Antioxidant Activity of Flavonoids Isolated from Licorice

Michael H. Gordon* and Jing An†

Department of Food Science and Technology, The University of Reading, Whiteknights, P.O. Box 226, Reading, Berks. RG6 6AP, United Kingdom

The antioxidant activity of solvent extracts from licorice, fractions purified by column and thin layer chromatography, and eight purified flavonoids has been assessed in lard at 100 °C using an accelerated test method, the Rancimat. The chloroform extract was more active than extracts with other solvents, and some fractions were higher in activity than α-tocopherol. High antioxidant activity was present in flavonoid mixtures covering a wide polarity range after column chromatography. The purified flavonoids isolated were considerably less active than α-tocopherol at a concentration of 175 ppm, with the most active being a 3-arylcoumarin with a protection factor of 2.7 compared with 6.2 for α-tocopherol. It is clear that the antioxidant activity of licorice extracts contains contributions from several components, although it is possible that the flavonoids isolated were not those with greatest antioxidant activity. Synergistic effects of flavonoid mixtures may be responsible for the high activity observed in mixtures.

Keywords: *Antioxidant; flavonoids; licorice*

INTRODUCTION

Licorice is an economically important plant that has been used for centuries as a medicine because of its wide-ranging therapeutic properties including relief of rheumatic and other pain and healing effect on ulcers (Fenwick *et al.*, 1990). The roots are currently used in the tobacco, confectionery, and pharmaceutical industries. Besides glycyrrhizin, which is a biologically active triterpene glycoside present at 5–24%, licorice also contains flavonoids and isoflavonoids, chalcones, and coumarins, with 45 phenolic constituents belonging to these classes listed in the review covering the composition of licorice by Fenwick *et al.* (1990).

Flavonoids are of considerable interest as natural plant components with antioxidant activity. They occur widely in about half of the 400 plant species cultivated or gathered as human food. A number of studies have shown that some flavonoids possess antioxidant activity (Dziedzic and Hudson, 1983; Dziedzic *et al.*, 1985; Nieto *et al.*, 1993; Pratt and Hudson, 1990; Wanasundara and Shahidi, 1994). In addition to their antioxidant properties, flavonoids are also of interest because of their possible role in reducing the incidence of coronary heart disease and cancer (Hertog *et al.*, 1993).

The relationship between structure and antioxidant activity of flavonoids has been studied by several groups (Letan, 1966; Hudson and Lewis, 1983; Shahidi *et al.*, 1991; Das and Pereira, 1990). A hydroxyl group in the 3-position, unsaturation at carbon-2, and a carbonyl group at carbon-4 of the pyrone ring are associated with activity. Hydroxyl groups in the ortho 3′,4′-positions also contribute to the antioxidant activity. Isoflavones are commonly more active than flavones because of the stabilizing effects of the 4-carbonyl and 5-hydroxyl groups on isoflavone radicals (Dziedzic and Hudson, 1983a). 3,4-Dihydrochalcones such as butein are more active than analogous flavones due to the ability of chalcone-derived radicals to achieve greater electron delocalization (Dziedzic and Hudson, 1983b). Metal chelation is an important mechanism in the antioxidant action of *o*-dihydroxyflavonoids (Hudson and Lewis, 1983; Shahidi *et al.*, 1991).

Of the flavonoids present in licorice, while glycycoumarin has no significant activity (Demizu *et al.*, 1988), licochalcone A and licochalcone B were comparable to vitamin E in antioxidant activity, whereas glabrene was 3 times as active, and glabridin had no significant activity (Okuda *et al.*, 1989).

Extracts of licorice are known to possess antioxidant activity (Maruzen Chemical Co. Ltd., 1983, 1985; Takagaki, 1989). This investigation was concerned with a study of the characteristics of extracts from licorice with antioxidant activity and the identity and properties of flavonoids isolated from these extracts.

MATERIALS AND METHODS

α-Tocopherol was supplied by Sigma Chemical Co. Ltd., Poole, U.K.

Pig fat purchased in a local slaughterhouse was rendered in the laboratory.

Solvent Extraction of Licorice Root. Licorice root, imported from China, was purchased from Brome and Schinimr Ltd., Romsey, U.K. This was a commercial sample used for the preparation of herbal extracts. Extraction was performed on several batches of the licorice root on a scale of 0.5–5 kg.

