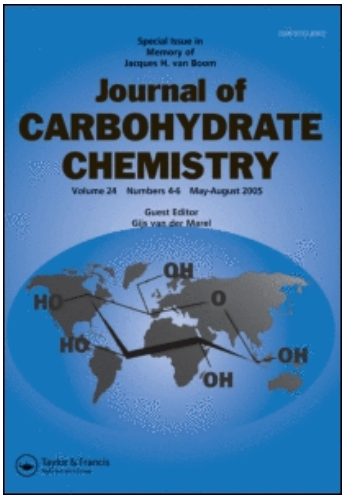


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

Structural Studies of Trisaccharide of Leptaculatin

Sanjay Srivastava[a]; Desh Deepak[a]; Anakshi Khare[a]
[a] Khare's School of Natural Products Department of Chemistry, Lucknow University, Lucknow, India

To cite this Article Srivastava, Sanjay , Deepak, Desh and Khare, Anakshi(1994) 'Structural Studies of Trisaccharide of Leptaculatin', Journal of Carbohydrate Chemistry, 13: 1, 75 — 80
To link to this Article: DOI: 10.1080/07328309408009179
URL: http://dx.doi.org/10.1080/07328309408009179

J. CARBOHYDRATE CHEMISTRY, 13(1), 75-80 (1994)

STRUCTURAL STUDIES OF TRISACCHARIDE OF LEPTACULATIN

Sanjay Srivastava, Desh Deepak, and Anakshi Khare*

Khare's School of Natural Products
Department of Chemistry, Lucknow University,
Lucknow - 226007, India

Received August 20, 1992 - Final Form August 30, 1993

ABSTRACT

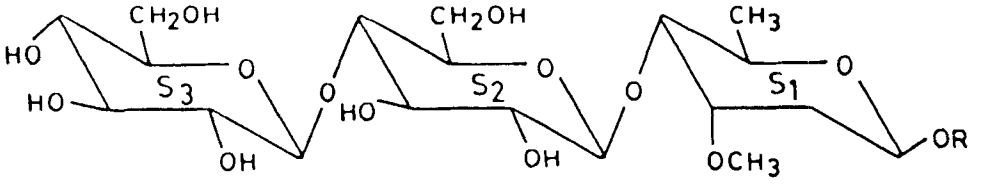
Leptatriose (**2**), a novel trisaccharide, was obtained from acid hydrolysate of Leptaculatin (**1**), isolated from *Leptadenia reticulata*. On the basis of physico-chemical results, the structure of **2** was established as *O*-ß-**D**-glucopyranosyl (1->4)-*O*-ß-**D**-glucopyranosyl (1->4)-ß-**D**-cymaropyranose.

INTRODUCTION

Oligosaccharides[1-2] and oligoglycosides[3-4] of 2-deoxy sugars have been reported from plants of Asclepiadaceae family. The naturally occurring oligosaccharides possess immuno-modulating, anticomplementary, anti-tumour and anti-cancer activities.[5] In continuation of our work on plants of family Asclepiadaceae,[6] Leptatriose (**2**), a novel trisaccharide was isolated from the acid hydrolysate of **1** obtained from chloroform and chloroform-ethanol (4:1) mixed extract of *L. reticulata*.

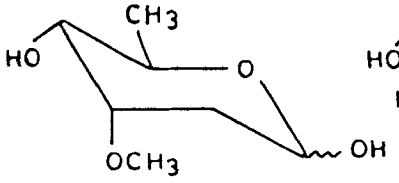
RESULTS AND DISCUSSION

Leptaculatin (**1**), mp 107-110 °C, $[\alpha]_D$-5.8 °, $C_{40}H_{66}O_{16}$ on acid hydrolysis with 0.05N H_2SO_4[7] yielded a C_{21} steroid and chromatographically pure syrupy sugar **2**, $[\alpha]_D$-53.57 °, which reduced Fehling's solution and gave positive tests in

