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ACYLATED FLAVONOID GLYCOSIDES AND ACCOMPANYING PHENOLICS FROM LICORICE*

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Key Word Index—*Glycyrrhiza uralensis*; Leguminosae; licorice; acylated flavonoid glycoside; vestitol glucoside; licorice-glycoside A–E; ferulic acid; *p*-coumaric acid; indole-3-carboxylic acid; conformation.

Abstract—Fifteen phenolic compounds including eight new flavonoid glycosides were isolated from Tohoku licorice (a kind of commercial licorice, which is regarded as the underground part of *Glycyrrhiza uralensis*). The structures of the new compounds (3*R*)-vestitol 7-*O*-glucoside and acylated flavonoid glycosides (licorice-glycosides A, B, C1, C2, D1, D2 and E), were elucidated. The ^{1}H NMR and CD spectral data of the acylated chalcone apioglucosides suggested the existence of intramolecular stacking of the feruloyl or *p*-coumaroyl groups over the aglycone residue. © 1997 Elsevier Science Ltd

INTRODUCTION

Licorice, the underground part of *Glycyrrhiza* species, is one of the most frequently used natural medicines in traditional Asian medicine [1]. In particular, Tohoku licorice (licorice imported from the north-eastern region of China, of which the source plant has been regarded as *Glychyrrhiza uralensis* [2, 3]) is the licorice of choice in traditional Japanese medicine. Recently, various kinds of pharmacologically active licorice phenolics have been found [3–8]. Further investigation of phenolic components of Tohoku licorice led to the isolation of eight new flavonoid glycosides. Seven of which have both flavonoid aglycone and hydroxycinnamic acid residues in their molecules. This paper deals with structures of these new glycosides.

RESULTS AND DISCUSSION

Tohoku licorice was extracted with methanol, and the ethyl acetate-soluble portion of the methanol extract subjected to counter-current distribution. Further separation of fractions by droplet counter-current chromatography (DCCC), followed by reversed-phase liquid chromatography gave compounds **1**–**8**, together with 3′,4′,7-trihydroxyflavone (**9**), glycyroside (**10**) [9], carpusin (**11**) [10], isoliquiritin (**12**), neoisoliquiritin (**13**) [11], liquiritin apioside (**14**) [12], isoliquiritin apioside (**15**) [13] and tetrahydroxymethoxychalcone (**16**) [14].

Compound **1**, $[\alpha]_D$ −35.5°, was obtained as colourless needles. The FAB mass spectrum showed $[M+H]^+$ and $[M+Na]^+$ ion peaks at *m/z* 435 and 457, respectively. These ion peaks correspond to the molecular formula $C_{22}H_{26}O_9$. The ^{1}H NMR spectrum showed signals at δ 4.23 (*ddd*, J = 2, 4, 10 Hz, H-2), 3.98 (*t*, J = 10 Hz, H-2), 3.46 (*dd*, J = 4, 5.5 Hz, H-3), 2.98 (*dd*, J = 10, 15.5 Hz, H-4) and 2.82 (*ddd*, J = 2, 5.5, 15.5 Hz, H-4), forming a CH_2-CH-CH_2 system, and two sets of ABX protons at δ 7.01 (*d*, J = 8.5 Hz, H-6′), 6.38 (*dd*, J = 2.5, 8.5 Hz, H-5′), 6.97 (*d*, J = 8.5 Hz, H-5), 6.57 (*dd*, J = 2.5, 8.5 Hz, H-6) and 6.50 (2H, *br*, H-3′ and H-8), indicating the presence of an isoflavan residue in the molecule. The spectrum also showed signals of seven protons due to a sugar moiety at δ 3.4–4.9, along with a methoxyl signal at δ 3.69 (3H, *s*). The ^{13}C NMR spectrum of **1** showed sugar carbon signals at δ 61.7 (C-6), 70.4 (C-4), 73.7 (C-2), 76.8, 76.9 (C-3 and C-5) and 101.4 (C-1), indicating that the sugar moiety in the molecule of **1** was β-glucopyranose. The β-orientation of the glycosidic linkage was also shown by the anomeric proton signal at δ 4.97 (*d*, J = 8 Hz) in the ^{1}H NMR spectrum. The location of the methoxyl group at C-4′ in the aglycone residue was shown by NOE correlations of the methoxyl group with H-5′ and H-3′ in the NOESY spectrum. Therefore, the aglycone of **1** is vestitol (**17**) [15]. The formation of vestitol (**17**) on the treatment of **1** with diluted HCl, substantiated this assignment. The CD spectrum of **1** showed a positive

* Part 5 in the series of 'Phenolic Constituents of Licorice'. For Part 4, see Hatano, T., Fukuda, T., Liu, Y.-Z., Noro, T. and Okuda, T., *Yakugaku Zasshi*, 1991, **111**, 311.

