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DISTRIBUTION OF *MANNO*-HEPTULOSE AND SEDOHEPTULOSE IN PLANTS*

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Key Word Index—*Gymnospermae, angiospermae*; *manno*-heptulose; sedoheptulose; distribution.

Abstract—Upon investigation of the distribution of heptuloses in plants, most of the plants which were positive to the preliminary PC of the heptulose were found to contain *manno*-heptulose by the confirmative tests with descending PC and GLC of TMS derivatives after purification on the thick paper chromatogram. The amounts of *manno*-heptulose in many plants were comparable to those of sedoheptulose, and often higher than that of the latter.

INTRODUCTION

AMONG higher carbon sugars, only sedoheptulose is known to be widely distributed in plants.[1] Its occurrence is expected on the basis of the photosynthesis and the pentose-phosphate cycle of sugar metabolism, although the investigations of the distribution of sedoheptulose have hitherto been carried out almost solely by PC.[1,2] The isolation of accumulated sedoheptulose has been limited to the representatives of a few families: Crassulaceae,[3] Saxifragaceae[4] and Primulaceae.[4,5] After isolating labelled sedoheptulose and coriose (D-*altro*-3-heptulose) from *Coriaria japonica* A. Gray,[6] we attempted to detect higher carbon sugars in other plant species using a combination of GLC and three types of PC because it was considered that the PC method hitherto applied to plant extracts may often be unsatisfactory in differentiating sedoheptulose from other heptuloses. We began the investigation with plants which are taxonomically related to the Coriariaceae† and then extended the survey to members of other families.

RESULTS AND DISCUSSIONS

Table 1 lists the plants whose aqueous extract after fermentation showed spots corresponding to heptuloses by ascending PC. The extracts which gave a positive preliminary test were further fractionated by preparative PC to remove the substances which caused tailing of the heptulose spots on the preliminary test and also to remove

* Part V in the series "Coriose and Related Compounds". For Part IV see OKUDA, T. and KONISHI, K. (1969) *Yakugaku Zasshi* **89**, 1407.

† Based on ENGLER, A. (1964) *Syllabus der Pflanzenfamilien*, 12 Auflage.

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TABLE 1. DISTRIBUTION OF SEDOHEPTULOSE AND *Manno*-HEPTULOSE IN LEAVES OF VARIOUS PLANTS

Families	Species	Month* collected	MH†	SH‡	MH/SH§
	Gymnospermae				
Pinaceae	*Cedrus deodara* Loud.	12	−	+	
Taxodiaceae	*Cryptomeria japonica* D. Don	12	±	±	
Cupressaceae	*Chamaecyparis obtusa* Endl.	12	−	+	
	Angiospermae				
	Dicotyledoneae				
Salicaceae	*Populus nigra* L. var. *italica* Muench.	7	+	+	3·4
Moraceae	*Ficus carica* L.	7	+	+	0·7
Theaceae	*Camellia japonica* L.	7	+	+	3.5
Hamamelidaceae	*Liquidambar formosana* Hance.	7	+	+	7·4
		12	+	+	
Crassulaceae	*Sedum lineare* Thunb.	7	+	+	0·05
		12			
	S. kamtschaticum Fisch.	11	+	+	0·01
Saxifragaceae	*Saxifraga stolonifera* Curtis	7	−	+	
	Chrysosplenium grayanum Maxim.	5	−	+	
	Hydrangea macrophylla Ser. forma *otaksa* Sieb. et Zucc.	7	+	+	1·4
Rosaceae	*Photinia glabra* Maxim.	7	+	+	19
Leguminosae	*Cassia tora* L.	7	+	−	
	Pueraria lobata Ohwi	7	+	−	
Geraniaceae	*Pelargonium inquinans* Ait.	7	+	−	
Euphorbiaceae	*Daphniphyllum macropodum* Miq.	3	+	+	
	Ricinus communis L.	7	+	−	
	Triadica sebifera Small	7	+	+	0·43
	Euphorbia helioscopia L.	7	+	+	0·05
	E. pekinensis Rupr. var. *japanensis* Makino	7	+	+	6·1
	E. jolkinii Boiss.	7			
		12	+	+	3·1
	E. lathyris L.	7	+	+	7·8
		12	+	+	
	E. supina Rafin.	7	+	+	0·26
	E. maculata L.	7	+	±	4·3
	Mallotus japonicus Muell. Arg.	7	+	+	2·8
		11	+	+	
	Aleurites fordii Hemsl.	7	+	+	0·34
Rutaceae	*Xanthoxylum piperitum* DC.	7	+	−	
	Phellodendron amurense Rupr.	7	+	−	
	Poncirus trifoliata Rafin.	7	+	−	
Meliaceae	*Melia azedarach* L. var. *japonica* Makino	6	+	+	0·17
		11	+	+	
Coriariaceae	*Coriaria japonica* A. Gray	6	+	+	0·09
Anacardiaceae	*Rhus javanica* L.	6	+	+	4·0
Balsaminaceae	*Impatiens balsamina* L.	7	+	−	
Aquifoliaceae	*Ilex rotunda* Thunb.	7	+	+	2·6
		2	+	+	
	I. oldhami Miq.	7	+	+	0·06
	I. pedunculosa Miq.	7	+	+	0·09
	I. latifolia Thunb.	11	+	+	2·1
	I. crenata Thunb. f. *bullata* Rehd.	7	+	+	0·03
	I. serrata Thunb. var. *sieboldii* Loesn.	7	+	+	1·0
Vitaceae	*Ampelopsis brevipedunculata* Tr.	7	+	−	
Malvaceae	*Hibiscus syriacus* L.	11	±	±	3·3
Primulaceae	*Lysimachia fortunei* Maxim.	8	+	+	0·86
Oleaceae	*Fraxinus longicuspis* Sieb. et Zucc. f. *yamatensis* Nakai	7	+	+	2·8
	Forsythia suspensa Vahl.	8	+	+	3·2

