EXPERIMENTAL[6]

Additional studies on original "neutral" fraction. The 1% acetic acid in 95% ethanol eluate of the alumina column chromatogram of this fraction noted earlier[3] was submitted to reverse-phase chromatography on mineral oil-treated paper at 37° with "peracid" developer.[7] In addition to the C_{16} to C_{24} saturated fatty acids reported earlier[3] this fraction contained myristic and lauric acids with R_f values of 0.65 and 0.82, respectively.

Additional studies on unsaponifiables from petroleum ether-soluble "neutral" fraction. This fraction was boiled with methanol and filtered hot as described earlier.[3] The filtrate deposited hexacosanol melting at 78° upon cooling. The hexacosanol was filtered, and the filtrate was cooled to −70°. The crystals which separated were filtered and chromatographed reverse-phase at 37° on mineral oil-impregnated paper with 85% acetic acid. The chromatograms indicated the presence of C_{26} and higher long-chain fatty alcohols. No individual spots were apparent, but the spot for long-chain fatty alcohols appeared as a continuous spot from the origin to R_f 0.34, the R_f for the C_{26} alcohol. Infrared spectra of this crystalline material indicated only long-chain fatty alcohols.

The methanol filtrate from the crystallization at −70° was chromatographed reverse-phase in the same manner. The chromatograms were dried and scanned under ultraviolet light before and after iodine vapor treatment and examined by means of the mercury stain.[8] Linoleic acid (R_f 0.66), oleic acid (R_f 0.57), arachidonic acid (R_f 0.40), and two unidentified unsaturated acids with R_f's 0.30 and 0.02 were found. Reverse-phase chromatography with the "peracid" developer at 37° indicated the presence of lauric acid (R_f 0.82) and capric acid (R_f 0.94) in this fraction.

Additional studies on acids from petroleum ether-soluble "neutral" fraction. Chromatography of the crude fraction on Whatman No. 1 paper in butanol–2% aqueous ammonia and in 10:3:3 butanol-pyridine-water and location of spots with bisdiazotized benzidine, diazotized *p*-nitroaniline, and Mäule reagents as described earlier[9] indicated the presence of vanillic, syringic, *p*-hydroxybenzoic, and ferulic acids.

The crude fraction was chromatographed on a column of alumina and eluted successively with petroleum ether (b.p. 65–110°), benzene, chloroform 95% ethanol, and 1% acetic acid in 95% ethanol as before.[3] Again, the 1% acetic acid in ethanol eluate contained almost the entire fraction. Reverse-phase chromatography with "peracid" developer at 37° indicated the presence of lauric acid in addition to the previously reported acids in this fraction.

Additional studies on the unsaponifiables from the saponification of petroleum ether-soluble "neutral" fraction with strong alkali. The oil filtrate and petroleum ether washings from the C_{27} alcohol reported previously[3] were evaporated to dryness and dissolved in anhydrous ether. Fractional crystallization by stepwise concentration and cooling of the ether solution yielded several crystalline fractions melting from 77 to 83°. Infrared spectra of all fractions were essentially identical and indicated only long-chain fatty alcohols. High-temperature reverse-phase chromatography by the Fiker and Hajek[4] method indicated the presence of C_{22}, C_{24}, C_{25}, and C_{26} long-chain saturated fatty alcohols. These fractions were also examined by the new reverse-phase procedure employing *p*-phenylazobenzoyl esters of the alcohols.

p-Phenylazobenzoyl ester chromatographic procedure. The esters were prepared by a modification of the procedure of Woolfolk, Beach, and McPherson.[10] A mixture of excess *p*-phenylazobenzoyl chloride, known or unknown alcohol, and pyridine was boiled under reflux for 4 hr. The red reaction mixture was poured with vigorous stirring into a mixture of ice and saturated sodium bicarbonate solution, and the mixture was allowed to stand a short while. The crystalline precipitate was filtered, washed with water, and air dried.

