Agriculture.
(2) Cf. K. R. Markham and T. J. Mabry, *Tetrahedron*, **24**, 823 (1968), for other examples.
(3) T. Tominaga, *J. Pharm. Soc. Jpn.*, **80**, 1202 (1960); *Chem. Abstr.*, **55**, 3577 (1961).
(4) T. Tominaga and K. Yoshimura, *J. Pharm. Soc. Jpn.*, **80**, 1337 (1960); *Chem. Abstr.*, **55**, 6526 (1961).
(5) T. Tominaga, *J. Pharm. Soc. Jpn.*, **80**, 1206 (1960).
(6) T. Tominaga and K. Yoshimura, *J. Pharm. Soc. Jpn.*, **80**, 1340 (1960).
(7) (a) J. W. Clark-Lewis, R. W. Jemison, and V. Nair, *Aust. J. Chem.*, **21**, 3015 (1968); (b) J. P. van der Merwe, D. Ferreira, E. V. Brandt, and D. G. Roux, *J. Chem. Soc., Chem. Commun.*, 521 (1972).
(8) J. W. Clark-Lewis, T. M. Spotswood, and L. R. Williams, *Aust. J. Chem.*, **16**, 107 (1963).
(9) D. M. X. Donnelly, A. K. Keenan, T. Leahy, E. M. Philbin, G. Janzso, F. Kallay, and I. Koczar, *Tetrahedron*, **28**, 2545 (1972).
(10) Cf. F. M. Dean, "Naturally Occurring Oxygen Ring Compounds," Butterworths, London, 1963, pp 350–352.
(11) J. W. Clark-Lewis and W. Korytnyk, *J. Chem. Soc.*, 2367 (1958).
(12) G. Snatzke, F. Snatzke, A. L. Tokes, M. Rakosi, and R. Bognar, *Tetrahedron*, **29**, 909 (1973).
(13) W. Gaffield, *Tetrahedron*, **26**, 4093 (1970).
(14) While 2,3-diaxial substituents have been observed[9] in some 4-oximinoflavans, no 2-axial aryl substituents have been found in either flavanones or 3-substituted flavanones and such a possibility seems extremely remote in the case of **3** and **4** according to the precepts of conformational analysis.
(15) L. Bartlett, D. N. Kirk, W. Klyne, S. R. Wallis, H. Erdtman, and S. Thoren, *J. Chem. Soc. C*, 2678 (1970).
(16) These configurations are opposite to those originally proposed in ref 5.
(17) The existence of stable rhamnosides of *cis*-3-hydroxyflavanones suggests that mild hydrolytic conditions, such as enzymatic, might yield the presently unknown *cis*-3-hydroxyflavanones. However, our cursory attempts to hydrolyze neoisoengeletin with fungal hemicellulase were unsuccessful.
(18) These configurations are opposite to those originally proposed in ref 6.
(19) We have studied the isomerization of **1** in aqueous pyridine at 75° and in ethanolic sodium acetate at room temperature for the reason that the original preparation[3] of **2**, **3**, and **4** was conducted under similar conditions.
(20) Cf. T. Tominaga, *J. Pharm. Soc. Jpn.*, **80**, 1212 (1960), for previous mechanistic discussions of the astilbin isomerization.
(21) J. W. Clark-Lewis and V. Nair, *Tetrahedron Lett.*, 5467 (1966).
(22) NMR spectra were measured on a Varian HA-100 spectrometer with tetramethylsilane (Me_4Si) as the internal standard. CD spectra were obtained with the aid of a Cary 6003 dichrometer.
(23) Cf. R. C. Cambie, *J. Chem. Soc.*, 848 (1959). The authors thank Professor Cambie for a generous sample of the bark.
(24) A. Streitwieser, L. Verbit, and P. Stang, *J. Org. Chem.*, **29**, 3706 (1964).
(25) Cf. E. S. Hand and R. M. Horowitz, *J. Am. Chem. Soc.*, **86**, 2084 (1964).

A Simple Method for Determining the Configurations of Tertiary Alcoholic Centers in Branched-Chain Carbohydrate Derivatives by Use of Europium(III)-Induced Shifts in the ^1H Nuclear Magnetic Resonance Spectrum[1-3]

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The addition of graduated amounts of a solution of tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III) [Eu(fod)$_3$] to solutions of the sugar derivatives methyl 3,4-*O*-isopropylidene-2-*C*-(2-methyl-1,3-dithian-2-yl)-β-D-ribopyranoside (**1**), methyl 2-*C*-(1,3-dithian-2-yl)-3,4-*O*-isopropylidene-β-D-ribopyranoside (**2**), 4,6-dideoxy-1,2-*O*-isopropylidene-3-*C*-(2-methyl-1,3-dithian-2-yl)-α-D-*ribo*-hexopyranose (**3**), 4,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexopyranose (**4**), methyl 6-deoxy-2,3-*O*-isopropylidene-4-*C*-(2-methyl-1,3-dithian-2-yl)-α-L-talopyranoside (**5**), methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (**6**), 3-*C*-(1,3-dithian-2-yl)-1,2:4,5-di-*O*-isopropylidene-β-D-psicopyranose (**7**), and 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**8**) produces displacements of the resonance frequencies of protons in approximate proportion to the amount of Eu(fod)$_3$ added. Comparison of the magnitudes of this proportionality (shift gradient) for each identifiable proton resonance of a tertiary alcoholic derivative (**1**, **3**, **5**, or **7**) with that of the corresponding proton resonance of an alcohol of known stereochemistry (**2**, **4**, **6**, or **8**, respectively) is used to relate the configuration of the tertiary alcoholic center at the chain-branched position to that of the reference compound; uniform correspondence of the entire set of shift gradients is taken as evidence that the same relative configuration prevails in both the chain-branched tertiary alcohol and the reference molecule, whereas any gross deviation from parallelism in magnitude of corresponding shift-gradient terms in the two sets of values indicates that the tertiary alcoholic center is epimeric to the corresponding center in the reference alcohol. Statistical analysis of these shift-gradient data further supports the configurational assignments.