The root was chopped into small pieces (*ca.* 5 cm long), powdered with a UDV cyclone sample mill, sieved, and freeze-dried before extraction. Dried licorice powder was extracted with hexane in a Soxhlet extractor (7 volumes). The powder was then extracted with methanol (7 volumes) to yield an extract corresponding to 29.7% by mass (m/m) of the original powder.

The methanol extract was then extracted twice with chloroform at room temperature using an ultrasonic bath. The yield of the chloroform extract was 4.5–5.1% (m/m).

Chromatography. The chloroform extract was separated by column chromatography on a chromatographic column packed with silica gel (Kieselgel 60, 70–230 mesh) of mass 300 g (20 cm × 4.5 cm i.d. column). The chloroform extract was applied to the top of the column and eluted with chloroform–methanol (100:7). An elution speed of 0.6 mL

* Author to whom correspondence should be addressed (fax 01734-310080; e-mail afsgmich@reading.ac.uk).

† Present address: NRC-PBI, 110 Gymnasium Place, Saskatoon, SK S7N 0W9, Canada.

0021-8561/95/1443-1784$09.00/0

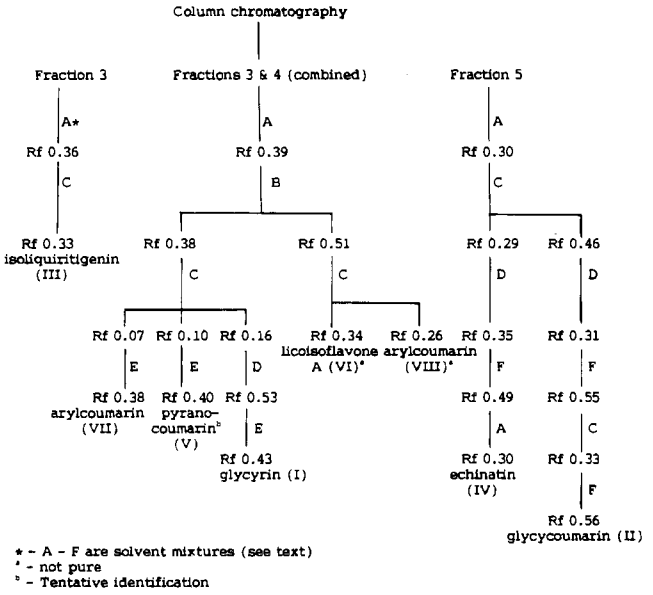


Figure 1. Isolation of flavonoids by TLC.

min^{-1} was found to give optimal separation. Fractions (18 mL) were collected with a fraction collector, Model 270, Chem Lab, United Kingdom. In a typical automated separation, 176 fractions were collected and analyzed by TLC, and fractions similar in composition were combined into six fractions (1–6).

Preparative thin layer chromatography (TLC) was performed with 20 × 20 cm plates coated in the laboratory with silica gel 60G with coatings of 0.5, 0.75, or 1 mm. Analytical TLC was performed with aluminum sheets (20 × 20 cm × 0.2 mm thick) or glass plates (20 × 20 cm × 0.25 mm thick) coated with silica gel 60 containing a fluorescent agent, supplied by Merck Ltd., Lutterworth, U.K. The solvents used for elution were as follows: system A, chloroform–methanol (100:9); system B, chloroform–ethyl acetate (3:1); system C, *n*-pentane–diethyl ether–acetic acid–methanol (75:25:6:2); system D, ammonia–acetone–chloroform (1:20:20); system E, chloroform–methanol (100:7); system F, chloroform–ethyl acetate–acetic acid (65:40:2.5).

The TLC procedures for the isolation of seven pure flavonoids and one impure flavonoid are shown in Figure 1. Purity was checked by TLC with three solvent mixtures and confirmed by spectroscopy.

