

Note

A rare flavonol glycoside from *Aerva tomentosa* Forsk as antimicrobial and hepatoprotective agent

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From the perianth lobes of *A. tomentosa*, kaempferide 3-*O*-(6''-*O*-acetyl-4'''-*O*- α -methylsinapyl)neohesperidoside has been isolated which has shown marked antimicrobial and hepatoprotective activities.

In continuation of our studies on flavonoids^{1a-f} as biologically active agents we now report the isolation of compound **1**, a rare acylated flavonol glycoside viz., kaempferide 3-*O*-(6''-*O*-acetyl-4'''-*O*- α -methylsinapyl)neohesperidoside from the perianth lobes of *Aerva tomentosa* Forsk belonging to Amarantaceae family. Compound **1** has been found to possess marked antimicrobial and hepatoprotective activities.

Results and Discussion

Compound **1** C₄₂H₄₇O₂₀, m.p. 186-88° from EtOAc

fraction gave green colour with alcoholic Fe³⁺, pink colour with Mg-HCl and greenish yellow fluorescence when fumed with NH₃. It responded to Wilson's boric acid and Gibb's tests but did not answer the Horhammer-Hansel test.

The UV spectrum and a positive Molisch test indicated **1** to be a flavonol glycoside with substitution at 3-hydroxyl. Hydrolysis of **1** with 5% H₂SO₄ gave compound **2** which was identified as kaempferide by UV, co- and mixed-chromatography on paper and by undepressed m.m.p. with an authentic sample. D-glucose and L-rhamnose were identified in the sugar components present in equimolar amounts in **1**. Product analysis of alkaline hydrolysis of **1** showed an acyl function in its sugar part.

The β -linkage of glucose moiety to 3-hydroxyl group was evident from the large coupling constant of H-1'' signal in ¹H NMR of **1**. The appearance of methyl protons of rhamnose at δ 1.10 ppm as a broad signal revealed the presence of neohesperidoside². The appearance of C-6''' signal at δ 21.60 ppm and C-6'' signal at δ 60.80 ppm in ¹³C NMR confirmed it. Again in ¹H NMR the signal at δ 1.89 ppm indicated the presence of 6''-*O*-acetyl group³. Another 3-proton signal at δ 1.50 ppm accounted for a methyl group at α -carbon of sinapic acid. One ethylenic proton at β -carbon and the two equivalent aromatic protons of sinapic acid resonated at δ 5.30 and 6.80 respectively.

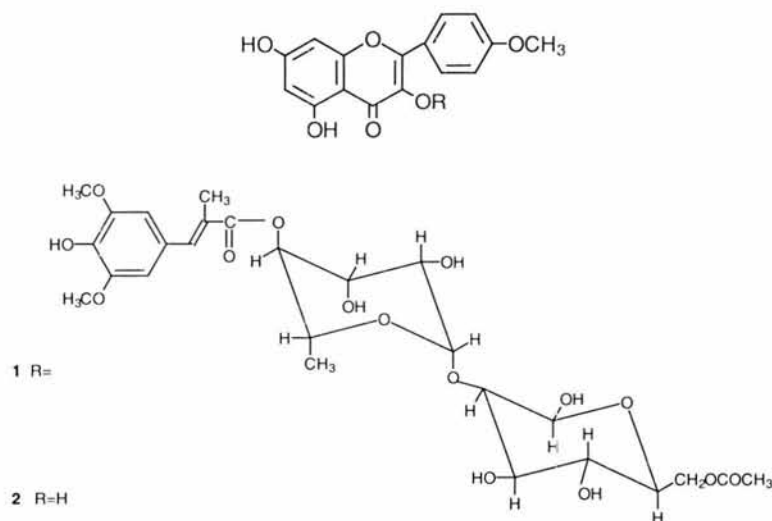


Table I—Antimicrobial activity of compound **1** against *Escherichia coli* and *Staphylococcus aureus*

DOSE μg/mL ↓	Hours →	% Inhibition					
		<i>E.coli</i>			<i>S.aureus</i>		
		24	48	72	24	48	72
50		64	67	67	70	76	76
100		79	84	84	85	86	86
200		89	93	93	90	96	96

The signal at δ 3.67 which integrated from nine protons represented three -OCH₃ groups, one at C-4' of the flavonoid nucleus and two at the sinapic acid residue.

