[CONTRIBUTION FROM THE INSTITUTE OF PAPER CHEMISTRY]

Studies on the Chemistry of Aspenwood. X.[1] Neutral Materials from the Benzene Extractives of *Populus tremuloides*[2]

IRWIN A. PEARL AND JAMES A. HARROCKS

Received August 25, 1960

The neutral materials from the benzene extractives of *Populus tremuloides* were divided into two fractions based on solubility in boiling methanol, and both fractions were saponified. The saponification products were fractionated to yield all members of the saturated fatty acid series from C_{12} to C_{28} including the odd-numbered acids with the single exception of C_{27}, linoleic and oleic acids, C_{24}, C_{26}, and C_{27} saturated fatty alcohols, and glycerol. In addition, an alcohol, $C_{32}H_{54}O$, and a steroidal compound, $C_{32}H_{56}O$, have been isolated and partially characterized, but their structures have not been proved.

Recent studies on the neutral fractions of the extractives of a spent sulfite liquor from the pulping of mixed aspenwoods (*Populus tremuloides*, *P. grandidentata*, and *P. tacamahaca*)[3] led to an investigation of the neutral materials present in the original woods which might be responsible for the appearance of some of the compounds found or which might be responsible for some of the properties exhibited by pulps of aspen origin. The present paper reports the results of studies on the neutral materials present in the benzene extractives of *P. tremuloides*, quaking aspen, one of the most important pulpwoods of the *Populus* genus used in the midwest.

Although many difficulties associated with aspen pulps have been attributed to the extractives of aspen,[4] very little has been reported on the chemical nature of these materials. Mutton[5] and Browning and Bublitz[4] noted the presence of linoleic and oleic acids in saponified quaking aspen extractives. Buchanan, Sinnett, and Jappe[6] saponified the neutral portion of the benzene extractives of quaking aspen and reported even-numbered saturated fatty acids from C_{16} to C_{24}, oleic and linoleic acids, and glycerol. These authors also reported the presence of sterol material.

Authentic *Populus tremuloides* wood, freshly cut in June was reduced to sawdust and extracted at room temperature with benzene, and the benzene extract was concentrated to approximately 20% solids. The yield of solids was 1.27% based on the oven-dry wood. The raw benzene concentrated extract was shaken in the presence of a little water with an excess of calcium hydroxide powder and centrifuged. The centrifugate was extracted with 1% sodium hydroxide solution, then with dilute hydrochloric acid, and finally with water to leave a benzene extract of neutrals containing 0.624% of the original oven-dry wood. The benzene was removed under reduced pressure, and the neutral residue was extracted exhaustively with boiling methanol to yield 72% methanol-soluble neutral material and 28% methanol-insoluble neutral material.

The methanol-soluble neutral material was saponified with ethanolic potassium hydroxide and separated into acids and unsaponifiable material. Glycerol was isolated from the saponification. The acid fraction was analyzed by means of reverse-phase and normal chromatography and found to contain saturated and unsaturated fatty acids, but no *p*-hydroxybenzoic acid. The saturated and unsaturated acids were separated, and methylated separately. Gas chromatography of the methyl esters of the saturated acids indicated all saturated acids from C_{15} to C_{28} with the single exception of C_{27}, but the acids below C_{20} were present in relatively small amounts. Gas chromatography of the unsaturated methyl esters indicated linoleic acid as the major component with a smaller amount of oleic acid.

The unsaponifiable material from the methanol-soluble neutral material was chromatographed on alumina and eluted successively with petroleum ether (b.p. 30–60°), benzene, chloroform, and 1% acetic acid in absolute ethanol. The petroleum ether eluate yielded 0.43% (methanol-soluble neutral material basis) of colorless waxy semi-solid which was composed of saturated aliphatic hydrocarbons as indicated by infrared analysis. The benzene eluate yielded 1.1% of solids which were recrystallized from methanol to yield crystalline C_{24}, C_{26}, and C_{27} saturated fatty alcohols. The chloroform eluate yielded 16.5% residue which was recrystallized from methanol to yield colorless crystals melting at 136–137° and having a rotation $[\alpha]_D^{25}$ −31° in chloroform. Liebermann-Burchard and digitonin tests indicated a sterol, and the infrared spectrum was virtually identical with that of β-sitosterol. The compound did not add bromine. The infrared spectrum of the benzoate was identi-

(1) For paper IX of this series, see I. A. Pearl and P. F. McCoy, *J. Org. Chem.*, submitted for publication.

