Potential Cancer Chemopreventive Flavonoids from the Stems of *Tephrosia toxicaria*

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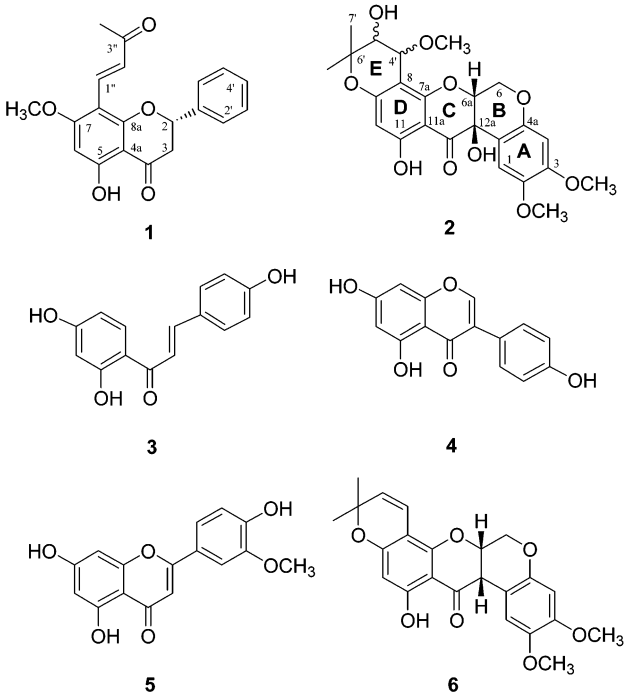
Received May 9, 2003

A new butenylflavanone, (2*S*)-5-hydroxy-7-methoxy-8-[(*E*)-3-oxo-1-butenyl]flavanone (**1**), and a new rotenoid, 4′,5′-dihydro-11,5′-dihydroxy-4′-methoxytephrosin (**2**), as well as three active flavonoids of previously known structure, isoliquiritigenin (**3**), genistein (**4**), and chrysoeriol (**5**), along with nine known inactive compounds, α-toxicarol (**6**), sumatrol, 6a,12a-dehydro-α-toxicarol, 11-hydroxytephrosin, obovatin, marmesin, lupenone, benzyl benzoate, and benzyl *trans*-cinnamate, were isolated from an ethyl acetate-soluble extract of the stems of *Tephrosia toxicaria*, using a bioassay based on the induction of quinone reductase (QR) in cultured Hepa 1c1c7 mouse hepatoma cells to monitor chromatographic fractionation. The structures of compounds **1** and **2** were elucidated by spectroscopic data interpretation. All isolates were evaluated for their potential cancer chemopreventive properties utilizing an in vitro assay to determine quinone reductase induction. Selected compounds were tested in a mouse mammary organ culture assay to evaluate the inhibition of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced preneoplastic lesions.

Tephrosia (Leguminosae) comprises about 400 species, and representatives of this large genus are distributed throughout warm regions of both hemispheres.[1] *Tephrosia toxicaria* (Sw.) Pers. is a tropical fish-poisoning plant growing in Sri Lanka and South America and is well-known as a source of rotenoids including deguelin, sumatrol, and toxicarol.[1−4] As part of our ongoing project directed toward the discovery of novel naturally occurring cancer chemopreventive agents from plants,[5,6] the stems of *T. toxicaria* were chosen for more detailed investigation, since its ethyl acetate-soluble extract significantly induced the enzyme quinone reductase (QR) in cultured Hepa 1c1c7 (mouse hepatoma) cells. Induction of QR is considered a major mechanism of protection against tumor initiation.[5,7]

Bioassay-guided fractionation of the ethyl acetate-soluble residue of the stems of *T. toxicaria*, using the QR induction assay, led to the isolation and characterization of a new butenylated flavanone (**1**) and a new rotenoid (**2**) and the identification of 12 known constituents including the chalcone isoliquiritigenin (**3**), the isoflavone genistein (**4**), the flavone chrysoeriol (**5**), and the rotenoid α-toxicarol (**6**). All of these isolates were evaluated for their potential cancer chemopreventive properties in the Hepa 1c1c7 QR induction model. Selected compounds were then evaluated in a mouse mammary organ culture assay, which is used in our program on cancer chemoprevention as a secondary discriminator to select in vitro-active compounds for additional biological testing.[5,6]