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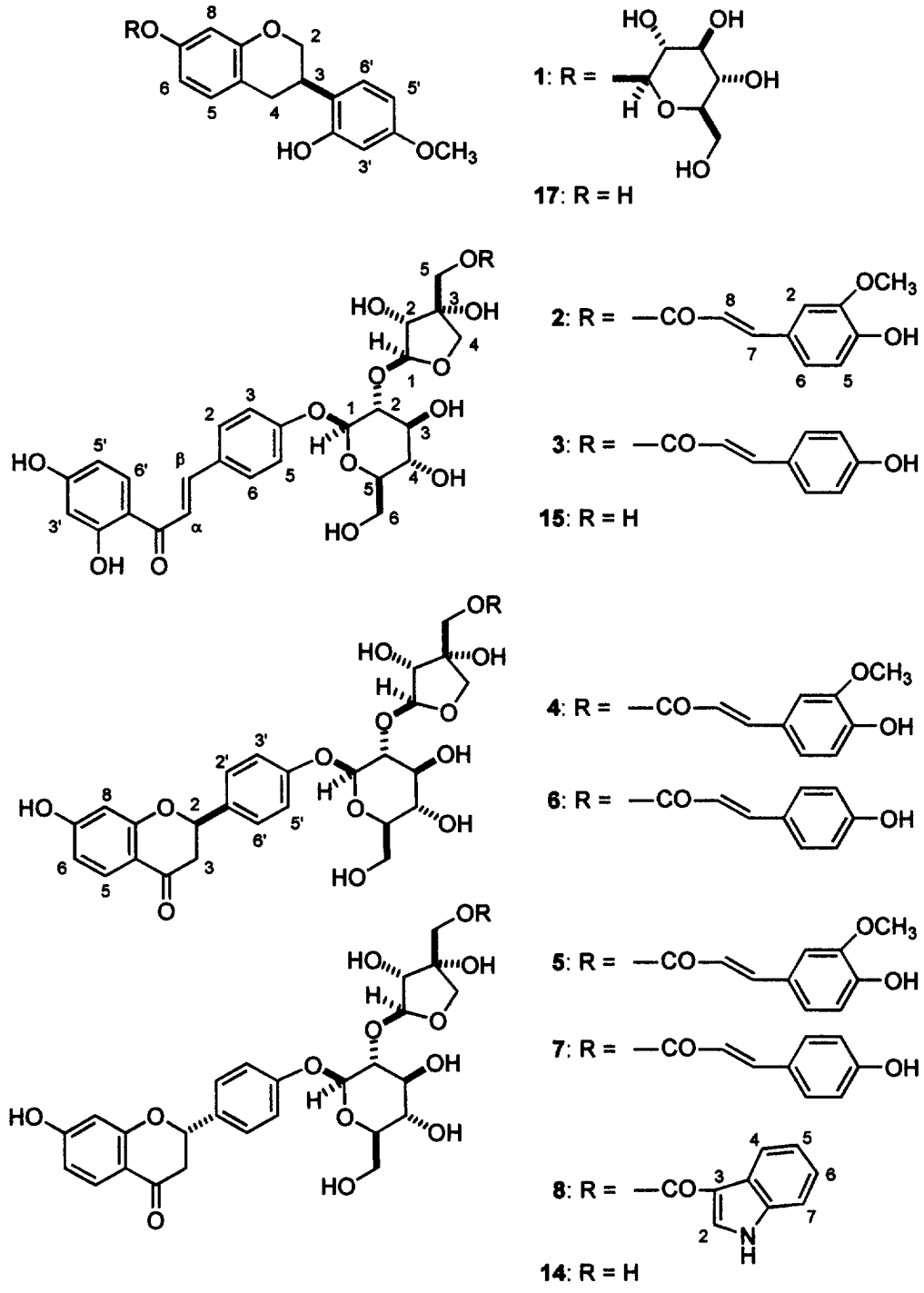
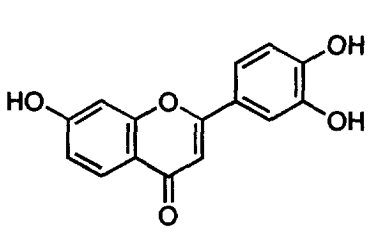


Cotton effect ([θ] $+2.5\times10^3$) at 285 nm, indicating the *R*-configuration at C-3 in the vestitol residue [16].

The position of the glucose moiety on the vestitol residue was assigned to be C-7, based on the downfield shifts of H-6 and H-8 among the vestitol protons in the ^{1}H NMR spectrum of **1**, relative to the corresponding protons of **17** [δ 6.27 (**17**) $\rightarrow$ δ 6.50 (**1**) (H-8); δ 6.35 (**17**) $\rightarrow$ δ 6.57 (**1**) (H-6)]. Downfield shifts of C-4a [δ 113.5 (**17**) $\rightarrow$ δ 116.4 (**1**)], C-6 [δ 107.9 (**17**) $\rightarrow$ δ 109.1 (**1**)] and C-8 [δ 102.8 (**17**) $\rightarrow$ δ 104.6 (**1**)], and a slight upfield shift of C-7 [δ 155.3 (**17**) $\rightarrow$ δ 155.1 (**1**)] among the vestitol carbons in the ^{13}C NMR spectrum also substantiated the assignment. Based on these data, the structure of (3*R*)-vestitol 7-*O*-β-D-glucopyranoside was assigned for compound **1**.