Acylation at C-4''' of rhamnose and C-6'' of glucose was indicated by downfield shifts of these carbons and upfield shifts of adjacent carbons in ¹³C NMR as compared to corresponding carbon resonances in literature⁴. Higher downfield shift experienced by C-4''' indicated the electron withdrawing nature of sinapyl residue as compared to lower downfield shift of C-6'' to which acetyl residue was attached. On the basis of the above, compound **1** was characterised as kaempferide 3-O-(6''-O-acetyl-4'''-O- α -methylsinapyl) neohesperidoside.

The antimicrobial efficacy of compound **1** against *Escherichia coli* and *Staphylococcus aureus* was studied. The perceived percentage of inhibition is shown in **Table I**.

The bacteriostatic effect of **1** is found to be dose dependent, independent of length of time and more active against *S. aureus* than *E. coli*.

Compound **1** possesses significant hepatoprotective activity also. The inference is drawn from the biochemical reversal of target enzymes SGOT, SGPT, serum LDH, serum alkaline phosphatase and serum cholesterol as shown in **Table II**.

The data were analyzed statistically using student's t-test. The test of significance between control and compound **1** shows that all the values are greater than the expected value of t at 1% level.

Experimental Section

General. Melting points were determined on a Thomas Hoover Unimelt capillary melting apparatus and are uncorrected. UV spectra were recorded using SP 1750 spectrophotometer. ¹H NMR and ¹³C NMR spectra were recorded on Bruker 270 and 67.89 MHz spectrometers respectively, with TMS as an internal standard. (chemical shifts in δ , ppm).

Collection of plant material

The perianth lobes of *A.tomentosa* (2kg) were collected from Rayanur in Karur district in 1999.

Extraction and isolation

The fresh perianth lobes were extracted with 85% ethanol under reflux. The concentrated aqueous extract after the removal of ethanol on successive fractionation with light petrol (60-80°), Et₂O and EtOAc yielded compound **1**.

Compound **1**, yield 120 mg; m.p. 186-88°; UV (MeOH): 253, 266, 323, 354; (+NaOMe): 245, 267, 405; (+AlCl₃): 266, 353, 390; (+AlCl₃ - HCl): 266, 350, 390; (+NaOAc): 276, 325, 385; (+NaOAc - H₃BO₃): 265sh, 325sh; 354; PMR (270 MHz, DMSO-d₆): 6.25 (d, *J*=2.5 Hz, 1H, H-6), 6.37 (d, *J*=2.5 Hz, 1H, H-8), 6.42 (d, *J*=8.5 Hz, 2H, H-3' and H-5'), 8.10 (d, *J*=8.5 Hz, 2H, H-2' and H-6'), 5.15 (m, 1H, H-1''), 4.90 (d, *J*=2.0 Hz 1H, H-1'''), 1.10 (m, 3H, rhamn-Me), 1.89 (br.s.3H, 6''-O-COCH₃), 1.50 (s, 3H, α -methylsinapyl - Me) 3.67 (s, 9H, three-OCH₃ groups; one at C-4' and two at sinapic acid), 5.30 - 6.80 (m, 3H, ethylenic H at β carbon and two equivalent aromatic protons of sinapic acid), ¹³C 145.2 (C-2), 130.0 (C-3), 178.0 (C-4), 160.8 (C-5), 99.6 (C-6), 169.2 (C-7), 94.4 (C-8), 157.2 (C-9), 104.4 (C-10), 121.6 (C-1'), 128.4(C-2'), 116.0(C-3'), 162.0(C-4'), 116.8(C-5') 128.0(C-6') 56.4 (4'-OCH), 100.8 (C-1''), 76.4 (C-2''), 76.0 (C-3''), 70.4 (C-4''), 74.0 (C-5''), 60.8 (C-6''), 18.0 (-OCOCH₃), 170.8 (-OCOCH₃), 100.7 (C-1'''), 68.0 (C-2'''), 66.0 (C-3'''), 72.5 (C-4'''), 62.0 (C-5'''), 21.6 (C-6'''), 166.4, 28.4, 125.6, 144.0, 130.8, 102.4, 147.6, 134.0, 53.5 (sinapyl

