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J. Nat. Prod., **1992**, 55 (3), 357-363• DOI: 10.1021/np50081a012 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 55, No. 3, pp. 357-363, March 1992

ISOLATION OF POTENTIAL CANCER CHEMOPREVENTIVE AGENTS FROM *ERIODICTYON CALIFORNICUM*

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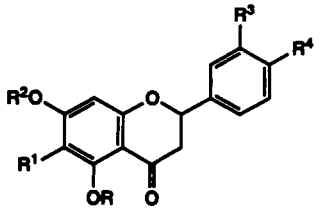
ABSTRACT.—Activity-based fractionation of *Eriodictyon californicum* resulted in the isolation of 12 flavonoids that inhibit the metabolism of the carcinogen benzo[*a*]pyrene by hamster embryo cells in tissue culture. One was identified as a new flavanone, 3′-methyl-4′-isobutyryleriodictyol [**1**], on the basis of spectroscopic analysis and alkaline hydrolysis. The seven other active flavanones were identified as eriodictyol [**2**], homoeriodictyol [**3**], 5,4′-dihydroxy-6,7-dimethoxyflavanone [**4**], pinocembrin [**5**], sakuranetin [**6**], 5,7,4′-trihydroxy-6,3′-dimethoxyflavanone [**7**], and naringenin 4′-methyl ether [**8**]. Four active flavones were also isolated: cirsimaritin [**9**], chrysoeriol [**10**], hispidulin [**11**], and chrysin [**12**]. The high inhibition of benzo[*a*]pyrene metabolism and the activation of benzo[*a*]pyrene to ultimate carcinogenic DNA-binding metabolites by cirsimaritin and chrysoeriol at a concentration of only 10 μg/ml indicates that these flavones warrant further investigation in vivo as potential chemopreventive agents.

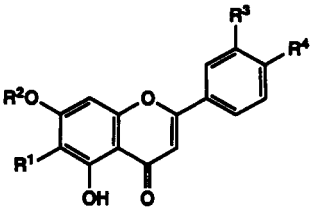
Dietary factors are now recognized as one of the major determinants in the incidence of human cancer (1–3). The dietary influence on cancer incidence in humans involves a complex interaction of a number of components that can cause or inhibit carcinogenesis. Dietary components have been related to a decreased incidence of certain tumors in humans (2,3). A number of compounds have been shown to have chemopreventive activity in animal bioassays, and many are already being tested in clinical trials in humans (4). Wattenberg (5) has described a number of mechanisms by which compounds can exhibit chemopreventive activity. Chemopreventive compounds include: agents that prevent carcinogen formation, agents that interfere with promotion and progression, and blocking agents that reduce the activation of carcinogens to their "ultimate" carcinogenic form that reacts with cellular macromolecules and initiates cancer induction. To isolate blocking agents from plants, a mammalian cell culture assay has been developed. This rapid bioassay measures the effect of test compounds and fractions on the metabolism of [^{3}H]-benzo[*a*]pyrene (B[*a*]P) to H_2O-soluble derivatives in Syrian hamster embryo cell cultures (6). It can be used to direct activity-based fractionation of potential cancer chemopreventive agents present in a variety of higher plants as well as to screen plant extracts for activity. This bioassay showed that an extract from *Eriodictyon californicum* Hook. & Arn. (Hydrophyllaceae), a sticky shrub from which dried leaves are used medicinally, exhibited reproducible inhibition of B[*a*]P metabolism. This manuscript describes the activity-directed fractionation of this plant extract and the isolation of a number of active flavonoids, two of which were very potent inhibitors of B[*a*]P metabolism and B[*a*]P-DNA binding.

RESULTS AND DISCUSSION

The dried leaves and stems of *E. californicum* (7) were percolated with 95% EtOH. The EtOH extract showed significant inhibitory activity in the B[*a*]P metabolism assay. Solvent partitioning of the concentrated crude EtOH extract between CH_2Cl_2 and H_2O resulted in localization of the activity in the CH_2Cl_2-soluble fraction. Further

partition of this fraction between hexane and 10% aqueous MeOH led to concentration of activity in the MeOH-soluble fraction. The latter was in turn chromatographed on columns and plates to isolate 12 active flavonoids, including a new flavanone. The structure of the new flavonoid was elucidated as 3′-methyl-4′-isobutyryleriodictyol [**1**]. The seven known flavanones were identified as eriodictyol [**2**], homoeriodictyol [**3**], 5,4′-dihydroxy-6,7-dimethoxyflavanone [**4**] (8), pinocembrin [**5**], sakuranetin [**6**], 5,7,4′-trihydroxy-6,3′-dimethoxyflavanone [**7**] (9), and naringenin 4′-methyl ether [**8**]. The four known flavones were identified to be cirsimaritin [**9**], chrysoeriol [**10**], hispidulin [**11**], and chrysin [**12**].