Licorice-glycoside A (**2**), $[\alpha]_D$ $-27.4°$, was obtained as a yellow amorphous powder. The $[M+H]^+$ and $[M+Na]^+$ ion peaks at *m/z* 727 and 749 in the FAB



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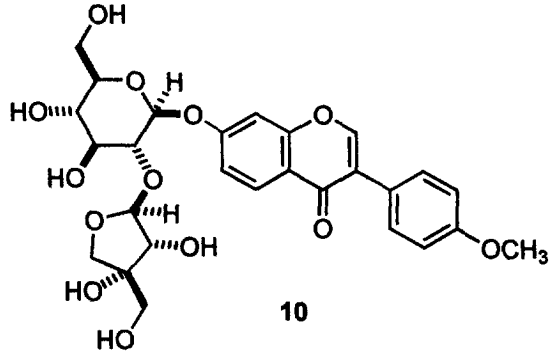

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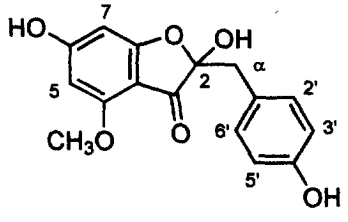

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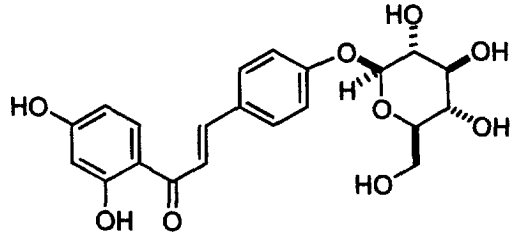

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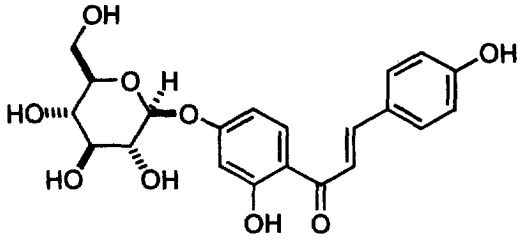

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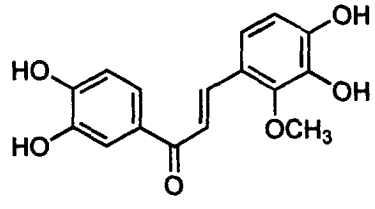

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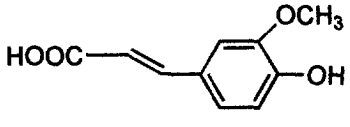

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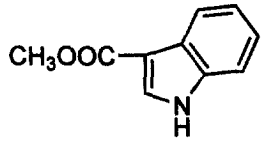

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mass spectrum suggested the molecular formula $C_{36}H_{38}O_{16}$ for this compound. The ^1H NMR spectrum (500 MHz, MeOH-d_4) of **2** suggested that this compound has a structure closely related to isoliquiritin apioside (**15**) [13], as revealed by the signals attributable to protons of isoliquiritigenin [δ 6.33 (1H, *d*, J = 2 Hz, H-3′), 6.43 (1H, *dd*, J = 2, 9 Hz, H-5′), 7.86 (1H, *d*, J = 9 Hz, H-6′) (A-ring), 7.53 (1H, *d*, J = 15.5 Hz, H-α), 7.67 (2H, *d*, J = 15.5 Hz, H-β) (*trans*-olefin), 7.09 (2H, *d*, J = 9 Hz, H-3, H-5) and 7.63 (2H, *d*, J = 9 Hz, H-2, H-6) (B-ring)] and sugar residues {δ 3.44 [1H, *m*, glucose (Glc) H-4], 3.49 (1H, *m*, Glc H-5), 3.67–3.77 (3H, *m*, Glc H-2, H-3, H-6), 3.93 [2H, *m*, Glc H-6, apiose (Api) H-4], 3.96 (1H, *s*, Api-2), 4.19 (1H, *d*, J = 11.5 Hz, Api-5), 4.23 (1H, *d*, J = 11.5 Hz, Api-5), 4.32 (1H, *d*, J = 10 Hz, Api-4), 5.14 (1H, *d*, J = 7.5 Hz, Glc H-1), 5.55 (1H, *br s*, Api-1)}. In addition to these signals, the spectrum also showed the signals of a feruloyl group {δ, 3.86 (3H, *s*, OCH_3), 6.20 [1H, *d*, J = 16 Hz, feruloyl (Fer) H-8], 6.77 (1H, *d*, J = 8 Hz Fer H-5), 7.00 (1H, *dd*, J = 2, 8 Hz, Fer H-6), 7.09 (1H, *d*, J = 8.5 Hz, Fer H-2) and 7.46 (1H, *d*, J = 16 Hz, Fer H-7)}. The presence of isoliquiritigenin, ferulic acid, glucose and apiose, in the molecule of **2** was also indicated by its ^{13}C NMR spectrum (see Experimental). Alkaline hydrolysis of **2** gave isoliquiritin apioside (**15**) and ferulic acid (**18**), along with small amounts of (2*RS*)-liquiritin apioside [(2*RS*)-**14**] which is regarded as a product of chalcone-flavanone isomerization.

The position of the feruloyl group in the molecule was shown to be at C-5 of the apiose residue by a remarkable downfield shift of the apiose H-5 signals in the ^{1}H NMR spectrum of **2** (in Me_2CO-d_6), relative to the corresponding signals of **15** [δ 3.51 (2H, *s*) (**15**) → δ 4.22, 4.16 (1H each, *d*, J = 11.5 Hz) (**2**)]. The signal of apiose C-5 in the ^{13}C NMR spectrum of **2** also showed a distinctive downfield shift, relative to the corresponding signal of **15** [δ 65.3 (**15**) → δ 67.5 (**2**)] (in Me_2CO-d_6), substantiating the location of the feruloyl group. Based on these data, licorice-glycoside A was assigned to have structure **2**.

