Phytochemistry, 1969, Vol. 8, pp. 235 to 241. Pergamon Press. Printed in England

EXTRACTIVES OF JACK PINE BARK: OCCURRENCE OF *CIS*- AND *TRANS*-PINOSYLVIN DIMETHYL ETHER AND FERULIC ACID ESTERS[1]

J. W. ROWE, CAROL L. BOWER and E. R. WAGNER

Forest Products Laboratory,[2] Forest Service, U.S. Department of Agriculture, Madison, Wisconsin 53705

(*Received* 28 *June* 1968)

Abstract—A new natural product, *cis*-pinosylvin dimethyl ether (3,5-dimethoxy-*cis*-stilbene) has been isolated from the benzene extract of the bark of jack pine (*Pinus banksiana*). The only other nonpolar benzenoid extractives isolated were *trans*-pinosylvin dimethyl ether, wax alcohol esters of ferulic acid, dehydroabietic acid and related diterpenes, and phlobatannin esters. Data are given also on the wax alcohols, free and esterified wax acids, and *n*-paraffins.

INTRODUCTION

IN THE course of our investigation on softwood bark extractives, we examined the benzene extract (4·2 per cent dry weight basis) of the bark of jack pine (*Pinus banksiana* Lamb.). The isolations of sterols,[3] four serratenediol-related triterpenes,[4] and (+)-13-epimanoyl oxide and (+)-manoyl oxide[5] have already been reported.

In this paper we report the isolation of the *n*-aliphatics and of the nonterpenoid benzenoid components of this extract: *cis*- (0·35 per cent)[6] and *trans*-pinosylvin dimethyl ether (1·8 per cent), wax alcohol esters of ferulic acid (2 per cent), and phlobatannin (8 per cent) esters. The only other benzenoid components consist of the related series of diterpenes: dehydroabietic acid (20 per cent), 7-ketodehydroabietic acid (trace), dehydroabietol (0·07 per cent), 7-ketodehydroabietol (0·005 per cent), 18-nordehydroabiet-4α-ol[7] (0·04 per cent), and what is presumably dehydroabiet-18,6α-olide (0·01 per cent). These will be the subject of a future paper.

Extraction with more polar solvents should remove benzenoid derivatives related to the flavonoids and condensed tannins. Hergert has detected quercetin, 6-methylquercetin, 6-methylmyricetin, dihydroquercetin, dihydromyricetin, catechol, and vanillin in the ethanol extract of this bark,[8] a pattern similar to that of ponderosa pine (*P. ponderosa*) bark.[9]

[1] For the previous paper in this series see "The structure and the stereochemistry of abieslactone", by S. UYEO, J. OKADA, S. MATSUNAGA and J. W. ROWE, *Tetrahedron* **24**, 2817 (1968).
[2] Maintained at Madison, Wis., U.S.A., in cooperation with the University of Wisconsin.
[3] J. W. ROWE, *Phytochem.* **4**, 1 (1965).
[4] J. W. ROWE and C. L. BOWER, *Tetrahedron Letters* 2745 (1965).
[5] C. L. BOWER and J. W. ROWE, *Phytochem.* **6**, 151 (1967).
[6] This and subsequent percentages relate to the benzene extract.
[7] This new diterpene and the related new diterpene, 18-norpimara-8(14),15-dien-4α-ol, which was also isolated from jack pine bark, are analogous to the reported 18-norisopimara-7,15-dien-4α-ol (P. K. GRANT, C. HUNTRAKUL and D. R. J. SHEPPARD, *Australian J. Chem.* **20**, 969 (1967)) and 19-norpimara-8(14),15-dien-3-one (T. NORIN and B. WINNELL, 155th Nat. Meeting Am. Chem. Soc., San Francisco, Calif., April 4, 1968).
[8] H. L. HERGERT, Rayonier Inc., private communication.
[9] A. B. ANDERSON, *J. Inst. Wood Sci.* **10**, 29 (1962).

RESULTS AND DISCUSSIONS

The benzene extract was separated into 34 per cent neutral and 66 per cent acidic fractions by extraction with alkali. Wax acids were separated by formation of their urea canal inclusion complexes. Cooling a hot methanolic solution of the remaining free acids resulted in the crystallization of wax alcohol esters of ferulic acid. The structure of these was proved by ethylation to the wax alcohol esters of 3,4-dimethoxycinnamic acid, and by hydrolysis to ferulic acid and the free wax alcohols. These wax alcohols were shown by gas–liquid chromatography (GLC) to consist of stearyl, arachidyl, behenyl, lignoceryl, and ceryl alcohols in almost the same proportion as in the wax alcohol mixture found in the unsaponifiable fraction. The free and esterified wax acids have a distribution of chain lengths similar to that of the wax alcohols. The composition of these *n*-aliphatics is compared in Table 1.

