Substitution Reactions of the 2-Aminophenoxaz-3-one System[1]

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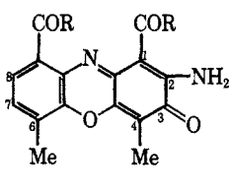
The nitration of a model 2-aminophenoxaz-3-one **2** and the bromination of a derived 2-(N,N-diacetyl)amino-3-hydroxyphenoxazine **16** are described as is a method for oxidizing the phenoxazine system back to the phenoxaz-3-one system. A novel reductive reaction of the N,N-diacetyl derivative **11** of **2** that leads to phenoxazines substituted at C-7 was observed.

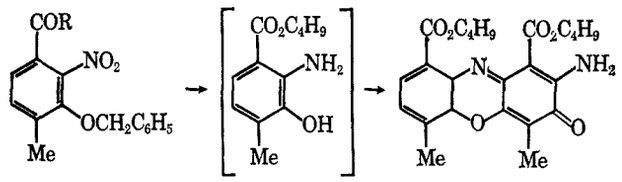
The chemical alteration of the naturally occurring actinomycins **1** is of obvious importance in studying structure-biological activity relationships in that class of compounds and in helping to clarify the mechanism of biological action of the compounds. Brockmann, in numerous papers, has elucidated much of the chemistry of the actinomycins and has reviewed these studies[2] that include work on the alteration of the C-2 amino group. Evidence exists that implicates the C-2 and C-3 functional groups in the interaction with deoxyribonucleic acid (DNA) that is probably the basis



1, R = a cyclic pentapeptide lactone
2, R = n-C_4H_9O

of the antibiotic's biological activity;[3] it would therefore be logical to attempt to change the tissue distribution of the molecule without destroying the basis for biological activity by altering the molecule at C-7 and C-8. A recent review of phenoxazinone chemistry[4] revealed no report of electrophilic substitution of a 2-aminophenoxaz-3-one which constitutes the chromophoric unit of the actinomycins, although it described the preparation of certain 7-substituted 2-aminophenoxaz-3-ones by condensation reactions. In order to establish the chemistry that could lead to C-7 and C-8 substituted analogs of **1** we chose, as a model substrate, the dibutyl ester **2** with the expectation that it would have favorable solubility in organic solvents. While the work was in progress, Müller[5] reported the biological activity of a number of 7-substituted actinomycins, without, however, giving any details of the preparation of these compounds.

Esterification of the acid **3**[6] by Brewster's method[7] gave an excellent yield of **4** which was then hydrogenated. The product **5**, without isolation, was oxidatively condensed with potassium hexacyanoferrate (III) to give **2** in yields up to 95% from **3**. The yield in the oxidation was significantly improved and the



3, R = OH
4, R = n-C_4H_9O

5 **2**

previous procedure[8] simplified by carrying out the reaction under essentially homogeneous conditions.

Direct electrophilic substitution of **2** was disappointing. Bromination in a variety of solvents yielded a very insoluble material that had incorporated three atoms of bromine. The ultraviolet spectrum of the product, however, was the same as that of **2** indicating that none of the bromine was covalently bound; the bromination product is probably a complex similar to pyridinium bromide perbromide.[9] Nitration of **2** in sulfuric acid afforded a good yield of a mixture of two products, the major of which could be purified by crystallization and shown by nmr analysis to be the 7-nitro derivative **8**; the minor product was the 8-nitro derivative. Attempted Friedel–Craft reactions or chlorosulfonylations of **2** were unsuccessful.

The Vilsmeier–Haack[10] reaction on **2** in an attempt to prepare the C-7 aldehyde gave an interesting result. The sole product, obtained in good yield, was the dichloroformamidine **7** (Scheme I). There is ample precedent for the formation of formamidines from aromatic amines with the phosphoryl chloride, N,N-dimethylformamide reagent,[11] but, because the amine group of **2** is part of a vinylogous amide system, it was surprising to observe formamidine formation. The formation of **7** is visualized as proceeding first to the formamidine **20** (Scheme II), followed by 1,8 addition of hydrogen chloride formed in the first step to form the intermediate **21**, which rearranges to the fully aromatized system **22** with final chlorine replacement to give the product **7**. This tendency in the phenoxaz-3-one system to aromatize by 1,8 addition was noted previously by Levine and Wani[12] who treated the dimethyl ester corresponding to **13** with thionyl chloride in the absence of pyridine and obtained the 2,7-dichlorophenoxazone by a further oxidation step; several other 1,8 additions are noted in the succeeding text.