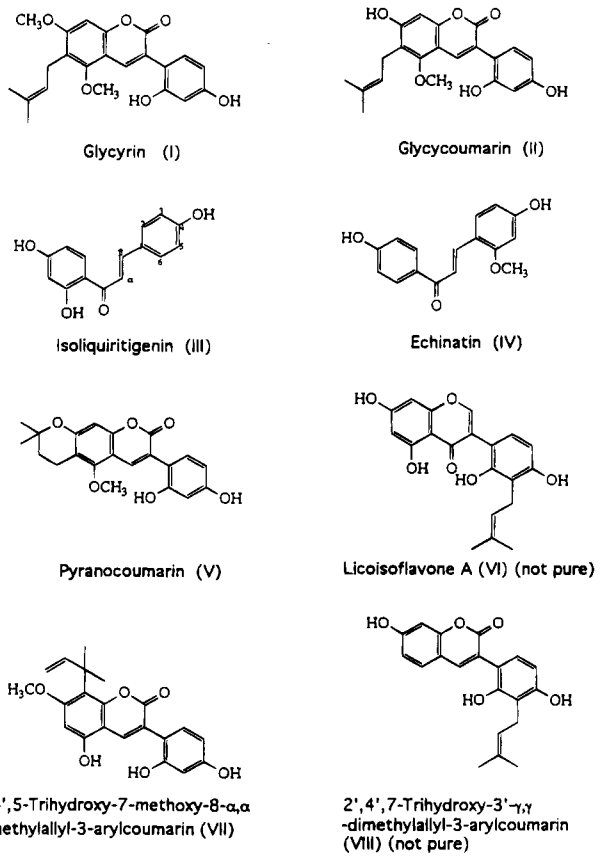
Spectral Data for Isolated Compounds (See Table 1 for UV Data and Chart 1 for Structures). Mass spectra were obtained by electron impact ionization at 70 eV and PMR spectra in $CDCl_3$ at 400 MHz.

I: mass spectrum found 382 (100), 367 (22), 351 (10), 314 (9), 327 (5), 269 (5); PMR spectrum δ 1.68 (s, 3), 1.78 (s, 3), 3.38 (d, 2, J = 7 Hz), 5.15 (t, 1, J = 7 Hz), 3.84 (s, 3), 3.91 (s, 3), 6.52 (m, 2), 6.72 (s, 1), 7.16 (d, 1, J = 8 Hz), 8.02 (s, 1).

II: mass spectrum found 368 (100), 313 (80), 350 (31), 308 (12), 270 (12), 285 (9); PMR spectrum δ 1.76 (s, 3), 1.85 (s, 3), 3.46 (d, 2, J = 7 Hz), 5.23 (t, 1, J = 7 Hz), 3.86 (s, 3), 6.56 (m, 2), 6.72 (s, 1), 7.18 (d, 1, J = 8 Hz), 8.01 (s, 1).

III: mass spectrum found 137 (100), 256 (M^+, 82), 120 (66), 163 (28), 91 (15), 239 (11).

Chart 1



IV: mass spectrum found 239 (100), 121 (58), 134 (15), 177 (13), 270 (M^+, 11).

V: mass spectrum found 368 (100), 313 (78), 270 (25), 354 (14), 297 (14), 339 (8); PMR spectrum δ 1.38 (s, 6), 1.85 (t, 2, J = 7 Hz), 2.82 (t, 2, J = 7 Hz), 3.88 (s, 3), 6.53 (m, 2), 6.65 (s, 1), 7.16 (d, 1, J = 8 Hz), 8.00 (s, 1).

VI: mass spectrum found 41 (100), 354 (65), 235 (57), 110 (46), 149 (39), 262 (24), 205 (24), 165 (23), 340 (22), 372 (M^+, 14); PMR spectrum δ 1.63 (s, 3), 1.73 (s, 3), 3.25 (d, 2, J = 7 Hz), 5.14 (t, 1, J = 7 Hz), 6.0 (d, 1, J = 2 Hz), 6.35 (broad d, 2), 6.81 (d, 1, J = 8 Hz), 7.82 (s, 1).

VII: mass spectrum found 368 (100), 353 (74), 176 (14), 123 (11), 140 (10), 191 (6); PMR spectrum δ 1.71 (s, 6), 3.69 (s, 3), 5.44 (d, 1, J = 10 Hz), 5.53 (d, 1, J = 18 Hz), 6.30 (s, 1), 6.43–6.50 (m, 2), 6.46 (dd, 1, J = 10, 11 Hz), 7.17 (d, 1, J = 8 Hz), 8.19 (s, 1).

