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CHEMICAL INDEXES CALCULATED FOR 8,11,13-TRIEN-ABIETANE DITERPENOIDS ISOLATED FROM *SWARTZIA* SPECIES

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Abstract: The disparity found in the molecular structures of compounds isolated from nine plants of the *Swartzia* genus indicates that the *Swartzia* species that furnished cassane diterpenoids and triterpenoidal saponins are more recent, since these metabolites have adopted the mevalonic acid route of formation, abandoning the shikimic acid/acetate route that produces the isoflavonoids found in the remaining species. Chemical indexes calculated from the molecular structure diversities of sixteen 8,11,13-trien-abietane diterpenoids isolated from *Swartzia langsdorffii* and *S. arborescens* indicate that *S. arborescens* is more recent than *S. langsdorffii*. The results suggest a more evolved position in *Swartzia* species of the section *Possira*.

Keywords: Leguminosae, Systematics of *Swartzia* genus, plant evolution, chemical index, chemotaxonomy.

Introduction

The genus *Swartzia* Schreb. belongs to tribe Swartzieae, subfamily Papilionoideae (Faboideae) of the Leguminosae (Fabaceae), and consists of about 180 species distributed in tropical America [1]. It was revised by Cowan [2], who recognized two sections, *Swartzia* sect. *Possira* (Aubl.) DC. and *Swartzia* sect. *Swartzia*, one subsection, and eight series.

Nevertheless, studies conducted after this review showed, directly or indirectly, the need of a revision of the infrageneric classification, due the artificiality of some series [3-10]. In fact, some of the Swartzia species are included in two distinct subsections or series and varieties of the same species have been placed in different series 11]. Cowan attempted to order the species in his monograph along putative phylogenetic lines, but his infrageneric groups were not intended to reflect common ancestry. Indeed, a systematic study of *Swartzia*, based on morphological and molecular characters, could provide an evolutionary basis to evaluate the infrageneric taxonomy of Cowan [2].

Recently, it was pointed out [**11]** that *Swartzia* should be retained without further division, despite its large number of species and considerable morphological diversity, because it was otherwise resolved as monophyletic, albeit with limited support. The present resolution of subclades of *Swartzia* should be used as a basis for revising the highly artificial infrageneric classification of Cowan [2].

Additionally, an updated monograph of the entire genus has taxonomic and phylogenetic significance because of the position of Swartzieae in the Papilionoideae classification. Molecular data strongly suggest that Swartzieae *sens. strict.* should be redefined to include only the genera

Swartzia, *Bobgunnia*, *Bocoa*, *Candolleodendron*, *Trischidium*, *Cyathostegia*, and *Ateleia* [1,12, 13].

Cladistic analyses showed the group of *Myrospermum*, *Myroxylon* and *Myrocarpus* of Sophoreae to be associated with the Aldinoid clade of the Swartzieae and closely to Swartzieae *sens. strict*. [12]. The reclassification of Swartzieae *sens. strict*., and realignment of the remaining swartzioid genera in other tribes, needs to be corroborated by further evidence [1, 13].

On the other hand, evolutionary changes in plants can also be evaluated through the chemical structures of the special metabolites found in them, based on the disparity (related to the biogenetic route) and the diversity of their molecular structures [14]. The methodology is very attractive because it does not imply knowledge of the complete biogenetic route of metabolites and has already indicated evolutionary tendencies for the *Swartzia* species that produce isoflavonoids [15]. In this paper, the methodology is used to suggest evolutionary directions for two *Swartzia* species that, instead of isoflavonoids [16-20], furnish terpenoids.

Methodology

Disparity Evaluation- The disparity of metabolites reported in *Swartzia* genera were evaluated by comparing their chemical structures since, according to Gottlieb's methodology [14], disparity in the molecular structure of special metabolites indicates an evolutionary move and occurs by the replacement of compounds generated from the shikimic acid/acetate biogenetic route by compounds generated from the acetated route (or mevalonic acid route).

Accordingly, the species were separated into two groups, those that furnished special metabolites originating from the shikimic acid/acetate route - the isoflavonoids, and those that furnished compounds formed by the mevalonic acid route - the terpenoids (Table 1).

Table 1. Correlation between chemical data reported for *Swartzia* studied phytochemically and botanic classification (Cowan 1967), phylogeny reported in the current literature (Torke & Schaal 2008).

