Results from bR-**2** were similar except that due to its photosensitivity, alkalinization of the medium was monitored against irradiation time; the extent of H^+ pumping remained constant at the level of blank and thus it is also due to residual bR^{LA}.

The results described show that fixed 13-ene structures inhibit proton translocation. It has been shown that bR^{LA} formed from 5,6-dihydro-,[32] phenyl-,[33] and 3-(diazoacetoxy)retinal[34] still retain the ability to pump protons although less efficiently. This suggests that the 13-ene plays a more important role than the ring site in initiating the translocation of protons across the membrane.

Acknowledgment. The studies were supported by NSF Grant CHE 81-10505.

Registry No. **1**, 86309-94-6; **2**, 86309-95-7; **3**, 3917-41-7; **4**, 86309-96-8; **5** (isomer 1), 86309-97-9; **5** (isomer 2), 86310-00-1; **6**, 86323-11-7; **7**, 86323-12-8; **8**, 765-76-4; **9**, 86309-98-0; **10**, 86309-99-1; hydrogen ion, 12408-02-5.

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Structure and Synthesis of 3-Deoxy-D-*glycero*-pentos-2-ulose, an Unusual Sugar Produced Enzymatically from (ADP-ribosyl)histone H2B

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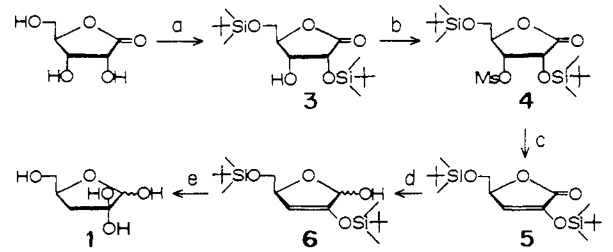
Poly(ADP-ribosylation) is a posttranslational covalent modification of histones and non-histone nuclear proteins including poly(ADP-ribose) synthetase itself in eukaryotic cells.[1] It is initiated by enzymatic reactions of NAD on reactive functional groups of proteins such as glutamate of histones[2,3] followed by elongation and branching. Evidence suggests the involvement of poly(ADP-ribosylation) in various biological functions.[4-6] Although poly(ADP-ribose) is known to have α-ribosyl linkages at its C-2′ elongation sites[7] and C-2″ branching sites,[8] the nature of the histone/poly(ADP-ribose) linkage is not fully understood.[2,3]

We have purified and characterized ADP-ribosyl protein lyase, an enzyme that cleaves the ADP-ribose/histone linkage to give, instead of the expected ADP-ribose, an unidentified ADP-X.[9,10] In contrast, nonenzymatic cleavage of (ADP-ribosyl)histones yielded ADP-ribose.[2,3,11,12] The sugar X obtained by successive degradation of ADP-X with phosphodiesterase and phosphatase retains the five carbons of the ribosyl nicotanamide portion of NAD as shown by ^{14}C-labeling studies[10] but differs from the common pentoses.[10] Sugar X (ca. 10 μg using ca. 100 rat livers)[10] was reduced by $NaBH_4$[13] to the pentitol (reduced X) whose R_f value on paper chromatogram (R_f 0.51; *n*-BuOH/AcOH/H_2O 52:13:35 v/v[14])[10] suggested it to be 3-deoxypentitol.

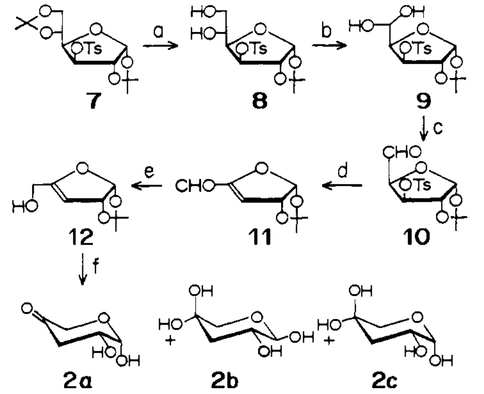
Two of the most plausible candidates for X,[15] 3-deoxy-D-*glycero*-pentos-2-ulose (**1**)[16,17] and -4-ulose (**2**)[16,18] were therefore

Scheme I



^{a} *t*-$BuMe_2SiCl$/Py; Me_2NPy, room temperature, 4 h. ^{b} MsCl/Py; Me_2NPy, room temperature, 2 h. ^{c} Et_3N/C_6H_6, reflux, 2 h, 80% over 3 steps. ^{d} DIBAL/CH_2Cl_2, −78 °C, Ar, 64%. ^{e} Bu_4NF/THF, room temperature, 30 min, 38%.

