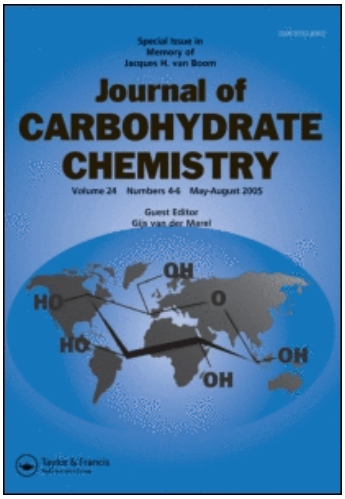

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Two-Dimensional Proton Chemical Shift Correlated NMR Spectroscopy of Digitoxose[1]

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TWO-DIMENSIONAL PROTON CHEMICAL SHIFT CORRELATED NMR SPECTROSCOPY OF DIGITOXOSE[1]

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ABSTRACT

The hydroxyl proton coupled ^{1}H NMR spectra of solutions of β-D-digitoxopyranose and of an equilibrated mixture of the four ring forms of D-digitoxose in dimethylsulfoxide-d_6 have been assigned completely by two-dimensional, proton chemical shift correlated NMR spectroscopy and spin decoupling at 400 MHz. Analysis of resolution enhanced, one-dimensional ^{1}H NMR spectra yielded an almost complete set of CH and OH proton-proton coupling constants for the four ring forms. The free aldehydo form of D-digitoxose in dimethylsulfoxide-d_6 solution has been detected by means of its characteristic H-1 quartet at δ 9.687. Quantitative analysis of the equilibrated mixture of the five forms of D-digitoxose gave the composition:- α-pyranose, β-pyranose, α-furanose, β-furanose, aldehydo form, 11.2, 67.3, 8.4, 13.0, and 0.1%, respectively. The $^4C_1$ chair conformations have been assigned to the α- and β-pyranose forms by analysis of the coupling constants and are discussed qualitatively in terms of their relative stabilities.

INTRODUCTION

The development of two-dimensional, nuclear magnetic resonance (2D NMR) techniques[2] has recently had a substantial impact on the spectroscopy of carbohydrates.[3-9] In fact, owing to the large number of inequivalent nuclei and well defined stereochemical and

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conformational variations that are present in carbohydrates, these substances serve as excellent models for demonstration of many of the 2D NMR techniques that are now available. 2D ^{1}H chemical shift COrrelated NMR SpectroscopY (2D COSY) has been implemented[10,11] as an elegant and efficient alternative to conventional, one-dimensional spin decoupling, and various modified 2D COSY experiments have been proposed and used for other purposes.[10,12]

Solutions of carbohydrates in dimethylsulfoxide (DMSO) are of significant interest to us for purposes of quantitative analysis and physical characterization; for example, the certification of D-glucose Standard Reference Materials.[9,13] In DMSO solution, the mutarotation of sugars is normally slower than in aqueous solution,[14] thus affording more time for initial analytical measurements. Additionally, the hydroxyl protons of sugars are usually in slow exchange in neutral DMSO solutions, so that the NMR signals of these protons are observed as separate multiplets. This phenomenon offers the possibility of a greater number of well dispersed analytical "handles" for DMSO solutions than for aqueous solutions, but has the disadvantage that coupling with the hydroxyl protons causes the spectra of many of the CH protons to be more complex.[14] Nevertheless, the hydroxyl proton coupling constants have intrinsic value and in some cases yield additional stereochemical information.[9]

This paper describes an investigation of D-digitoxose (2,6-dideoxy-D-*ribo*-hexose) and its various forms by ^{1}H NMR spectroscopy at 400 MHz. The 2D COSY method is used to delineate the assignments in the hydroxyl proton coupled spectra obtained from solutions of D-digitoxose in DMSO-d_6. In related work, the 2D J-resolved NMR technique has been applied to complete analysis of the ^{1}H NMR spectra of the hydroxyl proton coupled α- and β-D-glucopyranoses.[9]

RESULTS AND DISCUSSION

^{1}H NMR Spectroscopy of Solutions of Crystalline D-Digitoxose. Taken soon after dissolution of the sugar, the ^{1}H NMR spectrum of

a solution of a commercial sample of crystalline D-digitoxose in DMSO-d_6 showed the CH and OH proton signals of predominantly a single species. Interpretation of the vicinal CH proton-proton coupling constants of this species (*vide infra*) indicates that it is the β-pyranose form (β-p). Under high resolution conditions (low sugar concentration and resolution enhancement by Gaussian exponential filtering), the ^{1}H NMR spectrum (see FIG. 1) of β-D-digitoxopyranose shows evidence for at least two long range

