Production of Volatile Amines and Skatole at Anthesis in Some Arum Lily Species[1]

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Summary. The odoriferous compounds produced at the time of flowering by the inflorescences of 5 arum lily species were condensed in dry ice traps. The components of the condensates were separated by paper chromatography and identified on the basis of position and color reactions in comparison with known compounds. The concentration of free amino acids in the appendix of *Sauromatum guttatum* Schott increased 20-fold during flowering.

The spadix of some members of the *Araceae* has, when in bloom, a distinct, and often unpleasant odor. The stench in most cases is given off for only a few hours. Odor production apparently is correlated with a period of furious metabolic activity (6, 18) in the inflorescence. Light (10, 19) probably plays a role in initiation of this activity. Knoll (8) as well as Matile (10) suggested that the heat produced at anthesis as a result of respiratory activity is responsible for volatilization of odoriferous substances attractive to insect pollinators.

In this investigation, volatile, odoriferous constituents from 5 different arum lily species have been identified. Distinctive differences in the pattern of odoriferous compounds were observed between species.

Materials and Methods

Plant Material. *Arum dioscoridis* Sibth. and Sm. and *Sauromatum guttatum* Schott were grown in the greenhouse in Seattle. *Arum dioscoridis* bloomed in April while the *Sauromatum* flowering period was roughly September to February. The other 3 species of *Araceae* examined, *Arum italicum* Mill., *Dracunculus vulgaris* Schott, and *Hydrosme rivieri* (Durieu) Engl., were grown outside in the vicinity of the greenhouse. *Arum italicum* bloomed in May while the other 2 flowered in June.

Collection of Volatiles. Two methods were employed to collect volatiles. An entire warm, and odor-producing appendix was enclosed in a flask or cylinder. The water vapor given off by the rapidly respiring tissue condensed as droplets on the walls of the vessel. After 6 to 8 hours the condensate (containing many of the odoriferous compounds) was collected and stored at 5°. In the second method appendix tissue was removed on the morning of flowering and placed in a stoppered flask which was connected to a conical centrifuge tube imbedded in dry ice which served as a trap for constituents comprising the odor. The centrifuge tube was submitted to a slight suction to ensure movement of the atmosphere surrounding the appendix through the trap (ca. 60 ml/min).

Paper Chromatography. Whatman No. 4 filter paper was spotted with 0.25 ml of condensate or 10 μg of known compounds. The spots were dried without heat, the paper was equilibrated for 4 to 8 hours in an atmosphere saturated with the solvent, and descending chromatography was carried out until the solvent front had moved about 45 cms from the origin. The paper was dried at room temperature and sprayed with various reagents.

Solvent systems employed were those suggested by Blau (2): butanol-acetic acid-water (4:1:5, v/v/v) upper phase; butanol-acetic acid-water (4:1:1); butanol-acetic acid-water-pyridine (15:3:12:10); pyridine-*n*-amyl alcohol-water (7:2:1); butanol-HCl-water (7:2:1); 2-methoxyethanol-propionic acid-water (3:3:2); and isopropyl alcohol-ammonia-water (8:1:1).

The amine spots on the dried chromatography paper were sprayed with 0.5 % (w/v) ninhydrin (1, 2, 3-triketohydrindene hydrate) and 1.5 % (v/v) *s*-collidine in ethanol. The sprayed chromatogram was allowed to dry at room temperature and placed in an oven at 80 to 90° for 10 minutes to develop colors. For detection of compounds not sensitive to ninhydrin we substituted other reagents. Tertiary amines were detected with the Thies and Reuther reagent (17). Indoles were developed with Ehrlich reagent (3). Sulfanilic acid reagent (12) was employed as a test for indoles, phenolics, and imidazoles.

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Some 50 compounds were obtained from various commercial sources and compared with components of aroid condensates. Identifications were made on the basis of color comparisons and R_F in 5 solvent systems. Co-chromatography and 2-dimensional chromatography were also employed.

Free Amino Acids. Free amino acids were extracted according to Baptist (1). *Sauromatum* appendix tissue was ground with water in a mortar and centrifuged for 10 minutes at 18,000 × *g*. The supernatant fraction (200 ml) was brought to a boil and 5 drops of 10 % (v/v) acetic acid was added to precipitate the protein which was removed by centrifugation. The supernatant fluid was passed through a cation exchange column (acid form). The amino acids were eluted from the column with N NH_4OH, neutralized with HCl, and dried at 40° in a flash evaporator. The residue was dissolved in 2.5 ml of 0.2 M phosphate buffer (pH 2.3) and 2.5 ml of HCl (0.12 N) for each 3 g fresh weight of appendix tissue. Samples were analyzed in the Spinco amino acid analyzer (13).