Scheme II



^{a} TsOH/MeOH, reflux, 4 h. ^{b} $NaIO_4$/MeOH-H_2O, room temperature. ^{c} C_6H_6, reflux, 1 h. ^{d} Et_3N/C_6H_6, reflux, 1 h, Ar, 67% from **7**. ^{e} DIBAL/CH_2Cl_2, −78 °C, Ar, 80%. ^{f} AcOH-H_2O (2:1), room temperature, overnight, 76%.

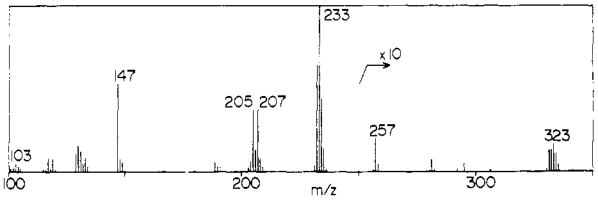


Figure 1. EI mass spectrum of a reduced X-d_2 Me_4Si derivative (erythro derivative; threo derivative showed almost identical spectrum).

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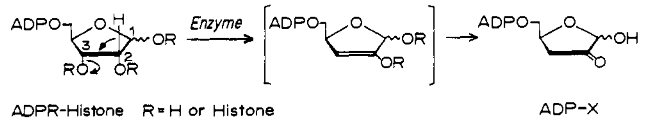
(14) This solvent system generally does not distinguish epimeric alditols such as ribitol, arabinitol, and xylitol.

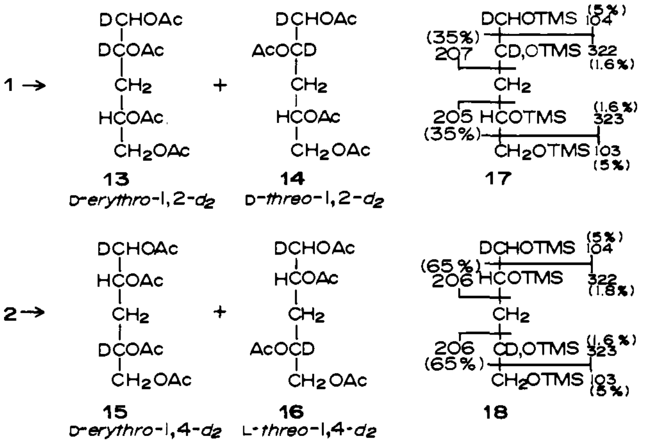
(15) The possibility of X being a 5-ulose was considered unlikely because 5-O is phosphorylated in the original (ADP-ribosyl)histone.

Scheme III



synthesized respectively from D-ribonolactone and D-glucose (via **7**[19]), as shown in Schemes I and II.[20]

The synthetic ulose and natural X were reduced with $NaBD_4$ in D_2O[13] and acetylated to give pentitols-d_2 **13/14** and **15/16** and



the ribo and arabino forms of reduced X-d_2 acetates. The retention times in the GC–MS[21] of **13/15**[22] (7.4 min) and **14/16**[22] (7.2 min) were identical with those of reduced X-d_2, while the MS patterns of all compounds were indistinguishable. The acetates **13–16** and reduced X-d_2 acetates were therefore deacetylated and trimethylsilylated[23] in situ to per-Me_4Si derivatives, which were then subjected to GC–MS[21] to locate the D atoms in reduced X-d_2. Ribo and arabino isomers appeared at t_R 7.8 and 8.3 min, respectively, with diagnostic fragments 205/207 (**17**) and 206 (**18**). The MS fragmentation pattern of reduced X-d_2 Me_4Si derivatives was identical with that of **17** (Figure 1). A D-*glycero* configuration can be assigned to C-4 in X since this chiral center is the D-ribose C-4 in the original (ADP-ribosyl)histone. Sugar X is thus 3-deoxy-D-*glycero*-pentos-2-ulose or its hydrate.

The mechanism of sugar X formation is unclear at this stage. Although an ester linkage between the terminal ribose and glutamate residue was suggested[2,3] for the enzyme substrate, the site of linkage in the ribose moiety is still unknown. However, elucidation of sugar X shows that the 3-hydroxy or 3-glutamyloxy group in (ADP-ribosyl)histone is eliminated by the enzyme to yield ADP-X (Scheme III).[10]

Acknowledgment. We are grateful to Drs. M. Hirama and J. Pawlak, SUNBOR, for valuable discussions on syntheses and MS, respectively.

Supplementary Material Available: ^1H NMR data of all synthetic intermediates, observed and simulated ^1H NMR spectra for reduced X-d_2 acetates, and synthesis of deoxypentitols (8 pages). Ordering information is given on any current masthead page.

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(16) These two structures have been proposed previously for some products in a complex reaction mixture obtained by γ-ray radiolysis of D-ribose: Von Sonntag, C.; Dizdaroglu, M. *Carbohydr. Res.* **1977**, *58*, 21.