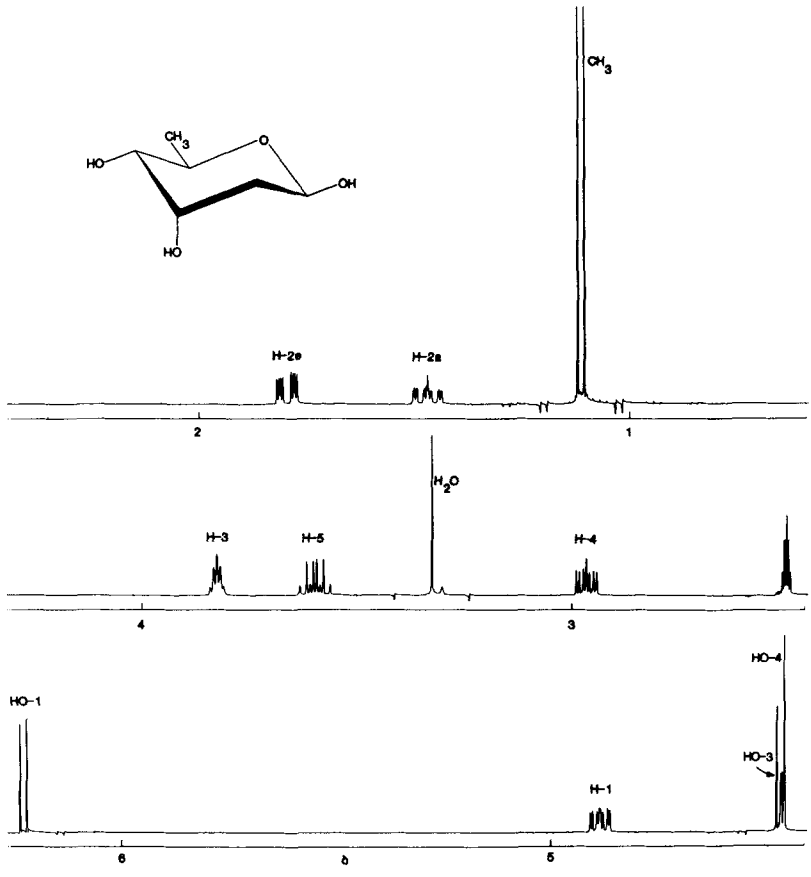
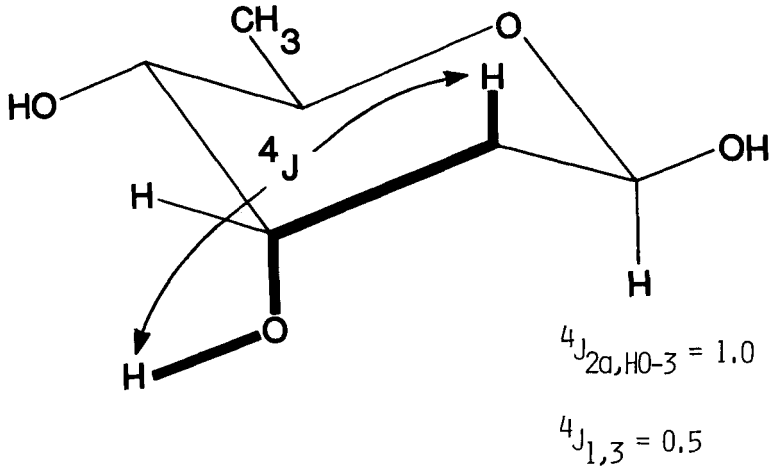


FIG. 1. ^{1}H NMR spectrum of a solution of β-D-digitoxopyranose in DMSO-d_6 at 400 MHz.

coupling constants since the H-1 signal appears as a tridecet and the HO-3 signal as a quartet. On addition of deuterium oxide to the solution, the hydroxyl signals disappear and are translocated to the HOD signal, and the H-2'(=H-2a) pentadecet collapses to an octet, because of removal of the coupling constant $^{4}J_{2a,HO-3}$ 1.0 Hz. Spin decoupling experiments confirmed the presence of this coupling and also that of a smaller one, $^{4}J_{1,3}$ 0.5 Hz. The magnitude of the coupling between H-2a and HO-3 suggests that H-2a, C-2, C-3, and HO-3 have a planar 'W' arrangement, in which HO-3 has a favored orientation with the OH bond trans coplanar to the



C-2--C-3 bond. The observation of a small value $J_{3,HO-3}$ 3.1 Hz is consistent with a gauche orientation (dihedral angle 60°) of H-3 and HO-3 in the β-p anomer. A similar long range coupling constant $^{4}J_{2,HO-1}$ 1.2 Hz has been reported for α-D-glucopyranose examined under the same conditions.[9] Further confirmation of the long range coupling between H-2a and HO-3 of β-D-digitoxopyranose was obtained by means of the 2D COSY method.

A pulse sequence for the 2D COSY NMR technique is shown in FIG. 2. The first π/2 pulse turns the magnetization vector from its equilibrium position along the z-axis into the x-y plane. The transverse magnetization evolves during the incrementable delay

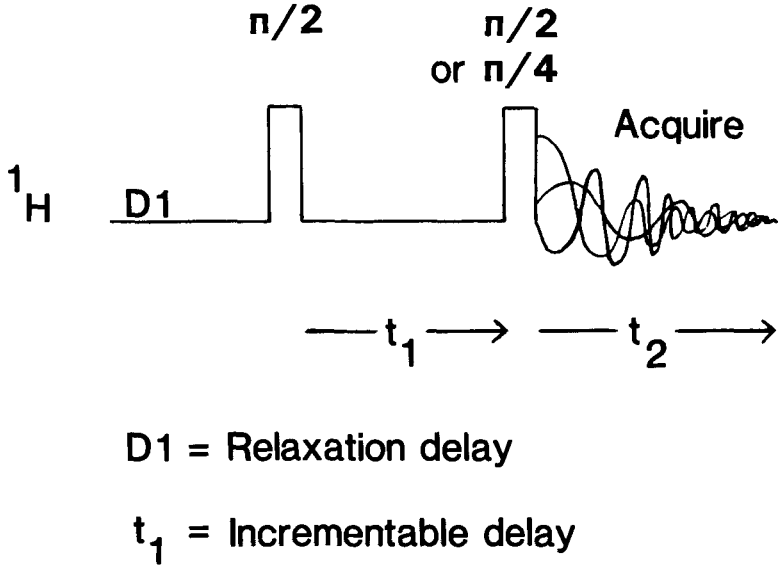


FIG. 2. Pulse sequence for 2D COSY ^{1}H NMR.

