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Chemical Constituents of Gentianaceae XXVIII: Flavonoids of *Enicostemma hyssopifolium* (Willd.) Verd.

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Abstract □ The whole plant of *Enicostemma hyssopifolium* (Willd.) Verd. (Gentianaceae) was collected at different growth stages and was shown to contain seven flavonoids: apigenin (I), genkwanin (II), isovitexin (III), swertisin (IV), saponarin (V), 5-*O*-glucosylswertisin (VI), and 5-*O*-glucosylisoswertisin (VII). Compounds VI and VII previously were unreported in nature. The yields of the flavonoids varied with the growth stage. The biochemical and chemotaxonomic significance of these results is appraised.

Keyphrases □ Flavonoids—isolation from *Enicostemma hyssopifolium*, identification □ Medicinal plants—isolation of flavonoids from *Enicostemma hyssopifolium*, identification □ Gentianaceae—isolation of flavonoids from *Enicostemma hyssopifolium*, identification □ *Enicostemma hyssopifolium*—isolation and identification of flavonoids

The genus *Enicostemma* (Gentianaceae) is monotypic; the only recorded species is *E. hyssopifolium* (Willd.) Verd. (synonymous with *E. littorale* Blume) (1). The plant is found throughout India up to 450 m (1500 ft). Extracts of the plant are used in Indian medicine to treat cardiac dropsy, rheumatism, and certain mental disorders. Significant antipsychotic (3), anti-inflammatory (4), and anthelmintic (5) activities were reported for its major alkaloid, gentianine; the corresponding heteroside, swertiamarin, was reported to produce central nervous system depressant (6) and cardiostimulant (7) activity.

The chemicals previously reported in this species were monoterpene alkaloids (8–12), heterosides (6, 7, 10, 13, 14), and a triterpene (14). This paper describes the isolation and characterization of the flavonoids of this species. The biochemical significance of the chemical constituent changes during vegetation is appraised in light of its separation as a monotypic genus.

EXPERIMENTAL[1]

All solutions were dried with anhydrous sodium sulfate. Silica gel[2] (60–120 mesh) was used for column chromatography. TLC was performed on silica gel[3] with chloroform–acetic acid (98:2, Solvent 1) and *n*-butanol–acetic acid–water (4:1:2, Solvent 2). Iodine vapor and ferric chloride solution were used for staining. Glucose was detected by partition paper chromatography[4] using sodium metaperiodate–benzidine reagent for staining (15).

Dried and milled whole plants of *E. hyssopifolium*[5] at the flowering stage (5.2 kg) were extracted continuously with light petroleum ether (bp 60–80°) in a soxhlet apparatus for 30 hr. The defatted plant material was extracted with ethanol for 30 hr. The petroleum ether extract contained alkanes and alkanols and was not processed further. The alcoholic extract was concentrated under reduced pressure and processed as shown in Scheme I.

The relative percent yields of the individual compounds at various stages of plant development are given in Table I. Determination of the mixed minor entities was accomplished by preparative layer chromatography of mixture extracts and absorptiometry of the layers after the components in these layers were identified by the usual methods.

Treatment of Fraction A—Fraction A was dissolved in 250 ml of hot ethyl acetate, the solution was concentrated, and a yellow solid (14.2 g) was separated. The solid showed two major spots on TLC at R_f 0.68 and 0.74 (Solvent 2). The two components were separated by fractional crystallization from methanol.

Swertisin (IV)—The sparingly methanol-soluble solid crystallized from methanol–dioxane as cream-colored crystals (7.65 g), mp 240–242°; $[\alpha]_D^{24}$ −9° (c 0.33, pyridine); UV: λ_{max} (ethanol) 272 (log ϵ 4.28) and 334 (4.38) nm; IR: ν_{max} 3360, 1668, and 1610 cm^{-1}; NMR (deuterodimethyl sulfoxide): δ 7.88 (2H, d, J = 9 Hz, H-2′,6′), 6.80 (2H, d, J = 9 Hz, H-3′,5′), 6.54 (1H, s, H-8), 6.50 (1H, s, H-3), 4.78 (1H, broad, glucosyl H-1), and 3.98 (3H, methoxyl). The hexaacetate, formed with acetic anhydride and pyridine under reflux, crystallized from alcohol as colorless needles, mp 150–151°; mass spectrum: *m/e* 698 (M$^+$, relative intensity 8%), 656 (22), 639 (12), 597 (20), 537 (12), 463 (80), 421 (98), 313 (100), 297 (60), and 139 (20).

