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Note

Isolation, stereochemical assignments and molecular mechanics calculation of ethyl β-L-arabinopyranoside

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Abstract

Ethyl β-L-arabinopyranoside (**1**) was isolated from the roots of *Hibiscus rosa-sinensis* L. as a natural monoglycoside. Its structure elucidation and unequivocal NMR signals assignments were achieved by a combination of DQF-COSY, HOHAHA, HMQC, and $^1J_{(C,H)}$, HMBC experiments with the aid of molecular mechanics calculations. © 1998 Elsevier Science Ltd. All rights reserved

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Many biologically active natural products are glycosides, including steroid and triterpenoid saponins [1,2]. The most difficult issue in the structure elucidation of these glycosides concerns the composition, interlinkage sequence and anomeric configurations of the glycone portion. Recent advances in NMR spectroscopy in the field of carbohydrate chemistry, however, have greatly facilitated the structure elucidation of these complex molecules. In the present paper, we describe the isolation, characterization and complete NMR assignment of natural ethyl β-L-arabinopyranoside (**1**) from *Hibiscus rosa-sinensis* L. (Malvaceae).

Compound **1** was isolated as colorless plates and assigned a molecular formula of $C_7H_{14}O_5$ (MW = 178) on the basis of a protonated molecular ion at *m/z* 179 observed in a positive-ion DCI mass spectrum, and was further supported by its ^{13}C (DEPT) NMR spectrum. The general spectral features of **1**, coupled with the hydroxyl group and glycosidic absorptions at 3500 and 1050 cm^{-1} in its IR spectrum, showed it to be an ethyl monosaccharide of a pentose. Compound **1** afforded a very well-resolved ^1H NMR spectrum which led to a de novo structure generation starting from the signal at the lowest field resonating at δ 5.27,

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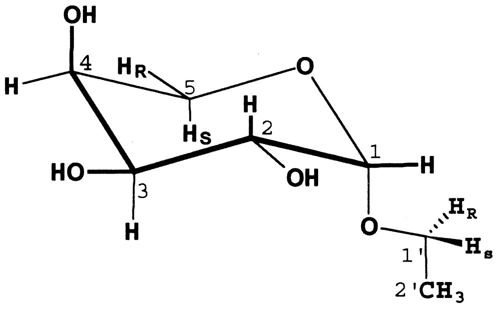
which is attributed to the anomeric proton (H-1). Following the connectivities from DQF-COSY and HOHAHA spectra, the two doublets of doublets at δ 4.60 ($J=3.4$, 9.3 Hz) and δ 4.47 ($J=3.4$, 9.3 Hz) were assigned to H-2 and H-3, respectively. These protons are axial based on their large coupling constants. On the other hand, the small coupling constant ($J=3.4$ Hz) observed for the anomeric proton indicated it to be equatorially oriented. The four-line signal at δ 4.37 (m) was assigned to H-4, since it coupled with both H-3 and the signals of the nonequivalent methylene proton pairs (H_2-5) at δ 4.08 (dd, $J=2.0$, 11.9) and δ 4.02 (dd, $J=2.6$, 11.9) from the HMQC spectrum. The presence of an ethyl group attached to C-1 was supported by the HMBC spectrum, in which ^{13}C–^{1}H long-range cross peaks were observed between C-1/H-1′ and C-1′/H-1.



Ethyl β-L-arabinopyranoside (1)

In recent years, the direct-bonded carbon–proton coupling constants $\{^{1}J_{(C,H)}\}$ of the anomeric carbon signals have been widely used in the structure elucidation of mono- and oligosaccharides, since these values are markedly anomeric configuration dependent [3]. A general rule has been established that a coupling constant of an axially oriented C–H bond is observed to be at 155–165 Hz, whereas that of an equatorially oriented C–H bond is ca. 10 Hz higher at 165–175 Hz [4–6]. The anomeric $^{1}J_{(C,H)}$ value of 169.5 Hz of compound **1** strongly favors its having a β-configuration.

