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Benzene-Alcohol-Soluble Extractives of Sitka Spruce

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Extraction of mature Sitka spruce woodmeal with benzene-alcohol (2:1) dissolved 2.0% of the wood. After precipitation of an ether-insoluble lignin-like fraction (0.2% of the wood), the extractives were separated into neutral, acidic, and phenolic portions by conventional methods. The phenolic fraction (0.36%) consisted of acetovanillone (0.144%), vanillin (0.144%), and vanillyl alcohol (0.072%). Tropolones were absent. The acidic fraction (0.27%) apparently contained no volatile or fatty acids but contained a lignin-like acid (methoxyl = 11%, neut. equiv. = 750). The cyclohexylamine salts of the acid fraction precipitated as an oil or solid from which a low melting acid fraction (m.p., *ca.* 50° neut. equiv. = *ca.* 300) could be regenerated.

Neutral compounds amounted to about 65% of the ether soluble extractives (1.17% of the wood) and consisted of volatiles (0.18%): a conjugated monoterpene diene, possibly caradiene, and an unsaturated alcohol; and nonvolatiles (0.97%): β-sitosterol (.09%) and l-2-octyl-β-sitosterol phthalate (0.90%). Phthalates have apparently been isolated from only one other plant source in trace amounts. The fact that one has been obtained here as one-half of the ether-soluble extractives emphasizes the marked chemical differences between extractives of Sitka spruce and those of more thoroughly investigated species.

The extractive components which occur in mature woody tissue have considerable interest in organic chemistry, in plant taxonomy and in the wood-based industries. Although they sometimes affect the course of pulping processes and the durability of the wood, some of the commercial woods of North America have not been investigated thoroughly,[1–3] among them Sitka spruce (*Picea Sitchensis*, Carr.). Previous reports on the extractive content of this species appear to be restricted to values of the content of total extractives (2.61%[4] or 2.3%[5]), to a portion of an investigation by Erdtman on the presence or absence of conidendrin in conifers,[6] and to a portion of an investigation of components of leaf oils of conifers by Lehman and Lynn.[7] Erdtman found no conidendrin present in Sitka spruce woodmeal, and the latter authors found in its leaf oils β-pinene, β-phellandrene, camphor, borneal, terpineol, and nonylic and butyric acids. Terpenes are reported to constitute over 40% of the steam volatile oil.[8]

The present investigation was carried out upon a sample of extractives obtained from mature Sitka spruce logs. The alcohol-benzene extract contained extractives amounting to 2% of the wood weight. The solution was concentrated on a cyclone separator and separated by conventional methods into four fractions, an ether-insoluble material and acidic, phenolic, and neutral ether-soluble materials (Fig. 1). The ether-insoluble fraction had the appearance of a native lignin fraction and was not investigated in detail.

A number of separate experiments were run on the acid fraction. The bicarbonate-soluble material was steam-distilled after acidification and no volatile acids were found to be present. However, when the residue from the steam distillation was extracted into ether a small amount of ether-insoluble acid was obtained. This amounted to five or six per cent of the original sodium bicarbonate-soluble material. It was a brown amorphous solid melting from 175 to 195° with a neutral equivalent of 771 to 773 and a methoxyl content of 11.2%. The ultraviolet absorption spectrum showed a minimum at 258 to 260 mμ and a maximum at 283 to 284 mμ. This is reminiscent of an ether-insoluble lactonic acid reported previously to be isolated after steam distillation of wood extractive acids[9,10] but appeared more ligninlike in character.

Separation of fatty and resin acids was attempted by several procedures. The resin acids were precipitated by cyclohexylamine from acetone solutions of the bicarbonate-soluble material, (acids), the alkali-soluble material (phenols and acids), and also the total ether-soluble material. From both the bicarbonate-soluble and alkali-soluble products only viscous oils were obtained. On precipitating cyclohexylamine salts from the total ether-soluble material, a light colored precipitate corresponding to 15% of the total was obtained. This is essentially the same quantity of acids as was obtained by bicarbonate extraction. The total acid fraction was therefore apparently precipitable by cyclohexylamine. The cyclohexylamine salt on acidification with boric acid yielded a yellow light

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(3) E. F. Kurth, *Chem. Revs.*, **40**, 33 (1947).

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(8) A. J. Lehman and E. V. Lynn, *J. Am. Pharm. Assoc.*, **20**, 29 (1931).

(9) H. Hibbert and J. B. Phillips, *Can. J. Research*, **4**, 1 (1931).