Methylation with ethereal diazomethane gave swertisin-4′,5-di-*O*-methyl ether, mp 298–300°. Acetylation of the di-*O*-methyl ether with acetic anhydride and pyridine under reflux afforded swertisin-di-*O*-methyl ether tetraacetate, mp 128–129°; mass spectrum: *m/e* 642 (M$^+$, 5%), 583 (100), 582 (22), 523 (8), 341 (55), 325 (62), 294 (5), and 139 (5). The physical and spectral properties of the parent compound and its derivatives were indistinguishable from those of swertisin (16, 17).

Isovitexin (III)—After separation of swertisin, the methanol mother liquor was concentrated and diluted with acetone. It was kept at ordinary temperature overnight, and a yellow solid precipitated. Repeated crystallization of the solid from acetone–methanol gave isovitexin (18, 19) as yellow needles (6.4 g), mp 229–230°; $[\alpha]_D^{28}$ +15.4° (c 0.37, ethanol); UV: λ_{max} (ethanol) 272 (log ϵ 4.45) and 338 (4.42) nm; IR: ν_{max} 3300 (broad), 1662, 1628, 1600, and 1588 cm^{-1}; NMR (deuterodimethyl sulfoxide): δ 7.95 (2H, d, J = 9 Hz, H-2′,6′), 6.95 (2H, d, J = 9 Hz, H-3′,5′), 6.72 (1H, s, H-3), 6.54 (1H, s, H-8), and 4.70 (1H, broad, glucosyl H-1); mass spectrum: *m/e* 414 (M − 18, 20%), 396 (8.5), 378 (14), 295 (18), 283 (100), and 165 (20). The 4′,5,7-tri-*O*-methyl ether tetraacetate, prepared as de-

[1] Melting points were taken on a Kofler block in open capillaries and are uncorrected. UV spectra were determined on a Cary model 14 recording spectrophotometer. IR spectra were taken in Nujol using a Perkin-Elmer model 337 spectrophotometer, and only the major bands are quoted. ^{1}H-NMR spectra were recorded on an XL 100 instrument with tetramethylsilane as the internal standard. Mass spectra were determined with an MS-9 or MS-50 spectrometer at 70 ev.
[2] British Drug Houses, Poole, England.
[3] Silica gel G, E. Merck, Darmstadt, West Germany.
[4] Whatman No. 50.
[5] The plants were collected from the Banaras Hindu University Campus area 12 times during June and August of 1973–1976. Voucher specimens have been preserved at the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University.

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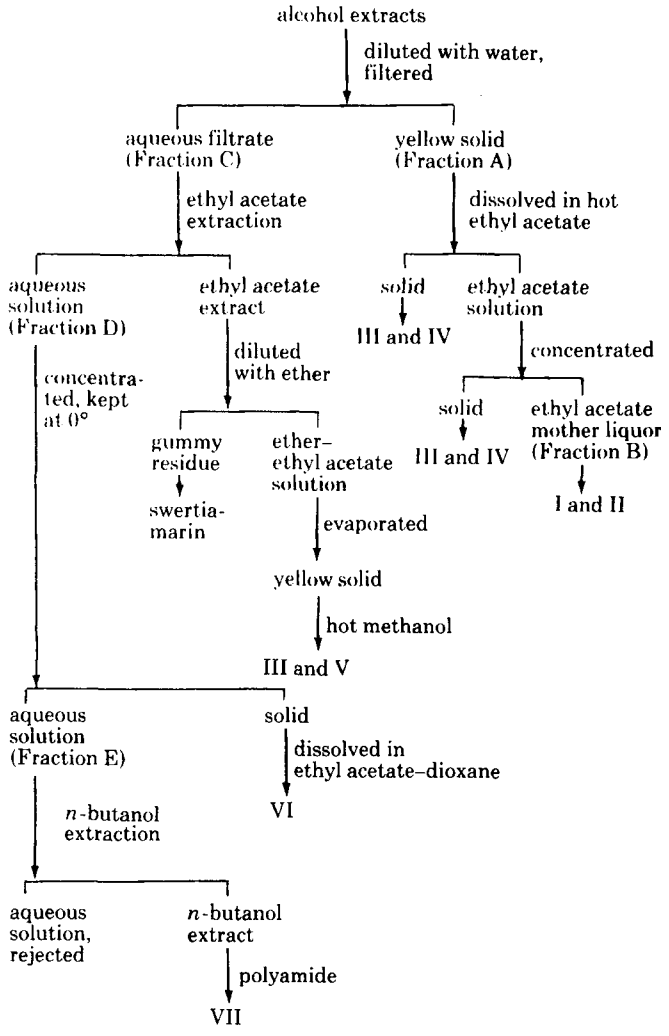


