was added, barium carbonate was removed by filtration through Celite, and the solution was treated with Amberlite IR-120 exchange resin (acid form). After concentration, the solution was added to the top of a column with Dowex 1-X4 exchange resin (acetate form). A neutral fraction (1.35 g.) was eluted with water after which an acid fraction (0.80 g.) was removed with 30% aqueous acetic acid.

Characterization of the Acid Fraction.—This fraction, which contained only one compound, was esterified with methanolic hydrogen chloride in the usual way and then reduced with lithium aluminum hydride (1.5 g.) in dry tetrahydrofuran (50 ml.). The disaccharide obtained was hydrolyzed with *N*-sulfuric acid, yielding a sirup (400 mg.) which contained equimolar quantities of a di-*O*-methylxylose and a tri-*O*-methylglucose. The mixture was resolved by paper chromatography (solvent B).

The xylose derivative (110 mg.) was obtained as a colorless sirup, $[\alpha]_D$ +21° (*c* 0.5 in water). Its rates of movement on the paper chromatogram (solvents B and C) and on the electrophoretogram were the same as those of an authentic specimen of 3,4-di-*O*-methyl-D-xylose. The 3,4-di-*O*-methyl-*N*-phenyl-D-xylosylamine[16] had m.p. 118–119°. Its infrared diagram was identical with that of an authentic sample.

The glucose derivative (150 mg) had $[\alpha]_D$ +75° (*c* 0.5 in water). The 2,3,4-tri-*O*-methyl-*N*-phenyl-D-glucosylamine[17] had m.p. and mixed m.p. 143–144°. Its infrared diagram was the same as that of an authentic specimen.

Characterization of the Neutral Fraction.—The neutral fraction was hydrolyzed with *N*-sulfuric acid to give a colorless sirup (1.20 g.) which crystallized immediately, m.p. 87–90°.[18] Paper chromatography (solvents B and C) and paper electrophoresis indicated the presence of only one compound. The 2,3-di-*O*-methyl-D-xylose had $[\alpha]_D$ +22.3° (*c* 2.3 in water).

(16) J. K. N. Jones and L. E. Wise, *J. Chem. Soc.*, 3389 (1952).

(17) S. Peat, E. Schluchterer, and M. Stacey, *J. Chem. Soc.*, 581 (1939).

(18) J. K. Hamilton, E. V. Partlow, and N. S. Thompson, *Tappi*, **41**, 811 (1958).

Anal. Calcd. for $C_7H_{14}O_5$: OMe, 34.8. Found: OMe, 34.5.

The 2,3-di-*O*-methyl-*N*-phenyl-D-xylopyranosylamine[19] had m.p. and mixed m.p. 124–125°. Its infrared diagram was identical to that of an authentic specimen.

Anal. Calcd. for $C_{13}H_{19}O_4N$: OMe, 24.5. Found: OMe, 24.2.

Periodate Oxidation of the Aldohexaouronic Acid.—Aliquots (30–40 mg.) of the hexaouronic acid were dissolved in 0.05 *M* sodium metaperiodate (50 ml.) and the reaction was allowed to proceed in the dark at 30° for various lengths of time. The results, given as moles per mole of hexaouronic acid, were as follows:

Time, hr.	15	24	48	72	96
Periodate consumed	5.70	6.62	7.02	7.13	7.05

For estimation of formic acid production, the same amounts of the hexaouronic acid were dissolved in water (10 ml.) and neutralized with 0.01 *N* sodium hydroxide. Periodate solution (50 ml.) was added and the oxidation was carried out as before. Excess periodate was destroyed by addition of excess ethylene glycol and formic acid was determined by titration with 0.01 *N* sodium hydroxide. The following moles of formic acid were produced per mole of hexaouronic acid:

Time, hr.	24	48	72	96
Formic acid	2.00	2.04	2.27	2.67

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(19) I. Ehrenthal, M. C. Rafique, and F. Smith, *J. Am. Chem. Soc.*, **74**, 1341 (1952).

