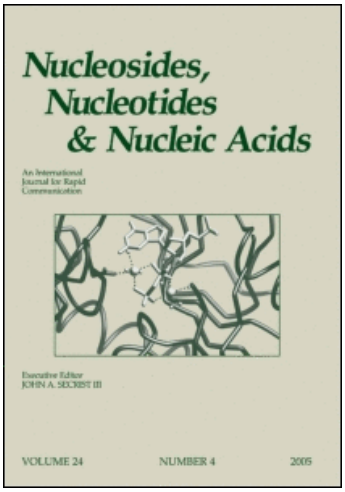

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6,7-Dimethyl-3-β-D-Erythrofuranosyl-1-Phenyl- and 1-p-Fluorophenyl-Pyrazolo[3,4-b]QUINOXALINE*

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6,7-DIMETHYL-3-β-D-ERYTHROFURANOSYL-1-PHENYL- AND 1-p-FLUOROPHENYL-PYRAZOLO[3,4-b]QUINOXALINE*

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Abstract - The C-nucleoside analogs 6,7-dimethyl-3-β-D-erythrofuranosyl-1-phenylpyrazolo[3,4-b]quinoxaline 4 and 3-β-D-erythrofuranosyl-1-p-fluorophenylpyrazolo[3,4-b]quinoxaline 8 were prepared by dehydration of the polyhydroxyalkyl chain of 6,7-dimethyl-1-phenyl-3-(D-arabino-tetritol-1-yl)-pyrazolo[3,4-b]quinoxaline 3 and 1-p-fluorophenyl-3-(D-arabino-tetritol-1-yl)-pyrazolo[3,4-b]quinoxaline 7, respectively. The structure and anomeric configuration of the products were determined by n.m.r. spectroscopy. The mass spectra and biological activities in connection with chemical constitution are discussed.

C-Nucleosides are a group of C-gylcosylated heterocycles in which the anomeric carbon is attached to the heterocycle by a carbon-carbon bond. This linkage is more stable towards hydrolytic and enzymatic reagents than the carbon-nitrogen bond of N-nucleosides, which makes C-nucleosides powerful tools for biochemical investigations and antimitotic or antiviral research[2]. Few members of this class of naturally occurring compounds such as showdomycin, formycin, and oxazinomycin are known and possess diverse biological properties

*C-Nucleoside pyrazolo[3,4-b]quinoxaline analogs Part III. For Part II, see ref. 1.

that are, in several instances, of medical significance. The frequently important biological properties of these substances have made them interesting targets for chemical synthesis, but as yet this has proved to be a more formidable task than the preparation of N-nucleosides.

The synthesis of C-nucleosides by dehydrative cyclization of the polyhydroxyalkyl chain of the saccharide heterocyclic derivative has been used recently[3] as a facile route for the synthesis of these compounds. The main problem militating against the extensive use of this reaction in the field of C-nucleosides has been that of determination of the anomeric configuration of the products. This problem has been solved recently[3,4] by high resolution n.m.r. spectroscopy.

Pyrazolo[3,4-b]quinoxaline derivatives are of potential biological interest. Some of these derivatives have pharmacological properties and show tuberculostatic activity *in vitro*[5]. The chemotherapeutic properties of saccharide pyrazolo[3,4-b]quinoxaline analogs have not been thoroughly investigated. Cyclization of their polyhydroxylakyl chain provides[6] a novel method for C-nucleoside pyrazolo[3,4-b]quinoxaline synthesis with modification of the biological activities of the precursor polyhydroxyalkyl analogs. Substitution at the base moiety is expected to exert analogous effect on the biological properties.

Organic fluoro compounds are of biological importance because of the extraordinary stability of the carbon-fluorine bond in biological systems[7]. The number of naturally occurring fluorine compounds are very rare and replacement of hydrogen atom by fluorine in naturally occurring compounds alters dramatically their biological properties. In this work the synthesis of two types of saccharide pyrazolo[3,4-b]-quinoxaline analogs have been explored, to study the relation between their chemical constitution and biological activities. The first type has two methyl substituents at the quinoxaline part of the pyrazolo-[3,4-b]quinoxaline base moiety. The second type contains fluorine substituent at the pyrazole part. The two types of saccharide pyrazolo-[3,4-b]quinoxaline analogs were converted into the corresponding C-nucleoside analogs, and their structure and anomeric configuration were determined by mass spectrometry and n.m.r. spectroscopy. The correlation between the modified chemical constitution and biological activity is discussed.

