with cold 95% ethanol. Two crystallizations from ethyl acetate gave pure D-rhamnose 2,4-dinitrophenylhydrazone, m.p. 165–166°. (The 2,4-dinitrophenylhydrazone of L-rhamnose is reported[10] to be 164–165°.)

Anal. Calcd. for $C_{12}H_{16}O_8N_4$: N, 16.27. Found: N, 16.01.

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The Synthesis of 1-(2-Aminonaphthyl)-β-D-glucopyranosiduronic Acid

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Bladder cancer in man and dogs exposed to 2-naphthylamine[1,2] probably occurs through the action of urinary metabolites of this compound. Accordingly, identification of urinary metabolites in the species which develop this disease is of particular interest. 1-(2-Aminonaphthyl)-β-D-glucopyranosiduronic acid has been recently identified as an important urinary metabolite of 2-naphthylamine in our laboratory in dog urine,[3] and by Levitz *et al.*[4] in human urine.

The present paper describes a convenient chemical synthesis of this material which until now has been prepared biosynthetically.[5]

The essential starting material methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate was prepared from glucuronolactone according to the procedure of Bollenback *et al.*[6]

Methyl [1-(2-nitronaphthyl) tri-*O*-acetyl-β-D-glucopyranosid]uronate was prepared by a modification of the procedure of Bollenback *et al.*[6] for the preparation of the corresponding *o*-nitrophenyl derivative. *N,N*-Dimethylformamide was used instead of acetone because of the insolubility of the potassium salt of *o*-nitronaphthol in this solvent. Although the presence of water and room temperature resulted in low yields of about 5%, consistently good yields of about 40% were obtained when the reaction was carried out with dry reagents in the cold.

Attempts at deacetylation of the nitronaphthyl derivative with methanolic sodium or barium methoxide resulted in turbid solutions which became increasingly yellow with time even in the refrigerator. This suggests that the nitro group is activating basic hydrolysis of the glycosidic linkage. The successful synthesis of the desired product was achieved by carrying out the reduction prior to deacetylation.

EXPERIMENTAL

Methyl [1-(2-nitronaphthyl)tri-O-acetyl-β-D-glucopyranosid]uronate. Methyl (tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate,[6] 2 g. (0.0051 mole) and the potassium salt of *o*-nitronaphthol,[7] 5 g. (0.0216 mole) (prepared from an acetone solution of *o*-nitronaphthol to which an equivalent amount of 5*N* potassium hydroxide was added) were dissolved in 200 ml. of *N,N*-dimethylformamide and allowed to react at 4° for 3 weeks. The dark yellow solution was diluted with an equal volume of chloroform. The unchanged potassium salt precipitated and was collected by centrifugation. The solution was then extracted with water, 2*N* sodium hydroxide, and finally with water until the extracts were nearly colorless. The chloroform layer was dried over anhydrous sodium sulfate and evaporated to dryness at 50° on the water pump. The oily yellow residue was dissolved in acetone and precipitated by addition of water. The oily precipitate was dissolved in a minimum volume of methanol and crystallized on cooling. The crystals were washed with ligroin (b.p. 90–120°) and dried to give faintly yellow crystals (1 g., 39% yield) melting at 150° (uncorrected, Fischer-Johns block).

The infrared spectrum of a 5% chloroform solution was compared with the spectrum of the corresponding *o*-nitrophenyl derivative.[6] The spectra were almost identical and exhibited the characteristic bands for methyl (2,3,4-tri-*O*-acetyl-β-D-glucosid)uronates.[8]

Methyl[1-(2-aminonaphthyl) tri-O-acetyl-β-D-glucopyranosid]uronate. Methyl [1-(2-nitronaphthyl) tri-*O*-acetyl-β-D-glucopyranosid]uronate, 1 g. (0.002 mole) in 180 ml. methanol, was catalytically hydrogenated with 100 mg. of 10% palladium-on-charcoal. The slightly yellow solution became colorless and highly fluorescent. The uptake of hydrogen was quantitative; calcd. 144 ml., found 148 ml. The solution was filtered and evaporated at 40° on the water pump. The residue consisted of light pink crystals melting at 160° (corr.). It was unnecessary to isolate the reduced compound prior to deacetylation.

1-(2-Aminonaphthyl) β-D-glucopyranosiduronic acid. Methyl [1-(2-nitronaphthyl) tri-*O*-acetyl-β-D-glucopyranosid]uronate, 1.5 g. (0.003 mole), was reduced as above. A tenfold excess of sodium methoxide was added to the filtered methanolic solution and allowed to react at 4° for 2 days. The solution was evaporated on the water pump and the residue dissolved in a minimal amount of water. The *p*H was adjusted to 4.0 and cooled. The pink crystals recrystallized from water[5] weighed 910 mg. (86% yield). The melting point was 178–180° (corr.); (reported[5]

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m.p. 178–180°). Mixed melting point with the biosynthetic preparation[9] was 176–178° (corr.).

Anal. Calcd. for $C_{16}H_{17}O_7N \cdot H_2O$: C, 54.40; H, 5.39; N, 3.96. Found: C, 55.06; H, 5.60; N, 3.62.

