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2-Deoxy-D-ribose. VII.[1] Crystalline 2-Deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl Chloride and Related Derivatives

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The two anomeric methyl 2-deoxy-D-ribofuranoside di-*p*-nitrobenzoates (II and III) have been obtained in crystalline form. Cleavage of the methoxyl group from these glycosides with hydrogen chloride serves as a practical means of preparing a new crystalline halide, 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride (IV). With silver *p*-nitrobenzoate this halide gives the previously reported 2-deoxy-β-D-ribofuranose tri-*p*-nitrobenzoate (V) in moderate yield; with methanol it is rapidly converted to methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-β-D-riboside (III) in high yield. Hydrolysis affords a crystalline 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribose (VI) which can also be made by the hydrolysis of two new glycosides, the anomeric isopropyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosides (VIII and IX).

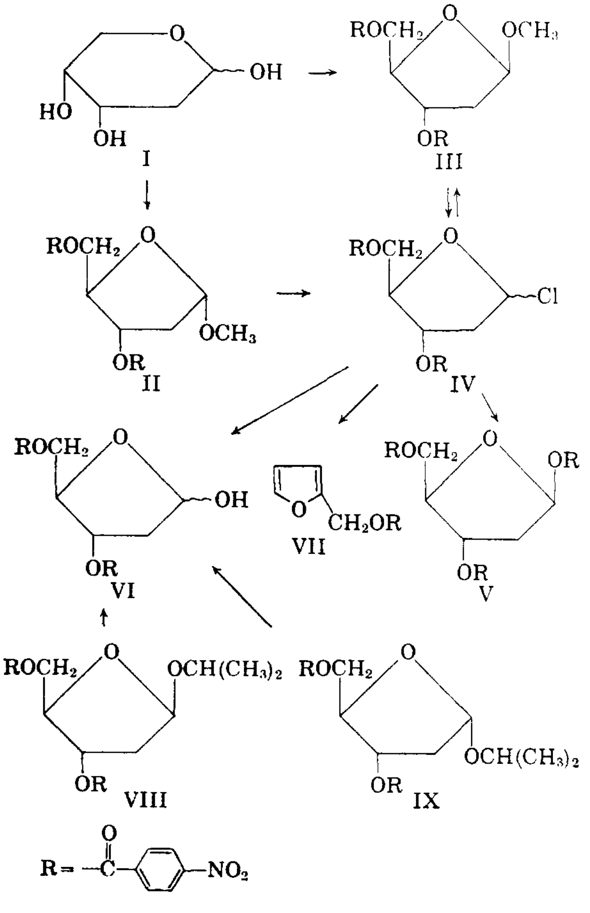
At its melting point, the chloride (IV) evolves hydrogen chloride and *p*-nitrobenzoic acid to afford furfuryl *p*-nitrobenzoate (VII).

In recent communications[2] we have described the preparation of the two anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates from a 2-deoxy-D-ribose mercaptal and the use of these esters for the synthesis of three 2′-deoxy-D-ribofuranosyladenines. An intermediate in these syntheses was 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride; in the earlier work no attempt was made to isolate this substance. We now wish to report that a comparatively simple process has allowed us to obtain this halide in crystalline form and to study some of its properties more closely.

The acid-catalyzed condensation of reducing sugars with simple alcohols to form alkyl or aralkyl glycofuranosides has long been used for the synthesis of a variety of glycofuranose derivatives. It has repeatedly been shown that acylated glycofuranosides may be cleaved with hydrogen halides in glacial acetic acid to afford acylated glycofuranosyl halides[3]; this process has recently been employed to obtain such products in crystalline form, notably in the D-arabinofuranose series[4] and in the 2-deoxy-D-ribofuranose series.[5]

In the course of the present work, crude methyl 2-deoxy-D-ribofuranoside (readily preparable from the free sugar, I) was *p*-nitrobenzoylated to yield two crystalline anomeric methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosides in a total yield of 91%. One of these isomers proved to be dextrorotatory ($[\alpha]^{20}_{\mathrm{D}}$ +115° in chloroform) while the other was weakly levorotatory ($[\alpha]^{20}_{\mathrm{D}}$ −5.4° in chloroform); in accord with the usual convention, the α-structure II will tentatively be assigned to the dextrorotatory and the β-structure III to the levorotatory isomer.

