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AN ISOPRENYLATED FLAVANONE FROM *GLYCYRRHIZA GLABRA* AND REC-ASSAY OF LICORICE PHENOLS†

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Key Word Index—*Glycyrrhiza glabra*; Fabaceae; licorice; kanzoh; roots; prenylated pyranoflavanone; kanzonol Z; kanzonol Y; absolute structure; rec-assay; *Bacillus subtilis*.

Abstract—A new prenylated 3-hydroxypyranoflavanone, kanzonol Z, was isolated from cultivated licorice, *Glycyrrhiza glabra*, and the structure elucidated from spectral evidence. The stereochemical structure of kanzonol Y previously isolated from the licorice has been shown to be (α*R*)-3,5′-diprenyl-α,2′,4,4′-tetrahydroxydihydrochalcone by Mosher's method. In a prescreening test for bioactive compounds amongst licorice phenols using a recombinationless mutant of *Bacillus subtilis* M45, seven compounds showed induction activities of DNA damage.

INTRODUCTION

The genus *Glycyrrhiza* consists of *ca*. 30 species in which three species, *G. glabra* in Europe and Asia and *G. uralensis* and *G. inflata* in China, are mainly used for licorice (kanzoh in Japanese). In Western countries, *G. glabra* has been used as flavouring for American-type tobaccos, chewing gums, candies, etc., as a sweetening agent and as a raw material for drugs, such as enoxolone and carbenoxolone. In Japan, *G. glabra* extract containing flavonoids, glabridin, glabrene, etc., is also used as a depigmentation agent in cosmetics and a flavonoid-rich fraction of *G. uralensis* is used as an anti-ulcer drug, Aspalon®. We have examined the phenolic constituents of Chinese licorice (*G. uralensis*, *G. inflata*, *G. aspera*, *G. eurycarpa*, *G. glabra*, etc.) and *G. glabra* cultivated in Japan [1]. In a previous paper, we reported on the isolation and characterization of new isoprenoid-substituted flavonoids, kanzonols U–Y and eight known compounds, from cultivated licorice [2]. In our further research on licorice, six known flavonoids and a new prenylated 3-hydroxypyranoflavanone, kanzonol Z (**1**) were isolated. In this paper, we report the structure of the new compound and the stereochemistry of kanzonol Y (**2**), previously isolated from the same material. We also describe the results from prescreening some licorice phenols using a recombinationless mutant of *Bacillus subtilis* (rec-assay) [3].

RESULT AND DISCUSSION

Four known flavonoids, medicarpin, liquiritigenin, (α*R*)-α,2′,4,4′-tetrahydroxydihydrochalcone and licuraside (isoliquiritigenin 4-*O*-apiosylglucoside) [4–6], were isolated from a methanol extract of the roots of *G. glabra* cultivated in Japan. Two known compounds, euchrenone a_5 and glyinflanin K [7, 8], were isolated from the acetone extract and the new compound (**1**) from the benzene extract.

Kanzonol Z (**1**), $C_{25}H_{26}O_5$, $[\alpha]_D$ −17°, was negative to the ferric chloride test on a TLC plate. Its UV spectrum resembled those of 5-unsubstituted flavanones [9]. The ^{1}H NMR spectrum of **1** showed AMX-type aliphatic and hydroxyl protons (H-2, H-3 and 3-OH), protons of a prenyl group and a 2,2-dimethylpyran ring, *ortho*-coupled aromatic protons (AX-type, A ring), ABX-type aromatic protons (B ring) and a hydroxyl proton (δ 8.46). In the ^{13}C NMR spectrum (Table 1), the oxygenated carbon atoms of the A and B rings were observed between δ 156.3 and 160.5, indicating that these carbons are located at a *meta*-position to each other or at an