TABLE 1. *n*-ALIPHATICS OF THE BENZENE EXTRACT

	(% of Extract)				
Alkyl chain length	Wax alcohols in unsaponifiables (4%)	Wax alcohol ferulates (2%)	Free wax acids (7%)	Wax acids in esters (7%)	Paraffins (0·06%)
16			0·3	0·02	
18	0·02	0·006	0·6	0·03	
19					
20	0·1	0·07	0·3	0·5	
21			0·06	trace	0·001
22	1·2	0·6	0·9	1·9	0·003
23	0·03		0·06	0·04	0·01
24	2·3	1·2	3·8	3·1	0·008
25			trace	trace	0·01
26	0·3	0·01	1·6	1·4	0·004
27					0·01
28			0·3		0·002
29					0·005
30					0·001
31					0·002

Wax alcohol esters of ferulic acid appear to be quite common in tree barks. In 1959, Brooker[10] reported the first isolation of wax alcohol ferulates (in the bark of *Phyllocladus glaucus*). However, in 1952, Hergert and Kurth[11] reported finding both wax alcohol and ferulic acid after saponification of the hexane extract of *Pseudotsuga menziesii* bark. Because ferulic acid is not soluble in hexane, it is to be expected that it was originally present as the wax alcohol ester. Since then, numerous reports indicating the presence of wax alcohol ferulates have appeared including *Pseudotsuga* spp.,[12] *Abies concolor* cork,[13] *Larix laricina*

[10] E. G. BROOKER, *New Zealand J. Sci.* **2**, 212 (1959).
[11] H. L. HERGERT and E. F. KURTH, *Tappi* **35**, 59 (1952).
[12] G. D. MANNERS, M.S. Thesis, Oregon State University (1965); G. D. MANNERS and H. AFT, 155th Nat. Meeting Am. Chem. Soc., San Francisco, Calif., April 4 (1968).
[13] H. L. HERGERT, *Forest Prod. J.* **11**, 335 (1958).

heartwood,[14] *L. lyallii* heartwood,[15] *A. amabilis* bark,[16,17] *Pinus palustris* bark,[17] and *P. sylvestris* bark.[18] We have also found compounds of this type in the ether extract of *Ulmus thomasii* heartwood.

Saponification of the neutral fraction of the benzene extract yielded 7 per cent homologous wax acids, 19 per cent unsaponifiables, and 8 per cent water-and-benzene-insoluble polyphenolic phlobatannins. The phlobatannins were not investigated further, because the presence of benzene-soluble phlobatannin esters is characteristic of a great many barks in which it is apparently produced by the phellogen as part of the production of suberin in the periderm.[19] The major portion of these phlobatannin esters is, of course, insoluble in inert solvents and gives rise on treatment with hot alkali to the "phenolic acids" and complex fatty constituents.

The sterols were removed from the unsaponifiables as their digitonides,[3] and then the *n*-aliphatics as their urea canal inclusion complexes. From the residual unsaponifiables, most of the stilbenes could be isolated in the form of their picric acid charge transfer complexes. Gas chromatography of the stilbenes showed that two constituents were present in the ratio of 1:10. Later chromatography of the residual unsaponifiables yielded a small additional amount of the same two stilbenes in the ratio of 2:3. The major stilbene was readily shown to be pinosylvin dimethyl ether (3,5-dimethoxy-*trans*-stilbene) by comparison of a crystalline sample with an authentic sample. The minor stilbene was eluted from an alumina column just before pinosylvin dimethyl ether, and was purified to analytical purity by preparative scale gas chromatography. The pure, oily, colorless 3,5-dimethoxy-*cis*-stilbene was characterized by its spectral and analytical properties, and the structure proved by isomerization to pinosylvin dimethyl ether.