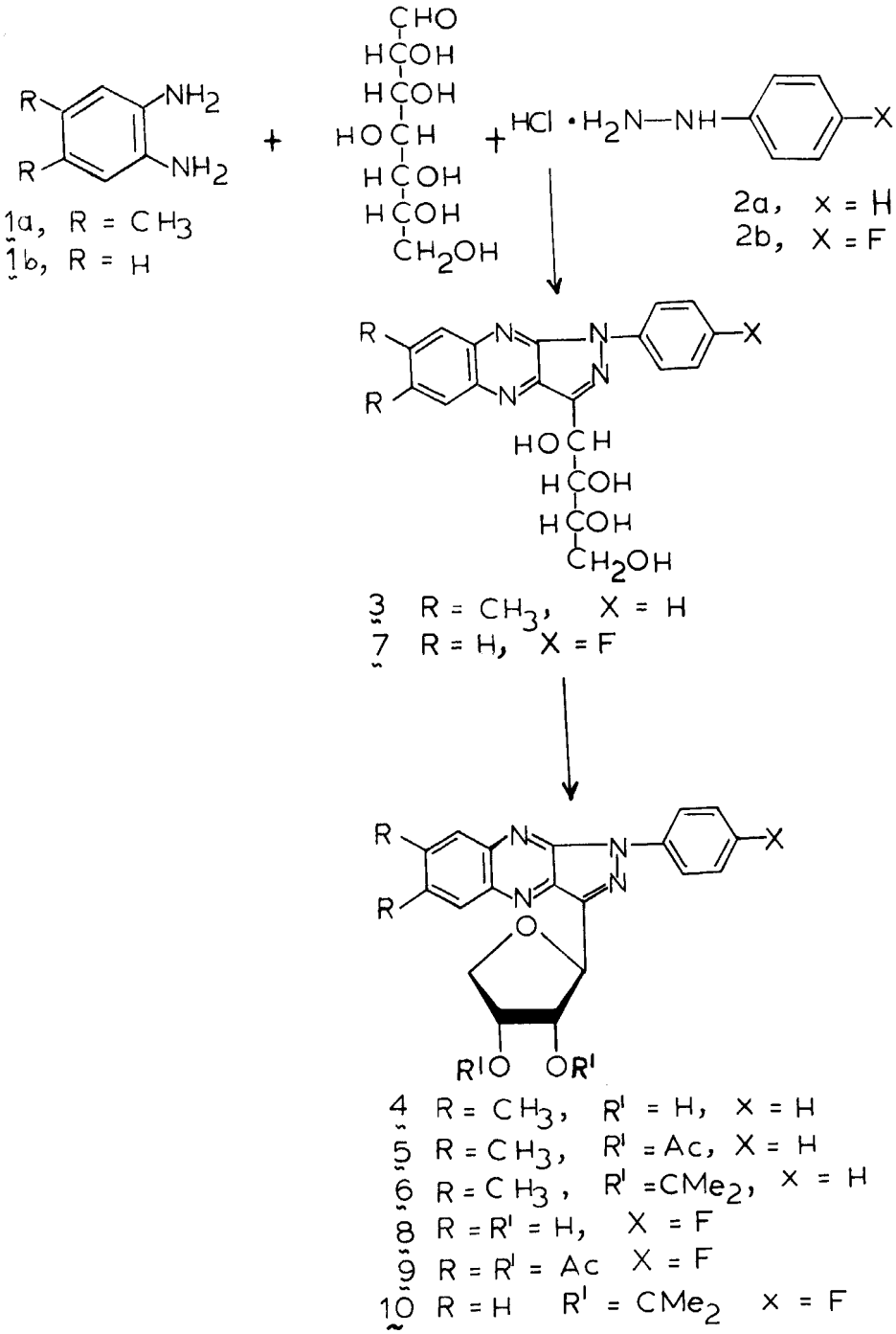
DISCUSSION

Condensation of 4,5-dimethyl-o-phenylenediamine 1a, D-glycero-D-gulo-heptose, and phenylhydrazine hydrochloride 2a afforded 6,7-dimethyl-1-phenyl-3-(D-arabino-tetritol-1-yl)-pyrazolo[3,4-b]quinoxaline 3, in a one flask reaction, similar to the reaction with o-phenylenediamine[6] (see Scheme 1). Refluxing compound 3 with methanolic sulfuric acid solution (with monitoring of the reaction by t.l.c.) afforded the C-nucleoside analog, namely, 6,7-dimethyl-3-(β-D-erythrofuranosyl)-1-phenylpyrazolo-[3,4-b]quinoxaline 4. Its n.m.r. spectrum showed the anomeric proton as a doublet at δ 5.19 having $J_{1',2'}$ 6.8 Hz. This coupling-constant value made the anomeric assignment of compound 4 uncertain[8,9], as it agrees with either a cis or trans arrangement for H-1' and H-2' of the D-erythrofuranosyl group. Acetylation of compound 4 gave the di-O-acetyl derivative 5, whose n.m.r. spectrum showed the anomeric proton as a doublet at δ 5.67, with little decrease in the value of the coupling constant ($J_{1',2'}$ 6.2 Hz). However, the isopropylidene derivative 6, showed the anomeric proton as a singlet which is unequivocally consistent[8,9] with the trans arrangement of H-1' and H-2', that is, the β-D-erythro configuration.

Additional evidence for the β-D-configuration was obtained from the value of the difference (Δδ) between the chemical shift of the methyl signals of the 2,2-dimethyldioxolane ring; the difference 0.20 (1.664-1.464) between the chemical shifts of the two methyl protons of 6 is consistent[10-12] with the β-D-configuration.

The higher negative specific rotation of the C-nucleoside 4, $[\alpha]_D^{20}$ -117.4°) compared to the precursor 3 ($[\alpha]_D^{20}$ +11.1°) confirms the β-D-configuration for compound 4, and illustrates the inversion in the configuration of C-1' of the precursor 3 during the dehydrative cyclization process. This inversion was confirmed by circular dichroism (c.d.) studies for the phenyl analog[6].

The second type of saccharide pyrazolo[3,4-b]quinoxaline analog was prepared by condensation of o-phenylenediamine 1b, D-glycero-D-gulo-heptose, and p-fluorophenylhydrazine hydrochloride 2b giving 1-p-fluorophenyl-3-(D-arabino-tetritol-1-yl)-pyrazolo[3,4-b]quinoxaline 7. Refluxing 7 with methanolic sulfuric acid solution, with monitoring of the reaction by t.l.c., afforded the C-nucleoside analog, namely, 3-β-D-erythrofuranosyl-1-p-fluorophenylpyrazolo[3,4-b]quinoxaline 8.



