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BIFLAVONOIDS FROM FRUITS OF POISON IVY *TOXICODENDRON RADICANS*

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Key Word Index—*Toxicodendron radicans*; Anacardiaceae; poison ivy; biflavonoids; 3′,8″-binaringenin, amentoflavone; tetrahydroamentoflavone.

During the course of our analytical work [1] with poison ivy (*Toxicodendron radicans*), an ethanolic extract of the fruits of the plant was prepared. Upon partitioning of the ethanol extract between chloroform and water, a brown colored amorphous residue was obtained in the interface. TLC on silica gel plates using 20% methanol in chloroform showed the presence of two major phenolic spots (R_f 0.55 and 0.62). Repeated chromatography of the residue obtained above results in the isolation of two compounds A and B. The structure determination of these two phenolic compounds is the subject of this note.

Compound A, mp 250–257° was optically inactive; gave orange color with Mg–HCl test and green color with $FeCl_3$. It formed a hexaacetate, mp 245–248° and a hexamethyl ether, MW 622 (Mass). This compound was characterized as amentoflavone (**1**) by comparing the spectral data with those reported in the literature [2]. Particular attention was drawn to the ^{13}C NMR, which was identical to that reported [3] for amentoflavone. In addition direct comparison was made with an authentic sample (mp, IR and ^1H NMR).

Compound B was isolated as light tan powder, which gave purple color with Mg–HCl and purple blue with $FeCl_3$; mp 234–239° dec.; $[\alpha]_D^{23}$ −19° (*c* 0.68, MeOH); IR, ν_{max}^{KBr} 3460 (br, OH) and 1650 (C=O) cm^{-1}. The UV spectrum [λ_{max}^{MeOH} 321 nm (log ε 4.43), 290 (4.52) and 228 (4.64)] with bathochromic shift in basic medium [$\lambda_{max}^{MeOH-NaOMe}$ 322 nm (log ε 4.72) and 227 (4.57)] was similar to that of naringenin; which also underwent bathochromic shift in the presence of NaOAc or $AlCl_3$ indicating the presence of OH groups at 5 and 7 positions [$\lambda_{max}^{MeOH-NaOAc}$ 322 nm (log ε 4.74) and 230 (5.12); $\lambda_{max}^{MeOH\ AlCl_3}$ 375 nm (log ε 3.99), 311 (4.69) and 227 (4.74)].

The ^1H NMR (60 MHz, DMSO-d_6) of compound B showed peaks at δ 7.25 (*m*, 4H), 6.89 (*d*, *J* = 8 Hz, 1H), 6.73 (*d*, *J* = 8 Hz, 2H), 6.12 (*s*, 1H), 5.95 (*s*, 2H), 5.47 (*br d*, *J* = 12 Hz, 2H), 3.18 (*br m*, 4H) and two D_2O exchangeable protons at 11.13 (*s*, 1H) and 11.25 (*s*, 1H) (two bonded phenolic OH groups). The mass spectrum showed a molecular ion at *m/e* 542, for $C_{30}H_{22}O_{10}$, indicating a biflavanone with 6 phenolic hydroxy groups. The fact that compound B had 6 OH groups and 10 aromatic protons along with UV similarities with naringenin indicated a binaringenin structure with a C-C linkage. The structure was proven to be 3′,8″-binaringenin (tetrahydroamentoflavone) as follows.

The ^1H NMR spectrum showed 7 aromatic protons as part of the A_2B_2 system, four of which were further downfield indicating that C-3′ position of one naringenin unit was substituted. Since it is known that, in the flavonoids, the C-6 proton appears up field from C-8 proton [4], the two protons singlet at δ 5.92 was assigned to the C-6 and C-6″ protons and the one proton singlet at δ 6.12 was assigned to H-8. Comparison of the ^{13}C NMR of compound B with that of rhusflavanone (6,8″-binaringenin) and naringenin (Table 1) clearly indicated a 3′,8″-linkage [3]. The chemical shift of C-3′ in the two model compounds was 115.4 and 115.2 ppm, respectively. The ^{13}C NMR spectrum of B showed a peak at 120.1 ppm which shows a shift of ~ 5 ppm units indicating a C-C linkage at C-3′. Also, the ^{13}C NMR spectrum of compound B showed 3 signals at 95.8, 95.7 and 95.1 ppm assigned to carbons 6,6″ and 8, respectively. The signal for C-8″ was found at 105.9 ppm again indicating a C-C linkage at that position since resonances for carbons 6 and 8, in 5,7-dihydroxyflavoids absorb between 90 and 100 ppm with C-6 about 0.9 ppm downfield from C-8 [3].