Configurational assignment of secondary alcoholic centers in carbohydrate molecules is often accomplished, after appropriate derivatization, by analysis of spin-coupling interactions observed in the NMR signal of the secondary CH proton.[4] Analogous molecules having tertiary alcoholic centers, which are accessible by the addition of carbon nucleophiles to a free carbonyl group in glycosulose derivatives,[5] have no proton at the newly formed asymmetric center and thus cannot be examined by the direct ^1H NMR technique. The stereochemistry at C-2 of methyl 2-*C*-formyl-β-L-arabinopyranoside was identifiable[5] because the formyl group forms an internal hemiacetal with the 4-hydroxyl group. Continued efforts in the same laboratory revealed that the change in electrophoretic mobility caused by complexation with benzeneboronic acid is configurationally determined and that the intramolecularly hydrogen-bonded O–H stretching frequency in the infrared spectrum is dependent upon the axial or equatorial disposition of the hydroxyl group involved; both of these observations can be applied to configurational elucidation[6] of a somewhat broader, but still limited range of examples.

Synthetic programs in our two laboratories have applied nucleophilic addition to free carbonyl groups in protected sugar derivatives as a general means of extending[7-9] or branching[9-12] the carbon chain of sugar molecules. The utility of these addends as potential intermediates in synthetic schemes has occasioned a renewal in our laboratories of the quest for convenient, general methods of assigning the relative configuration of the tertiary alcoholic center of molecules such as those formed in the quaternization reaction of glycosulose precursors.

^{13}C NMR spectroscopy has been employed successfully

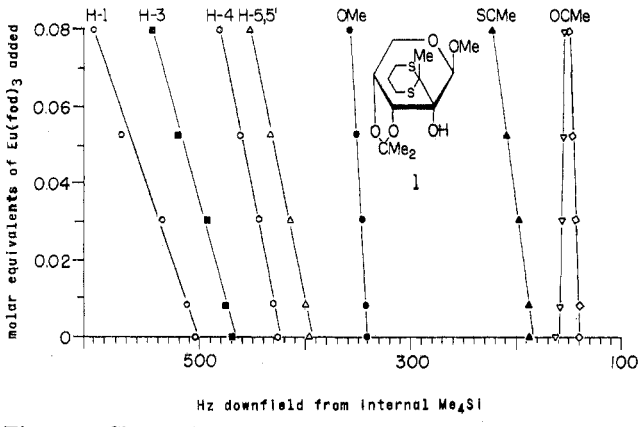

Figure 1. Chemical-shift values measured for solutions of methyl 3,4-*O*-isopropylidene-2-*C*-(2-methyl-1,3-dithian-2-yl)-β-D-ribopyranoside (1) in the presence of different concentrations of $Eu(fod)_3$.

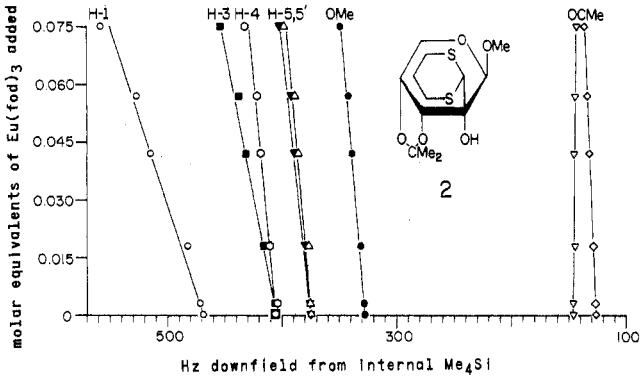

Figure 2. Chemical-shift values measured for solutions of methyl 2-*C*-(1,3-dithian-2-yl)-3,4-*O*-isopropylidene-β-D-ribopyranoside (2) in the presence of different concentrations of $Eu(fod)_3$.

in configurational elucidation of synthetic, tertiary alcoholic derivatives of carbohydrates;[13] this promises to be a general method, but it requires a number of reference compounds for comparison of chemical shifts, a fairly sizable sample, and a rather specialized item of spectroscopic equipment. Another method that has provided reliable identification of a few branched-chain tertiary alcoholic derivatives in which all the other substituent heteroatoms are derivatized[2,8] is lanthanide-shifted ^1H NMR spectroscopy.[14] The latter technique offers the advantages that it requires rather less sample and that it may be performed with relatively commonplace equipment; however, the simple relationship derived[15] to describe shifts caused by a pseudocontact (dipolar) mechanism, which was assumed[16] to apply to lanthanide ion–monofunctional ligand interactions, can apply to more extensively functionalized molecules only when the separation of substituents is sufficient to preclude[17] bidentate[18] coordination. Furthermore, direct, elementary interpretations of shifts produced by paramagnetic cations have subsequently been assailed as unsound,[19] and recent reports attest that neither the basic theory[20] of this effect nor the mechanism[21] by which it acts for europium(III) complexes is simple.