In order to provide a nucleus better activated towards electrophilic substitution **2** was first acetylated, then catalytically reduced. Reaction with acetyl

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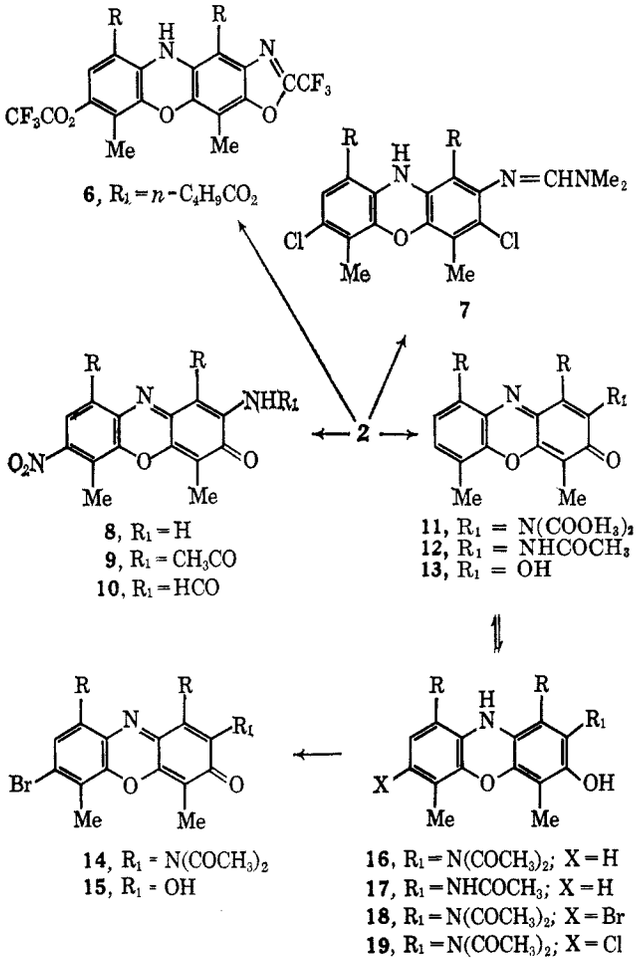
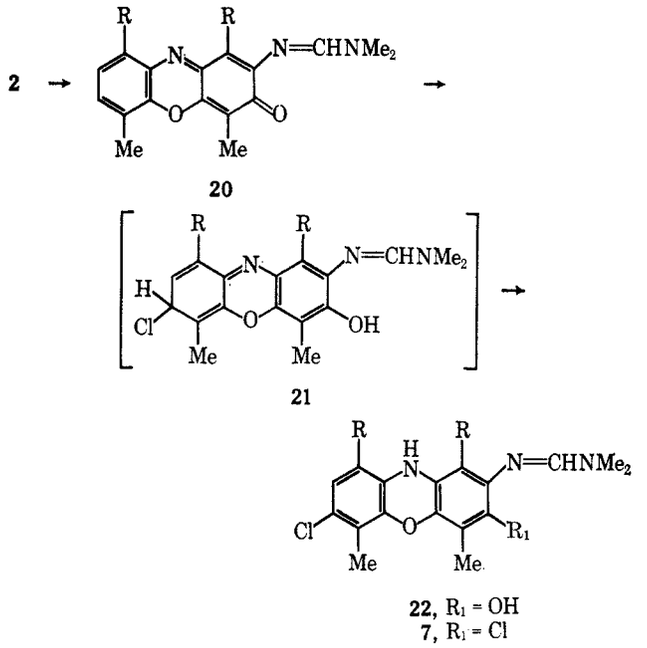
SCHEME I



SCHEME II



chloride in pyridine gave a mixture of the monoacetyl **12** and diacetyl **11** derivatives that could be separated easily by silicic acid chromatography. Very recently[13] the analogous diacetylation of an actinomycin derivative was reported; a monoacetyl compound similar to **12** has been described.[12] Catalytic hydrogenation of **11** furnished **16** as a bright yellow solid in contrast to the red **11**. Bromination of **16** in methylene chloride proceeded rapidly and yielded the 7-bromo derivative **18**. By way of contrast, however, catalytic hydrogenation of **12** afforded **17**, but attempts to brominate **17** simply oxidized it back to **12**; apparently a fully substituted 2-amino group in this phenoxazine system is required to stabilize it toward oxidation. The reaction of **11** with hydrochloric acid in an effort to hydrolyze the acetyl groups gave the 7-chlorophenoxazine **19** in a reaction similar to the conversion of **20** to **22**. The use of hydrogen bromide or hydrogen iodide with **11**, however, simply resulted in reduction to **16**.

