IV. A STUDY OF THE DETERMINATION OF PHENOL GROUPS IN VIRUS DERIVATIVES BY MEANS OF MODEL EXPERIMENTS WITH DERIVATIVES OF TYROSINE

BY GAIL LORENZ MILLER

(From the Department of Animal and Plant Pathology of The Rockefeller Institute for Medical Research, Princeton)

(Received for publication, August 7, 1942)

Measurements of phenol groups in acetyl derivatives of tobacco mosaic virus by Herriott's methods (1) have been found to be easily duplicated and apparently reliable (2, 3). The extension of the methods to certain other derivatives of the virus, however, led to unsatisfactory results, owing to the resistance of these derivatives to denaturation. It therefore became necessary to work out modified methods of analysis which would assure complete denaturation of each derivative (3). During the course of this work, it was observed that under the usual conditions of the pH 11 method (2) the carbobenzoxy, p-chlorobenzoyl, and benzenesulfonyl derivatives of the virus exhibited a much less complete recovery of chromogenic power than that shown by the acetyl virus. The phenylureido virus, on the other hand, exhibited in the pH 11 method even more chromogenic power than that of the normal virus. In an effort to explain these apparently anomalous results, studies on the behavior of the differently substituted phenolic linkages under the conditions of Herriott's methods were carried out by means of model experiments with the corresponding derivatives of free tyrosine.

The rates of saponification of disubstituted tyrosine derivatives at pH 11 were first determined. Complete hydrolysis of a given derivative was assumed to have been obtained when the chromogenic power reached a maximum and remained constant. The results are presented graphically in Fig. 1 from which it may be seen that the diphenylcarbamidotyrosine, a derivative which corresponded to the phenylureido virus, was completely saponified within 30 seconds and the diacetyltyrosine, within about 10 minutes. The dicarbobenzoxy and dibenzoyl derivatives, on the other hand, required nearly 30 minutes. In still greater contrast, the dibenzenesulfonyl derivative was not measurably saponified even after 60 minutes. When heated at 100° at pH 11, the dibenzenesulfonyltyrosine required over 30 minutes for saponification, whereas the other derivatives of tyrosine were saponified within 1 minute. Abderhalden and Bahn reported that the benzenesulfonyl-phenolic linkage of dibenzenesulfonyltyrosine is very stable to alkali (4). It was apparent from the above results that the low
chromogenic power exhibited by the carbobenzoxy, \( p \)-chlorobenzoyl, and benzenesulfonyl derivatives of tobacco mosaic virus when analyzed by the pH 11 method could have been due, in part at least, to the slow rate of saponification of the substituted phenolic linkages involved.

More detailed studies were next carried out with phenylcarbamido derivatives of tyrosine. The diphenylcarbamidotyrosine, after treatment at pH 11, was found to yield with the phenol reagent an amount of color which was about 16 per cent greater than that yielded by an equimolecular amount of tyrosine. The monophenylcarbamidotyrosine, on the other hand, yielded approximately 22 per cent less color than an equivalent amount of tyrosine. Since it was to be expected that aniline would be formed as a product of the saponification of the diphenylcarbamidotyrosine, it appeared that this substance was responsible for the extra chromogenic power possessed by the disubstituted tyrosine. In colorimetric tests carried out on aqueous solutions of doubly distilled aniline, the compound was found to give with the phenol reagent about 35 per cent of the color given by an equimolecular amount of tyrosine. It was apparent that this amount of color accounted for the difference in the chromogenic power of the monophenylcarbamidotyrosine and that of the saponified diphenylcarbamido derivative. It was therefore concluded that, by analogy, the extra color yielded by phenylureido derivatives of tobacco mosaic virus after treatment at pH 11 was also due to the presence of aniline which was formed during the saponification of the derivatives.

The direct application of the results of the model experiments to the determination of phenol groups in derivatives of tobacco mosaic virus did not give rise to completely satisfactory results in all cases. This was due to the secondary effect of the slow rate of denaturation of certain preparations of derivatives of the virus under the conditions of the pH 11 method (3). In the cases of the acetyl, carbobenzoxy, and \( p \)-chlorobenzoyl derivat-
vatives of the virus, it was found, however, that a complete denaturation and saponification could be obtained if the pH 11 treatment was carried out at 100° for a period of 2 minutes. This procedure appeared to be justified on the basis of the finding that under these more vigorous conditions samples of normal virus exhibited the same chromogenic power which they exhibited when denatured by acid or alkaline detergent at room temperature. Determinations of the phenylureido virus or benzenesulfonyl virus by the pH 11 method were carried out by saponification at room temperature for 2 or 3 hours (3). Because of the properties of the linkages involved, the appearance of an excess of chromogenic power in the phenylureido virus and a complete lack of recovery of chromogenic power in the benzenesulfonyl virus served as corroborative evidence for the substitution of phenolic linkages within these particular derivatives.

