butyric acid reacted with the same olefins to give a 47% yield of octyl ester of *n*-butyric acid, b. p. 221.8° at 735, $n^{20}D$ 1.4213, d^{20} 0.8593. With the octenes polymerization was a greater factor than it was with cyclohexene, but here also there was an absence of tar formation.

Reaction of Ethyl Alcohol with Acetic Acid.--Thirty-five grams (0.76 mole) of absolute ethanol was added over a period of twelve hours to a solution of 0.8 mole of glacial acetic acid in 7.5 moles of hydrogen fluoride, the reactants being kept at 0°. There was recovered a 17.5% yield of ethyl acetate (based on alcohol), b. p. 74–75.5° (735 mm.), $n^{20}D$ 1.3700–1.3740.

Hydrolysis of Ethyl Acetate.—A mixture of 106 g. of ethyl acetate and 18 g. of water (molar quantities) was stirred with 157 g. of hydrogen fluoride at ice temperatures for eleven hours. The products were poured onto ice and neutralized with solid sodium carbonate, the temperature not being allowed to exceed 5°. The liquid was then packed in ice as it was saturated with potassium carbonate. There was obtained 17 g. of liquid, b. p. 75.5–83° (735 mm.), $n^{20}D$ 1.3659. A 3,5-dinitrobenzoate derivative was prepared, m. p. 90°; mixed melting point with an authentic sample of the 3,5-dinitrobenzoate of ethyl alcohol, 89°. This quantity represents a 35% conversion to ethyl alcohol.

Reactions of Alcohols with Olefins.—To one mole of alcohol dissolved in 5–7 moles of hydrogen fluoride kept near 0° by external cooling was added dropwise one mole of olefin over a period of seven to eleven hours. The products were usually neutralized shortly after complete addition of the olefin, although in the case of dicyclohexyl ether, the yield was slightly increased by a few hours of additional stirring at 0°. After separation of layers and neutralizing with cold dilute sodium carbonate solution, the oil was dried over anhydrous potassium carbonate and distilled first through a Claisen flask to separate unreacted materials and polymer from the principal product. Then the ether or middle fraction was distilled through an efficient column. The following reactions were carried out: (1) cyclohexene and cyclohexanol; (2) cyclohexene and isopropyl alcohol; (3) propylene and ethyl alcohol (the propylene was passed through the alcohol solution under 10 mm. pressure for one and one-half hours).

From (1), using 5.5 moles of hydrogen fluoride, addition time of five hours and reaction time of eight hours, there were obtained a 12% yield of dicyclohexyl ether, 61.5% cyclohexyl fluoride (based on olefin), 43.5% recovered alcohol, and 1.6% residual tar.

From (2) using 7.5 moles hydrogen fluoride, and eleven hours addition and reaction time, there were obtained 45% low boiling materials, probably consisting of unreacted olefin, alcohol, and some cyclohexyl fluoride, 2% cyclohexanol, 3.5% dicyclohexyl ether, and 35% residual tar.

From (3) there were obtained only unreacted alcohol and high boiling polymer.

Summary

Secondary olefins reacted with acids in the presence of hydrogen fluoride to give good yields of esters. A tertiary olefin reacted unfavorably.

Hydrogen fluoride promoted the esterification of acetic acid with ethanol and also the reverse hydrolysis reaction.

The reaction of cyclohexene with cyclohexanol in the presence of hydrogen fluoride produced a small yield of dicyclohexyl ether. In other reactions involving secondary olefins, the desired ether was not produced.

STATE COLLEGE, PENNA. RECEIVED APRIL 22, 1941

[CONTRIBUTION FROM THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF WISCONSIN]

Quantitative Studies of the Oxidation of Fatty Acids with Hydrogen Peroxide and an Interpretation of the Reaction Mechanism*

BY ROVELLE H. ALLEN AND EDGAR J. WITZEMANN

More than thirty years ago Dakin reported a series of pioneering studies of the oxidation of fatty acids *in vitro*, that along with the fundamental work of Knoop laid the basis of our present conceptions of these processes as they occur both *in vitro* and *in vivo*. The new data here reported constitute an extension of a portion of these earlier results of Dakin.

