Carbohydrate Research, 53 (1977) 101–108
© Elsevier Scientific Publishing Company, Amsterdam – Printed in Belgium

OXIDATION OF A BRANCHED-CHAIN ALDITOL BY *Acetobacter suboxydans*: A STEREOSPECIFIC SYNTHESIS OF L-DENDROKETOSE

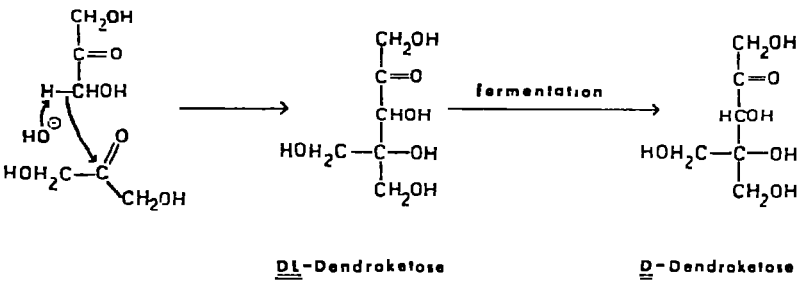
WALTER A. SZAREK, G. WAYNE SCHNARR, HAROLD C. JARRELL, AND J. K. N. JONES
Department of Chemistry, Queen's University, Kingston, Ontario K7L 3N6 (Canada)
(Received May 24th, 1976; accepted for publication July 12th, 1976)

ABSTRACT

A synthesis of L-dendroketose (**5**) has been achieved by microbiological oxidation by *Acetobacter suboxydans* of the branched-chain alditol 2-*C*-(hydroxymethyl)-D-*erythro*-pentitol (**4**). Treatment of the oxidation product with acetone, copper(II) sulfate, and sulfuric acid afforded the two di-*O*-isopropylidene-L-dendroketose derivatives **6** and **7**. Assignment of configuration at the branching carbon atom (C-4) and at the anomeric center in **6** and **7** was made on the basis of the carbon-13 magnetic resonance spectra of these derivatives.

DISCUSSION

In 1949 a preparation of a branched-chain sugar, by self-condensation of 1,3-dihydroxy-2-propanone in the presence of dilute alkali, was reported by Utkin[1]. The sugar was named dendroketose, and the L isomer was observed to be preferentially fermented by a mold, leaving the D isomer, 4-*C*-(hydroxymethyl)-D-*glycero*-pentulose, which could then be isolated (see Scheme 1). A project in this laboratory is concerned with stereospecific syntheses of derivatives of one enantiomer of



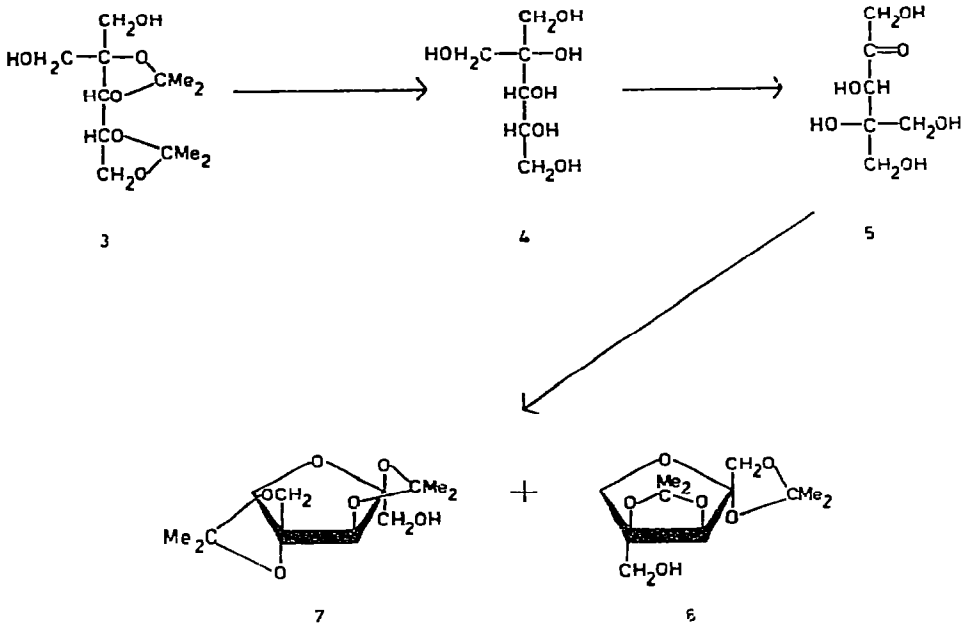
Scheme 1.

