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# L-Dianose, a new monosaccharide from *Dianthus chinensis* L.

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Plants of the dianthus group (Caryophyllaceae), commonly known as *ju mai*, are used in Chinese traditional medicine[1]. In 1984, pharmacological studies conducted by Hikino et al.[2] confirmed their analgesic activity. Later, the aerial parts of *Dianthus superbus* L. var. longicalycinus Williams were found by Oshima et al.[3–5] to contain nine new saponins, which have analgesic and antihepatotoxic activities[2]. Also, Bukharov et al.[6,7] have characterized several saponins from *D. deltoides* L. and our previous investigations[8,9] of *Dianthus chinensis* resulted in the isolation of dianchinenosides A, B, C, and D. As part of our continuing investigation of this plant, we isolated a new monosaccharide, provisionally named L-dianose.

A 95% ethanol extract of the dried aerial parts (6 kg) of *Dianthus chinensis* was partitioned between chloroform and water. The aqueous layer was successively extracted with ethyl acetate and *n*-butanol. The *n*-butanol-soluble fraction was subjected to Dianion HP-20 column chromatography and droplet counter-current chromatography, followed by medium pressure column chromatography, to afford $\alpha,\beta$-dianose. The requirement for several chromatographic steps made large-scale separation difficult. However, by extensive reliance on NMR analysis we were able to elucidate the structure and configuration of L-dianose.

Dianose (**1**) was obtained from aqueous methanol as colorless needless, mp 76-78°C (dec).

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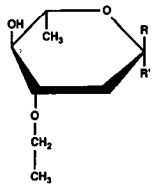


Dianose (1)
α anomer: R=OH, R'=H
β anomer: R=H, R'=OH

Both the $^{1}H$ and $^{13}C$ NMR spectra exhibited pairs of signals having an intensity ratio of 6:4, indicating the existence of two inseparable conformers or isomers. NMR resonances were identified for two anomeric centers, six oxymethines, two secondary methyls, two methylenes, and two ethoxy groups. In addition, the signal in the EIMS spectrum at $m/z$ 159 $(M - 17)^{+}$ corresponded to the loss of OH from the molecular ion. Those spectral features were suggestive of a 2,6-dideoxy monosaccharide having the molecular formula $C_8H_{16}O_4$.

In order to assign the NMR signals due to the respective components, $^{1}H$–$^{1}H$ COSY and $^{1}H$–$^{13}C$ COSY were determined. In the $^{1}H$–$^{1}H$ COSY spectrum two

TABLE I

$^{1}H$ NMR data for dianose in $CDCl_3$

| Position | δ | Multiplicity | J (Hz) | No of H | Correlation |
|---|---|---|---|---|---|
| α Anomer | | | | | |
| 1 | 5.33 | d | $J_{1,2ax}$ 2.8 | 1 | H-$2_{ax}$, $2_{eq}$ |
| $2ax$ | 1.54 | ddd | $J_{2ax,2eq}$ 12.9, $J_{2ax,3}$ 11.1, $J_{2ax,1}$ 3.6 | 1 | H-1, H-$2_{eq}$, H-3 |
| $2eq$ | 2.25 | ddd | $J_{2ax,2eq}$ 13.0, $J_{2eq,3}$ 4.6, $J_{2eq,1}$ 1.1 | 1 | H-1, H-$2_{ax}$, H-3 |
| 3 | 3.68 | ddd | $J_{2ax,3}$ 11.2, $J_{2eq,3}$ 4.6, $J_{3,4}$ 9.1 | 1 | H-$2_{ax}$, $2_{eq}$, H-4 |
| 4 | 3.15 | dd | $J_{3,4}$ 9.1, $J_{4,5}$ 9.3 | 1 | H-5, H-3 |
| 5 | 3.93 | dq | $J_{4,5}$ 9.3, $J_{5,6}$ 6.1 | 1 | H-4, H-6 |
| 6 | 1.28 | d | $J_{5,6}$ 6.1 | 3 | H-5 |
| 1′ | 3.48 | q | $J_{1'2'}$ 6.9 | 2 | H-2′ |
| 2′ | 1.22 | t | $J_{1',2'}$ 6.9 | 3 | H-1′ |
| β Anomer | | | | | |
| 1 | 4.80 | dd | $J_{1,2ax}$ 7.8, $J_{1,2eq}$ 2.0 | 1 | H-$2_{ax}$, $2_{eq}$ |
| $2ax$ | 1.44 | ddd | $J_{2ax,2eq}$ 12.6, $J_{2ax,3}$ 9.5, $J_{2ax,1}$ 7.7 | 1 | H-1, H-$2_{eq}$, H-3 |
| $2eq$ | 2.36 | ddd | $J_{2ax,2eq}$ 12.6, $J_{2eq,3}$ 4.8, $J_{2eq,1}$ 2.0 | 1 | H-1, H-$2_{ax}$, H-3 |
| 3 | 3.30 | ddd | $J_{2ax,3}$ 9.7, $J_{2eq,3}$ 4.8, $J_{3,4}$ 9.2 | 1 | H-$2_{ax}$, $2_{eq}$, H-4 |
| 4 | 3.13 | dd | $J_{3,4}$ 9.2, $J_{4,5}$ 9.3 | 1 | H-5, H-3 |
| 5 | 3.37 | dq | $J_{4,5}$ 9.3, $J_{5,6}$ 6.1 | 1 | H-4, H-6 |
| 6 | 1.33 | d | $J_{5,6}$ 6.1 | 3 | H-5 |
| 1′ | 3.47 | q | $J_{1'2'}$ 7.0 | 2 | H-2′ |
| 2′ | 1.24 | t | $J_{1',2'}$ 7.0 | 3 | H-1′ |

