+NaOMe 280, 332, 390; +$AlCl_3$ 280, 306, 348, 380; +$AlCl_3$+HCl 278, 304, 346, 380sh; +NaOAc 280, 306sh, 380; +NaOAc + H_3BO_3 274, 316, 340, 400sh. MS of the PM ether: EIMS 70 eV, *m/z* (rel. int.) 704 M^+ (10), 689 M-15 (32), 673 M-31 (100), 601 M-103 (18), 573 M-131 (18), 541 M-163 (41), 529 M-175 (45), 515 M-189 (23).

Acid isomerization. The sample (1 mg) was dissolved in MeOH–4 N HCl (1:1) (2 ml) and heated for 6 hr in a sealed tube under N_2 at 100°. After repeated evaporations, the residue was taken up in H_2O and extracted with *n*-BuOH. The products were identified by TLC (cellulose) in BAW.

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O-GLYCOSYLATED *C*-GLYCOSYLFLAVONES FROM *PASSIFLORA PLATYLOBA*

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Key Word Index—*Passiflora platyloba*; Passifloraceae; *C*-glycosylflavonoids; isovitexin 7-rhamnoglucoside; isomollupentin (6-*C*-arabinosylapigenin); isomollupentin 7-rhamnoglucoside; isovitexin; vitexin; esculetin.

Abstract—Five *C*-glycosylflavonoids including two new compounds, isovitexin 7-rhamnosylglucoside and isomollupentin 7-rhamnosylglucoside, were obtained from the leaves of *Passiflora platyloba*. The known flavonoids were isovitexin, vitexin and isomollupentin (6-*C*-arabinosylapigenin). In addition, the coumarin esculetin was isolated.

As part of a broad biochemical systematic investigation of the mostly New World genus *Passiflora* (Passifloraceae), we report here the flavonoid chemistry of *P. platyloba* Killip, a species which like other *Passiflora* taxa we have recently investigated [1–3], is rich in *C*-glycosylflavonoids. It is hoped that these chemical studies will ultimately provide some insight into the chemical basis for the choice of different *Passiflora* species as larval food plants by different species of neotropical butterflies of the genus *Heliconius*.

In the present investigation, the leaves of *P. platyloba* afforded isovitexin as the major compound along with vitexin, esculetin, isomollupentin (6-*C*-arabinosylapigenin) [4] and two new *C*-glycosylflavonoids, isovitexin 7-rhamnosylglucoside and isomollupentin 7-rhamnosylglucoside.

Isovitexin 7-rhamnosylglucoside

The colors of this new glycoside and its acidic hydrolytic product were purple when viewed on paper under UV light (366 nm) and yellow-green when exposed to NH_3/UV and sprayed with NA reagent. Acid hydrolysis both with 4 N HCl–MeOH (1 : 1) and with 0.1 N trifluoroacetic acid yielded isovitexin (UV and TLC comparison with standard sample) and equal amounts of rhamnose and glucose (TLC comparison with standard sugars). β-Glucosidase did not produce any hydrolysis, suggesting that glucose was not present as a terminal sugar. UV spectra showed a typical apigenin-type skeleton with free 5 and 4′-hydroxyl groups, however, the lack of band 3 in the sodium methoxide spectrum indicated a 7-OR substituent. The mass spectrum of the permethyl ether of the compound showed the characteristic fragmentation pattern of a PM 5,7-dihydroxy-6-*C*-glycosylflavone 7-*O*-glycoside [5] with two homologous series of peaks corresponding to the fragmentation of PM 6-*C*-glycosylflavones, the first series related to the molecular peak: M^+, 908; M − 15; M − 31; M − 163 (hM); M − 175 (iM) (from the 6-*C*-hexosyl residue) while the second series related to the aglycone AH^+, 516; AH − 31; AH − 47; AH − 63; AH − 163 (hAH); AH − 175 (iAH) (again from the 6-*C*-hexosyl residue). The nature of the 7-*O*-glycosyl residue is given by the difference M − A, 393 corresponding to a disaccharide containing one deoxyhexose and one hexose. As noted above, the hexosyl nature of the *C*-bound sugar is given by the difference AH − iAH, 175 and the apigenin nature of the flavone moiety by iAH, 341.

The spectral and hydrolytic data established the new glycoside as isovitexin 7-rhamnosylglucoside.

