



Fig. 5.—Absorption spectra of genistin and genistein.

A large Hilger double monochromator with quartz optics was employed to isolate spectral regions which varied between 6 and 13 Å. For genistin a single maximum was found at 2625 Å., with $\alpha = 90.5$. Recrystallization of the genistin did not affect the absorption spectrum. The absorption spectrum of the aglucone, genistein, was of the same general shape as that of genistin, but somewhat higher. The maximum occurred at 2625 Å., with $\alpha = 138$.

If one plots the absorption spectra data of Asahina and co-workers¹⁴ for iridin, irigenin, tectori-

(14) Y. Asahina, B. Shibata and Z. Ogawa, *J. Pharm. Soc. Japan*, **48**, 150, 1093 (1928).

din and tectorigenin in the same terms used in Fig. 5, a maximum is observed in the region of 2680 Å. for each of these compounds. A minimum occurs in the region of 2400 Å. and a shelf in the region of 3200 Å. Thus a similarity in the general shape of the curves exists between the absorption spectra of these compounds and those of genistin and genistein, although the maxima and minima do not occur at exactly the same wave lengths.

Summary

1. A crystalline pale yellow substance has been isolated from oil-free soybean meal. The data on this substance and its derivatives are in agreement with the data for genistin and its derivatives reported by Walz, who also isolated it from soybeans.

2. Additional evidence, that the sugar hydrolyzed from genistin is *d*-glucose, is presented.

3. The absorption spectra, a positive Wilson boric acid color reaction and a positive Asahina reduction test are recorded to further characterize genistin and its aglucone, genistein.

4. Crystallographic optical data are presented for genistin, genistein, genistin hexaacetate and genistin triacetate.

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[CONTRIBUTION FROM THE FOOD AND DRUG ADMINISTRATION, FEDERAL SECURITY AGENCY]

Preparation of the Dinitrochlorobenzenes from the Corresponding Dinitroanilines*

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In connection with clinical studies of the specificity of response of persons eczematously sensitized to aromatic compounds, the need arose for specimens of the dinitrochlorobenzenes. Synthesis of five of the six possible isomers was required since only 2,4-dinitrochlorobenzene was commercially available.

The 3,4-,¹ 2,5-² and 2,6-³ isomers are formed directly by the action of nitric acid on benzene derivatives. The last substance cited has also been prepared from tosyl chloride and 2,6-dinitrophenol.⁴ Chlorination of *m*-dinitrobenzene

has been reported to give 3,5-dinitrochlorobenzene.⁵ The Sandmeyer reaction has been used in the preparation of 3,5-,⁶ 2,5-,⁷ 2,3-,^{7a} 2,4-⁸ and 2,6-dinitrochlorobenzene⁹ from amino compounds.

For purposes of clinical study, the quantitative separation of the isomers was of more fundamental importance than the removal of unrelated impurities, and it was thought that the chances of satisfying this requirement would be increased by using syntheses which involved a plurality of steps between the isolation of the final product and the reaction which fixed the relationship of the substit-

* Not copyrighted.

(1) Laubenheimer, *Ber.*, **9**, 760 (1876); Mangini and Deliddo, *Gazz. chim. ital.*, **63**, 612 (1933).

(2) Kehrman and Grab, *Ann.*, **303**, 1 (1898).

(3) Borsche and Rantscheff, *ibid.*, **379**, 152 (1911).

(4) Kubota, *J. Chem. Soc. Japan*, **53**, 404 (1932); *C. A.*, **27**, 274 (1933).

(5) German Patent 108,165; *Chem. Zentr.*, **71**, I, 1115 (1900).

(6) Bader, *Ber.*, **24**, 1653 (1891); de Kock, *Rec. trav. chim.*, **20**, 111 (1901).

(7) (a) Hollemann and ter Weel, *ibid.*, **35**, 46-47 (1915); (b) Körner and Contardi, *Atti accad. Lincei*, **23**, I, 281 (1914).

(8) Hodgson and Walker, *J. Chem. Soc.*, 1620 (1933).

(9) Körner and Contardi, *Atti accad. Lincei*, **23**, II, 464 (1914).

uents appearing in the final product. Synthesis of the isomers via the dinitroanilines was, therefore, considered preferable to more direct methods.

Of the dinitroanilines, only the 3,5-isomer is sufficiently basic to permit diazotization under conditions approximating those usually employed, and when such treatment has been followed by introduction of chlorine by the Sandmeyer reaction, yields have been low.^{6,7a} The other isomers may be diazotized by nitrosylsulfuric acid in concentrated sulfuric acid. Not only solution of the amine, but a high concentration of base as ammonium salt, is achieved by use of this reagent. Communications of Blangey¹⁰ and Schoutissen¹¹ have made it apparent, however, that when this reagent is employed, diazotization is slow unless nitrous acid is freed from the nitrosylsulfuric acid combination. This may be accomplished by the addition of water,¹² pyridine¹³ or a weak acid, such as phosphoric¹¹ or acetic.¹² By using phosphoric acid for this purpose, Schoutissen was able to effect quantitative diazotization. This method has been employed to advantage in preparing the dinitrochlorobenzenes by means of the Sandmeyer reaction. In purifying the crude products, the use of concentrated sulfuric acid as a wash and the use of activated alumina as adsorbent were extremely useful in removing by-products. Only in the case of the 2,6-isomer was recrystallization necessary in order to obtain reasonable purity. The crude products, in general, contained extraneous materials having lower solubilities than the desired substances, and recrystallizations were inefficient unless the bulk of such impurities was removed by washing and adsorption procedures. The average yield of the six isomers was 70%, and in the case of each substance, the yield represented a significant improvement on those which have been previously described.¹⁴

