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ISOFLAVONES AND STILBENES FROM *JUNIPERUS MACROPODA*

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Key Word Index—*Juniperus macropoda*; Cupressaceae; isoflavones; irigenin; iridin; 5,7,3',5'-tetrahydroxy-4'-methoxyisoflavone; stilbenes; resveratrol; priceid.

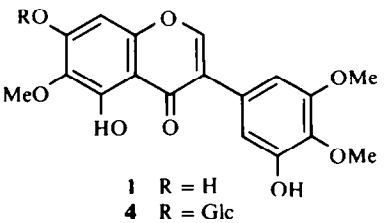
Abstract—An ethanolic extract of *Juniperus macropoda*, after chromatography, yielded three isoflavones, viz. irigenin, iridin and a new compound. In addition two stilbenes, resveratrol and piceid (resveratrol-3-*O*-β-D-glucoside), were isolated and characterized.

INTRODUCTION

Juniperus macropoda Boiss. is an aromatic plant, twigs of which are used as incense by the natives of Himachal Pradesh, India. Previous work on this plant includes the isolation of flavonoids [1] and biflavonoids [2,3] from berries and of essential oil [4] and other compounds [5,6] from leaves. In the present communication we report the isolation and characterization of two known and one new isoflavone from this species. This appears to be the first report of isoflavons in the Cupressaceae, a family mainly known for its biflavonoids. Amongst conifers, the Podocarpaceae is the only other family known to contain these substances. Besides isoflavones, two stilbenes, resveratrol and piceid, have also been obtained from this plant.

RESULTS AND DISCUSSION

From the defatted alcoholic extract of leaves of *Juniperus macropoda*, five compounds **1**–**5** were separated on Si gel column. **1**, mp 185° was identified as irigenin from its physical data, ^{1}H NMR and comparison with authentic sample. **2** mp 277–278°, a new isoflavone was assigned the structure 4H-1-benzopyran-4-one-5,7-dihydroxy-3-(3,5-dihydroxy-4-methoxy)-phenyl, i.e. 5,7,3',5'-tetrahydroxy-4'-methoxyisoflavone. It analysed for $C_{16}H_{12}O_7$ (M^+ 316). The ^{1}H NMR (Me_2CO-d_6) established its nature as an isoflavone. A sharp singlet at δ 8.0 was assigned to C-2 proton. Two *meta*-coupled doublets at δ 6.23 and 6.34 (J = 2.5 Hz each) represented as C-6 and C-8 protons. The UV spectrum by the application of diagnostic shift reagents also showed the presence of a 5,7-dihydroxy grouping. A two-proton singlet at δ 6.6 was assigned to the equivalent protons at the 2',6'-positions. This signal remained unsplit even after acetylation and methylation indicating the symmetrical nature of the B ring, although the signals shifted to δ 7.13 and 6.86, respectively. The structure was finally confirmed by oxidative degradation of the methyl ether of **2** with $KMnO_4$; one of the products formed was identified as 3,4,5-trimethoxybenzoic acid. Finally, the MS fragmentation pattern (see Experimental) fully confirmed the assigned structure for **2**. **3**, mp 257° (decomp.) was found to be resveratrol by the usual methods. ^{13}C NMR of **3** triacetate has also been measured and is reported here for the first time. **4**, mp 217° was identified as the isoflavone glycoside, iridin, which on hydrolysis yielded an aglycone identified as irigenin. It was finally confirmed as 7-*O*-β-D-glucoside of irigenin from spectral data, co-TLC, mp and mmp. **5**, mp 228–229°, gave a positive glycoside test. Acid hydrolysis resulted in the formation of resveratrol and glucose. The glucoside linkage was finally established at position 3, by methylating the glycoside and subjecting it to hydrolysis. The product of hydrolysis was identified as 3-hydroxy-5-4'-dimethoxystilbene from its spectral data. The structure of **5** was thus established as piceid.



