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ANTIMICROBIAL AGENTS FROM HIGHER PLANTS: PRENYLATED FLAVONOIDS AND OTHER PHENOLS FROM *GLYCYRRHIZA LEPIDOTA*

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Key Word Index—*Glycyrrhiza lepidota*; Leguminosae; prenylated flavanones; biphenyls; antimicrobial activity.

Abstract—Bioassay directed fractionation of extracts of American licorice, *Glycyrrhiza lepidota* (Leguminosae), resulted in identification of the known bibenzyl, 3,5-dihydroxy-4-(3-methyl-2-butenyl)-bibenzyl, and the known flavanones, glabranin and pinocembrin, as well as the isolation and structure determination of the new flavonol, glepidotin A and the new dihydroflavonol, glepidotin B as antimicrobial agents.

INTRODUCTION

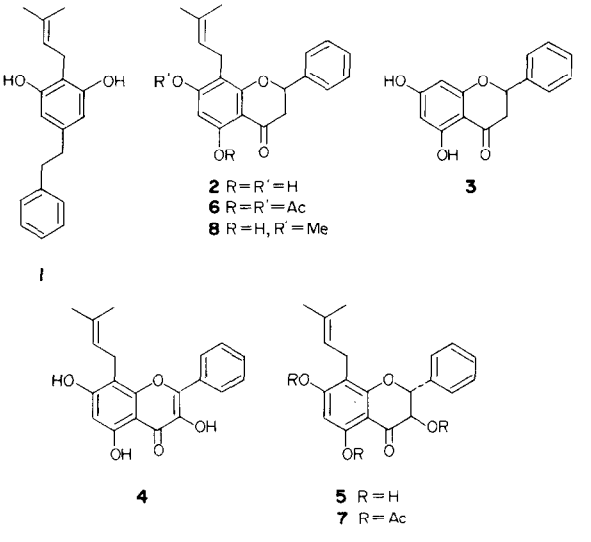
Glycyrrhiza species has been used by man for at least 4000 years [1]. Partly because of its present commercial value and partly because of continuing interest in the anti-inflammatory activity of extracts, there has been continuing interest in the secondary metabolites of various *Glycyrrhiza* species [2–13]. This work has resulted in the interesting observation that the constituents of the various *Glycyrrhiza* species examined differ fairly substantially from one another. In our own hands, several flavonoid antimicrobial agents were isolated and identified in *Glycyrrhiza glabra*, var. Spanish, and several of these were new to the literature [14, 15]. Naturally we were, therefore, attracted to *Glycyrrhiza lepidota* (American or wild licorice), the only species of this genus growing in Kansas, when we found that this plant had not been examined by modern methods [16] and was active *in vitro* in our screening system against *Staphylococcus aureus*, *Mycobacterium smegmatis* and *Candida albicans* [17].

Systematic bioassay-directed fractionation of the whole plant demonstrated that the bioactivity resided exclusively in the fraction containing acidic and phenolic substances. We report herein the identity of five active principles. Minor constituents are also present and it is our intention to discuss them in a subsequent report.

RESULTS AND DISCUSSION

Glycyrrhiza lepidota L. was collected near Goodland, Kansas, by Ralph Brooks of the Kansas Biological Survey, Lawrence, where there is a voucher specimen on deposit. The air-dried, ground whole plant (200 g) was exhaustively extracted with ethanol in a Soxhlet apparatus and then fractionated essentially as described previously [17]. The biological activity was associated with the acidic fraction. Silica gel chromatography produced active constituents, 3,4-dihydroxy-4-(3-methyl-2-butenyl)-bibenzyl (**1**), glabranin (**2**), pinocembrin (**3**), glepidotin A (**4**) and glepidotin B (**5**).

The bibenzyl, **1**, was readily identified as being the same as the substance previously encountered in *Helichrysum umbraculigerum* [18] and *Radula complanata* [19] based upon its physicochemical properties. Likewise, glabranin (**2**), well established as a constituent of *Glycyrrhiza glabra* [15, 20], was similarly identified. Confirmation was obtained by conversion to its monomethyl ether, 7-methylglabranin (**8**), with diazomethane [21]. Pinocembrin (**3**), known as a constituent of *G. glabra* [22] as well as other plants, was also identified by its physicochemical properties.



