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# METABOLISM OF SAFFLOR YELLOW B BY HUMAN INTESTINAL BACTERIA

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ABSTRACT.—Safflor yellow B [**1**], a *C*-glycosyl quinochalcone isolated from the flower petals of safflower (*Carthamus tinctorius*), was transformed to carthamin [**2**], hydroxysafflor yellow A [**3**], and safflor-metabolin [**4**] by anaerobic incubation with human intestinal bacteria including *Peptostreptococcus anaerobius*.

Recently, interest in drug and food colorants has been directed to the use of naturally occurring pigments. Of these, yellow and red pigments are obtained from the petals of safflower (Benibana in Japanese). The flower pigments are safely applicable to processed foods and soft drinks as naturally harmless color additives. Moreover, the decoction of the flower petals of safflower has been used in traditional Chinese medicine for the treatment of menopausal syndromes and irregular menstruation cycle (1,2) and as a sedative and anti-inflammatory (3,4). Saito and Fukushima (5–8) have studied the stability of carthamin red coloration in aqueous and organic solvents and the exogenous factors controlling it. They also reported an improved technique for large-scale isolation of *Carthamus* pigments from the floral extract. The chemistry of the flower pigments has been extensively studied by many workers (9–18). Takahashi and co-workers (12,13) and Obara and co-workers (14–18) reported the isolation and structure determination of carthamin, safflomin A, safflomin C, safflor yellow A, and safflor yellow B. However, nothing has been reported on the metabolism of these pigments by intestinal flora. Thus, it is deemed of interest to study the transformation of the flower pigments by human intestinal bacteria.

In the present paper, we report the metabolism of a *C*-glycosyl quinochalcone, safflor yellow B, by defined bacterial strains isolated from human feces and by fecal flora of humans.

## RESULTS

Various bacterial strains isolated from human feces were screened for their ability to metabolize safflor yellow B [**1**] by anaerobic incubation at 37° for 4 days (Table 1). All the bacterial strains had the ability to transform **1** to a metabolite **3**. Among them, *Bifidobacterium longum, Bifidobacterium pseudolongum, Gaffkya anaerobia, Peptostreptococcus anaerobius*, and *Streptococcus faecalis* produced three metabolites, **2**, **3**, and **4** ($R_f$ 0.6, 0.33, and 0.48, respectively, in solvent system A), when the products were analyzed by tlc-densitometry. However, without the bacteria, no products were formed by incubation of **1** under the similar experimental conditions.

For the purpose of isolating large amounts of metabolites and determining their structures, *Pe. anaerobisu* was selected to metabolize **1**. The metabolites thus obtained were characterized as follows.

Compound **2** was obtained as a minor metabolite and exhibited an ion peak at *m/z* 909 $[M-1]^-$ in the negative ion fabms, corresponding to the molecular formula $C_{43}H_{42}O_{22}$. The $^1$H-nmr spectrum showed signals due to a β-D-glucopyranose moiety with the anomeric proton at $\delta_H$ 3.88 ($J$=9 Hz); also the $^{13}$C-nmr spectrum (Table 2) showed only twenty-one signal peaks in spite of the molecular formula indicating 43

TABLE 1. Screening of Bacterial Strains Capable of Metabolizing Safflor Yellow B [1].[a]

