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Chemical Discontinuity in *Laeliinae bentham**

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Key Word Index—*Encyclia*; *Anacheilium*; *Hormidium*; Orchidaceae; druse-type pattern; dihydroflavonol; chemosystematics; phylogeny.

Abstract—A new dihydroflavonol glycoside, anacheiloside, has been characterized in several species of the family Orchidaceae. The distribution of the flavonoid in druse-type patterns in flowers fixed in ethanol was used as a criterion to restablish the genus *Anacheilium* and correlate phylogeny among *Encyclia, Anacheilium* and *Epidendrum*.

Introduction

Most taxonomists now attribute more importance to biological concepts, such as genetic information, physiology, ecology and phytochemistry, considering them essential for the creation or delineation of new taxa, instead of relying strictly on morphological characteristics. Several groups of plants have been studied following this approach, especially when it is difficult to define them precisely because morphological criteria were common among many species [1, 2].

These methods have the advantage in avoiding subjective opinions. For example, flavonoids [3] have been applied to help distinguish hybrid species. Ecological methods are also useful in studying species–species and species–habitat relationships [4] based on the presence of chemical components, allelochemicals, produced by plants (or animals). These have been used to study behaviour, classification and phylogeny. Recently Gottlieb *et al.* set up general principles to use allelochemicals to study evolution, systematics and ecology in plants [5].

In the Orchidaceae, some work has been done utilizing chemical constituents in order to study their taxonomy, evolution and ecology. Tosello *et al.* [6], using phenolic compounds from flowers, made a phylogenetic study of the genera *Cattleya* (Ldl.) and *Laelia* (Ldl.). Studies based on alkaloids, led to delimitation of sections in the genus *Pragmipedium* [7], affinities in the genus *Dendrobium* and correlations among the genera *Malaxi, Lipares* and *Hammabya* [8]. Williams has made use of flavonoids to clarify the relationship between orchids and other families of monocotyledons [9]. Chromatographical analysis has also been applied in studies of the floral fragrance of *Catasetum* and *Epidendrum,* as well in other tropical and European genera [10–13].

Recently our group has published some papers which throw some light on the *Epidendrum/Encyclia* controversy [14]. We found crystalline structures mainly in the flowers of the sub-genus *Osmophytum* (Ldl.) Dressler & Pollard. These crystals are very abundant in species of the drusa type and are distributed in all parts of the plant. The structures are not found in other plants of the same genus that did not show drusa type patterns. This difference was used to elevate the sub-genus *Osmophythum* to genus rank, re-establishing the genus *Anacheilium,* created in 1842 by Hoffmanseg to describe *Epidendrum cochleatum* [15]. Our earlier studies described the chemical analysis of the crystals [16]. Here we present the structure of the compound involved, anacheiloside, based on its ^{13}C NMR analysis as well as other spectroscopic data. We also present a list of plants which produce anacheiloside.

*This work is dedicated to the memory of the Brazilian orchidologist J. F. G. Pabst (deceased 1980).

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Results and discussion

When handling flowers preserved in alcohol, we noticed that some species belonging to the subgenus *Osmophytum* (Ldl.) Dressler & Pollard were covered with crystals spread over all parts of the flower (Fig. 1). An examination of herbarium material (Herbarium Bradeamum collection) confirmed the presence of these crystals throughout the plant, including pseudobulbs, leaves, stems as well as fruit capsules.

Histological sections of the stem in *Anacheilium fragrans, A. calamaria, A. inversa* and *A. vespa* showed the presence of druse-type crystals. At first we thought they may be calcium oxalate commonly found in plants, but histochemical tests proved this was not so. The presence of the crystals became a most important feature for their characterization. We have, therefore, extracted them and determined their structure in order to investigate their importance as a taxonomic marker. The crystals were easily extracted from the flower with a solution of 5% of sodium hydroxide in ethanol, followed by acidification of the extract with hydrochloric acid (pH=3) [15, 16]. The extract was left at 4° to precipitate the colourless crystals which were then centrifuged and washed thoroughly with water, ethanol and acetone. Hydrolysis (6 N CF_3CO_2H, 100°, 12 h) revealed the presence of equal amounts of D-(+)-rhamnose, D-(+)-glucose and diosmetin (**1**). The sugars were demonstrated to be linked to give 6-*O*-β-D-rhamnopyranosyl-D-

