Flavonoids from *Vernonia fasciculata* Michx. Isolation of Genkwanin and a New Flavone Disaccharide, Fasciculatin

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Genkwanin(4',5-dihydroxy-7-methoxyflavone) and a new flavone disaccharide, 4'-*O*-(2-*O*-rhamnosylglucosyl)-genkwanin, have been isolated from a chloroform extract of the leaves of *Vernonia fasciculata* Michx.

SIGNIFICANT *in vitro* activity of extracts of *Vernonia* amygdalina and *V.* hymenolepsis against cells derived from human carcinoma of the nasopharynx has been found by Kupchan and his co-workers.[1-3] During a search for physiologically active compounds from *V. fasciculata* Michx.,[4] a rare flavone, genkwanin, and a new flavone disaccharide, fasciculatin, have been isolated and characterized as follows.

Fasciculatin (1), a non-crystalline, yellow solid, $C_{28}H_{32}O_4$, exhibited colourations with magnesium–hydrochloric acid and iron(III) chloride and u.v. data (Table 1)

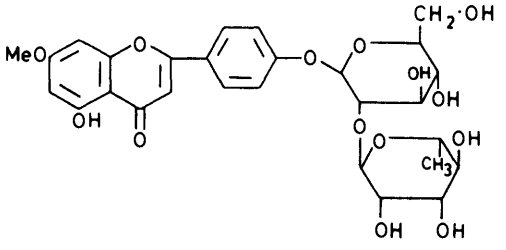
TABLE 1

U.v. data of fasciculatin

Shift reagent	Band I $\lambda_{max.}$/nm	Band II $\lambda_{max.}$/nm a	Band II $\lambda_{max.}$/nm b
EtOH	271	340	405sh
EtOH–NaOMe	278	355sh	394
EtOH–NaOAc	269	345	403sh
EtOH–$AlCl_3$	278	304 352	395sh
EtOH–$AlCl_3$–HCl	277	303 346	392sh

which suggested it to be a flavonoid glycoside.[5-7] Its glycosidic nature was confirmed by a positive Molisch test.

Paper chromatography of the aqueous portion of the acidic hydrolysate of fasciculatin against glucose, rhamnose, fructose, sucrose, lactose, D-ribose, D-mannose, sorbose, xylose, D-galactose, and various mixtures of these demonstrated the presence of rhamnose and



(1)

glucose in the sugar portion of the glycoside. The ethereal layer afforded an aglycone whose mass spectrum had a molecular ion at *m/e* 284 and whose u.v. spectrum in ethanol was typical of a flavone.[5]

^{1}H N.m.r. data are given in Table 2. The absence of a typical low-field singlet for H-2 of ring C at δ 8.85—8.70 excluded the possibility of fasciculation being an isoflavonoid.[5] A doublet at δ 1.30 is typical of the rhamnose methyl group.[5] The broad singlet at δ 3.84 (21 H)

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[1] S. M. Kupchan, R. J. Hemingway, D. Werner, and A. Karim, *J. Org. Chem.*, 1969, **34**, 3903.

[2] S. M. Kupchan, R. J. Hemingway, A. Karim, and D. Werner, *J. Org. Chem.*, 1969, **34**, 3908.

[3] S. M. Kupchan, R. J. Hemingway, D. Werner, A. Karim, A. T. McPhail, and G. A. Sim, *J. Amer. Chem. Soc.*, 1968, **90**, 3596.

[4] N. K. Narain, *Canad. J. Pharm. Sci.*, in the press.

[5] T. Mabry, K. R. Markham, and M. B. Thomas, 'The Systematic Identification of Flavonoids,' Springer-Verlag, New York, 1970.

[6] M. Shabbir and A. Zaman, *Tetrahedron*, 1970, **26**, 5041.

[7] L. Quijano, F. Malanco, and T. Rios, *Tetrahedron*, 1970, **26**, 2851.

