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BIOSYNTHESIS OF SESQUITERPENES IN *HYMENAEA* INFERRED FROM THEIR QUANTITATIVE CO-OCCURRENCE

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Abstract—Leaf pocket resins of 11 species of the tropical arborescent genus *Hymenaea* are virtually identical qualitatively, but of widely varying quantitative proportions. Within this large range of variability, several strong positive quantitative correlations between resin constituents were found, especially between caryophyllene and β-humulene and between γ-muurolene and δ-cadinene. These data lead to clarification of sesquiterpene biosynthetic routes in *Hymenaea*. In addition, quantitative relationships found among caryophyllene, α- and β-selinene, γ-muurolene and δ-cadinene are explained only with difficulty by long accepted biosynthetic pathways, and the intermediacy of germacrenes is suggested.

INTRODUCTION

The genus *Hymenaea* L. (Leguminosae, subfamily Caesalpinioideae, tribe Detarieae) includes fourteen species of resin-producing tropical trees found in habitats ranging from the true equatorial rainforest to dry thorn forest [1,2]. One species is found in rainforest along the eastern coast of Africa and the islands off that coast, whereas the other thirteen are Neotropical, ranging from central Mexico at about 23°N to Argentina at about 26°S. The single African species, *Hymenaea verrucosa*, has until a recent revision [3] been called *Trachylobium verrucosum*. Several studies of trunk resins and seed pod resins of both this species and some of the New World species have been made [4–10]. Moreover, as part of a long-range evolutionary study of the genus [11,12], we have made an extensive study of leaf pocket resins of seedlings of 11 species [13–15]. Leaf pockets are small ovoid to spheroid resin containers of about 100 μm in cross-sectional diameter, surrounded by a layer of secreting cells; their anatomy has been described in greater detail elsewhere [16]. The plants studied will be described in this report by their geographic population of origin, but all were grown from seed germinated and maintained under essentially identical environmental conditions in our greenhouses.

The leaf pocket resin of *Hymenaea* consists primarily of a mixture of sesquiterpene hydrocarbons, accompanied by trace amounts of low-boiling compounds, and occasionally some oxygenated sesquiterpenes [13]. We have isolated and spectrally characterized the individual components of leaf pocket resins of two *Hymenaea* species, the Old World *Hymenaea verrucosa* of Kenya [17] and the Neotropical *Hymenaea courbaril* of Mexico [18]. Finding the same set of sesquiterpenes in these two species, and the same major compounds in several other species, we have therefore accepted gas chromatographic identity of retention with the known compounds as sufficient identification in the other species and geographic populations analyzed.

The major compounds in *Hymenaea* leaf pocket resins are caryophyllene and α- and β-selinene; smaller proportions of α- and β-copaene, α-cubebene, β-humulene, γ-muurolene, and δ- and γ-cadinene occur, as well as a few unidentified minor components. It is the quantitative variation among these sesquiterpenes in the various *Hymenaea* species and populations that forms the basis for this report [13–15].

The difficulties in obtaining direct evidence of biosynthetic routes via the mevalonate pathway to specific lower terpenoids are well-known, and it now appears that compartmentalization of biosynthetic sites prevents significant incorporation of exogenous mevalonate or other precursors [19]. Consequently, hypothetical biosynthetic routes based mainly on chemical reasoning have been proposed to account for the structural classes of known compounds. For sesquiterpenes, the accepted scheme has usually assumed the derivation of these compounds from either *trans, trans-* or *trans,cis*-farnesyl pyrophosphate (FPP) [20]. This precursor is envisioned as

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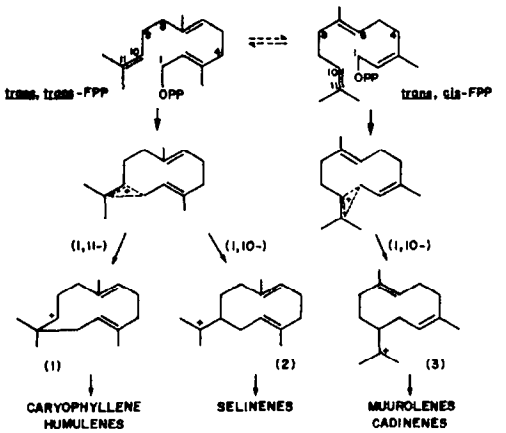

Fig. 1. Hypothetical biosynthetic routes to classes of sesquiterpenes in *Hymenaea*.

cyclizing to form several intermediates, from which the structures of known sesquiterpenes can be derived by suitable isomerizations, further cyclizations, and similar processes. The hypothetical pathways leading to the classes of sesquiterpenes known in *Hymenaea* are illustrated in Fig. 1.