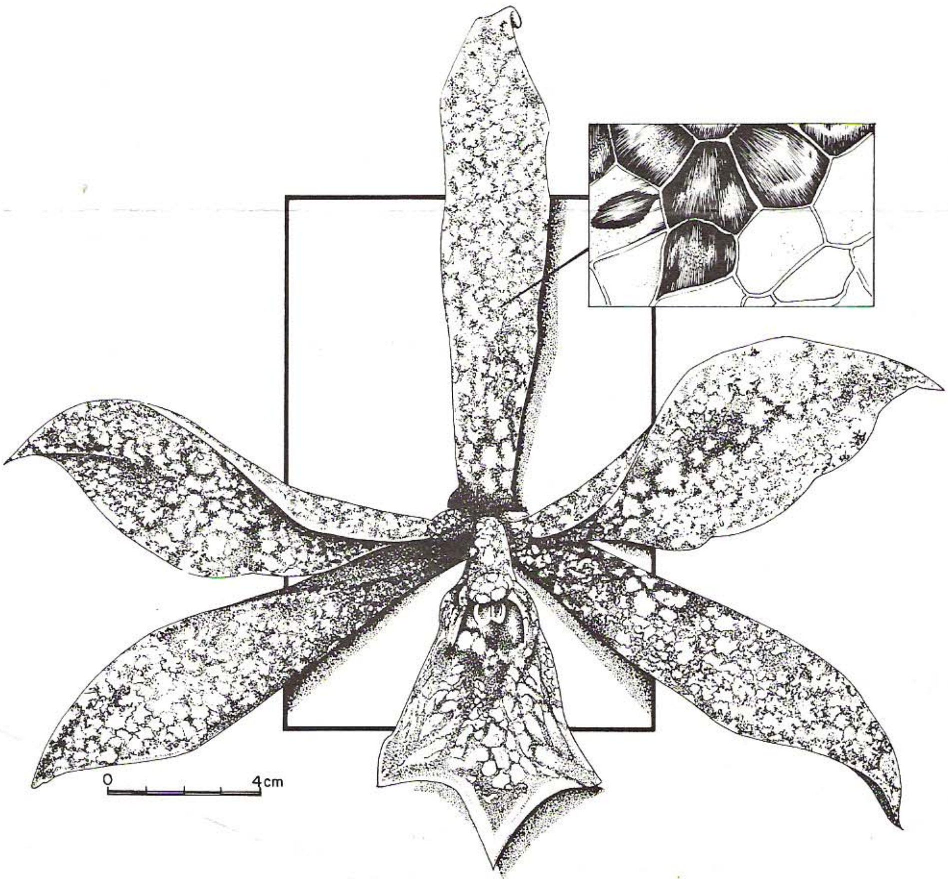
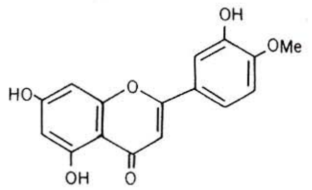


FIG. 1. *ANACHEILIUM ALLEMANOIDES* (VELL.) PABST, MOUTINHO & PINTO; FLOWER FIXED IN ETHANOL, SHOWING THE DRUSE-TYPE PATTERN SCATTERED IN ALL SEGMENTS. The section shows the crystal pattern.



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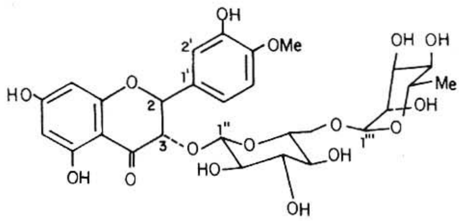

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glucopyranoside (I → aglycone). The ^{13}C NMR (DMSO-d_6) spectrum of the aglycone (Table 1) confirmed that it was diosmetin. However, the IR and UV spectra of the glycoside were not in agreement with this finding. The UV spectrum and the bathochromic effect caused by addition of base indicated that the aglycone was a dihydroflavonol suggesting that diosmetin was formed during the extraction or hydrolysis of the glycoside [17].

The ^{13}C NMR (DMSO-d_6) spectrum of anacheiloside revealed signals indicating that the sugar chain was linked to carbon-3 of the dihydroflavonol skeleton (Table 1). Based on the spectral data and comparison with literature, structure **2** was proposed for anacheiloside [18, 19].

TABLE 1. ^{13}C NMR SHIFTS OF DIOSMETIN (1) AND ANACHEILOSIDE (2)*

Carbon	1	Aglycone in 2	Carbon	Sugar chain in 2
2	78.5	161.3	1''	103.4
3	70.8	103.9	2''	73.1
4	197.0	182.0	3''	76.3
4a	100.6	105.5	4''	70.8
5	163.9	157.0	5''	75.6
6	96.4	94.6	6''	68.4
7	165.2	164.3	1'''	100.3
8	95.6	99.5	2'''	70.5
8a	162.5	163.0	3'''	69.4
1'	131.0	128.0	4'''	72.1
2'	114.2	112.2	5'''	68.4
3'	146.8	146.6	6'''	17.9
4'	149.7	147.9		
5'	114.0	118.0		
6'	118.8	119.0		

*The δ values are in ppm downfield from TMS. In DMSO-d_6 δ (TMS)=δ (DMSO-d_6)+39.5 ppm.

