Pergamon

PII: S0305-1978(97)00064-1

Biochemical Systematics and Ecology, Vol. 25, No. 8, pp. 757–766, 1997

0305-1978/97 $17.00+0.00

Polyhydroxyalkaloids in the Aroid Tribes Nephthytideae and Aglaonemateae: Phytochemical Support for an Intertribal Relationship

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Key Word Index—*Aglaonema*; *Anchomanes*; *Nephthytis*; *Amorphophallus*; Araceae; DMDP; polyhydroxyalkaloids; chemotaxonomy.

Abstract—Using living and herbarium material, a survey of polyhydroxyalkaloids in species of 52 genera of Araceae revealed the presence of 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) and α-homonojirimycin (HNJ) in leaves of *Nephthytis* Schott, *Anchomanes* Schott, *Pseudohydrosme* Engl. (tribe Nephthytideae), *Aglaonema* Schott and *Aglaodorum* Engl. (tribe Aglaonemateae). Levels were high in living plants, ranging from 0.1 to 1% dry weight DMDP and 0.04 to 0.6% HNJ. Isomers of HNJ, such as α-3,4-di-*epi*-homonojirimycin, were also present in the five genera. Seven of the eight *Nephthytis* species examined also contained deoxymannojirimycin at levels, in fresh material, of 0.1–0.2% dry weight. Lower levels of DMDP (mean 0.009%) and HNJ (mean 0.002%) were detected in species of *Amorphophallus* Blume & Decne and *Pseudodracontium* N.E. Br. (tribe Thomsonieae). The similarity in polyhydroxyalkaloid chemistry between Nephthytideae and Aglaonemateae concurs with recently published chloroplast restriction site data in suggesting a relationship between these tribes. © 1997 Elsevier Science Ltd

Introduction

Polyhydroxy derivatives of indolizidine, pyrrolizidine, piperidine and pyrrolidine have been detected in several families of dicotyledons, namely Leguminosae, Polygonaceae, Moraceae, Euphorbiaceae (Fellows *et al.*, 1992), Casuarinaceae (Nash *et al.*, 1994), Convolvulaceae (Molyneux *et al.*, 1995) and Myrtaceae (Wormald *et al.*, 1996). In some instances their occurrence has been of taxonomic significance. In Leguminosae their presence delimited proposed subgenera of *Lonchocarpus* Kunth (Evans *et al.*, 1985) and supported a relationship between *Castanospermum* A. Cunn. ex Hook. and *Alexa* Moq. (Nash *et al.*, 1988). In Euphorbiaceae their occurrence in *Omphalea* L. and *Endospermum* Benth. (Kite *et al.*, 1991) indicated a relationship that is now supported by DNA sequence evidence (M. Chase, pers. commun.).

Polyhydroxyalkaloids were reported for the first time in monocotyledons in 1995 following a survey of Araceae by high voltage paper electrophoresis in which ninhydrin staining revealed 2,5-dihydroxymethyl-3,4-dihydroxypyrrolidine (DMDP) (**1**) in species of several genera (Dring *et al.*, 1995). This preliminary survey indicated that a more detailed chemosystematic analysis of the family for polyhydroxyalkaloids would be profitable, particularly as high voltage electrophoresis does not adequately resolve several of the known structures and some polyhydroxyalkaloids do not stain with ninhydrin (Kite *et al.*, 1988). Furthermore, Asano *et al.* (1997) have recently isolated DMDP

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(*Received* 6 *March* 1997; *accepted* 9 *June* 1997)

and five other polyhydroxyalkaloid aglycones and two glycosides from an aroid. Thus, in this paper we report on a survey for polyhydroxyalkaloids in Araceae using gas chromatography-mass spectrometry (GC-MS) to provide chemical characters that may be suitable for incorporating into a cladistic analysis of the family (S. Mayo, J. Bogner, P. Boyce, J. Murata and J. Yokoyama, unpublished).

Materials and Methods

Plant material. All fresh leaf material was obtained from specimens growing at the Royal Botanic Gardens, Kew, except for a sample of *Aglaodorum griffithii* Schott which was provided by Munich Botanic Garden. The species representation of genera found to contain high levels of polyhydroxyalkaloids in the survey of living plants was broadened by using leaf material removed from herbarium specimens lodged in the Kew Herbarium (K). Where possible, small samples of type specimens were used to ensure nomenclatural accuracy.