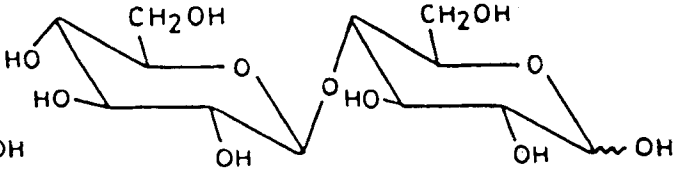


1 R = Aglycone

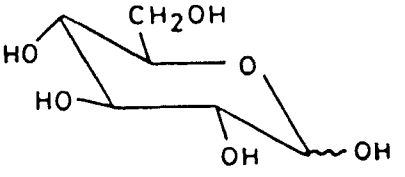
2 R = H



3



4



5

the xanthydrol, Keller-Kiliani and $NaIO_4$ reactions. The ^{13}C NMR of **1** contains three anomeric carbons at δ 104.2, 103.9 and 102.7 supplemented by three anomeric proton signals at δ 4.45(1H) and 4.32(2H) in the 400 MHz ^1H NMR spectrum of **1**. The mass ion peak at m/z 486 in the FAB mass spectrum of **1** also supported the nature of glycon **2** as a trisaccharide.

To identify the sugar units of the trisaccharide (**2**), Mannich hydrolysis[8] of **2** was done. After 5 days two spots were observed on the TLC which corresponded to D-cymarose (**3**) and cellobiose (**4**) (TLC, PC) on comparison with authentic samples. After 7 days the hydrolysis was

complete, affording a mixture of two sugars which were identified with authentic samples of **D**-cymarose (**3**) and **D**-glucose (**5**). For further characterization the sugars were converted to their respective phenylhydrazides which were found identical to **D**-cymaronic acid phenylhydrazide[9] (mp 151-153 °C) and **D**-gluconic acid phenylhydrazide[11] (mp 196-198 °C) respectively.

The configurations of the glycosidic linkages were assigned from the ^1H NMR spectrum of **1** at 400 MHz. For convenience, the one cymarose and two glucose units of **2** were designated as S_1, S_2 and S_3 respectively. A two proton doublet (J=8 Hz) at δ 4.32 in the spectrum could be assigned to the two identical anomeric protons of two glucose units. A one proton double doublet centered at δ 4.45 (J=9 and 2 Hz) was attributed to the anomeric proton of the **D**-cymarose residue (S_1). The large coupling constants (8 and 9 Hz) were typical of an axial orientation of anomeric proton in the $^4C_1$ (**D**) conformation[10] indicating ß-glycosidic linkages of S_1, S_2 and S_3. The characteristic methylene group signals of the 2-deoxy sugar unit were present at δ 2.28-2.34 (m) and 1.84-1.89(m) for equatorial and axial protons, respectively, along with other significant signals of **D**-cymarose and **D**-cellobiose moieties.

The structure of Leptatriose was further supported by the ^{13}C NMR data which was in close conformity with the ^1H NMR data. The downfield appearance of the three anomeric carbons showed ß-glycosidic linkages for the three sugar units. Other signals of trisaccharide moiety given below were also consistent with the derived structure (FIG. 1).

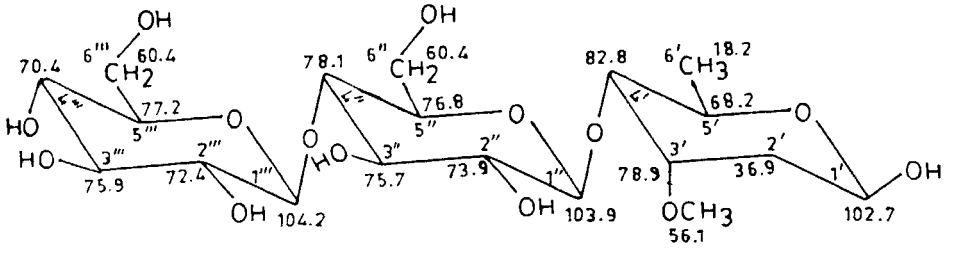


FIG. 1. ^{13}C NMR shifts of **2** (δ in $CDCl_3$)

The FABMS of **1** contained mass ion peaks at m/z 486 and m/z 469 which corresponded to the (trisaccharide) and (trisaccharide-OH). The other important fragments of the trisaccharide were m/z 437 (469-CH_3OH), 419 (437-H_2O), 401 (419-H_2O), 383 (401-H_2O) and 365 (383-H_2O). The important fragments obtained from the EIMS of **1**, were at m/z 450 (trisaccharide-$2H_2O$), 418 (450-CH_3OH), 400 (418-H_2O) and 382 (400-H_2O). The spectrum also contained the significant fragments of di- and monosaccharide units of **2**. The mass fragments thus account for the significant peaks in the spectrum that fully support the derived structure of **2**.

