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# SESQUITERPENES IN LEAF POCKET RESIN OF *HYMENAEA COURBARIL*

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**Key Word Index**—*Hymenaea courbaril*; Leguminosae; leaf pocket resin; sesquiterpenes; caryophyllene; α- and β-selinene; α-copaene.

**Abstract**—The major sesquiterpene hydrocarbons of *Hymenaea courbaril* leaf pocket resin were identified as caryophyllene and α- and β-selinene; α-cubebene, α-copaene, β-copaene, α- and β-humulene, γ-muurolene, and δ-cadinene were also present in smaller amounts.

## INTRODUCTION

THE EVOLUTION of the leguminous genus *Hymenaea* L. (Caesalpinioideae, Cynometreae),[1,2] which consists of a group of widely-distributed neotropical tree species, is being studied in our laboratory because of the relation of its resin to amber (fossil resin) from various Tertiary deposits in Mexico, Colombia and Brazil.[3] Seventeen species of *Hymenaea* are presently recognized, their distribution extending from central Mexico through Central America, the West Indies, and throughout South America except Chile and Uruguay. The resin of *Hymenaea*, known as 'Brazil copal', has been a commercially important product used in high grade varnishes. *Hymenaea* trees synthesize resin in all organs and secrete the material into intercellular spaces by two types of anatomical mechanisms.[4] In the trunk or root of the mature tree, cavities formed by the breakdown (lysigeny) of resin secretory cells coalesce into large resin-containing pockets. A natural or man-made opening in the bark provides an outlet for the viscous trunk or root resin. In addition to an early isolation of a diterpenoid resin acid from trunk resin of *H. courbaril*,[5] the most widely distributed species, we have since reported on the chemistry of trunk resins of eight species.[6,7]

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In leaves, floral parts and young stems or branches, resin is produced in parenchyma cells lining rounded pockets into which the resin is secreted. These pockets arise by schizogeny, the separation of cells permitting increased intercellular spaces surrounded by an epithelial layer of secretory cells.[4] Several thousand tiny pockets (approx. 50 × 50 × 70 $\mu$m) occur in a single leaf. As part of systematic investigations and studies of the role of environmental conditions on the synthesis and secretion of resins in *Hymenaea*, we have examined the composition of leaf pocket resins in nine species.[8,9] Here we report the isolation and identification of sesquiterpene hydrocarbons comprising the leaf pocket resin of *Hymenaea courbaril* L. Monoterpene hydrocarbons are absent (the leaves are not aromatic as in familiar terpenoid-producing plants of temperate regions) and preliminary investigation showed sesquiterpene hydrocarbons and a few oxygenated sesquiterpenoids to be the major leaf pocket resin constituents.

## RESULTS

An ether extract of fresh leaves was separated into acidic and neutral fractions. The neutral sample was chromatographed over silica gel to separate the hydrocarbon fraction, which was further separated by column chromatography on $AgNO_3$–silica gel. Isolated components were purified by GLC and identified primarily by IR (Table 1).

TABLE 1. SESQUITERPENE HYDROCARBONS IN LEAF POCKET RESIN OF *Hymenaea courbaril*

| Compound | Percentage* | Compound | Percentage* |
|---|---|---|---|
| α-Cubebene | 0·8 ± 0·1 | α- and β-Humulene | 5·7 ± 0·2 |
| α-Copaene | 9·4 ± 0·6 | γ-Muurolene | 3·5 ± 0·6 |
| Unidentified† | 3·4 ± 0·3 | β-Selinene | 18·4 ± 1·1 |
| β-Copaene | 5·7 ± 0·6 | α-Selinene | 17·6 ± 0·9 |
| Caryophyllene | 26·6 ± 1·5 | δ-Cadinene | 6·6 ± 1·2 |

* Mean (± s.e.) of 3 determinations by gas chromatography on 3 instruments, each with a different column and integrator. Expressed as percentage of total sesquiterpene hydrocarbons.

† GLC retention times suggest copacamphene.

An hypothetical scheme of sesquiterpene biogenesis[10] proceeds by cyclization of *trans,-trans-* and *trans,cis*-farnesyl pyrophosphates to six possible intermediate carbonium ions, transformations of which lead to the known compounds. Following this concept, in *Hymenaea courbaril* leaf pocket resin (considering only sesquiterpene hydrocarbons) the *trans,trans*-farnesol route appears to account for the greater proportion of the material (by about 2·5:1), with 1/10 (selinenes) and 1/11 (caryophyllene, humulene) initial cyclizations being approximately equally represented. Of the four possible cyclizations of *trans, cis*-farnesol (1/6, 1/7, 1/10, 1/11) only the 1/10 route has thus far been found to occur (cadinene, muurolene, copaenes).

In other studies we have examined leaf pocket resin composition in greenhouse-grown plants originating in twenty populations occupying differing habitats throughout the wide

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range of *H. courbaril* from central Mexico to Argentina. These will be discussed in detail elsewhere.

## EXPERIMENTAL

*IR spectra* were obtained neat or in $CCl_4$ using 0·5 mm microcells and a Perkin–Elmer 457 spectrophotometer. NMR spectra were obtained at 100 MHz or 60 MHz in $CDCl_3$ or $CCl_4$ with TMS internal standard. UV spectra were in EtOH. GLC for analytical purposes used 3% Carbowax 20M on Chromosorb G, 4 m × 3 mm, 102°, FID. For preparative GLC 1% Carbowax 20M on Chromosorb G, 18 m × 6 mm, 165°, was employed. MS determinations were at 70 eV.

*Sampling.* 169 g (fr. wt) of leaves of *Hymenaea courbaril* were obtained from a greenhouse-grown plant at Univ. of California, Santa Cruz (originating from Puerto Marquéz, Mexico; J. H. Langenheim No. 4969). Petioles and midribs were excluded, and the remaining leaf material was macerated and stirred with $Et_2O$ for 24 hr. Previous comparison of this method with extraction in a Soxhlet apparatus showed no significant difference in sesquiterpene hydrocarbons by GLC. On completion of detailed study of the genus now in progress, herbarium specimens fully covering all parts of the work will be deposited in the herbarium of the University of California, Berkeley.

*Separation.* The $Et_2O$ solution was filtered and the volume reduced, then exhaustively extracted with $Li_2CO$ (sat. aq.). The ethereal fraction was thoroughly washed, dried with $Na_2SO_4$, and $Et_2O$ removed by distillation. The remaining material was taken up in light petroleum (b.p. 37–55°) and chromatographed on silica gel, eluting with light petroleum. This hydrocarbon fraction (*ca.* 1·5 g) was column chromatographed on $AgNO_3$–silica gel[11] eluting with cyclohexane followed by $C_6H_6$–cyclohexane mixtures (1:50–1:5) then $Et_2O$–cyclohexane (1:20). Final purification of each compound from combined fractions was achieved by GLC. Detailed comparison of IR spectra with literature[12,13] was used for identification. α-Cubebene, β-copaene, α- and β-humulene (mixture), γ-muurolene, β-selinene, and α-selinene were identified by IR and MS. In addition to IR and MS, NMR and UV data confirmed identification of α-copaene, caryophyllene, and δ-cadinene. The only significant unidentified compound was apparently lost from the sample through a fraction collector malfunction; it is tentatively identified from GLC retention times as copacamphene.

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