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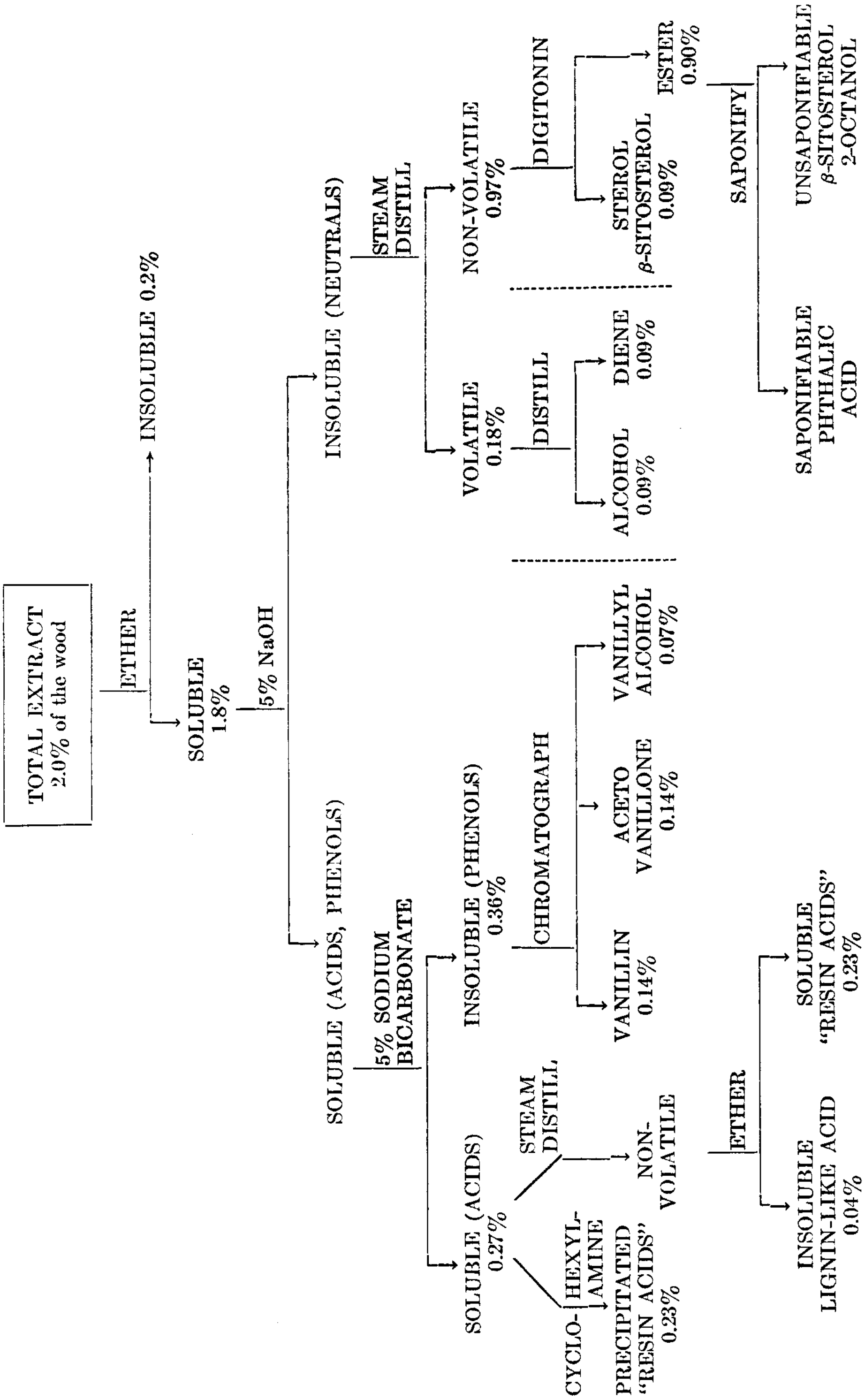

FIG. 1. SCHEME OF SEPARATION

colored solid with a melting range of 50–55° and with a neutral equivalent of 300. The melting point range appeared unusually low even for a gross mixture of resin acids. The product had an ultraviolet absorption spectrum showing several maxima. However, the product obtained on standard precipitations with diamylamine had litte absorption in the ultraviolet and when the total acid mixture was treated in a similar fashion with diethylamine to obtain neoabietic acid only a trace of precipitate was obtained. These and similar results probably indicate that the products decomposed on manipulation but new resin acids may be present. Several standard methods for obtaining urea inclusion compounds failed to separate any aliphatic acids. All the acids in Sitka spruce therefore appear to behave like resin acids except for the observed melting range. No volatile or aliphatic acids were found.

The phenolic fraction, which was bicarbonate-insoluble and alkali-soluble, was separated readily on alumina. The phenols were developed with chloroform and three distinct yellow bands appeared. The most strongly adsorbed top band, representing 40% of the mixture, was acetovanillone, the middle band, 20%, was vanillyl alcohol, and the lower band, 40%, vanillin. Identification was by means of qualitative tests, mixed melting points, and derivatives.

