

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

Ronald E. Bambury, Daniel T. Feeley, Gerald C. Lawton, John M. Weaver, and James Wemple*

SDS Biotech Corporation, 7528 Auburn Rd., Painesville, Ohio 44077, U.S.A.

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 methylation² or acylation⁴ 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 Vorbrueggen⁶ 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 approach⁷ 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**)

† 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%): ¹H n.m.r. ([²H₆]Me₂SO) 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); ¹³C n.m.r. ([²H₆]Me₂SO) 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); ¹H n.m.r. ([²H₆]Me₂SO) δ 4.18 (s, 3H), 8.22 (d, 1H, *J* 5 Hz), 8.52 (d, 1H, *J* 5 Hz); ¹³C n.m.r. ([²H₆]Me₂SO) 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).

to (**2a**) occurs over a period of several months, and rapidly when heated in dichloroethane in the presence of SnCl₄.

Both cyanopyridazine nucleoside derivatives, (**2a**) and (**3a**), proved to be highly sensitive to base. Treatment of the betaine, (**3a**), with NaOMe or NH₃ in methanol gave an immediate, intense blue colour and a complex reaction mixture. However, we have found that NaHCO₃ in methanol is a simple, efficient reagent for deblocking (**3a**) and other sensitive nucleoside triacetates. We attribute the success obtained with NaHCO₃–MeOH to the lower pH achievable under these conditions and we recommend the use of NaHCO₃ with other base-sensitive nucleosides. Thus 2.0 g of (**3a**) in MeOH (600 ml) was left in the presence of NaHCO₃ (4.0 g) at 0–5 °C for 6 days followed by neutralization with Amberlite^R IRC 50 and chromatography on silica gel (CHCl₃: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 SnCl₄ in dichloroethane at 0–5 °C for 1 h gave a 57% yield of (**4a**) which with NaHCO₃ 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 known⁹ 4,5-dichloro-1-methyl-3-oxidopyridazinium. The mesoionic isomer of the previously reported⁷ 4,5-dichloro-2-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)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 ED₅₀ 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-oxidopyridinidium 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

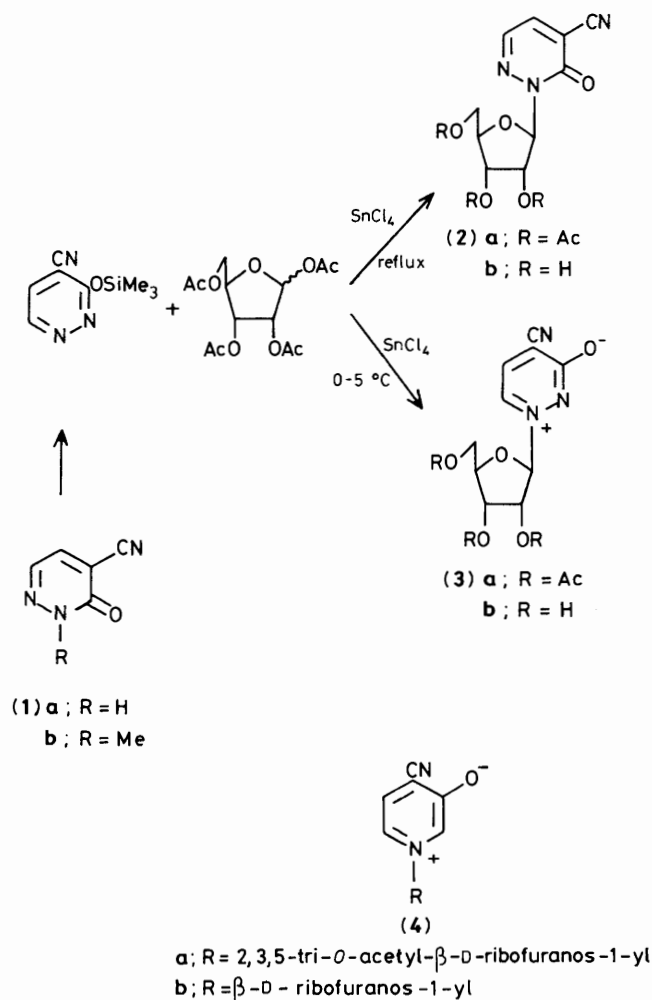
§ For (**3b**): ¹H n.m.r. ([²H₆]Me₂SO) δ 3.5–4.0 (m, 2H), 4.0–4.2 (m, 2H), 4.2–4.4 (m, 1H), 5.1–5.35 (m, 2H, D₂O exchangeable), 5.67 (d, 1H, *J* 1 Hz), 5.94 (d, 1H, *J* 5 Hz, D₂O exchangeable), 8.31 (d, 1H, *J* 5 Hz), 8.89 (d, 1H, *J* 5 Hz); ¹³C n.m.r. ([²H₆]Me₂SO) δ 165.8(s), 137.9(d), 127.9(d), 115.6(s), 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**): ¹H n.m.r. ([²H₆]Me₂SO) δ 3.6–3.8 (m, 2H), 4.0–4.25 (m, 3H), 5.25–5.5 (m, 2H, D₂O exchangeable), 5.66 (d, 1H, *J* 4 Hz) superimposed on a D₂O 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); ¹³C n.m.r. ([²H₆]Me₂SO) 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).

¶ Difco Laboratories, Detroit, Michigan.

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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