Journal of Natural Products
Vol. 53, No. 6, pp. 1471-1478, Nov-Dec 1990

MICROBIOLOGICAL TRANSFORMATION OF CHROMONE, CHROMANONE, AND RING A HYDROXYFLAVONES

ABDEL-RAHIM IBRAHIM[1] and YUSUF J. ABUL-HAJJ*

Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455

ABSTRACT.—Microbial transformation of chromone, chromanone, and 3 ring A hydroxyflavones (5-hydroxy-, 6-hydroxy-, and 7-hydroxyflavones) was attempted using thirty-two microorganisms. While chromone was not biotransformed, chromanone was transformed to chromone and chromanol by *Aspergillus niger* in 2% yield. Ring A hydroxylated flavones were transformed to the corresponding C-4′ hydroxylated metabolites, and the yields and rate of product formation were found to correlate with the distance between the C-4 carbonyl group and the hydroxyl group in ring A.

Flavonoids are widely distributed in plants and are ingested in large amounts in our diet; the estimated daily dietary intake is 1 g/day in the USA (1). Recent studies have shown diverse physiological and pharmacological activities of these natural products, including inhibition of cAMP phiosphdiesterase (2) as well as antiviral effects (3). Although flavonoids, as modulators of P-450 monooxygenase system (4) and inhibitors of tumor promoters (5), can be considered inhibitors of carcinogensis, many were found to be mutagenic in the Ames test (6). Therefore, an understanding of the metabolism of these compounds is extremely important, as some metabolites may be more mutagenic than the parent compound (7).

Studies on the in vivo metabolism of flavonoids in mammals were, to a large extent, hampered by their breakdown in the intestine via the microflora (8). However, hydroxylation (9) and conjugation (10) products were obtained using in vivo studies.

The use of microorganisms in the simulation of mammalian metabolism of different compounds is well documented in the literature (11,12). Microbial models were found to provide insights into the mechanisms of action, toxicity, pharmacological activity, and the metabolic pathway of drugs (13).

Very little work is known on the microbial transformation of flavonoids. Several metabolites of flavanone were reported from transformations using *Gibberella fujikuroi*; these transformations include hydroxylation, carbonyl reduction, ring C cleavage, and dehydrogenation products (14–17). However, only 4′-hydroxyflavone and salicylic acid were reported as metabolites of flavone by *Gibberella saubnetti* (18).

As part of an ongoing project aimed at understanding substrate specificity in microbiological transformation of flavonoids, we have recently reported the results of microbial transformation of flavone and isoflavone (19) as well as flavanone and isoflavanone (20). The results obtained from these studies showed the predominance of ring B hydroxylation at the 4′ and 3′,4′ positions. Furthermore, hydroxylations at C-2 and C-3 positions of ring C were observed only with substrates having a saturated C-2–C-3 bond. Thus the lack of hydroxylation of ring A in flavones suggests that the chromone ring system is the part of the flavonoid nucleus that interacts with the binding site of the enzyme, resulting in exposure of the 2 and 3 positions of ring C as well as ring B to the hydroxylating site of the enzyme. In order to test this model, biotransformation studies with chromone and chromanone were carried out to determine if indeed the chromone nucleus interacts specifically with the binding site of the enzyme. Furthermore, studies

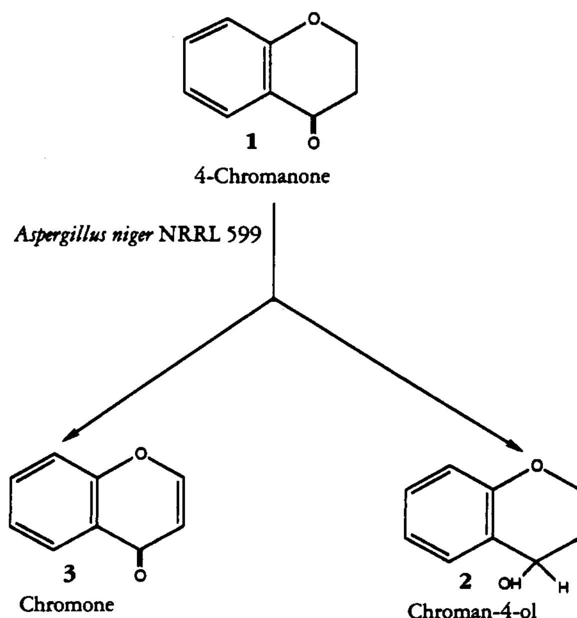
[1]This material is from a thesis submitted in partial fulfillment of the degree of Doctor of Philosophy, University of Minnesota, Minneapolis, Minnesota, 1989.

of three ring-A hydroxyflavones were carried out to determine whether a hydrophilic substituent in ring A influences the mode of hydroxylation by the enzyme.