Either pinosylvin or its monomethyl ether or both are very common constituents of pine heartwood (including jack pine heartwood).[20] However, the dimethyl ether has been reported in the heartwood of only *Pinus palustris*, *P. nigra*, *P. wallichiana* (formerly *P. griffithii* or *P. excelsa*), and *P. albicaulis*.[21,22] It has also been reported in the oleoresin from *P. cembra* var. *sibirica*,[23] southern pine wood rosin[24] (first reported natural occurrence), and pine tall oil.[25–28] Pinosylvin dimethyl ether is probably common in the genus *Pinus*, but the paper chromatographic proof of its presence is difficult.[29] Pinosylvin dimethyl ether has none of

[14] G. V. NAIR and E. VON RUDLOFF, *Can. J. Chem.* **37**, 1608 (1959).
[15] G. V. NAIR and E. VON RUDLOFF, *Can. J. Chem.* **38**, 177 (1960).
[16] H. L. HERGERT, NW Regional Amer. Chem. Soc. Meeting, Seattle, Wash., June (1959).
[17] H. L. HERGERT, 133rd Nat. Am. Chem. Soc. Meeting, San Francisco, Calif., Apr. 1958, p. 7E of abstracts.
[18] T. NORIN, see Ref. 7.
[19] L. M. SRIVASTAVA, in *International Review of Forestry Research* (edited by J. A. ROMBERGER and P. MIKOLA), Vol. 1, p. 203, Academic Press, New York (1964); W. JENSEN, K. E. KREMER, P. SIERILA and V. WARTIOVAARA in *The Chemistry of Wood* (edited by B. L. BROWNING) John Wiley, New York (1963).
[20] H. ERDTMAN, in *Chemical Plant Taxonomy* (edited by T. Swain) p. 89, Academic Press, New York (1963); H. ERDTMAN, B. KIMLAND and T. NORIN, *Botan. Mag. Tokyo* **79**, 499 (1966).
[21] G. BILLEK, in *Progress in the Chemistry of Organic Natural Products* (edited by L. ZECHMEISTER), Vol. 22, p. 115, Springer-Verlag, Vienna (1964).
[22] J. O. LINDSTROM and L. WESTFELT, *Ark. Kemi* **26**, 539 (1967).
[23] A. I. LISINA, N. K. KASHTANOVA, A. K. DZIZENKO and V. A. PENTEGOVA, *Izv. Sib. Otd. Akad, Nauk SSSR, Ser. Khim. Nauk* **1**, 165 (1967).
[24] R. F. B. COX, *J. Am. Chem. Soc.* **62**, 3512 (1940).
[25] H. ALBRECHT and E. H. SHEERS, *J. Am. Chem. Soc.* **76**, 603 (1954).
[26] J. B-SON BREDENBERG, *Soc. Sci. Fenn. Comment. Phys.-Math.* **24**, 1 (1960).
[27] H. P. KAUFMANN, *Fette, Seifen, Anstrichm.* **64**, 334 (1962).
[28] R. LUNDQUIST, *Ark. Kemi* **21**, 497 (1963).
[29] G. LINDSTEDT and A. MISIORNY, *Acta Chem. Scand.* **5**, 121 (1951).

the toxic decay-preventative properties of the free phenol or its monomethyl ether.[30] However, its presence might be explained by the fact that the enzyme systems in pines are apparently relatively nonspecific; thus they produce a series of closely related derivatives. Pinosylvin dimethyl ether may thus be a product of overmethylation by the same enzyme that converts pinosylvin to its monomethyl ether.

It has been claimed that the phenolics of pine bark are quite different from those of the heartwood, and that no traces of the heartwood flavonoids or stilbenes could be detected in the barks of *P. strobus*, *P. sylvestris*, and *P. banksiana*.[29] Our results suggest that for the stilbenes, at least, this is not true. Furthermore, pinosylvin and its monomethyl ether have been detected in the barks of *P. densiflora*, *P. thunbergii*, *P. armandii*, and *P. parviflora* (formerly *P. pentaphylla*).[31]

cis-Stilbenes have not been reported previously in *Pinus* species. However, *cis–trans* mixtures of polyhydroxystilbenes have been found in *Eucalyptus* species.[32] Thus, 3,5-dimethoxy-*cis*-stilbene is a new natural product, although it is known synthetically.[33] We suspect that *cis* stilbenes probably occur quite commonly in *Pinus* species, but that they have been overlooked because they are present in much smaller amounts, they lack the strong absorption in the u.v. at 299 nm, and the dimethyl ether, at least, is an oil.

