AAPH-mediated antioxidant reactions of secoisolariciresinol and SDG

Farah S. Hosseinian,[a] Alister D. Muir,[b] Neil D. Westcott[b] and Ed S. Krol*[a]

Received 29th November 2006, Accepted 22nd December 2006
First published as an Advance Article on the web 23rd January 2007
DOI: 10.1039/b617426d

Secoisolariciresinol (SECO **1**) is the major lignan found in flaxseed (*Linum usitatissimum* L.) and is present in a polymer that contains secoisolariciresinol diglucoside (SDG **2**). SECO, SDG and the polymer are known to have a number of health benefits, including reduction of serum cholesterol levels, delay in the onset of type II diabetes and decreased formation of breast, prostate and colon cancers. The health benefits of SECO and SDG may be partially attributed to their antioxidant properties. To better understand their antioxidant properties, SECO and SDG were oxidized using 2,2′-azobis-(2-amidinopropane), an *in vitro* model of radical scavenging. The major lignan radical-scavenging oxidation products and their formation over time were determined. SDG was converted to four major products (**11–14**), which were the result of a phenoxyl radical intermediate. One of these products (**13**), a dimer of SDG, decomposed under the reaction conditions to form two of the other major products, **12** and **14**. SECO was converted to five major products (**6–10**), two of which (**6** and **7**) were also the result of a phenoxyl radical intermediate. The remaining products (**8**, **9** and **10**) were the result of an unexpected alkoxyl radical intermediate. The phenol oxidation products were stable under the reaction conditions, whereas two of the alcohol oxidation products (**8** and **9**) decomposed. In general, only one phenol group on the lignans was oxidized, suggesting that the number of phenols per molecule may not predict radical scavenging antioxidant ability of lignans. Finally, SECO is a superior antioxidant to SDG, and it may be that the additional alcohol oxidation pathway contributes to its greater antioxidant ability.

Introduction

Consumption of flaxseed ((*Linum usitatissimum* L.) has been associated with a number of health benefits, including protection against cardiovascular disease in rodents,[1–4] rabbit[5] and humans,[6] hepatoprotection in rats[7,8] and protection against cancer in rodents.[9–14] Most of these beneficial effects have been linked to the lignans found in the hull portion of flaxseed. Secoisolariciresinol (SECO, **1**) (Fig. 1) is the major lignan found in flaxseed[15] and is present in flaxseed as the diglucoside (SDG, **2**), which is incorporated into a (hydroxymethyl)glutarate-linked polymer.[16,17] Other lignans, such as matairesinol (MAT, **3**) are present in smaller amounts. In animal models, SDG and SECO have been shown to reduce cancer,[9,12,18] cholesterol and atherosclerosis,[10,19] to protect against loss of lupus-induced renal function[20] and to delay the development of Type II diabetes.[21] Flaxseed lignans undergo metabolism in the human gastrointestinal tract to form the mammalian lignans enterodiol (END, **4**) and enterolactone (ENL, **5**) (Fig. 1), which have also been associated with decreased risk of coronary events,[22,23] inhibition of prostate cancer cell lines[24] and aromatase activity.[25,26]

The health benefits attributed to SECO and SDG have been proposed to be the result of estrogenic[27–30] or antioxidant properties.[31,32] Antioxidants are believed to play an important role in disease protection by reducing the effects of oxidative stress seen in processes such as cardiovascular disease, cancer and neurodegenerative diseases.[33] Phenolic antioxidants, such as vitamin E and butylated hydroxytoluene (BHT), neutralize radical species including lipid peroxides by forming stable, long-lived radicals which lead to stable, non-radical products;[34] SECO and SDG would be expected to behave in a similar manner. A possible mechanism for SECO/SDG scavenging of radical species is shown in Fig. 2. In the proposed scheme, a radical ($Y^{\bullet}$) abstracts a hydrogen atom from the lignan phenol hydroxyl, yielding a resonance-stabilized phenoxyl radical and YH. The lignan radical could then react with a second radical, including another lignan radical, to form a stable product. An ideal stoichiometric ratio of 4 would be expected for SECO, SDG and MAT, however radical scavenging antioxidant stoichiometries determined for a variety of 2-methoxy phenols (eugenol, 1.30,[35] 1.85;[36] 2-methoxy-4-methylphenol, 1.41;[35] zingerone, 1.85[36]) suggest that stoichiometric values closer to 2.6–2.8 might be anticipated for the lignans.

The antioxidant properties of SECO, SDG, MAT, END and ENL have been studied using several techniques. Inhibition of lipid peroxidation of 1,2-dilinoleoyl-*sn*-glycerol-3-phosphocholine (DLPC) induced by 2,2′-azobis(2-amidinopropane) (AAPH) was greater for SECO (1.5) than SDG (1.2).[37] These values are substantially less than 2.6–2.8 radicals scavenged per molecule, and both compounds are poorer antioxidants than BHT (2.0), which has only one phenol hydroxyl group. SECO was also found to be a comparable radical scavenger to a monophenolic analogue, 2-methoxy-4-methylphenol, using the Ferric Reducing/Antioxidant Power (FRAP) assay.[32] Data from a recent study by Eklund[38]

[a]*College of Pharmacy and Nutrition, University of Saskatchewan, 110 Science Place, Saskatoon, SK, S7N 5C9, Canada. E-mail: ed.krol@usask.ca; Fax: +1 (306) 966-6377; Tel: +1 (306) 966-2011*
[b]*Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK, Canada*

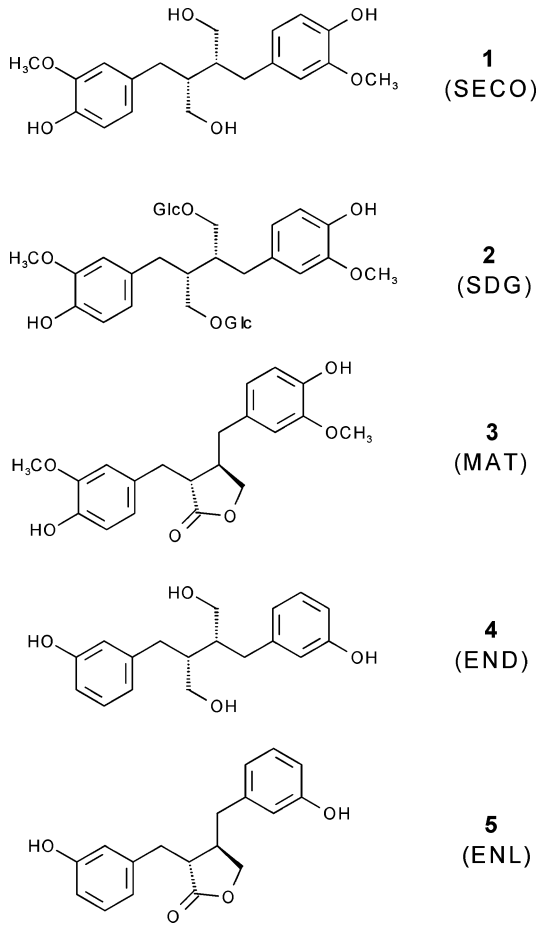

Fig. 1 Structures of the major flaxseed lignans secoisolariciresinol (SECO) **1** and secoisolariciresinol diglucoside (SDG) **2** (Glc = glucose), the minor lignan matairesinol (MAT) **3**, and their mammalian metabolites, enterodiol (END) **4** and enterolactone (ENL) **5**.

also suggests that lignan antioxidant capacity may not be directly related to the number of phenol hydroxyl groups per molecule.

The value for antioxidant scavenging of diphenylpicrylhydrazyl (DPPH) radicals by SECO is 2.3 times greater than BHT and 1.5 times greater than MAT,[38] indicating that the two primary hydroxyl groups on the butyl chain of SECO may also contribute to radical scavenging. Lignans with primary hydroxyl groups were also more effective inhibitors of radical oxygen species generated by polymorphonuclearleukocytes (PMNL-CLs)[31] and radical scavenging measured by the FRAP assay.[32] Inhibition of ammonium thiocyanate-mediated lipid peroxidation of linoleic acid showed that at 100 μM lignan, END was superior to ENL; however, at lower concentrations (10 μM) ENL was the superior antioxidant.[39]

This study is based on two hypotheses: (i) only one phenol per lignan molecule contributes to the radical-scavenging antioxidant capacity and (ii) primary alcohols on the butyl chain are involved in the antioxidant reactions of lignans. The first objective of this study was to determine the antioxidant reaction products for SECO and SDG using a model radical-generating system. A second objective was to determine whether the reaction products obtained could explain the difference in antioxidant stoichiometry between SECO and SDG, specifically whether the primary alcohols were involved in the antioxidant reactions.[37] The radical initiator AAPH was used by this group in the determination of the stoichiometric ratios of SECO and SDG.[37] AAPH undergoes thermal decomposition in solution to produce two carbon-centred amidino propane (AP) radicals, which can add O_2 to form a peroxyl radical, although carbon-centred radicals usually predominate. AAPH has solubility properties which allowed study of both SECO and SDG in the same reaction system, and it does not possess a chiral centre. This type of model system allows for study of the major radical-scavenging reactions of phenolic antioxidants.[38,40] The major AAPH reaction products of SECO and SDG were collected and their structures determined. This investigation differs in several ways from the earlier study reported by Eklund:[38] the oxidation products for SDG were determined as well as those for SECO, a time-course for the reaction of the lignans with AAPH was performed and a shorter reaction time was used (5 h *vs.* 72 h), allowing for the identification of transient products that could be involved in the antioxidant reactions.