Results and Discussion

Purification of the ethyl acetate-soluble fraction of the methanol extract of the stems of *T. toxicaria* using the quinone reductase assay to monitor fractionation led to the isolation of a new butenylflavanone, (2*S*)-5-hydroxy-7-methoxy-8-[(*E*)-3-oxo-1-butenyl]flavanone (**1**), and new rotenoid, 4′,5′-dihydro-11,5′-dihydroxy-4′-methoxytephrosin (**2**), as well as three active compounds of known structure, isoliquiritigenin (**3**),[8] genistein (**4**),[9] and chrysoeriol (**5**),[9] along with nine known inactive compounds, α-toxicarol

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10.1021/np0302100 CCC: $25.00
Published on Web 08/20/2003

Table 1. NMR Data for Compound **1** (in $CDCl_3$)[a]

position	δ_C		δ_H multiplicity (*J*, Hz)	$^1H-^1H$ COSY
2	79.6	d	5.54 dd (12.3, 3.3)	3α, 3*β*
3α	42.9	t	2.93 dd (17.2, 3.3)	2, 3*β*
3*β*			3.10 dd (17.2, 12.3)	2, 3α
4	196.2	s		
4a	103.0	s		
5	165.7	s		
6	93.1	d	6.15 s	
7	167.0	s		
8	104.4	s		
8a	161.7	s		
1′	137.8	s		
2′/6′	125.9	d	7.47 m	
3′/5′	129.02	d	7.40 m	
4′	129.00	d	7.40 m	
1″	132.9	d	7.81 d (16.5)	2″
2″	128.6	d	7.01 d (16.5)	1″
3″	199.8	d		
4″	27.5	q	2.27 s	
OCH_3-7	56.3	q	3.95 s	
OH-5			12.52 brs	

[a] Run at 500 MHz for ^1H (*J* values in parentheses) and 125 MHz for ^{13}C. Assignments are based on HMQC and HMBC experiments.

(**6**),[10] sumatrol,[4,10,11] 6a,12a-dehydro-α-toxicarol,[12] 11-hydroxytephrosin,[13] obovatin,[14] marmesin,[15] lupenone,[16] benzyl benzoate,[17] and benzyl *trans*-cinnamate.[17] Of these isolates, only sumatrol and α-toxicarol have been isolated from this plant previously. The structures of the known compounds were identified by physical (mp, $[\alpha]_D$) and spectroscopic data (^1H NMR, ^{13}C NMR, 2D NMR, and MS) measurement and by comparison with published values.

Compound **1** was obtained as a white amorphous powder and gave a protonated molecular ion at *m/z* 339.1221 $[M + 1]^+$ by HRFABMS, consistent with an elemental formula of $C_{20}H_{19}O_5$. The ^1H NMR spectrum of **1** (Table 1) showed resonances for an ABX system at δ_H 2.93 (1H, dd, $J = 17.2$, 3.3 Hz), 3.10 (1H, dd, $J = 17.2$, 12.3 Hz), and 5.54 (1H, dd, $J = 12.3$, 3.3 Hz), which is diagnostic for H-2 and H-3 of a flavanone nucleus.[18] The doublet resonances at δ_H 7.81 (1H, $J = 16.5$ Hz) and 7.01 (1H, $J = 16.5$ Hz) and the two singlet resonances at δ_H 6.15 (1H) and 2.27 (3H) suggested that **1** has a *trans*-3-oxo-1-butenyl group substituted at C-6 or C-8, by comparison with a 3-oxo-1-butenylated coumarin, toddalenone,[19] and a 3-oxo-1-butylated flavanone, 6-(3-oxobutyl)taxifolin.[20] Additional signals at δ_H 7.40−7.47 (5H, m) supported the presence of an unsubstituted benzene ring (B ring). Also, a singlet at δ_H 12.52 (OH-5), which was strongly hydrogen-bonded to the C-4 carbonyl group, and a singlet at δ_H 3.95 (3H, s, OCH_3-7) were observed in the ^1H NMR spectrum. These data suggested that **1** is a flavanone bearing one hydroxyl group, one methoxy group, and a *trans*-3-oxo-1-butenyl group, and these inferences were confirmed using the DEPT, COSY, and HMQC NMR techniques. The positions of the substituents were deduced as occurring at C-5 (hydroxyl), C-7 (methoxy), and C-8 (*trans*-3-oxo-1-butenyl group) using the HMBC NMR technique (Figure 1). The absolute stereochemistry at C-2 of compound **1** was established as *S*, on the basis of the observation of a positive Cotton effect at 310 nm (θ +0.16) and a negative Cotton effect at 290 nm (θ −1.00) in its circular dichroism spectrum.[21] Thus, the structure of the new compound **1** was elucidated as (2*S*)-5-hydroxy-7-methoxy-8-[(*E*)-3-oxo-1-butenyl]flavanone. There are several reports of natural compounds which have a 3-oxo-1-butenyl group.[19,20,22,23] However, to the best of our knowledge, compound **1** represents the first example of a flavonoid with a 3-oxo-1-butenyl group.