The question arises whether 3,5-dimethoxy-*cis*-stilbene is a true natural product or an artifact formed, perhaps, by the natural irradiation of the bark. This was answered by a study of the isomerization of 3,5-dimethoxy-*cis*-stilbene with iodine in acetic acid to pinosylvin dimethyl ether. The isomerization followed first-order kinetics and produced an equilibrium mixture of about 96 per cent *trans*–4 per cent *cis*. Analogous isomerization of pinosylvin dimethyl ether yielded a maximum of 1·3 per cent of the *cis* isomer. Although these figures are subject to considerable error because of side reactions (such as to form the corresponding dihydrophenanthrene and phenanthrene[34]), the equilibrium ratio is, nevertheless, far below our observed results in which the *cis* isomer comprises 17 per cent of the dimethoxystilbenes.

TABLE 2. SPECTRA OF 3,5-DIMETHOXYSTILBENE

Spectrum	*cis*	*trans*
I.r. (film) cm^{-1}	1605 (s), 1592 (s), 1493 (w), 1458 (m), 1427 (m), 1403 (w), 1339 (w), 1312 (w), 1290 (w), 1252 (w), 1203 (s), 1155 (s), 1064 (s), 921 (w), 857 (m), 831 (w), 774 (w), 725 (w), 695 (m), 678 (m),	1603 (s), 1595 (s), 1495 (w), 1457 (m), 1425 (m), 1348 (w), 1319 (w), 1293 (w), 1269 (w), 1235 (w), 1202 (s), 1151 (s), 1063 (m), 954 (m), 922 (w), 863 (w), 824 (m), 751 (m), 691 (m),
NMR ($CDCl_3$) τ		
MeO—	6·38 sharp singlet	6·22 sharp singlet
C_6H_5—	2·77 sharp singlet	2·65 complex multiplet
C_6H_3≡	3·62 multiplet	3·59 triplet + 3·33 doublet (J = 2 Hz)
—CH=CH—	4·43 sharp singlet	2·96 sharp singlet
U.v. (EtOH) nm (ϵ)	λ_{max} 277 (11,200)	λ_{shldr} 306 (29,900) λ_{max} 298·5 (30,300) λ_{shldr} 234 (18,850) λ_{max} 228 (19,400)

[30] K. O. FRYKHOLM, *Nature* **155**, 454 (1945).
[31] K. HATA and M. SOGO, *J. Jap. Forestry Soc.* **36**, 8 (1954).
[32] D. E. HATHWAY, *Biochem. J.* **83**, 80 (1962); W. E. HILLIS and A. CARLE, *Biochem. J.* **82**, 435 (1962).
[33] G. AULIN-ERDTMAN and H. ERDTMAN, *Berichte* **74B**, 50 (1941).
[34] F. B. MALORY, C. S. WOOD and J. T. GORDON, *J. Am. Chem. Soc.* **86**, 3094 (1964).

The spectral properties of the *cis* and *trans* isomers are dramatically different (Table 2). The differences in the u.v. spectra are typical of stilbene isomers in which the λ_{max} for the *trans* isomer is shifted 15–40 nm to longer wavelengths, and the value of the extinction coefficient is 1½–3 times as great.[35] The differences in the NMR spectra, especially for the splitting of the unsubstituted phenyl and the chemical shift of the vinylic hydrogens, parallel the observed differences in the spectra of *cis*- and *trans*-stilbene.[36]

EXPERIMENTAL

Isolation of Free Wax Acids

The wax (630 g) extracted from 15 kg of jack pine bark by benzene was dissolved in benzene and extracted exhaustively with several portions of cold 0·5 N NaOH. The alkaline extracts were washed several times with fresh benzene. Working up in the usual way yielded 215 g of neutrals and 413 g of acids.

The acids were dissolved in 400 ml of benzene and 4 l. of hot 95 per cent ethanol added. Urea (850 g) was then dissolved in the solution at reflux. Benzene (5 l.) was added to the refluxing solution that was then cooled overnight, and the urea canal inclusion complex[37] of the wax acids filtered off. The complex and the filtrate were each added to water and then extracted with benzene to yield the wax and the noncomplexing acids. The acids from the filtrate were treated once again in the same way with urea. The combined wax acids were again treated with urea.

The total yield was 365 g of noncomplexing acids and 44·5 g of wax acids. These yellow wax acids were crystallized from methanol and from hexane to yield colorless wax acids, m.p. 76–76·5° cor., neut. eq., 369·5. The i.r. spectrum was superimposable on that of lignoceric acid, and no color was produced with tetranitromethane.