Scheme I—Isolation of flavonoids of E. hyssopifolium

scribed, was identified by melting-point, mixed melting-point, and co-TLC analyses as the di-*O*-methyltetraacetate derivative of IV.

Treatment of Fraction B—The ethyl acetate mother liquor was evaporated. The residue was dissolved in chloroform and chromatographed over silica gel (30 × 3 cm). Elution was carried out with benzene, benzene–chloroform (1:1), and chloroform (1 liter each), and 100-ml fractions were collected.

Genkwanin (II)—On evaporation, the middle benzene–chloroform eluates (fractions 14–17) afforded genkwanin (20) as yellow needles (0.37 g), mp 285–286°; R_f 0.48 (Solvent 1); UV: λ_{max} (ethanol) 268 (log ϵ 4.40) and 337 (4.47) nm; IR: ν_{max} 3250, 1660, 1600, 1592, and 1498 cm^{-1}; mass spectrum: *m/e* 284 (M^+, 100%), 256 (5), 255 (22), 254 (5), 167 (18), 166 (19), 138 (10), 128 (20), 118 (12), and 95 (11). The diacetate, prepared with acetic anhydride and pyridine under reflux, crystallized from ethanol as colorless needles, mp 195–198°. Correspondence of these properties with those reported previously (20–22) established the identity as II.

Apigenin (I)—The chloroform eluates were combined and evaporated. The residue crystallized from aqueous pyridine to give apigenin as yellow needles (0.46 g), mp 340–343° dec.; R_f 0.3 (Solvent 1); UV: λ_{max} (ethanol) 265 (log ϵ 4.44) and 336 (4.48) nm. 4′,5,7-Tri-*O*-methyl ether, prepared with dimethyl sulfate and potassium carbonate in anhydrous acetone under reflux for 40 hr, crystallized from ethanol as light-yellow needles, mp 153°; NMR (deuterochloroform): δ 7.91 (2H, d, J = 9 Hz, H-2′,6′), 6.94 (2H, d, J = 9 Hz, H-3′,5′), 6.70 (1H, s, H-3), 6.48 (1H, d, J = 3 Hz, H-8), 6.22 (1H, d, J = 3 Hz, H-6), and 4.0–3.98 (9H, methoxyl protons); mass spectrum: *m/e* 312 (M^+, 100%), 297 (22), 295 (10), 269 (22), and 254 (11). The melting point and spectral properties of the flavone and its permethyl ether were indistinguishable from those reported for apigenin (20, 21).

Treatment of Fraction C and Isolation of Saponarin (V)—The aqueous filtrate was extracted with ethyl acetate (5 × 1 liter), and the ethyl acetate extract was worked up in the usual fashion to give a brown concentrate (200 ml). It was diluted with 200 ml of ether, and swertiamarin (6) was precipitated as a brown gum. The ethyl acetate–ether mother liquor was evaporated to give a dull-yellow solid (0.28 g), which showed two major spots on TLC at R_f 0.42 and 0.7 (Solvent 2). Trituration of the residue with hot methanol gave 0.1 g of swertisin as the sparingly methanol-soluble solid.

Table I—Flavonoids of *E. hyssopifolium* during Growth [a] (Relative Percent Yield)

Flavone	2–3 Weeks Old	6–8 Weeks Old	Flowering Stage	Fruiting Stage
I	44	20	3	5
II	32	28	2	4
III	–	12	35	–
IV	–	7	42	–
V	–	14	+	8
VI	–	+	5	40
VII	–	–	+	24
Unidentified phenolic constituents	24	17	12	19
Yield, %	0.08	0.22	0.37	0.30

[a] n = 3; + denotes 1–2%; – denotes absence.

