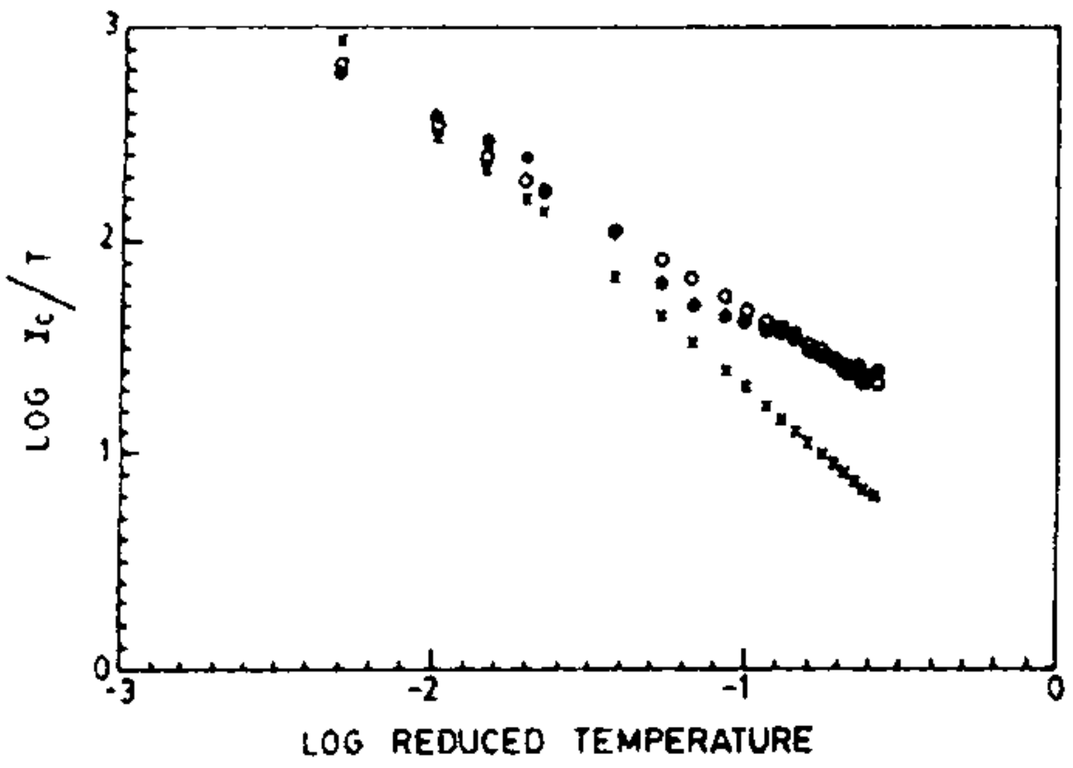

Figure 1. Intensity versus temperature (RUN I) ●–Experimental data points, × – Theo fit to $I_c/T = A_1 t^{-\gamma}$, o – Theo fit to $I_c/T = A_1 t^{-\gamma} + A_2 t^{-\gamma+\Delta}$.

very close to T_c may not show any evidence of corrections-to-scaling[5]. In figure 1, the fit with two correction terms is not shown, since, as seen from the tables, the improvement obtained with the second correction term $A_3 t^{-\gamma+2\Delta}$, is only marginal. It is also seen that the pure scaling term fits the data well much closer to T_c. Similar evidence of corrections-to-scaling terms in a binary liquid mixture has been found by A. Bourgou and D. Beysens[6].

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OROXINDIN, A RARE FLAVONE GLYCOSIDE FROM THE LEAVES OF *HOLMSKIOLDIA SANGUINEA*

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PLANTS belonging to the family Verbenaceae[1] are important from the point of view of medicinal properties[2,3] and the biosynthesis of rare and unusual flavonoids[1,4]. The medicinal uses of plants belonging to Verbenaceae may be attributed to their flavonoid content to some extent and hence an examination of the flavonoids of plants of this family has been considered worthwhile. As there is no record of any detailed chemical work on *Holmskioldia sanguinea*[5,6] (Verbenaceae), except the isolation of wogonin[7] from the aerial parts of this plant, a systematic study was undertaken and the results are presented here.

Shade-dried leaves of *H. sanguinea* (1 Kg) were extracted with hot 90% EtOH (3 × 3 l). The combined extract was concentrated *in vacuo* to 300 ml and the aq. concentrate was then extracted with petroleum ether (60–80), ether and ethyl acetate in succession.

The petroleum ether extract yielded a waxy solid, identified after chromatographic purification [Al_2O_3; petrol-benzene (1:4) eluate] as sitosterol, colourless needles (180 mg) from MeOH, m.p. 136–37°, $[\alpha]_D^{28} = -37°$ (c, 1.2, $CHCl_3$) (m.m.p., co-TLC and superimposable IR).

