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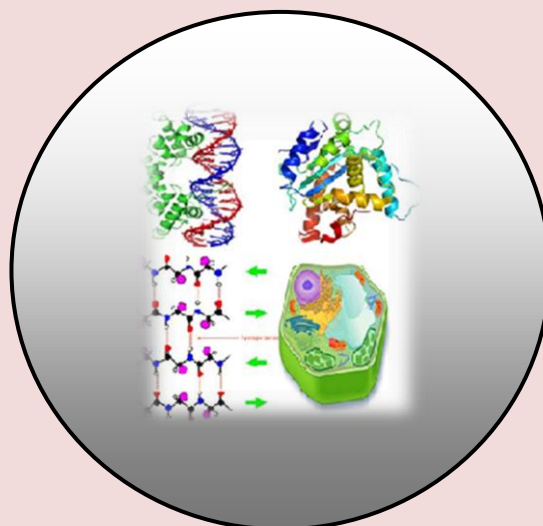
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Isolation of a Novel Pregnane Ester Glycosides from *Dregea lanceolata*

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ABSTRACT

A novel pregnane oligoglycoside namely Lancoside A was isolated from chloroform soluble extract of Dregea lanceolata (family: Asclepiadaceae). Their structures were elucidated by chemical transformation/degradation, ¹H, ¹³C and 2D NMR experiments as well as Mass spectrometry as 12-O-acetyl lineolon 3-O-β-D-cymaropyranosyl (1→4)-O-β-D cymaropyranosyl (1→4)-O-β-D-cymaropyranosyl (1→4)-O-β-D-mannopyranosyl (1→4) α-D-galactopyranoside.

Key words: Pregnane, Lancoside A, *Dregea lanceolata*, NMR and Mass spectrometry.

INTRODUCTION

Dregea lanceolata is a suberect, climbing shrub with lanceolate leaves belonging to family Asclepiadaceae. It is commonly grown in Maharastra. The genus *Dregea* is well known for their biological properties and chemical composition. There is a number of species of *Dregea* but most commonly available species include *Dregea sinensis*, *Dregea volubilis* and *Dregea lanceolata*. The *D. volubilis* traditionally used in Ayurveda to treat various diseases such as inflammation, piles, leucoderma, asthma, tumors, urinary discharge [Hossain et al., 2011]. All parts of this plant used traditionally and the methanolic extract of the leaves possess anti-inflammatory activity [Hossain et al., 2010].

Plants of family Asclepiadaceae are rich source of steroids (pregnane and cardenolides), terpenes and their glycosides. Numbers of pregnanes [Deepak et al., 1989] [Khare et al., 1986], their esters and glycosides [Deepak et al., 1997], have been isolated from various species of Asclepiadaceae family. The pregnane glycosides are comprise of mostly 2, 6-dideoxy sugars and normal sugars. Besides the traditional use of these plant as medicine, in present scenario these pregnane glycosides have shown antitumor, anticancer

[Hussain et al., 2015], anti-inflammatory, antimicrobial [Babu et al., 2008], anti-complementary [Piacente et al., 1998] antioxidant and antidyslipidemic activities [Sethi et al., 2008]. In search for more medicinally important pregnane glycosides, we have re-investigated the plant *Dregea lanceolata* and isolated a novel pregnane oligoglycoside namely Lancoside A.

EXPERIMENTAL

General experimental procedures

Melting points were recorded on Buchi melting point B-540 apparatus and are uncorrected. Optical rotations were measured with an automatic polarimeter AA-5 series of optical activity. The ^1H NMR, ^{13}C NMR spectra and 2D spectra were recorded with a AVANCE 400 MHz Bruker spectrometer in CDCl_3 using TMS as internal standard. The ES-MS spectra were recorded on AEI-MS-30 mass spectrometer. TLC was performed on silica gel-G (BDH), compound were detected by spraying with 50% aq. H_2SO_4 solution followed by heating. Column chromatography was performed over silica gel 60-120 mesh (BDH).

Plant extraction

Shade dried plant (10 kg) of *D. lanceolata* were extracted by the process employed for pregnane glycosides [Neupane et al., 2017] using 50-95% ethanol. Chloroform extract (2.2g) was fractionated by repeated column chromatography over Si gel using solvent mixture of C_6H_6 -EtOAc and CHCl_3 -MeOH as eluents afforded Lancoside A (40mg).