Studies on the Barks of the Family Salicaceae. V.[1] Grandidentatin, a New Glucoside from the Bark of *Populus grandidentata*

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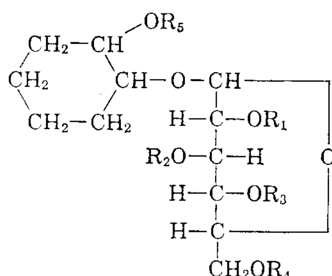
A new glucoside has been isolated from the bark of *Populus grandidentata*. This glucoside, which we have named grandidentatin, has been identified as *cis*-2-hydroxycyclohexyl 2-*O*-*p*-coumaroyl-β-D-glucopyranoside. Alkaline hydrolysis of grandidentatin yields *p*-coumaric acid, and the de-*p*-coumaroylated glucoside, grandidentin, which has been identified as *cis*-2-hydroxycyclohexyl β-D-glucopyranoside. Enzymatic hydrolysis of grandidentin with β-glucosidase yields glucose and *cis*-1,2-cyclohexanediol. Complete methylation of grandidentatin followed by complete hydrolysis with acid yielded 3,4,6-tri-*O*-methyl-D-glucose.

In the earlier preliminary evaluation of barks of several species of the genus *Populus*, a compound melting at 210–211°, which appeared to be a new glycoside, was obtained from the bark of *Populus grandidentata*, bigtooth aspen. This glycoside was obtained in very small yield by Craig machine fractionation of a fraction of hot water extractives which had been freed of most of its salicin, tremuloidin, and salireposide. This finding led to a more extensive investigation of the glucosides of *P. grandidentata* and an attempt to isolate a larger quantity of the new substance. The present paper reports the first studies on the reinvestigation of the bark of *P. grandidentata*. A thirty-four year old

(1) (a) For paper IV of this series, see I. A. Pearl, S. F. Darling, H. DeHaas, B. A. Loving, D. A. Scott, R. H. Turley, and R. E. Werth, *Tappi*, **44**, 475 (1961); (b) To be presented at the 141st National Meeting of the American Chemical Society, Washington, D. C., March 20–29, 1962.

bigtooth aspen was felled in northern Wisconsin in July, and both the brown broken up bark near the base of the tree and the greenish smooth bark higher up on the tree were collected separately and employed for these studies. The brown bark was extracted directly with ethanol, and the green bark was air-dried, Wiley milled, and used for hot-water extractions.

During the processing of the ethanol extractives in the same manner described previously for the extraction of tremuloidin from *Populus tremuloides*[2] crystals melting at 199–201° were obtained. The yield amounted to 0.03% based on the original ovendry *P. grandidentata* bark. Recrystallization of these crystals from water containing a little ethanol in the presence of decolorizing carbon yielded colorless needles melting at 201–202° and depressing a mixed melting point with tremuloidin. During the melting point determination of these colorless clear crystals, at about 140–150°, they became opaque and white without shrinking or melting. The new compound with specific rotation $[\alpha]^{30}_{D}$ −66.3° (c = 5.3 in methanol) was named "grandidentatin" (I).



I. R_1 = p-$HOC_6H_4CH{=}CHCO$; $R_2 = R_3 = R_4 = R_5$ = H
II. $R_1 = R_2 = R_3 = R_4 = R_5$ = H
III. R_1 = p-$CH_3COOC_6H_4{=}CHCO$; $R_2 = R_3 = R_4 = R_5 = CH_3CO$
IV. $R_1 = R_2 = R_3 = R_4 = R_5 = CH_3CO$
V. R_1 = p-$HOC_6H_4CH{=}CHCO$; $R_2 = R_3 = R_4 = R_5 = CH_3$
VI. $R_1 = R_2 = R_3 = R_4 = R_5 = CH_3$

Grandidentatin was also isolated from the smooth green bark of the same tree in 0.08% yield by hot-water extraction, purification, and ethyl acetate extraction as described in the earlier paper.[1] The ethyl acetate extract was evaporated to dryness and partitioned between equal volumes of ether and water. Concentration of the water layer yielded crystals of grandidentatin.

Hydrolysis of grandidentatin with barium hydroxide yielded p-coumaric acid and a new glucoside, "grandidentin" (II). Acid hydrolysis yielded p-coumaric acid, glucose, and an aglucone which has been identified as *cis*-1,2-cyclohexanediol. Acetylation of grandidentatin gave a crystalline pentaacetate (III). Although the de-p-coumaroylated grandidentatin, grandidentin (II), could not be obtained crystalline, it gave a crystalline acetate (IV) when acetylated with acetic anhydride and pyridine.

