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NEW 3-HYDROXYFLAVANONE (DIHYDROFLAVONOL) PHYTOALEXINS FROM THE PAPILIONATE LEGUME *SHUTERIA VESTITA*

JOHN L. INGHAM

Department of Botany, Plant Science Laboratories, University of Reading, Whiteknights, P.O. Box 221, Reading RG6 2AS, England

SATOSHI TAHARA

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University, Kita-ku, Sapporo 060, Japan

and STANLEY Z. DZIEDZIC*

Department of Food Science, Food Studies Building, University of Reading, Whiteknights, P.O. Box 226, Reading RG6 2AP, England

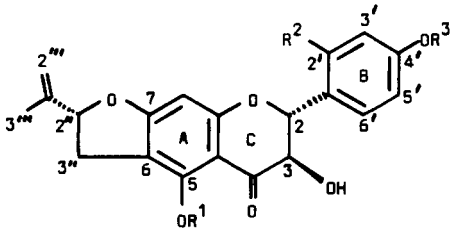
ABSTRACT.—Diffusates from the fungus-inoculated leaflets of *Shuteria vestita* have yielded four novel 3-hydroxyflavanone (dihydroflavonol) phytoalexins. From spectroscopic and chemical evidence, three of these phytoalexins have been identified as (2*R*,3*R*,2″*R*)—3,5,4′-trihydroxy-2′-isopropenyldihydrofurano(4″,5″; 6,7)flavanone (shuterol, **1**), (2*R*,3*R*)—3,5,7,4′-tetrahydroxy-6-(3,3-dimethylally)flavanone (shuterin, **2**), and (2*R*,3*R*,2″*R*)—3,5,2′,4′-tetrahydroxy-2″-isopropenyldihydrofurano(4″,5″; 6,7)flavanone (shuterone A, **3**). The fourth compound (shuterone B, **4**) is considered to be the 2*S*,3*R* stereoisomer of shuterone A.

A large number of papilionate legumes have now been found to produce antimicrobial chemicals (phytoalexins) in response to inoculation with fungi and bacteria (1,2). Although many of these compounds are isoflavonoids (2), it is known that other types of phytoalexin also accumulate in some leguminous species. Thus, stilbenoids (stilbenes or stilbene-2-carboxylic acids) occur in genera such as *Arachis* (1) and *Cajanus* (3), benzofurans in *Coronilla, Tetragonolobus,* and *Vigna* (1), chromones in *Lathyrus* (4), and furanoacetylenes in both *Vicia* and *Lens* (5,6). However, despite their widespread occurrence in higher plants, flavonoid (as opposed to isoflavonoid) derivatives have been implicated as legume phytoalexins on relatively few occasions, the chemicals concerned being liquiritigenin (7,4′-dihydroxyflavanone) and possibly the corresponding chalcone (isoliquiritigenin) in *Medicago sativa* and *Medicago lupulina* (7), odoratol and methylodoratol, two unusual α-hydroxydihydrochalcones, in *Lathyrus odoratus* (8), and pinostrobin chalcone in *Cajanus cajan*(3). In addition, liquiritigenin, isoliquiritigenin, and 2′-*O*-methylisoliquiritigenin have also been obtained as stress metabolites from *Pisum sativum* seedlings treated with aqueous $CuCl_2$ (9,10).

