Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.47; H, 7.20.

Isotenulin.—The method of Barton[8] was followed using tap water to convert 2 g. of tenulin to isotenulin, yielding 1.8 g., m.p. 161–163°, lit.[9] m.p. 160–161°; $[\alpha]^{26}D$ +9° (EtOH); λ_{max} 225 mμ (ε 8800); ν 1775 (γ-lactone), 1705 and 1590 (cyclopentenone), 1750, and 1230 cm.$^{-1}$ (acetate).

Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24. Found: C, 66.52; H, 7.14.

Dihydroisotenulin.—Isotenulin (2.1 g.) was reduced catalytically over 200 mg. of 10% palladium on carbon in methanol yielding 1.9 g. of dihydroisotenulin, m.p. 147–149° crystallized from cyclohexane–CH_2Cl_2 (lit.[9] m.p. 148–149°.

Anhydrodesacetyldihydroisotenulin.—Dihydroisotenulin (2 g.) was converted[13] through the intermediates desacetyldihydroisotenulin and its methanesulfonyl ester to anhydrodesacetyldihydroisotenulin (0.75 g.), m.p. 130–132°. Further crystallization from ether, filtration in benzene through Woelm activity II–III neutral alumina, and crystallization from hexane–CH_2Cl_2 raised the melting point to 143–144°, lit.[13] m.p. 143.5–144°; $[\alpha]^{26}D$ +170° (EtOH); λ_{max} 218–220 mμ (ε 14,800); ν 1755 (γ-lactone), 1739 (cyclopentanone), and 1668 cm.$^{-1}$ (double bond).

Anal. Calcd. for $C_{15}H_{20}O_3$: C, 72.55; H, 8.12. Found: C, 72.51; H. 8.08.

Isotenulin Oxide.—To 500 mg. of isotenulin in 12 ml. of methanol, cooled to −7°, was added a mixture of 0.5 ml. of 30% hydrogen peroxide, 0.5 ml. of water, and 106 mg. of sodium carbonate. The mixture was stirred for 10 min. before the addition of 20 ml. of water to complete precipitation of the solid. After filtering and drying, it weighed 400 mg. and melted indistinctly at 85–95°. It resisted all attempts at recrystallization and had ν 1750–1780, sh 1715 cm.$^{-1}$; λ_{max} 225 mμ (ε 1950).

Anal. Calcd. for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.52; H, 6.82.

Reduction of Helenalin Oxide to Helenalin with Chromous Chloride.—An 18-mg. sample of helenalin oxide[12] was reduced with 0.4 *N* chromous chloride solution in exactly the same manner as described for the reduction of II. The crude reaction product crystallized from the CH_2Cl_2 extract and after recrystallization from CH_2Cl_2 afforded 10 mg. of substance, m.p. 164–166°, which was shown to be identical with a known sample of helenalin by the usual criteria.

Acknowledgment.—We wish to express our appreciation to Dr. E. Schlittler for his interest and encouragement during our work on this problem. Thanks are due to various members of the Technical Services Section for the microanalyses and for spectroscopic and rotational determinations. We are especially indebted to Professor E. Wenkert of Indiana University for stimulating discussions and for making available to us decoupling n.m.r. data on certain of the substances mentioned.

Benzene Extractives of Lodgepole Pine Bark. Isolation of New Diterpenes[1]

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Lodgepole pine bark is unique in that over one-fourth is soluble in benzene. The benzene extract has been fractionated and the neutral materials extensively investigated. These consist of esterified wax and fatty acids, homologous wax alcohols, *n*-paraffins, sterols, and diterpenes. The most significant discovery is that 21% of the benzene extract consists of the new diterpene alcohol, 13-epimanool (**1**) ($\Delta^{8(20),14}$-labdadien-13α-ol). Three other new diterpenes with the labdane skeleton, hydroxyepimanool (**2**), contortolal (**3**), and contortadiol (**4**), were also isolated.

Pinus contorta Dougl., commonly known as lodgepole pine, is an important commercial western tree and one of the oldest species on the North American continent.[3] It grows from Alaska and the Yukon Territory to Baja California, Mexico, and from the Pacific Coast to the eastern edge of the Rocky Mountains. Some botanists have claimed there are a number of varieties of lodgepole pine, especially the inland form, which shows some difference from the coastal form.[4] The "Checklist of Native and Naturalized Trees of the United States (Including Alaska)," however, lists all varieties as *P. contorta*.[5]

Lodgepole pine has a thin brown bark with many loose scales. Hergert has investigated this bark[6] and found it to be a rich source of flavonoids, especially myricetin, whose isolation from this bark has been patented.[7] Quercetin, dihydroquercetin, dihydromyricetin, aromadendrin, and pinobanksin were also found in lesser amounts. A low yield of a good tannin has also been obtained from this bark.[8]

The turpentine of lodgepole pine has been investigated and found to consist almost entirely of *l*-β-phellandrene with small amounts of *l*-α-pinene sometimes present.[4] Hergert has also identified small amounts of β-pinene, Δ^3-carene, and β-camphene in the turpentine by gas chromatography.[9] Schorger reported that the oleoresin contains abietadienic acids, and that the needle and twig oil contains *l*-α-pinene, *l*-β-pinene, *l*-phellandrene, camphene, dipentene, *l*-borneol, bornyl acetate, methylchavicol (?), cadinene, and a trace of furfural.[10]

During a study of the chemical composition of common North American pulpwood barks,[11] it was found

(1) Presented at the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., April, 1963, p. 10D of abstracts. Part of these results are included in the undergraduate thesis of J. H. S. at the University of Wisconsin. Previous paper in this series: W. C. Nickles and J. W. Rowe, *Forest Prod. J.*, **12**, 374 (1962).

(2) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

(3) D. A. Zischke, "Lodgepole Pine," U. S. Forest Products Laboratory Rept. No. 2052, 1956.

(4) N. T. Mirov, "Composition of Gum Turpentines of Pines," U.S.D.A. Tech. Bull. No. 1239, U. S. Government Printing Office, Washington, D. C., 1961, p. 117, and references cited therein.

(5) E. L. Little, Jr., U.S.D.A. Handbook No. 41, U. S. Government Printing Office, Washington, D. C., 1953, p. 263.

(6) H. L. Hergert, *J. Org. Chem.*, **21**, 534 (1956).

(7) H. L. Hergert, U. S. Patent 2,870,165 (Jan. 20, 1959); Canadian Patent 612,236 (Jan. 10, 1961).

(8) Oregon State Board of Forestry, Biennial Report of State Forester to Governor, 1948/49–1949/50, 1950, p. 65.

(9) H. L. Hergert, private communication.

(10) A. W. Schorger, *J. Ind. Eng. Chem.*, **7**, 24 (1915).

(11) Y.-P. Chang and R. L. Mitchell, *Tappi*, **38**, 315 (1955).

that lodgepole pine bark from the Rocky Mountains was unique in that 28.7% was soluble in benzene whereas most softwood barks contain only 2–5% benzene extractives.[12] This intriguing property led us to include lodgepole pine in a survey being made at the U. S. Forest Products Laboratory on the benzene extractives of pine bark. Chemotaxonomic indications had suggested that pines might be rewarding as a source for new diterpenes. The ubiquity of diterpene resin acids in pines was significant since biosynthetic theory indicates that diterpene alcohols should be the precursors of resin acids.[13] Expectations were amply fulfilled when it was found that new diterpene alcohols, all possessing the labdane skeleton,[14] were major constituents of lodgepole pine bark.

The benzene extract was fractionated as shown in Fig. 1. The unsaponifiable fraction of the benzene extract, the sterol fraction, the terpenoid fraction, and an ethanol extract of the benzene-extracted bark were submitted to the Cancer Chemotherapy National Service Center. No confirmable activity was found.

Thorough extraction of the benzene extract with alkali removed the free acids.[15] No attempt was made to prevent saponification of labile esters, which are undoubtedly present, especially as triglycerides. Saponification yielded the combined acids. The sodium salts of the wax acids produced by saponification were quite insoluble in water and satisfactory extraction could be carried out only after precipitation and filtration.