In Dakin's studies the materials were placed in a distillation apparatus and in the course of the distillation volatile materials passing over were condensed or absorbed. The multiplicity of products obtained and the lack of suitable analytical methods made quantitative studies impractical at that time. By placing the condenser in the refluxing position, the nature of the end-products was so greatly simplified that an approximately quantitative account can now be given of the fate of the fatty acids oxidized.

Dakin had suggested that perhaps such oxidations could be influenced by "a suitable catalyst,"[1] without realizing that the ammonia of his ammonium salt was just such a catalyst, as was

* The first part of this paper is an abstract of a part of the Ph. D. dissertation of R. H. Allen.

(1) Dakin, *J. Biol. Chem.*, **4**, 227 (1908).

learned by one of us later.[2] Glycine, ammonium glycolate and salts of other organic acids act similarly catalytically. The use of alkali phosphates as buffers soon revealed their catalytic action with hydrogen peroxide. These effects could first best be clarified by using glucose as a substrate,[3] and the results were later confirmed by Spoehr,[4] Harden and Henley[5] and Warburg and Yabusoe[6] in various ways. Later these effects that had first been observed with butyric acid could be reported.[7] It should perhaps also be recalled in this connection that without such catalysts the oxidation of these substances by hydrogen peroxide is negligible. This is also true of the substances and systems described in this paper, although all of these negative data were omitted.

Methods

Oxidation Systems and Solutions.—Solutions of the various fatty acids were prepared so as to contain 0.25 g. of the acid in question in 5 cc. of solution. Owing to the limited solubility of caproic, caprylic and capric acids in water, these acids were converted into the sodium or ammonium salts, respectively, depending upon which system was to be used. To compensate for the alkali so added, since oxidation was very slow under these conditions in the sodium phosphate system, an equivalent amount of sodium acid phosphate was introduced to bring about the more favorable hydrogen ion concentration, necessary for effective oxidation. That is to say, although sodium dihydrogen phosphate alone does not catalyze the reaction the presence of some of it is desirable, for reasons that will be given below.

Oxidation Apparatus and Procedure.—The oxidations in the three systems were carried out in the closed system, previously described,[8] as follows:

(1) With the disodium hydrogen phosphate system: 5 cc. of the fatty acid solution, 50 cc. of 0.3 *M* disodium phosphate, and 20 cc. of Merck 30% hydrogen peroxide were used, and enough water to make the volume 100 cc. The mixture was heated in a water-bath at 90° for about three hours, while passing a current of carbon dioxide-free air through the apparatus.

(2) With the ammonia system: 5 cc. of the fatty acid solution was neutralized with the amount of 0.59 *N* ammonium hydroxide specified in the tabulated data. The volume was made up to 90 cc., and 10 cc. of Merck 30% hydrogen peroxide was added. The mixture was treated as above.

(3) With the diammonium phosphate system: 5 cc. of the fatty acid solution containing one equivalent of 0.1 *N* sodium hydroxide, 50 cc. of 0.3 *M* diammonium hydrogen phosphate and 20 cc. of 30% hydrogen peroxide were used. The mixture was treated as in (1) above.

Methods of Analysis.—The analytical methods are necessarily reported by giving the principle involved, and in general terms, because space would not permit detail.

Analysis was begun during the oxidation by collecting the carbon dioxide evolved, and the oxidation mixture was then worked up as previously reported.[8] The volatile acid recovered was determined and carefully characterized by fractionation according to Virtanen's method as previously used.[9] In this way 80% or more of the fatty acid used could usually be accounted for as carbon dioxide and acetic acid.

Attempts to determine the fate of the unaccounted-for portion of the fatty acid used were laborious and the results are in some degree uncertain. Aliquot portions of the distillates were titrated by the Lieben iodoform method; other portions were treated with alkaline silver oxide to remove "aldehydes," and subsequently again titrated by the Lieben method. Other portions pretreated in the same way were allowed to react with mercuric sulfate and sulfuric acid according to Denigès' procedure,[10] under which conditions the mercury compound of acetone is precipitated, while the derivatives of other expected ketones (methyl ethyl, methyl propyl and methyl amyl ketones) are soluble in the hot solutions. Thus the acetone complex filtered off gave a measure of the true acetone present, and the difference between this and the Lieben iodoform titration of the silver oxide resistant material gave an indication of the amount of higher ketones. Because we could not differentiate the small amounts of other ketones found, this portion is called "methyl ethyl ketone," and constitutes a rough estimate of the ketones found other than acetone. Similarly the difference between the first Lieben iodoform titration and that obtained after the silver oxide treatment was considered to be largely due to aldehydes, and was calculated as "acetaldehyde." This too is a rough approximation. Fortunately these "undetermined" portions of the products rarely exceed 10% of the material oxidized, but even in this form the data are of some value for comparative purposes.

