AURAPIN, A NEW DIHYDROFLAVONOL GLYCOSIDE AND OTHER FLAVONOIDS FROM ACINOS ALPINUS

Pietro Venturella*, Aurora Bellino, Maria Luisa Marino,
and Marco Sorrentino
Institute of Organic Chemistry, University of Palermo
20, via Archirafi - 90123 Palermo, Italy

Abstract - A new dihydroflavonol glycoside has been isolated from Acinos alpinus and identified as 3-O-rhamnosyl-5-hydroxy--7,4'-dimethoxyflavanone on the basis of chemical and spectroscopic evidence.
Three known flavonoids (naringenin, taxifolin and neoponcirin) were additionally isolated from same source.

In continuation of our search on the flavonoids from Labiatae family[1], we report here the isolation and structure elucidation of a new dihydroflavonol glycoside which we name aurapin (I) (3-O-rhamnosyl-5-hydroxy-7,4'-dimethoxyflavanone) and of three other previously known flavonoids: 5,7,4'-trihydroxyflavanone (naringenin) (II)[1,2]; 3,5,7,3',4'-pentahydroxyflavanone (taxifolin) (III)[3]; and 7-O-rutinosyl-5-hydroxy-4'-methoxyflavanone (neoponcirin) (IV)[1,4] from Acinos alpinus, a species growing in the mountains of Madonie (Sicily).
The air dried aerial parts of the plant were milled and successively extracted with light petroleum ether, ethyl acetate, ethanol and acetone.
By chromatography on silica gel we have isolated from the petroleum ether extract, ursolic and oleanolic acid which characterized by physicochemical methods[1,5]; the compounds (II) and (III) isolated from the ethylacetate extract and (I) and (IV) from ethanol and acetone extracts.
Aurapin (I) has molecular formula $C_{23}H_{26}O_{10}$, mp 132-133° (from MeOH-H_2O); UV (MeOH) λ_{max}(logε) 228,292 nm (4.16; 4.03); (MeOH + $AlCl_3$) 228,329 nm (4.10; 4.20); no bathochromic shift in the presence of NaOAc; IR (nujol) 1630 cm^{-1} (C=O); 3455-3450 cm^{-1}(OH).
The NMR spectrum (FT 80A Varian DMSO) of (I) has the following signals: δ 1.18 (d, J = 6.0 Hz, CH_3); 3.90 (s, 2 OCH_3); 4.01 (d, J = 5.8 Hz, H-1", proton anomeric); 4.85 and 4.25 (2d, J_{23} = 11 Hz, H-2 and H-3); 6.38 and 6.47 (2d, J_{68} = 2 Hz, H-6 and H-8); 11.87 (OH-5).
Since the UV spectrum was inaltered after addition of NaOAc, the 7-hydroxyl is substituted[6]. The NMR signal at 11.87δ and the usual bathochromic shifts of band I (37 nm) on addition of $AlCl_3$ established the presence of a hydroxyl group at C-5. That the substance was a dihydroflavonol was evident from NMR spectrum which exibited the typical AB system of H-2 and H-3; the A ring was unsubstituted at 6- and 8-positions (meta coupled doublet, J = 2 Hz); the ring B was monosubstituted at the 4'-position, A_2B_2 system (6.80δ, H-3' and H-5'; 7.30δ, H-2' and H-6').

Signal for the anomeric proton of the sugar was observed at 4.01δ (J = 5.8 Hz)· the signal was agreement with sugar attached at C-3[7].

This observation was confirmed when acid hydrolysis with 5% H_2SO_4 of the compound (I) yielded an aglycone $C_{17}H_{16}O_6$, mp 189-191, (from MeOH-H_2O) identified as 3,5--dihydroxy-7,4'-dimethoxyflavanone (V) (7,4'-dimethoxyaromadendrin)[8], m/e 316 (M^+) (requires C 64.55; H 5.10; Found 64.58; H 5.28); UV (MeOH)λ_{max} (log ε) 228,292 (4.24; 4.08); IR (nujol) 1629 (C=O), 3450 (OH); NMR (FT80 Varian, DMSO): δ 5.06 and 4.52 (2d, J_{23} = 11.5 Hz, H-2 and H-3); 5.20 ($W_{\frac{1}{2}}$ = 5.0 Hz,OH-3); 5.90 and 6.10 (2d, J_{68} =1.5 Hz, H-6 and H-8); 6.80 and 7.29 (J = 8.5 Hz, H-2',3',5',6'); 11.87 (OH-5); 3.98 and 3.91 (2s, 2 OCH_3).

The hydrolysate, after neutralization and concentration, gave a single spot corresponding to rhamnose on a paper chromatogram (Whatman n.4 and two different solvent systems: ethylacetate-pyridine-water (12:5:4) and isopropanol-water) (4·1)). The sugar was located by spraying with aniline phthalate and identified unambiguously by direct comparison of osazone (mp and mixed mp, 181°) with those of authentic L-rhamnose (standard conditions[9]).

