Flavonoid Composition of *Aechmea* and *Billbergia:* Two Closely Allied Ornamental Bromeliads[1]

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Abstract. *Aechmea* and *Billbergia* are 2 morphologically similar bromeliads which are considered to be closely related. Chromatographic comparisons of the leaf extracts of *Aechmea glomerata* Hook. and *Billbergia vittata* Brongn. support a close relationship of these 2 species, i.e., the common occurrence of apigenin, luteolin, tricin and quercetin among the flavones/flavonols, delphinidin, cyanidin and peonidin among the anthocyanidin glycosides and several phenolic acid derivatives. Apigenin and luteolin glycosides are predominant in both species although there is considerable variation with respect to glycosidic types. *Billbergia vittata* is characterized by the major flavone, scutellarein glucoside[5] (6-hydroxyapigenin glucoside) and *Aechmea glomerata* contains apigenin 7-apiosylglucoside as the major compound. These major flavones appear to distinguish the one species of *Aechmea* which was examined from one species of *Billbergia*.

Aechmea and *Billbergia* are 2 of the ornamental bromeliads highly prized for their attractive flowers and leaves, the latter characterized by a thick, leathery texture. The 2 genera share many morphological characters and are considered to be closely related. There is considerable ambiguity in the recognition of their various species, many of which are referred to either genus: *Aechmea fasciata* Baker. = *Billbergia fasciata* Lindl. = *Billbergia rhodocyana*, Lem.. *Aechmea zebrina* = *Billbergia zebrina*. *Billbergia clavata longifolia* is also considered an *Aechmea* (1). The 2 genera are distinguished mainly by their flowers, the aechmeas bearing smaller flowers slightly exserted from the calyx, with conspicuous sharp pointed bracts and sepals, short filaments and small anthers. Descriptions of these 2 genera are given in the Standard Cyclopedia of Horticulture (1). The use of biochemical markers in cultivar identification (4) and in the chemosystematics of cultivated plants (3) has been frequently discussed (7). Flavonoid compounds have been employed in several studies to distinguish taxa particularly where obvious morphological differences are lacking (5).

Our purpose was to compare the flavonoid patterns of *Aechmea glomerata* and *Billbergia vittata* and identify as many of the flavonoids as possible.

Materials and Methods

The plants chosen for this study were *Aechmea glomerata* Hook. and *Billbergia vittata* Brongn. They were obtained from the Montreal Botanical Gardens[6] and maintained in the University greenhouse. Leaves were selected at random to include old and young stages and were extracted immediately after harvest.

Extraction and general preparative procedure. Leaf material weighing about 300 g was chopped into small pieces and homogenized with small volumes of 80% EtOH repeatedly. The extracts were pooled, filtered and concentrated *in vacuo* to a small volume. The concentrate was extracted with light petroleum ether to remove the chlorophyll and lipids and the resulting fraction was taken up in boiling water, mixed thoroughly with celite filter aid and the slurry filtered 2-3x under vacuum. The final filtrate was subjected to liquid-liquid extraction with ethyl acetate (EtOAc) for over 50 hr (6). The EtOAc layer containing the yellow pigments (flavones, flavonols) was separated from the aqueous phase containing the pink anthocyanins. The 2 layers were concentrated individually *in vacuo* and used for chromatography. The anthocyanin fraction was acidified with 1% HCl to stabilize the color prior to chromatography.

Acid hydrolysis: A portion of the EtOAc extract was concentrated to dryness, re-extracted into 80% EtOH and hydrolyzed with an equal volume of 2N HCl at 100°C for 1 hr (2), in order to obtain the total flavone/flavonol and possibly other aglycones. The aglycones upon re-extraction in EtOAc were chromatographed on Whatman 3 MM chromatographic paper in 3 solvent systems: Forestal (concentrated HCl, glacial acetic acid and water 3:30:10 v/v/v), PhOH (Phenol, water 3:1 v/v) and TBA (t-butyl alcohol, glacial acetic acid and water 3:1:1 v/v/v). Authentic samples of apigenin and quercetin were used as standard markers.

Acid hydrolysis of the aqueous anthocyanin fraction was carried out with an equal volume of 2N HCl in a boiling water bath for 45 minutes in the dark (10). The anthocyanidins were extracted in isoamyl alcohol and chromatographed in the Forestal solvent system, along with an authentic sample of cyanidin as the marker.