Extraction and purification of polyhydroxyalkaloids. Freeze-dried leaf tissue (200 mg) or 2–50 mg of herbarium specimen was ground in 70% (v/v) aqueous methanol (5 or 1 ml, respectively) in a pestle and mortar and left in the aqueous methanol for 24 h at room temperature (18–22°C). After removing the residue by centrifugation, the extract was made aqueous by rotary evaporation, re-clarified by centrifugation, and passed through a 0.5 ml column of 100–200 mesh Dowex 50 (H^+) ion exchange resin. The resin was washed with 10 ml of water and eluted with 5 ml of 2 M aqueous ammonia. The ammonia was quickly removed by centrifugal evaporation and the sample was freeze dried then taken up in water for storage at −20°C.

GC-MS analysis. An aliquot of the sample containing the compounds extracted from 10 mg of dry tissue (or the entire sample if less than 10 mg was extracted) was freeze dried into a reaction vial and then heated in 20 µl of Sigma-Sil A (a mixture of trimethylchlorosilane, hexamethyldisilazane and pyridine in the ratio 1:3:9; Sigma Chem. Co. Ltd) at 70°C for 30 min to convert any polyhydroxyalkaloids present to volatile trimethylsilyl derivatives. This solution was then analysed by making a 1 µl injection into a Perkin-Elmer 8500 GC equipped with a Finnigan-MAT ion trap detector (800 series).

Chromatographic separation of compounds was achieved using a 25 m×0.2 mm (i.d.)×0.25 µm BPX5 (SGE Ltd) capillary column and an oven programme of 120°C (2 min), 120–160°C (30°C min^{-1}), 160–200°C (4°C min^{-1}), 200–300°C (8°C min^{-1}), 300°C (13 min) with a helium carrier gas pressure of 20 psi. The ion trap detector was set to record full scan, electron impact mass spectra (m/z 50-650) every second. The relative retention times and mass spectra of any peaks in the total ion chromatogram were compared to those of known polyhydroxyalkaloids and the spectra of unmatched peaks were scrutinized to determine whether they showed fragmentation patterns expected for polyhydroxyalkaloid-type structures (e.g. typical losses of 103 and 90 from the molecular ion). To detect low levels of certain known polyhydroxyalkaloids, the baseline of the chromatogram was also searched for their characteristic M-CH_2OTMS ions: 348 (common six-carbon tetrahydroxypiperidines and pyrrolidines), 450 (seven-carbon pentahydroxypiperidines) and 374 (eight-carbon tetrahydroxypyrrolizidines). Known polyhydroxyalkaloids were quantified against isolated standards of the same type.

Results and Discussion

The species from Kew's living collection of Araceae that were screened for polyhydroxyalkaloids are listed in Tables 1 and 2. These provided representatives of 32 of the 40 tribes recognized by Grayum (1990) and examples of all of his five subfamilies. The tribes not sampled were Calleae, Callopsideae, Monotrichardieae, Zantedescieae, Bognereae, Peltandreae, Arophyteae and Zomicarpeae. *Acorus* L. (Acoraceae), which is no longer considered to belong to Araceae (Grayum, 1987), was also screened.

As reported previously (Dring *et al.*, 1995), leaves of living specimens of *Aglaonema* Schott (tribe Aglaonemateae *sensu* Grayum, 1990), *Anchomanes* Schott and *Nephthytis* Schott (tribe Nephthytideae *sensu* Grayum, 1990) were found to be rich in DMDP (**1**), as was *Aglaodorum griffithii*, the monotypic representative of the only other genus of Aglaonemateae; levels ranged from 0.1 to 1% dry weight (Table 2). Additionally, these taxa were also found to contain α-homonojirimycin (HNJ) (**2**) at levels of 0.04–0.6% dry weight, and various isomers of HNJ. These HNJ isomers have recently been isolated and characterized by Asano *et al.* (1997) from an aroid they identified as *Aglaonema treubii* Engl. (however, this is a dubious name that was assigned to a no-longer extant

