to that of the material originating from the methylated oxidized cellulose. Specifically, the β-form was found 4–6.2 cm. from the top of the column and the α-form 9.5 to 13 cm. from the top of the column.

Examination of Fraction A.—Fraction A, the chloroform extract of the aqueous solution of the hydrolyzed methylated oxidized cellulose was concentrated to dryness under reduced pressure at room temperature and then extracted with petroleum ether (b.p. 30–60°). The petroleum ether extract on being concentrated to dryness and treated with aniline in ethanol yielded the anilide of tetramethyl-D-glucose, m.p. 97°, in agreement with reported constants[29] (yield 10 mg.). The compound showed no depression in melting point on admixture with an authentic sample. The material that was insoluble in petroleum ether was dissolved in ether, filtered and allowed to stand several weeks at 0°. The solution deposited crystals (1 g.) which were identified as 2,3,6-tri-*O*-methyl-α-D glucose, m.p. 120–123°, $[\alpha]^{20}D$ +70° (H_2O, *c* 1),[30] after recrystallization from ether.

(29) J. C. Irvine and Agnes M. Moodie, *J. Chem. Soc.*, **93**, 95 (1908); M. L. Wolfrom and W. L. Lewis, THIS JOURNAL, **50**, 837 (1928).

(30) H. C. Carrington, W. N. Haworth and E. L. Hirst, *ibid.*, **55**, 1084 (1933).

Anal. Calcd. for $C_9H_{18}O_6$: C, 48.6; H, 8.12. Found: C, 48.7; H, 8.03.

Examination of Fraction B.—Fraction B was dissolved in ether, filtered and allowed to stand several weeks at 0°. The solution deposited crystals (16 g.) which were identified as 2,3,6-tri-*O*-methyl-α-D-glucose, m.p. 119–121°, $[\alpha]^{20}D$ +70° (H_2O, *c* 1). The filtrate from the crystals on being allowed to stand at 0° continued to deposit additional quantities of 2,3,6-tri-*O*-methyl-α-D-glucose over a period of weeks.

Acknowledgment.—We should like to express our appreciation to Drs. N. K. Richtmyer and H. G. Fletcher, Jr. of the National Institutes of Health for several very helpful discussions. We should also thank Dr. A. Patchornik of the National Institutes of Health for determining the carboxylic acid and carboxyl content of samples of methylated oxidized cellulose and Mr. C. H. Penning of Eastman Products, Inc., Kingsport, Tenn.. for the oxidized cellulose acetate.

INDIAN HEAD, MD.

[CONTRIBUTION FROM THE BOTANICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF TOKYO]

A New Phenolic Glycoside in *Viburnum furcatum* Blume[1]

BY SHIZUO HATTORI AND HIDEMASA IMASEKI

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A phenolic glycoside was isolated from the leaves of *Viburnum furcatum* Blume. The chemical structure of the glycoside has been shown to be *p*-vinylphenol D-apio-(D or L)-furanosyl-1,6-β-D-glucopyranoside, and the name "furcatin" is proposed for it.

In the course of experiments designed to isolate chlorogenic acid isomers as the agents responsible for the color change to brown of the leaves of the genus *Viburnum* (Caprifoliaceae), a phenolic glycoside has been isolated from the leaves of *Viburnum furcatum* Blume. This glycoside is new and the authors wish to propose for it the name "furcatin."

Furcatin crystallizes as colorless needles of bitter taste and is very easily soluble in ethanol, methanol and water and insoluble in ether, benzene and petroleum ether. It is also soluble in ethyl acetate saturated with water, but is slightly soluble in water-free ethyl acetate. Concentrated sulfuric acid dissolved furcatin and gave a deep red solution which did not fluoresce under ultraviolet light. Furcatin decolorized dilute potassium permanganate and bromine solutions, thus showing the presence of an aliphatic unsaturated bond in the molecule. On the other hand, negative reactions with ferric chloride or diazotized *p*-sulfanilic acid, 2,4-dinitrophenylhydrazine or acid fuchsin decolorized with sodium sulfite, hydroxylamine–ferric salt and hydroiodic acid by Zeisel's method indicated that furcatin did not possess free phenolic, carbonyl, carboxyl and methoxyl groups. Lead acetate did not give an insoluble lead salt with furcatin.