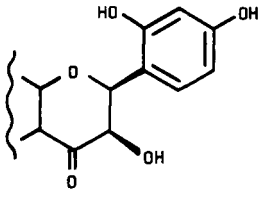
Previous studies have demonstrated that species belonging to the genus *Glycine* (Leguminosae-Papilionoideae; tribe Phaseoleae; subtribe Glycininae) characteristically produce isoflavonoid (6a-hydroxypterocarpan) phytoalexins when inoculated with microorganisms or treated with $CuCl_2$ (11-13). Species from other genera (e.g., *Cologania, Dumasia, Neonotonia, Pseudovigna, Pueraria,* and *Teramnus*) currently assigned to the subtribe Glycininae (14) also exhibit a strong tendency to accumulate isoflavonoid (pterocarpan and/or isoflavone) phytoalexins (15,16). However, we have now discovered that *Shuteria vestita* Wight & Arn., an Asian legume closely related to species of *Cologania* and *Dumasia*, affords four nonisoflavonoid phytoalexins (shuterol, shuterin, shuterone A, and shuterone B) following inoculation of its leaflets with a conidial suspension of the fungus *Helminthosporium carbonum* Ullstrup. In this paper we present spectroscopic and chemical evidence to show that these *Shuteria* phytoalexins are new 3-hydroxyflavanones (dihydroflavonols) possessing structures **1-4**, respectively.

RESULTS AND DISCUSSION

EtOAc extracts of diffusates from *H. carbonum*-inoculated *Shuteria* leaflets were chromatographed (silica gel tlc) to yield four spectroscopically (uv in MeOH) similar compounds, shuterol (**1**), shuterin (**2**), shuterone A (**3**), and shuterone B (**4**), all of which strongly inhibited the growth of *Cladosporium herbarum* Fr. on thin-layer plates (17). In contrast, diffusates collected from H_2O-treated (control) leaflets were apparently devoid of antifungal material. The formulation of phytoalexins **1-4** as flavanones rather than isoflavanones was based on the results of a Shinoda color test. Thus, when treated in MeOH solution with granular Mg and concentrated HCl (see Experimental), **1** and the model compounds naringenin (5,7,4′-trihydroxyflavanone) and dihydroquercetin (=taxifolin, 3,5,7,3′,4′-pentahydroxyflavanone) immediately afforded intense purple-pink colors indicative of the flavanone ring system (18). Shuterin, shuterone A, and shuterone B responded in an identical fashion whereas MeOH solutions of three reference isoflavanones, dalbergioidin (5,7,2′,4′-tetrahydroxyisoflavanone), cajanol (5,4′-dihydroxy-7,2′-dimethoxyisoflavanone), and the 3-hydroxy derivative bolusanthin (3,5,7,3′-tetrahydroxy-4′-methoxyisoflavanone; M.J. O'Neill, personal communication) remained colorless when tested under comparable conditions. From bathochromic uv shifts observed in MeOH+NaOAc, and MeOH+$AlCl_3$, it was evident that while flavanones **1-4** were hydroxylated at C-5, only **2** (shuterin) possessed an underivatized hydroxyl group at C-7 (19).



1 $R^1=R^2=R^3=H$
3 $R^1=R^3=H$; $R^2=OH$
5 $R^1=H$; $R^2=OCH_3$; $R^3=CH_3$
6 $R^1=R^3=CH_3$; $R^2=OCH_3$



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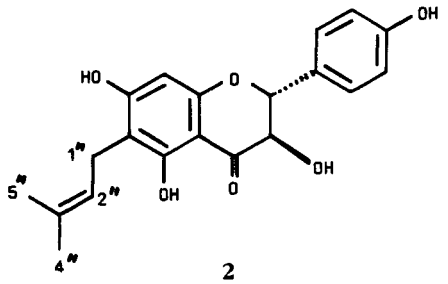

2

In the ^{1}H-nmr spectrum of **1** (shuterol, $[M]^+$ 354), two doublets (both $J=11.6$ Hz) at δ5.08 (1H) and 4.64 (1H) were assigned respectively to the *trans*-diaxially orientated protons (H-2 and H-3) in the heterocyclic (C) ring of a 3-hydroxy substituted flavanone (20) (Table 1). An aromatic singlet, subsequently assigned to H-8, was also apparent (δ5.96, 1H), as was a set of aliphatic proton signals (δ2.86dd, $J=15.4$ and 7.6 Hz, 1H; 3.31dd, $J=15.4$ and 9.5 Hz, 1H; 5.39br t, $J=$ca 8.3 Hz, 1H; 4.93br s, 1H; 5.08br s, 1H; 1.76s, 3H) attributable, as in the isoflavonoid (pterocarpan) phytoalexins apiocarpin (21) and glyceollin III (22), and the lupin root isoflavone lupinisoflavone A (23), to an isopropenyl-dihydrofuran side attachment. The four re-