VIII: mass spectrum found 38 (100), 153 (57), 253 (34), 283 (34), 338 (M^+, 30), 187 (19), 295 (14), 354 (9, impurity?); PMR spectrum δ 1.67 (s, 3), 1.82 (s, 3), 3.37 (d, 2, J = 7 Hz), 5.31 (m, 1), 6.83 (s, 1), 6.9 (d, 1, J = 8 Hz), 7.03 (dd, 1, J = 2, 8 Hz), 7.22 (d, 1, J = 8 Hz).

HPLC Analysis. HPLC analysis was performed with a Spherisorb ODS1 column (5 μm, 25 cm × 4.6 mm i.d.) using a Philips Model PU 4100 pump and a Philips PU 4120 diode array detector. The solvent gradient was 55% solvent 1–45% solvent 2 changing to 25% solvent 1–75% solvent 2 in 30 min.

Table 1. UV Spectral Data for Isolated Compounds

	λ_{max} (nm)	λ_{max} with shift reagents				
		$NaOCH_3$	$AlCl_3$	$AlCl_3$ + HCl	NaOAc	NaOAc + H_3BO_3
I	351.5	384.6, 332, 293.4	356.9	356.9	352.3	352.3
II	354	407, 269	354	356	399	356
III	368, 240	429, 276, 246	425, 241	420, 239	405, 310	376
IV	369, 308	429, 343	368	358	409	372
V	348	384, 328, 294	351	351	344	344
VI	265	325, 276	362, 308, 273	362, 308, 273	328, 273	328, 273
VII	356, 267	423, 287	357, 267	357, 267	353, 267	353, 267
VIII	345, 260	387, 276	355	355	364, 270	345, 260

Solvent 1 was 97% water–3% acetic acid, and solvent 2 was 97% acetonitrile–3% acetic acid. The flow rate was 1 mL/min.

Oil Stability. Induction periods of antioxidants added to lard (2.5 g) were determined with a Rancimat, Model 617 (Metrohm AG, Herisan, Switzerland), using air bubbled through the sample at 100 °C and monitoring of the electrical conductivity of an aqueous solution of the effluent as described previously (Gordon and Weng, 1992).

RESULTS AND DISCUSSION

Identification of Isolated Compounds. Identification was based on the UV, PMR, and mass spectra of the compounds. In addition, the effect of shift reagents on the UV spectra (Table 1) was used to confirm the positions of substituents as described by Markham (1982).

Compounds **I–IV** were identified as glycyrin, glycycoumarin, isoliquiritigenin, and echinatin by comparison of the spectral data with those reported in the literature by Kinoshita *et al.* (1978a), Demizu *et al.* (1988), Bate-Smith and Swain (1953), and Furuya *et al.* (1971), respectively. The spectra of samples **III** and **IV** were also identical with those of authentic samples. The spectra of compound **V** allowed it to be tentatively identified as pyranocoumarin by comparison with the data of Hatano *et al.* (1989) [although note the resonance of the aromatic proton in the A-ring (H-8) occurs at δ 6.65 in **V** compared with δ 6.50 in the literature data, which may be due to the different solvent used]. The spectral data for **VI** were consistent with the sample containing licoisoflavone A by comparison with the data of Kinoshita *et al.* (1978b); however, there were several extra resonances in the PMR spectrum, and it appears that the compound was impure. The spectral data for **VII** showed similarities with those reported for licoarylcoumarin by Hatano *et al.* (1989). However, differences in the PMR data, especially the resonances of the protons on the unsaturated carbons of the prenyl group which were at δ 5.44, 5.53, and 6.46, compared with the values of δ 4.92, 5.01, and 6.38 reported for licoarylcoumarin, indicate that **VII** was an isomer of licoarylcoumarin with the methoxy and hydroxyl groups at C-7 and C-5, respectively, whereas these substituents are at C-5 and C-7, respectively, in licoarylcoumarin. Compound **VII** is therefore a previously unreported arylcoumarin with the systematic name of 2′,4′,5-trihydroxy-7-methoxy-8-(α,α-dimethylallyl)-3-arylcoumarin. Sample **VIII** was tentatively identified as 2′,4′,7-trihydroxy-3′-(γ,γ-dimethylallyl)-3-arylcoumarin. Similarities in the UV spectra of **VII** and **VIII** suggest both compounds are 3-arylcoumarins, and a molecular ion at 338 in the mass spectrum of **VIII** is consistent with the presence of a prenyl and three hydroxyl groups. A peak in the mass spectrum at 354 and additional resonances in the PMR spectrum indicate that this compound was not pure. Resonances at δ 6.83 (1H, s), 6.9 (1H, d, J = 8 Hz), 7.03 (1H, dd, J = 2, 8 Hz), 7.22 (1H, d, J = 8 Hz), 7.45 (1H, d, J = 8 Hz), and 7.8 (1H, d) were assigned to protons H-4, H-5, H-6, H-8, H-5′, H-6′, respectively, in structure **VIII**. However, additional resonances were present, and the identification of the structure of **VIII** is tentative.