| Bacterial strain | Compound | | | |
|---|---|---|---|---|
| | **2** | **3** | **4** | **1** |
| *Bacteroides fragilis* ssp. *thetaotus*[b] | — | 0.50 | 0.11 | 0.04 |
| *Bifidobacterium adolescentis*[b] | — | 0.02 | 0.05 | 0.01 |
| *Bifidobacterium breve* S-2 KZ 1287[b] | — | 0.18 | 0.08 | 0.04 |
| *Bifidobacterium bifidium* aE319[b] | — | 0.07 | — | — |
| *Bifidobacterium longum* IV-55[b] | 0.01 | 0.07 | 0.48 | 0.36 |
| *Bifidobacterium pseudolongum* PNC-2-9-G[b] | 0.01 | 0.29 | 0.27 | 0.41 |
| *Clostridium butyricum*[b] | — | 0.08 | 0.08 | — |
| *Clostridium innocuum* ES 24-06[b] | — | 0.46 | 0.19 | 0.38 |
| *Clostridium innocuum* KZ-633[b] | — | 0.29 | 0.09 | 0.11 |
| *Clostridium perfringens* To-23[b] | — | 0.26 | — | 0.11 |
| *Escherichia coli* 0-127[b] | — | 0.25 | — | 0.02 |
| *Fusobacterium nucleatum* G-470[b] | — | 0.21 | 0.23 | — |
| *Gaffkya anaerobia* G-0608[b] | 0.01 | 0.16 | 0.32 | 0.07 |
| *Klebsiella pneumoniae* ATCC 13883 | — | 0.23 | 0.10 | 0.02 |
| *Lactobacillus acidophilus* ATCC 4356 | — | 0.20 | — | — |
| *Lactobacillus brevis* II-46[b] | — | 0.06 | 0.21 | — |
| *Lactobacillus fermentum* ATCC 9338 | — | 0.10 | — | — |
| *Lactobacillus plantarum* ATCC 14917 | — | 0.05 | — | — |
| *Lactobacillus xylosus* ATCC 155775 | — | 0.08 | 0.05 | 0.03 |
| *Peptostreptococcus anaerobius* 0240[b] | 0.01 | 0.68 | 0.15 | 0.13 |
| *Peptostreptococcus intermedius* EBF 77/2[b] | — | 0.14 | — | — |
| *Proteus mirabillis* S2[b] | — | 0.43 | 0.06 | 0.03 |
| *Ruminococcus* sp. P01-3[b] | — | 0.10 | 0.36 | — |
| *Streptococcus faecalis* II-136[b] | 0.01 | 0.20 | 0.09 | — |
| *Veillonella parvula* ssp. *parvula* ATCC 10790 | — | 0.14 | — | — |

[a]Each bacterium was anaerobically incubated in 0.1 M phosphate buffer (pH 7.3) containing safflor yellow B [1] (0.94 μmol/ml) for 4 days at 37°. Table entries are concentrations, μ mol/ml.

[b]Cultures are deposited at Research Instiute for Wakan-Yaku, Toyama Medical and Pharmaceutical University.

carbons, suggesting that **2** is a symmetrical structure with an $sp^2$ methine carbon at $\delta_c$ 126.1 (d, C-16) connecting two equivalent monoglucosyl quinochalcone units. Based on the foregoing findings, **2** was deduced as carthamin, and we subsequently confirmed its structure by comparing its spectral data with those of an authentic sample (10–13).

Compound **3** was obtained as a major metabolite from an aqueous layer after cc and subsequent preparative tlc. The $^1$H-nmr spectrum of **3** showed the presence of two β-D-glucopyranose units with the anomeric protons $\delta_H$ 3.64 ($J$=10 Hz) and 4.21 ($J$=10 Hz), pair of two equivalent aromatic protons at $\delta_H$ 6.77 and 7.40 (AB-type, $J$=8.5 Hz) and trans olefinic protons at $\delta_H$ 7.28 and 7.42 ($J$=16 Hz). Further, a characteristic downfield singlet at $\delta_H$ 18.6 assignable to a hydroxyl proton (at C-3) chelated with the neighboring carbonyl group. The $^{13}$C-nmr spectrum of **3** (Table 2) showed only 27 signal peaks, and the negative ion fabms spectrum exhibited an ion peak at $m/z$ 611 $[M-1]^-$, corresponding to the molecular formula $C_{27}H_{32}O_{16}$, suggesting the cleavage of the quinochalcone dimer **1** to a monomeric unit. Based on these findings, **3** was deduced to be hydroxysafflor yellow A, and we finally confirmed its structure by direct comparison of its spectral data with those of the authentic sample isolated from safflower (19).

