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Amino Acids and Sugars in Nectar, and Their Putative Evolutionary Significance*

By

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Abstract: Individual amino acids and sugars from flower nectar of 32 plant species with different pollination systems were quantified and compared. Data show that there is no correlation between sugar and amino acid concentration. Furthermore there is no correlation between composition and concentration of amino acids and evolutionary advancement, nor any direct relation with pollination systems. However, higher sugar concentrations are often linked with more advanced morphological characters. Nectars from pierced or damaged flowers or nectars contaminated with pollen exhibit modifications and increases in amino acid composition. The presence of proline probably indicates such pollen contamination. Most pollinating animals depend on flower nectar in their energetic requirements, yet innumerable alternative amino acid and protein sources exist. Future research has to consider the relationship between nutritional requirements of pollinating animals and dependence on flower nectars.

It is not surprising that nectar, which in addition to pollen and fatty oil (VOGEL 1974, 1975, SIMPSON & al. 1977) is one of the major primary attractants and rewards of flowers to their pollinators, has already been the object of considerable ecological, chemical and phylogenetical investigations. A compilation of the literature on nectaries and nectar

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* Dedicated to Prof. Dr. L. VAN DER PIJL, Den Haag, in honour of his 80th birthday.

(BROWN 1961, 1963) demonstrates a long-standing and wide-spread interest in these organs and their fluids by anatomists, physiologists, floral biologists and bee-keepers.

In recent years an increasing emphasis has been given to quantify the energy-providing sugars, amino acids and other chemicals of nectar for comparison with foraging and other behavior of flower visitors (HEINRICH & RAVEN 1972, HEINRICH 1975 a, 1975 b, BAKER 1978, BAKER & BAKER 1973 a, 1973 b, 1975, 1977, 1979, BAKER & al. 1973, etc.). The belief is that the visiting of flowers by animals, changes in insect activities during anthesis, flower preferences and flower constancy, as well as competition between plant species for visitors (see e.g. HEINRICH & RAVEN 1972, VOGEL 1978, YANAGIZAWA & GOTTSBERGER in press, etc.) can be better understood when explained on the basis of nectar (as well as pollen and oil) consumption and nutritional and energetic requirements of animals.

Since 1972, H. and I. BAKER have been focusing on nectar amino acids (as well as proteins, lipids, ascorbic acid and alkaloids) based on analyses of several hundred plant species from temperate and tropical regions. Although amino acid concentrations vary greatly, patterns were seen in relation to the pollination systems and taxonomic position of the plants. The mean values for nectar from species with "advanced" morphological characters or pollination systems normally showed higher amino acid concentrations than from those with more "primitive" characters.

Nectar production and concentration can be subject to considerable fluctuations (even within individual flowers) as the result of sometimes subtle changes in the environment, such as wind, temperature, relative humidity, cloudiness, soil moisture, the position of the flower on the plant, shading, age of the flower, seed development, pollinator activity and/or reabsorbation (WOOD 1961, LÜTTGE 1961, PERCIVAL 1965, MASSENGALE & al. 1968, KUGLER 1970, FREE 1970, CRUDEN 1976, CORBET 1978, CORBET & al. 1979 a, 1979 b, BAKER 1978: 65, Table 3.3, G. HEINRICH 1975). Therefore, sugar and amino acid concentration can also fluctuate to some degree, making it difficult for the flower ecologist to collect comparable nectar samples between and within species.

Although the present authors do not deny that nectar amino acids indeed may have some importance for nutrition for some flower visiting animals (see e.g. CAMARGO & al., in press), we doubt that amino acid concentrations reflect evolutionary advancement or are directly connected with the pollination systems. "Whether the quantity and composition of nectar amino acids are of importance as differentiating character in pollination syndromes, seems uncertain" (FAEGRI & VAN DER PIJL 1979: 67).

This article proposes to contribute to the subject of the relationship between amino acid and sugar concentrations and plant evolutionary taxonomy in a twofold way: 1) To give comparative quantitative data of individual amino acids and sugars of several species with different pollination systems. 2) To provide the amino acid and sugar concentrations of nectar from different flowers collected at the same time from a single plant. Uniformly quantified data are compared to show the variation between individual flowers, thus providing a base and validity for comparison of concentrations and composition of nectar chemicals as a whole.

Material and Methods

A total of 49 nectar samples from 32 different species was investigated. Of these, 17 are native species from the cerrado, forest and swamp vegetation near the city of Botucatu (22° 45′ S, 48° 25′ W), State of São Paulo (sample numbers 3 to 12, 13–24, 25 to 28, 41, 42, 44, 45, 47, 48); 12 species are cultivated ornamentals, also collected in Botucatu (nos. 1, 2, 30 to 33, 35, 38, 39, 40, 43, 46, 49); three samples are of two native (nos. 29, 36) and one cultivated (no. 34) species from the region of the Serra do Cipó, State of Minas Gerais; and one species (no. 37, also the only one with extra-floral nectaries) is from coastal forests at Bertioga, State of São Paulo.—Voucher herbarium specimens are deposited in the herbaria UB, BOTU and NY.

These species were grouped and classified according to their most common pollinator types. This was possible through previous field observations (see GOTTSBERGER 1972, SILBERBAUER-GOTTSBERGER 1972, SILBERBAUER-GOTTSBERGER & GOTTSBERGER 1975, and unpublished results, SAZIMA & SAZIMA 1975, SAZIMA 1981). Special cases include (1) *Norantea brasiliensis* with extra-floral nectaries, which was considered among bat pollinated "flowers" (compare VOGEL 1968, 1969); and (2) *Crotalaria anagyroides* with flowers that are frequently visited and pollinated by long-tongued bees (YANAGIZAWA & GOTTSBERGER in press), but also permits short-tongued bees to exploit its nectar and is therefore considered among the short-tongue bee class.