RESULTS AND DISCUSSION

Screening of chromone and chromanone with 32 microorganisms showed poor utilization of these molecules. None of the microorganisms used in this study metabolized chromone. On the other hand, chromanone screening showed only trace amounts of metabolites. *Aspergillus niger* NRRL 599 (the microorganism showing highest yield of products) produced two chromanone metabolites in 2% yield. While the exact reason for poor utilization of chromones by microorganisms is not fully understood, it is quite possible that the absence of ring B may influence the binding and/or biological transport through fungal cells or may simply indicate that the chromone nucleus is involved primarily in binding to the enzyme.

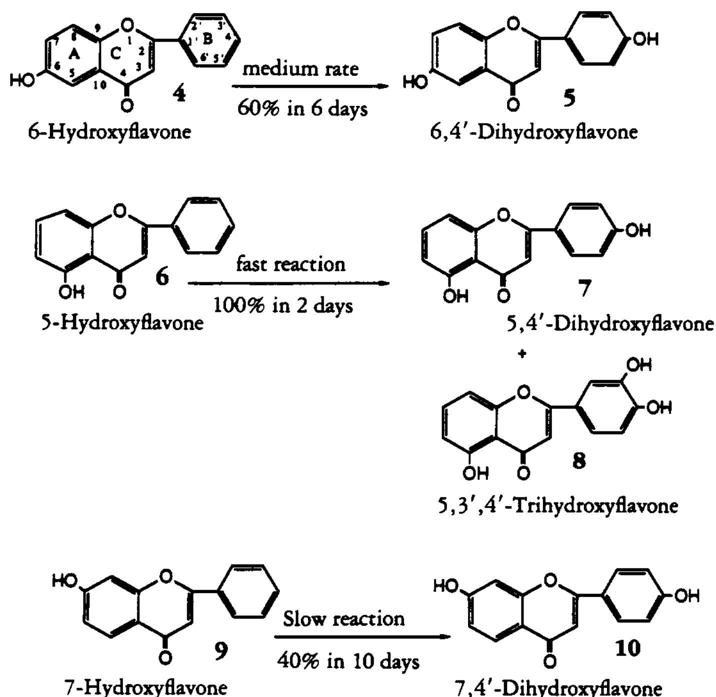
Preparative incubation of chromanone [**1**] with *A. niger* NRRL 599 produced chroman-4-ol [**2**] and chromone [**3**] in 2% yield (Scheme 1). The absence of a carbonyl absorption in the ir spectrum of compound **2** which appears at 1687 cm^{-1} in the chromanone spectrum and the presence of a broad band at 3366 cm^{-1} for a hydroxyl group indicated a reduction of the carbonyl group in the substrate. In ms, the molecular ion at *m/z* 150 and other fragments at 121, 122, and 123 are consistent with spectral data for chroman-4-ol. ^{1}H-nmr showed a quartet at 4.785 ppm that integrated for one proton and was assigned to the C-4 proton of the metabolite. The additional coupling to this proton led to a multiplet signal for H-3. Furthermore, the shielding of H-5 from 7.87 ppm in chromanone to 7.31 ppm in **2** agrees with the deshielding effect of the carbonyl. ^{13}C spectral data of **2** showed a signal for a carbon attached to a hydroxyl at 63.26 ppm instead of the carbonyl signal at 191 ppm in chromanone. All ^{13}C-nmr signals were identical to those reported for chroman-4-ol (21). The identity of the metabolite was further confirmed by direct comparison to a synthetic sample prepared by $NaBH_4$ reduction of chromanone.



SCHEME 1. Products of microbial metabolism of chromanone.

The ir spectrum of compound **3** showed a change in carbonyl absorption from 1687 cm^{-1} in chromanone to 1648 cm^{-1} in the metabolite, indicating dehydrogenation of chromanone. Ms fragments at *m/z* 146 [M]$^+$ and *m/z* 120 indicated the loss of 2 mass units from the substrate, suggesting that compound **3** is chromone. Further support for the identity of compound **3** was obtained from ^{1}H- and ^{13}C-nmr data as well as comparison to a synthetic sample of chromone (22).

