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SESQUITERPENES IN LEAF POCKET RESINS OF *COPAIFERA* SPECIES

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Abstract—Four sesquiterpene leaf resin components were isolated and identified from *Copaifera* leaf resin. Additional GC and mass spectrometric evidence support the close similarity of *Copaifera* leaf pocket resin composition with that of the related genus, *Hymenaea*.

INTRODUCTION

Analysis of the sesquiterpenes in the genus *Copaifera* is part of a long-range investigation of the evolution of tropical leguminous resin-producing genera [1, 2]. *Copaifera* is an amphi-Atlantic leguminous genus and a member of the subfamily Caesalpinioideae and the tribe Detarieae. At present *ca* 30 species of *Copaifera* are recognized to occur in the New World [3, 4] and four in Africa [5]. A monographic revision is also currently in progress [Langenheim, J. H. and Freitas da Silva, M., unpublished results]. *Copaifera* is related to the genus *Hymenaea*, the resin chemistry, systematics and ecology of which have been studied in some detail in our laboratory [6–16]. In Africa, *Hymenaea* occurs exclusively in eastern coastal areas, whereas *Copaifera* is distributed only in the west; however, in the New World the two genera have essentially the same distribution with numerous species co-occurring throughout the lowland tropical ecosystems [17]. These distributional relationships, the occurrence of resin from both genera as amber (fossilized resin) and their general resin chemistry make the comparison of their evolution particularly interesting [1, 16, 17]. There are both striking similarities as well as differences in the resin chemistry and secretory structures of all organs of the two genera. Leaf resin in both genera is secreted from epithelial cells which line small, ovoid, schizogenously-produced pockets [18, 19]. Ten of *ca* 15 hydrocarbons have been identified from *Hymenaea* leaf resins with all species containing essentially the same compounds [6]. The leaf resin constituents are generally comprised of sesquiterpene hydrocarbons, although in some cases there may be a few oxygenated sesquiterpenoids. All of the sesquiterpenes identified in *Hymenaea* have also been identified in *Copaifera* wood resin [2, 20].

RESULTS AND DISCUSSION

Leaf resins representative of *Copaifera* were obtained from *C. officinalis* L. and *C. venezuelana* var. *laxa* Xena and Arroyo by steam distillation. Resin from *C. officinalis* was initially separated by prep. GC and the isolated components identified using IR. Only two compounds, γ-cadinene and caryophyllene were sufficiently purified in this case for conclusive IR identification due to the difficulty of separating the structurally closely related resin components by prep. GC alone. Therefore, in continued work, resin isolated from *Copaifera venezuelana* was first separated by CC on silver nitrate impregnated silica gel; then isolated compounds were further purified by prep. GC. Two additional sesquiterpenes, cyperene and α-copaene, were thus isolated and identified by IR. Further confirmation of the identities of these four compounds was obtained by GC and GC/MS of the purified components and by analysis of resin mixtures in which they occur.

On the basis of GC retention times established during routine analysis of many different samples separated on analytical packed Carbowax columns, the constituents of *Copaifera* appear to be the same as those in *Hymenaea* leaf resins; cochromatographed samples of *Hymenaea* and *Copaifera* leaf resins on these packed columns further show that the retention times are virtually the same for all of the constituents previously identified in *Hymenaea*. Additionally, analytical GC on glass open-tubular columns of polar (Carbowax 20M) and apolar (SP-2100) phases and GC/MS were used to compare identified *Hymenaea* leaf sesquiterpenes with the remaining unisolated components of *Copaifera*. In this system peaks corresponding to α-cubebene, α-copaene and β-humulene occur in resins from both genera. Further indications for the presence of γ-muurolene and β-selinene were found with the open tubular Carbowax column. However, some discrepancies for these compounds occurred when compared on the SP-2100 open tubular column. γ-Muurolene was not distinguishable on this column, which could be due either to lack of resolution on this apolar phase or to some alteration of γ-muurolene. Also, only α-selinene was resolved on this column. The presence of both selinene isomers—with different ones appearing on the two columns—may be explained by on-column interconversion, which has sometimes been observed on Carbowax packed columns in our previous studies of *Hymenaea*.

EXPERIMENTAL

Sampling. 765 g (dry wt) leaves of *Copaifera officinalis* collected near Mochima, El Sucre, Venezuela (collection No. JHL 6124)

and 896 g (dry wt) *Copaifera venezuelana* collected near Machiques, Zulia, Venezuela (collection No. JHL 6139), were powdered in a Waring blender and steam distilled using a Likens–Nickerson apparatus modified according to Maarse and Kepner [21].

Separation. The resin distillate from *Copaifera* was separated by prep. GC using 10% Carbowax 20M, 4 m × 5 mm, isothermal at 165°, TC. Resin from *Copaifera venezuelana* was initially passed through a basic alumina column eluting with *n*-pentane to remove oxygenated compounds. It was then separated on $AgNO_3$–Si gel eluting with light petrol (bp 37–55°), followed by cyclohexane–petrol mixtures (1:100–1:1) then C_6H_6–*n*-pentane mixtures (1:100–1:1) and finally Et_2O–C_6H_6 mixtures (1:100–1:5). These fractions were finally purified using prep. GC. Collected fractions were analysed by analytical GC (2.5% Carbowax 20M on Gas Chrom Q; 8 m × 0.3 mm; 130°; N_2 26 ml/min; FID).

Further analytical GC was carried out on WCOT columns coated with Carbowax 20M [22], 60 m × 0.3 mm; and SP-2100; 31 m × 0.25 mm, splitting injector temp. 165°, split ratio 1:50, column temp. 120.5°, isothermal, N_2 12.5 cm/sec, FID temp. 200°. GC/MS was carried out with the above columns, column temp. 135°, H_2 20 cm/sec, Finnigan 3200, ion source temp. 200°, 70 eV, Series 6000 data system. In addition to analysis of isolated sesquiterpenes, resin samples from *C. officinalis* (collection No. JHL 6124 and 6126) were compared with a pooled resin sample from *H. courbaril* L. from Guerrero, Mexico (collection Nos. JHL 4969, 5014, 5015) by open tubular GC and GC/MS.

IR spectra were obtained neat using 0.1 mm microcells and a Perkin-Elmer 237-B spectrophotometer grating IR (Barnes 0.1 mm microcell with beam condenser). Detailed comparison of IR spectra with literature was used for identification [23].

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