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AERVANONE, A NEW FLAVANONE FROM *AERVA PERSICA*

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Key Word Index—*Aerva persica*; Amaranthaceae; flavanone; *C*-glycoside; 8-galactosyl-7,4′-dihydroxyflavanone.

Aerva persica Burm. f is a small herb growing wild in the western Rajasthan. Its roots and flowers are reported to possess medicinal properties against rheumatism and kidney troubles [1]. While ascorbic acid has been reported in the leaves [2], no other chemical work has been done on this plant. We report the isolation of a new compound from the alcoholic extract, which on the basis of colour tests, subsequent chemical studies, and spectral data is a flavanone and has been designated as aervanone (**1**).

The elemental analysis and molecular ion peak of **1** in the mass spectrum, M^+ 418, indicated the molecular formula $C_{12}H_{22}O_9$. The IR spectrum of the compound indicated the presence of polyhydroxy alcoholic groups (3450 cm^{-1}), phenolic groups (3380 cm^{-1}), and flavanone carbonyl groups (1670, 1513 cm^{-1}). Other peaks in the IR, at 2900 due to the carbon chain of a galactosyl unit, between 1100 and 1300 due to OH groups and between 1500 and 1600 cm^{-1} due to an aromatic ring containing phenolic groups, were also structurally indicative. It did not contain any methoxyl, methylenedioxy, or *C*-methyl groups. It formed a hexaacetate and since the hexaacetate did not show any absorption band in the 3200–3700 cm^{-1} region, it was concluded that aervanone contains only six hydroxyl groups. The formation of *O*-dimethylaervanone with ethereal diazomethane and *O*-diethylaervanone with ethyl iodide and K_2CO_3 showed that two of the hydroxyl groups are phenolic. The *O*-dimethyl ether readily formed a tetraacetate.

Aervanone was unaffected by aq. $NaHCO_3$ solution, but it dissolved in Na_2CO_3 solution, suggesting the presence of strongly acidic phenolic groups, e.g. at the 7 and 4′ positions [3]. In its UV spectrum at 274 nm the band showed a bathochromic shift on adding NaOMe (298 nm) and NaOAc (288 nm), and thus indicated the presence of a 7-hydroxyl group. Further, a bathochromic shift of the band at 312 was good evidence for a 4′-hydroxyl group [4, 5]. The UV spectrum remain unchanged on the addition of $AlCl_3$ indicating the absence of a 5-hydroxyl function [6]. Further evidence for the absence of a 5-hydroxyl group was obtained by the strong fluorescence given by aervanone and its methoxy derivative in UV light [7, 8].

The results of chemical degradation supported the spectral results. **1** resisted acid (HCl) hydrolysis, but on treatment with dilute HNO_3, it gave 4-hydroxy-3,5-dinitrobenzoic acid. *O*-Dimethylaervanone on warming with 1 N NaOH, gave *p*-methoxyacetophenone and anisic acid. These results clearly confirm the presence of only a 4′-hydroxyl group in ring B of aervanone.

Further, the periodate oxidation of *O*-dimethylaervanone and the hydrolysis of the oxidation products showed the presence of glyceraldehyde and pyruvaldehyde, thereby indicating the presence of a sugar moiety in the molecule. The nitric acid oxidation of *O*-dimethylaervanone gave pale cream needles (recrystallized from alcohol) besides 3-nitroanisic acid. The cream needles melted at 255–257° and readily formed a 2,4-dinitrophenyl-hydrazone, mp 304–305°. It also gave the test for an aromatic aldehyde group. The C, H analysis and molecular ion peak (M^+ 309) suggested the molecular formula $C_{18}H_{13}O_5$. It is presumably 8-formyl-7,4′-dimethoxyflavanone, since similar products are obtained from the nitric acid and periodate oxidation of bayin [9] (8-*C*-glucosyl-7,4′-dihydroxyflavone) and vitexin [10] (8-*C*-glucosyl-5,7,4′-trihydroxyflavone). Thus the presence of glyceraldehyde in the periodate oxidation mixture of aervanone may be attributed to the formyl group which is clearly produced by the scission of 1:2 glycol system of aervanone, with sodium metaperiodate.