Glepidotin A (**4**), $C_{20}H_{18}O_5$, was established to be a 5,7-dihydroxyflavonol in part by the close correspondence of its UV spectra in methanol, methanolic base, and aluminum chloride–hydrochloric acid with those of galangin [23]. Other key observations reinforcing this position were the formation of a triacetate, the characteristic separation of the signals for the unsubstituted B ring into two multiplets in the ^{1}H NMR spectrum [24], a characteristic C-4 carbonyl signal at δ176.32 in the ^{13}C NMR spectrum [26, 28, 29], and peaks at *m/z* 105 and 77 in the mass spectrum [25]. The remaining atoms (C_5H_9) clearly consisted of a carbon-attached prenyl group based

upon the presence of the characteristic ^1H and ^{13}C NMR signals (see Experimental) [26] and the presence of diagnostic $[M-15]^+$ and $[M-55]^+$ (*m/z* 283, base peak) in the mass spectrum. The prenyl group could only be located at C-6 or C-8 based upon the mass spectral and ^1H NMR results. Placement at C-6 was ruled out by divergence of the properties of **4** from those recorded for a synthetic product assigned that structure previously [24]. Placement at C-8 was also favored because of co-occurrence with glabranin (**2**), the rather dramatic downfield shift associated with H-6 in the ^1H NMR spectrum upon acetylation ($\Delta\delta = -0.67$) [27, 30], and a negative Gibbs test on the part of **4**.

The correctness of the structural assignment was made certain by oxidation of glepidotin B to glepidotin A with iodine according to the method of Mahesh and Seshadri [31]. As will be seen below, glepidotin B triacetate has been directly interconverted with glabranin diacetate (**6**). The absolute stereochemistry was established to be 2(*R*),3(*R*) by CD measurements (positive extrema at 330 mm and negative at 290) [36].

Glepidotin B (**5**), $C_{20}H_{20}O_5$, had a UV spectrum like that of a dihydroflavanol and the two strong hydrogen bonded hydroxyl groups in the ^1H NMR spectrum were consistent with an IR carbonyl frequency at 1635 cm^{-1}. Upon acetylation (triacetate formed) the carbonyl frequency shifted to 1700 cm^{-1}. The ^1H NMR spectrum contained the expected signals for the AB pair represented by H-2 (δ4.62, $J = 11.6$ Hz) and H-3 (δ5.15, $J = 11.6$ Hz) shifting to δ5.32 and 5.67, as expected, upon acetylation. The appropriate dihydroflavonol ^{13}C NMR signals (C-2, δ84.3; C-3, δ73.2; and C-4, δ197.7) and multiplicities (SFORD = *d*, *d* and *s*, respectively) were also observed [26].

The presence of a prenyl group to account for the remaining atoms was clear from the ^1H and ^{13}C NMR spectra and the MS (electron impact and chemical ionization) wherein the usual losses of $[M-Me]^+$ and $[M-C_4H_7]^+$ were prominent. A major retro-Diels–Alder fragment at *m/z* 220 also signified that ring B was unsubstituted.

Based upon these considerations, structure **5** was put forward. This was confirmed upon treatment of glepidotin B triacetate (**7**) with chromous chloride whereupon glabridin diacetate was produced. Thus, the structures of these new *Glycyrrhiza* products is unequivocably established by both spectroscopic and chemical means.

It is interesting to note that the *G. lepidota* products encountered to date are almost all prenylated at C-8 and that ring B is unsubstituted. This is an unusual structural pattern amongst *Glycyrrhiza* products. The antimicrobial potency of these isolates is set forth in Table 1. The activity is broad spectrum, including Gram-positive, Gram-negative, acid-fast and yeast micro-organisms. The potency is, however, not particularly strong. These results extend the relatively small number of flavonoids known to be active antimicrobial agents [32–34].