Results and discussion

Products of AAPH-mediated oxidation of flaxseed lignans

The progress of the AAPH-mediated oxidations was monitored by HPLC–UV (280 nm), and the peak areas of the major HPLC peaks were plotted against time. There were 5 major peaks for SECO (Fig. 3a) and 4 major peaks for SDG (Fig. 4a). The incubations were terminated after 5 h (SECO) and 4 h (SDG) when approximately 10% of the starting material remained and it became difficult to observe the starting lignans in the HPLC baseline. For SECO, two of the major HPLC peaks were more polar than SECO (**6**, **10**) and three were less polar (**7**, **8**, **9**). Compounds **8** and **9** formed rapidly and began to decompose after 90 min, whereas compounds **6**, **7** and **10** formed slowly throughout the time-course until the reaction was terminated. For SDG, two of the compounds formed were more polar than SDG (**11**, **12**) and two compounds were less polar (**13**, **14**). Compound **13** formed rapidly and began to decompose after 20 min, whereas compounds **11**, **12** and **14** formed more gradually through the incubation, with **12** being the major product after 4 h. The oxidation products for both reactions were collected by semi-preparative HPLC and their structures determined using NMR and MS. Additional minor oxidation products were not collected. Approximately 10% of SECO-containing oxidation products and 29% of SDG-containing oxidation products were lost during purification.

Additional AAPH-mediated oxidations were carried out on transient products formed during the initial incubations. An AAPH-mediated oxidation of **8** resulted in the formation of compounds **9**, **10** and SECO (Fig. 3b). This experiment could not be carried out with compound **9** due to a lack of product. An AAPH-mediated oxidation of **13** resulted in the formation of compound **12** and SDG (Fig. 4b).

Products from phenol OH oxidation

The SECO oxidation products **6** and **7** (Fig. 5) and all four of the SDG oxidation products (Fig. 6) resulted from oxidation of a phenol hydroxyl.[41] Products **6** and **7** were determined to be the

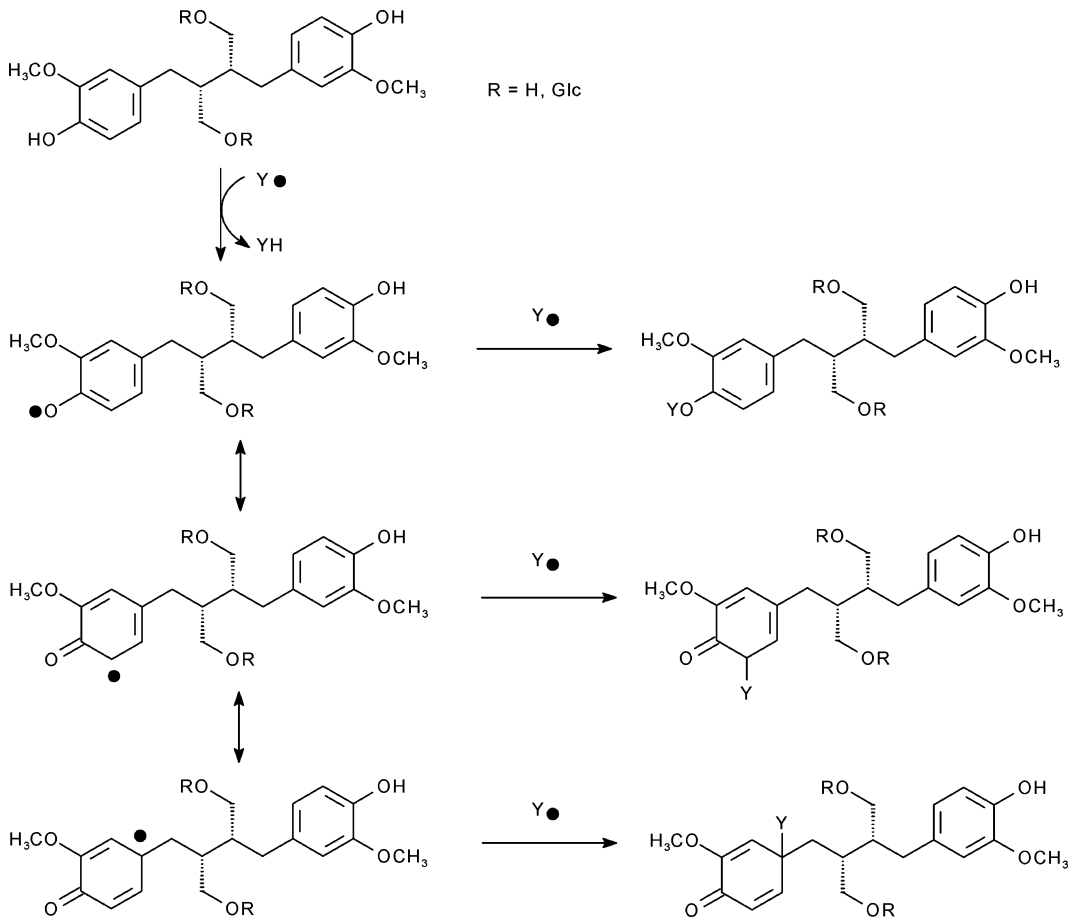

Fig. 2 Proposed antioxidant reactions of SECO/SDG.

same as previously identified AAPH reaction products.[38] Products **6** and **12** both result from substitution of AP for a phenol hydrogen on SECO and SDG, respectively. The SECO oxidation product **7** and the early forming SDG product **13** are C-5–C-5 dimers of SECO and SDG, respectively. Compounds **6**, **7**, **12**, **13** and **14** are all consistent with the antioxidant products predicted in Fig. 2 and should provide net radical scavenging.

The SDG dimer **13** decomposes to the AP substitution product **12** and SDG under oxidative conditions, whereas the SECO carbon–carbon dimer **7** appears stable. This suggests that an unexpected oxidative cleavage of the carbon–carbon bond in **13** occurs and that SDG is recycled, which may further contribute to the radical scavenging properties of SDG. A similar reaction, the conversion of the di-phenol dihydrodivanillin to the phenol vanillin has been reported (Fig. 7), although this reaction was catalyzed by a fusant of *Fusobacterium varium* and *Enterococcus faecium* (FE7).[42] The initial carbon–carbon dimerization products (**7** and **13**) would possess a di-keto structure that isomerizes to the more stable aromatic ring.[43] Thus, it is tempting to rationalize this oxidative carbon–carbon bond cleavage as occurring *via* the di-keto structure **13a** (Fig. 8), although the carbon–carbon bond lengths for **13** and **13a** would be comparable.[44,45] It is not clear why **7**, the SECO carbon–carbon dimer, does not decompose *via* this pathway, although the steric bulk of the glucose groups in **13** may be destabilising. Due to the limited amount of product isolated, an AAPH-mediated oxidation of **7** could not be performed to determine if this is a general phenomenon. Synthetic pathways that would produce larger quantities of **7** would aid in further study of this intermediate.

The ESI-MS spectrum of compound **11** (*m*/*z* 717 $[M - H]^-$, 718 $[M + H]^+$) was consistent with the addition of two oxygen atoms. NMR experiments indicated that **11** was nearly identical to the NMR spectrum for SDG, with small downfield shifts in the ^{13}C spectrum at the 2, 4 and 5 carbons. A peroxide test revealed 1.7 ± 1.9 peroxide equivalents per mole, suggesting two peroxide groups in the molecule (Fig. 6). Although peroxides are frequently unstable, **11** appears to be stable under these reaction conditions. In spite of this stability, **11** could still have a pro-oxidant effect. Compound **11** is the only product in this study in which both phenoxyl groups undergo reaction. It is worth noting that **11** could not be detected from the oxidation of **13**, which is surprising, as liberation of SDG from **13** should have led to formation of **11**. Compound **11** appears as a shoulder of **12** on the HPLC chromatogram and may not have been sufficiently resolved in the oxidation of **13**.

The mass spectrum of compound **14** (*m*/*z* 701 $[M - H]^-$), indicates the addition of an oxygen atom to SDG. NMR revealed that one of the aromatic rings remained unchanged while the other possessed a carbonyl carbon at C-4 (δ 171). Downfield shifts for C-2, C-3, C-5 and C-6, and a large upfield shift at C-1 (δ 133.9 to δ 73.4) were indicative of a cyclohexadienone structure with a hydroxyl substituted at C-1.

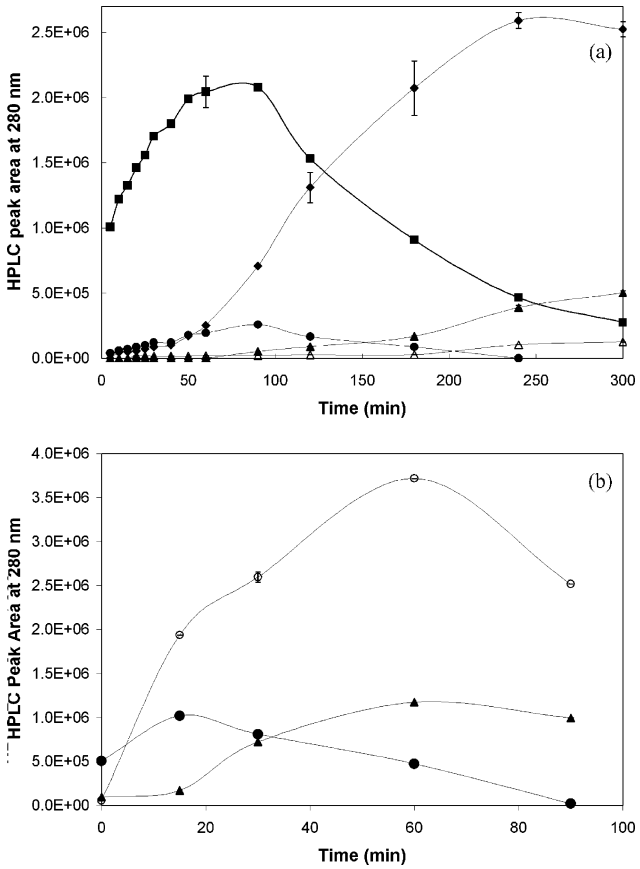

Fig. 3 (a) Graph of product formation *versus* time for the reaction of secoisolariciresinol (0.01 mol dm^{-3}) and AAPH (0.1 mol dm^{-3}) in acetonitrile at 60 °C. Each time point represents the HPLC–UV peak area at 280 nm. Error bars are standard deviations from the mean for at least 3 replicates. Compound **6** (R_t 18.6 min,◆), **7** (R_t 26.7 min, △), **8** (R_t 25.6 min, ■), **9** (R_t 28.9 min, ●), **10** (R_t 19.6 min, ▲). (b) Graph of product formation *versus* time for the reaction of **8** (0.01 mol dm^{-3}) and AAPH (0.1 mol dm^{-3}) in acetonitrile at 60 °C. Each time point represents the HPLC–UV peak area at 280 nm. Error bars are standard deviations from the mean for at least 3 replicates. SECO (R_t 21.4 min, ○), **9** (R_t 28.9 min, ●), **10** (R_t 19.6 min, ▲).