1 was subjected to ferric chloride oxidative hydrolysis [11] resulting into an insoluble compound **2** and the filtrate gave tests for reducing sugar. The paper chromatography of the filtrate showed it to contain D-galactose and D-lyxose and thus confirmed the presence of a galactose unit at C-8 position.

2 gave colour tests for flavanone and a blue fluorescence in UV. The elemental analysis and the molecular ion peak (M^+ 272) indicated the molecular formula $C_{15}H_{12}O_5$. On alkaline hydrolysis it gave *p*-hydroxyacetophenone and *p*-hydroxybenzoic acid confirming the 4′-OH group in B-ring. The isolation of 2,3,4-trihydroxybenzoic acid and 2,3,4-trihydroxyacetophenone from the alkaline hydrolysis mixture confirmed the 7,8-OH groups in the ring A of **2**. It was also found to give a bathochromic shift of 288 and 324 nm bands in the UV spectrum on adding NaOMe (310 and 342 nm) and NaOAc (306 and 342 nm) respectively, indicating the presence of —OH groups at 7 and 4′ positions. Thus **2** is 7,8,4′-trihydroxyflavanone.

The structure of aervanone was further supported by its mass spectrum. The molecular ion peak at M^+ 418 and other peaks clearly confirmed its proposed

structure. For example peaks at *m/e* 400, 382, 364 and 346 showed the loss of one, two, three and four water molecules showing a polyhydroxy moiety, representing the sugar moiety in the molecule [12]. The base peak at *m/e* 269 further confirms that the galactosyl unit is present in aervanone as *C*-galactosyl [12] and not as *O*-galactosyl because in the latter case an intense peak at *m/e* 256 would have appeared. Further a peak at *m/e* 120 clearly showed it to be a flavanone and not a flavone due to *p*-hydroxyphenyl ethene fragment from the B-ring [13]. A peak at *m/e* 390 (M−CO) also showed the presence of the carbonyl group of flavanone. An important peak at *m/e* 298 may be due to the loss of $C_4H_8O_4$ fragment from the sugar moiety of the molecule which after rearrangement loses a CHO fragment to give the base peak at *m/e* 269.

The structure of **2** as 7,8,4′-trihydroxyflavanone was also confirmed from its mass fragmentation. The molecular ion peak was the base peak at *m/e* 272 with other major peaks corresponding to M−H, M−CO, ring A and ring B. An important peak at *m/e* 120 due to *p*-hydroxyphenylethene clearly indicated it to be flavanone having *p*-hydroxyl group in ring B [13, 14]. Other peaks were in complete agreement with the proposed structure.

The NMR spectrum of the TMS ether of **1** fully confirms its proposed structure. Only two aromatic protons of ring A showed, proton 5 as doublet at δ 7.6–7.8 ($J = 9$ Hz) and proton 6, again a doublet, at δ 6.4 ($J = 9$ Hz). The position of C-5 proton near δ 8.0, as its characteristic feature was due to its deshielding by the C-4 carbonyl group and, it showed, due to *ortho* coupling with C-6 proton. This shows also that the *C*-glycosyl unit is present at C-8 and not at C-6 [15]. The signal pattern due to ring B protons clearly showed it to contain a hydroxyl group at 4′-position, as H-2′ and H-6′ proton appeared as a doublet at δ 7.2–7.3 ($J = 7$ Hz) while proton H-3′ and H-5′ appeared as a doublet between δ 6.8 and 6.7 ($J = 8$ Hz). Further, that the molecule is a flavanone was confirmed by the signals shown due to C-2 and C-3 protons. The signal for C-2 proton appeared as two doublets near δ 5.2, one doublet may be due to *J cis* (5 Hz) and the other due to *J trans* (11 Hz) of its coupling with the C-3 protons. The C-3 protons couple with each other in addition to their spin–spin interaction with C-2 proton and appeared as overlapping signals between δ 2.6 and 2.9 ($J = 17$ Hz). The 6 protons of galactosyl moiety appeared between the δ 3.4 and 3.7 multiplet while the single proton attached to C-1 of sugar unit appeared at δ 4.8 as a doublet ($J = 6$ Hz).

