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THREE ISOFLAVONE GLYCOSIDES FROM *JUNIPERUS MACROPODA*

M L SETHI, S C TANEJA, K L DHAR and C K ATAL

Regional Research Laboratory, Jammu, Tawi, India

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Key Word Index—*Juniperus macropoda*, Cupressaceae, leaves, isoflavone glycosides

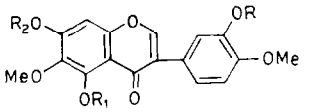
Abstract—Three new isoflavone glycosides have been characterized from the leaves of *Juniperus macropoda* 5,7-dihydroxy-6,3′,4′-trimethoxyisoflavone 7-glucoside, 5,7,4′-trihydroxy-6,3′,5′-trimethoxyisoflavone 7-glucoside and 5,7,3′-trihydroxy-6,4′-dimethoxyisoflavone 7-diglucoside

In continuation of our work on the leaf isoflavonoids of *Juniperus macropoda* Boiss (Cupressaceae), we now report the characterization of three new isoflavone glycosides This is the first report of isoflavone glycosides in *Juniperus* species We previously identified the isoflavones junipegenin A, B and C from the same plant [1, 2]

From the defatted alcoholic leaf extract of *J macropoda*, three isoflavone glycosides (**1–3**) were isolated and purified by repeated CC

1 was identified as the 7-O-β-D-glucoside of junipegenin B, 5,7-dihydroxy-6,3′,4′-trimethoxyisoflavone [2], from spectral and chemical studies It gave a positive Molisch test The ^{1}H NMR (DMSO-d_6) of **1** displayed in addition to signals for three methoxy groups, a sharp singlet at δ 8 42 for a C-2 proton Two overlapping multiplets lying between δ 7 0 and 7 25 integrating for three protons were assigned to the protons at C-2′, C-5′ and C-6′ A singlet at δ 6 90 integrating for one proton represented the C-8 proton A doublet at δ 5 30 (J = 7 00 Hz) integrating for a single proton was assigned to the C-1″ proton of glucose indicating a β-linkage [3] A singlet for nine protons at δ 3 83 represented three *O*-methyl groups A downfield singlet at δ 12 86 exchangeable with D_2O was attributed to phenolic protons UV shifts with aluminium chloride confirmed the presence of a hydroxy group at C-5 while the absence of a sodium acetate shift indicated the C-7 hydroxyl was substituted The IR (KBr) spectrum displayed bands at 3450 (–OH) and 1650 (>C=O) cm^{-1} Acetylation of **1** gave a penta-acetate **1a** In the ^{1}H NMR ($CDCl_3$) of **1a** all the signals remained unchanged except for a downfield displacement of the signal for the C-8 proton to δ 7 06 indicating a free phenolic group only in the A ring Acid hydrolysis of **1** gave glucose and an aglycone **1b** The ^{1}H NMR ($CDCl_3$) and IR of **1b** and its derivatives the 5, 7-diacetate (**1c**) and the 5, 7-dimethyl ether (**1d**) are in complete accord with junipegenin B and its corresponding derivatives [2] Identity of **1b** was finally confirmed by its mmp with an authentic sample of junipegenin B Since junipegenin B has free 5- and 7-hydroxyls the glucose in **1** must be attached to one of these positions A bathochromic shift of 6 nm in UV with aluminium chloride indicating a free hydroxyl at C-5 leaves only the 7-position free for glucosylation This was further confirmed by methylation and subsequent acid hydrolysis of **1** to yield **1e** which was identified as 7-hydroxy 5,6′,3′,4′-tetramethoxyisoflavone by spectral data