The combined acids were readily fractionated into three groups by treatment with urea to form the channel inclusion complex.[16] Table I gives the combined

TABLE I

LODGEPOLE PINE BARK COMBINED ACIDS

Saturated acid	%	Unsaturated acid	%
Myristic	0.2	Palmitoleic	1.4
Palmitic	5.2	Oleic	16.8
Stearic	0.4	Linoleic	19.1
Arachidic	7.4	5,9,12-Octadeca-trienoic	10.7
Heneicosanoic	0.1		
Behenic	7.9	Linolenic	1.4
Tricosanoic	0.1	Complex unsaturated higher acids	22.2
Lignoceric	5.1		
Cerotic	0.4		
Total	26.8	Total	71.6

analysis of this fraction. Apparently two different sets of acids are present. The first are long chain wax acids, especially behenic and lignoceric acids (Table II), which are probably part of the waxes in the suberized cell walls of the corky periderm. The second are typical fatty acids, which are most likely present as triglycerides in the phloem. The presence of only traces of stearic acid is usual in tree fats.[17] Some of the unsaturated acids are of higher molecular weight and

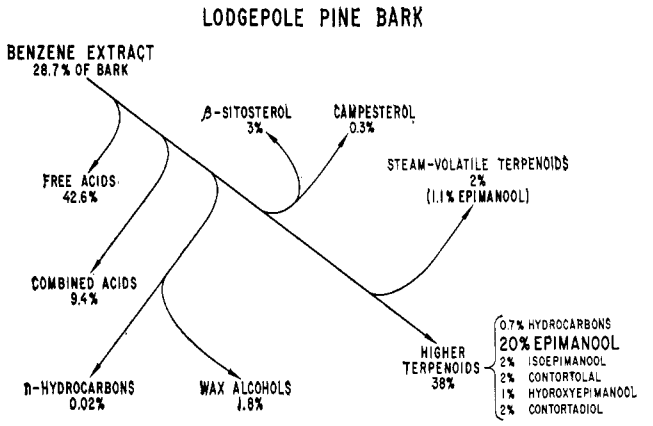


Figure 1.

may consist of a mixture of unsaturated wax and terpenoid acids. One major component of the unsaturated acids was shown to be the recently discovered *cis*-5,9,12-octadecatrienoic acid, a major component in tall oil and pine wood.[18] The unsaturated fatty acids were further confirmed by classical permanganate oxidation,[19] and the saturated acids were further confirmed by infrared analysis of the band progression frequencies of their barium salts.[20] The ultraviolet spectrum indicated that a small amount (~2% of the combined acids) of conjugated trienic acids were also present, perhaps formed by the alkaline rearrangement of double bonds in linolenic acid during saponification.[19]

TABLE II

SATURATED *n*-ALIPHATICS FROM LODGEPOLE PINE BARK

C_n	Paraffins, %	Wax alcohols, %	Combined wax acids, %
C_{16}			19
C_{18}	0.3	0.1	1.4
C_{19}	3.9		
C_{20}	3.8	24	28
C_{21}	17	0.3	0.4
C_{22}	8.6	47	30
C_{23}	26	0.4	0.4
C_{24}	8.9	27	19
C_{25}	9.8		
C_{26}	5.9	0.9	1.4
C_{27}	6.1		
C_{28}	3.5		
C_{29}	1.8		

The *n*-aliphatics were separated from the unsaponifiable fraction by formation of the urea channel inclusion complex.[16] Chromatography separated this into a small amount of *n*-paraffins and a major amount of wax alcohols. No trace of unsaturated or carbonyl components was detected. Gas chromatography showed

(12) However, Hergert[6] found that the amount of benzene extract from three samples of West Coast lodgepole pine varied from 5.7–9.8%.

(13) L. Ruzicka, *Pure Appl. Chem.*, **6**, 493 (1963), and references cited therein.

(14) Nomenclature is based on the unknown hydrocarbon labdane [J. D. Cocker and T. G. Halsall, *J. Chem. Soc.*, 4262 (1956)].

(15) The free acids will be the subject of a future paper when research on the development of a gas chromatographic method for the quantitative analysis of mixed fatty and resin acids is completed. See initial paper: F. H. M. Nestler and D. F. Zinkel, *Anal. Chem.*, **35**, 1747 (1963).

(16) H. Schlenk and R. T. Holman, *J. Am. Chem. Soc.*, **72**, 5001 (1950).

(17) D. B. Mutton, "Wood Extractives and Their Significance to the Pulp and Paper Industries," W. E. Hillis, Ed., Academic Press, New York, N. Y., 1962, p. 331; M. A. Buchanan, "The Chemistry of Wood," B. L. Browning, Ed., John Wiley and Sons, Inc., New York, N. Y., 1963, p. 335.

(18) Y. Aho, O. Harva, and S. Nikkilä, *Teknillisen Kemian Aikakausilehti*, **19**, 390 (1962); O. Lehtinen, V. Kärkkäinen, and M. Antila, *Suomen Kemistilehti*, **35B**, 179 (1962); E. Elomaa, T. Lehtinen, and J. Alhojärvi, *ibid.*, **36B**, 52 (1963).

(19) M. L. Meara, "Modern Methods of Plant Analysis," Vol. II, K. Paech and M. V. Tracey, Ed., Springer-Verlag, Berlin, 1955, pp. 337–339, 354–357.

(20) R. A. Meiklejohn, R. J. Meyer, S. M. Aronovic, H. A. Schuette, and V. A. Meloche, *Anal. Chem.*, **29**, 329A (1957); V. F. Wenzel and U. Schiedt, *Z. Naturforsch.*, **12B**, 71 (1957).

that both fractions consisted of a mixture of homologs, as shown in Table II. The hydrocarbon chromatogram also showed five minor peaks totaling 1.3% which could correspond to paraffins with a small amount of branching. The wax alcohols and the esterified wax acids have a similar distribution with the even chain lengths predominating, as is to be expected. The paraffins, on the other hand, have a similar distribution but with the odd chain lengths predominating, as is to be expected for a biogenesis *via* decarboxylation of wax acids.

The sterols were readily separated from the unsaponifiable fraction *via* precipitation of the digitonides. β-Sitosterol is the major sterol, although, surprisingly, pure β-sitosterol has apparently been obtained only very recently.[21] The β-sitosterol, as well as all available comparison samples, showed about 10% of a lower molecular weight impurity on gas chromatography. This ubiquitous impurity in β-sitosterol preparations has recently been identified as campesterol,[22] and comparison with an authentic sample indicated that this was indeed the minor sterol. Another peak in the gas chromatogram of the lodgepole sterols had a retention time identical with an authentic sample of α_1-sitosterol.[23,24] No trace of stigmasterol could be detected. No attempt was made to detect saturated sterols which are normally present in small amounts in sitosterol preparations.[25a] Strong indications were also found of two β-sitosterol autoxidation products, stigmasta-3,5-dien-7-one[26] and 7-keto-β-sitosterol. Formation of these from β-sitosterol parallels the behavior of cholesterol on autoxidation.[25b]

To summarize, the 3.9% of sterols in the benzene extract consists of 2.9% β-sitosterol, 0.2% campesterol, 0.1% α_1-sitosterol, 0.1% 7-keto-β-sitosterol, and 0.01% stigmasta-3,5-dien-7-one.

Steam distillation of the remaining part of the unsaponifiable fraction removed only a small amount of material. Chromatography separated this into 17% of hydrocarbons and 83% of polar compounds. Gas chromatography of the hydrocarbons by Dr. E. von Rudloff at the Prairie Regional Laboratory, National Research Council, Saskatoon, indicated that they were a mixture of sesquiterpenes. At least three cadinenes (one predominates) and lesser amounts of α- and β-caryophyllene and cedrene were tentatively identified by retention characteristics on Apiezon N, Carbowax 20M, and neopentylglycol adipate polyester. The infrared spectrum suggests the presence of compounds such as γ- and γ_1-cadinene.[27] The polar fraction was shown to consist of one major component identical with 13-epimanool (**1**) which was later isolated from the nonvolatile higher terpenoids.

The oily, nonvolatile, pale yellow, higher terpenoids constitute 38% of the benzene extract and, therefore, contribute significantly to the high benzene solubility of this bark. Column chromatography succeeded in separating these into six major fractions, each of which was shown by gas and thin layer chromatography to contain a major diterpene component. These major components are shown in the order of elution in Fig. 1.

The oily hydrocarbon fraction can not be dehydration products of **1** since the ultraviolet spectrum showed only a high end absorption, while dehydration products of **1** must contain a conjugated diene. Since no crystalline hydrochloride could be obtained and the gas chromatogram indicated that this small fraction was a complex mixture, no further work was carried out on it.