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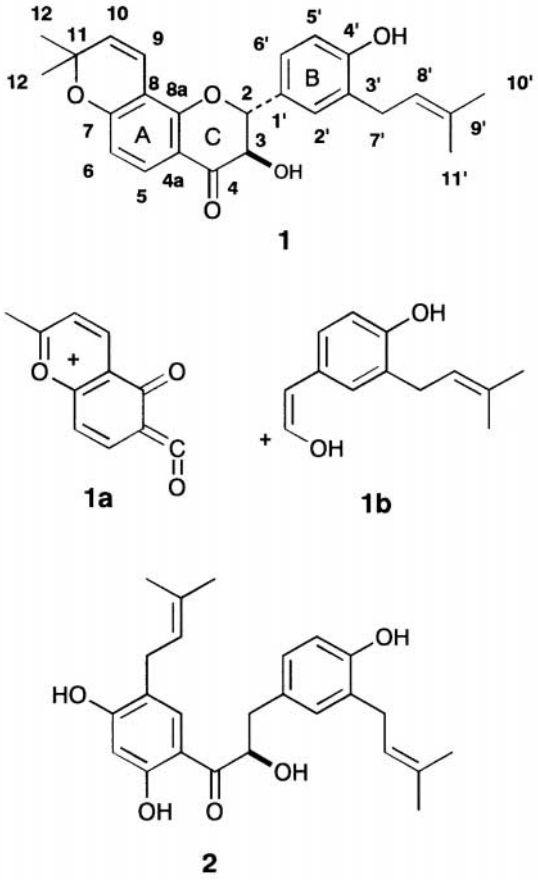
†Part 22 in the series "Phenolic constituents of *Glycyrrhiza* Species". For part 21, see the article by Fukai *et al.* [Fukai, T., Cai, B.-S., Horikosi, T. and Nomura, T., *Phytochemistry*, 1996, **43**, 1119.].

Table 1. ^{13}C NMR data of kanzonol Z (**1**) and 3-hydroxylgrabrol (**3**) in Me_2CO-d_6 (125 MHz)

C	1	3	C	1	3
2	85.1	84.9	1′	128.5	128.4
3	73.8	73.9	2′	130.2†	130.1
4	193.3	193.7	3′	129.2	129.5
4a	113.8	113.1	4′	156.3	156.1
5	128.5	126.6	5′	115.5	115.3
6	111.9	111.0	6′	127.5	127.4
7	158.5*	162.0*	7′	29.1	29.1
8	110.1	116.5	8′	123.6	123.6
8a	160.5*	162.9*	9′	132.5	132.4
9	116.1	22.6	10′	17.8	17.9
10	130.3†	122.8	11′	25.9	25.9
11	78.4	131.8			
12	28.2	17.9			
13	28.5	25.9			

*, †These signals may be interchanged in each compound.

isolated position [10]. The methylene carbon of the prenyl group (C-7′) was observed at δ 29.1, indicating that one of the *ortho*-positions of the prenyl group is substituted by an oxygen function and that the other is unsubstituted [11]. The chemical shifts of the carbon atoms of the B ring of **1** resembled those of 3-hydroxyglabrol [**3**, (2*R*,3*R*)-3′,8-di-prenyl-3,4′,7-trihydroxyflavanone] but those of the A ring carbons of **1** were different from those of **3** (Table 1). The EI-mass spectrum of **1** gave characteristic fragment ions at *m*/*z* 187 (**1a**) and 203 (**1b**). The absolute configuration of **1** was assigned as 2*R*,3*R*, because its CD spectrum showed three positive Cotton effects at 228, 252 and 345 nm and a negative Cotton effect at 311 nm [12]. Thus, the structure of kanzonol Z is deduced as (2*R*,3*R*)-3,4′-dihydroxy-11,11-dimethylpyrano[b-7,8]-3′-prenylflavanone (**1**).



The isolation and structural determination, without stereochemistry, of kanzonol Y (**2**) from cultivated *G. glabra* has been reported recently [2]. The CD curves of **2** resembled to those of synthetic (α*R*)-α,2′,4,4′-tetrahydroxydihydrochalcone [5]. Nevertheless, the CD spectrum of 2′,4,4′-tri-*O*-methyl kanzonol Y (**2a**) was different from that of synthetic (α*R*)-4,4′-dimethoxy-α-hydroxy-2′-methoxymethyldihydrochalcone in shape (bathochromic shift and additional two Cotton effects compared with that of the synthetic compound) and in molecular ellioticity values. Thus, the chirality of the secondary hydroxyl group of **2** was confirmed using Mosher's method by conversion to (*R*)- and (*S*)-MTPA (2-methyl-2-phenyl-2-trifluoromethylacetyl) esters of **2a** and application of the MTPA determination rule [13]. The positive $\Delta\delta_H$-values [$\Delta\delta = \delta_{(S)} - \delta_{(R)}$] observed for the signals of the B ring protons and one of β-protons (Fig. 1) indicated (α*R*)-stereochemistry in **2a**. Certainty of the application of the method for **2a** was confirmed by the following ^1H NMR data. When the (*S*)- and (*R*)-MTPA esters have one of two stable conformations

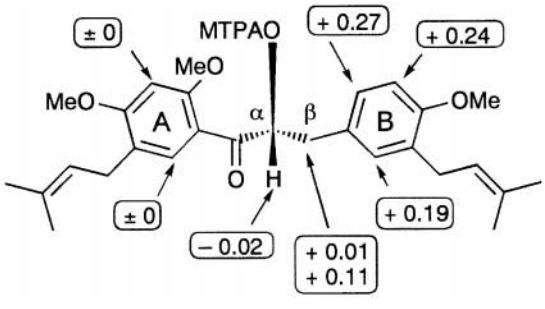


Fig. 1. MTPA ester of compound **2a** ($\Delta\delta_H$-values in ppm).

