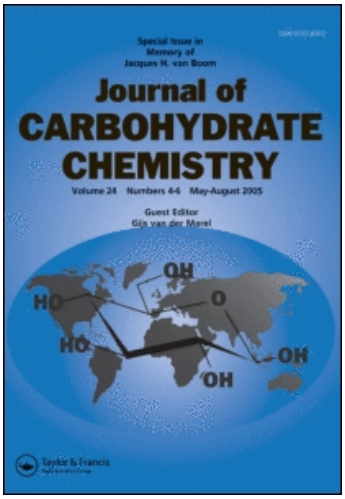

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STRUCTURE OF 1,5-ANHYDRO-D-FRUCTOSE: X-RAY ANALYSIS OF CRYSTALLINE ACETYLATED DIMERIC FORMS[1]

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ABSTRACT

Acetylation of 1,5-anhydro-D-fructose under acidic conditions gave two crystalline acetylated dimeric forms, which by X-ray analysis were shown to be diastereomeric spiroketals formed between C-2 and C-2'/C-3'. The structures of the compounds differed only at the configuration at C-2. Acetylation or benzoylation of 1,5-anhydro-D-fructose in pyridine yielded 3,6-di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-enos-2-ulopyra-nose or crystalline 1,5-anhydro-3,6-di-*O*-benzoyl-4-deoxy-D-*glycero*-hex-3-enos-2-ulo-pyranose.

INTRODUCTION

Since 1,5-anhydro-D-fructose (**1a**) was first synthesised in 1980[2] it has been found to be a precursor of the antibiotic microthecin in fungi[3] and has received a lot of attention because it is a degradation product from α-1,4-glucans.[4,5,6] In addition, it has

Table 1. Composition of amorphous 1,5-anhydro-D-fructose (**1a**) in pyridine[a]

Time	% (mole) **1a**	% (mole) **4a**	% (mole) **5a**
0	35	43	22
2 days	31	20	49
4 days	28	9	63
7 days	28	3	69

a. Calculated on the basis of ^{1}H NMR data (500 MHz) by comparison of the relative integrals of H-1 (4.85 ppm), H-3′ (4.89 ppm) or H-4′ (5.12 ppm) for **4a**, H-3′ (4.75 ppm) for **5a** and H-3 (4.82 ppm) for **1a**. Solvent signals at 7.19, 7.55 and 8.71 ppm.

been reported that 1,5-anhydro-D-fructose is an intermediate in the glycogen degrading pathway[7] leading to the formation of 1,5-anhydro-D-glucitol, which constitutes the major polyol in cerebrospinal fluid and plasma, closely related with diabetic conditions.[8,9] The chemical properties of 1,5-anhydro-D-fructose have, however, only been scarcely investigated, mainly because the availability of the compound has been limited. Chemical synthesis involves a multistep procedure[2,10] while the enzymatic approaches require enzymes[5] that are non-redundant in the cells, and not commercially available yet. Our recent access to 1,5-anhydro-D-fructose (**1a**) is based on the enzymatic approaches using transgenic lyase,[11] and this has prompted us to investigate its structure and chemical properties.

RESULTS AND DISCUSSION

It has already been shown that 1,5-anhydro-D-fructose in aqueous solution exists as the hydrated form **2**.[12] The structure of anhydrous **1a** has, however, not been studied, but the existence of dimeric forms of 1,5-anhydro-D-fructose has been mentioned.[2] We have now found that 1,5-anhydro-D-fructose exists mainly as two different dimeric forms in non aqueous solvents such as dimethyl sulfoxide or pyridine, as seen from the ^{1}H and ^{13}C NMR spectra in these solvents. Furthermore we found that an equilibrium between the dimers exists in pyridine. The composition of the mixture was changed from one dimer, **4a**, to the other, **5a**, the latter being the main component present, when the equilibrium was reached within a week (Table 1).