(17) Only one anomer was detected: ^1H NMR (D_2O, 360 MHz) 4.13 (br s, 1-H), 4.01 (dddd, $J_{4,5} = 4.0$, $J_{4,5'} = 6.8$, $J_{3,4} = 8.6$, $J_{3',4} = 3.8$ Hz, 4-H), 3.63 (dd, $J_{5,5'} = 11.7$ Hz, 5-H), 3.51 (dd, 5′-H), 2.01 (dd, $J_{3,3'} = 14.5$ Hz, 3-H), 1.95 (dd, 3′-H); CD (0.03% in D_2O) $\Delta\epsilon_{213} = +0.013$, $\Delta\epsilon_{260} = -0.005$, $\Delta\epsilon_{277} = +0.004$, $\Delta\epsilon_{310} = -0.003$; UV (0.03% in H_2O) λ_{max} 269 nm (ϵ 75).

(18) A 1:2:1 mixture of **2a**, **2b**, and **2c** was obtained after deblocking: ^1H NMR (D_2O, 360 MHz) (**2a**) 4.91 (d, $J_{1,2} = 4.9$ Hz, 1-H), 4.42 (d, $J_{5,5'} = 19.2$ Hz, 5-H), 4.38 (d, 5′-H), 4.38 (d, 5′-H), 4.01 (ddd, $J_{2,3(a)} = 8.0$, $J_{2,3(e)} = 4.2$ Hz, 2-H), 2.74 (dd, $J_{3,3'} = 16.4$ Hz, 3(e)-H), 2.68 (dd, 3(a)-H) (**2b**) 4.63 (d, $J_{1,2} = 6.7$ Hz, 1-H), 3.70 (dd, $J_{3(e),5(e)} = 2.98$ $J_{5,5'} = 11.6$ Hz, 5(e)-H), 3.62 (ddd, $J_{2,3(a)} = 10.5$, $J_{2,3(e)} = 4.7$ Hz, 2-H), 3.54 (d, 5(a)-H), 2.28 (ddd, $J_{3,3'} = 13.0$ Hz, 3(e)-H), 1.80 (dd, 3(a)-H) (**2c**) 5.12 (d, $J_{1,2} = 3.2$ Hz, 1-H), 3.92 (ddd, $J_{2,3(a)} = 10.6$, $J_{2,3(e)} = 4.6$ Hz, 2-H), 3.79 (d, $J_{5,5'} = 11.7$ Hz, 5(a)-H), 3.41 (dd, $J_{3(e),5(e)} = 2.4$ Hz, 5(e)-H), 2.08 (ddd, $J_{3,3'} = 12.8$ Hz, 3(e)-H), 1.97 (dd, 3(a)-H); CD (0.2% in H_2O) $\Delta\epsilon_{265} = -0.017$, $\Delta\epsilon_{300}$ (sh) $= -0.005$; UV (0.2% in H_2O) λ_{max} 270 nm (sh, ϵ 20). The ratio of **2a/2b/2c** is ca. 0.5:2:1 from ^1H NMR in D_2O at the same concentration.

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(20) ^1H NMR were taken for all synthetic intermediates (cf. supplementary material).

(21) OV-1 Glass-packed column, 1 m × 0.6 cm OD, 64 mL/min He, oven temperature 100–170 °C, linear temperature gradient 4 °C/min (for acetates) or 2 °C/min (for TMS derivatives).

(22) Authentic 3-deoxypentitols were separately synthesized in order to distinguish ribo and arabino isomers.

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Hydrogen-Bond Stabilization of Oxygen in Hemoprotein Models

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Chemically synthesized compounds[1-3] permit the analysis of the relationship[4] of the stereochemical features to the kinetic and thermodynamic properties of the oxygenated hemes. The bent structure of the dioxygen molecule relative to the symmetry axis of the hemes proposed early by Pauling[5] is now demonstrated for oxymyoglobin[6] and oxyhemoglobin.[7] The stabilization of this conformation by hydrogen bond with the distal histidine residue[5] is however still questionable, at least for its energetic contribution. Such a bond has been recently observed in sperm-whale oxymyoglobin by neutron diffraction[8] and is likely to occur in human oxyhemoglobin, at least in the α subunits.[7] The bent oxygen structure has been also observed in model compounds.[9-11] The NMR investigation of new compounds including amide groups in the vicinity of the oxygen binding site[3] is now reported. It indicates a direct interaction of these groups with the oxygen molecule consistent with hydrogen-bond formation. This bond adds a free energy contribution for oxygen binding that makes the affinity of the model compounds comparable to that of the natural oxygen carriers.

The models are built up from 5,10,15,20-tetraphenylporphyrin. They include two chains bridged between opposite meso phenyl groups over both faces of the porphyrin ring. A pyridine molecule is inserted within one of these chains and acts as the proximal base. It is coordinated on the central iron(II) atom. The chain bridged over the distal face of the heme protects it from the irreversible oxidation of the oxygen complex into μ-oxo dimers.

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