When **2** was heated with trifluoroacetic anhydride that contained sodium trifluoroacetate the product was a yellow solid that is formulated as the 7-trifluoroacetate **6** on the basis of elemental analysis and its nmr spectrum. Presumably, trifluoroacetylation of the 2-amino group of **2** is followed by 1,8 addition of trifluoroacetic acid, then rearrangement and dehydration to give the oxazole ring. Levine and Wani[12] described similar oxazolo[4,5-*b*]phenoxazines. The yellow crystalline product **6** was unstable and decomposed to a dark red solid after 5–6 weeks at room temperature.

The oxidation of **16** to **11** could not be accomplished with a variety of common oxidizing agents including permanganate ion, dichromate ion, ferric chloride, or potassium hexacyanoferrate (III). Benzoyl peroxide in hot benzene was an excellent reagent for the oxidation and, presumably, other free-radical oxidants would be equally useful. The oxidation of the 7-bromophenoxazine **18** to **14** was carried out similarly.

We were interested in hydrolyzing the N-acetyl groups in **18** and **19** to give back the parent 2-aminophenoxaz-3-one system, but this was not possible. The instability of the phenoxaz-3-one compounds to basic reagents necessitated deacetylation by an acid-catalyzed reaction that resulted in the formation of 2-hydroxyphenoxaz-3-ones. Thus, **2** was hydrolyzed to **13** with refluxing 50% aqueous acetic acid, the same conditions under which **18** could be converted to **15**. When **11** was heated with aqueous acetic acid, **12** was formed as an intermediate that could be converted to **13** with 25% aqueous *p*-toluenesulfonic acid. The conversion of the 2-hydroxyphenoxaz-3-one system to the 2-aminophenoxaz-3-one system by a two-step chlorination–amination sequence has been described.[2,12]

The 7-nitrophenoxaz-3-one **8** could be acetylated to **9** and could be treated with formic-acetic anhydride to give **10**. Catalytic hydrogenation of **9** in acetic anhydride and pyridine yielded a triacetyl compound that we formulate as **23** although other structures are

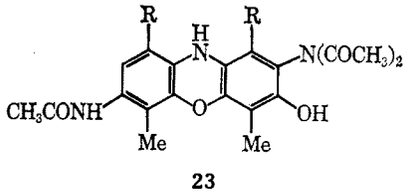


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TABLE I

NMR AND ULTRAVIOLET SPECTRA

Compd	τ, CDCl₃ 4-Me	6-Me	8-H	7-H	λ_{max}^{MeOH}, mμ (ε × 10⁻³)
2	7.82	7.52	2.53[a]	2.80[a]	433 (29.9), 238 (38.1)
6	7.82	8.15	2.93	...	434 (17.5), 252 (31.2), 228 (35.7)
7	7.82	7.86	2.65	...	407 (13.3), 235[b] (34.5)
8	7.80	7.32	1.85	...	434 (23.0), 305 (7.54), 228 (26.4)
c	(7-NO_2) 7.80	7.32	1.85	...	
	(8-NO_2) 7.79	7.47	...	1.92	
9					404 (23.9), 227 (27.7)
10					402 (12.1), 238[b] (27.8)
11	7.84	7.52	2.47[a]	2.59[a]	383 (14.2), 260[b] (15.0), 226[b] (19.8)
12	7.82	7.52	2.82[a]	3.00[a]	
13					438 (13.5), 231 (24.7)
14	7.82	7.45	2.22	...	493 (9.44), 373 (13.1), 258 (13.6), 225 (17.8)
15					419 (9.32), 363 (9.32), 229 (32.1)
16	7.78	7.91	2.75[a]	3.58[a]	397 (12.0), 230[b] (30.1), 213[b] (33.3)
17	7.75	7.89	2.75[a]	3.62[a]	408 (11.9), 234 (28.7)
18	7.78	7.84	2.45	...	403 (14.1), 237[b] (32.8)
19	7.75	7.84	2.63	...	400 (10.9), 235 (29.0)
d					429 (22.3), 237 (30.4)

[a] Geometrical center of a doublet. [b] A shoulder. [c] Product from nitration of 2 containing the 7- and 8-nitro isomers. [d] Product from the bromination of 2.

possible. The triacetate could be oxidized easily with benzoyl peroxide as shown by the characteristic yellow-to-red color change, but it gave a mixture of products according to thin layer chromatography. The ease of oxidation suggests that neither the 3-hydroxyl group nor N-10 is acetylated.

