ANTIMICROBIAL AGENTS FROM HIGHER PLANTS, GLYCYRRHIZA GLABRA L. (VAR. SPANISH). I. SOME ANTIMICROBIAL ISOFLAVANS, ISOFLAVENES, FLAVANONES AND ISOFLAVONES.

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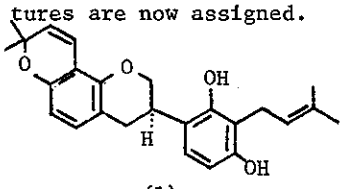
The isolation, structure determination and bioactivity of antimicrobial isoflavans hispaglabridin A (1), hispaglabridin B (2) and 4'-O-methylglabridin (3) from Glycyrrhiza glabra L. (Spanish variety) are reported. Isolation of previously known substances glabridin (5), glabrene, glabrol, formononetin and phaseollinisoflavan are also described.

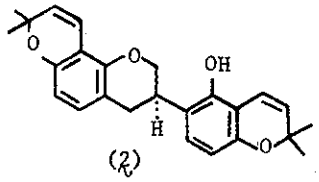
In our screening program for antimicrobial agents from higher plants,[1] ethanolic extracts of the powdered roots of commercial Glycyrrhiza glabra L. (var. Spanish) posessed reproducible in vitro activity against Staphylococcus aureus (ATCC 13709), Mycobacterium smegmatis (ATCC 607) and Candida albicans (ATCC 10231). The Russian variety was inactive. Fractionation and silica gel chromatography produced numerous active fractions including four substances previously found in G. glabra [glabridin (5)[2] (an isoflavan), glabrene[3] (an isoflavene), glabrol[2] (a flavanone) and formononetin[4,5] (an isoflavone)] as well as phaseollinisoflavan, previously known as a phytoalexin from Phaseolus vulgaris infected with tobacco necrosis virus.[6] None of these substances was previously suspected to be antimicrobially active (see table).

Glabridin, glabrol and phaseollinisoflavan were identical with authentic samples and the others were identified from their physical and spectral properties and those of their acetates and methyl ethers.

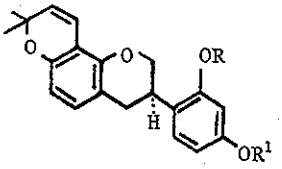
Three new bioactive isoflavans, hispaglabridin A (1), hispaglabridin B (2) and 4'-O-methylglabridin (3) were also isolated and the indicated structures are now assigned.



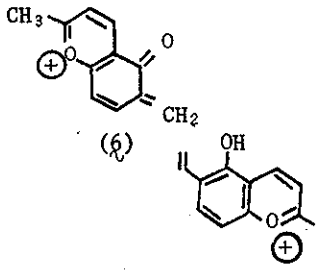
(1)



(2)



(3), R=H, R^1=CH_3
(4), R=R^1=CH_3
(5), R=R^1=H



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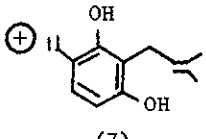

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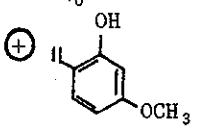

(9)

Hispaglabridin A, m.p. 132-133° (from cyclohexane), $C_{25}H_{28}O_4$(anal. C,H; M^+=392 (23%)), $[\alpha]_D^{25}$ -8.23 (c=2.43,chf), gave a positive Gibbs test (purple-blue) indicating a free p-position to a phenolic OH group[8] and the pmr spectrum contained peaks characteristic of the chromene ring (100 mHz,$CDCl_3$; 1.38δ, s,$(CH_3)_2C$; 5.51δ,d,J = 10Hz, $H_\beta C$=; 6.65δ,d,J = 10Hz, $H_\alpha C$=), the ABMXX' system of the C_2-C_4 portion of the isoflavan moiety (2.58δ,1H,d,J = 6Hz; 2.88δ,1H,d, J = 10Hz; 3.30-3.50δ,1H,m; 3.97δ,1H,t,J = 10Hz; 4.36δ,1H,dd,J = 10,4Hz),[2,9] the γ,γ-dimethylallyl group (1.75δ,s,CH_3; 1.81δ,s,CH_3; 3.42δ,br.d., J = 7Hz, CH_2; 5.25δ,br.t., J = 7Hz,CH=), two sets of o-aromatic hydrogens (6.32δ,d,J = 8Hz,HC=; 6.35δ,d,J = 8Hz,HC= and 6.78δ,d,J = 8Hz,2xHC=) and two exchangeable phenolic hydrogens at 4.87 and 5.42δ. The UV spectrum (λ_{max}^{MeOH} 281nm (log ε 4.05), 290 infl. (3.95) and 3.12 (3.41) is nearly identical with that of glabridin.[2]

Prominent ratio Diels-Alder peaks at m/e 173 (100%,6) and 204(5%,7) were consistent with this view and in conjunction with the pmr and Gibbs test data suggested formula 1 as most probable. Consistent with this view but not uniquely requiring it was the conversion of glabridin (5) with 3-methylbut-2-en-1-ol and $BF_3 \cdot Et_2O$ in dioxane[10,11] to a mixture of four products one of the monoisopentenylated materials of which was isolated by silica gel chromatography and found to be identical (ir,uv,tlc,pmr,ms and ord[9,14]) with hispaglabridin A.

