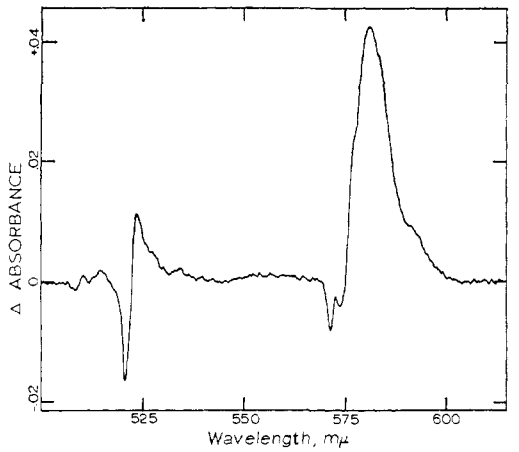

Figure 2. Difference spectrum of neodymium(III) ion in bovine serum albumin solution (sample beam) *vs.* neodymium(III) ion in water (reference beam). Concentrations of neodymium ion are identical in both cells and with those in Figure 1. This spectrum was obtained using a 0–0.1 A slide wire for the Cary 14.

maleate at pH 5.6 give nearly identical difference spectra. However, ligands which must have a different coordination symmetry, such as EDTA, or ligands which contain different coordinating groups, such as lysine and tris(hydroxymethyl)aminomethane, exhibit difference spectra in sharp contrast with the one where only simple carboxylic acid groups can bind the metal ion. These difference spectra are characterized by the appearance of new peaks in the 575-nm region as well as by change in extinction coefficients.

α-Aminocarboxylic acids such as alanine or glycine at pH 5.6 produce a difference absorption spectrum of the metal ion distinct from that of simple carboxylic acids or the other nitrogen ligands mentioned above. At this pH the amino acids are in the zwitterion forms and hence there should be little or no binding to the protonated amino group. This positive charge near the carboxyl group makes the binding of Nd^{3+} to the carboxyl weaker than with other simple carboxyl groups. This is then reflected in the spectrum observed.

When amino acids with functional side chains, which can also coordinate to neodymium(III), are used as ligands, a difference spectrum is obtained which is characteristic of the particular ligand. For example, if glutamate at pH 5.6 (where both carboxyl groups are ionized) is the ligand, a difference spectrum is obtained which is almost identical with the one in Figure 2. Additional changes are seen in the glutamate–neodymium(III) difference spectrum as the pH is adjusted to 3.0. At this pH the γ-carboxyl group is protonated, but the α-carboxyl group is still ionized. This difference spectrum at pH 3.0 is much smaller than at pH 5.6 and is nearly identical with the one obtained using alanine as a ligand. These changes reflect the difference in binding strengths of simple carboxylic acids as compared to carboxylic acids with a positive charge nearby.

If histidine at pH 5.6 is used as a ligand of neodymium(III), a difference spectrum is obtained which is again nearly identical with that of alanine. At this pH the imidazole group is protonated and hence would not be expected to bind to Nd^{3+}. At pH 7.0 the imidazole group is largely unprotonated and consequently may interact with the metal ion. We see changes in the difference spectrum at pH 7.0 over that at pH 5.6 which probably reflect this binding to the imidazole group at the higher pH. Since our protein–neodymium(III) difference spectrum was obtained at pH 5.6, it is very unlikely that protonated amino groups or imidazole groups bind the neodymium ion in the protein. Details of these and additional spectra will be presented in a later publication.

Possible ligands of neodymium in the protein are carboxyl groups, hydroxyl groups, tyrosyl hydroxyl groups, sulfhydryl groups, or the peptide linkage itself. At pH 5.6 our spectroscopic evidence with a variety of model compounds indicates that neodymium is bound to BSA only through interactions with carboxyl groups.

We would like to suggest that it should be possible to generally use rare earth metal ions to probe the binding sites of the calcium ion in proteins since the chemistry and size of these ions are very similar. Our studies indicate that much information concerning the coordinating ligands of rare earth metal ions may be gained by carefully looking at the absorption spectrum of the metal. Other methods which utilize the varied spectral and magnetic properties of the rare earth metal ions, such as circular dichroism, esr, nmr, fluorescence, etc., should likewise be applicable to the study of binding sites of these metal ions. In addition the gradual variation in size of the lanthanide ions across the series should allow a definitive test to be made of the effect of ionic radius on the binding to a protein.

