Recently, it has been shown by Srivastav and Kumar[10] that the deviation near $K_m(T)$ could be accounted by including an interference term ξ in the analysis which is taken as a constant (0.15 for Ge) but it appears to be contradictory since ξ depends on T considerably, as evident from their equation (20). From the present work it is concluded that the deviation near $K_m(T)$ could be attributed to the neglect of the cross term, between defect and anharmonic parameters, which varies as Dw^3T. The present analysis is also valid for other non-metallic solids doped with isotopic impurities.

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A NEW FLAVONE GLYCOSIDE FROM *MELIA AZEDARACH* LINN.

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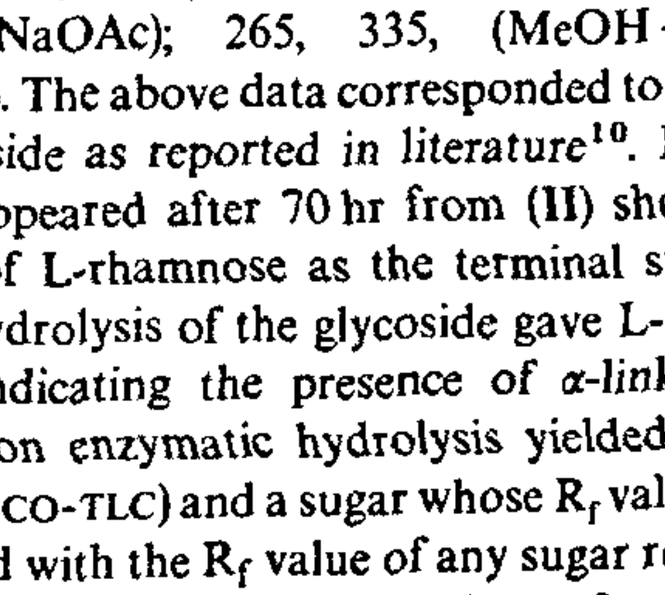
FROM the stem bark of *Melia azedarach*, we report the isolation and characterization of a new flavone glycoside; 4′-5-dihydroxy flavone-7-O-α-L-rhamnopyranosyl- (1 → 4)-β-D-glucopyranoside (I) on the basis of spectral and chemical evidence.

The air-dried and powdered stem-bark of *M. azedarach*[1,2] (5 kg) was extracted five times with rectified spirit under reflux for 20 days. The total spirit extract (40 lit.) was concentrated under reduced pressure to a small volume (400 ml) and poured into water (1 lit.). The water insoluble fraction was extracted with different organic solvents in the order of their increasing polarities. Ethyl acetate extract gave a compound which after purification by column chromatography (SiO_2-gel; EtOAc: Me_2CO; 8:2) yielded a reddish-yellow crystalline product (Me_2CO:MeOH), (yield 1.6 g), mp, 208–10° which was found to be a single entity on PC [R_f, 0.92, (*n*-BAW 4:1:5) and 0.78 (15% gl AcOH)] and TLC [R_f, 0.85, (MeOH:$CHCl_3$, 5:3) and 0.77 (MeOH:$CHCl_3$, 4:7)], IR ν_{max}^{KBr} 825, 665, 1050, 1125, 1375, 1380, 1460, 1510, 1610, 1665, 3360–3400 (br) cm^{-1}; UV λ_{max}, 265, 315 (MeOH); 260 (sh), 280, 300, 335, 380 (MeOH + $AlCl_3$); 277, 295 (sh), 360 (MeOH + NaOAc); 270, 332 (NaOMe); [Found: C, 56.20; H, 5.19; $C_{27}H_{30}O_{14}$ reqd; C, 56.05; H, 5.20%]. It gave Shinoda's[3] and Molisch's tests for a flavone glycoside. Acid-hydrolysis (50 ml; 7% H_2SO_4) of the glycoside (900 mg) gave an aglycone, mp, 347–48°, D-glucose (R_f; 0.18, *n*-BAW, 4:1:5 and CO-PC)[4] and L-rhamnose (R_f; 0.38; *n*-BAW, 4:1:5 and CO-PC).[4]