TABLE 1.—*cont.*

Families	Species	Month* collected	MH†	SH‡	MH/SH§
	Olea europaea L.	7	+	+	5·4
Caprifoliaceae	*Lonicera japonica* Thunb.	11	+	−	
	Monocotyledoneae				
Stemonaceae	*Stemona japonica* Miq.	7	−	+	
Iridaceae	*Crocus sativus* L.	2	+	+	6·0

* For instance, 12 means collected in December.
† *manno*-Heptulose.
‡ Sedoheptulose.
§ The ratio of *manno*-heptulose to sedoheptulose is based on the peak area in GLC, and calculated using the calibration graph.

myo-inositol which shows an almost identical GLC retention time to that of *manno*-heptulose. Sedoheptulose and *manno*-heptulose were isolated after preparative PC and characterised by descending PC and by GLC using the corresponding TMS derivatives.

Most of the plants which were positive in the preliminary test contained both *manno*-heptulose and sedoheptulose (Table 1). It is notable that plants of the Euphorbiaceae (11 species) and Aquifoliaceae (6 species) contain *manno*-heptulose. Some plants contain only *manno*-heptulose whilst other plants contain only sedoheptulose; although the number of the latter is smaller. However, most of the plants studied contained both *manno*-heptulose and sedoheptulose, and more than half of these plants contained larger amounts of *manno*-heptulose than those of sedoheptulose (see ratio Table 1). Our results clearly indicate that *manno*-heptulose is a widely distributed monosaccharide in nature although previous work has indicated that it is a rare sugar, being found only in *Persea gratissima*[7] and a few other plants.[8] *manno*-Heptulose is presumed to be D-*manno*-heptulose based on its probable route of biosynthesis in the plant and also on the fact that only the D-series of this sugar has been previously isolated.

EXPERIMENTAL

Preparation of plant extracts. Leaves were extracted with boiling H_2O for 30 min immediately after collection. The soln was concentrated *in vacuo* and treated with baker's yeast overnight and filtered. The filtered soln was evaporated *in vacuo* and the residue extracted 2× with boiling MeOH for 1 hr. The MeOH soln was evaporated and the syrupy residue submitted to the preliminary test. The extracts which were positive to the preliminary test were then further fractionated by preparative PC.

PC of the extracts. Heptuloses were detected using orcinol–Cl_3CCOOH–*n*–BuOH (saturated with water) (1 : 30 : 240, w/w/v). (a) The preliminary test was carried out with *n*–BuOH–pyridine–H_2O (6 : 4 : 3) using the ascending method. The R_f values of sedoheptulose and *manno*-heptulose were not consistent but were in the range 0·48 and 0·43 respectively. (b) Preparative PC was carried out on thick cellulose papers (40 × 40 × 0·7 cm) using *n*–BuOH–EtOH–H_2O (4 : 1·2 : 1). Heptuloses were detected by the orcinol reagent on a few strips cut from the paper. The region of the same R_f on the remainder of paper was then removed and extracted with MeOH. The syrupy residue obtained on evaporation of the MeOH soln was submitted for the final PC and GLC. (c) Final PC was carried out using the descending method with EtOAc–EtOH–H_2O (8 : 2 : 1).

GLC of the extracts. TMS derivatives of the syrups were prepared with trimethylchlorosilane–hexamethyldisilazane–pyridine (1 : 2 : 10).[9] GLC was carried out with a Shimadzu 5A instrument equipped with FID

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using glass columns (2 m × 3 mm i.d.) packed with 3% OV17 on 80–100 mesh Chromosorb W HMDS treated, and 1·5% SE30 on 60–80 mesh Chromosorb W HMDS treated. Oven temp.: 150° for OV17, and 170° for SE30; carrier gas: N_2. R_t relative to α-D-glucose : *manno*-heptulose (1·87 on OV17 and 2·13 on SE30), sedoheptulose (1·71 on OV17 and 1·87 on SE30).

Calibration and calculation. The ratio of *manno*-heptulose to sedoheptulose was determined on SE-30 at 170°. Calibration was obtained by chromatographing 9 different ratios of the two sugars and plotting the ratio of the peak area of *manno*-heptulose to sedoheptulose against the ratio of the amount of the two sugars. The ratio of the two sugars in each sample extract was determined by applying this calibration to the ratio of the peak areas: (weight of *manno*-heptulose/weight of sedoheptulose)/(peak area of *manno*-heptulose/peak area of sedoheptulose) = 0·86.