X-ray powder diffraction data[15]: 7.80 m, 6.89 m, 5.97 w, 5.57 w, 5.21 m, 4.92 vs (1), 4.18 m, 3.86 m, 3.46 s (2), 3.30 s, 3.06 s (3), 2.78 w, and 2.69 w.

Anal. Calcd. for $C_8H_{16}N_6O_6$: C, 32.87; H, 5.52; N, 28.75. Found: C, 32.84; H, 5.65; N, 28.79.

Absorption Spectrum of the Copper Complex.—A solution of D-*arabino*-hexosulose disemicarbazone (292 mg.) in water (10 ml.) was treated with a solution of cupric chloride (133 mg.) in ethanol (10 ml.) and the volume was brought to 25 ml. with ethanol. The absorbance of the green solution [λ_{max}^{EtOH} 740 mμ, (log ε 1.69)] remained constant for 24 hr.

D-*lyxo*-Hexosulose Disemicarbazone.—D-*lyxo*-Hexosulose sirup (1.0 g.) was treated as above and the product was isolated in the same manner: yield, 0.25 g. of disemicarbazone which was recrystallized from water; m.p. 235° dec.; $[\alpha]^{21}D$ −2° (*c* 1.9, water); λ_{max}^{EtOH} 292 mμ; λ_{max}^{KBr} 3.1 (OH), 5.9, 6.0 ($CONH_2$), 6.3 μ (C=N); X-ray powder diffraction data[15]: 4.78 m, 4.25 s (3), 3.99 s (2), 3.65 m, 3.24 vs (1), 3.15 w, 2.85 w, 2.48 w, and 2.33 w.

Anal. Calcd. for $C_8H_{16}N_6O_6$: C, 32.87; H, 5.52. Found: C, 32.35; H, 5.47.

L-*xylo*-Hexosulose Disemicarbazone.—L-*xylo*-Hexosulose sirup (10 g.) was treated as above: yield, 0.75 g. of disemicarbazone which was recrystallized from water; m.p. 225° dec.; $[\alpha]^{20}D$ −16° (*c*, 0.45, water); λ_{max}^{EtOH} 292 mμ (log ε 4.69); λ_{max}^{KBr} 3.0 (OH), 5.95, 6.05 ($CONH_2$), 6.33 μ (C=N); X-ray powder diffraction data[15]: 7.38 m (3), 5.91 w, 5.32 w, 4.86 m, 4.23 w, 3.67 s (2), 3.45 vs (1), 3.14 m, 3.02 vw, 2.50 w, and 2.29 w.

Anal. Calcd. for $C_8H_{16}N_6O_6$: C, 32.87; H, 5.52; N, 28.75. Found: C, 32.86; H, 5.98; N, 28.66.

D-*threo*-Pentosulose Disemicarbazone.—D-*threo*-Pentosulose sirup (1.0 g.) yielded 0.15 g. of disemicarbazone which was recrystallized from water: m.p. 232° dec.; $[\alpha]^{22}D$ +3° (*c* 1.5, water); λ_{max}^{EtOH} 292 mμ; λ_{max}^{KBr} 2.9 (OH), 5.9, 6.0, ($CONH_2$), 6.33 μ (C=N); X-ray powder diffraction data[15]: 10.92 m, 7.34 m, 5.47 w, 5.07 m, 4.21 s, 4.05 w, 3.88 s (2), 3.55 s (3), 3.46 s, 3.30 vs (1), 2.99 m, and 2.97 m.

Anal. Calcd. for $C_7H_{14}N_6O_5$: C, 32.05; H, 5.38. Found: C, 31.62; H, 5.61.

Action of Nitrous Acid on D-*arabino*-Hexosulose Disemicarbazone.—A solution of the disemicarbazone (0.5 g.) in water (25 ml.) was treated at 50° with sodium nitrite (5.5 g.) in 25 ml. of water followed by the dropwise addition of 15% hydrochloric acid (12 ml.). After 30 min. the hexosulose solution was neutralized and refluxed for 10 min. with *o*-phenylenediamine (0.2 g.) and 1 ml. of acetic acid. The quinoxaline derivative separated on cooling and was recrystallized from ethanol: m.p. 188°, $[\alpha]^{22}D$ −76.6° (*c* 1.84, 5 *N* hydrochloric acid) (lit.[17] m.p. 187–188°, $[\alpha]D$ −75.2°).