TABLE 1. SPECIES OF ARACEAE AND ACORACEAE ANALYSED IN WHICH POLYHYDROXYALKALOIDS WERE NOT DETECTED

Species	Kew accession No.	Species	Kew accession No.	Species	Kew accession No.
Acorus calamus L.	1969-18624	*Caladium bicolor* (Aiton) Vent	1969-6199	*Pothos scandens* L.	1970-1498
A. calamus 'Variegatus'	1969-19633	*Cercestis congensis* Engl.	1970-388		
Alocasia macrorrhizos (L.) G. Don	1981-422	*Culcasia saxatilis* Chev.	1965-5001	*Remusatia vivipara* (Roxb.) Schott	1983-1625
	1977-3382	*Dieffenbachia picta* (Lodd.) Schott	1969-51697	*Rhaphidophora africana* N.E. Br.	1976-787
A. micholitziana Sander	1967-28901	*D. oerstedtii* Schott	1969-11744	*Schismatoglottis wallichii* Hook.	1982-4988
Ambrosina bassii L.	1991-122	*Dracontium foecundum* Hook.	1980-1642	*Scindapsus pictus* Hassk.	1976-1047
Amydrium medium (Zoll & Mor.) Nicolson	1978-451	*Dracunculus canariensis* Kunth	1988-418	*Spathicarpa hastifolia* Hook.	1979-3384
		D. vulgaris Schott	1986-6255	*Spathiphyllum blandum* Schott	1982-2039
Anadendrum microstachyum (de Vriese & Miq.) Backer & Alderw	1982-4984	*Eminium spiculatum* (Blume) Kunze	1985-2308	*S. cannifolium* (Dryand.) Schott	1974-3588[a]
		Epipremnum pinnatum (L.) Engl.	1978-158		1979-3137
Anaphyllum wightii Schott	1984-4519	*Gonatopus boivinii* (Decne.) Engl.	1933-73501	*S. cochlearispathum* Engl.	1994-2591
Anthurium acaule (Jacq.) Schott	1980-1617	*Gymnostachys anceps* R. Br.	1979-3277	*S. friedrichsthalii* Schott	1983-807
A. erskinei Mayo	1974-786	*Hapaline benthamiana* Schott	1983-1979	*S. patinii* N.E. Br.	1973-13212
A. pentaphyllum (Aubl.) G. Don	1980-1530	*Holochlamys beccarii* Engl.	1970-1498	*S. wallisii* Regel	1969-1738
Anubias afzelii Schott	1969-17733	*Homalomena rubescens* (Roxb.) Kunth	1968-38220	*S.* aff. *wallisii* Regel	1982-2036
Arisaema amurense Maxim.	1989-3587	*Lagenandra ovata* (L.) Thwaites	1967-10201	*Stenospermation multiovulatum* N.E. Br.	1962-56708
A. erubescens (Wall.) Schott	1990-3537	*L. toxicaria* Dalz.	1962-73501	*Stylochaeton salaamicus* N.E. Br.	1968-30106
A. echinatum (Wall.) Schott	1988-833	*Lasia spinosa* (L.) Thwaites	1980-1630	*Symplocarpus foetidus* (L.) Salisb.	1969-18003
A. flavum (Forssk.) Schott	1983-5842	*Lysichiton americanum* Hultén & H. St. John	1994-529	*Synandrospadix vermitoxicus* (Griseb.) Engl.	1973-19870
A. tortuosum (Wall.) Schott	1989-2944				
Arisarum simorrhinum Durieu	1960-18802	*L. camtschatcense* (L.) Schott	1936-36401	*Syngonium* sp.	1984-2208
A. vulgare Targ. & Tozz.	1972-10427	*Monstera* sp.	1983-4494	*S. podophyllum* Scott	1973-13217
Arum alpinum Schott & Kotschy	1978-4801	*M. adansonii* Schott	1977-741	*Urospatha sagittifolia* (Rodsch.) Schott	1965-50024
A. italicum Mill.	1973-15808	*Philodendron imbe* Schott	1981-380		
A. nigrum Schott	1978-2628	*P. micans* C. Koch	1968-38203	*Xanthosoma brasiliense* (Desf.) Engl.	1958-79707
Biarum dispar (Schott) Talavera	1991-123	*Pinellia pedatisecta* Schott	1962-24001	*X. sagittifolium* Schott	1958-69910
B. tenuifolium (L.) Schott	1958-43601	*Pistia stratiotes* L.	1963-41001	*Zamioculcas lanceolata* Peter	1967-49404

[a]Polyhydroxyalkaloids detected at trace levels in initial analysis of this accession.

