Phytochemistry, Vol. 25, No. 10, pp. 2335–2337, 1986.
Printed in Great Britain.

0031–9422/86 $3.00 + 0.00
Pergamon Journals Ltd.

SOLUBLE CARBOHYDRATES OF LIVERWORTS

AKIHIKO MATSUO*, DAISUKE TAKAOKA† and HIROSHI KAWAHARA†

Department of Chemistry, Faculty of Science, Hiroshima University, Naka-ku, Hiroshima 730, Japan; †Department of Chemistry, Faculty of General Education, Ehime University, Matsuyama 790, Japan

(*Revised received* 10 *February* 1986)

Key Word Index—Liverworts; bryophytes; carbohydrates; absolute configuration; HPLC analysis; chemotaxonomy.

Abstract—Five soluble carbohydrates, (−)-L-bornesitol, (−)-D-mannitol, (+)-D-glucose, (+)-sucrose and (+)-trehalose, have been isolated as their acetyl derivatives from the liverwort, *Mylia taylorii*. The absolute configuration of these carbohydrates is the same as that of sugars found in vascular plants, although the liverwort sesquiterpenoids are usually enantiomeric relative to those of vascular plants. The soluble carbohydrates in seven other liverwort species have also been analysed by HPLC.

INTRODUCTION

Liverworts contain several characteristic oil bodies, in each cell of the gametophytic plant, and sesquiterpenoids and diterpenoids as their major lipophilic constituents. An important biochemical feature of these sesquiterpenoids is that almost all are enantiomeric with respect to those in vascular plants [1–3].

Comparatively little is known about the soluble carbohydrates in liverworts: the pioneering work by Ono and Yoshimura showed the presence of glucose, fructose and sucrose in many species [4–6]. Subsequently, Lewis *et al.* have analysed the soluble sugars in a range of liverworts by gas and paper chromatography, identifying volemitol, mannitol, glucose, fructose, sucrose and trehalose [7, 8]. However, there is no report of the isolation of soluble carbohydrates from liverworts [9] and no record of their absolute configuration. Here, we describe the isolation from the liverwort, *Mylia taylorii* (Hook.) S. Gray, and the structural elucidation including their absolute configuration of (−)-L-bornesitol, (−)-D-mannitol, (+)-D-glucose, (+)-sucrose and (+)-trehalose. We also report the analysis of the soluble sugars of seven other liverwort species.

RESULTS AND DISCUSSION

Structural determination of the soluble carbohydrates from the liverwort Mylia taylorii

The ethanol extract of the liverwort *Mylia taylorii* was re-extracted with ether to remove lipophilic components, and the water soluble fraction was concentrated *in vacuo* to a syrup. After confirming the absence of compounds having acetyl groups in the syrup by IR and NMR spectrometry, the syrup was firstly acetylated with acetic anhydride in pyridine to facilitate the isolation of each sugar constituent. The crude acetylated products, when chromatographed on a silica gel column, yielded five acetylated carbohydrates.

Spectroscopic data (CIMS, ^{1}H NMR, IR) of the first compound, $C_{17}H_{24}O_{11}$, mp 142–143.5°, $[\alpha]_D$ −9.3°, suggested it was a cyclitol derivative having five acetyl and one methoxy groups (see Experimental). The compound was converted, by treatment with hydroiodic acid followed by acetylation with acetic anhydride in pyridine, into hexa-*O*-acetyl-*myo*-inositol, $C_{18}H_{24}O_{12}$, mp 217.5–218.5°. The melting point and spectroscopic properties were identical with literature data [10]. Alternatively, the pentaacetyl derivative was hydrolysed with hydrochloric acid to yield (−)-L-bornesitol, $C_7H_{14}O_6$, mp 207–208°, $[\alpha]_D$ −30.3° [11].