1 R=R^1=R^2=H, R^3=OMe, R^4=OCOCH(CH$_3$)$_2$
2 R=R^1=R^2=H, R^3=R^4=OH
2a R=R^2=Ac, R^1=H, R^3=R^4=OAc
3 R=R^1=R^2=H, R^3=OMe, R^4=OH
4 R=R^3=H, R^1=OMe, R^2=Me, R^4=OH
5 R=R^1=R^2=R^3=R^4=H
6 R=R^1=R^3=H, R^2=Me, R^4=OH
7 R=R^2=H, R^1=R^3=OMe, R^4=OH
8 R=R^1=R^2=R^3=H, R^4=OMe

9 R^1=OMe, R^2=Me, R^3=H, R^4=OH
10 R^1=R^2=H, R^3=OMe, R^4=OH
11 R^1=OMe, R^2=R^3=H, R^4=OH
12 R^1=R^2=R^3=R^4=H

Compound **1** was crystallized from MeOH as needles (mp 149–150°). Hrms gave the molecular ion peak at 372.1207 corresponding to molecular formula $C_{20}H_{20}O_7$ (calcd 372.1209). ^{1}H nmr showed three double doublets at δ 5.55 (1H, dd, J = 12.8, 2.7 Hz, H-2), 3.32 (1H, dd, J = 17.1, 12.8 Hz, H-3$_{trans}$) and 2.76 (1H, dd, J = 17.1, 2.7 Hz, H-3$_{cis}$), indicating the flavanone skeleton of **1**. The 5,7-substitution pattern of the A ring could be judged from a pair of doublets at δ 5.94 (1H, d, J = 2.2 Hz) and 5.90 (1H, d, J = 2.2 Hz) which were assigned to H-8 and H-6, respectively. Two signals which appeared at δ 7.29 (1H, s) and 7.11 (2H, s) indicated the di-substitution pattern of the B ring. A singlet (1H) at δ 12.13 could be assigned to the 5-OH proton, and a three-proton singlet at δ 3.79 could be assigned to a methoxyl group. Two signals at δ 2.82 (1H, m) and 1.23 (6H, d, J = 7.5 Hz) and the carbon signals at 174.3 (C-1″), 33.1 (C-2″), and 18.7 (C-3″, -4″) ppm in the ^{13}C nmr indicated the presence of an isobutoxyl group in **1**.

The ^{13}C nmr of **1** gave 19 signals, including three signals belonging to the isobutyl group. Among them ten signals of C-2–C-10 and an aromatic methoxyl group were very similar to those of homoeriodictyol, but the chemical shift differences of the B ring for these compounds were obvious. Interpretation of ^{1}H and ^{13}C nmr as well as the fragmentation pattern in hrms of **1** confirmed the 5,7-dihydroxy substitution pattern of the A ring. Therefore, the B ring must contain a methoxyl group and an isobutoxyl group. Comparing the B ring signals of **1** with those of homoeriodictyol and considering the chemical shift effects of the acetylation of the aromatic hydroxyl group on the B ring aromatic carbons, the 3′-methoxyl-4′-isobutoxy substitution pattern was favored. Further confirmation of the B-ring substitution pattern was provided by alkaline hydrolysis of **1** to yield a compound which was identical to an authentic sample of

homoeriodictyol in all respects (ms, uv, ^{1}H nmr, ^{13}C nmr, co-tlc). Thus **1** was identified as 3′-methyl-4′-isobutyryleriodictyol. The identities of the other known compounds were determined by ms, uv, and ^{1}H and ^{13}C nmr (10,11).

The effect of these compounds on metabolism of [^{3}H]-B[*a*]P to H_2O-soluble metabolites in hamster embryo cell cultures is given in Table 1. In this assay a compound that decreased B[*a*]P metabolism by 20% is defined as an active compound. At 20 μg/ml medium all 12 compounds were active inhibitors of B[*a*]P metabolism. Those with the greatest inhibitory activity were tested at lower doses. Both cirsimaritin [**9**] and chrysoeriol [**10**] had high inhibitory activity (greater than 60% inhibition) at concentrations of 5 μg/ml medium. This activity is much greater than that of the isoflavone biochanin A which was isolated from *Trifolium pratense* (4). These two flavones, cirsimaritin [**9**] and chrysoeriol [**10**], were more active than their corresponding flavanones 5,4′-dihydroxy-6,7-dimethoxyflavanone [**4**] and homoeriodictyol [**3**]. The effect on activity of substituents in various positons varied between the flavone and flavanone series.