dendroketose, including nucleoside analogs. There has been considerable interest in recent years in the synthesis of branched-chain sugars, particularly because of their occurrence in several antibiotics[2]. The present publication describes a synthesis of

L-dendroketose by way of a microbiological oxidation of a branched-chain alditol by *Acetobacter suboxydans*. The microbiological oxidation* of sugars and their derivatives by the acetic acid bacteria[4], *Acetobacter suboxydans*, has been studied extensively. In the pH range 5–6.5, the oxidative specificity towards alditols has been formulated as the Bertrand–Hudson rule[5]. Thus, a polyhydric alcohol containing the structural unit **1** is oxidized mainly to afford the ketose **2**.

1 **2**

2-*C*-(Hydroxymethyl)-2,3:4,5-di-*O*-isopropylidene-D-*erythro*-pentitol (**3**) was prepared, as described[6] previously, by treatment of 2,3:4,5-di-*O*-isopropylidene-*aldehydo*-D-arabinose with formaldehyde in the presence of sodium hydroxide. Acid-catalyzed hydrolysis of **3** afforded syrupy 2-*C*-(hydroxymethyl)-D-*erythro*-pentitol (**4**). On the basis of the Bertrand–Hudson rule, the branched-chain alditol **4** was considered to be a possible substrate for oxidation by *Acetobacter suboxydans* to yield 4-*C*-(hydroxymethyl)-L-*glycero*-pentulose (**5**, L-dendroketose). Indeed, compound **4** was oxidized during 5 days to afford a compound that migrated in paper chromatography



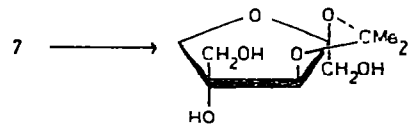
3 **4** **5**

7 **6**

*For a survey of microbiological, alcohol dehydrogenation, see ref. 3.

at the same rate as an authentic sample of DL-dendroketose[1], and which could be acetonated by three procedures (see Experimental section) to give the 1,2:3,4- and 2,3:4,4′-di-*O*-isopropylidene derivatives (**6** and **7**, respectively) of L-dendroketose.

The configuration at the branching carbon atom (*C*-4) and at the anomeric center in compounds **6** and **7**, and the complete structure of **7**, were assigned by carbon-13 magnetic resonance spectroscopy. An earlier publication[7] from this laboratory described the solution of such stereochemical problems by application of this technique to a series of dendroketose derivatives; one of the compounds studied was 1,2:3,4-di-*O*-isopropylidene-DL-dendroketose, and, on the basis of the results obtained in that study, the structure of the L isomer prepared in the present work can be assigned as shown by formula **6**. The ^{13}C chemical shifts of the di-*O*-isopropylidene derivatives **6** and **7** and of three additional, relevant compounds are documented in Table I. The three additional compounds are the mono-*O*-isopropylidene derivative



8

8, obtained by partial, acid-catalyzed hydrolysis of **7**, 4-*C*-(hydroxymethyl)-2,3-*O*-isopropylidene-α-L-*erythro*-pentulofuranose[7,8] (**9**), and 3-*C*-(hydroxymethyl)-1,2-*O*-isopropylidene-β-L-threofuranose[7] (1,2-*O*-isopropylidene-D-apio-β-L-furanose) (**10**). The chemical-shift data for compounds **9** and **10** are those reported in the earlier publication[7]. A comparison of the chemical shifts of the signals in the spectra of compounds **8**–**10** establishes that the dendroketose derivatives **8** and **9** are epimeric at C-4. Thus, the C-1 and C-2 chemical shifts in the spectrum of **8** are almost identical to those of the corresponding signals in the spectrum of **9**, whereas the C-3, C-4, and C-4′ chemical shifts in the spectrum of **8** are nearly identical to those of the corresponding signals (C-2, C-3, and C-3′, respectively) in the spectrum of **10**. Clearly, the establishment of the structure of the mono-*O*-isopropylidene derivative **8** permits formulation of the di-*O*-isopropylidene precursor **7** as shown. The signal assignments in the ^{13}C n.m.r. spectrum of compound **7** could be unequivocally made from the proton-coupled spectrum.