The neutral compounds amounted to 65% of the ether-soluble extractives. Steam distillation separated about one sixth of the neutral materials as a volatile fraction. The volatile fraction distilled into two fractions of almost equal size boiling at 35–38° and 55–60° at 1 mm. The first fraction, redistilled at 74 to 77°, 10-mm. pressure, was a light yellow oil which formed fine needle-like crystals at about 10°. The oil had a refractive index of 1.4945 and a density of 0.79 at 25°, was unsaturated to bromine and permanganate, and gave a negative test for alcohol and for carbonyl. The ultraviolet absorption spectrum had a peak at 265 mμ with an absorption coefficient of approximately 70. This material was hydrogenated under a slight pressure using platinum on charcoal as catalyst and absorbed 1.96 moles of hydrogen per 10 carbon atoms. On filtration of the hydrogenated solution and evaporation of ethanol, a yellow solid which had a menthol-like odor was obtained in insufficient quantities for other tests.

These results can be interpreted in the following manner: The boiling point and density are in a range suitable for a monoterpene but low for a sesquiterpene. Quantitative hydrogenation indicates two double bonds per C_{10} unit. The absorption coefficient and wave length of maximum absorption are typical of a conjugated diene, with both double bonds in a single cyclohexane ring since acyclic conjugated dienes or those with double bonds not in the same ring absorb between 217 and 250 mμ (Woodward's rules).[11] The molecular refraction for acyclic, monocyclic, and bicyclic monoterpene conjugated dienes are 47.46, 45.26, and 43.77. The values obtained from the experimental refractive index and density (for the corresponding assumed molecular weights of 138, 136, and 134) are 46.19, 45.52, and 44.85. The best agreement is therefore for the monocyclic diene; however, a 2% or 3% increase in our semimicro determination of density, the value of which appeared to be low, would make the bicyclic dienes a possibility as well. Known monocyclic dienes such as α-terpinene and the phellandrenes having conjugated double bonds do not have similar physical properties and the corresponding saturated hydrocarbons are liquids. However, some saturated bicyclic monoterpenes such as camphane and isocamphane are fragrant solids. The four adjacent carbon atoms of the diene system must not include a bridgehead carbon atom (Bredt's rule). If the unknown is bicyclic, only two adjacent carbon atoms can be common to both rings. Therefore, of the seven principal bicyclic monoterpene skeletons only that of carane has a suitable structure. This reasoning leads to the conclusion that the diene most probably is the unknown caradiene. However, carane prepared previously by the hydrogenation of carene and by other methods is a liquid. This is in conflict with our supposition but Simonsen suggests that the caranes obtained are probably mixtures of stereoisomers.[12]

The higher boiling fraction of the steam-volatile neutrals was an unsaturated alcohol, described in the Experimental section. It was obtained in rather small quantity and not completely identified.

The nonvolatile neutral fraction comprised the largest portion of the extractives of Sitka spruce and amounted in fact to about 55% of the ether-solubles. The nonvolatile material gave a negative ceric nitrate alcohol test and a negative dinitrophenylhydrazine carbonyl test. Permanganate and bromine tests for unsaturation were inconclusive. The Lieberman-Burchard test for sterols was positive but the material was clearly a mixture from its behavior on crystallization.

Nine grams of the nonvolatile neutral fraction was treated with digitonin and approximately seven tenths of a gram of the free sterol precipitated as the digitonide. This accounted for about 7% of the total neutral fraction or 4.5% of the total extractives. The remaining nonvolatile neutral compound was isolated by distilling off under vacuum the alcohol from which the digitonide had been precipitated. Two grams of this material was dissolved in petroleum ether and chromatographed on alumina. On development with ben-

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[11] R. B. Woodward, *J. Am. Chem. Soc.*, **63**, 1123 (1941); **64**, 72 (1942).

[12] J. L. Simonsen, *The Terpenes*, Vol. II, Cambridge University Press 1949, p. 61 *ff*.

zene one sharp yellow-brown band appeared which was eluted to yield 1.9 grams or 95% as a yellow semisolid material. This material had a saponification equivalent ranging from 269 to 272 and was titrated and shown to have no detectable acidity. By ether extraction of the alkaline solution a solid alcohol was recovered. This, as well as the free sterol, was identified as β-sitosterol by precipitation with digitonin, the Leiberman-Burchard sterol test, ultraviolet absorption spectrum in 97% sulfuric acid, and agreement between the melting points for the purified β-sitosterol and its acetate and benzoate with the literature values. No evidence was found for the presence of any other sterol than β-sitosterol in the ester or as free sterol.

