**, 718–720.
[17] J. M. Tedder, *Chem. Rev.* **1955**, 787–827.
[18] J. W. Clark-Lewis, D. C. Skingle, *Aust. J. Chem.* **1967**, *20*, 2169–2190.
[19] H. Kawamoto, F. Nakatsubo, K. Murakami, *J. Wood Chem. Technol.* **1989**, *9*, 35–52.
[20] B. Nay, J. F. Peyrat, J. Vercauteren, *Eur. J. Org. Chem.* **1999**, *9*, 2231–2234.
[21] B. R. Brown, M. J. Fuller, *J. Chem. Res.* **1986**, 140–141.
[22] G. P. Schiemenz, U. Schmidt, *Liebigs Ann. Chem.* **1982**, 1509–1513.
[23] L. Jurd, *Tetrahedron* **1969**, *25*, 1407–1416.
[24] H. Wagner, L. Farkas, in *The Flavonoids* (Eds.: J. B. Harborne, T. J. Mabry, H. Mabry), Chapman and Hall, London, **1975**, p 127–213.
[25] H. Kawamoto, F. Nakatsubo, K. Murakami, *Mokuzai Gakkaishi* **1991**, *37*, 488–493.

Received July 30, 1999
[O99480]