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α-HOMONOJIRIMYCIN [2,6-DIDEOXY-2,6-IMINO-D-GLYCERO-L-GULO-HEPTITOL] FROM OMPHALEA DIANDRA L.: ISOLATION AND GLUCOSIDASE INHIBITION

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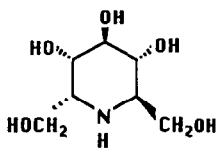
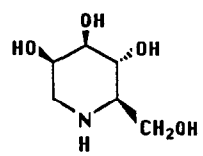
The isolation of α-homonojirimycin [2,6-dideoxy-2,6-imino-D-glycero-L-gulo-heptitol] from Omphalea diandra is described; α-homonojirimycin is an inhibitor of several α-glucosidases.

Alkaloidal glycosidase inhibitors, which are polyhydroxylated piperidines, pyrrolidines, octahydroindolizines and pyrrolizidines, have been found in a number of plants and microorganisms.[1,2] In the angiosperms (flowering plants) these compounds have been reported in the families Leguminosae, Moraceae and Polygonaceae.[3] Recently, the pyrrolidine glycosidase inhibitor DMDP [2,5-dideoxy-2,5-imino-D-mannitol] (3) was also found[4] in a neotropical liana, Omphalea diandra L., from the family Euphorbiaceae.

This paper describes the isolation from Omphalea diandra L. of α-homonojirimycin [2,6-dideoxy-2,6-imino-D-glycero-L-gulo-heptitol] (1) and its ability to inhibit glycosidases; this is the first report of a naturally occurring azapyranose analogue of a heptose. Omphalea diandra has also been shown to contain deoxymannojirimycin [1,5-dideoxy-1,5-imino-D-mannitol] (2), an inhibitor of glycoprocessing mannosidase I and mammalian α-L-fucosidase,[5] previously isolated from the legume Lonchocarpus sericeus.[6]



(1)



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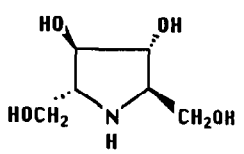
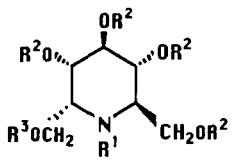


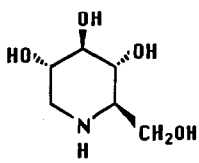
(3)

Isolation. Leaves of Omphalea diandra L. were collected from plants growing wild in the Republic of Panama and oven dried at 60°C. Finely powdered leaf material was extracted into 70% aqueous methanol for 2 h at room temperature and the concentrated extract was passed down a column of Dowex 50 (H^+) ion exchange resin. Homonojirimycin was then eluted with 1 M pyridine, was freeze dried and the residue dissolved in water. Contaminating amino acids were removed by washing the solution through Amberlite CG-400 (OH-) ion exchange resin with water. Further purificaton by adsorption onto aluminum oxide 90 (Merck) and elution with acetone gave α-homonojirimycin, m.p. 206°-207°C, $[\alpha]^{20}$ (c, 0.54 in H_2O) +88.2° (589), +92.0° (578), +103.9° (546), +169.2° (436) and +249.9° (365); m/z (DCI NH_3): 194 ($M+H^+$, 100%). ^{13}C NMR (D_2O with dioxane as a internal standard): 54.77 (d), 57.06 (t), 57.57 (d), 67.72 (t), 72.26 (d), 72.77 (d) and 75.00 (d). This material had ^1H and ^{13}C NMR spectra which were superimposable on those of an authentic sample of synthetic α-homonojirimycin, m.p. 198°-199°C, $[\alpha]_D^{20}$ +79.1° (c, 2.03 in H_2O), prepared by hydrogenation of 2,6-dideoxy-2,6-[phenylmethoxycarbonyl]imino-1,3,4,5-tetra-O-benzyl-D-glycero-L-gulo-heptitol (4)[7] or semi-synthetically from nojirimycin.[8] Levels of homonojirimycin in O. diandra leaves were estimated from GC analysis to be 0.3-0.4% of dry weight.[9]

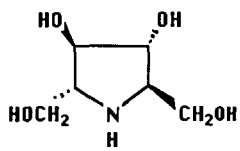
After homonojirimycin had been eluted from the column of Dowex 50 (H^+) ion exchange resin by aqueous pyridine, further elution with 2 M aqueous ammonia gave a mixture[10] containing deoxymannojirimycin (2) and DMDP (3).



(1) $R^1 = R^2 = R^3 = H$



(6)



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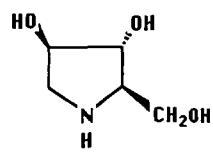
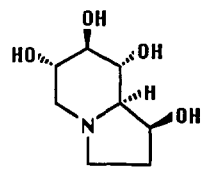


(7)

(4) $R^1 = COOCH_2Ph$; $R^2 = CH_2Ph$; $R^3 = H$

(5) $R^1 = R^2 = H$; R^3 = ß-D-glucopyranosyl



(8)

Among naturally occurring azapyranose and azafuranose α-glucosidase inhibitors, α-homonojirimycin (1) is structurally related to deoxynojirimycin (6) in the same way that DMDP (3) is related to 1,4-dideoxy-1,4-imino-D-arabinitol (7),[11] first isolated from Angylocalyx.[12] The ß-D-glucopyranosyl derivative (5) of homonojirimycin was designed[7,8] as a synthetic transition state inhibitor of α-glucohydrolases and has been shown to be an effective means of reducing the hyperglycemic response to an oral sucrose or starch load and may

therefore have potential as a drug for the treatment of diabetes mellitus.[13] Also, several α-glucosidase inhibitors, including castanospermine (8), isolated from the seeds of the Australian legume Castanospermum australe,[14] inhibit human immunodeficiency virus syncytium formation and virus replication;[15] such compounds may have potential as antiretroviral agents.[16]

α-Homonojirimycin was tested as a possible inhibitor of glycosidase activity in homogenates of mammalian (mouse) and insect (Spodoptera littoralis, fifth and sixth instar larvae) gut, using both synthetic (p-nitrophenyl glycopyranosides) and natural substrates. The assay methods employed have been described elsewhere.[2,17]

TABLE. Concentration of α-homonojirimycin (M) giving 50% inhibition of glycosidase activity [N.I. = less than 50% inhibition at 3.3×10^{-4}M]

	Gut homogenate from:	
Substrate	Mouse	Spodoptera littoralis
p-nitrophenyl α-D-glucopyranoside	2.2×10^{-7}	1.3×10^{-5}
p-nitrophenyl ß-D-glucopyranoside	1.4×10^{-4}	N. I.
p-nitrophenyl α-D-galactopyranoside	5.3×10^{-5}	N. I.
maltose	7.2×10^{-7}	1.3×10^{-4}
sucrose	8.1×10^{-8}	2.2×10^{-4}
lactose	5.5×10^{-5}	1.9×10^{-4}
trehalose	1.8×10^{-5}	3.7×10^{-5}

Thus , α-homonojirimycin (1) was found to be a potent inhibitor (Table) of digestive α-glucosidase activity in mouse but, with the exception of trehalase, to be relatively weak against comparable activity in Spodoptera. In this respect, α-homonojirimycin resembles deoxynojirimycin (6),[1,17] its potential as an antiviral and anti-diabetic agent is under investigation.

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