In light of the foregoing evidence, the structure of **2** was established as *O*-ß-**D**-glucopyranosyl-(1->4)-*O*-ß-**D**-glucopyranosyl-(1->4)-ß-**D**-cymaropyranose.

EXPERIMENTAL

General Procedures. The general procedures were as described in reference 9. The FAB and EI mass spectra (MS) were recorded with a JEOL mass spectrometer, model JMS-SX102 FAB with a DA 6000 Data system, and JEOL mass spectrometer D-300 with a IMA-2000 Data system, respectively.

Isolation of Leptatriose. Shade dried plant *Leptadenia reticulata* was extracted by the method reported earlier.[9] Leptaculatin (**1**) was isolated from a mixed chloroform and chloroform-ethanol (4:1) extract. Mild hydrolysis with acid afforded a trisaccharide (**2**) which was chromatographed over silica gel to give leptatriose (**2**) (12 mg), $[\alpha]_D$-53.57 ^{o} (*c* 0.2; methanol). The latter compound gave blue coloration with the vanillin-perchloric acid spray reagent, gave positive tests in the xanthydrol and Keller-Kiliani reactions and reduced Fehling's solution; it also gave a positive reaction with $NaIO_4$. ^1H NMR data (400 MHz, $CDCl_3$): δ 1.29 (d, 3H, $J_{5,Me}$=6 Hz, Me, S_1), 1.84-1.89 (m, 1H, H-2 ax., S_1), 2.28-2.34 (m, 1H, H-2 eq., S_1), 3.13-3.18 (m, 1H, H-4, S_1), 3.29-3.36 (m, 2H, H-2, S_2, S_3), 3.37-3.41 (m, 1H, H-4, S_3), 3.41-3.45 (m, 2H, H-3, S_2, S_3), 3.46-3.50 (m, 2H,

H-5, S_2, S_3), 3.52 (s, 3H, MeO, S_1), 3.56-3.58 (m, 1H, H-4, S_2), 3.58-3.63 (m, 2H, H-3, H-5, S_1), 3.68-3.72 (m, 2H, H-6, S_2, S_3), 3.74-3.78 (m, 2H, H-6, S_2, S_3), 4.32 (d, 2H, $J_{1,2}$=8 Hz, H-1, S_2, S_3), 4.45 (dd, 1H, $J_{1,\ 2CH2}$=9 and 2Hz, H-1, S_1). ^{13}C NMR - ^{13}C chemical shifts are given in FIG. 1. FABMS m/z 486 [trisaccharide]$^+$, 469 [trisaccharide-OH]$^+$, 437 [469-CH_3OH]$^+$, 419 [437-H_2O]$^+$, 401 [419-H_2O]$^+$, 391 [469-H_2O-$C_2H_4O_2$]$^+$, 383 [401-H_2O]$^+$, 365 [383-H_2O]$^+$, 355 [391-$2H_2O$]$^+$, 313 [391-H_2O-CH_2OHCHO]$^+$, 281 [313-CH_3OH]$^+$. EIMS m/z 486 (not observed), 450 [trisaccharide-$2H_2O$]$^+$, 436 [trisaccharide-CH_3OH-H_2O]$^+$, 418 [436-H_2O; 450-CH_3OH]$^+$, 400 [418-H_2O]$^+$, 382 [400-H_2O]$^+$ 353 [trisaccharide-$C_5H_9O_4$]$^+$, 342 [trisaccharide-S_1]$^+$, 325 [342-OH]$^+$, 324 [trisaccharide-S_3]$^+$, 321 [353-CH_3OH]$^+$, 317 [353-$2H_2O$]$^+$, 307 [325-H_2O; 324-OH]$^+$, 303 [321-H_2O]$^+$, 289 [307-H_2O]$^+$, 285 [303-H_2O]$^+$, 278 [324-OCHOH]$^+$, 277 [321-CH_3CHO]$^+$, 271 [289-H_2O; 317-HOCHO]$^+$, 264 [342-$C_2H_4O_2$]$^+$, 259 [277-H_2O]$^+$, 257 [289-CH_3OH]$^+$, 256 [324-CH_3OH-$2H_2O$]$^+$, 248 [324-CH_3OH-CH_3CHO]$^+$, 239 [257-H_2O; 285-HOCHO]$^+$, 231 [277-HOCHO]$^+$, 229 [289-$C_2H_4O_2$]$^+$, 228 [264-$2H_2O$]$^+$, 213 [259-HOCHO]$^+$, 210 [228-H_2O]$^+$, 209 [$C_7H_{13}O_7$], 199 [259-$C_2H_4O_2$]$^+$, 197 [229-CH_3OH]$^+$, 191 [$C_8H_{15}O_5$]$^+$, 180 [S_2; S_3]$^+$, 163 [209-HOCHO; 180-OH]$^+$, 162 [S_1]$^+$, 159 [191-CH_3OH]$^+$, 145 [S_1-OH; 191-HOCHO; 163-H_2O]$^+$, 131 [191-$C_2H_4O_2$]$^+$, 127 [145-H_2O]$^+$, 115 [159-CH_3CHO]$^+$, 113 [145-CH_3OH]$^+$, 103 [$C_4H_7O_3$]$^+$, 95 [127-CH_3OH; 113-H_2O]$^+$, 87 [$C_4H_7O_2$].

