Phytochemistry, Vol. 22, No. 10, pp. 2215–2217, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

C-GLYCOSYLFLAVONES FROM *SIPHONOGLOSSA SESSILIS*

RICHARD A. HILSENBECK and TOM J. MABRY

Department of Botany, University of Texas at Austin, Austin, TX 78712, U.S.A.

(*Received* 14 *March* 1983)

Key Word Index—*Siphonoglossa sessilis*; Acanthaceae; chemotaxonomy; *C*-glycosylflavones; embigenin; embinin; vicenin-2; mass spectrometry; permethylation; ^{1}H NMR.

Abstract—Two known *C*-glycosylflavones, embinin and vicenin-2, and two new *C*-glycosylflavones, 8-*C*-glucosylapigenin 7,4′-dimethyl ether (isoembigenin) and 2″-*O*-glucosyl-6-*C*-glucosylapigenin 7,4′-dimethyl ether (embinoidin) were obtained from *Siphonoglossa sessilis*.

INTRODUCTION

In connection with a monographic treatment of the genus *Siphonoglossa* Oerst. (Acanthaceae) flavonoid studies of *Siphonoglossa*, *Justicia* and species of related genera have been undertaken. *C*-Glycosylflavones were first described in the Acanthaceae from the genus *Ecbolium* [1], and it was previously reported that *Siphonoglossa ramosa* Oerst., the type species of *Siphonoglossa*, contains 6-*C*-glycosylapigenin derivatives (including embinin) as its major flavonoid constituents [2]. *Siphonoglossa* has been considered closely related to *Odontonema* in the tribe Odontonemeae, Odontoneminae [3] until recently when it was shown that *Siphonoglossa* should be placed in the tribe Justicieae, close to *Justicia* [4]. We report here the detailed flavonoid chemistry of *Siphonoglossa sessilis* (Jacq.) Oerst. ex Radlkofer, a species which has been classified both in *Justicia* [5] and *Siphonoglossa* [3].

RESULTS AND DISCUSSION

An aqueous methanolic extract of stem and leaf material of *S. sessilis* afforded one major flavonoid glycoside, **1** and three minor ones, **2**–**4**. Prolonged acid hydrolysis failed to produce an aglycone from any of the glycosides suggesting that all are *C*-glycosylflavones. All compounds appeared purple when viewed on paper in UV light indicating a free 5-hydroxyl and all except **4** were unaffected by ammonia indicating that in **1**–**3** the 4′-hydroxyl is substituted [6]. Identifications were essentially based on UV, ^{1}H NMR and mass spectra.

UV spectral analysis following standard procedures [6] indicated that **1** was based on an apigenin-like skeleton. Sodium methoxide and sodium acetate shift reagents indicated that the compound had substituents on the 7- and 4′-hydroxyls [6]. Compound **1** (R_f 0.86, TBA and 0.89, acetic acid) on treatment with 0.1 N TFA (mild hydrolysis), produced a new *C*-glycosylflavone (R_f 0.79, TBA and 0.64, acetic acid) with the liberation of rhamnose, indicating that rhamnose was attached via an *O*-linkage [7]. UV spectra of the new compound indicated two important structural features: the 7- and 4′-hydroxyls were still substituted indicating methoxyl groups at these positions; thus, the rhamnose moiety must have been attached to a *C*-linked sugar [8]. Prolonged acid treatment of **1** gave a mixture of two isomeric *C*-glycosides via the Wessely–Moser rearrangement [8], neither of which corresponded to the original compound. The 6-*C* isomer, the one with the highest R_f value, was chromatographically and spectrally identical to the new compound derived via 0.1 N TFA hydrolysis. The 8-*C* isomer with lower mobility in both solvents (R_f 0.69, TBA and 0.38, acetic acid) was identical with **3**.