The infrared absorption spectrum of grandidentin indicated no aromatic bands, and hydrolysis of grandidentin with β-glucosidase yielded glucose and an optically inactive aglucone which gave no color reactions characteristic of phenolic compounds. The aglucone was identified as *cis*-1,2-cyclohexanediol by analysis, mixed melting point, and identity of infrared absorption spectrum with that of authentic material. Although *cis*-1,2-cyclohexanediol has been synthesized, it has not been isolated previously from combination in a plant product. It should be noted, however, that the crystals of *cis*-1,2-cyclohexanediol found as the aglucone of grandidentatin in this study were identical with crystals found in an earlier study of *Populus trichocarpa* in which the compound was found in its uncombined form.[1] It is interesting to note that very recently Valenta, Khaleque, and Rashid[3] found *cis*-1,2-cyclohexanediol in the scent glands of the Canadian beaver. These authors speculated that this compound was probably produced by the beaver by the reduction of pyrocatechol or its derivatives, compounds known to be present in beaver glands. Inasmuch as aspen bark is one of the important foods of the beaver, the presence of *cis*-cyclohexane-1,2-diol in this bark both *per se* and in combination appears to be a more likely explanation for its occurrence in the scent glands of the beaver.

In an attempt to determine the location of the p-coumaroyl group in grandidentatin, the compound was submitted to the methylation procedure employed earlier for the location of the benzoyl substitution in tremuloidin.[2] After complete methylation by repeated treatment with methyl iodide and silver oxide, the product was hydrolyzed to yield predominantly a tri-O-methylglucose. The fact that a tri-O-methylglucose was obtained instead of a tetra-O-methylglucose indicates that one of the hydroxyl groups of the glucose was probably substituted by the p-coumaroyl group in the original grandidentatin. This is strong evidence that the p-coumaroyl group in the original grandidentatin is present on the glucose moiety and not on the aglucone. Paper chromatography and comparison with known tri-O-methyl-D-glucoses indicated that the methylated glucose obtained by hydrolysis of methylated grandidentatin was probably 3,4,6-tri-O-methyl-D-glucose. The tri-O-methyl-D-glucose was isolated from a large-scale paper chromatographic experiment, recrystallized, and identified as 3,4,6-tri-O-methyl-D-glucose by mixed melting point and identity of infrared absorption spectrum with that of authentic material. Thus, the p-coumaroyl substitution of grandidentatin is on the 2-O-position of the glucose. The pres-

(2) I. A. Pearl and S. F. Darling, *J. Org. Chem.*, **24**, 731 (1959).

(3) Z. Valenta, A. Khaleque, and M. H. Rashid, *Experientia*, 130 (1961).

ent finding of a glucoside with *p*-coumaroyl substitution on the glucose moiety apparently is the first such compound reported from a plant species.

The change in physical condition during melting noted above for grandidentatin crystals indicated the presence of moisture, and moisture determination indicated one mole of water of hydration. Grandidentatin has been identified as *cis*-2-hydroxycyclohexyl 2-*O*-*p*-coumaroyl-β-D-glucopyranoside and grandidentin as *cis*-2-hydroxycyclohexyl β-D-glucopyranoside.

Experimental[4]

Isolation of Grandidentatin (I).—The concentrated ethanol extract of 8500 g. (ovendry basis) of brown bark from a 34-yr. old *Populus grandidentata* tree felled in northern Wisconsin on July 8, 1958, was evaporated to dryness in a rotating evaporator under diminished pressure to leave 274 g. of residue containing considerable oily material. The residue was boiled with 7 l. of water, cooled, and filtered through a Celite pad. The clear filtrate was treated with an excess of basic lead acetate and filtered. The filtrate was saturated with hydrogen sulfide, boiled to remove the excess, and filtered hot. The almost colorless filtrate was concentrated in a circulating evaporator under reduced pressure to 1000 ml. and allowed to stand and cool. The crystals which separated were filtered and washed with boiling water to yield 2.5 g. (0.03% on original ovendry brown bark basis) of grandidentatin melting at 199–200°.