Based on the foregoing NMR spectral data, compound **1** could be either one of two enantiotropic isomers: ethyl β-L-arabinopyranoside in the $^{4}C_1$ conformation, or its mirror image, ethyl β-D-arabinopyranoside in the $^{1}C_4$ conformation. In this case, the structure of **1** could be conclusively assigned to ethyl β-L-arabinopyranoside (**1**) on the basis of its significant dextrorotary rotation, $[\alpha]_D$ $+298.8$, analogous to that of methyl β-L-arabinopyranoside with the same sign of rotation at $[\alpha]_D$ $+239.4$ [7].

Having established the structure of **1**, there remains the task of assigning the NMR resonances of the two sets of diastereotopic methylene protons H_2-5 and H_2-1′. It was reported that the $^{1}J_{(C,H)}$ coupling of the equatorial H-5 proton (149–150 Hz) in pentopyranosyls are larger than that for the corresponding axial H-5 protons (140–143 Hz) [8]. Thus, the doublet of doublets signal at δ 4.08 with a $^{1}J_{(C,H)}$ value of 140 Hz, was assigned to the *pro*-S H-5, while the axial counterpart, resonating at δ 4.02, was assigned to the *pro*-R H-5.

The stereochemical assignment of the two diastereotopic methylene proton pairs at C-1′, H-1′ *pro-R* and H-1′ *pro-S* (referred to as $H\text{-}1'_R$ and $H\text{-}1'_S$, respectively) were achieved using ROESY experiments with the aid of molecular mechanics calculations. It is notable that only one proton signal of the methylene group at C-1′ showed an ROE correlation peak with H-1, suggesting that the free rotation around the glycosidic bond is hampered. Consequently, the favorable conformation could be "frozen out", in which one of the prochiral methylene proton H_2-1′ is more proximate to H-1 in the NMR time scale. This is consistent with the results from the molecular mechanics calculations used for the conformational analysis of **1**. The calculations were carried out using the MacroModel/Batchmin program (version 4.5) [9] and the Amber force field in the software package [10]. Initially, a random structure of **1** was constructed and then energy minimized. Next, 5000 steps of a Monte Carlo conformational search were conducted to find low-energy conformations. The search converged rapidly, and the lowest energy conformer was obtained. The range of various vicinal coupling constants predicted from the lowest energy conformation (Fig. 1), using the generalized Karplus equation [4], was in good agreement with those obtained from the NMR experiments.

In order to assign $H\text{-}1'_S$ and $H\text{-}1'_R$, 1000 ps of molecular dynamics simulations were carried out with a solvation model (GB/SA water) [4]. According to the final averaged distances of H-1 and $H\text{-}1'_S$, and H-1 and $H\text{-}1'_R$ in the 1000 ps time period, given by the simulation as 2.71 and 3.07 Å, respectively, it seemed reasonable to assign the multiplicity resonating at δ 3.52 to $H\text{-}1'_S$, and that at δ 3.84. to $H\text{-}1'_R$. Thus, all of the NMR signals of compound **1** have been unequivocally assigned.

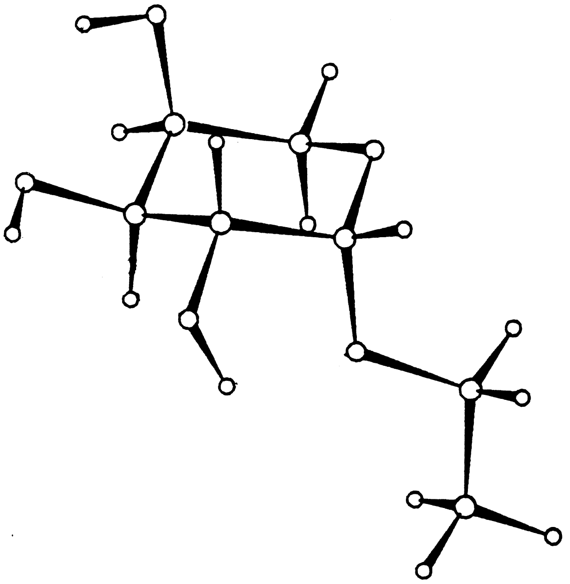


Fig. 1. Ball-and-stick representation of ethyl *β*-L-arabinopyranoside (**1**) for the computer-generated lowest energy conformation.