Furcatin was easily hydrolyzed by dilute mineral acids into reducing sugars and an oily aglycone, but was not hydrolyzable by apricot emulsin. The aglycone purified by steam distillation was a colorless oil of a peculiar aromatic odor and turned brown in the air in a few days. It has free phenolic hydroxyl group, since it reacted with diazotized sulfanilic acid and ferric chloride yielding orange and faint blue-violet colors, respectively. The aglycone also gave a deep red coloration with concd. sulfuric acid and a cornflower blue coloration with concd. hydrochloric acid. By catalytic hydrogenation, one mole of furcatin absorbed one mole of hydrogen. This also showed that furcatin has a double bond.

Methylation of the aglycone with dimethyl sulfate gave an oily methyl ether of an anise-like odor, which yielded *p*-methoxybenzoic acid and an alkaline-soluble, acid-insoluble gas by oxidation with dilute potassium permanganate. This indicates that the aglycone is a simple phenol which has an unsaturated side chain at the *p*-position. Although the gas evolved was not examined qualitatively, it must be carbon dioxide. Oxidation of the methyl ether with Beckmann mixture and ozone gave anisaldehyde and formaldehyde. Therefore it can be deduced that the carbon dioxide or formaldehyde formed by oxidation of the methyl ether arose from a terminal carbon atom of the two-carbon side chain. On the other hand, the carboxyl carbon atom of anisic acid is derived from the second carbon atom of the side chain. These results mean that the aglycone has a vinyl group as a side chain at the *p*-position. Therefore, it should be considered that the aglycone was *p*-vinylphenol.

H. Schmidt and P. Karrer[2] are the first to isolate from plant material (*Papaver somniferum* L.)

(1) Part of a thesis by H. Imaseki in partial fulfillment of the requirement for a D.Sc. degree, December, 1958.

(2) H. Schmidt and P. Karrer, *Helv. Chim. Acta*, **28**, 722 (1945).

p-vinylphenol as colorless needles of m.p. 73.5°. Having not yet succeeded in obtaining the aglycone in a crystalline form, the authors, in comparison of its nature with the description by Schmidt and Karrer, concluded that the aglycone of furcatin is *p*-vinylphenol.

Sugar components in the acid hydrolysate were examined by paper chromatography, and two kinds of sugar were detected. R_f values of these sugars were (I) 0.20, (II) 0.32 in 1-butanol–acetic acid–water (4:1:2), and (I) 0.35, (II) 0.53 in 80% phenol: where glucose was 0.20 and 0.31 and apiose was 0.33 and 0.52 in these solvents. respectively. The lower spot (I) seems to be glucose and the higher spot (II) coincides with apiose which was obtained from the partial hydrolysate of a flavone glycoside apiin, in its R_f values and characteristic yellow coloration with Bacon–Edelman benzidine reagent.[3] Glucose was confirmed as the phenylosazone and apiose as the *p*-bromophenylosazone.

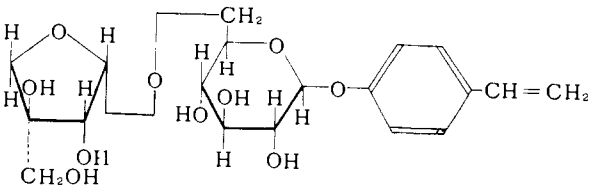
It is interesting that furcatin contains apiose as one of its sugar constituents. Apiose is a rarely occurring branched chain pentose which was discovered in apiin.[4] and its presence has been demonstrated in only a few plants.[5a,b] However. in any case. free apiose in plant materials has not been detected. Even in the leaves of *V. furcatum* and parsley. apiose was not present in a free form.

Furcatin was partially hydrolyzed by acid under mild conditions and liberated only apiose. and a glucoside was obtained. The glucoside was further hydrolyzed by acid yielding glucose and an aglycone. *p*-vinylphenol. Quantitative determination of glucose indicated that the glucoside was *p*-vinylphenol monoglucoside. The glucoside was hydrolyzed by apricot emulsin heated to have no remaining α-glucosidase activity. This indicates that the glucoside is a β-glucoside.

