Phytochemistry, 1980, Vol. 19, pp. 293–297. © Pergamon Press Ltd. Printed in England.

C-GLYCOSYLFLAVONES FROM *ZEA MAYS* THAT INHIBIT INSECT DEVELOPMENT

CARL A. ELLIGER, BOCK G. CHAN, ANTHONY C. WAISS, JR., ROBERT E. LUNDIN and WILLIAM F. HADDON

Western Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Berkeley, CA 94710, U.S.A.

(*Received* 26 *April* 1979)

Key Word Index—*Zea mays*; Gramineae; maize; flavone C-glycosides; *Heliothis zea*; corn earworm; larval growth inhibitors; host plant resistance; keto sugars.

Abstract—A new C-glycosylflavone isolated from corn silk inhibits the growth and development of the corn earworm, *Heliothis zea*. This new compound was shown to be a 2″-*O*-α-L-rhamnosyl-6-*C*-(6-deoxy-*xylo*-hexos-4-ulosyl)luteolin. Also found co-occurring in corn silk were minor amounts of the corresponding 6-*C*-glycosylated analogs of chrysoeriol and apigenin.

INTRODUCTION

Resistance in maize, *Zea mays* L., toward the corn earworm, *Heliothis zea* (Boddie), has been associated, in part, with toxicity of the silks of certain corn varieties to larvae of *H. zea* [1, 2]. Examination of extracts from silk of the line 'Zapolote Chico' revealed that methanol-soluble material served to severely inhibit growth of earworm larvae on standard artificial diets [3] to which this this component had been added at a level corresponding to that found in the original plant material. Chromatographic separation afforded one major constituent. This compound was shown to be a flavonoid, and it possessed nearly all of the inhibitory activity exhibited by the crude methanol extract. We have termed this compound maysin and present evidence that its structure is 2″-*O*-α-L-rhamnosyl-6-*C*-(6-deoxy-*xylo*-hexos-4-ulosyl)-luteolin (**1**). Also present in the extract at lower concentration are the C-glycosylflavones **2** and **3**; the latter substance is of lower activity in the bioassays and therefore is less significant as a possible insect resistance factor.

RESULTS AND DISCUSSION

Maysin

The UV spectrum of maysin (Table 1) shows typical flavonoid absorption and gives UV shifts characteristic of the basic luteolin structure with the usual diagnostic reagents [4]. ^{1}H NMR suggested the presence of two sugar moieties, each bearing a *C*-methyl group (Table 2). Treatment of maysin with N HCl (80°, 30 min) yielded rhamnose. The 'aglycone' after hydrolysis appeared to be seriously rearranged but still clearly possessed one sugar residue. Enzymic digestion of maysin with naringinase cleanly yielded derhamnosylmaysin (**4**). Both **1** and **4** showed carbonyl absorptions at 1725 cm^{-1} (KBr) in their IR spectra. Low resolution CI-mass spectral determinations upon the respective per-trimethylsilyl ethers of **1** and **4** indicated appropriate MWs for the parent compounds. High resolution mass measurements (EI) upon compound **1**–TMSi ether fragment ions were not satisfactory for determination of elemental composition because instrument resolution dropped off at such high masses. However, the value obtained for M^+ minus −Me (loss of methyl from TMSi) for compound **4**–TMSi ether was in agreement with the chemical formula for the proposed structure. The ^{1}H NMR spectra of **1** and **4** are consistent with attachment of the '*C*-sugar' residue at position 6. Thus, of the five flavone protons, three are accounted for in the ABX system of the B-ring. Singlets at *ca* 6.5 ppm for H-3 and H-8 point to the absence of a proton at position 6 of the A-ring which appears at about δ 6.3 in the examples of the 8-*C*-glycosyl substituted vitexin and orientin [5, 6]. In isovitexin and iso-orientin the H-8 proton appears at 6.56 and 6.54 ppm, respectively [5, 6]. In agreement with the assignment of sugar linkage at C-6 is the observation of positive Cotton effect at 265 nm ([θ] = +4320, MeOH) for compound **4**. Circular dichroism studies by Gaffield and Horowitz of *C*-glycosylflavones have shown that a positive Cotton effect at 250–270 nm indicates C-6 attachment while a negative value implies C-8 linkage of the glycosyl residue [7].