TABLE 1. Effect of Isolated Flavonoids **1–12** on Benzo[*a*]pyrene Metabolism in Hamster Embryo Cell Cultures.[a]

Compound	% Change in H_2O-Soluble Benzo[*a*]pyrene Metabolites		
	Dose of Test Compound		
	20 μg/ml	10 μg/ml	5 μg/ml
1	−52		
2	−55	−40	−16
3	−36		
4	−36		
5	−66	−29	−8
6	−52		
7	−32	−19	−9
8	−63	−28	−11
9	−92	−85	−69
10	−96	−85	−60
11	−92	−75	−18
12	−86	−44	−14

[a]The results are the % change in H_2O-soluble benzo[*a*]pyrene metabolites relative to a solvent-treated control sample. All samples represent the average of three wells per plate.

The binding of polycyclic hydrocarbons to DNA correlates with their carcinogenic activity. To determine whether the potent inhibitors of B[*a*]P metabolism cirsimaritin [**9**] and chrysoeriol [**10**] were also effective inhibitors of B[*a*]P-DNA binding, their effects on B[*a*]P-DNA adduct formation were examined in hamster embryo cell cultures. At 10 μg/ml, cirsimaritin [**9**] inhibited total B[*a*]P-DNA binding by 71%, and chrysoeriol [**10**] inhibited total B[*a*]P-DNA binding by 32% (Table 2). Both compounds were effective inhibitors of DNA adduct formation by the most carcinogenic isomer of the ultimate carcinogenic metabolite of B[*a*]P, (+)-*anti*-B[*a*]P-7,8-diol-9,10-epoxide{7*R*,8*S*,9*S*,10*R* configuration; (+)-*anti*-B[*a*]PDE}. Cirsimaritin [**9**] reduced the level of the (+)-*anti*-B[*a*]PDE-DNA adduct from 33 pmol/mg DNA in the control to 12 pmol/mg DNA in the cirsimaritin-treated group (Table 2). Chrysoeriol was less effective but still reduced the level of this adduct to 23 pmol/mg DNA (Table

TABLE 2. Summary of Benzo[*a*]pyrene Binding to DNA in Co-Treatments with Cirsimaritin [**9**] and Chrysoeriol [**10**].

Group[a]	Total Level of Binding pmole/mg DNA	(+)Anti-B[*a*]PDE-dGuo Adduct pmole/mg DNA
A	66.4	32.8
B	19.4	11.5
C	44.9	22.5

[a]Treatment groups A, Benzo[*a*]pyrene (0.5 μg/ml) + DMSO; B, benzo[*a*]pyrene (0.5 μg/ml) + Cirsimaritan [**9**] (10 μg/ml); C, Benzo[*a*]pyrene (0.5 μg/ml) + Chrysoeriol [**10**] (10 μg/ml).

2). Thus both compounds are able to reduce the metabolic activation of B[*a*]P to ultimate carcinogenic DNA-binding metabolites.

These results indicate that *E. californicum* contains a number of flavones and flavanones that inhibit the metabolism of the carcinogen B[*a*]P in mammalian cells. The most active inhibitors, cirsimaritin [**9**] and chrysoeriol [**10**], also inhibited the activation of B[*a*]P to DNA-binding ultimate carcinogenic metabolites and warrant further investigation in animal models as potential chemopreventive agents that block polycyclic hydrocarbon activation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Fischer & Johns melting point apparatus, and are uncorrected. Uv spectra were obtained on a Beckman uv 5260 spectrophotometer. Mass spectra were measured on a Finnigan 4023 machine. Hreims were measured on a VG 70-250 S or a Kratos MS-30 Mass Spectrometer. The ^{1}H nmr and ^{13}C nmr were recorded in DMSO-d_6 with TMS as standard on a Bruker AM-270 or Bruker AM-500 Spectrometer. Cc was performed using Aldrich Si gel 60 (230–400 mesh) and Polyclar AT. Tlc was carried out on Whatman Si gel 60A K6F precoated plates. The spots on tlc were determined under uv light (360 nm).

PLANT MATERIAL.—Twigs and leaves of *E. californicum* were collected and identified by Dr. Richard Spjut of the World Botanical Associates (WBA). A voucher specimen is deposited in the WBA Herbarium, Laurel, Maryland.

B[*a*]P METABOLISM AND DNA BINDING ASSAYS.—The effects of test compounds on B[*a*]P metabolism were measured as described previously (12). Briefly, hamster embryo cell cultures in 12-well tissue culture plates were exposed to a nontoxic dose of the test compound (5 to 20 μg/ml medium) and [^{3}H]-B[*a*]P (0.5 μg/ml) for 24 h. Culture medium was then removed and extracted with a mixture of $CHCl_3$, MeOH, and H_2O. The radioactivity in the $CHCl_3$ phase and in the MeOH/H_2O phase was determined by liquid scintillation counting, and the percentage of B[*a*]P converted to H_2O-soluble metabolites was calculated. The effect of selected test compounds on B[*a*]P-DNA adduct formation was evaluated by treating 175 cm^2 flasks of hamster embryo cells with 0.5 μg B[*a*]P and 10 μg test compound per ml medium for 24 h. The B[*a*]P-DNA adducts were analyzed by the [^{35}S]phosphorothioate postlabeling-hplc procedures described previously (13).