Table 2. ^{13}C NMR data[a]

	C-1/C-1′	C-2/C-2′	C-3/C-3′	C-4/C-4′	C-5/C-5′	C-6/C-6′
1a[b]	73.3	205.7	81.1	74.6	82.7	62.5
2[c]	72.6	93.5	77.7	69.8	81.5	62.0
3b[d]	71.2	187.5	143.6	132.6	72.4	64.2
3c[d]	71.6	187.6	144.1	133.1	72.7	64.9
4a[b]	71.4; 72.9	109.4/101.3	75.8/88.0	70.1; 71.1	82.0; 83.0	63.2; 63.6
4b[c]	69.4; 71.7	109.4/105.5	71.5/83.4	68.9/70.7	77.2/75.6	62.5/63.3
5a[b]	70.3; 73.4	110.0/101.9	76.3/89.3	70.8; 71.7	81.4; 83.0	62.6; 62.7
5b[c]	69.4; 72.1	110.1/105.1	73.7/83.2	68.9/69.3	76.7/75.9	62.2/63.0

a. δ-Values (ppm) for **1a**, **4a**, **4b**, **5a**, **5b** (125.8 MHz), **2**, **3c** (62.9 MHz), **3b** (50.3 MHz); data for **1a**, **4a** and **5a** were obtained from a mixture of the compounds (Table 1); signals from **3b** and **3c** were assigned by DEPT spectra and the signals from **2**, **4b**, **5b** were assigned by C-H correlated NMR; unless "/" the signals might be interconverted.
b. In C_5D_5N (solvent peaks at 123.5, 135.5 and 149.9 ppm).
c. In D_2O (dioxane internal standard at 67.4 ppm).
d. In $CDCl_3$ (solvent peak at 77.0 ppm).
e. In C_6D_6 (solvent peak at 128.0 ppm).

The structures of the dimers could not be deduced unambiguously from the NMR spectra, but significant differences in the ^{13}C NMR chemical shifts between the signals from C-2 and C-2′ (*ca.* 8 ppm) and between those from C-3 and C-3′ (*ca.* 12 ppm) in both **4a** and **5a** were observed (Table 2). To obtain derivatives of the dimeric forms of anhydrofructose, **1a** was acetylated under different conditions. Under acidic conditions (Ac_2O, aq. $HClO_4$) a reaction product was obtained from which an acetylated dimer **4b** could be crystallised directly from Et_2O (46 %). The mother liquor contained the triacetate of anhydrofructose, **1b**, the dimer **4b** and an isomeric dimer **5b** in the ratio *ca.* 3:3:4. An equilibrated mixture of **1a** in pyridine was concentrated and subsequently acetylated under acidic conditions. Workup gave dimer **5b** by direct crystallisation from Et_2O (30 %). The mother liquor contained the two dimeric acetates **4b** and **5b** in about equal amounts. ^{13}C and ^{1}H NMR spectra are given in Tables 2 and 3. X-Ray analysis[13] of the acetylated dimers **4b** and **5b** showed that both compounds were spiroketals formed between the C-2 and C-2′/C-3′(Figure 2). The only difference between the structures **4** and **5** is the configuration at the spiro-carbon C-2. These structures explain the differences between the carbon chemical shifts mentioned above: C-2 carries two alkoxy groups in contrast to C-2′ which carries one alkoxy and one acyloxy group, giving a

Table 3. ^{1}H NMR data of isolated compounds[a]