Acetylation of D-*arabino*-Hexosulose Disemicarbazone and Reaction with Nitrogen Trioxide.—The disemicarbazone (3.5 g.) was acetylated by stirring with a mixture of 15 ml. of acetic anhydride and 30 ml. of pyridine until complete dissolution occurred (4 days). Since the acetylated disemicarbazone was water soluble, the reaction mixture was evaporated to dryness and the acetate, which could not be obtained in a crystalline form, was subjected to thin layer chromatography [silica gel G, methanol–benzene (1:9)] to reveal eight spots on spraying with sulfuric acid. The crude acetate was taken up in acetic acid and a stream of nitrogen trioxide was passed into the solution for 5 hr. at 5° after which the mixture was left overnight at room temperature. The reaction mixture was then evaporated, taken up in chloroform, washed with water and then with aqueous sodium hydrogen carbonate, and evaporated to dryness, yielding a nearly colorless, hygroscopic sirup which distilled at 140° (0.5 mm.): it appeared as a single spot (R_F 0.88) on a paper chromatogram developed with 1-butanol–ethanol–water (4:1:5); $[\alpha]^{22}D$ +28° (*c* 3.8, chloroform); λ_{max}^{KBr} 5.7 (O-Ac), 6.29 μ (C=N).

Anal. Calcd. for $(C_{14}H_{20}N_2O_8)_2 \cdot H_2O$: C, 47.59; H, 5.99; N, 7.93; CH_3CO, 48.73. Found: C, 47.13; H, 5.63; N, 7.82; CH_3CO, 48.67.

The above substance yielded with phenylhydrazine and acetic acid 3,4,5,6-tetra-*O*-acetyl-D-*arabino*-hexose phenylosazone[11] which was converted with sodium hydroxide in acetone to Percival's dianhydrophenylosazone,[12] m.p. and m.m.p. 238°.

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Reduction Potential and Effect of *ortho* Substituents on Dimerization of Aromatic Nitroso Compounds

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It has been known for many years that *ortho* groups larger than hydrogen favor dimerization of aromatic nitroso compounds.[1-3] For example, in a 0.1 *M* benzene or chloroform solution at 20°, nitrosomesitylene is[4,5] about 70% dimer, whereas *p*-bromonitrosobenzene,[4,5] *p*-methylnitrosobenzene,[5] and nitrosobenzene itself[4,5] are close to 100% monomer. Various explanations have been offered.[3,6-8] Luttke[7] has presented a detailed theory in which he attributes increased dimerization of *ortho*-substituted compounds to steric inhibition of resonance in the dimers. It is the purpose of the present Note to show that di-*ortho*-substituted nitroso *monomers* are subject to a sizeable steric effect and to suggest that steric inhibition of resonance *in the monomers* is the cause of the greatly increased dimerization of these compounds.

Some years ago, Lutz and Lytton[9] measured the reduction potentials, $E°$, of a number of monosubstituted nitrosobenzenes. The effect of a substituent in *meta* or *para* position varied with the electronic nature of the group, but every substituent in the *ortho* position made reduction take place more readily. This observation suggests a steric effect. It was of interest, therefore, to measure polarographically the reduction potentials of some di-*ortho*-substituted nitrosobenzenes and, for comparison, of related compounds with one substituent in the *para* position only. The results are presented in Table I.

The second column lists observed half-wave potentials, the third the difference between the potential and that for nitrosobenzene itself. A more positive potential means that the compound is more easily reduced. Although the absolute values of the reduction potentials listed are not directly comparable to those reported by Lutz and Lytton,[9] since the latter authors' values refer to acidic solutions and were obtained by the more accurate potentiometric titration method,