The physical constants and spectral data of the other four acetyl carbohydrates, which were isolated in the pure state by column chromatography, were determined: the ^{1}H NMR spectra of the second and third carbohydrates gave, respectively, six acetyl methyls suggesting acetyl derivatives of monosaccharides, and the fourth and fifth compounds were acetates of disaccharides having eight acetyl methyl groups. From the above spectroscopic properties as well as the retention times of the original sugars (see Table 1), the structures of these carbohydrates were deduced to be acetyl derivatives of mannitol, glucose, sucrose and trehalose. Finally, their structures and absolute configurations were elucidated as the acetyl derivatives of (−)-D-mannitol [12], (+)-D-glucose [13], (+)-sucrose [14] and (+)-trehalose [15] by coincidence of the spectral data and the physical constants including the optical rotations with those of the acetates prepared from the authentic sugars. These results were also compatible with the reported values. Three remaining carbohydrates on the chromatogram were identified by comparing the retention times of HPLC of the original sugars and of GC of the TMS derivatives with those of authentic samples to be fructose, galactose and *myo*-inositol, respectively.

This is the first instance that these carbohydrates have been isolated from liverworts, although they were obtained as acetyl derivatives. Optical rotations of these liverwort carbohydrates showed the same sign as those of

*To whom correspondence should be addressed.

Table 1. Distribution pattern of the soluble carbohydrates in some liverworts*

Sugar	R_t (min)	M.t.†	M.v.	S.s.	S.p.	J.i.	J.t.	M.e.	C.n.
Rhamnose	5.90								2.5
Fucose	7.20								5.5
Xylose	8.60			9.9				7.4	
Arabinose	9.30		2.0						
Fructose	9.90	29.0	19.4		14.8	30.0	58.7	6.9	41.0
Unidentified	10.30							27.1	
Glucose	11.36	21.6‡	14.0‡	61.4	19.2	17.1	22.3	7.9	43.1
Mannitol	11.36	10.8‡	8.6‡						
Galactose	12.40	2.8					7.7	5.9	4.9
Bornesitol	13.90	15.8	54.8						1.2
Sucrose	14.90	14.3		28.7	59.8	33.3	8.8	38.1	
Unidentified	17.60					10.7			
Trehalose	24.00	5.6			6.2	6.8	2.6	3.2	1.7
myo-Inositol	30.20	1.1	0.6			1.3			

*The relative concentration (%) is obtained from the peak areas of the chromatogram.

†The species name and locality of the liverworts examined are as follow. *M.t.*: *Mylia taylorii* (Hook.) S. Gray (collected at Ôtoyo, Kôchi-ken). *M.v.*: *Mylia verrucosa* Lindb. (collected at Tônaru, Ehime-ken). *S.s.*: *Scapania stephanii* K. Muell. (collected at Mt. Kuishi, Kôchi-ken). *S.p.*: *Scapania parvidens* Steph. (collected at Mt. Kuishi, Kôchi-ken). *J.i.*: *Jungermannia infusca* (Mitt.) Steph. (collected at Mikawa, Yamaguchi-ken). *J.t.*: *Jungermannia torticalyx* Steph. (collected at Iwakuni, Yamaguchi-ken). *M.e.*: *Marsupella emarginata* var. *patens* N. Kitag. (collected at Iwakuni, Yamaguchi-ken). *C.n.*: *Calypogeia neesiana* (Mass. et Car.) K. Muell. subsp. *subalpina* (Inoue) Inoue (collected at Shiga, Nagano-ken).

‡The relative content of these compounds is calculated from the gas chromatogram of their TMS derivatives.

the known compounds obtained from vascular plants, confirming the absolute configurations as the normal, not enantiomeric form.

Distribution patterns of soluble carbohydrates of eight liverwort species

We also investigated the carbohydrates of seven other liverworts belonging to the Jungermanniales. The water soluble fractions of these were prepared by the procedures described above. Analysis was by HPLC; identification of the carbohydrates, based on comparison of the retention times with those of authentic specimens, was then confirmed by co-chromatography with the known compounds. However, the two peaks of glucose and mannitol were not separated by this column but were distinguished by GC analysis of their TMS derivatives.

