Anal. Calcd. for $C_{10}H_8ClNO_2S_2$: C, 43.87; H, 2.95. Found: C, 44.17; H, 3.08.

Attempts to replace the chlorine atom of the amide by heating with aqueous ammonia and cuprous chloride at 180-190° for 4 hours gave a black product from which no crystalline material could be isolated. Unchanged amide was recovered after refluxing with sodium amide in benzene for 8 hours.

5-Bromo-2-thiophenesulfonamide[15] was recovered unchanged after heating with aqueous ammonia at 150° for 2 hours. When cupric oxide was present a dark product was obtained from which only a small amount of starting material could be isolated. A similar result was obtained when the amide was heated with sodium amide in liquid ammonia for 2 hours at 90°. Likewise only starting material was isolated after 5-iodo-2-thiophenesulfonamide[15] was refluxed with sodium amide in benzene for 6 hours.

5-Nitro-3-thiophenesulfonyl Chloride and 5-Nitro-3-thiophenesulfonamide.—One-half of a solution of 39 g. (0.3 mole) of 2-nitrothiophene in 100 cc. of chloroform was added dropwise with stirring and refluxing to 93 g. (0.8 mole) of chlorosulfonic acid in 100 cc. of chloroform. An additional 93 g. of chlorosulfonic acid then was added in one lot to the flask and the remainder of the 2-nitrothiophene solution was added dropwise. The addition required about 40 minutes, and refluxing on the steam-bath was continued for 10 hours.

After cooling, the mixture was poured into a separatory funnel. On standing two layers formed with the acid layer on the bottom. The two layers were added separately to ice and water mixtures. The aqueous layers were extracted with several portions of chloroform, and the chloroform extracts were combined and washed with water. The chloroform solution was dried over anhydrous sodium sulfate, treated with Norit, filtered and distilled on a steam-bath under reduced pressure to remove the chloroform. The residue was transferred to a modified Claisen flask, and the fraction distilling at 146-151° (5-7 mm.) was collected to give 45 g. (74%) of 5-nitro-3-thiophenesulfonyl chloride as a yellow oil. Even after further purification and cooling, it would not crystallize. Stadler's product[6] was a viscous oil.

For converting the sulfonyl chloride to the amide, the best results were obtained by saturating a solution of 10 g. of the sulfonyl chloride in 50 cc. of acetone with ammonia, removing the ammonium chloride by filtration, diluting with 15 cc. of water, and evaporating until all of the acetone was removed. The residue of 5-nitro-3-thiophenesulfonamide weighed 7.7 g. Crystallization from acetone-water (1:1) gave 5.6 g. (60%) of light yellow crystals melting at 169.5-172°. After three recrystallizations from water the product melted at 171-173°. Stadler[6] reported 172-173° for his product.

Anal. Calcd. for $C_4H_4N_2O_4S_2$: C, 23.07; H, 1.94. Found: C, 23.22; H, 1.82.

When 13.5 g. of the sulfonyl chloride was heated with 10 g. of aniline for 15 minutes on the steam-bath, a solid was obtained which was crystallized from benzene to give 12.5 g. of yellow crystals melting at 129-133°. After three recrystallizations it melted at 135-136°.

Anal. Calcd. for $C_{10}H_8N_2O_4S_2$: C, 42.24; H, 2.84. Found: C, 42.66; H, 2.73.

5-Amino-3-thiophenesulfonamide.—A solution of 2 g. of 5-nitro-3-thiophenesulfonamide in 50 cc. of absolute alcohol was reduced with hydrogen in the presence of Raney nickel using a small-scale apparatus.[16] The calculated amount of hydrogen was absorbed in 6 hours. After removal of the catalyst (dry weight 2.2 g.) the solution was concentrated to 10 cc. Cooling gave 1.0 g. of grayish-yellow crystals. Further concentration of the mother liquor gave a small amount of crystals contaminated with a red oil. Repeated crystallization from ethyl alcohol-isopropyl alcohol (1:1) gave a product melting at 158° with decomposition.