The mass spectrum of the permethyl (PM) ether of **1** gave a molecular ion $[M]^+$ at *m/z* 704, with the virtual absence of peaks at *m/z* 689 $[M-15]^+$ and 673 $[M-31]^+$, replaced instead by the ions $[SO]^+$ (*m/z* 515) and $[S]^+$ (*m/z* 499) derived from the elimination of a PM 2″-*O*-rhamnosyl residue without and with the oxygen atom of the glycosidic bond, respectively [9]. The difference $[M-S]$ (*m/z* 205) identified rhamnose as the 2″-*O*-glycosyl attached to a 6-*C*-hexosyl moiety [9]. Identification of the hexosyl moiety as glucose was confirmed by mass spectrometry of the PM derivative of the 6-*C* isomer, produced via prolonged acid hydrolysis; giving $[M]^+$ at *m/z* 530, followed by intense peaks at *m/z* 515 $[M-15]^+$ and 499 $[M-31]^+$ with the parent peak at *m/z* 355 $[M-175]^+$ in accord with the scheme of Bouillant *et al.* [10, 11]. Since an $[M-17]^+$ peak, characteristic of 6-*C*-galactosylapigenin, was not observed this is consistent with the assignment of glucose as the *C*-linked hexose at the 6-position [10]. Further proof of glucose as the *C*-linked hexose in **1** (and **2**) was the co-TLC of the PM 6-*C* isomer with PM isovitexin on Si gel in two solvent systems; chloroform–ethyl acetate–acetone, 5:4:1 and 5:1:4. Thus, the 6-*C* isomer is identified as 6-*C*-glucosylapigenin 7,4′-dimethyl ether. The compound, called embigenin, has never been reported as occurring naturally although it has previously been isolated as a hydrolysis product [12]. Coupled with hydrolysis and UV spectral data the mass spectral results demonstrated that **1** is 2″-*O*-rhamnosyl-6-*C*-glucosylapigenin 7,4′-dimethyl ether. The compound has previously been isolated from *Iris* and was named embinin [12–14]. ^{1}H NMR analysis of the trimethylsilyl ether (TMSi) derivative of **1** in carbon tetrachloride confirmed the other spectral data demonstrating an apigenin-type B-ring pattern plus the presence of a singlet for H-8, and the absence of a signal

for H-6. Two H-1 sugar protons were present as were 10 protons between δ3.3 and 3.65 representing the rhamnosyl and glucosyl moieties. Two singlets at δ3.85 and 3.75 each integrating for three protons were assigned to methoxyl groups. ^{1}H NMR in deuterated benzene produced methoxyl singlets at δ3.45 and 3.30 (Δ0.40 and 0.45) in accord with their assignment to the 7- and 4'-positions, respectively [7].

On prolonged acid hydrolysis **2** gave the same isomeric products as **1**, but with the liberation of glucose. UV spectra of **2** were also identical with those of **1**. Mass spectrometry of the PM derivative of **2** gave an $[M]^+$ at m/z 734 again with the virtual absence of the $[M-15]^+$ and $[M-31]^+$ peaks. Present were the ions $[SO]^+$ (m/z 515) $[S]^+$ (m/z 499) as the base peaks; these were derived by elimination of a PM 2''-*O*-glucosyl residue without and with the oxygen atom of the glycosidic bond [9]. This result showed that **2** is the 2''-*O*-glucoside of embigenin. There was insufficient material of this compound for ^{1}H NMR. The name embinoidin is proposed for **2** as it represents a new *C*-glycosylflavone.