Identification of the sugar attached at C-6 as the deoxyhexosulose shown in Fig. 1 is consistent with observation of a non-flavonoid IR C=O band (1725 cm^{-1}) as well as the ^{13}C NMR signal appearing at 205.7 ppm which indicates the presence of an aliphatic ketone [8]. A C-13 chemical shift of 205.5 ppm has been observed for the keto group of methyl α-L-*threo*-pentopyranosid-4-ulose [9]. Derhamnosylmaysin also formed an oxime. The ^{1}H NMR spectrum of **4** in pyridine (see Experimental), which is more highly resolved than that obtained

Table 1. UV spectra of flavone *C*-glycosides*

Compound	MeOH	NaOMe	$AlCl_3$	$AlCl_3$–HCl	NaOAc	NaOAc–H_3BO_3
1	245 sh, 258, 272, 352	235 sh, 271, 413	278, 300 sh, 330, 426	265, sh, 280, 298, 365, 388	273, 279, 327, 396	268, 306 sh, 380, 425 sh
2	271, 332	278, 335 sh, 392	276, 302, 352, 375 sh	279, 300, 339, 380 sh	278, 300 sh, 338 sh, 384	270, 345
3	250 sh, 272, 346	265, 278, 340 sh, 410	280, 300 sh, 366, 390	280, 300 sh, 360, 388	278, 320, 401	272, 352
4	247 sh, 258, 272, 352	272, 313, 416	278, 333, 427	270 sh, 280, 297, 365, 388	274, 280, 330 sh, 394	270, 308 sh, 380, 425 sh

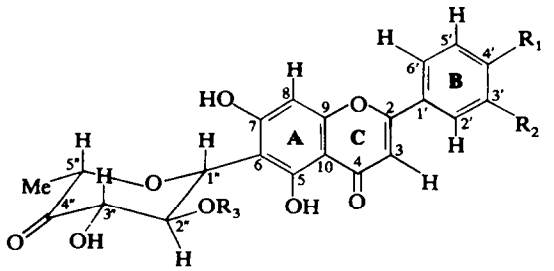
* All UV spectra were recorded using standard procedures [4].

Table 2. ^{1}H NMR data for compounds **1** and **4***

Proton	**1**	**4**
	Flavonoid signals	
H-3 and 8	6.48 (*s*), 6.50 (*s*)	6.45 (*s*), 6.48 (*s*)
H-2′ and 6′	7.33, 6 lines, AB of ABX	7.30, 6 lines, AB of ABX
H-5′	6.87, 3 lines, X of ABX $J_{2',6'}=2$; $J_{5',6'}=9$	6.85, 3 lines, X of ABX $J_{2',6'}=2$; $J_{5',6'}=9$
	4-Ketofucosyl signals	
H-1″	5.34, *d*, $J=9$	5.26, *d*, $J=9$
H-2″	4.75, *t*, $J_{1'',2''}=J_{2'',3''}=9$	4.62, *t*, $J_{1'',2''}=J_{2'',3''}=9$
H-3″	4.40, *br d*, $J_{2'',3''}=9$	4.30, *d*, $J_{2'',3''}=9$†
H-5″	4.22, *br q*, $J_{5'',6''}=6$	4.23, *q*, $J_{5'',6''}=6$†
C-5″-Me	1.26, *d*, $J=6$	1.28, *d*, $J=6$
	Rhamnosyl signals	
H-1‴	5.16, *d*, $J=2$	
H-2‴	3.77, *dd*, $J_{1''',2'''}=J_{2''',3'''}=2$	
H-3‴ and 4‴	3.0–3.5, complex	
H-5‴	2.60, *dq*, $J_{5''',6'''}=6$, $J_{4''',5'''}=9$	
C-5‴-Me	0.78, *d*, $J=6$	

* Values in δ CD_3OD at 90 MHz, J = coupling constant in Hz. Spectra recorded on Varian EM-390 at 34°. Abbreviations: *s*, singlet: *d*, doublet: *t*, triplet; *dd*, double doublet; *dq*, double quartet.

† Poorly resolved.