In several instances "acetone" was recovered from the "mercury" precipitate and converted into its *p*-nitrophenylhydrazone. The product proved to be identical with that derived from pure acetone, in mixed melting point tests. The same precipitate obtained in the oxidation of valeric acid in the ammonia system, gave a *p*-nitrophenylhydrazone, m. p. 144° (known product from acetone m. p. 148.5°), and 145° in the mixed melting point test, thus showing a slight impurity. This result seemed important because acetone was not expected as a principal product for fatty acids other than butyric acid.

Experimental Results

The analytical data obtained in the oxidation of the five fatty acids listed, in the three oxidation systems, are summarized in Tables I, II and III,

(2) Witzemann, *J. Biol. Chem.*, **35**, 83–100 (1918); **49**, 123–141 (1921); THIS JOURNAL, **49**, 987–992 (1927).

(3) Witzemann, *J. Biol. Chem.*, **45**, 1–22 (1920).

(4) Spoehr, THIS JOURNAL, **46**, 1494 (1924).

(5) Harden and Henley, *Biochem. J.*, **16**, 143 (1922).

(6) Warburg and Yabusoe, *Biochem. Z.*, **146**, 380–386 (1924).

(7) Witzemann, THIS JOURNAL, **48**, 202, 208, 211 (1926); as well as *J. Biol. Chem.*, **107**, 475 (1934).

(8) Witzemann, *ibid.*, **107**, 475–487 (1934).

(9) Virtanen, *Soc. sc. Fennica, Commentationes Phys.-Math.*, **1**, 36 (1923); Virtanen and Pulki, THIS JOURNAL, **50**, 3138 (1928); *cf.* Witzemann, *J. Biol. Chem.*, **95**, 219–245 (1932).

(10) Van Slyke, *ibid.*, **32**, 455 (1917); Denigès, *Compt. rend.*, **126**, 1868 (1898); **127**, 963 (1898).

TABLE I

OXIDATION OF FATTY ACIDS (Na_2HPO_4–H_2O_2 SYSTEM)

Expt.	Acid used	Amt., g.	Time, hr.	Concn. of H_2O_2, %	Per cent. of C in acid used recovered as CO_2	Acetic acid	Acetone	"Ethyl methyl ketone"	"Acetaldehyde"	Total recovery
	Acetic	0.25	...	...	79.6	24.7	..	...	...	104.3
1	Butyric	.25	3.5	8.0	70.9	13.4	25.9			110.2
2	Butyric	.25	6.0	8.0	70.0	12.9	20.8			103.7
3	Butyric	.25	3.0	8.0	60.2	8.5	15.9		7.2	91.8
4	Butyric	.25	3.0	8.0	80.7	4.4	10.8		3.8	99.7
5	Butyric	.25	3.0	3.2	62.3	4.2	13.9		6.8	87.2
1	Valeric	.25	6.0	6.0	71.5	16.2	19.4			107.2
2	Valeric	.25	3.0	3.0	71.1	13.2	12.0		3.6	99.8
	Caproic	.50	3.0	8.0	60.1	14.9	5.3	2.7	5.3	88.3
	Caprylic	.50	3.0	8.0	57.0	14.1	5.0	2.1	5.8	84.1
1	Capric	.50	3.0	8.0	75.5	9.3	6.2	0.4	4.5	95.9
2	Capric	.25	3.0	8.0	79.4	6.8	4.0	1.1	3.8	95.1

TABLE II

OXIDATION OF FATTY ACIDS (AMMONIA–H_2O_2 SYSTEM)