(2) A portion of a thesis submitted in partial fulfillment of the requirements of The Institute of Paper Chemistry for the Ph.D. degree from Lawrence College, Appleton, Wis., June, 1960.

(3) I. A. Pearl and P. F. McCoy, *J. Org. Chem.*, submitted for publication.

(4) B. L. Browning and L. O. Bublitz, *Tappi*, **36**, 418 (1953).

(5) D. B. Mutton, *Tappi*, **41**, 632 (1958).

(6) M. A. Buchanan, R. V. Sinnett, and J. A. Jappe, *Tappi*, **42**, 578 (1959).

cal with that of β-sitosterol benzoate, but that of the acetate was somewhat different from that of β-sitosterol acetate. Carbon and hydrogen analyses on the parent compound, the acetate, and the benzoate indicated a formula $C_{32}H_{56}O$ for the unknown sterol.

The methanol-insoluble neutral material was chromatographed directly on alumina, and the column was eluted successively with benzene, chloroform, and 1% acetic acid in absolute ethanol. The materials recovered in the three eluates represented 67, 23, and 10%, respectively, of the original methanol-insoluble neutral material. Only the benzene eluate was processed. The solids were saponified with ethanolic potassium hydroxide and separated into acids and unsaponifiable material amounting to 25 and 41.5% of the methanol-insoluble neutrals, respectively. No glycerol was found as a product of this saponification. The acid fraction was fractionated into saturated and unsaturated acids by crystallization from ether at −70°. Preliminary fractionation of the insoluble saturated acids by crystallization from ether at −40° yielded three fractions. Reverse-phase paper chromatography indicated that two of these fractions contained varying amounts of arachidic, behenic and lignoceric acids, and the third contained palmitic acid almost entirely. These fractions were converted to their methyl esters and submitted to gas chromatography which indicated that the first two fractions contained all saturated fatty acids from C_{12} to C_{24} including all odd-numbered acids. Gas chromatography of the third fraction indicated mostly palmitic acid with smaller amounts of stearic and arachidic acids. The unsaturated acids appeared to be a complex mixture containing oleic, linoleic, linolenic, and other acids.

The unsaponifiable material from the methanol-insoluble neutral material was dissolved in methanol and chilled to 0°. A small amount of crystalline material separated, and this was filtered. The product melted at 100°, gave Liebermann-Burchard and digitonin tests characteristic of sterols, but was not investigated further. The clear methanolic filtrate, upon standing at room temperature, gradually deposited colorless crystals. These crystals were recrystallized several times from methanol to yield crystals melting at 164–165° and having a rotation $[\alpha]_D^{25}$ +16.7 in chloroform. Liebermann-Burchard and digitonin tests for sterols were negative, and unsaturation was indicated by addition of bromine. Infrared and ultraviolet absorption curves indicated an hydroxyl group, but no carbonyl group, aromatic nuclei, or conjugated unsaturation. Ultimate analyses on the compound and its acetate indicated a formula $C_{32}H_{54}O$ for the alcohol.

Although not all components of both fractions have been identified, the boiling methanol fractionation procedure appears to have accomplished adequate separation of some of the materials. All glycerides were found in the methanol-soluble fraction, and all of the new sterol was also found in this fraction. The new unidentified alcohol was found only in the methanol-insoluble portion even though the long-chain saturated aliphatic alcohols were identified only in the methanol-soluble fraction. The separations in the cases of the unsaturated and saturated fatty acids appeared to be on the basis of molecular weight rather than on type.

The finding of all of the saturated fatty acids containing odd-numbered carbon chains from C_{13} to C_{25} was surprising. In the past it was a generally accepted fact that only saturated acids containing an even number of carbon atoms exist in natural fats and waxes,[7,8] but this view has changed somewhat in recent years with the identification of saturated acids with odd-numbered chains in fats of animal origin such as human hair fat,[9] butterfat,[10] ruminant body fats,[11–13] and shark liver oil.[14] In the case of fats and waxes of vegetable origin, James and Martin[15] suggested the possible occurrence of a nonadecanoic acid in olive oil, and very recently Cooke and Hansen[16] reported the finding of *n*-heptadecanoic (margaric) acid in the tall oil from *Pinus radiata*, a New Zealand conifer. The present paper is the first report of a series of saturated fatty acids with odd-numbered carbon chains in a plant extract and the first report of any such acid in a dicotyledonous wood. It is interesting to note that very recently, in a similar study on the fatty acids of a European birchwood (*Betula verrucosa*), Selleby[17] found only saturated acids with even-numbered carbon chains from C_{12} to C_{24} and no odd-numbered chains whatsoever.