EXPERIMENTAL

Extraction and fractionation. Dried and powdered whole plant material (200 g) of *Glycyrrhiza lepidota* was extracted with 90% EtOH for 48 hr in a Soxhlet. The EtOH extract was concd under red. pres. at 40° to give a dark brown residue (25 g). This was suspended in 5% HCl (800 ml) and extracted with $CHCl_3$ (3 × 1.6 l.). The combined $CHCl_3$ layers were back-washed with 5% HCl and dried. Filtration and evaporation of the $CHCl_3$ fraction gave a dark brown residue (13.6 g) which showed activity against *Staphylococcus aureus*, *Mycobacterium smegmatis* and *Candida albicans* at 100 mg/ml.

The aq. acid layer was made alkaline with conc. NH_4OH (pH 10) and extracted with $CHCl_3$. Evaporation of the dried $CHCl_3$ layer gave a brown oily residue (100 mg) which was antibacterially inactive. The aq. alkaline layer was acidified with HCl, evaporated to dryness and found to be antibacterially inactive.

The bioactive residue (13.6 g) was dissolved in 10% NaOH (600 ml) and partitioned with $CHCl_3$ (5 × 1 l.). The $CHCl_3$ layer was washed well with H_2O (5 × 3 l.), dried and filtered. The $CHCl_3$ was removed under vacuum. The dark green residue (2.6 g) which resulted was inactive.

The aq. NaOH layer was acidified with $CHCl_3$ (5 × 1.5 l.). The organic layer was washed well with H_2O, dried and filtered. The solvent was removed under red. pres. to give a dark brown residue (10 g) which was antibacterially active (100 mg/ml vs three micro-organisms).

The bioactive residue (10 g) was dissolved in $CHCl_3$ (50 ml) and adsorbed on Si gel (20 g). The adsorbed material was transfered to a Si gel column (500 g) packed in hexane–EtOAc (10:1). Elution with hexane through EtOAc yielded a number of semicrystalline fractions which were combined based upon TLC monitoring. Fractions of 40 ml were taken.

The semicrystalline residue (150 mg) from fractions 31–35, on

Table 1. Antimicrobial activity of *Glycyrrhiza lepidota* constituents

Compound	Micro-organism* [min. inhibitory concn (μg/ml)]					
	1	2	3	4	5	6
3,5-Dihydroxy-4-(3-methyl-2-butenyl)-bibenzyl (**1**)	12.5	i	i	50	12.5	50
Glabranin (**2**)	12.5	i	i	i	12.5	50
Pinocembrin (**3**)	100	i	i	i	100	100
Glepidotin A (**4**)	25	i	i	25	25	25
Glepidotin B (**5**)	25	i	i	100	25	50
Streptomycin sulfate	5	5	5	2.5	1.25	i

*1, *Staphylococcus aureus* ATCC 13709; 2, *Escherichia coli* ATCC 9637; 3, *Salmonella gallinarum* ATCC 9184; 4, *Klebsiella pneumoniae* ATCC 10031; 5, *Mycobacterium smegmatis* ATCC 607; 6, *Candida albicans* ATCC 10231.

crystallization from cyclohexane, gave needles of bibenzyl, **1** (65 mg); mp 80–81°; UV λ_{max}^{MeOH} nm (logε): 269 (3.08), 275 (3.10), 282 (3.11), 293 (2.80); $\lambda_{max}^{HCl-MeOH}$ nm (log ε): 269 (3.59), 274 (3.62), 282 (3.66), 295 (3.38); $\lambda_{max}^{NaOH-MeOH}$ nm (log ε): 284 (3.94), 291 (3.96), 330 (3.68); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600, 3400, 2930, 2860, 1640, 1580, 1420 (*br*), 1360, 1340 (*br*), 1145; ^{1}H NMR ($CDCl_3$) (80 Hz): δ1.72 (3H, *s*), 1.74 (3H, *s*), 2.80 (4H, *s*), 3.375 (2H, *d*, J = 7 Hz), 5.23 (1H, *t*, J = 7 Hz), 5.07 (2H, *s*), (D_2O exchange), 6.23 (2H, *s*), 7.21 (5H, *s*); ^{13}C NMR: δ 154.91, 154.84, 141.79, 141.59, 135.23, 128.85 128.50, 128.42, 128.37, 128.30, 128.25, 126.15, 126.03, 125.96, 121.79, 111.08, 108.48, 100.41, 37.52, 37.48, 25.78, 23.37; MS *m/z* (rel. int.): 283 (17.6), 282 (66.1), 267 (7.3), 228 (17.8), 227 (77.4), 192 (8.4), 191 (42.9), 147 (12.9), 105 (14.2), 92 (10.6), 91 (100), 77 (14.9), 69 (11.5). Anal. calcd. for $C_{19}H_{22}O_2$: C, 80.86; H, 7.80. Found: C, 80.90; H, 8.07%.