Large scale incubations of compound **4** (6-hydroxyflavone) with *Streptomyces fulvissimus* NRRL 1453B gave 6,4′-dihydroxyflavone [**5**] in 15% yield (Scheme 2). A



SCHEME 2. Products of microbial transformation of ring A hydroxyflavone by *Streptomyces fulvissimus*.

molecular ion at *m/z* 254 and two retro-Diels-Alder fragments at *m/z* 136 and 118 indicated a ring B hydroxylation product. ^{1}H nmr of **5** was different from that of 6-hydroxyflavone only in the H-3′, H-5′ signal, which was shifted upfield (Table 1). This shielding effect is consistent with a hydroxy group at C-4′ (23). The symmetry of the C-2′, C-6′ signal on the one hand and the C-3′, C-5′ signal on the other confirmed blocking of C-4′ position. Further support came from ^{13}C-nmr data (Table 2) which showed that the C-4′ signal was shifted from δ 131 in 6-hydroxyflavone to δ 160 in 6,4′-dihydroxyflavone while the C-3′, C-5′ was shielded from δ 129 to δ116. These changes were consistent with C-4′ hydroxylation.

Preparative incubations of 5-hydroxyflavone [**6**] and 7-hydroxyflavone [**9**] with *S. fulvissimus* NRRL 1453B gave rise to 5,4′-dihydroxyflavone [**7**] and 7,4′-dihydroxyflavone [**10**] in 36% and 5% yields, respectively (Scheme 2). Both ^{1}H nmr and ^{13}C nmr (Tables 1 and 2) displayed shifts in proton and carbon signals analogous to those reported previously for 6,4′-dihydroxyflavone.

Compound **8** was obtained as a minor metabolite in the biotransformation of 5-hydroxyflavone by *S. fulvissimus* NRRL 1453 B (yield = 2%). Mass spectral data of this

TABLE 1. ^{1}H nmr Chemical Shifts of Compounds **4**, **5**, **6**, **7**, **9**, and **10**.

Proton	Compound					
	4	**5**	**6**	**7**	**9**	**10**
H-2						
H-3	6.94	6.76	7.1	6.9	6.9	6.7
H-4						
H-5	7.3 (s)	7.3 (s)			7.9 (d) (J=9.83)	7.8 (d)
H-6			6.8 (d) (J=8.11)	6.8 (d)	6.9 (d) (J=10.63)	
H-7	7.3 (d) (J=9)	7.2 (d) (J=8.56)	7.7 (t)	7.6 (t)		
H-8	7.6 (d) (J=9)	7.6 (d) (J=8.92)	7.2 (d) (J=7.93)	7.1 (d)	7.0 (s)	6.95 (s)
H-9						
H-10						
H-1′						
H-2′	8.1 (d) (J=9.5)	7.9 (d) (J=8.93)	8.1 (d)	8.0 (d)	8.1 (d) (J=7.86)	7.9 (d) (J=8.15)
H-3′	7.6 (m)	6.9 (d) (J=8.63)		7.0 (d)	7.6 (m)	6.9 (d) (J=7.88)
H-4′	7.6 (m)	—	7.7 (m)	—	7.6 (m)	—
H-5′	7.6 (m)	6.9 (d) (J=8.63)	—	7.0 (d)	7.6 (m)	6.9 (d) (J=7.88)
H-6′	8.1 (d) (J=9.5)	7.9 (d) (J=8.93)	8.1 (d)	8.0 (d)	8.1 (d) (J=7.86)	7.9 (d) (J=8.15)

metabolite shows a molecular ion at *m/z* 270 as well as two retro-Diels-Alder fragments at *m/z* 137 and 136 consistent with introduction of two hydroxy groups in ring B. In ^{1}H nmr, the H-2′ displayed a shielded singlet, which agrees with a C-3′ hydroxy group. On the other hand, the H-5′ and H-6′ signals gave two AB doublets, which is consistent with C-4′ hydroxylation.

The rate of C-4′ hydroxylation of the three ring A hydroxyflavones was found to depend on the proximity of the hydroxy group to the C-4 carbonyl. The highest rate of transformation was observed when the hydroxy group was closest to the carbonyl group and was even higher than the rate of transformation of flavone. The dependence of the enzymatic hydroxylation on the position of the hydroxy group in the substrate correlates well with the reported importance of the polar groups as sites for binding to the enzyme (24–27). It is most likely that a single enzyme may be involved in 4′-hydroxylation of flavonoid compounds using the same microorganism. Whether different microorganisms accomplish 4′-hydroxylation using the same enzyme or more than one enzyme remains to be determined.

TABLE 2. ^{13}C nmr Chemical Shifts (δ) of Compounds **4**, **5**, **6**, **7**, **9**, and **10**.