Each nectar collection was made very carefully by inserting a capillary glass or plastic tube into the nectar of a producing flower. Maximal caution was taken to avoid the harming of tissues of the flower parts or mixing the nectar with pollen from its own flower. The nectar amount of each sample (normally a mixture from several flowers and, therefore, in itself a mean) was measured immediately after withdrawal from the flowers by the aid of a micropipette, mixed with a known volume of alcohol (70% for preservation) and kept within a sealed glass container until analyses. The sample (recorded by nos. 13–24) represents the mean of 12 individually analyzed nectar samples of identical volume from separate flowers of the same tree collected at the same time. Calculation of sugar and amino acid amount per ml nectar was possible since nectar and alcohol volumes of all 49 samples had been measured separately before mixing.

Nectar was collected during the time of activity of both the flower and its pollinator. In the case of diurnal flowers samples were collected during the daytime; nocturnal ones were collected during the night or early in the morning. We

avoided collecting during adverse weather conditions, such as during rain or very hot hours.

Sugar Determination: The carbohydrates were analysed by two different methods: (a) total carbohydrates according to the anthron method (DUBOIS & al. 1956)—total sugar (II); (b) identification of saccharides after separation by thin-layer chromatography (HANSEN 1975), and then quantitative determination using the UV test before and after enzymatic hydrolysis (Boehringer Test Combination no. 15 824). The sum of the identified individual sugars is given as total sugar (I).

Differences in the total sugar content (I) and (II) result from the fact that the mild enzymatic method (b) records only saccharides, whereas the phenol-sulphuric acid method (a) results in a partial hydrolysis of polysaccharides. Therefore, in general the anthron method (a) gives about 20% higher values. All values are calculated in mg per ml nectar volume.

Amino Acid Determination: Amino acids have been determined directly in the alcoholic solution of the nectar samples (and are presented as μg per ml nectar volume) using the amino acid analyser Jeol JCH-6AH, modified for special application (for details see LINSKENS & SCHRAUWEN 1969, SCHRAUWEN & LINSKENS 1974, WELTE & al. 1971).

Results

The results of quantitative sugar determination by methods I and II are shown in Table 1. The sample 13–24 is the mean value of the 12 individual samples, separately shown also in Table 2. The total concentration of sugars varies from 117.5 mg/ml to 629.4 mg/ml nectar amount (method I) or from 121.0 mg/ml to 723.0 mg/ml nectar amount (method II).

The total sugar amount of investigated samples (from method I) and the mean values per pollinator type are shown in Table 3. A ranking of sugar concentrations from the highest to the lowest mean values has the following sequence: The highest values were found to occur for long-tongued and short-tongued bee flowers, followed by hummingbird, moth, bat, Old World bird and mixed insect pollinated flowers. The more convincing data result from means obtained from the higher number of samples. In only two cases is the "mean" taken from only one sample, which obviously has limited confidence. The 10 most sugar-concentrated samples include 5 bee and 5 bird pollinated species and the least concentrated include one mixed insect flower type, 4 bat and 5 bird flower types (see Table 3).

In the nectar samples three sugars could be identified: Glucose, fructose and saccharose. In some cases additional weak spots could be observed on the thin-layer plates, but could not be identified with the available 35 reference sugars at our disposition. The amounts of those unknown spots are very small and are either artefacts of the separation procedure or still unknown compounds.

Saccharose is the dominant sugar in 28 samples (25 with concealed and 3 with exposed nectar). Six samples (4 with concealed and 2 with open nectar) have glucose as dominant sugar. Three samples from flowers with open nectar apparently have balanced glucose-fructose dominated nectars. One sample shows glucose, fructose and saccharose in about the same concentration.

In 4 cases investigation of the nectar-sugar concentration was repeated for the same species. Samples 6 and 7 are both from *Pyrostegia venusta*; 11 and 12 from *Caryocar brasiliense*; 32 and 33 from *Hibiscus rosa-sinensis;* 34 and 35 from *Malvaviscus arboreus*. Data in Table 1 show a sizable difference in concentration and also composition of nectar-sugars in these samples although the dominating sugars in each of both samples remain the same. Table 2 shows 12 samples from *Caryocar brasiliense* flowers for which the possibilities of differences in concentration and/or composition of nectar-sugars through collecting from different individuals or at different times is excluded. The lowest concentration (relying on results from method I) of sugars is 252.4 mg/ml nectar amount (no. 22) and the highest 322.1 mg/ml (no. 19). The proportion of individual sugars is not absolutely constant and it was even found that in 5 samples the slightly dominating sugar is fructose, whereas in 7 samples glucose is dominant over fructose.

In accordance with BAKER & BAKER's (1973 a, 1973 b, 1975) comparison of phyletic significance of amino acid concentrations, identical characters were selected to show the mean values of sugar concentration. The following pairs of primitive/advanced characters are used: woody or herbaceous species, actinomorphic or zygomorphic flowers, hypo- and perigynous or epigynous ovaries, choripetalous or sympetalous flowers with many or few stamens, and exposed or concealed nectaries (Table 4). The first character listed always is meant to be the more primitive. In the case of the character "many stamens", however, a secondary polyandrous androecium has to be considered as more advanced than many flowers bearing fewer stamens (for discussion see e.g. LEINS 1964, 1971, KUBITZKI 1973, STEBBINS 1974, EHRENDORFER 1977, GOTTSBERGER 1977). Many of the mentioned characters do not apply to *Norantea* because of its extrafloral nectaries, therefore its data are excluded from calculations.