Antioxidant Activity of Extracts and Purified Compounds. Powdered licorice root was extracted with hexane, followed by methanol, and the methanol extract was re-extracted with chloroform. It was found that the chloroform extract was more effective as an antioxidant than the fractions extracted with hexane or methanol (Table 2).

Table 2. Induction Periods of Lard Containing Licorice Extracts Assessed by the Rancimat at 100 °C

	induction period (h) for additive concentration of			
	0.05%	0.1%	0.2%	control
hexane extract	2.2	5.2	8.8	1.7
methanol extract		3.5	3.7	1.3
chloroform extract	11.2	23.6		1.4
BHA	19.4	28.0		0.9
BHT	2.1	6.8		0.9

Table 3. Antioxidant Activity of Chloroform Extract Separated into Fractions by Column Chromatography (Assessed in Lard at 100 °C by the Rancimat)

		induction period (h)		
	analytical TLC R_f value	lard + additive (0.1%)	lard control	protection factor (PF)[a]
fraction 1	0.61–0.92	28.1 ± 5.3[b]	4.8	4.85
fraction 2	0.44–0.88	36.4 ± 1[c]	4.8	6.6
fraction 3	0.44–0.64	46.5 ± 0.8[c]	5.4	7.6
fraction 4	0.32–0.48	44.2 ± 0.5[b]	5.7	6.75
fraction 5	0.2–0.39	48.9 ± 0.2[b]	5.5	7.9
fraction 6	0.1–0.34	44.5 ± 2.7[c]	5.7	6.8

[a] PF = [IP (lard + additive) − IP (lard)]/IP (lard). [b] Range of three determinations. [c] Range of two determinations.

Table 4. Antioxidant Activity of TLC Bands Isolated by Chloroform Extraction and TLC (Chloroform:Methanol = 100:9)

			induction period (h)		
band	R_f	color under UV at 365 nm	lard + additive (0.05%)	lard	PF
1	0.49	blue	4.4	2.4	0.8
2	0.41	green	21.4	2.7	6.9
3	0.39	blue	22.8	2.5	8.1
4	0.17	green	11.9	2.4	4.0

The chloroform extract was only slightly less active than BHA at 0.1% concentration but was significantly less active than BHA at 0.05% concentration (Table 2).

Six combined fractions isolated by silica gel chromatography on the chloroform extract, covering a range of polarities, were used as additives to lard at 0.1% concentration and tested with the Rancimat at 100 °C. The induction periods (IP) and the protection factors (PF) for each additive are given in Table 3. Comparison of the PF value is preferable to that of the IP, when samples from different runs on the Rancimat are being compared due to small variations in conditions between runs.

It was found that despite the wide range of polarity of the fractions separated by column chromatography, all of the fractions had good antioxidant activity when tested in lard at 0.1% concentration. Fractions 3 and 5 were most active with PF values of 7.6 and 7.9 compared with 0.02% tocopherol, which showed a PF value of 6.15.

The finding that fractions varying widely in polarity possessed good antioxidant activity suggested that there was no single antioxidant present among the licorice flavonoids, but instead there were several flavonoids with good antioxidant activity.

Further separation of column chromatography fractions 3 and 4 (combined) by preparative TLC with chloroform–methanol (100:9) gave four bands with R_f values 0.49, 0.41, 0.39, and 0.17 (Table 4). Bands 2 and 3 were highly effective antioxidants with PF values of 6.9 and 8.1, which were both higher than the value for α-tocopherol. Additional separation of bands 2 and 3 by further preparative TLC with chloroform–ethyl acetate (3:1) gave band 3-3, which had a strong anti-