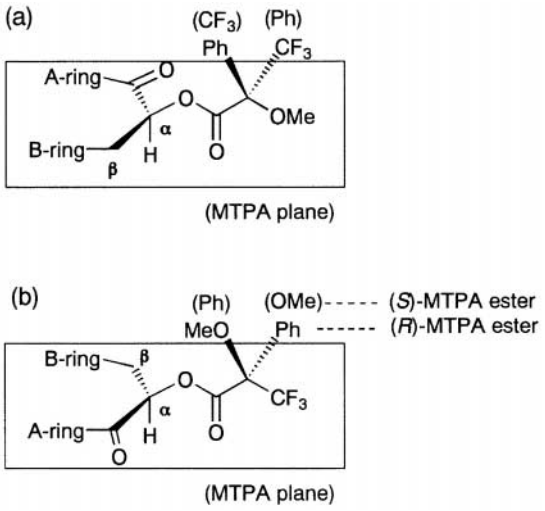


Fig. 2. Stable conformations (a) and (b) of compound **2a**.

[Fig. 2(a)], the methoxyl groups of the MTPA moieties exist on the MTPA-plane and come close to the ester carbonyl [13]. Thus, the chemical shifts of the methoxyl groups of the esters must be almost the same as those of the α-protons ($\Delta\delta_H$: −0.02 ppm). While the signal of the methoxyl protons of the (*S*)-MTPA ester was observed at δ 3.44, that of the (*R*)-MTPA ester appeared more downfield (δ 3.65) due to the anisotropic effect of the carbonyl group of the dihydrochalcone moiety (the methoxyl group of *N*-(*R*)-MTPA–L-valine methyl ester appears at δ *ca* 3.4 and *N*-(*S*)-MTPA–L-valine methyl ester is δ *ca* 3.55 in $CDCl_3$ [14]). Therefore, the conformation of the MTPA esters of **2a** was the other stable conformation [Fig. 2(b)], as well as that of MTPA derivatives of aromatic amino acid esters and an amine, L-phenylalanine ester, L-tryptophan ester and L-phenylalaninol [14]; the MTPA determination rule can be applied to the esters of **2a**. Thus, the structure of kanzonol Y was determined to be (α*R*)-3, 5′-diprenyl-α,2′,4,4′-tetrahydroxydihydrochalcone (**2**).

Rec-assay was developed by Kada *et al.* for screening chemical and environmental mutagens. Recombinationless mutant cells of *Bacillus subtilis* (M45) are more sensitive to the cell-killing action of chemical mutagens, e.g. mytomycin C, *N*-nitroso-*N*-methylurethane, etc., than are the wild-type bacteria (H17) [3]. The assay is also useful for prescreening anti-cancer drugs, such as dynemicines [15, 16]. For the constituents of plants, the assay was modified and used exclusively for the detection of antimutagenic compounds [17]. Since the sensitivity of the rec-assay to chemicals having induction activity of DNA damage is higher than that from other screening techniques, this method may be useful for prescreening of bioactive compounds in crude drugs, as well as in microorganisms. We tried the application of the rec-assay (unmodified) for the detection of bioactive phenolic compounds obtained from licorice (Table 2).

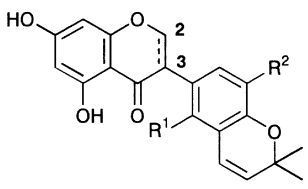
Sixty-nine licorice phenols out of a total of 108 compounds showed inhibitory activity against the growth of both *Bacillus subtilis* H17 and M45 (75 μg per disk). Seven compounds, licoisoflavanone (**4**), licoisoflavone B (**5**), semilicoisoflavone B (**6**), gancaonin C (**7**), isoliquiritigenin (**8**, 2,4,4′-trihydroxychalcone) and 6- or 8-prenylated eriodictyol (**9** and **10**) showed positive results in the rec-assay and 18 compounds weak activities (represented with ± in Table 2). In the prescreening of licorice phenols (Table 2), isoliquiritigenin (**8**) showed a relatively high activity in the rec-assay. The compound also showed activity at a lower concentration (38 μg per disk) but show did not any at low concentration (19 μg per disk). Many bioactivities of this compound, e.g. inhibition of tube formation from vascular endothelial cells on rats, preventive effect on ulcer formation induced by severe necrotizing in rats and anti-platelet action, have been reported [65–67]. From the above relationships between the result of the rec-assay and the known bioactivities, the rec-assay may also be useful as prescreening bioactive compounds from plants.