Means of sugar concentration for all woody species together are lower than that for the herbaceous species. This applies also for most of the other characters considered primitive, such as actinomorphic, choripetalous flowers, many stamens and exposed nectar. The only exception is for the position of the ovaries, where species with inferior ovary (epigyny) have a lower sugar concentration than species with the ovary in the original superior or medium position.

Table 1. Results of quantitative sugar determination (in mg/ml nectar volume) by methods I and II

Plant	No.	Enzymatic Glucose	Fructose	Saccharose	Total I	Anthron Total II	Enz/Anth %
Acanthaceae							
Jacobinia carnea (LINDL.) NICHOLSON	1	84.4	72.3	366.0	522.7	517.0	101
Sanchezia nobilis HOOK.	2	38.7	69.6	268.7	377.0	407.0	93
Bignoniaceae							
Anemopaegma laevis DC.	3	76.3	104.6	131.8	312.7	434.5	72
Distictella elongata (VAHL) URB.	4	7.2	50.4	349.2	406.2	540.0	85
Fridericia speciosa MART.	5	49.4	75.4	278.2	403.0	533.0	75
Pyrostegia venusta (KER.-GAWL.) MIERS	6	70.2	72.5	420.0	562.7	570.0	99
	7	19.0	32.7	258.9	310.6	317.3	98
Zeheyra digitalis (VELL.) HOEHNE	8	31.0	36.2	214.3	281.4	297.0	95
Bombacaceae							
Eriotheca gracilipes (SCHUM.) ROBYNS	9	53.8	51.6	342.9	448.3	462.0	97
Caesalpiniaceae							
Bauhinia rufa (BONG.) STEUD.	10	58.1	34.9	39.5	132.5	137.5	96
Caryocaraceae							
Caryocar brasiliense CAMB.	11	80.3	64.2	20.4	164.9	233.0	71
	12	140.8	126.4	75.6	342.8	440.0	78
	13–24 mean	132.4	128.4	29.0	289.7	378.9	76
Chrysobalanaceae							
Licania humilis CHAM. & SCHLECHT.	25	28.8	32.7	56.0	117.5	121.0	97
Fabaceae							
Crotalaria anagyroides H. B. K.	26	53.9	71.5	468.6	594.0	594.0	100
Erythrina crista-galli L.	27	146.3	141.0	235.0	522.3	539.5	97
Lobeliaceae							
Siphocampylus sulphureus WIMM.	28	57.2	96.8	237.6	391.6	539.0	73
Lythraceae							
Lafoensia pacari ST. HIL.	29	113.1	107.7	5.6	226.4	252.0	89

Malvaceae							
Abutilon megapotamicum (SPR.) ST. HIL. et HAUD	30	121.3	128.3	125.3	374.9	451.0	83
Abutilon striatum DICKS.	31	75.9	61.6	313.5	451.0	401.5	112
Hibiscus rosa-sinensis L.	32	130.7	120.6	3.4	254.7	297.0	85
Hibiscus rosa-sinensis L.	33	129.8	110.0	32.9	272.7	313.5	87
Malvaviscus arboreus CAV.	34	15.1	44.6	86.2	145.9	171.7	85
Malvaviscus arboreus CAV.	35	42.0	103.0	171.3	316.3	385.0	82
Pavonia montana GARCKE	36	120.2	103.0	105.0	328.2	377.7	87
Marcgraviaceae							
Norantea brasiliensis CHOISY	37	62.1	63.8	12.3	138.2	172.3	80
Mimosaceae							
Inga sp.	38	78.7	79.0	205.2	362.9	370.5	98
Musaceae							
Musa cf. *ensete* I. F. GMELIN	39	93.5	40.7	104.5	238.7	319.0	75
Onagraceae							
Fuchsia regia (VAND.) MUNZ.	40	14.3	60.5	145.2	220.0	247.5	89
Orchidaceae							
Habenaria ornithoides BARB. RODR.	41	3.1	6.7	265.5	275.3	371.4	74
Passifloraceae							
Passiflora alata DRYAND	42	142.2	148.2	339.0	629.4	732.0	86
Proteaceae							
Grevillea forsterii hort.	43	4.4	5.5	226.6	236.5	352.0	67
Rubiaceae							
Tocoyena formosa SCHUM.	44	3.3	12.1	234.3	249.7	286.0	87
Solanaceae							
Cestrum corymbosum SCHLECHT.	45	29.9	43.2	85.8	158.9	208.0	76
Datura cf. *arborea* L.	46	4.8	6.0	235.2	246.0	240.0	102
Tiliaceae							
Luehea speciosa WILLD.	47	67.3	60.2	158.3	285.8	297.0	96
Luehea cf. *speciosa* WILLD.	48	72.5	71.4	253.9	397.8	429.0	93
Zingiberaceae							
Costus sp.	49	2.1	4.3	523.0	529.4	577.5	93

Table 2. Results of quantitative sugar determination (mg/ml nectar) by methods I and II of 12 *Caryocar brasiliense* samples

No.	Glucose	Fructose	Saccharose	Total I	Total II	T I/T II %
13	106.9	111.3	72.5	290.7	378.0	77
14	125.2	131.5	27.5	284.2	367.5	77
15	136.1	142.4	13.9	292.4	399.0	73
16	137.8	133.3	10.3	281.4	378.0	74
17	143.2	146.0	10.3	299.5	367.5	82
18	106.9	107.5	44.7	259.1	336.0	77
19	146.8	144.3	31.0	322.1	399.0	81
20	136.0	122.2	20.6	278.8	388.5	72
21	134.2	126.0	20.6	280.8	357.0	79
22	128.7	116.8	6.9	252.4	346.5	73
23	139.7	118.7	58.6	317.0	388.5	82
24	146.8	140.5	31.1	318.4	441.0	72