The CD spectrum of **2** showed a positive couplet Cotton effect centred at around 330 nm ($[\theta]_{358}$ $+3.4\times10^3$, $[\theta]_{316}$ -1.8×10^3). Since the corresponding couplet was not observed for **15**, it was attributed to the interaction of the aglycone (isoliquiritigenin) residue and the acyl (feruloyl) group. On the other hand, the signals of the B-ring and olefin protons, and H-6′ (A-ring) of the isoliquiritigenin residue in the ^{1}H NMR spectrum of **2** showed noticeable upfield shifts, relative to the corresponding signals of **15** [$\Delta\delta$ 0.06 (H-2, H-6), 0.04 (H-3, H-5), 0.08 (C_α-H, C_β-H) and 0.07 (H-6′); $\Delta\delta = \delta_H$ (of signals of **15**) $-\delta_H$ (of signals of **2**), in Me_2CO-d_6]. These upfield shifts, attributable to the anisotropic effect, suggested that the acyl group and the aglycone residue stack on each other as exemplified by the formula in Fig. 1, in a way analogous to that observed for the intramolecular stacking of acylated anthocyanins [17]. The couplet Cotton effect described above was explainable by the spatial positions of the two moieties shown in Fig. 1, where the two groups are close enough to interact with each other.

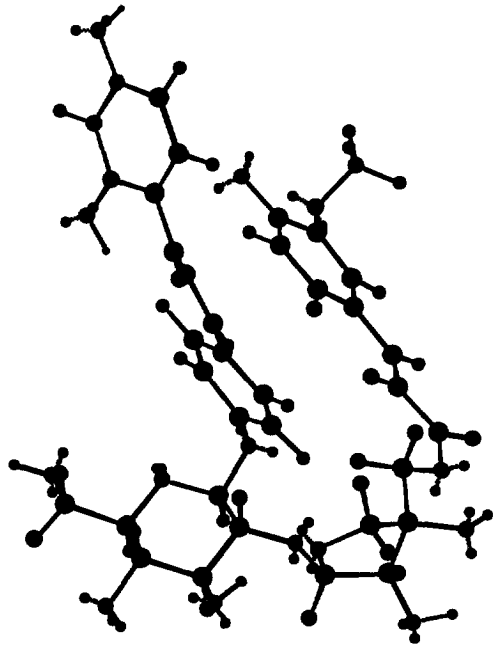

Fig. 1. A plausible stereostructure of licorice-glycoside A (**2**) where the aglycone and acyl residues are stacked onto each other, to show the couplet Cotton centred at 330 nm in the CD spectrum.

Licorice-glycoside B (**3**), $[\alpha]_D$ $-39.8°$, was obtained as a yellow amorphous powder, The FAB mass spectrum showed ion peaks at *m/z* 697 ($[M+H]^+$) and *m/z* 719 ($[M+Na]^+$), which correspond to the molecular formula $C_{35}H_{36}O_{15}$. The ^{1}H NMR spectrum of **3** showed signals attributable to the protons of a *p*-coumaroyl group [δ 6.19 (1H, *d*, J = 16 Hz, H-8), 6.84 (2H, *d*, J = 8.5 Hz, H-3, H-5), 7.44 (2H, *d*, J = 8.5 Hz, H-2, H-6) and 7.50 (1H, *d*, J = 16 Hz, H-7)], along with those of the aglycone and sugar residues. The ^{1}H and ^{13}C NMR data for the aglycone and sugar residues were practically the same as the corresponding ones of **2**, indicating that **3** has the structure in which the *p*-coumaroyl group was substituted for the feruloyl group in **2**. Structure **3** was thus assigned for licorice-glycoside B.

The CD spectrum of **3** showed a couplet centred at around 320 nm ($[\theta]_{349}$ $+2.0\times10^3$, $[\theta]_{311}$ -9.3×10^3), reflecting the interaction of the chromophores of the isoliquiritigenin residue and the *p*-coumaroyl group. Changes in the ^{1}H chemical shifts of the isoliquiritigenin protons, similar to those observed for **2**, were also shown by the ^{1}H NMR spectrum of **3** [upfield shifts relative to the corresponding signals of **15**: $\Delta\delta$ 0.06 (H-2, H-6), 0.04 (H-3, H-5), 0.09 (C_α-H, C_β-H) and 0.07 (H-6′); in Me_2CO-d_6], owing to the anisotropic effect of the acyl (*p*-coumaroyl) group stacked over the chalcone residue.

Licorice-glycoside C1 (**4**), $[\alpha]_D$ $-9.9°$, was obtained as a pale-yellow amorphous powder. The FAB mass spectrum of **4** showed ion peaks at *m/z* 727 ($[M+H]^+$) and 749 ($[M+Na]^+$), indicating the molecular formula $C_{36}H_{38}O_{16}$. The ^{1}H NMR spectrum of **4** showed signals of three aromatic protons forming an ABX system [δ 7.69 (*d*, J = 8.5 Hz, H-5), 6.55 (*dd*, J = 2 8.5 Hz, H-6) and 6.39 (*d*, J = 2 Hz, H-8)], four aromatic protons forming an A_2B_2 system [δ 7.40 (*d*, J = 8.5 Hz, H-2′, H-6′) and 7.09 (*d*, J = 8.5 Hz, H-3′ and H-5′)] and aliphatic protons attributable to a CH_2-CH system [δ 2.94 (*dd*, J = 13, 16.5 Hz, H-2), 2.64 (*dd*, J = 3, 16.5 Hz, H-2) and 5.38 (*dd*, J = 3, 13 Hz, H-3)], including the presence of a flavanone structure in the molecule. The substitution pattern of the aglycone residue is that of liquiritigenin. The spectrum also showed signals ascribable to protons of glucopyranose, apiofuranose and feruloyl residues, and the chemical shifts of these protons were closely similar to those of the corresponding protons of **2**. Therefore, structure **4**, in which liquiritigenin is substituted for isoliquiritigenin in the molecule of **2**, was assigned for licorice-glycoside C1. The CD spectrum of **4** showed a positive Cotton effect at 332 nm and a negative Cotton effect at 304 nm, indicating the *R*-configuration at C-2 of the flavanone residue [18].