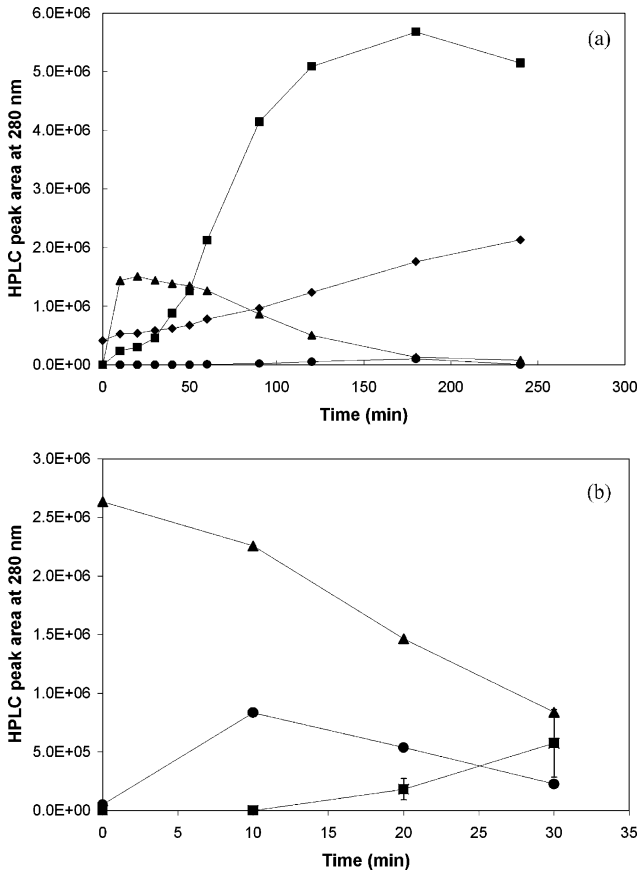

Fig. 4 (a). Graph of product formation *versus* time for the reaction of SDG (0.01 mol dm^{-3}) and AAPH (0.1 mol dm^{-3}) in acetonitrile at 60 °C. Each time point represents the HPLC–UV peak area at 280 nm. Error bars are standard deviations from the mean for at least 3 replicates. Compound **11** (R_t 12.1 min,◆), **12** (R_t 12.9 min, ■), **13** (R_t 14.3 min, ▲), **14** (R_t 17.0 min, ●). (b) Graph of product formation *versus* time for the reaction of **13** (0.01 mol dm^{-3}) and AAPH (0.1 mol dm^{-3}) in acetonitrile at 60 °C. Each time point represents the HPLC–UV peak area at 280 nm. Error bars are standard deviations from the mean for at least 3 replicates. **12** (R_t 12.9 min, ■), **13** (R_t 14.3 min, ▲), SDG (R_t 13.3 min, ●).

Eklund reported two additional products not observed in this study, a dimethyl furanone of SECO (**15**), formed from further reaction of compound **6**, and lariciresinol **16** (Fig. 9).[38] Lariciresinol was not observed in the present study, although it is possible that small amounts may have been formed but not detected under the analytical conditions used. NMR data was previously presented only for the dimethyl furanone of SECO; therefore, the present study is the first to provide unambiguous evidence for the structures of the products of AAPH-mediated oxidation of SECO.

Products from alcohol OH oxidation

Mass spectral data for the transient intermediate **8** (*m/z* 721 $[M - H]^-$, 723 $[M + H]^+$) suggested that compound **8** may also be a dimer of SECO. In contrast to compound **7**, no loss of proton signals was observed in the aromatic or aliphatic regions. The ^{13}C signals for aliphatic carbons C-7, C-8 and C-9 were shifted downfield with respect to the same carbons in SECO, and are consistent with the formation of a dimer of SECO linked by an O–O bond between alcohol groups (Fig. 10). The time-courses for oxidation of both SECO and **8** indicate the aliphatic peroxyl linkage is only moderately stable under these reaction conditions.

ESI-MS results for compound **9** (*m/z* 393 $[M - H]^-$, 395 $[M + H]^+$) indicate the addition of two oxygen atoms to SECO. The NMR results showed that the compound maintained the symmetry seen with SECO. Downfield ^1H and ^{13}C chemical shifts at positions 7, 8 and 9, compared to SECO, suggest the formation of two aliphatic peroxides (1.8 ± 0.1 peroxide equivalents). Finally, compound **10** (*m/z* 477 $[M - H]^-$, 479 $[M + H]^+$) is consistent with the substitution of an AP on one phenol and the presence of two aliphatic peroxides (1.7 ± 0.09 peroxide equivalents). NMR data showed that the ^1H and ^{13}C signals at the alkyl positions 8 and 9 for product **10** are shifted downfield from the same positions in **6**.

Compounds **8**, **9** and **10** differ from any of the other oxidation products observed in this or previous studies[38] in that they are derived from an alkoxyl radical (**1c**, Fig. 10). This is surprising,

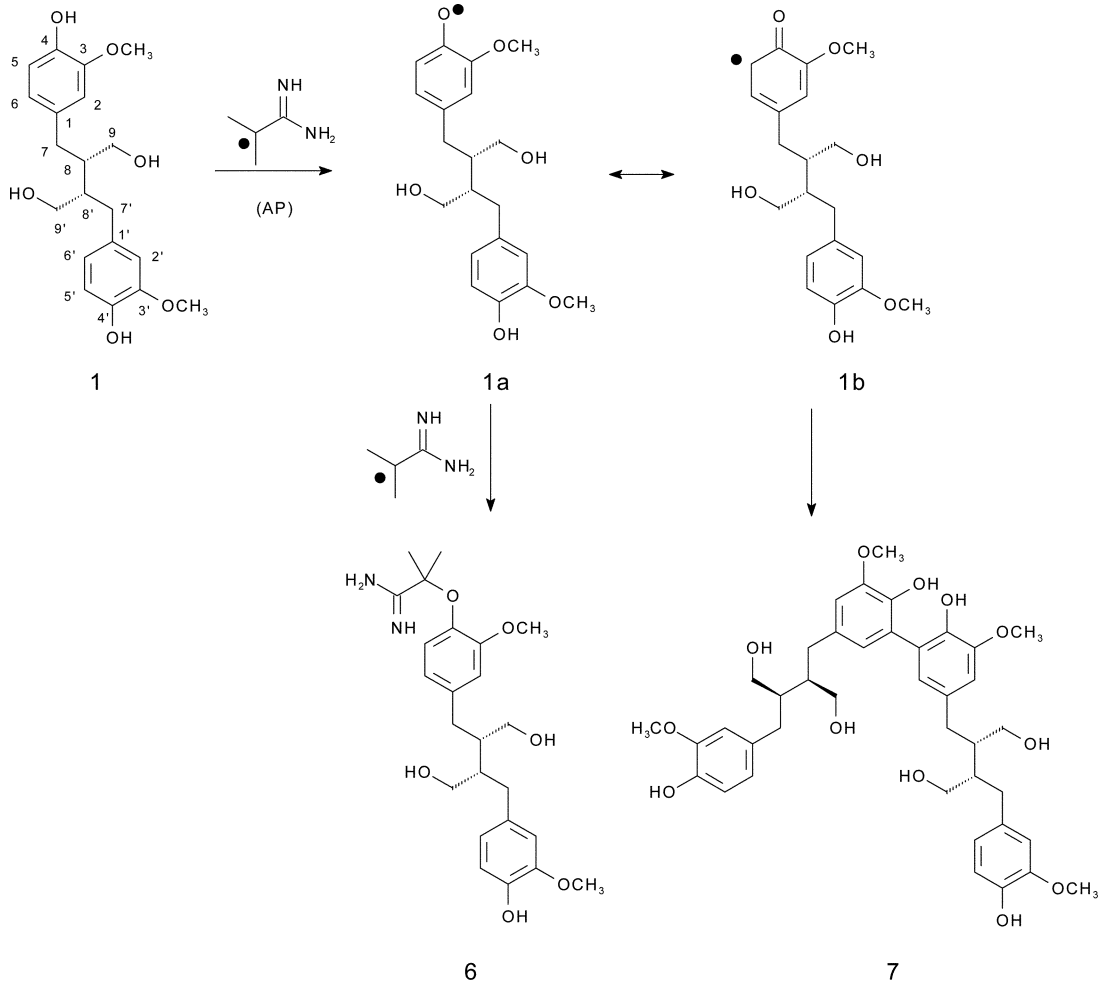

Fig. 5 Proposed pathway for the formation of AAPH-mediated aromatic oxidation products of secoisolariciresinol.

given that the O–H bond energy of an aliphatic alcohol (*ca.* 104 kcal mol^{-1})[46] is much greater than that for a phenol O–H (*ca.* 73 kcal mol^{-1}).[47] The SECO alkoxyl radical **1c** could be formed either by direct $H^{\bullet}$ abstraction or by deprotonation followed by a one-electron oxidation. Since ionized O–H bonds are more susceptible to oxidation, intramolecular hydrogen-bonding between the aliphatic alcohols may contribute to this process by decreasing the pK_a of the alcohol.[48] Alternatively, **1c** may be formed by an intramolecular or intermolecular $H^{\bullet}$ transfer from the aliphatic hydroxyl to a phenoxyl radical **1a**. Products **9** and **10** are most likely formed by further oxidation of **1c**, with the additional oxygen atoms derived from either solvent (H_2O) or atmosphere (O_2). The data in Fig. 3a and 3b suggest that an AP radical adds to the phenoxyl radical of **9** (**9a**, Fig. 10) to yield **10**, as opposed to initial formation of **6** followed by subsequent oxidation of the aliphatic alcohols. Alkoxyl radical formation may provide an explanation for the greater stability of **7** compared to **13**, as alkoxyl radical formation in **7** may compete with the carbon–carbon bond cleavage reaction.