Isomollupentin 7-rhamnosylglucoside

The colors of the second new glycoside and its acidic hydrolytic product were identical to those of isovitexin 7-rhamnosylglucoside suggesting a second apigenin-type compound. Acid hydroxysis both with 4 N HCl–MeOH (1 : 1) and with 0.1 N trifluoroacetic acid established the presence of equal amounts of rhamnose and glucose (TLC comparison) and the aglycone was found to be isomollupentin (6-*C*-arabinosylapigenin) by UV and TLC comparison. The UV spectral data supported an apigenin skeleton with 7-*O*-substitution, i.e. no band 3 in the sodium methoxide spectrum. β-Glucosidase hydrolysis did not liberate glucose, suggesting that glucose was not a terminal sugar. The mass spectrum of the permethyl ether of the compound again showed the characteristic fragmentation pattern of a PM 5,7-dihydroxy-6-*C*-glycosylflavone 7-*O*-glycoside: M^+, 864; M − 131 (iM); A, 471; AH − 15; AH − 31; AH—119 (hAH); AH − 131 (iAH). The difference M − A, 393 again identified the 7-*O*-glycosyl residue as a disaccharide containing one deoxyhexose and one hexose and the importance of the ion *m/z* 189 showed the deoxyhexose to be at the non-reducing end of the disaccharide [6]. The pentosyl nature of the C-bound sugar was given by the difference AH − iAH, 131 and the apigenin nature of the flavone moiety by iAH, 341.

These spectral and hydrolytic data established the structure of the new compound as isomollupentin 7-rhamnosylglucoside.

EXPERIMENTAL

Plant material. Leaf material was collected from plants grown in Dr. L. E. Gilbert's greenhouse collection, Department of Zoology, University of Texas at Austin. The rootstock is from Guanacasta, Costa Rica (voucher no. 70406).

Extraction and isolation. The dried and powdered leaves of *Passiflora platyloba* (31 g) were extracted with 96% EtOH in a Soxhlet. The aq. soln obtained after concentrating the extract to small vol. was extracted with C_6H_6, $CHCl_3$ and EtOAc: yields of syrups were 3, 2 and 3 g, respectively, with the remaining aq. layer affording 1.6 g of syrup.

The $CHCl_3$ concentrate was fractionated on a Polyclar column (2 × 30 cm). Elution was initiated with Egger's solvent ($CHCl_3$–MeOH–MeEtCO–Me_2CO, 4 : 2 : 0·5 : 0·1) and the polarity of the eluate was increased by reducing the amount of $CHCl_3$. The compounds were obtained in the following order: esculetin (5 mg), isomollupentin (6-*C*-arabinosylapigenin) (5 mg), isovitexin (55 mg) and vitexin (10 mg). The concd EtOAc extract was also fractionated on a Polyclar column (2 × 30 cm); elution was started with EtOH with the polarity being increased by the addition of H_2O. The two new glycosides which were obtained from the column as a mixture were separated on a microcrystalline cellulose column with 15% aq. Me_2CO. Isomollupentin 7-rhamnosylglucoside (4 mg) and isovitexin 7-rhamnosylglucoside (5 mg) were obtained.

Acid hydrolyses. The samples were dissolved separately in MeOH–4N HCl (1 : 1) and refluxed for 1 hr. After repeated evaporations of the solvent, each residue was taken up in H_2O and extracted with *n*-BuOH. The aglycones were identified in the *n*-BuOH extracts by TLC (Si gel) in EtOAc–pyridine–H_2O–MeOH (80 : 12 : 10 : 5); the sugars were identified by TLC on Na_2HPO_4 (0.2 M)-impregnated Si gel plates in Me_2CO–H_2O (9 : 1) against standard markers; R_f: 6-*C*-xylosylapigenin, 0.74; 6-*C*-arabinosylapigenin, 0.77; glucose, 0.13; and rhamnose, 0.74. The flavones and sugars were detected with bisdiazotized benzidine–Na_2CO_3 and aniline malonate, respectively. The samples were also hydrolysed by adding 1 ml of 0.1 N TFA and refluxing for 1 hr over a H_2O bath.

