CYTOLOGICAL AND BIOCHEMICAL ANALYSES OF *ANTHURIUM ANDRAEANUM* AND CLOSELY RELATED TAXA

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ABSTRACT

Karyotypes and meiotic configurations of Anthurium andraeanum and closely related taxa were analyzed. The karyotypes of Anthurium species with the exception of A. nymphaeifolium and A. wallisii commonly consisted of four large metacentric or submetacentric chromosomes, two fairly large acrocentric chromosomes, two satellite chromosomes and twenty two smaller chromosomes. A slight variation in the karyotypes of A. nymphaeifolium and A. wallisii suggested the presence of chromosomal rearrangement in the genus.

All taxa showed 15 pairs of chromosomes at prometaphase I of meiosis in pollen mother cells. Four large chromosomes formed two distinct ring bivalents and the rest of the chromosomes appeared as rings or rod bivalents. Cytological analysis of A. amnicola showed that the species exhibited a similar chromosome complement to that of A. andraeanum and closely related taxa.

Hybrid analysis revealed close genomic relationships among the parental taxa. Regular bivalent formation was found at prometaphase I of meiosis in pollen mother cells. However, reduction of pollen fertility estimated by pollen stainability in interspecific hybrids indicated genetic divergence of species. The genetic

distictiveness of two previously defined species groups (Sheffer and Kamemoto's groups V and VI) was indicated by virtual male sterility in intergroup interspecific hybrids.

Anthurium amnicola showed its genetic closeness to species in group VI. Mean configurations at prometaphase I showed 15 bivalents in various hybrids of A. amnicola, and pollen fertility of those hybrids were very similar to that of intragroup interspecific hybrids (VI x VI).

Several flavonoid compounds were characterized from leaves of Anthurium species. The major flavonoid from A. andraeanum was tentatively identified as acacetin C-glycoside. Anthurium formosum contained flavonols which were useful in assessing phylogenetic relationships of taxa. Co-pigmentation effects of those flavonols were also noticed. Anthurium amnicola (A417) was found to contain acacetin 6-C-glycoside as the major compound while apigenin seemed to be the most common flavonoid in other species.

Flavonoid profiles revealed by HPLC chromatograms showed that each species displayed a specific flavonoid pattern. Flavonoid data could be used for species identification. Numerical methods were applied to evaluate phenetic and phylogenetic relationships among A. andraeanum and closely related taxa. The phenogram constructed on the basis of flavonoid data indicated that

flavonoid analysis aided in dividing morphologically similar taxa into subgroups. Cladistic analysis supported close relationships among the three taxa in Sheffer and Kamemoto's group V.

Two anthocyanins were isolated and identified from lavender spathes of A. amnicola. The major pigment was cyanidin 3-rutinoside and the minor pigment was identified as peonidin 3-rutinoside. In the survey of anthocyanins in selected Anthurium taxa and interspecific hybrids cyanidin 3-rutinoside appeared as the most common anthocyanin. Presence of pelargonidin in A. andraeanum and peonidin in A. amnicola as the important pigment indicated the independent biochemical advancement from cyanidin in the two taxa.

The biochemical studies on A. amnicola and its hybrids were conducted to investigate factors responsible for their colors. The concentration of cyanidin 3-rutinoside, presence of co-pigment, and pH of tissues were found to be important in color determination of those plants.

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I. INTRODUCTION

The genus Anthurium which comprises over 700 species is one of the largest and the most taxonomically complex genera in the family Araceae (Sheffer and Croat, 1983). The distribution of this genus includes South America (eg. Ecuador and Colombia), Central America (eg. Panama and Costa Rica) and Mexico.

All species are herbaceous perennials. The genus is characterized by the possession of the spathe, a modified leaf, which subtends a cylindrical inflorescence called a spadix. The inconspicuous flowers are arranged in helixes on a fleshy axis. Each flower is bisexual and typically consists of one ovary, four stamens and four tepals. Leaves are simple or compound with netted veins.

The first extensive classification of Anthurium species was done by Engler (1905). Engler divided 486 species into 18 sections on the basis of the number of ovules per locule, leaf shapes and textures, inflorescence shapes and berry shapes. Although his work has been widely accepted among many taxonomists, numerous new species described recently have not been placed in any of Engler's sections.

Anthurium andraeanum Linden Andre, characterized by its showy spathe, is the most important flower crop in Hawaii. It was a member of Engler's Section

Belolonchium, but in 1983 Croat and Sheffer replaced this species in Section Calomystrium. Extensive hybridization within A. andraeanum has produced a multitude of spathe colors which are broadly classified into red, orange, pink, coral, and white. The major commercial cultivars are red-colored 'Ozaki', 'Kozohara' and 'Kaumana' and orange-colored 'Nitta'. Cultivars with light shades are less common. Intraspecific hybridizations have been made not only to improve quality of anthurium of various spathe colors, but also to increase productivity and to create new forms (Kamemoto and Nakasone, 1955, 1963).

Sheffer and Kamemoto (1977) performed interspecific hybridizations involving A. andraeanum and closely related species to improve existing cultivars as well as to create new types. Biochemical and cytological evaluations of those interspecific hybrids and their parental species are of value in furthering the anthurium breeding program.

Anthurium amnicola, a recently discovered lavender dwarf species from Panama (Dressler, 1978, 1980) has shown its potential uses in horticulture. Several interspecific hybrids of the species have exhibited attractive purple or violet spathes which contribute a whole new range of colors in anthuriums.

The present study was initiated to determine the chromosomal relationships among A. andraeanum and closely

related species, to measure the degree of fertility in their hybrids, to provide flavonoid analysis of selected species, and to clarify pigment systems of selected species and hybrids especially focusing on A. amnicola.

II. LITERATURE REVIEW

2.1 Classification of *Anthurium*

The genus *Anthurium* is one of the most taxonomically complex genera in the family Araceae due to the large number of species in the genus and the great diversity in their morphological expression (Croat and Bunting, 1978).

The first attempt to classify *Anthurium* species was made by Schott in 1860. He described 180 species and divided them into 28 sections according to floral and vegetative characters.

Later in 1905 Engler characterized 486 species including those described by Schott and classified the genus into 18 sections. Engler's classification was based primarily on the number of ovules per locule. The other important characters used in his categorizations of the sections were leaf shapes and textures, spathe and spadix shapes and the shape of berry.

In 1978 Croat and Baker attempted to clarify the key to Engler's Section *Polyphyllium* which was distinguished from other sections by a unique growth habit. It was noted that the most important and the most easily observed character of the section was the lack of 1-ribbed cataphylls.

A taxonomic revision of the Section *Tetraspermium* was done by Sheffer *et al*. (1980b). One of the complex

species in the section, Anthurium scandens was further studied and it was pointed out that polyploidy of the species made many taxonomists split variants of the taxon into various species rather than subspecies (Sheffer et al., 1980b).

In 1983 Croat and Sheffer modified Engler's classification system and rearranged several species. Anthurium andraeanum and A. kamemotoanum for example, were placed in Section Calomystrium instead of Section Belolonchium in Engler's system. Two new species, A. amnicola and A. antioquiense were placed in Section Porphyrochitonium where the well-known cultivated species, A. scherzerianum, represented the section with elongated non-cordate leaf blades and a compressed stem.

Tentative groupings of Anthurium species were proposed by Sheffer and Kamemoto (1976b). The system consists of six morphological groups using the major characters of Engler's classification system; the number of ovules per locule, color and shape of the berry, shape of inflorescence, and shape and texture of the leaf. Cross compatibility studies among 57 species confirmed the distinction of these six groups with the exception of groups V and VI which were closely related.

Group I was distinguished from Group II using Engler's major key character, the number of ovules per locule. The species in Group I contained three or more

seeds per berry while others contained two or less. Group III and Group IV were Engler's sections Pachynerium and Schizoplacium, respectively. Group V and Group VI, two largest groups were divided according to leaf texture, berry shape and berry color.

Anthurium species exhibit such a diverse morphological structure that Croat and Bunting (1978) tried to standardize terminology for species descriptions. They listed five aspects to consider in describing Anthurium species: structural description; habitat; geographical distribution; illustrations and additional discussions which include common name, local uses, reproductive biology, cytology, anatomy, pollen morphology and other data available to characterize the species. They further suggested, in detail, types of measurements for examining each character.

Croat in 1980 discussed aspects of flowering behavior as possible taxonomic characters for species separation in Anthurium. Significant differences among species examined were found in many aspects such as the rate of development of flowers, the amount of stigmatic fluid, the degree of exsertion of stamens, the kind and time of occurrence of flower scent, and changes in pollen color.

The description of new and previously documented species are available in many floras. Thirty seven

Panamanian species, for example, were characterized by Standley (1944), and more recently Dodson and Gentry (1978) documented 14 species in the flora of Rio Palenque. Croat in 1981 described 10 new species from Central America. In addition, Anthurium species have been documented in some other countries such as Peru (MacBride, 1936), Mexico (Matuda, 1954), Brazil (Reitz, 1957) and Guatemala (Standley and Steyermark, 1958).

2.2 Chromosome studies in Anthurium

Nearly 100 species in the genus Anthurium have had their chromosomes studied. In 1983 Sheffer and Croat surveyed the previous chromosome counts in the genus, added new counts, and determined the chromosome number of 86 Anthurium species. Other cytological works were done earlier by Gaiser (1927), Ito (1942), Simmonds (1954), Pfitzer (1957), Sharma and Bhattacharyya (1961) and Marchant (1973) and Sheffer and Kamemoto (1976a).

The somatic chromosome numbers reported in the genus range from 24 to ca. 124. The most common number is 2n=30. Four polyploid series proposed by Sheffer and Kamemoto (1976a) are: (1) 20 - 40, (2) 24 - 30 - 48 - 84, (3) 28 - 56 and (4) 30 - 60 - 90 - ca. 124. The basic numbers of 5, 6 and 7 are suggested for the genus because of the presence of somatic chromosome numbers 20, 24 and 28 (Sheffer and Kamemoto, 1976a). Sheffer and Kamemoto

(1976a) considered 15 as a secondary basic number (X_2) which has evolved from species with n=5, 6 and 7. They also pointed out the frequent occurrence of B chromosomes or supernumerary chromosomes in section Cardiolonchium. Seven species in this section reported to have B chromosomes were A. crystallinum, A. forgetii, A. magnificum, A. regale, A. splendidum, A. walujewii, and A. warocqueanum (Sheffer and Kamemoto, 1976a).

The somatic chromosome number of Anthurium andraeanum has been recorded as 2n=30 and 32. Two extra chromosomes in the count of 32 are possibly loosely attached satellites (Sheffer and Kamemoto,1976a) or B chromosomes.

The karyotype and meiotic analysis of A. andraeanum 'Kaumana' and 'Uniwai' were done by Kaneko and Kamemoto (1978). The karyotypes of both cultivars showed four large metacentric chromosomes, two fairly large satellite chromosomes and twenty-four smaller chromosomes. The study of meiotic configurations revealed about the same degree of irregularity in those two cultivars. The meiotic irregularity might indicate a hybrid origin of A. andraeanum cultivars (Kaneko and Kamemoto, 1978).

In A. warocqueanum cytological examination was focused on the meiotic behavior and transmission mechanism of B chromosomes (Kaneko and Kamemoto, 1979; Marutani and Kamemoto, 1983). When two or more B chromosomes were present in a plant, two meiotic B

chromosomes tended to pair at metaphase I and normally divided at anaphase I, resulting in the transmission of B chromosomes to subsequent generations. The univalent B chromosomes, on the other hand, lagged, formed micronuclei, and were eventually eliminated. With a high number of B chromosomes, bivalent formation of A chromosomes was found to be reduced. However, there were no obvious effects of B chromosomes on leaf length, leaf width, increase in stem length, and flowering date (Marutani and Kamemoto, 1983).

In the survey of chromosome numbers of 63 Anthurium species, Sheffer and Kamemoto (1976a) noted the occurrence of polyploidy in 16 species. Tetraploidy was the common level of polyploidy, although A. supianum was a hexaploid with 2n=ca. 90 and A. scandens exhibited diploid (2n=24) as well as tetraploid (2n=48) and octaploid (2n=84) chromosome levels. In A. gracile diploids (2n=20), tetraploids (2n=40) and hexaploids (2n=60) were recorded (Sheffer and Croat, 1983).

The first report of a triploid Anthurium crystallinum was made by Ali (1979). The plant was considered to be an autotriploid because meiosis was highly irregular and resulted in a maximum of ten trivalents at metaphase I. It was assumed that the triploid arose by the union of diploid and haploid gametes.

2.3 Hybridization in *Anthurium*

2.3.1 Intraspecific hybridization

Intraspecific hybridization within *A. andraeanum* has been done extensively by anthurium breeders in Hawaii. In 1950 the anthurium breeding program was initiated at the University of Hawaii to improve commercial cut flower cultivars through hybridizing available clones of *A. andraeanum*. Major plant characteristics considered in selection are: flower yield, sucker production, shape and color of spathe, shape and position of spadix, vegetative characters such as the length of peduncle, and disease resistance, particularly anthracnose caused by the fungus *Colletotrichum gloeosporioides*. Yield is one of the most important characters in commercial cut flower production (Kamemoto and Nakasone, 1955, 1963).

Although in recent years tissue culture technique has made it possible to have rapid propagation of slow growing anthuriums in some cases (Pierik, 1975, 1976; Pierik and Steegmans, 1976; Kunisaki, 1980), high sucker production is still important in order to increase the number of outstanding plants for commercial use.

Anthracnose is a serious disease in anthurium production in Hawaii. The fungus *Colletotrichum gloeosporioides* causes spadix rot. The disease is also called black nose. Since most commercial cultivars are susceptible to anthracnose (Kamemoto *et al.*, 1968)

resistant varieties have to be bred.

Recently a bacteria blight has become the most serious problem in the Hawaiian anthurium industry. The causal organism was identified as Xanthomonas campestris pv. dieffenbachiae (Nishijima et al. 1980). Anthuriums have shown various responses to this organism so that selection of resistant varieties can be one of the objectives in the anthurium breeding program.

Along with producing commercially promising selections, intraspecific hybridization among various clones of A. andraeanum has also provided genetic information on anthuriums. Two major characters studied for their modes of inheritance are anthracnose resistance and spathe color. In the study of anthracnose resistance Aragaki et al. (1968) developed a technique for testing pathogenecity of the causal agent on anthurium spadices. The result of controlled crosses showed that anthracnose resistance was inherited quantitatively. In general, crosses between resistant plants resulted in a predominantly resistant progeny and susceptible x susceptible gave predominantly susceptible progeny (Kamemoto et al., 1977)

In early years genetic studies of spathe color was conducted only by visual means. Kamemoto and Nakasone (1963) proposed the multiple allelic system of spathe color inheritance where both red and orange were dominant

to white, and red was dominant to orange. The presence of modifying genes was also discussed (Kamemoto *et al.*, 1968). Later, complementary gene action with multiple genes for color inheritance was proposed by Sheffer and Kamemoto (1977).

Recently, a technique for biochemical studies of spathe pigments was developed and applied to explain the inheritance of spathe color (Iwata *et al.*, 1979; Iwata, 1980). Two anthocyanins found in the spathe were controlled independently by two different genes. Within a gene intra-allelic interaction was not complete, producing various concentrations of a pigment. Recessive epistasis was also observed. The detailed biochemical studies on spathe color will be reviewed in later sections.

2.3.2 Interspecific hybridization

Early records of interspecific hybrids in the genus *Anthurium* were documented by Engler (1905). Parental taxa involving interspecific hybrids with *A. andraeanum* included *A. lindenianum*, *A. ornatum*, *A. nymphaeifolium*, *A. veitchii*, *A. magnificum*, *A. warocqueanum*, *A. walujewii*, *A. spendidum* and a hybrid of *A. subsignatum* and *A. nymphaeifolium*.

In 1969, Rapsey and Carr reported various anthurium hybrids growing in Trinidad and Tobago for commercial

use.

For many years numerous interspecific hybridizations have been performed in the anthurium breeding program at the University of Hawaii. Sheffer and Kamemoto (1976b) attempted 1592 cross- and self-pollinations among 56 species and studied cross compatibility in the genus Anthurium. The species were divided into six morphological groups based primarily on Engler's classification system. It was clear that the intragroup interspecific crossing resulted in much higher success than the intergroup interspecific pollinations. The result indicated a close relationship among species within each group.

Among many species two horticulturally important plants, A. andraeanum (group VI) and A. scherzerianum (group I), were examined in the interspecific pollination study (Sheffer and Kamemoto, 1976b, 1977; Kamemoto and Sheffer, 1978). It was thought that the hybrids involving these two species could become good horticultural cultivars.

Sheffer and Kamemoto (1976b) reported that only 6 out of 51 different interspecific crossings were successful in producing hybrids with A. andraeanum 'Uniwai'. The six species belonged to group VI, including A. concinnatum, A. roseospadix (A. hoffmannii), A. lindenianum, A. kamemotoanum (A. micromystrium), A.

nymphaeifolium and A. formosum (A. pichinchae). In 1977 they reported studies on interspecific hybridization involving A. andraeanum and closely related species focusing on gene exchange between A. andraeanum and species in group V which contained attractive velvety leaves. However none of the crosses were successful. Anthurium subsignatum (A. grandifolium), A. garagaranum (A. subsignatum) and A. cerrocampanense (A. walujewii) were found to be species intermediate between group V and Group VI.

On studies of interspecific hybrids Sheffer and Kamemoto (1977) discussed the mode of inheritance in spathe and spadix color and leaf peltation. It was suggested that more than one gene were involved in anthocyanin production in both the spathe and spadix. Leaf peltation seemed to be controlled by one or two genes.

Anthurium scherzerianum was crossed successfully to A. wendlingerii (Kamemoto and Sheffer, 1978). Fertile hybrids indicated a close relationship of the two species and showed their potential use in horticulture.

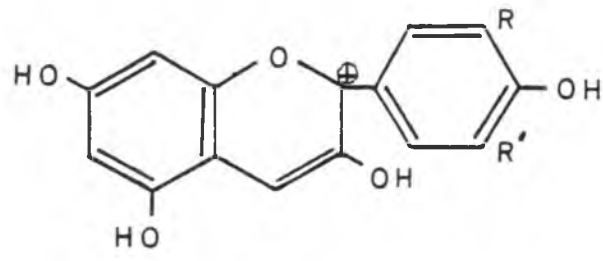
2.4 The flavonoids

The flavonoids are phenolic compounds which occur in various parts of higher plants. They are basically derived from flavan or 2-phenyl-benzopyran and are classified according to the oxidization level of the central pyran ring. Reviews on flavonoids in terms of their properties, functions, distribution in nature and biosynthesis are available in the literature (Harborne, 1967, 1973, 1976; Harborne and Mabry, 1982; Swain, 1976a, 1976b; Wong, 1976).

2.4.1 Flavonoids and flower color

Anthocyanins are a class of flavonoid compounds responsible for most scarlet, red, mauve, purple and blue pigments in flower, leaves, and fruits. They are water soluble and exist in the cell sap as glycosides of anthocyanidin which is a hydroflavylium ion (Harborne, 1967).

Three major anthocyanidins include pelargonidin, cyanidin and delphinidin which provide plant tissues the colors of orange, red-magenta and purple-blue, respectively. These pigments differ in structure only by the number of hydroxyl groups attached to the B ring of the basic skeleton. Three other common anthocyanidins are peonidin (red-magenta), petunidin (purple-blue), and malvidin (purple-blue) which contain at least one



Name	R	R'	Color
Pelargonidin	H	H	Orange
Cyanidin	OH	H	Red-Magenta
Delphinidin	OH	OH	Purple-Blue
Peonidin	OCH_3	H	Red-Magenta
Petunidin	OCH_3	OH	Purple-Blue
Malvidin	OCH_3	OCH_3	Purple-Blue

Figure 1. Structures and visual colors of six major anthocyanidins. (Bayer et al., 1966)

methoxyl group in the B ring (Harborne, 1967). The structures of six common anthocyanidins are shown in Figure 1 (Bayer et al., 1966).

Colors of anthocyanins can be modified by various factors. The concentration and kinds of anthocyanins are the most important factor influencing flower color. Stewart et al. (1980b) demonstrated that amount and proportion of anthocyanins affected color of poinsettia bracts. Pink bracts contained more cyanidin 3-glycosides than pelargonidin 3-glycosides, whereas in red bracts the ratio of cyanin to pelargonin was relatively low. In addition there were more glucosides than rutinosides in pink bracts.

A reddening effect on flower shades may be observed by methylation of some of hydroxy groups of the anthocyanidin. For instance, peonidin (=3′-O-methyl cyanidin), petunidin (=3′-O-methyl delphinidin) and malvidin (=3′,5′-O-dimethyl delphinidin) are slightly redder or less blue than cyanidin and delphinidin (Asen, 1976).

Co-pigmentation, color shift of anthocyanins resulting from associations with other compounds, was first suggested by Robinson and Robinson (1931). Asen (1976) discussed various factors responsible for diverse flower colors in nature and emphasized that co-pigmentation was the most important phenomenon which

explained a wide range of coloration in flowers.

Many examples have been reported on the co-pigmentation effect of anthocyanins. Asen et al. (1971b), for example, discussed that the orange sport of Red Wing azalea contained only cyanidin glycosides, while the Red Wing azalea exhibited co-pigmentation of the same cyanidin glycosides with flavonol glycosides. Kasha et al. (1982) summarized color shift of anthocyanins by flavonols and postulated breeding of blue hemerocallis by hybridizing purple cultivars with delphinidin and flavonol containing cultivars.

The degree of co-pigmentation effect depends on the type of anthocyanins. In Fuchsia hybrida Yazaki (1976) demonstrated that malvin showed greater blueing effects of a co-pigment (spiraeoside) than pelargonin, peonin and cyanin. In general delphinidin type (3′,4′,5′-trisubstituted) anthocyanins exhibited more co-pigmentation effect than others.

The amount of anthocyanins and the molar ratio of co-pigments to anthocyanins is another important factor determining the co-pigmentation effect. Asen et al. (1972) reported that both the concentration of cyanidin 3, 5-diglucoside and the molar ratio of quercitrin to the cyanin greatly affected the degree of co-pigmentation. They also found that the greatest color shift occurred by the association of aurone, flavonols or C-glycosyl

flavones with the anthocyanin.

The hydrogen bonding between anthocyanins and co-pigments stabilized the purple anhydrobase form of anthocyanin, resulting in both increase in color intensity and color shift (Asen, 1972). In 1981 Chen and Hrazdina suggested that the important hydrogen bondings were formed between the carbonyl group of the anthocyanin anhydrobase and aromatic hydroxy groups. The hydroxyl group at the 7-position of flavonoid molecule was most important for complex formation. The number of OH groups in the B-ring and unsaturation at C_2- C_3 in the heterocyclic ring is another important factor.

In 1983 Brouillard discussed the hydrophobic stacking process of anthocyanin molecules themselves and/or anthocyanin-copigment complexes to reduce the hydration reaction. It was hypothesized that the stacking process could prevent the pyrylium ring from water attack and subsequent color loss.

The cell sap pH can be one of the major factors determining flower color. Asen et al. (1971a) found the association of the higher pH of petal cells with the blueing of flower color in aging roses. In vitro the color of aluminum-cyanidin 3-glucoside complex changed from red to blue-violet as pH increased from 2.21 to 6.16 (Asen et al., 1969).

The highest pH recorded in floral epidermal cells was

7.5 from 'Heavenly Blue' morning glory (Stewart et al., 1975; Asen et al., 1977). During opening of flower bud of the morning glory, the pH of epidermal cells increased from ca. 6.5 to 7.5 as the flower color changed from strong reddish-purple to light blue (Asen et al., 1977).

As petals of Fuchsia hybrida aged, the color changed from blue-violet to purple-red, decreasing in pH from 4.8 to 4.2 (Yazaki, 1976). Yazaki (1976) quantified free amino acids and other non-volatile organic acids in both young and old petals. It was found that the total amount of amino acids was much higher in young petals. In addition the concentration of asparagine was especially high in young petals and its drastic decrease was observed in old tissues. Contrastingly the amount of organic acids such as aspartic, tartaric and malic acids increased as the flower became old. Thus, it was concluded that the increase in amount of organic acids caused the decrease of pH in old petals, and consequently reddening in the flower color in Fuchsia hybrida.

Chelation of anthocyanins with metals such as aluminum, molybdenum, iron and magnesium also produces bluer flower shade. The crystalline blue pigment in cornflower was found to be an iron complex of four molecules of cyanin and three molecules of flavone (Asen and Jurd, 1967). Yasuda (1970) demonstrated that iron was an important component beside cyanin and tannic

substance in the bluish spherule in epidermal tissues of rose petals.

Bayer et al. (1966) studied structural aspects of metal-anthocyanin complex formation and discussed that only glycosides of cyanidin, delphinidin and petunidin could form metal complexes. Those anthocyanins contained an ortho-dihydroxy group in B ring, where a metal chelation occurred.

Co-occurrence of anthocyanins with yellow pigments of chlorophyll modifies the visual color of anthocyanins. The yellow pigment can be carotenoids or members of flavonoids such as chalkones, aurones and flavonols.

The chalkones and aurones are yellow pigments having a restricted distribution in nature in about nine families (Harborne, 1976). The two pigments are often called the anthochlor pigments.

The flavonols contribute to yellow flower color when they are present in certain forms, e.g. methylated form. The flavonols as well as flavones can be co-pigments to enhance yellow pigments of chalkones and aurones or to modify anthocyanin pigments as described above.

2.4.2 Flavonoid biosynthesis and genetics of flower color.

The basic pathway of flavonoid biosynthesis has been established although the details have not been understood yet (Wong, 1976). Three major stages in the pathway include (1) formation of the basic $C_6C_3C_6$ skeleton, (2) formation of different classes of flavonoids, and (3) formation of individual compounds within a flavonoid class.

The basic skeleton is formed from a combination of the acetate-malonate and shikimic acid pathways. In the second stage a flavonoid, chalkone, takes an important role as the common intermediate of all other classes of flavonoids. Individual compounds are synthesized through a series of complex mechanisms such as hydroxylation, methylation, glycosylation and acylation. The general pathway of flavonoid biosynthesis is shown in Figure 2 (Ebel and Hahlbrock, 1982).

Recently much work of flavonoid biosynthesis has been concentrated on detailed studies of the enzymology by manipulating mutants and cell suspension cultures (Griesbach, 1979; Ebel and Hahlbrock, 1982. In 1982 Jourdan and Mansell isolated three distinct glucosyl transferases which are involved in the biosynthesis of flavonol triglycerides in Pisum sativum. The reaction sequence was determined as flavonol ⟶

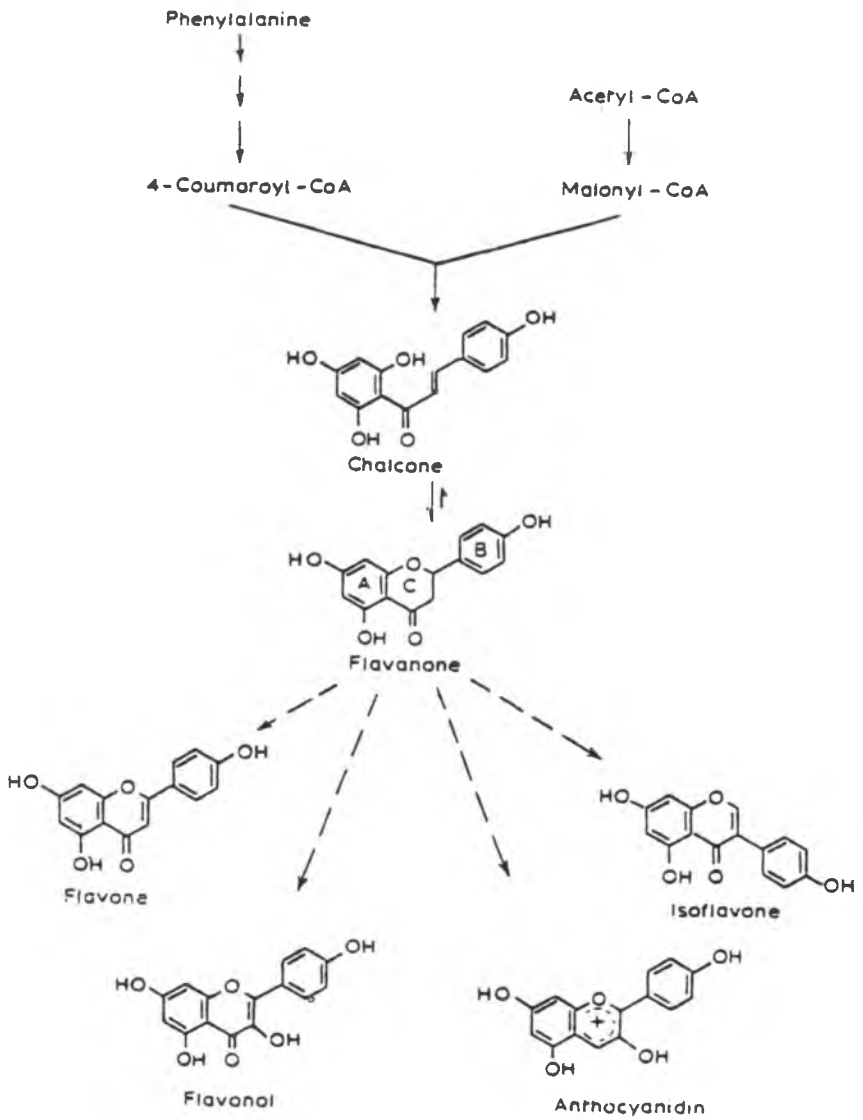


Figure 2. The general pathway of flavonoid biosynthesis (Ebel and Hahlbrock, 1982)

flavonol-3-monoglucoside→flavonol-3-diglucoside→ flavonol-3-triglucoside. Saleh (1979) reviewed various aspects of flavonoid O-glycosyltransferases including properties of enzymes and genetic control, and the biosynthetic pathway to C-glycosides was also proposed. In addition much more attention has been given to various environmental factors which regulate flavonoid biosynthesis (Wong, 1976). Examples of such factors are light, wounding and infection of tissues, and hormonal substances.

The genetic study of flower color has been done in several plant species. Harborne (1967) summarized various genes responsible for flavonoid biosynthesis in 14 species for which color inheritance studies had been done thoroughly. Important ornamental plants in his survey included Antirrhinum majus (snapdragon), Cyclamen cultivars, Dahlia variabilis, Dianthus caryophyllus (carnation), Impatiens balsamina, Lathyrus odoratus (sweet pea), Matthiola incana (stock), Petunia hybrida, Primula sinensis (chinese primrose), and Streptocarpus hybrida (cape primrose).

Major steps where genes controlled flower color formation consisted of formation of various flavonoid classes (eg. flavonols, flavones and anthocyanins), hydroxylation of B ring in anthocyanins, methylation, glycosylation (attachment of sugars) and acylation

(attachment of a cinnamic acid residue). In petals of Silene dioica, for example, there were six genes (C, A, P, M, N, and Ac) responsible for anthocyanin biosynthesis in addition to two genes, I1 and I2, controlling the intensity of flower color (Kamsteeg, et al., 1979). Without the C gene no anthocyanins were produced in any parts of a plant. Gene A governed the formation of anthocyanins in petals. Two glycosylation genes, M and N, controlled glucosylation of 5-hydroxyl group and rhamnosylation of the 3-O-bound glucose, respectively. Finally, genes Ac and P are involved in the acylation of anthocyanins.

In addition to the Mendelian type of inheritance study, there were some genetic experiments incorporated with enzymology studies. Spribille and Forkmann (1982) studied the activity of a key enzyme of flavonoid biosynthesis in different genotypes of Antirrhinum majus. It was demonstrated that flower extracts from the plants having the Niv gene exhibited high levels of chalcone synthase activity whereas in the chalk-white 'Nivea' mutant (niv/niv) chalcone synthase activity was nonexistant. The results clearly showed that the activity of the key enzyme, chalcone synthase, was controlled by the gene Niv in Antirrhinum majus.

2.4.3 Distribution and chemotaxonomy.

The taxonomic significance of flavonoids has been recognized in many plants. Harborne (1973) discussed four general conclusions drawn from the surveys of flavonoids in plants.

First, the more complex flavonoid compounds were present in the evolutionary advanced plant taxa. While the primitive plants such as mosses showed few simple flavonoid types, the highly specialized angiosperms contained a whole range of flavonoids.

Secondly, there was a striking difference between woody and herbaceous forms of plants in terms of flavonoid profiles in leaves. The woody plants showed frequent occurrences of flavonol, myricetin, leucoanthocyanidins and cyanidin. The herbaceous forms, in contrast, were characterized by the presence of flavone, delphinidin, and pelargonidin, and the absence of myricetin and leucoanthocyanidins.

Third, flower color (i.e. anthocyanin type) often correlated with the type of pollination. Bee-pollinated flowers in the Boraginaceae, for example, had been selected for their blue flowers in which delphinidin was the major pigment. By contrast, the bird-pollinated plants such as Bignoniaceae often showed red and orange flowers with pelargonidin and apigenin pigments. The biosynthetically simplest anthocyanidin, cyanidin, was

often found uniformly in the wind-pollinated Gramineae.

Lastly, there were certain flavonoids which occurred in specific groups of plants. Isoflavonoids, for instance, were present only in the Leguminosae and took important roles in the biosystematics of the family as well as the physiological investigations of phytoalexins.

Recent applications of flavonoid compounds as taxonomic characters have been reported at various ranks of taxa such as family, genus, and species, of which greater taxonomic value is realized below the genus level (Radford, et al., 1974).

Several cases have been reported that flavonoid analysis have helped to support the existing classification system at subfamily or tribal level, e.g. tribes in the family Umbelliferae (Saleh et al., 1983). In the family Limnanthaceae, Parker and Bohm (1979) studied 19 taxa and proposed the presence of three phyletic lines instead of two, which was the previously accepted number of genera in the family.

The flavonoid analysis was also utilized in taxonomical groupings of species within a genus. For example, sectional classification of the genus Limnanthes (Limnanthaceae) became possible by investigating flavonoid profiles of 19 species (Parker and Bohm, 1979). Parker and Bohm (1979) found that the separation of the section Inflexae from Reflexae was successful by

comparing petal flavonoids but not by the analysis of whole-plant flavonoids. It was assumed that the evolution of petal flavonoids was associated with an outcrossing breeding system to attract pollinators. Even after the outbreeding trait had been lost, the flavonoid compositions in the petals remained the same. Thus the data on petal flavonoids agreed with the natural classification system. Flavonoid variation in different organs was also reported in Eriastrum densifolium (Polemoniaceae) (Smith and Patterson, 1981). Smith and Patterson suggested functional differences of leaf and corolla flavonoids and emphasized the importance of using same tissues in a chemotaxonomic work.

Extensive chemotaxonomic work of the genus Dahlia (Compositae) was done by Giannasi (1975). Flavonoid analysis was applied to compare three sections, Entemophyllon, Dahlia, and Pseudodendron in addition to interspecific comparisons within each section. It was proven that flavonoids could be used as effective taxonomic markers at both taxonomic levels. 6-methoxy flavones were unique to the section Entemophyllon separating it from the other two sections which contained flavonols. At the interspecific level, all species were characterized by their own flavonoid profiles of the various tissues examined.

In the genus Ruellia (Acanthaceae) the groups of

blue flowered species and that of red flowered species showed distinct differences in flavonoid content (Bloom, 1976). Some other recent examples of flavonoid analysis for interspecific comparisons included Rhododendron (Ericaceae) (King, 1977), Thymus (Labiatae) (Adzet and Martinez, 1981), Glandularia (Verbenaceae) (Umber, 1980), Leucocyclus (Compositae) (Valant-Vetschera, 1982), and Luffa (Cucurbitaceae) (Schilling and Heiser, 1981).

An interesting comparison of leaf flavonoids in Coreopsis nuecensoides and C. nuecensis (Compositae) was done by Smith and Crawford (1981). Two species had been recognized as reproductively isolated taxa due to chromosomal translocations. Although the morphological expressions of the two species were very similar, flavonoid analysis showed distinct chemical constituents. It was hypothesized that major flavonoid differences between the two taxa were due to changes in genetic regulation after chromosomal rearrangement.

In recent years infraspecific variation in flavonoid chemistry has gained the attention of chemotaxonomists. It should be noted that the degree of flavonoid variation among populations of a species depends on the taxon which is investigated (Harborne, 1975). In Brickellia cylindracea (Compositae) two completely isolated populations showed identical flavonoid profiles, and therefore Timmermann and Mabry (1983) treated the two

groups as one taxon. Contrastingly chemical variation in Notholaena standleyi (Peteridaceae) suggested that the taxon could be divided into three subgroups (Seigler and Wollenweber, 1983).

A taxonomic rank may be shifted when flavonoid analysis shows the distinctiveness of a taxon. Smith (1980) biochemically examined the Pityrogramma triangularis complex and suggested that P. triangularis var. pallida should be treated as the species P. pallida due to the presence of flavones which were not isolated from the other variants.

In conjunction with cytology, flavonoid analysis can reveal the relationship of various groups within a species. In 1983 Soltis et al. applied flavonoid analysis to examine three cytotypes (diploid, triploid, and tetraploid) of Galax urceolata (Diapensiaceae). The taxon was characterized by possession of a relatively uniform flavonoid profile among sixty populations as well as three cytotypes. The result supported the hypothesis that the tetraploid was an autotetraploid.

Recently the rapid identification of horticultural cultivars has become possible by using high performance liquid chromatographic (HPLC) techniques for separation of flavonoids. The procedures successfully resolved quantitative differences of anthocyanins and flavonols from poinsettia (Euphorbia pulcherrima) bracts of various

cultivars, and that those chemical compounds could be used as taxonomic markers (Asen, 1979; Stewart *et al.*, 1979, 1980). Similar HPLC procedures for cultivar separation have also been applied to roses (Asen, 1982) and geranium (Asen and Griesbach, 1983).

2.5 Anthocyanins and other flavonoids in *Anthurium*

One of the major characteristics of the genus *Anthurium* is the possession of a spathe and spadix. A spathe is a modified leaf positioned at the base of the spadix (inflorescence). The spathe is often pigmented in various shades.

The first biochemical study of spathe color was done by Robinson and Robinson (1932). They found that the orange-scarlet spathe of *A. scherzerianum* contained pelargonidin 3-pentoseglycoside. Later Harborne (1967) reported the presence of pelargonidin 3-rutinoside in the same species.

In a survey of the anthocyanins of tropical plants Forsyth and Simmonds (1954) identified three cyanidins and one pelargonidin in *A. andraeanum* and a cyanidin in *A. gracile*. In *A. andraeanum* both pelargonidin and cyanidin 3-rutinoside were also isolated from spathes of cultivars by Lowry (1972).

Recently a biochemical study on the spathe color of *A. andraeanum* cultivars was conducted in the anthurium

breeding program at the University of Hawaii (Iwata et al., 1979; Iwata, 1980). Iwata et al. (1979) reported the presence of both pelargonidin and cyanidin 3-rutinoside in the red cultivars, 'Ozaki', 'Kaumana', 'Kansako No.1', and 'Nakasawa', and in the pink cultivar, 'Marian Seefurth'. As the color of the spathe became lighter the concentrations of each pigment decreased. The amount of cyanin was much higher than the amount of pelargonin (Iwata, 1980).

In some orange cultivars, like 'Nitta', pelargonin was the only pigment isolated whereas in other orange anthuriums, such as 'UH 139', a trace of cyanin was detected in addition to a large amount of pelargonin. In coral cultivars pelargonin was the only detectable pigment. No anthocyanin pigments were observed in the white spathe (Iwata, 1980).

On analysis of segregation patterns of offspring in controlled crosses, a scheme of inheritance of pigments in Anthurium andraeanum cultivar was proposed by Iwata in 1980. She suggested that because of the presence of two anthocyanins in red and pink spathes, two major genes were responsible for anthocyanin production. The gene M controlled production of cyanidin 3-rhamnosylglucoside and the gene O was responsible for production of pelargonidin 3-rhamnosylglucoside. She further discussed the recessive epistasis exhibited by the O locus over the

M locus. The proposed genotypes for each spathe color are as follows:

Phenotype	Genotype
Red group (red and pink)	MMOO, MMOo, MmOO, MmOo
Orange group (orange and coral)	mmOO, mmOo
White	mmoo, Mmoo, MMoo

The interspecific cross between Anthurium andraeanum 'Nitta' (orange) and A. roraimense (green) also revealed the complementary gene action of M and O loci(Iwata, 1980). The uniform F_1 progeny with red spathe contained both cyanin and pelargonin pigments. It was assumed that the genotypes of two parents, 'Nitta' and A. roraimense, and their offspring were mmOO, MMoo, and MmOo, respectively.

Iwata (1980) also discussed the gene dosage effect in anthocyanin production. It was suggested that the dominant homozygote possessed the most intense concentration of pigment, and the heterozygote had an intermediate concentration. The recessive homozygote contained nearly no pigments. The effect of the M gene was greater than that of the O gene. A series of genotypes was postulated according to the concentration of anthocyanin pigments in spathes: mmoo<MMoo<mmOo<mmOO<MmOo<MmOO<MMOo<MMOO. Three

genotypes, mmoo, Mmoo, and MMoo showed white color whereas MMOO exhibited the highest concentration of anthocyanins, and was visually a dark red color.

Few reports on non-cyanic flavonoids in Anthurium have been documented. Iwata (1980) found a flavone, acacetin 7-glycoside in A. andraeanum spathes. The role of the flavone was suggested to be stabilization of anthocyanins. No flavonols, chalcones, or aurones were detected (Iwata, 1980).

In 1981 Williams et al. surveyed leaf flavonoids in the family Araceae. Flavone C-glycosides were found to be the major flavonoid constituents of the family and the majority of Anthurium species studied showed the presence of the compounds. Additionally methylated flavones and two flavonols, quercitin and kaempferol were detected in some species of Anthurium.

III. MATERIALS AND METHODS.

3.1 Plant Material

Anthurium species and their interspecific hybrids used in this study were available at the University of Hawaii. Species were collected from Panama and other Central American and Caribbean countries, or from private and commercial sources. Species hybrids were produced earlier by Kamemoto and Sheffer (Sheffer and Kamemoto, 1977; Sheffer, 1974; Kamemoto, unpublished data).

Species relationships of taxonomic groups V and VI described by Sheffer and Kamemoto (1976b) were investigated because of their significance in the anthurium breeding program. Species in group VI which includes *A. andraeanum* have showy spathes and cordate leaves. In contrast, plants in group V often have inconspicuous flowers but attractive velvety foliage. The common chromosome number of the species in group VI is 2n=30 while that of group V is 2n=30, plus an occasional B chromosome.

A recently discovered lavender dwarf species from Panama, *A. amnicola* Dressler (Dressler, 1978; 1980) was also examined cytologically and biochemically. Various hybrids resulting from *A. amnicola* as a parent were studied to reveal genome relationships of parental plants and to clarify pigment systems of the spathe.

Sources of Anthurium species and cultivars used in this study are listed in Table 1, and various intergroup and intragroup interspecific hybrids are tabulated in Table 2. The list of Anthurium amnicola hybrids examined cytogenetically and/or biochemically is shown in Table 3.

3.2 Cytological studies

Karyotype analysis and somatic chromosome counts were done by sampling actively growing root tips. Samples were pretreated in 0.002 M 8-hydroxyquinoline solution for 3 to 5 hours at 18C, fixed in 6:3:1 (chloroform: 95% ethyl alcohol: glacial acetic acid) modified Carnoy's solution for 15 minutes at 18C, hydrolyzed in 1N hydrochloric acid for 15 minutes at about 50C, and stained with 1% aceto-orcein.