We are currently investigating the possibility of replacing specifically bound calcium ion with neodymium ion in enzyme systems.

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4,6-Di-*O*-acetyl-*aldehydo*-2,3-dideoxy-D-*erythro*-*trans*-hex-2-enose. A Probable Reason for the "al" in Emil Fischer's Triacetyl Glucal[1]

Sir:

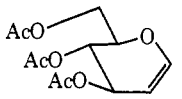
There is probably no sugar derivative more versatile in its service to carbohydrate chemists than "triacetyl glucal" (3,4,6-tri-*O*-acetyl-1,2-dideoxy-D-*arabino*-hex-1-enopyranose) (**1**).[2] Obtainable from D-glucose in a simple direct sequence,[3] it is featured frequently in studies relating to the still enthralling problem of re-

(1) We are indebted to a referee for the following comment: "The authors will be missing a wonderful opportunity if they fail to point out . . . that they may have come as close as will ever be possible to solving a scientific mystery of nearly sixty years' standing: the nature of the aldehyde-like impurity in crude glucal which induced the discoverers of this material to give it a name ending in "-al."

(2) (a) E. Fischer and C. Zach, *Sitzungsber. Kgl. Preuss. Akad. Wiss.*, **16**, 311 (1913); (b) E. Fischer, *Chem. Ber.*, 47, 196 (1914); (c) E. Fischer, M. Bergmann, and H. Schotte, *ibid.*, **53**, 509 (1920).

(3) W. Roth and W. Pigman, *Methods Carbohyd. Chem.*, 2, 405 (1963).

actions at the anomeric center.[4] When conformational aspects of shikimic acid needed clarification, triacetyl glucal was an accessible model, adequately suited to nmr study.[5] Chemical transformations based upon it are legion. Along with the other glycals,[6] the family of unsaturated carbohydrates of which it was the forerunner, it has been transformed into a host of biologically and chemically important substances.[4,6,7] It is therefore interesting, in the light of the foregoing, that the structure originally conceived by Emil Fischer, its discoverer, was not **1**. His impure preparation, apart from decolorizing bromine, gave a positive fuschin–SO_2 test, and in consummation, he wondered whether the substance was a derivative of the unsaturated aldehyde **2**.[2a] He postulated **3**,[2b] and although



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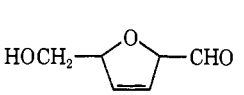

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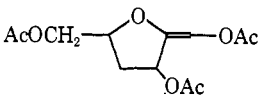
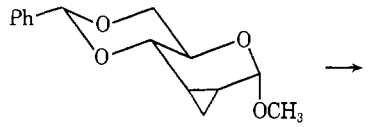


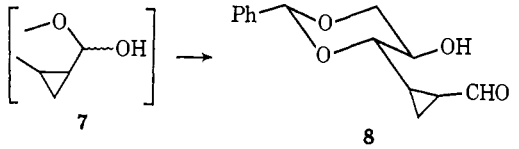
3

he did not remain fooled for long,[2c,8] he had already enshrined his conviction in the suffix of the name he chose—acetoglucal.[2a] In this communication we suggest 4,6-di-*O*-acetyl-*aldehydo*-2,3-dideoxy-D-*erythro*-*trans*-hex-2-enose (**4**) as the aldehyde responsible for the anomaly, and describe its isolation and characterization as its acetylated counterpart, **5**.

