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The Occurrence of the Human Glycoconjugate C^2-α-D-Mannosylpyranosyl-L-tryptophan in Marine Ascidians

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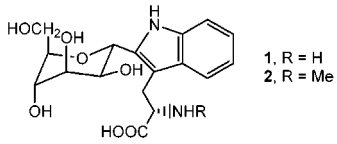
ABSTRACT



The *C*-glycoconjugate C^2-α-D-mannosylpyranosyl-L-tryptophan (1), a metabolite known to be generated in humans through a novel posttranslational process, has been isolated from marine ascidians *Leptoclinides dubius* and *Pharyngodictyon cauliflos* and its N^{α}-methyl derivative (2) from *Ritterella rete*.

The posttranslational modification of proteins by the attachment of carbohydrates to the amino acids constitutes one of the most important ways to modify the structure and activity of the protein. This process usually leads to *N*- and *O*-glycosidation,[1] and it has not been until quite recently that *C*-glycosidation has been detected as a posttranslational protein modification, representing a novel enzymic pathway in tryptophan metabolism in humans. The three cases known to date describe the presence of a C^2-mannosylpyranosyl-tryptophan fragment in RNase from human urine and erytrocytes,[2] in the recombinant human interleukin IL-12[3] and more recently in a protein possesing multiple C^2-mannosylatedtryptophan residues from the human complement system.[4a] The structural motif necessary for this modification to take place has been studied and determined to be a Trp-Aaa-Aaa-Trp peptidic fragment in the first two cases,[4b] but this was found to be not mandatory in the third one.[4a] The intracellular nature of the process has been demonstrated,[5] the enzyme involved identified,[6] and the structure, conformation, and stereochemistry of the glycoconjugate proven by NMR studies[7] and synthesis.[8]

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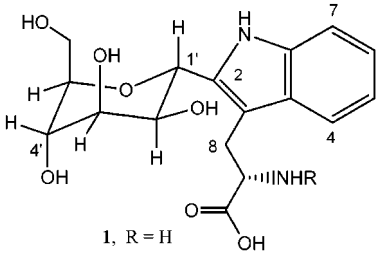
These facts, together with the absence of reports of the occurrence of **1** in nature and in common foods[7a] despite the large number of tryptophan derivatives known, pointed to C^2-α-D-mannosylpyranosyl-L-tryptophan as an apparently exclusive human metabolite. Nevertheless, the existence of *C*-mannosyltransferase activity in man and a few mammals[5] and the presence of the Trp-Aaa-Aaa-Trp structural fragment in many proteins suggests that more examples of *C*-mannosylation might be found in other organisms. In fact, a method for the detection of C^2-α-mannosylpyranosyl-tryptophan in living organisms based on a specific monoclonal antibody has been developed.[9]

In this Letter we wish to communicate the isolation of C^2-α-D-mannosylpyranosyl-L-tryptophan (**1**) from the New Caledonian marine ascidians[10] *Leptoclinides dubius* and *Pharyngodictyon cauliflos* and that of its N^{α}-methyl derivative **2** from *Ritterella rete*.



1, R = H

2, R = Me

This paper constitutes the first report of the presence of these compounds in nonhuman organisms.

Results. As a result of our interest in marine natural products and particularly in those derived from amino acids,[11] we became interested in marine ascidians. These animals constitute a well-known source of nitrogen-containing metabolites derived from amino acids,[12] and in 1996 we reported the isolation from *Leptoclinides dubius* of several new small peptides, some of which were tryptophan-derived.[10×bb] The results described in the introduction of this Letter prompted us to carry out a reexamination of the more polar fractions of that organism and of other ascidians. Specimens of *L. dubius* (340 g of lyophilized organism) were extracted and fractionated as previously described[10a] to give an *n*-BuOH fraction, which was desalted through an XAD-2 column (1.18 g), chromatographed on Sephadex LH20 ($MeOH/CH_2Cl_2$ 1:1), and then submitted to RP-HPLC (μ-Bondapak NH_2 column, MeOH as mobile phase, flow rate 2 mL/min) to give compound **1** (3 mg, retention time 14 min). On using a similar isolation procedure, the methanol extract (15.7 g) of the marine ascidian *Pharyngodictyon cauliflos* (580 g of lyophilized organism)[10b] gave 150 mg of a desalted *n*-BuOH fraction, which was first submitted to Sephadex LH20 (MeOH) and then to RP-HPLC (μ-Bondapak NH_2, MeOH/MeCN 8:2, flow rate 2 mL/min) to yield **1** (5 mg, retention time 8 min). Compound **1** was obtained as an amorphous solid with an $[\alpha]_D$ of +50° (*c* 4×10^{-4}, MeOH). The NMR (^1H, ^{13}C, DEPT, $^1H-^1H$ COSY, TOCSY, HMQC, HMBC, and NOESY in D_2O), UV, and HREIMS data of **1** matched perfectly with those reported for C^2-α-D-mannosylpyranosyl-L-tryptophan.[7a,8] In accordance with the above, the circular dichroism spectra of **1** showed a positive Cotton effect at $\lambda_{max} = 223$, which is coincident with that of L-tryptophan.

The same procedure applied to the *n*-BuOH fraction (2.2 g desalted) of *Ritterella rete* (3.1 kg of lyophilized organism)[10c] gave, after purification by RP-HPLC (Nucleosil C_{18}, $MeOH/H_2O$ 95:5 + 0.01% TFA, flow rate 1 mL/min), compound **2** (5 mg, retention time 30 min).

