A NEW METABOLITE FROM *STREPTOMYCES HYGROSCOPICUS*

II. IDENTIFICATION AS 1-DEOXY-D-*THREO*-PENTULOSE

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Mass spectrometric studies show this metabolite to be a 1-deoxy-pentulose. The stereochemistry was established by reduction to the corresponding 1-deoxy-D-pentitols.

The production of a new substance, inhibitory against a test strain of *Mycobacterium avium*, from a new soil isolate of *Streptomyces hygroscopicus* has been reported by SLECHTA and JOHNSON.[1] Studies which led us to conclude that this substance is 1-deoxy-D-*threo*-pentulose are reported in this paper.

Elemental analyses and high resolution mass spectroscopy require a molecular formula of $C_5H_{10}O_4$. The mass spectrum of this compound as such did not show a molecular ion at *m/e* 134. However an ion at *m/e* 135 having the elemental composition of $C_5H_{11}O_4$ was present. This therefore corresponds to $M^+ + 1$, as is sometimes observed for polyhydroxylated compounds. The molecular weight was further confirmed by the mass spectra of the triacetyl derivative, **2**, $M^{+\cdot}=260$ and *tris*-trimethylsilyl derivative, $M^{+\cdot}=350$. These data also showed that three of the oxygens were present as hydroxyls. The PMR spectra with a singlet (3H) at 2.15 ppm, suggested that the fourth oxygen was present in an acetyl moiety, but the remainder of the spectrum was not completely analyzed, since it

Table 1. High resolution data of selected ions from 1-deoxy-D-*threo*-pentulose

Found	Theory: ion
135: 135.0659	135.0657: $C_5H_{11}O_4$
117: 117.0547	117.0552: $C_5H_9O_3$
116: 116.0473	116.0473: $C_5H_8O_3$
103: 103.0394	103.0395: $C_4H_7O_3$
91: 91.0394	91.0395: $C_3H_7O_3$
74: 74.0368	74.0367: $C_3H_6O_2$
71: 71.0133	71.0133: $C_3H_3O_2$

Fig. 1. Fragmentation of 1-deoxy-pentulose

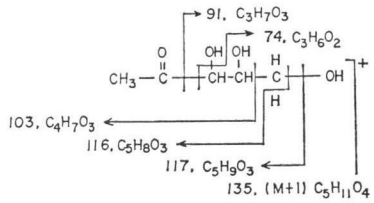
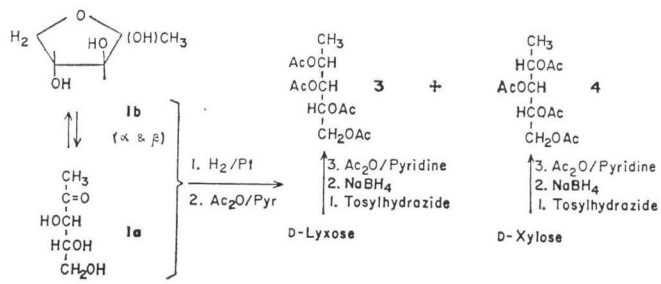


Fig. 2. Reduction products of 1-deoxy-D-*threo* pentulose



was complicated by a mixture of isomeric forms.

An analysis of the fragmentation pattern, further elaborated by peak matching (Table 1), unequivocally required a 1-deoxy-pentulose structure, **1a** or **1b** (Figs. 1, 2).

The reduction products of the pentulose provided the key to the stereochemistry of carbons 3 and 4. A platinum-catalyzed hydrogenation gave unequal amounts of two epimeric 1-deoxy-pentitols. Their tetraacetyl derivatives were separated chromatographically over silica. The major (crystalline) component was found to be identical with a sample of 1-deoxy-D-lyxitol-tetraacetate (**3**) prepared[8] from D-lyxose, and the syrupy minor component was identical with 1-deoxy-D-xylitol tetraacetate (**4**) prepared from D-xylose. PMR spectra, GLC retention times[2] and optical rotations were used to establish these identities. The configuration of **1**, then, is D-*threo*. The corresponding properties of 1-deoxy-D-ribitol (**5**) were dissimilar from those of **3** and **4**, further excluding the alternate *erythro* configuration.

Rather surprisingly, the predominant form of this sugar in both dimethyl sulfoxide-D_6 and D_2O is as the open chain ketone, **1a**. However, the PMR spectra of these solutions suggest the presence of about 20% of a mixture of the α and β anomers of the cyclic form **1b**, as shown by two methyl singlets at 1.23 and 1.31 ppm, the latter predominating over the former. For the crystalline material the large carbonyl absorption at 1710 cm^{-1} also suggests a substantial portion to be in the keto form. The tetraacetate (**2**) is in the keto form exclusively.

The occurence of 1-deoxy pentuloses as compounds of biological origin has not been previously reported.[4] However, a methyl ketose structure is present in another product from *Streptomyces hygroscopicus*, *viz* the 6-deoxy-D-arabino hexofuranos-5-ulose moiety of hygromycin A.[5] This is suggestive of a common biosynthetic step or series of steps but the stereochemical differences seem to preclude that the deoxy-pentulose is a precursor or degradation product of the arabinulose.