The green bark from the same tree (3000-g. ovendry basis) was extracted directly with hot water by the general glucoside extraction procedure described earlier,[1] and the purified aqueous extract was concentrated to 2000 ml. The concentrated aqueous solution was extracted exhaustively with ethyl acetate, and the ethyl acetate extract was evaporated to dryness in a rotating vacuum evaporator. The viscous residue was covered with 1000 ml. of water and shaken with 1000 ml. of ether. The aqueous layer was removed and concentrated to 250 ml. and allowed to cool. The crystals which separated were filtered and washed with boiling water to yield 2.1 g. of grandidentatin melting at 199–201°. The yield from this green bark amounted to 0.07% on the ovendry basis.

More recent studies have indicated that the simplest procedure for the isolation of grandidentatin from green smooth bark of *P. grandidentata* is to extract the bark directly with hot water, process the water extract in the usual manner, and concentrate the purified aqueous extract in a circulating vacuum evaporator at 20–25°. Under these conditions, the grandidentatin separates during concentration and may be filtered in quite pure form directly from the concentrated aqueous solution. This procedure yields grandidentatin in substantially the same amounts as the ethyl acetate extraction procedure described, but with much less manipulative effort.

The crude grandidentatin was recrystallized from water containing a little ethanol in the presence of a little decolorizing carbon to give colorless prisms melting at 201–202° and having a specific rotation in methanol of $[\alpha]^{30}D$ −66.3 (*c*, 5.3). During the melting point determination, the colorless transparent needles became opaque and white at about 140–150° without shrinking. The infrared absorption curve contained bands at 2.95, 3.13, 3.43, 5.83, 6.21, 6.59, 6.89, 7.25, 7.52, 7.97, 8.54, 9.30, 9.65, 10.0, 10.15, 10.90, 11.20, and 11.95 μ. Grandidentatin on paper chromatograms, when sprayed with diazotized *p*-nitroaniline followed by sodium carbonate, appeared as light violet spots with strong blue-white fluorescence under ultraviolet light.

Anal. Calcd. for $C_{21}H_{28}O_9.H_2O$: C, 57.00; H, 6.83. Found: C, 56.87, 57.08; H, 6.74, 6.86.

Acetylation of grandidentatin with acetic anhydride and pyridine and recrystallization from ethanol yielded crystals of grandidentatin pentaacetate (III) melting at 167–169°, $[\alpha]^{30}D$ −8.37° (*c*, 3.64 in chloroform). The infrared absorption spectrum contained bands at 2.95, 3.42, 5.75, 6.10, 6.24, 6.62, 6.99, 7.30, 7.63, broad 8.00–8.32, 8.60, 9.19, broad 9.50–9.65, 10.15, 10.40, 11.02, and 11.96 μ.

Anal. Calcd. for $C_{31}H_{38}O_{14}$: C, 58.67; H, 6.04. Found: C, 58.58, 58.79; H, 5.85, 5.92.

Alkaline Hydrolysis of Grandidentatin.—To a mixture of 20 ml. of clear saturated barium hydroxide solution and 100 ml. of water was added 1.0 g. of grandidentatin. The yellow mixture was heated to boiling with constant swirling, and, just at the boiling point, a clear, yellow solution was formed. The solution was boiled 12 min., and the resulting light yellow solution was cooled to room temperature and exactly neutralized with 1 *N* sulfuric acid. Barium sulfate was removed by centrifugation, and the precipitate was washed three times. The colorless filtrate and washings were passed through a regenerated column of Amberlite IR-120 cation-exchange resin to yield a bitter tasting clear solution free of barium. This was extracted thoroughly with ether and then evaporated to dryness in a rotating vacuum evaporator to give grandidentin (II) as an almost colorless oil which would not crystallize. The infrared absorption curve of grandidentin had bands at 2.95, 3.43, 6.12, 6.90, 7.10, 7.21, 8.35, 8.60, broad 9.30–9.80, 10.15, 10.52, 11.28, 11.74, and 12.20 μ. Grandidentin on paper chromatograms gave no colors with diazotized spray reagents and was found by spraying with the modified silver spray.[2]

Grandidentin was acetylated in the usual manner with acetic anhydride in pyridine, and the product was recrystallized from dilute ethanol to yield glistening needles of grandidentin pentaacetate (IV) melting at 121–122° and having a specific rotation $[\alpha]^{30}D$ −58.2° (*c*, 2.2 in chloroform). Its infrared absorption curve contained bands at 2.95, 3.40, 5.71, 6.15, 6.96, 7.29, broad 8.00–8.20, 8.52, 9.18, 9.50, 9.65, 10.20, 10.42, 11.05, and 14.14 μ.