Mild acid hydrolysis of Lancoside A

To a solution of J (15 mg) in 80% aq. 1,4-dioxane (1ml) was added 0.05 N H_2SO_4 (1ml) and the solution was left at room temperature. After five days two new spots (3 and 4) were appeared out of which one polar spot 3 could not be identified and the compound with faster mobility was identified as aglycon 4, however the polar spot could be expected as pentasaccharide 3. After seven days two new spots 5 and 6 were appeared out of which 6 was having the comparable mobility with the authentic sample of cymarose supporting that the cymarose [2, 6-dideoxy-3-O-methyl-D-ribohexose] was next sugar from the reducing end, while the other new spot may be of the tetrasaccharide 5. After ten days one more spot 7 was appeared which was having the faster mobility than the compound 5 supporting the fact that 7 may be a trisaccharide and the sugar was broken from the reducing end of 4 which may be cymarose 6. After twelve days one more spot was developed on the TLC plate which was faster in mobility with the trisaccharide 7 which was developed by partridge reagent may be the disaccharide 8. The disaccharide was converted to its methyl glycoside which on acid hydrolysis gave D-galactose and methyl mannoside (9 and 10) (TLC, PC, mp, mmp, $[\alpha]_D$). The reaction was completed in 14 days leaving 4 four spots on TLC.

Methanolysis of lancoside A by Zemplen method [Zemplen and Kiss, 1927].

To a solution of 1 (2 mg) in absolute MeOH (0.5 ml) was added sodium methoxide (0.05 ml) and the mixture was kept at room temp. After 30 min. it showed one spot of lower mobility i.e.; 2 (TLC) CHCl_3 -MeOH, 94:6). When the reaction was complete (TLC), it was neutralized with IR 120 H resin and filtered. MeOH was removed under reduced pressure yielding the product 11 (1.2 mg).

Acetylation of lancoside A

Compound 1 (15gm) was acetylated with pyridine (2ml) and acetic anhydride (2ml) at 60°C and the mixture was kept overnight, yielding acetate 2 (14.8 gm), ^1H NMR- δ 2.08-2.17 (8xOAc), 3.19-3.21 m (H-4 of S_1 , S_2 , S_3), 3.44 m (H-4 of S_4) 3.52 m (H-3 of aglycon).

Lancoside A (1)

Colorless crystals, mp 116-120°C, $[\alpha]_D^{25} +30.20^\circ$; ^1H NMR(400MHz, CDCl_3): δ 5.46 (1H, m, 6-H), 5.34 (1H, t, H-12) 4.83 (1H, dd, $J = 8.8$, 2Hz, H-1 in S_1), 4.79 (1H, d, $J = 2.8$ Hz, H-1 in S_5), 4.75 (1H, dd, $J = 8$, 2Hz, H-1 in S_2), 4.59 (1H, d, $J = 7.6$ Hz, H-1 in S_4), 4.48 (1H, dd, $J = 8$, 2Hz, H-1 in S_3), 3.66 (3H, s, $\text{S}_1\text{-OCH}_3$), 3.43 (3H, s, $\text{S}_2\text{-OCH}_3$), 3.38 (3H, s, $\text{S}_3\text{-OCH}_3$), 2.17 (3H, s, 21- CH_3), 1.96 (3H, s, 12- OCH_3), 1.36-1.26 (3H, d, sec cym- CH_3), 1.12 (3H, s, 18- CH_3), 1.10 (3H, s, 19- CH_3). ^{13}C NMR (400MHz, CDCl_3): δ 36.1 (C-I), 29.7 (C-2), 78.6 (C-3), 39.2 (C-4), 139.6 (C-5), 122.1 (C-6), 27.7 (C-7), 73.0 (C-8), 47.5 (C-9), 43.2 (C-10), 22.5 (C-11), 68.4 (C-12), 54 (C-13), 83.9 (C-14), 33.0 (C-15), 25.4 (C-16), 55.7 (C-17), 10.6 (C-18), 17.8 (C-19), 16.4 (C-20), 22.5 (C-21). 95.88 (C-1 S_1), 35.40 (C-2 S_1), 77.30 (C-3 S_1), 82.60 (C-4 S_1), 68.50 (C-5 S_1), 18.6 (C-6 S_1), 57.2 (OCH3 S_1), 99.62 (C-1 S_2), 35.50 (C-2 S_2), 77.30 (C-3 S_2), 82.5 (C-4 S_2), 68.40 (C-5 S_2), 18.4 (C-6 S_2), 57.8 (OCH3 S_2), 100.04 (C-1 S_3), 35.50 (C-2 S_3), 78.10 (C-3 S_3), 82.40 (C-4 S_3), 68.20 (C-5 S_3), 18.4 (C-6 S_3), 58.2 (OCH3 S_3), 101.34 (C-1 S_4), 71.40 (C-2 S_4), 73.09 (C-3 S_4), 68.20 (C-4 S_4), 78.50 (C-5 S_4), 71.9 (C-6 S_4), 99.14 (C-1 S_5), 68.40 (C-2 S_5), 70.70 (C-3 S_5), 70.70 (C-4 S_5), 71.30 (C-5 S_5), 68.08 (C-6 S_5).

