Diacetate of I. One hundred sixteen grams of I was combined with 100 g. of acetic anhydride and 200 ml. of xylene. The acetic acid formed was distilled as an azeotrope with xylene, through a 1.5-ft. Vigreux column. The excess anhydride and xylene were removed by vacuum distillation. The remaining residue amounted to 137.3 g. against 143.5 g. theoretical. It was fractionated without a column: 90% boiled at 186–189° at 0.6 mm. n_D^{20} 1.5230. On saponification it assayed 98.1% as the diacetate of I (II).

Anal. Calcd. for $C_{28}H_{36}O_4$: C, 77.03; H, 8.313. Found: C, 76.96; H, 8.203.

2-(2-Methyl-1-propenyl)-3-isopropyl-1,5-diphenyl-1,5-pentanediol (purified) (III). Fourty-four grams of II was saponified with a solution of 22.5 g. of potassium hydroxide (85%), 55 ml. of methanol, and 55 ml. of water by refluxing under agitation for 8 hr. The reaction product was cooled, 50 ml. of benzene and 50 ml. of water were added, and the organic layer was separated and washed with successive 50-ml. portions of water neutral to litmus. The solvent was distilled in a vacuum and the remaining residue of 35.5 g. was fractionated without a column. 85% boiled at 204–206° at 1.1 mm. n_D^{20} 1.550 (III). A modified Rast method[1] gave a molecular weight of 348 against 352 theoretical.

Anal. Calcd. for $C_{24}H_{32}O_2$: C, 81.77; H, 9.15. Found: C, 81.60; H, 9.20.

RESEARCH LABORATORIES OF FRITZSCHE BROTHERS, INC.
PORT AUTHORITY BUILDING
76 NINTH AVENUE
NEW YORK 11, N. Y.

16-Hydroxylated Steroids. XVIII.[1] 16-Hydroxylated 19-Norandrogens

SEYMOUR BERNSTEIN AND E. W. CANTRALL

Received January 16, 1961

Little information is available on the biological effect of C-16-hydroxylation of androgens in regard to their androgenic and protein anabolic activities.[2] It was decided therefore to prepare the C-16-hydroxylated derivatives of several 19-norandrogens, in particular, the 16α-hydroxy derivatives of 19-nortestosterone and 17α-methyl-19-nortestosterone.

The synthesis of 16α-hydroxy-19-nortestosterone (II) was readily accomplished as follows. Estriol 3-monomethyl ether (I)[3] on Birch reduction according to the procedure of Wilds and Nelson[4] followed by treatment of the intermediate dihydro product with hydrochloric acid in refluxing methanol gave the desired 16α-hydroxy-19-nortestosterone (II).[5]

16α-Hydroxy-17α-methyl-19-nortestosterone (VII) was synthesized in the following manner. 3-Methoxy-1,3,5(10),16-estratetraen-17-ol acetate (III)[6] was treated with perbenzoic acid in benzene followed by a mixture of glacial acetic acid and perchloric acid to give in 45% yield 16α-acetoxy-3-methoxy-1,3,5(10)-estratrien-17-one (IV). Reaction of the latter with methylmagnesium bromide afforded a mixture of diols, 16α,17β-dihydroxy-3-methoxy-17α-methyl-1,3,5(10)-estratriene (V) (38% yield) and 16α,17α-dihydroxy-3-methoxy-17β-methyl-1,3,5(10)-estratriene (VI) (47% yield). While C-17-ketones unsubstituted at C-16 undergo nucleophilic attack with the entering group coming in from the rear or α-face,[7] the formation of V and VI in approximately equal amounts indicates that the orienting effect of the C-13-methyl group is considerably altered by the presence of a 16α-acetoxy group. The stereochemistry of the two diols V and VI is supported by their mode of formation and by the fact that VI forms an acetonide derivative VIII while V under the same conditions is recovered unchanged.