EXPERIMENTAL

General methods. — Melting points were determined on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer Model 141 automatic polarimeter at 23 ± 3°. T.l.c. was performed with Silica Gel G as the adsorbent in the following solvent systems (v/v): (*A*) 8:1 ethyl acetate–ethanol; (*B*) 5:2 petroleum ether–ethyl acetate; (*C*) 1:3 petroleum ether–ethyl acetate. The term "petroleum ether" refers to the fraction of b.p. 60–80°. The developed plates

TABLE I

CARBON-13 CHEMICAL-SHIFT DATA[a]

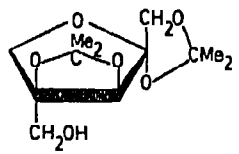
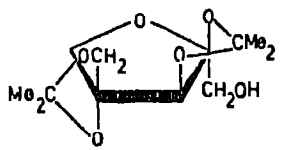
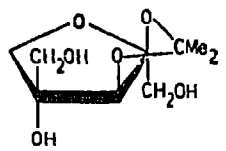
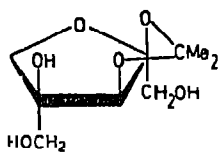
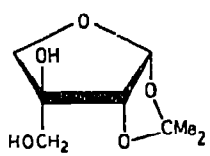
Compound		*Carbon atom*								
		1	*2*	*3*	*3'*	*4*	*4'*	*5*	*Me_2 -OCO-*	*Me groups*
	6[b]	69.3	113.7[c]	86.4	—	92.7	64.1	73.9	112.9[c] 111.9[c]	27.8, 27.6 26.6, 26.4
	7	63.2	115.2	84.2	—	88.2	65.3	74.4	112.7 111.1	27.6, 26.7 26.2
	8	63.7	114.9	85.1	—	82.4	62.0	74.7	112.8	27.4, 26.5

TABLE I (*continued*)

Compound		*Carbon atom*								
		1	*2*	*3*	*3'*	*4*	*4'*	*5*	*Me_2 -OCO-*	*Me groups*
	9[a]	63.8	114.3[c]	79.9	—	79.4	63.4	71.7	112.5[c]	27.2
	10[d]	106.1	84.3	82.2	62.3	73.2	—	—	112.6	26.9, 26.4

[a]In p.p.m. downfield from internal tetramethylsilane; spectra were recorded in chloroform-*d*. [b]Data given are the same as those reported for the DL modification in ref. 7. [c]Assignments for these peak positions may be reversed. [d]Data given are those reported in ref. 7.

were air-dried, and compounds located by heating the plates at ~ 150° after they had been sprayed with 10% aqueous sulfuric acid containing 1% of cerium sulfate and 1.5% of molybdic acid. Column chromatography was performed on silica gel (70–230 mesh). Paper chromatography was performed by the descending method at room temperature on Whatman No. 1 chromatography paper in the following solvent systems: (I) 7:1:2 (v/v) 1-propanol–ethyl acetate–water; (II) 0.55% (w/v) solution of benzeneboronic acid in 9:2:2 (v/v) ethyl acetate–acetic acid–water[9]. Sugars were detected on paper chromatograms by the following spray reagents: (*i*) aqueous sodium metaperiodate, silver nitrate, and sodium hydroxide solutions[10], (*ii*) triphenyltetrazolium chloride[11]. Carbon-13 magnetic resonance spectra were recorded in chloroform-*d* on a Bruker HX-60 spectrometer equipped with an FT60M Fourier transform accessory at 15.1 MHz, with tetramethylsilane as internal standard; chemical shifts are given in parts per million downfield from tetramethylsilane. Proton-coupled spectra were recorded employing the usual gated-decoupling technique.

2-C-(Hydroxymethyl)-D-erythro-*pentitol* (**4**). — A solution of 2-*C*-(hydroxymethyl)-2,3:4,5-di-*O*-isopropylidene-D-*erythro*-pentitol[6] (**3**) (2.0 g) in 50 ml of 4:1 (v/v) ethanol–water was shaken with Rexyn-101 (H^+) resin at room temperature until t.l.c. (solvent *A*) revealed only the presence of one major component (R_F 0.09) and a trace of another component (R_F 0.55); paper chromatography (solvent I, spray *i*) revealed the presence of one major component ($R_{SORBOSE}$ 1.22) and traces of two faster-moving components. The resin was removed by filtration, and the filtrate was neutralized with a basic ion-exchange resin; concentration of the solution afforded a syrupy product. Column chromatography (solvent *A*) afforded an analytically pure sample of the alditol **4** as a syrup; $[\alpha]_D$ + 14.8 ± 1° (*c* 0.88, water).