From the saponification of the nonvolatile neutral material with potassium hydroxide in 95% ethanol there was also obtained a potassium salt which precipitated from the cold basic solution and which amounted to 10% of the fraction. The potassium salt was acidified and yielded an acid melting at 205–207° which had a neutral equivalent of 81 to 81.5. The product of reaction with thionyl chloride proved to be an anhydride melting at 130°. The acid also formed a *p*-bromophenacyl ester melting at 153° and a mixed melting point of the unknown with phthalic acid was undepressed. The ultraviolet absorption spectrum of the isolated acid was essentially identical with that of phthalic acid. Ultraviolet absorption and saponification also established the expected amount of the phthalic acid in the purified ester.

When the alkaline solution from the saponification of one gram of pure ester was distilled a water-white, water-immiscible liquid was also obtained weighing approximately one tenth of a gram. It boiled between 180 and 185°, had a density of 0.820, a refractive index of 1.4244, a melting point of approximately −30°, a specific rotation of −60°. Oily 3,5-dinitrobenzoates and phenylurethanes were formed and a solid hydrogen phthalate melting at 74 to 75°. The corresponding properties of 2-octanol[13] are: b.p., 179–180°, d^{25} 0.817, n^{25} 1.4244, m.p., −31.6°, m.p. of derivative 75°. The specific rotations, $[\alpha]^{25}_{D}$, have been variously reported from ±8 to ±9°. The nonvolatile neutral compounds present in Sitka spruce are therefore essentially a mixture of a small amount of β-sitosterol and l-2-octyl-β-sitosterol phthalate.

There are several substantial differences between the extractives of Sitka spruce and other related wood species. The carboxylic acid fraction amounts to only 15–20% of the total ether-soluble fraction which is much less than is usually reported for soft woods. Resin and fatty acid fractions often account for around 70% of the extractives from other spruce species. The phenolic fraction on the other hand is considerably greater than is usually found in related species. The three phenolic compounds isolated constitute 15 to 20% of the total ether-soluble extractives.

The neutral fraction of this species is most unusual. The most remarkable fact is that so large a percentage of the extractive fraction should be a phthalate ester. Neither phthalic acid nor any phthalic ester seems to have been found in nature except for a single report of trace amounts of dibutyl phthalate found in Zinfandel grapes by Haagen-Smit and co-workers.[14] This appears also to be the first report of l-2-octanol as well although other naturally occurring octanols have been found. It will be interesting to learn whether the postulated presence of caradiene proves correct. These results once more emphasize the remarkable differences found between the extractive fractions of closely related species.

EXPERIMENTAL

Preparation of extract. The Sitka spruce logs were supplied by the Columbia Cellulose Co., Ltd., of Prince Rupert, B. C. They came from trees approximately 75 years old from an upland stand of 700 feet elevation where they occurred in mixture with Hemlock, near Terrace, B.C.

The whole logs were split, chipped, and hammer-milled to a coarse meal. Eighty-five pounds of the meal in 10-pound batches was extracted twice by immersion for 24 hr., with occasional stirring, in 15 gal. of alcohol-benzene (1:2) at room temperature. The solvent was drawn off and concentrated in a steam-jacketed vacuum flash evaporation apparatus (Scientific Glass Co., Inc., Cat. J-52, #J-1563), at the rate of just under 2 gal. per hr.

A total of approximately 700 g. of alcohol-benzene-soluble extraneous components was thus obtained, in the form of a 30% concentrate in alcohol-benzene. This total extractive concentrate, a dark brown slurry, was freed from solvent by vacuum distillation to yield the free extractives as a viscous semisolid mass. This was stirred with five to ten times its weight of ethyl ether, in order to separate the ether-soluble material. The precipitated ether-insoluble material constituted 8 to 10% of the total benzene-alcohol extract and resembled a native lignin fraction. It had a methoxyl content of 14.3% (analysis by Dr. K. Ritter, Basel, Switzerland) and the ultraviolet absorption spectrum showed a maximum at 282 mμ. The maxima reported for softwood lignins fall in a range of 280 to 284 mμ.

The ether-soluble fraction constituted approximately 90% of the total extract or 1.8% of the air-dried weight of the wood.

Separation of alkali-soluble, bicarbonate-soluble, and neutral fractions. In a typical separation, 30.5 g. of the total ether-soluble extractives was dissolved in 150 ml. of ethyl ether and shaken three times in a separatory funnel with 100-ml. portions of 5% sodium hydroxide solution. The sodium hydroxide solutions were combined and acidified to *p*H 4 to 5 with sodium dihydrogen phosphate, then extracted with 250-ml. portions of ethyl ether. The ether solutions were combined, dried over magnesium sulfate, and the ether evaporated to yield 14.2 g. (46.5%) of total acidic material, including phenolics. Under slightly different conditions, 65% of the extractives were recovered as brown viscous neutral material not extracted by 5% alkali from the original ether extract.