maining signals appeared as two low-field doublets with chemical shift values and coupling constants (δ6.89, 2H, and δ7.42, 2H; both J=8.4 Hz) resembling those respectively obtained in acetone-d_6 for H-3′/5′ and H-2′/6′ of naringenin [δ6.95d, J=8.5 Hz, 2H, and δ7.40d, J=8.5 Hz, 2H (16)] and its 6-isopentenyl derivative [δ6.90d, J=8.8 Hz, 2H, and δ7.39d, J=8.8 Hz, 2H (24)]. These data clearly suggest that shuterol also has a monohydroxylated (C-4′ OH) B-ring, and it follows, therefore, that in addition to the hydroxyl group at C-5, ring A must carry the isopropenyl-dihydrofuran substituent. This was readily placed at C-6/C-7 (linear attachment) from the blue-green color slowly given by **1** on thin-layer plates treated with methanolic Gibbs reagent/NH_3 vapor [cf. the identical response previously noted for the dimethyl ether of lupinisoflavone A (23)], a result consistent with the presence of an unsubstituted (C-8) position *para* to the H-bonded (C-5) hydroxyl group (25).

The absolute stereochemistry of shuterol at C-2 and C-3 was established by comparison of its cd spectrum with that of *trans*-(2*R*,3*R*)-dihydroquercetin which, in line with similar 3-hydroxyflavanones, exhibits a positive Cotton effect (+8210 at $[\theta]_{331}$), and a negative Cotton effect (−29940 at $[\theta]_{296}$) (26,27). Cd values obtained for **1** ($[\theta]_{334}$=+8800, and $[\theta]_{299}$=−31200) were comparable with those given by dihydro quercetin, and, hence, shuterol similarly possesses the 2*R*,3*R* absolute configuration. Finally, cd measurement of the osmate ester of **1** (see Experimental for details) gave a negative [θ] value (−3100) at 474 nm indicative of an *R* sidechain (C-2″) stereochemistry as in the model compound 2′*R*-rotenone} $[\theta]_{474}$=−5200 (16); reported, −5800 (22)}. In contrast, lupinisoflavone A (23) of previously unknown stereochemistry gave $[\theta]_{480}$=+2100 and thus has the alternative 2″*S* absolute configuration. The structure of shuterol, a major phytoalexin produced by *S. vestita* leaflets, can therefore be represented by **1**.

The presence of an isopentenyl (3,3-dimethylallyl) side chain in compound **2** (shuterin, $[M]^+$356) was evident from the diagnostic ^{1}H-nmr signals at δ1.75s and 1.61s (both 3H, 2×CH_3), 3.25br d (2H, CH_2) and 5.26br t (1H, CH), and the prominent ms fragments at *m/z* 295 (M^+-H_2O-43) and 282 (M^+-H_2O-56). As mentioned earlier, with NaOAc the 297 nm methanolic uv maximum shifted bathochromically to 332 nm [C-7 hydroxyl group (19)], but in other respects this minor flavanone resembled shuterol (**1**). Like **1**, the cd spectrum of **2** ($[\theta]_{334}$=+8900, and $[\theta]_{295}$=−21000) confirmed the expected 2*R*,3*R* absolute stereochemistry, while ^{1}H-nmr chemical shift values (Table 1) similarly indicated that the B- and C-rings of both compounds were identical. The 3,3-dimethylallyl side chain was provisionally placed at C-6 (ring A) by analogy with shuterol, a decision supported by the observation that on thin-layer plates **2** gradually afforded a dark blue Gibbs test color [C-8 *para* to C-5 hydroxyl unsubstituted (25)]. The structure of shuterin is thus defined as (2*R*,3*R*)-3,5,7,4′-tetrahydroxy-6-(3,3-dimethylallyl)flavanone (**2**). Although shuterin has not previously been reported as a natural product, its regio-isomer 3,5,7,4′-tetrahydroxy-8-(3,3-dimethylallyl)flavanone has recently been obtained by Bohlmann *et al.* (28) from the roots of *Marshallia obovata* (Compositae).