ISOLATION.—Plant material (800 g) was ground and percolated with 95% EtOH to give a brown extract (263 g), which in turn was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 fraction (49.5 g) was further partitioned between hexane and 10% aqueous MeOH. The MeOH extract (44.7 g) was separated on a Sephadex LH-20 column to obtain 12 fractions. From fractions 8 to 10, 3.4 g of compound **3** was obtained. Fractions 7 and 8 (7.19 g) were chromatographed on a Si gel column eluted with $CHCl_3$ with increasing proportions of MeOH, yielding 26 fractions. Compound **7** (40 mg) was obtained from fraction 3, 85 mg of **9** from fractions 11–13, 85 mg of **10** from fractions 15 and 16, 50 mg of **11** from fractions 18–20, and 47 mg of **2** from fractions 21–23.

Fractions 3 and 4 were combined and separated by preparative tlc on Si gel plates to obtain 6 fractions (13–18). Compound **5** (3 mg) and compound **4** (19 mg) were obtained from fractions 13 and 15, respectively. Fraction 14 was passed through a Sephadex LH-20 column to get 7 fractions (19–25). Fraction 21 was chromatographed on a Polyclar AT column, eluted with $CHCl_3$ and $CHCl_3$-MeOH (99:1 and 98:2) to

collect 13 fractions (26–38). Compound **1** (15 mg) was obtained from fraction 36. Fraction 23 was chromatographed on a Polyclar AT column and eluted with $CHCl_3$ and $CHCl_3$-MeOH (99:1), and 10 fractions were collected (39–48) according to the bands on the column under uv 360 nm. Compound **6** (18 mg), compound **8** (6 mg), and compound **12** (2 mg) were obtained.

3′-Methyl-4′-isobutyryleriodictyol [**1**].—Colorless prisms (recrystallized from MeOH): mp 149–150°; $C_{20}H_{20}O_7$; hreims (rel. int. %) *m/z* $[M]^+$ 372.1207 (8) (calcd 372.1209), $[M-C_4H_7O]^+$ 302.0786 (67) (calcd 302.0790), $[M-C_4H_7O-1]^+$ 301.0702 (15) (calcd 301.0712), 285 (4), 179 (8), 166 (13), 153 (25), 152 (4), 150 (25), 149 (2), 137 (67), 135 (10), 124 (6), 71 (37); ^1H nmr (500 MHz, DMSO-d_6) δ 1.23 (6H, d, $J=7.5$ Hz, 3″-,4″-Me), 2.76 (1H, dd, $J=17.1$, 2.7, H-3_{cis}), 2.82 (1H, m, H-2″), 3.32 (1H, dd, $J=17.1$, 12.8 Hz, H-3_{trans}), 3.79 (3H, s, 3′-OMe), 5.55 (1H, dd, $J=12.8$, 2.7 Hz, H-2), 5.90 (1H, d, $J=2$ Hz, H-6), 5.94 (1H, d, $J=2$ Hz, H-8), 7.11 (2H, s, H-5′, -6′), 7.29 (1H, s, H-2′), 12.13 (1H, s, 5-OH); ^{13}C nmr (125 MHz, DMSO-d_6) δ 195.7 (C-4), 174.3 (C-1″), 167.0 (C-7), 163.4 (C-5), 162.6 (C-9), 150.8 (C-3′), 139.4 (C-4′), 137.4 (C-1′), 122.6 (C-5′), 118.8 (C-6′), 111.3 (C-2′), 101.5 (C-10), 96.0 (C-6), 95.1 (C-8), 78.1 (C-2), 55.9 (3′-OMe), 42.0 (C-3), 33.1 (C-2″), 18.7 (C-3″); uv λ max (MeOH) 319, 290, (+NaOMe) 327, 248, (+$AlCl_3$) 378, 310, 275 sh, (+$AlCl_3$/HCl) 374, 308, 275 sh, (+NaOAc) 323, 272, (+NaOAc/H_3BO_3) 326 sh, 288.

Compound **1** (5 mg) was dissolved in 1.5 ml MeOH, and 1 ml of 0.2 N KOH methanolic solution was added. The reaction mixture turned orange-yellow and was monitored by tlc. After 4 h, the MeOH solution was passed through a Sephadex LH-20 column (2 × 12 cm), eluted with MeOH. A fraction that appeared as a yellow band on the column was collected and acidified with 1 N HCl. The solvent was removed under high vacuum, and the hydrolysis product was crystallized from MeOH (yield 3.8 mg) and identified as homoeriodictyol [**3**] by ^1H and ^{13}C nmr, uv, eims, and co-tlc with an authentic sample.