The residue from fractions 36–38 contained two compounds by TLC ($CHCl_3$). The residue was boiled with hot C_6H_6 and filtered × 5. The combined solvent was removed from the filtrate under red. pres. and the residue was crystallized twice from C_6H_6 and petrol to give glabranin (**2**) as crystalline material (35 mg), mp 169–170°; UV λ_{max}^{MeOH} nm (log ε): 209 (4.17), 294 (4.24), 336 (3.59); $\lambda_{max}^{HCl-MeOH}$ nm (log ε): 294 (4.25), 337 (3.57); $\lambda_{max}^{NaOH-MeOH}$ nm (log ε): 248 (3.91), 332 (4.46); $\lambda_{max}^{AlCl_3-MeOH}$ nm (log ε): 317 (4.43), 392 (3.60); $\lambda_{max}^{AlCl_3-HCl-MeOH}$ nm (log ε): 315 (4.40), 393 (3.61); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3240 (*br*), 2940, 1645, 1500, 1440, 1380, 1340, 1300, 1120, 1100, 1070; ^{1}H NMR ($CDCl_3$): δ1.71 (3H, *s*), 1.72 (3H, *s*), 2.97 (1H, *d*, J = 11.2 Hz), 2.88 (1H, *d*, J = 4.6 Hz), 3.32 (2H, *d*, J = 7.4 Hz), 5.16 (1H, *t*, J = 7.4 Hz), 5.27 (1H, *t*, J = 7.4 Hz), 5.37 (1H, *dd*, J = 4.6, 11.2 Hz), 6.03 (1H, *s*), 6.32 (1H, *s*, D_2O exchangeable), 7.42 (5H, *s*), 11.98 (1H, *s*, D_2O exchangeable); ^{13}C NMR: δ18 (*q*), 22 (*q*), 26 (*t*), 43 (*t*), 79 (*d*), 97 (*d*), 103 (*s*), 106 (*s*), 122 (*d*), 126 (*d*), 128 (*d*), 135 (*s*), 139 (*s*), 160 (*s*), 162 (*s*), 164 (*s*), 196 (*s*); *m/z* 325 (23.2), 324 (100), 291 (4.6), 281 (20.9), 270 (4.6), 269 (23.2), 256 (13.9), 220 (6.9), 219 (11.6), 205 (58.1), 192 (25.5), 191 (11.6), 179 (13.9), 178 (9.3), 177 (51.1), 165 (44.1), 149 (9.3), 104 (13.9), 103 (11.6), 91 (11.6), 77 (16.2), 69 (32.5), 55 (23.2); chemical ionization MS: *m/z* (rel. int.): 326 (24.5), 325 (100), 269 (45.2). Anal. calcd. for $C_{20}H_{20}O_4$: C, 74.10; H, 6.22. Found: C, 73.40; H, 6.35%.

