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PLANT ANTIMUTAGENS, 6.[1] INTRICATIN AND INTRICATINOL, NEW ANTIMUTAGENIC HOMOISOFLAVONOIDS FROM *HOFFMANOSSEGGIA INTRICATA*

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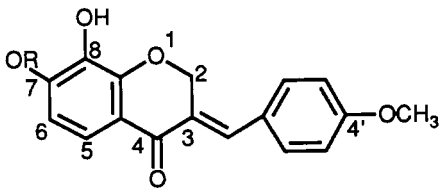
ABSTRACT.—Intricatin [**1**] and intricatinol [**2**] are new homoisoflavonoids isolated from a desert plant *Hoffmanosseggia intricata*, which was collected in Baja California, Mexico. The structures of the compounds were elucidated using a variety of spectroscopic techniques. The structure of **1** was shown to be 7,4′-dimethoxy-8-hydroxyhomoisoflavone, and **2** was shown to be 4′-methoxy-7,8-dihydroxyhomoisoflavone. Both compounds **1** and **2** displayed activity in the inhibition of the mutagenicity of 2AN toward *Salmonella typhimurium* (T98). Compound **2** was much more active than **1** in the case of the inhibition of the mutagenicity of AAF toward *S. typhimurium* (T98) and the inhibition of EMS towards *S. typhimurium* (T100). Compounds **1** and **2** are the first examples of antimutagenic activity in homoisoflavones.

Two new closely related homoisoflavonoids, intricatin [**1**] and intricatinol [**2**], have been isolated from the roots of *Hoffmanosseggia intricata* Brandegee (Fabaceae), guided by the inhibition of the mutagenicity of 2-aminoanthracene (2AN) toward *Salmonella typhimurium* (T-98) by crude and purified extracts and concentrates (1–5). Homoisoflavonoids, with one expection, have previously been reported to occur only in plants which are members of the Liliaceae family (6–10). This paper reports the characterization and antimutagenic or toxic properties of new homoisoflavones **1** and **2** isolated from a nonrelated family, Fabaceae.



1 R = Me
2 R = H

EXPERIMENTAL

ANTIMUTAGENIC TESTING.—The procedures for determining the inhibition of the mutagenicity of 2AN toward cultures of *S. typhimurium* (T-98) by crude plant extracts or purified products and toxicity determinations have been described in previous papers (1–5). Assays for antimutagenicity of pure compounds toward 2AN and acetylaminofluorene (AAF), which require metabolic activation, and ethyl methanesulfonate (EMS), which does not, have also been described (1–5).

GENERAL ISOLATION AND CHARACTERIZATION METHODS.—Melting points were determined on a Kofler hotstage microscope and are uncorrected. ^{1}H- and ^{13}C-nmr spectra were obtained with a Bruker WM250 spectrometer using TMS as an internal standard and $CDCl_3$ as a solvent. High resolution mass spectra were obtained with an AEI MS-902 instrument. Uv spectra were obtained in MeOH with a Varian 2290-UV-VIS spectrometer and ir spectra with Perkin-Elmer 467 Grating spectrometer. Standard chromatography was carried out on Si gel E. Merck 230–40 mesh or Baker Flash chromatography Si gel using, in general, CH_2Cl_2 as eluent with a gradient of 0.5–10.0% MeOH. For tlc determinations precoated Si gel plates were utilized; normal phase EM precoated Si gel 60, F254, usual solvent 10% MeOH in

[1]For Part 5 of this series, see Manikumar *et al.* (5).

CH_2Cl_2; reversed-phase, Baker precoated Si gel C_{18}-F plates, usual developer 5–10% H_2O in MeOH. Exposure of plates to iodine vapor was used as a general detection agent; alternatively, spraying with phosphomolybdate reagent followed by heating was utilized.

PLANT MATERIALS.—Roots of *H. intricata* were collected in March 1980, in Baja California, Mexico, by Richard Spjut, Plant Medicinal Resources Laboratory ARS. A voucher specimen is deposited in the herbarium of the National Arboretum, ARS.