Chromatography and characterization of flavonoids. The major portion of the EtOAc not used for hydrolysis was spotted on many sheets of Whatman 3MM chromatographic paper and developed with TBA for the first direction and 15% HOAc (glacial acetic acid, water 15:85 v/v) for the second direction (13). The individual compounds were exposed to NH_3, viewed under UV, and the spots cut out. These were then transferred to small labeled Erlenmeyer flasks containing 80% EtOH and agitated for 1–2 hr on mechanical shakers. The eluates of individual compounds from several chromatograms were pooled, concentrated, and purified by re-chromatography on Whatman No: 1 using BAW (n-butanol, glacial acetic acid and water 4:1:5 v/v/v) and 15% HOAc for the first and second dimensions respectively. The purified compounds were eluted with spectrophotometric quality (Baker Instra Analyzed) MeOH, keeping the elution time to a maximum of 5 minutes, and subjected to a systematic procedure of ultraviolet spectral analysis (13). The Rf values of individual compounds were determined by paper chromatography in various solvents: BAW, TBA, HOAc and PhOH.

The purified, individual compounds were also subjected to acid hydrolysis for determining the aglycones and sugars, if the compound was available in sufficient quantity. Conditions of hydrolysis were the same as described earlier except that 0.2N HCl was used. The aglycones were identified by paper chromatography in TBA, BAW and Forestal solvent systems. Sugars were separated on Avicel (microcrystalline cellulose) TLC plates in ethyl acetate, pyridine, water (4:2:4 v/v/v) and detected by aniline phthalate spray reagent (12).

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[5] Tentative identification.
[6] The gift of the plants from Montreal Botanical Gardens is gratefully appreciated.
[7] The author has confirmed the occurrence of the same compound in leaf extracts of the pineapple, *Ananas sativus*, another genus of *Bromeliaceae*.
[8] Spot numbers as in Figs. 1 and 2.

The anthocyanin fraction was also concentrated and chromatographed on several sheets of Whatman No:3MM chromatography paper, using BAW and 15% HOAc solvent systems. The individual anthocyanins were eluted in 0.1% HCl, hydrolyzed with 0.2N HCl, and anthocyanidins extracted in 0.01% HCl in MeOH (Baker Instra Analyzed) for spectral characterization.

Results

The chromatographic patterns derived from crude methanolic extracts of *Aechmea glomerata* (Fig. 1) and *Billbergia vittata* (Fig. 2) were similar with regard to the blue and blue-green fluorescent compounds (lower bottom and lower right). They were also similar with respect to anthocyanins (Figs. 1 and 2) (upper right) except that *B. vittata* had fewer pink spots than *A. glomerata*. The remaining yellow fluorescent and dark absorbing compounds were identified as glycosides and aglycones of flavonols and flavones.

The distribution patterns of the main flavones and flavonols in the EtOAc extracts of *A. glomerata* (Fig. 3) and *B. vittata* (Fig. 4) were similar to those obtained in Figs. 1 and 2. However, only 4 flavonoid aglycones were present on the chromatogram from the total hydrolysis of the EtOAc extracts of both *B. vittata* and *A. glomerata*. On the basis of their Rf values in various solvents, the 4 compounds were recognized as quercetin, luteolin, apigenin and a fourth, bright yellow spot corresponding with the flavone, tricin. (Table 1)

The compounds occurring in *A. glomerata* included several glycosides of apigenin and luteolin and, on the basis of their chromatographic and spectral characteristics, the various spots shown in the figures were identified as follows: spot A_3 = luteolin 7-apiosylglucoside; spot A_6 = luteolin 7-rhamnoglucoside; spot A_8 = isovitexin; spot A_9 = apigenin 7-apiosylglucoside; spot A_{10} = apigenin 7-glucoside (Tables 3 and 4). Luteolin 7-apiosylglucoside and apigenin 7-apiosylglucoside were further confirmed by co-chromatography with standards from celery and parsley extracts (9).

Spectral and chromatographic characteristics of compound A_{12} corresponded to the flavone tricin (9, 15). The very low mobility in 15% HOAc and its high Rf in BAW and TBA suggested the compound was an aglycone. Compounds A_2 and A_4 were not available in sufficient quantity for all tests but spectral properties of A_4 in MeOH and shifts with $AlCl_3$ and H_3BO_3 indicated a flavonol with 3′4′-dihydroxy groups on the B-ring (possibly quercetin glycoside). Compounds A_1, A_5, A_7 and A_{11} were present only in traces and were not studied further.

