FLAVONOIDS OF *DESMANTHUS ILLINOENSIS*

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ABSTRACT.—Rutin, quercitrin, myricitrin, kaempherol-3-*O*-glucoglucosyl (1-2) and two gallic esters of myricitrin attached in positions 2'' and 4'' to rhamnose were isolated from *Desmanthus illinoensis*. Biological activity and ^{13}C-nmr data for the last four flavonoids are reported.

Desmanthus illinoensis (Michaux), distributed over most of the southern United States, was collected in Oktibbeha County, Mississippi, and identified at the Botanical Institute of Mississippi State University. No chemical work has previously been reported on this plant which belongs to the family Leguminosae. *D. illinoensis* was selected for study because of its allelopathic activity in our bioassay (unpublished data). During our search for the active compounds, several flavonoids were isolated, identified and tested for growth inhibition and antibacterial properties. Acyl glycoside flavonoids isolated from *D. illinoensis* have previously been reported in the Crassulaceae and Pinaceae families (1-2).

We present the first record in the literature of ^{13}C-nmr data for the two gallic esters of myricitrin and the diglucoside of kaempferol.

MATERIALS AND METHODS

EXTRACTION AND CHROMATOGRAPHY.—Leaf material of *Desmanthus illinoensis* was freeze dried, powdered (50 g) and extracted in a Soxhlet with two liters of methanol for 8 hours. The solvent was evaporated and the residue (40 g) was redissolved in methanol and chromatographed on a polyamide column (Machere-Nagel) L=150 cm, Ø=4 cm eluted with methanol-water (5:5). Several bands were collected. The first was composed of sugars, the second contained flavonoid A along with other materials, and the third gave flavonoid B (impure). Bands IV, V, VI, VII and VIII contained, respectively, flavonoids C, C+D, D, E and F. All the fractions were rechromatographed separately on a sephadex LH 20 column, L=150 cm. Ø=2 cm eluted with methanol. In both cases each fraction was monitored by cellulose tlc: *t*butanol-acetic acid-water, 3:1:1 and 15% acetic acid.

ACETYLATION.—Ten mg of flavonoid were dissolved in 15 ml of acetic anhydride containing 1.5 ml of pyridine and allowed to stand overnight at 25°. The product was poured into water, and the solvent was removed by vacuum and the product crystallized from methanol-water (40:60).

BIOASSAY.—Root and stem growth determinations were made according to Nicollier and Thompson (3). Antibacterial properties were established by the method of Sikorowksi *et al.* (4). Its effect on the larval growth of the tobacco budworm, *Heliothis virescens*, was determined according to the methods of Hedin *et al.* (5).

ANALYTICAL.—The ^{1}H-nmr spectra were measured in $CDCl_3$ with TMS as an internal standard and ^{13}C-nmr in MeOD on a Varian CFT-20, mass spectra EI were made on a HP-5985B.

RESULTS AND DISCUSSION

Flavonoid A was hydrolyzed to kaempferol and glucose, indicative of a glucoside of kaempferol. The high Rf value (0.70) on cellulose tlc with 15% acetic acid indicated the presence of two sugars on flavonoid A. The ^{13}C spectrum (see table 1) suggested that the sugars are attached to one another. One anomeric carbon is shifted upfield 101.4 ppm, whereas the other anomeric carbon is 104.29 ppm which corresponds to the shift of a C-1' glucose. The upfield shift of the first

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TABLE 1. ^{13}C-nmr of myricitrin, gallic acid ester in 2" and 6" of myricitrin and kaempferol 3-O-diglucoside.

Compounds	Carbon No. and Shifts (ppm from TMS, solvent MeOD)														
	2	3	4	5	6	7	8	9	10	1'	2'	3'	4'	5'	6'
Myricitrin	158.4	134.53	177.96	162.78	99.86	165.39	94.42	157.5	105.02	122.59	109.49	146.43	137.02	146.43	109.49
Gallic acid ester 2' of myricitrin	159.71	135.03	178.63	162.37	99.20	165.03	94.14	157.76	105.20	121.25	109.88	146.14	137.23	146.14	109.88
Gallic acid ester 4" of myricitrin	159.06	136.12	179.30	162.80	99.49	165.44	94.36	158.20	105.67	121.2	110.3	146.45	137.57	146.45	110.3
Kaempferol 3-O-diglucoside	155.91	131.85	177.0	161.2	99.73	163.9	94.51	155.91	104.64	122.17	131.57	115.88	155.91	115.88	131.85