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TABLE I

Nitroso-benzene	$E_{1/2}$ (s.c.e.), v.[a]	$\Delta E_{1/2}$, v.[b]	$\Delta E_{1/2}$ steric, v.	$\Delta\Delta F°$ steric, kcal./mole	% monomer[c] Exptl.	Calcd.
Unsubstituted	−0.20	0.00	0	0	99.4[d]	99.999[f]
					100[e]	
4-Cl-	−0.19	+0.01	0	0	(99.4)[g]	99.999[f]
					(100)[e]	
4-CH_3-	−0.24	−0.04	0	0	100[h]	99.999[f]
2,6-Di-Cl-	−0.08[i]	+0.12	+0.10	−4.6[j]	20.4[k]	
2,4,6-Tri-Cl-	−0.07[i]	+0.13	+0.10	−4.6[j]	34.8[l]	
					33.5[k]	
2,4,6-Tri-CH_3-	−0.21[i]	−0.01	+0.11	−5.0[j]	30.4[l]	
					31.2[e]	

[a] In 50% acetone–50% pH 7.0 phosphate buffer (by volume), at 30°, *vs.* standard calomel electrode. Half-wave potentials were reproducible to ±0.01 v. Solutions, 1×10^{-3} to 5×10^{-5} *M* in nitroso compound. [b] $E_{1/2} - E_{1/2}$ C_6H_5NO. [c] At 20°. [d] Ref. 5, 0.0248 *M* in $CHCl_3$. [e] Ref. 4, 0.1 *M* in C_6H_6. [f] For 0.1 *M* solution. [g] Ref. 5, 0.04 *M* in $CHCl_3$. [h] Ref. 5, 0.0385 *M* in $CHCl_3$. [i] A second wave was observed at more negative potential, corresponding to reduction of the dimer, as shown by variation in height of the waves with temperature and concentration. A detailed study of the polarographic behavior of these and other nitroso compounds will be presented elsewhere. [j] Calculated from eq. 1 for a two-electron reduction. [k] Present work, 0.1 *M* in C_6H_6. [l] Ref. 5, 0.1 *M* in $CHCl_3$.

whereas the data in Table I refer to neutral solutions and were obtained polarographically, the conclusion that *ortho* groups make the nitroso monomer more easily reduced than expected is quite strikingly verified. An apparent exception is nitrosomesitylene, which is reduced at about the same potential as nitrosobenzene; but it turns out, as shown below, that this is because competing steric and electronic factors fortuitously cancel. Nitrosomesitylene is subject to a steric effect as large or larger than that present in 2,6-dichloro- and 2,4,6-trichloronitrosobenzenes.

The electrochemical reduction of nitrosobenzene is known[10] to be a reversible, two-electron process, leading to phenylhydroxylamine: $Ar\cdot NO + 2e + 2H^+ = Ar\cdot NHOH$. It is also known[11] that the polarographic reduction of nitrosobenzene is reversible and leads to the same product, with $E_{1/2} = E°$. Therefore $E°$ or $E_{1/2}$ values give a quantitative measure of the difference in stability of hydroxylamine and nitroso monomer, *via* the well-known thermodynamic equation.

$$\Delta F° = -n\mathfrak{F}E° \qquad (1)$$

An analysis of the data in the second and third columns of the table was carried out along lines suggested by Taft.[12] The electronic effect of a substituent was supposed to be given by the difference between $E_{1/2}$ for the *para*-substituted compound and $E_{1/2}$ for nitrosobenzene itself. Since resonance effects should be about the same in *ortho* and *para* positions, any extra *ortho* effect was taken to be steric. The fourth column of Table I lists the portion of the entry in the third column left after subtracting the electronic effect of each substituent (*e.g.*, three times the increment for *p*-Cl was subtracted from $\Delta E_{1/2}$ for 2,4,6-trichloronitrosobenzene to give the value of "$\Delta E_{1/2}$ steric" in the fourth column). The fifth column expresses the resulting differences in kilocalories. It is clear that a steric effect, presumably steric inhibition of resonance, makes di-*ortho*-chloro and di-*ortho*-methyl monomers less stable than expected, relative to arylhydroxylamine, by 4–5 kcal./mole. Dipole–dipole interactions cannot be the major factor, since methyl is as effective as chlorine. Apparently, these *ortho* groups are large enough to force the —N=O group of the monomer out of the plane of the benzene ring.

A steric effect of 4–5 kcal./mole on monomer stability should have twice as large an effect on $\Delta F°$ for the monomer–dimer equilibrium, since dissociation of 1 mole of dimer produces 2 moles of monomer. The predicted difference of 8–10 kcal./mole in $\Delta F°$ dissociation of tetra-*ortho*-substituted dimers and non-*ortho*-substituted dimers would correspond to an almost billion-fold difference in dissociation constants.