Evidence suggests, however, that induced shifts measured at low relative concentrations of the lanthanide ion are linearly related to this concentration,[22,23] and that the conformation of a tertiary alcoholic product of nucleophilic addition to a glycopyranosidulose derivative is not altered significantly from that of the identically derivatized secondary alcoholic analog having the same relative configuration.[2,8] Assuming these two conditions, it is possible to evade the complications inherent in a direct interpretation of the lanthanide-induced shift by comparing the induced shifts resulting from an arbitrary amount of added lanthanide (the shift gradients) for protons occupying analogous locations in the unknown tertiary alcoholic derivative and in a second molecule that is presumed to possess the same relative stereochemistry; if the configurations are the same, the interactions with the paramagnetic center will be similar, and the set of shift gradients measured for the sample will be linearly related to those measured for the reference, whereas if the configurations are different, different interactions will prevail in the two examples and comparison of the two sets of shift gradients will reveal that they are not linearly related. As common factors contribute to produce the induced shifts in complexes of configurationally and conformationally related molecules with $Eu(fod)_3$, an interpretation may be based confidently upon the simple, direct observation either of two sets of shift gradients that are systematically related, in pairs, by a common factor, or of two sets of shift-gradient values that are not related in any systematic manner.

This relationship may be stated algebraically as follows (eq 1), in which $\Delta\delta_{U(i)}$ and $\Delta\delta_{R(i)}$ are, respectively, the shift

$$\Delta\delta_{U(i)} = k\Delta\delta_{R(i)} \quad (1)$$

gradient of the ith proton in a (configurationally) unknown molecule (U) and that of the corresponding proton in a reference molecule (R, of known configuration), and k is a proportionality constant that relates all such pairs of shift gradients measured for the two molecules. The method thus consists of a visual estimate of the uniformity of k; if k is close to unity and deviates little from this value it is reasonable to conclude that the conformations are in direct correspondence, whereas if k exhibits major variations from a uniform value near unity, the conformations differ, reflecting a configurational difference.

Experimental Section

The 100-MHz NMR spectra of compounds **2–6** were recorded at ~30° on solutions of ~50 mg of sample dissolved in 0.3 ml of carbon tetrachloride, and spectra of compounds **1, 7,** and **8** were recorded under similar conditions in chloroform-*d*, by using a Varian HA-100 spectrometer in the frequency-sweep mode. Five percent Me_4Si was present in each solution as an internal reference and lock signal. Graduated amounts of a saturated solution of tris(6,6,7,7,8,8,8 - heptafluoro - 2,2 - dimethyl - 3,5 - heptanedionato)europium[24](III) [$Eu(fod)_3$, Aldrich] in carbon tetrachloride were added dropwise (with vigorous mixing) between spectral acquisitions on each sample until sufficient information had been accumulated; no extraordinary precautions were taken to exclude water or air. Integration of the *tert*-butyl proton resonance of $Eu(fod)_3$ was used to verify the relative concentration of shift reagent added to the sample. Data for compound **2,** which are reported in ref 2, were acquired under the same conditions.

Discussion

Figures 1–8 illustrate the dependence of chemical shifts of identifiable proton magnetic resonance signals upon the concentration of tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)europium(III)[24] added to solutions of methyl 3,4-*O*-isopropylidene-2-*C*-(2-methyl-1,3-dithian-2-yl)-β-D-ribopyranoside (**1**), methyl 2-*C*-(1,3-dithian-2-yl)-3,4-*O*-isopropylidene-β-D-ribopyranoside (**2**), 4,6-dideoxy-1,2-*O*-isopropylidene-3-*C*-(2-methyl-1,3-dithian-2-yl)-α-D-*ribo*-hexopyranose (**3**), 4,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexopyranose (**4**), methyl 6-deoxy-2,3-*O*-isopropylidene-4-*C*-(2-methyl-1,3-dithian-2-yl)-α-L-talopyranoside (**5**), methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (**6**), 3-*C*-(1,3-dithian-2-yl)-1,2:4,5-di-*O*-isopropylidene-β-D-psicopyranose (**7**), and 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**8**), respec-

Table I
Shift Gradients Measured[a] for Compounds 1-8 Complexed with Eu(fod)$_3$

Signal	1[b]	2[c]	3[c]	4[c]	5[c]	6[c]	7[b]	8[b]
H-1	12	12	6	20	12	2.7	7	1.2
H-1′							2.8	1.3
H-2			13	34	10	4		
H-3	9	6.5		48	11	10		17.2
H-4	6.5	3.6		16		11	11	17
H-4′				35				
H-5	6.5	4.0	7.5	15	10	5.5		6.8
H-5′		3.5						
H-6			2	12	2.4	1.6		4.5
H-6′								4.2
OH			15	170	17	24	45	25
OMe	2	2.7			2.6	1.5		
SCH		15.5					24	
SCMe	5		4		6			
OCMe	1.5	0.4	2.5	6.5	2.6	2.4	4	4.2
	−0.6	−1.3	0.5	4	0.2	2.3	2.5	3.8
							1	1.2
							−4.3	0.5

[a] In parts per million per molar equivalent of Eu(fod)$_3$ added. [b] In chloroform-*d*. [c] In carbon tetrachloride.

tively. The slopes describing the best (visual) straight-line fits for these data are recorded in Table I as shift gradients ($\Delta\delta$), expressed in parts per million per molar equivalent of Eu(fod)$_3$ added.

