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TWO ISOFLAVONES FROM *JUNIPERUS MACROPODA*

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Key Word Index—*Juniperus macropoda*; Cupressaceae; isoflavones; junipegenin-B; junipegenin-C; partial synthesis.

Abstract—Two new isoflavones, junipegenin-B and -C, have been isolated from ethanolic extracts of *Juniperus macropoda* and their structures assigned. One of them, junipegenin-C, has been synthesized from iridin.

INTRODUCTION

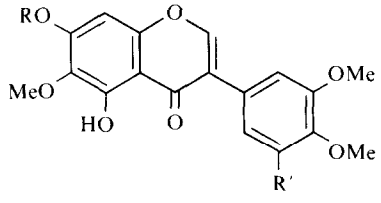
In a recent communcation [1], we reported the presence of a new isoflavone (which we now designate as junipegenin-A) from the ethanolic extract of the leaves of *Juniperus macropoda* Boiss. (Cupressaceae). In the present paper we report the isolation and characterization of two further isoflavones, junipegenin-B and -C, from the same extract.

RESULTS AND DISCUSSION

The isolation of junipegenin-B and -C, having very close R_f on TLC, materialized by repeated column chromatography on Si gel of the mother liquor left after the crystallization of irigenin from irigenin-rich fractions.

Junipegenin-B, mp 188–189° is assigned the structure 5,7-dihydroxy-6-methoxy-3-(3,4-dimethoxy)-phenyl-4H-1-benzopyran-4-one. The above structure is assigned on the basis of the following spectral and chemical studies. It analysed for $C_{18}H_{16}O_7$ (M^+ 344). The ^1H NMR spectrum (60 MHz, $CDCl_3$) established its nature as an isoflavone. A sharp singlet at δ 7.86 is assigned to C-2 proton. The C-8 proton is located at δ 6.50. In the diacetate this signal shifts to δ 7.20. Weakly coupled singlets at δ 7.10 and 6.97 integrating for one and two protons are identified for the 2′ and 5′, 6′ protons, respectively.

The UV spectrum indicates the presence of 5,7-dihydroxy grouping by the application of shift reagents. The structure is further confirmed by the oxidative degradation of junipegenin-B diacetate by K MnO_4 in acetone. One of the products of oxidation has been identified as veratric acid. Thus junipegenin-B is 5,7-dihydroxy-6,3′,4′-trimethoxy isoflavone (**1**).



1 R = R′ = H
2 R = H, R′ = OMe
3 R = Glc, R′ = OH

Junipegenin-C, needles from ethyl acetate, mp 233–235°, analysed for $C_{19}H_{18}O_8$ (M^+ 374). The ^1H NMR ($CDCl_3$ + DMSO-d_6) displays a singlet at δ 8.03 integrating for a single proton and is assigned to the C-2 proton. A singlet at δ 6.46 integrating for one proton is assigned to C-8. A singlet at δ 6.80 which integrates for two protons is assigned to the 2′, 6′ protons in the ring B. The C-8 proton shifts to δ 7.20 in the ^1H NMR spectrum of junipegenin-C diacetate. The presence of a 5,7-dihydroxy system is evident from the UV spectra recorded with various diagonistic shift reagents. On the basis of spectral data, junipegenin-C is 5,7-dihydroxy-6,3′,4′,5′-tetramethoxy isoflavone (**2**).

This structure is confirmed by its partial synthesis from iridin (**3**). **3** on methylation with CH_2N_2, followed by acid hydrolysis resulted in the formation of **2**. Its identity was confirmed by co-TLC, mmp, ^1H NMR and superimposable IR with junipegenin-C.

EXPERIMENTAL

All mps are uncorr. The irigenin-rich fractions from column chromatography of defatted alcoholic extract were pooled together. The mother liquor (10 g) left after the repeated crystallization of irigenin displayed two spots of almost identical R_f on the chromatoplates ($CHCl_3$–EtOAc, 3:1). Separation of these compounds was achieved by repeated column chromatography over Si gel using $CHCl_3$–MeOH in different proportions.

Junipegenin-B (**1**). Crystallizes from MeOH as golden yellow plates (80 mg), mp 188–189°, analysed for $C_{18}H_{16}O_7$. Found: C, 62.83; H, 4.65. Requires: C, 62.79; H, 4.65%. UV (MeOH) max nm: 268, 340 sh; + NaOMe 275, 345; + dry $AlCl_3$ 275, 315; + $AlCl_3$/HCl 275, 375; + NaOAc 281, 350. IR (KBr) cm^{-1}: 3370 (—OH), 1650 (C=O), 1575, 1522, 1450, 1365, 1150, 1145, 1065, 1015, 997, 865, 825, 805, 665. ^1H NMR (60 MHz, $CDCl_3$): δ 3.96 (6H, *s*, 3′4′-OMe), 4.06 (3H, *s*, 6-OMe), 6.50 (1H, *s*, C-8), 6.97 (2H, *s*, 5′, 6′-H), 7.10 (1H, *s*, 2′-H), 7.86 (1H, *s*, 2-H), 13.0 (2H, *br s*, phenolic H) which disappears on D_2O exchange. MS M^+ *m/e* (rel. int.): 344 (100), 329 (51.0), 326 (42.95), 315 (8.05), 301 (61.07), 172 (16.51), 162 (3.8), 157 (4.02).