The methyl esters, m.p. 54–54·5° cor., were formed by treatment with diazomethane and gas chromatographed on 7 per cent EGIP (Ethylene Glycol IsoPhthalate) on Gas Chrom P at 220° to give the results shown in Table 1.

Isolation of Wax Alcohol Ferulate

The noncomplexing acids were dissolved in 4 l. of hot methanol and cooled overnight in the deep freeze to give 15·5 g of a half-crystalline mass. This was chromatographed on 450 g of silica in a 31 mm tube. Four per cent methanol in CCl_4 eluted 12·6 g of low melting crystals that were crystallized alternately from benzene–methanol and hexane for analysis, m.p. 62–64° cor. (Found: C, 77·23; H, 10·90; MeO, 5·73. Calc. for $C_{34}H_{58}O_4$: C, 76·93; H, 11·01; MeO, 5·85 per cent.)

The i.r. spectrum (melted film) showed a phenol (3400 cm^{-1}), an ester (1709 and 1264 cm^{-1}), all bands characteristic of ferulate as present in authentic ethyl ferulate, and $-(CH_2)_x-$ (719 cm^{-1}). The u.v. spectrum in absolute ethanol had the same shape as that of authentic ethyl ferulate and showed $\epsilon_{200\,nm} = 16{,}800$, λ_{shldr} 217 nm ($\epsilon = 12{,}600$), λ_{max} 235 nm ($\epsilon = 10{,}600$) λ_{shldr} 298 nm ($\epsilon = 11{,}000$), and λ_{max} 325 nm ($\epsilon = 15{,}700$). The NMR spectrum ($CDCl_3$) showed a doublet at τ 2·37 ($J = 16$ Hz) for phenyl-CH=, and a doublet at τ 3·72 ($J = 16$ Hz) for =CH—COOR. The coupling constant indicates a *trans* double bond. Also present is a doublet at τ 3·00 for three aromatic hydrogens, an ill-defined quartet centered at τ 5·82 for $-CH_2O-$, a a sharp singlet at τ 6·08 for CH_3O-, a large sharp singlet at τ 8·73 for methylene, and an unresolved weak triplet for the aliphatic methyl at τ 9·12. The NMR spectrum was identical to that of authentic ethyl ferulate except for the expected difference in the methylene region.

Methylation of Wax Alcohol Ferulate

The wax alcohol ferulates (4·50 g) were methylated with an excess of diazomethane in methanol–ether in the refrigerator over the weekend. The crude product was chromatographed on 140 g of alumina (Woelm, neutral, activity II). Light petroleum–benzene (4:1 and 1:1) eluted 3·68 g of wax alcohol 3,4-dimethoxycinnamates that were crystallized twice from benzene–methanol and twice from hexane for analysis, m.p. 62·5–80°. (Found: C, 77·69; H, 11·50; MeO, 11·71. Calc. for $C_{35}H_{60}O_4$: C, 77·15; H, 11·10; MeO, 11·38 per cent.)

The u.v. spectrum was identical to that of authentic methyl 3,4-dimethoxycinnamate with maxima at 325, 236, and 218 nm and a shoulder at 297 nm. The i.r. spectrum was very similar to that of authentic methyl 3,4-dimethoxycinnamate except for the presence in the former of the methylene bands at 2907, 730 and 719 cm^{-1}.

[35] D. F. DeTar and L. A. Carpino, *J. Am. Chem. Soc.* **78**, 475 (1956).
[36] Varian Associates, NMR Spectra Catalog, compiled by N. S. Bhacca *et al.*, Nos. 305 and 306.
[37] E. von Rudloff, *Chem. & Ind.* 338 (1951).

Saponification of Wax Alcohol Ferulate

The wax alcohol ferulate (6·47 g) was saponified by refluxing 4 hr with 2 N ethanolic KOH in N_2. The solution was then poured into water, and the wax alcohols extracted with benzene. Acidification of the aqueous layer and extraction with ether gave crude ferulic acid whose u.v. and thin-layer chromatogram on silica were identical to that of authentic ferulic acid.

The wax alcohols (3·66 g) were treated with urea as before, the filtrate discarded, and the urea canal inclusion complex decomposed to yield 3·22 g of waxy material. This was rapidly chromatographed through 10 g of alumina (Woelm, neutral, activity II). Benzene and ether eluted 3·14 g of wax alcohols that were crystallized once from hexane for analysis, m.p. 71·5–73·5° cor.