Shuterone A ($[M]^+$ 370), the third and most abundant *Shuteria* phytoalexin, was identified as **3** by a combination of spectroscopic and chemical techniques similar to those described for **1** and **2**. Signals attributable to an isopropenyl-dihydrofuran group and to two *trans*-diaxially orientated protons (δ5.50d, J=11.7 Hz, H-2, and δ4.87d, J=11.7 Hz, H-3) were immediately revealed by the ^{1}H-nmr spectrum (Table 1) as was a 1H singlet with a δ value (5.95) resembling that given by H-8 of shuterol (δ5.96). Although the A- and C-rings of shuterone A were readily formulated as in **1**, it was clear from the ^{1}H-nmr signals at δ6.46 (incomplete doublet), 6.43 (dd, J=8.8 and 2.4 Hz), and 7.33 (d, J=8.8 Hz) that ring B was disubstituted. When treated with CH_2N_2,

TABLE 1. ^{1}H-nmr Data (δ values) for the Flavanone Phytoalexins (**1-4**) Isolated from Leaflets of *Shuteria vestita*[a]

Proton[b]	Compound			
	Shutерol (**1**)	Shuterin (**2**)	Shuterone A (**3**)	Shuterone B (**4**)
FLAVANONE NUCLEUS				
H-2	5.08d (*J*=11.6)	4.97d (*J*=11.5)	5.50d (*J*=11.7	5.66d (*J*=2.0)
H-3	4.64d (*J*=11.6)	4.53d (*J*=11.5)	4.87d (*J*=11.7	4.24d (*J*=2.0)
H-8	5.96s	5.99s	5.95s	6.03s
H-2′	7.42d (*J*=8.4, 2H)	7.40d (*J*=8.5, 2H)	——	——
H-6′			7.33d (*J*=8.8)	7.39d (*J*=8.8)
H-3′	6.89d (*J*=8.4, 2H)	6.87d (*J*=8.5, 2H)	6.46d[c]	6.46d[c]
H-5′			6.43dd (*J*=8.8 and 2.4)	6.40dd (*J*=8.8 and 2.4)
SIDE STRUCTURE				
a) Isopentenyl				
H-1″	——	3.25br d (*J*=7.3, 2H)	——	——
H-2″	——	5.26br t (*J*=ca 7.3)	——	——
H-4″/5″	——	1.75s (3H) 1.61s (3H)	——	——
b) Isopropenyldihydrofuran				
H-2″	5.39br t (*J*=ca 8.3)	——	5.40br t (*J*=ca 8.3)	5.40br t (*J*=ca 8.3)
-3″a	2.86dd (*J*=15.4 and	——	2.87dd (*J*=15.1 and	2.87dd (*J*=15.1 and
H	7.6)	——	7.8)	7.8)
-3″b	3.31dd (*J*=15.4 and 9.5)		3.31dd (*J*=15.1 and 9.8)	3.32dd (*J*=15.1 and 9.8)
H-2‴	4.93br s 5.08br s	——	4.93br s 5.08br s	4.93br s 5.09br s
H-3‴	1.76s (3H)	——	1.76s (3H)	1.77s (3H)

[a]Spectra were determined at 100 mHz in acetone-d_6 (internal standard, TMS) using a JEOL FX-100 spectrometer. Except where indicated by 2H or 3H in parentheses, all signals integrated for one proton. Coupling constants (*J*) are in Hz.

[b]For flavanone nucleus and side structure numbering systems, see **1** and **2**.