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(13) G. L. Dorough, H. B. Glass, T. L. Gresham, G. B. Malone, and E. E. Reid, *J. Am. Chem. Soc.*, **63**, 3100 (1941).

(14) A. J. Haagen-Smit, F. N. Hirosawa, and T. H. Wang, *Food Research*, **14**, 472 (1949).

Repeated separations of phenols and acids were carried out as follows: Thirty g. of the sodium hydroxide–soluble material was dissolved in 300 ml. of ether and extracted in a separatory funnel with three 250-ml. portions of 5% sodium bicarbonate. The sodium bicarbonate solutions were combined and acidified to a *p*H of 4 to 5 with sodium dihydrogen phosphate, then extracted with three 250-ml. portions of ether. The ether solutions were combined and dried over magnesium sulfate, and the ether evaporated off to yield approximately 12 to 13 g. of sodium bicarbonate-soluble material.

The carboxylic acids. (*1*) *The resin acids.* In the first trials, portions of the total ether-soluble material were dissolved in five times their weight of boiling acetone, about 5% (of the total weight) of cyclohexylamine was added and the resulting solution was allowed to cool overnight at 0°. A pale yellow-brown precipitate was filtered off which amounted to 15% of the total ether-soluble material, calculated as free resin acids, or roughly the same as the bicarbonate-soluble fraction. The precipitated salts were taken up in saturated boric acid solutions and shaken with ethyl ether. The ether layers were separated, dried over magnesium sulfate, and the ether evaporated off to yield a yellow-brown solid with a melting range of 50–55°. This had a neutral equivalent of 300.

The ultraviolet absorption spectrum of this material showed maxima at 244, 248, 254, and 260 mμ, with absorption coefficients (α) ($\alpha = \frac{D}{C}$, D is optical density, C is concentration in g./l.) of 37.5, 47.5, 52.5, and 32, respectively, and an inflection point at 238 mμ with an absorption coefficient of 29.

The acid fraction obtained from the cyclohexylamine precipitation was dissolved in boiling acetone and an equimolar amount of diamylamine added. On cooling the solution overnight at 0°, a yellow precipitate was obtained. This was recrystallized from acetone to a constant melting point of 50–52°, then shaken as before with ether and saturated boric acid solution. Upon evaporation of the ether, a solid amounting to 16% of the total acid fraction was obtained and was recrystallized from ethanol to a constant melting point of 58°. An ultraviolet absorption spectrum of the material showed negligible absorption.

When the total "resin acid" mixture was treated in a similar fashion with diethylamine, a specific precipitant for neoabietic acid, only a trace of precipitate was obtained.

When the alkali-soluble or the bicarbonate-soluble fraction was dissolved in boiling acetone and treated as before with cyclohexylamine, only gummy, viscous oils were obtained upon cooling. These could not be induced to crystallize either by supercooling in Dry Ice–acetone or by trituration with nonsolvents such as petroleum ether or ethyl ether.

(*2*) *The fatty and volatile acids.* In several separate trials, 1 g. of the sodium hydroxide–soluble or sodium bicarbonate-soluble material, as obtained above, was added to 30 ml. of hot methanol containing 5 g. of urea or 20 ml. containing 3 g. of urea. When this solution was cooled, no solid appeared.

Two g. of the sodium hydroxide–soluble or sodium bicarbonate–soluble material was ground for 2 hr. with 10 g. of urea moistened with methanol, then extracted with petroleum ether to remove uncomplexed material. Examination of the residual urea crystals showed no evidence of complex. The crystals were dissolved in dilute hydrochloric acid to destroy any complex, if present, and the acidic solution extracted with petroleum ether. The petroleum ether solution was dried over $MgSO_4$ and evaporated to leave negligible material.

The sodium bicarbonate–soluble material was steam-distilled, in portions of 20 to 30 g., for 5 hr. or more until approximately one l. of distillate was collected. This was extracted with three 250-ml. portions of ether, the ether solutions combined and dried over magnesium sulfate. No acidic product was obtained. When the nonsteam-volatile acids were extracted with ether, a small amount of ether-insoluble acid was obtained. This amounted to 5 to 6% of the original sodium bicarbonate–soluble material. It was a brown amorphous solid which melted from 175–195°, and had a neutral equivalent of 771 to 773. The ultraviolet absorption spectrum of this material showed a minimum at 258 to 260 mμ, α of 14, and a maximum at 283 to 284 mμ, with an α of 22. Analysis showed a methoxyl content of 11.20, 11.12% (analysis of Dr. K. Ritter, Bazel, Switzerland). Its appearance was similar to that of a lignin fraction.

