Journal of Natural Products
Vol. 50, No. 2, pp. 178-180, Mar-Apr 1987

IRISONES A AND B: TWO NEW ISOFLAVONES FROM *IRIS MISSOURIENSIS*

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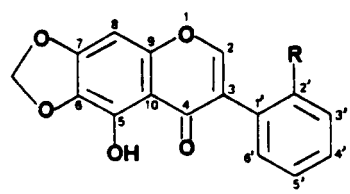
ABSTRACT.—Two new isoflavones, irisones A [**1**] and B [**2**], together with the known compound, 5,7-dihydroxy-2′,6-dimethoxy isoflavone, were isolated from the roots of *Iris missouriensis*. With the help of 2D-homonuclear-*J*-correlation and 2D-nOe experiments the structure of irisone A was deduced to be 5-hydroxy-2′-methoxy-6,7-methylenedioxy isoflavone. The structure of irisone B was elucidated by spectral analyses as 2′,5-dihydroxy-6,7-methylenedioxy isoflavone and was confirmed by chemical conversion to irisone A.

Extracts of the roots of *Iris missouriensis* Nutt. (Iridaceae) have been found to enhance the survival of P-388-infected mice, and we have reported the isolation and structure elucidation of active cytotoxic principles (1-3). Further investigation of a $CHCl_3$ extract led to the isolation of two new isoflavones, irisone A [**1**] (0.00005% yield) and irisone B [**2**] (0.00003% yield), and a known compound, 5,7-dihydroxy-2′,6-dimethoxy isoflavone (0.00006% yield). Here we report the isolation and structural elucidation of irisones A and B.

A molecular formula of $C_{17}H_{12}O_6$ for irisone A [**1**] was suggested by the molecular ion at *m/z* 312 and the number of carbons in the ^{13}C-nmr spectrum. The presence of an hydroxyl and an unsaturated carbonyl group (3400 and 1689 cm^{-1}, respectively) was indicated by the ir spectrum of **1**. These data, together with the mass fragment ions at *m/z* 181 and 131 (4) and uv absorption maxima at 269 and 335 nm (sh), suggested the presence of an isoflavone moiety. This conclusion was further substantiated by a ^{1}H-nmr signal at δ 7.89, which is typical for the 2-hydrogen of an isoflavone (δ 7.8-8.1) (4).

The 2D-homonuclear-*J*-correlation spectrum of **1** revealed couplings between the aromatic signals at δ 7.00 (H-3′) and 7.39 (H-4′). The latter signal (H-4′) was also coupled to signals at δ 7.03 (H-5′), which in turn was coupled to another aromatic proton at δ 7.32 (H-6′). These couplings led to the conclusion that irisone A has a 2′-substituted phenyl moiety.

In the ^{1}H-nmr spectrum of irisone A, signals for a methoxy group (δ 3.82), a methylenedioxy group (δ 6.10), and a 5-hydroxyl group (δ 12.80) were observed. The placement of the methoxyl group at the C-2′ position was confirmed by the 2D-nOe spectrum of **1**, in which a nOe was observed between the methoxy signal at δ 3.82 and the aromatic signal at δ 7.00, leading to the assignment of the broad doublet at δ 7.00 to H-3′. The rest of the aromatic protons could be easily assigned from the 2D-homonuclear-*J*-correlation spectrum. Thus, a doublet of doublet of doublets at δ 7.39 was assigned to H-4′, a doublet of doublet at δ 7.32 to H-6′, and a doublet of doublet



1 R=OCH_3
2 R=OH

of doublets at δ 7.03 to H-5′. Due to the presence of the 5-hydroxyl group, the methylenedioxy group could only be located at either the C-6 and C-7 or the C-7 and C-8 positions. The chemical shifts of the hydrogen and carbon signals (δ 6.52 and 89.11 ppm, respectively) for the A ring of irisone A were compatible with those at C-8 (δ 6.5-6.9 and 90-96 ppm) but not at C-6 (δ 6.2-6.4 and 97-100 ppm) in the spectra of related flavonoids (5-7). Thus, the methylenedioxy group is placed at the C-6 and C-7 positions. Therefore, the structure of irisone A was elucidated as 5-hydroxy-2′-methoxy-6,7-methylenedioxy isoflavone [**1**].