t_1, at the end of which a second $\pi/2$ (or $\pi/4$) pulse mixes the magnetizations and hence the chemical shifts of the homonuclear spins. A free induction decay (FID) signal is acquired during the time t_2, for each discrete value of t_1. The resonances detected by the first Fourier transformation of the FIDs in the t_2 dimension are modulated as a function of t_1 by all frequencies (due to both chemical shifts and coupling constants) that are generated by the first pulse.

For parallel transitions, i.e., those that generate resonances in the same multiplet, the chemical shifts that undergo mixing are identical and the resulting multiplets in the 2D spectrum have the same chemical shift in both dimensions. Thus one part of the 2D COSY spectrum consists of a set of resonances along the diagonal, that correspond to the normal one-dimensional spectrum. This effect may be seen in the contour plot of the ^{1}H 2D COSY spectrum of β-D-digitoxopyranose that is shown in FIG. 3.

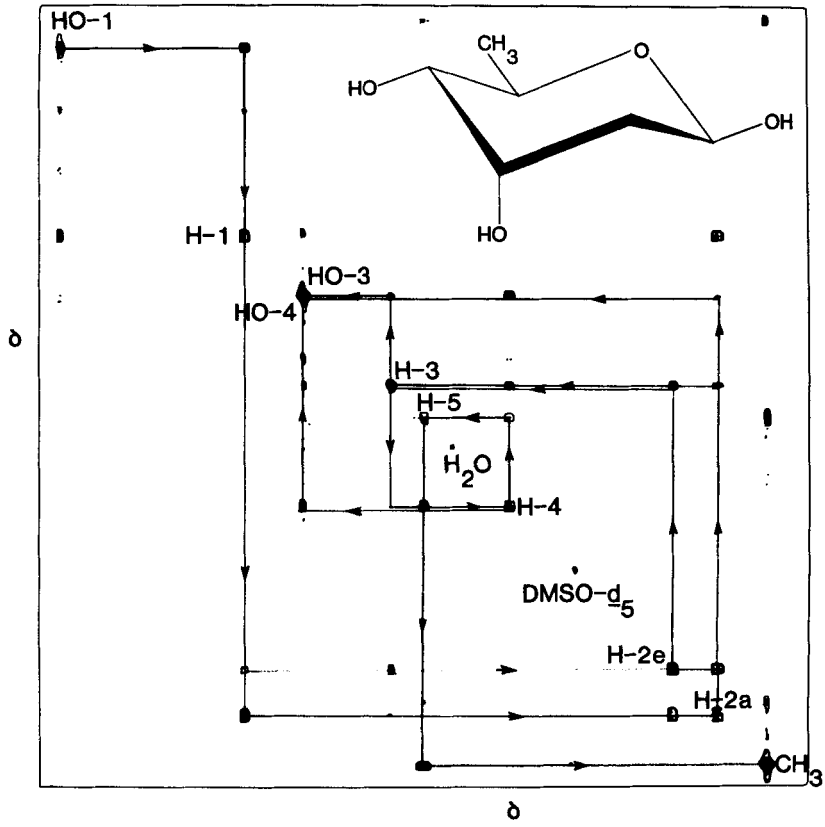


FIG. 3. Contour plot of the 2d COSY ^{1}H NMR spectrum of β-D-digitoxopyranose in DMSO-d_6 at 400 MHz, with the spin coupling connectivity pathway indicated.

Other transitions of spin coupled nuclei are either progressively or regressively connected through a common energy level, and mixing of the magnetizations of these chemically shifted nuclei (e.g., A and X) produces resonances in the 2D COSY spectrum that have different chemical shifts in each dimension, e.g., coordinates δ_A,δ_X, and δ_X,δ_A. These resonances therefore occupy off-diagonal positions in the 2D COSY spectrum and are known as cross peaks.[2] The observation of cross peaks signifies spin coupling connectivity between the related multiplets on the diagonal, thus affording a method for spectral assignment.[10]

In FIG. 3, the connectivity pathway between the HO-1 and CH_3 protons is illustrated for the case of a single anomer (β-p). Noteworthy is the observation of a cross peak for H-2a and HO-3, thus providing further verification of their long range coupling. The smaller coupling between H-1 and H-3 was not indicated by this experiment, although modified procedures are available for emphasis of long range coupling constants.[10,12] The chemical shifts measured by first order analysis of the well dispersed spectrum (FIG. 1) of the β-p anomer are shown in Table 1 and the coupling constants in Tables 2 and 3. The large value $J_{1,2'}$ 9.6 Hz indicates that H-1 and H-2' have a trans-diaxial orientation and, therefore, that the preponderant anomer in crystalline D-digitoxose is the β-pyranose form. The remaining vicinal CH proton-proton coupling constants (Table 2), in particular the value $J_{4,5}$ 9.4 Hz confirm the $^4C_1$ chair conformation for the β-p anomer (in solution).