Anal. Calc. for $C_6H_{14}O_6$: C, 39.6; H, 7.8. Found: C, 39.9; H, 7.8.

Oxidation of **4** *by* Acetobacter suboxydans. — To a solution of compound **4** (4.6 g) in tap water (200 ml) were added D-glucose (0.1 g), potassium dihydrogenphosphate (0.1 g), and yeast extract powder (1.0 g, ICN Pharmaceuticals, Inc.). The solution was divided into 50-ml portions, and each was placed in a 250-ml Erlenmeyer flask. The solutions were autoclaved at 15 lb.in^{-2} for 15 min, cooled to room temperature and then inoculated with ~2 ml of a 48-h culture of *Acetobacter suboxydans* (A.T.C.C. No 621H) grown in D-glucitol solution; within 2 days the broths became very turbid. The progress of the oxidation was monitored by paper chromatography (solvent II, spray *i* or *ii*), which showed that the starting material ($R_{SORBOSE}$ 2.24) was converted into a single component ($R_{SORBOSE}$ 2.00). The oxidation was complete within 5 days; growth was then terminated by the addition of ethanol (2 volumes) and a small amount of decolorizing carbon. The mixtures were filtered, and the filtrates were combined and concentrated to a syrup (5.4 g); this syrup, which consisted of the new compound in addition to L-sorbose and the other residues from the oxidation mixtures, was employed, without fractionation, in the acetonation experiments. The new compound migrated in paper chromatography (solvent I, spray *i* or *ii*) at the same rate as an authentic sample of DL-dendroketose[1] ($R_{SORBOSE}$ 1.26).

Preparation of di-O-isopropylidene-L-dendroketose derivatives **6** *and* **7** — (*a*) A mixture of the crude syrup obtained by oxidation of the sample of the alditol **4** derived from 18.5 g of **3**, Rexyn-101 (H^+) resin (~100 ml, washed with anhydrous acetone), and molecular sieves (Davison, 3 Å pore size) in anhydrous acetone (600 ml) was shaken for 5 days at room temperature; t.l.c. (solvent *B*) showed that all of the starting material (R_F 0.00) had been consumed and revealed the presence of two new major components (R_F 0.40 and 0.33). The mixture was filtered, the filtrate was neutralized with a basic ion-exchange resin, and the solution was evaporated to a syrup; fractionation (solvent *B*) on silica gel afforded the di-*O*-isopropylidene-L-dendroketose derivatives **6** and **7**. The component having R_F 0.40 was obtained crystalline and was identified as 4-*C*-(hydroxymethyl)-1,2:3,4-di-*O*-isopropylidene-*β*-L-*erythro*-pentulofuranose (**6**, 1,2:3,4-di-*O*-isopropylidene-L-dendroketose). Recrystallization from petroleum ether gave **6** as white needles (2.7 g, 14% from compound **3**), m.p. 87–88°, $[\alpha]_D$ +117 ± 1° (*c* 1.2, acetone); lit.[1] for the D enantiomer, m.p. 89°, $[\alpha]_D^{10}$ −121° (acetone). The ^{13}C n.m.r. spectra of the compound obtained in this experiment and of 1,2:3,4-di-*O*-isopropylidene-DL-dendroketose (see ref. 7) were identical.

The component having R_F 0.33 was also obtained crystalline and was identified as 4-*C*-(hydroxymethyl)-2,3:4,4′-di-*O*-isopropylidene-*β*-D-*threo*-pentulofuranose (**7**, 2,3:4,4′-di-*O*-isopropylidene-L-dendroketose). Recrystallization from petroleum ether (b.p. 30–60°) gave **7** as white needles (1.7 g, 9% from compound **3**), m.p. 58–59°, $[\alpha]_D$ −63.2° (*c* 1.6, acetone). Utkin[12] has reported the preparation of a 2,3:4,4′-di-*O*-isopropylidene derivative of D-dendroketose, m.p. 63.5–64.5°, $[\alpha]_D^{17}$ +60.5° (acetone). Carbon-13 chemical-shift data for compound **7** are given in Table I.

Anal. Calc. for $C_{12}H_{20}O_6$: C, 55.4; H, 7.8. Found. C, 55.1; H, 7.7.