Among the *B. vittata* compounds that were characterized (Tables 3, 4) were: spot B_{5a} = 7-glycoside of luteolin; spot B_{6a} = 7-diglucoside of luteolin; spot B_{8a} = 7-rutinoside of apigenin and spot B_{9a} = 7-glucoside of apigenin; spot B_{12} corresponded in spectral and chromatographic properties with the flavone tricin [7] and is the same as compound A_{12} in *A. glomerata*. The most interesting compound was B_{4a}. It had a characteristic dark brown appearance under UV, little affected by NH_3 vapors. The MeOH spectrum exhibited a characteristic peak at 285 nm, suggestive of a 6-hydroxyl group (9) and the λmax of Band II indicated apigenin (340 nm). The compound yielded glucose upon hydrolysis and was therefore a glycoside, with the sugar attached to the 7-position (NaOAc shift = 0). The H_3BO_3 shift indicated absence of 3′4′ vicinal hydroxyl groups. The amount of shift (48 nm) of

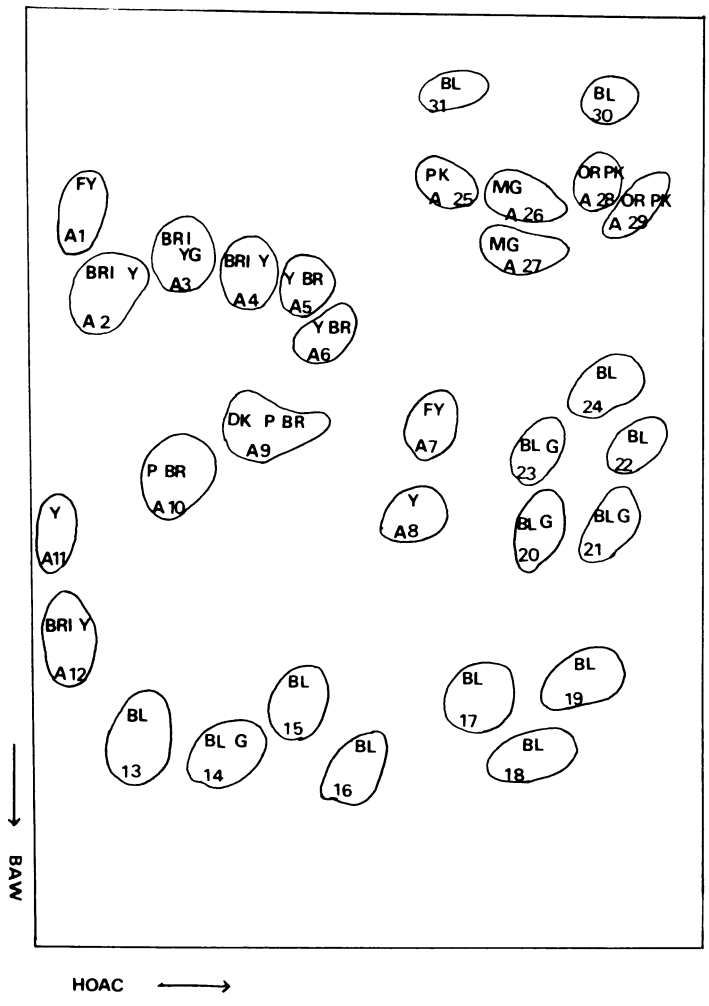

Fig. 1. Chromatographic profile of *Aechmea glomerata*. Colors denote reaction with NH_3 as viewed under UV. *Key:* BL = Blue; BR = Brown; G = Green; MG = Magenta; OR = Orange; P = Purple; PK = Pink; Y = Yellow. BRI = Bright; DK = Dark; F = Faint. Solvent system: BAW/HOAc.