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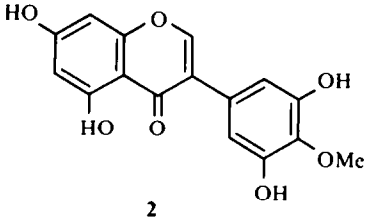
4 R = Glc



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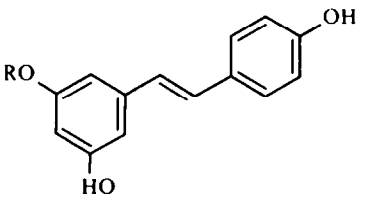

3 R = H
5 R = Glc

Table 1. ^{13}C NMR of resveratrol triacetate

S. No.	Carbon	Multiplicity	Integration (NNE mode)	Chemical shift
1.	1 and 5	*d*	2	127.623
2.	2 and 4	*d*	2	121.861
3.	3	*s*	1	150.410
4.	6	*s*	1	134.439
5.	7	*d*	1	129.623
6.	8	*d*	1	129.613
7.	9	*s*	1	139.499
8.	10 and 14	*d*	2	116.888
9.	11 and 13	*s*	2	151.288
10.	12	*d*	1	114.373
11.	15	*s*	1	169.336
12.	17 and 19	*s*	2	168.926
13.	16, 18 and 20	*q*	3	21.090

EXPERIMENTAL

All mps are uncorr. ^1H NMR was recorded on Varian A-60T spectrometer and ^{13}C NMR on JEOL FX-100 model with TMS as int. standard. The leaves of *Juniperus macropoda* were collected from the north-west Himalayan region at an elevation of 3000–3500 m above sea level. The air-dried leaves were first defatted and extracted with $CHCl_3$ followed by EtOH. The EtOH extract was concd to ca 1/3 of its vol. and kept at 0°. A crystalline solid, **4** settled (*ca* 12%). It was separated and repeatedly crystallized from aq EtOH mp 217°, analysed for $C_{24}H_{26}O_{13}$ and identified as iridin from its ^1H NMR, IR, UV, mmp and co-TLC with an authentic sample. The remaining extract was vacuum-dried. 30 g of extract were subjected to column chromatography over Si gel (2.5 kg) and eluted with a $CHCl_3$–MeOH mixture in different proportions. **1** was obtained from $CHCl_3$ MeOH (97:3) fractions, mp 185°, analysed for $C_{18}H_{16}O_8$. Triacetate, mp 128° was analysed for $C_{24}H_{22}O_{11}$ and was identified as irigenin by mp, mmp, ^1H NMR and superimposable IR with an authentic sample.

2 was isolated from $CHCl_3$–MeOH (95:5) fractions, crystallized from a $CHCl_3$–MeOH mixture, creamish yellow plates, mp 277–278°, analysed for $C_{16}H_{12}O_7$. UV λ_{max}^{MeOH} nm: 261, 330 sh; +NaOMe 271, 326; +$AlCl_3$ 271, 314, 370; +$AlCl_3$/HCl 271, 312, 374; +NaOAc 269, 326. IR (KBr) cm^{-1}: 3310 (OH), 1655 (>C = O), 1610, 1595, 1570, 1430, 1355, 1255, 1150 1190, 1040, 830, 813, 750, 715, 650. ^1H NMR: (60 MHz, Me_2CO-d_6): δ 3.86 (3 H, *s*, 4'-OMe), 6.23 (1 H, *d*, J = 2.5 Hz, 5-H), 6.34 (1 H, *d*, J = 2.5 Hz, 8-H), 6.60 (2 H, *s*, 2',6'-H), 8.00 (1 H, *s*, 2-H), 12.8 (1 H, *s*, -OH) which disappears on D_2O exchange. MS M$^+$ *m/e* (rel. int.): 316 (100%), 301 (66.66), 245 (44.44), 164 (6.52), 153 (57.40), 149 (51.18), 136 (8.70), 121 (25.92), 114 (13.76), 107 (7.24).