[c]Incomplete doublet. Coupling constant (*J*) not determined.

shuterone A was mainly converted to the phenolic (C-5 hydroxyl free) dimethyl ether **5** ([M]$^+$ 398), thereby allowing two hydroxyl groups to be situated on ring B. One of these was assigned to C-4′ as in shuterol (**1**) and shuterin (**2**) whilst the other was placed at C-2′ following a ^{1}H-nmr comparison with dihydroquercetin which has hydroxyl groups (ring B) at the alternative C-3′ and C-4′ positions. Thus, whereas the three B-ring protons of shuterone A afforded signals at δ6.43 (H-5′), 6.46 (H-3′), and 7.33 (H-6′), those of dihydroquercetin and its 6-methyl derivative (29) resonated as a complex low-field multiplet between δ6.80 and 7.10 (H-2′/5′/6′). The possibility that shuterone A might be hydroxylated at C-2′ and C-5′ was discounted because on thin-

layer plates the compound rapidly afforded an intense purple-blue (gradually becoming deep blue) Gibbs test color inconsistent with this B-ring substitution pattern.

Finally, evidence for the 2*R*,3*R*,2″*R* absolute stereochemistry was provided by the cd curve of **3** ($[\theta]_{332}=+6000$, and $[\theta]_{298}=-17700$) and its osmate ester derivative ($[\theta]_{474}=-2400$) (see configuration discussion for shuterol, **1**). Shuterone A is thus 2′-hydroxyshuterol (**3**), the linear side structure disposition being supported by the slow, blue-green Gibbs test color given by the dimethyl derivative (**5**) (23).

The remaining phytoalexin, shuterone B (**4**; $[M]^+$ 370), was an isomer of shuterone A which it resembled in many respects (see Experimental section). Chemical shift values (^{1}H nmr) obtained for the side structure and aromatic ring protons were identical, or in very close agreement, with those assigned to the corresponding protons of **3**, but differences in the heterocyclic ring signals of **3** and **4** were readily apparent (Table 1). In **3**, the H-2 and H-3 protons each resonated as a doublet with $J=11.7$ Hz reflecting the *trans*-diaxial orientation normally found in naturally occurring 3-hydroxyflavanones (dihydroflavonols). However, both H-2 (δ5.66d) and H-3 (δ4.24d) of shuterone B had a coupling constant of 2.0 Hz which suggests that in this compound the C-ring protons are related in a *cis* axial-equatorial fashion.

Although the absolute configuration at C-2″ was not determined because of a lack of material for conversion to the osmate ester, cd measurements involving the underivatized flavanone gave a negative first Cotton effect ($[\theta]_{346}=-8500$) and a positive second Cotton effect ($[\theta]_{314}=+10100$). If the *cis*-orientation at C-2/C-3 is assumed to be correct, these data provide evidence for the 2*S*,3*R* absolute stereochemistry shown in **4**. Methylation of shuterone B afforded a phenolic, weakly Gibbs-positive (blue-green) dimethyl ether ($[M]^+$ 398), thereby permitting the alkyl side attachment to be situated linearly as in shuterol and shuterone A.

Diffusates from *H. carbonum*-inoculated *Shuteria* leaflets invariably contained shuterone A (**3**) as the major antifungal component [ca 55 μg/ml based on $\epsilon=17700$ at 294 nm for 6-isopentenylnaringenin (24)]. Shuterol (**1**) likewise accumulated in considerable quantities (about 35 μg/ml), but shuterin (**2**; about 12 μg/ml) and shuterone B (**4**; about 5 μg/ml) were both relatively minor diffusate constituents. In the phytoalexin synthesizing leaf tissues of *S. vestita*, **2** is presumably converted to **1** by side-chain cyclization as suggested for the formation of glyceollin III from 3,6a,9-trihydroxy-2-(3,3-dimethylallyl)pterocarpan (glyceocarpin) (2,13) in soybean (*Glycine max*). Subsequent 2′-hydroxylation of **1** would then yield **3**. Alternatively, it is possible that the latter flavanone might arise from the 2′-hydroxy analogue of **2**, although no evidence was obtained during the present study to indicate that such a compound occurred in *S. vestita* diffusates.