Thus aervanone has been characterized as 8-*C*-β-D-galactosyl-7,4′-dihydroxyflavanone.

EXPERIMENTAL

The IR spectra were recorded in KBr and NMR measurements were run in CCl_4 with TMS as an internal standard [20].

Isolation. The roots of the plant (16 kg) were collected from the grounds of Jodhpur University, New Campus and nearby areas. The dried powder of the roots was extracted with C_6H_6 and alcohol in a Soxhlet. The alcoholic extract was concd and extracted with petrol. It was then diluted and precipitated with a saturated lead acetate and the mixture was kept refridgerated overnight. The ppt. was filtered, dried in air and extracted successively with C_6H_6 (A) and alcohol (B). The C_6H_6 extract (A) was mixed with the C_6H_6 root extract, after comparing the two on TLC.

The alcoholic extract (B) was concd and adsorbed on a small amount of Si gel, which was fed into a column (150 × 5 cm) of Si gel (BDH England, deactivated with 2 N HCl) and eluted with C_6H_6–alcohol mixtures, 4:1 and 7:3. The first ten fractions of 30 ml each gave a small amount of a red brown compound which was discarded. The next 120 fractions with C_6H_6–alcohol (7:3) gave aervanone as a pale cream compound which was recrystallized (7.5 g) from MeOH.

Aervanone. Mp 257–258°, M⁺ 418 (Found: C, 60.31; H, 5.27. $C_{21}H_{22}O_9$ requires: C, 60.23; H, 5.26%). R_f: 0.56 in TBA (*t*-BuOH–HOAc–H_2O, 3:1:1) and 0.36 in 15% aq HOAc. It gave: colour reactions for a phenolic flavanone and fluoresced blue in UV light, changing to a blue-green in the presence of NH_3. It showed pronounced shifts in the UV spectrum by adding sodium acetate and sodium methoxide to the spectral solution.

Aervanone hexaacetate. This was obtained by acetylating **1** (100 mg) with Py–Ac_2O (3:1) as colourless crystals (85 mg), mp 162–163°, M⁺ 670, (Found: C, 59.20; H, 5.11; acetyl, 52.83. $C_{33}H_{34}O_{15}$ requires: C, 59.10; H, 5.07; acetyl, 52.90%). IR (KBr) 1625, 1655, 1735 cm^{-1}.

O-Dimethylaervanone. Compound **1** (1.5 g) was methylated with diazomethane in Et_2O and recrystallized from Me_2CO as colourless needles (1.45 g), mp 248–249°, M⁺ 446. It formed a tetraacetate $C_{31}H_{34}O_{13}$ with Py–Ac_2O as colourless crystals, mp 225–226°.

O-Diethylaervanone. Compound **1** (350 mg) was ethylated with EtI and K_2CO_3 in dry butanone and recrystallized from MeOH as colourless crystals (250 mg), mp 242–244°, $C_{25}H_{30}O_9$.

Nitric acid oxidation of aervanone. Compound **1** (500 mg) was heated with 2.5 N HNO_3 on a water bath. On standing overnight the mixture deposited yellow crystals of 4-hydroxy-3,5-dinitrobenzoic acid, $C_7H_4N_2O_7$, mp 240–241° (dec.), identified by mmp and TLC with an authentic sample.

Alkaline fission of O-dimethylaervanone. By alkaline fission of *O*-dimethylaervanone with 1 N NaOH, *p*-methoxyacetophenone (superimposable IR comparable mp of semicarbazone and 2:4-DNP with authentic sample) and *p*-anisic acid (IR and mmp) were obtained.