The nmr spectra of the various compounds were of great help in assigning structures. Table I details the nmr data and the ultraviolet spectra. As examples of the use of nmr in the work, the spectrum of the nitration product **8** indicated only a single aromatic proton and showed the C-6 methyl as shifted downfield τ 0.20 unit and the C-8 proton shifted downfield about τ 0.9 unit from the positions in **2** as would be expected for an adjacent nitro group; the C-6 methyl in the bromo derivative **14** was also shifted downfield from the position in **11** as would be predicted for **7** substitution. Satisfactory integrated intensities of protons were observed for all compounds.

Application of some of the chemistry that has been developed using **2** as a model is being extended to the actinomycins **1**.

Experimental Section[14]

***n*-Butyl 3-Benzyloxy-2-nitro-*p*-toluate (4).**—To a solution of 3.0 g (10.5 mmoles) of 3-benzyloxy-2-nitro-*p*-toluic acid (**3**) in 50 ml of pyridine was added 4.0 g (21.0 mmoles) of *p*-toluenesulfonyl chloride. The mixture was cooled to 0°; then 1-butanol, 0.96 ml (10.5 mmoles) was added to the stirred solution. After 4 hr the mixture was evaporated to approximately one-fourth of its volume and the residue was dissolved in 75 ml of chloroform and washed with 75-ml portions of water, 1 *N* HCl, water, saturated sodium bicarbonate solution, and water, then dried, and evaporated; the product was a light yellow oil, 3.20 g (80%).

Anal. Calcd for $C_{19}H_{21}NO_5$: C, 66.5; H, 6.17; N, 4.08. Found: C, 66.5; H, 6.20; N, 4.04.

2-Amino-1,9-di-*n*-butyloxycarbonyl-4,6-dimethylphenoxaz-3-one (2).—A solution of the butyl ester **4**, 2.80 g, in 25 ml of absolute ethanol was hydrogenated at atmospheric pressure using 200 mg of 5% Pd–C. When the theoretical volume of hydrogen had been consumed the mixture was filtered through Celite and the filtrate was immediately poured into a solution of potassium hexacyanoferrate (III), 16.6 g, in 200 ml of phosphate buffer (pH 6.99). Enough dioxane (*ca.* 100 ml) was added to maintain the mixture near homogeneity. After stirring for 1 hr at room temperature the mixture was filtered, the filter cake was dissolved in chloroform, and the solution was dried. After evaporation the residue was recrystallized from benzene giving 1.30 g (73%) of dark red needles, mp 148–148.8° dec.

Anal. Calcd for $C_{24}H_{28}N_2O_6$: C, 65.4; H, 6.41; N, 6.36; O, 21.8. Found: C, 65.9; H, 6.23; N, 6.31; O, 22.1.

In larger runs a Parr apparatus was used for the hydrogenation and yields of up to 95% of usable material were obtained.

2-N,N-Diacetylamino-1,9-di-*n*-butyloxycarbonyl-4,6-dimethylphenoxaz-3-one (11) and 2-Acetamido-1,9-di-*n*-butyloxycarbonyl-4,6-dimethylphenoxaz-3-one (12).—The amino diester **2**, 6.00 g (13.6 mmoles), was suspended in a mixture of 250 ml of benzene and 30 ml of dry pyridine, then cooled to 0°. Acetyl chloride, 50 ml was added in portions to the cooled mixture after which the reaction was heated at 55° for 18 hr. The mixture was evaporated and a chloroform solution of the residue was washed with 1 *N* HCl (until acidic), saturated aqueous bicarbonate (until basic), dried, and evaporated. Recrystallization from ethyl acetate–cyclohexane gave 2.26 g of the red-orange monoacetyl compound, **12**, mp 233–233.5, homogeneous on tlc.

Anal. Calcd for $C_{26}H_{30}N_2O_7$: C, 64.7; H, 6.27; Ac, 8.90; N, 5.81. Found: C, 64.5; H, 6.26; Ac, 8.93; N, 5.82.

The filtrate from the above crystallization was evaporated and the residue dissolved in benzene and chromatographed on 150 g of silica gel. Elution with 15% ether–benzene (v/v) gave the diacetyl **11** which after recrystallization from ethyl acetate–cyclohexane gave 2.57 g (35%) of dark red solid, mp 162–163, homogeneous on tlc.

Anal. Calcd for $C_{28}H_{32}N_2O_8$: C, 64.8; H, 6.04; Ac, 16.1; N, 5.34. Found: C, 64.8; H, 6.21; Ac, 15.6; N, 5.56.

The remainder of material was eluted from the column with ethyl acetate and after recrystallization gave an additional 0.76 g of the monoacetyl **12**, raising the yield to 46%.