Isovitexin 7-O-rhamnosylglucoside. UV λ_{max}^{MeOH} nm: 334 (1), 270 (1.1); + NaOMe 393 (1), 295 (sh), 272 (0.7); + $AlCl_3$ and $AlCl_3$–HCl 384 (sh), 340 (1), 302 (0.5), 276 (1.05); + NaOAc 400 (sh), 335 (1), 268 (1.1) and + NaOAc–H_3BO_3 335 (1), 269 (1.1). (Relative absorptivities are given for each λ_{max} relative to the longest wavelength band as 1.) TLC, cellulose plates: 40% HOAc, 0.88; 15% HOAc, 0.93; 5% HOAc, 0.56. PM ether: TLC, Si gel: R_f 0.11 ($CHCl_3$–EtOAc–Me_2CO, 5 : 4 : 1); MS of PM ether: EI/MS 70 eV *m/z* (rel. int.) M^+, 908 (18); (M − 15) 893 (24); (M − 31), 877 (81); (M − 163), 745 (10); (M − 175), 733 (10); 717 (18); 673 (16); A, 515 (31); (AH − 15), 501 (35), (AH − 31), 485 (100); (AH − 47), 469 (34); (AH − 63), 453 (85); (AH − 163), 353 (45); (AH − 175), 341 (82).

Isomollupentin 7-O-rhamnosylglucoside. UV λ_{max}^{MeOH} nm: 334 (1), 270 (0.95); + NaOMe 393 (1), 308 (sh), 272 (0.5); + $AlCl_3$ and $AlCl_3$–HCl 378 (sh), 352 (1), 300 (0.5), 276 (0.8); + NaOAc 394 (sh), 338 (1), 270 (0.95) and + NaOAc–H_3BO_3 335 (1), 270 (0.9). TLC, cellulose plates: 40% HOAc, 0.88; 15% HOAc, 0.93; 5% HOAc, 0.60. PM ether: TLC, Si gel: R_f 0.06 ($CHCl_3$–EtOAc–Me_2CO, 5 : 4 : 1); MS of PM ether: EI/MS 70 eV, *m/z* (rel. int.) M^+, 864 (11); (M − 15), 849 (20); (M − 31), 833 (70); 803 (24); (M − 119), 745 (12); (M − 131), 733 (5); 673 (7); 629 (13); A, 471 (22); (AH − 15), 457 (52); (AH − 31), 441 (100); (AH − 47), 425 (35); (AH − 63), 409 (71); (AH − 119), 353 (50); (AH − 131), 341 (57); (AH − 145), 327 (33); 189 (79).

Vitexin, esculetin and isomollupentin were identified by UV, MS and standard sample comparisons.

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FLAVONE 5-*O*-GLUCOSIDES FROM *DAPHNE SERICEA*

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Key Word Index—*Daphne sericea*; Thymelaeaceae; flavones; flavone 7- and 5-*O*-glucosides; isovitexin; coumarins; a steroidal glycoside; antitumor activity.

Abstract—The aerial parts of *Daphne sericea* yielded two new flavonoids, luteolin 7-methyl ether 5-β-D-glucoside and luteolin 7,3′-dimethyl ether 5-β-D-glucoside, as well as luteolin 7-methyl ether, isovitexin, apigenin and its 7-β-D-glucoside.

INTRODUCTION

Since *Daphne mezereum* (family Thymelaeceae) was previously found to contain the antileukemic diterpenoid mezerein [1–3], an extract of the aerial parts of another *Daphne* sp., namely *D. sericea* Vahl, was tested for antitumor activity and its chemical constituents investigated. Except for two new flavone 5-O-glucosides, the compounds isolated from *D. sericea* are typical of other *Daphne* sp. For example, in other studies the coumarins umbelliferone, daphnerotin, daphnetin and 7-hydroxycoumarin 8-β-D-glucoside were isolated from *D. mezereum* [4] as was the toxic diterpene daphnetoxin [5]. *D. odora* afforded the flavones luteolin and apigenin [6] and the coumarins daphnin and daphnetin [7], two compounds which were also found in both *D. pontica* [8] and *D. acuminata* [9]. The latter species also contained daphnetin 8-β-D-glucoside [10]. *D. cannabina* yielded daphnerotin and β-sitosterol [11], while the diterpenoid yuanhuacine has been isolated from *D. genkwa* [12].

RESULTS

The aqueous layer which remained after partitioning the concentrate from the ethanol extract of *Daphne sericea* with organic solvents was tested for its antitumor activity and found to be active against the 3PS *in vivo* test system. However, the compounds so far isolated from this fraction, including the coumarins daphnerotin and daphnin, sitosteryl 3-β-D-glucoside and several flavonoids, apparently do