Photomicrographs of selected mitotic chromosomes were taken and karyotypes were made by cutting each chromosome from a print that was enlarged from a photomicrograph negative and arranging the chromosomes in descending order of length.

For meiotic analysis, spadices were fixed in the same Carnoy's mixture mentioned above for at least 24 hours at room temperature (ca. 24C) and stored in the freezer. Anthers were dissected out from the spadix and were squashed in 45% acetic acid and the pollen mother cells were stained with 1% aceto-carmine. The meiotic

Table 1. List of *Anthurium* taxa examined and their source.

Taxon	Engler's[z] section	Acc. and[y] cross No.	Source
Group VI[x]			
A. andraeanum Andre[v]	*Calomystrium*		
'Uniwai'			UH
'Kaumana'			Grower
'Marian Seefurth'			UH
'Manoa Mist'			UH
A481		A481	Ecuador
'Nitta'			Grower
'UH515'			UH
A. caperatum Croat & Baker	*Polyneurium*	A227-1	Panama
		A227-2	Panama
A. concinnatum Schott	*Belolonchium*[w]	A212	Costa Rica
A. formosum Schott	*Calomystrium*	A287	Panama
		A290	Panama
		A291	Panama
A. kamemotoanum Croat sp.ined	*Calomystrium*	A288	Panama
		567	A288 selfed

Table 1. (Continued)

Taxon	Engler's[z] section	Acc. number[y]	Source
A. lindenianum C. Koch & Augustin	*Calomystrium*	A170	Grower
		A185	Grower
		A220-1	Colombia
		A220-2	Colombia
A. nymphaeifolium C. Koch & Bouche	*Calomystrium*	A213-1	Venezuela
		A213-2	Venezuela
		A213-3	Venezuela
A. roraimense N. E. Brown	*Calomystrium*	A189	Panama
A. roseospadix Croat sp.ined.	*Calomystrium*	A289	Panama
		A292	Panama
		A293	Panama
Group V[x]			
A. cerrocampanense Croat	*Cardiolonchium*[w]	A225-1	Panama
		A225-2	Panama
A. subsignatum Schott	*Semaeophyllium*	A183	Panama

Table 1. (Continued)

Taxon	Engler's section[z]	Acc. number[y]	Source
A. garagaranum Standley	*Semaeophyllium*	A262	Panama
		A263	Panama
A. wallisii Masters	*Polyneurium*	A286	Panama
Other group			
A. amnicola Dressler[u]	*Porphyrochitonium*[w]	A417	Panama
		A430	Panama
'Calypso'			Trinidad
'Trinidad'			Trinidad

z Sheffer and Croat, 1983.
y Accession and cross numbers refer to Dr. H. Kamemoto's accession and cross numbers.
x Sheffer and Kamemoto, 1976b.
w Croat and Sheffer, 1983.
v Sheffer *et al.*, 1980a.
u Dressler, 1978, 1980.

Table 2. List of interspecific hybrids utilized in cytological and/or biochemical study.

Cross	Cross and plant number
Intragroup crosses:	
VI x VI[z]	
A. andraeanum 'Uniwai	
x *concinnatum* (A212)	RS987-2, -6[y]
x *formosum* (A290)	RS1205-7
x *formosum* (A290)	RS1183-5
x *kamemotoanum* (A288)	RS532-5, RS1249-1, -9, -10
x *lindenianum* (A220-2)	RS1360-1, -4
x *roseospadix* (A289)	RS691-8
x *roseospadix* (A292)	RS985-4
x *roseospadix* (A293)	RS675-2
A. andraeanum 'UH515'	
x *roraimense* (A189)	x 534
A. andraeanum 'Nitta'	
x *roraimense* (A189)	x 434
A. concinnatum (A212)	
x *roseospadix* (A292)	RS1227-1, -3

Table 2. (continued)

Cross	Cross and plant number
A. formosum (A287)	
x *concinnatum* (A212)	RS174-6, -7
A. formosum (A291)	
x *roseospadix* (A292)	RS1224-2, -5
A. kamemotoanum (A288)	
x *formosum* (A291)	RS1228-3, -8, -10, -13
x *lindenianum* (A220-2)	RS1293-5, -9
A. lindenianum (A170)	
x *formosum* (A290)	RS1212-3, -6
x *roseospadix* (A292)	RS986-7
x *lindenianum* (A220-2)	RS1361-1, -5
A. lindenianum (A220-2)	
x *kamemotoanum* (A288)	RS1327-1, -2
A. lindenianum (A220-1)	
x *roseospadix* (A292)	RS972-3
A. nymphaeifolium (A213-3)	
x *formosum* (A287)	RS251-1, -5

Table 2. (continued)

Cross	Cross and plant number
A. roraimense (A189) x *andraeanum* 'Manoa Mist'	426-73[x]
A. roseospadix (A289) x *kamemotoanum* (A288)	RS1325-2, -14
(VI x VI) x VI[z] (*A. andraeanum* 'Uniwai' x *kamemotoanum* (A288)) x *formosum* (A290)	392-1[x], -9, -25, -42, -56, -66, -75, -99
V x V[z]	
A. cerrocampanense (A225-1) x *garagaranum* (A262)	RS1130-5, -6, -8
A. cerrocampanense (A225-1) x *garagaranum* (A263)	RS511-4, -5
A. subsignatum (A183) x *garagaranum* (A263)	RS1354-4, -5

Table 2. (continued)

Cross	Cross and plant number
Intergroup Crosses:	
VI x V[z]	
A. lindenianum (A220-2)	
x *cerrocampanense* (A225-1)	RS970-4
A. formosum (A287)	
x *cerrocampanense* (A225-2)	RS1564-1, -2, -4
A. kamemotoanum (A288)	
x *wallisii*? (A286)	RS1253-1, -2, -4
V x VI[z]	
A. cerrocampanense (A225-1)	
x *concinnatum* (A212)	RS1235-1, -3
A. garagaranum (A263)	
x *lindenianum* (A185)	RS427-3, -4, -8
A. subsignatum (A183)	
x *nymphaeifolium* (A213-1)	RS1306-1, -2, -4, -5, -8
x *caperatum* (A227)	RS86-2

z Sheffer and Kamemoto, 1976b.
y RS cross numbers refer to Dr. R. Sheffer's crosses.
x Cross and plant numbers refer to Dr. H. Kamemoto's cross numbers.

Table 3. List of *Anthurium amnicola* hybrids examined in cytological and biochemical study.

Cross	Cross number[z] or Acc. number
A. amnicola (A417)	
x *formosum* (A287)	568
(A291)	572
x *lindenianum* (A220-2)	569, 575
x(*kamemotoanum* x *formosum*)	573
A. andraeanum	
x *amnicola*	A494
A. lindenianum (A170)	
x *amnicola* (A417)	552
'Calypso'	
x *amnicola* (A417)	531
'Trinidad'	
x *amnicola* (A417)	561
392-42	
[('Uniwai' x *kamemotoanum*) x *formosum*]	
x *amnicola* (A417)	587

z Cross and accession numbers refer to Dr. H. Kamemoto's cross and accession numbers.

cells were examined under a light microscope and photographed.

The pollen grains were stained with 1% aceto-carmine for about 10 minutes and examined under a light microscope to determine the percentage of stained pollen grains as a possible indicator of pollen fertility of the plant. The viability of the pollen was also evaluated by using the Nitro-blue tetrazolium test (Nitro-BT) described by Hauser and Morrison (1964). The chemical constituents in Nitro-BT incubation medium consisted as follows:

0.2M sodium succinate	1ml.
0.06M Phosphate buffer (pH=7.4)	1ml.
Nitro-blue tetrazolium	1mg.
Sodium amytal	1mg.
Deionized water	1ml.

Pollen grains were kept in Nitro-BT medium for about 5 hours and then were examined to determine the percentage of stainability.

For both staining techniques, 1000 grains per spadix were examined to estimate the pollen viability.

3.3 Flavonoid analysis

3.3.1 Anthocyanins

3.3.1.1 Isolation and spectral measurements of anthocyanins

Because of its unique spathe color Anthurium amnicola

was the first plant to be examined. Anthocyanin pigments were extracted from freshly harvested spathe in 0.1% HCl-MeOH. The extracts were filtered through Whatman No. 1 paper and were concentrated to a small volume by a rotary evaporator. Preparative paper chromatography was done by using 46cm x 54cm Whatman 3 MM paper with the solvent HOAc (acetic acid: water: conc. HCl = 15:85:3 vol/vol). Two clearly separated pigments were eluted individually in 0.01% HCl-MeOH. The degree of purity of each isolated pigment was resolved by high performance liquid chromatography (HPLC) (Beckman Model 110A) on a reverse phase C18-5s 250mm x 4mm ods column (Bio-Rad). The solvent consisted of 6% acetone, 10% acetic acid and 84% distilled water. The flow rate was 1.0ml/min. and absorption was detected at 530nm.

The absorption spectra of isolated anthocyanins were measured on an Ultraviolet-visible (UV-Vis) spectrophotometer (Hewlett-Packard Model 1030B) with and without a few drops of 5% $AlCl_3$ in 95% ethanol.

3.3.1.2 Authentic pigments

Various authentic anthocyanins were isolated from plant materials and were compared with pigments extracted from anthurium spathes, spadices, and leaf tissues by using HPLC and measurements of absorption spectra.

Two anthocyanins, cyanidin 3-rutinoside and

pelargonidin 3-rutinoside were obtained from the spathe of Anthurium andraeanum 'Kaumana' (Iwata, 1979). Five anthocyanins detected from bracts of Euphorbia pulcherrima were cyanidin 3-glucoside, 3-galactoside, and 3-rutinoside, and pelargonidin 3-glucoside and 3-rutinoside (Asen, 1979). From Bing cherries (Prunus avium L. var. Bing), cyanidin 3-glucoside, cyanidin 3-rutinoside and peonidin 3-rutinoside were isolated (Lynn and Luh, 1964).

Identification of anthocyanins was done by comparing Rf values and retention time of authentic pigments on thin layer chromatography(TLC) and HPLC, respectively. For TLC commercial plates (0.25mm MN 300 cellulose Analtech, Inc.) were used with solvent systems of Buff CH_3CN (acetonitrile: 0.1M ammonium acetate pH=4.0 7:3 vol/vol) and BAW (n-butanol: glacial acetic acid: water 4:1:5 vol/vol) (Iwata, 1979).

3.3.1.3 Qualification and quantification of anthocyanins

Weighed tissue (ca. 1gm)was shredded and blended in 10ml of 0.1% HCl-MeOH for about 30 sec. Extracts were filtered with Whatmans No. 1 paper and dried. Pigments were eluted in 1 to 5ml of solvent used in HPLC and prior to injection the sample was passed through 0.5µm membrane filter.

Two HPLC systems were used to resolve anthocyanins in

spathes, spadices and leaf tissues of various Anthurium species, cultivars and hybrids. The first system had the same solvent described above whereas in the second system solvent contained a slightly different proportion of acetone: acetic acid: distilled water(6:12:82). Retention time was much shorter with the second solvent but resolution was better with the first solvent.

Relative concentration was determined by calculating the area under each peak and expressed by absorbance (area) x solvent volume (ml)/ fresh weight (gm). When an integrator was available, absorbance of each pigment was estimated by reading the area calculated by the integrator.

Anthocyanin pigments were classified according to the size of peak on the chart and determined that if the peak was distinctively large, the anthocyanin was called a major pigment and if there was secondary major peak, it was called a minor pigment and finally if a very small peak was detected it was classified as a trace pigment. The concentration of a major pigment in a plant was not necessarily the same concentration of a major pigment of the other plant. Major, minor and trace pigments were relative to one another within the same tissue of the same plant.

3.3.1.4 Correlation analysis

The correlation analysis between various anthocyanins occurring in anthuriums was done in order to see any relationship among pigments. SAS, a computer software system was used for this analysis.

3.3.2 Flavanones, flavones and flavonols

3.3.2.1 Isolation and identification

Freshly harvested leaves were blended in 70% methanol and filtered through Whatman No. 1 paper. Sep-pak C18 cartridges (Waters Associates) were used to remove chlorophylls (Eskins and Dutton, 1979) and to purify samples for HPLC chromatography. Prior to injection samples were filtered through 0.5 um membrane filter.

Flavonoids were separated by using HPLC on a reverse-phase 250 mm x 10 mm, Ultrasil-ODS (10 µl). Two solvents, water (pump A) and methanol (pump B) were used with the elution profile as follows:

Time	Solvent % in pump B
0-1 min	10 %
1-3 min	10-30 %
3-13 min	50-75 %
13-35 min	75 %

The flow rate was 3.5 ml/min and the detector was set at 340 nm.

The compounds at major peaks were collected, dried by

rotary-evaporator, and eluted in methanol. The degree of purity of compound was examined by HPLC on a reverse-phase column, 10 ul Lichrosorb RP18 (250 mm x 4.6 mm) with the same elution profile as above except that the pump A contained 5% formic acid and the flow rate was 2.5 ml/min. When a compound was not pure enough, paper chromatography was used to purify samples.

Descending two-dimensional paper chromatography was applied by using 46 cm X 54 cm Whatman 3 MM paper with the first solvent TBA (tertiary butanol: glacial acetic acid: water = 3:1:1) and the second solvent 15% acetic acid (Mabry et al., 1970; Markham, 1982). Absorption spectra of pure or nearly pure compounds were analyzed by using UV spectrophotometer. Five shift reagents (sodium methoxide, sodium acetate, aluminum chloride, hydrochloric acid and boric acid) were used to characterize flavonoids. Effects of shift reagents were observed according to the standard procedures of Markham (1982) and Mabry et al.(1970).

Two dimensional paper chromatography described above was performed to obtain additional information on individual compounds. Each compound was visualized under UV light with and without ammonia vapor and Rf values of the compound were recorded for both solvents TBA and 15% acetic acid. Data were compared with reports by Mabry et al.(1970).

Acid hydrolysis was done by using 2N HCl at ca. 80C for 1 hour. Hydrolyzed products were resolved by HPLC, isolated and analyzed for their properties by using the same procedure described above.

Authentic standards from fruits of Fortunella margarita were compared with flavonoids from anthuriums.

3.3.2.2 Flavonoid profile on HPLC

Five grams of fresh leaf tissue was blended in 20ml of70% methanol for about 2 min and filtered with Whatman No. 1 paper. Prior to HPLC injection, Sep-pak and 0.5 µm membrane filter were used to purify samples. The HPLC system used was the same as mentioned in Sec 3.3.2.1 with 5% formic acid in pump A and methanol in pump B. A reverse-phase column, 10 ul Lichrosorb RP18 (250 mm x 4.6 mm) was used. Ten µl of sample was injected. The detector was set at 340 nm with the scale of 0.2. The speed of paper chart was 30 cm/hr.

3.4 pH and color measurements

For pH measurement about 0.3 g of shredded tissue was homogenized in 10 ml distilled water for ca. 30 sec. pH of the solution was determined by using pH meter.

Three methods were used for color determination. Visual color scoring was the simplest method to measure the coloration of plant tissues. Color was also

determined by matching with Royal Horticultural Society Color Chart (RHSCC). The most objective color determination was performed by using Hunterlab colorimeter. Hunterlab coordinates (L, a and b) were recorded. L (luminosity) measured intensity of color with the scale from 0 to 100, where 0 was totally black and 100 was white. Two color quality parameters a and b indicated difference in green (negative number)-red (positive number) and blue (negative)-yellow (positive), respectively. The third color index $\tan^{-1}(a/b)$ was calculated to combine the two color parameters a and b.

3.5 Co-pigmentation

A major flavonol was isolated from leaves of Anthurium formosum (A290) by HPLC and paper chromatography. Two mg of dried flavonol compound was dissolved in 1ml citrate-phosphate buffer solution (pH=5.66). Cyanidin 3-rutinoside was isolated from spathes of A. andraeanum 'Kaumana'. The same weight (2 mg) of the pigment was also dissolved in 1 ml citrate-phosphate buffer solution. The concentration of cyanidin 3-rutinoside was slightly lower than 5×10^{-3} M. Combined pigment solution was prepared by adding 2 mg of cyanidin 3-rutinoside and 2 mg of flavonol in the same buffer solution (1 ml).

Absorption spectra of three solutions were compared in order to detect any co-pigmentation effects of

flavonol such as an enhancement of absorption and a shift of absorption maxima. Color photography of three solutions was also taken to find visual difference in color.

3.6 Taxonomic studies

3.6.1 Plant taxa and characters

Thirty two taxa which include Anthurium species and various cultivars are listed in Table 1 (Sec 3.1). Four plants excluded from taxonomic studies were A. formosum (A287), A. kamemotoanum (A288), and two plants of A. cerrocampanense (A225-1, and A225-2).

A total of 26 morphological characters were investigated and were coded according to the type of character. Measurements of characters were divided into four classes and were changed to additive binary codes. Characters such as the shape of the blade or scent of spadix were coded as non-additive binary forms. Sixty two binary codes were obtained after transformation of the original data. Table 4 shows morphological characters and character states evaluated in the study.

Flavonoid data were taken from HPLC chromatograms of each taxon. A peak at a certain retention time was considered a character and the height of a peak was recorded as a character state. Although the total of 111 peaks was observed, 11 or 13 peaks at longer retention

Table 4. Morphological characters and character states used in taxonomic studies.

Structure	Character	Character state
Petiole	1-length	<100 mm = 000, <400 mm = 100, <700 mm = 110, >700 mm = 111
	2-diameter	<3 mm = 000, <6 mm = 100, <9 mm = 110, >9 mm = 111
	3-cross-section shape	round = 100, canaliculate or slightly canaliculate = 010, angular = 001
Geniculum	4-length	<15 mm = 000, <35 mm = 100, <50 mm = 110, <50 mm = 111
	5-diameter	<4 mm = 000, <7 mm = 100, <10 mm = 110, >10 mm = 111
	6-cross-section shape	round = 100, canaliculate or slightly canaliculate = 010, angular = 001
	7-color	green = 0, colored = 1
Blade	8-length	<200 mm = 000, <400 = 100, <600 mm = 110, >600 = 111
	9-width	<100 mm = 000, <250 = 100, <400 mm = 110, >400 = 111
	10-shape	ovate = 1000, narrow trianglar ovate = 0100, obpyriform-hastate = 0010, lanceolate = 0001
	11-texture	thin = 0, thick = 1

Table 4. (Continued).

Structure	Character	Character state
Peduncle	12-length	<200 mm = 000, <350 mm = 100 <500 mm = 110, >500 mm = 111
	13-diameter	<3 mm = 000, <5 mm = 100, <7 mm = 110, >7 mm = 111
	14-cross-section shape	round = 0, angular = 1
Spathe	15-length	<50 mm = 000, <100 = 100, <150 mm = 110, >150 mm = 111
	16-width	<50 mm = 000, <75 mm = 100, <100 mm = 110, >100 mm = 111
	17-color	green=0, colored = 1
	18-shape	linear = 10000 linear-lanceolate = 01000 lanceolate = 00100 ovate = 00010, cordate = 00001
	19-reflexed?	not reflexed or hooded = 0 reflexed = 1
Spadix	20-length	<50 mm = 000, <90 mm = 100 <130 mm = 110, >130 mm = 111
	21-diameter	<5 mm = 000, <10 mm = 100, <15 mm = 110, >15 mm = 111
	22-color	green = 0, colored = 1
	23-shape	not tapered = 0, tapered = 1
	24-stalked	stalked = 0, not stalked = 1
	25-scent	absent = 00, present sweet = 10 present moldy = 01
	26-position	upright = 0, reclining = 1

time had to be deleted in order to run PAUP and Wagner 78 computer programs, respectively. The two programs took a maximum of 100 characters.

3.6.2 Phenetic and cladistic analysis

Phenetic similarity based on morphological data was estimated by using the coefficient of simple matching while the coefficient of Jaccard was used for flavonoid data. Phenograms were developed after cluster analysis via the unweighted pair group method using arithmetic averages (UPGMA). The computer program SAHN was used for those analyses.

Cladistic studies were done by constructing Wagner networks without any assumptions of evolutionary character directions. Two Wagner networks were developed from flavonoid data: coded data (<10, <100, <1000, <10000 and >10000) (Wagner 78 computer program) and absence vs presence (PAUP computer program). The PAUP program was used to construct a Wagner network based on binary coded morphological data.

IV. RESULTS AND DISCUSSION

4.1 Cytological studies on *Anthurium* species, cultivars and hybrids

4.1.1 Karyotypes of *Anthurium* species and cultivars

Somatic chromosomes of *Anthurium* species and cultivars were investigated in order to confirm chromosome counts of parental taxa used in artificial hybridization and to obtain cytological data on other plants which had not been reported previously. All chromosome counts for species and cultivars showed 2n=30 except two plants, *A. garagaranum* (A263) and *A. wallisii* (A286), which had one and two small accessary or B-chromosomes, respectively (Table 5 and Figures 3 - 32). Both taxa belong to Sheffer and Kamemoto's group V where the occurrence of B-chromosomes is common (Sheffer and Kamemoto, 1976b).

The B-chromosomes in *A. wallisii* are smaller than the smallest A-chromosome pair. Meiotic configuration in pollen mother cells revealed that the two small chromosomes did not pair with A-chromosomes.

The size of the extra chromosome in *A. garagaranum* (A263) is similar to the smallest A-chromosome pair. A hybrid of A263 and *A. subsignatum* (2n=30) showed a meiotic configuration of 15 bivalents and one univalent (see Sec 4.1.3). It is assumed that the extra chromosome

Table 5. Somatic chromosome numbers of *Anthurium* species and cultivars.

Species and cultivar	Accession number[z]	Chromosome number
Group VI[y]:		
A. andraeanum	A481	30
'Uniwai'		30
'Kaumana'		30
'Marian Seefurth'		30
'Manoa Mist'		30
'Nitta'		30
A. caperatum	A227-1	30
	A227-2	30
A. concinnatum	A212	30[x]
A. formosum	A287	30
	A290	30
	A291	30
A. kamemotoanum	567-70 (A288 selfed)	30
A. lindenianum	A170	30
	A185	30
	A220-1	30
	A220-2	30
A. nymphaeifolium	A213-1	30
	A213-2	30
	A213-3	30
A. roraimense	A189	30
A. roseospadix	A289	30
	A292	30
	A293	30

Table 5. (Continued)

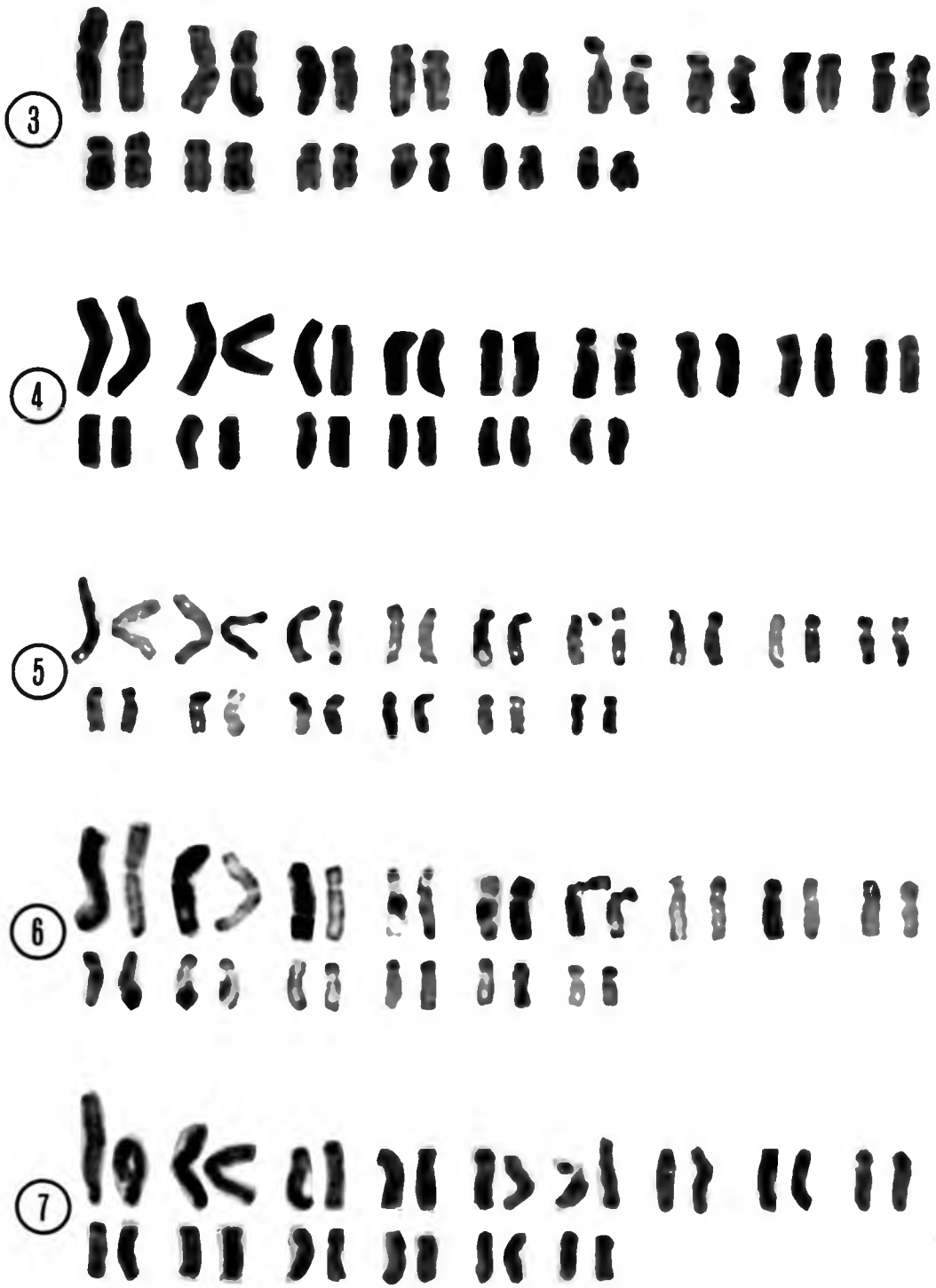
Species and cultivar	Accession number[z]	Chromosome number
Group V[y]:		
A. cerrocampanense	A225-1	30+2B[x]
	A225-2	30+2B[x]
A. subsignatum	A183	30
A. garagaranum	A262	30
	A263	30+B
A. wallisii	A286	30+2B
Others:		
A. amnicola	A417	30
'Calypso'		30
'Trinidad'		30

z Accession numbers refer to Dr. H. Kamemoto's accession numbers.
y Sheffer and Kamemoto, 1976b.
x Kamemoto, unpublished data.

Figures 3-7. Karyotypes of *Anthurium andraeanum*. (2200X)

Figure:

3. *A. andraeanum* 'Kaumana' 2n=30.
4. *A. andraeanum* 'Marian Seefurth' 2n=30.
5. *A. andraeanum* 'Manoa Mist' 2n=30.
6. *A. andraeanum* 'Uniwai' 2n=30.
7. *A. andraeanum* (A481) 2n=30.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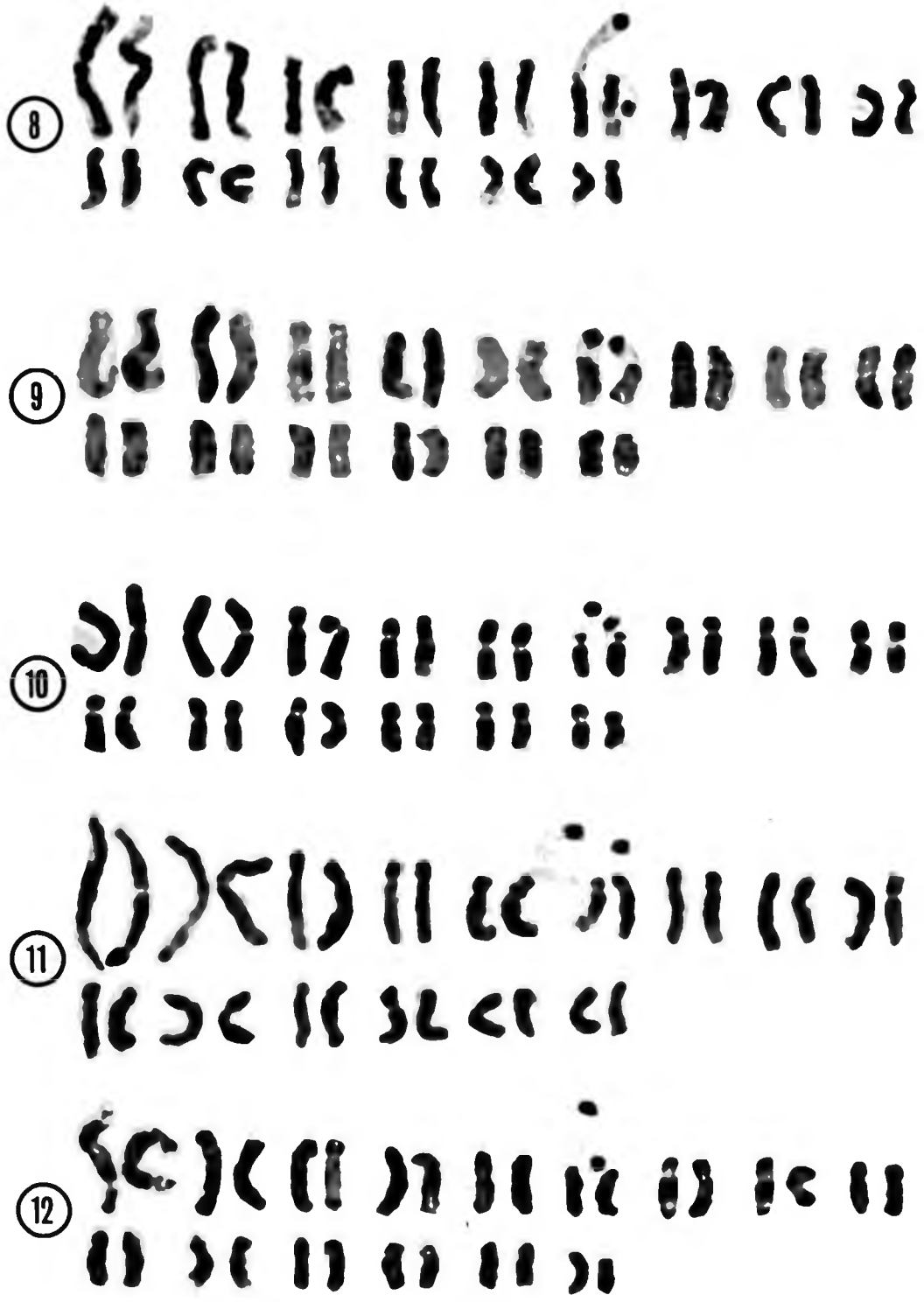
Figures 8-12. Karyotypes of *Anthurium* species. (2200X)

Figure:

8. *A. andraeanum* 'Nitta' 2n=30.
9. *A. lindenianum* (A170) 2n=30.
10. *A. lindenianum* (A185) 2n=30.
11. *A. lindenianum* (A220-1) 2n=30.
12. *A. lindenianum* (A220-2) 2n=30.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Figures 13-17. Karyotypes of *Anthurium* species. (2200X).

Figure:

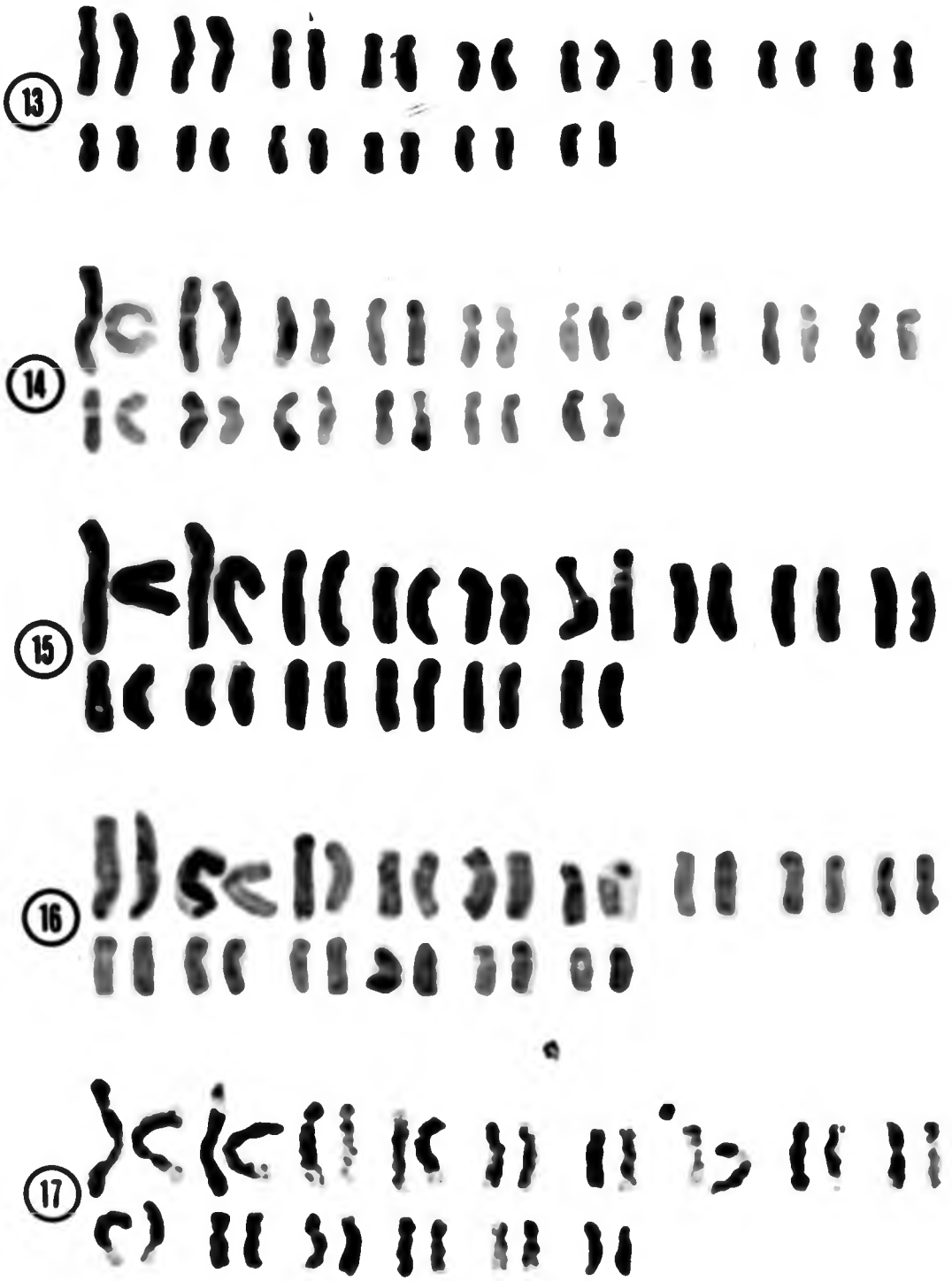
13. *A. caperatum* (A227-1) 2n=30.

14. *A. caperatum* (A227-2) 2n=30.

15. *A. concinnatum* (A212) 2n=30.

16. *A. formosum* (A290) 2n=30.

17. *A. formosum* (A291) 2n=30.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Figures 18-22. Karyotypes of *Anthurium* species. (2200X)

Figure:

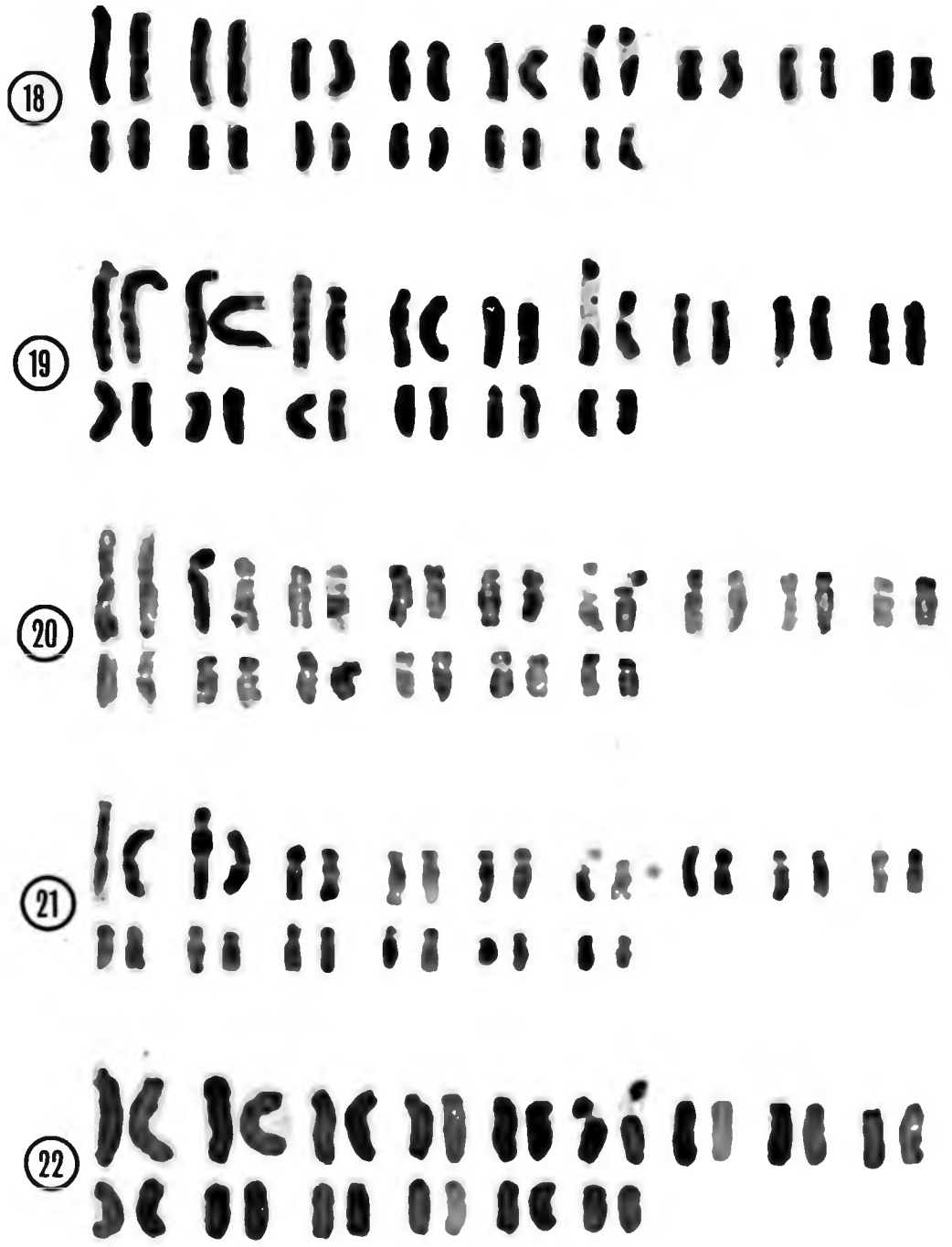
18. *A. kamemotoanum* (567-70) 2n=30.

19. *A. roraimense* (A189) 2n=30.

20. *A. nymphaeifolium* (A213-1) 2n=30.

21. *A. nymphaeifolium* (A213-2) 2n=30.

22. *A. nymphaeifolium* (A213-3) 2n=30.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Figures 23-27. Karyotypes of *Anthurium* species (2200X).

Figure:

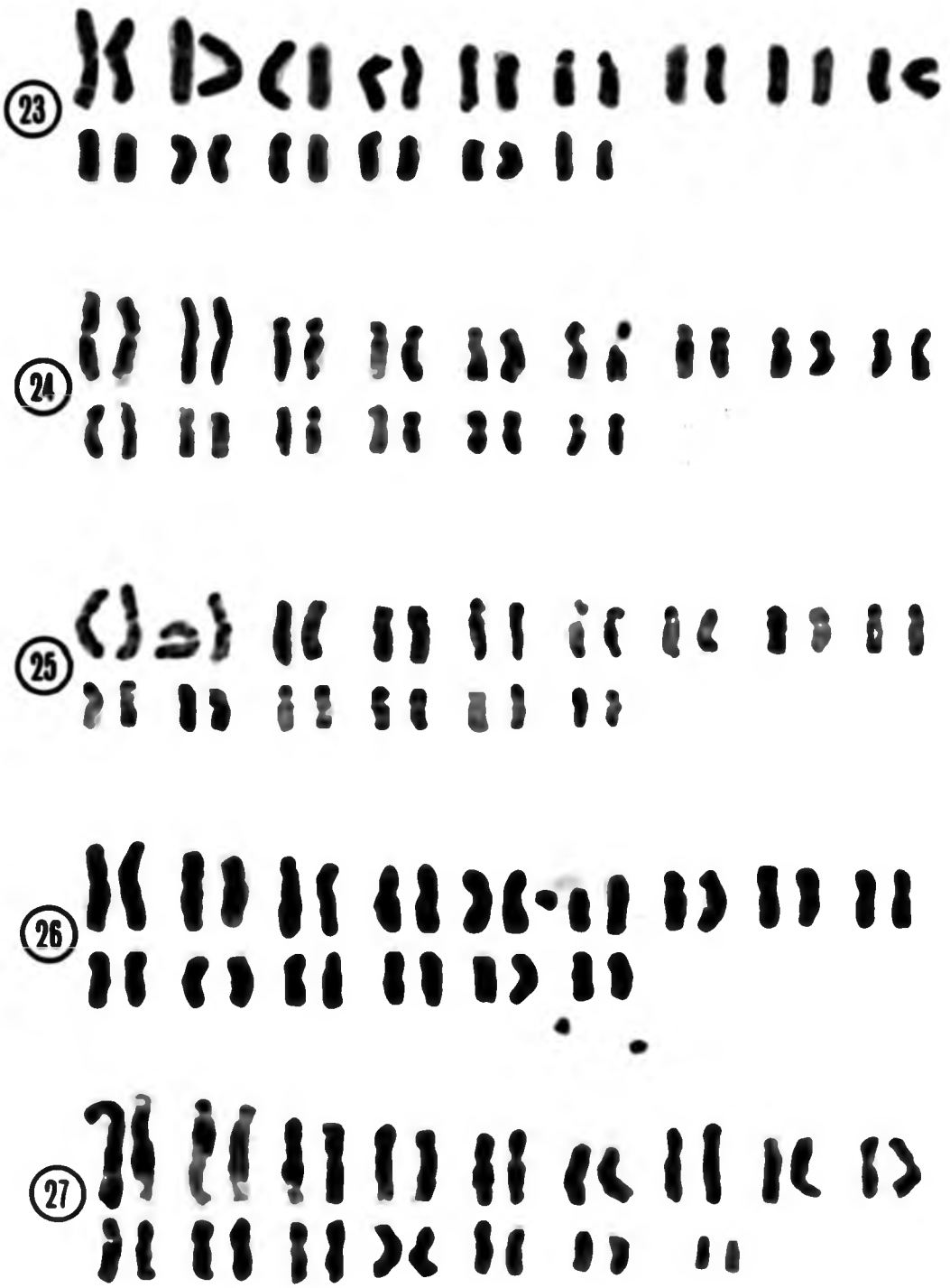
23. *A. roseospadix* (A289) 2n=30.

24. *A. roseospadix* (A292) 2n=30.

25. *A. roseospadix* (A293) 2n=30.

26. *A. subsignatum* (A183) 2n=30.

27. *A. wallisii* (A286) 2n=30+2B.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Figures 28-32. Karyotypes of *Anthurium* species and cultivars (2200X).