Compounds	Sugar Carbons No. and Gallic acid Shifts (ppm from TMS)														
	Glor R C-1	C-2	C-3	C-4	C-5	C-6	Gl or gallic acid C_1	C_2	C_3	C_4	C_5	C_6	C=0 R		
Myricitin	103.22	71.60	71.91	73.10	71.60	17.27									
Gallic acid ester 2" of myricitrin	99.29	72.91	70.10	73.23	71.60	17.01	121.25	109.11	145.70	139.28	145.70	109.11	166.83		
Gallic acid ester 4" of myricitrin	103.29	71.96	70.69	75.19	69.25	17.00	121.77	109.42	146.04	139.57	146.04	109.42	168.15		
Kaempferol 3-O-diglucoside	101.40	80.10	74.97	69.70	77.27	61.53	104.29	74.07	75.96	70.89	77.51	61.95			

anomeric carbon, indicative of a sugar in the C–2' position, is supported by a downfield shift of 74.07–80.10 ppm, further evidence for the C(2')-glycosidic linkage (6). The other assignments for the carbon shifts were made according to Wenkert and Gottlieb (7). The proton magnetic resonance spectrum gives a doublet at 6.05 ppm for H–C(1') with a coupling constant of 15 Hz (β configuration) and is shifted downfield, evidence that the glucosides are attached in the 3 position (8). The second H–(C–1") is at 5.1 ppm the normal shift for the glucose attached to another sugar.

The flavonoids E and F gave the same compounds upon hydrolysis, gallic acid, myricetin, and rhamnose, which suggest gallic acid esters of myricitrin. The Rf values for these flavonoids, as determined on cellulose tlc, 15% acidic acid were: E, 0.30 and F, 0.25. The ^{13}C-nmr data (see table 1) have significant differences in their shifts only for the sugar spectrum. The assignments of the resonances have been made according to myricitrin proton coupled spectra by use of a gating system and according to the published ^{13}C-nmr data of related flavonoids (7). The carbon anomeric of rhamnose in E was 99.29 ppm and, in F, 103.29 ppm. The first shift, upfield, is indicative of a substitution at the C–2" position, being shifted +1 ppm downfield compared with myricitrin. In the case of flavonoid F, gallic acid is attached in the C–4" position as indicated by a downfield shift of 75.19 ppm compared to 73.10 ppm for myricitrin. The position of gallic acid is indicated by examination of the protons H–C(2") and H–C(4") of the two acetylated flavonoids. E shows a broad signal at 5.88, integrated as two protons H–C(1") and H–C(2"), but F shows two separate signals 5.89 H–C(1") and 5.82–5.76 H–C(4"). Therefore, gallic acid is in the 2 positions for the flavonoid E and in the 4 position for the flavonoid F.

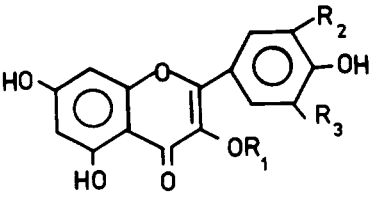

FIG. 1. A: R_1=glucoglucosyl (1–2), R_2=R_3=H
kaempferol-3-O-diglucoside
B: R_1=rhamnoglucosyl (1–6), R_2=OH, R_3=H
Rutin
C: R_1=rhamnose, R_2=OH, R_3=H
Quercitrin
D: R_1=rhamnose, R_2=R_3=OH
Myricitrin
E: R_1=gallic acid ester of rhamnose (1–2) R_2=R_3=OH
Gallic ester of myricitrin=desmanthin-1
F: R_1=gallic acid ester of rhamnose (1–4) R_2=R_3=OH
Gallic ester of myricitrin=desmanthin-2