Eriodictyol [**2**].—Colorless needles (recrystallized from MeOH): mp 274–276°; $C_{15}H_{12}O_6$; hreims (rel. int. %) *m/z* $[M]^+$ 288.0629 (58) (calcd 288.0633), 287 (24), 271 (7), 179 (25), 166 (42), 153 (100), 152 (11), 136 (52), 135 (7); ^1H nmr (500 MHz, DMSO-d_6) δ 2.66 (1H, dd, $J=18.4$, 3.1 Hz, H-3_{cis}), 3.17 (1H, dd, $J=18.4$, 13.4 Hz, H-3_{trans}), 5.36 (1H, dd, $J=13.4$, 3.1 Hz, H-2), 5.86 (1H, s, H-8), 5.87 (1H, s, H-6), 6.73 (2H, s, H-5′, -6′), 6.86 (1H, s, H-2′), 12.13 (1H, s, 5-OH); ^{13}C nmr (125 MHz, DMSO-d_6) δ 196.0 (C-4), 166.5 (C-7), 163.4 (C-5), 162.8 (C-9), 145.5 (C-4′ or C-3′), 145.0 (C-3′ or C-4′), 129.4 (C-1′), 117.8 (C-6′), 115.3 (C-5′ or C-2′), 114.2 (C-2′ or C-5′), 101.7 (C-10), 95.6 (C-6), 94.9 (C-8), 78.3 (C-2), 42.0 (C-3); uv λ max (MeOH) 317 sh, 286, (+NaOMe) 323, 245, (+$AlCl_3$) 373, 310 (+$AlCl_3$/HCl) 375, 310, (+NaOAc) 320, 285 sh, (+NaOAc/H_3BO_3) 327 sh, 285.

Acetylation of compound **2** afforded **2a**: hreims (rel. int. %) *m/z* $[M]^+$ 456.1042 (0.3) (calcd 456.1056), 414 (17), 372 (11), 330 (38), 288 (26), 287 (10), 271 (1), 179 (1), 166 (13), 153 (12), 152 (9), 136 (12), 135 (2); ^1H nmr (500 MHz, $CDCl_3$) δ 2.30 (6H, s, 2 × OAc), 2.31 (3H, s, OAc), 2.38 (3H, s, OAc), 2.79 (1H, dd, $J=18$, 3 Hz, H-3_{cis}), 3.00 (1H, dd, $J=18$, 13 Hz, H-3_{trans}), 5.47 (1H, dd, $J=13$, 3 Hz, H-2), 6.55 (1H, d, $J=2$ Hz, H-6), 6.79 (1H, d, $J=2$ Hz, H-8), 7.25 (1H, d, $J=9$ Hz, H-5′), 7.28 (1H, d, $J=2$ Hz, H-2′), 7.31 (1H, dd, $J=9$, 2 Hz, H-6′); ^{13}C nmr (125 MHz, $CDCl_3$) δ 188.4 (C-4), 169.0 (OO*C*Me), 167.9 (OO*C*Me), 167.8 (OO*C*Me), 163.0 (C-9), 156.1 (C-7), 151.3 (C-5), 142.5 (C-3′, -4′), 136.9 (C-1′), 124.1 (C-6′), 123.9 (C-5′), 121.4 (C-2′), 111.9 (C-10), 110.7 (C-6), 109.0 (C-8), 78.6 (C-2), 45.1 (C-3), 21.0 (*Me*COO), 20.9 (*Me*COO), 20.5 (*Me*COO).

Homoeriodictyol [**3**].—Colorless needles (recrystallized from MeOH): mp 227–229°; $C_{16}H_{14}O_6$; hreims (rel. int. %) *m/z* $[M]^+$ 302.0777 (12) (calcd 302.0790), 301 (8), 285 (3), 179 (6), 166 (7), 153 (23), 152 (2), 150 (27), 149 (2), 137 (37), 135 (12), 124 (6); ^1H nmr [500 MHz, $(CD_3)_2CO$] δ 2.73 (1H, dd, $J=17.1$, 2.7 Hz, H-3_{cis}), 3.21 (1H, dd, $J=17.1$, 12.8 Hz, H-3_{trans}), 3.88 (3H, s, 3′-OMe), 5.43 (1H, dd, $J=12.8$, 2.7 Hz, H-2), 5.96 (2H, br s, H-6, -8), 6.88 (1H, d, $J=8.1$ Hz, H-5′), 6.98 (1H, dd, $J=8.1$, 1.8 Hz, H-6′), 7.18 (1H, d, $J=1.8$ Hz, H-2′), 12.18 (1H, s, 5-OH); ^{13}C nmr (125 MHz, DMSO-d_6) δ 196.3 (C-4), 166.6 (C-7), 163.5 (C-5), 162.9 (C-9), 147.6 (C-3′), 147.0 (C-4′), 129.5 (C-1′), 119.6 (C-6′), 115.2 (C-5′), 111.3 (C-2′), 101.8 (C-10), 95.8 (C-6), 95.0 (C-8), 78.6 (C-2), 55.8 (3′-OMe), 42.1 (C-3); uv λ max (MeOH) 319 sh, 283, (+NaOMe) 318, 250, (+$AlCl_3$) 375, 310, (+$AlCl_3$/HCl) 375, 308, (+NaOAc) 320, 280; (+NaOAc/H_3BO_3) 350 sh, 287.