Figure:

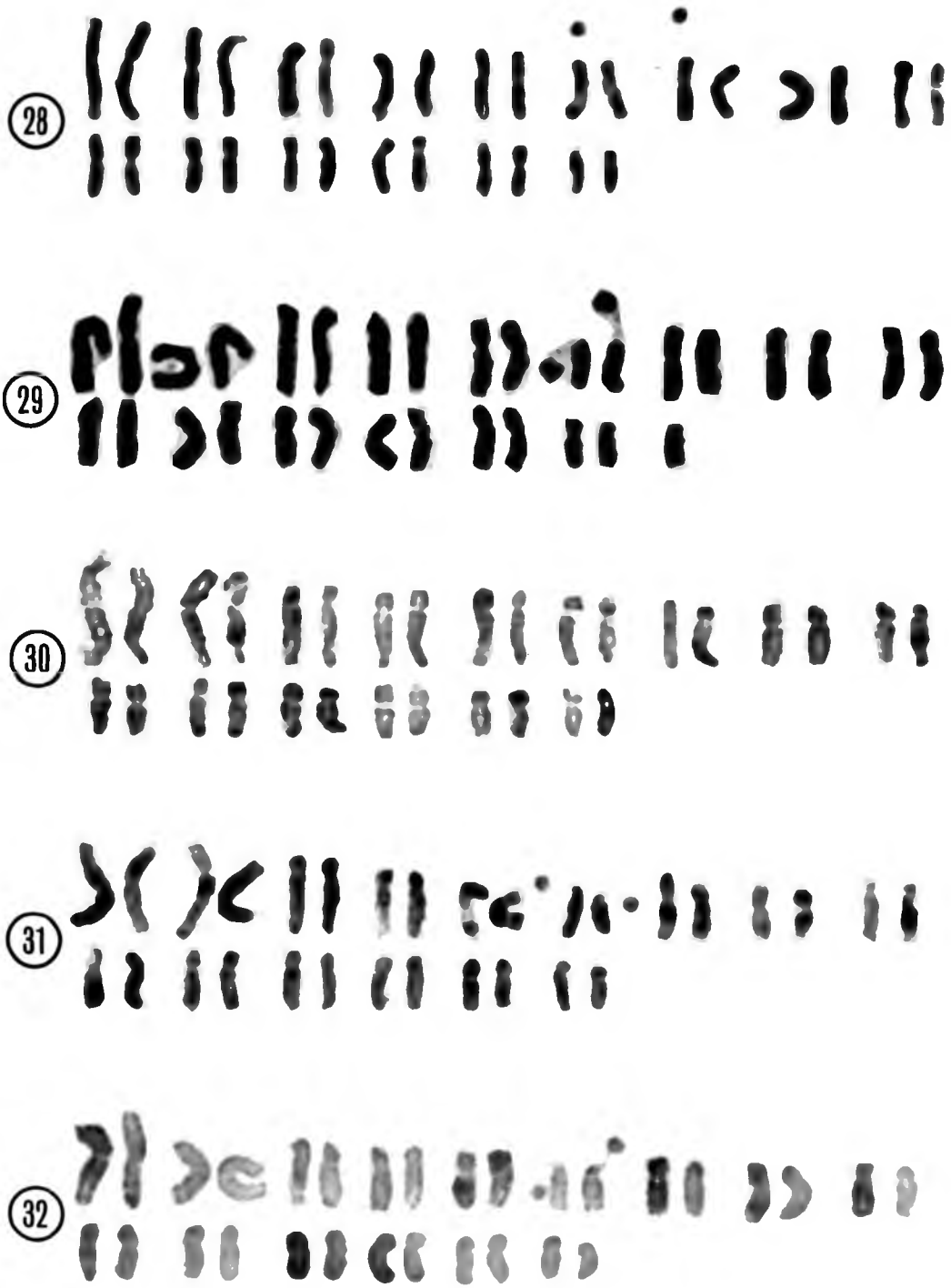
28. *A. garagaranum* (A262) 2n=30.

29. *A. garagaranum* (A263) 2n=30+1B.

30. *A. amnicola* (A417) 2n=30.

31. 'Calypso'

32. 'Trinidad'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from A263 was transmitted to the hybrid. Failure of trivalent formation strongly suggests that the extra chromosome is a B-chromosome.

The summary of karyotype analyses of Anthurium species and cultivars is shown in Table 6. Thirteen taxa commonly contain four large metacentric or sub-metacentric chromosomes, two fairly large acrocentric chromosomes, two satellite chromosomes and twenty two smaller chromosomes.

Two species, A. nymphaeifolium and A. wallisii display distinct differences in appearance of the karyotype. In A. nymphaeifolium two of the four large chromosomes are acrocentric rather than metacentric, and the fairly large acrocentric chromosomes present in other species are lacking. Anthurium wallisii contains one pair of the fairly large metacentric chromosomes instead of the fairly large acrocentric chromosomes.

The karyotype of the species in group V and group VI clearly shows the common presence of two pairs of large chromosomes. An earlier report on karyotypes of somatic chromosomes of two A. andraeanum (group VI) cultivars, 'Kaumana' and 'Uniwai', also showed 4 large chromosomes (Kaneko and Kamemoto, 1978). In addition, there were two species in group V which showed a similar karyotype; A. crystallinum (Ali, 1979) and A. warocqueanum (Kaneko and Kamemoto, 1979). Anthurium amnicola, 'Calypso', and

Table 6. Summary of karyotype analyses of Anthurium species and cultivars.

Species and cultivars	Number of chromosomes[z]						
	Total	Large M or SM	Large AC	Fairly large M or SM	Fairly large AC	Satellite	Medium and small
A. andraeanum	30	4	0	0	2	2	22
A. caperatum	30	4	0	0	2	2	22
A. concinnatum	30	4	0	0	2	2	22
A. formosum	30	4	0	0	2	2	22
A. kamemotoanum	30	4	0	0	2	2	22
A. lindenianum	30	4	0	0	2	2	22
A. nymphaeifolium	30	2	2	0	0	2	24
A. roraimense	30	4	0	0	2	2	22
A. roseospadix	30	4	0	0	2	2	22
A. subsignatum	30	4	0	0	2	2	22
A. garagaranum	30	4	0	0	2	2	22
A. wallisii	30	4	0	2	0	2	22
A. amnicola	30	4	0	0	2	2	22
'Calypso'	30	4	0	0	2	2	22
'Trinidad'	30	4	0	0	?	2	22

[z] M is metacentric; SM is sub-metacentric; AC is acrocentric.

'Trinidad' also display a similar chromosome complement as that of A. andraeanum.

The fact that A. nymphaeifolium and A. wallisii show slightly different chromosomal organization may suggest that these taxa were derived from some species with the typical karyotype found in taxa of group V and VI by chromosomal rearrangement.

4.1.2 Meiotic analysis and pollen stainability of Anthurium species and cultivars

A summary of meiotic analysis of PMCs and pollen stainability of Anthurium species and cultivars is presented in Table 7. Figures from 33 to 56 show chromosome configurations at prometaphase I or metaphase I of meiosis and some other meiotic stages in the pollen mother cells.

All species and cultivars showed 15 pairs of chromosomes at prometaphase I. A. wallisii showed 15 pairs plus two paired of univalent B-chromosomes.

Among the 15 pairs there were two distinct ring bivalents formed by the two sets of large chromosomes which were revealed in the karyotypes of the somatic chromosomes. The rest of the thirteen pairs appeared as ring or more frequently rod bivalents. It was noticed that at late metaphase I one or occasionally two chromosome pairs in some taxa had already started

Table 7. Mean chromosome configurations at prometaphase I of meiosis, frequency of tetrad formation at the end of meiosis and pollen stainability of *Anthurium* species and cultivars.

Species and cultivar	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Pollen Stainability(%)[z] (1000 pollen/spadix) Aceto-carmine	Nitro-BT
Group VI				
A. andraeanum				
'Kaumana'	15II	68.0	63.8(3)	56.3(3)
'Marian Seefurth'	15II	99.5	76.7(2)	58.4(2)
'Uniwai'	15II	99.0	male sterile	
'Manoa Mist'	15II	97.0	75.4(2)	52.6(2)
A. caperatum				
A227-1	15II	98.0	86.8(1)	61.7(1)
A227-2			64.4(2)	57.3(1)
A. concinnatum				
A212	15II	100.0	95.5(2)	90.4(2)
A. formosum				
A290	15II	93.0	70.6(1)	
A291			89.9(1)	

Table 7. (continued)

Species and cultivar	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Pollen Stainability(%) (1000 pollen/spadix) Aceto-carmine	Nitro-BT
A. kamemotoanum (A288 selfed)				
567-70		51.5		
567-50			62.7(1)	29.5(1)
567-33	15II	92.5	78.4(1)	
A. lindenianum				
A170	15II	96.5	50.7(2)	45.4(2)
A185	15II	90.0	82.8(2)	70.0(2)
A220-1	15II		male sterile?	
A220-2	15II	97.0	72.2(3)	62.5(2)
A. nymphaeifolium				
A213-1	15II	96.0	78.3(3)	48.2(2)
A213-2			31.1(3)	23.3(3)
A213-3	15II	97.0	63.4(2)	54.8(2)

[z]

Table 7. (continued)

Species and cultivar	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Pollen Stainability(%)[z] (1000 pollen/spadix) Aceto-carmine	Nitro-BT
A. roraimense				
A189	15II	100.0	53.0(1)	45.8(1)
A. roseospadix				
A289	15II	79.5	59.0(1)	44.9(1)
A292	15II	78.0	48.1(1)	46.3(1)
A293	15II	86.0	63.9(1)	32.7(1)
Group V				
A. subsignatum				
A183	15II	93.5	71.7(1)	54.4(1)
A. garagaranum				
A262	15II	100.0	37.9(1)	34.6(1)
A. wallisii				
A286	15II(+2B)	99.0		

Table 7. (continued)

Species and cultivar	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Pollen Stainability(%)[z] (1000 pollen/spadix) Aceto-carmine	Nitro-BT
Others:				
A. amnicola				
A417	15II	95.5	87.5(2)	61.4(1)
A430	15II	97.5	82.5(1)	63.8(1)
'Calypso'	15II	99.0	48.6(2)	30.6(2)
'Trinidad'	15II	99.0	46.5(2)	29.7(2)

[z] The first number indicates the percentage of pollen stained with 45% aceto-carmine and Nitro-Blue tetrazolium (Nitro-BT), and the number in parentheses indicates the number of spadices examined.

Figures 33-41. Meiotic configuration (PMC) of *Anthurium* species (1650X).

Figure:

33. *A. andraeanum* 'Manoa Mist' 2n=15II.

34. *A. andraeanum* 'Marian Seefurth' 2n=15II.

35. *A. andraeanum* 'Uniwai' 2n=15II.

36. *A. andraeanum* 'Kaumana' 2n=15II (with one loosely paired chromosomes indicated by an arrow).

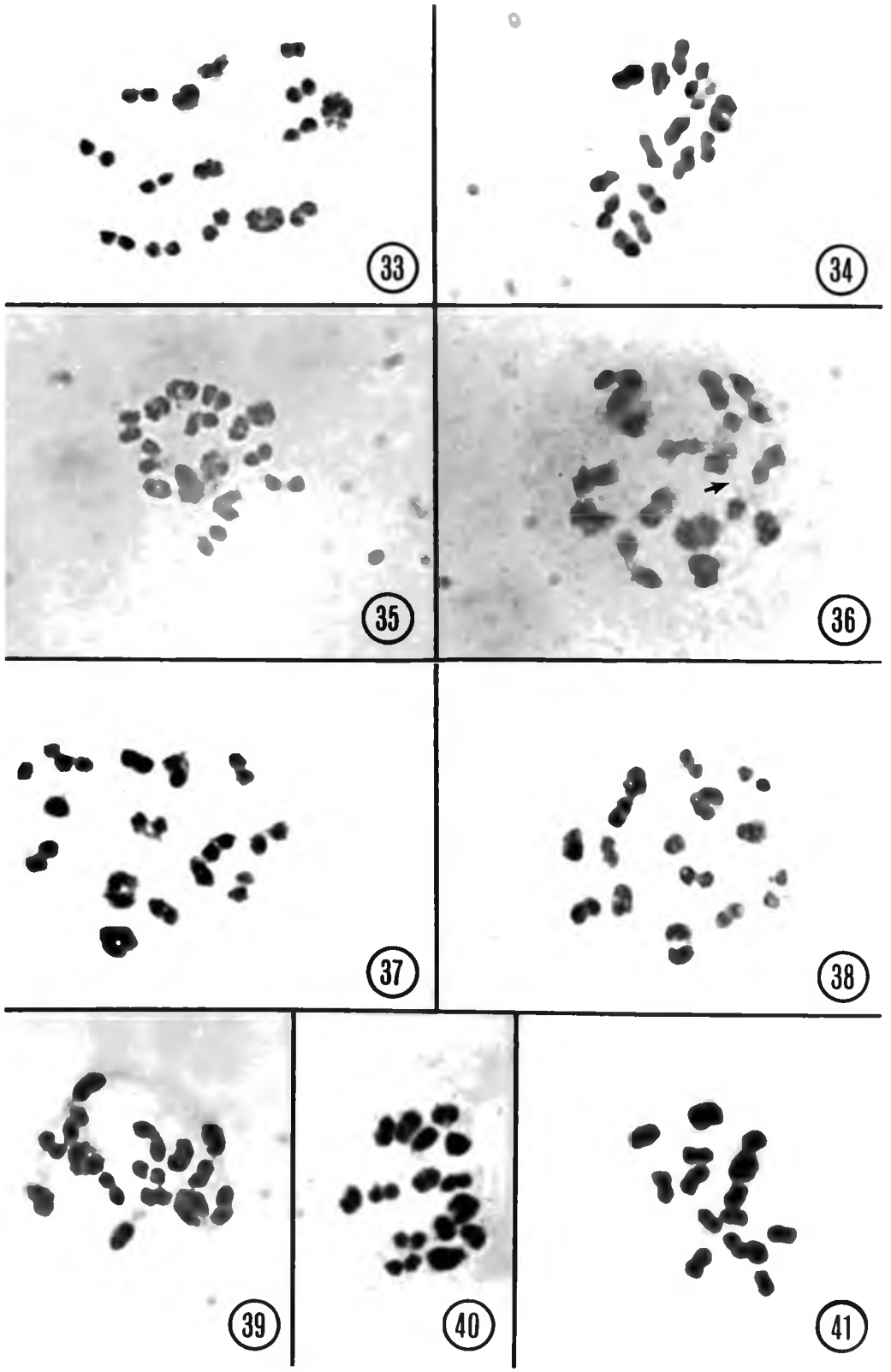
37. *A. concinnatum* (A212) 2n=15II.

38. *A. roraimense* (A189) 2n=15II.

39. *A. formosum* (A290) 2n=15II.

40. *A. caperatum* (A227-1) 2n=15II.

41. *A. kamemotoanum* (567-70) 2n=15II.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Figures 42-48. Meiotic configuration (PMC) of *Anthurium* species (1650X).

Figure:

42. *A. lindenianum* (A170) 2n=15II.

43. *A. lindenianum* (A220-1) 2n=15II.

44. *A. lindenianum* (A220-2) 2n=15II.

45. *A. roseospadix* (A289) 2n=15II.

46. *A. roseospadix* (A292) 2n=15II.

47. *A. roseospadix* (A293) 2n=15II; the arrows indicate loosely paired chromosomes.

48. *A. roseospadix* (A293) 2n=15II; the arrow indicates a pair with late terminalization of chiasmata.

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Figures 49-56. Meiotic configuration (PMC) of *Anthurium* species and cultivars (1650X).

Figure:

49. *A. nymphaeifolium* (A213-1) 2n=15II.

50. *A. nymphaeifolium* (A213-3) 2n=15II.

51. *A. wallisii* (A286) 2n=15II.

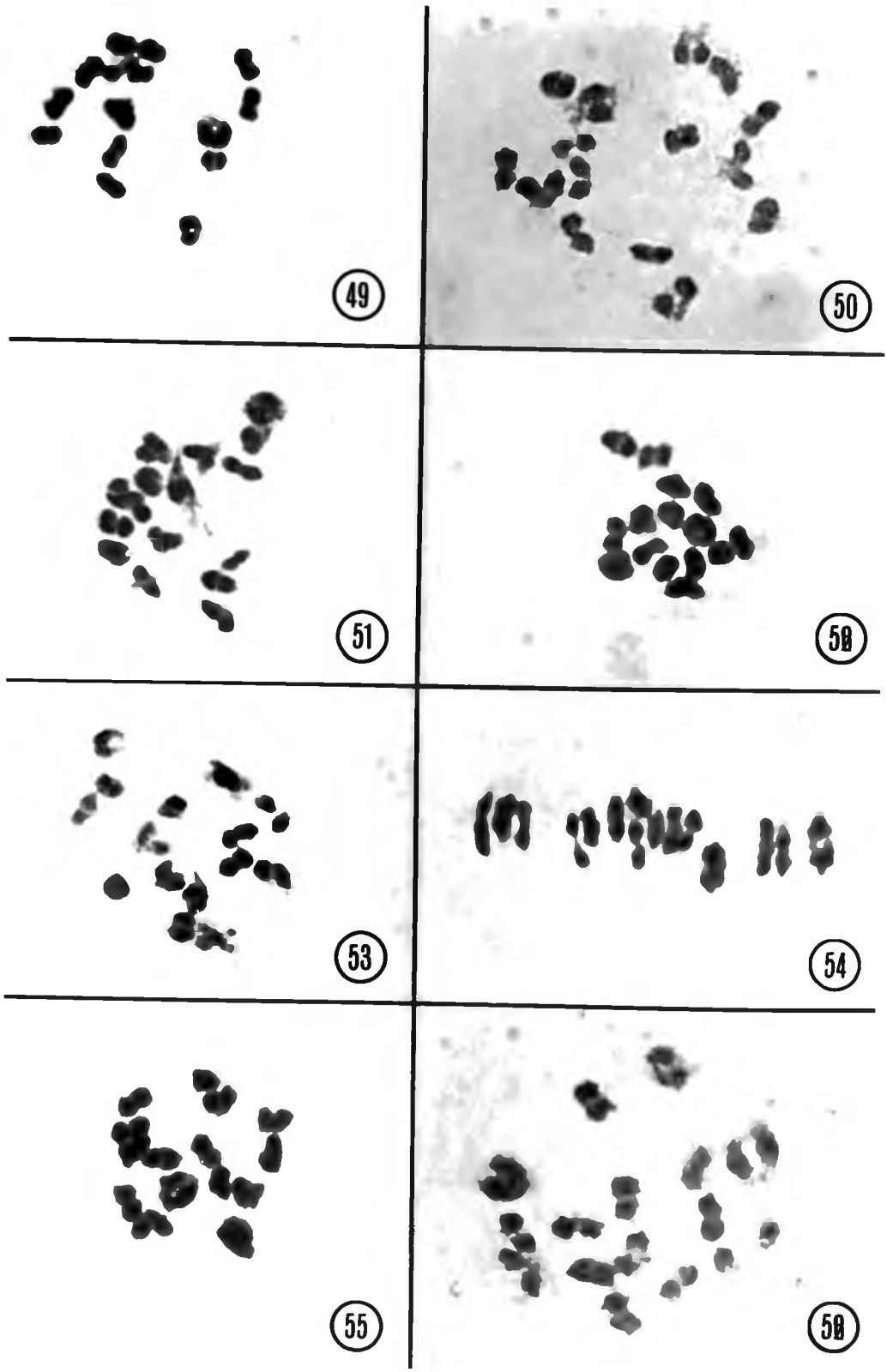
52. *A. subsignatum* (A183) 2n=15II.

53. *A. garagaranum* (A262) 2n=15II.

54. *A. amnicola* (A417) 2n=15II.

55. 'Calypso' 2n=15II.

56. 'Trinidad' 2n=15II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separating from each other. Sometimes they looked like two univalents in close proximity to one another. This phenomenon may lead to misinterpretation of meiotic configurations of pollen mother cells in Anthurium. In the present investigation, however, the mean configuration was determined by observing chromosomes at prometaphase I of meiosis.

Difference in meiotic analysis of A. andraeanum 'Kaumana' and 'Uniwai' between the previous study by Kaneko and Kamemoto (1979) and the present investigation may be due to: (1) the observation of chromosome pairing at slightly different stages of meiosis; (2) environmental conditions such as temperature or seasonal differences which might affect meiotic pairing; or (3) the loss of B-chromosomes in 'Uniwai' through clonal propagation.

'Kaumana' showed 15 pairs at prometaphase I. However, it was rather difficult to resolve meiotic configuration of this plant because chromosomes exhibited stickiness. At late metaphase I or early anaphase I some bivalents had separated much earlier than others. This irregular movement of chromosomes led to the formation of tetrads with micronuclei.

Table 7 shows the percentage of tetrad formation among 200 sporads for each plant. Most taxa exhibited very high percentages of regular tetrad formation.

However, A. andraeanum 'Kaumana' had 68% of its cells producing normal tetrads. Other individuals which showed a relatively low percentage of tetrad formation were A. kamemotoanum (567-70) and A. roseospadix (A289, A292 and A293).

Within A. andraeanum and A. kamemotoanum meiosis may vary among individuals as shown among cultivars of A. andraeanum or seedlings in A. kamemotoanum. It can be concluded that although some particular individuals have irregular meiotic activity, meiosis of the two species, in general, is normal. It is considered that such irregularities are due to genetic or environmental factors and can be a characteristic of each individual.

Anthurium roseospadix produced about 80% tetrads. Observation of meiosis revealed the formation of micronuclei due to lagging of chromosomes at anaphase I or anaphase II. At metaphase I there were a couple of pairs with late chiasma terminalization as well as early separation of homologous pairs.

Pollen stainability was used to estimate the fertility of each plant. With aceto-carmine, pollen stainability ranged from 31.1% to 95.5%. Most plants produced more than 50% stainable pollen grains but a few showed fairly low pollen stainability. A. nymphaeifolium (A213-2) and A. garagaranum (A262) exhibited 31.1% and 37.9% pollen stainability, respectively. It was not

possible to obtain pollen grains from A. lindenianum (A220-1) and A. andraeanum 'Uniwai'.

Another pollen stainability test was done by examining the activity of an enzyme, succinate dehydrogenase, using Nitro-Blue tetrazolium (Nitro-BT). There was a slightly lower percentage of stained pollen with this staining method than with aceto-carmine because Nitro-Blue tetrazolium could discriminate between pollen grains which had specific oxidative metabolism whereas aceto-carmine simply stained nuclear and some cytoplasmic materials in pollen grains.

4.1.3 Meiotic analysis and pollen stainability of inter- and intragroup interspecific hybrids

Results of the meiotic analysis of hybrids are given in Table 8 and Figures 57 to 95. Meiosis in all interspecific hybrids was characterized by regular bivalent formation, indicating genomic similarity of those Anthurium taxa involved in hybridization. The degree of normal tetrad formation was in most cases as high as those of the parental species (Table 7).

A lower percentage of normal tetrad production was found in two intragroup interspecific hybrids. Two plants of A. formosum (A291) x A. roseospadix (A292) produced 78.0 (RS1224-2) and 70.0% (RS1224-5) normal tetrads and two hybrid progenies between A. kamemotoanum

Table 8. Mean chromosome configurations at prometaphase I of meiosis, frequency of tetrad formation at the end of meiosis, number of spadices producing pollen per plant and pollen stainability of *Anthurium* hybrids.

Cross	Cross and plant no.	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Number of spadices producing pollenz	Pollen stainability (1000 pollen grains/spadix)	
					Aceto-carmine	Nitro-BT
Intragroup crosses:						
VI x VI						
A. andraeanum 'Unival'						
x *concinnatum* (A212)	RS987-2	15II	99.5	6(6)	33.7(2)	26.0(2)
	RS987-6	15II	99.5	6(6)	31.7(5)	27.2(2)
x *formosum* (A287)	RS1183-5	15II	99.5	0(5)		
(A290)	RS1205-7	15II	96.0	3(3)	1.9(2)	0.6(2)
x *kamemotoanum* (A288)	RS532-5		97.5	2(3)	35.3(2)	23.0(2)
	RS1249-1	15II	97.5	0(6)		
	RS1249-9	15II	100.0	2(2)	39.5(1)	23.1(1)
	RS1249-10	15II	96.0	2(7)	25.6(2)	17.7(1)
x *lindenianum* (A220-2)	RS1360-1	15II	98.5	0(4)		
	RS1360-4	15II	98.5	0(7)		
x *roseospadix* (A289)	RS691-8	15II	86.5	2(4)	63.2(2)	47.3(2)
(A292)	RS985-4	15II	95.0	0(4)		
(A293)	RS675-2	15II	90.0	0(3)		
A. concinnatum (A212)						
x *roseospadix* (A292)	RS1227-1	15II	100.0	9(9)	28.8(5)	17.0(2)
	RS1227-3	15II	97.5	7(7)	15.9(2)	7.1(2)
A. formosum (A287)						
x *concinnatum* (A212)	RS174-6	15II	98.0	4(4)	24.0(2)	18.4(2)
	RS174-7	15II	99.5	5(5)	11.8(2)	9.3(2)
A. formosum (A291)						
x *roseospadix* (A292)	RS1224-2	15II	78.0	5(5)	47.9(1)	48.1(1)
	RS1224-5	15II	70.0	3(3)	26.5(2)	5.5(2)

z The first number indicates the number of spadices which produced pollen grains, and the number in parenthesis indicates the number of spadices observed.

y The first number indicates the percentage of pollen stained with 45% aceto-carmine and tetrazolium Nitro-Blue(Nitro-BT), and the number in parenthesis indicates the number of spadices examined.

Table 8. (continued)

Cross	Cross and plant no.	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Number of spadices producing pollen[z]	Pollen stainability (1000 pollen grains/spadix)	
					Aceto-carmine	Nitro-BT
A. kamomoanum (A288)						
x formosum (A291)	RS1228-3		99.0	1(1)		
	RS1228-8	15II	97.5	1(1)	16.7(1)	14.9(1)
	RS1228-10	15II	98.0	2(2)	21.3(2)	11.8(1)
	RS1228-13	15II	99.0	1(1)	32.2(3)	24.3(2)
x lindenianum (A220-2)	RS1293-5	15II	78.0	6(6)	32.2(3)	24.3(2)
	RS1293-9	15II	73.0	5(5)	31.2(4)	27.0(2)
A. lindenianum (A170)						
x formosum (A290)	RS1212-3	15II	99.8	4(4)	35.3(2)	19.9(2)
	RS1212-6	15II	99.0	5(5)	21.4(2)	16.0(2)
x roseospadix (A292)	RS986-7	15II	88.0	7(7)	48.9(2)	27.3(2)
x lindenianum (A220-2)	RS1361-1	15II	100.0	10(10)	22.1(3)	17.9(2)
	RS1361-5	15II	99.0	6(6)	22.1(3)	17.8(2)
A. lindenianum (A220-2)						
x kamomoanum (A288)	RS1327-1	15II	99.5	6(6)	33.4(3)	28.7(2)
	RS1327-2	15II	99.0	4(4)	30.4(1)	24.4(1)
A. lindenianum (A220-1)						
x roseospadix (A292)	RS972-3	15II		0(1)		
A. nymphaefolium (A213-3)						
x formosum (A287)	RS251-1	15II	96.0	7(7)	30.3(3)	29.2(2)
	RS251-5	15II	98.5	2(2)	15.4(2)	13.1(2)
A. roralmense (A189)						
x andraanum 'Manoa Mist'	426-73	15II	100.0	6(7)	38.5(2)	27.6(2)
A. roseospadix (A289)						
x kamomoanum (A288)	RS1325-2		98.0	2(2)	35.0(1)	42.3(1)
	RS1325-14	15II	99.0	1(1)	55.5(1)	

z The first number indicates the number of spadices which produced pollen grains and the number in parentheses indicates the number of spadices observed.

y The first number indicates the percentage of pollen stained with 45% aceto-carmine and tetrazolium Nitro-Blue(Nitro-BT), and the number in parentheses indicates the number of spadices examined.

Table 8. (continued)

Cross	Cross and plant no.	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Number of spadices producing pollen[z]	Pollen stainability (1000 pollen grains /spadix)	
					Aceto-carmine	Nitro-BT
(VI x VI) x VI						
(andreanum x kamemotoanum) x formosum						
('Umiwai' x A288) x A230	392-1	15II	99.5	5(5)	43.9(3)	33.9(2)
	392-9	15II	98.5	1(4)	5.2(1)	2.7(1)
	392-25	15II	98.5	5(5)	20.0(1)	14.7(1)
	392-42	15II	99.0	3(3)		
	392-56	15II	99.5	0(4)		
	392-66	15II	92.0	5(5)	19.1(2)	11.7(2)
	392-75	15II	99.0	4(4)	34.3(1)	22.4(1)
	392-99	15II	90.5	4(6)	14.6(1)	8.9(1)
V x V						
A. carrocampanense (A225-1)						
x garagaranum (A263)	RS511-4	15II+1B	97.5	1(1)	49.3(1)	8.6(1)
	RS511-5	15II+1B	96.0	1(1)	24.5(1)	1.0(1)
x garagaranum (A262)	RS1130-6	15II+1B	84.5	2(2)	84.8(1)	78.7(1)
	RS1130-8	15II+1B	92.5	1(1)	70.5(1)	62.5(1)
	RS1130-5			1(1)	60.4(1)	53.6(1)
A. subsignatum (A183)						
x garagaranum (A263)	RS1354-4			1(1)	52.5(1)	34.3(1)
	RS1354-5	15II+1B	95.5	4(4)	41.8(1)	25.6(1)
Intergroup crosses:						
VI x V						
A. lindenianum (A220-2)						
x carrocampanense (A225-1)	RS970-4	15II+1B?	35.0	0(4)		
A. kamemotoanum (A288)						
x wallisii (A286)	RS1253-4		61.5			
	RS1253-2	15II+1B	91.0			
	RS1253-1	15II+1B	96.5	1(1)	3.2(1)	0.0(1)

z The first number indicates the number of spadices which produced pollen grains and the number in parentheses indicates the number of spadices observed.

y The first number indicates the percentage of pollen stained with 45% aceto-carmine and tetrazolium Nitro-Blue(Nitro-BT), and the number in parentheses indicates the number of spadies examined.

Table 8. (continued)

Cross	Cross and plant no.	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Number of spadices producing pollenz	Pollen stainability (1000 pollen grains/spadix)	
					Aceto-carmine	Nitro-BT
A. formosum (A287)						
x cerrocampanense (A225-2)	RS1564-1	15II+1B	94.0	0(1)		
	RS1564-2	15II+2B	90.5	3(5)	0.0(1)	0.0(1)
	RS1564-4	15II+1B	91.0	0(1)		
V x VI						
A. cerrocampanense (A225-1)						
x concinnatum (A212)	RS1235-3	15II	91.0	1(3)	6.1(1)	3.7(1)
	RS1235-1	15II+1B	96.5	0(2)		
A. subsignatum (A183)						
x nymphaeiphotium (213-1)	RS1306-1	15II	75.0	2(6)	0.8(1)	0.1(1)
	RS1306-2	15II	77.5	0(2)		
	RS1306-4			0(3)		
	RS1306-5	15II	99.0	0(2)		
	RS1306-8			2(2)	1.5(2)	0.1(2)
x caperatum (A227)	RS86-2			0(2)		
A. garagaranum (A263)						
x lindenianum (A185)	RS427-3	15II	97.0	2(2)	1.2(2)	1.2(1)
	RS427-4		75.0	2(2)	1.0(1)	0.1(1)
	RS427-8	15II	97.0	1(1)	10.3(1)	6.3(1)

z The first number indicates the number of spadices which produced pollen grains and the number in the parenthese indicates the number of spadices observed.

y The first number indicates the percentage of pollen stained with 45% aceto-carmine and tetrazolium Nitro-Blue(Nitro-BT), and the number in parentheses indicates the number of spadices examined.

Figures 57-65. Meiotic behavior (PMC) of interspecific hybrids of Anthurium (1650X).

Figure:

57. Meiotic configuration of RS987-6 (A. andraeanum 'Uniwai' X A. concinnatum (A212)) 2n=15II.

58. Meiotic configuration of RS1249-1 (A. andraeanum 'Uniwai' X A. kamemotoanum (A288)) 2n=15II.

59. Meiotic configuration of RS1205-7 (A. andraeanum 'Uniwai' X A. formosum (A290) 2n=15II.

60. Meiotic configuration of RS1183-5 (A. andraeanum 'Uniwai' X A. formosum (A287) 2n=15II.

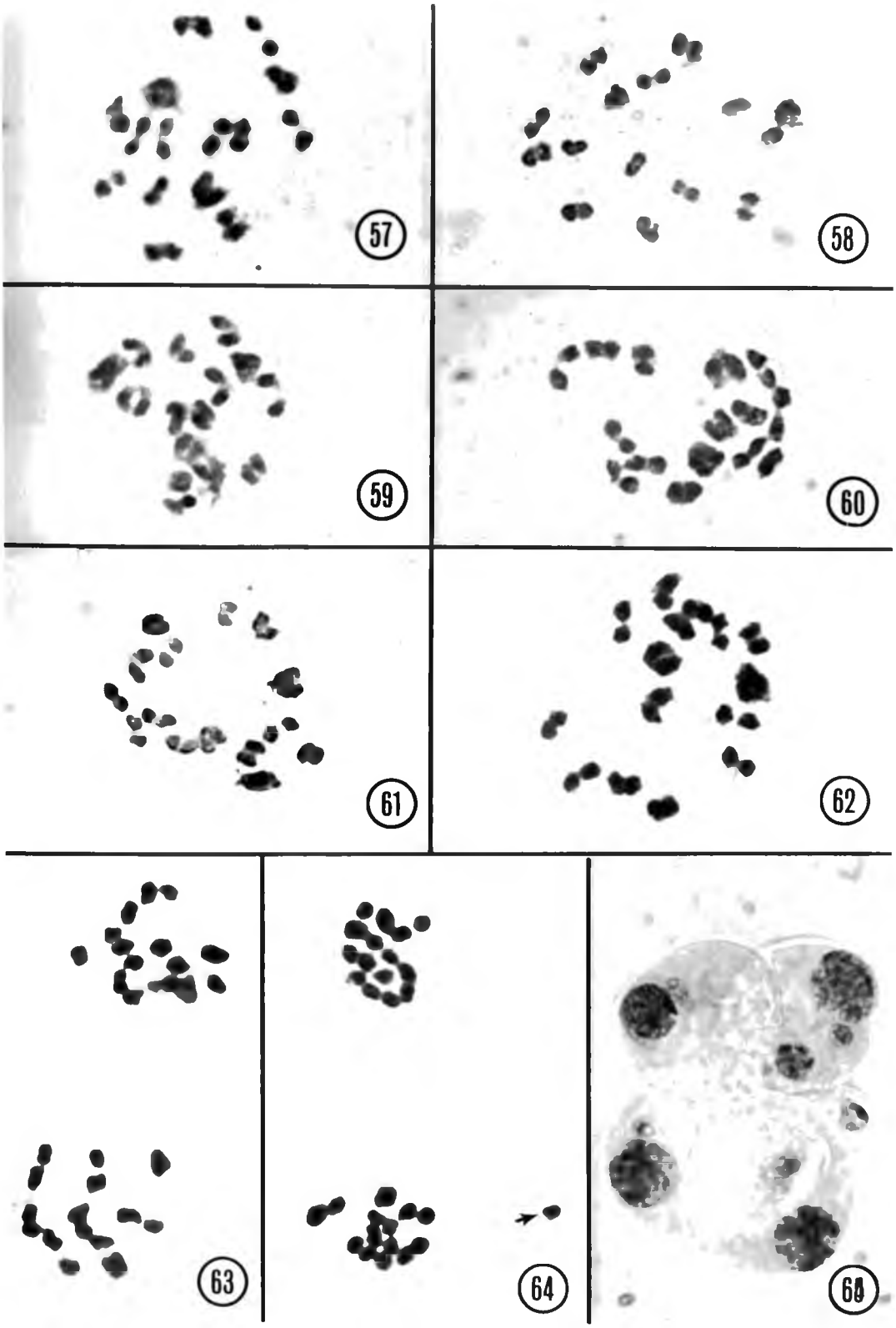
61. Meiotic configuration of RS1360-1 (A. andraeanum 'Uniwai' X A. lindenianum (A220-2) 2n=15II.

62. Meiotic configuration of RS675-2 (A. andraeanum 'Uniwai' X A. roseospadix (A293) 2n=15II.

63. Anaphase I of RS675-2, showing regular movement of chromosomes.

64. Anaphase I of RS675-2, showing one chromosome lagging indicated by an arrow.

65. Tetrad formation with micronuclei at the end of meiosis of RS675-2.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Figures 66-73. Meiotic behavior (PMC) of interspecific hybrids of Anthurium (1650X).

Figure:

66. Meiotic configuration of RS1227-3 (A. concinnatum (A212) X A. roseospadix (A292)) 2n=15II.

67. Meiotic configuration of RS174-6 (A. formosum (A287) X A. concinnatum (A212)) 2n=15II.

68. Meiotic configuration of RS174-7 (A. formosum (A287) X A. concinnatum (A212)) 2n=15II.

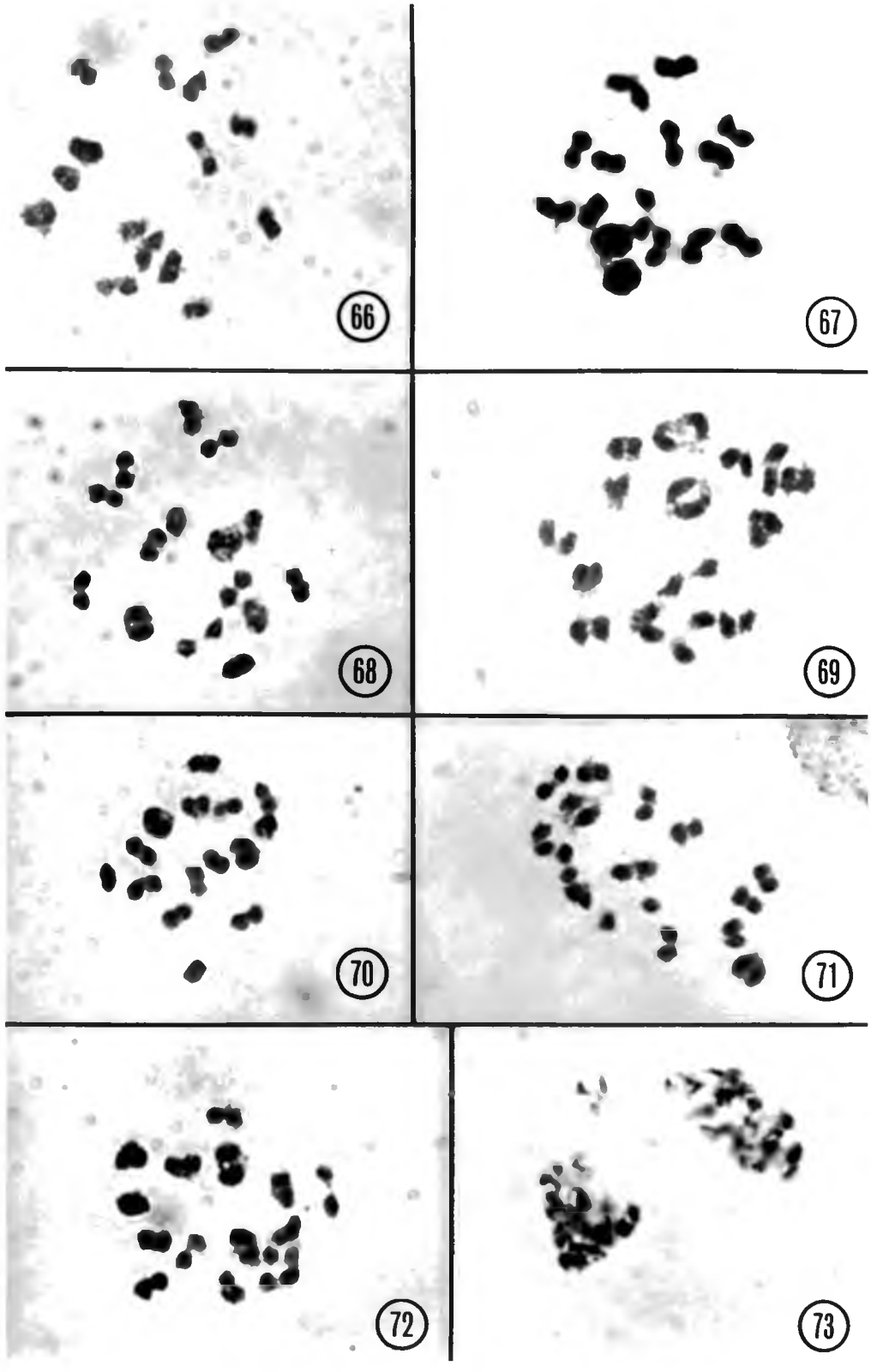
69. Meiotic configuration of RS1224-2 (A. formosum (A291) X A. roseospadix (A292)) 2n=15II.

70. Meiotic configuration of RS1228-13 (A. kamemotoanum (A288) X A. formosum (A291)) 2n=15II.

71. Meiotic configuration of RS1293-5 (A. kamemotoanum (A288) X A. lindenianum (A220-2)) 2n=15II.

72. Meiotic configuration of RS1293-9 (A. kamemotoanum (A288) X A. lindenianum (A220-2)) 2n=15II.

73. Late anaphase I of RS1293-5 (A. kamemotoanum (A288) X A. lindenianum (A220-2)), showing lagging chromosomes.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Figures 74-79. Meiotic configuration (PMC) of inter- and intraspecific hybrids of Anthurium (1650X).