The oily epimanool fraction constituted half of the nonvolatile terpenoids and was shown by gas and thin layer chromatography to be quite pure. Its infrared spectrum clearly indicated a tertiary allylic hydroxyl plus an exocyclic methylene and a vinyl group in a typically terpenic skeleton whose retention time on gas chromatography was as expected for a diterpene alcohol. The n.m.r. spectrum of the 3,5-dinitrobenzoate was most informative, clearly showing three tertiary methyl groups, a typical vinyl AB_2 system, the twin bands for an exocyclic methylene, and an unsplit band for a methyl geminal to oxygen. A proton count indicated an empirical formula of $C_{20}H_{34}O$ for the alcohol, which is as expected for a diterpene alcohol with two rings and two double bonds. These properties strongly suggested a compound like manool, a supposition strongly supported when manool trihydrochloride, identical with an authentic sample,[28] was formed on treatment with hydrogen chloride in acetic acid. Repeated attempts to crystallize this fraction had failed, but seeding with authentic manool resulted in the immediate formation of crystals which were recrystallized to constant melting point and optical rotation from hexane and acetone. However, these physical properties differed from those reported for manool (**7**).[29-33]

It was originally thought that the sample was merely impure since the infrared and ultraviolet spectra were identical with those of authentic manool, the mixture melting point was undepressed, and thin layer chromatograms of the product and authentic manool under a variety of conditions were identical, as were gas chromatograms under a variety of conditions including those in which decomposition occurred. However, the 3,5-dinitrobenzoate was found to be definitely different from that prepared from authentic manool, although their n.m.r. spectra were identical. Since the hydrochlorides were identical and preparation of the hydrochloride has been shown to lead to allylic rearrangement of the side chain,[28] the product can only be 13-epimanool. The absolute configuration of sclareol (**10**) has been determined,[34] and, since sclareol has been

(21) J. A. Steele and E. Mosettig, *J. Org. Chem.*, **28**, 571 (1963).

(22) M. J. Thompson, S. J. Louloudes, W. E. Robbins, J. A. Waters, J. A. Steele, and E. Mosettig, *Biochem. Biophys. Res. Commun.*, **9**, 113 (1962).

(23) K. Schreiber and G. Osske, *Experientia*, **19**, 69 (1963).

(24) α_1-Sitosterol has also been reported in the tall oil of *Pinus densiflora* [S. Ito, *Nippon Daigaku Kogaku Kenkyusho Iho*, **No. 13**, 114 (1956)].

(25) F. Radt, "Elsevier's Encyclopaedia of Organic Chemistry," Vol. 14, Elsevier Publishing Co., New York, N. Y., 1954-1959: (a) p. 1827s; (b) pp. 2682s, 2695s, and 2454s.

(26) R. A. Abramovitch and R. G. Micetich, *Can. J. Chem.*, **40**, 2017 (1962).

(27) V. Herout and V. Sykora, *Tetrahedron*, **4**, 246 (1958).

(28) R. M. Carman, *ibid.*, **18**, 285 (1962). Manool trihydrochloride is a mixture of C-13 epimers; $[\alpha]_D$ varies from +20 to +40° and the melting point runs as high as 128°.

(29) G. Büchi and K. Biemann, *Croat. Chem. Acta*, **29**, 163 (1957).

(30) G. Ohloff, *Helv. Chim. Acta*, **41**, 845 (1958); *Ann.*, **617**, 134 (1958).

(31) C. Enzell, *Acta Chem. Scand.*, **15**, 1303 (1961); H. S. Barreto and C. Enzell, *ibid.*, **15**, 1313 (1961).

(32) C. Enzell and H. Erdtman, *ibid.*, **11**, 902 (1957).

(33) J. R. Hosking and C. W. Brandt, *New Zealand J. Sci. Technol.*, **17**, 750 (1936).

(34) M. Soucek and P. Vlad, *Chem. Ind.* (London), 1946 (1962); *Collection Czech. Chem. Commun.*, **28**, 1211 (1963).

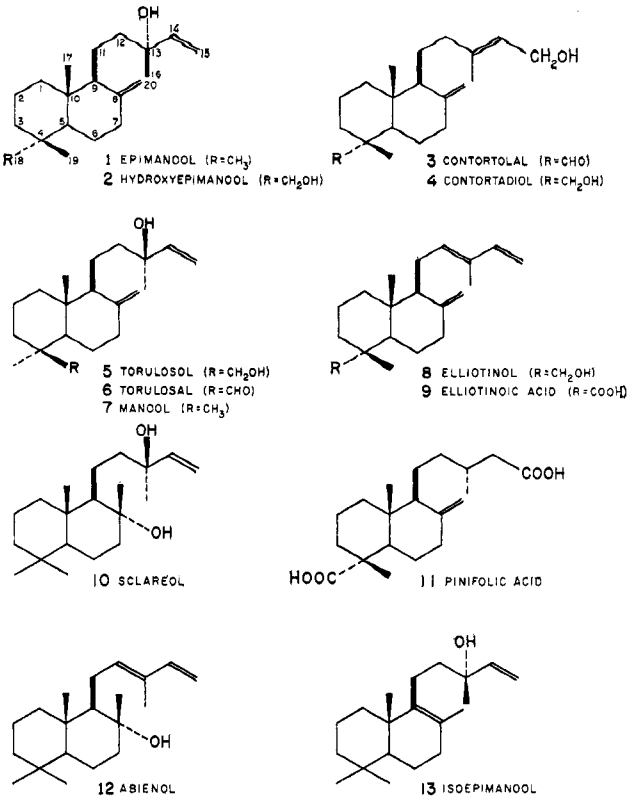
converted into manool,[29] the absolute configuration of 13-epimanool must, therefore, be **1** ($\Delta^{8(20),14}$-labdadien-13α-ol) which has a 13*S* configuration.

The mass spectrum of 1 provided further confirmation.[35] It was practically identical with that of manool.[31] The intensities of peaks formed by cleavage at C-13, however, showed definite differences in agreement with the difference in configuration. Epimanool has a more positive rotation than manool ($\Delta[M]_D$ +52°). A change from a 13*R* to a 13*S* configuration has been observed to result in a higher rotation in analogous cases. Thus, (*R*)-(−)-linalool → (*S*)-(+)-linalool has $\Delta[M]_D$ +70°; (13*R*)-sclareol (**10**) → (13*S*)-13-episclareol has $\Delta[M]_D$ +49°.[36,37] Unfortunately an attempted synthesis[36] of 13-epimanool was unsuccessful so that no definite correlation is possible.

The third fraction following 13-epimanool on the chromatogram was an oily mixture whose infrared spectrum was similar to that of 13-epimanool except that the band characteristic of the exocyclic methylene was absent while a new band appeared at 1380 cm.$^{-1}$ characteristic of a vinylic methyl.[36] In addition, the intensity of the end absorption in the ultraviolet was increased. The major diterpene component is assumed to be 13-epiisomanool (**13**), an oily compound which is known synthetically.[36] This could be either a native constituent of lodgepole pine bark, or an artifact produced by migration of the exocyclic double bond into the ring during fractionation of the benzene extract.

The fourth fraction was a small amount of oily mixture with bands in the infrared for an aldehyde, hydroxyl, and exocyclic methylene. The major component was named contortolal and isolated in the form of its semicarbazone. The semicarbazone gave a very characteristic n.m.r. with bands for two tertiary methyls a vinylic methyl, a single vinylic proton, and a doublet for an exocyclic methylene. Another doublet suggested a vinylic CH_2O group in which a single peak expected for two equivalent hydrogens was being split by an adjacent vinylic proton. That this is indeed the case was confirmed by spin–spin decoupling at 100 Mc.,[38] which showed that the triplet for the vinylic proton collapsed to a sharp singlet when irradiated with the frequency of the CH_2O, and that the doublet for the CH_2O collapsed to a singlet when irradiated with the frequency of the vinylic proton. The vinylic proton showed no indication of coupling with any other protons, especially those of the vinylic methyl that must be on the same double bond. Likewise, the vinylic methyl was a sharp singlet whose τ-value was the same at both 60 and 100 Mc. That the adjacent vinylic methyl and vinylic proton do not couple indicates the biogenically preferable *trans* relationship of these two groups as illustrated in **3**. In confirmation, the chemical shift of the vinylic methyl is exactly as has been predicted from model systems in which the vinylic

CHART I



methyl and proton are *trans* rather than *cis*.[39] Phytol has a similar side chain and the n.m.r. resonances for the vinylic proton, vinylic methyl, and vinylic CH_2O of phytol[40] have the same form and position as in contortolal semicarbazone. Another resonance in the n.m.r. was characteristic for one uncoupled proton on a carbon–nitrogen double bond. The resonances due to NH and NH_2 of the semicarbazone were readily identified since they exchanged with deuterium.

Taken all together, the proton count coupled with the analytical and spectral data show that contortolal is a dicyclic diterpene containing an exocyclic methylene, two tertiary methyls, a tertiary aldehyde group, and a *trans*-$C(CH_3)$=CH—CH_2OH grouping. This strongly suggests a labdane type of skeleton in which the side chain is allylicly rearranged from the vinyl carbinol as present in **1**. A structure such as **3** (15-hydroxy-$\Delta^{8(20),13}$-labdadien-18-al) satisfactorily incorporates all the available data and, although unknown as a natural product, possesses structural similarities with several labdane diterpenes and is biogenetically attractive. Placement of the tertiary aldehyde group at 4α rather than 4β or at 10 is done solely on the basis that all known pine diterpenes, such as the resin acids, have oxidized methyl groups only at this position. It should be possible to correlate this suggested structure with other labdane diterpenes, especially contortadiol (**4**).

