New anti-emetic flavanone glycoside from cotoneaster affinis

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ABSTRACT

The present paper deals with the isolation Characterization of a new compound along with Genkwanin and Kaempferol from the sapwood and anti-emetic activity determination of I. From the methanolic extract, genkwanin, kaempferol, naringenin and a new derivatives I were isolated. The structure of the known compounds were determined by spectroscopic methods and by direct comparison (m.m.p., co-TLC, Superimposible IR) with authentic samples. The new compound I was purified by acetylated and characterized with the help of NMR and MS datas.

INTRODUCTION

A new flavanone glycoside, 5-O-methyl naringenin-7-O-α-L-rhamnopyranoside 1 as well as the known flavonoids genkwanin, kaempferol and naringenin were isolated from stem sapwood of Cotoneaster affinis. The structure of 1 was established by UV, MS and NMR spectroscopy and Chemical studies. Compound (1) was tested for anti-emetic activity on copper sulphate induced emesis in young Chicks. It showed anti-emetic effects.

Cotoneaster affinis is an erect deciduous shrub belonging to the Rosaceae. It occurs in temperate Himalaya. This species has never been subjected to phytochemical or biological investigation although other cotoneaster species have been studied and many of these produced compounds (triterpenes, flavonoids) with anti-viral and anti-spasmodic activities.

EXPERIMENTAL

Material and method

Plant material cotoneaster affinis stem sapwood was procured from Dhanolti (Tehri), Uttrakhand (India) in May 2005 and authenticated by Botany Department of H N B Garhwal University Campus, Badhsahithaul, Tehri, Uttrakhand (India). A voucher specimen is available in the herbarium of Botany department.

The plant material was defatted with light petroleum (60-80°C) followed by exhaustive extraction with MeOH. The extract was concentrated under reduced pressure afforded a light yellow solid mass. The solid mass on column chromatography (CHCl₃-MeOH) afforded genkwanin (120mg), kaempferol (140mg) naringenin (90mg) and Compound 1 (160mg).

The melting points are uncorrected IR spectra were recorded on KBr discs (JASCO-IR-810 Spectrophotometer). The ¹H NMR and ¹³C NMR Spectra were obtained on a JEOL JNM-MH 200 at 300 MHz and JNM-FX 100 at 100MHz in DMSO, D₆ and CDCl₃ using TMS as internal Standard (Chemical shift in
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δppm). Mass spectra (70eV JEOL JMS-DX 300 Spectrometer) were taken with a direct inlet. Column chromatography was carried out on Kiesel gel (230-240 mesh MERK). TLC was performed on Kieselgel 60Gc(Merk) UV spectra (MeOH) were obtained Hitachi 320 Spectrophotometer.

Genkwanin m.p. 284-286°C, kampferol 277-279°C and naringenin m.p. 248-249°C, all are known compounds. Their spectral data are in agreement with published results for them[6]. Finally the identities of these compounds were confirmed by comparison (m.m.p., Co-TLC) with authentic samples.

Compound (1) (130mg) was purified by acetylation (Ac₂O/ Pyridine). The acetate 2m.p. 194-196°C (MeOH) was refluxed for 10min with 1ml of 10% KOH in MeOH (2ml). The reaction mixture was concentrated and submitted to preparative TLC(CHCl₃; MeOH, 18:2) to recover the deacetylated compound 1(25mg): m.p. (MeOH) 207°C, EIMS : m/z 432(M⁺) of aglycone, IR νmax cm⁻¹: 3420, 2950, 1685, 1610, 1350, 1210i, ¹H NMR(D₆-DMSO): 2.70(s, 1H, 3-Heq), 3.30(d J =2Hz, 3-Hax), 3.80(s, 1H, OCH₃), 5.60(s, 1H, OH), 6.15(s, 1H, J= 2Hz, H-8), 6.83 (d, 2H, J=9Hz, H-2' and 6'), rhammethyl proton : 1.78(3H, d, J= 6.2Hz), anomeric protons : 6.57(1H, br s, C-1" -H of rhamnose). ¹³C NMR: TABLE 1.