Cowan 1967			Torke & Schaal 2008	Araujo 2007; Braz Filho et al. 1980; Osawa et al. 1992; Dubois et al. 1995, 1996	Magalhães et al. 2005; Orphelin et al. 1996	Borel et al. 1987; Abdel-Kader et al. 2000; Magalhães et al. 2003
Species	Section	Series	Clade	Isoflavonoids	Diterpenoids	Saponins
S. ulei	Swartzia	Benthamianae		+		
S. laevicarpa	Swartzia	Benthamianae	benthamioid	+		
S. leiocalycina	Swartzia	Recurvae	recurvoid	+		
S. polyphylla	Swartzia	Orthostyleae	orthostyloid	+		
S. schomburgkii	Swartzia	Orthostyleae	orthostyloid			+
S. apetala	Swartzia	Tounateae	tournateoid	+		+
S. langsdorffii	Swartzia	Recurvae	acutifolioid		+	+
S. arborescens	Possira	Possira	possiroid		+	
S. simplex	Possira	Possira / Unifoliolatae	possiroid			+

Diversity Evaluation- For contextualization and better visualization, the biodiversity observed for ten 8,11,13-trien-abietane diterpenoids isolated from S. *langsdorffi*, **A**, **B**, and **1-4** [21], including a pair of epimers, **5-8** [22] is explained through a series of methylation and oxidation reactions rationalized using a proposed biogenetic route (Fig. 2).

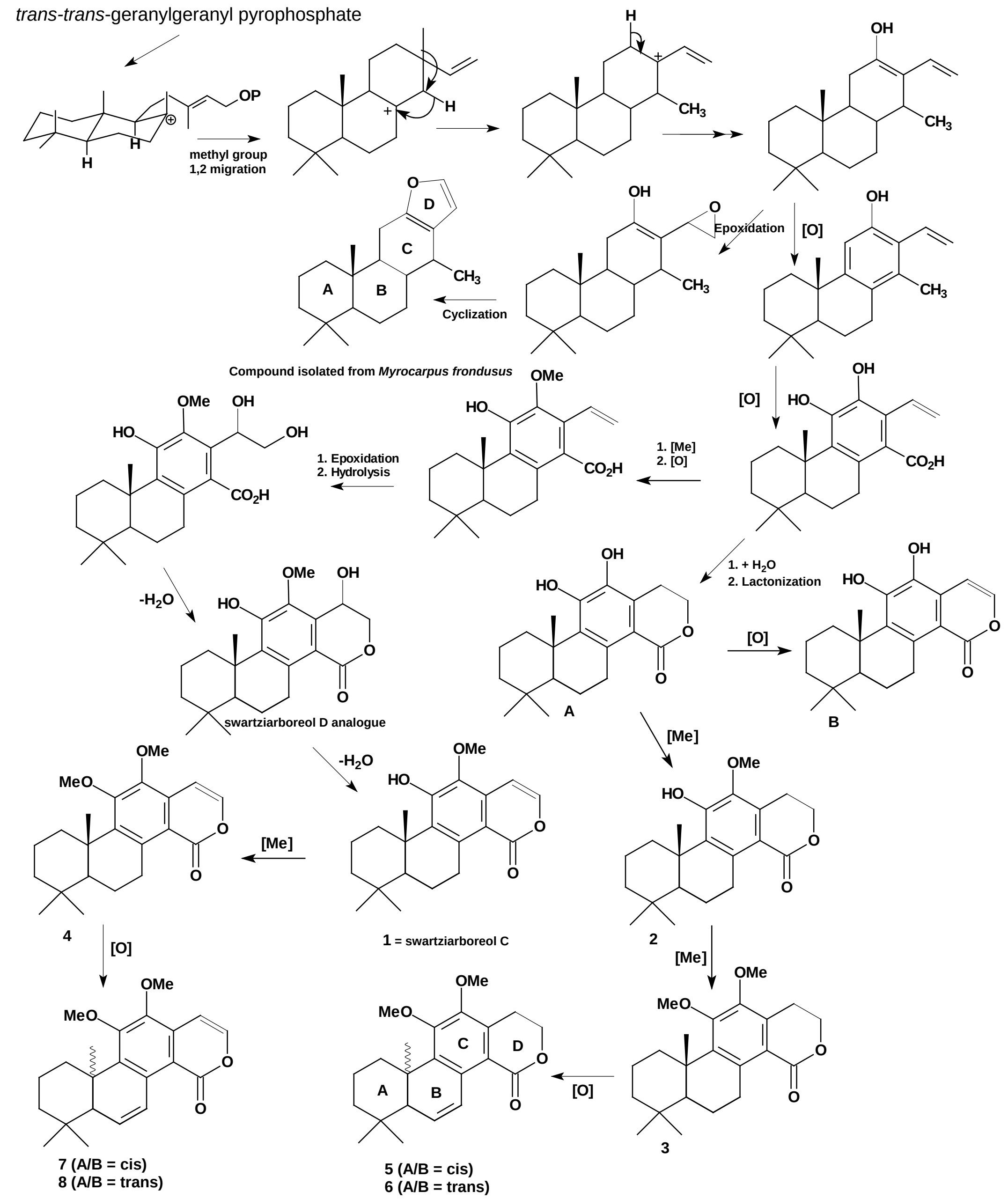


Figure 2. Biogenetic proposal rationalized for 8,11,13-trien-abietane diterpenoids isolated and identified from the native Brazilian tree *Swartzia langsdorffi* Raddii (Magalhães et al. 2005; Santos *et al.* 2007).