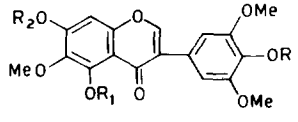
The second isoflavone glycoside was assigned structure **2** on the basis of chemical and spectral studies UV shifts with aluminium chloride ascertained the presence of a free hydroxyl at C-5, while with sodium acetate no shift was observed IR (KBr) displayed bands at 3400 (–OH) and 1660 (>C=O) cm^{-1} The ^{1}H NMR (DMSO-d_6) showed in addition to signals for three methoxy groups, a sharp singlet for one proton at δ 8 42, which was assigned to the C-2 proton A singlet at δ 6 85 integrating for three protons was assigned to three unsubstituted aromatic protons at C-8, C-2′ and C-6′ in the isoflavone system A doublet at δ 5 10 was assigned to the C-1″ (J = 7 Hz) proton of the sugar moiety, also indicating a β-linkage [3] A singlet at δ 3 83 integrating for nine protons was attributed to three *O*-methyl groups A broad downfield singlet at δ 12 66 for two protons was assigned to phenolic protons Acetylation of **2** gave a hexa-acetate (**2a**) The ^{1}H NMR ($CDCl_3$) of **2a** displayed a singlet at δ 2 16 integrating for 12 protons assigned to four acetate functions on the glucose moiety and two close singlets at δ 2 40 and 2 50 each integrating for three protons are due to aromatic –OCOC$\underline{H}_3$ functions A singlet for one proton at δ 7 87 was assigned to the C-2 proton and a singlet for one proton at δ 7 10 for the C-8 proton A sharp singlet integrating for two protons at δ 6 73 was attributed to protons at C-2′ and C-6′ in the B ring Acid hydrolysis of **2** gave glucose and an aglycone **2b** The ^{1}H NMR (Me_2CO-d_6) of **2b** displayed a singlet at δ 8 0 for the C-2 proton Another singlet integrating for two protons at δ 6 66 was assigned to symmetrical protons at C-2′ and C-6′ The signal for the C-8 proton was located at δ 6 33 A singlet at δ 3 73 for nine protons was attributed to three *O*-methyl groups A broad singlet at δ 12 80 represented phenolic protons Acetylation of **2b** gave a triacetate **2c** The ^{1}H NMR



1 R = Me, R_1 = H, R_2 = Glc
1a R = Me, R_1 = Ac, R_2 = Glc $(OAc)_4$
1b R = Me, R_1 = R_2 = H
1c R = Me, R_1 = R_2 = Ac
1d R = R_1 = R_2 = Me
1e R = R_1 = Me, R_2 = H
3 R = R_1 = H, R_2 = DiGlc
3a R = R_1 = Ac, R_2 = DiGlc $(OAc)_7$
3b R = R_1 = R_2 = H
3c R = R_1 = R_2 = Ac
3d R = Me, R_1 = R_2 = H

(Me_2CO-d_6) of **2c** showed a singlet at δ 7 33 for one proton assigned to the C-8 proton, a downfield shift of δ 1 0 The signal for C-2′ and C-6′ protons in **2c** remained unsplit and at approximately the same position as in **2b** This clearly indicates that the B ring is symmetrical and that there is a free hydroxyl at position C-4′ flanked by two methoxyls at C-3′ and C-5′ From the above data **2b** has been assigned as 5,7,4′-trihydroxy-6,3′,5′-trimethoxyisoflavone The structure was further confirmed by the selective methylation of **2** at C-4′ with diazomethane followed by acid hydrolysis to give **2d** Spectral data of **2d** was found to be identical in all respects with junipegenin C (5,7-dihydroxy-3′,4′,5′,6-tetramethoxyisoflavone) [2] The position of glycosylation was ascertained by methylation of **2** with dimethyl sulphate and subsequent acid hydrolysis to yield **2e** A positive sodium acetate shift in the UV spectrum indicated a free hydroxyl at C-7 Further, **2e** is identical in all respects (mmp, co-TLC) with the 5, 3′-dimethyl ether of irigenin (lit mp 218–219° [4]) **2e** is therefore identified as 7-hydroxy-5,6,3′,4′,5′-pentamethoxyisoflavone From the above data, it is clear that the only position for glycosylation in **2** is at the 7-position, thus, **2** is the 7-glucoside of **2b**

