Mesoionic Pyridazine and Pyridine Nucleosides. An Unusual Biologically Active Nucleoside Metabolite

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An *in vivo* process in mice leading to the mesoionic 3-oxidopyridazinium riboside, (**3b**), can also be accomplished *via* a kinetically controlled silyl Hilbert–Johnson reaction.

Unknown among an impressive array of pyrimidine, pyridine, pyridazine, and related monoheterocyclic nucleoside derivatives are mesoionic structures.[1] Bicyclic mesoionic purine nucleosides, first characterized by Jones and Robins,[2] have been found in the case of 7-methylguanosine to occur naturally in RNA isolated from several sources.[3] The lack of attention given to mesoionic nucleosides is due in part to the absence of a general approach to such compounds. Known methods for the few bicyclic examples involve methylation[2] or acylation[4] of a suitable nucleoside derivative. We report here the first examples of mesoionic systems structurally related to biologically important pyrimidine and pyridine nucleosides and a simple, efficient way to prepare them.

These nucleoside systems were discovered as a result of a metabolite study on 4-cyanopyridazin-3(2*H*)-one, (**1a**),[5] a compound which we found to have antibacterial activity against a systemic *Escherichia coli* infection in mice. Nucleoside metabolites were considered, including the betaine, (**3b**), and the non-mesoionic isomer, (**2b**). The betaine assignment was confirmed by isolation of (**3b**) from urine of animals dosed orally with (**1a**).† The silyl Hilbert–Johnson procedure developed by Niedballa and Vorbrueggen[6] has proved exceedingly useful for the preparation of a wide variety of non-mesoionic nucleoside systems. O and N-2 glycoside derivatives of pyridazin-3(2*H*)-ones have been studied extensively[7,8] and the silyl Hilbert–Johnson synthesis of non-mesoionic pyridazinone nucleosides has been reported in which conditions call for heating a solution of silylated base and acylated sugar in dichloroethane. We have found that mesoionic systems are easily accessible by using a silyl Hilbert–Johnson reaction under conditions which permit kinetic control. When the literature approach[7] was applied to (**1a**), using a 15 min reflux period, the normal riboside triacetate, (**2a**), was formed as expected (51%).‡ However, when the same reaction was carried out at ice bath temperature or even at room temperature, the betaine, (**3a**), was obtained instead in 85% yield: m.p. 168—170 °C (decomp.) (from MeOH); (KBr) 2225 (w), 1750 (s), 1610 cm^{-1} (s); λ_{max} (MeOH) 223 (log 4.28), 363 nm (3.64). At room temperature a slow rearrangement of (**3a**) to (**2a**) occurs over a period of several months, and rapidly when heated in dichloroethane in the presence of $SnCl_4$.

Both cyanopyridazine nucleoside derivatives, (**2a**) and (**3a**), proved to be highly sensitive to base. Treatment of the betaine, (**3a**), with NaOMe or NH_3 in methanol gave an immediate, intense blue colour and a complex reaction mixture. However, we have found that $NaHCO_3$ in methanol is a simple, efficient reagent for deblocking (**3a**) and other sensitive nucleoside triacetates. We attribute the success obtained with $NaHCO_3$–MeOH to the lower pH achievable under these conditions and we recommend the use of $NaHCO_3$ with other base-sensitive nucleosides. Thus 2.0 g of (**3a**) in MeOH (600 ml) was left in the presence of $NaHCO_3$ (4.0 g) at 0—5 °C for 6 days followed by neutralization with Amberlite^R IRC 50 and chromatography on silica gel ($CHCl_3$: MeOH, 3 : 1) to give (**3b**) (34%) : m.p. 168—170 °C (decomp.).§

Using this low temperature silyl Hilbert–Johnson approach it is possible to prepare a variety of mesoionic nucleosides. For example, reaction of the silyl derivative of 3-hydroxypyridine-4-carbonitrile with ribose tetra-acetate and $SnCl_4$ in dichloroethane at 0—5 °C for 1 h gave a 57% yield of (**4a**) which with $NaHCO_3$ in MeOH at room temperature gave (**4b**), m.p. 176—178 °C.§ The mesoionic riboside triacetate derivative of 4,5-dichloropyridazin-3(2*H*)-one has been prepared for spectral comparison with the known[9] 4,5-dichloro-1-methyl-3-oxidopyridazinium. The mesoionic isomer of the previously reported[7] 4,5-dichloro-2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranos-1′-yl)pyridazin-3-one has also been synthesized.

The 3-oxidopyridazinium riboside, (**3b**), has pronounced biological activity. Treatment of a systemic *E. coli* infection in mice subcutaneously with synthetic (**3b**) afforded an ED_{50} of 25—50 mg/kg. In Davis minimal media,¶ *in vitro*, (**3b**) has a minimum inhibitory concentration of less than 0.78 p.p.m. against *E. coli* under aerobic conditions. The isomeric N-2 substituted riboside, (**2b**), was inactive as was the 3-oxidopyridinium riboside, (**4b**). Also noteworthy is the role played by the cyano function. The cyano group is considered a bioisostere of the carbonyl oxygen atom.[10] 5-Cyanouridine derivatives are known,[11] however, pyrimidine nucleoside analogues in which the 2- or 4-amino group or carbonyl oxygen atom is

† The urine metabolite was identical (n.m.r., i.r., and u.v. spectroscopy, t.l.c., mixed m.p.) with the synthetic material. The metabolite and all other compounds with the exception of (**2b**) (amorphous) gave acceptable microanalytical data.