Approximately 100 μg. of the crystalline ester was spotted on Whatman No. 1 paper impregnated with mineral oil (7 g. of mineral oil dissolved in 100 ml. of ether). The paper was developed with glacial acetic acid saturated with mineral oil at 37° for 15 hr. Compounds appeared as weak orange spots in ordinary light, but, under ultraviolet light, the compounds gave dark spots against a light background, and were discernible even in small concentration. During the 15-hr. developing time, the solvent front moved off the paper, thus precluding the possibility of R_f determination. Instead, mobilities of individual compounds were recorded with reference to stearyl (octadecyl) alcohol and are denoted R_{st} values. The R_{st} values were determined for the *p*-phenylazobenzoyl esters of several authentic long-chain saturated fatty alcohols[3] as follows: C_{26}, 0.24; C_{24}, 0.41; C_{22}, 0.53; C_{20}, 0.76; and β-sitosterol, 0.42.

Chromatography of the 77–83° melting crystalline fractions by this procedure confirmed the occurrence of the alcohols noted by the Fiker and Hajek procedure.

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Methyl Glycoside Formation from β-D-Glucopyranose Pentanitrate

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By reaction of tetra-*O*-acetyl-α-D-glucopyranosyl chloride with silver nitrate, Skraup and Kremann[2] obtained tetra-*O*-acetyl-β-D-glucopyranosyl nitrate, a compound showing a close similarity to the corresponding chloride,[3] for like the latter it readily anomerizes in polar solvents to the α-D form. Tetra-*O*-acetyl-α-D-glucopyranosyl nitrate itself resembles the acetylated glucosyl halides in that it reacts with methanol under the influence of a variety of bases to give methyl tetra-*O*-acetyl-β-D-glucopyranoside[4,5] and with sodium acetate in acetic anhydride to give penta-*O*-acetyl-β-D-glucopyranose.[4]

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Unlike tetra-*O*-acetyl-β-D-glucopyranosyl nitrate, β-D-glucopyranose pentanitrate[6] is stable in polar solvents and no evidence of facile anomerization has been reported. This may be due in part to the known inability of the nitrate ester group to participate in the displacement reaction at a neighboring carbon atom,[7,8] as the participation of the acetate ester group at C-2 is believed to provide important anchimeric assistance in the solvolysis of 1,2-*trans*-poly-*O*-acylglycosyl halides.[9]

These considerations suggest that β-D-glucopyranose pentanitrate may be expected to react with hydroxylic compounds with Walden inversion at C-1 to give rise to α-D-glucopyranosides not readily accessible from the usual poly-*O*-acylglycosyl halides.

Accordingly β-D-glucopyranose pentanitrate was allowed to react with boiling methanol in the presence of silver carbonate. At intervals samples were withdrawn for examination and the nitrate group was removed by reductive hydrogenolysis.[10] The resulting aliquot portions were subjected to paper chromatography which indicated the relative amounts of D-glucose and D-glucoside in them. As the time allowed for reaction increased, the relative intensities of the spots observed on the chromatograms changed until finally only one spot corresponding to a methyl glucoside could be detected. In order to isolate the glucoside formed, a further experiment was performed under similar conditions for a period of time sufficient to allow complete reaction. The solution was filtered and evaporated to yield a sirup which could not be induced to crystallize. Methyl α-D-glucopyranoside tetranitrate is known as crystals of low melting point.[11] The sirup was reductively denitrated and the product crystallized readily to give methyl α-D-glucopyranoside as the only product isolated.

The rate of this solvolytic reaction was found to be low and attempts to react β-D-glucopyranose pentanitrate with hydroxylic compounds in inert solvents showed that the material is insufficiently reactive to produce any detectable glycoside formation in reasonable periods of time. At elevated temperatures decomposition of the nitrate exceeds the rate of glycoside formation and extension of the reaction to the synthesis of α-D-linked disaccharides appears to be impracticable.

