Carbohydrate Research, 63 (1978) 315–317

Note

The isolation and characterization of crystalline D-*arabino*-hexulosonic acid (2-keto-D-gluconic acid)

HERMANN* FAUBL AND CARL A. KOLB
Pfizer Inc., Groton, Connecticut 06340 (U.S.A.)
(Received September 15th, 1977; accepted for publication, October 13th, 1977)

The first successful isolation of the free acid form of D-*arabino*-hexulosonic acid (**1**, sometimes referred to as 2-keto-D-gluconic acid), an oxidation product of D-glucose, is reported. This conversion can be effected by a number of bacterial species[1,2], particularly by pseudomonads. Solutions of **1** are utilized as synthetic intermediates in the commercial production of D-*erythro*-hex-2-enono-1,4-lactone (erythorbic acid)[3,4]. Although the structure and some properties of **1** were established years ago[5,6], this important D-glucose metabolite has hitherto resisted crystallization[6,7].

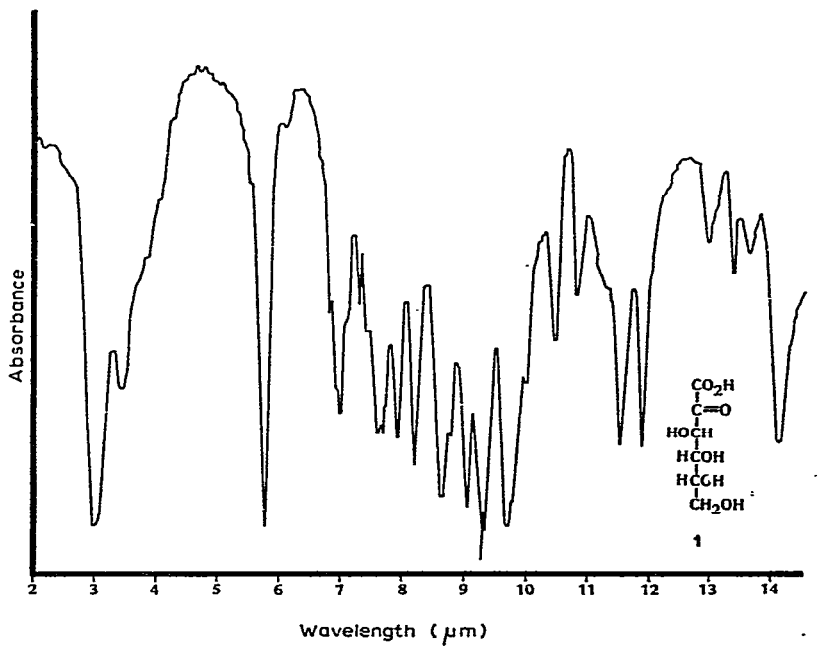

Fig. 1. Infrared spectrum of D-*arabino*-hexulosonic acid in a KBr pellet.

*Present address: Clinton Corn Processing Co., Clinton, Iowa 52732, U.S.A.

Solid, metal salts of **1** are preparable[5,8], and have provided a source for solutions of the free acid when it has been needed.

By carefully cooling concentrated solutions of **1** (see Experimental section), solid **1** was obtained as white crystals. The neutralization equivalent, infrared spectrum, elemental analysis, and sharp melting-point of these crystals are consistent with the presence, in the solid form, of only one isomer of the nonhydrated keto acid. The carbonyl stretching mode occurs at 5.75 μm in the infrared spectrum of **1** (KBr pellet); this wavelength is consistent with either an α-dicarbonyl functionality or a carboxylic acid with a hemiacetal in the α-position. Because of the sharpness of the carbonyl band in this spectrum, it may be concluded that the solid exists in a cyclic, hemiacetal form. The ^{13}C-n.m.r. spectrum of **1** is consistent with its being an ~7:2:1 mixture of cyclic isomers in aqueous solution; this may serve to explain the failure of prior attempts at crystallization, because, in effect, a tautomeric mixture is dealt with.

The crystalline **1** was transformable into erythorbic acid[4]. Furthermore, the solid **1** was homogeneous by thin-layer chromatography, and had an R_F value identical to that of the (unisolated) **1** present in the erythorbic acid process-stream.

EXPERIMENTAL

Isolation and characterization of D-arabino-*2-hexulosonic acid* (**1**). — The action of a strain of *Pseudomonas fluorescens* was used for the oxidation of D-glucose. (The commercial process affords an aqueous solution of **1** that is converted, without isolation, into erythorbic acid.) A 5% (w/w) solution of **1**, thus prepared, was treated with decolorizing carbon at room temperature, and then concentrated to 60–65 wt% in a rotary evaporator. This concentrate was allowed to cool *very slowly* (during 4 h) to 15°, and the suspension of crystals was filtered, giving a crystalline solid that was washed with ice-cold water (to remove the mother liquor). Although this washing resulted in a considerable lowering of the yield, it led to analytically pure **1**, with 20–30% recoveries after vacuum drying. The crystalline **1** appeared as random-shaped plates under the microscope, and had m.p. 142–143° (corr.), $[\alpha]_D^{21}$ −90.2° (*c* 1.01% w/w, in water); for the i.r. spectrum (recorded with a Perkin–Elmer Model 21 spectrometer), see Fig. 1; the ^{1}H-n.m.r. spectrum of a solution of **1** in D_2O (with 2,2-dimethyl-2-silapentane-5-sulfonic acid as the internal standard) was recorded with a Varian Model T-60 spectrometer: multiple peaks between δ 3.52 and 4.45.

Anal. Calc. for $C_6H_{10}O_7$: C, 37.12; H, 5.19. Found: C, 37.35; H, 5.04. Potentiometric titration with NaOH, neutralization equivalent: Calc. for $C_6H_{10}O_7$: 194. Found: 196.

ACKNOWLEDGMENT

We thank Professor D. Horton for helpful assistance in preparing the manuscript of this Note.

REFERENCES

1 O. M. Neijssel and D. W. Tempest, *Arch. Microbiol.*, 105 (1975) 183–185.
2 T. Chiyonobu, E. Shinagawa, O. Adachi, and M. Ameyama, *Agric. Biol. Chem.*, 39 (1975) 2425–2427.
3 F. Smith, *Adv. Carbohydr. Chem.*, 2 (1946) 79–106.
4 H. Ohle, H. Erlbach, and H. Carls, *Ber.*, 67 (1934) 324–332.
5 H. Ohle, *Ber.*, 58 (1925) 2577–2584.
6 H. Ohle and R. Wolter, *Ber.*, 63 (1930) 843–852.
7 W. Heimann and F. Reiff, *Pharm. Zentralhalle*, 93(3) (1954) 97–99.
8 H. Ohle and G. Berend, *Ber.*, 60 (1927) 173–187.