Because these hypothetical biosynthetic schemes have not been amenable to experimental verification, indirect means of substantiating or modifying the proposed routes must be used. The indirect approach to biosynthesis used in this report involves the quantitative co-occurrence of compounds, and was developed by Zavarin [21]. He noted that not only do particular pairs of compounds tend to occur together, but there are often linear quantitative correlations between amounts of compounds, expressible by the general linear equation

$$Y = A + BX$$

Three special cases of this relationship are of interest:

(1) If B is zero, then Y is *independent* of X; this implies that biosynthetic mechanisms leading to these compounds are not closely linked, and quantities of compounds X and Y are controlled independently.

(2) If A is zero, changes in Y are *proportional* to changes in X (i.e. the two compounds are present in a fixed ratio). In this case, the biosynthetic paths are apparently very closely linked, as the enzyme system involved seemingly can exercise little control over the relative quantities of the two.

(3) If $B = -1$, then one compound is increased at the expense of the other, and the relationship may be termed *substitutional*. This type of correlation suggests partial restriction of enzyme systems acting to produce the two compounds.

Zavarin discusses possible reasons for existence of correlations of these types, and concludes that they most likely result from linkage of biosynthetic reactions, resulting in an apparent gradation in ability of enzyme systems to independently control the synthesis of individual compounds. Two interpretative rules are postulated:

I. Compounds in a proportional or substitutional relationship should be biosynthetically closer to each other than to a compound to which they possess an independent relationship.

II. Compounds in a proportional relationship should be biosynthetically closer to each other than to compounds to which they possess a substitutional or independent relationship.

Zavarin also points out that intermediate degrees of correlation between compounds are difficult to assess, and that biosynthetic speculations must therefore be based mainly on relationships approaching the extreme or special cases outlined above. He also cautions that biosynthetic inferences based upon this method should be considered in conjunction with all available other types of evidence [21].

RESULTS AND DISCUSSION

In *Hymenaea*, close proportionality was observed between caryophyllene and β-humulene. The relevant linear regression and correlation statistics for these two compounds in the *Hymenaea* populations studied are summarized in Table 1. Very strong positive correlations exist between caryophyllene and β-humulene in most geographic populations studied, and in those where this is not the case, usually the 2 compounds were present with such narrow ranges that the true relationship between them was difficult to establish. For example, in 8 plants analyzed of *H. courbaril* of Acaponeta, Mexico, the caryophyllene range was 25·0–37·2% (all but 1, 30·3–37·2) and the β-humulene range 3·8–5·2%.

A comparison of the regressions of β-humulene on caryophyllene in the *Hymenaea* populations studied was made by analysis of covariance (Table 2). The F-test for the comparison of slopes among all populations ($F =$ 1·03 with 29 and 142 degrees of freedom) is well below the corresponding 1% probability F-value of 1·85, and indicates that there is no significant difference among the populations in slope of their regression lines. There are, however, differences among the populations in β-humulene content, as indicated by the F-test of the adjusted populational means of β-humulene (i.e. the populational values adjusted by regression on caryophyllene).

When the mean contents of each analyzed population for β-humulene are plotted against mean contents of caryophyllene, the degree of proportionality between the compounds is again evident (Fig. 2). Two populations, *Hymenaea parvifolia* and *H. reticulata*, are very atypical in their ratios of these two compounds; both are relatively low in caryophyllene content, but appear to contain unusually large amounts of β-humulene. The evidence suggests, therefore, that the gas chromatographic peak accepted as β-humulene in these two populations may in part be due to some other sesquiterpene with retention properties nearly identical to β-humulene under the analytical conditions employed. We hope to clarify this in future work.