The third new isoflavone glycoside was assigned structure **3** on the following basis It analysed for $C_{29}H_{34}O_{17}$, M^+ at *m/z* 654 (FD mode) Spectral studies and chemical reactions established its nature as an isoflavone glycoside A positive UV shift with aluminium chloride showed the presence of a free hydroxyl at C-5 IR (KBr) revealed prominent bands at 3350–3450 (–OH) and 1650 (>C=O) cm^{-1} The ^1H NMR (DMSO-d_6) of **3** displayed a sharp singlet at δ 8 32 for a C-2 proton and a four proton multiplet centred at δ 7 03 for a C-8 proton in ring A and C-2′, C-5′ and C-6′ protons in the B ring A multiplet at δ 5 1 integrating for two protons represents C-1″ and C-1‴ protons of a diglucoside Two singlets at δ 3 90 and 3 87 for three protons each are due to *O*-methyl protons at C-4′ and C-6′ Acetylation of **3** gave a nona-acetate **3a** Two of the acetoxyl signals in ^1H NMR ($CDCl_3$) of **3a** at δ 2 36 and 2 50 are identified for phenylacetates The other signals centred at δ 1 97–2 16 integrating for 21 protons are for the acetoxyl protons of the sugar moiety From the ^1H NMR and the molecular formula of **3** and its acetate it is clear that **3** is a diglycoside of an isoflavone Acid hydrolysis of **3** gave glucose and an aglycone, **3b**, which analysed for $C_{17}H_{14}O_7$ (M^+ at *m/z* 330) Hydrolysis of **3** with emulsin gave glucose and **3b** suggesting it to be an *O*-glycoside with a β-linkage In the ^1H NMR ($CDCl_3$) of **3b** a sharp singlet at δ 6 46 integrating for one proton was assigned to the C-8 proton A multiplet centred at δ 7 00 for three protons was assigned to C-2′, C-5′ and C-6′ protons A downfield singlet at δ 7 80 integrating for one proton represents the C-2 proton Acetylation of **3b** furnished a triacetate **3c** In the ^1H NMR ($CDCl_3$) of **3c** an *ortho*- and *para*-coupled doublet integrating for one proton at δ 7 00 (J = 8 00 and 0 5 Hz) is assigned to the C-5′ proton A multiplet centred at δ 7 20 integrating for two protons is ascribed to the C-2′ and C-8 protons Another *ortho*- and *meta*-coupled doublet for one proton at δ 7 30 (J = 8 0 and 2 5 Hz) is attributed to the C-6′ proton The position of the methoxyl group at C-4′ and hydroxyl group at C-3′ in the B ring is ascertained from ^1H NMR of **3b** and **3c**, which have been discussed above Had the positions of hydroxyl and methoxyl been reversed, we would have observed a downfield shift for the C-5′ proton only, while signals for C-2′ and C-6′ protons would have remained practically at the same positions as in **3b**, which is not so in this case This fact was further corroborated when **3c** was oxidized with potassium permanganate in acetone to give a compound, mp 254–256°, identified (co-TLC and mmp) as isovanillic acid (lit mp 255–257°) Thus, the substitution pattern of the B-ring of **3** is confirmed Further proof for the structure of **3b** comes from selective methylation of **3** at C-3′ with diazomethane and subsequently acid hydrolysis to yield **3d**, which was found to be identical with the known compound junipegenin B [2] (co-TLC, mmp and superimposable IR) Evidently junipegenin B is the 3′-monomethyl ether of **3b** Moreover methylation of **3b** with diazomethane gave its 7, 3′-dimethyl ether, mp 178–179° which is identical with the 7-monomethyl ether of junipegenin B (co-TLC and mmp) [2] **3** on permethylation with silver oxide and methyl iodide in DMF [5] and subsequent acid hydrolysis yielded methylated sugars and the 5,3′-dimethylether of **3b** From ^1H NMR and UV spectra with diagnostic shift reagents, this compound was identified as 7-hydroxy-5,6,3′,4′-tetramethoxyisoflavone, which was identical with **1e**, obtained by the methylation and acid hydrolysis of **1** This fixes the position of the sugar moiety at C-7 The methylated sugars were identified as 2,3,4-tri-*O*-methyl glucose and 2,3,4,6-tetra-*O*-methyl glucose This clearly proved that the sugar moiety in **3** is a diglucoside which is attached at C-7 through a β