Compound **4** was obtained from an EtOAc-soluble fraction. The negative ion fabms exhibited an ion peak at $m/z$ 449 $[M-1]^-$, corresponding to the molecular formula $C_{21}H_{22}O_{11}$. The $^1$H-nmr and $^1$H-$^1$H COSY spectra of **4** showed the presence of a β-D-

TABLE 2. $^{13}$C-nmr Spectral Data for Metabolites **2**–**4** Obtained from Safflor Yellow B [**1**].[a]

| Carbon No. | Compound | | |
|---|---|---|---|
| | **2**[b] | **3**[b,c] | **4**[b] |
| C-1 | 189.5 (s) | 188.9 (s) | 190.3 (s) |
| C-2 | 108.9 (s) | 106.1 (s) | 105.1 (s) |
| C-3 | 191.6 (s) | 195.3 (s) | 195.3 (s) |
| C-4 | 87.7 (s) | 84.5 (s) | 85.3 (s) |
| C-5 | 186.9 (s) | 182.8 (s) | 183.4 (s) |
| C-6 | 111.6 (s)[d] | 99.2 (s) | 91.6 (d) |
| C-7 | 182.3 (s) | 178.9 (s) | 180.9 (s) |
| C-8 | 120.4 (d) | 123.2 (d) | 132.2 (d) |
| C-9 | 140.7 (d) | 135.6 (d) | 136.4 (d) |
| C-10 | 126.4 (s) | 127.3 (d) | 127.2 (s) |
| C-11 | 130.5 (d) | 129.2 (d) | 129.5 (d) |
| C-12 | 115.7 (d) | 115.6 (d) | 115.5 (d) |
| C-13 | 159.6 (s) | 158.3 (s) | 158.5 (s) |
| C-14 | 115.7 (d) | 115.6 (d) | 115.5 (d) |
| C-15 | 130.5 (d) | 129.2 (d) | 129.5 (d) |
| C-16 | 126.1 (d) | | |
| C-17 | 114.7 (s)[d] | | |
| Sugar | | | |
| C-1′ | 83.8 (d) | 85.5 (d) | 85.0 (d) |
| C-2′ | 69.3 (d) | 69.7 (d) | 69.2 (d) |
| C-3′ | 78.3 (d) | 78.2 (d) | 78.1 (d) |
| C-4′ | 69.7 (d) | 69.8 (d) | 69.9 (d) |
| C-5′ | 80.4 (d) | 80.6 (d) | 79.2 (d) |
| C-6′ | 61.9 (t) | 61.1 (t) | 59.9 (t) |
| C-1″ | | 73.8 (d) | |
| C-2″ | | 68.6 (d) | |
| C-3″ | | 78.6 (d) | |
| C-4″ | | 70.9 (d) | |
| C-5″ | | 79.2 (d) | |
| C-6″ | | 61.5 (t) | |

[a]δ values in ppm. Multiplicities of carbon signals were determined by the mean of DEPT method and are indicated as s, d, and t in DMSO-$d_6$.
[b] $^1$H-$^1$H COSY was measured.
[c] $^1$H-$^{13}$C COSY was measured.
[d]May be interchanged.

glucopyranose moiety with the anomeric proton at $\delta_H$ 3.60 (1H, d, $J$=10 Hz), a pair of two equivalent AB-type aromatic protons at $\delta_H$ 6.80 and 7.43 ($J$=9 Hz), and transolefinic protons at $\delta_H$ 7.31 and 7.45 ($J$=9 Hz), indicating that the cinnamoyl side chain was still intact, and a singlet at $\delta_H$ 4.65 (unexchangeable with $D_2O$) attributable to H-6, suggesting the cleavage of *C*-glucosyl bond at C-6. This was further confirmed by the analysis of the $^{13}$C-nmr spectrum of **4**, which showed only 6 peaks at $\delta_c$ 59.9 (t), 69.2 (d), 69.9 (d), 78.1 (d), 79.2 (d), and 85.0 (d) for a glucose unit, eight quaternary $sp^3$ carbons at $\delta_c$ 190.3, 105.1, 195.3, 85.3, 183.4, 180.9, 127.2, and 158.5 assignable to C-1, C-2, C-3, C-4, C-5, C-7, C-10, and C-13, respectively, and three $sp^2$ methines at $\delta_c$ 91.6, 123.6, and 136.4 for C-6, C-8, and C-9, respectively (Table 2). Consequently, the structure of **4** was determined to be a quinochalcone monoglucoside, named safflor-metabolin.