Licorice-glycoside C2 (**5**), $[\alpha]_D$ $-31.1°$, showed an $[M+H]^+$ ion peak at *m/z* 727 in the FAB mass spectrum, indicating the molecular formula $C_{36}H_{38}O_{16}$. Although the ^{1}H NMR spectrum of **5** was closely

similar to that of **4**, the CD spectrum of **5** showed a negative Cotton effect at 331 nm and a positive Cotton effect at 307 nm. Therefore structure **5**, the C-2 epimer of **4**, was assigned for licorice-glycoside C2.

Licorice-glycoside D1 (**6**), $[\alpha]_D$ −42.9°, and D2 (**7**), $[\alpha]_D$ −23.4°, were respectively obtained as a pale-yellow amorphous powder. These two compounds both showed a $[M+H]^+$ ion peak at *m/z* 697 in their FAB mass spectra, indicating that both of them have the molecular formula $C_{35}H_{36}O_{15}$. Their ^1H NMR spectra, which were similar to each other, showed signals ascribed to the protons of *p*-coumaroyl groups [δ 6.23 (1H, *d*, *J* = 16 Hz, H-8), 6.83 (2H, *d*, *J* = 8.5 Hz, H-3, H-5), 7.45 (2H, *d*, *J* = 8.5 Hz, H-2, H-6) and 7.54 (1H, *d*, *J* = 16 Hz, H-7), (**6**); δ 6.23 (1H, *d*, *J* = 16 Hz, H-8), 6.83 (2H, *d*, *J* = 8.5 Hz, H-3, H-5), 7.46 (2H, *d*, *J* = 8 Hz, H-2, H-6) and 7.55 (1H, *d*, *J* = 16 Hz, H-7) (**7**)], along with glucopyranose, apiofuranose and liquiritigenin protons. Based on the similarity of the chemical shifts of the protons of the flavanone aglycone and sugar residues to those of the corresponding protons of **4** and **5**, **6** and **7** were assigned as liquiritin apiosides with a *p*-coumaroyl group at the apiose 5-OH, and stereoisomers at liquiritigenin C-2. The CD spectra of **6** and **7** showed Cotton effects at around 320 nm with the signs reversed to each other {$[\theta]_{305}$ -9.7×10^3, $[\theta]_{332}$ $+1.1\times10^4$ (**6**); $[\theta]_{305}$ $+1.1\times10^4$, $[\theta]_{330}$ -3.7×10^3 (**7**)}, respectively, indicating the 2*R* (**6**) and 2*S* (**7**) configurations of the liquiritigenin residues. Therefore, the structures of licorice-glycosides D1 and D2 were represented by **6** and **7**, respectively.

Licorice glycoside E (**8**), $[\alpha]_D$ −52.9°, was obtained as a pale-yellow amorphous powder. The ^1H NMR spectrum showed the signals of five protons in the aromatic region [δ 8.04 (1H, *m*), 7.99 (1H, *br s*), 7.46 (1H, *m*) and 7.11 (2H, *m*)], together with the signals of liquiritigenin, glucopyranose and apiofuranose protons (see Experimental). Although the chemical shifts of the aglycone and sugar residues were closely similar to those of **5** and **7**, the spectrum of **8** did not show hydroxycinnamoyl protons. On the other hand, the high-resolution FAB mass spectrum of **8** indicated the molecular formula $C_{35}H_{35}O_{14}N$. Therefore, **8** has the structure in which an indole-2-carboxyl or indole-3-carboxyl group is substituted for the hydroxycinnamoyl group in the molecules of **5** and **7**. Methanolysis of **8** gave liquiritin apioside (**14**) and methyl indole-3-carboxylate (**19**). The CD spectrum of **8** showed a positive Cotton effect at 332 nm and a negative Cotton effect at 307 nm, indicating the *S*-configuration at C-2 of the flavanone aglycone residue. Based on these data, structure **8** was assigned for licorice-glycoside E.

Compound **8** is a rare example of the flavonoid glycosides with a indole-3-carboxyl group, and compounds **2**–**7** are the first examples of flavonoid glycosides with the hydroxycinnamoyl groups from licorice, although the presence of hydroxycinnamic acids in licorice was suggested previously [19].

EXPERIMENTAL

General. ^1H (500 MHz) and ^{13}C (126 MHz) NMR: Varian VXR 500, and chemical shifts are given in δ values (ppm); FAB-MS: VG-70 SE mass spectrometer using 3-nitrobenzyl alcohol as the matrix agent. Electrospray ionization mass spectra (ESI MS): Micromass Autospec OA-Tof mass spectrometer, with 50% aq. MeOH containing 0.1% ammonium acetate as a solvent; DCCC: 95 glass tubes (3.2 mm i.d. × 120 cm) connected with Junflon tubes (1.0 mm i.d.), with a solvent system, $CHCl_3$–MeOH–H_2O (7:13:8); RP-HPLC: YMC J'sphere ODS-H80 column (4 mm i.d. × 25 cm) with the solvent system MeCN–H_2O–HOAc (5:14:1) at 40° in an oven. Detection was effected by UV absorption at 280 nm, and the flow rate was set at 1.0 ml min^{-1}. Molecular models were constructed with an Insight II/DISCOVER system (BIOSYM) on a COMTEC 4D PRC^2 XZ computer, and printed out using a Chem3D software (Cambridge) for Apple Macintosh.

