Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

Note

The isolation of both a *talo*-heptulose and an *allo*-heptulose from the avocado. Some new paper-chromatographic data on heptuloses

INGVAR JOHANSSON* AND NELSON K. RICHTMYER

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md. 20014 (U. S. A.)

(Received December 19th, 1969)

In 1960, Charlson and Richtmyer[1] reported the isolation of a *talo*-heptulose from the avocado. They were unable to induce crystallization in their small sample by inoculation with synthetic D-*talo*-heptulose, but proof of its structure as a *talo*-heptulose seemed reliable, because, upon degradation with oxygen in cold alkaline solution, it yielded a lactone that was indistinguishable from D-talonolactone on paper chromatograms; the D configuration was tentatively assigned to the *talo*-heptulose, because all other naturally occurring higher-carbon ketoses then known (as well as those since discovered) belong to the D configurational series. In 1966, Begbie and Richtmyer[2] reported the isolation of crystalline D-*allo*-heptulose from primula roots.

Having new supplies of both L-*allo*- and D-*talo*-heptulose available for comparisons, we decided to try to confirm, by gas–liquid chromatography (g.l.c.), the presence of a *talo*-heptulose in the sample (X) isolated[1] from the avocado. The data obtained from studies with four different columns (see the Experimental section) demonstrated conclusively that the avocado sample did indeed contain a *talo*-heptulose; moreover, it also contained an *allo*-heptulose, as well as small proportions of other constituents, presumably polyhydric alcohols.

Because we knew that the *allo*- and *talo*-heptuloses are not readily separated from each other by paper chromatography, we tested a number of solvent systems to see if one could be found that would separate them. One system (described in the Experimental section), composed of butanone, acetic acid, and saturated aqueous boric acid, cleanly separated the two heptuloses in 64 hours; and, when the avocado sample (X) was treated similarly on a paper chromatogram, two spots, respectively having the same mobilities as L-*allo*-heptulose and D-*talo*-heptulose, were visibilized with an orcinol spray that gives a characteristic color with these heptuloses. During the course of our search, we measured the mobilities, in five solvent systems**, of the six heptuloses that were available to us, as well as that of sedoheptulosan

*Fellow in the Visiting Program of the National Institutes of Health, February, 1968 to September, 1969; present address: Swedish Forest Products Research Laboratory, Stockholm, Sweden.
**For the mobilities of some heptuloses in other solvent systems, see Ref. 3.

(2,7 anhydro-β-D-*altro*-heptulopyranose) compared to that of D-*manno*-heptulose (see Table I).

TABLE I

MOBILITIES OF SOME HEPTULOSES IN DIFFERENT SOLVENT-SYSTEMS

Heptulose	*Solvent system*				
	1	*2*	*3*	*4*	*5*
L-*galacto*-	0.90	0.91	0.95	0.97	1.21
D-*gluco*-	**1.00**	**1.00**	**1.00**	**1.00**	**1.00**
D-*manno*-	1.01	0.97	1.04	1.00	1.42
D-*altro*-	1.17	1.03	1.26	1.15	1.54
L-*allo*-	1.23	1.17	1.38	1.28	1.58
D-*talo*-	1.28	1.17	1.48	1.32	2.05
D-*talo* / L-*allo*-	1.04	1.02	1.07	1.03	1.24
Sedoheptulosan / D-*manno*-Heptulose	1.00	1.10	1.06	1.36	1.47

In summary, the presence of a *talo*-heptulose in the avocado has been confirmed both by g.l.c. and by paper chromatography, and the presence of an *allo*-heptulose in a plant has been shown for the second time. It is likely that these avocado heptuloses belong to the D configurational series.

EXPERIMENTAL

General. — G.l.c. was performed with an F & M Model 5750 Research Gas Chromatograph equipped with a flame-ionization detector; the columns were 200 × 0.5 cm, and the flow rate was 75 ml of helium/min. The crystalline heptuloses were first dissolved in water, and the solution was kept overnight to equilibrate and then freeze-dried (so as to be similar to the sample from the avocado). The per(trimethylsilyl (TMS) derivatives were prepared by adding pyridine and Regisil [bis(trimethylsilyl)trifluoroacetamide]* to the lyophilized compound, and letting the mixture stand overnight. Details of the individual columns are as follows. Column A (copper) was packed with 15% by weight of 2,2-dimethyl-1,3-propanediol (neopentyl glycol) succinate polyester on Chromosorb W (80–100 mesh); column B (copper), with 10% by weight of neopentyl glycol sebacate polyester on Chromosorb W (80–100 mesh); and column C (stainless steel), with 1% by weight of SE-30 (a methylsilicone polymer) on Gaschrom P (80–100 mesh). For columns A, B, and C, the column temperature

*Tri-Sil Z [*N*-(trimethylsilyl)imidazole in pyridine solution] was also tried, but it was unsatisfactory, because it gave "ghost peaks" when injected into the polyester columns; these peaks were probably due to products formed by cleavage of some of the polyester linkages under the influence of the basic, imidazole derivative.

was 180°. Column D (stainless steel) was packed with 10% by weight of SE-30 on Chromosorb W (80–100 mesh); the column temperature was 205°.