Our previous work was carried out with flowers of *Anacheilium vespa, A. calamarium, A. inversum* and *A. camposportoi.* We have now shown that several other species of *Osmophytum* (Table 2) that show the druse-type pattern contain anacheiloside (**2**) [16, 20]. Two species, *Encyclia triptera* (Brogn.) Dressler and *E. sessiliflora* (Edwalle) Pabst, had traces of crystals and revealed a great morphological similarity to *E. fragrans.* However, no crystals were found in the vegetative part of the plants and only a few are scattered in the flowers. A more elaborate study of those plants might well lead to the establishment of a new infra-specific group. We believe this small group should be separated from *Epidendrum* as well as from *Encyclia* and *Anacheilium* and should be placed into a small independent genus, *Hormidium,* as initially proposed by Lindley [21]. Two other orchids of the genus, *Encyclia regnelliana* and *E. hoehnei,* were not examined.

Conclusion

Much has been written about the value of definitive morphological characteristics to justify the

TABLE 2. SPECIES OF *ANACHEILIUM* HOFFMAGG WITH DRUSE TYPE PATTERN IN THE FLOWERS [16]

A. alagoense (Pabst) Pabst, Moutinho & Pinto
A. allemanii (Barb. Rodr.) Pabst, Moutinho & Pinto
A. allemanoides (Hoehne) Pabst, Moutinho & Pinto
A. calamarium (Lindl.) Pabst, Moutinho & Pinto
A. caetense (Bicalho) Pabst, Moutinho & Pinto
A. cochleatum (L.) Hoffmagg
A. campos-portoi (Pabst) Pabst, Moutinho & Pinto
A. faresianum (Bicalho) Pabst, Moutinho & Pinto
A. faustum (Rchb. F. ex cogn.) Pabst, Moutinho & Pinto
A. fragrans (S.W.) Acuña
A. grammatoglossum (Rchb. F.) Pabst, Moutinho & Pinto
A. glumaceum (Lindl.) Pabst, Moutinho & Pinto
A. hartwegii (Lindl.) Pabst, Moutinho & Pinto
A. inversum (Lindl.) Pabst, Moutinho & Pinto
A. kaustkyi (Pabst) Pabst, Moutinho & Pinto
A. lividum (Lindl.) Pabst, Moutinho & Pinto
A. moogenii (Pabst) Pabst, Moutinho & Pinto
A. papilio (Vell.) Pabst, Moutinho & Pinto
A. radiatum (Lindl.) Pabst, Moutinho & Pinto
A. suzanense (Hoehne) Pabst, Moutinho & Pinto
A. vespa (Vell.) Pabst, Moutinho & Pinto
A. widgrenii (Lindl.) Pabst, Moutinho & Pinto

elevation of a group of plants to generic rank. However, the presence of **2** in only a few species of *Encyclia* make them biochemically distinct from the others and demonstrates the possibility that the systematics of the genus might need to be changed.

The presence of anacheiloside (**2**) in the flowers of this group of plants may have a great influence on their floral biology. They are fluorescent under UV light and probably more distinctive to insects under a dense forest canopy. Evidence that certain flavonoids act as nectar guides in flowers of various plants has been accumulating [22]. Anacheiloside may also play a role in the survival of the flowers, as a feeding deterrant to mammals and insects.

Although, it may well be difficult to establish a phylogenetic order among the genera examined, we believe that *Hormidium* and *Anacheilium* are more closely related to *Epidendrum* than to *Encyclia.* This is more evident for *Anacheilium* whose druse-type pattern are always present, whereas they occur only occasionally in *Epidendrum.* It is important to appreciate that the accumulation of flavonoid as crystals in druse-type plants does not simply represent a chemical novelty, but is a feature which may be of wider taxonomic significance.

Experimental

Extraction and separation. The glycoside was obtained from flowers and purified as described in the text. Analysis of the sugar chain was by GLC on 3% ECNSS-M on Chromosorb W. The temperature was programmed from 120° to 170° at 4°/min.

Anacheiloside (**2**), m.p. 255–256°; PC, R_f=0.3, *n*-BuOH–30% Na_2CO_3 (1 : 1); IR ν_{max}^{KBr} cm^{-1}: 341 (br), 1664 (s), 1614 (s), 1302 (m), 1440 (w), 1256 (m), 1200 (w), 1179 (w), 1080 (m), 834 (w), 807 (w) and 755 (w) cm^{-1}; m/e, 302 (25), 301 (10), 256 (10), 150 (30), 138 (30), 57 (8), 43 (base); exact mass: *m/z* 300.0690 [M−326], calculated for $C_{16}H_{12}O_6$. UV λ_{max}^{MeOH} nm: 280, 323; base: 285, 357. ^{13}C NMR: Table 1.

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