The finding of linoleic acid as the major constituent of the methanol-soluble portion is in agreement with the results of Buchanan, Sinnett, and Jappe[6] who reported this acid as the major acid found on the saponification of the total extract of *P. tremuloides*. The ratio of glycerol found to

(7) K. S. Markley, *Fatty Acids*, Interscience, New York, 1947, p. 22.
(8) A. W. Ralston, *Fatty Acids and Their Derivatives*, Wiley, New York, 1948, p. 11.
(9) A. W. Weitkamp, A. M. Smiljanic, and S. Rothman, *J. Am. Chem. Soc.*, 69, 1936 (1947).
(10) R. P. Hansen, F. B. Shorland, and N. J. Cooke, *Nature*, 179, 98 (1957).
(11) R. P. Hansen, F. B. Shorland, and N. J. Cooke, *Biochem. J.*, 65, 181 (1957).
(12) F. B. Shorland and A. S. Jessop, *Nature*, 176, 737 (1955).
(13) M. J. Chisholm and C. Y. Hopkins, *Can. J. Chem.*, 35, 1434 (1957).
(14) I. M. Morice and F. B. Shorland, *Biochem. J.*, 61, 453 (1955).
(15) A. T. James and A. J. P. Martin, *Biochem. J.*, 63, 144 (1956).
(16) N. J. Cooke and R. P. Hansen, *Chem. & Ind. (London)*, 1516 (1959).
(17) L. Selleby, *Svensk Papperstidn.* 63, 81 (1960).

total acids in the methanol-soluble fraction was much lower than that expected for triglycerides and indicated the presence in this fraction of fatty acid esters of materials other than glycerol, in this case, fatty alcohols and sterol.

The C_{24}, C_{26}, and C_{27} saturated fatty alcohols found in this study indicate that *P. tremuloides* is partly responsible for the same alcohols found earlier in the spent sulfite liquor from the pulping of mixed aspenwoods.[3] Although not necessarily of significance, it is interesting to note that Hossfeld and Hunter[17] recently isolated the C_{26} saturated alcohol (ceryl) from *P. tremuloides* bark.

The sterol material melting at 136–137° had a rotation, infrared spectrum, melting point, and melting point of its benzoate essentially identical with those of β-sitosterol. However, the melting point and the infrared absorption spectrum of its acetate were quite dissimilar from those of β-sitosterol acetate. Carbon and hydrogen data on the sterol, acetate, and benzoate indicate a formula $C_{32}H_{56}O$ or $C_{32}H_{58}O$. The presence of the sterol nucleus and accompanying unsaturation indicated by the positive Liebermann-Burchard test suggest a formula $C_{32}H_{56}O$ for a monohydroxy, 32-carbon sterol. The two additional hydrogens suggested by the formula $C_{32}H_{58}O$ would demand either a saturated molecule or the opening of one of the rings of the sterol nucleus. The former possibility is ruled out by the positive Liebermann-Burchard test, and the latter by the similarity of the infrared spectra with those of β-sitosterol and its derivatives.

A few years ago Perilä[18] isolated a sterol glucoside from the closely related European trembling aspen, *Populus tremula*. Hydrolysis of this glucoside yielded a sterol having the same melting point and melting point of its acetate as the instant sterol. Perilä reported analyses for the sterol glucoside and glucoside tetraacetate, but not for the sterol and sterol acetate. On the basis of these analyses and several qualitative tests, Perilä demonstrated that the sterol was not β-sitosterol, and suggested that the compound was a sitosterol or mixture of sitosterols. Perilä's quantitative data for the sterol glucoside (C, 71.5; H, 10.3) are in better agreement with the monohydrate of a glucoside of a $C_{32}H_{56}O$ sterol (calcd. for $C_{38}H_{68}O_7$: C, 71.65; H, 10.76) than for the suggested sitosterol glucoside (calcd. for $C_{35}H_{60}O_6$: C, 72.87; H, 10.48). Therefore, it is possible that Perilä's sterol is identical with the sterol isolated from *P. tremuloides*. It should be noted, however, that Perilä's analytical data for the acetylated glucoside do not agree with those calculated for a $C_{32}H_{56}O$ glucoside tetraacetate.

The similarity of the $C_{32}H_{54}O$ empirical formula for the nonsterol optically active alcohol isolated from the methanol-insoluble fraction with that of the sterol from the methanol-soluble fraction suggests a possible relationship between the two, but such a relationship must await further information on the structures of the two compounds.