	2[b]	**3b**[c]	**3c**[c]	**4b**[d]	**5b**[d]
H-1a	3.75 (d) $J_{1a,1b}$ 12.5	4.42 (d) $J_{1a,1b}$ 16.5	4.55 (d) $J_{1a,1b}$ 16.5	4.59 (d)* $J_{1a,1b}$ 12.5	4.80 (d)* $J_{1a,1b}$ 12.5
H-1b	3.45 (d)	4.21 (dd) $J_{1b,5}$ 2.0	4.36 (dd) $J_{1b,5}$ 2.0	3.63 (d)*	3.63 (d)*
H-3	3.56 (d) $J_{3,4}$ 9.0	-	-	5.56 (d) $J_{3,4}$ 10.0	5.45 (d) $J_{3,4}$ 9.5
H-4	3.43 (dd) $J_{4,5}$ 9.5	6.58 (d) $J_{4,5}$ 2.0	6.88 (d) $J_{4,5}$ 2.0	5.50 (dd) $J_{4,5}$ 10.0	5.38 (dd) $J_{4,5}$ 9.5
H-5	3.39 (ddd) $J_{5,6a}$ 2.0 $J_{5,6b}$ 6.5	4.76 (dddd) $J_{5,6a}$ 6.0 $J_{5,6b}$ 4.0	5.02 (dddd) $J_{5,6a}$ 6.0 $J_{5,6b}$ 4.0	3.29 (ddd) $J_{5,6a}$ 5.5 $J_{5,6b}$ 2.5	3.30 (ddd) $J_{5,6a}$ 5.0 $J_{5,6b}$ 2.0
H-6a	3.89 (dd) $J_{6a,6b}$ 12.5	4.37 (dd) $J_{6a,6b}$ 12.0	4.67 (dd) $J_{6a,6b}$ 12.0	4.21 (dd) $J_{6a,6b}$ 12.5	4.22 (dd) $J_{6a,6b}$ 12.5
H-6b	3.68 (dd)	4.20 (dd)	4.55 (dd)	4.14 (dd)	4.08 (dd)
H-1′a				3.89 (d)* $J_{1'a,1'b}$ 12.0	4.15 (d)* $J_{1'a,1'b}$ 12.0
H-1′b				3.07 (d)*	3.31 (d)*
H-3′				4.71 (d) $J_{3',4'}$ 6.0	4.56 (d) $J_{3',4'}$ 5.0
H-4′				5.57 (dd) $J_{4',5'}$ 9.0	5.10 (dd) $J_{4',5'}$ 6.5
H-5′				3.51 (ddd) $J_{5',6'a}$ 6.5 $J_{5',6'b}$ 3.5	3.52 (ddd) $J_{5',6'a}$ 6.0 $J_{5',6'b}$ 3.5
H-6′a				4.31 (dd) $J_{6'a,6'b}$ 12.5	4.26 (dd) $J_{6'a,6'b}$ 12.0
H-6′b				4.16 (dd)	4.07 (dd)

a. δ-Values (ppm) and coupling constants (*J*, Hz) for **3b** (200 MHz), **3c** (250 MHz), **2**, **4b**, **5b** (500 MHz); signals from **2**, **4b**, **5b** were assigned by 2D-COSY NMR; * H-1a and H-1b may be reversed with H-1′a and H-1′b, respectively.
b. In D_2O (acetone as internal standard at 2.23 ppm).
c. In $CDCl_3$ (solvent peak at 7.27 ppm).
d. In C_6D_6 (solvent peak at 7.16 ppm).

relatively larger downfield shift of the C-2 signal. C-3, in contrast, is acetylated and C-3′ is monoalkylated, causing a relative downfield shift of the C-3′ signal. Similar arguments also hold for the non acetylated dimers **4a** and **5a**, which are formed when 1,5-anhydro-D-fructose **1a** is dissolved in non-aqueous solvents.

*We have thus proved, that the dimeric structures of 1,5-anhydro-D-fructose (**1a**) are two C-2 isomeric spiroketals **4a** and **5a**. The ketal is formed between C-2 and C-2′/C-3′.*

For both dianhydrides **4b** and **5b,** the pyranosering possessing the spirocarbon C-2 adopts a chair $^4C_1$-conformation, as seen from the X-ray crystal structures as well as