These results suggest that the sugar moiety of furcatin is apiosylglucose like that of apiin. For direct confirmation. enzymatic hydrolysis with a cell-free enzyme extract from the leaves of *V. furcatum* produced from furcatin an aglycone and a reducing disaccharide consisting of glucose and apiose.

The bond of apiose to glucose was determined by means of periodic acid oxidation. Since apiosylglucose was not obtained from the enzymatic hydrolysate as pure crystals. oxidation was applied to furcatin itself. In this case, there remains a possibility that periodic acid may act on the aglycone moiety. However, the effect of periodic acid was eliminated by a finding that the glucoside was theoretically oxidized. Furcatin consumed 4 moles of periodic acid and produced 2 moles of the acid: this indicates that the sugar moiety of furcatin is apiofuranosyl-1,6-glucopyranose.

Thus, the authors concluded that the glycoside, furcatin. is *p*-vinylphenol D-apio-(D or L)-furanosyl-1,6-β-D-glucopyranoside, and the following formula is proposed.



Configuration shown by dotted lines has not been elucidated.

Distribution of furcatin in 11 species of *Viburnum* was examined by means of paper chromatography. Modified Cifonelli Smith's reagent[6] was used as spray reagent: the paper chromatogram was first sprayed with a 0.3% periodic acid solution and then Horrocks benzidine reagent[7] was sprayed. Furcatin was developed by the successive treatment as a white or yellow spot on a dark blue background having R_f values of 0.76 (*1*-butanol–acetic acid–water 4:1:2) and 0.75 (80% phenol). Plants tested were: *Viburnum sieboldii, V. dilatatum, V. awabuckii. V. tomentosum. V. japonicum, V. erosum* var. *punctatum, V. wrightii, V. phlebotrichum, V. brachyandrum, V. sargenti* and *V. carlesii* var. *bitchuense.* However. it was found that none of these contained furcatin.

C_6–C_2-compounds hitherto known in plants are only styrene (in styrax oil) and *p*-vinylphenol. Schmidt and Karrer. however. considered that *p*-vinylphenol isolated by them was not contained in poppy. but secondarily derived from *p*-coumaric acid by decarboxylation during the isolation. The fact that furcatin was contained in a relatively high concentration suggests a possibility of wider occurrences of C_6–C_2-compounds in nature. though the carbon skeleton is derived *in vivo* from phenylpropane compounds.

Experimental

Isolation.—The leaves were collected in the campus of the Nikko Botanical Garden, University of Tokyo, in July and August of 1956. Thirty two and one-half kg. of fresh leaves were divided into batches of about 2 kg. Each batch of the leaves was extracted with 13 l. of boiling water two times for 1 hr. each time. The water extracts were filtered while hot and concentrated to a heavy sirup (about 200 ml.) under reduced pressure. The concentrate was poured into about 600 ml. of methanol under strong stirring. The brown precipitate was filtered with a Büchner funnel and further extracted twice with 500 ml. of methanol, followed by filtration. The combined methanol extract was concentrated and the residue was dissolved in 300 ml. of water. The water solutions thus obtained from every batch were combined and repeatedly extracted with ethyl acetate in 500-ml. portions. The ethyl acetate was dehydrated over sodium sulfate by standing overnight, concentrated to about 300 ml. per 500-ml. portion of the water solution and allowed to stand for some time. A deposited crystalline substance was recrystallized several times from ethyl acetate which contained a little water and furcatin was obtained as colorless needles of m.p. 158°, yield 82 g., $[\alpha]_{D17}$ −101.2° (*c* 2, in water); ultraviolet absorption: λ_{max} 222 mμ, *E* 8960; λ_{max} 275 mμ, *E* 1020.

Anal. Calcd. for $C_{19}H_{26}O_{10}$: C, 55.11; H, 6.32. Found: C, 55.10; H, 6.51.

Hydrolysis of Furcatin by Mineral Acid.—A 5% solution of furcatin in 2% hydrochloric acid was heated over a boiling water-bath for 1 hr. The solution showed milky turbidity after heating for some time and then became clear forming a brown oily aglycone, *p*-vinylphenol, on the surface. The ether extract from the hydrolysate was concentrated and the residue was purified by steam distillation.

