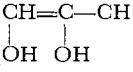
approximately the same, whereas, for an enol spectrum and the spectra of the irradiated sugars, the maximum is higher at the higher pH value.

Reductones were shown to be present in irradiated crystalline sugars, but not in irradiated sugar solutions. These results suggest that any reductones or their precursors formed by irradiation of sugars in aqueous solution may be destroyed as a result of the greater mobility of the sugar molecules in solution as compared to the crystal. Of course, it is not known whether reductones exist as such in the irradiated sugar crystal, or whether they are formed from a precursor, such as a stable free radical, when the crystal is dissolved. Irradiation of anhydrous (liquid) glycerol produced no reductones.

The spectra of irradiated crystalline sugars do not resemble that of glucoreductone  , which has absorption maxima at 263 mμ below pH 5 and at 293 mμ above this value, with a molecular absorbance coefficient of 1.8×10^4. However, it was shown by calculation that the ultraviolet absorption due to any glucoreductone present would be masked by the observed absorption bands. This calculation was based on the assumption that the reductone content of irradiated crystalline sugars, measured by titration with Tillman's reagent, was glucoreductone.

The presence of hydrogen and methanol in irradiated fructose also indicates that irradiation degradation involves a dehydrogenation. Enediols are formed on irradiation of hexonic acid lactones by dehydrogenation (*3*).

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FEED ADDITIVES ANALYSIS

Colorimetric Determination of 3,5-Dinitrobenzamide in Feeds by the Janovsky Reaction

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An adaptation of the Janovsky reaction for dinitro compounds is presented for the determination of microgram quantities of 3,5-dinitrobenzamide. The method is intended for use with animal feeds containing this medicament. Extraction of the feedstuff with acetone followed by a reaction with concentrated ammonium hydroxide to produce a colored solution is the basis for the method. An alternative method, which also produces a stable color, using anhydrous liquid ammonia is described.

THE INCREASING USE of organic chemicals as medicaments in the feed industry has created the need for methods of analysis which are specific for a compound.

The compound, 3,5-dinitrobenzamide, is now being marketed as a medicinal additive for poultry feeds. It was necessary to devise a method for rapid routine assay of microgram quantities of this material, because it is subject to control under the Texas Feed Control Law.

In 1886, Janovsky and Erb (*8*) investigated the various bromo and nitro derivatives of azobenzenes and produced color in acetone with potassium hydroxide. Janovsky (*7*) in 1891 extended this work to include a study of dinitro compounds. He reported on the colors that formed when dinitrobenzenes were dissolved in acetone and treated with an alkali. Through the years several investigators have experimented with this reaction (*3, 6, 7, 10, 11*). Various reactions mechanisms have been proposed (*1, 5, 9*) and special conditions suggested for making the test.

A variety of other methods, for the determination of other specific compounds, mainly dinitrophenols, have been published. These, however, do not fall within the scope of the colorimetric reaction being studied here and are not cited individually. They include titrimetric, gravimetric, and colorimetric methods.

Dinitrobenzamides were not included in the above tests. A new application of the Janovsky reaction is reported and also a modification which may present a broader application of the color test.

A method for the analysis of 3,5-dinitrobenzamide (*4*) has been proposed and subjected to study by a group from the Association of Official Agricultural Chemists. This method uses diethylamine reagent and dimethyl sulfoxide to produce a colored product.

Experimental

The first attempt at a color test for the compound 3,5-dinitrobenzamide utilized the classical approach of dissolving 1 to 2 mg. in 10 ml. of acetone, followed by the addition of 2 to 3 ml. of a 5% sodium hydroxide solution. This resulted in the formation of a blue color which faded immediately.

Previous information suggested that the presence of water might be detrimental to the formation of a stable color. To add an alkaline medium in an anhydrous condition, liquid anhydrous ammonia was added to an acetone solution of 3,5-dinitrobenzamide. The first few drops of liquid ammonia had no effect on the solution, because of the volatilization of the ammonia. Upon further addition of the ammonia a magenta color formed. The tube was stoppered lightly to allow the escape of gas and then was set aside. The color faded slowly to light pink, but as the

solution returned to room temperature, the color increased in intensity to a maximum after about 8 hours. This color was then stable and had absorption maxima at 518 and 390 mμ.

As only a few laboratories are provided with a source of anhydrous ammonia, it was felt that a more general method was needed for routine work. Even though it had been previously reported that ammonium hydroxide was ineffective (*5*), it was decided to test its value.

Thus, when 1 to 2 mg. of 3,5-dinitrobenzamide in 10 ml. of acetone was treated with concentrated ammonium hydroxide, a blue color developed. The first milliliter or two had no effect, but when the volume was increased to 5 ml. the color was formed. The intensity of the color increased over a 45-minute period. The blue solution had an absorption maximum at 575 to 580 mμ.