The aglycone was recovered as usual and crystallized from EtOAc:petroleum ether as yellow needles; $C_{15}H_{10}O_5$ (M^+ at m/e 270); IR ν_{max}^{KBr}, 1050, 1220, 1385, 1460, 1500, 1600, 1660, 1605 and 3440 (br) cm^{-1}; UV λ_{max}, 265, 325 (MeOH); 264, 375 (MeOH + NaOMe) 276, 345 (MeOH + $AlCl_3$); 260, 335 (H_3BO_3 – NaOMe); 286, 336 (MeOH + NaOAc) nm; MS at m/e; 270 (100%), 269 (13%), 242 (19%), 152 (16%), 149 (10%), 146 (18%), 118 (16%) and 117 (22%);[5] and ^{1}H NMR (δ, $CDCl_3$), 6.22 (d, J = 2 H_z, H-6), 6.48 (d, J = 2 H_z, H-8), 6.70 (s, H-3), 7.90 (d, J = 9 H_z, H-2′ and H-6′), 6.95 (d, J = 9 H_z, H-3′ and 5′), 12.90 (s, OH); [Found: C, 66.60; H, 3.68; $C_{15}H_{10}O_5$; reqd; C, 66.66; H, 3.70%]; triacetate (100 mg of the aglycone + 5 ml of Ac_2O + 5 ml C_6H_5N; yield 60 mg); [Found; C, 63.60; H, 4.00; OAc, 32.60; $C_{21}H_{16}O_8$ reqd., C, 63.63; H, 4.04; 3XOAc, 32.57%]; trimethyl ether (100 mg of the aglycone + 5 ml of Me_2SO_4 + 2 g K_2CO_3; yield, 65 mg), mp, 155–56°; [Found: C, 69.20; H, 5.10; OMe, 29.78; $C_{18}H_{16}O_5$ reqd; C, 69.23; H, 5.12; 3XOMe, 29.80%]. The aglycone gave a bathochromic shift with $AlCl_3$ (λ_{max} 276, 345 nm) and NaOAc (λ_{max} 286, 336 nm) which were clear indication for the presence of two hydroxyls at C-5 and C-7 respectively. KOH degradation (50%)[6] of the aglycone yielded phloroglucinol, mp, 216–17° (lit. mp, 217°, mmp and CO-TLC) and *p*-hydroxybenzoic acid, mp, 212–13° (lit. mp, 214°, mmp and CO-TLC), while $KMnO_4$ oxidation afforded *p*-hydroxybenzoic acid (mmp and CO-TLC) as their identifiable oxidation products. Thus, the structure of the aglycone was assigned as apigenin, which

was finally confirmed with the authentic sample of apigenin (lit. mp, 347–48°, mmp and CO-TLC)[7]. Periodate oxidation[8(a,b)] consumed 3 moles of periodate with the production of 2 mol of the formic acid per 1 mol of the glycoside, suggesting the presence of a disaccharide in pyranose structure. The glycoside (200 mg) in Me_2CO (25 ml) was completely methylated with Me_2SO_4 (10 ml) K_2CO_3 (3 g) followed by acid-hydrolysis (7% H_2SO_4) afforded apigenin 4'-5-dimethyl ether, mp 160–62° (dec) [lit. mp, 160–63° (dec)] (mmp and CO-TLC)[9], 2,3,6-tri-O-methyl-D-glucose (R_G value, 0.83 *n*-BuOH : EtOH; H_2O, 5 : 1 : 4; and CO-PC)[4] and 2,3,4-tri-O-methyl-L-rhamnose (R_G value, 1.01 in *n*-BuOH : EtOH : H_2O, 5 : 1 : 4 and CO-PC)[4] respectively, showing the attachment of the sugars with 7-position of the aglycone part. The partial hydrolysis of the glycoside (200 mg) with H_2SO_4 (7% H_2SO_4, 25 ml) at room temperature, liberated L-rhamnose (CO-PC) after 50 hr and (II). The (II) had mp, 110–13° (dec), $C_{21}H_{20}O_{10}$; TLC; R_f 0.60 ($CHCl_3$: MeOH, 7 : 3) and 0.43 (Me_2CO : MeOH; 9 : 1); IR ν_{max}^{KBr}, 3400–3350 (br), 2900, 1660, 1600, 1500, 1450, 1375, 1380, 1220, 1060, 820, 660 and 610 cm^{-1}; UV λ_{max} 267, 335 (MeOH); 242 (sh), 268, 300 (sh), 380 (MeOH + NaOMe); 275, 298, 350 (MeOH + $AlCl_3$); 275, 297, 340, 370 (MeOH + $AlCl_3$ + HCl); 267, 385 (MeOH + NaOAc); 265, 335, (MeOH + NaOAc + H_3BO_3). The above data corresponded to apigenin-7-O-glucoside as reported in literature[10]. D-glucose (CO-PC) appeared after 70 hr from (II) showing the presence of L-rhamnose as the terminal sugar. The diastase hydrolysis of the glycoside gave L-rhamnose (CO-PC) indicating the presence of α-linkage. The glycoside on enzymatic hydrolysis yielded apigenin (mmp and CO-TLC) and a sugar whose R_f value did not correspond with the R_f value of any sugar reported in literature[4]. The hydrolysate, obtained after removing the apigenin on treatment with diastase solution afforded L-rhamnose (CO-PC) and D-glucose (CO-PC) confirming the presence of α-linkage between L-rhamnose and D-glucose and β-linkage between D-glucose and apigenin.

Hence, the glycoside was assigned a structure (I).



Structure 1

(I); R = rhamnosyl – glucoside
(II); R = glucose.

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PROTECTIVE EFFECT OF *MYCOBACTERIUM HABANA* IN MICE AGAINST INFECTION WITH INDIGENOUS STRAINS OF *M. TUBERCULOSIS*

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VIABLE *Bacillus Calmette Guerin* (BCG) is widely used in the prophylaxis of tuberculosis and is the only vaccine available for this purpose. However, there are several practical and theoretical objections for its use. These include the loss of sensitivity of tuberculin as a diagnostic and epidemiological tool and the suspected