Methylation of *p*-Vinylphenol.—*p*-Vinylphenol, equivalent to 2 g. of furcatin, was dissolved in 15 ml. of dried

(3) J. S. D. Bacon and J. Edelman, *Biochem. J.*, **48**, 114 (1951).
(4) E. Vongerichten, *Ann.*, **318**, 121 (1901).
(5) (a) D. J. Bell, F. A. Isherwood and N. E. Hardwick, *J. Chem. Soc.*, 3702 (1954); (b) A. D. Patrick, *Nature*, **178**, 216 (1949).
(6) J. A. Cifonelli and F. Smith, *Anal. Chem.*, **26**, 1132 (1954).
(7) R. H. Horrocks, *Nature*, **164**, 444 (1949).

acetone, then 1.5 g. of potassium carbonate and 1.0 ml. of dimethyl sulfate were added and the reaction mixture was boiled for 3 hr. When the reaction was over, water was added until potassium carbonate dissolved and the solution was twice extracted with ether. The ether extract was washed successively with 2% sodium hydroxide and water, and the solvent was evaporated. The *p*-vinylphenol methyl ether was a colorless volatile liquid of an anethol-like odor.

Permanganate Oxidation of *p*-Vinylphenol Methyl Ether.—The methyl ether, equivalent to 2 g. of furcatin, was suspended in a small amount of water, then 50 ml. of 5% potassium permanganate solution was added drop by drop with continuous stirring. When the addition was over, a faint red color of permanganate remained for longer than 30 min., and it disappeared by heating the mixture at about 60° for 5 min. Manganese dioxide that formed was filtered and repeatedly washed with hot ethanol. The filtrate was concentrated to about 3 ml. and added to 20 ml. of 10% hydrochloric acid. Colorless crystals were precipitated with generation of a considerable amount of gas, possibly carbon dioxide. The crystals were recrystallized from dilute ethanol and obtained as colorless needles of m.p. 180.5°. The melting point was not depressed by admixing with authentic anisic acid.

Chromic Acid Oxidation of the Methyl Ether.—Ten ml. of Beckmann mixture (1.5 g. of potassium dichromate, 2 g. of sulfuric acid and 10 ml. of water) was carefully added to the methyl ether (about 0.4 g.) on a bath at 55–60°. After adding the reagent, the bath temperature was raised to 70°, when needle crystals began to deposit, and the reaction mixture was immediately cooled with tap water and extracted with benzene. After washing the benzene extract with 2% sodium hydroxide and water, benzene was evaporated under reduced pressure and the residual liquid of an anise-like odor was dissolved in ethanol. 2,4-Dinitrophenylhydrazine (0.2 g.) dissolved in 5 ml. of ethanol containing 1.0 ml. of concd. sulfuric acid was added to the solution. Red precipitates were recrystallized several times from ethyl acetate and obtained as red needle crystals of m.p. 245.5°. The mixed melting point with authentic anisaldehyde 2,4-dinitrophenylhydrazone (m.p. 245°) was unchanged.

Ozonolysis of the Methyl Ether.—Ozonized oxygen which contained 110 mg. of ozone per min. of streaming was conducted for 1 hr. to a glacial acetic acid solution (5 ml.) of the methyl ether (0.7 g.). The oxygen was passed through a water trap. The ozonized reaction mixture was further aerated for 2 hr. and the waste air was also passed through a water trap. When the combined trap water was added to a mixture of 0.5 g. of 2,4-dinitrophenylhydrazine, 15 ml. of water and 4 ml. of 70% sulfuric acid, a yellow precipitate was obtained. Recrystallization of the precipitate from ethanol gave yellow needle crystals of m.p. 163°. The melting point corresponds to that of the 2,4-dinitrophenylhydrazone of formaldehyde, and the mixed melting point was not depressed.

***p*-Vinylphenol Benzoate.**—Dehydrated *p*-vinylphenol, equivalent to 1 g. of furcatin, was dissolved in 3 ml. of pyridine and 0.5 ml. of benzoyl chloride was added. The mixture was heated for 5 min. and blended with ice-water. The benzoate was recrystallized from ethanol and obtained as colorless needles, m.p. 72.5°.