After separation of swertisin, the methanol mother liquor was evaporated to give a hygroscopic solid (0.11 g), mp 228–231°; R_f 0.4 (Solvent 2); $[\alpha]_D^{28}$ −8.5° (c 0.45, water) and −73.4° (c 0.55, pyridine); UV: λ_{max} (ethanol) 270 and 335 nm; NMR (deuterodimethyl sulfoxide): δ 7.95 (2H, d, J = 8 Hz, H-2′,6′), 6.98 (2H, d, J = 8 Hz, H-3′,5′), 6.92 (1H, s, H-8), 6.68 (1H, s, H-3), 4.95 (1H, broad, *O*-glucosyl H-1), and 4.8 (1H, d, J = 10 Hz, C-glucosyl H-1). Treatment with acetic anhydride and pyridine afforded a decaacetate, which crystallized from hexane–methylene chloride as a glassy solid, mp 142–144°. The physical and spectral properties of the compound and its acetate derivative were identical to those reported for saponarin (20, 23).

Treatment of Fraction D—The aqueous mother liquor was concentrated at 30° under reduced pressure to about 25 ml and kept at 0° for ~2 weeks, during which time a light-yellow solid precipitated. The solid was collected by filtration and dissolved in ethyl acetate–dioxane.

Swertisin-5-O-β-D-glucoside (VI)—On concentration and cooling, the ethyl acetate–dioxane solution gave an amorphous solid, which crystallized from methanol–dioxane as ivory-colored needles (0.93 g), mp 200–202°; R_f 0.32 (Solvent 2); $[\alpha]_D^{28}$ −77° (c 0.73, pyridine); UV: λ_{max} (ethanol) 271 (log ϵ 4.26) and 335 (4.30) nm; UV: λ_{max} (ethanol–0.2% sodium acetate) 262 sh, 268, and 388 nm; IR: ν_{max} 3300 (broad), 1668, 1662, 1630, 1612, 1295, 1255, 1020, and 838 cm^{-1}; NMR (deuterodimethyl sulfoxide): δ 7.98 (2H, d, J = 8.5 Hz, H-2′,6′), 6.95 (2H, d, J = 8.5 Hz, H-3′,5′), 6.82 (1H, s, H-8), 6.38 (1H, s, H-3), 5.0 (1H, broad, *O*-glucosyl H-1), 4.80 (1H, d, J = 10 Hz, C-glucosyl H-1), 3.94 (3H, methoxyl), and 3.35 (complex multiplet, 12 glucosyl protons plus water); ^{13}C-NMR (deuterodimethyl sulfoxide): δ 161.40 (C-2), 103.07 (C-3), 182.30 (C-4), 156.90 (C-5), 90.88 (C-6), 161.42 (C-7), 104.8 (C-8), 159.86 (C-9), 109.57 (C-10), 121.08 (C-1′), 128.46 (C-2′), 116.08 (C-3′), 164.05 (C-4′), 116.02 (C-5′), 128.46 (C-6′), 56.28 (methoxyl-C), and 56.0–100.5 (12 lines, C,*O*-glucosyl-C) (24).

Anal.—Calc. for $C_{28}H_{32}O_{15}\cdot H_2O$: C, 53.67; H, 5.43. Found: C, 52.99; H, 5.86.

Hydrolysis of VI with Emulsin—Compound VI, 52 mg, was dissolved in 20 ml of pH 5 aqueous buffer (0.5 *M* sodium acetate adjusted to pH 5 with acetic acid) to which 20 mg of powdered emulsin[6] was added. The mixture was kept at ambient temperature overnight. The reaction product was concentrated and subjected to paper chromatography[7] along with glucose, rhamnose, and glucuronic acid. Only glucose was detected as the sugar component in the hydrolyzed product. The aglucone was extracted with ethyl acetate, the solvent was removed, and the residue was crystallized from methanol–dioxane. Direct comparison of the aglucone by melting-point, mixed melting-point, and co-TLC analyses with swertisin established that they were identical.