Expt.	Acid used	Amt., g.	Time, hr.	Equivs. of NH_3 used	Concn. of H_2O_2, %	Per cent. of C in acid used recovered as CO_2	Acetic acid	Acetone	"Ethyl methyl ketone"	"Acetaldehyde"	Total recovery
	Acetic	0.25				33.3	72.6				105.9
1	Butyric	.25	3.0	1.0	1.5	43.6	21.2	32.9			97.8
2	Butyric	.25	6.0	1.0	1.5	46.3	28.5	26.2		11.1	112.3
3	Butyric	.25	3.0	2.0	3.0	55.7	23.1	16.7		4.9	100.6
1	Valeric	.251	6.0	1	4.4	68.3	11.0	12.0		3.7	95.3
2	Valeric	1.004	3.0	2	3.2	54.6	27.2	8.2		3.7	93.9
3	Valeric	0.502	3.0	2	3.0	56.7	27.4	6.0	1.8	3.1	95.2
4	Valeric	.502	3.0	2	3.0	54.0	26.1	6.5	3.1	4.6	94.5
	Caproic	.500	3.0	2	3.0	54.7	23.2	4.6	2.9	4.1	89.6
	Caprylic	.500	3.0	2	3.0	59.2	19.8	5.4	3.4	8.2	96.0
	Capric	.25	3.0	4	3.0	66.1	22.0	5.2	Small amt.	4.8	98.1

TABLE III

OXIDATION OF FATTY ACIDS ($(NH_4)_2HPO_4$–H_2O_2 SYSTEM)

Expt.	Acid used	Amt., g.	Time, hr.	Equivs. of NaOH used	Concn. of H_2O_2, %	Per cent. of C in acid used recovered as CO_2	Acetic acid	Acetone	"Ethyl methyl ketone"	"Acetaldehyde"	Total recovery
	Acetic	0.25				49.5	61.6				111.1
1	Butyric	.25	3	1.0	6.0	57.5	30.7	12.8		5.2	106.3
2	Butyric	.25	3	1.0	6.0	54.6	23.5	15.9		5.2	99.2
1	Valeric	.49	3	1.0	6.0	55.1	24.7	6.5	3.8	3.4	93.6
2	Valeric	.49	3	1.0	6.0	54.0	27.0	7.0	0.6	4.0	92.7
	Caproic	.50	3	1.0	8.0	58.8	23.7	5.2	0.0	3.6	91.4
	Capric	.25	3	1.0	8.0	70.3	19.4	5.0	Trace	2.6	97.5

respectively. At the head of each table a typical result obtained with acetic acid is given for comparison. The amounts of both the fatty acid and hydrogen peroxide used are given. The products of oxidation are not reported as grams of the products recovered, but for convenience have been converted into the per cent. of the carbon present in the original acid used, recovered in the form of the product in question. Thus with butyric in Table I 60 to 80%, out of a possible 100%, was recovered as carbon dioxide, and the result shows complete oxidation to this extent. Similarly 4 to 13% of the carbon appeared as acetic acid. This corresponds to an 8 to 26% yield, out of a possible 100% yield assuming that each molecule of butyric acid could yield only one molecule of acetic acid under these conditions. Likewise 10 to 25% of the carbon, out of a possible 75% conversion, appeared as acetone. Thus adding these values together it is evident that the original butyric acid was recovered to the extent of 87 to 110% in the form of oxidation products.

The details of the analyses cannot be given, but it is important to point out that the conditions

of oxidation were so selected that the acids subjected to oxidation were completely converted into something else. This was established by routinely determining the distillation constants of the volatile acids recovered, by the Virtanen method cited above. Thus although the products account for only 87% of the butyric acid used, in one instance, there was no reason to believe that the missing 13% was unoxidized butyric acid. The series of results as given were selected to display typical results, and to show the normal range of experimental error.

Discussion of the Tabulated Data.—The oxidations were so conducted as to bring about relatively complete oxidation, and no attempt was made to interrupt the process to obtain the possible intermediate products. The products found were carbon dioxide, acetic acid and ketones (mainly acetone) and small quantities of aldehydes.

Although the results with the disodium phosphate system were anticipated, on the basis of earlier data, this was not the case with the other two systems. In all instances the greater part of the fatty acid was oxidized completely to carbon dioxide. In general in these three systems fatty acids are more effectively oxidized, to the end-products carbon dioxide and water, than in any known *in vitro* system that could be of physiological interest.