SCHEME 1

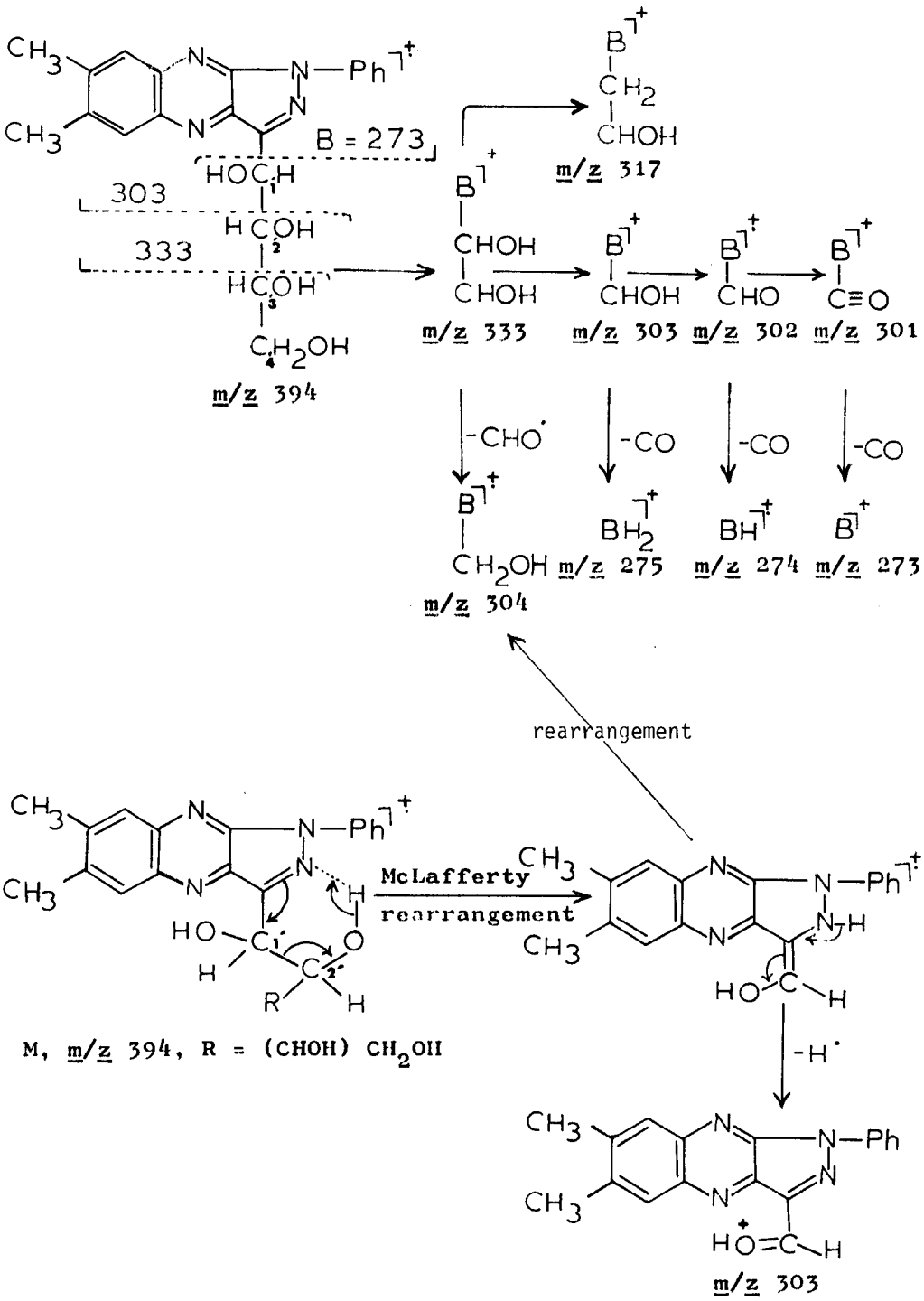
Its n.m.r. spectrum showed the anomeric proton at δ 5.25 having $J_{1',2'}$ 7.0 Hz, which cannot ascertain[8,9] the anomeric configuration. Acetylation of 8 afforded the di-O-acetyl derivative 9, whose n.m.r. spectrum showed the anomeric proton as a doublet at δ 5.68 with a little decrease in the value of the coupling constant ($J_{1',2'}$ 6.6 Hz). However, the isopropylidene derivative 10, showed the anomeric proton as a singlet at δ 5.85 which unequivocally ascertain[8,9] the β-D-configuration. The difference, $\Delta\delta$ = 0.202 (1.665 - 1.463), between the two methyl signals of the 2,2-dimethyldioxolane ring of compound 10 confirmed the β-D-configuration.

The high negative specific rotation for compound 8 ($[\alpha]_D^{20}$ -124.4°) supports the β-D-configuration and the inversion of C-1' of the acyclic precursor 7 ($[\alpha]_D^{20}$ +11°).