The C_6H_6 insoluble residue from fractions 36–38 obtained during isolation of glabridin was washed with small portions of cold $CHCl_3$. The residue was dissolved in hot EtOAc and to this soln cyclohexane was added. After 48 hr, yellow crystals of glepidotin A appeared; (90 mg); mp 200–201°; UV λ_{max}^{MeOH} nm (log ε): 216 (4.49), 239 (sh) (4.23), 272 (4.32), 324 (4.11), 360 (4.06); $\lambda_{max}^{HCl-MeOH}$ nm (log ε): 216 (4.53), 240 (sh) (4.23), 271 (4.34), 324 (4.14), 360 (4.10); $\lambda_{max}^{NaOH-MeOH}$ nm (log ε): 235 (4.43), 287 (4.24), 417 (4.15); $\lambda_{max}^{AlCl_3-MeOH}$ nm (log ε): 228 (4.32), 253 (4.25), 276 (4.30), 358 (4.07), 420 (4.21); $\lambda_{max}^{AlCl_3-HCl-MeOH}$ nm (log ε): 229 (4.31), 252 (4.25), 276 (4.26), 353 (4.04), 419 (4.20); IR ν_{max}^{KBr} cm^{-1}: 3380 (*br*), 3320 (*br*), 3010, 2960, 1650, 1640, 1605, 1570, 1430, 1420, 1370, 1310, 1280, 1210, 1160, 1110, 1070, 1060, 990, 970, 890, 845, 770, 760; ^{1}H NMR (Me_2CO): δ1.65 (3H, *s*), 1.78 (3H, *s*), 2.91 (*br s*), 3.39 (2H, *d*, J = 7.2 Hz), 5.27 (1H, *t*, J = 7.2 Hz), 6.63 (1H, *s*), 7.55 (3H, *m*), 8.22 (2H, *m*), 9.6 (1H, *br s*), 12.3 (1H, *br s*); ^{13}C NMR (DMSO) δ176.32 (C-4), 162.23 (C-7), 154.62 (C-5), 154.39 (C-9), 154.51 (C-2), 137.14 (C-3), 131.20 (C-3″), 130.64 (C-1′), 129.82 (C-4′), 128.52 (C-2′), 127.59 (C-3′), 122.44 (C-2″), 110.51 (C-10), 103.16 (C-6), 92.99 (C-8), 25.56 (C-4″), 21.19 (C-1″), 17.76 (C-5″); MS *m/z* (rel. int.): 338 (28.7), 323 (6.9), 295 (44.3), 296 (10.6), 284 (16.1), 283 (100), 165 (8.2), 154 (10.7), 123 (10.4), 105 (39.7), 78 (12.8), 77 (40.8), 69 (16.2), 55 (10.9); chemical ionization MS (CH_4) *m/z* (rel. int.): 339 (100), 338 (40.1), 284 (18), 283 (92.6), 105 (10.6). Anal. calcd. for $C_{20}H_{18}O_5$: C, 70.99, H, 5.37. Found: C, 70.60; H, 5.38%.

Fractions 41–43 gave a white residue (70 mg) after removal of solvent. This was redissolved in hot $CHCl_3$ (5 ml) and applied to a Si gel column (15 g) set with $CHCl_3$ and eluted with $CHCl_3$–EtOAc. Fractions 7–10, containing pure compound, were mixed and the solvent was removed under vacuum. The residue was redissolved in hot C_6H_6 and kept overnight to give white crystals of glepidotin B (80 mg); mp 172–173°; UV λ_{max}^{MeOH} nm (log ε): 214 (sh) (4.48), 233 (sh) (4.23), 297 (4.26), 334 (3.67); $\lambda_{max}^{HCl-MeOH}$ nm (log ε): 215 (4.48), 233 (4.24), 297 (4.26), 334 (3.49); $\lambda_{max}^{NaOH-HCl-MeOH}$ nm (log ε): 250 (3.96), 333 (4.45); $\lambda_{max}^{AlCl_3-MeOH}$ nm (log ε): 315 (4.22); $\lambda_{max}^{AlCl_3-HCl-MeOH}$ nm (log ε): 320 (4.32); IR ν_{max}^{KBr} cm^{-1}: 3500, 3200, 2920, 1635, 1585, 1480, 1450, 1350, 1300, 1270, 1180, 1110 1070, 990, 810, 740, 680; ^{1}H NMR ($CDCl_3$): δ1.64 (3H, *s*), 1.75 (3H, *s*), 3.27 (2H, *d*, J = 7.2 Hz), 4.62 (1H, *d*, J = 11.6 Hz), 5.15 (1H, *d*, J = 11.6 Hz), 5.13 (1H, *t*, obscured by 5.15), 6.06 (1H, *s*), 7.36–7.65 (5 ArH, *m*), 9.8 (1H, *br*), 11.93 (1H, *s*, D_2O exchangeable); MS *m/z* (rel. int.): 341 (34.8), 340 (100), 325 (10.3), 307 (9.3), 285 (20.3), 255 (19.5), 243 (8.7), 221 (49.6), 220 (18.9), 205 (17.7), 203 (13.6), 165 (72.3), 91 (35.3); chemical ionization MS (CH_4), *m/z* (rel. int.): 341 (100), 285 (20.5). Anal. calcd. for $C_{20}H_{20}O_5$: C, 70.08; H, 6.18. Found: C, 70.01, H, 5.93%; CD $[\theta]_{MeOH}^{max}$ 222 nm (+48 300), 290 (−21 550), 330 (+10 800).