Aside from carbon dioxide the main product was acetic acid, although in the case of butyric acid this was sometimes exceeded by the acetone found. Comparative studies brought out the fact that acetic acid is more resistant to oxidation than any of the other fatty acids tried; it is however oxidized and typical data are included in each table that show its relative susceptibility under the conditions in question. A comparison of these results shows that a larger proportion of any acetic acid formed, as an intermediate step, would be recovered as such from the ammonia system.

The third expected type of product of oxidation was the ketone, derived from the β-keto acid corresponding to the fatty acid used. With his technique Dakin obtained and characterized the expected series of ketones. Our analytical procedures were set up so that we too might catch, characterize and determine these. In general the recovery of the expected methyl ketones, other than acetone, failed or was consistently poor. The results with valeric acid, in which case methyl ethyl ketone was expected, were especially carefully obtained, because here the only other ketone to be expected was acetone, but the expected ketone was not an important product. If these ketones are formed in appreciable quantities, as was observed by Dakin,[1] under other conditions, they are also susceptible to oxidation under our conditions.[11]

Because of the unexpected completeness of the oxidations the results reported are of no great value in interpreting the intermediate mechanism. Some aspects of this problem were covered in earlier studies referred to above, in which it was for instance demonstrated for the first time that the carbon atoms can drop off in pairs and be recovered as oxalic acid in potassium permanganate oxidations.[12] Similarly evidence of the formation of short chain fatty acids like butyric acid was expected but not obtained.

Interpretation of the Catalytic Effects Observed.—Ever since these catalytic effects with ammonia and phosphates were first encountered, their interpretation has been a challenging question that cannot be answered unambiguously even now. Separate detailed studies on the behavior of these and related compounds were made and no clear basis for an interpretation could be obtained by the aid of the first five ideas listed below:

(a) There is a direct increase in the oxidizing power or action of the hydrogen peroxide.

(b) The catalysts liberate oxygen in an "active" form.

(c) The phosphate forms a substitution or addition compound with the peroxide that is a stronger oxidizing agent than the peroxide itself.

(d) The phosphate forms an easily oxidizable complex with the substrate, *i. e.*, a reducing compound.

(e) The substrate and the peroxide form a complex that breaks down to carbon dioxide and water in the presence of the catalyst.

Finally one more idea was tried and gave a satisfactory basis for an interpretation.

(f) Following Wieland's conception, the hydro-

(11) In later work Mr. Harold Wooster of this Laboratory oxidized acetone and propyl methyl ketone in quantitative experiments, in the sodium phosphate system. Aside from difficulties due to volatility, acetone was relatively easily oxidized and yielded some acetic acid and much more carbon dioxide. Because of its higher boiling point, propyl methyl ketone offered less difficulties, but the end-products were also acetic acid and carbon dioxide. The ketones were quantitatively recovered unchanged, or in the form of these products, so that no more than traces of other products could have been formed.

(12) Witzemann, *J. Biol. Chem.*, **95**, 219, 247 (1932).

gen peroxide functions as a hydrogen acceptor and the catalysts facilitate this effect.

With this idea the known facts fall into order. The peroxide reacts thus when it is merely decomposed catalytically

(1) $HOOH \longrightarrow O{:}O + 2H$ (oxidation)
(2) $HOOH + 2H \longrightarrow 2H_2O$ (reduction)

If, as Wieland considered, reaction (1) is the slower of the two, the catalysts could facilitate it directly, or in the presence of an organic substrate furnish hydrogen from another source for reaction (2). Such an interpretation raises two definite questions.

Can it be shown that hydrogen peroxide reacts with hydrogen derived from an organic compound to complete reaction (2)? Although formic acid is not oxidized by hydrogen peroxide, it could conceivably be oxidized to carbon dioxide by a simple dehydrogenation.

The test showed that the oxidation of 0.25 g. of formic acid in 5 cc. of water with 75 cc. of 0.166 *M* disodium phosphate and 10 cc. of 17% hydrogen peroxide was complete in about two and one-half hours, in the conditions described above. Three additional portions of formic acid were added and the 1.0 g. of formic acid so added yielded 0.96 g. of carbon dioxide (theory 0.956 g.). Less than 0.005 g. of unoxidized formic acid was recovered. The above conditions were selected so that sodium bicarbonate was not formed in appreciable amounts, because it so actively catalyzes the decomposition of hydrogen peroxide. The acid phosphate exercises a sparing or negative catalytic effect on the peroxide and saves it for the oxidation of the added substrate.

