FLAVONOIDS OF *VITEX NEGUNDO*

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Plants belonging to the genus *Vitex*, (Verbenaceae) are said to possess hormone-like properties (1). An insect molting hormone, 20-hydroxy ecdysone, was isolated from *Vitex megapotamica* (2). The earlier work on *Vitex negundo* reported the isolation of organic acids (3, 4), glucosides (5), essential oils, alkaloids (6–9) and flavonoids (10, 11) from leaves and β-sitosterol and *n*-alkanes (12) from the seeds of the plant. The leaves of the plant were reported to produce an antiarthritic effect (13).

In this study two new flavonoid glycosides were isolated from the stem bark of *Vitex negundo* (14).

EXPERIMENTAL

PLANT MATERIAL.—The plant was collected in April and May, 1977, at Jabalpur (Bhedaghat), Madhya Pradesh, India, and botanically identified by the Botany Department of the University of Jabalpur.

EXTRACTION AND ISOLATION OF THE FLAVONOIDS.—The air dried stem bark of *Vitex negundo* L. (5 kg) was extracted successively with petroleum ether, ethyl acetate, and methanol. The ethyl acetate extract was concentrated under reduced pressure and subjected to column chromatography on silica gel. The column was eluted with mixtures of benzene, chloroform, and methanol. The fraction eluted with chloroform-methanol (1:3) gave a mixture of two compounds, which, on repeated column chromatography on silica gel and elution with ethyl acetate gave compound A (**1**). Further elution of the column with ethyl acetate-methanol (1:1) gave compound B (**3**).

COMPOUND A (**1**).—Compound A, when crystallized from ethanol gave the following analytical data, mp 285° (dec). It gave a positive test for sugar and flavonoids. The ν max (KBr) of compound A showed peaks at (cm^{-1}) 3340 (OH) and 2885 (OCH_3). It did not give a bathochromic shift with $AlCl_3$, indicating a substituted 5-OH group. The absence of a signal at ca 13.0 ppm in the pmr spectrum of **1** confirmed the substitution at 5-OH. This signal was present in the spectrum of **2** (compound A after the removal of rhamnose by acid hydrolysis).

Anal. calcd for $C_{31}H_{38}O_{17}\cdot H_2O$: C, 53.23; H, 6.06. Found: C, 53.14; H, 5.71.

On acid hydrolysis, **1** yielded (−)-L-rhamnose. The pmr spectrum of the acetate of **1** ($CDCl_3$, 60 MHz, TMS internal standard) gave ppm 3.85 (*s*, 9H, 3 x $-OCH_3$), 3.80 (*s*, 3H, $-OCH_3$), 2.40 (*s*, 3H, $-COCH_3$, phenolic); seven alcoholic acetyl group signals appeared between 1.65–2.10 ppm indicating the presence of two sugar units. The signal for the 2"-O-acetyl group appeared in the region of 1.80–1.83 ppm (15–16). The other signals were at 1.1 ppm (*d*, 3H, J = 6.5 Hz, C-Me of rhamnose moiety), and 6.38 (*s*, 1H, H-3). On acid hydrolysis the rhamnose moiety was completely removed, and the acetate (**2a**) of the resulting compound (**2**) showed signals at ($CDCl_3$, TMS internal standard) ppm 3.85 (*s*, 9H, 3 x $-OCH_3$), 3.80 (*s*, 3H, $-OCH_3$), 2.39 (*s*, 3H, $-COCH_3$, phenolic), 7.0 (*s*, 2H, C-2', C-6'), 6.3 (*s*, 1H, H-3). Four alcoholic acetyl protons appeared at 1.65–2.10 ppm indicating that one glucosyl unit was still present in the compound. Five sugar protons appeared in the region of 4.8–5.2 ppm.

No sugar was released, even on prolonged boiling with acid, from compound (**2**) which confirmed the presence of a C-glycoside. Reductive cleavage of compound (**2**) with hydroiodic acid and phenol gave trimethoxy wogonin as confirmed by comparison of spectral shifts. Oxidation of (**2**) with $FeCl_3$ (17) gave D(+) glucose. The uv spectral shift studies showed that in (**2**) the 5-OH and 7-OH were free. The compound (**2**), after removal of rhamnose, gave a bathochromic shift with $AlCl_3$ and CH_3COONa. Glycosylation of the flavone caused a hypsochromic shift of 10 nm, which also confirmed a free 5-OH.

COMPOUND B.—The green colored compound B (**3**) had a mp 226–7° (dec). It showed a typical flavone carbonyl peak at 1645 cm^{-1} and an intense ester peak at 1740 cm^{-1} in the ir spectrum. It gave a bluish green color with alcoholic $AlCl_3$. It gave an uv absorption nm (log ϵ) λmax (EtOH), 285 (4.3), 340 (4.1); $+AlCl_3$, 287, 354. On acid hydrolysis, compound B gave glucose and the aglycone. The aglycone was identified as acerosin by comparison with an authentic sample (5,7,3'-trihydroxy 6,8,4'-trimethoxy flavone), the mixed mp gave no depression 241–42°; and by spectral studies. Acerosin gave a pink color with Mg-HCl, and a green color with alcoholic $AlCl_3$. The Gibb's

test was positive, and the gossypetone reaction was negative. On the addition of aqueous Na_2CO_3, the color changed from green to yellow. The uv absorption of the aglycone nm (log ϵ) λ max (EtOH), 280 (4.15), 345 (4.18); +$AlCl_3$, 300, 360; +NaOAc 285, 325; +NaOAc +H_3BO_3, 285, 330; νmax (KBr) 3500, 1665, 1614, 1587, 1570, 1490, 1462, 1424, 1388, 1352, 1300 (sh), 1278, 1220, 1192, 1140, 1113, 1058, 1019, 908, 877, 850, 828, 728, 782, 756, 732 cm^{-1}. Partial methylation of the aglycone gave 5-*O*-desmethyl nobiletin. The mixture mp 145° gave no depression.