The i.r. spectrum was identical to that of authentic lignoceryl alcohol. The NMR spectrum ($CDCl_3$) showed a sharp triplet at τ 6·36 (J=6 Hz) for a $—CH_2O—$, a sharp singlet at τ 8·73 for approximately 22 methylene groups, and an unresolved triplet at τ 9·12 for the end methyl. No color was produced with tetranitromethane. The mixture was analyzed by GLC on a 2·5 per cent SE-30 column, and the peaks identified by a log plot based on known standards. Results are given in Table 1.

Isolation of Wax Acids from Esters

The neutrals from the benzene extract (215 g) in 400 ml of benzene were added to 2·5 l. of 2·5 N ethanolic NaOH and refluxed under nitrogen for 6 hr. The solution was then poured into water, and the aqueous layer extracted repeatedly with benzene. The benzene layers were extracted repeatedly with fresh dilute alkali. Emulsions and interfacial precipitates caused considerable difficulty. Either sodium chloride or ethanol or both were added at various times to aid separation. The benzene layers yielded 120 g of unsaponifiables.

The combined aqueous layers containing the interfacial precipitates were acidified and extracted with benzene. Filtration of the two-phase system gave 50 g of a reddish brown phlobatannin insoluble in water or benzene but soluble in hot ethanol. Completing the extraction in the usual way yielded 45 g of wax acids that were purified via the urea canal inclusion complex as before. Decomposition of the complex yielded 42 g of colorless crystals that were crystallized from methanol and from hexane for analysis, m.p. 75·5–76·5° cor., neut. eq. 371·3. The i.r. spectrum was superimposable on that of lignoceric acid, and no color was produced with tetranitromethane.

The methyl esters were formed by treatment with diazomethane and gas chromatographed on the same column as before to yield the results shown in Table 1.

Isolation of the n-Aliphatics from the Unsaponifiables

The unsaponifiables (120 g) were treated twice with excess digitonin to remove 13 g of sterols.[3] The sterol-free unsaponifiables were then treated twice with urea as were the free acids, and the combined *n*-aliphatics treated again with urea to yield 25·6 g of *n*-aliphatics. These were chromatographed on 800 g of alumina (Woelm, neutral, activity II). Light petroleum eluted 800 mg of a hydrocarbon fraction. Light petroleum–benzene (9:1) eluted 500 mg of a dirty oil. More polar solvents then eluted 23·3 g of crystalline wax alcohol.

The hydrocarbon fraction in *n*-hexane was purified by filtration through a column of alumina (Woelm, alkaline, activity I) to yield 380 mg of a colorless wax that was crystallized from benzene–methanol, m.p. 60–70·5°. (Found: C, 85·17; H, 14·75 per cent.) The i.r. spectrum was as expected. The *n*-paraffins were analyzed by GLC on 7 per cent EGIP on Gas Chrom P at 220°, and the peaks identified by a log plot based on known standards. Results are given in Table 1.

The wax alcohol was crystallized from hexane, m.p. 73·5–74·5°. The i.r. spectrum was identical to that of the wax alcohol from the ferulate esters. They were analyzed by GLC as before to give the results shown in Table 1.

Isolation of Pinosylvin Dimethyl Ether

The unsaponifiables after removal of sterols and *n*-aliphatics (81 g) were dissolved in 200 ml of CCl_4 and added to 1·5 l. of a saturated solution of picric acid in 95 per cent ethanol at 25°. The solution was placed in the refrigerator overnight whereupon orange crystals deposited. These were filtered and washed with CCl_4, m.p. 107·5–109° (reported for pinosylvin dimethyl ether picrate, 109–110°).[25] The crystalline product was partitioned between aqueous ammonia and ether, and the ether extract dried and evaporated to yield 10·35 g of brownish oil.

Examination of the u.v. spectrum showed it to be only about 90 per cent pure. Gas chromatography (see 3,5-dimethoxy-*cis*-stilbene) showed about 10 per cent of the *cis* isomer present. The crude pinosylvin dimethyl ether was crystallized from hexane, decolorized with active carbon, recrystallized from hexane and then from methanol for analysis, m.p. 56–57° cor., undepressed on admixture with an authentic sample from Professor H. Erdtman. (Found: C, 80·18; H, 6·74; MeO, 25·66. Calc. for $C_{16}H_{16}O_2$: C, 79·97; H, 6·71; MeO 25·83 per cent.) The i.r., NMR, and u.v. spectra (Table 2) were superimposable on those of an authentic sample.