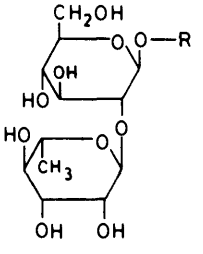
which overlapped with the methoxy-singlet at δ 4.0 was also taken as due to the sugar portion. The nature of the sugar–sugar linkage was deduced by comparison of the rhamnose methyl signal with the corresponding

TABLE 2

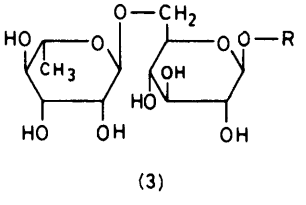
60 MHz ^{1}H N.m.r. spectra of fasciculatin in $(CD_3)_2SO$

δ Multiplicity	J/Hz	Intensity	Assignment
1.3 (d)	12	3 H	Rhamnose CH_3
3.8br (s)		21 H	Sugars
4.0br (s)		3 H	OCH_3
6.3 (d)	2.5	1 H	H-6
6.5 (s)	2	2 H	H-3, H-8
7.6 (d)	8.5	2 H	H-3′, H-5′
7.8 (d)	8.5	2 H	H-2′, H-6′

signals of neohesperidoside (2) [δ 1.2 (d)] and rutinoside [5,8,9] (3) [δ 0.80—0.95br (s)] to be of the neohesperidoside type.



(2)



(3)

The arrangement of methoxy-, hydroxy-, and sugar substituents on the aglycone was determined from u.v. data. Fasciculatin exhibited considerable shifts (Table 1) in the presence of aluminium chloride, indicating that the hydroxy-group is at C-5.[5,10] In contrast, a small bathochromic shift occurred with sodium acetate, indicating the absence of a free hydroxy-group at the 7-position in ring A. The considerable bathochromic shifts of bands I and IIa in the presence of sodium ethoxide indicate that C-4 carries either a free OH group or an OR group in which R is easily displaced by ethoxide.

The i.r. spectrum had bands at 3 375 (OH) and 1 650 and 1 600 cm^{-1} (chromone), similar to literature values of 3 400, 1 650, and 1 600 cm^{-1} for 4′-*O*-galactosyl genkwanin [11] and 3 400 and 1 655 cm^{-1} for glucose attached to a flavone.[10]

In view of the substituents present, only two aglycones are likely, acacetin (4′-methoxy-5,7-dihydroxyflavone) and genkwanin (4′,5-dihydroxy-7-methoxyflavone); the mass spectral fragmentation pattern is most consistent with genkwanin, as can be seen by comparing retro-Diels–Alder fragments [12,13] of genkwanin and acacetin. The aglycone was further identified unambiguously by direct comparison (m.p. and mixed m.p. and R_F) with those of authentic genkwanin. Confirmation of the 1,2-linkage in the disaccharide came from the mass spectrum [14] of the perdeuteriomethylated derivative [15] of fasciculatin, which showed typical peaks at *m/e* 712, 411, 301, 274, 214, and 198.

EXPERIMENTAL

Plant Material.—The plant material was collected near Sandwich, Illinois, during September and October 1970, 1971, and 1972. The plant was air-dried and ground in Wiley hand and electric mills equipped with a 2 mm pore screen.

General.—M.p.s were taken with a Fisher-Johns apparatus. I.r. spectra were recorded with a Beckman IR-8 or IR-12 or a Perkin-Elmer 237B spectrophotometer, for KBr pellets or melts, unless otherwise indicated. N.m.r. spectra were taken with a Varian A-60 or JEOL 100 MHz instrument, for solutions in $(CD_3)_2SO$ with Me_4Si as internal standard. U.v. spectra were measured for solutions in MeOH or EtOH with a Cary 14 or 15 or a Perkin-Elmer 202 spectrophotometer. Mass spectra were obtained with a Perkin-Elmer–Hitachi RMU-6E spectrometer at 70 eV.