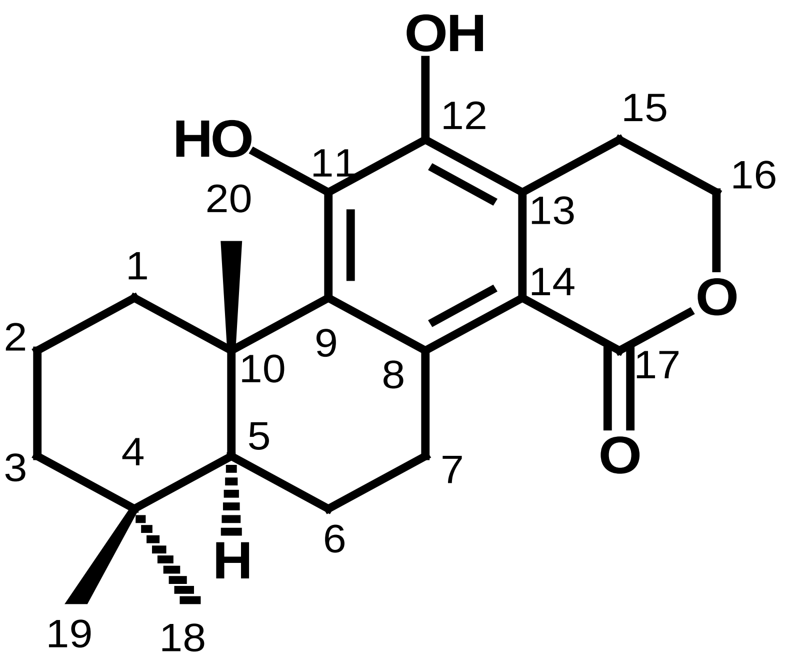
The initial part of proposed route is very similar to that reported for the C-12 methoxy derivative of ferruginol [23] including a 1,2-methyl *si* migration. However, the shift now proposed occurs between C-13 and C-14, and not between C-13 and C-15, and is reinforced by the detection of C-14 methylated 8,11,13-trien-abietane compounds in the species *Myrocarpus frondosus* [24], a species of the Sophoreae tribe, which is closely related to *Swartzieae sens. strict.*.

Some cyclization processes are proposed leading to the compounds that were isolated from *Swartzia* species and from the species *Myrocarpus frondosus*. The metabolites carbon skeletal difference is in ring D, as, in case of the metabolites furnished by *M. frondosus* , ring D would be formed by an oxidative cyclization reaction between C-16 and C-12, allowed by the absence of the protective C-12 methoxy group of swartziarboreols. In the case of swartziarboreols, however, oxidative steps would occur before a final cyclization reaction between C-16 and C-17 that would give rise to the isocoumarin ring D. Then, the other compounds would be generated by a series of oxidative and methylation processes.

However, when Gottlieb's chemical index methodology is applied, a knowledge of such biogenetic pathways of special metabolites is not necessary since the evolutionary evaluation is made by means of two chemical indexes, the oxidative evolutive advance index, EA_O, and the skeleton complexity evolutive advance index, EA_E, of each plant species.

The indexes EA_O and EA_E are found by calculating the averages: $EA_O = (\Sigma\ O) / N$ and $EA_E = (\Sigma\ E) / N$, where the value N refers to the number of metabolite occurrences, and the O and E values are the chemical indexes that quantify the oxidative degree of the molecular structures (O) and the carbon skeleton specialization (E) of each metabolite.