RESULTS AND DISCUSSION

Chloroform soluble extract of *Dregea lanceolata* was fractionated on a silica gel afforded compound 1.

LANCOSIDE A (1)

Lancoside A (1), mp 116-120°C, $[\alpha]_D^{25} 30.20^\circ$, $\text{C}_{56}\text{H}_{90}\text{O}_{25}$, m/z 1162 $[\text{M}]^+$ gave positive Liebermann Burchardt test [Abisch and Reichstein, 1960], Xanthidrol test [Tschesche et al., 1953], Keller Killiani test [Nagata et al., 1957], Partridge test [Partridge and Westall, 1948] and tetra-nitro methane test [Ostromisslensky, 1910] with olifinic bond indicating it to be steroidal glycoside of 2,6-dideoxy hexose(s) and normal sugar. It underwent reduction with NaBH_4 reagent showing the presence of carbonyl group and its nature as Ketomethyl chain was confirmed by its characteristic colour reaction with sodium nitroprusside. It also gave alkaline hydrolysis indicated the presence of ester function in the molecule.

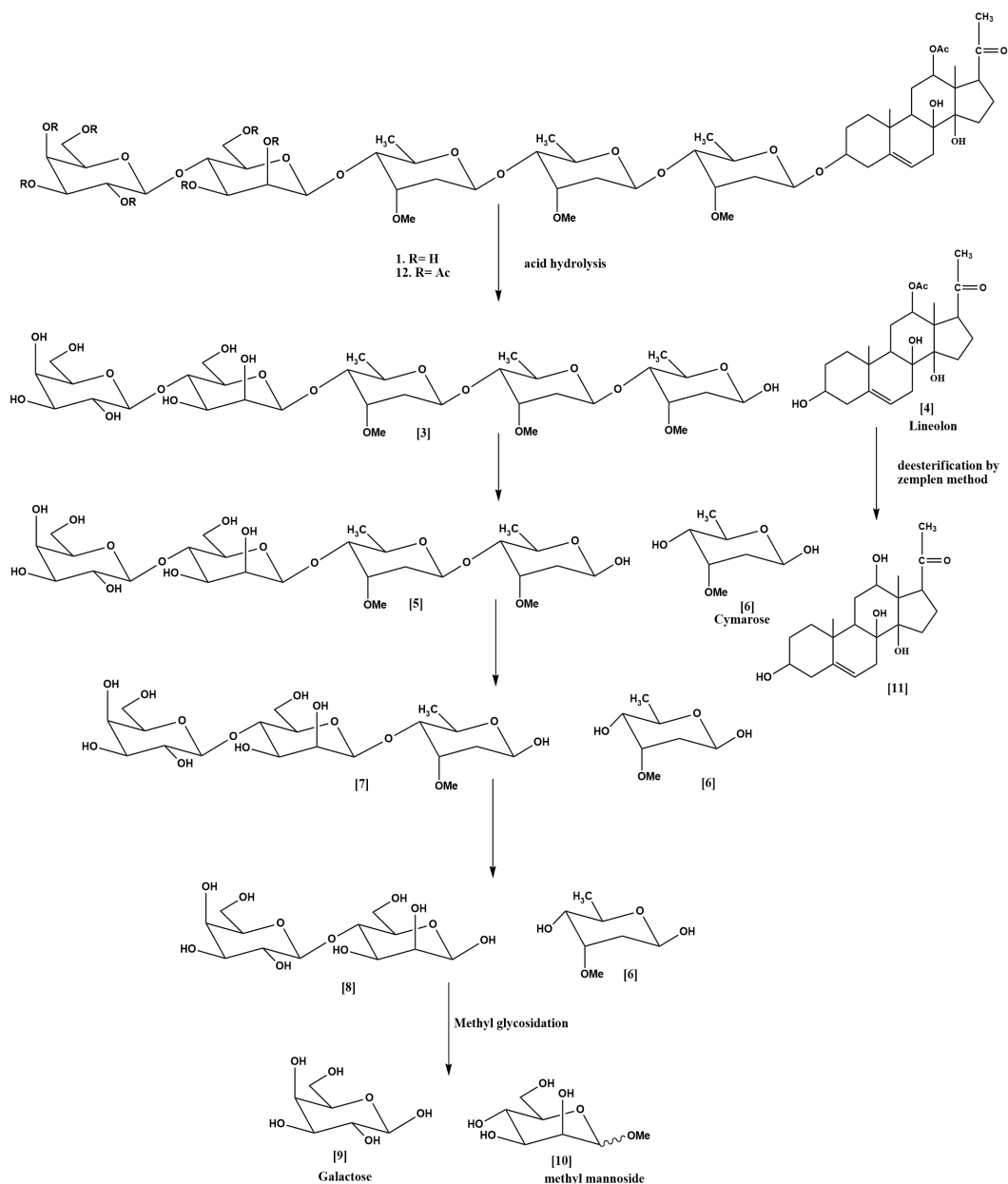
The ^1H NMR spectrum of 1 showed five anomeric proton signals at δ 4.83, 4.79, 4.75, 4.48 and 4.59 of one proton each suggesting that it to be a pentaglycoside. The pentaglycoside nature of 1 was further ascertained by the presence of five anomeric carbon signal at δ 101.34, 100.04, 99.62, 99.14 and 95.88 in ^{13}C NMR spectrum. HSQC spectrum of 1 showed five crossed peak in anomeric region at δ 101.34 x 4.59, 100.04 x 4.48, 99.62 x 4.75, 99.14 x 4.79 and 95.88 x 4.83 also concluded that compound 1 was a pentaglycoside.