Figure 1 shows the time course of the metabolism of safflor yellow B [**1**] by *Pe. anaerobius*, indicating that safflor yellow B [**1**] was almost consumed by the bacterium

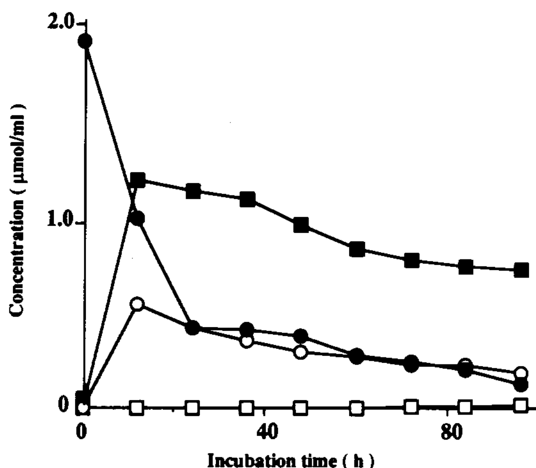



FIGURE 1. Time course of metabolism of safflor yellow B by *Peptostreptococcus anaerobius*: □, carthamin [2]; ■, hydroxysafflor yellow A [3]; ○, safflor-metabolin [4]; ●, safflor yellow B [1].

3 days after the start of incubation. Hydroxysafflor yellow A [3] and safflor-metabolin [4] increased progressively and reached a maximum concentration after 12 h, while carthamin [2] was detected as a minor metabolite after 12 h by tlc.

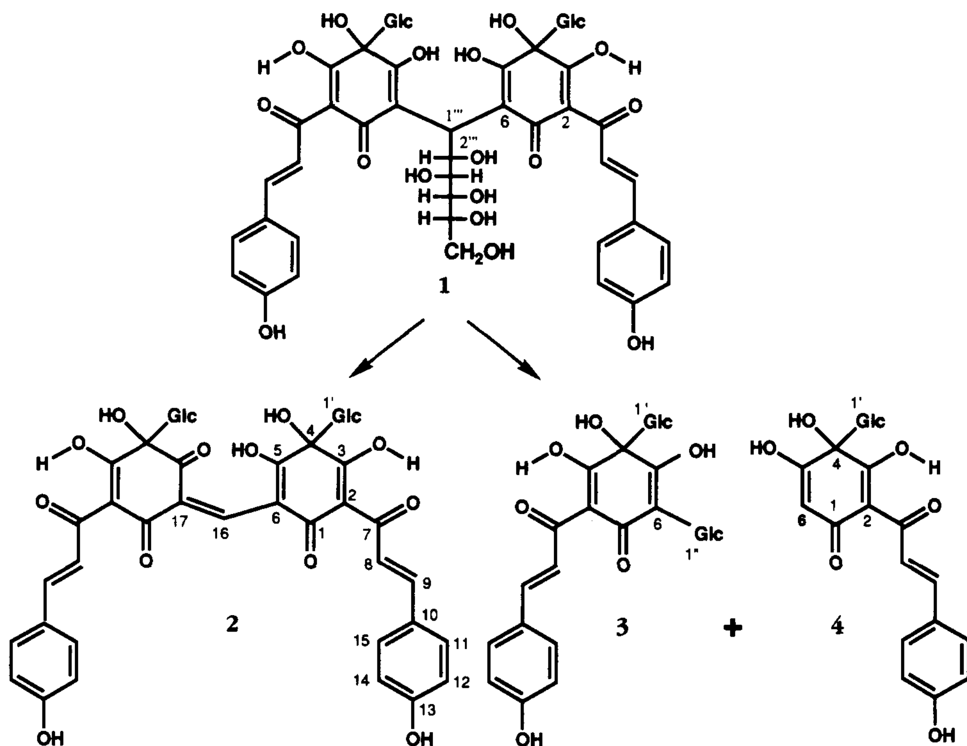
Anaerobic incubation of safflor yellow B [1] with a human fecal suspension revealed that **1** was metabolized in a similar manner, and two metabolites were isolated ($R_f$ 0.67 and 0.15, respectively in solvent system C). Though the metabolites were obtained in extremely poor recovery, these were identified as hydroxysafflor yellow A [3] and safflor-metabolin [4] by direct comparison of the $R_f$ values and $^1$H-nmr spectral data with those of authentic samples.