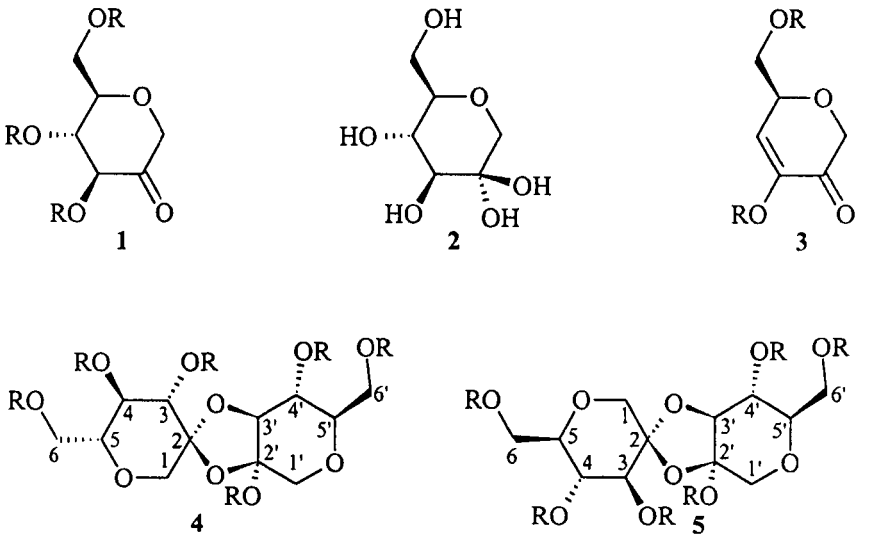

Figure 1.

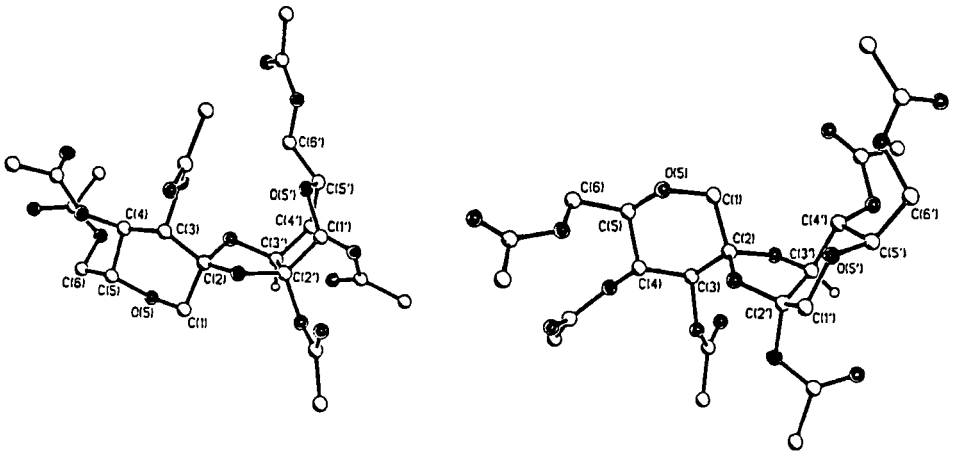

Figure 2.

X-Ray structures of **4b** and **5b** (the only illustrated hydrogen: H-3′)[13] (black atoms correspond to the oxygens).

from ^{1}H NMR data (Table 3). The other pyranosering is in both compounds a distorted chair. **5b** seems to have a more favoured conformation than **4b** since by equilibration of 1,5-anhydro-D-fructose (**1a**) in pyridine, **5b** this is the predominant dimeric form after 7 days (Table 1).

Direct acetylation of **1a** in pyridine using Ac_2O gave the acetylated unsaturated ketone **3b** as an oil (55%) while benzoylation yielded the corresponding benzoate **3c** (56%, 19% crystalline). In both acylations, the acylated dimers **4** and **5** were found in the crude products, thus explaining the rather low yields of **3b** and **3c**. These conjugated ketones are interesting chiral synthons, which have been used in natural product synthesis.[14,15]

EXPERIMENTAL

General methods. 1,5-Anhydro-D-fructose was freeze-dried from an aqueous solution and residual water coevaporated with toluene 3 times before use. All solvents distilled before use. ^{1}H NMR and ^{13}C NMR spectra were obtained on Bruker instruments AC 200, AC 250 (recorded at ambient temperature) and AM 500 (recorded at 300 K). For NMR spectra the solvent peak was used as a reference. For ^{1}H NMR in D_2O, acetone (δ = 2.23 ppm) was used as internal reference and for ^{13}C NMR dioxane (δ = 67.4 ppm). Melting points are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Microanalyses were carried out by The Microanalytical Department, University of Copenhagen and the Research Institute for Pharmacy and Biochemistry, Prague. TLC was performed on precoated kieselgel 60 F_{254} and was visualised by spraying with a mixture of 1.5% $(NH_4)_2MoO_2$, 1% $Ce(SO_4)_2$ and 10% H_2SO_4, followed by heating. Flash chromatography was performed with silica gel 60 (Merck, 40-63 μm and Grace AB Amicon, 35-70 μm). Evaporations were performed on a rotary evaporator at a temperature below 45 °C.