Phenolic constituents. Ether extraction of sodium bicarbonate suspensions of the sodium hydroxide–soluble material, as outlined in the separation of the carboxylic acid fraction, served to separate the phenolic material, amounting to 50 to 60% of the sodium hydroxide–soluble portion.

In appearance, the phenolic material was an amorphous yellow solid having an odor reminiscent of vanillin. It melted over the range of 70° to 80°. A ferric chloride test was positive, producing a pinkish coloration.[15] Reaction with 2,4-dinitrophenylhydrazine indicated the presence of a carbonyl fraction.[15]

One g. of the phenolic mixture was dissolved in the minimum volume of chloroform necessary for solution, (*ca.* 5 ml.) and added to a chromatographic grade, neutralized alumina column, measuring approximately 3 × 50 cm. Upon development with a large volume of chloroform three distinct yellow bands appeared. The column was extruded, the bands were cut out and each extracted with ethanol. The alcohol extracts were evaporated to yield 0.4 g. (40% of the mixture) from the top, 0.2 g. from the middle somewhat diffuse band, and 0.4 g. from the bottom band.

The material constituting the bottom band of the chromatogram melted in the range of 75–80°, had a vanillin-like odor and gave a positive Tollen's aldehyde test,[15] and a pink color characteristic of vanillin when treated with resorcinol and hydrochloric acid.[16] (The materials constituting both the top and middle bands gave negative Tollen's tests.)

When the unknown was dissolved in 50% aqueous ethanol and heated with potassium cyanide ammonium carbonate, a hydantoin which melted at 275–276° with decomposition, was obtained. (Vanillin hydantoin 276°.) The unknown formed a *p*-nitrobenzoate of m.p. 123–124°. Vanillin *p*-nitrobenzoate melts at 124.5°. A mixed melting point of the purified unknown, m.p. 80–81°, with vanillin, m.p. 81°, showed no depression.

The material forming the middle band of the chromatogram melted in a crude state at 95–105°. After repeated recrystallizations from aqueous ethanol it had a constant melting point of 113–114° and showed no depression with vanillyl alcohol. Its identity was confirmed by preparation of a benzoate,[15] recrystallized from aqueous alcohol, m.p. of 120–121° and an acetate recrystallized from aqueous alcohol, m.p. 47–48°.

The product from the top band of the chromatogram gave a positive carbonyl test with 2,4-dinitrophenylhydrazine, but negative Schiff's and Tollen's aldehyde tests.[15] When repeatedly recrystallized from aqueous ethanol, the melting point rose slightly to a constant value of 113–115°. No depression was shown in a mixed melting point of the unknown phenol with acetovanillone, melting at 113–114°. The substance also reacted with aqueous copper acetate to form a yellow-green complex, as reported by Mulliken[17] for acetovanillone. Its identity with acetovanillone was confirmed by the preparation of a benzoate[15] m.p. of 105–106° and an acetate[15] m.p. 57–58°.

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[15] N. D. Cheronis and J. B. Entrikin, *Semimicro Qualitative Organic Analysis*, Thomas V. Crowell Co., New York, 1947.

[16] Official and Tentative Methods of Analysis, Assoc. of Official Agric. Chemists, 320.

[17] S. P. Mulliken, *A Method for the Identification of Pure Organic Compounds*, Vol. I, John Wiley, New York, 1904, p. 95.

The neutral compounds. (1) *Steam distillation.* Ten g. of the total neutral material was distilled with steam for 8 hr. The distillate was extracted three times with 100-ml. portions of ether, the ether solutions combined and dried over magnesium sulfate. Upon evaporation of the ether 1.5 g. of a pungent-smelling yellow oil, about 10% of the total ether-soluble extractives, was obtained. Vacuum distillation of the oil in a Todd column gave two fractions of almost equal size: (1) a fraction boiling at 35–38° at 1-mm. pressure, (2) a fraction boiling at 55–60° at 1 mm.

A. The first fraction, redistilled at 74–77°, 10-mm. pressure, was a light yellow oil, more than half of which formed fine needle-like crystals at about 10°. The oil had a refractive index, n^{25}_{D}, of 1.4945 and a density of 0.790 (25°). Bromine and permanganate tests for unsaturation[15] were positive, a ceric nitrate test for alcohols was negative. Tollen's aldehyde and 2,4-dinitrophenylhydrazone tests were also negative. The ultraviolet absorption spectrum had a peak at 265 mμ with an absorption coefficient, α, of approximately 70, both indicating a conjugated diene.

This material (51.38 mg.) was hydrogenated under a slight pressure of hydrogen using 10 mg. of 5% platinum on charcoal catalyst in a system similar to that described by Vandenheuvel.[18] Sixteen and two-tenths ml. of hydrogen at standard temperature and pressure was consumed in 3 hr. Therefore, 70.3 g. of the sample was equivalent to 1 mole of hydrogen or 1.96 double bonds per ten carbon atoms. On filtration of the hydrogenated solution and evaporation of the ethanol, a yellow solid which had a menthol-like odor was obtained in insufficient quantity for other tests.

