JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2006; **49**: 479–487.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1057

Research Article

Synthesis of deuterated dihydrochalcones

Daniel J. Comeskey[1,*], Janine M. Cooney[2] and Daryl D. Rowan[1]
[1] *The Horticulture and Food Research Institute of New Zealand Ltd, Tennent Drive, Private Bag 11030, Palmerston North, New Zealand*
[2] *The Horticulture and Food Research Institute of New Zealand Ltd, East Street, Private Bag 3123, Hamilton, New Zealand*

Summary

The dihydrochalcones phloretin and phloridzin are major phenolic constituents of apple fruit. Phloretin-d_4, deuterated at both the α and β positions, was prepared by hydrogenolysis of naringenin and by deuterium exchange from unlabelled phloretin using Pd/C and sodium formate with methanol-d_1 as the source of deuterium. Deuterated derivatives of the glycosides, phloridzin and naringin dihydrochalcone, were similarly prepared. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: dihydrochalcone; phloretin; phloridzin; naringin dihydrochalcone; deuterium; synthesis

Introduction

Dihydrochalcones are a class of 'minor flavanoids' that widely occur in nature both as the glycosides and free aglycones.[1] New dihydrochalcones, variously methoxylated[2] and with chromyl,[3] galloyl, caffeoyl and hexahydroxydiphenoyl ester[4] and C-β-glucopyranosyl derivatives[5] continue to be reported. The dihydrochalcones, phloretin (3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one, Figure 1(a)) and phloridzin (1-[4,6-dihydroxy-2-*O*-(β-D-glucopyranosyl)phenyl]-3-(4-hydroxyphenyl)propan-1-one, Figure 1(b)) are found throughout the apple tree[1] and are regarded as the characteristic phenolics of apple fruit and of apple products, with concentrations of phloridzin in fruit ranging from 0.1 to 190 mg/kg.[6] Phloridzin has also been reported from various *Malus*, *Prunus* and *Populus* species[1] and from strawberry.[7] Phloridzin is reportedly repellent to blackbirds, its consumption may

*Correspondence to: D. J. Comeskey, The Horticulture and Food Research Institute of New Zealand Ltd, Private Bag 11030, Palmerston North, New Zealand. E-mail: dcomesky@hortresearch.co.nz

Contract/grant sponsor: Foundation for Research Science and Technology; contract/grant number: COX0220

Received 11 December 2005
Revised 12 December 2005
Accepted 13 January 2006

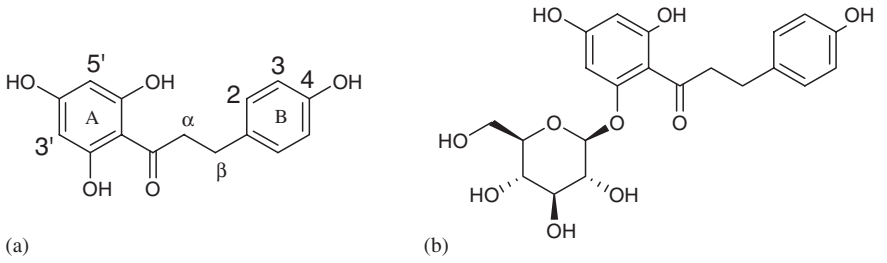


Figure 1. Structures of phloretin (a) and phloridzin (b). The numbering scheme follows Bohm[1]

confer deterrence to insects feeding on apple[1] and it has strong antioxidant activity.[8]

The biological activity of polyphenolic antioxidants and their possible role in promoting health, when consumed as part of a normal diet, is the subject of much recent research. Apples and apple products are the major source of polyphenolic dihydrochalcones in the human diet with 250 ml of apple juice or cider estimated to supply 1–5 mg of phloretin and a whole dessert apple (c. 100 g) supplying about 1 mg.[6] Phloridzin competitively inhibits glucose uptake by the sodium glucose cotransporter 1(SGLT1) in the small intestine,[9] has been classified as an anti-diabetic agent[10,11] and continues to be used as a research tool in the study of diabetes.[12] In rats, phloretin is excreted in the urine principally as phloretic acid, (4-hydroxyphenol)proprionic acid, and related metabolites presumably as a result of microbial metabolism in the gut.[13] In plasma, phloridzin occurs largely as phloretin conjugates.[14]