(35) A paper on the mass spectrum of carbodicyclic diterpenes is being prepared by C. Enzell.

(36) D. B. Bigley, N. A. J. Rogers, and J. A. Barltrop, *J. Chem. Soc.*, 4613 (1960); *Chem. Ind.* (London), 1570 (1962).

(37) D. P. Popa and G. V. Lazur'evskii, *Zh. Obshch. Khim.*, **33**, 303 (1963).

(38) N. S. Bhacca, M. E. Wolff, and R. Kwok, *J. Am. Chem. Soc.*, **84**, 4976 (1962), and references cited therein.

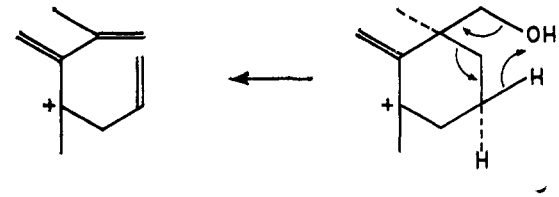
(39) R. B. Bates, R. H. Carnighan, R. O. Rakutis, and J. H. Schauble *Chem. Ind.* (London), 1020 (1962).

(40) Compare 2,2,4-trimethylpentane-1,3-diol. N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, "NMR Spectra Catalog," Varian Associates, Palo Alto, Calif., 1962, No. 217, phytol, No. 346. E. Wenkert and P. Beak [*Tetrahedron Letters*, **No. 11**, 358 (1961)] also report that axial and equatorial CH_2OH groups at C-4 both give AB quartets, but with different chemical shifts.

The fifth fraction was a small amount of an oil from which crystals could be obtained. The infrared spectrum strongly resembled 13-epimanool except that additional bands were present at 3643 and 1017 cm.$^{-1}$ characteristic of a primary hydroxyl group. The n.m.r. spectrum showed bands characteristic of two tertiary methyls, a vinyl AB_2 system, an allylic methyl, an exocyclic methylene, and a typical AB quartet for a tertiary CH_2O group. This strongly suggests a hydroxymanool or hydroxy-13-epimanool. A compound of this type, torulosol (**5**), has been reported by Enzell in *Cupressus torulosa*.[31] It co-occurs with the corresponding aldehyde, torulosal (**6**), and manool. Comparison of torulosol and our compound was most interesting. They have a similar optical rotation and R_f, and have almost identical infrared, mass and n.m.r. spectra. Although the melting points are almost the same, a mixture melting point is, however, sharply depressed. The mass spectra differ from one another mainly in small differences in intensity such as would be explained if our compound were epimeric with torulosol at both C-13 and C-4.[35] Thus, the axial 4β-CH_2OH group of torulosol appears to be more readily lost than the equatorial 4α-CH_2OH group of the hydroxyepimanool, while the axial 4-methyl of hydroxyepimanool appears to be more readily lost than the equatorial 4-methyl in torulosol. Similarly, the n.m.r. spectra are identical except that the signals for the 4-CH_2O and 4-methyl show a different chemical shift, although this is only by 2 c.p.s. Dr. Enzell attributed the nonequivalence of the 4β-CH_2O protons in torulosol to hindered rotation caused by the 10β-methyl, but according to Dr. Hollis of Varian Associates, 4α-CH_2O protons would also be expected to be nonequivalent due to steric hindrance at the adjacent carbon.[40] Thus, this compound would appear to be best described as 18-hydroxy-13-epimanool **2** ($\Delta^{8(20),14}$-labdadiene-13α,18-diol). Assignment of a 13*S* epimeric configuration as in the 13-epimanool is mostly by analogy. However, 13-episclareol has been recently found to co-occur with sclareol (**10**) in *Salvia sclarea*.[37] Future research should allow this to be correlated with both 13-epimanool and contortadiol (**4**) below. Dehydration should lead to elliotinol[41] from *Pinus elliottii*, which we believe should have the structure **8**. The corresponding acid, elliotinoic acid, was also recently reported in the same species[42]; we believe this should have the structure **9**. This is the 4-epimer of communic acid ($\Delta^{8(20),12,14}$-labdatrien-19-oic acid) from *Juniperus* spp. barks.[43]

The sixth and last fraction was a complex oily mixture of alcohols from which the major crystalline component was isolated *via* its di-*p*-phenylazobenzoate. The infrared spectrum indicated that the ester had no free hydroxyl groups, but did have an exocyclic methylene. The n.m.r. spectrum of the ester was similar to that of hydroxymanool and contortolal semicarbazone, showing two tertiary methyls, a doublet for the exocyclic methylene, the typical AB quartet for a tertiary CH_2O group, a vinylic methyl, a vinylic proton, and a doublet for a vinylic CH_2O as before. Spin–spin decoupling at 100 Mc.[38] clearly showed that the single vinylic proton was coupled to the vinylic CH_2O, but that there was no coupling to the vinylic methyl, thus indicating a *trans* $-C(CH_3)=CH-CH_2OR$ grouping. This, together with a proton count and the analytical data, clearly suggests a structure such as **4**, possessing the allylicly rearranged side chain as in contortolal and the biogenetically preferable 4α-CH_2OH as in **2**. In analogy with contortolal, we have named this compound contortadiol ($\Delta^{8(20),13}$-labdadiene-15,18-diol). It is thus the C-4 epimer of agathadiol ($\Delta^{8(20),13}$-labdadiene-15,19-diol), which is formed from torulosol by allylic rearrangement of the side chain.[31]

Comparison of contortadiol and agathadiol was most informative. According to physical properties, mixture melting point, infrared spectra, and thin layer chromatography, these two compounds are identical. However, the mass spectra, although very similar, did show definite differences in intensity. Of particular significance, the intensity of the *m/e* 135 peak is about five times as great (Σ% basis) in agathadiol as is to be expected since this peak is due to an ion originating by a dehydration which is more favorable for an axial CH_2OH group.[35]



It is significant to note that since the stereochemistry at C-4 apparently has no effect on the optical rotation the similarity of $\Delta[M]_D$ (**7** → **1**) +57° and $\Delta[M]_D$ (**5** → **2**) +37° supports the assigned stereochemistry at C-13 in 18-hydroxy-13-epimanool.

The genus *Pinus* is characterized by the presence of mono- and diterpenes. Although most pine diterpenes possess the tricyclic abietane or pimarane skeleton, the presence of dicyclic labdane diterpenes is to be expected biogenetically.[13] In addition to elliotinol and elliotinoic acid, which were mentioned previously, two further labdane diterpenes have been reported in pines, pinifolic acid (**11**)[44] and abienol (**12**),[45] both found in *P. sylvestris*.

This is the first reported isolation of 13-epimanool, 18-hydroxyepimanool, contortolal, and contortadiol, although current investigations indicate that these new labdane diterpenes are present in other pine barks as well. Manool itself has not been previously reported in the Pinaceae. It is normally considered characteristic of many *Dacrydium* spp. where it is found together with related labdane diterpenes as well as the primarane diterpenes, isopimaric acid and rimuene, and the abietane diterpenes, ferruginol and sugiol.[46] Manool has also been reported in *Cupressus sempervirens*[39] and *C. torulosa*.[31]

(41) E. M. Roberts and R. V. Lawrence, Abstracts of Papers, Division of Organic Chemistry, 131st National Meeting of the American Chemical Society, Miami, Fla., April, 1957, p. 20-O.

(42) N. M. Joye and R. V. Lawrence, *J. Org. Chem.*, **28**, 3274 (1963).

(43) V. P. Arya, C. Enzell, H. Erdtman, and T. Kubota, *Acta Chem Scand.*, **15**, 225 (1961).

(44) C. Enzell and O. Theander, *ibid.*, **16**, 607 (1962).

(45) G. V. Pigulevskii, V. G. Kostenko, and L. D. Kostenko, *Zh. Obshch. Khim.*, **31**, 3143 (1961).

(46) W. Karrer, "Konstitution und Vorkommen der organischen Pflanzenstoffe," Birkhäuser Verlag, Basel, 1958, pp. 782–788.

Experimental

Unless otherwise stated, melting points were determined in evacuated capillaries in a copper block and are corrected, optical rotations were determined with a Rudolph Model 80 polarimeter in chloroform, ultraviolet spectra with a Beckman DK-2 recording ratio spectrometer in ethanol, infrared spectra with a Baird Atomic spectrophotometer as a liquid film or in potassium bromide, and the n.m.r. spectra in deuteriochloroform at 60 Mc. with tetramethylsilane as an internal standard. Gas chromatographic data were mostly supplied by Mr. Robert F. Sweeny, Applied Science Laboratories, State College, Pa. Column chromatography was carried out using thirty times the amount of Woelm alumina, neutral, activity II.