Compound **3** failed to give an aglycone or sugars on prolonged acid treatment but did produce its 6-*C* isomer, embigenin, via the Wessely–Moser rearrangement. By producing the more mobile isomer in both solvents on prolonged acid treatment, **3** was initially characterized as an 8-*C*-glycoside [8]. Compound **3** had R_f values and UV spectra identical with the 8-*C* isomer derived by acid treatment of **1**. The band I absorption maximum in methanol was at 321 nm, a value comparable to that of acacetin with the 7-position substituted [6]. Sodium methoxide and sodium acetate shift spectra indicated the 7- and 4'-hydroxyls were indeed substituted and remained so after acid hydrolysis, indicating methoxyl groups at these positions. The permethyl ether of **3** co-chromatographed with PM vitexin on Si gel plates in chloroform–ethyl acetate–acetone (5:4:1 and 5:1:4) indicating glucose as the *C*-linked hexose, and also gave the mass spectrum of a PM 8-*C*-glucosylapigenin (vitexin): $[M]^+$ at m/z 530 (100) followed by relatively low intensity peaks $[M-14]^+$ at m/z 516 and $[M-29]^+$ at m/z 501 indicating that **3** was an 8-*C*-glucoside based on vitexin [10] but with 7- and 4'-methoxyls. The other main diagnostic peak was $[M-175]^+$ at m/z 355 (90) indicating glucose as the 8-*C*-linked sugar. ^{1}H NMR of the TMSi derivative of **3** in carbon tetrachloride exhibited an apigenin B-ring pattern, a singlet for H-6, and other signals for a *C*-glucosyl H-1 and six additional glucosyl protons. Signals for two methoxyl groups appeared at δ3.9 and 3.8. The name isoembigenin is proposed for **3** as it represents a new *C*-glycosylflavone.

On prolonged acid treatment of **4** there was no change in R_f values or the liberation of any sugars in accord with **4** being a symmetrical di-*C*-glycosylflavone [7, 10]. On exposure to ammonia the compound turned green indicating a free 4'-hydroxyl. From R_f (0.29, TBA and 0.50, acetic acid) it was suspected that **4** was vicenin-2 [7] (6,8-di-*C*-glucosylapigenin). The UV spectra with diagnostic reagents indicated that **4** was an apigenin type compound with free 7- and 4'-hydroxyl groups. The mass spectrum of the PM derivative of compound **4** was as follows: $[M]^+$ m/z 748, $[M-15]^+$ m/z 733 and the parent peak $[M-31]^+$ at m/z 717 (100). Other diagnostic peaks were observed as previously reported [10]. PM of **4** co-chromatographed on Si gel plates in chloroform–ethyl acetate–acetone (5:4:1 and 5:1:4) with PM of standard vicenin-2, thus indicating glucose as the hexose *C*-linked at both the 6- and 8-positions. ^{1}H NMR analysis of the TMSi derivative confirmed the identification as vicenin-2 [6, 15].

When several apigenin- and luteolin-based *C*-glycosides were previously reported from the genus *Ecbolium* [1] it was suggested that this occurrence was atypical for the Acanthaceae and that the aglycone luteolin might be more characteristic for the family [16]. As a result of this and other recent studies [17], [Hilsenbeck, R. A.; and R. Long, R. Mansell and J. Saunders, unpublished], *C*-glycosylflavones may be much more common than previously suspected. *Siphonoglossa sessilis* afforded two 6-*C*-, an 8-*C*- and one di-*C*-glycosylapigenin. Three of the flavones, including the major compound, are methoxylated at both the 7- and 4'-positions. *Siphonoglossa* is a relatively advanced member of the Acanthaceae, properly belonging to the tribe Justicieae [4] generally regarded as the most derived tribe in the family [3, 18]. If *C*-glycosylflavones represent 'primitive' characters [19, 20], then *C*-glycosylflavone methyl ethers may indicate 'advanced' states of these 'primitive' characters.

Since *S. sessilis* has also been classified in the genus *Justicia* [5], the present results and those of our ongoing chemotaxonomic studies should prove significant in the generic classification, disposition and delimitation of *Siphonoglossa* as well as *Justicia*. Considering that embinin has been found in *Siphonoglossa ramosa* [2], the type species of the genus, the inclusion of *S. sessilis* in *Siphonoglossa* [3] seems justified. However, due to the great morphological diversity in *Justicia* and the dearth of chemical information about this large genus, the possibility that *Siphonoglossa*, including *S. sessilis*, may have to be classified as a natural group at the sectional level within *Justicia* must remain open.