(18) O. Perilä, *Suomen Kemistilehti*, 28B, No. 3, 109 (1955).

EXPERIMENTAL[19]

Isolation of neutral materials. Several authentic trembling aspen (*Populus tremuloides*) trees were felled in northern Wisconsin in June, cut into four-foot bolts, barked immediately, and stored in warm circulating air. In batches of 30 pounds, the wood was reduced to sawdust and subjected immediately to extraction with benzene at room temperature in a stainless steel extractor. The benzene extract was concentrated under reduced pressure to approximately 20% solids. All benzene extracts were combined to give benzene solution containing 1258 g. of solids representing 1.27% of the oven-dry weight of the wood employed. After experiencing exceptional emulsion difficulties with direct extraction of the benzene extract with 1% sodium hydroxide solution, the benzene solution was shaken with an excess of calcium hydroxide powder to which a small amount of water had been added. The suspension was agitated for 1 hr. and centrifuged. The supernatant liquid was decanted, and the residue was washed with benzene by stirring and recentrifuging. The combined centrifugate and washings were extracted with 1% sodium hydroxide solution, and the benzene solution was then washed with dilute hydrochloric acid and finally with water until neutral. The resulting benzene extract was evaporated under reduced pressure to leave 616.4 g. of neutral material representing 0.624% of the original oven-dry wood. The neutral residue was extracted exhaustively with boiling methanol, and the methanol removed under reduced pressure to yield 445.5 g. of methanol-soluble neutral material as a light yellow viscous oil with a characteristic wood odor. This yield amounted to 72% of the total neutral material and 0.45% of the original oven-dry wood. The methanol-insoluble fraction amounted to 172.5 g. of yellow viscous oil and represented 28% of the total neutral material and 0.174% of the original oven-dry wood.

Saponification of methanol-soluble neutral material. The fraction was saponified with excess 5% ethanolic potassium hydroxide by boiling under reflux for 16 hr. The ethanol was removed by distillation at atmospheric pressure, and the volume was maintained by addition of water. The resulting aqueous alkaline solution was acidified with dilute sulfuric acid and extracted exhaustively with ether. The residual aqueous solution was neutralized with sodium bicarbonate and evaporated to dryness. The residue was extracted with 95% ethanol, and the extract evaporated to dryness. The process was repeated, and the resulting product was dissolved in water. The water solution was deionized with Amberlite MB-3 mixed bed resin, and the deionized solution was evaporated under reduced pressure to leave 6.86 g. of a thick sirup representing 1.54% of the methanol-soluble fraction. The thick sirup was identified as glycerol by paper chromatography along with glycerol on Whatman No. 1 paper with 8:2:1 ethyl acetate–pyridine-water and spraying with a permanganate-periodate spray. The sirup was acrylated with *p*-nitrobenzoyl chloride in pyridine, and the resulting *p*-nitrobenzoate did not depress a mixed melting point of 188° with authentic glycerol tri-*p*-nitrobenzoate prepared in the same manner.

Acids from methanol-soluble neutral material. The ether solution obtained above was extracted with 1% sodium hydroxide solution and then with water. The combined

(19) All melting points are uncorrected. Analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Colo.

extract and washings were acidified with dilute sulfuric acid and extracted with ether. The ether solution contained 237.5 g. of acids corresponding to 53.3% of the methanol-soluble fraction. This ether was extracted first with 8% sodium bicarbonate and then with 1% sodium hydroxide solutions. Both extracts were acidified and re-extracted with ether to yield 103.8 g. (44% of acids) of bicarbonate-soluble acids and 133.7 g. (56%) of alkali-soluble acids. Normal and reverse-phase paper chromatography of the bicarbonate-soluble fraction indicated only saturated and unsaturated fatty acids and no *p*-hydroxybenzoic acid. No further work was done on this fraction.

The alkali-soluble acid fraction deposited crystals upon standing. The crystals were filtered and recrystallized from dilute ethanol to give colorless crystals melting at 74°. Reverse-phase chromatography on mineral oil-impregnated paper with 85% acetic acid at 37° and detection with the mercury reagent[6] indicated a mixture of saturated fatty acids. The crystals were methylated with methanol and sulfuric acid, and the methyl esters were subjected to gas chromatography in a Barber-Coleman Gas Chromatograph with a 6 ft. by 0.25 in. column of Johns Manville Chromsorb W at 213° standardized with authentic myristic (C_{14}), *n*-pentanoic (C_{15}), palmitic (C_{16}), and stearic (C_{18}) acids. Observed retention times indicated the presence of all the saturated fatty acids from C_{15} to C_{28} with the single exception of C_{27}. Although the conditions employed with this gas chromatograph did not permit precise quantitative evaluation of the experiments, the relative peak areas for the various saturated fatty acid methyl esters gave the relative order of magnitude of the fatty acid components in this sample. The saturated fatty acids below C_{20} were present in such small amount that relative areas could not be obtained with any degree of accuracy. The data for acids from C_{20} to C_{28} are given in Table I.