Figure:

74. RS1212-3 (A. lindenianum (A170) X A. formosum (A290)) 2n=15II.

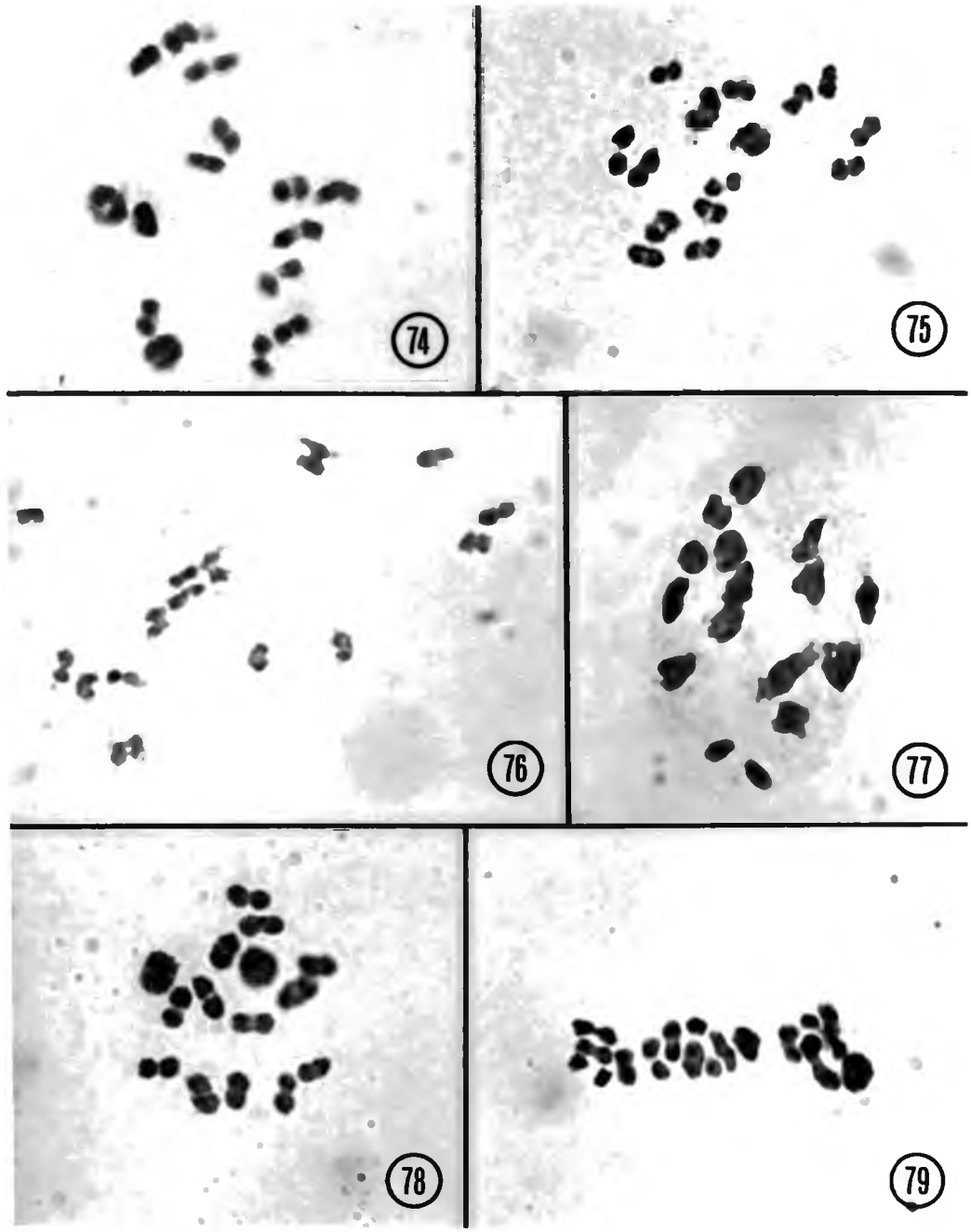
75. RS1212-6 (A. lindenianum (A170) X A. formosum (A290)) 2n=15II.

76. RS986-7 (A. lindenianum (A170) X A. roseospadix (A292)) 2n=15II.

77. RS1361-1 (A. lindenianum (A170) X A. lindenianum (A220-2)) 2n=15II.

78. RS1327-1 (A. lindenianum (A220-2) X A. kamemotoanum (A288)) 2n=15II.

79. RS972-3 (A. lindenianum (A220-1) X A. roseospadix (A292)) 2n=15II.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Figures 80-87. Meiotic configuration (PMC) of interspecific hybrids of Anthurium (1650X).

Figure:

80. RS251-5 (A. nymphaeifolium (A213-3) X A. formosum (A287)) 2n=15II.

81. 426-73 (A. roraimense (A189) X A. andraeanum 'Manoa Mist') 2n=15II.

82. RS1325-14 (A. roseospadix (A289) X A. kamemotoanum (A288)) 2n=15II.

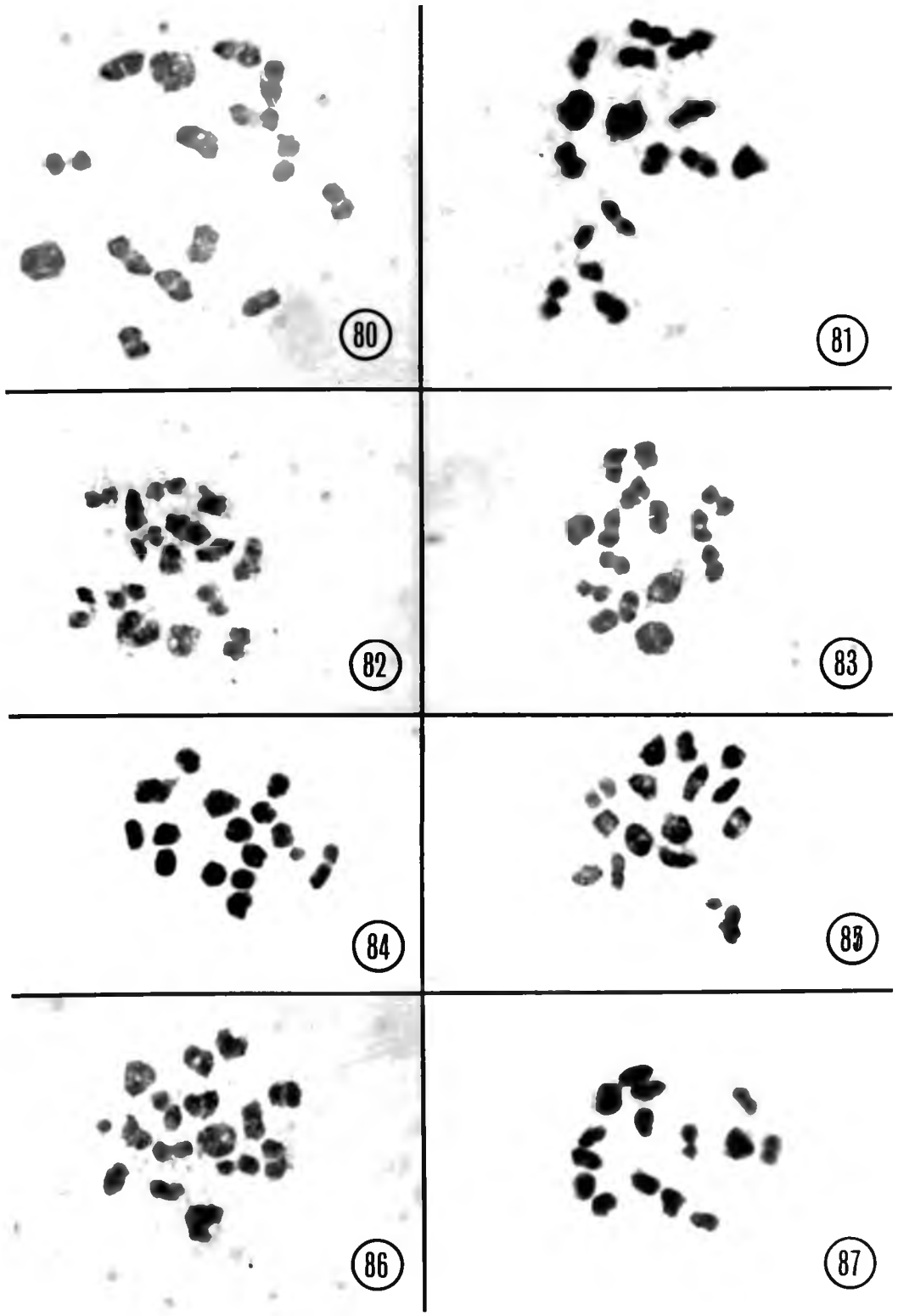
83. 392-9 ([A. andraeanum 'Uniwai' X A. kamemotoanum (A288)] X A. formosum (A290)) 2n=15II.

84. RS511-4 (A. cerrocampanense (A225-1) X A. garagaranum (A263)) 2n=15II+1B.

85. RS1130-8 (A. cerrocampanense (A225-1) X A. garagaranum (A262)) 2n=15II+1B.

86. RS1253-1 (A. kamemotoanum (A288) X A. wallisii (A286)) 2n=15II+1B.

87. RS1253-2 (A. kamemotoanum (A288) X A. wallisii (A286)) 2n=15II.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Figures 88-95. Meiotic configuration (PMC) of interspecific hybrids of *Anthurium* (1650X).

Figure:

88. RS1354-5 (*A. subsignatum* (A183) X *A. garagaranum* (A263)) 2n=15II+1B.

89. RS1564-1 (*A. formosum* (A287) X *A. cerrocampanense* (A225-2)) 2n=15II+1B.

90. RS1564-2 (*A. formosum* (A287) X *A. cerrocampanense* (A225-2)) 2n=15II+2B.

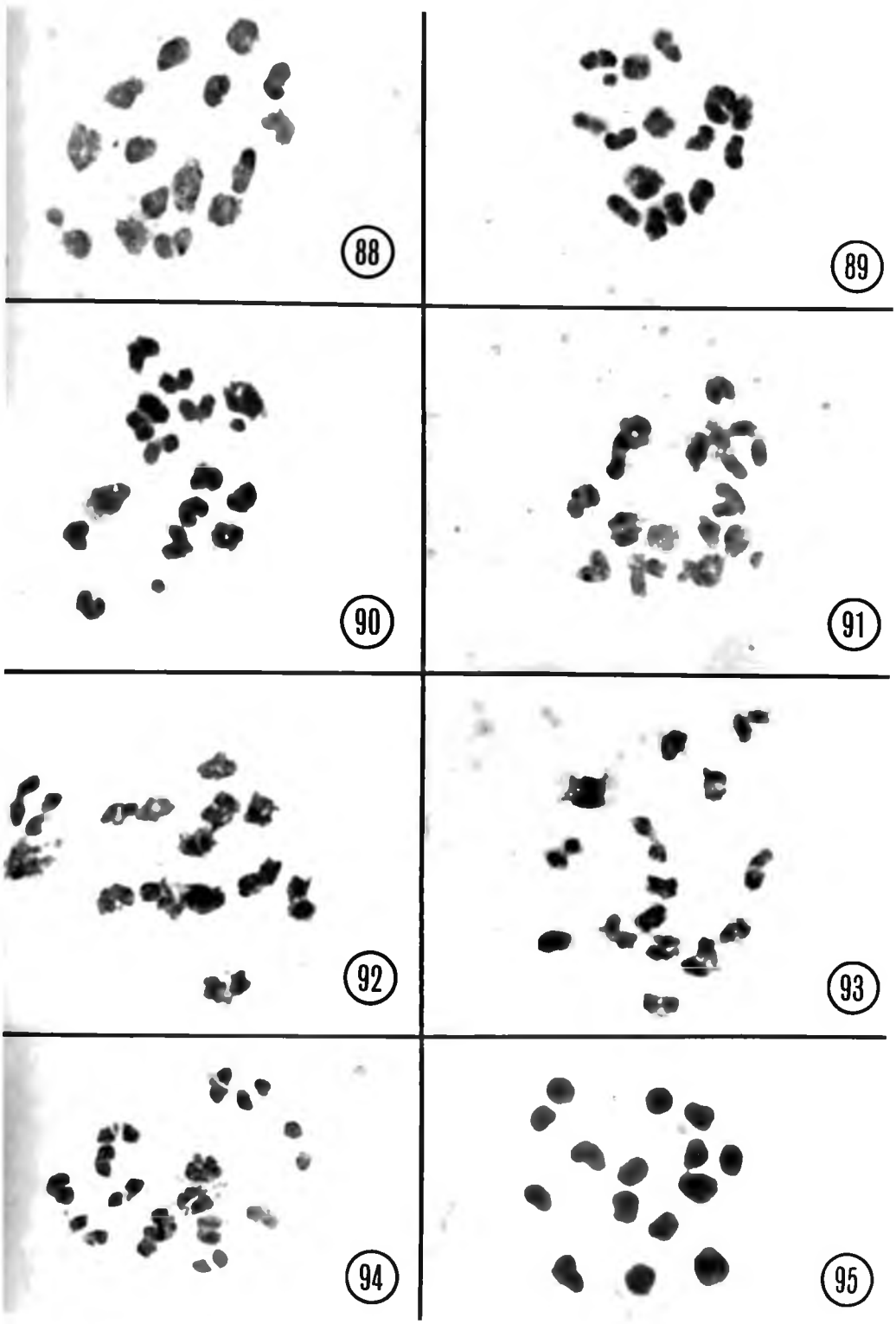
91. RS1235-1 (*A. cerrocampanense* (A225-1) X *A. concinnatum* (A212)) 2n=15II+1B.

92. RS1235-3 (*A. cerrocampanense* (A225-1) X *A. concinnatum* (A212)) 2n=15II.

93. RS1306-2 (*A. subsignatum* (A183) X *A. nymphaeifolium* (A213-1)) 2n=15II.

94. RS1306-5 (*A. subsignatum* (A183) X *A. nymphaeifolium* (A213-1)) 2n=15II.

95. RS427-3 (*A. garagaranum* (A263) X *A. lindenianum* (A185)) 2n=15II.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(A288) and A. lindenianum (A220-2), RS1293-5 and -9, displayed 78.0 and 73.0% normal tetrad production, respectively. Among intergroup interspecific hybrids, some individuals of A. subsignatum (A183) x A. nymphaeifolium (A213-1), A. garagaranum (A263) x A. lindenianum (A185), A. kamemotoanum (A288) x A. wallisii (A286), and A. lindenianum (A220-2) x A. cerrocampanense (A225-1) showed a reduction of regular tetrad formation. Anthurium lindenianum x A. cerrocampanense (RS970-4) produced the lowest percentage of normal tetrads (35.0%).

Various factors may be responsible for this phenomenon. Abnormal meiotic behavior independent of chromosomal homology may be due to unfavorable interaction of parental genes (Stebbins, 1971; Grant, 1971). Meiotic irregularity could be controlled by a combination of nuclear genes and cytoplasmic factors. In addition meiotic activities may be affected by environmental factors such as temperature.

When a parental plant contained B-chromosomes, its hybrids often included B-chromosomes in meiosis. However, since those Bs appeared in low number, they did not seem to have any great effects on meiotic activities.

A drastic difference between intergroup interspecific hybrids and intragroup interspecific hybrids became apparent when the degree of pollen grain production and pollen stainability were investigated. In crosses among

species in group VI, almost all hybrids showed the presence of pollen grains in each spadix except crosses involving A. andraeanum 'Uniwai' and A. lindenianum (A220-1) crosses. Male sterility in various hybrids of 'Uniwai' and A220-1 as a parent was probably inherited from those parental plants.

Excluding 'Uniwai', A220-1 and their various hybrids, a summary of pollen stainability is tabulated to clarify differences between parental taxa and interspecific hybrids(Table 9). Parental species are divided according to Sheffer and Kamemoto's system (group VI and V) and interspecific hybrids are grouped into two categories; intragroup crosses (VI x VI and V x V) and intergroup crosses (VI x V and V x VI). The table clearly shows a reduction of pollen stainability in interspecific hybrids. Comparison of pollen stainability of parental taxa in group VI with crosses of VI x VI demonstrates a 50% reduction of stained pollen grains in hybrids. These results indicate that there is some degree of genetic differences among species in group VI.

Pollen stainability of intergroup interspecific hybrids was much less than that of intragroup interspecific hybrids. Almost 100% of the spadices observed in this study successfully produced pollen grains in intragroup interspecific hybrids, whereas intergroup interspecific hybrids showed about 35% of

Table 9. Summary of pollen stainability of Anthurium species in group VI and V, and intra- and intergroup crosses.[z]

Group or Groups crossed	Frequency of spadices produced pollen (%)	Pollen stainability (%)			
		Aceto-carmine		Nitro-BT	
		Mean	Range	Mean	Range
Group VI		67,6(35)[y]	31.1 -95.5	52.5(29)	23.3 - 90.4
Group V		54.8(2)	37.9 - 71.7	44.5(2)	34.6 - 54.4
Intragroup crosses:					
VI x VI	99.1(110)[y]	28.4(50)	11.8 - 63.2	20.5(39)	5.5 - 48.1
V x V	100.0(11)	54.7(7)	24.5 - 84.8	37.8(7)	1.0 - 78.7
Intergroup crosses:					
VI x V	33.3(12)	1.6(2)	0.0 - 3.2	0.0(2)	-
V x VI	35.7(28)	3.2(8)	0.8 - 10.3	1.7(7)	0.1 - 6.3

z Table does not include A. andraeanum 'Uniwai', A. lindenianum A220-1, and their hybrids with either plant as a parent.

y The numbers in parenthesis indicate the number of spadices examined.

spadices with pollen. Pollen stainability tests indicated that those intergroup interspecific hybrids were virtually male sterile. The data suggest genetic divergence between group VI and Group V.

4.1.4 Meiotic analysis and pollen stainability of Anthurium amnicola hybrids.

Meiotic behavior in various interspecific hybrids was found to be regular (Table 10). Mean configurations at prometaphase I showed 15 bivalents, suggesting that A. amnicola was very closely related to A. andraeanum, A. formosum, A. lindenianum, A. kamemotoanum and 'Calypso'.

The percentage of normal tetrad production in hybrids was very high except in the hybrids between A. amnicola (A417) and A. lindenianum (A220-2). In these hybrids one or two pairs of chromosomes terminalized much faster than others at an early stage of meiosis and in some PMCs laggards were seen at anaphase I. Meiotic behavior in A. amnicola hybrids are illustrated in Figures 96 - 101.

Pollen stainability ranged from 7.4% to 65.7% for aceto-carmine and 0% to 60% for Nitro-Blue tetrazolium test. Reduction of pollen fertility was apparent. However, a hybrid plant of A. andraeanum x A. amnicola (A494) showed a high pollen stainability (65.7% for aceto-carmine and 60.0% for Nitro-BT). The overall pollen fertility in various A. amnicola hybrids was

Table 10. Mean chromosome configurations at prometaphase I of meiosis, frequency of tetrad formation at the end of meiosis, and pollen stainability of *Anthurium amnicola* hybrids.

Cross	Cross and plant no. or acc. no.	Mean configuration per PMC (25 cells/plant)	Tetrad formation(%) (200 cells/plant)	Pollen stainability(%)[z] (1000 pollen grains/spadix)	
				Aceto-carmine	Nitro-BT
A. amnicola (A417)					
× *formosum* (A287)	568-24	15II	97.0		
	568-25	15II	99.0		
× *formosum* (A291)	572-8	15II	95.5	7.6(1)	0.0(1)
	572-9	15II	93.0		
	572-18			11.7(1)	1.7(1)
× *lindenianum* (A220-2)	569-1	15II	82.0	27.0(1)	21.4(1)
	569-4			21.4(1)	23.8(1)
	575-18			27.8(1)	30.8(1)
	575-27		88.0		
	575-31			21.4(1)	20.7(1)
× RS 1228-8 (*kamemotoanum* × *formosum*)					
	573-2			13.1(1)	6.1(1)
	573-6			12.2(1)	9.0(1)
A. andraeanum					
× *amnicola*	A494	15II	97.5	65.7(1)	60.0(1)
A. lindenianum (A170)					
× *amnicola* (A417)	552-38			23.4(1)	
	552-72	15II		16.2(1)	16.7(1)
	552-73	15II	97.0		
	552-94			7.4(1)	
	552-104			14.0(1)	11.2(1)
'Calypso'					
× *amnicola* (A417)	531-14	15II	94.0	3.3(1)	

[z]The first number indicates the percentage of pollen stained with 45% aceto-carmine and tetrazolium Nitro-Blue(Nitro-BT), and the number in parentheses indicates the number of spadices examined.

Figures 96-101. Meiotic bahavior (PMC) of *Anthurium amnicola* hybrids (1650X).

Figure:

96. Meiotic configuration of 568-24 (*A. amnicola* (A417) X *A. formosum* (A291)) 2n=15II.

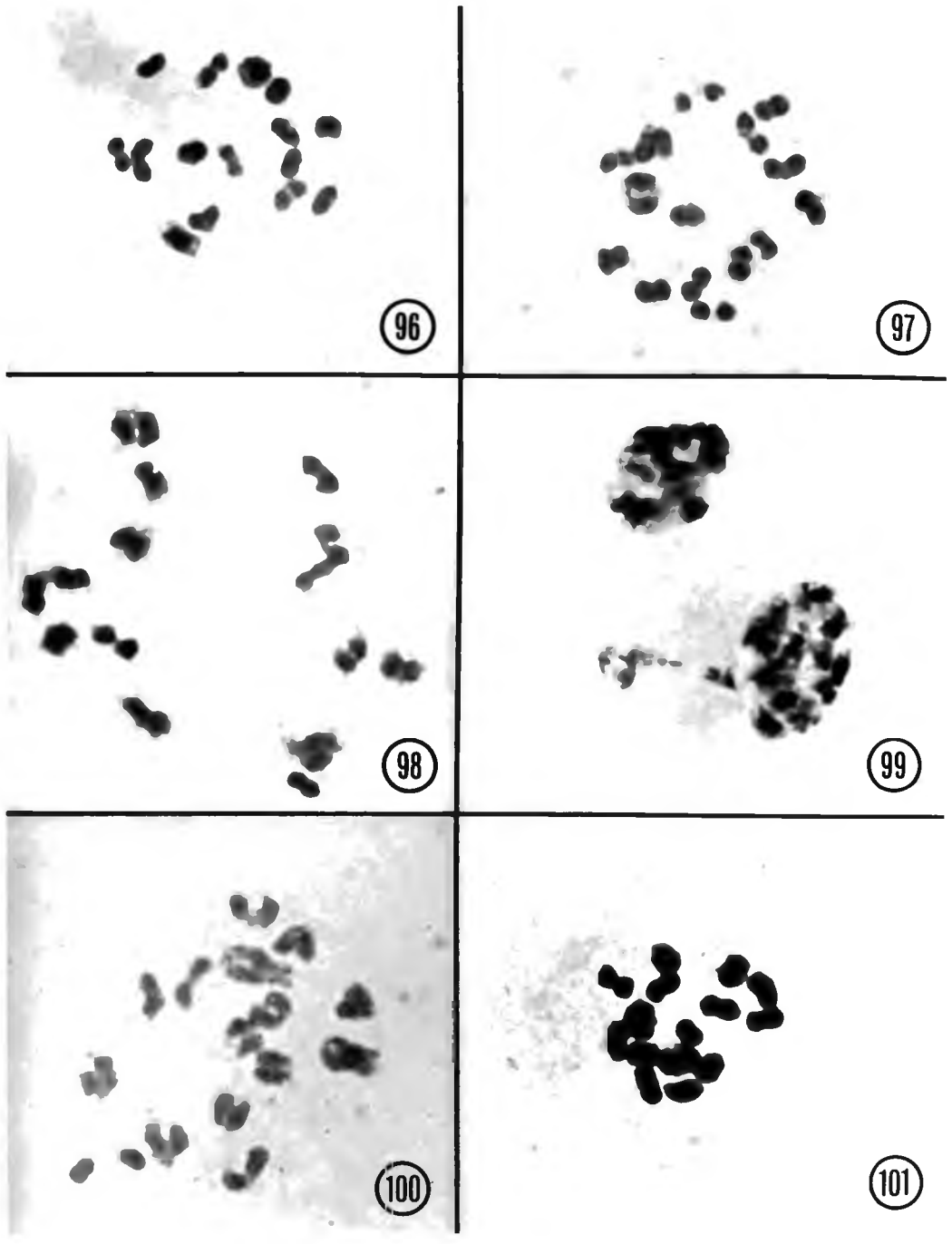
97. Meiotic configuration of A494 (*A. andraeanum* X *A. amnicola*) 2n=15II.

98. Meiotic configuration of 569-1 (*A. amnicola* (A417) X *A. lindenianum* (A220-2)) 2n=15II.

99. Late anaphase I of 569-1 of (*A. amnicola* (A417) X *A. lindenianum* (A220-2)), showing lagging chromosomes.

100. Meiotic configuration of 552-73 (*A. lindenianum* (A170) X *A. amnicola* (A417)) 2n=15II.

101. Meiotic configuration of 531-14 (`Calypso' X *A. amnicola* (A417)) 2n=15II.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101

similar to that of intragroup interspecific hybrids (Table 8). These results suggest that A. amnicola is genetically close to taxa in group VI.

In the anthurium breeding program at the University of Hawaii, further crossings of A. amnicola hybrids with other taxa or other hybrids have been achieved. Table 11 lists successful interspecific hybrids with A. amnicola. The highest number of taxa involved in hybridization was 5. The data again strongly suggest genomic similarity of A. amnicola, A. andraeanum, A. lindenianum, A. formosum, and A. kamemotoanum.

4.1.5 Discussion

The karyotypes of all 15 Anthurium taxa examined appear to be very similar and consist of two distinctively large chromosome pairs, one pair of satellite chromosomes, and twelve pairs of fairly large, medium and small chromosomes. According to Stebbins (1971), these karyotypes can be classified as asymmetrical because of great differences in relative size between the chromosomes of the complement. The smallest chromosome pairs are about half the size of the largest pairs. At meiosis it seems that these small chromosome pairs terminalize much faster than larger ones and the earlier separation of the small homologous chromosomes may cause misinterpretation of meiotic

Table 11. List of successful interspecific hybrids with *Anthurium amnicola*.[z]

Number of Taxa involved	Cross
2	*A. lindenianum* x *A. amnicola*
	A. amnicola x *A. lindenianum*
	(*A. lindenianum* x *A. amnicola*) x *A. amnicola*
	(*A. lindenianum* x *A. amnicola*) x *A. lindenianum*
	A. amnicola x *A. formosum*
	(*A. amnicola* x *A. formosum*) x *A. formosum*
	(*A. amnicola* x *A. formosum*) x *A. amnicola*
	A. andraeanum x *A. amnicola*
	'Calypso' x *A. amnicola*
	'Trinidad' x *A. amnicola*
3	(*A. andraeanum* x *A. lindenianum*) x *A. amnicola*
	(*A. kamemotoanum* x *A. formosum*) x *A. amnicola*
	(*A. andraeanum* x *A. kamemotoanum*) x *A. amnicola*
4	[(*A. andraeanum* x *A. kamemotoanum*) x *A. formosum*)] x *A. amnicola*
	[(*A. andraeanum* x *A. lindenianum*) x *A. amnicola*] x [*A. andraeanum* x (*A. lindenianum* x *A. kamemotoanum*)]
	[(*A. andraeanum* x *A. lindenianum*) x *A. amnicola*] x *A. formosum*
	[(*A. andraeanum* x *A. lindenianum*) x *A. amnicola*] x [*A. andraeanum* x (*A. lindenianum* x *A. kamemotoanum*)]
	[((*A. andraeanum* x *A. kamemotoanum*) x *A. formosum*) x *A. amnicola*] x *A. formosum*
5	[(*A. andraeanum* x *A. lindenianum*) x *A. amnicola*] x [*A. andraeanum* x *A. kamemotoanum*) x *A. formosum*]

[z] Kamemoto, unpublished data.

configuration.

A slight morphological variation in karyotypes is seen in two species, A. nymphaeifolium (group VI) and A. wallisii (group V). Anthurium nymphaeifolium contains one pair of large acrocentric chromosomes instead of large metacentric chromosomes (Table 6). It can be assumed that these species have been derived from their closely related taxa which contain the 'basic' karyotype found in species of group VI and V through chromosomal rearrangement such as translocation. Unfortunately, meiotic analysis of various hybrids of two species fails to resolve the origin of chromosomal variation. For instance, the hybrid A. nymphaeifolium (A213-3) x A. formosum (A287) had the mean configuration of 15 II at prometaphase I and showed regular meiotic division at later stages (Table 8).

The regular bivalent formation at meiosis in interspecific hybrids suggests close genomic relationship among taxa. Nevertheless, the reduction in pollen fertility in hybrids indicates the existence of some genetic barriers between taxa. Striking differences in pollen stainability between intragroup interspecific hybrids and intergroup interspecific hybrids (Table 9) indicates genetic diversity between groups.

The taxonomic treatment of A. amnicola can be a subject of controversy. Dressler (1978) first suggested

placing this Panamanian species in Engler's Section Urospadix and in group II of Sheffer and Kamemoto's classification.

Recently Croat and Sheffer (1983) revised Engler's classification system and placed A. amnicola and its closely related species, A. antioquiense, in Section Porphyrochitonium. This section was divided into two sub-groups depending on crossability of taxa. Anthurium amnicola and A. antioquiense were grouped together because they were crossable between themselves as well as with A. andraeanum (Section Calomystrium). The other group included A. scherzerianum and A. wendlingerii which were also intercrossable (Kamemoto and Sheffer, 1978) but exhibited a genetic divergence from A. andraeanum and its closely related taxa (Sheffer and Kamemoto, 1976b).

The present study confirms the close genomic relationship of A. amnicola with four taxa, A. andraeanum, A. formosum, A. kamemotoanum, and A. lindenianum which belong to Engler's Section Calomystrium (Croat and Sheffer, 1983) and Sheffer and Kamemoto's group VI. Although morphologically A. amnicola is similar to those members of Section Porphyrochitonium, genomic closeness strongly suggests that A. amnicola should be included in the group with A. andraeanum and closely related taxa. Genetic relationship among species should be considered in classification of Anthurium.

4.2 Flavonoid analysis of selected *Anthurium* species

4.2.1 Identification of flavonoids in *Anthurium* species

The various properties of the major flavonoids of selected *Anthurium* species are summarized in Table 12-19. Table 21 indicates a possible identity of each compound.

Six flavonoids isolated from the leaves of *A. andraeanum* 'Uniwai' were characterized (Table 12 and 13) and four compounds were identified as either apigenin or acacetin derivatives. The major flavonoid resolved in HPLC with the retention time of 17.0 min was either acacetin C-diglycoside of C-monoglycosyl acacetin O-monoglycoside. A comparison of this compound with standards isolated from the fruits of *Fortunella margarita* and their hydrolyzed products (Table 20) was not successful in identifying the exact nature of the compound. It was assumed that the sugar attached to acacetin at either position 6-C or 8-C was not glucose because the hydrolyzed product of the compound differed from either acacetin 8-C-glucoside or 6-C-glucoside.

The compound with the retention time of 12.9 min showed spectral maxima and color reaction similar to apigenin derivatives (Mabry *et al.*, 1970) The compound with the retention time =18.7 min was identified as acacetin 7-O-diglycoside. Two hydrolyzed products of the compound has retention times of 19.1 min and 28.0 min

Table 12. Retention time (HPLC), color, Rf values (PC) and products of hydrolysis of the major flavonoids from Anthurium andraeanum 'Uniwai'.

Compound Number	Retention time (min)	Color		Rf value (x100)		Products of acid hydrolysis
		UV	UV+NH_3	TBA	HOAc	
1	12.9	Q	YG	72	87	several compounds
2	17.0	Q	dullY	75	90	acacetin monoglycoside (major)
3	18.7					acacetin 7-O-glucoside and acacetin
4	20.3					
5a	22.8	C	FlYG	36	78	
5b	22.8	C	FlYG	33	48	

Key: Q = quenching, YG = yellow green, dullY = dull yellow, C = colorless, FlYG = fluorescent yellow green.

Table 13. Retention time (HPLC) and spectral maxima for the major flavonoids of *Anthurium andraeanum* 'Uniwai'.

Compound number	Retention time (min)	Absorption maxima in nm					
		MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+NaOAc/H_3BO_3
1	12.9	332	384	378sh[z]	378sh	398	332
		267	272	350	345	332	270
				302	301	270	
				278	278		
2	17.0	330	370	374sh	374sh	330	330
		272	320sh	349	345	272	272
			295	301	301		
				280	280		
2 (hydrolyzed)	18.0	330	370sh				
		272	320sh				
			295				
3	18.7	327	372	380	380	330	330
		267	320sh	338	334	267	267
			292	297	297		
				274	274		
3 (hydrolyzed)	19.1 28.0						
4	20.3	324	380	324	324	324	324
		274	326sh	270	270	270	270
			294				
5a+5b	22.8	322	315sh	326	326	320	320
		280	290	280sh	280sh	280sh	280sh

[z]sh = shoulder.

Table 14. Retention time (HPLC), color, Rf values (PC) and products of acid hydrolysis for the major flavonoids of Anthurium formosum.

Compound number	Retention time(min)	Color		Rf value (x100)		Products of acid hydrolysis
		UV	UV+NH_3	TBA	HOAc	
1	5.7	C	FlVio	72	89	
2	7.3	Q	Vio	86	58	
3	9.2	Or	FlY	39	34	same as the original compound
4	9.5	Or	FlY	41	46	same as the original compound
5	10.6	FlBl	FlBl	70	84	

Key: C = colorless, FlVio = fluorescent violet, Q = quenching, Vio = violet, Or = orange, FlY = fluorescent yellow, FlBl = fluorescent blue.

Table 15. Retention time (HPLC) and spectral maxima for the major flavonoids of Anthurium formosum.

Compound number	Retention time (min)	Absorption maxima in nm					
		MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+NaOAc/H_3BO_3
1	5.7	284	324	354	350	328	287
				302	300	287sh[z]	
2	7.3	285	324	362	289	330	284
				302		284	
3	9.2	363	387	412	400	378	420sh
		310	340	350	334	260	362
		254	301sh	288sh	278sh		314
			270	264	261		257
4	9.5	362	379	410	401	386	372
		312	340sh	350	336	333	318
		255	301	320sh	278sh	262	260
			268	288sh	262		
				265			
5	10.6	318	318	318	318	318	318
		243sh	243sh	243sh	243sh	243sh	243sh

[z]sh = shoulder.

Table 16. Retention time (HPLC), color, Rf values (PC) and spectral maxima for the major flavonoids of Anthurium lindenianum (A220-1) and A. concinnatum (A212)

Compound number	Retention time (min)	Color		Rf value (x100)		Absorption maxima in nm	
		UV	UV+NH_3	TBA	HOAc	MeOH	+NaOMe
A. lindenianum (A220-1)							
1	12.4	Q	Y	51	78	332 269	392 328 277
2	15.9	Q	YG	67	61		
A. concinnatum (A212)							
1	9.6	Q	YG	28	51		
2	10.2	Q	YG	31	53	330 272	396 323 280
3	11.6	Q	Y	30	55	327 271	398 324 281

Key: Q = quenching, Y = yellow, YG = yellow green.

Table 17. Retention time (HPLC), color, Rf values (PC) and spectral maxima for the major flavonoids of *Anthurium nymphaeifolium* (A213-3) and *A. wallisii* (A286).

Compound number	Retention time (min)	Color		Rf value (x100)		Absorption maxima in nm	
		UV	UV+NH_3	TBA	HOAc	MeOH	+NaOMe
A. nymphaeifolium							
1	8.4	Q	Y	30	55	325	395
						278	327sh[z]
							282
2	9.6	Q	Y	28	53	325	398
						272	330
							281
3	10.2	Q	Y	32	53	330	398
						272	330
							281
4	11.6	Q	Y	30	55		
A. wallisii							
1	7.4					325	392
						267	345sh
							292
							270
2	12.1					325	396
						265	320
							275

[z]sh = shoulder.
Key: Q = quenching, Y = yellow.

Table 18. Retention time (HPLC), color, Rf value (PC) and spectral maxima for the major flavonoids of Anthurium amnicola (A417).

Compound number	Retention time (min)	Color		Rf value (x100)		Absorption maxima (nm)		Products of acid hydrolysis
		UV	UV+NH_3	TBA	HOAc	MeOH	+NaOMe	
1		FlY	Y	31	11			
2		FlBl	BlGr	45	84			
3	4.5-7.0	Bl	FlBl	47	93			
4		Y	Y	62	82			
5		Vio	Vio	70	90			
6	9.7					325 270	390 292sh[z] 276sh	
7	12.6					325 270	395 322 278	
8	13.7	Q	dullY	46	73	324 270	365 291 242sh	acacetin 6-C-glucoside (major) acacetin 8-C-glucoside (minor)

[z]sh = shoulder.

Key: FlY = fluorescent yellow, Y = yellow, FlBl = fluorescent blue, BlGr = blue green, Bl = blue, Vio = violet, Q = quenching, dullY = dull yellow.

Table 19. Retention time (HPLC), color, and Rf value (PC) for the major flavonoids of Anthurium kamemotoanum (567-70).

Compound number	Retention time (min)	Color		Rf value (x100)	
		UV	UV+NH_3	TBA	HOAc
1	10.8	FlVio	Vio	13	33
2	10.8	FlVio	FlY	19	13
3	12.6	Q	dullY	65	50
4	13.2	FlVio	FlVio	27	21

Key: FlVio = fluorescent violet, Vio = violet, Q = quenching, FlY = fluorescent yellow, dullY = dull yellow.

Table 20. Retention time (HPLC), color, Rf value (PC) and spectral maxima for authentic pigments and their hydrolyzed products from fruits of Fortunella margarita.

Compound	Retention time (min)	Color		Rf value (x100)		Absorption maxima in nm	
		UV	UV+NH_3	TBA	HOAc	MeOH	+NaOMe
Isolated compounds:							
Naringin 7-neohesperidoside	11.2					282	
Acacetin 8-C-neohesperidoside	13.8	Q	dullY	75	76	323	370
						270	295sh[z]
							278
Acacetin 6-C-neohesperidoside	16.5	Q	dullY	64	83	325	370
						270	300sh
							272
Acacetin 7-O-neohesperidoside	18.6	Q	dullY	67	54	324	352sh
						267	290
Hydrolyzed products:							
Acacetin 8-C-glucoside	14.4						
Acacetin 6-C-glucoside	17.5						
Acacetin 7-O-glucoside	19.1						
Acacetin	28.0						

[z]sh = shoulder.
Key: Q = quenching, dullY = dull yellow.

Table 21. characterization of major flavonoids of Anthurium species.

Plant	Compound number	Retention time (min)	Characterization
A. andraeanum 'Uniwai'	1	12.9	apigenin glycoside
	2(major)	17.0	acacetin C-diglycoside
	3	18.7	acacetin 7-O-diglycoside
	4	20.3	acacetin glycoside?
A. formosum (A290)	1	5.7	naringin derivative
	2	7.3	naringin derivative
	3	9.2	flavonol C-monoglycoside
	4(major)	9.5	flavonol C-monoglycoside
	5	10.6	4'-methoxyflavone derivative
A. lindenianum (A220-1)	1(major)	12.4	apigenin derivative
	2	15.9	apigenin derivative

Table 21.(Continued)

Plant	Compound number	Retention time (min)	Characterization
A. concinnatum (A212)	1	9.6	apigenin derivative
	2	10.2(major)	apigenin derivative
	3	11.6	apigenin derivative
A. nymphaeifolium (A213-3)	1	8.4(major)	apigenin derivative
	2	9.6	apigenin derivatives correspond to compounds found in A. concinnatum
	3	10.2	
	4	11.6	
A. wallisii (A286)	1	7.4	apigenin derivative
	2	12.1	apigenin derivative
A. kamemotoanum (567-70)	3	12.6(major)	apigenin derivative
A. amnicola (A417)	7	12.6	apigenin derivative
	8	13.7(major)	acacetin 6-C-diglycoside (6-C-glucose)

which corresponded to two hydrolyzed products of acacetin 7-O-neohesperidoside, acacetin 7-O-glucoside (retention time=19.1 min) and acacetin (retention time=28.0 min). The sugar attached to the basic acacetin structure at the position 7-O was glucose although the other sugar linked to glucose remained unidentified.

Five pigments from the leaves of Anthurium formosum (A290) were examined for their properties (Table 14 and 15). Two compounds with short retention times (5.7 min and 7.3 min) were presumed to be flavanone naringin derivatives. Two flavonols found in this species exhibited such distinct color reaction that orange spots in UV changed to fluorescent yellow after fuming with ammonia. Acid hydrolysis with 2N HCl failed to cleave any sugars from those compounds; therefore, the two compounds were tentatively identified as C-monoglycosylflavonols. Color reaction (fluorescent blue in UV to fluorescent blue in UV/NH_3) and absorption maxima with various shift reagents revealed that the compound with retention time of 10.6 min was a 4'-methoxyflavone derivative (Mabry et al., 1970).

Major flavonoids isolated from A. lindenianum (A220-1), A. concinnatum (A212), A. nymphaeifolium (A213-3) and A. wallisii (A286) commonly showed characteristics of flavone apigenin in color reaction and UV spectra (Table 16 and 17). Quenching spots in UV

turned yellow or yellow green after fuming with ammonia. Spectral maxima of these compounds are 325-332 nm (Band I) and 265-278 nm (Band II). Addition of NaOMe resulted in Band I bathochromic shift (60-73 nm) with an increase in intensity, indicating that there is -OH at 4′ position (Mabry et al., 1970). Three flavones from A. concinnatum (A212) and compounds from A. nymphaeifolium (A213-3) with the retention time of 9.6, 10.2 and 11.6 min were co-chromatogramed in HPLC. It was concluded that those flavones with the same retention time were identical.

The leaves of Anthurium amnicola (A417) are found to contain acacetin glycoside, apigenin derivative and other compounds which have retention times earlier than 10 min (Table 18 and 21). The major flavonoid, an acacetin glycoside (retention time=13.7 min) was hydrolyzed with 2N HCl to produce acacetin 6-C-glucoside (major) and acacetin 8-C-glucoside (minor). It was assumed that the compound was an acacetin 6-C-diglycoside with glucose attached at 6-C position or 6-C-glucosyl acacetin O-monoglycoside. 6-C-glucosylglycoside The presence of acacetin 8-C-glucoside after acid hydrolysis might be due to isomerization of acacetin 6-C-glucoside (Chopin and Bouillant, 1975). The compound with retention time=12,6 min was identical with the one with the same retention time of A. kamemotoanum (Table 21). The spectral analysis indicates that this compound is an apigenin derivative.

Presence of C-glycosylflavonoids in Anthurium species was expected because Williams et al. (1981) pointed out the predominant occurrence of flavone C-glycosides in the family Araceae. Two different acacetin C-glycosides were found in leaves of A. andraeanum 'Uniwai' and A. amnicola (A417) as their major components. C-flavonols were characteristic flavonoids in A. formosum while apigenin derivatives were commonly found in some other species.

4.2.2 Flavonoid profile on HPLC of selected Anthurium species

HPLC resolved a great variation of flavonoid composition among species (Fig. 102-127). Anthurium formosum had a large amount and number of flavonoids in leaf tissues (Fig. 111 and 112) whereas in A. caperatum, virtually no flavonoids were detected at 340 nm (Fig. 109).

Generally, each species displayed a specific flavonoid profile. Seven plants of A. andraeanum consistently contained acacetin C-glycoside (retention time=17.0 min) as the major compound and the secondary flavonoid, apigenin derivative (retention time=12.9 min) appeared in relatively the same concentration.

Anthurium lindenianum had two types of chromatograms. The first type seen in A220-1 and A220-2 was characterized by the presence of apigenin derivative

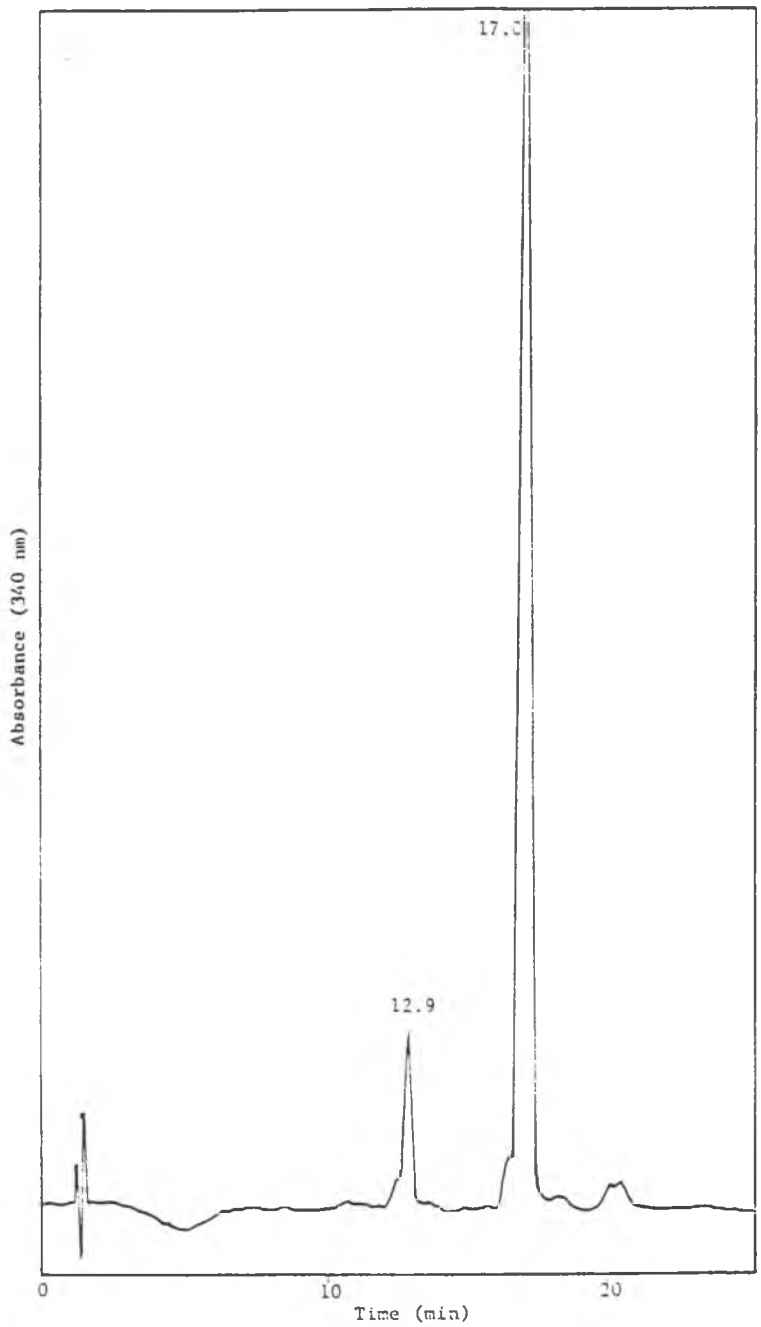

Fig. 102. HPLC resolution of flavonoids in leaves from Anthurium andraeanum (A481).