In order to exclude the possibility that the *cis*-diol had not arisen from IV *via* initial attack of the Grignard reagent on the ester carbonyl followed by rearrangement of the intermediate ketol to the more stable[3a] 17β-hydroxy-3-methoxy-1,3,5(10)-estratrien-16-one (X) and subsequent attack at C-16 to give the isomeric diol XI,[8] the latter was prepared for comparison with VI. The base catalyzed rearrangement of IV according to the procedure of Leeds, Fukushima, and Gallagher[3a] afforded X in 45% yield. The latter was treated with methylmagnesium bromide to give 16β,17β-dihydroxy-3-

(1) Paper XVII, S. Bernstein, M. Heller, and S. M. Stolar, *Chem. & Ind. (London)*, 516 (1961).

(2) The preparation of both C-16-epimers of 16-hydroxytestosterone has been described. A Butenandt, J. Schmidt-Thomé, and T. Weiss, *Ber.*, **72B**, 417 (1939), have reported on the synthesis of 16-hydroxytestosterone in nine steps from dehydroisoandrosterone. Although the stereochemistry of the product was not discussed, it was presumably 16β-hydroxytestosterone. This assignment of configuration follows from an analogous set of transformations known to produce the methyl ether of 16-epiestriol in which the C-16, and 17-hydroxyl groups are *cis* to each other and β. In this connection, see M. N. Huffman and H. H. Darby, *J. Am. Chem. Soc.*, **66**, 150 (1944), and M. N. Huffman and M. H. Lott, *J. Am. Chem. Soc.*, **69**, 1835 (1947); **75**, 4327 (1953).

The 16α-hydroxy epimer has been prepared by microbiological hydroxylation of testosterone with *Streptomyces roseochromogenus* [J. Fried, D. Perlman, A. F. Langlykke, and E. O. Titus, U. S. Patent **2,855,410** (October 7, 1958)], and by an elaborate synthesis from methyl 3β-hydroxy-16,17-seco-5-androstene-16,17-dioate [W. J. Adams, D. K. Patel, V. Petrow and I. A. Stuart-Webb, *J. Chem. Soc.*, 297 (1956)].

(3) (a) N. S. Leeds, D. K. Fukushima, and T. F. Gallagher, *J. Am. Chem. Soc.*, **76**, 2943 (1954); (b) A. Butenandt and E. L. Schäffler, *Z. Naturforsch.*, 1, 82 (1946); and (c) S. A. Thayer, L. Levin, and E. A. Doisy, *J. Biol. Chem.*, 91, 655 (1931).

(4) A. L. Wilds and N. A. Nelson, *J. Am. Chem. Soc.*, **75**, 5366 (1953).

(5) The preparation of this compound by an identical pathway has been described by O. Schindler, *Helv. Chim. Acta*, **42**, 1955 (1959). Our work was completed prior to this publication.

(6) W. S. Johnson and W. F. Johns, *J. Am. Chem. Soc.*, **79**, 2005 (1957).

(7) L. F. Fieser and M. Fieser, *Steroids*, Reinhold Publishing Corp., New York, N. Y., 1959, pp. 467–8.

(8) (a) D. A. Tyner, U. S. Patent **2,949,476** (August 16, 1960); and (b) H. Mori, K. Yasuda, and S. Wada, *J. Pharm. Soc. Japan*, **78**, 813 (1958).