As summarized in Table 1, compound **1** exhibited diagnostic HOHAHA, ROESY and HMBC correlations profiles. As exemplified by the HOHAHA spectrum, and in contrast to D-glucopyranose [12] in which the anomeric proton showed crosspeaks up to H_2-6, the anomeric proton H-1 of L-arabinose showed crosspeaks only up to H-4 rather than H_2-5 because of the small coupling constants between H-4 and H_2-5. The NMR profiles obtained in this work could be of use in the characterization of glycosides containing L-arabinose.

The synthesis of compound **1** was reported by Birch and Lindley [13], but neither experimental details nor physical data were published. To our knowledge, this is the first report on the isolation and structure elucidation of **1** as a naturally occurring compound.

1. Experimental

General experimental procedures.—The melting point was determined with a Kofler hot-stage apparatus and is uncorrected. The optical rotation was measured with a Perkin–Elmer model 241 polarimeter. The IR spectrum was obtained with a Midac Collegian FT–IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were measured with Me_4Si as the internal standard, using a Bruker Advance DPX-300 instrument at 300 MHz and 75.6 MHz, respectively. DQF-COSY, HOHAHA (mixing time 50 ms), ROESY (mixing time 200 ms), HMQC, HMBC experiments were also performed on this instrument using standard pulse sequences. The one-bond coupling constants between the carbon atoms and protons ($^1J_{(C,H)}$) have been measured with a 500 MHz Varian Unity-plus spectrometer at the National High Magnetic Field Laboratory, FL, USA, using two-dimensional inverse heteronuclear shift correlation through multiple quantum coherence (HMQC) techniques.

Extraction and isolation. The dried roots (500 g) of *Hibiscus rosa-sinensis* L. (Malvaceae) (People's Republic of China) were extracted with methanol (3×2 L). The combined MeOH extract was concentrated under reduced pressure to a syrup, which was subjected to silica gel column chromatography, eluting with $CHCl_3$ and an increasing gradient of MeOH (0–5%). From the $CHCl_3$ fraction was isolated the known compound, *β*-sitosterol (12 mg), which was identified by direct TLC and mmp comparison with an authentic sample from our laboratory. Compound **1** (25 mg) was obtained as colorless plates from the (97:3) $CHCl_3$–MeOH fraction.

Ethyl β-L-*arabinopyranoside* (**1**). mp 139–140 °C, $[\alpha]_D^{25}$ +298.8 (*c* 0.5, MeOH), IRν_{max} (cm^{-1}): 3600–3100 (OH), 1050, 980, 902; DCIMS *m/z* 179 $[M+H]^+$, 133 (bp) $[M+H-EtOH]^+$; ^{1}H and ^{13}C NMR data are shown in Table 1.

Table 1
^{1}H, ^{13}C, $^1J_{(C,H)}$ data and HOHAHA, ROESY and HMBC correlations of ethyl *β*-L-arabinopyranoside (**1**)

	^{13}C	^{1}H	$^1J_{(C,H)}$ (Hz)	HOHAHA (H)	ROESY (H)	HMBC (C)
1	100.86	5.27 (d, 3.4)	169.5	2, 3, 4	2, $1'_S$	1′, 3
2	70.63	4.60 (dd, 3.4; 9.3)	144.3	1, 3, 4, 5	1	3*
3	71.01	4.47 (dd, 3.4; 9.3)	139.6	1, 2, 4, 5	/	2
4	70.19	4.37 (m)	143.1	1, 2, 5	/	/
5	64.12	*R*: 4.02 (dd, 2.6; 11.9)	150.4	4, 5_S	5_S	1, 4
		S: 4.08 (dd, 2.0; 11.9)	140.4	4, 5_R	5_R	1, 4
1′	63.63	*R*: 3.84 (dq, 7.1; 9.7)	144.7	2	$1'_S$, 2′	1*
		S: 3.52 (dq, 7.1; 9.7)	144.7	2	$1'_R$, 2′, 1	1*
2′	15.35	1.15 (t, 7.1)	125.2	$1'_R$, $1'_S$	$1'_R$, $1'_S$	1′

Note: * indicates low-intensity cross peaks; coupling constants are in parentheses.

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