‡ For (**2a**) : λ_{max} (MeOH) 324 nm (log ε 3.65). (**2a**) was converted into (**2b**) using NaOMe in MeOH (53%) : ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 3.3—3.7 (m, 2H), 3.8—4.5 (m, 3H), 4.68 (t, 1H, *J* 6 Hz), 5.13 (d, 1H, *J* 6 Hz), 5.41 (d, 1H, *J* 5 Hz), 6.29 (d, 1H, *J* 4 Hz), 8.22 (s, 2H); ^{13}C n.m.r. ([$^2H_6$]Me_2SO) δ 157.0(s), 139.5(d), 136.6(d), 114.3(s), 114.0(s), 90.5(d), 85.4(d), 73.4(d), 70.6(d), 62.0 p.p.m. (t); ν (KBr) 3450 (br.), 2240(w), 1665 cm^{-1}(s); δ_{max} (MeOH) 329 nm log ε 3.67). 4-Cyano-1-methyl-3-oxidopyridazinium was prepared by treatment of the *O*-trimethylsilyl derivative of (**1a**) with methyl fluorosulphonate in dichloroethane at 0—5 °C (30%) : m.p. 200—203 °C (decomp.) (EtOH); ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 4.18 (s, 3H), 8.22 (d, 1H, *J* 5 Hz), 8.52 (d, 1H, *J* 5 Hz); ^{13}C n.m.r. ([$^2H_6$]Me_2SO) 166.1(s), 138.2(d), 131.9(d), 115.6(s), 111.1(s), 52.1 p.p.m.(q); ν (KBr) 2235(w), 1610 cm^{-1}(s); λ_{max} (MeOH) 222 (log ε 4.31), 3.62 nm (log ε 3.63).

§ For (**3b**) : ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 3.5—4.0 (m, 2H), 4.0—4.2(m, 2H), 4.2—4.4 (m, 1H), 5.1—5.35 (m, 2H, D_2O exchangeable), 5.67 (d, 1H, *J* 1 Hz), 5.94 (d, 1H, *J* 5 Hz, D_2O exchangeable), 8.31 (d, 1H, *J* 5 Hz), 8.89 (d, 1H, *J* 5 Hz); ^{13}C n.m.r. ([$^2H_6$]Me_2SO) δ 165.8(s), 137.9(d), 127.9(d), 115.6(s), 112.7(s), 102.7(d), 86.1(d), 76.2(d), 68.2(d), 59.5 p.p.m.(t); ν (KBr) 3390(s), 2235(w), 1610(s) cm^{-1}; λ_{max} (MeOH) 223 (log ε 4.28), 363 nm (3.65). For (**4b**): ^{1}H n.m.r. ([$^2H_6$]Me_2SO) δ 3.6—3.8 (m, 2H), 4.0—4.25 (m, 3H), 5.25—5.5 (m, 2H, D_2O exchangeable), 5.66 (d, 1H, *J* 4 Hz) superimposed on a D_2O exchangeable m at 5.7—5.9(1H), 7.60(dd, 1H, *J*6, 1 Hz), 7.80 (d, 1H, *J* 6 Hz), 8.10 (d, 1H, *J* 1 Hz, *J* 1 Hz); ^{13}C n.m.r. ([$^2H_6$]Me_2SO) 167.8(s), 135.9(d), 130.5(d), 117.2(s), 116.2(d), 112.5(s), 99.3(d), 87.9(d), 77.8(d), 70.3(d), 60.6 p.p.m.(t); ν(KBr) 3350(br.), 3160, 2235(w), 1610 cm^{-1}; λ_{max} (MeOH) 231 (log ε 4.31), 254 (sh), 373 nm (3.85).

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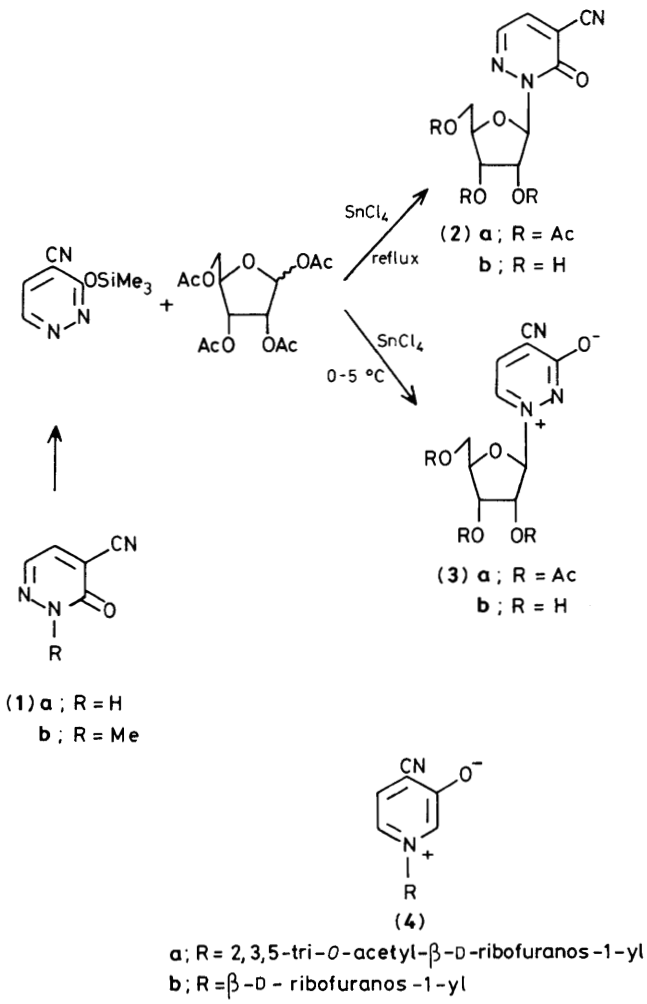
replaced by a cyano group have not been reported, although an attempt has been made to prepare two examples.[12] Such compounds are of interest whether or not they are mesoionic in character.[13]



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