## DISCUSSION

Safflower (*Carthamus tinctorius* L., Compositae) is one of the common crude drugs used in traditional Chinese medicine. We have isolated three *C*-glycosides from the dried florets of this plant, safflor yellow B [1], carthamin [2], and hydroxysafflor yellow A [3]. Among these pigments, carthamin [2] was unstable in aqueous solutions and its discoloration proceeded more prominently at 37° even in phosphate buffer. Because of this we could not investigate its metabolism by human intestinal bacteria. Further study on the metabolism of stable pigments, **1** and **3**, indicated that neither human feces nor a defined bacterium had the ability to metabolize **3** under the conditions used (data not shown), while safflor yellow B [1] is readily transformed to hydroxysafflor yellow A [3] in addition to safflor-metabolin [4]. Anaerobic incubation of **1** with *Pe. anaerobius* revealed that about 85% of **1** resulted in formation of three metabolites, carthamin [2], hydroxysafflor yellow A [3], and safflor-metabolin [4]. It is supposed that **3** and **4** were produced in equimolar quantities. However, **4** might be metabolized further to other unidentified compounds, so it was detected in relatively low yield. In the major metabolic processes, the reaction may proceed through elimination of a quinochalcone moiety followed by cyclization of the polyol chain to give a pyranose form in the counter part of it. On the other hand, in the minor metabolic processes, **1** may be subjected to elimination of the polyol residue to give the red dimer **2** (Scheme 1).

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Specific rotations were measured with a Jasco DIP-4 auto-



SCHEME 1. The possible metabolic pathways of safflor yellow B [**1**].

matic polarimeter at 25°. Ir spectra were measured on a Hitachi 260–10 infrared spectrometer. Uv spectra were taken on a Shimadzu UV-2200 UV-VIS spectrophotometer. $^1$H- and $^{13}$C-nmr spectra were measured with a JEOL GX-400 spectrometer in DMSO-$d_6$ with TMS as an internal standard, and chemical shifts were recorded in δ values. $^1$H-$^1$H shift correlation spectroscopy (COSY) and $^1$H-$^{13}$C COSY were performed with the usual pulse sequence, and data processing was done with the standard JEOL software. Fabms was measured with a JEOL JMS DX-300 mass spectrometer. Densitometric profiles were recorded on a Shimadzu CS-910 dual wavelength tlc scanner equipped with a Shimadzu C-R6A chromatopac. Sephadex LH-20 (Pharmacia LKB, Sweden) was used for cc. Merck Kieselgel 60 $F_{254}$ (layer thickness 0.25 mm, 0.5 mm) was used for tlc and preparative tlc, respectively, with solvent system A, BuOH–HOAc–$H_2O$ (4:1:2) and solvent system B, EtOAc-MeOH-$H_2O$ (100:16:12), and Kieselgel RP-18 $F_{254}$ S (layer thickness 0.25 mm) with solvent system C, MeOH-$H_2O$ (1:1). Spots were detected under a uv lamp or by spraying with $Ce(SO_4)_2/H_2SO_4$.

PLANT MATERIAL.—The dried flower petals of *Ca. tinctorius* were purchased from Tochimoto Tenkaido Co. (Osaka, Japan), and a voucher specimen is deposited at the Museum of Materia Medica of Toyana Medical and Pharmaceutical University, Toyama.

ISOLATION OF SAFFLOR YELLOW B [**1**] AND OTHER PIGMENTS FROM THE FLOWER PETALS OF SAFFLOWER.—The powdered flower petals (3 kg) of *Ca. tinctorius* were extracted with 60% $Me_2CO$ (5×5 liters) in dark at room temperature for 5 days. The combined $Me_2CO$ solutions were concentrated in vacuo to 3 liters and mixed with cellulose powder (3 kg) to give a reddish brown paste. The paste was washed with $H_2O$ and filtered in vacuo.

The aqueous filtrate (10 liters) was concentrated in vacuo and lyophilized. The lyophilizate (352 g) was dissolved in MeOH (1500 ml) and filtered. EtOAc (3 liters) was added to the filtrate with occasional shaking to give a yellow precipitate (252 g). The precipitate was chromatographed on a column of Sephadex LH-20 (70×6 cm) with 50% MeOH as eluent (flow rate, 8 ml/min), and fractions (300 ml each) were collected. Hydroxysafflor yellow A [**3**] ($R_f$ 0.33; yield 2.8 g, 0.1% w/w from the dried powder of flower petals) was isolated as yellow powder from fractions 2–6 followed by preparative tlc using solvent system A. Safflor yellow B [**1**] was obtained in a yield of 1.5 g (0.05% w/w from the dried powder) as powder, $[\alpha]^{25}$D +208° ($c$=0.1, MeOH), fabms $m/z$ 1061 [M−H]$^-$, from fractions 7–14 by preparative tlc with solvent system A ($R_f$ 0.42). The $^1$H and $^{13}$C nmr of **1** were in agreement with the data reported for safflor yellow B (13).