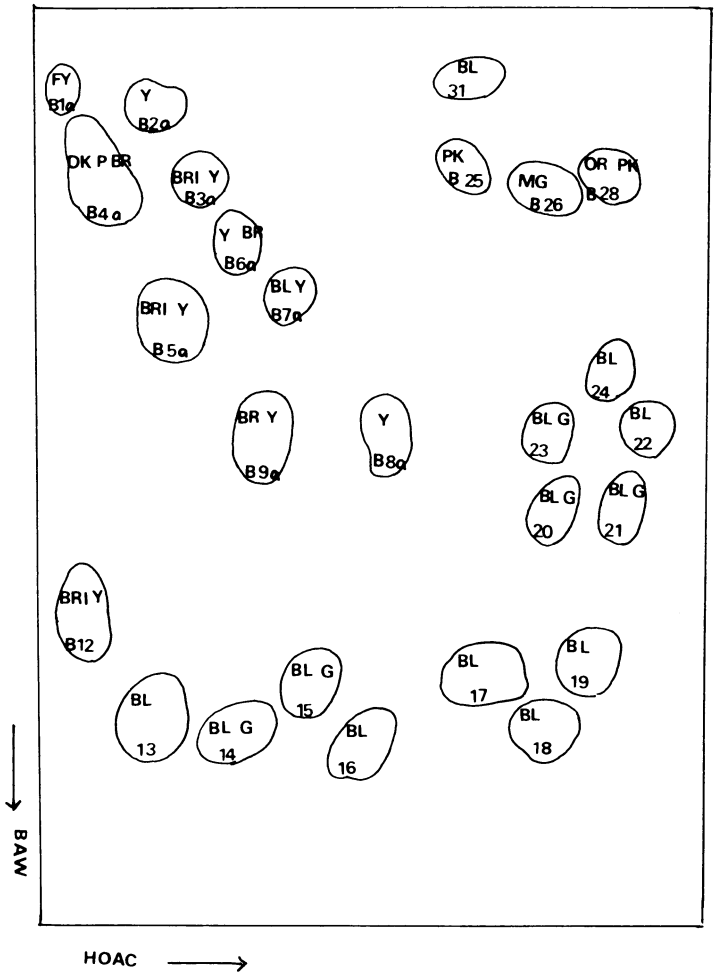

Fig. 2. Chromatographic profile of *Billbergia vittata*. Color key as in Fig. 1. Solvent system: BAW/HOAc. Compounds are numbered B1a, B2a, ... to distinguish them from *Aechmea* compounds with which they do not correspond.

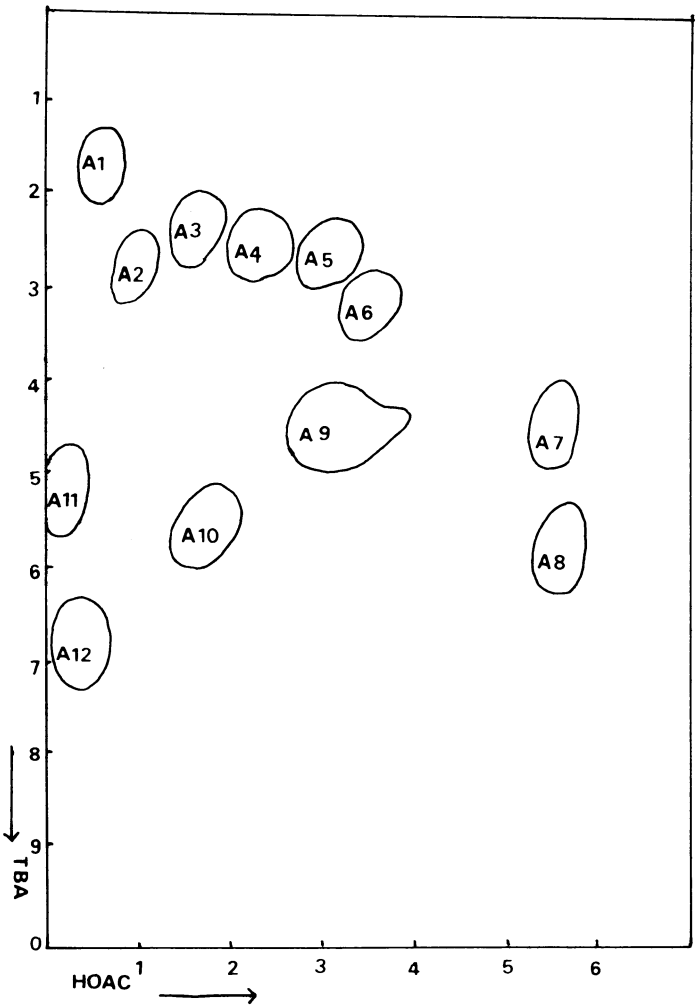

Fig. 3. Chromatogram of the total ETOAc extract of *Aechmea glomerata* in TBA/HOAc solvent system.