EXPERIMENTAL

Plant material. Siphonoglossa sessilis Oerst. ex Radlkofer was collected by the senior author (R.A.H.) in a dry, tropical forest northwest of Santiago, Dominican Republic. Voucher specimens are deposited in the Herbarium of the University of Texas at Austin, Plant Resources Center (LL, TEX), Hilsenbeck 780.

Extraction and isolation. Ground, air-dried stem and leaf material (149 g) was extracted repeatedly with excess vols. of 85% aq. MeOH followed by extraction with 50% aq. MeOH. The combined extracts were concd *in vacuo* to a thick syrup (*ca* 24 g). This aq. syrup was partitioned against CH_2Cl_2. The CH_2Cl_2 fraction (*ca* 15 g) was examined by PC and TLC and discarded as it contained no detectable flavonoids. The remaining H_2O fraction (9 g) was placed on a Polyclar column packed in 20% aq. MeOH and eluted with a MeOH–H_2O gradient (20–80% MeOH). Three flavonoid containing fractions were obtained, each concd *in vacuo* and taken up in 50% aq. MeOH. Each fraction was then further purified by 1D-PC on Whatman 3 mm paper using both *t*-BuOH–HOAc–H_2O (3:1:1) (TBA) and 15% HOAc (HOAc) as solvents. The bands corresponding to **1**–**4** were eluted over 48 hr with 50% MeOH. Final purification was on a Sephadex LH 20 (Pharmacia) column with 60% aq. MeOH.

Hydrolysis conditions. (a) Mild acid hydrolysis: dry sample dissolved in 0.1 N TFA, flask was sealed and placed in steam bath for 1.5 hr. (b) Prolonged acid hydrolysis: dry sample dissolved in 2 N HCl; flask was sealed and placed in steam bath for 2.5 hr.

Sugar analysis. Sugars were recovered from the hydrolysed flavonoids after repeated evaporations *in vacuo* of the hydrolysis

solution. The residue was taken up in H_2O and the aq. soln extracted with EtOAc. Sugars present in the H_2O fraction were identified by TLC on cellulose against standard markers in pyridine–EtOAc–HOAc–H_2O (36:36:7:21) and detected by spraying with aniline phthalate.

Derivatization techniques. (a) Permethylation: The sample (2–3 mg) was evaporated to dryness in a small flask which was then covered with Al foil. To this was added 1 ml DMF followed by Et_2O-washed NaH powder in 1 ml DMF, with the addition of 10–15 drops MeI. The flask was sealed and allowed to stand overnight. Excess MeI was removed *in vacuo* and after the addition of H_2O the permethyl ether was isolated by extraction with Et_2O, followed by thick layer chromatography on Si gel plates developed in EtOAc. The major fluorescent band in UV (356 nm) was eluted with 100% MeOH. (b) Trimethylsilation: as described in ref. [7].

Spectroscopy. (a) UV spectroscopy: as described in ref. [6]. (b) MS: by direct insertion of PM samples into the ion source, 70 eV, EIMS. (c) ^{1}H NMR spectroscopy: as described in ref. [6], spectra recorded at 90 MHz.

Compound **1** (embinin) (205 mg). UV λ_{max}^{MeOH} nm: 272, 328; + NaOMe 294, 345 sh, 390 sh; + $AlCl_3$ 260 sh, 278, 299, 348, 375 sh; + $AlCl_3$ + HCl 263 sh, 281, 300, 345, 380 sh; + NaOAc 272, 329; + NaOAc + H_3BO_3 272, 333. PC: R_f 0.86 (TBA), 0.89 (HOAc). Permethyl ether: EIMS 70 eV, *m/z* (rel. int.) 704 $[M]^+$ (6), 689 $[M-15]^+$ (< 1), 673 $[M-31]^+$ (< 1), 559 $[SO_i]^+$ (5), 545 $[SO_j]^+$ (14), 529 $[SO_k]^+$ (3), 515 $[SO]^+$ (65), 499 $[S]^+$ (100), 467 $[S-32]^+$ (7), 397 $[f]^+$ (7), 355 $[i]^+$ (8), 341 $[j]^+$ (74), 339 $[j-2H]^+$ (10), 325 $[k]^+$ (20), 312 $[1+1]^+$ (7), 311 $[l]^+$ (9). TMSi ether: ^{1}H NMR, 90 MHz (CCl_4), δ7.7 (2H, *d*, *J* = 9 Hz, H-2′, H-6′), 6.85 (2H, *d*, *J* = 9 Hz, H-3′, H-5′), 6.45 (1H, *s*, H-8), 6.2 (1H, *s*, H-3), 4.85 (1H, *d*, Rha H-1), 4.7 (1H, *d*, Glc H-1), 3.85 (3H, *s*, OMe-7), 3.75 (3H, *s*, OMe-4′), 3.65–3.3 (10H, *m*, sugar H's). Mild acid hydrolysis of **1** gave embigenin. PC: R_f 0.79 (TBA), 0.64 (HOAc). Prolonged acid hydrolysis of compound **1** gave embigenin and its 8-*C* isomer which was identical with **3**.