Isolation of the Neutral Benzene Extract of Lodgepole Pine (*Pinus contorta* Dougl.) Bark.—Bark was collected at the Kremmling, Colo., sawmill of the Nebraska Bridge Co. from the upper portion of butt logs that were cut 2 days before on the eastern side of the summit of Gor Pass in Arapaho National Forest, Colo. After partial air drying, the bark was reduced on a hammer mill with a 4-mm. screen, air-dried to approximately 5% moisture, and exhaustively extracted in a Lloyd extractor with benzene. The benzene extract was filtered through Celite and then extracted with warm 1 *N* sodium hydroxide. Both the aqueous phase, which contained suspended solids and emulsion, and the benzene phase were washed several times with fresh solvent. The combined aqueous phases and the combined benzene phases were worked up as usual to yield free acids and neutrals in the ratio 1:1.35.

Isolation and Fractionation of the Combined Acids.—The neutral fraction of the benzene extract (115.2 g.) was saponified by refluxing for 5 hr. with 1 l. of 2 *N* ethanolic sodium hydroxide. The solution was then concentrated under vacuum and 1.2 l. of benzene and 1.2 l. of a saturated sodium chloride solution were then added with vigorous agitation. The mixture was filtered and the precipitated sodium salts thoroughly washed with benzene and salt solution.

The benzene layer of the filtrate was washed with dilute alkali removing benzene-soluble sodium salts, and worked up in the usual way to yield 88.5 g. of the unsaponifiable fraction. The precipitated sodium salts were suspended in aqueous acid and similarly extracted with benzene to yield 4.76 g. The combined aqueous layers from the filtration analogously yielded 16.07 g. No phlobaphenes were produced.

The acid fractions were combined and added to a refluxing solution of 250 g. of urea in 1200 ml. of 95% ethanol. Benzene (600 ml.) was slowly added to the refluxing solution which was then allowed to cool overnight. The urea complex was filtered, washed with fresh benzene, suspended in water, and extracted with benzene as usual to yield 4.185 g. of combined wax acid fraction.

The filtrate was concentrated to 200 ml. and cooled overnight in the refrigerator. Filtration of the complex and working up as before yielded 5.62 g. of combined fatty acid fraction. The filtrates were poured into water and extracted with benzene to yield 9.85 g. of combined unsaturated acid fraction.

Combined Wax Acids.—The white, crystalline, combined wax acid fraction was crystallized from hexane and methanol and dried for analysis, m.p. 68–71°. The infrared spectrum was identical with that of behenic acid. The infrared spectrum of the barium salt indicated behenic acid plus homologs.[20] The ultraviolet spectrum showed only a very weak end absorption, and the iodine number was 0.2.

Anal. Calcd. for $C_{22}H_{44}O_2$: C, 77.58; H, 13.02; neut. equiv., 340.6. Found: C, 77.57; H, 13.12; neut. equiv., 339.4.

Gas chromatography of the methyl esters on 7% ethylene glycol isophthalate (EGiP) on Gas Chrom P at 224° gave the following results (methyl ester, t_R' in minutes, % total area): C_{18}, 7.5, 0.2; C_{20}, 12.0, 35.4; C_{21}, 15.3, 0.5; C_{22}, 19.8, 37.5; C_{23}, 25.5, 0.6; C_{24}, 33.1, 24.0; C_{26}, 58, 1.8. Peak assignments were checked against known wax acid esters.

Combined Fatty Acids.—The combined fatty acid fraction was an almost colorless mobile oil that solidified in the refrigerator. It gave a brown color with tetranitromethane, and had an iodine number of 118 and a neut. equiv. of 224. The infrared spectrum was identical with oleic acid. The ultraviolet spectrum showed a strong end absorption plus weak peaks at 259, 268, and 279 mμ ($E^{1\%}_{1cm}$ 30, 39, and 31, respectively). Gas chromatography of the methyl esters on 15% ethylene glycol succinate (EGS) on Gas Chrom P at 188° gave the following results (methyl ester, t_R' in minutes, % total area): myristate, 3.8, 0.7; palmitate, 5.9, 17.9; palmitoleate, 7.0, 4.7; stearate, 9.5, 1.1; oleate, 11.0, 57.9; linoleate, 13.2, 11.7; unsaturated lignocerate (?), 42, 0.7; unsaturated cerotate (?), 90, 5.2; and traces of laurate, lauroleate, and linolenate. Retention times for the major components were checked against known samples.

A portion of the acids (2.268 g.) was dissolved in 1 l. of ice–water containing 6 g. of potassium hydroxide and crushed ice. The solution was stirred while 300 ml. of 1% potassium permanganate solution were added rapidly. Sulfurous acid and dilute hydrochloric acid were added after 5 min. and the product was filtered, dried, and placed in a Soxhlet extractor. Extraction with petroleum ether (b.p. 30–80°) yielded 426 mg. of saturated fatty acids; extraction with ethyl acetate yielded 1.329 g. of dihydroxy fatty acids; and extraction with water and with ethanol yielded 55 mg. of tetrahydroxy fatty acids.

The colorless saturated fatty acids, m.p. 52–53.5°, neut. equiv. 283, iodine number 6, had no absorption in the ultraviolet. The acid and its barium salt had infrared spectra identical to those of palmitic acid and its salt.[20]

The dihydroxy fatty acids were recrystallized from ethyl acetate to yield colorless crystals, m.p. 122–126° (reported[19] for *cis*-9,10-dihydroxystearic acid, 133°), which had an infrared spectrum (Nujol) as expected and gave no color with tetranitromethane.

Anal. Calcd. for $C_{18}H_{36}O_4$: C, 68.31; H, 11.47; neut. equiv., 317. Found: C, 68.19; H, 11.36; neut. equiv., 319.

The colorless tetrahydroxy fatty acids, m.p. 172.5–174.5° (reported[19] for *cis*-9,10,12,13-tetrahydroxystearic acid, 172°), had the expected infrared spectrum (Nujol).

Anal. Calcd. for $C_{18}H_{36}O_6$: C, 62.04; H, 10.41; neut. equiv., 349. Found: C, 61.90; H, 10.51; neut. equiv., 350.

Combined Unsaturated Acids.—The combined unsaturated acid fraction was a dark viscous oil which gave only a very strong end absorption in the ultraviolet, iodine number 166.5, neut. equiv. 291. Its infrared spectrum resembled that of oleic acid. Gas chromatography of the methyl esters on 15% EGS on Gas Chrom P at 188° gave the following results (methyl ester, t_R' in minutes, % total area): unknown, 7.9, 3.5; linoleate, 13.1, 31.4; *cis*-5,9,12-octadecatrienoate, 14.8 21.4; linolenate, 16.9, 2.8; plus a series of eight unknown peaks with longer retention times totaling 40.8%. Retention times were checked against known samples of each of the C_{18} esters.

Hydrogenation of the methyl esters (Adams' catalyst in acetic acid), followed by rechromatography as before, gave the following results (methyl ester, t_R' in minutes, % total area): C_{16}, 5.9, 1.0; C_{18}, 9.5, 56.9; C_{20}, 15.7, 10.1, C_{22}, 27.5, 4.2; unknown, 29.4, 10.5; unknown, 41, 8.6; unknown, 72, 8.7. Peak assignments were checked with known fatty acid esters.

***n*-Aliphatics from Unsaponifiable Fraction.**—The unsaponifiable fraction (17.2 g.) in 200 ml. of benzene was added to 50 g. of finely powdered urea moistened with 10 ml. of methanol. The mixture was shaken intermittently for 48 days after which the crystals were filtered washed with benzene, and recrystallized from benzene–ethanol. The complex and filtrate were worked up as usual by extraction with benzene from an aqueous suspension to yield 685 mg. of white waxy *n*-aliphatics and 65 mg. of impure *n*-aliphatics, respectively.

The filtrate from the treatment with urea was shaken for 10 days with 20 g. of active expanded urea.[47] The mixture was filtered and both crystals and filtrate worked up by adding to water and extracting with benzene to yield 20 mg. of impure *n*-aliphatics and 16.0 g. of *n*-aliphatic-free unsaponifiables.

The pure *n*-aliphatics (685 mg.) gave no color with tetranitromethane and showed no carbonyl absorption in the infrared. Chromatography on alumina yielded 16 mg. of hydrocarbons eluted with petroleum ether and 663 mg. of wax alcohols eluted mostly with benzene.

The hydrocarbon fraction was absorbed onto 1.4 g. of alumina (alkaline, activity 1) and eluted with petroleum ether to yield 6 mg. of pure *n*-paraffins. This was crystallized from benzene–methanol for analysis, m.p. 50–56.5°.

Anal. Found: C, 85.05; H, 14.74.