The alkoxyl radical-derived products found for SECO, but not for SDG, may contribute to the greater antioxidant capacity observed for SECO in the inhibition of AAPH-mediated lipid oxidation.[37] Even though the SECO peroxyl dimer **8** is transient under these conditions, its rapid formation may result in a significant contribution to the initial inhibition of lipid oxidation. Regeneration of SECO from **8** may further contribute to antioxidant ability over a longer duration of time. Even though lipid alkoxyl radicals have been reported to be 10^4–10^6 times more reactive towards hydrogen atom abstraction than a lipid peroxyl radical,[47] the stoichiometric results for lignans with and without the butyl chain alcohols suggest that alkoxyl radical formation of these lignans is not pro-oxidant. The alkyl–peroxyl oxidation products, **9** and **10**, might also be expected to provide a pro-oxidant effect. However, the primary breakdown product of the di-alkyl peroxide **9** is the AP substituted di-alkyl peroxide **10**, which was shown to be stable under the reaction conditions. There was no indication that the peroxide groups on **9** and **10** were involved in further reactions, although this system was not designed to measure redox cycling reactions, a potential fate for these compounds.

Conclusion

The results in the present study combined with data from the literature suggest that the difference in antioxidant stoichiometry between SDG and SECO is less dependent on the difference in

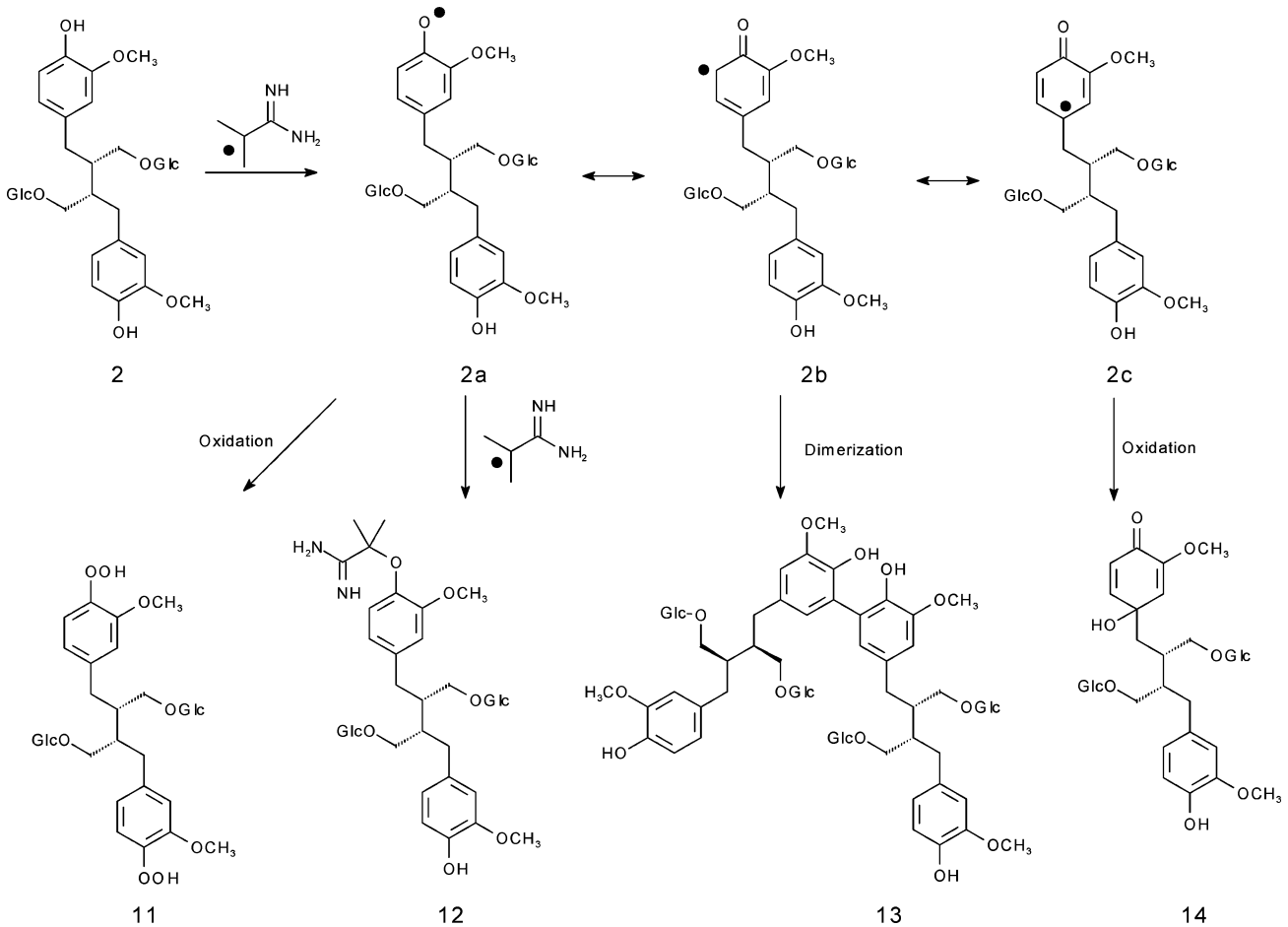

Fig. 6 Proposed pathway for the formation of AAPH-mediated SDG oxidation products.

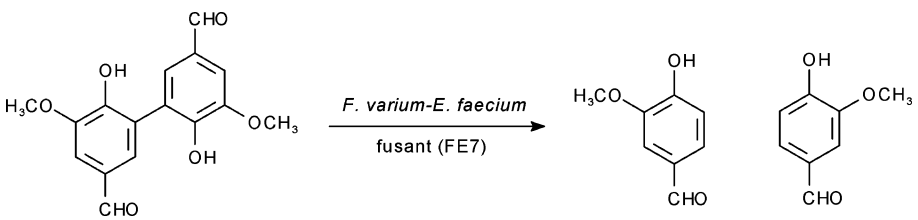

Fig. 7 Enzyme-mediated carbon–carbon bond cleavage in the conversion of dihydrodivanillin to vanillin.[42]

solubility, and more dependent on the participation of the primary alcohol groups on SECO in radical-scavenging antioxidant reactions. One previous antioxidant study suggested that steric hindrance from the glucose groups on SDG may interfere with radical scavenging by the phenol hydroxyl groups, a possibility that cannot discounted as a contributing factor.[39] No products arising from oxidation of the alcohol groups on SECO were reported by Eklund, which was likely due to the longer reaction time (72 h *vs.* 5 h in the present study) which would favour formation of the more stable, phenol radical-derived oxidation products.[38] It would be useful to further determine how the aliphatic hydroxyl groups on SECO contribute to antioxidant stoichiometry and radical scavenging reactions by blocking the phenol or the alcohol groups on SECO.

The antioxidant stoichiometries for both SECO and SDG using AAPH to catalyze lipid peroxidation are far below the predicted ideal values.[37] The results from the present study confirm that the stoichiometric ratios for both lignans cannot be predicted based on the total number of phenol OH groups, as only one phenol per lignan molecule is involved in radical scavenging reactions. Most of the oxidation products in the present study are stable and seem resistant to oxidation of the second phenol. This could be the result of an intramolecular interaction between the oxidized and reduced phenol rings which somehow prevents further oxidation, although this would require further investigation. It is worth noting that when DDPH scavenging was used as a measure of lignan stoichiometry, the stoichiometric factor of 4.5 for SECO more closely agrees with the predicted stoichiometry.[38] This may

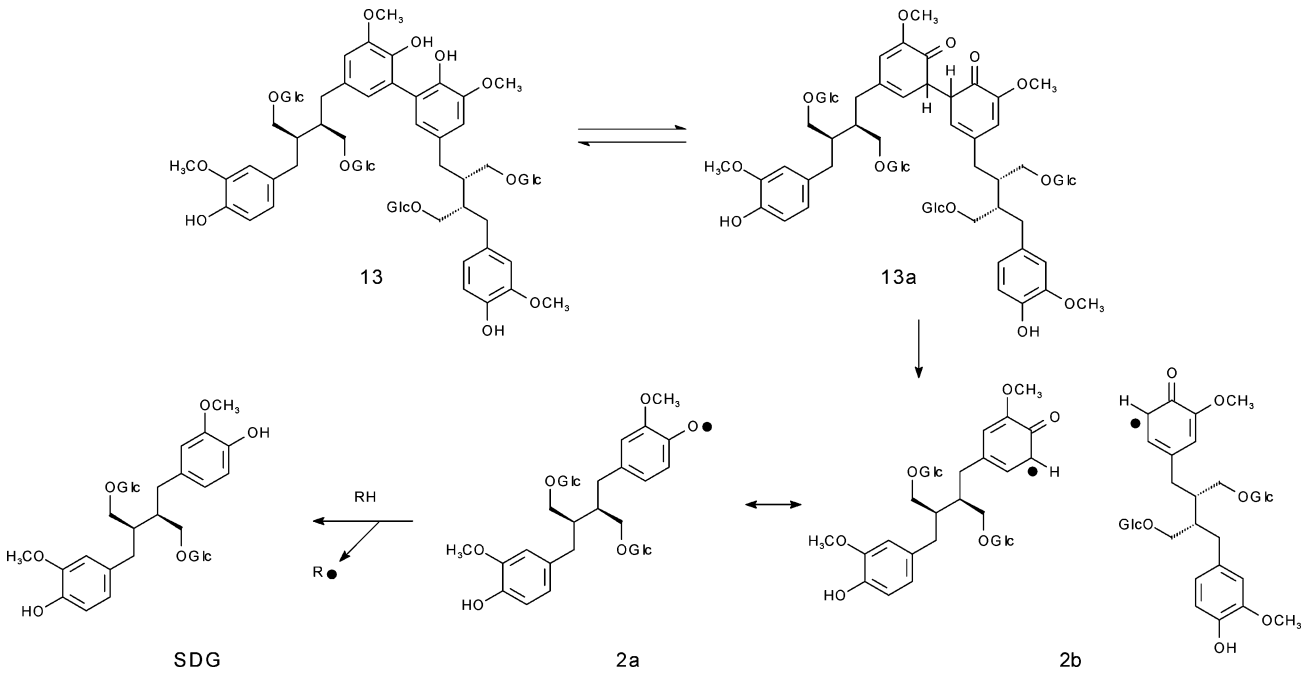

Fig. 8 Proposed pathway for the AAPH-mediated decomposition of SDG dimer **12**.