Anal. Calcd. for $C_4H_6N_2O_2S_2$: C, 26.95; H, 3.39; N, 15.72. Found: C, 26.89; H, 3.36; N, 15.59.

Attempts to reduce 5-nitro-3-thiophenesulfonanilide gave a red viscous oil from which only an amorphous brown solid could be obtained.

Summary

5-Amino-3-thiophenesulfonamide has been prepared together with several new compounds obtained as intermediates in its preparation or in attempts to prepare 5-amino-2-thiophenesulfonamide.

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[15] Steinkopf, Jacob and Penz, *Ann.*, **512**, 136 (1934).

[16] Noller and Barusch, *Ind. Eng. Chem., Anal. Ed.*, **14**, 907 (1942), **18**, 730 (1946).

STANFORD, CALIF.

RECEIVED JUNE 12, 1950

[CONTRIBUTION FROM THE NORTHERN REGIONAL RESEARCH LABORATORY[1]]

Phosphorylation of Starch

BY ROLLAND LOHMAR, JOHN W. SLOAN AND C. E. RIST

As part of a general program on the reaction of starch with polyfunctional reagents, we have prepared esters by treating several starches with phosphorus oxychloride in pyridine. The literature contains several references[2,3] to esters of amylaceous polysaccharides prepared in this manner. Those derivatives were reported to be soluble in water. Starch,[4] "soluble starch,"[5] and various starch materials[6,7,8,9] have been phosphorylated in aqueous media to give soluble products containing up to 2% phosphorus.

In this paper we describe the preparation of water-insoluble phosphates of a variety of starches by reaction with phosphorus oxychloride in pyridine. We found that the pretreatment given the starches controlled their reactivity and the nature of the products formed.

Starches which had been oven-dried or dried

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[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Article not copyrighted.

[2] H. Vogel, *Ber.*, **72**, 2052 (1939).

[3] P. Karrer, H. Koenig and E. Usteri, *Helv. Chim. Acta*, **26**, 1296 (1943).

[4] H. K. Barrenscheen and J. Pany, *Biochem. Z.*, **219**, 364 (1930).

[5] J. Kerb, *ibid.*, **100**, 3 (1919).

[6] H. Pringsheim and K. Goldstein, *Ber.*, **56**, 1520 (1923).

[7] P. Koets, *Proc. Acad. Sci. Amsterdam*, **38**, 63 (1935).

[8] P. Koets and H. R. Kruyt, *C. A.*, **32**, 1543 (1938); *Kolloid-Beihefte*, **47**, 100 (1937).

[9] M. Samec, "Kolloidchemie der Stärke," Theodor Steinkopff, Dresden-Blasewitz, 1927, pp. 25-28.

azeotropically with benzene gave soluble products whereas starches dried by azeotropic distillation with pyridine gave insoluble products. This difference is attributed specifically to an activating effect by pyridine. Oven-dried corn starch, after treatment with pyridine under reflux for 15 minutes, led to insoluble phosphates. Natural moisture corn starch gave soluble products, whereas after treatment with refluxing pyridine it yielded insoluble products. This activation by pyridine occurred with potato and Lintner's soluble starch as well as with corn starch. Hence, the difference between our products and Karrer's,[3] for example, was attributed to the pretreatment given the starch. Gelatinization in liquid ammonia[10] appeared to have the same effect on reactivity as pyridine pretreatment. It must be emphasized, however, that our pretreatment technique did not involve gelatinization. The granules were swollen only about 50% in volume and still retained their characteristic birefringence and shape.

When normal moisture was removed from the starch, the product was obtained in the granule form; otherwise, the starch gelatinized in the pyridine–water–phosphorus oxychloride mixture. Phosphates prepared from pregelatinized starches or prepared under conditions such that gelatinization occurred during the esterification were also insoluble in water.