5,4′-Dihydroxy-6,7-dimethoxyflavanone [**4**].—$C_{17}H_{16}O_6$: hreims (rel. int. %) *m/z* $[M]^+$ 316.0947 (82) (calcd 316.0947), 301 (7), 196 (89), 181 (100), 168 (12), 153 (30), 120 (21), ^1H nmr (DMSO-d_6) δ 2.70 (1H, dd, $J=18.4$, 3.1 Hz, H-3_{cis}), 3.27 (1H, dd, $J=18.4$, 13.4 Hz, H-3_{trans}), 3.63 (3H, s, OMe), 3.81 (3H, s, OMe), 5.46 (1H, dd, $J=13.4$, 3.1 Hz, H-2), 6.25 (1H, s, H-8), 6.79 (2H, d, $J=9.2$ Hz, H-3′, -5′), 7.32 (2H, d, $J=9.2$ Hz, H-2′, -6′), 9.63 (1H, br s, OH), 11.94 (1H, br s, 5-OH); ^{13}C nmr (DMSO-d_6) δ 197.3 (C-4), 160.5 (C-7), 158.4 (C-5), 157.5 (C-4′), 153.8 (C-9), 129.6 (C-6), 128.6 (C-1′), 128.1 (C-2′,6′), 115.0 (C-3′,5′), 102.4 (C-10), 91.9 (C-8), 78.6 (C-2), 59.9 (OMe), 56.1 (OMe), 42.0 (C-3); uv λ max (MeOH) 338, 289, (+NaOMe) 371, 292, (+$AlCl_3$) 385, 310, (+$AlCl_3$/HCl) 384, 309, (+NaOAc) 326, 286, (+NaOAc/H_3BO_3) 340, 285.

Pinocembrin [**5**].—$C_{15}H_{12}O_4$: hreims (rel. int. %) *m/z* $[M]^+$ 256.0722 (94) (calcd 256.0735), 255 (48), 239 (5), 228 (9), 179 (80), 166 (1), 153 (24), 152 (90), 124 (60), 121 (72), 104 (100), 103 (31), 91 (36); ^{1}H nmr (DMSO-d_6) δ 2.72 (1H, dd, J = 18, 3 Hz, H-3_{cis}), 3.23 (1H, dd, J = 18, 13 Hz, H-3_{trans}), 5.58 (1H, dd, J = 13, 3 Hz, H-2), 5.90 (1H, d, J = 2.1 Hz, H-6), 5.93 (1H, d, J = 2.1 Hz, H-8), 7.55–7.41 (5H, m, H-2′, -3′, -4′, -5′, -6′), 12.13 (1H, s, 5-OH); ^{13}C nmr (DMSO-d_6) δ 195.5 (C-4), 166.6 (C-7), 163.5 (C-5), 162.6 (C-9), 138.6 (C-1′), 128.7 (C-3′, -4′, -5′), 126.4 (C-2′, -6′), 101.6 (C-10), 95.8 (C-6), 94.9 (C-8), 78.2 (C-2), 42.0 (C-3); uv λ max (MeOH) 320 sh, 290, (+NaOMe) 315, 247, (+$AlCl_3$) 375, 305, 252, (+$AlCl_3$/HCl) 375, 305, 254, (+NaOAc) 320, 248, (+NaOAc/H_3BO_3) 320 sh, 290.

Sakuranetin [**6**].—$C_{16}H_{14}O_5$: hreims (rel. int. %) *m/z* $[M]^+$ 286.0839 (89) (calcd 286.0841), 285 (44), 269 (6), 193 (29), 180 (27), 167 (100), 166 (32), 138 (21), 120 (47), 119 (12); ^{1}H nmr (DMSO-d_6) δ 2.72 (1H, dd, J = 18, 3 Hz, H-3_{cis}), 3.26 (1H, dd, J = 18, 13 Hz, H-3_{trans}), 3.79 (3H, s, OMe), 5.48 (1H, dd, J = 13, 3 Hz, H-2), 6.08 (1H, d, J = 2.3 Hz, H-6), 6.10 (1H, d, J = 2.3, H-8), 6.80 (2H, d, J = 9.2 Hz, H-3′, -5′), 7.33 (2H, d, J = 9.2 Hz, H-2′, -6′), 9.62 (1H, br s, OH), 12.11 (1H, br s, 5-OH); ^{13}C nmr (DMSO-d_6) δ 196.7 (C-4), 167.4 (C-7), 162.8 (C-5), 157.7 (C-9), 128.7 (C-1′), 128.2 (C-2′, -6′), 115.1 (C-3′, -5′), 102 (C-10), 94.6 (C-6), 93.8 (C-8), 78.5 (C-2), 55.8 (OMe), 42.0 (C-3); uv λ max (MeOH) 318 sh, 284, (+NaOMe) 355, 285, (+$AlCl_3$) 375, 310, (+$AlCl_3$/HCl) 375, 308 (+NaOAc) 323 sh, 286, (+NaOAc/H_3BO_3) 323 sh, 286.