The behavior of each of the two anomers with hydrogen chloride in glacial acetic acid was studied polarimetrically. With 1.6 molar equivalents of hydrogen chloride at 20°, each glycoside mutarotated rapidly to nearly the same final rotation. With a much larger excess of hydrogen chloride, the two glycosides again behaved alike but the end



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[1] 2-Deoxy-D-ribose. VI. H. B. Wood, Jr., and H. G. Fletcher, Jr., *J. Org. Chem.*, **26**, 1969 (1961).

[2] R. K. Ness and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **81**, 4752 (1959); **82**, 3434 (1960).

[3] *Cf.* H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **75**, 2624 (1953); R. K. Ness, H. W. Diehl, and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **76**, 763 (1954).

[4] R. K. Ness and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, **80**, 2007 (1958).

[5] M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959); M. Hoffer, *Chem. Ber.*, **93**, 2777 (1960).

rotation was somewhat more dextrorotatory. In view of these observations, subsequent studies were undertaken with mixtures of II and III and a crystalline 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride (IV) obtained in 76 to 86% yield. A relatively simple process for making this halide from 2-deoxy-D-ribose (I) is described in the experimental part of this paper. The new halide has a specific rotation of +112° in methylene chloride; however, in view of the even higher rotation of what is presumed to be 2,6-dideoxy-3,4-di-*O*-*p*-nitrobenzoyl-β-D-*ribo*-hexosyl chloride[6] and other rotatory anomalies associated with certain derivatives of the 2-deoxyaldoses,[7] further evidence seems required here before assignment of an anomeric configuration to this halide.

While the pure halide could be stored at room temperature over soda lime and phosphorus pentoxide for some weeks without significant decomposition, the substance proved, as might be expected, a highly reactive one. With silver *p*-nitrobenzoate it gave 2-deoxy-1,3,5-tri-*O*-*p*-nitrobenzoyl-β-D-ribofuranose (V)[2] in 44% yield; a small amount of more dextrorotatory material, closely resembling the anomeric ester, was also obtained.

With methanol in the absence of an acid acceptor, the halide underwent solvolysis with great rapidity, giving methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-β-D-riboside (III) in 86% yield.

Hydrolysis of the halide afforded a crystalline, dextrorotatory 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribose (VI) which, on *p*-nitrobenzoylation afforded both of the anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates. The same di-*p*-nitrobenzoate (VI) was made by an alternative method. 2-Deoxy-D-ribose was treated with isopropyl alcohol and hydrogen chloride to give a mixture of glycosides which was subjected to the action of periodate to remove any pyranosides present and then *p*-nitrobenzoylated. Two anomeric isopropyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosides (VIII and IX) were isolated; the dextrorotatory one ($[\alpha]^{20}_{D}$ +109.9° in chloroform) is tentatively assigned structure IX while the levorotatory one ($[\alpha]^{20}_{D}$ −16.4° in chloroform) is assumed to correspond to structure VIII. Each of the anomers was found to hydrolyze in aqueous dioxane, 0.1N in sulfuric acid, to give a solution of the same rotation. Hydrolysis of a mixture of the two anomers gave 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribose (VI), identical with the ester obtained by the hydrolysis of the chloride (IV).

In Table I values of 2B, derived from three anomeric pairs of 2-deoxy-D-ribofuranose derivatives listed, are shown. The agreement among these three is normal for such comparisons and suggests in these cases that the assignment of anomeric configuration is at least consistent.

TABLE I

DERIVATIVES OF 2-DEOXY-3,5-DI-*O*-*p*-NITROBENZOYL-D-RIBOSE

Substituent and Configuration at C-1	$[\alpha]^{20}_{D}$ ($CHCl_3$)	$[M]^{20}_{D}$	2B
Methyl α	+115°	+51,300	48,900
Methyl β	−5.4°	−2,400	
Isopropyl α	+109.9°	+52,100	44,320
Isopropyl β	−16.4°	−7,780	
p-Nitrobenzoyl α	+70.7°[a]	+41,100	51,040
p-Nitrobenzoyl β	+17.1°[a]	+9,940	

[a] Ref. 2.