Methylation of VI—Compound VI, 48 mg, was methylated by repeated addition of ethereal diazomethane daily for 7 days. After the usual workup, the methyl ether was obtained as a light-yellow solid.

[6] Sigma Corp.
[7] Whatman No. 1.

Isovitexin-4′,7-di-O-methyl Ether—The methyl ether of VI was dissolved in 20 ml of 10% aqueous acetic acid and kept at ambient temperature overnight. The solvent was removed under high vacuum, and a straw-colored residue was obtained. It was triturated with hot methanol. The methanol solution was concentrated and afforded 38 mg of a microcrystalline solid, mp 268–270°; R_f 0.42 (Solvent 2); UV: λ_{max} (ethanol) 268 (log ϵ 4.28) and 324 (4.36) nm; UV: λ_{max} (ethanol–aluminum chloride) 278, 300, and 345 nm (indicating the presence of a C_5-OH group); mass spectrum: *m/e* 460 (M^+, 3%), 445 (7), 442 (100), 431 (7), 430 (8), 422 (3), 417 (5), 311 (34), 296 (17), 281 (24), and 179 (7). The physical and spectral properties of the compound suggested its identity as isovitexin-4′,7-di-*O*-methyl ether.

Isovitexin-4′,5,7-tri-O-methyl Ether Tetraacetate—Further methylation of isovitexin-4′,7-di-*O*-methyl ether with excess ethereal diazomethane followed by acetylation with acetic anhydride and pyridine on a steam bath for 4 hr afforded isovitexin-tri-*O*-methyl ether tetraacetate, mp 127–129° (25); mass spectrum: *m/e* 642 (M^+, 3%), 583 (100), 541 (2), 523 (10), 481 (8), 463 (18), 421 (12), 408 (2), 341 (68), 339 (44), 31 (22), 294 (2), 179 (2), and 139 (25).

Permethylation of VI—The diglucoside, 24 mg, in 5 ml of *N,N*-dimethylformamide was treated with 1 ml of methyl iodide and 60 mg of sodium hydride under nitrogen. After 1 hr at ambient temperature, the reaction mixture was diluted with 50 ml of water and extracted with chloroform. The chloroform layer was worked up in the usual fashion, and the chloroform concentrate was subjected to preparative layer chromatography with Solvent 1 as the developer. The blue fluorescent zone at R_f 0.5 under a shortwave UV lamp was cut out and eluted with chloroform. Evaporation of the solvent from the chloroform solution gave a pale-yellow gum, which crystallized from benzene as yellow needles, mp 168–171°; mass spectrum (no M^+ was observed, only fragment ions were recorded): *m/e* 516 (relative intensity 12%), 501 (22), 485 (20), 469 (8), 341 (100), 311 (5), 101 (55), and 88 (47).

Treatment of Fraction E—Fraction E was extracted with *n*-butanol (4 × 250 ml). The residue from the butanol extract was dissolved in ethyl acetate and subjected to preparative layer chromatography on polyamide[8] plates (20 × 20 cm) with methanol–acetic acid–water (90:5:5) as the developer.

Isoswertisin-5-O-β-D-glucoside (VII)—The zone at R_f 0.4 was eluted with methanol, and the solvent was evaporated. The residue crystallized from methanol–dioxane as light-yellow microcrystals (0.1 g), mp 212–215°; R_f 0.37 (Solvent 2); $[\alpha]_D^{28}$ −91° (c 0.51, pyridine); UV: λ_{max} (ethanol) 270 (log ϵ 4.23) and 336 (4.26) nm; UV: λ_{max} (ethanol–0.2% sodium acetate) 262 sh, 268, and 390–395 nm; IR: ν_{max} 3300 (broad), 1665, 1610, 1502, 1362, 1295, 1258, 1212, 1196, 1035, and 842 cm^{-1}; NMR (deuterodimethyl sulfoxide): δ 7.92 (2H, d, J = 8.5 Hz, H-2′,6′), 6.95 (2H, d, J = 8.5 Hz, H-3′,5′), 6.34 (1H, s, H-6/H-3), 6.32 (1H, s, H-3/H-6), 5.0 (1H, broad, *O*-glucosyl H-1), 4.78 (1H, d, J = 10 Hz, C-glucosyl H-1), 3.92 (3H, methoxyl), and 3.50 (complex multiplet, 12 glucosyl protons plus water).