The oxidative degree indexes (O) were calculated by the equation, $O = (x-h)/ n$, where the letters n, h and x refer, respectively, to the total number of carbon atoms (n), the number of C-H bonds (h) and the number of C-heteroatom bonds (x) of the terpenoid skeleton, while the carbon skeleton specialization indexes (E) were calculated by comparing the carbon skeleton of each diterpenoid chemical structure in focus with the carbon skeleton of a hypothetic common precursor (compound **A**; Fig. 1), by using the equation: $E = (q + f + c + u) / n$, where the letters n, q, f, c, and u, represent, respectively, the total number of carbon atoms (n), the number of C-C bonds broken (q), the number of C-C bonds formed (f), the number of rings formed with a heteroatom, (c), and the number of additional carbon atoms (u). Table 2 shows the O and E values found for ten compounds identified from *S. langsdorffii*. The same calculations were applied to the five diterpenoids reported for *S. arborescens* [25] (Table 3). Finally, the oxidative advance index, EA_O, and the skeleton specialization advance index, EA_E, of each species are displayed in Table 4.



A = common biogenetic precursor

Figure 1. Hypothetic common precursor which has been used to calculate skeleton complexity evolutive advance (EA_c) values for all swartziarboreols identified.

Table 2. Oxidative evolutive index values (O), calculated for swartziarboreols isolated and identified in the extracts of *S. langsdorffii* (Magalhães et al. 2005; Santos et al. 2007).

Compound	O*	E**
A	-0.90	0.00
B	-0.80	0.00
1	-0.86	0.00
2	-0.95	0.00
3	-0.91	0.00
4	-1.00	0.00
5	-1.00	0.00
6	-1.00	0.00
7	-0.91	0.00
8	-0.91	0.00

* O = x-h/ n, where the letters refer, respectively, to the total carbon atoms (n), the C-H bonds (h) and the C-heteroatom bonds (x) of the diterpenoid skeleton. ** E = Carbon skeleton specialization chemical index.

Table 3. Oxidative evolutive index values (O), calculated for the swartziarboreols **A-E** isolated and identified in the extracts of *S.arborescens* (Orphelin et al. 1996).

Compound	O*	E**
swartziarboreol A	-0.62	0.00
swartziarboreol B	-0.52	0.00
swartziarboreol C	-0.71	0.00
swartziarboreol D	-0.57	0.00
swartziarboreol E	-0.57	0.00

* O = x-h/ n, where the letters, refer, respectively, to the total carbon atoms (n), the C-H bonds (h) and the C-heteroatom bonds (x) of the diterpenoid skeleton. ** E = Carbon skeleton specialization chemical index.

Table 4. Values for oxidative evolutive advance and skeleton complexity evolutive advance, EA_o and EA_E, calculated using the chemical index values found for the metabolites identified in the species *S. langsdorffii* and *S. arborescens.*

Species	EA_o^{a}	EA_E^{b}
S. langsdorffii	-0.94	0.00
S. arborescens	-0.60	0.00

^{a} $EA_o = \Sigma$ O/n $^b EA_E = \Sigma$ E/n; n = metabolite occurrences.

Results and Discussion

Disparity Evaluation- Comparing the chemical structures of metabolites reported for *Swartzia* species (Table 1) it is possible to observe two groups characterized by the presence of isoflavonoids or terpenoids. Phytochemically studied *Swartzia* species that furnished diterpenoids and triterpenoidal saponins (*S. arborescens* [25], *S. simplex* [26], *S. schomburgkii* [27] and *S. langsdorffii* [21, 22, 28]) are more recent concerning the evolutionary aspects, since their metabolites were generated by the mevalonic acid route while the remaining species produced isoflavonoids that are formed by the shikimic acid/ acetate route.

Considering the clades obtained by **Torke & Schaal** [12], the presence of these substances could be used, in addition to other data, to define the *Swartzia* clades: possiroid and acutifolioid clades characterized by terpenoids, and orthostyloid and benthamioid clades characterized by flavonoids (Table 1). In this sense, the systematic position of *S. schomburgkii* is doubtful, appearing with 58% maximum parsimony bootstrap support in a combined chloroplast and nuclear sequences tree [12, Fig. 3]. In the trees obtained in separate searches of combined chloroplast sequence data and ITS sequences, *S. schomburgkii* and *S. polyphylla* remain in distinct clades [12, Fig. 1 and 2]. If the absence of diterpenoids in *S. simplex* would be confirmed, this would point out the applicability of subcategories in *Possira* classification.

Diversity Evaluation- The second evaluation was made by analysis of the diversity of the chemical structures of fifteen 8,11,13 *-trien-*-abietane diterpenoids identified from the species *S. arborescens* and *S. langsdorffi*.

Different from isoflavonoid chemical index calculations, the chemical indexes for terpenoids, as mentioned above, is evaluated by the oxidative degree (O) and by the carbon skeleton specialization (E) of their molecular structures. Table 2 shows the values of the chemical indexes obtained for *S. langsdorffii* metabolites. The same rationalization and kind of calculations led to the values displayed in Table 3 for the metabolites of *S. arborescens*.

