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RESIN ACIDS FROM TWO AMAZONIAN SPECIES OF *HYMENAEA*

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Key Word Index—*Hymenaea oblongifolia*; *Hymenaea parvifolia*; Leguminosae; resin acids; diterpenoids; guamáic acid.

Abstract—The major diterpene acid constituents of two Amazonian species of *Hymenaea* have been isolated and identified. A new resin acid, guamáic acid, is described.

INTRODUCTION

RESIN acids from the neotropical leguminous genus *Hymenaea* [Caesalpinioideae, Detarieae (Cynometreae)]* are of possible chemotaxonomic and evolutionary significance.[6] Our interest in them resulted initially from discovery that fossil resin from various Tertiary deposits in Mexico, Colombia and Brazil was derived from *Hymenaea*.[7] Seventeen species of *Hymenaea* are presently recognized, their distribution extending from central Mexico, throughout the West Indies and South America except Chile and Uruguay; the center of distribution appears to be in the Amazon Basin.[8,9] The closest relative of *Hymenaea* is *Trachylobium*, a monotypic genus restricted to the east African coast (Kenya, Tanzania and Mozambique) and the adjacent islands of Madagascar, Zanzibar, Mauritius and the Seychelles.

Both *Hymenaea* and *Trachylobium* synthesize resin in all organs.[10] However, the material is secreted by two different anatomical mechanisms and resin from different organs is characterized by a different set of terpenoids.[1] Sesquiterpenes are secreted into schizogenously-formed pockets in the leaves.[11] In the trunk or root of the mature tree, resin consisting primarily of diterpenoids is secreted into cavities produced by breakdown (lysigeny) of resin secretory cells. As a result of this lysigeny large quantities of resin may accumulate and are exuded to the outside when a natural or man-made opening occurs in

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* In our previous work,[1] we have used the tribal designation of Cynometreae which Leonard[2] had emended from Bentham.[3] Recently Heywood[4] has presented de Candolle's name Detarieae,[5] which apparently has priority over Bentham's name Cynometreae.

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[2] J. LÉONARD, *Memoire Classe des Sciences de l'Academie Royale de Belgique* XXX (2), 1 (1957).

[3] G. BENTHAM, *Hooker's J. Bot.* 2, 74 (1840).

[4] V. H. HEYWOOD, in *Chemotaxonomy of the Leguminosae* (edited by J. B. HARBORNE, D. BOULTER and B. L. TURNER), p. 1, Academic Press, London (1971).

[5] A. DE CANDOLLE, *Prodr.* 2, 94 (1825).

[6] J. B. HARBORNE, in *Chemotaxonomy of the Leguminosae* (edited by J. B. HARBORNE, D. BOULTER and B. L. TURNER), p. 277, Academic Press, London (1971).

[7] J. H. LANGENHEIM, *Science* 163, 1157 (1969).

[8] J. H. LANGENHEIM, Y. T. LEE and S. S. MARTIN, *Am. J. Bot.* 57, 754 (1970).

[9] J. H. LANGENHEIM, Y. T. LEE and S. S. MARTIN, *Am. J. Bot.* 58, 466 (1971).

[10] J. H. LANGENHEIM, *J. Arnold Arbor.*, 48, 203 (1967).

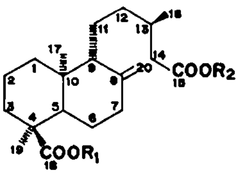
[11] S. S. MARTIN, J. H. LANGENHEIM and E. ZAVARIN, *Phytochem.* 11, 3049 (1972).

the bark. This trunk and root resin has been of commercial importance; that from *Hymenaea* is called 'Brazilian copal' and that from *Trachylobium* is known as 'Zanzibar copal'.