Table 3. Comparison of means of amino acids (μg/ml nectar) and sugars (mg/ml nectar) for plant species grouped according to their pollinators. Samples excluded from calculation ("proline" and pierced flower specimens) are given in brackets; see text

Pollination by:	Amino acids	Sugars
Short-tongued bees		
Crotalaria anagyroides	65.95	594.0
Eriotheca gracilipes	8.91	448.3
Means	**37.43**	**521.15**
Long-tongued bees		
Anemopaegma laevis	92.06	312.7
Costus sp.	9.79	529.4
Distictella elongata	11.82	406.2
Passiflora alata	(80.5)	629.4
Means	**37.89**	**469.43**
Flies, wasps, butterflies and bees (mixed)		
Licania humilis	28.54	117.5
Moths (hovering and settling)		
Datura cf. *arborea*	42.6	246.0
Habenaria ornithoides	113.71	275.3
Inga sp.	34.17	362.9
Tocoyena formosa	24.75	249.7
Means	**53.80**	**283.48**

Table 3 (continued)

Pollination by:	Amino acids	Sugars
Old World birds		
Musa cf. *ensete*	(1446.7)	238.7
Grevillea forsterii	42.4	236.5
Means	**42.4**	**237.6**
Hummingbirds		
Abutilon megapotamicum	31.9	374.9
Abutilon striatum	38.83	451.0
Erythrina crista-galli	(1890.9)	522.3
Cestrum corymbosum	77.82	158.9
Fridericia speciosa	65.52	403.0
Fuchsia regia	52.76	220.0
Hibiscus rosa-sinensis	34.53	254.7
Hibiscus rosa-sinensis	31.35	272.7
Jacobinia carnea	57.34	522.7
Malvaviscus arboreus	85.9	145.9
Malvaviscus arboreus	30.5	316.3
Pavonia montana	61.6	328.2
Pyrostegia venusta	5.45	562.7
Pyrostegia venusta, pierced flower	(91.63)	310.6
Sanchezia nobilis	(257.87)	377.0
Zeheyra digitalis	15.84	281.4
Means	**45.33**	**343.89**
Bats		
Bauhinia rufa	17.93	132.5
Caryocar brasiliense	179.6	164.9
Caryocar brasiliense	729.9	342.8
Caryocar brasiliense, shown separately	(1735.2)	(289.7)
Lafoensia pacari	19.8	226.4
Luehea speciosa	93.82	285.8
Luehea cf. *speciosa*	127.44	397.8
Norantea brasiliensis	6.05	138.2
Siphocampylus sulphureus	22.0	391.6
Means	**149.56**	**260.0**

The variation of individual amino acids in the samples is shown in Table 5. The concentration of the amino acids is in general low: in many cases it is around limits of traceability (below 0.1 μg/ml nectar amount). Values $\leqslant 2\,\mu$g/ml therefore have only a limited confidence. The table is blank where there was no trace detectable with the methods used.

The collection of nectar was carefully done, to avoid contamination. Further evidence of purity is the absence of the amino acid proline, which is always present in pollen in huge amounts (LINSKENS & SCHRAUWEN 1969, STANLEY & LINSKENS 1974) and would have been

Table 4. Sugar (mg/ml nectar) and amino acid (μg/ml nectar) concentrations (means) for species (samples) grouped according to "primitive" or "advanced" characters

Plant characters	Sugars	Amino acids
Woody	332.15	75.78
Herbaceous	443.07	52.86
Actinomorphic	299.23	91.30
Zygomorphor	381.04	43.32
Hypo- and perigynous	337.93	78.14
Epigynous	317.45	44.60
Choripetalous	328.53	90.26
Sympetalous	344.58	40.85
Many stamens	310.82	107.73
Few stamens	350.82	43.90
Exposed nectar	260.87	168.19
Concealed nectar	351.81	49.82

present in the nectar samples if contamination had occurred. The amino acid values determined, therefore, must be the actual constituents of genuine nectar. There are, however, 7 samples in which proline was detected in traceable to moderate concentrations. The 4 samples (nos. 2, 27, 39 and 42) in which proline concentration was above 5 μg/ml were separated and their amino acid concentration and composition compared separately. Possibly these nectar samples contain amino acids from nectar plus pollen and/or damaged tissues, since the whole amino acid concentration was abnormally high. Sample 7 (a repetition of *Pyrostegia venusta*) was from strongly pierced flowers and with amino acid values 17 times higher than from unpierced flowers (sample no. 6). This sample from damaged flowers was excluded as were the 12 *Caryocar* samples (no. 13–24) which are considered separately.

The first and somewhat unexpected result demonstrated was that there is **no** correlation between sugar and amino acid concentration. This can be seen by a direct comparison of data from Tables 1, 2 and 5. Samples with low amino acid concentration may have either a low or a high sugar concentration as may the samples with a high concentration of amino acids. No regularity can be observed on the level of individual samples, nor when comparing the pollinator types (see Table 3). For example, the short-tongued bee flowers with the highest sugar amount show a low concentration of amino acids; bat pollinated flowers with low sugar values, however, are the most concentrated in amino acids.