Thus Wieland's equations in this case become:

(1) $O{=}C(H){-}OH \longrightarrow O{=}C{-}O{-}$ (or CO_2) $+ 2H$

($HOOH \longrightarrow O_2 + 2H$)

(2) $H_2O_2 + 2H \longrightarrow 2H_2O$

This reaction scheme is to suggest that the overall velocity of the reaction is facilitated by using formic acid as the hydrogen donor to replace in part, at least, the similar but slower reaction of hydrogen peroxide itself. Here the disodium phosphate catalyzes reaction (1) at least, and may also facilitate reaction (2). Wieland used iron salts in a similar way with formic acid, but less successfully, and so the relation of his results with iron salts to these effects was not obvious at first.

This brings us to the second question: Can hydrogen peroxide acting in the same or a different way complete the oxidation of the hydrogen donor to carbon dioxide and water?

The most difficultly oxidizable compound that we have investigated is acetic acid. A typical oxidation was set up as follows: 60 cc. of 0.166 *M* disodium phosphate, 10 cc. of 0.166 *M* monosodium phosphate, 0.256 g. of acetic acid in 5 cc. and 10 cc. of 17% hydrogen peroxide were used. After heating for twelve hours, another 5 cc. of hydrogen peroxide was added and heating continued for six hours more. In this way 0.365 g. of carbon dioxide, or 97.0% of the calculated amount was recovered. The same amount of acetic acid was again added and the treatment repeated, and gave 0.374 g. of carbon dioxide (99.3%). Not over 0.03 g. of unchanged acetic acid was recovered from both oxidations.

This question is thus also answered in the affirmative and these data amplify those given in the tables above. The succession of events in such a dehydrogenation of acetic acid is not known, but the conceptions of Wieland for the interpretation of these effects are no doubt the best we have. Moreover, it seems clear that the reaction results can be interpreted without assuming a demonstrable increase in the oxidation potential due to the formation of a perphosphate, for instance. Thus we could consider that the phosphate increases the "activity" or lability of the hydrogen of the substrate,[13] and that the phosphate thus plays the role of the little understood dehydrogenase systems in the living organism.

It is presumed that the ammonia-containing systems function in a somewhat analogous manner.

Summary

Using a reflux condensation system six lower fatty acids (acetic, butyric, valeric, caproic, caprylic, and capric acids) were subjected to oxidation by hydrogen peroxide in three different catalytic systems (disodium phosphate, ammonium hydroxide and diammonium phosphate solutions). The major product was carbon dioxide, the yield being 50 to 80% of the calculated amount. Acetic acid, a resistant intermediate

(13) This would in some respects be promotion of oxidation by intensification of reducing power, which it is not always realized is an old practice. Thus a glucose solution will not react with air nor with cupric hydroxide. If pre-treated with alkali it readily reacts with both. The products formed in the pretreatment are more labile, and the difference in properties may be expressed as an intensification of the reducing power of glucose, or a greater lability of hydrogen.

product, was also an important end-product. Acetone was found with all acids except acetic acid, in appreciable amounts, and this was not expected, except with butyric acid. The products recovered and determined analytically gave a good "accounting" of the acids subjected to oxidation. Negligible oxidation occurs in the absence of the catalysts.

The interpretation of the catalytic effects presents serious difficulties, in view of certain experimental data mentioned, if it is assumed that the rather feeble oxidizing power of hydrogen peroxide is intensified by these substances. When, however, it was considered that they promote the reduction of hydrogen peroxide by supplying hydrogen from another source (the organic acids used) to reduce it to water, and that the final oxidation of the substrate to carbon dioxide is a secondary reaction depending on the addition of water and is not due primarily to the peroxide itself, all of the known facts fall into order.