Results

Volatiles from 4 different species of *Araceae* are compared in table I. Information is summarized from 75 chromatograms developed under similar conditions. The condensate obtained in 4 hours from an appendix weighing 30 g had a total volume of about 2 ml. This was mostly water. Concentrations (μl/ml of condensate) for compounds from the condensates were estimated from known concentrations of commercially obtained amines giving spots of similar size and color. A given R_F in table I may, in some instances, represent a different compound, e.g. an R_F of 0.20 for *Hydrosme* probably represents histamine rather than cadaverine, a conclusion based on color and movement in other solvent systems. However, parallel R_Fs in general indicate the same compound. An unidentified amine (R_F of ca. 0.55) not present in *Sauromatum* condensates is present in both *Arum italicum* and *Hydrosme rivieri*.

We were reasonably certain that the volatiles were produced by the plant tissue and not by bacterial action as no amine could be detected from aroid appendix on either the day preceding or the day following spathe opening and odor production.

A summary of information obtained from condensates for 5 species of *Araceae* is presented in table II. No identification of odoriferous compounds from *Arum dioscoridis* or *Dracunculus vulgaris* has appeared in the literature. T. A. Smith (personal communication) has detected putrescine and agmatine in another species, *Arum maculatum*. *Dracunculus vulgaris* had a strong orange-pink spot with the Thies-Reuther reagent (17) which corresponded to trimethylamine. A faint trimethylamine spot was also detected in *Sauromatum guttatum* condensate. Skatole co-chromatographed with a component from condensates of *Arum italicum*, *Hydrosme rivieri*, and *Arum dioscoridis*; R_F of 0.96 when chromatographed on Whatman No. 1 paper in butanol-acetic acid-water (4:1:1). The blue color characteristic of skatole with Ehrlich reagent in each case quickly appeared and then faded rapidly. The skatole concentration of *Arum dioscoridis* was several-fold that of either *A. italicum* or *Hydrosme*. An unidentified compound (R_F of 0.58), yellow with Ehrlich reagent, was observed in the above system with all aroids tested. An

Table I. *Volatile Amines from 4 Aroids*

Compound	Known amines R_F*	Color**	approx. conc μl/ml	R_F* of condensate constituents from *Arum italicum*	*Dracunculus vulgaris*	*Hydrosme rivieri*	*Sauromatum guttatum*
Isoamylamine	0.83	P	1	...	0.84	0.80	0.83
Isobutylamine	0.77	P	5	0.77	...	0.74	0.77
Diethylamine	0.69	Br-G-P	10	0.72	...	...	0.69
Ethylamine	0.57	P	5	0.60	0.58	0.62	0.58
?		(P)	(1)	0.53	...	0.55	...
Dimethylamine	0.50	R	10	0.48	0.49	...	0.50
Methylamine	0.44	Br-P	5	0.44	0.44	0.46	0.45
2-Aminoethanol	0.41	P	2	...	...	0.40	0.41
1,2-Propanediamine	0.37	Br-P	1	0.38	...	...	0.38
1,6-Hexanediamine	0.34	P	1	0.36	...	...	0.34
Ammonia (acetate)	0.31	Br-P	15	0.31	0.29	0.30	0.31
Agmatine	0.28	P	10	0.25	...	0.24	0.26
Cadaverine	0.20	P	1	0.20	...		0.21
Histamine	0.20	Br	1			0.20	
Putrescine	0.17	P	1	0.15	...	...	0.17
?		(G-P)	(1)	...	...	0.13	0.13
?		(G-P)	(1)	0.09	...	...	0.08

* R_F = ± 0.01. Known amines and a sample of 0.25 ml of condensate from each species were chromatographed descending on Whatman No. 4 paper in butanol-acetic acid-water (4:1:5), upper phase, at room temperature.

** Colors: B = blue, Br = brown, G = gray, P = purple, R = red. Chromogenic reagent = ninhydrin. Colors listed are the same for both known and unknown substance.

unidentified bright orange spot with sulfanilic acid reagent was detected in condensates from *Hydrosme rivieri* and *Arum italicum*. The spot did not react with ninhydrin, Ehrlich reagent, or Thies-Reuther reagent.

Preliminary work with gas chromatography has confirmed the presence of putrescine and isobutylamine in *Sauromatum* condensates. Ethanol has been shown by gas chromatography to be produced by poorly aerated appendices of *Arum italicum, Dracunculus vulgaris*, and *Sauromatum guttatum*.

Amines, theoretically, could be produced from

Table II. *Compounds Identified by Paper Chromatography in Condensates from 5 Aroids*

Known compound	Solvents*	*Arum dioscoridis***	*Arum italicum*	*Dracunculus vulgaris*	*Hydrosme rivieri*	*Sauromatum guttatum*
Agmatine	5		±		+	+
2-Aminoethanol	5				+	+
Ammonia	5	+	+ (7)	+	+ (7)	+ (7)
Cadaverine	5		±			+
Diethylamine	5		±			+
Dimethylamine	5	?	+	+		+
Ethylamine	5		± (15)	+	+	+
1,6-Hexanediamine	1		+			+
Histamine	5				+	
Isoamylamine	2	?		+	+	+
Isobutylamine	5		+ (7)		+ (7)	+
Methylamine	5	+	+ (16)	+	+	+
1,2-Propanediamine	1		±			+
Putrescine	5		+			+
Skatole	3	+	+		+	
Trimethylamine	3			+	(7)	+ (14)
A*** = ninhydrin-pos.			3		3	2
B = Ehrlich-pos.			1	1	1	1
C = sulfanilic-pos.			1		1	1

* Number of different solvent systems in which condensates and known compounds were compared.
** Symbols used: + = present; ? = probably present; ( ) = reported in the literature for the species.
*** A, B, and C = unidentified spots.

