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L-Galactose as a natural product: isolation from a marine octocoral of the first α-L-galactosyl saponin

Marcelino Gutiérrez,[a] Todd Capson,[b] Héctor M. Guzmán,[b] Emilio Quiñoá[a] and Ricardo Riguera[a,*]

[a]*Departamento de Química Orgánica, Instituto de Acuicultura y Unidad de RMN de Biomoléculas asociada al CSIC, Universidad de Santiago de Compostela, E-15782 Santiago de Compostela, Spain*
[b]*Smithsonian Tropical Research Institute, 2072 Balboa, Ancon, Republic of Panama*

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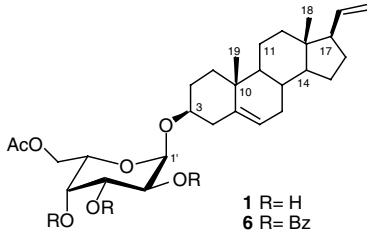
Abstract—Saponine **1** (6′-*O*-acetyl-3β-pregna-5,20-dienyl-α-L-galactopyranoside), that contains a L-galactose moiety linked to the aglycone through an infrequent α-glycosidic bond, has been isolated from the marine octocoral *Muricea* c.f. *purpurea*. This constitutes the first report on the occurrence of L-Gal as a nonpolymeric natural product. A CD procedure for the absolute stereochemical assignment of saponins, based on the CD analysis of its perbenzoylated derivative, is proposed.

The presence of L-sugars in nature is considered to be a rare occurrence when compared to the massive presence of D-sugars in both terrestrial and marine environments. Nevertheless, there are some exceptions such as arabinose, fucose and rhamnose that are peculiar amongst the simple monosaccharides because they occur more commonly in the L- than in the D-configuration.[1]

An extensive literature search has shown us that L-galactose (L-Gal) is a rare sugar that, in terrestrial organisms, has been found as a component of polysaccharides from some plants[2–5] and in snail galactans.[6] In the marine environment, L-Gal has been detected in red seaweeds,[7] in several species of tunicates[8–11] and in sulfated glycoproteins from soft corals.[12] In the case of the polysaccharides from red seaweeds, it has been shown that L-Gal may appear in place of or jointly with D-Gal,[7] while in tunicates, the polysaccharides that present L-Gal are devoid of D-Gal.[8–11]

Apart from polysaccharides and glycoproteins, another important class of natural products that contain sugars are the saponins. These metabolites are widely distributed in many terrestrial plants and in certain marine organisms, particularly echinoderms.[13,14] The immense majority of the sugars found in the marine saponins belong to the D series.[13,15] Nevertheless, in a very small number of cases, the presence of L-sugars has been reported (e.g., L-fucose,[16] L-arabinose[17] and L-digitalose[18]), but up till now, no saponin—either from terrestrial or marine sources—has ever been identified containing L-Gal.



As a result of our interest on marine natural products showing relevant bioactivities, we have recently focused our attention on octocorals, marine invertebrates that constitute a well-known source of acetogenins, sesquiterpenoids, diterpenoids and prostanoids.[19] In this letter we wish to communicate the isolation of saponin **1** (6′-*O*-acetyl-3β-pregna-5,20-dienyl-α-L-galactopyranoside) from the marine octocoral *Muricea* c.f. *purpurea*. This

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* Corresponding author. Tel./fax: +34 981591091; e-mail: ricardo@usc.es

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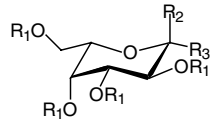
constitutes the first report on the occurrence of L-Gal as a nonpolymeric natural product.

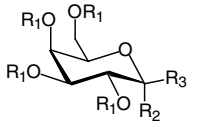
In this study, fresh specimens (0.9 kg) of *Muricea* c.f. *purpurea*, collected at Coiba National Park at the Pacific Coast of Panama, were soaked in methanol, homogenized and filtered. The residue was sequentially washed with methanol and dichloromethane. The extracts were pooled and concentrated in vacuo at 25 °C yielding 21.0 g of crude extract.

This material was subjected to solvent partition as previously described[20] to give a dichloromethane fraction (2.18 g) that was subjected to vacuum liquid chromatography (VLC) on silica gel. Elution with dichloromethane–methanol mixtures yielded eight fractions. The third fraction (81 mg) was dissolved in diethyl ether and cooled down till a precipitate appeared. The soluble material was concentrated and submitted to normal phase HPLC (ethyl acetate–acetone 9:1 as mobile phase) to give compound **1** (6 mg).

Compound **1** was obtained as a pale yellow glassy solid with an $[\alpha]_D$ −115 (*c* 0.6, $CHCl_3$) and its structure was established by extensive MS (LREIMS, HRESI-TOF MS) and NMR (^1H, ^{13}C, DEPT, COSY, HMQC, HMBC, NOESY and ^1H decoupling) analysis.