TABLE II

$^{13}C$ NMR data for L-dianose and L-oleandrose

| Position | Chemical shifts ($\delta$) | | | |
|---|---|---|---|---|
| | L-Dianose [a] | | L-Oleandrose [b] | |
| | $\alpha$ | $\beta$ | $\alpha$ | $\beta$ |
| 1 | 92.00 | 93.94 | 93.78 | 95.87 |
| 2 | 34.83 | 37.30 | 36.82 | 39.01 |
| 3 | 76.68 | 78.97 | 79.88 | 82.07 |
| 4 | 75.13 | 76.04 | 77.38 | 77.97 |
| 5 | 67.69 | 71.82 | 70.56 | 74.44 |
| 6 | 17.86 | 17.82 | 19.57 | 19.57 |
| 1′ | 64.26 | 64.17 | 58.85 | 58.85 |
| 2′ | 15.44 | 15.36 | | |

[a] At 100 MHz in $CDCl_3$. [b] At 25 MHz in $D_2O$, from G. Berti et al.[11].

sets of crosspeaks could be distinguished, one attributable to **1**$\alpha$ and one to **1**$\beta$ and leading to the assignments given in Table I. Following this the $^1H-^{13}C$ COSY could be used to assign sets of carbon signals to **1**$\alpha$ and **1**$\beta$, respectively, as recorded in Table II. The ethoxy groups were considered to be attached to the carbon atoms having the lowest chemical shifts among the oxymethine positions, namely C-3 in both anomers (Table II), and this assignment completed the elucidation of the structure of dianose.

The configurations at oxygen-substituted C-3, C-4, and C-5 of **1**$\alpha$ and **1**$\beta$ were established via $^1H-^1H$ coupling constants. In **1**$\alpha$, the $J$ values between H-2 and H-3 are 11.2 and 4.6 Hz, indicating that OH-3 is equatorial; the coupling value of 9.1 Hz between H-3 and H-4 suggests that OH-4 is also equatorial. Finally, the coupling constant of 9.3 Hz between H-4 and H-5 shows the terminal methyl group to be equatorial. The $J_{1,2}$ values of 3.6 and 1.1 Hz for the major component and 7.7 and 2.0 Hz for the minor component characterize these as the $\alpha$ and $\beta$ anomers, respectively.

According to this characterization, dianose is the ethyl analogue of the *O*-methyl sugar L-oleandrose, which occurs in cardiac glycosides and also was synthesized[10]. The $^{13}C$ NMR data (Table II) for L-oleandrose support this conclusion. In view of the L configuration of natural oleandrose, we tentatively assign dianose to the L series of sugars. To our knowledge, this is first example of an *O*-ethyl monosaccharide in a natural product.

## EXPERIMENTAL

*Plant material.*—Aerial parts of *Dianthus chinensis* L. were collected by one of authors (H. Li) from Nansan mountain, Dongliao, P.R. China in September 1989. The material was identified by Professor C.K. Xie, Department of Pharmacognosy,

School of Pharmacy, West China University of Medical Sciences, Chengdu, China. A reference specimen is deposited there.

*General experimental procedure.*—Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Electron ionization mass spectrometry was conducted on a Jeol JMS D-300 mass spectrometer at 70 eV energy. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a Jeol GX-400 ($^{1}H$ at 400 MHz, $^{13}C$ at 100 MHz) or a Bruker AC 300 ($^{1}H$ at 300 MHz) FT-NMR spectrometer. Standard Jeol pulse sequences were used for the $^{1}H$–$^{1}H$ COSY and $^{1}H$–$^{13}C$ COSY experiments. Chemical shifts are expressed in $\delta$ (ppm) downfield from $Me_4Si$ as an internal standard, and coupling constants are reported in hertz (Hz). Thin-layer chromatography was carried out on Silica Gel 60 $F_{254}$, and spots were detected by spraying the plates with 10% $H_2SO_4$ followed by heating. Dianion HP-20 (Mitsubishi Kasei) was used for column chromatography. Droplet countercurrent chromatographic separation was achieved on a Tokyo Rikakikai apparatus. Medium pressure liquid chromatography was carried out on a silica gel (BW-820MH, Fuji Division) column (CQ-3, 10 mm i.d. × 250 mm).

*Isolation of dianose.*—Dried aerial parts (6 kg) of *Dianthus chinensis* were extracted with 95% EtOH (2.5 L) four times under reflux for 1 h. The combined EtOH extract was concentrated under reduced pressure, water was added to the concentrate, and the aqueous suspension was extracted successively with $CHCl_3$, EtOAc, and *n*-BuOH (500 mL, three times). The *n*-BuOH layer was evaporated in vacuo to give a residue (59 g), which was applied to a column of Dianion HP-20 (4.5 kg) and eluted with 30, 50, 70, and 100% MeOH to give 118 fractions (400 mL each). Fraction 4 gave a residue (1.35 g) that was subjected to droplet countercurrent chromatography using 78:32:10 MeOH–$CHCl_3$–$H_2O$. Fractions 12 and 13 furnished impure dianose (72.4 mg), which was further purified by medium pressure liquid chromatography with 1:4 MeOH–$CHCl_3$ to afford dianose (52.7 mg), colorless needless ($H_2O$ and MeOH); mp 76–78°C; EIMS: *m/z* (%) 159 (6.2), 132 (4.9), 119 (5.0), 101 (34.6), 88 (100), 73 (27.8), 60 (73.7), and 45 (39.1); $^{1}H$ NMR and $^{13}C$ NMR see Tables I and II.

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