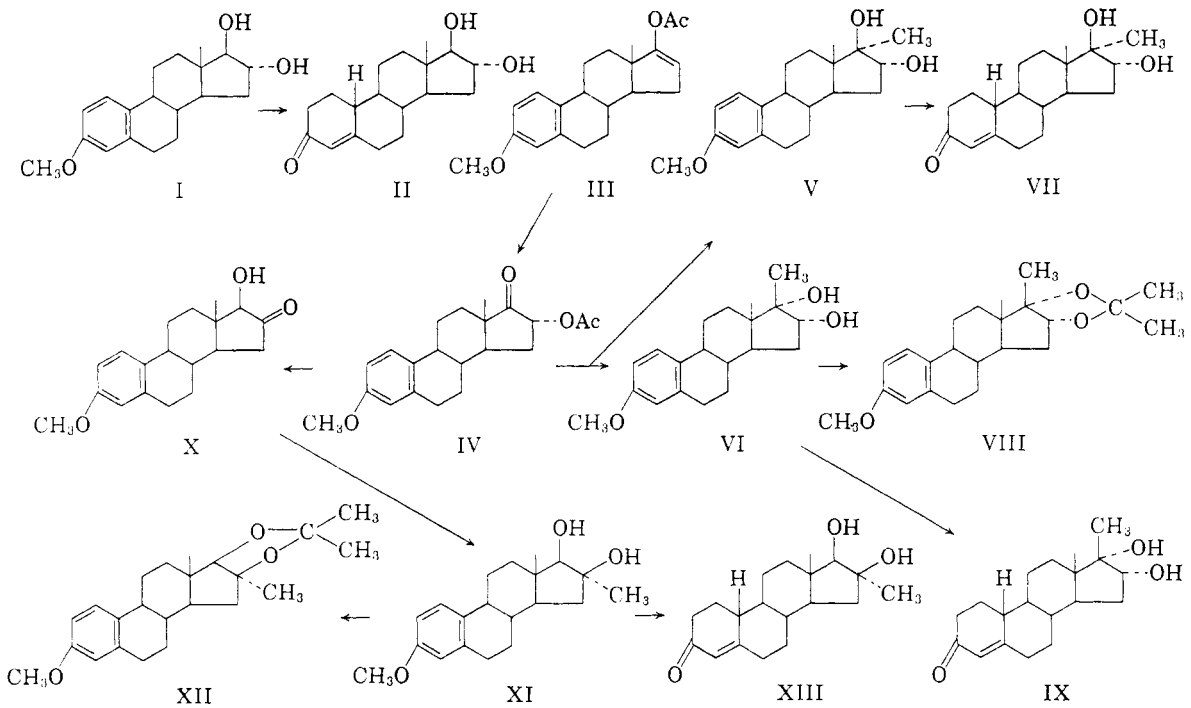

TABLE I

PHYSICAL CONSTANTS OF ISOMERIC DIOLS AND ACETONIDES

Diol			Acetonide		
Compound No.	M.P.	$[\alpha]_D$(CHCl$_3$)	Compound No.	M.P.	$[\alpha]_D$(CHCl$_3$)
VI	154.5–155	+30.5	VIII	162.5–163	+70.5
XI	174–177	+68	XII	142.5–144.5	—
	179–181[8a]	+71		141–143.5[8a]	+109
	171–174[8b]	+77		136–138[8b]	—

methoxy-16α-methyl-1,3,5(10)-estratriene (XI). A comparison of the physical constants (Table I) of the two *cis*-diols VI and XI and their respective acetonides (VIII and XII) shows that they are not identical.

Reduction of the diols V and VI with lithium and liquid ammonia followed by treatment of the intermediate dihydro compound with acid gave 16α-hydroxy-17α-methyl-19-nortestosterone (VII) and 16α,17α-dihydroxy-17β-methyl-19-nor-4-androstenone (IX), respectively. Similarly the diol XI gave 16β-hydroxy-16α-methyl-19-nortestosterone (XIII).

Bioassays.[9] The diols II, VII, IX, and XIII in a modified androgenic protein anabolic assay[10] were found to have little if any activity as androgens and protein anabolic agents.

(9) The assays were carried out by the Dept. of Pharmacological Research, Experimental Therapeutics Research Section of these laboratories.

(10) L. G. Herschberger, E. G. Shipley, and R. K. Meyer, *Proc. Soc. Exptl. Biol. Med.*, **83**, 175 (1953).