Similar considerations, of which the more decisive were the positive (blue) Gibbs test[12] the presence of two chromene rings in the pmr (1.40δ, s,2x$(CH_3)_2C$; 5.47 and 6.57δ,d,J = 9.5Hz,HC=CH;5.50 and 6.60δ,d,J = 10Hz, HC=CH) and retro Diels-Alder fragments 6 (100%) and 8 (67%) in the mass spectrum led to the assignment of structure 2 to hispaglabridin B, m.p. amorphous, $[\alpha]_D^{25}$ -25.7(c=2.35,chf), anal($C_{25}H_{26}O_4$:C,H; M^+=390 (29%)), λ_{max}^{MeOH} 280nm(log ε 4.17), 290 infl.(4.11), 309(3.67), etc. In support of this, pyridine catalyzed condensation of 3-hydroxy-3-methyl-1,1-dimethoxybutane[13] with glabridin (5) produced hispaglabridin B (tlc,ir,uv,pmr,ord[9,14] and ms).

The structure of 4'-O-methylglabridin (3); m.p. 120-121°; $[\alpha]_D^{28}$ +10.2 (c=1.04,chf); $C_{21}H_{22}O_4$ (anal:C,H; M^+ 338 (25%)); λ_{max}^{MeOH} 281nm (log ε 4.07), 290sh(3.96), 312(3.34); pmr(100 mHz, $CDCl_3$:1.40δ(6H,s), 2.90(2H,AB of ABMXX'), 3.3-3.6(1H, M of ABMXX'), 3.70 (3H,s), 4.05(1H,X of ABMXX'), 4.38 (1H, X' of ABMXX'), 5.20(1H,s,exchangeable), 5.50(1H,d,J = 10Hz), 6.29(1H,d, J = 2Hz), 6.32(1H,d,J = 8Hz), 6.41(1H,dd,J = 8,2Hz), 6.61(1H,d,J = 10Hz), 6.77 (1H,d,J = 8Hz), 6.96(1H,d,J = 8Hz)) was assigned based upon these spectral considerations, the positive Gibbs test, the presence of ions 6(100%) and 9 (m/e 150,22%) in the mass spectrum and conversion of glabridin (5) to 3 with diazomethane (6hr., room temperature. The less hindered OH is doubtless the

more reactive) and the identity of monomethyl 3 and dimethyl 5 (4, prepared by heating with Me_2SO_4 and K_2CO_3 in acetone).

The antibacterial potencies of these materials is set forth in the table in comparison with streptomycin sulfate standard.[1] The substantial potency of many of these agents against Staphylococcus aureus and Mycobacterium smegmatis is noteable and, in our experience, unusual for plant products. Glabrol, in particular, is highly potent. Only glabridin (5) and phaseollinisoflavan showed activity against Candida albicans but at a level insufficient for further interest.

Table. In Vitro Agar-Dilution Streak Antimicrobial Potency

Substance	*Minimum Inhibitory Concentration (mcg/ml)*					
	Organism No.					
	1	2	3	4	5	6
Hispaglabridin A (1)	3.12	i	i	i	3.12	i
Hispaglabridin B (2)	6.25	i	i	i	3.12	i
4'-O-Methylglabridin (3)	6.25	i	i	i	3.12	i
Glabridin (5)	6.25	i	i	i	6.25	25
Glabrol	1.56	i	i	i	1.56	i
Phaseollinisoflavan	25	i	i	i	12.5	50
Glabrene	25	i	i	i	25	i
Formononetin	i	i	i	i	i	i
Streptomycin Sulfate	5	5	50	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), i = no inhibition at 100 mcg/ml.

References and Notes

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14. ORD: 1(c=0.000036,MeOH), $[\phi]_{306}$ 0, $[\phi]_{295}$ +2722, $[\phi]_{289}$ 0, $[\phi]_{278}$ -7623, $[\phi]_{268}$ -1851; 2(c=0.000043,MeOH), $[\phi]_{310}$ -8344, $[\phi]_{300}$ -10883, $[\phi]_{292}$ 0, $[\phi]_{280}$ +14511; 3 (c=0.000048,MeOH), $[\phi]_{310}$ +3520, $[\phi]_{296}$ +5280, $[\phi]_{284}$ 0, $[\phi]_{274}$ -1410, $[\phi]_{264}$ 0; Synthetic 1, prepared from 5 (c=0.000078,MeOH), $[\phi]_{308}$ 0, $[\phi]_{296}$ +3062, $[\phi]_{288}$ 0, $[\phi]_{279}$ -4287, $[\phi]_{270}$ -2754. Synthetic 2, prepared from 5, (c=0.0000531,MeOH), $[\phi]_{310}$ -5875, $[\phi]_{300}$ -8225, $[\phi]_{292}$ 0, $[\phi]_{280}$ +13220, $[\phi]_{264}$ 0.

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