If *H. parvifolia* and *H. reticulata* are excluded, the correlation coefficient for caryophyllene and β-humulene in the remaining populations is 0·931; their regression equation is shown in Fig. 2. Their relationship is essentially proportional, with a low regression constant (A) approaching zero, and a positive slope. According to Rule II, these compounds appear to be closely linked in their biosynthesis. They have frequently been found to occur together on a qualitative basis in many other plant taxa, and it has therefore been assumed that they arise

Table 1. Linear regression analysis for caryophyllene and β-humulene (x,y) in *Hymenaea*

Hymenaea population	Locality	Sample number *n*	Correlation coefficient *r*	Constant *a*	Slope *b*	Standard error
H. courbaril	Brasília, D. F., Brazil	5	0·967	1·67	0·092	0·28
	Ceará, Brazil	7	0·957	1·73	0·081	0·82
	Vitória, Bahia, Brazil	8	0·950	1·20	0·113	0·68
	Palhão Reserve, Pará, Braz.	8	0·878	0·18	0·146	0·36
	Belém, Pará, Brazil	5	0·708	1·38	0·077	0·28
	Curuá-Una, Pará, Brazil	9	0·911	0·50	0·126	0·60
	Puerto Rico	8	0·894	1·96	0·120	0·51
	Honduras	4	0·987	−2·46	0·202	0·21
	Cañas, Costa Rica	8	0·510	2·55	0·104	0·97
	Alajuela, Costa Rica	8	0·669	0·78	0·150	1·25
	Puerto Marquéz, Mexico	10	0·715	0·84	0·133	1·04
	Tepic, Mexico	8	0·862	2·87	0·054	0·32
	Acaponeta, Mexico	8	−0·054	4·69	−0·006	0·46
	Venezuela	8	0·734	−0·23	0·198	0·63
H. courbaril var. *altissima*	São Paulo, S. P., Brazil	3	0·999	0·42	0·144	0·10
H. courbaril var. *subsessilis*	Manaus, Amazonas, Brazil	6	0·212	4·68	0·018	0·54
H. courbaril var. *stilbocarpa*	Brasília, D. F., Brazil	8	0·824	1·07	0·142	0·94
	Vitória, Bahia, Brazil	8	0·632	2·42	0·062	1·12
	Moji-Guaçu, S. P., Brazil	8	0·751	2·44	0·070	1·83
H. oblongifolia	Belém, Pará, Brazil	8	0·968	1·86	0·121	0·21
H. parvifolia	Manaus, Amazonas, Brazil	8	0·362	3·82	0·097	3·90
H. rubriflora	Recife, Pb., Brazil	3	0·991	−9·78	0·414	0·48
H. reticulata	Manaus, Amazonas, Brazil	5	−0·177	15·19	−0·080	2·64
H. eriogyne	Picos, Piauí, Brazil	3	0·999	3·30	0·064	0·76
H. velutina	Picos, Piauí, Brazil	5	0·622	0·58	0·047	0·33
H. stigonocarpa	Goiás, Brazil	5	0·462	1·09	0·066	1·02
H. stigonocarpa (?)	Assaré, Ceará, Brazil	4	0·967	3·10	0·087	0·37
H. martiana	Luziânia, Goiás, Brazil	8	0·387	1·93	0·056	0·57
H. aurea	Una, Bahia, Brazil	8	0·969	−1·74	0·214	0·56
Hymenaea sp.*	Osa Peninsula, Costa Rica	8	0·976	0·04	0·193	0·25

* Undescribed species; see refs. [5,6].

Table 2. Analysis of covariance comparison of regression lines for caryophyllene (independent) and β-humulene (dependent) content in *Hymenaea* populations

Source of variance	*d.f.*	*SS*	*MS*	*F*
Equality of adjusted group means	29	873·97	30·14	22·13
Zero slope	1	205·25	205·25	150·71
Error	171	232·89	1·36	
Equality of slopes	29	40·47	1·40	1·03 NS
Error	142	192·42	1·36	

from the same intermediate. Although caryophyllene can theoretically be derived from (1,11-) cyclization of *trans, cis*-FPP (Fig. 1), the demonstration of the all-*trans* configuration of α-humulene double bonds [22,23] does not permit simple derivation from this intermediate. It is therefore assumed in most biosynthetic schemes that both caryophyllene and α- and β-humulene arise from (1,11-) cyclization of *trans, trans*-FPP [20]. The above evidence in *Hymenaea* for proportional co-occurrence of caryophyllene and β-humulene is consistent with this interpretation.