Merck silica gel HF-254 was used in t.l.c. and column chromatography. T.l.c. plates (0.25—0.75 mm thick) were air-dried and activated at 100 °C overnight. Spots were located by u.v. illumination, iodine complexation, or spraying with concentrated H_2SO_4 followed by heating at 100 °C for 5—10 min.

Column chromatography was performed with Merck silica gel HF-254, silica 7G, or neutral alumina. All samples for analysis were dried *in vacuo* over calcium chloride–silica gel.

Extraction.—Dried ground leaves (5.4 kg) were extracted (Soxhlet) in portions (each 500 g) for 8 days with chloroform (total 5 l). Concentration *in vacuo* at 50 °C gave a syrupy mass, which was exhaustively extracted with hot 50% ethanol. The aqueous alcoholic extract was filtered twice to remove a red gum then concentrated *in vacuo* and cooled overnight at room temperature to yield a red precipitate (A) (130.5 g). The precipitate when treated with chloroform left an insoluble material (B) (0.5 g). Evaporation of the filtrate to dryness gave a solid (C) (130 g).

Isolation of Genkwanin.—Genkwanin was isolated by column chromatography of fraction (C) on silica gel ($CHCl_3$ as eluant). It appeared as essentially a single spot on silica gel t.l.c. with only traces of impurities. Preparative t.l.c. [silica gel (0.75 mm); MeOH; multiple elution] followed by recrystallization from hot aqueous methanol gave a yellow solid (40 mg), m.p. 292—295° (lit.,[16] 282—283°; lit.,[17] 287—290°; lit.,[18] 283—284°; lit.,[11] 280°; lit.,[19] 282°; lit.,[20] 285°; lit.,[21] 273—275°), $\nu_{max.}$ (KBr) 3 270 (OH),

[8] J. P. Kutney, W. D. C. Warnock, and B. Gilbert, *Phytochemistry*, 1970, **9**, 1877.
[9] R. T. Sherwood and M. Shamma, *Phytochemistry*, 1973, **12**, 2275.
[10] N. Shkattaev and G. K. Nikonov, *Khim. prirod. Soedinenii*, 1972, **5**, 648.
[11] S. C. Sharma, Y. N. Shukla, J. S. Tandon, M. M. Dhar, *Phytochemistry*, 1974, **13**, 527.
[12] 'Spectral Data of Natural Products,' vol. I, ed. K. Yamaguchi, Elsevier, New York, 1970, p. 95.
[13] D. G. Kingston, *Tetrahedron*, 1971, **27**, 2691.
[14] R. D. Schmid, *Tetrahedron*, 1972, **28**, 3259.
[15] J. S. Brimacombe, B. D. Jones, M. Stacey, and J. J. Willard, *Carbohydrate Res.*, 1966, **2**, 167.
[16] R. N. Goel and T. R. Seshadri, *Tetrahedron*, 1959, **5**, 91.
[17] N. Rarasimhachari and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 1950, **32A**, 17.
[18] G. Zemplen, L. Mester, and E. Moczar, *Acta Chim. Acad. Sci. Hung.*, 1957, **10**, 369.
[19] M. Hasegawa and T. Shirato, *J. Amer. Chem. Soc.*, 1955, **77**, 3557.
[20] N. Rarashimhachari and T. R. Seshadri, *Proc. Indian Acad. Sci.*, 1949, **30A**, 271.
[21] N. Kawano, H. Miura, and E. Matsuishi, *Chem. and Pharm. Bull. Japan*, 1966, **14**, 299.

1 692 and 1 650 (chromone), 1 575, 1 488, 1 440, 1 379, 1 330, 1 210, and 825 cm^{-1}; $\lambda_{max.}$ (EtOH) 268 (ε 21 520) and 335 (26 634); δ 3.8 (3 H, s, OCH_3), 6.4 (1 H, d, *J* 2.5 Hz, H-6), 6.8 (1 H, d, *J* 2.5 Hz, H-8), 6.8 (1 H, s, H-3), 7.0 (2 H, d, *J* 8.5 Hz, H-3′ and -5′), 8.0 (2 H, d, *J* 8.5 Hz, H-2′ and -6′), 10.4 (1 H, s, 4′-OH), and 13.0 (1 H, s, 5-OH); *m/e* 284, 283, 255, 241, 212, 166, 121, and 118 (Found: C, 67.0; H, 4.3. Calc. for $C_{16}H_{12}O_5$: C, 67.0; H, 4.2%). The acetate had m.p. 200° (from methanol) (lit.,[22] 194—195°; lit.,[23] 204°).