Anal. Calcd. for $C_{15}H_{12}O_2$: C, 80.31; H, 5.39. Found: C, 80.29; H, 5.93.

Partial Hydrolysis of Furcatin.—A 5% solution of furcatin in 1% hydrochloric acid was heated at 80° for 30 min. Long needle crystals deposited from the hydrolysate after cooling and these were recrystallized from water; m.p. 148°.

Anal. Calcd. for $C_{14}H_{18}O_6 \cdot {}^1/_2H_2O$: C, 57.72; H, 6.53; H_2O, 3.1; glucose, 61.8. Found: C, 57.90; H, 6.32; H_2O, 3.4 (dried under reduced pressure over P_2O_5 at 118°); glucose content (detd. by Sumner's method[8] after acid hydrolysis under normal conditions), 61.5.

The partial hydrolysate of 2 g. of furcatin, from which the glucoside had been filtered off, was extracted three times with ethyl acetate and neutralized with sodium hydroxide. The neutralized solution was incubated with 2 g. of bakers' yeast at 30° for 12 hr., and concentrated to about 3 ml. after filtration. The concentrate was heated on a boiling water-bath for 40 min. with a mixture of *p*-bromophenylhydrazine (2 g.), 50% acetic acid (12 ml.) and water (30 ml.). Yellow crystals which formed were collected by suction, washed with petroleum ether (b.p. 30–60°) and recrystallized from ethanol; m.p. 209.5°. Authentic apiose *p*-bromophenylosazone (m.p. 209°) was prepared from apiose which was obtained from the partial hydrolysate of apiin by means of the same operation described above. The mixed melting point was unchanged.

Hydrolysis of *p*-Vinylphenol Glucoside.—One gram of the glucoside was hydrolyzed with 2% hydrochloric acid. The hydrolysate was shaken with ether, neutralized with sodium hydroxide and concentrated to about 3 ml. The concentrate was added to a mixture of phenylhydrazine hydrochloride (1 g.), sodium acetate (1.5 g.) and water (7 ml.), and the reaction mixture was heated on a water-bath of 90° for 30 min. The crystalline mass was recrystallized from ethanol and obtained as yellow needles of m.p. 206.5°. The mixed melting point with the authentic glucosazone was not depressed.

Hydrolysis of *p*-Vinylphenol Glucoside by Emulsin.—Apricot emulsin (prepared from purchased seeds of apricot) was dissolved in acetate buffer (0.1*M*, *p*H 4.7) to make a 5% solution and heated to 62° for 5 min. The emulsin solution thus treated did not have α-glucosidase activity. A mixture of the emulsin solution (1 ml.) and 1% solution of the glucoside (1 ml.) was incubated at 31° for 24 hr. under toluene. Semi-quantitative estimation on a paper chromatogram showed that about 40% of the glucoside was hydrolyzed by the emulsin.

Hydrolysis of Furcatin by Cell-free Enzyme Extract.—About 50 g. of the leaves of *V. furcatum* were chopped and homogenized with 200 ml. of ice-cold water in a Waring blendor. The resultant homogenate was centrifuged at 5,000 r.p.m. for 15 min. after filtration through cotton-cloth and the supernatant was dialyzed for 48 hr. in a refrigerator. A mixture of 1 ml. of the cell-free extract, 0.5 ml. of acetate buffer (0.1*M*, *p*H 4.7) and 0.5 ml. of 2% furcatin solution was incubated at 29° for 20 hr. under toluene and its concentrate was qualitatively tested by paper chromatography. Only a reducing sugar of the same R_f value as sucrose in 1-butanol–acetic acid–water (4:1:2) was detected on the chromatogram, but any monosaccharide such as glucose or apiose was not detected. Eluates of the sugar zones obtained from large scale chromatograms were hydrolyzed by acid yielding both glucose and apiose.