EXPERIMENTAL

Methods. Paper chromatography was performed in a glass tank employing Whatman No. 1 filter paper developed by a descending front of 1-butanol saturated with water. The air-dried chromatograms were sprayed with ammoniacal silver nitrate solution and heated at 110° for 3–5 min. to produce brown or black spots indicating reducing sugars or *cis*-glycols. Solutions were evaporated under reduced pressure.

Preparation of "active" silver carbonate.[12] A solution of 16 g. of anhydrous sodium carbonate in 75 ml. of water was added dropwise to a mechanically stirred solution of 80 g. of silver nitrate in 200 ml. of water. Careful control of the rate of addition ensured that the silver oxide precipitate, which formed where the drops fell, was only transitory. A solution of 10 g. of anhydrous sodium bicarbonate in 125 ml. of water was then added in two or three portions; the mixture foamed and the precipitate became yellow. The solid was recovered by filtration and washed at least twelve times by stirring with water and refiltering (the last filtrate gave a negative sodium flame test). The silver carbonate was dried over calcium chloride (not under reduced pressure) in the dark; yield 63 g. Best results were obtained if the reactions were carried out in the dark at about 25°.

Reaction of β-D-glucopyranose pentanitrate with methanol. β-D-Glucopyranose pentanitrate[6] (0.15 g.), dried over phosphorus pentoxide, was dissolved in absolute methanol (15 ml.), and stirred under reflux with 2 g. of calcium sulfate (Drierite, previously heated overnight at 200°) with precautions to exclude moisture. After 15 min., the addition of the first of several portions of freshly prepared "active" silver carbonate (see above) was made; over the period of reaction further portions were added (a total of 3 g.). At intervals, 1-ml. aliquots of the solution were removed, filtered, and evaporated. Each sirupy residue was dissolved in ethyl acetate, extracted with water, and the organic layer evaporated. The residue, dissolved in absolute ethanol (approximately 5 ml.), was refluxed with freshly prepared Raney nickel (0.2 g.) for 30 min. to remove nitrate ester groups. After filtration and evaporation of the filtrates to low volume, the resulting material was applied to the starting line of a paper chromatogram, which was developed and sprayed. Examination of the paper showed that the sample removed after 5 hr. gave a spot corresponding to methyl α-D-glucopyranoside (R_f 0.21) and only a trace corresponding to D-glucose (R_f 0.08). After 13.5 hr., no glucose could be detected and the only spot appeared at R_f 0.21. After allowing the reaction to proceed for 20 hr., the remainder of the mixture was cooled, filtered, and evaporated to a sirup, which could not be induced to crystallize.

Preparation of methyl α-D-glucopyranoside. β-D-Glucopyranose pentanitrate (2 g.) was dissolved in absolute methanol (75 ml.) and stirred for 30 min. under reflux with Drierite (2.5 g.) and a few glass beads. At this time, the first of a series of additions of silver carbonate was made; a total of 5 g. being added in portions during a period of 19 hr. The mixture was cooled, filtered, and evaporated. The residue was partitioned between ethyl acetate and water. The sirup (1.79 g.), obtained on evaporation of the organic layer, was hydrogenated[10] in absolute ethanol solution (50 ml.) with the aid of 10% palladium-on-charcoal catalyst (1 g.) employing 40 min. shaking with hydrogen at 45 p.s.i. Filtration and evaporation of the filtrate yielded a white crystalline mass, which on crystallization from ethanol yielded methyl α-D-glucopyranoside; yield 550 mg., m.p. 165–166°, mixed melting point with authentic material undepressed, $[\alpha]_D^{25}$ +158° (*c* 1.0, water). Concentration of the liquor gave a further crop; yield 23 mg. (80% total), m.p. 163°.

Attempted reaction of β-D-glucopyranose pentanitrate with methanol at low concentration. β-D-Glucopyranose pentanitrate[6] (207 mg.) was dissolved in acetone (0.5 ml.), previously distilled from potassium permanganate and from Drierite, containing methanol (0.2 ml., 5 equivalents) and the solution was stirred and refluxed with Drierite (2 g.) and "active" silver carbonate (total of 1.0 g.) as above. Portions were

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removed periodically, denitrated and examined by paper chromatography as described above. The reaction was terminated after 96 hr., at which time no methyl D-glucoside could be detected in the products. Reactions at higher temperatures effected decomposition of the nitrate derivative.