Our interest arose from the observation that the cyclopropanated pyranoside **6** was converted by refluxing aqueous dioxane in 1 hr to the δ-hydroxy aldehyde **8**.[9] The ease of the hydrolysis was ascribed to



6



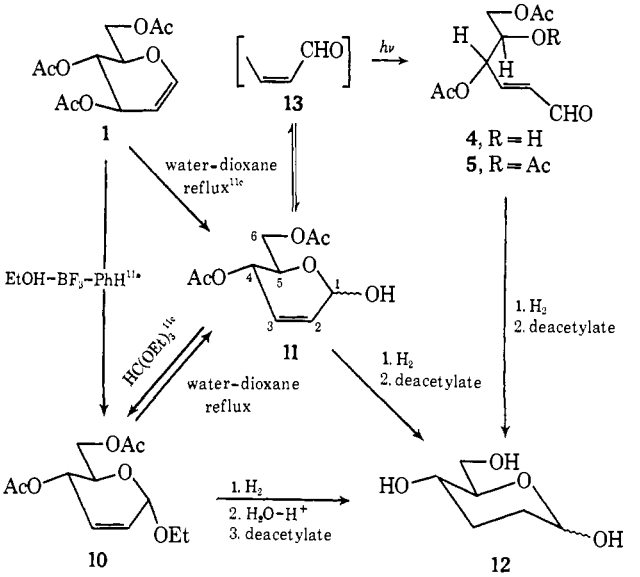
7 **8**

formation of a cyclopropylcarbinyl oxocarbonium ion[10] generated upon removal of the methoxyl substituent from the anomeric center of **6**. The complete absence of the hemiacetal **7**, the presumed intermediate, was attributed to the torsional strain inflicted upon the anomeric center in this [4:1:0]bicycloheptyl arrangement. Support for these conclusions would be forthcoming if another glycoside, also reactive but relatively unstrained, gave the hemiacetal in preference to the acyclic aldol upon hydrolysis. Accordingly, methyl 4,6-*O*-benzylidene-2,3-dideoxy-α-D-*erythro*-hex-2-enopyranoside (**9**), the 2-olefin equivalent of **6**, was hydrolyzed. However, liberation of benzaldehyde defeated the purpose of the experiment. Olefin **10**[11] was therefore chosen in the expectation that the allylic *O*-acetyl (C-4) group would be less vulnerable to hydrolysis than an allylic *O*-benzylidene group of **9**. In addition, the hemiacetal arising from it should be **11**, well known as the product of reacting triacetyl glucal (**1**) with boiling water.[1,11b,11c]

Nmr monitors indicated that refluxing a 7.7×10^{-2} *M* solution of **10** in water–dioxane (1:1)[12] caused disappearance of the ethoxy aglycon and the anomeric proton[13] during 14 hr. The pattern for the olefinic protons[13] had changed only slightly suggesting that **11** comprised at least 75% of the reaction product.[14] However, the presence of an aldehyde was apparent from a signal for one-tenth of one proton at τ 0.38[15] and this was substantiated by a positive Schiff test. The hydrolysis was therefore allowed to proceed for a further 10 hr at which time an optimum situation existed in which the ratio of aldehyde to **11** had grown to 1.5, both substances accounting for 68% of the nmr spectrum.

We confirmed the earlier reports[1,11b,11c] that refluxing a solution of **1** in aqueous dioxane for 15 min furnishes **11**, which after treatment with ethyl orthoformate affords **10** (see Scheme I).[11b,11c] However, the aldehyde

Scheme I



described above was also present in about 5–10%. After a total reaction time of 1.5 hr, the aldehyde and **11** (3:1) accounted for 93% of the nmr spectrum.[16]

(4) R. U. Lemieux and S. Levine, *Can. J. Chem.*, **40**, 1926 (1962); **42**, 1473 (1964); R. U. Lemieux and B. Fraser-Reid, *ibid.*, **42**, 532 (1965); **43**, 1460 (1965); R. U. Lemieux and A. R. Morgan, *ibid.*, **43**, 2191, 2199, 2205 (1965); R. J. Ferrier, *J. Chem. Soc.*, 5443 (1964); R. J. Ferrier and N. Prasad, *ibid.*, *C*, 570, 581 (1969).

(5) L. D. Hall and L. F. Johnson, *Tetrahedron*, 20, 833 (1964); L. D. Hall and J. F. Manville, *Advan. Chem. Ser.*, No. 74, 228 (1968).

(6) B. Helfrich, *Advan. Carbohyd. Chem.*, 7, 210, (1952).