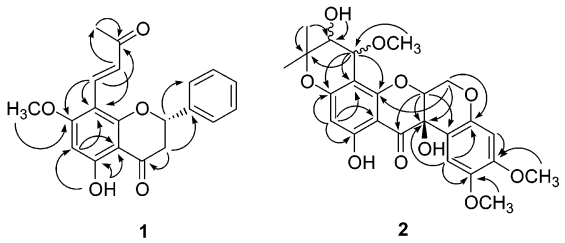

Figure 1. Selected correlations observed in the HMBC NMR spectra of **1** and **2**.

Compound **2** was obtained as a colorless solid and gave a protonated molecular ion M^+ at *m/z* 474.1512 by HREIMS, consistent with an elemental formula of $C_{24}H_{26}O_{10}$. The general features of its MS and NMR spectral data suggested a rotenoid structure for **2**.[24,25] The LREIMS of **2** showed prominent peaks at *m/z* 208 (100%) and 207 (30%), indicative of a typical cleavage of the intact A- and B-ring fragments from a 6a,12a-saturated rotenoid.[13,25] Comparison of ^1H and ^{13}C NMR spectral data of **2** and 11-hydroxytephrosin[13,24] showed that the A−D ring proton and carbon resonances were similar but that points of difference were evident in the E rings. This was confirmed by observed correlations in their 2D NMR spectra ($^1H-^1H$ COSY, HMQC, HMBC, and NOESY). In the ^1H NMR spectrum of **2** (Table 2), three protons at δ_H 4.71 (1H, overlapped), 4.69 (1H, dd, J = overlapped, 2.5 Hz), and 4.49 (1H, d, $J = 11.8$ Hz), assignable to H-6 and H-6a of a rotenoid system,[24] were observed. This was supported by the ^1H NMR spectrum of **2** using pyridine-d_5 as solvent (Experimental Section), which gave clear separations of the proton signals for H-6 and H-6a at δ_H 5.02 (1H, d, $J = 2.4$ Hz, H-6a), 4.91 (1H, d, $J = 12.0$ Hz, H-6*β*), and 4.81 (1H,

Table 2. NMR Data for Compound **2** (in acetone-d_6)[a]

position	δ_C		δ_H multiplicity (*J*, Hz)	position	δ_C		δ_H multiplicity (*J*, Hz)
1	112.3	d	6.83 s	11a	103.8	s	
1a	109.6	s		12	197.2	s	
2	144.9	s		12a	67.8	s	
3	152.8	s		4′	71.7	d	4.43 d (4.6)
4	101.9	d	6.51 s	5′	72.9	d	3.67 dd (7.8, 4.6)
4a	149.9	s		6′	80.5	s	
6α	64.4	t	4.49 d (11.8)	7′	27.8	q	1.38 s
6*β*			4.69 dd (overlapped, 2.5)	8′	21.8	q	1.27 s
6a	76.8	d	4.71 d (overlapped)	OCH_3-2	56.8	q	3.65 s
7a	161.8	s		OCH_3-3	56.0	q	3.78 s
8	100.8	s		OCH_3-4′	60.4	q	3.51 s
9	163.3	s		OH-11			11.83 brs
10	97.7	d	5.81 s	OH-12a			5.76 brs
11	164.5	s		OH-5′			3.98 brd (7.8)

[a] Run at 500 MHz for ^1H (*J* values in parentheses) and 125 MHz for ^{13}C. On addition of D_2O, the OH signals disappeared and the H-5′ signal collapsed to a sharp doublet ($J = 4.5$ Hz). Assignments are based on COSY, HMQC, and HMBC experiments.