Methyl 3,4-*O*-Isopropylidene-2-*C*-(2-methyl-1,3-dithian-2-yl)-β-D-ribopyranoside (1). The chemical-shift alterations induced by addition of Eu(fod)$_3$ to solutions of methyl 3,4-*O*-isopropylidene-2-*C*-(2-methyl-1,3-dithian-2-yl)-β-D-ribo- (or arabino-) pyranoside (1), for which it is desired to determine the configuration generated at C-2 by addition[11] of the 2-methyl-1,3-dithian-2-yl anion to the carbonyl group of methyl 3,4-*O*-isopropylidene-β-D-*erythro*-pentopyranosid-2-ulose, and for the configurationally related, similarly derivatized analog, methyl 2-*C*-(1,3-dithian-2-yl)-3,4-*O*-isopropylidene-β-D-ribopyranoside (2), for which the configuration has been determined[2,13] already, are displayed in Figures 1 and 2, respectively. Complete separation of signals is effected in the spectrum of 2 by Eu(fod)$_3$, so that a complete set of shift gradients is measurable. In the spectrum of 1, the resonances of the methylene protons (H-5 and H-5′) were not separable, and the data in Figure 1 for these signals, as well as the shift gradient recorded in Table I, represent a composite of these two signals; all other signals were separated. It was possible to identify each isolated signal for both 1 and 2 by inspection on the basis of initial chemical shift, intensity, multiplicity, and line spacings, and, accordingly, to determine the shift gradient associated with each signal. The close overall similarity of all shift gradients for 1 and 2 (Figures 1 and 2) indicates the same relative (ribo) stereochemistry for both compounds, so that the D-ribo configuration may be confidently assigned to 1.

The shift gradients of the isopropylidene methyl groups are quite small, so that slight changes in their value exert profound influence upon the ratio of two such values. For shift gradients of magnitude greater than 1 ppm per molar equivalent of Eu(fod)$_3$ added, however, the ratios of corresponding signals in 1 and 2 (the nominal constant k in eq 1) range from a maximum of 1.8 (H-4) to a minimum of 0.75 (OMe); this approximates fairly closely to a constant value of unity, and the discrepancies may be amplified by the fact that a different solvent (CCl_4 for 2, $CDCl_3$ for 1) was used for the two determinations. One isopropylidene methyl resonance of both 1 and 2 experiences an upfield shift under the influence of Eu(fod)$_3$; this constitutes confirmatory evidence for the configurational identity of 1 with 2 because both *C*-methyl resonances of an analogue having arabino stereochemistry undergo displacement[2] to lower field.

4,6-Dideoxy-1,2-*O*-isopropylidene-3-*C*-(2-methyl-1,3-dithian-2-yl)-α-D-*ribo*-hexopyranose (3). Addition of Eu(fod)$_3$ to solutions of 4,6-dideoxy-1,2-*O*-isopropylidene-3-*C*-(2-methyl-1,3-dithian-2-yl)-α-D-*ribo*- (or *xylo*-) hexopyranose (3), which, like 1, contains an asymmetric, tertiary alcoholic group of stereochemistry to be determined, and 4,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexopyranose (4), which is the immediate precursor[12] of the glycosulose from which 3 is prepared, was slightly less successful in producing a completely separated NMR spectrum than in the preceding example. Nonetheless, seven of the proton resonances of 3 and all of the proton resonances of 4 were identified, and the slopes of the straight lines fitted to the induced-shift data plotted in Figures 3 and 4 for these two derivatives are recorded in Table I as shift gradients for the respective signals. Comparison of the slopes of corresponding lines in these two figures (particularly the OH resonances) reveals that, in contrast to the preceding example, the values of shift-gradient ratios (the nominal constant k in eq *1*) for 3 and 4 range from 0.09 (for the OH signal) to 0.5 (for the C-6 methyl resonance). This discrepancy (a factor of 5.5) alone is sufficient to indicate that 3 and 4 are configurationally dissimilar; in addition, the observation that the average ratio (k_{av}) is considerably smaller than unity signals a major alteration in the characteristics of the two ligand–lanthanide complexes, affording further evidence for the interpretation of different relative stereochemistry in 3 and 4. As 3 was prepared from 4 by a two-step sequence of oxidation followed by nucleophilic addition that should affect only the underivatized hydroxyl group, net inversion is indicated, and 3 is assigned the D-ribo configuration.

Methyl 6-Deoxy-2,3-*O*-isopropylidene-4-*C*-(2-methyl-1,3-dithian-2-yl)-α-L-talopyranoside (5). Chemical shifts measured for the proton resonances of a methyl 6-deoxy-2,3-*O*-isopropylidene-4-*C*-(2-methyl-1,3-dithian-2-yl)-α-L-hexopyranoside (5) having either the talo or the manno configuration are plotted in Figure 5 as a function of the relative concentration of Eu(fod)$_3$ added. Similar

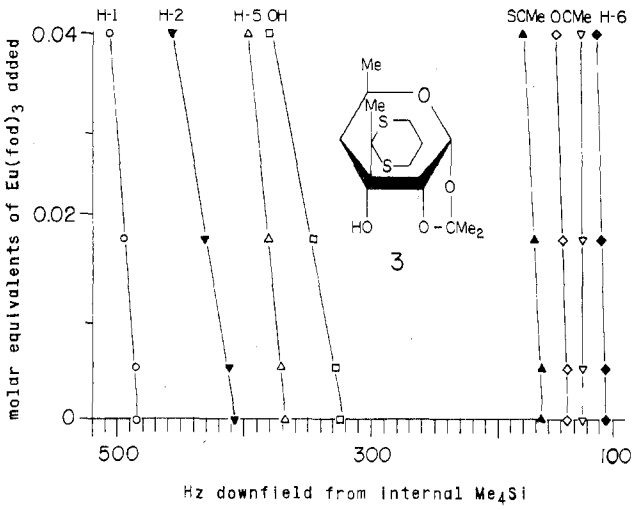

Figure 3. Chemical-shift values measured for solutions of 4,6-dideoxy-1,2-*O*-isopropylidene-3-*C*-(2-methyl-1,3-dithian-2-yl)-α-D-*ribo*-hexopyranose (3) in the presence of different concentrations of Eu(fod)$_3$.