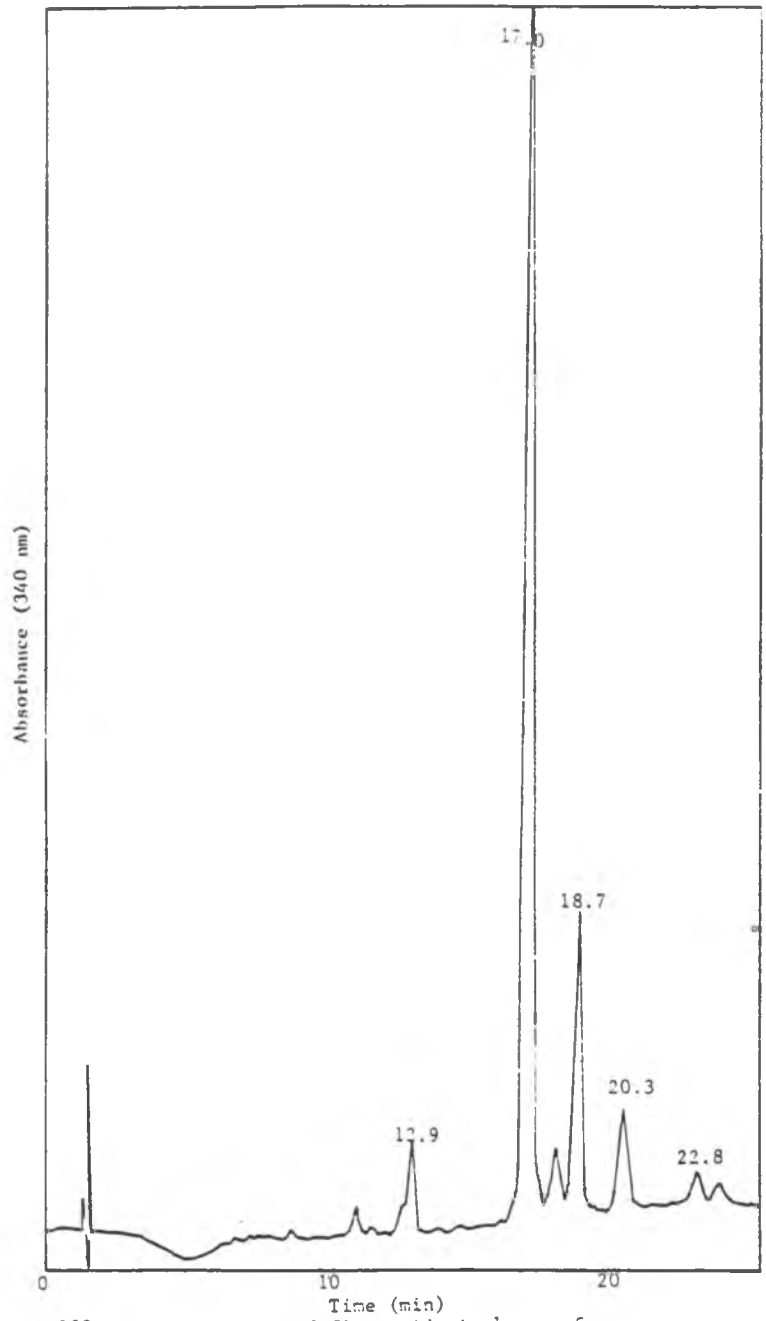

Fig. 103. HPLC resolution of flavonoids in leaves from Anthurium andraeanum 'Uniwai'.

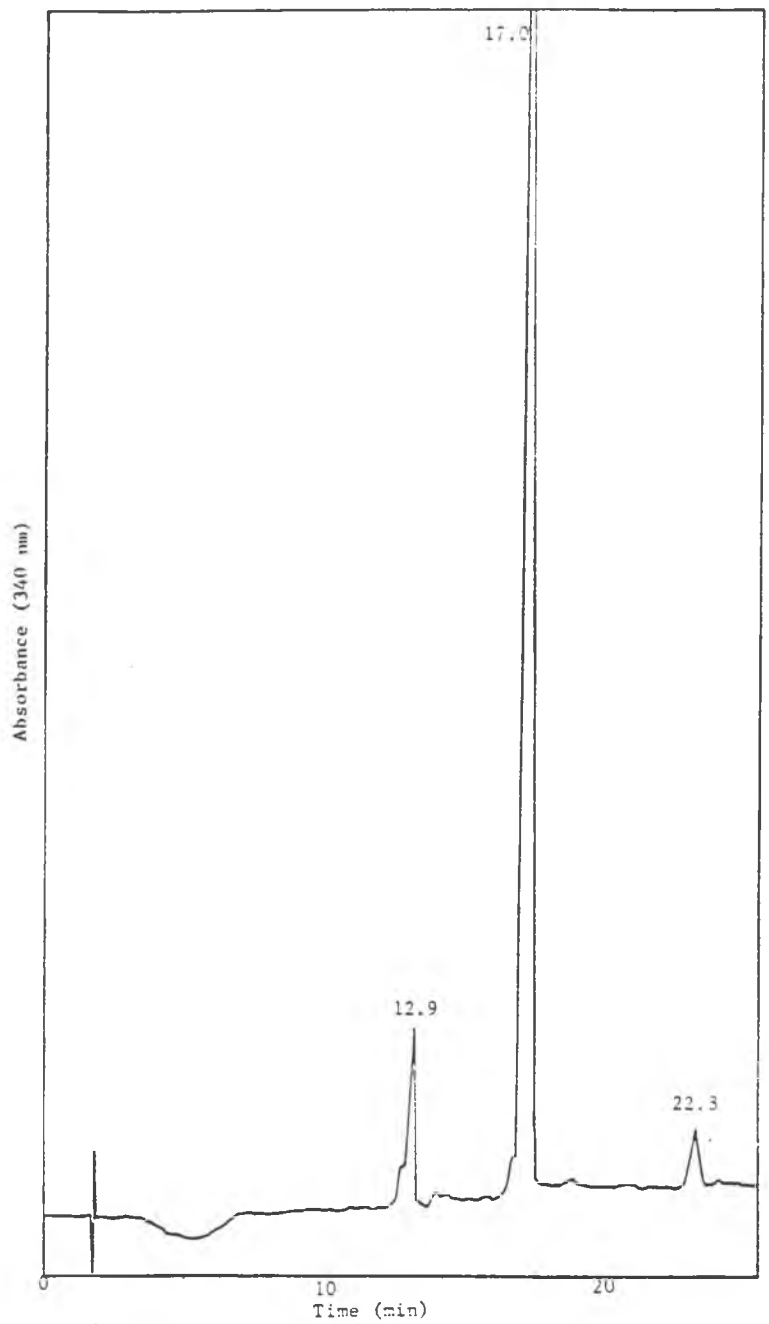

Fig. 104. HPLC resolution of flavonoids in leaves from Anthurium andraeanum 'Kaumana'.

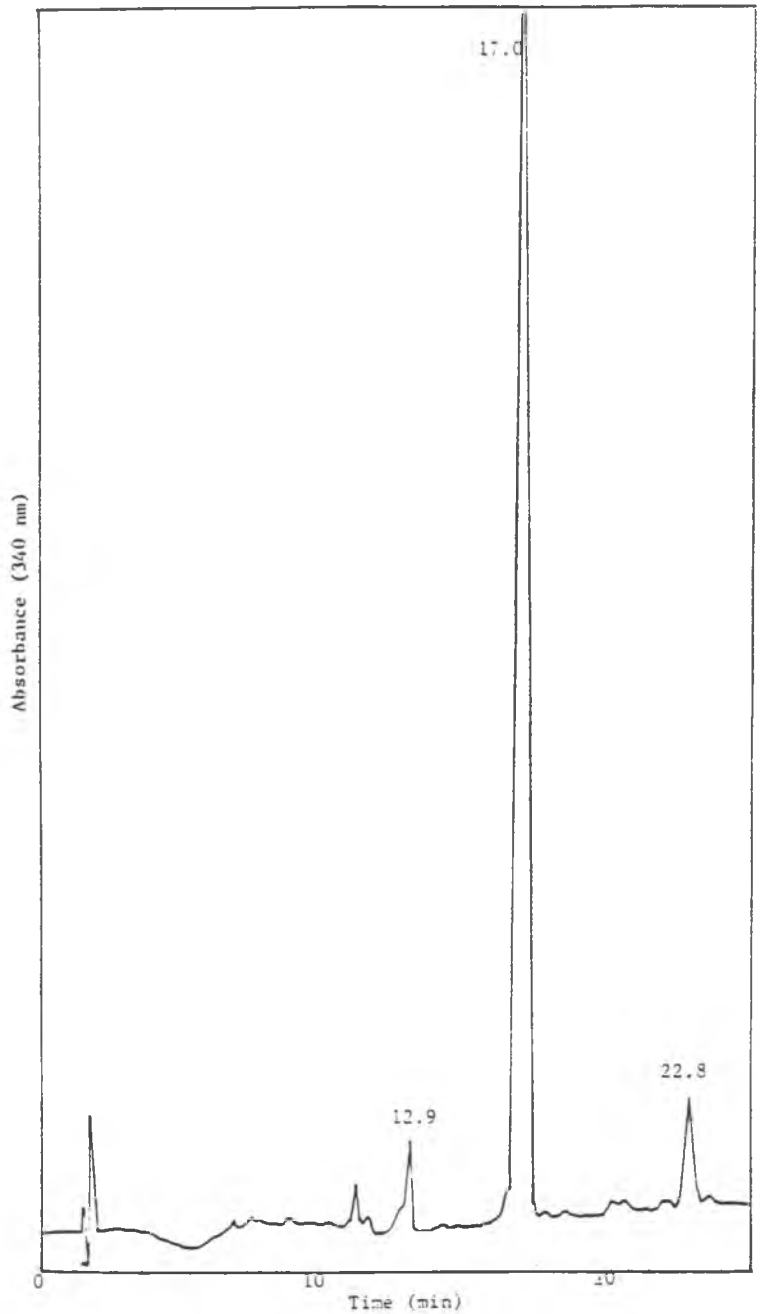

Fig. 105. HPLC resolution of flavonoids in leaves from Anthurium andraeanum 'Manoa Mist'.

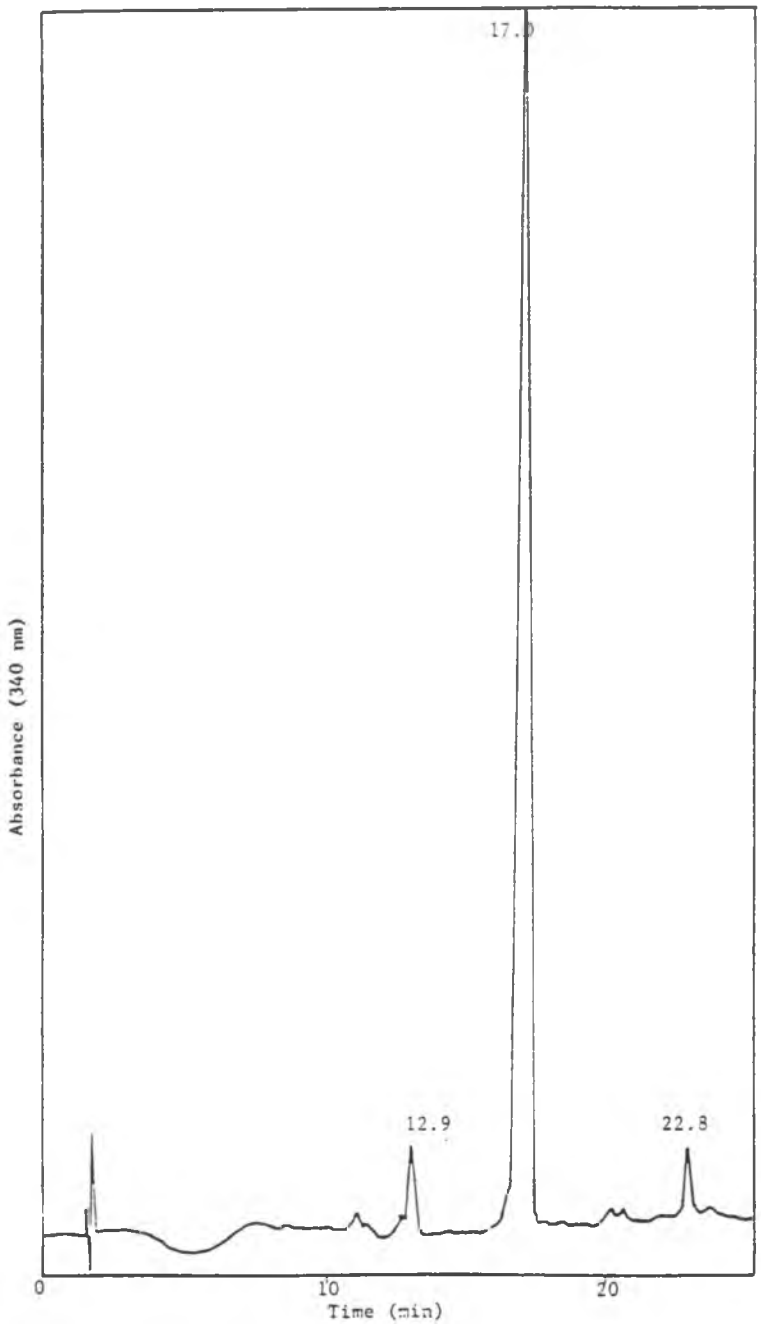

Fig. 106. HPLC resolution of flavonoids in leaves from Anthurium andraeanum 'Marian Sheefurth'.

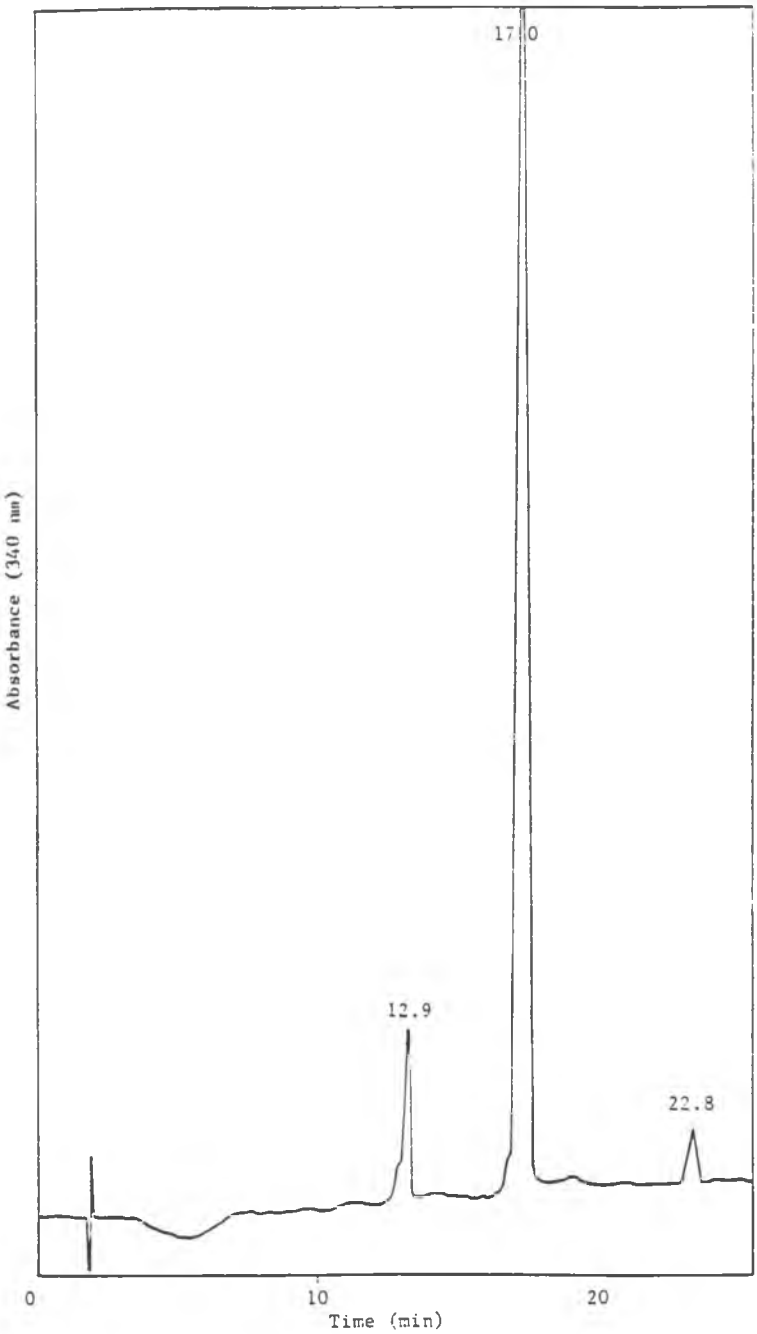

Fig. 107. HPLC resolution of flavonoids in leaves from Anthurium andraeanum 'Nitta'.

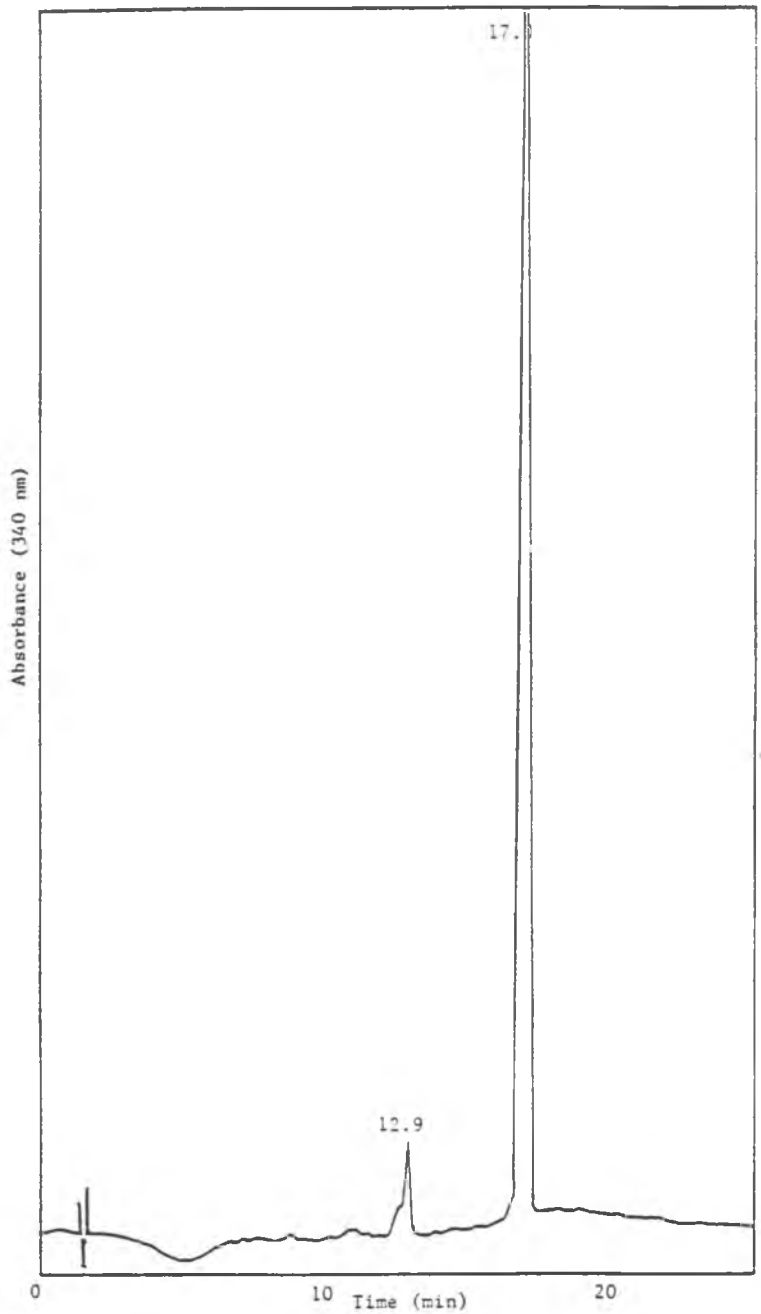

Fig.108.HPLC resolution of flavonoids in leaves from Anthurium andraeanum 'UH515'.

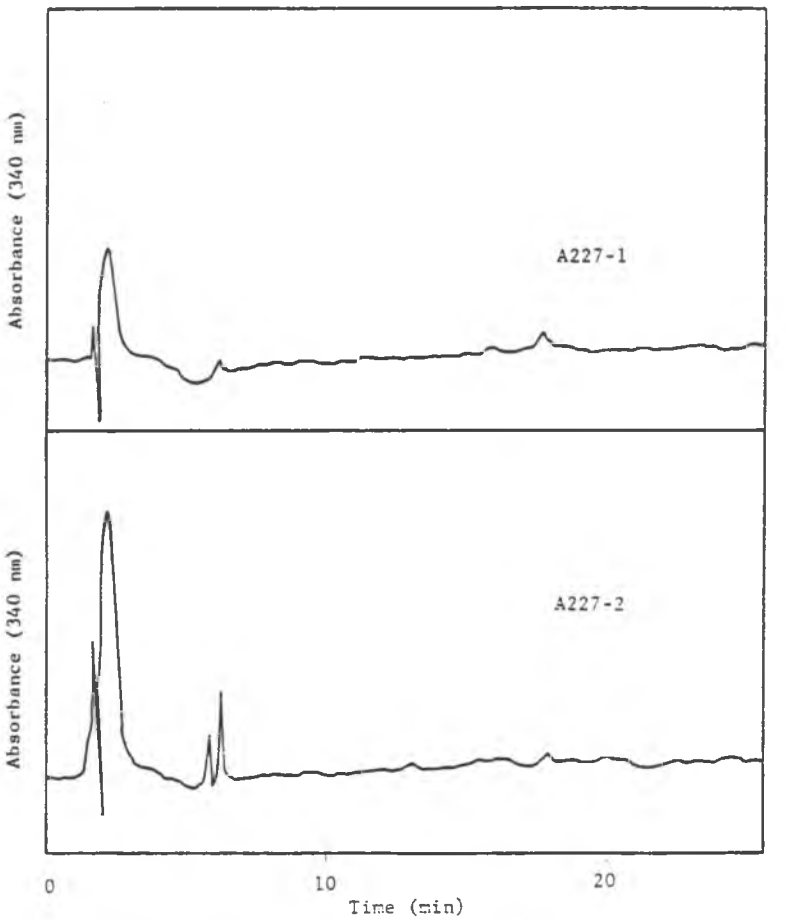

Fig. 109. HPLC resolution of flavonoids in leaves from Anthurium caperatum (A227-1 and A227-2).

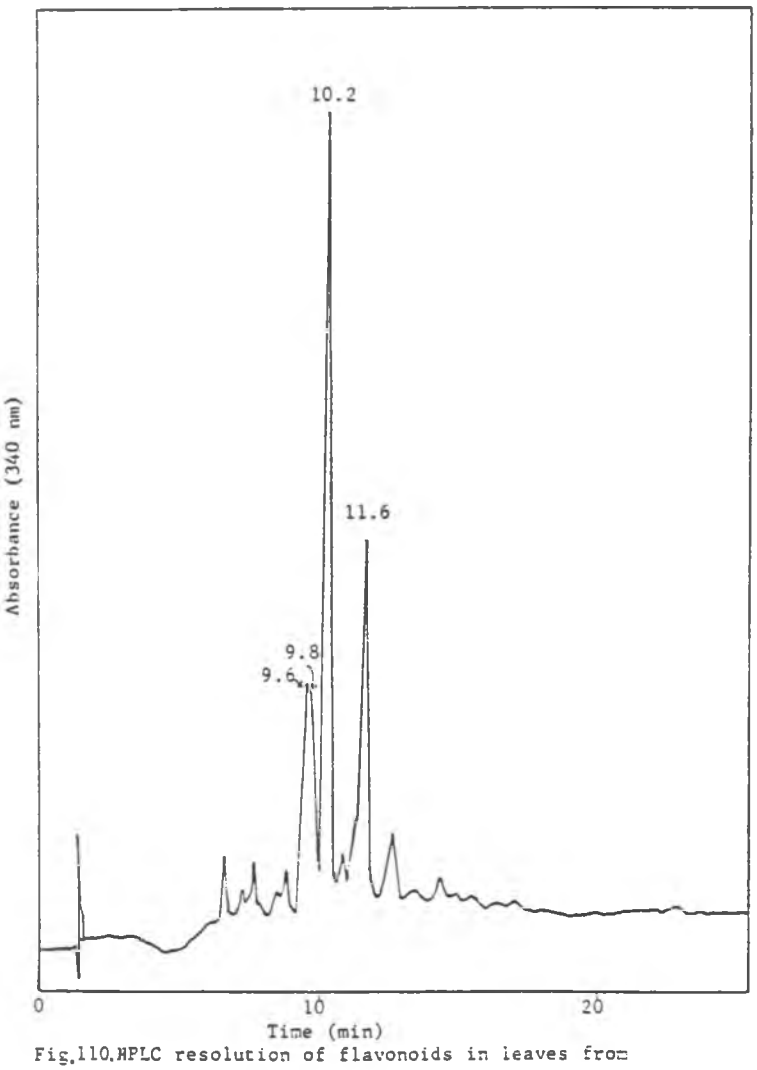

Fig.110.HPLC resolution of flavonoids in leaves from Anthurium concinnatum (A212).

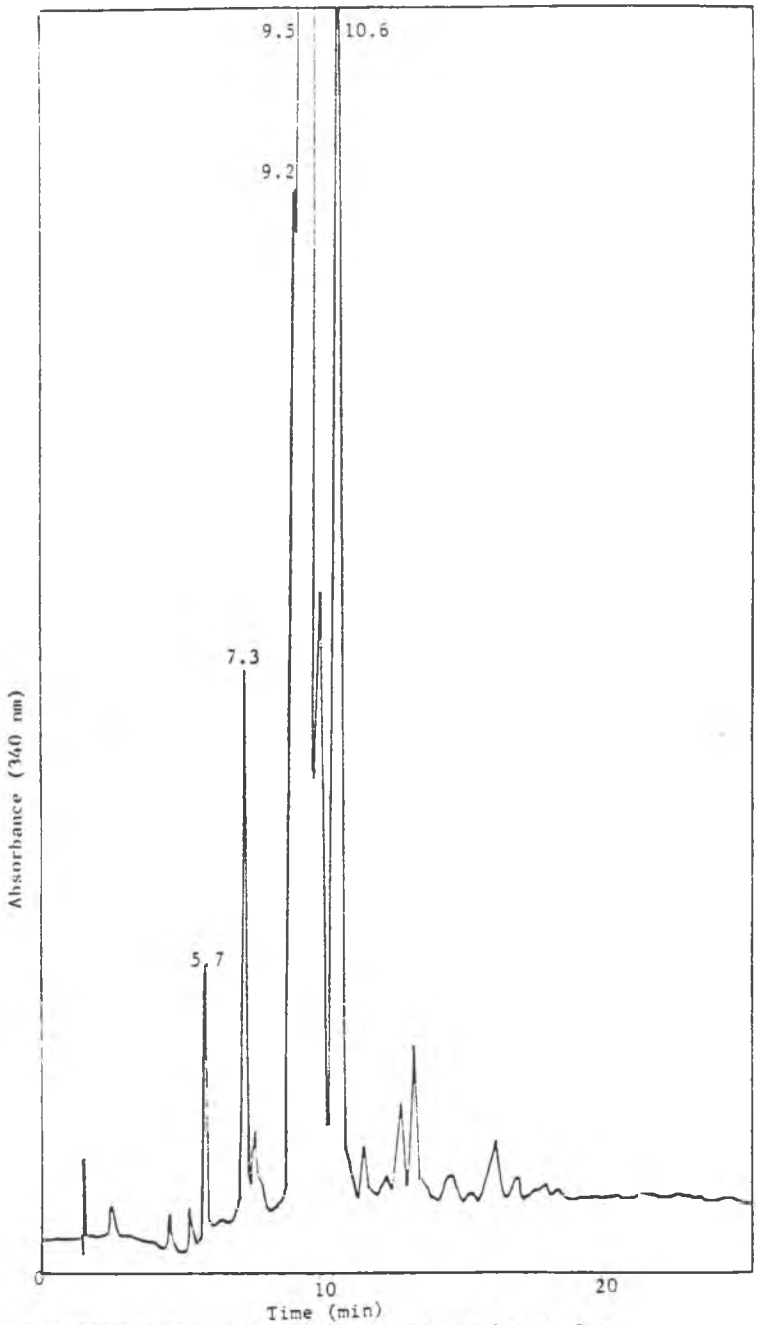

Fig.111.HPLC resolution of flavonoids in leaves from Anthurium formosum (A290).

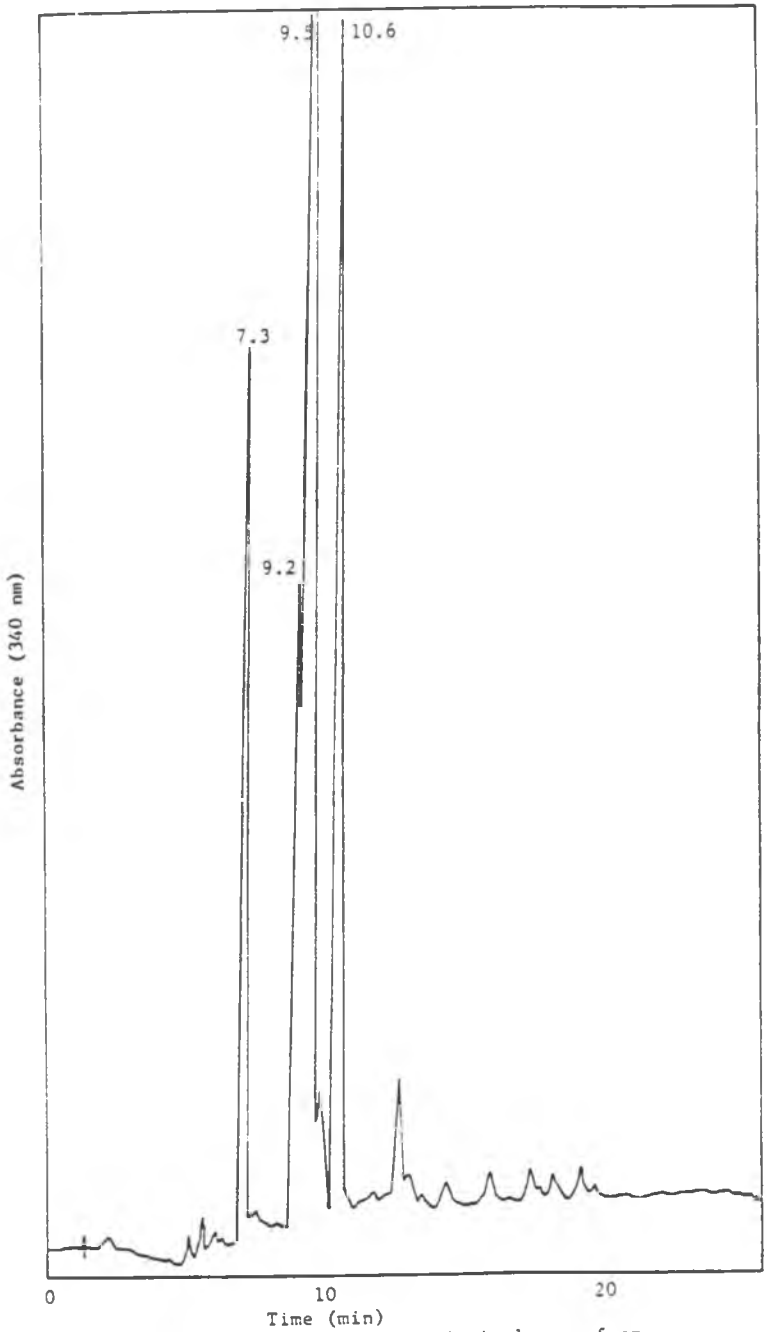

Fig. 112. HPLC resolution of flavonoids in leaves from Anthurium formosum (A291).

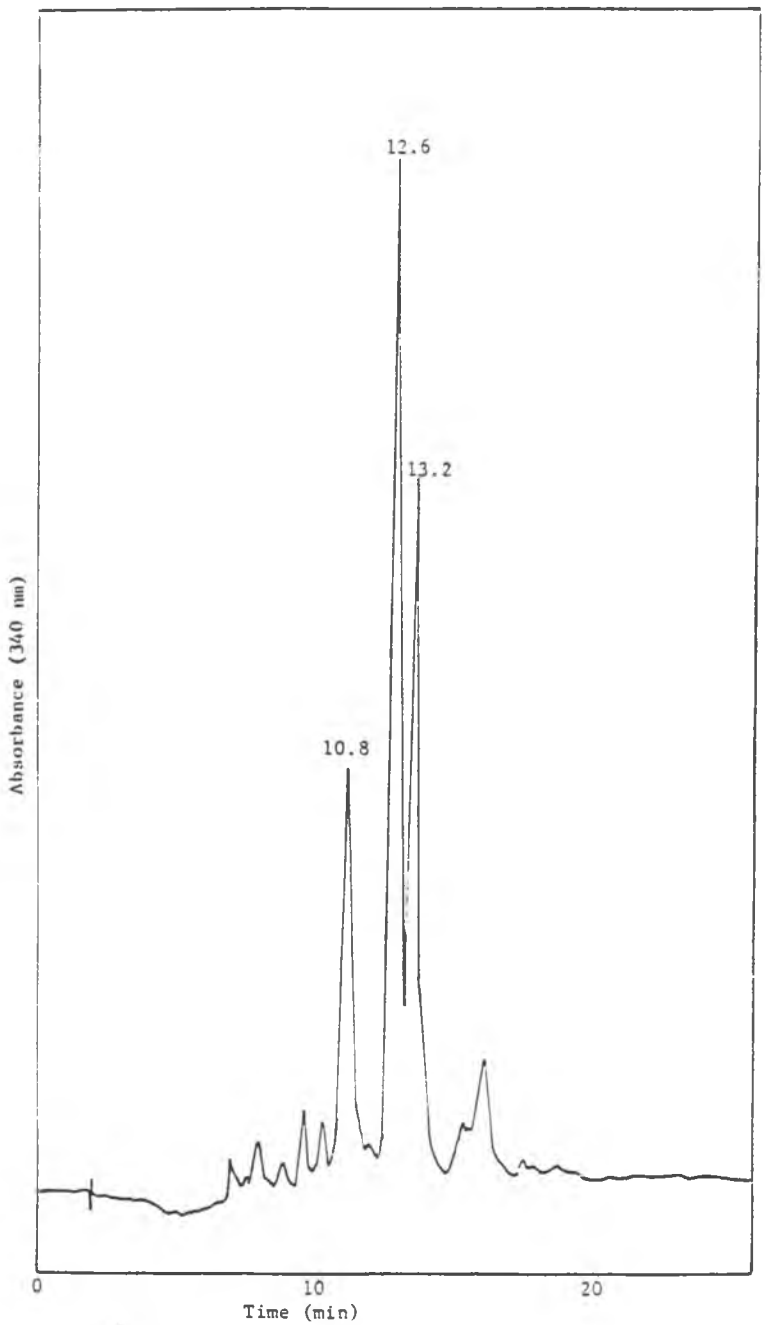

Fig. 113. HPLC resolution of flavonoids in leaves from Anthurium kamemotoanum (567-70).

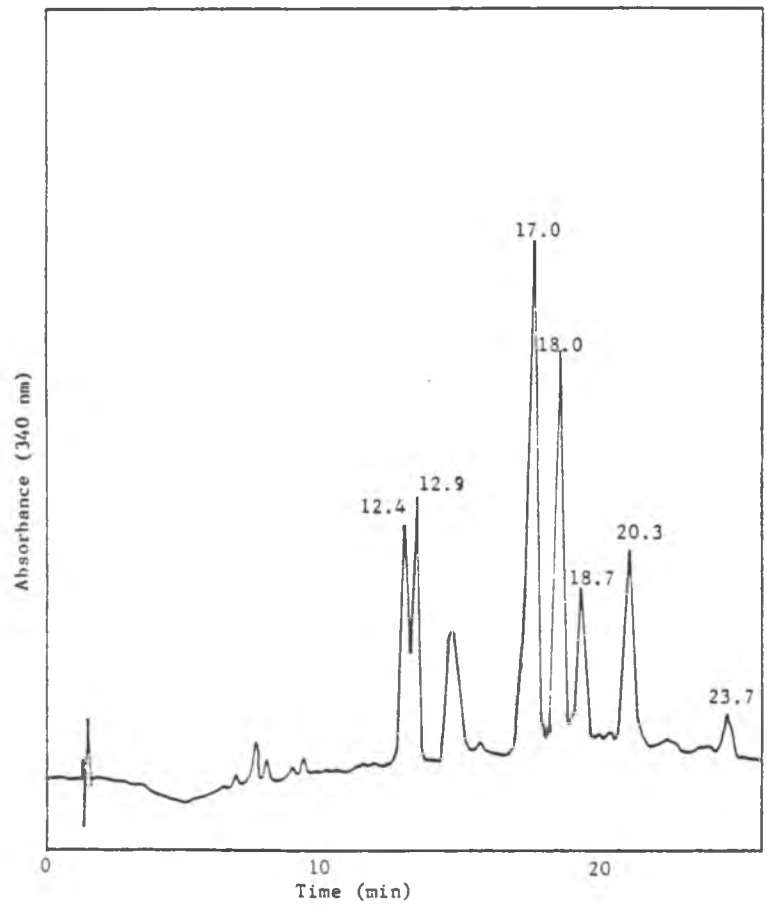

Fig. 114. HPLC resolution of flavonoids in leaves from Anthurium lindenianum (A170).

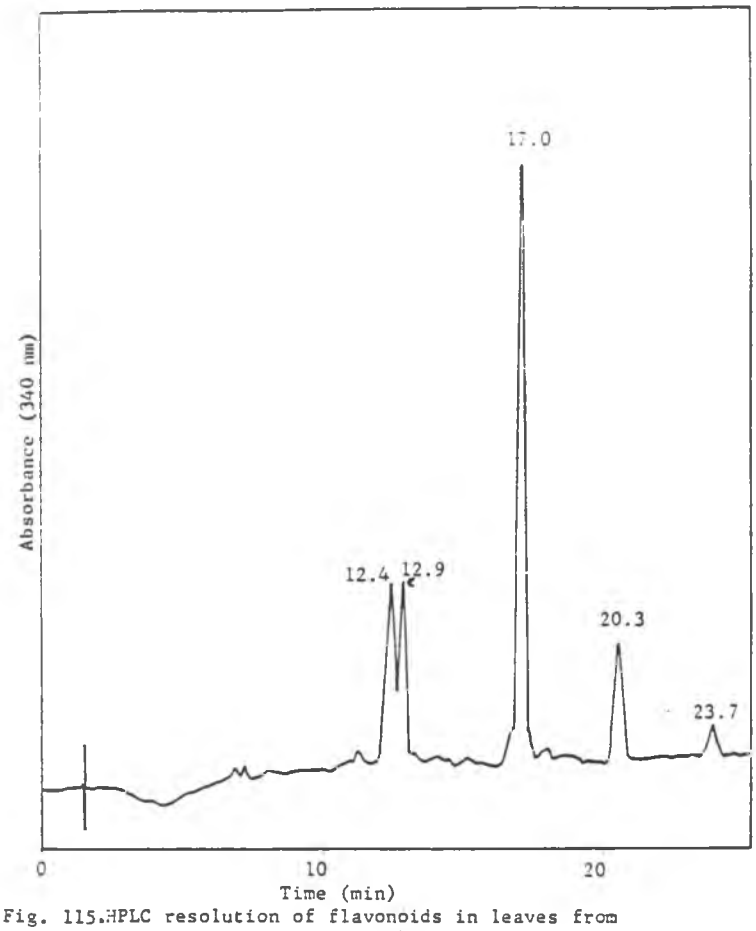

Fig. 115. HPLC resolution of flavonoids in leaves from Anthurium lindenianum (A185).