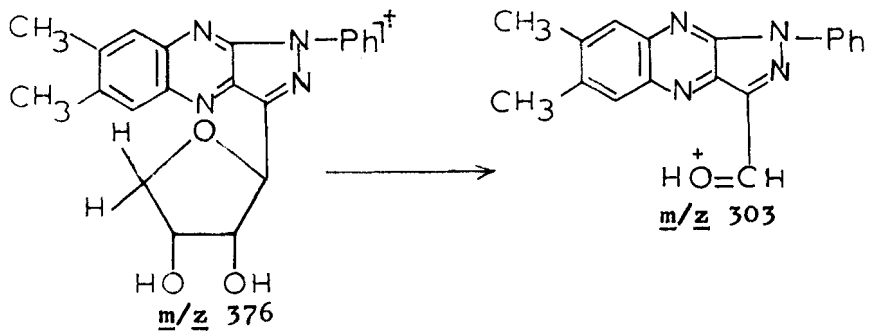
From the present results it seems that the dehydrative cyclization of saccharide pyrazolo[3,4-b]quinoxaline analogs is a stereospecific process and the stereo-course of the reaction is not affected by substitution in the base moiety. The stereoselective dehydration process[1,6] produces the favored β-anomer having the trans arrangement between the base moiety and 2'-hydroxyl group of the furanosyl group formed.

The mass spectrum of compound 3, showed molecular ion M of low intensity at m/z 394. The fragment at m/z 333 is obtained by $C_{2'}$-$C_{3'}$-cleavage of the polyhydroxyalkyl chain. The abundant fragments at m/z 303 ($BCH\overset{+}{O}H$) and 304 (BCH_2OH) are formed by $C_{1'}$-$C_{2'}$-cleavage of the polyhydroxyalkyl chain by McLafferty rearrangement of the molecular ion (see Scheme 2). The peak at m/z 303 corresponding to ($BCH\overset{+}{O}H$) which has a stable benzylic structure was shown as the base peak. The fragments at m/z 273 (B), 274 (BH), and 275 (BH_2) are obtained by B-$C_{1'}$-cleavage of the polyhydroxyalkyl chain and hydrogen transfer to the base moiety which are common for the framentation of polyhydroxyalkyl nitrogen heterocyclic analogs, than for the cyclized C-nucleoside derivatives (see the Experimental Section). The BH_2 fragment usually shows higher abundance compared to the other base fragments.

The mass spectrum of the C-nucleoside 4, showed the molecular-ion peaks M and (M + 1) at m/z 376 and 377, respectively. The base peak occured at m/z 303, corresponding to $BCH\overset{+}{O}H$, and is formed as shown in Scheme 3. This fragment is characteristic for C-nucleosides and is an indication of the carbon-carbon linkage[13]. The fragments at m/z 303



SCHEME 2



SCHEME 3

(BCHOH), 304 (BCH_2OH), 248 (BH_2 - HCN), 275 (BH_2), 274 (BH), and 273 (B) are characteristic for 6,7-dimethylpyrazolo-[3,4-b]quinoxaline analogs.

The di-O-acetyl derivative 5, showed the molecular ion peak M at m/z 460. The base peak was shown at m/z 341 corresponding to (M - H - 2 OAc) and is considered, together with the abundant peak at m/z 342 (M - 2 OAc), indicative of the furanosyl group and are common for the fragmentation of the acetyl derivatives of C-nucleoside pyrazolo[3,4-b] quinoxaline analogs[6]. The acetylium ion (CH_3CO), which is characteristic for acetyl derivatives was shown as a high abundance peak at m/z 43. The peaks at m/z 303 (BCHOH), 304 (BCH_2OH), 275 (BH_2), 274 (BH), 273 (B), and 248 (BH_2 - HCN) were of less abundance than that for compound 4.

The mass spectrum of the isopropylidene derivative 6 did not show molecular ion (M) or the ion (M - CH_3). However, the ions (M - CH_3COCH_3) and (M - $CH_3C{=}OHCH_3$), observed for the phenyl analog[6], were shown at m/z 358 and 357, respectively. The base peak was shown at m/z 43 corresponding to the acetylium ion CH_3CO^+.

The mass spectrum of compound 7, showed the molecular ion at m/z 384. Its fragmentation pattern is identical to that of compound 3. The fragment obtained by $C_{2'}$-$C_{3'}$-cleavage of the polyhydroxyalkyl chain was shown as a weak peak at m/z 323. The abundant fragments at m/z 293 and 294 corresponding to B'CHOH and B'CH_2OH were obtained by identical $C_{1'}$-$C_{2'}$-cleavage and McLafferty rearrangement as indicated for compound 3 (see Scheme 2). The peak at m/z 293 was shown as the base peak. The fragments B', B'H, B'H_2, obtained by B-$C_{1'}$-cleavage of the polyhydroxy-

alkyl chain and subsequent hydrogen transfer to the base moiety, were shown at m/z 263, 264, and 265, respectively. The $B'H_2$ fragment showed the highest abundance between the base fragments. The fluorine substituent was detected by the presence of the fragments at m/z 121 (C_6H_4FCN), 111 ($C_6H_4FNH_2$), 110 (C_6H_4FNH), 109 (C_6H_4FN), 96 (C_6H_5F), and 95 (C_6H_4F). The corresponding fragments obtained by loss of fluorine atoms were also observed (see Experimental Section).

The di-O-acetyl derivative 9, did not show molecular ion, and the highest mass fragment at m/z 391 corresponds to (M - OAc). The fragment (M - H- 2 OAc) characteristic for furanosyl pyrazolo[3,4-b] quinoxaline C-nucleoside acetyl derivatives[6], was shown at m/z 331. Fragments corresponding to $B'CHOH^+$ and $B'CH_2OH$ were of less abundance than that for compound 7. The base peak was shown at m/z 43 corresponding to the CH_3CO group.