Under optimum conditions the sensitivity of the color test was determined to have an $E^{1\%}_{1\,cm.}$ value of 436. This showed that the method should be fully applicable for determining very small amounts. The molar extinction coefficient is 8.8×10^4 using the molecular weight of 211 for 3,5-dinitrobenzamide at 580 mμ.

The following data were obtained, upon further experimentation.

A qualitative test for the amide portion of the molecule using hydrogen peroxide and ferric chloride gave a positive result, but the sensitivity appeared to be low.

The use of *N*,*N*-dimethylformamide, in place of acetone (*2*), as a solvent was tried. Upon addition of concentrated ammonium hydroxide, a pink-purple color developed, and with anhydrous ammonia a deep purple color was obtained. Both of these colors faded rather rapidly (3 to 5 minutes) and were not judged satisfactory for use because of the previous results with acetone.

Charcoal effectively removes all dinitrobenzamide from an acetone solution and a column packed with a magnesium oxide–Celite mixture retains all the 3,5-dinitrobenzamide at the top of the column. This is recognized by the narrow blue band that forms at the top of the column.

The dry compound itself when treated with anhydrous ammonia developed a deep blue colored solution in the liquid ammonia. Thus it is shown that the role of the acetone, or other carbonyl compounds, is not essential to color formation, but serves only as an excellent solvent and leads to a stable color.

With anhydrous ammonia, the maxima were at 518 and 390 mμ. The solutions contain no apparent yellow, yet the absorbance at 390 mμ is just as strong as at 518. This is perhaps a further characteristic of this compound.

Table I. Recovery of 3,5-Dinitrobenzamide from Blank Feed

DNBA, γ per Ml. of Final Dilution	Absorbance Values[a] Pure material	From feed	Recovered, %
1.6	0.062	0.062	100.0
3.2	0.127	0.130	102.2
4.8	0.202	0.198	98.0
6.4	0.260	0.262	100.8
8.0	0.330	0.320	97.0
		Average	99.8

[a] Measured at 580 mμ.

To establish the optimum volume ratio of acetone to ammonium hydroxide several combinations were prepared and a standard curve was plotted for each. The *E* values were essentially the same for all the combinations. The criteria for the choice of conditions are in the stability of the color formed and the length of time required for maximum color to develop. These conditions are set forth later in the method.

Because the product is marketed in such a way as to produce feeds or feed concentrates containing between 0.025 and 0.150%, the method was designed to give final aliquots containing the necessary amount of medicaments per unit of volume.

Method

Standards and standard curves. Dissolve 100 mg. of 3,5-dinitrobenzamide in a 100-ml. volumetric flask with acetone, make to volume, and dilute with acetone 10 ml. of the above solution to 100 ml. (100 γ per ml.). To prepare dilutions for a standard curve, take 0-, 1-, 2-, 3-, 4-, and 5-ml. aliquots from the 100 γ per ml. of standard solution and dilute each to 50 ml. with acetone in a volumetric flask.

Remove 20 ml. from each solution and transfer to 25-ml. volumetric flasks. Fill each to the mark with concentrated ammonium hydroxide. Stopper well and mix. Set the flasks aside under a towel for 45 minutes for maximum color development. Read the absorbance at 580 mμ. Plot concentrations *vs.* absorbance readings.

Analysis of Sample

For samples containing 0.025% 3,5-dinitrobenzamide, weigh a 2.0-gram sample into a 100-ml. volumetric flask. Make to volume with absolute methanol and shake well. Continue shaking intermittently for 30 minutes. Filter a portion and transfer a 20.0-ml. aliquot to a 50-ml. beaker.

Evaporate the alcohol to dryness on a steam bath and add 10 ml. of acetone. Warm the beaker to dissolve the residue and carefully transfer the contents to a 25-ml. volumetric flask. Add 5 ml. of concentrated ammonium hydroxide to the beaker and swirl to dissolve the remainder of the residue. Transfer this to the volumetric flask and rinse off the beaker with another 10-ml. portion of acetone. After the second acetone rinse has been added to the volumetric flask, fill the flask to the mark with acetone. This will give a 5 γ per ml. final solution of 3,5-dinitrobenzamide.

For samples containing 0.150% 3,5-dinitrobenzamide, weigh a 2.0-gram sample into a 100-ml. volumetric flask. Make to volume with absolute methanol, shake intermittently for 30 minutes, then filter. Transfer 10.0 ml. to a 50-ml. volumetric flask. Fill to mark with absolute methanol, take a 20-ml. portion, and treat as before. This will give a 6 γ per ml. final solution of 3,5-dinitrobenzamide.