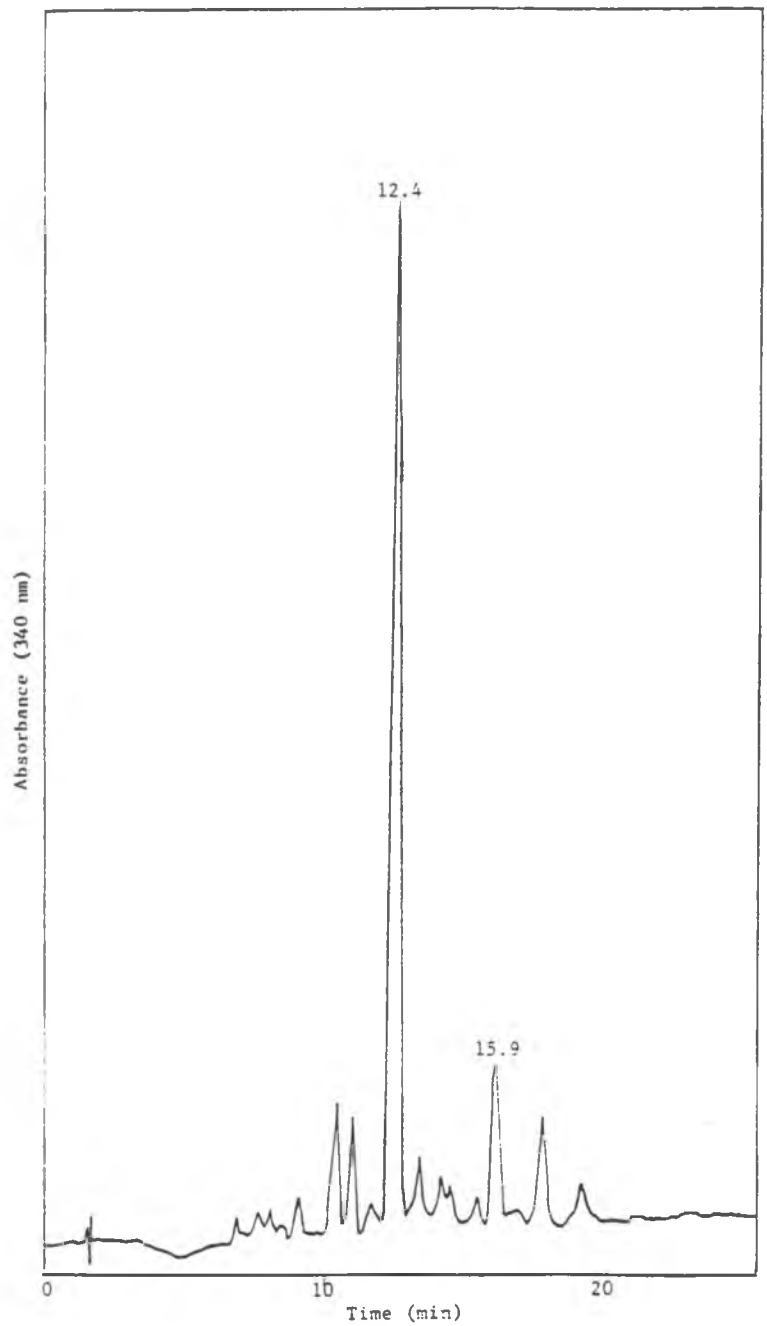

Fig. 116. HPLC resolution of flavonoids in leaves from Anthurium lindenianum (A220-1)

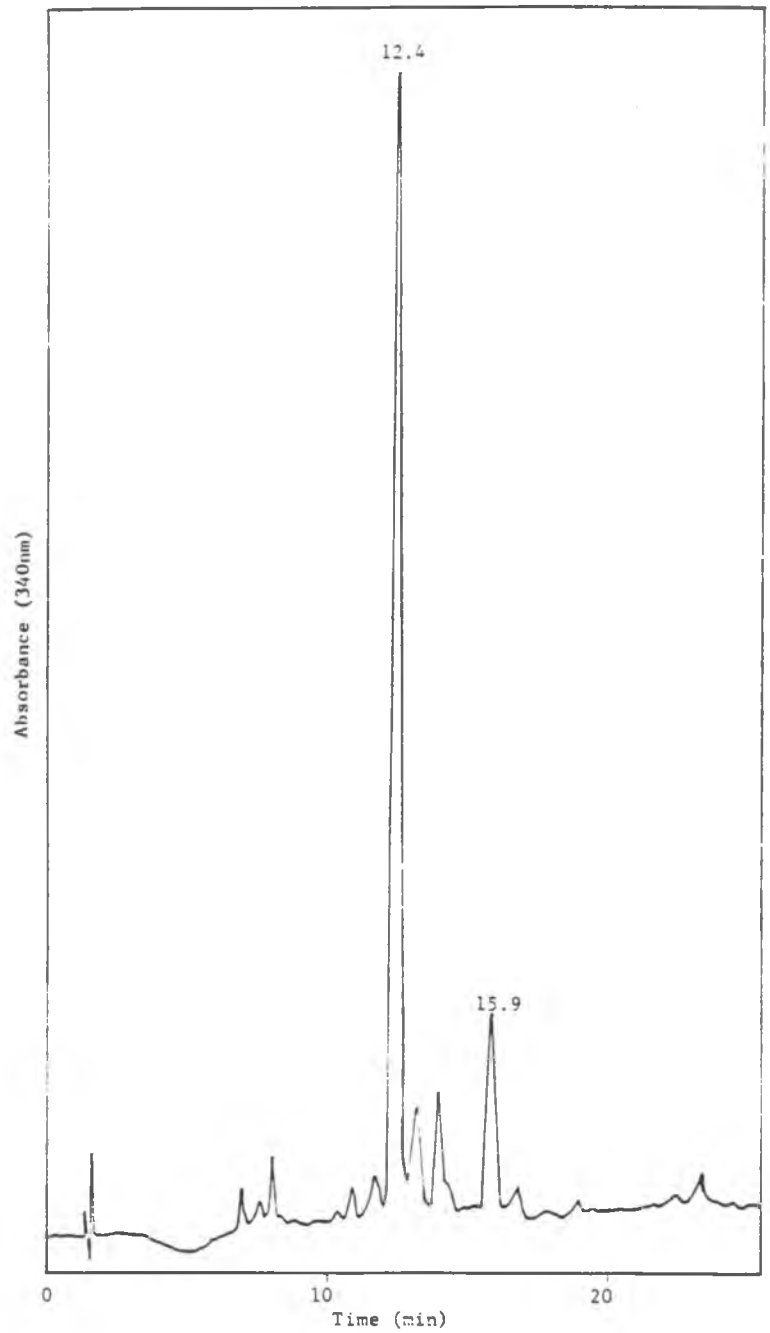

Fig. 117. HPLC resolution of flavonoids in leaves from Anthurium lindenianum (A220-2)

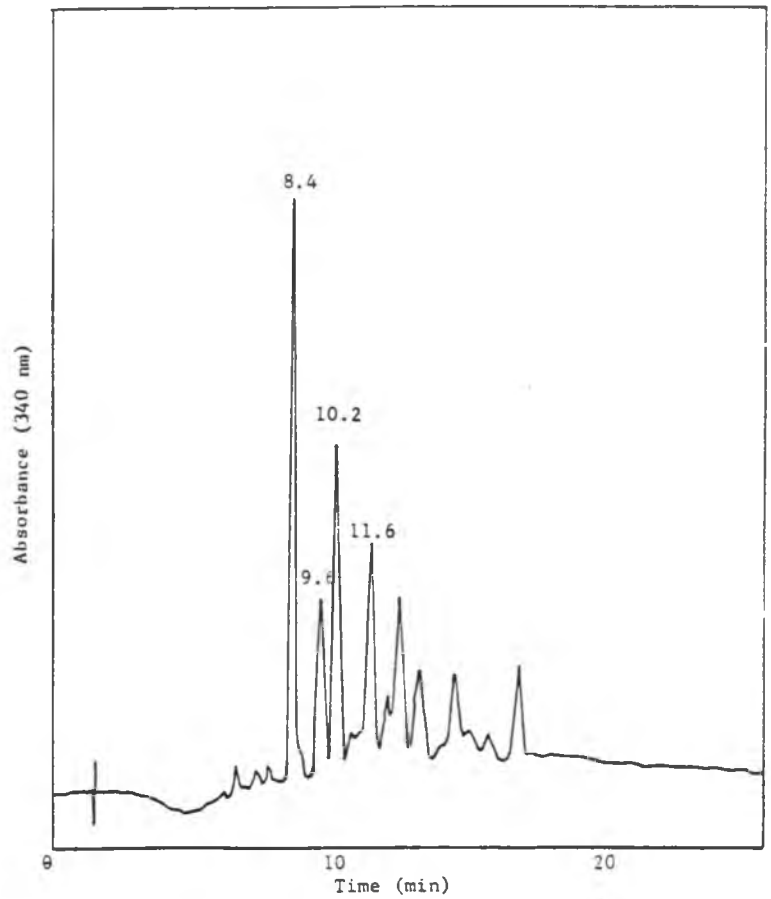

Fig. 118. HPLC resolution of flavonoids in leaves from Anthurium nymphaeifolium (A213-1).

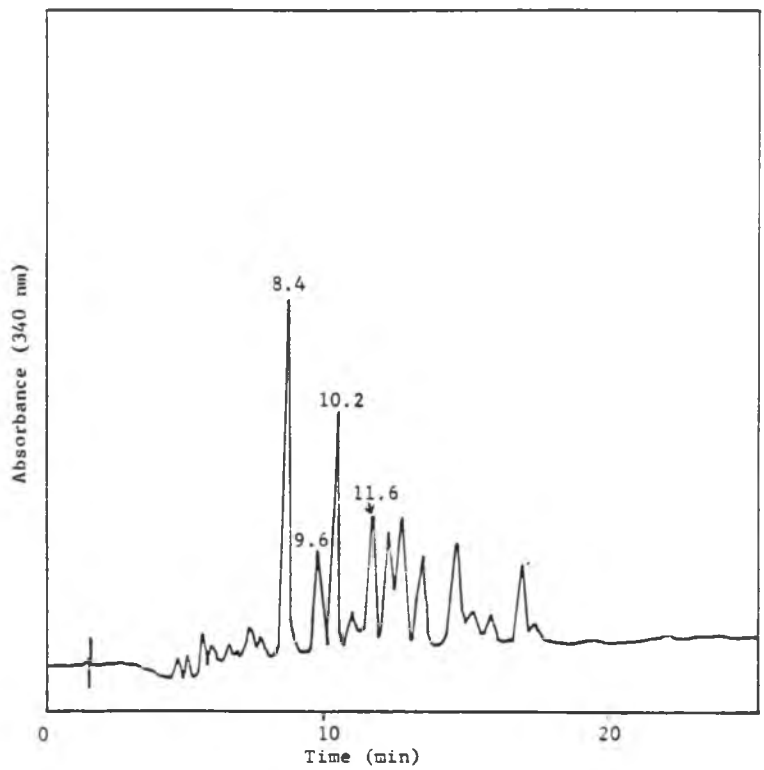

Fig. 119. HPLC resolution of flavonoids in leaves from Anthurium nymphaeifolium (A213-2).

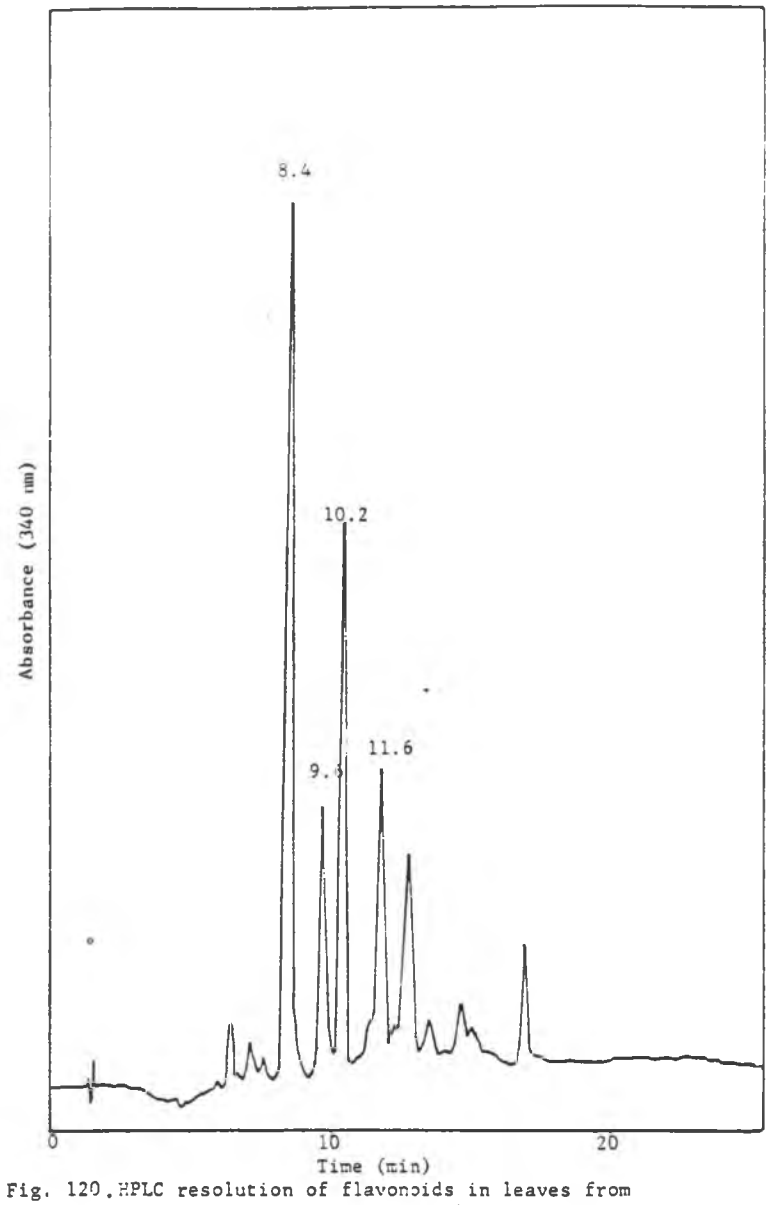

Fig. 120. HPLC resolution of flavonoids in leaves from Anthurium nymphaeifolium (A213-3).

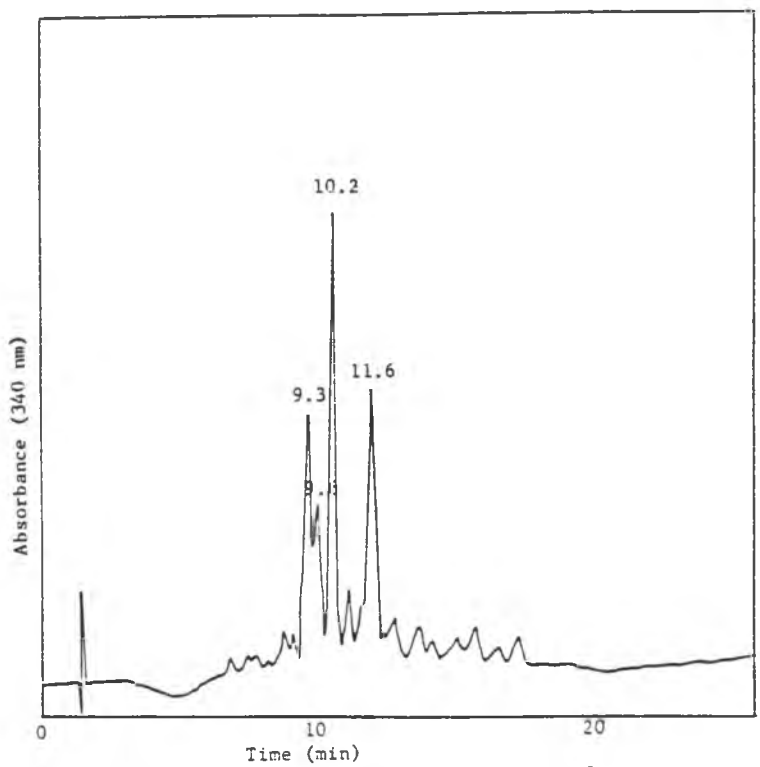

Fig. 121. HPLC resolution of flavonoids in leaves from Anthurium roraimense (A189).

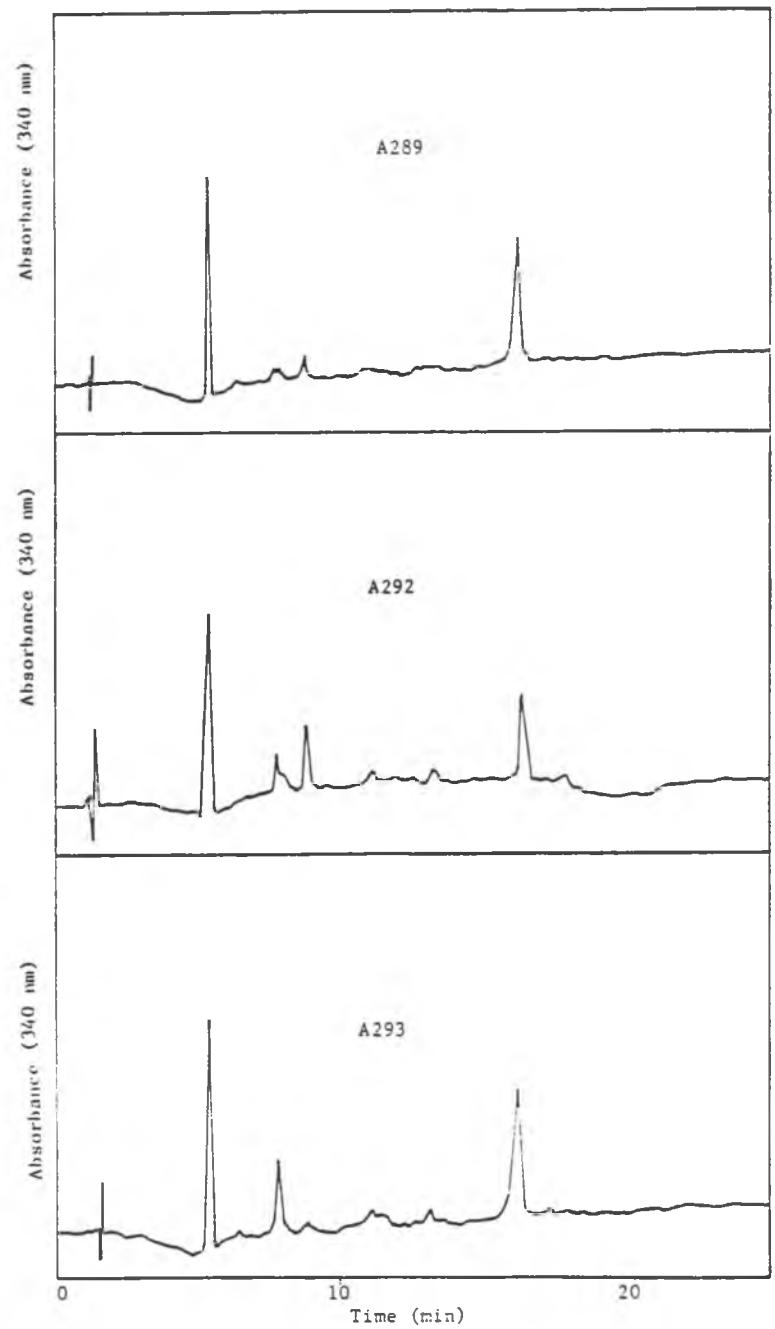

Fig. 122. HPLC resolution of flavonoids in leaves from Anthurium roseospadix (A289, A292 and A293).

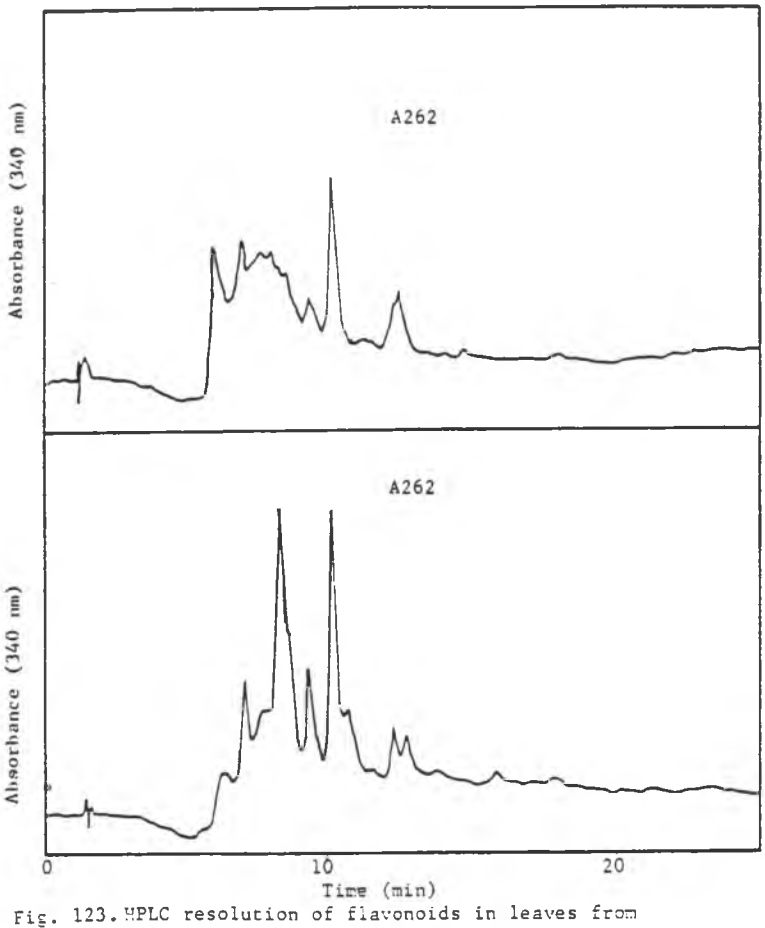

Fig. 123. HPLC resolution of flavonoids in leaves from Anthurium garagaranum (A262 and A263).

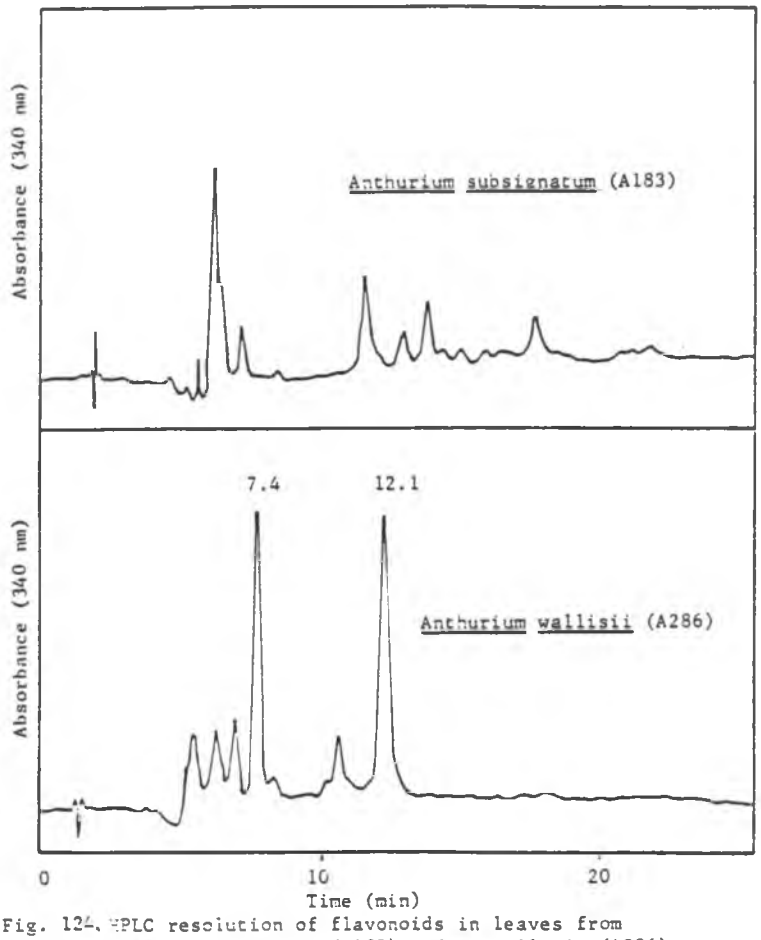

Fig. 124. HPLC resolution of flavonoids in leaves from Anthurium subsignatum (A183) and A. wallisii (A286).

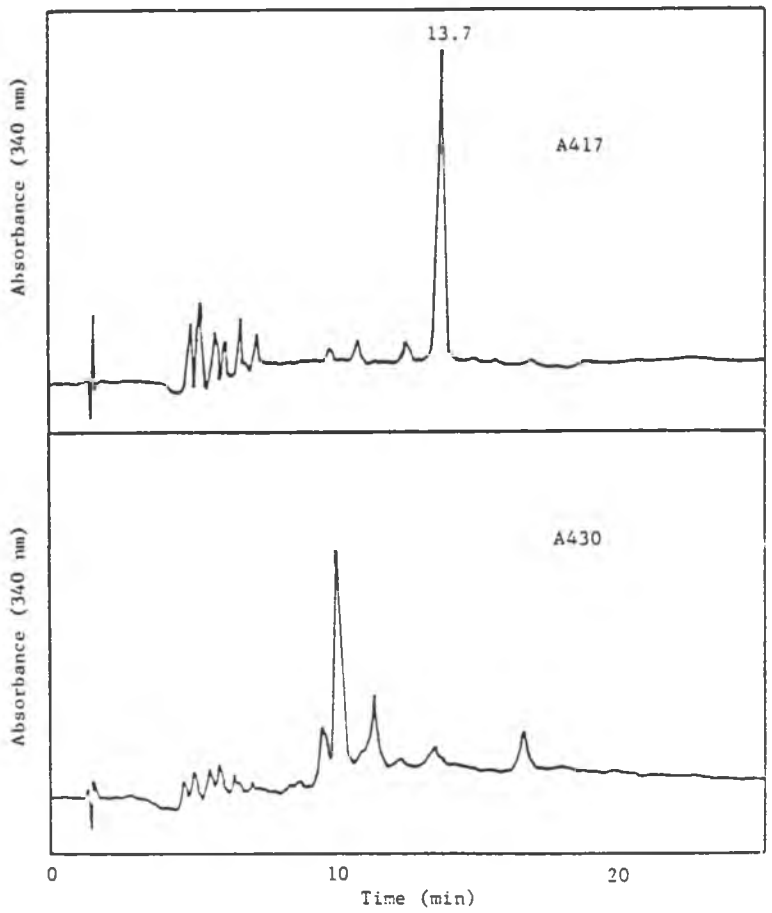

Fig. 125. HPLC resolution of flavonoids in leaves from Anthurium amnicola (A417 and A430).

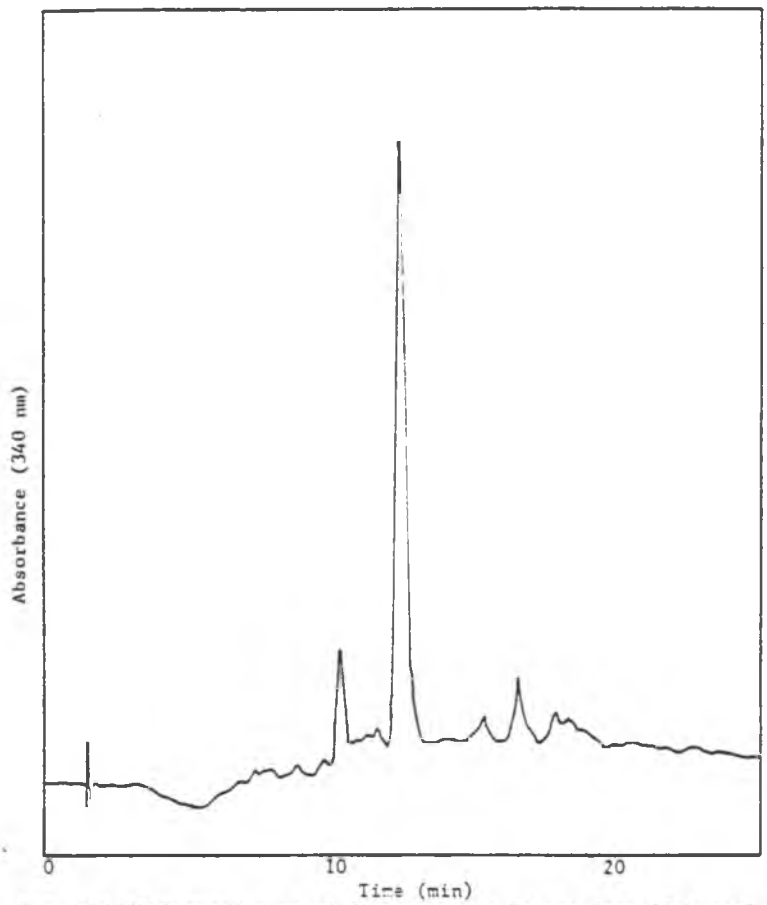

Fig. 126. HPLC resolution of flavonoids in leaves from 'Calypso'.

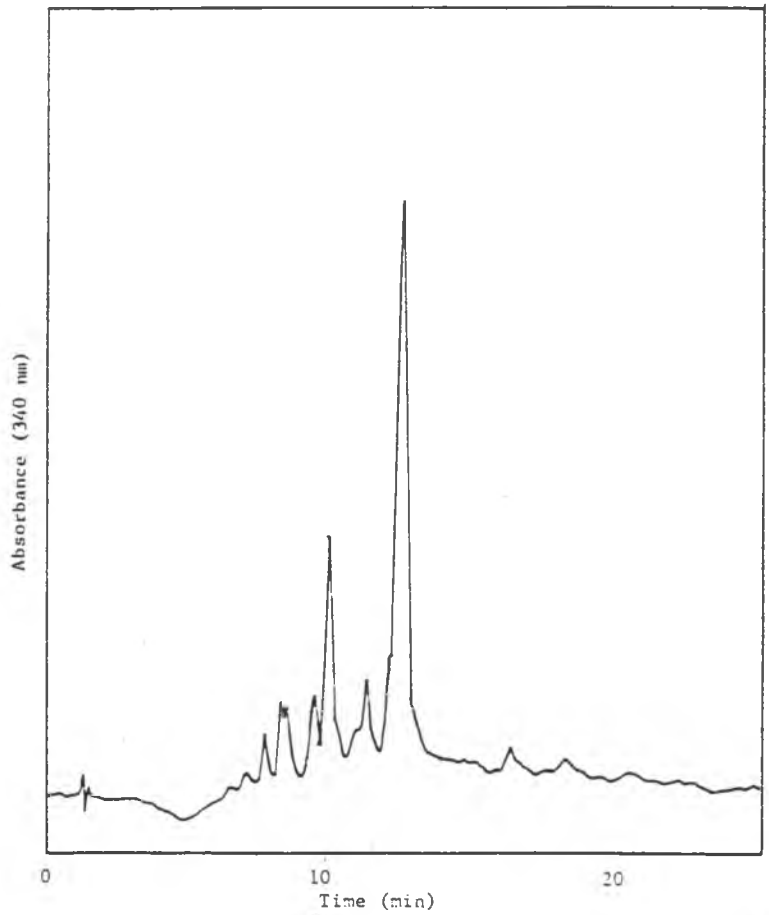

Fig. 127. HPLC resolution of flavonoids in leaves from 'Trinidad'.

(retention time=12.4 min) as the major flavonoid, and the second type contained compounds found in A. andraeanum such as acacetin C-glycoside (retention time=17.0 min). Two plants, A170 and A185 showed the second type of chromatogram. It is uncertain at this point whether A. lindenianum has two chemotypes or there are any taxonomic misplacement of these plants.

Interspecific variation as well as relative uniformity within a species could make flavonoid analyses useful in species identification of Anthurium.

4.2.3. Taxonomic studies on selected Anthurium species using morphological and flavonoid data

Numerical methods were used to evaluate similarities and dissimilarities (phenetic analysis) among A. andraeanum and closely related species on the basis of flavonoid characters which were examined in the previous section (Sec 4.2.2). In addition, morphological expression of taxa were compared to see any differences in taxonomic analysis. Cladistic analyses based on flavonoid data as well as morphological data were done to obtain any hypothetical phylogenetic relationship among taxa.

4.2.3.1 Phenetic analysis

The phenograms in Fig 128 and 129 show a measure of

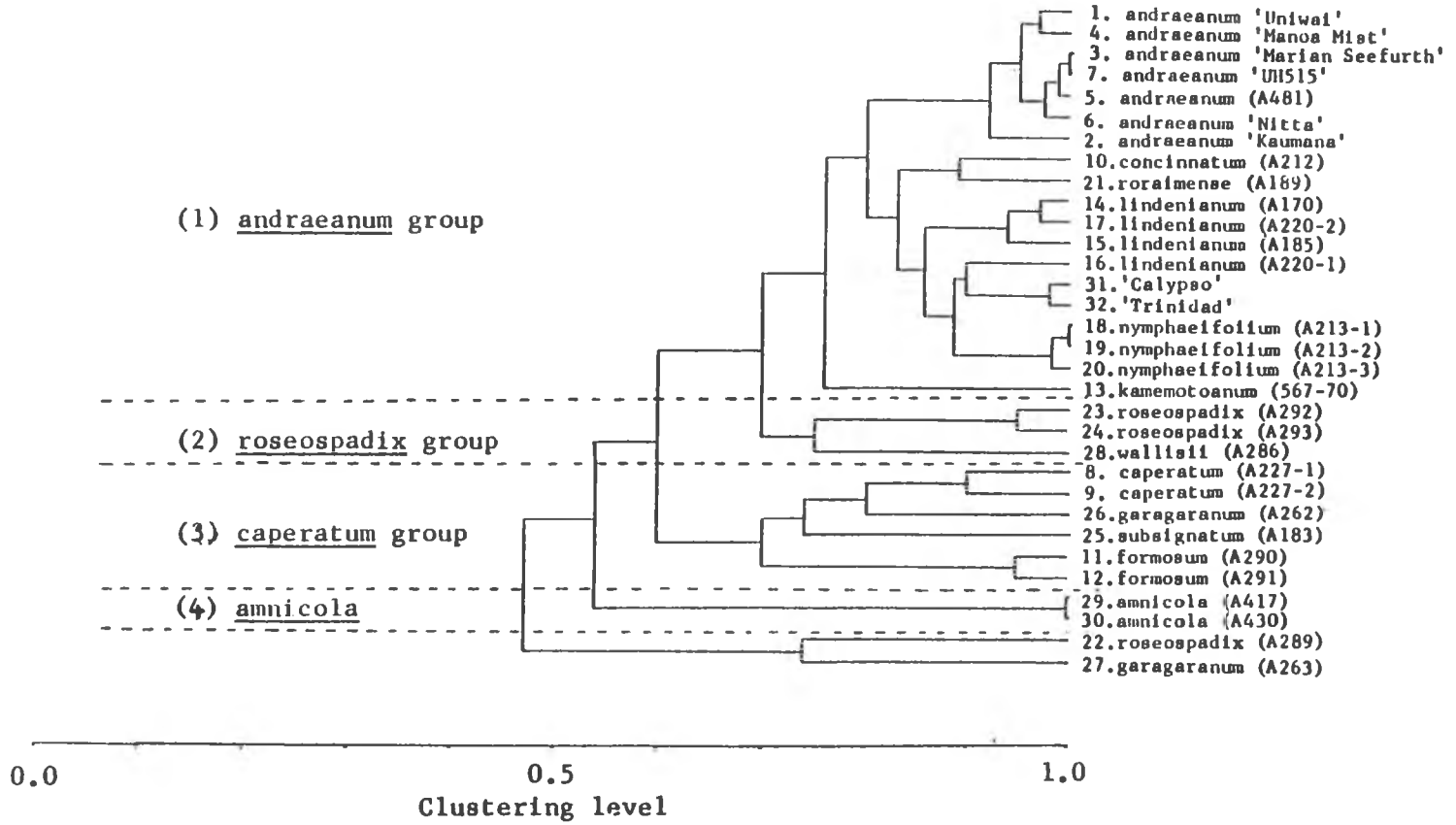

Figure 128. Phenogram of 32 Anthurium taxa based on morphological characters: S_{SM}(simple matching coefficient), UPGMA cluster analysis.

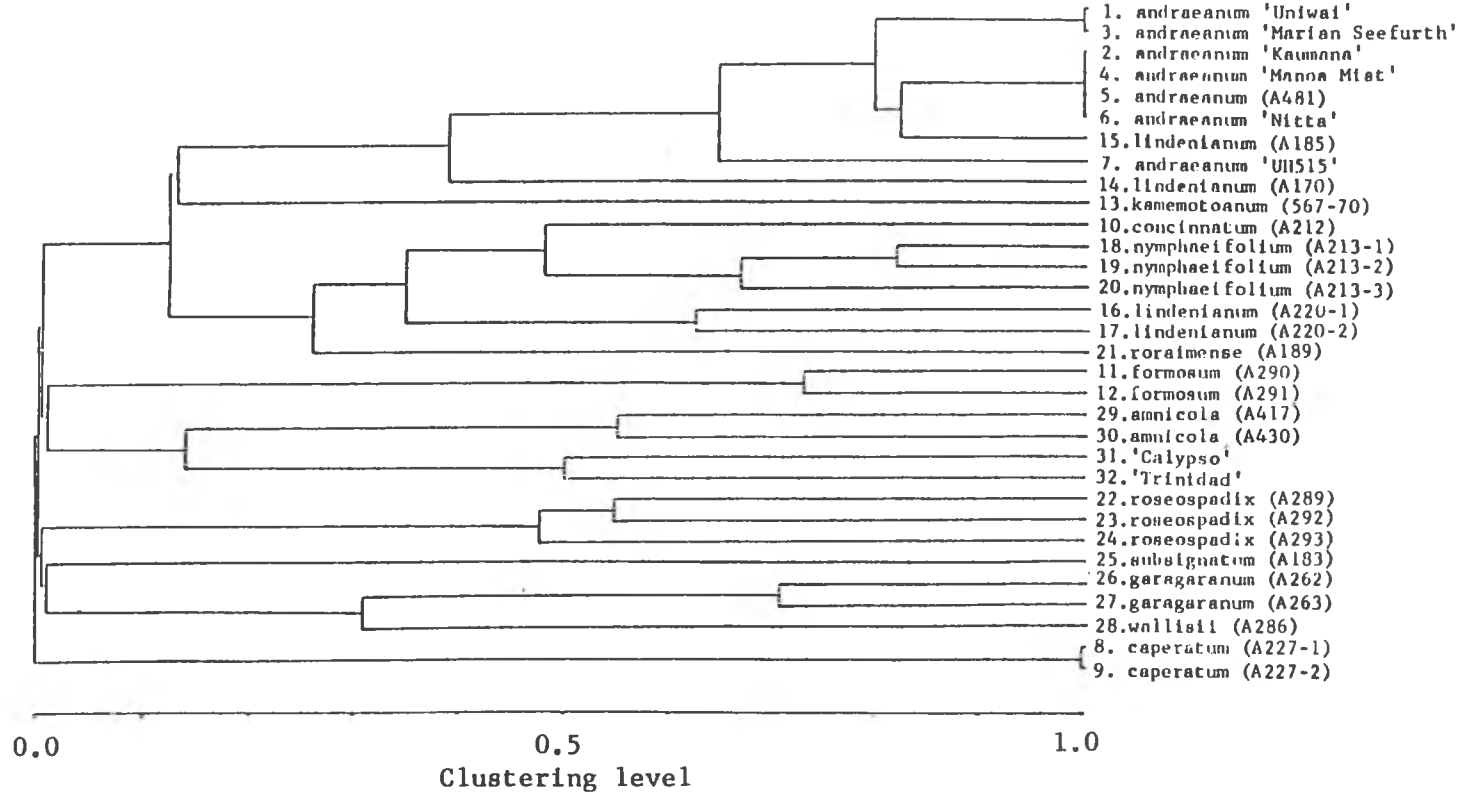

Figure 129. Phenogram of 32 Anthurium taxa based on flavonoid data: S_j (Jaccard coefficient), UPGMA cluster analysis.

similarity in morphological characters and flavonoids, respectively. Figure 128 indicates that morphological data tends to cluster OTU's (taxa) that belong to the same species and also shows relatively strong similarity (at 0.47 level) among all taxa examined. Four major groupings based on morphological characters at about 0.7 clustering level are : (1) andraeanum group which includes andraeanum, concinnatum, roraimense, lindenianum, nymphaeifolium, kamemotoanum, 'Calypso' and 'Trinidad'; (2) roseospadix group which includes roseospadix (except A289) and wallisii; (3) caperatum group which contains caperatum, garagaranum(except A263), subsignatum, and formosum; and (4) amnicola. Two OTU's, A289 and A263, show differences from the others because they have 18 missing values for inflorescence characters which seem to have influenced this cluster analysis.

A phenogram of 32 Anthurium taxa based on flavonoid data is shown in Figure 129. In general the clustering level based on flavonoid data was lower than that of morphological data and yet a higher level of clustering was seen among some taxa on the basis of flavonoid profile. This indicates that flavonoid analysis is useful to divide morphologically similar taxa into subgroups.

The highest level of clustering (at 1.0) was seen between A. andraeanum 'Uniwai' and 'Marian Seefurth',

and among A. andraeanum 'Kaumana', 'Manoa Mist', A481 and 'Nitta'. Two taxa of A. caperatum (A227-1 and A227-2) were also grouped by themselves at 1.0 level. When there were more than one representative of a species, the clustering among them occurred at level of 0.5 or above.

Similarity of flavonoid constituents between A. lindenianum (A170 and A185) and A. andraeanum was again demonstrated in the phenogram. In earlier sections the HPLC flavonoid profile and identification of compounds indicated that A170 and A185 were more similar to A. andraeanum than A. lindenianum A220-1 and A220-2. In the phenogram taxa of A. andraeanum , A170 and A185 clustered at 0.4 level. A220-1 and A220-2 showed their close relationship with A. nymphaeifolium (A213-1, -2 and -3) and A. concinnatum. 'Calypso' and 'Trinidad' were clustered at 0.5 level. They showed their similarity on both morphological and flavonoid characters.

The phenogram of 32 Anthurium taxa based on flavonoid data clearly indicates that the flavonoid patterns are species-specific except for A. lindenianum. This suggests that flavonoid profile may be of considerable value in species identification in the genus Anthurium.

4.2.3.2 Cladistic analyses.

Figures 130 and 131 represent the shortest Wagner networks calculated from two forms of flavonoid data.

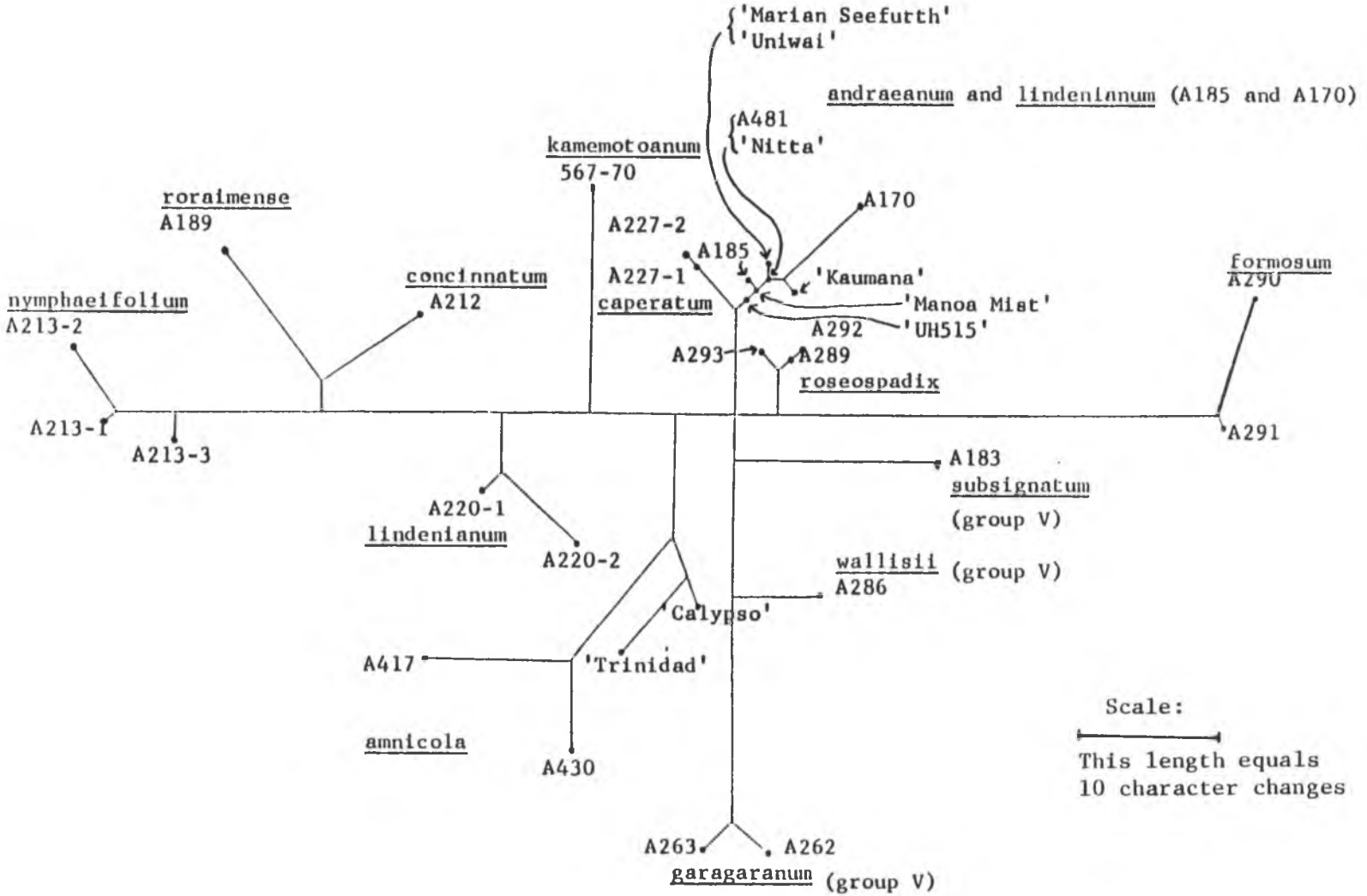

Fig. 130. Wagner network for 32 Anthurium taxa based on flavonoid data: coded data with 98 characters; Wagner 78 computer program. The length of branch is proportional to the number of character changes (see scale).