The structure of D-digitoxose is closely related to that of 2-deoxy-D-erythro-pentose, from which the 2,6-dideoxyhexose may be derived merely by substitution of a hydrogen atom at C-5 by a methyl group. Previous work has shown that 2-deoxy-β-D-erythro-pentopyranose adopts the alternative $_4C^1$ chair conformation, both in aqueous solution,[15] and in the solid state.[16] Thus the observation that β-D-digitoxopyranose in DMSO-d_6 solution favors the $^4C_1$ conformation exclusively may be attributed to the conformational locking effect of an equatorial methyl group.

^{1}H NMR Spectroscopy of Equilibrated Solutions of D-Digitoxose. Following dissolution of crystalline D-digitoxose in DMSO-d_6, the solution mutarotates fairly rapidly (2-3 days) to give an equilibrated mixture of five different forms. The hydroxyl proton coupled ^{1}H NMR spectrum of the four major components of the mixture is shown in FIG. 4, in which may be seen four HO-1 doublets in the vicinity of δ 6 and four H-1 multiplets around δ 5. These signals obviously represent the four ring forms of the sugar. The fifth species in the equilibrated mixture was detected as a

TABLE 1. Proton Chemical Shifts[a] of Digitoxose.

Anomer-ring form	H-1	H-2	H-2'[b]	H-3	H-4	H-5	CH_3	HO-1	HO-3	HO-4	HO-5
α-pyranose	4.973do	∿1.87m[d]	1.723sx	3.883q	3.042o	3.921o	1.142d	6.022d	4.831d	4.616d	---
β-pyranose	4.900o	1.820o	1.485td[e]	3.847qi	2.990sp	3.608o[d]	1.122d	6.231d	∿4.46q[d]	4.474d	---
	4.886td[e,f]	1.794o	1.467pd[e]	3.825qi	2.965sp	3.597o	1.110d	6.228d	4.460q	4.466d	---
α-furanose	5.294sx	2.122sp	1.647o	4.103de	3.653q	3.503m[g]	1.058d	5.791d	4.678d	---	4.505d
β-furanose	5.370q	∿1.87m[d]	1.87m[d]	4.292n	3.433q	3.524m[g]	1.089d	6.139d	4.793d	---	∿4.47d[d]

[a] Ppm from internal tetramethylsilane, measured from one-dimensional spectra at 400 MHz. Unless stated otherwise, the chemical shifts are reported for an equilibrated solution of D-digitoxose (2 mol L^{-1}) in DMSO-d_6, with a digital resolution of 0.0003 ppm/point.

[b] The prime refers to the methylene proton at higher field. For the α- and β-pyranose forms, H-2 corresponds to H-2e (equatorial H-2) and H-2' to H-2a (axial H-2).

[c] Signal multiplicities: do, dodecet; m, multiplet; sx, sextet; q, quartet; o, octet; d, doublet; td, tridecet; qi, quintet; sp, septet; pd, pentadecet; de, decet; n, nonet.

[d] Incompletely resolved.

[e] Incompletely resolved hexadecet.

[f] Measured for a non-equilibrated solution of β-D-digitoxose (10 mg) in DMSO-d_6 (0.5 mL), with a digital resolution of 0.0002 ppm/point.

[g] Unresolved and obscured by water signal; these chemical shifts were obtained from per-O-deuterated material.

TABLE 2. CH Proton-Proton Coupling Constants (Hz) of Digitoxose.[a]

Anomer-ring form	$J_{1,2}$	$J_{1,2'}$	$^4J_{1,3}$	$^2J_{2,2'}$	$J_{2,3}$	$J_{2',3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
α-pyranose	2.0	3.7	0.6	14.1	3.6	3.2	3.0	9.0	6.3
β-pyranose	2.1	9.6	0.5	13.4	3.5	2.7	3.1	9.4	6.2
α-furanose	5.6	2.3	---	13.5	7.7	3.6	4.3	4.4	6.5
β-furanose	4.7	4.7	---	---[b]	6.1	4.4	2.9	6.1	6.4

[a]For solutions in DMSO-d_6, measured at 400 MHz, with digital resolution, 122 mHz/point.

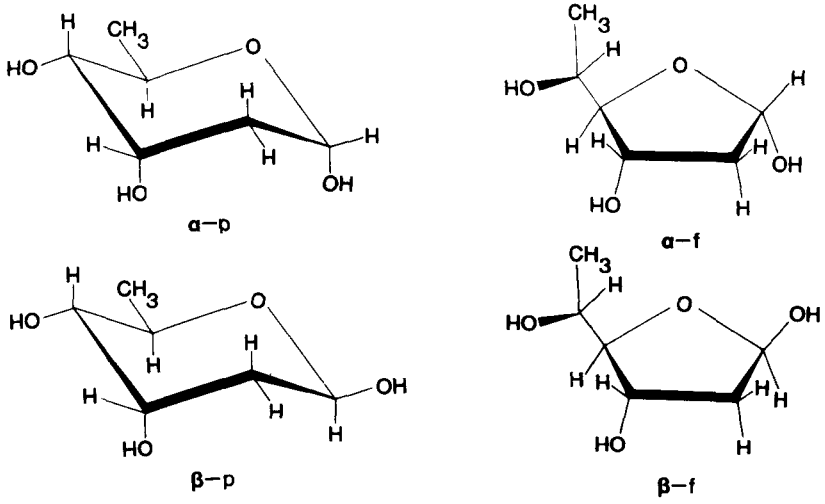
[b]Obscured.