TABLE 2. LEVELS (% DRY WEIGHT) OF POLYHYDROXYALKALOIDS IN LIVING SPECIMENS OF NEPHTHYTIDEAE, AGLAONEMATEAE AND THOMSONIEAE

Species	Kew acc. no.	Origin	Month collected	Polyhydroxyalkaloid conc. (%) DMPD	HNJ	*diepi*HNJ	DMJ
Nephthytis afzelii Schott	1982-4608	Côte d'Ivoire	Nov	1.0	0.2	0.03	0.2
	1982-4608	Côte d'Ivoire	Dec	0.6	0.1	0.07	0.2
N. gravenreuthii (Engl.) Engl.	1992-51	Cameroon	Dec	0.5	0.1	0.01	0.2
N. poissonii (Engl.) N.E. Br.	1954-20901	(not known)	Aug	0.2	0.3	0.02	0.2
	1957-43801	Nigeria	Nov	0.2	0.1	0.01	0.1
Anchomanes difformis (Blume) Engl.	1991-1894	Cameroon	Aug	0.2	0.1	0.01	
A. welwitschii Rendle	1953-40702	(not known)	Aug	0.6	0.4	0.01	
Aglaonema sp. nov.	1982-49681	Malaysia (Peninsular)	Nov	1.0	0.04	0.1	
A. commutatum Schott var. *maculatum* (Hook.) Nicolson	1953-45801	Philippines	Aug	0.3	0.3	0.04	
	1968-20002	Philippines	Aug	0.5	0.08	0.3	
A. modestum Schott ex Engl.	1968-38227	Philippines	Nov	1.0	0.6	0.001	
A. nitidum (Jack) Kunth var. *nitidum* f. *nitidum*	1963-45512	Malaysia (Peninsular)	Aug	0.3	0.2	0.001	
	1981-4213	Malaysia (Peninsular)	Nov	0.1	0.02	0.02	
A. pictum (Roxb.) Kunth	1981-4215	Indonesia (Sumatra)	Dec	1.0	0.02	0.001	
A. simplex Blume	1982-4990	Burma	Dec	0.1	0.04	0.08	
Aglaodorum griffithii Schott	—[a]	Malaysia (Sarawak)	Oct	0.2	0.04	0.09	
Amorphophallus bulbifer (Roxb.) Blume	1990-994	Indonesia (Sumatra)	Aug	+[b]	+		
	1990-994	Indonesia (Sumatra)	Jun	0.004	0.002	+	
	1990-994	Indonesia (Sumatra)	Jun	+	+		
A. curvistylus Hett.	1982-4581	Thailand	Jun	0.003			
A. fallax (Serebryanyi) Hett.	1993-3899	Vietnam	Sep	0.003	+	+	
A. gallaensis (Engl.) N.E. Br.	1956-32601	Kenya	Aug	0.004	0.002	+	
A. konjac C. Koch	1985-3802	China	Jun	0.02	+		
	1985-3802	Thailand	Jun	0.02			
A. krausei Engl.	1983-8101	Thailand	Jun	+	+		
	1994-3547	Thailand	Sep	0.002	0.005	+	
A. lambii Mayo & Widjaja	1992-62	Malaysia (Sabah)	Jun	+			
A. paeoniifolius (Dennst.) Nicolson	1994-3577	India	Sep	0.003	0.002	+	
A. prainii Hook.	1994-3546	Indonesia (Sumatra)	Sep	0.04	0.008	+	
A. salmoneus Hett.	1984-8058	Philippines	Jun	0.05	+		
	1984-8058	Philippines	Jun	0.01	+		
A. sutepensis Gagnep.	1983-1698	Thailand	Jun	0.006			
A. variabilis Blume	1982-8085	Indonesia (Java)	Jun	+	+		
A. yuloensis H. Li	1994-3575	China	Sep	0.003	0.001	+	
Pseudodracontium anomalum N.E. Br.	1984-4105	Thailand	Sep	0.006	0.02	0.001	

[a]Provided by Munich Botanic Garden, ref Bogner 1767. [b] + = detected at less than 0.001%.