On the other hand, the cellulose paste after washing with $H_2O$ was eluted with $Me_2CO$, and the red eluate was evaporated to dryness in vacuo. The residue was applied to a column of Sephadex LH-20 (65×2.5 cm) using $H_2O$ containing increased proportions of $Me_2CO$ as eluent to give two fractions. The second fraction was evaporated to afford carthamin [**2**] ($R_f$ 0.49 in solvent system A; yield 900 mg, 0.03% w/w from the dried powder) as reddish brown powder. The $^1$H- and $^{13}$C-nmr spectra were in agreement with those of an authentic sample.

SCREENING OF DEFINED BACTERIAL STRAINS FOR THEIR ABILITY TO METABOLIZE SAFFLOR YELLOW B [**1**].—Precultured bacterial strains (0.2 ml each) were individually incubated for 24 h at 37° in general anaerobic medium (GAM, Nissui Co., Japan) broth (10 ml) under anaerobic conditions in an anaerobic jar in which the air had been replaced with oxygen-free $CO_2$. The culture was diluted tenfold with the same broth and incubated for further 24 h. The pellets obtained by centrifugation at 7800×*g* for 10 min were washed twice with a saline solution, and the bacterial cells were suspended in 0.1 M phosphate buffer (10 ml, pH 7.3). Safflor yellow B [**1**] (10 mg) was added, and the mixture was incubated under the same anaerobic conditions for 4 days at 37°. The incubation mixture was concentrated in vacuo, dissolved in MeOH (2 ml), and filtered through a column of Sephadex LH-20 (10×0.5 cm). The filtrate was evaporated in vacuo to give a residue, and the residue was dissolved in MeOH (0.5 ml). Aliquots (5 μl and 10 μl) of the solution were separately applied to Kieselgel RP-18 plates which were developed with solvent system C. The spots of metabolites were quantitatively analyzed with a tlc scanner at 400 nm and 500 nm (reference wavelengths of 500 nm and 625 nm for yellow and red metabolites, respectively) by using calibration lines obtained with the isolated metabolites. The calibration lines were linear in the range of 0.25–1.0 μg/spot.

METABOLISM OF SAFFLOR YELLOW B [**1**] BY *PE. ANAEROBIUS*—A precultured bacterial suspension (100 ml) of *Pe. anaerobius* was added to GAM broth (900 ml) and cultured overnight at 37° under anaerobic conditions. The bacterial culture was centrifuged at 7800×*g* for 10 min, and pellets were washed twice with saline and suspended in 0.1 M phosphate buffer (900 ml, pH 7.3). Safflor yellow B [**1**] (900 mg) was added to the bacterial suspension, and the mixture was anaerobically incubated for 4 days at 37°. The mixture was then acidified to pH ca. 3 with 5% HCl and extracted with EtOAc (500 ml×4). The EtOAc layer was washed with $H_2O$ and evaporated in vacuo to give a residue. The residue (125 mg, 5%) was applied to a column of Sephadex LH-20 (25×2 cm), and elution with 50% MeOH afforded metabolites in mixture. The crude metabolites dissolved in $H_2O$ were passed through a column of cellulose powder (100 mg), and the column was eluted with 50% MeOH to give compound **2** (3.5 mg). The aqueous filtrate was subjected to preparative tlc with solvent system B to give compound **4** ($R_f$ 0.35, 15 mg).

The aqueous layer after EtOAc extraction was evaporated in vacuo to give a residue, which was applied to a column of Sephadex LH-20 (45×1.5 cm). Elution with 50% MeOH afforded crude compound **3**, which was further purified by preparative tlc (solvent system A, $R_f$ 0.33) to give a pure compound (37 mg).