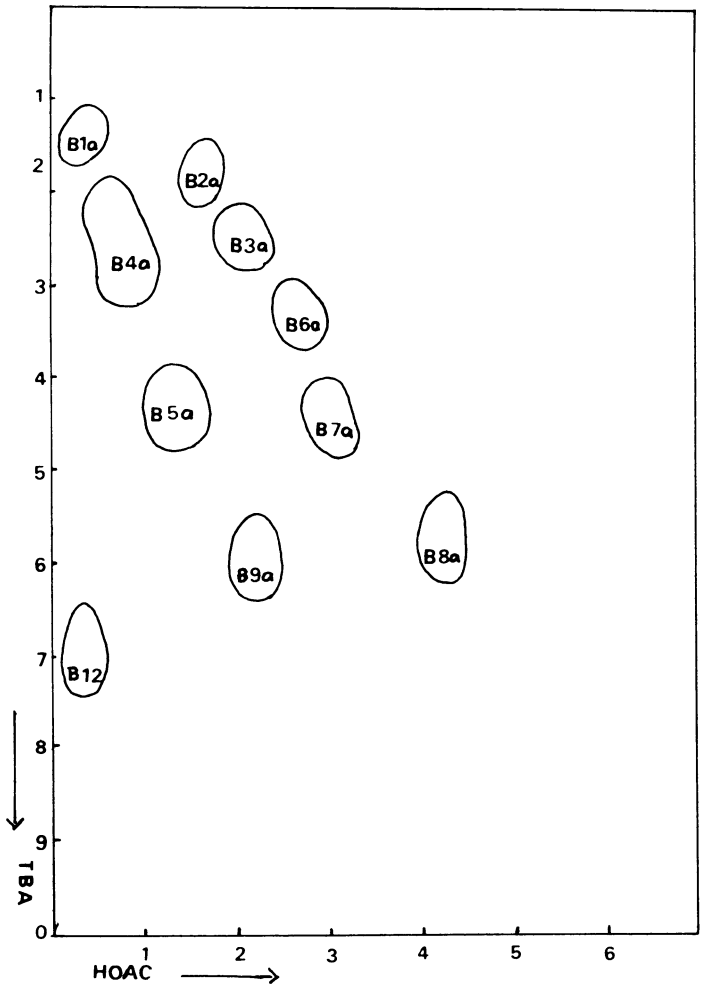

Fig. 4. Chromatogram of the total ETOAc extract of *Billbergia vittata* in TBA/HOAc system.

Table 1. Chromatographic properties of compounds obtained from acid hydrolysis of the total EtOAc fraction.

Sample	Color of spot in UV + NH_3	Rf values (× 100)			
		Forestal[z]	PhOH[z]	TBA[z]	Identification
Aechmea	1. fluorescent yellow	43	32	53	Quercetin
	2. dull yellow	66	59	75	Luteolin
	3. yellow	74	—	68	Tricin
	4. brown-yellow	85	89	89	Apigenin
Billbergia	1. fluorescent yellow	41	30	52	Quercetin
	2. dull yellow	64	60	75	Luteolin
	3. yellow	73	—	69	Tricin
	4. brown-yellow	84	89	87	Apigenin
Standard Markers:					
Apigenin	brown-yellow	83	89	87	
Quercetin	bright yellow	41	29	57	
Literature values:					
Luteolin	yellow	66	66	77	
Tricin	yellow-green	72	87	68	

[z] Forestal: concentrated HCl, acetic acid, water 3:30:10 v/v/v. PhOH: phenol, water 3:1 v/v. TBA: tertiary butyl alcohol, acetic acid, water 3:1:1 v/v/v.

Band I with NaOMe also corresponded with the value indicated in literature for scutellarein (9, 14), a 6-hydroxy derivative of apigenin. This compound appears to be unique to *B. vittata* and appeared on the chromatograms of another unknown species of *Billbergia*. Compounds B_{2a} and B_{3a} were faint spots but spectral characteristics suggested flavonol glycosides. This finding was substantiated by the presence of quercetin on the Forestal chromatograms. Spots B_{1a} and B_{7a} were too faint to elute and were not studied.

Table 2. Properties of anthocyanidins obtained by acid hydrolysis of the total anthocyanin (aqueous) fraction.

Sample	Rf values (× 100)		
	Color of spot in UV + NH_3	Forestal	Identification
Aechmea	1. bluish-pink	34[z] (32)[y]	Delphinidin
	2. magenta	53 (49)	Cyanidin
	3. orange-pink	69 (63)	Peonidin
	4. orange-pink	73 (68)	Pelargonidin
Billbergia	1. bluish-pink	35 (32)	Delphinidin
	2. magenta	52 (49)	Cyanidin
	3. orange-pink	68 (63)	Peonidin
Standard Markers:			
Cyanidin (from red rose)	pink	53	

[z] Observed values.
[y] Values in parenthesis obtained from literature. (10)

The compounds identified in *A. glomerata* and *B. vittata* corresponded closely in both Rf values and spectral properties with respective standards (Tables 3 and 4).