2-Deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride (IV) melts at 107–109°, hydrogen chloride being evolved and a higher-melting solid being precipitated. Quantitative investigation of this reaction showed a weight loss close to that expected for one mole of hydrogen chloride and led to the isolation of approximately one mole of *p*-nitrobenzoic acid. From the remainder of the material was isolated, in low yield, furfuryl *p*-nitrobenzoate[8] (VII). Presumably this product arose from one or two *cis* eliminations[9]; pyrolytic eliminations of this type have not, to our knowledge, been observed with acylated glycosyl halides before. However, some of the cardiac glycosides (2,6-dideoxyhexosides) have been found to give 6-deoxy-D-*ribo*-hexo-pyranos-1-ene (digitoxal) on pyrolysis,[10,11] and a nonpyrolytic dehydrohalogenation of a 2-deoxyglycopyranosyl halide has been observed.[6]

EXPERIMENTAL[12]

The anomeric methyl 2-deoxy-3,5-di-O-p-nitrobenzoyl-D-ribosides (II and III). A solution of 5.00 g. of 2-deoxy-D-ribose in 90 ml. of absolute methanol was treated with 10 ml. of 0.1% (w./v.) (0.0274N) hydrogen chloride in methanol and the rotation of the resulting reaction mixture observed polarimetrically at 20° (4-dm. tube). After *ca.* 2 hr. the rotation attained a maximum (α^{20}_{D} +7.70°) and the mixture was then neutralized with 1 g. of silver carbonate. The insoluble salts were removed by filtration with Celite and the filtrate concentrated *in vacuo* to a sirup which was treated with a suspension of 5.2 g. of sodium metaperiodate[13] in 200 ml. of phosphate buffer (0.1M, *p*H 7.0). The suspension (*p*H 6.4 to 6.8) was stirred for 18 hr. at room temperature and then concentrated *in vacuo* to dryness. Absolute

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[6] W. W. Zorbach and T. A. Payne, *J. Am. Chem. Soc.*, **82**, 4979 (1960).

[7] J. J. Fox and I. Wempen, *Advances in Carbohydrate Chem.*, **14**, 340 (1959); R. U. Lemieux, and M. Hoffer, *Can. J. Chem.*, **39**, 110 (1961).

[8] R. D. Kleene and S. Fried, *J. Am. Chem. Soc.*, **62**, 3516 (1940).

[9] *Cf.* C. H. DePuy and R. W. King, *Chem. Revs.*, **60**, 431 (1960).

[10] A. Windaus and G. Schwarte, *Nachr. Ges. Wiss. Göttingen. Math. Physik. Klasse*, 1 (1926); *Chem. Abstr.*, 21, 3618 (1927).

[11] F. Micheel, *Ber.*, **63**, 347 (1930).

[12] Melting points are corrected.

[13] In subsequent preparations where a mixture of the anomeric glycosides was employed for the preparation of the halide IV, it was found that this periodate oxidation, designed to remove traces of pyranosides, could be omitted.

ethanol (250 ml.) was evaporated *in vacuo* from the residue to remove water and the product extracted with warm benzene (10 × 50 ml.). The combined extracts were filtered and concentrated to dryness; the residue (4.76 g.) was dissolved in 50 ml. of water and the solution passed through an equal volume of Amberlite IRA-400-OH$^-$ to remove periodate oxidation products. The combined effluent and washings (450 ml.) were concentrated *in vacuo* (60–80° bath) to a sirup (4.00 g., 72%). A portion (0.9866 g.) of this sirup was dissolved in 5.0 ml. of dry pyridine and, after cooling, treated with 3.0 g. of *p*-nitrobenzoyl chloride. The reaction mixture was stirred at room temperature overnight and the excess acyl chloride then decomposed with a little water. Dissolved in methylene chloride, the crude product was washed successively with water, cold 3*N* sulfuric acid, and saturated aqueous sodium bicarbonate. Magnesium sulfate was used to dry the solution which was then concentrated *in vacuo* to a sirup which, from 125 ml. of absolute ethanol, afforded 1.26 g. (42% based on the crude glycoside) of crystalline methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-β-D-riboside, m.p. 138–141°. Recrystallization from 100 parts of absolute ethanol and then from 55 parts of benzene–pentane (1:1) gave the pure ester, melting at 143–144° and showing $[\alpha]^{20}_{D}$ −5.4° in chloroform (*c* 1.16).

Anal. Calcd. for $C_{20}H_{18}N_2O_{10}$ (446.36): C, 53.81; H, 4.06; N, 6.28. Found: C, 53.59; H, 4.09; N, 6.03.

The main filtrate from the above preparation was concentrated to dryness and the residue dissolved in a mixture of 100 ml. of ether and 50 ml. of pentane. The solution was seeded[14] and cooled to −5°, more pentane (100 ml.) being gradually added as crystallization progressed: 1.45 g., 49%, based on the crude methyl 2-deoxy-D-ribofuranoside. The combined, over-all yield from 2-deoxy-D-ribose was 66%. The crude α-ester (m.p. 80–*ca.* 88°) was recrystallized from ether–pentane and then from ether–hexane: m.p. 83–85°, $[\alpha]^{20}_{D}$ +115° in chloroform (*c* 1.84).