Paper chromatography in 1-butanol–1-propanol–water; 2/1/1, gave R_f = 0.15; mixed chromatogram with biosynthetic compound gave one fluorescent spot R_f = 0.19. When large amounts were chromatographed, an additional slower moving fluorescent spot was observed. This could be decreased but not removed by repeated recrystallizations. Paper electrophoresis on Whatman 3 MM, *p*H 9.2, 0.05*M* borate, 500 volts, for 3.5 hr. gave one major fluorescent spot which moved 7 cm. toward the anode. The biosynthetic preparation had the same mobility. The minor component moved 1 cm. toward the cathode.

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(9) The author is indebted to Dr. Mortimer Levitz for a sample of this preparation.

Products of the Oxidation of Nitrolutidine and Nitrocollidine

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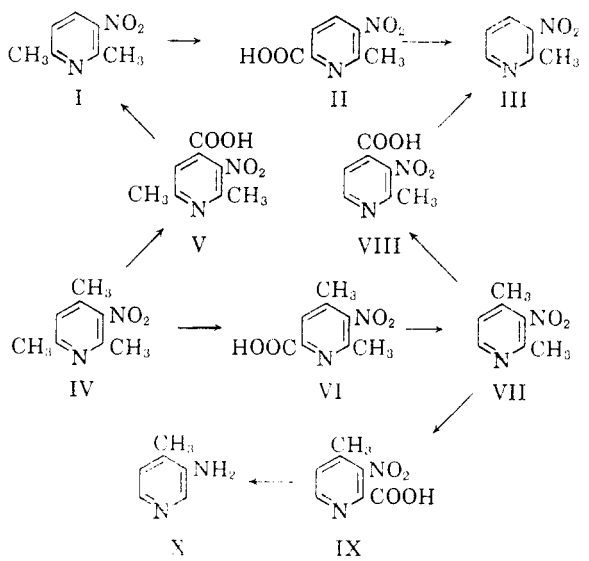
The nitration of 2,6-lutidine using the method of Plazek[2] gave an 80% yield of 3-nitro-2,6-lutidine (I). Upon treating this product with an amount of potassium permanganate calculated to oxidize one methyl group, an acid (II) was obtained in 17% yield which was then decarboxylated in 97% yield to 3-nitro-2-methylpyridine (III). Again, oxidation of this material gave 17% of a nitropyridinecarboxylic acid which was shown to be 3-nitro-2-pyridinecarboxylic acid[3a] by comparison with the corresponding amino compound of known structure prepared by Sucharda.[3b] Kogl and co-workers[4] oxidized 3-nitro-2,4,6-collidine (IV) to a mixture of three acids; the main product melted at 136°, which they assumed to be 3-nitro-2,4-dimethylpyridine-6-carboxylic acid (VI). We oxidized the nitrocollidine and obtained two acids. The third acid, if present, was in an amount not detected by our method. The acid melting at 138° was the main product and was decarboxylated to a



nitrolutidine (VII) which on further oxidation gave two products both nitromethylpyridinecarboxylic acids. The lower melting (IX) of these two acids was then decarboxylated and the product proved to be 3-nitro-4-methylpyridine by reduction to 3-amino-4-methylpyridine (X) and comparison with a known sample. The higher melting acid (VIII) was decarboxylated and the product identified as 3-nitro-2-methylpyridine (III). Thus it is definitely established that the original main oxidation product of nitrocollidine is 3-nitro-2,4-dimethylpyridine-carboxylic acid (VI) as it is the only one that could produce the obtained result. The second acid melted at 238° which was an indication that it might be a 4-carboxylic acid, namely, 3-nitro-2,6-dimethylpyridine-4-carboxylic acid (V). This was confirmed when the product of decarboxylation was shown to be 3-nitro-2,6-lutidine (I).

EXPERIMENTAL

3-Nitro-2,6-lutidine. 2,6-Lutidine (174 g.) was nitrated by the method of Plazek[2] to give 200 g. (81%) of 3-nitro-2,6-lutidine (I), boiling at 105° (10 mm) and melting at 38°.

3-Nitro-2-methylpyridine-6-carboxylic acid (II). One hundred grams of 3-nitro-2,6-lutidine in 1.5 l. of water was heated to 90° and treated with 200 g. of solid potassium permanganate in small portions over a period of 1.5 hr. After removal of the manganese dioxide, the filtrate was extracted with ether to recover 50 g. of unchanged nitro compound. The aqueous solution was evaporated to low volume and acidified. Ten grams of acid was recovered melting at 126°.

Anal.[5] Calcd. for $C_7H_6O_4N_2$: C, 46.16; H, 3.32. Neut. equiv., 182. Found: C, 45.96; H, 3.37. Neut. equiv., 185.

3-Nitro-2-methylpyridine. 3-Nitro-2-methylpyridine-6-carboxylic acid (14.2 g.) was decarboxylated by heating under nitrogen (bath temp. 205°) under 320 mm. pressure. There was obtained 10.5 g. (97.6%) of 3-nitro-2-methylpyridine[3a] (III) boiling at 104° (15 mm) and melting at 28.5°. The

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