11 From 16.—The reduced **16**, 250 mg, was dissolved in 15 ml of hot benzene and three 30-mg portions of benzoyl peroxide were added during 1 hr. The solution was diluted with 15 ml of benzene, cooled to room temperature, and washed with three 30-ml portions of saturated aqueous bicarbonate. After drying and evaporation, the residue from the benzene extract was recrystallized from ethyl acetate–cyclohexane giving 210 mg

(14) Melting points are uncorrected and were obtained with a Thomas–Hoover Capillary Melting point apparatus. Magnesium sulfate was used as the drying agent and all evaporations were conducted *in vacuo*. Nuclear magnetic resonance spectra were determined on a Varian HA-100 or A-60 instrument and deuteriochloroform was used as a solvent with tetramethylsilane as an internal reference. Thin layer chromatography (tlc) was carried out on Brinkman silica gel HF, 0.25 mm thick. Visible and ultraviolet detection was used. The solvent system benzene–ether, 7:3 (v/v), was used for tlc. Gallard-Schlesinger silica gel (90–200 mesh) was used for column chromatography.

(84%) of **11**, identical with the compound prepared above by melting point, infrared spectrum, and tlc.

1,9-Di-*n*-butyloxycarbonyl-2-hydroxy-4,6-dimethylphenoxaz-3-one (13). A.—The amino diester **2**, 0.750 g (1.70 mmoles), was dissolved in 100 ml of glacial acetic acid then 100 ml of water was added and the mixture was refluxed for 4 hr and allowed to stand overnight at room temperature. The mixture was diluted with 100 ml of water and extracted with 100 ml of chloroform. The chloroform extract was dried and evaporated and the residue was recrystallized from benzene giving 0.669 g (89%) of dark red solid, mp 231–231.8.

Anal. Calcd for $C_{24}H_{27}NO_7$: C, 65.3; H, 6.17; N, 3.17. Found: C, 64.9; H, 5.79; N, 3.23.

B. From 11.—The diacetyl **11**, 300 mg, was hydrolyzed in acetic acid–water as described above. The product, according to tlc, was the monoacetyl **12**. Hydrolysis was continued with 15 ml of 25% *p*-toluenesulfonic acid in water (w/w) at reflux for 2.5 hr. Chloroform was added and, after separation of the layers, the chloroform extract was washed with saturated aqueous bicarbonate, dried, and evaporated. The residue was recrystallized from benzene giving 150 mg (60%) of solid identical with **13** as prepared above, according to melting point and infrared spectrum.

Reaction of 2 with Bromine.—To a solution of 166 mg of **2** in 10 ml of chloroform was added dropwise a solution of 0.2 ml of bromine in 5 ml of chloroform. The precipitate, which formed immediately, was collected and recrystallized from chloroform giving 166 mg of dark red solid, mp > 300°.

Anal. Calcd for $C_{24}H_{28}N_2O_8 \cdot Br_3$: C, 42.4; H, 4.15; Br, 35.3; N, 4.12. Found: C, 42.8; H, 4.40; Br, 35.2; N, 4.16.

2-N,N-Diacetylamino-1,9-di-*n*-butyloxycarbonyl-3-hydroxy-4,6-dimethylphenoxazine (16).—A solution of 2.00 g of the diacetyl **11** in 25 ml of acetic acid was hydrogenated at atmospheric pressure using 200 mg of 5% Pd–C. When the uptake of hydrogen ceased the mixture was diluted with 100 ml of chloroform and filtered through Celite. The filtrate was evaporated and the residue was recrystallized from benzene giving 1.49 g (74%) of yellow solid, mp 176–177.

Anal. Calcd for $C_{28}H_{34}N_2O_8$: C, 63.9; H, 6.51; N, 5.32. Found: C, 63.9; H, 6.38; N, 5.37.

2-Acetamido-1,9-di-*n*-butyloxycarbonyl-3-hydroxy-4,6-dimethylphenoxazine (17).—A solution of 511 mg of the acetamido **12** in 10 ml of glacial acetic acid was hydrogenated and worked up as in the preparation of **16**, above. Recrystallization from cyclohexane gave 329 mg of yellow solid, mp 126–127. A second crop of 80 mg was obtained for a total yield of 80%.

Anal. Calcd for $C_{26}H_{32}N_2O_7$: C, 64.4; H, 6.66; N, 5.78. Found: C, 64.6; H, 6.55; N, 5.83.

