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A NEW α-GLUCOSIDASE INHIBITING DITHIADIAZETIDIN DERIVATIVE FROM *SYMPLOCOS RACEMOSA*

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Abstract – The phytochemical investigation of the *n*-butanol soluble fraction of *Symplocos racemosa* Roxb. resulted in the isolation of a new dithiadiazetidin derivative; symploate (**1**). Its structure was established through various 1D and 2D NMR techniques together with high-resolution mass spectrometric techniques and spectral evidences. The symploate (**1**) showed moderate inhibitory activity against α-glucosidase in a concentration-dependent fashion with an IC_{50} value of 691.1 ± 3.29 μM.

INTRODUCTION

Symplocos racemosa Roxb. (Lodh) belongs to the family Symplocaceae, which is a unigeneric family of about 290 species. Lodh has a wide range of usage in Ayurveda and Unani medicines. Its bark is described as an emmenagogue tonic for the persons of plethoric constitution and is useful in bowel complaints and ulcers.[1] Its decoction is used as a gargle for giving firmness to bleeding and spongy gums.

It cures watery eyes, opthalmia and is good for all diseases of the eye. It also cures “Kapha” biliousness, diseases of the blood, dysentery, inflammations, vaginal discharges, leprosy, useful in abortions and miscarriages and is good for ulcers in the vagina. The bark, mixed with sugar, is given in menorrhagia due to relaxation of the uterine tissue. The bark is also prescribed in the treatment snake-bite and scorpion-sting.[1]

α-Glucosidase catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the uptake dietary carbohydrates and suppress postprandial hyperglycemia, and could be useful to treat diabetic and /or obese patients.[2] α-Glucosidase inhibitors are effective in lowering the insulin release, insulin requirement and some lower plasma lipids. They have also been used as inhibitors of tumor metastasis, antiobesity drugs, fungistatic compounds, insect antifeedents, antivirals and immune modulators.[3] Inhibition of α-glucosidase causes abnormal functionality of glycoproteins because of incomplete modification of glycans. Suppression of this processing is to be expected for antiviral activity and decreasing of growth rate of tumors.

RESULTS AND DISCUSSION

From the *n*-butanol soluble fraction of *Symplocos racemosa* Roxb., a new dithiadiazetidin derivative; symploate (**1**) was isolated as a pale yellowish solid. Its EI-MS spectrum showed a bunch of molecular ion peak at *m/z* 546 and its HR-EI-MS revealed the presence of (M+2) peak at *m/z* 548.4 and (M+4) peak at *m/z* 550.4, together with the M^+ ion peak at *m/z* 546.3. The Lassaigne test (Sodium fusion test), was positive for nitrogen and sulfur but negative for halogens. When the isotopic pattern for (M + 2) and (M+4) peaks was measured, their percentage relative abundance was found to be 13.99 % and 1.16 % respectively, which indicated the presence of two sulfur atoms in the molecule. Its HR-MS showed the M^+ ion peak at *m/z* 546.404097 which suggested the molecular formula as $C_{28}H_{54}N_2O_4S_2$ and indicated three degrees of unsaturation. Furthermore, the calculated isotopic pattern of the molecular ion region of $C_{28}H_{54}N_2O_4S_2$ is in favorable agreement with the relative intensities measured for *m/z* 546 – *m/z* 550. The EI-MS spectrum showed a fragment peak at *m/z* 273, which was the exact half of the M^+ ion peak and hence suggested its dimeric nature. The uniform difference of 14 amu between a numbers of ion peaks showed the presence of a long aliphatic chain, while the loss of 32 amu and 34 amu can be accounted for the loss of S and H_2S respectively. The fragment ions at *m/z* 375 and 204 represented the cleavage of C-O bonds in the molecule. The ^{1}H-NMR spectrum showed two doublets of triplet (2H each) at δ 3.15 and 3.31 respectively for the protons of two (-N-C$\underline{H}_2$-) groups and an overlapped doublet of doublet (4H) at δ 3.02 for two (-C$\underline{H}_2$-COO) groups. The formers and the latter also caused a cross peak in the ^{1}H-^{1}H COSY spectrum, which indicated that the two methylene groups were adjacent to each other in the molecule. In the ^{1}H-NMR a triplet (4H) at δ 4.14 was assigned to two (COOC$\underline{H}_2$-) groups while a six-proton triplet at δ 0.85 was due to two terminal methyl groups. The quintet at δ 1.56 was integrated for four protons assignable to two (O-CH_2-C$\underline{H}_2$-CH_2) groups and the broad clustered singlet at δ 1.25 was due to 32 protons, revealing the presence of 16-methylene groups in the molecule. The ^{13}C-NMR spectrum displayed fourteen carbon resonances and the DEPT experiments revealed that out of these fourteen resonances only one was a quaternary carbon and one was a methyl carbon while all the remaining signals were of methylene carbons. These half number of