EXPERIMENTAL[11]

16α-Hydroxy-19-nortestosterone (II). To a solution containing 1.0 g. of estriol monomethyl ether (I) in 65 ml. of tetrahydrofuran was added 80 ml. of liquid ammonia and 1.05 g. of lithium wire cut into small pieces. The resulting solution was stirred for 1 hr. in a Dry Ice–acetone bath, when 12 ml. of absolute ethanol diluted with 12 ml. of tetrahydrofuran was added dropwise, with stirring, over 14 min. The cooling bath was removed, and most of the ammonia was evaporated by warming the reaction mixture in a bath of warm water. The reaction mixture was partitioned between 150 ml. of ether–methylene chloride (2:1) and water. The aqueous phase was extracted with 3 × 90 ml. portions of ether–methylene chloride (2:1). The combined extract was washed with water and saturated sodium chloride solution and evaporated to a semicrystalline solid *in vacuo*. The latter crystallized from methanol to yield 705 mg. (70%) of the intermediate dihydro derivative, 16α,17β-dihydroxy-3-methoxy-2,5(10)-estradiene, m.p. 154–162°; ν_{max} 1700 and 1675 cm.$^{-1}$

(11) All melting points are uncorrected and, unless noted otherwise, were determined on a Kofler block. Specific rotations are for chloroform solution at 25°. The infrared spectra were determined in pressed disks of potassium bromide; the ultraviolet spectra were measured in methanol. Unless noted otherwise, the term "petroleum ether" refers to the fraction of b.p. 60–70°.

To a solution containing 603 mg. of the latter in 50 ml. of methanol was added 2.5 ml. of water and 1.25 ml. of concd. hydrochloric acid solution. The resulting solution was heated to reflux for 20 min. and neutralized with sodium acetate. The reaction mixture was evaporated *in vacuo*, and the residue was partitioned between ether–methylene chloride (2:1) and water. The organic phase was washed successively with 5% potassium bicarbonate, water, and saturated sodium chloride solutions. On evaporation of the extract, an oil was obtained which crystallized from acetone–petroleum ether to give 440 mg. (76%), m.p. 185–187°. A sample for analysis was recrystallized thrice more from the same solvents and had m.p. 187–188°; $[\alpha]_D$ + 25°; λ_{max} 240 mμ 17,000); ν_{max} 1668, 1623, and 1065 cm.$^{-1}$ (lit.[5] m.p. 191–193°; $[\alpha]_D$ + 33°].

Anal. Calcd. for $C_{18}H_{26}O_3$ (290.39): C, 74.44; H, 9.03. Found: C, 74.42, 74.34; H, 9.35, 9.12.

16α-Acetoxy-3-methoxy-1,3,5(10)-estratrien-17-one (IV). To a solution containing 7.65 g. of 3-methoxy-1,3,5(10),16-estratetraen-17-ol acetate (III) in 25 ml. of benzene was added 110 ml. of a 0.35 *M* solution of perbenzoic acid in benzene. The resulting solution was let stand 24 hr. at room temperature. The reaction mixture was washed successively with ice-cold 5% sodium hydroxide, water, and saturated sodium chloride solutions. The benzene solution was evaporated to a solid *in vacuo*, and the latter was dissolved in 90 ml. of glacial acetic acid and treated with a solution containing 1.5 ml. of 72% perchloric acid and 8.5 ml. of glacial acetic acid. The mixture was let stand 30 min. at room temperature and was diluted with 500 ml. of ether and washed successively with ice-cold 10% potassium carbonate, water, and saturated sodium chloride solutions. The ether solution on evaporation gave a red glass which was dissolved in benzene and filtered through a 50-g. column of magnesium silicate to give 4.84 g. of a yellow semicrystalline solid. The latter was crystallized from acetone–petroleum ether to yield 3.50 g. (45%), m.p. 156–158°.

An analytical specimen was prepared from the product obtained in another run, and had m.p. 161.5–162.5°; $[\alpha]_D$ +139°; ν_{max} 1760, 1753, 1258 (shoulder), 1235 and 1223 cm.$^{-1}$

Anal. Calcd. for $C_{21}H_{26}O_4$ (342.42): C, 73.66; H, 7.66. Found: C, 73.16; H, 7.78.

