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GLEPIDOTIN C: A MINOR ANTIMICROBIAL BIBENZYL FROM *GLYCYRRHIZA LEPIDOTA*

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Key Word Index—*Glycyrrhiza lepidota*; Leguminosae; bibenzyl; antimicrobial activity.

Abstract—Further examination of chromatographic residues from an earlier study of the antimicrobial constituents of American licorice, *Glycyrrhiza lepidota*, resulted in the isolation of a small amount of a new, weakly active, bibenzyl named glepidotin C. Spectroscopic measurements led to the assignment of the structure of this compound as 2-(2-hydroxy-3-methylbut-3-enyl)-5-(1-phenylethyl)-1,3-benzenediol.

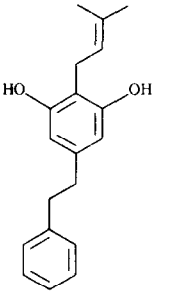
INTRODUCTION

In a previous study of the antimicrobial constituents of *Glycyrrhiza lepidota* (Leguminosae; American or wild licorice) growing in western Kansas, bioassay-directed fractionation resulted in identification of the known bibenzyl 2-(3-methyl-2-butenyl)-5-(1-phenylethyl)-1,3-benzenediol (**1**) and the known flavanones glabranin (**2**) and pinocembrin (**3**). In addition, the isolation and structure determination of the then new flavanol glepidotin A (**4**) and the then new dihydroflavonol glepidotin B (**5**) was also described [1]. A subsequent re-examination of the chromatographic residues and mother liquors revealed the presence of a small amount of yet another antimicrobial substance, the novel bibenzyl glepidotin C (**6**) whose properties and structural assignment we report herein.

RESULTS AND DISCUSSION

Continued examination of the more polar fractions of the chromatogram, which had led to the discovery of the five *G. lepidota* constituents described previously by us [1], revealed the presence of a very minor additional bioactive component. Further chromatographic resolution resulted in molecular homogeneity. Fortunately its structure was readily established from its spectral properties as only 5 mg were available for experimentation.

Glepidotin C (**6**), $C_{19}H_{22}O_3$, was clearly an asymmetrical bibenzyl based upon the presence of the characteristic isolated two methylene system resonating at δ2.77 and 2.87 in the ^{1}H NMR spectrum and at 37.50 in the ^{13}C NMR as well as the prominent (base peak) tropylium ion peak at 91 atomic mass units in the mass spectrum. The ^{1}H and ^{13}C NMR spectra agreed that the pendant phenylethyl group is unsubstituted. The UV spectrum of glepidotin C is quite similar to that of **1**, previously isolated from this plant, suggesting the presence of the same chromophoric units, and thus, the same ring system. In agreement, the 2,5-dialkyl-1,3-benzenediol moiety required by this inference was clearly discernible in the ^{1}H and ^{13}C NMR spectra of **6** and of its acetate ester. The remaining elements, C_5H_9O, were present in a modified prenyl unit, revealed from its spectral properties to be an unusual but well precedented 2-hydroxy-3-methylbut-3-enyl moiety [2]. The spectral properties of the triacetyl ester (**7**), as compared with that of **6**, showed clearly a secondary allylic alcohol moiety in **6**. The benzylic methylene resonates at δ2.77 in **6** and this shifts to δ2.89 on acetylation. Both of these signals are partially obscured



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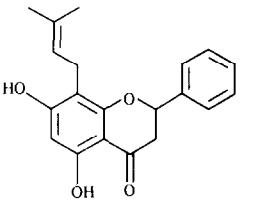

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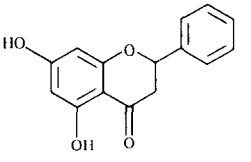

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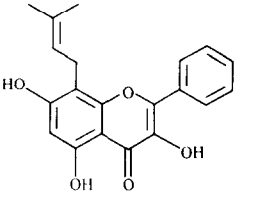

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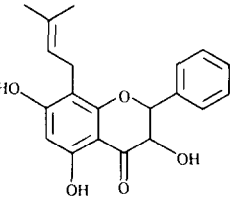

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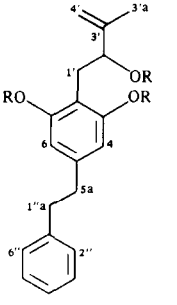

6 R = H
7 R = Ac

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by other proton peaks. The C-2′ methine signal of **6** is at δ4.33 and this shifts to δ5.31 (*dd*, $J = 7.95$, 6.5 Hz) in the acetate, as expected for this transformation. A vinyl methylene results in broad singlets at δ4.87 and 5.00 in **6** and closely overlapping signals at δ4.83 in the acetate. The C-methyl group is a singlet at δ1.84 in **6** and 1.77 in **7**. The ^{13}C NMR signals of **6** at 29.20 (C-1′), 77.98 (C-2′), 147.18 (C-3′), 110.60 (C-4′) and 18.37 (C-3′a) are in full agreement with this formulation. Furthermore, a HOMCOR* pulse sequence related, i.a. the signals for H-1′, H-2′, H-3′a and H-4′ as contiguous. The nearest neighbour relationships between H-5a, H-4, H-6 and H-2″, H-6″, and H-1″a were also seen in this experiment. Additional evidence was obtained for the location of the modified prenyl side chain through a (Selective) INEPT* experiment [3]. Irradiation of the H-1′ proton at 3.09 resulted in polarization transfer to the C-1 and C-3 carbons as revealed by an increase in the intensity of these signals. Further, all of the carbons bearing attached protons were correlated using a HETCOR* pulse sequence. These spectral and chemical properties are in agreement with formulation **6** for glepidotin C making it a closely related oxygenated analogue of **1** isolated previously by us from this plant and by others from *Helichrysum umbraculigerum* [4] and *Radula complanata* [5]. Too little material was available to permit the establishment of absolute configuration for glepidotin C.