**TABLE I**

*Relative Chromogenic Powers of Different Derivatives of Tyrosine*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative chromogenic power (pH 11 method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyrosine</td>
<td>100</td>
</tr>
<tr>
<td>N-Glycyltyrosine</td>
<td>89</td>
</tr>
<tr>
<td>N-Phenylcarbamidotyrosine</td>
<td>78</td>
</tr>
<tr>
<td>O,N-Diacetyltyrosine</td>
<td>78</td>
</tr>
<tr>
<td>O,N-Dibenzyoltyrosine</td>
<td>80</td>
</tr>
<tr>
<td>O,N-Dicarbobenzyoxtyrosine</td>
<td>75</td>
</tr>
<tr>
<td>N-Carbobenzyoxtyrosine</td>
<td>78*</td>
</tr>
<tr>
<td>N-Chloroacetyltyrosine</td>
<td>83*</td>
</tr>
<tr>
<td>N-Carbobenzyoxytyrosylglycine</td>
<td>87*</td>
</tr>
</tbody>
</table>

* Data of Tracy and Ross (5).

It was observed in the course of the experiments with the derivatives of tyrosine that the chromogenic values given by the various derivatives in the pH 11 method did not coincide with that given by free tyrosine. Because of the stability of the linkage between the various substituent radicals and the amino group of the tyrosine, the chromogenic power of the saponified disubstituted derivatives was due in general to the monosubstituted derivatives which were formed. In Table I, the relative chromogenic powers of the different tyrosine derivatives, together with that of a sample of glycyltyrosine, are compared with the chromogenic power of free tyrosine. Similar data obtained by Tracy and Ross (5) for a number of other derivatives of tyrosine also are included in Table I. Without exception, the derivatives developed less color with the Folin reagent than did equivalent amounts of tyrosine. Tracy and Ross concluded that, in general, substitution diminishes the color developed by
tyrosine with the phenol reagent. Our present data serve as additional
evidence for this generalization. It was of particular significance in this
connection that tyrosine appears to possess a diminished chromogenic
power when linked within the intact protein molecule. For example, it
was found by Herriott that intact proteins when treated with the Folin
reagent yielded on the average only 59 per cent of the color to be expected
from their known content of tyrosine and tryptophane (1). Other investi-
gators have obtained similar results (2, 5-7). In view of the data obtained
with tyrosine derivatives of known structure, it might well be anticipated
that the units of tyrosine which occur within protein molecules would
exhibit a diminished reaction with the Folin reagent.

EXPERIMENTAL

Preparation of O,N-Diphenylcarbamidotyrosine—5 gm. of tyrosine dis-
solved in a mixture of 100 cc. of 2 per cent sodium hydroxide and 100 cc.
of 1 m dipotassium phosphate were treated with continuous stirring at 0°
with 8 cc. of phenyl isocyanate added in 2 cc. portions at 10 minute
intervals. 12 cc. of 10 per cent sodium hydroxide were added gradually
during the reaction to maintain the pH at 8 to 9. Diphenylurea was
extracted with ether and the aqueous layer was acidified with hydrochloric
acid. The precipitate which formed was filtered and dried. The yield,
15.8 gm., was practically quantitative. When crystallized from hot 95
per cent ethyl alcohol, the compound separated as spear-shaped plates,
which, after a second recrystallization, melted at 205-206° with efferves-
cence. Neutralization equivalent, 421; calculated for diphenylcarbamido-
tyrosine, 419. C 65.6, H 5.0, N 10.0 per cent; theory, C 65.9, H 5.0,
N 10.0 per cent. The high sensitivity of the phenylcarbamido-phenolic
linkage to alkali probably accounted for the failure of Gaunt and Wormall
to obtain this compound under the conditions they employed (8).