When various species of starch were phosphorylated at 70°, the extent of phosphorylation was dependent on granule size—the larger granule starches being less reactive, as shown by a lower titratable acidity (Table I). When the granule was disrupted by gelatinization in aqueous media or liquid ammonia, that difference was not apparent. Likewise, when the reaction temperature was raised to the reflux point of the medium, no difference was found. The products, however, tended to be tan to dark brown, whereas those prepared at lower temperatures were light-colored.

TABLE I

PROPERTIES OF PHOSPHATES OF VARIOUS STARCHES[a]

Species[b]	Swelling, volume increase in hot water, %	Acidity, m.e./g.
White potato	sol	0.2
Tapioca	370	1.0
Sweet potato	340	1.6
Wheat	170	2.9
Waxy corn	0	5.9
Corn	0	5.9

[a] The starches were dried azeotropically with pyridine, then heated in pyridine with 3 moles of $POCl_3$ for 3.5 hours at 70°. [b] In order of decreasing granule size.

Insoluble products were obtained by treating corn starch with 0.25 to 9 moles of phosphorus oxychloride per anhydroglucose unit for 0.25–5 hours at 40–115°. The maximum substitution of phosphorus occurred at 1.5–3 moles at 70° for 1 to 3 hours (Table II). At higher ratios of phosphorus oxychloride, degradation apparently caused part of the product to be water-soluble, regardless of the pretreatment.

TABLE II

PHOSPHORYLATION OF PYRIDINE ACTIVATED CORN STARCH AT 70°

$POCl_3$/anhydroglucose unit, moles	Time, hr.	P, %	Cl, %	Acidity M.e./g. 1st	Acidity M.e./g. Total	Linkage of P, % Single	Linkage of P, % Double	Linkage of P, % Triple
0.125[a]	3	0.45	nil	0.14	0.28	97	0	0
0.75	3	7.0	2.6	2.02	3.30	52	37	11
1.50	3	12.2	6.4	3.42	5.73	59	28	13
3	0.5	6.9	2.8	2.07	3.26	54	40	7
3	1[b]	2.8	0.5	0.78	1.33	61	26	13
3	1	10.8	5.3	3.07	5.06	57	31	12
3	1[c]	12.1	11.0	3.33	5.76	62	23	15
3	2	11.8	6.9	3.44	5.76	61	30	10
3	4	12.4	7.8	3.51	5.98	62	26	12
3	6	12.5	8.5	3.60	6.11	62	27	11
3	10	12.3	8.7	3.59	6.14	64	26	10

[a] This product gels in hot water. [b] 40°. [c] 115°.

Granule forms of starch phosphates did not stain blue with iodine and birefringence was lost to some degree. The phosphates and their salts were remarkably free flowing and resistant to swelling in hot water, the extent of swelling decreasing with increased phosphorus content. When the phosphorus content was low, the sodium salts were more resistant to swelling than the acid forms. Both the sodium salts and the acid forms were hygroscopic to about the same extent as starch, sorbing about 10% moisture under ordinary laboratory conditions.

The crude starch phosphates contained pyridine which was very firmly held, presumably by salt formation with the phosphate groups, since it could be displaced by metallic cations. It was conveniently removed by water washing after neutralization of the crude acid form to a *p*H of 8 with sodium hydroxide. The free acid could be regenerated from the salt by treatment with hydrochloric acid.

Organically bound chlorine was present in the regenerated as well as in the crude products, those prepared at higher temperatures having a relatively higher chlorine content. It did not appear that the ester groups had been replaced by chlorine through the action of pyridine hydrochloride, in analogy with the replacement of tosyloxy groups in α-glucosides.[11] Treatment of starch phosphate with pyridine hydrochloride at 110° does not produce such exchange. Reid, *et al.*,[12] found that exchange apparently occurs in the preparation of cellulose phosphate. These divergent results with starch and cellulose are in agreement with the

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(10) J. E. Hodge, S. A. Karjala and G. E. Hilbert, THIS JOURNAL, in preparation.