Anal. Calcd. for $C_{20}H_{18}N_2O_{10}$ (446.36): C, 53.81; H, 4.06; N, 6.28. Found: C, 53.62; H, 4.16; N, 6.14.

Behavior of the anomeric methyl 2-deoxy-3,5-di-O-p-nitrobenzoyl-D-ribosides (II *and* III) *with hydrogen chloride in glacial acetic acid.* Methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-α-D-riboside (II, 43.0 mg.) was dissolved in 0.50 ml. of glacial acetic acid in a 0.50-dm. polarimeter tube and the solution treated with 1.50 ml. of acetic acid containing 5.7 mg. (1.6 mole equivalents) of hydrogen chloride. Observed at 20°, the solution showed the following rotations: +1.19° (1.3 min.), +0.89° (22.5 min.), +0.71° (57 min.), and +0.61° (196 min.). Assuming complete conversion to the halide, the latter value corresponds to $[\alpha]^{20}_{D}$ *ca.* +56°.

Methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-β-D-riboside (III, 42.2 mg.) was treated in the same fashion to provide the following rotational values: −0.03° (1 min.), +0.26° (7.3 min.), +0.53° (67.5 min.), +0.56° (103 min.), +0.57° (139 min.), and +0.54° (270 min.). The value at 139 min. corresponds to $[\alpha]^{20}_{D}$ *ca.* +54° assuming complete conversion of the glycoside to the chloride.

Under strictly comparable conditions (2 ml. of solution in 0.5-dm. tube) but with 25 molar equivalents of hydrogen chloride, a sample (42.3 mg.) of the α-glycoside mutarotated: +0.88° (0.5 min.), +0.76° (2.5 min.), and +0.75° (17 min.). Assuming complete conversion of the glycoside to the chloride, the latter value corresponds to $[\alpha]^{20}_{D}$ *ca.* +70°. Similarly, the β-glycoside (42.2 mg.) with 25 molar equivalents of hydrogen chloride gave: +0.35° (0.5 min.), +0.71° (3 min.), +0.73° (4.3 min.), and +0.74° (7, 10 min.). Assuming complete conversion, the final value corresponds to $[\alpha]^{20}_{D}$ *ca.* +69°. Prepared as described below, pure 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride shows $[\alpha]^{20}_{D}$ +61° in glacial acetic acid (*c* 2.73).

2-Deoxy-3,5-di-O-p-nitrobenzoyl-D-ribosyl chloride (IV). For the preparation of comparatively large quantities of the mixed anomeric methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosides, suitable for conversion to the chloride (IV), the following procedure was developed.

A solution of 20.0 g. of 2-deoxy-D-ribose in 360 ml. of absolute methanol was treated with 2.75 ml. of 0.667*N* methanolic hydrogen chloride and the rotation of the resulting mixture observed at 20°. After 39 min. a maximum ($[\alpha]^{20}_{D}$ = +8.4°, 4 dm.) was attained and the reaction halted through the addition of 100 ml. of pyridine. The mixture was concentrated *in vacuo* (60° bath) to a sirup which was diluted with 50 ml. of pyridine and the solution reconcentrated in order to complete the removal of the methanol. The amorphous residue was dissolved in 120 ml. of dry pyridine, the solution cooled and, while being stirred vigorously, treated with 70 g. of *p*-nitrobenzoyl chloride, the halide being added over the course of 8 min. and the reaction temperature being held below 40°. After standing overnight at room temperature, the reaction mixture was cooled to 0° and treated with 5 ml. of water. Ten minutes later, more water (900 ml.) was added and the aqueous layer extracted with methylene chloride, the extract then being used to dissolve the precipitated sirup. The methylene chloride solution was washed successively with aqueous sodium bicarbonate, water, cold 3*N* sulfuric acid, and aqueous sodium bicarbonate. Removal of solvent *in vacuo* left a friable mass which was held at 0.2 mm. for 1 hr.: 66.7 g., quantitative.[15] The product was dissolved in one part of warm ethyl acetate and the solution diluted with two parts of warm absolute ethanol. After standing overnight at −5°, the crystalline material was removed and washed with 2:1 ethanol–ethyl acetate, followed by ethanol and then pentane. After drying, the mixture of anomeric methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosides weighed 51.8 g. (78%) and showed a rotation +43.3° in chloroform.