Carbon	Compound					
	4	**5**	**6**	**7**	**9**	**10**
C-2	162.2	162.7	164.1	164.7	162.8	162.7
C-3	107.5	107.5	105.7	103.3	106.6	104.5
C-4	177.0	176.8	183.2	182.9	176.4	176.2
C-5	105.5	103.8	159.8	159.4	126.5	126.4
C-6	149.4	149.2	111.0	110.8	115.1	114.9
C-7	124.2	122.7	136.0	135.7	162.0	162.5
C-8	119.9	119.6	107.6	107.4	102.6	102.5
C-9	154.9	154.7	155.9	155.8	157.5	157.4
C-10	123.1	124.2	110.1	109.9	116.1	116.1
C-1′	131.4	121.7	130.5	120.9	131.3	121.9
C-2′	126.2	128.2	126.6	128.8	126.2	128.1
C-3′	129.2	115.9	129.2	116.0	129.1	115.9
C-4′	131.6	160.9	132.3	161.5	131.6	160.7
C-5′	129.2	115.9	129.2	116.0	129.1	115.9
C-6′	126.2	128.2	126.6	128.8	126.2	128.1

The results obtained in this study suggest that a three-point attachment model (Figure 1) may be involved in the microbiological hydroxylation of flavonoids. In this model, it is proposed that the chromone ring system orients itself so as to maximize hydrophilic binding between its hydroxyl, carbonyl, and ether functions and three hydrophilic sites on the enzyme which correspond to positions 1, 4, and 5 of the flavonoid nucleus. The lack of hydroxylation of chromone and chromanone suggests that the benzo-γ-pyrone ring system serves only as a binding function. While the presence of a 5-hydroxy group leads to tight binding and efficient hydroxylation, the hydroxy groups at either the 6 or 7 positions of flavone result in decreased binding to the enzyme, which may be in part due to decreased interaction between the hydroxy group and the corresponding site on the enzyme. Furthermore, the increased hydrophilic character of the 6-hydroxy- and 7-hydroxyflavones around the hydrophobic region of the enzyme results in increased repulsive forces, leading to decreased enzyme activity. Studies aimed at determining the importance of the oxygen functions of the chromone nucleus for anchoring the molecule in the proper orientation to allow for maximum interaction between ring B of flavone and the hydroxylating site of the enzyme are currently under investigation.

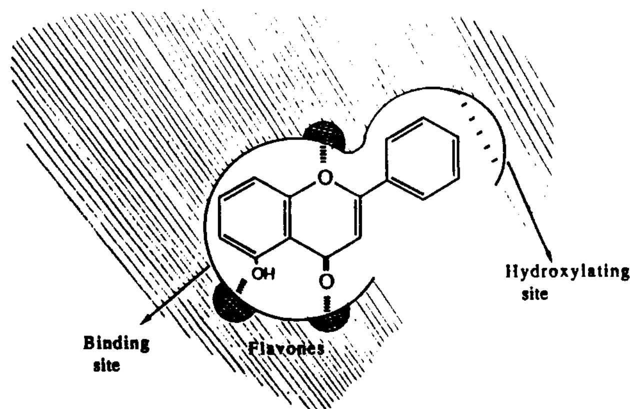


FIGURE 1. Proposed model for the interaction of flavonoid nucleus with hydroxylase.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fischer Jones hot plate apparatus and are uncorrected. Ir spectra were obtained on 5 DXC Ft-ir spectrophotometer using KBr discs. Mass spectra were determined on an AEI MS-30 mass spectrometer. ^{1}H nmr and ^{13}C nmr were recorded on a Nicolet NT-300W3 spectrometer operating at 300 and 78 MHz, respectively. Uv spectra were taken on Beckman Du70 spectrophotometer. Tlc was performed on precoated Si gel and alumina on plastic sheets, 40 × 80 mm, with fluorescent indicator, Machery-Nagel, Germany. Chromanone was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, and all ring-A hydroxylated flavonoids were obtained from Indofine Chemical Company, Somerville, New Jersey.

MICROORGANISMS.—Microorganisms were either purchased from American Type Culture Collection (ATCC) or obtained from Northern Regional Research Laboratories (NRRL). Eleven genera (32 species) of microorganisms, which include *Absidia, Aspergillus, Penicillium, Rhizopus, Curvularia, Cunninghamella, Fusarium, Gliocaldium, Thamnidium, Trichoderma*, and *Streptomyces* were used.