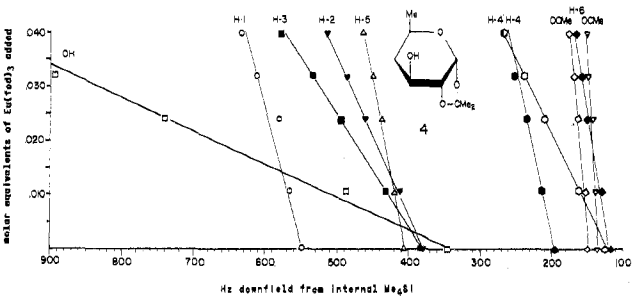

Figure 4. Chemical-shift values measured for solutions of 4,6-dideoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexopyranose (4) in the presence of different concentrations of Eu(fod)$_3$.

data are presented in Figure 6 for methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (6), which is the precursor of **5** in a two-step scheme[12] of oxidation of the 4-hydroxyl group followed by addition of the 2-methyl-1,3-dithian-2-yl anion to the newly generated carbonyl group. Shift-gradient values, determined as the slopes of the respective straight lines drawn through the data points in these two plots, are recorded in Table I; every signal in both spectra was identified so that a complete set of data was obtained. Comparison of shift-gradient values for the various protons occupying similar positions in the two molecules reveals a range from 4.4 (for H-1) to 0.7 (for the OH proton), the discrepancy amounting to a factor of 6.3 (4.4/0.7). By consideration of the very small shift-gradient values of the isopropylidene methyl groups (which are potentially hypersensitive to slight errors of measurement), the discrepancy is raised to a factor of nearly 50, and direct consideration of the *C*-methyl resonances reveals that both *C*-methyl signals of **6** experience nearly equal displacements downfield by Eu(fod)$_3$, whereas one of the two *C*-methyl resonances of **5** is virtually unshifted. Each of these observations supports the same conclusion, namely, that the configurations of **5** and **6** are not identical and that, therefore, **5** had the L-talo configuration.

3-*C*-(1,3-Dithian-2-yl)-1,2:4,5-di-*O*-isopropylidene-β-D-*ribo*-hexulopyranose (7). Relatively poor signal separation is produced by graduated additions of Eu(fod)$_3$ to a solution of 3-*C*-(1,3-dithian-2-yl)-1,2:4,5-di-*O*-isopropylidene-β-*ribo*- (or *arabino*-) hexulopyranose[12] (**7**) in chloroform-*d*, although it was possible to identify all of the 12 proton resonances except for H-4, H-5, and the two H-6

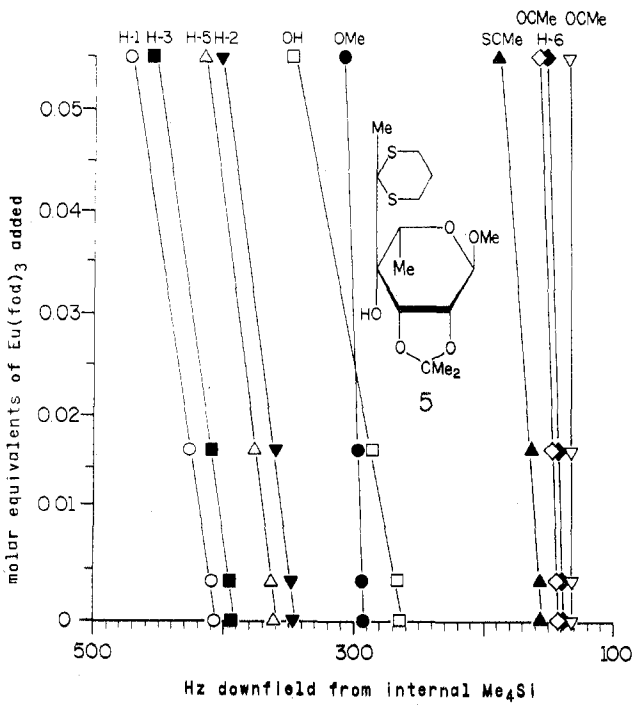

Figure 5. Chemical-shift values measured for solutions of methyl 6-deoxy-2,3-*O*-isopropylidene-4-*C*-(2-methyl-1,3-dithian-2-yl)-α-L-talopyranoside (5) in the presence of different concentrations of Eu(fod)$_3$.

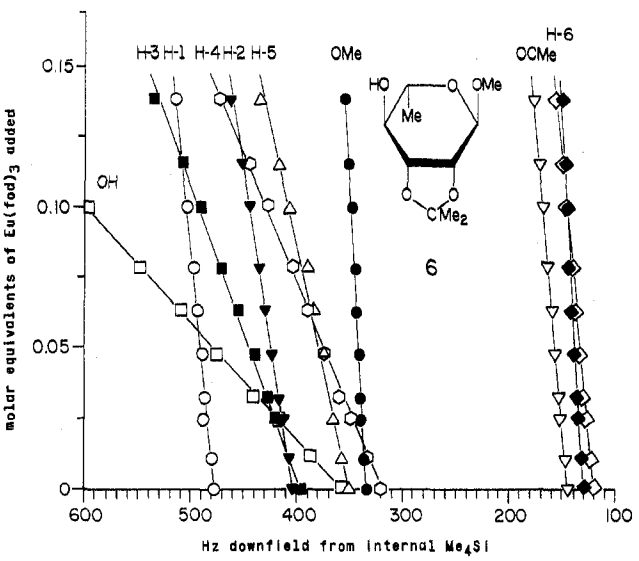

Figure 6. Chemical-shift values measured for solutions of methyl 6-deoxy-2,3-*O*-isopropylidene-α-L-mannopyranoside (6) in the presence of different concentrations of Eu(fod)$_3$.