carbon resonances as compared to the calculated molecular formula $C_{28}H_{54}N_2O_4S_2$, also confirmed the dimeric nature of the molecule. In the ^{13}C-NMR spectrum, the quaternary carbon at δ 171.7 revealed the presence of an ester carbonyl, which was also supported by the IR spectrum absorption band at 1740 cm^{-1}. A downfield shift of C-3 (δ 47.2) indicated that a nitrogen atom was directly linked with this methylene group and due to this downfield shift the adjacent methylene was shifted ~7 ppm upfield as compared to the respective signal of reported octacosanyl hexadecanoate.[5] All these assignment were further confirmed by long rang HMBC correlations as shown in Figure 2. The IR absorption at 1365 cm^{-1} showed the (C-N) stretching, while absorption at 793 cm^{-1} indicated the (N-S) stretching in the molecule. Hence from the above spectral evidences it was concluded that the dimmer was linked by nitrogen to nitrogen and sulfur to sulfur, forming a four membered cyclic dithiadiazetidin ring[6] as shown in the Figure 1.

The symploate (**1**) displayed moderate inhibitory potential against α-glucosidase in a concentration-dependent fashion with an IC_{50} value of 691.1 ± 3.29 μM. Acarbose and deoxynojirimycin were used as positive controls and their IC_{50} values were found to be 780.4 ± 2.88 μM and 425.6 ± 8.14 μM respectively.

Table 1. NMR spectral data (in C_5D_5N) for compound (**1**).[a]

Position	δ_H	δ_C [b]
1	-	171.7
2	3.02 (dd, *J* = 6.7, 7.2)	27.4
3	3.15 (dt, *J* = 6.5, 6.7)	47.2
	3.31 (dt, *J* = 6.5, 7.2)	
1'	4.14 (t, *J* = 6.7)	65.2
2'	1.56 (quint. *J* = 6.9)	28.8
3'	1.22-1.26 brs	26.1
4'	1.22-1.26 brs	29.9
5'	1.22-1.26 brs	29.6
6'	1.22-1.26 brs	29.7
7'	1.22-1.26 brs	29.8
8'	1.22-1.26 brs	29.5
9'	1.22-1.26 brs	32.0
10'	1.22-1.26 brs	22.9
11'	0.85 (t, *J* = 6.9)	14.3

[a] All spectra were recorded at 400 MHz (^{1}H) and 125 MHz (^{13}C); assignment were aided by 2D NMR COSY, HMQC and HMBC spectral experiments.

[b] ^{13}C NMR multiplicities were determined by DEPT 135°.