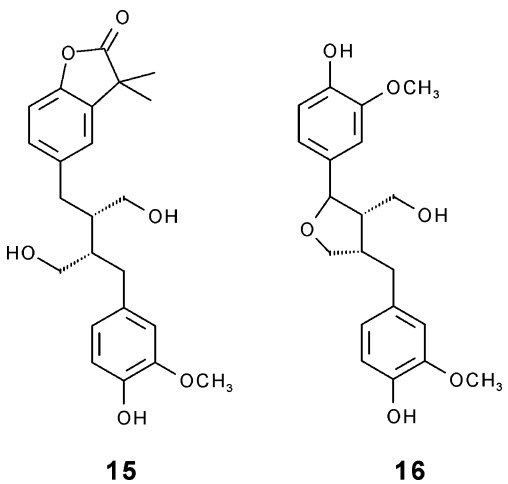

Fig. 9 Compounds **15** and **16** as identified by Eklund[38] from the AAPH-mediated oxidation of SECO in 1 : 1 THF–H_2O (70 °C, 72 h).

indicate that reaction with the stable DPPH radical is a better measure of the total number of oxidizable hydrogen atoms per molecule than the AAPH method.

Fujisawa *et al.* used AIBN to measure the antioxidant stoichiometries of C-5–C-5 dimers of 2-methoxyphenols such as eugenol (2.58) and 2-methoxy-4-methylphenol (2.56), finding them to be approximately twice that of the mono-phenols.[35] This suggests that dimers **7** and **13** from this study may also contribute to the antioxidant stoichiometry of SECO and SDG, respectively. Further study of the antioxidant properties of these metabolites will be necessary to determine whether the lignan dimers behave in a similar manner. It would also be valuable to determine whether dissociation of the carbon–carbon dimers is a general phenomenon under oxidative conditions, by studying **6** and model compounds such as the dimer of 2-methoxy-4-methylphenol.

Experimental

Chemicals and reagents

Secoisolariciresinol and SDG were isolated and purified from flaxseed as previously reported.[49] (*R*,*R*)-(+)-SECO ($[a]_D^{22}$ = +35 (*c* 0.20, methanol) and (*R*,*R*)-(+)-SDG ($[a]_D^{22}$ = +0.02 (*c* 0.20, methanol) were >99% pure by analytical high-performance liquid-chromatography (HPLC). Optical rotation was measured on a Jasco P-1010 polarimeter (glass cell, length: 100 mm) (Jasco Corp., Tokyo, Japan). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Monomer, Polymer and Dajac Inc. (Feasterville, PA). Hydrogen peroxide, ammonium thiocyanate, ferrous chloride, formic acid, trifluoroacetic acid and deuterated methanol (CD_3OD) were purchased from Sigma-Aldrich Canada (Oakville, ON). Methanol and acetonitrile were HPLC grade (EMD Chemicals, Norwood, OH). All other solvents were analytical grade. Water was purified using a Millipore Super-Q water system with one carbon cartridge followed by two ion exchange cartridges (Millipore Corp., Billerica, MA).

Liquid chromatography–mass spectrometry (LC-MS) analysis

LC-MS was performed with a Quattro-LC instrument (Micromass UK Limited, Manchester, UK) equipped with an electrospray (ES) source in both positive and negative modes using acetonitrile as mobile phase. LC analysis was performed applying the same gradient as analytical HPLC except 0.05% formic acid was used instead of 0.05% trifluoroacetic acid.

NMR analysis

NMR data was obtained on a Bruker AVANCE DPX-500 spectrometer operating at 500 MHz and 125 MHz for proton and carbon, respectively. CD_3OD was used as solvent. Residual

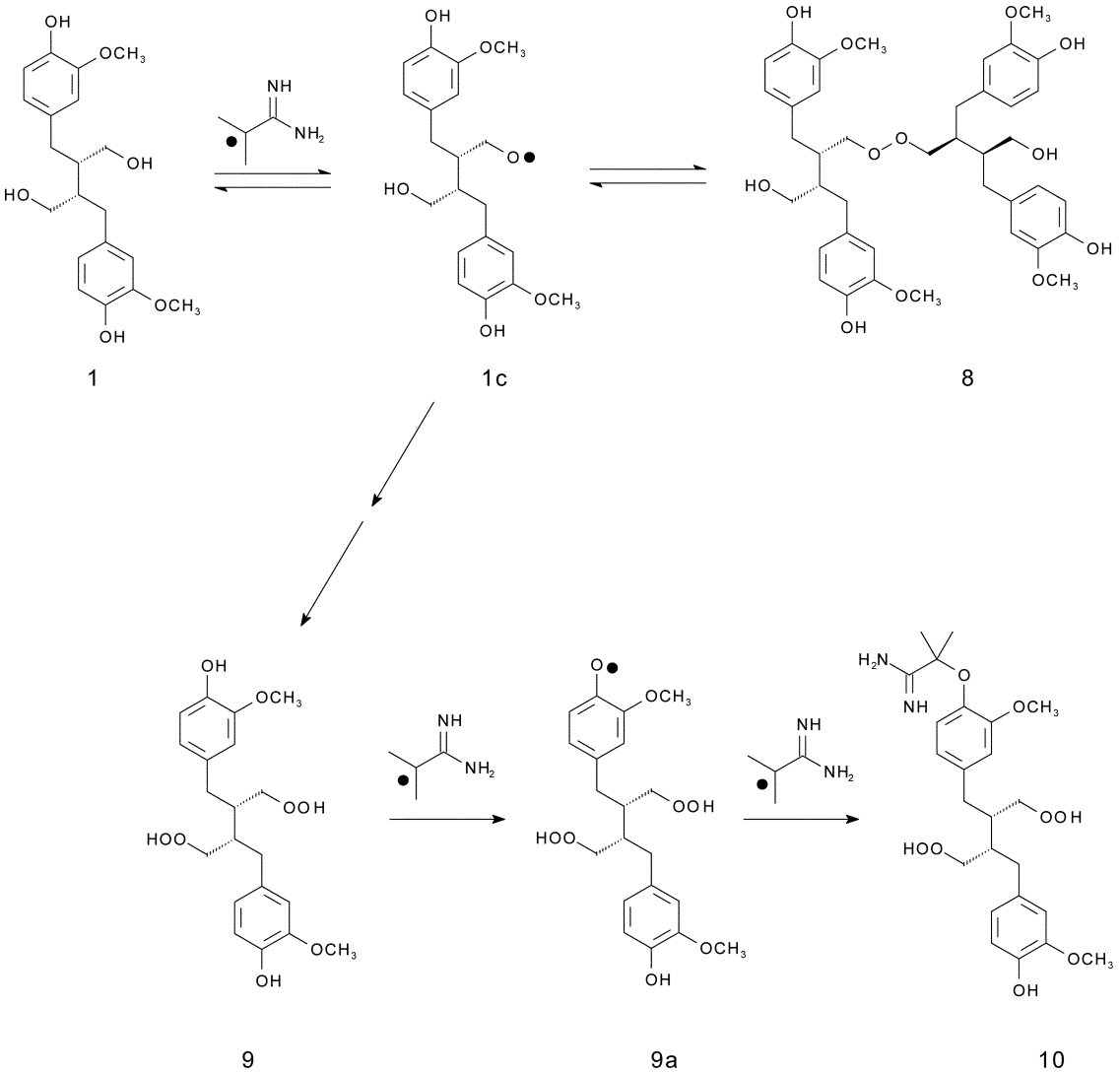

Fig. 10 Proposed pathway for the formation of AAPH-mediated aliphatic oxidation products of secoisolariciresinol.

signals from CD_3OD (3.30 ppm (^1H) and 49.0 ppm (^{13}C)) served as internal standards. Programs for 2-D experiments were available from the software package XWINNMR, provided by Bruker. COSY, HMQC and HMBC experiments were performed with gradient pulses. The Distortionless Enhancement by Polarization Transfer (DEPT) experiment together with 2-dimensional-NMR (2D-NMR) experiments including: Correlation Spectroscopy (COSY), Heteronuclear Multiple Quantum Correlation (HMQC), Heteronuclear Multiple Bond Correlation (HMBC) and Nuclear Overhauser Effect Spectroscopy (NOESY) experiments were performed with gradient pulses.