TABLE 3. OH Proton Coupling Constants (Hz) of Digitoxose.[a]

Anomer-ring form	$J_{1,HO-1}$	$^4J_{2a,HO-3}$	$J_{3,HO-3}$	$J_{4,HO-4}$	$J_{5,HO-5}$
α-pyranose	7.3	---	5.4	7.0	---
β-pyranose	6.4	1.0	3.1	7.0	---
α-furanose	5.6	---	5.4	---	7.2
β-furanose	5.5	---	4.6	---	---[b]

[a]For solutions in DMSO-d_6, measured at 400 MHz, with digital resolution, 122mHz/point.

[b]Obscured.



very weak quartet (J 1.8 and 3.4 Hz) at δ 9.687 that is undoubtedly the H-1 signal of the aldehydo form of digitoxose. The observation of this form as a free aldehyde rather than as an aldehydrol may be attributed to the relatively anhydrous conditions in the solvent. The signals of the aldehydo form are beyond the dynamic range of the 2D COSY experiments reported herein and, therefore, these signals are not discussed further, except for the purpose of

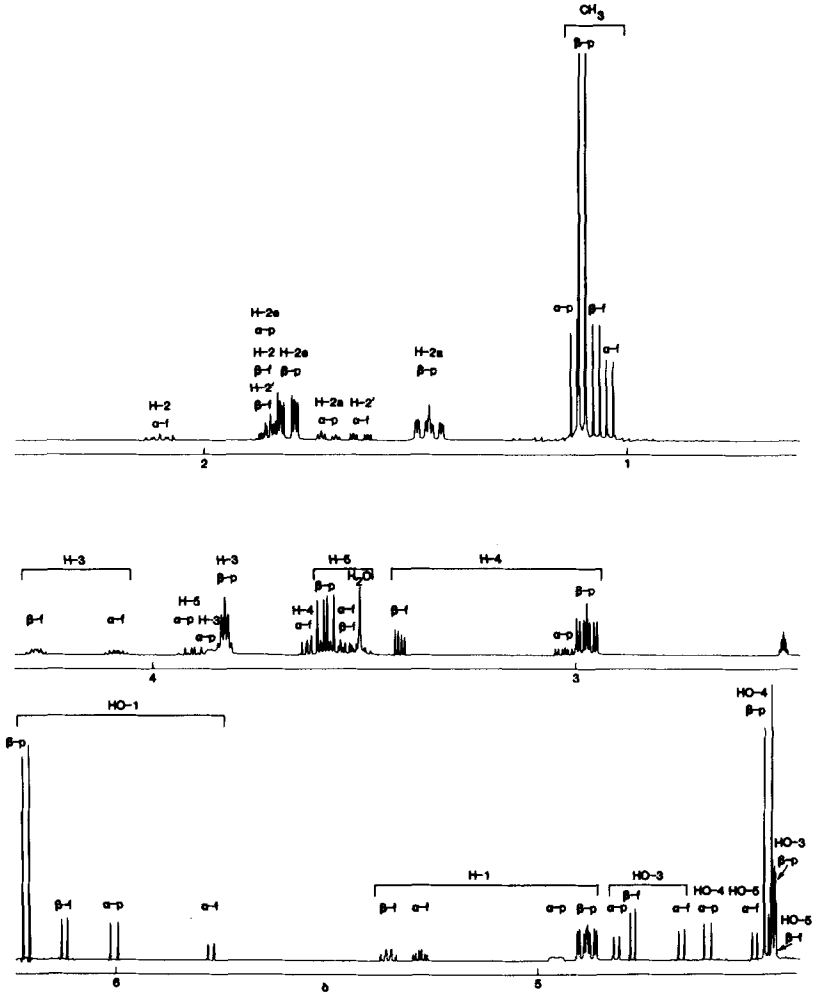


FIG. 4. Hydroxyl proton coupled, ^{1}H NMR spectrum of an equilibrated mixture of the ring forms of D-digitoxose in DMSO-d_6, measured at 400 MHz. The signals of the aldehydo form are not shown.

quantitative analysis of the mixture. The aldehydo forms of digitoxose and other sugars will be the subject of a future communication.

The ^1H NMR spectrum (Fig. 4) of the equilibrated mixture of D-digitoxose species is quite complex and it was here that the full power of the 2D COSY method became useful. If the equivalent protons of a methyl group are considered as a single spin group, then each ring form of digitoxose gives rise to a 10 spin system of protons, and complete assignment of the proton spectrum of four ring forms by conventional spin decoupling techniques would require 40 separate experiments. In practice, most of the spin coupling connectivity information for the four ring forms was obtained from a single 2D COSY experiment. A limited number (four) of spin decoupling experiments was done to resolve certain ambiguities in the two-dimensional data. However, in spectra that contain overlapping multiplets or multiplets of similar chemical shift, assignments made exclusively by spin decoupling methods may also be expected to suffer from some ambiguities, owing to the presence of non-selective off-resonance effects.