Acetylation (Ac_2O–C_5H_5N) gave the diacetate, colourless needles from MeOH, mp 162–163°, analysed for $C_{22}H_{20}O_9$. ^1H NMR (60 MHz, $CDCl_3$): δ 2.40 and 2.46 (2 × 3H, 2 × Ar. OCOMe), 3.90 (3H, *s*, 6-OMe), 3.96 (6H, *s*, 3′,4′-OMe), 6.97 (2H, *br s*, 5′,6′-H), 7.06 (1H, *s*, 2′-H), 7.20 (1H, *s*, 8-H), 7.83 (1H, *s*, 2-H).

Methylation of **1** with CH_2N_2 furnished the 7-methyl ether, colourless needles from MeOH, mp 178–179°. Analysed for $C_{19}H_{18}O_7$. ^1H NMR ($CDCl_3$): δ 3.96 (12H, *s*, 4 × Ar–OMe), 6.46 (1H, *s*, 8-H), 6.96 (2H, *br. s*, 5′,6′-H), 7.13 (1H, *s*, 2′-H), 7.86 (1H, *s*, 2-H). Methylation of **1** with DMS–K_2CO_3–Me_2CO gave the 5,7-dimethyl ether, colourless plates from MeOH, mp 185–186°. Analysed for $C_{20}H_{20}O_7$. ^1H NMR ($CDCl_3$): δ 3.90 (9H, *s*, 3 × Ar–OMe), 3.93 (6H, *s*, 2 × Ar–OMe), 6.66 (1H, *s*, 8-H), 6.96 (2H, *s*, 5′,6′-H), 7.16 (1H, *s*, 2′-H), 7.80 (1H, *s*, 2-H).

Oxidative degradation of junipegenin-B diacetate. The diacetate (30 mg) was subjected to oxidative degradation by $KMnO_4$ in Me_2CO. Among other products, a compound crystallized from H_2O (7.0 mg), mp 180–181°, analysed for $C_9H_{10}O_4$, and was identified as veratric acid by co-TLC, mmp and superimposable IR.

Junipegenin-C. Crystallized from EtOAc as fine needles, mp 233–235°, analysed for $C_{19}H_{18}O_8$. Found: C, 61.08; H, 4.82. Requires: C, 60.96; H, 4.81%. UV (MeOH) max nm: 267,327 sh; + NaOMe 273, 334; + NaOAc 271, 330; + $AlCl_3$ 272, 316; + $AlCl_3$/HCl 272, 318. IR (KBr) cm^{-1}: 3450, 1670, 1625, 1590, 1460, 1260, 1225, 1190, 1019, 805, 720. ^1H NMR (60 MHz, $CDCl_3$ + DMSO-d_6): δ 3.96 (12H, *m*, 4 × Ar–OMe), 6.53 (1H, *s*, 8-H), 6.80 (2H, *s*, 2′,6′-H), 8.03 (1H, *s*, 2-H), 12.80 (1H, *s*, phenolic H) disappears on D_2O exchange. MS M^+ *m/e* (rel. int.): 374 (100), 359 (54.47), 356 (15.12), 341 (18.37), 187 (5.69), 178 (4.06), 177 (6.50).

Acetylation (Ac_2O–C_5H_5N) furnished the diacetate, needles from MeOH, mp 161–162°, analysed for $C_{23}H_{22}O_{10}$. ^1H NMR (CCl_4): δ 2.46 and 2.53 (2 × 3H, 2 *s*, 2 × Ar–OCOMe), 3.93 (12H, *m*, 4 × Ar–OMe), 6.63 (2H, *s*, 2′,6′-H), 7.10 (1H, *s*, 8-H) and 7.80 (1H, *s*, 2-H).

Partial synthesis of junipegenin-C from iridin. Iridin (100 mg) was dissolved in a minimum quantity of MeOH and a solution of CH_2N_2 in dry ether was slowly added to it. The reaction mixture was kept at 0° for 2 hr and after processing yielded a product which was hydrolysed with 7% methanolic HCl. The product of hydrolysis crystallized from EtOAc as fine needles, mp 231–232°. Co-TLC, mmp and superimposable IR showed the semisynthetic product to be identical with junipegenin-C in all respects.

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REFERENCE

1. Sethi, M. L., Taneja, S. C., Agarwal, S. G., Dhar, K. L. and Atal, C. K. (1980) *Phytochemistry* **19**, 1831.