CULTURE AND FERMENTATION SCREENING PROCEDURES.—Microorganisms used in this study were maintained on Sabourad dextrose or potato dextrose agar (Difco) slants and stored in screw-capped culture tubes at 4°. Microorganisms were grown in a medium composed of 1% glucose, 1% glycerol, 0.5% yeast extract, 0.5% peptone, 0.5% K_2HPO_4, 0.5% HaCl in distilled H_2O; pH was adjusted to 6 before autoclaving. Biotransformation experiments were performed in shake culture flasks by using two-stage fer-

mentation procedures. After 24 h of incubation in the above medium, 5 ml of stage I was used as inoculum for fresh stage II culture (50 ml/250 ml flask). After 24 h of incubation in stage II, substrate was added (0.2–0.25 mg/ml) as a solution in DMF (200 mg/ml). Culture controls consisted of fermentation blanks in which organisms were grown under identical conditions but without substrate. Substrate controls consisted of sterile medium containing the same amount of substrate and incubated under the same conditions. Samples (4 ml) of substrate containing stage II fermentations were withdrawn at various time intervals and extracted with 4 ml $CHCl_3$. Concentrated $CHCl_3$ extracts were spotted on tlc (0.25 mm), which was developed by C_6H_6-EtOAc (1:1) or hexane-Et_2O (2:1). Chromatograms were visualized by fluorescence quenching under uv light and by spraying with $AlCl_3$ or exposure to NH_3 vapors and rechecking under uv light.

Preparative scale fermentations were conducted on 1–2 g of substrate. Transformation continued for 2–10 days, depending on the period required for maximum accumulation of metabolites. Stage II fermentations were carried out in several 2-liter Erlenmeyer flasks, each containing 400 ml of culture. Substrate was dissolved in DMF (1 g/1 ml) and distributed evenly among 24-h-old stage II culture. The mycelium was filtered from the fermentation medium by use of cheesecloth, and the filtrate was extracted three times with an equal volume of $CHCl_3$. The cells were lyophilized and extracted with Me_2CO, and the Me_2CO extract was concentrated and extracted with EtOAc. All extracts ($CHCl_3$ and EtOAc) were washed with H_2O and dried over anhydrous Na_2SO_4. Si gel cc was used to purify products of ring-A hydroxyflavones, using a gradient of $CHCl_3$/MeOH. Metabolites of chromanone were purified on an alumina column using a C_6H_6/EtOAc gradient.

SYNTHESIS OF CHROMONE [**3**].—A solution of chromanone (3.7 g) in dry dioxane (50 ml) was heated with 2,3-dichloro-5,6-dicyanoquinone (6.4 g) in an H_2O bath for 8 h. The hydroquinone was filtered and washed with $CHCl_3$. The filtrate and $CHCl_3$ washings were washed with H_2O and dried over anhydrous Na_2SO_4. Cc on Si gel using a hexane/Et_2O gradient and recrystallization of the product from hexane gave chromone as needles in 70% yield, mp 55–56°.

CHROMANONE [**1**].—Mol wt 148; mp 37°; ir (KBr) 1687, 1604, 1479, 1467, 1459, 1320, 1300, 1257, 1216, 1148, 1119, 1039, 765, 557 cm^{-1}; ms (direct probe) *m/z* $[M]^+$ 148, 120, 92, 74, 63, 50, 39; ^1H nmr ($CDCl_3$) δ 7.87 (1H, d, H-5, J=9.9), 7.443 (1H, t, H-7), 6.96 (2H, m, H-6, H-8), 4.509 (2H, t, H-2), 2.784 (2H, t, H-3); ^{13}C nmr ($CDCl_3$) δ 191.703 (C-4), 161.764 (C-9), 135.881 (C-7), 127.055 (C-5), 121.285 (C-6, C-10), 117.795 (C-8), 66.938 (C-2), 37.732 (C-3); uv λ max 319.5, 246, 220.

CHROMAN-4-OL [**2**].—Compound **2** was obtained from transformation of chromanone by *A. niger* NRRL 599 in 2% yield: mp (hexane) 75–76°; ir (KBr) 3366, 1611, 1588, 1490, 1469, 1456, 1444, 1419, 1313, 1272, 1253, 1222, 1200, 1187, 1120, 1092, 1061, 1046, 1029, 1061, 1046, 1029, 982, 875, 778, 760, 755 cm^{-1}; ms (direct probe) *m/z* $[M]^+$ 150, 131, 121, 105, 94, 77, 65, 51, 39; ^1H nmr ($CDCl_3$) δ 7.311 (1H, d, H-5, J=8.34), 7.209 (1H, t, H-7), 6.924 (1H, t, H-6), 6.841 (1H, d, H-8, J=8.13), 4.785 (1H, q, H-4), 4.27 (2H, m, H-2), 2 (2H, m, H-3), 1.6 (4-OH); ^{13}C nmr ($CDCl_3$) δ 154.555 (C-9), 129.687, 129.566 (C-5, C-7), 124.278 (C-10), 120.555 (C-6), 117.056 (C-8), 63.257 (C-4), 61.89 (C-2), 30.827 (C-3).