2-N,N-Diacetylamino-7-bromo-1,9-di-*n*-butyloxycarbonyl-4,6-dimethylphenoxazine (18).—A solution of 0.5 ml of bromine in 10 ml of methylene chloride was added in portions to a solution of 337 mg of the reduced diacetyl **16** in 15 ml of the same solvent until the dark green color (presumably the protonated form) persisted for 10 min. The solution was washed with a mixture of saturated aqueous sodium thiosulfate and saturated aqueous sodium bicarbonate, dried, and evaporated. Recrystallization of the residue from benzene gave 308 mg (78%) of a yellow solid, mp 216.5–217.

Anal. Calcd for $C_{28}H_{33}BrN_2O_8$: C, 55.5; H, 5.49; Br, 13.2; N, 4.63. Found: C, 55.6; H, 5.46; Br, 13.7; N, 4.76.

2-N,N-Diacetylamino-7-bromo-1,9-di-*n*-butyloxycarbonyl-4,6-dimethylphenoxaz-3-one (14).—To 30 ml of a hot benzene solution of 355 mg of the reduced bromo **16** was added *ca.* 20 mg of benzoyl peroxide. The solution was refluxed for 2 hr during which time two more portions of benzoyl peroxide were added. The solution was cooled to room temperature, washed with three 25-ml portions of saturated aqueous sodium bicarbonate, dried, and evaporated. The red, oily residue was recrystallized from cyclohexane giving, in two crops, 302 mg (85%) of a red solid, mp 141–143.

Anal. Calcd for $C_{28}H_{31}BrN_2O_8$: C, 55.7; H, 5.18; Br, 13.2; N, 4.64. Found: C, 56.1; H, 5.29; Br, 13.6; N, 4.71.

2-Amino-1,9-di-*n*-butyloxycarbonyl-7-nitrophenoxaz-3-one (8).—To 25 ml of concentrated sulfuric acid cooled to 0° was added 3.00 g of the aminodiester **2**, followed by the dropwise addition of a solution of 0.35 ml of fuming nitric acid in 2 ml of sulfuric acid. After stirring for 30 min at 0° the mixture was poured into a well-stirred mixture of 250 ml each of ice water and chloroform. After separation of the layers the chloroform layer was washed with saturated aqueous sodium bicarbonate (until basic), dried, and evaporated. The residue was recrystallized from benzene–ethanol giving a first crop of 1.50 g; this darkens at 164–165 but does not melt to 300°. A tlc showed this material to be homogeneous.

Anal. Calcd for $C_{24}H_{27}N_3O_8$: C, 59.4; H, 5.61; N, 8.66. Found: C, 59.7; H, 5.65; N, 8.51.

The mother liquor from the above crystallization yielded a second crop of 1.30 g of solid. A tlc of the material showed three spots at R_f 0.83 (7-nitro, **8**), 0.75 (8-nitro), and 0.60 (starting material, **2**). This mixture was renitrated as above except that the reaction was allowed to proceed for 1 hr. After work up as before a tlc showed no starting material and recrystallization gave 0.74 g of pure **8**. The mother liquor from this crystallization was evaporated and the residue recrystallized from ethyl acetate giving 496 mg, mp 189–191 with darkening from 175°. The nmr of this material showed a ratio of 7-nitro derivative **8** to the 8-nitro of 1.63. The total yield of pure **8** was 68% and of total nitro compounds was 83%.

2-Acetamido-1,9-di-*n*-butyloxycarbonyl-4,6-dimethyl-7-nitrophenoxaz-3-one (9).—The nitro compound **8**, 617 mg, was suspended in a mixture of benzene, 35 ml, and pyridine, 3 ml, then was cooled to 0°. Acetyl chloride, 30 ml, was added in portions to the stirred, cooled mixture after which the reaction was allowed to proceed at 55° for 18 hr. Work-up was the same as in the preparation of **11**. A tlc showed two spots, presumably the mono- and diacetyl compounds. Removal of the second acetyl group was accomplished by dissolving the solid in 30 ml of ethyl acetate containing a little 1-butanol and stirring overnight with neutral alumina, 3 g. After filtration the filtrate appeared homogeneous on tlc. Recrystallization from ethyl acetate–cyclohexane gave 326 mg (49%) of orange solid, mp 194–195.

Anal. Calcd for $C_{26}H_{29}N_3O_9$: C, 59.2; H, 5.54; N, 7.97. Found: C, 59.2; H, 5.67; N, 7.94.