Surprisingly, when dividing the samples as to their "primitive" or "advanced" morphological state (see Table 4), without exception, the lower means are always from samples representing the "more advanced" character. Even when excluding the second high value of *Caryocar* (no. 12) (which modifies the value of the means), the principal result remains unchanged. The lack of correlation between sugar and amino acid concentration is shown also in a scatter diagram (Fig. 1). A convincing

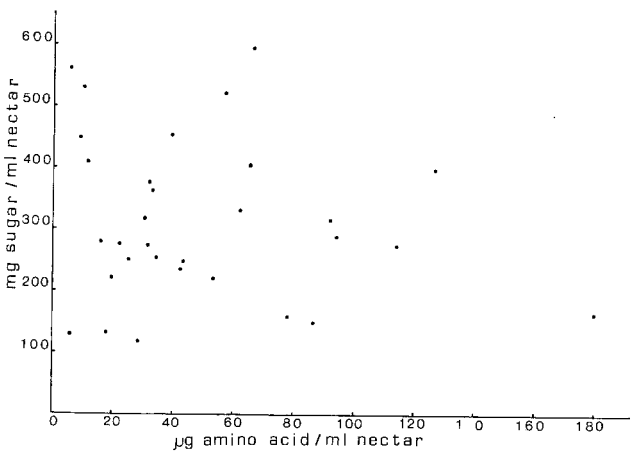

Fig. 1. Scatter diagram showing the lack of correlation between sugar and amino acid concentration for 28 species (31 nectar samples). Sample numbers 2, 7, 12, 13–24, 27, 39 and 42 ("proline" and pierced flower specimens) excluded

demonstration of relative independence of sugar and amino acid concentration is the comparison (Tables 2 and 6) of the 12 *Caryocar* samples 13–24. A low amino acid concentration (sample no. 21) is not necessarily correlated (not even in individual flowers) with low sugar content; samples number 18, 20, 22 contain less sugar but much more amino acids than no. 21.

The lowest amino acid concentrations (means) are found in nectar of a mixed insect pollinated flower type. Short- and long-tongued bee species show about the same concentrations. Somewhat more concentrated are nectars from Old World bird and hummingbird pollinated species, followed by moth pollinated species. Bat flowers show the highest amino acid amount in their nectar (Table 3). The results of means and ranges are shown in graphical form in Fig. 2 and are compared with results from BAKER & BAKER (1973 a, 1973 b, 1975) and

Table 5. Results of quantitative amino acid determi

Plant No.	MetSO	Asp	Thr	Ser	Glu	Gly	Ala	Val	GalNH$_2$	Met	Ileu
1		5.72	7.48	19.7		10.8	8.03				2.20
2		19.0	100	26.8	82.1		8.58	7.59			
3	7.37	11.9	5.17	6.16	19.5	4.07	5.83	16.0			2.42
4		1.5	0.96	1.86	1.14	1.38	1.14				
5		7.54		12.48	8.84	6.76	6.76	12.48			
6		2.39				3.06					
7		7.70	19.3	14.3	24.0	8.24	7.83				
8						8.25	7.59				
9						4.62	4.29				
10		1.21	2.86	3.74		1.54	1.10	4.07			
11	2.3	6.7	54.6	8.6	31.5	3.7	4.0	3.0	7.0	0.2	0.7
12		16.4	90.2	45.1	86.1	24.6	20.5	24.6			8.2
13–24			682.0	70.8	311.0	36.6	25.3	16.0			4.8
25				10.5		5.72	7.15				
26		8.14	11.5	7.15	7.26	3.63	3.85	6.16			1.10
27		104	727		36.6	8.66	131	169			170
28		1.1	2.1	3.3	2.9	2.8	1.7	4.0			+
29		1.2	1.8	3.6	6.0		4.2				
30		4.40	4.18	4.51	2.86	4.07	2.75	6.71			1.21
31		6.71	4.62	7.81	3.63	4.07	3.52	7.37			1.10
32		7.8	8.58	2.64	4.07	2.09	2.20	5.28			
33		7.37	3.74	1.21	5.06	1.54	1.21	3.85			
34		6.9	6.9	20.6		13.7	13.7	17.2			
35		10.7	1.10	3.52	5.28	2.75	1.76	5.39			
36		3.4	6.8	13.7		10.3	6.9	10.3			
37		0.38	0.35	0.90	+	0.93	0.46	1.30			0.31
38		24.6				2.51					1.47
39		163	699	157	166	15.6	42.1	53.2	16.2		22.3
40		3.74	10.3	8.91	7.04	7.04	1.65	7.59			1.43
41		4.28	3.82	11.9	44.2	6.11	18.6	13.5			+
42		3.3	21.9	8.0	12.0	3.7	4.2	4.0			1.2
43		3.4	2.2	4.3	4.7	2.6	3.4	3.7		+	+
44		4.95	3.52	7.48	4.07	+					1.54
45		5.46	16.4	7.80	11.2	7.80	4.94	12.0			1.82
46		4.80	7.26	4.38	8.76	1.98	2.10	3.36			0.60
47		5.17	34.7	13.5	21.2	5.06	6.71				
48						10.0	6.16				1.87
49						3.52	6.27				

BAKER (1978). It is demonstrated that (with the exception of bat pollinated flowers) the amino acid concentration values are lower than those obtained by these authors. Only the 4 "proline" specimens give higher amino acid values. The bat pollinated species with the highest mean value shows 149.56 μg/ml amino acids per nectar amount, whereas the mean values of the "proline" specimens is as high as 918.84 μg/ml.

