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TERPENOIDS OF *PINUS STROBUS* CORTEX TISSUE

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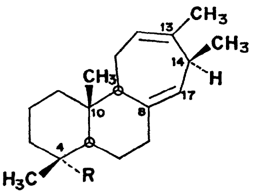
Key Word Index—*Pinus strobus*; Pinaceae; cortex tissue; terpenoids; 3β-methoxy-14-serraten-21-one; strobol; strobal; manoyl oxide; *cis*- and *trans*-abienol; polyprenol.

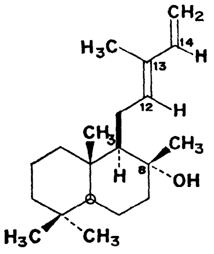
Abstract—The diterpenes, strobol, strobal, manoyl oxide, and *cis*- and *trans*-abienols, were isolated as major constituents of the extract of *Pinus strobus* L. cortex tissue. The known triterpene, 3β-methoxy-14-serraten-21-one, was also found. A polyprenol was isolated from the needle extractives.

Previous investigation of the diterpene resin acids of eastern white pine, *Pinus strobus* L., has resulted in the isolation and structure determination of a new resin acid, strobic acid[1] [14*S*,17-cyclolabda-8(17),12-dien-18-oate],[2] (I). The discovery that this novel diterpene contained a seven-membered ring prompted examination of the major constituents in the neutral fraction of *P. strobus* cortex extracts.

As most diterpene resin acids found in pine are accompanied by trace amounts of the corresponding aldehydes and alcohols, it was not unexpected that strobal (II) and strobol (III) occur in tissues containing strobic acid. However, the high relative level of strobal and strobol (26 and 32%, respectively, by weight of strobic acid) was surprising.



(I) R = COOH
(II) R = CHO
(III) R = CH2OH



(IV)

Lithium aluminum hydride conversion of strobic acid and strobal to alcohols that were identical in all respects with natural strobol confirmed the structures and stereochemistry of the strobal and strobol. *cis*-Abienol is widely distributed in the firs, but there is only one report of the isolation of the *trans* isomer, and that being from Canada balsam.[3] We

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

[1] D. F. Zinkel and B. P. Spalding, *Tetrahedron*; *Tetrahedron Letters* 2459 (1971).

[2] 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).

[3] R. M. Carman and N. Dennis, *Austral. J. Chem.* **21,** 823 (1968). P. F. Vlad, A. G. Russo and C. K'uang-fang, *Zh. Obshch. Khim.* **39,** 451 (1969) have shown that the 'isoabienol' isolated from *Abies siberica* [M. A. Chirkova *et al., Khim. Prir. Soedin* **2,** 99 (1966)] was actually (impure) *trans*-abienol.

now report the second observation of the co-occurrence of these isomeric abienols as well as the first occurrence of either isomer in the pines. Although we were concerned that the *trans*-abienol might be an artifact, scrutiny of our isolation and handling procedures and conditions (e.g. our laboratories are equipped with low-actinic fluorescent lights) gives no reason to doubt that it occurs naturally.

The triterpene, 3β-methoxy-14-serraten-21-one, was isolated as a minor component of the cortex extractives. This triterpene has been found in the bark of a number of pines[4–6] and of Sitka spruce.[7]

Preliminary study of *P. strobus* needle extractives (same trees from which the cortex was obtained) showed the presence of strobal as a minor component.[8] More interesting, however, was a waxy material obtained from a silica column and further purified from component *n*-octacosanol by column chromatography on silver nitrate–silica. The material was identified as a polyprenol on the basis of the NMR spectrum (C_6D_6):[9] δ 5·25 (broad multiplet, olefinic H), 4·00 (broad doublet, $J = 7$, $=CH-C\underline{H}_2OH$), 2·12 ($-C\underline{H}_2-$), 1·71 (*Z*, $C=C-C\underline{H}_3$), and 1·58 (E-ω-terminal methyl). Calculations based on the integration data for the olefinic, methylene, and methyl hydrogens compared with that for the hydroxy α-methylene hydrogens show the material to be a C_{90} polyprenyl containing 18 isoprene units (or a homologous series of polyprenols averaging 18 units). Detailed examination of the NMR spectrum shows the OH-terminal residue to be *Z* and that nearly if not all other double bonds are also *Z*.