In the following discussion, retention times (r.t.) of the TMS derivatives are all relative to that of the tetra-TMS ether of TMS α-D-glucoside.

Results with column A. — The TMS derivative of D-*talo*-heptulose showed two peaks having r.t. 1.30 and 1.53; that of L-*allo*-heptulose showed a single peak having r.t. 1.34; and a mixture of the two showed peaks having r.t. 1.32 (obviously a combination of the 1.30 and 1.34 peaks) and 1.52. The TMS derivative of the avocado syrup (X) showed peaks having r.t. 1.33 and 1.54; the latter r.t. clearly indicates the presence of *talo*-heptulose in the mixture.

Results with column B. — The TMS derivative of D-*talo*-heptulose again showed two peaks, having r.t. 1.31 and 1.56; that of L-*allo*-heptulose showed a single peak having r.t. 1.42; and a mixture of the two showed all three peaks (though with considerable overlapping) having r.t. 1.34 (as a shoulder), 1.42, and 1.58. The TMS derivative of the avocado syrup (X) showed the same three peaks having r.t. 1.34 (as a shoulder), 1.41, and 1.59.

Results with column C. — The TMS derivative of D-*talo*-heptulose here showed three peaks having r.t. 1.32, 1.64, and 1.89; that of L-*allo*-heptulose now showed two peaks having r.t. 1.62 and 1.81; and a mixture of the two showed distinct peaks having r.t. 1.33, 1.64, and 1.81 followed by a slight shoulder undoubtedly derived from the peak having r.t. 1.89 (D-*talo*-heptulose). The TMS derivative of the avocado syrup (X) showed an almost identical pattern, with peaks having r.t. 1.31, 1.66, 1.81, and a shoulder having a slightly higher r.t.

Results with column D. — The TMS derivative of D-*talo*-heptulose again showed three peaks, having r.t. 1.29, 1.54, and 1.60, the last being a shoulder; that of L-*allo*-heptulose showed two distinct peaks having r.t. 1.54 and 1.75; and a mixture of the two showed four peaks having r.t. 1.29, 1.56, 1.62 (shoulder), and 1.76. The TMS derivative of the avocado syrup (X) showed an almost identical pattern of four peaks having r.t. 1.29, 1.57, 1.63 (shoulder), and 1.77.

Paper chromatography.— Paper chromatography was performed on Whatman No. 1 filter paper by the descending method at room temperature. The solvent systems used were (*1*), 6:4:3 (v/v) butyl alcohol–pyridine–water; (*2*), 40:11:19 (v/v) butyl alcohol–ethyl alcohol–water; (*3*), 72:20:23 (v/v) ethyl acetate–pyridine–water (upper phase); (*4*) 18:3:1:4 (v/v) ethyl acetate–acetic acid–formic acid–water; and (*5*) 9:1:1 (v/v) butanone–acetic acid–saturated aqueous boric acid. The spray reagent consisted of 2% of orcinol and 3% of concentrated hydrochloric acid in butyl alcohol, and the chromatograms were heated for 3 min at 100–110°; all of the sugars mentioned gave a blue color, except D-*manno*-heptulose, which gave a greenish blue color. Chromatograms developed with solvent (*5*) were preferably sprayed several times with methanol and allowed to dry each time in a hood (to remove the boric acid as methyl borate*)

*This procedure was suggested by Dr. C. P. J. Glaudemans of this laboratory.

before being sprayed with the orcinol–hydrochloric acid reagent; otherwise, it was necessary to spray and heat several times with the latter reagent to bring out the color.

Each of the other heptuloses was compared directly with D-*gluco*-heptulose as the standard. D-*talo*-Heptulose was also compared directly with L-*allo*-heptulose, and sedoheptulosan directly with D-*manno*-heptulose. The values recorded in Table I are the averages obtained from six spots on each chromatogram, and from chromatograms developed for various lengths of time; thus, in solvent (*1*), the chromatograms were developed for 24 and 40 h; solvent (*2*), 24 and 47 h; solvent (*3*) 16, 24, and 40 h; solvent (*4*), 17, 25 and, 41 h; and solvent (*5*) 24 h (except for the D-*talo*- *versus* L-*allo*-heptulose, for which the chromatograms were developed for 16, 24, 40, and 64 h). In addition to the solvent systems listed in Table I, two others were tried; in 4:1:1 (v/v) butyl alcohol–ethyl alcohol–water (24, 72, and 144 h), the *talo*-:*allo*-heptulose ratio was 0.97, and in 12:5:4 (v/v) ethyl acetate–pyridine–4% aqueous boric acid (16 and 24 h), the ratio was 1.05.

REFERENCES

1 A. J. CHARLSON AND N. K. RICHTMYER, *J. Amer. Chem. Soc.*, 82 (1960) 3428.
2 R. BEGBIE AND N. K. RICHTMYER, *Carbohyd. Res.*, 2 (1966) 272.
3 G. R. NOGGLE, *Arch. Biochem. Biophys.*, 43 (1953) 238; E. A. MCCOMB AND V. V. RENDIG, *ibid.*, 95 (1961) 316.