Anal.—Calc. for $C_{28}H_{32}O_{15}\cdot H_2O$: C, 53.67; H, 5.43. Found: C, 53.18; H, 5.82.

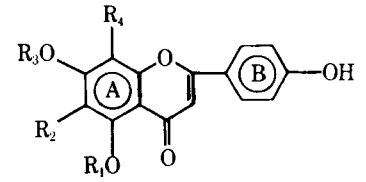
Hydrolysis of VII—Compound VII, 23 mg, was hydrolyzed with 10 mg of emulsin as described when glucose was detected as the only glucone moiety present. The aglucone, mp 295–298°, showed UV: λ_{max} (ethanol) 272 (log ϵ 4.17) and 335 (4.30) nm, identical to that reported for isoswertisin (17). It afforded a hexaacetate, mp 128–131°.

Methylation of VII—Compound VII, 22 mg, was methylated with excess ethereal diazomethane. After the usual workup, the methyl ether was obtained as an amorphous solid (21 mg).

Vitexin-4′,7-di-O-methyl Ether—The methyl ether of VII was hydrolyzed with acetic acid as described, and vitexin-4′,7-di-*O*-methyl ether was obtained as yellow microcrystals (9 mg), mp 260–262°; UV: λ_{max} (ethanol) 270 (log ϵ 4.27) and 324 (4.22) nm; UV: λ_{max} (ethanol–aluminum chloride) 277, 300, 345, and 383 nm.

Vitexin-4′,5,7-tri-O-methyl Ether Tetraacetate—Further methylation of vitexin-4′,7-di-*O*-methyl ether with ethereal diazomethane and subsequent acetylation afforded vitexin-4′,5,7-tri-*O*-methyl ether tetraacetate (26), mp 220–221°; mass spectrum: *m/e* 642 (M^+, 100%), 583 (8), 541 (12), 523 (18), 481 (5), 463 (4), 341 (35), 339 (11), 311 (28), 294 (6), 179 (5), and 139 (10). The mass fragmentation data of this compound are consistent with those reported for vitexin-4′,5,7-tri-*O*-methyl ether tetraacetate (16).

Permethylation of VII—Compound VII was permethylated as described for VI. The derivative crystallized from acetone–benzene as a light-brown solid, mp 122–125°; mass spectrum (no M^+ was observed, only fragment ions were recorded): *m/e* 516 (relative intensity 80%), 486 (5), 341 (100), 327 (22), 311 (8), 101 (70), and 88 (68). The mass spectral data were consistent with those reported (27) for the permethyl ether of vitexin-5-*O*-glucoside.



I: $R_1 = R_2 = R_3 = R_4 = H$
II: $R_1 = R_2 = R_4 = H$, $R_3 = CH_3$
III: $R_1 = R_3 = R_4 = H$, R_2 = glucosyl
IV: $R_1 = R_4 = H$, R_2 = glucosyl, $R_3 = CH_3$
V: $R_1 = R_4 = H$, $R_2 = R_3$ = glucosyl
VI: $R_1 = R_2$ = glucosyl, $R_3 = CH_3$, $R_4 = H$
VII: $R_1 = R_4$ = glucosyl, $R_3 = CH_3$, $R_2 = H$

RESULTS AND DISCUSSION

Seven flavonoids (22) were isolated from the whole plants of *E. hyssopifolium* at different vegetation periods by solvent extraction and column and preparative layer chromatography. These compounds, apigenin (I), gerkwanin (II), isovitexin (III), swertisin (IV), saponarin (V), swertisin-5-*O*-β-D-glucoside (VI), and isoswertisin-5-*O*-β-D-glucoside (VII), were characterized by chemical transformation and the UV, IR, NMR, and mass spectra of the compounds and their methyl ether and acetate derivatives. The structural elucidation of the two new compounds (VI and VII) that were not encountered before in nature or prepared synthetically is described.