(7) For some leading references see (a) R. J. Ferrier, *ibid.*, 20, 67 (1965); (b) R. J. Ferrier, *ibid.*, 24, 199 (1969); (c) R. D. Guthrie, R. J. Ferrier, and M. J. How, *Carbohyd. Chem.*, **1**, 126 (1968); (d) R. D. Guthrie, R. J. Ferrier, M. J. How, and P. J. Sommers, *ibid.*, 2, 127 (1969).

(8) The fact that the structure in ref 2c is represented as a furan rather than a pyran is unimportant here, for it was then thought that hexoses existed primarily as five-membered rings.

(9) B. Fraser-Reid and B. Radatus, *Can. J. Chem.*, **48**, 2146 (1970).

(10) B. Fraser-Reid and B. Radatus, *ibid.*, 47, 4905 (1969).

(11) (a) R. J. Ferrier and N. Prasad, *J. Chem. Soc. C*, 570, 575 (1969); (b) S. Laland, W. G. Overend, and M. Stacey, *ibid.*, 738 (1950); (c) M. Bergmann, *Justus Liebigs Ann. Chem.*, **443**, 223 (1925).

(12) The distilled water used had a pH of 6.23.

(13) For **10** in $CDCl_3$ (TMS): H-1 τ 5.0 (s); H-3 and H-4 4.95 (s).

(14) Nmr estimation based on intensity of H_3–H_4 signal[13] relative to six acetyl protons.

(15) L. M. Jackman, "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Macmillan, New York, N. Y., p 62.

The aldehyde was obviously α,β-unsaturated judging from the nmr and uv spectra of the hydrolysate,[17] and the value of J_{23} (15.0 Hz) indicated *trans*-olefinic protons[18] as in **4**. However, it could not be separated from **11** either by chromatography or distillation. The mixture was therefore acetylated at 0° with acetic anhydride and pyridine to enable fractionation by reverse-phase chromatography[19] whereby compound **5** was obtained as an oil: λ_{max} 217 mμ; $[\alpha]D^{23}$ +12.0° (*c* 2.32, $CHCl_3$). Its 100-MHz nmr spectrum in CCl_4 (TMS)[20] showed: H-1, τ 0.43 (doublet); H-2, 3.30 (quartet); H-2, 3.81 (quartet of doublets); H-4, 4.30 (triplet of doublets); H-5, 4.82 (multiplet); H-6, 5.75 (quartet); H′-6, 5.81 (quartet). The coupling constants (Hertz) observed[20] were: J_{12} = 7.2; J_{23} = 15.5; J_{24} = 1.5; J_{34} = 5.0; J_{45} = 5.0; J_{56} = 4.0; $J_{56'}$ = 5.3; $J_{66'}$ = 11.5. For the 2,4-dinitrophenylhydrazone of **5**: mp 108–109°; λ_{max} 370 mμ (ε 22,500). *Anal.* Calcd for $C_{18}H_{20}N_4H_{10}$: C, 47.79; H, 4.42; N, 12.39. Found: C, 47.39; H, 4.06; N, 12.49.

Hydrogenation of an ethanolic solution of **5** at ice–salt temperature using 5% palladized carbon ceased after 1 mol of hydrogen had been consumed. The saturated aldehyde produced (nmr, CCl_4, TMS, τ 0.22, –CHO)[18] was characterized as its 2,4-dinitrophenylhydrazone: mp 123–124°; λ_{max} 356 mμ (ε 18,200). *Anal.* Calcd for $C_{18}H_{22}N_4O_{10}$: C, 47.57; H, 4.84; N, 12.34. Found: C, 47.43; H, 4.68, N, 12.20. Upon deacetylation the aldehydo group vanished suggesting that hemiacetal **12** may have formed. This was confirmed by converting olefin **10** into **12** by an alternative route involving (i) hydrogenation, (ii) mild acid hydrolysis, and (iii) deacetylation. This information also indicated that no epimerization had occurred in the formation of **4** from **11**.