Luttke and Keussler[4] and Havinga, *et al.*,[5] have made spectrophotometric measurements on solutions of nitrosomesitylene and 2,4,6-trichloronitrosobenzene in benzene and chloroform from which equilibrium constants and per cent of monomer and dimer can be calculated. These results and also their data on nitrosobenzene, *p*-methylnitrosobenzene, and *p*-bromonitrosobenzene are listed in column 6 of Table I. (The entry opposite *p*-chloronitrosobenzene is actually for *p*-bromonitrosobenzene.) Measurements in this laboratory have confirmed the earlier work[5] on 2,4,6-trichloronitrosobenzene and provided values for per cent monomer and dimer and the dissociation constant of 2,6-dichloronitrosobenzene. It appears to make little difference whether the solvent is chloroform or benzene. From these results it is possible to calculate the following values of $\Delta F°$ dimer dissociation in kcal./mole at 20°: nitrosomesitylene, +2.1,[5] +2.07[4]; 2,4,6-trichloronitrosobenzene, +1.9,[5] +1.97 (present work); 2,6-dichloronitrosobenzene, +2.66 (present work).

If a steric effect of 8–10 kcal./mole on monomer stability, suggested by the polarographic data, is the chief factor responsible for greater dimerization of di-*ortho*-substituted aromatic nitroso compounds, it should be possible to calculate per cent monomer at equilibrium for non-*ortho*-substituted compounds from equilibrium data on the di-*ortho*-substituted compounds. By subtracting twice "$\Delta\Delta F°$ steric" (column 5, Table I) from $\Delta F°$ dissociation of each *ortho*-substituted dimer, theoretical values were computed of per cent monomer at equilibrium (in 0.1 *M* solution at 20°) for each non-*ortho*-substituted compound. The calculation did not take into account other factors which must have some influence on the equilibria, but, in spite of this omission, calculated values (column 7) agree well with experimental (column 6). It may be concluded that most of the effect of *ortho* groups on dimerization is due to steric inhibition of resonance in the monomers. It is interesting that the magnitude of this effect can be deduced from variations with structure of $E_{1/2}$ for reduction of monomer to arylhydroxylamine.

Other substituent effects are not negligible, as shown by the difference of 0.7 kcal. in $\Delta F°$ dissociation of 2,6-dichloro and 2,4,6-trichloro dimers, but they are an order of magnitude smaller than the 8–10-kcal. steric effect.

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Experimental

The nitroso compounds were prepared and purified by methods described in the literature.[5,13,14] The polarographic current–voltage curves were obtained with a Sargent Model XXI polarograph, using a conventional H-cell fitted with a standard calomel electrode. Buffer solutions were made up at 30° by mixing 50.00 ml. of 0.2000 *M* aqueous KH_2PO_4 and 29.63 ml. of 0.2000 *M* aqueous NaOH and diluting to the mark in a 100.0-ml. volumetric flask. The apparent pH of the resulting solutions was 7.00 ± 0.03. To 50.00 ml. of this buffer in a 100.0-ml. volumetric flask was added an aliquot of acetone stock solution of nitroso compound, and the mixture was diluted to the mark with acetone. Solutions were flushed in the H-cell with purified nitrogen for 3–6 min. before recording the polarogram. Temperatures were controlled to 30 ± 0.1° by immersing the H-cell in a constant-temperature bath. Various capillary drop times and concentrations between 1×10^{-3} and 5×10^{-5} *M* gave the same half-wave potentials. The dropping mercury electrode at a pressure of 60.0 cm. had a drop time of 3.60 sec. (open circuit) in 50% acetone–50% pH 7 phosphate buffer at 30°. The value of m was 2.69 mg. sec.$^{-1}$ with a calculated value of $m^{2/3}t^{1/6}$ of 2.40 mg.$^{2/3}$ sec.$^{-1/2}$.