EXPERIMENTAL

The non-volatile ether extract of fresh eastern white pine cortex tissue was separated (DEAE-Sephadex)[10] into neutral (24%) and fatty resin acid (48%) fractions. The neutral fraction was saponified using a modification of AOCS method Cd-6-38.[11] DEAE-Sephadex fractionation of the acidified product yielded an acid fraction consisting of the usual fatty acids found in pine (determined by GLC on DEGS) and a non-saponifiable fraction (NS) corresponding to 80% of the original neutrals. The NS fraction was chromatographed on silica and the eluate combined into three major fractions (*A*, *B*, *C*).

Fraction *A* was chromatographed on a 40% $AgNO_3$–silica column using stepwise gradient elution with Et_2O–light petrol. Manoyl oxide was the first significant constituent (6% of NS) eluted. Its identity was established by comparison of its NMR and GLC (DEGS and SE-30) characteristics with those for authentic manoyl oxide. Further elution produced the major component (19% of NS), strobal (II), which was crystallized from light petrol.: m.p. 78–78·5° (evac. capillary, corr.); $[\alpha]_D^{20}$ $-17·1°$ (*c* 1·9, $CHCl_3$); NMR ($CDCl_3$) δ 9·22 (*s*, $-C\underline{H}O$), 5·47 (*d*, $J = 7$, one olefinic H), 5·43 (*t*, $J = 7$, one olefinic H), 2·70 (*m*, one H at C-14), 1·66 (*s*, C-13 methyl), 1·16 (*d*, $J = 7$, C-14 methyl), 1·06 (*s*, C-4 methyl) and 0·88 (*s*, C-10 methyl); ν_{max}^{film} 2715, 2725 (2 bands, –CHO stretching) and 1721 cm^{-1} (C=O): (Found: C, 83·77; H, 10·62. $C_{20}H_{30}O$ required: C, 83·87; H, 10·56%). $LiAlH_4$ reduction of II and chromatography on silica yielded strobol (III); $[\alpha]_D^{23}$ $-35·8°$ ($CHCl_3$). Trace amounts of abietal, dehydroabietal, neoabietal, communal, and isopimaral were obtained by preparative GLC (DEGS) of fractions of the eluate from the $AgNO_3$–silica column. All were identified by GLC and NMR; UV added confirmatory evidence for the first three of these aldehydes.

Fraction *B* contained a material (~1% of NS) that was insoluble in pentane. This insoluble material was filtered and recrystallized from Et_2O–light petrol. It was identified as 3β-methoxy-14-serraten-21-one by TLC, GLC (SE-30), NMR, and IR comparison with authentic compound.[4]

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[4] J. W. ROWE and C. L. BOWER, *Tetrahedron Letters* 2745 (1965).

[5] J. W. ROWE, personal communication.

[6] T. NORIN and B. WINELL, unpublished.

[7] J. P. KUTNEY, I. H. ROGERS and J. W. ROWE, *Tetrahedron* **25**, 3731 (1969).

[8] The strobic acid content of the cortex resin acids was 17% but was only 2% of the needle resin acids (see D. F. ZINKEL and B. P. SPALDING, *Phytochem.* **11**, 425 (1972); and Ref. 1.

[9] For a detailed treatment of the NMR of polyprenols, see J. FEENEY and F. W. HEMMING, *Anal. Biochem.* **20**, 1 (1967).

[10] D. F. ZINKEL and J. W. ROWE, *Anal. Chem.* **36**, 1160 (1964).

[11] *Official and Tentative Methods of the American Oil Chemists Society*, 3rd Edn.