protons; data for the eight identifiable signals (plus H-2′ of the 1,3-dithianyl group) are plotted in Figure 7 as a function of the number of molar equivalents of Eu(fod)$_3$ added. Figure 8 displays the chemical shifts obtained by similar examination of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (8), which is the antecedent of **7** in the synthetic sequence[12] of oxidizing the 3-hydroxyl group to a ketonic function and adding the 1,3-dithian-2-yl anion to the carbonyl group to produce the tertiary alcoholic branched-chain alcohol **7**; this may be formed with net retention or inversion of configuration at C-3, depending upon the stereochemistry of the attack.

Shift-gradient values in Table I [the slopes of lines in Figures 7 and 8, expressed in parts per million per molar equivalent of Eu(fod)$_3$] exhibit a number of gross, qualitative disparities for corresponding signals in **7** and **8**. The

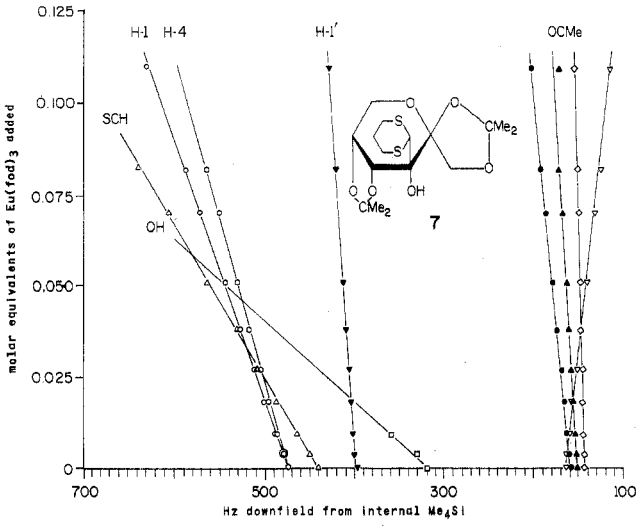

Figure 7. Chemical-shift values measured for solutions of 3-*C*-(1,3-dithian-2-yl)-1,2:4,5-di-*O*-isopropylidene-β-D-psicopyranose (**7**) in the presence of different concentrations of $Eu(fod)_3$.

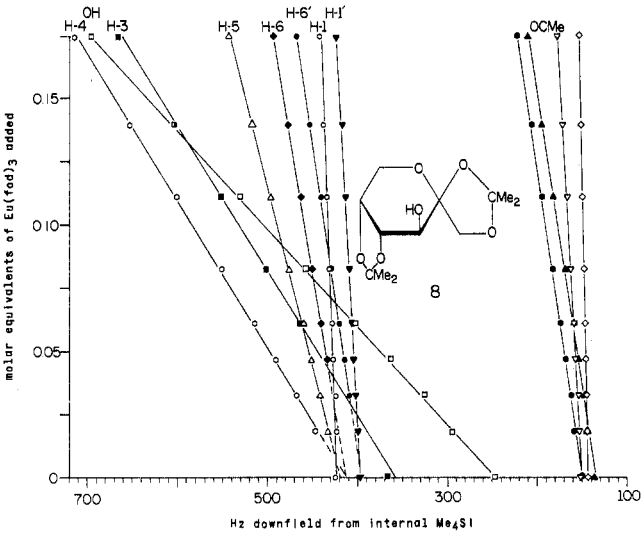

Figure 8. Chemical-shift values measured for solutions of 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose (**8**) in the presence of different concentrations of $Eu(fod)_3$.

H-4 signal of **7** shifts only 0.65 as strongly as the H-4 signal of **8**, whereas the lower field H-1 signal of **1** is shifted six times as strongly as the corresponding signal in the spectrum of **8**, a discrepancy factor exceeding 9. Furthermore, both H-1 signals of **8** are displayed by a similar amount, whereas the H-1 resonance of **7** is shifted 2.5 times more strongly than the H-1′ signal, and the resonances of all four *C*-methyl groups of **8** are displaced to low field by $Eu(fod)_3$ whereas one *C*-methyl resonance of **7** experiences *a strong upfield shift*. It is, therefore, clear that the conversion of **8** into **7** is accomplished with effective inversion of configuration at C-3 and that **7** does indeed possess the ribo configuration.

No special attempt was made to exclude water from the solvents or from **1–8**, although conditions used in preparation of solutions were uniform, so that numerical values reported in Table I represent lower limit approximations to true induced shifts of the 1:1 complexes with $Eu(fod)_3$. Within a set of values for a given compound, however, the deviation from true values will take the form of a multiplicative factor common to all members of that set. Thus, systematic comparison of corresponding terms within two sets of shift gradients measured for configurationally related molecules should compensate both for the vicissitudes of direct interpretation of induced shift magnitudes and for minor, systematic interferences arising in the course of experimentation.