EXPERIMENTAL

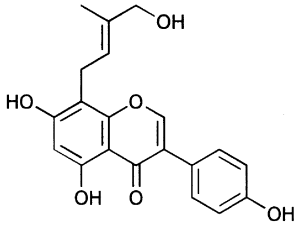
General procedures and instruments used are described in our previous papers [23, 52].

Isolation of flavonoids

Plant materials, extraction and fractionation of the benzene extract using CC on silica gel were as reported in our previous paper [2]. The MeOH



4 R^1 = OH, R^2 = H, no $\Delta^{2,3}$
5 R^1 = OH, R^2 = H, $\Delta^{2,3}$
6 R^1 = H, R^2 = OH, $\Delta^{2,3}$



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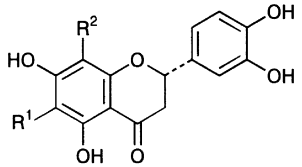

9 R^1 = - $CH_2CH{=}CMe_2$, R^2 =H
10 R^1 =H, R^2 = - $CH_2CH{=}CMe_2$

Table 2. Inhibitory activity against *Bacillus subtilis* H17 and rec-assay of licorice phenols (75 μg/disk)

Trivial name	Inhibition for H17*	Rec-assay (M45-H17)†	Source‡	Refs.
Gancaonin I	+ +	−	Northwest licorice	[18]
Kanzonol U	+	−	*G. glabra*	[2]
Kanzonol V	+	−	*G. glabra*	[2]
(Pterocarpan)				
Shinpterocarpin	+ +	−	*G. glabra* (Chinese)	[19]
Edudiol	+ +	±	*G. glabra* (Kirghyz)	[20]
1-Methoxyphaseollidin	+ +	−	*G. uralensis*	[21]
Medicarpin	+	−	*G. pallidiflora*	[22]
1-Methoxyficifolinol	+	−	*G. uralensis*	[21]
Kanzonol P	+	−	*G. uralensis*	[23]
(Isoflavanone)				
Licoisoflavanone (**4**)	+ +	+	Xinjiang licorice	[24]
Glyasperin B	+ +	−	*G. aspera*	[25]
Glyasperin J	+ +	±	*G. aspera*	[26]
No name (**11**)	+ +	−	Northwest licorice	[27]
No name (**12**)	+ +	−	*G. aspera*	[25]
Kanzonol G	+	−	*G. uralensis*	[28]
Glyasperin K	+	−	*G. aspera*	[29]
Glyasperin M	−	−	*G. aspera*	[29]
(Isoflavan)				
Glabridin	+ + +	±	*G. glabra*	[30]
4′-*O*-Methylglabridin	+ +	±	*G. glabra*	[31]
Glyasperin D	+ +	±	*G. aspera*	[25]
Licoricidin	+ +	−	*G. uralensis*	[32]
Kanzonol R	+ +	−	*G. glabra* (Chinese)	[23]
Kanzonol X	+ +	−	*G. glabra*	[2]
Glyasperin C	+ +	−	*G. aspera*	[25]
Hispaglabridin A	+	−	*G. glabra*	[31]
Kanzonol H	+	−	*G. uralensis*	[28]
Gancaonin Y	+	−	Tiexin gancao	[33]
Kanzonol I	−	−	*G. uralensis*	[28]
Glyinflanin K	−	−	*G. inflata*	[8]
Licorisoflavan A	−	±	*G. uralensis*	[1]
Glyasperin G	−	±	*G. aspera*	[26]
(Isoflav-3-ene)				
Glabrene	+ +	±	*G. glabra*	[34]
Dehydroglyasperin C	+ +	−	*G. aspera*	[1]
(Isoflavone)				
Licoisoflavone B (**5**)	+ +	+	Xinjiang licorice	[24]
Semilicoisoflavone B (**6**)	+ +	+	*G. uralensis*	[21]
Isoderrone	+ +	±	*G. inflata*	[8]
Wighteone	+ +	−	*G. glabra*§	[1]
Licoisoflavone A	+	−	Northwest licorice	[35]
Licoricone	+	−	*G. uralensis*	[36]
Glabrone	+	±	*G. glabra*	[34]
Gancaonin C (**7**)	+	+	*G. uralensis*§	[37]
Angustone B	+	−	*G. inflata*	[8]
Gancaonin G	+	−	Northwest licorice	[18]
Gancaonin H	+	−	Northwest licorice	[18]
8-(γ,γ-Dimethylallyl)- wighteone	+	−	*G. inflata*	[8]
Gancaonin B	−	−	*G. uralensis*§	[37]
Formononetin	−	−	licorice	[38]
Genistein	−	−	*G. glabra*§	[39, 40]
Afromosin	−	−	*G. pallidiflora*	[22]
(3-Arylcoumarin)				
Glycycoumarin	+ +	−	*G. uralensis*	[41]
Kanzonol W	+	−	*G. glabra*	[2]
Glycyrin	−	−	Northwest licorice	[42]
Gancaonin W	−	−	Northwest licorice	[43]