Also occurring together in a strongly proportional relationship in most *Hymenaea* populations studied are γ-muurolene and δ-cadinene. In a few populations these compounds did not occur in amounts sufficient to be accurately determined, and these groups are excluded from the comparison. Only two of the remaining 27 populations had a correlation coefficient for these compounds of less than 0·82, only five less than 0·90. Comparing populational means, a high positive correlation ($r = 0·947$) was found between γ-muurolene and δ-cadinene among the *Hymenaea* groups. The plotted data and linear regression equation are shown in Fig. 3. The generally accepted biosynthetic sequence for these two compounds assumes their derivation from an intermediate resulting from (1,10-) cyclization of *trans, cis*-FPP (Fig. 1). It has been suggested by several workers that

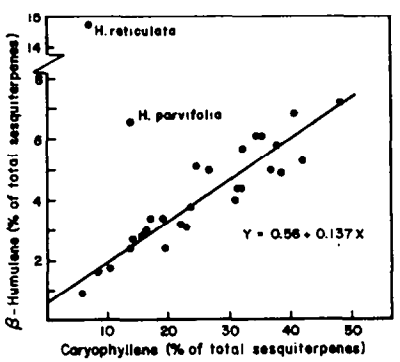

Fig. 2. Relationship of caryophyllene and β-humulene content in *Hymenaea*. Each point is the mean of a population.

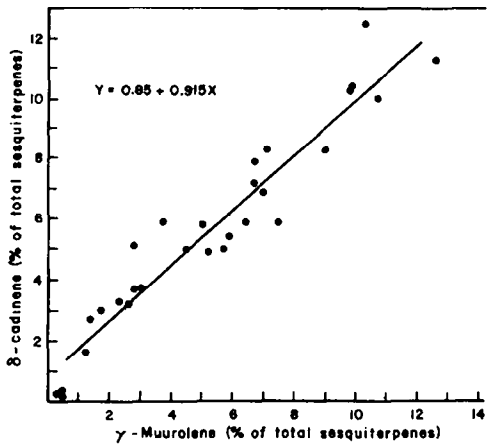

Fig. 3. Relationship of γ-muurolene and δ-cadinene content in *Hymenaea*. Each point is the mean of a population.

an intermediate cation (Fig. 4, structure 4) is formed by a 1,3-hydride shift from structure 3 of Fig. 1, and that four classes of closely related bicyclic sesquiterpenes are derived from this intermediate [24–26]. These classes are termed the bulgarane, amorphane, cadinane and muurolane groups, based upon the stereochemistry of their ring junctions relative to the isopropyl substituent (Fig. 4). In *Hymenaea*, only representatives of the latter two groups have yet been found. The structure of γ-muurolene is readily derived from structure 6 (Fig. 4) by loss of a proton. The structure of δ-cadinene, on the other hand, is such that it could conceivably arise from either structure 5 or 6. The best evidence bearing on this problem is that of Ohta *et al.* [24], who on the basis of acid catalyzed isomerization reactions concluded that δ-cadinene probably is derived via the muurolane group. The proportional relationship of δ-cadinene and γ-muurolene in *Hymenaea*, according to Rule II, strongly supports their close biosynthetic linkage, but does not *per se* provide evidence as to the probable biosynthetic route for δ-cadinene.

The copaenes and cubebenes are believed to be derived from the muurolane structural group [24–26]. The quantitative co-occurrence of α-copaene and α-cubebene in *Hymenaea* shows a positive correlation ($r = 0{\cdot}520$ for all analyzed populations), although the small amounts in which α-cubebene was usually present make it subject to greater quantitative analytical error. Similarly, α-copaene and β-copaene were weakly positively correlated ($r = 0{\cdot}287$) when comparing means of all studied *Hymenaea* populations. Because these compounds occur in *Hymenaea* in low concentrations with limited ranges, their relationships could not clearly be discerned.