Acetylation of (V) with acetic anhydride and piridine afforded the 3,5-diacethoxy--7,4'-dimethoxyflavanone (VI), mp 129-131°, (from MeOH-H_2O) confirming the presence of two OH groups; methylation with diazomethane yielded unreacted product.

On the contrary, methylation of (V) with dimethyl sulphate in acetone solution afforded 3-hydroxy-5,7,4'-trimethoxyflavanone (VII) (dihydrokaempferol trimethyl ether[8], mp 150-151°), m/e 330 (M^+) showing the typical fragmentation (m/e 181, base peak), reported by J.W.Clark-Lewis[10]. UV (MeOH)λ_{max} 257,308,352 nm (log ε 4.0; 3.72-4.04); no bathochromic shift in the presence of NaOAc and $AlCl_3$; NMR (FT80 Varian, $CDCl_3$) δ 4.96 and 4.42 (2d, J_{23} = 12 Hz, H-2 and H-3); 3.88 and 3.81 (2s, 3 OCH_3); 6.12 and 6.20 (2H, ring A); 5.16 ($W_{\frac{1}{2}}$ = 5.0 Hz, OH-3); 6.8 and 7.3 (4H, ring B).

For acetylation the compound (VII) gave 3-acethoxy-5,7,4'-trimethoxyflavanone (VIII), mp 124-125°.

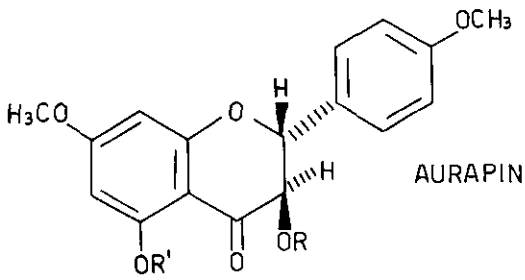
The structure of the aglycone (V) has been tested by catalytic dehydrogenation of trimethyl ether (VII) to the corresponding flavonol 3-hydroxy-5,7,4'-trimethoxyflavone (yellow needles, mp 149-150° [8]; the dehydrogenation is carried out at 180° with a palladium-charcoal catalyst employing cinnamic acid as a hydrogen acceptor[11].

The 3-hydroxy-5,7,4'-trimethoxyflavone was prepared, for direct comparison, by a treatment of 2-hydroxy-4,6,4'-trimethoxyxhalcone, prepared by condensing 2-hydroxy-4,6-dimethoxyacetophenone with anisaldehyde, with alkaline hydrogen peroxide[12].

Confirmation of the glycoside structure is given also by comparison of the NMR spectra of (I) and (V) compounds. In fact, in compound (I) the signal of the broad one-proton singlet at 5.20δ due to the hydroxyl proton is not present and signals protons concerning C-2 and C-3 appear at fields much lower in (I) than in (V).

Based on these observation, aurapin (I) has the rhamnose molecule attached at 3 position and the structure of 3-O-rhamnosyl-5-hydroxy-7,4'-dimethoxyflavanone

should be assigned



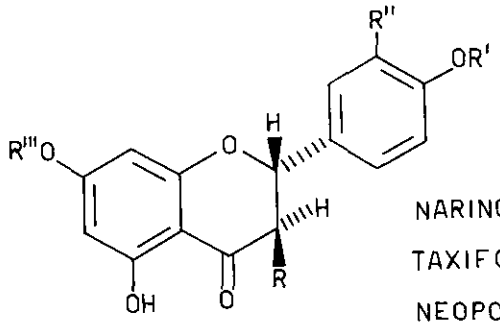
AURAPIN (I) R = RHAMNOSYL; R' = H

(V) R = R' = H

(VI) R = R' = Ac

(VII) R = H; R' = CH_3

(VIII) R = Ac; R' = CH_3



NARINGENIN (II) R = R' = R'' = R''' = H

TAXIFOLIN (III) R' = R''' = H; R = R'' = OH

NEOPONCIRIN (IV) R = R''' = H; R' = CH_3;

R'' = RUTHINOSYL

The following known flavonoids (II), (III) and (IV) were additionally isolated. The first of these was identified as naringenin (II) spectroscopically (UV, IR, NMR and MS) and by comparison of mixed mp with an authentic sample[1].
The second compound melted at 230-232° (from EtOH), M^+ 304; the UV, IR, NMR and MS agreed with those of an autenthic sample of taxifolin (III). The identity was confirmed by methylation with dimethyl sulphate· it gave a product (mp 169-170°) undepressed by an autenthic sample of 5,7,3',4'-tetramethoxydihydroquercetin.
The compound (IV), on the basis of the chemical and spectroscopic investigation, was identified as 7-O-rutinosylisosakuranetin (neoponcirin) and its agreed with those recorded in literature[1,4].

To our knowledge, the only variety of Acinos studied is Acinos thymoides, Moensch, from which Sergienko[13] has isolated poncirin and acinoside.

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