PREPARATION OF AN INTESTINAL BACTERIAL MIXTURE.—Fresh feces obtained from a healthy subject were thoroughly suspended in 0.1 M phosphate buffer, pH 7.3, and filtered through layers of gauze to remove the sediments. The filtrate was kept under $O_2$-free $CO_2$ by bubbling for 3 min and used for the following experiment as an intestinal bacterial mixture.

INCUBATION OF SAFFLOR YELLOW B [**1**] WITH AN INTESTINAL BACTERIAL MIXTURE.—Safflor yellow B [**1**] (120 mg) was incubated with an intestinal bacterial mixture (150 ml) for 7 days at 37° in an anaerobic jar in which the air had been replaced by $O_2$-free $CO_2$. The incubation mixture was acidified with 5% HCl and extracted with EtOAc (50 ml×4). According to the above-mentioned method, metabolites **3** and **4** were isolated and purified by preparative tlc (solvent system A).

*Compound* **2**.—Reddish brown powder with metallic luster (1.5 mg, 0.16%): $[\alpha]^{25}$D −57.3° (*c*=0.2, 50% $Me_2CO$); ir ν max (KBr) cm$^{-1}$ 3400 (OH), 2900, 1620 (conjugated C=O), 1600, 1500, 1400 (aromatic); uv λ max (MeOH) nm (log ε) 280 (4.80), 380 (6.83), 520 (4.40); fabms *m/z* $[M-1]^-$ 909; $^1$H nmr (DMSO-$d_6$) $\delta_H$ 3.07 (1H, m, H-4′), 3.20 (1H, t, *J*=9 Hz, H-3′), 3.41 (1H, t, *J*=9 Hz, H-2′), 3.51 (1H, m, H-5′), 3,53 (1H, m, H-6′), 3.77 (1H, m, H-6′), 3.88 (1H, d, *J*=9 Hz, H-1′), 5.49 (1H, s, H-16), 6.87 (2H, d, *J*=8.5 Hz, H-12 and H-14), 7.38 (1H, d, *J*=16.5 Hz, H-9), 7.58 (2H, d, *J*=8.5 Hz, H-11 and H-15), 7.65 (1H, d, *J*=16.5 Hz, H-8), 9.37 (1H, s, 13-OH), 19.79 (1H, s, 3-OH); $^{13}$C-nmr see Table 2.

*Compound* **3**.—Yellow amorphous powder (37 mg, 4.1%): $[\alpha]^{25}$D −54.3° (*c*=0.1, MeOH); ir ν max (KBr) cm$^{-1}$ 3400, 2950, 1640 (conjugated C=O), 1610, 1520, 1450; uv λ max (MeOH) nm (log ε) 280 (4.63), 400 (4.43); fabms *m/z* $[M-1]^-$ 611; $^1$H nmr (DMSO-$d_6$) $\delta_H$ 2.91 (1H, m, H-4′), 2.95 (1H, m, H-5′), 3.05 (1H, m, H-5″), 3.08 (1H, m, H-4″), 3.10 (1H, m, H-3′), 3.13 (1H, m, H-3″), 3.31 (1H, m, H-2′), 3.38 (1H, m, H-6′), 3.40 (1H, m, H-6″), 3.60 (1H, m, H-6″), 3.61 (1H, m, H-6′), 3.64 (1H, d, *J*=10 Hz, H-1′), 4.01 (1H, s, 2″-OH), 4.14 (1H, m, H-2″), 4.14 (1H, s, 3″-OH), 4.21 (1H, d, *J*=10 Hz, H-1″), 4.44 (1H, s, 6″-OH), 4.64 (1H, s, 4-OH), 4.69 (2H, s, 2′-OH and H-4″), 4.81 (1H, s, 4′-OH), 4.88 (2H,

s, 3′ and 6′- OH), 6.77 (2H, d, J=9 Hz, H-12 and H-14). 7.28 (1H, d, *J*=16.5 Hz, H-9), 7.4 (2H, d, *J*=9 Hz, H-11 and H-15), 7.42 (1H, d, *J*=16.5 Hz, H-8), 8.29 (1H, s, 13-OH), 9.75 (1H, s, 5-OH), 18.61 (1H, s, 3-OH); $^{13}$C nmr see Table 2.