Isolation of phenolic constituents from licorice. Licorice roots from the North-eastern region of China (Tohoku kanzo) (3 kg) purchased from Tochimoto-tenkai-do Co., Ltd., Osaka, Japan, were soaked in MeOH (7 l × 3), and the insoluble materials were filtered off. A portion (126 g) of the MeOH extract (550 g) was suspended in H_2O (200 ml), and then extracted with EtOAc (200 ml × 4) and *n*-BuOH (200 ml × 3), successively. The EtOAc extract (31 g) was subjected to counter-current distribution [EtOAc–*n*-PrOH–H_2O (4:2:7), *r* = 3, *n* = 3], to separate 6 frs. The fr. 6 with the least polarity (29 g) was then subjected to counter-current distribution [*n*-Hexane–EtOH–H_2O–EtOAc (5:4:1:2), *r* = 5, *n* = 5], to give 10 frs. The most polar fr. (20 g) was subjected to DCCC [3.2 mm i.d. × 1.2 m × 95 glass tubes, $CHCl_3$–MeOH–H_2O (7:13:8), descending method], to yield 19 frs. Frs showed similar HPLC patterns were combined and further chromatographed over YMC Gel ODS AQ 120-S50 with aq. MeOH to give 3′,4′,7-trihydroxyflavone (**9**) (2.4 mg), glycyroside (**10**) (3.0 mg), licorice-glycoside C1 (**4**) 12.2 mg), licorice-glycoside C2 (**5**) (14.0 mg), vestitol glucoside (**1**) (4.5 mg), licorice-glycoside A (**2**) (21.2 mg), licorice-glycoside E (**8**) (6.4 mg), carpusin (**11**) (3.6 mg), tetrahydroxymethoxychalcone (**16**) (33.7 mg), isoliquiritin (**12**) (24.0 mg), neoisoliquiritin (**13**), (7.1 mg), liquiritin apioside (**14**) (36.1 mg), isoliquiritin apioside (**15**) (38.0 mg), licorice-glycoside D2 (**7**) (6.6 mg), licorice-glycoside D1 (**6**) (5.0 mg) and licorice-glycoside B (**3**) (18.0 mg).

(3R)-*Vestitol* 7-O-*glucoside* (**1**). Needles (from MeOH–H_2O), mp 143–144°. $[\alpha]_D$ −35.5° (MeOH, *c* 1.0). High-resolution ESI MS *m/z*: 452.1962 ($[M+NH_4]^+$; $C_{22}H_{26}O_9+NH_4$ requires: 452.1921); FAB MS *m/z*: 435 ($[M+H]^+$), 457 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 206 (4.68), 223 (4.22), 280 (3.83), 285 (3.80); CD (MeOH) [θ] (nm): $+3.0\times10^4$ (207), -8.7×10^3 (214), -9.3×10^3 (231), $+2.5\times10^3$ (285).

Acid hydrolysis of **1**. Compound **1** (0.5 mg) in 5%

H_2SO_4 (0.2 ml) in a sealed tube was heated on a boiling-H_2O bath for 3 hr. The reaction mixt. was then analyzed by HPLC [MeCN–HOAc–H_2O (8:1:11)], to show the presence of **17** (R_t 7.94 min) in the mixt.

Licorice-glycoside A (**2**). Yellow amorphous powder, $[\alpha]_D$ −27.4° (MeOH, *c* 1.0): (Found: C, 56.9; H, 5.5. $C_{36}H_{38}O_{16}\cdot 2H_2O$ requires: C, 56.7; H, 5.6%). FAB MS *m/z*: 727 ($[M+H]^+$), 749 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 205 (4.59), 219 (sh, 4.45), 234 (4.37), 315 (sh, 4.40), 330 (4.56), 368 (sh, 4.40); CD (MeOH) [θ] (nm): -1.2×10^3 (238), $+4.4\times10^2$ (255), -1.8×10^3 (316), $+3.4\times10^3$ (358); ^{1}H NMR δ (MeOH-d_4, 27°): see text. δ (Me_2CO-d_6): 3.45 [1H, *m*, glucose (Glc) H-4], 3.55 (1H, *m*, Glc-5), 3.69 (2H, *m*, Glc H-3, H-6), 3.71 (1H, *m*, Glc H-2), 3.88 (3H, *s*, OCH_3), 3.88 (1H, overlapped with OCH_3; Glc H-6), 3.88 [1H, *d*, *J* = 9 Hz, apiose (Api) H-4], 3.97 (1H, *s*, Api H-2), 4.16 (1H, *d*, *J* = 11.5 Hz, Api H-5), 4.22 (1H, *d*, *J* = 11.5 Hz, Api H-5), 4.26 (1H, *d*, *J* = 9 Hz, Api H-4), 5.12 (1H, *d*, *J* = 7.5 Hz, Glc H-1), 5.55 (1H, *br s*, Api H-1), 6.26 [1H, *d*, *J* = 16 Hz, feruloyl (Fer) H-8], 6.35 [1H, *d*, *J* = 2 Hz, isoliquiritigenin (IL) H-3′], 6.45 (1H, *dd*, *J* = 2, 9 Hz, IL H-5′), 6.83 (1H, *d*, *J* = 8 Hz, Fer H-5), 7.06 (1H, *dd*, *J* = 2, 8 Hz, Fer H-6), 7.10 (2H, *d*, *J* = 9 Hz, IL H-3, H-5), 7.26 (1H, *d*, *J* = 2 Hz, Fer H-2), 7.50 (1H, *d*, *J* = 16 Hz, Fer H-7), 7.73 (2H, *s*, IL H-2, H-6), 7.73 (2H, *d*, *J* = 16.5 Hz, IL C_α-H, C_β-H), 8.04 (1H, *d*, *J* = 9 Hz, IL H-6′), 13.6 (1H, *s*, IL 2-OH); ^{13}C NMR (Me_2CO-d_6) δ: 56.3 (OCH_3), 62.5 (Glc C-6), 67.5 (Api C-5), 71.6 (Glc C-4), 75.2 (Api C-4), 77.1 (Glc C-2), 77.8 (Glc C-3), 78.2 (Api C-2), 78.6 (Glc C-5), 78.7 (Api C-3), 99.7 (Glc C-1), 103.7 (IL C-3′), 108.7 (IL C-5′), 109.8 (Api C-1), 111.3 (Fer C-2), 114.5 (IL C-1′), 115.4 (Fer C-8), 116.1 (Fer C-5), 117.4 (IL C-3, C-5), 119.6 (IL C-α), 123.9 (Fer C-6), 127.3 (Fer C-1), 129.7 (IL C-1), 131.3 (IL C-2, C-6), 133.3 (IL C-6′), 144.5 (IL C-β), 146.0 (Fer C-7), 148.7, 150.1 (Fer C-3, C-4), 160.5 (IL C-4), 165.6 (IL C-2′), 167.2 (IL C-4′), 167.6 (Fer C-9), 192.7 (IL C=O).