MADISON, WIS. RECEIVED APRIL 12, 1941

[CONTRIBUTION FROM THE COLLEGE OF PHARMACY, UNIVERSITY OF ILLINOIS]

Derivatives of N^1-Phenylsulfanilamide. II

BY G. L. WEBSTER AND S. D. GERSHON

In an earlier report, Webster and Powers[1] described a series of N^1-phenylsulfanilamide[2] derivatives in which a hydrogen atom of the phenyl group had been replaced by a nitro, amino or hydroxyl group. In this paper we describe disubstituted derivatives in which two hydrogen atoms of the phenyl group have been replaced by nitro and amino, nitro and hydroxy, amino and hydroxy, nitro and acetoxy, hydroxy and acetylamino, acetoxy and acetylamino, two acetylamino or two amino groups with the N^4-nitrogen acetylated or unacetylated. In addition to the sulfanilamide derivatives we describe eight disubstituted phenyl-4-acetylamino (or amino)-benzenesulfonates.

Most of the N^4-acetylsulfanilamide derivatives were prepared by the general method described in the experimental part. N^4-Acetyl-N^1-2-nitro-4-hydroxyphenylsulfanilamide and 3′-nitro-4′-aminophenyl-4-acetylaminobenzenesulfonate were obtained from 3-nitro-4-aminophenol in 41% and 10% yields, respectively. When the sodium acetate was replaced by an equivalent amount of sodium bicarbonate, the principal product was the sulfonate (80% yield of crude material). In the case of 5-nitro-2-aminophenol, 18% of the sulfonanilide and 11% of the sulfonate were obtained using sodium acetate. When sodium bicarbonate was used, quantitative yields of the sulfonate were obtained.

The pharmacology of the compounds described is being investigated by Dr. Perrin H. Long.

(1) Webster and Powers, THIS JOURNAL, **60**, 1553 (1938).

(2) The nomenclature has been changed to conform to that suggested by Crossley, Northey and Hultquist, *ibid.*, **60**, 2217 (1938).

Experimental Part

2-Amino-4-acetylaminophenol.—Twenty grams of 2-nitro-4-acetylaminophenol[3] was reduced catalytically (platinum) in 150 cc. of hot alcohol. Eleven grams of white crystalline product, dec. 221–222°, was obtained after crystallization from 0.5% aqueous sodium hydrosulfite. When the product was crystallized from water, using decolorizing charcoal, tan-colored crystals formed which decomposed at the same temperature.[4]

Anal. Calcd. for $C_8H_{10}O_2N_2$: N, 16.86. Found: N, 16.92, 16.96.

The 2-amino-4-acetylaminophenol was acetylated to 2,4-di-(acetylamino)-phenol, m. p. 221° (lit. 220–222°),[5] and 2,4-di-(acetylamino)-1-acetoxybenzene, m. p. 184–185° (lit. 180–182°).[5]

3-Amino-4-acetylaminophenol.—Five grams of 1-acetylamino-2-amino-4-acetoxybenzene[6] was suspended in 25 cc. of water. Thirty cc. of 10% sodium hydroxide was added, the mixture stirred until solution was complete, and filtered. The filtrate was neutralized with 35% acetic acid and placed in an ice box overnight. The red crystals (2.4 g., m. p. 187.5–188.5°) were crystallized from water, using decolorizing charcoal. One and one-tenth grams of white crystalline material, m. p. 191°, was obtained.

Anal. Calcd. for $C_8H_{10}O_2N_2$: N, 16.86. Found: N, 16.70

The monoacetyl compound was acetylated to 3,4-di-(acetylamino)-phenol, m. p. 212° (lit. 214–215°),[6] and to 3,4-di-(acetylamino)-1-acetoxybenzene, m. p. 185–186° (lit. 187–188°).[6]

Preparation of N^4-Acetyl-N^1-disubstituted Phenylsulfanilamides.—One-tenth mole of disubstituted aniline

(3) Girard, *Bull. soc. chim.*, [4] **35**, 772 (1924).

(4) This compound has been reported with a decomposition point of 248° [Höchster Farbw., German Patent 164,295; *Chem. Zentr.*, **76**, II, 1701 (1905)] and 249° [Cassella and Co., German Patent 162,069; *Chem. Zentr.*, **76**, II, 865 (1905)].

(5) Kehrmann and Bahatrian, *Ber.*, **31**, 2399 (1898).

(6) Fieser and Martin, THIS JOURNAL, **57**, 1836 (1935).