The spectrophotometric measurements were carried out with a Beckman DU equipped with a double set of thermospacers through which water from a constant-temperature bath was circulated. A hole through the lid of the cell compartment allowed a thermometer to be placed so that its bulb was immediately adjacent to the cell containing the solution. Matched 1.00-cm. silica cells were used and temperatures were maintained constant to within ±0.05°. Optical densities were determined at or near the wave length of maximum absorption due to the characteristic[4] n–π transition of aromatic nitroso monomers. Optical densities were reproducible to within ±0.5% or better except at the lowest concentrations where reproducibility was somewhat less satisfactory. ϵ_0, the molar extinction coefficient, was determined by linear extrapolation (Ostwald dilution law) of the usual[4] ϵ *vs.* $\epsilon^2 \cdot C^0$ plots (where C^0 is concentration of total nitroso compound, all figured as monomer) to zero concentration. The resulting values of ϵ_0 were then used in the calculation of equilibrium constants and per cent monomer at 20°. The optical densities (1-cm. cell, 775 mμ) and concentrations in benzene (moles/liter) for 2,4,6-trichloronitrosobenzene were 0.6720, 0.0250; 0.3980, 0.0125; 0.2250, 0.00625; 0.1223, 0.003125 (at 25°); 0.5150, 0.0200 (at 20°), which led to ϵ_0 = 43.7 l./mole-cm. and K_c = 3.38×10^{-2} moles/l. (at 20°). For 2,6-dichloronitrosobenzene (1-cm. cell, 790 mμ) in benzene the corresponding quantities were 0.4530, 0.0200; 0.2185, 0.0100; 0.1645, 0.0050; 0.0925, 0.0025 (at 30°); 0.2590, 0.0125 (at 20°), giving ϵ_0 = 44.0 l./mole-cm. and K_c = 1.048×10^{-2} moles/l. (at 20°).

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A New Preparation of Gentiobiose[1]

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Determination of the structure of complex carbohydrates by chemical and biochemical methods has necessitated the preparation of oligosaccharides for reference purposes and for use as substrates to ascertain the action pattern of carbohydrases.[2] Oligosaccharides have been obtained by direct extraction of natural products, by acid hydrolysis of polysaccharides, by chemical or enzymic synthesis, and also by enzymic degradation of polysaccharides.

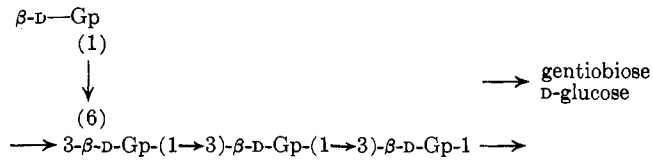
Although gentiobiose can be isolated directly from the gentian root[3,4] and prepared synthetically by chemical[5-7] and enzymatic methods,[8,9] it remains nevertheless relatively inaccessible and expensive.

The recent observation[10] that a β-D-(1→3)-glucanase, isolated from culture filtrates of *Basidiomycete* sp. QM 806,[11] completely hydrolyzes the extracellular D-glucan elaborated by a fungus of the Fungi imperfecti group to give 1 molecular proportion of gentiobiose and 2 molecular proportions of D-glucose suggested that this might form the basis of a new method for making gentiobiose.

It is of interest to note that the β-D-glucan produced by *Claviceps purpurea* behaves similarly when treated with an *exo*-laminarase preparation to give gentiobiose and glucose in a molar ratio of about 1:3.[12] Likewise sclerotan, a glucan generated by *Sclerotina libertiana*, gives glucose and gentiobiose when treated with an enzyme, a β-D-(1→3)-glucanase, isolated from cultures of this organism.[13,14]

The imperfecti D-glucan referred to above has been shown[10] to be composed of a chain of β-(1→3)-linked D-glucose units, one out of every three of which is attached to a single glucose unit by a (1→6) linkage (Scheme I). This polysaccharide was allowed to react

SCHEME I



with the *Basidiomycete* β-D-(1→3)-glucanase until maximum reducing power had been attained. The mixture of gentiobiose and glucose was then separated by charcoal column chromatography[15] either before or after treatment with yeast and the gentiobiose was crystallized as the methanolate. Since the glucan used in this work is only one of a number of structurally related polysaccharides it seems that any one of them should provide an excellent source of gentiobiose.

Experimental

Preparation of β-D-(1→3)-glucanase.—*Basidiomycete* sp. QM 806 was grown in a modified medium similar to that described by Reese and Mandels[8] with the exception that glucose rather than starch was used as the source of carbohydrate. The crude culture filtrate (1 l.) was centrifuged, concentrated 50-fold *in vacuo*

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