Isolation of Fasciculatin.—Fraction (B) contained four compounds [silica t.l.c. (MeOH)], which were separated by preparative t.l.c. [0.5 mm; MeOH–C_6C_6 (80 : 20)]. The third fraction, *fasciculatin*, crystallised from methanol–water as a yellow powder (12 mg), m.p. >300° (decomp.); $\nu_{max.}$ (KBr) 3 375, 1 650, 1 620, 1 575, 1 480, 1 425, 1 375, 1 338, 1 250, 1 240, 1 210, 1 175, 1 150, 1 110, 1 060, 1 010, and 825 cm^{-1}; $\lambda_{max.}$ (EtOH) 271, 340, and 405sh nm; for n.m.r. see Table 2; *m/e* (perdeuteriomethylated derivative) 712, 411, 301, 274, 214, 213, and 198. The fourth fraction (27 mg) appeared to be polymeric material; $\nu_{max.}$ (KBr) 3 400, 3 300, 1 600, 1 475, 1 425, 1 372, 1 100, and 800 cm^{-1}; $\lambda_{max.}$ (MeOH) 213, 230sh, 275sh, and 400sh nm; δ 1.03 (3 H, t), 1.51 (1 H, s), 2.01 (1 H, s), 3.45 (6 H, s), and 3.50 (6 H, s).

Acidic Hydrolysis of Fasciculatin.—Fasciculatin (3.4 mg) was heated with 8% hydrochloric acid (3 ml) and a little methanol (to complete dissolution) for 3 h. The solution was extracted with ether, and the aqueous layer was concentrated for paper chromatography. The ethereal layer was concentrated to give a solid which was further dried *in vacuo* and was identical (m.p., R_F, and mass spectra) with genkwanin.

The aqueous layer was chromatographed on Whatman No. 1 paper in (a) ethyl acetate–pyridine–water (12 : 5 : 4) [5] and (b) ethyl acetate–isopropyl alcohol–water (3 : 1 : 1) with the following sugars as standards: glucose, rhamnose, fructose, sucrose, lactose, D-ribose, D-mannose, sorbose, xylose, D-galactose, and mixtures of these. The chromatograms were developed with (a) *p*-anisidine hydrochloride (1 g) and sodium hydrogen sulphite (0.1 g) in methanol (10 ml) diluted to 100 ml with n-butyl alcohol, [5,24] and (b) aniline hydrogen oxalate spray at 120—130 °C for 10—15 min.

Perdeuteriomethylation of Fasciculatin.[14,15]—Fasciculatin (5.4 mg) under nitrogen was dissolved in dimethylformamide (3 ml) and ether-washed sodium hydride (60 mg) was added. The solution was cooled and trideuteriomethyl iodide (3 ml) was added slowly. The solution solidified in about 50 min and was set aside at ambient temperature for 78 h. Methanol (3 ml) was added carefully to destroy the excess of hydride. When effervescence had ceased, the solution was partitioned between chloroform (30 ml) and water (30 ml); the separated organic layer was washed with water, filtered, and concentrated under vacuum to give a crystalline residue (3.2 mg). The main compound was separated by t.l.c. and a detailed mass spectral study was made.

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[22] B. S. Joshi, V. N. Kamat, and T. R. Govindachari, *Tetrahedron*, 1967, **23**, 261.

[23] M. Hasegawa and T. Shirato, *J. Amer. Chem. Soc.*, 1952, **74**, 6114.

[24] J. B. Pridham, *Analyt. Chem.*, 1956, **28**, 1967.