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GYMNOSPERMAE

PINACEAE

OCCURRENCE OF ANTICOPALIC ACID IN *PINUS MONTICOLA*

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Abstract—Anticopalic acid, a labdane diterpene not previously reported in the Gymnospermae, was found to be a major resin acid in the bark and in the wood of western white pine, *Pinus monticola* Dougl.

Copalic acid [*ent*-8(17), *E*-13-labdadien-15-oic acid][1] was first isolated from the trunk resin of *Hymenaea courbaril* as a mixture of double bond isomers (35% copalic acid).[2] The structure was deduced and confirmed by synthesis of the enantiomeric methyl ester from sclareol.[2,3] Copalic acid was later found in the trunk resin of *Trachylobium verrucosum*[4] and, with double bond isomers, in the wood of *Oxystigma oxyphyllum*;[5,6] in one instance,[5] the copalic acid was shown to be admixed with about one-fourth as much of its enantiomer, anticopalic acid (I). All the above plants are members of the Leguminosae, but in this work we report the occurrence of anticopalic acid in the gymnosperm *Pinus monticola* Dougl. (western white pine) bark and wood extracts and the first isolation of the acid in the form of its pure methyl ester. This is the fourth resin acid having a labdane skeleton to be isolated from the genus *Pinus*; the previous three are communic (elliotinoic),[7] pinifolic,[8] and antidaniellic (lambertianic).[9,10]

As a continuation of a detailed investigation of the benzene extract of western white pine bark,[11] an analysis was made of the fatty and resin acids.[12] The gel-permeation chromatogram (GPC is used to simplify the GLC analysis by providing a preliminary separation[13] of resin acids from fatty acids) showed the presence of an unexpected major component with a retention volume less than that of methyl pimarate, the earliest to elute of the common resin acids. The GLC retention characteristics on three different column packings, however,

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* Maintained at Madison, Wis., U.S.A. in cooperation with the University of Wisconsin.

[1] The systematic nomenclature follows the recent proposals of a committee chaired by Dr. J. W. Rowe, *The Common and Systematic Nomenclature of Cyclic Diterpenes*, Forest Products Laboratory, USDA, Madison, Wis. 1968; (with Addenda and Corrigenda, February 1969).

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[3] G. Ohloff, *Liebigs Ann. Chem.* **617**, 134 (1958). (See Ref. 2, *footnote* 20.)

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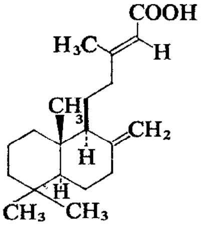
[11] W. C. Nickles and J. W. Rowe, *Forest Prod. J.* **12**, 374 (1962).

[12] J. W. Rowe, unpublished.

[13] D. F. Zinkel and L. C. Zank, *Anal. Chem.* **40**, 1144 (1968).

corresponded closely to methyl isopimarate. Further chromatography of the appropriate GPC fractions on silver nitrate-alumina followed by GPC and preparative GLC resulted in the isolation of a pure compound with physical and spectral properties in excellent agreement with those reported for the purest methyl copalate.[4] The rotation, however, shows that our compound is the enantiomer, methyl anticopalate. The resin acid fraction of the bark extract was estimated, via a combination of silver nitrate-column and GLC, to contain 26% anticopalic acid and about 50% isopimaric acid; also found were 15% dehydroabietic, 5% sandaracopimaric, 1% each of abietic and 6,8,11,13-abietatetraen-18-oic, and a trace of pimaric acid.

We have also isolated anticopalic acid from the ether extract of western white pine wood; the total resin acid fraction contained 55% anticopalic acid and 13% isopimaric acid as estimated by quantitative infrared analysis using the 1653 cm^{-1} C=C stretching band in conjunction with GLC of the appropriate GPC fractions. Although the resin acids of western white pine wood extract have been analyzed,[14] neither the GLC nor the TLC procedures as used were adequate for detecting anticopalic acid. The various labdane resin acids may provide an important phytochemical key to the classification of the pines.