Alkaline hydrolysis of **2**. Compound **2** (1 mg) was treated with 0.2% MeONa in a mixt. of MeOH and H_2O (5:1) (1.5 ml) under N_2 at room temp. overnight. The soln was then acidified with HOAc and evapd. The residue was analysed by HPLC [MeCN–HOAc–H_2O (18:5:77)] to show the presence of ferulic acid (**18**) (R_t 8.44 min) and isoliquiritin apioside (**15**) (R_t 13.87 min), together with liquiritin apioside (**14**) (R_t 5.10 min). Treatment of 5 mg of **2** in an analogous way gave 1 mg of **15** which was identified by its ^{1}H NMR spectrum, and a trace amount of **14** which showed no distinctive Cotton effects in the region of 200–400 nm.

Licorice-glycoside B (**3**). Yellow amorphous powder, $[\alpha]_D$ −39.8° (MeOH, *c* 1.04). High-resolution ESI MS *m/z*: 697.2191 ($[M+H]^+$, $C_{35}H_{36}O_{15}+H$ requires: 697.2132); FAB MS *m/z*: 697 ($[M+H]^+$), 719 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 206 (4.61), 228 (4.42), 318 (4.59), 359 (4.45); CD (MeOH) [θ] (nm): -2.0×10^3 (243), -9.3×10^2 (311), $+2.0\times10^3$ (349).

Licorice-glycoside C1 (**4**). Pale yellow powder, $[\alpha]_D$ −9.9° (MeOH, *c* 1.0). (Found: C, 56.2; H, 5.3. $C_{36}H_{38}O_{16}\cdot 2H_2O$ requires: C, 56.7; H, 5.6%.) FAB MS *m/z*: 727 ($[M+H]^+$), 749 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 205 (4.41), 218 (4.33), 231 (4.28), 279 (4.11), 322 (4.17); CD (MeOH) [θ] (nm): -1.8×10^4 (211), -9.1×10^3 (235), $+1.3\times10^4$ (304), 3.4×10^3 (332).

Alkaline hydrolysis of **4**. Compound **4** (1 mg) was treated with 0.2% MeONa in a mixt. of MeOH and H_2O (5:1) (1.5 ml) under N_2 at room temp. overnight. The soln was acidified with HOAc and evapd. HPLC analysis of the residue [MeCN–HOAc–H_2O (18:5:77)] showed production of ferulic acid (**18**) (R_t 8.44 min) on this reaction.

Licorice-glycoside C2 (**5**). Pale-yellow amorphous powder, $[\alpha]_D$ −31.1° (MeOH, *c* 1.0). (Found: C, 55.6; H, 5.2; $C_{36}H_{38}O_{16}\cdot 3H_2O$ requires: C, 55.4; H, 5.7%.) FAB MS *m/z*: 727 ($[M+H]^+$), 749 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 205 (4.59), 217 (4.62), 230 (4.47), 280 (4.31), 322 (4.37); CD (MeOH) [θ] (nm): $+1.3\times10^4$ (214), $+1.1\times10^4$ (234), -6.2×10^3 (307), $+1.1\times10^4$ (331).

Licorice-glycoside D1 (**6**). Pale-yellow amorphous powder, $[\alpha]_D$ −42.9° (MeOH, *c* 1.04). High-resolution ESI MS *m/z*: 714.2326 ($[M+NH_4]^+$, $C_{35}H_{36}O_{15}+NH_4$ requires: 714.2398); FAB MS *m/z*: 697 ($[M+H]^+$), 719 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 218 (4.54), 228 (4.44), 280 (4.34), 314 (4.40); CD (MeOH) [θ] (nm): $+1.9\times10^4$ (213), $+1.3\times10^4$ (234), -9.7×10^3 (305), $+1.1\times10^4$ (332).