1 $R_1=R_2=OH$, $R_3=\alpha$-Rha
2 $R_1=OH$, $R_2=H$, $R_3=\alpha$-Rha
3 $R_1=OH$, $R_2=OMe$, $R_3=\alpha$-Rha
4 $R_1=R_2=OH$, $R_3=H$

Fig. 1.

in CD_3OD, consists of non-overlapping multiplets which permit unambiguous assignment of position and configuration of the ring protons for the C-6 bound sugar. Protons 1″, 2″ and 3″ must have the all-axial relationship ($J_{a,a}=9$ Hz) [10] and must be coupled in the order shown as is evidenced by collapse of H-1″ and H-3″ doublets upon irradiation at H-2″. The keto function is thereby assigned to position 4″. The methyl group on position 5″ is likely to be equatorial as is indicated by the following arguments: A coupling of *ca* 0.8 Hz between H-3″ and H-5″ is observed which is similar to the value reported for diaxial protons flanking the carbonyl group in substituted cyclohexanones [11]. Long range coupling between equatorial and axial protons α to C=O did not occur in these cyclohexanone systems. Reduction of **4** with $NaBH_4$ gave two epimeric *C*-glycosyl products which were separated chromatographically. The ^{1}H NMR spectra (in C_5D_5N) indicated only differences in configuration at C-4″ consistent with formation of 6-*C*-quinovosyl- and 6-*C*-fucosyl-luteolin. Carbonyl absorption at 1725 cm^{-1} in the IR was absent.

Attachment of rhamnose to the C-6 sugar residue and not directly to a flavone oxygen was established by consideration of the UV shifts of **1** (Table 1). The enzymic hydrolysis product **4** shows essentially the

same diagnostic behavior. Permethylation of the flavone —OH's of **1** followed by acid hydrolysis gave a rhamnose-free product which did not display base-induced bathochromic shifts. Maysin and derhamnosylmaysin are sensitive to the usual methods of acetylation and permethylation because of the presence of the keto-group at position 4″. A product was isolated from attempted acetylation (pyridine–Ac_2O) of **4** which displayed coupling constants in the ^{1}H NMR for ring protons of the keto-sugar that clearly indicated epimerization at C-3″ and C-5″ ($J_{3'',5''} = 1.5$ Hz) with diequatorial orientation of the respective protons [11]. No identifiable product of acetylation could be obtained from maysin. The products of borohydride reduction are more tractable. Maysin, upon reduction, yields two chromatographically separable products which undergo methylation and acetylation without stereochemical alteration. The product of methylation (CH_2N_2 followed by MeI, Ag_2O in DMF) was resistant to hydrolysis of the *O*-glycosidic linkage under conditions which maintained integrity of the remainder of the molecule. This prevented direct establishment of the point of attachment of rhamnose. Examination of the ^{1}H NMR spectrum of the acetylated reduction product revealed no acetyl signals in the range δ 1.70–1.85 ppm. Since a C-2″ acetoxyl would be expected to give rise to a signal in this region [12–14], it is concluded that rhamnose is instead attached at position 2″. Acetylation of 6-*C*-quinovosyl- and 6-*C*-fucosyl-luteolin yielded acetates that exhibited the required high field acetyl signals (δ 1.79 ppm) in the region expected for 6-*C*-glycosylflavones [12, 14].

Maysin analogs

From chromatographic fractions preceding maysin were isolated small amounts of compounds **2** and **3**. Compound **2** exhibits UV absorptions consistent with monohydroxylation of the B-ring in position 4′ (substituted apigenin). The ^{1}H NMR spectra are identical to those of **1** except for signals arising from the A_2B_2 system of the B-ring (δ, CD_3OD: 6.93 and 7.84, broadened doublets, $J = 9$ Hz, 2H) in which the lower field doublet is assigned to protons 2′ and 6′. Compound **3** also shows UV absorptions and base shifts characteristic of a flavone bearing a free -OH group at position 4′; the longer wavelength of band I absorption is consistent with further oxygenation of ring-B, but no borate shift of significance was observed. The ^{1}H NMR spectrum in CD_2OD closely resembles that of **1**; however, the presence of an aromatic methoxyl (δ, CD_3OD, 3.92, *s*, 3H) is indicated. Signals at δ 6.90 (*br d*, $J = 9$ Hz, 1H) and 7.46 (complex, 2H) for B-ring protons are assigned to positions 5′ and 2′, 6′, respectively, in agreement with methoxylation at 3′.

