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ISOFLAVANS AND A STILBENE FROM WOOD OF THE DECAY-RESISTANT TROPICAL TREE *DIPHYSA ROBINIOIDES*

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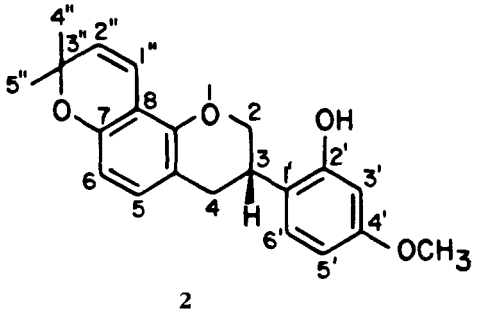
We previously reported (1) isolation of the biflavanone chamaejasmin from wood of *Diphysa robinioides* Benth. (Leguminosae). Work on this species, known as "guachipelin," has continued, and this report describes additional wood components as well as their antimicrobial activity.

The major component (about 3% of the dry weight) was *trans*-3,3′,4′,5,5′-pentahydroxystilbene (**1**). This stilbene has previously been found in the heartwood of *Schotia brachypetala* (2,3), in *Vouacapoua* species (4), and in *Maclura pomifera* (5,6). A fraction containing the stilbene was shown (6) to inhibit wood decay fungi, and the antimicrobial activity of this and other stilbenes (7) is well-known.

Two isoflavans, **2** and **3**, were also isolated. Structure **2** is that of (−)-4′-*O*-methylglabridin (8), and our high field ^{1}H-nmr and tlc Rf values corresponded to those of a standard sample of (+)-4′-*O*-methylglabridine. The ^{13}C-nmr spectrum, not previously reported (8), was also in accord with the structure, and it is given in Table 1. In the original work (8), relative placement of the OH and OCH_3 groups was in part inferred from a



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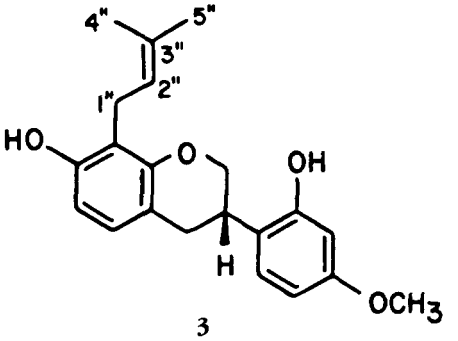

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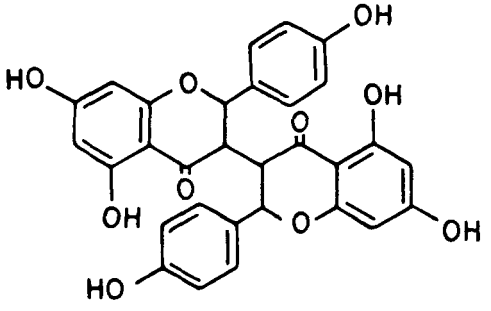
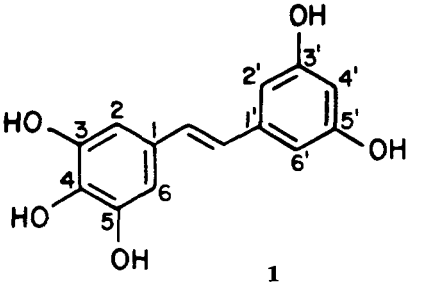


chamaejasmin



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sluggish CH_2N_2 reaction (suggesting a hindered OH) and a positive Gibbs test. Irradiation of the 4′-OMe resonance pro-