Table II—Hepatoprotective activity of compound **1**

Treatment	SGOT μ mol/mL	SGPT μ mol/mL	Serum LDH μ mol/mL	Serum alkaline phosphatase KA/100mL	Serum cholesterol mg%
Control (H ₂ O)	24.66 ± 0.13	23.16 ± 0.90	168.70 ± 1.60	6.08 ± 0.09	50.70 ± 0.70
CCl ₄	79.85 ± 0.27	78.50 ± 0.70	293.67 ± 0.76	10.65 ± 0.40	99.0 ± 1.00
CCl ₄ + Compound 1	30.00 ± 0.50	33.00 ± 1.20	201.01 ± 0.60	7.70 ± 0.20	63.33 ± 0.45

-OCO-, α -CH₃, α -C, β -C Ar-C₁, C₂ and C₆, C₃ and C₅, C₄, two OCH₃ at C-3 and C-5 respectively).

Hydrolysis of compound 1

Acid hydrolysis. The glycoside (100 mg) was dissolved in 1 mL methanol and heated with 1 mL 5% H₂SO₄ at 95° for 1 hr. The reaction mixture after usual work-up yielded compound 2.

Compound 2, yield 30mg, m.p. 227-39°; UV (MeOH): 267, 320, 367; (+NaOMe): 280, 411; (+AlCl₃): 271, 305, 350, 423; + (AlCl₃-HCl) 257, 270, 347, 422; (+NaOAc) 274, 384 (+NaOAc-H₃BO₃):268, 349, 367 triacetate (Ac₂O, Py), m.p. 193-95°.

Alkaline hydrolysis. The concentrated aqueous solution of compound 1 (2 mL) was mixed with 2 M NaOH (5 mL) in a 10 mL syringe and the mixture was left in the syringe (with all air expelled) for 2 hr at room temperature. The mixture was ejected into a vial containing 2 M HCl (6 mL) and evaporated to dryness *in vacuo*. The dried mass was extracted with warm ether. The residue from ethereal solution fluoresced blue on paper under UV and turned bluish green when fumed with NH₃; UV (MeOH) 239, 325 and + NaOMe 239, 350 The observations were reminiscent of a carboxylic acid. The remaining residue containing deacylated glycoside exhibited the same UV (MeOH) as compound 1 suggesting the attachment of acyl function to the sugar.⁵

Antimicrobial studies were done using serial dilution method. *Escherichia coli* of gram negative group and *Staphylococcus aureus* of gram positive group were taken as test organisms. The percentage of bacterial growth inhibition produced by the compound 1 was calculated from the measure of the turbidity of the control and the turbidity of specific treatment.

SGOT, SGPT, serum LDH, serum alkaline phosphatase and serum cholesterol were determined

in vitro using the methods of Reitman and Frenkal⁶, Wooten⁷, Kind and King⁸. Assay was done using the diagnostic kit supplied by Span Diagnostics Pvt. Ltd., India and Erma photocolormeter. Healthy male wistar rats weighing between 150 and 200g were used. For each experiment, the animals have been randomly selected into groups consisting of six animals. CCl₄ was administered at 0.1mL/100 g body weight; aqueous solution of compound 1 at 1% concentration as 1mL/100g body weight. At the end of the treatment schedule of one week the animals were sacrificed by decapitation and their serum was subjected for biochemical profile.

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