(11) K. Hess and W. Eveking, *Ber.*, **67**, 1908 (1934); K. Hess and H. Stenzel, *ibid.*, **68**, 981 (1935).

(12) J. D. Reid, L. W. Mazzeno, Jr., and E. M. Buras, Jr., *Ind. Eng. Chem.*, **41**, 2831 (1949).

postulated analogy with the work of Hess[11] on tosyl esters. Hess found that there is a tendency for the ester groups to be more readily replaced by chlorine in the tosylation of methyl-α-glucoside than in methyl-β-glucoside. Hess also found that tritosyl starch (a β-glucoside) could be prepared almost chlorine-free, while tosylated cellulose (an α-glucoside) contained up to one chlorine atom for every two tosyloxy groups. Starch phosphate was quantitatively recovered unchanged after treatment with 0.2 *N* ethanolic potassium hydroxide for sixty-two hours at room temperature. Boiling, aqueous, half-normal alkali reduced the chlorine content, but the phosphorus content was increased and the recoveries were low, indicating a loss of that part of the molecule not substituted by phosphate. Reid, *et al.*,[12] point out the high stability of chlorine in similarly prepared cellulose phosphates. Pentachloro starch has been found to be very stable.[13]

The amounts of phosphorus linked singly and multiply to the starch were calculated from the two inflection points obtained when pyridine-free corn starch phosphates were titrated electrometrically with barium hydroxide (Fig. 1). When sodium hydroxide was used, the second inflection point was obscure. This is in agreement with the results obtained with cellulose phosphates.[12] Briggs and Hanig,[14] however, found two well defined inflection points when they used sodium hydroxide to titrate electrodialyzed and pasted potato starches. Natural potato starch, however, contains much less phosphate than the samples prepared by our method. Kumler and Eiler[15] have shown that the remaining hydrogens of mono- and dialkyl phosphates have progressively increased dissociation. The amounts of phosphorus singly and doubly linked to the carbohydrate may be calculated on the assumption that, similarly, the first inflection point occurs after titration of the remaining hydroxyl group of doubly-linked phosphate and the first hydroxyl group of singly-linked phosphate; between the first and second inflection points the remaining hydroxyl group of singly-linked phosphate is titrated. The curve of Fig. 1 indicates inflection points at 3.42 and 5.73 m.e./g. (third entry of Table II). The difference, 2.31 m.e. or 7.16% phosphorus, corresponds to singly-linked phosphate. This value, subtracted from the figure represented by the first inflection point, corresponds to 1.11 m.e. or 3.44% phosphorus in the doubly-linked state. Thus 10.6% out of 12.2% phosphorus is accounted for. The remainder, 1.6%, probably represents phosphorus in triply-linked phosphate. The relative amounts of phosphorus linked in these three ways are listed in Table II.

Several ion exchange experiments were run, using 0.1 *N* calcium chloride as the test solution. The capacity of the column was in agreement with that obtained from the first inflection point in the electrometric titration and remained constant over a period of 20 cycles. Insoluble metallic salts of starch phosphates were readily prepared by treating the starch phosphates with aqueous solutions of metallic salts.

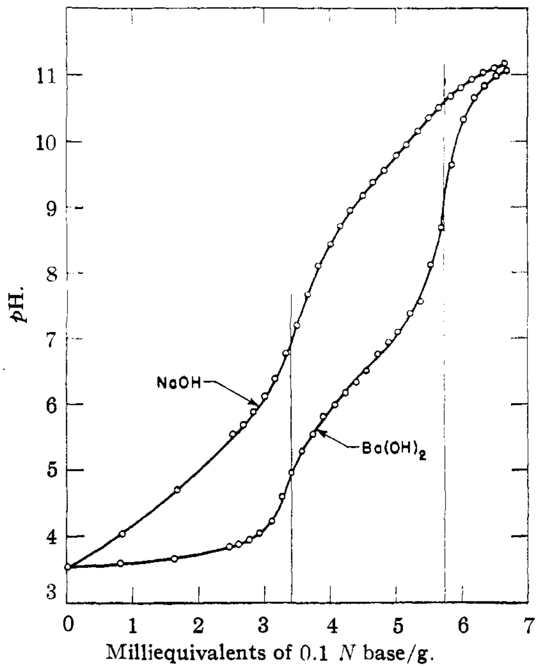

Fig. 1.—Electrometric titration of starch phosphate, 12.2% P.