To further understand the biological activity of this class of compounds, isotopically labelled derivatives would be useful. Methods for the synthesis of labelled phenolics generally involve hydrogen exchange under acidic,[15] basic or supercritical[16,17] conditions with a back exchange step sometimes being used to remove unwanted label from the most labile positions.[18] Many complex phenolics do not survive these reaction conditions.[15] Krishnamurty and Sathyanarayan[19] reported the synthesis of dihydrochalcones from flavanones by catalytic hydrogenation using sodium formate and Pd/C. This method seemed applicable to the incorporation of an isotope label (deuterium or tritium) in the non-exchangeable β position of the dihydrochalcone skeleton. We report here the further development of this chemistry to synthesize deuterated dihydrochalcones both by hydrogenolysis from the corresponding flavanones and by deuterium exchange from dihydrochalcone aglycones or glycosides using methanol-d_1 as the deuterium source.

Results and discussion

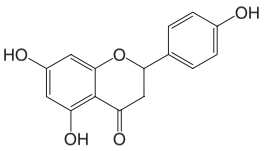
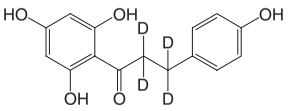
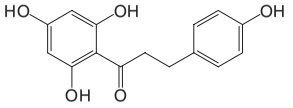
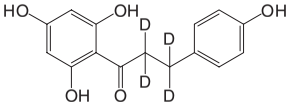
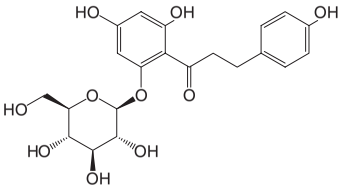
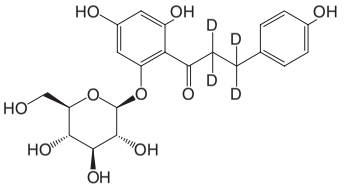
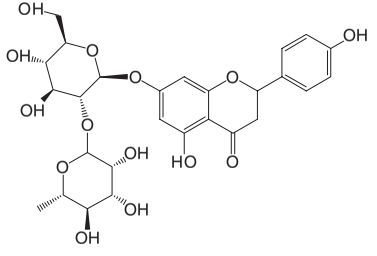
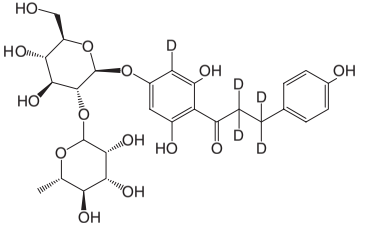
Reaction of naringenin (4′,5,7-trihydroxyflavan-4-one, **2**) with sodium formate-d_1 (DCOONa) and formic acid-d_2 (DCOOD) in refluxing isopropanol gave phloretin **1a** in 33% yield as reported[19] but with little incorporation of deuterium (< 15% by MS) and with less than 5% deuteration α or β to the carbonyl group as measured by NMR. Most of the deuterium exchange had occurred on ring A at C3′ and C5′ presumably by deuterium exchange occurring under the highly basic conditions occurring at completion of the reaction. Repetition of the reaction in methanol-d_1, and optimization of the reaction workup, gave deuterated phloretin **1a-*d*$_4$** (Table 1) in 84% yield (99% pure by HPLC). FDMS gave a molecular ion cluster centred at m/z 278.1108 indicating incorporation of four deuterium atoms. Integration of residual signals in the ^{1}H NMR at δ 3.28 and 2.84 ppm showed 88 and 93% deuterium incorporation, respectively, at positions α and β to the carbonyl group. Some additional deuteration into ring A (25% distributed between H3′ and H5′) was also observed. Reaction in methanol-d_1 using non-deuterated sodium formate and with omission of the formic acid[20] similarly gave **1a-*d*$_4$** with the same level of deuterium incorporation.