The infrared spectrum was as expected with a strong band progression doublet at 729 and 719 cm.$^{-1}$. There was no absorption in the ultraviolet (isooctane). The gas chromatographic

(47) M. H. Gorin and L. Rosenstein, U. S. Patent 2,785,151 (March 12, 1957).

analysis (6% EGiP on Gas Chrom P at 220°) gave the following results (hydrocarbon, t_R' in minutes, % total area): C_{18}, 0.7, 0.3; C_{19}, 0.9, 3.9; C_{20}, 1.3, 3.8; C_{21}, 1.8, 17.1; C_{22}, 2.5, 8.6; C_{23}, 3.4, 25.7; unknown, 4.5, trace; C_{24}, 5.0, 8.9; unknown, 6.5, 0.3; C_{25}, 7.0, 9.8; unknown, 9.3, 0.4; C_{26}, 10.0, 5.9; unknown, 13.4, 0.4; C_{27}, 14.4, 6.1; unknown, 19.4, 0.25; C_{28}, 21.0, 3.5; unknown, 28.2, trace; C_{29}, 30.5, 1.8; C_{30}, 44.9, 1.85; C_{31}, 65.4, 1.55. The slope of the log plot was checked against several standards, and cochromatography with known C_{24} was used to establish definite identification.

The wax alcohols were crystallized from hexane for analysis, m.p. 69–70°.

Anal. Calcd. for $C_{22}H_{46}O$: C, 80.90; H, 14.20. Found: C, 81.22; H, 14.36.

The infrared spectrum was identical with that of behenyl alcohol. Gas chromatographic analysis (0.75% G.E. SE-30 silicone gum rubber on Gas Chrom P at 200°) gave the following results (alcohol, t_R' in minutes, % total area): C_{18}, 3.0, 0.07; C_{20}, 4.9, 24.3; C_{21}, 6.6, 0.3; C_{22}, 8.9, 47.2; C_{23}, 12.0, 0.4; C_{24}, 15.9, 26.7; C_{26}, 29, 0.9. Peak assignments were confirmed by chromatography of pure behenyl and lignoceryl alcohols.

Sterols from Unsaponifiable Fraction.—The *n*-aliphatic-free unsaponifiables (16.0 g.) were added to a solution of 5.2 g. of digitonin in 400 ml. of 95% ethanol. The solution was taken to dryness under vacuum and 400 ml. of benzene added to the residue. After refluxing for 1 hr., the fine suspension of digitonin and digitonides was filtered and washed thoroughly with fresh benzene. The filtrate was again treated with digitonin (recovered from first precipitation) similarly to yield 14.3 g. of *n*-aliphatic- and sterol-free unsaponifiables (terpene fraction).

The digitonin precipitates were each refluxed briefly with 50 ml. of pyridine, cooled, and 400 ml. of ether–benzene then added with thorough shaking. After settling for 1 hr., the supernate was decanted and the gelatinous precipitate of digitonin was filtered and thoroughly washed with benzene. The ether–benzene solutions of the sterols were washed with dilute acid, dilute alkali, and water yielding 1.10 g. of sterols from the first precipitation and 90 mg. from the second.

The sterols were purified by reprecipitation of the digitonide as before. They showed a high end absorption in the ultraviolet plus shoulders at 281 mμ (ε 71) [reported[26] for stigmasta-3,5-dien-7-one, λ_{max} 278 mμ (ε 20,000)] and at 232 mμ (ε 379) [predicted[25b] for 7-keto-β-sitosterol, λ_{max} 237 mμ (ε 13,000)]. The infrared spectrum was completely identical with that of β-sitosterol. No trace of a peak at 968 cm.$^{-1}$ as expected for the *trans* disubstituted double bond of stigmasterol could be seen.[48] Gas chromatography (1% SE-30 on Gas Chrom P at 228°) showed 90% of a single component with the same elution time (27.5 min.) as pure β-sitosterol, 6% of a peak eluted earlier with the same elution time (22 min.) as campesterol, and 4% of a peak eluted later with the same elution time (35 min.) as α_1-sitosterol.[23] No peak was present at the position (24 min.) expected for stigmasterol.

A portion of the sterols (260 mg.) were chromatographed on alumina. Petroleum ether–benzene mixtures eluted 17 mg. with λ_{max} 282 mμ (ε 2260), ν_{max} 1653 cm.$^{-1}$ (conj. C=O). Methylene chloride–95% ethanol eluted 29 mg. of material with λ_{max} 236 mμ (ε 2075), ν_{max} 1672 cm.$^{-1}$ (conj. C=O). The main fraction (202 mg.) eluted with benzene gave infrared and ultraviolet spectra identical with those of β-sitosterol. The center cut was recrystallized from methanol for analysis, m.p. 139–140°, $[\alpha]^{23}D$ −30° (*c* 1.4), reported[21] for pure β-sitosterol, m.p. 139–140°, $[\alpha]D$ −33°. A mixture melting point was undepressed. Gas chromatography as before showed 94% β-sitosterol plus 6% campesterol.

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15. Found: C, 83.82; H, 12.23.

Steam-Volatile Terpenoids.—The terpene fraction free of sterols and *n*-aliphatics (14.3 g.) was steam distilled for 5 hr. The distillate was extracted with benzene in the usual manner to yield 675 mg. of a pale yellow oil whose ultraviolet spectrum showed only a strong end absorption. Chromatography on 125 g. of alumina (activity I) readily separated this into 113 mg. of colorless oily methanol-insoluble hydrocarbons and 556 mg. of a pale yellow polar oil.

The hydrocarbons had very strong bands for methylene and a trisubstituted double bond, ν_{max} 3067, 1646, 885, 833, 792 cm.$^{-1}$; a sharp doublet at 1384 and 1365 cm.$^{-1}$ indicated an isopropyl group. Gas chromatography (SE-30) indicated a mixture of mostly sesquiterpenes with a major component constituting about one-quarter of the fraction. The polar fraction had an infrared spectrum almost identical with 13-epimanool. Gas chromatography as below showed 68% 13-epimanool as the only major constituent.

Chromatography of Nonvolatile Terpenoids.—The nonvolatile terpenoids (50 g.) were chromatographed on alumina. Petroleum ether eluted 1.0 g. of a colorless oily hydrocarbon fraction. The ultraviolet spectrum showed only a very strong end absorption at 210 mμ ($E^{1\%}_{1cm}$ 1084). The infrared spectrum had characteristic bands at 1387, 1376, 1368 (CH_3), 3056, 1641, 1410, 995 910 (-CH=CH_2), and 961 cm.$^{-1}$ (*trans* -CH=CH-). Gas chromatography (10% G.E. XE-60 nitrile silicone gum on Gas Chrom P, temperature programmed) showed this fraction to be a complex mixture with diterpenes predominating. No crystalline product could be obtained when this fraction in glacial acetic acid was treated with anhydrous hydrogen chloride for 5 hr. at 15°.

The second fraction, eluted with petroleum ether–benzene mixtures, was 24 g. of pale yellow oily epimanool (**1**) fraction. The ultraviolet spectrum showed only a strong end absorption at 210 mμ (ε 3110). The infrared spectrum had characteristic bands at 1643, 1406, 992, 916, 886 (-CH=CH_2 and >C=CH_2), and 1385 and 1364 cm.$^{-1}$ (>$C(CH_3)_2$). A single-beam, double-pass infrared spectrum in carbon tetrachloride was run on a Perkin-Elmer 112 spectrophotometer using a calcium fluoride prism. A single, strong, sharp hydroxyl band was present at 3610 cm.$^{-1}$ for the tertiary allylic hydroxyl; a single sharp vinylic C–H stretching band was present at 3084 cm.$^{-1}$. Gas and thin layer chromatography under a variety of conditions as below showed this fraction to be essentially homogeneous. All attempts to crystallize this fraction failed until a hexane solution was seeded with authentic manool. Repeated recrystallization from both hexane and from acetone failed to change the melting point or optical rotation significantly. An analytical sample of 13-epimanool (**1**) had m.p. 36.5–38.5°, $[\alpha]^{23}D$ +51° (*c* 1.3); reported[29–33] for manool, m.p. 53°, $[\alpha]D$ +30 to 33°. A mixture of two parts 13-epimanool and one part authentic manool melted at 41–49°.

Anal. Calcd. for $C_{20}H_{34}O$: C, 82.69; H, 11.80. Found: C, 82.72; H, 11.68.