Oxidation of Furcatin with Periodic Acid.—Oxidation was carried out under standard technique.[9] A reaction mixture of 40 ml. of 0.04*M* periodic acid ($1.6 \times 10^{-3}M$), 7 ml. of 0.03 *M* furcatin ($2.1 \times 10^{-4}M$) and 33 ml. of water was incubated at 28°. From time to time, aliquots of 2 ml. and 5 ml. were withdrawn and titrated for periodic acid remaining by 0.01*N* iodine solution and for acid produced by 0.01*N* sodium hydroxide to methyl red, respectively. After 20 hr., when the reaction stopped, the periodic acid consumed was 4.0 moles (theor. 4.0 moles) and acid produced was 1.8 moles (theor. 2.0 moles) per mole of furcatin. The neutralized reaction mixture which was titrated for the total acidity was added with an excess of 0.01*N* arsenite solution prior to distillation. Formaldehyde produced from the apiose moiety was qualitatively proved in the above distillate by the chromotropic acid reaction, which is a specific reaction for formaldehyde.

To examine the effect of periodic acid on the aglycone, a parallel experiment with *p*-vinylphenol glucoside was carried out in a mixture of 40 ml. of 0.04*M* periodic acid ($1.6 \times 10^{-3}M$) and 40 ml. of the 0.01*M* glucoside ($4 \times 10^{-4}M$). The periodic acid consumed and acid produced were 2.0 moles (theor. 2.0 moles) and 0.9 mole (theor. 1.0 mole) per mole of the glucoside, respectively.

Catalytic Hydrogenation of Furcatin.—Palladium-charcoal prepared according to Gattermann[10] was used as catalyst. Hydrogen obtained from Kipp's apparatus was washed successively with alkaline sodium sulfite, lead acetate and alkaline potassium permanganate solution. The suspension of 0.1 g. of catalyst in 20 ml. of water was mechanically shaken with hydrogen until absorption of the gas ceased. Then a solution of 0.107 g. of furcatin in 30 ml. of water was added to the catalyst suspension and shaken with hydrogen at 26°, 758 mm. After 50 min.,

(8) J. B. Sumner, *J. Biol. Chem.*, **69**, 287 (1925).

(9) J. R. Dyer, "Methods of Biochemical Analysis," Interscience Publishers, Inc., New York, N. Y., 1954, Vol. III, p. 111.

(10) L. Gattermann and H. Wieland, "Die Praxis des Organischen Chemikers," Walter De Gruyter, Berlin, 34 Aufl., 1952, pp. 330.

gas absorption stopped. The volume of hydrogen absorbed was 6.0 ml. at the above condition. This corresponds to 0.93 mole of hydrogen per mole of furcatin.

Furcatin Hexaacetate.—Two hundred mg. of furcatin was kneaded with 2 ml. of acetic anhydride and 5 drops of pyridine. When the crystals dissolved, the mixture was heated on a boiling water-bath for 5 min. and poured into ice-water with stirring. The separated acetate was recrystallized from ethanol and obtained as colorless needles which melted at 116–116.5°.

Anal. Calcd. for $C_{31}H_{38}O_{16}$: C, 55.85; H, 5.71. Found: C, 55.96; H, 5.83.

***p*-Vinylphenol Glucoside Tetraacetate.**—Sixty five mg. of the glucoside was acetylated with 1 ml. of acetic anhydride and 5 drops of pyridine as in the case of furcatin hexaacetate. Recrystallization was repeated from ethanol and the acetate was obtained as colorless needles of m.p. 142.5°.

Anal. Calcd. for $C_{22}H_{26}O_{10}$: C, 58.66; H. 5.82. Found: C, 59.11; H; 6.44.

Acknowledgment.—The authors wish to express their sincere gratitude to Prof. S. Shibata and his research fellows. of Faculty of Pharmaceutical Science. University of Tokyo. for their kind permission for the use of the ozonization apparatus. Thanks are also due to Mr. S. Nakamura and the members of the Nikko Botanical Garden for their kindness in collecting plant material.

HONGO, TOKYO, JAPAN

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Distribution of Formyl Groups in Amylose Monoformate[1]

BY ROY L. WHISTLER AND HUGH J. ROBERTS

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Amylose and starch monoformates are acetylated and the formyl groups are found to hydrolyze easily and quantitatively without appreciable loss or wandering of the acetyl groups. The monoformates, prepared by formylation in 90% formic acid. have approximately 70% of the primary hydroxyl groups formylated.