2 (α-L) R_1= Bz; R_2= OMe; R_3= H
3 (β-L) R_1= Bz; R_2= H; R_3= OMe



4 (α-D) R_1= Bz; R_2= OMe; R_3= H
5 (β-D) R_1= Bz; R_2= H; R_3= OMe

Thus, its HRESI-TOF MS showed a pseudomolecular ion at *m/z* 527.29799 corresponding to a $C_{29}H_{44}O_7Na$—$[M+Na]^+$—formula. The overall data from the 1D and 2D NMR experiments clearly suggested the presence of a 6′-*O*-acetylgalactopyranoside linked to position 3 of a C_{21} pregnane skeleton. Consequently, compound **1** was a saponin made of an acetylated galactose with a pregnane aglycone[22,23] (Table 1). In order to check if the sugar moiety was a member of the D or L series, we resorted to the circular dichroism (CD) study of the perbenzoylated α and β methyl glycosides. These derivatives should show Davidov splitting due to exciton coupling, characteristic for the L- and the D-galactose, therefore allowing the safe assignment of the absolute configuration of the sugar.

Thus, 2 mg of **1** were submitted to methanolysis followed by perbenzoylation,[21] affording a mixture of **2** and **3** that were separated by normal phase HPLC.

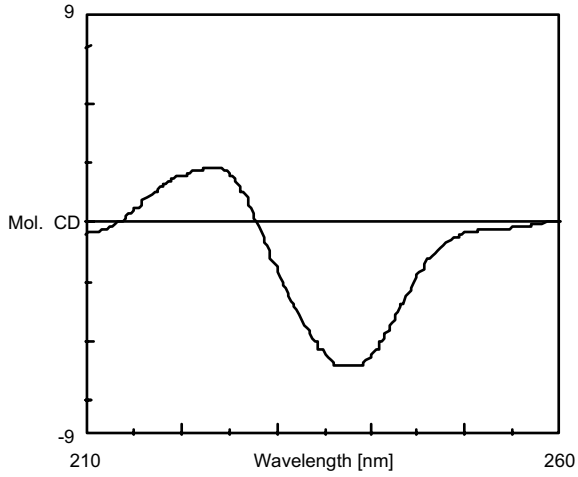
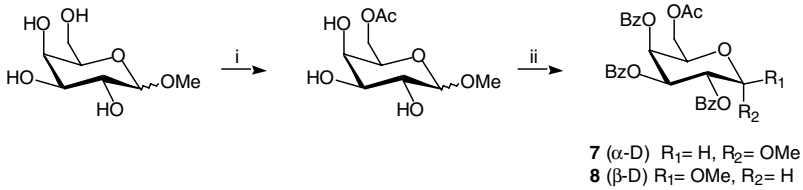


Figure 1. CD spectrum (MeCN) of methyl tetra-*O*-benzoyl-α-L-galactopyranoside **2**. The CD spectrum of methyl tetra-*O*-benzoyl-α-D-galactopyranoside **4** has been recently published and can be found in the Supporting Information of Ref. 21.

Table 1. ^{13}C and ^1H NMR for compound **1** in $CDCl_3$ at 62.5 and 750 MHz, respectively

Position	^1H NMR (m, *J* in Hz)	^{13}C NMR (m)	Position	^1H NMR (m, *J* in Hz)	^{13}C NMR (m)
1	1.08 (m)	37.3 (t)	14	1.00 (m)	55.8 (d)
	1.88 (m)		15	0.96 (m)	24.8 (t)
2	1.57 (m)	29.5 (t)	16	1.54 (m)	27.2 (t)
	1.94 (m)			1.81 (m)	
3	3.49 (m)	78.5 (d)	17	1.95 (m)	55.3 (d)
4	2.23 (t, 12.0)	38.7 (t)	18	0.60 (s)	12.7 (q)
	2.34 (dd, 3.0, 12.7)		19	1.01 (s)	19.34 (q)
5		139.9 (s)	20	5.76 (ddd, 8.2, 9.7, 17.2)	139.74 (d)
6	5.36 (d, 4.5)	122.2 (d)	21	4.96 (bd, 9.7)	114.5 (t)
7	1.50 (m)	31.9 (t)	C(O)CH_3	2.07 (s)	20.8 (q)
	2.02 (m)		C(O)CH_3		170.9 (s)
8	1.71 (m)	31.9 (d)	1′	5.05 (d, 3.7)	97.3 (d)
9	0.96 (m)	50.3 (d)	2′	3.77 (dd, 3.0, 9.8)	69.4 (d)
10		36.8 (s)	3′	3.75 (dd, 2.2, 10.2)	71.1 (d)
11	1.47 (m)	20.6 (t)	4′	3.96 (bs)	68.8 (d)
	1.56 (m)		5′	4.05 (t, 6.0)	68.0 (d)
12	1.71 (m)	37.3 (t)	6′	4.22 (dd, 7.5, 11.2)	63.3 (t)
13		43.3 (s)		4.37 (dd, 2.25, 11.2)	



Scheme 1. Synthesis of the CD standards 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (**7**) and 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-β-D-galactopyranoside (**8**). Reagents and conditions: (i) AcCl, *sym*-collidine, −40 °C/25 °C, 3/1 h sequentially; (ii) BzCl, pyridine, 25° C, 16 h.