Examination of the u.v. spectrum of the picric acid-free filtrate from crystallization of the picrate indicated that precipitation of the pinosylvin dimethyl ether was not complete.

Isolation of 3,5-*Dimethoxy*-cis-*Stilbene*

The picric acid-free filtrate (70·6 g) was dissolved in 750 ml of hot hexane and cooled to −20° overnight. Filtration gave 26·7 g of material called hexane-insoluble neutral terpenoids plus 43·9 g of what was called hexane-soluble neutral terpenoids. The hexane-soluble neutral terpenoids were chromatographed on alumina as described yielding first terpene hydrocarbons and then a mixture of (+)-manoyl oxide and (+)-13-epimanoyl oxide.[5] Immediately following this, light petroleum and light petroleum–benzene (9:1) eluted a yellow oil (6 g) that by gas GLC was shown to contain 3·2 g of *trans*- and *cis*-pinosylvin dimethyl ether in the ratio of 3:2. GLC columns were 2·5 per cent SE-30 on 70/80 Anakrom ABS and 6 per cent DEGS (Diethylene Glycol Succinate) on 1 per cent polyvinylpyrrolidinone pretreated 70/80 Anakrom ABS in ¼-in. o.d., 6-ft stainless-steel columns at about 203° and a flow rate of about 60 ml of He per min. The *cis* isomer had an elution time of 2·2 min from SE-30 and 14·0 min from DEGS. The *trans* isomer had an elution time of 4·9 min from SE-30 and 55 min from DEGS.

The first 4 g of this yellow oil, which were the richest in the *cis* isomer, were fractionally distilled at 0·24 mm to yield 1·235 g of material boiling sharply at 156° that analyzed for 63 per cent *cis* plus 9 per cent *trans*. The *trans* isomer distilled at above 200°.

This enriched fraction was further purified by preparative scale GLC on 3 per cent SE-30 that effectively removed the *trans* isomer, and then on 7 per cent EGiP to remove the remaining impurities (especially manoyl and epimanoyl oxides). The chromatographically pure 3,5-dimethoxy-*cis*-stilbene was distilled for analysis, b.p. 123° at 0·02 mm Hg (reported: 150° at 0·1 mm of Hg),[33] $n_D^{27°}$ 1·3161, $d_4^{27°}$ 1·11, $M_D^{27°}$ 42·4. (Found: C, 80·36; H, 6·93; MeO, 25·73. Calc. for $C_{16}H_{16}O_2$: C, 79·97; H, 6·71; MeO, 25·83 per cent.) The mass spectrum showed the molecular ion as the base peak at *m/e* 240. The u.v., i.r. and NMR spectra are given in Table 2.

Proof of Structure of 3,5-*Dimethoxy*-cis-*Stilbene*

Pure 3,5-dimethoxy-*cis*-stilbene (29 mg) was dissolved in 87 ml of glacial acetic acid with a crystal of I_2 and refluxed under nitrogen for 33 hr. Samples (2 ml each) were taken at 0·5, 1, 2, 4, 6, 8, 23, 27, 31, and 33 hr and extracted with alkali. The isomerization was followed by GLC on SE-30 and area *cis* isomer/area *cis* plus *trans* isomers was plotted against time.

The results were observed to follow first-order kinetics, although the data at longer times were rendered uncertain by the observation that side reactions were also slowly taking place. The initial slope of the plot showed that the rate constant for the forward reaction was 0·3 hr^{-1}. From the final slope, the equilibrium constant was seen to be approximately 21. The rate constant for the reverse reaction is therefore approximately 0·015 hr^{-1}. The u.v., i.r. and NMR spectra of the product at the end of the isomerization were identical with those of pinosylvin dimethyl ether, but showed the presence of impurities. In particular, the u.v. spectrum, which was superimposable on that of pinosylvin dimethyl ether, nevertheless had a molar absorptivity that was only 52 per cent of that of the pure compound.

Pure pinosylvin dimethyl ether was analogously isomerized. After 24 hr, 1·3 per cent of the stilbenes were the *cis* isomer. After 48 hr, this decreased to 0·3 per cent.