Oxidation of SECO and SDG with AAPH

Oxidation of SECO or SDG was performed based on modifications of a method in the literature.[40] Pilot studies were carried out to optimize the reaction conditions for AAPH-catalyzed oxidation of SECO and SDG. A time-course study up to 72 h was performed, and it was determined that >90% of SECO and SDG had been consumed after 5 h and 4 h respectively, and sufficient amounts of the major oxidation products were observed to allow for isolation and purification. Briefly, SECO or SDG (final concentration 0.01 M) was dissolved in CH_3OH–H_2O (50 : 50 , v/v) and incubated with AAPH (final concentration 0.1 M) also dissolved in CH_3OH–H_2O (50 : 50, v/v) at 60 °C, using 10 mL screw-capped test tubes fitted with rubber septa. The progress of the reaction was monitored using analytical HPLC (see below). Aliquots (20 μL) were removed through the septum *via* syringe at 5.0 min intervals for 1 h, and at 30.0 min intervals to the end of the reaction. Aliquots were analyzed directly by HPLC. Incubations were carried out until approximately 10% of the starting material remained and significant levels of oxidation products were observed (5 h for SECO, 4 h for SDG). AAPH in the absence of SECO or SDG was used as control. The AAPH-mediated oxidations of both SECO and SDG were scaled up (by a factor of 30) in order to collect sufficient quantities of each oxidation product for further purification and structural determination. The 10 : 1 ratio of AAPH–SECO or AAPH–SDG was maintained. The SECO–AAPH reaction was heated in the dark at 60 °C for 5 h, using a shaking water bath. The SDG–AAPH reaction was heated in the

dark at 60 °C for 4 h, using a shaking water bath. The oxidation products were isolated and purified using preparative and semi-preparative HPLC (see below). LC-MS and NMR were used for structural characterization.

Analytical HPLC

Reaction progress of both SECO and SDG oxidations was monitored using an Alliance HPLC (Waters Inc., Milford, MA) on a symmetry C18 reverse-phase column (3 × 150 mm, 5 μm, Waters Inc.). Mobile phase consisted of 0.05% trifluoroacetic acid in H_2O (solvent A) and 0.05% trifluoroacetic acid in acetonitrile (solvent B). Gradient elutions were used at a flow rate at 0.4 mL min^{-1}. SECO gradient: 90% A for 10 min, decreasing to 60% A over 20 min, then decreasing to 40% A over 10 min, returning to 90% A over 10 min and isocratic at 90% A for 5 min. SDG gradient: 90% A for 5 min, decreasing to 60% A over 15 min, returning to 90% A over 10 min and isocratic at 90% A for 5 min. Peaks were detected at 280 nm using a 996 UV-Vis photodiode array detector (Waters Inc.). Full spectral scans (200–400 nm) were also collected.

Preparative HPLC

Separations were performed on a C18 (Prep Nova-Pak®, Waters Inc.), 6 μm, 250 × 10 mm reverse-phase column with a flow rate of 20 mL min^{-1}; other parameters were similar to the analytical HPLC conditions with the exception of using methanol instead of acetonitrile. For the SECO oxidation, 23 fractions were collected; fractions 2–6 and 17–22 were enriched with the compounds of interest. These enriched fractions were subjected to further purification using semi-preparative HPLC. For the SDG oxidation, only semi-preparative HPLC was necessary for the isolation of oxidation products.

Semi-preparative HPLC

Separations were performed on a C18, 5 μm, 300 × 10 mm reverse phase column with a flow rate of 3.0 mL min^{-1}. Gradient elutions were used to isolate both SECO and SDG oxidation products; other parameters were similar to the analytical HPLC protocol. SECO gradient: 80% A for 10 min, decreasing to 60% A over 40 min, isocratic for 5 min, then decreasing to 20% A over 5 min, isocratic for 1 min and returning to 80% A over 4 min.

Peroxide value test

Peroxides were determined using an ammonium thiocyanate assay.[50] The assay was conducted by adding 2.5 mL of 75% ethanol, 0.05 mL of ammonium thiocyanate solution (30% w/v in H_2O), and 0.05 mL of ferrous chloride (0.1% w/v in H_2O) to 100 μL of sample in ethanol. In a 3 mL test tube, the mixed solution was incubated at room temperature (23 °C) in the dark for 3 min. The absorbance of the reaction mixture was measured at 500 nm against 75% ethanol as a blank. Peroxide concentrations were determined according to the equation obtained from the standard curve of H_2O_2. To obtain the standard curve, H_2O_2 at concentrations ranging from 5–40 μg mL^{-1} were added to the reaction mixture in a reference cell consisting of 2.5 mL of 75% ethanol, 0.05 mL of ammonium thiocyanate solution (30% w/v in H_2O), and 0.05 mL of ferrous chloride to a final volume of 3 mL. Peroxides were calculated from the ratio of concentration of each sample to reference. Results are an average of four individual readings (from four separate tubes) at 500 nm, using a UV-Vis spectrophotometer (Agilent 8453, Canada) with Agilent Chem Station software.

AAPH/lignan oxidation products

Secoisolariciresinol 4-*O*-(1-amidinopropyl) adduct (6). ESI-MS *m/z* 445 (80%) $[M - H]^-$, 447 (40%) $[M + H]^+$, λ_{max} (CH_3OH)/nm 202, 258 (ε/dm^3 mol^{-1} cm^{-1} 3694). δ_H (500 MHz, CD_3OD) 1.56 (6H, s, AP CH_3), 1.91 (1H, br s, H-8), 1.96 (1H, br s, H-8′), 2.63 (2H, m, H-7′), 2.68 (2H, m, H-7), 3.59 (2H, m, H-9), 3.61 (2H, dd, J = 4.5 Hz, 10.5 Hz, H-9′), 3.69 (3H, s, 3′-OCH_3), 3.71 (3H, s, 3-OCH_3), 6.51 (1H, br d, J = 8.0 Hz, H-6′), 6.64 (1H, d, J = 7.9 Hz, H-5′), 6.65 (1H, br s, H-2′), 6.67 (1H, br d, J = 8.2 Hz, H-6), 6.73 (1H, br s, H-2), 6.91 (1H, d, J = 8.0 Hz, H-5). δ_C (125 MHz, CD_3OD) 28.1 (AP CH_3), 36.0 (C-7′), 36.2 (C-7), 44.0 (C-8′), 44.5 (C-8), 56.2 (3-OCH_3), 56.3 (3′-OCH_3), 62.0 (C-9), 62.2 (C-9′), 80.1 (AP C=N), 113.5 (C-5′), 115.1 (C-2), 115.9 (C-2′), 122.6 (C-6′), 124.6 (C-6), 125.1 (C-5), 133.9 (C-1′), 140.8 (C-1), 140.8 (C-4), 145.4 (C-4′), 148.8 (C-3′), 154.1 (C-3), 176.6 (AP C-2).

Secoisolariciresinol C-5a dimer (7). ESI-MS *m/z* 721 (100%) $[M - H]^-$, 723 (30%) $[M + H]^+$, λ_{max} (CH_3OH)/nm 206, 280 (ε/dm^3 mol^{-1} cm^{-1} 6412 l). δ_H (500 MHz, CD_3OD) 1.95 (4H, br t, J = 6.1 Hz, H-8,8′, 8″, 8‴), 2.58 (4H, m, H-7′,7‴), 2.60 (4H, m, H-7,7″), 3.60 (8H, dd, J = 4.5, 10.5 Hz, H-9,9′,9″,9‴), 3.66 (6H, s, 3′-OCH_3, 3‴-OCH_3), 3.77 (6H, s, 3-OCH_3, 3″-OCH_3), 6.47 (2H, dd, J = 1.5, 8.0 Hz, H-6′,6‴), 6.54 (2H, d, J = 1.5 Hz, H-6,6″), 6.54 (2H, d, J = 1.5 Hz, H-2′,2‴), 6.59 (2H, d, J = 8.0 Hz, H-5′,5‴), 6.60 (2H, d, J = 1.5 Hz, H-2, 2″). δ_C (125 MHz, CD_3OD) 36.0 (C-7′,7‴), 36.2 (C-7,7″), 44.0 (C-8,8″), 44.1 (C-8′,8‴), 56.2 (3′-OCH_3, 3‴-OCH_3), 56.4 (3-OCH_3, 3″-OCH_3), 62.1 (C-9, 9′,9″,9‴), 113.3 (C-5′,5‴), 115.8 (C-2′,2‴), 115.9 (C-2,2″), 122.7 (C-6′,6‴), 123.2 (C-6,6″), 127.0 (C-5,5″), 133.4 (C-1′,1‴), 133.8 (C-1,1″), 145.4 (C-4′,4‴), 146.7 (C-4,4″), 148.8 (C-3′,3‴), 149.3 (C-3,3″).

Secoisolariciresinol peroxyl dimer (8). ESI-MS *m/z* 721 (100%) $[M - H]^-$, 723 (50%) $[M + H]^+$, λ_{max} (CH_3OH)/nm 206, 280, (ε/dm^3 mol^{-1} cm^{-1} 6413). δ_H (500 MHz, CD_3OD) 1.83 (2H, br t, J = 6.1 Hz, H-8′,8‴), 2.20 (2H, t, J = 6.1 Hz, H-8,8″), 2.62 (4H, m, H-7, 7′,7″,7‴), 3.61 (4H, dd, J = 4.7, 10.8 Hz, H-9′,9‴), 3.65 (4H, dd, J = 4.5, 10.5 Hz, H-9,9″) 3.69 (6H, s, 3′-OCH_3, 3‴-OCH_3), 3.77 (6H, s, 3-OCH_3, 3″-OCH_3), 6.47 (2H, dd, J = 1.5, 8.0 Hz, H-6′,6‴), 6.48 (2H, d, J = 1.5 Hz, H-6,6″), 6.54 (4H, d, J = 1.5 Hz, H-2, 2′,2″,2‴), 6.58 (4H, d, J = 8.0 Hz, H-5,5′,5″,5‴). δ_C (125 MHz, CD_3OD) 36.0 (C-7′,7‴), 39.9 (C-7,7″), 44.6 (C-8′,8‴), 47.6 (C-8,8″), 56.4 (3′-OCH_3, 3‴-OCH_3), 56.5 (3-OCH_3, 3″-OCH_3), 62.2 (C-9′,9‴), 74.4 (C-9,9″), 113.1 (C-5′,5‴), 113.2 (C-5,5″), 115.5 (C-2,2″), 115.8 (C-2′,2‴), 122.5 (C-6′,6‴), 122.6 (C-6,6″), 133.6 (C-1,1″), 133.9 (C-1′,1‴), 145.4 (C-4,4′,4″,4‴), 148.8 (C-3′,3‴), 149.3 (C-3,3″).