Incorporation of four deuterium atoms into **1a-*d*$_4$** indicated that more complex chemistry than simple hydrogen transfer[19] had occurred. Deuterium exchange adjacent to the carbonyl group can be accounted for by the highly alkaline conditions (pH > 12) present at the end of the reaction but the presence of two deuterium atoms β to the carbonyl group suggested palladium benzylic catalysed exchange had also occurred. To test this, the reaction was repeated using phloretin **1a** as the substrate. Reaction of phloretin **1a** with sodium formate in refluxing methanol-d_1 gave **1a-*d*$_4$** in 84% yield (99% pure by HPLC) with 91% incorporation of deuterium α and β to the carbonyl group as judged by ^{1}H NMR.

Deuteration resulting from deuterium transfer from methanol-d_1 implies formate[19] is not the sole hydrogen donor in this reaction. Following Rajagopal and Spatola,[20] an alternative scheme, involving addition of methanol-d_1 to a reduced palladium species (Figure 2), is proposed to account for the extensive deuteration observed in the reaction.

The deuterium atoms α to the carbonyl group are potentially exchangeable and the removal of the deuterium from this site by back exchange was briefly investigated. Reaction of **1a-*d*$_4$** with 1.0 M sodium hydroxide in methanol for 24 h at room temperature reduced deuteration at the α position from 88 to 75%. Deuteration at the β position was unchanged. Under prolonged or harsher reaction conditions **1a-*d*$_4$** showed decomposition. The deuteration α to the carbonyl group was surprisingly stable implying participation by palladium in the exchange reaction.

Table 1. Percent deuteration as determined by ^{1}H NMR of synthesized dihydrochalcones

Substrate	Product	% Deuteration
 **2**	 **1a-d_4**	88(Hα), 93(Hβ)
 **1a**	 **1a-d_4**	91(Hα), 91(Hβ)
 **1b**	 **1b-d_4**	94(Hβ)
 **3**	 **3-d_5**	96(Hα), 95(Hβ)

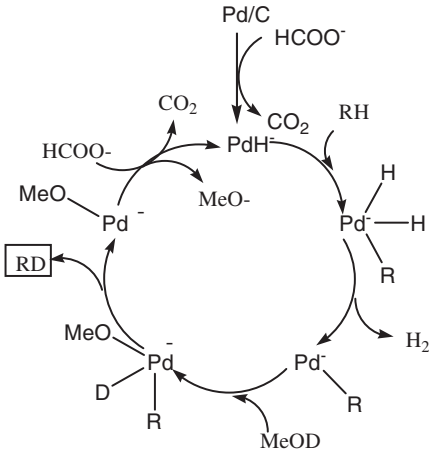


Figure 2. Mechanism proposed for the deuterium exchange of dihydrochalcones using Pd/C and sodium formate in methanol-d_1

The generality of this deuterium exchange reaction was tested using the glycosides phloridzin **1b** and naringin (4′,5-dihydroxy-7-*O*-(α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranosyl)flavan-4-one **3**) (Table 1). Reaction of **1b** with Pd/C and sodium formate in methanol-d_1 gave deuterated phloridzin **1b-*d*$_4$** in 29% isolated yield (95% pure by HPLC). FDMS gave the most abundant molecular ion at m/z 440 corresponding to the incorporation of four deuterium atoms. A fragment ion at m/z 278 confirmed deuteration incorporation into the aglycone. ^{1}H NMR analysis showed 94% deuterium incorporation at the β position; however, residual signals for the α protons were obscured by signals from H3 of glucose. Irradiation of the β protons at δ 2.87 in a TOCSY experiment confirmed their attachment to a weak doublet centred at δ 3.46. Integration of signal intensities also indicated some deuterium incorporation into the A ring (57 and 20% incorporation at H3′ and H5′, respectively). Extensive deuteration of **1b-*d*$_4$** at the α position was confirmed by LC-MS/MS. Thus negative ionization of **1b-*d*$_4$** gave a pseudomolecular ion m/z 439 $(M\text{-}H)^-$ which was fragmented with an initial loss of glucose to give an aglycone ion (ms^2) at m/z 277 ($C_{15}H_9D_4O_5^-$). This daughter ion was in turn fragmented (ms^3) to give two fragment ions derived from ring A at m/z 125 ($C_6H_5O_3^-$) and m/z 169 ($C_8H_5O_4D_2^-$).