EXPERIMENTAL

INSTRUMENTATION.—Uv spectra were recorded using either a Pye-Unicam SP 1800 or a Hitachi EPS-3T instrument. Ms and cd data were obtained respectively on a JEOL JMS-D300 spectrometer (70 eV; direct inlet system) and a JASCO J-20A Automatic Recording Spectropolarimeter. Details of ^{1}H-nmr instrumentation are given in Table 1.

PLANT MATERIAL.—Seeds of *S. vestita* (originally collected during 1978 near the town of Yercaud in southern India) were obtained from the Krukoff Seed Collection (Ref. No. 130) held at the Royal Botanic Gardens, Kew, England. Only one seed germinated successfully, the resulting plant being grown under conditions similar to those previously described (30). Phytoalexins **1-4** were isolated from detached, fungus-inoculated leaflets using the standard drop-diffusate procedure (31,32). Cultures of *H. carbonum* (phytoalexin inducing fungus) and *C. herbarum* (tlc bioassay fungus) were maintained as reported elsewhere (17).

ISOLATION AND PURIFICATION OF PHYTOALEXINS **1-4**.—Diffusates (48 h) collected from *H. carbonum*-inoculated *Shuteria* leaflets (31,32) were shaken (3×) with equal volumes of EtOAc, and the organic

fractions were then combined and reduced to dryness in vacuo (40°). Si gel tlc (Merck pre-coated plates, glass-backed, F-254, layer thickness 0.25 mm) of the residue in $CHCl_3$-MeOH (20:1, v/v) afforded three well separated bands at Rf 0.46 (SHU-B1), 0.27 (SHU-B2), and 0.16 (SHU-B3). After elution (MeOH), the components of these bands were purified as follows: (a) SHU-B1; Si gel tlc in *n*-pentane-Et_2O-glacial HOAc-MeOH (PEAM; 75:25:6:2, v/v; Rf 0.40) followed by elution (MeOH) and further tlc in C_6H_6-MeOH (BM; 9:1, v/v; Rf 0.36) gave pure shuterol (**1**), (b) SHU-B2; tlc in PEAM (Rf 0.34) and BM (Rf 0.25) as described for SHU-B1 yielded shuterin (**2**), and (c) SHU-B3; tlc in PEAM (Rf 0.12) and BM (Rf 0.20) gave single bands, but with $CHCl_3$-Me_2CO-EtOAc-MeOH (15:5:5:1, v/v) separation occurred to afford shuterone A (**3**; Rf 0.34) and very small amounts of shuterone B (**4**; Rf 0.40). Compounds **1-4** did not accumulate when droplets of deionized H_2O were incubated on detached *Shuteria* leaflets.