Acidic Hydrolysis of Compound (1). Compound (1) (5mg) was heated with aq 10% HCl(2ml) in a sealed tube at 100°C for 3hr to afford the aglycone (5-methoxy naringenin) m.p. 286-259°C. Identified with the help of spectral studies[6] and by direct comparison (m.m.p., co-TLC and Superimposable IR) with an authentic sample. The neutralized and concentrted aqueous hydrolysate showed the presence of L-rhamnose (PC, solvent EtOAc- C₅H₅N - H₂O, 10:4:3 RF Value 0.42).

Anti-emetic assay

The young chicks were divided into 1-3 groups consisting of six each and each young chick was set aside for 10min to stabilize in large beaker at 25°C. The sample solution was administered orally at a dose 10ml/kg. After 10min, copper sulpher anhydride was administered orally at a dose of 50 mg/kg, then the number of chicks retching (an emetic action without vomiting gastric materials) was recorded during rest 10min. The results were judge by decrease in number of retching compared with those of the controls. The inhibition (%) was calculated as follows:

Inhibition % = [(A-B)/ A]×100

Where A is the frequency of retching in control group and B is the frequency of retching after sample treatment.

RESULTS AND DISCUSSION

Acidic hydrolysis of compound (1) gave rhamnose (co-PC, co-TLC) and an aglycone which gave a positive Shinoda’s test for flavanones[7]. The presence of methoxy group was determined with the help of Zeisel’s...
The aglycone was treated with HI to afford naringenin, the identification of which along with its acetate was established with the help of spectral studies. The 1H NMR spectra of 1 and 2 supported the presence of 4,5,7 trihydroxy flavanone (naringenin) skeleton. The presence of one aromatic (2.30 ppm) and three alcoholic (δ 2.06, 2.10, 2.11) acetoxyl and an aromatic methoxyl group (δ 3.81) was revealed by 1H NMR spectrum of 2. Therefore 1 should be a mono-O-glycosylated mono-O-methylated naringenin. The chemical shift for the protons of B and C ring of 2 were in good agreement with those of triacetyl naringenin, where as H-6 and H-8 of 1 where observed to be shifted upfield. Accordingly the OH group at C-4′ must be unsubstituted. The existence of a methoxy group on a ring was confirmed by the presence of ions at m/z, 286, 167 and 120 in the EIMS of 1. FAB-MS of 1 showed ion peaks at m/z (M+ 432), 286(M+ Sugar unit). Thus on basis of hydrolytic and spectral data 1 was a mono-O-glycosylated, mono-O-methylated naringenin. The chemical shift of sugar moiety in the 13C NMR spectra of 1 and 2 supported the identification of attached sugar as rhamnose (δ 1.78 due to the secondary methyl). The position of the methoxy group was determined as follows: the C-10 (δ 106.5ppm) scareely shifted on acetylation (δ 107.0) in the 13C NMR of 2 on other hand both C-6 and C-8 (δ 93.7 and 95.1) shifted upfield (δ 95.8 and 98.8). More over the chemical shift of C-10 was consistent with that of 5-methylated flavanoids like wise the shift of C-1" was consistent with 7-O-rhamnoside. Thus the structure of the aglycone was elucideted as 5-O-methyl naringenin and that of 1, a new flavanone glycoside, 5-O- methyl naringenin-7-O-α-L-rhamnopyranoside.

The compound was tested for anti-emetic activity on copper sulphate induced emesis in young chicks. It showed significant inhibition at a dose of 50 mg/kg (TABLE 2).

ACKNOWLEDGMENT

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REFERENCES


TABLE 2 : Anti-emetic effects of compound (1) on CuSO4 induced emesis in young chicks

<table>
<thead>
<tr>
<th>Compound</th>
<th>Does (mg/kg)</th>
<th>no of chicks</th>
<th>No of retching (mean ± S.E.M)</th>
<th>Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>6</td>
<td>75.2 ± 4.80</td>
<td></td>
</tr>
<tr>
<td>D- 5- O-methyl</td>
<td>10</td>
<td>6</td>
<td>68.6 ± 4.00</td>
<td>8.8</td>
</tr>
<tr>
<td>Naringenin-7- O-α-L-rhamnoside</td>
<td>20</td>
<td>6</td>
<td>64.3 ± 5.91</td>
<td>14.5</td>
</tr>
<tr>
<td>Rhamnopyranoside</td>
<td>50</td>
<td>6</td>
<td>37.4 ± 3.35*</td>
<td>50.2</td>
</tr>
</tbody>
</table>

OR O OR O OR O OR O

1 : R = H
2 : R = Ac

rham (146)