A vigorously stirred suspension of 5 g. of the above product in 30 ml. of glacial acetic acid was cooled in an ice bath while a brisk stream of anhydrous hydrogen chloride was passed in for a period of 3 min.[16] The rate of solution of the starting material depends upon the proportion of anomers present, the α-form dissolving more rapidly than the β. After *ca.* 6 min. (and sometimes before all of the starting material had completely dissolved) the product began to crystallize. After 20 min., 30 ml. of ether was added and, with continued cooling and stirring, hydrogen chloride was again rapidly added (1 min.). Ten minutes later, 30 ml. of pentane was added and, after 5 min., the mixture was brought to room temperature and filtered, the crystalline chloride being washed with ether and then with pentane. The following data are derived from a number of such preparations: weight, 3.83 to 4.32 g. (76–86%); m.p. (dec.) 105–107° (16° per min.); $[\alpha]^{20}_{D}$ +106–108° in dry methylene chloride. As made thus, the product is sufficiently pure for the majority of uses. Upon recrystallization from 10 parts of dry methylene chloride–pentane (1:1), pure 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride is obtained as fine needles melting with decomposition at 107–109° (in bath at 95°, 16° per min.) and showing $[\alpha]^{20}_{D}$ +112° in methylene chloride (*c* 0.82) which had been dried over Drierite.

Anal. Calcd. for $C_{19}H_{15}ClN_2O_9$ (450.79): C, 50.62; H, 3.35. Found: C. 50.75; H, 3.43.

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[14] Seeds of methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-α-D-riboside were first obtained by leaving a sample of the material in ethanolic solution for 3 days at −5°.

[15] This glassy solid was quite suitable for direct conversion into the glycosyl chloride. However, conversion of it into the crystalline mixture of anomers, as described here, proved to be desirable inasmuch as this facilitated the subsequent preparation of small batches of the glycosyl chloride as needed.

[16] Owing to the insolubility of III in ether, the conditions used by Hoffer *et al.* (ref. 5) for the preparation of some analogous chlorides proved unsuitable and were modified as described here. We are indebted to Dr. W. F. Scott of Hoffman-La Roche, Inc., for certain details before publication (ref. 5).

*Reaction of 2-deoxy-3,5-di-O-p-nitrobenzoyl-*D*-ribosyl chloride* (IV) *with silver p-nitrobenzoate.* The crystalline chloride (344.2 mg.) was added to a stirred suspension of 500 mg. of silver *p*-nitrobenzoate in 10 ml. of dimethylformamide. After 3 days the insoluble salts were removed by filtration and washed with methylene chloride. The combined filtrate and washings were concentrated under reduced pressure to remove the methylene chloride and the remaining dimethylformamide solution was then poured into 125 ml. of water. The solid formed was removed by filtration, dissolved in methylene chloride, and the solution dried with magnesium sulfate. After removal of the solvent under reduced pressure, the residue was dissolved in 5 ml. of hot butanone; while slowly cooling, crystallization occurred. Several days later the product was collected by filtration and washed with butanone followed by ethanol and by pentane: 196.7 mg. (44%), m.p. 125–133°, $[\alpha]^{20}_{D}$ +18.0° (*c* 0.36, chloroform). After solution of 155 mg. in hot benzene and seeding with the anhydrous form[2] of the β-tri-*p*-nitrobenzoate, both solvated and anhydrous forms appeared on cooling. Rewarming slightly for several minutes dissolved the solvated form which did not reappear on cooling while the anhydrous form remained: m.p. 173–175° (in bath at 160°, 8°/min.); $[\alpha]^{20}_{D}$ +16.8° (*c* 0.89, chloroform). From the original mother liquor, by the addition of pentane, additional fractions were obtained: fraction 2, 65.1 mg. (14.8%), $[\alpha]^{20}_{D}$ +54.3° (chloroform); fraction 3, 17.8 mg. (4.0%), $[\alpha]^{20}_{D}$ +44.1° (chloroform) and fraction 4, 27.7 mg. (6.3%), $[\alpha]^{20}_{D}$ +62.4° (chloroform). Pure 2-deoxy-α-D-ribofuranose tri-*p*-nitrobenzoate shows $[\alpha]^{20}_{D}$ +70.7° in chloroform.[2]

*Reaction of 2-deoxy-3,5-di-O-p-nitrobenzoyl-*D*-ribosyl chloride* (IV) *with methanol.* A solution of the chloride (48.0 mg.) in 10.0 ml. of dry methylene chloride showed a rotation corresponding to $[\alpha]^{20}_{D}$ +109°. Within 30 sec. after the addition of 10.0 ml. of absolute methanol mutarotation had ceased, the new rotation being equivalent to $[\alpha]^{20}_{D}$ +1.7° on the assumption of complete conversion to the methyl glycoside. Concentration of the reaction mixture *in vacuo* afforded a crystalline residue which was washed with absolute ethanol: 41.0 mg. (86%), m.p. 141–143°, $[\alpha]^{20}_{D}$ −3.0° (chloroform, *c* 0.90). Mixed with authentic methyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-β-D-riboside, it melted at 141–144°.