TABLE I

COMPOSITION OF SATURATED FATTY ACIDS FROM THE METHANOL-SOLUBLE FRACTION

Acid	Total Area, %
C_{20}—Arachidic	2.0
C_{21}—*n*-Heneicosanoic	1.4
C_{22}—Behenic	15.1
C_{23}—*n*-Tricosanoic	9.0
C_{24}—Lignoceric	34.0
C_{25}—Pentacosanoic	4.8
C_{26}—Cerotic	30.0
C_{28}—Montanic	3.0
Total	99.3

The oily mother liquor from which the 74°-melting saturated fatty acids crystallized were submitted to reverse-phase paper chromatography on mineral oil-treated paper as before, and the chromatograms were examined by means of ultraviolet light before and after treatment with iodine vapors. The material appeared to be mostly unsaturated fatty acids. The methyl esters were prepared and determined by gas chromatography. Data indicated that the major component of the fraction was linoleic acid with lesser amounts of oleic acid and stearic acid.

Unsaponifiables from methanol-soluble neutral material. The ether solution remaining after alkaline extraction of the acids was evaporated to dryness to yield 98.9 g. of unsaponifiable material representing 22.2% of the methanol-soluble fraction or 0.1% of the original oven-dry wood. This unsaponifiable material was chromatographed on alumina[20] and developed successively with petroleum ether (b.p. 30–60°), benzene, chloroform, and 1% acetic acid in 95% ethanol as described earlier.[3] The petroleum ether eluate yielded 1.91 g. of residue which appeared from infrared spectra to be a mixture of unsaponified esters and hydrocarbon material. The fraction was saponified again, and the unsaponifiable material again chromatographed. This time the petroleum ether eluate gave a colorless residue with the consistency of petroleum jelly. Infrared analysis indicated only saturated aliphatic hydrocarbons.

The benzene eluate yielded 4.9 g. of residue accounting for 1.1% of the methanol-soluble fraction. The material was dissolved in methanol and allowed to stand. After several days the methanol solution deposited crystals which had an indefinite melting point. Infrared and paper chromatographic analysis indicated long-chain fatty alcohols. The mixture was acetylated with acetic anhydride in pyridine, and the acetates were chromatographed in the gas chromatograph. Data indicated the presence of C_{24}, C_{26}, and C_{27} saturated fatty alcohols.

The chloroform eluate yielded 73.5 g. of crystalline material amounting to 16.5% of the methanol-soluble fraction. The crude product was recrystallized several times from methanol to yield colorless plates melting at 136–137° and having a rotation $[\alpha]_D^{25}$ −31° (*c* = 2 in chloroform). A mixture with authentic β-sitosterol[21] (m.p. 138–139°) melted at 134–135°. Liebermann-Burchard and digitonin tests for sterols were positive. An infrared absorption spectrum was essentially identical with that of authentic β-sitosterol.

Anal. Calcd. for $C_{32}H_{56}O.{}^1/_2H_2O$: C, 82.51; H, 12.34. Found: C, 82.19; H, 12.35.

The product was acylated with benzoyl chloride in pyridine, and the benzoate was recrystallized from methanol-benzene to give colorless crystals melting at 147–148° and having a rotation $[\alpha]_D^{25}$ −14.3° (*c* = 2 in chloroform). A mixed melting point with the benzoate prepared in the same manner from authentic β-sitosterol (m.p. 143–144°, $[\alpha]_D^{25}$ −13.4°) was 144–145°. The infrared spectra of the two benzoates were essentially identical.

Anal. Calcd. for $C_{39}H_{60}O_2$: C, 83.51; H, 10.78. Found: C, 83.32; H, 10.98.

The sterol was acetylated with acetic anhydride in pyridine, and the acetate was recrystallized from methanol to yield colorless leaflets melting at 117–118° and having a rotation $[\alpha]_D^{25}$ −28.4° (*c* = 2 in chloroform). The acetate prepared in the same manner from authentic β-sitosterol melted at 132–133° and had a rotation $[\alpha]_D^{25}$ −39.1 (*c* = 2 in chloroform). The infrared spectra of the two acetates were quite dissimilar in the regions of 7.9–8.1, 9.5–10, 10.5–11, and 12–13 microns.