Acetylation. (Ac_2O–C_5H_5N) gave the tetraacetate, crystallized from MeOH, colourless needles, mp 190–191°, analysed for $C_{24}H_{20}O_{11}$. ^1H NMR (60 MHz, $CDCl_3$): δ 2.4 (12 H, *s*, 4 × Ar·OCOMe), 3.83 (3 H, *s*, 4'-OMe), 6.83 (1 H, *d*, J = 2.3 Hz, 8-H), 7.13 (2 H, *s*, 2',6'-H), 7.26 (1 H, *d*, J = 2.3 Hz, 6-H), 7.9 (1 H, *s*, 2-H).

Methylation. MeI–K_2CO_3–Me_2CO, gave fine colourless needles from Me_2CO–solvent Et_2O, mp 156–157°, analysed for $C_{20}H_{20}O_7$. ^1H NMR (Me_2CO-d_6): δ 3.80 (3 H, *s*, 4'-OMe), 3.86 (6 H, *s*, 3'- and 5'-OMe), 3.93 (6 H, *s*, 5,7-OMe), 6.53 (2 H, *dd*, J = 2.3 Hz and 5 Hz, 6-H and 8-H), 6.86 (2 H, *s*, 2 = H and 6'-H), 8.03 (1 H, *s*, 2-H).

Oxidative degradation of **2** *methyl ether.* **2** methyl ether (100 mg) was subjected to oxidative degradation ($KMnO_4$–Me_2CO) by the procedure of ref. [7]. The product (30 mg) crystallized from Me_2CO, mp 171–172°, showed IR (KBr) cm^{-1}: 3450 (OH), 1685 (>C = O), 1587, 1468, 1425, 1325, 1270, 1222, 1180, 1122, 1000, 860, 760, 715. ^1H NMR (Me_2CO-d_6): δ 3.90 (3 H, *s*, 4-OMe), 3.96 (6 H, *s*, 3,5-OMe), 7.40 (2 H, *s*, Ar–H, β to a carbonyl function). The oxidation product was identified as 3,4,5-trimethoxybenzoic acid by comparision with authentic sample, mmp and co-TLC.

3: Isolated from $CHCl_3$–MeOH (23:2) fractions, crystallized from aq. alcohol, mp 257°. MS M$^+$ 228, calc. for $C_{14}H_{12}O_3$. Triacetate (Ac_2O–C_5H_5N), crystallized from MeOH, mp 118.5–119.5°. IR (KBr) cm^{-1}: 3230 (OH), 1605, 1585, 1510, 1460, 1437, 1380, 1145, 1007, 985, 965 and 830. ^1H NMR (Me_2CO-d_6): δ 6.00 (1 H, *dd*, J = 2.5 and 5.0 Hz, 4-H), 6.30 (2 H, *d*, J = 2.0 Hz, 2-H and 6-H), 6.56 (2 H, *d*, J = 8 Hz, 3'-H and 5'-H), 6.66 (2 H, *d*, J = 2 Hz, ethylenic protons), 7.16 (2 H, *d*, J = 8 Hz, 2'-H and 6'-H). Identified as resveratrol from ^1H NMR, UV, superimposable IR, mmp and co-TLC with authentic sample. ^{13}C NMR of resveratrol triacetate (Table 1) with probable assignments is reported for the first time.

5 was isolated from $CHCl_3$ MeOH (17:3) fractions, crystallized from MeOH–$CHCl_3$, mp 228 229°, analysed for $C_{20}H_{22}O_8$. Dimethyl ether (diazomethane) mp 167°. On hydrolysis (3% aq. H_2SO_4) gave resveratrol and glucose. Acid hydrolysis of **5** dimethyl ether gave a product mp 116–117°, which was identified as 3-hydroxy-5,4'-dimethoxystilbene. The structure of **5** was thus established as resveratrol 3-*O*-β-D-glucoside.

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