TABLE 1. Nmr Spectral Data for **2** and **3**

Atom	^{13}C nmr (67.5 MHz, $CDCl_3$)		^{1}H nmr (360 MHz, $CDCl_3$)
	Compound		Compound
	2	**3**	**3**
C2	69.99	70.05	4.37 ddd, *J* 10.4, 3.3, 2.0 4.02 t, *J* 10.4
C3	31.75	31.81	3.47, heptet
C4	30.64	31.05	3.00 dd, *J* 10.9, 15.7 2.89 dd, *J* 15.7, 5.3
C4a	114.37	114.31	
C5	129.08	128.02	6.40 d, *J* 8.2
C6	102.85	102.11	6.81 d, *J* 8.2
C7	150.29	159.15	
C8	109.87	114.31	
C8a	154.19	154.13	
C1′	119.86	119.97	
C2′	149.57	152.20	
C3′	105.56	106.02	6.34 d, *J* 2.4
C4′	151.62	153.48	
C5′	108.65	108.06	6.47 dd, *J* 8.5, 2.5
C6′	128.85	127.50	7.01 d, *J* 8.5
C1″	116.88	22.53	3.40 d, *J* 7.2
C2″	128.03	122.07	5.25 t, *J* 7.2
C3″	75.70	134.16	
C4″	27.84[a]	17.97	1.80 s
C5″	27.61[a]	25.91	1.73 s
OMe	55.43	55.40	3.75 s
^{1}H nmr nOe's observed for **2**:			^{1}H nmr nOe's observed for **3**:
1″ 2″, 5 6, 4 6′ 5′ 6′, 4′OMe 3′ and 5′			5 6, 4 6, 4 6′, 5′ 6′, 4′OMe 3′ and 5′

[a]Interchangeable.

duced nOe enhancements in two aromatic proton resonances, and, hence, there must be two aromatic protons *ortho* to the OCH_3 group. This would not be true if the OH and OCH_3 positions were reversed. This reinforced the structure of (−)-4′-*O*-methylglabridin as **2**.

Flavan **3**, which we have named (−)-4′-*O*-methylpreglabridin, was shown to have the molecular formula $C_{21}H_{24}O_4$ by hrms. The ^{1}H- and ^{13}C-nmr spectra (Table 1) and mass spectrum were closely analogous to those for **2**. The main differences were those expected if we were dealing with the ring-opened prenyl phenol rather than the chromene **2**. In Table 1, the main nmr spectral changes were those listed for carbons 1″-5″ and their associated protons. The only other differences were shifts in the carbon resonances for C-7 and C-8, again expected on the basis of change from **2** to **3**. The ^{13}C-nmr spectrum was particularly instructive. The equivalent C-4″ and C-5″ resonances in **2** at 27.61 and 27.84 ppm were changed to 17.97 (C-4″ *cis* to C-1″) and 25.91 (C-5″). The quaternary C-3″ next to oxygen at 75.70 in **2** was replaced by a singlet at 134.16 ppm, as expected for the prenyl C-3″ carbon of **3**. In the ^{13}C-nmr spectrum of **3**, the expected 22.53 ppm resonance of C-1″ was now visible. Thus, the ^{13}C resonances were exactly those for an aryl prenyl moiety (9). The ^{1}H-nmr resonances for C-2 to C-6 and C-3′, C-5′, and C-6′ for **3** were essentially identical in chemical shift and coupling constant to those

reported (8) for (+)-4-*O*-methylglabridin and determined by us for **2**. In addition, to assure the relative placement of all groups, nOe experiments were performed, with the results given in Table 1.

It is clear from the literature data (2-7) and the large amount (3% dry weight) of **1** present in the wood of *D. robinioides*, that this stilbene is the major contributor to the resistance of the wood toward fungal attack. Antimicrobial activity for (+)-**2** was previously reported (8), and, hence, similar studies were conducted[2] on chamaejasmin (1) and (−)-**3**. with the results given in Table 2. The activity of (−)-4′-*O*-methylpreglabridin (**3**) was comparable to that reported (8) for (+)-**2** with the exception that modest activity was noted for **3** against *Candida albicans*, while (+)-**2** was inactive. Chamaejasmin was somewhat less active than **3** against two organisms.

evaporated to yield 12, 184, and 79 g of residue, respectively. The petroleum ether residue was chromatographed on Al_2O_3 (Act. I) eluting with $CHCl_3$, followed by $CHCl_3$-MeOH increasing from 5 to 35% MeOH. Early fractions were rich in a mixture of **2** and **3**, which were then separated and purified by preparative layer chromatography ($CHCl_3$-MeOH, 95:5). This yielded 60 mg of **2**, Identified by comparison (mp, optical rotation, 360 MHz ^{1}H nmr, and tlc) with a standard sample. The plc also yielded 20 mg of pure **3** as an oil. The Et_2O residue was similarly chromatographed, but with a final elution of $CHCl_3$-MeOH (2:2). Fractions eluted with $CHCl_3$-MeOH (85:10 and 95:15) yielded 4 g of chamaejasmin (1), while subsequent fractions yielded 19 g of the stilbene **1**.