Flavonoids such as naringenin, found in dormant peach buds, and 5,4'-dihydroxy-7-methoxy flavanone isolated from *Betula verrucosa*, antagonize the action of gibberellins (9–10). Isoflavones, genistein 7-glucoside, and genistein C-monoglucoside (from *Lupinus luetus*) are characterized as endogenous growth regulators of yellow lupine (11). The leaves of *Kalanchoe glossfeldiana* contain a specific flowering inhibitor which was isolated and identified as gallic acid (12). Testing the hypothesis that some allelochemical may affect both host-plant specificity and plant-plant interaction, several flavonoids isolated from *D. illinoensis* were tested for growth regulating activity (see fig. 2), antibacterial property (table 2), and for resistance on larval growth of the tobacco budworm (table 3). The results of these three tests show that the gallic acid ester of myricitrin (2") seems to be a key factor in the multiple activities of this plant. The importance

of the linkage in the 2" position of the gallic ester is also shown. The ED_{50} value for this gallic acid ester of myricitrin in the tobacco budworm larval inhibition assay was 0.04, similar to that reported for tannins, flavonoid and anthocyanidin (5). Figure 2 shows that some compounds have more activity at low concentrations than at higher concentrations; for instance, the strong activity of gallic acid, myricitrin, and myricetin may reflect the high degree of oxidation of these molecules.

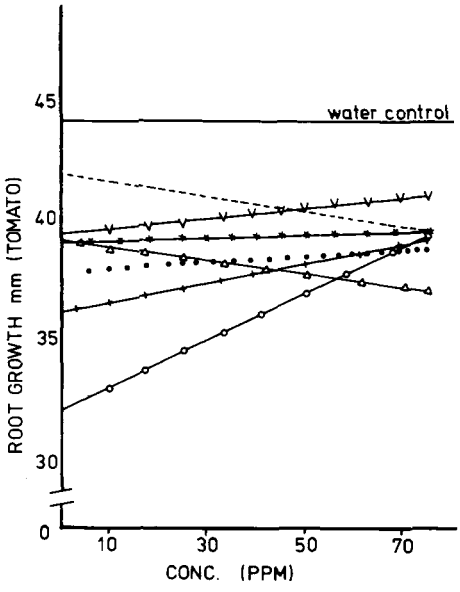

FIG. 2. 1. Quercetin -△-△-
2. Quercitrin -★-★-
3. Rutin -●-●-
4. Myricetin -+-+-
5. Myricitrin -o-o-
6. Gallic acid -v-v-
7. Gallic acid ester in 2" of myricitrin - - - - -
8. Gallic acid ester in 4" of myricitrin no activity.

IDENTIFICATION (FIG. 1):

Flavonoid A (10 mg), mp: 150°; uv max (MeOH): 331, 267; $AlCl_3$: 416, 346, 300 sh, 274; $AlCl_3$+HCl: 390, 342, 272; NaOMe: 397, 322, 273; NaOAC: 367 sh, 322, 273. Hydrolysis with 2N HCl gave the aglycone kaempferol and sugars. After neutralizing, the aq. phase was chromatographed on cellulose tlc [isoamly-alcohol-pyridine-water (7:7:2)] and found to give only glucose. ^{1}H-nmr (TMS, $CDCl_3$): 8.01–7.9 (dd, 2H, H-2'-H-6' $J=27$ and $J=9$ Hz), 6.90–6.80 (d, 1H,

TABLE 2. Antibacterial property of *Desmanthus illinoensis* constituents.

Bacteria	Myricitrin	Gallic acid ester		Quercetin	acid
		In 2" of myricitrin	In 4" of myricitrin		
Pseudomonas mallophilia...	−	++[a]	−	++	+
Bacillus sphaericus.....	−	+	−	−	+
Bacillus thuriengensis.....	+	++	+	−	
Bacillus subtilis.....	+	++	+	+	++

[a]100 μmg applied to bacteriological test disc paper produced a 10 mm clear zone on a nutrient agar innoculated with the test bacteria.

TABLE 3. Inhibition of tobacco budworm larval growth by some *Desmanthus illinoensis* constituents, ED_{50} as percent of diet.

Gallic acid	18.50
Quercetin[a]	0.05
Myricitrin	0.11
Gallic acid ester in 2" of myricitzin	0.04
Gallic acid ester in 4" of myricitrin	0.09

[a]Using the value of Hedin et al. (5).