Compound **2** was isolated as a colorless solid with an $[\alpha]_D = +95°$ (*c* 7×10^{-3}, MeOH). The NMR spectral data (in methanol-d_4) of **2** (Table 1) were very similar to those of **1** but showed the signals corresponding to an additional methyl group at $\delta_H = 2.47$ (s, 3 H) and $\delta_C = 33.81$ (q), which must be linked to a nitrogen (NMe). The attachment of the methyl group to the N^{α} of the tryptophan unit was deduced from the HMBC correlations of the NCH_3 protons with C-9 and of H-9 with the NCH_3 carbon and by the downfield shift of C-9 of **2** in relation to **1**. Furthermore, the N−H signal at $\delta_H = 11.84$ (br s) observed when the spectrum was recorded in pyridine-d_5 rules out the attachment of the methyl group to the N^1 of the indole. The presence in **2** of an α-mannose unit in a $^1C_4$ chair conformation was deduced from the large vicinal coupling between H-1′ (t, $J = 9.7$ Hz) and H-2′ and by the NOE between H-1′ and H-6′ due to the axial orientation of the hydroxymethyl group.

The intense NOE correlation between H-6′ and H-4′ and the small coupling constants of H-4′ (br d, $J = 3.2$ Hz) are indicative of an equatorial position for H-4′. The pseudomolecular ions $[M + H]^+$ at *m*/*z* 381 and $[M + Na]^+$ at *m*/*z* 403 in the (+)-LRFABMS, corresponding to the molecular formula $C_{18}H_{24}N_2O_7$, confirmed the suggested structure. On the basis of these data, compound **2** is C^2-α-D-mannosylpyranosyl-L-N^{α}-methyltryptophan.

The isolation of **1** and its N^{α}-methyl derivative **2** from these three ascidians is remarkable not only because it is the first report of the presence of these *C*-glycoconjugates in nonhuman living organisms but also because it points to ascidians as new sources for those compounds[13] and suggests that *C*-mannosylation is a more general process than previously believed.

The possibility that these organisms possess the enzymes capable of performing the mannosylation of tryptophan in the peptides, and therefore that **1** and **2** are the products of

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(13) Cases are known where metabolites reported from marine macroorganisms have been proved to proceed from their symbionts instead. This possibility should also be considered in this case.

Table 1. ^{13}C NMR (125 MHz), ^{1}H NMR (500 MHz), HMBC, and NOESY Spectral Data of Compound **2**

position	δ_C[a]	δ_C[b]	mult	δ_H mult (*J* in Hz)[a,c]	δ_H mult (*J* in Hz)[b]	HMBC[b,d]	NOESY[b]
1				11.84 br s			
2	136.53	136.01	s			H1′, H2′, H8	
3	108.04	108.83	s			H1′, H8, H9	
3a	128.30	128.51	s			H4, H5, H7, H8	
4	118.95	119.50	d	7.96 d (7.7)	7.79 d (7.7)	H6	H5
5	119.06	120.39	d	7.12 t (7.7)	7.11 t (7.7)	H7	H4, H6
6	121.84	123.43	d	7.20 t (7.7)	7.19 t (7.7)	H4	H5, H7
7	111.78	112.27	d	7.46 d (7.7)	7.41 d (7.7)	H5	H6
7a	136.82	137.82	s			H4, H6	
8	26.79	27.89	t	3.94 dd (15.1, 5.3) 3.85 dd (15.1, 7.4)	3.64 m 3.24 dd (14.9, 10.1)	H9	H9, H1′
9	63.61	66.19	d	4.47 br t (6.3)	3.64 m	H8, N*Me*	H8, N*Me*
10	172.62	173.39	s			H8, H9	
1′	65.48	65.64	d	5.87 d (9.2)	5.09 d (9.7)	H2′, H3′, H5′	H2′, H6′, H8
2′	68.55	69.16	d	5.08 dd (9.2, 3.6)	4.30 dd (9.7, 3.2)	H1′, H3′, H4′	H1′, H3′
3′	72.59	72.24	d	4.79 t (3.6)	4.11 t (3.2)	H1′, H2′, H4′, H5′	H2′, H4′, H5′
4′	70.69	71.13	d	4.57 dd (3.6, 2.1)	3.95 br d (3.2)	H3′, H5′, H6′	H3′, H5′, H6′
5′	81.92	81.81	d	4.68 m	4.04 dd (8.8, 3.2)	H1′, H4′, H6′	H3′, H4′, H6′
6′	60.01	60.44	t	4.93 dd (11.9, 8.8) 4.28 dd (11.9, 4.0)	4.38 dd (12.3, 8.8) 3.71 dd (12.3, 3.2)	H5′	H1′, H4′, H5′
NMe	32.85	33.81	q	2.83 s	2.47 s	H9	H9

[a] Recorded in pyridine-d_5. [b] Recorded in methanol-d_4. [c] Recorded at 60 °C. [d] Protons correlated to carbon resonances in ^{13}C column.

a posttranslational process similar to that in humans, has not been proved warrants further investigation. In any case, we believe this report should stimulate further work on the still unknown specific role of *C*-mannosylation in Nature.

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Supporting Information Available: NMR spectra (^{1}H, ^{13}C, DEPT, $^{1}H-^{1}H$ COSY, HMQC, HMBC, and NOESY in methanol-d_4) of compound **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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