EXTRACTION AND ISOLATION.—Roots (0.2 kg) were extracted with hot 95% EtOH. The EtOH extract was concentrated and the residue partitioned between H_2O and $CHCl_3$. After concentration of the $CHCl_3$ fraction, the crude residue (8.7 g) was tested for inhibition of 2AN mutagenicity at 0.6 mg/plate. Two separate assays showed inhibition of 38 and 50%. The $CHCl_3$ residue was nontoxic at 0.6 mg/plate. The $CHCl_3$ residue (4.0 g) was chromatographed on 50 g of Baker Si gel and was eluted initially with 0.5% MeOH in CH_2Cl_2 (fractions 1–50). Fractions 30–50 yielded inhibitory material (0.3 g). Further elution with 2% MeOH in CH_2Cl_2 yielded inhibitory material (0.06 g) in fractions 76–120. The material from fractions 30–50 was crystallized from aqueous MeOH, yielding 0.044 g of yellow crystals of a new compound designated intricatin [**1**] (7,4′-dimethoxy-8-hydroxyhomoisoflavone), 62% inhibition of 2AN at 0.3 mg/plate, nontoxic at 0.6 and 0.3 mg/plate. The more polar fraction was crystallized from aqueous MeOH, yielding 0.12 g of yellow needles of a new compound designated intricatinol [**2**] (4′-methoxy-7,8-dihydroxyhomoisoflavone), toxic at 0.6 mg, nontoxic at 0.3 mg/plate, 87% inhibition of 2AN at 0.3 mg/plate.

Intricatin [**1**].—Mp 157–159°, uv λ max (MeOH) nm (log ε) 347 (4.41), 237 sh (4.27), (MeOH + NaOH) 393 (4.48), 324 (4.20), 269 (3.99); ir ν max ($CHCl_3$) 3510 (OH), 1660 (C=O), 1600 (aromatic), 1470, 1300, 1175, 840, 820 cm^{-1}; ^1H nmr (Me_2CO-d_6) δ 3.82 (3H, s, OMe), 3.88 (3H, s, OMe), 5.48 (2H, d, J = 2 Hz, H-2), 6.65 (1H, d, J = 8.8 Hz, H-6), 7.06 (2H, d, J = 8.8 Hz, H-3′, -5′), 7.43 (2H, d, J = 8.8 Hz, H-2′, -6′), 7.61 (1H, d, J = 8.8 Hz, H-5), 7.72 (1H, broad triplet, H-9); hrms *m/z* (rel. int. %) $[M]^+$ 312.0990 ($C_{18}H_{16}O_5$ = 312.0998) (100), 297 (15), 167 (55), 146 (70), 121 (35).

Intricatinol [**2**].—Mp 196–198°, uv λ max (MeOH) nm (log ε) 351 (4.44), 234 sh (4.24), (MeOH + NaOH) 396 (4.33), 320 (4.17), 286 (3.93); ir ν max (KBr) 3375, 1660, 1600, 1270, 840, 815 cm^{-1}; ^1H-nmr (DMSO-d_6) 2 broad OH peaks at δ 10.0 and δ 8.7 exchanged with D_2O, 3.83 (3H, s, OMe), 5.39 (2H, d, J = 2 Hz, H-2), 6.57 (1H, d, J = 8.5 Hz, H-6), 7.06 (2H, d, J = 8.5 Hz, H-3′, -5′), 7.27 (1H, d, J = 8.5 Hz, H-5), 7.42 (2H, d, J = 8.5 Hz, H-2′, -6′), 7.65 (1H, broad s, H-9); hrms *m/z* (rel. int. %) $[M]^+$ 298.0845 ($C_{17}H_{14}O_5$ = 298.0841) (30), 153 (65), 152 (40), 146 (100).

RESULTS AND DISCUSSION

The uv and ir spectra of intricatin [**1**] and intricatinol [**2**] are similar to those described for the structurally similar homoisoflavones bonducellin (9) and 8-methoxybonducellin (10). Hrms of **1** gave a molecular formula of $C_{18}H_{16}O_5$ in accord with the assigned structure. Ions at *m/z* 312 $[M]^+$, 297 $[M-15]^+$, and 281 $[M-31]^+$ are characteristic. Of greater diagnostic value, however, was a strong fragment peak at *m/z* 167 indicative of retro-Diels-Alder rearrangement with one OH and one OMe moiety in ring A (11). Hence, the remaining OMe group must be in ring B.