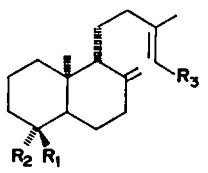
The close morphological relationship which has been noted between particularly the Amazonian species of *Hymenaea* and *Trachylobium* is also reflected in both the leaf pocket sesquiterpenes and the diterpenoid resin acid components.[12] A preliminary investigation of the resin acids of eight *Hymenaea* species and *Trachylobium verrucosum* (Gaertn.) Oliv. showed that differences in resin composition among certain *Hymenaea* species are at least as large as those found between the genera.[13] Here we report the isolation and characterization of the major diterpenoid trunk resin acids from two species of *Hymenaea*, *H. oblongifolia* Huber and *H. parvifolia* Ducke, distributed only within the Amazonian region.[1]

RESULTS

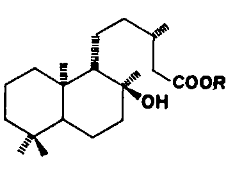
Two major constituents have been isolated from trunk resin samples of *H. oblongifolia*. By GLC these represent about 75% of the total resin acids. One of these compounds, *enantio*-pinifolic acid (*enantio*-labd-8(20)-en-15, 18-dioic acid, Ia), has been previously isolated from the trunk resin of *Trachylobium verrucosum*.[14] The physical and spectral properties of Ib are in agreement with the literature values,[14] and (except for the sign of rotation) are also in close agreement with properties of the enantiomer, dimethyl pinifolate, $\overline{\text{Ib}}$.[*,15,16]



(Ia) $R_1 = H$; $R_2 = H$
(Ib) $R_1 = Me$; $R_2 = Me$



(IIa) $R_1 = COOH$; $R_2 = Me$; $R_3 = COOH$
(IIb) $R_1 = COOMe$; $R_2 = Me$; $R_3 = COOMe$
(IIc) $R_1 = Me$; $R_2 = COOMe$; $R_3 = COOMe$



(IIIa) R = H
(IIIb) R = Me

We have given the name guamáic acid (*enantio*-labd-8 (20),13-dien-15,18-dioic acid, IIa) to the second acid isolated from *H. oblongifolia*. The common name is derived from the Área de Pesquisas de Ecológicas Guamá (Guamá Reserve), where this *H. oblongifolia* resin was collected. Esterification of IIa yielded dimethyl guamáate, IIb, a colorless liquid. Its IR spectrum showed absorption at 1747 (carbonyl), 1648, 1240 and 890 cm^{-1} (olefin). The mass spectrum of IIb was very similar to the published spectrum of dimethyl agathate ($\overline{\text{IIc}}$),[16] and showed the molecular ion at m/e 362 with prominent ions at m/e 302, 189, 121 (100%). The NMR spectrum of IIb showed two high field 3-hydrogen singlets at δ 0·71 and 0·96. A prominent 3H doublet (J 1·5 Hz) was centered at δ 2·11. Two 1H signals at δ 4·52 and 4·83 ($=CH_2$) and a 1H multiplet centered at δ 5·57 are attributable to olefinic protons. The position of the C-4 ester group can be inferred from spectral data; the strong IR absorption[17] at 1240 cm^{-1}, together with the C-10 methyl resonance in the NMR spectrum (δ 0·71),[18]

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[*] A bar placed over a numeral denotes an enantiomeric relationship.

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[13] S. S. MARTIN, J. H. LANGENHEIM and A. CUNNINGHAM, *Am. J. Bot.* **58**, 479 (1971).

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[16] C. R. ENZELL and R. RYHAGE, *Arkiv Kemi* **23**, 367 (165).

[17] S. BORY and M. FETIZON, *Bull. Soc. Chim. Fr.* 570 (1964).

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indicate that the C-4 carboxyl group is equatorial. The stereochemistry of the side chain (C-13 methyl *trans* to C-14 hydrogen) has been assigned on the basis of the chemical shift of the C-13 methyl group (δ 2·11), which is almost identical to that reported for dimethyl agathate (δ 2·12, C-13 methyl *trans* to C-14 hydrogen) but different from that expected (δ 1·90) for the *cis* isomer.[19] A *trans-anti* configuration of the *A*/*B* rings (*trans A*/*B* junction, C-9 hydrogen *trans* to C-10 methyl) is assumed in agreement with biogenetic considerations and consistent observation in this diterpene series.[20,21]