Saponification of compound B with 1% KOH gave a compound which was identified as acerosin—5-O-glucoside, mp 260° (dec).

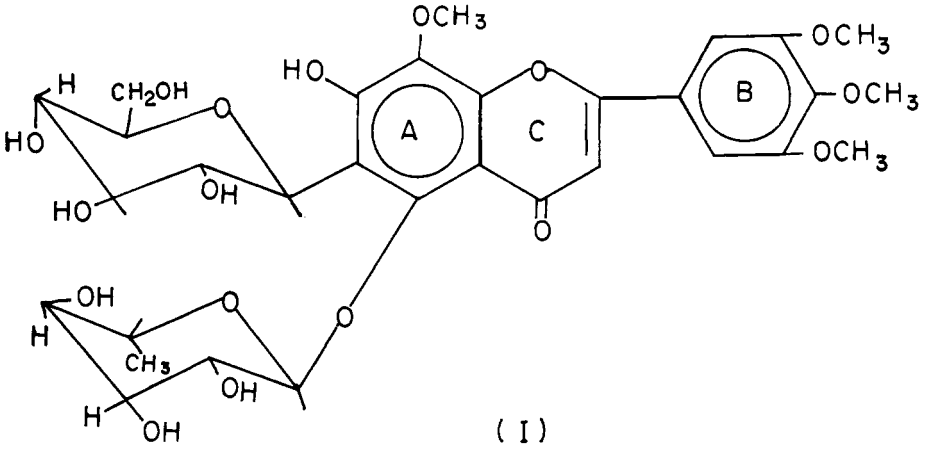
Anal. Calcd for $C_{35}H_{28}O_{18}$: C, 50.00; H, 5.07. Found: C, 50.40; H, 5.47.

DISCUSSION

The positive test for sugar and flavonoid showed that compound A is a flavonoid glycoside. The absence of a signal at about 13.0 ppm in the pmr spectrum of **1** confirmed the substitution at 5-OH. The signal was present in the spectrum of **2**. The pmr spectrum of acetylated C-glycosylflavonoid showed a signal for the 2″-*O*-acetyl group at 1.80–1.83 ppm, which is characteristic feature of 6-C-glucosylflavonoids (15–16).

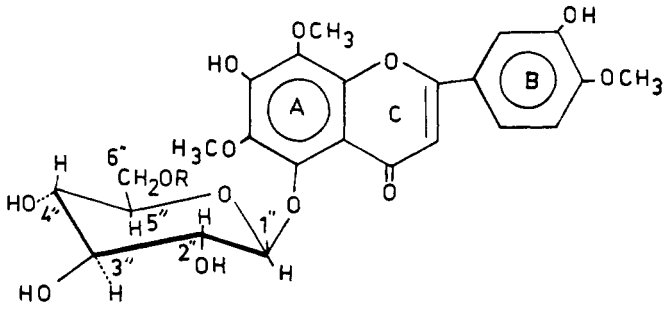
The rhamnose moiety was completely removed by acid hydrolysis, and the pmr spectrum of **2a** indicated that one glucose unit was still present in the compound. No sugar was released, even on prolonged boiling with acid, from compound **2** (the compound left after removal of rhamnose by acid hydrolysis); hence, the compound is a C-glycoside. Reductive cleavage of compound **2** afforded trimethoxy wogonin, and $FeCl_3$ oxidation gave D(+) glucose. Acid hydryolysis released rhamnose; hence, the 5-OH was substituted by rhamnose, and the C-glycoside released glucose. Thus compound A (**1**) is 6-C-glucosyl-5-*O*-rhamnopyranosyl trimethoxy wogonin.

In the case of compound B an intense peak at 1740 cm^{-1} indicated the presence of an ester group, which was confirmed by saponification. Saponification of compound B gave acerosin-5-*O*-glucoside. From the above observations, compound B was considered to be acerosin-5-*O*-glucoside-monoacetate. The position of the acetyl group on the glucose was found to be at C–6 as follows: Acetylation of one hydroxyl at C–2, C–3 or C–4 of the glucose in the nmr one proton shifted down field by 1.0–1.2 ppm, whereas substitution at C–6 shifted two protons down field by 0.5 ppm.



(I)

(FIGURE 1)



(a) R = Ac

(b) R = H

(3)

(FIGURE 3)

In the case of the pmr spectrum of the acetate of compound B (a), two protons were shifted from 3.7 to 4.2 ppm, hence the compound B has the structure (**3**).

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