2 R = R_1 = H, R_2 = Glc
2a R = R_1 = Ac, R_2 = Glc$(OAc)_4$
2b R = R_1 = R_2 = H
2c R = R_1 = R_2 = Ac
2d R = Me, R_1 = R_2 = H
2e R = R_1 = Me, R_2 = H

linkage and the linkage between the two glucose units is 1→6 Hence, the structure finally assigned to **3** is 5, 7,3′-trihydroxy-6,4′-dimethoxyisoflavone 7-*O*-β-D-(1→6)diglucoside

EXPERIMENTAL

All mps are uncorr ^{1}H NMR was recorded on a Varian T-60A spectrometer with TMS as int standard

The EtOH leaf extract of *Juniperus macropoda* was concd and vacuum dried The dry EtOH extract was refluxed with EtOAc for 30 min and the EtOAc-soluble part separated 85 g of the EtOAc-insoluble EtOH extract was subjected to CC over Si gel (6 5 kg) and eluted successively with $CHCl_3$–MeOH mixtures of differing proportions **1** was obtained from $CHCl_3$–MeOH (17 3) fractions, needles from MeOH, mp 244–246°, analysed for $C_{24}H_{26}O_{12}$ (M^+ *m/z* 506) UV λ_{max}^{MeOH} nm 266, 330 (sh) MS M^+ at *m/z* 506 (0 7%), 344 (100), 329 (51 0), 326 (42 95), 315 (8 05), 301 (61 07), 172 (16 51), 162 (3 80), 157 (4 02) Acetylation of **1** (Ac_2O–C_5H_5N) gave the penta-acetate **1a**, crystallized from MeOH as colourless needles, mp 166–167°, analysed for $C_{34}H_{36}O_{17}$ ^{1}H NMR (60 MHz, $CDCl_3$) δ 7 80 (1H, *s*, H-2), 7 06 and 6 91 (4H, *m*, H-2′, H-5′, H-6′, H-8), 5 10–5 60 (4H, *m*, –CHO–), 4 30 (2H, *m*, —CH_2OAc), 3 90 (6H, *s*, 2×OMe), 3 80 (3H, *s*, 1×OMe), 2 43 (3H, *s*, Ar O $COCH_3$), 2 10 (12H, 2×*s*, 4×$OCOCH_3$) **1** on hydrolysis with 7% H_2SO_4 in MeOH gave a sugar and an aglycone **1b**, yellow plates from MeOH, mp 190–191°, analysed for $C_{18}H_{16}O_7$ (M^+ *m/z* 344) UV λ_{max}^{MeOH} nm 268, 340 (sh), +NaOMe 275, 345, +$AlCl_3$ 275, 315, +$AlCl_3$–HCl 275, 315, +NaOMe 281, 350 IR ν_{max}^{KBr} cm^{-1} 3370 (–OH), 1650 (>C=O), 1575, 1522, 1450, 1365, 1150, 1145, 1065, 1015, 997, 865, 825, 805, 665 ^{1}H NMR (60 MHz, $CDCl_3$) δ 3 96 (6H, *s*, OMe-3′, OMe-4′), 4 06 (3H, *s*, OMe-6), 6 50 (1H, *s*, H-8), 6 97 (2H, *s*, H-5′, H-6′), 7 10 (1H, *s*, H-2′), 7 86 (1H, *s*, H-2), 13 0 (2H, *br s*, phenolic H which disappears on D_2O exchange) MS M^+ at *m/z* (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) **1b** was identified as junipegenin B by co-TLC, superimposable IR and mmp with an authentic sample The sugar was identified as glucose by co-PC (BAW 4 1 5) Acetylation of **1b** gave a 5,7-diacetate (**1c**) which crystallized from MeOH as colourless needles, mp 162–163°, analysed for $C_{22}H_{20}O_9$ ^{1}H NMR ($CDCl_3$) δ 2 40 and 2 46 (2×3H, 2×*s*, 2×Ar $OCOCH_3$), 3 90 (3H, *s*, OMe-6), 3 96 (6H, OMe-3′, OMe-4′), 6 97 (2H, *br s*, H-5′, H-6′), 7 06 (1H, *s*, H-2′), 7 20 (1H, *s*, H-8), 7 83 (1H, *s*, H-2) Methylation of **1b** gave the 5, 7-dimethyl ether (**1d**), colourless plates from MeOH, mp 185–186°, analysed for $C_{20}H_{20}O_7$ ^{1}H NMR ($CDCl_3$) δ 3 90 (9H, *s*, 3×Ar OMe), 3 93 (6H, *s*, 2×Ar OMe), 6 66 (1H, *s*, H-8), 6 96 (2H, *s*, H-5′, H-6′), 7 16 (1H, *s*, H-2′), 7 80 (1H *s*, H-2) **1** on methylation (DMS + K_2CO_3 + Me_2CO) followed by acid hydrolysis yielded **1e**, plates from MeOH, mp 224–225°, which analysed for $C_{19}H_{18}O_7$ ^{1}H NMR ($CDCl_3$) δ 3 90 (6H, *s*, 2×Ar OMe), 3 93 (3H, *s*, Ar OMe), 4 03 (3H, *s*, Ar OMe), 6 73 (1H, *s*, H-8), 6 93 (2H, *br s*, H-2′, H-5′), 7 13 (1H, *m*, H-6′), 7 78 (1H, *s*, H-2) UV λ_{max}^{MeOH} nm 260, 320 (sh), +$AlCl_3$ 260, 320, +$AlCl_3$–HCl 260, 320, +NaOAc 267, 328