Anal. Calcd. for $C_{34}H_{58}O_2$: C, 81.86; H, 11.72. Found: C, 81.84; H, 11.99.

The acidic ethanol eluate of the alumina column was not investigated further at this time.

Preliminary fractionation of methanol-insoluble neutral material. The crude fraction was chromatographed on a column of alumina and developed successively with benzene, chloroform, and 1% acetic acid in 95% ethanol. Yields obtained in this separation were benzene eluate, 67%; chloroform eluate, 23%; and acidified ethanol eluate, 10%, all based on the methanol-insoluble neutral material. The chloroform and acidified ethanol eluates were not investigated further at this time. All studies on the methanol-insoluble neutral material reported herein were made on the benzene eluate which was obtained as an almost colorless, waxy solid melting at 60°.

Saponification of methanol-insoluble neutral material. The solids obtained upon removal of the solvent from the benzene eluate were saponified with excess 5% ethanolic potassium hydroxide in the presence of a little benzene by boiling under reflux for 24 hr. The ethanol and benzene

(20) The alumina used for all chromatographic separations was Fisher Adsorption Grade Alumina 80-200 mesh.

(21) Purchased from Aldrich Chemical Co., Milwaukee, Wis., and recrystallized from methanol.

were removed by boiling at atmospheric pressure, and the volume was maintained with water. The aqueous alkaline solution was acidified with dilute sulfuric acid and extracted with ether. The aqueous solution was tested for glycerol as described for the methanol-soluble neutral material, but no glycerol was found.

Acids from methanol-insoluble neutrals. The ether solution was extracted with 1% sodium hydroxide solution, and the alkaline extract was acidified with dilute sulfuric acid and re-extracted with ether. The ether solution contained 29 g. of acids amounting to 25% of this fraction of methanol-insoluble neutrals. Reverse-phase chromatography indicated saturated and unsaturated long-chain fatty acids. The ether was removed to yield the mixture of acids as a yellow oil solidifying at about room temperature.

The saturated acids were separated from the unsaturated acids by dissolving the mixture in anhydrous ether, chilling to −70°, and centrifuging at 2000 r.p.m. for 1 min. The supernatant liquid was decanted, and the operation was repeated several times until the residue gave no test for unsaturated acids when spotted on paper and examined under ultraviolet light after exposure to iodine vapors according to Mangold, Lamp, and Schlenk.[22] An equimolar mixture of palmitic and oleic acids was separated completely by this procedure after only two crystallizations. This acid fraction yielded 7.9 g. (27%) of saturated acids and 21 g. (73%) of unsaturated acids.

Saturated fatty acids from methanol-insoluble neutral materials. This fraction was obtained as white crystalline material melting at 51°. Reverse-phase chromatography indicated primarily palmitic acid with small amounts of other saturated fatty acids. Crystallization from anhydrous ether at −40° gave a solid fraction melting at 61–65°. The mother liquor was concentrated to half volume and again chilled to yield another crop of crystals melting at 60–63°. The mother liquor was evaporated to leave a residue melting at 52°. Reverse-phase chromatography indicated that the first two crystalline fractions contained varying amounts of arachidic, behenic, and lignoceric acid as the chief components, and the last fraction comprised essentially palmitic acid with smaller amounts of stearic and arachidic acids.

The two crystalline fractions, representing only a small portion of the entire saturated fatty acid fraction, were mixed, converted to their methyl esters, and subjected to gas chromatography. Observed retention times indicated the presence of all the saturated fatty acids from C_{12} to C_{24} including all odd-numbered acids. The quantitative data obtained from the relative peak areas of the chromatogram are given in Table II. Data for C_{12}, C_{13}, and C_{14} acids are not included because the small areas could not be determined accurately.

TABLE II

COMPOSITION OF CRYSTALLIZED SATURATED FATTY ACIDS FROM THE METHANOL-INSOLUBLE FRACTION

Acid	Total Area, %
C_{15}—Pentadecylic	1.8
C_{16}—Palmitic	6.3
C_{17}—Margaric	1.6
C_{18}—Stearic	13.7
C_{19}—Nonadecylic	1.0
C_{20}—Arachidic	30.9
C_{21}—*n*-Heneicosanic	4.2
C_{22}—Behenic	21.6
C_{23}—*n*-Tricosanoic	9.1
C_{24}—Lignoceric	9.7
Total	99.9

(22) H. K. Mangold, B. G. Lamp, and H. Schlenk, *J. Am. Chem. Soc.*, **77**, 6070 (1957).