CHROMONE [**3**].—Compound **3** was produced from transformation of chromanone by *A. niger* NRRL 599 in 2% yield: mp (hexane) 55–56°; ir (KBr) 1648, 1616, 1600, 1566, 1461, 1405, 1345, 1316, 1255, 1235, 1190, 1128, 864, 841, 775, 757, 526, 470 cm^{-1}; ms (solid probe) *m/z* $[M]^+$ 146, 118, 120, 92, 89, 63, 50, 38; ^1H nmr ($CDCl_3$) δ 8.207 (1H, d, H-5, J=8.07), 7.848, (1H, d, H-2, J=5.99), 7.668 (1H, t, H-7), 7.44 (2H, m, H-6, H-8), 6.309 (1H, d, H-3, J=6.01).

5-HYDROXYFLAVONE [**6**].—Mol wt 238; mp 158–160°; ir (KBr) 3425, 1654, 1615, 1587, 1475, 1468, 1450, 1413, 1256, 1226, 800, 753 cm^{-1}; ^1H nmr (DMSO-d_6) δ 12.665 (5-OH), 8.121 (2H, d, H-2′, H-6′), 7.65 (4H, m, H-3′, H-4′, H-5′, H-7), 7.209 (1H, d, H-8, J=7.93 Hz), 7.124 (1H, s, H-3), 6.829 (1H, d, H-6, J=8.11); ^{13}C nmr (DMSO) δ 183.207 (C-4), 164.08 (C-2), 159.759 (C-5), 155.906 (C-9), 135.99 (C-7), 132.334 (C-4′), 130.537 (C-1′), 129.175 (C-3′, C-5′), 126.617 (C-2′, C-6′), 110.978 (C-6), 110.132 (C-10), 107.589 (C-8), 105.672 (C-3); uv spectrum λ max 335, 269, 204.5 nm.

5,4′-DIHYDROXYFLAVONE [**7**].—Compound **7** was produced from transformation of 5-hydroxyflavone by *S. fulvissimus* NRRL 1453 B in 36% yield (after 2 days of incubation): mp 258° (mp was compared to that of an authentic sample, 239–240°, obtained from Indofine Chemical, Somerville, New Jersey); ir (KBr) 3345, 1656, 1557, 1475, 1444, 1384, 1266, 1247, 1225, 1181, 831 cm^{-1}; ms (direct probe) *m/z* $[M]^+$ 254, 226, 197, 169, 137, 136, 121, 118, 108, 93, 79, 65, 52, 39; ^1H nmr (DMSO-d_6) δ 12.821 (1H, s, 5-OH), 10.5 (4′-OH), 7.971 (2H, d, H-2′, H-6′), 7.638 (1H, t, H-7), 7.14 (1H, d, H-

8), 6.946 (2H, d, H-3′, H-5′), 6.921 (1H, s, H-3), 6.784 (1H, d, H-6); ^{13}C nmr (DMSO) δ 182.942 (C-4), 164.674 (C-2), 103.337 (C-3), 159.386 (C-5), 110.843 (C-6), 135.665 (C-7), 107.364 (C-8), 155.777 (C-9), 120.917 (C-1′), 128.776 (C-2′, C-6′), 116.037 (C-3′, C-5′), 109.913 (C-10), 161.528 (C-4′); uv λ max 327.5, 268, 220.5, 201 nm.

5,3′,4′-TRIHYDROXYFLAVONE [**8**].—Compound **8** was produced from transformation of 5-hydroxyflavone by *S. fulvissimus* NRRL 1453 B in 2% yield (after 2 days incubation): mp 295°; ir (KBr) 3405, 1633, 1591, 1560, 1547, 1523, 1473, 1447, 1433, 1295, 1285, 1257, 1238, 1141, 1124, 1103, 1098, 1057, 1048, 1031, 1010, 838, 622 cm^{-1}; ms (direct probe) *m/z* [M]$^+$ 270, 242, 213, 137, 134, 122, 108, 53, 39, 27; ^{1}H nmr ($CDCl_3$/CD_3OD) δ 7.6 (1H, d, H-7, J=8.24 Hz), 7.548 (1H, s, H-2′), 7.43 (1H, d, H-6′), 7.05 (1H, d, H-8, J=8.32), 6.95 (1H, d, H-5′, J=8.81), 6.803 (1H, d, H-6, J=8.21), 6.649 (1H, s, H-3).