Table II
Correlation Coefficients (r Values) and Best-Fit Slopes (k_{av}) Determined by Linear Regression Analysis[a] of Data in Table I and Ref 2

Related			Unrelated		
Pair	r	k_{av}	Pair	r	k_{av}
1–2	0.903	0.885	**3–4**	0.746	0.058
9–10[b]	0.96	1.16	**5–6**	0.778	0.505
9–11[b]	0.98	1.24	**7–8**	0.861	1.403
12–13[b]	0.89	1.14	**12–14**[b]	0.81	0.80
			15–16[b]	−0.05	−0.04

[a] Calculated with a Hewlett-Packard 9100B calculator by using the stock program. [b] These compounds are methyl 4,6-*O*-benzylidene-2-deoxy-3-*C*-(1,3-dithian-2-yl)-α-D-*ribo*-hexopyranoside (**9**) and its 3-*C*-(1-butyl) analog (**10**), methyl 4,6-*O*-benzylidene-2-deoxy-α-D-*ribo*-hexopyranoside (**11**), methyl 2-*C*-(1,3-dithian-2-yl)-3,4-*O*-isopropylidene-β-D-ribopyranoside (**12**), methyl 2-*C*-deuterio-3,4-*O*-isopropylidene-β-L-ribopyranoside (**13**), methyl 3,4-*O*-isopropylidene-β-D-arabinopyranoside (**14**), 3-*C*-(1,3-dithian-2-yl)-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (**15**), and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**16**); see ref 2.

Linear Regression Analysis of the Data. As eq 1 is of the form $y = mx + b$, linear regression analysis would be expected to provide a statistical measure whereby this approach might be evaluated. Table II presents the results of such an analysis of the data of Table I, and incorporates also the data for five pairs of compounds discussed in ref 2.

The range of the correlation factors is rather low, but it must be borne in mind (a) that the sample population is relatively small and that the probable errors of measurement are admittedly[2] rather substantial, and (b) that the character of the molecules examined deviates from randomness sufficiently that a correlation coefficient (r) as high as 0.75 was calculated even for the data of **4** and **5**, which would otherwise be almost completely uncorrelated. An r value of 1.0 would be required for identical coordination behavior by a pair of compounds. Of the four sample pairs from the present study in Table II, only **1–2** displays both a large r value (>0.9) and a k_{av} value (derived from regression analysis of data fit to eq 1), close to unity (1 ± 0.2); this corroborates the conclusion that **1** and **2** are configurationally related. The configurationally different pairs **3–4** and **5–6** exhibit significantly smaller r values, on the order of 0.75 (a value which was also found for the configurationally *and* structurally unrelated pair **4–5**). The value 0.86 of r for the pair **7–8** is relatively large, but the value of k_{av} (1.4) is far enough from unity to warrant the conclusion that the configurations are different. Similar analysis of data in ref 2 reveals r (k_{av}) values of −0.05 (−0.04) and 0.81 (0.80) for the two examples having dissimilar configurations, as compared with 0.96 (1.16), 0.98 (1.24), and 0.89 (1.14) for the three pairs that are configurationally related.

The foregoing examples demonstrate clearly (a) that *relative* (but not necessarily *absolute*) shift gradients for corresponding protons within a conformationally homogeneous series of derivatives having similar substitution are closely similar, and (b) that this property affords a reliable method of determining directly the configuration of nonprotonated carbon atoms (as in synthetic, branched-chain sugars) simply by comparing shift gradients found for the configurationally unknown molecule with those of a related compound (frequently a precursor in the synthesis) whose

configuration is known. Supporting evidence may be derived from a linear regression treatment of the shift-gradient data.