Reaction of 16α-acetoxy-3-methoxy-1,3,5(10)-estratrien-17-one (IV) *with methylmagnesium bromide.* A solution containing 3.43 g. of 16α-acetoxy-3-methoxy-1,3,5(10-estratrien-17-one (IV) in 70 ml. of benzene was made anhydrous by distilling 30 ml. of solution. The system was placed under a nitrogen atmosphere, and 50 ml. of *ca.* 3.0*M* solution of methylmagnesium bromide in di-*n*-butyl ether was added dropwise with stirring over 25 min. The reaction mixture was heated to reflux under nitrogen for 12 hr., cooled, and treated with a solution containing 10 ml. of acetone in 50 ml. of benzene. The inorganic salts formed dissolved upon the addition of 75 ml. of ice-cold 10% sulfuric acid solution. The organic phase was washed successively with water, 5% sodium bicarbonate, water and saturated sodium chloride solutions, and evaporated to afford 3.0 g. of a viscous oil. The latter was submitted to partition chromatography on diatomaceous earth (700 g.) with the solvent system, chloroform–petroleum ether: methanol-water (50,200,110,20) (1 hold-back volume = 1300 ml.)

Evaporation of Fraction A (*ca.* second half of the first hold-back volume) gave 1.5 g. (47%) of 16α,17α-dihydroxy-3-methoxy-17β-methyl-1,3,5(10)-estratriene (VI). One recrystallization from methanol gave 1.15 g. (36%) of product, m.p. 152.5–154°. A sample for analysis was recrystallized twice from methanol and had m.p. 154.5–155°; $[\alpha]_D$ +30.5°.

Anal. Calcd. for $C_{20}H_{28}O_3 \cdot {}^1/_4\ H_2O$: C, 74.86; H, 8.96. Found: C, 75.06; 75.30; H, 9.12, 9.13.

Evaporation of Fraction B (*ca.* third hold-back volume) afforded 1.2 g. (38%) of 16α,17β-dihydroxy-3-methoxy-17α-methyl-1,3,5(10)-estratriene (V). A single recrystallization from acetone–petroleum ether gave 863 mg. of product, m.p. 185–187°. A sample from another run was recrystallized four times for the same solvent pair and had m.p. 184–185.5°; $[\alpha]_D$ +32°.

Anal. Calcd. for $C_{20}H_{28}O_3$ (316.42): C, 75.91; H, 8.92. Found: C, 75.51; H, 9.13.

16α,17α-Isopropylidenedioxy-3-methoxy-17β-methyl-1,3,5-(10)-estratriene (VIII). To a solution containing 606 g. of VI in 20 ml. of acetone was added 4 drops of a 72% perchloric acid solution. The resulting solution was let stand for 4 hr. at room temperature and was then diluted with water. The product was extracted into ether, and the extract was washed with sodium bicarbonate solution, water, and saturated saline. The dried extract was evaporated to afford a semicrystalline solid which crystallined from aqueous acetone to yield 566 mg. (83%) of VIII, m.p. 158–164°. A sample for analysis was recrystallized several times from aqueous acetone and had m.p. 162.5–163°; $[\alpha]_D$ +71°.

Anal. Calcd. for $C_{23}H_{32}O_3$ (356.49): C, 77.49; H, 9.05. Found: C, 77.65; H, 9.26.

16α-Hydroxy-17α-methyl-19-nortestosterone (VII). Treatment of 1.0 g. of V with lithium and liquid ammonia as described above (*cf.* the preparation of II) gave 743 mg. of the dihydro derivative, m.p. 153–60°; ν_{max} 1670 and 1700 cm.$^{-1}$ The latter (490 mg.) was treated with hydrochloric acid in refluxing methanol as described above to give a viscous oil. Crystallization from ethyl acetate afforded 176 mg. (38%), m.p. 193–199°. An additional 23 mg. of VII, m.p. 195–199°, was obtained from the mother liquors. A sample for analysis was crystallized several times from aqueous acetone and had m.p. 198–200°; $[\alpha]_D$ ±0°; λ_{max} 240 mμ (ε 16,900); ν_{max} 3490, 1653, and 1615 cm.$^{-1}$

Anal. Calcd. for $C_{19}H_{28}O_2$ (304.41): C, 74.96; H, 9.25. Found: C, 74.80; H, 9.31.