The saturated fatty acid fraction recovered from the evaporated ether mother liquor, and representing a majority of the saturated fatty acids of the methanol-insoluble fraction appeared from reverse-phase chromatograms to be slightly impure palmitic acid. However, the molecular weight of 320 indicated by neutralization equivalent was too high for the calculated value of 256, and suggested the presence of nonacidic materials. The entire fraction was dissolved in benzene, chromatographed on alumina, and developed with five column volumes of benzene and then with five column volumes of 1% acetic acid in 95% ethanol. Evaporation of the benzene eluate yielded a colorless semi-crystalline mass which appeared by infrared analysis to be a mixture of saturated aliphatic hydrocarbons. The acidic ethanol extract was evaporated to dryness under reduced pressure, and the residue was extracted with petroleum ether (b.p. 30–60°). Evaporation of the petroleum ether yielded a crystalline residue melting at 51–54°, having an infrared absorption curve identical with that of palmitic acid, and molecular weight of 259 (Calcd.: 256.4). The low melting point (lit. m.p. 62–63°) indicated that the product could not be pure palmitic acid. The material was methylated and chromatographed on the gas chromatograph to give retention times and peak areas corresponding with a mixture of 77% palmitic acid, 10% stearic acid, and 13% arachidic acid. A synthetic mixture with this composition was prepared and found to melt at 54–55°. A mixed melting point of this mixture with the purified saturated acid fraction was not depressed.

Unsaturated fatty acids from methanol-insoluble neutral material. Reverse-phase paper chromatography indicated the presence of oleic, linoleic, linolenic, and other unsaturated acids as well as small amounts of saturated acids including palmitic and stearic. The gas chromatogram of the methylated fraction was complex, and the peaks showed considerable scatter with no clear indication of an homologous series. No further work was done on this fraction.

Unsaponifiable material from methanol-insoluble neutral material. The ether solution remaining after alkaline extraction of the acids was evaporated to dryness to yield 48 g. of unsaponifiable material representing 41.5% of the methanol-insoluble fraction. The crude residue was dissolved in methanol, chilled to 0°, and filtered to yield a few milligrams of colorless crystals melting at 100° and giving a positive Liebermann-Burchard test for sterols. No further characterization of this material was made. The methanolic filtrate was allowed to stand at room temperature. After several days, the mass of crystals which had separated was filtered and recrystallized from methanol to yield colorless crystals melting at 164–165° and having a rotation $[\alpha]_D^{25}$ +16.7 (c = 2 in chloroform). Liebermann-Burchard and digitonin tests were negative for sterols. Unsaturation was indicated by absorption of bromine from a carbon tetrachloride solution. The ultraviolet absorption spectrum contained no maxima. The infrared absorption curve indicated an hydroxyl group, but no carbonyl groups, aromatic nuclei, or conjugated unsaturation. Absorption bands were noted at 2.92, 3.42, 6.10, 6.86, 7.25, 9.65, 10.05, and 11.35 microns.

Anal. Calcd. for $C_{32}H_{54}O \cdot {}^1/_2H_2O$: C, 82.87; H, 11.95. Found: C, 82.85; H, 11.83.

The alcohol was acetylated with acetic anhydride and pyridine, and the acetate was recrystallized from ethanol to give colorless crystals melting at 164–165° and depressing greatly the melting point of a mixture with the parent alcohol. The infrared absorption curve of the acetate contained bands at 2.95, 3.45, 5.75, 6.88, 7.30, 8.03, 8.70, 9.10, 9.70, 10.16, and 11.10 microns.

Anal. Calcd. for $C_{34}H_{56}O_2$: C, 82.20; H, 11.36. Found: 82.24; H, 11.34.

An attempted acetyl analysis of the acetate by direct saponification followed by a determination of the volatile acids produced indicated that 3 moles of acid had been liberated instead of the expected 1 mole. The low oxygen

content of the acetate (less than 7%) precluded the presence of additional acyl groups.

Infrared spectra. Infrared absorption spectra were obtained with a Perkin-Elmer model 21 recording spectrophotometer using a sodium chloride prism.

Acknowledgment. The authors wish to thank Dr. Marion A. Buchanan for assistance with the gas chromatographic analyses and Mr. Lowell Sell for the infrared spectra reported in this paper. The authors also wish to thank Dr. H. A. Schuette of the University of Wisconsin for a sample of authentic *n*-pentadecanoic acid.