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References and Notes

(1) For the previous, related paper, see ref 2.
(2) S. D. Gero, D. Horton, A. M. Sepulchre, and J. D. Wander, *Tetrahedron*, **29**, 2963 (1973); see also D. Horton and J. D. Wander, *Carbohydr. Res.*, **39**, 141 (1975).
(3) Supported, in part, by Grant No. GP-33524 (The Ohio State University Research Foundation Project 3443A1) from the National Science Foundation, Washington, D.C., and by Grant No. GM-11976 (OSURF Project 1820) from the U.S. Public Health Service.
(4) B. Coxon, *Methods Carbohydr. Chem.*, **6**, 513 (1973); L. D. Hall, *Adv. Carbohydr. Chem.*, **19**, 51 (1964).
(5) J. S. Burton, W. G. Overend, and N. R. Williams, *Chem. Ind. (London)*, 175 (1961); B. Flaherty, W. G. Overend, and N. R. Williams, *J. Chem. Soc. C*, 398 (1966).
(6) R. J. Ferrier, W. G. Overend, G. A. Rafferty, H. M. Wall, and N. R. Williams, *Proc. Chem. Soc., London*, 133 (1963).
(7) D. Horton and J. M. J. Tronchet, *Carbohydr. Res.*, **2**, 315 (1966); J. L. Godman, D. Horton, and J. M. J. Tronchet, ibid., **4**, 392 (1967); D. Horton, J. B. Hughes, and J. K. Thomson, *J. Org. Chem.*, **33**, 728 (1968); D. Horton and F. O. Swanson, *Carbohydr. Res.*, **14**, 159 (1970); D. Horton and A. Liav, ibid., **24**, 105 (1972); R. Hems, D. Horton, and M. Nakadate, ibid., **25**, 205 (1972).
(8) D. Horton and J. K. Thomson, *Chem. Commun.*, 1389 (1971).
(9) A. M. Sepulchre, G. Vass, and S. D. Gero, *C. R. Acad. Sci., Ser. C*, **274**, 1077 (1972).
(10) D. Horton and E. K. Just, *Carbohydr. Res.*, **18**, 81 (1971); D. C. Baker, D. K. Brown, D. Horton, and R. G. Nickol, ibid., **32**, 299 (1974).
(11) A. M. Sepulchre, A. Gateau-Olesker, G. Lukacs, G. Vass, S. D. Gero, and W. Voelter, *Tetrahedron Lett.*, 3945 (1972).
(12) A. M. Sepulchre and S. D. Gero, unpublished data.
(13) A. M. Sepulchre, B. Septe, G. Lukacs, S. D. Gero, W. Voelter, and E. Breitmaier, *Tetrahedron*, **30**, 905 (1974).
(14) P. Girard, H. Kagan, and S. David, *Bull Soc. Chim. Fr.*, 4515 (1970); see also L. D. Hall, *Adv. Carbohydr. Chem. Biochem.*, **29**, 11 (1974).
(15) H. M. McConnell and R. E. Robertson, *J. Chem. Phys.*, **29**, 1361 (1958).
(16) C. C. Hinckley, *J. Am. Chem. Soc.*, **91**, 5160 (1969).
(17) I. Fleming, S. W. Hanson, and J. K. M. Sanders, *Tetrahedron Lett.*, 3733 (1971).
(18) N. S. Bhacca, J. Selbin, and J. D. Wander, *J. Am. Chem. Soc.*, **94**, 8719 (1972).
(19) I. M. Armitage, L. D. Hall, A. G. Marshall, and L. G. Werbelow, *J. Am. Chem. Soc.*, **95**, 1437 (1973).
(20) B. Bleaney, *J. Magn. Reson.*, **8**, 91 (1972).
(21) J. Reuben, *J. Magn. Reson.*, **11**, 103 (1973).
(22) I. M. Armitage, G. Dunsmore, L. D. Hall, and A. G. Marshall, *Can. J. Chem.*, **50**, 2119 (1972).
(23) V. G. Gibb, I. M. Armitage, L. D. Hall, and A. G. Marshall, *J. Am. Chem. Soc.*, **94**, 8919 (1972).
(24) R. E. Rondeau and R. E. Sievers, *J. Am. Chem. Soc.*, **93**, 1522 (1971).

Syntheses of 2-Substituted 1,N^6-Ethenoadenosines[1]

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The syntheses of some 2-substituted (–SH, –SC_2H_5, –SO_3^-, –OH, –$NHNH_2$, –N_3, and –NH_2) 1,N^6-ethenoadenosines are described. The fluorescent properties of the derivatives were affected by the substituents. While substituents –SH, –SC_2H_5, –NH_2, –$NHNH_2$, and –N_3 quenched the fluorescence, substituents –OH and –SO_3^- enhanced the fluorescence.

1,N^6-Ethenoadenosine[1] (ε-adenosine, **1**) is a fluorescent analog of adenosine. The phosphate derivatives of ε-adenosine have been found to be useful substrates in numerous enzyme reactions.[2] However, the fluorescence emission maximum of these derivatives is 410 nm and they are not suitable for cytochemical investigation where either tissues or cells possess autofluorescence in this range. The synthesis of a new fluorescent adenosine analog, 2-aza-ε-adenosine (**3**), that could be useful for such purposes was therefore undertaken in our laboratory. The synthesis of this new compound and its properties have recently been reported.[3] In several instances, the phosphate derivatives of this new fluorescent adenosine analog have been found to be better substrates than the corresponding ε analogs.[4,5] Furthermore, compound **3** was found to be cytotoxic against a mammary tumor cell line.[6] Since the synthetic objectives in our laboratory are to provide, first, fluorescence nucleosides and nucleotides that could be useful probes for protein–oligonucleotide interaction,[7,8] second, fluorescent nucleoside substrates that can be used as histochemical or cytochemical substrates for localizing enzymes at cellular level,[9,10] and third, potential chemotherapeutic agents, the preparation of other 2-substituted ethenoadenosines is therefore of interest. The present paper reports the synthesis and some properties of these new nucleoside derivatives.

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The preparation of **2** was described in our recent paper[6] (Scheme I). Conversion of **2** to 1,N^6-etheno-2-mercaptoadenosine (2-mercapto-ε-A, **4**) was accomplished by carbon disulfide in pyridine. The ultraviolet absorption spectrum of the nucleoside **4** showed a maximum (pH 7) at 317 nm, but when alkylated as in **5**, the uv spectrum (Table I) showed a hypsochromic shift of 35 nm to 282 nm (pH 7). Thus, it is likely that **4** exists predominantly in the thiono form in neutral solution, as in the case of 6-mercaptopurine riboside[11] and 2-mercaptoinosine.[12] When **2** was heated with urea at 150° under nitrogen, 1,N^6-etheno-2-hydroxyadenosine (2-hydroxy-εA, **6**) was isolated as the major product. This compound, however, does exist in the enol form, as its ir spectrum shows no carbonyl absorption. Compound **6** is also a good fluorescent compound with emission maximum at 430 nm and an excitation maximum at 315 nm (Table II). While the mercapto derivative **4** is nonfluorescent, the quantum yield of **6** was 0.68 at pH 5.5. The fluorescence of this compound is unique among all other fluorescent ε-adenosine derivatives. These compounds are usually quenched in acidic solution because of the protonation of the imidazole ring,[1b] but the fluorescence of **6** was quenched at alkaline pH as well as acidic pH (Figure 1). Two pK_a values of 2.40 and 6.75 were found from the titration curve. The low pK_a was due to the protonation of the imidazole ring and the higher pK_a corresponded to the ionization of the phenolic acidic proton, as shown in Scheme