6-HYDROXYFLAVONE [**4**].—Mol wt 238; mp 231–232°; ir (KBr) 3476, 1627, 1594, 1577, 1567, 1475, 1455, 1404, 1376, 1383, 773 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 10.033 (6-OH), 8.056 (2H, d, H-2′, H-6′, J=9.5), 7.637 (1H, d, H-8, J=9 Hz), 7.571 (3H, m, H-3′, H-5′, H-4′), 7.326 (1H, s, H-5), 7.251 (1H, d, H-7, J=9 Hz), 6.942 (1H, s, H-3); ^{13}C nmr (DMSO) δ 177.014 (C-4), 162.199 (C-2), 154.885 (C-9), 149.393 (C-6), 131.644 (C-4′), 131.355 (C-1′), 129.22 (C-3′, C-5′), 126.246 (C-2′, C-6′), 124.232 (C-7), 123.107 (C-10), 119.887 (C-8), 107.456 (C-3), 105.526 (C-5); uv λ max 349, 303, 269.5, 203.5 nm.

6,4′-DIHYDROXYFLAVONE [**5**].—Compound **5** was obtained from transformation of 6-hydroxyflavone by *S. fulvissimus* NRRL 1453 B in 15% yield: mp 338° (dec) (compared to that of an authentic sample, 340°, obtained from Indofine, Somerville, New Jersey); ir (KBr) 3267, 1640, 1621, 1614, 1596, 1585, 1476, 1446, 1376, 1359, 1254, 1248, 1181, 627 cm^{-1}; ms (direct inlet) *m/z* 254, 239, 226, 197, 168, 138, 120, 108, 89, 80, 52, 44, 29; ^{1}H nmr (DMSO-d_6) δ 7.943 (2H, d, H-2′, H-6′, J=8.93), 7.59 (1H, d, H-8, J=8.92), 7.292 (1H, s, H-5), 7.212 (1H, d, H-7, J=8.56), 6.91 (2H, d, H-3′, H-5′, J=8.63), 6.758 (1H, s, H-3); ^{13}C nmr (DMSO) δ 176.799 (C-4), 162.711 (C-2), 160.871 (C-4′), 154.718 (C-9), 149.207 (C-6), 128.186 (C-2′, C-6′), 124.171 (C-10), 122.729 (C-7), 121.713 (C-1′), 119.625 (C-8), 115.928 (C-3′, C-5′), 107.526 (C-3), 103.843 (C-5); uv λ max 326, 274, 227, 203 nm.

7-HYDROXYFLAVONE [**9**].—Mol wt 238; mp 240–241°; ir (KBr) 1633, 1625, 1613, 1584, 1576, 1551, 1512, 1498, 1455, 1384, 1359, 1259, 842, 774, 686 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 10.4 (7-OH), 8.06 (2H, d, H-2′, H-6′, J=7.86), 7.905 (1H, d, H-5, J=9.83), 7.556 (3H, m, H-3′, H-5′, H-4′), 7.016 (1H, s, H-8), 6.944 (1H, d, H-6, J=10.63), 6.906 (1H, s, H-8); ^{13}C nmr (DMSO) δ 176.415 (C-4), 162.792 (C-2), 161.951 (C-7), 157.495 (C-9), 131.551 (C-4′), 131.271 (C-1′), 129.084 (C-3′, C-5′), 126.546 (C-5), 126.163 (C-2′, C-6′), 116.121 (C-10), 115.095 (C-6), 106.61 (C-3), 102.551 (C-8); uv λ max 307, 250.5, 212 nm.

7,4′-DIHYDROXYFLAVONE [**10**].—Compound **10** was produced from transformation of 7-hydroxyflavone by *S. fulvissimus* NRRL 1453 B in 5% yield: mp 320–322° (compared with that of an authentic sample, 324–325°, obtained from Indofine Chemical, Somerville, New Jersey); ir (KBr) 3219, 1631, 1603, 1575, 1561, 1504, 1449, 1386, 1274, 1251, 1237, 1225, 1182, 827 cm^{-1}; ms (direct probe) *m/z* 254, 226, 197, 184, 168, 151, 137, 118, 108, 89, 80, 77, 65, 51, 39, 29; ^{1}H nmr (DMSO-d_6) δ 10.5 (7-OH), 7.9 (2H, d, H-2′, H-6′, J=8.15), 7.847 (1H, d, H-5, J=8.76), 6.95 (1H, s, H-8), 6.914 (2H, d, H-3′, H-5′, J=7.876), 6.707 (1H, s, H-3); ^{13}C nmr (DMSO) 176.243 (C-4), 162.721 (C-2), 162.487 (C-7), 160.695 (C-4′), 157.412 (C-9), 128.057 (C-2′, C-6′), 126.403 (C-5), 121.865 (C-1′), 116.05 (C-10), 115.931 (C-3′, C-5′), 114.851 (C-6), 104.514 (C-3), 102.471 (C-8); uv λ max 328, 253, 230, 205.5 nm.