The structural similarity between irisone A [**1**] and irisone B [**2**] was revealed by comparison of the ir, uv, and ^{1}H-nmr spectra of these two compounds. In the ^{1}H-nmr spectrum of irisone B the signals for a methylenedioxy group (δ 6.24), a hydrogen bonded hydroxyl at C-5 (δ 12.82), a 2-hydrogen (δ 8.34), a 2′-substituted B ring (δ 7.33, 4′-H; 7.36, 6′-H; 7.02, 3′-H; 6.98, 5′H), and an 8-hydrogen (δ 6.78) were observed. However, the absence of a methoxyl signal and the appearance of a D_2O exchangeable singlet at δ 8.40 indicated the presence of a 2′-hydroxy substituent on the B ring of irisone B rather than a 2′-methoxyl functionality as found in irisone A. This was further supported by a difference of 14 mass units between the molecular ion of irisone A (*m/z* 312) and that of irisone B (*m/z* 298). Finally, the structure of irisone B was confirmed as 2′,5-dihydroxy-6,7-methylenedioxy isoflavone [**2**] by its chemical conversion to irisone A. Treatment of irisone B with CH_2N_2 gave a single product, which was in every respect (mp, uv, ms, ^{1}H nmr) the same as irisone A.

When evaluated for cytotoxic activity utilizing cultured P-388 cells, all three isolates were found to be inactive, having ED_{50} values greater than 50 μg/ml.

EXPERIMENTAL

GENERAL METHOD.—Melting points were determined by means of a Kofler hotstage apparatus and are uncorrected. The uv spectra were obtained with a Cary Model 118 spectrophotometer and ir spectra with a Nicolet MX-1 interferometer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded with Nicolet NMC 360 and NMC 200 spectrometers. TMS was used as an internal standard, and chemical shifts are reported on the ppm scale. Low-resolution mass spectra were obtained with a Varian MAT-112S double focusing mass spectrometer.

PLANT MATERIAL.—The collection of the plant material has been described previously(1-3).

EXTRACTION AND ISOLATION OF ISOFLAVONES.—Extraction of the roots of *I. missouriensis* has been described previously (3). The $CHCl_3$ extract (36 g) obtained from 1.3 kg of the dried roots was chromatographed over Si gel (1.3 kg) with gradient elution from $CHCl_3$ to MeOH to afford 30 fractions (2 liters each). Fractions 15 to 20, obtained from eluting the column with 5% MeOH, were combined (14 g) and rechromatographed over Si gel (100 g) pre-packed in hexane-EtOAc-HCOOH (50:5:1). Eluting with hexane-EtOAc-HCOOH (10:1:0.01) gave 28 fractions (65 ml each). Fractions 11 to 18 were pooled (1.5 g) and further chromatographed over Si gel (25 g). Elution with hexane-EtOAc-formic acid (10:1:0.01) afforded 15 fractions. Fractional crystallization of pooled fractions 6 to 8 with a mixture of $CHCl_3$ and MeOH gave 5 mg of irisone A [**1**] and 3 mg of irisone B [**2**]. Crystallization of combined fractions 10 and 11 from a mixture of MeOH and $CHCl_3$ gave 6 mg of 5,7-dihydroxy-2′,6-dimethoxy isoflavone.