1,9-Di-*n*-butyloxycarbonyl-2-formamido-4,6-dimethyl-7-nitrophenoxaz-3-one (10).—Formic–acetic anhydride was prepared by mixing 14.3 ml of acetic anhydride and 6.1 ml of formic acid at 0° then stirring at room temperature for 15 min and finally at 50° for 15 min. The mixed anhydride was cooled to 0°, 445 mg of the nitro **8** was added, and the reaction was allowed to proceed at 60° for 20 hr. After evaporation the residue was recrystallized from acetone giving a first crop, 219 mg, of red-brown solid, mp 274, sinters at 230°, and a second crop 96.3 mg, mp 230, resolidifies and melts at 274°. These two crystalline forms were identical in infrared spectra and on tlc. The combined yield was 67%. The first crop was used for analysis.

Anal. Calcd for $C_{25}H_{27}N_3O_9$: C, 58.5; H, 5.30; N, 8.18. Found: C, 59.2; H, 5.67; N, 7.94.

N-(3,7-Dichloro-1,9-di-*n*-butyloxycarbonyl-4,6-dimethylphenoxazin-2-yl)-N′,N′-dimethylformamidine (7).—To 10 ml of N,N-dimethylformamide (DMF) cooled to −15° was added 0.50 ml of phosphoryl chloride and the solution was stirred in the cold for 1 hr. The amino diester **2**, 500 mg, was dissolved in 4 ml of DMF and was added to the cold solution of the complex, then the resulting mixture was stirred at room temperature for 3 hr. After this time the reaction mixture was poured into an ice-cold mixture of 25 ml each of chloroform and saturated aqueous bicarbonate. After separation the bicarbonate extract was washed with three 10-ml portions of chloroform. The combined chloroform extracts were dried and evaporated. The residue was recrystallized from cyclohexane giving 293 mg of bright yellow solid, mp 145–146°. A second crop of 35.4 mg was obtained for a total yield of 53%.

Anal. Calcd for $C_{27}H_{31}Cl_2N_3O_5$: C, 58.9; H, 6.04; Cl, 12.9; N, 7.63. Found: C, 59.2; H, 5.79; Cl, 13.0; N, 7.74.

2-N,N-Diacetyl-1,9-di-*n*-butyloxycarbonyl-7-chloro-3-hydroxy-4,6-dimethylphenoxazine (19).—The N,N-diacetyl **11** was dissolved in mixture of chloroform, 15 ml, and 1-butanol, 10 ml, and dry hydrogen chloride gas was passed through the solution. After 15 min the solution was evaporated; the residuew as dissolved in chloroform and washed with saturated aqueous bicarbonate. The chloroform extract was dried and evaporated, and the residue was recrystallized from ethyl acetate–cyclohexane giving 174 mg (56%) yellow solid, mp 203–204.5.

Anal. Calcd for $C_{28}H_{33}ClN_2O_8$: C, 59.9; H, 5.93; Cl, 6.32; N, 4.99. Found: C, 60.2; H, 6.26; Cl, 5.85; N, 4.99.

7-Bromo-1,9-di-*n*-butyloxycarbonyl-2-hydroxy-4,6-dimethylphenoxaz-3-one (15).—The bromo diacetyl **14**, 200 mg, was dissolved in 15 ml of acetic acid followed by the addition of 15 ml of water. The mixture was refluxed overnight, then diluted

with 30 ml of water and extracted with chloroform. The chloroform extract was washed with saturated aqueous bicarbonate, dried, and evaporated. The oily residue was dissolved in benzene and passed through a short column of silica gel. The benzene eluent was evaporated and the residue was recrystallized from cyclohexane giving 50 mg (27%) of red-brown solid.

Anal. Calcd for $C_{24}H_{26}BrNO_7$: C, 55.4; H, 5.04; N, 2.69. Found: C, 55.9; H, 5.04; N, 2.90.

4,6-Di-*n*-butyloxycarbonyl-8-trifluoroacetoxy-2-trifluoromethyl-9,11-dimethyl-5H-oxazolo[4,5-*b*]phenoxazine (6).—The amino diester **2**, 520 mg, was dissolved in 20 ml of trifluoroacetic anhydride that contained 1 g of dry sodium trifluoroacetate. The mixture was stirred at 45° for 18 hr, then an equal volume of benzene was added, and the mixture was evaporated. The residue was dissolved in chloroform, washed with water, dried, and evaporated. Recrystallization of the residue from ethyl acetate–*n*-hexane gave 599 mg (79%) of brilliant yellow solid, mp 201–202.

Anal. Calcd for $C_{28}H_{26}F_6N_2O_8$: C, 53.2; H, 4.14; F, 18.0; N, 4.43. Found: C, 53.2; H, 4.31; F, 17.7; N, 4.63.