16α,17α-Dihydroxy-17β-methyl-19-nor-4-androsten-3-one (IX). The Birch reduction of 2.0 g. of VI as described above (*cf.* preparation of II) gave on crystallization of the crude product from methanol 1.53 g. (76%) of the intermediate dihydro product, m.p. 169–176°; ν_{max} 1695 and 1667 cm^{-1}. Similarly, treatment of 0.50 g. of the latter with acid as described above followed by crystallization from aqueous acetone gave 337 mg. (70%) of IX, m.p. 171–178°. A specimen for analysis was recrystallized several times from acetone–petroleum ether, m.p. 189–190°; $[\alpha]_D$ −4.5°; λ_{max} 240 mμ (ε 17,600); ν_{max} 3500, 3290, 1660, and 1622 cm^{-1}.

Anal. Calcd. for $C_{19}H_{28}O_3$ (304.41): C, 74.96; H, 9.27. Found: C, 74.58; H, 9.46.

17β-Hydroxy-3-methoxy-1,3,5(10)-estratrien-16-one (X). To a solution containing 5.0 g. of 16α-acetoxy-3-methoxy-1,3,5(10)-estratrien-17-one (IV) in 1100 ml. of methanol–benzene (10:1) was added 400 ml. of a 0.1*N* solution of sodium hydroxide. The reaction mixture was let stand at room temperature for 15 hr., was acidified with a few ml. of acetic acid, and concentrated *in vacuo* to *ca.* 500 ml. The product precipitated as an orange, oily solid which was extracted with 375 ml. of ether–methylene chloride (2:1). The extract was washed successively with 5% sodium bicarbonate solution, water, and saturated saline. On evaporation the extract gave an amber oil which was dissolved in acetone and decolorized with charcoal. The product crystallized from acetone–petroleum ether to yield 1.98 g. (45%) of oily crystals, m.p. 158–166° (capillary). A single recrystallization from aqueous acetone raised the melting point to 164–170° (capillary). (Lit. m.p. 169–169.5°,[3a] 167–168°.[12])

16β,17β-Dihydroxy-3-methoxy-16α-methyl-1,3,5(10)-estratriene (XI). The reaction of 2.18 g. of X with methylmagnesium bromide as described above (*cf.* the preparation of V and VI) afforded a semicrystalline mass which crystallized from benzene to yield 1.83 g. (80%) of XI, m.p. 174–177°; $[\alpha]_D$ + 68°.

16β,17β-Isopropylidenedioxy-3-methoxy-16α-methyl-1,3,5-(10)-estratriene (XII). Treatment of 101 mg. of XI with acetone and perchloric acid as described above (*cf.* the

(12) M. N. Huffman, *J. Biol. Chem.*, **169**, 167 (1947).

preparation of the acetonide VIII) gave 75 mg. of XII from aqueous ethanol, m.p. 142.5–144.5° (lit. 141–143.5°,[8a] 136–138°[8b]).

16β-Hydroxy-16α-methyl-19-nortestosterone (XIII). Reduction of 1.5 g. of XI with lithium and liquid ammonia as described above (*cf.* the preparation of II) gave a solid which crystallized from methylene chloride–methanol to give 1.14 g. (75%) of the dihydro product as colorless needles, m.p. 183–195°; ν_{max} 1698 and 1669 cm.$^{-1}$ Treatment of 500 mg. of the latter with hydrochloric acid in refluxing aqueous methanol as described above gave a semicrystalline solid which crystallized from acetone–petroleum ether to give 400 mg. (84%) of XIII, m.p. 175.5–179°. A sample for analysis was recrystallized thrice from acetone–petroleum ether and had m.p. 176–179.5°; $[\alpha]_D$ + 34°; λ_{max} 240 mμ (ϵ 16,800); ν_{max} 1670, 1618, 1263, 1208, 1130, and 1063 cm.$^{-1}$

Anal. Calcd. for $C_{19}H_{28}O_3$ (304.41): C, 74.96; H, 9.27. Found: C, 74.75; H, 9.31.

Acknowledgment. We wish to thank Louis M. Brancone and associates for the elemental analyses, William Fulmor and associates for the infrared and ultraviolet absorption spectra and optical rotation data, and C. Pidacks for the partition chromatography.