Table 3. Biological Activity of Compounds from *Tephrosia toxicaria* in the Quinone Reductase (QR) Induction and Mouse Mammary Organ Culture (MMOC) Assays

compound	QR[a]			MMOC (%)[b]
	CD (μM)	IC_{50} (μM)	CI	
1	10.7	>14.9	>1.4	0.0
2	6.7	9.1	1.4	ND[c]
3	3.9	36.3	10.1	*d*
4	22.9	45.2	2.0	ND[c]
5	11.7	36.0	3.1	ND[c]
6	>23	>47	ND[e]	80.0
6a,12a-dehydro-α-toxicarol	>23	>47	ND[e]	19.0
11-hydroxytephrosin	>23	>47	ND[e]	60.0
sumatrol	>23	>47	ND[e]	49.9
sulforaphane[f]	0.43	11.0	25.0	83.7

[a] CD, concentration required to double QR activity; IC_{50}, concentration inhibiting cell growth by 50%; CI, chemoprevention index (=IC_{50}/CD). Compounds with CD values of <10 μg/mL are considered active. [b] Inhibition of 7,12-dimethylbenz[*a*]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. Selected compounds were tested at concentrations of 10 μg/mL. On the basis of historical controls, inhibition of >60% (at 10 μg/mL) is considered significant. [c] Not determined since the amount of available compound was insufficient. [d] Recently determined in our laboratory to exhibit 76.0% inhibition in this assay at 10 μg/mL. [e] Not determined since the exact CD value of compound was not accurately evaluated. [f] Sulforaphane was used as a positive control and was tested at a concentration of 1 μg/mL.[7,41,42]

dd, J = 12.0, 2.4 Hz, H-6α). The chemical shift and multiplicity of H-6a and the small coupling constant of 2.4 Hz between H-6a and H-6 suggested a *cis* B/C ring fusion and the β configuration of H-6a.[25−27] A singlet at δ_H 11.83 (1H, OH-11), which was chelated to the C-12 carbonyl group, three one-proton singlets for an aromatic ring at δ_H 6.83 (H-1), 6.51 (H-4), and 5.81 (H-10), and three three-proton singlets at δ_H 3.78 (OCH_3-3), 3.65 (OCH_3-2), and 3.51 (OCH_3-4′) were observed in the ^{1}H NMR spectrum of **2**. The ^{1}H NMR spectrum of **2** also showed resonances for a dioxygenated dimethylchromene moiety (ring E) at δ_H 4.43 (1H, d, J = 4.6 Hz, H-4′), 3.67 (1H, dd, J = 7.8 and 4.6 Hz, H-5′), 1.38 (3H, s, H-7′), and 1.27 (3H, s, H-8′).[25] After addition of D_2O, the signal for H-5′ of **2** collapsed to a sharp doublet (J = 4.6 Hz), with the disappearance of the signal at δ_H 3.98 (brd, J = 7.8 Hz), indicating a 5′-hydroxyl group. The HMBC correlations (Figure 1) for H-1/C-2, C-3, C-1a, C-4a, and C-12a; H-6a/C-12, C-1a, and C-12a; H-10/C-8, C-9, C-11, and C-11a; H-4′/C-8, C-9, C-7a, C-6′, and C-4′-OMe; OMe-2/C-2; and OMe-3/C-3 confirmed the assignments of all proton and carbon resonances and the location of the methoxy and hydroxyl groups. Although their absolute stereochemistry could not be ascertained, H-4′ and H-5′ were shown to be *cis*-oriented on the basis of the observed coupling constant between these two protons ($J_{4',5'}$ = 4.6 Hz).[25] Therefore, the structure of this new rotenoid (**2**) was elucidated as 4′,5′-dihydro-11,5′-dihydroxy-4′-methoxytephrosin.