In an attempt to increase the yield of phloridzin **1b-*d*$_4$**, shorter reaction times and more mild conditions were investigated. Comparable deuterium incorporation and recoveries were obtained after 30 min in refluxing methanol-d_1. No deuterium exchange was observed after 4 h of reaction at room temperature.

Reaction of naringin **3** under the standard reaction conditions gave deuterated naringin dihydrochalcone (1-[2,6-dihydroxy-4-*O*-(α-L-rhamnopyranosyl (1 → 2)-β-D-glucopyranosyl)-3-$^{2}H_1$-phenyl]-3-(4-hydroxyphenyl)-[2,2,3,3]-$^{2}H_4$-propan-1-one **3-d_5**) in 27% yield (98% pure by HPLC). FDMS gave the molecular ion as the sodium adduct at *m/z* 610.2145 ($(M+Na)^+$, $C_{27}H_{30}D_5O_{14}Na$) together with a less abundant ion cluster centred at *m/z* 587 (M^+) and a major fragmentation ion centred at *m/z* 278.1099 ($C_{15}H_{10}D_4O_5$) confirming deuteration of the aglycone. LCMS analysis of **3-d_5** showed a narrower distribution of deuterated species with a pseudomolecular ion cluster centred at *m/z* 587 $(M\text{-}H)^-$, six mass units above that of non-deuterated naringin dihydrochalcone ($(M\text{-}H)^-$, *m/z* 581). The reason for the difference in isotope distributions between these two MS methods is not known but the formation of both M^+ and $(M+H)^+$ ions during FDMS has been reported.[21]

Based on the LCMS results, negative ion LC-MS/MS of both **3-d_5** and of non-deuterated naringin dihydrochalcone was used to search for any additional sites of deuteration in **3-d_5**. Fragmentation of the deuterated pseudomolecular ion *m/z* 587 $(M\text{-}H)^-$ gave prominent daughter ions at *m/z* 479 (loss of 108, $C_7H_4D_2O$, ring B) and at *m/z* 466 (loss of 121, presumably a sugar fragment $C_4H_7D_1O_4$) ms^3 fragmentation of the ion at *m/z* 466 containing five deuterium atoms gave fragment ions at *m/z* 357 (loss of 108, $C_7H_4D_2O$), at *m/z* 278 (aglycone, $C_{15}H_9D_5O_5^-$) and at *m/z* 277 (aglycone, $C_{15}H_9D_4O_5^-$) consistent with partial additional deuteration on ring A of the dihydrochalcone and complete deuteration at both the α and β positions.

Detailed ^1H NMR analysis of **3-d_5** in d_6-acetone (using COSY, TOCSY, HSQC and HMBC experiments and both deuterated and non-deuterated compounds to aid assignments) showed 96 and 95% deuteration at Hα and Hβ, respectively, and also indicated extensive deuteration in ring A (81% on average at H3′and H5′). No further deuteration was detected elsewhere in the molecule. The origins of the higher mass ions recorded by LCMS with **3-d_5** remain unknown.