(*b*) A mixture of the crude, oxidation product (derived from 18.5 g of **3**), copper(II) sulfate (40 g), and concentrated sulfuric acid (6 ml) in anhydrous acetone (700 ml) was shaken for 60 h at room temperature. The mixture was neutralized with concentrated aqueous ammonia, filtered, and the filtrate concentrated to a syrup; t.l.c. (solvent *B*) revealed the presence of two major components having the same R_F values as those obtained in experiment (*a*). The crude syrup was dissolved in dichloromethane, and the solution was washed with water (2 × 75 ml), dried (magnesium sulfate), and evaporated. Fractionation (solvent *B*) on silica gel afforded compounds **6** (1.3 g, 7% from compound **3**) and **7** (0.8 g, 4.5% from compound **3**).

(*c*) A mixture of the crude, oxidation product (derived from 3.3 g of **3**), finely powdered, anhydrous zinc(II) chloride (1.5 g), and copper(II) sulfate (4 g) in anhydrous acetone (100 ml) was stirred for 6 days at room temperature. The reaction mixture was processed as described in experiment (*b*) to again afford compounds **6** (~0.1 g) and **7** (~0.6 g).

*4-C-(Hydroxymethyl)-2,3-O-isopropylidene-β-*D-threo-*pentulofuranose* (**8**). — A solution of compound **7** (390 mg) in 9:1 (v/v) acetone–water was stirred with Rexyn-101 (H^+) resin at room temperature until t.l.c. (solvent *C*) revealed that all of the starting material (R_F 0·80) had been consumed and only one new component

(R_F 0.25) had been formed. The resin was removed by filtration, and the filtrate was neutralized with 0.1M sodium hydroxide solution and evaporated to a syrup. Acetone was added, the mixture was filtered, and the filtrate was evaporated to afford the mono-*O*-isopropylidene, derivative **8** as a syrup (225 mg, 68%), $[\alpha]_D$ −14.3° (*c* 0.6, chloroform). Carbon-13 chemical-shift data for compound **8** are given in Table I. A 60-MHz p.m.r. spectrum of the product in chloroform-*d* showed two, 3-proton singlets at δ 1.37 and 1.53 p.p.m. attributable to one *O*-isopropylidene group.

Anal. Calc. for $C_9H_{16}O_6$: C, 49.1; H, 7.3. Found: C, 49.4; H, 7.2.

ACKNOWLEDGMENTS

The authors are grateful to the National Research Council of Canada for financial support in the form of scholarships (to G.W.S. and H.C.J.) and grants (to W.A.S. and J.K.N.J.). They also thank Dr. B. K. Hunter for recording the ^{13}C n.m.r. spectra and Dr. D. M. Vyas for helpful discussions.

REFERENCES

1 L. M. Utkin, *Dokl. Akad. Nauk SSSR*, 67 (1949) 301–304.
2 J. S. Brimacombe, *Angew. Chem. Int. Ed. Engl.*, 10 (1971) 236–248; H. Grisebach and R. Schmid, *ibid.*, 11 (1972) 159–173; W. A. Szarek, in G. O. Aspinall (Ed.), *MTP International Review of Science, Organic Chemistry Series One, Vol. 7, Carbohydrates*, Butterworths, London, 1973, pp. 85–90; W. A. Szarek and D. M. Vyas, *ibid*, *Series Two*, 1976, pp. 105–108.
3 G. S. Fonken and R. A. Johnson, *Chemical Oxidations with Microorganisms*, Marcel Dekker, New York, 1972, pp. 185–209.
4 T. Asai, *Acetic Acid Bacteria*, University of Tokyo Press, Tokyo, 1968.
5 R. M. Hann, E. B. Tilden, and C. S. Hudson, *J. Am. Chem. Soc.*, 60 (1938) 1201–1203.
6 H. C. Jarrell, W. A. Szarek, J. K. N. Jones, A. Dmytraczenko, and E. B. Rathbone, *Carbohydr. Res.*, 45 (1975) 151–159.
7 D. M. Vyas, H. C. Jarrell, and W. A. Szarek, *Can. J. Chem.*, 53 (1975) 2748–2754.
8 H. C. Jarrell, M.Sc. Thesis, Queen's University, 1974.
9 E. J. Bourne, E. M. Lees, and H. Weigel, *J. Chromatogr.*, 11 (1963) 253–257.
10 T. Yamada, M. Hisamatsu, and T. Motoji, *J. Chromatogr.*, 103 (1975) 390–391.
11 W. E. Trevelyan, D. P. Procter, and J. S. Harrison, *Nature*, 166 (1950) 444–445.
12 L. M. Utkin, *Zh. Obshch. Khim.*, 25 (1955) 530–536; *Chem. Abstr.*, 50 (1956) 3228*f*.