Methylation of glabranin. Compound **2** (15 mg) was methylated with ethereal CH_2N_2 in the usual fashion. The monomethyl ether was recovered from the reaction mixture after removal of the solvent and recrystallization from cyclohexane (14 mg); mp 123–124°; ^{1}H NMR: δ1.62 (6H, *s*), 3.05 (2H, m), 3.22 (2H, *d*, J = 7 Hz), 3.82 (3H, *s*), 5.15 (1H, *t*, J = 7 Hz), 5.42 (1H, *q*, J = 12 Hz), 6.08 (1H, *s*), 7.4 (5H, *s*), 12.13 (1H, *s*); MS *m/z* (rel. int.): 338 (97.7), 323 (56.8), 295 (19.3), 283 (19.3), 270 (22.7), 219 (100), 206 (30), 191 (60), 179 (34.4), 91 (18.8), 77 (34.4), 69 (44.4), 55 (24.5).

Fractions 48–53 were evaporated and the residue crystallized × 3 from EtOAc–hexane to give pure pinocembrin (**3**), mp 191–192°; UV λ_{max}^{MeOH} nm (log ε): 246 (3.71), 323 (4.46); $\lambda_{max}^{MeOH-HCl}$ nm (log ε): 229 (4.17), 290 (4.46); $\lambda_{max}^{MeOH-NaOH}$ nm (log ε): 246 (3.87), 324 (4.41); IR ν_{max}^{KBr} cm^{-1}: 3010, 1630, 1600, 1580, 1480, 1300, 1150, 1080, 820, 760; ^{1}H NMR: δ2.78 (1H, *dd*, J = 3.9, 17 Hz), 3.10 (1H, *dd*, J = 11.9, 17 Hz), 5.5 (1H, *dd*, J = 3.9, 11.9 Hz), 6.03 (1H, *s*), 7.35–7.57 (5H, *m*), 12.13 (1H, *s*, exchangeable); MS *m/z* (rel. int.): 256 $[M]^+$ (86), 179 (100), 152 (78), 124 (52), 104 (22), 96 (27), 78 (37), 77 (32).

Glepidotin A triacetate. Compound **4** (40 mg) was dissolved in pyridine (6 ml) and to this, Ac_2O (7 ml) was added. The reaction mixture was kept at room temp. overnight and then poured into cold H_2O (50 ml). The white ppt obtained was collected after filtration and successive washings with cold H_2O. The residue was dried and crystallized from EtOH to give crystals (40 mg) mp 169–170°; UV λ_{max}^{MeOH} nm (log ε): 296 (4.20), 253.5 (4.33); IR ν_{max}^{KBr} cm^{-1}: 3400 (*br*), 2910, 1765, 1640, 1610, 1440, 1365, 1180, 1150, 1050; ^{13}C NMR (DMSO): δ170.25 (C-4), 132.7 (C-3), 148.13 (C-2), etc.; ^{1}H NMR ($CDCl_3$): δ1.67 (3H, *s*), 1.74 (3H, *s*), 2.29 (3H, *s*), 2.33 (3H, *s*), 2.45 (3H, *s*), 3.34 (2H, *d*, J = 7 Hz), 5.02 (1H, *t*, J = 7 Hz), 7.30 (1H, *s*), 7.53–7.41 (3H, *m*), 7.74–7.90 (2H, *m*); MS *m/z* (rel. int.): 464 (0.5), 422 (17.1), 380 (61.0), 337 (46.8), 325 (38.3), 323 (10.1), 321 (22.9), 295 (100), 286 (18.8), 283 (98.2), 165 (9), 105 (59.3), 77 (63), 69 (19.7).