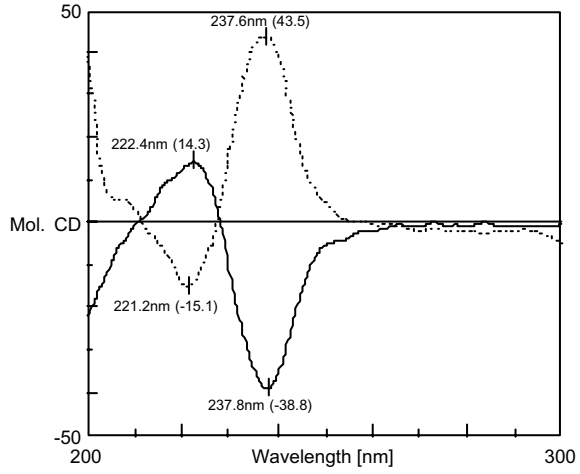


Figure 2. CD spectrum (MeCN) of 6′-*O*-acetyl-2′,3′,4′-tri-*O*-benzoyl-3β-pregna-5,20-dienyl-α-L-galactopyranoside (**6**) [continuous line]. *Idem* for 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-galactopyranoside (**7**) [dashed line].

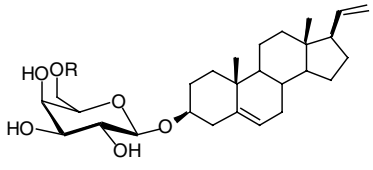
These derivatives showed retention times identical to those of the synthetic glycosides **4** and **5**, respectively (prepared by an analogous procedure from commercial β-D-galactose pentaacetate). When the CD spectra were compared, those of **2** and **3** were found to be the exact mirror images of those of the standard D-derivatives **4** and **5** [opposite signs for the absorption maxima at λ_{max} 223 (+2)/237 (−6) nm, see Fig. 1]. These results unambiguously prove that **2** and **3** belong to the L series and that the sugar present in **1** is L-Gal.

Once the absolute configuration of the sugar was known, we decided to check if this CD procedure for assignment could be directly applied to the intact saponin.

Thus, a small sample (2 mg) of **1** was perbenzoylated as before affording **6**. Its CD spectrum presents a Davidov splitting indicative of negative chirality and is presented in Figure 2. For CD comparison, authentic samples of methyl 6-*O*-acetyl-2,3,4-tri-*O*-benzoyl-α-D-galactopyranoside (**7**), and of its β-anomer **8**, were prepared as shown in Scheme 1 from methyl α/β-D-galactopyranosides.[†] The key step in their preparation was the selective acetylation of the primary alcohol at C-6 in the presence of the unprotected secondary alcohols by means of AcCl in *sym*-collidine (−40 and 25 °C, 3 and 1 h consecutively) with no formation of secondary acetates in a detectable amount.[24] The ^{1}H NMR data of **7** matched perfectly the data of the sugar part of **6** confirming it as a α-galactopyranoside.

Figure 2 shows that the CD spectra of **6** is a perfect mirror image of that of the α-D-derivative **7** [λ_{max} 222 (+14)/237 (−38) nm for **6**; λ_{max} 221 (−15)/237 (+43) nm for **7**, $c = 10^{-4}$ M], thus corroborating the L stereochemistry for the sugar and demonstrating that analysis of the CD of the perbenzoylated saponin constitutes a very useful tool for identification of the sugar, especially in those cases where the amount of sample is very small.[‡]

The isolation of **1** from this octocoral is remarkable not only because it is the first report of the presence of an L-galactose unit in a nonpolymeric natural product but also because the glycosidic linkage is α, being this also a quite unusual fact in saponins. Actually, the related saponin **9**,bearing D-galactose bound through a β-glycosidic linkage to the same aglycone, had been already isolated from a gorgonian and its configuration determined by X-ray studies.[25] Other three glycosides possessing the 6-*O*-acetyl-β-D-galactopyranoside moiety bounded to cembrane-type diterpenes (calyculaglycosides A, C and D) have also been identified.[26]



9 R= H
10 R= Ac

Finally, the recent report[27] of the isolation from the octocoral *Eunicea laciniata* of a saponin (**10**) containing a β-6-*O*-acetylgalactose unit is noteworthy although, unfortunately, the chirality of the sugar was not established.[§]

Our finding suggest that L-sugars, and particularly L-Gal, may be more widespread metabolites than previously believed and that careful examination of the

[†] Prepared from methanolysis of commercial β-D-galactose pentaacetate as previously shown.

[‡] For a collection of CD spectra of derivatized sugars, see Ref. 28.

[§] The authors in Ref. 27 seem to assume a 'D' configuration for the galactose by analogy to compound **9**, but they do not present any experimental evidence.

chirality of sugar units in saponins—particularly in those from octocorals—could possibly render more examples. CD spectroscopy of the directly derivatized saponins may become a very useful tool for this search.

In any case, we believe this report should stimulate further work on occurrence of L-sugars as natural products.

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