5,7,4′-Trihydroxy-6,3′-dimethoxyflavanone [**7**].—Colorless needles (recrystallized from MeOH): mp 230–232°; $C_{17}H_{16}O_7$; hreims (rel. int. %) *m/z* $[M]^+$ 332.0890 (96) (calcd 332.0896), 331 (6), 317 (6), 315 (2), 289 (3), 209 (4), 196 (1), 183 (56), 182 (100), 167 (56), 154 (13), 150 (44), 149 (2), 137 (16), 135 (23); ^{1}H nmr (500 MHz, DMSO-d_6) δ 2.69 (1H, dd, J = 17.1, 2.7 Hz, H-3_{cis}), 3.15 (1H, dd, J = 17.1, 12.8 Hz, H-3_{trans}), 3.65 (3H, s, 6-OMe), 3.76 (3H, s, 3′-OMe), 5.39 (1H, dd, J = 12.8, 2.7 Hz, H-2), 5.96 (1H, s, H-8), 6.85 (1H, d, J = 9 Hz, H-5′), 6.90 (1H, s, H-2′), 6.92 (1H, d, J = 9 Hz, H-6′), 12.17 (1H, s, 5-OH); ^{13}C nmr (125 MHz, DMSO-d_6) δ 196.6 (C-4), 159.4 (C-7), 157.8 (C-5), 154.9 (C-9), 147.8 (C-3′), 146.4 (C-4′), 131.2 (C-6), 128.9 (C-1′), 117.5 (C-6′), 113.9 (C-5′), 112.1 (C-2′), 101.8 (C-10), 94.9 (C-8), 78.1 (C-2), 59.9 (6-OMe), 55.7 (3′-OMe), 42.0 (C-3); uv λ max (MeOH) 324 sh, 286, (+NaOMe) 325, 245 sh, (+$AlCl_3$) 313, 290 sh, (+$AlCl_3$/HCl) 313, 288 sh, (+NaOAc) 326, 281 sh, 254 sh, (+NaOAc/H_3BO_3) 329, 288.

Naringenin 4′-methyl ether [**8**].—$C_{16}H_{14}O_5$: hreims (rel. int. %) *m/z* $[M]^+$ 286.0834 (46) (calcd 286.0841), 285 (22), 269 (4), 179 (12), 153 (7), 152 (10), 134 (100), 133 (3), 124 (10), 121 (57); ^{1}H nmr (DMSO-d_6) δ 2.73 (1H, dd, J = 18, 3 Hz, H-3_{cis}), 3.27 (1H, dd, J = 18, 13 Hz, H-3_{trans}), 3.77 (3H, s, OMe), 5.50 (1H, dd, J = 13, 3 Hz, H-2), 5.89 (2H, br s, H-6, -8), 6.98 (2H, d, J = 9.3 Hz, H-3′, -5′), 7.44 (2H, d, J = 9.3 Hz, H-2′, -6′), 12.14 (1H, br s, 5-OH); ^{13}C nmr (DMSO-d_6) δ 195.9 (C-4), 166.7 (C-7), 163.4 (C-5), 162.8 (C-9), 159.4 (C-4′), 130.7 (C-1′), 128.1 (C-2′, -6′), 113.9 (C-3′, -5′), 101.7 (C-10), 95.8 (C-6), 95.0 (C-8), 78.1 (C-1), 55.2 (OMe), 42.0 (C-3); uv λ max (MeOH) 317 sh, 287, (+NaOMe) 322, 275 sh, (+$AlCl_3$) 375, 311, (+$AlCl_3$/HCl) 375, 310, (+NaOAc) 322, 275 sh, (+NaOAc/H_3BO_3) 325, 289.

Cirsimaritin [**9**].—$C_{17}H_{14}O_6$: hreims (rel. int. %) *m/z* $[M]^+$ 314.0779 (100) (calcd 314.0790), 313 (20), 299 (86), 285 (18), 271 (19), 268 (12), 254 (5), 181 (19), 153 (29), 119 (13); ^{1}H nmr (DMSO-d_6) δ 3.72 (3H, s, OMe), 3.90 (3H, s, OMe), 6.82 (1H, s, H-3), 6.90 (1H, s, H-8), 6.92 (2H, d, J = 9.6 Hz, H-3′, -5′), 7.94 (2H, d, J = 9.6 Hz, H-2′, -6′), 12.91 (1H, s, 5-OH); ^{13}C nmr (DMSO-d_6) δ 182.0 (C-4), 164.0 (C-2), 161 (C-4′), 158.5 (C-7), 152.5 (C-5,9), 131.9 C(-6), 128.3 (C-2′, -6′), 121.0 (C-1′), 115.8 (C-3′, -5′), 105.0 (C-10), 102.6 (C-3), 91.5 (C-8), 59.9 (6-OMe), 56.3 (7-OMe); uv λ max (MeOH) 339, 274, (+NaOMe) 383, 272, (+$AlCl_3$) 359, 298, 265 sh, (+$AlCl_3$/HCl) 356, 298, 265 sh, (+NaOAc) 384, 340 sh, 273, (+NaOAc/H_3BO_3) 335, 274.