Glepidotin B triacetate. To a soln of glepidotin B (20 mg) in pyridine (2 ml) was added Ac_2O (5 ml) and the soln was heated at 85° for 6 hr under N_2. After cooling, the reaction mixture was poured over crushed ice and filtered to remove the product as a gum. This was dissolved in $CHCl_3$, washed with H_2O, dried and evaporated. The amorphous residue was filtered through Si gel using $CHCl_3$ to give pure **7**, 22 mg (82%), mp 122–124°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1760, 1750, 1700, 1600, 1350; ^{1}H NMR ($CDCl_3$): δ1.65 (3H, *s*), 1.70 (3H, *s*), 1.95 (3H, *s*), 2.25 (3H, *s*), 2.35 (3H, *s*), 3.15 (2H, *br d*, J = 8 Hz), 4.85–5.05 (1H, *m*), 5.32 (1H, *d*, J

= 15 Hz), 5.67 (1H, *d*, *J* = 15 Hz), 6.72 (1H, *s*), 7.37 (5H, *s*); *m/z* 438, 423.

Glabridin diacetate. This was prepared as just described, starting with 12 mg glabridin (**2**). Chromatography over Si gel with hexane–EtOAc (5:1) gave 12.5 mg (83%) of product, further purified by crystallization from cyclohexane, mp 84–85°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1760, 1680, 1600, 1360, 1170, 1120; ^{1}H NMR ($CDCl_3$): δ 1.63 (3H, *s*), 1.70 (3H, *s*), 2.28 (3H, *s*), 2.37 (3H, *s*), 2.68 (1H, *dd*, *J* = 16.25, 4.27 Hz), 3.00 (1H, *dd*, *J* = 16.25, 11.97 Hz), 3.12 (2H, *d*, *J* = 8 Hz), 4.75–5.00 (1H, *m*), 5.38 (1H, *dd*, *J* = 11.97, 4.27 Hz), 6.72 (1H, *s*), 7.37 (5H, *s*); *m/z* 408, 365.

Conversion of glepidotin B triacetate to glabridin diacetate. A soln of glepidotin B triacetate (50 mg) in 17 ml Me_2CO was thoroughly flushed with N_2 and treated, drop-wise, with a soln of chromium (II) chloride in aq. HCl [35] until TLC examination showed the absence of starting material whereupon the Me_2CO was removed under red. pres. The resulting soln was diluted with brine, extracted with $CHCl_3$, dried, evaporated, chromatographed through Si gel (hexane–EtOAc, 5:1) to give glabridin diacetate (36 mg, 74%) identical in mp, mmp, co-TLC and IR with that prepared directly from glabridin as described.

Oxidation of glepidotin B to glepidotin A. Glepidotin B (40 mg), fused KOAc (240 mg) and HOAc (2.4 ml) were heated under reflux and a soln of I_2 (40 mg) in HOAc (1.6 ml) was added during the course of 1 hr to the boiling soln. The mixture was refluxed for 3 hr, after which most of the HOAc was removed under red. pres. and the residue treated with H_2O (10 ml) satd with SO_2. The brownish yellow solid obtained (10 mg) was chromatographed through a Si gel column (25 g) using CH_2Cl_2. Yellow crystalline glepidotin A (5 mg) was recovered after crystallization from CH_2Cl_2 and cyclohexane. The mp, mmp, TLC and IR were identical with those of glepidotin A.

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