Enantio-13-epilabdanolic acid (IIIa) has been isolated as the major resin acid in a trunk resin sample of *H. parvifolia* (62% of total resin acids by GLC). The mass spectrum of the ester (IIIb) showed the molecular ion at *m*/*e* 338 and was very similar to a published spectrum for labdanolic acid.[16] Ester IIIb showed m.p. 70–71° and $[\alpha]_D$ −3·5° (lit.[22] m.p. 74–75°, $[\alpha]_D$ −3°), and an IR spectrum identical to that published for methyl 13-epilabdanolate.[23]

EXPERIMENTAL

NMR spectra were obtained at 60 or 100 MHz in $CDCl_3$ or CCl_4 with TMS as an internal standard. Rotations were obtained on a Jasco J20 spectropolarimeter in $CHCl_3$. GLC for quantitative purposes used 2% QF-1 on Chromosorb G, 3·5 m × 6 mm, 170°, FID. MS determinations were at 70 eV.

Samples. Hardened masses of exuded trunk resin of *Hymenaea oblongifolia* (J. H. Langenheim No. 5604 and 5607) were collected at the Guamá Reserve, Belém, Pará, Brazil. *Hymenaea parvifolia* resin (J. H. Langenheim No. 5623) was collected at the Palhão Reserve near Santarém, Pará, Brazil. Upon conclusion of a detailed study of the genus *Hymenaea* now in progress, herbarium specimens documenting these collections will be deposited in the herbarium of the University of California, Berkeley.

Extraction and isolation. Powdered samples of hardened trunk resin were extracted with Et_2O and the Et_2O soluble portion was partitioned with saturated Li_2CO_3 solution. The aqueous phase was adjusted to pH 3 with HOAc and the acids were again extracted into Et_2O. Evaporation of the Et_2O resulted in yields of 20–27% of resin acids. Channel-layer chromatography of the crude acid fraction followed by methylation (CH_2N_2) and TLC of the methyl esters on $AgNO_3$-silica gel was used to isolate the individual esters. GLC was used for estimation of proportions of the individual compounds.

Dimethyl enantio-pinifolate (Ib). Colorless liquid; $[\alpha]_D$ −23° (*c* 0·9, $CHCl_3$) (lit.[14] for dimethyl *enantio*-pinifolate $[\alpha]_D$ −26°, for dimethyl pinifolate[15] $[\alpha]_D$ +27°;) ν_{max}^{Kbr} (cm^{-1}) 1725, 1645, 1241, 891; NMR (CCl_4) δ 0·70 (*s*, 3H), 1·13 (*s*, 3H), 0·95 (*d*, *J* 5·5 Hz, 3H), 4·50 and 4·80 (each 1H, $=CH_2$); MS *m*/*e* 364 (M^+), 305, 304, 121 (100%).

Dimethyl guamáate (IIb).* Liquid from TLC distilled at pot temp. of 140° (100 μm); $[\alpha]_D$ −41° (*c* 0·9, $CHCl_3$), ν_{max}^{KBr} (cm^{-1}) 1747, 1648, 1240, 890; NMR (CCl_4) δ 0·71 (*s*, 3H), 0·96 (*s*, 3H), 2·11 (*d*, *J* 1·5 Hz, 3H) 4·52 and 4·83 (each 1H, $=CH_2$); MS *m*/*e* 362 (M^+), 180, 121 (100%).

Methyl enantio-13-epilabdanolate (IIIb). The ester from TLC was distilled at a pot temp. of 120° (35 μm) and recrystallized from hexane to give colorless needles, m.p. 70–71°, $[\alpha]_D$ −3·5° (*c* 1·1, $CHCl_3$) (lit.[18,22] for methyl *enantio*-13-epilabdanolate m.p. 70–71°, $[\alpha]_D$ −3°); ν_{max}^{KBr} (cm^{-1}) 3400, 1730, 1441, 1156, 940; NMR ($CDCl_3$) δ 1·15 (*s*, 3H), 0·96 (*d*, *J* 6 Hz, 3H), 0·79 (*s*, 6H); MS *m*/*e* 338 (M^+), 149, 137, 97, 82 (100%).

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Note added in proof. Dehydropinifolic acid, the enantiomer of guamaic acid, has been isolated from *Pinus silvestris* by Norin, Sundin and Theander [*Acta Chem. Scand.* **25,** 607 (1971)].

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