Anal. Calcd. for $C_{22}H_{32}O_{12}$: C, 54.09; H, 6.60. Found: C, 53.90, 53.68; H, 6.55, 6.41.

The Amberlite IR-120 column employed above for the removal of barium was eluted with 2% sodium hydroxide solution, and the alkaline eluate was acidified with 6 *N* sulfuric acid and extracted with ether. The ether extract was combined with the ether extract of the original deionized hydrolyzate, and the combined extract was evaporated to dryness in a rotating vacuum evaporator to yield 0.307 g. of *p*-coumaric acid melting at 213–214° and not depressing a mixed melting point with authentic *p*-coumaric acid. The recovered *p*-coumaric acid amounted to 83%.

Enzymatic Hydrolysis of Grandidentin (II) and Isolation of Grandidentol and Glucose.—The grandidentin obtained above was dissolved in 70 ml. of 0.01 *M* sodium acetate buffered with acetic acid to pH 5 and containing 0.13 g. of β-glucosidase[5] and allowed to stand at 37° for 16 hr. The mixture was concentrated almost to dryness in a rotating vacuum evaporator, and the residue was extracted twice with ether. The ether was dried and evaporated under reduced pressure to leave a colorless crystalline residue. The residue was recrystallized by dissolving in a small amount of dry ether, filtering, treating with a little petroleum ether (b.p. 30–60°), and allowed to stand in a refrigerator. The aglucone, grandidentol, was obtained as colorless transparent square plates melting at 94–95° and not depressing a mixed melting point with authentic *cis*-1,2-

(4) All melting points are uncorrected. Analyses were performed by Huffman Microanalytical Laboratories, Wheatridge, Colo. and Geller Microanalytical Laboratories, Bardonia, N. Y. Infrared absorption spectra were determined by Mr. Lowell Sell of The Institute of Paper Chemistry Analytical Department.

(5) Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

cyclohexanediol.[6] Grandidentol sublimes without decomposition to yield the same square plates. The infrared absorption spectrum of grandidentol is identical with that of authentic *cis*-1,2-cyclohexanediol.

Anal. Calcd. for $C_6H_{12}O_2$: C, 62.04; H, 10.41. Found: C, 61.95, 62.13; H, 10.01, 10.19.

In an identical experiment performed on 0.40 g. of grandidentatin, the sirupy residue left after ether extraction of the grandidentol was extracted twice with 25-ml. portions of boiling methanol. The methanol solution was filtered to remove suspended β-glucosidase and evaporated to dryness in a rotating evaporator to leave a colorless sirup. The sirup was covered with 3.0 ml. of methanolic hydrochloric acid and treated with 0.13 g. of *p*-nitroaniline and 2 microdrops of concentrated hydrochloric acid. The mixture was boiled under reflux for 15 min. and allowed to stand a few minutes. The fine yellow needles which separated were filtered and recrystallized twice from methanol to give yellow needles melting at 184–186° and not depressing the melting point of a mixture with authentic *N*-(*p*-nitrophenyl)-α-D-glucopyranosylamine prepared from authentic D-glucose by the procedure of Weygand, Parkow, and Kuhner.[7] The infrared absorption spectrum, containing bands at 3.00, 3.45, 6.23, 6.63, 6.72, 7.02, 7.48, 7.61, 7.82, 8.38, 8.55, 9.00, 9.24, 9.81, 10.28, 11.23, 11.55, 11.91, 13.30, and 14.45 μ, was identical with that of the authentic product.

Methylation of Grandidentatin.—Grandidentatin (3.0 g.) was methylated by means of methyl iodide and silver oxide by the method and in the apparatus described earlier for the methylation of tremuloidin.[2] The course of the methylation was followed by infrared spectral examination. Eight methylations were required before the hydroxyl band at 3 μ disappeared from the infrared absorption spectrum of the methylated product, thus assuring complete methylation of grandidentatin to V. During the course of this extended methylation, the silver oxide caused some hydrolysis of *p*-coumaroyl groups with the formation of a small amount of pentamethylgrandidentin (VI). The presence of VI was confirmed by acid hydrolysis of a sample of the completely methylated product and the finding of tetramethylglucose in the hydrolyzate by paper chromatography.