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Molecular Rotations of Poly-*O*-acetyl (or Benzoyl) Carbohydrates in Relation to Their Structures. The Rules Which Even D-Mannose Derivatives Obey

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The molecular rotation of a poly-*O*-acetyl- or -benzoylglycopyranosyl halide is plotted against the atomic refraction of the halogen. It becomes obvious that straight lines can be obtained here and, moreover, if a definite proper value of the abscissa is given to hydrogen, the molecular rotations of the corresponding "hydrides" can exist on the above straight lines. Next, the inclinations of these lines are discussed from the viewpoint of the structural formulas of their corresponding compounds and a new empirical rule is obtained. D-Mannose derivatives show no optical abnormalities throughout the course of this study.

Brauns found[1] that, for the poly-*O*-acetylglycopyranosyl halides of four monosaccharides (glucose, fructose, xylose, and arabinose), the differences in specific rotation (but not the molecular rotation) for Cl − F, Br − Cl, and I − Br are proportional to the corresponding differences in atomic diameters. He proved afterwards however, that this rule is not applicable to the mannose derivatives.[2] Concerning the hepta-*O*-acetylglycopyranosyl halides of disaccharides, Brauns concluded[3] that the derivatives of melibiose and maltose follow the atomic dimension relationship, whereas those of the other three [gentiobiose, cellobiose, and 4-*O*-(β-D-glucopyranosyl)-α-D-mannose] agree with this relationship only when the fluorine derivatives are excluded.

In 1924, Hudson reported[4] that the difference between the molecular rotation of a poly-*O*-acetylglycopyranosyl halide and half the sum of the molecular rotations of anomers of the corresponding acetates is approximately constant for a definite kind of halogen, regardless of the parent sugar. In this case also, a deviation was noticed in the mannose derivatives. Later, Hudson used[5] the value of the molecular rotation of the 1,5-anhydride of the corresponding poly-*O*-acetylalditol, in place of the above-mentioned half of the sum.

Korytnyk recalculated[6] the partial molecular rotation of the (C-1–Cl) moiety in poly-*O*-acetylaldopyranosyl chloride molecules, and he also pointed out that the values in both D-mannose and D-xylose derivatives are different from those in the other sugar derivatives.

In this article, the author has first compared the values of the molecular rotation, $[M]^{20}D$ (in chloroform), not only of the poly-*O*-acetylglycopyranosyl halides but also of the poly-*O*-benzoylglycopyranosyl

(1) D. H. Brauns, *J. Am. Chem. Soc.*, **45**, 2381 (1923).
(2) D. H. Brauns, *ibid.*, **53**, 2004 (1931).
(3) D. H. Brauns, *ibid.*, **51**, 1820 (1929).
(4) C. S. Hudson, *ibid.*, **46**, 462 (1924).
(5) H. G. Fletcher, Jr., and C. S. Hudson, *ibid.*, **71**, 3682 (1949).
(6) W. Korytnyk, *J. Chem. Soc.*, 650 (1959).

TABLE I

$[M]^{20}D$ (IN CHLOROFORM) OF POLY-*O*-ACETYLALDOPYRANOSYL COMPOUNDS (MONOSACCHARIDES)

Derivative of	Compd	$[M]^{20}D$ for X at C-1 H	F	Cl	Br	I
2,3,4-Tri-*O*-acetyl-						
β-L-arabinose	1	193.1[a]	384.5[b]	720.2[b]	961.8[c]	1309.4[b]
α-D-xylose	2	0.0[d]	187.1[e]	504.5[f]	718.6[f]	v
β-D-xylose	2′	0.0[d]	v	−415.5[g,w]	v	v
β-D-ribose	3′	0.0[h]	v	−499.8[i,w]	−709.8[i,w]	v
α-D-rhamnose	4	−131.9[k]	v	392.2[l]	582.7[m]	v
2,3,4,6-Tetra-*O*-acetyl-						
α-D-galactose	5	163.2[n]	v	651.0[g,w]	892.3[o]	v
β-D-galactose	5′	163.2[n]	v	54.6[g]	v	v
α-D-glucose	6	129.3[p]	315.6[f]	615.6[f]	813.4[f]	1087.8[f]
β-D-glucose	6′	129.3[p]	70.1[q]	−29.3[g]	v	v
α-D-mannose	7	−140.9[r]	75.3[s]	328.6[g]	541.1[s]	872.9[s]
β-D-mannose	7′	−140.9[r]	v	−125.1[g,w]	v	v
α-D-talose	8	−53.8[t]	v	v	681.0[u]	v

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