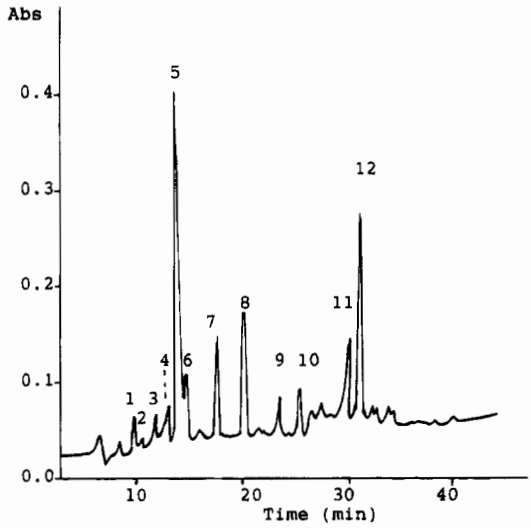


Figure 2. HPLC chromatogram of TLC band 3-3.

Table 5. Antioxidant Activity of Purified Bands from Further TLC (Isolation as in Table 3 + Additional TLC Separation)

band	R_f	color under UV at 365 nm	induction period (h): lard + additives (0.05%)	induction period (h): lard	PF
3-1	0.59	dark	12.1	2.5	3.9
3-2	0.51	blue	13.1	2.0	5.55
3-3	0.45	dark	26.1	2.4	9.9
3-4	0.38	green	10.5	2.0	4.25
2-1	0.42	green	9.5	2.3	3.2

Table 6. Antioxidant Activity of Purified Compounds Isolated from Licorice

compound	induction period (h): lard + additive (0.0175%)	induction period (h): lard control	PF
glycyrin (**I**)	4.6	2.4	0.9
glycycoumarin (**II**)	3.5	2.8	0.3
isoliquiritigenin (**III**)	3.0	3.0	0
echinatin (**IV**)	3.5	2.8	0.4
pyranocoumarin (**V**)	6.9	2.4	1.9
liscoisoflavone A (**VI**)	6.9	2.4	1.9
new arylcoumarin (**VII**)	4.0	2.4	0.6
3-arylcoumarin (**VIII**) (not pure)	8.9	2.4	2.7
α-tocopherol	21.1	3.0	6.2
column chrom fraction 2	8.4	2.8	2.1

oxidant effect with a PF of 9.9 (Table 5). Insufficient material was present in this band to allow identification of the active constituent. Isoliquiritigenin (**III**) was isolated from this band, but when tested as a pure compound, it was found to have weak antioxidant activity (Table 6). HPLC analysis of band 3-3 (Figure 2) indicated that more than 12 compounds were present in this sample. Diode array spectra (Table 7) indicated the presence of several flavonoid classes in this mixture, but identification of the components was not possible. The UV spectrum of peak 1 had the characteristics of a 3-arylcoumarin, whereas peaks 2–4, 6, 7, and 10 could be either flavanones, dihydroflavonols, or dihydrochalcones. Peak 8 was characteristic of a pterocarpene, while peak 9 may correspond to an isoflavonoid. Peak 12, a major component, was a mixture including isoliquiritigenin and a flavone, flavonol, or chalcone, and peaks 5, the main component, and 11 were unidentified mixtures (see Figure 3 for UV spectra of peaks 5 and 12).

Band 3-4 yielded **I**, **V**, and **VII** on further TLC separation, and band 3-2 yielded **VI** and **VIII** on further

Table 7. HPLC Photodiode Array UV Absorption Data for Band 3-3

HPLC peak	retention time (min)	λ_{max} (A)[a]
1	10.0	240 (0.11), 264 (0.02), 289 (0.01), 335 (0.02)
2	10.5	242 (0.36), 288 (0.28)
3	11.1	242 (0.62), 283 (0.21)
4	12.1	242 (0.48), 288 (0.13)
5	13.2	242 (0.96), 251 (0.88 sh), 297 (0.93), 327 (1.0)
6	13.7	242 (0.48), 291 (0.32), 324 (0.11), 335 sh (0.10)
7	16.8	240 (0.41), 270 (0.28), 347 (0.10)
8	20.3	242 (0.52), 327 (0.83), 338 (0.69)
9	24.2	240 (0.39), 270 (0.12), 292 sh (0.06)
10	26.8	242 (0.48), 291 (0.13)
11	31.6	240 (0.57), 272 sh (0.11), 319 (0.09), 341 (0.10), 359 (0.10)
12	33.4	242 (0.77), 261 sh (0.45), 316 (0.34), 351 (0.84), 366 (0.69)

[a] A is the absorbance relative to the most intense peak of component 5. sh = shoulder.