The identification of sugars, genin and their sequence in 1 was identified by very mild acid hydrolysis by 0.005 N H_2SO_4 [Rangaswami and Reichstein, 1949]. For convenience the sugars present were designated as S_1 , S_2 , S_3 , S_4 and S_5 . After five days two spots, 3 and 4 were appeared, out of which one polar spot 3 could not be identified and the compound with faster mobility was identified as aglycon 4, however the polar spot could be expected as pentasaccharide 3 whereas component with faster mobility was identified as genin 4 mp 196–198°C, $[\alpha]_D 49.50$. After seven days two new spots 5 and 6 were appeared out of which 6 was having the comparable mobility with the authentic sample of cymarose [Krasso et al., 1963] supporting that the cymarose was next sugar from the reducing end, while the other new spot may be of the tetrasaccharide 5.

After ten days one more spot 7 was appeared which was having the faster mobility than the compound 5 supporting the fact that 7 may be a trisaccharide and the sugar was broken from the reducing end of 4 which may be cymarose 6. After twelve days one more spot was developed on the TLC plate which was faster in mobility with the trisaccharide 7 which was developed by partridge reagent may be the disaccharide 8. The disaccharide was converted to its methyl glycoside which on acid hydrolysis gave D- galactose (Urashima et al. 2004) and methyl mannoside (9 and 10) (TLC, PC, mp, mmp, $[\alpha]_D$) (scheme-1).

Table 1. ^{13}C NMR data of Lancoside.