Table III. *Free Amino Acids from* Sauromatum *Appendix*

Amino acid	µmoles/g dry wt Appendix 0−1*	0	0 + 1	% of total amino acids 0−1*	0	0 + 1
Alanine	2.94	13.40	21.70	24.8	22.8	9.6
β-Alanine	0.36	5.16	8.68	3.0	8.8	3.8
α-Aminoadipic acid	...	...	tr.			
α-Aminobutyric acid	...	...	tr.			
γ-Aminobutyric acid	0.40	2.31	5.86	3.4	3.9	2.6
Aspartic acid	0.04	0.16	3.78	0.3	0.3	1.7
Glutamic acid	3.87	11.22	41.92	32.6	19.1	17.8
Glutamine	0.85	7.95	79.07	7.1	13.5	34.8
Glycine	0.37	2.02	2.17	3.1	3.4	1.0
Histidine	...	0.10	3.69	...	0.2	1.6
Isoleucine	0.27	0.87	5.77	2.2	1.5	2.5
Leucine	0.27	1.92	7.64	2.2	3.3	3.4
Lysine	0.40	0.10	3.91	3.4	0.2	1.7
Methionine	0.13	0.48	2.99	1.1	0.8	1.3
Ornithine	...	tr.	tr.			
Phenylalanine	0.13	1.35	5.73	1.1	2.3	2.5
Proline	tr.	tr.	4.34	tr.	tr.	1.9
Serine	1.52	6.12	10.55	12.8	10.4	4.7
Threonine	0.20	1.51	5.47	1.7	2.6	2.4
Tyrosine	0.13	0.93	0.87	1.1	1.6	0.4
Valine	tr.	3.30	14.41	tr.	5.6	6.4
Totals	11.86	56.88	228.52	100	100	100

* 0 = day of spathe opening, heat and odor production, female flower anthesis, etc. 0 − 1 and 0 + 1 represent the day before and the day after these events, respectively.

amino acids (7). Therefore we followed changes in free amino acid content of *Sauromatum* appendix during the flowering process. The total μmoles of amino acids per g dry weight of *Sauromatum* appendix increased 20-fold from the day preceding anthesis to the day after flowering (table IV). The burst of amino acid increase is as striking as the sudden appearance of the odor. Glutamine increased nearly 100-fold in amount during this same period. After anthesis over one-third of all the amino acids was glutamine. Of the 21 free amino acids detected, alanine, β-alanine, glutamine, glutamic acid, serine, and valine accounted for 77 to 80 % of the total at each flowering stage. Growth of the inflorescence had ceased when the changes in amino acids described took place.

Discussion

2-Aminoethanol, cadaverine, diethylamine, dimethylamine, 1,6-hexanediamine, histamine, isoamylamine, 1,2-propanediamine, and skatole (see table II) have not previously been reported from the family *Araceae*. Three of these compounds, diethylamine, 1,6-hexanediamine, and 1,2-propanediamine, have not, to our knowledge, been reported from any higher plant (5, 9, 20). Diamines (e.g. cadaverine, putrescine, hexanediamine, and 1,2-propanediamine), although very common in animals, have apparently never before been reported in association with flowers. Ammonia (7), histamine (21), trimethylamine, and methylamine (16) are apparently of widespread occurrence through the plant kingdom. The distinctive smell of a particular arum lily would seem to depend on concentration and occurrence of several amines rather than the presence of one particular or unusual compound.

During the *Sauromatum* flowering period, after the inflorescence had ceased growth, the level of free amino acids in the appendix increased tremendously. Gadal and Brunel-Capelle (4) have recently published an analysis of the amino acids from post-anthesis appendix tissue of *Arum italicum* Mill, which essentially agrees with our results. M. E. Davies (personal communication) has evidence from *Arum maculatum* L. that valine is, or could be, made in the spadix itself during the flowering process. In addition, an active transaminase system in *Sauromatum* appendix was demonstrated by Hess (6). The change in free amino acid composition was not a reflection of the total protein amino acid spectrum, thus the free amino acid content is probably not the result of a general protein breakdown. However, selective hydrolysis of certain proteins cannot be ruled out.

Klein and Steiner (7) suggested that the volatile amines produced at anthesis in many aroids could be formed by a simple decarboxylation of the corresponding amino acid. In support of this idea Simon (11) did demonstrate valine decarboxylation to isobutylamine by extracts of *Arum maculatum* spadix. From our experiments it would appear that a large supply of amino acids is available for possible conversion to amines and skatole. However, some preliminary experiments (although not recorded in this article) which we did on amino acid decarboxylation were essentially negative. Thus, it would appear that apart from Simon's work (11), which accounts only for isobutylamine, the mechanism for the origin of amines in aroid appendices is still a mystery.

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