Experimental

General

Reagents were obtained from the Aldrich Chemical Co. (Milwaukee, WI) and Kodak (naringin) and were used without further purification unless otherwise stated. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker 400 and 500 NMR spectrometers. Chemical shifts (δ) are in parts per million relative to acetone-d_6 at 2.15 ppm for ^1H and at 30.67 ppm for ^{13}C. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Field desorption mass spectra (FDMS) were recorded on

a Waters GCT time of flight mass spectrometer equipped with a FD probe operating with an extraction voltage of 12 kV and ramping the emitter current from 0 to 70 μA over 8 min. Pentafluorochlorobenzene was used as the lock mass. LCMS-MS spectrometry was carried out using a LCQ Deca ion trap mass spectrometer fitted with an ESI interface (ThermoQuest, Finnigan, San Jose, CA, USA) and coupled to a Surveyor™ HPLC and PDA detector. Analysis was by direct infusion of the sample at 10 μl/min. Full-scan mass spectral data were acquired in the negative mode. MS/MS data were acquired by isolation and fragmentation of the M-1 parent ion to give ms^2 data, followed by isolation and fragmentation of the most intense daughter ion to give ms^3 spectra.

Phloretin-d_4 **1*a*-$\mathbf{d_4}$**. Typically to a solution of phloretin **1a** or naringenin **2** (50 mg) in methanol-d_1 (3 ml) was added sodium formate (50 mg) and palladium on charcoal powder (50 mg). The reaction mixture was stirred at reflux for 4 h before being filtered through a plug of celite with methanol washings. The solution was acidified with 1 M HCl and the methanol removed *in vacuo*. The resulting aqueous solution was extracted with ethyl acetate (50 ml), and the resulting organic layer washed with water, saturated brine, and then dried over magnesium sulphate, filtered and the solvent removed *in vacuo*. The crude product was purified by column chromatography on silica, eluting with methanol/dichloromethane (6:94) to give **1a-*d*$_4$** (42 mg, 84%) as a white solid. ^{1}H NMR δ 7.06 (2H, d, J = 8.8 Hz, H2, H6), 6.72 (2H, d, J = 8.8 H3, H5), 5.93 (1.75H, s, H3′, H5′) 3.28 (0.24H, s, Hα), 2.84 (0.14H, s, Hβ) ppm.[22] ^{13}C NMR δ 205.6, 165.4, 165.2, 156.3, 133.3, 130.1, 115.9, 105.1, 95.7 ppm. FDMS m/z 278.1108 (M^+, $C_{15}H_{10}D_4O_5$ requires 278.1092, isotopic distribution d_2:d_3:d_4:d_5:d_6 = 5:36:100:28:4).

Back exchange of phloretin-d_4. To a solution of **1a-*d*$_4$** (30.0 mg, 0.11 mmol) in methanol (5 ml) at room temperature was added NaOH (200 mg, 5 mmol). After stirring for 24 h at room temperature, the reaction mixture was neutralized with 1 M HCl, the methanol removed *in vacuo* and the residue extracted with ethyl acetate. The ethyl acetate phase was washed with water then brine, then dried over magnesium sulphate and the solvent removed *in vacuo* to give a white solid (20.9 mg, 70%). ^{1}H NMR δ 7.09 (2H, d, J = 8.8 Hz, H2, H6), 6.74 (2H, d, J = 8.8 Hz, H3, H5), 5.95 (1.86H, s, H3′, H5′) 3.28 (0.50H, s, Hα), 2.85 (0.14H, s, Hβ) ppm. FDMS m/z 277.1020 (M^+, isotopic distribution d_1:d_2:d_3:d_4:d_5 = 12:86:100:49:10).

Deuterated glycosides **1b-*d*$_4$**, **3-*d*$_5$**. Typically to a solution of substrate (500 mg) in methanol-d_1 (20 ml) was added sodium formate (500 mg) and palladium on charcoal powder (500 mg). The reaction mixture was stirred at reflux for

30 min before being filtered through a plug of celite, the solution was acidified with 1 M HCl and the methanol removed *in vacuo*. The crude product was filtered through reverse-phase silica with water, followed by water/methanol (1:1). The water/methanol fraction was further purified by chromatography on silica eluting with methanol/dichloromethane (1:9). Removal of solvent *in vacuo* gave a glassy solid that was recrystallized from water to give the product as a white solid.