Matching of the integrated intensities of the multiplets in the one-dimensional spectrum (FIG. 4) allowed many of the resolved multiplets to be identified as originating from one particular ring form or another. A contour plot of the 2D COSY spectrum of the four ring forms of equilibrated D-digitoxose is shown in FIG. 5. To avoid confusing the diagram, the connectivity pathway is only shown for the α-furanose anomer (α-f), although a similar pathway was constructed for each ring form in turn. In this way, the multiplet locations of the ring forms in the equilibrated mixture were assigned completely and measurement of the high resolution, one-dimensional spectra yielded the chemical shifts, and CH and OH proton-proton coupling constants reported in Tables 1, 2, and 3, respectively.

The α-pyranose anomer (α-p) was assigned as the form that displayed similar coupling constants (except $J_{1,2'}$) to the β-p form characterized already. The vicinal CH proton-proton coupling

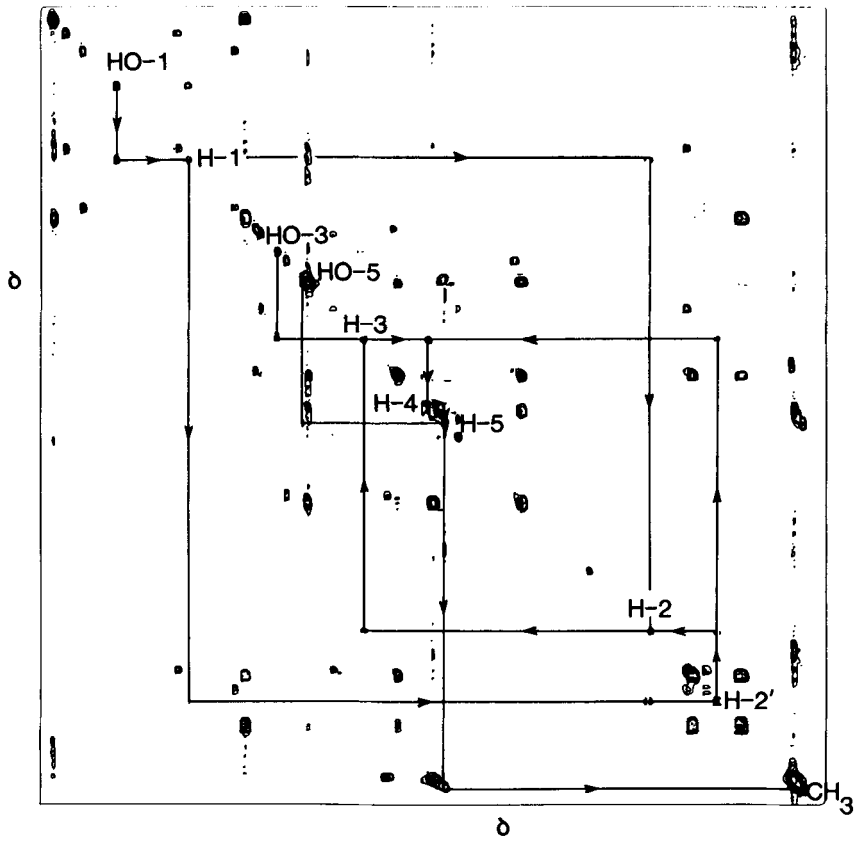


FIG. 5. Contour plot of the 2D COSY ^{1}H NMR spectrum of an equilibrated mixture of the ring forms of D-digitoxose in DMSO-d_6 at 400 MHz.

constants of the α-p form are also consistent with the $^{4}C_{1}$ chair conformation, although in view of the fact that H-1 and H-3 have a planar 'W' arrangement in this conformation, it is surprising that the value $^{4}J_{1,3}$ 0.6 Hz for the α-p form is only slightly larger than that of the β-p form. It is possible that the $^{4}C_{1}$ conformation of the α-p anomer does not have ideal geometry because of distortion induced by a syn-axial 1,3 interaction between the DMSO-d_6-solvated hydroxyl groups at C-1 and C-3. Although the

value $J_{1,HO-1}$ 7.3 Hz of the α-p form is larger than that (6.4 Hz) of the β-p form (Table 3), it has recently been observed that the value $J_{1,HO-1}$ 4.8 Hz of α-D-glucopyranose is smaller than that (6.6 Hz) of β-D-glucopyranose. Thus, an earlier suggestion[17] that equatorial H-1 protons have relatively large couplings of 6.5-8 Hz with HO-1, whereas the corresponding couplings of axial H-1 protons are smaller (4.5-5 Hz) does not appear to be generally valid.

The α-furanose form (α-f) was assigned as the component of the equilibrated mixture that displayed the smaller coupling constants ($J_{1,2'}$ 2.3 and $J_{2',3}$ 3.6 Hz) between one of the protons at C-2 (H-2') and H-1 and H-3, respectively, since smaller couplings would be expected for these trans pairs of protons than for the cis pairs of protons H-1, H-2 and H-2, H-3 ($J_{1,2}$ 5.6 and $J_{2,3}$ 7.7 Hz). This assignment identifies H-2 as the hydrogen atom above the furanoid ring (i.e., the one with the L configuration) and H-2' as the atom below the ring. The assignments of the α-f and β-f forms are also consistent with assignments derived previously for the α and β forms of 2-deoxy-D-erythro-pentofuranose on the basis of both ^{1}H NMR and polarimetric studies.[15]

The coupling constants $J_{1,2}$ 2.4 and $J_{1,2'}$ 5.3 Hz reported[15] for 2-deoxy-α-D-erythro-pentofuranose are very similar to those found for α-D-digitoxofuranose and those reported for 2-deoxy-β-D-erythro-pentofuranose ($J_{1,2} = J_{1,2'}$ 4.5 Hz) are closely similar to the values measured for β-D-digitoxofuranose (see Table 2), although the labeling of H-2 and H-2' is reversed.