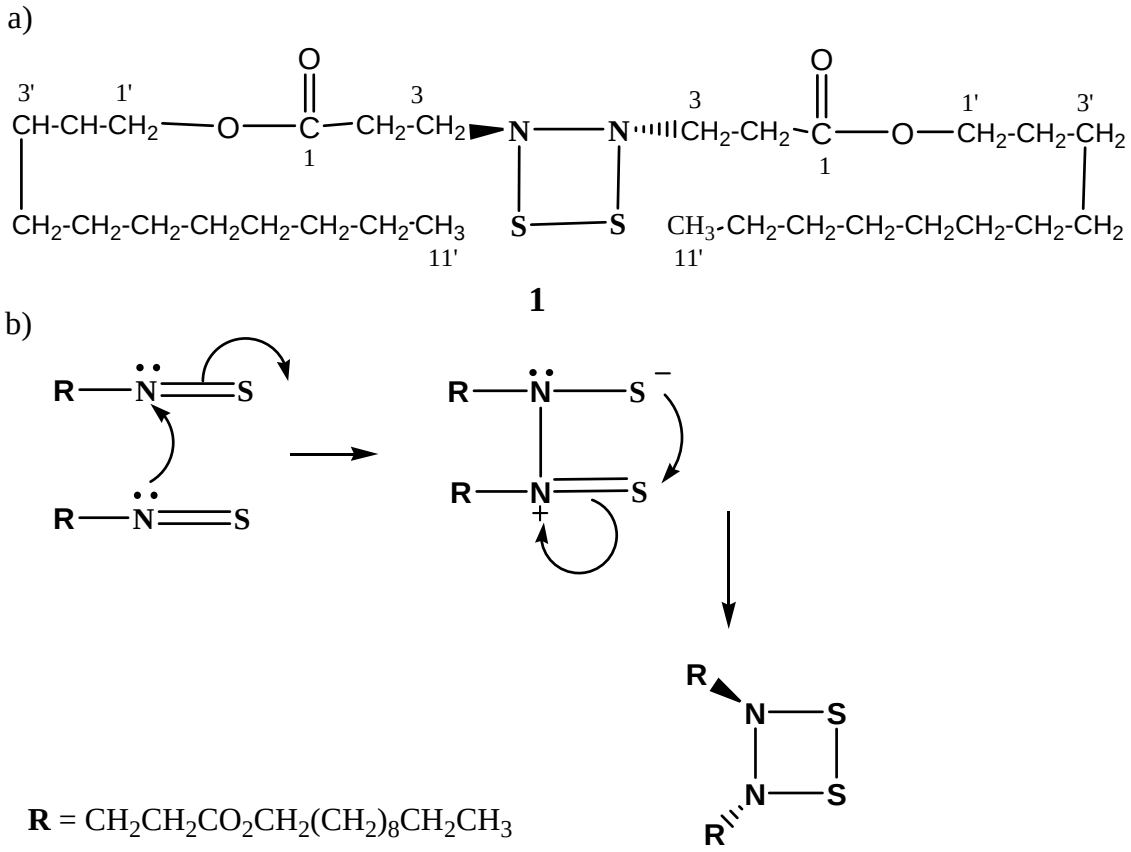
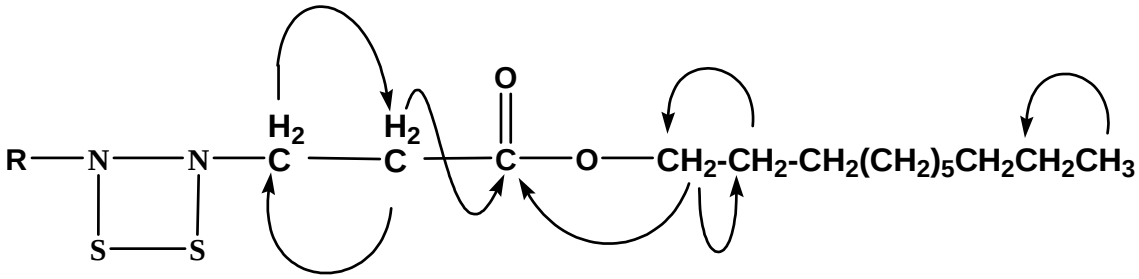