The infrared spectrum was unchanged from that of the oily epimanool fraction and was identical with that of authentic manool. Thin layer chromatography of the epimanool, authentic manool, and a mixture of the two at varying concentrations on alumina and silica gel plates gave identical R_f values of 0.6 and 0.8, respectively, when developed with 4:1 benzene–anhydrous ether; no separation of the mixture could be detected. Gas chromatography of the epimanool, authentic manool, and a mixture of the two on 3% SE-30 on Gas Chrom P at 150 (t_R' 45 min.), 170 (t_R' 22 min.), and 185° (t_R' 12 min.), and on 3% ethylene glycol succinate silicone (EGSS-X) on Gas Chrom P, temperature programmed, all gave a single peak with the same retention time for all three samples. Gas chromatography on 3% and 10% XE-60 on Gas Chrom P at 150 and 210° caused considerable decomposition, especially at the higher temperature, but the decomposition patterns were identical for all three samples. The mass spectrum of 13-epimanool was practically identical with that of manool itself[31] except that the intensities of those peaks arising from cleavage at C-13 were altered. In particular, the intensity of the *m/e* 71 ion (CH_3-$\overset{+}{C}$(OH)-CH=CH_2) was markedly lower in 13-epimanool than in manool.[35]

The third fraction eluted from the chromatogram with benzene was 6.0 g. of oily isoepimanool (**13**) fraction. The ultraviolet spectrum showed only a strong end absorption at 210 mμ (ε 7090). The infrared spectrum had characteristic bands at 3023, 1632, 1401, 996, and 913 (-CH=CH_2), 1380 (=C-CH_3), and 3291 cm.$^{-1}$ (OH). Thin layer chromatography showed a major and two minor spots more polar than 13-epimanool. Gas chromatography (10% XE-60 on Gas Chrom P) indicated that this fraction contained one major and eight minor constituents.

The fourth fraction, eluted from the chromatogram with benzene–ether mixtures, was 3.1 g. of yellow oily contortolal (**3**) fraction. The ultraviolet spectrum showed only a strong end absorption at 210 mμ (ε 5640). The infrared spectrum had characteristic bands at 3077, 1645, 870 (>C=CH_2), and 1712 and 2710 cm.$^{-1}$ (-CHO). Thin layer and gas chromatography

(48) J. A. Campbell, D. A. Shepherd, B. A. Johnson, and A. C. Ott, *J. Am. Chem. Soc.*, **79**, 1127 (1957).

indicated that this fraction contained one major and one minor constituent.

The fifth fraction, eluted from the chromatogram with ether and 100:1 ether–methanol, was 2.7 g. of yellow oily hydroxyepimanool (2) fraction. Although thin layer chromatography showed only a single spot, gas chromatography (10% XE-60 on Gas Chrom P) indicated six minor coproducts in addition to the major constituent. Careful crystallization from heptane–methylene chloride yielded the major constituent, 18-hydroxy-13-epimanool (2), as white crystals which were recrystallized from heptane–methylene chloride and methanol–methylene chloride to constant melting point for analysis, m.p. 113–114.5°, $[\alpha]^{22}D$ +43° (*c* 1.3).

Anal. Calcd. for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18. Found: C, 78.71; H, 11.26.

The ultraviolet spectrum showed only a strong end absorption at 210 mμ (ε 3265). The infrared spectrum had ν_{max} 3050, 1640, 1410, 999, 919, 890 (–CH=CH_2 and >C=CH_2), and 3302 and 1017 cm.$^{-1}$ (–OH). An infrared spectrum in carbon tetrachloride was run on a Perkin-Elmer 421 spectrophotometer, ν_{max} 3085 (=CH_2), and a doublet at 3643 and 3617 cm.$^{-1}$ (C-18 and C-13 OH, respectively). Except for the bands at 3643 and 1017 cm.$^{-1}$ for primary hydroxyl,[49] the spectra were very similar to those of 13-epimanool. The spectrum was also very similar to that of torulosol (5), m.p. 110–111°, $[\alpha]D$ +31°.[31] However, a mixture melting point was depressed to 89–101°. Thin layer chromatography on alumina of these two compounds gave identical results (R_f 0.59 when developed with 100:1 benzene–methanol). The mass spectra of both compounds were practically identical except for minor differences in intensity for those peaks resulting from cleavage at C-13 or from removal of one of the groups at C-4.[35]

The n.m.r. spectrum indicated 34 protons, was practically identical with that of torulosol, and showed only the expected differences from that of manool. Characteristic resonances were present at τ 9.32 and 9.03 (C-17 and C-19 methyls), τ 8.67 (C-16 allylic methyl), and τ 5.19 and 5.48 (C-20 exocyclic methylene). A typical vinyl AB_2 system was present between τ 3.8 and 5.1, while a typical AB quartet was observed for the C-18 CH_2O group at τ 6.16, 6.34, 6.53, and 6.72.

The sixth and last fraction, eluted from the chromatogram with ether–95% ethanol, was 13 g. of yellow oily contortadiol (4) fraction. The ultraviolet spectrum showed only a strong end absorption at 210 mμ (ε 6100). The infrared spectrum had characteristic bands at 3041, 1637, 886 (>C=CH_2), and 3436 and 1027 cm.$^{-1}$ (–CH_2OH). Although thin layer chromatography indicated that this fraction had a major and one minor constituent, gas chromatography (10% XE-60 on Gas Chrom P) indicated that this fraction was more complex with five minor constituents in addition to the major one.

Manool Trihydrochloride.—Crude 13-epimanool (568 mg.) in 10 ml. of glacial acetic acid was saturated with anhydrous hydrogen chloride for 5 hr. at 15°. The white precipitate was filtered and washed with 10:1 glacial acetic acid–concentrated hydrochloric acid to yield 451 mg., m.p. 114–119°. Repeated recrystallization from hexane yielded an analytical sample, m.p. 118–120.5°, $[\alpha]^{23}D$ +22° (*c* 0.8). A mixture melting point with authentic manool trihydrochloride, m.p. 117–121°, was undepressed. No color was produced with tetranitromethane, the Beilstein test was positive, and the infrared spectrum was superimposable on that of authentic manool trihydrochloride.[28]

Anal. Calcd. for $C_{20}H_{35}Cl_3$: C, 62.90; H, 9.24. Found: C, 63.10; H, 9.32.

Manool and 13-Epimanool 3,5-Dinitrobenzoates.—The 13-epimanool ester was prepared in the usual fashion with an excess of 3,5-dinitrobenzoyl chloride in pyridine at room temperature for 3 days. The product was recrystallized from methylene chloride–methanol and methylene chloride–hexane to constant melting point, m.p. 116.5–118° with decomposition, $[\alpha]^{22}D$ +33° (*c* 1.0). Owing to instability of the ester at 100°, it was at no time heated above 60°.

Anal. Calcd. for $C_{27}H_{36}O_6N_2$: C, 66.92; H, 7.49; N, 5.78; mol. wt., 485. Found: C, 66.25; H, 7.90; N, 5.80; mol. wt. (vapor pressure osmometer), 461.

Manool 3,5-dinitrobenzoate was prepared similarly except that the crude ester was first purified by chromatography on alumina. The product was subsequently crystallized to constant melting point as before, m.p. 95–96° without decomposition, $[\alpha]^{22}D$ +8° (*c* 1.3); reported[30] melting point for manool 3,5-dinitrobenzoate is 95–97°.

The infrared spectra of both esters resembled each other closely. Comparison thin layer chromatography on alumina gave spots with an identical R_f 0.75 when developed with 1:1 petroleum ether–benzene. However, a mixture melting point was sharply depressed to 79–80.5°.

The n.m.r. spectra were identical. Three tertiary methyl peaks were present at τ 9.32, 9.20, and 9.12, while a fourth methyl group at τ 8.25 was characteristic of a tertiary methyl geminal to oxygen. A doublet was present at τ 5.45 and 5.13 for the exocyclic methylene group. A typical vinyl AB_2 pattern was present at τ 3.60, 3.77, 3.90, 4.07, 4.60, 4.65, 4.82, and 4.88. The ninth AB_2 line that would be expected around τ 5.64 could not be detected, but this resonance is normally very low when δ_ν/J_{AB} is high as in the present case. The total number of protons was 36, three of which were represented by aromatic protons of the 3,5-dinitrobenzoate group and appeared well downfield. Brief heating at 140° decomposed 13-epimanool 3,5-dinitrobenzoate *via* elimination of 3,5-dinitrobenzoic acid. The n.m.r. spectrum indicated that extensive rearrangement of the double bonds occurred concurrently.

Contortolal Semicarbazone.—The contortolal fraction (3.1 g.) in ethanol was refluxed for 1 hr. with an excess of semicarbazide hydrochloride and sodium acetate. The reaction mixture was cooled, poured into water, and extracted with benzene to yield 4.0 g. of product which was chromatographed on alumina (activity III). Benzene–methanol 10:1 eluted 1.5 g. of an essentially pure derivative which was crystallized with difficulty from benzene–acetonitrile to constant melting point for analysis, m.p. 157–159°, $[\alpha]^{21}D$ +22° (*c* 1.2). Thin layer chromatography on alumina gave a single spot, R_f 0.80, when developed with 5:1 benzene–methanol; ν_{max} 3280-3510 (OH and NH), 1681 (C=O), 1580 (C=C and C=N), 995 (primary OH), 885 (>C=CH_2), and 763 cm.$^{-1}$ (CH=N).