Mannich Hydrolysis of 2. To a solution of **2** (8 mg) in acetone (1 mL) concd. HCl (0.01 mL) was added. After 5 days, two new spots were shown which were found identical with **D**-cymarose and cellobiose (TLC, PC). After 7 days, the hydrolysis was complete showing two spots on TLC which were found identical with **D**-cymarose and **D**-glucose. The usual work up afforded two chromatographically pure sugars identified as **D**-glucose (**5**) (3.8 mg) $[\alpha]_D$+52 ^{o} (*c*, 0.13, H_2O), and **D**-cymarose (**3**) (2.1 mg) $[\alpha]_D$+49.4 ^{o} (*c*, 0.11, H_2O) by comparison with authentic samples.

D-Cymaronic Acid Phenylhydrazide. A solution of **3** (2 mg) in H_2O (0.4 mL) was oxidized with Br_2 (6 µL) using the

usual method[9] yielding a syrupy lactone which on treatment with phenylhydrazine yielded the known crystalline **D**-cymaronic acid phenylhydrazide (0.6 mg) mp 151-153 °C (mmp).

D-Gluconic Acid Phenylhydrazide. A solution of **5** (3.5 mg) in H_2O (0.5 mL) was oxidized with Br_2 (7 µL) using the usual method[11] yielding syrupy lactone. This lactone on reaction with phenylhydrazine yielded known **D**-gluconic acid phenylhydrazide (1.5 mg) mp 196-198 °C (mmp).

ACKNOWLEDGEMENT

Authors are thankful to U. G. C. and D. S. T. for financial assistance and R. S. I. C., C. D. R. I., Lucknow for successful running of NMR and Mass spectra.

REFERENCES

1. K. N. Tiwari, A. Khare, and M. P. Khare, *J. Carbohydr. Chem.*, **3**, 315 (1984).

2. K. N. Tiwari, N. K. Khare, A. Khare, and M. P. Khare, *Carbohydr. Res.* **129**, 179 (1984).

3. C. Jijun, Z. Zhuaugxin, and Z. Jun, *Acta Bot. Yunn.*, **12**, 197 (1990).

4. H. Mitsuhashi, and K. Hayashi, *Shoyaku. Zasshi*, **39**, 1 (1985).

5. R. Srivastava, and D. K. Kulshreshtha, *Phytochemistry*, **28**, 2877 (1989).

6. K. Prakash, A. Sethi, Desh Deepak, A. Khare, and M. P. Khare, *Phytochemistry*, **30**, 297 (1991).

7. S. Rangaswami, and T. Reichstein, *Helv. Chim. Acta*, **32**, 939 (1949).

8. C. Mannich, and G. Siewert. *Ber. Dtsch. Chem. Ges.*, **75**, 737 (1942).

9. N. K. Khare, M. P. Khare, and A. Khare, *Phytochemistry*, **23**, 2931 (1984).

10. H. Allgeier, *Helv. Chim. Acta*, **51**, 311 (1968).

11. *Carbohydrates*, P. M. Collins, Ed.; Chapman and Hall Chemistry Source Books: London, 1987, p 244.