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N,N'-Diethylene-*N''*-ethyl-*N''*-(1,3,4-thiadiazol-2-yl)phosphoramide

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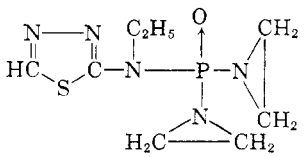
Carcinostatic activity has been reported for many derivatives of diethylenephosphoramide[1] and also for 2-ethylamino-1,3,4-thiadiazole.[2] It was therefore of interest to synthesize and test *N,N'* - diethylene - *N''* - ethyl - *N''* - (1,3,4 - thiadiazol - 2 - yl)phosphoramide (I), a potential "dual antagonist" incorporating these two active moieties



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in one molecule. The product showed substantial activity against transplanted mouse tumors Sarcoma 180, 6C3HED lymphosarcoma, and C3H mammary adenocarcinoma by both oral and intraperitoneal administration. The synthesis is described below, and details of the testing will be reported elsewhere.[3] The compound is now undergoing clinical evaluation.

EXPERIMENTAL

N-Ethyl-*N*-(1,3,4-thiadiazol-2-yl)amidophosphoryl chloride was prepared by refluxing 16.4 g. (0.1 mole) of 2-ethylamino-1,3,4-thiadiazole hydrochloride[4] with 50 ml. of phosphorus oxychloride for 6 hr. and then removing excess phosphorus oxychloride by distillation under reduced pressure. The residual oil was washed with cold petroleum ether, (b.p. 30–60°), dried, and dissolved in 350 ml. of warm dry benzene. This solution was added slowly to a mixture of 9.5 g. (0.22 mole) of ethylenimine, 30.3 g. (0.3 mole) of triethylamine and 50 ml. of dry benzene at 10°. Agitation was continued for 2 hr. without cooling, after which the precipitated triethylamine hydrochloride was filtered off. The benzene was removed from the filtrate under reduced pressure, and the crude *N,N'*-diethylene-*N''*-ethyl-*N''*-(1,3,4-thiadiazol-2-yl)phosphoramide (19.7 g.) was purified by recrystallization from hexane; m.p. 95–96.5°.

Anal. Calcd. for $C_8H_{14}N_5OPS$: C, 37.1; H, 5.44; N, 27.0; S, 12.4. Found: C, 37.4; H, 5.67; N, 27.0; S, 12.6.

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The Pyrolysis of 2-Methylnaphthalene

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The wide occurrence of traces of benzo(g,h,i)-perylene in many petroleum products, such as waxes and solvents,[1] in which other six ring aromatic hydrocarbons are seldom found, suggested the possibility that this compound might be produced in the thermal procedures (*e.g.*, distillation) by which these products are manufactured. It is possible that benz(g,h,i)perylene is formed by condensation of smaller molecules, which process could be studied by pyrolysis of likely precursors. Pyrolysis of simple compounds has produced polycyclic aromatic hydrocarbons in small amounts.[2] Recently Badger and Kimber have studied the pyrolysis of tetralin[3] and of indene[4] which yielded, among other compounds, benzo(a)pyrene and benzo(j)fluoranthene.

The pyrolysis of 2-methylnaphthalene was studied, naphthalenes being common constituents of many petroleums. The vaporized methylnaphthalene was passed through a heated copper tube, and the material leaving the tube collected and analysed by chromatography.

Most of the 2-methylnaphthalene passed through the tube unchanged, even at 950°. The only product in amounts large enough for crystallization was 2,2'-binaphthyl. Some 1,2'-binaphthyl was present but could not be isolated and, like the other products, was identified by absorption spectroscopy. The identity of most of the products was confirmed by fluorescence spectroscopy. The calculated yields

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