ACKNOWLEDGMENTS

The authors thank Mr. Tom Krick for technical help in some nmr measurements.

LITERATURE CITED

1. V.L. Singleton, *Adv. Food Res.*, **27**, 149 (1981).
2. J.E. Ferrel, P.D. Changsing, G. Laew, R. King, and T.E. Mansour, *Mol. Pharmacol.*, **16**, 556 (1979).
3. T.N. Kaul, E. Middleton, and P.L. Agra, *J. Med. Virol.*, **15**, 71 (1985).
4. M. Huang, E.F. Johnson, U.M. Eberhand, D.R. Coop, M.J. Coon, and A.H. Conney, *J. Biol. Chem.*, **256**, 10897 (1981).
5. H. Nishino, M. Nagao, T. Fujihiki, and T. Sugimura, *Cancer Lett.*, **21**, 1 (1983).
6. B.N. Ames, *Science*, **221**, 1256 (1983).

7. L.F. Bjeldanes and G.W. Chang, *Science,* **197**, 577 (1977).
8. L.A. Griffiths, in: "Flavonoids: Advances in Research." Ed. by J. Harborne and T. Mabry, Chapman and Hall, London and New York, 1982, pp. 681–718.
9. L.A. Griffiths and S.E. Smith, *Biochem. J.*, **128**, 901 (1972).
10. A.M. Hackett and L.A. Griffiths, *Xenobiotica,* **15**, 907 (1985).
11. R.V. Smith, D. Acosta, and J.P. Rosazza, *Adv. Biochem. Eng.*, **5**, 70 (1977).
12. P.J. Davis, S.K. Yang, and R.V. Smith, *Xenobiotica,* **15**, 1001 (1985).
13. J.B. Reighard and J.E. Knapp, *Pharm. Int.*, **7**, 92 (1986).
14. S.R. Udupa, A. Banerji, and M.S. Chadha, *Tetrahedron,* **25**, 5415 (1969).
15. S.R. Udupa, A. Banerji, and M.S. Chadha, *Tetrahedron Lett.*, **37**, 4003 (1969).
16. S.R. Udupa and M.S. Chadha, *Indian J. Biochem. Biophys.*, **9**, 141 (1972).
17. S.R. Udupa and M.S. Chadha, *Indian J. Biochem. Biophys.*, **15**, 329 (1978).
18. S.R. Udupa and M.S. Chadha, *Indian J. Biochem. Biophys.*, **15**, 333 (1978).
19. A.S. Ibrahim and Y.J. Abul-Hajj, *Xenobiotica,* **20**, 363 (1990).
20. A.S. Ibrahim and Y.J. Abul-Hajj, *J. Nat. Prod.*, **53**, 644 (1990).
21. T.N. Huckerby and G. Sunman, *J. Mol. Struct.*, **56**, 87 (1979).
22. C.G. Shanker, B.V. Mallaiah, and G. Srimannarayana, *Synthesis,* 310 (1983).
23. T.J. Mabry, K.R. Markham, and M.B. Thomas, "Systematic Identification of Flavonoids," Springer-Verlag, Berlin, 1970.
24. D.R. Brannon, F.E.W. Parrish, J.B. Wiley, and L. Long, *J. Org. Chem.*, **32**, 1521 (1976).
25. A.M. Bell, P.C. Cherry, I.M. Clark, W.A. Denny, E.R. Jones, G.D. Meakins, and P.D. Woodgate, *J. Chem. Soc., Perkin Trans. 1,* 2081 (1972).
26. A.M. Bell, W.A. Denny, E.R. Jones, G.D. Meakins, and W.E. Muller, *J. Chem. Soc., Perkin Trans. 1,* 2759 (1972).
27. J.W. Brown, W.A. Denny, E.R. Jones, G.D. Meakins, Y. Morisawa, and A. Penlebury, *J. Chem. Soc., Perkin Trans. 1,* 1493 (1973).

Received 2 April 1990