Table 2 (*continued*)

Trivial name	Inhibition for H17*	Rec-assay (M45-H17)†	Source‡	Refs.
(2-arylbenzofuran)				
(Dibenzoylmethane)				
Glyinflanin A	+ +	–	*G. inflata*	[44]
Glyinflanin B	+ +	–	*G. inflata*	[44]
Glyinflanin C	+ +	–	*G. inflata*	[44]
2′-O-Methyllicodione	–	–	*G. pallidiflora*	[45]
(Chalcone)				
Isoliquiritigenin (**8**)	+ +	+ +	*G. glabra*	[46, 47]
Licochalcone A	+ +	–	Xinjiang licorice	[48]
Kanzonol B	+	±	*G. eurycarpa*	[49]
Kanzonol Y	+	–	*G. glabra*	[2]
No name (**13**)	+	–	*G. inflata*	[50]
Echinatin	–	±	Xinjiang licorice	[48]
Licuraside	–	–	*G. glabra*	[6]
Glyinflanin G	–	–	*G. inflata*	[8]
Licochalcone B	–	–	Xinjiang licorice	[48]
Paratocharpin B	–	–	*G. inflata*	[1]
Paratocharpin C	–	–	*G. inflata*	u.d.
(Flavonol)				
Licoflavonol	+ +	–	Northwest licorice	[51]
3-*O*-metylgancaonin P	+ +	–	*G. eurycarpa*§	[52]
Topazolin	+	–	*G. aspera*	[25]
Glyasperin A	+	–	*G. aspera*	[25]
Kaempferol	–	–	*G. macedonica*§,‖	[53, 54]
Quercetin	–	–	*G. macedonica*§,‖	[53, 54]
Fisetin	–	–	*G. uralensis*	[55]
Kumatakenin	–	–	Northwest licorice	[51]
No name (**14**)	–	–	*G. uralensis*§	[50]
No name (**15**)	–	–	*G. eurycarpa*§	[52]
Glepidotin A	–	–	*G. lepidota* (whole plants)¶	[57]
Gancaonin P	–	–	*G. urlensis*§	[58]
(Flavone)				
Gancaonin O	+	–	*G. uralensis*§	[58]
Gancaonin Q	–	–	*G. uralensis*§	[58]
Prenyllicoflavone A	–	–	*G. glabra*	[19]
(Flavanone)				
Pinocembrin	+ +	±	*G. glabra*¶	[59, 60]
Sigmoidin B	+ +	–	*G. uralensis*§	[37]
Paratocarpin L	+ +	–	*G. inflata*	[8]
6-Prenylnaringenin	+ +	–	*G. eurycarpa*§	[52]
Glabrol	+ +	±	*G. glabra*	[30]
3-Hydroxyglabrol (**3**)	+ +	–	*G. glabra*	[31]
Kanzonol S	+ +	–	*G. eurycarpa*§	[52]
No name (**9**)	+ +	+	*G. eurycarpa*§	[52]
No name (**10**)	+ +	+	*G. eurycarpa*§	[52]
Naringenin	+	±	*G. glabra*§,‖	[39, 40]
Liquiritigenin	–	–	*G. glabra*	[61, 62]
Eriodictyol	–	–	*G. eurycarpa*§	[52]
Euchrenone a_5	–	–	*G. inflata*	[8]
Gancaonin E	–	–	*G. uralensis*§	[37]
Isobavachin	–	–	*G. pallidiflora*	[22]
Sophoraflavanone B	–	–	*G. eurycarpa*§	[52]
Kanzonol Z (**1**)	–	–	*G. glabra*	
Ovaliflavanone B	–	–	*G. pallidoflora*	[45]
(Dihydrostilbene)				
Gancaonin R	+ +	±	*G. uralensis*§	[63]
Gancaonin S	+ +	±	*G. uralensis*§	[63]