BIOLOGICAL ACTIVITY

Biological activity studies in vitro showed cytotoxicity against KB cells (a human epidermoid carcinoma of the nasopharynx, cell culture) for the fluorinated C-nucleoside 8, whereas the C-nucleoside 4 was inactive. Biological studies in vivo against P388 mouse lympocytic leukemia cells for compounds 3, 4, 7, and 8, did not show activity. Against the leukemia screening test was 3PS31, the acyclic precursors 3 and 7 did not show activity, however, the C-nucleoside analog 4, showed toxisity at 50, 25, and 12.5 mg/kg doses, and the fluorinated analog 8, indicated toxisity at 200, 100, 50 mg/kg doses.

These results indicate that modification of the structure of the polyhydroxyalkyl-pyrazolo[3,4-b]quinoxaline analogs by cyclization of the polyhydroxyalkyl chain to attain the C-nucleoside structure, enhanced the biological activity. On the other hand, modification of the base moiety by substitution at the quinoxaline part by two methyl groups, or at the pyrazole part by fluorine atom, showed the same effect.

EXPERIMENTAL

General. Evaporations were performed under diminished pressure below 60°. T.l.c. was conducted on silica gel (Kieselgel G, Merck) with 3:1 benzene-ethanol. I.r. absorption spectra were recorded with

a unicum SP 1025 and Perkin-Elmer 467 instruments. N.m.r. spectra were recorded with a Nicolet 470 MHz instrument, using internal tetramethylsilane as the reference. Mass spectra were recorded with a Finnigan 6100 Data System Gas-Chromatograph/e.i.-c.i. spectrometer. Combustion analyses were performed in the department of Chemistry, Purdue University.

6,7-Dimethyl-1-phenyl-3-(D-arabino-tetritol-1-yl)-pyrazolo[3,4-b]quinoxaline (3). A solution of D-glycero-D-gulo-heptose[14] (10 g) in water (1300 mL) was heated with 4,5-dimethyl-o-phenylenediamine (1a; 6.5 g), phenylhydrazine hydrochloride (2a, 35 g), and acetic acid (11 mL) in a sealed flask for 10 h in a boiling water-bath. The flask was cooled, opened, and the yellow precipitate was filtered off, washed with water, 50% ethanol, and ether, and dried; yield 7 g. The mother liquor was further heated for 24 h, in a sealed flask, giving further 3.2 g; total yield 10.2 g (54%). Recrystallization from propyl alcohol gave yellow needles, m.p. 237-239°; R_F 0.38; $[\alpha]_D^{20}$ +11.1° (c 1.9, pyridine); ν_{max}^{KBr} 3340 (OH), and 1605, 1560 cm^{-1} (C=N); mass-spectral data (selected ions): m/z 394 (1, M), 334 (1, M - $CHOCH_2OH$), 333 (2, M - $CHOHCH_2OH$), 317 (2, BCH_2CHOH), where B=6,7-dimethyl-1-phenylpyrazolo(3,4-b)quinoxaline, 305 (10), 304 (56, BCH_2OH), 303 (100, $BCHOH^+$), 302 (11, BCHO), 301 (2, BCO), 288 (2, BCH_3), 287 (5, BCH_2), 276 (7), 275 (30, BH_2), 274 (11, BH), 273 (25, B), 260 (6, BH_2 - CH_3), 258 (BH - CH_3), 249 (3, BH_2 - CN), 248 (17, BH_2 - HCN), 246 (B - HCN), 233 (4, B - CN_2), 170 (2, B - PhCN), 156 (3, B - CN_2Ph), 117 (2, $PhCN_2$), 104 (2, PhCNH), 103 (3, PhCN), 91 (4 PhN), 77 (11, Ph), and 43 (30, CH_3CO). For n.m.r. spectral data see Table 1.

Anal. Calc. for $C_{21}H_{22}N_4O_4$: C, 63.45; H, 5.62; N, 14.20. Found: C, 63.76; H, 5.80; N, 14.00.

6,7-Dimethyl-3-(β-D-erythrofuranosyl)-1-phenyl-pyrazolo[3,4-b]quinoxaline (4). Asuspension of 3 (2 g) in 8% methanolic sulfuric acid solution (500 mL, made by adding 40 mL conc. sulfuric acid to 500 mL methanol) was boiled under reflux with stirring, for 49 h; complete dissolution occurred after 28 h. The reaction was monitored by t.l.c.; complete dehydration was found after 60 h (only one spot). The solution was poured into hot water, and the methanol was evaporated under diminished pressure, and the yellow precipitate obtained was collected, washed with water until neutral, and dried; yield 0.4 g (21%). It was