Licorice-glycoside D2 (**7**). Pale-yellow amorphous powder, $[\alpha]_D$ −23.4° (MeOH, *c* 1.0). High-resolution ESI MS *m/z*: 714.2459 ($[M+NH_4]^+$, $C_{35}H_{36}O_{15}+NH_4$ requires 714.2398); FAB MS *m/z*: 697 ($[M+H]^+$), 719 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 218 (4.66), 228 (4.56), 280 (4.46), 314 (4.54); CD (MeOH) [θ] (nm): -1.4×10^4 (210), -7.0×10^3 (237), $+1.1\times10^4$ (305), -3.7×10^3 (330).

Licorice-glycoside E (**8**). Pale-yellow amorphous powder, $[\alpha]_D$ −52.9° (MeOH, *c* 1.0). High-resolution FAB MS *m/z*: 694.2160 ($[M+H]^+$; $C_{35}H_{35}O_{14}N+H$ requires: 694.2136). FAB MS *m/z*: 694 ($[M+H]^+$), 716 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 206 (4.68), 223 (4.22), 280 (3.83), 285 (3.80); CD (MeOH) [θ] (nm): $+2.2\times10^4$ (214), -6.8×10^3 (226), $+6.2\times10^3$ (245), -1.1×10^4 (307), $+6.5\times10^3$ (332).

Methanolysis of **8**. Compound **8** (*ca* 1 mg) was treated with 0.07% MeONa in MeOH (1.5 ml) under N_2 at room temp. for 3 days. The soln was then acidified with HOAc and evapd. The residue was dissolved in H_2O and the soln was passed through a Sep-pak C18 cartridge (Waters), and the adsorbed materials were eluted with increasing concns of MeOH in H_2O, to give liquiritin apioside (**14**) (0.7 mg) and methyl indole-3-carboxylate (**19**) (0.5 mg). These were identified by comparison of their ^{1}H NMR spectra with those of the samples obtained from licorice (for **14**) or prepd from indole-3-carboxylic acid (for **19**).

Liquiritin apioside (**14**) [12]. ^{1}H NMR (Me_2CO-d_6+D_2O) δ: 2.70 (1H, *dd*, *J* = 3, 17 Hz, LG H-3a),

2.94 (1H, *br*, LG H-3b), 3.44 (1H, *m*, Glc H-4), 3.50 (1H, *m*, Glc H-5), 3.53 (2H, *s*, Api H-5), 3.65–3.70 (3H, *m*, Glc H-2, H-3, H-6), 3.76 (1H, *d*, $J = 9$ Hz, Api H-4), 3.86 (1H, *dd*, $J = 2$, 12 Hz, Glc H-6), 3.94 (1H, *s*, Api H-2), 4.08 (1H, *d*, $J = 9$ Hz, Api H-4), 5.02 (1H, *d*, $J = 8.5$ Hz, Glc H-1), 5.49 (1H, *dd*, $J = 3$, 13 Hz, LG H-2), 5.50 (1H, *br s*, Api H-1), 6.39 (1H, *d*, $J = 2$ Hz, LG H-8), 6.55 (1H, *dd*, $J = 2$, 8.5 Hz, LG H-6), 7.09 (2H, *d*, $J = 8.5$ Hz, LG H-3′, H-5′), 7.48 (2H, *d*, $J = 8.5$ Hz, LG H-2′, H-6′), 7.71 (1H *d*, $J = 8.5$ Hz, LG H-5).

Methyl indole-3-carboxylate (**20**). ^{1}H NMR (Me_2CO-$d_6 + D_2O$) δ: 3.82 (3H, *s*, $COOCH_3$), 7.19 (2H, *m* , H-5, H-6), 7.50 (1H, *dd*, $J = 2.5$, 6 Hz, H-7), 8.01 (1H, *s*, H-2), 8.09 (1H, *dd*, $J = 2.5$, 6 Hz, H-4).

Carpusin (**11**) [10]. Microcrystalline powder, mp 213°. FAB MS *m/z*: 303 ($[M+H]^+$), 325 ($[M+Na]^+$); UV λ_{max}^{MeOH} nm (log ε): 209 (4.24), 224 (4.10), 293 (4.06), 320 (3.80); ^{1}H NMR (Me_2CO-$d_6 + D_2O$) δ: 3.05 (2H, *s*, C_α-H), 3.72 (3H, *s*, OCH_3), 5.89 (2H, *m*, H-5, H-7), 6.61 (2H, *d*, $J = 8.5$ Hz, H-3′, H-5′), 7.00 (2H, *d*, $J = 8.5$ Hz, H-2′, H-6′); δ (Me_2CO-$d_6 + C_6D_6$, *ca* 10:1): 3.09 (2H, *m*, C_α-H), 3.70 (3H, *s*, OCH_3), 5.83 (1H, *d*, $J = 2$ Hz, H-5), 5.89 (1H, *d*, $J = 2$ Hz, H-7), 6.58 (2H, *d*, $J = 8.5$ Hz, H-3′, H-5′), 7.01 (2H, *d*, $J = 8.5$ Hz, H-2′, H-6′). Upon irradiation of the methoxyl signal, NOE with H-5 was observed in the NOE difference spectrum, while the H-7 signal did not show a NOE. ^{13}C NMR (Me_2CO-d_6) δ: 41.6 (C-α), 55.3 (OCH_3), 91.6 (C-7), 92.3 (C-5), 102.9 (C-2), 106.4 (C-3a), 115.4 (C-3′, C-5′), 125.4 (C-1′), 132.3 (C-2′, C-6′), 156.9 (C-4, C-7a), 160.5 (C-4′), 169.7 (C-6), 173.7 (C-3).

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