(continued on next page)

Table 2 (*continued*)

Trivial name	Inhibition for H17*	Rec-assay (M45-H17)†	Source‡	Refs.
(Dihydrophenancerene)				
Gancaonin V	+ +	–	*G. uralensis*§	[63]
Gancaonin U	+	±	*G. uralensis*§	[63]
(Coumestan)				
Glycyrol	–	–	*G. uralensis*	[64]
Isoglycyrol	–	–	*G. uralensis*	[64]
Gancaonin F	–	–	Northwest licorice	[18]

*Diameter of inhibition zone, +: less than 11 mm, + + : between 11 and 18 mm, + + + : more than 18 mm. Diameter of inhibition zone (for both M45 and H17) for kanamycin (10 μg/disk) is 22 mm.

†Difference in diameter of inhibition zone between *Bacillus subtilis* M45 (rec. –) and H17 (rec. +); –: diameters are same, ±: less than 2 mm, +: between 2 and 5 mm, + + : more than 5 mm. A positive control is mitomycin C (0.75 μg/disk); the difference of inhibition zone is 6 mm.

‡First isolation or HPLC detection from licorice. The other sources (*Glycyrrhiza* species) and some revised structures have been described in Ref. [1].

§Isolated from aerial part.

||Commercial available test reagents were used.

extract of roots was partitioned between H_2O and EtOAc. Licurasid (150 mg) was isolated from the H_2O layer (16.5 g) by a published procedure [19]. Medicarpin (49 mg), liquiritigenin (16 mg) and (α*R*)-α,2′,4,4′-tetrahydroxychalcone (2 mg) were isolated from the Me_2CO extract of the roots followed by silica gel CC (*n*-hexane–EtOAc) and then prep. TLC ($CHCl_3$–EtOAc, 5:1, *n*-hexane–EtOAc, 7:3, $CHCl_3$–MeOH, 9:1). Kanzonols U (1 mg), V (0.5 mg), glabrone (2 mg), glyinflanin K (2 mg), shinpterocarpin (3 mg), 4′-*O*-methylglabridin (0.5 mg), euchrenone a_5 (1.5 mg), hispaglabridin A (1 mg) and **16** (1 mg) were isolated from the Me_2CO extract of the roots using a published method [2]. Kanzonol Z (**1**, 6 mg) was isolated from fr. 8 (mother liquor of glabrene) of the CC of the benzene extract of roots [2] by prep. TLC ($CHCl_3$–EtOAc, 5:1, 7:1, $CHCl_3$–MeOH, 20:1). Kanzonol Y (**2**, 15 mg) was isolated from 50 g of commercial licorice extract (P-TH®; CH_2Cl_2-sol. parts of *G. glabra* extract, Marzen Phar. Co., Lot No. 51 126 139) by the method described previously [2].

Bioassay

Biological activities were examined by the rec-assay system using a paper disk method. Each sample was dissolved in MeOH (2 mg ml^{-1}) and then 38 μl of the soln was adsorbed on a paper disk (1.5 mm thick, 8 mm diameter, Toyo Roshi Kaisha). *Bacillus subtilis* H17 (wild, rec. +) and M45 (recombinationless mutant, rec. –) were preincubated in nutrient broth and incubated in nutrient agar (Eiken Chemical Co.) for 24 h. Mitomycin C was used as a positive control and kanamycin (aq. soln) as a negative control in the rec-assay (Table 2). Positive compounds were re-tested 3–5 times.