Secoisolariciresinol peroxide (9). ESI-MS *m/z* 393 (30%) $[M - H]^-$, 395 (10%) $[M + H]^+$, λ_{max} (CH_3OH)/nm 202, 280 (ε/dm^3 mol^{-1} cm^{-1} 3209). δ_H (500 MHz, CD_3OD) 2.20 (2H, m, H-8,8′), 2.61 (4H, m, H-7,7′), 3.65 (6H, OCH_3), 4.35 (2H, dd, J = 4.9, 11.1 Hz, H-9b,9′b), 4.42 (2H, dd, J = 5.0, 11.0 Hz, H-9a,9′a), 6.60 (2H, d, J = 8.0 Hz, H-5,5′), 6.64 (2H, d, J = 2.0 Hz, H-2,2′), 6.65 (2H, dd, J = 2.0, 8.0 Hz, H-6,6′). δ_C (125 MHz, CD_3OD)

39.0 (C-7a,7′a,7b,7′b), 47.7 (C-8,8′), 56.3 (OCH_3), 74.3 (C-9a,9′a,9b,9′b), 113.4 (C-5,5′), 115.6 (C-2,2′), 124.8 (C-6,6′), 133.6 (C-1,1′), 145.5 (C-4, 4′), 148.8 (C-3,3′).

Secoisolariciresinol peroxide, 4-*O*-(1-amidinopropyl) adduct (10). ESI-MS *m/z* 477 (100%) $[M - H]^-$, 479 (30%) $[M + H]^+$, λ_{max} (CH_3OH)/nm 202, 266 (ε/dm^3 mol^{-1} cm^{-1} 3795). δ_H (500 MHz, CD_3OD) 1.56 (6H, s, AP CH_3), 2.18 (2H, m, H-8,8′), 2.73 (2H, m, H-7′), 2.74 (2H, m, H-7), 3.70 (2H, m, H-9), 3.70 (3H, s, 3′-OCH_3), 3.71 (3H, s, 3-OCH_3), 3.75 (2H, m, 9′), 6.54 (1H, br d, $J = 8.0$ Hz, H-6′), 6.65 (1H, d, $J = 7.9$ Hz, H-5′), 6.66 (1H, br s, H-2′), 6.69 (1H, br d, $J = 8.2$ Hz, H-6), 6.71 (1H, br s, H-2), 6.92 (1H, d, $J = 8.0$ Hz, H-5). δ_C (125 MHz, CD_3OD) 28.1 (AP CH_3), 40.0 (C-7), 40.1 (C-7′), 47.6 (C-8), 47.7 (C-8′), 56.2 (3-OCH_3), 56.3 (3′-OCH_3), 74.3 (C-9, 9′), 80.1 (AP C=N), 113.4 (C-5′), 114.9 (C-2), 115.8 (C-2′), 124.5 (C-6), 124.7 (C-6′), 125.3 (C-5), 133.8 (C-1′), 140.8 (C-1), 141.5 (C-4), 145.5 (C-4′), 148.8 (C-3′), 154.2 (C-3), 176.6 (AP C-2).

SDG peroxide (11). ESI-MS *m/z* 717 (100%) $[M - H]^-$, 719 (30%) $[M + H]^+$, λ_{max} (CH_3OH)/nm 202, 269 (ε/dm^3 mol^{-1} cm^{-1} 3208). δ_H (500 MHz, CD_3OD) 2.11 (2H, t, $J = 5.6$ Hz, H-8,8′), 2.59 (2H, dd, $J = 8.3$, 13.7 Hz, H-7a,7a′), 2.66 (2H, dd, $J = 6.5$, 13.7 Hz, H-7b,7b′), 3.10–3.90 (glucose), 3.46 (2H, dd, $J = 5.4$, 9.8 Hz, H-9a,9a′), 3.71 (3H, s, OCH_3), 4.05 (2H, dd, $J = 5.4$, 9.8 Hz, 9b,9b′), 4.21 (2H, d, $J = 7.7$ Hz, glucose—anomeric H), 6.54 (2H, dd, $J = 2.0$, 8.0 Hz, H-6,6′), 6.57 (2H, d, $J = 2.0$ Hz, H-2,2′), 6.63 (2H, d, $J = 8.0$ Hz, H-5,5′). δ_C (125 MHz, CD_3OD) 35.3 (C-7a,7a′), 35.6 (C-7b,7b′), 41.2 (C-8,8′), 56.3 (OCH_3), 62.8–78.2 (glucose), 71.2 (9a,9a′,9b,9b′), 104.7 (glucose—anomeric carbon), 114.8 (C-2,2′), 117.1 (C-5,5′), 123.1 (C-6,6′), 133.9 (C-1,1′), 149.4 (C-4,4′), 149.7 (C-3,3′).

SDG 4-*O*-(1-amidinopropyl) adduct (12). ESI-MS *m/z* 771 (100%) $[M + H]^+$, λ_{max} (CH_3OH)/nm 206, 280 (ε/dm^3 mol^{-1} cm^{-1} 3765). δ_H (500 MHz, CD_3OD) 1.56 (6H, s, AP CH_3), 1.91 (1H, br s, H-8), 1.96 (1H, br s, H-8′), 2.63 (2H, m, H-7′), 2.68 (2H, m, H-7), 3.59 (2H, m, H-9), 3.61 (2H, dd, $J = 4.5$ Hz, 10.5 Hz, H-9′), 3.69 (3H, s, 3′-OCH_3), 3.73 (3H, s, 3-OCH_3), 6.51 (1H, br d, $J = 8.0$ Hz, H-6′), 6.64 (1H, d, $J = 7.9$ Hz, H-5′), 6.65 (1H, br s, H-2′), 6.67 (1H, br d, $J = 8.2$ Hz, H-6), 6.73 (1H, br s, H-2), 6.91 (1H, d, $J = 8.0$ Hz, H-5). δ_C (125 MHz, CD_3OD) 28.0 (AP CH_3), 35.5 (C-7′), 35.8 (C-7), 41.2 (C-8′), 41.4 (C-8), 56.0 (3-OCH_3), 56.1 (3′-OCH_3), 62.7–78.1 (9,9′-glucose carbons), 70.7 (C-9), 71.0 (C-9′), 80.1 (AP C=N), 104.4 (9′-*O*-glucose—anomeric carbon), 104.6 (9-*O*-glucose—anomeric carbon), 113.8 (C-2′), 114.1 (C-5′), 114.6 (C-2), 122.7 (C-6′), 124.8 (C-6), 124.9 (C-5), 133.9 (C-1′), 140.7 (C-1), 145.3 (C-4′), 146.1 (C-4), 148.6 (C-3′), 154.0 (C-3), 176.5 (AP C-2).

SDG C-5a dimer (13). ESI-MS *m/z* 1371 (100%) $[M - H]^-$, λ_{max} (CH_3OH)/nm 202, 281 (ε/dm^3 mol^{-1} cm^{-1} 6405). δ_H (500 MHz, CD_3OD) 2.1 (4H, br t, $J = 6.1$ Hz, H-8,8′,8″,8‴), 2.61 (2H, m, H-7′,7‴), 2.63 (2H, m, H-7,7″), 3.1–3.90 (glucose), 3.43 (4H, dd, $J = 4.7$, 10.8 Hz, H-9b,9b′,9b″,9b‴), 3.71 (6H, s, 3′-OCH_3, 3‴-OCH_3), 3.73 (6H, s, 3-OCH_3, 3″-OCH_3), 4.04 (4H, dd, $J = 4.5$, 10.5 Hz, H-9a,9a′,9a″,9a‴), 4.24 (1H, d, $J = 7.7$ Hz, 9-*O*-glucose—anomeric H), 4.28 (1H, d, $J = 7.7$ Hz, 9′-*O*-glucose—anomeric H), 6.57 (2H, dd, $J = 1.5$, 8.0 Hz, H-6′,6‴), 6.58 (2H, d, $J = 1.5$ Hz, H-2′,2‴), 6.61 (2H, d, $J = 1.5$ Hz, H-6,6″), 6.64 (2H, d, $J = 8.0$ Hz, H-5′,5‴), 6.65 (2H, d, $J = 1.5$ Hz, H-2,2″). δ_C (125 MHz, CD_3OD) 35.5 (C-7′,7‴), 35.6 (C-7,7″), 41.3 (C-8, 8′,8″,8‴), 56.2 (3′-OCH_3, 3‴-OCH_3), 56.5 (3-OCH_3, 3″-OCH_3), 62.8–78.2 (glucose), 71.2 (C-9,9′,9″,9‴), 104.7 (glucose—anomeric carbon), 113.6 (C-2′,2‴), 115.6 (C-2,2″), 115.8 (C-5′,5‴), 122.9 (C-6′,6‴), 125.4 (C-6,6″), 126.9 (C-5,5″), 134.0 (C-1,1′,1″,1‴), 145.4 (C-4′,4‴), 146.5 (C-4,4″), 148.8 (C-3′,3‴), 149.3 (C-3,3″).