With respect to anthocyanins, *A. glomerata* contained glycosides of delphinidin (spot no:25)[8], cyanidin (spots 26 and 27), peonidin (spot 28) and pelargonidin (spot 29) while in *B. vittata*, glycosides of delphinidin (spot No:25), cyanidin (spot 26) and peonidin (spot 28) were present (Table 5). The aglycone moieties were determined after acid hydrolysis of the above compounds, chromatography and spectral characterization. The sugar residues were not determined. It is obvious that a delphinidin and at least 1 cyanidin glycoside (Rf BAW/HOAc = 16/50) as well as a peonidin glycoside (Rf BAW/HOAc = 20/60) occurred in both *A. glomerata* and *B. vittata*. In addition, *A. glomerata* yielded one other glycoside of cyanidin and pelargonidin. These results confirmed the earlier data obtained from acid hydrolysis of the total anthocyanin fraction of each plant (Table 2).

Although the phenolic acids were not investigated separately, some of the intensely fluorescent compounds (spots No:14, 16, 18)[8] occurring on the original chromatograms (Figs. 1 and 2) were eluted for spectral analysis. The spectra of all 3 compounds exhibited a characteristic peak between 310 and 335 nm with a prominent shoulder at 295 nm, diagnostic of cinnamic acids (8). The 3 compounds had the following spectral characteristics in MeoH:225, 290^{sh}, 310 nm; 225,285^{sh}, 325 nm and 230,290^{sh}, 320 nm. The first compound was possibly a derivative of p-coumaric acid and the latter 2 appeared to be caffeic acid derivatives.

Table 3. Rf values (× 100) of the flavonoids (in EtOAc fractions) of *Aechmea glomerata* and *Billbergia vittata*, in various solvent systems.

Compounds (Spot no.)	Color of spot in UV & NH_3	Hydrolyzed compounds							Identification
		BAW[z]	TBA[y]	HOAc[y]	PhOH[y]	Forestal[y]	BAW	TBA	
Aechmea									
A3	yellow-green	40	25	23	56	68	—	—	Luteolin 7-apiosylglucoside
		42	—	23	50	66	77	78	Literature value
A4	fluorescent yellow	33	25	25	—	41	64	—	———
A6	yellow-brown	49	31	34	—	68	74	—	Luteolin 7-rutinoside
		—	26	30	—	66	77	—	Literature value
A8	yellow	60	56	59	82	—	—	—	Isovitexin
		56	57	55	79	—	—	—	Literature value
A9	purplish-brown	54	43	40	71	80	84	85	Apigenin 7-apiosylglucoside
		57	—	42	75	83	88	87	Literature value
A10	brown-yellow	64	57	19	75	80	—	84	Apigenin 7-glucoside
		65	61	23	78	83	89	87	Literature value
A12	bright-yellow	75	68	02	—	73	71	75	Tricin
		73	68	05	—	72	68	73	Literature value
Billbergia									
B2a	yellow	11	18	15	52	—	—	—	———
B3a	bright-yellow	23	22	44	—	—	—	—	———
B4a	dark brown	25	24	08	—	57	60	74	Scutellarein glycoside
		—	—	—	—	60	66	—	Literature value
B5a	bright-yellow	43	47	15	60	69	80	69	Luteolin 7-glucoside
		44	43	15	56	66	78	66	Literature value
B6a	yellowish-brown	37	33	28	—	63	—	—	Luteolin 7-diglucoside
		40	—	29	54	66	—	—	Literature value
B8a	yellow	58	55	43	—	80	—	—	Apigenin 7-rutinoside
		58	52	46	—	83	88	87	Literature value
B9a	yellow-brown	62	59	23	82	80	—	—	Apigenin 7-glucoside
		65	61	23	78	83	88	87	Literature value
B12	bright-yellow	74	69	04	—	69	75	67	Tricin
		73	68	05	87	72	73	68	Literature value
Standard									
Apigenin		—	—	—	—	84	89	87	
Quercetin		—	—	—	—	41	66	60	

[z] BAW: n-butanol acetic acid, water 4:1:5 v/v/v.
[y] Solvent compositions as described in Table 1.

Table 4. Spectral properties of flavonoid compounds of *Aechmea glomerata* and *Billbergia vittata*.