*The anomeric isopropyl 2-deoxy-3,5-di-O-p-nitrobenzoyl-*D*-ribosides* (VIII and IX). Ten grams of 2-deoxy-D-ribose was dissolved in 180 ml. of isopropyl alcohol which had been dried over Drierite. The solution was left overnight to allow mutarotation and then 20 ml. of 1% (w./v.) hydrogen chloride in isopropyl alcohol was added. The optical rotation of the solution rose rapidly, reaching a maximum in 20 min.; at the end of 30 min. the acid was neutralized through the addition of 4 g. of silver carbonate. After stirring for 1.5 hr. the suspension was filtered through a bed of Celite and concentrated in *vacuo* to give 12.78 g. of sirup. Any residual sugar and any pyranoside were removed by dissolving the sirup in 400 ml. of 0.1*M* phosphate buffer (*p*H 7.0) and adding 100 ml. of 0.5*M* aqueous sodium metaperiodate. After standing overnight, the solution was concentrated *in vacuo* and the residue dried by evaporating (*in vacuo*) 300 ml. of absolute alcohol therefrom. When thoroughly dried, the residue was extracted with warm benzene (5 × 100 ml.). The combined extracts were filtered and then concentrated to yield a yellow sirup (12.13 g.). This was dissolved in 150 ml. of water and the solution passed through a column (2.8 × 16 cm.) of Amberlite IRA-400-OH$^-$. The solution and washings were then concentrated *in vacuo* (40° bath) and the sirupy residue dried by evaporating absolute alcohol therefrom: 10.63 g. (81%). The sirup was dissolved in 50 ml. of dry pyridine and the pyridine removed *in vacuo*. It was then dissolved in 250 ml. of dry pyridine and the solution cooled to 0°. *p*-Nitrobenzoyl chloride (33.7 g., 3 molar equivalents) was added and the mixture left in an ice bath for 1 hr. After standing at room temperature overnight it was cooled to 0° and treated with 3 ml. of water. After 0.5 hr., the mixture was diluted with 1000 ml. of methylene chloride and washed successively with water (3 × 2000 ml.), 2*N* sulfuric acid (2 × 1000 ml.), saturated aqueous sodium bicarbonate (2 × 1000 ml.) and, finally, water (2000 ml.). Moisture was removed with sodium sulfate, the solution filtered through decolorizing carbon, and then concentrated *in vacuo* to a yellow sirup (27.85 g.). Attempts to obtain crystals from this product directly were unsuccessful. It was therefore dissolved in 200 ml. of cyclohexane–benzene (1:1) and the solution passed through a column (3.8 × 26 cm.) of alumina (Woelm, neutral, Grade II, 250 g., under cyclohexane). The column was washed with 1500 ml. of benzene and the combined effluents were concentrated *in vacuo* to give 23.85 g. of crystalline product. This was dissolved in 40 parts of ether, an equal volume of pentane added and the solution seeded. After 24 hr. at room temperature and a further day at −5° the product was removed: 18.12 g. On concentration the mother liquor yielded 4.24 g. more material. These two crops, consisting of the two anomeric isopropyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosides, were combined: 22.36 g., 63%, based on 2-deoxy-D-ribose. Separation of the two anomers was achieved through the differences between their rates of solution in dry ether. The compact needles of the α-anomer were less rapidly dissolved by ether; recrystallization from ether–pentane and then from methanol (30 parts) gave pure *isopropyl 2-deoxy-3,5-di-O-p-nitrobenzoyl-α-*D*-riboside* (IX), melting at 111–112°, $[\alpha]^{20}_{D}$ +109.9° (*c* 1, chloroform).

Anal. Calcd. for $C_{22}H_{22}N_2O_{10}$ (474.42): C, 55.69; H, 4.68; N, 5.91. Found: C, 55.82; H, 4.91; N, 5.64.