Twelve carbohydrates were identified in the eight liverworts and Table 1 records their relative contents calculated from peak areas of the chromatograms. Bornesitol has been found in three liverworts for the first time, although the occurrence of the other eleven compounds in liverworts has already been recorded [9]. Glucose, fructose and sucrose were detected in almost all of the liverworts. Rhamnose, fucose, xylose, arabinose, galactose and *myo*-inositol as well as bornesitol are not distributed so widely in the liverworts studied and they are diagnostic compounds of particular species. Species of the same genus generally have similar patterns of the carbohydrates. Chromatograms of two liverworts, *J. infusca* and *M. emarginata* var. *patens*, included unknown peaks and their structural determination is in progress.

EXPERIMENTAL

General procedure. Mps: uncorr.; IR: $CHCl_3$ and/or KBr; ^1H NMR: $CDCl_3$ and/or D_2O with TMS as the internal standard; CIMS: reactant gas NH_3, ionization voltage 200 eV; SIMS: matrix glycerine, primary ion beam 8 kV, secondary ion beam 3 kV; GLC: glass column (3 mm × 3 m) packed with OV-1 (3%) and/or FFAP (10%) on Chromosorb AW (80–100 mesh), column temp. 280° and/or 240°, respectively; CC: Merk Kieselgel 60; TLC and prep. TLC: Merk Kieselgel 60 PF_{254}, spots were visualized under UV radiation and/or sprayed 10% H_2SO_4 in EtOH and heated at 120°.

HPLC analysis of the soluble carbohydrates. HPLC analysis of the syrups was carried out by the following conditions and a differential refractometer was used for detection of the constituents. Column: Shodex RS_{pak} DC-613 (6 mm × 150 mm); column temp.: 68°; mobile phase: MeCN and H_2O (4:1); flow rate: 13 ml/min.

Material and its extraction. The liverwort *M. taylorii* was collected at Ôtoyo in Kôchi-ken. The whole plant (2.2 kg), after washing with H_2O and drying in the shade for several days, was extracted with EtOH. The solvent was distilled off under red. pres. and the residue then extracted with ether to remove lipophilic constituents. The soluble part was concd *in vacuo* to give a syrup (13.0 g). For analysis of the soluble carbohydrates by HPLC, the other seven liverworts were collected in southwest Japan as shown in Table 1 and each syrup was obtained by the same procedure as the case of *M. taylorii*.

Isolation of the soluble carbohydrates from M. taylorii. The syrup (6.8 g) thus obtained was dissolved in pyridine (50 ml) and excess of Ac_2O was added with stirring, and the mixture was allowed to stand overnight at room temp. The reaction product

was poured into ice water and extracted with Et_2O. After recovery in the usual way, the crude acetylated products were chromatographed through a silica gel column using a mixed solvent of hexane–EtOAc (7:3) to isolate the following five compounds. The spectral data obtained (CIMS, ^1H NMR and IR) were identical to published results.

(−)-*Penta*-O-*acetyl*-L-*bornesitol*: mp 142–143.5° (from hexane); $[\alpha]_D^{25}$ −9.3° (*c* 1.0; $CHCl_3$). (+)-*Hexa*-O-*acetyl*-D-*mannitol*: mp 125–126° (from EtOH) (lit. [12] 125°); $[\alpha]_D^{25}$ +23° (*c* 4.5; $CHCl_3$) (lit. [12] +25°). (+)-*Hexa*-O-*acetyl*-D-*glucose*: mp 114–115° (from EtOH) (lit. [13] 114°); $[\alpha]_D^{25}$ +103° (*c* 1.5; $CHCl_3$) (lit. [13] +102°). (+)-*Octa*-O-*acetyl-sucrose*: mp 89–90° (from EtOH) (lit. [14] 89°); $[\alpha]_D^{25}$ +58° (*c* 2.2; $CHCl_3$) (lit. [14] 89°). (+)-*Octa*-O-*acetyl-trehalose*: mp 104–105° (from EtOH) (lit. [15] 100°); $[\alpha]_D^{25}$ +154° (*c* 2.0; $CHCl_3$) (lit. [15] +162°).