Finally, dehydrogenation of B (I_2/HOAc/KOAc) [5] resulted in the formation of a biflavone which was purified by preparative TLC, the hexaacetate of which was

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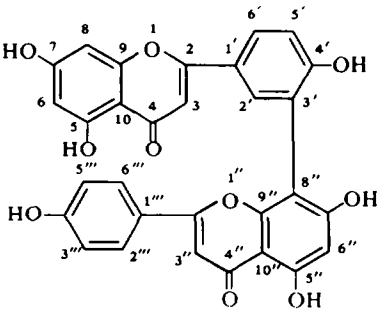
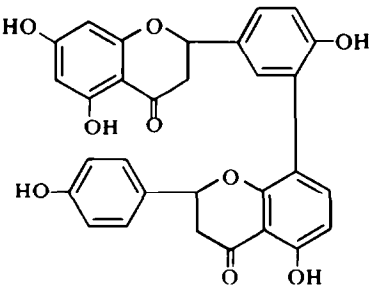
Table 1. Comparison of the ^{13}C NMR chemical shifts of 3',8''-binaringenin, rusflavanone and naringenin

Carbon No.	Chemical shift (ppm) Naringenin	Rhusflavanone	3',8''-Binaringenin	Carbon No.	Chemical shift (ppm) Rhusflavanone	3',8''-Binaringenin
2	78.4	78.6[a]	78.7[g]	2''	77.9[a]	77.9[g]
3	42.0	42.3[b]	42.3[b]	3''	42.1[b]	41.6[h]
4	196.2	196.4[c]	196.7[e]	4''	196.3[c]	196.2[e]
5	162.9	161.8	163.6	5''	161.8	162.5
6	95.9	101.2	95.8[i]	6''	95.7	95.7[i]
7	166.7	165.2	166.7[f]	7''	165.2	164.7[f]
8	95.0	94.6	95.1	8''	100.3	105.9
9	163.6	162.7	157.4	9''	161.6	155.9
10	101.8	101.8[d]	102.1[j]	10''	101.1[d]	101.9[j]
1'	128.9	129.2	131.2	1'''	129.2	129.1
2'	128.2	128.1	126.9	2'''	127.6	127.8
3'	115.2	115.4	120.1	3'''	115.3	115.1
4'	157.8	157.7	160.2	4'''	157.3	163.1
5'	115.2	115.4	115.1	5'''	115.3	115.1
6'	128.2	128.1	128.3	6'''	128.1	127.8

Values with the same superscript could be interchanged.



Amentoflavone **1**



3',8''-Binaringenin **2**

identical (mp, mmp, IR, UV, ^1H NMR, MS) with amentoflavone hexacetate. Thus B is 2,3,2'',3''-tetrahydroamentoflavone or 3',8''-binaringenin (**2**). The large coupling constant for the 2,2'' protons indicates that the phenyl rings in both cases are in the equatorial conformation. This is the first isolation of 3',8''-binaringenin from nature, although Rao *et al.* [6] reported the isolation of two bichalcones from the methylated mixture of a phenolic fraction obtained from *Semecarpus anacardium* and indicated that these two bichalcones could be derived from this biflavanone.

EXPERIMENTAL

Mps are uncorr. IR spectra were determined in KBr pellets. All spectra were recorded using DMSO-d_6 as solvent ($CDCl_3$ for acetates) and TMS as internal standard.

Plant material and extraction of the biflavonoids. The fruits of poison ivy (650 g) (*Toxicodendron radicans*) were collected in October 1975 in Oxford, Mississipi. Herbarium specimens were stored in the Herbarium, Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677. The fresh fruits were extracted with EtOH (15.1) and the EtOH extract concd. The concentrate (31 g) was partitioned between $CHCl_3$ (3 × 250 ml) and H_2O (100 ml) and the dark brown residue (5.5 g) obtained in the interface was used for the isolation of 3',8''-binaringenin and amentoflavone.

Isolation of 3',8''-binavingenin and amentoflavone. The brown residue obtained above (1.1 g) was chromatographed on a column (3 × 39 cm) of Si gel 60-PF (75 g, processed to 60 mesh) using 10% MeOH in $CHCl_3$ where the biflavanone (458 mg) was eluted first, followed by amentoflavone. Further purification was carried out by re-chromatography using the same system.

Dehydrogenation of binaringenin. Binaringenin (50 mg), KOAc (0.4 g) and I_2 (90 mg) were refluxed in HOAc (10 ml) for 4 hr. After cooling, the reaction mixture was poured into H_2O (30 ml) and the ppt. extracted with EtOAc. Evapn of the solvent afforded a dark brown residue which was extracted with CCl_4 to remove excess I_2 and purified by prep-TLC using $CHCl_3$-EtOAc (1:1). Acetylation of the purified product (Ac_2O/Py) followed by crystallization from $CHCl_3$–EtOH afforded needle crystals of a hexaacetate (18 mg) which was identical (mp, mmp, IR, UV and ^1H NMR) with amentoflavone hexaacetate.

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