Compounds (Spot No.)	MeOH[z]	NaOMe[z]	$AlCl_3$[z]	$AlCl_3$/HCl[z]	NaOAc[z]	NaOAc/H_3BO_3[z]	Identification[y]
Aechmea							
A_2	270 380	275 410	ND	ND	ND	ND	Flavonol
A_3	255 268 348	240^{sh} 265 390	274 300^{sh} 335^{sh} 420	275 290 355 390	265 390 430^{sh}	268 390	Luteolin 7-glycoside
A_4	268 380	270 280^{sh} 400	275 300^{sh} 425	258 275 300 390	258 270 390	270 390 430^{sh}	Flavonol
A_6	269 350	274 305^{sh} 385	260^{sh} 278 290^{sh} 340 420	273 295 350 385	270 370	270 370	Luteolin 7-glycoside
A_8	272 336	278 332 395	278 303 345 385	278 303 345 385	279 303^{sh} 375	274 346^{sh} 406^{sh}	Isovitexin
	271 336	278 329 398	278 304 352 382	280 302 344 380	279 303 385	274 345 408^{sh}	Literature value
A_9	269 290^{sh} 339	270 300^{sh} 380	260^{sh} 279 290^{sh} 350 385	260^{sh} 279 288^{sh} 350 387	268 338	268 338	Apigenin 7-glycoside
A_{10}	269 334	238^{sh} 273 300^{sh} 380	275 293 345 385	280 290 335 385	273 355	270 339	Apigenin 7-glucoside
	268 333	245^{sh} 269 301^{sh} 386	275 300 348 386	277 299 341 382	267 355 387	267 340	Literature value
A_{12}	248 265 353	265 275 412	260^{sh} 270 290 350 390	275 290^{sh} 350 390	267 390	265 370 430^{sh}	Tricin
	244 269 350	263 275 416	258^{sh} 277 303 366^{sh} 393	277 302 360 386	264 276^{sh} 321 414	270 304^{sh} 350 422^{sh}	Literature value
Billbergia							
B_3a	272 375	275 410	ND	ND	ND	ND	Flavonol
B_4a	283 342	265 300^{sh} 390	275^{sh} 300 390	258 295 375	285 350	280 345	6-OH apigenin 7-glycoside
	286 339	Δ +47					Literature value
B_5a	258 267 350	265 300^{sh} 390	273 300^{sh} 335^{sh} 425	258 272 298^{sh} 355 ^{sh} 390	262 390	262 390	Luteolin 7-glucoside
	255 267 348	263 300^{sh} 394	274 298^{sh} 329^{sh} 432	273 294^{sh} 358 387	259 266^{sh} 405	259 372	Literature value
B_6a	255 267 345	265 290^{sh} 330^{sh} 400	275 300^{sh} 420	258 275 290^{sh} 370	258 268 347	265 360	Luteolin 7-glycoside
B_8a	265 342	272 330^{sh} 395	272 300^{sh} 370 425^{sh}	272 300^{sh} 355 400	268 348 400^{sh}	268 345	Apigenin 7-glycoside
B_9a	268 336	265 385	275 298^{sh} 345 380	272 300^{sh} 330 380	268 340 365^{sh}	268 340	Apigenin 7-glycoside
	268 333	269 301^{sh} 386	276 300^{sh} 348 386	277 299 341 382	256^{sh} 267^{sh} 387	268 340	Literature value
B_{12}	248^{sh} 265 350	265 274^{sh} 410	260^{sh} 270 290 350 390	275 290 350 390	265 390	265 370 420^{sh}	Tricin
	244 269 299^{sh} 350	263 275^{sh} 416	258^{sh} 277 303 366^{sh} 393	277 302 355 386	264 276^{sh} 321 414	270 304^{sh} 350 422^{sh}	Literature value

[z] Reagents as described by Mabry (13).
[y] Identifications based on spectral data and comparison with literature data where available.

Table 5. Properties of anthocyanidins by acid hydrolysis of anthocyanins eluted from the chromatograms.

Compound	Visible color of spot	Rf values (× 100)		Spectral properties		Identification
		BAW/HOAc (glycoside)	Forestal	λmax in 0.01% MeOH-HCl nm.	$AlCl_3$ shift Δnm.	
Aechmea						
A 25	bluish-pink	15/40	35	276 546	Δ25	Delphinidin
			32	277 546	Δ23	Literature value
A 26	magenta	16/50	55	277 535	Δ15	Cyanidin
			50	277 535	Δ18	Literature value
A 27	magenta	21/51	53	277 534	Δ16	Cyanidin
			50	277 535	Δ18	Literature value
A 28	orange-pink	20/60	64	278 530	Δ0	Peonidin
			63	277 532	Δ0	Literature value
A 29	orange-pink	21/65	68	278 525	Δ0	Pelargonidin
			68	277 520	Δ0	Literature value
Billbergia						
B 25	bluish-pink	15/42	33	276 546	Δ25	Delphinidin
B 26	magenta	16/50	54	277 534	Δ18	Cyanidin
B 28	orange-pink	20/60	63	276 532	Δ0	Peonidin
Standard: Cyanidin (from red rose)	pink		53	277 534	Δ18	