2*R*,3*R*-SHUTEROL (**1**).—Color and rate of color development on thin-layer plates treated with diazotized *p*-nitroaniline reagent (33), or Gibbs reagent/NH_3 vapor (23): orange/rapid, and blue-green/slow, respectively. Appearance on chromatograms viewed under long wavelength uv (ca 365 nm) light: blue-black fluorescence gradually changing to dull yellow after fuming (15-20 sec) with NH_3 vapor. Uv λ max nm MeOH 206 sh (83%), 220 (100%), 227 sh (94%), 237 sh (70%), 242 sh (60%), 298 (67%), 343 sh (10%); MeOH+NaOH, 207, 245, 298, 355; MeOH+$AlCl_3$, principal maximum 324. The MeOH spectrum of **1** was unaffected by addition of powdered NaOAc; ms *m/z* 355, 354 ($[M]^+$; $C_{20}H_{18}O_6$), 337, 336 (M^+-H_2O), 326, 325 (M^+-CHO), 322, 321 (M^+-H_2O-CH_3), 294, 293, 233, 232, 220, 219 (A-ring fragment from RDA fission; base peak) 217, 192, 189, 177, 175, 161, 136 (B-ring fragment from RDA fission), 134, 107. For ^{1}H nmr data, see Table 1. Cd (underivatized **1**) $[\theta]_{334}$+8800, $[\theta]_{321}$ 0, $[\theta]_{299}$−31200, $[\theta]_{276}$ 0, $[\theta]_{263}$+4500 (0.60 mg in 24 ml of MeOH determined at 23°). Comparable cd data obtained for 2*R*,3*R*-dihydroquercetin (3,5,7,3′,4′-pentahydroxyflavanone) were $[\theta]_{331}$+8200, $[\theta]_{314}$ 0, $[\theta]_{296}$−29900, $[\theta]_{275}$ 0, $[\theta_{256}$+5000 (3.0 mg in 150 ml of MeOH; 23°) [reported, $[\theta]_{328\text{-}330}$+6620, $[\theta]_{295}$−23200, $[\theta]_{255}$+3970 (26)]. Whitish plates from Me_2CO, mp (uncorr.) 171-173° (micro hot-plate method).

2*R*,3*R*-SHUTERIN (**2**).—Color and rate of response with diazotized *p*-nitroaniline reagent, and Gibbs reagent/NH_3 vapor: orange-yellow/rapid, and dark blue/slow, respectively. Long wavelength uv fluorescence: blue-black, unaffected by fuming (15-20 sec) with NH_3 vapor. Uv λ max nm MeOH, 205 (100%), 230 sh (61%), 243 sh (36%), 297 (38%), 343 sh (9%); MeOH+NaOH, 208, 249, 333; MeOH+NaOAc, 257 sh, 332 (addition of solid boric acid regenerated the MeOH spectrum); MeOH+$AlCl_3$, principal maximum 322. Ms *m/z* 357, 356 ($[M]^+$; $C_{20}H_{20}O_6$), 339, 338 (M^+-H_2O), 323 (M^+-H_2O-CH_3), 295 (M^+-H_2O-C_3H_7), 282 (M^+−H_2O-C_4H_8), 271, 234, 232, 231, 221, 217, 203, 194, 179, 165 (A-ring fragment from RDA fission; base peak), 151, 139, 136 (B-ring fragment from RDA fission), 134. For ^{1}H-nmr data, see Table 1. Cd $[\theta]_{333}$+8900, $[\theta]_{315}$ 0, $[\theta]_{295}$−21000, $[\theta]_{279}$ 0, $[\theta]_{256}$+7400 (0.25 mg in 9 ml of MeOH determined at 23°).