APPLETON, WIS.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

1-Acrylamido-1-deoxy-D-glucitol, 1-Deoxy-1-methacrylamido-D-glucitol and Their Polymerization[1]

ROY L. WHISTLER, HANS P. PANZER, AND HUGH J. ROBERTS

Received July 8, 1960

1-Acrylamido-1-deoxy-D-glucitol and 1-deoxy-1-methacrylamido-D-glucitol are synthesized from 1-amino-1-deoxy-D-glucitol (D-glucamine) and the appropriate acid anhydride. In an alternate synthesis acryloyl chloride replaces acrylic anhydride, and D-glucamine oxalate may replace the free base. The amides are relatively resistant to alkaline hydrolysis.

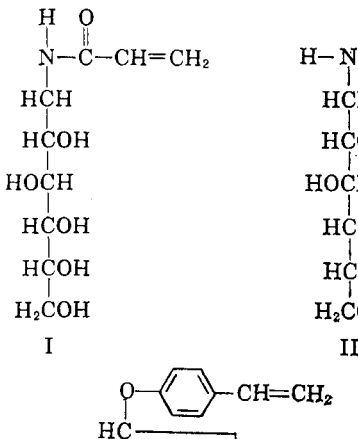
Polymerization of the amides is initiated by high energy radiation, by decomposition of peroxidic or azo-type catalysts, and by redox catalyst systems. The polymers are water-soluble and form viscous solutions which gel with borate ion. Copolymerization of the amides with other vinyl monomers also occurs.

1-Acetamido-1-deoxy-D-glucitol is synthesized and characterized.

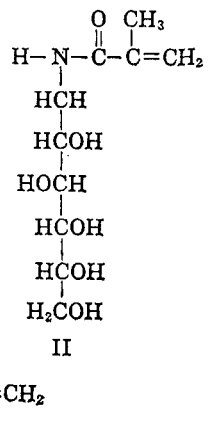
A small group of neutral synthetic high polymers receive industrial attention because of their hydrophilic nature. Among these are polyacrylamide, polyvinyl alcohol, and polyvinylpyrrolidone. A polymer of still greater hydrophilicity might consist of a hydrocarbon chain with a sugar unit attached to alternate carbon atoms. Such a structure might also evidence some polysaccharide-like characteristics. To examine these possibilities, work was undertaken to synthesize and polymerize sugar-substituted vinyl monomers. A straight chain sugar might be expected to confer greater water solubility than the ring form of the sugar. Consequently, the monomers chosen were the *N*-acryloyl and *N*-methacryloyl derivatives of 1-amino-1-deoxy-D-glucitol (D-glucamine), a commercially available sugar.[2] Both 1-acrylamido-1-deoxy-D-glucitol (I) and 1-deoxy-1-methacrylamido D-glucitol (II) should produce polymers more water-soluble than the polymer[3,4] from *p*-vinylphenyl glucoside (III) which is soluble only in the low molecular weight range.

N-Acylation of D-glucamine occurs readily in methanol at −10° to 0° using the acid anhydride as the acylating agent. However, with commercial acrylic or methacrylic anhydrides, the *N*-acetyl derivative is produced simultaneously due to acetyl impurity. In an alternate synthesis of the acrylamide derivative, acrylic anhydride is replaced by acryloyl chloride, which is easily prepared from benzoyl chloride and acrylic acid,[5] and D-glucamine may be replaced by its oxalic acid salt.

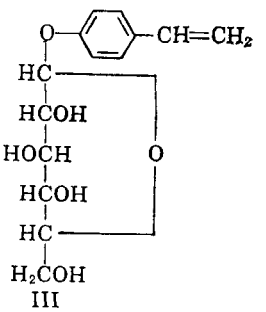
Each of the *N*-acyl derivatives of D-glucamine is water-soluble, neutral, and nonreactive toward



I



II



III

(1) Presented, in part, before the Division of Carbohydrate Chemistry at the 135th Meeting of the American Chemical Society, Boston, Mass., April 1959; Journal Paper No. 1632 of the Purdue Agricultural Experiment Station.

(2) R. B. Flint and P. L. Salzberg, U. S. Pat. 2,016,962 (1935); available from Corn Products Company, New York, N. Y.

(3) B. Helferich and H.-J. Höfmann, *Chem. Ber.*, **85**, 175 (1952).

(4) B. Helferich and K.-H. Jung, *Z. physiol. Chem.*, **311**, 54 (1958).

(5) G. H. Stempel, Jr., R. P. Cross, and R. P. Mariella, *J. Am. Chem. Soc.*, **72**, 2299 (1950).