A useful simplification of the ^{1}H NMR spectrum of the equilibrated mixture of digitoxose forms was obtained by deuterium exchange of the hydroxyl protons prior to spectroscopy of the solution in DMSO-d_6 (see FIG. 6). This procedure effectively removed the OH coupling constants and yielded a spectrum resembling that of digitoxose equilibrated in deuterium oxide. The chemical shifts of the simplified, but still overlapping H-5 multiplets of the α-f and β-f forms were measured in this way (Table 1). The quartet and triplet band shapes of the hydroxyl uncoupled H-1 multiplets

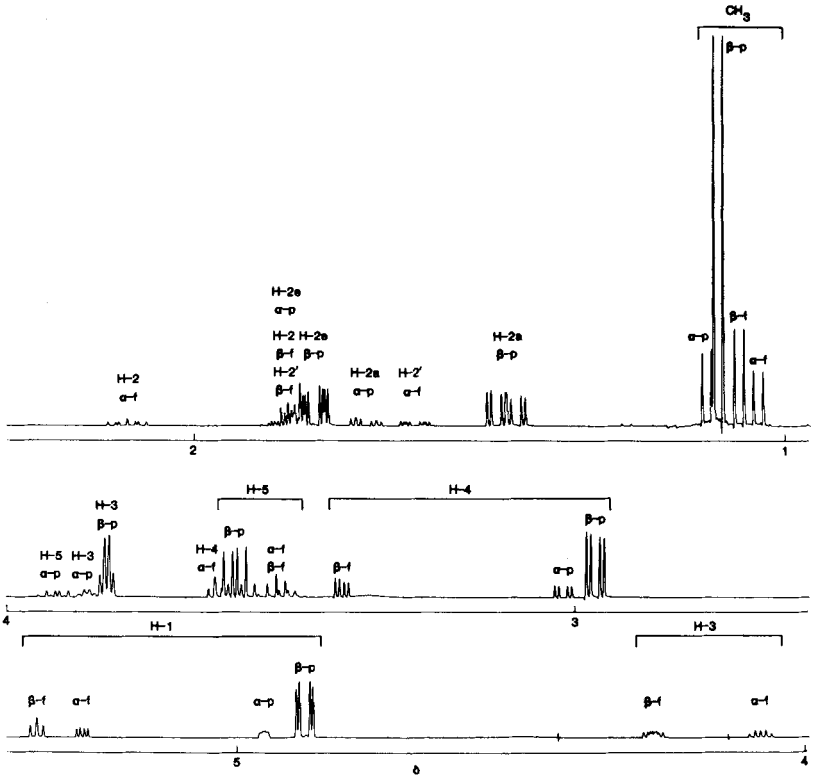


FIG. 6. ^{1}H NMR spectrum of an equilibrated mixture of per-O-deuterated ring forms of D-digitoxose in DMSO-d_6 at 400 MHz.

are generally characteristic of the anomeric configurations of 2-deoxy-α- and β-D-erythro-pentofuranose derivatives.[18]

Quantitative Analysis of Equilibrated D-Digitoxose. The presence of a large number of well resolved multiplets in the hydroxyl proton coupled spectrum (FIG. 4) of the equilibrated mixture of D-digitoxose anomers allowed a multi-parameter, quantitative analysis to be performed; i.e., one in which a number (in this case, 5-8) of the proton signals of each ring form were

integrated following acquisition of the spectrum under non-saturating conditions. The results of this analysis are shown in Table 4. The composition data show that the β-p form is by far the most stable form in DMSO-$\underline{d}_6$ solution, in spite of the fact that the $^4\underline{C}_1$ conformation favored by this form must be destabilized by the anomeric effect. This lends further weight to the argument that the α-p form must be even more destabilized by its *syn*-axial 1,3 interaction. By contrast, the α-p form of 3-deoxy-D-*ribo*-hexose (in which HO-3 is not present) is relatively more stable (β-p:α-p ratio, 40:26).[19]

The proportions of the D-digitoxose ring forms measured by ^{1}H NMR (Table 4) are in good agreement with those determined by ^{13}C NMR.[20] The *aldehydo* form has the expected low concentration (0.1%).

EXPERIMENTAL

Preparation of Solutions. Crystalline D-digitoxose (Aldrich[21] Gold Label grade, 99+%) was dried under vacuum at 50 °C for five

TABLE 4. Composition of an Equilibrated Solution of Digitoxose.[a]

Species	Percent Composition[b]
α-pyranose	11.2 ± 0.3
β-pyranose	67.3 ± 0.4
α-furanose	8.4 ± 0.2
β-furanose	13.0 ± 0.2
aldehydo-form	0.1[c]

[a] 2 mol L^{-1} in DMSO-$\underline{d}_6$, equilibrated for two months.

[b] For the ring forms, the composition reported is the mean of the integrals of 5-8 different proton signals of each form, plus or minus one standard error. Methyl proton signals were not used because of significant peak overlaps.