I

EXPERIMENTAL

The free fatty acid-resin acid fraction of the benzene extract of western white pine bark,[12] obtained by DEAE-Sephadex ion exchange,[15] was methylated (CH_2N_2) and fractioned by GPC chromatography.[13] The GPC fractions that showed the presence of an unknown material (TLC on $AgNO_3$-alumina,[16] 3:1 petroleum-ethyl ether: R_f unknown 0·4; R_f isopimarate 0·1) and that had GPC retention characteristics between those of the fatty acid and the resin acid esters were combined. GLC retention characteristics for this material and methyl isopimarate were almost identical for three different liquid phases.

TABLE 1

	GLC*(r_{pim})		
	DEGS	SE-30/EGiP	OV-225
Methyl anticopalate	1·42	1·23	1·37
Methyl isopimarate	1·45†	1·21†	1·38

* DEGS and SE-30/EGiP conditions as described[17]; 5% OV-225 on 70–80 Anakrom ABS, 6 ft × 1/4 in. o.d., column oven at 200°, flow rate of 125 ml/min, $t_{pimarate}$ = 17·7 min.

† Data from Ref. 17.

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Chromatography of the 179 mg of combined material on 18 g of 40% $AgNO_3$–alumina in a 1·2 cm i.d. column with petroleum ether–benzene (10:1) yielded 97 mg of isopimarate-free product. Further purification by GPC followed by preparative GLC on 5% OV-225 yielded 42 mg of a colorless oil that showed no impurities on GLC or $AgNO_3$–alumina–TLC. The compound was distilled at a pot temperature of 143° (0·05 mm). (C, 79·15; H, 10·93Calc. for $C_{21}H_{34}O_2$: C, 79·19; H, 10·76%); $[\alpha]_D^{22} + 46°$ (c0·5, $CHCl_3$) (lit.[4] for methyl copalate, $[\alpha]_D$-45°); η_D^{21} 1·5152 (lit.[5] η_D^{20} 1·5130); $\lambda_{max}^{isooctane}$ 217·9 ($\epsilon = 15700$) and 200·6 nm ($\epsilon = 17000$) (conditions, see Burgstahler *et al.*[18]; ν_{max}^{film} 3041, 1650, 890 (olefin) and 1727, 1228, 1151 cm^{-1} (ester); NMR ($CDCl_3$), τ4·33 (quartet, $J = 1$, one olefinic H), 5·15 and 5·49 (singlets, $=CH_2$), 7·33 (sharp singlet, Me ester), 7·86 (doublet, $J = 1$, $CH_3C = C$, *cis* to ester), and 9·13, 9·20, and 9·32 (sharp singlets, 3 tertiary Me); *m/e* 318 (98%, M^+), 303 (100%, M^+-CH_3), 287 (12%, M^+-CH_3OH), 244 (35%, M^+-CH_3COOCH_3), 205 (71%, $C_{15}H_{25}^+$), 204 (60%, $C_{15}H_{24}^+$), 137 (78%, $C_{10}H_{17}^+$), 114 (80%, $C_6H_{10}O_2^+$), 95 (100%, $C_7H_{11}^+$), and 81 (99%, $C_6H_9^+$). Cf. reported spectra.[2,4,5] Anticopalic acid was isolated as the methyl ester (CH_2N_2) in a similar manner from western white pine wood (sample from the Clearwater National Forest in Idaho).

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ANGIOSPERMAE

APOCYNACEAE

CONSTITUENTS OF *ANODENDRON AFFINE*

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Plant. Anodendron affine (Hook. et Arn.) Durce.

Previous work. Alkaloid[1] and flavonoid.[2]

Stems. The MeOH extract concentrated, diluted with H_2O and filtrated. The filtrate fractionated with Et_2O and $CHCl_3$. The aqueous layer concentrated to dryness and extracted with $CHCl_3$–MeOH (2:1) by heating at 100°. The precipitate extracted with benzene and separated into acidic and neutral fractions with aq. alkali.

β-Sitosterol $C_{29}H_{50}O$ (m.p., mixed m.p., i.r. and TLC of alcohol and acetate): from neutral fraction, chromatographed on silica gel.

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