The fine needles of the β-anomer dissolved more rapidly in dry ether and were recovered from solution by the addition of an equal volume of pentane. Recrystallized from ether–pentane and then from methanol (20 parts), the *isopropyl 2-deoxy-3,5-di-O-p-nitrobenzoyl-β-*D*-riboside* (VIII) melted at 103–104° and showed $[\alpha]^{20}_{D}$ −16.4° in chloroform (*c* 1.4).

Anal. Calcd. for $C_{22}H_{22}N_2O_{10}$ (474.42): C, 55.69; H, 4.68; N, 5.91. Found: C, 55.99; H, 4.87; N, 5.90.

*2-Deoxy-3,5-di-O-p-nitrobenzoyl-*D*-ribose* (VI) (a) *from isopropyl 2-deoxy-3,5-di-O-p-nitrobenzoyl-*D*-ribosides* (VIII and IX). Aqueous dioxane (80% dioxane), 0.1*N* in sulfuric acid, was used at 50° to hydrolyze the glycosides. In preliminary experiments it was found that each of the anomeric glycosides (VIII and IX) attained a rotation of $[\alpha]^{20}_{D}$ *ca.* 55°[17] after about 20 hr. under these conditions. Prolonged hydrolysis (2 days) was found to lead to the liberation of *p*-nitrobenzoic acid.

A mixture (10.00 g.) of the two anomeric isopropyl 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosides was dissolved in 200 ml. of dioxane; to this solution was added 25 ml. of water and 25 ml. of *N* sulfuric acid. The mixture was heated for 20 hr. at 50°, cooled, and treated with 500 ml. of methylene chloride. The solution was then washed successively with water (1000 ml.), saturated aqueous sodium bicarbonate (100 ml.) and water (1000 ml.). Moisture was removed with sodium sulfate and the solution concentrated to a yellow sirup which was dissolved in 85 ml. of methanol–benzene (9:1). After 3 days at room temperature, the solution had deposited 5.91 g. of crystalline material; dried at 100° for 5 hr., this sintered at 135°, gave a cloudy melt at 137–139° and a clear melt at 141°. Recrystallization failed to improve these values. In dioxane (*c* 0.6) the ester rotates $[\alpha]^{20}_{D}$ +62°, no mutarotation being observed in 18 hr. or after the addition of aqueous pyridine. Concentration of the mother liquor and crystallization as described above afforded a second crop (1.41 g.) of slightly less pure material, raising the total yield to 80%.

Anal. Calcd. for $C_{19}H_{16}N_2O_{10}$ (432.34): C, 52.78; H, 3.73; N, 6.48. Found: C, 52.63; H, 3.60; N, 6.23.

b) *From 2-deoxy-3,5-di-O-p-nitrobenzoyl-*D*-ribosyl chloride* (IV). The chloride (436.5 mg.) was added to a stirred suspension of 0.4 g. of silver oxide in 4 ml. of dioxane and 0.3 ml. of water. After 30 min., the mixture was diluted with meth-

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(17) Based on assumption of complete hydrolysis.

ylene chloride and dried with magnesium sulfate. Upon filtration and removal of the solvent by concentration under reduced pressure, the resulting sirup was dissolved in a minimum quantity of absolute methanol and seeded. After 6 days the crystalline product (211 mg., 50%) was collected. It showed a rotation of +58° (dioxane) and melted at 132–135° yielding a cloudy melt which became clear at 138°. There was no depression of the melting point when admixed with the 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribose prepared as described above.

p-Nitrobenzoylation of 2-deoxy-3,5-di-O-p-nitrobenzoyl-D-ribose (VI). One gram of 2-deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribose (2.3 mmoles) was added to an ice-cold solution of 0.86 g. of *p*-nitrobenzoyl chloride (4.6 mmoles) in 15 ml. of pyridine. The solution was left at 0° for 10 min. and then at room temperature overnight. It was then cooled, treated with a little water, and diluted with 100 ml. of methylene chloride. The solution was washed successively with water, 3*N* sulfuric acid, saturated aqueous sodium bicarbonate, and water. It was then dried with sodium sulfate and concentrated to a sirup (1.34 g., 100%) which showed $[\alpha]^{20}_{D}$ +58° in chloroform (*c* 2). If this consisted solely of the anomeric 2-deoxy-D-ribofuranose tri-*p*-nitrobenzoates,[2] its rotation indicates that it contained 76% of the α-anomer. From chloroform–pentane solution the material deposited 740 mg. of crystalline product. Recrystallized from chloroform–pentane the ester (590 mg., 44%) was dried *in vacuo* overnight at 100°: m.p. 160–161°, $[\alpha]^{20}_{D}$ +71.6° (chloroform, *c* 0.5). Mixed with an authentic sample of 2-deoxy-α-D-ribofuranose tri-*p*-nitrobenzoate it melted at 160–162°. Ness and Fletcher[2] reported $[\alpha]^{20}_{D}$ +70.7° (chloroform) for the substance.