Table II. Recovery of 3,5-Dinitrobenzamide Added to Poultry Feeds

(200 γ added)

Sample No.	Recovery γ	%
1	195.00	97.50
2	197.50	98.60
3	205.50	102.40
4	207.50	103.50
5	197.50	98.60
6	206.50	103.20
7	197.00	98.50
8	206.00	103.00
9	197.50	98.60
10	194.50	97.40
11	207.00	103.30
12	206.00	103.00
	Average	100.60

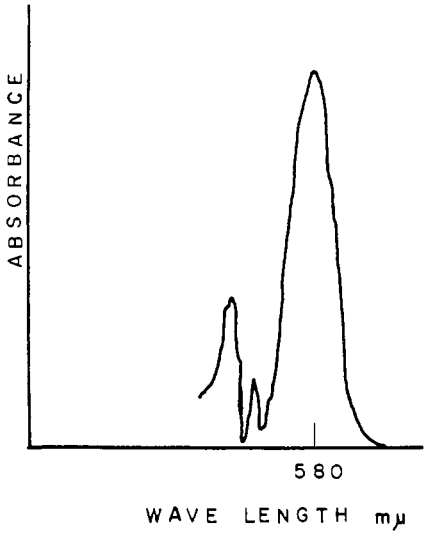

Figure 1. Absorption curve for 3,5-dinitrobenzamide in acetone with ammonium hydroxide

Results

Table I presents data showing recovery of 3,5-dinitrobenzamide added to a feed as compared to the color developed by the pure material. The method used was exactly as previously described. The absorption maxima were determined using a Beckman DK-2 ratio-recording spectrophotometer (Figure 1).

The data in Table II were intended to show that the source of feed has little effect on the recovery of added 3,5-dinitrobenzamide. The presence of other medications was not detrimental to the results. Samples 1 and 5 contained 3-nitro-4-hydroxyphenylarsonic acid, *N*,*N'*, di-(3-nitrobenzenesulfonyl) ethylenediamine, and N^4-acetyl-*N*-(4-nitrophenyl) sulfanilamide. Sample II contained 3 - nitro - 4 - hydroxyphenylarsonic acid and nitrophenide [bis(3 nitrophenyl)-disulfide]. All the feeds shown are poultry feeds chosen to be representative of the extremes normally encountered. Two milliliters of a 100-γ-per-ml. solution of 3,5-dinitrobenzamide in acetone were added to 2.0 grams of each of the samples in Table II.

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FISH STORAGE EFFECTS

Composition Changes in Puget Sound Pink Salmon during Storage in Ice and in Refrigerated Brine

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To determine the changes taking place in fish flesh under different storage conditions, the composition and quality of Puget Sound pink salmon were determined shortly after capture, during storage in ice, and during storage in chilled brine. Large variations were found in the dorsal, ventral, and belly flap parts, but variations were small in the light meat or steak sections. Fish about ready to spawn showed a great depletion of the oil reserves in all parts of the fish. Storage of fresh and of brine chilled specimens in ice caused extensive leaching of the ash, sodium, and potassium contents. Storage in brine greatly increased the content of ash and of sodium. Such changes would have special significance in the preparation of a "dietetic" or low-sodium product.

SALT-WATER FISH is usually preserved from the time of capture until delivery to the processor or consumer by ice or more recently by refrigerated brine (*6*, *7*). Although brine is more commonly used with tuna (*5*), in this study, pink salmon, owing to its ready availability to the Seattle laboratory, was used to compare the effect of the two methods on keeping quality. The physical and chemical changes taking place in the flesh soon after capture, during storage in ice, and during storage in chilled brine were investigated. Storage periods of about 1 and 2 weeks were used, because it was felt these periods represent the maximum lengths of time fish would be retained on board ship or on land before being processed.

Description of Specimens

Puget Sound pink salmon (*Oncorhynchus gorbuscha*) captured with gill nets were placed in ice at the landing dock and brought immediately to the Seattle laboratory, where they were prepared for analysis. The physical data (Table I) for the specimens showed wide variation. Males and females were about equal in number. In size, they varied from small to large for the males (54 to 66 cm.) and from medium to large for the females (54 to 59 cm.). They ranged from a bright silvery green to a dark sand color with numerous watermarks. In the females, the roe varied from small and compact to large and separating. The hump and nose hook were slight in some males and very large in others.

Table I. Physical Data for Pink Salmon Used in Tests

Factor	Males	Females
Quantity	11	13
Length, cm.	54–66	54–59
Weight, grams	1845–3500	1990–2490
Color	Bright to dark	Bright to dull
Roe	Small	Medium
hump and hook	to large	to large

Preparation of Samples

Samples were prepared from fresh fish, from fish stored in ice, and from fish stored in chilled brine.

Fresh. For the chemical analyses, seven samples were taken from each of six male and six female fish. The whole, cold fish was dipped in scalding water for a few seconds to loosen the skin, which then was peeled off. The fish was eviscerated, and the head removed. Three steak sections were taken from the nape, center, and tail. From the two remaining portions, the dorsal, lateral, dark muscle, and belly flaps were removed from the light meat. These in-