The potential of compounds **1**−**6** and the additional rotenoids 6a,12a-dehydro-α-toxicarol, 11-hydroxytephrosin, and sumatrol to induce QR activity in Hepa 1c1c7 cells is summarized in Table 3. The chalcone, isoliquiritigenin (**3**),[8] exhibited the most potent QR activity (CD value 3.9 μM). There are several recent reports on this compound in terms of its cancer chemopreventive potential,[28,29] and isoliquiritigenin (**3**) was recently isolated by our group as a potent QR inhibitor with a significant response in the MMOC assay (76.0% inhibition at 10 μg/mL) from the fruits of *Dipteryx odorata* (Tonka bean).[30] The two new compounds (**1** and **2**) and two known flavonoids, genistein (**4**) and chrysoeriol (**5**), significantly induced QR activity, with CD values ranging from 6.7 to 22.9 μM. Genistein (**4**) is one of the predominant soy isoflavones and has demonstrated a wide variety of biological effects including tyrosine kinase inhibitory activity, chemoprotectant activities against cancer, and phytoestrogenic activity, which make it worthy of further investigation as a cancer chemopreventive agent.[31−35] Chrysoeriol (**5**) has been shown to inhibit the metabolism of the carcinogen benzo[*a*]pyrene in hamster embryo cells in tissue culture.[36] Thus, the present in vitro test data on these flavonoids are consistent with recent results on their cancer chemopreventive potential. The known compounds α-toxicarol (**6**), 6a,12a-dehydro-α-toxicarol, 11-hydroxytephrosin, sumatrol, obovatin, marmesin, lupenone, benzyl benzoate, and benzyl *trans*-cinnamate were inactive against QR, since they all exhibited CD values of >10 μg/mL.

Selected compounds were then tested in a mouse mammary organ culture (MMOC) model (Table 3). Compound **1** was inactive in this assay at a dose of 10 μg/mL. Since 4′,5′-dihydro-11,5′-dihydroxy-4′-methoxytephrosin (**2**), isoliquiritigenin (**3**), genistein (**4**), and chrysoeriol (**5**) were isolated in the present investigation in only low yields, the compound quantities available did not permit their evaluation in this assay. However, as mentioned above, isoliquiritigenin (**3**) has been found to be active in the MMOC assay in recent work in our laboratory.[30] In contrast, genistein (**4**) failed to show any efficacy in inhibiting mammary alveolar lesions in the MMOC assay when tested previously.[37] Although the known rotenoids 6a,12a-dehydro-α-toxicarol, 11-hydroxytephrosin, sumatrol, and α-toxicarol (**6**) obtained in this study were not active as QR inducers, they were evaluated in the MMOC assay, both because they were isolated from *T. toxicaria* stems in high yields and because the rotenoid deguelin was found to be active in this same assay[38] and subsequently found to exhibit cancer chemopreventive activity in skin and mammary carcinogenesis inhibition assays in animal models.[39] However, only one of the four rotenoids evaluated was deemed active in the MMOC assay, with α-toxicarol (**6**) found to exhibit 80.0% inhibition of DMBA-induced preneoplastic lesions at a dose of 10 μg/mL. Accordingly, of the isolates obtained from *T. toxicaria* in the present study, isoliquiritigenin (**3**) and α-toxicarol (**6**) both seem worthy of additional biological testing to more fully evaluate their potential as cancer chemopreventive agents.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 241 polarimeter. UV spectra were recorded with a Beckman DU-7 spectrometer. IR spectra were run on an ATI Mattson Genesis Series FT-IR spectrometer. NMR experiments were conducted on Bruker DPX-300 and DRX-500 MHz spectrometers with tetramethylsilane (TMS) as internal standard. FABMS and HRFABMS were obtained on a VG 7070E-HF sector-field mass spectrometer, and EIMS and HREIMS on a Finnigan MAT 95 sector-field mass spectrometer operating at 70 eV. Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness), with compounds visualized by dipping plates into 10% (v/v) H_2SO_4 reagent (Aldrich, Milwaukee, WI) followed by charring at 110 °C for 5−10 min. Silica gel (Merck 60A, 70−230 or 200−400 mesh ASTM) was used for column chromatography. Preparative TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness). All solvents used for chromatographic separations were purchased from Fisher Scientific (Fair Lawn, NJ) and distilled before use.

Plant Material. The stems of *Tephrosia toxicaria* (Sw.) Pers. were collected in Rio Curanja, Purús District, Purús Province, Peru, in November 1998 by two of us (J.S.V. and J.G.G.). A voucher specimen has been deposited at the Field Museum of Natural History, Chicago, IL (accession no. Schunke 286).