Kanzonol Z (**1**)

M.p. 141–145.5°C (uncorr.). $[\alpha]_D^{24}$ −17° (*c* 0.058, MeOH). UV λ_{max}^{MeOH} nm (log ε): 206 (4.29), 233 (4.11), 251 (4.10), 268 (4.13), 310 (*sh* 3.60). EI-MS (probe) 70 eV *m*/*z* (rel. int.): 507 $[M + 1]^+$ (4), 406 $[M]^+$ (14), 391 (7), 388 (4), 377 (18), 373 (7), 203 (100), 187 (93), 175 (15), 160 (15); HR-MS *m*/*z*: 406.1776 $[M]^+$ ($C_{25}H_{26}O_5$ requires: 406.1773). ^{1}H NMR (500 MHz, Me_2CO-d_6): δ 1.42, 1.44 (each 3H, *s*, 11-Me), 1.70, 1.71 (each 3H, 9′-Me), 3.36 (2H, *br d*, *J* = 7 Hz, H_2-7′), 4.39 (1H, *d*, *J* = 3 Hz, 3-OH), 4.59 (1H, *dd*, *J* = 3 and 12 Hz, H-3), 5.08 (1H, *d*, *J* = 12 Hz, H-2), 5.39 (1H, *br t*, *J* = 7 Hz, H-8′), 5.71 (1H, *d*, *J* = 10 Hz, H-10), 6.52 (1H, *br d*, *J* = 10 Hz, H-9), 6.52 (1H, *br d*, *J* = 8.5 Hz, H-6), 6.89 (1H, *d*, *J* = 8 Hz, H-5′), 7.28 (1H, *dd*, *J* = 2 and 8 Hz, H-6′), 7.35 (1H, *d*, *J* = 2 Hz, H-2′), 7.64 (1H, *d*, *J* = 8.5 Hz, H-5), 8.42 (1H, *br s*, 4′-OH); (400 MHz, $CDCl_3$): δ 1.45, 1.47 (each 3H, *s*, 11-Me), 1.78, 1.79 (each 3H, *br s*, 9′-Me), 3.41 (2H, *br d*, *J* = 7 Hz, H_2-7′), 3.72 (1H, *d*, *J* = 2 Hz, 3-OH), 4.54 (1H, *dd*, *J* = 2 and 12 Hz, H-3), 5.01 (1H, *d*, *J* = 12 Hz, H-2), 5.36 (1H, *m d*, *J* = 7, H-8′), 5.56 (1H, *d*, *J* = 10 Hz, H-10), 6.54 (1H, *dd*, *J* = 1 and 9 Hz, H-6), 6.58 (1H, *dd*, *J* = 1 and 10 Hz, H-9), 6.87 (1H, *d*, *J* = 8 Hz, H-5′), 7.33 (1H, *dd*, *J* = 2 and 8 Hz, H-6′), 7.30 (1H, *d*, *J* = 2 Hz, H-2′), 7.72 (1H, *d*, *J* = 9 Hz, H-5). CD (*c* 1.18×10^{-4} mol l^{-1}, MeOH): $[\theta]_{228}$ + 10400 (max), $[\theta]_{236}$ + 7700 (valley), $[\theta]_{252}$ + 16800 (max), $[\theta]_{275}$ 0, $[\theta]_{285}$ −7400 (sh), $[\theta]_{311}$ −15300 (max) $[\theta]_{331}$ 0, $[\theta]_{345}$ + 8100 (max), $[\theta]_{383}$ 0.

Kanzonol Y trimethyl ether (**2a**)

Kanzonol Y (**2**, 10 mg) was methylated with Me_2SO_4 (0.5 ml)/dry K_2CO_3 1 g) in Me_2CO (10 ml). The reaction mixt. was purified by prep. TLC