[c] Obtained from two equilibrated solutions by integration of the *aldehydo* proton quartet.

days and portions were dissolved in aliquots (0.5 mL) of fresh DMSO-$\underline{d}_6$. ^{1}H NMR spectra of these solutions indicated that the crystalline D-digitoxose contained greater than 99% of the β-pyranose anomer. A per-O-deuterated mixture of D-digitoxose anomers and ring forms was prepared by successive lyophilizations of the crystalline sugar with aliquots of deuterium oxide (3 mL, 99 atom % D, 2 mL, 100 atom % D; and 1 mL, 100 atom % D). The lyophilizate (50 mg) was then dissolved in DMSO-$\underline{d}_6$ (0.55 mL).

NMR Spectroscopy. ^{1}H NMR spectra were recorded at 400 MHz and at 27 °C by use of a Bruker Instruments Model WM-400 spectrometer in the pulse-Fourier transform mode. One dimensional spectra were acquired by means of the Bruker FTQNMR and DISNMRP programs using spectral widths of 3, 4, or 5 kHz and a 32,768 point data set, resolution enhanced, and then zero filled to 65,536 points. The use of a spectral width of 5 kHz was necessary for detection of an unfolded H-1 signal of the aldehydo form of D-digitoxose. Resolution enhancement was performed by Gaussian, exponential filtering of the FID signal, using a line broadening of -1.0 Hz and a Gaussian broadening fraction of 0.3. For quantitative analysis, a 20° pulse (width 3.0 μs) was used together with a total relaxation delay of 8.05 s. Before integration of each multiplet, the baseline surrounding it was individually corrected.

2D COSY ^{1}H NMR spectra were obtained by means of the Bruker DISNMRP program, using an initial data matrix ($\underline{t}_1$ x $\underline{t}_2$) of 256 x 1,024 points that was zero filled to 512 x 1,024 points, and then doubly transformed to give a matrix (real part, $\underline{f}_1$ x $\underline{f}_2$) of 512 x 512 points, for which the spectral width was 2,304 Hz in both dimensions (digital resolution, 4.5 Hz/point = 0.011 ppm/point). The π/2 pulse width was 14 μs and a minimum relaxation delay of 5.22 s was used together with either 16 or 64 scans for each value of the incrementable delay $\underline{t}_1$.

ACKNOWLEDGMENT

The spectra were recorded at the high field NMR facility of the National Measurement Laboratory.

REFERENCES AND FOOTNOTES

1. Presented at the 186th National Meeting of the American Chemical Society, Washington, D. C., August 29, 1983.

2. W. P. Aue, E. Bartholdi, and R. R. Ernst, J. Chem. Phys., 64, 2229 (1976).

3. L. D. Hall, S. Sukumar, and G. R. Sullivan, J. Chem. Soc. Chem. Commun., 292 (1979); L. D. Hall and S. Sukumar, J. Am. Chem. Soc., 101, 3120 (1979); L. D. Hall, G. A. Morris, and S. Sukumar, J. Am. Chem. Soc., 102, 1745 (1980); L. D. Hall and G. A. Morris, Carbohydr. Res., 82, 175 (1980).

4. R. C. Bruck and M. A. Bruck, J. Biol. Chem., 257, 3409 (1982).

5. J. Dabrowski, H. Egge, and U. Dabrowski, Carbohydr. Res., 144, 1 (1983).

6. T. A. W. Koerner, Jr., J. H. Prestegard, P. C. Demou, and R. K. Yu, Biochemistry, 22, 2676 (1983).

7. W. Curatolo, L. J. Neuringer, D. Ruben, and R. Haberkorn, Carbohydr. Res., 112, 297 (1983).

8. L. Szilagyi, Carbohydr. Res., 118, 269 (1983).

9. B. Coxon, Anal. Chem., 55, 2351 (1983).

10. A. Bax and R. Freeman, J. Magn. Reson., 44, 542 (1981); see also G. Batta and A. Lipták, J. Am. Chem. Soc., 106, 248 (1984).

11. G. Wagner, A. Kumar, and K. Wüthrich, Eur. J. Biochem., 114, 375 (1981).

12. A. Bax, "Two-Dimensional Nuclear Magnetic Resonance in Liquids", Reidel, Dordrecht, 1982, p. 69.

13. B. Coxon and R. Schaffer, Anal. Chem., 43, 1565 (1971).

14. B. Casu, M. Reggiani, G. G. Gallo, and A. Vigevani, Tetrahedron Lett., 2839 (1964).

15. R. U. Lemieux, L. Anderson, and A. H. Conner, Carbohydr. Res., 20, 59 (1971).

16. S. Furberg, Acta. Chem. Scand., 14, 1357 (1960).

17. W. Pigman and H. S. Isbell, Advan. Carbohydr. Chem., 23, 11 (1968).

18. L. B. Townsend in "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 2; W. W. Zorbach and R. S. Tipson, Eds., Wiley, New York, NY, 1973, p. 300.

19. S. J. Angyal and V. A. Pickles, Carbohydr. Res., 4, 269 (1967).

20. B. Coxon, unpublished results.

21. Certain commercial equipment, instruments, or materials are identified in this paper to specify adequately the experimental procedure. Such identification does not imply recommendation by the National Bureau of Standards, nor does it imply that the materials or equipment are necessarily the best available for the purpose.