The mean concentration of individual amino acids for all insect, all vertebrate and all animal pollinated species together is shown in Table 7 in relation to the "proline" specimens. The most concentrated amino

nation (μg/ml nectar) from 49 samples (cf. Table 1)

Leu	Tyr	$EtNH_2$	Gaba	Orn	Lys	His	Arg	$GluNH_2$	Pro	Phe	Cyst	$GlucNH_2$	Total
3.41													57.34
									13.8				257.87
1.54		3.41			3.52		5.17						92.06
					0.66						3.18		11.82
										10.66			65.52
													5.45
				7.29	2.97								91.63
													15.84
													8.91
			2.42		0.99								17.93
0.9	1.6	0.9	2.1	35.9	9.8	0.5	5.6						179.6
8.2		12.3		61.5	324		8.2						729.9
7.1	2.7			261.1	156.1		158.9		1.2	1.0			1734.6
2.20	2.97												28.54
0.66				3.30	6.05					7.15			65.95
64.3	52.7			6.84	238	29.3	68.0		18.3	67.2			1890.9
+		2.6	+	1.5	+	+							22.0
		3.0											19.8
1.21													31.9
													38.83
			0.99		0.88								34.53
												7.37	31.35
				6.9									85.9
													30.5
		3.4	3.4	3.4									61.6
0.29	+	0.60	+	0.53	+	+			+	+			6.05
0.91									4.68				34.17
11.7	17.7	3.9	0.9	2.8	5.0	11.9			29.0	24.2	5.2		1446.7
1.43				1.65	1.98								52.76
+		6.10	5.2										113.71
1.0	+		0.6	2.6	+	1.4			16.6	+			80.5
+		2.4	+	6.4	2.6					4.4	2.3		42.4
1.21					1.98								24.75
2.86		1.56	5.98										77.82
0.36				1.80	3.30					3.90			42.6
				4.84	2.64								93.82
2.31				56.8	10.0		40.3						127.44
													9.79

acids are not always the most common ones. The succession of the most abundant amino acids in insect pollinated species is (in decreasing order) glu, asp, ser, ala, val, gly, etc., whereas for frequency the succession is gly, ala, asp, ser, leu, etc. In vertebrate animal pollinated species the succession of abundance is lys, thr, glu, ser, orn and that of frequency ala, asp, gly, ser, thr, and so on. A similar comparison for the "proline" specimens shows that they present a much higher individual amino acid concentration. The insect pollinated species have the maximum mean values of amino acids per sample around 8, 6 and 4 μg/ml nectar;

Table 6. Results of quantitative amino acid determination

	13	14	15	16	17
Met SO					
Asp					
Thr	1311	800	657	560	798
Ser	108	81.5	69.1	56.7	76.4
Glu	396	343	299	276	330
Gly	59.0	53.1	39.7	25.0	33.0
Ala	25.0	26.0	25.8	23.3	27.1
Val	22.9	6.93	26.0	27.5	30.0
Gal NH_2					
Met					
Ileu	3.57	4.41	5.46	3.57	5.67
Leu	6.09	6.09	6.30	6.30	7.77
Tyr	13.2				
Et NH_2					
Gaba					
Orn	390	328	404	198	162
Lys	138	193	115	154	165
His					
Arg	164	222	98.5	127	156
Glu NH_2					
Pro	14.5				
Phe					11.7
Cyst					
Total	**2651.26**	**2064.03**	**1745.86**	**1457.37**	**1802.64**

vertebrate pollinated samples around 16, 11 and 9 μg/ml; and the "proline" specimens with values around 296, 138 and 74 μg/ml nectar in a decreasing order. Histidine, which is absent in insect pollinated species and only present in traces in the vertebrate species, as well as tyrosine, which in both groups shows up in only very small amounts, appear quite prominently in the "proline" species.

Not even in flowers of the same species does there seem to be an absolute consistency of individual amino acids. Comparison of data from Table 5 for the pairs 32/33, 34/35 or 11/12 indicate that some amino acids are more prevalent than others. In all 12 samples of *Caryocar* (Table 6), with exception of no. 21 in which the total amount of amino acids is lower (without visible correlation to sugar concentration), the amino acids thr, ser, glu, gly, ala, ileu, leu, orn and lys are present. There is a considerable individual variation of concentrations, which are apparently independent from nectar sugar amounts. Samples 13–20 contain valine, which is lacking in samples 21–24. Notable are also the

(μg/ml nectar) of 12 *Caryocar brasiliense* samples

18	19	20	21	22	23	24
733	1007	851	41.8	568	444	413
79.6	88.4	115		62.0	58.4	54.4
312	460	404	174	237	297	204
45.2	36.5	48.7	20.0	28.6	23.5	27.3
24.4	33.6	28.8	19.3	23.3	28.4	18.7
22.3	37.0	19.1				
4.62	9.87	4.83		6.51	5.25	3.78
8.82	12.8	8.82		10.0	7.77	4.62
8.19	10.9					
437	154	421	67.8	215	108	248
150	358	184	31.7	66.8	242	75.4
146	485	212	25.2	22.9	208	40.1
1971.13	**2693.07**	**2297.25**	**379.8**	**1240.11**	**1422.32**	**1089.3**

presence or absence of tyr and phe in different samples, whereas the presence of proline in sample 13 possibly can be explained by a slightly contaminated nectar of this flower through its own pollen.

The "essential" amino acids for insects, histidine and methionine (see BAKER & BAKER 1975), are not present in nectar samples of the insect pollinated flowers. These two amino acids are found only in very low concentrations in the "vertebrate pollinated" samples. The "quasi essential" and "common" amino acids ser, gly, ala, asp, glu are more or less frequent and abundant in both insect and vertebrate pollinated species.