The pentane soluble portion of fraction *B* was chromatographed on 10% $AgNO_3$-silica using a stepwise gradient of Et_2O–light petrol. The first (3·3% of NS) of two major constituents to elute was identified as *cis*-abienol (*Z*-12,14-labdadien-8-ol:[2] $[\alpha]_D^{23}$ +20·8° ($CHCl_3$) [lit.,[3] $[\alpha]_D^{21}$ +22° ($CHCl_3$)]: and λ_{max}^{238} ($\epsilon = 17\,400$, isooctane) [lit.,[3] λ_{max}^{238} ($\epsilon = 19\,800$, EtOH)]; NMR (60 MHz, $CDCl_3$) AB_2 pattern[12] with the chemical shift of A at δ 6·86 (*q* of peaks at 7·14, 6·96, 6·86 and 6·68, H at C-15), 5·18 (*t* of peaks at 5·33, 5·18 with double intensity peak at 5·03, B_2 hydrogens at C-16), 5·50 (*t*, $J = 7$, H at C-12), 1·78 (*d*, C-13 methyl), 1·18 (*s*, C-8 methyl) and three methyl singlets at 0·87, 0·83 and 0·80. Comparison of NMR (also see published data[13,14]) and IR spectra with those for authentic *cis*-abienol confirmed the identification. The second constituent (2·6% of NS) that eluted was found to be *trans*-abienol (*E*-12,14-labdadien-8-ol, IV);[2] $[\alpha]_D^{23}$ +19·9° ($CHCl_3$) [lit., $[\alpha]_D$ +20° ($CHCl_3$)[3], $[\alpha]_D$ +25° ($CHCl_3$)[14]]; λ_{max}^{233} ($\epsilon = 24\,500$, isooctane) [lit., $\epsilon = 27\,100$[3] and $\epsilon = 27\,500$,[14] EtOH]; NMR[15] (60 MHz, $CDCl_3$) AB_2 pattern[12] with the chemical shift of A at δ6·30 (*q* of peaks at 6·60, 6·41, 6·30 and 6·13, H at C-15), 5·04 (*q* of peaks at 5·18, 4·99, 4·90 and 4·81, B_2 hydrogens at C-16), 5·58 (*t*, $J = 7$, H at C-12), 1·78 (*d*, $J \sim 1$, C-13 methyl, 1·18 (*s*, C-8 methyl), and three methyl singlets at 0·87, 0·84 and 0·80.

Fraction *C* was chromatographed on 10% $AgNO_3$–silica using a stepwise gradient of Et_2O–light petrol. Strobol (III) having $[\alpha]_D^{23}$ −36·7° was isolated as the major component (23% of NS).

$LiAlH_4$ reduction of methyl strobate yielded strobol: $[\alpha]_D^{23}$ −36·3° (*c*, 5·5, $CHCl_3$). It was sublimed for analysis: (Found: C, 83·20; H, 11·29. $C_{20}H_{32}O$ required: C, 83·28; H, 11·18%); NMR ($CDCl_3$) δ 5·45 (*d*, $J = 7$, one olefinic H), 5·41 (*t*, $J = 6$, one olefinic H), 3·25 (*AB* quartet, $J = 11$, $-C\underline{H}_2OH$), 2·72 (*m*, one H at C-14), 1·67 ((*s*, C-13 methyl), 1·18 (*d*, $J = 7$, C-14 methyl), 1·06 (*s*, C-4 methyl), and 0·88 (*s*, C-10 methyl); CD (*c*, 0·013, isooctane) $[\theta]_{260}$ ±0°, $[\theta]_{230\cdot5}$ −5800°, $[\theta]_{225}$ ±0°, $[\theta]_{215}^{inflection}$ +17 600°, $[\theta]_{210}$ +21 700°, $[\theta]_{203\cdot5}$ ±0°, $[\theta]_{198}$ −40 000°.

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[12] We have approximated the complex *ABB'* vinyl group as an AB_2 system and determined the chemical shifts for the *A* and *B* hydrogens according to H. J. Bernstein, J. A. Pople and W. G. Schneider, *Can. J. Chem.* **35,** 65 (1957).

[13] R. M. Carman, *Austral. J. Chem.* **19,** 1535 (1966).

[14] J. S. Mills, *J. Chem. Soc.* **C,** 2514 (1967).

[15] There is a greater discrepancy between our NMR data ($CDCl_3$) and that (CCl_4) of Carman and Dennis[3] than can be attributed to solvent effects. However, our comparative data for the *cis*- and *trans*-abienols is consistent and parallels data published for the *cis*- and *trans*-communates, B. R. Thomas, *Acta Chem. Scand.* **20,** 1074 (1966). Professor Carman (private communication) agrees as to the greater reliability of our data. Mills[14] also presents data for the *cis*- and *trans*-abienols. Although his chemical shifts and our figures agree within 0·1 ppm (apart from a printer's error for the C-15 quartet of *trans*-abienol which should read τ3·73 not 3·37), inspection of the NMR spectra, kindly furnished by Mr. Mills, disclosed some scale calibration errors which render his figures less sure.