Phloridzin **1b-*d*$_4$**(145 mg, 29%) ^{1}H NMR δ 7.13 (d, 2H, J = 6.5 Hz, H2, H6), 6.75 (d, 2H, J = 6.5 Hz, H3, H5), 6.29 (0.80H, br s, H5′), 6.02 (0.43H, d, J = 2.1 Hz, H3′), 5.13 (1H, d, J = 5.7 Hz, Glu-H1), 3.92 (1H, dd, J = 2.5, 11.9 Hz, Glu-H6a), 3.74 (1H, dd, J = 5.7, 11.9 Hz, Glu-H6b), 3.57 (3H, m, Glu-H2, Glu-H4, Glu-H5), 3.46 (1H, m, Glu-H3), 2.87 (0.13H, s) ppm. ^{13}C NMR δ 161.3, 155.4, 132.4, 129.3 (C2), 115.0 (C3), 105.4, 101.2, 97.2 (C3′), 94.4 (C5′), 77.4, 77.1, 73.5, 70.3, 61.7 ppm. FDMS m/z 440.1703 (M^+, $C_{21}H_{20}D_4O_{10}$ requires 440.1621), 422.1554 (M^+-H_2O, $C_{21}H_{18}D_4O_9$ requires 422.1515), 278.1099 ($C_{15}H_{10}D_4O_5$ requires 278.1092); LCMS-MS m/z 439 ($M\text{-}H^-$, isotopic distribution d_2:d_3:d_4:d_5 = 9:36:100:19); 277 (ms^2), 169, 125 (ms^3).

Naringin dihydrochalcone **3-*d*$_5$**. (137 mg, 27%). ^{1}H NMR δ 7.09 (2H, d, J = 8.5 Hz, H2, H6), 6.75 (2H, d, J = 8.5 Hz, H3, H5), 6.10 (0. 38H, s, H3′, H5′), 5.35 (1H, d, J = 1.7 Hz, Rha-H1), 5.08 (1H, d, J = 7.4 Hz, Glu-H1), 3.98 (1H, dq, J = 6.2, 9.5 Hz, Rha-H5), 3.94 (1H, dd, J = 1.7, 3.4 Hz, Rha-H2), 3.91 (1H, dd, J = 2.3, 11.9 Hz, Glu-H6a), 3.73 (1H, dd, J = 5.8, 11.9 Hz, Glu-H6b), 3.71 (1H, m, Glu-H3), 3.68 (1H, dd, J = 7.4, 9.9 Hz, Glu-H2), 3.63 (1H, dd, J = 3.4, 9.5 Hz, Rha-H3), 3.56 (1H, ddd, J = 2.3, 5.8, 9.6 Hz, Glu-H5), 3.48 (1H, t, J = 9.6 Hz, Glu-H4), 3.47 (1H, t, J = 9.5 Hz, Rha-H4), 1.26 (3H, d, J = 6.2 Hz, Rha-H6) ppm. ^{13}C NMR δ 207.1, 165.9 (C2′, C6′), 165.2 (C4′), 157.4 (C4), 134.3 (C1), 131.2 (C2, C6), 117.0 (C3, C5), 107.5 (C1′), 102.6 (Rha-C1), 100.0 (Glu-C1), 97.3 (C3′, C5′), 79.8 (Glu-C3), 78.7 (Glu-C5), 78.6 (Glu-C2), 74.9 (Rha-C4), 73.3 (Rha-C3), 72.9 (Rha-C2), 72.5 (Glu-C4), 70.2 (Rha-C5), 63.5 (Glu-C6), 19.4 (Rha-C6) ppm. FDMS m/z 610.2145 ($M + Na^+$, $C_{27}H_{30}D_5O_{14}Na$ requires 610.2160, isotopic composition d_2:d_3:d_4:d_5:d_6:d_7:d_8:d_9 = 16:54:89:100:83:51:21:8). LCMS-MS m/z 587 ($M\text{-}H^-$, isotopic distribution d_3:d_4:d_5:d_6:d_7 = 5:31:86:100:14), 466 (ms^2), 357, 277 (ms^3).