Like caryophyllene, also proposed to be synthesized from *trans*, *trans*-FPP, but via (1,10-) cyclization (Fig. 1), are α- and β-selinene (reported together in this study as α + β-selinene, as no significance appeared to exist with respect to the isomer present, wide variations in their proportions occurring within a given population). A proportional relationship betwwen α + β-selinene and caryophyllene might be expected on the basis of their presumed derivation from a common carbonium ion (Fig. 1), but in most *Hymenaea* populations a substitutional relationship exists. This could be due in part to the fact that these two compounds are almost always the two major leaf pocket resin constituents, and that a decrease (or increase) in one results in an apparent increase (or decrease) in the other as a result of normalization of the quantitative data to 100% total sesquiterpenes. The influence of this effect is minimized in *Hymenaea* however, by the fact that the leaf pocket resin is of predominantly sesquiterpene hydrocarbon composition. Therefore, the proportions determined include essentially all of the products derived from farnesyl pyrophosphate, and an increase in one compound and decrease in another in one sample or population relative to another is probably real, not an artifact of normalization. Furthermore, in certain *Hymenaea* populations, quantitative leaf pocket resin compositions of two distinct types appeared to exist, between which α + β-selinene content varies greatly, but caryophyllene content does not vary accordingly. Details are available elsewhere [13,14], but as an example, in the Palhão Reserve, Pará, Brazil population of *Hymenaea courbaril* (Table 3, part A), four analyzed individuals had a mean α + β-selinene content of 71·3% and a mean caryophyllene content of 7·2% (compositional type I, Table 3). The mean total of these and the other minor components theoretically derived from *trans*, *trans*-FPP was 79·8%. The other four individuals analyzed from this population had a mean α + β-selinene content of just 25·8%, but caryophyllene content rose only to a mean of 13·3% (Type II in Table 3). The compounds presumably derived from *trans*, *trans*-FPP in this compositional group amounted to only 41·2%, whereas γ-muurolene and δ-cadinene combined had increased almost 15-fold from their proportion in the Type I plants, and now constituted nearly 38% of the total sesquiterpenes. The same division of analyzed plants into these two distinct resin compositional types was also found in four other populations of *Hymenaea courbaril*, and in the single population analyzed of each of two other species [13]. The existence of these large intrapopulational compositional differences (as well as significant differences among populations and species) were of obvious importance in recognition and computation of quantitative correlations between pairs of compounds, discussed above.

Also shown in Table 3, part B, is an example of a third resin compositional type, found along with some individuals of compositional type II in the *H. courbaril* var. *stilbocarpa* population of Moji-Guaçu, São Paulo, Brazil. From the preceding example of an inverse relationship between α + β-selinene and (γ-muurolene + δ-

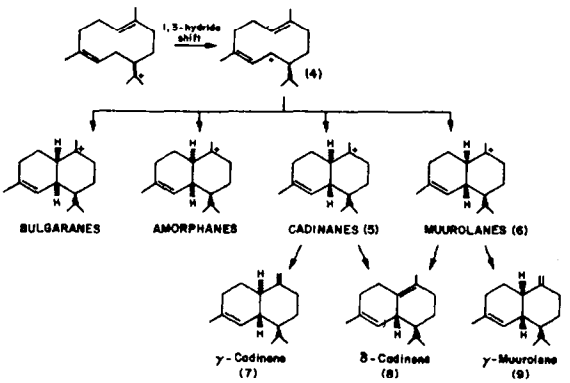

Fig. 4. Hypothetical biosynthetic routes to muurolanes, cadinanes, amorphanes and bulgaranes.

Table 3. Proportions of primary leaf pocket resin constituents in two *Hymenaea* populations showing division into three compositional types. Data are percentages of total sesquiterpenes

Population	Type	Sample	Caryophyllene	γ-Muurolene δ-Cadinene	α + β-Selinene
A.		1	9·3	1·7	69·3
H. courbaril		2	7·9	2·9	71·1
Palhão Reserve,	I	3	6·9	3·1	73·7
Pará, Brazil		4	4·5	2·5	70·9
		5	16·4	28·0	34·3
	II	6	15·8	40·5	19·6
		7	11·3	46·9	18·8
		8	9·7	35·1	30·5
B.		1	27·4	25·7	29·3
H. courbaril var.		2	24·9	27·6	23·5
stilbocarpa	II	3	21·0	17·0	42·0
Moji-Guaçu, S. P.,		4	20·2	23·4	37·2
Brazil		5	15·8	36·4	24·5
		6	78·1	4·0	8·6
	III	7	73·7	0·6	11·5
		8	71·7	3·5	10·3

cadinene), it might logically be expected that still lower selinene contents should be accompanied by still greater (γ-muurolene + δ-cadinene). In fact the opposite is true in plants grouped as Type III in Table 3; α + β-selinene content is lower than in Type II, but (γ-muurolene + δ-cadinene) is also very low, and instead caryophyllene content is very high. To explain these observations biosynthetically, one could postulate an altered ratio of cyclization of the carbonium ion leading via two routes to caryophyllene and α + β-selinene respectively (Fig. 1). The approximately ten-fold decrease in (γ-muurolene + δ-cadinene) content accompanying the large increase in caryophyllene, however, requires an assumption of a second type of biosynthetic alteration linked to the first if the derivation of these compounds from *trans, cis*-FPP is accepted. An alternative biosynthetic hypothesis which would account for these observations will be discussed below.