Alkaline Hydrolysis of Methylated Grandidentatin.—A solution of the entire methylation product (about 3 g.) in 60 ml. of dry methanol was treated with a solution of 0.3 g. of sodium in 30 ml. of dry methanol. The yellow solution was boiled under reflux for 10 min., cooled, and diluted with 90 ml. of water. The methanol was removed by evaporation under reduced pressure in a rotating evaporator, and a voluminous crystalline precipitate separated. The precipitate was filtered, washed with water, and recrystallized from dilute methanol to yield fine white needles of methyl *p*-methoxycinnamate melting at 86–88° and not depressing the melting point of a mixture with authentic material.[8] Its infrared absorption spectrum, containing bands at 2.90, 5.81, 6.09, 6.21, 6.33, 6.59, 6.96, 7.50, 7.75, 7.96, 8.30, 8.39, 8.55, 9.00, 9.75, 9.87, 10.15, 10.40, 10.72, 11.54, 11.92, 12.15, and 13.00 μ, was identical with that of the authentic compound.

The alkaline aqueous filtrate was acidified with dilute hydrochloric acid. The precipitate was filtered and recrystallized from dilute ethanol in the presence of decolorizing carbon to yield colorless needles of *p*-methoxycinnamic acid melting at 169–170° to an opaque liquid which became clear at 182° and identical with authentic material prepared from authentic methyl *p*-methoxycinnamate[8] by alkaline hydrolysis. A mixed melting point was not depressed. The infrared absorption spectrum contained bands at 2.90, 3.90, 5.91, 6.14, 6.24, 6.59, 6.90, 6.99, 7.60, 7.75, 7.97, 8.20, 8.40, 8.54, 8.95, 9.72, 10.25, 10.69, 11.60, 12.10, 12.90, and 14.61 μ. *p*-Methoxycinnamic acid fluoresces strongly blue under ultraviolet light.

The slightly acid aqueous filtrate was freed of hydrochloric acid with Amberlite IR-4B in the acetate form, and the resulting solution was evaporated to dryness in a vacuum rotating evaporator. The residue was extracted thoroughly with anhydrous ether, and the ether was evaporated to leave the de-*p*-coumaroylated completely methylated grandidentatin as a colorless viscous sirup. A sample of this sirup (0.70 g.) was dissolved in 5 ml. of *N* hydrochloric acid and heated on the steam bath for 4 hr. An aliquot of the hydrolyzate containing approximately 250 mg. of solids was streaked on five 8-in. wide Whatman 3 *M* papers, previously washed with ethyl acetate–acetic acid–water (9:2:2). The papers were developed with the same solvent. One-fourth inch strips were cut from each paper for monitoring, and the located bands of trimethylglucose were cut from the papers and separated from the trace of dimethylglucose and small amount of tetramethylglucose. The bands containing trimethylglucose were eluted with ethanol in a Soxhlet apparatus, and the ethanolic eluate was evaporated to leave a colorless sirup which crystallized after standing several weeks. The crystalline material was recrystallized from a small amount of purified diisopropyl ether to yield fine colorless needles melting at 91–92° which did not depress the melting point of a mixture with authentic 3,4,6-tri-*O*-methyl-D-glucopyranoside[9] and whose infrared absorption spectrum, containing bands at 3.01, 3.46, 6.14, 6.88, 7.28, 7.44, 7.60, 7.89, 8.01, 8.40, 8.90, 9.04, 9.33, 9.46, 9.70, 10.47, 10.66, and 11.01 μ, was identical with that of the same trimethylglucose.

Infrared Spectra.—Infrared absorption spectra were obtained with a Perkin-Elmer Model 21 recording spectrophotometer using a sodium chloride prism and potassium bromide pellets prepared by hand grinding with sample before pressing.

(6) N. A. Milas and S. Sussman, *J. Am. Chem. Soc.*, **59**, 2345 (1937).

(7) F. Weygand, W. Parkow, and P. Kuhner, *Chem. Ber.*, **84**, 594 (1951).

(8) W. Will, *Ber.*, **20**, 294 (1887).

(9) Kindly supplied by Dr. N. K. Richtmyer, National Institutes of Health, Bethesda, Md.