Compound **2** (embinoidin) (5 mg). UV λ_{max}^{MeOH} nm: 272, 328; + NaOMe 294, 345 sh, 390 sh; + $AlCl_3$ 260 sh, 278, 299, 348, 375 sh; + $AlCl_3$ + HCl 263 sh, 281, 300, 345, 380 sh; + NaOAc 272, 329; + NaOAc + H_3BO_3 272, 333. PC: R_f 0.73 (TBA), 0.87 (HOAc). Permethyl ether: EIMS 70 eV, *m/z* (rel. int.) 734 $[M]^+$ (3), 719 $[M-15]^+$ (< 1), 703 $[M-31]^+$ (< 1), 703 $[M-31]^+$ (< 1), 559 $[SO_i]^+$ (5), 545 $[SO_j]^+$ (10), 529 $[SO_k]^+$ (6), 515 $[SO]^+$ (65), 513 $[SO-2]^+$ (2), 499 $[S]^+$ (100), 467 $[S-32]^+$ (18), 397 $[f]^+$ (12), 355 $[i]^+$ (19), 341 $[j]^+$ (70), 339 $[j-2H]^+$ (20), 325 $[k]^+$ (36), 311 $[l]^+$ (19). Mild acid hydrolysis of **2** gave embigenin. Prolonged acid hydrolysis of **2** gave embigenin and its isomer.

Compound **3** (isoembigenin) (14 mg). UV λ_{max}^{MeOH} nm: 268, 321; + NaOMe 287, 368; + $AlCl_3$ 275, 302, 338, 383; + $AlCl_3$ + HCl 277, 302, 336, 382; + NaOAc 269, 324; + NaOAc + H_3BO_3 268, 325. PC: R_f 0.69 (TBA), 0.38 (HOAc). Permethyl ether: EIMS 70 eV, *m/z* (rel. int.) 530 $[M]^+$ (100), 516 $[M-14]^+$ (25), 501 $[M-29]^+$ (7), 397 $[M-133]^+$ (13), 369 $[M-161]^+$ (42), 355 $[M-175]^+$ (90), 341 $[M-189]^+$ (61), 325 $[M-205]^+$ (42), 311 $[M-219]^+$ (41). TMSi ether: ^{1}H NMR, 90 MHz (CCl_4) δ7.9 (2H, *d*, *J* = 9 Hz, H-2′, H-6′); 6.9 (2H, *d*, *J* = 9 Hz, H-3′, H-5′); 6.3 (1H, *s*, H-6); 6.2 (1H, *s*, H-3); 4.85 (1H, *d*, Glc H-1); 3.9 (3H, *s*, OMe-7); 3.8 (3H, *s*, OMe-4); 3.75–3.3 (6H, *m*, glucose H's). Mild acid hydrolysis of **3** produced no change in R_f value and no sugars. Prolonged acid hydrolysis produced the 6-*C* isomer, embigenin.