B. The higher boiling fraction of the steam volatile compounds gave a positive permanganate unsaturation test and also a positive ceric nitrate alcohol test.[15] On reaction with phenyl isocyanate a solid was formed which seemed to be a mixture of two compounds, one melting around 190°, the other about 240°. This oil had a refractive index, n^{25}_{D}, of 1.4473, and a density of 0.845 at 25°.

(2) *The nonsteam volatiles.* The residue after steam distillation above was extracted with ether (250 ml.) three times, the ether dried over magnesium sulfate and evaporated at the water pump to yield *ca.* 8 g. of viscous brown material, about 55% of the total ether-soluble alcohol-benzene–extracted compounds. This material was soluble in ether, petroleum ether, benzene, ethanol, and chloroform, sparingly soluble in methanol, and insoluble in water. It gave a negative ceric nitrate alcohol test and a negative dinitrophenylhydrazine carbonyl test.[15] Permanganate and bromine tests for unsaturation were inconclusive.[15] A Lieberman-Burchard color test for sterols[19] was positive.

Three grams of the total nonvolatile neutral compounds was saponified by the method of Jamieson[20] for 3 hr. Upon cooling fine needle-like crystals were formed in the aqueous ethanol and filtered off. The alkaline solution was extracted three times with 50 ml. of ether, the ether solutions combined, dried over magnesium sulfate and the ether evaporated to yield 2.7 g. of unsaponifiable material.

The crystals previously filtered off were recrystallized from 95% ethanol to fluffy white needles which did not melt at temperatures up to 250°, but gave a positive flame test for potassium.[19] It was acidified with 10% hydrochloric acid and extracted with ether. The ether extract on evaporation and recrystallization from water yielded an acid of m.p. 208–210° and neut. equiv. 81.5. The acid on reaction with excess thionyl chloride and ice cold excess concentrated ammonium hydroxide failed to form the amide.[21] A light tan nitrogen-free solid was formed, melting at 130–131° and corresponding to phthalic anhydride m.p. 131°.

A solid *p*-bromophenacyl ester was obtained from the acid which had a constant melting point of 153–154°. The ultraviolet absorption spectra of phthalic acid and the acid isolated from saponification were essentially identical and a mixed melting point was undepressed.

The unsaponifiable fraction, a viscous brown oil, gave a positive Lieberman-Burchard sterol test. Accordingly, it was treated with digitonin, a specific precipitant for sterols, as described by Hibbert and Phillips.[9] The digitonide was decomposed by heating with excess pyridine, the pyridine was stripped off under vacuum and the residue was extracted with ether, leaving behind the insoluble digitonin.[9] On evaporation of the ether, the crude sterol was obtained melting over the range 112–125°.

The acetate of the sterol was formed in very poor yield by heating the dry digitonide in 5 ml. of acetic anhydride until complete dissolution, cooling and adding 30 ml. of 60% aqueous ethanol to the cold solution. The acetate thus precipitated had a constant melting point of 118–119° after repeated recrystallizations from 95% ethanol. The benzoate, after repeated recrystallizations from 95% ethanol, had constant m.p. 139–140°. The sterol itself was recrystallized from methanol to constant m.p. 134–135°. The corresponding melting points for β-sitosterol are 118–119° for the acetate, 141–142° for the benzoate, and 135.5–136° for the free sterol.[22] The ultraviolet absorption spectrum of the sterol in 97% H_2SO_4[23] corresponded closely to that of β-sitosterol reported by Bernstein and Lenhard.[23]

When 0.9 g. of the total nonvolatile neutral material was treated with digitonin before saponification, approximately 0.7 g. of β-sitosterol was isolated and identified as before. This corresponds to about 5% of the total extractives as free sterol.

The remaining nonvolatile neutral compounds were obtained by distilling off under vacuum the alcohol from the solution from which the digitonide had been precipitated. A 2-g. sample of this material was dissolved in approximately 20 ml. of petroleum ether and chromatographed on a 3.5 × 40 cm. column packed with alumina. On development with benzene a sharp yellow-brown band appeared and was eluted. The benzene was evaporated from this eluate fraction to yield 1.9 g. or 95% of a yellow semisolid material. This material had saponification equivalents ranging from 269 to 272, indicating a molecular weight of 540 or greater, depending on the purity of the chromatographic fraction which could not be recrystallized.