When tested using an agar dilution-streak assay, glepidotin C showed very weak antimicrobial activity (50 μg/ml) against *Mycobacterium smegmatis* (ATCC 607). It was inactive against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella gallinarum*, *Klebsiella pneumoniae*, *Candida albicans* and *Pseudomonas aeruginosa*. This level of activity makes it unworthy of further study as a potential agent for treatment of human bacterial diseases.

Gelpidotin C represents another example of the comparative wealth of novel antimicrobial agents elaborated by higher plants, presumably for their self protection but whose antimicrobial spectra are broad enough to include detectable potency against human pathogens. There are, as yet, comparatively few bibenzyls known but their apparently common possession of antimicrobial activity makes them comparatively easy to detect and the future should see an expanded list. The once common statement that secondary metabolites were useless metabolic flotsam can perhaps be seen, in retrospect, as describing the contemporary state of our knowledge and methodology rather than a fundamental truth.

* Copies of these spectra were provided to the referees and are available from the authors upon request.

EXPERIMENTAL

Plant material. Glycyrrhiza lepidota was collected near Goodland, KS. It was worked-up as described in ref. [1].

Fractions 54–61 were evapd to give the residue (28 mg). This was subjected to silica gel CC (10 g) set with hexane–EtOAc (4:1) while collecting 2 ml fractions. Fractions 11 and 12 contained glepidotin C with some minor impurities. This was purified further by PTLC using the solvent system hexane–EtOAc (4:1).

Glepidotin C (**6**). Colourless crystals from C_6H_6, *n*-C_6H_{14} (5.3 mg), mp 112°. $[\alpha]_D + 2.64°$ (MeOH; *c* 0.113); CD (MeOH) 210 nm ($\Delta\varepsilon + 3.24$). UV λ_{max}^{MeOH} nm (log ε): 262 (3.25), 268 (3.39), 273 (3.40), 281 (3.41); $\lambda_{max}^{MeOH-HCl}$ 261 (3.44), 268 (3.50), 274 (3.53), 280 (3.51), $\lambda_{max}^{MeOH-NaOH}$ 267 (3.59), 291 (3.66). IR $\nu_{max}^{CH_2Cl_2}$ cm^{-1}: 3360, 3289, 2920, 2855, 1628, 1433, 1315, 1062, 902, 864, 819. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.84 (3H, *s*, Me-3′a), 2.77 (3H, *m*, H-1′, 5a), 2.87(2H, *m*, H-1″a), 3.09 (1H, *dd*, $J = 14.77$, 2.05 Hz, H-1′), 4.33 (1H, *br d*, $J = 8.4$ Hz, H-2′), 4.87 (1H, *br s*, H-4′), 5.00 (1H, *br s*, H-4′), 6.30 (2H, *s*, H-4, 6), 7.18 (3H, *m*, Ar-H), 7.27 (2H, *m*, Ar-H). ^{13}C NMR (125.75 MHz, $CDCl_3$) ppm: 18.4 (C-3′a), 29.2 (C-1′), 37.5 (C-1″a, C-5a), 78.0 (C-2′), 108.8 (C-4,6), 110.5 (C-2), 110.6 (C-4′), 125.9 (C-4″), 128.3, 128.4 (C-2″, 3″, 5″, 6″), 141.8, 142.1 (C-1″, C-5), 147.2 (C-3′), 155.5 (C-3, 1); HRMS *m/z* (rel. int.): 298.15769 (M^+, 3.9%), calc. for $C_{19}H_{22}O_3$ 298.15677); EIMS *m/z* (rel. int.): 298 (3.9), 280 (4.0), 265 (4.1), 228 (36.8), 227 (66.4), 137 (23.2), 91 (100).

Glepidotin C triacetate (**7**): To glepidotin C (1 mg), one drop of Ac_2O and one drop of pyridine were added and the soln was left overnight at room temp. The solvent was removed *in vacuo*, to give glepidotin C triacetate. ^{1}H NMR (500 MHz, $CDCl_3$): δ1.77 (3H, *s*, Me-3′), 1.98 (3H, *s*, -OAc), 2.34 (6H, *s*, Ar-OAc), 2.69 (1H, *dd*, $J = 13.75$, 6.5 Hz, H-1′), 2.89 (5H, *m*, H-1′, 5a, 1″a), 4.83 (2H, *m*, H-4′), 5.32 (1H, *dd*, $J = 7.95$, 6.5 Hz, H-2′), 6.81 (2H, *s*, H-4, 6), 7.21 (5H, *m*, Ar-H), MS *m/z* (rel. int.) 313 (23.05), 312 (13.04), 296 (20.4), 295 (100), 263 (19.57), 166 (41.42), 124 (24.0), 98 (22.48), 96 (27.35).

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