Carbon	Chemical shifts of Lancoside (δ)	Carbon	Chemical shifts of Lancoside (δ)
1	36.1	D-Cym (S-2)	
2	29.7	1	99.62
3	78.6	2	35.50
4	39.2	3	77.30
5	139.6	4	82.5
6	122.1	5	68.40
7	27.7	6	18.4
8	73.0	OMe	57.8
9	47.5	D-Cym (S-3)	
10	43.2	1	100.04
11	22.5	2	35.50
12	68.4	3	78.10
13	54.0	4	82.40
14	83.9	5	68.20
15	33.0	6	18.4
16	25.4	OMe	58.2
17	55.7	D-Mann (S-4)	
18	10.6	1	101.34
19	17.8	2	71.40
20	16.4	3	73.09
21	22.5	4	68.20
D-Cym (S-1)		5	78.50
1	95.88	6	71.9
2	35.40	D-Gal (S-5)	
3	77.30	1	99.14
4	82.60	2	68.40
5	68.50	3	70.70
6	18.6	4	70.70
OMe	57.2	5	71.30
		6	68.08



Scheme 1. Acid hydrolysis of Lancoside 1.

The genin 4, $C_{23}H_{34}O_6$, mp 196–198°C, $[\alpha]_D$ 49.5° on deesterification by Zemplen method (Zemplen et al. 1926) gave 11 $C_{21}H_{32}O_5$ m.p. 171°C which has the comparable mobility with authentic sample of lineolon [Warshina and Noro, 1994] confirming that 4 was esterified lineolon. The structure of genin 4 was further confirmed by the 1H NMR of compound 1 at 400 MHz which showed four singlet of three protons each at δ 1.12, 1.10 for C-18 and C-19 methyl and 2.17, 1.96 for $COCH_3$ group at C-21 and acetyl group at C-12 along with H-12 methine proton triplet at δ 5.34 confirmed that genin was 12-O-acetyl lineolon.

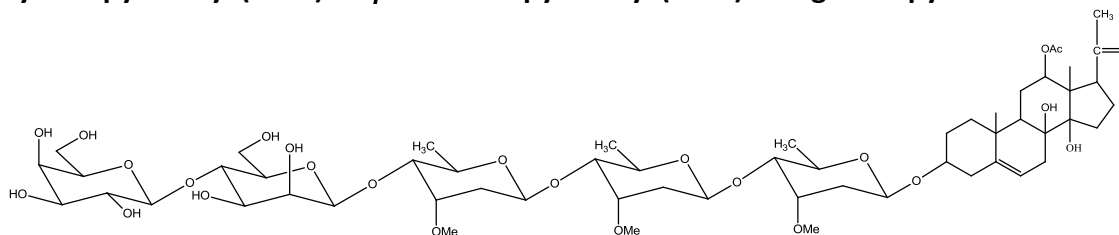
The 1H NMR spectrum of the glycoside 1 showed the configuration of glycosidic linkages. The configuration of five anomeric protons in the five sugar unit could be easily derived from the value of their coupling constants of the signals at δ 4.83 (1H) as double doublet