The hydroxy aldehyde **13** is undoubtedly the substance initially formed from **11** and subsequently isomerized to **4**. Thus, when the hydrolysis of **1** was done (a) in the presence of 10 mol % of hydroquinone or (b) in the dark, compound **4** was, respectively, completely absent or present in drastically reduced quantities. The inhibition was not in the conversion of compound **11** to **13** since **6** was still completely converted to **8** in the presence of hydroquinone. Evidently small amounts of **13** in equilibrium with **11** undergo photochemical (or less efficiently, thermal) conversion to the more stable trans isomer[21] **4**, and it is the latter process that is inhibited by hydroquinone.

Further study of equilibria of the type **10** ⇌ **13** ⇌ **4** is under way and will be reported in due course.

Acknowledgments. We acknowledge generous financial support from the National Research Council of Canada and Bristol Laboratories.

(16) Although Bergmann represented his "diacetyl pseudoglucal" (**11**) as the open chain form (structure II in ref 11c), it is clear from the text that he believed that the compound existed as, and certainly reacted from, the cyclic form. In this he was indeed correct for under his reaction conditions (15-min reflux) the hydrolysate actually comprised 90–95% of the cyclized structure **11**. (Note that he also represented the "dihydro pseudoglucal" (**12**), 2-deoxyglucose, and glucose itself as open chain structures (VII, VI, and V, respectively.)

(17) Nmr features of 4 recognizable in the mixture ($CDCl_3$, TMS): H-1, τ 0.38 (d); H-2, 3.73 (quartet of doublets); H-3, 3.01 (q); J_{12} = 7.5, J_{23} = 15.0, J_{34} = 5.0 Hz; μ_{max} ($CHCl_3$) 3.58 3.70, 5.88; λ_{max} (H_2O) 217 mμ.

(18) Reference 15, p 87.

(19) B. Wickberg, *Acta Chem. Scand.*, **12**, 615 (1958).

(20) We are grateful to our colleague, Dr. K. Shaw, for this determination using an instrument kindly made available by Professor L. W. Reeves. The coupling constants were read directly from the spectrum.

(21) P. G. Wagner and G. S. Hammond, *Advan. Photochem.*, **5**, 21 (1968).

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Additions and Corrections

Conformational Analysis. LVII. The Calculation of the Conformational Structures of Hydrocarbons by the Westheimer–Hendrickson–Wiberg Method [*J. Amer. Chem. Soc.*, **89**, 4345 (1967)]. By NORMAN L. ALLINGER, MARY ANN MILLER, FREDERIC A. VANCATLEDGE, and JERRY A. HIRSCH, Department of Chemistry, Wayne State University, Detroit, Michigan 48202.

The van der Waals equation in Table II should read

$$E_v = -2.25\epsilon(d^*/r)^6 + 8.28(10)^5(\epsilon)\exp(-r/0.0736d^*)$$

The correct form of the equation was used for all of the calculations reported. We are indebted to Dr. Steven D. Stellman for calling this misprint to our attention.

On the Question of Bridge-Proton Absorptions in the Nuclear Magnetic Resonance Spectra of Norbornene and Related Systems [*J. Amer. Chem. Soc.*, **90**, 3724 (1968)]. By ALAN P. MARCHAND and JOSEPH E. ROSE, Department of Chemistry, The University of Oklahoma, Norman, Oklahoma 73069.

The captions under Figures 5 and 6 have been reversed. As they appear in the paper, Figure 5 shows the nmr spectrum of isodrin (IX); Figure 6 shows the nmr spectrum of aldrin (VIII).

Ligand Penetration Rates into Metal Ion Coordination Spheres. Aluminum(III), Gallium(III), and Indium(III) Sulfates [*J. Amer. Chem. Soc.*, **90**, 6967 (1968)]. By JOHN MICELI and JOHN STUEHR, Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106.

The quantity F_4, defined as $1 + [C_H(1 + d \ln \gamma_{SO_4}/d \ln C_{SO_4})(K'_{14} + C'_H) + K'_{14}C'_{SO_4}]/[K'_a(K'_{14} + C'_{MeOH}) + C'_H(K'_a + C'_{SO_4})]$ was omitted in the follow-