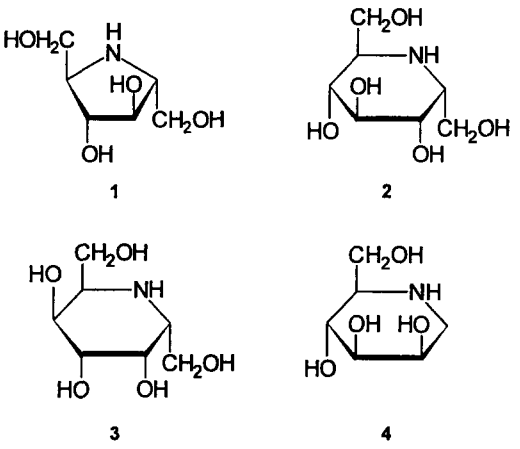

FIG. 1. STRUCTURES OF SOME POLYHYDROXYALKALOIDS IN ARACEAE: (**1**) 2,5-DIHYDROXYMETHYL-3,4-DIHYDROXY-PYRROLIDINE (DMDP); (**2**) α-HOMONOJIRIMYCIN (HNJ); (**3**) α-3,4-DI-*EPI*-HOMONOJIRIMYCIN (DI*EPI*-HNJ); (4) DEOXY-MANNOJIRIMYCIN (DMJ).

cultivated specimen). The most abundant of the HNJs in the living taxa that we examined were always HNJ itself and the epimer α-3,4-di-*epi*-homonojirimycin (di*epi*-HNJ) (**3**). The other HNJ isomers were present at much lower levels and were not quantified. A further polyhydroxyalkaloid, deoxymannojirimycin (DMJ) (**4**), was found in the living material of *Nephthytis* that was examined; in these species it was present at 0.1–0.2% dry weight (Table 2 and Fig. 1). Also evident in some analyses were the glycosides of HNJ that have been isolated by Asano *et al.* (1997). Unfortunately, the mass of the trimethylsilyl derivatives of these glycosides exceeded the molecular weight limit of the mass spectrometer we used and so they could not be detected reliably.

The production of polyhydroxyalkaloids appeared to be a consistent character of the above genera, as they were present in herbarium material of all species examined of *Aglaonema* (19 of 21 recognized species), *Anchomanes* (6 of *c.* 10 recognized species) and *Nephthytis* (8 of *c.* 10 recognized species) (Table 3). Although levels of polyhydroxyalkaloids were generally much lower in old herbarium specimens compared to living material, the consistency of the GC-MS profiles was remarkable considering the age of some of the type specimens analysed (e.g. the holotype of *Aglaonema hookerianum* Schott collected in 1850). The one discrepancy was the failure to detect DMJ in *Nephthytis bintulensis* A. Hay, J. Bogner & P.C. Boyce, a species only discovered in 1994 and unusual in being the only representative of the genus known from south-east Asia (*Nephthytis* is otherwise restricted to west Africa). The use of herbarium material also enabled examination of a specimen of oligotypic *Pseudohydrosme gabunensis* Engl., the remaining genus in Nephthytideae (*sensu* Grayum, 1990). This also contained polyhydroxyalkaloids: 1% DMDP, 0.2% HNJ, 0.3% di*epi*-HNJ and the various other HNJ isomers.

Polyhydroxyalkaloids were not detected in any of the other aroids examined except in species of *Amorphophallus* Blume & Decne, *Pseudodracontium* N.E. Br. (the two genera of tribe Thomsonieae: *sensu* Grayum, 1990), and in one analysis of *Spathiphyllum cannifolium* (Dryand.) Schott. In *Amorphophallus* and *Pseudodracontium*, DMDP, HNJ and di*epi*-HNJ were detected erratically and at lower concentrations than