Since the carbon skeleton is the same for all the isolated swartziarboreols, the carbon skeleton specialization index (E) is zero for all of them, and the value of $EA_E = \Sigma$ E/N is also zero. The differences between metabolites are related only to the oxidation indexes (O) calculated for each metabolite (Tables 2 and 3). Finally, Table 4 shows the EA_O and EA_E values found for each species, and indicate that, according to the chemical index methodology, *S. arborescens* is a more recent evolutionarily species than *S. langsdorffii*.

Conclusions

When considering chemical index methodology for taxonomic problem elucidation, the idea is to show a relationship between the plant evolutionary order found by the use of chemical indexes and the plant evolutionary order already described in the literature based on plant morphology and other data.

The use of the chemical index methodology demonstrates that molecular structure differences observed for the metabolites isolated from the two species that produce 8,11,13-trien-abietane diterpenoids, the native Brazilian tree *Swartzia langsdorffi* Raddii, included in the series *Recurvae* of *Swartzia* sect. *Swartzia*, and *S. arborescens* (Aubl.) Pittier (*Swartzia* sect. *Possira*), are related to oxidative processes and indicated an evolutionary direction.

Based on morphological data of *S. langsdorffii*, such as pattern venation, slender, axillary and shorter than leaf raceme, calyx shape, and androecium characteristics, and given the presence of terpenoids and terpenoidal saponins, we observe that this species is related to *S. arborescens* and to *S. simplex*, both pertaining to the section *Possira*.

Then, even though the aromatic diterpenoids found in *S. langsdorffii* were very similar to those isolated from *S. arborescens*, the chemical index values obtained for the two species allow suggesting that *S. arborescens* is evolutionarily more recent than *S. langsdorffii*. Those results corroborate the Cowan [2] evolutive line.

The **Torke & Schaal's** molecular phylogeny of the neotropical species of *Swartzia* [12] identifies 11 nonoverlapping clades, few of which correspond exactly to previously published taxa [2]. The grouping of the clades orthostyloid and benthamioid is a sister of the remaining *Swartzia* and, as mentioned above, is characterized by isoflavonoids. Thus, the presence of terpenoids in the clades possiroid and acutifolioid could be taken as an evolutionary novelty.

A weighted cluster analysis based on wood anatomical characters, which shows seven distinct groups within the Brazilian species of *Swartzia* [29], add data to this recent cladistic analysis and can be correlated with our results. The authors pointed out some similarity of their wood groups with Cowan's [2] classification. Although the species, which were studied phytochemically, are not enough, the results can be useful to indicate phyllogenetic and taxonomic relationships in *Swartzia*. The correlation of chemical data and data reported by Angyalossy-Alfonso & Miller [29] suggest an evolutionary tendency from group 4 (where are the species of orthostyloid and benthamioid clades) to group 5 (where *S. arborescens* is included). In this sense, for example, the multiseriate rays in *Swartzia* can be interpreted as derived from the exclusively uniseriate rays.

Considering the presence of metabolites as one discriminating character for *Swartzia* sect. *Swartzia*, it seems that there are two subgroups: the orthostyloid clade characterized by isoflavonoids not containing a tetracyclic ring system and benthamioid, recurvoid and tournateoid clades characterized by the occurrence of pterocarpenoids.

Finally, our analysis compares favourably to some clades within cladistic analysis using molecular data of **Torke & Schaal [12]**. The two main clades within *Swartzia* that were recovered by **Torke & Schaal**, the most interior node including most species and nine of the 11 supported subclades of *Swartzia* and other with orthostyloid and benthamioid clades, also finds support from the phytochemical data shown in this paper.

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Resumo: A análise das estruturas moleculares dos compostos isolados de nove plantas do gênero *Swartzia* mostrou que, na produção de metabólitos especiais, algumas plantas abandonaram a rota do ácido shikimic/acetato e adotaram a rota do ácido mevalônico. Esta mudança é fator indicativo de que essas espécies são evolutivamente mais recentes. Já a diversidade de estruturas moleculares encontrada nos dezesseis diterpenoides 8,11,13-trien-abietanos, chamados de swartziarboreols, identificados em *S. arborescens* e *S. langsdorffii*, permitiu atribuir a cada uma dessas espécies um índice químico, mostrando que os de *S. arborescens* são mais recentes do que os de *S. langsdorffii*. Os resultados sugerem que as espécies da seção *Possira* devam ocupar uma posição derivada em *Swartzia*.

Palavras-chave: Leguminosas, Sistematica do gênero *Swartzia*, evolução de plantas, índices químicos, quimiotaxonomia.

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