recrystallized from methanol-benzene to give yellow needles, m.p. 211-213°; R_F 0.65; $[\alpha]_D^{20}$ -117.4° (c 1.1, pyridine); ν_{max}^{KBr} 3360 (OH), 1600, 1560 (C=N), and 1530, 755 cm^{-1} (Ph); mass spectral data (selected ions): m/z 377 (4, M + 1), 376, M), 318 (5, $BCHOHCH_3$), 317 (22, $BCHOHCH_2$), 316 (9), 304 (23, BCH_2OH), 303 (100, $BCH\overset{+}{O}H$), 302 (4, BCHO), 301 (3, BCO), 288 (3, BCH_3), 287 (BCH_2), 275 (8, BH_2), 274 (8, BH), 273 (21, B), 272 (2, B - 1), 249 (3, BH_2 - CN), 248 (14, BH_2 - HCN), 246 (4, B - HCN), 333 (3, B - CN_2), 170 (2, B - PhCN), 156 (2, B - $PhCN_2$) 149 (5), 129 (4), 117 (2, $PhCN_2$), 104 (3, PhCNH), 103 (4, PhCN), 91 (5, $PhNH_2$), 77 (15, Ph), 43 (18). For n.m.r. spectra data see Table 1. Anal. Calc. for $C_{21}H_{20}N_4O_3$: C, 67.01; H, 5.36; N, 14.88. Found: C, 67.05; H, 5.43; N, 14.80.

3-(2,3-Di-O-acetyl-β-D-erythrofuranosyl)-6,7-dimethyl-1-phenylpyrazolo[3,4-b]quinoxaline (5). Compound 4 (0.3 g) was refluxed with acetic anhydride (10 mL) for 4 h, the solution was evaporated to dryness and traces of acetic anhydride removed by repeated addition and evaporation of toluene. The residue was recrystallized from methanol, to give yellow needles of 5, yield 0.26 g (71%); m.p. 130°, $[\alpha]_D^{20}$ -68.9° (c 0.3, chloroform); ν_{max}^{KBr} 1740 (OAc), 1600, 1560 (C=N), and 1500, 758 cm^{-1} (Ph); mass-spectral data (selected ions): m/z 460 (2, M), 401 (1, M - OAc), 400 (6, M - AcOH), 342 (24, M - 2 OAc), 341 (100, M - H - 2 OAc), 340 (3, M - 2 AcOH), 329 (7), 316 (3), 315 (4), 313 (6), 312 (7), 304 (3, BCH_2OH), 303 (13, $BCH\overset{+}{O}H$), 287 (5, BCH_2), 275 (2, BH_2), 274 (5, BH), 273 (15, B), 248 (6, BH_2 - HCN), 149 (6), 115 (17), 111 (4), 100 (6), 98 (5), 83 (10), 81 (7), 77 (9), 73 (10), 71 (12), 69 (17), 60 (8, AcOH), 57 (22), 56 (7), 55 (21), 44 (7), 43 (93, CH_3CO), and 42 (7, CH_2CO). For n.m.r. spectral data see Table 1.

Anal. Calc. for $C_{25}H_{24}N_4O_5$: C, 65.21, H, 5.25; N, 12.17. Found: C, 65.27; H, 5.54; N, 12.01.

6,7-Dimethyl-3-(2,3-O-isopropylidene-β-D-erythrofuranoryl)-1-phenylpyrazolo[3,4-b]quinoxaline (6). A solution of 4 (100 mg) in dry acetone (50 mL) was treated with p-toluenesulfonic acid (300 mg), with stirring. After 24 h, t.l.c. showed the reaction to be complete (one spot, R_F 0.72). The mixture was poured into a cold solution of sodium hydrogen carbonate, and the resulting precipitate was filtered off, washed with water, and dried; yield 100 mg (90%). It was recrystallized from methanol to give yellow needles, m.p. 195-197°; ν_{max}^{KBr} 1600,

1560 (C=N), 1500, 760 (Ph), and 1370 cm^{-1} (CMe_2); mass-spectral data (selected ions): m/z 358 (2, M - CH_3COCH_3), 357 (8, M - $CH_3\overset{+}{C}{=}OHCH_3$), 330 (4), 329 (18), 316 (9, $BCOCH_3$), 304 (2, BCH_2OH), 303 (22, $BCH\overset{+}{O}H$), 302 (24, BCHO), 301 (7, BCO), 275 (1, BH_2), 274 (10, BH), 273 (41, B), 272 (1, B - 1), 246 (2, B - HCN), 170 (9, B - PhCN), 156 (7, B - $PhCN_2$), 117 (3, $PhCN_2$), 104 (5, PhCNH), 103 (12, PhCN), 102 (4), 101 (7), 99 (5), 91 (17, $PhNH_2$), 90 (3, PhNH), 78 (7), 77 (80, Ph), 73 (13), 69 (22), 65 (11), 60 (13), 59 (36), 57 (43), 56 (10), 51 (23), 45 (14), 44 (29), 43 (100, CH_3CO), and 41 (53). For n.m.r. spectral data see Table 1.

Anal. Calc. for $C_{24}H_{24}N_4O_3$: C, 69.21; H, 5.81; N, 13.43. Found. C, 68.99; H, 6.03; N, 13.60.