Experimental

Nitration of *m*-Nitroacetanilide.—*m*-Nitroacetanilide was prepared in quantitative yields from *m*-nitroaniline by the procedure of Kaufmann, *et al.*¹⁵; m. p. 151.5–152°.¹⁸

(10) Blangey, *Helv. Chim. Acta*, **8**, 780 (1925); **21**, 1579 (1938).

(11) Schoutissen, *THIS JOURNAL*, **55**, 4531 (1933).

(12) Misslin, *Helv. Chim. Acta*, **3**, 626 (1920).

(13) Krishna and Bhatia, *Proc. 15th Ind. Sci. Cong.*, 152 (1928); *C. A.*, **25**, 2981 (1931); de Milt and Van Zandt, *THIS JOURNAL*, **58**, 2044 (1936).

(14) Hodgson and Walker⁸ mention an 80% yield of 2,4-dinitrochlorobenzene by means of the Sandmeyer reaction, but the preparation is not described in detail, and the yield apparently refers to an unpurified product.

(15) Kaufmann, Hüsey and Luterbacher, *Ber.*, **42**, 3480 (1909).

(16) All melting points are uncorrected.

Three hundred grams of product was nitrated according to Macciotta¹⁷ by dissolving in 750 cc. of nitric acid (sp. gr. 1.5) and adding the solution to a stirred mixture of sulfuric and glacial acetic acids maintained at –5–0°. During this addition, which required ninety minutes, the nitric acid solution of *m*-nitroacetanilide became darker, and its temperature rose to 45°. It is probably advantageous to prepare this solution in small portions and to keep it cold during the process. The crude product, obtained by pouring the reaction mixture over 2000 g. of ice, still retained considerable water after drying in a vacuum desiccator four days over calcium chloride. Fourteen hundred cc. of boiling benzene–acetone (2:1) was required to dissolve the product in this condition. Although Macciotta used this solvent exclusively in separating the three isomeric products, in this work it was found to have a too effective solvent action on the later fractions obtained. It was most useful in separating the least soluble 2,3-dinitroacetanilide from the other products. The crude 3,4-isomer was best separated by recrystallization from toluene. The following amounts of pure isomers were obtained after recrystallizations from ethanol: 2,3-, 115 g. (30%), m. p. 189.5–190°; 2,5-, 45 g. (12%), m. p. 122°; 3,4-, 33 g. (8.8%), m. p. 146.5–147°. In addition to these, there was obtained a dark brown solid which was not investigated further. Macciotta has noted that Wender's method¹⁸ for nitrating *m*-nitroacetanilide is undesirable because of the resinous nature of the crude reaction product. Work in this laboratory has confirmed this observation. However, in the procedure described above, if the reaction mixture is not properly poured over ice, the product may separate in the form of lumps containing occluded acids. Such material cannot be washed properly, and when heated with solvents undergoes darkening and resinification.

Hydrolysis of Dinitroacetanilides.—This was accomplished essentially by the method described by Holleman and ter Weel⁷ for 2,3- and 2,5-dinitroacetanilide. 2,3-Dinitroaniline, m. p. 125–126°, and 2,5-dinitroaniline, m. p. 138°, were obtained in respective yields of 60 and 94%. 3,4-Dinitroaniline resulted from heating a solution of 25 g. of the acetyl derivative in 65 cc. of concd. sulfuric acid at 115° for twenty minutes and pouring the resulting solution into 500 cc. of ice and water. The precipitate was washed with water and recrystallized from ethanol. By working up the mother liquor, a further crop was obtained: total yield 18.4 g. (90%); m. p. 155–156°.

2,4-Dinitroaniline.—Eastman Kodak Co. material, "M.P. 175–176°" was used.

3,5-Dinitroaniline.—This substance was prepared from 3,5-dinitrobenzoyl chloride via the azide.¹⁹ The yield of product recrystallized from methanol and melting at 161.5–162.5° was 58% (based on the acyl halide). This synthesis was found much more convenient than the partial reduction of 1,3,5-trinitrobenzene.²⁰

2,6-Dinitroaniline.—Chlorobenzene was sulfonated and nitrated in one step.²¹ The resulting 2,6-dinitrochlorobenzene-4-sulfonic acid was warmed with ammonium

(17) Macciotta, *Ann. chim. applicata*, **29**, 81 (1939).