The ether extract yielded a yellow crystalline substance (120 mg), m.p. 202–204°. It gave pink colour with Mg-HCl and yellow colour with alkali. It was purple under UV and UV/NH_3 and had UV (λ_{max}): 275, 340 (MeOH), 292, 333 sh, 395 ($AlCl_3$), 283, 355 (NaOAc) 275, 340 (NaOAc/H_3BO_3) and 283, 375 nm (NaOMe); IR: 3200 (br), 1660, 1610, 1580, 1510, 1450, 1420, 1360, 1275, 1020, 840, 760, 680 and 660 cm^{-1}; 100 MHz PMR spectrum in DMSO-d_6: δ 12.52 (s, 1H, 5–O<u>H</u>), 7.9 – 8.2, centred at 8.0 (m, 2H, 2′ and 6′–<u>H</u>), 7.4–7.8, centred at 7.6 (m, 3H, 3′4′ and 5′–<u>H</u>), 7.01 (s, 1H, 3–<u>H</u>), 6.34 (s, 1H, 6–<u>H</u>) and 3.88 (s, 3H, 8–OC<u>H</u>$_3$); MS: (m/z) 284 (M^+, 65), 269 (M–CH_3, 100), 241(269–28, 42), 167 (A-ring fragment, 12), 139 (167–28, 70), 77 (B-ring fragment, 10%). With acetic anhydride and pyridine it yielded a diacetate, m.p. 150–52°, and identified as 5,7-dihydroxy-8-methoxyflavone (wogonin) which was confirmed by comparison (m.m.p., co-TLC, IR) with an authentic sample of wogonin.

Ethyl acetate extract gave a yellow substance which when crystallized from MeOH yielded yellow needles

(110 mg), m.p. 209–10° (Found: C, 57.20; H, 4.60. Calc. for $C_{22}H_{20}O_{11}$: C, 57.39; H, 4.34%). It gave yellow colour with alkali, pink colour with Mg-HCl, brown colour with Fe^{3+} and positive Molisch's test suggesting that it could be a flavonoid glycoside. It had $[\alpha]_D^{28} = -50°$ (c, 1.0. C_5H_5N); UV (λ_{max}): 275, 340 (MeOH), 280, 330 sh, 348, 390 sh ($AlCl_3$), 275, 340 (NaOAc), 275, 340 (NaOAc/H_3BO_3) and 280, 388 nm (NaOMe); IR: 3400 (br), 2925, 1720, 1640, 1600, 1480, 1250, 1100 (br), 1040, 940, 860, 780 and 695 cm^{-1}; PMR (90 MHz, DMSO-d_6): δ 11.4 (br s, 2H, COOH and 5-OH), 7.4–8.2, centred at 8.0 and 7.6 (pair of m, 5H, H–2′, 3′, 4′, 5′ and 6′), 6.9 (s, 1H, 3–H), 6.66(s, 1H, 6–H), 4.0 (s, 3H, OCH_3) and 3.6 (br s, H of sugar/water).

The glycoside showed high R_f in water, with marked decrease in 5% HOAc (typical of glucuronides)[8]. It resisted mild acid hydrolysis (1 N HCl, 1 hr). On refluxing with 2 N HCl for 3 hr, as well as when treated with the enzyme β-glucuronidase, it underwent hydrolysis and yielded an aglycone and D-glucuronic acid in equimolar ratio. The aglycone was identified as wogonin.

The glycoside on acetylation ($Ac_2O + C_5H_5N$, 28°, 24 hr) gave the lactone acetate, C_{28} H_{24} O_{13}, m.p. 189–90°, $[\alpha]_D^{28} = -96°$ ($CHCl_3$), PMR (90 MHz, $CDCl_3$): δ 7.3–8.0, centred at 7.9 and 7.5 (pair of m, 5H, H, 2′, 3′, 4′, 5′ and 6′), 6.9 (s, 1H, 3–H), 6.6 (s, 1H, 6–H), 5.74 (s, 1H, 1″–H), 5.52 (d, J = 4Hz, 1H, 2″–H), 5.0 (t, J = 4Hz, 1H, 4″–H) 4.4 (d, J = 4Hz, 1H, 5″–H), 4.02 (s, 3H, 8–OCH_3), 2.44 (s, 3H, 5–$OCOCH_3$), 2.24 and 2.18 (s, each, 3H each, 4″ and 2″–$OCOCH_3$); MS: (m/z) 526 (lactone acetate–$CH_2 = CO$), 326 (5-acetyl aglycone), 284 (aglycone), 269 (284–15), 255, 243, 167 (A-ring), 139 (167–28), 111 and 97.

Identical λ_{max} of the glycoside and its aglycone, as also the absence of any shift in the NaOAc spectrum of the glycoside, established the involvement of 7–OH in glycosylation[4,9]. The enzyme hydrolysis showed that the sugar was β-linked, while the PMR spectrum revealed its pyranoside structure. Thus the flavonoid has been characterized as 5-hydroxy-8-methoxy-7-O-β-D-glucopyranuronosylflavone (oroxindin). The identity was unequivocally established by direct comparison (m.m.p., co-TLC, IR) with an authentic sample of oroxindin obtained from *Oroxylum indicum*[10].

Oroxindin has been isolated for the first time from a plant belonging to the family Verbenaceae. Its earlier occurrence has been reported only in two other plants, *Oroxylum indicum*[10] (Bignoniaceae) and *Scutellaria galericulata*[11] (Labiatae). It is interesting to note that the flavonoid wogonin, devoid of any B-ring oxygenation (possessing unsubstituted B-ring) occurs along with its glycoside, oroxindin in *H. sanguinea*, a member of the Verbenaceae in the Tubiflorae, as unsubstituted B-ring flavones have been reported mostly in the Tubiflorae[4,11].

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INHIBITION OF ASCORBATE AUTOXIDATION BY HUMAN BLOOD

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HUMAN and rat blood sera have been shown to contain powerful protective factors against *in vitro* lipid auto-