The ^1H-nmr spectrum at 250 MHz (Me_2CO-d_6) revealed the presence of two methoxy groups with signals at δ 3.88 and δ 3.82. One methoxy group was assigned to the 4′ position in agreement with literature (6–10) and the other to ring A based on the mass spectral fragmentation pattern (11). A perturbed AA′, BB′ system had two protons centered at δ 7.43 and two at δ 7.06 which were assigned to H-2′, -6′ and H-3′, -5′, respectively, similar to the data reported for bonducellin (9,10). The spectra showed two ortho coupled protons (J = 8.5 Hz) at δ 7.61 and δ 6.65 assignable to H-5 and H-6, respectively. A proton due to H-9 appeared at δ 7.72 as a broad triplet as a result of coupling with the two methylenic protons at position 2, which appeared as a doublet at δ 5.48 (J = 2 Hz). The position of the H-9 proton is indicative of a trans double bond at this position (6). In $CDCl_3$, a D_2O exchangeable proton (8-OH) appeared at δ 6.3. The location of the methoxy groups at C-7 and C-4′ was deduced from the C_6H_6-induced shifts of methoxy groups. Only methoxy groups ortho to an aromatic proton are shifted upfield by 0.5 ppm in C_6D_6 compared to $CDCl_3$ (12). We found both

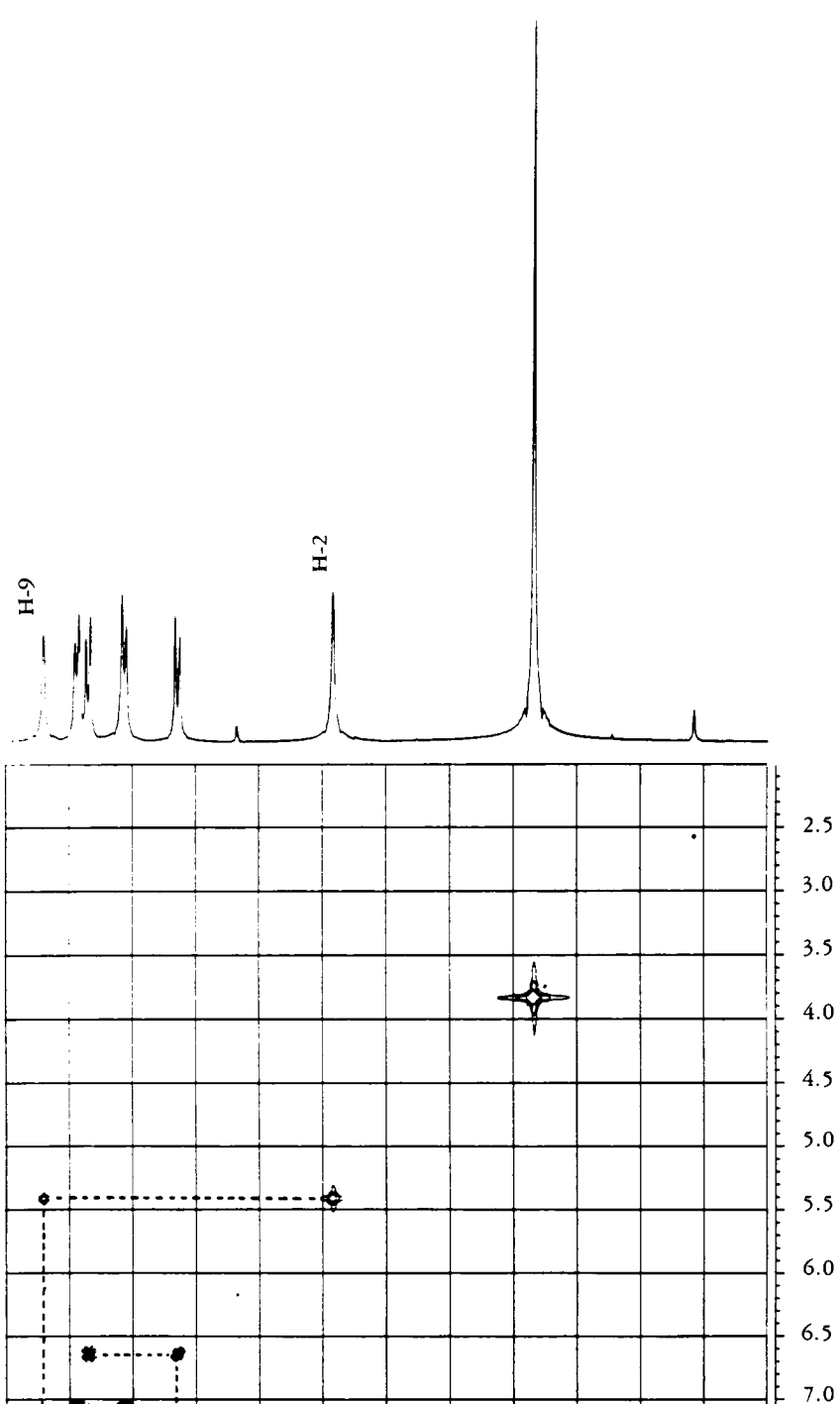


FIGURE 1. Long range 2D proton correlation (COSYLR) of intricatinol [**2**].

methoxy groups at C-7 and C-4′ shifted upfield by 0.5–0.6 ppm, confirming the structure of **1** as 7,4′-dimethoxy-8-hydroxyhomoisoflavone.