Periodic acid oxidation of aervanone. Compound **1** (1 g) on oxidation with periodic acid gave pale yellow needles, mp 240–241°, M⁺ 309, $C_{18}H_{13}O_5$ which formed diacetate, mp 210° and di-*O*-methyl ether, mp 233–235° and gave a positive test for aromatic aldehyde group. It appeared to be 8-formyl-7,4′-dihydroxyflavanone (Found: C, 70.1; H, 4.15. $C_{18}H_{13}O_5$ requires: C, 69.90; H, 4.21%).

Periodate oxidation of O-dimethylaervanone and the hydrolysis of oxidation product. *O*-Dimethylaervanone (500 mg) in 30% MeOH was first treated with $NaIO_4$ (0.5 N, 18 ml) and then the mixture was hydrolysed with 1 N HCl and filtered [16]. The filtrate formed a yellow-orange precipitate with 2,4-dinitrophenylhydrazine which showed two spots on silica TLC in C_6H_6–EtOAc (4:1) having R_fs 0.12 and 0.42. The mixture was chromatographed over Si gel with C_6H_6–EtOAc (4:1). From the first 10 fractions of 10 ml, the 2,4-dinitrophenylhydrazone of glyceraldehyde was obtained which was identified by mp and mmp (166–167°). The last 20 fractions gave 2,4-dinitrophenylhydrazone of pyruvaldehyde, which recrystallized from MeOH as orange needles, mp and mmp

300–301°.

Acid hydrolysis of aervanone. Compound **1** remained unaffected on heating with 1 N HCl at 100° for 2 hr.

Ferric chloride oxidative hydrolysis. Compound **1** (3 g) and ferric chloride (2.5 g) in water (25 ml) were refluxed for 6–8 hr [11]. The mixture was cooled, adjusted the pH to 8.0 with an aq. solution of NaOH and a ppt. was removed by centrifugation. The ppt. (ii) thus obtained was recrystallized from EtOH, mp 105–106° (1.4 g) and was identified as 7,8,4′-trihydroxyflavanone [17, 18] (mp lit. [18] 104–105°), M^+ 272: (Found: C, 66.0; H, 4.5. $C_{15}H_{12}O_5$ required: C, 66.18; H, 4.41%). NMR(CCl_4^1): δ 7.3 (H-2′), 7.2 (H-6′), 6.8 (H-3′), 6.7 (H-5′), 6.3 (H-6), 7.6 (H-5). It formed a triacetate with Py–Ac_2O, mp 166–167° (lit. [18] 165–166°). The supernatant was adjusted to pH 7.0 with aq. HCl, and was extracted with pentyl alcohol several times. The pale yellow aq. solution was passed successively through Amberlite resin IR-120 (H) and IR-4B (OH) until all ferrous and chloride ions were removed. The neutral solution was concd and subjected to PC in phenol (90%)–H_2O (89:11) when the presence of D-galactose (R_f 0.44) and D-lyxose (R_f 0.05) was confirmed by comparing the two with authentic specimens.

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A NEW PRENYLATED FLAVANONE FROM *TEPHROSIA VILLOSA*

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INTRODUCTION

Past work on pods of *Tephrosia villosa* has yielded four rotenoids—villosin, villosone, villol and villinol along with 6a, 12a-dehydrosumatrol (villosal) and 12a-hydroxysumatrol (villosinol) [1]. We now report the structure elucidation of a new flavanone from the roots of *Tephrosia villosa*.

RESULTS AND DISCUSSION

Elemental analysis and M^+ at *m/e* 338 led to the molecular formula $C_{21}H_{22}O_4$ of compound **1**. It gave a positive ferric chloride test, a colourless monoacetate **2** with Ac_2O–Py at room temperature and a monomethyl ether **3** with Me_2SO_4–K_2CO_3–Me_2CO indicating the presence of a phenolic hydroxyl group.