TABLE 3. LEVELS (% DRY WEIGHT) OF POLYHYDROXYALKALOIDS IN HERBARIUM SPECIMENS OF NEPHTHYTIDEAE AND AGLAONEMATEAE

Species	Collector's reference (type)	Origin	Coll. date	Polyhydroxyalkaloid conc. (%)			
				DMDP	HNJ	*diepi*HNJ	DMJ
Nephthytis afzelii Schott	Barker 1440	Liberia	Oct 1952	0.9	0.03	0.07	0.1
N. bintulensis A. Hay, J. Bogner & P.C. Boyce	Boyce 727	Malaysia (Sarawak)	Mar 1994	1.0	0.06	0.1	
N. constricta N.E. Br.	Mann 1839 (Holotype)	Equatorial Guinea (Bioko)	1867	0.005	0.003	+[a]	+
N. gravenreuthii Engl.	Preuss 299 (Isotype)	Cameroon	1890	0.2	0.05	0.05	0.03
N. hallaei Bogner	Bogner 750 (Isotype)	Gabon	Oct 1973	0.8	0.02	0.02	0.2
N. liberica N.E. Br.	Cult. Kew (Holotype)	Liberia	Aug 1887	0.4	0.03	0.01	0.2
N. poissonii N.E. Br.	Onochie FHI 20679	Nigeria	Jan 1949	0.3	0.1	0.01	0.2
N. swainei Bogner	Swaine 4433F (Isotype)	Ghana	Jun 1981	0.4	0.1	0.05	0.3
Anchomanes abbreviatus Engl.	Bally 12178	Kenya	Apr 1960	0.8	0.5	0.05	
A. difformis Engl.	Stubbings 109	Nigeria	Jan 1958	0.9	0.1	0.03	
	Pitz 2006	Nigeria	Apr 1977	0.01	0.02	+	
A. giganteus Engl.	Louis 2787	Zaire	Oct 1936	0.06	0.004	+	
A. nigritianus Rendle	Bogner 640	Gabon	Oct 1973	0.3	0.03	0.03	
A. petiolatus Hutch.	Bougley 163	Equatorial Guinea (Bioko)	Jul 1951	0.5	0.2	0.05	
A. welwitschii Rendle	Milne-Redhead 2676A	Zambia	Oct 1937	0.07	0.02	0.01	
Pseudohydrosome gabunensis	Bogner 664	Gabon	1973	1.0	0.2	0.3	
Aglaonema brevispathum Engl.	Cult. Hong Kong B.G. (Holotype)	China (Hong Kong)	May 1888	0.01	0.001	+	
A. cochinchinensis Engl.	Kerr 19527	Thailand	Jul 1930	0.009	0.02	0.005	
A. commutatum Schott	Meijer	Sri Lanka	Jun 1970	0.01	0.01	+	
A. costatum N.E. Br.	*Curtis* 2813	*Malaysia (Peninsular)*	Mar 1892	0.02	0.006	+	
A. crispum Nicolson	Galston	Sri Lanka	Jul 1926	0.2	0.01	0.01	
A. densinervium Engl.	Ridsdale *et al.* 5413	Philippines	Apr 1986	0.03	0.003	0.002	
A. hookerianum Schott	Hokker & Thomson 8 (Holotype)	India	Jun 1850	0.06	0.05	0.002	
A. marantifolium Blume	Borssum 3107	Indonesia (Java)	Mar 1956	0.008	0.005	+	
A. modestum Schott	Kerr 1160	Thailand	May 1910	0.01	0.004	+	
A. nebulosum N.E. Br.	Nicolson 1344	Malaysia (Sarawak)	Aug 1961	0.001	+	+	
A. nilidum Kunth	Nur SFN2906 (Isotype of fma *curtisii*)	Thailand	Feb 1927	0.001	+	+	
A. ovatum Engl.	Kerr 21776	Laos	Apr 1932	0.08	+	+	
A. philippinense Engl.	Merrill 7347	Philippines	Feb 1934	0.02	0.002	+	
A. pictum Kunth	Horsfield (Holotype)	Indonesia (Java)	Aug 1856	+	+	+	
A. pumilum Hook.	Griffith (Holotype)	Burma	1867	0.06	0.02	0.005	
A. rotunda N.E. Br.	Cribb	Indonesia (Sumatra)	Dec 1983	0.1	0.009	+	
A. simplex Blume	Griffith 5985 (Holotype)	Malaysia (Peninsular)	Aug 1963	0.02	0.004	+	
A. stenophyllum Merr.	Nicolson 789	Philippines	Oct 1960	0.006	0.003	+	
A. tenuipes Engl.	Kerr 10765	Thailand	Jul 1926	0.03	0.02	0.007	
Aglaodorum griffithii Schott	Ridley	Malaysia (Peninsular)	Jul 1890	0.001	0.002	0.004	

[a] + = detected at less than 0.001%.