Experimental

Preparation of Insoluble Starch Phosphate.—A mixture of 250 g. of corn starch (10.3% moisture) and 1.5 liters of pyridine was heated until 500 cc. of distillate had been collected. After addition of another 1.5 liters of pyridine, 190 cc. (1.5 moles per anhydroglucose unit) of phosphorus oxychloride was added at room temperature. The mixture was heated at 70° for 3 hours. cooled and filtered. The cake was slurried in water and washed further with water. To convert to the sodium salt, 5% sodium hydroxide was added to a slurry of the crude acid form to a *p*H of 8. After washing and drying, 375 g. of the sodium salt of starch phosphate containing 11.2% phosphorus was obtained. Conversion of a portion to the free acid form was accomplished by washing the salt with *N* hydrochloric acid. The free acid contained 12.2% phosphorus (Table II and Fig. 1).

Treatment with Pyridine Hydrochloride.—Twenty grams of starch phosphate (9.97% moisture), containing 10.4% P and 4.7% Cl and having a total acidity of 4.95 m.e./g. was suspended in 300 cc. pyridine and dried by distilling 150 cc. through a Vigreux column. Forty-eight grams pyridine hydrochloride was added at 40° and the mixture was heated to 110° for 1 hour. The product was filtered, washed with 5% sodium hydroxide, then *N* hydrochloric acid and finally with water until the filtrate gave a negative test for chloride. The air-dried product, 16.5 g. (9.25% moisture), represented an 83% recovery.

Anal.: P, 11.5; Cl, 4.8; acidity, 5.24 m.e./g.

Swelling.—Two grams of starch phosphate (air-dried) and 35 cc. of water were stirred in a 40-cc. centrifuge tube on the steam-bath for 20 minutes. The tubes were then allowed to stand at room temperature and the volumes were read to the nearest 0.5 cc. after 24 hours.

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[13] H. N. Barham, E. S. Stickley and M. J. Caldwell, THIS JOURNAL, **68**, 1018 (1946).

[14] D. R. Briggs and M. Hanig, *Cereal Chem.*, **23**, 277 (1946).

[15] W. D. Kumler and J. J. Eiler, THIS JOURNAL, **65**, 2355 (1943).

Acknowledgment.—The authors are indebted to C. H. Van Etten and Mrs. Mary Wiele of the Analytical and Physical Chemical Division of this Laboratory for the phosphorus and chlorine analyses.

Summary

1. Starch granules show increased chemical reactivity after heating in pyridine.

2. The reactivity of various species of starch decreases with increasing granule size.

3. Insoluble non-swelling phosphates may be obtained in the granular form by treating activated starch granules with phosphorus oxychloride in pyridine.

4. Characterization of the phosphate linkages in starch phosphates by electrometric titration shows varying amounts of singly-, doubly- and triply-linked phosphate, depending on the reaction conditions.

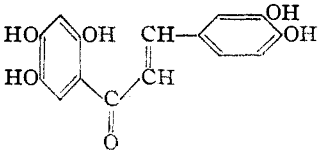
PEORIA, ILLINOIS RECEIVED APRIL 20, 1950

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA]

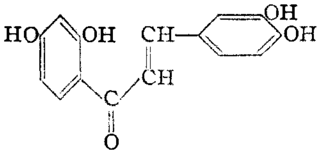
Anthochlor Pigments. VI. The Pigments of *Coreopsis Stillmanii*

BY MARGARET K. SEIKEL[1] AND T. A. GEISSMAN

The anthochlor pigment of *Coreopsis Stillmanii*[2] has been isolated in the form of its octaacetate and has been shown to be a glycoside of 3,4,2′,4′,5′-pentahydroxychalcone (I). It will be called stillopsin (II).