Conclusion

Pd/C catalysed deuterium exchange using methanol-d_1 as the deuterium source provides ready access to a variety of complex deuterated dihydrochalcones glycosides. As this labelling method is also amenable to the incorporation of tritium, it should be widely useful for studying biological activity of this interesting class of flavonoids.

Acknowledgements

We wish to thank Martin Hunt for FDMS and the Foundation for Research Science and Technology for financial support under Contract COX0220.

References

1. Bohm BA. The minor flavanoids. In *The Flavanoids Advances in Research since 1986*, Harbourne JB (ed.). Chapman & Hall: London, 1993; 399–406.
2. Kamperdick C, Van NH, Van Sung TV. *Phytochemistry* 2002; **61**(8): 991–994.
3. Kumar JK, Narender T, Rao MS, Rao PS, Toth G, Balazs B, Duddeck H. *J Braz Chem Soc* 1999; **109**(4): 278–280.
4. Tanaka T, Uehara R, Nishida K, Kouno I. *Phytochemistry* 2004; **65**: 1–7.
5. Ogawa K, Kawasaki A, Omura M, Yoshida T, Ikoma Y, Yano M. *Phytochemistry* 2001; **57**(5): 737–742.
6. Tomas-Barberan FA, Clifford MN. *J Sci Food Agric* 2000; **80**(7): 1073–1080.
7. Hilt P, Schieber A, Yildirim C, Arnold G, Klaiber I, Conrad J, Beifuss U, Carle R. *J Agric Food Chem* 2003; **51**(10): 2896–2899.
8. Yang W-M, Liu J-K, Qin X-D, Wu W-L, Chen Z-H. *Z Naturforsch* [*C*] 2004; **59c**: 481–484.
9. Alvarado F, Crane RK. *Biochim Biophys Acta* 1964; **93**: 116–135.
10. Rossetti L, Smith D, Shulman GI, Papachristou D, Defronzo RA. *J Clin Investig* 1987; **79**: 1510–1515.
11. Starke A, Grundy S, McGarry JD, Unger RH. *Proc Natl Acad Sci USA* 1985; **82**(5): 1544–1546.
12. Zhao H, Yakar S, Gavrilova O, Sun H, Zhang Y, Kim H, Setser J, Jou W, LeRoith D. *Diabetes* 2004; **53**: 2901–2909.
13. Monge P, Solheim E, Scheline RR. *Xenobiotica* 1984; **14**: 917–924.
14. Crespy V, Aprikian O, Morand C, Besson C, Manach C, Demigne C, Remesy C. *J Nutr* 2001; **131**(12): 3227–3230.
15. Tuck KL, Tan H-W, Hayball PJ. *J Label Compd Radiopharm* 2000; **43**: 817–823.
16. Yao J, Evilia RF. *J Am Chem Soc* 1994; **116**(25): 11229–11233.
17. Hibbs MR, Yao J, Evilia RF. *High Temp Mater Sci* 1996; **36**: 9–14.
18. Wähälä K, Rasku S. *Tetrahedron Lett* 1997; **38**: 7287–7290.
19. Krishnamurty HG, Sathyanarayan S. *Synthetic Commun* 1989; **19**(1 and 2): 119–123.
20. Rajagopal S, Spatola AF. *J Org Chem* 1995; **60**(5): 1347–1355.
21. Matich AJ, Bunn BJ, Hunt MB, Rowan DD. *Phytochemistry*: http://www.sciencedirect.com/science?_ob=Mlmg&_imagekey=B6TH7-4J6W780-2-9&_cdi=5275&_user=706881&_orig=search&_coverDate=02%2F07%2F2006&_sk=999999999&_view=c&wchp=dGLbVIb-zSkWb&md5=1a1628cb1324f98fdb7a940c419faf42&ie=/sdarticle.pdf
22. Markham KR, Geiger H. 1H nuclear magnetic resonance spectroscopy of flavonoids and their glycosides in hexadeuterodimethylsulfoxide. In *The Flavonoids Advances in Research Since 1986*, Harbourne JB (ed.). Chapman & Hall: London, 1993; 441–497.