in genera of Nephthytideae and Aglaonemateae (mean dry-weight levels among the samples analysed were 0.009% DMDP and 0.002% HNJ). In an initial analysis of *S. cannifolium* trace levels of DMDP (<0.001% dry weight) were recorded. Dring *et al.* (1995) also reported the presence of DMDP in *S. cannifolium*, but we could not confirm our initial finding in a critical re-examination of this and seven other accessions of *Spathiphyllum* Schott (Table 1). We cannot exclude the possibility that *Spathiphyllum* might produce polyhydroxyalkaloids, since DMDP and HNJs could often not be detected in leaf samples of *Amorphophallus* collected at the end of the growing season. Another possibility is that these genera may be producing novel polyhydroxyalkaloids that are not detectable by the methods used. When considering the use of our present data as chemotaxonomic characters, it is the accumulation of DMDP, HNJ and, to a lesser extent, di*epi*-HNJ that appears significant, and in this respect the genera in tribes Nephthytideae and Aglaonemateae are distinct from all other genera examined in consistently containing high levels throughout the growing season. Dring *et al.* (1995) also reported compounds producing yellow ninhydrin chromophores in species of several other aroid taxa, notably *Lysichiton camtschatcense* (L.) Schott, *L. americanum* Hultén & H. St John and *Symplocarpus foetidus* (L.) Salisb. We could not detect any known polyhydroxyalkaloids in these three species by GC-MS analysis and so these ninhydrin-yellow compounds require further investigation to determine their identity.

Higher level taxonomic problems in Araceae lie mainly with tribal relationships and the grouping of tribes into subfamilies (Grayum, 1990). The concepts of generic relationships within tribes have remained largely stable since Engler's classification (Engler, 1920), although one exception has been the affinities of *Nephthytis, Anchomanes* and *Pseudohydrosme* Engl. Engler placed *Nephthytis* with *Cercestis* Schott in his Nephthytideae, and *Anchomanes* and *Pseudohydrosme* with *Amorphophallus* and *Pseudodracontium* in his Pythonieae (=Thomsonieae); both tribes he considered to be in the subfamily Lasioideae. In his thesis on aroid phylogeny, Grayum (1984) viewed the vegetative similarities between *Anchomanes/Pseudohydrosme* and *Amorphophallus/Pseudodracontium* as being striking but superficial and classified the latter genera in his subfamily Aroideae. He also considered that *Pseudohydrosme, Anchomanes, Nephthytis* and *Cercestis* showed "philodendroid" alliances, so placed them in his subfamily Philodendroideae. Later, on the basis of the presence of root resin canals in *Cercestis* but not in *Nephthytis* (French, 1987), Grayum (1990) moved *Cercestis* into its own tribe close to *Culcasia* P. Beauv. and also included *Anchomanes* and *Pseudohydrosme* in Nephthytideae. Bogner and Nicolson (1991) concurred with this inclusion but retained Nephthytideae in Lasioideae and also kept *Cercestis* in the tribe. We could not detect polyhydroxyalkaloids in *Cercestis* and so this chemical data supports Grayum's delimitation of Nephthytideae rather than Bogner and Nicolson's. Two recent cladistic analyses of Araceae, one using morphological and anatomical characters (Mayo *et al.*, unpublished) and one using chloroplast DNA restriction sites (French *et al.*, 1995), also suggest an alliance between *Cercestis* and *Culcasia*, with both being close to Nephthytideae (*sensu* Grayum). Further information on the relationships between these genera may come from anthocyanin pigment analysis. Williams *et al.* (1981) found that both *Cercestis* and *Anchomanes* contained the rare pigment cyanidin 3-gentiobioside, but these authors did not examine the anthocyanins of *Nephthytis* or *Culcasia*.

A close alliance between *Aglaonema* and *Aglaodorum* Engl. in Aglaonemateae has been upheld in several aroid classifications as has their position in Philodendroideae

(Engler, 1920; Grayum, 1990; Bogner and Nicolson, 1991). Only Grayum, therefore, has placed Aglaonemateae in the same subfamily as Nephthytideae and even his classification suggests that they have closer relationships with other tribes than with each other. Thus, on the basis of current classifications, the presence of high levels of DMDP and HNJ in both Aglaonemateae and Nephthytideae would appear to be a case of parallel evolution. However, the chloroplast DNA restriction site analysis of French *et al.* (1995) showed unexpectedly strong support for *Aglaonema, Aglaodorum, Nephthytis* and *Anchomanes* forming a four-genus clade within Philodendroideae (*Pseudohydrosme* was not examined). These authors commented that this arrangement was not congruent with any previous phylogeny.