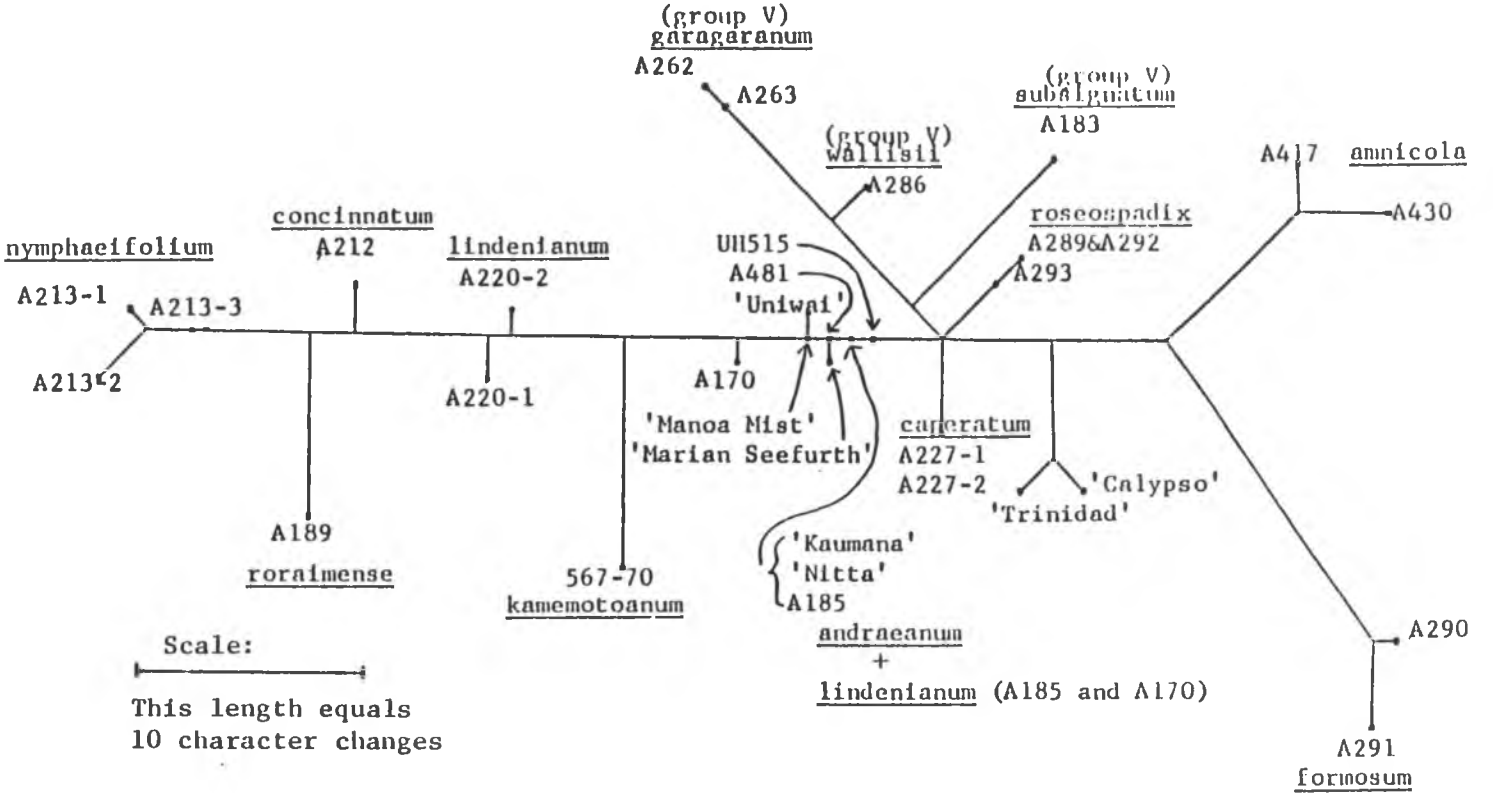

Fig. 131. Wagner network for 32 Anthurium taxa based on flavonoid data:
Presence vs absence (100 characters); PAUP computer program.
The length of branch is proportional to the number of character changes (see scale).

The network in Fig.130 is constructed by using coded data. The original continuous data were classified into five groups (<10, <100, <1000, <10000 and >10000) according to the concentration of each compound. The second network in Fig. 131 is formed on the basis of presence vs absence of flavonoid compounds. The two networks were very similar except position of A. amnicola (A417 and A430), 'Calypso' and 'Trinidad'. In Wagner network based on coded data those plants were placed on the branch between A. formosum (A290 and A291) and A. nymphaeifolium (A213-1, -2, and -3). In contrast on the basis of presence and absence data, A. amnicola, 'Calypso' and 'Trinidad' were closer to A. formosum.

Anthurium andraeanum cultivars consistently show their close relationships on two networks. In addition, there are close relationships among A. nymphaeifolium (A213), A. roraimense (A189) and A. concinnatum (A212) and among species in Sheffer and Kamemoto's group V (A. garagaranum, A. wallisii. and A. subsignatum). Anthurium formosum and A. nymphaeifolium appear as two distantly related taxa.

Figure 132 represents the Wagner network based on morphological data. Three species in Sheffer and Kamemoto's group V again show their closeness. The fact that A. caperatum and A. formosum are placed near group V rather than close to taxa in group VI may indicate that

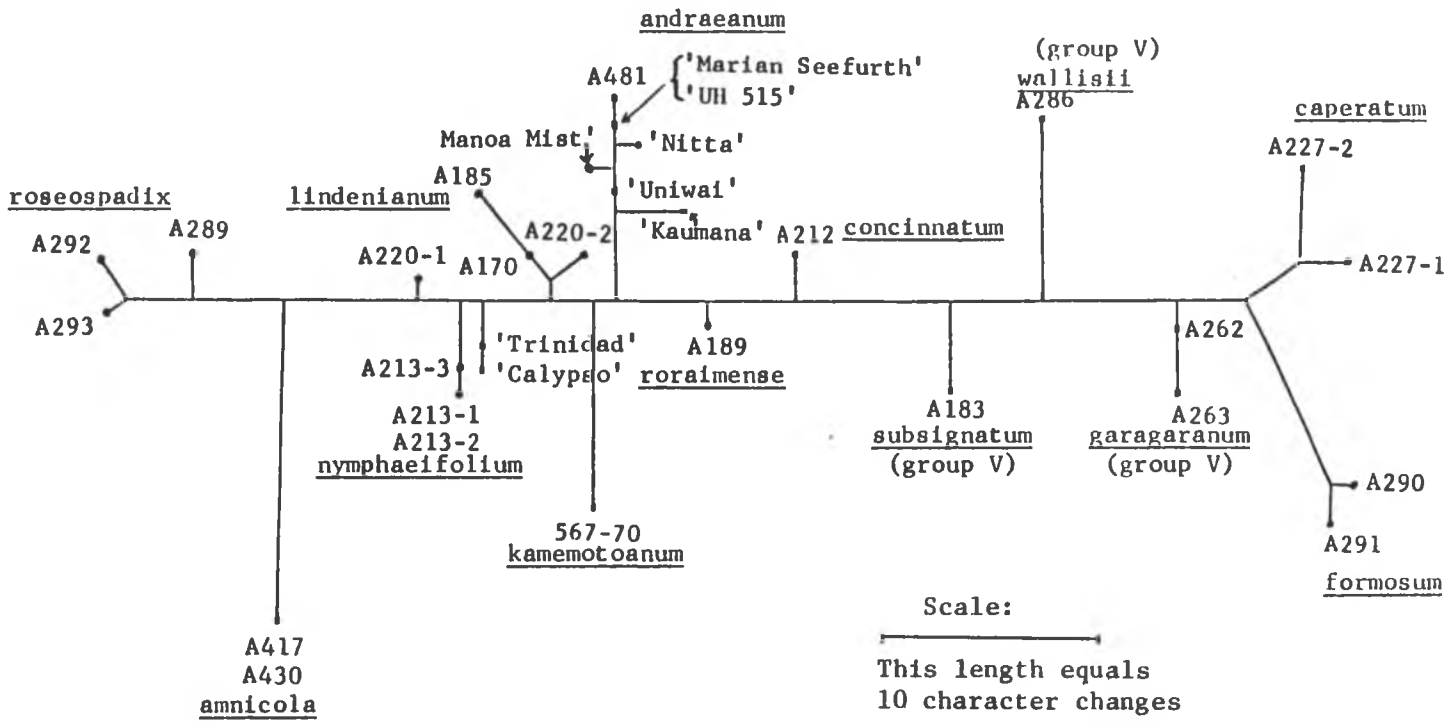

Fig. 132. Wagner network for 32 Anthurium taxa based on Morphological characters: coded data (62 characters); PAUP computer program. The length of branch is proportional to the number of character changes (see scale).

both are species intermediate between the two groups.

4.2.3.3 Discussion

Phenetic analyses resolve similarity or dissimilarity between taxa. It is clearly demonstrated that in most cases flavonoids occur in species specific ensembles, and may be useful for species identification.

Comparison of two phenograms based on morphological characters and flavonoid analysis demonstrates that the type of character used in cluster analysis may change the result of groupings. Although morphological characters tend to group taxa with a high degree of similarity together, flavonoid data separate each species. Biochemically two plants of A. lindenianum (A170 and A185) show a high degree of similarity to A. andraeanum.

Examinations of two Wagner networks give a clue of phylogenetic relationships among taxa although additional information such as identification of flavonoid compounds and cytological studies are necessary to provide knowledge of evolutionary trends. Preliminary flavonoid study has shown that the major compounds in A. formosum are flavonols, while other taxa do not contain flavonols but flavones. It is generally known that the presence of flavonols and the absence of flavones is a primitive state of leaf flavonoid character (Harborne, 1977). Therefore, it can be assumed that A. formosum is a

primitive species among the group of plants examined in this study. Numerical analyses confirm that A. amnicola is unique for its morphological feature. On the basis of flavonoid data the species shows close relationship with 'Calypso' and 'Trinidad' (Fig. 130).

Numerical methods show their usefulness to clarify phenetic relationships among A. andraeanum and closely related species. Basically, the phenograms agreed with Sheffer and Kamemoto's grouping except some species were misplaced. Those taxa were considered species intermediate between groups.

4.3 Anthocyanins in selected *Anthurium* species, cultivars and hybrids

4.3.1 Identification of major anthocyanins in anthuriums

A total of 10 different anthocyanins were detected in tissue from spathes, spadices and young leaves of anthuriums by using HPLC. *Anthurium amnicola* contained two major pigments, one of which (magenta pigment) appeared in higher concentration than the other (pink pigment).

Figure 133 shows HPLC chromatograms of *A. amnicola*. Both spathes and spadices exhibited similar chromatograms. The major (A6) and minor (A10) pigments were compared with authentic anthocyanins isolated from *A. andraeanum* 'Kaumana' and *Prunus avium* 'Bing' in terms of color, retention time in HPLC, Rf value in TLC and spectral measurements (Table 22; Figures 134 - 135). Data clearly showed that the major anthocyanin (A6) is cyanidin 3-rutinoside. The pink pigment (A10) corresponds to peonidin 3-rutinoside from *Prunus avium*.

Figure 136 (1) and (2) show HPLC chromatograms of a hybrid, 531-14 ('Calypso' x *A. amnicola*) and *A. andraeanum* 'Nitta'. The hybrid contains anthocyanins A1, A2, A3, A4, A5, A6, A9 and A10 with A6 being a major pigment, whereas orange spathe of 'Nitta' includes A2, A6, A7, A8 and A9. The concentration of A9 is extremely high compared to other pigments.

Table 22. Color, retention time (HPLC), Rf value (TLC) and spectral measurements of anthocyanins from Anthurium amnicola and authentic pigments.

Anthocyanin	Color		Retention time in min (HPLC) (system 1)	Rf value (x 100)		Spectral measurement	
	Visual	UV		CH_3CN	BAW	Absorption max. (nm)	$+AlCl_3$ (nm)
Two pigments from A. amnicola:							
Pigment 1 (A6)	Magenta	Dull	20.0	50	52	532	570
Pigment 2 (A10)	Pink	Faint	38.8	56	55	526	526
Authentic pigments from:							
A. andraeanum 'Kaumana'							
Cyanidin 3-rutinoside	Magenta	Dull	20.0	50	52	532,280 (Iwata,1980)	
Pelargonidin 3-rutinoside	Orange	Dull	32.4	59	57	512,270 (Iwata,1980)	
Prunus avium 'Bing'							
Cyanidin 3-glucoside	Magenta		17.0			532	572
Cyanidin 3-rutinoside	Magenta		20.0				
Peonidin 3-rutinoside	Pink		38.8			526	526

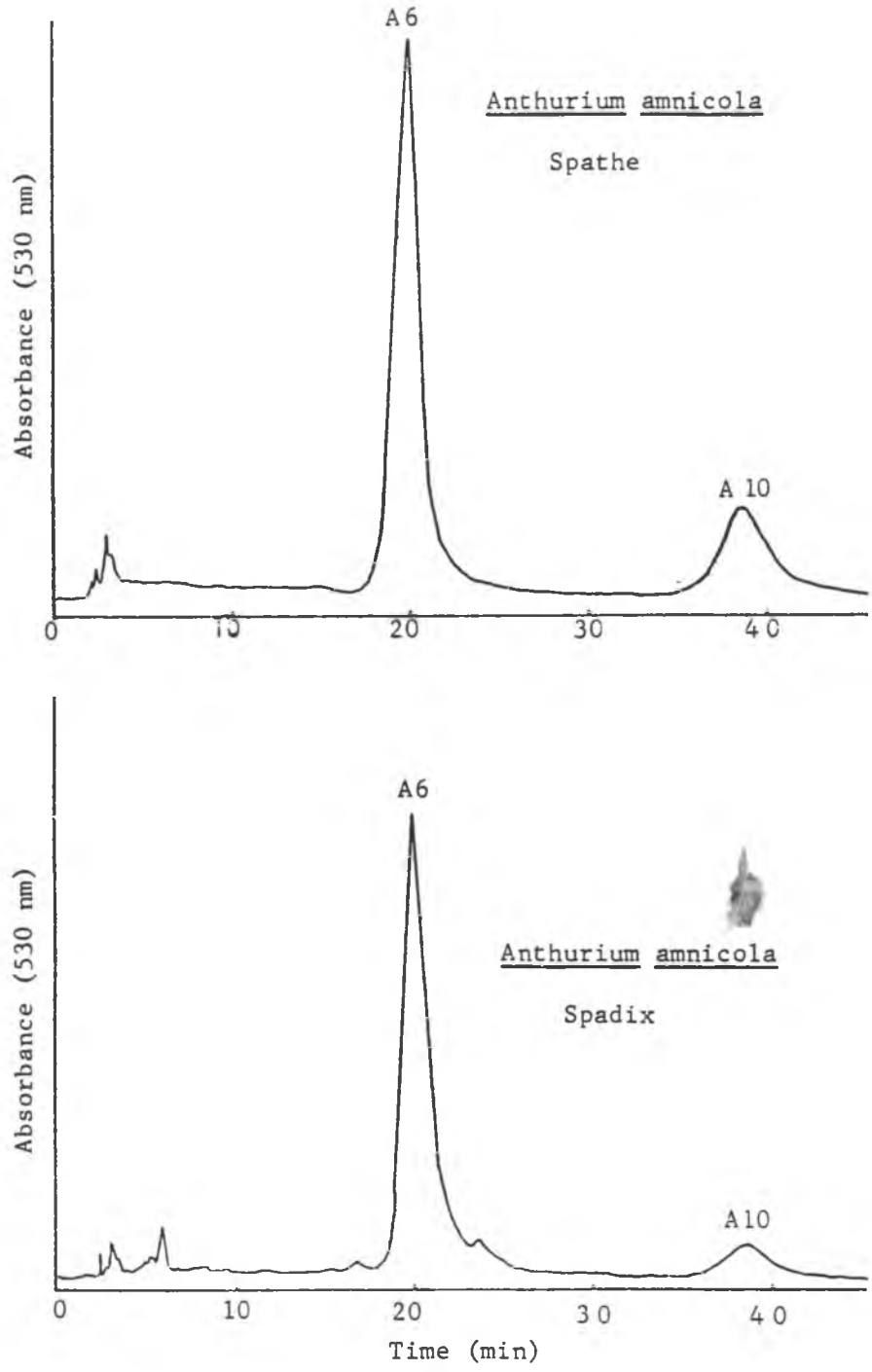

Figure 133. HPLC resolution of anthocyanins in Anthurium amnicola.

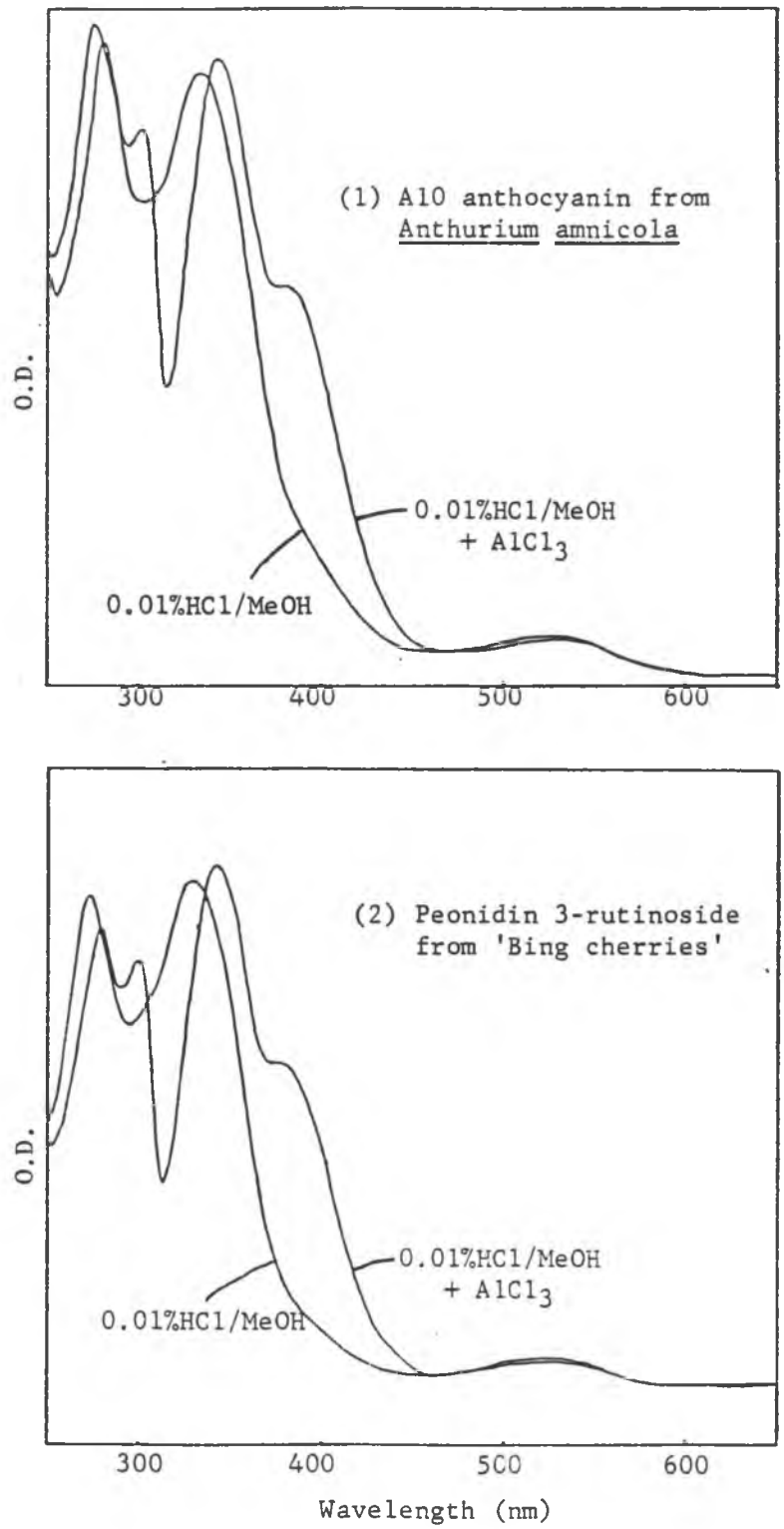

Figure 134. Spectra of (1) A10 anthocyanin from Anthurium amnicola and (2) peonidin 3-rutinoside from 'Bing cherries'.

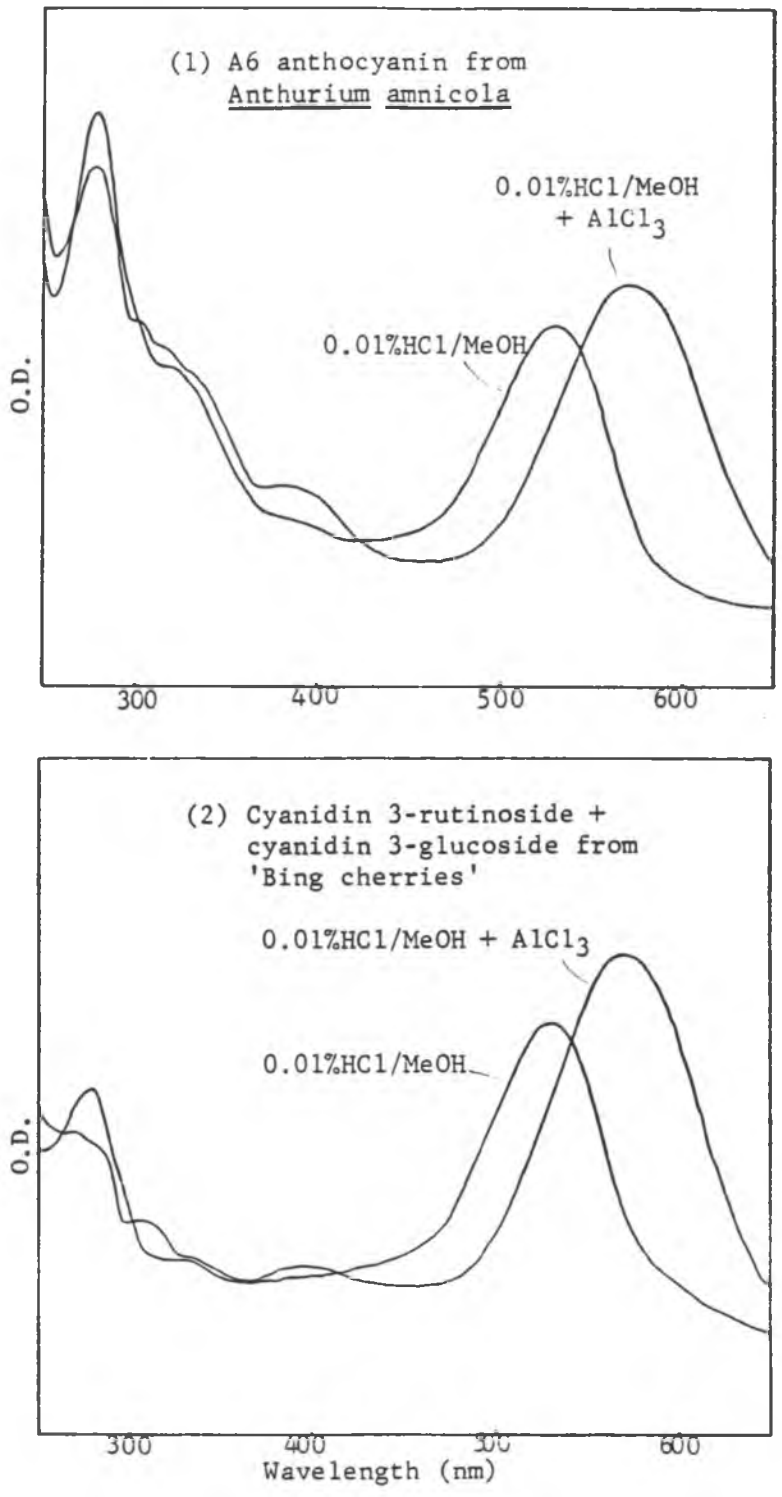

Figure 135. Spectra of (1) A6 anthocyanin from Anthurium amnicola and (2) a mixture of cyanidin 3-rutinoside and cyanidin 3-glucoside from 'Bing cherries'.

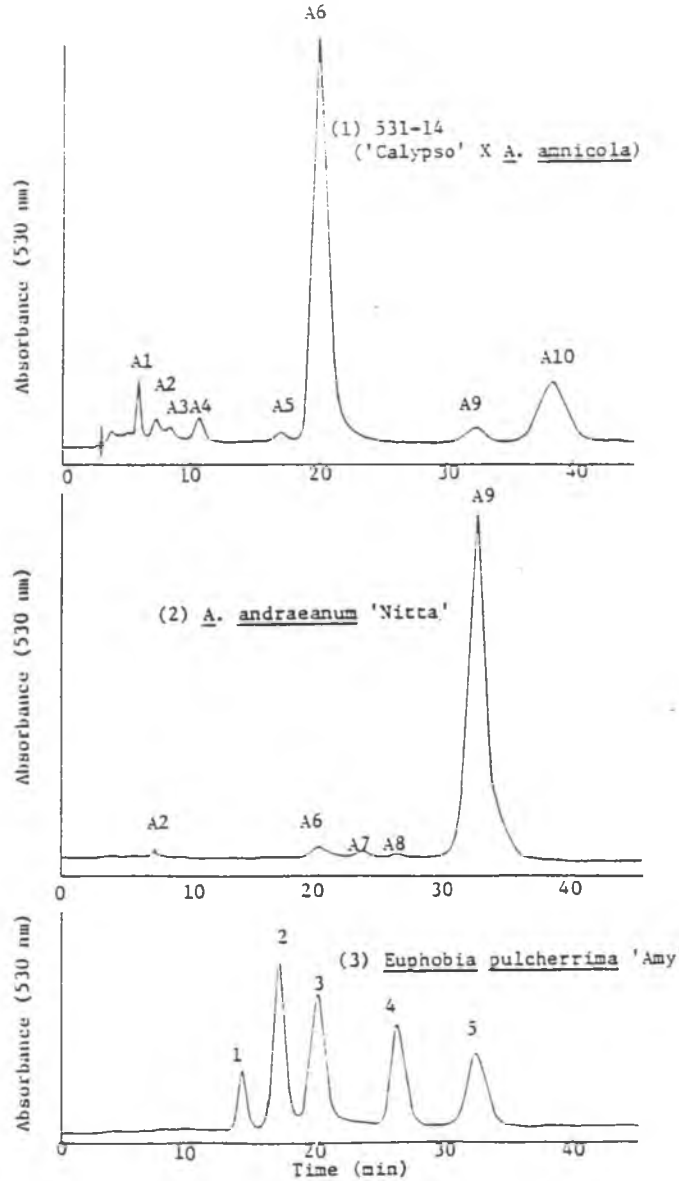

Figure 136. HPLC resolution of anthocyanins in (1) spathe of 531-14 ('Calypso' x Anthurium amnicola); (2) spathe of Anthurium andraeanum 'Nitta'; and bracts of Euphorbia pulcherrima 'Amy': 1=cyanidin 3-galactoside, 2=cyanidin 3-glucoside, 3=cyanidin 3-rutinoside, 4=pelargonidin 3-glucoside, and 5=pelargonidin 3-rutinoside.

In addition to 'Kaumana' and 'Bing' cherries, bracts of Euphorbia pulcherrima 'Amy' were examined to identify pigments in anthuriums. Figure 136 (3) presents five anthocyanins found in poinsettia (Asen, 1979). Comparison on retention time (HPLC) between pigments in anthuriums (A1 - A10) and anthocyanins in poinsettia is shown in Table 23. By matching retention time of authentic samples in HPLC chromatograms, A5 and A6 were identified as cyanidin 3-glucoside and cyanidin 3-rutinoside, respectively. A8 and A9 were identified as pelargonidin 3-glucoside and pelargonidin 3-rutinoside, respectively. A10 was identified earlier as peonidin 3-rutinoside (Table 22). The minor pigments A1, A2, A3, A4, A7 remain unknown.

A correlation analysis was done in order to measure the degree of association among ten different anthocyanins occurring in anthuriums. Simple correlation coefficients (r) in Table 24 indicate that there are very high correlations between A1 and A2 (0.92), A1 and A5 (0.76), A1 and A6 (0.90), A2 and A5 (0.71). A2 and A6 (0.81), A3 and A4 (0.89), A3 and A10 (0.64), A4 and A10 (0.68), A5 and A6 (0.69), A5 and A7 (0.74), and A8 and A9 (0.99).

The high degree of association among A1, A2, A5 (cyanidin 3-glucoside) and A6 (cyanidin 3-rutinoside) may suggest that A1 and A2 are possibly other cyanins with

Table 23. Comparison on retention time (HPLC system 1) between anthocyanins (A1 - A10) in anthuriums and pigments from bracts of Euphorbia pulcherrima 'Amy'.

Anthocyanin	Retentin time (min)
Anthurium spp.:	
A1	6.0
A2	7.2
A3	8.4
A4	10.8
A5	17.0
A6	20.0
A7	23.4
A8	26.2
A9	32.4
A10	38.8
Euphorbia pulcherrima:	
Cyanidin 3-galactoside	14.2
Cyanidin 3-glucoside	17.0
Cyanidin 3-rutinoside	20.0
Pelargonidin 3-glucoside	26.2
Pelargonidin 3-rutinoside	32.4

Table 24. Simple correlation coefficients (r) among 10 different anthocyanins detected in anthuriums.

Anthocyanin	Anthocyanin								
	A1	A2	A3	A4	A5	A6	A7	A8	A9
A2	0.92***								
A3	0.17	0.09							
A4	0.16	0.14	0.89***						
A5	0.76***	0.71***	-0.01	-0.04					
A6	0.90***	0.81***	-0.03	-0.06	0.69***				
A7	0.39*	0.35*	-0.08	-0.10	0.74***	0.49**			
A8	-0.07	-0.11	-0.05	-0.07	-0.06	-0.04	0.03		
A9	-0.04	-0.08	-0.04	-0.06	-0.04	-0.06	0.07	0.99***	
A10	0.41*	0.30	0.64***	0.68***	0.39*	0.30	0.27	-0.09	-0.07

*, **, *** Significant at 5% (*), 1% (**) and 0.1% (***).

different kinds or numbers of sugar attached to the basic structure of cyanidin. A8 (pelargonidin 3-glucoside) shows the highest degree of association (r=0.99) with A9 (pelargonidin 3-rutinoside). A3, A4 and A10 (peonidin 3-rutinoside) might belong to one group (peonin). A7 showed very high or fairly high correlation with A1, A2, A5, and A6, and could be another cyanin or might possess the similar structure to cyanin.

4.3.2 Anthocyanins in selected Anthurium species and cultivars

Distributions of anthocyanins were surveyed in five species and two horticultural cultivars, 'Calypso' and 'Trinidad'. Because the color of spathe in A. andraeanum 'Uniwai' changes as a flower ages, pigments were analyzed at three differnt stages of flower development.

Results of anthocyanin analysis are presented in Table 25. It was found that cyanidin 3-rutinoside is the common anthocyanin which occurs in all colored tissues except in orange and coral spathes which have pelargonidin 3-rutinoside as the major pigment.

Both 'Calypso' and A. kamemotoanum have cyanidin 3-glucoside as another important pigment and the occurrence of peonidin 3-rutinoside as a minor pigment is a characteristic of A. amnicola. White spathes of A. lindenianum and green spathes of A. roraimense do not

Table 25. Anthocyanins in some Anthurium species and cultivars.

Species and cultivar	Tissue	Color	Pigment[z]		
			Major	Minor	Trace
A. andraeanum					
'Kaumana'	Spathe	Dark red	Cy3R	Pel3R,Cy3R	A1,A2,A4,Peo3R
'Uniwai'	Spathe				
	Young	White	-	-	-
	Old	Pink	Cy3R		
	Older	Light red	Cy3R	-	-
'Nitta'	Spathe	Orange	Pel3R	Cy3R	A2,A7,Pel3G
'UH515'	Spathe	Coral	Pel3R	-	Cy3R,A7,Pel3G
A. kamemotoanum					
A288 selfed	Spathe	Dark red	Cy3R,Cy3G	-	-
	Spadix	Dark red-purple	Cy3R	-	-
A. lindenianum					
A170	Spathe	White	-	-	-
A. roraimense					
A189	Spathe	Green	-	-	-
A. amnicola					
A417	Spathe	Lavender	Cy3R	Peo3R	-
	Spadix	Purple	Cy3R	Peo3R	A1,A5,A7
'Calypso'	Spathe	Light red	Cy3R	Cy3G,Pel3R	A1,A2,Peo3R
	Spadix	Purple	Cy3R	Cy3G,Pel3R,A1,A2	Peo3R
'Trinidad'	Spathe	Light pink	Cy3R	Pel3R	Cy3G,A1,A2
	Spadix	Purple	Cy3R	Pel3R,A2	A1,A4,Peo3R

[z]Cy3R = cyanidin 3-rutinoside; Cy3G = cyanidin 3-glucoside; Pel3R = pelargonidin 3-rutinoside; Pel3G = pelargonidin 3-glucoside; Peo3R = peonidin 3-rutinoside; A1, A2 and A7 = cyanidin glycoside?; A3 and A4 = peonidin glycoside?

contain any pigments.

The common presence of cyanidin 3-rutinoside, cyanidin 3-glucoside and pelargonidin 3-rutinoside was noted in the survey of anthocyanin pigments in the family Araceae by Williams *et al*. (1981). In addition Chang and Collins (1970) identified peonidin 3-rutinoside in Symplocarpus foetidus which is a member of the family Araceae.

Generally, spathes and spadices of the same plant show similar pattern of anthocyanin distribution if tissues are pigmented. For instance, in *A. amnicola* there are two important anthocyanins in both tissues: cyanidin 3-rutinoside (major) and peonidin 3-rutinoside (minor). Nevertheless, difference in visual color between two tissues was evident. It might be due to co-pigmentation effects and/or difference in pH which modified the color of the anthocyanin-copigment complex.

It is not unusual to observe color changes in spathes of anthuriums during flower development. One phenomenon is simple color fading which may be a result of anthocyanin breakdown. Contrastingly in some plants like 'Uniwai' pigment concentration may increase with aging of spathe. The pink pigment in old spathes of 'Uniwai' was found to be cyanin 3-rutinoside, the common pigment in anthuriums. The amount of this pigment increases as a flower ages. Some physiological factors such as a

hormonal change by age and/or changes in environmental conditions like increases in light intensity and temperature may trigger such drastic change in spathe color of 'Uniwai'.

4.3.3 Anthocyanins in selected interspecific hybrids.

Results of anthocyanin analysis of some interspecific hybrids are presented in Tables 26 and 27. Cyanidin 3-rutinoside is, again, found to be the most common pigment occurring in highest concentrations. Pink tinged spathe and spadix of RS987-6 (A. andraeanum 'Uniwai' x A. concinnatum) is due to the presence of cyanidin 3-rutinoside in very small amounts.

Assuming that A1 is a cyanin, hybrids between A. amnicola and A. formosum have predominantly cyanins (cyanidin 3-rutinoside, cyanidin 3-glucoside and A1). The production of peonidin 3-rutinoside as the second important pigment is observed in crosses of A. amnicola with A. lindenianum (A170) and 'Calypso', although cyanidin 3-rutinoside is always the major pigment.

The presence of various anthocyanins from tissues of interspecific hybrids indicates that the genetic mechanism of anthocyanin production is complicated. Further investigations such as evaluation of backcrosses and sib-crosses are necessary in order to reveal genetics of anthocyanin production in anthurium hybrids.

Table 26. Anthocyanins in some interspecific hybrids in *Anthurium*.

Cross	Cross no.	Tissue	Color	Pigment[z] Major	Minor	Trace
A. andraeanum 'Uniwai'						
X *concinnatum* (A212)	RS987-6	Spathe	Yellow-white pink tinged	-	-	Cy3R
		Spadix	Yellow-white pink tinged	-	-	Cy3R
X *formosum* (A287)	RS1183-5	Spathe	Greyed pink-purple	Cy3R	-	Pel3R,A7,A1, A2,Cy3G
		Spadix	Greyed pink-purple	Cy3R	-	Peo3R,A2,A1, A7,Cy3G
X *formosum* (A290)	RS1205-7	Spathe	Pink	Cy3R	A1,A4,A2, Cy3G	A7
		Spadix	Light greyed purple	-	-	Cy3R
X *kamemotoanum* (A288)	RS532-5	Spathe	Light red	Cy3R	A1,Pel3R	Cy3G,Peo3R A2,A3
A. andraeanum 'UH515'						
X *roraimense* (A189)	534	Spathe	White	-	-	-
		Spathe	Light red	Cy3R	Pel3R	A1,Cy3G
		Spathe	Red	Cy3R	Pel3R,A7	A1,A2,Cy3G
A. andraeanum 'Nitta'						
X *roraimense* (A189)	434	Spathe	Red	Cy3R	Pel3R	-

[z] Cy3R=cyanidin 3-rutinoside; Cy3G=cyanidin 3-glucoside; Pel3R=pelargonidin 3-rutinoside; Peo3R=peonidin 3-rutinoside.

Table 26. (Continued)

Cross	Cross no.	Tissue	Color	Pigment[z] Major	Minor	Trace
A. kamemotoanum (A288) X formosum (A291)	RS1228-13	Spathe	Very dark red	Cy3R	A1,A2	Cy3G,Pea3R, Pel3R
		Spadix	Dark red-purple	Cy3R	Cy3G,A1,A2	A7,Pel3R, Pea3R
(A. andraeanum'Uniwai' X kamemotoanum (A288)) X formosum (A290)	392-1	Spathe	Very dark red	Cy3R	Pel3R,A1	A2,Cy3G,Pea3R
		Spadix	Dark red-purple	Cy3R	A1,A2,Cy3G	Pel3R
	392-42	Spathe	Dark red	Cy3R	A1,A2,Pel3R	Cy3G,Pea3R
	392-66	Spathe	Very dark red	Cy3R	A1,A2,Cy3G, Pea3R	Pea3R
		Spadix	Dark red-purple	Cy3R	A1,A2,Cy3G, Pea3R	Pel3R
A. nymphaeifolium (A213-3) X formosum (A287)	RS251-1	Spathe	White	-	-	-
			(pink tinged)	(Cy3R,A1)-		(A2,A4,Pel3R)
		Spadix	White	-	-	-
		Young leaf		Cy3R	-	A1,A4,Cy3G, Pel3R
A. subsignatum (A183) X nymphaeifolium (A213-1)	RS1306-2	Spathe	Yellow-green	-	-	-
			(pink tinged)	-	-	(Cy3R,Cy3G)
		Spadix	Dark red-purple	Cy3R	Pea3R,Pel3R	A7,A1,Cy3G

[z] Cy3R=cyanidin 3-rutinoside; Cy3G=cyanisin 3-glucoside; Pel3R=pelagonidin 3-rutinoside; Pea3R=peonodon 3-rutinoside.

Table 27. Anthocyanins in Anthurium amnicola hybrids.

Cross	Cross no.	Tissue	Color		Pigment[z]		
			Visual	RHS[y]	Major	Minor	Trace
A.amnicola (A417)							
X formosum (A287)	568-2	Spathe	Red-purple	70B	Cy3R	Cy3G,A1	Peo3R,Pel3R,A2
	568-25	Spathe	Red-purple	70B	Cy3R	A1,Cy3G	Peo3R,Pel3R,A2
	568-40	Spathe	Red-purple	70B	Cy3R	A1,Cy3G	Peo3R,Pel3R,A2
X formosum (A291)	572-7	Spathe	Red-purple	70B	Cy3R	A1,Cy3G	Peo3R,A2
	572-24	Spathe	Red-purple	70B	Cy3R	A1	Peo3R,A2,Pel3R
A. lindenianum (A170)							
X amnicola (A417)	552-88	Spathe	Pink	58B	Cy3R	Peo3R,A1	A2,A3,A4,Cy3G, Pel3R
		Spadix	Purple	79D	Cy3R	Peo3R,A1	A2,A3,A4,Cy3G, Pel3R
	552-38	Spathe	Pink	58D	Cy3R	Peo3R,A1	A2,A4,Cy3G, Pel3R
'Calypso'							
X amnicola (A417)	531-14	Spathe	Light red	53D	Cy3R	Peo3R,A1	A2,A3,A4,Cy3G, Pel3R
		Spadix					
		Upper	Red	53C	Cy3R	Peo3R,A1, A4	A2,A3,Cy3G, Pel3R
		Lower	Purple	78C	Cy3R	Peo3R,A1 A2,A4	A3,Cy3G,Pel3R

[z] Cy3R=cyanidin 3-rutinoside; Cy3G=cyanidin 3-glucoside; Peo3R=peonidin 3-rutinoside; Pel3R=pelargonidin 3-rutinoside.

[y] RHS=Royal Horticultural Society Color Chart.

4.4 Anthocyanins and other pigment controlling factors in various hybrids of *Anthurium andraeanum* 'Uniwai', *A. kamemotoanum*, *A. formosum*, *A. amnicola* and *A. lindenianum*

4.4.1 Anthocyanins

Data on Royal Horticultural Society Color Chart (RHSCC), colorimeter readings, pH and relative concentrations of anthocyanins of the spathes from 'Calypso', 'Trinidad', *A. amnicola* (A417) and various hybrids were used to examine factors responsible for color variation in anthuriums (Appendix I and II). The visual color of spathes in the plants studied ranged from pink, red and red-purple to lavender. Matching each spathe color with RHSCC showed a color range from 46B to 84B. According to RHSCC, 46B to 53D belong to the red group, 58D to 70c to the red-purple group, 77B to 78D to the purple group, 80B and 80C to the purple-violet group and 84B to the violet group (Appendix III). Letters A,B,C, and D indicate intensity of color (measure of luminance). The letter A designates the darkest color while the letter D indicates the lightest.

The concentration of anthocyanins was found to be an important factor for color variation in anthuriums. All anthocyanins except pelargonidin 3-rutinoside were pink or magenta in color when they were isolated in 0.01% HCl-MeOH solution. Pelargonidin 3-rutinoside was an orange pigment. In all colors, cyanidin 3-rutinoside

occurred in the highest concentration. The percentage of this pigment in spathes ranged from 70.6% to 97.0% (Appendix II) and influence of other pigments did not seem as important as cyanidin 3-rutinoside. The lavender spathe of Anthurium amnicola contained 74.3% cyanidin 3-rutinoside and 22.6% peonidin 3-rutinoside.