2*R*,3*R*-SHUTERONE A (**3**).—Color and rate of response with diazotized *p*-nitroaniline reagent, and Gibbs reagent/NH_3 vapor: orange/rapid, and purple-blue/rapid, respectively. Long wavelength uv fluorescence as given for shuterol (**1**); uv λ max nm MeOH, 207 (100%), 218 sh (75%), 237 sh (48%), 243 sh (40%), 297 (43%), 344 sh (9%); MeOH+NaOH, 208, 244 sh, 298, 359; MeOH+$AlCl_3$, principal maximum 322. The MeOH spectrum of **3** was unaffected by addition of powdered NaOAc. Ms *m/z* 371, 370 ($[M]^+$; $C_{20}H_{18}O_7$), 353, 352 (M^+-H_2O), 341 (M^+-CHO), 337 (M^+-H_2O-CH_3), 247, 220, 219 (A-ring fragment from RDA fission; base peak), 203, 193, 192, 177, 152 (B-ring fragment from RDA fission), 151, 150, 124, 123. For ^{1}H-nmr data, see Table 1. Cd (underivatized **3**) $[\theta]_{333}$+9200, $[\theta]_{318}$ 0, $(\theta]_{298}$−23500, $[\theta]_{283}$ 0, $[\theta]_{265}$+5600 (2.15 mg in 72 ml of MeOH determined at 23°). *Dimethyl ether* **5** (CH_2N_2; Rf 0.87 in $CHCl_3$-Me_2CO-EtOAc-MeOH (CAEM; 100:10:10:1, v/v). Color and rate of response with Gibbs reagent/NH_3 vapor: blue-green/slow. Long wavelength uv fluorescence: dark purple; uv λ max nm MeOH, 217 sh, 225 sh, 236 sh, 243 sh, 297; MeOH+NaOH, ca 240 sh, 288 sh, 299, 364; MeOH+$AlCl_3$, principal maximum 321. Ms *m/z* 399, 398 ($[M]^+$; $C_{22}H_{22}O_7$), 380 (M^+-H_2O), 370, 369 (M^+-CHO), 342, 341, 261, 260, 231, 220, 219 (A-ring fragment from RDA fission; base peak), 180 (B-ring fragment from RDA fission), 178, 151. *Trimethyl ether* **6** (CH_2N_2; Rf 0.81 in CAEM). No response with Gibbs reagent/NH_3 vapor. Long wavelength uv fluorescence: blue-white; uv λ max nm MeOH, 223 sh, 236, 243 sh, 285. The MeOH spectrum of **6** was unaffected by alkali. Ms *m/z* 412 ($[M]^+$; $C_{23}H_{24}O_7$), 394 (M^+-H_2O), 384, 383 (M^+-CHO), 356, 355, 245, 234, 233 (A-ring fragment from RDA fission; base peak), 180 (B-ring fragment from RDA fission), 178, 151.

2*S*,3*R*-SHUTERONE B (**4**).—Color with diazotized *p*-nitroaniline reagent and Gibbs reagent/NH_3 vapor, and long wavelength uv fluorescence, as recorded for shuterone A (**3**). Uv maxima (MeOH, MeOH+NaOH, and MeOH+$AlCl_3$) and ms data obtained for **4** were also comparable with those given by **3**. For ^{1}H-nmr data, see Table 1. Cd $[\theta]_{346}$−8500, $[\theta]_{331}$ 0, $[\theta_{314}$+10100, $[\theta]_{260}$ 0, $[\theta]_{215}$−20100 (0.48 mg in 9 ml of MeOH determined at 23°).

PREPARATION AND CD DETERMINATION OF THE OSMATE ESTERS OF **1** AND **3**.—Stock solutions of CH_2Cl_2+pyridine (3.05 ml+0.1 ml; *A*) and osmic acid+CH_2Cl_2 (4.7 mg in 125 μl; *B*) were first prepared. A dry sample of shuterol (**1**; 0.60 mg) or shuterone A (**3**; 0.50 mg) was dissolved in *A* (63 μl), and *B* (10 μl) was then added. After incubation for 30 min at 23°, the mixture was diluted to 2.8 ml with CH_2Cl_2. The cd curve of the resulting osmate ester was immediately measured over the range 470-480 nm on a spectropolarimeter (see Instrumentation) zeroed with a blank consisting of *A*+*B*. For comparative purposes the cd curve of a comparably treated sample of 2′*R*-rotenone was also determined. Like 2′*R*-rotenone [$[\theta]_{474}$−5200; reported, −5800 (22)], both shuterol (**1**; $[\theta]_{474}$−3100) and shuterone A (**3**; $[\theta]_{474}$−2400) were found to posses the *R* side-chain (C-2″) stereochemistry.

SHINODA TEST.—Each *Shuteria* phytoalexin (200 μg) was transferred in MeOH (0.1-0.15 ml) to a specimen tube, and four small Mg metal granules were added followed by concentrated HCl (1 drop). Rapid effervescence accompanied by the development of a deep purple-pink color indicated that all four compounds were flavanone derivatives. The model flavanones dihydroquercetin and naringenin responded similarly, but no color was produced when the isoflavanones dalbergioidin, cajanol, and homoferreirin were tested under identical conditions.

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