(18) Wender, *Gazz. chim. ital.*, **19**, 225 (1889).

(19) Blanksma and Verberg, *Rec. trav. chim.*, **53**, 988 (1934).

(20) Flürscheim, *J. prakt. Chem.*, **71**, 537 (1905).

(21) Lindemann and Wessel, *Ber.*, **58**, 122 (1925).

hydroxide²² to form the ammonium salt of 2,6-dinitroaniline-4-sulfonic acid. Desulfonation of this substance by refluxing three hours with 60% sulfuric acid gave the desired 2,6-dinitroaniline. For each gram of final product, melting at 138.5–139°, 10 g. of chlorobenzene was required.

Nitrosylsulfuric Acid.—Three hundred cc. of concentrated sulfuric acid was cooled to 0°, and 23.4 g. of finely powdered 97% sodium nitrite added slowly while the suspension was mechanically stirred. The suspended matter dissolved as the mixture was occasionally agitated while being allowed to warm to room temperature. The resulting solution had a density of 1.86, and each cc. was equivalent to 0.0733 g. of sodium nitrite.

Diazotization and Sandmeyer Reaction.—The dinitroaniline (9.0 g.) was dissolved in 45 cc. of concentrated sulfuric acid and the solution cooled to –5°. Forty-seven cc. of nitrosylsulfuric acid solution was added by means of a pipet while the solution was mechanically stirred. The temperature rose to 5–10° during the addition. After the temperature had fallen to –5°, 180 cc. of 85% phosphoric acid was added dropwise, with stirring. The temperature rise was pronounced during addition of the first 60 cc. of this reagent, and very slow addition was necessary to maintain a temperature of –2 to +2°. The entire addition required about ninety minutes, after which the mixture was stirred an additional hour at 0°. The mixture was then poured in a thin stream and with stirring onto 300 g. of cracked ice. All resulting diazonium solutions were clear at this point with the exception of that from 2,6-dinitroaniline. In this case, a small amount of whitish suspended matter was present.

Cuprous chloride was prepared from 4.4 g. of sodium hydroxide, 6.1 g. of reagent Na₂S₂O₆, 31.6 g. of copper sulfate and 8.2 g. of sodium chloride by the procedure described in "Organic Syntheses."²³ The product was dissolved in 300 cc. of concentrated hydrochloric acid, the solution cooled to 10°, and the cold diazonium solution slowly added to it with stirring. Precipitate formation and vigorous evolution of nitrogen occurred at this point. After standing at 10° for fifteen minutes, the mixture was diluted with 500 cc. of water, heated to 80°, cooled and extracted with benzene. Emulsions which formed at this point were eliminated by filtering off suspended matter. The red-brown benzene extract was concentrated to 90 cc. and extracted with three 5-cc. portions of concentrated sul-

furic acid. This treatment removed considerable colored matter from the benzene solution which, after washing with water, was evaporated to partial solidification and the residue dissolved in 20–25 cc. of benzene. Addition of 20–35 cc. of petroleum ether (E. and A., "acc. to A.O.C.S. and N.C.P.A.") caused turbidity or precipitation, and the mixture was allowed to stand about thirty minutes to permit further deposition. Of the six isomers, only 2,6-dinitrochlorobenzene precipitated in preference to other substances at this point, and in this case more benzene was added until the system was homogeneous. Under a pressure of ca. 70 mm., the supernatant liquid was forced through a column of activated alumina ("according to Brockmann") 51 × 0.9 cm. Rinsings and pure solvent mixture were passed through the column, and the effluent collected in convenient fractions until the properties of the residue obtained therefrom indicated the presence of extraneous matter. The chromatograms were characterized by several highly colored bands in the top 2 cm. of column, and broader bands below this region. The lower region of the column, temporarily containing a dinitrochlorobenzene, was light gray in the case of the 3,5-isomer, and varying shades of yellow with the other isomers.

TABLE I

Dinitrochlorobenzene	Yield, %	M. p. residue, °C.	M. p. recrystallized residue, °C.
2,4-	66	51–52	51.5–52
3,4-	77	40.5–41	41
2,5-	65	59.5–60	59.5–60
3,5-	71	54–54.5	54.5–55
2,3-	75	73–75	75.5–76
2,6-	63	86–87	87–87.5

With the exception of 2,6-dinitrochlorobenzene, Table I lists the yields obtained by this process, and compares the melting points of residues corresponding to such yields with melting points of material recrystallized from methanol. The residue of the 2,6-isomer amounted to 7.7 g. and melted at 65–82°. It was dissolved in 15 cc. of warm benzene, and 45 cc. of petroleum ether was added. After cooling, there resulted 6.3 g. of the product listed in the table.

Summary

A method has been described for obtaining improved yields of purified dinitrochlorobenzenes from the corresponding dinitroanilines.

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(22) Ullmann and Kuhn, *Ann.*, **366**, 102 (1909).

(23) "Organic Syntheses," Coll. Vol. I, p. 163, John Wiley and Sons, Inc., New York, N. Y., 1932.