2 was isolated from $CHCl_3$–MeOH (3 1) fractions crystallized from MeOH, mp 292–293°, analysed for $C_{24}H_{26}O_{13}$ (M^+ *m/z* 522) UV λ_{max}^{MeOH} nm 269, 330, +NaOMe 270, 335, +$AlCl_3$ 276, 328, +$AlCl_3$–HCl 276, 334 IR ν_{max}^{KBr} cm^{-1} 3400 (–OH), 1660 (>C=O), 1615, 1580, 1535, 1520, 1455, 1430, 1370, 1305, 1235, 1200, 1180, 1120, 1020, 890, 820, 725, 690, MS M^+ at *m/z* 522, 360, 348, 331, 317, 271, 231, 229, 185, 183, 173, 163 **2** on acetylation (Ac_2O–pyridine) gave a hexa-acetate **2a**, colourless needles, mp 112–113°, analysed for $C_{36}H_{38}O_{19}$ IR ν_{max}^{KBr} cm^{-1} 1750 (O–C(=O)–Me), 1635 (>C=O), 1595, 1485, 1430, 1365, 1300, 1220, 1130, 1090, 1040, 1000, 955, 900, 845, 820, 730, 620 Hydrolysis of **2** with 7% H_2SO_4 in MeOH yielded a sugar and an aglycone, **2b** colourless needles from MeOH, mp 224–225°, analysed for $C_{18}H_{16}O_8$ (M^+ at *m/z* 360) The sugar was identified as glucose by PC (BAW 4 1 5) UV λ_{max}^{MeOH} nm 270, 335, +NaOMe 268, 336, +NaOAc, 273, 336, +$AlCl_3$ 275, 362, +$AlCl_3$–HCl 275, 360 Acetylation of **2b** gave a triacetate **2c**, mp 214–215° IR ν_{max}^{KBr} cm^{-1} 1760 (–O COMe), 1640 (>C=O), 1615, 1590, 1450, 1415, 1360, 1275, 1235, 1170, 1120, 1070, 1005, 960, 875, 830, 790, 730, 690, 640 Selective methylation of **2** with CH_2N_2, followed by its hydrolysis with 6% HCl yielded **2d**, mp 233–235° and was found to be identical with junipegenin C (co-TLC and mmp) **2** (100 mg) on methylation (DMS + K_2CO_3 + Me_2CO) followed by acid hydrolysis yielded **2e**, mp 218–219°, analysed for $C_{20}H_{20}O_8$