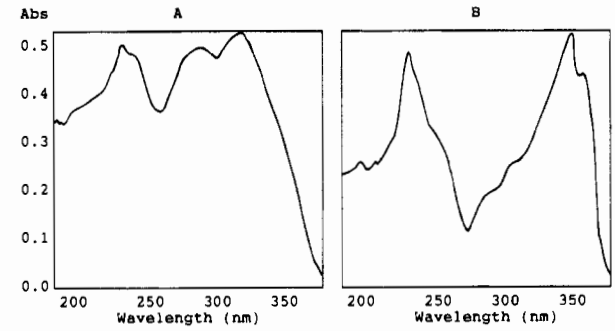


Figure 3. UV spectra of (A) peak 5 and (B) peak 12 of HPLC chromatogram of TLC band 3-3.

TLC separation. Compounds **II** and **IV** were isolated by TLC from column chromatography fraction 5. All of the isolated compounds **I**–**VIII** had moderate antioxidant activity when assessed by the Rancimat at a concentration of 0.0175% in lard at 100 °C. The most active purified compounds **V** and **VIII** were comparable in activity to column chromatography fraction 2. Insufficient material remained for investigation of the effects of higher concentrations of these flavonoids, but literature reports indicate that the antioxidant activity of flavonoids increases with concentration at least up to 0.1% (Dziedzic and Hudson, 1983a,b). This increase in activity with concentration was observed for column chromatography fraction 2, which increased in antioxidant protection factor from 2.1 to 5.9 with an increase in concentration from 0.0175% to 0.05%. The failure to isolate any components with strong antioxidant activity from the active extracts indicates that either the most active components were not isolated or the activity of licorice extracts is due to the contribution from a number of components. Since the antioxidant activities of the column chromatography fractions 2–6 were comparable (Table 3), despite these fractions containing components with widely differing R_f values, it appears clear that several components contribute to the activity of licorice extracts.

An examination of the effect of concentration on antioxidant activity of flavonoids isolated from licorice may well be justified. Product **VIII** had the highest antioxidant activity of the purified products that were assessed. Although this sample contained a 3-arylcoumarin which was tentatively identified as 2,4,7-trihydroxy-3′-(γ,γ-dimethylallyl)-3-arylcoumarin, the product clearly contained significant amounts of impurities, and it is possible that the antioxidant activity is due to an unidentified component of the mixture. Strong anti-

oxidant activity has been observed in flavonols such as quercetin, flavones such as luteolin, and chalcones such as butein (Hudson and Lewis, 1983). 3-Arylcoumarins such as **I**, **II**, **V**, **VII**, and **VIII** lack both a fixed hydroxyl group in a position to form a strong hydrogen bond to the carbonyl group and an *o*-dihydroxy structure that may contribute to a metal chelating activity. Genistein, which is an isoflavone, has been observed to have weak antioxidant activity in lard with PF = 0.48 at 0.02% concentration (Dziedzic and Hudson, 1983a), but sample **VI**, which contains mainly licoisoflavone A, has a higher antioxidant activity.

ACKNOWLEDGMENT

We acknowledge the assistance of Dr. J. Ingham, who supplied authentic samples of isoliquiritigenin and echinatin and assisted in spectral interpretation.

LITERATURE CITED

Bate-Smith, E. C.; Swain, T. The isolation of 2,4,4′-trihydroxychalcone from yellow varieties of *Dahlia variabilis*. *J. Chem. Soc.* **1953**, 2185–2187.

Das, N. P.; Pereira, T. A. Effects of flavonoids on thermal autoxidation of palm oil: structure-activity relationships. *J. Am. Oil Chem. Soc.* **1990**, *67*, 255–258.

Demizu, S.; Kajiyama, K.; Takahashi, K.; Hiraga, Y.; Yamamoto, S.; Tamura, Y.; Okada, K.; Kinoshita, T. Antioxidant and antimicrobial constituents of licorice. *Chem. Pharm. Bull.* **1988**, *36*, 3474–3479.

Dziedzic, S. Z.; Hudson, B. J. F. Hydroxy isoflavones as antioxidants for edible oils. *Food Chem.* **1983a**, *11*, 161–166.