Returning to consideration of the Type I and Type II plants occurring together in the Palhão Reserve, Pará, Brazil population of *H. courbaril* (Table 3, Part A), if γ-muurolene and δ-cadinene are derived from *trans, cis*-FPP, and α + β-selinene, caryophyllene, and β-humulene from *trans, trans*-FPP, it is surprising that (δ-cadinene + γ-muurolene) content rises so dramatically in accompaniment to an α + β-selinene decrease. Biosynthetically, a shift in relative proportions of *trans, trans*- and *trans, cis*-FPP could account for the increased proportion of γ-muurolene and δ-cadinene, but it is not obvious why there would be only a small change in content of caryophyllene, and an enormous change in selinene content. In other words, because the selinene-to-caryophyllene ratio is not fixed, another biosynthetic alteration linked to the altered proportion of FPP isomers would be required, increased (1,10-) cyclization of *trans, trans*-FPP accompanying a shift to greater content of *trans, cis*-FPP (at whatever point this might occur). This explanation, like that for the Type II and Type III compositions in the other example of Table 3, is awkward, and one is led to seek an alternative biosynthetic hypothesis.

A class of sesquiterpenes with ten-membered rings, the germacranes, can theoretically be derived either by double bond isomerization of the intermediate carbonium ion arising from (1,10-) cyclization of *trans, cis*-FPP (Figure 4, structure 7), or by (1,10-) cyclization of *trans, trans*-FPP [27]. There have now been four germacratrienes isolated, and all have been shown to be readily rearranged by the action of heat, silica gel, or light [28]. Germacrene A co-occurs with β-selinene and β-elemene, and was found to undergo facile Cope rearrangement to β-elemene, which in turn rearranged on silicic acid chromatography to β-selinene [29]. Germacrene B has been found to occur with selina-3,7(11)-diene, selina-4(14),7(11)-diene, α-selinene (selina-3,11-diene) and β-selinene (selina-4(14),11-diene) in *Humulus lupulus* [30]. It was noted that many hop varieties contained all four selinenes and germacrene B, but many others contained only α- and β-selinene without germacrene B and the other two selinene isomers. Hartley and Fawcett also suggested that in the living plant, germacrene B might rearrange to form the four isomeric selinenes, "...due to the presence of α- and β-acids in the same resin glands as essential oils." Germacrene C has been shown to rearrange to yield selina-4,6-diene and selina-4(14),6-diene on standing over silica gel [31]. It was also noted that biogenetic differences in *Kadsura japonica* led to production of germacrene C in the fruits, and germacrene D in leaves and stem. Germacrene D, most interestingly of all, has been shown to isomerize readily on treatment with silica gel or heat, yielding a mixture of γ-muurolene, α-muurolene, δ-cadinene, γ-cadinene, and α-amorphene, while photoisomerization resulted in the production of β-bourbonene [32].

The frequent close associations of germacrenes and selinenes suggests that they may be biosynthetically closely related, either both being derived from a common intermediate or the germacrenes being biosynthetic intermediates from which the selinenes or other compounds are formed. Using these assumptions, a plausible biosynthetic sequence could be postulated for *Hymenaea* in which all the resin constituents could be derived from *trans, trans*-FPP (Fig. 5). If an intermediate obtained from (1,10-) cyclization gave rise to both germacrenes and selinenes, it is conceivable that further *in vivo* rear-

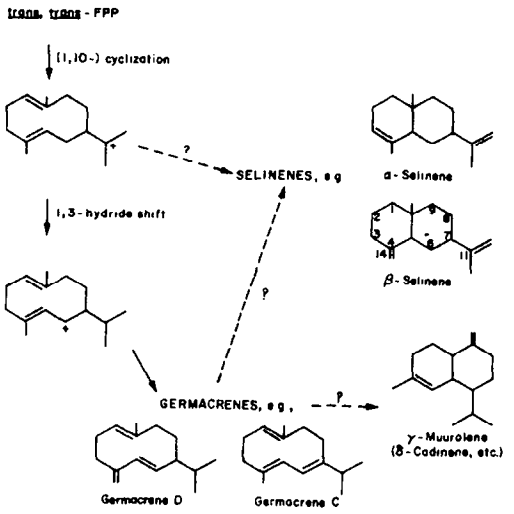

Fig. 5. An hypothetical pathway of sesquiterpene biosynthesis in *Hymenaea*.