Biogenetic origin of the keto-sugar

The carbon-linked sugar residue seems highly unusual at first glance. However, a likely pathway of biogenesis (Fig. 2) may proceed from uridine diphosphate-glucose. UDP-D-glucose is converted by higher plants into UDP-L-rhamnose [15]. Similarly, the corresponding thymidine derivatives are utilized by certain microorganisms [16]. In these examples, conversion of the sugar takes place by oxidation of the hydroxyl group on C-4 to ketone and transformation of the pendant hydroxymethyl group into methyl. In the pathway leading to rhamnose, subsequent epimerizations and reduction of the 4-keto group afford nucleotide-linked L-rhamnose. It can be seen that interaction of suitable flavone (or flavanone) precursors with the UDP- or TDP-6-deoxy-*xylo*-hexos-4-ulose will give instead *C*-glycosylated products of the type isolated. Final *O*-glycosylation completes the sequence.

Varietal and seasonal variations in flavonoids

We have examined methanol extracts from silks of a number of sweet corn and field corn varieties and have observed considerable differences in flavonoid content although definitive analyses of components were not performed. Thus, the variety 'Jubilee' had essentially no flavone content while Zapolote Chico silk samples from a number of sources differed in concentration over a six-fold range (by UV of total extract). We also note time dependent variation: Silks from 'Golden cross Bantam' declined in flavone content by about a factor of five over about a one month period even

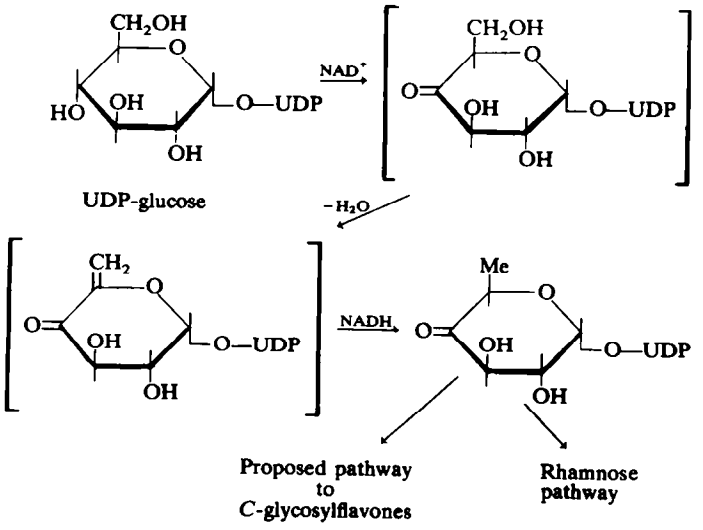


Fig. 2. Proposed biosynthetic pathway leading to maysin and analogs.

though material was collected at about the same interval after protrusion of silk from the immature ear. The same decline was noted in the type 'super stiff stalk'; on the other hand, the flavone content remained constant in 'Zapolote Chico and a number of experimental crosses with advancing maturity. It is clear that this phenomenon is highly significant in host plant resistance if the population of pests and egg laying behavior of adults happens to coincide with high content of larva-inhibiting factors.

EXPERIMENTAL

Bioassays. Tests for growth inhibiting activity were carried out on artificial diets by the method of Chan [3] using first-instar larvae of *Heliothis zea*. The various extracts were applied on α-cellulose and incorporated into the diet mixture at levels approximately equal to the amount found in the corn silk. Chromatographic fractions were assayed similarly.

Isolation of flavones. Samples of corn silk were freeze-dried and finely ground. In a typical extraction, 100 g of silk (var. Zapolote Chico, Missouri, 1976 season) was milled with 3 × 500 ml portions of Me_2CO using a high speed grinder followed by filtration. Subsequent extraction, 3 × 500 ml MeOH, and filtration gave a brown gum which was separated from sugar components by redissolving in *ca* 250 ml H_2O and selective adsorption of phenolic constituents upon Amberlite XAD-2 resin (100 ml settled vol.). The resin was washed 3 × 100 ml H_2O, and crude flavonoids were then desorbed using 3 × 100 ml MeOH. Chromatography upon Sephadex LH-20, 75 × 850 mm column, with MeOH gave enriched maysin (**1**), 0.9 g (elution vol. 4.43–4.65 l.) flanked by related compounds. Further chromatography on LH-20, 50 × 950 mm column, gave purer material (elution vol. 2050–2300 ml) which crystallized from MeOH–Me_2CO, mp *ca* 225° dec. MS of TMSi ether [17], CI, isobutane *m/e* = 1152, $C_{27}H_{28}O_{14}$ $(TMSi)_8$. The fraction of material eluting from the large column before **1** was repeatedly rechromatographed on LH-20/MeOH (50 × 950 mm) to give nearly pure samples of **2** and **3** (elution vols. 2000–2150 and 1850–2000 ml, respectively).