($J = 8.8$ and 2 Hz) of S_1 , 4.75 (1H) as double doublet ($J = 8$ and 2 Hz) of S_2 , 4.48 (1H) as double doublet ($J = 8\text{ Hz}$ and 2 Hz) of S_3 , 4.59 (1H) as doublet ($J = 7.6\text{ Hz}$) of S_4 and 4.79 (1H) as doublet with $J = 2.8\text{ Hz}$ of S_5 . The larger value of coupling constant of three 2, 6-dideoxy sugars S_1 , S_2 , S_3 were typical of axial configuration of hexopyranose in 4C_1 (D) conformation indicating β glycosidic linkage in all three cymarose units while the two normal sugars present in pentaglycoside which gave their anomeric signals at δ 4.79 as doublet ($J = 2.8\text{ Hz}$) and δ 4.59 as doublet ($J = 7.6$) showed that the first sugar S_4 (mannose) was having the β glycosidic linkage and other monosaccharide S_5 (galactose) was having α glycosidic linkage. The ${}^1\text{H}$ NMR also contained a multiplet of one proton at δ 5.46 which was assigned to the vinylic proton present at C-6 of the aglycon moiety. The singlet of three protons at δ 1.96 along with a downfield shifted methine proton triplet at δ 5.34 was due to acetyl group present at H-12 of aglycon confirming that acetyl group was present at H-12 of aglycon which was substantiated by a cross peak at δ 5.34×1.6 in ${}^1\text{H}$ - ${}^1\text{H}$ COSY spectrum showed the presence of methylene group at C-11 of the aglycon. It also showed a singlet at δ 2.17 for COCH_3 group present at C-21 of aglycon. ${}^1\text{H}$ NMR also contained a multiplet at δ 3.52 which was due to H-3 proton of aglycon which also has cross peaks in the methylene region in the COSY spectrum. The two singlet's of three protons each at δ 1.12 and δ 1.10 were assigned for two angular methyl group present at C-10 and C-13. The presence of three methoxy groups of sugars S_1 , S_2 and S_3 present in 1, were confirmed by the presence of three singlet's of three protons each at δ 3.66 , δ 3.43 and δ 3.38 in its ${}^1\text{H}$ NMR spectrum. The doublets of secondary methyl of cymarose gave the ${}^1\text{H}$ MNR signals in the region δ 1.36 - 1.26 . The presence of two carbon chain at C-17 position of the aglycon as ketomethyl group was confirmed by appearance of three protons singlet at δ 2.17 in the ${}^1\text{H}$ NMR spectrum of 1. The ring methine protons of S_1 , S_2 , S_3 , S_4 and S_5 were assigned on the basis of TOCSY experiment by comparison with the reported values in literature. The above assignments of the sugar ring methine protons were also supported and confirmed by COSY, HSQC and TOCSY experiments of 1. The other characteristic carbon signals of 1 were also supported the derived structure, which were shown in Table 1.

To confirm the glycosidic linkages of 1, it was acetylated with Ac_2O in pyridine at 100°C which afforded an amorphous octa-O-acetyl derivative (12). The structure of 12 was finally elucidated by the analysis of its ${}^1\text{H}$ NMR. It showed the presence of eight acetyl groups twenty four protons in the region δ 2.17 - 2.083 which accounted for the seven hydroxy group present in five sugar units and one acetate was present at position 12 of aglycon moiety. The chemical shift of H-3 methine proton of the aglycon remain unchanged in the ${}^1\text{H}$ NMR spectra of compound 1 and its acetylated derivative 12 indicated that it was involved in glycosidic linkage with cymarose. The absence of any downfield shift in the H-4 methine proton of cymarose upon acetylation at δ 3.19 , 3.21 , 3.20 for S_1 , S_2 , S_3 sugars respectively showed that C-4 of all cymarose units were involved in glycosidic linkages. Further the C-4 of S_4 (mannose) which came at δ 3.44 in ${}^1\text{H}$ NMR of 12 did not show any downfield shift, also confirmed that C-4 of mannose was involved in glycosidic linkage by (1 $\rightarrow$ 4) with the S_5 (galactose). The ES mass spectrum of 1 displayed highest mass ion peaks at m/z 1201 and m/z 1185 which were due to $[\text{M} + \text{K}]^+$ and $[\text{M} + \text{Na}]^+$ respectively. The $[\text{M}]^+$ of compound was also found at m/z 1162 .

The sugar sequence was confirmed via the fragmentation path which showed ion at m/z 1000[1162-S5], 838[1000-S4], 694[838-S3], 550[694-S2] and 406[550-S1] (Scheme-3). The mass peak at m/z 406 corresponded to genin which further fragmented to give other respective fragment peak at m/z 391 (406 - Me), m/z 363 (406 - COCH₃), m/z 345 (363 - H₂O), m/z 388 (406-H₂O), m/z 328 (388-AcOH), m/z 225 (268-COCH₃), m/z 207 (225-H₂O) were supported the presence of keto methyl unit, two methyl, one acyl and two hydroxyl group in genin.

In the light of foregoing evidence, the structure of novel compound 1 was established as **12-O-acetyl lineolon 3-O- β -D-cymaropyranosyl (1 $\rightarrow$ 4)-O- β -D-cymaropyranosyl (1 $\rightarrow$ 4)-O- β -D-cymaropyranosyl (1 $\rightarrow$ 4)-O- β -D-mannopyranosyl (1 $\rightarrow$ 4) α -D-galactopyranoside.**



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