1-p-Fluorophenyl-3-D-arabino-tetritol-1-yl)-pyrazolo-[3,4-b]-quinoxaline (7). A solution of D-glycero-D-gulo-heptose (2.8 g) in water (250 mL) was heated with o-phenylenediamine (6b, 1.6 g), p-fluorophenylhydrazine hydrochloride (2b, 10 g), and acetic acid (5 mL) in a sealed flask for 6 h and treated as compound 3; yield 2.5 g (51%). Recrystallization from propyl alcohol gave yellow needles, m.p. 232-234°, R_F 0.47; $[\alpha]_D^{20}$ +11° (c 1.9, pyridine); ν_{max}^{KBr} 3320 (OH), 1570 (C=N), 1270 cm^{-1} (C-F); mass-spectral data (selected ions): m/z 384 (1, M), 335 (1), 334 (1, B'CHOHCH$_2$OH, where B' = 1-p-fluorophenylpyrazolo [3,4-b]quinoxaline), 323 (1, B'CHOHCHOH), 307 (1, B'HCH_2OH), 306 (1, B'CH_2CHO), 305 (1, B'CH_2CO), 295 (7, B'HCH_2OH), 294 (61, B'CH_2OH), 293 (100, B'CHOH), 292 (7, B'CHO), 291 (B'CO), 277 (B'CH_2), 266 (8), 265 (41, B'H_2), 264 (5, B'H), 263 (18, B'), 250 (5), 249 (1), 239 (3, B'H_2 - CN), 238 (33, B'H_2 - HCN), 237 (3), 236 (3), 191 (2), 144 (2), 143 (2), 110 (2, C_6H_4FNH), 109 (2, C_6H_4FN), 103 (1, PhCN), 102 (2), 96 (1, C_6H_5F), 95 (8, C_6H_4F), 92 (2, PhNH), 91 (2, PhN), 90 (4), 77 (2, Ph), and 43 (5). For n.m.r. spectral-data, see Table 2.

Anal. Calc. for $C_{19}H_{17}N_4O_4F$: C, 59.37; H, 4.46; N, 14.58. Found: C, 59.63; H, 4.52; N, 14.33.

3-β-D-Erythrofuranosyl-1-p-fluorophenylpyrazolo-[3,4-b]quinoxaline (8). A suspension of compound 7, (2 g) in 8% methanolic sulfuric acid solution (400 mL) was boiled under reflux with stirring, for 48 h (with monitoring of the reaction by t.l.c.); after 48 h, t.l.c. revealed the absence of the starting material and formation of one more mobile spot R_F 0.55. The solution was diluted with hot water and the methanol was

TABLE 1. Chemical shifts (δ) and first-order coupling constant (J Hz) for Compounds (3)-(6).

δ	3	4	5	6
Sugar protons				
H-1'	a 5.24dd b 5.25d $J_{1',2'}$9.4	5.19d $J_{1',2'}$6.8	c 5.67s $J_{1',2'}$6.2	c 5.83s
H-2'	a 4.41d b 4.60d $J_{1',2'}$9.4 $J_{2',3'}$0	a 4.91m b 4.91t $J_{2',3'}$5.0	6.27t $J_{2',3'}$5.4	5.57d $J_{2',3'}$6.0
H-3'	a 4.04m b 4.04t	a 4.46m 4.45d	5.88m	5.27t $J_{3',4'}$0
H-4'	3.54m	4.35dd $J_{3',4'}$4.6	4.66dd $J_{3',4'}$4.5	4.19m
H-4"	$J_{3',4'}$6.4 $J_{3',4"}$7.5 $J_{4',4"}$10.5	3.86dd $J_{3',4"}$2.1 $J_{4',4"}$9.4	4.14d $J_{4',4"}$10	$J_{3',4"}$4.6 $J_{4',4"}$10.4

removed by evaporation under diminished pressure. The yellow precipitate obtained was collected, washed thoroughly with water until neutral, and dried yield 1.8 g (95%). It was recrystallized from methanol-benzene, to give yellow needles, m.p. 210-212°; $[\alpha]_D^{20}$ -124.4° (c 1.3, pyridine). For n.m.r. spectral data see Table 2.

Anal. Calc. for $C_{19}H_{15}N_4O_3F$: C, 62.29; H, 4.13; N, 15.29. Found: C, 62.18; H, 4.42; N, 15.20.

3-(2,3-Di-O-acetyl-β-D-erythrofuranosyl)-1-p-fluorophenylpyrazolo-[3,4-b]quinoxaline (9). Compound 8 (160 mg) was refluxed with acetic anhydride (10 mL) for 2 h, and the solution was treated as described for 5. The yellow product, yield 150 mg (77%), was recrystallized from

TABLE 1 (continued)

δ	3	4	5	6
OH	5.78d	5.27d		
(OAc)	J 5.0	J 6.2	2.188	
	5.61t	5.14d	2.079	
(CMe_2)	J 4.3	J 3.7		1.664
	J 5.1t			1.464
	J 8.4			Δδ 0.200
	4.25d			
	J 7.4			
6,7-Dimethyl-1-phenylpyrazolo[3,4-b]quinoxaline protons				
H-5	8.45s	8.39s	8.45s	8.44s
H-8	8.43s	8.37s	8.43s	8.42s
6-CH_3	2.52s	2.50s	2.56s	2.55s
7-CH_3	2.50s	2.48s	2.54s	2.54s
H-(o)	8.06s	8.01s	8.04s	8.02s
	7.99s	7.96s	7.97s	7.96s
H-(m)	7.63t	7.64t	7.56t	7.57t
H-(p)	7.33t	7.36t	7.34t	7.33t

a. In dimethyl sulfoxide-d_6. b. CD_3CO_2D added. c. in $CDCl_3$.

methanol-chloroform, to give yellow needles m.p. 163-164°; $[\alpha]_D^{20}$ -103.4° (c 1.7, chloroform); ν_{max}^{KBr} 1775 (OAc), 1520, 760 (Ph), and 1260, 1225 cm^{-1} (C-F); mass-spectral data (selected ions): 391 (0.1, M - OAc), 390 (1, M - AcOH), 331 (17, M - H - 2 Ac), 302 (2, $B'H_2CH_2OH$), 294 (0.3, $B'CH_2OH$), 293 (2, $B'CHOH^+$), 265 (1, $B'H_2$), 264 (1, B'H), 263 (3, B'), 238 (3, $B'H_2$ - HCN), 116 (2, $C_6H_4CN_2$), 115 (19), 109 (C_6H_4FN), 95 (C_6H_4F), 90 (3, C_6H_4N), 77 (1, Ph), and 43 (100, CH_3CO). For n.m.r. spectral data, see Table 2.