Conversion of (−)-*penta*-O-*acetyl*-L-*bornesitol into hexa*-O-*acetyl*-myo-*inositol*. The pentaacetyl compound (105 mg) was refluxed with HI (55%, 2 ml). After cooling the reaction mixture, the acid was removed by distillation under red. pres. Excess of Ac_2O was added under cooling to the residue dissolved in pyridine (20 ml) and was stirred overnight at room temp. The product, hexa-*O*-acetyl-*myo*-inositol, was obtained as crystals by treating in the usual way: mp 217.5–218.5° (from EtOH).

Deacetylation of penta-O-*acetyl bornesitol*. The pentaacetate (435 mg) was refluxed with EtOH (20 ml) and 2 N HCl (15 ml). The organic solvent was distilled off under red. pres. and the residue was passed through a small column of active carbon. The product, bornesitol, was obtained by crystallization from EtOH: mp 207–208° (lit. [11] 205–205.5°); $[\alpha]_D$ −30.3° (*c* 3.1; H_2O) (lit. [11] −32.6°); found: C, 43.36; H, 7.45. $C_7H_{14}O_6$ requires C, 43.29; H, 7.27%.

Acknowledgements—We thank Drs. H. Inoue, T. Seki and Prof. N. Kitagawa for collection and identification of the liverworts.

REFERENCES

1. Matsuo, A. (1982) *J. Hattori Bot. Lab.* **53**, 295.
2. Matsuo, A., Nozaki, H., Kubota, N., Uto, S. and Nakayama, M. (1984) *J. Chem. Soc. Perkin Trans.* 1, 203.
3. Matsuo, A., Nakayama, N. and Nakayama, M. (1985) *Phytochemistry* **24**, 777.
4. Ono, M. and Yoshiura, H. (1958) *J. Jap. Bot.* **33** 59
5. Ono, M. (1958) *J. Jap. Bot.* **33**, 285.
6. Ono, M. (1958) *J. Jap. Bot.* **33**, 367.
7. Lewis, D. H. (1970) *Trans. Br. Bryol. Soc.* **6**, 108.
8. Suleiman, A. A. A., Bacon, J., Christie, A. and Lewis, D. H. (1979) *New Phytol.* **82**, 439.
9. Huneck, S. (1983) in *New Manual of Bryology* (Shuster, R. M., ed.) Vol. 1, p. 1. Hattori Bot. Lab., Miyazaki, Japan.
10. Lemieux, R. V., Kullnic, R. K., Bernstein, H. J. and Shneider, W. G. (1957) *J. Am. Chem. Soc.* **79**, 1005.
11. Post, G. G. and Anderson, L. (1962) *J. Am. Chem. Soc.* **84**, 478.
12. Hough, L. and Richardson, A. C. (1967) in *Rodd's Chemistry of Carbon Compounds* (Coffey, S., ed.) 2nd edn, Vol. 1, Part F, p. 1. Elsevier, Amsterdam.
13. Hough, L. and Richardson, A. C. (1967) in *Rodd's Chemistry of Carbon Compounds* (Coffey, S., ed.) 2nd edn, Vol. 1, Part F, p. 67. Elsevier, Amsterdam.
14. Linstead, R. P., Rutenberg, A., Dauben, W. G. and Evans, W. L. (1940) *J. Am. Chem. Soc.* **62**, 3260.
15. Hudson, C. S. and Johnson, J. M. (1915) *J. Am. Chem. Soc.* **37**, 2748.