Hydrolyses. Acid hydrolyses were done in N HCl at *ca* 80° on a steam bath. Enzymic hydrolyses were carried out with Sigma Naringinase in H_2O adjusted to pH 3 with citric acid. Identification of rhamnose in the hydrolysates was done by removal of flavone from the soln with Woelm polyamide followed by concn to dryness and PC (*i*-PrOH–HOAc–H_2O, 3:1:1) vs known sugars. Conversion of the sugar to the TMSi derivative [17] followed by GLC isothermally at 175° on 1% OV-101/chromasorb W using N_2 carrier and FID confirmed identification of rhamnose.

The monoglycosylated flavone (**4**) resulting from enzyme hydrolysis was purified by chromatography on Sephadex LH-20/MeOH, 50 × 950 mm (elution vol. 2850–3100 ml) followed by crystallization from MeOH–Me_2CO to give material of mp 217–220° dec. (Found: C, 58.7; H, 4.40. $C_{21}H_{18}O_{10}$ requires: C, 58.61; H, 4.22%). High resolution MS of **4**-$(TMSi)_6$ gave: $M^+ - (-Me) = 847.3036$. $C_{38}H_{63}O_{10}Si_6$ requires: 847.3040 (loss of —Me from TMSi group). Conversion of **4** to the oxime in MeOH–H_2O (NaOAc) [18] gave product having mp *ca* 310° dec. (MeOH). (Found % Kjeldahl N: 3.01. $C_{21}H_{19}NO_{10}$ requires: N, 3.14%).

IR of **4**: ν_{max}^{KBr} cm^{-1}: 1725 (carbonyl of hexosulose). 90 MHz ^1H NMR spectrum of **4** in C_5D_5N: δ 1.46 (3H, *d*, *J* = 6 Hz, C-5″ Me), 4.42 (^1H, *dq*, $J_{5'',6''} = 6$, $J_{3'',5''} = 0.8$ Hz, H-5″), 4.92 (1 H *dd*, $J_{2'',3''} = 9$, $J_{3'',5''} = 0.8$ Hz, H-3″), 5.57 (1 H, *t*, $J_{1'',2''} = J_{2'',3''} = 9$ Hz, H-2″), 6.02 (1 H *d*, $J_{1'',2''} = 9$ Hz, H-1″), 6.64 (1 H, *s*, H-8), 6.78 (1 H, *s*, H-3), 7.14 (1 H, *d*, $J_{5',6'} = 9$ Hz, H-5′), 7.41 (1 H, *dd*, $J_{5',6'} = 9$, $J_{2',6'} = 2$ Hz, H-6′), 7.73 (1 H, *d*, $J_{2',6'} = 2$ Hz, H-2′). Irradiation at H-5″ and H-3″ yielded a sharpened doublet for H-3″ and a sharpened quartet for H-5″ respectively.

$NaBH_4$ *reduction of derhamnosylmaysin* (**4**). In 30 ml MeOH were dissolved 250 mg of **4**, and 4 × 100 mg portions of $NaBH_4$ were added with stirring at 30 min intervals. The mixture was acidified with HOAc, evapd under red. pres., and extracted repeatedly with EtOAc. The EtOAc-soluble material was chromatographed on Sephadex LH-20/MeOH, 50 × 920 mm, to give two major fractions: 6-*C*-quinovosyl-luteolin, 83 mg (elution vol. 2450–2775 ml) and 6-*C*-fucosyl-luteolin, 107 mg (elution vol. 2900–3250 ml).

Crystallization from MeOH gave 6-*C*-quinovosyl-luteolin, mp 221–222°. (Anal. calc. for $C_{21}H_{20}O_{10}$: C, 58.34; H, 4.66. Found: C, 58.1; H, 4.90%). Similarly obtained from MeOH–H_2O was 6-*C*-fucosyl-luteolin, mp 235–236°. (Anal. calc. for $C_{21}H_{20}O_{10}$: C, 58.34; H, 4.66. Found: C, 57.8; H, 4.91%). Examination of the IR spectra (KBr) showed the absence of carbonyl absorption at 1725 cm^{-1} for the reduction products.