The ease with which formyl groups may be introduced and removed from starch and the suggestion that starch monoformates are extensively esterified at carbon atom C6 make the monoester interesting as an intermediate in the preparation of specific starch derivatives. The approximate monoester is easily prepared by reaction of starch at room temperature with an excess of 90% formic acid.[2–5] Wolff and co-workers[5] show that this is a reversible reaction. Browning and Sell[6] and Wolff and co-workers[5] further show that increased concentration of formic acid leads to more rapid esterification and to higher degrees of substitution. although a D. S. (average degree of substitution) above 2.3 is not obtained. By use of periodate oxidation[2,5] to determine the number of hydroxyl groups simultaneously free on carbon atoms C2 and C3 in starch monoformates evidence is obtained which suggests that the formoxy groups reside almost entirely on primary carbon atoms. However, Moe. Miller and Buckely[3] point out that if the periodate oxidation of starch monoformate is conducted in a buffered system at *p*H 4.2–4.3. only 0.6 mole of periodate is consumed per mole of anhydro D-glucose unit as contrasted with 1.0–1.8 moles consumed in an unbuffered system. and conclude that in the latter system hydrolysis of formate ester groups occurs. Tarkow and Stamm[7] show that starch monoformate is produced when the molar ratio of anhydrous formic acid to starch is about 2:1. and conclude that only the primary hydroxyls of starch are esterified under these conditions.

Because of the potential usefulness of a 6-*O*-formyl starch in this Laboratory. the monoformate ester is reinvestigated. As is described,[2,5,8] the monoformates are readily obtained by reaction of corn amylose or corn starch in 90% formic acid. Treatment of the formates with acetic anhydride in pyridine produces the fully esterified mixed esters.[8] It is now found, however. that the formyl groups can be removed from the acetate-formates by piperidine-catalyzed hydrolysis in aqueous acetone without significant loss of acetyl groups. It is further shown that the hydroxyl groups in the resultant acetates are those which were originally formylated since little or no acetyl wandering occurs in the deformylation. Such derivatives of amylose and starch are shown in Table I.

TABLE I

ESTERS OF AMYLOSE AND STARCH

Polysaccharide	Formate ester Formyl groups per D-glucose unit	Mixed acetate-formate Acetyl groups per D-glucose unit	Mixed acetate-formate Total D. S.	Acetate ester[a] Acetyl groups per D-glucose unit
Amylose	0.83	2.16	2.99	2.21
Amylose	1.05	1.94	2.99	1.98
Amylose	1.18	1.77	2.95	1.79
Amylose	1.38	1.60	2.98	1.67
Starch	1.08	1.91	2.99	1.88

[a] Prepared by deformylation of the acetate-formate. Values given are for total acyl calculated as acetyl.

The mixed acetate-formates undergo piperidine-catalyzed hydrolysis at a rapid initial rate as shown in Fig. 1. Then as the amount of acid released becomes equivalent to the formate originally present in the ester. the rate sharply decreases to a low constant value. Extrapolation of the linear

(1) Presented before the Division of Carbohydrate Chemistry at the 135th Meeting of the American Chemical Society, Boston, Mass., April, 1959; Journal Paper No. 1393 of the Purdue Agricultural Experiment Station.

(2) D. Gottlieb, C. G. Caldwell and R. M. Hixon, THIS JOURNAL, **62**, 3342 (1940).

(3) O. H. Moe, S. E. Miller and M. I. Buckley, *ibid.*, **73**, 4185 (1951).

(4) R. F. Nickerson, *Textile Res. J.*, **21**, 195 (1951).

(5) I. A. Wolff, D. W. Olds and G. E. Hilbert, THIS JOURNAL, **79**, 3860 (1957).

(6) B. L. Browning and L. O. Sell, *Textile Res. J.*, **23**, 939 (1953).

(7) H. Tarkow and A. J. Stamm, *J. Phys. Chem.*, **56**, 266 (1952).

(8) I. A. Wolff, D. W. Olds and G. E. Hilbert. *Ind. Eng. Chem.*, **49**, 1247 (1957).