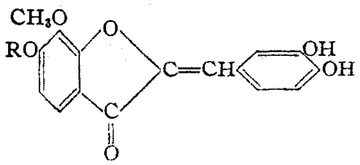


I



III

This isolation represents the first time that the polyhydroxychalcone (I) has been discovered among plant pigments. Anthochlor pigments previously identified include the chalcone, butein (III), found in yellow *Dahlia variabilis*,[3] *Coreopsis Douglasii*,[4] and in *Coreopsis gigantea*,[5] a hexoside of butein, coreopsin, found in *Cosmos sulphureus*[6] and in *Coreopsis gigantea*,[5] and the benzalcoumaranone glucoside leptosin (V), and its aglucone, leptosidin (IV), found in *Coreopsis grandiflora*, Nutt.[7] Recent work[8] has shown that the anthochlor pigment of yellow *Antirrhinum majus* is a benzalcoumaranone derivative. The chalcone nucleus is present in isocarthamin (isolated from safflower[9,10]), pedicin and its ethers (from the leaves of *Didymocarpus pedicellata*[10,11]), and isosalipurposide (from *Salix purpurea L.*[12]).



IV, R = H, leptosidin
V, R = glucosyl ($C_6H_{11}O_5$), leptosin

Hitherto the method of distinguishing between the two types of anthochlor pigments required a study of the products obtained on acidic hydrolysis of glycosides or acetylated pigments. Under these conditions pigments with a chalcone nucleus have yielded chalcones or flavanones, readily identified by color tests, while those with a benzalcoumaranone nucleus retained their initial structure. Absorption spectra have now been found to differentiate the two types readily. The differentiation is best with the acetates, either of the aglycones or the glycosides (Figs. 1 and 2 and Table I), and since the acetates of the pigments are commonly prepared for the purpose of identification, purification or isolation, this method is experimentally the most suitable. With chalcone itself and with the acetyl derivatives of polyhydroxychalcones and of their glycosides, Band I, the longer wave length band,[13] has one maximum. With the corresponding compounds of the benzalcoumaranone series, it has two maxima. With the free polyhydroxy aglycones the differentiation is not as clear-cut because the second maximum has become merely a shoulder,[14] but Band I is at longer wave lengths for the benzalcoumar-

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[1] Wellesley College, Wellesley, Mass. Sarah Berliner Research Fellow, 1948–1949, of the American Association of University Women.

[2] Gertz, *Kgl. Fysiograf. Sällskap. Lund, Förh.*, **8**, 65 (1938).

[3] Price, *J. Chem. Soc.*, 1017 (1939). It probably occurs also in *Butea frondosa*, see Perkin and Hummel, *ibid.*, **85**, 1459 (1904), and Lal and Dutt, *J. Indian Chem. Soc.*, **12**, 262 (1935) [*C. A.*, **29**, 6602 (1935)].

[4] Geissman, THIS JOURNAL, **63**, 656 (1941).

[5] Geissman, *ibid.*, **63**, 2689 (1941).

[6] Geissman, *ibid.*, **64**, 1704 (1942).

[7] Geissman and Heaton, *ibid.*, **65**, 677 (1943); **66**, 486 (1944).

[8] Seikel and Geissman, *ibid.*, **72**, 5725 (1950).

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[11] Sharma and Siddiqui, *J. Indian Chem. Soc.*, **16**, 1 (1939) [*C. A.*, **33**, 5824 (1939)]; Rao and Seshadri, *Proc. Indian Acad. Sci.*, **27**, 375 (1948).

[12] Zemplén, Bognar and Székeley, *Ber.*, **76**, 387 (1943).

[13] This designation follows that used by Skarzynski for flavones; *Biochem. Z.*, **301**, 150 (1939).

[14] In the one free glycoside studied, leptosin (see footnote 15), this maximum is just barely retained as a distinct entity.