Anal. Calcd. for $C_{21}H_{35}N_3O_2$: C, 69.77; H, 9.76; N, 11.62. Found: C, 69.93; H, 9.74; N, 11.56.

The n.m.r. spectrum confirmed the presence of 35 protons. Characteristic resonances were present at τ 9.45 and 8.97 (C-17 and C-19 methyls), τ 8.33 (C-16 vinylic methyl), τ 5.82 and 5.94 (C-15 vinylic CH_2O), τ 5.16 and 5.48 (C-20 exocyclic methylene), triplet at τ 4.63 (C-14 vinylic proton), and a sharp singlet at τ 3.98 (C-18 CH=N). The amide nitrogens resonated at τ 1.2 (NH) and 4.5 (NH_2) and were identified by the fact that they exchanged with deuterium in deuterium oxide. The NH exchanged very rapidly, while the NH_2 was completely exchanged after 1 hr. at room temperature. The hydroxyl proton was not visible, being no doubt hidden among the high field resonances as would be expected in the case where hydrogen bonding is not likely. The n.m.r. spectrum at 100 Mc. was very informative. The NH_2 and the vinylic proton triplet were now cleanly resolved from one another. Spin–spin decoupling clearly showed that this triplet for one vinylic proton was coupled with the vinylic CH_2O doublet at τ 4.82 and 5.94. Irradiating at τ 5.88 caused the triplet for a vinylic proton to collapse to a sharp singlet. Likewise, irradiating at τ 4.63 caused the doublet for the vinylic CH_2O to collapse to a singlet. The τ-values for the two angular methyls and even for the vinylic methyl were unchanged and no indication of coupling between the adjacent vinylic proton and vinylic methyl could be observed.

Contortadiol Di-*p*-phenylazobenzoate.—The contortadiol fraction (2.2 g.) was dissolved in 120 ml. of pyridine. *p*-Phenylazobenzoyl chloride (16 g.) was added and the solution heated on the steam bath for 4 hr. Cooling, destruction of the excess acid chloride with water, and extraction in the usual way yielded 4.1 g. of product that was chromatographed on 410 g. of alumina. Benzene eluted 0.49 g. of a crystalline product which was homogeneous according to infrared spectra and thin layer chromatography on alumina (R_f 0.60 when developed with 2:1 benzene–petroleum ether). No other major crystalline product was obtained. The ester was crystallized from benzene–heptane and benzene–methanol to constant melting point for analysis, m.p. 170–173° with decomposition, $[\alpha]^{22}D$ −11° (*c* 1.1); ν_{max} 1713 (C=O), and 1406 and 889 cm.$^{-1}$ (>C=CH_2).

Anal. Calcd. for $C_{46}H_{30}N_4O_4$: C, 76.42; H, 6.97; N, 7.75. Found: C, 75.97; H, 7.11; N, 7.55.

The n.m.r. spectrum confirmed the presence of 50 protons, 18 of which appeared well downfield in the region for aromatic protons. Characteristic resonances were present at τ 9.23 and

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8.91 (C-17 and C-19 methyls), and τ 5.15 and 5.43 (C-20 exocyclic methylene). An AB quartet was present at τ 5.38, 5.56, 5.81, and 5.99 for a C-18 CH_2O as in 18-hydroxy-13-epimanool (2), but further downfield owing to formation of the ester group. A multiplet for a single C-14 vinylic proton was present at τ 4.6 as in contortolal semicarbazone. Analogously, the C-16 vinylic methyl appeared at τ 8.21, and a doublet appeared at τ 5.08 and 5.19 for the vinylic C-15 CH_2 in which the signal for the two equivalent protons was being split by coupling to the adjacent vinylic proton at C-14. This was confirmed as before by spin–spin decoupling at 100 Mc. in which irradiation at τ 5.13 (the midpoint of the C-15 doublet) caused the multiplet for the single C-14 vinylic proton to collapse to a sharp singlet, while irradiation at τ 4.55 caused the C-15 doublet to collapse to a singlet at τ 5.13 which overlapped one of the exocyclic methylene resonances. Again no indication of coupling was observed between the C-16 vinylic methyl and the C-14 vinylic proton.

Saponification of Contortadiol Di-*p*-phenylazobenzoate.—The ester (1.652 g.) in 80 ml. of benzene was added to a solution of 6.95 g. of sodium hydroxide in 200 ml. of 95% ethanol. The mixture was refluxed for 2 hr. under nitrogen and cooled; the precipitated sodium *p*-phenylazobenzoate was filtered and washed with benzene. The filtrates were extracted as usual to yield 0.708 g. of an almost colorless oil.

This was crystallized from methylene chloride–hexane and acetone to constant melting point for analysis, m.p. 106–107.5°, $[\alpha]^{22}D$ +31° (*c* 0.9).

Anal. Calcd. for $C_{20}H_{34}O_2$: C, 78.38; H, 11.18. Found: C, 78.56; H, 11.41.

The ultraviolet spectrum showed only a strong end absorption, 210 mμ (ϵ 5600). The infrared spectrum had characteristic bands at 3270 and 1027 ($-CH_2OH$), and 3050, 1641, and 898 cm.$^{-1}$ ($>C{=}CH_2$). An infrared spectrum in carbon tetrachloride was run on a Perkin-Elmer 421 spectrophotometer, ν_{max} 3078 ($=CH_2$), and a doublet at 3634 and 3617 cm.$^{-1}$ (C-18 and C-15 OH, respectively).

Comparison with a sample of agathadiol (m.p. 107–108° $[\alpha]D$ +31°)[31] showed that the mixture melting point was undepressed and the infrared spectra were superimposable. Thin layer chromatography on alumina (R_f 0.83) and on silica gel (R_f 0.57) showed no difference between them, and mixtures were not separated. The plates were developed with 3:1 benzene–1,2-dimethoxyethane and the spots were detected with iodine vapor. However, the mass spectra, although very similar, did show definite differences. On a Σ% basis, the peaks at *m/e* 55, 82, 86, 96, 122, and 160 are clearly more intense in the spectrum of contortadiol, whereas the peaks at *m/e* 135, 277, and 306 are more intense in the spectrum of agathadiol.

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Preparation and Reactions of Hydrazino Perfluoroaromatic Compounds

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Nucleophilic attack by hydrazine on perfluoroaromatic systems has yielded versatile, difunctional, hydrazino perfluoroaromatic intermediates which have been employed in the synthesis of several disubstituted perfluoroaromatic compounds.

Nucleophilic attack by hydrazine on hexafluorobenzene has been reported[2,3] to yield pentafluorophenylhydrazine. In our studies, disubstitution was encountered when 4 moles of anhydrous hydrazine were employed (Table I).

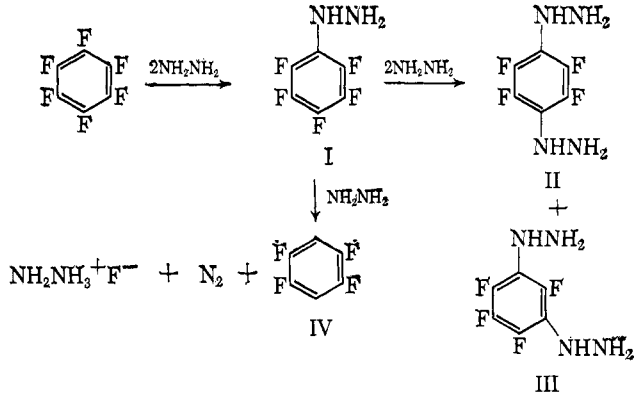
TABLE I

PRODUCTS OBSERVED[a]

Solvent	I	II	III	IV	N_2
Dioxane	24	12.5	12.5	Detected	27
Tetrahydrofuran	32	35	0	Detected	23

[a] Mole %.

When the reaction was conducted in *p*-dioxane, a 25% yield of 1:1 mixture[4] of the isomeric dihydrazinotetrafluorobenzenes II and III was obtained. An efficient means of separation of this nearly intractable mixture has not been found. When tetrahydrofuran (THF) was employed as the solvent, a 35% yield of only one isomer, *p*-dihydrazinotetrafluorobenzene (II), was realized. Pentafluorophenylhydrazine (I) was isolated from both of these reactions and was shown to be the intermediate, since it yielded the same products in essentially the same ratio when treated with additional anhydrous hydrazine.



The low yield of disubstituted product(s) has been attributed to the consumption of hydrazine, which is capable of acting as a base rather than a nucleophile, in the intramolecular oxidation–reduction of the intermediate pentafluorophenylhydrazine (I). Nitrogen and 1,2,4,5-tetrafluorobenzene (IV) are shown by us

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(4) As determined by F^{19} n.m.r.