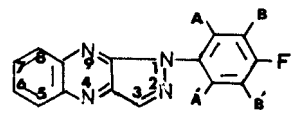
Anal. Calc. for $C_{23}H_{19}H_4O_5F$: C, 61.33; H, 4.25; N, 12.44. Found: C, 61.26; H, 4.38; N, 12.24.

1-p-Fluorophenyl-3-(2,3-O-isopropylidene-β-D-erythrofuranosyl)-pyrazolo[3,4-b]quinoxaline (10). Compound 8 (150 mg) was dissolved in

TABLE 2. Chemical shifts (δ) and first-order coupling constant (J Hz) for compounds (7)-(9).

	3	8	9	10
Sugar protons				
H-1'	a 5.60dd b 5.60d $J_{1',2'}$ 2.6	a 5.31d b 5.25d $J_{1',2'}$ 7.0	c 5.68d $J_{1',2'}$ 6.6	c 5.85s
H-2'	a 4.18m b 4.18dd $J_{2',3'}$ 8	a 4.91dd b 4.91dd $J_{2',3'}$ 4.7	6.26t $J_{2',3'}$ 5.8	5.57d $J_{2',3'}$ 6.2
H-3'	a 3.50m b 3.50dd J 5.9, 10.7	a 4.50m b 4.47dd $J_{3',4'}$ 4.4	5.89dd $J_{3',4'}$ 4.9	5.26m $J_{3',4'}$ 0
H-4'	3.73m $J_{4',4''}$ 14.3	4.38dd $J_{3',4'}$ 4.5	4.67dd $J_{3',4'}$ 4.6	4.20m $J_{3',4''}$ 3.6 $J_{4',4''}$ 10.6
H-4"		3.89dd $J_{3',4''}$ 2.9 $J_{4',4''}$ 14.3	4.19dd $J_{3',4''}$ 3.1 $J_{4',4''}$ 10.2	
OH	5.41d J 7.2	5.31d J 6.5		
(OAc)	4.80d J 5.5	5.25d J 7.0	2.09s	
(CMe_2)	4.73d J 6.5		2.20s	1.665s 1.463s
	4.40t J 5.6, 5.5			Δδ 0.202

TABLE 2 (continued)

	3	8	9	10
1-p-Fluorophenylpyrazolo[3,4-b]quinoxaline.				
H-5	8.45d $J_{5,6}$5.8	8.38d $J_{5,6}$4.8	8.42d $J_{5,6}$4.7	8.41d $J_{5,6}$5.3
H-6	8.30d $J_{6,7}$8.5	8.28d $J_{6,7}$8.7	8.32d $J_{6,7}$8.5	8.32d $J_{6,7}$8.4
H-7	8.21d	8.19d	8.21d	8.21d
H-8	8.43d $J_{7,8}$4.8	8.36d $J_{7,8}$4.6	8.40d $J_{7,8}$4.5	8.40d $J_{7,8}$4.4
H-A	7.98t J 7.3, 7.7	8.96t J 8.3	7.88t J 7.3, 8.4	7.88t J 7.4, 7.5
H-A'	7.88t J 7.7, 7.3	7.87t J 8.2	7.79t J 8.3, 7.3	7.79t J 7.7, 7.2
H-B,B'	7.50t $J_{H,F}$8.7	7.49t $J_{H,F}$8.8	7.28t $J_{H,F}$8.6	7.28t $J_{H,F}$8.4

a. In dimethyl sulfoxide. b. CD_3CO_2D added. c. In $CDCl_3$.

dry acetone and treated with p-toluenesulfonic acid (150 mg) as described for 6. It gave yellow precipitate; yield 120 mg (75%) which was recrystallized from dilute methanol, to give yellow needles, m.p. 128-130°; R_F 0.71. For n.m.r. spectral data see Table 2.

Anal. Calc. for $C_{22}H_{19}N_4O_3F$: C, 65.02; H, 4.71; N, 13.61. Found. C, 65.20; H, 4.82; N, 13.61.

Biological tests. Compounds 4 and 8 were tested in vitro against KB cells, by the Cell Culture Laboratory of the Purdue University Cancer Center. The compounds were tested as a suspension in dilute aqueous dimethyl sulfoxide. Compound 8 showed activity at 100 μg/mL dilution, and compound 4 was inactive.

Compounds 3, 4, 7, and 8 were tested in vivo against P388 mouse lymphocytic leukemia cells, and were inactive, as indicated from the NIH screening data summary (SDS). The leukemia screen (3PS31) test results, did not show activities for compounds 3 and 7. However compound 4 indicated activity at 50, 25, and 12.5 mg/kg doses, whereas compound 8 was active at 200, 100, and 50 mg/kg doses. The P388 and (3PS31) test results were obtained through the Screening Program of Drug Evaluation Brnach, Division of Cancer Treatment, National Cancer Institute, National Institute of Health, Bethesda, Maryland 20205.

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