3,4,6,2′,4′,6′-Hexa-*O*-acetyl-1,5-Anhydro-D-fructose Hydrate 1′,5′-Anhydro-D-fructose Hydrate (2*S*,2′*S*)-2,2′:2,3′-Dianhydride (4b). To a stirred solution of acetic anhydride (20 mL, 212 mmol) and 60% aqueous perchloric acid (5 drops from a pasteur pipette) at 0 °C, amorphous 1,5-anhydro-D-fructose (**1a**) (0.983 g, 6.06 mmol) was added

and the reaction mixture was stirred at ambient temperature. After 2 h, water (10 mL) was slowly added at 0 °C and the pale yellow solution was stirred for ½ h at ambient temperature. Dichloromethane (100 mL) and water (100 mL) were added and the organic phase was washed with water (100 mL) and saturated aqueous sodium bicarbonate (100 mL), dried (magnesium sulphate) and concentrated to a foam (1.59 g). By addition of diethyl ether the acetylated dimer **4b** crystallised (0.803 g, 46%, mp 133-138 °C). Two recrystallisations from hexane-ethyl acetate afforded an analytical sample: mp 141-143 °C; $[\alpha]_D$ -54.0° (*c* 1.02, $CHCl_3$); ^1H NMR, Table 3; ^{13}C NMR, Table 2.

Anal. Calcd for $C_{24}H_{32}O_{16}$: C, 50.00; H, 5.60. Found: C, 49.67; H, 5.55.

The mother liquor was concentrated to a foam, which was shown by ^1H NMR to contain **1b**, **4b** and **5b** in the ratio 3:3:4.

3,4,6,2′,4′,6′-Hexa-*O*-acetyl 1,5-Anhydro-D-fructose Hydrate 1′,5′-Anhydro-D-fructose Hydrate (2*R*,2′*S*)-2,2′:2,3′-Dianhydride (5b). Amorphous 1,5-anhydro-D-fructose (**1a**) (0.529 g, 3.26 mmol) was dissolved in pyridine (20 mL) and stirred for a week. The yellow solution was concentrated and coevaporated with toluene three times to give a foam. Acetic anhydride (10 mL, 106 mmol) was added at 0 °C, followed by a few drops of 60% aqueous perchloric acid until acidic (approx. pH 3) and kept at ambient temperature for 2 h. Water (5 mL) was then added and the mixture was stirred for ½ h. Workup as described for **4b** afforded a yellow syrup (0.72 g). By addition of diethyl ether, the acetylated dimer **5b** crystallised (0.284 g, 30%, mp 137-141 °C). Two recrystallisations from hexane-ethyl acetate afforded an analytical sample: mp 142-143 °C; $[\alpha]_D$ -31.5° (*c* 1.07, $CHCl_3$); ^1H NMR, Table 3; ^{13}C NMR, Table 2.

Anal. Calcd for $C_{24}H_{32}O_{16}$: C, 50.00; H, 5.60. Found: C, 49.71; H, 5.50.

The mother liqour was concentrated to a residue, which was shown by ^1H NMR to contain **4b** and **5b** in a 1:1 ratio.

3,6-Di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-enos-2-ulopyranose (3b). To a stirred solution of pyridine (20 mL, 248 mmol) and acetic anhydride (6 mL, 64 mmol) at 0 °C, 1,5-anhydro-D-fructose (**1a**) (0.708 g, 4.37 mmol) was added as an amorphous solid. After stirring at ambient temperature for 2 h the homogeneous solution was concentrated. The residue was dissolved in dichloromethane (50 mL), washed with 4 M aqueous hydrochloric acid (10 mL) and saturated aqueous sodium bicarbonate (10

mL), dried (magnesium sulphate) and concentrated to a syrup (1.31 g). Chromatography (25 g silica, hexane-ethyl acetate, 1:1) afforded **3b** as a syrup (0.55 g, 55%): $[\alpha]_D$ - 43.7° (*c* 1.71, $CHCl_3$); ^1H NMR, Table 3; ^{13}C NMR, Table 2.