Chrysoeriol [**10**].—$C_{16}H_{12}O_6$: hreims (rel. int. %) *m/z* $[M]^+$ 300.0633 (100) (calcd 300.0634), 272 (3), 257 (6), 229 (6), 153 (17), 148 (10), 136 (8), 133 (7), 124 (5), 115 (8); ^{1}H nmr (DMSO-d_6) δ 12.95 (1H, s, 5-OH), 7.55 (1H, d, J = 9.6 Hz, H-6′), 7.53 (1H, s, H-2′), 6.92 (1H, d, J = 9.6 Hz, H-5′), 6.87 (1H, s, H-3), 6.48 (1H, br s, H-8), 6.17 (1H, br s, H-6), 3.88 (3H, s, OMe); ^{13}C nmr (DMSO-d_6) δ 181.5 (C-4), 164.4 (C-7), 163.4 (C-2), 161.5 (C-5), 157.3 (C-9), 150.7 (C-3′), 147.9 (C-4′), 121.4 (C-1′), 120.2 (C-6′), 115.7 (C-5′), 110.3 (C-2′), 103.1 (C-3), 103.2 (C-10), 98.8 (C-6), 93.9 (C-8), 55.9 (OMe); uv λ max (MeOH) 345, 264, (+NaOMe) 404, 328 sh, 274 sh, 263, (+$AlCl_3$) 388, 356 sh, 294, 273, (+$AlCl_3$/HCl) 384, 355, 292 sh, 273, (+NaOAc) 398, 319, 272, (+NaOAc/H_3BO_3) 348, 268.

Hispidulin [**11**].—$C_{16}H_{12}O_6$: hreims (rel. int. %) *m/z* $[M]^+$ 300.0636 (100) (calcd 300.0634), 285 (61), 282 (45), 257 (60), 254 (13), 228 (3), 200 (2), 167 (15), 153 (8), 139 (21), 119 (22); ^{1}H nmr (DMSO-d_6) 13.06 (1H, br s, 5-OH), 7.90 (2H, d, J = 9.5 Hz, H-2′, -6′), 6.91 (2H, d, J = 9.5 Hz, H-3′, -5′), 6.75 (1H, s, H-8), 6.57 (1H, s, H-3), 3.74 (3H, s, OMe); ^{13}C nmr (DMSO-d_6) 181.9 (C-4), 163.7 (C-2), 160.9 (C-4′), 157.1 (C-7), 152.6 (C-5), 152.3 (C-9), 131.3 (C-6), 128.2 (C-2′, -6′), 121.2

(C-1′), 115.8 (C-3′, -5′), 103.9 (C-10), 102.3 (C-3), 94.1 (C-8), 59.8 (OMe); uv λ max (MeOH) 334, 272, (+NaOMe) 393, 324, 275, (+$AlCl_3$) 360, 301, 278 sh, (+$AlCl_3$/HCl) 354, 299, 276 sh, (+NaOAc) 385, 330 sh, 295, 273, (+NaOAc/H_3BO_3) 339, 274.

Chrysin [**12**].—$C_{15}H_{10}O_4$: hreims (rel. int. %) *m/z* $[M]^+$ 254.0586 (100) (calcd 254.0579), 226 (21), 179 (5), 152 (29), 124 (24), 113 (14), 102 (8); uv λ max (MeOH) 314 sh, 267, (+NaOMe) 325, 275, (+$AlCl_3$) 380, 311, 280, (+$AlCl_3$/HCl) 375, 309, 280, (+NaOAc) 325, 273, (+NaOAc/H_3BO_3) 326, 266. Co-tlc with an authentic sample showed identical R_f values (10,11).

ACKNOWLEDGMENTS

This work was supported by Grant CA-38151 from the National Cancer Institute of the National Institutes of Health. Nmr spectra were obtained on a Bruker 250 and 270 MHz spectrometer by John Miller of the College of Pharmacy, The Ohio State University. High-field nmr on a Bruker AM-500 (supported by RR01458) was performed by Dr. Charles E. Cottrell and high resolution ms on a VG 70-250S by Dr. C.R. Weisenberger and Mr. David H. Chang of the Campus Chemical Instrumentation Center of the Ohio State University.

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Received 16 September 1991