On standing, the mother liquors from the first crystallization above deposited 223 mg. of crystalline material. This was recrystallized from chloroform–pentane and dried at 100° *in vacuo* overnight: 154 mg. (11%), m.p. 162–164°, resolidifying and then melting at 168–170°, $[\alpha]^{20}_{D}$ +15.3° (chloroform, *c* 0.5). 2-Deoxy-β-D-ribofuranose tri-*p*-nitrobenzoate[2] melts at 172–173° and shows $[\alpha]^{20}_{D}$ +17.1° in chloroform.

Pyrolysis of 2-deoxy-3,5-di-O-p-nitrobenzoyl-D-ribosyl chloride (IV). 2-Deoxy-3,5-di-*O*-*p*-nitrobenzoyl-D-ribosyl chloride (164.9 mg.) was placed in a tared flask which was then evacuated to *ca.* 35 mm. and immersed in an oil bath (110–112°) for 2.5 min. On reweighing, the material was found to have lost 13.0 mg. or 7.9%. Loss of one mole equivalent of hydrogen chloride is calculated as 8.1%. The residue was dissolved in methylene chloride–ether solution and the solution extracted with sodium bicarbonate solution. Acidification of the aqueous extract yielded 57.1 mg. (93%) of *p*-nitrobenzoic acid, melting at 238–240°. The organic layer was dried with sodium sulfate, filtered through a little decolorizing carbon and concentrated *in vacuo* to a sirup (80.9 mg.). Several milliliters of hot absolute alcohol was added, the mixture centrifuged and the alcoholic layer decanted; on standing it deposited 11.2 mg. (12%) of crystalline material melting at 74–76°. Mixture with an authentic sample of furfuryl *p*-nitrobenzoate[8] (m.p. 76–77°) did not depress this melting point. In chloroform solution the infrared spectra of the product and of the authentic material were identical.

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[CONTRIBUTION FROM THE FRUIT AND VEGETABLE CHEMISTRY LABORATORY, WESTERN UTILIZATION RESEARCH AND DEVELOPMENT DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE]

Flavonoids of Citrus. V. Structure of Limocitrin[1]

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Limocitrin, a new flavonol isolated from lemons, is shown to be 3′,8-dimethoxy-3,5,7,4′-tetrahydroxyflavone. A synthesis of 5-*O*-methyllimocitrin is described and spectral data are presented for limocitrin and a number of related compounds.

Extracts of lemon peel (*Citrus limon*) contain a number of phenolic glycosides which can be hydrolyzed enzymatically by crude preparations of hemicellulase. The product of this hydrolysis is a mixture of polyphenols from which various flavonoid aglycones, coumarins and substituted cinnamic acids have been separated and identified.[2] Many of these compounds were not previously recognized as constituents of the lemon, although they had been obtained earlier from other plant sources. Several of the compounds are new, however, and one of these will be described here.

The crude hydrolytic products can be conveniently divided into an ether soluble and ether insoluble fraction. On standing for several days the ether soluble fraction deposits a small amount of a pure, crystalline yellow solid, m.p. 274–275°. A slightly better yield of the substance is obtained by chromatographing this fraction on a column of silicic acid which is eluted with chloroform-acetone. The compound can be recognized as a flavonol by the magenta color obtained on reduction with magnesium in hydrochloric acid and by its ultraviolet spectra in ethanol, and in ethanol saturated with aluminum chloride (Table I). We propose to name the new flavonol *limocitrin.*

Limocitrin contains two methoxyl and four hydroxyl groups as shown by analyses of the compound itself and its tetraacetyl and tetraethyl derivatives. The location of one of the methoxyl groups at the 3′-position and one of the hydroxyl groups at the 4′-position followed from the identification of vanillic acid among the alkaline cleavage products.

Methylation of limocitrin with excess methyl sulfate yields a permethyl ether which has the same ultraviolet spectrum as that recorded[3] for 3,5,7,8,3′,4′ - hexamethoxyflavone (gossypetin hex-

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[1] A preliminary account of this work has appeared: R. M. Horowitz, *J. Am. Chem. Soc.*, **79**, 6561 (1957).

[2] R. M. Horowitz and B. Gentili, *J. Org. Chem.*, **25**, 2183 (1960).