($CHCl_3$–EtOAc, 5:1) to give 4 mg of trimethyl ether (**2a**). EI-MS, *m/z*: 452 $[M]^+$. ^1H NMR (400 MHz, Me_2CO-d_6): δ 1.68, 1.71 (each 3H, *br d*, J = 1 Hz, Me), 1.69, 1.70 (each 3H, *br s*, Me), 2.51 (1H, *dd*, J = 8 and 14 Hz, H-β), 2.98 (1H, *dd*, J = 3.5 and 14 Hz, H-β), 3.23, 3.25 (each 2H, *br d*, J = 7 Hz, H_2-7 and 7′), 3.78, 3.99, 4.05 (each 3H, *s*, OMe), 3.95 (1H, *d*, J = 7 Hz, α-OH), 5.25, 5.27 (each 1H, *br t*, J = 7 Hz, H-8 and 8′), 6.77 (1H, *s*, H-3′), 6.78 (1H, *d*, J = 8 Hz, H-5), 6.93 (1H, *d*, J = 2 Hz, H-2), 6.99 (1H, *dd*, J = 2 and 8 Hz, H-6), 7.63 (1H, *s*, H-6′); (400 MHz, $CDCl_3$): δ 1.69, 1.71 (each 3H, *br s*, Me), 1.72, 1.74 (each 3H, *br d*, J = 1 Hz, Me), 2.58 (1H, *dd*, J = 8 and 14 Hz, H-β), 3.05 (1H, *dd*, J = 3.5 and 14 Hz, H-β), 3.25, 3.28 (each 2H, *br d*, J = 7 Hz, H_2-7 and 7′), 3.80, 3.93, 3.96 (each 3H, *s*, OMe), 5.23-5.31 (2H, *m*, H-8 and 8′), 6.43 (1H, *s*, H-3′), 6.74 (1H, *d*, J = 8 Hz, H-5), 6.93 (1H, *d*, J = 2 Hz, H-2), 6.99 (1H, *dd*, J = 2 and 8 Hz, H-6), 7.73 (1H, *s*, H-6′). CD (c 4.03×10^{-4} mol l^{-1}, MeOH): $[\theta]_{233}$ + 920 (max), $[\theta]_{243}$ + 550 (valley), $[\theta]_{246}$ + 600 (max), $[\theta]_{254}$0, $[\theta]_{270}$−2500 (max), $[\theta]_{285}$−250, $[\theta]_{302}$−930 (max), $[\theta]_{315}$0, $[\theta]_{329}$ + 1700 (max), $[\theta]_{369}$0.

*(**R**)- and (**S**)-MTPA esters of kanzonol Y trimethyl ether (**2b** and **2c**)*

A soln of **2a** (0.9 mg) and (*R*)-MTPA-Cl (6 μl) in dry pyridine (0.1 ml) was stood at room temp. After 14 h, 3-dimethylamino-*n*-propylamine (3.5 μl) was added to the mixt. and the solvent evapd. The product was purified by prep. TLC ($CHCl_3$–EtOAc, 19:1) to give (*R*)-MTPA ester (**2b**, 0.2 mg). The trimethyl ether (**2a**, 3 mg) was acylated with (*S*)-MTPA-Cl (20 μg), as described above, to give 1.2 mg of (*S*)-MTPA ester (**2c**).

(***R***)-*MTPA ester* (**2*b***). ^1H NMR (500 MHz, $CDCl_3$): δ 1.68 (6H, br s, Me_2), 1.70, 1.72 (each 3H, *br s*, Me), 2.84 (1H, *dd*, J = 10 and 14.5 Hz, H-β), 3.13 (1H, *dd*, J = 2 and 14.5 Hz, H-β), 3.17, 3.25 (each 1H, *br dd*, J = 7 and 13 Hz, H-7), 3.25 (2H, *br d*, J = 7 Hz, H_2-7′), 3.79, 3.93, 4.00 (each 3H, *s*, OMe), 5.22, 5.25 (each 1H, *br t*, J = 7 Hz, H-8 and 8′), 6.33 (1H, *dd*, J = 2 and 10 Hz, H-α), 6.45 (1H, *s*, H-3′), 6.55 (1H, *d*, J = 8.5 Hz, H-5), 6.87 (1H, *d*, J = 2 Hz, H-2), 6.79 (1H, *dd*, J = 2 and 8.5 Hz, H-6), 7.78 (1H, *s*, H-6′), MTPA moiety: 3.65 (3H, *s*, OMe), 7.22–7.37 (5H, *m*, C_6H_5).

(***S***)-*MTPA ester* (**2*c***). ^1H NMR (500 MHz, $CDCl_3$): δ 1.70 (6H, *br s*, Me_2), 1.66, 1.72 (each 3H, *br s*, Me), 2.85 (1H, *dd*, J = 10 and 14.5 Hz, H-β), 3.24 (1H, *dd*, J = 2 and 14.5 Hz, H-β), 3.25, 3.29 (each 2H, *br d*, J = 7 Hz, H_2-7′ and 7), 3.84, 3.93, 4.05 (each 3H, *s*, OMe), 5.26 (2H, *br t*, J = 7 Hz, H-8 and 8′), 6.31 (1H, *dd*, J = 2 and 10 Hz, H-α), 6.45 (1H, *s*, H-3′), 6.79 (1H, *d*, J = 8.5 Hz, H-5), 7.06 (1H, *d*, J = 2 Hz, H-2), 7.06 (1H, *dd*, J = 2 and 8.5 Hz, H-6), 7.78 (1H, *s*, H-6′), MTPA moiety: 3.44 (3H, *s*, OMe), 7.21–7.35 (5H, *m*, C_6H_5).

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