An ultraviolet absorption spectrum was taken of the purified phthalate ester. The extinction coefficient for the ester at the wave length 282 mμ was that to be expected of a phthalate ester of about 650 molecular weight approximately corresponding to one sterol unit per phthalic acid residue. However, when the sample was titrated it was shown to have no detectable acidity and therefore was not a half ester of phthalic acid.

When the pure ester was saponified with alcoholic potassium hydroxide followed by ether extraction of the alkaline solution, a solid alcohol was recovered. From one gram of ester approximately 0.6 g. of a yellow-white solid alcohol was obtained with a m.p. range 110–120°. It gave a positive Lieberman-Burchard color test[19] and showed the same absorption spectrum, in 97% sulfuric acid, as the β-sitosterol obtained as the free sterol, and from saponification of the total nonvolatiles.

When the alkaline solution from the saponification of 1 g. of ester was distilled, a water-white, water-immiscible liquid was obtained, approximately 0.1 g. It boiled between 180 and 185° and had a density, at 25° of 0.820. The refractive

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index, n^{25}_{D}, was 1.4244. When frozen in Dry Ice–acetone, the melting point was approximately −30°. The optical rotation of this alcohol, $[\alpha]^{25}_{D}$, was −6.0°, c = 4.56 in alcohol. Attempted preparations of the 3,5-dinitrobenzoate and phenylurethane of the alcohol led only to uncrystallizable oils. The hydrogen phthalate[24] melted at 74–75°. l-2-Octyl hydrogen phthalate melts at 75°.[21]

SYRACUSE 10, N. Y.

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Ring D Steroid Oximes and the Beckmann Rearrangement

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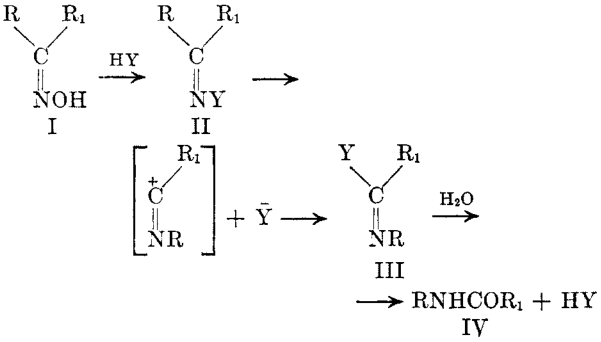
Whereas simple ring D and ring A steroid oximes yield the corresponding oxime acetates on subjection to mild acetylating conditions, 3β-hydroxyandrostan-16,17-dione 16-oxime gives a pure crystalline compound which was characterized by its chemical properties and behavior as an intermediate in a Beckmann Rearrangement. Rearrangement could be completed under appropriate, though equally mild, conditions. Reasons for the unexpected rearrangement under such mild conditions are advanced.

The mechanism of the Beckmann Rearrangement still stimulates considerable experimental investigation despite the ninety years that have elapsed since the original discovery of the reaction. Mesenheimer, Kuhara, and Chapman have laid the foundations of our knowledge and outlined the generalizations for understanding this reaction.[3] As stated by various authors,[4,5] Beckmann Rearrangement with a variety of acid reagents essentially involves ester formation between an oxime (I) and the reagent HY. By means of ionization a process of *trans* interchange then takes place yielding an imidoyl ester (III) from the oxime ester (II). The amide (IV), the end-product of the reaction, arises from (III) by ill-defined steps, one of which undoubtedly is hydrolytic, and the acid reagent is regenerated.



A recent paper by Stephen and Staskun[6] departs from the classical formulations of this reaction and proposes a new mechanism for amide formation under entirely anhydrous conditions. Our contribution in this field arose during the course of investigations on the structure of Heard's lactone.[7] These investigations involved an attempt to prepare 3β-acetoxy-16-acetoximinoandrostan-17-one (VI) from 3β-hydroxyandrostan-16,17-dione 16-oxime (V) by allowing the latter to stand overnight in a mixture of acetic anhydride and pyridine at room temperature. We have acetylated the oximes of cholestanone, testosterone, and epiandrosterone under these same conditions. However, while many stable acetates of alpha oximino ketones have been prepared,[8] the product obtained from V was not VI, as expected, but has been formulated as having the structure VII.

After recrystallization from anhydrous ether VII was obtained as colorless feathery needles. While stable in the dark, it gradually turns yellow on exposure to light. It has an absorption peak in the ultraviolet at 223 mμ as compared with the peak at 240 mμ exhibited by V. This hypsochromic shift of 17 mμ could not be explained by mere acetylation of the oxime hydroxyl—as in VI—, especially since we observed that similar acetylation of testosterone oxime causes a bathochromic displacement of the absorption maximum of 5 mμ. (Table I). When dissolved in alcohol the absorption

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(3) See, for example, B. Jones, *Chem. Revs.*, **35**, 335 (1944).

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