ORGANIC CHEMICAL RESEARCH SECTION
LEDERLE LABORATORIES
A DIVISION OF AMERICAN CYANAMID CO.
PEARL RIVER, N. Y.

Studies on the Chemistry of Aspenwood. XIII.[1] Further Studies on the Neutral Extractives of Commercial Aspen Spent Sulfite Liquor[2]

IRWIN A. PEARL AND OLIVER JUSTMAN

Received January 16, 1961

In a previous paper on the neutral extractives of a spent sulfite liquor from the commercial ammonia-base pulping of peeled mixed aspenwood (*Populus tremuloides, P. grandidentata,* and *P. tacamahaca*)[3] the finding of esters of long-chain fatty alcohols, sterols, glycerol, and phenolic acids with saturated and unsaturated long-chain fatty acids and phenolic acids together with some of their constituent components was reported. Studies on crude "neutrals" and saponified "neutrals" were reported. The present paper reports additional studies on these same materials.

Re-evaluation of the fatty-acid fraction of the original "neutrals" indicated the presence of myristic and lauric acids in addition to the previously reported palmitic, stearic, arachidic, behenic, and lignoceric saturated fatty acids reported earlier. The original "neutrals" were re-examined for phenolic acids, but none could be found by means of paper chromatographic procedures.

Further studies on the saponification of the petroleum ether-soluble "neutrals" with both *N* and 2*N* ethanolic potassium hydroxide were made. Lauric and capric acids were found in addition to previously reported saturated fatty acids. The presence of C_{22}, C_{24}, C_{25}, and C_{26} long-chain saturated fatty alcohols was indicated by the high-temperature reverse-phase chromatographic procedure of Fiker and Hajek[4] and confirmed by low-temperature reverse-phase chromatography of *p*-phenylazobenzoic acid esters of the mixed alcohols.

Even after saponification with strong ethanolic alkali, the "neutral" fraction obtained by continuous extraction with ether or ethyl acetate contained substantial amounts of acidic materials. Linoleic, oleic, arachidonic, lauric, and capric acids in addition to two unidentified unsaturated fatty acids were found in the mother liquors from the crystallization of the long-chain fatty alcohols in these "neutral" fractions. Thus, it is apparent again that the so-called "neutral" fraction obtained by extraction of an alkaline saponification mixture with an immiscible solvent may actually contain acidic materials in the form of fatty acids. Although the distribution coefficient of these fatty acid compounds between aqueous alkaline solution and ethyl acetate or ether is in favor of the aqueous alkaline solution, continuous extraction with the immiscible solvent will remove a portion of these acids.

The acids and phenols fraction obtained after saponification of the original "neutrals" was found to contain substantial amounts of vanillic, syringic, *p*-hydroxybenzoic, and ferulic acids in addition to several unidentified phenolic acids. Thus, it appears that the extractives of the mixed aspens employed in the original sulfite cook contain esters of fatty acids with all of these phenolic acids, and that the ester linkage is between the carboxyl group of the fatty acids and the phenolic group of the phenolic acids. Furthermore, the carboxyl groups of the phenolic acids must be esterified with aliphatic long-chain alcohols. Apparently, these esters are hydrolyzed to a certain extent during the sulfite cook because all the same products were found free in the ether extractives of the original liquor before saponification,[5] but some pass through the sulfite cook and can be hydrolyzed only by subsequent saponification with strong alkali.

(1) For paper XII of this series, see I. A. Pearl and L. R. Busche, *Tappi*, **43**, 970 (1960).

(2) This paper represents a portion of the results obtained in the research program sponsored by the Sulphite Pulp Manufacturers' Research League and conducted for the League by The Institute of Paper Chemistry. Acknowledgment is made by the Institute for permission on the part of the League to publish these results.

(3) I. A. Pearl and P. F. McCoy, *J. Org. Chem.*, **26**, 550 (1961).

(4) S. Fiker and V. Hajek, *Chem. Listy*, **52**, 549 (1958).

(5) I. A. Pearl and D. L. Beyer, *J. Org. Chem.*, **26**, 546 (1961).