Quinone Reductase Assay. For the evaluation of plant extracts, fractions, and pure isolates as inducers of quinone reductase (QR), cultured mouse Hepa 1c1c7 cells were used as described previously.[7,40−42]

Mouse Mammary Organ Culture Assay. The inhibition of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced preneoplastic lesion formation in mouse mammary organ culture (MMOC) was performed as described previously.[37,38]

Extraction and Isolation. The dried and milled plant material (1080 g) was extracted with MeOH (3 × 4 L) by maceration. The extracts were combined and concentrated in vacuo at 40 °C. The concentrated extract was suspended in 90% MeOH and then partitioned with petroleum ether (3 × 1 L) to afford a petroleum ether-soluble syrup (D001) on drying. Next, the aqueous methanol extract was concentrated and suspended in H_2O (1 L) and partitioned again with EtOAc (3 × 1 L) to give an EtOAc-soluble extract (D002) and an aqueous residue (D003). The CD values (μg/mL) of the solvent partitions, D001, D002, and D003, were 3.9, <2.5, and >10, respectively.

On the basis of the above activity results, the EtOAc-soluble extract (D002, 17.2 g) was chromatographed over silica gel as stationary phase using a $CHCl_3$−MeOH gradient (from 1:0 to 0:1 v/v) as mobile phase to afford 17 pooled fractions (fractions 4−20). Of these, fractions 4, 11, 12, and 15 showed the most potent QR-inducing activity (CD values 3.9, 3.5, 4.2, and 7.7 μg/mL, respectively). Fraction 4 [eluted with $CHCl_3$; 409 mg] was chromatographed over silica gel (petroleum ether−$CHCl_3$, 24:1 → 0:1 v/v) resulting in nine subfractions (fractions 21−29). Benzyl benzoate (19 mg, 0.0018%)[17] and benzyl *trans*-cinnamate (10 mg, 0.00093%)[17] were isolated from fraction 22 by preparative TLC, developed with petroleum ether−EtOAc (49:1; R_f = 0.62 and 0.38, respectively). Lupenone (8 mg, 0.00074%)[16] and obovatin (28 mg, 0.0026%)[14] were purified by recrystallization (from acetone and from MeOH, respectively) from fractions 23 and 24, respectively. The new compound **1** (2.0 mg, 0.00019%) was isolated from fraction 27 by preparative TLC, developed with petroleum ether−$CHCl_3$−acetone (5:4:1; R_f = 0.52). Fraction 5 (eluted with $CHCl_3$) was purified over a further silica gel column, with petroleum ether−EtOAc (19:1 → 3:1 v/v) used as solvent system, yielding, in turn, the known rotenoids sumatrol (100 mg, 0.0093%),[4,10,11] α-toxicarol (**6**; 250 mg, 0.023%),[10] 6a,12a-dehydro-α-toxicarol (80 mg, 0.0074%),[12] and 11-hydroxytephrosin (70 mg, 0.0065%).[13]

Two other active fractions, 11 and 12 [eluted with $CHCl_3$−MeOH (199:1 v/v), 1.2 g], were combined and then chromatographed over silica gel ($CHCl_3$−MeOH, 99:1 v/v), resulting in 10 subfractions (fractions 41−50). Marmesin (2.5 mg, 0.00023%)[15] was obtained from fraction 47 by recrystallization from MeOH. Fraction 49 was subjected to Sephadex column chromatography followed by preparative TLC, developed with $CHCl_3$−MeOH (24:1, R_f = 0.67), to give the new compound **2** (1.7 mg, 0.00016%).

Fraction 15 [eluted with $CHCl_3$−MeOH (49:1 v/v), 810 mg] was purified over a Sephadex column, with $CHCl_3$−MeOH (1:1 v/v), to give isoliquiritigenin (**3**; 1.6 mg, 0.00015%),[8] genistein (**4**; 0.7 mg, 0.000065%),[9] and chrysoeriol (**5**; 1.8 mg, 0.00017%).[9]