*Compound* **4**.—Yellow amorphous powder (15 mg, 1.6%): $[\alpha]^{25}D$ −61.4° (*c*=0.1, MeOH); ir $\nu$ max (KBr) $cm^{-1}$ 3400, 2900, 1620, 1600, 1520, 1420, 1380; uv $\lambda$ max (MeOH) nm (log $\epsilon$) 275 (4.49), 400 (4.36); fabms *m/z* $[M-1]^-$ 449; $^1$H nmr (DMSO-$d_6$) $\delta_H$ 2.97 (1H, t, *J*=8.5 Hz, H-5′). 3.07 (1H, m, H-4′), 3.33 (1H, m, H-2′), 3.47 (1H, m, H-6′), 3.58 (1H, m, H-6′), 3.60 (1H, d, *J*=10 Hz, H-1′), 4.65 (1H, s, 6′- OH), 4.65 (1H, s, H-6), 6.80 (2H, d, *J*=9 Hz, H-11 and H-15), 7.31 (1H, d, *J*=16.5 Hz, H-9), 7.43 (2H, d, *J*=9 Hz, H-12 and H-14), 7.45 (1H, d, *J*=16.5 Hz, H-8), 9.82 (1H, s, 5-OH), 18.51 (1H, s, 3-OH); $^{13}$C nmr see Table 2.

TIME COURSE OF THE METABOLISM OF SAFFLOR YELLOW B [**1**].—Safflor yellow B [**1**] (50 mg) was incubated with a precultured bacterial suspension of *Pe. anaerobius* (100 ml) under anaerobic conditions for 4 days. An aliquot (10 ml) was taken out at 12-h intervals and evaporated to dryness in vacuo. The residue was dissolved in MeOH (0.5 ml) and analyzed by tlc densitometry.

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## LITERATURE CITED

1. T. Namba, "Colored Illustrations of Wakan-Yaku," Hoikusha Co., Osaka, 1980, p. 101.
2. "Dictionary of Chinese Materia Medica," Publishing House of Science and Technology of Shang Hai, Shang Hai, 1985, p. 992.
3. N. Kutsuna, S. Fujii, K. Kitamura, K. Komasu, and K. Nakano, *Yakugaku Zasshi,* **108**, 1108 (1988).
4. Y. Kasahara, K. Kumaki, T. Sato, and S. Katagiri, *Shoyakugaku Zasshi,* **43**, 331 (1989).
5. K. Saito and A. Fukushima, *Food Chem.*, **26**, 125 (1987).
6. K. Saito and A. Fukushima, *Acta Soc. Bot. Pol.*, **55**, 639 (1986).
7. K. Saito and A. Fukushima, *Food Chem.*, **32**, 297 (1989).
8. K. Saito and A. Fukushima, *Biochem. Physiol. Pflanzen,* **184**, 145 (1989).
9. C. Kuroda, *Nippon Kagaku Zasshi,* **51**, 237 (1930).
10. C. Kuroda, *J. Chem. Soc.*, 752 (1930).
11. T.R. Seshadri and R.S. Thakur, *Curr. Sci.*, **29**, 54 (1960).
12. Y. Takahashi, T. Miyasaka, S. Tasaka, I. Miura, S. Urano, M. Ikura, K. Hikichi, T. Matsumoto, and M. Wada, *Tetrahedron Lett.*, **23**, 5163 (1982).
13. Y. Takahashi, K. Saito, M. Yanagiya, M. Ikura, K. Hikichi, T. Matsumoto, and M. Wada, *Tetrahedron Lett.*, **25**, 2471 (1984).
14. H. Obara and J. Onodera, *Chem. Lett.*, 201 (1979).
15. H. Obara, J. Onodera, and Y. Kurihara, *Bull. Chem. Soc., Jpn.*, **44**, 289 (1971).
16. H. Obara, J. Onodera, Y. Kurihara, and F. Yamamoto, *Bull. Chem. Soc. Jpn.*, **51**, 3627 (1978).
17. J. Onodera, H. Obara, M. Osone, Y. Maruyama, and S. Sato, *Chem. Lett.*, 433 (1981).
18. J. Onodera, H. Obara, R. Hirose, S. Matsuba, N. Sato, S. Sato, and M. Suzuki, *Chem. Lett.*, 1571 (1989).
19. M.R. Meselhy, S. Kadota, Y. Momose, A. Kusai, M. Hattori, and T. Namba, *Chem. Pharm. Bull.*, in press.

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