Anal. Calcd for $C_{10}H_{12}O_6$: C, 52.63; H, 5.30. Found: C, 51.78; H, 5.46.

By TLC the acetylated dimers were recognised as by-products in the crude reaction mixture.

1,5-Anhydro-3,6-di-*O*-benzoyl-4-deoxy-D-*glycero*-hex-3-enos-2-ulopyranose (3c). To a stirred solution of benzoyl chloride (3.70 mL, 31.9 mmol) and pyridine (10 mL, 124 mmol) at 0 °C, 1,5-anhydro-D-fructose (**1a**) (0.292 g, 1.80 mmol) was added as an amorphous solid. The mixture was stirred for 1 h at 0 °C and then allowed to come to ambient temperature during 2 h; ice was carefully added to the pale brown suspension and the mixture was stirred for ½ h. Dichloromethane (60 mL) was added and the organic phase was washed with water (2×30 mL), 4 M aqueous hydrochloric acid (30 mL) and saturated aqueous sodium bicarbonate (30 mL), dried (magnesium sulphate) and concentrated to a pale brown syrup (0.95 g), which was purified by chromatography (22 g silica, hexane-ethyl acetate, 4:1) to give a colourless syrup (0.355 g, 56 %). Crystallisation from methanol afforded 0.121 g (19%) of **3c**, mp 98-101 °C. Two recrystallisations afforded an analytical sample: mp 101-102 °C; $[\alpha]_D$ - 16.50° (*c* 1.32, $CHCl_3$) [Lit.[2] mp 104-105 °C, $[\alpha]_D$ -16° (*c* 1, $CHCl_3$)]; ^1H NMR, Table 3; ^{13}C NMR, Table 2.

Anal. Calcd for $C_{20}H_{16}O_6$: C, 68.18; H, 4.58. Found: C, 68.03; H, 4.92.

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13. Compound **4b** ($C_{24}H_{32}O_{16}$; M_r = 576.50) crystallised in the monoclinic space group $P2_1$ with cell dimensions a = 9.7459(13), b = 8.5473(11), c = 16.3936(20) Å, β = 91.969(3)°, V = 1364.8(3) Å^3 and Z = 2. Data were collected at 120 K on a SMART diffractometer using Mo$K\alpha$ radiation. The crystal-to-detector-distance was 4.5 cm. 4523 reflections were measured to $2\theta_{max}$ = 50°. The structure was solved by direct methods (SHELXTL) and refined with full matrix least-squares giving R = 0.1538 [2313 refl., $I > 2\sigma(I)$] and $wR2$ = 0.3784 (4523 refl.). The quality and the reflecting power of the crystals were rather poor.
    Compound **5b** ($C_{24}H_{32}O_{16}$; M_r = 576.50) crystallised in the orthorhombic space group $P2_1\,2_12_1$ with cell dimensions a = 8.2431(3), b = 17.2219(4), c = 18.8824(4) Å, V = 2680.58(13) Å^3 and Z = 4. Data were collected at 120 K on a SMART diffractometer using Mo$K\alpha$ radiation. The crystal-to-detector-distance was 4.5 cm. 4710 unique reflections were measured to $2\theta_{max}$ = 50°. The structure was solved by direct methods (SHELXTL) and refined with full matrix least-squares giving R = 0.1280 [2475 refl., $I > 2\sigma(I)$] and $wR2$ = 0.3354 (4710 refl.). The quality and the reflecting power of the crystals were rather poor.
    Tables of atomic coordinates, bond lengths, and bond angles have been deposited with the Cambridge Crystallographic Data Center. These tables may be obtained on request from the Director, Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 IEZ, UK.
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