The ratio of isolated yields given for chamaejasmin, **1**, **2**, and **3** represent reasonable estimates of the relative amounts of each substance in *D. robinioides* wood. Since some chromatographic fractions remained as mixtures, the isolated yields from the Et_2O fraction quoted are minimum absolute yields. The EtOAc fraction also contained large amounts of stilbene **1**. Total concentration was estimated at 3%.

TRANS-3,3′,4′,5,5′-PENTAHYDROXYSTILBENE (**1**).—Mp 238° (uncorr.), lit. (2,4) mp 245°; ms *m/z* 260 (M^+, 100%), 245 (1.5%). Pentamethoxyderivative: ms *m/z* 330 (M^+, 100%), 315 (54%); mp 112-114°; lit. (3) mp 134-135°. Recrystallization did not change our mp. ^{1}H nmr of **1** (270 MHz, $CDCl_3$) with data from Drews and Fletcher (3) in parentheses: C-4′, 1H, 6.28t, *J* 2Hz (6.28t); C2′, 6′, 2H, 6.54d, *J* 2Hz (6.50d); C2, 6, 2H, 6.65s, (6.60s); olefinic protons (1H, 6.79d, *J* 16Hz and 1H, 6.89d, *J* 16Hz (6.82s, 2H). ^{13}C nmr C3,5 158.47s; C3′5′ 145.92s; C4 140.20s; C1 133.43s; C1′ 129.10s; C olefinic 129.10d; C olefinic 128.94; C2,6 or C2′,6′ 105.75d; C2′,6′ or C2,6 104.82d; C4′

TABLE 2. In vitro Antimicrobial Activity of *Diphysa robinioides* Isolates

Substance	Minimum Inhibitory Concentration (μg/ml)						
	Organism Number[a]						
	1	2	3	4	5	6	7
(−)-4′-*O*-Methylpreglabridin (**3**)	6.25	i[b]	i	i	6.25	12.5	i
(+)-4′-*O*-Methylglabridin [(+)-**2**][c]	6.25	i	i	i	3.12	i	i
Chamaejasmin	12.5	i	i	i	25.0	i	i
Streptomycin sulfate	5	5	50	2.5	1.25	i	i

[a]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, and 7=*Pseudomonas aeruginosa* ATCC 27853.

[b]i=inactive at highest level tested.

[c]Data from Mitscher *et al.* (8).

EXPERIMENTAL

EXTRACTION AND ISOLATION.—Dried and ground wood (2.2 kg) of *D. robinioides* (1) was macerated at room temperature several times with EtOH, filtered, and the EtOH distilled off in vacuo to leave 450 g of gummy residue. This was suspended in a solution of H_2O-EtOH (4:1) and then extracted successively with petroleum ether, Et_2O, and EtOAc. These solutions were

[2]Bioassay details are described in Mitscher *et al.* (8). We are indebted to Professor Mitscher for assay of **3** and chamaejasmin.

101.66d. Nmr data were also obtained for the pentamethoxyderivative: ^{1}H nmr 3.82 (6H, OMe), 3.87 (3H, OMe), 3.91 (6H, OMe), 6.39t (1H, H4′), 6.66d (2H, H2′,6′), 6.73s (2H, H2,6), 6.93d (2H, olefinic), 7.01d (2H, olefinic); ^{13}C nmr 160.78 (C3,5), 153.19 (C3′,5′), 139.06 (C4), 137.95 (C1), 132.70 (C1′), 129.02 (olefinic C), 128.02 (olefinic C), 104.44 (C2,6 or C2′,6′), 103.62 (C2′,6′ or C2,6), 99.89 (C4′), 60.94 (C4 OMe), 56.16 and 55.34 (C3,5 and C3′,5′ OMes).

(−)-4′-*O*-METHYLPREGLABRIDIN (**3**).—Hreims 340.1674 corresponds to $C_{21}H_{24}O_4$ to −0.1 ppm. Eims (*m/z*, rel intensity) 340(28), 338(12), 323(23), 191(51), 190(4), 173(27), 150(100), 135(42); $[\alpha]^{25}D$ −7.6 c 0.25 (EtOH); uv ν max (EtOH) 284 sh, 280, 216 nm and ν max (EtOH, OH^-) 294, 228 nm; ^{1}H- and ^{13}C-nmr data in Table 1.

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