The uv and ir spectra of **2** closely resembled those of **1** and 8-methoxybonducellin (10). Hrms showed that the molecular formula of **2** was $C_{17}H_{14}O_5$. A strong fragment of *m/z* 153 was indicative of a retro-Diels-Alder rearrangement with two OH moieties in ring A (11).

The nmr spectrum (250 MHz) of **2** in DMSO-d_6 revealed the presence of one methoxy group at δ 3.83 assigned to the C-4′ position and two D_2O exchangeable hydroxyl groups at δ 10.0 and 8.7. A perturbed AA′, BB′ system had two protons centered at δ 7.42 and two at δ 7.06 which were assigned to the 2′, 6′ and 3′, 5′ positions, respectively. There were two ortho coupled protons ($J = 8.5$ Hz) centered at δ 7.27 and δ 6.57. These signals corresponded to H-5 and H-6, respectively. The two aliphatic protons at position 2 appeared as a narrow doublet at δ 5.39 ($J = 2$ Hz), and H-9 appeared at δ 7.65. The relationship of H-2 and H-9 protons was confirmed by long range 2D-proton correlation spectra (COSYLR) (Figure 1). Hence the structure of **2** is 7,8-dihydroxy-4′-methoxyhomosioflavone.

Intricatin [**1**] was nontoxic at 0.6 and 0.3 mg/plate; intricatinol [**2**] was toxic at 0.6 mg but nontoxic at 0.3 mg/plate. Table 1 shows the inhibition by **1** and **2** of the mutagenicity toward of *S. typhimurium* of 2AN, AAF, and EMS. The first two require metabolic activation but the latter is a direct acting mutagen which is known to alkylate the various DNA bases (13). Compound **1** was active in the inhibition of the mutagenicity of 2AN toward *S. typhimurium* (T98) but did not inhibit the mutagenicity of AAF, *S. typhimurium* (T98), or EMS, *S. typhimurium* (T100). The lack of dose response for **1** shown in Table 1 at doses of 300–37.5 μg is anomalous; usually a dose response is observed over this dose range (cf. Table 1, compound **2**). Compound **1** was inactive in inhibition of the mutagenicity of AAF toward *S. typhimurium*. The dihydroxy analogue **2** was in general more broadly active than **1** in the inhibition of the mutagenicity of 2AN, AAF, and EMS (Table 1). The greater potency of **2** may be due to the presence of a dihydroxy moiety in ring A. In a previous study with flavonoids (2), we found that hydroxylated flavonoids were generally more active than their methylated analogues.

TABLE 1. Inhibition of Mutagenicity of Various Mutagens by Intricatin [**1**] and Intricatinol (**2**).

Compound	Mutagen[a]								
	2AN[a,b] Positive Control 2.5 μg/plate 1794 Colonies/plate[c]			AAF[a,b] Positive Control 25 μg/plate 1131 Colonies/plate[c]			EMS[a] Positive Control 750 μg/plate 2046 Colonies/plate[c]		
	Dose μg/plate	Colonies[c] #/plate	Inhibition[d] %	Dose μg/plate	Colonies[c] #/plate	Inhibition %	Dose μg/plate	Colonies[c] #/plate	Inhibition %
1	300	673	62	300	1047	7	300	1126	45
	150	279	84	150	1341	0	150	1491	27
	75	151	92	75	1162	0	75	1966	4
	37.5	543	70	37.5	1035	8	37.5	1854	9
2	300	226	87	300	335	70	300	526	74
	150	773	57	150	518	54	150	363	82
	75	1141	36	75	590	48	75	537	74
	37.5	1654	8	37.5	840	26	37.5	1438	30

[a]2AN = 2-aminoanthracene; AAF = acetylaminofluorene; EMS = ethyl methanesulfonate.
[b]Requires metabolic activation.
[c]Average of 3 plates.
[d]$\%\ \text{Inhibition} = \frac{\text{Colonies in positive control} - \text{Colonies in experimental plate}}{\text{Colonies in positive control}} \times 100.$

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