Flavonoid VI—Compound VI ($C_{28}H_{32}O_{15}\cdot H_2O$), mp 200–202°, showed UV absorption maxima in neutral ethanol and in the presence of the usual shift reagents (21), characteristic of 4′,5,7-trioxygenated flavones with a free 4′-hydroxyl group (21). The 100-MHz PMR spectrum of the compound in deuterodimethyl sulfoxide exhibited signals due to 12 glucosyl protons, one aromatic methoxyl proton, two glucose anomeric protons, and six aromatic protons ascribable to C-3, C-8, and C-2′,3′,5′,6′-H (21). Its ^{13}C-NMR spectrum, interpreted on the basis of two recent reports of the ^{13}C-spectra of hydroxy–methoxy flavones (27, 28), also was consistent with this assignment.

On hydrolysis with emulsin, the compound gave IV and glucose. Methylation with ethereal diazomethane and subsequent acid hydrolysis afforded isovitexin-4′,7-di-*O*-methyl ether, which showed a mass spectrum consistent with this structure (16, 18). Likewise, the mass spectrum of the permethyl ether of the parent compound produced fragment ion peaks characteristic of 6-C,*O*-diglucosyl flavones (29). Based on these observations, the flavonoid was identified as 5-*O*-β-D-glucopyranosyl-6-C-β-D-glucopyranosyl genkwanin (VI).

Flavonoid VII—Compound VII ($C_{28}H_{32}O_{15}\cdot H_2O$), mp 212–215°, showed a close similarity in its UV absorption spectra in neutral ethanol and in the presence of the usual shift reagents (21) with those of VI. In the 100-MHz PMR spectrum, a significant difference from that of VI was discernible in the A-ring proton singlet, which appeared near the signal due to C_3-H. Therefore, the A-ring proton was assigned to C_6-H. The downfield shift was due to the glucosyloxy function at C-5. On hydrolysis with emulsin, it afforded isoswertisin and glucose. Methylation with ethereal diazomethane followed by acid hydrolysis under mild conditions yielded vitexin-4′,7-di-*O*-methyl ether. The permethyl ether of the parent compound produced fragment ion peaks in its mass spectrum that are characteristic of 8-C,*O*-diglucoside flavones (29). Based on these observations, the compound was identified as 5-*O*-β-D-glucopyranosyl-8-C-β-D-glucopyranosyl genkwanin (VII).

The glycoflavones (III–VII) were absent in the seedling stage (2–3 weeks) of the plant. At this stage, only the free flavones (I and II) were detected in the roots, stalks, and leaves of *E. hyssopifolium*. From the onset of maturity (6–8 weeks), biosynthesis of the C-glycoflavones (III and IV) started. At the flowering stage, the free flavones were largely replaced by these entities. At the fruiting stage, III and IV were represented by the C,*O*-diglycoflavones (V–VII). However, at this stage, I and II reappeared as minor entities.

Since previous results of biosynthetic experiments suggested that C-glycosylation of flavones takes place at the chalcone stage (30), it seemed plausible that elaboration of the free flavones (I and II) in *E.*

[8] Powder D, Riedel, Haen AG.

hyssopifolium was interrupted during the early growth of its stalk and leaves and substituted by the synthesis of the C-glycoflavones (III and IV). Toward the end of vegetation, synthesis of the free flavones revived, while the C-glycoflavones were further *O*-glycosylated to afford V–VII. This time lag in the *O*-glycosylation is surprising because *O*-glycosides are less toxic to plants than the free phenols (31); the reaction, therefore, was expected at an early stage of plant growth but was not observed in *E. hyssopifolium*. However, a similar ontogenic variation in the content of free xanthones and of xanthone-*O*-glycosides was reported recently in *Swertia angustifolia* (32).

Another noteworthy feature of *E. hyssopifolium*, whose certain chemical characters, *e.g.*, monoterpene alkaloids, heterosides, and flavonoids, are similar to those of *Gentiana* species (12), is the apparent complete absence of xanthones. Xanthones occur almost ubiquitously in *Gentiana* species (33, 34). From the chemotaxonomic point of view, this observation is significant since *E. hyssopifolium* was known previously as *Gentiana verticillata* Linn. but later was separated from the *Gentiana* genus on other taxonomic grounds (1). The chemical results reported in this paper support this separation of *E. hyssopifolium* as a monotypic species.

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