Discussion

It is obvious from our data that *A. glomerata* and *B. vittata* have in common, a large number of compounds among each of the 3 classes of phenolic compounds: anthocyanins; flavones/flavonols and phenolic acids. The flavones appear to be the predominant flavonoids in both plants and are represented by apigenin, luteolin and tricin. It appears that apigenin and luteolin have been particularly exploited in both species in the synthesis of various glycosides. *A. glomerata* has luteolin and apigenin apiosylglycosides as well as isovitexin and among these, apigenin 7-apiosylglucoside is the most prominent compound. *B. vittata* has none of the above 3 glycosides but on the other hand, is characterized by the prominent brown compound tentatively identified as the glucoside of scutellarein which is absent in *A. glomerata*.

A close relationship between the 2 species is suggested by the number and types of compounds common to both. Although variation is seen in the glycosidic types especially of the flavones, the aglycones are the same. The major difference appears to be the presence of scutellarein glucoside in *B. vittata* and apigenin 7-apiosylglucoside in *A. glomerata*. The only report on some of the chemical constituents of the *Bromeliaceae* is that by Hegnauer (11). Our investigation on the flavonoid composition of 2 taxa of *Bromeliaceae* suggests that a detailed analysis of the flavonoids of various species in both genera would be of taxonomic value.

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Photosynthesis in the Rose; Effect of Light Intensity, Water Potential and Leaf Age[1]

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Abstract: The net rate of $^{14}CO_2$ uptake was determined on individual leaves of *Rosa hybrida*, cv. 'Forever Yours', budded on *Rosa manetti* and grown in gravel. Rose leaves were found to reach an average peak of 11 mg CO_2 dm^{-2} hr^{-1} about 6 days after the red color disappeared on the leaf underside, or 32 days after harvesting the previous flower on the parent cane. Thereafter, CO_2 uptake declined during 14 days to 5 to 6 mg CO_2 dm^{-2} hr^{-1}. At ambient CO_2 concentrations of 500 ppm, the maximum net uptake was near 3400 ft-c. However, internal plant water potential influenced the CO_2 uptake by reducing it at each increase of radiant energy. This resulted in light saturation at lower energies the lower the plant water potential. Radiant energy affected both net CO_2 uptake and water potential. Wilting was generally observed to occur at about −13 bars, and maximum rates of CO_2 uptake were found at potentials of −8 bars or higher, over a range of 350 to 450 microeinsteins, or 3000 to 3500 ft-c.

There is inadequate information on basic physiological processes in the rose. In order to intelligently manipulate environment for maximum production, one of the important factors to elucidate is how the rate of photosynthesis may vary with changes in the environment. Provided sufficient, accurate information is obtained, it may be possible to predict the environment required for the rose to produce at its genetic potential. This study reports on effects of radiant intensity, water potential, and changes in net CO_2 uptake with leaf age.

Materials and Methods

Rosa hybrida, cv. 'Forever Yours', budded on *R. manetti*, were established in a granitic gravel in 15 liter, plastic containers, and grown in a fiberglass-covered greenhouse. Temperatures were 16.7°C nights and 22.2°C days. Forced-air ventilation began between 25.6 and 26.7°C. The total ventilation time during this study (October to May), was less than 20 hours. Relative humidity during the daylight hours was maintained near 70% with high pressure mist. CO_2 was injected at the same time at sufficient rates to maintain 500 ppm under conditions of maximum solar radiation when the ventilation system was off. The plants were automatically irrigated 2 to 5 times daily, depending upon the season, using a nutrient solution devised by Sadisaviah (9).

Technique. The net CO_2 uptake determination method we used has been described by Shmishi (8). Briefly, leaf sections were exposed to flowing $^{14}CO_2$, total CO_2 level 500 ppm, for about 30 seconds. One cm diameter leaf sections were excised, digested to remove the ^{14}C, and the resultant activity determined by liquid scintillation counting. The method was deliberately chosen for its versatility in the field, although precision may be decreased. Supplemental studies included examination of radiation differences within the greenhouse as the result of location, calibration for the loss of counting sensitivity ("quenching") due to the technique used for incorporating ^{14}C in the scintillation fluid, radiation transmittance through the plastic $^{14}CO_2$ leaf applica-

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[3] Model 756, Weston Instrument Co., Monterey Park, CA.

[4] Lampda Instrument Co., Lincoln, NE., Sensor Mod. No. L1-190S.

[5] Model IT-2, Barnes Engineering Co., Stamford, CN.