Discussion

Nectar secretion is such a subtle process of minimum, maximum, optimum, increase and decrease of secretion activity that by the relatively rough method of collecting "at the maximum" of nectar

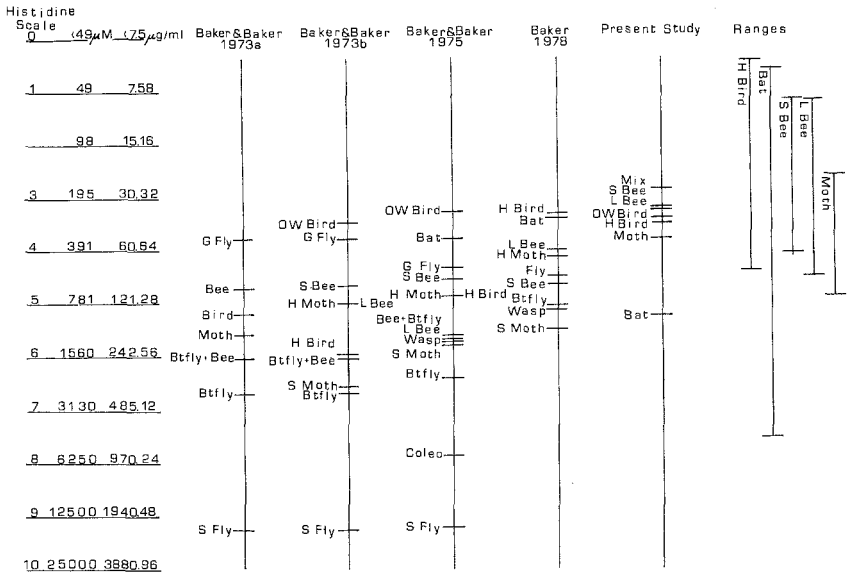

Fig. 2. Comparison of means of amino acid values for plant species grouped according to their pollinators. Means and ranges of the present study are given in µg/ml nectar; means of BAKER & BAKER (1973 a, 1973 b, 1975) in the "histidine scale"; and of BAKER (1978) in µM. Note that values are relative and double between units in the first column to facilitate graphing. Abbreviations: Pollinated by bees = *Bee*; long-tongued bees = *L Bee;* short-tongued bees = *S Bee*; beetles (*Coleoptera*) = *Coleo*; birds = *Bird*; hummingbirds = *H Bird*; Old World birds = *OW Bird*; butterflies = *Btfly*; generalized flies = *G Fly*; (specialized) carrion/dung flies = *S Fly*; mixed insects (flies, wasps, butterflies, bees) = *Mix*; moths = *Moth*; hawkmoths = *H Moth*; settling moths = *S Moth*; wasps = *Wasp*

production, comparable data for nectar constituents are not easily or likely to be obtained. Investigations which focus on one or a few species clearly show that considerable differences, at least for the sugar amounts, can be obtained during different stages of this ephemeral and rhythmic process (for comparison see PERCIVAL 1965, KUGLER 1970, FREE 1970, CORBET 1978, CORBET & al. 1979 a,, 1979 b, etc.). Nectar secretion is dependent to a large extent on the physiological state of the plant (HUBER 1956) as well as the more or less pronounced autonomous rhythm corresponding to the periodicity of the pollination process (FAEGRI & VAN DER PIJL 1979: 66).

Future research certainly will have to focus much more on all these processes to allow a more valid comparison of nectar constituents. The contribution of the present study lies in the direct comparison of

Table 7. Mean value (µg/ml nectar) of concentrations of individual amino acids for insect, vertebrate and all animal pollinated species (n = samples) in relation to "proline" specimens. See text

Amino acids	Insects (n = 10)	Vertebrates (n = 22)	All (n = 32)	"Proline" (n = 4)
Met SO	0.74	0.1	0.3	
Asp	6.02	4.9	5.25	72.33
Thr	3.22	11.77	9.1	296.1
Ser	4.94	8.45	7.35	138.82
Glu	8.49	9.11	8.92	74.03
Gly	3.35	6.07	5.22	6.99
Ala	4.92	4.97	4.95	46.47
Val	3.9	5.86	5.25	58.44
Gal NH_2		0.32	0.22	4.05
Met		0.01	0.01	
Ileu	0.71	0.86	0.81	48.38
Leu	0.69	0.94	0.86	19.25
Tyr	0.3	0.07	0.14	17.6
Et NH_2	0.95	1.22	1.13	0.97
Gaba	0.52	0.68	0.63	0.38
Orn	0.51	8.16	5.77	3.06
Lys	1.55	16.04	11.51	60.75
His		0.02	0.02	10.65
Arg	0.52	2.46	1.85	17.0
Glu NH_2				
Pro	0.47		0.15	19.42
Phe	1.11	0.68	0.81	22.85
Cyst	0.32	0.10	0.17	1.3
Gluc NH_2		0.33	0.23	

quantitatively determined sugar and amino acid amounts in nectar samples.

Data on sugar composition of samples confirm that in general sugars from concealed nectaries tend to be dominated by saccharose, whereas in the more open flowers the invert sugars glucose and fructose tend to be more prominent (PERCIVAL 1961, VAN HANDEL & al. 1972, GOTTSBERGER & al. 1973, KÄPYLÄ 1978, etc.). Although the total mean of sugar concentration is higher for nectar from insect pollinated flowers than for vertebrate pollinated ones (347.89 against 280.49 mg/ml nectar amounts), there are individual cases where especially hummingbird pollinated flowers nearly equal the highest values of bee flowers. Recently BAKER (1975) critized PERCIVAL's (1974) results based on some hummingbird flowers from southeast Jamaica with highly sugar concentrated nectar (for discussion see also BOLTEN & FEINSINGER 1978, PYKE & WASER 1981). High sugar concentrations, however, might not

limit hummingbird feeding (see also GOTTSBERGER & al. 1973: data on *Palicourea rigida*), since hummingbird flowers show more variation in their sugar concentration than that for bee flowers.