90 MHz ^1H NMR spectrum of 6-*C*-quinovosyl-luteolin in C_5D_5N; δ 1.63 (3 H, *d*, *J* = 6 Hz, C-5″ Me), 3.95 (2 H, Complex, H-4″ and H-5″), 4.30 (1 H, complex, H-3″), 5.20 (1 H, *t*, *J* = 9 Hz, H-2″), 5.52 (1 H, *d*, *J* = 9 Hz, H-1″), 6.62 (1 H, *s*, H-8), 6.78 (1 H, *s*, H-3), 7.20 (1 H, *d*, *J* = 9 Hz, H-5′), 7.44 (1 H, *dd*, $J_{5',6'} = 9$, $J_{2',6'} = 2$ Hz, H-6′), 7.80 (1 H, *d*, *J* = 2 Hz, H-2′).

90 MHz ^1H NMR spectrum of 6-*C*-fucosyl-luteolin in C_5D_5N: δ 1.46 (3 H, *d*, *J* = 6 Hz, C-5″ Me), 3.90 (1 H, *dq*, $J_{5'',6''} = 6$, $J_{4'',5''} = 0.5$ Hz, H-5″), 4.18 (2 H, complex, H-3″ and H-4″), 5.03 (1 H, *t*, *J* = 9 Hz, H-2″), 5.56 (1 H, *d*, *J* = 9 Hz, H-1″), 6.54 (1 H, *s*, H-8), 6.78 (1 H, *s*, H-3), 7.25 (1 H, *d*, *J* = 9 Hz, H-5′), 7.50 (1 H, *dd*, $J_{5',6'} = 9$, $J_{2',6'} = 2$ Hz, H-6′), 7.83 (1 H, *d*, *J* = 2 Hz, H-2′).

REFERENCES

1. Straub, R. W., Fairchild, M. L., Zuber, M. S. and Keaster, A. G. (1973) *J. Econ. Entomol.* **66,** 534.
2. Starks, K. J., McMillian, W. W., Sekul, A. A. and Cox, H C (1965) *Ann, Entomol. Soc. Am.* **58,** 74.
3. Chan, B. G., Waiss, A. C., Jr., Stanley, W. L. and Goodban, A. E. (1978) *J. Econ. Entomol.* **71,** 366.
4. Mabry, T. J., Markham, K. R. and Thomas, M. B. (1970) *The Systematic Identification of Flavonoids*, pp. 41–61. Springer, New York.
5. Horowitz, R. M. and Gentili, B. (1964) *Chem. Ind.* 498.
6. Koeppen, B. H. and Roux, D. G. (1965) *Biochem. J.* **97,** 444.
7. Gaffield, W. and Horowitz, R. M. (1972) *J. Chem. Soc. Chem. Commun.* 648.
8. Levy, G. C. and Nelson, G. L. (1972) *Carbon-13 Magnetic Resonance for Organic Chemists*, pp. 109–135. Wiley-Interscience, New York.
9. Szarek, W. A. and Schnarr, G. W. (1977) *Carbohydr. Res.* **55,** C5.
10. Jackman, L. M. and Sternhell, S. (1969) *Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*, p. 288. Pergamon Press, Oxford.
11. Cuvelier, C., Ottinger, R. and Reisse, J. (1972) *Tetrahedron Letters* 277.
12. Gentili, B. and Horowitz, R. M. (1967) *J. Org. Chem.* **33,** 1571.

13. Hillis, W. E. and Horn, D. H. S. (1964) *Aust. J. Chem.* **18,** 531.
14. Holdsworth, D. K. (1973) *Phytochemistry* **12,** 2011.
15. Barber, G. A. (1963) *Arch. Biochem. Biophys.* **103,** 276.
16. Glaser, L. and Kornfeld, S. (1961) *J. Biol. Chem.* **236,** 1795.
17. Sweeley, C. C., Bently, R., Makita, M. and Wells, W. W. (1963) *J. Am. Chem. Soc.* **85,** 2495.
18. McElvain, S. M. (1953) *The Characterization of Organic Compounds*, p. 204. MacMillan, New York.

NOTE ADDED IN PROOF

We have isolated from certain lots of Zapolote Chico silks a compound isomeric to maysin which is not susceptible to hydrolysis by naringinase. Because of nearly identical spectroscopic properties we consider this material to differ from maysin only in configuration at the anomeric position of rhamnose.