Dziedzic, S. Z.; Hudson, B. J. F. Polyhydroxychalcones and flavanones as antioxidants for edible oils. *Food Chem.* **1983b**, *12*, 205–212.

Dziedzic, S. Z.; Hudson, B. J. F.; Barnes, G. Polyhydroxydihydrochalcones as antioxidants for lard. *J. Agric. Food Chem.* **1985**, *33*, 244–246.

Fenwick, G. R.; Lutomski, L.; Nieman, C. Liquorice, Glycyrrhiza glabra L. composition, uses and analysis. *Food Chem.* **1990**, *38*, 119–143.

Furuya, T.; Matmumoto, K.; Hikichi, M. Echinatin, a new chalcone from tissue culture of *Glycyrrhiza echinata*. *Tetrahedron Lett.* **1971**, 2567–2569.

Gordon, M. H.; Weng, C-C. Antioxidant properties of extracts from tanshen (Salvia miltiorrhiza Bunge). *Food Chem.* **1992**, *44*, 119–122.

Hatano, T.; Yasuhara, T.; Fukuda, T.; Noro, T.; Okuda, T. Phenolic constituents of licorice II. *Chem. Pharm. Bull.* **1989**, *37*, 3005–3009.

Hertog, M. G. L.; Feskens, E. J. M.; Hollman, P. C. H.; Katan, M. B.; Kromhout, D. Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen elderly study. *Lancet* **1993**, *342*, 1007–1011.

Hudson, B. J. F.; Lewis, J. I. Polyhydroxy flavonoid antioxidants for edible oils. *Food Chem.* **1983**, *10*, 47–55.

Kinoshita, T.; Saitoh, S.; Shibata, S. A new 3-arylcoumarin from licorice root. *Chem. Pharm. Bull.* **1978a**, *26*, 135–140.

Kinoshita, T.; Saitoh, S.; Shibata, S. A new isoflavone from licorice root. *Chem. Pharm. Bull.* **1978b**, *26*, 141–143.

Letan, A. Relation of structure to antioxidant activity of quercetin and some of its derivatives. 1. Primary activity. *J. Food Sci.* **1966**, *31*, 518–522.

Markham, K. R. *Techniques of Flavonoid Identification*; Academic Press: London, 1982.

Maruzen Chemical Co. Ltd. Food antioxidants from liquorice. *Chem. Abstr.* **1984**, *100*, 119655r.

Maruzen Chemical Co. Ltd. Food antioxidants from liquorice extracts and gallic acid. *Chem. Abstr.* **1985**, *103*, 86795t.

Nieto, S.; Garrido, A.; Sanhueza, J.; Loyola, L. A.; Morales, G.; Leighton, F.; Valenzuela, A. Flavonoids as stabilizers of fish oil: an alternative to synthetic antioxidants. *J. Am. Oil Chem. Soc.* **1993**, *70*, 773–778.

Okuda, K.; Tamura, Y.; Yamamoto, M.; Inoue, Y.; Takagaki, R.; Takahashi, K.; Demizu, S.; Kajiyama, K.; Hiraga, Y.; Kinoshita, T. Identification of antimicrobial and antioxidant constituents from licorice of Russian and Xinjing origin. *Chem. Pharm. Bull.* **1989**, *37*, 2528–2530.

Pratt, D. E.; Hudson, B. J. F. Natural antioxidants not exploited commercially. In *Food Antioxidants*; Hudson, B. J. F., Ed.; Elsevier Applied Science: London, 1990; pp 171–192.

Shahidi, F.; Wanasundara, P.; Hong, C. Antioxidant activity of phenolic compounds in meat model systems. In *Phenolic Compounds in Food and Their Effects on Health I*; ACS Symposium Series 506; American Chemical Society: Washington, DC, 1991; pp 214–222.

Takagaki, P. Antioxidant from liquorice roots and its use as a stabilizer for hardened oil. *Chem. Abstr.* **1989**, *110*, 153082t.

Wanasundara, U. N.; Shahidi, F. Stabilization of canola oil with flavonoids. *Food Chem.* **1994**, *50*, 393–396.

Received for review November 2, 1994. Revised manuscript received April 21, 1995. Accepted April 28, 1995.[⊗] We acknowledge the British Council for a grant.

JF940621+

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1995.