Preparation of N-Phenylcarbamidotyrosine—5 gm. of tyrosine dissolved
in 200 cc. of 1 per cent sodium hydroxide were treated with continuous
stirring at 0° with 4 cc. of phenyl isocyanate added in 0.5 cc. portions at
5 minute intervals. 4 cc. of 10 per cent sodium hydroxide were added
during the reaction to maintain the solution strongly alkaline to phenol-
phthalein. The mixture was washed with ether, filtered, and acidified
with hydrochloric acid. The product separated as an oil which crystal-
lized as long, flat needles on cooling and stirring. The yield was 7.4 gm.
(80 per cent of the theory). After repeated recrystallization from dilute
alcohol, the compound softened at 104° and melted with effervescence at
106-110°. Beilstein (9) has given a melting point of 104° for N-phenyl-
carbamidotyrosine. Neutralization equivalent, 298; calculated for
N-phenylcarbamidotyrosine, 300.

Preparation of Diacetyl, Dicarbobenzoxy, Dibenzoyl, and Dibenzenesulfonyl
Derivatives of Tyrosine—A sample of diacetyltyrosine, prepared by treating tyrosine with ketene (10), was obtained through the courtesy of Dr. R. M. Herriott. The dicarbobenzyxoy, dibenzyol, and dibenzenesulfonyl derivatives of tyrosine were prepared by procedures described elsewhere (4, 11-14).

Measurements of Rates of Saponification of Tyrosine Derivatives—For solutions of the diacetyl, dibenzyol, and dibenzenesulfonyl derivatives, weighed samples were dissolved in small volumes of acetone, neutralized with the calculated amounts of 0.05 N sodium hydroxide, and diluted with water to the desired final volumes. For the solution of the diphenylcarbamido derivative, the weighed sample was suspended in a little dilute acetone and was dissolved by the addition of the minimum required amount of 0.05 M disodium phosphate. For the preparation of the solution of dicarbobenzyxoytyrosine, a much larger amount of acetone was required in order to keep the neutralized derivative in solution. The acetone did not interfere with the color tests. Each of the solutions was made up to contain an amount of derivative approximately equivalent to 0.1 mg. of tyrosine per cc.

To 1 cc. aliquots of each solution was added 0.1 cc. of 0.2 N sodium hydroxide. The mixtures were allowed to stand at 25° or at 100° for the desired period of time and were then neutralized with 0.1 cc. of 0.2 N hydrochloric acid. 0.2 cc. of 10 per cent sodium dodecyl sulfate and 0.6 cc. of water were added, followed by 1 cc. of Folin reagent and 2 cc. of alkaline phosphate buffer. The alkaline buffer and Folin reagent were prepared as described in a previous paper (3). The colors were measured in the Klett-Summerson colorimeter at the end of 15 minutes.

The use of sodium dodecyl sulfate (3) in the place of urea formerly employed (2) has no special significance as far as the above experiments are concerned. It was of interest to observe, however, that the detergent possessed a remarkable dispersing power for the tyrosine derivatives which were relatively insoluble in water.

SUMMARY

Carbobenzyxoy, p-chlorobenzyol, and benzenesulfonyl derivatives of tobacco mosaic virus were found to give a less complete recovery of chromogenic power under the usual conditions of the pH 11 method of Herriott than did the acetyl derivative of the virus. By means of model experiments with the corresponding derivatives of tyrosine, it was demonstrated that the different substituent radicals on the phenolic group varied considerably in their rates of saponification. The application of the findings to the measurement of phenolic groups in derivatives of tobacco mosaic virus was discussed.

The phenylureido derivative of tobacco mosaic virus was found to yield
more color in the pH 11 method than did normal virus. By means of studies on the chromogenic power of pure aniline and on the behavior of N phenylcarbamido and O,N-diphenylcarbamido derivatives of tyrosine, this result was demonstrated to be due to the formation of aniline during the treatment at pH 11.

Monosubstituted tyrosine derivatives were found to yield with the phenol color reagent less color than did free tyrosine. An analogy was pointed out between this property of known derivatives of tyrosine and the similar low chromogenic power of tyrosine when present in protein linkage.

BIBLIOGRAPHY

DERIVATIVES OF TOBACCO MOSAIC VIRUS: IV. A STUDY OF THE DETERMINATION OF PHENOL GROUPS IN VIRUS DERIVATIVES BY MEANS OF MODEL EXPERIMENTS WITH DERIVATIVES OF TYROSINE

Gail Lorenz Miller


Access the most updated version of this article at http://www.jbc.org/content/146/2/345.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/146/2/345.citation.full.html#ref-list-1