H–3', $J=2.8$ Hz), 6.82–6.78 (d, 1H, H–5', $J=2.7$ Hz), 6.35 (d, 1H, H–8, $J=2$ Hz), 6.27–6.24 (d, 1H, H–6, $J=2$ Hz), 6.05–6.01 (d, H–C–1" 3-glucosyl, $J=15$ Hz), 5.1 [6.5, 1H, H–C(1" ') 3-glucosyl], 3.96–3.82 (bs, 12 H, 3 diglucosyl). ^{13}C-nmr see table 1. The structure as it was discussed above is kaempferol-3-*O*-gluco-glucosyl (1–2).

Flavonoid B (10 mg): Rutin: uv spectra and ^{1}H-nmr data were similar to those published for rutin (6).

Flavonoid C: Quercitrin (3 g): uv, ^{1}H and ^{13}C-nmr spectra were similar to those published for quercitrin (8).

Flavonoid D: Myricitrin (2 g): uv, ^{1}H-nmr spectra were similar to those of a pure sample; the ^{13}C-nmr spectrum is given in table 1.

Flavonoid E (2 g), mp; 196° uv max (MeOH) 354, 297 sh, 269; $AlCl_5$: 426, 304, 273; $AlCl_3$+HCl: 403, 359, 275; NaOMe: 332, 267, 256 sh, NaOAc: 358, 322, 272. Acid hydrolysis with 2N HCl gave myricetin (uv identical with pure material) and gallic acid. Cellulose tlc of the neutralized hydrolysate (same solvent as above) gave rhamnose. ^{1}H-nmr (acetylated, $CDCl_3$); 7.79–7.70 (d, 4H, H–2', H–6' and H–2', H–6' of gallic acid part, $J=7.5$ Hz), 7.27–7.25 (d, 1H, H–8, $J=2$ Hz), 6.85–6.82 (d, 1H, H–6, $J=2$ Hz), 5.88 (6.5, 2H, H–C(1') and H–C(2')), 5.25–5.23 (m, 2H, H–C(3") and C(4")), 5.07–4.94 (d, 1H, H–C(5')), 2.40 (s, 3H, OAC–C–5), 2.30 (s, 21H, 7 Ac), 2.11 (s, 3H, OAC sugar), 2.08 (s, 3H, OAc sugar), 1.00–0.92 (d, 3H, CH_3-rhamnosyl, $J=6.1$ Hz). The ms gave important fragments at: *m/e*: 318, 302, 170, 153, 126. And after acetylation: *m/e*: 528, 486, 278, 236. For the ^{13}C-nmr see table 1. The flavonoid E was identified as gallic acid ester 2" of myricitrin.

Flavonoid F (100 mg) mp: 176°; uv max (MeOH) 405, 358, 307 sh, 270; NaOMe 324, 266; NaOAc 378, 322, 272. Hydrolysis of this compound gave myricetin with the same uv and ^{1}H-nmr as an authentic sample and gallic acid. Cellulose tlc of the neutralized hydrolysate (same solvent as above) gave rhamnose. ^{1}H-nmr (acetylated, $CDCl_3$): 7.71–7.68 (d, 4H, H–2', H–6' and H–2" and H–6" of gallic acid part), 7.25 (bs, 1H, H–8), 6.85–6.83 (d, 1H, H–6, $J=2$ Hz), 5.89 (bs, 1H, H–C(1')), 5.82–5.76 (m, 1H, H–C(4")), 5.28–5.20 (m, 2H, H–C(2")), 5.08 (bs, 1H, H–C(5")), 2.42 (s, 3H, OAc-C(5)), 2.33 (s, 21H, 7 OAc aromatic), 2.9 (s, 3H, OAc-sugar C(2')), 1.96 (s, 3H, OAc-sugar C(3")), 1.00–0.92 (d, 3H, CH_3-rhamnosyl $J=6.2$ Hz).

The mass spectrum gave the following fragments: *m/e* 318, 302, 170, 153, 137, 126 and from the acetylated compound 528, 495, 296, 279, 237, 153. From all the data and according to the discussion above, flavonoid F was deduced to be the gallic acid ester 4" of myricitrin.

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