Irisone A [**1**].—Recrystallized from EtOAc as colorless needles: mp 186-189°; uv (EtOH) λ max (log ε) 269 (3.86), 337 sh (3.44) nm; NaOMe 279 (3.98), 360 sh (3.16) nm; $AlCl_3/H_3BO_3$, 340.5 sh (3.18), 271.5 (3.90) nm; ir (Neat) ν max 3400, 1689, 1630, 1579, 1462, 1325, 1283, 1051, 1025, 923, 733, 727 cm^{-1}; ^{1}H nmr ($CDCl_3$, 360 MHz) δ 12.80 (1H, s, D_2O exchangeable, 5-OH), 7.89 (1H, s, 2-H), 7.39 (1H, ddd, *J*=8.6, 7.6 and 1.8 Hz, 4′-H), 7.32 (1H, dd, *J*=7.2 and 1.8 Hz, 6′-H), 7.03 (1H, ddd, *J*=7.6, 7.2 and 1.08 Hz, 5′-H), 7.00 (1H, br. d, *J*=8.6 Hz, 3′-H), 6.52 (1H, s, 8-H), 6.10 -2H, s, -O-CH_2-O-), 3.82 (3H, s, OCH_3); ^{13}C nmr ($CDCl_3$, 90 MHz) δ 154.39 (C-1), 120.16 (C-3), 180.83 (C-4), 153.74 (C-5), 142.37 (C-6), 157.26 (C-7), 89.11 (C-8), 153.32 (C-8), 108.21 (C-10), 119.16 (C-1′), 154.39 (C-2′), 111.14 (C-3′), 131.51 (C-4′), 120.49 (C-5′), 130.04 (C-6′), 55.62 (OCH_3), 102.50 (-O-CH_2-O-); ms (70 ev) *m/z* 312 (M^+, $C_{17}H_{12}O_6$), 281, 181, 131.

Irisone B [**2**].—Recrystallized from EtOAc as colorless plates: mp 230-233°; uv (EtOH) λ max (log ε) 267 (3.76), 329 sh (3.22) nm; NaOMe 272.5 (2.78), 352.0 sh (3.25) nm; $AlCl_3$/HCl 281 (3.79), 317.0

sh (3.48) nm; NaOAc/H_3BO_3 268.5 (3.91), 333.0 (3.38) nm; ir (neat) ν max 3310, 3088, 1677, 1677, 1623, 1558, 1279, 1234, 1098, 1050, 744 cm^{-1}; ^1H nmr (acetone-d_6, 200 MHz) δ 12.82 (1H, s, D_2O exchangeable, 5-OH), 8.40 (1H, s, D_2O exchangeable, 2'-OH), 8.34 (1H, s, 2-H), 7.36 (1H, dd, J=7.4 and 1.2 Hz, 6'-H), 7.33 (1H, ddd, J=8.0, 7.4 and 1.2 Hz, 4'-H), 7.02 (1H, br. d, J=8.0 Hz, 3'-H), 6.98 (1H, dt, J=1.2 and 7.4 Hz, 5'-H), 6.78 (1H, s, 8-H), 6.24 (2H, s, -O-CH_2O-); ms (70 ev) *m/z* 298 (M^+, $C_{16}H_{10}O_6$), 281, 181, 180, 118.

5,7-Dihydroxy-2',6-dimethoxy isoflavone.—Crystallized from a mixture of $CHCl_3$ and MeOH as pale yellow prisms: mp 190-193°; uv (EtOH) λ max (log ϵ) 262.5 (3.76), 333 sh. (3.11) nm. The compound was identified by comparison with published data (4).

CONVERSION OF IRISONE B TO IRISONE A.—CH_2N_2 in Et_2O was mixed with 1.5 mg of irisone B [**2**] for 3 h at room temperature. Evaporation of solvent from the reaction mixture under reduced pressure gave a white solid, which was in every respect (mp, ir, ^1H nmr, and ms) the same as irisone A [**1**].

CYTOTOXIC ACTIVITY.—Cytotoxic activity was assessed utilizing cultured P-388 cells, essentially by protocols developed by the National Cancer Institute (8) as described previously (9). All three isolates were inactive.

ACKNOWLEDGMENTS

The authors are grateful to Ms. Dorothy Guilty for aid in the preparation of this manuscript and the Nuclear Magnetic Resonance Laboratory of the Research Resources Center, University of Illinois at Chicago, for providing equipment and assistance during the course of this study. This work was supported, in part, by grant CA 33047 from the NCI. S.M.W. is the recipient of a fellowship from the Graduate College of the University of Illinois at Chicago. J.M.P. is the recipient of a Research Career Development Award from the NCI, 1984-1989.

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Received 9 June 1986