(2*S*)-5-Hydroxy-7-methoxy-8-[(*E*)-3-oxo-1-butenyl]flavanone (1): white amorphous powder; mp 156−157 °C; $[\alpha]^{20}_D$ −98° (*c* 0.1, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 237 (3.63), 269 (3.78), 300 (4.11), 323 (4.10) nm; CD ($CHCl_3$; 20 °C; $\Delta\epsilon$) 291 (−1.00), 310 (+0.16) nm; IR ν_{max} (NaCl) 3378, 3023, 2932, 2848, 1683, 1613, 1596, 1512, 1446, 1233, 1111, 834, 755 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and ^{1}H−^{1}H COSY data, see Table 1; FABMS *m*/*z* 339 ([M + 1]$^+$, 16), 154 (100), 136 (73); HRFABMS *m*/*z* 339.1221([M + 1]$^+$, calcd for $C_{20}H_{19}O_5$, 339.1233).

4′,5′-Dihydro-11,5′-dihydroxy-4′-methoxytephrosin (2): colorless solid; $[\alpha]^{20}_D$ +1.7° (*c* 0.1, acetone); UV (EtOH) λ_{max} (log ϵ) 243 (4.33), 306 (4.63) nm; IR ν_{max} (NaCl) 3468, 2975, 2931, 2848, 1640, 1617, 1582, 1508, 1458, 1344, 1268, 1199, 1173, 1110, 755 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ 12.64 (1H, brs, OH-11), 7.20 (1H, s, H-1), 6.74 (1H, s, H-4), 6.48 (1H, brd, *J* = 8.8 Hz, OH-5′), 6.28 (1H, s, H-10), 5.02 (1H, d, *J* = 2.4 Hz, H-6a), 4.91 (1H, d, *J* = 12.0 Hz, H-6β), 4.81 (1H, dd, *J* = 12.0, 2.4 Hz, H-6α), 4.67 (1H, d, *J* = 4.5 Hz, H-4′), 4.00 (1H, dd, *J* = 8.6, 4.5 Hz, H-5′), 3.75 (3H, s, MeO-3), 3.63 (3H, s, MeO-2), 3.42 (3H, s, MeO-4′), 1.60 (3H, s, H-7′), 1.54 (3H, s, H-8′); ^{1}H NMR and ^{13}C NMR (in acetone-d_6), see Table 2; LREIMS *m*/*z* 474 ([M]$^+$, 30), 235 (33), 208 (100), 207 (30), 193 (7), 165 (7); HREIMS *m*/*z* 474.1512 ([M]$^+$, $C_{24}H_{26}O_{10}$, calcd 474.1526).

Isoliquiritigenin (3): pale yellow powder; mp 188−191 °C; EIMS *m*/*z* 256 ([M]$^+$, 100), 239 (15), 163 (29), 137 (73), 120 (35), 107 (13); ^{1}H NMR data were in agreement with the reported literature values.[8]

Genistein (4): pale yellow needles; mp 288−290 °C; EIMS *m*/*z* 270 ([M]$^+$, 100), 253 (17), 213 (10), 137 (22), 112 (11); ^{1}H and ^{13}C NMR data were in agreement with the reported literature values.[9]

Chrysoeriol (5): pale yellow needles; mp >300 °C; EIMS *m*/*z* 300 ([M]$^+$, 100), 257 (8), 229 (6), 153 (16), 148 (9), 133 (7), 114 (6); ^{1}H and ^{13}C NMR data were in agreement with the reported literature values.[9]

α-Toxicarol (6): yellowish crystals; $[\alpha]^{20}_D$ +34.4° (*c* 0.5, $CHCl_3$); mp 102−104 °C; EIMS *m*/*z* 410 ([M]$^+$, 63), 395 (38), 203 (16), 192 (100), 179 (16), 177 (14), 149 (3); ^{1}H and ^{13}C NMR data were in agreement with the reported literature values.[10]

Acknowledgment. We thank Dr. K. Fagerquist, Mass Spectrometry Facility, Department of Chemistry, University of Minnesota, Minneapolis, MN, and Dr. J. A. (Art) Anderson, Research Resources Center (RRC), University of Illinois at Chicago (UIC), for the mass spectral data. We are grateful to the RRC, UIC, for providing spectroscopic equipment, and to Dr. R. A. Kleps of the RRC, UIC, for facilitating the running of the 500 MHz NMR instrument. This research was supported by program project P01 CA48112, funded by the National Cancer Institute, NIH, Bethesda, MD.

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NP0302100