Compound **4** (vicenin-2) (12 mg). UV λ_{max}^{MeOH} nm: 273, 333; + NaOMe 280, 332, 395; + $AlCl_3$ 272, 300, 345, 380; + $AlCl_3$ + HCl 269, 292, 333, 370; + NaOAc 280, 334, 394; + NaOAc + H_3BO_3 274, 329 sh, 347, 410 sh. PC: R_f 0.29 (TBA), 0.50 (HOAc). Permethyl ether: EIMS 70 eV, *m/z* (rel. int.): 748 $[M]^+$ (9), 733 $[M-15]^+$ (41), 717 $[M-31]^+$ (100), 701 $[M-47]^+$ (15), 685 $[M-63]^+$ (8), 645 $[M-103]^+$ (23), 615 $[M-133]^+$ (9), 585 $[M-163]^+$ (56), 573 $[M-175]^+$ (76), 559 $[M-189]^+$ (13), 543 $[M-205]^+$ (12), 541 $[M-207]^+$ (16). TMSi ether: ^{1}H NMR, 90 MHz (CCl_4) δ7.9 (2H, *d*, *J* = 9 Hz, H-2′, H-6′); 6.9 (2H, *d*, *J* = 9 Hz, H-3′, H-5′); 6.5 (1H, *s*, H-3); 4.8–4.2 (2H, *m*, Glc H-1's); 3.8–3.3 (12H, *m*, Glc H's). Prolonged acid hydrolysis of **4** produced no change in R_f value and no sugars.

Acknowledgements—We thank Mark Leidig for mass spectral measurements and Susan P. McCormick and Sharon J. Wright for their advice and assistance. This study was partially supported by the National Science Foundation (Grant DEB 8014249) awarded to R.A.H. and the Robert A. Welch Foundation (Grant F-130) awarded to T.J.M.

REFERENCES

1. Nair, A. G. R., Ramesh, P. and Subramanian, S. S. (1975) *Phytochemistry* **14**, 1644.
2. Hilsenbeck, R. A. (1980) in *Botanical Society of America*, Miscellaneous Publication Vol. **158**, p. 51. Vancouver.
3. Lindau, G. (1895) *Nat. Pflanzenfam*, **IV** (3b), 274. Wilhelm Engelmann, Leipzig.
4. Henrickson, J. and Hilsenbeck, R. A. (1979) *Brittonia* **31**, 373.
5. Lindau, G. (1900) in *Symb. Antill.* (Urban, I., ed.) Vol. **II**, 170. Asher, Amsterdam.
6. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*. Springer, New York.
7. McCormick, S. P. (1982) Ph.D. dissertation, University of Texas at Austin.
8. Chopin, J. and Bouillant, M. L. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds.) Chapter 12. Academic Press, New York.
9. Bouillant, M. L., Besset, A., Favre-Bonvin, J., and Chopin, J. (1978) *Phytochemistry* **17**, 527.
10. Bouillant, M. L., Favre-Bonvin, J. and Chopin, J. (1975) *Phytochemistry* **14**, 2267.
11. Bouillant, M. L., Besset, A., Favre-Bonvin, J. and Chopin, J. (1980) *Phytochemistry* **19**, 1755.
12. Kawase, A. and Yagishita, K. (1968) *Agric. Biol. Chem.* **32**, 537.
13. Hirose, Y., Hayashi, K. A., Wagishi, E. and Shibata, B. (1962) *Kamamoto Pharm. Bull.* **5**, 48.
14. Arisawa, M., Morita, N., Kondo, Y. and Takemoto, T. (1973) *Yakugaku Zasshi*, **93**, 1655.
15. Seikel, M. K. and Mabry, T. J. (1965) *Tetrahedron Letters* 1105.
16. Nair, A. G. R. (1965) *Curr. Sci.* **34**, 79.
17. Meagher, M. B. (1974) Ph.D. dissertation, University of South Florida.
18. Bremekamp, C. E. B. (1965) *Bull. Bot. Surv. India* **7**, 21.
19. Harborne, J. B. (1967) *Comparative Biochemistry of the Flavonoids*. Academic Press, London.
20. Swain, T. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H., eds.) Chapter 20. Academic Press, New York.