rangement of a germacrene might yield the observed γ-muurolene and δ-cadinene proportional co-occurrence. A decrease in ring closure of the intermediate and shift to greater production of germacrenes would account for the observed increase in γ-muurolene and δ-cadinene accompanying dramatic decreases in α + β-selinene in some *Hymenaea* populations. Alternatively, if germacrenes were viewed as the precursors of both selinenes and muurolenes/cadinenes, a similar biosynthetic alteration, perhaps in the proportions of two germacrene isomers produced, could equally well lead to altered, substitutional-type ratios between the two compound classes. Likewise, either of the alternatives for selinene and cadinene-muurolene production would provide a ready explanation for the observed leaf resin compositional relationships of some *Hymenaea* individuals, those of Type III having very high caryophyllene contents but low α + β-selinene and (γ-muurolene + δ-cadinene). In this case the biosynthetic alteration would appear to be at the point of cyclization of the acyclic precursor. A shift to greater (1,11-) cyclization as opposed to (1,10-) cyclization would leave the selinene to (γ-muurolene + δ-cadinene) ratio largely unaffected, but increase the proportion of caryophyllene (and, proportionally, β-humulene). Yet another possibility is that germacrenes are not actual intermediates, but that cation 2 of Fig. 2 is transformed via a non-classical carbonium ion to a *trans, cis*-carbonium ion serving as a precursor for cadinanes and muurolanes. Such a mechanism has been outlined by Hirose [33].

In view of the thermal instability of germacrenes, if selinenes and muurolenes/cadinenes were derived via such a route in *Hymenaea*, it is necessary to consider that the observed leaf pocket resin compositions might in fact be artifacts of isolation. Several lines of evidence argue against this likelihood, although further attempts to examine the possibility may be indicated. Extracts of leaf pocket resins of *Hymenaea* were made at room temperature, concentration of solutions was made at room temperature, and gas chromatography was conducted at relatively low temperatures (119° for quantitative compositional analyses), although a heated injector was employed. Germacrene D was stable to gas chromatography at 140° [24], and germacrene B to 125° on PEGA stationary phase [30]. Germacrene A was stable on GLC on 5% Carbowax 20M (the same stationary phase used in the present study) at 110°, injector unheated [29]. Other than the heated injector employed in these studies of *Hymenaea*, the GLC conditions themselves would not appear likely to affect a possible germacrene constituent of the resin. Furthermore, reruns of a given sample were always consistent in compositional data, within the limits of instrumental variation, and resamplings of the same plant always yielded nearly identical quantitative results. If rearrangement of a labile intermediate on sample preparation or gas chromatography were occurring, one would not expect such reproducible results.

Still another possibility is that photoisomerization of a sensitive intermediate might be occurring in the plant. While this cannot be excluded, it would seem unlikely inasmuch as the large compositional variations were found in plants germinated and maintained side-by-side under as nearly identical environmental conditions as exist under greenhouse growth. Again, constancy of composition within the individual plant, whether on shaded or exposed leaves, also argues against this possibility. The most probable conclusion, it would thus appear, is that a true biosynthetic difference exists among plants showing the differing types of leaf pocket resin composition, all three of which have been found in one population of *H. courbaril* [13]. Thus far, compositional differences of the type described have been found in several Brazilian populations of *H. courbaril* and its varieties [14], and in the Brazilian *H. aurea* and *H. parvifolia* [13,15].

The attraction of the germacrene intermediacy hypothesis is that it enables us to account for the observed variations in quantitative leaf pocket resin composition in all our *Hymenaea* populations with only a single relatively simple biosynthetic alteration being required to explain a set of observations. This hypothesis seems to fit our data better than requiring the linking, in some inexplicable way, of more than one biosynthetic alteration as necessary for the long-accepted hypothetical biosynthetic pathway.

EXPERIMENTAL

Details of sesquiterpene isolation, identification, and resin composition in 11 *Hymenaea* species have been described elsewhere [13–15,17,18]. Sampled plants were germinated and grown under essentially identical environmental conditions in the greenhouses of the University of California, Santa Cruz. Plants were grown on a day/night temp. cycle averaging about 80° day and 70° night, kept well-watered, and relative humidity was maintained above 50% (usually above 70%) by misting units. Photoperiod was that existing naturally at Santa Cruz, California (36° 58′ N). Specimens documenting these collections will be deposited in the Herbarium, University of California, Berkeley. Et_2O extracts of leaves were quantitatively analyzed for sesquiterpenes by GLC (2% Carbowax 20M on Chromosorb G 80/100, AW, DMCS, 3 mm × 4 m, FID). Computations were made on PDP-11 and IBM 360 computers of the University of California, Santa Cruz.