Table 28 shows simple correlation coefficients between color measurements and various anthocyanins. Significant negative correlations between luminance and anthocyanins (A1, A2, and cyanidin 3-rutinoside) and total anthocyanin content indicate that the greater the amount of anthocyanins present in spathe, the darker the color. Figures 137 and 138 illustrate the relationship between luminance and concentrations of cyanin 3-rutinoside and total anthocyanin content. As the amount of anthocyanin increases, the value of luminance decreases; that is, the color becomes darker. Cyanidin 3-rutinoside is also found to affect the quality of spathe color. Table 28 shows significant positive correlation between cyanidin 3-rutinoside and color measurements, a, b and $\tan^{-1} a/b$. The relationship between those variables are also illustrated in Figures 139, 140 and 141. As the concentration of cyanidin 3-rutinoside increases, redness (a) also increases. On the other hand, with smaller amount of pigment, the value of b becomes smaller: i.e. color is bluer. The value

Table 28. Simple correlation coefficients (r) between color measurements and pH and various anthocyanins in spathe.

pH and anthocyanin	Color measurement			
	L (luminance)	a (green-red)	b (blue-yellow)	$\tan^{-1}(a/b)$
pH	.325	-.426*	-.537**	-.437*
Anthocyanin A1	-.567**	.406*	.204	.218
Anthocyanin A2	-.580**	.380*	.326	.332
Anthocyanin A4	.011	-.094	-.160	-.069
Cyanidin 3-glucoside	-.237	.289	.376*	.193
Cyanidin 3-rutinoside	-.685**	.649**	.606**	.550**
Pelargonidin 3-rutinoside	-.270	.305	.383*	.284
Peonidin 3-rutinoside	-.302	.110	.074	.112
Total anthocyanin content	-.695**	.640**	.595**	.539**

*, ** Significant at the 5% (*) and 1%(**) levels.

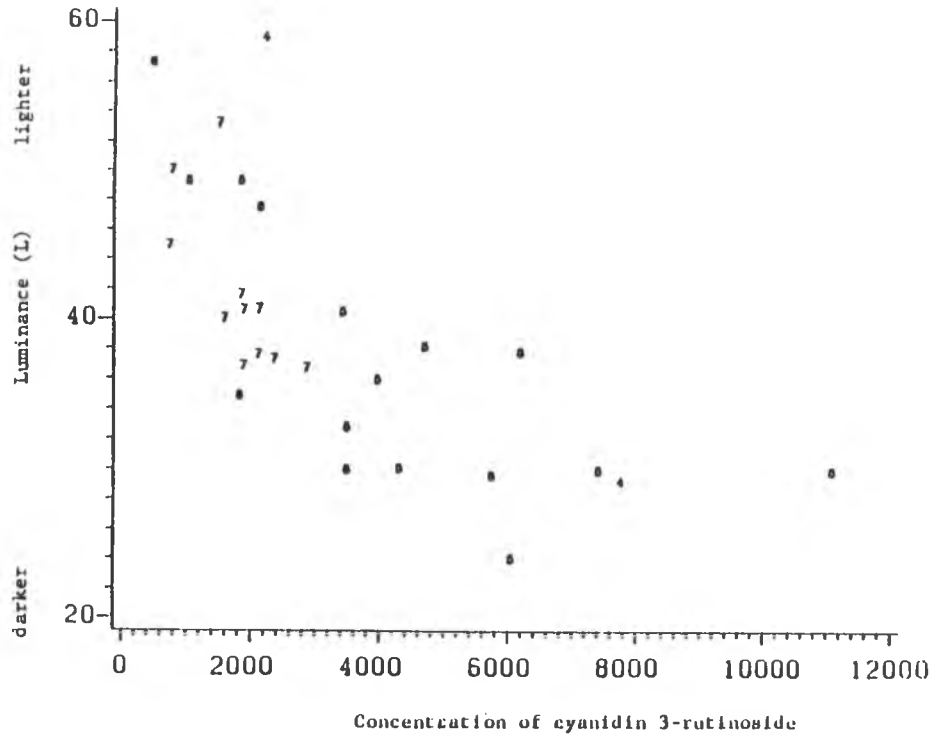

Figure 137. The relationship between luminance (L) and concentration of cyanidin 3-rutinoside of spathe. Concentration of cyanidin 3-rutinoside is expressed by (area of peak on HPLC chromatogram X volume of solvent)/ fresh weight of tissue. Numbers show RHS color codes:4=46B and 49B; 5=51A-59C; 6=63D; 7=70B-78D; and 8=80B-84B.

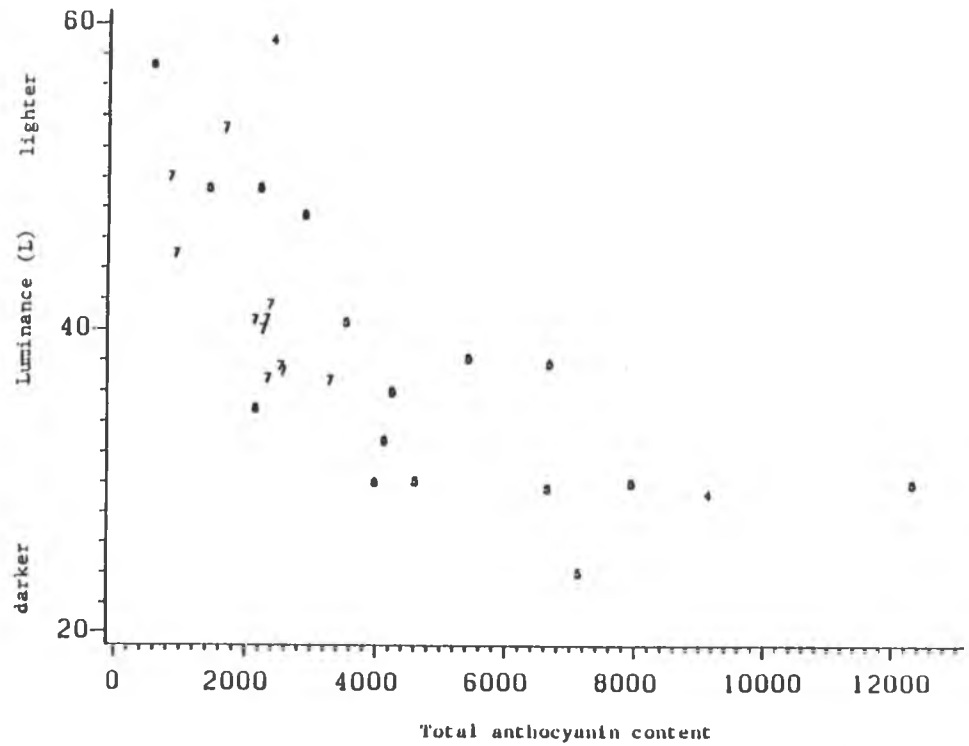

Figure 138. The relationship between luminance (L) and total anthocyanin content of spathes. Total anthocyanin content is expressed by (the sum of areas of peaks on HPLC chromatogram X volume of solvent)/fresh weight of tissue. Numbers show RHS color codes: 4=46B and 49B; 5=51A-59C; 6=63D; 7=70B-78D; and 8=80B-84B.

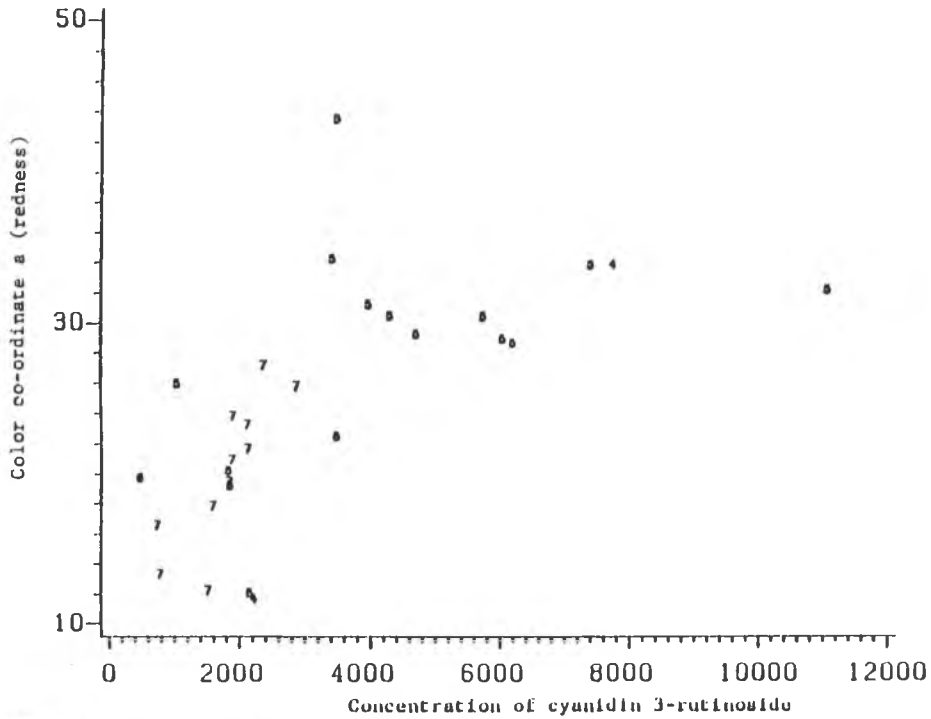

Figure 139. The relationship between color co-ordinate a (green-red) and concentration of cyanidin 3-rutinoside of spathe. Concentration of cyanidin 3-rutinoside is expressed by (area of peak on HPLC chromatogram X volume of solvent)/ fresh weight of tissue. Numbers show RHS color codes: 4=46B and 49B; 5=51A-59C; 6=63D; 7=70B-78D; and 8=80B-84B.

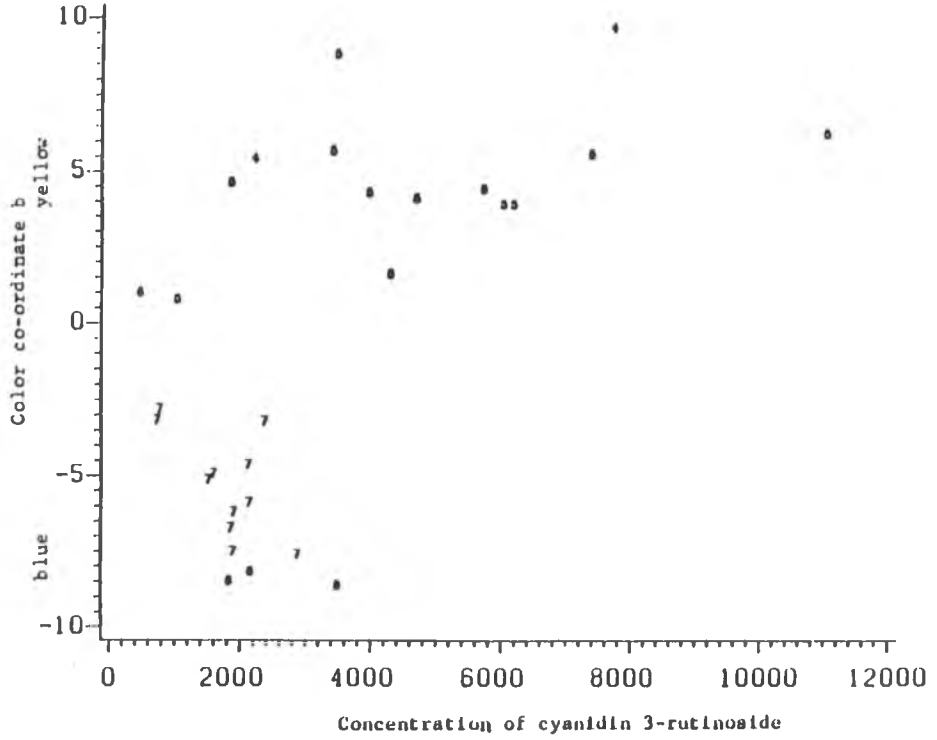

Figure 140. The relationship between color co-ordinate b (blue-yellow) and concentration of cyanidin 3-rutinoside. Concentration of cyanidin 3-rutinoside is expressed by (area of peak on HPLC chromatogram X volume of solvent)/ fresh weight of tissue. Numbers show RHS color codes: 4=46B and 49B; 5=51A-59C; 6=61D;7=70B-78D; and 8=80B-84B.

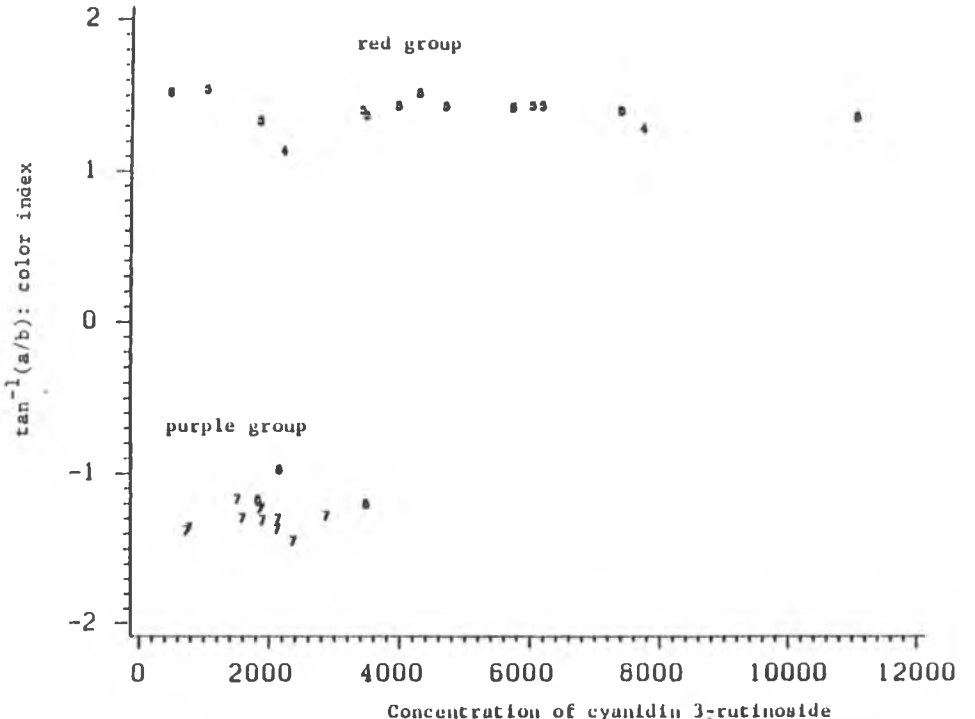

Figure 141. The relationship between color index $\tan^{-1}(a/b)$ and concentration of cyanidin 3-rutinoside of spathe. Concentration is expressed by (area of peak on HPLC chromatogram X volume of solvent)/ fresh weight of tissue. Numbers show RHS color codes: 4=46B and 49B; 5=51A-59C; 6=63D; 7=70B-78D; and 8=80B-84B.

$\tan^{-1}$ a/b combines two color parameters a and b and produces one color index. It is found that by using this parameter various spathe shades are divided into two color groups, red and purple. The first group having positive number of $\tan^{-1}$ a/b is called the red group. Colors with the negative number of $\tan^{-1}$ a/b belong to the purple group. Figure 141 shows the relationship of this color index and the concentration of cyanidin 3-rutinoside. It is noticed that the purple group generally contained less amount of the pigment.

The results indicate that cyanidin 3-rutinoside is the major anthocyanin in all colors of spathes, and the concentration of this pigment influences not only color intensity but also kinds of shade. The higher the concentration of cyanidin 3-rutinoside, the darker the color of the spathe. The purple spathes contain relatively small amounts of anthocyanins. The fact that pink (red group) and lavender spathes (purple group) have similar amounts of anthocyanins suggests that additional factors are responsible for color variation of spathes. It should be noted that the presence of an orange pigment, pelargonidin 3-rutinoside, blends with a magenta pigment, cyanidin 3-rutinoside, producing redder and less purplish shades. However, in this study, the influence of pelargonin was not clearly defined.

Peonidin 3-rutinoside is a pink pigment and a

methylated form of cyanidin 3-rutinoside (a magenta pigment) at position 3′. Absence of pelargonidin 3-rutinoside and a larger proportion of peonidin 3-rutinoside (22.6%) in the spathe of A. amnicola could be one of the important factors in the production of lavender color.

4.4.2 pH

pH is found to be one of factors which may contribute color variation in anthuriums. Table 28 indicates a negative linear correlation between pH and color measurements suggesting that with high pH the color of spathe becomes bluer and less reddish. Figure 142 shows a moderate linear relationship between a color coordinate a and pH. When pH is higher the value of a becomes smaller and therefore redness is decreased. The relationship between pH and another color coordinate b is illustrated in Figure 143. The moderate linear relationship of two variables demonstrates that generally a bluer spathe has a higher pH. In Figure 144 two color groups, red and purple, show differences in pH. The range of pH of the red group is lower than that of the purple group.

Spectral measurements of two isolated pigments and the pigment in intact cells of Anthurium amnicola are compared in Table 29. Considerable difference in

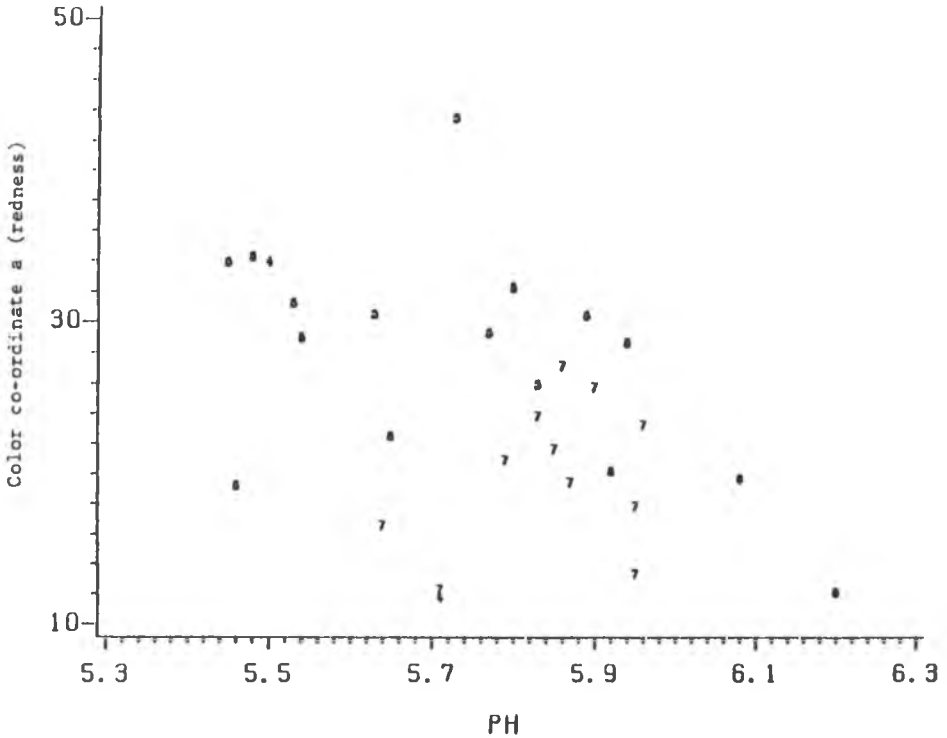

Figure 142. The relationship between color co-ordinate a (green-red) and pH of spathes. Number show RHS color codes: 4=46B and 49B; 5=51A-59C; 6=63D; 7=70B-78D; and 8=80B-84B.

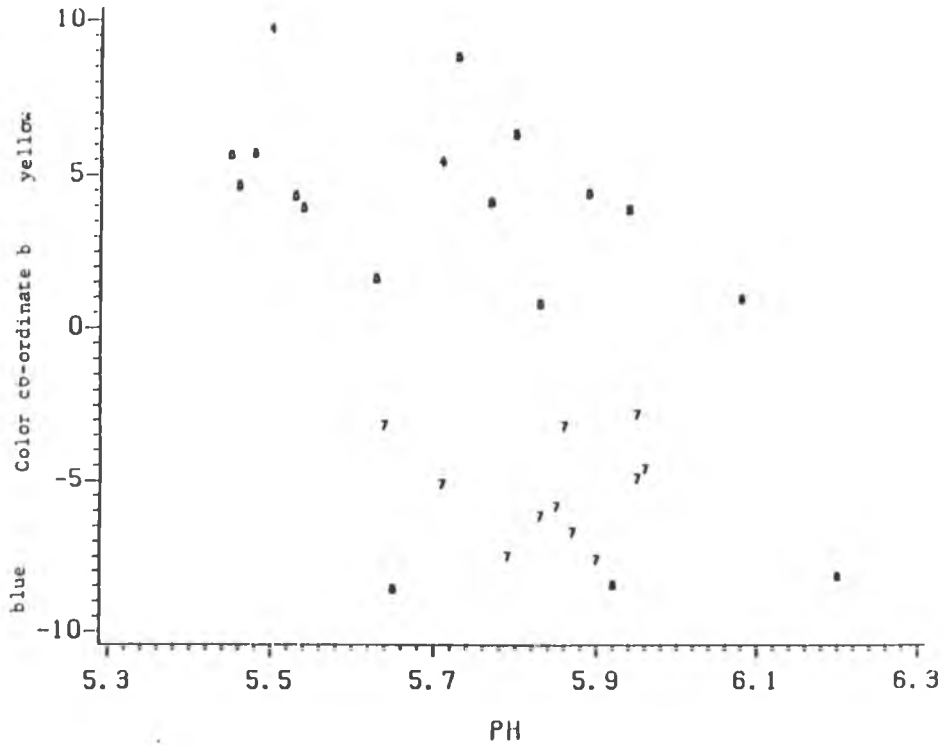

Figure 143. The relationship between color co-ordinate b (blue-yellow) and pH of spathe. Number show RHS color codes: 4=46B and 49B; 5=51A-59C; 6=63D; 7=70B-78D; 8=80B-84B.

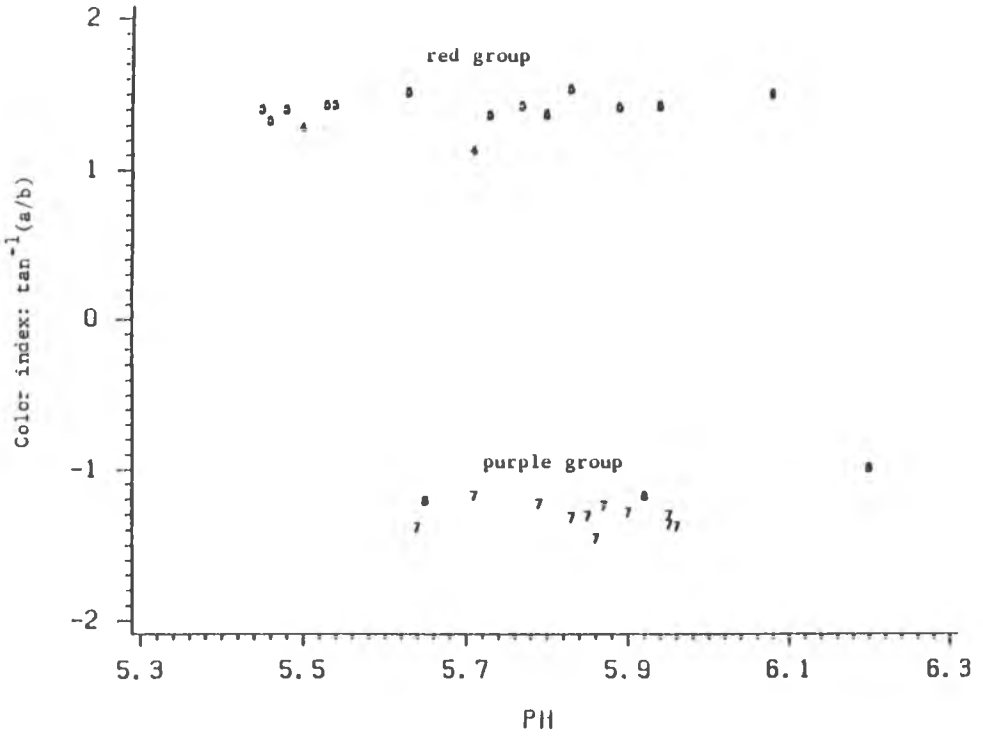

Figure 144. The relationship between color index $\tan^{-1}(a/b)$ and pH of spathe. Numbers show RHS color codes: 4=46B and 49B; 5=51A-59C; 6=63D; 7=70B-78D; 8=80B-84B.

Table 29. Spectral measurements of two isolated anthocyanins and pigments in intact cells of Anthurium amnicola.

Pigment	pH	Color	Absorption maximum (nm)
Isolated in 0.01% HCl-MeOH:			
Cyanidin 3-rutinoside		magenta	532
Peonidin 3-rutinoside		pink	526
Intact cells:			
Cyanidin 3-rutinoside + Peonidin 3-rutinoside	6.2	lavender	552

absorption maxima was obtained between intact cells (552 nm) and two pigments (532 and 526 nm). The two pigments were isolated in 0.01% HCl-MeOH solution which is very acidic, while the pH of intact cells was determined as 6.2. The results suggest that anthocyanins in anthuriums may become bluer with increased pH as found in the spathe of A. amnicola.

4.4.3 Co-pigmentation

According to Asen (1976), co-pigmentation is the most important phenomenon which contributes to a wide range of flower colors. Visual observation of spathe color in various hybrids of Anthurium amnicola and A. kamemotoanum is summarized in Table 30 and 31. All A. amnicola hybrids except A. amnicola x A. formosum have light pink or red spathes. Two crosses, 568 and 572 (A. amnicola x A. formosum), produced offspring with red-purple spathe (Table 30). A comparison of spathe color of A. kamemotoanum crosses again revealed that A. formosum hybrids exhibit darker and/or purplish spathes. Since only A. formosum contains a large amount of flavonol (Sec. 4.2) and flavonols are one of the most effective co-pigments in modifying color of anthocyanins in flowers (Asen et al., 1972), it can be assumed that co-pigmentation effects are operating in A. formosum hybrids.

Table 30. Spathe color of various Anthurium amnicola hybrids.

Cross	Cross no.	Spathe color
lindenianum x amnicola (white) (lavender)	552	light pink
amnicola x lindenianum	569	light pink
	575	light pink
'Calypso' x amnicola (light red)	531	red
'Trinidad' x amnicola (light pink)	561	light pink
amnicola x formosum	568	red-purple
(white with pink tinged)	572	red-purple

Table 31. Spathe color of various Anthurium kamemotoanum hybrids.

Cross	Cross and plant no.	Spathe color
andraeanum 'Uniwai' X kamemotoanum (white) (very dark red)	RS532 RS1249	light red light red
roseospadix X kamemotoanum (white, pink tinged)	RS1325	red
lindenianum X kamemotoanum (white)	RS1327	red
kamemotoanum X lindenianum	RS1293	red
kamemotoanum X formosum (white, pink tinged)	RS1228	very dark red (red-purple)
('Uniwai' X kamemotoanum) X formosum (light red)	392-9 392-42	light pink-purple very dark red (red-purple)

The major flavonol of A. formosum was partially identified as C-monoglycosylflavonol. It was easily isolated by using HPLC and paper chromatography. Spectrums of the flavonol, cyanidin 3-rutinoside, and a mixture of two compounds are compared in Figure 145. It is clearly shown that the absorption maximum of cyanidin 3-rutinoside is shifted by +15 nm in the presence of flavonol. A slight increase in absorbance is also noticed. This co-pigmentation effect of flavonol from A. formosum is illustrated in visual observation of three solutions (Figure 146). A mixture of cyanidin 3-rutinoside and flavonol appears much darker (at the center) than cyanidin rutinoside alone (left) in citric acid-Na HPO buffer solution (pH=5.66). The flavonol itself is colorless (right).

It should be noted that the lavender of A. amnicola is the closest color to blue in anthuriums, and yet this species does not contain flavonols which have co-pigmentation effects. In addition, although a flavonol from A. formosum gives a purplish shade to spathes, it does not produce a bluer color but produces red-purple as seen in hybrids of A. amnicola and A. formosum. It may be that flavonols are co-pigments which form reddish anthocyanin-copigment complexes (Asen, 1976). Asen et al. (1972) have shown that 6-C-glycosylflavone produces much bluer

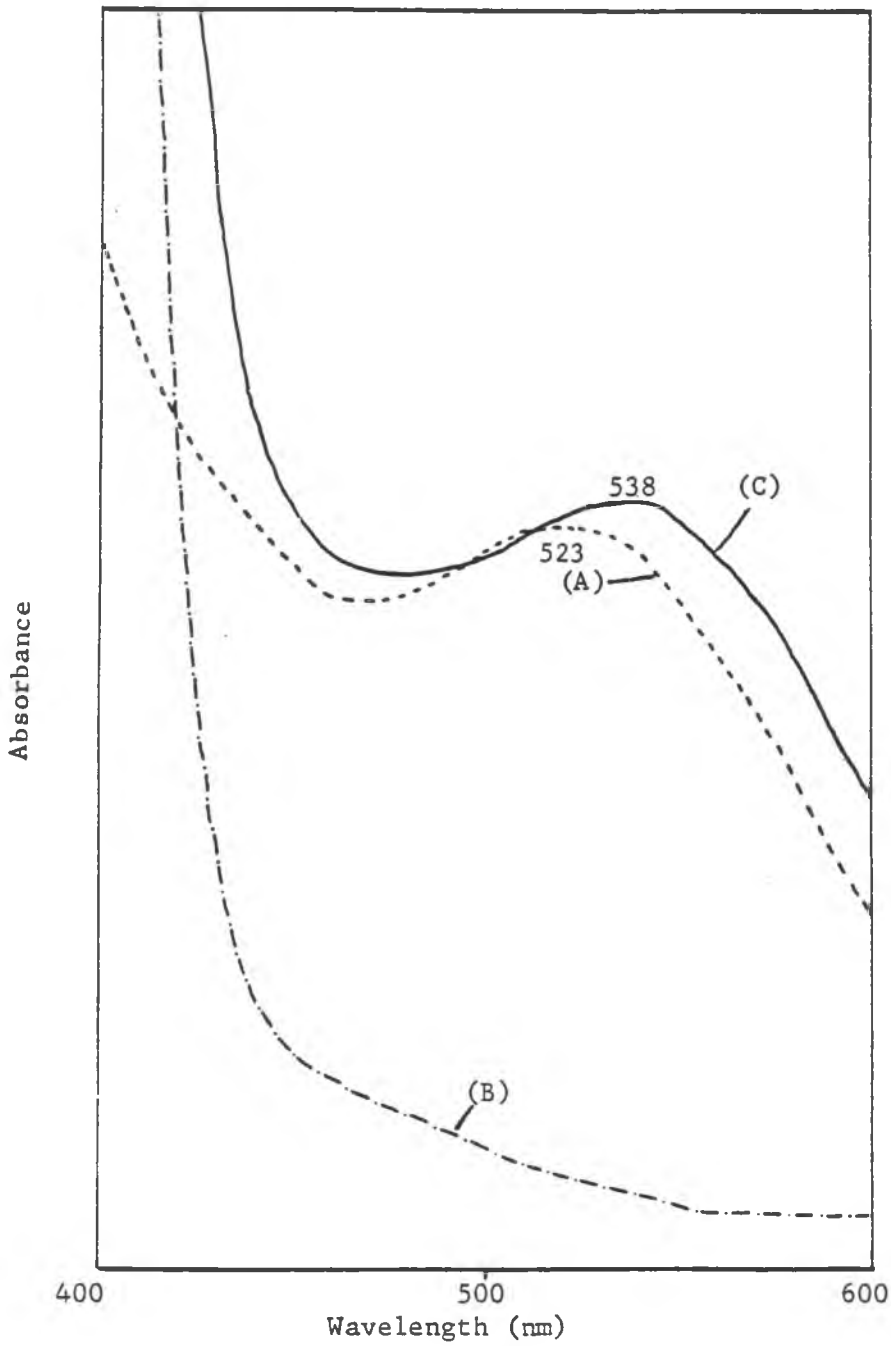

Figure 145. Absorption spectra of cyanidin 3-rutinoside (A), flavonol from Anthurium formosum (B) and a mixture of two compounds (C) in citrate-phosphate buffer solution (pH=5.66).

Figure 146. Comparison on visual color of cyanidin 3-rutinoside (left), flavonol from Anthurium formoum (right), and a mixture of cyanidin 3-rutinoside and flavonol (center) in citrate-phosphate buffer solution (pH=5.66).

anthocyanin-copigment complex than flavonols. The fact that A. amnicola contains a 6-C-glycosylflavone, acacetin 6-C-diglycoside as the major flavonoid in leaves (Sec 4.2) and spathes (unpublished data) may suggest that this compound could have co-pigmentation effect to produce lavender spathes at a high pH of 6.2. On the other hand, A430, another specimen of A. amnicola does not show clearly the presence of the same compound (Sec 4.2) and the concentration of flavonoids in two plants of A. amnicola is relatively low compared to that of A. formosum and some other species. It is not possible to conclude whether any significant co-pigmentation effects exist in A. amnicola because co-pigmentation does not occur if the concentration of pigments is too low (Asen et al., 1972; Asen, 1976).

4.5 General discussion

A better understanding of evolutionary relationships between Anthurium andraeanum and closely related taxa was one of the objectives of the study. Hybrid analysis indicated a close genomic relationship among parental taxa. A slight variation in karyotypes of A. nymphaeifolium and A. wallisii from other species suggested existence of chromosomal evolution in the genus Anthurium. However, regular bivalent formation at meiosis in species hybrids failed to elucidate mechanisms of evolution. Different chromosome staining techniques such as Giemsa stains may give further information on the cytogenetics of the genus.

Although there are high degrees of crossability among taxa and regular bivalent formation in meiosis of hybrids, reduction of pollen stainability in interspecific hybrids indicated the presence of genetic barriers among species. In addition, a drastic decrease in the number of spadices with pollen grains and the percentage of stainable pollen grains in intergroup interspecific hybrids suggest a genetic divergence between Sheffer and Kamemoto's group V and VI (Sheffer and Kamemoto, 1976b).

Flavonoid analysis aided in revealing phylogenetic relationships of Anthurium taxa. Harborne (1977) pointed out three evolutionary trends in leaf flavonoids: loss of

proanthocyanin, replacement of B-ring trihydroxylation by di- or mono-hydroxylation and replacement of flavonol by flavone. Anthurium formosum was found to be unique in possessing 'primitive' flavonoid compounds, specifically flavonols, while other taxa contained flavones or in some species there were no major compounds detected by HPLC. It was therefore assumed that A. formosum was a primitive species whereas others were advanced taxa.

Apigenin derivatives were commonly found in species in group VI (A. andraeanum, A. lindenianum, A. concinnatum, A. nymphaeifolium, and A. kamemotoanum) and a species in group V, A. wallisii. Anthurium amnicola (A417) also contained an apigenin derivative (Table 21). Presence of acacetin glycosides in A. andraeanum and A. amnicola suggested that the two species might be advanced taxa possessing 4'-methoxyl compound (acacetin) as a major flavonoid instead of 4'-hydroxyl compound (apigenin) because in the biosynthetic pathway, methylation is one of the last steps in forming an individual flavonoid.

The anthocyanin study also pointed out that A. andraeanum and A. amnicola had advanced characters. According to Harborne (1977), pelargonidin types occur in advanced plants in tropics as a replacement of cyanidin, the most primitive pigment. Since Anthurium andraeanum with an orange spathe is a typical representative of the

taxon and no other colored spathes have been found in nature, it is assumed that one of the evolved characters of the taxon is possession of pelargonidin. On the other hand, A. amnicola showed a different direction of anthocyanin evolution. O-methylation is a trend in evolutionary advancement (Harborne, 1977) and a peonidin typically found in A. amnicola is a methylated form of cyanidin.

Complex glycosylation is another trend in flavonoid evolution. A variety of apigenin derivatives present in some taxa may indicate that complex glycosylation has occurred during speciation of these taxa.

Numerical studies based on flavonoids support genetic distinctiveness between group V and VI. Three species in group V, A. subsignatum, A. garagaranum, and A. wallisii always exhibited a close relationship. In addition, a close relationship among A. nymphaeifolium, A. roraimense and A. concinnatum was also noticed.

In spite of chromosomal similarity, Anthurium species display a wide range of flavonoid profiles. It can be concluded that speciation of these taxa has occurred mainly at the genic level. Chromosomal repatterning exists in the genus, however, it does not seem to be a major mechanism of evolution.

In anthurium breeding, the ease of interspecific hybridization enhances color variation of plants. The

present biochemical studies provided useful information on the pigment system of A. amnicola and its hybrids. The concentration and kinds of anthocyanin present in tissues were the most important factor for color determination of anthuriums. Cyanidin 3-rutinoside appeared to be the major anthocyanin and the concentration of the pigment greatly affected the shade of tissues. Peonin 3-rutinoside was identified from A. amnicola but the color of the pigment itself was close to cyanin 3-rutinoside and the color variation caused by this pigment seemed very slight. As Iwata et al. (1979) pointed out, the pelargonidin 3-rutinoside was responsible for orange spathe of A. andraeanum. A trace of pelargonidin 3-rutinoside was detected in some A. amnicola hybrids. It was assumed that the orange pigment could give redder or less purplish shade but among A. amnicola hybrids influence of pelargonidin 3-rutinoside was far less than that of cyanidin 3-rutinoside.

Co-pigmentation effects and pH are other important factors which contribute to color variation of anthuriums. Generally when pH is higher, the color of spathe is more purplish. The lavender spathe of A. amnicola exhibited the highest pH(6.2) in the study.

Flavonols from A. formosum were found to have co-pigmentation effects. Color enhancement as well as color shift (red to red-purple) of cyanidin 3-rutinoside

by a flavonol were clearly demonstrated. Other flavonoids or phenolic compounds in tissues may have some influences on color variation. In fact purplish color of spadix may be due to co-pigmentation effects and/or influence of pH on anthocyanin-copigment complex. In preliminary experiments the pH of the spadix was relatively high and the presence of different phenolic compounds were detected in the survey by using paper chromatography.

Hybridization for bluer anthuriums seems possible if new germplasm with suitable co-pigments are introduced. Tissues should have a lower amount of free organic acids in order to have a higher pH. Kinds of co-pigments and the concentration of both anthocyanins and co-pigments are other factors to be considered. By incorporating these factors it should be possible to create new types of anthuriums.

Appendix I. RHS color code, colorimeter readings and pH of spathe from 'Calypso', 'Trinidad', Anthurium annicola (A417), and various hybrids.

Plant	RHS[z]	Color measurement				pH
		L (luminance)	a (green-red)	b (blue-yellow)	$\tan^{-1}(a/b)$	
'Calypso'	46B	29.23	33.95	9.76	1.2909	5.50
'Trinidad'	49B	58.96	11.73	5.46	1.1351	5.71
A417	84B	47.49	12.08	-8.16	-0.9769	6.20
392-66	53A	24.00	28.97	3.94	1.4356	5.54
392-42	53B	35.95	31.23	4.33	1.4330	5.53
RS532-5	52A	32.78	43.52	8.84	1.3704	5.74
RS1183-5	51C	49.29	19.23	4.66	1.3330	5.46
587-1	77B	36.81	20.94	-7.50	-1.2269	5.79
587-9	59C	37.80	28.69	3.94	1.4343	5.94
587-10	70C	44.93	16.56	-3.15	-1.3828	5.64
587-14	80C	34.88	20.22	-8.46	-1.1745	5.92
587-15	51A	38.13	29.31	4.13	1.4308	5.77
587-28	51A	40.50	34.27	5.71	1.4057	5.48
587-39	78D	50.02	13.33	-2.79	-1.3645	5.95
587-40	78C	40.04	17.85	-4.92	-1.3018	5.95
587-44	78D	53.22	12.24	-5.12	-1.1746	5.71
587-55	59D	30.06	30.50	1.64	1.5171	5.63
587-59	53D	29.94	33.89	5.65	1.4056	5.45
587-66	53C	29.58	30.46	4.45	1.4257	5.89
587-67	78C	41.61	19.48	-6.71	-1.2391	5.87
587-71	53B	29.93	32.30	6.37	1.3761	5.80
587-73	80B	29.99	22.53	-8.61	-1.2058	5.65
568-2	70B	36.70	25.80	-7.60	-1.2843	5.90
568-25	70B	37.61	23.29	-4.61	-1.3754	5.96
568-40	70C	37.29	27.20	-3.19	-1.4541	5.86
572-7	70B	40.67	21.67	-5.87	-1.3063	5.85
572-24	70B	40.63	23.86	-6.18	-1.3174	5.83
552-38	58D	49.28	25.96	0.80	1.5400	5.83
552-85	63D	57.28	19.75	1.08	1.5197	6.08

[z]Royal Horticultural Society Color Chart.

Appendix II. Concentration of anthocyanins and the percentage of cyanindin 3-rutinoside in spathes of 'Calypso', 'Trinidad', Anthurium annicola and various hybrids.

Plant	Relative concentration of anthocyanin[z]								% CR
	A1	A2	A4	CG	CR	PEL	PEO	TOT	
'Calypso'	112	167	0	506	7750	405	189	9129	84.7
'Trinidad'	48	32	0	0	2217	97	0	2394	92.6
A417	58	22	11	0	2148	0	652	2891	74.3
392-66	392	379	0	0	6040	80	230	7121	84.8
392-42	68	77	0	8	3974	13	106	4246	93.6
RS532-5	143	151	0	61	3488	119	158	4120	84.7
RS1183-5	121	111	0	8	1851	116	0	2207	83.9
587-1	118	0	0	0	1888	17	303	2326	81.2
587-9	125	0	0	0	6194	303	38	6660	93.0
587-10	0	0	0	0	737	36	147	920	80.1
587-14	67	109	0	0	1825	0	142	2143	85.2
587-15	236	0	0	0	4702	48	430	5416	86.8
587-28	69	0	0	0	3426	28	0	3423	97.2
587-39	8	0	0	0	778	0	42	828	94.0
587-40	96	0	36	0	1592	35	495	2254	70.6
587-44	63	0	0	0	1512	0	89	1664	90.9
587-55	100	92	28	0	4301	31	53	4605	93.4
587-59	220	0	0	0	7408	0	311	7939	93.3
587-66	90	125	0	0	5742	57	628	6642	86.4
587-67	91	90	0	0	1853	177	149	2360	78.5
587-71	127	197	0	0	11084	115	779	12302	90.1
587-73	143	105	0	0	3484	182	60	3974	87.7
568-2	125	120	0	0	2369	54	112	3280	87.5
568-25	288	0	24	0	2122	18	72	2524	84.1
568-40	112	0	0	0	2366	56	28	2562	92.3
572-7	64	0	0	0	2133	29	81	2307	92.5
572-24	109	0	0	0	1900	11	108	2128	89.3
552-38	60	0	23	0	1035	82	226	1426	72.6
552-85	58	0	0	0	465	0	41	564	82.4

[z]Relative concentration of anthocyanin = $\frac{\text{(area of a peak x volume of solvent)}}{\text{fresh weight}}$

Abb.: CG=cyanidin 3-glucoside, CR=cyanidin 3-rutinoside, PEL=pelargonidin 3-rutinoside, PEO=peonidin 3-rutinoside.

Appendix III. RHS code, color, and RHS color group.

RHS code	color	RHS color group
46B	red	red
49B	light pink	red
51A	light pink	red
51C	pink	red
52A	red	red
53A	dark pink	red
53B	dark pink	red
53C	red	red
53D	red	red
58D	pink	red-purple
59C	red-purple	red-purple
63D	pink	red-purple
70B	lilac purple	red-purple
70C	purple	red-purple
77B	light violet purple	purple
78C	purple	purple
78D	light purple	purple
80B	purple	purple-violet
80C	light purple	purple-violet
84B	lavender	violet

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