Figure 1 a) Structure of compound (1) b) its proposed pathway



R = $CH_2CH_2CO_2CH_2(CH_2)_8CH_2CH_3$

Figure 2 Important HMBC corellations of 1

EXPERIMENTAL

General

Silica gel (70-230 mesh) and (230-400 mesh) was used for column chromatography (CC) for flash chromatography (FC) respectively. TLC was performed on pre-coated silica gel G-25-UV_{254} plates. Detection was carried out at 254 nm, and by ceric sulphate reagent. The optical rotations were measured on a JASCO-DIP-360 digital polarimeter. The UV and IR Spectra were recorded on Hitachi-UV-3200 and JASCO-320-A spectrophotometers, respectively. ^{1}H-NMR, ^{13}C-NMR, COSY, HMQC and HMBC spectra were run on Bruker spectrometers operating at 500, 400 and 300 MHz. The chemical shifts are given for δ in ppm and coupling constants in Hz. EI-MS and FAB^{-}MS spectra were recorded on a JMS-HX-110 spectrometer, with a data system.

Plant material

The plant *Symplocos racemosa* (Symplocaceae) was collected from Abbottabad, Pakistan, in August 2002, and identified by Dr. Manzoor Ahmed (Taxonomist) at the Department of Botany, Post-Graduate College, Abbottabad, Pakistan. A voucher specimen (No. 6453) has been deposited at the herbarium of the Botany Department of Post-Graduate College, Abbottabad, Pakistan.

Extraction and purification

The shade-dried ground bark (30 kg) was exhaustively extracted with methanol (50 mL) at rt. The extract was evaporated to yield the residue (818 g). The whole residue was dissolved in 1500 mL of water for 1 h and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. The *n*-butanol soluble extract (23 g) was subjected to CC over a silica gel column using $CHCl_3$ with gradient of methanol up to 100 %. Eleven fractions (Frs. 1-11) were collected. The Fr. 1 was loaded on silica gel (flash silica 230-400 mesh) and eluted with MeOH: $CHCl_3$ (0.5: 95.5) to afford compound (**1**).

Symploate; Undecyl 3-{4-[3-oxo-3-undecyloxypropyl]-1,3,2,4-dithiadiazetidin-2-yl}propanoate (**1**). Pale yellowish solid (20.7 mg): $C_{28}H_{54}N_2O_4S_2$; $[\alpha]^{23}_{D}$ – 2.5 ^{0} (*c* = 0.078, C_5H_5N); IR ν_{max} (KBr): 2919, 2850, 1740, 1365, 1238, 1029, 988, 793; ^{1}H and ^{13}C NMR: Table 1; FAB-MS (Neg. ion mode) m/z 545 $[M-H]^{-}$; HR-MS: 546.404097; HR-EIMS: 550.4 $[M + 4]^{+}$, 548.4 $[M + 2]^{+}$, 546.3 $[M]^{+}$; EIMS *m/z*: 546 $[M]^{+}$, 514 $[M- S]^{+}$, 441 $[M- HSNH (CH)_2COH]^{+}$, 375, 361, 329, 275 $[C_{14}H_{27}NHO_2SH]^{+}$, 273 $[C_{14}H_{27}NO_2S]^{+}$, 239 $[C_{14}H_{27}NO_2S – H_2S]^{+}$, 204, 193, 178, 143, 121 $[HSNH (CH)_2CO_2H]$, 119 $[SN(CH)_2CO_2H]$, 105, 57 (C_4H_9) (100 %).

α-Glucosidase Inhibition Assay:

α-Glucosidase (E.C.3.2.1.20) enzyme inhibition assay has been performed according to the slightly

modified method of Matsui *et al*. α-Glucosidase (E.C.3.2.1.20) from *Saccharomyces sp*. purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). The inhibition has been measured spectrophotometrically at pH 6.9 and at 37°C using 0.7 mM *p*-nitrophenyl α-D-glucopyranoside (PNP-G) as a substrate and 250 m units/mL enzyme, in 50 mM sodium phosphate buffer containing 100 mM NaCl. 1-Deoxynojirimycin (0.425 mM) and acarbose (0.78 mM) were used as positive controls. The increment in absorption at 400 nm due to the hydrolysis of PNP-G by α-glucosidase was monitored continuously with the spectrophotometer (Molecular Devices USA).[7]

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