Given that both molecular and chemical evidence suggest a close alliance between Nephthytideae and Aglaonemateae, we have re-examined the strength of morphological and anatomical synapomorphies for these tribes. Analysis of the data of Grayum (1990) and French (1985) reveals that genera of both tribes share a combination of four character states (absence of endosperm, uniovulate locules, apical anther dehiscence and non-anastomosing laticifers) that is otherwise only found in *Cercestis, Amorphophallus, Pseudodracontium, Dieffenbachia* Schott and possibly *Ulearum* Engl. (laticifers were not examined by French). All of these characters are amongst those that Grayum (1990) considered to be key taxonomic indicators and thus there does appear to be some morphological support for a relationship between Aglaonemateae and Nephthytideae. Grayum himself, in a "quasi-cladistic" analysis of his key characters, found that his *Nephthytis*-alliance was sister to his *Aglaonema*-alliance (Grayum, 1990).

It is interesting to note that *Amorphophallus* and *Pseudodracontium* (Thomsonieae) also share the above four morphological character states, given the occurrence of low levels of DMDP and HNJs in these genera. As mentioned previously, Engler's tribe Thomsonieae included *Anchomanes* and *Pseudohydrosme* in his Lasioideae due to similar gross vegetative features, and he linked Thomsonieae to tribe Lasieae (Engler, 1911). Subsequent authors have restricted Thomsonieae to *Amorphophallus* and *Pseudodracontium* and placed the tribe in Aroideae, linking it with tribe Arinae on the basis of numerous shared floral characteristics (Grayum, 1990; Bogner and Nicolson, 1991). Grayum (1990) points out that the only apomorphy shared by Thomsonieae and Lasieae, that is not also shared with Arinae, is the lack of seed endosperm. However, if one argues that the presence of polyhydroxyalkaloids links Thomsonieae with Nephthytideae and Aglaonemateae, more supporting synapomorphies have to be rejected. Accordingly, an unweighted cladistic analysis of morphological and anatomical characters by Mayo *et al.* (unpublished) places Thomsonieae sister to Nephthytideae; however, the choroplast DNA restriction site data of French *et al.* (1995) support the currently held view of Thomsonieae being part of Aroideae.

The restricted occurrence of known polyhydroxyalkaloids in Araceae reflects the situation in other plant families which produce these compounds, such as Euphorbiaceae where they are only reported from *Omphalea* and *Endospermum* (Kite *et al.*, 1991), and Myrtaceae where they are only present in a few genera (E. Porter, unpublished). Such restricted occurrences of nitrogen-containing compounds is sometimes associated with ecological or phytogeographical factors; the accumulation of 4-methyleneglutamic acid by some species of the legume *Caesalpinia* L. (*Poincianella* group) but not others, is one example (Kite and Lewis, 1994). But in Araceae, Euphorbiaceae and Myrtaceae, polyhydroxyalkaloid-producing taxa do not appear to share ecological

requirements or geographical ranges that are distinct from taxa in which these compounds are absent, and thus the evolution of this character in a common ancestor seems a more likely explanation for their distribution.

In conclusion, we would recommend that in future cladistic analyses of Araceae our polyhydroxyalkaloid data are included as characters and that the states are coded as "1" (DMDP and HNJs present) in Thomsonieae, "2" (DMDP and HNJs accumulated) in Aglaonemateae and Nephthytideae, and "0" (DMDP and HNJs absent) in all other taxa examined in this study. The congruence of this data with the chloroplast restriction site analysis of French *et al.* (1995) appears to provide a persuasive argument for a taxonomic relationship between Nephthytideae and Aglaonemateae and it illustrates the importance that chemical characters can have in complementing macromolecular and morphological data in modern systematic studies.

Acknowledgements—We wish to thank staff of the Alpine and Tropical Sections at the Royal Botanic Gardens, Kew, who care for the living aroid collection, Josef Bogner (Munich Botanic Garden) for the sample of *Aglaodorum griffithii*, Wilbert Hetterscheid (The Netherlands) for donating live material of *Amorphophallus* to RBG Kew for chemotaxonomic studies, and Dr Robert Nash (Institute of Grassland and Ecological Research, Aberystwyth, U.K.) for a sample of di*epi*-HNJ.

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