Only one of the 1-deoxy-pentuloses appears to have been synthesized. This was the enantiomer of our compound, obtained as the tribenzoate[6] and triacetate[7] of 1-deoxy-L-*threo*-pentulose, prepared from L-threonamide.

Experimental

Melting points are uncorrected. Infrared spectra were obtained on a Perkin-Elmer Infracord Model 137B. Optical rotations were determined on a Perkin Elmer 141 polarimeter. PMR spectra were obtained on a Varian T-60 instrument and chemical shifts were measured to an internal TMS standard. A Varian MAT CH-5 D.F. spectrophotometer was used for the mass spectra. Gas-liquid chromatograms were obtained on an HP 5830A.

Compound **1a**, **1b**

Isolation of the material used in this work was reported in the previous paper. M.W. calcd. for $C_5H_{10}O_4$: 134, Found: $M^+ + 1$, 135.

Compound **2**

A solution containing 510 mg (3.8 mmol) of **1**, 5 ml of pyridine, and 2 g (20 mmol) of acetic anhydride was heated 10 minutes at 80°C, then stored 16 hours at ambient temperature. Following evaporation on a rotary evaporator, the residue was redissolved in methylene chloride. This solution was washed with cold 0.3 N sulfuric acid, cold 5% sodium bicarbonate solution, and water, dried over magnesium sulfate and evaporated, affording 300 mg of colorless syrup, $[\alpha]_D + 52°$ (*c* 1, $CHCl_3$). A rotation of $[\alpha]_D - 51°$ is reported[7] for triacetyl-1-deoxy-L-*threo*-pentulose. M.W. calcd. for $C_{11}H_{16}O_7$: 260, Found: $M^{+\cdot} = 260$. The PMR spectrum: s (6H)

2.07 ppm, s (6H) 2.25 ppm, octet (2H) 4.3 ppm, d (1H) 5.33 ppm, octet (1H) 5.66 ppm.

Preparation of Compounds **3** and **4** from Compound **1**

A mixture of 5 g of compound **1**, 500 mg of platinum oxide catalyst, 100 ml of methanol was shaken 5 hours on a Parr hydrogenator under 40 psig of hydrogen. After filtration it was concentrated in a rotary evaporator to a syrupy residue. This was then dissolved in 100 ml of pyridine and 30 g of acetic anhydride, heated at 80°C for 0.5 hour and stored 4 hours at room temperature. After washing a methylene chloride solution of the residue successively with cold 5% bicarbonate, 0.5 N sulfuric acid, and water, evaporation afforded a crystalline-syrup mixture (9 g). A 3 g aliquot of this was chromatographed on 210 g of silica gel (Silica Gel 60, 0.063~0.200 mm, E. Merck), eluted with a solution of ethyl acetate-Skellysolve B (1:2, v/v). After the column hold-up was discarded collection of 20-ml fractions was begun. The fractions were analyzed by tlc using silica gel GF, 250 microns, (Analtech Uniplates) developed with ethyl acetate-Skellysolve B (1:2).

Compound **3** was isolated by evaporation from tubes 70~95, crystallized on vacuum drying, mp. 52~54°C, 2.3 g.

Anal. Calcd. $C_{13}H_{20}O_8$, C, 51.31; H, 6.63. Found C, 51.44; H. 6.89. $[\alpha]_D$+47 (*c* 1, $CHCl_3$).

Compound **4** was isolated similarly fractions 121~141 as a colorless syrup, $[\alpha]_D$+8° (*c* 1, $CHCl_3$), 0.3 g.

Anal. Calcd. for $C_{13}H_{20}O_8$, MW 304. Found: M^++1, 305.

Synthesis of Compounds **3**, **4**, and 1-Deoxy-D-ribitol Tetraacetate **5** from their Respective Pentoses

The toluene-*p*-sulfonylhydrazones of D-lyxose, D-xylose, and D-ribose were prepared by the literature procedure.[8]

The 1-deoxypentitols were prepared by using the GAGLIOTI[9] method as applied by DEBELDER.[3] The pentitols were not fully purified but were acetylated in the crude state and then purified chromatographically by the previously described procedure. Rotations of the acetates of 1-deoxy-D-lyxose and 1-deoxy-D-xylose were comparable to those of **3** and **4**. The PMR spectra of compound **3**, **4** and **5** were readily distinguishable from each other in the 3.0~6.0 ppm region. The PMR spectra of the synthetic 1-deoxy-D-lyxose tetraacetate was identical to that of compound **3**. The PMR spectrum of 1-deoxy-D-xylose tetraacetate was identical with that of compound **4**.

Table 2. Gas-liquid chromatography of 1-deoxy-pentitol tetraacetates

Compound	Relative retention
3 Prepared from **1**	1.00
3 Prepared from D-lyxose	1.00
4 Prepared from **1**	1.28
4 Prepared fram D-xylose	1.28
1-Deoxy-D-ribitol tetraacetate (**5**)	0.94

Gas-Liquid Chromatography of the 1-Deoxy-pentitol Tetraacetates

Gas chromatography was run on a 90×0.45 cm glass column packed with 10% LAC-466 on Anachrome (90/100 mesh). The injection port and detector temperatures were 250°C. The column was operated isothermally at 190°C with a flow rate of 53 ml/min. The relative retention times are given in Table 2.

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