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REFERENCES

1. Lee, Y. T. (1973) Ph.D. Dissertation, University of California, Santa Cruz.
2. Lee, Y. T. and Langenheim, J. H. (1975) *University of California Pubs. in Botany* **69**, (in press).
3. Lee, Y. T. and Langenheim, J. H. (1974) *Brittonia* **26**, 3.
4. Nakano, T. and Djerassi, C. (1961) *J. Org. Chem.* **26**, 167.
5. Hugel, G. and Ourisson, G. (1965) *Bull. Soc. Chim. Fr.*, 2903.
6. Hugel, G., Oehlschlager, A. C. and Ourisson, G. (1966) *Tetrahedron* **Suppl. 8**, Pt. I, 203.
7. Cunningham, A., Martin, S. S. and Langenheim, J. H. (1973) *Phytochemistry* **12**, 633.
8. Khoo, S. F., Oehlschlager, A. C. and Ourisson, G. (1973) *Tetrahedron* **29**, 3379.
9. Martin, S. S. and Langenheim, J. H. (1974) *Phytochemistry* **13**, 523.
10. Cunningham, A., Martin, S. S. and Langenheim, J. H. (1974) *Phytochemistry* **13**, 294.
11. Langenheim, J. H. (1969) *Science* **163**, 1157.
12. Langenheim, J. H. (1972) in *Tropical Forest Ecosystems in Africa and South America: A Comparative Review* (Meggers, B. J., Ayensu, E. S. and Duckworth, W. D., eds.), pp. 89–104, Smithsonian Institution Press, Washington, D.C.
13. Martin, S. S. (1973) Ph.D. Dissertation, University of California, Santa Cruz.
14. Martin, S. S., Langenheim, J. H. and Zavarin, E. (1974) *Biochem. Syst. and Ecol.* **2**, 75.
15. Martin, S. S., Langenheim, J. H. and Zavarin, E., in preparation.
16. Langenheim, J. H. (1967) *J. Arnold Arboretum* **48**, 203.
17. Martin, S. S., Langenheim, J. H. and Zavarin, E. (1973) *Biochem. Syst.* **1**, 35.
18. Martin, S. S., Langenheim, J. H. and Zavarin, E. (1972) *Phytochemistry* **11**, 3049.
19. Loomis, W. D. and Croteau, R. (1973) in *Terpenoids: Structure, Biogenesis, and Distribution* (Runeckles, V. C. and Mabry, T. J., eds.), pp. 147–185, Academic Press, New York.
20. Parker, W., Roberts, J. S. and Ramage, R. (1967) *Quart. Rev.* **21**, 331.
21. Zavarin, E. (1970) *Phytochemistry* **9**, 1049.
22. McPhail, A. T., Reed, R. I. and Sim, G. A. (1964) *Chem. Ind.* 1964, 976.
23. Hartsuck, J. A. and Paul, I. C. (1964) *Chem. Ind.* 1964, 977.
24. Ohta, Y., Ohara, K. and Hirose, Y. (1968) *Tetrahedron Letters* **39**, 4181.
25. Ohta, Y. and Hirose, Y. (1969) *Tetrahedron Letters* **20**, 1601.
26. Anderson, N. H. and Syrdal, D. D. (1970) *Phytochemistry* **9**, 1325.
27. Roberts, J. S. (1971) in *Terpenoids and Steroids* (Overton, K. H. *et al.*, eds.), Vol. 1, pp. 51–123, The Chemical Society, London.
28. Šorm, F. (1971) *J. Agr. Food Chem.* **19**, 1081.
29. Weinheimer, A. J., Youngblood, W. W., Washecheck, P. H., Karns, T. K. B. and Ciereszko, L. S. (1970) *Tetrahedron Letters* 1970, 497.
30. Hartley, R. D. and Fawcett, C. H. (1969) *Phytochemistry* **8**, 1793.
31. Morikawa, K. and Hirose, Y. (1969) *Tetrahedron Letters* 1969, 1799.
32. Yoshihara, K., Ohta, Y., Sakai, T. and Hirose, Y. (1969) *Tetrahedron Letters* 1969, 2263.
33. Hirose, Y. (1969) Abstr. of Papers, AGFD 28, 158th *Am. Chem. Soc. Natl. Mtg.*, New York, Sept. 1969.