SDG 4-OH-dienone adduct (14). ESI-MS *m/z* 701 (100%) $[M - H]^-$, λ_{max} (CH_3OH)/nm 202, 276 (ε/dm^3 mol^{-1} cm^{-1} 3209). δ_H (500 MHz, CD_3OD) 2.11 (1H, br s, H-8′), 2.25 (1H, br s, H-8), 2.61 (1H, m, H-7′), 2.67 (1H, m, H-7), 3.11–3.91 (glucose), 3.59 (2H, m, H-9), 3.61 (2H, dd, $J = 4.5$, 10.5 Hz, H-9′), 3.72 (3H, s, 3′-OCH_3), 3.74 (3H, s, 3-OCH_3), 4.04 (1H, m, 9-*O*-glucose—anomeric H), 4.23 (1H, m, 9′-*O*-glucose—anomeric H), 6.54 (1H, br d, $J = 8.0$ Hz, H-6′), 6.60 (1H, br d, $J = 8.2$ Hz, H-6), 6.61 (1H, d, $J = 2.0$ Hz, H-2′), 6.64 (1H, d, $J = 7.9$ Hz, H-5′) 6.65 (1H, d, $J = 2.0$ Hz, H-2), 6.91 (1H, d, $J = 10.0$ Hz, H-5). δ_C (125 MHz, CD_3OD) 35.6 (C-7′), 37.6 (C-7), 41.2 (C-8′), 42.2 (C-8), 56.7 (3′-OCH_3), 56.9 (3-OCH_3), 62.3–78.2 (glucose), 71.2 (C-9′), 71.6 (C-9), 73.4 (C-1), 104.8 (9, 9′-*O*-glucose—anomeric carbon), 113.5 (C-2′), 115.4 (C-5′), 122.9 (C-6′), 127.6 (C-5), 129.7 (C-6), 133.9 (C-1′), 137.6 (C-2), 146.3 (C-4′), 147.4 (C-3′), 150.5 (C-3), 171.0 (C-4).

Acknowledgements

We would like to thank K. Thompson for her assistance in the use of the mass spectrometer (Agriculture and Agri-Food Canada, Saskatoon Research Station) and the Plant Biotechnology Institute, Saskatoon, SK, for the use of their NMR facilities. Dr D. E. Ward and Dr A. Tsopmo provided helpful comments on the NMR spectral interpretations. This work was funded by the Saskatchewan Flax Development Commission, the Health Services Utilization and Research Commission, Agriculture and Agri-Food Canada, the University of Saskatchewan and the Natural Sciences and Engineering Research Council.

References

1 H. Adlercreutz, P. I. Musey, T. Fotsis, C. Bannwart, K. Wahala, T. Makela, G. Brunow and T. Hase, *Clin. Chim. Acta*, 1986, **158**, 147–154.
2 R. T. Talom, S. A. Judd, D. D. McIntosh and J. R. McNeill, *Life Sci.*, 1999, **64**, 1415–1425.
3 S. J. Bhathena, A. A. Ali, C. Haudenschild, P. Latham, T. Ranich, A. I. Mohamed, C. T. Hansen and M. T. Velasquez, *J. Am. Coll. Nutr.*, 2003, **22**, 157–164.
4 S. J. Bhathena, A. A. Ali, A. I. Mohamed, C. T. Hansen and M. T. Velasquez, *J. Nutr. Biochem.*, 2002, **13**, 684–689.
5 M. Athar, H. Raza, D. R. Bickers and H. Mukhtar, *J. Invest. Dermatol.*, 1990, **94**, 162–165.
6 D. J. A. Jenkins, C. W. C. Kendall, E. Vidgen, S. Agarwal, A. V. Rao, R. S. Rosenberg, E. P. Diamandis, R. Novokmet, C. C. Mehling, T. Perera, L. C. Griffin and S. C. Cunnane, *Am. J. Clin. Nutr.*, 1999, **69**, 395–402.
7 S. J. Hemmings and L. Barker, *Cell Biochem. Funct.*, 2004, **22**, 113–121.
8 S. J. Hemmings, N. Westcott, A. Muir and D. Czechowicz, *Cell Biochem. Funct.*, 2004, **22**, 225–231.
9 H. Abou-Gazar, E. Bedir, S. Takamatsu, D. Ferreira and I. A. Khan, *Phytochemistry*, 2004, **65**, 2499–2505.
10 T. A. Berkhout, H. M. Simon, D. D. Patel, C. Bentzen, E. Niesor, B. Jackson and K. E. Suckling, *J. Biol. Chem.*, 1996, **271**, 14376–14382.
11 B. A. Evans, K. Griffiths and M. S. Morton, *J. Endocrinol.*, 1995, **147**, 295–302.
12 S. E. Rickard, Y. V. Yuan, J. Chen and L. U. Thompson, *Nutr. Cancer*, 1999, **35**, 50–57.
13 C. Dabrosin, J. Chen, L. Wang and L. U. Thompson, *Cancer Lett.*, 2002, **185**, 31–37.

14 C. P. Cox and K. L. Wood, *Thromb. Res.*, 1987, **47**, 249–257.
15 J. E. Bakke and H. J. Klosterman, *Proc. N. D. Acad. Sci.*, 1956, **10**, 18–22.
16 J. D. Ford, K. S. Huang, H. B. Wang, L. B. Davin and N. G. Lewis, *J. Nat. Prod.*, 2001, **64**, 1388–1397.
17 A. Kamal-Eldin, N. Peerlkamp, P. Johnsson, R. Andersson, R. E. Andersson, L. N. Lundgren and P. Aman, *Phytochemistry*, 2001, **58**, 587–590.
18 D. Li, J. A. Yee, L. U. Thompson and L. Yan, *Cancer Lett.*, 1999, **142**, 91–96.
19 K. Prasad, *Atherosclerosis*, 2005, **179**, 269–275.
20 W. F. Clark, A. D. Muir, N. D. Westcott and A. Parbtani, *Lupus*, 2000, **9**, 429–436.
21 K. Prasad, *J. Lab. Clin. Med.*, 2001, **138**, 32–39.
22 M. Vanharanta, S. Voutilainen, T. A. Lakka, M. van der Lee, H. Adlercreutz and J. T. Salonen, *Lancet*, 1999, **354**, 2112–2115.
23 F. Bonnet and R. Gilbert, *Lancet*, 2000, **355**, 1642–1643.
24 X. Lin, B. R. Switzer and W. Demark-Wahnefried, *Anticancer Res.*, 2001, **21**, 3995–3999.
25 T. H. Makela, K. T. Wahala and T. A. Hase, *Steroids*, 2000, **65**, 437–441.
26 C. Wang, T. Makela, T. Hase, H. Adlercreutz and M. S. Kurzer, *J. Steroid Biochem. Mol. Biol.*, 1994, **50**, 205–212.
27 P. Pietinen, K. Stumpf, S. Mannisto, V. Kataja, M. Uusitupa and H. Adlercreutz, *Cancer Epidemiol. Biomarkers Prev.*, 2001, **10**, 339–344.
28 M. E. Martin, M. Haourigui, C. Pelissero, C. Benassayag and E. A. Nunez, *Life Sci.*, 1996, **58**, 429–436.
29 T. den, I. L. Keinan-Boker, P. V. Veer, C. J. Arts, H. Adlercreutz, J. H. Thijssen and P. H. Peeters, *Cancer Epidemiol. Biomarkers Prev.*, 2001, **10**, 223–228.
30 W. E. Ward, Y. V. Yuan, A. M. Cheung and L. U. Thompson, *J. Toxicol. Environ. Health, Part A*, 2001, **63**, 53–65.
31 K. Prasad, *Int. J. Angiol.*, 2000, **9**, 220–225.
32 H. B. Niemeyer and M. Metzler, *J. Food Eng.*, 2003, **56**, 255–256.
33 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, New York, 1999, pp. 1–936.
34 D. C. Liebler, *CRC Crit. Rev. Toxicol.*, 1993, **23**, 147–169.
35 T. Atsumi, S. Fujisawa, K. Satoh, H. Sakagami, I. Iwakura, T. Ueha, Y. Sugita and I. Yokoe, *Anticancer Res.*, 2000, **20**, 2519–2524.
36 W. BrandWilliams, M. E. Cuvelier and C. Berset, *Lebensm.-Wiss. Technol.*, 1995, **28**, 25–30.
37 F. S. Hosseinian, A. D. Muir, N. D. Westcott and E. S. Krol, *J. Am. Oil Chem. Soc.*, 2006, **83**, 835–840.
38 P. C. Eklund, O. K. Langvik, J. P. Warna, T. O. Salmi, S. M. Willfor and R. E. Sjoholm, *Org. Biomol. Chem.*, 2005, **3**, 3336–3347.
39 D. D. Kitts, Y. V. Yuan, A. N. Wijewickreme and L. U. Thompson, *Mol. Cell. Biochem.*, 1999, **202**, 91–100.
40 A. Arora, S. Valcic, S. Cornejo, M. G. Nair, B. Timmermann and D. C. Liebler, *Chem. Res. Toxicol.*, 2000, **13**, 638–645.
41 B. Halliwell, M. A. Murcia, S. Chirico and O. I. Aruoma, *Crit. Rev. Food Sci. Nutr.*, 1995, **35**, 7–20.
42 W. Chen, M. Ohmori, K. Ohmiya and S. Shimizu, *J. Ferment. Technol.*, 1988, **66**, 341–346.
43 R. F. Moore and W. A. Waters, *J. Chem. Soc.*, 1954, 243–246.
44 R. B. Corey, *Annu. Rev. Biochem.*, 1951, **20**, 131–148.
45 A. A. Zavitsas, *J. Phys. Chem. A*, 2003, **107**, 897–898.
46 T. G. Denisova and E. T. Denisov, *Kinet. Catal.*, 2006, **47**, 121–130.
47 N. V. Yanishlieva-Maslarova, 'Inhibiting Oxidation', in *Antioxidants in food. Practical applications*, ed. J. Pokorny, N. Yanishlieva and M. Gordon, Woodhead Publishing Limited, Cambridge, 2001, pp. 22–70.
48 S. V. Jovanovic, S. Steenken, Y. Hara and M. G. Simic, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2497–2504.
49 N. D. Westcott and A. D. Muir, *Process for Extracting Lignans From Flaxseed*, Agriculture and Agri-Food, Canada, *Can. Pat.*, 5705618, 1998.
50 A. N. Wijewickreme, Z. Krejpcio and D. D. Kitts, *J. Food Sci.*, 1999, **64**, 457–461.