3 was isolated from $CHCl_3$–MeOH (7 3) fractions, colourless needles from MeOH, mp 251–252° UV λ_{max}^{MeOH} nm 265, 326, +NaOMe 268, 326, +NaOAc 265, 326, +$AlCl_3$ 275, 314, 360, +$AlCl_3$–HCl 275, 314, 360 MS (FD mode) M^+ at *m/z* 654, 474, 330, 315, 312, 183, 167, 149, 135, 105, 69 Acetylation of **3** yielded a nona-acetate, colourless needles from MeOH, mp 125–126°, analysed for $C_{47}H_{52}O_{26}$ ^{1}H NMR (60 MHz, $CDCl_3$) δ 8 03 (1H, *s*, H-2), 7 5 (1H, *dd*, *J* = 8, 2 5 Hz, H-6′), 7 3 (2H, *s*, H-2′, H-8), 7 06 (1H, *d*, *J* = 8 0 Hz, H-5′), 4 9–5 50 (*m*, –CHOAc and CHO-protons of sugar moiety), 4 33 (*m*, 2×CH_2OAc), 3 90 and 3 83 (6H, 2×*s*, 2×OMe), 2 50 and 2 36 (6H, 2×*s*, 2×Ar OCOMe), 1 97–2 16 (21H, overlapped singlets, 7×OCOMe) Hydrolysis of **3** with 6% H_2SO_4 in MeOH gave glucose and an aglycone **3b**, colourless plates from MeOH, mp 93–94° Hydrolysis of **3** with emulsin yielded glucose and **3b**, mp 93–94°, UV λ_{max}^{MeOH} nm 267, 365, +NaOMe 270, 336, +NaOAc 270, 344, +$AlCl_3$ 275, 386, +$AlCl_3$–HCl 278, 368 IR ν_{max}^{KBr} cm^{-1} 3470 (–OH), 1630 (>C=O), 1580, 1490, 1455, 1410, 1375, 1285, 1190, 1165, 1130, 1060, 1020, 995, 905, 875, 830, 810, 795, 750, 735, 675, 600 MS M^+ at *m/z* 330, 315, 312, 183, 167, 149, 135, 105, 69 Acetylation of **3b** yielded a triacetate **3c**, colourless needles from MeOH, mp 166–167° Selective methylation (CH_2N_2) of **3**, followed by hydrolysis with 7% HCl in MeOH yielded **3d**, yellow plates from MeOH, mp 188–189° Permethylation of **3** followed by acid hydrolysis yielded methylated sugars and the 5, 3-dimethyl ether of **3b**, crystals from MeOH, mp 224–225°, which was identical with **1e** The methylated sugars were isolated by CC and identified as 2, 3, 4-tri-*O*-methyl-D-glucose by co-PC (*n*-BuOH–EtOH–H_2O, 5 1 4) and 2, 3, 4, 6-tetra-*O*-methyl-D-glucose, crystals from petrol, mp 94–96° (lit mp 96° [6]) and by comparison with authentic samples

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