The whole problem of amino acids in nectar has been discussed in considerable length by BAKER (1978) and BAKER & BAKER (1973 a, 1973 b, 1975). Our data, however, conflict with the results of these authors. Their method was a comparison of sample spots with the "histidine scale" which provided estimates of amino acid concentrations and which was felt to be free from gross errors (BAKER & BAKER 1975: 104). The weak positive correlation between sugar content of nectar samples and their "histidine scale" scores could not be detected in the present study. Apparently there is neither a correlation between sugar and amino acid values on the level of the individual samples, nor when considering the means of the pollinator types. There was also no evidence of increase in amino acid concentration from plants with "primitive" to others with "advanced" character states; in fact, the opposite result was obtained. This might not be absolutely significant, since a numerical increase of samples investigated might provide a more balanced result.

A comparison of data in Fig. 2 shows that the results of BAKER & BAKER (1973 a, 1973 b, 1975) and BAKER (1978) change considerably from publication to publication. The relative position of one or the other pollinator type (e.g. compare long- and short-tongued bee, butterfly, moth or hummingbird pollinated flowers in the 1973 b, 1975 and 1978 papers) also varies greatly. In general (excluding bats) their results gave higher values than ours.

In an earlier publication (BAKER & BAKER 1975: 116) it was stated that: "Proline, which is so abundant in many pollens is also rather infrequent in nectar...", whereas in a later paper: "Arginine, alanine, serine, threonine, and proline [emphasis ours] are the most commonly occurring nectar amino acids, and frequently they are the most abundant in a nectar" (BAKER 1978: 70). When discussing butterfly flowers, which were found to have the second most concentrated nectar in amino acids after the carrion or dung fly flowers, it was stated that: "Even so, it must be noted that some very characteristic 'butterfly' flowers give only moderate "histidine scale" scores when their nectar is collected very carefully" (BAKER & BAKER 1975: 111). One might wonder if pure nectar always was collected without pollen pollution. Proline is always very prevalent in pollen and plays a metabolic role in pollen tube growth (LINSKENS & SCHRAUWEN 1969, STANLEY & LINSKENS 1974: 155); thus a few pollen grains probably increase the amino acid amount in nectar through rapid diffusion. Future research may show that high amino acid values of nectar are artefacts due to mixture with pollen.

Our data show that except for bat flowers, mean values for amino acids are lower and do not show characteristic patterns for different pollinator classes. The 4 "proline" samples may have resulted from (1) damage to flower tissues during collection of nectar (e.g. flowers like those of *Erythrina crista-galli* are very stiff and break easily when collecting), or (2) contamination prior to collection. Furthermore, BAKER & BAKER (1975: Table 8) cite histidine as one of the most frequently occurring "essential" amino acids for insects (at least in nectar of bee and butterfly flowers); however, histidine does not appear in our data in any considerable amount or frequency except for the "proline" samples.

The importance of the sugars in nectar of flowers seems well established and understood. They provide energy for the pollinating animals and their larvae. On the other hand the mere presence or absence of amino acids in nectar does not yet explain in a satisfactory way their role in nutrition for flower visiting animals. It is significant that phloem-sap, which is the source both for sugars and amino acids, seems to have a concentration of ninhydrin-positive substances (of which amino acids are the major fraction) that can be 5 000 times higher than in the nectar itself. In a very instructive way this demonstrates that these substances are actively filtered out, whereas carbohydrate concentration in phloem-sap and nectar is quite identical or even higher in nectar (FREY-WYSSLING & AGTHE 1950, LÜTTGE 1961). To LÜTTGE (1961) nectaries are organs specialized for secretion of sugars. LÜTTGE (1961: 195, Abb. 2) and LÜTTGE & HIGINBOTHAM (1979: 340–341) also show that anatomically more primitive nectaries are filtering these ninhydrin-positive substances less than the anatomically more advanced ones. Thus in the more primitive nectaries more concentrated amino acids are found, whereas the more advanced plant species with more elaborated nectaries, exhibit reduced amino acid concentration (but see KOPTUR & al. 1982). This is in opposition to conclusions drawn by BAKER & BAKER (1973 a, 1973 b, 1975). In our samples the more advanced morphological characters nearly always were connected with nectar of higher sugar concentration, but lower amino acid concentrations. There is, however, a very wide variation of amino acid concentration between individual plant species, which until now has not yet been compared with the anatomy of flower nectaries.

Possibly, during the evolution of Angiosperms and more advanced pollinators, sugars in flower nectars became richer and/or more balanced for an adequate energy requirement of principal flower visitors. On the other hand, innumerable alternative nutrition sources exist from which concentrated amino acids can be obtained. Most bees are known to collect and to store pollen grains and to add them to honey for their

larvae and own alimentation. Beetles gnaw from flower tissues and eat pollen grains (as do also syrphids), which are rich amino acid sources. Flower birds and bats do not need to rely on amino acids of the nectar since insects are a much richer alternative food source. Even butterflies have several amino acid alternatives (see BAKER & BAKER 1975: 101), such as the often cited example of *Heliconius* collecting pollen, steeping it in nectar, and subsequently ingesting the amino acids that diffuse from the grains (GILBERT 1972). This is actually indicative of flower nectars that apparently are not rich enough in amino acids for the insect's nutritional requirements.

Adequate understanding and analysis of nectar constituents is an emerging field of research. More detailed studies in the future will hopefully show the real significance of amino acids and other chemicals in nectar.

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